Performance Evaluation of Continuous and Fed Batch Sequential Moving Bed Reactors for Removals of Phenol, Thiocyanate and Ammonia-Nitrogen from Wastewater

Thesis
Submitted in Partial
Fulfillment of the Requirements for the Degree of

Doctor of Philosophy

By

Biju Prava Sahariah
(Roll No. 08615203)

Centre for the Environment
Indian Institute of Technology Guwahati

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Dedicated

To

My Parents and Well Wishers

Centre for the Environment
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Certificate

It is certified that the work contained in this thesis entitled "Performance Evaluation of Continuous and Fed Batch Sequential Moving Bed Reactors for Removals of Phenol, Thiocyanate and Ammonia-Nitrogen from Wastewater" by Biju Prava Sahariah (Reg No. 08615203) has been carried out under my supervision in Centre for the Environment, Indian Institute of Technology Guwahati. I am forwarding her thesis to submit for the award of degree of Doctor of Philosophy from this institute. I certify that she has fulfilled all the requirements according to the rules of this institute regarding the investigations embodied in her thesis and this work has not been submitted elsewhere for a degree.

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Statement

I hereby declare that the work contained in this thesis entitled "Performance Evaluation of Continuous and Fed Batch Sequential Moving Bed Reactors for Removals of Phenol, Thiocyanate and Ammonia-Nitrogen from Wastewater" is carried out by me at Centre for the Environment, Indian Institute of Technology Guwahati, under the supervision of Dr. S. Chakraborty.

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ABSTRACT

Biological treatment process using microbes as treatment agent is economical and generates no undesirable by-products compared to costly physicochemical process like advanced oxidation or adsorption and stripping processes which generate secondary wastes. Wastewater generated from industries like petrochemicals, chemical industries, synthetic fuel processing, coal gasification etc. contain phenol, thiocyanate, ammonia and pyridine etc. Simultaneous presence of these pollutants make treatment challenging due to their inhibitory nature and demand higher oxygen in aerobic process whereas anaerobic process becomes highly sensitive and requires higher hydraulic retention time; a combination of anaerobic-anoxic-aerobic treatment could achieve the desirable results through partial treatment of organic matter in anaerobic reactor and release to anoxic unit, where the organic compounds and nitrate/nitrite are simultaneously removed. Finally, aerobic unit further oxidized residual pollutants like COD and NH_4^+-N.

In the present work moving bed reactor (MBR) system is selected since it is a combination of conventional activated sludge process and fluidized bed system, where mixed heterogenous biomass was grown on sponge cube of 1 cm^3 dimension and acclimatized with gradual increase in feed concentration. MBR is expected to be advantageous being less prone to clogging though retain the other benefits of attached growth system. Design data on application of anaerobic-anoxic-aerobic MBR system for removal of phenol, thiocyanate and ammonia containing wastewater are very limited. Two types of sequential MBR system: operated in continuous mode (CMBR; R1-R2-R3) and fed batch mode (FMBR; B1-B2-B3) were used with feed concentrations, hydraulic retention time (HRT), fill time, and cycle time as variable parameters (maximum feed phenol, thiocyanate and ammonia considered was 2500, 800 and 600 mg/L, respectively). Effect of pyridine on FMBR was also evaluated. Shock load and real wastewater were applied to evaluate the performance. Finally pollutant removal kinetics and predominant microorganisms in each reactor were identified.

R1 showed moderate removal of phenol and COD whereas B1 showed considerable phenol and COD removal with consistently low SCN^- and no NH_4^+-N removal. Probably, the microorganisms in R1/B1 did not support degradation of SCN^-. Specific methanogenic activity

(SMA) was achieved up to feed SCN^- 200 mg/L in R1/B1. Increased HRT enhanced the pollutant removal efficiencies. Phenol and COD removal in R1 was significantly inhibited at feed phenol, SCN^- and NH_4^+-N concentration 1500, 200, and 500 mg/L or above whereas inhibition in B1 was observed at phenol, thiocyanate and pyridine concentrations of 2000, 400 and 50 mg/L, respectively. Increased fill time negatively affected phenol/COD removal; however, instant fill was strongly unfavorable and gradual short fill facilitated performance of B1. Increased cycle time enhanced pollutant removal efficiency in B1 by increasing reactor HRT. In both R1 and B1 *Lactobacillus sp.* and *Streptococcus sp.* were common.

Influent to R2/B2 constituted by effluent from R1/B1 and recycle from R3/B3 (recycle ratio 1) with additional nitrate. Simultaneous phenol, thiocyanate, COD and nitrate removals were achieved. NH_4^+-N concentration increased along with sulfate due to thiocyanate degradation. SCN^- concentration of 54-400 mg/L in influent caused no significant inhibitory effect on phenol, thiocyanate and COD removal in R2/B2; however higher phenol concentration (above 468 mg/L in R2 and 511 mg/L in B2) showed negative effect on SCN^- removal. Contribution of R2 and B2 in SCN^- removal was significantly high compared to R3 and B3. In FMBR system B1 highly contributed in total phenol and COD removal, whereas in CMBR system both R2 and R3 were significantly responsible for phenol and COD removals. It was observed that nitrate- nitrogen was essential for SCN^- removal. COD:N removal ratio was 3-7 in R2 and 2.2-6 in B2. R2 showed presence of *Streptococcus sp., Neisseria sp., Corynebacterium sp.* and *Citrobacter sp.*. Similar to thiocyanate removal, B2 showed higher pyridine removal compared to B1 and B3. Pyridine caused no inhibitory affect in B2 in terms of phenol/COD removal though at high concentration (127 mg/L) it affected SCN^- removal. Increased cycle time enhanced pollutant removal whereas change in fill time showed insignificant affect on performance of B2 and the optimum fill time was 3-5 hour. *Pseudomonas sp., Enterobacter sp., E. coli* and *Citrobacter sp.* were observed in B2.

Phenol and thiocyanate concentration up to 468 and 122 mg/L, respectively caused no inhibitory on phenol, SCN^- and COD removals; however, higher influent NH_4^+-N of 360 mg/L or more significantly affected NH_4^+-N removal efficiency of R3. Lower reactor HRT (R3: 0.75 day) and phenol, thiocyanate and NH_4^+-N loading to R3 beyond 0.450, 0.061 and 0.284 g/L.day, respectively affected NH_4^+-N removal efficiency. Cycle time and fill time above 24 hour and 5 hour, respectively showed no major change in effluent profile of B3. B3 brought

down phenol, COD and SCN^- irrespective of HRT, presence of SCN^- and pyridine. Influent NH_4^+-N concentration ≥325-350 mg/L and HRT ~1.25 day significantly affected NH_4^+-N removal efficiency in B3. B3 always received low influent phenol, thiocyanate and COD concentration compared to R3. In B3 the threshold levels were lower than R3 being 0.001, 0.006 and 0.249 g/L.day, respectively for phenol, thiocyanate and NH_4^+-N loading indicating B3 was more sensitive to pollutant loading compared to R3. In R3 predominant microorganisms were *Staphylococcus sp., Lactobacillus sp., Neisseria sp.* and *Citrobacter sp.*. *Pseudomonas sp., Lactobacillus sp.* and *Citrobacter sp.* were observed in B3.

R1 recovered from shocks of phenol: 3000 mg/L; SCN^-:1000 mg/L, where as shocks by phenol: 3500 mg/L; SCN^-:1200 mg/L affected severely and achieved normal state only after stop of feed for 2-4 days. R2 was quite robust to sustain shock loads. R3 severely became vulnerable for nitrification with insignificant effect on phenol, COD or SCN^- removal. FMBR system failed to recover second SCN^- shock. B2 acted in very robust way when exposed to phenol shock and regain a new steady state after normal feed addition and stop for 4 days.

When coke oven wastewater spiked with synthetic pollutants were added to CMBR system, phenol/COD removal in R1 decreased than normal operation and performance of R2 and R3 were same in terms of phenol/COD removal. SCN^- removal in R2 dropped immediately and nitrification in R3 also fallen with addition of real wastewater.

R1 followed Bhatia et al. model for phenol and COD removal whereas R2, R3, B1, B2 and B3 followed the modified Stover- Kincannon model for pollutants removals.

Both CMBR and FMBR system successfully brought down phenol, thiocyanate and COD to discharge levels. NH_4^+-N being most tricky pollutant, other conventional methods like adsorption can be adopted for lower level of effluents released by the systems to reach the discharge limit.

Key words: Moving bed reactor, Biodegradation, Phenol, Thiocyanate, HRT, Cycle time, Fill time, Shock load

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ABBREVIATION

%	percentage
COD	Chemical oxygen demand
CMBR	Continuous moving bed reactor
FMBR	Fed batch moving bed reactor
FA	Free ammonia (mg/L)
h	hour
HRT	Hydraulic retention time
Is	Concentration of inhibitors (mg/L)
k	Substrate utilization rate (/day) according to first order kinetic model
K_b	Saturation constant (g/L.day)
Ks	Half saturation constant (mg/L)
K_i	Inhibitory constant Haldane model (mg/L)
KI	Inhibitory constant Bhatia et al model (L/mg)
NH_4^+-N	Ammonia nitrogen
NO_3^--N	Nitrate nitrogen
NO_3^--N	Nitrite nitrogen
N_R	Nitrification rate (g/L.day)
Q	Flow rate (L/day)
q_{max}	Maximum substrate utilization rate (g/L.day); Haldane model
R	Recycle ratio
R^2	Correlation coefficient
R_{max}	Maximum substrate utilization rate (g/L.day)
S_0	Influent concentration (mg/L)
S_e	Effluent concentration (mg/L)
SCN^-	Thiocyanate
T	Temperature in °C
UN	Unaccounted nitrogen (%)
χ^2	Chi square

CHAPTER 1

INTRODUCTION

Limited usable water resources require more advanced technology to preserve the water quality. Increasing industrialization and urbanization has mostly exploited and polluted the water resource. Presence of toxic pollutants like phenol, thiocyanate (SCN^-) and ammonia (NH_4^+-N) severely deteriorate water quality and affect aquatic flora-fauna. High concentration of these pollutants are generated in several wastewaters like petrochemicals, chemical industries, synthetic fuel processing, coal gasification, photofinishing, herbicide production and metal separation etc in association with other phenolic derivatives like cresol, catechol, resornicol etc., sulfides and chlorides, as well as small amounts of pyridine, cyanide and suspended solids etc (Neufeld and Valiknac, 1979; Kelly & Baker, 1990; Zhang et al. 1998; Li et al. 2003; Vázquez et al. 2006a; Sirianutapiboon et al. 2007; Li et al. 2011). These wastewaters demand adequate treatment before discharging to the receiving water. Organic pollutants have traditionally been removed from industrial effluents by costly physical and chemical processes, although biological methods have also been applied with reduced capital and operating costs (Mahajan, 1989).

Biodegradation, a potential biological treatment technique is based on the ability of microorganism (generally microorganism) to convert organic pollutants to water, carbon dioxide and biomass under aerobic or anaerobic condition. In most cases no undesirable by-products or secondary emissions, like in chemical scrubbing or thermal waste gas treatment, are generated (Patterson, 1985). Biodegradation is reported to be a reliable option for treatment of phenol and thiocyanate containing wastewater (Fang et al. 1996; Chen et al. 2008). However, at high concentrations inhibition occurs at degradation of either of the pollutant (Banerjee, 1996); whereas both are inhibitor for nitrification (Neufeld et al. 1986; Kumar et al. 2000; Kim et al. 2007; 2011 a) and causing treatment process challenging. A combination of anaerobic, anoxic, aerobic treatment for these multiple pollutant containing wastewater could achieve the desirable results instead of a single unit. Anaerobic reactor can act as partial removal of organic matter like phenol and COD and helps in readily oxidization by the subsequent treatment process (Yu et al. 1996). In the anoxic unit, organic compounds and nitrate (NO_3^--N) are simultaneously removed through denitrification process and residual COD and NH_4^+-N are further oxidized in the aerobic unit with generation of NO_3^--N . Several studies were carried out in anaerobic-anoxic-aerobic system with suspended growth reactors, fixed-bed biofilm reactors, fluidized bed reactors and membrane based reactors (Li et al. 2003; Jeong and Chung, 2006 a-b; Chakraborty and Veeramani, 2006; Zhao et al. 2009).

Modification of suspended growth reactors with additional media for support of microbial growth is proved to be very suitable for treatment of various industrial effluents (Johnson et al. 2000; Sigrun et al. 2002). Moving bed reactor (MBR), a combination of conventional activated sludge process and fluidized bed system, where biomass is grown on small carrier elements like sponge cubes having density less than water, is a completely mixed and continuously operated biofilm reactor (Ødegaard, 2006; Chen et al. 2008). The biofilm containing carriers (bed) move in the reactor due to the effect of aeration (for aerobic system) and mechanical stirrer or simply due to movement of water and gas. MBR reactor possesses various advantages over suspended-growth wastewater treatment systems, like (i) the treatment plant require less space and compactness due to the availability of the biofilm media with high specific surface area holding higher biomass; (ii) the final treatment results is less dependent on biomass separation since the biomass concentration

to be separated is 10 times lower than suspended growth system and (iii) lower sensitivity and better recovery from shock loadings (Sigrun et al. 2002). Also, the MBR is highly favorable for slow growing nitrifying bacteria and supposed to be robust for toxic and changing wastewater stream.

Mode of operation is also an important parameter which decides performance of bioreactor. Most studies with phenol, SCN^- and NH_4^+-N used reactors with continuous mode of operation in suspended growth system or fixed growth system (Chakraborty and Veeramani, 2006; Jeong and Chung, 2006a-b; Zhao et al. 2009). Fed batch operation of biological reactor is a promising method for treatment of high strength and /or toxic wastewater (Bali and Sengul, 2002). In fed batch operation, wastewater is fed to the reactor either intermittently or continuously with a low flow rate and contrary to continuous system, effluent is not withdrawn until the reactor is full and reaction is completed.

In this study, moving bed reactor (MBR) was operated with mixed microbial culture in presence of multiple pollutants like phenol, thiocyanate and NH_4^+-N at different pollutant loadings and operating conditions to evaluate the performance of the MBR system. Study was conducted both in anaerobic-anoxic-aerobic continuous moving bed reactor (CMBR) and fed batch moving bed reactor (FMBR) system.

Organization of the Thesis

The presentation of the work has been divided into five chapters. The current **Chapter 1** presents a general introduction of the present work. While literature that supports the present work is presented in **Chapter 2** along with objective and scope of the study. Details of the materials and methods adopted in the present study are described in **Chapter 3.** It elaborates the details of the sequential continuous moving bed bioreactor (CMBR) and fed batch moving bed bioreactor (FMBR). It also provides technical information about the analytical methods adopted in the present work. **Chapter 4** contains the results and discussions of experiments carried out with acclimatized mixed culture. This chapter addresses the efficiency of the biological system in treating phenol, thiocyanate and ammonia both in continuous and fed batch system. This chapter emphasizes the performance of CMBR and FMBR at different pollutant concentration and operational conditions. Finally it presents and discusses the efficiency of the acclimatized culture to

treat actual industrial wastewater supplemented with phenols, thiocyanate and ammonia-N and also stability profile during shock load condition. **Chapter 5** draws summary and appropriate conclusion based on the previous results and discussion and also provides some useful recommendations for future research in the relevant field.

CHAPTER 2

LITERATURE REVIEW

2.1 TYPE OF WASTEWATER

Toxic pollutants such as phenol, thiocyanate and ammonia are commonly present in various industrial wastewaters for example, coal gasification, coal carbonization, synthetic fuel processing operations, coke oven, petrochemical etc. (Zhang et al. 1998, Li et al. 2003, Vázquez et al. 2006a; Kim et al. 2008 a and b). Table 2.1 gives typical concentrations of pollutants of these industrial wastewaters. Direct discharge of these pollutants to the receiving water bodies can seriously affect the aquatic life, due to the toxic nature of these pollutants. The discharge of these pollutants in receiving water is also limited by current legislation.

2.2 CHARACTERISTICS AND TOXICITY OF POLLUTANTS

In present study major pollutants considered are phenol, thiocyanate, pyridine and two forms of nitrogen: ammonia and nitrate. The physical-chemical properties of these pollutants are enlisted in Table 2.2. Source, toxicity and permissible limits of these pollutants are discussed in the present section.

Table 2.1 Pollutant composition in industrial wastewater

Parameters (mg/L)	**Industry**		
	Petrochemical (Jingbo et al. 2009)	Coal gasification (Li et al. 2011)	Coke oven (Chang et al. 2008; Marañón et al. 2008a)
COD	9750	7000-14000	930-6500
Suspended solids	<150	2-10	19-3300
NH_3-N	10-30	50-1000	492-2195
NO_3^--N	-	-	-
NO_2^--N	-	-	-
Total nitrogen	-	100-600	-
Phenol	1500-2000	190-3500	400-1200
Thiocyanate		200-500	275-947
pH	-	7-9	6.8-8.2
Oil and grease	<50	-	-

Units are in (mg/L), except pH

Table 2.2 Physical and chemical properties of the pollutants studied (HSDB, 1989)

Parameters	**Phenol**	**Thiocyanate (as Potassium thiocyanate)**	**Ammonium (as Ammonium chloride)**	**Pyridine**
Molecular weight	94.11	97.18	53.49	79.1
Melting point (°C)	40.91	173.2	338	-41.6
Boiling point (°C)	181.7	500	-	115.2-115.3
Density (g/cm^3)	1.07	1.886	1.5274	0.982
Dissociation constant (pK_a)	9.95	-	9.245	5.23
Solubility (Distilled H_2O) at 20°C	1 g/ 15mL	32.5 g/15 mL	5.92 g/15mL	Miscible with water
Vapor pressure (mmHg)	0.35 at 25 °C	-	1 at 160 °C	20.8 at 25 °C

2.2.1 Phenol (C_5H_5OH)

Phenol is an aromatic molecule containing hydroxyl group attached to the benzene ring structure. The origin of phenol in the environment is both industrial and natural. The presence of phenol in water imparts carbolic odor to receiving water bodies (Ghadhi and Sangodkar, 1995). In coal gasification, synthetic fuel processing, coal carbonization wastewaters phenol is present along with its various derivatives like catechol, cresols etc. (ortho, meta and para) (Podkoscielny et al. 2003; Juang and Tsai, 2006; Yan et al. 2006). In addition to these, phenol is well known pollutant associated with various other industries like paper and pulp mills, coal mines, petroleum refineries, resin and plastic, rubber reclamation plants, foundry operation, leather and textile manufacturing, wood preservation plants, various chemical and petrochemical industries, pharmaceutics and agro-industrial operation as well as their wastewaters (Paula and Young, 1998; Sarfaraz et al. 2004; Jeong et al. 2006a; Eiroa et al. 2008).

Phenol is an antiseptic agent and is used in surgery, which indicates that they are also toxic to many microorganisms (EPA, 1979). Owing to the toxic effects on several biochemical functions of human being and fish including permeabilisation of cellular membranes and cytoplasmic coagulation, phenolic contaminants can damage sensitive cells and thus cause profound health and environmental problems (Nuhoglu and Yalcin, 2005). Phenol may be carcinogenic, mutagenic and teratogenic and its derivatives are classified as hazardous materials. Acute exposure of phenol causes burning of skin, central nervous system disorders, myocardial depression, cardiac toxicity including weak pulse, reduced blood pressure and leads to collapse and coma. Muscular convulsions, muscle weakness and tremors are also observed. It causes liver and kidney damages. Ingestion of 5–500 mg, accidental or intentional, has been reported to be fatal in infants and deaths in adults have resulted after ingestion of 1–32 g (Agency for Toxic Substance and Disease Registry 2003). At 48 h experimental period, LC_{50} values for *Daphnia sp.* were reported to be 13-23 mg/L and 3.9 mg/L for acute and chronic toxicity level, respectively (Hermens et al. 1984; Gersich et al. 1986; Cowgill and Milazzo, 1991). The increasing presence of phenols represents a significant environmental toxicity hazard. Therefore, the development of methods for removing phenols from industrial wastewater has generated huge interest.

2.2.2 Thiocyanate (SCN^-)

Thiocyanate is a C_1 sulfur compound and is produced as a natural metabolite in biological cyanide detoxification processes and is widely used in many industries: photofinishing, herbicide and insecticide production, dyeing, acrylic fiber production, thiourea production, gold and silver extraction, electroplating, soil sterilization and corrosion inhibition (Kelly & Baker, 1990). Concentrations of thiocyanate in effluents from gold ore concentrators have been reported to range from 168 to 680 mg/L (Lanno and Dixon, 1994). The main sources of thiocyanate along with phenol and ammonia in the environment are wastewater discharged from coal conversion (e.g. coking, refining, gasification and liquefaction) processes in which thiocyanate is generated by reaction of free cyanide with sulfur (Stratford et al. 1994).

Thiocyanate inhibits several enzyme systems and is particularly inhibitory to Mg^{2+} ATPases (Katz and Epstein, 1971). Thiocyanate is very stable compound and thus difficult to destroy (Boucabeille et al. 1994) and its toxicity increases at high concentration (more than 0.3 g/L as SCN^-). Besides, thiocyanate is reported to be toxic to microorganisms at relatively low concentrations of 58–116 mg/L (Wood et al. 1998). Thyroid function is depressed on chronic exposure to thiocyanate (Filove, 1993). At 96 h experimental period, LC_{50} values for *Daphnia sp.* were reported to be 0.554-33.467 mg/L and 1.43 mg/L for acute and chronic toxicity level, respectively and toxicity was increased by both low pH values and higher temperatures (Watson and Mally, 1987; Zhang et al. 1998). It has been reported that a concentration greater than 15 mg SCN^-/100 mL in mammalian blood is critically toxic (Paruchuri et al. 1990).

2.2.3 Ammonia-nitrogen, Nitrate and Nitrite-nitrogen

Ammonia **(**$NH_3 + NH_4^+$) in environment is excreted by animals and produced as a result of the decomposition of organisms and sewage by microorganisms. Ammonia nitrogen is one of the main pollutant in wastewater from fertilizer and coal gasification industries, petroleum refineries, petrochemical plants, landfill leachate etc. (Yu et al. 2003; Chen et al. 2008). Nitrate/ nitrite **(**NO_3^- and NO_2^-) are mainly produced as secondary pollutant in aerobic treatment unit through nitrification. Many industries produce wastes that contain high concentrations of nitrate. During the production of cellophane, explosives, fertilizer,

pectin, and in metals furnishing industries, nitrate wastes containing greater than 1000 mg/L NO_3^--N are generated (Fernández-Nava et al. 2010).

Ammonia is known to be toxic to aquatic life and often results in increase oxygen demand of the receiving waters (Arthur et al. 1987; Tchobanglous et al. 2004). Ammonia toxicity expressed as total ammonia ([NH_3+ NH_4^+], mg/L), increases with water pH because ammonia enters organisms as NH_3 and the proportion of NH_3 increases with increase in water pH. The pK_a of ammonia/ammonium reaction is around 9.5 and varies with ionic strength, pressure and temperature. Compiled normalized data on acute toxicity in various species of fish indicates that the effect of increased temperature on ammonia is minor between 3 and 30 °C in freshwater systems (USEP, 1985). The nature of ammonia toxicity seems to be similar in fish and mammals. Convulsion, coma and death take place in both groups of animals soon after ammonia intoxication. Most biological membranes are permeable to ammonia but relatively impermeable to ammonium ions. The LC_{50} value (95% confidence interval) reported for acute toxicity of ammonia (expressed as un-ionized ammonia nitrogen, NH_3-N) to *Daphnia magna* at 48h experimental period was 2.94 (2.70 to 3.22) mg NH_3-N/L. The 21-day chronic toxicity value, reported was between 0.42 and 0.87 mg NH_3-N/L (Gersich and Hopkins, 1986).

Nitrite-nitrogen (NO_2^--N) is a highly toxic compound for fish, benthic fauna, plants, bacteria, plankton, nitrifiers and methanogens (De Beer et al. 1997). Nitrate-nitrogen (NO_3^--N) enrichment of receiving waters should be avoided since drinking water containing high amount of nitrate is reported to increase the probability of infant methaemoglobinaemia (blue baby diseases) and gastric cancers (Dahab et al. 1994). Higher amounts of nitrate when discharged into the environment can cause serious problems such as the eutrophication of rivers and deterioration of water sources, as well as hazards to human health. Furthermore, nitrates can also form nitrosamines and nitrosamides, potentially carcinogenic compounds (Ono et al. 2000).

2.2.4 Pyridine (C_5H_5N)

Pyridine is a heterocyclic volatile aromatic compound and is a weak organic base, colorless liquid with a threshold odor concentration of 58.6 mg/L and an odor index of 2390 (Mohan et al. 2004; Pandey et al. 2006). Pyridine and its derivatives are an important class of

aromatic nitrogen heterocyclic compounds. The pyridine ring occurs in nature in the form of pyridine coenzymes, plant alkaloids and natural products and is released to environment on the death and decay of the host species. Coal gasification, processing of oil shale and pesticide use are anthropogenic sources of pyridine and its derivatives. Pyridine is the parent of a series of chemicals, as an industrial solvent in paint and rubber preparation, raw material of herbicide synthesis. It is also used directly in the denaturation of alcohol and to make many different products such as medicines, vitamins, dyes, adhesives and in water proofing of fabrics (Mohan et al. 2005; Lataye et al. 2006). These pyridine compounds inevitably find their way into effluents.

Pyridine is classified as a hazardous substance in the USEPA list of priority pollutants because of its recalcitrant, toxic and teratogenic nature (Lataye et al. 2006; Padoley et al. 2006). It is mildly toxic if inhaled; its vapor is skin and severe eye irritant and exposure to it can cause gastrointestinal upset, liver and kidney damage, headache, nervousness, dizziness, insomnia, nausea, frequent urination, and dermatitis. Carcinogenic toxicity of pyridine and its derivatives are well reported (Lee et al. 1994; Ren et al. 2006; Wang et al. 2008). LC_{50} for *Daphnia* ranges 70-240 mg/L.

2.2.5 Permissible limit for pollutants for discharging into water body

In Indian standard, maximum allowable limit of phenol concentration is 0.001 mg/L for drinking water and 1 mg/L for industrial effluent discharge into surface waters (Mukherjee et al. 2007; Vasu, 2008). Effluent phenol concentration is fixed at 0.0035 mg/L for petroleum oil refineries by Central Pollution Control Board (CPCB), India. Permissible discharge level for ammonia and total nitrogen is 15 mg/L and 50 mg/L, respectively by CPCB, India. However no report is available on permissible limit of thiocyanate and pyridine. The World Health Organization has limited thiocyanate concentration in drinking water to 0 mg/L. Occupational Safety and Health Administration (OSHA) and American Conference of Governmental Industrial Hygienists (ACGIH) recommended that the average exposure limit of pyridine over a 10-h work shift is 5 mg/L (ATSDR, 1992).

2.3 TREATMENT TECHNOLOGIES FOR REMOVALS OF PHENOL, THIOCYANATE, AMMONIA-N, NITRATE-N AND PYRIDINE

Phenol, thiocyanate, ammonia, nitrate and pyridine are toxic pollutants and in order to minimize the severe and irreversible damage to aquatic system, treatment of these pollutants is needed to bring down to permissible limits for safe disposal of wastewater (Poots et al. 1978). Both physicochemical and biological treatment methods are available for removal of these pollutants.

2.3.1 Physicochemical processes

Physicochemical treatment processes commonly used in wastewater treatment include adsorption, air stripping, electro chemical oxidation, advance oxidation process etc. Adsorption, a separation method in which the contaminants, dissolved in water phase, are transferred to the surface of adsorbent, where it is accumulated for subsequent extraction or destruction of the contaminants. Activated carbon adsorption is a conventional wastewater treatment method used for the removal of recalcitrant organic compounds as well as residual inorganic compounds such as nitrates, sulfides and heavy metals. The adsorption process is widely applied for control of color and odors, removal of organic compounds. Numerous literatures reported treatment of phenols and cresols by adsorption (Kennedy et al. 2007; Singh et al. 2008; Liu et al. 2008; Hadjar et al. 2011; Zhu et al. 2011). Adsorption process applied for removal of other pollutant like thiocyanate (Namasivayam and Kumar, 2007), ammonia (Du et al. 2005; Przepi´orski, 2006; Kleemann et al. 2000) and pyridine (Mohan et al. 2004; Ocampo-Perez, 2010) are also reported.

Another physicochemical process air stripping, involves the transfer of volatile organics from liquid phase to the air phase by greatly increasing the air/water contact area. Typical aeration methods include packed towers, diffusers, trays and spray aeration etc. (Metcalf and Eddy, 2003). If air emissions are not required to be regulated, air stripping is by far the simplest and cheapest solution for the removal of volatile compounds from water. Ammonia wastewater treatment by air stripping has been reported by researchers (Quan et al. 2009; Guˇstin and Marinˇsek-Logar, 2011).

The use of electrochemical oxidation for the destruction of organic compounds in aqueous solutions has been tried on bench and pilot plant scale operation (Borras et al. 2003;

Quiroz et al. 2005; Wang et al. 2006; Flox et al. 2009), but is not used commercially because of its high operating cost. In this process electrons are generated or assimilated by the electrodes and thus it supplies a clean reactant and does not increase the number of chemical molecules involved in the process. The electrochemical oxidation of organic compounds is thermodynamically favored against the competitive reaction of oxygen production by oxidation of water. However, the kinetics of oxidation of water is much faster than the kinetics of oxidation of the organic compounds, among other reasons because of its higher concentration (Boudenne et al. 1996; Brillas et al. 1998).

Advanced oxidation processes (AOP) refers specifically to processes in which oxidation of organic contaminants occurs primarily through reactions with hydroxyl radicals (Glaze et al. 1995). It involves two stages of oxidation: (1) the formation of strong oxidants (e.g., hydroxyl radicals) and (2) the reaction of these strong oxidants with organic contaminants in water (Alnaizy and Akgerman, 2000). In water treatment applications, AOPs usually refer to a specific subset of processes that involve O_3, H_2O_2, and/or UV light. All these processes can produce hydroxyl radicals, which can react with and destroy a wide range of organic contaminants, including phenol and substituted phenols. Phenol removal by ozonization was reported in many literatures (Chang et al. 2008; Amin et al. 2010). Coelho et al. (2006) reported AOP for petroleum wastewater. Kusic et al. (2006) reported phenol removal through Fenton oxidation. Collado et al. (2010) reported catalytic wet oxidation of thiocyanate. Stapleton et al. (2006) and Padoley et al. (2011) reported pyridine removal by photocatalytic oxidation and Fenton oxidation, respectively.

2.3.2 Limitations of physicochemical treatment techniques

- Adsorption
  - The pollutant in the wastewater is selectively transferred into the solid phase (adsorbent) instead of eliminating it from the wastewater.
  - Produces a large amount of solid waste, which further requires a safe disposal.
- Electrochemical oxidation
  - Expensive in comparison with other processes and the mechanism in water is rather complex and becomes uneconomical process with lack of feasibility towards commercialization.

- ❖ Moreover, the effluent needs to be a conductor and therefore, a salt should be added in the effluent in case it does not have good conductivity

➢ Advanced oxidation processes

- ❖ Supplement oxidant such as O_3 and H_2O_2 are required to achieve a maximum removal efficiency, which results in increased cost
- ❖ Rigorous studies are needed to determine the optimum dosage.

➢ Air stripping

- ❖ Transfer of pollutant from liquid phase to gaseous phase and air pollution problem.

2.3.3 Biological treatment process

In biological treatment process the main treatment mechanism is bioremediation, which is defined as the elimination, attenuation or transformation of polluting or contaminating substances by the use of biological processes (Lynch and Moffat, 2005). Biological methods or bioremediation processes appear to be a potentially economical, energy efficient and environmentally friendly approach (Vidali, 2001; Shim et al. 2002). In biological process the most important factors are (a) microorganisms capable of degrading the target pollutants are required, (b) the microorganisms must have mechanisms for capturing the energy released during the degradation process, and (c) the environmental conditions must be appropriate for maintenance of active, if not growing, microorganisms (Liu et al. 1994). Bacteria may be divided further into three groups according to their response to free molecular oxygen. Biological treatment process can be either aerobic (molecular oxygen is the electron acceptor) or anaerobic (in absence of oxygen). Anaerobic treatment offers numerous significant advantages, such as low sludge production, low energy requirement, and possible energy recovery (Ghosh and Pohland, 1974; van Staikenburg, 1997). Another biological treatment environment, named anoxic condition can be developed where organic compounds are used as an electron donor, while nitrate or nitrite is used as an electron acceptor. Therefore, organic compounds and nitrate/ nitrite can be simultaneously removed under anoxic/denitrifying conditions. Many organic compounds, which are non-biodegradable under aerobic condition or slowly degradable under anaerobic conditions, can be effectively utilized by denitrifying microbes as a carbon source (Zoh et al. 1997).

2.3.4 Biodegradation of Pollutant

Treatment of phenol, thiocyanate and ammonia is elaborately studied in individual or in common using various bioreactors. Individual biodegradation of these pollutants are discussed in present section.

2.3.4.1 Phenol biodegradation

Many microorganisms are capable of degrading phenol through the action of variety of enzymes. Wide range of bacteria like *Pseudomonas sp., Bacillus sp., Acinetubacter sp., Corynbacterium sp., Enterobacter sp., Alcaligenes sp. Streptomyces sp., Serratia sp.,* are reported to be efficient in phenol degradation in anaerobic, anoxic and aerobic environments (An et al. 2001; Prieto et al. 2002; Neumann et al. 2004; Fang et al. 2006; Nilotpala and Ingle, 2007; Jiang et al. 2007; Ho et al. 2009**).** Loh et al. (2000) reported biodegradation of phenol from wastewater is generally more cost effective than the physicochemical treatment process. Efficient phenol removal is reported in temperature of psychrophilic, mesophilic and or thermophilic conditions (Scully et al. 2006; Fang et al. 1996; 2006).

Cleavage of the aromatic ring is typically achieved via the ortho (intradiol) or meta (extradiol) pathways (Yang and Humphrey, 1975). The most well known key intermediates resulting from the biodegradation of aromatic compounds are catechol, protocatechuic acid and gentisic acid. These intermediates further undergo ring fission following the Krebs cycle to yield other metabolites, such as pyruvic acid, acetic acid, succinic acid and acetyl-CoA (Loh and Chua, 2002). Degradation of phenol follows a sequence of (a) hydroxylation to catechol, (b) ring cleavage via catechol-2,3-dioxygenase to 2-hydroxymuconic semialdehyde (HMSA) for meta-pathway, and via catechol-1,2-dioxygenase to cis, cis-muconate for ortho pathway, (c) HMSA is either oxidized to 4-oxalocrotonate or hydrolyzed to 2-oxopent-4-enoate in case of meta and cis, cis-muconate gets converted into muconolactone for ortho-cleavage (Kwon and Yeom, 2009; Cao and Loh, 2008). Phenol degradation in anaerobic, anoxic and aerobic environment is given in equation 2.1a, 2.1.b and 2.1.c with generation of final product bicarbonate, water and carbon dioxide etc (Fang et al. 1996; Chakraborty and Veeramani, 2006).

$$C_6H_5OH + 17H_2O \rightarrow 6HCO_3^- + 34H^+ \quad \text{(Eq.2.1a)}$$

$$C_6H_5OH + 5.6NO_3^- + 0.2H_2O \rightarrow 6HCO_3^- + 0.4H^+ + 2.8N_2 \quad \text{(Eq.2.1b)}$$

$$C_6H_5OH + 7O_2 \rightarrow 3H_2O + 6CO_2 \quad \text{(Eq. 2.1c)}$$

$\Delta G^o_{(aq)}$ for reaction shown in equation 2.1 (a), 2.1 (b) and 2.1 (c) are (-) 2549 kJ.mol^{-1}, (-) 2802 kJ.mol^{-1} and (-) 2866 kJ.mol^{-1}, respectively, suggesting phenol degradation in anaerobic, anoxic and aerobic environments are thermodynamically favorable and aerobic treatment of phenol is the most favorable.

2.3.4.2 Thiocyanate degradation

Thiocyanate wastewater is usually treated by an activated sludge process, where microbial activity degrades this substance as source of nitrogen, sulfur carbon and, energy (Kim and Katyama, 2000). Thiocyanate degradation capacity was initially reported to be limited to strains of neutrophilic *Thiobacilli sp.* by various researchers (Katayama & Kuraishi, 1978; Smith and Kelley, 1988). However, several new thiocyanate-oxidizing bacteria are identified being capable of growth on thiocyanate at high pH and presence of high concentration of salt (Sorokin et al. 2001). Two major pathways for thiocyanate metabolism have been identified. The first reaction pathway involves carbonyl sulfide as an intermediate, whereas cyanate is an intermediate in the second pathway. Hung and Palvosthis, (1997) reported thiocyanate biodegradation in aerobic condition to proceed as follows: first, thiocyanate is hydrolyzed to cyanate (OCN^-) and sulfide (S^{2-}); second, cyanate is hydrolyzed to ammonium (NH_4^+) and bicarbonate (HCO_3^-) ions; finally, sulfide is oxidized to sulfate (SO_4^{2-}). Thus, the overall degradation reaction is expressed as equation (2.2a).

$$SCN^- + 3H_2O + 2O_2 \rightarrow HCO_3^- + NH_4^+ + SO_4^{2-} + H^+ \quad \text{(Eq. 2.2a)}$$

($\Delta G^o_{(aq)}$ = -824.65 kJ.mol^{-1})

In absence of oxygen, mineralization of SCN^- generates sulfide, ammonia and HCO_3^- as shown in equation 2.2 (b) (Hung and Pavlostathis, 1997). Not much information is available about the possibility of anaerobic growth with thiocyanate.

$$SCN^- + 2H_2O \rightarrow CO_2 + NH_4^+ + S^{2-} \quad \text{(Eq. 2.2b)}$$

$(\Delta G^o_{(aq)} = -9.1\ kJ.mol^{-1})$

An early publication of De Kruyff et al. (1957) reported that *Thiobacillus denitrificans* can grow with thiocyanate, aerobically or anaerobically, in the presence of nitrate as the electron acceptor, reducing the latter completely to N_2, while *Thiobacillus thioparus* only reduced nitrate to nitrite in the presence of thiocyanate. Andreoni et al. (1988) reported thiocyanate-dependent denitrification by a mixed bacterial population in a thiocyanate waste-treatment plant. Sorokin et al. (2007) reported anaerobic thiocyanate oxidation by denitrifying isolates proceeded through intermediate cyanate, similar to aerobic thiocyanate degradation utilizing halo-alkaliphiles genus *Thioalkalivibrio* with ammonia, sulfate and nitrogen gas as the final products as shown in equation 2.2 (c-e).

$$5SCN^- + 8NO_3^- + H_2O \rightarrow 5SO_4^{2-} + 4N_2 + CNO^- + 2H^+ \quad \text{(Eq. 2.2c)}$$

$$5CNO^- + 5HCO_3^- + 10H^+ \rightarrow 5NH_3 + 10CO_2 \quad \text{(Eq. 2.2d)}$$

$$SCN^- + 1.6NO_3^- + 0.2H_2O + 1.6H^+ + HCO_3^- \rightarrow SO_4^{2-} + NH_3 + 2CO_2 + 0.8N_2 \quad \text{(Eq. 2.2e)}$$

$(\Delta G^o_{(aq)} = -824.01\ kJ.mol^{-1})$

There are differences between autotrophic and heterotrophic metabolism of thiocyanate as well as differences within each group of organisms in anoxic environment. The autotrophic isolate studied by Youatt (1954) used the cyanate pathway, whereas the isolate studied by Katayama and Kuraishi, (1978) used the carbonyl sulfide pathway. Mason et al. (1994) isolated two heterotrophs *Acinetobacter jeunii* and *Pseudomonas fluorescens* that were able to metabolize thiocyanate. They suggested that the former organism used the carbonyl sulfide pathway and the latter the cyanate pathway. Sorokin et al. (2007) isolated a new species from a hypersaline lake and named *Thiohalophilus thiocyanooxidans* which was an incomplete denitrifier able to use thiocyanate as an electron donor and nitrite as an electron acceptor. *Thiohalophilus thiocyanooxidans* followed both carbonyl sulfide pathway and cyanate pathway for thiocyanate degradation through generation of carbonyl sulfide or cyanate as intermediate (Sorokin et al. 2007).

2.3.4.3 Nitrogen removal

(a) Ammonia

Conventional microbial nitrogen removal is based on autotrophic nitrification and heterotrophic denitrification. The removal involves (i) aerobic nitrification (i.e., the conversion of NH_4^+ to NO_2^- and further to NO_3^- by ammonia oxidizing bacteria (AOB) and nitrite oxidizing bacteria (NOB), respectively) with molecular oxygen as the electron acceptor and (ii) anoxic denitrification i. e., the reduction of nitrate or nitrite to N_2, mostly catalyzed by heterotrophic bacteria. AOB and NOB are slow growers and influenced by many environmental factors such as pH, temperature, C/N ratio, unsaturated fatty acids, dissolved oxygen, ammonia, and nitrite concentrations (Stephen et.al. 1998; Svenson et.al. 2000; Avrahami et.al. 2003). The fraction of nitrifying bacteria in the biomass is important in nitrogen removal systems, since nitrifiers are considered poor competitors for oxygen compared to heterotrophs (Rittmann et. al. 1999). The relevant nitrification reactions are as shown in equation 2.3 (a) - (c)

$$NH_4^+ + 1.5O_2 \rightarrow NO_2^- + 2H^+ + H_2O \qquad \text{(Eq. 2.3a)}$$

$$NO_2^- + 0.5O_2 \rightarrow NO_3^- \qquad \text{(Eq. 2.3b)}$$

$$NH_4^+ + 2O_2 \rightarrow NO_3^- + 2H^+ + H_2O \qquad \text{(Eq. 2.3c)}$$

($\Delta G^o_{(aq)} = -204.54$ kJ.mol^{-1})

(b) Nitrate and nitrite removal

Biological denitrification is a reliable method for nitrogen removal from wastewater. The anoxic denitrification (i.e., the conversion of NO_3^- and NO_2^- to gaseous nitrogen) is accomplished with a variety of electron donors in presence of heterotrophic denitrifying bacteria using nitrate/nitrite as electron acceptor (Grabinska-Loniewska, 1991; Tam et al. 1992; Akunna et al. 1993). This process results in simultaneous removal of nitrate/nitrite and organic/ or inorganic matter used as electron donor.

The anoxic denitrification involves the following reactions as shown in equation [2.3(d) and (e)]

$$2NO_3^- + 10H^+ + 10\ e^- \rightarrow N_2 + 2OH^- + 4H_2O \qquad \text{(Eq. 2.3d)}$$

($\Delta G^o_{(aq)} = -1040.04$ kJ.mol^{-1})

$2NO_2^- + 6H^+ + 6\,e^- \rightarrow N_2 + 2OH^- + 2H_2O$ (Eq. 2.3e)

($\Delta G^o_{(aq)} = -714.36$ kJ.mol^{-1})

As nitrification and denitrification are carried out under different conditions and by different microorganisms, experience shows that these processes have to be separated in time or space to function effectively. The conventional nitrification/denitrification reactions have been known for a long time. The nitrification reaction consumes a large amount of oxygen, requiring 4.2 g of oxygen for each gram of ammonium nitrogen nitrified (EPA, 1975). During denitrification, the requirement of organic carbon is significant. For example, 2.47 g of methanol is required per gram of nitrate nitrogen for complete denitrification (McCarty et al. 1969). Therefore if the electron donor is present in adequate amount in wastewater there is feasibility of high denitrification efficiency with high nitrogen removal.

2.3.4.4 Pyridine degradation

Chemically, the pyridine ring is susceptible to reduction and these characteristic have been exploited by microorganisms for evolving mechanisms for pyridine ring degradation (Liu et al. 1994). Two general strategies of bacterial pyridine degradation involve (i) hydroxylation reactions, followed by reduction, and (ii) (aerobic) reductive pathway not initiated by hydroxylations (Kaiser et al. 1996).

Pyridine mineralization in anaerobic, anoxic and aerobic conditions is reported to be occurred as shown in Equation 2.4 a-c (Liu et al. 1994)

$C_5H_5N + 4.5H_2O + H^+ \rightarrow 2.25CO_2 + 2.75\,CH_4 + NH_4^+$ (Eq. 2.4a)

($\Delta G^o_{(aq)} = -169$ kJ/mole)

$C_5H_5N + 4.4NO_3^- + 5.4H^+ \rightarrow 5CO_2 + 2.2N_2 + 3.2H_2O + NH_4^+$ (Eq. 2.4b)

($\Delta G^o_{(aq)} = -2277$ kJ/mole)

$C_5H_5N + 6O_2 + 3H^+ \rightarrow 5CO_2 + + 2H_2O + NH_4^+$ (Eq. 2.4c)

($\Delta G^o_{(aq)} = -2682.4$ kJ/mole)

Adav et al. (2007) observed no inhibitory effect of pyridine on phenol degradation in aerobic environment upto pyridine concentration of 1500 mg/L. Li et al. (2001) reported complete removal of pyridine in anoxic environment within 12-24 h at initial pyridine concentration of 20-100 mg/L in absence of any other pollutant. Li et al. (2009) also reported complete removal of pyridine by an aerobic strain *Streptomyces* within 8 days from initial concentration of 2000 mg/L at pH of 7. Sun et al. (2011) observed simultaneous degradations of pyridine and phenol by an aerobic strain *Rhodococcus*, using phenol as carbon source and pyridine as the nitrogen source. Pyridine and its derivatives are reported to be toxic to the anaerobic process (Gijzen et al. 2000). Blum et al. (1986) reported pyridine have inhibitory effect on anaerobic digestion in presence of phenol.

2.3.5 Bioreactors used for treatment of pollutants in single and combination

Both suspended growth and attached growth bioreactors were used for removal of phenol, thiocyanate, ammonia, nitrate and pyridine. In suspended system, microbes are kept suspended in the liquor whereas in attached growth system, some carrier material is provided in the reactors for use as a bed for microbial growth. Bioreactors were operated in aerobic anaerobic and anoxic environment either as single units or in series of sequence.

2.3.5.1 Suspended growth system

A. Suspended growth aerobic reactor

Activated sludge process (ASP) is the simplest form of suspended growth aerobic process. ASPs are widely used for treatment of various industrial wastewaters including removals of phenol, thiocyanate and ammonia-N present in single or in combination (V´azquez, et al. 2006a, 2006b; Sharma et al. 2012). Yamagishi et al. (2001) achieved complete removal of phenol from a phenol concentration of 300-1900 mg/L along with simultaneous nitrification in an ASP with cross flow filtration with phenol and ammonia–N wastewater. Phenol removal rate and nitrification rate were 0.30 and 0.20 g/L.day, respectively and denitrification efficiency recorded was 0-13% and also observed emission of nitrous oxide in the aeration period. Amor et al. (2005) found ASP worked satisfactorily up to phenol concentration 1000 mg/L and above 1000 mg/L the level of nitrification decreased at ammonia-N concentration 350 mg/L. They observed in batch assays that, phenol was

completely biodegraded at concentrations ranging from 100 to 2500 mg/L, showing more inhibition on nitrification process at higher initial phenol concentrations. However in the activated sludge reactor, at applied ammonium loading 0.14 g NH_4^+-N/L.day (350 mg NH_4^+-N/L) and at increased phenol (35-2800 mg/L), they achieved high phenol removal efficiencies, above 99.9% along with ~99.8% ammonia removal with no inhibition by phenol on nitrification.

The highest biodegradation rate of thiocyanate reported using the suspended sludge system was 1.7 g/L.day when there was no toxic substance such as phenol and cyanide (Plessis, 2004). Hung and Palvostathis (1997) achieved complete oxidation of thiocyanate to ammonia, bicarbonate and sulphate, partial inhibition on nitrification at low HRT of 0.32 days in an ASP. Lee et al. (2008) observed decrease in thiocyanate removal efficiency of an ASP from 99% to 17% at influent concentration 500 mg/L when reactor SRT was decreased from 3.0 day to 0.8 day. They observed a sudden drop of removal efficiency from 96% to 43% between 2.0 and 1.8 day SRTs. Kwon et al. (2002) studied thiocyanate degradation in presence of phenol (625 mg/L) at varied pH using single culture *of Acremonium strictum*. Maximum thiocyanate degradation was achieved at pH 6, though degradation rate started to be inhibited above thiocyanate 4000 mg/L and thiocyanate was completely degraded up to 7400 mg/L within 85 h in shake-flask cultures.

Kim et al. (2008b) observed thiocyanate, phenol, and cresol inhibited nitrification process at concentration above 200, 200 and 100 mg/L, respectively and ammonia above 350 mg/L showed substrate inhibition towards nitrification in ASP. Free cyanide and thiocyanate were efficiently degraded in ASP system from wastewater containing phenol, thiocyanate, ammonia and cyanide. The system exhibited thiocyanate degradation and nitrification inhibition by free ammonia. The main factor was expected to be pH, which mainly control NH_3/NH_4 equilibrium in water or wastewater.

Vázquez et al. (2006b) investigated simultaneous removals of ammonia, organic matter (particularly phenol) and thiocyanate at higher concentration of 504-2340 mg/L, 119-350 mg/L and 180-370 mg/L respectively in a single step ASP and reported that bicarbonate addition enhanced the removal of pollutant and nitrification efficiency with increase of pH up to 6 and 8 for thiocyanate and phenol, respectively and high concentration of ammonia affected the kinetics of SCN^- biodegradation. Vázquez et al. (2006a) during study of a two

steps ASP system, observed removals of COD, phenol and SCN^- in the first step ASP while the second ASP efficiently removed ammonia. The best results were obtained when the HRT was 98 and 86 hours for first step and second step ASP respectively.

The most obvious problems faced in conventional ASP system used for treatment of phenol, thiocyanate and ammonia in combination are the toxicity and inhibitory nature of the pollutants towards each other, washout of biomass, poor settle ability due to occurrence of filamentous microorganisms, inhibition to nitrifiers by toxic compounds, high oxygen consumption and energy demand leading to high operational cost and many times ammonia inhibition. Thereby making conventional single stage activated sludge process inadequate to handle these wastewaters (Vázquez et al. 2006a; Kim et al. 2008b).

B. Suspended growth anaerobic reactor

Anaerobic removal of phenol is widely reported in a wide range of influent concentration, temperature (psychophilic, mesophilic and thermophilic) and HRT (Fang et al. 1996, 2006; Ramakrishnan and Gupta, 2006; Boubaker and Ridha, 2007; Bajaj et al. 2009). Blum et al. (1986) studied 24 constituents from coal conversion wastewater in serum bottle and anaerobic filter for biodegradability and toxicity assay based on gas production by degradation. They reported that anaerobic filters were superior to batch serum bottle cultures because they were able to acclimate more quickly and to higher concentrations of phenolics. Simultaneous degradations up to 98% of phenol and 20% of m- cresol without a carbohydrate co-substrate, for wastewaters containing up to 900 mg/L of phenol and 320 mg/L of m-cresol in an up flow anaerobic sludge blanket (UASB) reactor operated at 37^oC, one-day hydraulic retention and effluent recycle is reported by Zhou and Fang, (1997). Further increases of phenol and m-cresol concentration in influent impaired the phenol-degrading activity of the biomass. However biomass was able to regain activity once the phenolic concentrations were lowered. In treating a wastewater containing 600 mg/L of phenol, m-cresol had a threshold toxicity of 600-800 mg/L in a continuous reactor; but in a batch reactor the toxicity was progressive with an IC_{50} value of 330 mg/L. Hung and Palvostathis (1997) investigated the fate of SCN^- and cyanate (OCN^-) under methanogenic conditions at 35 °C with an organic mixture. Results revealed that thiocyanate was stable under those conditions, and had no adverse effect on methanogenesis at a concentration as

high as 145 mg/L. In contrast, cyanate at a concentration as low as 12.6 mg/L inhibited methanogenesis that can be recovered though repeated cyanate additions cause profound inhibition. The addition of 2,4-dinitrophenol, carbonyl cyanide *m*-chlorophenyl hydrazone (CCCP), and mercuric chloride (Hg_2Cl_2) completely inhibited the activity of the ammonium oxidizing sludge.

C. Anoxic reactor

Phenol removal in anoxic environment is widely reported by many researchers in single (Ramakrishna and Gupta, 2008; Bajaj et al. 2010**)** or in presence of thiocyanate and ammonia (Kim et al. 2008a; Zhao et al. 2009). Report on thiocyanate removal in anoxic environment with generation of ammonia and sulfate is reported though limited (De Kruyff et al. 1957; Smith and Kelley, 1988; Sorokin et al. 2001, 2004). Li et al. (2001) conducted batch experiments to investigate anoxic biodegradation of five refractory nitrogenous heterocyclic compounds, i.e. pyridine, indole, quinoline, isoquinoline, 2-methyl quinoline, from coke plant wastewater. These compounds were effectively degraded by the denitrifying organisms in acclimated activated sludge within 2.5 days.

D. Sequencing Batch Reactor (SBR)

SBR is the concept of biological reactors characterized by exposure of the microorganisms to periodically changing environment such as i) high and low substrate concentration, ii) batch wise introduction of wastewater and withdrawal of the treated water causing periodic changes in the water level in the reactor or iii) execution of metabolic processes followed by solid liquid separation in the same tank. Typically activated sludge is used to perform the metabolic process. In SBR each cycle with intermittent aeration is used resulting in presence of anaerobic phase beneficial for organic matter degradation, anoxic phase for denitrification with simultaneous nitrogen gas and organic matter removal and finally the aeration phase for nitrification.

Sarafaz et al. (2004) developed anoxic granules for phenol degradation and it was maintained for long time without loss of its phenol degradation activity at 6 h cycle time in a SBR. They achieved more than 80% phenol and nitrate removal up to influent phenol 1050 mg/L and COD/NO_3 ratio observed to 3.4. However phenol removal efficiency

steeply decreased to 56% at influent phenol concentration of 1150 mg/L. Sirianuntapiboon et al. (2007) also reported SBR system was capable for removal of organic matter, nitrification and denitrification in a single reactor. They observed that thiocyanate (SCN) compounds in photo-processing wastewater (PPWW) could be treated by SBR system without any release of thiocyanate to the atmosphere during the aeration step and SCN^- loading greater than 0.084 g/L.day showed negative effects on the growth of bio-sludge and removal efficiencies of the system. The required acclimatization period of the system also increased with an increase in SCN^- concentration or loading. Marañón et al. (2008a) reported COD, thiocyanate and phenol removals of 80-90%, 95% and >97%, respectively from industrial effluent containing 1100-1700 mg/L COD, 210-487 mg/L thiocyanate and 185-253 mg/L phenol with no ammonia removal at HRT more than 4.8 days in SBR system. Kargi et al. (2004) observed phenol concentration above 400 mg/L adversely affected the nutrient removal at a four stage nutrient removing SBR system operated at anaerobic- aerobic- anoxic-aerobic stage with HRT of 1h/3h/1h/1h and settling phase ¾ h with SRT 10 days. Staib and Lant (2007) reported thiocyanate degradation with generation of end products ammonium and sulfate in two SBRs while treating coke oven wastewater in presence of phenol and cyanide. They observed no inhibition of phenol on thiocyanate as phenol degradation was much quicker compared to thiocyanate though CN^- significantly inhibited thiocyanate degradation. Liu et al. (2005) reported ammonium oxidation rate and nitrate production rate decreased in the presence of phenol in a SBR with microbial granule. Nitrifying activity was recovered completely after degradation of phenol when initial phenol concentrations were not higher than 10 mg/L.

2.3.5.1.1 Limitations of suspended growth reactors

Suspended growth system is a prevalent biological process treating various wastewaters, which is unable to remove ammonium in presence of toxic and inhibitory compounds (Kumar et al. 2000). Also, high hydraulic residence time (HRT) must be adopted in treating wastewater to achieve simultaneous biological oxidation of organic and nitrogenous compounds when present in a single sludge system because of nitrification inhibition (Staib and Lant, 2007). Meanwhile, high sludge recycle ratio should be adopted in an activated sludge systems to keep high biomass concentration in the reactor. However,

high sludge recycling often leads to the growth of filamentous bacteria, which promotes sludge bulking, scum formation and increased sludge wasting (Soddell and Seviour, 1990). Kim et al. (2007) reported nitrifying bacteria are washed out easily in treating coal process wastewater using a suspended growth activated sludge process because of the fast growth of competitive microorganisms at higher temperature under increased concentrations of phenols and thiocyanate. To avoid these above mentioned problems, attached growth techniques in aerobic, anaerobic or anoxic environments are adopted to have high amount of microbial agent and high treatment efficiency.

2.3.5.2 Attached growth system

In attached growth systems microbes exists in an anchored form, either on the surface of an inert carrier or attached to one another. The carrier could be the wall of the reactor, baffles provided for this purpose etc (Ødegaard et al. 2006). Bioreactors implying biofilm systems play important roles in detoxification of hazardous organic contaminants such as volatile aromatic hydrocarbons, chlorinated solvents, phenolics and chlorinated aromatics (Hosseini and Borghei, 2005). In comparison with the suspended-growth wastewater treatment systems, the advantages of the attached growth systems are: (1) the treatment plant require less space and compactness due to the availability of biofilm media with high specific surface area; (2) the high biomass hold up in biofilm enables the process to be operated significantly at higher hydraulic/organic loading and the final treatment results is less dependent on biomass separation since the biomass concentration to be separated is 10 times lower than suspended growth system and (3) the attached biofilm acts as buffer to reduce the concentration of toxic chemicals thereby providing advantage for the treatment of low biodegradable industrial wastewater containing recalcitrant compounds and also (4) provides lower sensitivity and better recovery from shock loadings and supposed to be robust for toxic and changing wastewater stream where slowly growing organisms with special metabolic capabilities are to be protected from washout (Wilderer et al. 1993; Zhang et al. 1998; Ødegaard et al. 2006). Biofilm processes have proved to be reliable for organic carbon and nitrogen removal without some of the problems of activated sludge processes (Yang et al. 2009). Various biofilm processes such as trickling filter, rotating biological contactor, packed bed reactor, fluidized bed reactor and moving bed biofilm

reactor etc are in use for industrial wastewater treatment process and have been studied to improve the treatment performance of the industrial and domestic wastewater removing organics and improving nitrification process (Li et al. 2003; Jeong and Chung, 2006a; Lai et al. 2008).

Tziotzios et al. (2005) studied phenol removal using same inoculums in two systems, one operated as suspended growth system and other as a packed bed system. The later was capable of higher phenol removal almost 12.65 times than the former resulting in less reaction time requirement. Ramos et al. (2007) found submerged fixed film reactor having good capacity for eliminating high concentration of phenol (1000 mg/L) which also implied high COD removal in presence of total nitrogen 400 mg/L at HRT 1 day with air flow and recirculation. The fixed film system also exhibited advantages over suspended growth system like higher concentration of active biomass on immobilized media. However the system could not remove total nitrogen more than 63%. Zaiat et al. (2001) reported utilization of biofilm, where microorganisms were immobilized and this technology seems to be promising, since it eliminates uncertainty as to the granulation phenomenon, solves the problem of solids retention and may eliminate the sedimentation step, hence reducing total cycle period and extending the range of possible uses of sequencing batch biofilm reactor (SBBR). Goh et al. (2009) reported SBBR with polyurethane sponge cubes showed better performance than sequencing batch reactor (SBR) as SBBR achieved almost complete removal of ammonia nitrogen removal while SBR achieved average 86% when both were used in simultaneous removal of p-nitrophenol and ammonia-nitrogen. Dictor et al. (1997) observed maximum thiocyanate biodegradation rate 5.3 g/L.day using heterotrophic microorganisms and packed-bed reactor charged with pumice stone and zeolite. Mudliar et al. (2008) reported almost 98% of pyridine removal in a rotating rope bioreactor from pyridine loading of 0.78 g/L.day that was higher than completely mixed activated sludge process reported by Padoley et al. (2006). Banerjee (1996) studied four- stage across the flow laboratory scale RBC reactor for treatment of simulated steel plant wastewater containing phenol and thiocyanate. The treatment revealed that the toxicants were removed in sequence. Phenol was mostly removed in the earlier (first and second) stages and thiocyanate was removed in the later (third and fourth) stages. Mohan et al. (2007) studied SBBR for low biodegradable

composite chemical wastewater (low COD/BOD ratio ~ 0.3) with fixed packed bed system operated at anoxic – aerobic –anoxic microenvironment. The reactor was capable of removing 88-55% COD and 89-75% BOD at 1 day operational HRT and organic loading of 0.90-4.76 g COD/L.day, which was more than suspended growth system operated at same conditions.

In fixed bed attached growth system the carrier material is kept fixed to the system and thus microbes get attached and remain in contact with the substrate with the flow of influent whereas in case of moving bed biofilm system the carrier material along with microbes keep moving through out the reactor due to influent flow or external mixing. The moving bed biofilm reactor (MBBR) process was introduced about 30 years ago and it has since become popular in Europe (Ødegaard, 2006). The basic idea of the MBBR is to have a continuous operated biofilm reactor with a high density of biomass and without backwashing or sludge return. Moving bed reactor is a combination of conventional activated sludge process and fluidized bed system, where biomass is grown on small carrier elements like sponge etc. having density less than water and some inert material like sand, basalts, granulated activated carbon, kaldane paricles and particles of polymers etc (Ødegaard et al. 1994). Chu and Wang (2011) reported better performance by reactor with sponge cube than that of biodegradable polymer as carrier. MBR is less prone to clogging; biomass in the effluent is less than suspended growth system and is suitable for slow growing nitrifying bacteria (Chen et al. 2008). Contrary to most biofilm reactors, the moving bed reactor utilizes the whole tank volume for biomass growth, and would be more efficient and stable to remove toxic pollutants from the wastewater. MBRs are reported for treatment of wastewater from poultry processing, pulp and paper industry, refinery and slaughterhouse, coke industry and also landfill leachate etc. (Johnson et al. 2000; Sigrun et al. 2002; Rusten et al. 2003; Chen et al. 2008). Moussavi et al. (2009) used a moving-bed sequencing batch reactor (MSBR) for treatment of high concentration of phenol (50-3000 mg/L) from synthetic wastewater. The system revealed efficient phenol and COD removals when operated at a single reactor with 30% carrier material without any recycle. The activity of the biofilm involved in the phenol degradation was almost eight times more than that of the suspended biomass. The inhibition concentration of phenol was found to be 3000 mg/L. The optimum HRT for MSBR was 40 h at which removal efficiency of phenol

and COD were greater than 99% and reactor was resistant to shock loading at various operational conditions. Borghei et al. (2008) studied performance of moving bed biofilm reactor (MBBR) in terms of phenol-COD to total-COD ratio. Results showed that the system performance was significantly affected by the ratio and system HRT. However the system was stable against hydraulic and toxic load and it can recover to steady state condition after 24 h. The system performance was better at HRT higher than 1 day. Canziani et al. (2006) reported 30-40% saving of the carbon required for denitrification when MBBR as denitrification unit was installed after membrane bioreactor treating landfill leachate as the nitrification was partially completed to nitrite saving oxygen requirement. Li et al. (2011) used a laboratory-scale moving bed biofilm reactor (MBBR) with a volume of 4 L to study the biodegradation of coal gasification wastewater. Maximum removal efficiencies of 81%, 89%, 94% and 93% were obtained for COD, phenols, SCN^- and NH_4^+ -N, respectively. They observed that NO_2^- -N accumulation induced increase of effluent COD concentration when the hydraulic residence time (HRT) decreased. Phenols removal was not affected when the HRT decreased from 2-1.5 days. Effluent SCN^- and NH_4^+ -N concentration increased with the decrease of the HRT, and decreased gradually when the HRT returned to 2 days. Yang et al. (2009) studied a moving bed membrane bioreactor filled with carriers instead of activated sludge for simultaneous removal of organic carbon and nitrogen in wastewater and compared its performance with a conventional membrane bioreactor at various influent COD/TN ratios of 8.9-22.1. The moving bed membrane bioreactor system demonstrated better performance on nitrogen removal at different COD/TN ratios compared to conventional membrane bioreactor. Also, multifunctional microbial reactions in the carrier, such as simultaneous nitrification and denitrification (SND), play important roles in nitrogen removal. They observed from the specific oxygen utilization rate that the biofilm has a better microbial activity than an activated sludge. Nevertheless, the membrane fouling behavior was more severe in the moving bed membrane bioreactor than in the other due to a thick and dense cake layer formed on the membrane surface.

2.3.5.3 Sequential bioreactor system

When wastewater contains multiple pollutants like phenol, thiocyanate, ammonia-nitrogen, pyridine etc. degradation of all pollutants in ASP require high amount of oxygen demand. Anaerobic reactor alone is unable to meet the discharge limit and ammonia removal (Chuang and Ouyang, 2000; Mosquera-Corral et al. 2003). In that case, a combination of anaerobic, anoxic, aerobic treatment for removals of these pollutants frequently could achieve the desired results compared to single aerobic, anaerobic or anoxic reactor (Chakraborty and Veeramoni, 2002; Li et al. 2003; Jeong and Chung, 2006b). Wang et al. (2011) investigated effect of step feeding in a two-continuous mesophilic UASB system with real coal gasification wastewater. They observed that after the anaerobic digestion with step-feed, the aerobic effluent COD concentration decreased from 270 ± 9 to 215 ± 10 mg/L. The results suggested that step-feed enhanced the degradation of refractory organics in the second reactor.

Joeng et al. (2006a) investigated simultaneous removals of COD, CN^-, SCN^-, ammonia and TN using fluidized biofilm process with aerobic -anoxic reactor arrangement. Though the wastewater contained hardly biodegradable compounds and toxic compounds the system was able to achieve 93-99% of all pollutant removal respectively at HRT 1 day. Kim et al. (2008b) operated a lab scale serial anoxic-aerobic reactor in continuous mode and the system efficiently and economically treated actual coke wastewater at HRT above 11.9 hour along with inorganic carbon, chloride, sulphate, fluoride etc. Chen et al. (2008) treated landfill leachate using a MBBR system with an anaerobic-aerobic arrangement for simultaneous removal of COD and ammonium. Anaerobic system primarily removed COD and aerobic MBBR acted as COD polishing and ammonium removal step. More than 97% of total NH_4^+-N removal efficiency could be achieved when the HRT of the aerobic MBBR was more than 1.25 days.

The anaerobic-anoxic–aerobic process is often the preferential choice to simultaneously remove these pollutants from wastewater (Peng et al. 2006; Vaiopoulou et al. 2007; Zheng and Li, 2009; Sahariah and Chakraborty, 2011). In this process, the anaerobic unit mainly performs as pretreatment or partial treatment (Fang and Yu, 2000; Li et al. 2003). In the anoxic unit, organic compounds are oxidized by nitrate while nitrate is reduced to nitrogen gas and discarded from the system and finally, the pollutants such as residual COD and

ammonia were further oxidized in the aerobic unit. A fraction of the treated effluent was commonly required to return to anoxic unit to enhance the denitrification reaction (Kim et al. 2008a; Zheng and Li, 2009). All the three units work fulfilling the pre-requirement of each other and finally give complete treatment of the wastewater. Phenolic compounds and other refractory compounds with inhibitory effect on nitrification are expected to be partially degraded making easily biodegradable organic acids for the anoxic reactor and so they are expected to get removed in the upstream reactors. NH_4^+-N will be converted to nitrate-N under aerobic environment. This nitrified effluent is to be recycled to the upstream anoxic reactor for denitrification and nitrogen removal from the system. With alteration of the position of the aerobic reactor to the front of the series, the nitrifying bacteria might get affected by high toxicity of influent pollutant such as phenol/thiocyanate and more aeration will be required for the treatment thus increasing the cost. Also the treated water when passed to anoxic unit might be in deficiency of organic compound for nitrate/nitrite removal and in need of addition of external organic carbon source. Also the effluent from anoxic/anaerobic reactor might not be in disposable quality in terms of odor/ color or DO content.

Li et al. (2003) reported among two multistage biofilm system operated in anoxic-aerobic and anaerobic – anoxic- aerobic stage with coke wastewater in similar operating conditions such as HRT and wastewater characteristics, the later performed in better way in terms of total nitrogen removal than the anoxic-aerobic system though both systems represented similar COD and ammonia removal. Zhang et al. (1998) studied anaerobic-anoxic-aerobic fixed biofilm system for coke plant wastewater and reported that biodegradable phenolics were removed at lower efficiency while complicated and high molecular weight organics were removed at higher efficiencies by the anaerobic treatment in comparison to anoxic treatment and the biodegradability of anaerobic effluent was higher than that of anoxic effluent. Chakraborty and Veeramani (2002) studied feasibility of a three stage anaerobic-anoxic-aerobic system with heterogeneous culture for handling complex wastewater and nitrification and denitrification without adding external carbon source and observed that the system performed efficiently and almost complete phenol removal and denitrification was achieved in anoxic reactor as it was fed with effluent of anaerobic reactor mixed with recycle from aerobic reactor.

Zheng and Li (2009) studied a lab-scale anaerobic-anoxic-aerobic system with three cycles for treatment of high-strength coking effluent with 8 000–15000 mg/L COD in presence of high concentration of phenol 1200-1700 mg/L and 700–1800 mg/L ammonia-nitrogen using recycle to both anaerobic and anoxic reactors. They achieved more than 90% COD and 100% phenol removals while ammonia-nitrogen removal was only 60%. They also observed that the modification of HRT (18-30 days) did not influence the overall phenol and COD removal efficiency but significantly influenced the distribution of COD and ammonium removals among the reactors. Zhao et al. (2009) studied a laboratory-scale anaerobic–anoxic–aerobic membrane bioreactor system for treatment of heavily loaded and toxic coke plant wastewater. They observed that system was more efficient and reliable in pollutants and acute toxicity reduction than the conventional anaerobic–anoxic–aerobic system tested in parallel as control especially at high and varying loading rates.

2.4 SUBSTRATE REMOVAL KINETICS

Modeling is a valuable tool for designing and operation of biological treatment plants and modeling of wastewater treatment plants can be used for process optimization and testing to meet effluent quality requirements at a reasonable cost. Development of kinetic models is a useful attempt that reduces laborious and complex experimental data to simple and convenient mathematical expressions. Moreover, model results can be evaluated for different operating data before transferring the concepts to a fullscale plant (Yetilmezsoy, 2007).

Various kinetic models like Haldane's inhibition model, modified Stover Kincannon model, Grau second order model, Bhatia et al toxicity model and first order kinetic models were applied for to determine substrate removal kinetics in CMBR and FMBR reactors. The model with best fit was selected on the basis of highest correlation coefficient (R^2) and lowest chi square (χ^2) value

1. Haldane' inhibition model

$$\frac{ds}{dt} = \frac{q_{max} S_e}{K_s + S_e + (S_e^2/K_i)} \qquad \text{(Eq. 2.5)}$$

where q_{max} is maximum degradation rate (/day), s is substrate concentration at any time t (mg/L), S_e is effluent concentration (mg/L), K_s and K_i are half saturation constant (mg/L) and inhibitory constant (mg/L), respectively. Root mean square error RMSE of predicted and experimental q_{max} value was calculated using equation 2.5b.

$$RMSE = \sqrt{\frac{\sum_{i=1}^{n}(observedq_i - predictedq_i)^2}{n}} \quad \text{(Eq.2.6)}$$

where n = Number of observations.

2. Modified Stover-Kincannon model

In Modified Stover-Kincannon model substrate utilization rate is expressed as organic loading rate by monomolecular kinetic for biofilm reactor (Ahn and Forster, 2000; Kuscu and Sponza, 2009). However due to difficulties in measuring the active surface which supports the biofilm growth, the effective volume of the reactor can be used (Borghei and Hosseiny, 2004). At steady state it is expressed as

$$\frac{ds}{dt} = \frac{\left(\frac{R_{max} Q S_0}{V}\right)}{\left(K_b + \frac{QS_0}{V}\right)}$$

Substituting ds/dt as $Q(S_0-S_e)/V$ and reversing the expression

$$\frac{V}{Q(S_0 - S_e)} = \frac{\left(K_b + \frac{QS_0}{V}\right)}{\left(R_{max}\frac{QS_0}{V}\right)}$$

$$\text{or,} \quad \frac{HRT}{(S_0 - S_e)} = \frac{K_b}{R_{max}}\left(\frac{V}{QS_0}\right) + \frac{1}{R_{max}} \quad \text{(Eq. 2.7)}$$

where, V is volume of the reactor (L), Q is flow rate (L/day), HRT is hydraulic retention time (=V/Q), S_0 and S_e are influent and effluent substrate concentrations at steady state (mg/L), K_b is saturation value constant (g/L.day) and R_{max} is maximum substrate utilization rate (g/L.day). From the straight line plot of $HRT/(S_0 - S_e)$ vs $\{V/(Q.S_0)\}$, R_{max} and K_b can be determined from slope and intercept. Equation (2.7) can be rewritten as equation 2.8 for effluent prediction.

$$S_e = S_0 - \frac{(R_{max}\, S_0)}{(K_b + \frac{QS_0}{V})} \qquad \text{(Eq. 2.8)}$$

3. Inhibition kinetics due to toxicity

The linearized form of Bhatia et al (1985) model considering the process inhibition due to toxicity is given in equation (2.8).

$$\frac{(HRT \times S_e)}{(S_0 - S_e)} = \frac{1}{R_{max}} + (\frac{K_i}{R_{max}}) I_S \qquad \text{(Eq. 2.9)}$$

where, R_{max} is the maximum substrate utilization rate (/day), K_i is the inhibition coefficient (L/mg) and I_S is the inhibitor concentration in effluent (mg/L). Equation (2.9) can be rearranged to predict effluent substrate concentration as given in equation (2.10).

$$S_e = \frac{S_0}{\{1 + \frac{(R_{max}\, HRT)}{(1 + \frac{I_S}{K_i})}\}} \qquad \text{(Eq. 2.10)}$$

4. Grau second order kinetic model

$$\frac{S_0 \times HRT}{(S_0 - S_e)} = n \times HRT + m \qquad \text{(Eq. 2.11)}$$

S_0, S_e and HRT are as described above. The dimensionless kinetic coefficients m, and n can be calculated form the linear plot of $\frac{S_0 \times HRT}{(S_0 - S_e)}$ vs HRT (Grau et al. 1975; Kuscu and Sponza, 2009). The model effluent value (predicted) was calculated using following equation (equation 2.12)

$$S_e = S_0 - (\frac{S_0 \times HRT}{n \times HRT + m}) \qquad \text{(Eq. 2.12)}$$

5. First order kinetic model

$$\frac{S_0 - S_e}{HRT} = k \times S_e \qquad \text{(Eq. 2.13)}$$

The value of k, degradation rate (/day) can be obtained from the slope of the line by plotting $(S_0 - S_e)/HRT$ versus S_e (Metcalf and Eddy, 2003; Kuscu and Sponza, 2009). The model effluent value (predicted) was calculated using following equation (equation 2.13)

$$S_e = \frac{S_0}{(1 + HRT\ k)} \qquad \text{(Eq. 2.14)}$$

Chi square value was calculated for validation of model using following equation (2.15)

$$\chi^2 = \sum \frac{(\text{Experimental value} - \text{Predicted value})^2}{\text{Predicted value}} \qquad \text{(Eq. 2.15)}$$

2.5 OBJECTIVE AND SCOPE OF THE STUDY

Biological treatment utilizing consortia of heterogeneous microorganisms is the most cost effective one for removal of various recalcitrant inorganic and organic compounds discharged from several industrial effluents. Process effluents from coal gasification, synthetic fuel processing operations, coal carbonization, coke oven form integrated steel plant etc. generate wastewater containing high concentration of phenol along with inorganics like ammonia-nitrogen (NH_4^+-N), and thiocyanate (SCN^-) as major pollutants (Zhang et al. 1998; Li et al. 2003; Vázquez et al. 2006). Efficient treatment of wastewaters contaminated with such pollutants is highly essential prior to their discharge into the environment.

A combination of anaerobic, anoxic, aerobic treatment for this type of wastewater could achieve the desirable results instead of a single treatment unit. Several reactor configurations in this mode are used, like suspended growth, fixed film, membrane process, fluidized process. The suspended systems contain less biomass and prone to high toxicity and wash out whereas fixed film system is prone to clogging. The membrane system and the fluidized process though very efficient are reported to be expensive in terms of establishment cost and operating cost, respectively. Moving bed reactor (MBR) being a combination of conventional activated sludge process and fluidized bed system, where biomass is grown on small carrier elements like sponge etc. turn into an efficient

and emerging technology for treatment of industrial wastewaters. MBR is less prone to clogging; uses the whole tank volume and possesses advantages of attached growth systems like biomass in the effluent is less than suspended growth system and is suitable for slow growing nitrifying bacteria (Chen et al. 2008).

Mode of operation of biological reactor is an important parameter that influences reactor performance. In continuous feeding system, influent is continuously added to the reactor and effluent is released. Feed is diluted with large volume of liquid present in the reactor, and thus helps in reduction of toxicity. In fed batch operation, feed is added to the reactor for smaller duration, thus exerting concentration gradient which is beneficial for performance and also higher substrate load to the reactor results more toxicity. Also, as influent is added in smaller duration, pumping time requirement is reduced and makes the process simpler and cost effective. Fill time and cycle time of a fed batch/batch reactor plays a major role in feed strategy (Ratusznei et al. 2003; Sahinkaya and Dilek, 2007; Damasceno et al. 2007). Cycle period of the reactor also play major role in the performance of the reactor controlling exposure of the organic load/ intensity and the length of reaction time.

Though adequate literatures are available on biodegradation of phenol, thiocyanate and ammonia in single step ASP and/or multiple steps ASP and sequencing batch rector (SBR), design data on application of anaerobic-anoxic-aerobic MBR system for removals of phenol, thiocyanate and ammonia containing wastewater are very limited. We developed two types of sequential MBR system: operated in continuous mode (CMBR) and fed batch mode (FMBR). Various operational and parameter wise parameters were considered to evaluate the performance of moving bed bioreactor system in continuous and fed batch mode. The objective of the study is to evaluate the performance of anaerobic-anoxic-aerobic moving bed reactors while treating high concentration of phenol, SCN^-, NH_4^+-N and pyridine from simulated industrial wastewater using continuous and fed batch reactor system.

The detail methodology to fulfill the objective of study is as given below

- ✓ Fabrication of sequential reactor system
- ✓ Development of heterogeneous culture

- ✓ Study in sequential continuous moving bed reactor (CMBR) with following variable parameters
  - a) thiocyanate concentration
  - b) hydraulic retention time
  - c) phenol concentration
  - d) ammonia concentration
- ✓ Study in sequential fed batch moving bed reactor (FMBR) with following variable
  - a) thiocyanate concentration
  - b) filling time
  - c) hydraulic retention time
  - d) cycle time
  - e) phenol concentration
  - f) pyridine concentration
- ✓ Determination of substrate removal kinetics in CMBR and FMBR systems.
- ✓ Study the affect of shock loads in CMBR and FMBR systems.
- ✓ Treatment of actual coke oven wastewater.
- ✓ Isolation and identification of predominant microorganisms reactors of CMBR and FMBR systems.

CHAPTER 3

MATERIALS AND METHODS

In this section various materials and equipment used and various methodologies exercised in the present investigation are described.

3.1 Materials

3.1.1 Chemicals and Reagents

All the chemicals used were either of analytical grade (AR) or laboratory grade (LR). Phenol, potassium thiocyanate, ammonium chloride, potassium nitrate, pyridine, p-cresol, m-cresol, o-cresol used as feed in the study were of analytical grade and obtained from Merck and CDH (India). Potassium di hydrogen phosphate, Di potassium hydrogen phosphate for phosphate buffer; $MgSO_4.7H_2O$, $CaCl_2.2H_2O$, $FeCl_3.6H_2O$, $CuCl_2$, $ZnCl_2$, $NiCl_2.6H_2O$ and $CoCl_2$ used for trace metal solution was analytical grade and purchased from Merck India. Yeast extract used as nutrient in feed was purchased from CDH, India. Potassium di chromate, ferrous ammonium sulfate, ferroin solution, ammonium hydroxide, 4-aminoantipyrene, potassium ferric-cyanate, ferric nitrate, ethanol, 80% phosphoric aicid, glacial acetic acid, tri sodium citrate, sodium hydroxide, sodium bi carbonate used for

various analysis were of analytical grade. Chemicals and reagents used for biochemical tests and microscopic analysis such as peptone, sucrose, beef extracts, sucrose, agar, potassium iodide, iodine, crystal violet, safranin O etc. were laboratory grade and purchased from Sigma-aldrich and Loba chemicals. HPLC grade acetone, acitonitrile were used as solvent for HPLC (99.5% purity). For preparation of feed to the reactors tap water was used whereas for reagent preparation, stock/standard solution preparation and analysis of samples deionized water or Millipore water were used wherever applicable.

3.1.2 Biomass support material: Sponge cubes

Sponge sheet (polyurethane foam) was procured from local market and cut into cube sizes of approximately 1 cm × 1 cm × 1 cm, washed with tap water, and dried overnight at 70°C in a hot air oven before being used as biomass support material in the reactors. The porosity of sponge was estimated from the ratio of void space volume and sample sponge volume. For sponge density analysis weights of five sponge cubes, ten sponge cubes, twenty sponge cubes were taken for average weight of each sponge cube. Density of sponge cube was determined from weight and volume of each sponge cube. Sponge cube was cut in $1cm^3$ (1cmx1cmx1cm) having six sides with each cube has surface area of 6 cm^2. Sponge cubes had porosity 0.81, density 0.051 g/cm^3 and specific surface area 600 m^2/m^3. About 120 gm of oven dried sponge cube were placed in each reactor of CMBR and 100 g sponge was added in FMBR manually, more or less uniformly in layers starting from bottom.

3.2 Experimental Methodologies

In this section, description on various experimental set-ups, reactor specification, feeding and operating conditions are given in detail. The reactor system was maintained at a constant temperature (30 ± 3 °C) using a temperature controlled blower in a closed room.

3.2.1 Seed sludge

Sludge (total solids of 40 g/L and volatile solids of 15 g/L) collected from one anaerobic biogas plant fed with kitchen waste located at IIT Guwahati was used as inoculums for anaerobic and anoxic reactors (3L sludge for 5 L reactors). Sewage collected from IIT

Guwahati sewage treatment plant (total solids 7.0 g/L and volatile solids of 4.5 g/L) was used as inoculums for the aerobic reactors (3L sewage for 5 L reactors).

3.2.2 Synthetic feed

The study was conducted with synthetic feed containing thiocyanate (SCN^- as KSCN), phenol (C_6H_5OH) and ammonia (NH_4^+–N as NH_4Cl). Nitrate (NO_3^--N as KNO_3) was added in recycle from aerobic reactor to anoxic reactor. Freshly prepared feed was purged with N_2 gas. Feed pH was maintained at 7.5 ± 0.2 by using phosphate buffer (using KH_2PO_4 72.3 g/L and K_2HPO_4 104.5 g/L). This also worked as a phosphorus source to microorganisms. Yeast extract of 10-20 mg/L and trace metals solution of 1 mL/L feed were added as nutrients. The composition of stock trace metal solution was: $MgSO_4.7H_2O$: 10,000 mg/L, $CaCl_2.2H_2O$: 10,000 mg/L, $FeCl_3.6H_2O$: 5,000 mg/L, $CuCl_2$: 1000 mg/L, $ZnCl_2$: 1000 mg/L, $NiCl_2.6H_2O$: 500 mg/L, $CoCl_2$: 500 mg/L (Chakraborty and Veeramani, 2006).

3.2.3 Reactor operation

Two sequential anaerobic-anoxic-aerobic moving bed reactor systems were used for the present study. One was operated as continuous fed moving bed reactor (CMBR) and another as batch fed moving bed reactor (FMBR). At each parameter variation, steady state data was achieved characterized by consistency of effluent quality of the three–stage system after a transient period of 7–12 days. Steady state data was collected for 12–15 days and considered to analyze the performance of each reactor.

3.2.3.1 Continuous moving bed reactor (CMBR)

3.2.3.1.1 Acclimatization of CMBR

Two glass aspirator bottle of (5 L working volume) labeled R1A and R2A were used for anaerobic acclimatization of 3L screened seed sludge added with 60 g sponge cube each and final volume was made to 5 L with tap water. In R1A and R2A, 500 mg/L of dextrose was added daily for initial 21 days. Along with dextrose, the synthetic feed also included ammonium chloride (50 mg NH_4Cl/L) as nitrogen source; phosphate buffer (dissolving 104.5 g/L K_2HPO_4 and 72.3 g/L KH_2PO_4) of pH 6.8; trace metals solution (1mL/L) and

yeast extract 20 mg/L as nutrients. Phosphate buffer of 1 mL/L of feed was added as phosphorus source and to maintain reactor pH. Concentration of phosphate buffer, yeast extract and trace metal solution remained unchanged through out the study. Synthetic feed was dissolved in tap water and purged with nitrogen gas before addition to reactors.

For the first 21 days R1A and R2A were operated only with 500 mg/L of dextrose as carbon source. On day-22, feed dextrose concentration was increased to 1000 mg/L daily. From day 30 onwards, phenol 10 mg/L and NH_4^+-N 10 mg/L, and thiocyanate 5 mg/L were introduced along with 1000 mg/L dextrose. Dextrose concentration was decreased gradually and dextrose concentration in feed became nil on day-90 onwards and R1A and R2A were operated only with phenol (250 mg/L), NH_4^+-N (100 mg/L), thiocyanate (50 mg/L) and other nutrients. With each feed R1A and R2A was operated for 3-5 days before going for next increment. In feed of R1A and R2A, phenol, thiocyanate and NH_4^+-N concentrations were gradually increased to 2500, 110 and 500 mg/L to overcome the toxic effect on microorganisms on 217 days through out acclimatization period.

Similarly for anoxic sludge acclimatization, two aspirator bottles R3A and R4A were prepared with sludge (3L), sponge cube (60 gm) and tap water to 5 L each. For the initial 21 days in anoxic reactors were fed daily with 500 mg/L of dextrose and 70 mg/L of nitrate-nitrogen. Same amount of phosphate buffer, yeast extracts and trace metal solutions were added in the feed of anoxic reactors like anaerobic reactors. On day-22 onwards, feed dextrose and nitrate (NO_3^--N) concentrations were increased to 1000 mg/L and 250 mg/L, respectively (COD:NO_3^--N of 4.24). From 30th day onwards, phenol of 5 mg/L, 5 mg/L SCN^- and 5 mg/L NH_4^+-N were added in anoxic reactors along with 1000 mg/L of dextrose and 250 mg/L of NO_3^--N. Feed dextrose concentration was decreased in R3A and R4A along with increase in phenol concentration in the feed. On day-79, feed phenol, thiocyanate and NH_4^+-N concentration became 500 mg/L, 25 and 50 mg/L and dextrose concentration became 200 mg/L. On day-97 onwards anoxic reactors were operated with 500 mg/L phenol, 40 mg/L thiocyanate and 200 mg/L NH_4^+-N and 450 mg/L of NO_3^--N without any dextrose in the feed (COD:NO_3^--N ratio of 2.64). In three-stage system, effluent of anaerobic reactor is expected to be diluted with nitrified effluent of aerobic reactor so that denitrification can take place in anoxic reactor. Due to this, anoxic reactor was acclimatized up to maximum phenol concentration of 1250 mg/L, 50 mg/L SCN^- and

250 mg/L NH_4^+-N and acclimatization period was 140 days like anoxic reactors. Feed to R3A and R4A was also purged with nitrogen gas to make oxygen free.

In aerobic reactors (R5A and R6A) sponge of 60 g in each reactor was added in 5L of screened sewage. Compressed air was added in R5A and R6A for supply of oxygen which caused mixing of reactor content. Synthetic feed consisting of ammonia-nitrogen (using ammonium chloride) was added in aerobic reactors. Concentration of ammonia-nitrogen, phenol and thiocyanate was increased gradually from 10 mg/L to 250 mg/L, 500 mg/L and 25 mg/L in 100 days.

3.2.3.1.2 Reactor assembly of CMBR system

Three PVC (polyvinyl chloride) columns, each of diameter 15 cm and height 118 cm were used as reactors for CMBR system. It consisted of three reactors in series, maintained under anaerobic (R1), anoxic (R2) and aerobic (R3) environments. After acclimatization contents (liquid + biomass + sponge) of two 5L batch anaerobic reactors (R1A and R2A) were added in anaerobic reactor (R1). Similarly contents of two anoxic (R3A and R4A) and aerobic (R5A and R6A) reactors were added in R2 and R3, respectively. A schematic representation of the experimental assembly of CMBR system is shown in Figure 3.1.

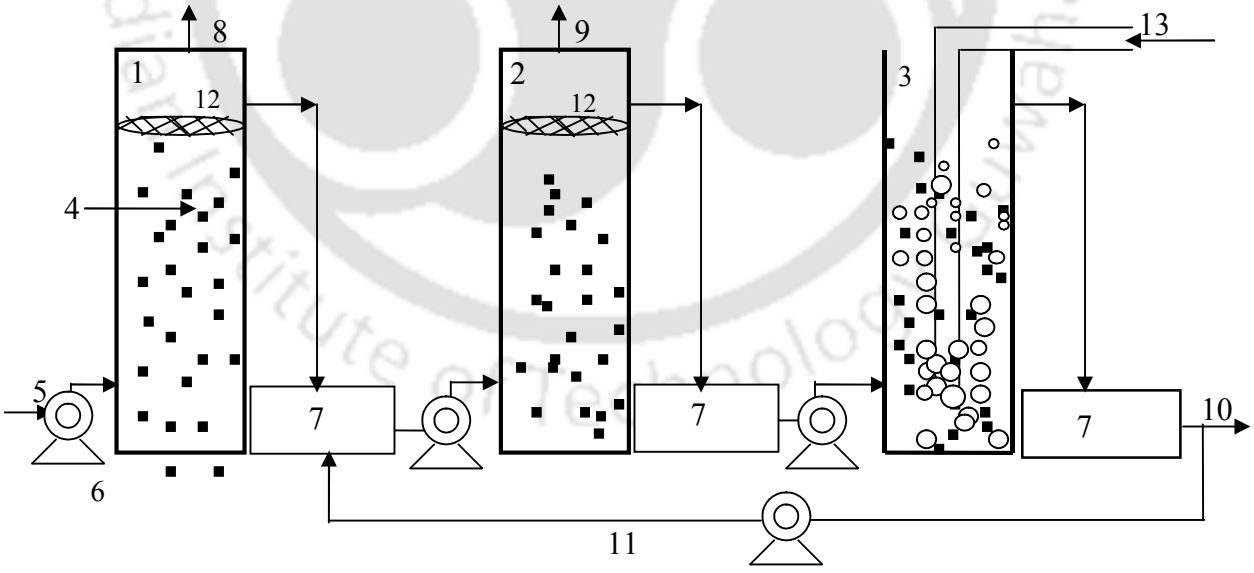

Figure 3.1 Schematic of the experimental set up of the moving bed reactor system with its various components: (1) Anaerobic reactor, (2) Anoxic reactor, (3) Aerobic reactor, (4) Sponge cube (5) Influent, (6) Peristaltic pump (7) Clarifier, (8) Biogas collection pipe, (9) Gas outlet (10) Effluent reservoir, (11) Recirculation to anoxic reactor with KNO_3 addition, (12) Separator with nylon mesh (13) Compressed air supplier

Working volume (liquid, sponge and biomass) of each reactor was made as 15 L with tap water. In each 15 L reactor total amount of sponge cubes were 120 g and total volume of sponge cube in each reactor was 2360 cm^3, which was 15.7% of working volume of each reactor, giving number of sponge cubes in each reactor as 2353. All reactors were operated in up flow mode and influent were added 10 cm height from the bottom. Each reactor was connected with a clarifier of volume 500 mL. Mixed liquor from each reactor was allowed to flow to the clarifier for settling of biomass. From the clarifier clear supernatant was pumped to the next reactor and settled biomass was returned to the reactor. In R1 and R2, mixing was achieved only by the upflow motion of the influent. In R3, compressed air (0.15 L/min) was supplied for aeration which also provided mixing. Hydraulic retention time (HRT) was maintained adjusting the influent flow rate and calculated using equation (3.3a). Also, treated effluent from third reactor R3 was partially recycled to R2 at recycle ratio 1 through out the study (Eq.3.3b). In this recycle, 1000 mg/L NO_3^--N (as KNO_3) was added, to supply adequate nitrate in anoxic reactor in order to maintain the anoxic condition. The effluent from R2 was pumped to third reactor R3 (flow rate according HRT). The influent flow rate to R2 and R3 were twice of feed flow rate. Dissolved oxygen concentrations (mg/L) in the reactors were: 0 (R1 and R2) and 4.0-4.8 (R3). The reactor system was maintained at a constant temperature (30 ± 3 °C) using a temperature controlled blower. Plastic pipes were connected to R1 and R2 which were maintained in a water seal for collection of biogas by water displacement method.

$$\text{HRT (day)} = \frac{\text{Reactor volume (L)}}{\text{Feed flow rate (L/day)}} \qquad \text{(Eq.3.3 a)}$$

$$\text{Recycle ratio (R)} = \frac{\text{Recycled flow rate (L/day)}}{\text{Influent Flow rate (L/day)}} \qquad \text{(Eq.3.3 b)}$$

$$\text{Influent concentration to anoxic reactor} = \frac{Q\,(\text{Effluent concentrations of anaerobic reactor}) + RQ\,(\text{Effluent concentrations of aerobic reactor})}{(Q+RQ)} \qquad \text{(Eq.3.4)}$$

3.2.3.1.3 Experiments in CMBR

Four sets of experiments were carried out in continuous moving bed system (CMBR) varying the feed composition of phenol, thiocyanate and ammonia at a time maintaining

the other parameter constant. The operating conditions and feed composition during the experiments were as shown in Table 3.1. In experiments, feed thiocyanate was varied from 0-600 mg/L; in experiment 2, HRT of reactors were varied (total HRT 3-8 days); in experiment 3, feed phenol concentration was varied from 1000-2500 mg/L and in experiment 4, feed NH_4^+-N was modified from 100-600 mg/L.

Table 3.1 Operational schedule and conditions of CMBR system

Experiment	Days	Feed (mg/L)			Feed flow rate (Q) (L/day)	HRT (day)			
		Phenol	NH_4^+-N	SCN^-		Anaerobic (R1)	Anoxic (R2)	Aerobic (R3)	Total
1	140-190	2500	500	0	7.5	2	1	1	4
	210-236	2500	500	110					
	237-285	2500	500	200					
	286-330	2500	500	450					
	331-388	2500	500	600					
2	389-435	2500	500	600	10.0	4	2	2	8
	436-465	2500	500	600	6.0	3	1.5	1.5	6
	466-505	2500	500	600	5.0	2.5	1.25	1.25	5
	540-592	2500	500	600	3.7	1.5	0.75	0.75	3
3	610-660	1000	500	800	5.0	3	1.5	1.5	6
	661-710	1500	500	800					
	711-750	2000	500	800					
	751-794	2500	500	800					
4	805-850	1500	100	800	5.0	3	1.5	1.5	6
	851-893	1500	300	800					
	894-934	1500	500	800					
	935-985	1500	600	800					

Feed thiocyanate in three-stage system was gradually increased from 110 to 200 mg/L with increment of 10-20 mg/L/day without changing other feed parameters. Feed SCN^- was further increased to 450 and 600 mg/L in a likely manner and operated for almost 30 days.

