STUDIES ON THE USE OF THERMOALKALI TREATED RICE STRAW AS CARBON SOURCE FOR OXYANIONS REMOVAL BY BIOLOGICAL PROCESS

Thesis

Submitted to
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Of

Doctor of Philosophy

by

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Under the guidance of

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My family and supporters

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DECLARATION

I certify that

- The matter embodied in this thesis entitled **"Studies on the Use of Thermoalkali Treated Rice Straw as Carbon Source for Oxyanions Removal by Biological Process"** is the result of an investigation carried out by me at the Department of Civil Engineering, Indian Institute of Technology Guwahati, Assam, India, under the supervision of **Dr. Pranab Kumar Ghosh**.
- The work has not been submitted by any other institute for any other degree or diploma.
- I have followed the guidelines of the thesis provided by the institute in writing the thesis.
- I have confirmed the norms and guidelines given in the Ethical Code of the institute.
- Keeping with the general practice of reporting scientific observations acknowledgments has been made wherever the work of other investigations is referred.

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TH-2881_146104040

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TH-2881_146104040

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TH-2881_146104040

ABSTRACT

Rice straw is one of the most abundant, renewable lignocellulosic crop residues, biodegradable, and waste material all over the world. Burning in situ, rice straw leads to air pollution. Wastewater pollution is a significant issue. Hence it is the uttermost requirement to sustainably utilize agricultural waste i. e. rice straw as a carbon source in the biological wastewater treatment methods. With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. Oxyanions such as sulfate, nitrate, arsenate, etc. are some of the common water pollutants. However, so far the utilization of rice straw has been reported for various purposes such as bio-ethanol production, H^+ production, and methane generation. Despite having great potential, its performance on oxyanion removal has not been explored so far. In the present study, the utilization of rice straw as a carbon source in biological systems for the removal of oxyanions has been tried. The target oxyanions in this study are sulfate, nitrate, and arsenic.

The present work can be broadly divided into five major parts viz; i) collection, processing, and study on the effects of thermoalkali (here pre-treatment) methods on the hydrolysis and change in physicochemical properties of rice straw; ii) use of the raw rice straw and thermoalkali pre-treated rice straw as a carbon source in biological reduction of oxyanions in batch mode; iii) Fabrication, operation and performance evaluation of an attached growth continuous flow bioreactor on oxyanions reduction using fermented liquor of pre-treated rice straw (RS-NaOH); iv) characterization of bio solids, and v) determination of bacterial community structure in bioreactors used.

The present study demonstrates that the hot air oven pretreatment was more effective than the autoclave pretreatment in all cases based on the hydrolyzate and compositional characteristics of rice straw. Based on the hydrolysis of rice straw during the pretreatment, the optimized temperature and time were 85^{o}C and 240 min in the case of the hot air oven and 121^{o}C and 20 min in the case of the autoclave. The optimum doses of alkali were 2.5% (w/w) $Ca(OH)_2$, 1% (w/w) NaOH, and 0.4% (v/v) ChOH for both of the cases in a hot air oven and autoclave in terms of COD, TRS, and VFA production. The maximum COD, TRS, VFA %, and hydrolysis rate % in the hot air oven treatment (at 85^{o}C corresponding to 240 min) with 1% (w/w) NaOH was 44±1.8 %, 27±3.1%,

TH-2881_146104040

3±0.01%, and 26.8 % respectively. Effectiveness of reacting agents was in the sequence of NaOH > ChOH > $Ca(OH)_2$. Production of COD, TRS, and VFA from rice straw hydrolyzate and alteration in various parameters (lignocellulosic compounds, CHNS %, residual char %, increment in Cr Index %, biodegradability fraction, and hydrolysis rate %) is suggesting that rice straw hydrolyzate, as well as amorphous rice straw both of them, has enough potential to be used as a carbon source for the microorganism.

Based on batch studies it can be summarised rice straw can efficiently remove the oxyanions (single/multiple) from the wastewater containing sulfate (1000-3000 mg/L), nitrate (500-2000 mg/L), and arsenic (500-2000 µg/L) with the removal efficiency of 62-95% and 64-94 %, 89-94 % respectively within 40 days of HRT using biological process.

Performance evaluation of continuous reactor was investigated for simultaneous removal of sulfate, nitrate, and arsenic using the biological process in packed bed continuous reactor. This reactor can treat the wastewater with initial sulfate, nitrate, and arsenic concentration of 1500 mg/L, 100-1000 mg/L, and 1000-2000 µg/L leaving the final effluent concentration in the range of 150-524 mg/L, 20-140 mg/L, and 54-230 µg/L respectively. This reactor and the process adopted in this research work are suitable for industrial application and for treating wastewater containing sulfate, nitrate, and arsenic.

The characterization of bio solids from reactors SA3, SAN3, and PBCR confirmed the bio precipitation as As-S, As_2S_3, and As_4S_3 were the removal mechanisms of arsenic and sulfate. FETEM analysis suggested the formation of stable bio-sludge in the bioreactor, which is of stable nature, thus the sludge-handling problem can be expected to be low. The belt structure showed distinctive clear lattice fringes with a d-spacing of 0.299 nm, 0.32 nm, and 0.27 nm of bio solids from SA3, SAN3, and attached growth reactor (PBCR), respectively. The crystalline nature of the obtained precipitates suggests the stable nature of bio solids which ensures their safe disposal in a landfill.

Metagenomic studies (16S rRNA analysis, TRFLP test, and Taxonomic profiling) of the raw paper mill sludge and reactors SN3, SA3, and SAN3, confirm the presence of sulfate, nitrate, and arsenic removing microorganisms.

Overall, rice straw was found to be efficient enough for treating the sulfate, nitrate, and arsenic polluted wastewater in batch and continuous reactors.

TH-2881_146104040

Keywords: Arsenic; Arsenosulfide; Rice Straw; Nitrate; Orpiment; Realgar; Thermoalkali Treatment, Sulfate

TH-2881_146104040

List of Abbreviations/Annotations

SN	Symbols	Full Form
1	AC	Acclimatization Reactor
2	As	Arsenic
3	As-S	Arseno-Sulfide
4	AAS	Atomic Absorption Spectroscopy
5	APHA	American Public Health Association
6	BOD_5^{20}	Biological Oxygen Demand
7	COD	Chemical Oxygen Demand
8	ChOH	Choline Hydroxide
9	C	Carbon
10	^oC	Degree Celsius
11	CO_2	Carbon Dioxide
12	gm	Gram
13	g/L	Gram per Litre
14	EDX	Energy Dispersive X-Ray Spectroscopy
15	FESEM	Field Emission Scanning Electron Microscopy
16	FETEM	Field Emission Transmission Electron Microscope
17	FTIR	Fourier Transmission Infrared Spectroscopy
18	H, H^+	Hydrogen, Hydrogen Ion
19	HRT	Hydraulic Retention Time
20	HRTEM	High Resolution Transmission Electron Microscopy
21	h	Hour
22	λ	Wavelength
23	IFFT	Inverse Fast Fourier Transform
24	L	Litre
25	min	Minute
26	mL	Milliliter
27	mg/gm	Milligram/ Gram
28	mg/L	Milligram/ Litre
31	MLSS	Mixed Liquor Suspended Solids
32	MLVSS	Mixed Liquor Volatile Suspended Solids
33	NO_3^-	Nitrate
34	PBCR	Packed Bed Continuous Reactor
35	PCR	Polymerase Chain Reaction

TH-2881_146104040

36	PUF	Polyurethane Foam
37	RNA	Ribose Nucleic Acid
38	RPM	Revolution per Minute
39	RS-WTA	Without Thermoalkali Treated, Rice Straw Slurry
40	RS-$Ca(OH)_2$	$Ca(OH)_2$ (2.5% w/w) Treated Rice Straw Slurry
41	RS-NaOH	NaOH (1 % w/w) Treated Rice Straw Slurry
42	RS-ChOH	ChOH (0.4% v/v) Treated Rice Straw Slurry
43	R1	Batch Reactor Operated at pH 3 using RS-WTA as Carbon Source [Sulfate 2000 mg/L]
44	R2	Batch Reactor Operated at pH 5 using RS-WTA as Carbon Source [Sulfate 2000 mg/L]
45	R3	Batch Reactor Operated at pH 7 using RS-WTA as Carbon Source [Sulfate 2000 mg/L]
46	R4	Batch Reactor Operated at pH 9 using RS-WTA as Carbon Source [Sulfate 2000 mg/L]
47	R- $Ca(OH)_2$	Batch Reactor Operated using RS-$Ca(OH)_2$ as Carbon Source [Sulfate 2000 mg/L]
48	R-NaOH	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L]
49	R-ChOH	Batch Reactor Operated using RS-ChOH as Carbon Source [Sulfate 2000 mg/L]
50	S1	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 1000 mg/L]
51	S2	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L]
52	S3	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 3000 mg/L]
53	N1	Batch Reactor Operated using RS-NaOH as Carbon Source [Nitrate 500 mg/L]
54	N2	Batch Reactor Operated using RS-NaOH as Carbon Source [Nitrate 1000 mg/L]
55	N3	Batch Reactor Operated using RS-NaOH as Carbon Source [Nitrate 2000 mg/L]
56	SN1	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 500 mg/L]

TH-2881_146104040

57	SN2	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 1000 mg/L]
58	SN3	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 2000 mg/L]
59	SA1	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Arsenic 500 µg/L]
60	SA2	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Arsenic 1000 µg/L]
61	SA3	Batch Reactor Operated using RS-NaOH as Carbon Source [Sulfate 2000 mg/L; Arsenic 2000 µg/L]
62	SAN1	Batch Reactor Operated using RS-ChOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 500 mg/L; Arsenic 2000 µg/L]
63	SAN2	Batch Reactor Operated using RS-ChOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 1000 mg/L; Arsenic 2000 µg/L]
64	SAN3	Batch Reactor Operated using RS-ChOH as Carbon Source [Sulfate 2000 mg/L; Nitrate 2000 mg/L; Arsenic 2000 µg/L]
65	S0N0	Batch Reactor Operated using RS-NaOH as Carbon Source [Without External Addition of Oxyanions]
66	CR	Batch Reactor Operated without Rice Straw [Sulfate 2000 mg/L; Nitrate 2000 mg/L; Arsenic 2000 µg/L]
67	SO_4^{2-}	Sulfate
68	SAED	Selected Area Electron Diffraction
69	SRB	Sulfate Reducing Bacteria
70	T-RFLP	Terminal Restriction Fragment Length Polymorphism
71	TGA	Thermo gravimetric Analysis
72	TRS	Total Reducing Sugar
73	VFA	Volatile Fatty Acids
74	w/v	Weight/ Volume
75	v/v	Volume/ Volume
76	XRD	X-Ray Powder Diffraction

TH-2881_146104040

List of Content

TH-2881_146104040

TH-2881_146104040

TH-2881_146104040

TH-2881_146104040

List of Figure

TH-2881_146104040

TH-2881_146104040

TH-2881_146104040

TH-2881_146104040

List of Table

TH-2881_146104040

CHAPTER 1

INTRODUCTION

Rice straw is one of the most abundant, renewable lignocellulosic crop residues, biodegradable, and waste material all over the world. Burning in situ, rice straw leads to air pollution. Wastewater pollution is a significant environmental problem. With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. Sulfate, nitrate, arsenate, etc. are some of the common water pollutants. Hence it is the uttermost requirement to sustainably utilize agricultural waste i. e. rice straw as a carbon source in the biological wastewater treatment methods.

1.1. Rice Straw Production and its Management

Rice straw is one of the most abundant, renewable lignocellulosic crop residues, biodegradable, and waste material all over the world. Rice straw is produced as a byproduct of rice production at harvest. For every kg of rice grain obtained from the field, 1 to 2 kg of straw is produced (Ghasemi et al., 2013). Rice straw has become a major source of environmental and social problems. It is traditionally disposed of, by burning in situ, causing real harmful environmental implications through producing huge black clouds of smoke, the production of CO_2, that is, causing serious environmental and safety problems, such as air pollution and fire disaster.

1.2. Oxyanions and Conventional Removal Methods

Wastewater pollution is a significant environmental problem. With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. Oxyanions such as sulfate, nitrate, arsenate, etc. are some of the common water pollutants. Sulfate, nitrate, and arsenic may come into the environment from various industrial sources such as tannery industries, nuclear industries, and semiconductor manufacturing industries respectively (Bhatnagar and Sillanpää, 2011; Boshoff et al., 2004; Sarkar and Paul, 2016). There are various harmful impacts of this oxyanion on the environment.

TH-2881_146104040

Several treatment technologies are available for the removal of oxyanions. The conventional water treatment process is used for the removal of inorganic pollutants through physical, chemical, and biological processes. The physical and chemical treatment process involves coagulation and precipitation, adsorption, ion exchange, etc. Membrane separation processes such as reverse osmosis (RO), nano-filtration (NF), ultra-filtration (UF), microfiltration (MF), dialysis (D), Donnan dialysis (DD) and electrodialysis (ED), foam flotation, and solvent extraction are also practiced. Conventional water treatment processes, such as coagulation and precipitation, are very effective but limited due to ferric or alum dose applications and the requirement of frequent backwashes when high coagulant doses are used. In the chemical oxidation and adsorption process, efficient control of the pH, temperature, and oxidation step is required. Similarly in physical membrane processes, inorganic anions cannot be destroyed and have been argued as costly solutions. Recently, reverse osmosis and ion exchange have been demonstrated in the simultaneous removal of multiple contaminants (Matos et al., 2006). However, high energy input and post-treatment demand make it an unreliable solution to be used as a scale-up solution (Nerenberg and Rittmann, 2004).

The biological reduction is most appreciable due to its low capital cost, and it offers selective removal of the target anion from water using anoxic bacteria. These microorganisms grow under appropriate micro environmental conditions (pH, oxidation-reduction potential, temperature, etc.) by utilizing anions as electron acceptors and organic (heterotrophic microorganisms) or inorganic (autotrophic microorganisms) compounds as electron donors. Electron donors serve as carbon sources for microorganism for their growth. They should be having low-molecular-weight organic compounds. In most cases, synthetic organic compounds have been used as electron donors, especially small molecular weight compounds, such as lactate, acetate, propionate, pyruvate, and butyrate (Harada et al., 1994; Okabe and Characklis, 1992; Visser et al., 1993). These compounds are known to be fermented products recovered from carbohydrates, proteins, and other constituents of dead biomass by using anaerobic bacterial degradation (Widdel et al., 2007). These carbon sources are a costlier solution for treating wastewater in large quantities. Thus there is a need for a sustainable treatment solution.

TH-2881_146104040

1.3. Rice Straw as an Alternative Carbon Source

As rice straw primarily contains cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%) and the ash content of the straw is (10–17%), its utilization has been reported for various purposes such as bio-ethanol production, H^+ production, and methane generation. Rice straw (waste biomass) can be converted into these valuable products (biofuel/bioethanol/H+) by adopting thermochemical or biological processes (Chang et al., 2011; Lei et al., 2010; Yoswathana et al., 2010). In biological processes, bacteria convert the rice straw or thermochemically treated rice straw into various products through anaerobic digestion and fermentation. Anaerobic digestion of rice straw is a well-established technology and practiced worldwide, for the production of biofuels/other valuable products, in which organic waste matter is converted to energy forms with the assistance of microbes. To enhance the digestion process of rice straw term alkali treatment can be beneficial. Therefore the lacuna found after a thorough literature survey is that, no studies have been carried out on the use of rice straw as a carbon source for the removal of oxyanion sulfate, nitrate, and arsenic.

1.4. Thesis Organization

The whole thesis is subdivided into different modules named as chapters dealing with the key components of research such as “Introduction”, “Literature survey”, “Materials and Methods”, “Analytical Procedure”, “Results and Discussion”, “Conclusions and Scope of Future Studies” etc. A separate materials and methods chapter has been added to describe general key methods and materials, which were commonly applied in almost all the objectives and research activities. Nevertheless, each objective and research activities have its specific materials and methods section, which are provided in the corresponding chapters.

Chapter 1: Introduction

This chapter gives a brief description of rice straw production and management issues. Burning in situ, rice straw leads to air pollution. Wastewater pollution is a significant environmental problem. With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. Oxyanions such as sulfate, nitrate, arsenate, etc. are some of the common water pollutants. The biological processes serve as a very effective and economical method as

TH-2881_146104040

compared to the costs and additional units required when physicochemical methods are employed. However, the main drawback of existing biological treatment systems is the cost of carbon sources. Thus to minimize the cost of carbon sources, the utilization of agricultural waste (rice straw) can become a sustainable solution for treating wastewater. Hence it is the uttermost requirement to sustainably utilize agricultural waste i. e. rice straw as a carbon source in the biological wastewater treatment methods. All this information is highlighted in this section.

Chapter 2: Literature Review

This chapter includes a detailed description of the worldwide production of rice straw and its management, the burning of rice straw, and its harmful environmental impact. Rice straw composition, why thermochemical/alkali treatment of rice straw is important before using it as a carbon source for microorganisms, how rice straw is useful for producing various valuable products, oxyanion, and their conventional treatment options and comparison of these methods. Furthermore, how rice straw can be useful as an alternative source of carbon source for the removal of oxyanions in the biological process. A summary of the literature and research needs is given in the last part of this chapter.