Influent concentration to R2 was calculated using equation 3.4. Performance of the CMBR was also evaluated by varying the hydraulic retention time (HRT) from 3-8 days at constant feed of phenol, thiocyanate and ammonia concentration of 2500 mg/L, 600 mg/L and 500 mg/L, respectively. Similar to thiocyanate phenol concentration in the CMBR was varied from 1000 to 2500 mg/L maintaining thiocyanate and ammonia concentration fixed at 800 mg/L and 500 mg/L, respectively during the experiments. Feed ammonia in CMBR was varied from 100-600 mg/L and performance was evaluated in presence of thiocyanate 800 mg/L and phenol 1500 mg/L, respectively.

3.2.3.2 Fed batch moving bed reactor (FMBR)

3.2.3.2.1 Acclimatization of culture

Acclimatization of culture was conducted separately under anaerobic, anoxic and aerobic environments in six 5 L batch reactor (two for each). Sludge from biogas plant (volatile solids of 15 g/L) was used for seeding of anaerobic and anoxic reactor after screening and screened effluent (volatile solids of 4.5 g/L) from sewage treatment plant was used as inoculums for aerobic reactor like CMBR system. For anaerobic and anoxic acclimatization, each reactor was added with 3L of screened seed sludge and 50 g of sponge cube for immobilization of microbes and final volume of 5L was made by adding tap water. Initially anaerobic reactors were fed for 15 days with dextrose (1000 mg/L) and NH_4^+-N (5 mg/L) along with phosphate buffer 1mL/L, trace metal solution 1mL/L and yeast extract 20 mg/L. Phenol and NH_4^+-N was increased and dextrose was decreased stepwise up to 500, 200 and 100 mg/L, respectively in 101 days and then SCN^- 5 mg/L was added in feed. Phenol, SCN^- and NH_4^+-N in anaerobic reactors was increased to 1500 mg/L, 100 mg/L 500 mg/L, respectively in 170 days with zero dextrose in feed. Anoxic reactors were acclimatized similarly like anaerobic reactors with 750 mg/L phenol, 250 mg/L NH_4^+-N, 50 mg/L SCN^- and 500 mg/L NO_3^--N in 113 days. Feed of anaerobic and anoxic reactors were purged with N_2 gas through out acclimatization period. Two aerobic reactors with 3L screened effluent from sewage treatment plant added with 50 g sponge cube each. Total volume was made 5L with tap water and were acclimatized with NH_4^+-N (initially 5 mg/L) along with phosphate buffer, trace metal solution 1mL each per liter feed and yeast extract 20 mg/L NH_4^+-N were gradually increased to 250 mg/L in 83 days.

3.2.3.2.2 FMBR Reactor Assembly

Three PVC (polyvinyl chloride) columns, each of diameter 15 cm and height 118 cm were used as reactors for this study. Working volume of each reactor was 10L. The acclimatized sludge maintained under anaerobic (B1), anoxic (B2) and aerobic (B3) environments were added to reactors and connected in series and operated under fed batch mode from 113th day at total HRT 10 days. Each reactor contained 100 g of sponge cubes. Total volume of sponge cube in each reactor was 1967 cm^3, which was 19.67% of working volume of each reactor. Decanted time for B1 was 15 min whereas for B2 and B3 it was 20-30 min through out the study. Decanted volume from each reactor was calculated from decided HRT of the reactor (equation 3.5). Effluent was first withdrawn in three 10L plastic buckets and then influent was fed to the reactors. Dissolved oxygen in B3 was 3-4.5 mg/L.

$$\text{HRT (day)} = \frac{\text{Reactor volume (L)}}{(\text{Volume decanted per cycle in L})(\text{No. of cycles per day})} \qquad \text{(Eq.3.5)}$$

3.2.3.2.3 Performance evaluation of FMBR system at varied feed concentration and operating condition

In each cycle, from B1, required amount of supernatant was withdrawn and was replaced with fresh feed purged with N_2 gas was added through a multi channel peristaltic pump. B1 was directly fed with synthetic feed. Similarly, supernatant each from B2 and B3 were withdrawn at a cycle. Working volume of each reactor was constant at 10L. Collected effluent from B1 was mixed with effluent of B3 (recycle ratio of 1) with external addition of 1000 mg/L NO_3^-–N and fed to B2. Effluent of B2 was directly fed to B3 after necessary pH adjustment with 1N HCl or 1N $NaHCO_3$ through multi channel peristaltic pump. All reactors were operated in up flow mode and maintained at a temperature controlled room (30 ± 2 °C). B1 and B2 were kept closed at both upper and lower ends and mixing was achieved by up flow motion of the influent and the gas generated, whereas in B3 compressed air was supplied continuously for aeration and provided mixing too.

Acclimatized batch reactors were started with feed of phenol 1500, SCN^- 100, NH_4^+-N 500 and COD 4500 mg/L. Three-stage system was operated for almost 30 days at this feed. Thereafter feed SCN^- was increased to 200 mg/L with increment of 10 mg/L/day without changing other feed parameters. Feed SCN^- was further increased to 400 and 800 mg/L in a

likely manner. Similarly for phenol study influent phenol was varied from1000 mg/L to 2500 mg/L in step manner. Influent concentration to B2 was calculated using equation 3.4. The various feed and operating conditions during these studies are given in Table 3.2.

Table 3.2 Operational schedule and conditions of FMBR system

Experiment	Days	Feed (mg/L)			Fill time, B1 (h)	Cycle time	HRT (day)			
		Phenol	SCN^-	Pyridine			B1	B2	B3	Total
1	200-250	1500	100	NA	2	24	5	2.5	2.5	10
	251-300	1500	200							
	301-340	1500	400							
	341-388	1500	800							
2	389-440	1500	100		instant	24	5	2.5	2.5	10
	441-490	1500	800		1					
	491-520	1500	800		1.5					
	521-570	1500	800		2.5					
	571-620	1500	800		3.7					
3	621-680	1500	800		1.25	24	5	2.5	2.5	10
	681-720	1500	800		1.5		4	2	2	8
	721-758	1500	800		2.08		3	1.5	1.5	6
	759-805	1500	800		2.5		2.5	1.25	1.25	5
4	806-853	1500	800		2.08	18	2.25	1.12	1.12	4.5
	854-880	1500	800		2.08	24	3	1.5	1.5	6
	881-925	1500	800		2.08	30	3.75	1.87	1.87	7.5
	926-950	1500	800		2.08	36	4.5	2.25	2.25	9
5	951-980	1000	800		2.08	24	3	1.5	1.5	6
	981-1000	1500	800		2.08					
	1001-1030	2000	800		2.08					
	1031-1062	2500	800		2.08					
6	1064-1100	1500	800	25	2.08	24	3	1.5	1.5	6
	1101-1140	1500	800	50	2.08					
	1141-1178	1500	800	100	2.08					
	1179-1220	1500	800	250	2.08					

Fill time, HRT and cycle time were made variable parameters for this study with constant influent concentration. Various operating conditions during the experiments are given in Table 3.3-3.5. During fill time variation study, volume decanted from B2 and B3 and fill time were twice of B1 and HRT of each reactor was constant (total 10 days). However, during cycle time variation, with increase in cycle time, number of cycles decreased per day and hence, HRT increased (total 4.5-9 days), (equation 3.5). Similar to thiocyanate phenol and pyridine concentration in the FMBR was varied from 1000 to 2500 mg/L and 25-250 mg/L in respective studies maintaining other constituents fixed during the experiments.

Table 3.3: Operating conditions of Sequential fed batch MBR (FMBR) system during fill time variation

Reactor	Fill time (h)					Reaction time (h)				
Anaerobic reactor (B1)	1 (33.33)	1.5 (22.22)	2.5 (13.3)	3.7 (9)	0[a] (NA[b])	22.75	22.25	21.25	20.05	23.75
Anoxic (B2)	2 (33.33)	3 (22.22)	5 (13.3)	7.4 (9)	0[a] (NA[b])	21.5	20.5	18.5	16.1	23.75
Aerobic (B3)	2 (33.33)	3 (22.22)	5 (13.3)	7.4 (9)	0[a] (NA[b])	21.5	20.5	18.5	16.1	23.75

[a] Instantaneous fill, NA[b] Not applicable as influent was added instantaneously;
Values in parenthesis indicate flow rate (mL/min).

Table 3.4: Operating conditions of Sequential fed batch MBR (FMBR) at HRT variation study

HRT variation	Anaerobic (B1)				Anoxic (B2)				Aerobic (B3)			
HRT (day)	5	4	3	2.5	2.5	2	1.5	1.25	2.5	2	1.5	1.25
Fill period (h)	1.25	1.5	2.08	2.5	2.5	3.12	4.16	5	5	6.2	8.33	10
Amount withdrawn (L)	2	2.5	3.33	4	4	5	6.66	8	4	5	6.66	8
Cycle period (h)	24				24				24			
Flow during fill (L/h)	1.6				1.6				0.8			

Table 3.5: Operating conditions of sequential fed batch MBR (FMBR) system during cycle period variation study

cycle variation	Anaerobic (B1)				Anoxic (B2)				Aerobic (B3)			
Cycle time (h)	18	24	30	36	18	24	30	36	18	24	30	36
Flow during fill period (L/h)	1.6				1.6				0.8			
Amount withdrawn (L)	3.33				6.66				6.66			
Fill period (h)	2.08				4.16				8.33			
HRT (day)	2.25	3.00	3.75	4.50	1.13	1.50	1.88	2.25	1.13	1.50	1.88	2.25
Reaction time (h)	15.67	21.67	27.67	33.67	13.34	19.34	25.34	31.34	9.17	15.17	21.17	27.17

3.2.4 Substrate removal kinetics study in CMBR and FMBR

Substrate removal kinetics in CMBR and FMBR system were studied with experimental values from feed concentration and operational condition variation study.

3.2.5 Performance of the moving bed systems at shock load

The key problems of any industrial wastewater treatment involves irregular organic loading due to change in process conditions. In order to evaluate performance of MBR system at sudden increase in influent feed, both CMBR and FMBR were subjected to sudden increase in influent concentration as shock loading. Shock loading experiments were performed at two conditions by sudden increase in phenol (2500 to 3000-3500 mg/L) and thiocyanate (600 to 1000-1200 mg/L) concentration in CMBR and FMBR. Samples

were taken at regular time interval and analyzed for various parameters like phenol, COD, thiocyanate, ammonia nitrogen and nitrate-nitrite- nitrogen etc.

3.2.6 Treatment of real wastewater

To evaluate the performance of the moving bed reactor system on removal of phenolics, pyridine and thiocyanate, two coke oven wastewater was collected from a coke production industry at Assam, India. The wastewater was collected from inlet point to the treatment plant and stored in refrigerator at 4^{o}C. The wastewater was initially characterized for its phenol content, chemical oxygen demand (COD), pH, Dissolved Oxygen (DO), heavy metals along with other parameters

In Figure 3.2 to 3.3 the picture of experimental set is given. Figure 3.2 is the set up before use for the experiment and Figure 3.3 contain both the CMBR and FMBR set used for the study.

Figure 3.2 Photograph of the CMBR set up used in the study.

Figure 3.3 Photograph of the CMBR/FMBR used in the study.

3.3 Analytical Methods

3.3.1 Wastewater parameter

In general, standard techniques as detailed in standard methods (APHA, 1998) has been followed unless otherwise specified. Samples of reactor effluents were collected and centrifuged at 7500 rpm prior analysis as per respective methods. Influent and effluent pH was measured using a digital pH meter with a sensitivity of 0.01 with temperature correction facility. The instrument was calibrated periodically with standard buffer solutions. Temperature was measured with thermometer. Dissolved oxygen concentration was measured using a digital DO meter with a sensitivity of 0.01. Conductivity, oxidation reduction potential etc intermittently measured using a multi-parameter water quality measurement kit (Model 6920-M Yellow Springs, Ohio-USA).

Phenol was estimated by colorimetric method using 4-aminoantipyrene measuring absorbance at 500 nm. Chemical oxygen demand (COD) was estimated by closed refluxed method and titrating samples by ferrous ammonium sulphate. Thiocyanate was measured by colorimetric method using ferric nitrate in acidic pH and absorbance was measured at 460 nm. Ammonia-nitrogen was measured by phenate method at 640 nm using sodium nitroprusside. Volatile fatty acid was measured by titrimetric method.

Nitrate-nitrogen concentration was determined by using ultra violet screening method measuring absorbance at 220 and 275 nm in UV-spectrophotometer and Ion chromatograph. Wavelength of 220 nm was used to obtain NO_3-N reading and a wavelength of 275 nm to determine interference due to dissolved organic matter. Nitrate ions were also measured using ion Chromatograph equipped with a Dual 3 column (250 mm×4 mm), a RP guard column, and a conductivity detector. Samples taken during the experiments were centrifuged at 8000 rpm for 10 min and were filtered through a C-18 reverse-phase cartridge and then through 0.45µm filter for analysis. NaOH (5mM) served as the eluent and sulfuric acid (2.0mM) as the regenerant in the chromatogram analysis. Nitrite-nitrogen (NO_2^--N) was estimated by colorimetric method using N (1-Naphthyl) ethylene-diamine dichloride) at 543 nm. Sulfate was analyzed by turbidity method using barium chloride.

Concentration of pyridine was estimated using reverse phase HPLC equipped with a UV-VIS detector at wavelength 254 nm and an Onsphere C-18 column (Varian, particle size 5 µm, length 15 cm, diameter 4.6 mm) using acitonitrile: water (80%:20%) as the mobile phase. About 300 µl of sample was filtered through sterile syringe filter (PTFE filter media of 13 mm diameter, pore size 0.20 µm, Pall, USA) to remove biomass or any other particulate matters. The retention time of pyridine was found to be 1.4 min at flow rate of 0.8 mL/min.

For the SEM (scanning electron microscope) photograph, fragments of sponge cube containing the microbial bio-film were sampled at various sampling points of the moving bed reactor and cut into small thin pieces. The sample was mounted on aluminum stubs and then coated with gold using sputter coater (Edward, USA). The stubs were then introduced into the specimen chamber of LEO 1430vp for scanning. Figure 3.4 (a) and (b)

shows the SEM image of clean sponge cube before use and sponge cube with biomass after use in reactor.

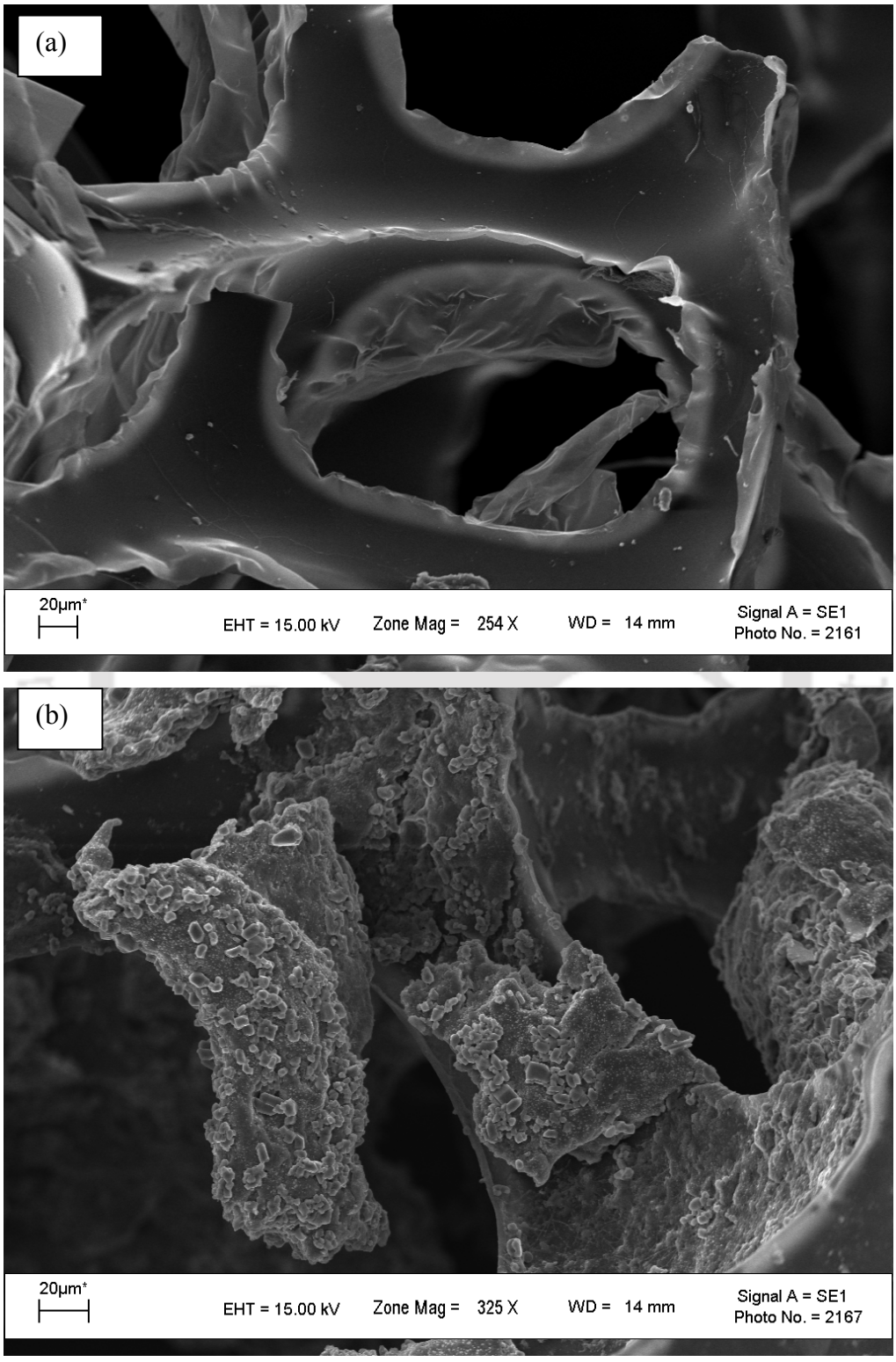

Figure 3.4 SEM image of sponge cube (a) before use (b) with biomass

Concentrations of heavy metals, such as cobalt, strontium, lead and Nickel etc, in the real wastewater were measured using atomic absorption spectrometer (AAS) (Varian, USA, Model No. Spectra AA 240 FS) at their respective absorbance maxima.

3.3.2 Biomass concentration

For measuring suspended biomass, known amount of mixed liquor was taken. Total volatile solid (VS) were measured after drying mixed liquor and sponge cubes at 103^{o}C and ignited at 550^{o}C, respectively.

For attached biomass randomly 3-4 sponge cubes were taken. Rinsed with distilled water thoroughly and dried in 105^{o}C and then ignited at 550^{o}C according to APHA 1998. During the initial days operation four sponge cubes were collected from top (near effluent port) and middle (one foot below the effluent point) of anaerobic, anoxic and aerobic reactors and rinsed with distilled water. Biomass in sponge was determined from weight difference of the rinsed liquor after drying in oven (110 °C) and muffle furnace (550 °C) and calculated based in sponge cube number.

3.3.3 Chemical characteristics of sludge

Sludge was collected from reactors R1, R2 and R3 and digested using concentrated nitric acid and analysed for various parameters.

3.3.4 Specific methanogenic activity (SMA)

SMA analysis was carried out with sludge from R1/B1 in a procedure similar to Isa et al.(1997). A known amount of sludge with VSS of 1-2 g/L was taken from anaerobic reactor and poured into serum bottles. Feed was added to each of serum bottle identical to synthetic feed purged with nitrogen gas used in the study varying thiocyanate concentration. Each test was carried out for three times. Supernatant was decanted, sludge was washed and fresh feed was given after the completion of each cycle. Data of third feeding was considered as data for actual specific methanogenic activity of the anaerobic reactor sludge. Biogas generation in R1 was measured by using water displacement method.

3.3.5 Enrichment, isolation and identification of microorganisms

Isolation of pure culture was carried out using pour-plate method. From each reactor randomly two sponge cubes were collected, dipped in distilled water separately for 5 minutes and scraped using a spatula. Then this water was serially diluted with distilled water upto10^5 times by serial dilution method. One mL of diluted culture was poured to Petri dish containing peptone beef extract and agar and incubated at 35°C for 24 hours. Discrete and well separated single colony was collected by sterile inoculating loop and transferred separately to conical flask containing peptone (5 g/L), beef extract (3 g/L), synthetic feed, phosphate buffer (1 mL/L), trace metal solution (1 mL/L) in a total volume of 75 mL. Synthetic feed of anaerobic culture was prepared by adding phenol (1000 mg/L), SCN^- (500 mg/L) and NH_4^+-N (500 mg/L). For anoxic culture synthetic feed contained phenol (500 mg/L), SCN^- (250 mg/L), NH_4^+-N (250 mg/L) and NO_3^--N (500 mg/L). For aerobic reactor culture, synthetic feed contained phenol (100 mg/L), SCN^- (100 mg/L) and NH_4^+-N (250 mg/L).

All conical flasks were incubated for two days. After two days 1 mL of culture was taken from conical flask and used for each biochemical test separately. All the dilution, inoculation and transfer of culture were carried out in sterile condition. The identification of microorganisms was carried out by Morphological and biochemical tests along with microscopic observations. Biochemical tests were conducted according to Cappuccino and Sherman (1998). In biochemical test for identification of strain, gram reaction test, fermentation test (lactose, dextrose, sucrose and inulin), H_2S production, nitrate reduction, indole production, Methyl red test, Vogues Proskauer test, catalase, oxidase and citrate tests were carried out.

Various instruments used during the study is mentioned with their make etc. in Table 3.6

Table 3.6 Instruments and equipments used in the present investigation

Instruments/ Equipments	Parameters tested/ Measured	Model/Manufacturer Specification
Digital pH meter	pH	Orion 3 star –QY-14478, Thermo Scientific, Singapore
Dissolved oxygen meter	DO	Orion 3 star –QY-14478, Thermo Scientific, Singapore
Electronic balance	Weight	Sartorius, BT-224S (4 decimal)
Centrifuge	Separation of suspended solids	Remi C-24-BL Mumbai, India
COD digester	COD	HACH, DRB-200, USA
UV-visible spectrophotometer	Phenol, Thiocyanate, Ammonia-nitrogen, Nitrate, Nitrite-N	Model lambda-45, Perkin Elmer, USA,
Ion Chromatograph	Nitrate-N, Sulfate, Chloride	(Metrohm 792 AG, Herisau, Switzerland
Nephelo metric turbidity meter	Sulfate, turbidity	Systronics 132
Oven	MLSS, Drying other materials	Tonco-PLT-125, India
Autoclave	Sterilization	Equitron-7407PAD India
Laminar Air flow hood	To transfer pure culture in aseptic manner	Clean air system, CAH -1800 Chennai, India
BOD incubator	To prepare culture in agar media	Delux model- IK-120 Delhi, India
High performance liquid Chromatography	Phenol, pyridine, m, o, p- cresol	Varian Prostar 210
Atomic Absorption spectrophotometer	Pb, Co, Cr and Cd	Varian, USA, Model No.Spectra AA 240FS
Air compressor	Aeration in aerobic reactors	Sonee SSY-8
Water purification system	To provide distilled water and Millipore water	Sartorius –AG, arium -611uv, 61315. Germany
Peristaltic Pump	Feeding to reactors	Miclin India limited PP10
Hot air blower	Maintain temperature	Philips

CHAPTER 4

RESULTS AND DISCUSSION

Present study was conducted using moving bed anaerobic–anoxic–aerobic reactor system in continuous and fed batch mode for removals of phenol, thiocyanate, ammonia–nitrogen containing wastewater. Concentration of pollutants in feed and various operational parameters like hydraulic retention time (HRT), fill time, cycle time (fed batch mode) were considered to evaluate the performance of moving bed bioreactor system.

The performance of reactors was evaluated in terms of pollutant removal (%) and pollutant removal rate (g/L.day). Pollutant loading and removal rate (g/L.day) were calculated as shown in equation (4.1 & 4.2).

$$\text{Loading rate} = \frac{S_0}{\text{HRT} \times 1000} \qquad \text{(Eq.4.1)}$$

$$\text{Removal rate} = \frac{(S_0 - S_e)}{\text{HRT} \times 1000} \qquad \text{(Eq.4.2)}$$

where, S_0 and S_e are influent and effluent, concentration in mg/L.

4.1 PERFORMANCE OF SEQUENTIAL ANAEROBIC–ANOXIC–AEROBIC CONTINUOUS MOVING BED REACTOR (CMBR) SYSTEM

The CMBR system was operated with varied feed concentration of thiocyanate, phenol and ammonia–N. Effect of hydraulic retention time (HRT) being the most profound operating condition was also evaluated. During the experiments except the varied parameter the other experimental conditions were maintained same for a particular set of experiment.

4.1.1 Performance of CMBR system at varied feed thiocyanate (SCN^-) concentration

Feed SCN^- concentration was varied from 0–600 mg/L in five experimental runs. After the first experimental run (absence of SCN^- in feed), it was added in feed gradually and increased till 110 mg/L maintaining all other parameters constant (total HRT 4 days and feed phenol 2500, NH_4^+–N 500 mg/L). In subsequent experimental runs feed SCN^- concentrations was increased in a likely manner to 200, 450 and 600 mg/L. It was observed that after each modification in feed SCN^- concentration, the three–stage system attained steady state as characterized by consistency in effluent parameters, after a transient period of 7–10 days. Steady state data was collected for 12–15 days and considered to analyze the performance of each reactor.

Figure 4.1 shows influent/effluent profile in CMBR during feed SCN^- variation study.

4.1.1.1 Performance of anaerobic CMBR (R1) at varied feed thiocyanate

Steady state performance of R1 at varied influent thiocyanate concentrations is presented in Tables 4.1 (a) and (b) as average values along with standard deviation. Thiocyanate loading rates were 0.055, 0.1, 0.225 and 0.30, g SCN^-/L.day. In R1 SCN^- removal increased from 4.5% to 7.7% with increase in SCN^- loading up to 0.225 g SCN^-/L.day and thereafter decreased to 4.7% at SCN^- loading of 0.30 g/L.day. Figure 4.2 shows that maximum SCN^- removal rate of 0.017 g SCN^-/L.day was achieved in R1 at loading of 0.225 g SCN^-/L.day. Hung and Pavlostathis (1998) observed no degradation of SCN^- by anaerobic microorganisms at feed SCN^- of 145 mg/L and the probable reason was reported as absence of appropriate enzymes. Present result was better than literature findings, though removal of SCN^- was low in R1.

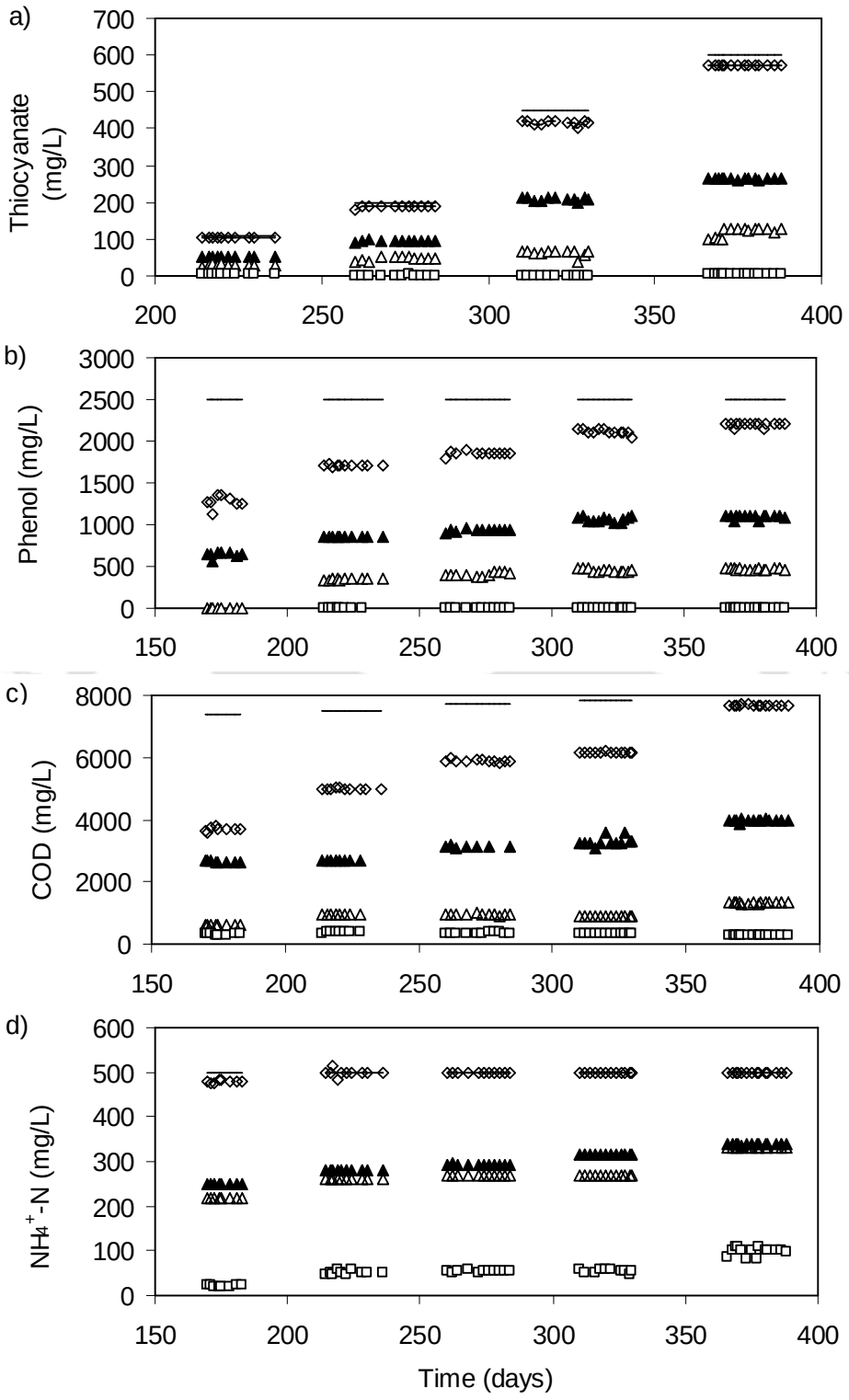

Figure 4.1 Pollutant profile in sequential CMBR a) Thiocyanate, b) Phenol, c) COD and d) NH_4^+-N during feed SCN^- variation study

Influent phenol concentration was fixed at 2500 mg/L with loading rate of 1.25 g/L.day. In R1 removal of phenol decreased from 48% to 12.6% (almost 73% decrease) with increase in feed SCN^- concentration from 0–600 mg/L [Table 4.1 (a)]. For removal of COD, the effect was similar in nature but more profound. During feed SCN^- variation study, average feed COD were 7400–7980 mg/L (Feed COD was principally contributed from phenol with 1mg phenol contributed 2.38 mg COD and 1 mg SCN^- contributed 1.11 mg COD). COD removal decreased form 50% to 3.7% (more than 92% decrease) with increase in feed SCN^- from 0–600 mg/L. In Figure 4.2, it can be seen that both phenol and COD removal rates showed declining trends (phenol removal rate 0.158–0.61 g/L.day; COD removal rate 0.148–1.85 g/L.day) with increase in loading of SCN^- in R1. These results show that SCN^- inhibited degradations of phenol and COD in anaerobic environment. Previous literatures clearly reported inhibitory effect of SCN^- on degradation of phenol in aerobic environment (Banerjee, 1996). However, no literature report is available on the effect of SCN^-, on degradation of phenol in anaerobic environment. Table 4.1 (a) shows that at all levels of feed SCN^-, removal of NH_4^+–N in R1 was almost nil. In anaerobic condition NH_4^+–N removal might occur due to incorporation of nitrogen in biomass.

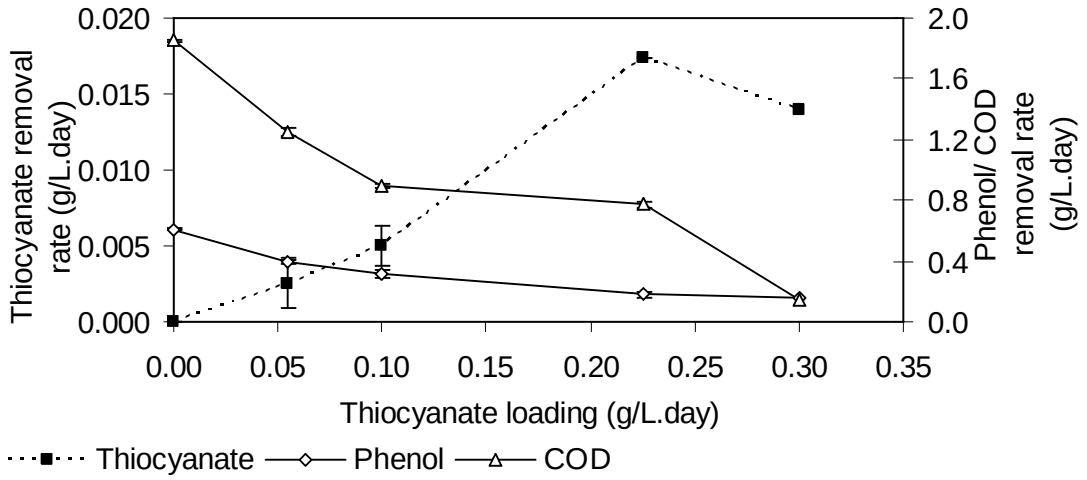

Figure 4.2 Effect of thiocyanate loading on performance of R1

In Figure 4.3 fractional removals of feed SCN^-, feed phenol and feed COD by R1 are shown. It can be seen that the removal of total feed SCN^- by R1 was very low at all levels of feed SCN^-. However, R1 had significant role in fractional removals of feed phenol and COD only when feed SCN^- was low. This decreased with increase in feed SCN^-

concentration. In R1, pH decreased from 7.5 to 6.8–6.9 and effluent VFA (volatile fatty acid) concentration observed was 175–379 mg/L as acetic acid.

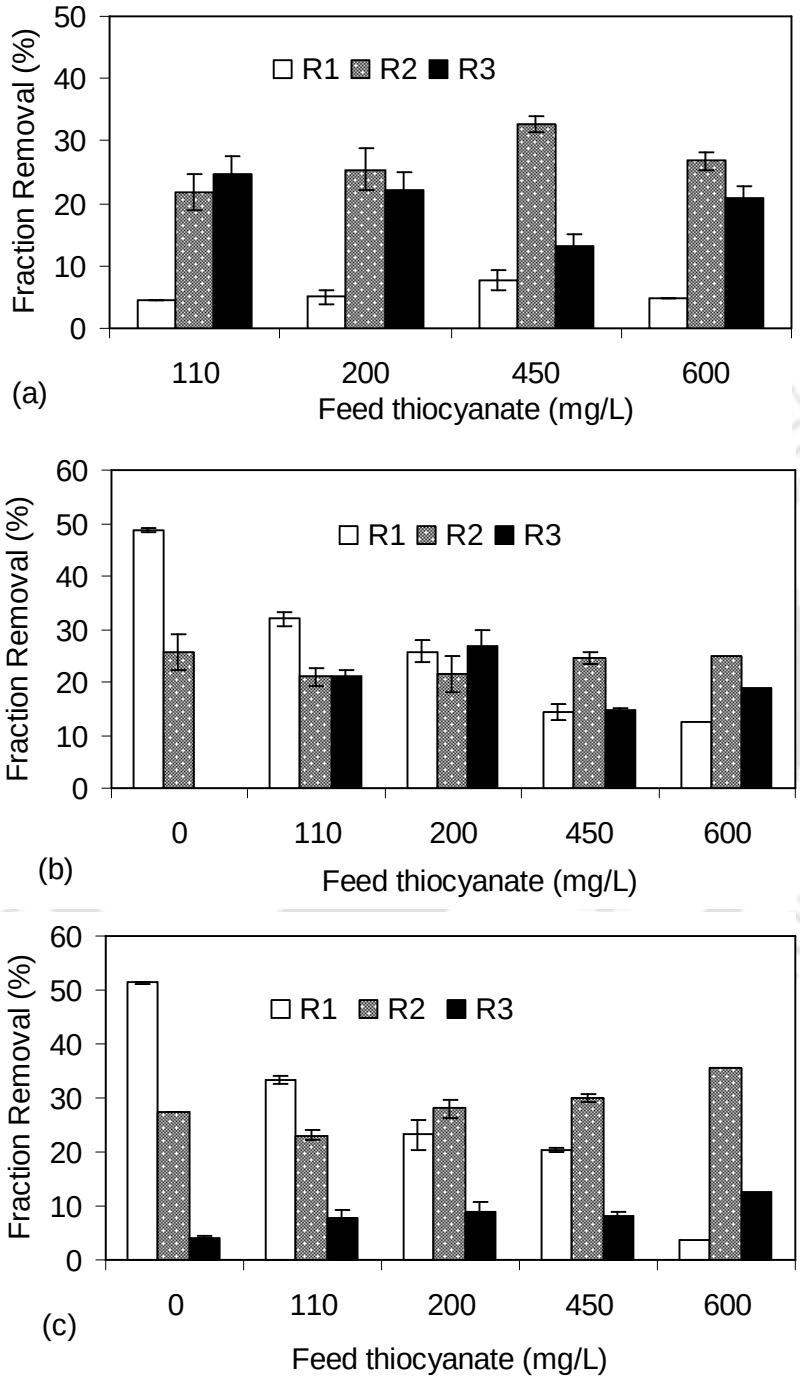

Figure 4.3 Fractional removal of (a) Thiocyanate (b) Phenol and (c) COD in individual reactor at varied feed thiocyanate concentration

Table 4.1 (a): Average performance of anaerobic CMBR (R1) at feed SCN^- concentration variation

SCN^-			Phenol			COD			NH_4^+–N	
Feed	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e
0	–	–	2500	1280 (76)	48.8	7400	3700 (65)	50.0	500	480 (15)
110	105 (0)	4.5		1701 (8.9)	31.9	7500	5000 (11)	33.0		500 (17.5)
200	190 (5.7)	5.0		1858 (33)	25.7	7700	5900 (56)	23.4		505 (4.2)
450	415.3 (6.6)	7.7		2137 (51)	14.5	7850	6309 (292)	19.6		503 (27.9)
600	572 (0)	4.7		2184 (37)	12.6	7980	7685 (28)	3.7		500 (0)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

Numbers in parenthesis indicate standard deviation values

Table 4.1 (b) shows that biogas generation in R1 decreased with increase in feed SCN^-, and no gas produced at feed SCN^- of 450 mg/L and above. Specific methanogenic activity (SMA) of R1 biomass was studied at varying concentration of feed SCN^-. In absence of SCN^-, SMA activity in R1 sludge was 0.349 gCH_4–COD/gVSS.day and decreased to 0.1019 gCH_4–COD/gVSS.day and 0.036 gCH_4–COD/gVSS.day when feed SCN^- concentrations of 110 mg/L and 200 mg/L, respectively was added to biomass taken from R1. No SMA was observed when feed SCN^- was 450 mg/L and above, suggesting SCN^- inhibited methanogenic activity at feed $SCN^- \geq 450$ mg/L. Fang et al. (1996, 2006) reported SMA of 0.19 and 0.24 gCH_4–COD/gVSS.day during degradation of phenol at temperatures of 26 and 37ºC, respectively. Tay et al. (2001) reported SMA of 0.17 g CH_4–COD/gVSS.day at initial phenol of 2000 mg/L using phenol as the sole carbon source. SMA observed in the present study in absence of SCN^- in feed at initial phenol of 2500 mg/L was higher than these reported values. As no SMA was achieved at high SCN- concentration, biomass yield was calculated and found in ranged of 0.3-0.7 g VSS/g COD removed/day during the study.

Table 4.1 (b): Average performance of anaerobic reactor (R1) at feed SCN^- concentration variation

SCN^- Feed (mg/L)	Biogas (mL/day)	SMA (g CH_4–COD/ g VSS. day)	pH S_e	TVS (mg/L)	VFA S_e
0	344	0.349	6.7	12600	379 (14)
110	156	0.102	6.8	10200	175 (4)
200	56	0.036	6.9	11000	288 (45)
450	0	ND	6.8	10750	194 (40)
600	0	ND	6.7	10500	285 (31)

S_e: Effluent (mg/L), ND: not detectable; VFA: Volatile fatty acid as acetic acid (mg/L)

Numbers in parenthesis indicate standard deviation values

Total volatile solids (TVS) in R1 was almost 12600 mg/L in absence of thiocyanate and decreased little to 10,000–11,000 mg/L with addition of SCN^- then remained stable at 10700– 10500 mg/L at maximum feed SCN^-. The ratio of attached biomass to suspended biomass in R1 was 7.7 in absence of SCN^-. This ratio increased to 13.5 with addition of 110 mg/L thiocyanate as biomass in suspension decreased from 1400 mg/L to 700 mg/L (decreased by almost 50%) and then this ratio decreased to 10.6–10.2 at feed thiocyanate more than 200 mg/L. This indicates higher amount of biomass was in attached condition to sponge cube than the suspended biomass concentration through out the study.

4.1.1.2 Performance of anoxic CMBR (R2) at varied influent thiocyanate

Anoxic reactor (R2) received its influent consisted of effluent from R1 and recycled effluent from R3 in a recycle ratio of 1. Influent concentration of R2 was calculated using equation 3.4. R2 was operated at HRT of 1 day during feed SCN^- variation study. Nitrate (NO_3^-–N of 1000 mg/L) was added in the recycle flow from R3. The anoxic reactor performance is shown in Tables 4.2 (a) and (b).

In R2, influent SCN^- was 54, 96, 208 and 288 mg/L, with respective loadings of 0.054, 0.096, 0.208 and 0.288 g SCN^-/L day during the study. R2 was responsible for removal of

44–70% of influent SCN^- [Table 4.2 (a)]. Figure 4.4 shows that with increase in SCN^- loading in R2, SCN^- removal rate in R2 increased almost linearly form 0.024 to 0.166 g/L.day with a slope of 0.61. Figure 4.3 (a) shows that almost 21–32% of feed SCN^- was removed in R2, which initially increased with increase in feed SCN^- up to 450 mg/L and with further increase in feed SCN^- it decreased slightly to 27%. In published literatures reports on degradation of SCN^- in anoxic environment is quite contradictory.

Kim et al. (2008a), reported phenol and COD removal of 69–32% and 42–31% at a phenol loading rate of 0.06–0.25 g/L.day and COD loading of 1.03–1.86 g/L.day, respectively without any SCN^- removal in presence of 82–128 mg/L NH_4^+–N in the anoxic unit of a pre–denitrification system. Shieh and Richards (1988) too reported no thiocyanate removal in anoxic reactor. However, Andreoni et al. (1988) first reported SCN^- degradation by mixed denitrifying cultures with end product as sulfate. Sorokin et al. (2004) also reported SCN^- removal in anoxic reactor by sulfur oxidizing autotrophic bacteria, *Thialkalivibrio thiocyanodenitrificans*, where SCN^- was used as an electron donor with nitrate/nitrite as electron acceptors and end products were sulfate and ammonia (Eq.2.2d). Grigor'eva et al. (2009) also observed ammonium and sulfate as end products of thiocyanate degradation under both aerobic and denitrifying condition. However, these reported studies were carried out without any phenol in medium. Present investigation shows that SCN^- can be efficiently biodegraded by mixed denitrifying cultures in presence of phenol. Influent and effluent sulfate values in R2 are presented in Table 4.2 (a).

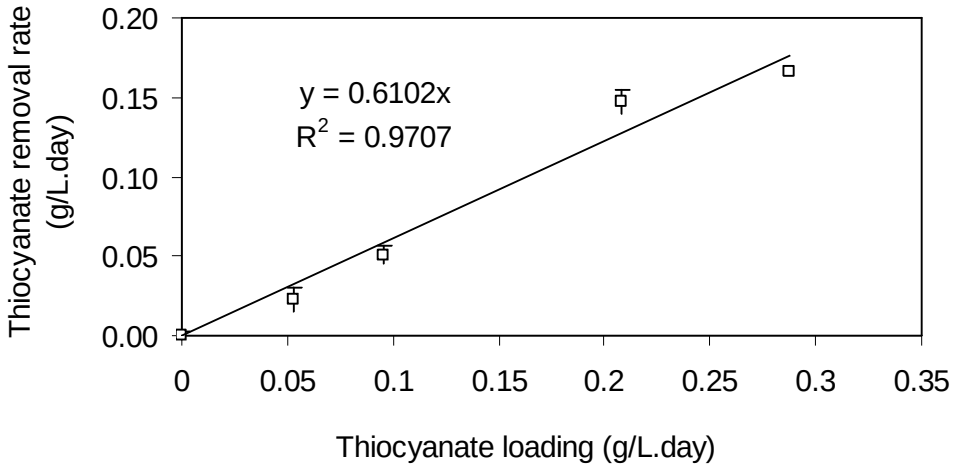

Figure 4.4 Effect of thiocyanate loading on removal rate in R2

In R2, some sulfate entered as influent from R3 with the recycle (95–365 mg/L). Table 4.2 (a) shows that 140–600 mg/L sulfate got released in R2 effluent with 45 –235 mg/L of sulfate generation in R2. Generation of sulphate in R2 was possible only from SCN^- degradation. Based on the generation of byproducts, the stoichiometric equation of SCN^- degradation in anoxic reactor is proposed below (Sorokin et al. 2004):

$$SCN^- + 1.6NO_3^-\text{-}N + 0.2H_2O + 1.6H^+ + HCO_3^- \rightarrow SO_4^{2-} + NH_3 + 2CO_2 + 0.8N_2 \qquad \text{(Eq.4.3.a)}$$

It was calculated that Gibb's free energy change at standard state ($\Delta G^o_{(aq)}$) for this reaction was (–)824.01 kJ, suggesting feasibility of the reaction at standard state. The stoichiometric equation for SCN^- removal in aerobic environment is as equation 4.3 (b) (Staib and Lant, 2007) with $\Delta G^o_{(aq)}$ of (–)828.34 kJ, suggesting aerobic SCN^- degradation is thermodynamically more feasible than anoxic one.

$$SCN^- + 2O_2 + 2H_2O \rightarrow SO_4^{2-} + NH_4^+ + CO_2 \qquad \text{(Eq.4.3.b)}$$

Table 4.2 (a): Average performance of anoxic CMBR (R2) at feed SCN^- concentration variation

SCN^-			Phenol			COD			Sulfate				
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Gen	Th SO_4^{2-}	Err
–	–	–	644	0	100	2005	620 (21)	69.0	–	–	–	–	–
54 (0.1)	30 (3.1)	44.3	850 (4.7)	344 (18)	59.5	2682	950 (10)	65.0	95 (0.8)	140 (3.5)	45	39.6	7.2
96 (2.3)	45 (5.7)	52.6	929 (16.8)	410 (27)	55.9	3127	947 (43)	70.0	136 (8)	210 (12)	74	83.2	–9.2
208 (3.7)	61 (8.5)	70.6	1069 (25.9)	450 (23)	57.9	3326	915 (30)	72.5	353 (19)	590 (27)	237	242.5	–5.3
288 (0.8)	122 (12)	57.6	1093 (19.3)	468 (12)	57.2	3980	1317 (30)	67.0	365 (13)	600 (23)	235	274.0	– 38.3

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%) Gen: Generation (mg/L);

Th. SO_4^{2-}: Theoretical sulfate generated (1.65x SCN^- removed) mg/L;

Err: Error, experimental value–theoretical value, (mg/L)

Numbers in parenthesis indicate standard deviation values.

Maximum error in sulfate generation was observed to be (–) 38 mg/L compared to the theoretical sulfate generation at maximum thiocyanate loading of 0.28 g/L.day. Balance in thiocyanate degradation and sulfate generation is rarely reported. Mahmood et al. (2008) observed less generation of sulfate during oxidation of sulfide in anoxic reactor at high pH (> 8.0) and higher sulfide loading though sulfide removal was steady. Buisman et al. (1990) also reported accumulation of other sulfur compound such as thiosulfate, polysulfides in anoxic environment in same condition of high pH and high sulfide loading. In the present study lower generation of sulfate than that of theoretical value at higher loading of thiocyanate might be due to conversion of sulfate to other undetected sulfur compound like polysulfides etc.

Influent phenol concentrations to R2 were 640, 851, 929, 1069 and 1093 mg/L [Table 4.2 (a)] with loading rates of 0.64, 0.851, 0.929, 1.069 and 1.093 g/L.day, respectively. Reactor R2 removed 56–60% of influent phenol in presence of 54–288 mg/L SCN^-. Figure 4.5 shows that phenol removal rate in R2 decreased little due to presence of SCN^- in influent of R2.

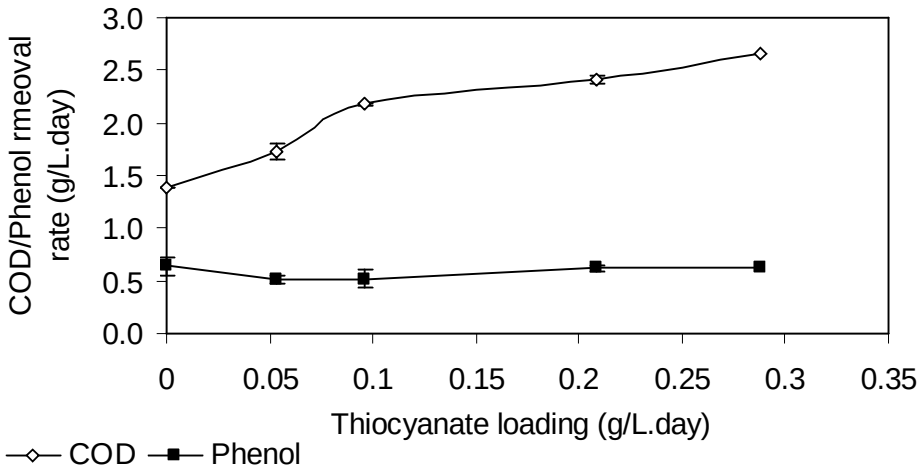

Figure 4.5 Effect of thiocyanate loading on phenol and COD removal rates in R2

The average influent COD to R2 were 2005, 2682, 3127, 3326 and 3980 mg/L. COD loading rates were 2.05, 2.68, 3.12, 3.32 and 3.98 g/L.day. R2 removed 64–72% of influent COD showing 23–35% of total feed COD removal. It was observed that with increase in SCN^- loading, COD removal rate in R2 increased, suggesting that the inhibitory affect of SCN^- on phenol and COD removal was absent in R2 unlike upstream reactor R1. Figure

4.3 shows that fractional phenol and COD removal by R2 was in the range of 20–25% and 25–35% respectively, irrespective of feed SCN^-.

Influent ammonia to R2 ranged from 251–338 mg/L [Table 4.2(b)] and the loadings were 0.251, 0.281, 0.292, 0.314 and 0.338 g/L.day. In R2, some amount of NH_4^+–N was expected to be generated due to degradation of SCN^- (0.24 g of NH_4^+–N/g SCN^- removed) and 7.5–2.6% of NH_4^+–N removal occurred which might be due to incorporation in the synthesis of biomass.

Table 4.2 (b): Average performance of anoxic CMBR (R2) at SCN^- concentration variation

Feed SCN^-	SCN^-	NO_x^-–N					NH_4^+–N			COD: N rem$^{\Psi}$	COD_B	TVS_C
		NO_3^-–N		NO_2^-–N		Rem						
	S_0	$S_0^{\#}$	S_e	S_0	S_e		S_0^A	S_e	Rem			
0	–	727	316 (6)	0	0	56.6	251	220	12.35	3.36	15.0	10580
110	54 (0.1)	617 (9)	47 (2)	2	0	92.4	281 (8.8)	260 (7.6)	7.42	3.03	5.6	9125
200	96 (2.3)	627 (3)	45 (1)	3.5	0	92.8	292 (1.8)	267 (7.5)	8.45	3.76	23.9	9432
450	208 (3.7)	624 (6)	120 (9)	29	0	81.6	314 (11)	270 (4.2)	14.10	4.91	41.7	10420
600	288 (0.8)	612 (8)	129 (9)	50	0	80.5	338 (5.5)	330 (12)	2.60	5.60	49.8	11355

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

$^{\#}$ In R2 influent 500 mg/L of NO_3^-–N was added externally;

^{A} Influent NH_4^+–N of R2 = {Effluent NH_4^+–N of (R1+R3)/2 + 0.24x (SCN^- removed in R2)}.

$^{\Psi}$: as per equation (4.5); COD_B: COD fraction (%) for biomass;

$_C$TVS: Biomass as Total volatile solids in sponge + suspension (mg/L);

Numbers in parenthesis indicate standard deviation values.

With introduction of SCN^- in R2, total volatile solids (TVS) initially found to decrease from 10500 mg/L to 9125 mg/L. However it increased to 11300 mg/L while high concentration of SCN^- was present in the influent to R2. In absence of SCN^- in R2, high

ratio of attached biomass to suspended biomass was observed (14:1). With increase in influent SCN^- the ratio continuously decreased. Up to influent SCN^- concentration 96 mg/L the ratio was 9.2 and it decreased to 4.1 and 3.6 at further increase in influent SCN^- concentration to 208 and 288 mg/L, respectively. Sludge rising in R2 was observed regularly due to production of nitrogen gas inside the reactor. The entrapped gas was released by inserting a thick bamboo stick to the interior of the reactor and again closing R2 at the upper end.

In the present study, NO_3^-–N concentration in recycle (effluent of R3) was inadequate, so additional nitrate (1000 mg/L NO_3^-–N) was supplied externally in recycled effluent of R3 in order to maintain strict anoxic condition in R2. Previous literatures reported commencement of methanogenic condition in anoxic reactor, after depletion of nitrate/nitrite (Karim and Gupta, 2003). Influent NO_3^-–N to R2 were 727, 617, 627, 624 and 612 mg/L with volumetric loadings of 0.612– 0.727 g/L.day. From Table 4.2 (b), it can be seen that with increase in feed SCN^-, influent NO_3^-–N to R2 decreased, whereas influent NO_2^-–N increased. In R2, though complete removal of NO_2^-–N was achieved, removal of NO_3^-–N was incomplete, ensuring anoxic condition in R2. In absence of SCN^- NOx–N (NO_3^-–N + NO_2^-–N) removal was only 56% which further increase to 80–92% with addition of SCN^- in R2. Equation 4.3 shows that 0.38 g NO_3^-–N was required for each g of SCN^- oxidation in anoxic environment. Theoretically, for each gram of phenol, required NO_3^-–N is 0.83 g [equation 2.1 (b)]. Stochiometrically 2.86 g and 1.71 g COD are consumed for removal of one g of NO_3^-–N and NO_2^-–N, respectively [equation 4.4 (a–c)] (Sarfaraz et al. 2004). However, the actual ratio may be higher than this stoichiometric value, since some amount of COD is also consumed for synthesis of biomass.

$$O_2 + 4H^+ + 4e^- \rightarrow 2H_2O \qquad \text{(Eq.4.4.a)}$$

$$NO_3^- + 6H^+ + 5e^- \rightarrow 0.5N_2 + 2H_2O \qquad \text{(Eq.4.4.b)}$$

$$NO_2^- + 4H^+ + 3e^- \rightarrow 0.5N_2 + 2H_2O \qquad \text{(Eq.4.4.c)}$$

The ratio of COD consumed for unit amount of nitrate was calculated using equation 4.5, with the correction of NO_3^-–N consumed for SCN^- oxidation (0.38 mg/mg SCN^-), COD consumed for nitrite reduction (1.71 mg/mg nitrite) and COD generated from SCN^-(1.1 mg/mg SCN^-.

$$COD{:}N_{rem} = \frac{(Influent_{COD} - Effluent_{COD}) - 1.1x\,(Influent_{SCN^-} - Effluent_{SCN^-}) - 1.71x\,(Influent_{NO_2^-N} - Effluent_{NO_2^-N})}{(Influent_{NO_3^-N} - Effluent_{NO_3^-N}) - 0.38x\,(Influent_{SCN^-} - Effluent_{SCN^-})}$$

(Eq. 4.5)

Table 4.2 (b) shows that the COD/NO_3^-–N_{rem} ratio was 3.03–5.6 in R2 during variation of feed SCN^- study. When the theoretical value of 2.86 was divided with this value of COD/N_{rem}, (3.0–5.6), it provided 0.50–0.94. This suggest that 50 to 94% COD was utilized for reduction of NO_3^-–N and residual 6–50% COD was consumed for synthesis of biomass in R2. With higher amount of COD removed, COD fraction available for biomass synthesis (COD_B) in R2 increased. Considering 1.42 as COD of the biomass ($C_5H_7NO_2$), the observed yield of biomass in R2 was 0.04–0.35 (Metcalf and Eddy, 2003). Tuesseau–Vuillemin et al. (2003) reported bacterial growth yield greatly varies from 0.2 to 0.6 and mostly considered to be 0.4. Chakraborty and Veeramani (2006) reported bacterial yield of 0.22–0.24.

Another study was conducted to find out the necessity of nitrate in thiocyanate degradation and therefore separate six 5 L aspirator bottle, acclimatized bacteria similar to R2 and B2 operated at a draw and fill mode with 16% sponge cube were applied in presence of phenol. Nitrate concentration was varied as 0, 50, 75, 100, 200 and 350 mg/L along with thiocyanate and phenol concentration 40 and 200 mg/L, respectively and reactor HRT maintained at 5 days. The variation of influent NO_3^-–N showed the most profound effect on SCN^- removal [Table 4.2 (c)]. With increase in feed NO_3^-–N concentration from 50 to 350 mg/L, removal of SCN^- in the reactor increased from 67% to 95% from influent of 40 mg/L which was only 5% in absence of NO_3^-–N. Sulphate was observed in the effluent and increased from 44 to 60 mg/L, with increase in influent NO_3^-–N and was near to the theoretical sulphate generation from thiocyanate degradation. Effluent NH_4^+–N also increased to 11-14 mg/L from influent of 10 mg/L. With increase in influent NO_3^-–N, denitrification efficiency increased from 88% to 92%, up to feed NO_3^-–N of 100 mg/L and beyond this denitrification efficiency decreased probably due to less availability of electron donor, since phenol and SCN^- were kept constants at 200 and 40 mg/L, respectively. The study shows that thiocyanate degradation is feasible in anoxic environment and nitrate is essential.

Table 4 2 (c): Performance of 5L anoxic batch reactor at varying NO_3^-–N concentration

NO_3^-–N			COD			Phenol		SCN^-		NH_4^+–N	TVS (mg/L)	Sulfate		
S_0	S_e	Rem	S0	S_e	Rem	S_e	Rem	S_e	Rem	S_e		S_e	Th SO_4^{2-}	Err
50	6 (2.1)	88.0	700	242.0 (15)	65.43	79.0 (1.6)	60.5	13.00 (1.2)	67.5	12.0 (0)	5211 (440)	44.0 (2)	44.5	–0.5
75	6 (3)	92.0	710	152.0 (30)	78.59	8.8 (1.7)	95.6	9.35 (3)	76.6	13.0 (10)	7202 (555)	45.0 (2)	50.5	–4.5
100	8 (0.9)	92.0	720	138.0 (22)	80.83	0.6 (0)	99.7	6.35 (1.3)	84.1	14.0 (0)	8220 (548)	50.0 (3)	55.5	–4.5
200	38 (2)	81.0	720	135.0 (17)	81.25	1.3 (0)	99.3	1.90 (1)	95.2	12.0 (0)	9432 (600)	58.0 (2.3)	62.8	–3.2
350	127.0 (3)	63.7	720	120.0 (22)	83.33	0.8 (0)	99.6	1.65 (0)	95.4	12.0 (0)	9440 (600)	60.0 (2.5)	63.2	–3.2
0	0	NA	700	550.0 (20)	21.43	120.0 (1.1)	40.0	38.00 (1)	5.0	11.0 (0)	3930 (580)	–	–	–

S_0: Influent (mg/L); S_e: Effluent (mg/L); Rem: Removal (%) Err: Error (mg/L);
Influent phenol, SCN^- and NH_4^+–N were constant at 200, 40 and 10 mg/L, respectively.
Th SO_4^{2-}: Theoretical sulfate generation (mg/L); [1.65* (SCN– removed (mg/L)]

4.1.1.3 Performance of aerobic CMBR (R3) at varied influent thiocyanate

Average steady state performance of aerobic CMBR, R3 is shown in Tables 4.3 (a) and (b). Influent SCN^- concentrations to R3 were 30 to 122 mg/L with SCN^- loading of 0.03 to 0.122 g/L.day. Removal efficiencies were 97–98% with effluent SCN^- from 0.8–3.8 mg/L. With increase in SCN^- loading, removal rate in R3 increased linearly [Figure 4.6 (a)]. Maximum SCN^- degradation rate observed in R3 in this study was 0.118 g/L.day at loading of 0.122 g SCN^-/L.day. This removal rate was little lower than R2, where maximum SCN^- removal rate achieved was 0.166 g/L.day. Sirianuntapiboon et al. (2007) achieved 82% removal of SCN^- in an aerobic fed batch reactor at loading of 0.084 g SCN^- /L.day while treating photo–processing wastewater containing SCN^-, glucose and urea.

Vázquez et al. (2006b) reported 90% removal of SCN^- while treating coke wastewater in an activated sludge process at SCN^- loading of 0.02 g/L.day at HRT of 9.83 days in presence of 177 mg/L of phenol. The performance of R3 was higher than these reported values.

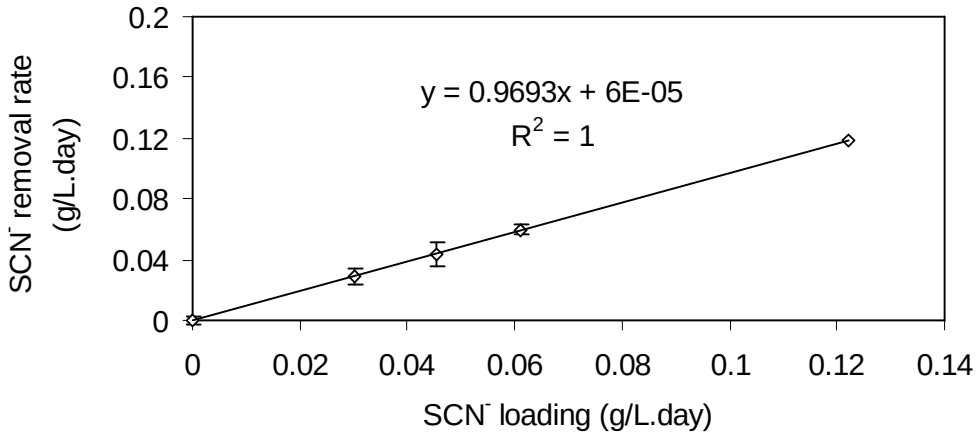

Figure 4.6 (a) Effect of thiocyanate loading on SCN^- removal rate in R3

In the three–stage system anoxic CMBR showed less SCN^- removal efficiency (44–70%) than aerobic CMBR (almost 97%) at all concentrations of feed SCN^-. R3 contributed 13–24% of total SCN^- removal during the study [Figure 4.3 (a)]. However, upstream anoxic reactor was responsible to bring down SCN^- concentration considerably from feed. This facilitated SCN^- removal in R3 as it was able to overcome the toxicity of feed SCN^- when present at high concentrations. In R3, SCN^- was found to oxidized and converted to sulfate as suggested by previous researchers shown in equation 2.2d (Hung and Pavlostathis, 1997).

In R3 sulfate balance was carried out [Table 4.3 (a)] considering stoichiometric evolution of sulfate (1.65 mg/mg of SCN^- degradation) (equation 4.3b). Actual sulfate generation was much less than theoretical sulfate values and higher error was observed when feed SCN^- was maximum at 600 mg/L. Similar to anoxic environment generation of other intermediate sulfur compound such as thiosulfate, polysulfides, element sulfur etc. were probably occurred in R3 as reported in literatures (Janssen et al. 1997; Krishnakumar et al. 2005).

Influent phenol loading in R3 was 0.344 to 0.47 g/L.day. Phenol removal in R3 was more than 99% [Table 4.3 (a)] and it seemed that SCN^- concentration up to 122 mg/L did not

inhibit phenol degradation. Influent COD to R3 was 620–1318 mg/L with loading of 0.62–1.318 g COD /L.day. COD removal efficiencies in R3 were within the range of 50–79% and it increased with increase in COD loading. In R3, maximum phenol and COD removal rate 0.467 and 1.04 g/L.day, respectively was observed at maximum phenol and COD loading in presence of maximum SCN^- loading [Figure 4.6 (b)].

Table 4.3 (a): Performance of aerobic CMBR (R3) at feed SCN^- concentration variation

Feed SCN^-	SCN^-			Phenol			Sulfate					TVS[#]
	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Gen	Th. SO_4^{2-}	Err	
0	–	–	–	0	–	–	–	–	–	–	–	10120 (158)
110	30 (3.1)	0.8 (0.2)	97.3	344 (81.7)	1.1 (0.4)	99.9	140	190 (7)	50	48.2	1.8	9845 (140)
200	45 (5.7)	1.6 (1.1)	96.5	410 (67.9)	1.4 (0.5)	99.7	210	272 (16.2)	62	72.2	–10.2	11912 (133)
450	61 (8.5)	1.4 (0.8)	97.6	450 (73.3)	1.0 (0.4)	99.8	590	705 (7.8)	115	98.3	16.7	12408 (175)
600	122 (12)	3.8 (0.6)	96.9	468 (12.5)	1.5 (0.6)	99.7	600	730 (26)	130	195.0	–65	12667 (251)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Err: Error (mg/L).

Numbers in parenthesis indicate standard deviation values.

Gen: Sulfate generated (mg/L) = (Influent SO_4^{2-} – Effluent SO_4^{2-});

Th. SO_4^{2-}: Theoretical sulfate generated (1.65x SCN^- removed) mg/L.

[#]TVS: Biomass as Total volatile solids [in sponge + suspension (mg/L)]

Biomass concentration in R3 during feed SCN^- variation study was observed to increase from 9845 mg/L to 12667 mg/L when higher amount of COD and NH_4^+–N was entering R3. The attached biomass to suspended concentration ratio was 8.8–7.7 when R3 was receiving 30–60 mg/L thiocyanate in influent. The ratio found to drastically decrease to 3.5 with increase in influent SCN^- to 122 mg/L in R3. Higher detachment of biomass to

suspended liquor might have occurred in high SCN^- concentration as total biomass was not affected and observed to increase.

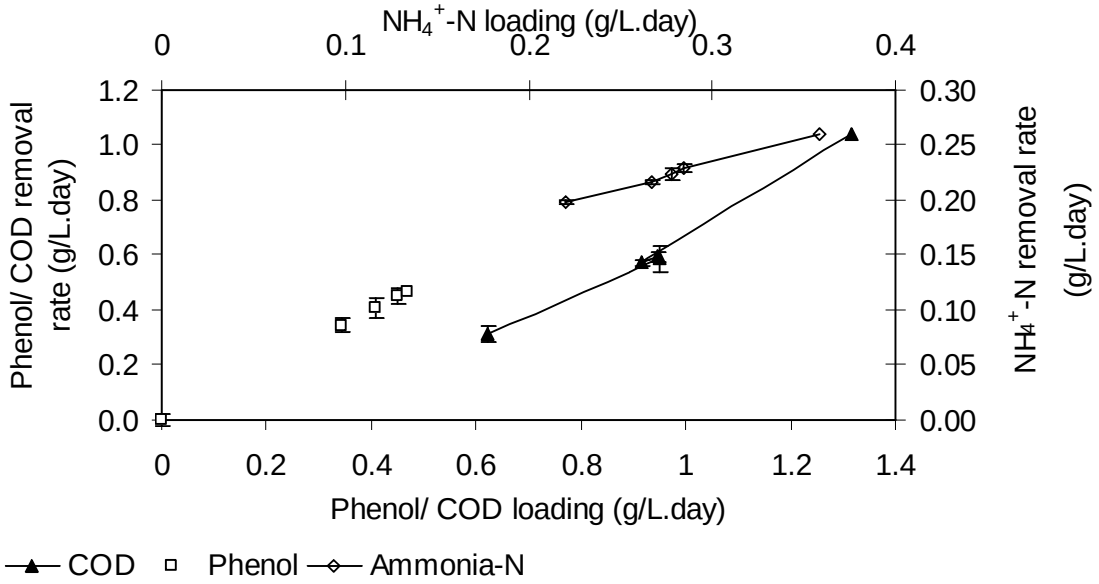

Figure 4.6 (b) COD and NH_4^+-N removal rate in R3 at varied loading at thiocyanate variation study

Influent NH_4^+–N concentration to R3 was comprised of NH_4^+–N released from R2 and NH_4^+–N generated in R3 from degradation of SCN^- (0.24 g NH_4^+–N from one gram of SCN^- removed) and ranged from 220 to 358 mg/L [Table 4.3 (b)]. In absence of thiocyanate in feed, R3 showed maximum NH_4^+–N removal efficiency of 90%. Ammonia–N removal efficiency was 80% (influent 267 mg/L) when influent phenol and SCN^- to R3 were 344 and 30 mg/L. Table 4.3(b) shows that NH_4^+–N removal in R3 was not affected when influent phenol and SCN^- increased to 450 and 61 mg/L, respectively at influent NH_4^+–N of 270 mg/L. However, NH_4^+–N removal decreased to 72% when influent phenol and SCN^- were 468 and 122 mg/L, respectively at influent NH_4^+–N of 358 mg/L. Inhibition of phenol on NH_4^+–N removal is reported by various authors and can starts from concentration as low as 5 mg/L (Neufeld and Valiknac, 1979; Jeong et al. 2006b; Kim et al. 2007; Eiroa et al. 2008). Kim et al. (2008b) reported inhibition of phenol and thiocyanate on NH_4^+–N removal at 200 mg/L each and substrate inhibition occurs at 350 mg/L NH_4^+–N and thiocyanate inhibition was mainly due to the NH_4^+–N generation from thiocyanate degradation resulting higher NH_4^+–N concentration. NH_4^+–N removal rate in R3 increased from 0.198 to 0.260 g/L.day with increase in increase NH_4^+–N loading from

0.220 to 0.358 g/L.day as shown in Figure 4.6 (b). Jeong and Chung (2006b) observed maximum ammonia removal rate of 0.28 g/L.day in a fluidized bed aerobic reactor using wastewater containing COD, phenol, SCN^-, CN^- and NH_4^+–N concentration of 2200–2400, 625, 360–390, 17–19 and 220–250 mg/L, respectively. Vázquez et al. (2006b) observed NH_4^+–N removal efficiency of 65% from influent NH_4^+–N of 1095 mg/L along with phenol concentration of 280 mg/L in absence of thiocyanate in aerobic suspended growth reactor operated at 4 days HRT. The present NH_4^+–N removal rate was comparable with literature values.

Table 4.3 (b): Performance of aerobic CMBR (R3) at feed SCN^- concentration variation

SCN^-	COD			NH_4^+–N			NO_x–N				N_R	COD/ NH_4^+–N	FA	UN
							NO_3^-–N		NO_2^-–N					
S_0	S_0	S_e	Rem	S_0^A	S_e	Rem	S_0	S_e	S_0	S_e				
0	620 (21)	310 (8.6)	50	220	22.0	90.0	316 (6.7)	455 (8)	0	0	0.14	2.81	11.9	24
30	950 (5.8)	364 (6.67)	61.6	267 (7.6)	50.7	81.0	47 (2.3)	235 (5)	0	4	0.19	3.56	23.4	0.5
45	947 (42.6)	354 (8.6)	62.6	267 (7.5)	54.3	80.4	45 (1.3)	255 (6)	0	7	0.22	3.41	24.4	0
61	915 (0.3)	342 (0.7)	62.6	270 (4.2)	55.0	80.6	120 (11)	247 (13)	0	58	0.19	3.22	30.3	11
122	1318 (28.5)	275 (9.3)	79.1	358 (12)	98.0	72.0	129.0 (13)	225 (9)	0	100	0.20	3.68	40.7	9

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%)

[A] Influent NH_4^+–N of R3 = {Effluent NH_4^+–N of R2 + 0.24 x (SCN^- removed in R3)}.

N_R: Nitrification rate (g/L.day); FA: Free ammonia (mg/L); UN: Unaccounted nitrogen (%);

Numbers in parenthesis indicate standard deviation values.

In R3, NH_4^+–N is oxidized to NO_2^-–N and NO_3^-–N. Nitrification rate in R3 was calculated based on generation of NO_3^-–N and NO_2^-–N and reactor HRT. Nitrification rate in R3 was

0.14–0.22 g/L.day and it decreased when feed SCN^- was increased above 200 mg/L. In R3, accumulation of nitrite at high influent thiocyanate was observed [Table 4.3 (b)]. The main factors that enhance nitrite build–up include temperature 35–40°C (Peng and Zhu, 2006), hydraulic retention time (HRT) of 12 h or less than that (Yamamoto et al. 2006), DO concentration between 0.5– 1.5 mg/L (Bae et al. 2001; Ruiz et al. 2006) and FA concentration around 5 mg/L or above (Abeling and Seyfried, 1992; Aslan and Dahab, 2008). As high pH was observed in R3, free ammonia (FA) concentration was calculated using equation 4.6 (Anthonisien, 1976, Vadivelu et al. 2007). The FA concentration increased from 12 mg/L to 40 mg/L during the study with increase feed SCN^-, influent and effluent ammonia in R3 at pH 8.1–8.4 (considering pH of R3 effluent).

$$\text{Free ammonia (mg/L)} = \frac{[NH_4^+\text{-N}]x10^{pH}}{\exp[6334/(273+T)]+10^{pH}} \qquad \text{(Eq. 4.6)}$$

Negative effect of nitrite on NH_4^+–N removal in aerobic reactor are also reported in previous literatures (Philips and Verstraete, 2001; Fux et al. 2006). Philips and Verstraete, (2001) observed activated sludges regularly exposed to NO_2^-–N concentrations exceeding 50 mg/L showed 40% inhibition on nitrification rates. In present study, temperature of the reactor room was controlled within 30±2 ^oC, HRT and dissolved oxygen was maintained 1 day and 3–4 mg/L which was high enough than reported value and not responsible for nitrite buildup. However the FA concentration in this case was higher and might be the cause of nitrite accumulation. Higher accumulation of nitrite 58–100 mg/L occurred when FA concentration calculated was 30–40 mg/L. Inhibitory effect of FA to nitrite oxidizing bacteria and ammonia oxidizing bacteria reported to occur at FA concentration of 0.1–4.0 mg/L and 10–150 mg/L, respectively (Anthonisen, 1976; Bae et al. 2001; Liu et al. 2001). The accumulated nitrite in combination with higher concentration of phenol and thiocyanate affected NH_4^+–N removal in R3.

A balance was made on amount of NH_4^+–N removed in R3 and amount of NH_4^+–N oxidized to NO_2^-–N and NO_3^-–N and calculated as unaccounted nitrogen (UN). The unaccounted nitrogen fraction in R3 was 0.5–24%. This could be probably due to incorporation of nitrogen in biomass, suggesting that NH_4^+–N consumption for synthesis of heterotrophic biomass. Influent COD to NH_4^+–N ratio in R3 was 2.8 to 3.68 suggesting

opportunities for the growth of heterotrophs (Hankai et al. 1990; van Neil et al. 1993; Cheng and Chen, 1994).

Clogging in aerobic reactor was absent during the experiment. However sometime the sponge cubes get stuck to the out let discontinuing the release of effluent and make the reactor over flow. The sponge cubes were then removed manually and normal reactor operation was achieved again.

4.1.1.4 Overall performance of sequential CMBR system at varied thiocyanate concentration

The feed and final effluent of R3 was considered to estimate overall performance of three–stage CMBR system. The overall performance at varying feed SCN^- concentrations is shown in Figure 4.7 in terms of COD, SCN^-, NH_4^+–N and phenol removals. It can be seen that phenol and SCN^- removals were complete and independent of feed SCN^- concentration. COD removal was around 95–96% with effluent COD 275–364 mg/L (from influent 7500–7980 mg/L) irrespective of influent SCN^- concentration. NH_4^+–N removal in three–stage system was constant at 89% up to feed SCN^- of 450 mg/L and decreased to 80% when feed SCN^- was 600 mg/L. Total nitrogen (TN) in influent and effluent of CMBR system was estimated using following Equation 4.7.

$$TN = \sum(SCN^-–N + NH_4^+–N + NO_3^-–N + NO_2^-–N) \quad \text{(Eq. 4.7)}$$

Feed TN were 1527, 1548, 1608 and 1644 mg/L at feed SCN^- of 110, 200, 450 and 600 mg/L respectively (considering influent NO_3^-–N of 1000 mg/L added in the recycle of R3). Figure 4.7 shows that TN removal was almost 80% up to feed SCN^- of 200 mg/L and decreased to 77% when feed SCN^- was 450 mg/L and above. In R3 effluent TN was in the form of remaining NH_4^+–N and little residual SCN^-–N (as unoxidized–N) and oxidized nitrogen (NO_3^-–N and NO_2^-–N). Amount of feed TN as remaining fraction of unoxidized–N (%) and oxidized–N (%) in the effluent with feed thiocyanate is shown in Figure. 4.7. Unoxidized nitrogen fraction increased in the final effluent with increased in feed SCN^-. Present result shows that the effect of feed SCN^- on phenol and COD removals in anaerobic reactor was very profound. However, the response of feed SCN^- on the overall performance of the three–stage CMBR system was quite insignificant, as anoxic reactor

was highly efficient and helped to improve the overall performance of the three–stage system.

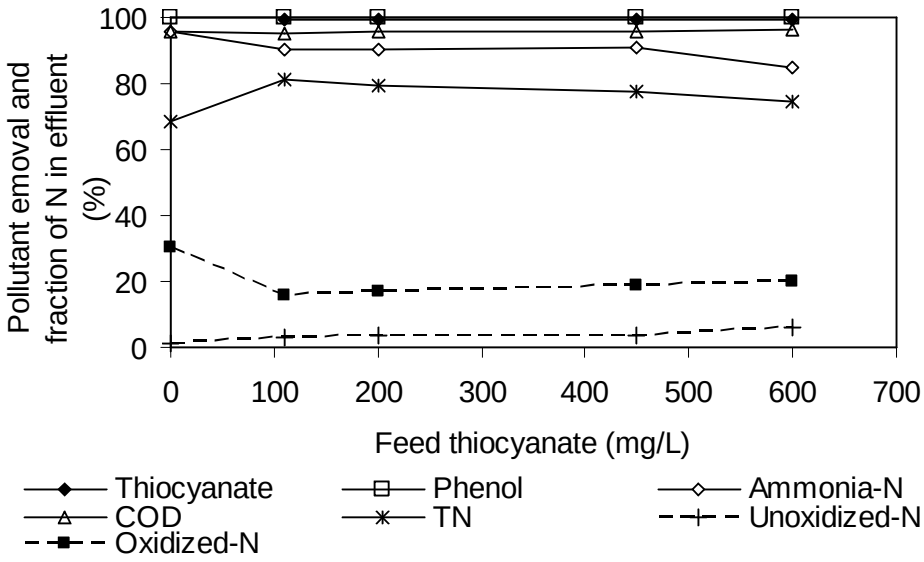

Figure 4.7 Effect of feed thiocyanate on performance of three-stage CMBR system

4.1.2 Performance of CMBR system at varied hydraulic retention time (HRT)

HRT of CMBR system was varied at five levels of 3–8 days at a constant feed concentration (phenol 2500, SCN^- 600, NH_4^+–N 500 mg/L in feed and NO_3^-–N 1000 mg/L in the recycle) and performance of the system was evaluated. R1 was operated at HRT of 1.5–4 days, whereas HRT of R2 and R3 were varied from 0.75–2 days resulting total HRT of CMBR system 3–8 days. In the present section the performance of anaerobic, anoxic and aerobic reactors at varying HRT is discussed. The influent/effluent profile of various pollutants in CMBR at varying HRT is shown in Figure 4.8.

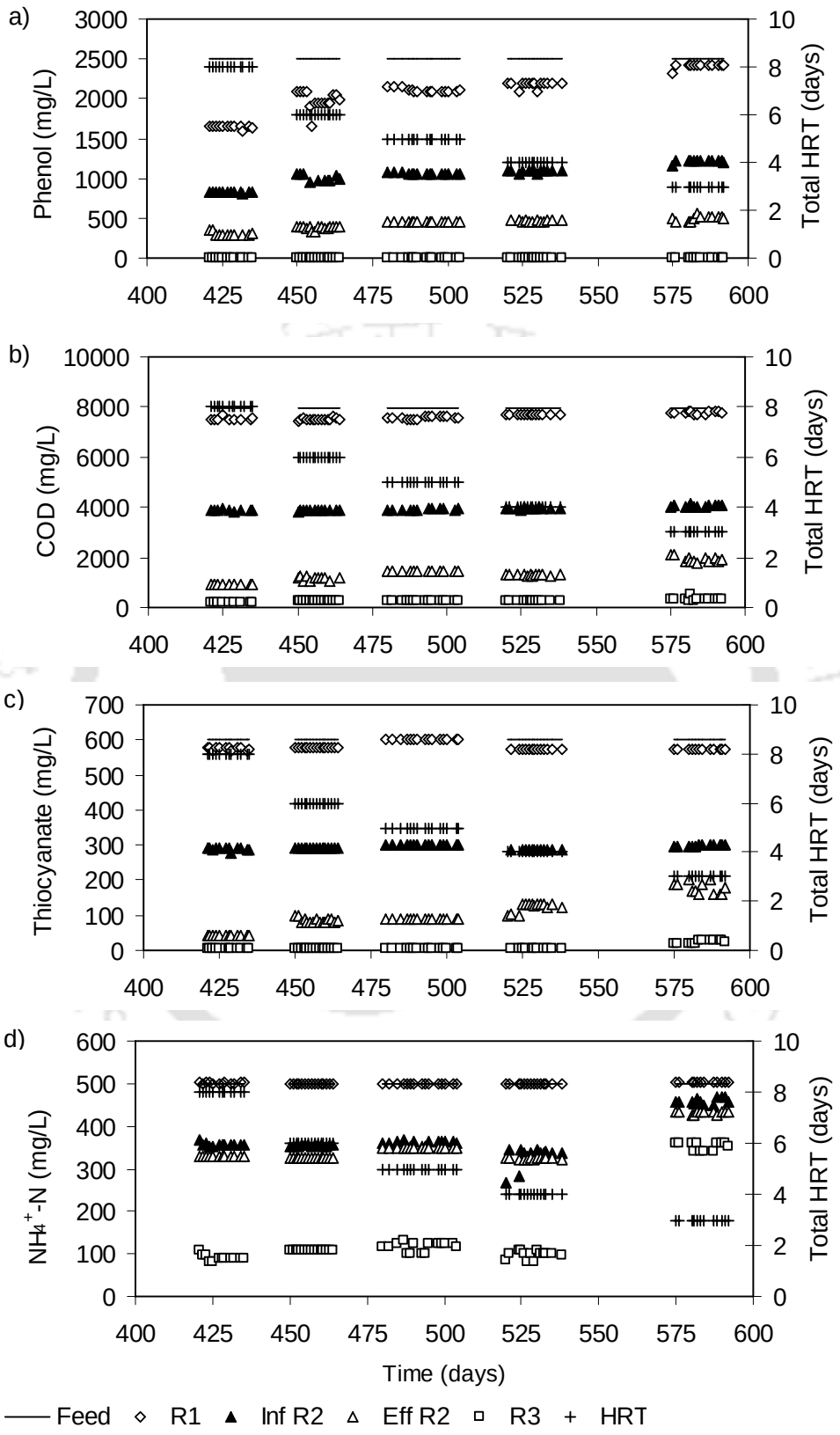

Figure 4.8 Pollutant profile in CMBR during HRT variation study

4.1.2.1 Performance of anaerobic CMBR (R1) at HRT variation

The anaerobic CMBR was operated at HRT of 1.5, 2, 2.5, 3 and 4 days. The average performance of R1 at varying HRT is given in Table 4.4.

With increase in HRT from 1.5 to 4 days, SCN^- removal in R1 increased from 4% to 12% (influent SCN^- 600 mg/L). Corresponding loading was 0.15–0.4 g SCN^-/L.day and removal rate was 0.018–0.016 g SCN^-/L.day.

Influent phenol concentration was constant at 2500 mg/L and phenol loading to R1 varied from 0.625 to 1.667 g/L.day. Phenol removal efficiency increased with increase in HRT from 3% at 1.5 days HRT to 34% at 4 days HRT. Phenol removal rates in R1 decreased with increase in loadings. Maximum phenol removal rate was 0.214 g phenol/L.day at phenol loading of 0.625 g phenol/L.day during the study (Figure 4.9). Fang et al. (2006) reported decrease in phenol removal efficiency from 99% to 77% in an UASB reactor with decrease in HRT from 2.5 days to 1.11 day at loading rate of 0.24–0.51 g phenol/L.day in absence of other toxic compound. Bajaj et al. (2009) reported maximum phenol removal rate of 3.7 g/L.day at a hydraulic retention time of 2.5 days and an organic loading 5.3 g phenol/L.day. In the present study the low removal rate of phenol might be associated with the presence of toxic compounds like SCN^- and ammonia–N.

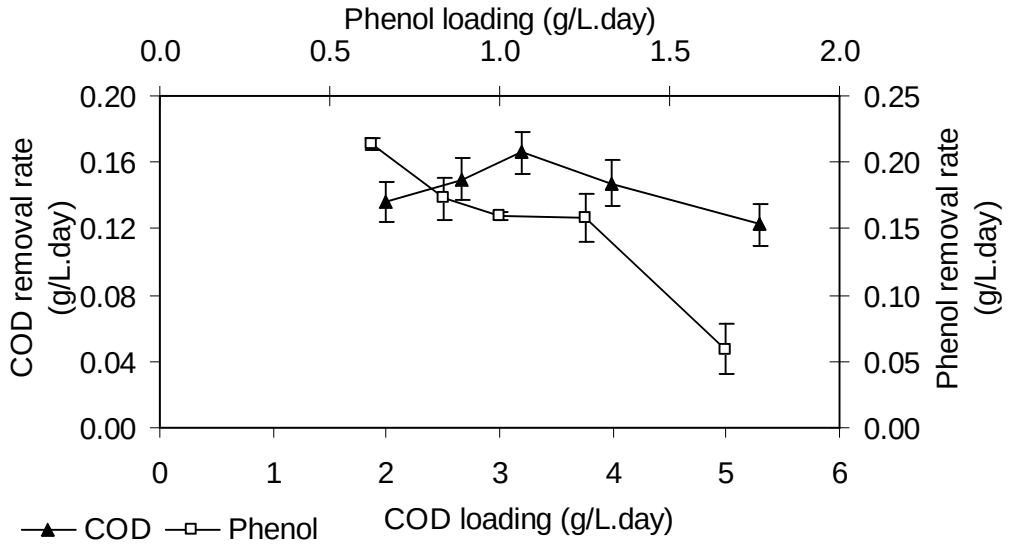

Figure 4.9 Effect of COD and phenol loading on removal rates in R1 at varying reactor HRT

The average feed COD was 7980 mg/L contributed mainly from phenol and SCN^- in the synthetic feed. COD loading rate was 1.99–5.30 g/L.day. R1 showed accelerated COD

removal efficiency with increase in reactor HRT. Up to HRT of 1.5–2 days, COD removal was low (2.3–3.7%) and then increased to 5.2% and 6.8% respectively, at HRT of 3 and 4 days. COD removal rate was 0.136 g/L.day at COD loading of 1.995 g/L.day which then increased to 0.167 g/L.day with increased COD loading up to 3.192 g/L.day. However beyond that loading, COD removal rate in R1 decreased (Figure 4.9). Zheng and Li (2009) reported increase in phenol (29–38%) and COD (27–38%) removals from influent phenol and COD concentration of 690–740 mg/L and 5720–6020 mg/L, respectively with increase in reactor HRT in anaerobic unit of a anaerobic–anoxic–aerobic system (HRT 18 days–30 days).

Table 4.4 Average performance of anaerobic CMBR (R1) at HRT variation

R1 HRT (day)	SCN^-		Phenol		COD			NH_4^+–N	TVS	VFA	pH
	S_e	Rem	S_e	Rem	S_0	S_e	Rem	S_e			S_e
1.5	575	4.1	2411 (30.1)	3.5	7980	7766 (96)	2.3	505	9622 (401)	275 (50)	7.2
2	572	4.6	2184 (37.5)	12.6		7685 (28)	3.7	500	10500 (522)	280 (31)	6.8
2.5	560	6.67	2100 (20)	16		7565 (60)	5.2	500	10100 (432)	288 (70)	6.9
3	550	8.3	1982 (120)	20.7		7530 (187)	5.6	505	10100 (455)	265 (48)	6.9
4	525	12	1644 (16)	34.2		7436 (81)	6.8	505	10300 (388)	300 (85)	6.8

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

TVS: Total volatile solids (mg/L); VFA: Volatile fatty acids (mg/L as acetic acid);

Numbers in parenthesis indicate standard deviation values

Feed: phenol 2500 mg/L, COD 7980 mg/L, NH_4^+–N 500 mg/L, SCN^- 600 mg/L.

Besides, phenol, COD and SCN^-, no removal of NH_4^+–N was observed in R1 at all HRT levels. No specific methanogenic activity (SMA) was detected in R1 sludge as already observed in previous studies. Feed pH decreased little from 7.5 to 6.8. Total biomass (biomass in sponge + biomass in suspension) in R1 during HRT study was observed to be

in the range of 9600–10,500 mg/L (Table 4.4). Suspended biomass concentration increased from 900 to 1100 mg/L and hence the attached to suspended biomass ratio slightly reduced from 10 to 8 at HRT 2.5 days or more. In R1, VFA concentration observed was 265–300 mg/L as acetic acid.

4.1.2.2 Performance of anoxic CMBR (R2) at HRT variation

The CMBR system was operated at a constant recycle ratio of 1 to R2. HRT of anoxic reactor was half of HRT of R1. HRT of R2 varied from 0.75–2 days. Average performance of R2 is shown in Tables 4.5 (a) and (b). With increase in HRT and total system HRT, phenol removal efficiency in R1 increased, resulting less influent phenol to downstream anoxic reactor. Influent phenol to R2 decreased with increase in HRT from 1206 mg/L to 823 mg/L and it removed 57–62% of influent phenol [Table 4.5 (a)] which was 20–28% of total phenol removal by the system. Figure 4.10 shows that phenol removal rate in R2 increased almost linearly with a slope 0.56 (R^2 of 0.99) with increase in phenol loading to R2. Maximum phenol removal rate was 0.939 g/L.day at phenol loading of 1.608 g/L.day.

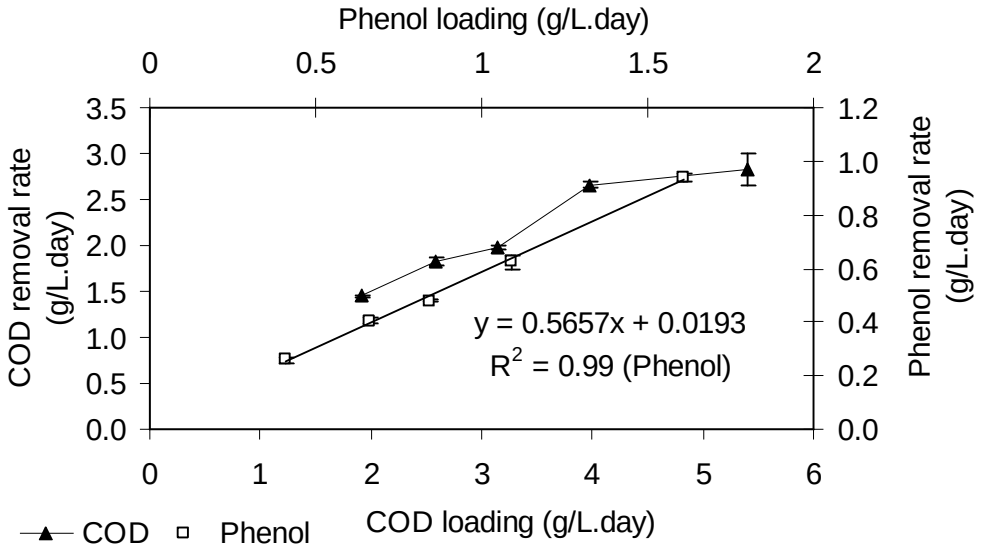

Figure 4.10 COD and phenol removal rates in R2 at varied loadings

Average influent COD to R2 were 4053–3815 mg/L at 0.75–2 days HRT. R2 removed 52–76% influent COD (26–36% of total COD removal). COD loadings were 1.91–5.40 g COD/L.day. With decrease in reactor HRT, COD loading increased and COD removal

rates showed a high increasing trend up to loading of 3.98 g COD/L.day (Figure 4.10). Maximum COD removal rate in R2 was 2.83 g COD/L.day and observed at the highest COD loading of 5.4 g COD/L.day. Kim et al. (2008a) reported phenol and COD removal rates of 0.041–0.08 g phenol/L.day and 0.44–1.34 g COD/L.day, respectively with decrease in HRT and increase in loading from 0.06–0.25 g phenol/L.day and 1.03–2.84 g COD/L.day, respectively in anoxic reactor. The present anoxic MBR was capable of removing more phenol and COD than the reported values.

Table 4.5 (a): Performance of anoxic CMBR (R2) at reactor HRT variation

HRT (day)	Phenol			COD			SCN^-			NH_4^+–N		
	S_0	S_e	Rem	S_0	S_e	S_0	S_0	S_e	Rem	S_0[A]	S_e	Rem
0.75	1206 (15)	502 (39)	58.4	4053 (32)	1931 (212)	52.3	300 (2.7)	177 (16)	41	408	401 (54)	1.7
1	1093 (19)	468 (12.5)	57.2	3980 (39)	1318 (28.5)	66.9	288 (0.8)	122 (12)	57.6	340	330 (25)	2.9
1.25	1050 (12)	450 (5)	57.2	3920 (0)	1455 (0)	62.9	302 (0)	90 (0)	70.2	349	338 (22)	3.0
1.5	991 (40)	385 (23)	61.2	3885 (177)	1150 (71.2)	70.4	277 (0.3)	86 (7.6)	68.9	351	330 (26)	5.9
2.0	823 (8.3)	307 (32)	62.7	3815 (42)	914 (20)	76.0	264 (7)	40 (0)	84.8	351	335 (26)	4.6

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%).

[A] Influent NH_4^+–N of R2 = {Effluent NH_4^+–N of (R1+R3)/2 + 0.24 x (SCN^- removed in R2)}.

Numbers in parenthesis indicate standard deviation values.

In R2, influent SCN^- was 264–300 mg/L and R2 removed 41–84% of it [Table 4.5 (a)]. With increase in SCN^- loading from 0.132 to 0.40 g/L.day, R2 showed decreasing removal efficiency from 84% to 41% (Figure 4.11). Thiocyanate removal rate in R2 initially increased from 0.112–0.166 g/L.day with increase in thiocyanate loading from 0.132–0.288 g/L.day and maximum SCN^- removal rate achieved was 0.166 g/L.day at loading of 0.288 g SCN^- /L.day and remained constant with further increase in SCN^- loading up to

0.4 g/L.day. R2 was responsible for 21–37% of total SCN^- removal during the study. Due to SCN^- degradation some amount of NH_4^+–N (30–55 mg/L) generated in R2. The influent NH_4^+–N in R2 ranged from 340–408 mg/L and almost 1.7– 6% NH_4^+–N removal occurred.

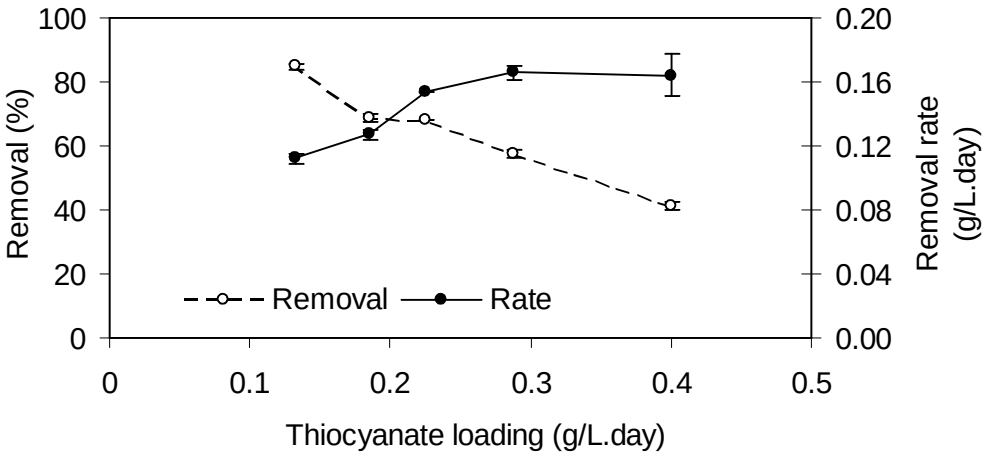

Figure 4.11 Effect of thiocyanate loading on SCN^- removal efficiency and removal rate in R2 at varying reactor HRT

Average influent NO_3^-–N concentration to R2 was 600–620 mg/L and influent NO_2^-–N to R2 was 22–70 mg/L. The overall NOx–N loadings in R2 was 0.335–0.83 g NO_x^-–N/ L.day at varying reactor HRT. NO_3^-–N removal achieved in R2 was 65–78% whereas NO_2^-–N was removed completely comprising total NOx–N removal of 69–80%. With decrease in reactor HRT and increase in loadings, NO_x^-–N removal rate increased almost linearly from 0.263–0.643 g/L.day in R2 (Figure 4.12). R2 contributed 29–33% of total nitrogen removal from CMBR system. COD/N_{rem} ratio increased from 4.7 to 7.2 with increase in HRT from 0.75 to 1.5 day but further increase in HRT to 2 days resulted in decrease COD/N_{rem} ratio to 6.8. At higher HRT, high amount of COD, SCN^- were removed from influent and COD/N_{rem} ratio increased (equation 4.5). The COD available for biomass synthesis was 39–60% and the biomass yield coefficient was 0.28–0.41 being higher at higher HRT. The suspended biomass in R2 was maximum (3710 mg/L) at 0.75 days HRT and beyond that it reduced to 3000–3300 mg/L. Attached to suspended biomass ratio was the minimum of 2.5 at lowest HRT and it increased to 3 at HRT 1 day or above. The total volatile solid concentration in R2 was 13010, 12900, 12672, 13270 and 13350 mg/L at HRT 0.75, 1, 1.25, 1.5 and 2 days, respectively.

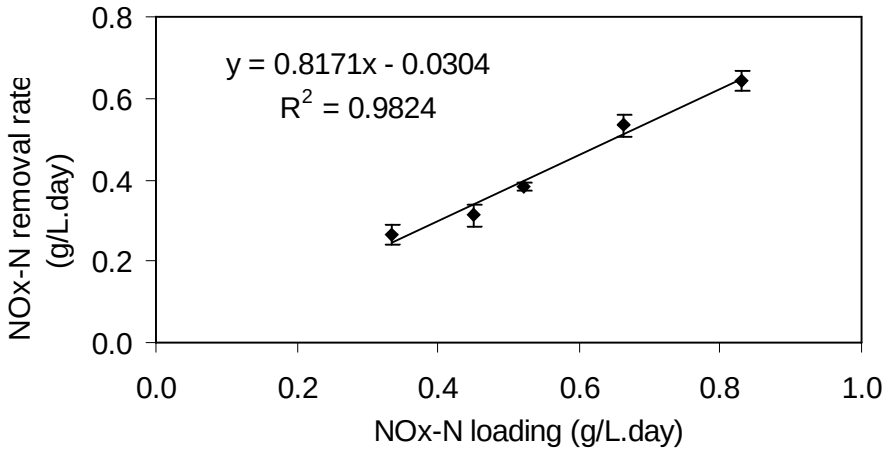

Figure 4.12 Effect of total nitrate/ nitrite-nitrogen loading on denitrification rate in R2

Table 4.5 (b): Performance of anoxic CMBR (R2) at reactor HRT variation

HRT (day)	NO_x^-–N					COD: N_{rem}	COD_B	Sulfate				
	NO_3^-–N		NO_2^-–N		Rem							
	S_0[A]	S_e	S_0	S_e				S_0	S_e	Gen	Th. SO_4^{2-}	Err
0.75	600	140 (28)	22	0	77.5	4.7	39	388	550 (27)	162	202	–40
1	612	129.0 (13)	50	0	80.5	5.7	49	365	600 (23)	235	273	–38
1.25	615	169.7 (10)	35	0	73.8	5.9	51	438	747 (95)	309	349	–40
1.5	620	210 (11)	62.5	0	69.2	7.2	60	452	757 (26)	303	314	–11
2.0	600	144 (29)	70	0	78.6	6.8	58	448	808 (37)	360	369	–9

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%). Err: Error (mg/L)

[A] In R2 influent 500 mg/L of NO_3^-–N was added externally.

COD_B: COD fraction for biomass (%);

Gen: Sulfate generated (mg/L) {= Influent SO_4^{2-} – Effluent SO_4^{2-}}

Th. SO_4^{2-}: Theoretical sulfate generated (1.65x SCN^- removed) mg/L);

Numbers in parenthesis indicate standard deviation values.

The effluent sulfate from R2 was always higher than the influent sulfate in recycle as ~160–360 mg/L sulfate was generated in R2. Sulfate generation in R2 increased with increase in SCN^- removal towards higher reactor HRT [Table 4.5 (b)]. During low HRT, sulfate generation in R2 was lower than theoretical sulfate value. At lower reactor HRT, thiocyanate loading and removal rates in R2 increased and this influenced sulfate generation due to formation of other sulfur products as higher error observed during SCN^- variation study at high influent SCN^- concentration.

4.1.2.3 Performance of aerobic CMBR (R3) at HRT variation

The aerobic reactor, R3 was operated at various HRT of 0.75–2 days as in R2 to study the effect of HRT variation on reactor performance. The feed to the CMBR system was maintained constant during HRT variations; however influent quality of R3 varied due to change in performance of upstream reactors R1 and R2 with varied HRT. Average performance of R3 is shown in Tables 4.6 (a) and (b).

Influent NH_4^+–N to R3 were 343–437 mg/L considering NH_4^+–N generated from SCN^- degradation (8.8–36.5 mg/L). Figure 4.13 shows that with increase in loading up to 0.358 g NH_4^+–N/L.day, removal efficiency was 72% with removal rate of 0.258 g NH_4^+–N/L.day. NH_4^+–N removal decreased to 42% at loading of 0.583 g NH_4^+–N/L.day though NH_4^+–N removal rate remained constant at 0.247 g NH_4^+–N /L.day at 0.75 day HRT. At low HRT, microorganisms in R3 were shocked and their activities decreased. Li et al. (2011) reported NH_4^+–N removal efficiency of 89% in an aerobic moving bed biofilm reactor in presence of phenol and thiocyanate concentration of 408 and 123 mg/L, respectively at two days HRT (NH_4^+–N removal rate of 0.097 g/L.day from NH_4^+–N loading of 0.109 g/L.day). In present study, higher NH_4^+–N removal rate was observed in R3 at higher NH_4^+–N loading in presence of higher phenol and thiocyanate loading though removal efficiency decreased releasing higher effluent NH_4^+–N concentration. Also the effluent phenol and SCN^- concentration from R3 was comparatively lower than that of reported by Li et al. (2011). Free ammonia concentration was 39–61 and maximum FA concentration during the shortest HRT. During short HRT study, R3 was receiving very high amount of influent NH_4^+–N and effluent NH_4^+–N was also maximum which led to increased amount of FA. From NH_4^+–N oxidation, 200–230 mg/L nitrate and 44–140 mg/L nitrite evolved in R3.

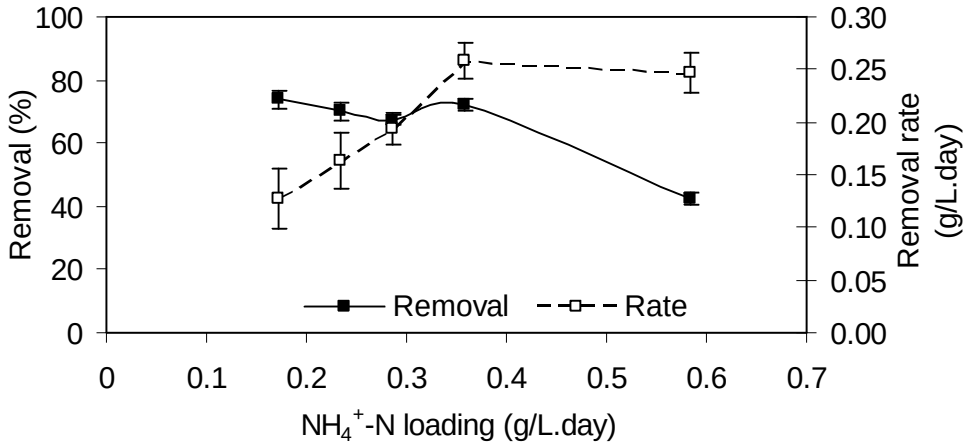

Figure 4.13 Effect of NH_4^+-N loading on removal efficiency and rate in R3

Table 4.6 (a): Performance of aerobic CMBR (R3) at HRT variation

HRT (day)	NH_4^+–N			NO_3^-–N		NO_2^-–N		N_R	COD: NH_4^+–N_{inf}	pH	FA	UN	TVS (mg/L)
	S_0[A]	S_e	Rem	S_0	S_e	S_0	S_e						
0.75	437	252 (10)	42.4	140	200 (16)	0	44 (12)	0.138	4.41	8.4	61.5	11	12682 (436)
1	358	100 (10)	72.1	129	225 (9)	0	100 (3.6)	0.190	3.68	8.3	40.7	5.7	12660 (252)
1.25	358	117 (12)	69.7	170	230 (11)	0	70 (8.3)	0.104	4.05	8.4	42.4	28	12045 (431)
1.5	349	105 (0)	70.0	210	240 (0)	0	125 (8.3)	0.103	3.28	8.4	40.6	20	11656 (535)
2.0	343	90 (9)	73.8	144	200 (54)	0	140 (10)	0.100	2.66	8.4	38.7	7.5	11200 (648)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

[A] Influent NH_4^+–N of R3 = {Effluent NH_4^+–N of R2 + 0.24x (SCN^- removed in R3)}.

N_R: Nitrification rate (g/L.day); FA: Free Ammonia (mg/L); UN: Unaccounted nitrogen (%); Numbers in parenthesis indicate standard deviation values.

Table 4.6 (a) shows with increase in reactor HRT from 0.75 to 2 days, there was more generation of nitrite–nitrogen (NO_2^-–N) in R3 that increased from 44 to 140 mg/L. In

present study there was higher amount of FA which might have led to the accumulation of the nitrite in R3. Unaccounted nitrogen was 6–28% during the study. Table 4.6 (a) shows that nitrification rate in R3 was 0.10–0.19 g/L.day.

Influent SCN^- to R3 were 177, 122, 90, 86 and 40 mg/L at HRT of 0.75, 1, 1.25, 1.5 and 2 days, respectively [Table 4.6 (b)]. The reactor was responsible for high SCN^- removal (86–97%) which was 6–25% of total SCN^- removal during the study. Up to loading of 0.122 g SCN^-/L.day, SCN^- removal was 91–97% and decreased to 86% at loading of 0.236 g SCN^-/L.day. SCN^- removal rates in R3 increased linearly from 0.018 to 0.202 g/L.day with increase in loading of SCN^- (Figure 4.14).

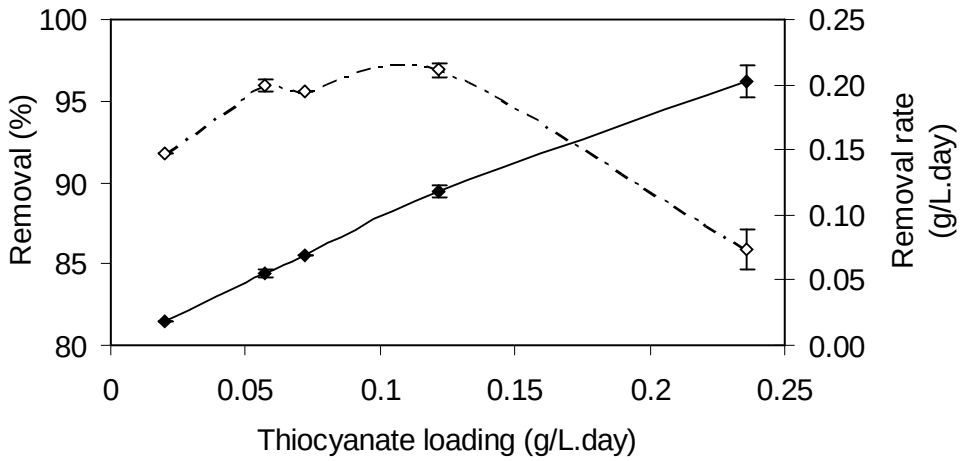

Figure 4.14 Effect of thiocyanate loading on removal efficiency (dotted line) and rate (solid line) in R3

Influent phenol to R3 was 307–502 mg/L [Table 4.6 (b)]. Influent phenol to R3 was lower at higher HRT, as in higher HRT; R2 showed higher phenol removal efficiency. Phenol removal efficiency of R3 remained almost unchanged with varied HRT and decreased loading. More than 99% of phenol was removed through out the study with 1.5–1 mg/L phenol in the R3 effluent. R3 was responsible for 12–20% of phenol removal from feed phenol concentration 2500 mg/L. Phenol removal rate in R3 at varying HRT was 0.153–0.667 g/L.day at phenol loadings of 0.153–0.669 g phenol/L.day (Figure 4.15).

The average influent COD to R3 were 914–1931 mg/L. COD removal in R3 was 78–82%, with effluent COD of 195–340 mg/L. R3 was accounted for 9–19% of feed COD removal (7980 mg/L) at varying HRT. COD loading in R3 at varying reactor HRT was 0.457–2.574 g COD/L.day. Figure 4.15 shows that with increase in COD loadings, removal efficiency

was insignificantly affected in R3, where as rate increased linearly from 0.36–2.12 g COD/L.day. Hosseni and Borghei (2005) reported decrease in COD removal efficiency from ~95% to 75% when COD loading rate was increased 0.2–0.6 g/L.day by decreasing HRT in a moving bed reactor treating phenol COD.

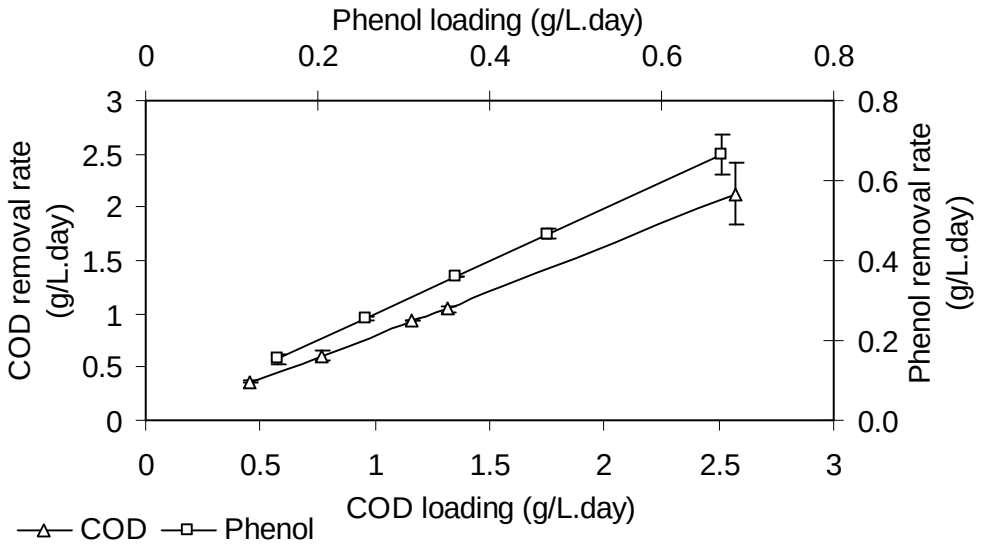

Figure 4.15 Effect of COD and phenol loading on removal rate of aerobic reactor (R3)

There was high influent COD: NH_4^+–N in R3 (2.6–4.4) during the study. The high ratio at short HRT (1.25–0.75 day) ~4 facilitated the growth of heterotrophs in R3. Suspended biomass concentration in R3 was fluctuating within 2880 to 3380 mg/L and attached biomass was observed to decrease continuously with increase in HRT. The ratio of attached to suspended biomass initially increased from 2.8 to 3.5 with increase in HRT from 0.75 day to 1 day and then continuously decreased to 2.4 at higher HRT (2 days). Lowest suspended biomass concentration of 2880 mg/L in R3 at HRT 1 day resulted in high ratio of 3.5. Nearly 11000–12600 mg/L total biomass was monitored in R3 that decreased slightly with increase in HRT. At higher HRT, R3 received lower amount of COD compared to lower HRT which might be the reason of decrease in biomass in R3 during higher HRT.

Sulfate generation with the removal of SCN^- in R3 at all levels of HRT was observed. The influent sulfate from anoxic reactor was 550–808 mg/L and effluent sulfate was 730–905 mg/L. In Table 4.6 (b), sulfate evaluation based on SCN^- removed was carried out. Sulfate

generation was near to theoretical generation except at HRT 1 day study where higher error of (–) 65 mg/L was achieved with low sulfate generation.

Table 4.6 (b): Performance of aerobic CMBR (R3) at HRT variation

R3	SCN^-			Phenol			COD			Sulfate				
HRT (day)	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Gen	Th. SO_4^{2-}	Err
0.75	177	25 (5.5)	85.8	502	2.0 (0)	99.6	1931	340 (66)	82.4	550 (17)	776 (49)	226	250	–24
1	122	3.8 (0.6)	96.9	468	1.5 (0.6)	99.7	1317	275 (9.3)	79.1	600 (23)	730 (26)	130	195	–65
1.25	90	4 (0)	95.5	450	1.2 (0)	99.7	1455	275 (0)	81.1	747 (95)	876 (84)	129	142	–13
1.5	86	3.5 (0.7)	95.9	385	1.0 (0)	99.7	1150	240 (0)	79.1	757 (26)	905 (36)	148	136	12
2.0	40	3.3 (0.4)	91.7	307	1.0 (0)	99.7	914	195 (14)	78.6	808 (37)	896 (36)	88	60	28

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%); Gen (generation (mg/L);
Th. SO_4^{2-}: Theoretical sulfate generated (1.65 x SCN^- removed) mg/L); Err: Error (mg/L)
Numbers in parenthesis indicate standard deviation values.

4.1.2.4 Overall performance of CMBR system at varied HRT

In Figure 4.16 overall removal efficiency is shown with respect to total HRT. It can be seen that SCN^- removal efficiency increased from 96% to 99% when total HRT was increased from 3 to 4 days and efficiency remained constant. Phenol removal in CMBR system was independent of total HRT as 99.9% removal was achieved at HRT of 3–8 days. COD removal was 95% at total HRT of 3 days and increased slightly to 96–97% when total HRT was 4–8 days. NH_4^+–N removal was only 60% when total HRT was 3 days. There was significant increase in NH_4^+–N removal to 81–86% when total HRT increased from 4 to 8 days. Influent total nitrogen (TN) was ~1644 mg/L. TN removal was independent of total HRT and fluctuated within 71–74%. Zheng and Li (2009) achieved more than 90% COD and complete phenol removals with only 60% ammonia–nitrogen

removal in a lab–scale anaerobic–anoxic–aerobic system during treatment of high strength coking effluent with 8000–15000 mg/L COD, phenol 1200–1700 mg/L and 700–1800 mg/L ammonia–nitrogen using recycle to both anaerobic and anoxic reactors. They observed that the modification of HRT (18–30 days) did not influence the overall phenol and COD removal efficiency but significantly influenced the distribution of COD and ammonium removals among the reactors. In CMBR system also the though pollutants removals were influence by the reactor HRT in individual reactor; the overall performance remained almost same at HRT 4 days or more.

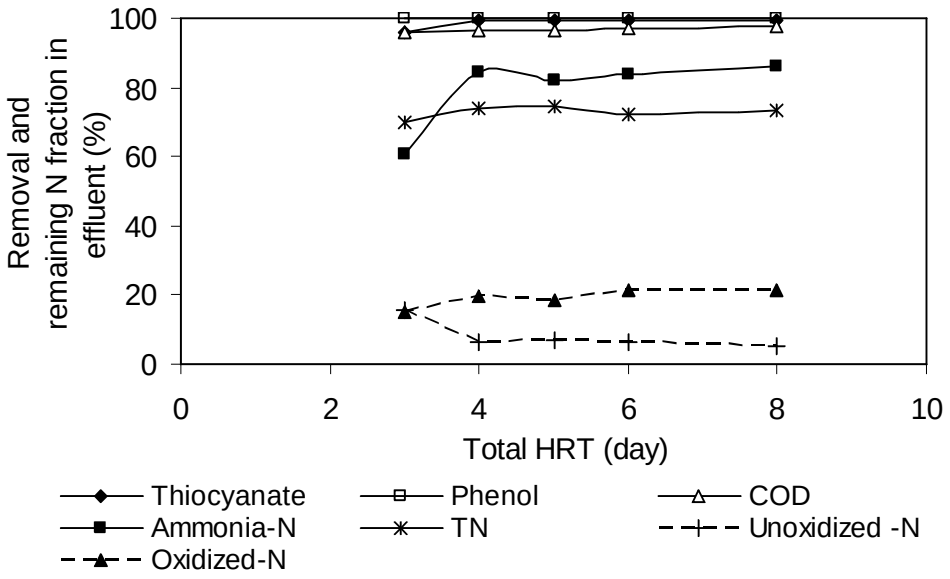

Figure 4.16 Overall performance of three stage CMBR at varied HRT (Unoxidized-N and oxidized-N are expressed as remaining fraction in effluent)

Effluent unoxidized–N was 15% of feed TN at total HRT of 3 days and decreased significantly 6% at total HRT of 4 days and thereafter remained constant. Oxidized–nitrogen was only 14% (200 and 44 mg/L of NO_3^-–N and NO_2^-–N) of feed TN at total HRT of 3 days. With increase in HRT to 4 days and 6 days, oxidized–N fraction increased to 18% and increased further to 21% at total HRT of 6–8 days. This is because with increase in total HRT more NH_4^+–N was converted to oxidized–nitrogen species and fraction of feed NH_4^+–N in effluent decreased and oxidized nitrogen increased, though TN removal was not affected much.

4.1.3. Performance of CMBR system at varied concentration of feed phenol

Four experiments were conducted from 610th day to 794th day at varying feed phenol of 1000, 1500, 2000 and 2500 mg/L along with 800 mg/L SCN^- and 500 mg/L NH_4^+–N at total HRT of 6 days (R1: 3 days; R2: 1.5 day and R3: 1.5 day) and influent/ effluents profiles of pollutants studied are shown in Figure 4.17.

4.1.3.1. Effect of feed phenol on performance of anaerobic CMBR (R1)

Phenol concentration in feed was varied at four levels: 1000, 1500, 2000 and 2500 mg/L during the study and feed SCN^- and NH_4^+–N were constant at 800 and 500 mg/L, respectively with feed COD of 4200–8150 mg/L. Steady state performance of R1 is presented in Table 4.7. With increase in influent phenol concentration from 1000 to 2500 mg/L, phenol removal in R1 decreased from 42% to 4.9% releasing 580–2378 mg/L phenol in the effluent. Phenol loading rates to R1 increased from 0.333 to 0.833 g/L.day. With increase in feed phenol, influent COD increased from 4200 to 8150 mg/L and corresponding loading rate was 1.4–2.72 g/L.day. It can be seen from Table 4.7 that with increase in feed COD, removal in R1 continuously decreased from 10% to 6%.

Table 4.7: Performance of anaerobic CMBR (R1) at feed phenol concentration variation

Phenol			SCN^-	COD			NH_4^+–N	pH	TVS (mg/L)	VFA
S_0	S_e	Rem	S_e	S_0	S_e	Rem	S_e	S_e		S_e
1000	580 (5)	42.0	800	4200	3750 (67)	10.7	500	6.7	9505	315 (41)
1500	934 (25)	37.7		5400	4904 (60)	9.2		6.8	10338	400 (57)
2000	1580 (36)	21.0		6710	6272 (144)	6.5		6.9	10854	327 (47)
2500	2378 (0)	4.9		8150	7644 (35)	6.2		6.8	10772	281 (31)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

Numbers in parenthesis indicate standard deviation values

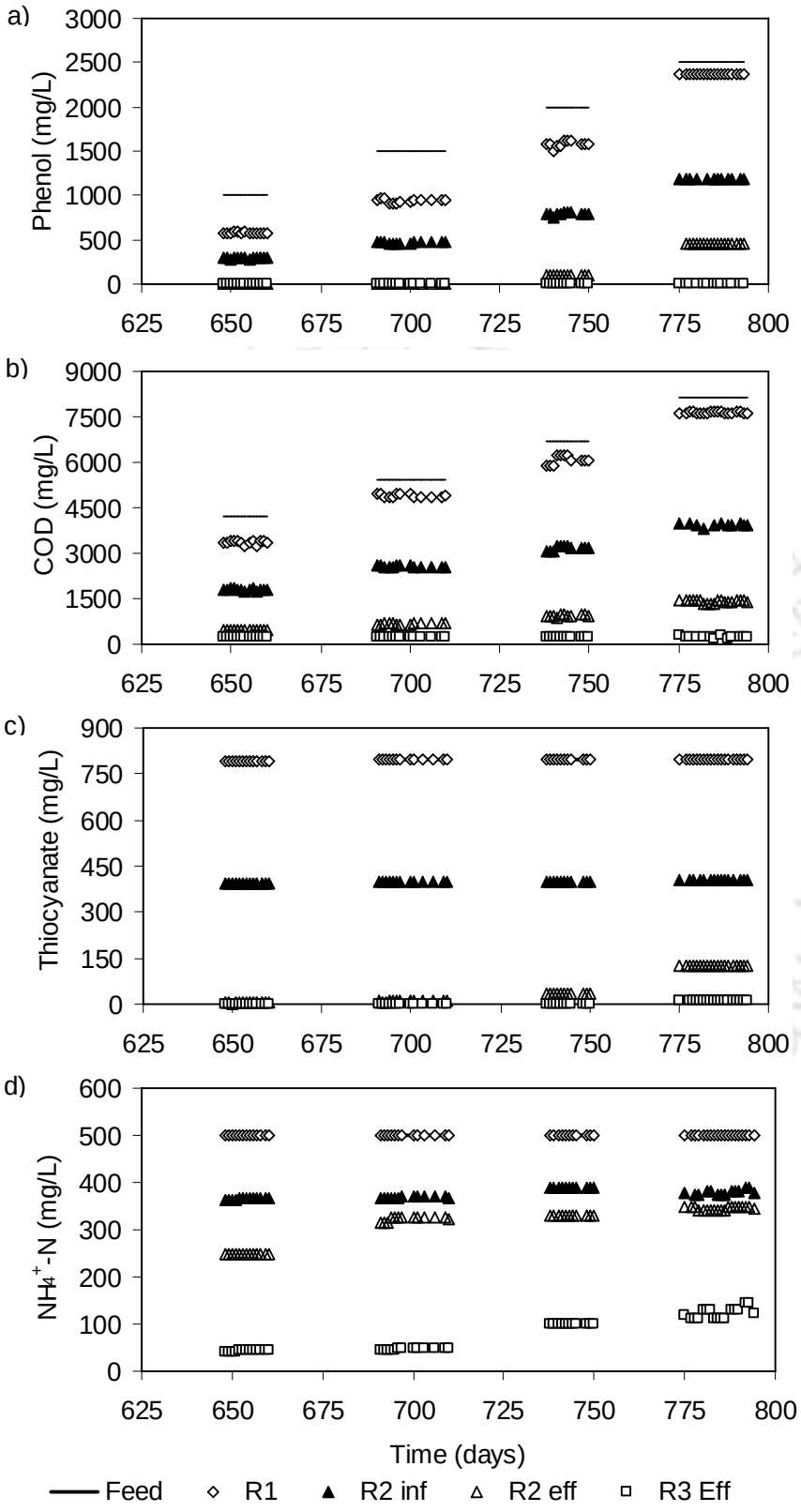

Figure 4.17 Pollutant profile in CMBR during feed phenol variation study

Figure 4.18 shows that maximum phenol and COD removal rates achieved in R1 were 0.188 g phenol/L.day and 0.168 g COD/L.day at respective loadings of 0.50 g/L.day and 2.72 g COD/L.day. In Figure 4.19 (a) and (b) fractional phenol and COD removal by three reactors are presented. It can be seen that when feed phenol concentration was low (1000–1500 mg/L), fractional phenol removal by R1 was comparatively higher than R2. However, when feed phenol was 2000 mg/L and more, this fractional phenol removal in R1 was less than R2. Contribution of R1 in COD removal remained less than R2 through out this study.

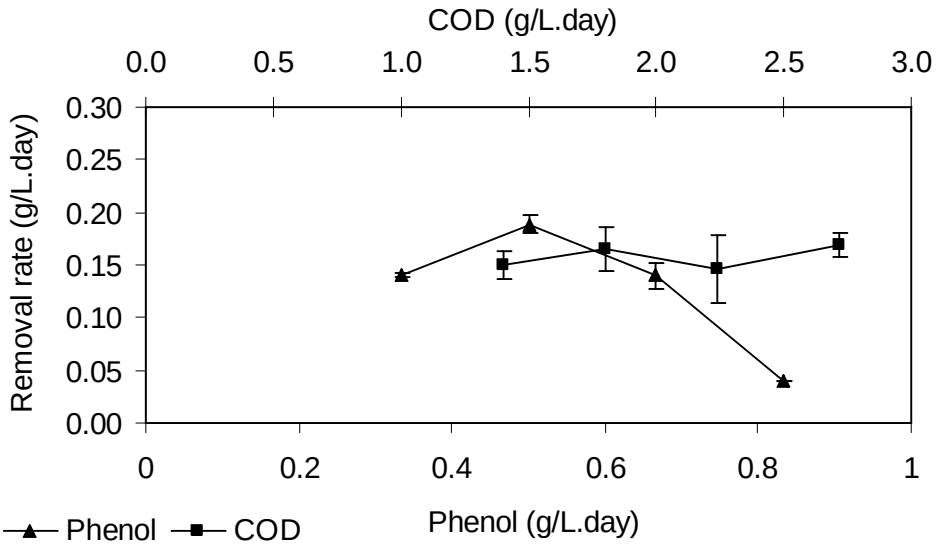

Figure 4.18 Performance of anaerobic CMBR (R1) at varied phenol and COD loading

Bajaj et al. (2009) reported 94% phenol removal at loading of 1.5 g phenol/L.day in a fixed film reactor and with further increase of phenol loading, phenol removal decreased. Ramakrishna and Gupta (2006) also reported very high phenolics and COD removal efficiencies of 93% and 88%, respectively at loading of 2.24 g COD/L. day in a hybrid upflow anaerobic sludge blanket reactor. The higher performance of these reported literatures might be due the absence of toxic compounds other than phenol. In the present study other two inhibitory substances, thiocyanate and ammonia–nitrogen were present which probably the cause of less efficiency of R1 as compared to reported anaerobic reactors. Zheng and Li (2009) reported phenol and COD removals of 29–38% and 27–38%, respectively by anaerobic reactor of a anaerobic/anoxic/aerobic suspended growth

system at loadings of 0.046–0.081 g phenol/L.day and 0.35–0.63 g COD/L.day in presence of SCN^- and NH_4^+–N in feed wastewater. In the present study, anaerobic reactor was given phenol/COD concentrations much higher than this reported literature. Wang et al. (2011a) reported COD and phenol removals of 53.7% and 51.0% at organic loading rates of 1.67 g COD/L.day and 0.32 g phenol/L.day, respectively from coal gasification wastewater containing ~ 47 mg/L thiocyanate. R1 also exhibited similar phenol removal efficiency of 42% at phenol loading of 0.33 g/L.day. Figure 4.19 (a) and (b) shows that at low phenol concentration in the feed, COD removal in R1 was much less than phenol removal. The probable reason was accumulation of intermediates generated during phenol degradation as suggested by previous researchers (Bajaj et al. 2009).

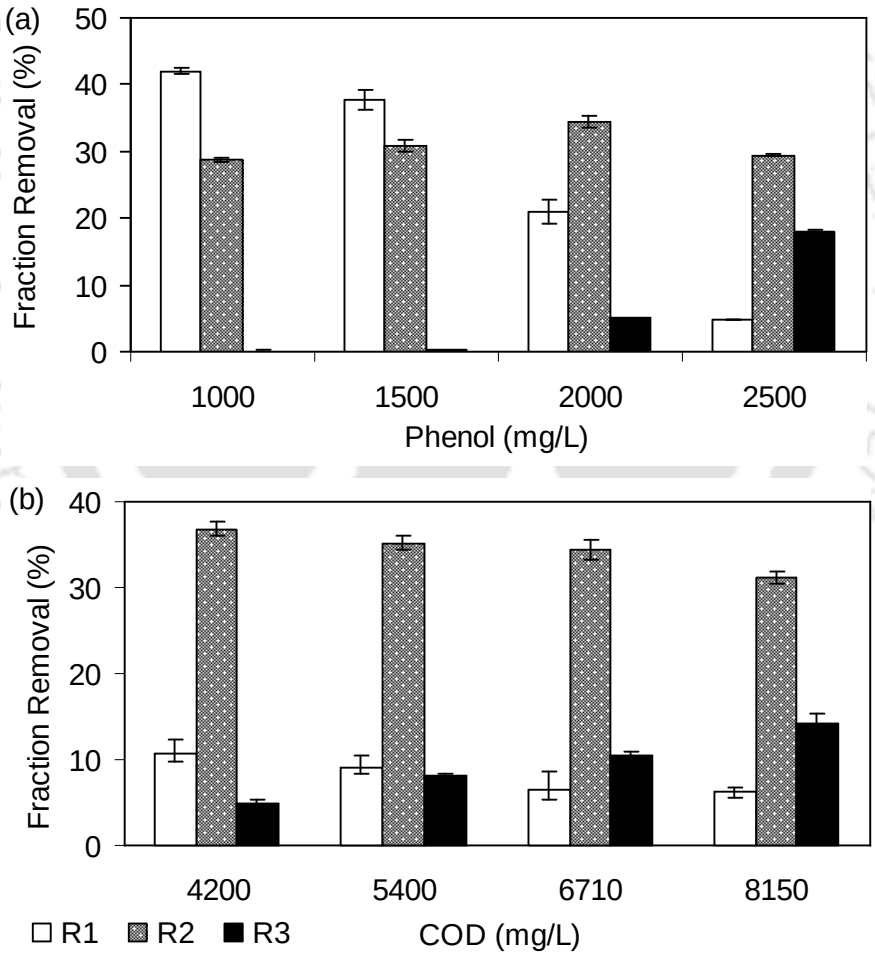

Figure 4.19 (a) Phenol (b) COD removal by R1, R2 and R3 at varied feed phenol concentration

Biomass concentration in the sponge increased from 8450 to 9880 mg/L with increase in feed phenol concentration whereas suspended biomass concentration fluctuated between 900–1060 mg/L and the ratio between attached to suspended biomass increased from 8 to 11. Total biomass (biomass in sponge + biomass in suspension) in R1 at varied feed phenol concentrations was observed to be increased from 9505 mg/L to 10772 mg/L (Table 4.7). In R1, pH decreased from 7.5 to 6.8–6.9 and VFA concentration observed was 281–400 mg/L as acetic acid that decreased with increase in phenol concentration indicating less biomass activity towards high feed phenol. No specific methanogenic activity (SMA) of anaerobic sludge from R1 was observed through out the study and gas generation was also below detectable level.

4.1.3.2 Effect of influent phenol concentration on performance of anoxic CMBR (R2)

Steady state performance of R2 is shown in Tables 4.8 (a) and (b). Influent phenol to R2 was 290–1190 mg/L at a constant reactor HRT of 1.5 days and phenol loading rates were 0.193, 0.311, 0.527 and 0.793 g/L.day. R2 satisfactorily removed phenol throughout the study. Nearly 62–99% phenol removal was achieved in R2 which was 28–34% of total phenol removal [Figure 4.19 (a)]. Phenol removal rate in R2 increased with increase phenol loading rate and maximum removal rate was 0.491 g/L.day at loading rate of 0.793 g/L.day [Figure. 4.20 (a)] indicating insignificant effect on phenol removal in R2.

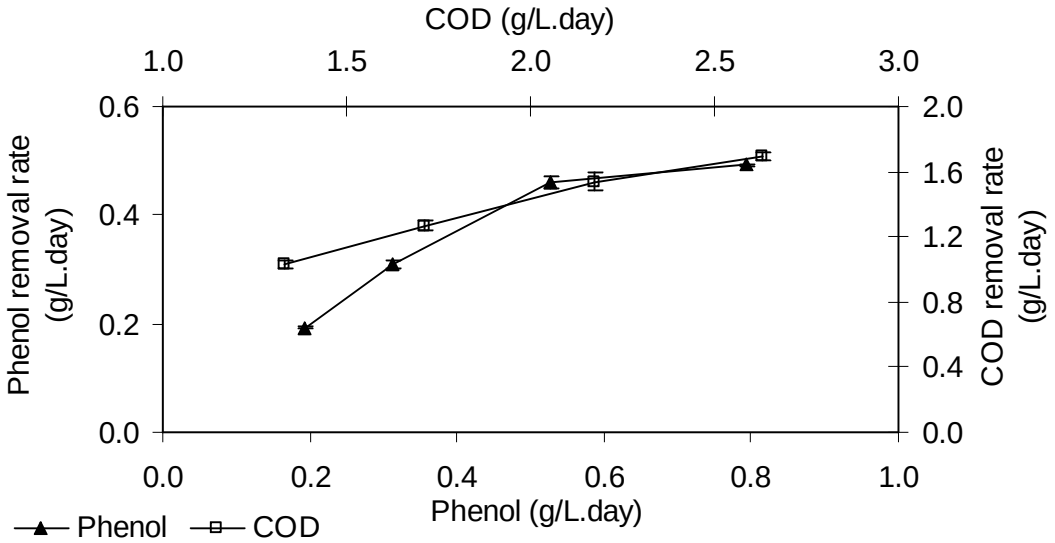

Figure 4.20 (a) Phenol and COD degradation in R2 at varied loading rate

With increase in feed phenol concentration, COD in R1 influent/effluent increased and correspondingly increased in R2 influent. The COD loading rate in R2 was 1.33–2.63 g/L.day. Almost 64–77% of influent COD was removed in R2 through out the study and it was observed that COD removal decreased with increase in COD loading. However in removal of COD, R2 contributed to a great extent as can be seen from Figure 4.19 (b) being 31–36% of total COD removal by the CMBR system and it was the higher than R1 and R3 throughout phenol variation study. Increased COD removal rate of 1.03–1.69 was achieved in R2 at COD loading of 1.33–2.63 g/L.day [Figure 4.20 (a)].

Table 4.8 (a): Performance of anoxic CMBR (R2) at influent phenol concentration variation

Phenol			COD			Thiocyanate			NO_3^-–N		NO_2^-–N		NO_x^-–N
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	S_0	S_e	Rem
290	2.25 (1.2)	99.2	1998	452 (14)	77.4	396	3.3 (0.8)	99	625	189 (7)	27	0	71.01
468	5 (0)	98.9	2567	666 (10)	74.0	401	10 (1.5)	97	623	173 (9.3)	50	0	74.4
790	100 (0)	87.3	3258	950 (26)	70.84	401	32 (0)	92	592	169 (7.8)	50	0	74.0
1190	452 (2.5)	62.0	3942	1400 (47)	64.49	406	125 (0)	69	590	111 (3.28)	48	1.8	82.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),
Numbers in parenthesis indicate standard deviation values.

No SCN^- removal occurred in the upstream reactor R1. The initial feed SCN^- got diluted 50% by the recycle from R3 and the feed SCN^- to R2 was 396–406 mg/L resulting in SCN^- loading rate of 0.264–0.271 g/L.day during the study. R2 released 3–32 mg/L SCN^- in its effluent comprising 99–92% SCN^- removal in presence of 290–790 mg/L phenol in its influent. Table 4.8 (a) shows that SCN^- removal decreased significantly (by 25%) when influent phenol concentration to R2 was 1190 mg/L. Figure 4.20 (b) shows that SCN^- removal rate decreased from 0.261 g/L.day to 0.187 g/L.day with increase in phenol

loading from 0.193–0.793 g/L.day at almost fixed SCN^- loading of 0.264–0.271 g/L.day. Staib and Lant (2007) observed no inhibition of phenol on thiocyanate up to phenol loading ~0.134 g/L.day as phenol degradation was much quicker compared to thiocyanate though CN^- significantly inhibited thiocyanate degradation. Figure 4.21(a) depicted that R2 had significant role for removing 35–49% of total SCN^- during the study.

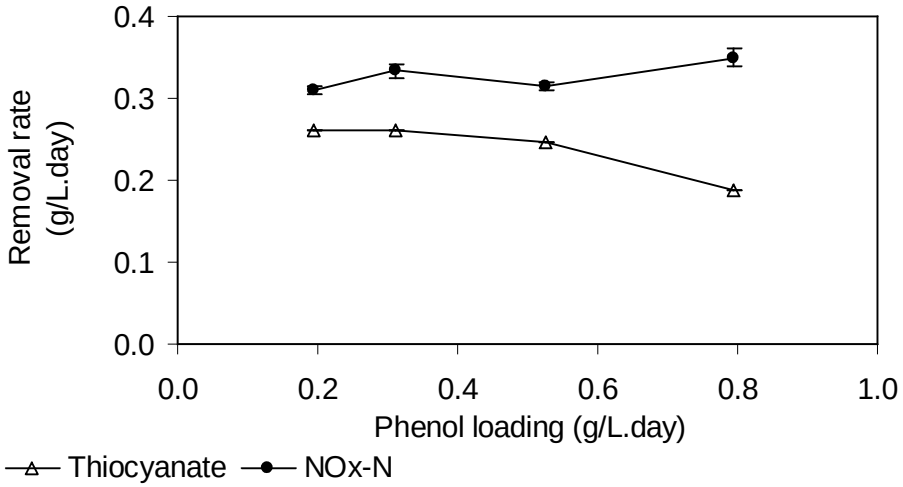

Figure 4.20 (b) Thiocyanate and NOx-N degradation in R2 at varied phenol loading rate

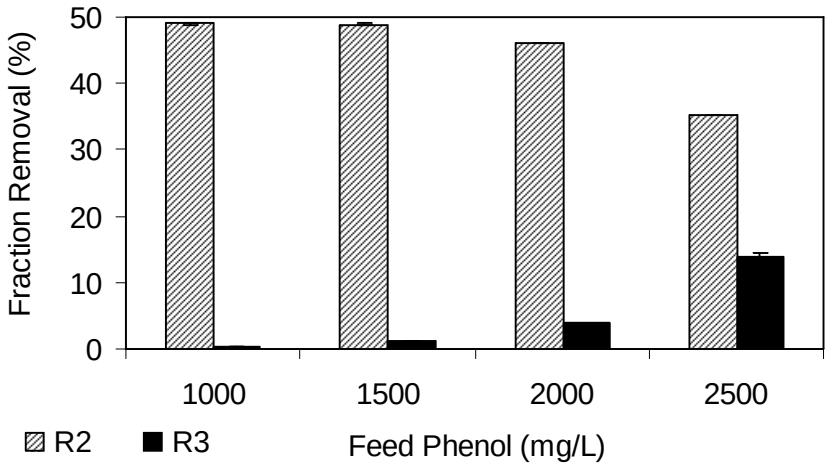

Figure 4.21 (a) Fraction of total SCN^- removal by R2 and R3 at varied initial feed phenol concentration

The NO_x–N (NO_3^-–N + NO_2^-–N) loading rate to R2 was 0.42–0.45 g NOx–N /L.day. Nitrite was completely utilized in R2 and effluent pH was 8.2–8.4 whereas influent pH which was ~7.5, which was probably due to denitrification (equation 2.1b and equation

2.2c) where bicarbonates ions are produced during anoxic phenol and thiocyanate degradation. NO_x–N removal efficiency in R2 increased from 71 to 82% with increase in feed phenol. Figure 4.20 (b) shows that NO_x–N removal rate was 0.308– 0.349 g/L.day and increased at phenol loading 0.193–0.793 g/L.day.

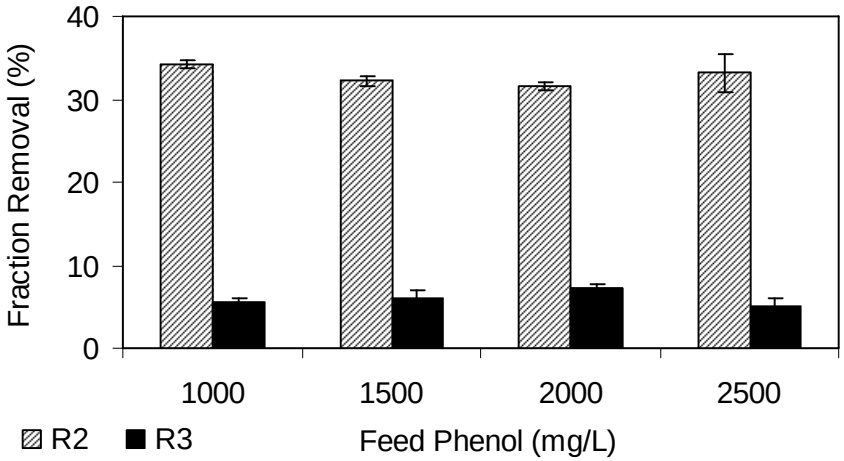

Figure 4.21 (b) Nitrogen removal by R2 and R3 at varied initial feed phenol concentration

The NO_x–N got reduced for COD and thiocyanate oxidation and with high phenol loading, higher loading of COD occurred in R2 consuming more NO_x–N. Figure 4.21 (b) shows that R2 was responsible for 31–34% nitrogen removal, which occurred through denitrification route. Ramos et al. (2007) also observed that anoxic reactor was mainly responsible for nitrogen removal while treating nitrogen and phenol from saline industrial wastewater in a pre–denitrification system consists of submerged fixed film reactor while phenol and COD removal occurred both in anoxic and aerobic reactor. Ramakrishnan and Gupta, (2008a) reported minimum COD/N ratio for 98% of phenolics removal from influent concentration of 752 mg/L at HRT of 1 day was 6.36. In the present study the influent COD/N ratio was 2.67–6.16 and high phenol and COD removals were achieved along with 71–82% NO_x–N removal. The COD/N_{rem} ratio in R2 at varied influent phenol concentration calculated using equation 4.5 was 3.7–6.4 [Table 4.8(b)]. Almost 45–77% COD was utilized for denitrification and 23–55% COD was utilized for biomass generation. With high phenol/ or COD loading in R2, COD utilized for generation of biomass (COD_B) increased and biomass yield coefficient increased from 0.16 to 0.35 with increase in influent phenol and COD.

Total biomass concentration (TVS) is shown in Table 4.8 (b) that increased from ~12690 mg/L to ~14790 mg/L with increase in influent phenol to 790 mg/L and it slightly decreased to 13800 mg/L when influent phenol was 1190 mg/L. Attached biomass concentration increased from 8000 mg/L to ~10000 mg/L with increase in influent phenol. Maximum suspended biomass observed was 4700 mg/L at low influent phenol (290 mg/L) that decreased to ~ 3700 mg/L and 3400 mg/L with increase in influent phenol. Attached to suspended biomass ratio in R2 initially increased from 1.7 to 3.5 when influent phenol was increased from 290–790 mg/L, however with further increase in influent phenol concentration to 1190 mg/L, the ratio decreased to 3.0 as attached biomass concentration slightly decreased.

In R2, nearly 9-31% NH_4^+–N removal was observed that decreased with increase in influent phenol. Along with NH_4^+–N entering from effluent of R1 and recycle from R3, some amount of NH_4^+–N was generated from degradation of SCN^- in R2.

Table 4.8 (b): Performance of anoxic CMBR (R2) at feed phenol concentration variation

Phenol	NH_4^+–N			COD : N_{rem}	COD_B	SO_4^{-2}					TVS (mg/L)	pH (S_e)
S_0	S_0^A	S_e	Rem			S_0	S_e	Gen	Th. SO_4^{2-}	Err		
290	365	250 (0)	31.6	3.72	23	461	920 (4.2)	458	647	–188	12690 (158)	8.1 ±0.2
468	367	322 (4.5)	12.3	4.6	38	454	888 (15)	434	645	–210	13629 (122)	8.4 ±0.2
790	391	345 (0)	11.7	6.4	55	448	860 (17)	402	608	–206	14795 (175)	8.4 ±0.2
1190	380	345 (5.1)	9.3	5.8	51	465	800 (0)	336	464	–128	13860 (139)	8.4 ±0.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);

[A] Influent NH_4^+–N of R2 = {Effluent NH_4^+–N of (R1+R3)/2 + 0.24x (SCN^- removed in R2)}.

COD_B: COD fraction (%) for biomass;

Th. SO_4^{2-}: Theoretical sulfate generation; Err: Error (mg/L)

Numbers in parenthesis indicate standard deviation values for TSS.

The effluent from R2 was rich in sulfate through out the study which was generated as a byproduct from oxidation of SCN^- in R2 along with ammonia–N (Equation 2.2e). The sulfate concentration in the effluent was 800–920 mg/L and the highest concentration was evolved when influent phenol concentration to R2 was the minimum. However sulfate generation was less in R2 than that of theoretical value showing higher error at low influent phenol study [Table 4.8 (b)]. During the thiocyanate and HRT variation study, error in sulfate generation was observed to increase when influent thiocyanate concentration was high (~288 mg/L) though thiocyanate removal occurred efficiently. During the phenol variation study, R2 always received higher concentration of thiocyanate (396–400 mg/L) and thiocyanate removal occurred through out the study and might be the reason for higher error in sulfate generation due accumulation as other intermediate sulfur compound. Influent pH to R2 was maintained fixed at 7.5± 0.5, and the effluent pH was always observed to be higher being 8.1–8.4.

4.1.3.3. Effect of influent phenol variation on performance of aerobic CMBR (R3)

Average steady state performance of R3 is shown in Tables 4.9 (a) and (b). R3 received a wide range of phenol concentrations comprising of minimum 2 mg/L to maximum phenol concentration of 452 mg/L, respectively when feed phenol in R1 was varied from 1000 mg/L to 2500 mg/L. The phenol loading rates were 0.001, 0.003, 0.06 and 0.301 g/L.day in R3 at fixed HRT of 1.5 days and the influent phenol concentration 2.25, 5, 100 and 452 mg/L, respectively. R3 released only 1–2 mg/L phenol in the effluent comprising almost 55–99% removal irrespective of influent phenol [Table 4.9 (a)]. Maximum phenol removal rate achieved was 0.30 at maximum loading of 0.301 g phenol/L.day (Figure 4.22). Contribution of R3 was 0.1 to 18% of total phenol removal which increased with increase in influent concentration of phenol [Figure 4.19 (a)].

R3 received nearly 3–125 mg/L SCN^- in its influent with loading of 0.001–0.083 g SCN^- /L.day which was accompanied with increased phenol and COD during the study. With increase in feed phenol concentration, phenol, COD and SCN^- removal efficiencies of upstream reactor R2 decreased, and more amount of SCN^- along with phenol and COD entered to R3. Almost 70–97% removal of SCN^- occurred releasing only 1 mg/L SCN^- in the effluent when influent SCN^- and phenol were 3–32 mg/L and 2–100 mg/L,

respectively. However R3 released nearly 13 mg/L SCN^- removing 90% of its influent SCN^- at influent SCN^- and phenol concentrations of 125 and 452 mg/L, respectively [Table 4.9 (a)]. Both high concentrations of phenol and SCN^- might have troubled the reactor in this situation as reported previous literature (Banerjee, 1996; Kim et al. 2007, 2008 a and b). Figure 4.21(a) shows that contribution of R3 in total SCN^- removal was only 0.5% when feed phenol was low as R2 almost completely taken care of the influent SCN^- releasing only 3 mg/L in effluent. Fractional SCN^- removal in R3 increased to 14% when feed phenol was 2500 mg/L. Thiocyanate removal rate in R3 increased from 0.001–0.075 g/L.day irrespective of phenol [Figure 4.22].

Table 4.9 (a): Performance of aerobic CMBR (R3) at feed phenol concentration variation

Phenol			Thiocyanate			NH_4^+–N			NO_3^-–N		NO_2^-–N		N_R
S_0	S_e	Rem	S_0	S_e	Rem	S_0[A]	S_e	Rem	S_0	S_e	S_0	S_e	
2.25	1 (0)	55	3.3	1 (0)	70	250	43 (2.5)	83	189	250 (16)	0	55	0.08
5	2 (0)	60	9.8	1 (0)	89	324	48 (2.2)	85	173	247 (10)	0	100	0.11
100	1 (0)	99	32	1 (0)	97	352	100 (0)	72	169	185 (4)	0	100	0.08
452	1.3 (0.6)	99	125	13 (0)	90	372	124 (13)	67	111	179 (8)	1.8	95	0.10

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of R3 = {Effluent NH_4^+–N of R2 + 0.24x (SCN^- removed in R3)};

N_R: Nitrification rate (g/L.day);

Numbers in parenthesis indicate standard deviation values.

The main role of R3 in the system was as a polishing unit and to achieve nitrification. NH_4^+–N loading was 0.167–0.248 g/L.day during the study. NH_4^+–N removal rate in R3 initially increased from 0.138 to 0.184 g/L.day when influent phenol was 2–5 mg/L and then decrease to 0.158–0.165 g/L.day at higher influent phenol concentration of 100–452 mg/L [Figure 4.22].

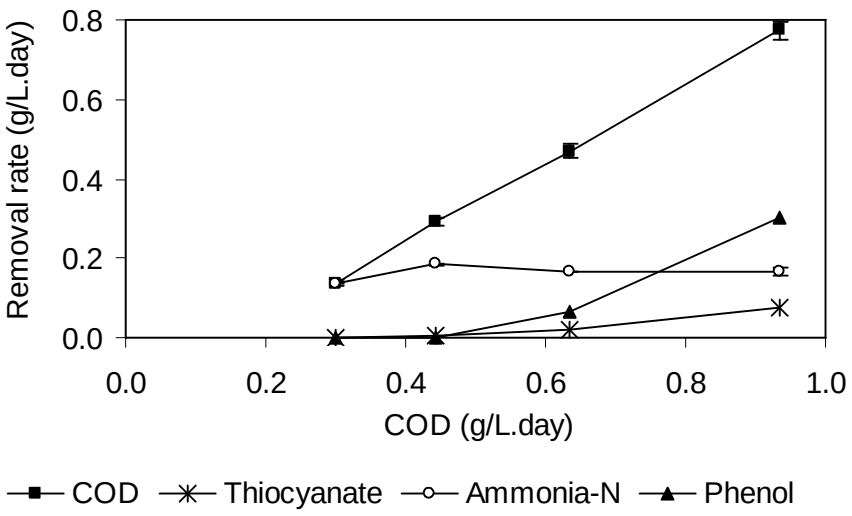

Figure 4.22 Performance of R3 at varied COD loading

R3 released 43–48 mg/L NH_4^+–N in its effluent with more than 80% removal efficiency when influent phenol, SCN^- and NH_4^+–N concentration were 2–5 mg/L, 3–9 mg/L and 250–324 mg/L, respectively [Table 4.9 (a)]. However at influent NH_4^+–N concentration of 352 mg/L and phenol concentration of 100 mg/L, the reactor failed to achieve the same performance level and released nearly 100 mg/L NH_4^+–N in its effluent and removal efficiency dropped to 72%. Also, highest influent NH_4^+–N concentration of 372 mg/L flowed to R3 when phenol and SCN^- concentration was also in their maximum concentration of 452 and 125 mg/L, respectively. In this situation NH_4^+–N removal achieved was least of 67% and R3 released a high concentration of NH_4^+–N in its effluent. Effluent nitrate concentration of R3 continuously decreased from 250 mg/L to 179 mg/L nitrite concentration of 55–100 mg/L was observed with increase in influent phenol. Phenol compound is known to negatively affect nitrifying bacteria even concentration as low as 5.6 mg/L (Dyreborg and Arvin, 1995; Chen et al. 2008). Kim et al. (2008a) reported decreased nitrification rate as initial phenol concentration was increased. Particularly, phenol and thiocyanate above 200 mg/L, there was significant inhibition on nitrification, and no nitrification occurred at phenol concentration 500 mg/L. Amor et al. (2005) reported high phenol removal efficiencies, above 99.9% along with ~99.8% ammonia removal with no inhibition by phenol on nitrification in an activated sludge reactor, at

applied ammonium loading 0.14 g NH_4^+–N/L.day (350 mg NH_4^+–N/L) and at increased phenol (35–2800 mg/L).

FA concentration was found to increase with increase in influent NH_4^+–N and ranged from 26–44 mg/L which also have inhibitory affect on nitrifying bacteria and responsible for nitrite accumulation. In present study during higher feed phenol study, influent phenol in R3 was accompanied with higher thiocyanate concentration, FA and nitrite which might have affected the nitrification rate in combination.

Contribution of R3 in removal of total nitrogen (TN) was very poor being 5–8%. It was mainly due to the nitrogen was transferring from one form (NH_4^+–N) to another (NOx^-–N) not getting eliminated from the reactor. The NH_4^+–N concentration was oxidized to either nitrate or nitrite and was available in the reactor. The unaccounted nitrogen fraction in R3 was 17–38% which was higher during lower influent phenol [Table 4.9 (b)]. Nitrification rate in R3 observed was 0.08–0.11 g/L.day [Table 4.9 (a)], which was lower than NH_4^+–N removal rate. This might be due to some nitrogen loss through denitrification during this condition. Helmer et al. (1999) reported that under low DO concentrations autotrophic ammonia–oxidizers might be the causative agents of nitrogen loss by performing aerobic/anoxic denitrification with nitrite as electron acceptor and ammonia as electron donor. In R3 same phenomena might have occurred with the biofilm towards interior of the sponge cube.

Nearly 451–1400 mg/L residual COD entered R3 and the loading rates were 0.33, 0.44, 0.63 and 0.93 g/L.day. Almost 46–82% removal of COD occurred releasing 230–245 mg/L of effluent COD and removal increased with increase in influent COD concentration. The contribution of R3 in COD removal increased from 5% to 14% of total COD removal when it received increased influent COD [Figure 4.19 (b)]. Figure 4.22 shows that COD removal rate increased linearly from 0.14–0.77 g/L.day with increase in COD loading rate 0.33–0.93 g/L.day. Due to high amount of influent COD, higher amount of heterotrophs made biofilm at the outer site of the sponge limiting oxygen content for the slow growing nitrifiers grown in the interior of the sponge cubes. This also could be another reason for less nitrification at higher influent phenol/COD to R3. From Table 4.9 (b), it can be seen that with increased phenol feed, COD to NH_4^+–N ratio in the influent increased from 1.80

to 3.68. This high ratio is prone to favor higher growth of heterotrophs in R3 as mentioned earlier (Hankai et al. 1990).

In R3, suspended biomass concentration was fluctuating in 3000–3600 mg/L during the study, whereas attached biomass concentration was observed to increase with increase in influent phenol. The attached to suspended biomass ratio was 2.3 to 2.8 at influent phenol 2–100 mg/L and increased to 3.2 when R3 received 452 mg/L influent phenol. Total biomass concentration in R3 increased from 11479 mg/L to 13070 mg/L during the study with increase in influent phenol to R3 [Table 4.9 (b)].

R3 released 897–930 mg/L of sulfate in its effluent with 3–130 mg/L sulfate generation irrespective of influent phenol. Low sulfate generation than theoretical value (higher negative error of –54 mg/L) was observed when R3 was receiving higher phenol, COD and thiocyanate in influent. This might be due to accumulation of other sulfur compound like polysulfide etc as reported earlier.

Table 4.9 (b): Performance of aerobic CMBR (R3) at feed Phenol concentration variation

Phenol	COD			COD : NH_4^+ –N	TVS	pH	FA	UN	SO_4^{2-}				
S_0	S_0	S_e	Rem						S_0	S_e	Gen	Th. SO_4^{2-}	Err
2.25	452	245 (0)	46	1.8	11479	8.4 ±0.2	26.3	31	920	923 (2.5)	3	4	-1
5	666	230 (0)	65	2.06	12280	8.4 ±0.2	33.2	25	888	907 (11)	19	14	5
100	950	245 (8)	74	2.88	12830	8.4 ±0.2	40	38	850	897 (32)	47	51	-4
452	1400	241 (26)	82	3.68	13070	8.4 ±0.2	44.3	17	800	930 (30)	130	184	-54

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L); Err: Error (mg/L);
UN: Unaccounted nitrogen (%); FA: Free Ammonia (mg/L)
Th. SO_4^{2-}: Theoretical sulfate generation (mg/L); TVS in mg/L
Numbers in parenthesis indicate standard deviation values.

4.1.3.4 Effect of feed phenol on overall performance of CMBR system

The feed and final effluent of R3 was considered to estimate overall performance of three–stage CMBR system. The overall performance of the three–stage CMBR at varying feed phenol concentrations is shown in Figure 4.23 in terms of phenol, COD, SCN^- and TN removals. Phenol and SCN^- removal were complete and independent of feed phenol concentration. COD removal was 94–97% with effluent COD 230–245 mg/L (from influent 4200–8150 mg/L) irrespective of influent phenol concentration. The total NH_4^+–N removal in three–stage system varied with feed phenol concentration. NH_4^+–N removal decreased from 93.7% to 82% with increased in feed phenol. Total nitrogen (TN) in influent of three–stage CMBR system was 1692 mg/L (considering influent NO_3^-–N of 1000 mg/L added in the recycle of R3) (equation 4.7). TN removal remained almost fixed at 77–76% with varied phenol loading. It was observed that during low phenol loading there was less NOx–N removal and high NH_4^+–N removal whereas at high phenol loading high NOx–N removal but less NH_4^+–N removal was achieved. Also higher fraction of oxidized nitrogen in effluent in low phenol study and low amount in high phenol study and vice versa was observed. Finally this resulted in balancing TN removal through out the study.

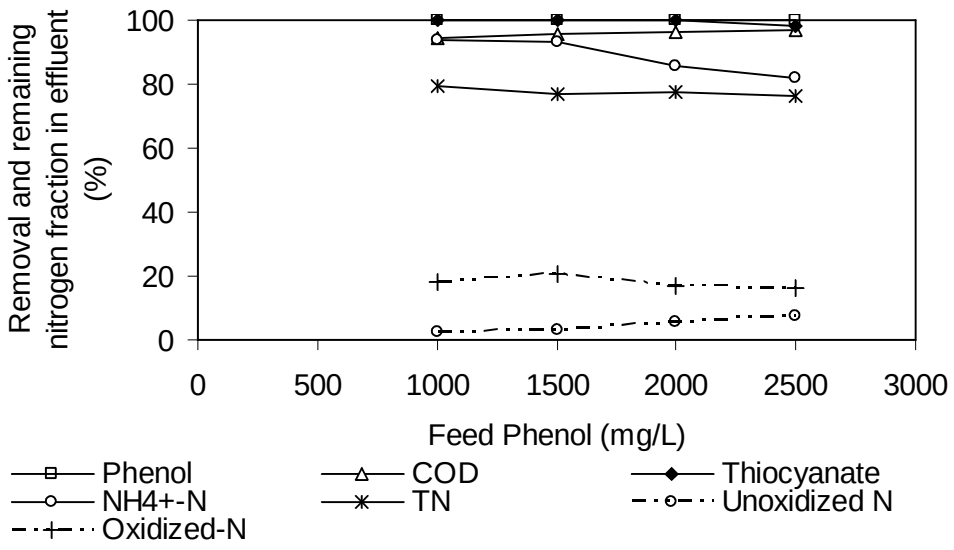

Figure 4.23 Effect of feed phenol on performance of CMBR system
(Solid lines removal; dotted line fraction of TN remaining in effluent)

4.1.4 Performance of CMBR system at varied feed ammonia–nitrogen concentration

Ammonia in wastewaters if discharged inappropriately has adverse environmental effects to aquatic systems. It is toxic to living organisms and causes eutrophication in water bodies (Wu et al. 2008). Performance of three staged CMBR was evaluated with feed NH_4^+–N as variable parameter. Four experimental runs were conducted from 800th to 985th day at varying feed NH_4^+–N concentration at 100, 300, 500, and 600 mg/L at constant total HRT of 6 days (R1: 3 days; R2 1.5 day and R3 1.5 day). Feed SCN^- and phenol were constant throughout the study at 800 and 1500 mg/L, respectively with COD 5400 mg/L. After each modification in feed NH_4^+–N concentration, the three–stage system attained steady state as characterized by consistency in effluent parameters, after a transient period of 7–10 days. Steady state data was collected for 12–15 days and considered to analyze the performance of each reactor and profile of various pollutant is shown in is shown in Figure 4.24.

4.1.4.1 Effect of feed ammonia–nitrogen on performance of anaerobic CMBR (R1)

Steady state performance of R1 at varied influent NH_4^+–N concentrations is presented in Table 4.10 as average values along with standard deviation. R1 showed moderate removal of phenol and COD whereas no removal of SCN^- or NH_4^+–N was observed. Effluent NH_4^+–N concentration during the initial studies were found to increase 4–6 mg/L in R1 that might be due to cell lysis or ammonification (Jokela and Rintala, 2003). Zheng and Li (2009) and Chen et al. (2008) reported 20% and 10–32% removal of NH_4^+–N from influent NH_4^+–N concentration of 700 mg/L and 350–390 mg/L, respectively as a result of biomass assimilation process in anaerobic reactor.

Phenol and COD loadings in R1 were constants at 0.5 and 1.8 g/L.day during feed NH_4^+–N variation study. Phenol removal decreased from 46 to 26% and R1 accounted for decrease in COD removal from 10% to 4% with increase in influent NH_4^+–N concentration from 100 to 600 mg/L (Table 4.10). Phenol removal rate in R1 decreased from 0.228 to 0.133 g/L.day with increase in NH_4^+–N loading. COD removal rate also decreased from 0.181-0.165 g/L.day and there was a drastic decrease in COD removal rate from 0.165 to 0.075 g/L.day with increase in NH_4^+–N loading above 0.167 g/L.day (Figure 4.25). This indicates the toxicity exerted by NH_4^+–N increases to R1 culture at increasing concentration.

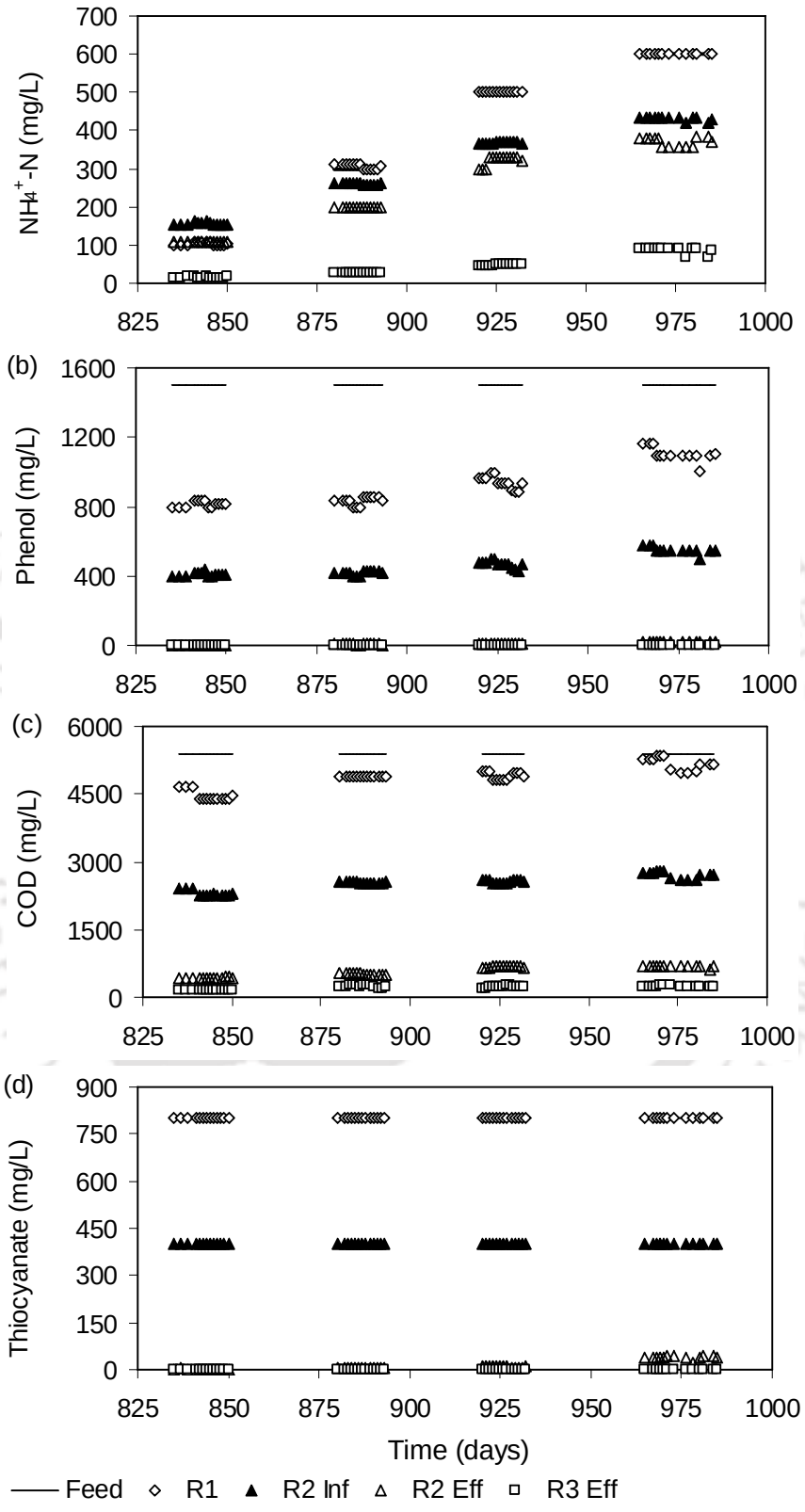

Figure 4.24 Pollutant profile in CMBR during feed NH_4^+-N variation study

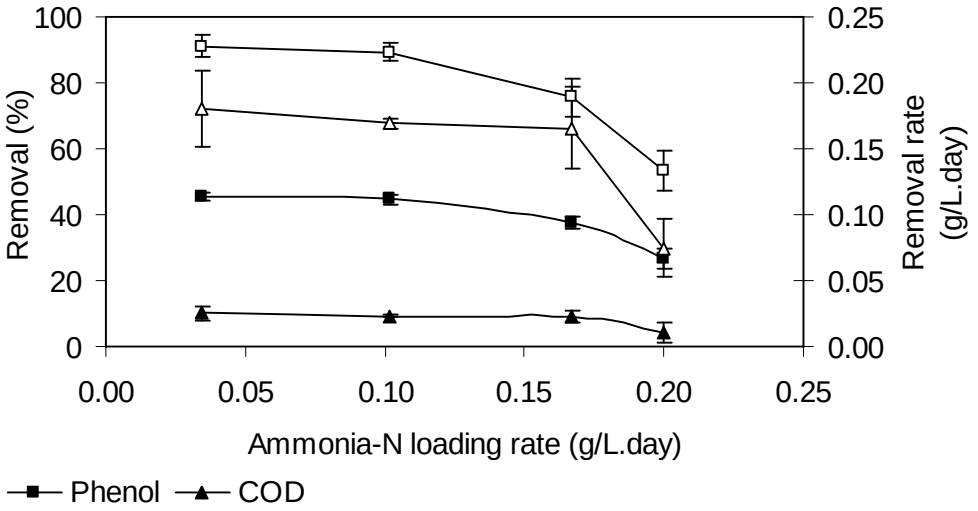

Figure 4.25 Phenol and COD removal (solid symbol) and removal rate (hollow symbol) in R1 at varied ammonia loading [Error bar suggests standard deviation]

Table 4.10: Performance of anaerobic CMBR (R1) at varied feed NH_4^+–N concentration

NH_4^+–N		Phenol			COD			SCN^-		pH	TVS (mg/L)	VFA
Feed	S_e	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	S_e		S_e
100	104 (5)	1500	816 (23)	45.60	5400	4858 (115)	10.04	800	800	6.8	10830	480 (13)
300	306 (5.1)		830 (20)	44.67		4893 (10)	9.39		800	6.9	10786	638 (20)
500	500 (0)		934 (25)	37.73		4904 (93)	9.19		800	6.8	10338	400 (57)
600	600 (0)		1100 (44)	26.67		5175 (303)	4.17		800	6.7	9060	650 (23)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

VFA: Volatile fatty acid as acetic acid (mg/L)

Numbers in parenthesis indicate standard deviation values.

Figure 4.25 also shows that phenol and COD removals were unaffected up to NH_4^+–N loading up to 0.1 g/L.day in R1, whereas the toxic affect was more profound above loading

of 0.167 g/L.day. Uludag–Demirer et al. (2008) and Sung and Liu (2003) observed that in absence of other toxic compounds like phenol and thiocyanate, the inhibition of ammonia in anaerobic reactors start likely from 1000 mg/L depending on the pH and temperature in synthetic wastewater treatment. However, in the present condition ammonia was associated with toxic compounds phenol and SCN^- and resulted in inhibition to the anaerobic culture at much less concentration of ammonia in feed. Figure 4.26 (a) shows contribution of R1 in total phenol removal remained higher than R2 up to feed NH_4^+–N concentration of about 500 mg/L. However the contribution of R1 in total COD removal decreased with increase in feed NH_4^+–N concentration [Figure 4.26 (b)]. Liu et al. (2012) reported ammonia inhibition also related to ratio of available soluble COD to total ammonia nitrogen. Higher ratio reveals less inhibition whereas the reverse causes inhibition of ammonia on COD removal and biogas generation. However, in the present study the ratio was 54–9 and it always higher than the reported ratio by Liu et al. (2012).

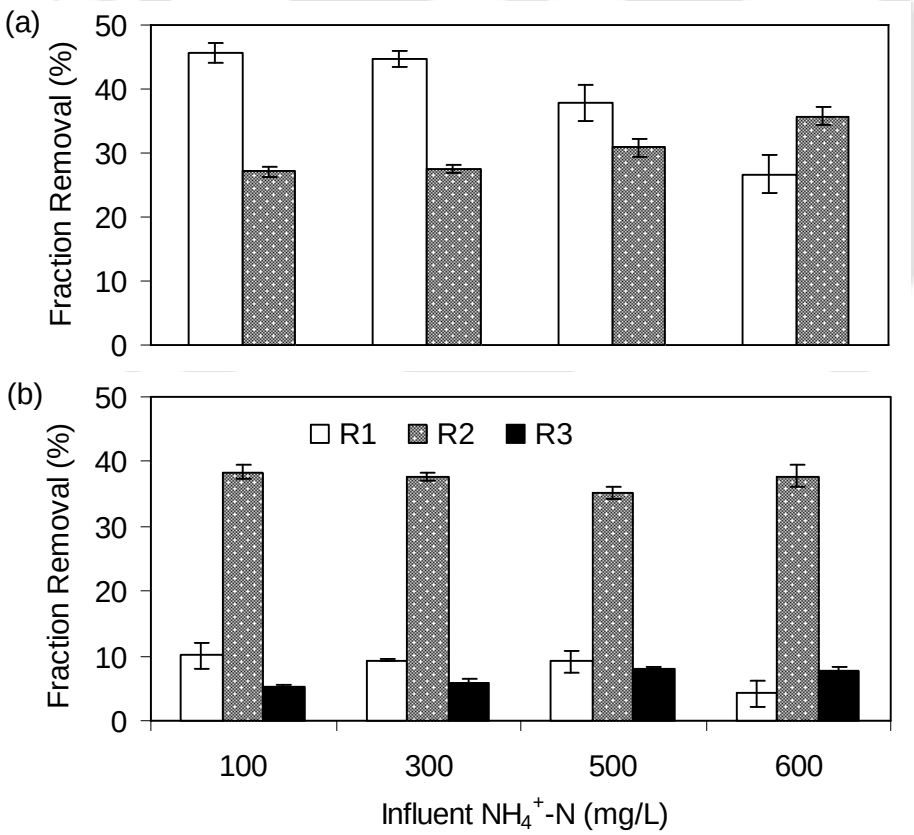

Figure 4.26 Fraction of a) Phenol and b) COD removal by R1, R2 and R3 at varied feed ammonia concentration [in figure (a) phenol removal was complete in R2 and no phenol enterd to R3]

Total biomass (biomass in sponge + biomass in suspension) concentration in R1 was observed to decrease from 10830 mg/L to 9060 mg/L with increase in feed NH_4^+–N and the change was significant only when feed NH_4^+–N was 600 mg/L. In R1, suspended and attached biomass concentrations were 930–1200 mg/L and 8100–9700 mg/L, respectively during the study, being higher concentrations of both attached and suspended biomass at low influent NH_4^+–N concentration in feed. When feed NH_4^+–N was low, there was more removal of phenol/COD and this was responsible for generation of higher biomass. Attached to suspended biomass ration was 8–10 during the study. In R1, pH decreased from 7.5 to ~6.9 and VFA (volatile fatty acid) concentration observed was 400–650 mg/L as acetic acid.

4.1.4.2. Effect of varied influent ammonia concentration on anoxic CMBR (R2)

Steady state performance of R2 is shown in Tables 4.11 (a) and (b). In R2, influent NH_4^+–N concentration varied from 160 to 419 mg/L with respective loading of 0.107 to 0.279 g/L.day. Nearly 65–332 mg/L NH_4^+–N entered to R2 from as influent from effluent of R1 and recycle from R3 and some amount of NH_4^+–N was generated from SCN^- degradation. Accounting all this as total influent NH_4^+–N, R2 was capable of removing 31–12% NH_4^+–N and released 110–370 mg/L NH_4^+–N in the effluent [Figure 4.24 (a) and Figure 4.27 (a)]. NH_4^+–N removal rate in R2 was 0.031–0.033 g/L.day. NH_4^+–N removal in anoxic environment can occur through anoxic ammonium oxidation or assimilatory removal for biomass as widely reported (Caffaz et al. 2006; Jung et al. 2007; Fernández et al. 2010).

COD in influent of R2 increased from 2502–2710 mg/L with corresponding loading rate 1.67–1.80 g/L.day. The COD removal accounted in R2 decreased from 82% to 75% with increase in influent COD and NH_4^+–N showing COD removal rate of 1.267–1.383 g COD/L.day during the study. This comprised of 35–37% of total COD removal. Figure 4.26 (b) depicted that contribution of R2 in the system for COD removal was always higher compared to R1 and R3, suggesting insignificant inhibition of NH_4^+–N for present studied concentration on phenol/ or COD degradation in R2 [Figure 4.27 (a) and (b)].

Influent nitrate and nitrite concentration to R2 was 600–635 mg/L and 32–59 mg/L, respectively resulting in influent NO_x^-–N loading to R2 0.421–0.462 g/L.day. The NO_x^-–N concentration in the recycle increased as R3 released higher concentration of nitrate/nitrite

in effluent with increase in influent NH_4^+–N during the study. Respective NO_x^-–N removal rate in R2 were observed as 0.295, 0.314, 0.334 and 0.375 g/L.day.

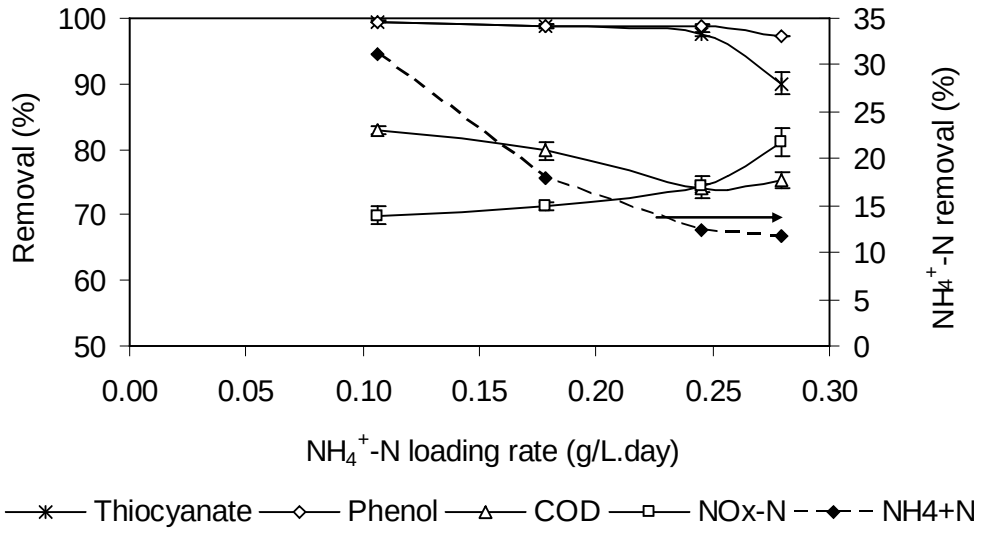

Figure 4.27 (a) Pollutant removal by R2 at varied NH_4^+-N loading

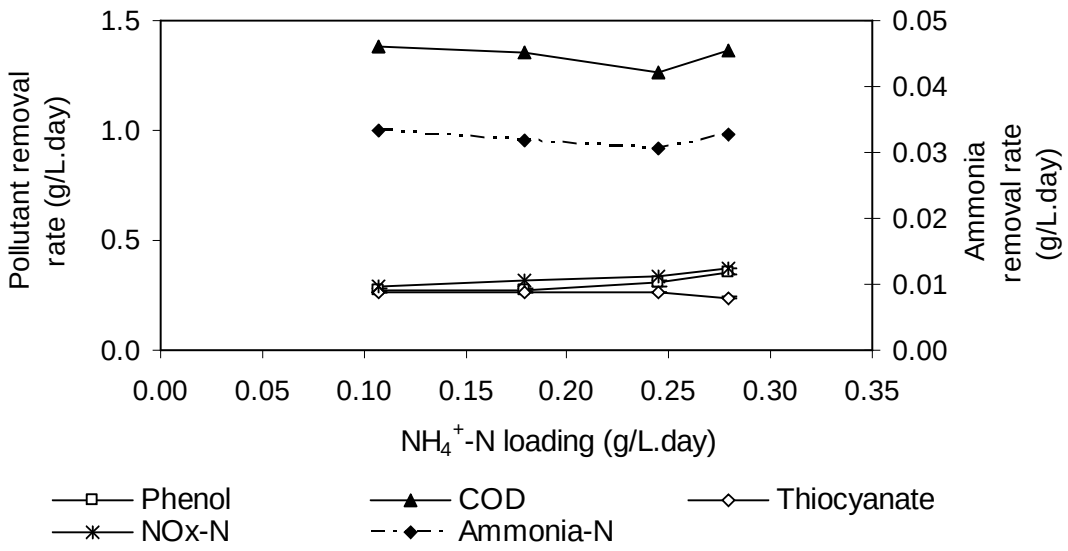

Figure 4.27 b) Pollutant removal rates in R2 at varied NH_4^+-N loading

Table 4.11 (a) shows that nitrite (NO_2^-–N) was completely utilized in R2. However, denitrification was incomplete as nitrate was detected in effluent of R2 and anoxic condition in R2 existed through out the study. Figure 4.28 (a) shows that R2 was more profoundly responsible for nitrogen removal which comprised of 37–45% of total nitrogen

removal during the study as denitrification was the main route of nitrogen removal from the system.

Table 4.11 (a): Performance of anoxic CMBR (R2) at feed NH_4^+–N variation

NH_4^+–N			COD			NO_3^-–N		NO_2^-–N			COD:	COD_B	TVS
S_0[A]	S_e	Rem	S_0	S_e	Rem	S_0	S_e	S_0	S_e	Rem	N_{rem}		(mg/L)
160	110 (0)	31.2	2502	428 (11)	82.90	600	190 (7.0)	32	0	69.96	6.1	53	12090 (236)
268	220 (0)	17.9	2542	513 (34)	79.82	620	189 (0.9)	40	0	71.36	5.4	47	13050 (286)
368	322 (13)	12.4	2561	666 (10)	74.10	635	173 (8.6)	50	0	74.74	4.6	37	13629 (75)
419	370 (14)	11.7	2710	670 (23)	75.28	635	131 (9.8)	59	0	81.12	4.2	32	11296 (92)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of R2 = {Effluent NH_4^+–N of (R1+R3)/2 + 0.24x (SCN^- removed in R2)}.

COD_B: COD fraction for biomass (%)

Numbers in parenthesis indicate standard deviation values

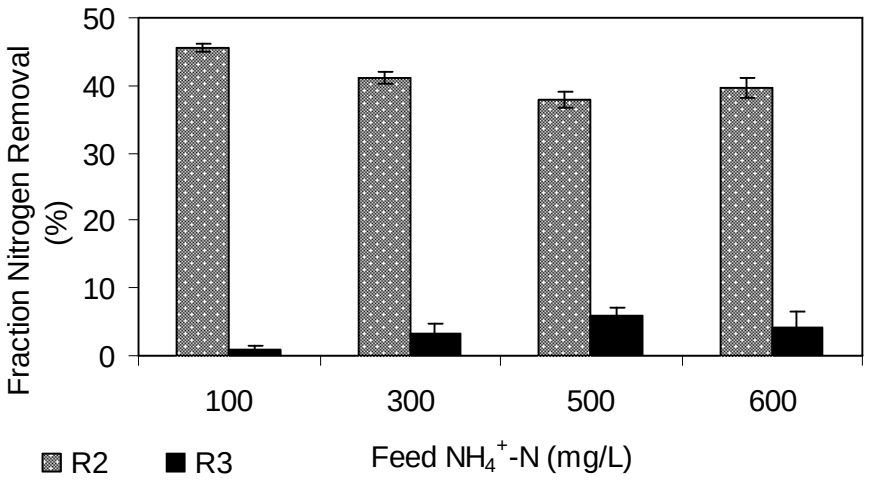

Figure 4.28 (a) Fraction nitrogen removal by R2 and R3 with varied feed ammonia concentration

COD in R2 got utilized for biomass synthesis and NO_x^-–N reduction. COD/N removed ratio was calculated using equation 4.5. With increased influent NH_4^+–N and COD:N

removed ratio was 6.1–4.2. COD fraction available for biomass decreased from 53% to 32% with decreased COD removal and the observed yield of biomass decreased from 0.37 to 0.23 with increase in influent NH_4^+–N in R2. The biomass strength in R2 during the feed NH_4^+–N variation study is given in Table 4.11 (a). Total biomass concentration decreased to ~11000 mg/L at maximum influent NH_4^+–N, which was 12000–13000 mg/L in low feed concentration. Attached biomass in sponge cube was 9000–9800 mg/L. With increase in influent NH_4^+–N 160–368 mg/L, suspended biomass initially increased from 3000–3700 mg/L, however decreased to 2000 mg/L with further increase of influent NH_4^+–N to 419 mg/L. Attached biomass to suspended biomass ratio was 2.2–4.6 being higher towards higher influent NH_4^+–N concentration.

Table 4.11 (b): Performance of anoxic CMBR (R2) at feed NH_4^+–N variation

NH_4^+ –N	Phenol			Thiocyanate			SO_4^{-2}					pH
S_0	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Gen	Th. SO_4^{2-}	Err	S_e
160	409	2 (0)	99.51	400	2 (0.5)	99.50	475	938 (16.8)	463	657	–194	8.1 ±0.2
268	416	5 (1.2)	98.80	401	5 (1.5)	98.75	465	920 (6.8)	455	652	–197	8.2 ±0.2
368	468	5 (0)	98.93	401	10 (1.5)	97.57	454	888 (34)	434	644	–210	8.4± 0.2
419	551	15 (0)	97.28	401	40 (6.7)	90.02	465	880 (5)	415	596	–181	8.4± 0.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);
Th. SO_4^{2-}: Theoretical sulfate generation {1.65*(SCN–removed)}, Err: Error (mg/L)
Numbers in parenthesis indicate standard deviation values.

With increase in feed NH_4^+–N, influent phenol and COD to R2 increased as R1 released increased amount of phenol in its effluent correspondingly. Influent phenol to R2 was 409–551 mg/L and corresponding phenol loading rate was 0.272–0.367 g/L.day. R2 satisfactorily removed 97–99% influent phenol and the contribution of R2 in total phenol

removal increased with increased influent phenol irrespective of ammonia loading. Phenol removal rate in R2 was 0.271–0.357 g/L.day. Eiroa et al. (2008) reported 91–90% phenol removal at phenol loading rate of 0.04–0.59 g/L.day in anoxic unit treating wastewater from resin producing industry containing 348–282 mg/L NH_4^+–N and NH_4^+–N removal in aerobic unit. In R2, influent pH was 7.5–7.7 and this increased to 8.1–8.4, which was due to denitrification.

SCN^- in influent of R2 was ~ 401 mg/L with loading rate 0.267 g/L.day. SCN^- degradation rate remained almost stable at 0.265– 0.261 g/L.day up to influent NH_4^+–N concentration 368 mg/L with SCN^- removal 99–97%. SCN^- degradation rate decreased to 0.241 g/L.day with 90% SCN^- removal when influent NH_4^+–N concentration was 419 mg/L at loading of 0.279 g NH_4^+–N/L.day. The decrease might be associated with combine inhibitory effect of NH_4^+–N and phenol. In present study, while R2 was receiving maximum NH_4^+–N loading (0.279 g/L.day), it was accompanied with maximum phenol loading of 0.367 g/L.day and resulted in decrease in SCN^- removal rate to 0.241 g/L.day and the removal efficiency decreased from 99% to 90%. Previous study with varied phenol HRT showed that phenol load more than 0.312 g/L.day caused decrease in thiocyanate removal in R2. However R2 remained responsible for 45–49% of total SCN^- removal with little decrease towards higher ammonia concentration [Figure 4.28 (b)]. R2 in present study sustained well to the toxic pollutants with better performance in terms of pollutant removal.

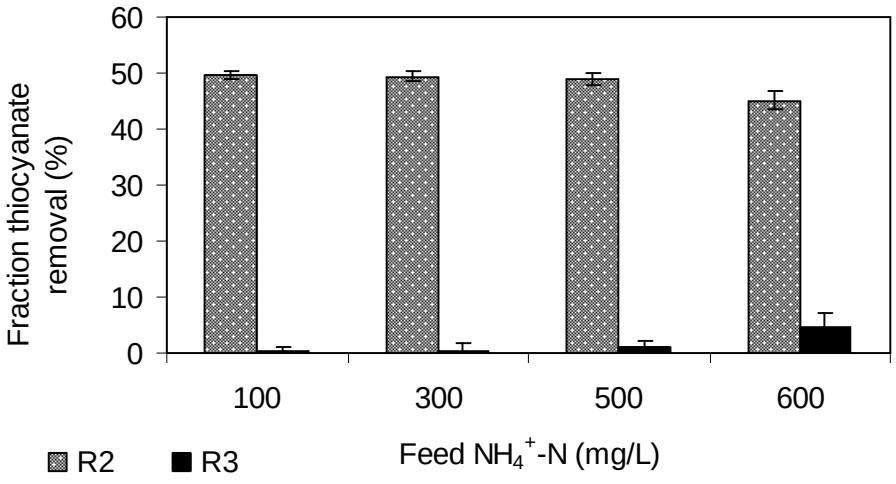

Figure 4.28 (b) Fraction thiocyanate removal by R2 and R3 with varied feed ammonia concentration

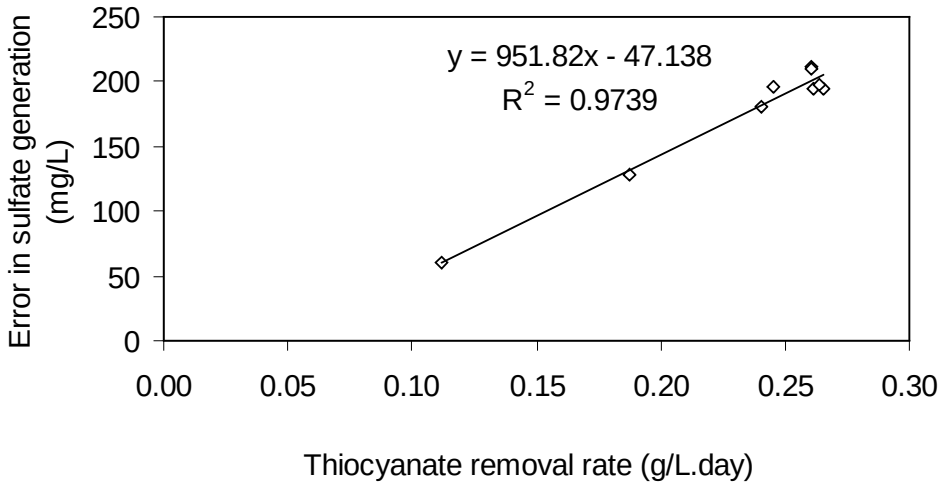

Figure 4.29 Error in sulfate generation against thiocyanate removal rate in R2

During the study, in R2, 415–460 mg/L sulfate evolved as byproduct from SCN^- biodegradation however, sulfate generation was lower to the theoretical sulfate generation value throughout the study. Figure 4.29 shows increase in error of sulfate generation with thiocyanate removal rate in R2. During other studies with feed thiocyanate, HRT and feed phenol variation also the error was observed to be higher in R2 while R2 was showing higher thiocyanate removal rate. Other sulfur compound might have accumulated in R2 rather than sulfate/sulfide as earlier reported (Buisman et al. 1990; Mahmood et al. 2008).