Chapter 3: Aim and Scope of Study

This chapter deals with the aim and scope of the study of this project work. The main objective of this research work is to utilize the rice straw as a carbon source for simultaneous removal of sulfate, nitrate, and arsenic from the contaminated wastewater by biological process under anaerobic conditions in batch and continuous reactor. Mechanisms of oxyanion removal were investigated through their characterization.

Chapter 4: Materials and Methods

A general discussion on the materials used and methodologies adopted have been decorated in this chapter to overview the instrumentation techniques and key analysis of the materials used to achieve different objectives of this research project.

This chapter deals with the analytical procedure and protocols used in this project work. Various materials procured from authentic sources and the development of new materials adopting self-developed techniques or adopted from highly cited literature to accomplish individual objectives have been systematically arranged in this chapter.

TH-2881_146104040

Methods related to system analysis, for example, hydrolyzate characteristics (COD, TRS, and VFA), wastewater characteristics [pH, COD, BOD, VFA, sulfate (SO_4^{2-}), sulfide (S^{2-}), nitrate (NO_3^-), nitrite (NO_2^-), and arsenic (As)], rice straw characteristics (cellulose, hemicellulose, lignin, surface area, FTIR, FESEM, XRD, TGA, CHNS %, Biodegradability fraction, Hydrolysis rate %), bio solids characterization (FESEM, XRD, EDX, and FETEM), and microbial characterization (using metagenomic studies; 16S rRNA, TRFLP analysis, Taxonomic profiling) of bio sludge have been elaborated to cover a maximum possible area of working principles used in the research activities.

Chapter 5: Results and Discussion

In the doctoral thesis, discussions with supporting figures and tables on the experimental results have been arranged in this chapter.

Chapter 6: Conclusion and Scopes of Future Study

The key conclusion and future scope drown from the present study are included in this chapter.

Apart from all the chapters, this thesis contains an abstract of the results of whole research activities carried out to accomplish the specific objectives and has been placed before all the chapters. The summary and conclusions obtained from various research activities can be found at the end of the chapters. Other necessary research elements such as bibliography, short forms, appendix (if any), units, etc. have been placed in appropriate places in the thesis.

TH-2881_146104040

TH-2881_146104040

CHAPTER 2

LITERATURE REVIEW

2.1. Oxyanions in the Environment

Oxyanions are the most common environmental problem. With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. Oxyanions such as sulfate, nitrate, arsenate, etc. are some of the common water pollutants. Sulfate, nitrate, and arsenic may come into the environment from various industrial sources such as tannery industries, nuclear industries, and semiconductor manufacturing industries respectively (Bhatnagar and Sillanpää, 2011; Boshoff et al., 2004; Sarkar and Paul, 2016). There is various harmful impact of these oxyanions on the environment.

2.2. Permissible and Discharge Limit of Oxyanions and Their Conventional Treatment Methods

Surface water quality standard for sulfate is 400 mg/L and 1000 mg/L for class C and class E respectively, for nitrate is 50 mg/L (class C) and class E (not specified) and for arsenic is 0.2 mg/L (class C) and class E (not specified) (IS: 2296, 2015), where class C is the source of drinking water with conventional treatment followed by disinfection and class E is the water for irrigation, industrial cooling, and controlled waste disposal.

The conventional treatment methods of oxyanion removal are physicochemical or biological. The physicochemical methods have several limitations such as high energy input and post-treatment demands, high volume of pollutant bearing unstable solids, frequent addition of chemicals, and efficient control of temperature and pH (Nerenberg and Rittmann, 2004).

2.3. Use of Various Carbon Sources for Oxyanions Removal

Biological treatment methods depend greatly on the carbon source, which is the sole source of energy and growth for the micro-organism. In this section, various types of carbon sources for oxyanions removal, their performances, limitations, and conclusive remarks on the acceptability of lignocellulosic waste material as a carbon source have been discussed.

TH-2881_146104040

The use a high-cost carbon source (synthetic carbon source) has been successfully employed for wastewater treatment. For example, Tan et al. (2018) reported 15 % sulfate, 79 % nitrate, and 61 % Se removal with an initial concentration of 1.5 g/L sulfates, 0.047 g/L nitrates, and 5-12 mg/L Se using lactate at HRT of 24 h in UASB at pH 5.5. Chung et al. (2006) reported 79 % sulfate, 100 % nitrate, and 90 % Se with initial 0.079 g/L sulfate, 0.005 g/L nitrate, and 0.55 mg/L Se using H_2 at HRT of 24 h in biofilm from the pilot plant at pH 7-9. Teclu et al. (2009) reported 36-49 % sulfate and 92-99 % As removal with the initial concentration of 0.175 g/L sulfate and 0.1-20 mg/L As using molasses in a batch reactor at pH 6.4.

Apart from this various low-cost carbon sources (lignocellulosic waste material) have also been employed for wastewater treatment. Greben et al. (2009) reported 84-91 % sulfate removal with the initial concentration of 0.175 g/L sulfate using grasses in a batch reactor at pH 6.5. Boshoff et al. (2004) reported 60-80 % sulfate removal with the initial concentration of 1.8 g/L sulfate using tannery effluent in UASB and stirred tank reactor (STR).

Neculita and Zagury (2008) reported >99 % sulfate and heavy metals removal Mn, (96.9) Ni (99.8), Cd (99.8), Zn (99.8), Fe (99.8) % with the initial concentration of 55±0.25 g/L sulfate along with heavy metals Mn (13.5), Ni (16.8), Cd (12.6), Zn (18.9), Fe (1670) mg/L using mixtures of waste materials (wood chips, maple, poultry, leaf compost) in a passive batch reactor at pH 5.4 of AMD. The performance of various high-cost and low-cost carbon sources for the removal of various pollutants are shown in table 2.3.1 and 2.3.2 respectively.

TH-2881_146104040

Table 2.3.1. Performance of the various high-cost carbon sources for the removal of various pollutants

SN	Carbon Source	Inoculum	Reactor Type	HRT (h)	pH_i	pH_f	Oxyanions			Heavy Metal		Reference
							Conc. (g/L)	Removal Rate (g/L/d)	Removal (%)	Conc. (mg/L)	Removal (%)	
							Sulfate	**Sulfate**	**Sulfate**			
1	Acetate and Peptone	Sludge from the wastewater treatment plant	CSTR	48-90	8.0	-	5	0.17-1.4	-	-	-	(Moosa et al., 2005)
2	Synthesis gas-fed	THIOPAQ granular sludge	GLB	1-3	7.3	-	5-30	15	88	-	-	(van Houten et al., 2006)
3	Ethanol	Mixed SRB	FBR	5.1	7	-	0.06	6.33	-	-	-	(Nagpal et al., 2000a)
4	H_2/CO_2	Neotrophilic digester sludge	MBR	18	5.0	-	4.32	5	99	-	-	(Bijmans et al., 2008)
5	Lactate	Water from the wetland filter of a mine site	Packed-bed	16.2	4.5	7	2.5	0.48	>82	Cu > (10) Zn > (10) Ni > (10) As > (10) Fe > (10)	Cu > (97) Zn > (97) Ni > (97) As > (77) Fe > (82)	(Jong and Parry, 2003)
6	Lactate	SRB enriched from wetland	UASB	35.5, 16,10	4.0	6.7	3.04	0.053-0.1	62-73	-	-	(Jong and Parry, 2006)

TH-2881_146104040

7	Methanol	Spent manure	Packed-bed	6.6	3.5	6.5	2.5	1.608	>95	Fe (100)	Fe (98)	(Tsukamoto and Miller, 1999)
8	Ethanol, Lactate, Glycerol	Derelict mine sites	Packed-bed	49.3	4.0	>6.5	1.44	0.25-0.3	-	-	-	(Kolmert and Johnson, 2001)
9	Ethanol	Sludge and mine sediment	FBR	6.5-21	3.0-3.2	-	2	2.2-4.3	-	Zn (200) Fe (100)	Zn >99 Fe >99	(Kaksonen et al., 2004)
10	Lactate	Sludge and mine sediment	UASB and FBR	16	2.5-3	7.4-8.5	1-2.2	-	35-95	Zn (350) Fe (57)	Zn >99 Fe >99	(Kaksonen et al., 2003)
11	Acetate, Lactate	Disintegrated granular sludge	Down flow FBR	24	4-6	-	2	0.42-1.5	-	-	-	(Montoya et al., 2013)
12	Methanol	Water from lignite mine	Packed-bed	12, 4.2	3.0	6.9	1.9-2.1	3.12	-	Fe (90) Al (40) Zn (1) Ni (0.3)	Fe >99 Al >99 Zn >99 Ni >99	(Glombitza, 2001)
13	Molasses	SRB	Batch	336	6.4	7.1	0.175	-	36-49	As (0.1-20)	As (92-99)	(Teclu et al., 2009)
14	Lactate	Mixed culture from mine tailing slurry	Batch	336	6.9	8.03	1.8	-	44	As (5) Sb (5) Fe(II) (200)	As (78-98) Sb (98-99) Fe(II) (65)	(Liu et al., 2018a)

TH-2881_146104040

15	Ethanol	Biomass from UASB reactor treating paper industry wastewater	UASB	0.5-1	3.5-4	8.0	2-2.5	4	88-98	As (0-10) Fe (100-300) Cu (10-30) Ni (5-15) Zn (25-50)	As (98-100) Fe (99) Cu (99) Ni (99) Zn (99)	(Sahinkaya et al., 2015)
16	Ethanol	SRBs	Column	4800	6.5	7	1	-	47-60	As (1) Se (0.2)	As (80-99) Se (90-97)	(Luo et al., 2008)
							Nitrate	**Nitrate**	**Nitrate**			
17	Glucose, Acetate, Citrate	Municipal sludge	Batch	96	-	-	0.351-0.386	0.12-0.20	80-94	-	-	(Yang et al., 2012)
18	Glucose	Soil	Batch	80	5-9	-	-	0.021	-	-	-	(Seenivasagan et al., 2017)
	Starch							0.022				
	Cellulose							0.017				
19	Starch	Groundwater and soil samples	Batch	72		-	0.5	0.7	86-99	-	-	(Ayyasamy et al., 2007)
							Sulfate + Nitrate	**Sulfate + Nitrate**	**Sulfate + Nitrate**			
20	Lactate	Anaerobic bacteria from UASB	UASB	24	5	7.4	Sulfate (1.5) Nitrate (0.047)	-	Sulfate (15) Nitrate (79)	Se (5-12)	Se (61)	(Tan et al., 2018)

TH-2881_146104040

21	H_2	Biofilm from pilot plant treating groundwater	H_2-MBfR	24	7-9	-	Sulfate (0.079) Nitrate (0.005)	-	Sulfate (79) Nitrate (100)	Se (0.55)	Se (90)	(Chung et al., 2006)
22	Ethanol	Digested sewage sludge	Bench-scale EGSB	15-22	8	-	Sulfate (1-3.6) Nitrate (0.3-1.2)	-	Sulfate (80) Nitrate (94)	-	-	(Liao et al., 2014)

UASB (Upflow Anaerobic Sludge Blanket), CSTR (Continuous Stirred Tank Reactor), EGSB (Expanded Granular Sludge Bed) Membrane-Biofilm Reactor (MBfR), Membrane Bioreactor (MBR), FBR (Fluidized Bed Reactor), GLB (Gas-Lift Bioreactor), pH i (Initial) and pH f (Final)

TH-2881_146104040

Table 2.3.2. Performance of the various low-cost carbon sources for the removal of various pollutants

SN	Carbon Source	Inoculum	Reactor Type	HRT (d)	pH_i	pH_f	Oxyanions			Heavy Metal		Reference
							Conc. (g/L)	Removal Rate (mg/L/d)	Removal (%)	Conc. (mg/L)	Removal (%)	
							Sulfate	**Sulfate**	**Sulfate**			
1	Softwood chippings, manure, anaerobically digested sludge, methanol	SRB	Column	0.5-0.6	3	7.4	0.200	-	35-95	Pb (5) Cu (0.5) Zn (5)	Pb (>99) Cu (>99) Zn (>99)	(Mayes et al., 2011)
2	Composted mixture of manure, hay, straw, corn cobs, wood chips	NR	Pilot scale continuous bioreactor	5-17	-	-	1.002	-	17	Al (7) Cd (0.3) Mn (26) Ni (0.9) Zn (317)	>95	(Dvorak et al., 1992)
3	Wood chips, maple, poultry, leaf compost	SRB from AMD	Passive batch	120	5.4	8.7	55±0.25	80-86	>99	Mn (13.5) Ni (16.8) Cd (12.6)	Mn (96.9) Ni (99.8) Cd (99.8) Zn (99.8) Fe (99.8)	(Neculita and Zagury, 2008)

TH-2881_146104040

										Zn (18.9) Fe (1670)		
4	Wood chips, leaf compost, poultry manure, oxidized mine tailings, and silica sand	SRB from sediment	Batch	41	5.5 -6	7.9± 0.1	2.020 -3.254	35.6-87.6	>99	Ni (0.8) Zn (7.3)	Ni (51–84) Zn (73–93)	(Cocos et al., 2002)
5	Sewage sludge, leaf mulch, wood chips, sheep manure, sawdust cellulose	Creek sediment	Batch	40-70	<6	6.5-7	1.2-4.8	0.14-4.23	>99	Fe (350-600) Ni (480) Cd (135) Zn (2.3)	Fe (> 90) Ni (>99) Cd (>99) Zn (>99)	(Waybrant et al., 1998)
6	Maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure, and	Creek sediment	Batch	70	8-8.5	7.1-8.8	2.944-4.608	5.5	-	Fe (1683 ± 35) Mn (14 ± 1) Cd (8.3 ± 2)	Fe (93-100) Mn (98-99) Cd (97–99) Ni (92–99) Zn (92-94)	(Zagury et al., 2006)

TH-2881_146104040

	conifer sawdust									Ni (15 ± 1) Zn-15 ± 3		
7	Mushroom compost [composted straw/ hay, horse, poultry, and cow manure, ground corncobs, gypsum, limestone (mix)]	SRB	Column	0.5	-	-	2	-	75	Fe (620) Cu (178) Zn (530) Al (278) Mn (191)	Fe >99 Cu >99 Zn >99 Al >99 Mn >91	(Hammack et al., 1994)
8	AMD	SRB from fresh cow manure	Bench scale	10	2.7 - 3.3	7.1-7.5	4.1-4.2	-	No Removal	Cu (27.83 - 29.93) Fe (183-192) Zn (23-25) Ni-(10.4-11.9)	Cu (91-97) Fe (52-99) Zn (35-96) Ni (17-97) Co (66-99) Mn (17-73)	(Choudhary and Sheoran, 2011)
	Cow manure								47			
	Sugarcane								23			
	Goat manure								54			
	Buffalo manure								53			
	Babool woodchips								20			
	Mango woodchips								22			
	Babool sawdust								24			
	Mango sawdust								23			

TH-2881_146104040

	Pearl millet fodder								16	Co (1.2-1.4) Mn (32.4-34.4)		
	Proso millet fodder								26			
9	Compost yard waste	SRB	Constructed wetland system	4-10	5.2	7.4	0.82	-	69.5	Cu-7.3 Ni-24.5 Co-1.2 Zn-1.4	Cu >98 Ni >99 Co > 96 Zn >95	(Eger, 1994)
	Manure, municipal sawdust compost								23			
	Horse manure, sawdust								29			
	Municipal compost waste, sawdust								54.3			
10	Food industry waste (lactose, cheese whey)	SRB from wastewater sludge	Batch	60	7	7.1	2-5	-	80-98	-	-	(Martins et al., 2009)
11	Tannery effluent	SRB	UASB and stirred tank reactor (STR)	-	-	7.5	1.8	400-600	60-80	-	-	(Boshoff et al., 2004)
12	Whey (mix)	SRB from cow manure	Bench scale	203	5.2	-	3.307	-	19-27	-	-	(Christensen et al., 1996)

TH-2881_146104040

13	Liquid whey	SRB from potable mineral water	Batch	17.7	5.5	7.1	1.6	-	23		-	(Luptakova and Macingova, 2012)
	Solid whey		Batch	17.7	5.5	NR	1.6	-	0	-	-	
14	Rice husk filtrate	SRB (*Dsm. Nigrificans*)	Batch	36	1	NR	0.55-1.2	NR	64-81	Fe (50-75)	85	(Chockalingam et al., 2005)
15	Cellulosic biomass (switch grass, sugarcane bagasse)	*Cellulose* Enzyme	Batch	-	2.1	5.4	NR	NR	NR	Fe (500)	62	(Magowo et al., 2015)
16	Grasses	Rumen microbes	Batch	78	6.5	7.6	2-6	364-1050	84-91	-	-	(Greben et al., 2009)
							Nitrate	**Nitrate**	**Nitrate**			
17	Wheat straw	Forest and garden soils	Batch	60	-	-	0.022	53	-	-	-	(Soares and Abeliovich, 1998)
18	Woodchips sawdust	Soil	Batch	80	6.5-7		0.15	29-140	-	-	-	(Hu et al., 2019)
19	Maize cops	NR	Denitrification beds	1.3-2.25	-	-	0.141-0.159	15-19	-	-	-	(Cameron and Schipper, 2010)
	Green waste							7.8-10.5				
	Wheat straw							5.8-7.8				
	Softwood							3-4.9				
	Hardwood							3.3-4.4				
20	Biodegradable polymer	Denitrifiers	Packed-bed bioreactor	0.04-0.08	-	-	0.05	640	-	-	-	(Shen et al., 2013)
21	Plant biomass	Gravel	Subsurface-flow constructed wetlands	5	-	-	0.05	64.8	-	-	-	(Wen et al., 2010)