4.1.4.3. Effect of varied influent ammonia concentration on aerobic CMBR (R3)

Average steady state performance of aerobic reactor R3 is shown in Tables 4.12 (a) and (b). Influent NH_4^+–N concentration in R3 was 110, 221, 324 and 380 mg/L along with some amount of NH_4^+–N generated in R3 from degradation of SCN^- (0.24 g NH_4^+–N from one gram of SCN^- removed). NH_4^+–N loading at R3 was 0.07–0.25 g/L.day which increased with increase in initial feed as there was negligible NH_4^+–N removal in the upstream reactors. Figure 4.30 describes NH_4^+–N removal and removal rate against NH_4^+–N loading in R3. NH_4^+–N removal increased initially 77–85% with increased loading up to 0.216 g NH_4^+–N/L.day. However at maximum loading of 0.252 g/L.day, NH_4^+–N removal in R3 decreased to 82%. NH_4^+–N removal rate in R3 increased from 0.06 g/L.day to 0.21 g/L.day with increase in NH_4^+–N loading rate from 0.05–0.21 g/L.day. Maximum

ammonia removal rate of 0.28 g/L.day at NH_4^+–N loading rate of ~0.33 g/L.day was observed in a fluidized bed aerobic reactor from wastewater containing phenol, SCN^- and CN^- and NH_4^+–N and COD of 2500 mg/L (Jeong et al. 2006b).

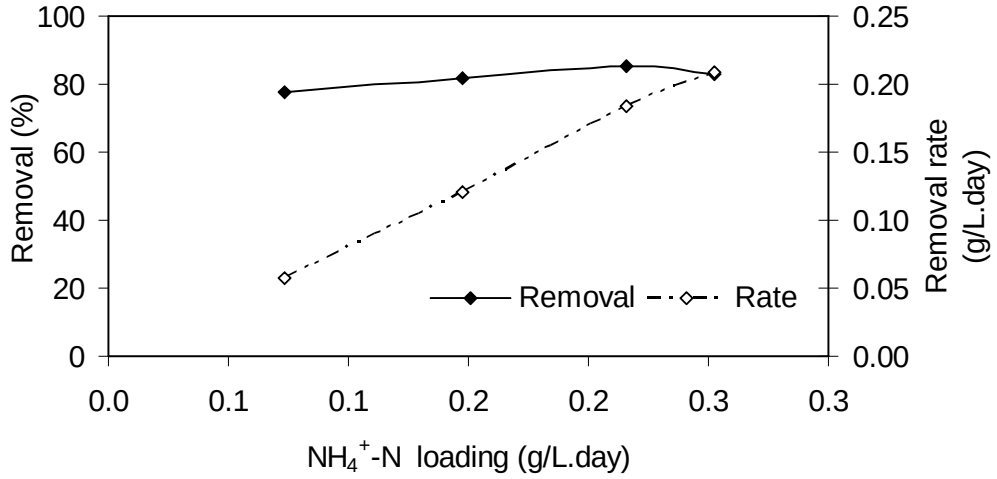

Figure 4.30 Performance of R3 at varied NH_4^+-N loading

Table 4.12 (a): Performance of R3 at feed NH_4^+–N concentration variation

Feed NH_4^+–N	NH_4^+–N			NO_3^-–N		NO_2^--N	N_R	FA	UN	COD : NH_4^+–N	TVS	pH
	S_0#	S_e	Rem	S_0	S_e	S_e						
100	110	25 (0)	77.27	190	200 (12)	65 (0)	0.06	4.2	7	3.8	11658	8.4 ±0.2
300	221	40 (0)	81.82	189	240 (21)	80 (0)	0.09	8.9	22	2.3	11700	8.4 ±0.2
500	324	48 (2.5)	85.09	173	247 (12)	100 (0)	0.12	24.6	28	2.0	12280	8.4 ±0.2
600	380	65 (7.8)	82.86	131	270 (48)	118 (0)	0.17	28.0	9	1.8	12900	8.4 ±0.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L)

Influent NH_4^+–N of R3 = {Effluent NH_4^+–N of R2 + 0.24x (SCN^- removed in R3)};

N_R: Nitrification rate (g/L.day);

FA: Free ammonia (mg/L); UN: Unaccounted nitrogen (%)

Numbers in parenthesis indicate standard deviation values

The respective nitrification rate (generation of NO_x^-–N) in R3 was 0.06–0.17 g/L.day, which increased with increase in NH_4^+–N loading rate. Though both the nitrification rate and NH_4^+–N removal rate in R3 increased with increase in NH_4^+–N loading rate, nitrification rate was found to be lower to NH_4^+–N removal rate. Some nitrogen might got lost from the reactor through incorporation to biomass or by volatilization shown as unaccounted nitrogen. In the present study, R3 received NH_4^+–N concentration of 380 mg/L, higher than reported threshold inhibitory concentration of 350 mg/L for nitrifying bacteria (Kim et al. 2008b) only during the experiment with initial feed of 600 mg/L NH_4^+–N. Accumulation of nitrite in R3 was observed and it increased from 65 mg/L to 118 mg/L with increase in influent ammonia. R3 was responsible for only 0.25–4.0% nitrogen removal [Figure 4.28 (b)] as R3 mainly contributed nitrification of NH_4^+–N rather than nitrogen removal from the system. FA concentration in R3 was found to increase from 4–28 mg/L with increase in feed NH_4^+–N and pH in the reactor [Table 4.12(a)].

Influent COD to R3 was 428–670 mg/L with loading of 0.285–0.446 g COD/L.day. COD removal remained almost constant at 63–65% with varied loading of NH_4^+–N in R3. COD removal rate in R3 increased from 0.187 g/L.day to 0.283 g/L.day with increase in feed NH_4^+–N and COD loading. Maximum COD removal rate observed in R3 was 0.291 g/L.day at COD and NH_4^+–N concentration of 666 and 324 mg/L, respectively during this study. Vázquez et al. (2006a) reported maximum COD removal rate of 0.76 g/L.day (removal of 58%) in an aerobic suspended growth reactor at influent COD of 1012 mg/L and maximum SCN^- degradation rate was 0.019 g/L.day at influent SCN^- of 210 mg/L in presence of phenol and NH_4^+–N. The COD to influent NH_4^+–N ratio in R3 decreased from 3.88–1.76 with increase in influent NH_4^+–N to R3. Total biomass observed during the study that increased from 11658 mg/L to 12900 mg/L with increase in influent COD and NH_4^+–N indicating the biomass was capable of tolerating the toxicity exerted by the pollutants in the present study. Suspended biomass concentration in R3 was observed to increase from 3000–4900 mg/L through out the study and attached biomass was 8600 mg/L up to influent NH_4^+–N 324 mg/L and then decreased slightly to 7900 mg/L when influent NH_4^+–N was maximum of 380 mg/L.

The influent phenol to R3 was only 2–15 mg/L with phenol loading of 0.001–0.01 g/L.day. Phenol removal in R3 was almost 50–93% releasing ~ 1 mg/L phenol in all the cases of

influent NH_4^+–N and it seemed that NH_4^+–N concentration up to 380 mg/L did not affect phenol degradation in R3 in present condition. Dyreborg and Arvin (1995) reported that pseudo–critical concentration of phenol was 3.7 mg/L for nitrifying bacteria.

Table 4.12 (b): Performance of R3 at feed NH_4^+–N concentration variation

NH_4^+–N	Phenol			COD			SCN^-			SO_4^{2-}				
S_0	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Gen	Th. SO_4^{2-}	Err
110	2	1 (0)	50	428	147 (7.5)	65.65	2	0 (0)	100	938	950 (10)	12	3	9
221	5	1 (0)	80	513	190 (12)	62.96	5	1 (0)	80	920	930 (15)	10	7	3
324	5	1 (0)	80	666	230 (13.4)	65.47	10	1 (0)	89	888	907 (11)	19	14	5
380	15	1 (0)	93	670	245 (13)	63.43	40	2 (0)	95	880	930 (15)	50	62	–8

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L), Err: Error (mg/L);
Th. SO_4^{2-}: Theoretical sulfate generation {1.65*(SCN–removed)};
Numbers in parenthesis indicate standard deviation values.

Influent thiocyanate to R3 was 2–40 mg/L and R3 efficiently removed influent thiocyanate releasing 0–2 mg/L thiocyanate in effluent. SCN^- loading to R3 was 0.001 to 0.026 g/L.day and removal efficiencies were 80–100% with effluent SCN^- from 0–2 mg/L. With increase in influent NH_4^+–N to 110–379 mg/L, SCN^- removal efficiency was not affected in R3 and it satisfactorily removed the SCN^-. High NH_4^+–N removal from high NH_4^+–N concentration suggested that there was very little or no inhibition by phenol, SCN^- and NH_4^+–N on nitrification and vice versa in present study. Maximum SCN^- degradation rate observed in R3 was 0.025 g/L.day at maximum loading of 0.026 g SCN^-/L.day. SCN^- degradation rates of 0.2 and 5.0 g/L.day, respectively in aerobic suspended growth and fluidized bed reactor were observed by Hung and Palvostathis, (1997) and Jeong and

Chung, (2006a). In a suspended growth aerobic reactor maximum SCN^- degradation rate was reported as 0.019 g/L.day at influent SCN^- of 210 mg/L in presence of phenol and NH_4^+–N by Vázquez et al. (2006a) which is close to SCN^- degradation rate observed in the present investigation. Banerjee (1996) observed maximum SCN^- degradation rate of 0.2 g/L.day in a rotating biological contactor in presence of phenol. However in present study R3 received very low amount of SCN^- than reported inhibitory value of 200 mg/L in its influent and successfully removed the same (Kim et al. 2008b).

In presence of varied influent NH_4^+-N, 3–62 mg/L sulfate evolved in R3 as byproduct from SCN^- biodegradation along with NH_4^+–N. During the study, sulfate generation was similar to the theoretical sulfate generation value with low error [Table 4.12 (b)].

4.1.4.4 Overall performance of three–stage CMBR at ammonia-N variation

The feed to R1 and final effluent of R3 was considered to estimate overall performance of three–stage CMBR system. It can be seen that phenol and SCN^- removals were complete and independent of feed NH_4^+–N concentration (Figure 4.31). COD removal was around 95–97% with effluent COD 147–245 mg/L (from influent 5400 mg/L) irrespective of influent NH_4^+–N concentration. NH_4^+–N removal in three–stage system was constant at 91–93% up to feed NH_4^+–N of 500 mg/L and decreased to 91% when feed NH_4^+–N was 600 mg/L. Total nitrogen (TN) in influent and effluent of three–stage CMBR system was estimated using equation 4.7 and feed TN were 1292, 1492, 1692 and 1692 mg/L at feed NH_4^+–N of 100, 300, 500 and 600 mg/L, respectively (considering influent NO_3^-–N of 1000 mg/L added in the recycle of R3). TN removal was almost 77–74% irrespective of feed NH_4^+–N. R2 and R3 competitively furnished denitrification and nitrification and resulted in stable total nitrogen removal as when there was higher nitrification or more NOx–N generation in R3 and the same was recycled to R2, denitrification occurred removing the NOx–N. Fraction of oxidized nitrogen increased the value of total nitrogen effluent in the final effluent. Present result shows that though increased feed NH_4^+–N concentration profoundly affected phenol and COD removal profile in R1, R2 strongly stabilized the system and overall phenol and COD removal by the system was 99 and 97–95%, respectively. Similarly overall SCN^- removal was always >99% by the CMBR

system as anoxic reactor was highly efficient and helped to improve the overall performance of the three–stage system.

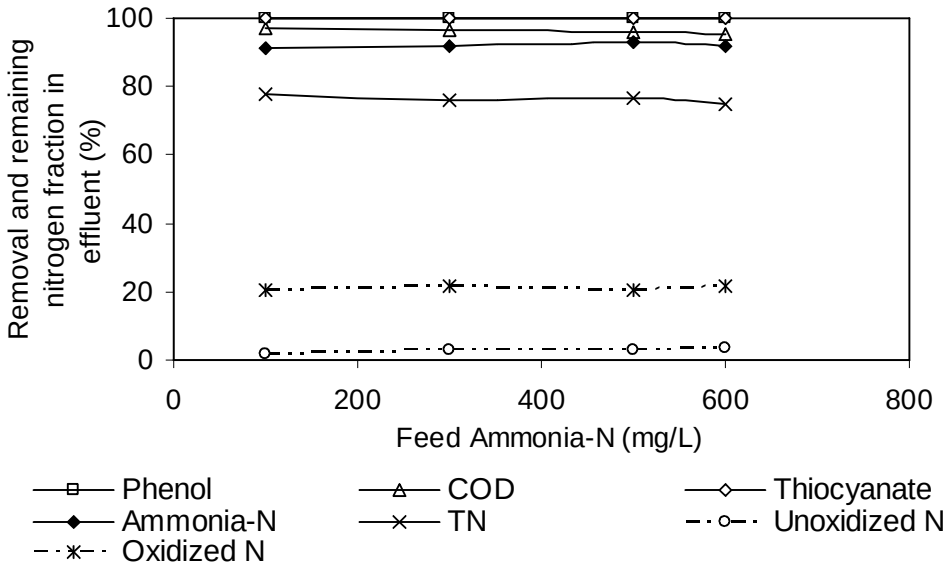

Figure 4.31 Overall performance of three stage CMBR at varied feed ammonia-N concentration

4.1.5 Summary of CMBR

4.1.5.1 Anaerobic CMBR (R1)

Acclimatization of anaerobic mixed culture with gradual increase to high influent concentration helped R1 to sustain toxic multi–components like phenol, thiocyanate and ammonia–nitrogen. Performance of CMBR was evaluated at varying concentrations of feed thiocyanate, phenol and ammonia–nitrogen and at varied HRT. In all studies, R1 showed removal of phenol and COD along with insignificant removal of thiocyanate and no removal of ammonia–nitrogen. Prolonged acclimatization did not improve thiocyanate and ammonia–nitrogen removal in R1. With introduction of thiocyanate, phenol and COD removals in R1 continuously decreased and specific methanogenic activity was nil when feed thiocyanate was higher than 200 mg/L. Phenol removal was comparatively higher (4–46%) than that of COD removal (2–33%) suggesting accumulation of intermediates. Maximum phenol removal rate observed in R1 was 0.399 g/L.day at phenol and SCN^-

loading of 1.250 g/L.day and 0.055 g SCN^-/L.day, respectively during overall study. In presence of high thiocyanate loading of ~0.270 g/L.day, phenol removal rate in R1 was low of ~0.15 g/L.day even at low phenol loading (0.333–0.833 g/L.day). Decreasing phenol loading at higher HRT also did not help to achieve high phenol removal rate in presence of high thiocyanate loading. With increase in feed phenol concentration, phenol and COD removals in R1 decreased, however the effect was less profound than affect of higher thiocyanate in feed. Phenol and COD removals in R1 decreased from 42% to 5% and 10% to 6%, respectively (decreased by 88% and 42%, respectively) with increase in phenol concentration 1000 mg/L to 2500 mg/L whereas with increase in influent thiocyanate from 110 to 600 mg/L phenol and COD removal decreased from 32% to 12% and 33% to 3%, respectively (decreased by 63% and 89%, respectively). Similarly increase in influent NH_4^+–N loading also exerted toxicity to phenol degraders though the affect was comparatively low to that of thiocyanate (phenol removal decrease by 50%). Therefore toxicity intense is SCN^-> phenol> NH_4^+–N to anaerobic phenol degrading microbes. At higher thiocyanate concentration more than 200 mg/L, biogas generation was absent and all COD removed might get incorporated to biomass. High amount of biomass concentration (8–9 g/L) was retained in sponge cube and higher ratio of attached to suspended biomass (10–8:1) was maintained in anaerobic reactor. No clogging or sludge rising effect was observed through out the study. Higher HRT of 2–3 days was observed as favorable for partial treatment of wastewater in anaerobic CMBR when high influent phenol and thiocyanate concentration are introduced in the reactor.

4.1.5.2 Anoxic CMBR (R2)

Anoxic reactor was prepared with mixed culture through acclimatization similar to R1. R2 received effluent of R1 containing SCN^-, phenol, COD and NH_4^+–N and recycle from R3. The recycle effect (recycle ratio of 1) decreased the toxicity of R1 effluent. As nitrate from recycle in R3 was inadequate based on stoichiometric requirement of COD and thiocyanate oxidation, nitrate was added externally to R2 during the study.

In R2, simultaneous degradation of phenol and thiocyanate occurred. Separate study confirmed that nitrate was essential for degradation of SCN^- in anoxic environment and 0.38 g NO_3^-–N was consumed for removal of each g of SCN^- and NH_4^+–N and sulfate

were end products. R2 was able to handle upto 0.4 g SCN^-/L.day without inhibition on removal of SCN^-. Maximum SCN^- removal rate observed in R2 was 0.265 g/L.day in presence of loadings of 0.277 g phenol/L.day, 0.267 g SCN^-/L.day and 0.178 g NH_4^+–N/L.day. It was observed that phenol loading in R2 above 0.5 g/L.day showed little inhibitory effect on thiocyanate removal rather than thiocyanate itself. The maximum SCN^- loading of 0.40 g/L.day did not show any inhibitory affect on phenol degradation and maximum phenol removal rate of 0.94 g/L.day was achieved at phenol loading of 1.61 g/L.day. NH_4^+–N removal in R2 was due to incorporation of nitrogen into biomass and it increased when phenol, thiocyanate and NH_4^+–N concentrations were low in influent. Sulfate balance between experimental and theoretical values showed higher error when thiocyanate removal rate was high, suggesting formation of other intermediate sulfur compounds. COD removal in R2 was 50–85% and maximum removal rate of 2.8 g/L.day was observed at loading of 5.4 g/L.day irrespective of phenol and SCN^-. The removal ratio of COD to NO_3^-–N was almost 3–7. In R2, with increase in influent COD, higher amount of COD removal was achieved and also more COD was channeled for synthesis of biomass. COD fraction for biomass ranged from 5% to 60%. With increase in influent thiocyanate suspended biomass concentration in R2 increased while attached biomass concentration decreased. Attached to suspended biomass concentration ratio significantly decreased from 8 to 3 with addition of increasing thiocyanate and remained 2–4 in rest of the studies with high influent thiocyanate. Nitrate–nitrogen removal was incomplete, though nitrite–nitrogen was exhausted completely. Maximum NO_x^-–N removal rate achieved in R2 was 0.61 g/L.day at maximum loading of 0.80 g nitrate/L.day at HRT of 0.75 day during HRT variation study. Sludge rising was regular in R2 but after releasing the entrapped gas the sludge was in normal mode.

Recommended HRT of R2 was 1–1.5 days for removals of 57–61% phenol, 61–70% COD, 57–69% thiocyanate, 67–70% COD and 3–6% NH_4^+–N from influent concentration of 822–1206 mg/L, 264–400 mg/L, 3815–4053 mg/L and 330–401 mg/L, respectively.

4.1.5.3 Aerobic reactor (R3)

R3 in the series was mainly placed for nitrification so that the nitrified effluent can be recycled to upstream R2 for denitrification and nitrogen removal from the system. Aerobic

mixed culture was acclimatized with ammonia and thiocyanate. R3 received low concentration of phenol and SCN^- in influent. Phenol removal was complete at the maximum loading of 0.669 g phenol/L.day. R3 remained unaffected with SCN^- load up to 0.122 g/L.day and releasing 1–3 mg/L SCN^-. Maximum SCN^- removal rate observed was 0.202 g/L.day. Residual phenol/COD/ thiocyanate were efficiently removed to the dischargeable level of ~1mg/L, ≤250 mg/L and ~1 mg/L in R3 through out the study at reactor HRT of 1 day or more.

R3 was mostly responsible for oxidation of NH_4^+–N to nitrate and nitrite. With increase in feed SCN^-/ NH_4^+–N, influent NH_4^+–N to R3 also increased as NH_4^+–N also evolved from thiocyanate degradation in R2 and R3. Maximum NH_4^+-N removal rate in R3 was 0.258 g/L.day at 1 day HRT, at NH_4^+–N loading of 0.358 g/L.day and further increase in NH_4^+-N loading resulted decrease in NH_4^+–N removal rate. Influent profile of NH_4^+-N, phenol and SCN^- in R3 greatly affected NH_4^+–N removal efficiency. Threshold phenol, thiocyanate and NH_4^+–N loading to achieve 80% NH_4^+–N removal efficiency in R3 were 0.450, 0.061 and 0.284 g/L.day, respectively. In R3, increase in pH was observed from 7.5 to 8.0 ± 0.4. Free ammonia 4-60 mg/L was observed which enhanced nitrite accumulation and affected NH_4^+–N removal in combination to higher concentration of phenol and thiocyanate.

Influent COD to R3 was quite high during high feed phenol, thiocyanate and low HRT studies with high value COD: NH_4^+–N ratio (3.7–4.4). Higher amount of biomass was observed in R3 in this condition. This might resulted in oxygen limitation to the biofilm located in the inner site of sponge cube and build up of anoxic/anaerobic environment. Reduction of some amount of nitrite/nitrate to nitrogen gas was responsible for rising of reactor pH and unaccounted nitrogen. In R3, at low thiocyanate in feed, higher amount of attached biomass was observed and with increase in feed thiocyanate suspended biomass concentration increased and also total biomass concentration increased. The attached to suspended biomass ratio decreased from 8 to 2–3 at high feed thiocyanate and remained 2–3 at other studies with high feed thiocyanate. Similar to R2, sulfate evolved in R3 from degradation of thiocyanate and no significant effect of phenol or HRT or NH_4^+–N was observed on sulfate generation other than thiocyanate loading.

4.2 PERFORMANCE OF SEQUENTIAL ANAEROBIC–ANOXIC–AEROBIC FED BATCH MOVING BED RECTOR (FMBR) SYSTEM

Fed batch moving bed reactor (FMBR) system consisting of sequential anaerobic (B1), anoxic (B2) and aerobic (B3) reactor was operated at varied feed concentration and operating conditions to evaluate the performance of the reactors. Feed thiocyanate, phenol and pyridine concentrations were varied at a time keeping other constituents and operating parameters constant. Similarly, reactor HRT, fill time and cycle time was varied at a time keeping feed concentrations constant. Dissolved oxygen concentrations (mg/L) in the bioreactors were: 0 (B1 and B2) and 3.5–4.0 (B3). Similar to CMBR, the FMBR system was also maintained at a constant temperature (30 ± 3 °C) using a temperature controlled blower. After each modification, the reactor attained steady state as characterized by consistency in effluent parameters, after a transient period of 10–12 days. Steady state data was collected for at least 10–15 days and analyzed to evaluate the performance of each reactor. The performance of the system is discussed below.

4.2.1 Performance of FMBR system at varied feed thiocyanate concentration

The study was conducted with synthetic feed containing varying thiocyanate concentration of 100, 200, 400 and 800 mg/L along with phenol (1500 mg/L) and NH_4^+–N (500 mg/L). NO_3^-–N (750 mg/L) was added in recycle of B3. Feed COD was in the range of 4400–5400 mg/L during the study. The experiment was carried out for almost 188 days (day 200th to 388th day). Feed pH was maintained at 7.5 ± 0.2 by using phosphate buffer (KH_2PO_4 72.3 g/L and K_2HPO_4 104.5 g/L). Yeast extract of ~20 mg/L and trace metals solution of 1 mL/L feed were added as nutrients. Total system HRT was 10 days (HRT of B1: 5 days; B2: 2.5 day and B3: 2.5 day).

4.2.1.1 Performance of anaerobic FMBR (B1) at varied feed thiocyanate

Steady state performance of B1 is shown in Table 4.13 and Figure 4.32 describes the pollutant profiles during the study. The reactor removed negligible amount of thiocyanate 12–0.75% with increasing influent of 100–800 mg/L. The microbes were not efficient in degrading thiocyanate in anaerobic condition like R1.

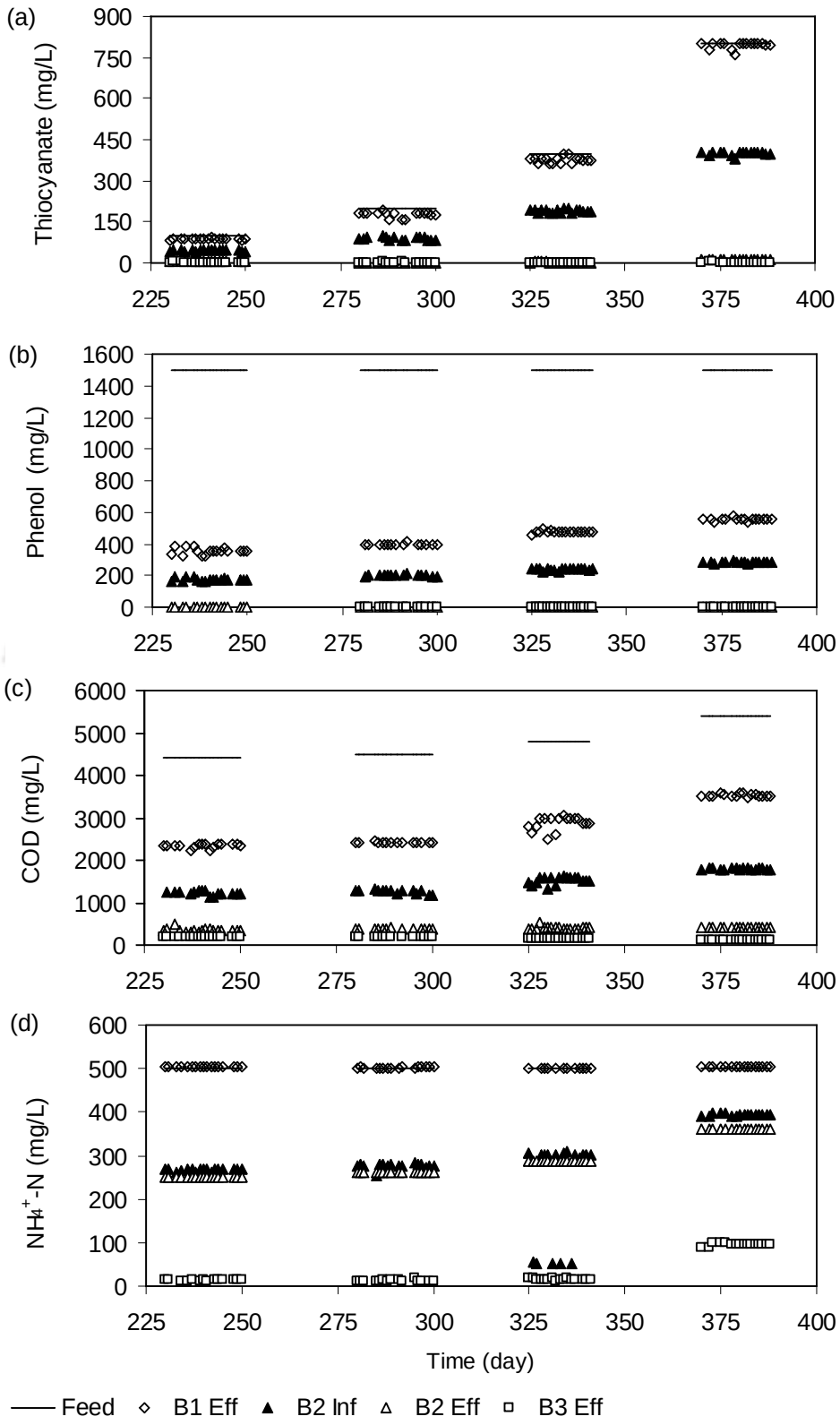

Figure 4.32 Feed and pollutant profile in FMBR during thiocyanate variation study

With increase in feed SCN^- from 100 to 800 mg/L phenol removal efficiency and removal rate in B1 decreased linearly from 77 to 63% (decrease by 18%) and 0.230 to 0.188 g/L.day, respectively (Figure 4.33), suggesting SCN^- had inhibitory effect on phenol degrading microbes.

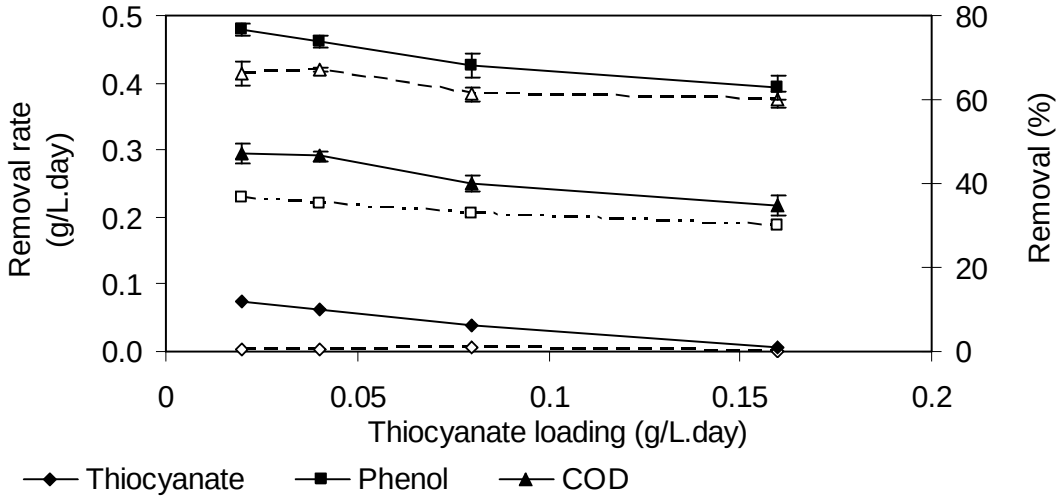

Figure 4.33 Performance of B1 as pollutants removal (solid line) and removal rate (dotted lines) at varied thiocyanate loading

. **Table 4.13: Performance of anaerobic FMBR (B1) at feed thiocyanate concentration variation**

SCN^-			Phenol		COD			NH_4^+–N	Gas mL/h	pH	SMA	TVS (mg/L)	VFA
S_0	S_e	Rem	S_e	Rem	S_0	S_e	Rem	S_e		S_e			
100	88 (3.9)	12	350 (21)	76.7	4400	2328 (49)	47.0	510	85	6.8± 0.2	0.029	10604	625 (40)
200	180 (9.6)	10	393 (30)	73.8	4500	2405 (16)	46.0	510	50	6.8± 0.2	0.028	10345	740 (48)
400	376 (14)	6	480 (9.4)	68.0	4800	2885 (183)	40.0	510	0	6.8± 0.2	ND	10740	630 (35)
800	794 (12)	0.75	558.6 (9.5)	62.7	5400	3527 (33)	34.6	510	0	6.8± 0.2	ND	10126	700 (58)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%); Feed phenol: 1500 mg/L; NH_4^+–N 500 mg/L; SMA: specific methanogenic activity (g CH_4–COD/ g VSS. day)

Numbers in parenthesis indicate standard deviation values

B1 was capable of removing 34–47% of influent COD 4400–5400 mg/L in presence of 100–800 mg SCN^-/L. Similar to phenol, removal of COD in B1 also decreased from 47% to 34% (decrease by 26%) with increase in influent SCN^- showing decrease COD removal rate of 0.375–0.414 g/L.day. The COD removal efficiency/ rate were mostly affected by thiocyanate loading above 0.04 g/L.day. Contribution of B1 in phenol and COD removal is significantly high compared to B2 and B3 [Figures 4.34 (a) & (b)]. The effluent NH_4^+–N of B1 (510 mg/L) was always higher than the influent NH_4^+–N concentration (500 mg/L). The possible reason may be ammonia generated from thiocyanate degradation. In B1, feed pH decreased from 7.5 to 6.8 ± 0.2.

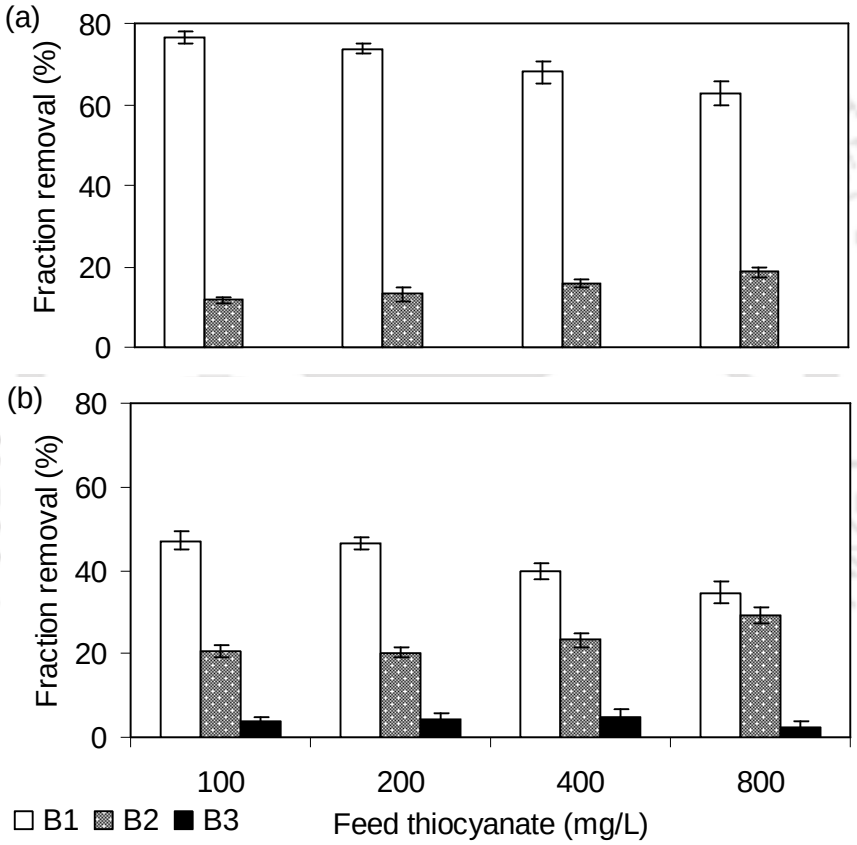

Figure 4.34 (a) Phenol (no phenol entred to B3) and (b) COD removal by B1, B2 and B3 at varied feed thiocyanate concentration

B1 sludge shows specific methanogenic activity only when feed SCN^- was 100–200 mg/L. Activity of 0.0285 and 0.0278 gCH_4–COD/ gVSS.day were recorded, respectively at feed SCN^- of 100 mg/L and 200 mg/L. No SMA was detected when thiocyanate in feed was 400 and 800 mg/L. The SMA values from B1 were found to be lower to that of SMA value of

R1 culture. In the present study, the higher thiocyanate concentration was found to have direct effect on biogas production as biogas generation was 50–85 mL/h in presence of 100 and 200 mg/L SCN^-, respectively and beyond it was absent. Biomass yield was 0.1-0.7 gVSS/gCODremoved /day. Biomass concentration through out the study was almost same at ~10000 mg/L. The ratio of attached biomass to suspended biomass decreased from 10.9 at 100 mg/L feed thiocyanate to 7.3 and 9.9 at higher feed SCN^- of 400 and 800 mg/L, respectively. Volatile fatty acid (VFA) concentration was 625–740 mg/L as acetic acid through out the study.

4.2.1.2 Performance of anoxic FMBR (B2) at varied influent thiocyanate

Influent to B2 constituted of effluent of B1 that got diluted 50% with recycle from B3 (equation 3.4) and this resulted in great decrease of toxicity from the individual pollutant and aided the reactor to perform efficiently. B2 was operated at HRT of 2.5 day and the average performance of B2 is presented in Tables 4.14 (a) and (b). Influent SCN^- concentration to B2 increased from 45 mg/L to 398 mg/L with increase in feed SCN^-. The corresponding loadings to B2 were 0.018, 0.036, 0.075 and 0.16 g SCN^-/L.day at feed SCN^- concentration 100, 200, 400 and 800 mg/L, respectively. Similar to R2, B2 showed removal of SCN^- in anoxic condition with generation of sulfate and ammonia–nitrogen and responsible for SCN^- removal in principal [Figure 4.35]. Thiocyanate removal efficiency of B2 was 91–98% and SCN^- removal rate in B2 increased linearly from 0.016 to 0.154 g/L.day with a slope of 0.98 with increase in loading [Figure 4.36 (a)]. Maximum SCN^- removal rate achieved in B2 was 0.154 g/L.day at loading of 0.16 g SCN^-/L.day. In CMBR system, in R2 maximum SCN^- removal rate was 0.165 g/L.day from thiocyanate loading 0.288 g/L.day during thiocyanate variation study. Maximum removal of 98% was achieved when influent SCN^- to B2 was up to 189 mg/L (when feed SCN^- was 400 mg/L) and SCN^- removal decreased to 96% at further increase in influent thiocyanate to 398 mg/L. B2 was responsible of 40–48% of total SCN^- removal which was higher than B1 and B3 through out the study [Figure 4.35].

B2 received 175–279 mg/L phenol in influent as major portion of phenol was removed in upstream reactor B1. Phenol loading rate was 0.07, 0.08, 0.096 and 0.112 g/L.day in presence of 44, 91, 189 and 398 mg/L influent SCN^-, respectively. B2 removed more than

99% influent phenol releasing ~1 mg/L phenol in effluent. Increased phenol removal rate 0.07–0.110 g/L.day was observed in presence of increased influent SCN^-.

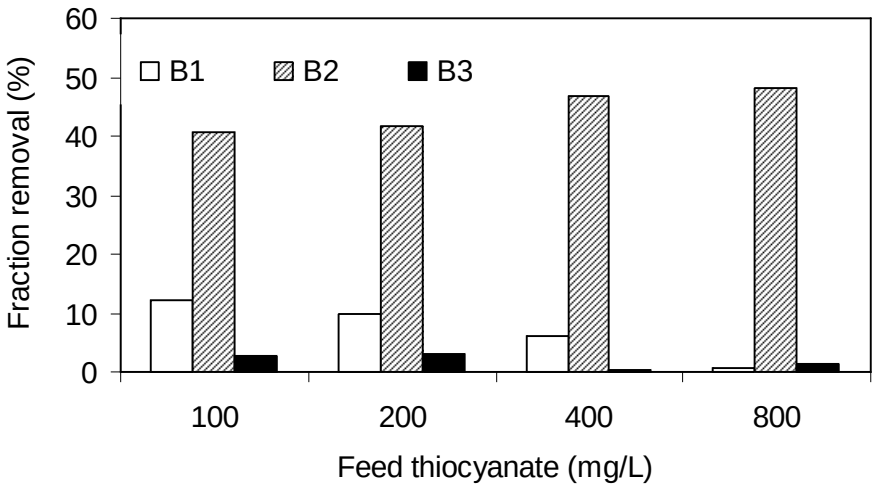

Figure 4.35 Thiocyanate removal by B2 and B3 at varied feed thiocyanate concentration

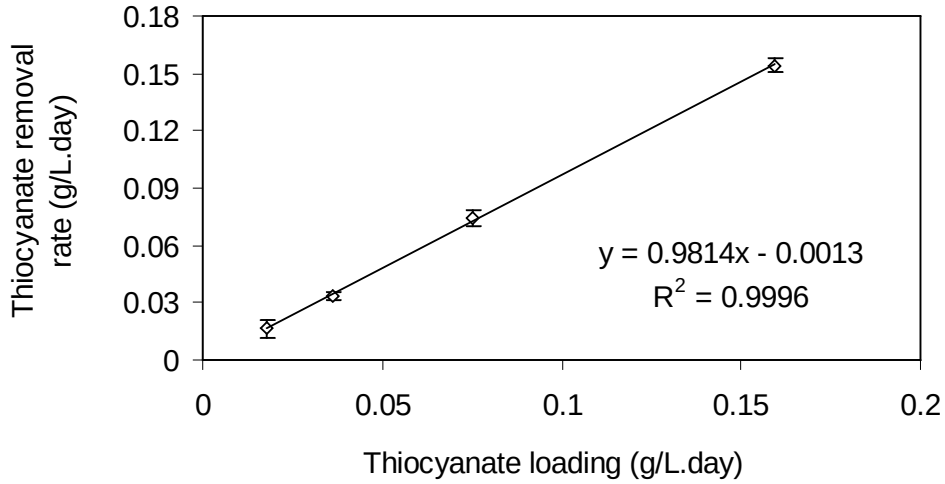

Figure 4.36 (a) Effect of thiocyanate loading on thiocyanate removal rate in B2

Influent COD to B2 was 1253–1823 mg/L with increased COD loading of 0.50–0.73 g/L.day. B2 showed increased COD removal efficiency of 70–86% with increase in influent SCN^-. Figure 4.36 (b) shows that COD removal rate in B2 increased from 0.361 to 0.633 g/L.day with increase in influent COD loading. B2 contributed 12–18% and 20–29% of total phenol and COD removal, respectively that increased with increased SCN^-, indicating thiocyanate inhibition on B2 in terms of phenol or COD removal was nil [Figure

4.34 (a) and (b)]. This was probably due to diluted concentration of SCN^- in influent as compared to B1.

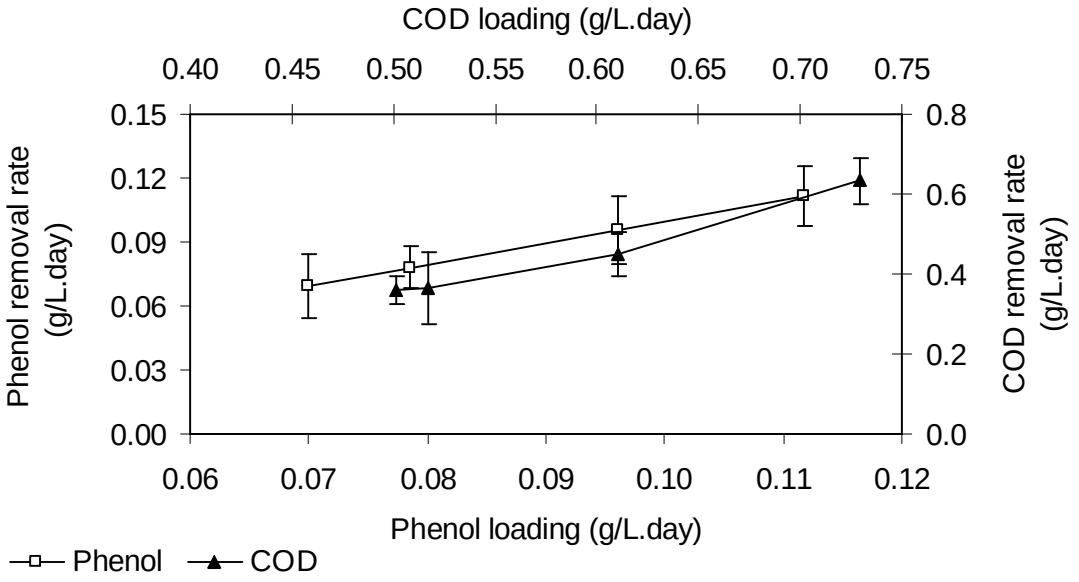

Figure 4.36 (b) Phenol and COD removal rates in B2

Table 4.14 (a): Performance of anoxic FMBR (B2) at feed thiocyanate variation

Thiocyanate			Phenol			COD			NH_4^+–N			TVS	pH
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem		
44.6	4.0 (1)	90.9	175.5	1	99.43	1253	350 (55)	72.06	271	250 (0)	7.8	10580	8.4
91	7.9 (1)	91.33	196.5	1	99.49	1293	380 (9)	70.62	282	260 (5)	7.9	11120	8.5
189	2.2 (0.5)	98.82	240	1	99.58	1527	405 (39)	73.47	308	288 (0)	6.4	12300	8.3
398	12 (0)	96.9	279.3	1	99.65	1823	240 (0)	86.8	395	360 (0)	8.9	13580	8.4

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B2 = {Effluent NH_4^+–N of (B1+B3)/2 + 0.24x (SCN^- removed in B2)}.

TVS: Biomass as Total volatile solids in sponge + suspension, (mg/L);

Numbers in parenthesis indicate standard deviation values.

Influent NH_4^+–N in B2 was 271–395 mg/L along with NH_4^+–N generated from degradation of SCN^- (0.24 mg ammonia from 1 mg SCN^-) in B2. At higher SCN^- loading

in B2, with higher SCN^- degradation more NH_4^+–N generation occurred and resulted in higher NH_4^+–N concentration. B2 removed 6.4–8.9% NH_4^+–N releasing higher NH_4^+–N concentration of 280–360 mg/L in effluent towards higher influent thiocyanate study.

The mixed liquor from B3 was added with external 750 mg/L NO_3^-–N and recycled to B2 to give complete anoxic environment. The influent NO_3^-–N and NO_2^-–N to B2 was 627–580 mg/L and 22–43 mg/L, respectively resulting total NOx–N loading rate 0.249–0.260 g/L.day in B2. B2 showed almost complete nitrite removal though nearly 249–370 mg/L nitrate–nitrogen remained in effluent. NOx–N removal increased from 43–60% with increase in influent SCN^-. NOx–N removal rate also increased from 0.112 to 0.149 g/L.day with increase in SCN^- loading (Figure 4.37). The main cause of incomplete denitrification in B2 might be due to lower availability of carbon source as compared to NO_3^-–N (Tam et al. 1992). However in the present study NOx–N removal was the main process of nitrogen removal from the system as contribution of B2 was significantly higher in total nitrogen removal being 24–29% compared to B3 (Figure 4.38).

Table 4.14 (b): Performance of anoxic FMBR (B2) at influent thiocyanate variation

SCN^-	NO_3^-–N		NO_2^-–N		NO_x^-–N	COD: N_{rem}	COD_B	SO_4^{-2}				
S_0	$S_0^{\#}$	S_e	S_0	S_e	Rem			S_0	S_e	Gen	Th SO_4^{-2}	Err
44.6	627	370 (36)	22	1 (0.5)	42.9	3.4	15	80	150 (3)	70	67	3
91	599	340 (37)	27	1.4 (0.2)	45.5	3.4	16	160	305 (19)	145	137	8
189	597	310 (23)	40	1.0 (0)	51.2	3.9	27	265	520 (38)	255	308	-53
398	580	249 (34)	42	0 (0)	60.0	5.8	51	620	1180 (37)	560	636	-76

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);

COD_B: COD fraction (%) for biomass;

#: NO_3–N 750 mg/L was added externally in the recycle from B3.

Th SO_4^{-2}: Theoretical sulfate generation (mg/L); Err: Error (mg/L)

Numbers in parenthesis indicate standard deviation values

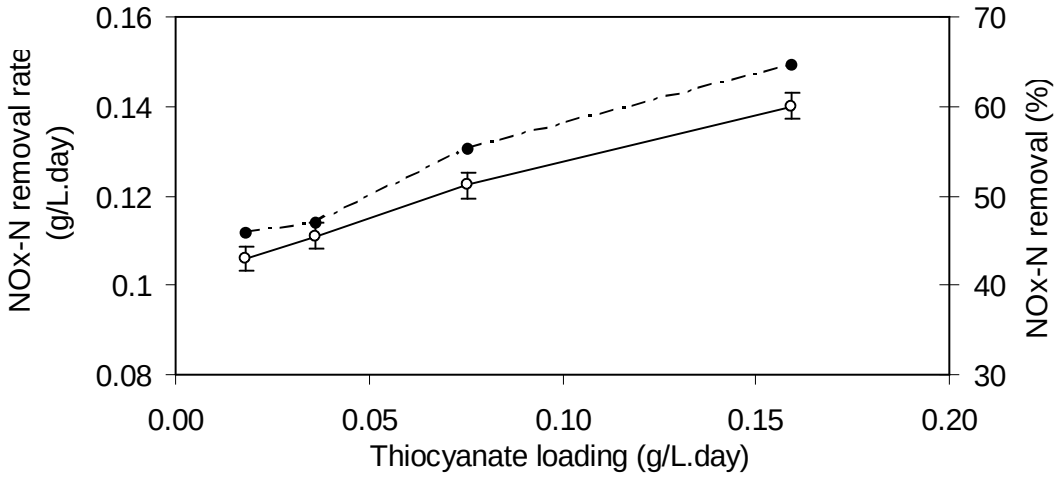

Figure 4.37 NOx-N removal (solid line) and removal rate (dotted line) in B2 at varied SCN^- loading

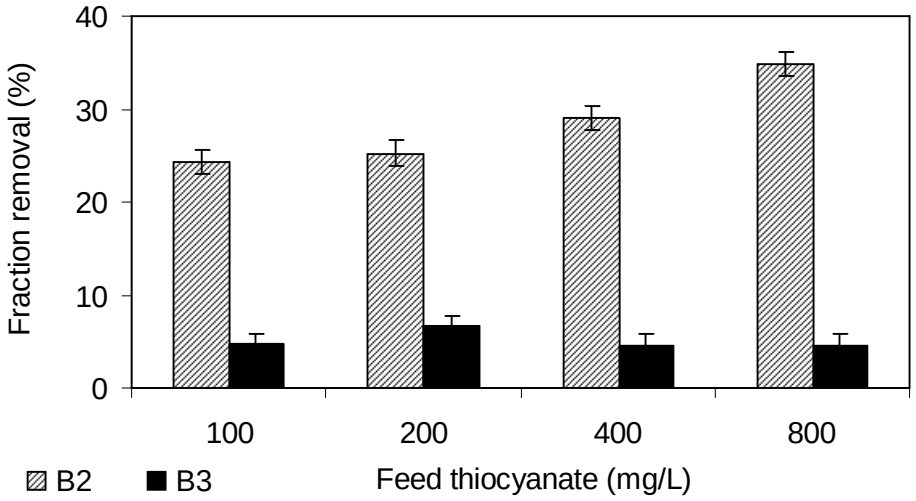

Figure 4.38 Nitrogen removal by B2 and B3 at varied feed thiocyanate concentration

In the present study COD/N_{rem} ratio calculated using equation 4.5 was 3.4–5.8 and found to increase with influent SCN^- in B2 and increased COD removal [Table 4.14 (b)]. By dividing with stoichiometric ratio 2.86 (Sarfaraz et al. 2004; Zhu et al. 2006), it was found that 52–85% of removed COD was utilized for nitrate reduction and remaining 15–51% COD was utilized for biomass synthesis in B2. Considering 1.42 as COD of the biomass ($C_5H_7NO_2$), the observed yield of biomass in B2 was 0.10–0.35 (Metcalf and Eddy, 2003). At influent SCN^-concentration 44 mg/L, suspended biomass in B2 was 1400 mg/L and increased to 3200–4000 mg/L at influent SCN^- 91 mg/L or above. Attached biomass

concentration initially decreased from 9100 to 7800 mg/L when influent SCN^- increased from 44 to 91 mg/L and then gradually increased to 8000–9780 mg/L in presence of 190–398 mg/L SCN^-. The ratio between attached to suspended biomass decreased from 6.4 to 2–2.5 with increase in with increased suspended biomass in liquor at high influent SCN^-. Total biomass concentration measured found to increase from 10.5 g/L to 13.5 g/L in presence of higher SCN^- concentration.

Nearly 70 to 560 mg/L sulfate generation occurred in B2. Increased sulfate concentration was observed in effluent with increased SCN^- removal in B2. However sulfate generation at higher influent thiocyanate concentration was lower to theoretical value and showed higher difference as was observed in case of R2 in CMBR with higher thiocyanate removal rate which might be due to accumulation of other intermediate compounds like thiosulfate and polysulfides etc.. The difference between experimental sulfate generation and theoretical sulfate generation is presented as error in Table 4.14 (b).

4.2.1.3 Performance of aerobic FMBR (B3) at varied influent thiocyanate

Influent SCN^- to B3 was only 4–12 mg/L and loading rate was 0.0016–0.0048 g/L.day. Average performance of B3 is given in Tables 4.15 (a) and (b). Almost 69–89% SCN^- removal occurred in B3 releasing 1–2 mg/L in the effluent. No phenol entered in B3 as phenol removal was almost complete in upstream reactor B1 and B2. Residual 240–405 mg/L COD released from B2 entered to B3 and loading was 0.096–0.162 g COD/L.day. B3, released 120–178 mg/L of COD in effluent. The COD removal rate in B3 was 0.048–0.095 g/L.day and independent of thiocyanate loading to B3 (Figure 4.39). Contribution of B3 in COD removal was very less (only 2–5% of total COD removed) [Figure 4.34(b)] and probable reason was that the biodegradable organics COD was already removed by upstream B1 and B2 releasing the unfavorable parts for B3.

With increase in feed SCN^-, more amount of NH_4^+–N was generated due to degradation of SCN^- in B2 and influent NH_4–N to B3 also increased [Table 4.15 (a)]. Influent NH_4^+–N concentration to B3 was 250–362 mg/L with corresponding loadings of 0.100–0.145 g/L.day. B3 removed nearly 94–95% of NH_4^+–N from influent concentration 250–289 mg/L and released only 13–16 mg/L NH_4^+–N in effluent. The removal efficiency decreased to 74% with effluent NH_4^+–N of 95 mg/L from influent of 362 mg/L.

Table 4.15 (a): Performance of aerobic FMBR (B3) at feed thiocyanate concentration variation

Thiocyanate			COD			NH_4^+–N			pH	COD: NH_4^+–N	TVS (mg/L)
S_0	S_e	Rem	S_0	S_e	Rem	S_0#	S_e	Rem	S_e		
4.05	1.23 (0.6)	69.56	350	178 (13)	49.10	250.6	13 (2.4)	94.8	7.8	1.39	11318
7.90	2.00 (1.2)	74.68	380	182 (7)	52.11	261.4	15 (5)	94.2	7.8	1.45	11791
2.23	1.35 (0.5)	83.13	405	168 (11)	58.52	289.6	16 (3)	94.5	7.8	1.39	12219
12.00	1.28 (1)	89.33	240	120 (0)	50.00	362.6	95 (3)	73.8	8.3	0.70	11974

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

Influent NH_4^+–N of B3 = {Effluent NH_4^+–N of B2 + 0.24x (SCN^- removed in B3)}.

Numbers in parenthesis indicate standard deviation values

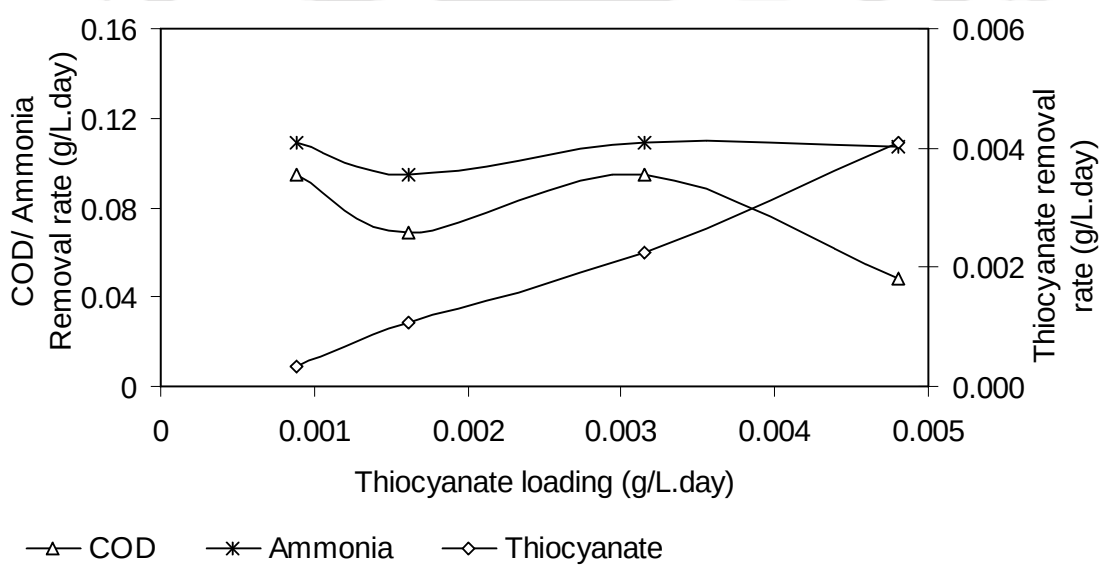

Figure 4.39 Pollutant removal rate in B3 at varied Thiocyanate loading

Figure 4.40 shows that maximum NH_4^+–N removal rate achieved in B3 was 0.107 g/L.day at NH_4^+–N loading of 0.145 g/L.day. At this condition thiocyanate loading was 0.0048

g/L.day and phenol was absent in B3. Though ammonia removal by nitrification is reported to be inhibited by phenol and thiocyanate, in the present study the possibility was less as influent phenol was nil and thiocyanate were very low to cause inhibition. Less ammonia removal of 74% at influent NH_4^+–N of 362 mg/L was probably due to substrate inhibition, since the threshold value to cause substrate inhibition is reported to be 350 mg/L (Kim et al. 2008b). During thiocyanate variation study in CMBR, it was observed that maximum NH_4^+–N removal rate achieved in R3 was 0.26 g/L.day at NH_4^+–N loading of 0.358 g/L.day in presence of SCN^- loading of 0.122 g/L.day and phenol loading of 0.468 g/L.day. This shows that R3 was more efficient in terms of NH_4^+–N removal than B3 in presence of phenol and thiocyanate.

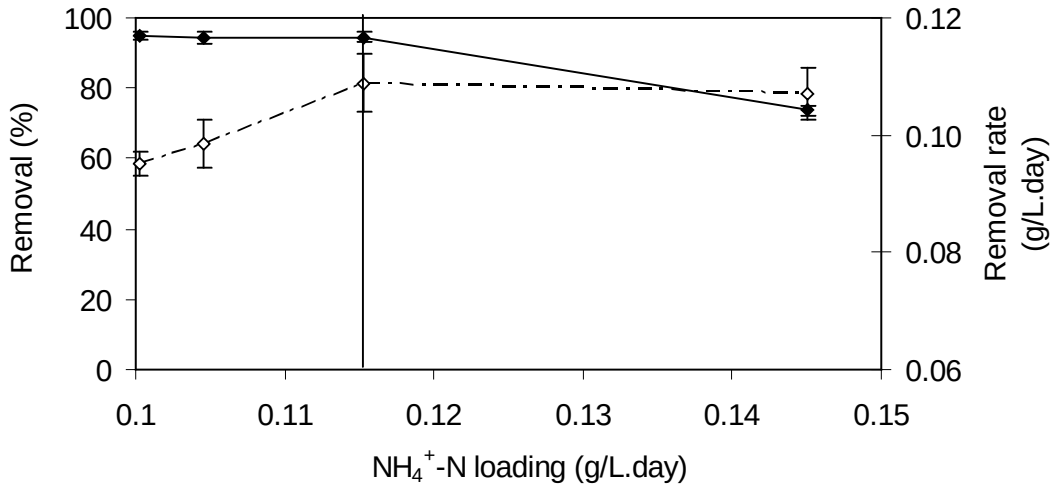

Figure 4.40 NH_4^+-N removal (solid line) and removal rate (dotted line) in B3 at varied NH_4^+-N loading

Oxidation of NH_4^+–N occurred in B3 and 370–505 mg/L nitrate and 45–85 mg/L nitrite got released in effluent [Table 4.15 (b)]. Nitrification rate in B3 was 0.064– 0.085 g/L.day that was less than that of R3 (HRT of R3 was 1 day). B3 showed high accumulation of NO_2^-–N in its effluent which ranged from 45–85 mg/L. In the present study free ammonia calculated using equation 4.6 was 6.8–11.8 mg/L which was suspected to be responsible for nitrite accumulation in B3 (Yang et al. 2004). Unaccounted nitrogen was 18–31% and found to be higher during low influent NH_4^+–N to B3, might be due to incorporation of NH_4^+–N to biomass or denitrification (Helmer et al. 1999). Influent COD to NH_4^+–N ratio was low at ~1.4 during feed SCN^- 100–400 mg/L and decreased to 0.7 when feed SCN^-

was 800 mg/L as during this condition high influent NH_4^+–N entered B3. There was no significant change in suspended and attached biomass profile in B3 with increased feed thiocyanate as very low amount of thiocyanate (4–12 mg/L) entered B3. Suspended biomass slightly increased from 2300 mg/L to 2780 mg/L and attached biomass was 8900–9400 mg/L and the ratio of attached to suspended biomass was 3.4–3.8 during the study. Total biomass concentration was 11300–12200 mg/L. Nearly 10–60 mg/L sulfate generation occurred in B3 that exceeded theoretical sulfate generation [Table 4.15 (b)]. This might be because of low sulfate detected in B2 than theoretical generation during the study that enhanced conversion of more sulfate in B3.

Table 4.15 (b): Performance of aerobic FMBR (B3) at feed SCN^- variation

SCN^-	NO_3^-–N		NO_2^-–N	N_R	SO_4^{-2}					FA	UN
S_0	S_0	S_e	S_e		S_0	S_e	Gen	Th SO_4^{-2}	Err		
4.05	370	505 (37)	45 (4)	0.072	150	160 (4.6)	10	5	5	6.8	22
7.90	340	448 (23)	55 (2.5)	0.064	305	320 (17)	15	10	5	7.1	31
2.2	310	444 (41)	80 (8)	0.085	520	530 (26)	10	1	9	7.8	19
12.00	249	370 (15)	85 (6)	0.082	1180	1240 (18)	60	18	42	11.8	18

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L); Err: Error: (mg/L)

Th SO_4^{-2}: Theoretical sulfate generation (1.65x SCN^- removed in B3);

N_R: Nitrification rate (g/L.day);

FA: Free ammonia (mg/L);

UN: Unaccounted nitrogen (%);

Numbers in parenthesis indicate standard deviation values

4.2.1.4 Overall performance of FMBR at varied feed thiocyanate

The initial synthetic feed to B1 and final effluents from B3 were considered for overall performance evaluation of the fed batch MBR system in the study. Effect of feed

thiocyanate on removal of SCN^-, phenol and COD were insignificant up to 800 mg/L thiocyanate in feed. Overall removal of SCN^-, phenol, NH_4–N and COD were 98.8–99.8%, 99.9%, 86—97% and 96–98%, respectively (Figure 4.41). Overall COD and SCN^- removal rate increased with increase in feed SCN^-. Total nitrogen in the system was calculated using equation 4.7 and it found to range from 1273 mg/L to 1441 mg/L in the influent and total nitrogen removal was 56–60% with increase in thiocyanate in the feed. Unoxidized nitrogen in the effluent was 1–6% whereas oxidized nitrogen 31–43% being higher during low feed thiocyanate and low influent NH_4^+–N to B3 as there was higher effluent NOx–N during this stage. Effluent NOx–N concentration in final effluent of FMBR (B3) was comparatively higher than CMBR (R3) system.

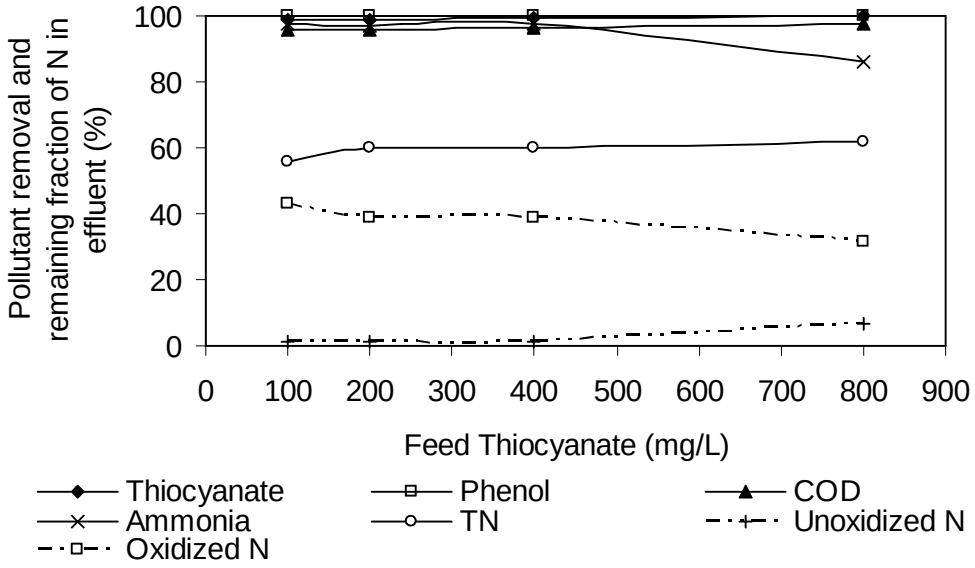

Figure 4.41 Performance of sequential FMBR at varied feed thiocyanate concentration

4.2.2 Performance of FMBR system at varied fill time

Fill time (t_f) is an important parameter regulating the intensity of exposure of influent to reactor microbes and influencing the total reaction time. Fill time in B1 was varied from 1 hour to 3.7 h regulating the influent flow rate 13.33–33.33 mL/min. In B2 and B3 same flow rate was maintained and fill times were 2–7.4 hour each, double to B1 as double

volume of influent to B2 and B3 were added and reactor HRT of each was half of B1. Fresh feed was prepared every day by dissolving phenol, thiocyanate and NH_4^+–N of 1500, 800, and 500 mg/L, respectively. Another study at instantaneous fill was carried out lowering the feed thiocyanate concentration to 100 mg/L to avoid strong toxic affect and damage to biomass, keeping other constituents constant. The experiment was executed for almost 230 days from 389th to 620th day. Performance of FMBR system at varied fill time is discussed in this section as per individual reactor.

4.2.2.1 Performance of anaerobic FMBR (B1) at varied fill time

Average performance of B1 at various fill time (t_f) is given in Table 4.16. Fill times were 1, 1.5, 2.5 and 3.7 h. With increase in fill time from 1 to 1.5 h, phenol removal in B1 decreased consistently from 82% to 70% and then decreased to 62% with further increase in fill time to 2.5–3.7 h. Similarly, COD removal was 70% at fill time 1–1.5 h and decreased to 53% (decrease by 24%) when fill time was increased to 2.5 and 3.7 h (Figure 4.42). Removal of SCN^- was very low (0.625%) up to filling time of 2.5 h and decreased further when filling time increased to 3.7 h. No removal of NH_4^+–N was observed in B1 during fill time variation.

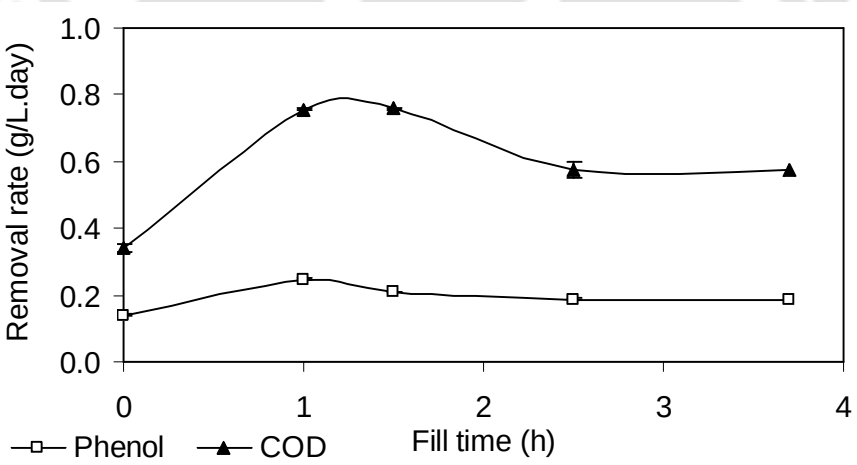

Figure 4.42 Effect of fill time on phenol and COD removal rate in B1

Another study was done at instantaneous fill time (1 min). In order to avoid toxicity of feed SCN^-, its concentration was now decreased to 100 mg/L. Performance of B1 was even lower than the longest fill time of 3.7 h (with removal of phenol 46%, COD 38% and no

removal of SCN^- and NH_4^+–N). Phenol removal rate increased from 0.14 g phenol/L.day to 0.248 g phenol /L.day when fill period was increased from instant to 1 h and remained constant. COD degradation rate also initially increased from 0.34 g COD/L.day at instant fill to 0.76 g COD/L.day at fill time 1–1.5 h and remained constant at 0.575–0.572 g/L.day when $t_f \geq 2.5$ h.

Table 4.16: Performance of anaerobic fed batch MBR (B1) at fill period variation

Fill Time (h)	SCN^-			Phenol			COD			pH	TVS (mg/L)
	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem		
1	800	795 (0)	0.625	1500	259 (7)	82.73	5400	1614 (24)	70.11	6.9	10425
1.5		795 (0)	0.625		450 (0)	70.00		1600 (5)	70.37	6.9	10538
2.5		795 (12)	0.625		558 (5)	62.70		2527 (33)	53.20	6.9	10395
3.7		798 (0)	0.25		560 (0)	62.67		2541 (5)	52.94	6.9	10450
Instant	100	100 (0)	0		800 (12)	46.67	4500	2800 (51)	37.78	6.8	10384

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),
Numbers in parenthesis indicate standard deviation values.

Figure 4.43 shows that fractional phenol and COD removals in B1 were higher than B2 throughout the fill variation study though with decreasing trend. This result suggests that longer fill time has detrimental affect on performance of anaerobic reactor treating toxic and inhibitory wastewater. It is obvious that during longer fill time feed flow rate (Q) to reactor decreased since same amount of feed was delivered to B1 for longer duration. Table 3.3 also shows that feed flow rate (Q) to B1 was only 9 mL/min at fill time of 3.7 h and increased to 33.3 mL/min at fill time of 1 h, suggesting that mass of substrate ($Q.S_0$, where S_0 is influent substrate concentration in mg/L) entering in the reactor was much less

during longer fill time. Thus toxicity of influent substrate on biomass was much less at longer filling time.

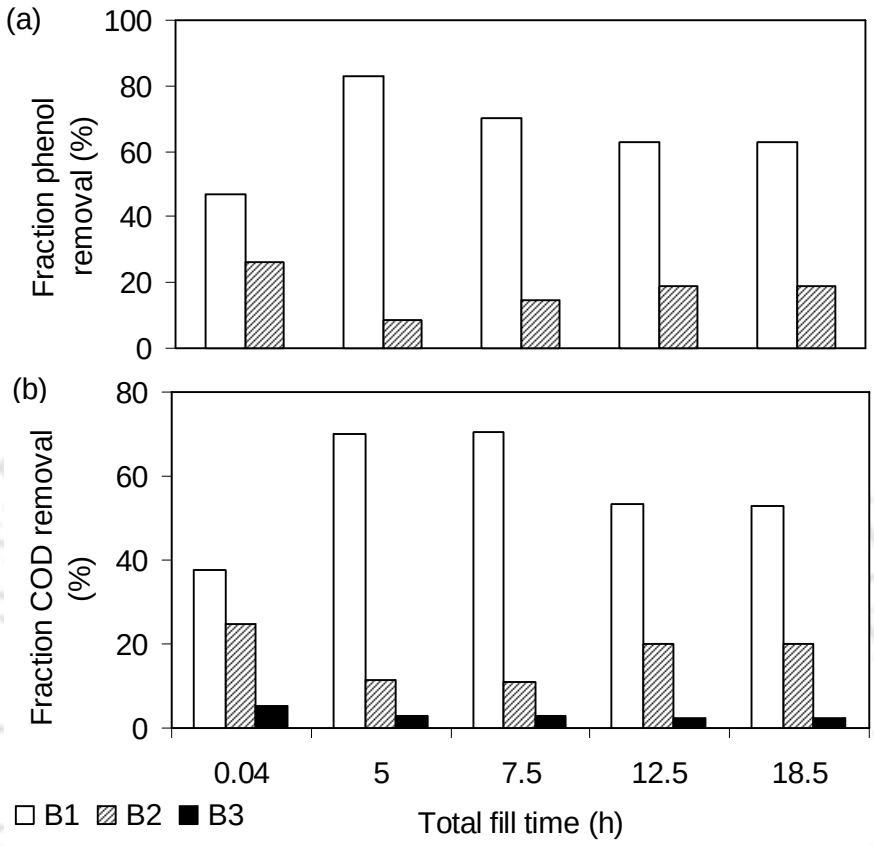

Figure 4.43 Fraction (a) Phenol and (b) COD removal by B1, B2 and B3 at varied Fill time

In a fed batch reactor the cycle time (t_c) is constituted by fill time (t_f), reaction time and decantation (t_d) time as shown in equation 4.9.

Cycle time (t_c) = $t_f + t_r + t_d$ (Eq. 4.9)

As shown in Table 3.3, at a constant cycle time of 24 h and decant time of 15 min, reaction times in B1 were 22.75, 22.25, 21.25, 20.05 h and 23.7 h at fill times of 1, 1.5, 2.5, 3.7 h and instant fill, respectively. At longer fill probably the less available contact time/reaction time between biomass and substrate was responsible for poor performance of B1. During instant fill substrate was delivered to B1 as pulse input, so concentration peak of substrate was very high and anaerobic biomass was suddenly exposed to very high concentrations of toxic substances due to high value of QS_0 (even though influent SCN^- was much less) and

could not withstand the toxicity, though the reaction time was the maximum (23.7 h). Present results shows that in anaerobic fed batch reactor handling toxic wastewater, gradual short fill was the best and gradual long fill did not provide any additional benefit. This result shows that optimization of filling time is necessary in order to obtain the efficient performance of the reactor. Previous literatures reported that longer fill time was extremely beneficial to anaerobic fed batch reactor operation for simple and rapidly acidified organic substrates like glucose, sucrose, acetate etc. (Shizas and Bagley, 2002). 2009). Ratusznei et al. (2003) reported that anaerobic fed batch reactor while treating low strength wastewater showed higher COD removal efficiency and more stability at fill time of 0.5 h as compared to 1 h and 3 h. Damasceno et al. (2007) reported 85–95% and 80–87% of total COD removal at organic loading of 2–4 g COD/L.day at 2 and 4 h fill time, respectively in an anaerobic sequencing batch biofilm reactor (ASBBR) operated at fixed 8 h cycle time. However, the reactor showed better performance at longer fill time 4 h, when higher loading of 8–12 g COD/L.day was applied. No literature report is available on effect of fill time on performance of anaerobic fed batch MBR treating phenol, thiocyanate and ammonia containing wastewater.

In B1, no gas production was observed throughout this study, which was probably due to effect of high concentration of thiocyanate in feed. Similar result was observed in anaerobic–anoxic–aerobic continuous system at feed SCN^- at or above 400 mg/L. In B1, suspended biomass concentration was more or less same being 890–970 mg/L during the fill variation study and total biomass was 10.5 g/L with attached to suspended biomass ratio of 10.7–9.8. No significant change was observed on biomass concentration in B1 during fill variation study.

4.2.2.2 Performance of anoxic FMBR (B2) at varied fill time

Average performance of B2 at varying fill time (2–7.4 h) is given in Tables 4.17 (a) and (b). Influent phenol concentrations were 129–280 mg/L during gradual fill times and highest influent phenol concentration of 400 mg/L entered at instant fill. Phenol loading rate in B2 was 0.052–0.112 g/L.day during gradual fill and 0.16 g/L.day at instant fill. Almost complete phenol removal of 99–100% was achieved in B2 during gradual filling which was 8–18% of total phenol removal [Figure 4.43 (a)]. Phenol removal rate also

increased with increase in fill time from 0.051–0.112 g phenol/L.day from higher phenol loading [Figure 4.44 (a)]. During instant fill phenol removal efficiency decreased slightly to 98% and this was almost 26% of total feed phenol removal [Figure 4.43 (a)]. Phenol removal rate in B2 was the maximum at 0.158 g phenol/L.day during instant fill.

Influent COD to B2 was 850–1450 mg/L and similar to phenol, COD removal increased from 71 to 81% with increase in fill time and loading rate of 0.343–0.534 g/L.day. The corresponding removal rate was 0.243–0.434 g/L.day. Maximum COD removal rate of 0.449 g/L.day was achieved when B2 received highest loading of 0.580 g/L.day at instant fill [Figure 4.44 (a)]. B2 removed 11–20% and ~25% of total influent COD during gradual fill and instant fill, respectively and released low amount of COD (240–328 mg/L) to B3.

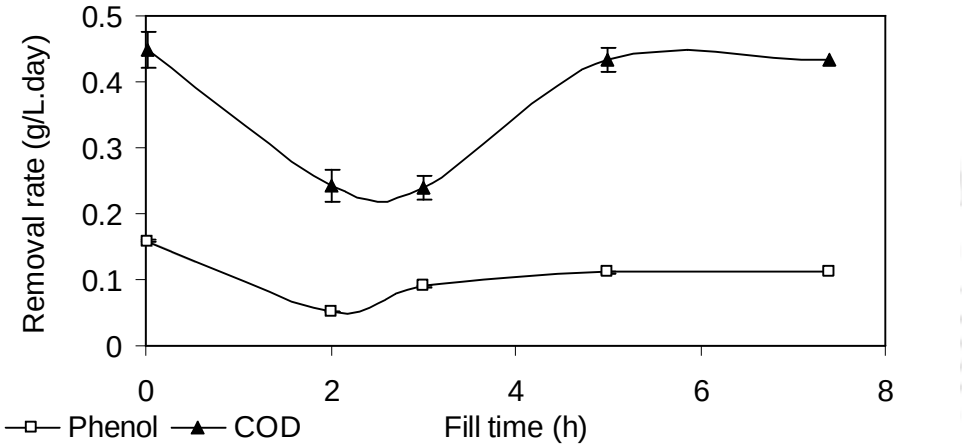

Figure 4.44 (a) Phenol and COD removal rate in B2 at varied fill time

As there was negligible SCN^- removal in B1, influent SCN^- to B2 was 50 mg/L with loading of 0.02 g SCN^-/L.day at instant fill and ~399 mg/L comprising loading of 0.159 g SCN^-/L.day, during gradual fills. During short gradual fill of 2–3 h, ~99% thiocyanate removal occurred in B2 and it decreased to ~97% with further increase in fill time to 5–7.4 h. This decreased further to 96% at instant fill. Thiocyanate removal rate was 0.154–0.158 during the study of gradual fill [Figure 4.44 (b)]. Contribution in total thiocyanate removal was 48–49% higher than B1 and B3 through out the study [Figure 4.45 (a)]. During instant fill time, performance of B2 did not deteriorate with 96%, 98% and 77% removal of SCN^-, phenol and COD, respectively. Reaction time in B2 varied from 16.1 h to 21.5 h. It seems that the lowest available reaction time was sufficient for the activity of anoxic culture. Moussavi et al. (2010) observed insignificant effect of increase of fill time from 1 h to 4 h,

on removal of phenol (influent 1000 mg/L) from saline wastewater in aerobic granular fed batch reactor. Similar observations were reported by Sarfaraz et al. (2004) with insignificant effect of decrease in fill time from 6h (3 h fill) on anoxic SBR treating phenolic wastewater at 12 h cycle period. Higher influent NH_4^+–N concentration 444–452 mg/L was observed in B2 during short gradual fill (2–3 h) as recycle from B3 was containing high NH_4^+–N concentration and also due to higher thiocyanate degradation during this stage. NH_4^+–N removal was 13% during instant fill from lowest influent concentration of 300 mg/L and it was 1–4% during gradual fill.

Table 4.17 (a): Performance of anoxic fed batch MBR (B2) at fill time variation

Fill period (h)	Phenol			COD			Thiocyanate			NH_4^+–N		
	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem
2	129	1 (0)	99.2	857	250 (3)	70.8	398	2 (0)	99.5	452	455 (11)	0
3	225	1 (0)	99.2	850	250 (9)	70.6	398	5 (0)	98.7	444	425 (12)	4.3
5	279	0 (0)	100	1323	240 (4)	81.87	398	12 (0)	97.0	392	388 (0)	1.2
7.4	280	0 (0)	100	1335	250 (4)	81.28	400	9 (0)	97.7	383	380 (9)	1.0
Instant	400	5 (0)	98.75	1450	328 (13)	77.38	49.5	2 (0)	96.0	346	300 (4)	13

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

[A] Influent NH_4^+–N of B2 = {Effluent NH_4^+–N of (B1+B3)/2 + 0.24 x (SCN^- removed in B2)}.

Numbers in parenthesis indicate standard deviation values.

With increasing fill time, influent NOx–N to B2 increased, suggesting higher amount of generation of NOx–N in downstream reactor B3. NOx–N loading rate in B2 was 0.268, 0.281, 0.303 and 0.313 g/L.day at fill time of 2, 3, 5 and 7.4 h, respectively. NOx–N removal rate in B2 was 0.175–0.196 g/L.day [Figure 4.44 (b)].

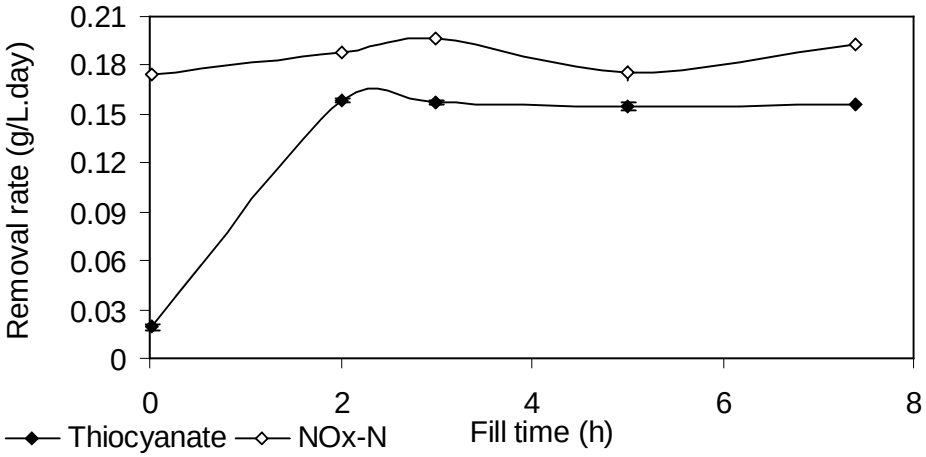

Figure 4.44 (b) Thiocyanate and Nox-N removal rate in B2 at varied fill time

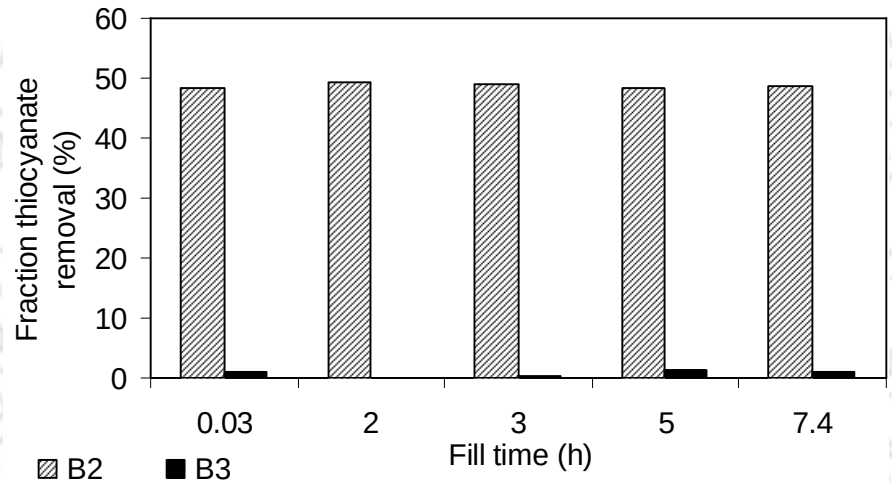

Figure 4.45 (a) Fraction Thiocyanate removal by B2 and B3 at varied Fill time

At instant fill, loading and removal rate of NOx–N in B2 was lowest of 0.258 and 0.173 g/L.day, respectively. The COD/N removed ratio calculated using equation 4.5 was 1.0–2.6 being lower to the stoichiometric ratio. Contribution of B2 in total nitrogen removal (26–31%) is shown in Figure 4.45 (b) and it was always higher than B3 through out the study. Fernández–Nava et al. (2010) reported the low COD consumption compared to nitrate removal can occur in anoxic reactor and might be due to intercellular storage as reported in other literatures (Dionisi et al. 2001; Qin et al. 2005). Abufayed and Schroeder (1986) also reported that the low COD:N_{rem} ratio might be attributed to the hydrolysis of

influent solids captured within reactor to generate a COD source not measured by filtered samples.

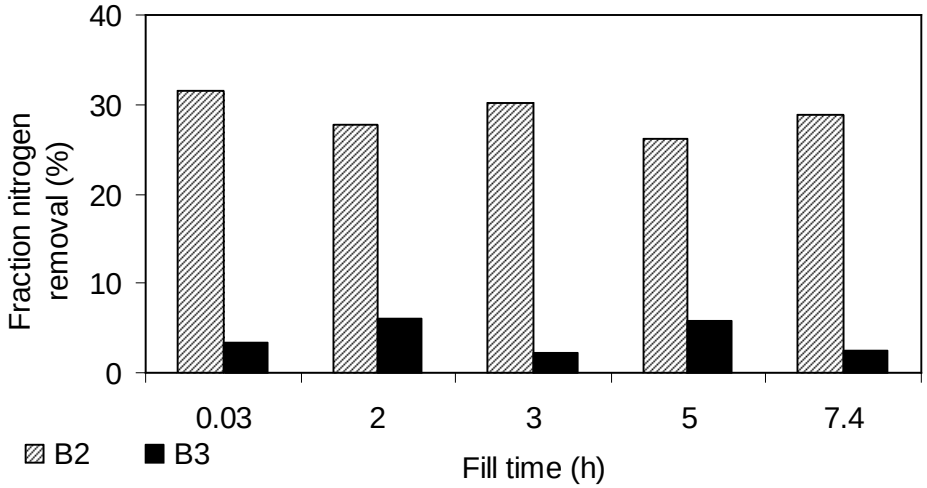

Figure 4.45 (b) Fraction Nitrogen by B2 and B3 at varied Fill time

Table 4.17 (b): Performance of anoxic fed batch MBR (B2) at fill time variation

Fill period (h)	Sulfate					NO_3^-–N		NO_2^-–N		NOx^-–N	COD :N_{rem}	TVS (mg/L)
	S_0	S_e	Gen	Th SO_4^{-2}	Err	S_0	S_e	S_0	S_e	Rem		
2	635	1180 (13)	545	653	–108	625	200 (4)	46	0.1	70.17	1.0	12410
3	640	1200 (0)	560	648	–88	655	211 (3)	47	0	69.96	1.0	12022
5	643	1197 (49)	554	636	–83	715	319 (25)	42	0	59.00	2.4	12650
7.4	624	1200 (46)	576	644	–68	740	300 (30)	42	0	61.66	2.0	12060
Instant	99	181 (3.5)	82	80	2	610	210 (5)	35	0.2	67.41	2.6	13178

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%); Gen: Generation (mg/L);
Th SO_4^{2-}: Theoretical sulfate generation; Err: Error (mg/L);
Numbers in parenthesis indicate standard deviation values

Nearly 82–576 mg/L sulfate was generated in B2 instead of theoretical sulfate generation value 80–664 mg/L. Higher negative error was observed during short fill with high thiocyanate removal. Biomass concentration in B2 was 12–13 g/L and the attached to suspended biomass concentration ratio was 1–2.3 as high amount of suspended biomass concentration was detected. Suspended biomass concentration during gradual fill in B2 was fluctuating between 3600–4000 mg/L. At instant fill higher amount of suspended biomass (6700 mg/L) was detected in B2.

4.2.2.3 Performance of aerobic FMBR (B3) at varied fill time

Average performance of B3 is presented in Tables 4.18 (a) and (b). B3 was operated essentially for the study of fill effect on nitrification or removal of NH_4^+–N, since phenol, SCN^- and COD entered to B3 was always in low range of 0–1 mg/L, 2–12 mg/L and 328–250 mg/L, respectively. Nearly 50–92% SCN^- removal occurred in B3 releasing ~1 mg/L SCN^- in its effluent. Influent COD to B3 was 240–250 mg/L during gradual fill and 328 mg/L at instant fill. B3 removed 48–69% bringing the effluent COD down to 100–130 mg/L throughout the study. Removal of SCN^-, phenol, and COD was not affected by the fill time.

Ammonia being the most critical pollutant to be removed in B3 was kept 500 mg/L in the feed through out the studies. This amount further increased with the NH_4^+–N generated from SCN^- degradation in B2 and final influent NH_4^+–N to B3 was 300–455 mg/L. The corresponding loading to B3 was 0.12, 0.182, 0.17, 0.156 ad 0.152 g NH_4^+–N /L.day at instant fill, 2 h, 3 h, 5 h and 7.4 h fill time, respectively. B3 received the lowest value influent NH_4^+–N (300 mg/L) during instant fill period but removal efficiency achieved was only 43%. Fill period of 2–3 h also did not improve NH_4^+–N removal efficiency in B3 as at short fill times 2–3 h, NH_4^+–N removal efficiency in B3 was ~54% and higher NH_4^+–N generated in effluent (195–210 mg/L). Effluent of B3 was recycled to B2 and influent NH_4^+–N to B2 also increased at short fill time. Since, anoxic reactor B2 was unable to remove NH_4^+–N, so influent NH_4^+–N to B3 increased with decrease in fill time. When fill period was increased to 5 h and 7.4 h, NH_4^+–N removal efficiency in B3 significantly increased to 75% and 80%, respectively. It seems that at longer fill time, nitrifying bacteria in B3 was gradually exposed to the influent NH_4^+–N and the toxicity of NH_4^+–N

decreased. The NH_4^+–N removal rate was 0.054 g/L.day at instant fill and it increased to 0.098–0.122 g/L.day at the increased gradual fill from higher NH_4^+–N loading than B3 received in instant fill (Figure 4.46). Tomei et al. (2004) reported long and aerated fill enhanced substrate removal by reducing the inhibitory action of the toxic compounds on biomass activity. The influent NH_4^+–N concentration in the present study was higher than substrate inhibition concentration during gradual fill period. Also during short gradual fill 2–3 h, influent NH_4^+–N to B3 was quite higher (426–455 mg/L) and might be responsible for low NH_4^+–N removal as bacteria could not sustain that high peak concentration.

Table 4.18 (a): Performance of aerobic fed batch MBR (B3) at fill time variation

Fill period (h)	SCN^-			Phenol			COD			NH_4^+–N			COD: NH_4^+ –N	pH
	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem		
2	2	1 (0)	50	1	0	100	250	100 (0)	60.0	455	210 (9)	53.8	0.60	8 ± 0.2
3	5	1 (0)	80	1	0	–	250	100 (0)	60.0	426	195 (7)	54.2	0.60	8 ± 0.2
5	12	1 (0)	94	–	–	–	240	120 (0)	50.0	390	95 (6)	75.6	1.12	8 ± 0.2
7.4	9	1 (0)	89	–	–	–	250	130 (6)	48.0	382	75 (7)	80.3	1.12	8 ± 0.2
Instant	2	1 (0)	50	5	1 (0)	80	328	100 (0)	69.5	300	170 (11)	43.4	1.09	8 ± 0.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),
Numbers in parenthesis indicate standard deviation values

Effluent nitrate in B3 increased from 220 mg/L to 480 mg/L with increase in fill time and nitrification rate was 0.03–0.11 g/L.day being higher at longer fill period [Table 4.18 (b)]. In all the fill period, the system showed incomplete nitrification process with high accumulation of nitrite (70–95 mg/L) in the effluent. Free ammonia calculated ranged 34–49 mg/L due to high pH in respective studies which was also suspected to be responsible

for incomplete nitrification and nitrite accumulation in B3. Unaccounted nitrogen calculated was 3–22% during the study.

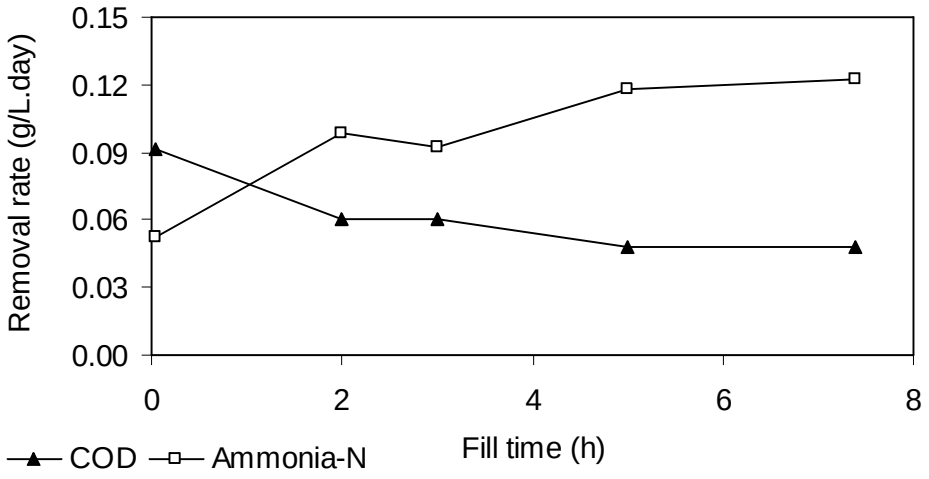

Figure 4.46 Effect of fill time on COD and NH_4^+-N removal rate in B3

Table 4.18 (b): Performance of aerobic fed batch MBR (B3) at fill time variation

Fill time (h)	NO_3^-–N		NO_2^-–N		N_R	FA	UN	TVS (mg/L)	Sulfate				
	S_0	S_e	S_0	S_e					S_0	S_e	Gen	Th SO_4^{-2}	Err
2	200	250 (24)	0.13	92 (3)	0.06	48.9	22	11500	1180	1270 (43)	90	2	88
3	211	310 (12)	0	95 (9)	0.08	45.7	3	12050	1200	1280 (46)	80	7	73
5	319	430 (45)	0	85 (0)	0.08	35.7	21	13000	1197	1287 (16)	90	18	72
7.4	300	480 (32)	0	85 (6)	0.11	33.6	3	12300	1200	1248 (32)	48	14	34
Instant	210	220 (13)	0.2	70 (5)	0.03	34.6	12	12840	181	198 (4.8)	17	2	15

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);
Th SO_4^{-2}: Theoretical sulfate generation (1.65 x SCN^- removed in B3); Err: Error (mg/L)
N_R: Nitrification rate (g/L.day); FA: mg/L; UN: Unaccounted nitrogen (%)
Numbers in parenthesis indicate standard deviation values

Nearly 198–1280 mg/L sulfate was released from B3 in effluent with 17–90 mg/L sulfate generation. Observed sulfate generation in B3 was higher during the study than that of theoretical sulfate generation. The suspended biomass concentration was 6342 mg/L being significantly higher at instantaneous fill than gradual fill. During gradual fill suspended biomass concentration was 3333–4782 mg/L increasing gradually with increase in fill time and total biomass was 11.5–13.0 g/L with attached to suspended biomass ratio of 1.0–2.4 during the study.

4.2.2.4 Overall performance of FMBR system at varied fill time

Overall performance of present fed batch MBR system was insignificantly affected by the fill time variation in terms of SCN^-, phenol and COD degradation (Figure 4.47).

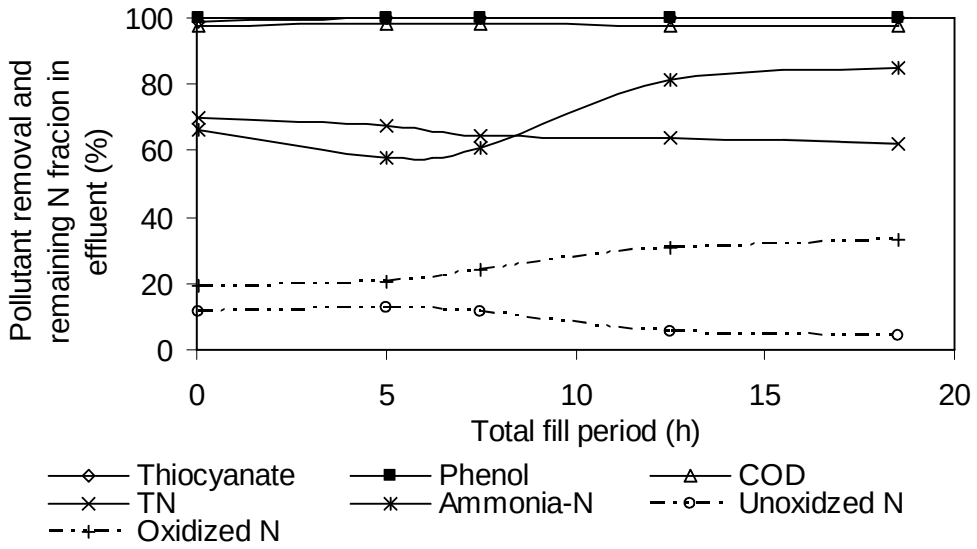

Figure 4.47 Performance of moving bed batch reactor system with varied fill period

More than 99–99.8% SCN^- was removed from 100–800 mg/L influent SCN^-. Phenol and COD removal were ~100% and 98%, respectively irrespective of fill period. Influent total nitrogen was 1524 mg/L and 1692 mg/L during instant fill and gradual fills, respectively. TN removal was 69% during instant fill and it was 65–62% during gradual fill. NH_4^+–N removal was significantly affected by fill time variation. Higher NH_4^+–N removal of 81–

85% was accounted during the longer fill whereas it dropped to 58–61% during the short total fill time of 7.5 h. Fraction of oxidized nitrogen in effluent increased compared to unoxidized nitrogen at longer fill. During short fill time studies, the system removed lower amount of NH_4–N in B3 but reduced higher NOx–N. During longer fill time the reverse scenario was seen retaining similar total nitrogen removal. It seems that total fill time of 12.5 h (Fill time of B1, B2 and B3 are 2.5h, 5h and 5h) is optimum for anaerobic–anoxic–aerobic moving bed fed batch reactor system, since above this fill time (fill time of 18.5 h), relative increase in oxidized nitrogen fraction and decrease in NH_4^+–N fraction were negligible, however pumping cost will increase.

4.2.3 Performance of FMBR system at varied HRT

Total HRT of FMBR system was varied from 5–10 days with constant feed concentration of phenol, thiocyanate, NH_4^+–N and NO_3^-–N in recycle to evaluate the effect of HRT on performance. HRT of B1 was 2.5–5 days and HRT of B2 and B3 were 1.25–2.5 days each. The experiment was conducted for 185 days from 621st to 805th day. Influent and effluent profile of various parameters during HRT variation study is shown in Figure 4.48.

4.2.3.1 Performance of anaerobic FMBR (B1) at varied HRT

HRT of B1 was 2.5, 3, 4 and 5 days with constant feed of SCN^-, phenol, NH_4^+–N and COD concentration at 800, 1500, 500 and 5400 mg/L, respectively. B1 removed 1.2–0.75% SCN^- irrespective of increasing HRT. Previous study with increased thiocyanate in FMBR showed low amount of thiocyanate removal (12%) in low influent concentration (100 mg/L). The anaerobic culture might not be efficient for thiocyanate removal from higher influent thiocyanate concentration though higher HRT was provided.

Feed phenol concentration during the study was maintained constant at 1500 mg/L and corresponding loading rate decreased from 0.6 to 0.3 g/L.day with increase in HRT from 2.5 to 5 days. Phenol removal was 50% at HRT of 2.5 days and increased to 53–63% with increase in HRT in B1 3–5 days HRT (Table 4.19).

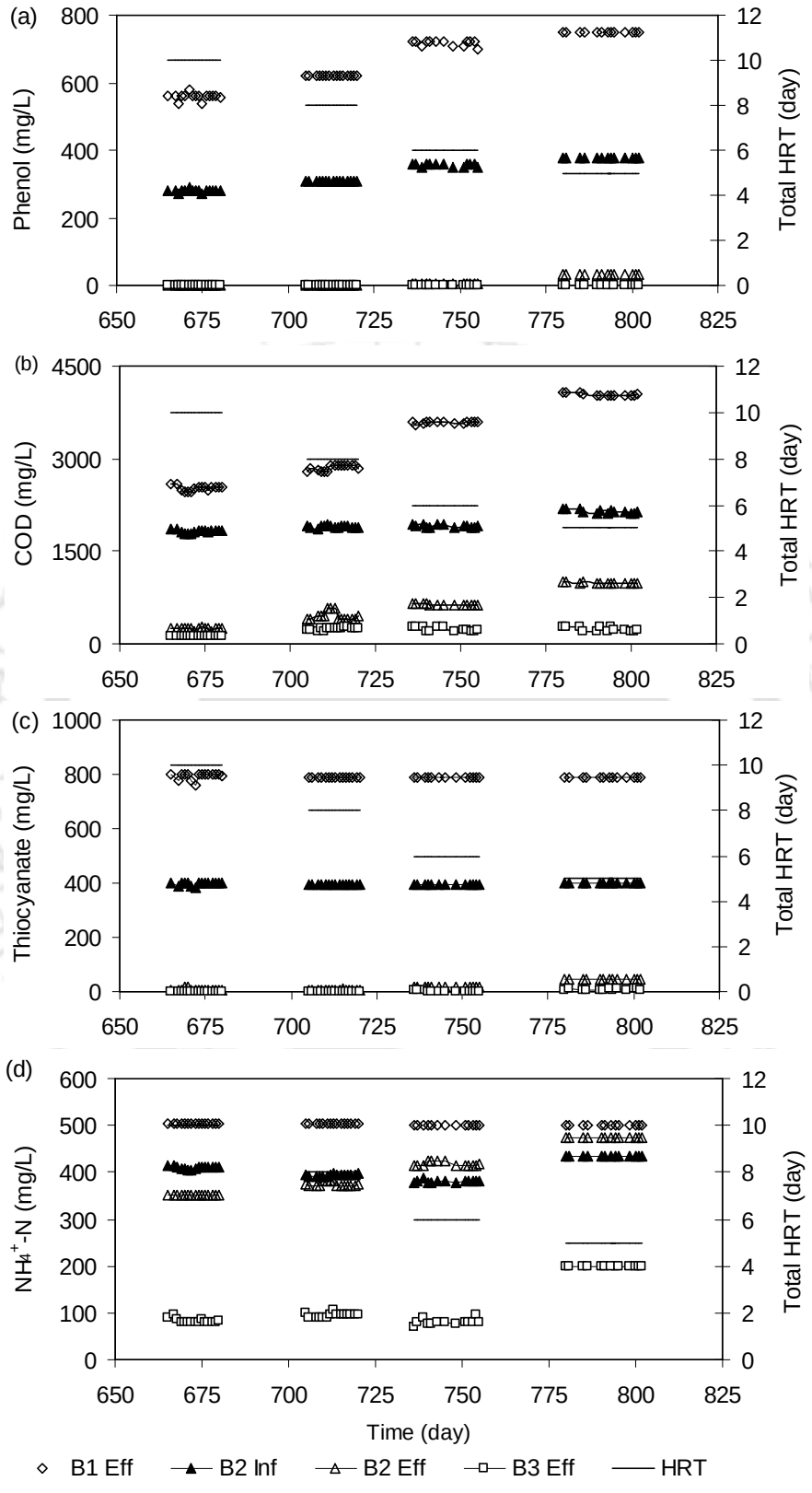

Figure 4.48 Pollutant profile (a) Phenol, (b) COD, (c) Thiocyanate and (d) NH_4^+-N in FMBR at HRT variation

Phenol removal rate increased from 0.19 –0.30 g/L.day with increased loading (Figure 4.49). Fang et al. (2006) reported that due to decrease in HRT from 2.5 day to 1.17 day, phenol loading increased from 0.25 to 0.54 g/L.day and removal decreased from 99% to 70% in an upflow anaerobic sludge blanket reactor (UASB) in absence of toxic components like thiocyanate and NH_4–N.

With increase in HRT, COD loading rate decreased from 2.16 to 1.08 g/L.day at constant influent concentration 5400 mg/L. Increased COD removal of 25 to 53% was achieved in B1 and maximum COD removal rate was 0.648 g/L.day at HRT 4 days. COD removal was only 25% when HRT was 2.5 days and it increased to 33% (increase by 32%) at HRT of 3 days and with further increase in HRT to 4 and 5 days improved COD removal efficiency in B1 of 48–53% was achieved. However COD removal rate increased initially from 0.546 to 0.648 g/L.day with increase in HRT 2.5–4.0 days and then decreased to 0.575 g/L.day at 5 days HRT with decreased COD loading (Figure 4.49).

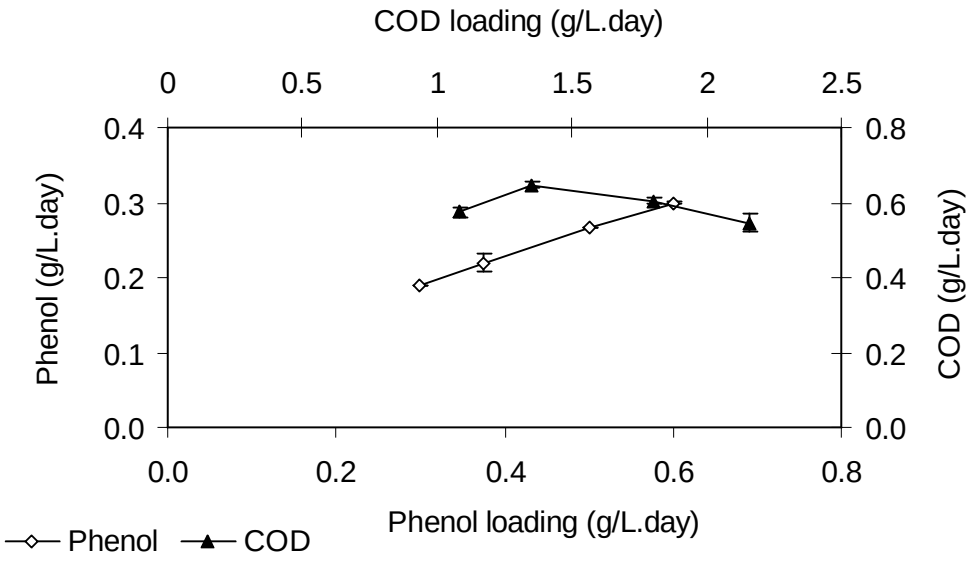

Figure 4.49 Effect of Phenol and COD loading on removal rates in B1 at varying reactor HRT

Table 4.19: Performance of anaerobic fed batch MBR (B1) at HRT variation

HRT (day)		SCN^-			Phenol			COD			pH	TVS
Total	B1	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem		(mg/L)
5	2.5	800	790 (0)	1.25	1500	750 (0)	50.00	5400	4035 (60)	25.27	6.5	10537 (300)
6	3		790 (0)	1.25		700 (36)	53.33		3587 (39)	33.57	6.9	10200 (335)
8	4		790 (0)	1.25		620 (0)	58.67		2808 (115)	48.0	6.6	10721 (330)
10	5		794 (12)	0.75		558 (9.5)	62.80		2527 (45)	53.20	6.8	10126 (300)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

Numbers in parenthesis indicate standard deviation values

Ramakrishnan and Gupta (2006) reported phenol and COD removal of 92% and 88% while treating phenolic wastewater by a hybrid anaerobic reactor (bottom UASB and anaerobic filter on the end) at phenol and COD loading rate of 0.45 g/L.day and 2.24 g/L.day, respectively. Boubaker and Ridha (2007) have reported that a maximum COD removal of ~90% could be achieved by increasing the HRT to 36 days at lower OLR (0.67 g/L.day). Present study with high feed phenol concentration in presence of toxic compounds and at low HRT shows better performance than suspended system though it was less to the reported attached growth system. No ammonia was removed in B1 even at higher HRT and no SMA was detected through out the study. Reactor pH was always observed to decrease to 6.8–6.9. Suspended biomass and attached biomass was fluctuating 920–1000 mg/L and 9200–9700 mg/L, respectively in B1 during the study. Total biomass concentration through out the study was 10.1 –10.7 g/L showing no significant affect of HRT on it and the ratio of attached biomass to suspended biomass was 9.6–10.2.

4.2.3.2 Performance of anoxic FMBR (B2) at varied HRT

B2 received effluent from B1 and recycled effluent from B3 (Eq.3.4) and average performance of B2 at 1.25–2.5 days HRT is presented in Tables 4.20 (a) and (b). B2 efficiently removed all the phenol along with COD and SCN^- removal.

Influent SCN^- ranged from 396–399 mg/L and loading increased from 0.16–0.32 g SCN^- /L.day with decreasing HRT from 2.5–1.25 days. B2 was the sole reactor efficiently degrading 88–97% of influent SCN^- with increasing HRT and released 12–45 mg/L SCN^- in its effluent [Table 4.20 (a)]. At minimum HRT, thiocyanate removal was only 88% releasing maximum of 45 mg/L thiocyanate in effluent. However, SCN^- removal significantly increased to 96–97% at increase in HRT of 1.5–2.5 days. It was 44–48% of total feed SCN^- removal. The removal rate increased from 0.154 to 0.283 g SCN^-/L.day with increased SCN^- loading and decreased HRT. Maximum SCN^- removal rate in B2 was 0.283 g/L.day at HRT 1.25 day at maximum loading indicating absence of substrate inhibition of thiocyanate in B2 (Figure 4.50).

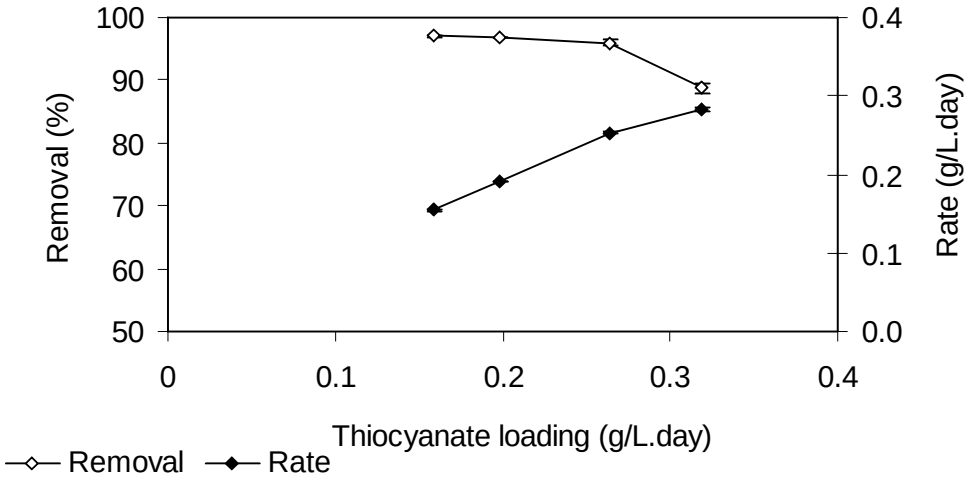

Figure 4.50 Thiocyanate degradation at varied loading in B2 at varying reactor HRT

With decreasing HRT, B2 received increased influent phenol concentration 279–376 mg/L. B2 removed ~92% influent phenol and released maximum of 30 mg/L phenol in effluent when HRT was minimum of 1.25 days. With increase in HRT to 1.5 days or more phenol removal in B2 increased to more than 99% and released only ~ 1 mg/L phenol. Phenol loading rate in B2 was 0.112–0.301 g/L.day and removal rate achieved was 0.111–0.278 g/L.day (Figure 4.51). Similarly, influent COD concentration in B2 increased from 1323 mg/L to 2135 mg/L with decreasing HRT and the corresponding loading rate was 0.529–1.71 g/L.day. The COD removal efficiency increased from 54 to 82% with increase in HRT. B2 showed increased COD removal rate from 0.433–0.922 g/L.day at increasing

loading rate (Figure 4.51). Contribution of B2 in total feed phenol and COD removal was 18–23% and 20–23%, respectively and B2 was efficiently removing phenol/COD or thiocyanate at a wide range of HRT in present study.

Table 4.20 (a): Performance of anoxic fed batch MBR (B2) at HRT variation

HRT (d)	SCN^-			Phenol			COD			NH_4^+–N			pH
B2	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem	
1.25	399	45 (0)	88.72	376	30 (0)	92.02	2135	983 (3.7)	54	435	390 (0)	10.10	8.4
1.5	396	16 (0)	95.96	350	1 (5)	99.71	1916	635 (8.9)	69	381	366 (0)	3.40	8.5
2	396	12 (2)	96.97	310	1 (0)	99.68	1525	450 (30)	70	395	380 (0)	6.20	8.3
2.5	398	12 (0)	96.98	279	1 (0)	99.64	1323	240 (22)	82	395	360 (0)	8.90	8.4

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B2 = {Effluent NH_4^+–N of (B1+B3)/2 + 0.24x (SCN^- removed in B2)}.

Numbers in parenthesis indicate standard deviation values

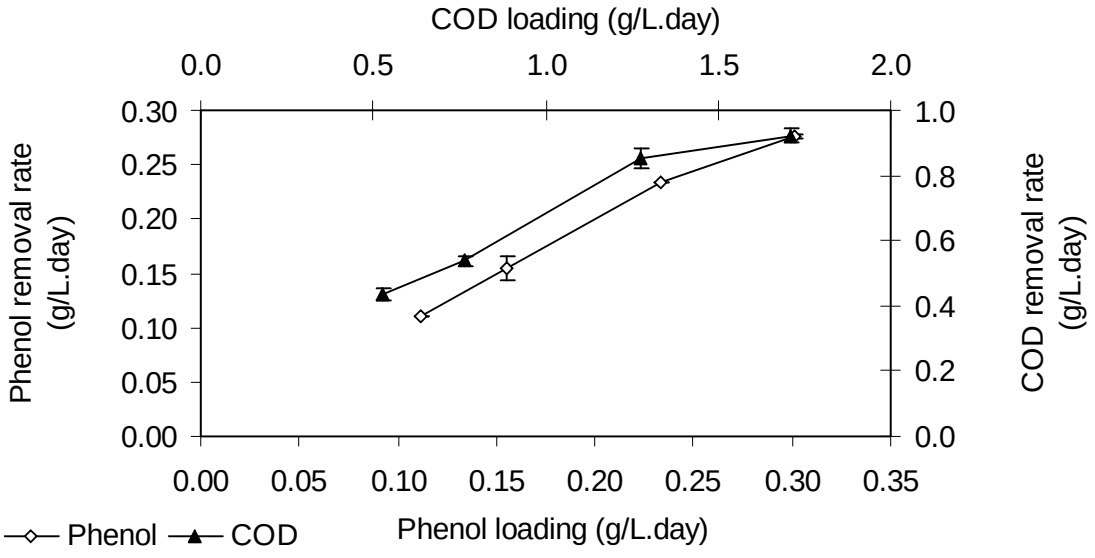

Figure 4.51 Effect of Phenol and COD loading on removal rates in B2 at varying reactor HRT

Influent NH_4^+–N in B2 was 381–435 mg/L that constituted from effluent of B1 and recycle from B3 along with NH_4^+–N generated from degradation of SCN^- in B2. At higher HRT slightly higher SCN^- degradation occurred increasing NH_4^+–N generation and at short HRT, recycle from B3 was rich in NH_4^+–N resulting higher final influent NH_4^+–N in B2. B2 removed 3–10% NH_4^+–N releasing 360–390 mg/L NH_4^+–N in effluent.

NO_3^-–N externally added in the recycle was 1000 mg/L and influent NO_3–N ranged between 590–715 mg/L. During higher HRT period, B3 released more NO_3^-–N in its effluent which enriched the recycle and hence total influent NOx–N to B2 increased. During short HRT 1.25–1.5 days NO_2^-–N 0.2–1.0 mg/L was detected in effluent of B2 whereas no nitrite was detected in effluent during longer HRTs. NOx–N removal efficiency in B2 initially increased from 63–69% with increase in HRT 1.25 to 2 days and then decreased to 58% with further increase in HRT to 2.5 days. During lower HRT, influent COD was higher which might have facilitated increased NOx–N removal. NOx–N removal rate was found to increase from 0.175–0.321 g/L.day with a slope of 0.68 with increase in NOx–N loading 0.303–0.506 g/L.day (Figure 4.52).

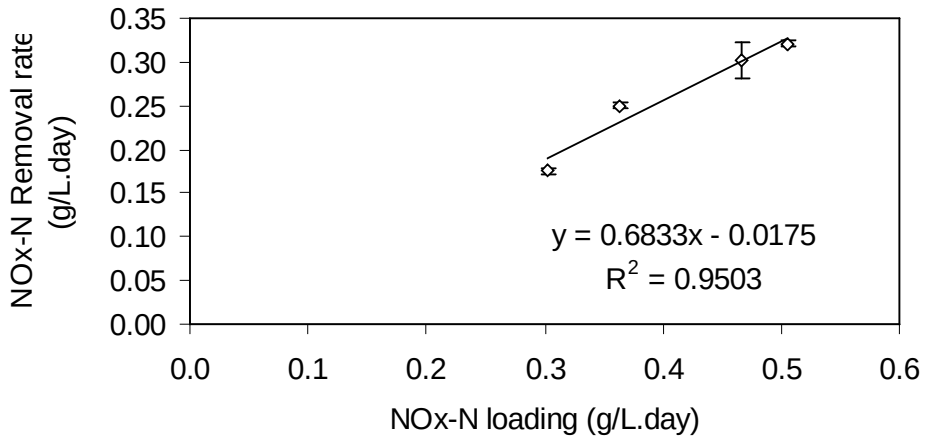

Figure 4.52 Effect of nitrate and nitrite -N loading on denitrification rate in B2 at varying reactor HRT

Contribution of B2 in total nitrogen removal increased from 26% to 31% with increase in reactor HRT (Figure 4.53). Also, the COD/N_{rem} ratio calculated using equation 4.5 was 2.0– 3.07 at HRT 1.25–2.5 days being low at higher HRT and the COD fraction calculated for biomass was low being 2–7% only. The biomass yield coefficient was only 0.01–0.04. In B2, suspended biomass was 3800– 4730 mg/L being higher at low HRT and attached

biomass was 8800–9700 mg/L during the study. The attached to suspended biomass ratio increased from 1.9 to 2.6 with increase in HRT as suspended biomass concentration slightly decreased. Total biomass concentration in B2 was ~13–13.7 g/L through out the study with higher ratio of attached biomass to suspended biomass at higher HRT.

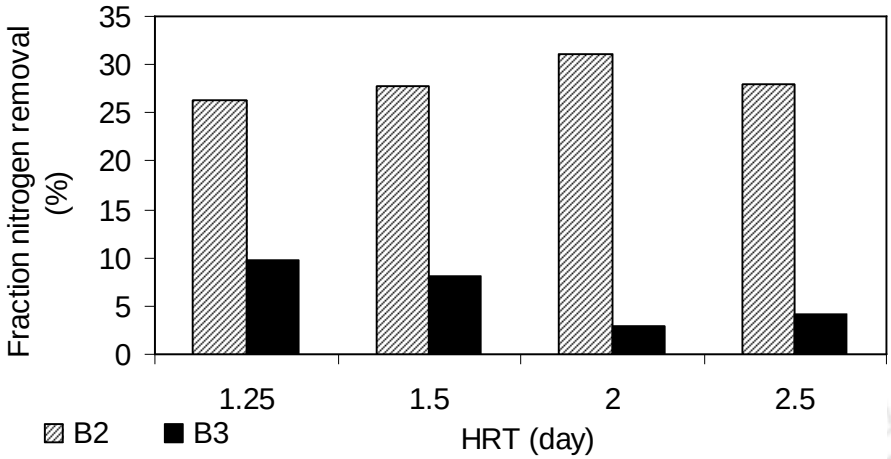

Figure 4.53 Contribution of B2 and B3 in nitrogen removal in FMBR

Table 4.20 (b): Performance of anoxic fed batch MBR (B2) at HRT variation

HRT (d)	Nitrate		Nitrite		NOx–N	Sulfate					COD: N_{rem}	COD_B	TVS (mg/L)
B2	S_0	S_e	S_0	S_e	Rem	S_0	S_e	Gen	Th SO_4^{-2}	Err			
1.25	590	230 (0.6)	42.5	1 (2)	63.50	405	760 (62)	355	584	–229	3.07	7	13740
1.5	664	245 (1.6)	36.0	0.2	65.00	485	932 (19)	447	626	–179	2.92	2	13172
2	678	225 (4.94)	47.3	0	68.91	488	960 (44)	471	633	–161	2.00	–	13110
2.5	715	319 (6.3)	42.5	0	58.00	644	1197 (36)	553	636	–82	2.40	–	13580

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);

Th SO_4^{-2}: Theoretical sulfate generation; Err: Error (mg/L)

COD_B: COD fraction (%) for biomass

Numbers in parenthesis indicate standard deviation values

Nearly 760–1197 mg/L sulfate was detected in effluent of B2 during the study and higher sulfate concentration was observed during higher HRT. Sulfate generation was 355–553 mg/L and increased with increase in HRT. However sulfate generation was lower to the theoretical sulfate generation through out the study. During lower HRT of 1.25– 2 days, though the reactor showed high thiocyanate removal as in higher HRT, the final product, sulfate generation was having large distance from theoretical sulfate generation.

4.2.3.3 Performance of aerobic FMBR (B3) at varied HRT

B3, the last reactor of the series received insignificant amount of phenol and thiocyanate along with 240–983 mg/L COD and average performance of B3 is presented in Tables 4.21 (a) and (b). Influent phenol was ~1 mg/L during reactor HRT of 1.5–2.5 days and B3 received 30 mg/L phenol when HRT was least of 1.25 days. Influent SCN^- in B3 was 12, 12, 16 and 45 mg/L when HRT was 2.5, 2, 1.5 and 1.25 days, respectively. Nearly 91–93% SCN^- removal occurred in B3 releasing 1 mg/L SCN^- in effluent at HRT 1.5–2.5 days and the removal decreased to only 82% when HRT was decreased to minimum of 1.25 day. Thiocyanate loading and removal rate was 0.005–0.036 g/L.day and 0.004–0.030 g/L.day, respectively. B3 removed phenol irrespective of HRT, might be due to low level of the pollutant [Table 4.21 (a)]. Removal rate of these pollutants increased with increased loading at decreasing HRT.

Influent COD to B3 was 983, 635, 450 and 240 mg/L at HRT 1.25, 1.5, 2 and 2.5 days, respectively. The corresponding loading rate increased from 0.096 g/L.day to 0.787 g/L.day when HRT was decreased from 2.5 days to 1.25 days. COD removal was 46–76% during the study and maximum COD removal achieved was ~76% when B3 received maximum loading of COD 0.787 g/L.day at HRT 1.25 days. Effluent COD from B3 was 120–235 mg/L, lower to permissible discharged limit through out HRT variation study. COD removal rate decreased from 0.598 g/L.day to 0.048 g/L.day with decreasing COD loading and increasing HRT (Figure 4.54).

High concentration of NH_4^+–N 362–400 mg/L entered B3 as insignificant amount of NH_4^+–N got removed in the upstream reactors. Influent NH_4^+–N to B3 was 400, 371, 372 and 362 mg/L at reactor HRT of 1.25, 1.5, 2 and 2.5 days, respectively. The NH_4^+–N loading rate also increased from 0.145–0.319 g/L.day when HRT was decreased from 2.5

to 1.25 days. NH_4^+–N removal was significantly affected by the HRT of B3. During HRT 1.5–2.5 days, B3 accounted 73–78% NH_4^+–N removal from influent NH_4^+–N. The removal was only 50% releasing 200 mg/L effluent NH_4^+–N when HRT was decreased to 1.25 days and it was receiving maximum influent concentration. Removal rate achieved was 0.109–0.194 g/L.day at HRT 1.5–2.5 days with increase in NH_4^+–N loading of 0.145–0.248 g/L.day; however it decreased to 0.159 g NH_4^+–N /L.day when HRT was decreased to 1.25 day and B3 received 0.319 g NH_4^+–N/L.day (Figure 4.54). The nitrification rate achieved was only 0.03 g/L.day at HRT 1.25 days and continuously increased to ~0.08–0.11 g/L.day with increase in HRT.

Table 4.21 (a): Performance of aerobic fed batch MBR (B3) at HRT variation.

HRT (day)	SCN^-			Phenol			NH_4^+–N			COD			COD /NH_4^+–N_{inf}
B3	S_0	S_e	Rem	S_0	S_e	Rem	S_0[A]	S_e	Rem	S_0	S_e	Rem	
1.25	45	8 (2.5)	82.85	30	2 (0)	93.33	400	200 (13)	50.00	983	236 (18)	75.99	2.4
1.5	16	1 (0.65)	93.75	1	1 (0)	–	371	80 (0)	78.50	635	245 (37)	61.42	1.7
2	12	1 (0)	91.67	1	1 (0)	–	372	100 (9.8)	73.20	450	242 (24)	46.06	1.2
2.5	12	1	91.67	1	0	–	362	95	73.80	240	120	50.00	0.7

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B3 = {Effluent NH_4^+–N of B2 + 0.24x (SCN^- removed in B3)}.

Numbers in parenthesis indicate standard deviation values

Higher COD to NH_4^+–N ratio of 2.4 was achieved at 1.25 days HRT which was only 0.7–1.2 at higher HRT [Table 4.21 (a)]. Suspended biomass in B3 was 3400–3450 mg/L at reactor HRT 1.25–2 days and decreased to 2780 mg/L at HRT 2.5 days. Contrary, the attached biomass in B3 was lower (~7050 mg/L) during short HRT (1.25–2 days) and higher (9190 mg/L) at long HRT. Total biomass concentration in B3 was 10.2–11.9 g/L and attached to suspended biomass ratio was 2.0–3.3 being maximum at higher HRT.

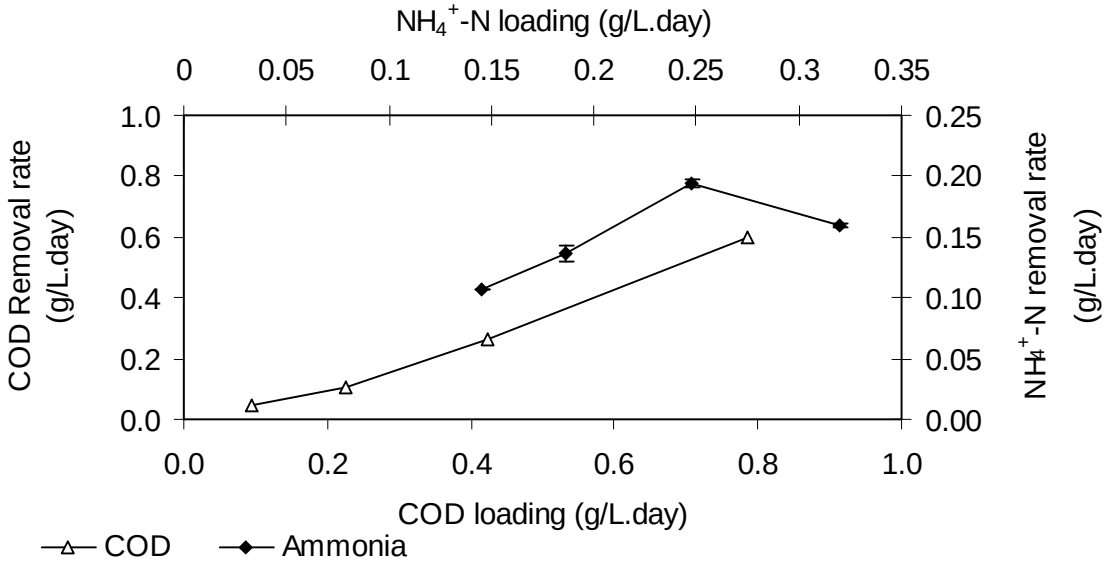

Figure 4.54 Effect of COD and NH_4^+-N loading on removal rate in B3

Table 4.21 (b): Performance of aerobic fed batch MBR (B3) at HRT variation

HRT (d)	Sulfate					Nitrate		Nitrite		N_R	FA	UN	TVS (mg/L)
B3	S_0	S_e	Gen	Th SO_4^{-2}	Err	S_0	S_e	S_0	S_e				
1.25	760	810 (17)	50	61	–11	230	180 (6.86)	3	85 (0)	0.03	64.2	50	10820
1.5	932	970 (33)	48	24	13	245	328 (4.54)	0	72 (6)	0.10	40.3	33	10221
2	960	977 (6)	17	18	–1	225	356 (17.6)	0	94 (2)	0.11	42.2	2	10506
2.5	1197	1287 (40)	90	18	72	319	430 (15)	0	85 (0)	0.08	33.7	14	11974

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L); Err: Error (mg/L)

Th SO_4^{-2}: Theoretical sulfate generation, (mg/L);

N_R: Nitrification rate (g/L.day);

FA: Free ammonia (mg/L); UN: Unaccounted nitrogen (%);

Numbers in parenthesis indicate standard deviation values.

In B3, higher concentration of free ammonia (40–64 mg/L) and nitrogen loss calculated as unaccounted nitrogen (33–50%) was observed at low HRT. At lower HRT, high amount of COD was entering compared to longer HRT and accumulation of nitrite was always observed in B3. Reduction of nitrite to nitrogen through denitrification at the interior of the sponge cube might have occurred causing nitrogen loss from the reactor. Li et al. (2011) reported maximum removal efficiencies of 81%, 89%, 94% and 93% for COD, phenols, SCN^- and NH_4^+–N, respectively during treatment of coal gasification wastewater in a laboratory–scale moving bed biofilm reactor. They observed that NO_2^-–N accumulation induced increase of effluent COD concentration when the hydraulic residence time (HRT) decreased. Also, phenols removal was not affected when the HRT decreased from 2–1.5 days though effluent SCN^- and NH_4^+–N concentration increased with the decrease of the HRT, and decreased gradually when the HRT returned to 2 days.

Higher amount of sulfate (810–1287 mg/L) was released from B3 and sulfate generation increased with increase in thiocyanate removal. Higher amount of sulfate compared to theoretical amount was detected in B3 which might be due to sulfur species generated in B2 got complete oxidation to sulfate in B3 [Table 4.21 (b)]

4.2.3.4 Overall performance of FMBR at varied HRT

Overall performance of FMBR system in terms of total COD, SCN^-, NH_4^+–N, total nitrogen and phenol removal are presented in Figure 4.55.

Phenol removal was complete up to 99.9% and COD removal was 96–97% and reached effluent discharged limit 1 mg/L and ≤ 250 mg/L, respectively. Thiocyanate removal was also more than 99.8% at total HRT 6 days and above but effluent contained almost 8 mg/L SCN^- when total HRT was only 5 days. Similarly, higher NH_4^+–N removal occurred at higher HRT and it was hampered during lower HRT. NH_4^+–N removal was 71% at total HRT 5 days and increased to more than 85–88% with increased total HRT of 6–10 days. Total nitrogen (TN) in influent and effluent of three–stage FMBR system was estimated using equation 4.7 and influent TN was ~1688 mg/L (considering influent NO_3^-–N of 1000 mg/L added in the recycle of B3). Figure 4.55 shows that TN removal was 63–72% and it increased with decrease in HRT. Unoxidized nitrogen fraction in final effluent was 5–12% being higher at low HRT whereas oxidized nitrogen fraction in effluent increased from

15% to 30% with increase in HRT as shown in Figure 4.55. The minimum HRT of the three–stage FMBR system should be maintained at 6 days or above to handle influent phenol, COD, SCN^- and NH_4^+–N of 1500, 5400, 800 and 500 mg/L, respectively.

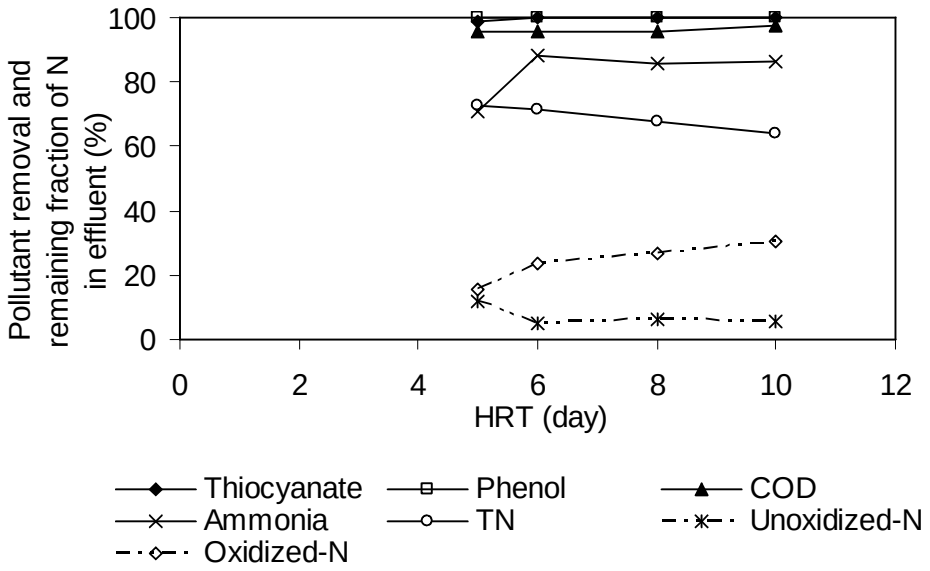

Figure 4.55 Effect of total HRT on performance of FMBR system

4.2.4 Performance of FMBR system at varied cycle time

Each reactor volume of B1–B2–B3 was maintained constant at 10L for the study of cycle period variation study with constant feed. The experiment was conducted almost 145 days from 806th day to 950th day. During cycle time variation, HRT of each reactor also changed according to equation 3.5, due to change in number of cycles per day and constant volume of influent/ effluent in each cycle. Decanted volume was 3.33 L for B1 and 6.66 L for B2 and B3 each.

4.2.4.1 Performance of anaerobic FMBR (B1) at varied cycle time

B1 showed removal of phenol and COD without any removal of SCN^- and NH_4^+–N during the study. Table 4.22 shows that when cycle time was increased from 18 to 36 h, reactor

HRT increased from 2.25 days to 4.5 days. Phenol and COD loadings decreased from 0.666 to 0.333 g phenol/L.day and from 2.4 to 1.2 g COD/L.day at constant influent concentration of 1500 and 5400 mg/L, respectively. With increase in cycle time form 18 to 36 h and reactor HRT, phenol removal increased from 50 to 81% and COD removal also increased from 24 to 41%. Up to cycle time 24 h, phenol removal was 50–53% and further increase in cycle time to 30–36 h, phenol removal in B1 increased from 71–81%. Similarly, COD removal was only 24% at minimum cycle time of 18 h, and it increased to 33–41% when cycle time was increased to 24 h and more. The maximum phenol removal rate of 0.333 g/L.day was achieved at the minimum cycle time of 18 h from maximum phenol loading of 0.666 g/L.day (Figure 4.56).

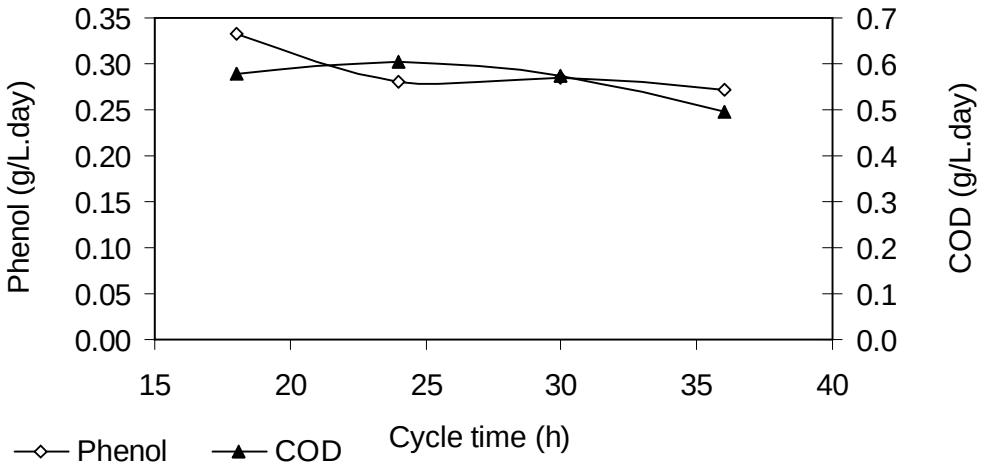

Figure 4.56 Phenol and COD removal rate in B1 at varied cycle time

COD removal rate was 0.495–0.603 g/L.day and maximum removal rate was observed to be 0.603 g/L.day at cycle time of 24 h at loading of 1.8 g COD/L.day (Figure 4.56). Influent concentration of the reactor was exposed to higher HRT at higher cycle time and it accelerated the performance of the same (Moussavi et al. 2009). Siman et al. (2004) and Oliveria et al. (2010) also reported an anaerobic sequencing biofilm batch reactor (ASBBR) performed better at longer cycle time (12 and 24 h) than short cycle time (8 h), allowing more time for the biomass to degrade the pollutants whose metabolic routes are slower and more complex when same organic load was introduced. B1 was significantly efficient for phenol and COD removal at cycle time 24 h or more.

Table 4.22: Performance of anaerobic fed batch MBR (B1) at cycle period variation

Cycle time (h)	HRT (day)	Phenol		COD				NH_4^+–N	SCN^-	pH	TVS (mg/L)
		S_e	Rem	S_0	Loading (g/L.day)	S_e	Rem	S_e	S_0	S_e	
18	2.25	750 (4)	50.00	5400	2.40	4097 (95.7)	24.13	500	800	7.0	9960
24	3.00	700 (18.8)	53.33		1.80	3587 (95.75)	33.57	500		6.8	10235
30	3.75	430 (3)	71.33		1.44	3250 (88)	39.81	500		6.8	10500
36	4.50	278 (7.6)	81.47		1.20	3170 (112)	41.30	500		6.8	10700

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%).

Influent phenol, SCN^- and NH_4^+–N was 1500, 800 and 500 mg/L, respectively.

Numbers in parenthesis indicate standard deviation values

Suspended biomass and attached biomass concentration in B1 was monitored and found to be stable at 900–1000 mg/L and 9000–10000 mg/L, respectively through out the study. Similarly the ratio of attached to suspended biomass was 9.5, 10, 10 and 9.2 at cycle time 18, 24, 30 and 36 h, respectively and total biomass in B1 was 10000–10580 mg/L.

4.2.4.2 Performance of anoxic FMBR (B2) at varied cycle time

Influent to B2 contained phenol, SCN^-, NH_4^+–N, NOx-N (NO_3^-–N + NO_2^-–N) and COD (Equation 3.4). Average performance of B2 at varied cycle time is shown in Tables 4.23 (a) and (b). HRT of B2 increased from 1.12 day to 2.25 days with increase in cycle period. With increase in cycle time, influent phenol and COD to B2 decreased as phenol and COD removal efficiencies in B1 improved and was responsible for lower influent phenol and COD concentration in B2. Influent phenol and COD to B2 varied from 139–375 mg/L and 1700–2200 mg/L, respectively with loadings of 0.062–0.333 g phenol/L.day and 0.755–1.961 g COD/L.day. Figure 4.57 shows that phenol removal was complete up to loading of 0.233 g phenol/L.day (cycle time 24–36 h) and decreased to 95% at loading of 0.333 g

phenol/L.day (cycle time 18 h). COD removal in B2 was ~80% at COD loading of 0.758–0.927 g COD /L.day (30–36 h cycle time) and decreased to 66% with increase in COD loading 1.276–1.961 g/L.day at low cycle time of 18–24 h. Maximum phenol and COD removal rates in B2 were 0.315 g/L.day and 1.295 g/L.day, respectively with maximum loading at cycle time 18 hour. Sarfaraz et al. (2004) reported decrease in phenol and COD removal in an SBR in short cycle period with phenol 550–950 mg/L with COD loading 1.5–4.6 g/L.day when treated at cycle period 12–6 h.

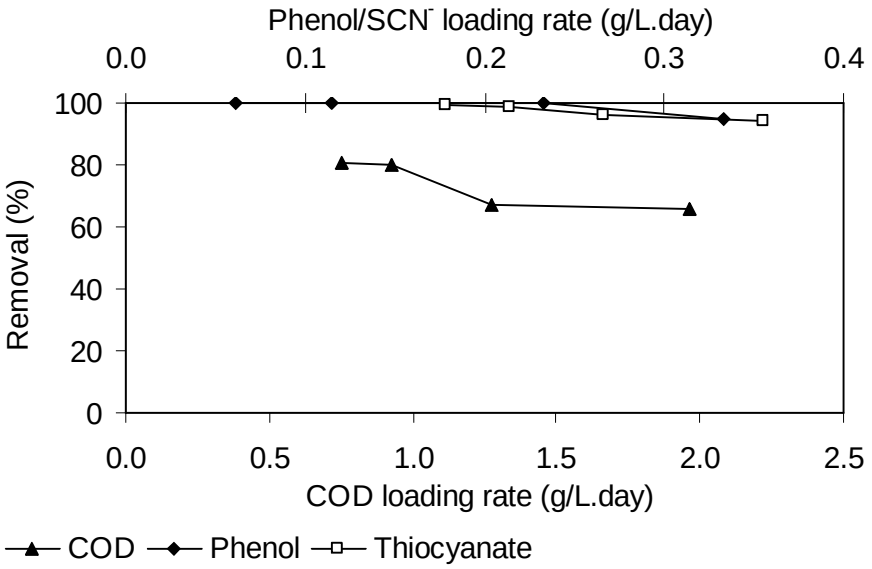

Figure 4.57 Phenol, thiocyanate and COD removals in B2 at varying cycle time

Influent SCN^- to B2 was almost constant at ~400 mg/L and thiocyanate loading was 0.18–0.35 g/L.day. Almost 99% removal efficiency was achieved up to loading of 0.213 g SCN^-/L.day at cycle time 30–36 h and SCN^- removal decreased to 94–96% at further increase in loading to 0.267–0.355 g SCN^-/L.day (Figure 4.57). Thiocyanate removal rate in B2 increased from 0.177 g/L.day to 0.335 g/L.day with increase in SCN^- loading. Maximum SCN^- removal rate was 0.335 g SCN^-/L.day at maximum loading of 0.355 g SCN^-/L.day at 18 h cycle period (Figure 4.58).

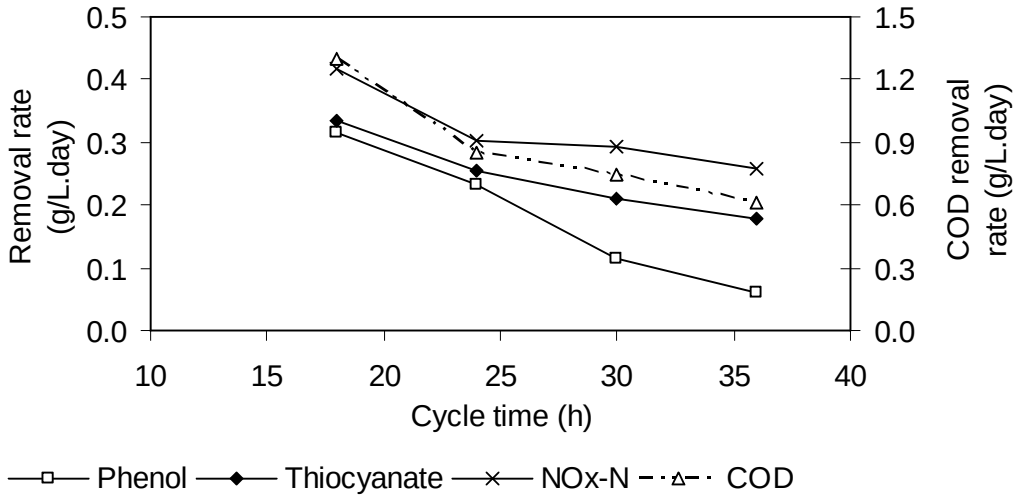

Figure 4.58 Pollutant removal rate in B2 at varied cycle time

Table 4.23 (a): Performance of anoxic fed batch MBR (B2) at cycle period variation

Cycle Time (h)	HRT (day)	Thiocyanate			Phenol			COD			NH_4^+–N		
		S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem
18	1.12	400	23 (5)	94.25	375	20 (2)	94.67	2208	750 (6)	66.04	396	380 (1)	4.1
24	1.50	400.5	16 (0)	96.00	350	0 (0)	100.00	1916	635 (7)	66.86	382	370 (3)	3.2
30	1.87	400.5	4 (2)	99.00	215	0 (0)	100.00	1740	350 (3)	79.89	380	370 (3)	2.67
36	2.25	400.5	2 (2)	99.50	139	0 (0)	100.00	1700	330 (5)	80.59	380	340 (11)	10.7

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%)

[A] Influent NH_4^+–N of B2 =

{Effluent NH_4^+–N of (B1+B3)/2 + 0.24x (SCN^- removed in B2)}.

Numbers in parenthesis indicate standard deviation values.

B2 received nearly 380–396 mg/L influent NH_4^+–N during the study and almost 3–10% NH_4^+–N removal occurred. Higher NH_4^+–N removal of 10% was observed at higher cycle time of 36 hour and released 340–380 mg/L NH_4^+–N in effluent.

Influent nitrate and nitrite to B2 was 618–707 mg/L and 30–36 mg/L, respectively. NOx^-–N loading rate with increased cycle time (increased HRT) decreased from 0.579 g/L.day to 0.327 g/L.day. NOx–N removal was observed to increase from 65 to 79% with increase influent concentration and cycle time and removal rate was 0.258–0.417 g/L.day. Incomplete denitrification in B2 suggests existing of anoxic condition in B2. It was probably due to lower availability of organic carbon in B2. Low COD:N_{rem} ratio towards longer cycle period more than 24 hour was observed. The COD:N_{rem} ratio found to decrease from 3.3 to 2.9 with increase in cycle period 18 hour to 24 hour and then further decreased to 2.2–2.5 showing more consumption of NO_3^-–N during the longer cycle time. COD fraction for biomass was 2–15% and biomass yield coefficient calculated was 0.04–0.11. Suspended biomass concentration was fluctuating between 3300–3700 mg/L being less (3300 mg/L) at 18 hour cycle. Attached biomass also remain stable at nearly 9000-9800 mg/L and the attached biomass to suspended biomass was 2.9 at cycle period 18 and 24 hour and then decreased to 2.4 at cycle period 30 hours and above. Total biomass concentration was ~12-13 g/L during the study.

Table 4.23 (b): Performance of anoxic fed batch MBR (B2) at cycle period variation

Cycle Time (h)	NO_3^-–N		NO_2^-–N		NO_x^-–N	COD / N_{rem}	pH	TVS (mg/L)	Sulfate				
	S_0	S_e	S_0	S_e	Rem				S_0	S_e	Gen	Th SO_4^{2-}	Err
18	618	180 (13)	35	3 (0)	70.85	3.3	8.1 ±0.2	13200	400	750 (27)	350	623	–272
24	664	245 (2)	36	2 (1)	64.71	2.9	8.2 ±0.2	13172	485	932 (29)	447	635	–187
30	700	180 (23)	32	5 (0)	74.73	2.5	8.4± 0.2	12100	540	1020 (49)	480	654	–174
36	707	150 (17)	30	5 (0)	78.98	2.2	8.4± 0.2	12400	605	1170 (17)	565	657	–92

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%); Gen: Generation (mg/L);

Th SO_4^{2-}: Theoretical sulfate generation; Err: Error (mg/L)

Numbers in parenthesis indicate standard deviation values.

Sulfate generation in B2 was observed to increase with increase in cycle time. Influent sulfate in recycle to B2 was 400–605 mg/L and B2 released 750–1170 mg/L with 350–565 mg/L sulfate generation. During low cycle period thiocyanate removal rate was significantly higher than that of longer cycle period and the error of experimental sulfate generation and theoretical sulfate generation was observed to be higher during this stage similar to CMBR study. The error was observed to decrease with increase cycle time.

4.2.4.3 Performance of aerobic FMBR (B3) at varied cycle time

Average performance of B3 at varied cycle time is presented in Tables 4.24 (a) and (b). B3, being the last reactor of the series received very less amount of SCN^- (2–23 mg/L) and residual COD (330–750 mg/L). HRT of B3 increased from 1.12–2.25 day with increase in cycle time 18–36 h and loading of pollutant to B3 decreased with increase in cycle time. Removal rate of basic pollutants entered in B3 decreased with decreased loading at higher cycle time as shown in Figure 4.59. The residual SCN^- was removed completely in B3 releasing ~ 1mg/L in effluent and no phenol was observed in influent or effluent.

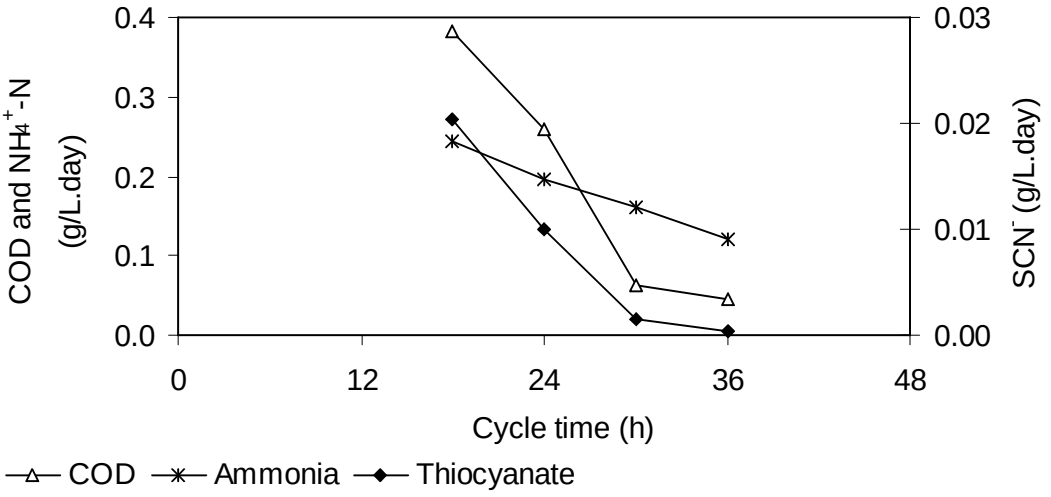

Figure 4.59 Pollutant removal rate in B3 at varied cycle time

Figure 4.60 shows that COD removal in B3 initially increased from 30% to 61% with increase in COD loading from 0.146 to 0.423 g/L.day. Further increase in loading to 0.666 g COD/L.day caused slight decrease in COD removal to 57% and effluent was less than 250 mg/L at cycle time 24–36 h.

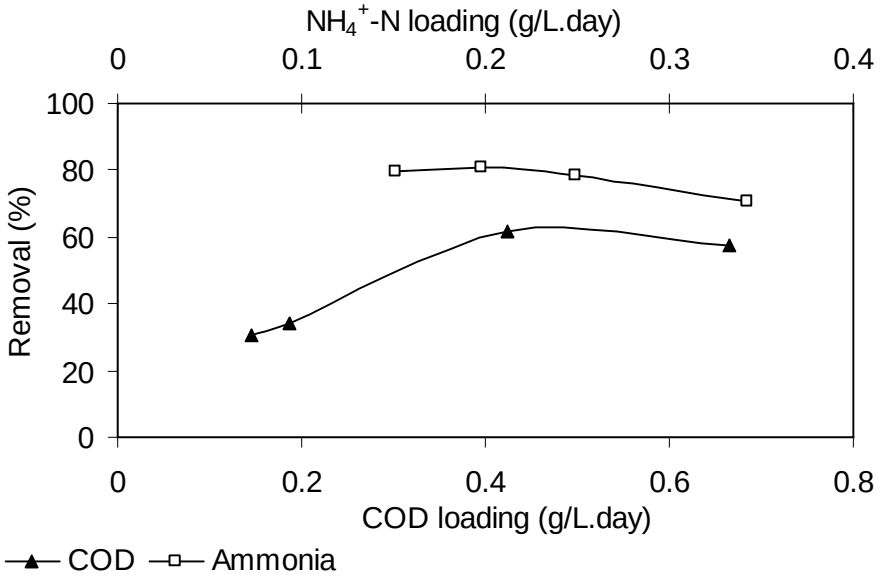

Figure 4.60 COD and Ammonia-N removal in aerobic FMBR (B3) at varied cycle time

Table 4.24 (a): Performance of aerobic fed batch MBR (B3) at cycle period variation

Cycle Time (h)	HRT (day)	SCN^-			COD			NH_4^+–N			COD: NH_4^+–N	TVS (mg/L)	pH
		S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem			
18	1.12	23	0 (0)	100	750	320 (63)	57.33	380	112 (1)	70.00	1.9	10500	8.2±0.2
24	1.50	5	1 (0)	80	635	245 (32)	61.42	370	80 (0)	78.38	1.7	9200	8.2±0.2
30	1.87	4	1 (0)	75	350	230 (28)	34.29	370	70 (0)	81.08	0.94	10200	8.2±0.2
36	2.25	2	1 (0)	50	330	230 (56)	30.30	340	70 (0)	79.41	0.97	10900	8.2±0.2

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B3 = {Effluent NH_4^+–N of B2 + 0.24x (SCN^- removed in B3)}.

Numbers in parenthesis indicate standard deviation values.

High concentration of NH_4^+–N (340–380 mg/L) entered in B3 as very low removal occurred in the upstream reactors. NH_4^+–N removal efficiency also followed similar trend like COD (Figure 4.60). It increased to 81% removal up to loading of 0.197 g NH_4^+–N

/L.day and then decreased to 70% at loading of 0.342 g/L.day. Cycle time of 30 h and HRT of 1.87 days provided maximum NH_4^+–N removal efficiency. NH_4^+–N removal in B3 was incomplete throughout the cycle time of 18–36 h. This might be due to higher concentration peak and the nitrifiers could not deal with the toxicity level. COD and NH_4^+–N removal rates increased and were 0.044–0.382 g/L.day and 0.120–0.243 g/L.day, respectively that increased with increase loading. Kim et al. (2008a) achieved 87% NH_4^+–N removal efficiency at loading of 0.17 g/L.day in aerobic reactor in anoxic–aerobic suspended growth system, which was comparable with present findings. However, the influent NH_4^+–N was much lower (82–128 mg/L) than present study (500 mg/L).

Table 4.24 (b): Performance of aerobic fed batch MBR (B3) at cycle period variation

Cycle Time (h)	HRT (day)	NO_3^-–N		NO_2^-–N		N_R	FA	Sulfate				
		S_0	S_e	S_0	S_e			S_0	S_e	Gen	Th SO_4^{-2}	Err
18	1.12	180	235 (7.8)	3	70 (0)	0.111	36.6	750 (27)	800 (23)	50	36.3	13
24	1.50	245	328 (17)	2	77 (0)	0.110	33.4	932 (2.9)	970 (33)	38	24.7	13
30	1.87	180	400 (12)	5	64 (3)	0.151	32.4	1020 (49)	1080 (11)	60	4.9	55
36	2.25	150	415 (12)	5	60 (10)	0.144	30.2	1170 (17)	1210 (59)	40	1.65	38

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: Generation (mg/L);
N_R: Nitrification rate (g/L.day); FA: Free ammonia (mg/L)
Th SO_4^{-2}: Theoretical sulfate generation; Err: Error (mg/L)
Numbers in parenthesis indicate standard deviation values.

B3 showed incomplete nitrification with high effluent NO_2^-–N at all cycle times. Nitrification rate in B3 was 0.111–0.151 g/L.day. Nitrification rate increased from 0.11 g/L.day at cycle time 18–24 h to 0.14–0.15 g/L.day at cycle time 30–36 h. In present study free ammonia (FA) calculated was 30–36 mg/L. Almost 34–41% unaccounted nitrogen was calculated at shorter cycle time of 18–24 h.

Influent COD/ NH_4^+–N ratio in B3 was ~1 at higher cycle time as almost equal amount of COD and NH_4^+–N entered to B3. However the ratio increased to 1.6–1.9 at cycle time 18–24 hour with higher COD influent. Higher concentration of suspended biomass ~4000 mg/L was observed in B3 at cycle time 30–36 hour which decreased to 2300–3200 mg/L at low cycle time. Contrary, attached biomass was fluctuating within 6500–7300 mg/L and tentative total biomass was 9200–10900 mg/L during the study. Attached to suspended biomass ratio was ~1.6 at cycle time 30–36 hour and 2.2–2.9 at cycle time 18–24 hour.

Influent sulfate to B3 was 750–1170 mg/L and B3 released 800–1210 mg/L sulfate with ~40–60 mg/L sulfate generation in B3. Higher sulfate generation was observed at longer cycle period. The error between experimental and theoretical sulfate generation was low in B3 compared to B2.

4.2.4.4 Overall performance of fed batch MBR system at varied cycle time

Overall performance of sequential anaerobic–anoxic–aerobic fed batch reactor system at varying cycle time was calculated from values of feed and final effluent of B3 and shown in Figure 4.61. Total HRT of the system was 4.5, 6, 7.5 and 9 day when cycle time was 18, 24, 30 and 36 h respectively. Phenol and SCN^- removal were complete irrespective of cycle time. COD removal increased slightly with increase in cycle time from 94 to 96%. Effect of cycle time was significant on removal of NH_4^+–N and it increased from 77.6 to 86% with increase in cycle time. Total nitrogen (TN) in feed was 1692 mg/L ($\sum NH_4^+$–N + NO_3^-–N + NO_2^-–N + SCN^-–N) throughout the study. Behavior of TN was different than other parameters as TN removal declined with increase in both cycle. TN is summation of unoxidized (NH_4^+–N) and oxidized nitrogen (NO_3^-–N and NO_2^-–N), and presence of former one is more objectionable than later one. With increase in cycle time oxidized nitrogen fraction increased from 18 to 28% and NH_4^+–N fraction decreased little from 6 to 4%. Increase of oxidized nitrogen and decrease of NH_4^+–N was almost negligible above cycle time of 30 h. Corresponding HRT of B1, B2 and B3 were 3.75, 1.88 and 1.88 days, respectively with total HRT of 7.51 days.

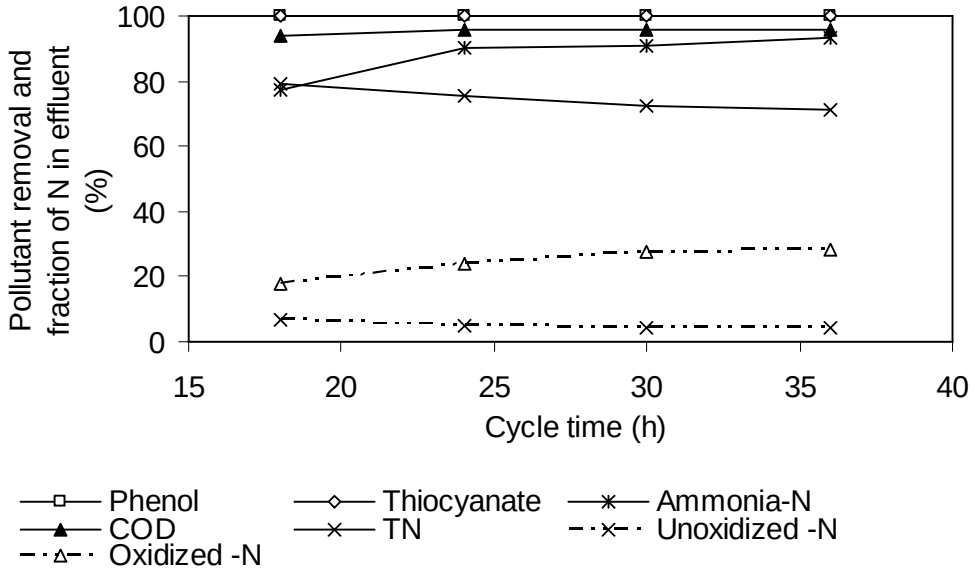

Figure 4.61 Total performance of FMBR at varied cycle time

4.2.5 Performance of FMBR system at varied concentration of feed phenol

The study was conducted with synthetic feed containing various phenol concentrations from 1000 mg/L to 2500 mg/L along with SCN^- (800 mg/L) and NH_4^+–N (500 mg/L). NO_3^-–N was added in recycle (1000 mg/L as 7201 mg KNO_3/L) from 951st to 1062nd day. Feed pH was maintained at 7.5 ± 0.2 by using phosphate buffer (KH_2PO_4 72.3 g/L and K_2HPO_4 104.5 g/L). Yeast extract of 20–50 mg/L and trace metals solution of 1 mL/L feed were added as nutrients. Feed COD was in the range of 4200–8150 mg/L during the study. Total system HRT was 6 days (HRT of B1: 3 days; B2: 1.5 day and B3: 1.5 day). Dissolved oxygen concentrations (mg/L) in the bioreactors were: 0 (B1 and B2) and 4–4.5 (B3). Influent and effluent profile of various parameters during feed phenol variation is presented in Figure 4.62.

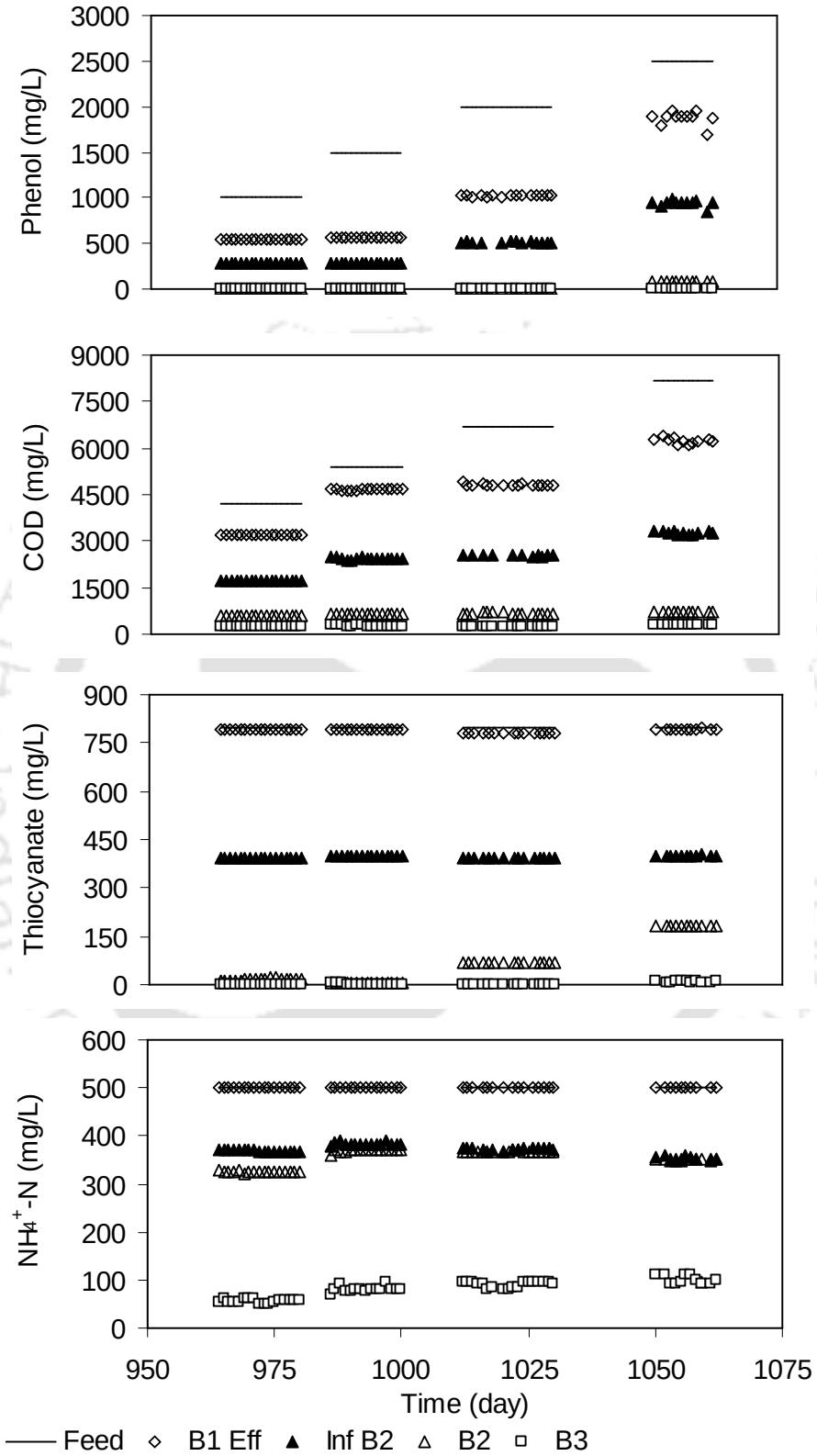

Figure 4.62 Pollutant profile in FMBR during phenol variation study

4.2.5.1 Performance of anaerobic FMBR (B1) at phenol variation

Steady state performance of B1 at varied influent phenol concentrations is presented in Table 4.25. The feed phenol concentration was varied at four levels (1000, 1500, 2000 and 2500 mg/L) and phenol loading was 0.333–0.833 g/L.day. Phenol removal in B1 was ~52–53% at influent phenol concentration 1000–1500 mg/L and decreased to 49–25% at influent phenol concentration 2000–2500 mg/L. Phenol removal rate initially increased from 0.173 to 0.327 g/L.day when influent concentration was increased from 1000–2000 mg/L and then decreased to 0.206 g/L.day with further increase in influent feed phenol (Figure 4.63). Contribution of B1 in phenol removal was higher than B2 and B3 when influent phenol concentration was 1000–2000 mg/L [Figure 4.64 (a)].

Table 4.25: Performance of anaerobic fed batch MBR (B1) at feed phenol variation

Phenol			SCN^-		COD			NH_4^+ –N	pH	TVS (mg/L)	VFA (mg/L)
S_0	S_e	Rem	S_e	Rem	S_0	S_e	Rem	S_e	S_e		
1000	480 (16)	52.00	790 (0)	1.25	4200	3210 (11)	23.57	500 (0)	6.8	10660 (350)	232 (50)
1500	700 (26)	53.33	790 (0)	1.25	5400	3587 (46)	33.57	500 (0)	6.9	10235 (335)	413 (15)
2000	1020 (10)	49.00	800 (0)	0.00	6710	4961 (35)	26.07	500 (0)	6.8	11016 (286)	698 (67)
2500	1881 (76)	24.76	800 (0)	0.00	8150	6241 (10)	23.42	500 (0)	6.7	10857 (404)	1500 (180)

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

Feed thiocyanate and NH_4^+–N were 800 and 500 mg/L, respectively at 3 days HRT

Numbers in parenthesis indicate standard deviation values.

With increase in feed phenol concentration, influent COD increased from 4200–8150 mg/L and corresponding loading rate was 1.400–2.716 g COD/L.day. COD removal in B1 increased 23% to 33% with increase in feed phenol concentration of 1000–1500 mg/L and then decreased to 23% with further increase in feed phenol/COD. Also, with increase in

feed phenol, COD removal rate increased from 0.330 g/L.day to 0.636 g/L.day with increase in COD loading. B1 removed insignificant amount of SCN^- (0–1.25%) and no ammonia removal was observed. In B1, feed pH decreased from 7.5 to 6.7–6.9. Specific methanogenic activity of B1 was not detected in present study.

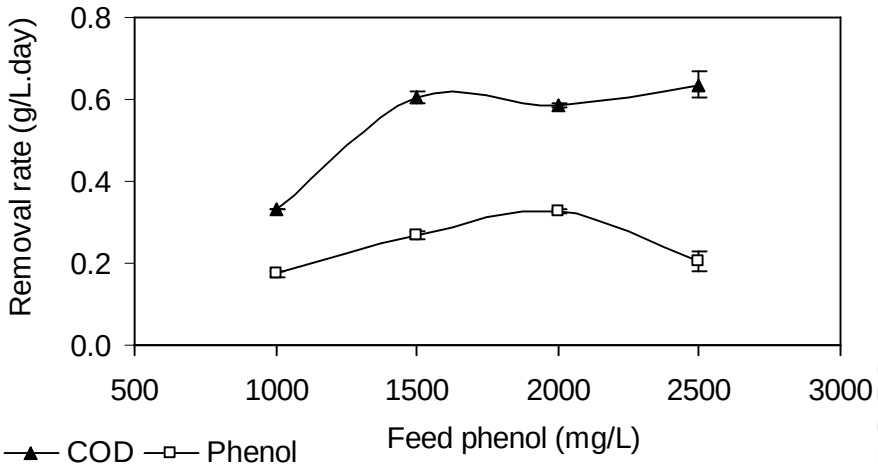

Figure 4.63 Effect of feed phenol on phenol and COD removal rate in B1

Tziotzios et al. (2005) reported that reactor operated at draw and fill mode achieved removal rates up to 12.65 g phenol/L.day, while the suspended growth continuous operation mode reactor achieved removal rates only 0.082 g phenol/L.day during treatment of feed phenol concentration 527 mg/L. Tay et al. (2001) reported the inhibitory nature of phenolic compounds at high concentration affects further enhancement of organic load with desired performance. Zhou and Fang (1997) reported simultaneous degradations up to 98% of phenol and 20% of m–cresol without any carbohydrate co–substrate, at phenol and m–cresol loading of 0.9 g/L.day and 0.320 g/L.day, respectively in an up flow anaerobic sludge blanket (UASB) reactor. Hirata et al. (2000) also reported COD reduction during anaerobic co–digestion of olive mill wastewater with pig slurry varied between 62% and 89% at OLRs of 0.20–0.44 g COD/L.day. In present study B1 showed higher phenol removal than that of suspended growth system even in presence of thiocyanate and ammonia.

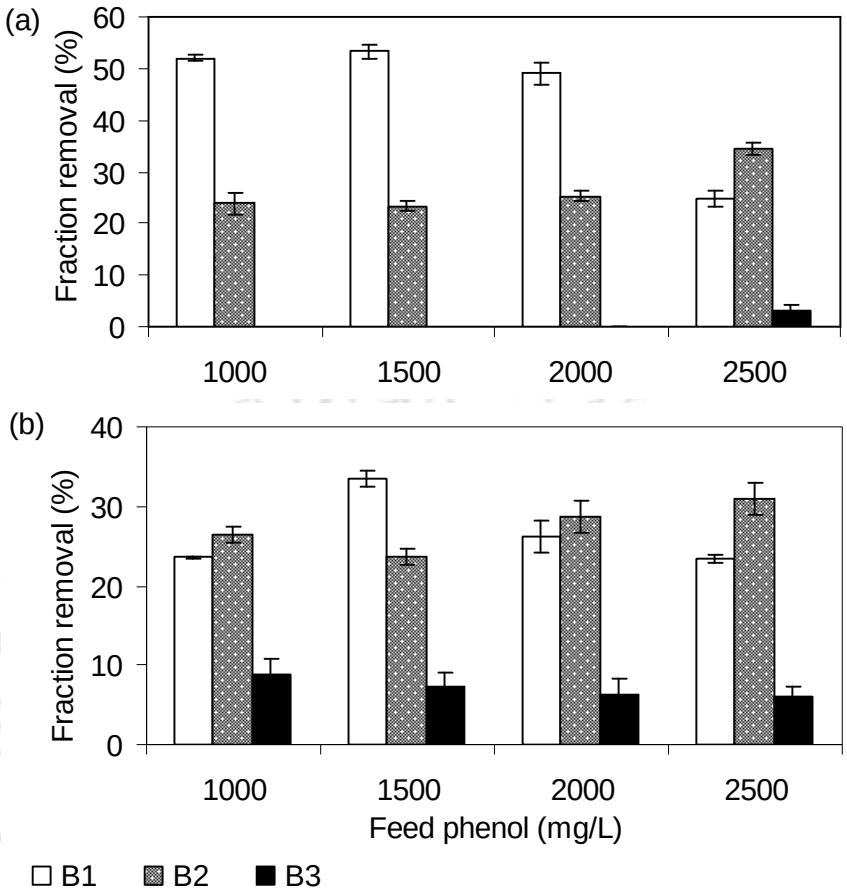

Figure 4.64 (a) Phenol and (b) COD removal by individual reactor at varied feed phenol concentration

Suspended and attached biomass concentration in B1 was 900–1000 mg/L and 9300–9890 mg/L, respectively during the feed phenol concentration variation study with insignificant change. The total biomass concentration was almost 10200–10800 mg/L with attached biomass to suspended biomass ratio of 9–10 thorough out the study. Immobilized microorganisms have been showed to be effective to treat phenol containing wastewater with little sludge production and have been receiving increasing attention.

4.2.5.2. Performance of anoxic FMBR (B2) at varied influent phenol

Influent of B2 consisted of effluent from B1 and recycle effluent from B3 (equation 3.4) at recycle ratio of 1. Steady state performance of B2 is given in Tables 4.26 (a) and (b). Influent phenol to B2 was 240-941 mg/L at a constant reactor HRT of 1.5 days. Up to influent phenol of 511 mg/L, phenol removal efficiency in B2 was 99% and decreased to

91% when influent phenol increased to 941 mg/L releasing 80 mg/L phenol. In B2, phenol loading was 0.160–0.627 g/L.day and removal rate linearly increased up to 0.574 g/L.day at highest loading of 0.627 g/L.day [Figure 4.65 (a)]. B2 removed 23–34% of total phenol during the study and it increased with increase in feed phenol concentration. Chakraborty and Veeramani (2006) reported phenol removal rate of 0.37 g/L.day in a suspended growth anoxic reactor at influent phenol loading of 0.45 g/L.day in presence of thiocyanate, cyanide and ammonia. Present study showed higher phenol removal rate.

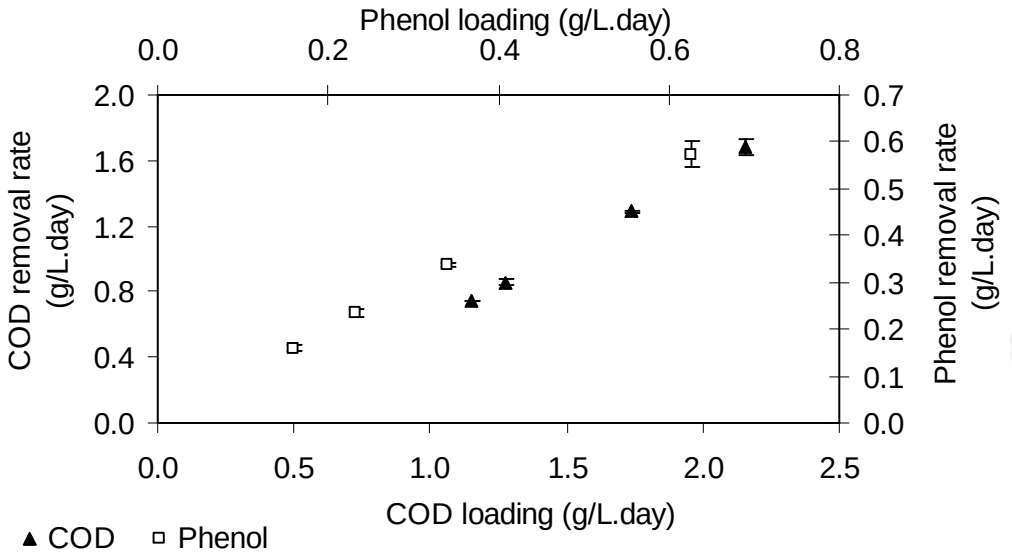

Figure 4.65 (a) Effect of phenol and COD loading rate in B2

With increase in feed phenol concentration, COD in B1 influent/effluent increased and correspondingly increased in B2 influent and COD loading rate was 1.151–2.158 g/L.day. COD removal increased 64–77% with increase influent concentration with maximum COD degradation rate 1.678 g/L.day at loadings of 2.158 g COD/L.day [Figure 4.65 (a)].

B2 removed thiocyanate simultaneously along with phenol. At a constant reactor HRT of 1.5 days and influent thiocyanate concentration 395–400 mg/L, thiocyanate removal in B2 decreased with increase in feed phenol. Thiocyanate removal in B2 decreased from 99% to 94% when influent phenol concentration increased from 240–511 mg/L and further decreased to 62% when influent phenol was 941 mg/L and phenol and SCN^- loading to B2 were 0.627 and 0.267 g/L.day, respectively. Thiocyanate removal rate decreased from 0.261 g/L.day to 0.167 g/L.day with increased phenol loading whereas thiocyanate loading

was almost constant at 0.263–0.267 g/L.day [Figure 4.65 (b)]. It is clear that phenol at higher concentration inhibited SCN^- removal in anoxic reactor.

Table 4.26 (a): Performance of anoxic FMBR (B2) at feed phenol variation

Phenol			Thiocyanate			COD			NH_4^+–N			TVS	pH
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem		
240	1 (0)	99.58	396	3 (1)	99.24	1728	616 (3)	64.37	372	325 (0)	12.5	12993 (455)	8.2
350	1 (0)	99.71	396	16 (0)	95.95	1916	635 (17)	66.86	381	370 (0)	2.9	13172 (995)	8.2
511	4 (0.5)	99.22	401	23 (0)	94.26	2604	673 (14)	74.16	356	366 (0)	5.1	13355 (274)	8.3
941	80 (0)	91.50	401	150 (3)	62.55	3238	720 (24)	77.76	360	350 (0)	2.8	12939 (223)	8.4

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

[A] Influent NH_4^+–N of B2 = {Effluent NH_4^+–N of (B1+B3)/2 + 0.24x (SCN^- removed in B2)}.

TVS: Biomass as Total volatile solids (mg/L)

Numbers in parenthesis indicate standard deviation values

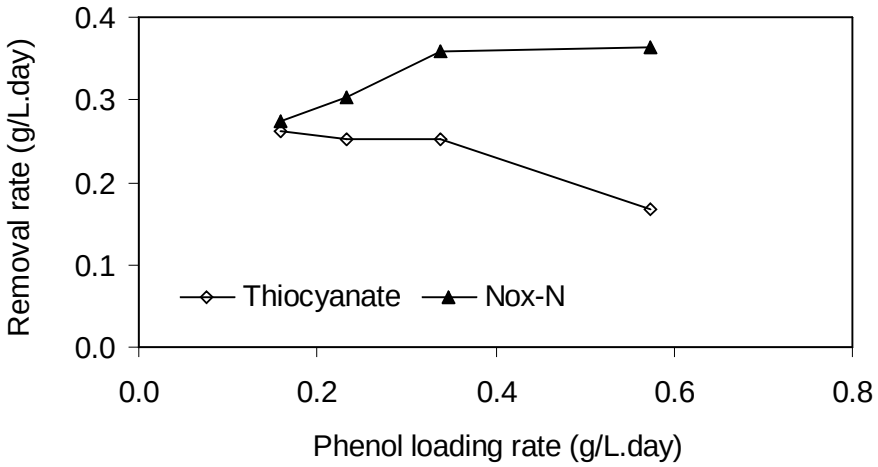

Figure 4.65 (b) Effect of phenol loading on thiocyanate and NOx-N removal rate in B2

From influent NH_4^+–N concentration 360–385 mg/L, nearly 2.8–12.5% NH_4^+–N removal occurred in B2 and maximum removal was observed in presence of low influent phenol and NH_4^+–N concentration of 240 mg/L and 325 mg/L, respectively.

COD generated from 1 g phenol and 1g SCN^- is equivalent to 2.38 g and 1.11 g, respectively. Theoretically, for each gram of phenol and SCN^-, required NO_3^-–N is 0.83 g and 0.38 g, respectively. In the present study, additional 1000 mg/L NO_3^-–N was supplied externally in recycle from B3. Influent NO_3^-–N and NO_2^-–N to B2 was 585–665 mg/L and 35–44 mg/L, respectively and the loading rate to B2 was 0.419–0.467 g NOx–N/L.day. In B2, nitrite was completely utilized whereas complete nitrate removal was not achieved and denitrification was incomplete throughout the study, indicating existence of complete anoxic condition. Effluent pH was 8.2–8.4 whereas influent pH which was 7.5–7.6, which was probably due to denitrification. NOx–N removal efficiency of B2 increased from 58% to 86% with increase in influent phenol and NOx–N removal rate was 0.273–0.362 g/L.day [Figure 4.65(b)]. Bajaj et al. (2010) reported 65% NO_3^-–N removal along with 81% COD removal together with phenol removal rate of 0.207 g/L.day at phenol loading rate 0.750 g/L.day. The organic loading rate of the denitrifying reactor was 4.3 g COD/L.day and HRT of 1 day. They also observed phenol degradation in batch assays under anoxic conditions and at low phenol concentrations (188 mg/L) preceded a removal rate of 1.2 g/L.day which decreased to 0.67 mg/L.day at high phenol concentration (847 mg/L).

Along with 2.8 to 12% NH_4^+–N removal, B2 contributed in 38 to 46% total nitrogen removal from the system. COD:N removed ratio in B2 increased from 2.9 to 5.3 with increase amount of COD removed at higher influent phenol study. COD fraction available for biomass synthesis also increased from 2% to 47% and the yield coefficient was 0.02-0.33.

Suspended biomass concentration in B2 was 3372–3475 mg/L at influent phenol 240–511 mg/L and then increased to 4411 mg/L when B2 received higher influent phenol concentration (941 mg/L). Contrary, attached biomass concentration was higher of 9300–9800 mg/L towards low influent phenol and lower of 8520 mg/L at high influent phenol concentration. Total biomass concentration in B2 was ~ 13000 mg/L during the study with attached biomass to suspended biomass ratio of 1.9–2.9 (higher at low influent phenol concentration). Attached biomass to suspended biomass ratio was 2.5–2.9 at influent phenol 240–511 mg/L and then decreased drastically to 1.9 when influent phenol concentration to B2 was 941 mg/L.

In present study, high amount of sulfate generation occurred (265–465 mg/L) from thiocyanate degradation through out wide range of influent phenol. However the sulfate generation was lower to theoretical sulfate generation from thiocyanate degradation might be due to accumulation of other sulfate products [Table 4.26 (b)].

Table 4.26 (b): Performance of anoxic FMBR (B2) at feed phenol variation

Phenol	NO_3^-–N		NO_2^-–N		NO_x^-–N	COD:	COD_B	SO_4^{2-}				
S_0	S_0	S_e	S_0	S_e	Rem	N_{rem}		S_0	S_e	Gen	Th SO_4^{-2}	Err
240	665	290 (3)	35	0	58.57	2.90	2.1	485	950 (26)	465	647	-182
350	664	245 (2)	36	2 (1)	65.0	2.91	2.2	485	932 (29)	447	626	-179
511	587	87 (5)	37	0	86.05	4.08	29	465	917 (11)	452	623	-170
941	585	85 (1)	44	0	86.49	5.35	47	475	740 (13)	265	413	-148

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%), Gen: generation (mg/L);
COD_B: COD fraction (%) for biomass;
Th SO_4^{-2}: Theoretical sulfate generation; Err: Error (mg/L)
Numbers in parenthesis indicate standard deviation values

4.2.5.3 Performance of aerobic FMBR (B3) at varied influent phenol concentration

Average steady state performance of B3 is shown in Tables 4.27 (a) and (b). B3 received maximum phenol concentration of 80 mg/L when feed phenol added was 2500 mg/L. Phenol removal in B3 was almost complete with effluent ~1 mg/L. Influent thiocyanate to B3 increased from 3 mg/L to 150 mg/L when feed phenol was increased from 1000 mg/L to 2500 mg/L. Influent COD to B3 was 616–720 mg/L with corresponding increased loading rate of 0.410–0.480 g/L.day. COD and thiocyanate in effluent was 235–245 mg/L and ~1 mg/L, respectively showing increased thiocyanate and COD removal efficiencies of 67–99% and 60–67%, respectively in presence of higher influent concentrations.

Maximum COD removal rate achieved in B3 was 0.323 g/L.day at COD loading rate of 0.480 g/L.day during the study (Figure 4.66).

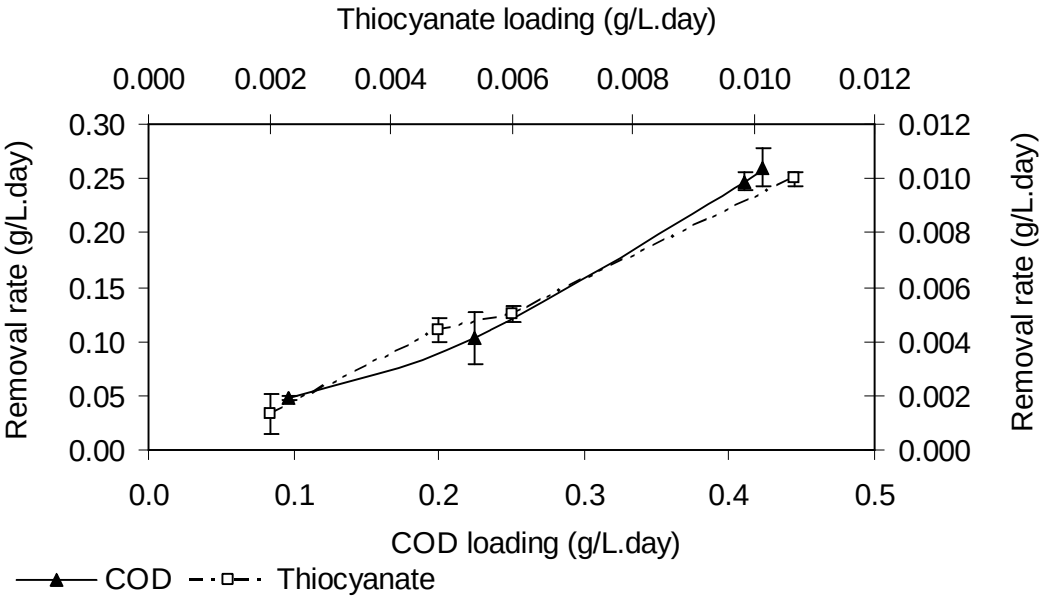

Figure 4.66 COD and thiocyanate removal rate in B3 corresponding to various loading during varied feed phenol

Maximum SCN^- removal rate of 0.099 g/L.day was achieved at loading rate of 0.10 g/L.day. Figure 4.66 shows that both COD and SCN^- removal rates in B3 increased almost linearly with increase in loadings of COD and SCN^-. Banerjee (1996), observed maximum SCN^- degradation rate of 0.2 g/L.day in a rotating biological contactor in presence of phenol. Sirianuntapiboon et al. (2007) reported COD and SCN^- removal efficiencies of ~96.0 and ~82%, respectively, under an SCN^- loading of up to 0.084 g/L.day in an SBR treating photo–processing wastewater (PPWW). They observed that removal efficiency was affected by SCN^- due to the decrease growth rate of nitrification bacteria and the removal efficiency could be recovered with increase in HRT or decrease in SCN^- loading. Contribution of B3 in thiocyanate and COD removal was 0.2–18.6% and 5–8%, respectively during the study.

Besides, influent NH_4^+–N received from B2, some amount of NH_4^+–N (~0.4–35 mg/L) was also generated in B3 from degradation of SCN^- and final influent NH_4^+–N accounted was 325–386 mg/L. NH_4^+–N loading to B3 was 0.217–0.257 g/L.day at constant HRT of 1.5 days. NH_4^+–N removal rate in B3 achieved was 0.180–0.195 g/L.day at loading rate of 0.217–0.249 g NH_4^+–N/L.day and it decreased to 0.190 g/L.day at further increase in

NH_4^+–N loading to 0.257 g/L.day in presence of phenol and thiocyanate concentration of 80 mg/L and 150 mg/L, respectively. With increase in feed phenol 1000 to 2500 mg/L (influent phenol to B3: 1–80 mg/L) effluent NH_4^+–N increase 55 to 100 mg/L (increase by 82%). Table 4.27 (a) shows that NH_4^+–N removal efficiencies in B3 continuously decreased from 83% to 74% with increased influent phenol and thiocyanate concentration.

Table 4.27 (a): Performance of aerobic FMBR (B3) at phenol concentration variation

Phenol			Thiocyanate			COD			NH_4^+–N			pH	COD: NH_4^+–N
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem	S_e	
1	0	100	3	1 (0)	67	616	245 (3)	60	325	55 (0)	83.1	8.1	1.9
1	0	100	16	1 (0)	94	635	245 (3.7)	61	374	80 (1.6)	78.6	8.1	1.7
4	1 (0)	75	23	1 (0)	96	673	247 (7.5)	63	371	90 (0)	75.8	8.3	1.8
80	1 (0)	99	150	1 (0)	99	720	235 (12)	67	386	100 (1.8)	74.1	8.4	1.8

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B3 = {Effluent NH_4^+–N of B2 + 0.24x (SCN^- removed in B3)}.

Numbers in parenthesis indicate standard deviation values

Decrease in NO_3^-–N concentration from 330 mg/L to 170 mg/L and nitrite concentration of 70–88 mg/L was observed in effluent of B3 during the study with increase in feed phenol from 1000–2500 mg/L. Nitrification rate in B3 was calculated based on generation of NO_3^-–N and NO_2^-–N and reactor HRT and found to be 0.073–0.115 g/L.day. Nearly 18–43 mg/L free ammonia was calculated in B3. Higher influent phenol and SCN^- concentration and free ammonia might be responsible for decrease NH_4^+–N removal efficiency and nitrite accumulation in B3 which is too a nitrification inhibitor. Higher unaccounted nitrogen of 29–50% was observed at low influent phenol study similar to the observation in feed phenol variation in CMBR. With increase in influent COD/phenol, influent NH_4^+–N to B3 also observed to increase and COD/NH_4^+–N ratio was 1.7–1.9

during the study. Suspended biomass concentration continuously decreased from 2815 to 2100 mg/L and increase in attached biomass 7340–8800 mg/L was observed with increase in influent COD to B3. Total biomass concentration in B3 increased from 10–11.9 g/L during the study and attached to suspended biomass ratio increased from 2.6 to 2.9, 3.3 and 4.2 during high feed phenol study.

Table 4.27 (b): Performance of aerobic FMBR (B3) at phenol concentration variation

Phenol	NO_3^-–N		NO_2^- –N	N_R	SO_4^{-2}					FA	UN	TVS (mg/L)
S_0	S_0	S_e	S_e		S_0	S_e	Gen	Th SO_4^{-2}	Err			
1	290	330 (10)	70 (0)	0.073	950	970 (13)	20	3.3	16	18.68	50	10163
1	245	328 (21)	72 (0.5)	0.103	932	970 (33)	38	24.75	13	22.27	39	10221
4	87	173 (18)	74 (0.6)	0.107	917	930 (13)	13	36.3	–23	33.94	29	11135
80	85	170 (7)	88 (0.6)	0.115	740	950 (0)	210	245.8	–36	43.35	13	11944

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L); Err: Error (mg/L)

Th SO_4^{-2}: Theoretical sulfate generation (1.65x SCN^- removed in B3);

N_R: Nitrification rate (g/L.day);

FA: Free ammonia (mg/L); UN: Unaccounted nitrogen (%)

Numbers in parenthesis indicate standard deviation values

4.2.5.4 Overall performance of three–stage FMBR system at varied feed phenol concentration

The feed and final effluent of B3 was considered to estimate overall performance of three–stage FMBR system. The overall performance of the three–stage FMBR at varying feed phenol concentrations is shown in Figure 4.67 in terms of COD, SCN^-, NH_4^+–N and phenol removal. Phenol and thiocyanate removal during the study was more than 99% and

COD removal increased from 94% to 97% with increase in influent COD releasing 245–235 mg/L effluent COD (from influent 4200–8150 mg/L) irrespective of influent phenol concentration. NH_4^+–N removal in three–stage system decreased 92% to 85% with increase in feed phenol. Feed TN was 1692 mg/L (considering influent NO_3^-–N of 1000 mg/L added in the recycle of B3). TN removal increased from 73% to 80% with increase in phenol concentration. Figure 4.67 shows that oxidized fraction of nitrogen was ~23% being higher towards low feed phenol study and decreased to 14–15% at high feed phenol and unoxidized nitrogen fraction was 3–5%. Present result showed that the inhibitory effect of feed phenol above 1500 mg/L on phenol and COD removal in anaerobic reactor was very profound in presence of SCN^- and NH_4^+–N. However, the response of increased feed phenol up to 1500 mg/L on the overall performance of the three–stage FMBR system was quite insignificant, as B2 was highly efficient and helped to improve the overall performance of the three–stage system.