TH-2881_146104040

22	Arundo donax, Glycyrrhiza glabra, Gracilaria verrucosa	NR	Batch	0.2-0.8	-	-	0.1	3-13	87-100	-	-	(Ovez, 2006)
							Sulfate + Nitrate	**Sulfate + Nitrate**	**Sulfate+ Nitrate**			
23	Control	Microbes	Batch	2.9	-	-	Nitrate (0.057) Sulfate (0.12)	Nitrate (0.02) Sulfate (0.01)	-	-	-	(Chang et al., 2016)
	Carnation straw							Nitrate (2.1) Sulfate (2.08)				
	Rose straw							Nitrate (1.2) Sulfate (1.03)				
	Lily straw							Nitrate (1.83) Sulfate (2.16)				
	Violet straw							Nitrate (1.42) Sulfate (1.3)				

UASB (Upflow Anaerobic Sludge Blanket), pH_i (Initial) and pH_f (Final), NR-Not Reported

TH-2881_146104040

Although, conventional high-cost carbon sources such as lactate, acetate, ethanol, and hydrogen (Chung et al., 2006; Jong and Parry, 2003; Montoya et al., 2013; Sahinkaya et al., 2015) are profound options for the oxyanion removal process. These compounds are known to be fermented products recovered from carbohydrates, proteins, and other constituents of dead biomass by using anaerobic bacterial degradation (Widdel et al., 2007). There are some limitations of these carbon sources. Hydrogen is an attractive electron donor for sulfate reduction. When H_2 is used as an electron donor, CO_2 must also be added to supply carbon for SRB. However, adding CO_2 normally leads to a decrease in the pH of the system because of the formation of carbonic acid, especially during the start-up period. Attention is required to prevent acidification (Van Houten et al., 1994). *Desulfovibrio* species growing on hydrogen seem to require at least an organic C_2-compound such as acetate in addition to carbon dioxide for cell synthesis (Widdel, 1988). Methanol is of particular interest as an electron donor because it is readily available and cost-effective. The growth of the sulfate reducers on methanol is slow, with a doubling time of around a day or more, compared with the growth of special methane bacteria or homoacetogens (Weijma and Stams, 2001). Methanol has been found to provide low rates of sulfate reduction, is not suitable for complete reduction of sulfate, and creates excessive methane formation under mesophilic conditions. Weijma et al. (2003) concluded that more than 90% of methanol used was converted to methane, whereas only 5–10% was used for sulfate reduction. Ethanol is another attractive electron donor. A sulfate conversion efficiency as high as 80% has been achieved at high sulfate loading rate values while using ethanol as an electron donor (Barnes et al., 1991). The complete oxidation of ethanol to CO_2 using SRBs alone was reported using cultures of *Desulfovibrio desulfuricans* and *Desulfobacter postgatei* (Nagpal et al., 2000b). However, a drawback of using this electron donor is a rather low growth rate of SRB on ethanol. The growth of SRB is inhibited by undissociated H_2S to a greater extent than the growth of hydrogenotrophic (Van Houten et al., 1994). The use of ethanol produces acetate which increases the COD of the effluent. Methanol is of particular interest as an electron donor because it is readily available and cost-effective. Molasses is widely available in large quantities from sugar-producing processes (Hilton and Archer, 1988). It has been used as an electron donor in sulfate reduction. Because of its low cost and ready availability, molasses is one of the most cost-effective electron donors. Another major drawback of using molasses is the high volatile fatty acid (VFA; acetate, propionate, and butyrate) content in the reactor. VFAs create a reactor souring

TH-2881_146104040

problem and negatively impact the growth of both methanogens and sulfate reducers (Lo et al., 1990). Addition of NaOH or $NaHCO_3$ is necessary to prevent acidification. Also, the quality of the effluent in terms of residual COD and color, must be considered when molasses is used. Lactate has been assessed as an organic substrate for enrichment of sulfate reducers (Zindel et al., 1988). However, complete lactate oxidation is not achieved by most *Desulfobacter* species and some *Desulfobacterium* species. *Desulfonema magnum* does not grow on lactate (Widdel, 1988). Acetate can be used as an electron donor and carbon source in the sulfate reduction process. Acetate production during the biological sulfate reduction is actually a major drawback of sulfate reducing reactors because SRB cannot completely oxidize acetate even with excess sulfate levels (Lens et al., 2002). The acetate remaining in the effluent contributes largely to the residual COD (Widdel, 1988). The gap identified in their research is that these carbon sources are a costlier solution for treating the wastewater in large quantity. Thus there is a need for a sustainable treatment solution to minimise the cost of treatment.

The research hypothesis is to replace the carbon source in the oxyanion removing biological system because it is important to find out an alternative carbon source or the electron donor for the microorganism. Substantial reduction in operating cost can be achieved by the application of wastes or by-products as an external carbon source for oxyanion removal. Furthermore, the disposal of agricultural wastes poses great economical and ecological problems. It should be emphasized that the utilization of these wastes not only avoids the use of an expensive refined carbon source but also replaces it with refuse, which is burdening our environment. A significant fraction of the operating cost of a sulfate reduction/denitrification unit is incurred by the carbon source. Hence, the policy of using alternative carbon sources (lignocellulosic waste material) is recommended. These carbon sources are mainly agricultural wastes, industrial effluents, or by-products. Since most of these carbon sources are complex carbon compounds. Hence, proper pretreatment which includes thermal hydrolysis, acid/base hydrolysis, and enzymatic hydrolysis is required which would enhance the digestion. The lab scale studies are needed to check the feasibility and sustainability of the process with alternative carbon sources for oxyanion removal.

TH-2881_146104040

2.4. Worldwide Production of Rice Straw and its Management

Rice straw is one of the most abundant renewable lignocellulosic crop residues, considered a waste material all over the world. Rice straw is produced as a byproduct of rice production at harvest. According to the reports, the total annual production of rice straw is around 227.78 Million tons worldwide, out of this, India produces around 60.08 Million tons (Sarnklong et al., 2010). For every kg of rice grain obtained from the field, 1 to 2 kg of straw is produced (Ghasemi et al., 2013). Worldwide production of (a) Rice straw (b) Rice husk (c) Rice is shown in figure 2.4. (i) (Sarnklong et al., 2010). Rice growing states in India are shown in figure 2.4. (ii), West Bengal is the state with the leading producer of rice (15.3 Million tons) in India, followed by the U.P. with 13.75 MT. The management of such a huge amount of rice straw has become a major challenge. Most farmers rely on the in situ burning of straw which creates numerous ecological, economic, environmental, and health issues. Uncontrolled burning of rice straw causes produces oxides of sulfur, CO_2, CO, and hydrocarbons and their accumulation to form black clouds of smoke that leads to air pollution and fire disaster (shown in figure 2.4. (iii)). Hence it is the uttermost requirement to sustainably utilize the unused rice straw. One of its uses may be as a carbon source in the biological process of wastewater treatment.

TH-2881_146104040

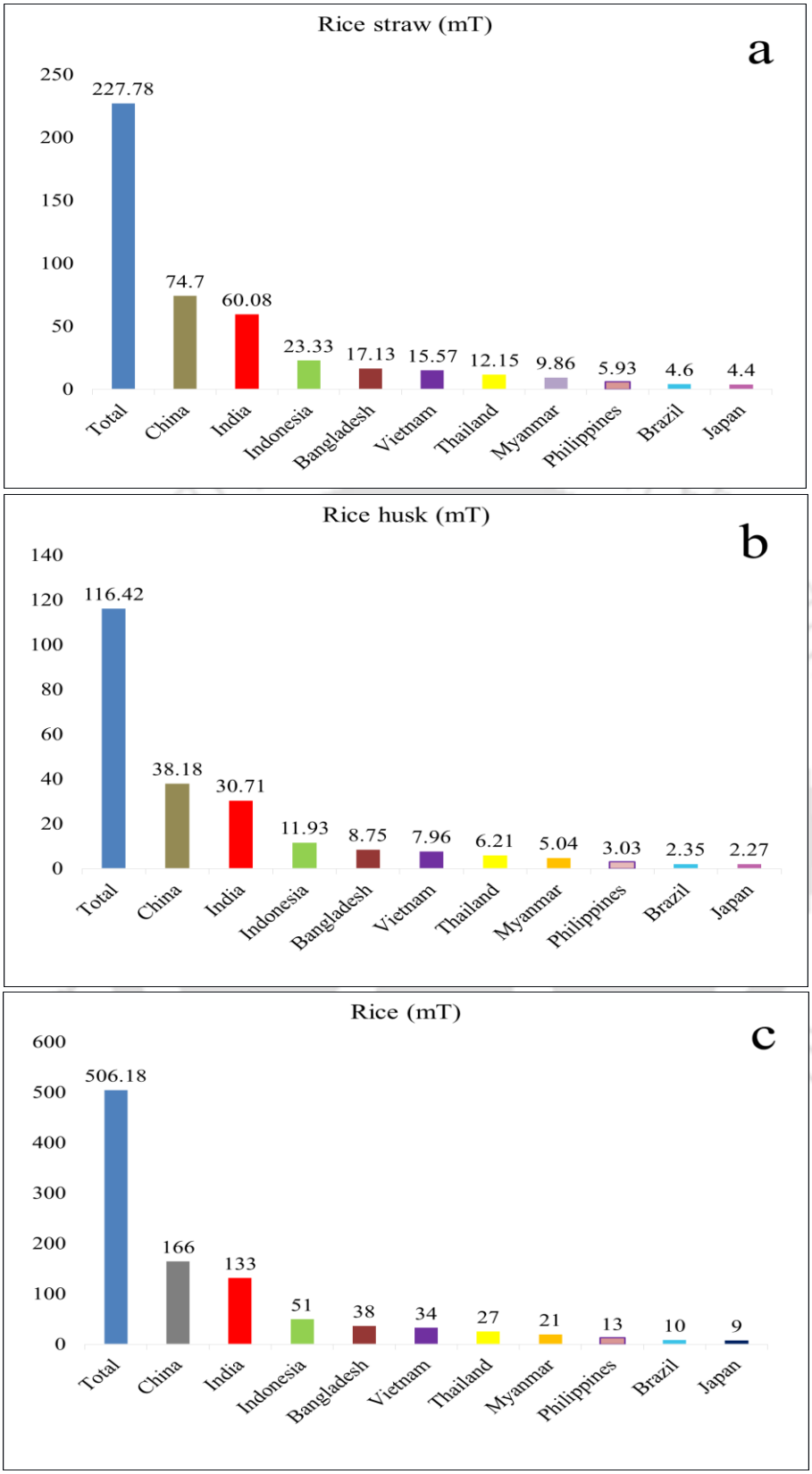

Figure 2.4. (i). Worldwide production of (a) Rice straw (b) Rice husk (c) Rice (Sarnklong et al., 2010)

TH-2881_146104040

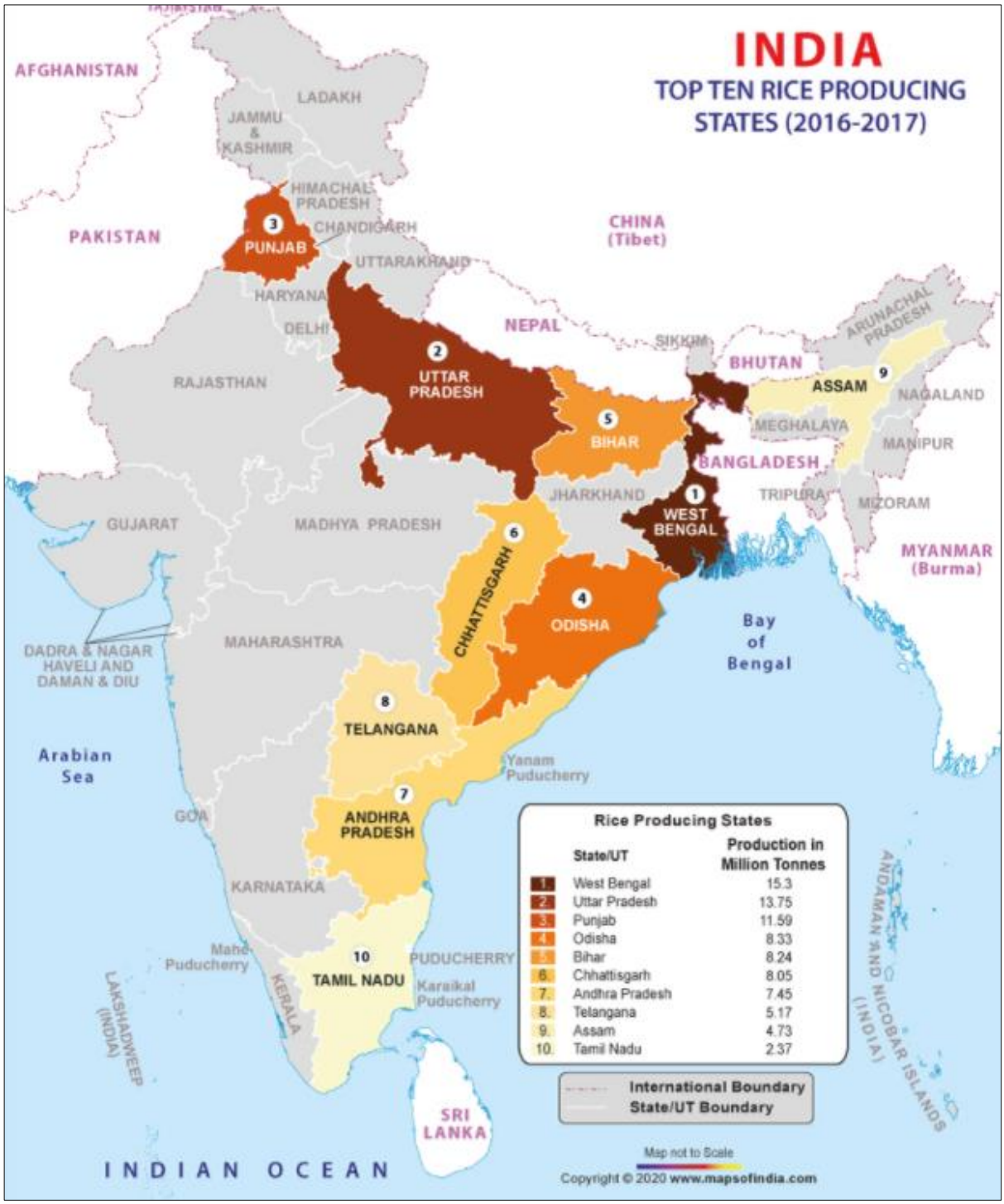

Figure 2.4. (ii). Rice growing states in India

TH-2881_146104040

Figure 2.4. (iii). Rice straw burning in the field

2.5. Rice Straw Composition

As rice straw primarily contains cellulose (32–47%), hemicellulose (19–27%), and lignin (5–24%) and ash content of the straw is (10–17%), as shown in figure 2.5 (Bhattacharyya et al., 2012). Silica covers 75–82% of ash content and silica incrustation protects the cell wall carbohydrates (Soest, 2006; Yoswathana et al., 2010). Carbohydrate contents of rice straw are in the form of glucose (41–43.4%), xylose (14.8–20.2%), arabinose (2.7–4.5%), mannose (1.8%), and galactose (0.4%) (Yoswathana et al., 2010). If cellulose in rice straw is completely degraded into glucose and hemicellulose into xylose, the theoretical reducing sugar yield of rice straw is (34.3 × 1.111 + 21.7 × 1.136)/0.875 = 71.7 g/100 g TVS (Cheng et al., 2011). The maximum reducing sugar yield has been reported i. e. 69.3 g/100 g TVS, which is 96.7% of the theoretical yield after providing an alkali pretreatment (Cheng et al., 2011).