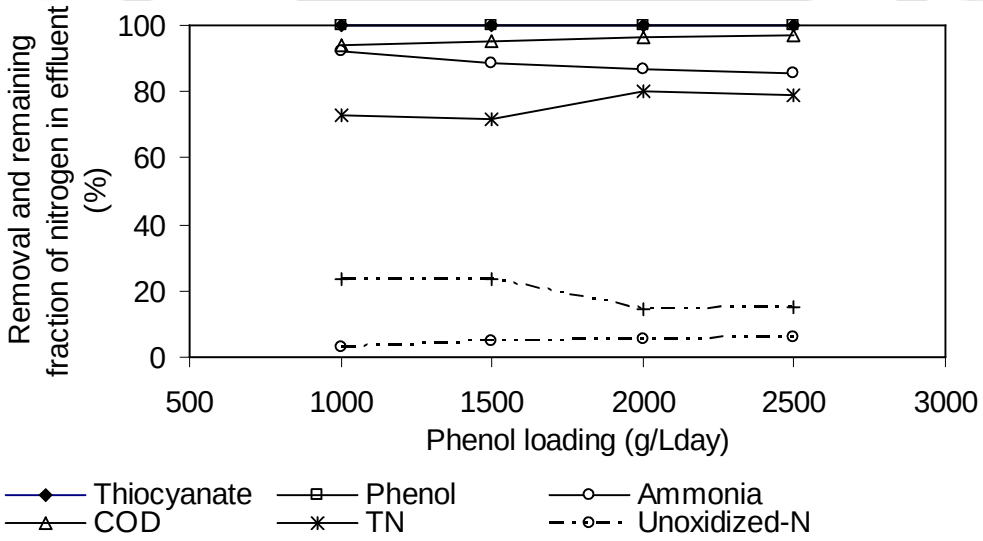

Figure 4.67 Overall performance of FMBR at varied phenol concentration

4.2.6 Performance of FMBR system at varied concentration of feed pyridine

Present study was carried out with feed pyridine as variable parameter in presence of phenol, thiocyanate and NH_4^+–N in sequential anaerobic–anoxic–aerobic FMBR system. Four experimental runs were conducted at varying feed pyridine concentration at 25, 50, 100, and 250 mg/L at constant total HRT of 6 days (B1: 3 days; B2 1.5 day and B3 1.5 day) from 1064th to 1220th day. Feed NH_4^+–N, SCN^- and phenol were constant throughout the study at 500, 800 and 1500 mg/L, respectively with COD 5400–5430 mg/L. Nitrate–N 1000 mg/L was added in the recycle that was supplied to anoxic reactor. To evaluate the effect of pyridine on the fed batch MBR system, pyridine was introduced to B1 at a concentration of 5 mg/L on 1064th day and gradually increased to 25 mg/L in 15 days. Stable effluent concentration was achieved with in 5 days and data were collected for 10–15 days. Then influent pyridine concentration was increased to 50 mg/L (1120th day) in a likely manner and later increased further to 100 mg/L (1155th day) and 250 mg/L (1195th day). The experimental values with feed pyridine variation observed during the study are shown in Figure 4.68 (a–f). It was observed that after each modification in feed pyridine concentration, the three–stage system released steady effluents after a transient period of 8–10 days. Steady state data was collected for 10–15 days and considered to evaluate the performance of each reactor for phenol, thiocyanate, COD, NH_4^+–N and NOx–N.

4.2.6.1. Performance of anaerobic FMBR (B1) at pyridine concentration variation

Steady state performance of B1 at various influent pyridine concentrations is presented in Table 4.28 as average values along with standard deviation. Liu et al. (1994) reported pyridine mineralization in anaerobic conditions with generation of carbon dioxide, methane and ammonia. During pyridine variation study, pyridine loading rate to B1 was 0.008–0.083 g/L.day. B1 removed 10–14% pyridine at influent concentration 25–100 mg/L and it decreased with increase in influent pyridine concentration from 25 mg/L to 50–100 mg/L and no pyridine removal occurred at influent pyridine concentration of 250 mg/L [Figure 4.69 (a)]. Pyridine and its derivatives are reported to be toxic to the anaerobic process (Liu et al. 1994). Blum et al. (1986) reported pyridine have inhibitory effect on anaerobic digestion in presence of phenol. However, fate of pyridine in presence of phenol, ammonia and thiocyanate in combination is not reported before.

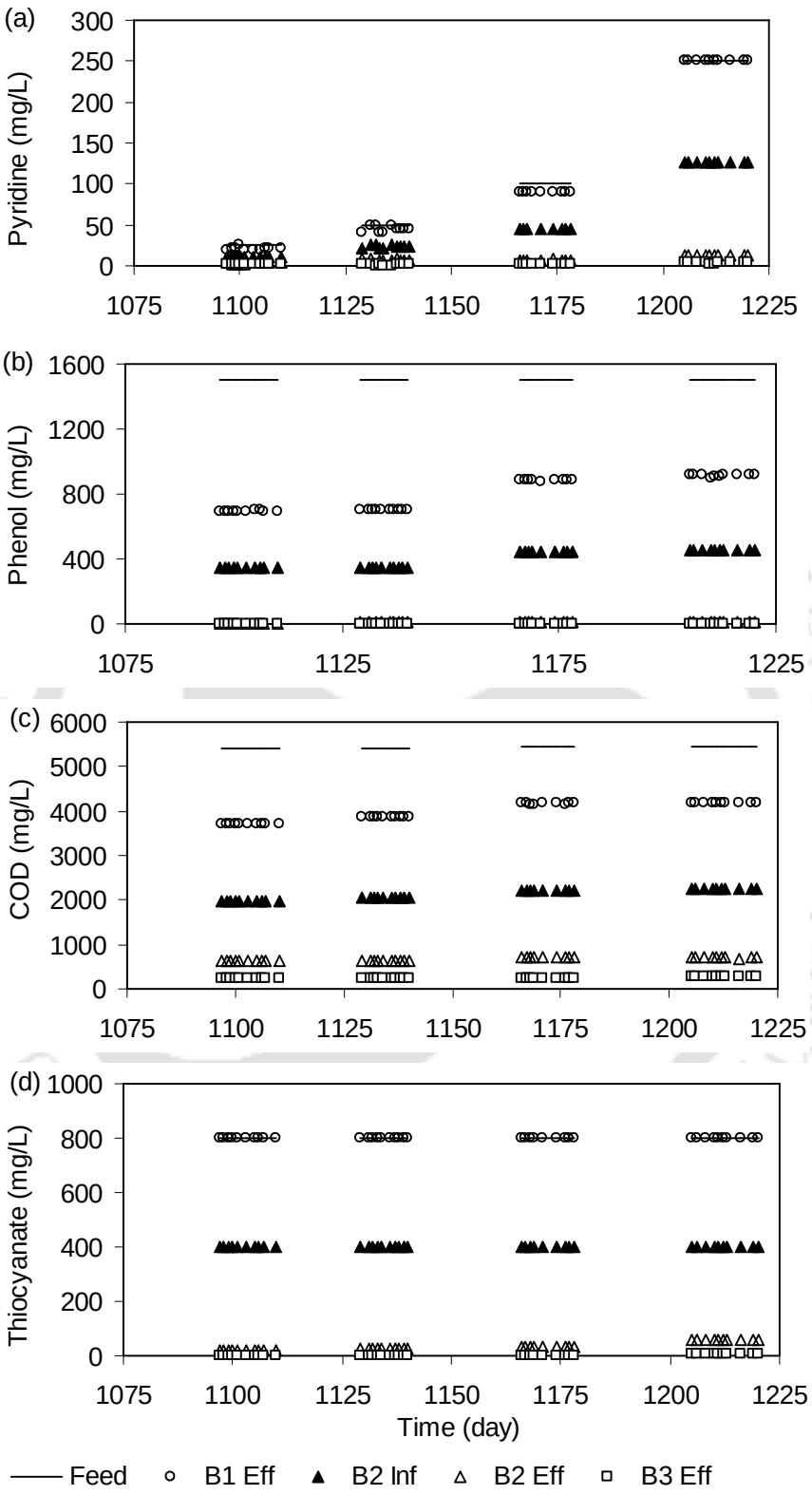

Figure 4.68 Pollutant profile in FMBR at varied feed pyridine concentration (a) Pyridine (b) Phenol (c) COD and (d) Thiocyanate

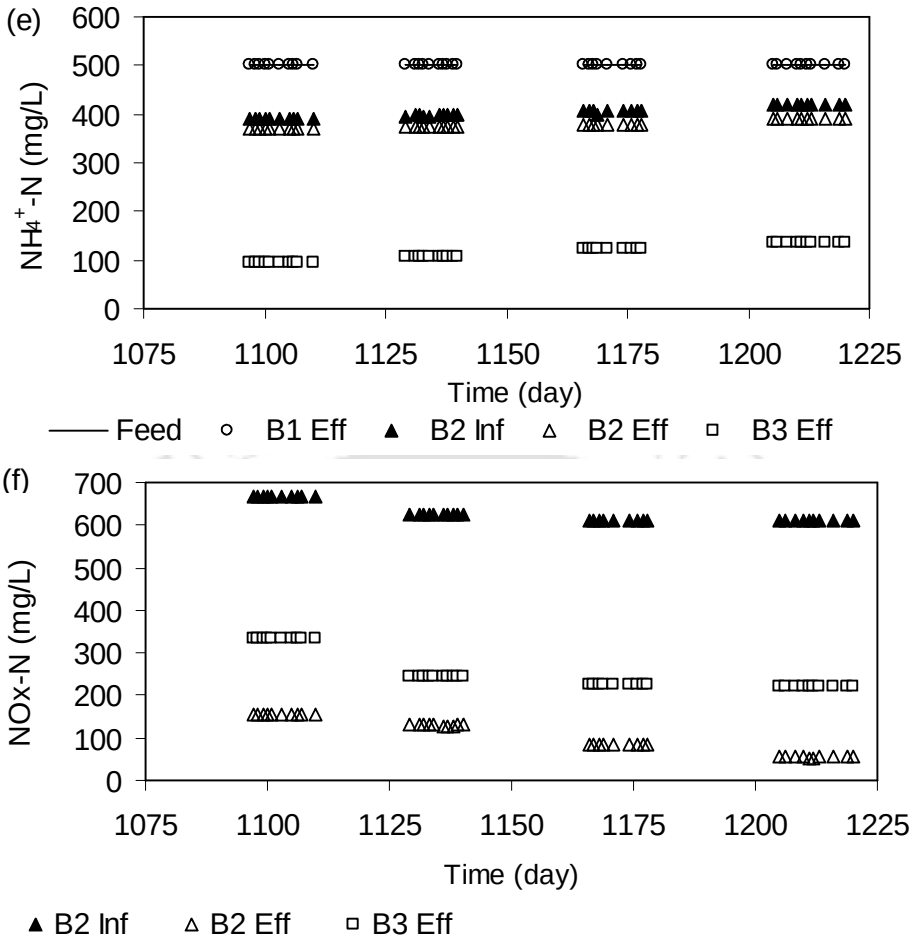

Figure 4.68 (e) NH_4^+-N profile and (f) NOx-N profile in FMBR at varied feed pyridine concentration

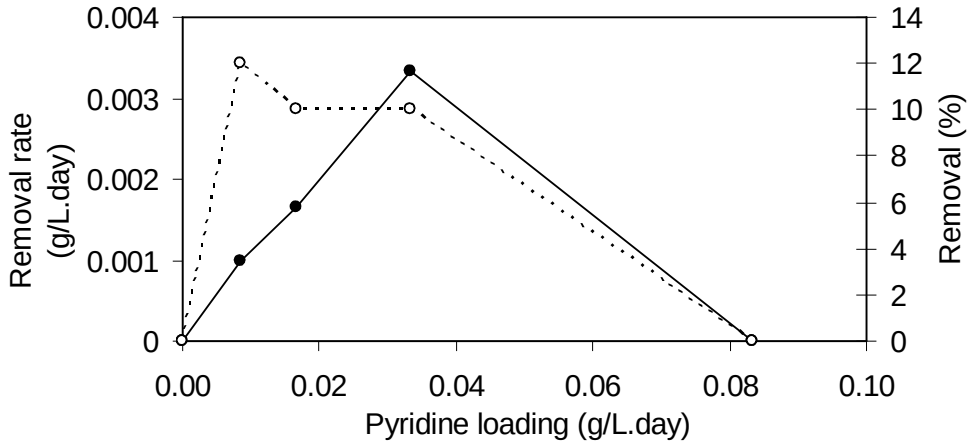

Figure 4.69 (a). Pyridine removal rate (solid line) and removal (dotted line) in B1

Influent phenol concentration to B1 was 1500 mg/L comprising loading rate 0.50 g phenol/L.day. Phenol removal of 53% was achieved up to influent pyridine concentration of 50 mg/L and then slightly decreased to 41% and 39% when pyridine was increased to 100 and 250 mg/L, respectively. Figure 4.69 (b) shows that phenol removal rate was 0.267–0.269 g/L.day at influent pyridine 25–50 mg/L and it decease to 0.203–0.195 g phenol/L.day with further increase in loading from 0.033 g pyridine /L.day to 0.083 g pyridine /L.day at influent pyridine concentration 100–250 mg/L.

COD removal in B1 was 31% in presence of 25 mg/L pyridine and decreased to 29% and then ~23% when pyridine was increased to 50 mg/L and 250 mg/L, respectively (Table 4.28). In absence of pyridine, B1 removed 53% and 33% of influent phenol (1500 mg/L) and COD (5400 mg/L), respectively during phenol variation study. Though feed pyridine up to 50 mg/L (loading 0.017 g/L.day) in present study did not have any negative effect on phenol removal, COD removal decreased to 29% (decreased by 12%) in B1. With increase in influent pyridine 25–100 mg/L, COD removal rate decreased from 0.567 to 0.417 g/L.day and maintained same at maximum influent pyridine concentration of 250 mg/L.

Table 4.28: Performance of anaerobic FMBR (B1) at pyridine concentration variation

Pyridine			Phenol		COD			SCN^-	NH_4^+–N		pH	TVS (mg/L)
S_0	S_e	Rem	S_e	Rem	S_0	S_e	Rem	S_e	S_0	S_e	S_e	
25	21 (1.9)	14.6	691 (9)	54	5400	3700 (41)	31.5	800	500	505 (0)	7.2	10870
50	45 (5)	10	700 (0)	53	5420	3850 (40)	29.0		500	510 (0)	7.2	9800
100	90 (0)	10	889 (10)	41	5430	4180 (30)	23.0		500	510 (0)	6.9	8033
250	250 (0)	0	915 (12)	39	5430	4200 (16)	22.7		500	505 (0)	6.9	7500

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

Influent phenol and thiocyanate was 1500 mg/L and 800 mg/L, respectively

Numbers in parenthesis indicate standard deviation values

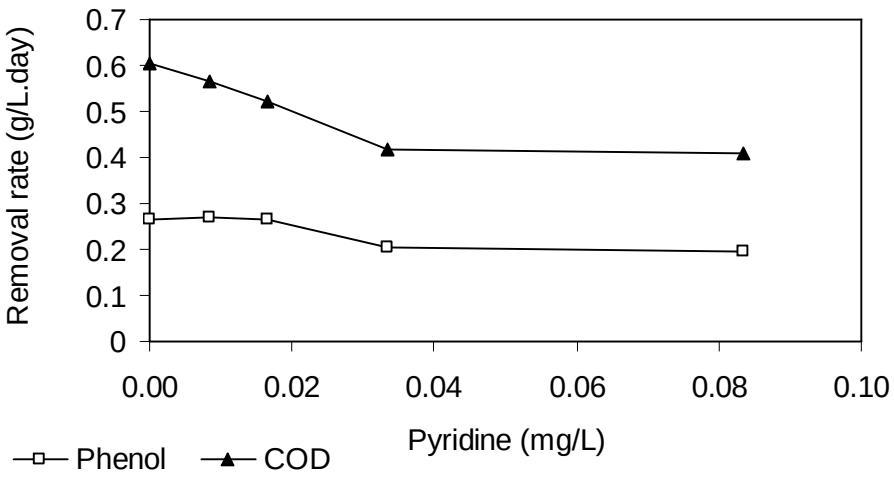

Figure 4.69 (b) Pollutant removal rate by B1 at varied pyridine loading

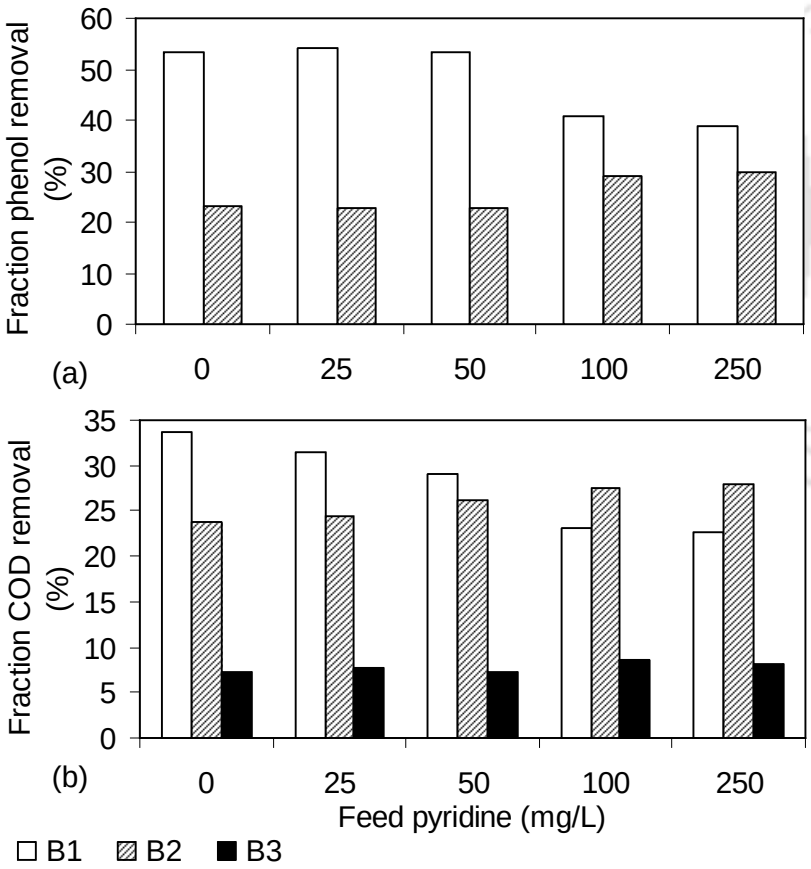

Figure 4.70 Fractional (a) Phenol (no influent phenol to B3) and (b) COD removals by B1, B2 and B3 at varied feed pyridine concentration

Wang et al. (2011b) observed maximum removal efficiencies of COD and total phenols of 55–60% and 58–63% respectively with real coal gasification wastewater in a two–continuous mesophilic UASB system with step feeding. They observed that after the anaerobic digestion with step–feed, the aerobic effluent COD concentration decreased from 270 ± 9 to 215 ± 10 mg/L suggesting enhancement of degradation of refractory organics in the second reactor. B1 received 5400–5430 mg/L COD in its influent resulting COD loading rate 1.80 g/L.day. Contribution of B1 in phenol removal was always higher compared to B2 in presence of increased pyridine [Figure 4.70 (a)]. However contribution in COD removal decreased compared to B2 at higher feed pyridine concentration of ≥100 mg/L [Figure 4.70 (b)].

Suspended biomass concentration in B1 initially increased from 1000 mg/L to 1260 mg/L with increase in feed pyridine from 25 to 100 mg/L and then drastically decreased to 700 mg/L at maximum feed pyridine concentration 250 mg/L. Attached biomass concentration in B1 continuously decreased from 9800 to 6800 mg/L with increase in feed pyridine. Total biomass concentration was observed to reduce from 10870 mg/L to 7500 mg/L in B1 with attached to suspended biomass ratio of 9.5 to 5.3. Study of effect of pyridine in anaerobic sludge in presence of thiocyanate and phenol in combination is not reported yet.

4.2.6.2 Performance of anoxic FMBR (B2) at varied pyridine concentration

In anoxic environment generation of carbon–dioxide and ammonia occurs from pyridine degradation (Equation 2.4.b). Average performance of B2 with pyridine is presented in Tables 4.29 (a) and (b). Influent pyridine to B2 was 12, 23, 46 and 127 mg/L with corresponding loading rates 0.008, 0.015, 0.031 and 0.084 g/L.day, respectively. Pyridine removal in B2 increased from 68% to 90% with increase in influent pyridine concentration and pyridine removal rate increased linearly from 0.054–0.077 g/L.day with a slope of 0.92 with increase in pyridine loading (Figure 4.71). The maximum pyridine removal rate achieved in B2 was 0.077 g/L.day. Literatures on removal of pyridine in anoxic environment are limited. Liu et al. (1994) reported anoxic removal of pyridine from initial concentration of 10 mg/L within 2–3 days by an acclimatized anoxic sludge. Li et al. (2001) reported complete removal of pyridine in anoxic environment within 12–24 h at initial pyridine concentration of 20–100 mg/L in absence of any other pollutant. In the

present study pyridine in B2 was associated with other pollutants such as phenol, thiocyanate and NH_4^+–N and B2 released only 3–12 mg/L in effluent from influent concentration of 12–127 mg/L at HRT of 1.5 days. B2 accounted for 31–49% of total pyridine removal during the varied pyridine concentration study which was higher than B1 and B3 (Figure 4.72).

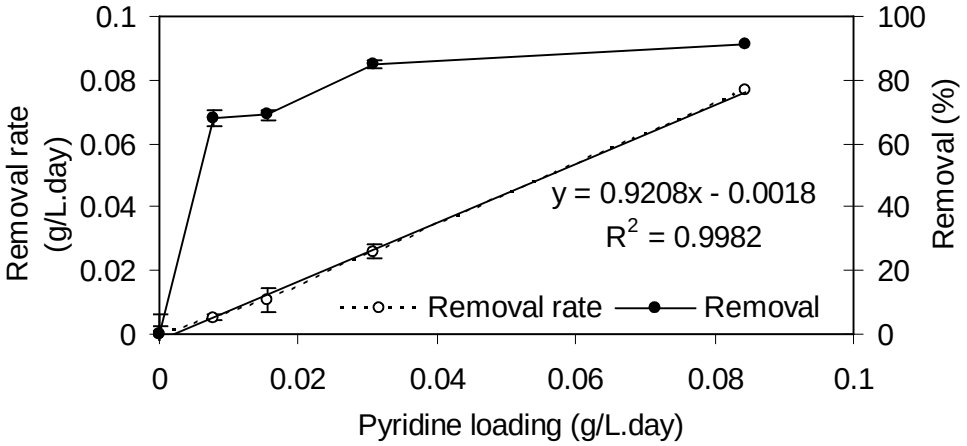

Figure 4.71 Pyridine removal and removal rate in B2 at varied pyridine loading

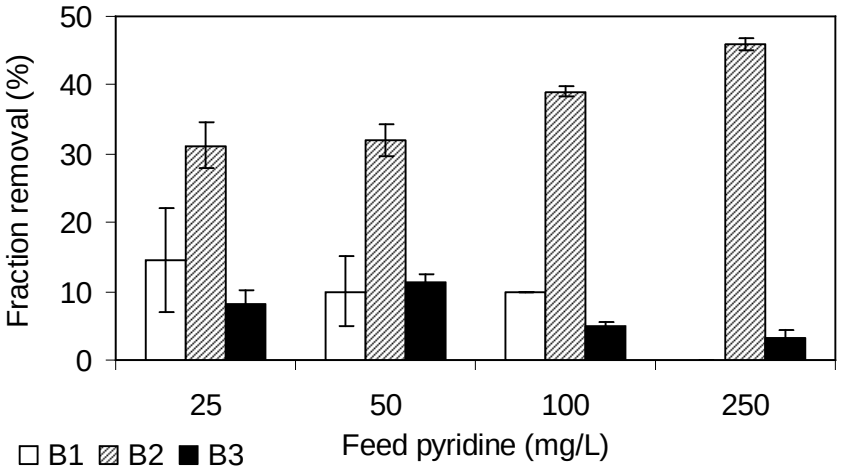

Figure 4.72 Fractional pyridine removal by B1, B2 and B3 at varied feed pyridine concentration

Influent phenol in B2 ranged from 346 to 458 mg/L. This increased with increase in feed pyridine concentration as B1 released higher amount of phenol in the effluent. Corresponding phenol loading rate was 0.231–0.305 g/L.day. In B2, almost 98–99%

phenol removal was achieved and effluent contained only 3–9 mg/L phenol. Figure 4.73 (a) shows that phenol removal rate was unaffected by pyridine loading up to loading of 0.084 g pyridine/L.day (maximum loading). Similar results were reported by Adav et al. (2007), where no inhibitory effect of pyridine on phenol degradation in aerobic environment was observed upto pyridine concentration of 1500 mg/L. B2 was responsible of 23-30% of total phenol removal throughout the study irrespective of feed pyridine.

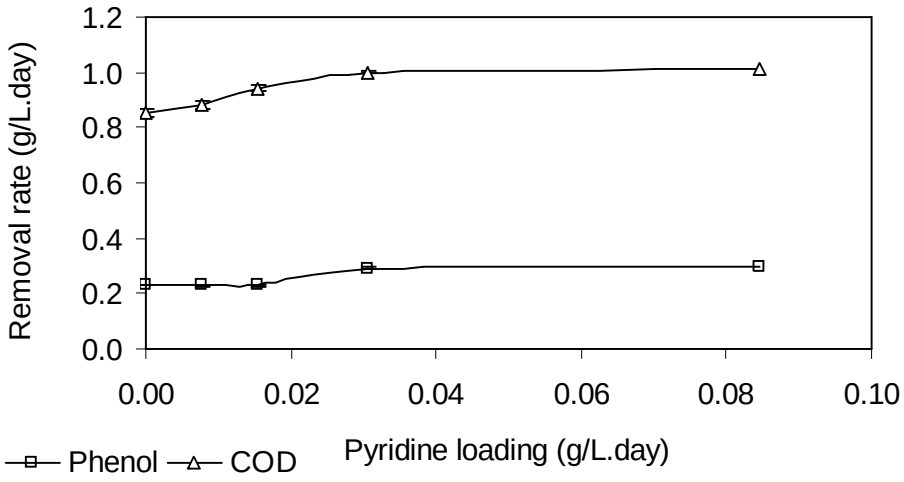

Figure 4.73 (a) Phenol and COD removal rate in B2 at varied pyridine loading

COD in influent of B2 increased from 1965 mg/L to 2240 mg/L with corresponding loading rate 1.31–1.493 g/L.day. COD removal accounted in B2 was 67–69% with increasing removal rate of 0.889–1.012 g/L.day. This comprised 24–28% of total COD removal. COD loading and removal rate in B2 initially increased with introduction of pyridine and B2 maintained almost stable performance at higher influent concentration indicating no inhibition of pyridine on COD removal in anoxic reactor up to loading of 0.084 g pyridine /L.day.

SCN^- in influent of B2 was 401–412 mg/L and loading rate was 0.267–0.274 g SCN^- /L.day. B2 removed 92–95% SCN^- in presence of 11–46 mg/L pyridine and beyond this pyridine concentration (127 mg/L), SCN^- removal decreased to ~86%. SCN^- degradation rate was 0.248–254 g/L.day up to pyridine loading of 0.307 g/L.day and decreased to 0.236 g/L.day when pyridine loading to B2 was 0.084 g/L.day [Figure 4.73 (b)]. In absence of pyridine B2 removed 96% of its influent SCN^- and pyridine in low concentration did not

show negative effect on thiocyanate removal efficiency. B2 was responsible for 44–48% of total SCN^- removal releasing 20–58 mg/L SCN^- in its effluent [Figure 4.74 (a)].

Influent NH_4^+–N to B2 was 389–422 mg/L. Influent NH_4^+–N in B2 from effluent of B1 and recycle from B3 was enhanced by NH_4^+–N generated from pyridine (0.17 mg NH_4^+–N/mg pyridine) and SCN^- degradation (0.24 mg NH_4^+–N/ mg SCN^-). B2 showed 4.9–7.7% NH_4^+–N removal releasing 360–390 mg/L NH_4^+–N in the effluent. This NH_4^+–N removal was probably due to incorporation in biomass.

Table 4.29 (a): Performance of anoxic FMBR (B2) at influent pyridine variation

Pyridine			Phenol			COD			Thiocyanate			NH_4^+–N		
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0^A	S_e	Rem
12	3 (0.5)	68.9	346	3 (1.3)	99.1	1965	631 (3.7)	67.9	401	20 (0.5)	95.0	389	360 (0)	4.9
23	7 (1)	68.7	351	7 (1)	98.1	2045	631 (8.9)	69.1	401	24 (1.9)	94.1	396	370 (0)	5.2
46	7 (0.6)	84.8	445	7 (2)	98.4	2217	720 (30)	67.5	402	30 (5.4)	92.5	407	376 (0)	6.7
127	12 (0)	90.5	458	9 (1.2)	98.0	2240	721 (22)	67.8	412	58 (5.6)	85.9	422	390 (0)	7.7

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%),

[A] Influent NH_4^+–N of B2 = {Effluent NH_4^+–N of (B1+B3)/2 + 0.24x (SCN^- removed in B2) + 0.17x (pyridine removed in B2)}.

Numbers in parenthesis indicate standard deviation values.

Influent NO_3^-–N to B2 was 628–582 mg/L along with 28–40 mg/L NO_2^-–N. Total NO_x^-–N loading to B2 was 0.407–0.443 g/L.day. Respective NO_x^-–N removal rate in B2 was 0.313–0.367 g/L.day. NO_x^-–N removal in B2 increased from 70% to 90% with higher loading of pyridine releasing 58–196 mg/L NO_x^-–N in its effluent. With increase in feed pyridine, influent NO_x^-–N to B2 decreased, which could be due to lower nitrification efficiency of the downstream reactor B3. Table 4.29 (b) shows that NO_2^-–N was almost completely exhausted in B2. B2 was responsible for 27–32% of total nitrogen removal during the study [Figure 4.73 (b)]. Stoichiometrically NO_3^-–N requirement for each gram

of pyridine was 0.78 g (equation 2.4). NO_x–N in B2 was utilized for pyridine and SCN^- oxidation and remaining was consumed for COD oxidation. COD/N_{rem} value was 3.02–3.6 (after correcting equation 4.5 with nitrate consumption for pyridine). Nearly 5.4–20.7% of COD was consumed for biomass synthesis and the yield coefficient was 0.04–0.15.

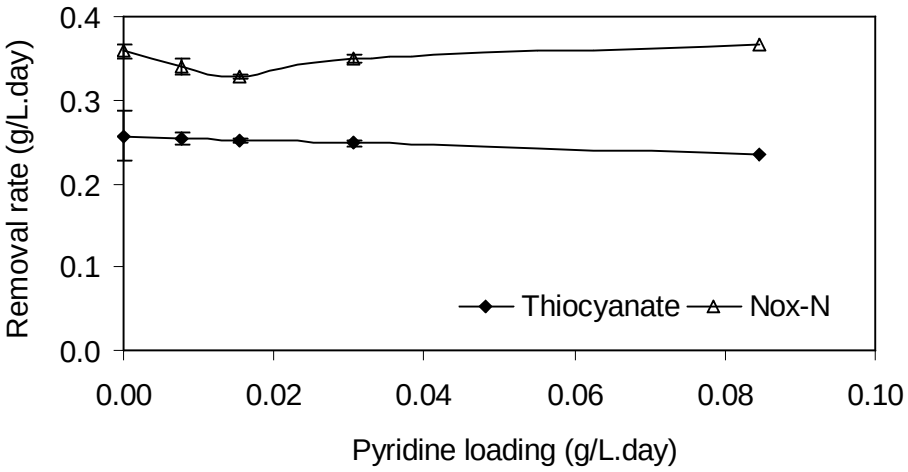

Figure 4.73 (b) Thiocyanate and NOx-N removal rate in B2 at varied pyridine loading

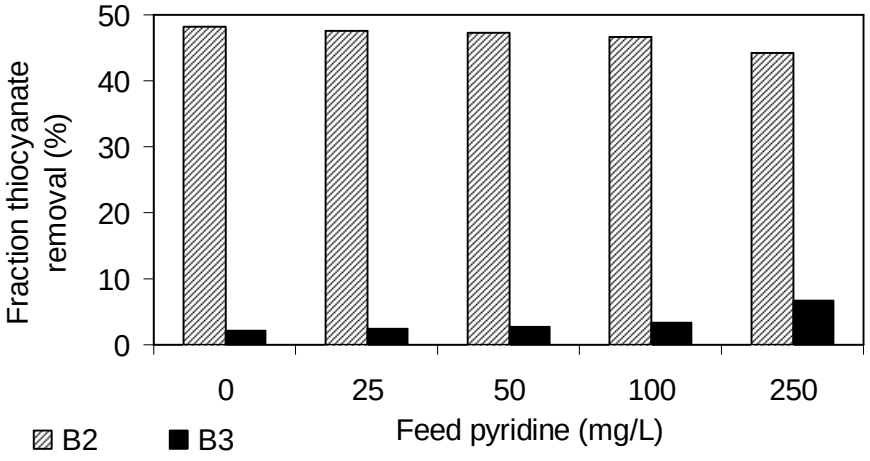

Figure 4.74 (a) Fractional thiocyanate removal by B2 and B3 at varied feed pyridine concentration

Suspended biomass in B2 increased from 3600 mg/L to 4110 mg/L whereas attached biomass concentration was ~8700–8300 mg/L with influent pyridine. Total biomass concentration in B2 was ~12 g/L during the study [Table 4.29 (b)] with decreased attached to suspended biomass ratio of 2.4 to 2.0. No literature is available on degradation of pyridine in anoxic environment in presence of phenol and thiocyanate in common and

present investigation shows simultaneous degradations of phenol and pyridine in anoxic reactor. B2 released 905–930 mg/L sulfate in effluent was detected showing 422–450 mg/L sulfate generation. Sulfate generation in B2 was lower than the theoretical sulfate generation value through out the study and might be influenced by thiocyanate removal rate similar to other studies [Table 4.29 (b)].

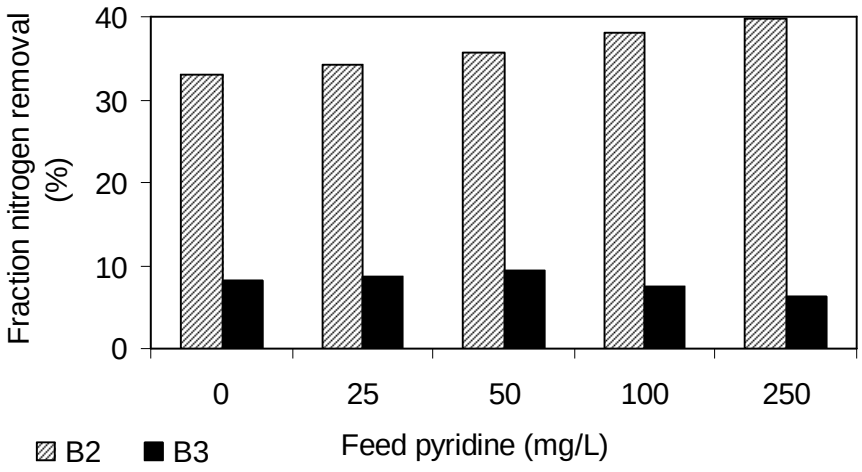

Figure 4.74 (b) Fractional nitrogen removal by B2 and B3 at varied feed pyridine concentration

Table 4.29 (b): Performance of anoxic FMBR (B2) at influent pyridine variation

Pyridine	Sulfate				NO_3^-–N		NO_2^-–N		NO_x^-–N	COD: N_{rem}	COD_B	TVS (mg/L)	pH
S_0	S_0	S_e	Gen	Err	S_0	S_e	S_0	S_e	Rem				
11	485	930 (9)	445	–183	628	196 (1.7)	37.5	0	70.6	3.02	5.4	12355	8.4
23	480	930 (8)	450	–172	584	130 (9.6)	39.5	1 (1)	79.0	3.12	8.4	12400	8.5
46	482	905 (6.3)	422	–192	584	85 (4.4)	27.5	1 (0.5)	86	3.18	10.2	12260	8.3
127	475	905 (4)	430	–153	582	58 (6)	27.5	1 (1.2)	90.3	3.60	20.7	12410	8.4

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%); Gen: Generation (mg/L);

Err: Error (= experimental value–theoretical vale), mg/L

COD_B: COD fraction (%) for biomass;

Numbers in parenthesis indicate standard deviation values

4.2.6.3 Performance of aerobic FMBR (B3) at varied feed pyridine concentration

Average steady state performance of aerobic reactor B3 is shown in Tables 4.30 (a) and (b). Very low amount of pyridine (3–12 mg/L) entered to aerobic reactor B3. The final effluent contained nearly 1.6–3.7 mg/L pyridine resulting 57–78% of pyridine removal. B3 removed 3–11% of total pyridine. Figure 4.75 (a) shows pyridine removal rate increased linearly with increased loading. Li et al. (2009) reported complete removal of pyridine by an aerobic strain *Streptomyces* within 8 days from initial concentration of 2000 mg/L at pH 7. In present study the influent pyridine concentration was far low than reported value.

With increase in initial influent pyridine from 25–250 mg/L, B2 released higher SCN^- and influent to B3 was 20–58 mg/L. B3 removed 83–92% of influent SCN^- and released 1–5 mg/L SCN^- in its effluent. Maximum SCN^- degradation rate observed in B3 was 0.035 g/L.day at pyridine loading of 0.008 g/L.day. In absence of pyridine, B3 released ~1 mg/L phenol in its effluent from 2–8 mg/L influent phenol and the same trend was observed in B3 in presence of pyridine with ~ 1 mg/L phenol in effluent. However, thiocyanate in effluent of B3 was observed to be 1–5 mg/L, being higher compared to the study in absence of pyridine.

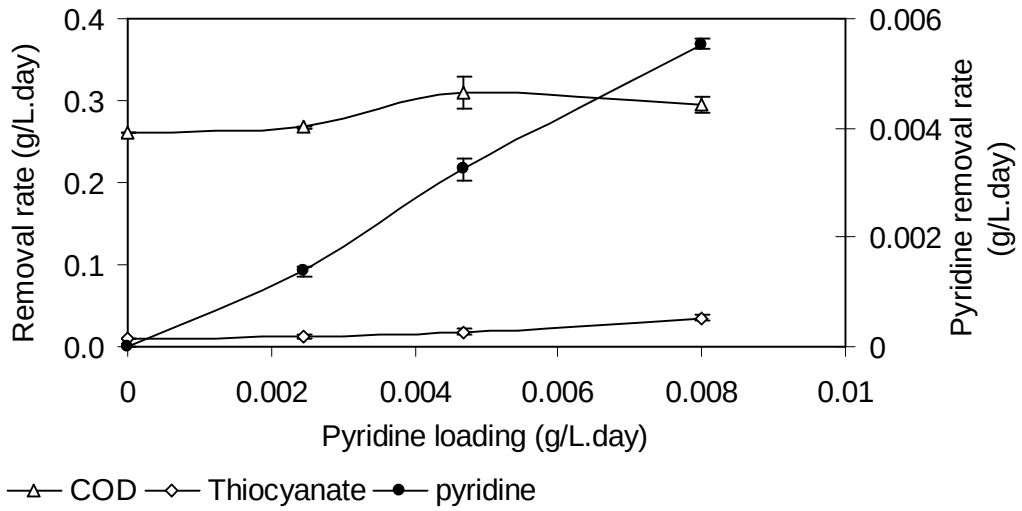

Figure 4.75 (a) Performance of B3 at varied pyridine loading

Influent COD to B3 was observed to be 630–721 mg/L with loading of 0.42–0.48 g COD/L.day. COD removal efficiencies achieved in B3 fluctuated within the range of 61–64%. COD removal rate in B3 increased with increase in pyridine loading and influent

COD concentration and maximum COD removal rate observed was 0.31 g/L.day [Figure 4.75 (a)].

The influent phenol to B3 was 3–9 mg/L and almost complete phenol removal occurred in B3 releasing ~ 1mg/L phenol in all the cases and it seemed that pyridine of 3–12 mg/L did not affect phenol degradation. Similarly no inhibitory effect of phenol on pyridine degradation was observed. Sun et al. (2011) also observed simultaneous degradations of pyridine and phenol by an aerobic strain *Rhodococcus*, using phenol as carbon source and pyridine as the nitrogen source. In the present study influent phenol concentration to B3 was only 3–9 mg/L, much less than inhibitory concentration of 500 mg/L to affect pyridine removal (Kim et al. 2006; Adav et al. 2007; Chandra et al. 2009).

Table 4.30 (a): Performance of aerobic FMBR (B3) at influent pyridine concentration variation

Pyridine			Thiocyanate			Phenol			COD			NH_4^+–N		
S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0	S_e	Rem	S_0[A]	S_e	Rem
3	1.6 (0)	56.3	20	1.5 (0)	92.4	3	1 (0)	66.7	630	230 (4)	63.4	374	93 (0)	75.3
7	1.6 (1.5)	78.5	24	2 (0)	91.5	7	1 (0)	85.1	631	230 (8)	63.5	382	107 (4.9)	72.0
7	2.1 (1.2)	69.4	30	3 (0)	91.0	7	1 (0)	85.7	720	255 (16)	64.6	387	123 (4.4)	68.2
12	3.7 (0.8)	69.1	58	5 (0)	91.3	9	1 (0)	88.9	721	280 (29)	61.2	404	136 (0)	66.3

S_0: Influent (mg/L), S_e: Effluent (mg/L), Rem: Removal (%);

[A] Influent NH_4^+–N of B3 =

{Effluent NH_4^+–N of B2 + 0.17 x (pyridine removed in B3) + 0.24x (SCN^- removed in B3)}.

Numbers in parenthesis indicate standard deviation values

Influent NH_4^+–N concentrations in B3 were 374, 382, 387, 404 mg/L along with some amount of NH_4^+–N generated in B3 from degradation of pyridine (0.3-1.4 mg NH_4^+–N/L) and SCN^- (4.4-12.7 mg/L). NH_4^+–N loading at B3 was 0.249–0.269 g/L.day. NH_4^+–N removal efficiency in B3 was 75% at influent concentration 374 mg/L which decreased to

66% when influent NH_4^+–N was 404 mg/L. NH_4^+–N removal rate decreased from 0.188 g/L.day to 0.178 g/L.day [Figure 4.75 (b)]**.** In the present study influent pyridine concentration was quite low (3–12 mg/L). However, influents NH_4^+–N to B3 were higher than the threshold value of 350 mg/L, responsible for decrease in nitrification (Kim et al. 2008b). B3 removed 74–78% NH_4^+–N when influent was 370–385 mg/L NH_4^+–N in absence of pyridine. The NH_4^+–N removal efficiency was observed to decrease 68–75% from same NH_4^+–N influent concentration (375–387 mg/L) in presence of pyridine.

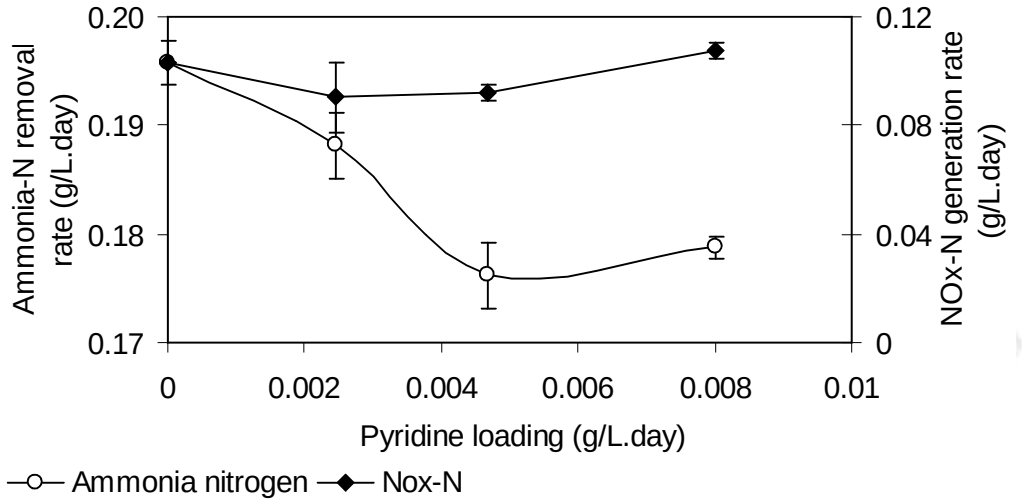

Figure 4.75 (b) Nitrogen profile in B3 at varied pyridine loading

In B3, nitrification was incomplete with generation of 55–75 mg/L NO_2^-–N. Figure 4.75 (b) shows that nitrification rate in B3 was 0.080–0.110 g/L.day, lower than NH_4^+–N removal rate might be due to loss of nitrogen through volatilization or incorporation to biomass and presented as unaccounted nitrogen in Table 4.30 (b). Hung and Palvostathis (1997) reported partial inhibition of nitrification with unaccounted nitrogen of 35–55% in an activated sludge reactor while treating thiocyanate. Free ammonia (FA) calculated from in B3 was observed to be 28–39 mg/L. FA is known as nitrification inhibitor at concentrations of 10–150 mg/L and 0.1–1.0 mg/L for *Nitrosomonas* (ammonia oxidizer) and *Nitrobacter* (nitrite oxidizer), respectively (Bae et al. 2001, Rodriguez et al. 2011). NO_2^-–N concentration of 50 mg/L and above also inhibits nitrification (Fux et al. 2004; Philips and Verstraete, 2001). Therefore in present study, influent NH_4^+–N, FA and nitrite might be responsible for low nitrification rather than pyridine /phenol or thiocyanate.

B3 released 950–970 mg/L sulfate in effluent with 30–60 mg/L sulfate generation from thiocyanate degradation. The calculated error to theoretical sulfate generation was low except maximum feed pyridine study.

Table 4.30 (b): Performance of aerobic FMBR (B3) at influent pyridine concentration variation

Pyridine	NO_3^-–N		NO_2^-–N	N_R	SO_4^{-2}					COD : NH_4^+–N	FA	UN	TVS	pH
S_0	S_0	S_e	S_e		S_0	S_e	Gen	Th SO_4^{-2}	Err					
3	196	257 (0)	75 (0)	0.10	930	970 (0)	40	30	10	1.68	28.2	42	9600	8.2
7	130	169 (8)	79 (0)	0.08	930	960 (1)	30	36.3	–5	1.65	35.9	44	9290	8.3
7	85	169 (4.1)	55 (0)	0.09	905	965 (23)	60	44.5	15	1.86	37.5	33	8930	8.3
12	58	165 (7)	55 (0)	0.10	905	950 (13)	45	87.5	–42	1.78	39.7	25	9080	8.4

S_0: Influent (mg/L), S_e: Effluent (mg/L), Gen: Generation (mg/L); Err: Error (mg/L);

Th SO_4^{-2}: Theoretical sulfate generation (1.65 x SCN^- removed in B3);

N_R: Nitrification rate (g/L.day):

FA: Free ammonia (mg/L);

UN: Unaccounted nitrogen (%);

Numbers in parenthesis indicate standard deviation values

In B3, the influent COD to NH_4^+–N ratio observed was 1.68–1.78 with influent COD 630–720 mg/L. Suspended and attached biomass concentration observed in B3 was 3100 –2800 mg/L and 6500 mg/L– 6300 mg/L during the study. Total biomass concentration remained almost stable within 9600–9080 mg/L showing attached to suspended biomass ratio of 2.1–2.6. This high ratio of COD: NH_4^+–N suggests presence of heterotrophs and unaccounted nitrogen could be either due to incorporation in the biomass.

4.2.6.4. Overall performance of three–stage FMBR at varied pyridine concentration

The overall performance of the three–stage FMBR at varying feed pyridine concentrations is shown in Figure. 4.76**.** The system efficiently removed 94–98% influent pyridine. Phenol and SCN^- removal were complete (>99%) and independent of feed pyridine and COD removal was around 95% with effluent COD 230–280 mg/L similar to study in absence of pyridine. NH_4^+–N removal decreased steadily with increase in feed pyridine from 90% to 84%. NH_4^+–N removal by the system was 85% in absence of pyridine. Feed TN were 1695, 1696, 1707 and 1734 mg/L at feed pyridine of 25, 50, 100 and 250 mg/L respectively (considering influent NO_3^-–N of 1000 mg/L added in the recycle of B3). TN removal increased from 75% to 79% with increased pyridine concentration. The fraction of nitrogen in effluent as NH_4^+–N and oxidized nitrogen is also shown in the figure. It can be seen that fraction of total nitrogen as NH_4^+–N in effluent increased with increase in feed pyridine concentration, where as the fraction of total nitrogen as oxidized nitrogen in effluent decreased. It could be noted that higher amount of oxidized nitrogen in feed suggests more stable effluent.

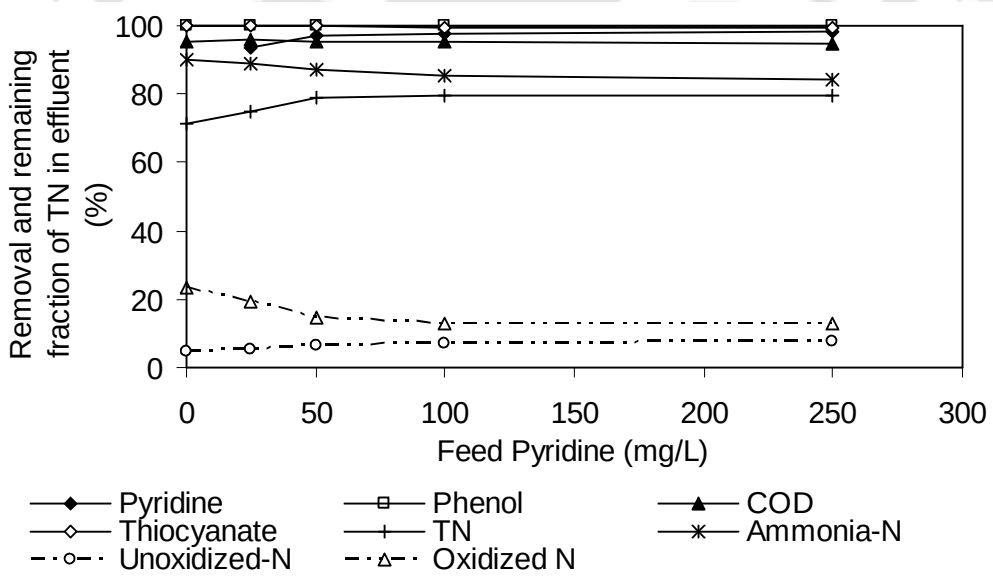

Figure 4.76 Performance of FMBR system at varied feed pyridine concentration

4.2.7 Summary FMBR performance

4.2.7.1 Fed batch anaerobic reactor (B1)

B1 was acclimatized with phenol, thiocyanate and ammonia and operated in fed batch mode. B1 showed significant removals of phenol and COD, which increased with reactor HRT and decreased with higher thiocyanate in feed indicating inhibition of thiocyanate on B1 culture similar to R1. Maximum phenol removal rate observed in B1 was 0.333 g/L.day at loading rate of 0.667 g phenol/L.day and beyond this loading phenol removal rate decreased. Phenol removal in B1 was 40% or more in all studies cases except study with maximum phenol concentration 2500 mg/L. Maximum COD removal rate observed in B1 was 0.648 g COD /L.day at COD loading of 1.350 g/L.day. COD removal in B1 remained almost unaffected at 23% or more at phenol, thiocyanate and COD loading up to 0.833 g/L.day, 0.267 g.L.day and 2.716 g/L.day, respectively. At same phenol, thiocyanate and COD loading to B1 and R1 (during phenol variation study), it was observed that B1 showed 25–52% phenol and more than 23% COD removal which was higher compared to R1 (phenol 5–42% and COD 6–10%). NH_4^+–N removal in B1 was absent. Low removal of SCN^- (0.75–12%) in B1 was observed during initial study with feed thiocyanate variation. SMA was inhibited at feed SCN^- 200 mg/L and above, similar to R1. Small amount of pyridine removal occurred only when influent pyridine concentration was less than 100 mg/L. Feed phenol of 2000 mg/L, thiocyanate 200 mg/L and pyridine of 50 mg/L inhibited phenol/COD removal in B1. In R1, feed phenol 1500 and SCN^- 200 mg/L were responsible for lower phenol/COD removals.

Increased cycle time enhanced pollutant removal efficiency in B1 by increasing reactor HRT. Increased fill time more than 1.5–2 h caused negative effect on phenol and COD removal efficiency in B1. With increase in fill time at constant cycle and decant time, reaction time decreased, suggesting microbes actually got less time to consume substrate in B1. However, instant fill was not favorable for the pollutant treatment in B1. It seems that gradual short fill was best for removal of toxic pollutants in B1.

Higher biomass concentration of ~10 g/L was observed in B1 with high attached to suspended biomass ratio of 7–10:1. No significant effect of HRT, phenol or thiocyanate was observed on biomass concentration. However with increase in feed pyridine,

suspended and attached biomass concentration in B1 continuously decreased and the ratio was observed to decrease from 9.5 to 5.3.

4.2.7.2 Fed batch anoxic MBR (B2)

Similar to B1, B2 was acclimatized with phenol, thiocyanate, and ammonia with additional nitrate. The influent of B2 comprised of effluent of B1 and recycle from B3 and hence almost all parameters were present in B2 influent. B2 was highly efficient and robust like R2 for simultaneous removal of phenol, COD, SCN^- and NO_x^-–N.

Maximum removal rate of 0.574 g phenol/L.day and 1.678 g COD/L.day were observed in B2 with maximum phenol and COD loading of 0.627 and 2.158 g/L.day, respectively. COD removal rate in B2 was affected at minimum HRT of 1.25 days. And there was no negative effect of thiocyanate and pyridine on phenol and COD removal rate as observed in B1. Similar to R2, B2 was responsible in principal for thiocyanate removal. B2 efficiently removed more than 90% influent thiocyanate (~400 mg/L) when influent phenol concentration was 511 mg/L or less and removal rate increased with increased loading irrespective of fill time/cycle time and HRT. Maximum thiocyanate removal rate of 0.335 g/L.day was observed at minimum cycle time of 18 h study at a maximum loading of 0.355 g SCN^-/L.day. During other studies conducted at 24 h cycle, the maximum thiocyanate removal rate in B2 was 0.283 g/L.day from maximum loading of 0.319 g SCN^-/L.day (at 1.25 day HRT during HRT variation study). However, higher loading of phenol >0.34 g/L.day significantly affected thiocyanate removal in B2. Pyridine showed little negative effect on thiocyanate removal rate at higher concentration (127 mg/L).

Sulfate was evolved in B2 from thiocyanate degradation and completely influenced by thiocyanate removal rate. Higher difference in experimental and theoretical sulfate generated value was observed at higher thiocyanate removal/loading rate. At higher thiocyanate removal there should be higher sulfate generation; whereas, in present study there might be accumulation of other intermediate compounds like polysulfides or thiosulfate at this condition. Almost 2–12% NH_4^+–N removal occurred in B2 during the study and clear effect of feed concentration or operating condition was not understood. B2 was mostly responsible for pyridine degradation which showed higher pyridine removal efficiency than B1 and B3. Pyridine degradation rate increased linearly with increased

loading and maximum pyridine degradation rate observed was 0.076 g/L.day at maximum pyridine loading of 0.085 g/L.day. To the best of our knowledge this is the first study to report simultaneous degradation of phenol, SCN^- and pyridine removal in anoxic environment. Almost in all cases B2 completely removed nitrite from its influent whereas complete nitrate removal was not achieved. Maximum NOx–N removal rate observed was 0.417 g/L.day at 18 h cycle time from loading of 0.579 g NOx–N/L.day in presence of COD loading of 1.960 g/L.day. Addition of pyridine too accelerated NOx–N removal rate in B2. COD:N removal ratio was within 2.2–6. The biomass yield coefficient in B2 was 0.01–0.33 and fraction COD available for biomass synthesis was 1–46%. Increased cycle time enhanced pollutant removal in B2 whereas change in fill time showed insignificant affect on performance of B2 and the optimum fill time was 3–5 h. However, instant fill did not cause adverse affect on the pollutant removal in B2. The total biomass concentration in B2 remained higher of 8–13 g/L throughout the study and higher values were observed at higher HRT. Attached biomass to suspended biomass concentration ratio decreased with increased influent thiocyanate from 10–3 and remained 1.9–2.9 during other studies of HRT, phenol or pyridine variation with high influent thiocyanate. No significant effect of increased pyridine was observed in biomass concentration of B2 as in B1.

4.2.7.3 Fed batch aerobic MBR (B3)

Aerobic FMBR (B3) was acclimatized with ammonia and thiocyanate for nitrification and was the last reactor in the series. During experimental condition influent to B3 comprised of effluent from B2 after necessary pH adjustment. Due to higher efficiency of B1 and B2, very less amount of phenol (2–30 mg/L; except influent phenol 80 mg/L when feed phenol was 2500 mg/L), thiocyanate (<50.0 mg/L; except highest phenol and pyridine study) and COD (220–750 mg/L; except low HRT of 1.25 day when B3 received 980 mg/L COD) was entering in it. Almost complete removal of phenol, thiocyanate occurred and B3 released effluent COD less than 250 mg/L except 280–320 mg/L COD when cycle period was 18 h and pyridine entered to B3. Pollutant removal rate in B3 increased with increase in loading and maximum phenol, thiocyanate and COD removal rate was 0.190 g phenol/L.day. 0.099 g SCN^-/L.day, and 0.598 g COD/L.day at their maximum loading of 0.0533 g/L.day, 0.10 g/L.day and 0.787 g/L.day, respectively.

B3 released only 12–16 mg/L NH_4^+–N in its effluent when influent NH_4^+–N was 250–290 mg/L. The effluent concentration increased to 55 mg/L or more and NH_4^+–N removal efficiency significantly decreased when influent NH_4^+–N to B3 increased to 325–360 mg/L or more. NH_4^+–N removal rate in B3 was observed to increase with increase in NH_4^+–N loading rate and maximum NH_4^+–N removal rate was 0.243 g/L.day from NH_4^+–N loading of 0.343 g/L.day at 18 h cycle time, however higher amount of effluent NH_4^+–N was released during this study. Addition of pyridine in feed also caused decrease in NH_4^+–N removal in B3. Threshold phenol, thiocyanate and NH_4^+–N loading to achieve 80% NH_4^+–N removal efficiency in B3 were 0.001, 0.006 and 0.249 g/L.day, respectively. Maximum nitrification rate of 0.151 g/L.day was achieved in B3 at 30 h cycle period. Accumulation of nitrite and high amount of FA 45–88 mg/L was accounted in B3 and it might have affected the nitrifying bacteria. Also, higher influent COD might have promoted the growth of hetertrophs which competed for substrate, oxygen and space with the nitrifiers. No major change in effluent profile of B3 was observed when cycle time and fill time was more than 24 hour and 5 hour, respectively.

Effect of operating condition or feed concentration on total biomass concentration in B3 remained insignificant through out the study and attached to suspended biomass concentration ratio fluctuated between 2–4.

4.3. BIO–KINETIC PARAMETER STUDY

The rate of substrate consumption was suggested to be the most important parameter of microbial performance (Prpich and Daugulis, 2005). Substrate removal kinetics in CMBR and FMBR system was evaluated based on experimental data. Various substrate degradation kinetic models like haldane model, Modified Stover –Kincnnon model, Grau second order model, Bhatia et al. model and first order kinetic model used in the present study are described in section 2.4. The best fit model was selected based on correlation coefficient and Chi square value (equation 2.15).

4.3.1 Kinetic study of CMBR system

4.3.1.1 Anaerobic CMBR (R1)

In anaerobic reactor R1, only phenol and COD removals were achieved. It was observed that modified Haldane's model (equation 2.5), Stover–Kincannon model (equation 2.7), Grau second order model (equation 2.11) and first order kinetic model (equation 2.13) provided very low correlation coefficient (0.4–0.6) for phenol and COD degradation in R1. Experimental data suggested that thiocyanate was responsible for decrease in phenol and COD removals in R1. The Bhatia et al (1985) model (equation 2.9) considering the process inhibition due to toxicity was used and the plots of (HRT x S_e) /(S_0 – S_e) versus effluent SCN^- for COD and phenol are shown in Figure 4.77.

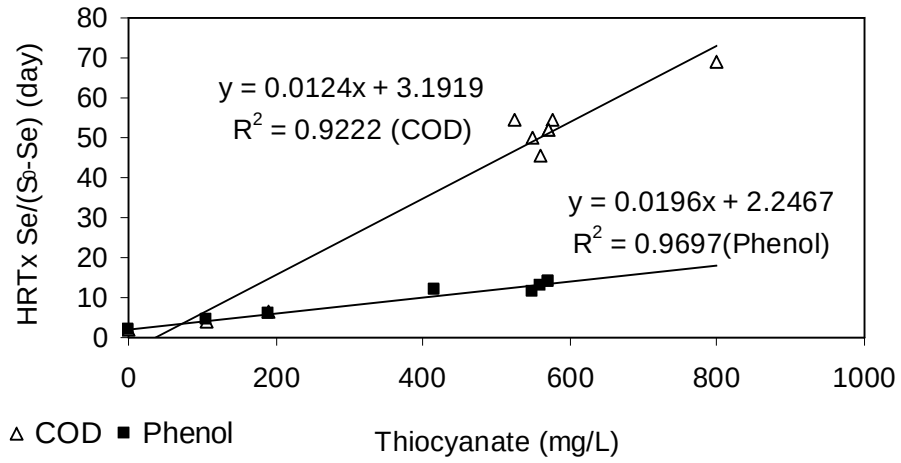

Figure 4.77 Estimation of R and KI of phenol and COD utilization in R1 using Bhatia et al model

The maximum rates of substrate utilization (R) for phenol and COD were 0.46 and 0.32 /day, respectively with inhibition coefficients (KI) of 0.008 and 0.005 L/mg. During treatment of distillery spent wash in an anaerobic hybrid reactor maximum rate of COD utilization was observed as 1.945 (Kumar et al. 2005), which was higher than the maximum COD degradation rate observed in R1. Effluent COD and phenol were calculated using Eq 2.10 and plotted in Figure 4.78 as experimental and model predicted effluent concentrations. Figure 4.78 shows that Bhatia et al model predicted experimental effluent COD and phenol with good agreement (χ^2: 243 and 216, respectively).

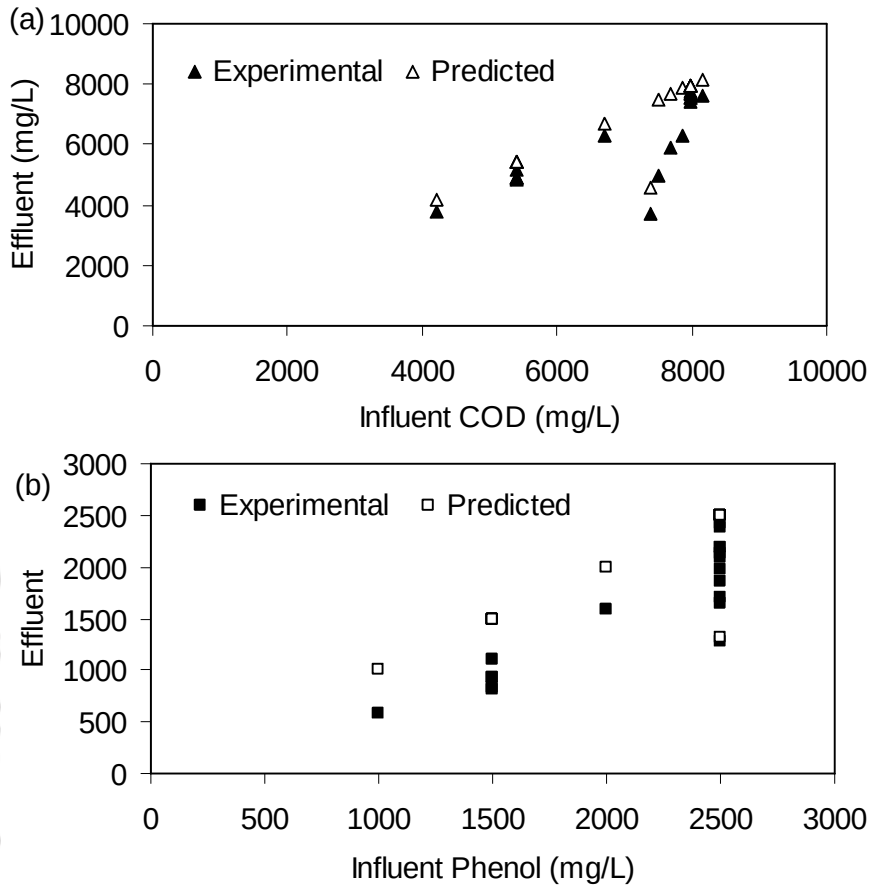

Figure 4.78 Comparison of predicted and experimental effluent (a) COD and (b) phenol in R1 according to Bhatia et al model

4.3.1.2 Anoxic CMBR (R2)

In R2 modified Stover Kincannon model showed very high correlation coefficient (0.95–0.98) as compared to other models for phenol, COD, thiocyanate and NOx-N removals (Table 4.31). Plots of $HRT/(S_0 - S_e)$ versus $\{V/(Q.S_o)\}$ according to modified Stover Kincannon model are shown in Figures 4.79 and 4.80. Table 4.31 also shows that χ^2 value for experimental and predicted effluent concentration was lower for this model compared to other models. Maximum substrate removal rate (R_{max}) achieved for phenol and COD were 1.08 and 7.89 g/L.day, respectively, suggesting COD removal was at much higher rate than phenol in R2. R_{max} value obtained for thiocyanate and NOx–N were 0.196 and 5.84 g/L.day, respectively. R_{max} achieved for COD degradation was higher in R2 compared to R1 and R3. In Figure 4.81 and 4.82 experimental and predicted effluent values are presented. Experimental and predicted values matched well for phenol, COD and SCN^-.

Grau second order model also gave high correlation coefficient for phenol and COD though it was less than modified Stover–Kincannon model.

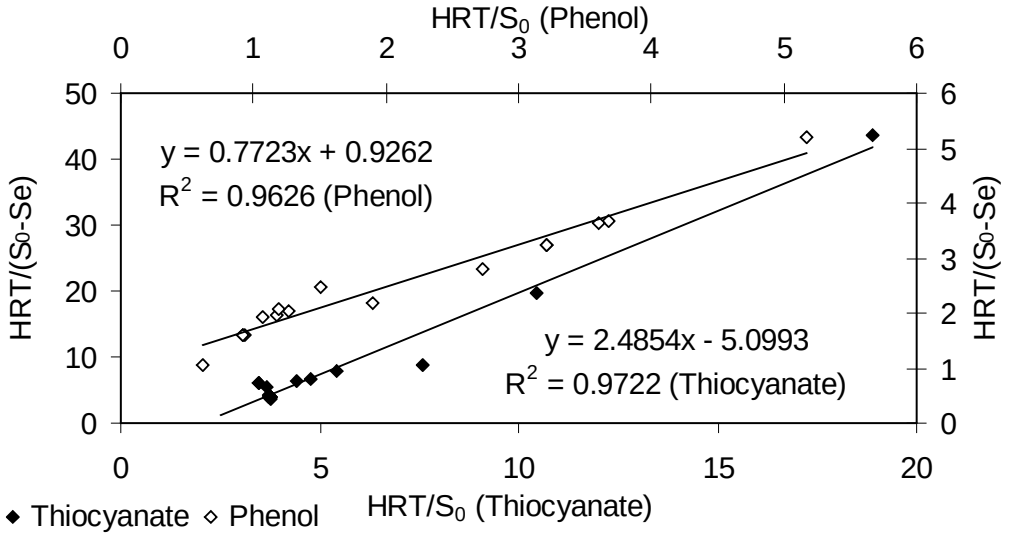

Figure 4.79 Estimation of R_{max} and K_b for phenol and thiocyanate utilization in R2 using Modified Stover-kincannon model

Kuscu and Sponza (2009) observed maximum COD utilization rate of 29.49 g/L.day in anaerobic migrating blanket reactor using modified Stover– Kincannon model during treatment of synthetic wastewater containing p–nitrophenol and glucose. Sandhya and Swaminatnan (2006) reported maximum substrate utilization rate of 31.69 g/L.day for textile wastewater in hybrid column upflow anaerobic fixed bed reactor. In anaerobic moving bed biofilm reactor and anaerobic filter higher substrate utilization rates of 89.30 and 86.21 g/L.day were observed during treatment of milk permeate and paper mill wastewater respectively (Yilmaz et al. 2008; Wang et al. 2009). In the present study the maximum COD removal rate observed in R2 was much lower than these reported values, which was probably due to presence of toxic substance like thiocyanate. Borghei and Hosseini (2004) achieved maximum substrate utilization rate (R_{max}) of 8.3 g/L.day with saturation value (K_b) 9.45 for simulated wastewater in a moving bed biofilm reactor from influent COD 750–4500 mg/L at 1 day HRT.

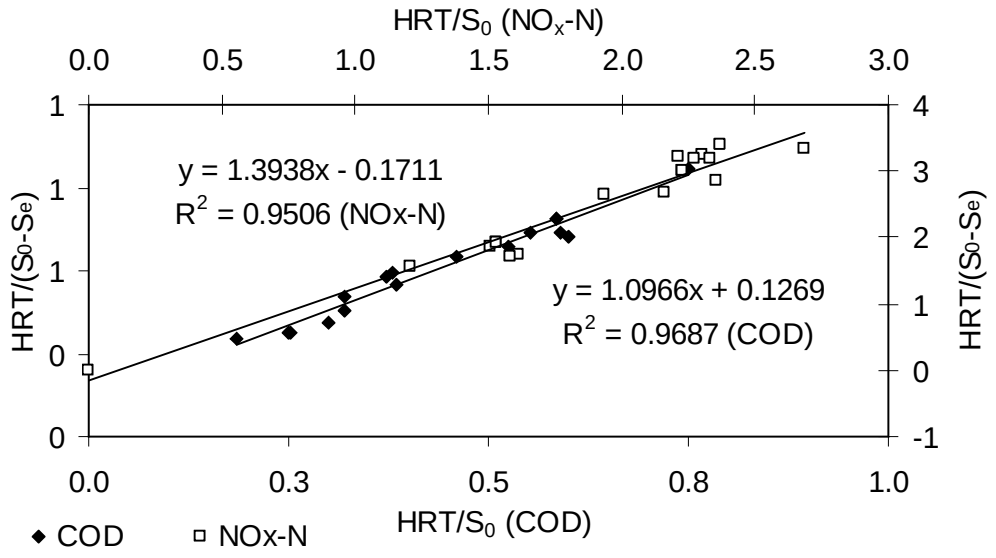

Figure 4.80 Estimation of R_{max} and K_b for COD and Nox-N utilization in R2 using Modified Stover-Kincannon model

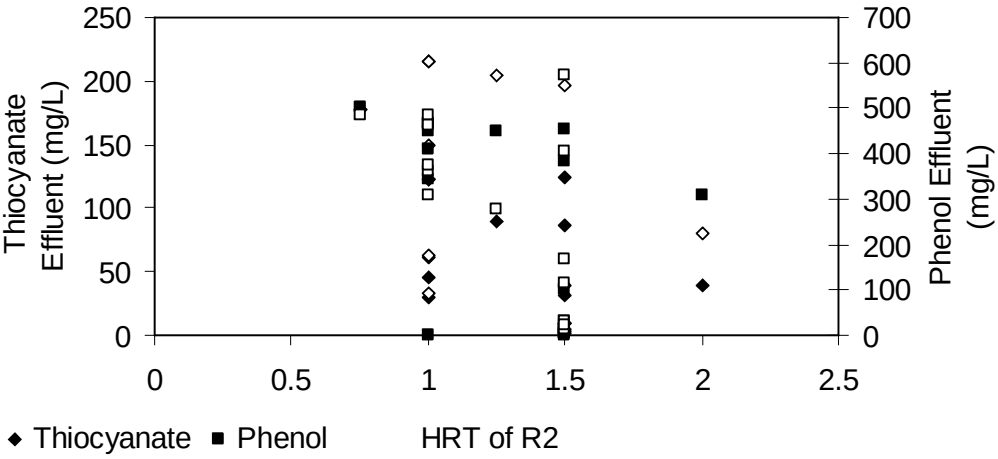

Figure 4.81 Comparison of predicted (hollow symbol) and experimental (solid symbol) thiocyanate and phenol effluent in R2 according to modified Stover-Kincannon model

Table 4.31: Kinetic parameters for substrate removal kinetics in CMBR

Parameter	Reactor	Modified Stover Kincannon				Grau second order				Bhatia et al.				First order kinetics		
		R_{max} (g/L.day)	K_b (g/L.day)	R^2	χ^2	m	N	R^2	χ^2	R (/day)	KI (L/mg)	R^2	χ^2	k (/day)	R^2	χ^2
Phenol	R1	0.59	0.56	0.06	–	39	7.9	0.04	–	**0.46**	**0.01**	**0.98**	**243**	0.013	0.6	–
	R2	**1.08**	**0.84**	**0.96**	**547**	0.41	1.27	0.72	998	–	–	–	–	0.002	0.75	–
	R3	**3.74**	**4.01**	**0.99**	**134**	0.06	1.08	0.93	186	–	–	–	–	0.332	0.80	546
COD	R1	0.13	0.19	0.04	–	43.7	26.8	0.79	804	**0.32**	**0.004**	**0.92**	**216**	–	–	–
	R2	**7.89**	**8.39**	**0.97**	316	0.423	1.10	0.90	462	–	–	–	–	0.01	0.44	–
	R3	**4.72**	**7.35**	**0.98**	**188**	0.093	1.342	0.80	559	–	–	–	–	0.411	0.46	–
Thiocyanate	R2	**0.196**	**0.11**	**0.95**	**283**	–	–	–	–	–	–	–	–	1.04	0.75	–
	R3	**0.215**	**1.61**	**0.99**	**34**	0.12	1.19	0.86	70	–	–	–	–	6.9	0.70	–
Nitrate –N	R2	**5.84**	**6.2**	**0.95**	**686**	0.19	1.45	0.92	737	–	–	–	–	0.88	0.46	–
Ammonia–N	R3	**5.23**	**6.59**	**0.98**	**78**	0.04	1.35	0.87	253	–	–	–	–	0.15	0.57	–

Highlighted values are best fit with experimental value

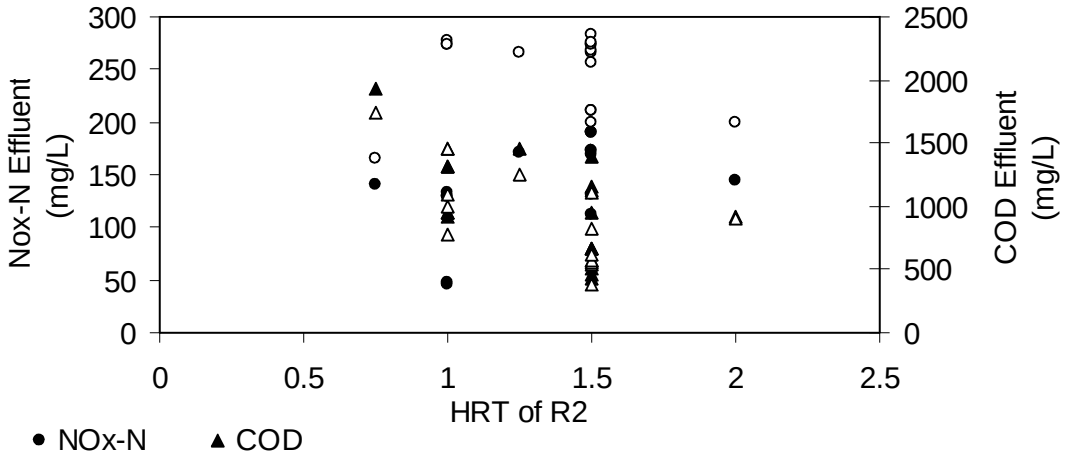

Figure 4.82 Comparison of predicted (hollow symbol) and experimental (solid symbol) Nox-N and COD effluent in R2 according to modified Stover-Kincannon model

4.3.1.3 Aerobic CMBR (R3)

In R3, Grau second order model and modified Stover Kincannon model both showed high correlation coefficients for removal of phenol, COD, NH_4^+–N and SCN^- with higher R^2 for the later model. Low χ^2 value for predicted and experimental effluent concentrations was also observed for modified Stover Kincannon model. The plots of $HRT/(S_0 - S_e)$ vs $\{V/(Q.S_0)$ for R3 are presented in Figure 4.83 and 4.84.

Effluent values were calculated using equation 2.8 and shown in Figure 4.85 and 4.86 and indicates well match between experimental and predicted effluent values. The maximum substrate removal rates were observed as 3.74, 4.72, 0.215 and 5.23 g/L.day respectively for phenol, COD, SCN^- and NH_4^+–N and given in Table 4.31 along with saturation constant. Based on the model, the maximum phenol and SCN^- removal rates in R3 were higher than R2. It suggests that R3 was more efficient than R2 for phenol and thiocyanate degradation due to aerobic environment. Rostron et al. (2001) achieved maximum ammonia removal rate of 0.7 g/L.day using polyvinyl alcohol encapsulated nitrifiers and 0.57 and 0.53 g/L.day by other media such as Linpor and Kaldane in a continuously stirred tank reactor at a loading rate of 0.5–1.0 g/L.day. In the present study R3 showed higher NH_4^+–N removal rate as compared to these literature values.

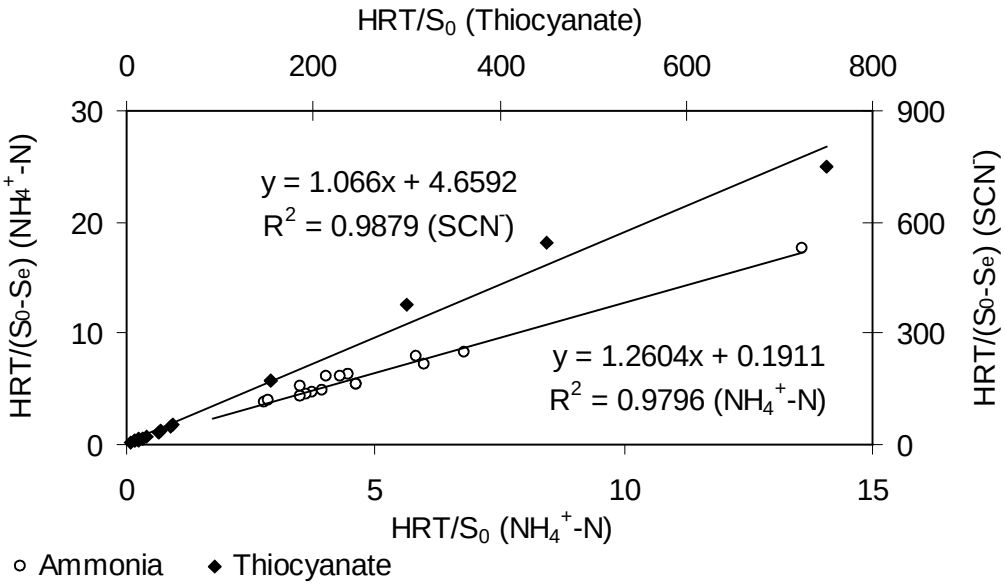

Figure 4.83 Estimation of R_{max} and K_b for NH_4^+-N and thiocyanate utilization in R3 using Modified Stover-Kincannon model

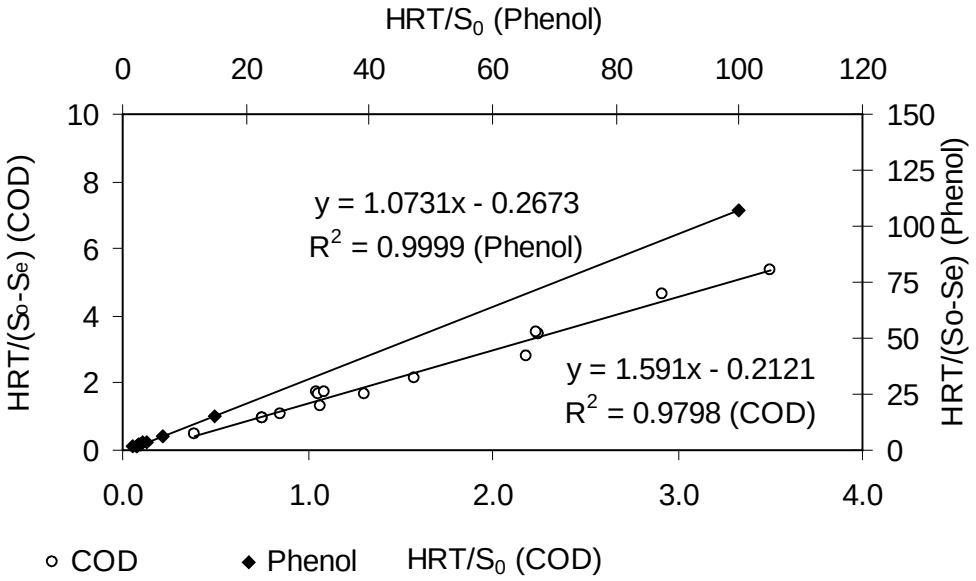

Figure 4.84 Estimation of R_{max} and K_b for Phenol and COD utilization in R3 using Modified Stover-Kincannon model

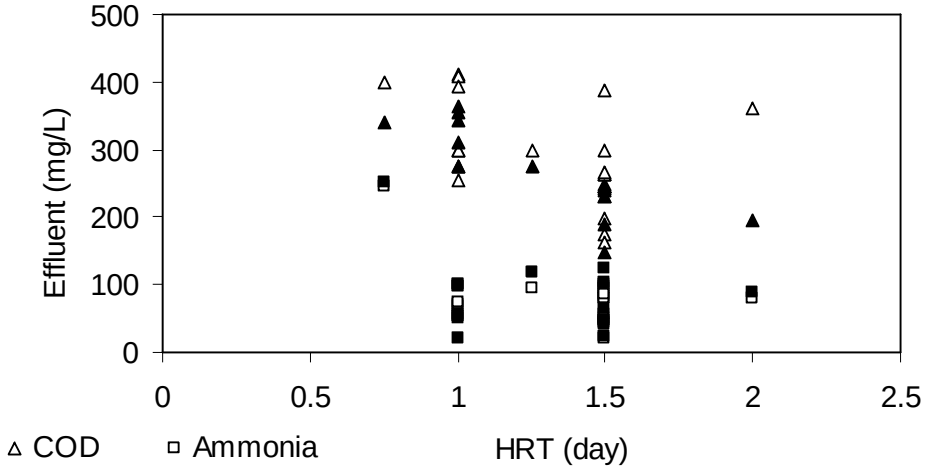

Figure 4.85 Experimental (solid symbol) and predicted (hollow symbol) COD and ammonia effluent in R3 according to modified Stover-Kincannon model

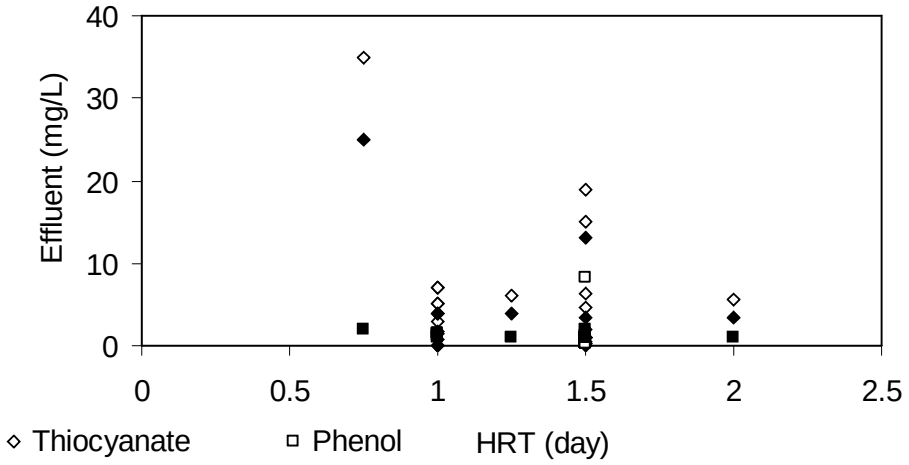

Figure 4.86 Experimental (solid symbol) and predicted (hollow symbol) thiocyanate and phenol effluent in R3 according to modified Stover-Kincannon model

4.3.2 Kinetic study of FMBR system

Haldane inhibition model, Stover–Kincannon model and Grau second order equation and First order kinetic equations were applied to evaluate the biokinetic parameter for the FMBR system in the present study. The values of biokinetic parameters are given in Table 4.32.

4.3.2.1 Anaerobic FMBR (B1)

In B1, when phenol and COD removal kinetics were plotted, the maximum correlation coefficient (R^2) of 0.955 and 0.954 was achieved for modified Stover–Kincannon model as compared to other models (Table 4.32). The maximum rate of substrate utilization (R_{max}) and saturation coefficient (K_b) for phenol was 0.69 g/L.day and 0.86 g/L.day, respectively estimated from Figure 4.87. For COD, R_{max} and K_b were 2.5 and 12.04 g/L.day and less than reported by Ahn and Forster (2000) who achieved R_{max} of 6.71 g/L.day and 3.86 g/L.day for paper and pulp and corrugated paper wastewater, respectively.

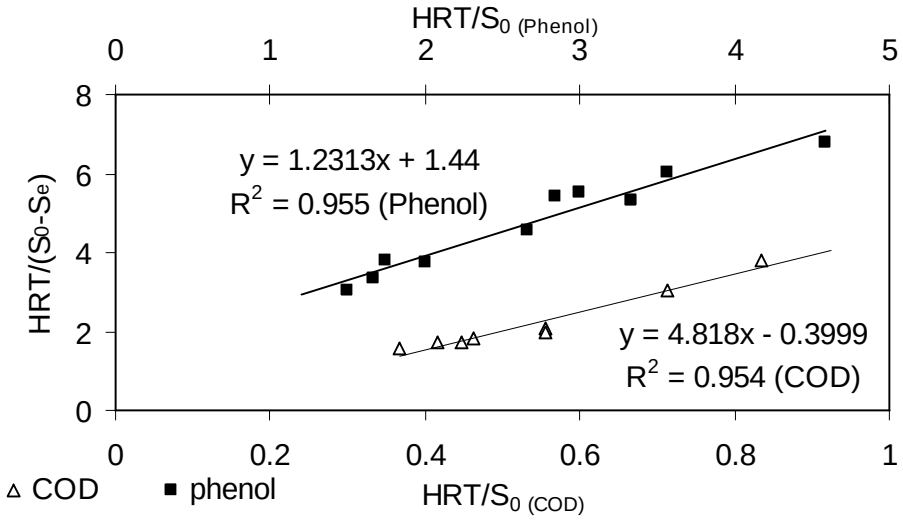

Figure 4.87 Estimation of R_{max} and K_b of phenol and COD utilization in B1 using modified Stover-Kincannon model

In Figure 4.88, experimental and predicted values of effluent phenol and COD for reactor R1 are presented. Turkdogan–Aydinol et al. (2011) achieved R_{max} and K_b of 1.99 and 1.536 g/L.day for municipal wastewater treatment in anaerobic biofilm reactor. Bhatia et al. and Haldane inhibition model also gave good fit for phenol and COD whereas Grau

second order model showed good fit for phenol degradation and no good fit was observed when first order kinetic model equation was applied.

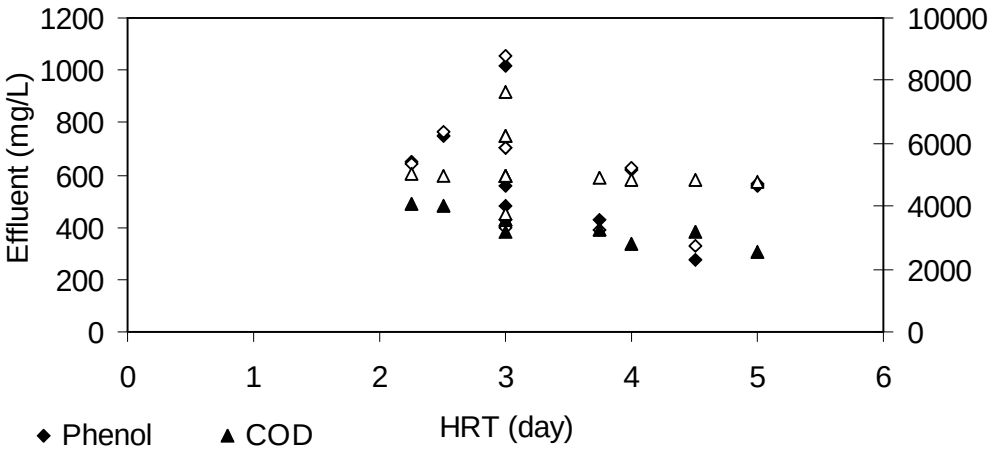

Figure 4.88 Comparison of predicted (hollow symbol) and experimental (solid symbol) effluent in B1 according to modified Stover-Kincannon model

4.3.2.2 Anoxic FMBR (B2)

In B2, almost all the model equation showed high R^2 for pollutant degradation. Best fit for phenol, COD, SCN^- and NO_x–N was achieved by modified Stover Kincannon equation with maximum R^2 value and the minimum χ^2 value (Table 4.32). Figure 4.89–4.90 shows plots of $HRT/(S_0 - S_e)$ versus HRT/S_0 for phenol, COD and SCN^- and NO_x–N.

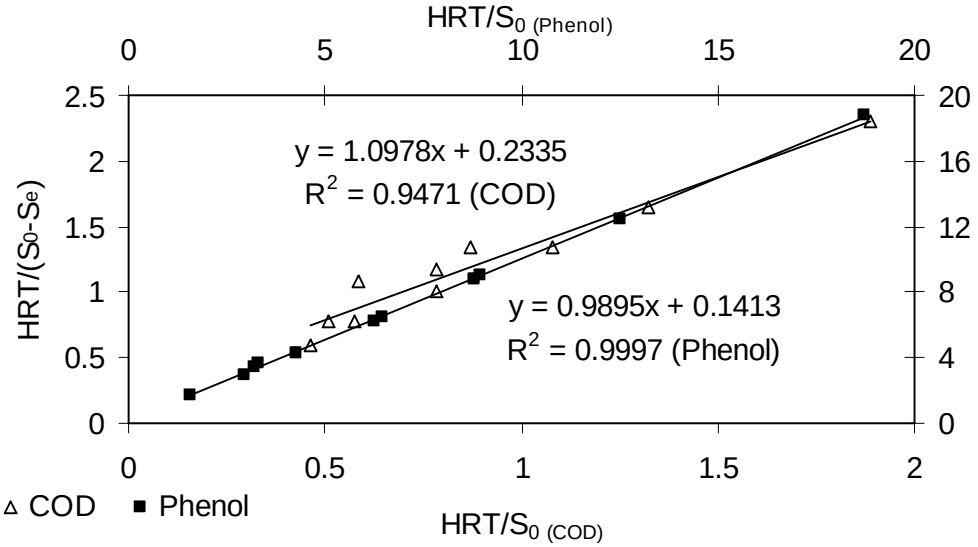

Figure 4.89 Estimation of R_{max} and K_b of phenol and COD utilization in B2 using modified Stover-Kincannon model

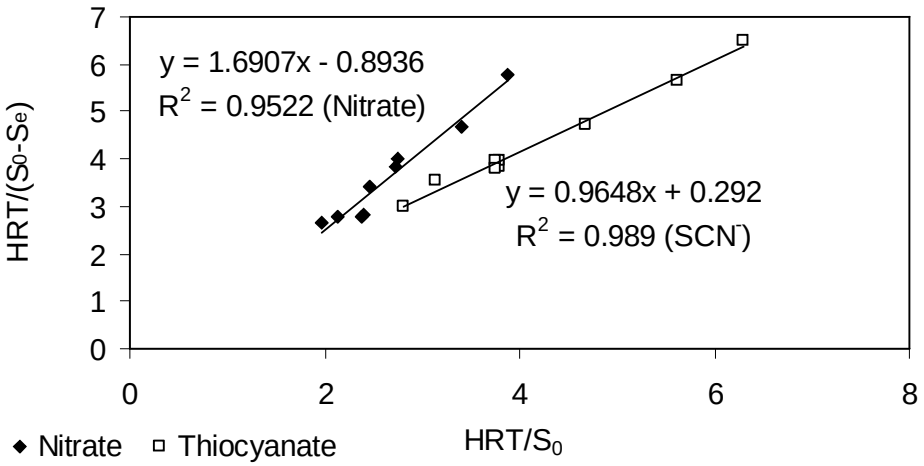

Figure 4.90 Estimation of R_{max} and K_b of thiocyanate and nitrate utilization in B2 using modified Stover-Kincannon model

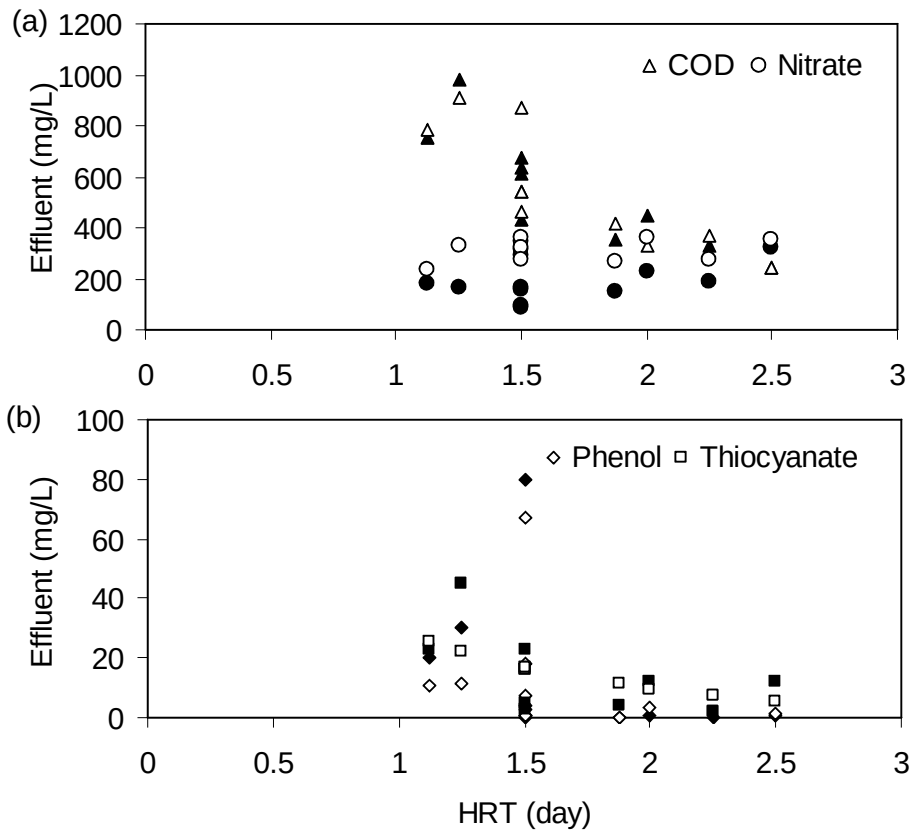

Figure 4.91 Comparison of predicted (hollow symbol) and experimental (solid symbol) effluent values in B2 according to modified Stover-Kincannon model

In the present study R_{max} of 7.09 g/L.day for phenol removal was higher than that of R2 (1.08 g/L.day), whereas R_{max} of 4.28 g/L.day for COD removal in B2 was lower than that

of R2 (7.87 g/L.day). Higher R_{max} of 3.42 g/L.day for thiocyanate degradation was achieved in B2. Figure 4.91 (a) and (b) present the comparison of experimental and model predicted effluent values with well match.