TH-2881_146104040

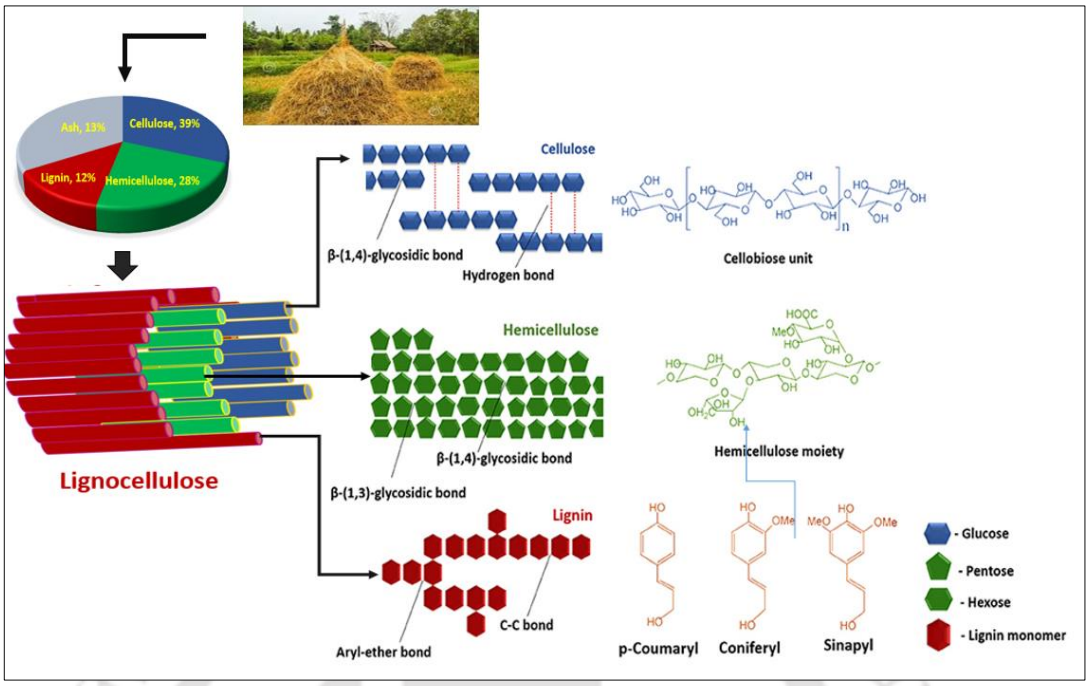

Figure 2.5. Rice straw composition

(i) Cellulose

Cellulose $(C_6H_{10}O_5)n$ is an organic compound or polysaccharide consisting of a linear chain of several hundred to many thousands of β (1→4) linked D-glucose units. Cellulose is an important structural component of the primary cell wall of green plants. Cellulose is derived from D-glucose units, which condense through β (1→4)-glycosidic bonds. This linkage motif contrasts with that for α (1→4)-glycosidic bonds present in starch and glycogen. Cellulose is a straight chain polymer. The multiple hydroxyl groups on the glucose from one chain form hydrogen bonds with oxygen atoms on the same or a neighbor chain, holding the chains firmly together side-by-side and forming microfibrils with high tensile strength. This confers tensile strength in cell walls where cellulose microfibrils have meshed into a polysaccharide matrix. Cellulose contains both crystalline and amorphous areas. The amorphous fraction of cellulose is structurally less compact and can be degraded more easily by enzymes/microorganisms than the crystalline parts (Yoswathana et al., 2010).

Alkaline hydrolysis allows the partial separation of the cellulose fibers from the cell wall. These treatments are usually made using diluted solutions of alkali at low or high temperatures, thereby it produces the amorphous cellulosic material (Ibrahim et al., 2011; Yoswathana et al., 2010). Valuable products (bioethanol; biofuels, acetic and

TH-2881_146104040

butyric acid, glucose, and ethanol) can be obtained from the cellulose through microbial fermentation and enzymatic hydrolysis (Liu et al., 2018b; Yang et al., 2015).

(ii) Hemicellulose

Hemicellulose $(C_5H_8O_4)n$ is one of several heteropolymers (matrix polysaccharides), such as arabinoxylans, present along with cellulose in almost all plant cell walls. Commonly, the formulates of hemicellulose are expressed as while cellulose is crystalline and strong, hemicelluloses have random, amorphous structure with little strength, they are easily hydrolyzed by dilute acid or base as well as a myriad of hemicellulase enzymes. Hemicelluloses are polysaccharides often associated with cellulose, but with distinct compositions and structures. Whereas cellulose is derived exclusively from glucose, hemicelluloses are composed of diverse sugars and can include the five-carbon sugars xylose and arabinose, the six-carbon sugars glucose, mannose, and galactose, and the six-carbon deoxy sugar rhamnose. Hemicellulose is amorphous and much more diverse, made up of glucose, mannose, galactose, xylose, arabinose, O-methyl-glucuronic acid, and galacturonic acid. This makes it a heteropolysaccharide. Hemicelluloses contain most of the D-pentose sugars, and occasionally small amounts of L-sugars as well. Xylose and mannose are in most cases the sugar monomer present in the largest amount, Not only regular sugars can be found in hemicellulose, but also their acidified forms, for instance, glucuronic acid and galacturonic acid can be present (Heinze et al., 2005). Unlike cellulose, hemicelluloses consist of shorter chains of 500–3,000 sugar units. In contrast, each polymer of cellulose comprises 7,000–15,000 glucose molecules (Gibson, 2012). Hemicelluloses are much more soluble and labile, that is, susceptible to chemical degradation than cellulose. Hemicellulose can be hydrolyzed easily as compared to cellulose by the addition of dilute base or acids as well as hemicellulase enzymes due to its feature of high solubility. Valuable products (ethanol; butanol; hydrogen) can be obtained from the hemicellulose through microbial fermentation and enzymatic hydrolysis (Li and He, 2016; Shin et al., 2010; Yang et al., 2015).

(iii) Lignin

Lignin $(C_{10}H_{12}O)n$ is an amorphous, irregular three-dimensional, and highly branched complex polymer made by cross-linking phenolic precursors (p-coumarylalcohol, coniferyl alcohol, and sinapyl), that provides rigidity and structural support, and remains

TH-2881_146104040

present in the integral cell wall of all vascular plants. Lignin is particularly important in the formation of cell walls and protects the plant. Lignin is rich in aromatic subunits. Lignin fills the spaces in the cell wall between cellulose and hemicellulose. Lignin can be hydrolyzed by alkali treatment (Ibrahim et al., 2011). Valuable products (sugar) can be obtained from the lignin through microbial fermentation and enzymatic hydrolysis. A large number of microbes have been described with lignin-degrading capabilities. Some produce large lignin degradation fragments whereas in other cases, smaller fragments with just a few aromatic ring structures are produced. The conversion of lignin degradation products into methane and carbon dioxide has been investigated (Khan and Ahring, 2019). The fermentative, nitrate, and sulfate reducing anaerobic bacteria are capable to degrade the lignin and aromatic compounds (Khan and Ahring, 2019).

2.6. Use of Rice Straw for Producing the Valuable Products

Various literature suggests that rice straw has enough potential to be used as a carbon source for the microorganism. In this section use of rice straw in various forms such as raw rice straw, pretreated rice straw, rice straw hydrolysate, and rice straw slurry for producing various valuable products, the effect of pretreatment on the composition of rice straw, and the final output of rice straw has been discussed.

After following the thermochemical (acidic or alkaline) pretreatment process two parts can be obtained first is the rice straw hydrolyzate and the second is the amorphous lignocellulosic material. The main constitutes of the rice straw hydrolyzate after the thermochemical pretreatment (acidic or alkaline) is total reducing sugars (xylose, glucose, and arabinose), furans (furfural and HMF), and carboxylic acids (acetic acid and formic acid) (Weng et al., 2010; Zhu et al., 2005), which is mainly due to partial fragmentation of the component. In other words, few researchers have expressed the composition of hydrolyzate in terms of the total reducing sugar (glucose, xylose, furfural, gellobiose, and arabinose) and VFA (ethanol, butanol, acetate, propionate, n-butyrate) and COD (Chang et al., 2011; Kumari and Singh, 2020; Nagaiah et al., 2016; Qi et al., 2021).

According to the reports rice straw hydrolyzate contains 23-27.6% COD, 12-21.4% TRS, and 0.42-2.5% VFA, glucose (0.2-0.85%), xylose (0.2-12%), furfural (0.83-1.51%), gellobiose (0.35-3.72%), and arabinose (0.31-2.31%), ethanol (0.07-0.35%),

TH-2881_146104040

butanol (0.093%), acetate (0.29-2.55%), propionate (0.01%), n-butyrate (0.03-0.13%) after acidic pretreatment (condition at 150 °C, 60 min in presence of acids; 100:30:0.9; sulfuric, nitric, and hydrochloric acid) (Chang et al., 2011). These constitutes can be used by microorganisms as a source of carbon or electron donor for wastewater treatment during the biological oxyanion reduction.

Few researchers have also utilized the whole "raw rice straw" (untreated) without giving any pretreatment for producing valuable products such as methane and bioethanol (Lei et al., 2010). Few researchers have utilized this "rice straw hydrolyzate" followed by a later enzymatic hydrolysis/fermentation process for producing various valuable products such as hydrogen (Chang et al., 2011). Furthermore, few researchers have utilized this solid residue of rice straw or "pretreated rice straw" after following an acidic/alkali/ammonia pretreatment process and then following enzymatic hydrolysis/fermentation/anaerobic digestion process for producing valuable products such as reducing sugar, bioethanol, hydrogen and methane formation (Chang et al., 2011; Lei et al., 2010; Yoswathana et al., 2010; Zhu et al., 2005). A list of various types of valuable products (hydrogen, ethanol, methane, etc.) obtained from rice straw (raw rice straw, pretreated rice straw, rice straw hydrolysate, and rice straw slurry) is shown in table 2.6.

(i) Use of Raw Rice Straw for Producing Valuable Products

Chen et al. (2012) reported that raw rice straw (90 gm TS/L, particle size <0.297 mm), can produce hydrogen (24.8 mL/gm TS) using the seed sludge (municipal wastewater) under dark fermentation at pH 6.5 and 55°C in batch test within 45 h. The study was conducted to identify the probable major metabolic pathway, the soluble metabolic products (SMPs) composition, and its concentration in the anaerobic fermentation of raw rice straw along with non-treated and heat-treated sludges (municipal wastewater treatment plants, paper manufacturing plants, and cow dung compost). The non-treated sludges after 240 h cultivation with rice straw showed SMPs concentration of 1244-2325 mg COD/L and ethanol (EtOH) concentration of 43-98 mg COD/L. The major SMP detected with non-treated sludges were acetate (HAc, 506-1698 mg COD/L), propionate (HPr, 104-358 mg COD/L), butyrate (HBu, 116-414 mg COD/L), and valerate (HVa, 19-70 mg COD/L). In contrast, the SMPs concentrations with heat-treated sludges showed a 114–391% increase as compared to non-treated sludges. The

TH-2881_146104040

heat-treated sludges after 240 h of cultivation on rice straw showed SMPs concentration of 5063-6429 mg COD/L and EtOH of 768-1042 mg COD/L. The major SMPs detected in the case of heat-treated sludges were HAc (3070-4261 mg COD/L), HPr (228-486 mg COD/L), HBu (531-566 mg COD/L), and HVa (64-303 mg COD/L). Their results reveal that HAc is the dominant SMP formed during the dark fermentation of rice straw. It is well reported that the fermentation pathway depends on the type of substrate and bacterial metabolism, and studies have shown different pathways of hydrogen production. They have reported acetate and butyrate as the main end-products of carbohydrate fermentation by Clostridium species and follow the butyrate pathway.

Lei et al. (2010) reported that raw rice straw (14 gm TS/L, 3-5 mm) can produce biogas or methane yields of (0.33-0.35) m^3/kg-VS loaded or (0.27-0.29) m^3 CH_4/kg-VS loaded and average methane contents of 75.9-78.2%. (75.9-78.2%) and VFA (1500-2000 mg/L) using the acclimated sludge under anaerobic digestion within 25-30 days of operation. Initial VFA content was 600-800 mg/L, meanwhile, the VFA concentrations increased and became relatively stable (around 1500 mg/L, within 20-25 days) and decreased substantially after the 60^{th} day, due to the adaptation and activity recovery of methanogenic bacteria and the subsequent balance of VFA generation and consumption in the reactors. The final VFAs remained between 650 and 700 mg/L (114-120 days). During this anaerobic digestion process, changes in pH were observed, the initial pH was 7.1–7.2, the intermediate pH was 5.8–6.3 (20-25 d), and the final pH was 6.8-7.2 (114-120 d).

(ii) Use of Pretreated Rice Straw for Producing Valuable Products

The use of pretreated rice straw for producing various valuable products and the effect of pretreatment agents on enhancement in biodegradability has been reported by various literature. Pretreatment methods are practiced to hydrolyze the components, increase the solubility, and produce the amorphous substrate through breakage of structural bonds are physical methods (e.g., milling, grinding, soaking in water in the presence or absence of acid or alkaline), physico-chemical methods (steam explosion with or without chemical, ammonia fiber explosion); and thermochemical methods (e.g., using acids, alkali, oxidizing agents, or organic solvents in presence or absence of heat) (Brodeur et al., 2011; Cheng et al., 2011; Kumar and Sharma, 2017; Murnen et al., 2007). There are various types of pretreatment agents such as acids, alkali, oxidizing

TH-2881_146104040

agents, and organic solvents available. The preferred chemical, such as $Ca(OH)_2$, NaOH, and ChOH, has been extensively used in improving biodegradability because of their low cost, high recoverability, safe handling, and minor environmental effects. Alkali (due to the presence of OH^-), has been proven to be the most promising, which has practical advantages when such pretreatment is to be followed by anaerobic digestion. The extent of hydrolysis depends upon various factors such as the size of material, time, temperature, amount, and type of reacting agents. The complex structure of the internally packed network between lignin, hemicellulose, and cellulose inhibits the effective conversion of rice straw to fermentable sugars (Vlasenko et al., 1997). Therefore, a pretreatment step is necessary for opening the structure of the lignocellulosic materials by introducing breakage in the lignin structure so that the cellulose fibrils become exposed. An optimal thermalkali treatment method is the one that can increase the surface area and porosity, reduces the crystallinity of cellulose, increases the cellulose content, and reduces the lignin and hemicellulosic content as well as partially hydrolyses (depolymerize) the material. The thermoalkali treatment mechanism of rice straw is shown in figure 2.6. Thermoalkali treatment helps to remove the lignin content, to break the inter/intra-chain hydrogen bonding between cellulose fibrils. Thermalkali treatment can break these bonds and partially converts complex organic carbons into simplest form of sugar such as glucose, xylose, and arabinose etc. and produces the amorphous solid material, which is easily digestible by the microorganisms (Cheng et al., 2011; Lei et al., 2010).

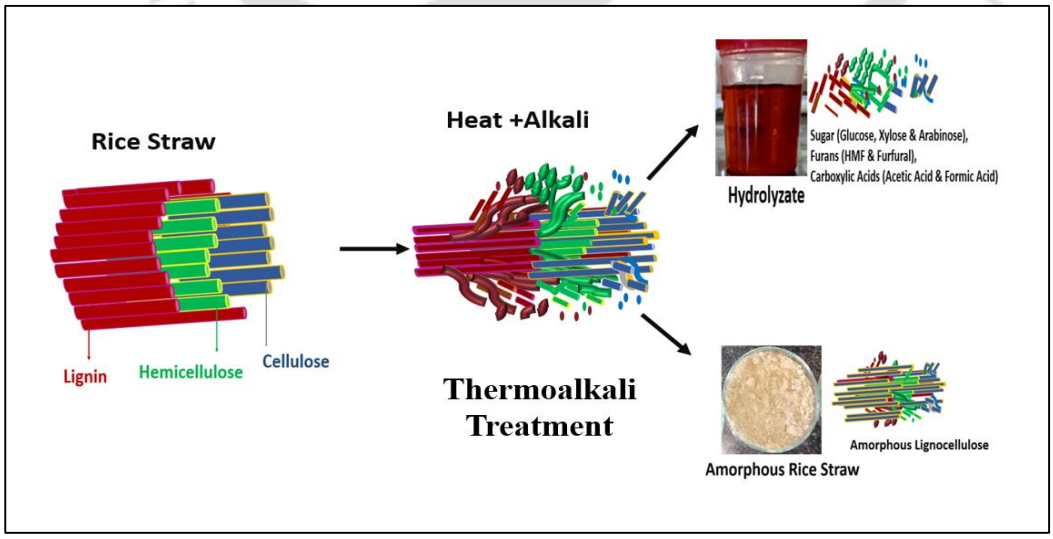

Figure 2.6. Thermoalkali treatment mechanism of rice straw

TH-2881_146104040

Song et al. (2013) reported that alkali (9.81 % $Ca(OH)_2$, at 5.89 d) pretreated rice straw can produce the methane yield of 225.3 mL/g VS using anaerobic digestion. Elsayed et al. (2018) reported that alkali (10 % NaOH, at 60°C, 48 h) pretreated rice straw (72.9 gm/L) can produce ethanol (87.4 gm/Kg) using the enzymatic hydrolysis within 48 h time. Cellulose and hemicellulose are converted mainly to glucose and xylose. Glucose yield, xylose yield, and total glucose conversion after enzymatic hydrolysis were 190.7 gm/Kg dry straw, 77.9 gm/Kg dry straw and 55.6 %, respectively. They have reported that the Cr I increased from 38.7 % in raw rice straw to 42.9, 52.9 and 52.3 % after alkali pretreatment, enzymatic hydrolysis and fermentation, respectively. This can be primarily attributed to the removal of hemicellulose and lignin in the amorphous region, rather than to structural changes in cellulose fibers. Alkaline pretreatment showed the highest significant lignin degradation which resulted in 0.8 % lignin content, with significant increase of cellulose content (65 %) representing 82.5 % over that of raw rice straw.

Kumar et al. (2016) reported increment in cellulose content (from 40.0±1.8 to 46.0±0.9 %), reduction in hemicellulose content (28.0±2.0 to 24.6±1.3 %) and reduction in lignin content (9.0±0.8 to 3.8±0.5 %) after ionic liquid pretreatment (at 60 °C, 12 h). Hideno et al. (2009) have reported an increment in crystallinity index (from 51.9 to 64.1 %) of rice straw from using hot compressed water pretreatment (180^{o}C) and they have reported a 67.5 % increment in total sugar yield during the enzymatic hydrolysis. Hsu et al. (2010) reported crystallinity index (from 57 to 58-65 %) was found to be increased after dilute acid pretreatment of rice straw. A maximum sugar yield of 83 % was achieved when the rice straw was pretreated with 1 % (w/w) sulfuric acid with a reaction time of 1-5 min at 160-180 °C, followed by enzymatic hydrolysis.

(iii) Use of Rice Straw Hydrolyzate for Producing Valuable Products

Nguyen et al. (2010) reported that pretreated rice straw hydrolyzate (combined pretreatment using ammonia; 10 % and ionic liquid, 20 ml at 100°C, 6 h) can be used for producing glucose (52-97 %) using the enzymatic hydrolysis within 50 h. Sen et al. (2016) showed that 0.8–1.0 M hydrochloric acid could give the maximum total sugar yield of 52.9 %, 2.8 g/L glucose, 14.5 g/L xylose, and 38.6 g/L total reducing sugar from 100 g/L rice straw with particle size 0.15 mm within hydrolysis time of 20 min. High volumetric hydrogen production of 771 mL/L and ethanol production of 1776 mg

TH-2881_146104040

COD/L was obtained from the pretreated rice straw hydrolyzate using enzymatic hydrolysis.

(iv) Use of Rice Straw Slurry for Producing Valuable Products

Jung et al. (2015) evaluated the feasibility of whole rice straw slurry (pretreated lignocellulose) saccharification and fermentation for producing ethanol from maleic acid-pretreated rice straw. The optimized conditions for pretreatment were to treat rice straw at a high temperature (190°C) with 1 % (w/v) maleic acid for a short duration (3 min ramping to 190 °C and 3 min holding at 190 °C). Enzymatic digestibility (based on theoretical glucose yield) of cellulose in the pretreated rice straw was 91.5 %. Whole slurry saccharification and fermentation of pretreated rice straw resulted in an 83.2 % final yield of ethanol based on the initial quantity of glucan in untreated rice straw. Their findings indicate that maleic acid pretreatment results in a high yield of ethanol from fermentation of the whole slurry even without conditioning or detoxification of the slurry. Additionally, the separation of solids and liquid is not required; therefore, the economics of cellulosic ethanol fuel production are significantly improved. They have demonstrated whole rice straw slurry saccharification and fermentation of pretreated lignocellulose, which has rarely been reported. They have reported that 40.6 % of lignin removal and 53.2 % (w/w) insoluble solids recovery yields after maleic acid pretreatment.

Bio-hydrogen production potential of wastes slurry (rice straw, rice husk, and rice bran) was compared by co-digestion with heat shocked sludge under mesophilic thermophilic temperatures by using an anaerobic bioreactor under no pH control conditions (Sattar et al., 2016). It was observed that the bio-hydrogen production potential increased with an increase in temperature from 37°C to 55°C for all wastes, except for rice waste. Although the highest experimental yield of 40.04 mL/VS was produced from thermophilic rice straw, the maximum bio-hydrogen production rate of 97.08 mL/h was observed from rice bran after 29.03 h under thermophilic conditions. The bio-hydrogen production period of rice bran was 33–38 % smaller than rice straw due to which the highest yield was observed from rice straw. The lignin content of the rice husk was 19.34%, which was double that of rice straw, and hence the rice husk produced the least experimental yield of 23.05 mL/VS under mesophilic conditions.

TH-2881_146104040

Table 2.6. Valuable products from the rice straw

SN	Pretreatment Agents	Pretreatment Conditions	Substrate used	Application/Process Involved; Time	Amount of Substrate	Unit	Products	Concentration	Unit	Reference
1	Without pretreatment	-	Raw rice straw (size 3-5 mm)	Anaerobic digestion with acclimated sludge; Time 25-30 d	14.60	g/L	Methane	75.9–78.2	%	(Lei et al., 2010)
2	Without pretreatment	-	Raw rice straw (size <0.297 mm)	Municipal wastewater; Time 45 h	90	g/L	VFA	1500-2000	mg/L	(Chen et al., 2012)
							H^+	24.8	mL/g TS	
3	Alkali (0.5% NaOH)	140^0C, 15 min	Pretreated rice straw	Enzymatic hydrolysis and anaerobic fermentation; Time 12 h	10-60	g/L	H^+	155	mL/kg VSS	(Cheng et al., 2011)
4	Alkali (1-5% NaOH) Acid (1-9% H_2SO_4)	160°C (5 bar) 200°C (15 bar), 40W, 10 min	Pretreated rice straw	Enzymatic fermentation; Time 6 d	NR	-	Ethanol	55-65	%	(Yoswathana et al., 2010)
5	Calcium capturing by carbonation (CO_2), (CaCCO) process	120°C, 1h	Pretreated rice straw	Enzymatic fermentation	70	g/L	Ethanol	74	% of theoretical yield	(Park et al., 2010)

TH-2881_146104040

6	Alkali (20 % NaOH)	80°C, 1 h, Steam-exploded	Pretreated rice straw	Enzymatic fermentation	NR	NR	Glucose	58.4	g/L	(Ibrahim et al., 2011)
7	Alkali (4% NaOH)	4h, Irradiation	Pretreated rice straw	Enzymatic hydrolysis	NR	NR	Reducing sugar	90	% of Rice straw	(Xin and Kumakura, 1993)
							Glucose	50		
8	Ammonia (10 %) and Ionic Liquid Solvent (20 ml)	100°C, 6h	Pretreated rice straw	Enzymatic fermentation; Time 50 h	0.1	g/mL	Glucose	52-97	%	(Nguyen et al., 2010)
9	Alkali (10 % NaOH)	60°C, 48 h	Pretreated rice straw	Enzymatic hydrolysis	72.9	g/L	Ethanol	87.4	gm/Kg Rice straw	(Elsayed et al., 2018)
10	Acid (100:30:0.9; Sulfuric, nitric, and hydrochloric acid)	150 °C, 60 min,	Rice straw hydrolyzate	Seed sludge; Time 150 h	0.83	mL/mL	H^+	10.48	Mmol/g Rice straw	(Chang et al., 2011)
11	Acid (0.8-1 M HCl)	121 °C, 20 min	Rice straw hydrolyzate	Enzymatic hydrolysis; Time 20-60 min	85	%	H^+	771	mL/L	(Sen et al., 2016)
12	Acid (1 % (w/v) maleic acid)	190°C, 3 min	Rice straw slurry	Enzymatic hydrolysis; Time 20-60 min	0.1	g/mL	Ethanol	83.2	%	(Jung et al., 2015)

NR-Not Reported

TH-2881_146104040

The optimum pH range of bio-hydrogen production from rice straw, bran, and husk was observed from pH 7 to pH 6. The average bio-hydrogen yield of wastes produced from rice crops was 30.36 and 33.16 mL/VS under mesophilic and thermophilic conditions, respectively.

2.7. Use of other Lignocellulosic Compounds for Producing the Valuable Products

Yang and Fang (2014) reported ultrasound-assisted enzymatic hydrolysis by cellulase, the total reducing sugar yield from rice hulls after the ChOH pretreatment (300 W/40 kHz, at 60 °C for 180 min) was 43.5% at only 3 h of hydrolysis time, and was greater than that from untreated rice hulls (24.0%), they have confirmed that the ultrasound and ChOH pretreatment enhanced the enzyme hydrolysis of rice hulls effectively.

Liew et al. (2011) demonstrated that using fallen leaves the greatest enhancement in methane yield was achieved with a NaOH loading of 3.5 %, which is 24-fold higher than that of the control (without NaOH addition). Their results suggest that pretreatment time, temperature, chemical concentration, and inoculum amount have more considerable effects on the digestibility of agricultural wastes. Kaar and Holtzapple, (2000) showed that pretreatment with $Ca(OH)_2$ at the loading of 0.075 g per g of dry biomass (120°C for 4 h) increased the enzymatic hydrolysis of corn stover to nine times higher than that generated by untreated corn stover. Li et al. (2019) showed that the methane yield from the wood waste after anaerobic digestion was increased by 38.5% using 2 % NaOH pretreatment (90°C for 2 h) compared with the untreated wood waste. Salehian et al. (2013) found that the yield of methane produced from pine wood pretreated with 8 % NaOH (100°C for 10 min) was increased from 65 mL CH_4/g VS to 178.2 mL CH_4/g VS after 45 days of incubation. Similarly, Mirahmadi et al. (2010) observed a 50% enhancement in methane production after the 7% NaOH pretreatment (100°C for 2 h) of birch. The hydrogen yield from wheat straw was improved (45 min of the pretreatment before enzymatic hydrolysis); it decrease inhibitor content in the feed and improves the hydrogen production by 158% (Wu et al., 2013). Romero et al. (2015) reported that hydrogen peroxide improves enzymatic hydrolysis of rape straw and conversion to ethanol from 44.2 % (1% H_2O_2, 30 min, 90 °C) to 77.2 % (5% H_2O_2, 90 min, 90 °C).

The literature survey suggests remarks that rice straw during enzymatic hydrolysis/ anaerobic digestion process led to a breakdown in various organic carbonic compounds

TH-2881_146104040

which can become carbon source for reducing the oxyanions (electron acceptor) from the wastewater. All these literature surveys suggest that rice straw has enough potential to be used as a carbon source for the microorganism, thereby biological oxyanion removal can be done.

2.8. Use of Rice Straw as a Carbon Source for Oxyanions Removal by Biological Process

When rice straw is applied as a carbon source during the anaerobic digestion process then fermentative bacteria will hydrolyze the organic matter and produces the simple monomers (sugars, volatile acids, and H^+), which will be consumed by sulfate reducing bacteria (SRB)/ nitrate reducing bacteria (NRB) for reducing the sulfate and nitrate (García de Lomas et al., 2006; Lovley and Chapelle, 1995). Once the sulfate is reduced to sulfide, it will help to precipitate the arsenic in form of arsenosulfide (realgar, orpiment, and dimorphites). The end product will the inert nitrogen gas and arsenosulfide. In such a way rice straw can be used as a sustainable carbon source for oxyanions removal from the containing wastewater using the biological process. Figure 2.8, represents the process mechanism for the use of rice straw as a carbon source for oxyanion removal by biological process.

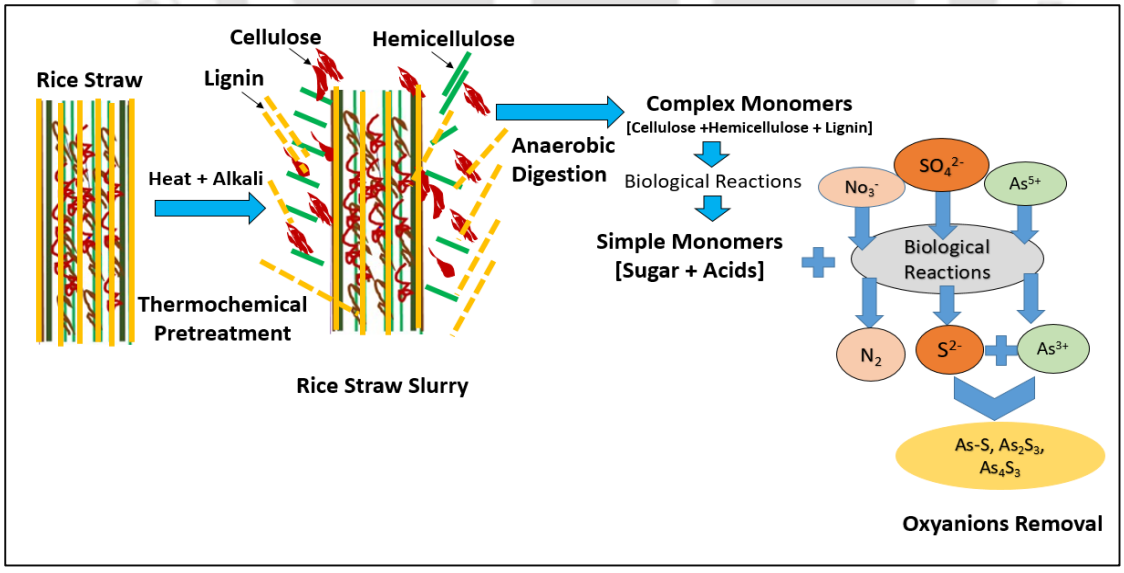

Figure 2.8. Process mechanism: use of rice straw as a carbon source for oxyanions removal by biological process

TH-2881_146104040

2.9. Summary of Literature and Research Needs

With the increase in human population and rapid industrialization, the generation of wastewater of various nature is increasing day by day. With the virtue of the advancement of biotechnology, biological processes have been widely practiced for the treatment of wastewater containing multiple pollutants. Oxyanions such as sulfate, nitrate, arsenate, etc. are some of the common water pollutants, which are electron acceptors, biologically reduced, and removed from contaminated water and wastewater. In biological processes, an external carbon source is required to be supplied to serve as an electron donor cum carbon source. The most frequently used carbon sources in the biological oxyanion removal process are lactate, acetate, propionate, pyruvate, and butyrate which can be grouped under the category of "high-cost carbon" sources. To minimize the treatment cost investigation of the suitability of waste material as the carbon source in a biological process is imperative. Several researchers have successfully employed agricultural wastes or complex organic matters such as tannery effluent, fresh alfalfa, hay, peat whey, cow manure, mushroom compost, paper sludge, poultry manure, etc. as carbon sources and/or electron donors in biological systems, which can be grouped under "low-cost carbon" sources.

Rice straw is an abundant lignocellulosic organic compound and waste material in the environment. Effective management and production of value-added products from the rice straw is the main concern nowadays. So far the rice straw has been extensively used for producing various valuable products (biofuel/bioethanol/H^+) through adopting the various thermochemical or biological processes, which confirms that rice straw has enough potential to be used as a carbon source as explained in section 2.6.

Biological processes utilize bacteria to convert the biomass into various products either through digestion/fermentation of organic matter directly or through thermochemical treatment and then the digestion/fermentation process. Anaerobic digestion of rice straw is a well-established mature technology practiced worldwide, for the production of biofuels/other valuable products, in which organic waste matter is converted to energy forms with the assistance of microbes. To enhance the anaerobic digestion of rice straw thermochemical treatment has been extensively used.

Although rice straw, one of the most abundant agricultural wastes in India, is a biodegradable lignocelluloses crop residue, its usage in biological processes is limited

TH-2881_146104040

mainly to composting and biogas production. However, thermoalkali pretreatment can enhance the digestibility of lignocelluloses and enhance bio-ethanol, and methane generation. Thus, despite having great potential, the use of rice straw as a carbon source cum oxygen donor in a biological system on oxyanions (electron acceptors) removal has not been explored so far.

The lacuna found in the various literature is that no studies have been carried out on the use of rice straw as a carbon source for oxyanion removal using a biological process. Thus it is imperative to sustainably utilize the rice straw as a carbon source for the pollutant/oxyanion removal from the wastewater using biological methods. This study in this area would be very much helpful for minimizing the cost factor. As well, this technology will give an alternative solution to two major environmental problems. First is that it will reduce the management and disposal problem of agricultural waste and second it will reduce the cost of carbon source addition during the biological treatment processes. In such a way rice straw can be used as a carbon source for oxyanions removal from the wastewater using the biological process.

The main goal is to assess the feasibility of thermoalkali pretreated rice straw as a carbon source in the biological batch and continuous reactor. The targeted oxyanions are sulfate, nitrate, and arsenic.

TH-2881_146104040

CHAPTER 3

AIM AND SCOPE OF STUDY

Aim of the Study:

The present study aims to assess the feasibility and efficiency of thermoalkali pretreated rice straw slurry as a carbon source for the biological reduction of oxyanions sulfate, nitrate, and arsenic by mixed bacterial culture.

Scope of the Study:

The following scopes have been identified to achieve the above objective:

i) Collection, processing, and study of the effects of thermoalkali (here pretreatment) methods on the hydrolysis, and change in physicochemical properties of rice straw.

ii) Collection, characterization, and acclimatization of a suitable biosludge to use as inoculum for bioreactors.

iii) Use of the raw rice straw and thermoalkali treated rice straw slurry as a carbon source in biological reduction of oxyanions in batch mode.

iv) Fabrication, operation, and performance evaluation of an attached growth continuous flow bioreactor on oxyanions reduction using liquor of partially digested rice straw slurry (RS-NaOH).

v) Characterization of bio solids formed in the bioreactors.

vi) Identification of microbial communities present in the bioreactors using 16S rRNA, TRFLP analysis, and Taxonomic profiling.

TH-2881_146104040

TH-2881_146104040

CHAPTER 4

MATERIALS AND METHODS

A successful experiment with highly perceptive results depends on the concrete organization of individual research components. In this chapter, various materials procured from authentic sources and the development of new materials adopting self-developed techniques or adopted from highly cited literature to accomplish individual objectives have been systematically arranged. In this section, various materials and equipment used and different methodologies, and exercises used in the present investigation are described.

All the glassware used during the study was of borosilicate and plastic ware of Tarson products. Deionized water or distilled water was used to prepare all the reagents. All the glassware used in this study was kept immersed in 20 mL of HCl per lit of distilled, followed by washing with tap water and deionized water. The glassware was kept in a hot air oven for drying at 70-75^{o}C for about 4 h before being used for any experiments. All the chemicals were of either analytical reagent (AR) or laboratory reagent (LR) grade. Sodium arsenate ($Na_2HASO_4.7H_2O$; 98.5% assay), Sodium nitrate ($NaNO_3$; 99% assay), Magnesium sulfate ($MgSO_4.7H_2O$; 99% assay), Calcium hydroxide ($Ca(OH)_2$; 96% assay), Sodium hydroxide (NaOH; 99% assay) and Choline hydroxide (ChOH, $C_5H_{15}NO_2$, 46 % solution) were of AR grade and procured from Hi-media (India) or Sigma Aldrich, USA. Whatman filter paper of grade 1 and syringe-driven filter of 0.45 µm were used whenever required. Each experiments and analysis were peformed in triplicate.