4.3.2.3 Aerobic FMBR (B3)

Modified Stover Kincannon model showed best fit for phenol, COD, NH_4^+–N and SCN^- degradation in B3 with maximum R^2 values. Estimation of R_{max} and K_b for COD and NH_4^+–N is shown in Figure 4.92. For NH_4^+–N removal kinetics in B3, modified Stover Kincannon model and Grau second order kinetic model both showed similar R^2 value of 0.96. However, Modified Stover Kincannon model showed lower χ^2 value than Grau second order model (Table 4.32).

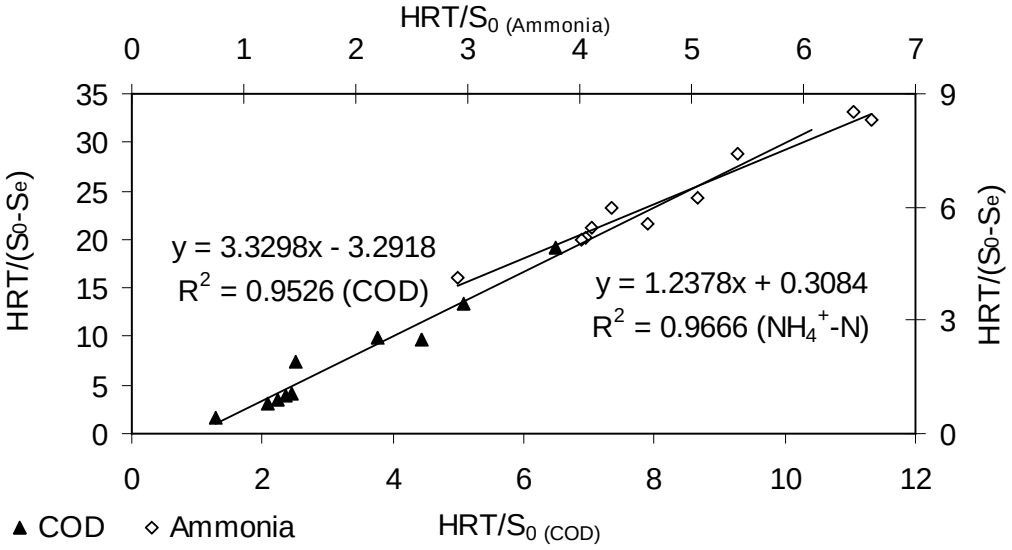

Figure 4.92 Estimation of R_{max} and K_b of COD and ammonia utilization in B3 using modified Stover-Kincannon model

Comparing values in Table 4.32 it can be seen that maximum phenol and COD utilization rate in B3 (0.08 and 0.3 g/L.day, respectively) were less than B2 and thiocyanate removal rate (9.17 g/L.day) was higher which was the maximum among all reactors studied. Maximum NH_4^+–N removal rate as predicted by the model was 3.24 g/L.day and it was lower than the value achieved for R3 in CMBR system (5.23 g/L.day) suggesting R3 was more efficient more ammonia removal than B3.

Table 4.32 Kinetic parameters for substrate removal kinetics in FMBR

Parameter	Reactor	Modified Stover Kincannon				Grau second order				Haldane inhibition				Bhatia et al.			
		R_{max} (g/L.day)	K_b (g/L.day)	R^2	χ^2	m	n	R^2	χ^2	q_{max} (g/L.day)	K_s (mg/L)	K_i (mg/L)	R^2	R (/day)	KI (L/mg)	R^2	χ^2
Phenol	B1	**0.69**	**0.86**	**0.95**	**57**	3.34	0.81	0.75	421	75	14104	8	0.82	0.704	0.1	0.87	350
	B2	**7.09**	**7.01**	**0.99**	**59**	0.13	0.94	0.98	144	141	14103	3	0.87	0.12	0.23	0.87	180
	B3	**0.08**	**0.109**	**0.99**	**13**	0.66	1.60	0.69	33	0.09	0.66	0.5	0.41	–	–	–	–
COD	B1	**2.56**	**12.04**	**0.95**	**314**	–	–	–	–	1.11	4558	6474	0.88	0.20	0.01	0.90	540
	B2	**4.29**	**4.71**	**0.95**	**198**	0.83	0.88	0.78	267	124	24660	15600	0.90	–	–		
	B3	**0.30**	**1.01**	**0.95**	**21**	0.96	2.73	0.56	769	98	12	0.79	0.70	–	–		
SCN^-	B2	**3.42**	**3.31**	**0.99**	**64**	0.11	0.67	0.98	2269	0.27	0.11	233	0.93	0.01	0.001	0.91	444
	B3	**9.17**	**17.83**	**0.97**	**3.8**	1.89	2.48	0.79	185	74	0.001	.004	0.05	–	–	–	–
Nitrate–N	B2	**1.18**	**1.89**	**0.95**	**717**	0.68	1.87	0.85	991	4.98	733	18	0.90	–	–	–	–
Ammonia–N	B3	**3.24**	**4.03**	**0.96**	**10**	0.02	1.33	0.96	107	0.225	19	781	0.46	–	–	–	–

Highlighted values are best fit with experimental value. RMSE for Haldane equation was 0.001–0.05

Compared with the maximum loading rate obtained in this study, the predicted substrate removal rates (R_{max}) in B3 were higher for phenol, COD and thiocyanate, suggesting B3 has higher potential in coping with this wastewater. The comparison of experimental and model predicted effluent values matched well (Figure 4.93).

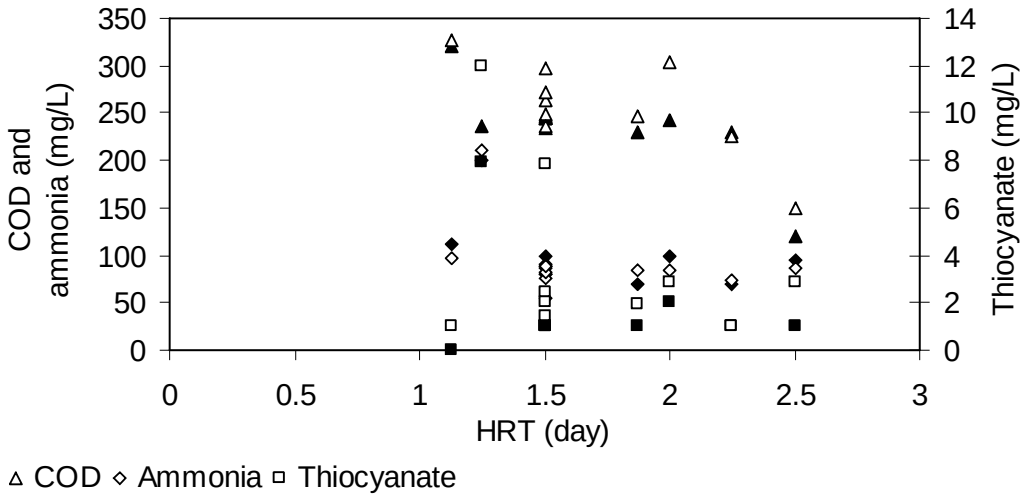

Figure 4.93 Comparison of predicted (hollow symbol) and experimental (solid symbol) effluent values in B3 according to modified Stover-Kincannon model

From the bio-kinetic parameter analysis using experimental data, most reactors followed modified Stover- Kincannon model for substrate utilization whereas R1 followed Bhatia et al (1985) inhibition model. First order kinetic model, Grau second order kinetic model and Haldane inhibition model also provided higher correlation coefficient (R^2) value; however modified Stover Kincannon model showed the maximum R^2 and lower χ^2 for R2, R3, and FMBR system. For CMBR system, R3 showed higher maximum substrate utilization rate (R_{max}) for phenol (3.74 g/L.day)) and thiocyanate (0.215 g/L.day), whereas R2 showed higher R_{max} for COD (7.89 g/L.day). Saturation value constant (K_b) was the maximum of 8.39 g/L.day for COD in R2 compared to all the substrates in R2 and R3.

In case of FMBR system higher R_{max} for phenol and COD were observed in B2 whereas B3 showed higher R_{max} for thiocyanate removal. Higher K_b value 17.83 g/L.day for thiocyanate in B3 was achieved compared to all the substrates in B1, B2 and B3.

4.4 THE PERFORMANCE OF SEQUENTIAL MOVING BED REACTORS AT SHOCK LOAD APPLICATION

Any industrial wastewater treatment plant undergoes different intermittent organic and pollutant loading conditions, when concentrations of its pollutants changes abruptly. Therefore shock loading generally refers to sudden increased input of pollutants to the reactor, which results in substrate accumulation and further inhibition; the degree of inhibition depends on overall microbial activity and the extent of shock loading (Veeresh et al. 2005). Because of the variable nature of industrial wastes, reactor stability to shock loading is one of the most important aspects of design.

4.4.1 Shock load applications on sequential CMBR system

Stability of sequential CMBR system was evaluated by phenol and thiocyanate shock application at two levels each and discussed in detail in following sections

4.4.1.1 Effect of phenol shock load on sequential CMBR system

Phenol shock load was applied at two levels: PSL–I and PSL–II. During both the studies other feed parameter and HRT were maintained constant. Before application of shock load, CMBR system was operated for 18 days at influent phenol concentration of 2500 mg/L along with 600 mg/L SCN^- and 500 mg/L NH_4^+–N at total HRT of 6 days (R1:3 days; R2: 1.5 days and R3: 1.5 days). Table 4.33 shows the average effluent concentrations and removal efficiencies in three reactors of CMBR system before application of phenol shock load (PSL–I).

Table 4.33 Average steady state effluent concentrations of the three–stage CMBR system before application of the first phenol shock loads (PSL–I)

Parameter	Feed	R1	R2 inf	R2	R3
Phenol	2500	1982 (20.7)	991.5	385 (61.2)	1.0 (99.7)
COD	7982	7580 (5.1)	3910	1150 (70.58)	240.0 (79.1)
SCN^-	600	580	291.8	85 (70.8)	3.5 (95.9)
NH_4^+–N	500	505	307.5	325	110 (68)
NO_x–N	0	–	695	220 (68.3)	390

Values in parenthesis show removal efficiency (%)

4.4.1.1.1 Performance of anaerobic CMBR (R1) after phenol shock load

Table 4.33 shows that before application of the first phenol shock load (PSL–I), effluent phenol of R1 was 1982 mg/L, with removal efficiency of 20%. PSL–I was given to R1 from day 1129th to 1131st. Feed phenol concentration was increased to 3000 mg/L from 2500 mg/L (increase of 20%). Figure 4.94 shows profile of effluent phenol concentration before and after PSL–I in reactor R1. Immediately after application of shock load on day 1129th, effluent phenol concentration started increasing to 2300–2890 mg/L from day 1130th to day 1131st. From day 1132nd onward, effluent phenol of R1 started decreasing and it required another 10 days to attain a new steady state with effluent phenol of 2120 mg/L with removal efficiency of 15%, against removal efficiency of 20% before application of shock load. During PSL–I, feed phenol concentration was increased by 20% and phenol removal efficiency of R1 declined by 25%.

Due to increase of feed phenol during shock load, feed COD also increased to 9550 mg/L from 7980 mg/L. Effluent COD started increasing from 7580 mg/L to 9330 mg/L till day 1134th (Figure 4.95). R1 required another 7 days to achieve the steady state condition with effluent COD of 7750 mg/L (removal of 2.9%). The affect of PSL–I was more profound on COD removal efficiency of R1, since it decreased by almost 43% (from 5.1% to 2.9%).

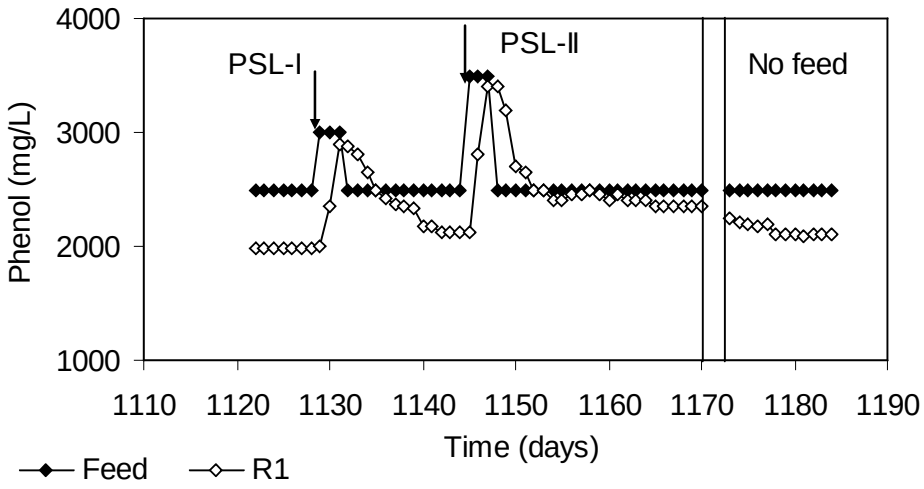

Figure 4.94 Feed and effluent phenol concentrations in R1 before and after application of phenol shock loads

On day 1145th, PSL–II was applied by increasing feed phenol to 3500 mg/L (40% increase from 2500 mg/L). R1 remained unstable from day 1145th to 1154th and then

released stable effluents with phenol of 2350–2400 mg/L and COD of 7750 mg/L, respectively. Corresponding removal phenol and COD efficiencies were 4–6% and 0.3–2.8%, respectively. Phenol and COD removal efficiencies of R1 did not improve after 25 days of operation (after PSL–II). In order to improve efficiencies, feed was stopped for two days (1171– 1172nd) day and then normal operation started. From 1178th day onwards, efficiencies of R1 improved with effluent phenol and COD of 2110 mg/L and 7700 mg/L, respectively with removal efficiencies of 15.6% and 3%. This efficiency was comparable with performance of R1 after PSL–I. However, R1 did not regain the pre–shock efficiency. Probably the two levels of phenol shock loads caused irreversible damage to phenol/COD degrading microbes in R1 as suggested by Buitrón et al. (2005).

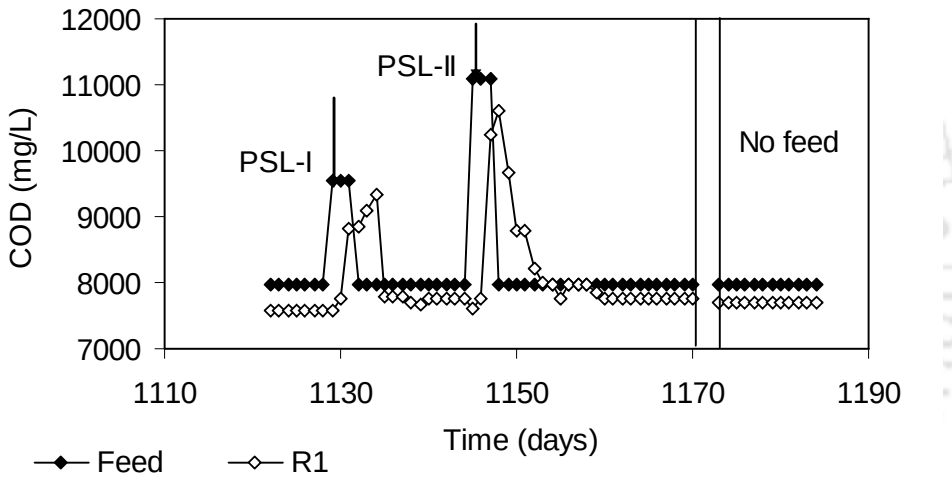

Figure 4.95 Influent and effluent COD concentrations in R1 after application of phenol shock loads

Ramakrishnan and Gupta (2008a) reported that the affect of phenol shock load of 2.5 times for 4 days (from initial concentration of 752 mg/L) on the performance of upflow hybrid anaerobic sludge blanket reactor was reversible. This was probably lower concentration of feed phenol both during regular and shock load study. In the present study, phenol concentration was much higher for both during shock load period and regular period as compared to this published value (Ramakrishnan and Gupta, 2008a). Bajaj et al. (2009) observed similar phenomenon after application of phenol shock load of 4700 mg/L (increasing from 3700 mg/L) in an anaerobic fixed bed reactor. Phenol and

COD removals declined after injection of phenol shock load and recovery was possible after providing no feed to reactor for one month.

4.4.1.1.2 Performance of anoxic CMBR (R2) after phenol shock load

Before application of PSL–I, R2 was receiving 991–1000 mg/L phenol in its influent and released ~385 mg/L showing removal efficiency of 61–62% (Table 4.33). Figure 4.96 shows influent and effluent phenol profile in R2. Immediately after application of PSL–I, influent phenol to R2 increased to 1176–1435 mg/L on 1130th–1132nd day. Thereafter it started decreasing steadily and influent phenol of R2 stabilized on day 1142nd onwards. With increase in influent, effluent phenol of R2 initially increased to 410–415 mg/L (from 385 mg/L) on day 1134–1136. However, it started decreasing and on day 1137–1140 onwards, R2 released effluent with stable phenol concentration of 390–370 mg/L.

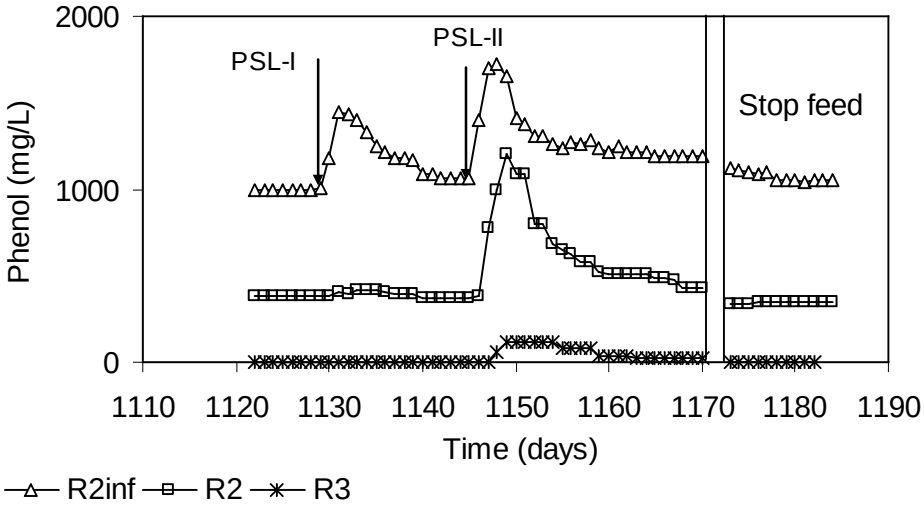

Figure 4.96 Influent and effluent phenol concentrations in R2 and R3 before and after application of phenol shock loads

Phenol removal efficiency of R2 was almost 67%, slightly higher than pre-shock condition. After application of PSL–II, influent phenol to R2 increased from 1061 to 1727 mg/L (66% increase) within 3 days. Influent phenol to R2 decreased slowly to 1187 mg/L within next 17 days (day 1165th). Effluent phenol of R2 initially increased and after 1152–1154th day R2 gradually regained its efficiency and on 1165–1170th day, R2 removed 60–64% with effluent phenol concentration of 430 mg/L. Phenol removal

efficiency improved further to 67% (effluent phenol 350 mg/L) on day 1174 onwards when feed was stopped for two days.

Figure 4.97 shows that influent COD to R2 increased from 3910 mg/L to 4770 mg/L (22% increase) in 4 days after application of PSL–I. Initially COD removal in R2 fluctuated from 63–65% with effluent COD of 1500–1300 mg/L. From 1139th day onward, effluent COD of R2 became stable with removal efficiency of 70% (effluent 1200 mg/L). R2 required almost 8 days to regain its COD removal efficiency. The second phenol shock load (PSL–II), showed more profound affect on initial COD removal efficiency of R2. With increase in influent COD from 3999 mg/L to 5540 mg/L (again 38% increase) on day 1148, COD removal efficiency decreased to 51%. It decreased further to 45–46% on day 1152–1153. R2 required almost 20 days after application of PSL–II to achieve COD removal efficiency of 66%. This improved further to 68% after feed was stopped and CMBR system was restarted.

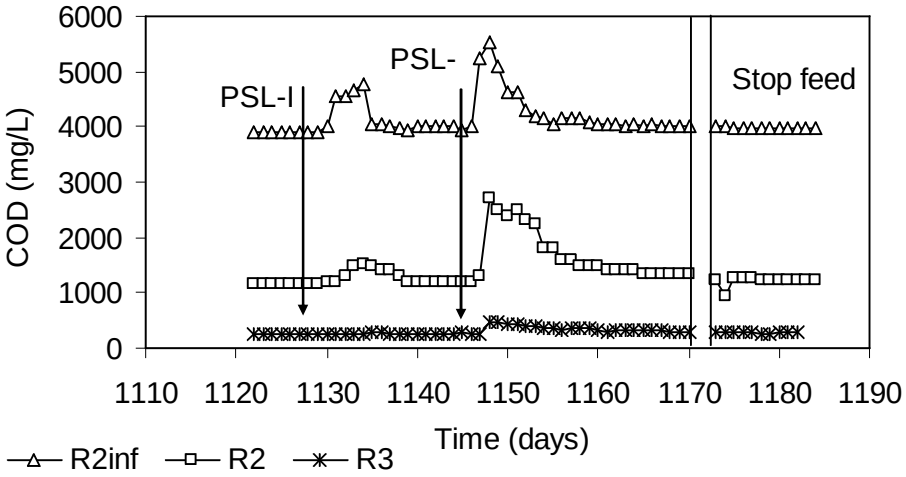

Figure 4.97 Influent and effluent COD concentrations in R2 and R3 after application of phenol shock loads

Feed SCN^- concentration to R1 was kept constant at 600 mg/L during the study. Figure 4.98 shows profile of SCN^- in feed and three reactors of CMBR. Before PSL–I, R2 received average influent SCN^- of 292 mg/L and almost 71% was removed with effluent SCN^- of 86 mg/L. After introduction of PSL–I, the influent SCN^- to R2 increased to 300 mg/L with effluent of 110–120 mg/L (removal 60–63%). R2 released steady effluent with 90 mg/L SCN^- from day 1136th onwards with 70% SCN^- removal efficiency. Influent SCN^- to R2 increased to 312–324 mg/L on 1149th to 1151st day after application of PSL–

II. Effluent SCN^- from R2 reached as high as 190 mg/L and removal dropped to 36–40%. In this period, the influent phenol was 1385–1727 mg/L and might be responsible for lower SCN^- removal by R2. However effluent SCN^- started decreasing from day 1151st onward and from 1166th day onwards (after 20 days of application of PSL–II) R2 released steady SCN^- concentration of 110 mg/L in its effluent and removal efficiency achieved was 63%. The cut down of feed on 1171st and 1172nd day further improved SCN^- removal efficiency to 70% with effluent of 90 mg/L, same as pre–shock condition Figure.4.98.

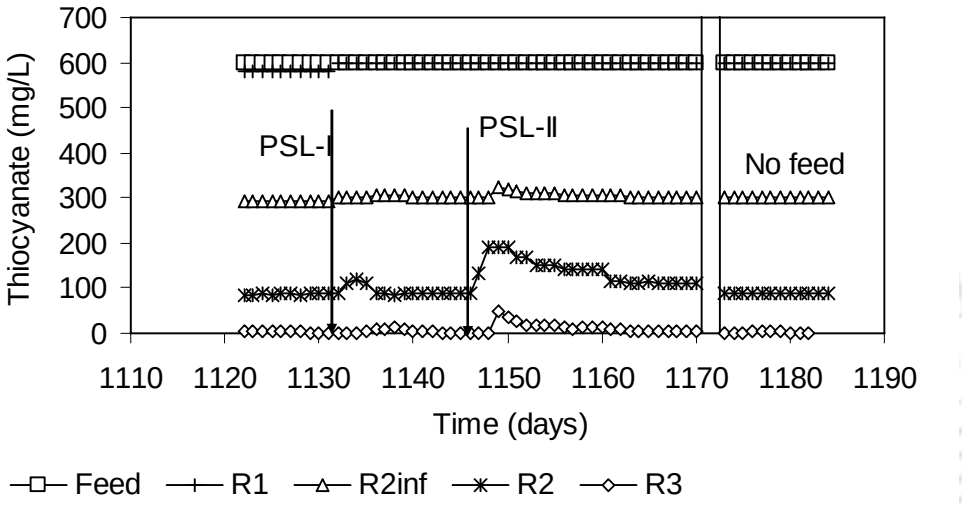

Figure 4.98 Thiocyanate removal efficiency in three-stage CMBR system after application of phenol shock load

In recycle to R2 1000 mg/L NO_3^-–N was added to maintain strict anoxic condition in R2. During introduction of PSL–I and PSL–II, feed phenol was increased and it was expected that higher phenol could be released from R1. Hence, concentration of NO_3^-–N was also increased to 1200 mg/L (from 1131st day to 1145th day) and 1400 mg/L (from 1146th day to 1171st day). Table 4.33 shows that before application of PSL–I, influent NOx–N to R2 was 695 mg/L with effluent of 210 mg/L and removal of 68%. After application of PSL–I, influent NOx–N to R2 fluctuated for a brief period and from day 1139th onward, it was stable to 711 mg/L. Figure 4.99 shows that effluent NOx–N decreased significantly after PSL–I to 95 mg/L from day 1135th onwards and decreased further with a stable value to 80 mg/L from day 1140th. Almost 87–90% denitrification efficiency was achieved after PSL–I. The higher NOx–N removal efficiency in R2 was probably due to higher influent

COD to R2 during this period. This was also confirmed from the ratio of COD:N_{rem}. Before application of PSL–I, it was 4.2. During initial 4–5 days after PSL–I, this ratio increased to 4.7–4.8 and became constant to 4.0–4.1 from day 1135th onward, suggesting drastic improvement in denitrification in R2 was associated with higher COD removal. After PSL–II, influent NOx–N to R2 increased to 800 mg/L. Influent COD: NOx–N ratio was 5–6:1 in this period. With higher COD loading denitrification efficiency increased to 87–89%. Therefore no negative affect of shock load was observed by phenol/COD shock load on denitrification.

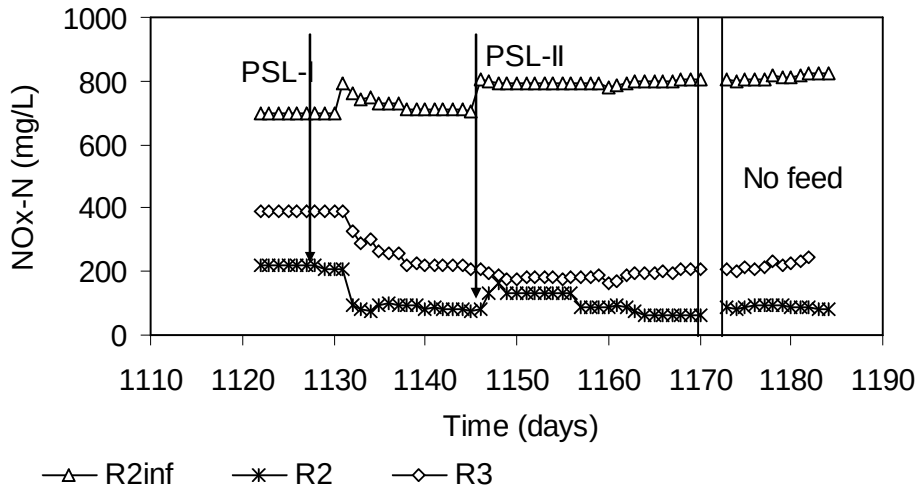

Figure 4.99 Nitrate-nitrogen removal efficiency in three-stage CMBR system after application of phenol shock load

4.4.1.1.3 Performance of aerobic CMBR (R3) after phenol shock load

Before application of PSL–I, influent phenol to R3 was 385 mg/L with removal efficiency of 99.7% (Table 4.33). After the first phenol shock load in R1, no significant changes were observed in terms of influent and effluent phenol profile of R3. Influent phenol concentration was 370–385 mg/L except on 1133rd –1135th day when R3 received 410–420 mg/L. R3 removed more than 99% influent phenol and released 1–3 mg/L. After PSL–II, on 1147th day, due to extensive release of phenol in R2 effluent, double amount of phenol i.e., 780 mg/L entered to R3. This value further increased to 990–1200 mg/L on 1148th –1149th day. Eventually R3 released higher amount of phenol 55–110 mg/L in its effluent during 1149th–1154th day and removal decreased to 83–94%.

However phenol concentration in influent to R3 decreased from 620 mg/L to 430 mg/L (1156th–1170th day) as upstream anoxic reactor R2, regained its efficiency and phenol in effluent of R3 also decreased to 20 mg/L with phenol removal efficiency 86–93%. After 2 days cut off of the system, the final effluent from R3 was 2–3 mg/L and removal efficiency achieved was more than 99%.

Before PSL–I, influent COD to R3 was 1150 mg/L and almost 79% of COD removal was achieved. Effect of PSL–I on COD profile of R3 was insignificant. After PSL–II, up to 1147th day influent COD was 1150–1300 mg/L and R3 released 240–260 mg/L COD in its effluent showing COD removal efficiency of 80%. On 1148th day, due to extensive release of phenol and COD in R2 effluent, influent COD increased to 2700 mg/L (almost two times from 1300 mg/L) and gradually decreased to 2500–2250 mg/L on 1149th – 1153rd day. During this period R3 shows 81–82% of COD removal efficiency. With time COD concentration in influent decreased to 1800–1350 mg/L and almost steady effluent COD was released at a concentration of 350–310 mg/L with a COD removal efficiency of 77–80%. After 2 days cut off of the system, the final effluent from R3 was 280 mg/L and removal efficiency achieved was more than 77%.

After the first shock load introduction to R1 (1129th day) thiocyanate profile in R3 was affected from 1132nd day. Prior to shock load, R3 was receiving 85–90 mg/L SCN^- and released average 3 mg/L in its effluent with a removal efficiency of 96–97%. On 1133rd–1135th day, SCN^- in the influent of R3 increased to 110–120 mg/L. R3 released 8–12 mg/L SCN^- during 1136th–1139th day when influent phenol and SCN^- were 390–370 and 90 mg/L, respectively and SCN^- removal efficiency dropped from 91% to 86%. From 1140th day, R3 regained its previous SCN^- removal efficiency of 98%. R3 required nine days (six times of reactor HRT) to regain the same SCN^- removal efficiency after application of first phenol shock load. On 1147–1148th– day influent SCN^- increased to 135–190 mg/L as the second phenol load was added to the upstream reactor. This influent strength prevailed for a long period of 1148th–1160th day as influent SCN^- slowly decreased from 190 to 140 mg/L (Figure 4.98). During this period R3 exhibited removal efficiency of 74–91%. On 1162nd day onwards stable influent with 115–110 mg/L SCN^- entered R3 and effluent was containing nearly 5 mg/L SCN^- showing SCN^- removal

efficiency of 95%. After 2 days cut off of the system, the final effluent from R3 was 1–3 mg/L and removal efficiency achieved was 96–99%.

Effect of first phenol shock load on NH_4^+–N removal efficiency of R3 was not profound. Figure 4.100 shows that after PSL–I, effluent NH_4^+–N of R3 increased little from 110 mg/L to 130 mg/L from day 26 onwards with decrease in removal from 68% to 62% (decrease by 8%). The effect of PSL–II was more significant. From 1147th day onwards influent NH_4^+–N concentration increased to 395 mg/L from 330 mg/L and increased further to 440 mg/L from day 1151st. From 1147th to 1162nd day, NH_4^+–N removal in R3 was only 36–38%. From day 1147th to 1149th, influent phenol to R3 was very high (780–1200 mg/L) due to less phenol removal in upstream reactor R2. This higher phenol was probably responsible for decrease in NH_4^+–N removal efficiency in R3. Due to effect of recycle, the higher amount of effluent NH_4^+–N released from R3 was recycled to R2 and again entered to R3. Due to combined effect of higher amount of NH_4^+–N and phenol, NH_4^+–N removal was significantly affected.

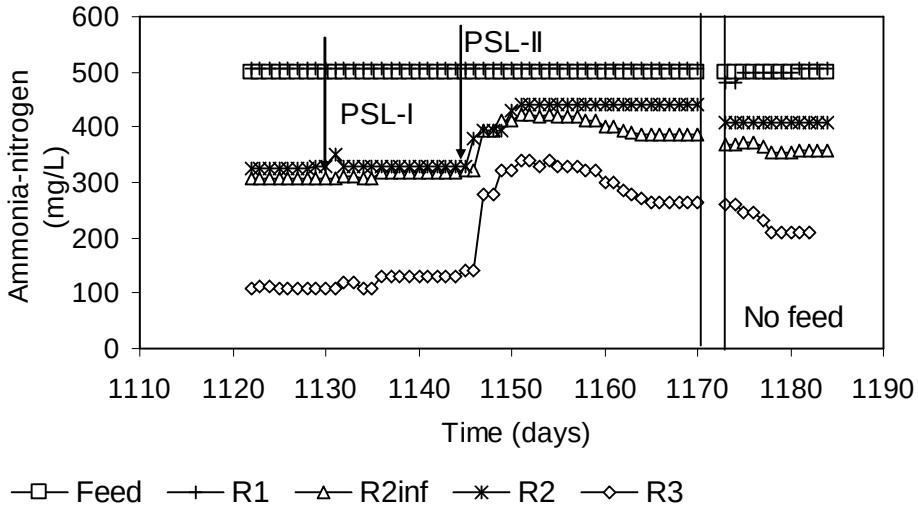

Figure 4.100 Ammonia-nitrogen removal efficiency in three-stage CMBR system after application of phenol shock load

Influent phenol to R3 decreased with time, as R2 was able to regain previous phenol removal efficiency from day 1169th onward. However, NH_4^+–N removal efficiency was consistently low to 42–43%. It seems that high amount of phenol and NH_4^+–N caused significant inhibition/washout of nitrifiers from aerobic reactor R3. Even after two days

shut down of the CMBR system, was unable to help R3 to regain previous NH_4^+–N removal efficiency.

NOx–N generated in R3 also significantly decreased after the shock loads (Figure 4.99). Simultaneously there was totally unsteady stage about the unaccounted nitrogen in R3. Original degradation time and the activity of microorganisms after the peak was not recovered in the subsequent cycles operated at normal condition. Lee and Park (1998) studied the effect of a sudden increase in influent phenol concentration in a three–stage suspended growth system. The system consisted of a first aerobic stage (carbon removal), second aerobic stage (nitrification) and third anoxic reactor (denitrification) with external carbon addition. They had reported that due to a sudden increase in phenol concentration (from 500 to1500 mg/L), in the feed, the first aerobic carbon removal stage regained its previous efficiency as soon as the phenol concentration came down. But the downstream nitrifying reactor was affected more and the inhibitory effect continued even after influent phenol concentration to the nitrifying stage returned to the initial level. Neufeld et al. (1986) had reported that phenol (above 1.4 mg/L), CN^- (above 0.2 mg/L) and SCN^- (above 236 mg/L) inhibit nitrification. Chakraborty and Veeramoni (2005) reported that nitrification efficiency was hindered after phenol shock load and the effect was irreversible aerobic reactor in a suspended growth sequential anaerobic-anoxic-aerobic system as aerobic nitrifying reactor was the most sensitive and prone to process upset due to overloading of phenol. In the present study the influent phenol concentration to the nitrifying reactor was much higher than this reported value (385–1200 mg/L), which caused irreversible damage to nitrifiers.

4.4.1.1.4 Overall performance of sequential CMBR system during phenol shock load

The feed and final effluent of R3 was considered to estimate overall performance of three–stage CMBR system during the shock load study. It was observed that the system regained its normal performance efficiency in terms of phenol COD and SCN^- removals. Total phenol removal after shock load decreased slightly from 99% to 95% from 1149th to 1154th day. The system regained it's the efficiency of more than 99% phenol removal from 1163rd day onwards. The inhibitory and toxic nature of phenolic compounds at high concentrations, even to granular sludge grown on phenol (Tay et al. 2001), prohibits

further enhancement of organic load with desired performance. Relatively long acclimatization period, small granule size and decrease phenol removal efficiency a higher loadings, sensitivity to temperature and shock loading and long recovery period s after shocks (Fang et al. 2006; Tay et al. 2001) are few problems associated with the treatment of phenol at high concentration.

Similarly thiocyanate removal decreased to 95–92% on 1149th to 1151st day during the high shock load period. However the removal efficiency increased to 97% from 1155th day and reached more than 99% from 1163rd day. Low COD removal efficiency by the system was exhibited during high shock load study when COD removal dropped to 93% (1148th–1153rd days). After normal feed was started total COD removal efficiency enhanced and the system was able to regain its normal performance value. The total NH_4^+–N removal in three–stage system continuously decreased from 86% to 55% from the initial of the shock load introduction and it was not recovered even after closing down the reactor for two days.

Total nitrogen (TN) in influent and effluent of three stage CMBR system was estimated from summation of SCN^-N, NH_4^+–N, NO_3^-–N and NO_2^-–N. Feed TN was 1644–2044 mg/L (considering influent NO_3^-–N of 1000, 1200 and 1400 mg/L added in the recycle of R3). TN removal remained almost 70% during normal condition and with high phenol loading total nitrogen removal increased significantly and reached 76–78% towards the end of the study. It was observed that during phenol loading there was high NOx–N removal and less NH_4^+–N removal whereas and fraction NOx–N removal was higher and it finally resulted in higher TN removal through out the study.

The present study suggested that the CMBR was able to completely regain its original performance within a short period of operation, under the two shock loads applied for phenol and thiocyanate removal. Hosseni and Borghei (2005) observed that moving bed biofilm reactor reached the steady state conditions after about 24 h (two cycle of hydraulic retention time) when a sudden shock of phenolic COD 200–1000 mg/L was applied apart from normal condition of 200 mg/L of phenolic COD. The performance of the R2 of CMBR under shock loads was better than many other continuous bioreactor systems studied on phenolics biodegradation (Xing–yu et al. 2007; Tomei et al. 2008; Cho et al. 2000).

4.4.1.2 Effect of thiocyanate shock load on sequential CMBR system

After phenol shock load study, once the performance of CMBR system stabilized, thiocyanate shock load was applied in two levels: first thiocyanate shock load (TSL–I) from 600 mg/L to 1000 mg/L and second thiocyanate shock load (TSL–II) from 600 mg/L to 1200 mg/L. Performance of the CMBR system after TSL–I and II are discussed in following sections. Table 4.34 shows average steady state effluent concentrations in CMBR before application of the first thiocyanate shock load (TSL–I).

Table 4.34 Average steady state effluent concentrations of the three–stage CMBR system before application of first thiocyanate shock loads (TSL–I)

Parameter	Feed	R1	R2inf	R2	R3
Before TSL–I					
Phenol	2500	2000 (20)	1000	350 (65)	2 (99.4)
COD	7980	7550 (5.3)	3897	1100 (71.8)	245 (77.7)
SCN^-	600	600	300	95 (68.3)	1 (98.9)
NH_4^+–N	500	505	369	350 (5.1)	135 (65.6)
NO_x–N	0	–	648	85 (87)	295

Values in parenthesis indicates removal efficiency

4.4.1.2.1 Response of anaerobic CMBR (R1) at thiocyanate shock load

TSL–I was given to R1 from day 1211th–1213th. Feed thiocyanate concentration was increased to 1000 mg/L from 600 mg/L (increase of 66%). Figure 4.101 shows profile of effluent thiocyanate concentration before and after TSL–I. Immediately after application of shock load on day 1211th, effluent SCN^- concentration increased to 980 mg/L from day 1213th to day 1214th.

From day 1215th onward, effluent SCN^- of R1 decreased to 650 and 598 mg/L and remained stable showing no SCN^- removal at all like steady state before TSL. On 1225th–1227th day (after 11 days of TSL–I), the CMBR system was subjected to second SCN^- shock load (TSL–II) of 1200 mg/L (100% increased from 600 mg/L). R1 showed unstable effluent concentration in terms of SCN^-, phenol and COD release. R1 released 720–1180 mg/L SCN^- in its effluent for another 2–3 days and after three days of TSL–II, effluent SCN^- of R1 became stable of 600 mg/L without any removal of SCN^-.

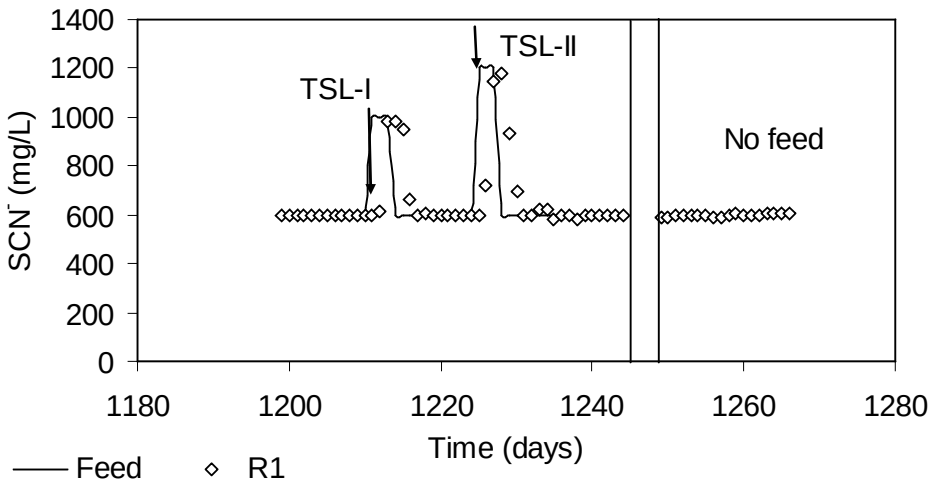

Figure 4.101 Thiocyanate profile in R1at application of SCN^- shock load

Table 4.34 shows that before application of the first thiocyanate shock load (TSL–I), effluent phenol of R1 was 2000 mg/L, with removal efficiency of 20%. Figure 4.102 shows that after application of TSL–1, effluent phenol of R1 increased from pre–shock value 2000 mg/L to 2340 mg/L (increase by 17%) during 1213th to 1215th day and then slowly decreased to a new steady state with effluent phenol of 2200 mg/L with removal efficiency of 12%, against removal efficiency of 20% before application of shock load. Due to application of TSL–I, phenol removal efficiency in R1 decreased by 40%.

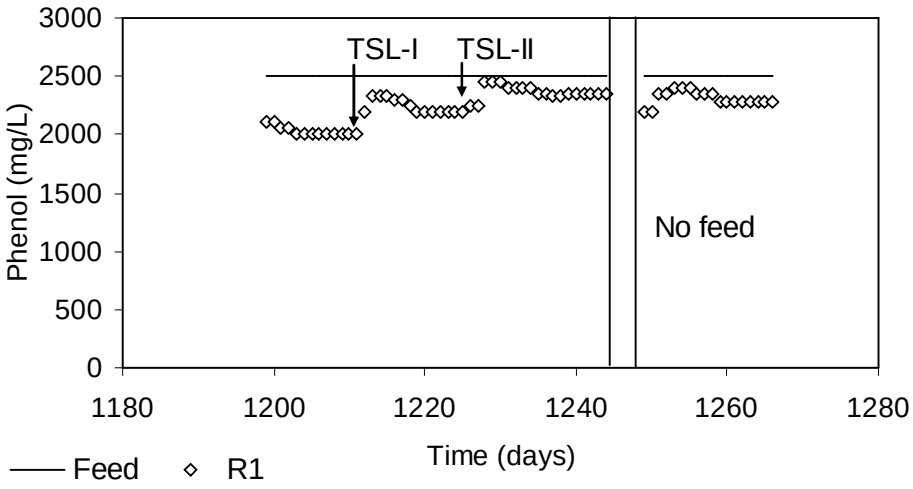

Figure 4.102 Phenol concentration profile in R1at application of SCN^- shock load

Due to increase of feed SCN^- during shock load, feed COD also increased from 7980 mg/L, to 8300 mg/L (Figure 4.103). Effluent COD started increasing from 7500 mg/L (removal 5.3%) to 8250 mg/L till day 1214th. R1 required another 4 days to achieve the steady state condition with little higher value of effluent COD of 7780 mg/L (removal of 2.5%). The affect of TSL–I was quite significant on phenol and COD removal efficiencies of R1, since these decreased by almost 40% and 52%, respectively.

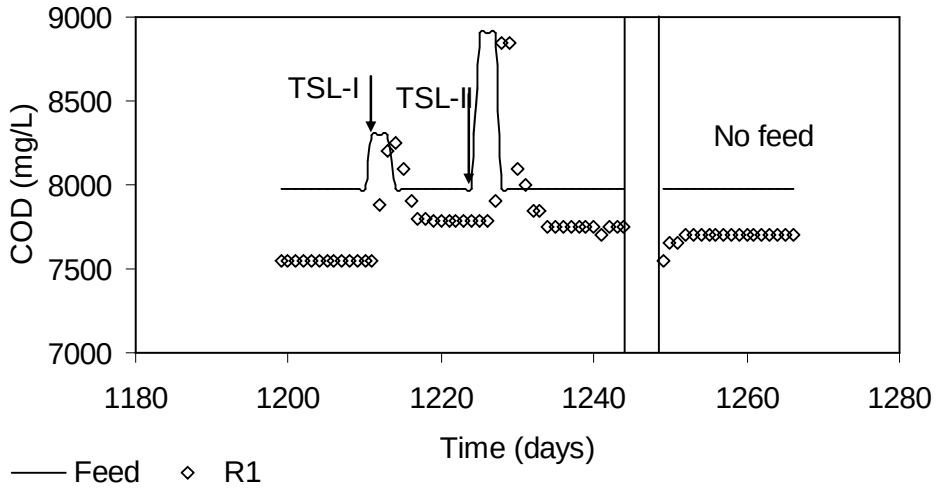

Figure 4.103 COD concentrations profile in R1at application of thiocyanate shock load

After application of TSL–II (1225th to 1227th day), effluent phenol and COD of R1 increased to 2450 mg/L and 8850 mg/L, respectively for the next two–three days and then decreased gradually. From day 1235th onwards R1 released stable phenol and COD concentration of 2350 mg/L and 7750 mg/L, respectively. Phenol removal decreased from 12 to 6%, whereas no change was observed in COD removal efficiency. Phenol removal efficiency in R1 did not improve after 17 days of regular operation (after TSL–II). Feed was stopped for four days (1245–1248th day) and then again regular reactor operation started. From 1252nd day and 1259th day onwards COD and phenol removal efficiencies improved slightly to 3.5% and 8.8% with effluent COD and phenol of 7700 mg/L and 2280 mg/L, respectively. This efficiency was nearer to the performance of R1 after TSL–I though it was inferior to the pre–shock condition performance. No literature report is available on performance of anaerobic reactor after thiocyanate shock. Probably,

high shock load of thiocyanate caused irretrievable damage to phenol degrading anaerobes in R1 deteriorating the performance of reactor.

4.4.1.2.2 Response of anoxic CMBR (R2) towards thiocyanate shock load

Prior to TSL–I, R2 received 300 mg/L SCN^- and almost 68% removal was achieved releasing 95 mg/L of SCN^- in the effluent (Table 4.34). Influent SCN^- to R2 increased to 471–491 mg/L on 1213–1215th day after application of TSL–I and then decreased to 300 mg/L in next four days. Effluent SCN^- initially increased to 120–170 mg/L with slight drop in removal efficiency. From 1221st day onwards (seven days after application of TSL–I), R2 released steady effluent concentration of 105 mg/L (removal efficiency 65%) showing decrease in removal efficiency by 4.4% than prior to TSL–I. There was sharp increase of influent SCN^- from 300 mg/L to 582–600 mg/L after application of TSL–II (100% increase). However within three days influent SCN^- to R2 decreased gradually to 360–316 mg/L (Figure 4.104).

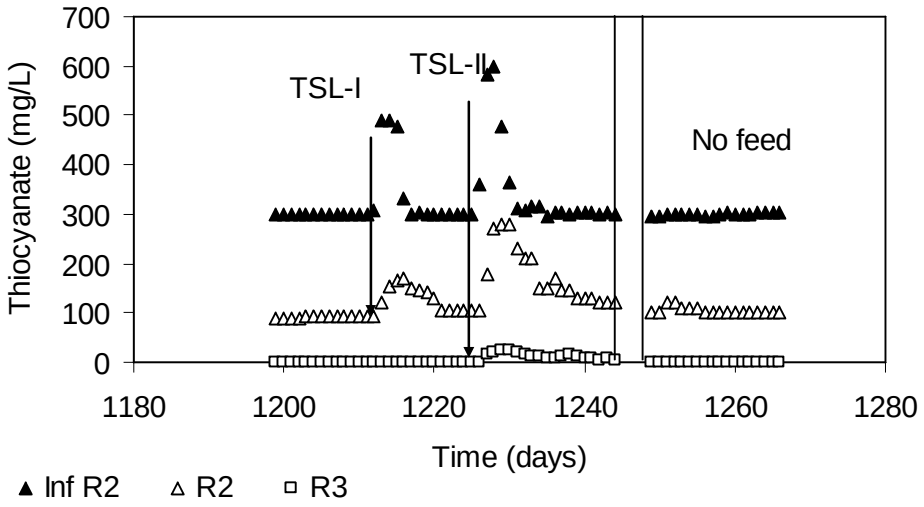

Figure 4.104 Thiocyanate profile in R2 and R3 at application of thiocyanate shock load

Immediately, after TSL–II, SCN^- removal efficiency decreased to 55% and decreased further to 22% on 1230th day. Then this improved slowly and R2 released stable effluent SCN^- concentration of 120 mg/L (removal 60%) from day 1242 onwards. When feed was stopped for four days, R2 regained almost the pre–shock SCN^- removal efficiency of

67% with effluent of 100 mg/L. It shows that even 100% increase of SCN^- shock to anoxic reactor, performance of R2 was reversible in terms of SCN^- removal efficiency.

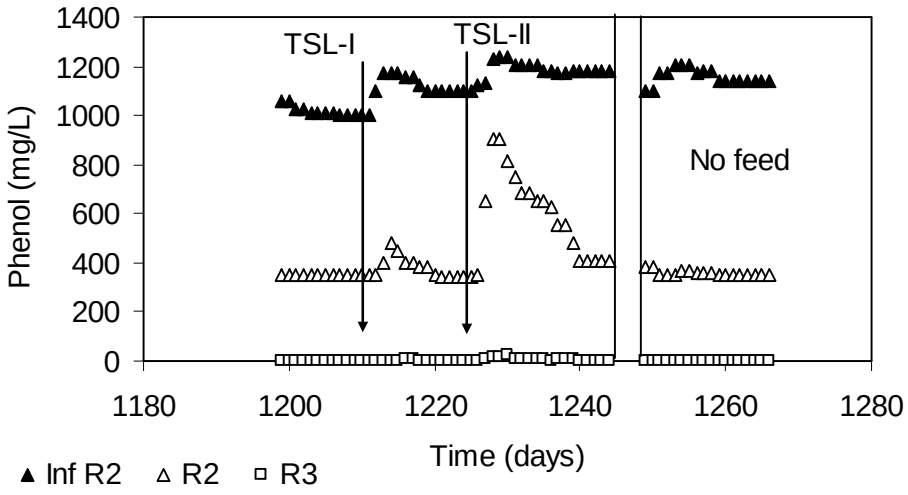

Figure 4.105 Phenol concentrations profile in R2 and R3 at application of SCN^- shock load

After application of TSL–1, influent phenol to R2 increased slightly to 1100–1171 mg/L (due to decrease in phenol removal efficiency in R1) from its regular concentration of 1000 mg/L on 1212–1215th day (10–17% increased) as shown in Figure 4.105. Phenol removal decreased little from 65% to 59% with effluent concentration of 350–480 mg/L during 1212–1215th day. From 1221st day onwards influent and effluent concentration remained stable at 1100 mg/L and 340 mg/L, respectively showing 69% phenol removal which was even higher than efficiency shown prior to TSL–I. After application of TSL–II in R1, influent phenol to R2 increased from 1100 mg/L to 1237 mg/L during 1228–1230th day. On 1228th–1229th day, phenol removal efficiency of R2 decreased to 27% releasing maximum of 900 mg/L phenol in effluent. During the next nine days (1230–1239th day), the effluent concentration decreased from 810 to 480 mg/L with increase in removal efficiency from 34% to 59%. R2 released stable phenol effluent concentration of 410 mg/L from its influent concentration of 1177 mg/L from 1240th day onwards (after 13 days of TSL–II). After 4 days cut off feed from 1245th to 1248th day, phenol removal efficiency of R2 increased further to 69% as it was after TSL–I.

Prior to TSL–I, R2 received average influent COD concentration of 3897 mg/L and released 1100 mg/L showing 71.8% COD removal efficiency. COD profile during TSL is

shown in Figure 4.106. After TSL–I application on 1211th day, influent COD to R2 increased to 4062–4265 mg/L for the next three days. Then influent COD decreased gradually from 4190 mg/L to 4012 mg/L and remained stable after seven days of TSL–I. During these seven days, R2 released increased amount of effluent COD of 1280–1350 mg/L and COD removal efficiency decreased to 65–66%. From 1221st day onwards, R2 released stable effluent concentration of 1310 mg/L with removal efficiency of ~ 67%. The effluent concentration from R2 increased by 19% showing decrease in removal by 6.7% after TSL–I.

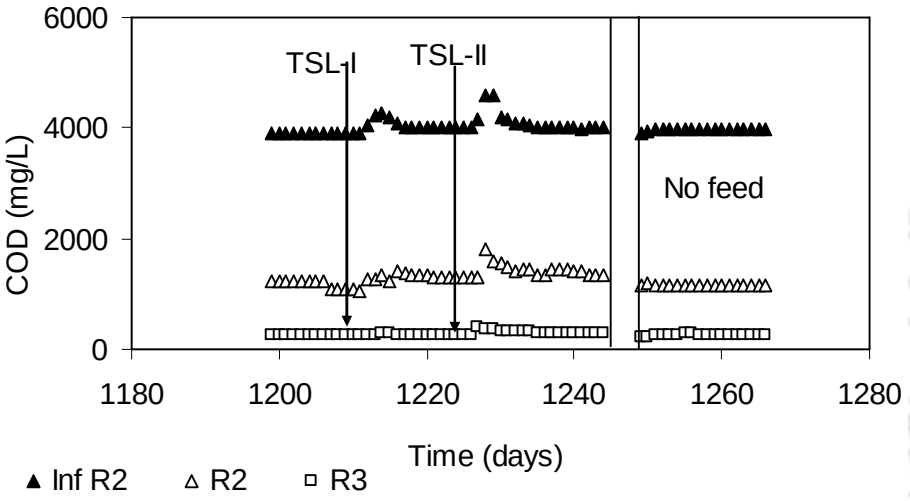

Figure 4.106 COD concentrations profile in R2 and R3 at application of SCN^- shock load

After application of TSL–II to R1 on 1225th–1227th day, influent COD concentration to R2 reached maximum of 4600 mg/L on 1228–1229th day (Figure 4.106). During these days R2 released higher amount of effluent COD (1600–1800 mg/L) showing decrease in removal efficiency to 60%. From 1230th to 1241st day, effluent COD from R2 fluctuated 1550–1400 mg/L with removal efficiency 63–64%. From 1242nd day onwards (fifteen days after TSL–II) R2 released stable effluent COD concentration of 1350 mg/L with 66% removal efficiency. The stop of feed from 1245th to 1248th day further enhanced COD removal efficiency of R2 to 71% with effluent concentration 1150 mg/L, which was similar to prior TSL–I condition.

With application of TSL–I, influent SCN^-, phenol and COD to R2 increased, hence NO_3^-–N in the recycle also was increased to 1200 mg/L (1212th –1226th day) and 1400 mg/L

(1227th to 1233rd day) from its normal concentration 1000 mg/L. R2 was receiving 648 mg/L NOx–N and removed 87% of it releasing 85 mg/L NOx–N in the effluent (Figure 4.107). After application of TSL–I, influent to R2 and effluent NOx–N fluctuated for 9–10 days and then it became stable with effluent NOx–N of 66 mg/L and removal efficiency of 90%. NOx–N removal efficiency in R2 increased by 3.4% from 87% to 90% after application of TSL–I.

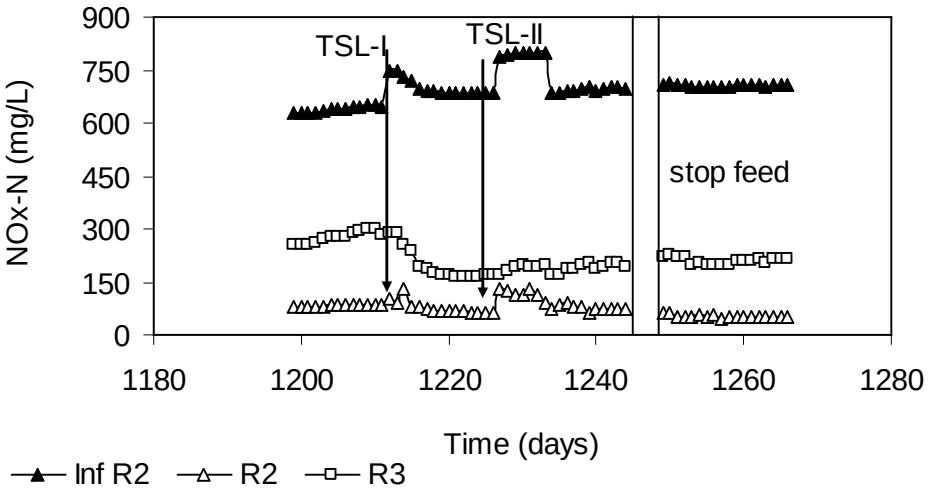

Figure 4.107 Influent and effluent of NOx-N concentrations in R2 and R3 at application of SCN^- shock load

Higher amount of phenol, COD and thiocyanate in this period resulted in increased requirement of NOx–N. Prior to TSL–I, COD:N removed ratio was 5.2 and with application of TSL–1, it increased to 5.5 and due to addition of external NOx–N, it decreased to 4.6–5. After TSL–II, influent NOx–N to R2 increased to 785 mg/L. With higher influent COD concentration during this period, NO_X–N removal efficiency was 84–88% during 1228th–1238th day and remained stable at 89% from 1239th day onwards. After cut down of feed for four days increased NOx–N removal efficiency was observed along with higher COD removal these days.

4.4.1.2.3 Response of aerobic CMBR (R3) at thiocyanate shock load

Table 4.34 shows that prior to TSL–I, R3 was in steady state receiving influent phenol, COD, SCN^- and NH_4^+–N concentrations of 350, 1100, 95 and 350 mg/L, respectively. After TSL–I, R3 received maximum phenol concentration of 400–480 mg/L and released

3–5 mg/L showing decrease in phenol removal efficiency 99.8–98.8% during 1213–1217th day. However, from 1219th day onwards R3 released steady effluent of 1 mg/L with removal efficiency 99.7%. After application of TSL–II, influent phenol to R3 drastically increased to 900 mg/L from 350 mg/L (increase by 171%). R3 released 15–25 mg/L phenol in its effluent and removal decreased to 96% during 1228th to 1230th day. From 1231st to 1239th day influent and effluent phenol of R3 slowly decreased from 780 to 450 mg/L and 10 to 5 mg/L, respectively with removal efficiency 98.6–98.9%. The stop of influent to R3 resulted in decrease in influent and similarly effluent phenol in R3 to 350 mg/L and 3 mg/L and increasing the removal efficiency to 99.2%. The effect of TSL–I and TSL–II was insignificant on R3 in terms of phenol removal efficiency as observed by Kim et al. (2011b). After application of TSL–I to R1, SCN^- concentration to R3 increased to 120–170 mg/L from its regular concentration of 95 mg/L (increased by 26–79%) during 1213–1216th day. From 1217th day onwards the influent concentration decreased to 105 mg/L on 1222nd day and remained stable. R3 released steady effluent of 2 mg/L SCN^- showing 98% removal during this period. Therefore TSL–I did not affect R3 in terms of SCN^- profile in this study. However after injection of TSL–II, influent and effluent SCN^- profile of R3 drastically increased from 180 to 280 mg/L (increase by 71–166% than prior TSL–II) and from 15 to 25 mg/L, respectively. Removal efficiency also dropped to 91% in R3. After being unstable for the next 12 days, R3 received steady influent SCN^- concentration of 120 mg/L and released 5 mg/L SCN^- in the effluent with removal efficiency of 95%. After 4 days cut off of feed, the final effluent from R3 was ~2 mg/L and removal efficiency achieved was 98% against 1 mg/L before injection of TSL–I. TSL–I did not cause any massive change in COD profile of R3. Influent COD increased from 1050 mg/L to 1250 mg/L and then further increased to 1400 mg/L on 1212th day and 1216th day. R3 released 255–280 mg/L COD in its effluent showing COD removal efficiency of 80–81%. The influent gradually decreased to 1310 mg/L on 1222nd day and remained stable with removal efficiency 81% releasing effluent COD concentration of 245 mg/L, same as before application of TSL–I. With application of TSL–II, influent COD concentration in R3 increased to 1600–1800 mg/L on 1228th – 1229th day (increase by 45–63% than prior TSL–I). Next 5 days from 1230th to 1234th day, R3 received unstable influent COD and removal was 78–79% releasing 350–380

mg/L effluent COD. From 1242nd day onwards, R3 released steady effluent of 280 mg/L. After four days cut off of feed to the CMBR system, the final effluent from R3 was 265 mg/L and removal efficiency achieved was ~77% similar to prior TSL application.

Clear effect of SCN^- shock load was observed on the performance of R3 in terms of NH_4^+–N profile (Figure 4.108). Prior to TSL–I, R3 exhibited steady performance of NH_4^+–N removal of 63% from influent NH_4^+–N concentration 372 mg/L (effluent 145 mg/L). From 1214th day onwards influent NH_4^+–N was observed to increase from 387–400 mg/L on 1214th –1218th day and it released 150 –280 mg/L NH_4^+–N and removal efficiency dropped to 54% (decrease by 14% prior TSL–I). However from 1219th day R3 released 140 mg/L NH_4^+–N showing NH_4^+–N removal efficiency 64–65%.

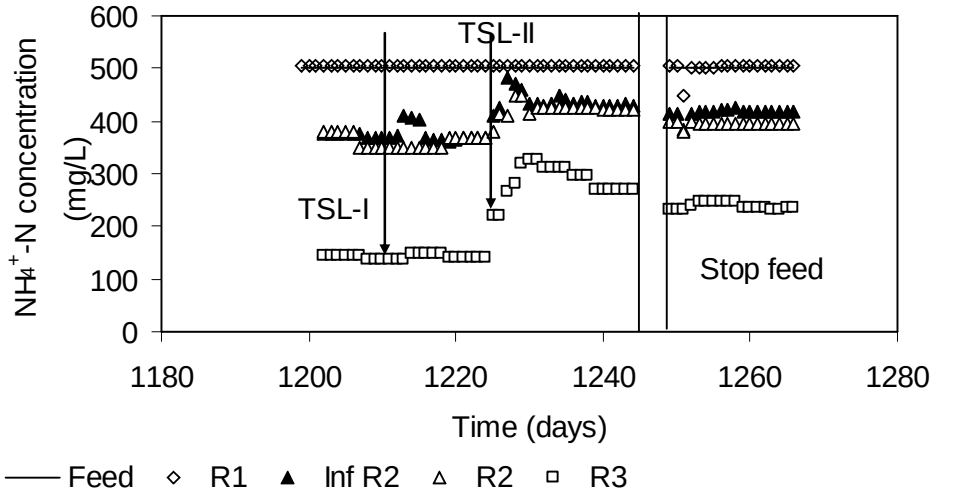

Figure 4.108 NH_4^+-N concentrations profile in three-stage CMBR system at application of SCN^- shock load

From 1226th to 1229th day the influent NH_4^+–N concentration to R3 increased to 405–512 mg/L due to after effect of application of TSL–II to R1. R3 released high NH_4^+–N in the effluent as 280–325 mg/L (increased by more than 100% as prior to TSL–II). NH_4^+–N removal decreased to 31–32% during 1230–1231st day. High discharge of effluent NH_4^+–N from R3 was recycled to R2 and again carried over to R3. Effluent from R3 was observed to be 310–270 mg/L with removal efficiency ~34–39% during 1232nd–1244th day. The 4 days cut off of feed was not able to help R3 to regain its previous NH_4^+–N removal efficiency as the influent was still high as 418–420 mg/L and removal achieved was only 45%. NOx–N generated also significantly decreased after the shock loads. The

effect of TSL–II on nitrification efficiency of R3 was irreversible probably due to high concentration of phenol, thiocyanate and NH_4^+–N itself. Higher COD caused generation of more amounts of heterotrophs and the slow growing and sensitive nitrifying bacteria could not compete with heterotrophs and were washed out from the reactor.

4.4.1.2.4 Overall performance of CMBR system during thiocyanate shock load

The overall performance of three–stage CMBR system during the shock load study is shown in Figure 4.109 in terms of phenol, COD, SCN^- and TN removals. CMBR system regained its normal performance efficiency in terms of phenol, COD, SCN^- and TN removals and effect of TSL-I (1000 mg/L) was insignificant on CMBR system. Total phenol removal after shock load was not affected and always more than 99% through out the study. After TSL–II, SCN^- removal immediately decreased to 96% though it regained its efficiency up to 99% during the next 7 days. COD removal efficiency was unaffected by TSL–I and was ~97%. However after TSL–II it slightly decreased to 95% and recovered to 96% during the following days as normal feed was started. The total NH_4^+–N removal in three–stage system decreased from 80% to 70%. Total nitrogen (TN) removal remained almost stable at 75%. Though the effect of TSL–I on NH_4^+–N removal efficiency was insignificant and CMBR was able to again previous efficiency, the affect of TSL–II on NH_4^+–N removal efficiency of CMBR system was irreversible.

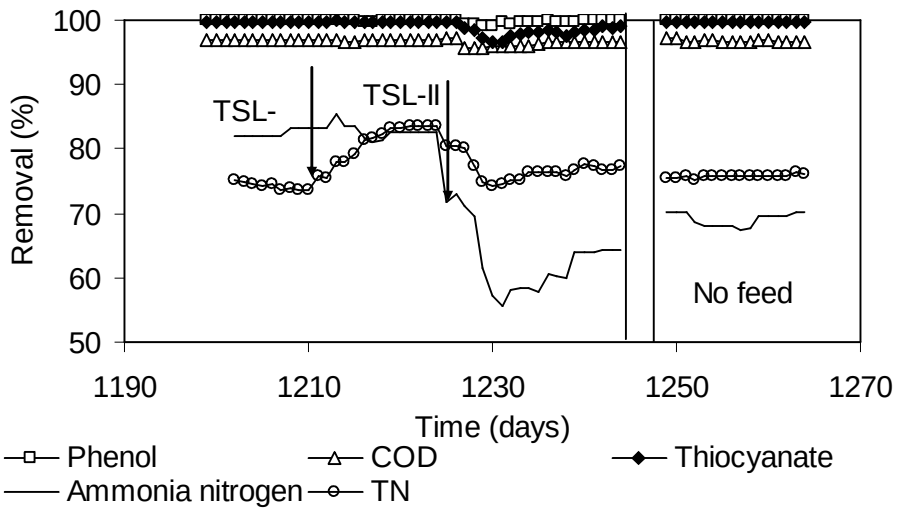

Figure 4.109 Overall performance of CMBR system at thiocyanate shock load

4.4.2 Shock load applications on sequential FMBR system

Performance of the FMBR under thiocyanate and phenol shock loading conditions was evaluated by monitoring the reactor performance on pollutant removal at two different shocks loadings. The system was initially operated with influent phenol concentration of 2500 mg/L along with thiocyanate and ammonia –nitrogen 600 mg/L and 500 mg/L, respectively at total HRT of 6 days (B1:3 days; B2: 1.5 days and B3: 1.5 days) prior to shock application. The reactor was brought back to a steady state condition at relatively lower pollutant loading with normal feed before being subjected to a high pollutant loading as shock load. Initially thiocyanate load of 1000 mg/L and 1200 mg/L was applied to FMBR and thereafter phenol shock application study was carried out by increasing feed phenol to 3000 mg/L and 3500 mg/L from normal phenol concentration of 2500 mg/L for three days each.

4.4.2.1 Effect of thiocyanate shock load on sequential FMBR system

On 1251st day thiocyanate concentration was increased to 1000 mg/L from initial 600 mg/L without any intermediate concentration increment keeping other parameter same. After three days (1251st –1253rd), it was again brought down to 600 mg/L. On 1265th day 1200 mg/L SCN^- was added to B1 and continued to 1267th day and decreased to 600 mg/L again along with other normal feed. Average steady state effluent profile in FMBR prior to thiocyanate shock application is presented in Table 4.35.

Table 4.35 Average steady state performance of the three–stage FMBR system before application of first thiocyanate shock load (TSL–I)

Parameter (mg/L)	Feed	B1	B2inf	B2	B3
Before TSL–I					
Phenol	2500	1881 (24.7)	941	80 (91.5)	1 (98.7)
COD	7980	6580 (17.5)	3197	680 (78.7)	235 (65.4)
SCN^-	600	600	301	75 (75.1)	1 (98.7)
NH_4^+–N	500	505	356	330 (7.5)	100 (71.2)
NO_x–N	0	–	667	90 (86.5)	335

Values in parenthesis indicates removal efficiencies

4.4.2.1.1 Performance of B1 after thiocyanate shock load

B1 was in operation with thiocyanate, NH_4^+–N and phenol concentration of 600 mg/L, 500 mg/L and 2500 mg/L, respectively in normal condition. Prior to TSL I, B1 removed 24.8% and 17.5% phenol and COD, respectively with no thiocyanate removal (Table 4.35). With application of TSL–I with thiocyanate concentration 1000 mg/L (increased by 66.7%), the corresponding SCN^- load to B1 became 0.33 g/L.day. No thiocyanate removal occurred in B1 and influent and effluent remained almost same (Figure 4.110). B1 started release increased phenol of 2320–2380 mg/L on 1253rd to 1254th day and remain unstable with 2380–2180 mg/L for next 6 days with removal 4.8–12.8% (Figure 4.111). From 1261st day onwards, B1 released stable effluent of 2110 mg/L showing phenol removal efficiency 15.6 % (decrease by 36.8% prior to TSL–I). On 1267th–1269th day TSL–II was applied (17 days after TSL–I) with increase in SCN^- concentration in feed to 1200 mg/L (100% increase). From 1270th day onwards normal feed was started. Phenol in effluent from B1 increased to 2460–2480 mg/L on 1270th –1275th day (increase by 14.2–14.9% than prior TSL–II) showing negligible phenol removal of ~0.8%. From 1276th day onwards (after 7 days of TSL–II), B1 gradually released decreased phenol concentration in effluent ranging 2460–2350 mg/L showing phenol removal efficiency 0.8–6.0% and from 1283rd day onwards B1 released stable phenol concentration of 2340 mg/L in effluent (removal 6.4%). The cut down of feed for 4 days from 1287th –1291st day improved the removal efficiency up to 15.6% as that was prior to TSL–II.

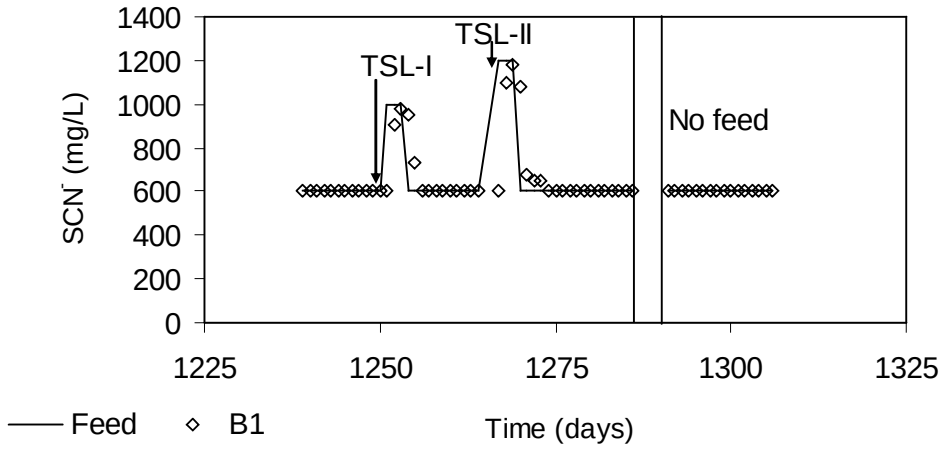

Figure 4.110 SCN^- profile in B1 before and after application of SCN^- shock load

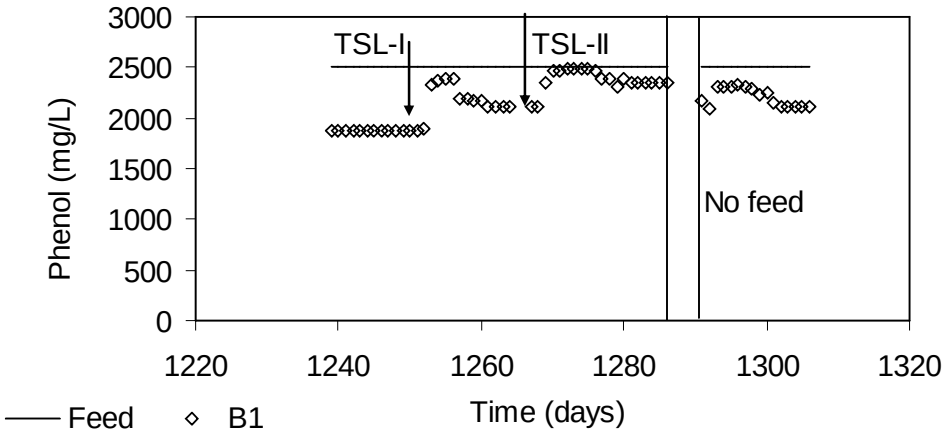

Figure 4.111 Phenol concentrations in B1 before and after application of SCN^- shock load

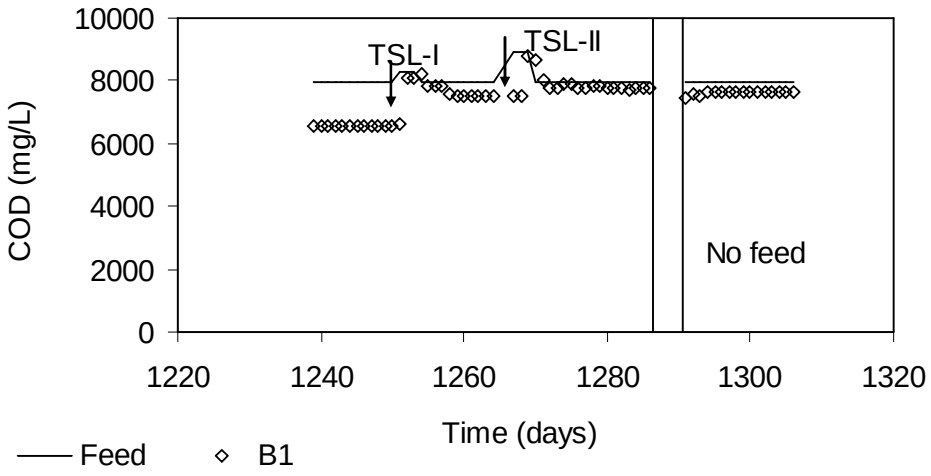

Figure 4.112 COD concentrations in B1 before and after application of SCN^- shock load

With application of TSL–1 and TSL–II, feed COD also increased to 8300 mg/L and 8900 mg/L, respectively. COD profile in B1 during TSL is shown in Figure 4.112. B1 released increased amount of COD 8100–8200 mg/L from 1253–1254th day and afterwards released unsteady effluent concentration for next four days (1254th –1258th day). From 1259th day onwards steady effluent COD of 7530 mg/L was observed (increase by 14% prior TSL–I) with removal efficiency of 5.6% (decrease by 68% prior to TSL–I). With application of TSL–II, the effluent COD further increased to as high as 8800 mg/L on 1269th day and remain unstable with 8660–7850 mg/L COD in effluent for next 9 days (1271st – 1278th day). After 12 days of TSL–II application, B1 released stable effluent of

7750 mg/L with removal efficiency 2.8%. The cut down of feed for four days (1287th to 1290th day) improved the COD removal efficiency of B1 to 4.5% which was 74% and 19% less than the performance shown by B1 prior to TSL–I and TSL–II condition, respectively.

4.4.2.1.2 Performance of B2 after thiocyanate shock load

Prior to TSL–I, B2 received average influent concentration of 301 mg/L and showed removal efficiency of 75.08% releasing average of 75 mg/L thiocyanate in its effluent. After the TSL–I from 1250th to 1252nd day, B2 received 456–491 mg/L SCN^- in its influent (increase by 34–38%) and profile is shown in Figure 4.113.

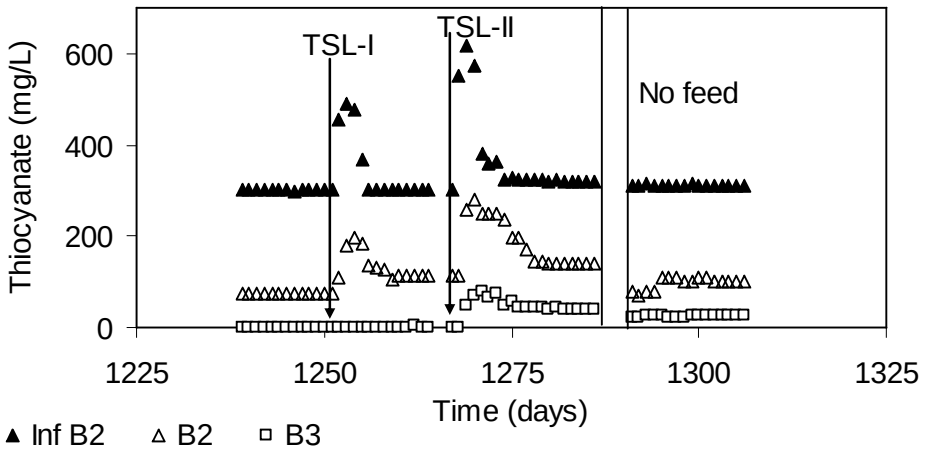

Figure 4.113 SCN^- profile in B2 and B3 before and after application of thiocyanate shock load

B2 released 110–190 mg/L SCN^- in its effluent and removal decreased to 58% (decrease by 22.67% from prior TSL–I). The high effluent value continued for next four days (1255–1258th day) after the influent SCN^- decreased 304–301 mg/L in B2 and removal achieved during this period was 49–58%. B2 started releasing decreased steady effluent of 115 mg/L SCN^- from 1260th day onwards and SCN^- removal efficiency of 61.8% was achieved (17.7% less than prior TSL–I condition). Influent SCN^- to B2 increased to 551–615 mg/L on 1268th to 1270th day when the reactor was subjected to TSL–II. The effluent SCN^- from B2 reached as high as 256–280 mg/L and removal dropped to 51–30%. However with addition of normal feed, from 1270th day onwards SCN^- in effluent started to drop and was 250–145 mg/L showing removal efficiency 27–55% (1271st to 1279th

day). B2 released steady thiocyanate concentration of 140 mg/L in its effluent and removal efficiency achieved was 56.2% from 1280th day onwards and the removal efficiency was found to further improve to 68% with the cut down of influent on 1287th to 1290th day.

In normal condition B2 was receiving 940 mg/L phenol in its influent and released 80 mg/L showing removal efficiency of 91%. When TSL–I was applied B2 received phenol concentration of 1160–1190 mg/L (increase by 23.4–26.5%) on 1253rd to 1256th day and B2 released higher concentration phenol, 200–380 mg/L in its effluent the respective phenol removal was 67–81% (decrease by 26.3–10.9%) as shown in Figure 4.114. This indicated B2 was affected by the thiocyanate shock. However after 7 days of unsteadiness, B2 showed receiving stable influent of 1056 mg/L releasing 100 mg/L phenol in its effluent with removal efficiency 89% from 1261st day onwards. On 1269th – 1271st day influent phenol to B2 increased from 1065 mg/L to 1320 mg/L due to TSL–II. Immediately the effluent phenol concentration rose to 660–880 mg/L and then gradually decreased from 760 mg/L to 380 mg/L in next 10 days (1272nd to 1281st day). The removal in this period was 31–68%. From 1282nd day B2 released steady effluent of 310 mg/L with removal efficiency 74.27% (17% less prior to TSL–II. The efficiency of phenol removal increased to a steady removal of 87% when B2 was stopped for four days and normal feed was added.

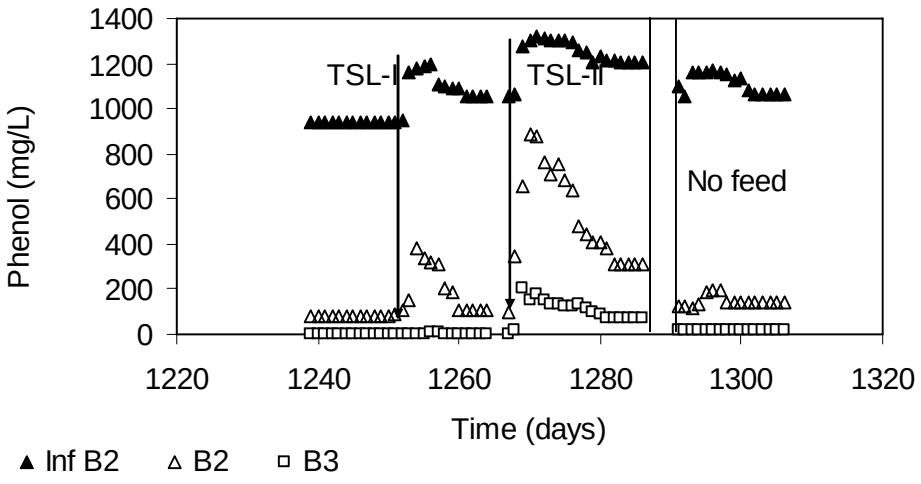

Figure 4.114 Phenol concentrations in B2 and B3 before and after application of SCN^- shock load

Influent and effluent COD concentration 3192 mg/L and 680 mg/L was monitored in B2 with COD removal efficiency 78.7% prior to TSL application. With application of TSL–I, influent COD to B2 increased to 4167–4295 mg/L on 1253rd to 1254th day and released 1480 mg/L COD with decreasing COD removal efficiency of 64% (decrease by 18.6%) as shown in Figure 4.115. From 1257th day onwards COD in the effluent reduced gradually to 1380 mg/L to 950 mg/L on 1261st day and corresponding removal was 66–75% and remained steady.

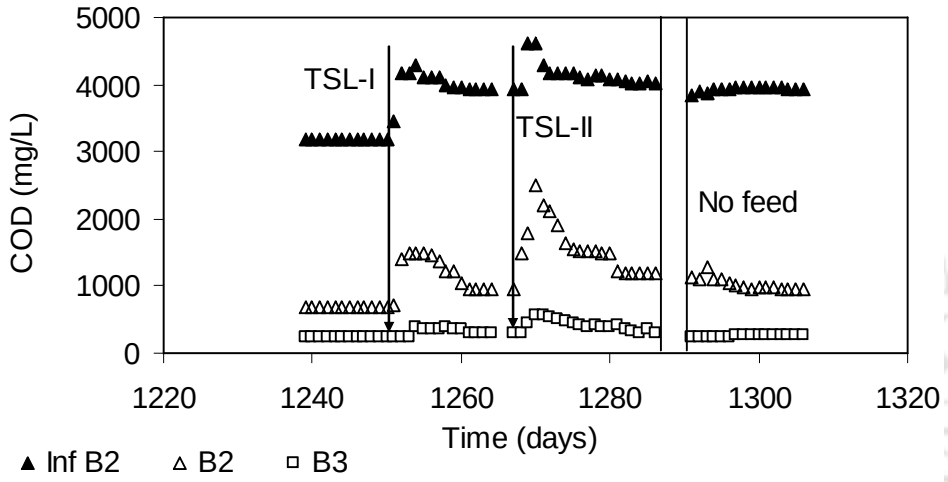

Figure 4.115 COD profile in B2 and B3 before and after application of SCN^- shock load

During TSL–II, B2 received increased influent COD concentration being maximum at 4620 mg/L on 1269th day. Higher COD concentration of 2200–2500 mg/L was released and COD removal efficiency of B2 decreased to 48–45%. After 12 days of unsteady condition, B2 started to release steady and decreased amount of COD concentration of 1200 mg/L with removal efficiency 70% from 1282nd day onwards.

After introduction of shocks to B1, the SCN^-, phenol and COD effluent also increased from B1 and eventually the influent concentration to B2 increased (Figure 4.116). Therefore NO_3–N was also increased from 1000 mg/L in normal condition to 1200 and 1400 mg/L in recycle after TSL–I and TSL–II, respectively. Denitrification efficiency was not affected by the shock load and contrary it enhanced the NOx–N removal efficiency in B2 (more than 82%). Mora et al. (2003) studied performance of sequencing batch reactors (SBR) treating sewage, through a process of endogenous biological

denitrification with molasses and nitrate, as carbon and nitrogen sources, respectively. Sudden changes (shock loading) of inorganic matter concentration was performed by quickly increased three fold in relation to the original concentration. They observed that SBR reactor withstand adequately moderate shock loading. With regard to substratum degradation, nitrate elimination achieved was approximately 80%. In present study the anoxic FMBR sustained toxic substance like thiocyanate and phenol and removal was higher enough than reported values.

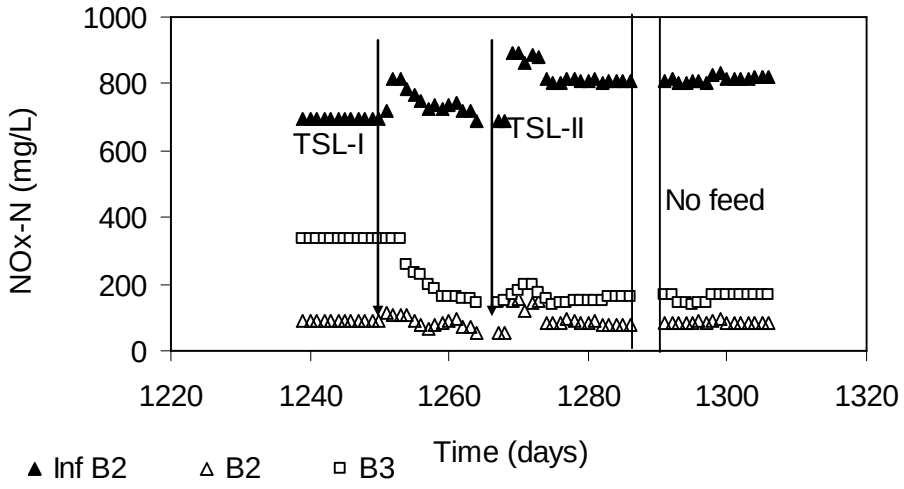

Figure 4.116 NOx-N concentrations in three-stage FMBR system after application of SCN^- shock load

4.4.2.1.3 Performance of B3 after thiocyanate shock load

Prior to TSL, B3 was receiving 75 mg/L SCN^- and released average 1–2 mg/L in its effluent with a removal efficiency of 98.7%. After TSL–I introduction, influent SCN^- in B3 increased to 180–198 mg/L on 1253rd –1254th day (increase by 140%). However the TSL–I application did not cause any change in the effluent profile and steady removal of 98.9–99% was maintained by B3. From 1269th day onwards B3 got exposed to high influent thiocyanate concentration of 256–280 mg/L due to TSL–II. This influent strength caused alteration in performance of B3 and 50–85 mg/L SCN^- was detected in the effluent and SCN^- removal decreased to 68–74% from 98%. However from 1275th–1279th day, influent SCN^- slowly decreased from 195–145 mg/L though the effluent still contained 40–45 mg/L SCN^- showing removal efficiency of 68%. After stop of feed for 4 days (1287–1290th day), influent SCN^- to B3 decreased and 1296th day onwards stable

influent SCN^- 80–100 mg/L entered B3 and effluent was containing nearly 22–25 mg/L showing SCN^- removal efficiency of 72–75%.

Prior to TSL, B3 received influent phenol 80 mg/L and was showing removal efficiency of 98%–99%. After TSL–I, there was no massive change observed in the effluent profile of B3 though the influent concentration increased to 150–380 mg/L (increased by 87–375% prior TSL–I). From 1254–1259th day, influent phenol decreased from 340 to 190 mg/L and released 3–8 mg/L in effluent phenol and removal was 99–97%. On 1260th day onwards B3 started receiving steady influent phenol concentration 110 mg/L and 97% of it was removed. Again due to TSL–II influent phenol increased in B3 on 1269th–1271st days and it was 660–890 mg/L and great portion was released in the effluent 150–180 mg/L with only 79% removal. B3 remained unstable for next 12 days with high influent phenol of 880–380 mg/L (1270th to 1281st day). From 1282nd day onwards B3 released stable phenol concentration of 70 mg/L in its effluent with 77% phenol removal efficiency (decrease by 22% and 13% prior and later TSL–I). After stopping of the system for four days the effluent phenol concentration came down to 15 mg/L and removal archived was 89% from 1298th day onwards.

Influent COD to B3 was ~ 680 mg/L and released 235 mg/L showing 65% COD removal. During TSL–I influent COD increased to up to 1480 mg/L on 1253rd day (increase by 117%) and it was 1480–950 mg/L for next 6–7 days. B3 showed higher COD removal and it was 65–75% during this period though B3 released higher effluent COD of 300 mg/L than pre shock condition. During TSL–II, B3 received highest COD concentration of 2500 mg/L (1270th day) and released 580 mg/L in its effluent with 76% removal. Influent COD decreased to 2200–1480 mg/L from 1271st day to 1280th day and B3 released 550–380 mg/L with 70–74% COD removal. After cut down of the reactor for four days, effluent COD concentration came down to 250–260 mg/L. Seetha et al. (2010) studied the effects of short–term organic shock loads on the performance of a laboratory scale two–stage activated sludge (AS)–biofilm reactor working at 6 h HRT and treating medium strength domestic wastewater by increasing the influent chemical oxygen demand (COD) to 2–4 times the normal values and each shock load was applied for a period of 6 h, after which normal loading conditions were resumed. The maximum effluent COD concentration obtained was 169, 169, 250 and 617 mg/L under the shock

loads of 808, 1170, 1358 and 1900 mg COD/L, respectively. The COD removal rate increased with increasing effective OLR. The system recovered quickly from shock loads; recovery time proportional to the magnitude of shock loads. In present study, though B3 was receiving high COD load for longer period it was recovering well in terms of COD removal.

Clear effect of thiocyanate which led to phenol shock load in B3 was observed on the performance of B3 in terms of NH_4^+–N removal (Figure 4.117 and 4.118).

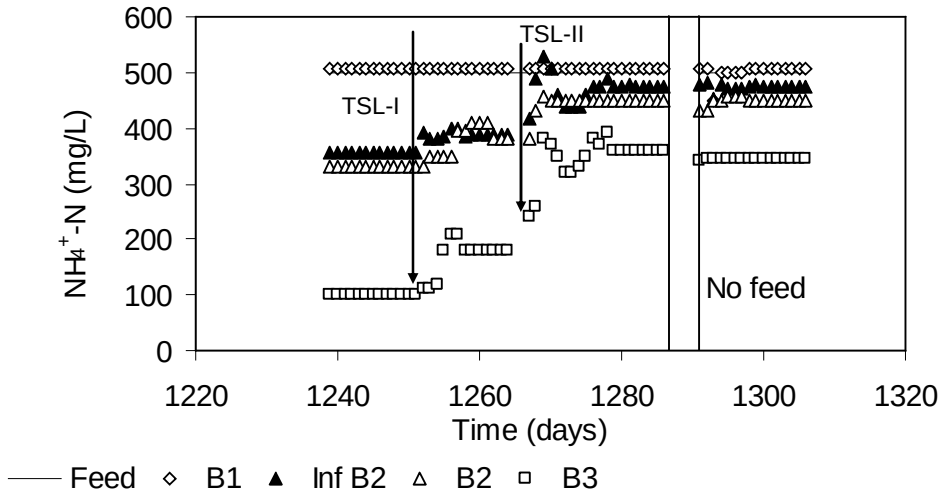

Figure 4.117 NH_4^+-N concentrations in three-stage FMBR system after application of SCN^- shock load

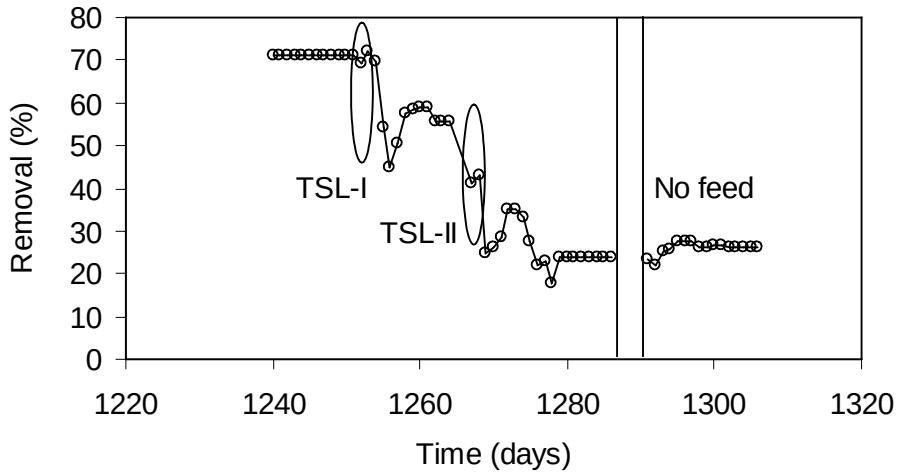

Figure 4.118 Ammonia removal by B3 during thiocyanate shock load study

Up to 22nd day, B3 was exhibiting its normal performance of NH_4^+–N removal of 71% from influent 347 mg/L. On 1253rd day onwards the influent NH_4^+–N started to increase more than 392 mg/L and continued to increase. B3 released 110–210 mg/L NH_4^+–N and removal efficiency decreased from 71% to 45% during 1254th day to 1257th day. From 1259th day B3 released steady effluent concentration of 180 mg/L with NH_4^+–N removal efficiency 55–58% (decrease by 29–22% than prior TSL–I). The nitrification condition was further deteriorated when effluent ammonia–nitrogen from B2 released in increasing concentration as high as 484–504 mg/L during TSL–II. Effluent from B3 was 380–320 mg/L and B3 totally failed to recover the nitrification efficiency even after stopping the reactor for 4 days showing only 24% NH_4^+–N removal. The final effluent contained ~345 mg/L ammonia nitrogen.