4.1. Collection, Processing, and Thermoalkali Treatment (here Pretreatment) of Rice Straw

Rice straw used in this project was collected from nearby paddy fields in Hazo, Assam, India. After cutting, milling, and sieving (as shown in figure 4.1), 150-300 µm size, termed raw rice straw (RRS) was used for further studies. The RRS was heated either in a hot air oven or in an autoclave, without or with the addition of varying doses of lime [$Ca(OH)_2$], caustic soda (NaOH), and choline hydroxide (ChOH) [$C_5H_{15}NO_2$] to determine the optimum conditions (temperature, heating duration) and doses of alkali

TH-2881_146104040

on hydrolysis of RRS by the thermoalkali treatment process. The thermoalkali treatment is also referred to as pretreatment of rice straw in this thesis.

In the preliminary thermoalkali pretreatment studies, two instruments have been used i. e. hot air oven and an autoclave. At first, rice straw (1g/100 mL distilled water) has been pretreated in a hot air oven at different temperatures (35, 65, 85, 95^{o}C at a fixed time 20 min) and at different time intervals of 20, 40, 60, 120, 240, 260 min (at the fixed temperature 85^{o}C). Subsequently at the optimized temperature (85^{o}C) and time (240 minutes) the experiments were performed in presence of different alkali. Variation in $Ca(OH)_2$ dose % (w/w) (0, 0.5, 2.5, 5, 7.5), NaOH dose % (w/w) (0, 0.25, 0.5, 1, 2.5, 5) and ChOH dose % (v/v) (0, 0.1, 0.2, 0.3, 0.4, 0.5) was done to optimise the alkali concentration, in hot air oven at the temperature 85^{o}C for 240 minutes. Similarly, in an autoclave, time varied from 5, 10, and 20 minutes (at a fixed temperature of 121^{o}C). And then alkali doses were varied at 121^{o}C for 20 minutes as stated above.

The studies carried out on thermoalkali pretreatment of rice straw are given in table 4.1, which were characterized following different methods and instruments described in section 4.6.

Figure 4.1. Rice straw collection and its pre-processing

TH-2881_146104040

Table 4.1. Details of thermoalkali pretreatment given to raw rice straw (RRS)

Variable			Hot Air Oven		Autoclave		Remarks	
			Time (min)	Temp (°C)	Time (min)	Temp (°C)	Hydrolyzate Analysis	Compositional Analysis
Temperature			20	35	-		COD, TRS, VFA	-
				65				
				85				
				95				
Time			20	85	5	121		
			60		10			
			120		20			
			240					
			260					
Alkali Dose	$Ca(OH)_2$ % (w/w)	0, 0.5, 2.5, 5, 7.5	240	85	20	121	COD, TRS, VFA	FTIR, FESEM, XRD, TGA analysis, CHNS %, Biodegradability Fraction, Hydrolysis Rate %, Weight Loss %, Lignocellulose
	NaOH% (w/w)	0, 0.25, 0.5, 1, 2.5, 5						

TH-2881_146104040

	ChOH % (v/v)	0, 0.1, 0.2, 0.3, 0.4, 0.5						Content of 0 % alkali, 2.5 % (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, and 0.4 % (v/v) of ChOH treated Rice Straw

TH-2881_146104040

4.2. Composition of Synthetic Feed Media

The composition of simulated synthetic media used in this project is given in table 4.2. The bicarbonate alkalinity was added to the reactor to maintain the pH of the content near 7.4±0.3.

Table 4.2. Composition of synthetic feed media

Major Nutrients		**Variable**	
Composition	**g/L**	**Composition**	**Concentration**
KH_2PO_4	0.5	$NaNO_3$ (as NO_3^-)	500-2000 mg/L
K_2HPO_4	0.1	$Na_2HAsO_4.7H_2O$ (as As)	500-2000 µg/L
NH_4Cl	0.3	$MgSO_4.7H_2O$ (as SO_4^{2-})	1000-3000 mg/L
$NaHCO_3$	0.5-0.8	*$C_6H_{12}O_6$	1500-4500 mg/L
		pH	3-9
Micro Nutrients of 1 mL/L			
Composition	**g/L**		
$CaCl_2.2H_2O$	0.05		
$NaMo_4.2H_2O$	0.03		
KCl	0.05		
$CoCl_2.6H_2O$	0.02		
$MgCl_2.6H_2O$	0.05		
$NiSO_4.6H_2O$	0.01		
$C_6H_7NaO_6$	0.05		
$C_2H_3NaO_2S$	0.05		

*Dextrose ($C_6H_{12}O_6$) is only used for the acclimatization process and during the operation of the PBCR only for phases 1, 2, and 3.

TH-2881_146104040

4.3. Collection, Processing, and Acclimatization of Mixed Bacterial Sludge

Bio sludge from an abandoned secondary treatment plant, which was used in treating a pulp and paper mill (Hindustan Pulp and Paper mill) wastewater was collected from Kagajnagar, Assam (26°15'25.7"N 92°14'49.6"E) as shown in figure 4.3.1. The collected bio sludge (paper mill sludge) was first screened through a fine mesh (2 mm) to eliminate any unwanted materials like leaves, tree bark, the undigested fiber of bamboo, etc.

Acclimatization of paper mill sludge was conducted in a series of plastic containers (Jerry can) of 5.0 L capacity, denoted as reactor AC. The working volume of the batch reactors was 4.4 L. It was then acclimatized at room temperature (30 ± 5^{o}C) for 40 days before being used as inoculum in bioreactors. The MLSS and MLVSS content in the sludge was 8.7-10.5 g/L and 6.6-7.5 g/L, respectively. The consortium was fed with 1500 mg/L COD (using dextrose), 1000 mg/L SO_4^{2-}, 100 mg/L NO_3^-, and 100 µg/L arsenic along with synthetic feed media. Bicarbonate alkalinity was added to the reactor to maintain the pH of the content to 7.4±0.3. Supernatant volume (40 %) was replaced with fresh feed media at the interval of 10 days. Nitrogen gas was supplied at the interval of 24 h for 30 min to reduce DO concentration in a bioreactor. Feed and nitrogen gas supply was done using the ports provided at the top of the reactor. The reactors were monitored on daily basis. Figure 4.3.2 shows the (a) schematic diagram and (b) lab-scale photograph of the acclimatization of seed sludge.

Figure 4.3.1. Sludge collection site

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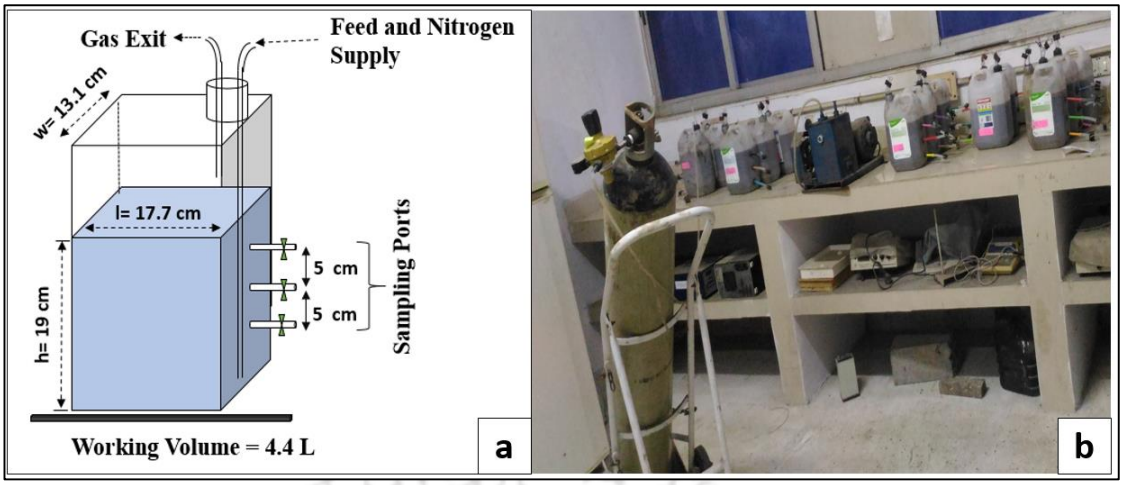

Figure 4.3.2. (a) Schematic diagram (b) Lab-scale photograph of acclimatization of seed sludge

4.4. Operation of Batch Reactor

A series of plastic containers (jerry cans) of 5 L capacity were used as batch reactors. Each batch reactor was having 3 outlets (sampling) ports 5 cm apart to take samples. Provision of nitrogen gas supply and its outlet along with other dissolved gases were made at the top of each batch reactor. For operating the batch reactors, rice straw slurry was used as a carbon source for the removal of oxyanions. For this purpose, four types of rice straw slurry were prepared: (i) rice straw (100 g/L) which was only soaked for 10 days in distilled water, denoted as RS-WTA (without thermoalkali treated rice straw), and rice straw (100 g/L) in presence of different alkali reagents, viz., (ii) $Ca(OH)_2$ (2.5 % w/w), (iii) NaOH (1% w/w), and (iv) ChOH (0.4% v/v) denoted as RS-$Ca(OH)_2$, RS-NaOH and RS-ChOH, were thermally treated at 85^oC for 240 min in a hot air oven. The slurry obtained after thermoalkali pretreatment of rice straw contains, the hydrolyzate, and solids portion of rice straw, both of them were characterized following different methods and instruments described in section 4.6, analytical procedure. The designation of rice straw slurry used as a carbon source for the microorganism is listed in table 4.4.1. Figure 4.4.1, represents the (a) schematic diagram and (b) images of thermoalkali treated rice straw slurry.

One liter of rice straw (mass of RS is 100 g/L) slurry (RS-WTA/ RS-$Ca(OH)_2$/ RS-NaOH/ RS-ChOH) and 250 mL of synthetic feed media solution (details are given in table 4.2 and 4.4.2) was added into the reactor. Subsequently, 3.15 L of inoculum was added to the batch reactors using a peristaltic pump (Model: PP10, Miclin India). After

TH-2881_146104040

the addition of inoculum, MLVSS concentration in the reactors was between 3140-4665 mg/L. The working volume in the reactors was 4.4 L. The bicarbonate alkalinity was added to the reactor to maintain the pH of the content near 7.4±0.3. In batch reactor (R1, R2, and R4), pH correction was done using 0.05 N H_2SO_4 and 0.05 N NaOH solution. After the inoculation reactor was purged with nitrogen gas for 30 min to establish the anoxic condition. All the batch reactors were operated at room temperature 30±5ºC. Intermediated shaking (at an interval of 6 h) was done on the reactor for the efficient hydrolysis and extraction of the soluble component from the rice straw. Nitrogen gas was supplied at the interval of 24 h for 30 min to reduce DO concentration in a bioreactor. Performance of the batch reactors was evaluated on one or more than one oxyanions removal using the above-mentioned carbon sources. Each set of experiments was conducted for about 40 days. Different batch reactors were used and their operating schedule is given in table 4.4.2. Figure 4.4.2 shows the (a) schematic diagram and (b) lab-scale photograph of the batch reactor. All the batch reactors were monitored at the interval of 5 days.

Figure 4.4.1. (a) Schematic diagram (b) Images of thermoalkali treated rice straw slurry

TH-2881_146104040

Table 4.4.1. Designation of rice straw slurry as a carbon source for the microorganism

Designation	**Mass of Rice Straw (g/L)**	**Specification**	**Case**
RS-WTA	100	Without thermo alkali treated rice straw slurry	Kept at room temperature along with distilled water for 10 days
RS-$Ca(OH)_2$		$Ca(OH)_2$ (2.5 % w/w) treated rice straw slurry	Heated at 85° C, for 240 min in hot air oven
RS-NaOH		NaOH (1 % w/w) treated rice straw slurry	
RS-ChOH		ChOH (0.4% v/v) treated rice straw slurry	

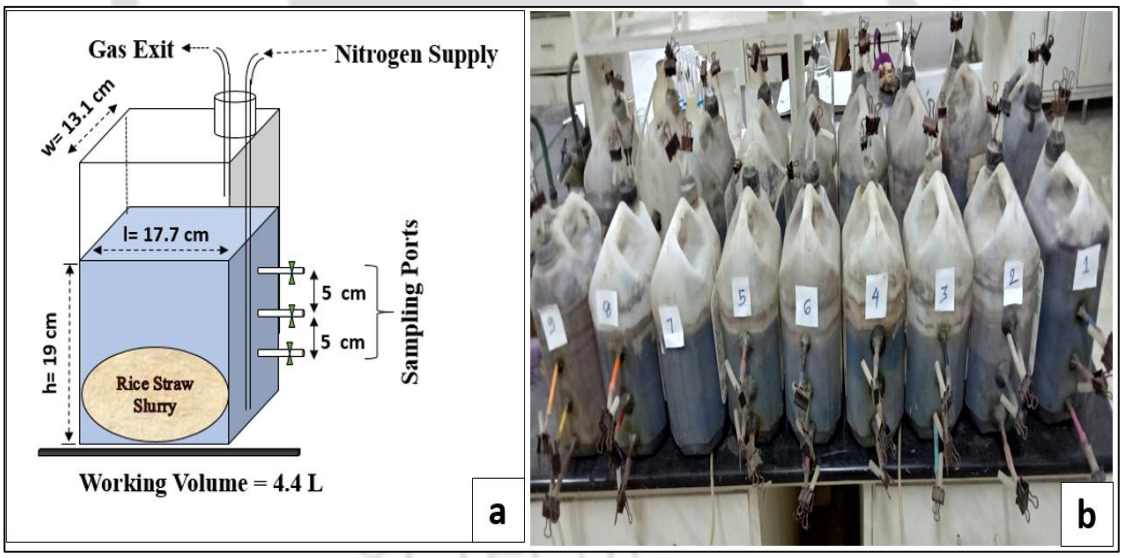

Figure 4.4.2. (a) Schematic diagram (b) Lab-scale photograph of batch reactor

TH-2881_146104040

Table 4.4.2. Operating schedule of batch reactor

Reactor Name	Oxyanion Concentration			Matrix Used	Purpose/Study Carried Out	Remarks
	Sulfate (mg/L)	Nitrate (mg/L)	Arsenic (µg/L)			
AC	1000	100	100	Paper mill sludge	Suspended growth used for the acclimatization of sulfate, nitrate, and arsenic	FESEM, EDX, XRD analysis, 16S rRNA, and TRFLP test of raw paper mill sludge before acclimatization
R1, (pH 3) **R2, (pH 5)** **R3, (pH 7)** **R4, (pH 9)**	2000	-	-	RS-WTA+ Inoculum	Effect of pH on performance of batch reactors using RS-WTA as carbon source	FESEM analysis of bio solids of reactors R1, R2, R3, and R4 and EDX analysis of reactor R3
R-Ca(OH)$_2$ **R-NaOH** **R-ChOH**	2000	-	-	RS-Ca(OH)$_2$ + Inoculum RS-NaOH+ Inoculum RS-ChOH+ Inoculum	Effect of various thermoalkali treatments on the performance of batch reactors using RS-Ca(OH)$_2$, RS-NaOH, RS-ChOH as carbon source	FESEM and EDX analysis of bio solids reactors of R-Ca(OH)$_2$, R-NaOH and R-ChOH
S1 **S2** **S3**	1000 2000 3000	-	-	RS-NaOH+ Inoculum	To remove the different concentrations of sulfate using RS-NaOH as a carbon source	FESEM and EDX analysis of bio solids of reactor S3

TH-2881_146104040

N1 **N2** **N3**	-	500 1000 2000	-	RS-NaOH+ Inoculum	To remove the different concentrations of nitrate using RS-NaOH as a carbon source	FESEM analysis of bio solids of reactor N3
SN1 **SN2** **SN3**	2000	500 1000 2000	-	RS-NaOH+ Inoculum	Simultaneous removal of sulfate and nitrate using RS-NaOH as a carbon source	FESEM, EDX analysis of bio solids and 16S rRNA, and TRFLP test of bio sludge of reactor SN3
SA1 **SA2** **SA3**	2000	--	500 1000 2000	RS-NaOH+ Inoculum	Simultaneous removal of sulfate and arsenic using RS-NaOH as a carbon source	FESEM, EDX, XRD, FETEM analysis of bio solids and Taxonomic profiling and TRFLP test of bio sludge of reactor SA3
SAN1 **SAN2** **SAN3**	2000	500 1000 2000	1000	RS-ChOH+ Inoculum	Simultaneous removal of sulfate, nitrate, and arsenic using RS-ChOH as a carbon source	FESEM, EDX, XRD, FETEM analysis of bio solids and 16S rRNA and TRFLP test of bio sludge of reactor SAN3
S0N0	-	-	-	RS-NaOH+ Inoculum	To observe the anaerobic digestion of RS-NaOH without the addition of oxyanions	FESEM analysis of bio solids of reactor S0N0
CR	2000	2000	1000	Inoculum	Simultaneous removal of sulfate, nitrate, and arsenic using inoculum	-

TH-2881_146104040

Reactors R1 (pH 3), R2 (pH 5), R3 (pH 7), and R4 (pH 9) were operated to observe the effect of pH on the performance of batch reactors using RS-WTA as a carbon source. Reactor R-$Ca(OH)_2$, R-NaOH, and R-ChOH were operated to observe the effect of various thermoalkali treatments on the performance of batch reactors using RS-$Ca(OH)_2$, RS-NaOH, and RS-ChOH as carbon sources. Reactors S1, S2, and S3 were operated to remove the different concentrations of sulfate using RS-NaOH as a carbon source. Reactor N1, N2, and N3 were operated to remove the different concentrations of nitrate using RS-NaOH as a carbon source. Reactor SN1, SN2, and SN3 were operated for simultaneous removal of sulfate and nitrate using RS-NaOH as a carbon source. Reactor SA1, SA2, and SA3 were operated for simultaneous removal of sulfate and arsenic using RS-NaOH as a carbon source. Reactor SAN1, SAN2, and SAN3 were operated for simultaneous removal of sulfate, nitrate, and arsenic using RS-ChOH as a carbon source. Reactor S0N0 was operated to observe the anaerobic digestion of RS-NaOH as a carbon source, without the addition of oxyanions. Reactor CR was operated for simultaneous removal of sulfate, nitrate, and arsenic using inoculum.

TH-2881_146104040

4.5. Fabrication, Operation, and Performance Evaluation of an Attached Growth Packed Bed Continuous Reactor (PBCR)

An attached growth packed bed continuous reactor (PBCR) was fabricated using a 1.2 m long, 10.3 cm internal diameter Perspex cylinder, and operated in continuous mode. Polyurethane foam of cuboid shape (approximate size of each is 2.5cm X 1.5cm X 1.5cm) was used as the packing media. The working volume of the reactor was 6.1 L. Detailed specifications of the attached growth packed bed continuous reactor are given in table 4.5.1. Figure 4.5.1 shows the (a) schematic diagram and (b) lab-scale images of the packed bed continuous reactor (PBCR) set up (when dextrose is used as a carbon source). Figure 4.5.2 shows the (a) schematic diagram and (b) lab-scale images of packed bed continuous reactor (PBCR) set up (when liquor after anaerobic digestion of RS-NaOH slurry is used as carbon source).

Table 4.5.1. Detailed specification of attached growth packed bed continuous reactor (PBCR)

Parameter	**Unit**
Column Height	120 cm
Inner Diameter	10.3 cm
Water Height	110 cm
Total Volume	9.1 L
Packed-Bed Height	65 cm
Volume Occupied by PUF	3L
Working Volume	6.1 L
Porosity of PUF	0.67
Bulk Density	30 g/L

- ***Screens***

Two nos. of perforated (about 70% perforation, 1 mm diameter holes) circular discs of 10.2 cm diameter of perspex sheets were placed at the top and bottom of the reactor bed. The main purpose of the bottom perforated disc was to support the PUF. The purpose of the top perforated disc is to prevent PUF from being washed out.

- ***Deflector Beam***

A hollow Perspex disc, with an external diameter of 10.2 cm (same as the internal diameter of the main reactor), and an internal diameter of 9.2 cm, tapered from the outer dia was fitted inside the main reactor, at the depth of 20 cm below from the top, was used as deflector beam to deflect the gasses at the top. Figure 4.5.3, shows the photograph of (a) deflector beam (b) screen (top) (c) screen (bottom).

TH-2881_146104040

- ***Sampling Ports***

Total of 11 nos. of sampling ports was provided (P1 to P11) at the center to center distance of 20 cm for the collection of samples at different depths of the reactor. The ports were closed with metallic clips except during sampling. Effluent from the reactor was collected into the effluent tank from the topmost port (P11).

- ***Biogas Release System***

Gas release system adopted in this study consisted of 2 no. of reagent bottles of capacity 1000 mL. The biogas was first allowed to pass through the empty bottle and then, through the partially filled with distilled water, before being released into the atmosphere. This system prevents contamination of reactor content from atmospheric gas as well accidental mixing up of distilled water in the second bottle with reactor content.

- ***Biomass Supporting Material***

Polyurethane foam (PUF) procured from the local market was used as a supporting material for microbial growth. PUF was cut into cuboid shapes of approximately 2.5 cm x 1.5 cm x 1.5 cm. The cuboid-shaped PUF was washed twice with deionized water, autoclaved (20 min, 120^{o}C), rewashed, and dried overnight at 70^{o}C, in a hot air oven before being used as supporting material in a packed bed continuous reactor (PBCR). Approximately, 270 g of oven-dried PUF was placed inside the reactor. The bulk density was approximately 30 g/L as determined by measuring the weight of PUF cuboids in a 1 L graduated cylinder. Void volume was 670 mL, which is equivalent to 0.67 as porosity.

- ***Inoculation and Start-up***

Seed sludge after the acclimatization was used to inoculate the packed bed continuous reactor (PBCR) with maintaining the MLVSS concentration of 7590-7865 mg/L using a peristaltic pump (Model: PP10, Maclin India). Any biomass washed out of the reactor along with the effluent was recycled back. After the inoculation, the reactor was purged with nitrogen gas for 30 min to establish the anoxic condition. Subsequently, the reactor was fed continuously with synthetic media (table 4.2) and influent pollutant concentration (table 4.5.2). The reactor was operated at room temperature 30±5^{o}C. After the inoculation, the reactor was fed with synthetic wastewater at a controlled flow rate

TH-2881_146104040

using the peristaltic pump from the bottom, in an upward direction. To maintain the HRT of 36, 24, 18, and 15 h, the flow rate was kept at 2.66, 4.23, 5.64, and 6.7 mL/min, respectively using a peristaltic pump.

- ***Operating Schedule of PBCR***

The performance of the reactor (PBCR) was evaluated for the removal of sulfate, nitrate, and arsenic on daily basis. The operation of the reactor was divided into four phases. In the first phase of the study, optimization of HRT was done using dextrose as a carbon source, in presence of sulfate (1500 mg/L), nitrate (100 mg/L), arsenic (1000 µg/L), and COD (3000 mg/L). In phase 2, the effect of varying concentrations of nitrate (100, 400, 600, 800, 1000 mg/L) addition on the reactor performance is monitored while feed sulfate (1500 mg/L) and arsenic (1000 µg/L) concentrations were fixed, along with COD concentration of 4500 mg/L. In phase 3, the effect of arsenic (1000, 1500, 2000 µg/L) addition on the reactor performance was monitored in presence of fixed sulfate (1500 mg/L), nitrate (800 mg/L), and COD (4500 mg/L) in the feed. In phase 4, the application of liquor of partially digested RS-NaOH slurry was added as a carbon source and simultaneous removal of the oxyanions (sulfate 1500 mg/L; nitrate 800 mg/L; arsenic 2000 µg/L) was monitored. The operation schedule of the PBCR operated in continuous mode is given in table 4.5.2.

Liquor from rice straw used in the PBCR was prepared through the following steps. First of all RS-NaOH slurry was prepared [as explained in section 4.4], then this slurry was anaerobically digested in batch reactor S0N0 along with synthetic feed media (as explained in section 4.4). Figure 4.5.4 shows the process adopted for the preparation of liquor to be used as a carbon source. The liquor (supernatant) after the anaerobic digestion from the batch reactor S0N0, was collected on the 10^{th} day of operation, the initial characteristics are given in table 4.5.3. The liquor thus collected was used as a carbon source in phase 4 in the attached growth reactor (PBCR) for its operation. The selection of liquor of S0N0 after 10 days of operation was due to the fact that after this period COD, BOD and VFA concentration was the maximum in the supernatant (performance of S0N0 is discussed in results and discussion section 5.4.8). The liquor of partially digested RS-NaOH slurry from the batch reactor S0N0 was collected in the influent tank (tank no. 1) and pumped into the attached growth bioreactor (PBCR) along with the feed media with the help of a peristaltic pump.

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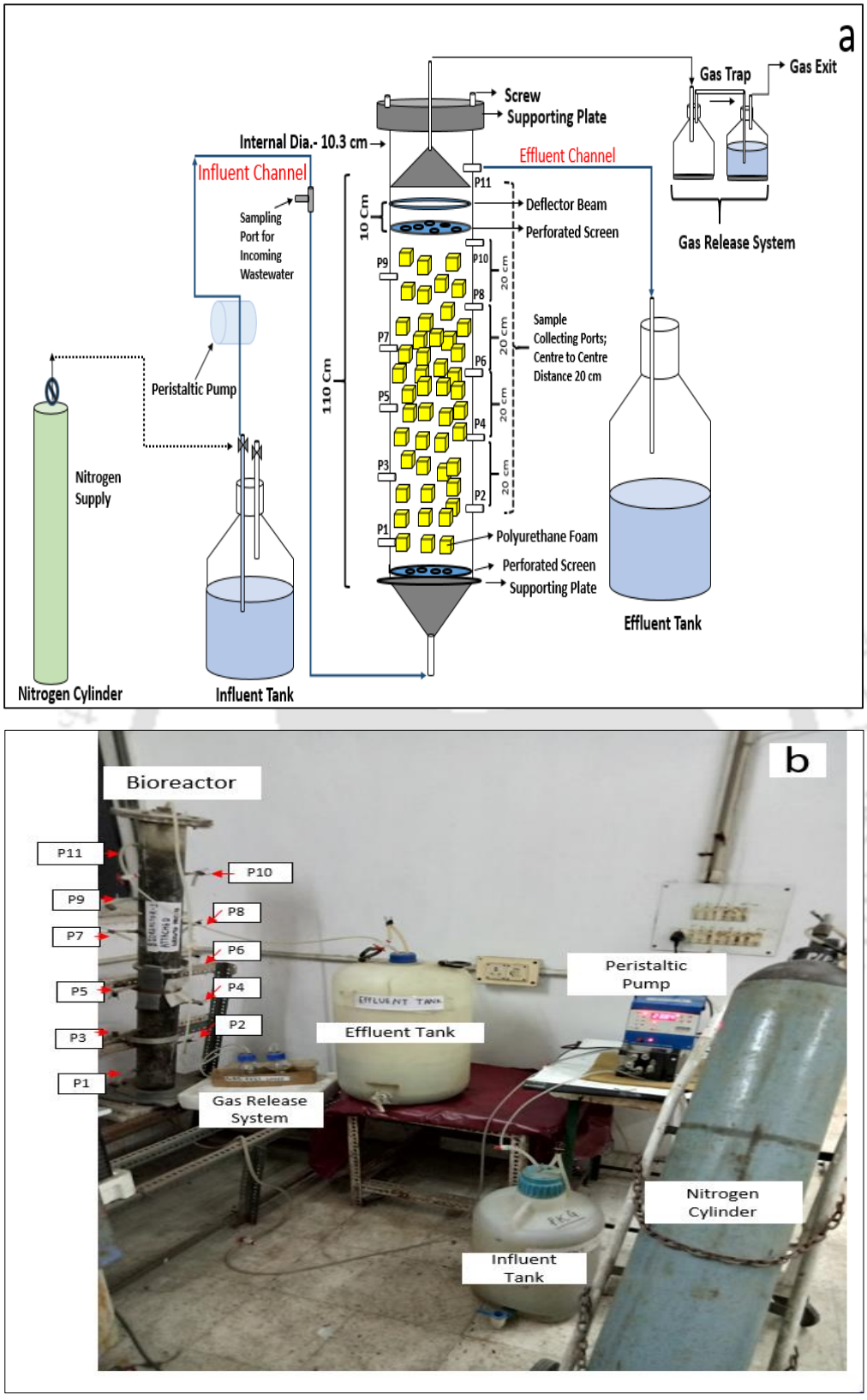

Figure 4.5.1. (a) Schematic diagram (b) Lab-scale images of packed bed continuous reactor (PBCR) set up (when dextrose used as carbon source)

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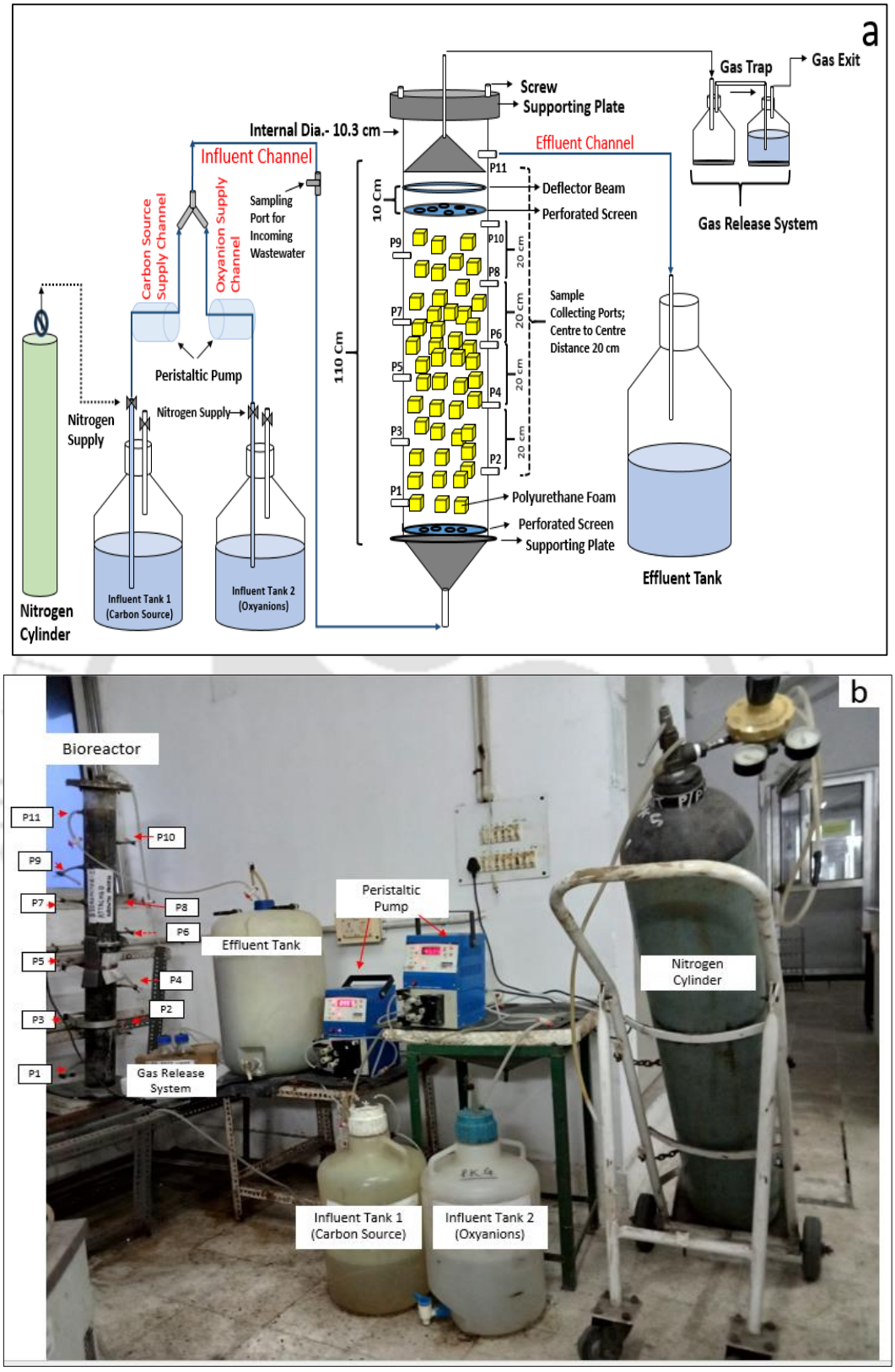

Figure 4.5.2. (a) Schematic diagram (b) Lab-scale images of packed bed continuous reactor (PBCR) set up (when liquor after anaerobic digestion of RS-NaOH slurry used as carbon source)

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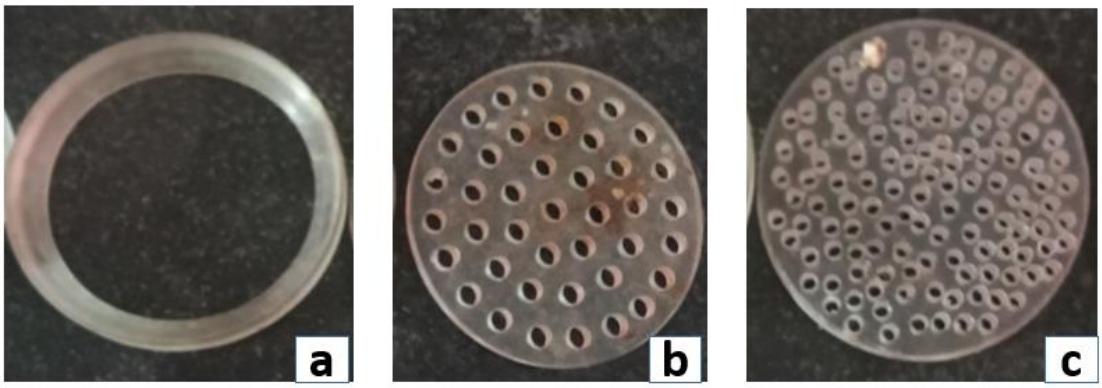

Figure 4.5.3. Photograph of (a) Deflector beam (b) Screen (top) (c) Screen (bottom)