4.4.2.1.4 Overall performance of FMBR system at thiocyanate shock load

It was observed that the system recovered high performance efficiency though it was slightly less than its normal performance efficiency in terms of phenol, COD and SCN^- removals (Figure 4.119).

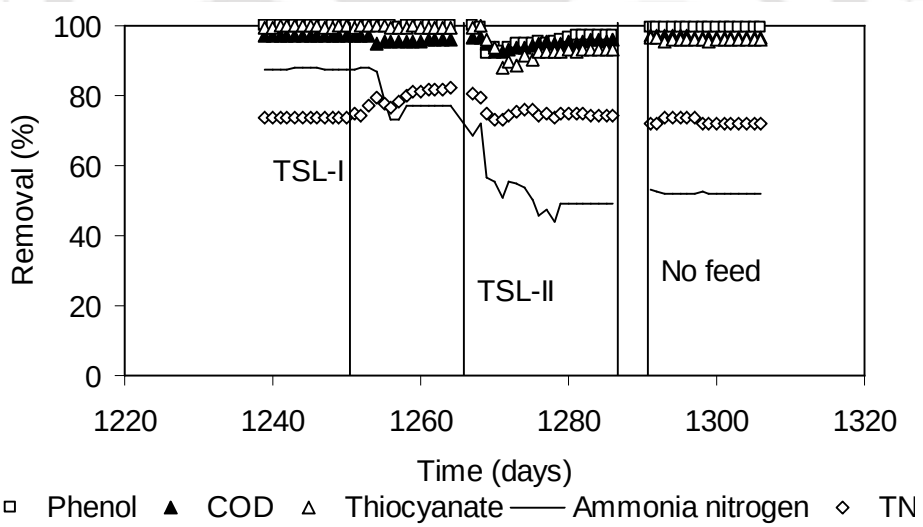

Figure 4.119 Overall performance of FMBR system on Thiocyanate shock load application

Total phenol removal after shock load decreased slightly from 99% to 98%. Similarly thiocyanate removal decreased to 99.75% to 96.88% after the high shock load period.

The system tackled the first shock of thiocyanate concentration in terms of phenol, thiocyanate and COD removal but its removal efficiency dropped than normal efficiency after the second shock. Denitrification efficiency was unaffected by the shocks. Ammonia removal was significantly affected and it was not recovered by the system in the long run. The total ammonia–nitrogen removal was 88% prior to TSL application and after TSL–I it decreased to 76% and further decreased to 52% after TSL–II. TN removal remained almost 72–74% during the study.

4.4.2.2 Effect of phenol shock application on FMBR system

Performance of the FMBR under phenol shock loading conditions was evaluated by monitoring the reactor performance on pollutant removal at two different phenol concentrations. Prior to phenol shock load application, the FMBR system was subjected to thiocyanate shock load (TSL) application and in that state, the B3 was totally deteriorated. To recover it, tap water enriched with TMS and yeast extract only was passed through the reactor instead of the effluent from B2 for 2 days. Also 3 L of sludge was withdrawn from B3 and 3L acclimatized sludge (with 300 mg/L NH_4^+–N, 100 mg/L SCN^- and 250 mg/L phenol) was added. The system was again started with influent phenol concentration of 2500 mg/L along with thiocyanate and ammonia–nitrogen 600 mg/L and 500 mg/L, respectively at total HRT of 6 days (B1:3 days; B2: 1.5 days and B3: 1.5 days). The system came to stabilization within 12–15 days of normal operation study from 1324th day onwards. On 1332nd day phenol concentration was increased to 3000 mg/L without any intermediate concentration increment keeping other parameter same and after three days (1332nd–1334th) it was brought down to 2500 mg/L. On 1348th day 3500 mg/L phenol was added to B1 and continued to 1350th day and decreased to 2500 mg/L again along with other normal feed as phenol shock load I and II (PSL I and PSL II). Average performance of FMBR prior to phenol shock application is presented in Table 4.36.

Table 4.36 Average steady state effluent concentrations of the three–stage FMBR system before application of phenol shock loads (PSL–I)

Parameter (mg/L)	Feed	B1	B2inf	B2	B3
Before PSL–I					
Phenol	2500	2100 (16)	1050	140 (86.7)	1 (99.3)
COD	7980	7400 (7.2)	3815	950 (75)	230 (75.8)
SCN^-	600	600	301	110 (63.4)	2 (99.3)
NH_4^+–N	500	505	353	328 (7.17)	115 (67.5)
NO_x–N	0	–	605	110 (83)	335

Values in parenthesis indicate and removal efficiencies (%)

4.4.2.2.1 Performance of B1 after phenol shock load

In normal condition, B1 released phenol and COD at concentration of 2100 and 7400 mg/L, with removal efficiency 16% and 7.2%, respectively (Table 4.36). At PSL–I from 1332nd to 1334th day, with increase in phenol concentration 2500 mg/L to 3000 mg/L (increase by 20%), the effluent phenol from B1 immediately increased to 2890 mg/L on 1334th day and continued to 1336th day. B3 remained unstable for another 9 days even after addition of normal feed up to 1345th day and from 1346th day onwards steady effluent phenol concentration of 2130 mg/L was observed with removal efficiency 14.8% (decrease by 7.5% prior PSL–I) as shown in Figure 4.120.

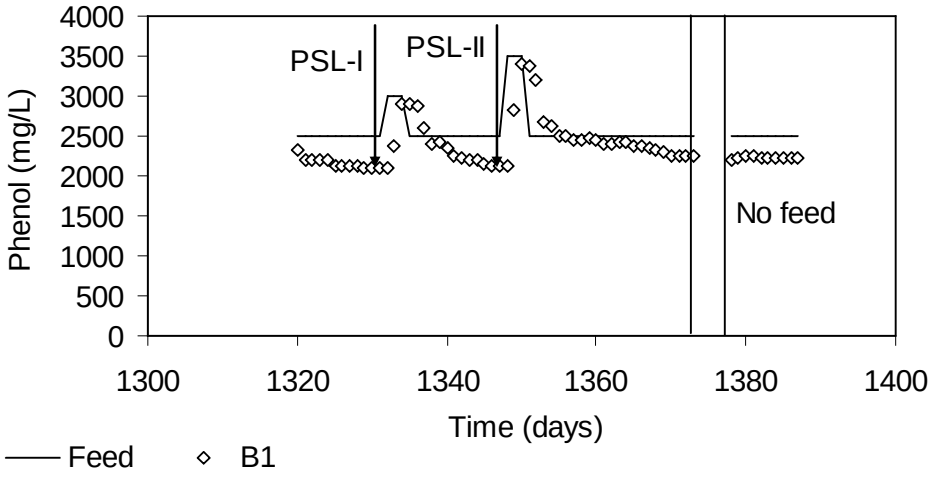

Figure 4.120 Phenol profile in B1 before and after application of phenol shock load

With application of PSL–II of phenol 3500 mg/L on 1348th to 1350th day (increase by 40% prior PSL), phenol effluent peak of 3400 mg/L was observed on 1350th day. Almost 2630–3400 mg/L phenol was released from B1 in decreasing trend during the next 3–4 days. From 1355th to 1373rd day B1 was releasing unstable phenol effluent of 2480–2260 mg/L and phenol removal in this period was 2–9%. With four days cut off of feed, B1 resumed stable effluent of 2230 mg/L with phenol removal efficiency 10% (decrease by 37% prior to PSL).

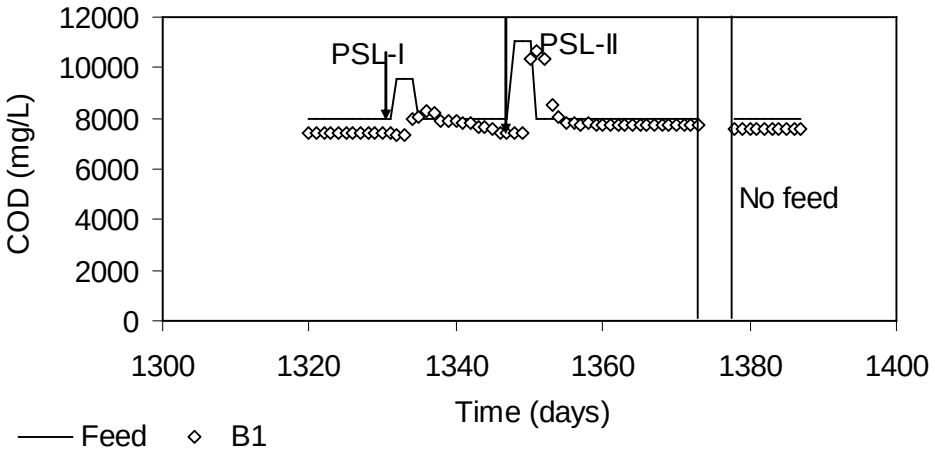

Figure 4.121 COD profile in B1 before and after application of phenol shock load

The respective influent COD also increased to 9550 mg/L during application of PSL–I. B1 released 8090–8290 mg/L effluent COD during 1336th to 1338th day and COD in the effluent gradually decreased 7600 mg/L during next 9 days (1337th to 1345th day) as shown in Figure 4.121. During this period B1 removed 1–6.6% of influent COD. From 1346th day onwards, stable COD concentration of 7450 mg/L was observed in B1 effluent. COD content in B1 effluent increased sharply to 10680–10350 mg/L on 1351st – 1352nd when PSL–II with COD loading rate 3.69 g/L.day was applied to B1. However, the effluent concentrations gradually decrease from 8035 mg/L to 7700 mg/L from 1354–1367th day. During this period COD removal accounted by B1 was only of 1.8% and gradually increased to 3.5% after 15 days of PSL–II and remained stable. After four days cut off of feed, there was not much improvement of effluent COD quality and removal was 4.6–5.4% only.

4.4.2.2.2 Performance of B2 after phenol shock load

Prior to PSL, B2 received average influent phenol concentration of 1050 mg/L and showed removal efficiency of 86.7% releasing average of 140 mg/L phenol in its effluent. After PSL–I day, B2 received 1190 mg/L which sharply increased to 1445 mg/L (increase by 27%) during 1334th to 1336th day (Figure 4.122). The influent decreased gradually to 1300–1066 mg/L during 1338th–1346th day as B1 slowly resumed its removal efficiency to some extent. Stable effluent of 105 mg/L from B2 was observed from 1341st day onwards after 6 days of shock application showing removal efficiency of 90% which was higher than prior PSL–I condition.

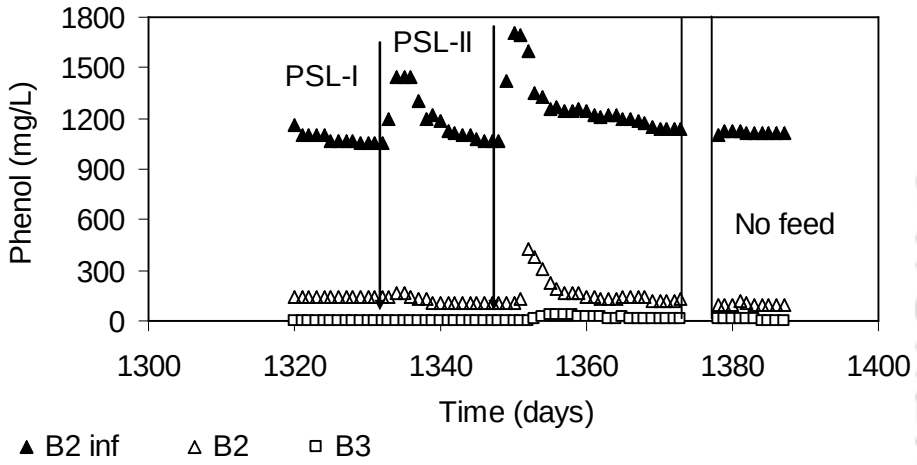

Figure 4.122 Phenol profile in B2 and B3 before and after application of phenol shock load

Influent phenol to B2 reached as high as 1416–1700 mg/L on 1349th to 1350th day while PSL–II was applied. Concomitantly B2 released higher effluent phenol showing decrease in removal efficiency than the pre shock condition during the following 3 days. Fang et al. (1996) and Tay et al. (2001) reported relatively long acclimatization period, decrease in removal efficiency at higher loadings, sensitivity to temperature and shock loading and long recovery periods after shocks are a few problems associated with the treatment of phenol at concentration. B2 gradually started releasing effluent phenol less than 200 mg/L after 9th day (from 1356th day) and slowly resumed its phenol removal efficiency up to 85% which finally increased to 89.6% during 1356th–1373rd day, after 15–21 days of shock application. Moreover the cut off of influent feed for 4 days further enhanced the removal efficiency to 91.5%.

In terms of COD profile, prior to PSL, B2 removed 75% of influent COD 3815 mg/L releasing ~950 mg/L COD in its effluent. With the application of PSL–I, influent COD to B2 increased to 4115 mg/L on 1350th day and the effluent COD was ~1400 mg/L. This influent concentration further increased to 5455–5322 mg/L during 1351st–1352nd day and B2 released higher effluent COD of 2600–2800 mg/L (Figure 4.123). From 1353rd to 1360th day B2 received decreased influent COD concentration of 4460 mg/L to 4020 mg/L and decrease in effluent COD was observed with removal efficiency of 41–53%. From 1361st day onwards B2 showed improved removal efficiency of 53–65% from stable influent concentration of 3990 mg/L. From 1370th day onwards B2 was observed to resume its COD removal efficiency which increased up to 71% and remained stable. The four day cut off of influent further resulted in improve COD removal efficiency of B2 up to 76% as was in pre shock condition.

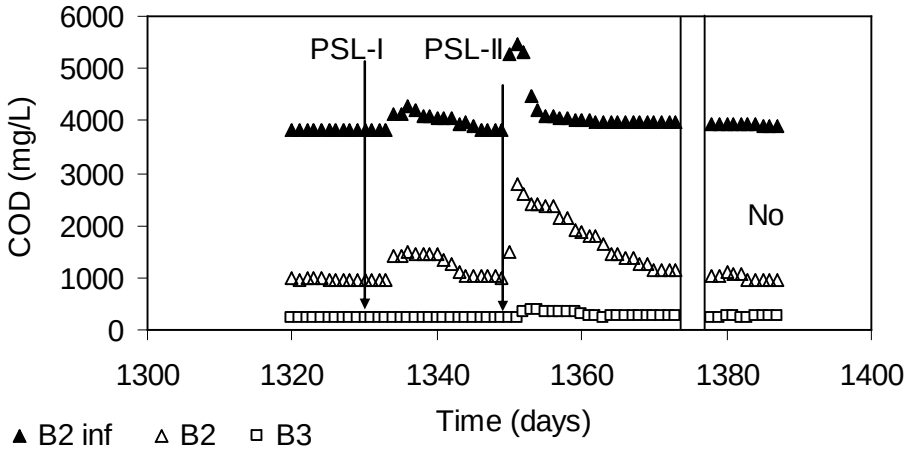

Figure 4.123 COD profile in B2 and B3 before and after application of phenol shock load

Nearly 300 mg/L SCN^- was observed in influent to B2 prior to phenol shock load application and B2 released nearly 110 mg/L thiocyanate in its effluent showing removal efficiency of ~63.4%. With application of PSL–1, B2 released 155–170 mg/L SCN^- and removal efficiency decreased to 49–42% (Figure 4.124). During 1335th to 1342nd day (in the next 10 days), SCN^- removal in B2 slowly recovered to 55% and remained stable (decrease by 13.2% than prior PSL–I). With application of the PSL–II, effluent SCN^- from B2 increased to ~190 mg/L and the SCN^- removal efficiency of B2 drastically decreased to 37%. With introduction of normal feed, B2 gradually started resuming its

efficiency and SCN^- released in effluent decreased to 170–140 mg/L showing 45–53% SCN^- removal after 14–18 days of second shock. This condition continued up to 1369th day and with cut off of feed during 1374th–1378th day, B2 showed SCN^- removal of 56–57%.

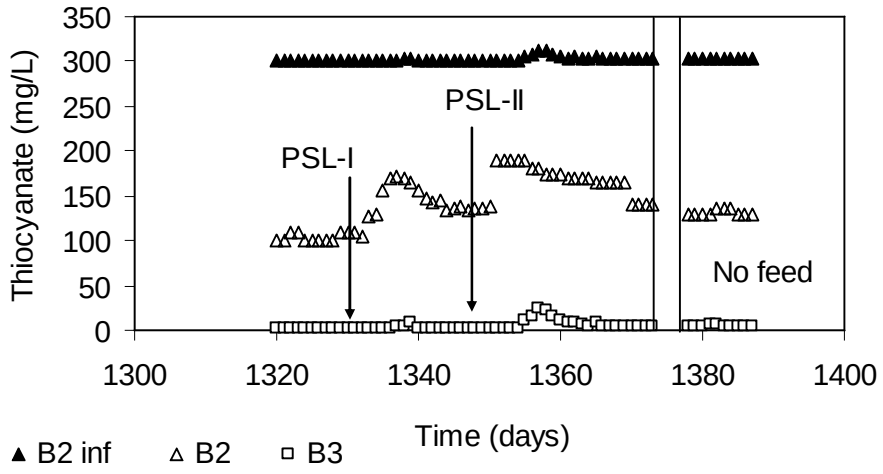

Figure 4.124 Thiocyanate profile in B2 and B3 before and after application of phenol shock load

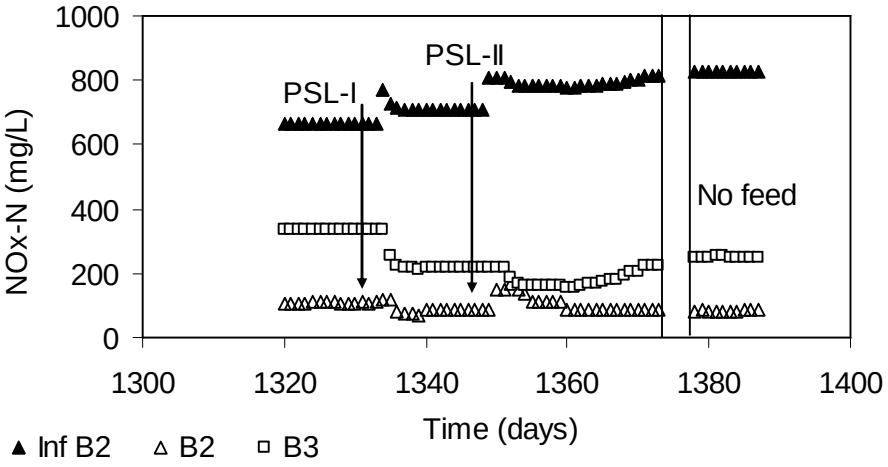

Figure 4.125 NOx-N in three-stage FMBR system at application of phenol shock load

In normal condition 1000 mg/L NO_3^-–N was added in the recycle and the denitrification efficiency observed was 88–89%. NO_3^-–N in recycle was increased to 1200 and 1400 mg/L after first and second shock, respectively as after introduction of shocks, the SCN^-, phenol and COD effluent also increased from B1 and eventually the influent concentration to B2 increased. Denitrification efficiency was not affected by the shock

load and contrary it enhanced the NOx–N removal efficiency in B2 (~ 90%) as can be seen in Figure 4.125.

4.4.2.2.3 Performance of B3 after phenol shock load

Prior to PSL, B3 was receiving influent phenol 140 mg/L and showing removal efficiency of ~99.3%. After the PSL–I, no massive change was observed in the influent and effluent profile of B3 as the upstream reactors were tackling the shock effectively. The influent phenol concentration was 130–145 mg/L on 1335th to 1336th day and effluent concentration slightly increased to 1.5–2 mg/L showing no adverse affect in this condition and removal efficiency sustained at ~99%. During PSL–II, the influent concentration touched the peak of 430 mg/L and gradually decreased to 120–125 mg/L in 20 days. B3 coped up with this concentration and stable effluent of 15–13 mg/L was observed with 89% phenol removal from 1366th day (removal decrease by 10.4% than prior to PSL).

In normal condition influent COD to B3 was 950 mg/L and B3 released 230 mg/L showing 75.8% COD removal. After PSL–I, influent COD increased to up to 1500 mg/L on 1336th day and it was ~1450 mg/L up to 1337–1340th day. During higher loading higher COD removal occurred in B3 and it was 83%. The effluent remained stable at 240–230 mg/L during the following days from influent COD concentration of 1250–1050 mg/L. During PSL–II, B3 received highest COD concentration of 2800 mg/L on 1351st day and released 340–380 mg/L in its effluent showing increment in COD removal efficiency up to 88–91% and remained unstable for next 16 days with reduced influent COD 2600–1260 mg/L. There was slight decrease in COD removal efficiency from 82–78%. Sable effluent COD concentration of 260–280 mg/L was detected in the B3 effluent from 1368th day. After cut down of the reactor for four days, effluent COD concentration came down to 255 mg/L with COD removal efficiency 75%.

Prior to PSL–I, ~ 115 mg/L SCN^- was entering B3 and showing removal efficiency of 99% it released average 1–2 mg/L in its effluent. After the PSL–I, thiocyanate profile in B3 was not much affected as influent SCN^- 150–198 mg/L received during the first shock period did not result any change in the effluent profile and steady removal of 98% was maintained by B3. During PSL–II application B3 released 11–24 mg/L SCN^- when peak

concentration of 190 mg/L SCN– entered B3 and removal efficiency dropped to 86% during 1351st to 1357th day. B3 took 8–10 days to recover up to 97% SCN– removal and release stable effluent concentration of 5 mg/L from influent concentration of 165–140 mg/L.

During pre shock period NH_4^+–N removal observed in B3 was ~ 67.5% in influent NH_4^+–N concentration of 353 mg/L. NH_4^+–N removal and NO_X–N generation in B3 got affected as shock load of phenol was applied to the upstream reactor. After PSL–I, B3 showed a decrease in ammonia removal from 67% to 59%. However from 1341st day onwards, B3 recovered its pre shock efficiency of ammonia removal though there was slight increase of effluent concentration to 125 mg/L (Figure 4.126). The affect of PSL–II was observed in B3 as influent NH_4^+–N concentration increased from 411 mg/L to 440 mg/L and simultaneously removal decreased drastically to 39% from 7th day of shock application (1341st to 1363rd day). No improvement was observed in NH_4^+–N removal for next 10 days in B3. From 1368th day onwards B3 exhibited slight improvement in NH_4^+–N removal from 39% to 41% releasing ~250 mg/L NH_4^+–N in effluent. The nitrification condition further improved after 4 days cut off of feed and NH_4^+–N removal increased to 46.9% in presence of 415 mg/L and released nearly 220 mg/L NH_4^+–N in effluent. Chakraborty and Veeramani (2005) also observed negative effect on aerobic reactor as decrease in nitrification when pulse phenol injection was applied in an anaerobic-anoxic –aerobic suspended growth system.

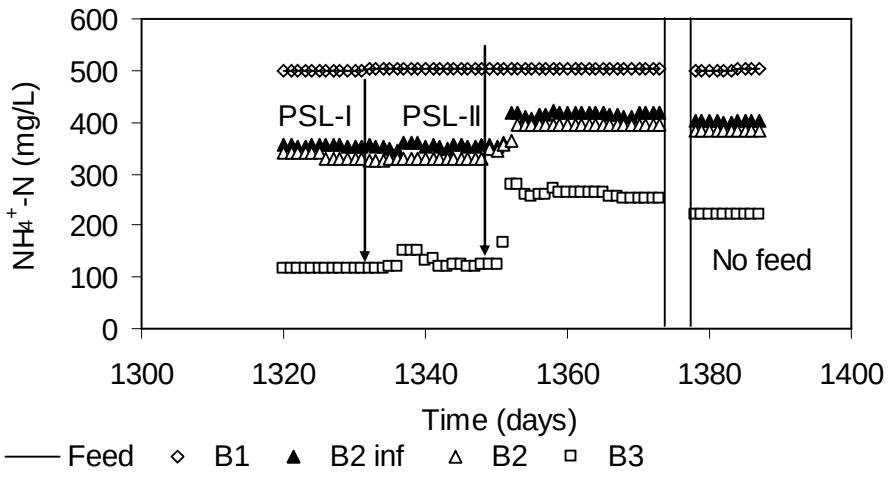

Figure 4.126 NH_4^+-N in three-stage FMBR system at application of phenol shock load

4.4.2.2.4 Overall performance of FMBR system during phenol shock load

For evaluation of overall performance of three–stage FMBR system at phenol shock load application, feed and final effluent of B3 was considered. The overall performance of the three–stage FMBR at varying feed phenol concentrations is shown in Figure 4.127 in terms of phenol, COD, SCN^- and TN removal. It was observed that the system recovered high performance efficiency and almost similar to normal performance efficiency in terms of phenol COD and SCN^- removal. Total phenol removal after second shock load slightly decreased from 99.9% to 99.0%. Similarly thiocyanate removal decreased to 97% to 96% after the first shock load and found to resume to 99.6% as in regular performance. Similar performance was observed during second shock operation though there was slight drop in SCN^- removal to 95% right after the shock load. The system tackled the first shock of phenol concentration in terms of phenol, thiocyanate and COD removal but its removal efficiency decreased than normal efficiency after the second shock which was reversible. Denitrification efficiency remained unaffected by the shocks. Ammonia removal was significantly affected and it was not recovered by the system in the long run up to its pre shock condition performance level. The total ammonia–nitrogen removal was 86% during pre shock period and PSL–I it decreased to ~84% and further decreased to 71% after PSL–II. TN removal remained almost 72–77% during the study.

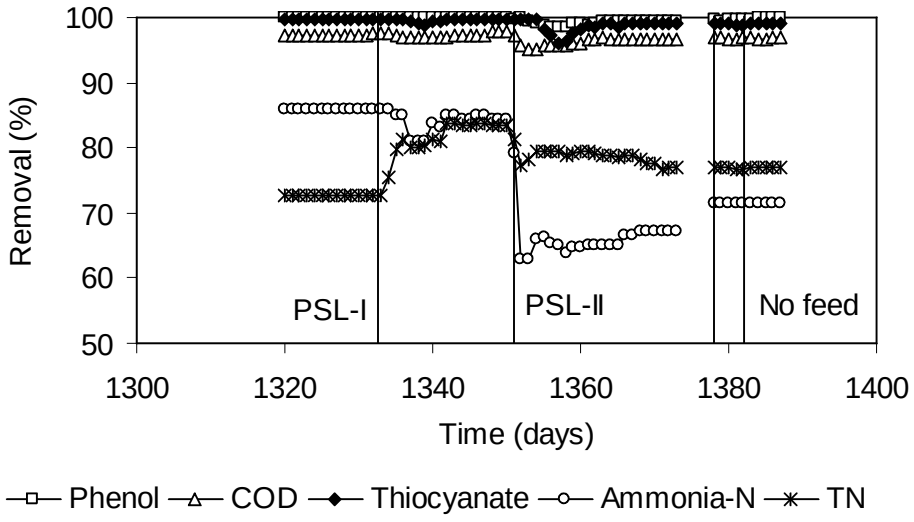

Figure 4.127 Overall performance of FMBR system during phenol shock load application

4.5 TREATMENT OF COKE OVEN INDUSTRIAL WASTEWATER

After evaluating the potential of CMBR in treating synthetic wastewater with different phenol/thiocyanate/ammonia with mixed substrate systems, its performance in raw coke oven wastewater treatment was evaluated. The characteristic of raw wastewater collected from Barnyhat, Guwahati, India is given in Table 4.37.

As the pH of the raw wastewater was 6.9, it was adjusted to 7.5 by using dilute $NaHCO_3$ solutions. Present study with synthetic wastewater in CMBR system was carried out at influent pH of 7.5. The pH of real wastewater was made similar to synthetic wastewater.

Table 4.37 Characteristics of the raw wastewater collected from coke oven industry

Parameter	Concentration in Wastewater	Parameter	Concentration in Wastewater
pH	6.9	Sulfide	3 mg/L
Conductivity	1.005 mS/cm	Phosphate	3 mg/L
DO	0.65 mg/L	Total solids#	28000 mg/L
Phenol	3 mg/L	Volatile solids	180 mg/L
COD	185 mg/L	Chloride	410 mg/L
BOD_5, 20°C	12 mg/L	Iron	15 mg/L
Ammonia–nitrogen	15 mg/L	Fluoride	32 mg/L
Thiocyanate	–	Lead	1 mg/L
Nitrate–N	2 mg/L	Cadmium	1.3 mg/L
Turbidity	>200/25* NTU	Cobalt	0.02 mg/L
Sulfate	130 mg/L	Oil and grease	58 mg/L

NTU* = Nephelo Turbidity Unit.

Total solids were settled and influent to CMBR was at 880 mg/L

Treatment of real coke wastewater is reported by various researchers using different configuration of reactor set up (Zhang et al. 1998; Li et al. 2003; Vázquez et al. 2006b; Marañón et al. 2008a, b; Lai et al. 2008; Zhao et al. 2009; Zheng et al. 2009; Wang et al. 2010; Li et al. 2011). From the Table 4.37 it is observed that the raw wastewater contains very low amount of phenol, and ammonia with negligible amount of thiocyanate compared to the synthetic wastewater used in the study along with some species of heavy

metals. Therefore phenol (1350 mg/L), ammonia–N (500 mg/L), thiocyanate (800 mg/L), pyridine (50 mg/L), cresols (m–, o– and p–) (50 mg/L each) were added to give the picture of real coal gasification/ synthetic fuel processing/ coke oven wastewater. Besides, organic carbon (as COD) of real wastewater was very low (185 mg/L). Hence, supplying the real wastewater may not provide adequate substrate to microbes in R1 and R2. Phenolics are the main organic constituents of coal gasification, coke oven wastewaters, accounting for about 80% of the total COD. Other organics include polynuclear aromatic hydrocarbons (PAHs) and heterocyclic compounds containing nitrogen, oxygen and sulfur (Zhang et al. 1998, Lee and Park, 1998). Inorganic constituents are mainly composed of thiocyanate and ammonia (Zhang et al. 1998, Kim et al. 2007, Kumar et al. 2003; Chakraborty and Veeramoni, 2006). Thus, these wastewaters are been considered the most toxic one to be treated before being discharged into the environments.

Before addition to the industrial wastewater, the system was step wise acclimatized with pyridine and cresols up to 50 mg/L each for 25 days. The real wastewater was having very high settleable suspended solids of 28,000 mg/L, and this was reduced to 880 mg/L after settling for 20 min to avoid the clogging of the reactors. Figure 4.128 demonstrated the phenolics removal efficiency (%) by anaerobic, anoxic and aerobic reactor.

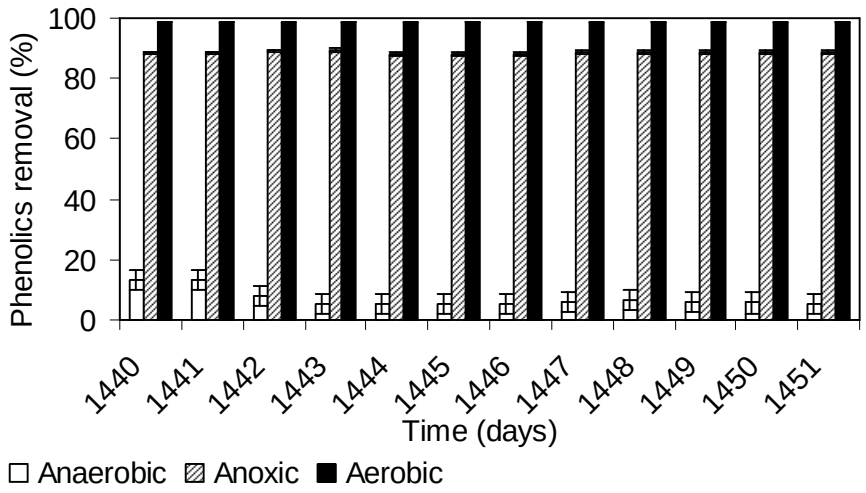

Figure 4.128 Phenolics removal profile by CMBR system from actual coke oven wastewater

From the Figure 4.128 it was observed that, for real wastewater spiked with phenol (1350 mg/L) and cresol (150 mg/L), removal of total phenolics by R1, R2 and R3 were 13.33,

88.47 and 98.67% respectively, in the beginning resulting in total phenol removal 99.93% at a HRT of 6 days. However, it decreased with time and after 10 days of operation phenolics removal in R1 decreased to only 5.33%, whereas no affect was observed either in anoxic reactor (R2) or aerobic reactor (R3). The total phenolics removal remained more or less constant at 99.93% through out the study at loading of 0.5 g/L.day. Ramakrishnan and Gupta (2008b) reported 96% phenolics removal using hybrid upflow anaerobic sludge blanket reactor (HUASB) for treatment of synthetic coal wastewater containing cresols with phenolic loading rate 0.0075 g/L.day. Zheng and Li (2009) achieved almost 100% phenol removal from influent phenol concentration of 1200–1700 mg/L while treating coke oven wastewater in an anaerobic/anoxic /aerobic system in presence of high concentration of ammonia–N.

No thiocyanate removal occurred in anaerobic reactor during the studies. Thiocyanate removal in R2 and R3 were 85.54% and 96.55% from influent concentration 400 and 54 mg/L, respectively. This resulted in the 99.75% total thiocyanate removal from the system at influent thiocyanate concentration 800 mg/L. Through out the study SCN^- loading rate in R2 was 0.267 g/L.day. After 5^{th} to 7^{th} day of real wastewater additions SCN^- removal in R2 decreased from 86% to 81%. However R2 regained its efficiency to 84% from 1457^{th} day onwards and remained static (Figure 4.129).

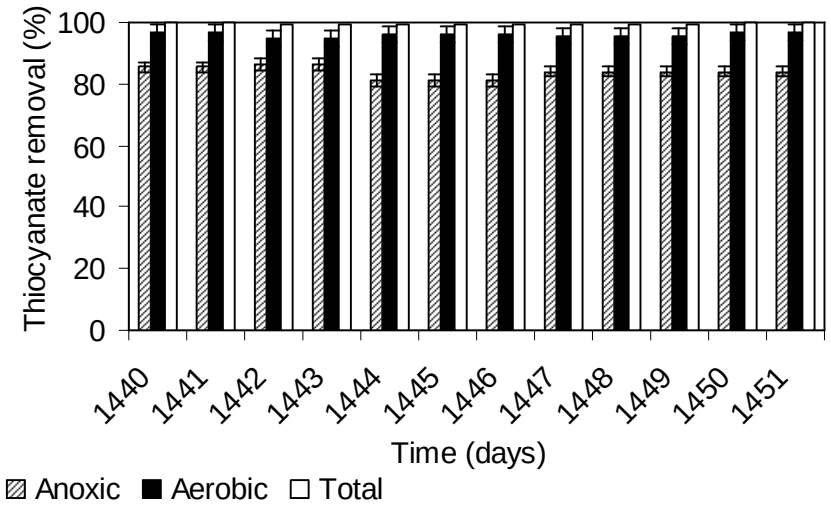

Figure 4.129 Thiocyanate removal profile by CMBR system from actual coke oven wastewater

The thiocyanate removal rate in R2 was 0.228 g/L.day in feed with tap water where as in case of real wastewater it dropped to 0.22 g/L.day and remained constant at 0.224 g/L.day with time. R3 released 3–4 mg/L SCN^- resulting in ~96.5% thiocyanate removal efficiency. On 1440th and 1445th day, SCN^- removal in R3 decreased to 89% releasing 8 mg/L SCN^- in its effluent. However R3 recovered its removal efficiency of 96.88% as soon as upstream reactor R2 regained its efficiency. Total SCN^- removal efficiency was always more than 99% from its influent concentration of 800 mg/L. Jeong and Chung (2006b) achieved 99% thiocyanate removal from real coke wastewater using anoxic–oxic–anoxic–oxic (AOAO) system at a loading rate of 0.12–0.82 g/L.day. Marañón et al. (2008b) reported 97% thiocyanate removal from coke wastewater having thiocyanate concentration 198–427 mg/L using three step ASP.

COD loading rate to R1 was 1.946 g/L.day as influent COD was maintained at 5840 mg/L during the study. The loading rate in R2 and R3 was 1.8–1.9 g/L.day and 0.80–0.83 g/L.day, respectively. With synthetic wastewater COD removal by R1, R2 and R3 were 8.4, 55.75 and 76%, respectively. This performance slightly dropped to 6.6% and 71.13% in R1 and R3 with no change in COD removal efficiency in R2 (Figure 4.130). The COD removal rate in R1 was 0.163 g/L.day for synthetic wastewater and found to decrease to 0.13 g/L.day when real coke wastewater was added to the reactor. Similarly removal rate in R2 was 1.05–1.07 g/L.day during operation with synthetic and real wastewater. It was observed that with time COD removal rate in R1 and R2 was recovered with real coke wastewater with time. However in R3 COD removal rate was not found to get recovered to its normal condition (operation with synthetic wastewater). It decreased from 0.63 g/L.day to 0.58 g/L.day and remained stable. Therefore R1, R2 and R3 exhibited steady COD removal and little drop in removal efficiency was recovered very soon while feed was prepared with real coke wastewater. The total removal was always stable at > 94%.

Peng et al. (2008) reported maximum COD and ammonia nitrogen removal efficiency of 91% and 96.8%, respectively during treatment of coking effluent in a biofilm system consists of one anaerobic biofilm reactor with two aerobic ones, all of which were filled with special carriers without effluent recirculation at HRT of 2.5 days. Zhao et al. (2009) reported COD, phenol, ammonia and total nitrogen removals of almost 89.8%, >99.9%,

99.5% and 71.5%, respectively in a laboratory–scale anaerobic–anoxic–aerobic membrane bioreactor system for treatment of heavily loaded and toxic coke plant wastewater at total HRT of 1.67 days.

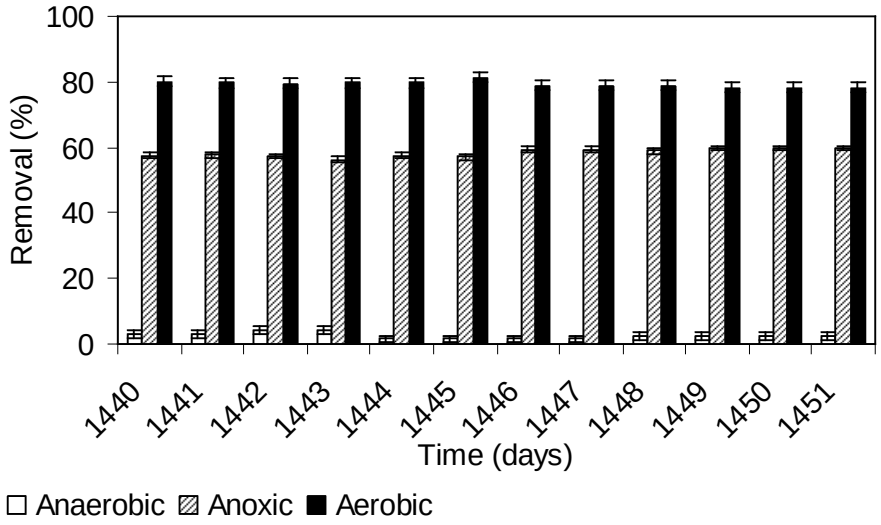

Figure 4.130 COD removal profile by CMBR system from actual coke oven wastewater

Pyridine removal in R1 was negligible and R2 and R3 played the major role in pyridine removal during the study. From influent concentration 50 mg/L, influent to R2 was about 25–28 mg/L. Figure 4.131 shows that pyridine removal by R2 and R3 were 53.8% and 66.67%, respectively constituting total pyridine removal 92%. After 5 days of real wastewater addition (1444th day), pyridine removal in R2 dropped to 33% though it remained unchanged in R3 till for 1447th day. From 1407th day onwards, R2 was observed to be regaining its removal efficiency whereas removal in R3 fallen to 55–57%. The total pyridine removal was observed to be 84–88% at the end of the study period. The denitrification efficiency in R2 remained through out the study with 93.58–93.85% NOx–N removal. Nitrite–nitrogen was completely consumed in both cases of synthetic or actual wastewater (Figure 4.132).

However nitrification in aerobic reactor fallen with addition of real wastewater from 71.32% for synthetic wastewater to 64.28% on 1444th day for real wastewater. There was nitrite accumulation indicating incomplete nitrification. In the present study denitrification remained major reason of total nitrogen removal and it was always higher than nitrification in R3. Total nitrogen removal by the system was 81–82% through out

the study as shown in Figure 4.132. Coke wastewater is reported to be inhibitory for nitrification because of presence of various toxic substances (Li et al. 2003; Jeong and Chung, 2006a and b; Marañón et al. 2008b). Toxicity exerted by other pollutants and heavy metals present in the real wastewater might be the reason for dropping performance during actual wastewater addition. The system was however able to regain its prior efficiency. The overall performance of the CMBR system with real coke wastewater is given in Table 4.38.

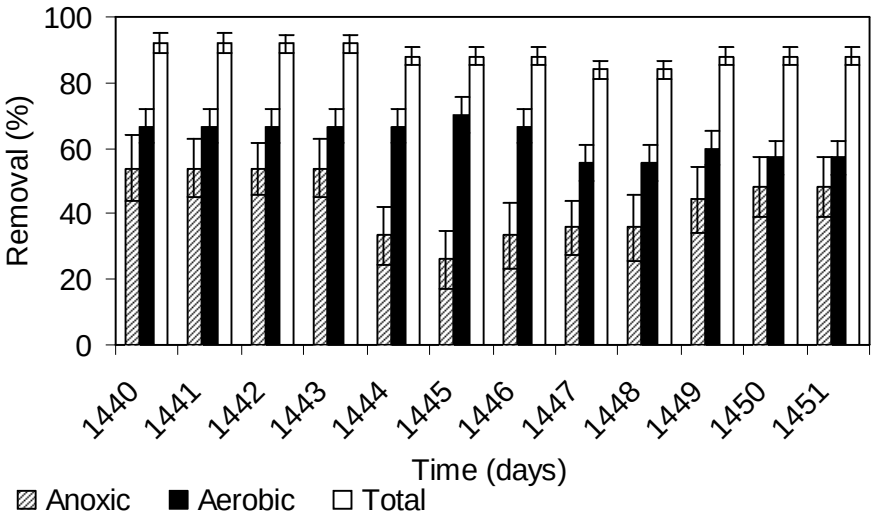

Figure. 4.131 Pyridine removal profile by CMBR system from actual coke oven wastewater

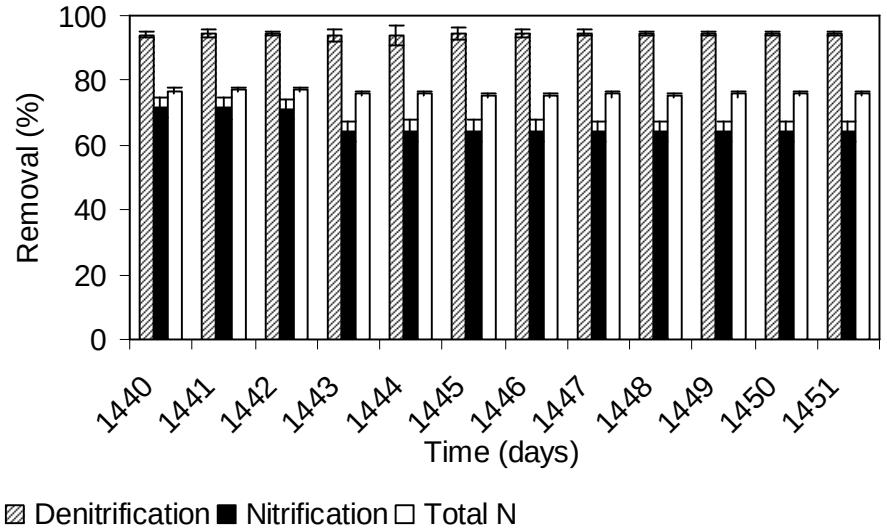

Figure 4.132 Nitrogen removal profile by CMBR system with actual coke oven wastewater

Table 4.38 Performance of the CMBR treating coke oven wastewater added with synthetics

Day	Influent (mg/L)					Effluent (mg/L)					Removal efficiency (%)				
	Phenolic*	SCN^-	Pyridine	COD	Total N	Phenolic	SCN^-	Pyridine	COD	Total N	Phenolic	SCN^-	Pyridine	COD	Total N
1400	1500	800	50	5840	1742	1	2	4	300	304.16	99.93	99.75	92.0	94.86	82.54
1401	1500	800	50	5840	1742	1	2	4	300	296.16	99.93	99.75	92.0	94.86	83.00
1402	1500	800	50	5840	1742	1	3	4	300	297.4	99.93	99.63	92.0	94.86	82.93
1403	1500	800	50	5840	1742	1	3	4	350	317.4	99.93	99.63	92.0	94.07	81.78
1404	1500	800	50	5840	1742	1	8	6	350	320.94	99.93	99.00	88.0	94.00	81.58
1405	1500	800	50	5840	1742	1	8	6	350	327.94	99.93	99.00	88.0	94.00	81.18
1406	1500	800	50	5840	1742	1	3	6	350	328.74	99.93	99.63	88.0	94.00	81.13
1407	1500	800	50	5840	1742	1	3	8	320	324.08	99.93	99.63	84.0	94.52	81.40
1408	1500	800	50	5840	1742	1	3	8	320	328.08	99.93	99.63	84.0	94.52	81.17
1409	1500	800	50	5840	1742	1	3	6	320	324.74	99.93	99.63	88.0	94.52	81.36
1410	1500	800	50	5840	1742	1	2	6	320	322.5	99.93	99.75	88.0	94.52	81.49
1411	1500	800	50	5840	1742	1	2	6	320	321.5	99.93	99.75	88.0	94.52	81.55

Phenolics* composed of phenol 1350 mg/L and p–cresol=o–cresol=m–cresol=50 mg/L;

Total HRT 6 days (R1–3 days, R2–R3 1.5 days each)

4.6. SLUDGE CHARACTERISTICS AND IDENTIFICATION OF PREDOMINANT BACTERIA SPECIE IN CMBR AND FMBR SYSTEM

4.6.1 Sludge characteristics in seed sludge

Before to the study with CMBR and FMBR sludge characteristics of the raw sludge applied for anaerobic, anoxic and aerobic reactor were carried out. It is shown in Table 4.39. These values may help to decide the proper disposal method of the excess sludge. It seems that potassium content of the sludge from all three reactors were quite high. In R1 sludge amount of nitrogen was maximum and decreased in downstream reactors.

Table 4.39: Characteristics of digested sludge from continuous reactors

Parameters	Na^+ (mg/g)	K^+ (mg/g)	Ca^{2+} (mg/g)	NH_4^+–N (mg/g)	P (mg/g)	COD (mg/g)	Cl^- (mg/g)	N:P:K
Anaerobic (R1)	0.346	0.377	0.131	0.472	0.029	0.734	0.251	1.25: 0.07:1
Anoxic (R2)	0.096	0.185	0.042	$7.8x10^{-3}$	0.055	1.04	0.296	0.042:0.2 9:1
Aerobic (R3)	0.329	1.02	0.161	0	0.064	0.599	0.129	0:0.06:1

Table 4.40 (a) and (b) presents the amount of biomass on sponge cube in R1, R2 and R3 from CMBR series and B1, B2 B3 in FMBR series, respectively. It can be seen that for R1, less amount of biomass was present in middle (282 g) as compared to top (453 g). However, this picture was reverse for R2 and R3, where amount of biomass in middle was much higher than top. The probable reason could be in R1; the toxic feed entered in the reactor at bottom and gradually moved upward direction along with the flow. Due to biodegradation of pollutants along the reactor length, the toxicity effect decreased from bottom to top. Hence, more biomass was present at top near effluent port as compared to middle of the reactor. However, in R2 and R3, the toxicity effect of wastewater was largely decreased (due to partial degradation in R1 and dilution effect) and more biomass was present in middle as compared to top as more substrate was available in the middle. Similarly in case of FMBR

system, biomass concentration was maximum in top for B1 whereas it was maximum in middle and bottom for B2 and B3, respectively

Table 4.40 (a): Biomass concentration on sponge cube of CMBR

VSS (g)	R1		R2		R3	
	Top	Middle	Top	Middle	Top	Middle
VSS /sponge cube (g)	0.1928	0.1198	0.1203	0.1336	0.0185	0.0253
Total biomass in reactor (g)	453.71	282.00	283.12	314.42	43.53	59.59
Average biomass in reactor (g)	367.86		298.77		51.56	

Table 4.40 (b): Biomass concentration on sponge cube of FMBR

VSS (g)	B1			B2			B3		
	Top	Middle	Bottom	Top	Middle	Bottom	Top	Middle	Bottom
VSS /sponge cube (g)	0.1823	0.1205	0.1034	0.1483	0.1889	0.1156	0.0133	0.0167	0.0412
Total biomass in reactor (g)	357.451	236.27	202.745	290.784	370.39	226.667	26.0784	32.745	80.784
Average biomass in reactor (g)	265.488			295.947			46.535		

4.6.2 Isolation and identification of microbes in CMBR

Predominant bacteria specie isolated from each reactor of CMBR and FMBR systems were identified from microscopic and biochemical tests as described below:

During isolation and identification of bacteria in R1, three gram positive facultative anaerobic bacterial strains were observed: two circular shaped bacteria (cocci) with grape

like structure (*Staphylococcus sp.*) and chain like structure (*Streptococcus sp.*) and one rod shaped bacteria (*Lactobacillus sp.*). The SEM photograph of microbial cultures in R1 is shown in Figure 4.133. Biochemical and microscopic characterization is presented in Table 4.41. Phenol removals by *Staphylococcus sp.* and *Lactobacillus sp.* are reported in presence of cyanide and in olive mill wastewater (Kowalska et al. 1998; Aouidi et al. 2009).

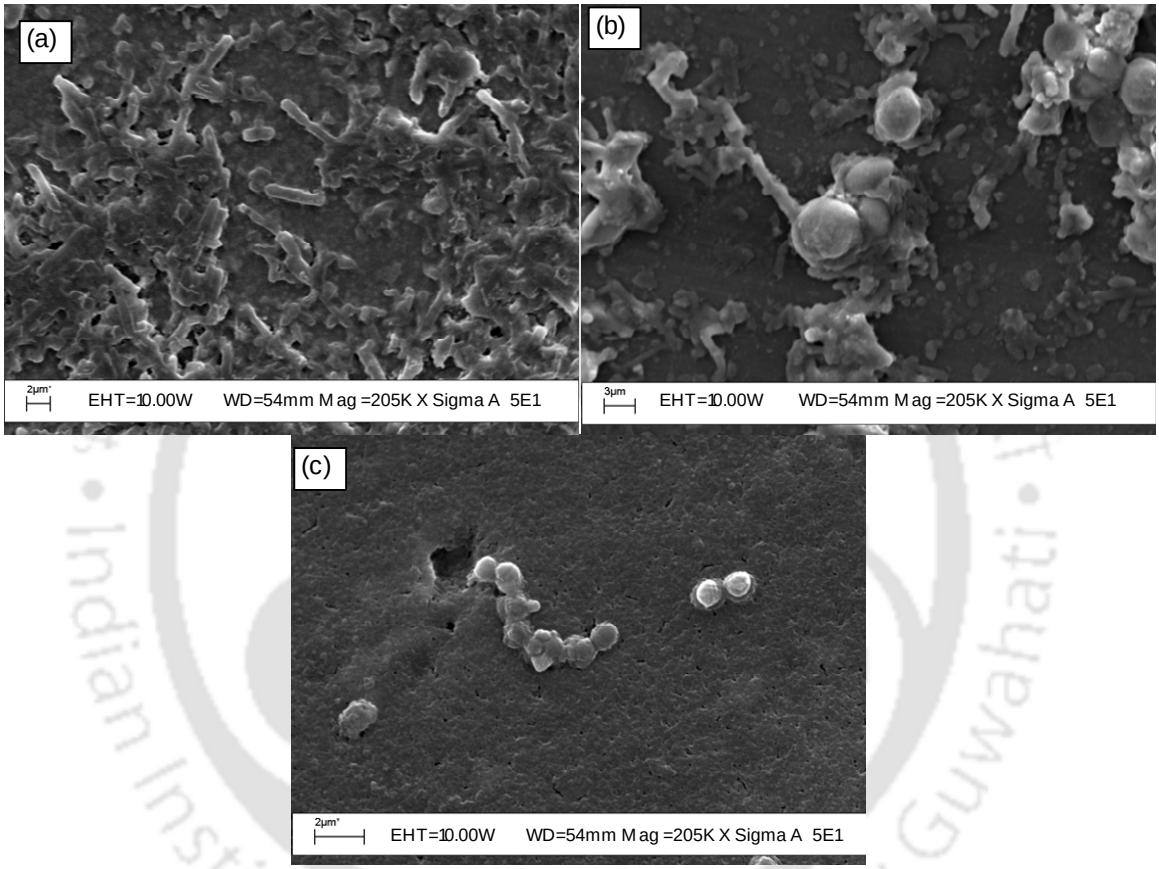

Figure 4.133 Isolated and identified bacteria specie in R1 (a) *Lactobacillus sp.* (b) *Streptococcus sp.* (c) *Staphylococcus sp.*

In R2 after isolation four morphologically distinct types of bacteria were observed: two rod shaped bacteria were observed: *Corynebacterium sp.* and *Citrobacter sp.* and two circular shaped bacteria: gram negative *Neisseria sp.* and gram positive *Streptococcus sp.* (Figure 4.134). Biochemical and microscopic characterization is presented in Table 4.42. All these bacteria are reported to have capability to grow in denitrifying condition (Gerardi, 2006).

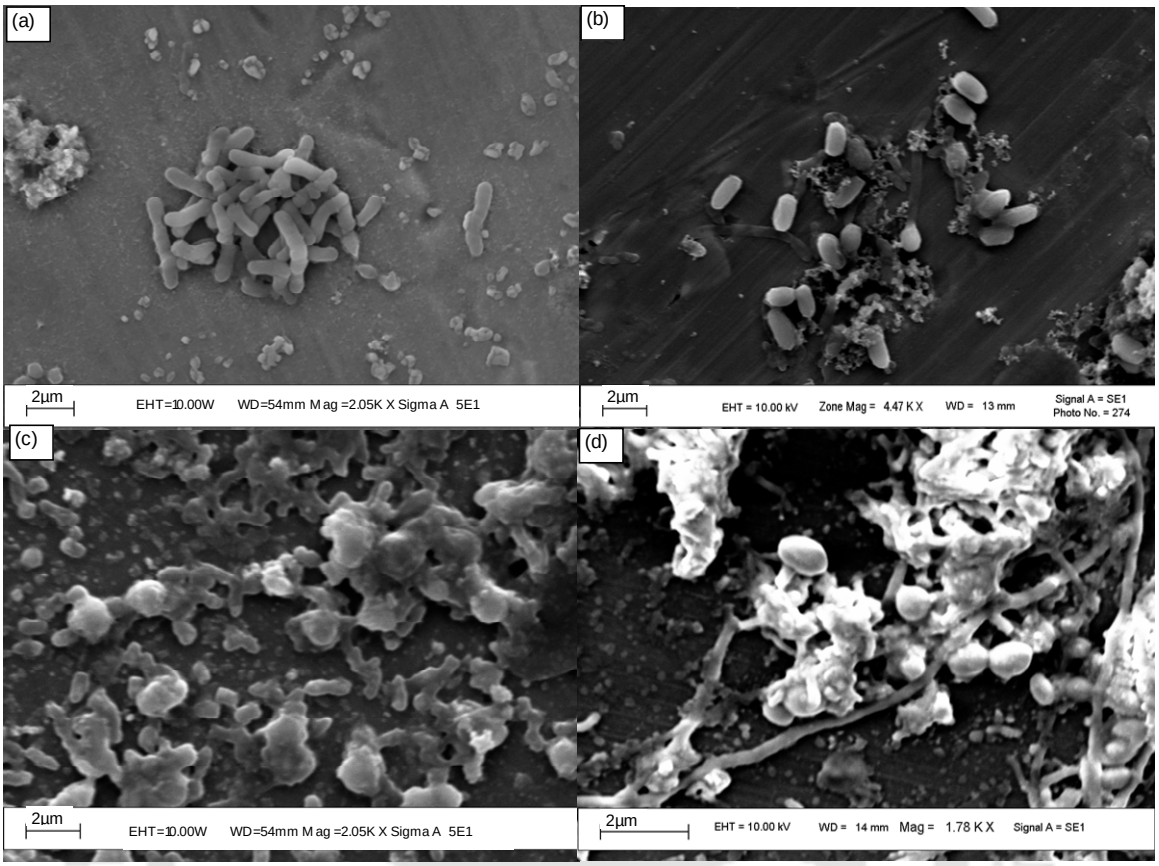

Figure 4.134 Isolated and identified bacteria specie in R2 (a) *Corynbacterium sp.* (b) *Cirtrobacter sp.* (c) *Neisseria sp*. (d) *Streptococcus sp.*

In the present study the anoxic reactor R2 received mixed feed containing phenol, SCN^-, ammonia and nitrate and removal of all four compounds were observed. Hence, from isolation study it was difficult to identify the microorganism responsible for removal of a specific pollutant. A qualitative study was conducted, where two synthetic feeds were prepared containing (a) phenol (100 mg/L) and NO_3^-–N (200 mg/L); (b) SCN^- (100 mg/L) and NO_3^-–N (200 mg/L) along with phosphate buffer, trace metal solution and yeast extract. In each feed, separately, one bacteria strain was inoculated. Performance of each bacteria strain with two different feed is shown in Table 4.43. It can be seen that all species were able to remove phenol and nitrate, with complete phenol removal in 136 hr. Though complete phenol removal was achieved by all strains in 136hr, denitrification was maximum of 58% for *Streptococcus sp.* and lowest for *Neisseria sp.* (only 36%). The anaerobic reactor (R1) and anoxic reactor (R2) were started with the same inoculum (anaerobic biogas plant). The

facultative anaerobic microorganisms *Streptococcus sp.* was common in both R1 and R2. It seems that *Streptococcus sp.* being facultative in nature was capable to degrade phenol both under anaerobic and anoxic environments depending on availability of external electron acceptor. Phenol degradation by bacteria *Corynebacterium sp.* In aerobic environment is supported by Ho et al. (2009). Present study shows that *Corynebacterium sp.* can degrade phenol under anoxic environment also. All four species were able to degrade SCN^- and degradation was maximum of 20% for *Streptococcus sp.*. Both *Neisseria sp.* and *Citrobacter sp.* showed less removal of SCN^- (10%). Further, denitrification efficiency was also very low (4–7%) when only SCN^- was present in the medium in absence of phenol. Probable reason could be that in absence of organic carbon source, denitrification was low by heterotrophic bacteria. Port et al. (1984) reported SCN^- degradation by *Neisseria sp.* where SCN^- was used as a source of sulfur. However, no literature information is available on thiocyanate degradation by *Corynebacterium sp.*, *Streptococcus sp, Neisseria sp.* and *Citrobacter sp.* under anoxic environment. However, present results suggest that SCN^- removal is possible in anoxic environment though denitrification efficiency becomes lower in absence of organic carbon.

In aerobic reactor R3, during isolation of bacteria, two rod shaped *Lactobacillus sp., Citrobacter sp.*, bacteria and two cocci named *Neisseria sp.* and *Staphylococcus sp.* were observed (Figure 4.135). Biochemical and microscopic characterization is presented in Table 4.44. Further, all four isolates were separately added in synthetic feed containing ammonia (100 mg/L NH_4^+–N). It was observed that in 240 hr, NH_4^+–N removal (%) by *Lactobacillus sp., Citrobacter sp.*, *Neisseria sp.* and *Staphylococcus sp.* were 40, 30, 50 and 35% respectively in aerobic environment. It was also observed that *Citrobacter sp.* converted NH_4^+–N completely to NO_3^-–N, whereas *Neisseria sp.*, oxidized 50% of NH_4^+–N to NO_3^-–N. *Lactobacillus sp.* and *Staphylococcus sp.* converted NH_4^+–N to NO_2^-–N with negligible amount of NO_3^-–N. It is interesting that *Citrobacter sp.* and *Neisseria sp.* showed both denitrification and nitrification abilities depending on environmental condition. Similar behavior is reported by *Pseudomonas pavonaceae* which showed switching behavior between denitrification and nitrate generation depending on environmental condition (Sakai et al. 1997).

Table 4.41: Biochemical Test Result for R1

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Inulin	Litmus	MR	VP	H_2S	Indole	Citrate	Nitrate	Oxidase	Catalase
Staphylococcus	+ve	Cocci	abundant growth	AG	AG	AG	AG	Acid	+ve	+ve	+ve	–ve	–ve	+ve	–ve	+ve
Lactobacillus	+ve	Rod	Thin, growth	AG	AG	AG	AG	Acid, curd	–ve	–ve	+ve	–ve	–ve	+ve	–ve	–ve
Streptococcus	+ve	Cocci	Do	AG	AG	AG	AG	Acid, curd	+ve	+ve	+ve	–ve	–ve	+ve	–ve	–ve

Table 4.42: Biochemical Test Result for R2

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Inulin	Litmus	MR	VP	H_2S	Indole	Citrate	Nitrate	Oxidase	Catalase
Corynebacterium	+ve	Rod	Thin, growth	AG	AG	AG	AG	alkaline	–ve	–ve	+ve	–ve	–ve	+ve	–ve	+ve
Citrobacter	–ve	Rod	Thin, growth	AG	AG	AG	AG	Acid, curd	–ve	–ve	+ve	–ve	+ve	+ve	+ve	+ve
Neisseria	–ve	Cocci	Thin, growth	AG	AG	AG	AG	Acid	–ve	+ve	+ve	–ve	+ve	+ve	–ve	+ve
Streptococcus	+ve	Cocci	Thin, growth	AG	AG	AG	AG	Acid, curd	+ve	+ve	+ve	–ve	–ve	+ve	–ve	–ve

Table 4.43: Performance of bacterial species isolated from anoxic reactor R2

Specie	Phenol and NO_3^-–N		SCN^- and NO_3^-–N	
	Phenol	NO_3^-–N	SCN^-	NO_3^-–N
	Removal (%)	Removal (%)	Removal (%)	Removal (%)
Corynebacterium	100	45	15	7.5
Citrobacter	100	51	10	4
Neisseria	100	36	10	6.5
Streptococcus	100	58	20	6

Initial phenol: 100, NO_3^-–N 200 and SCN^- 100 mg/L.Degradation time 136 hr.

Table 4.44: Biochemical Test Result for R3

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Inulin	Litmus	MR	VP	H_2S	Indole	Citrate	Nitrate	Oxidase	Catalase
Lactobacillus	+ve	Rod	Thin, growth	AG	AG	AG	AG	alkaline	–ve	–ve	+ve	–ve	–ve	+ve	–ve	–ve
Citrobacter	–ve	Rod	Thin, growth	AG	AG	AG	AG	Acid, curd	–ve	–ve	+ve	–ve	+ve	+ve	+ve	–ve
Neisseria	–ve	Cocci	Thin, growth	AG	AG	AG	AG	Acid	+ve	+ve	+ve	–ve	–ve	+ve	–ve	+ve
Saphylococcus	+ve	Cocci	abundant growth	AG	AG	AG	AG	Acid, curd	+ve	+ve	+ve	–ve	–ve	+ve	–ve	+ve

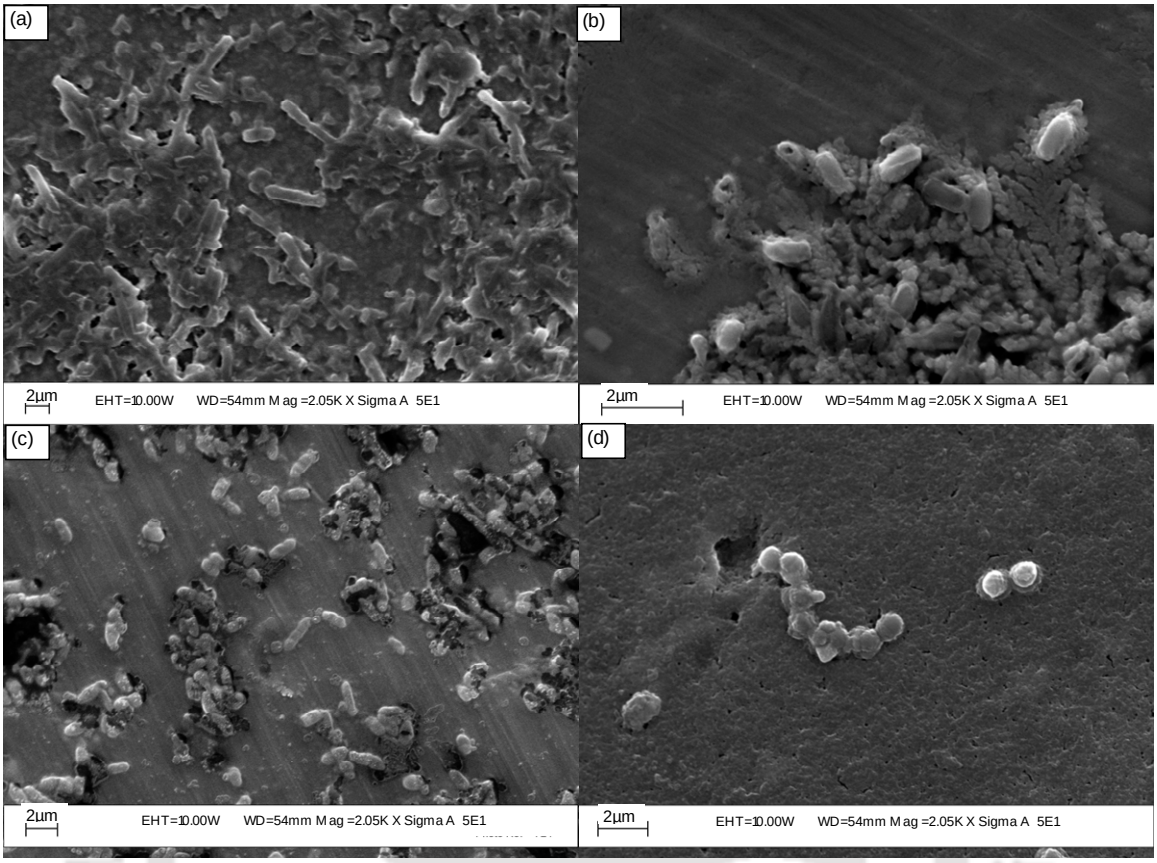

Figure 4.135 Isolated and identified bacteria specie in R3 (a) *Lactobacillus sp.* (b) *Cirtrobacter sp.* (c) *Neisseria sp*. (d) *Staphylococcus sp.*

4.6.3 Isolation and identification of bacteria in FMBR

Lactobacillus sp., *Streptococcus sp.* and *Escherichia coli* were isolated and identified through biochemical tests from the sludge of B1 as predominant microorganisms. SEM image is presented in Figure 4.136. Biochemical and microscopic characterization is presented in Table 4.45. Phenol removal by *Lactobacillus sp.* is reported in olive oil mill wastewater by Kowalaska et al. (1998) and degradation by *E. coli* in presence of glucose id reported by Movahedin et al. (2006).

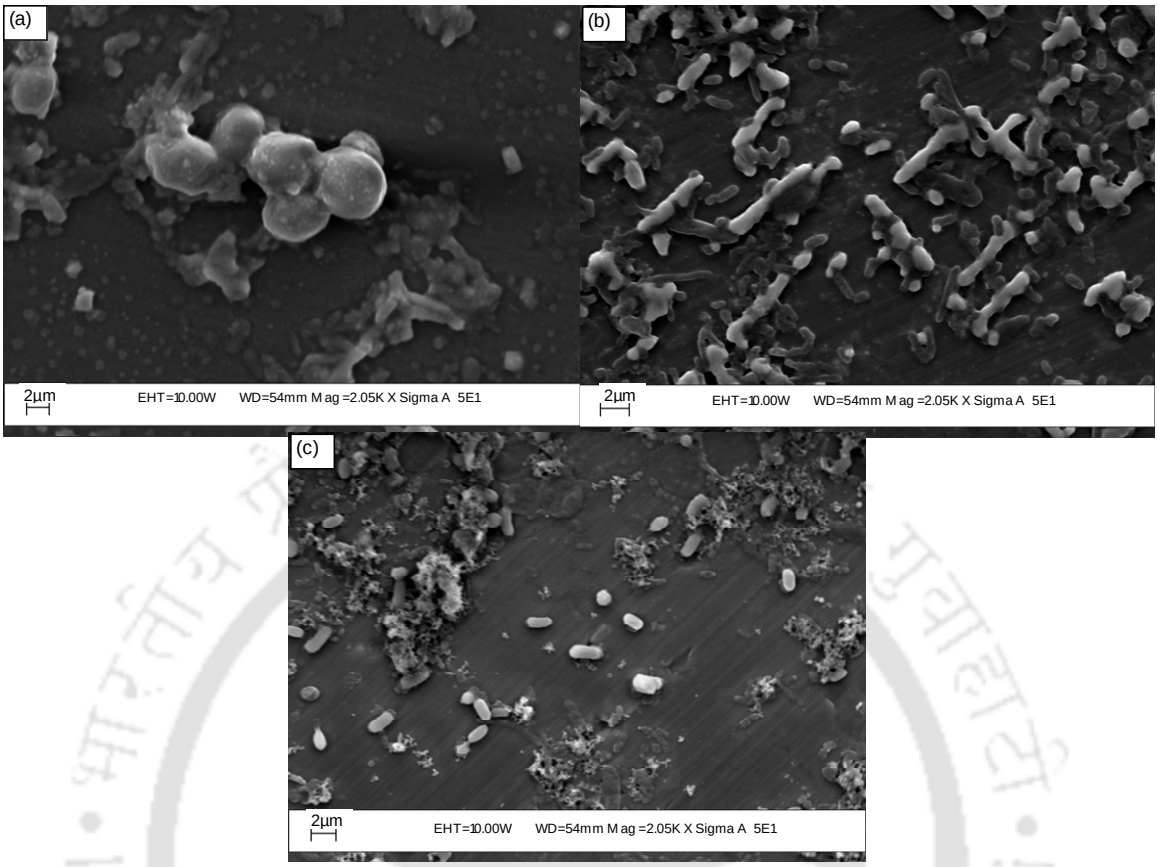

Figure 4.136 Isolated and identified bacteria specie in B1 (a) *Streptococcus sp.* (b) *Lactobacillus sp.* (c) *E. Coli*

Biochemical tests shows the presence of *Pseudomonas sp., Citrobacter sp., Enterobacter sp.* and *E.coli* in B2 as dominant species (Figure 4.137). Biochemical and microscopic characterization is presented in Table 4.46. Biodegradation of phenol containing wastewater by *Pseudomonas sp.* was reported in literatures (Sa, 2001, Saravanan, 2008). Thomas et al. (2002) reported phenol degradation by *Enterobacter sp*. in denitrifying condition whereas pyridine degradation by *Pseudomanas sp*. is reported by Padoley et al. (2006) and Mudliar et al. (2008).

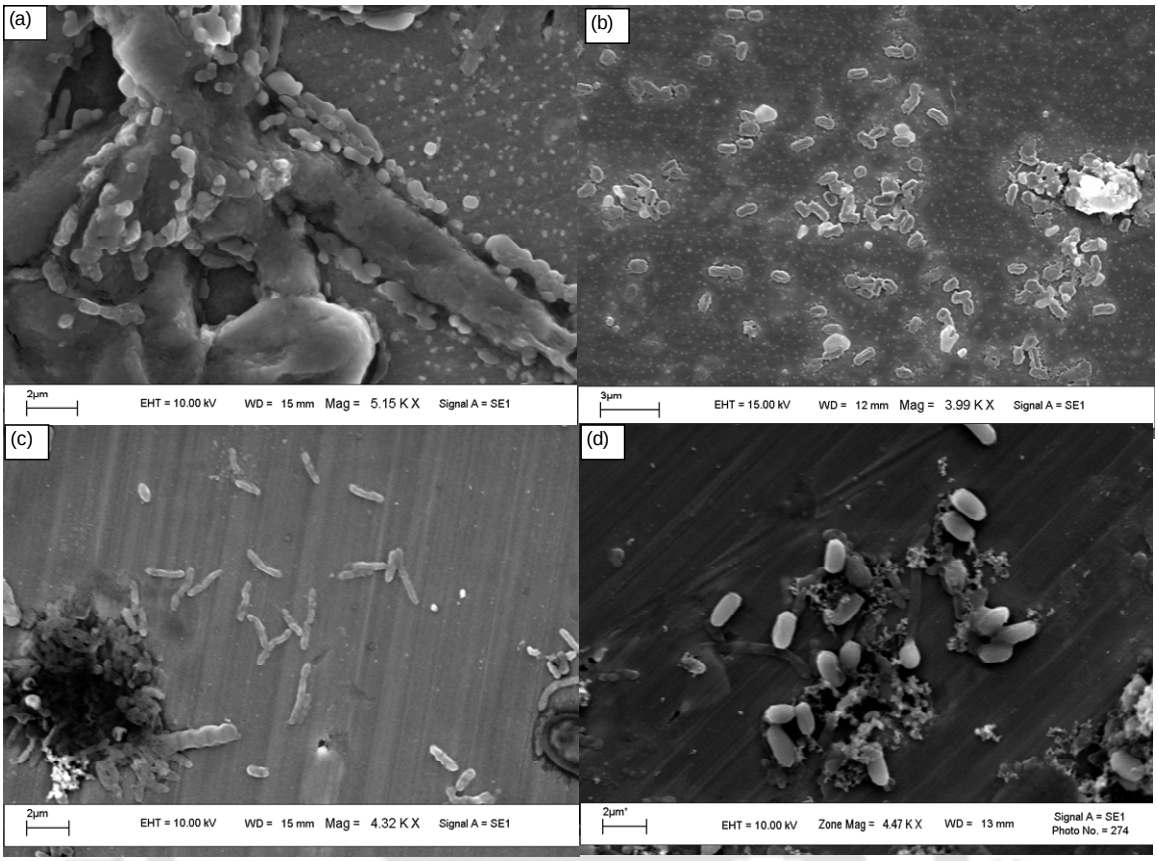

Figure 4.137 Isolated and identified bacteria specie in B2 (a) *Enterobacter sp.* (b) *Citrobacter sp.* (c) *Pseudomonas sp. (d) E. Coli*

Lactobacillus sp., Pseudomonas sp. and Citrobacter sp .was identified in the sludge of B3 (Figure 4.138). Biochemical and microscopic characterization is presented in (Table 4.47). This result also suggests presence of heterotrophs as dominant species in B3 like reactor R3. Influent COD to B3 was responsible for presence of heterotrophic microorganisms. Thiocyanate degradation by *Pseudomonas sp.* under aerobic and oxygen free (with nitrate) condition is reported by Grigor'eva et al. (2009) with production of sulfate and ammonia.

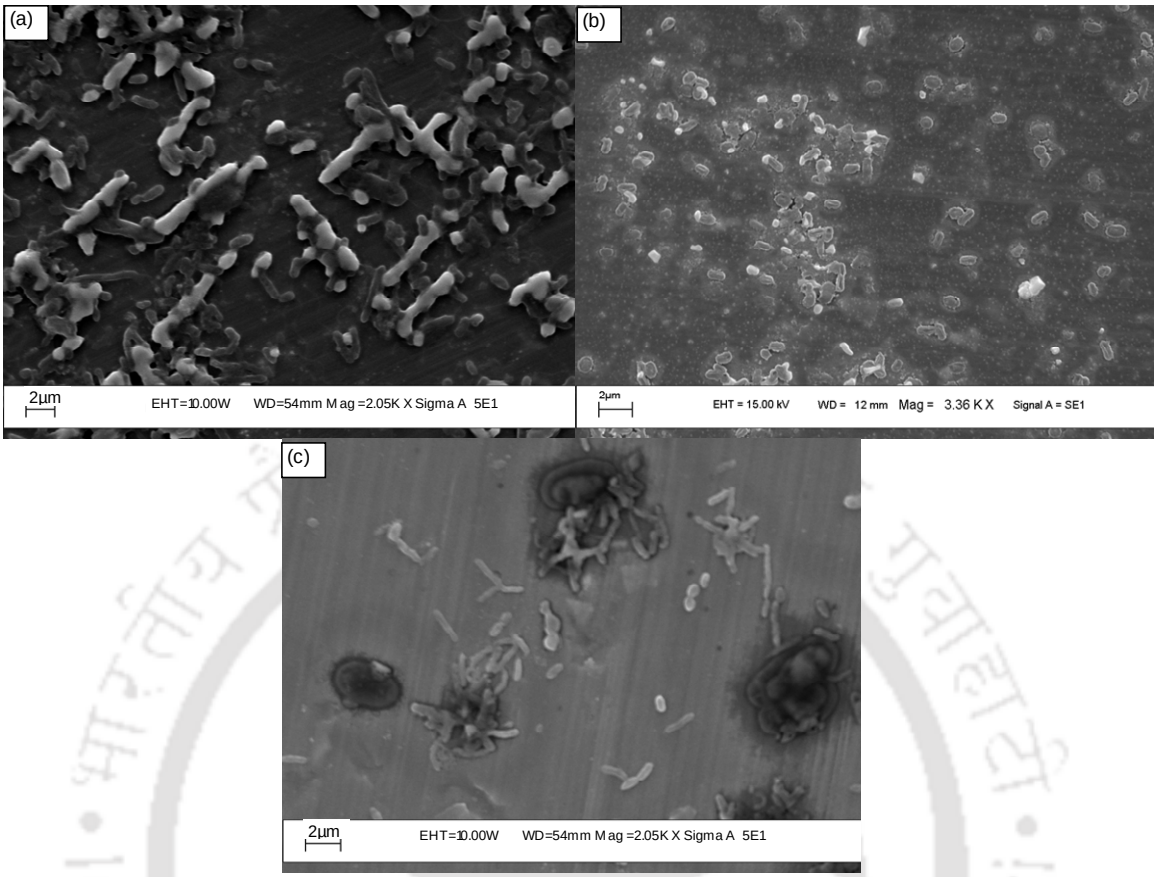

Figure 4.138 Isolated and identified bacteria specie in B3 (a) *Lactobacillus sp.* (b) *Citrobacter sp.* (c) *Pseudomonas sp.*

Table 4.45: Biochemical Test Result for B1

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Litmus	MR	VP	H_2S	Indole	Urease	Citrate	Nitrate	Oxidase	Catalase
Lactobacillus	+ve	Rod	Thin, growth	AG	AG	AG	Acid, curd	–ve	–ve	+ve	–ve	–ve	–ve	+ve	–ve	–ve
Streptococcus	+ve	Cocci	Do	AG	AG	AG	Acid, curd	+ve	+ve	+ve	–ve	–ve	–ve	+ve	–ve	–ve
Esherichia coli	–ve	Rod	White	AG	AG	A+ve	Acid curd	+ve	–ve	–ve	+ve	–ve	–ve	+ve	–ve	+ve

Table 4.46: Biochemical Test Result for B2

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Litmus	MR	VP	H_2S	Indole	Citrate	Nitrate	Urease	Oxidase	Catalase
Enterobacter	–ve	Rod	Abundant growth	AG	AG	AG	Acid	–ve	+ve	–ve	–ve	+ve	+ve	–ve	–ve	+ve
Citrobacter	–ve	Rod	Thin, growth	AG	AG	AG	Acid, curd	–ve	–ve	+ve	–ve	+ve	+ve	–ve	+ve	+ve
Pseudmonas	–ve	Rod	abundant growth	–ve	–ve	–ve	Rapid peptonization	–ve	–ve	–ve	–ve	+ve	+ve	–ve	+ve	+ve
Esherichia coli	–ve	Rod	White	AG	AG	A +ve	Acid curd	+ve	–ve	–ve	+ve	–ve	+ve	–ve	–ve	+ve

Table 4.47: Biochemical Test Result for B3

Specie	Gram	Shape	Colony	Lactose	Dextrose	Sucrose	Litmus	MR	VP	H_2S	Indole	Citrate	Nitrate	Urease	Oxidase	Catalase
Lactobacillus	+ve	Rod	Thin, growth	AG	AG	AG	alkaline	–ve	–ve	+ve	–ve	–ve	+ve	–ve	–ve	–ve
Citrobacter	–ve	Rod	Thin, growth	AG	AG	AG	Acid, curd	+ve	–ve	+ve	–ve	+ve	+ve	–ve	+ve	–ve
Pseudmonas	–ve	Rod	abundant growth	–ve	–ve	–ve	Rapid peptonization	–ve	–ve	–ve	–ve	+ve	+ve	–ve	+ve	+ve

CHAPTER 5

Conclusions and Future scope

In this chapter summary and conclusion of the research work carried out is presented. Two sequential anaerobic–anoxic–aerobic moving bed reactor systems operated at continuous mode (CMBR: R1-R2-R3) and fed batch mode (FMBR: B1-B2-B3) were applied for treatment of synthetic wastewater containing multiple pollutants phenol, thiocyanate and ammonia–nitrogen. Some studies were carried using pyridine in feed. Feed concentrations and operational condition such as HRT, cycle time and fill time (for FMBR only) were varied and performance of both the system was evaluated. Stability of both the systems was also evaluated by sudden increase of some feed pollutants. Major conclusions from this study are listed below:

1. Sequential anaerobic–anoxic–aerobic continuous and fed batch moving bed reactor systems were successfully operated with more than 99% of thiocyanate and phenol removals along with 90–95% COD and 75-90% total nitrogen removal from synthetic wastewater containing high concentration phenol, SCN^-, NH_4^+–N, COD and total nitrogen of 2500, 800, 500, 8150 and 1692 mg/L, respectively.

2. Minimum total HRT for efficient removal of high concentration phenol, SCN^-, NH_4^+–N, COD and total nitrogen of 2500, 800, 500, 8150 and 1692 mg/L, respectively should be of 4 days and 6 days for CMBR and FMBR, respectively and below this NH_4^+–N removal efficiency deteriorated significantly from influent concentration.
3. Anaerobic MBRs, R1 and B1 were placed in beginning of the sequence and received fresh influent through out the study. Both R1 and B1 were responsible for phenol and COD removals, which were significantly inhibited by thiocyanate in feed above 200 mg/L. Phenol and NH_4^+–N above 1500 and 500 mg/L, respectively also inhibited phenol/COD removals in R1 and B1. Very less thiocyanate removal and no NH_4^+–N removal was observed in anaerobic reactors. In presence of thiocyanate loading 0.267 g/L.day in both R1 and B1, maximum phenol and COD removal rates achieved in R1 were 0.188 g/L.day and 0.168 g/L.day at organic loading of 0.50 g phenol/L.day and 2.72 g COD/L.day, respectively; B1 showed maximum phenol and COD removal rates of 0.326 g/L.day and 0.636 g/L.day at loading of 0.667 g phenol/L.day and 2.72 g COD /L.day, respectively. This suggests that B1 was more efficient than R1 in normal operation.
4. In CMBR system, fraction phenol removal by R1 was comparatively lower than R2 except low influent phenol and NH_4^+–N studies whereas COD removal was always lower than R2. Fractional removals of phenol/COD in B1 were comparatively higher than that of B2 and B3 in most of the studies.
5. In sludge of anaerobic CMBR (R1) and FMBR (B1) specific methanogenic activity (SMA) decreased from 0.102 g CH_4–COD/ g VSS. day to 0.036 g CH_4–COD/ g VSS. day and 0.029 g CH_4–COD/ g VSS. day to 0.028 g CH_4–COD/ g VSS. day, respectively when feed thiocyanate was increased from 100 to 200 mg/L and no SMA was observed either in sludge of R1 or B1 at SCN^- concentration above 200 mg/L.
6. Anoxic MBRs, R2 and B2 received influent that constituted by partially treated effluent from anaerobic reactors and recycle from aerobic reactors at a ratio of 1:1. R2 and B2 exhibited simultaneous removals of phenol, COD, SCN^- and nitrate/nitrite. R2 and B2 were mainly responsible for thiocyanate removal from the system with generation of sulfate and NH_4^+–N as end products. Nitrate was essential for SCN^- degradation in anoxic condition. Fractional thiocyanate removals in R2 and B2 were

21–49.5% and 31–49.5%, respectively thorough out the study. With higher thiocyanate removal in both R2 and B2, higher difference in actual and theoretical sulfate generation was observed which might be due to formation of other products like thiosulfate and polysulfide etc.

7. With increase in phenol loading, though denitrification and COD removals increased, removal of SCN^- decreased in anoxic reactors. Higher influent phenol concentration more than 468 mg/L and 511 mg/L inhibited thiocyanate removal in R2 and B2, respectively. Increase in thiocyanate removal rate was observed in R2 and B2 with increase in thiocyanate loading (maximum loading in R2: 0.4 g/L.day; B2: 0.357 g/L.day) suggesting no substrate inhibition of thiocyanate in anoxic reactor.
8. Higher COD and nitrate–nitrogen removal ratio was observed in B2 and R2 towards higher COD loading and it was 3–7 in R2 whereas it was 2.2–6 in B2 due to low organic loading in B2.
9. Influent to aerobic reactors, R3 and B3 were constituted by effluent of R2 and B2, respectively. In most of the studies R3 received higher amount of phenol/thiocyanate and COD in influent whereas B3 received low influents of these pollutants and both R3 and B3 brought down the residual phenol, thiocyanate and COD to discharge limit of 1 mg/L, 1mg/L and ≤ 250 mg/L, respectively. Main target pollutant to be treated in aerobic reactors was NH_4^+–N. Threshold phenol, thiocyanate and NH_4^+–N loading to achieve 80% NH_4^+–N removal efficiency in R3 were 0.450, 0.061 and 0.284 g/L.day, respectively. Threshold phenol, thiocyanate and NH_4^+–N loading to achieve 80% NH_4^+–N removal efficiency in B3 were 0.001, 0.006 and 0.249 g/L.day, respectively. This indicates that B3 was more sensitive to pollutant loading compared to R3. In R3/B3, increase in pH was observed from 7.5 to 8.0 ± 0.4. Free ammonia also enhanced nitrite accumulation and affected NH_4^+–N removal in combination to higher concentration of phenol and thiocyanate.
10. At identical pollutant loading rate in CMBR and FMBR system, during increased feed phenol concentration studies, B1 showed higher removal compared to R1, whereas performance of R2 and R3 was similar to B2 and B3. Final effluent from CMBR contained phenol, thiocyanate, COD, NH_4^+–N and total nitrogen concentration of 1–2 mg/L, 1–13 mg/L, 230–245 mg/L, 43–123 mg/L and 347–398 mg/L, respectively. The

final effluent from FMBR contained phenol, thiocyanate, COD, NH_4^+–N and total nitrogen concentration of 0 –1 mg/L, ~1 mg/L, 235–245 mg/L, 55–100 mg/L and 337–480 mg/L, respectively.

11. Gradual short fill time (1–1.5 h) showed higher phenol and COD removals in B1 as compared to gradual long fill (2.5 and 3.7 h) and instantaneous fill. B2 was insignificantly affected during cycle time and fill time variation of 18–36 h and 2–7.4 h, respectively. Cycle time of 30 h or above, longer HRT and gradual long fill were beneficial for nitrification. Aerobic reactors are very much sensitive and the cultures need longer reaction time for nitrification to occur and low toxicity load when a number of pollutants are considered.
12. Pyridine concentrations of 25–250 mg/L were added in influent of FMBR system. Phenol and COD removal efficiency in B1 was significantly affected when feed pyridine concentration was higher than 50 mg/L. B2 showed higher efficiency in pyridine removal compared to B1 and B3. Though effect of increased pyridine concentration was insignificant on removals of phenol/ COD, thiocyanate removal in B2 was affected at higher influent pyridine concentration of 127 mg/L. In B3 NH_4^+–N removal decreased with increase in influent pyridine which was mainly due to increased influent NH_4^+–N concentration generated from thiocyanate and pyridine degradation.
13. Modified Stover–Kincannon model showed the best fit for substrate removal in R2 and R3 of CMBR. In R1, Bhatia et al model showed best fit for removals of phenol and COD. All reactors in FMBR system followed modified Stover Kincannonn model for pollutant degradation with maximum correlation coefficient though good correlation coefficient was achieved for Grau second order model too. Maximum phenol degradation rate (R_{max}) among all reactors was 7.09 g/L.day and achieved in B2. R_{max} for COD and nitrate was higher in R2 compared to B2 and R3 showed higher R_{max} for ammonia removal than B3 whereas both B2 and B3 showed higher R_{max} for thiocyanate removal than R2 and R3 with maximum R_{max} value of 9.17 in B3.
14. Shock loads were introduced in CMBR by sudden increase of phenol to 3000–3500 mg/L from 2500 mg/L and thereafter thiocyanate to 1000–1200 mg/L from 600 mg/L, the effect of first phenol and SCN^- shocks, on R1 was reversible (10–12 days), where

as the second shock caused more damage and the reactor achieved its normal state only after stop of feed for 2–4 days. Maximum influent phenol, COD and thiocyanate concentrations in R2 was 1770 mg/L, 5540 mg/L, (phenol shock load) and 600 mg/L (SCN^- shock load), respectively. R2 was quite robust to sustain both phenol and SCN^- shock loads and recovered within 15–22 days. The effect of phenol and SCN^- shocks of 3000 mg/L and 1000 mg/L, on R3 was negligible as influent profile to R3 was almost same. At higher phenol and thiocyanate shock, R3 severely became vulnerable for nitrification as it received instable and high influent phenol, thiocyanate and NH_4^+–N concentration and whereas phenol, COD or SCN^- removal remained unaffected.

15. Thiocyanate shock load application of 1000 and 1200 mg/L caused significant affect on phenol and COD removal efficiencies of B1. The steady effluent after 8 days of first SCN^- shock increased by 12% and 14% for phenol and COD, respectively. At phenol shock of 3000 mg/L and 3500 mg/L application to B1, effluent COD increased by 2% though effluent phenol decreased by 3% than the pre–shock condition. Though B2 regained a new stability state after first shock load application; second SCN^- application adversely affected phenol and thiocyanate removal. At phenol shock application, B2 acted in very robust way similar to R2. Both B1 and B2 regained their pre–shock efficiency after introduction of normal feed and stop for 4 days. During first thiocyanate and phenol shock application, phenol and thiocyanate removal efficiency of B3 remained unaffected though NH_4^+–N removal efficiency decreased by 24–30%. Higher shock of phenol and thiocyanate caused decrease in phenol and thiocyanate removal 20% and 28% whereas NH_4^+–N removal decreased by 71% and with stop of feed for 2–4 days also did not help B3 to regain its regular NH_4^+–N removal efficiency.
16. Presence of diverse microbes in series reactors facilitated the biodegradation of complex wastewater with phenol, thiocyanate and ammonia. From biochemical and microscopic analysis, R1 showed presence of three heterotrophic facultative anaerobic microorganisms like *Staphylococcus sp., Lactobacillus sp.* and *Streptococcus sp*. In R2 *Corynebacterium sp.* and *Citrobacter sp.,Neisseria sp.* and *Streptococcus sp.* were observed. Similarly in R3 predominant bacteria species were *Lactobacillus sp., Citrobacter sp.*, *Neisseria sp.* and *Staphylococcus sp.* In B1 the species observed were *Lactobacillus sp., Streptococcus sp.* and *Escherichia coli.* Biochemical tests confirmed

the presence of *Pseudomonas sp., Citrobacter sp., Enterobacter sp.* and *Escherichia coli* in B2 and in B3 the predominant identified bacteria were *Lactobacillus sp., Pseudomonas sp. and Citrobacter sp.*

17. When real coke oven wastewater containing some unusual compounds rather than the synthetic wastewater such as heavy metals, sulfides, oil– grease and fluorides etc with trace amount of phenol, SCN^-, pyridine spiked with synthetic pollutants, phenol (1350 mg/L), cresols (50 mg/L), SCN^- (800 mg/L), pyridine (50 mg/L), ammonia–N (500 mg/L); were added to CMBR system, phenol/COD removal in R1 decreased than synthetic wastewater treatment. Performance of R2 and R3 were same in terms of phenol/COD removal though SCN^- removal in R2 dropped immediately, R2 regained its efficiency in next 4–5 days onwards and remained static. Nitrification in R3 fallen from 71.32% to 64.28% on 5th day after addition of real wastewater and there was high nitrite accumulation up to 165 mg/L. The total phenolics, SCN^-, COD, pyridine and TN removal remained were 99.93, 99.63, 84.0, 94.5 and 81.4 %, respectively through out the study.
18. Both CMBR and FMBR system successfully brought down phenol, thiocyanate and COD to discharge levels. NH_4^+-N being most tricky pollutant were brought down to lower level of 100 mg/L or so but unable to reach the discharge level. Therefore, other conventional method such as adsorption etc can be adopted for the effluents NH_4^+-N released by the systems to reach the discharge limit.
19. Establishment and operation of the CMBR and FMBR systems include no sophisticated process or high maintenance cost. For proper functioning, the points for consideration are acclimatization of microbes to higher concentration through gradual increase of feed concentration and maintaining reactor pH. Through acclimation common bacteria from local anaerobic biogas plant and sewage treatment plant were capable of performing the required treatment activity. No mechanical device other than peristaltic pump and air compressor was utilized in the process making the system simple.

SUGESTION FOR FUTURE WORK

Based on the findings of the present investigation, the following suggestions are made for future studies

1. Aerobic reactor was observed as the most sensitive one both in continuous and fed batch system. Attempt should be made to improve efficiency of aerobic reactor either by bioaugmentation or some other technique.
2. Study of the moving bed reactor (MBR) with real industrial wastewater for field application.
3. Mathematical modeling of the multi substrate degradation and attached biomass growth in the MBR for better understanding and predictability of the system performance.
4. Microbial community analysis during long continuous operation of the bioreactor system and before and after shock load application.
5. Toxicity study on treated effluent of multi pollutant contaminated wastewater

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International peer reviewed journal

[1] B.P. Sahariah and S. Chakraborty, (2011). Kinetic analysis of phenol, thiocyanate and ammonia-nitrogen removals in an anaerobic-anoxic-aerobic moving bed bioreactor system, *Journal of Hazardous Materials* 190, 260-267.

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[5] B.J. Deka, B.P. Sahariah and S. Chakraborty, (2008). Anaerobic-anoxic-aerobic three stages attached growth system for removal of ammonia, *Journal of Environmental Research and Development* 3 (2), 442-448.

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