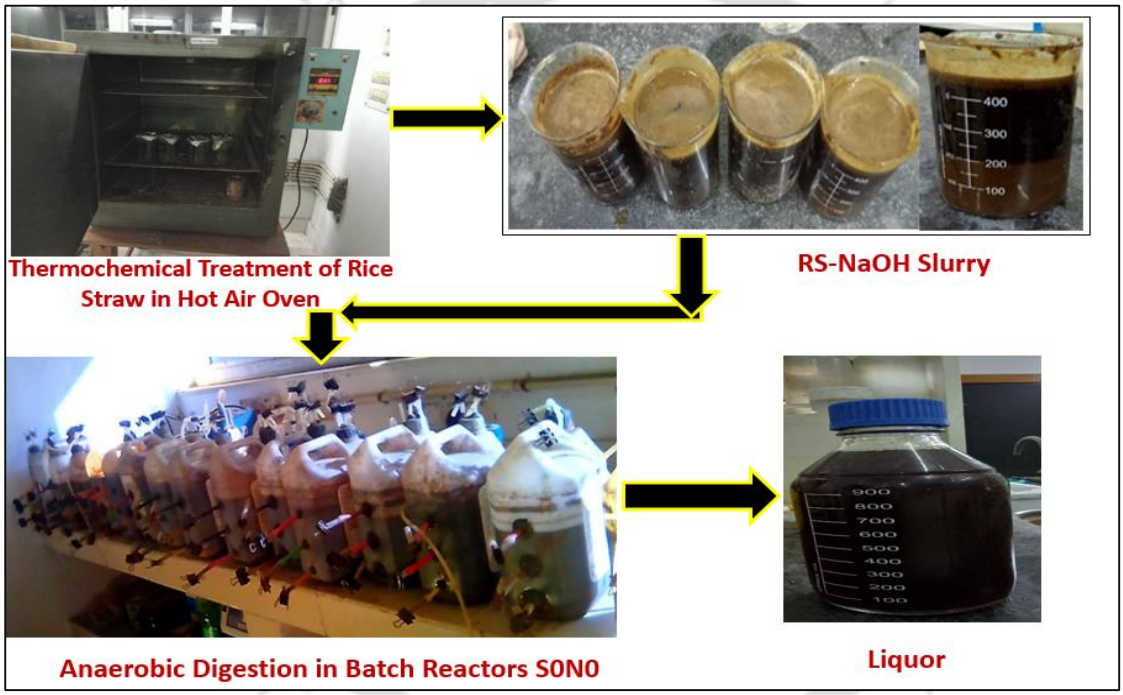

Figure 4.5.4. Process adopted for the preparation of liquor to be used as a carbon source

TH-2881_146104040

Table 4.5.2. Operating and feeding condition of attached growth packed bed continuous reactor (PBCR)

Carbon Source	Phase/ Purpose	No. of Day	HRT	COD (mg/L)	Sulfate (mg/L)	Nitrate (mg/L)	Arsenic (µg/L)	Remarks
Dextrose	Phase-1 (Effect of HRT)	1-30	36	3000	1500	100	1000	
		31-40	24					
		41-50	18					
		51-60	15					
		61-69	24					
	Phase-2 (Different Nitrate Concentration)	70-84	24	4500	1500	100	1000	Port profile at 105 d
		85-100				400		
		101-115				600		
		116-130				800		
		131-144				1000		Port Profile at 140 d
	Phase-3 (Different Arsenic Concentration)	145-160		4500	1500	800	1000	Port Profile at 190 d, and EDX, XRD, FESEM, FETEM Analysis
		161-175					1500	
		176-190					2000	
Liquor of Partially Digested RS-NaOH Slurry	Phase-4	191-230	24	-	1500	800	2000	Port Profile at 220 d, XRD, EDX Analysis

TH-2881_146104040

Table 4.5.3. Characteristics of liquor obtained from batch reactor S0N0

Parameter	Unit	Concentration
pH	-	6.35-6.89
TCOD	mg/L	13200-14428
COD	mg/L	11268-12345
BOD	mg/L	6124-6980
TRS	mg/L	4238-4450
VFA	mg/L	4050-4820

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TH-2881_146104040

4.6. Analytical Procedure

In this section, the methods and protocols that have been adopted for the analysis of various parameters have been elaborated.

4.6.1. Rice Straw Hydrolyzate Characteristics

After thermochemical treatment, samples were cooled and hydrolyzate samples (liquid) were filtered through Whatman filter paper (grade 1).

COD was estimated by following the standard method (Eaton et al., 2005) (5220 C-COD), and volatile fatty acids (VFA) were analyzed by following the direct titration method (Dilallo and Albertson, 1961).

Total reducing sugar (TRS) was analyzed by following the DNS (3,5- dinitrosalicylic acid) reagent method (Chang et al., 2011; Miller, 1959). To determine the reducing sugar concentration, 2 mL of the DNS solution was added to 2 mL of the filtered sample and the mixture was kept, in a boiling water bath for 10 min. This absorbance was measured by the spectrophotometer (Visiscan) at a wavelength of 550 nm to determine the yield of reducing sugar; glucose was used as the standard. The hydrolysis rate % was calculated using formulae proposed by (Zhao et al., 2010), where 0.9 is the ratio of mole weight of cellulose (162) to mole weight of glucose (180).

$$Hydrolysis\ Rate\ \% = \frac{Reducing\ Sugar \times 0.9}{Cellulose + Hemicellulose} \times 100$$

4.6.2. Rice Straw Compositional Characteristics

The solid portion of pretreated rice straw was washed with distilled water until the pH of washed water become near neutral and subsequently dried overnight at 80^{o}C, ground using mortar and pestle to obtain the fine powder, and stored in plastic bags for structural and compositional analysis.

(i) Moisture Content, Ash, Organic Matter, Organic Carbon, Weight Loss

The moisture content of rice straw was determined by drying samples at 100±5°C for 24 h in a hot air oven (Tiquia and Tam, 1998). Ash content was determined by igniting the dried sample in a muffle furnace at 500±50°C for 5 h (Tiquia and Tam, 1998), and the organic matter (OM) was calculated as the difference between ash and dry weight as a percentage. From the values of OM, the percentage of organic carbon (OC) was calculated by multiplying OM by 0.58 (Tiquia and Tam, 1998). The weight loss was

TH-2881_146104040

determined by weighing the samples before and after pretreatments, after drying in a hot air oven at 100 °C to a constant weight (Kaur and Phutela, 2016).

(ii) Cellulose

The cellulose content in rice straw was estimated by the anthrone reagent method after taking absorbance at 630 nm (Updegraff, 1969). Cellulose undergoes acetolysis with acetic-nitric reagent forming acetylated cellulodextrins which get dissolved and form the glucose molecules on the treatment with 67% H_2SO_4. This glucose molecule is dehydrated to form hydroxymethyl furfural which forms a green color product with anthrone reagent and the color intensity is measured at 630 nm. Two reagents are prepared likewise i. e. Acetic-Nitric reagent; (150 mL of 80% acetic acid and 15 mL of nitric acid) and anthrone reagent (200 mg of anthrone in 100 mL of 95% sulfuric acid). A powdered form of rice straw sample (0.5 g or 1 g) and 3mL acetic/nitric reagent were taken in the test tube and mixed in a vortex mixer. The mixture was placed in a boiling water bath at (~100°C) for 30 min. After this, the contents were cooled and centrifuged for 15-20 min, the supernatant was discarded, and the residue was washed with distilled water. In the residual portion, 10 mL of 67% sulfuric acid was added and kept for 1h at room temperature. 1mL of the above solution was diluted to 100 mL. 1mL of this diluted solution was mixed with 10 mL of anthrone reagent and heated in a boiling water bath (~100°C) for 10 min which will develop a green color. After this process samples were cooled and color intensity was measured at the wavelength of 630 nm using a spectrophotometer (Visiscan). The standard stock solution was prepared using cellulose powder.

(iii) Hemicellulose

Hemicellulose was analyzed according to the protocols suggested by (Goering and Van Soest, 1975). Two reagents are required for the estimation of hemicellulose i. e. NDS (neutral detergent solution) and ADS (acid detergent solution). Decahydronaphthalene, sodium sulfite, and acetone are also required.

NDS (Neutral Detergent Solution)

Disodium ethylenediamine tetraacetate (18.61 g) and sodium borate decahydrate (6.81 g) were dissolved in 200 mL of heated distilled water, followed by the addition of 150 mL solution containing 30 g of sodium lauryl sulfate and 10 ml of 2-ethoxy ethanol. To

TH-2881_146104040

this solution, 4.5 g of disodium hydrogen phosphate were added and the volume was adjusted to 1 L with the addition of distilled water.

ADS (Acid Detergent Solution)

In 1 L of the volumetric flask, 27.84 mL of sulfuric acid and 20 g of trimethyl ammonium bromide were added and finally, the volume was adjusted to 1 L with the addition of distilled water.

- **NDF (Neutral Detergent Fiber) Measurement**

1 g of the dried powdered sample was mixed with 2 mL decoline, 10 mL NDS (neutral detergent solution), and 0.5 g sodium sulfite, the mixture was heated in a boiling water bath (~100^0C) for 60 min. The content was filtered and washed thoroughly with the addition of hot water. Final washing was done with the addition of 10 mL of acetone and the content was centrifuged. The supernatant was discarded and the residual was transferred to a crucible and dried at 100^0C for 8 h. Finally, after cooling weight of the content was measured.

$$\text{NDF \%} = \frac{\text{Wt}_{\text{Crucible+Residue}} - \text{Wt}_{\text{Crucible}}}{\text{Initial Wt}} \text{X100}$$

- **ADF (Acid Detergent Fiber) Measurement**

1 g of the dried powdered sample was mixed with 10 mL ADS (acid detergent solution) and 0.5 g sodium sulfite, the mixture was heated in a boiling water bath (~100^0C) for 60 min. The content was filtered and washed thoroughly with the addition of hot water. Final washing was done with the addition of 10 mL of acetone and the content was centrifuged. The supernatant was discarded and the residual was transferred to a crucible and dried at 100^0C for 24 h. Finally, after cooling weight of the content was measured.

$$\text{ADF \%} = \frac{\text{Wt}_{\text{Crucible+Residue}} - \text{Wt}_{\text{Crucible}}}{\text{Initial Wt}} \text{X 100}$$

Hemicellulose % = NDF % – ADF %

NDF (g) = Hemicellulose + Cellulose + Lignin + Minerals

ADF (g) = Cellulose + Lignin + Minerals

(iv) Lignin and Biodegradability Fraction

TH-2881_146104040

Lignin content in rice straw was analyzed by following the procedure suggested by (Inari et al., 2007). In a 100 mL volumetric flask, 40 mL of distilled water and 0.5 g of rice straw were taken, heated at 80ºC for 3 h in a water bath with the addition of 0.5 g of sodium chlorite, and 0.1 mL of acetic acid thrice at the interval of 1 h. The suspension was filtered and the residue was washed with distilled water and dried at 100±5^{0}C (24 h). The weight loss expressed as a percentage is considered as lignin content.

The biodegradability fraction of rice straw was calculated using the empirical equation proposed by (Gibert et al., 2004).

$$B = -0.028 \times \text{Lignin Content (\%)} + 0.830$$

(v) CHNS Analysis

The carbon and hydrogen percentage of untreated and pretreated rice straw samples (dried powdered form) was analyzed in a CHNS analyzer (EuroEA3000 Elemental Analyser) at Biotech Park, Guwahati, India.

(vi) X-Ray Diffraction (XRD) Analysis

The crystallinity index of untreated and pretreated rice straw was determined using the X-Ray diffractometer (Rigaku TTRAX III). The X-Ray unit was operated at 25ºC (50 kV and 100 mA) with CuKα radiation (λ=1.542 Aº). The θ-2θ method was used to collect the diffraction spectra. The rice straw sample was scanned over the angular range (2θ) from 5 to 60, with a step size of 0.03 and a step time of 0.5 s. The crystallinity index (Cr I) was calculated by using the equation given below, where I_{cry} and I_{am} the intensity of crystalline and amorphous regions at 2θ = 22.480 and 17.10, respectively (Segal et al., 1959).

$$\text{Cr Index} = \frac{I_{cry} - I_{am}}{I_{cry}} X100$$

(vii) FTIR Analysis

The dried powdered sample (1mg) was mixed with 300 mg of KBr in a mortar and then the mixture was compressed for 3 min at 10 MPa, to set up the sample disc. The infrared spectrum was measured from the range of 4000 to 400 cm^{-1}, using the PerkinElmer Spectrum Version model with a resolution of 4 cm^{-1} (16 scans). The changes in the functional group and band position were analyzed by measuring the spectra and compared to each other.

TH-2881_146104040

(viii) Thermogravimetric (TGA) Analysis

Thermogravimetric analysis (TGA) is an analytical technique used to determine a material's thermal stability and its fraction of volatile components by monitoring the weight change that occurs as a sample is heated at a constant rate. The structural rigidity change on the substrate was probed using TGA of untreated and pretreated rice straw. The rice straw was exposed at a temperature range from 20˚C to 600˚C at a flow rate of 10.0 (K/min) using a thermal analyzer (NETZSCH STA 449 F3). The samples were weighed to about 6 mg and loaded into an alumina pan.

(ix) FESEM Imaging

The morphology of untreated and pretreated rice straw was studied with a scanning electron microscope (Zeiss, Sigma). The modifications of the morphology were studied by taking the images at several magnifications. The samples were made conductive by sputtering with gold and observed using a 5 kV voltage source.

(x) BET Surface Area

The BET surface areas of rice straw were measured by nitrogen adsorption-desorption isotherms at -196°C in a surface-area analyzer (Autosorb-IQ MP) (Wi et al., 2013). Before determination, the powdered form of rice straw sample (~0.8 g) was degassed for 1.5 h at 110°C under vacuum (5 mm Hg) to remove moisture. The total pore volume was assessed by converting the amount of nitrogen gas adsorbed to the volume (cm^3/g at STP) of liquid adsorbate, using single point adsorption (at a relative pressure circa 0.99).

4.6.3. Wastewater Characteristics

The wastewater sample was collected from the effluent ports and centrifuged (R-24 REMI, India) at 6000 RPM for 15 mins and filtered with a whatman filter paper (grade 1). The pH of the wastewater was measured using a digital pH meter (Systronics, India), with a sensitivity of 0.01. The instrument was calibrated before use with standard buffer solutions of pH 4, 7, and 9.2.

Wastewater samples were analyzed for sulfate (Nephelo-turbidity meter 132, Systronics, India), sulfide, nitrate (UV-Spectrophotometer CARY50BIO, Varian, the USA at 220 and 275 nm), nitrite (Spectrophotometer Visiscan at 543 nm), COD (Digestor Hach DRB 200 USA; Spectrophotometer Visiscan at 600 nm) and BOD

TH-2881_146104040

(Incubator ICT, India) and on the same day using standard method (Eaton et al., 2005) (4500E-SO_4^{2-}, 4500F-S^{2-}, 4500B-NO_3^-, 4500B-NO_2^-, 5220C-COD, 5210 B- BOD) respectively. BOD_5^{20} is represented as BOD in the entire thesis. The MLSS and MLVVS content were measured by following the standard methods (Eaton et al., 2005). VFA was estimated by the titration method proposed by (Dilallo and Albertson, 1961). Filtered samples were taken and initial pH was measured and pH was adjusted up to 3.3 using 0.05N H_2SO_4. Carefully boiled the sample minimum for 3 minutes in soxhlet apparatus and cooled at room temperature. Samples were back titrated against 0.05 N NaOH up to pH 4 and subsequently up to pH 7. The calculation for volatile acid alkalinity was done using the formula below given.

$$\textbf{Volatile Acid Alkalinity} = \frac{\textbf{Volume of 0.05N consumed for titration from pH 4 to 7}}{\textbf{Volume of Sample}} \textbf{X 2500}$$

Volatile acid concentration

- Case 1. If volatile acid alkalinity >180 mg/L

Volatile acid concentration =Volatile acid alkalinity X 1.5

- Case 2. If Volatile acid alkalinity < 180 mg/L

Volatile acid concentration = Volatile acid alkalinity X 1

Arsenic concentration was estimated according to the guideline of the AAS manual using a hydride generation atomic absorption spectrophotometer (VGA-77 and SpectrAA 55B, Varian, USA) having an electrodeless discharge lamp at 193.7 nm wavelength, at a flow rate of 0.50 mL/min to generate arsenic hydrides, with argon gas into a quartz cell at 900 °C. Before the measurements, all samples (filtered through 0.45 µm) were mixed with 8.3 mL of HCl, subsequently, 1 g potassium iodide was added as a reducing agent and the final volume of 100 mL was maintained by adding deionized water. Samples, blanks, and standards subject to the same conditions were analyzed.

The standard calibration curve of COD, sulfate, nitrate, nitrite, TRS, and cellulose is shown in appendix A1, A2, A3, A4, A5, and A6 respectively.

4.6.4. Mineralogical Studies

Collection of Bio solids

Bio solids from the batch and continuous reactor were collected in a nitrogen-filled container to minimize the contact with air and were spun at 6000 rpm for 10 min

TH-2881_146104040

followed by lyophilization at −105 °C to ensure no oxidation of arsenic sulfides. The freeze-dried samples were used for FESEM, EDX, XRD, and TEM analysis.

(i) FESEM and EDX Analysis

Frieze dried bio solids were analyzed for FESEM and EDX analysis. Energy-Dispersive X-Ray spectroscopy (EDX) is an X-Ray technique used to identify the elemental composition of materials. It is an analytical technique used for the elemental analysis or chemical characterization of the sample. Frieze dried sample was lightly dusted onto the carbon tape of SEM stub surface and coated with gold using a Scancoat Six SEM sputter coater system.

(ii) X-Ray Diffraction (XRD) Analysis

Frieze dried bio solids were used as a sample in XRD analysis (Rigaku TTRAX III diffractometer). X-Ray powder diffraction was used to check the existence and crystalline form of the solids phase generated in bioreactors. The analysis was carried out with a PAN analytical X'pert PRO-MPD diffractometer equipped with a monochromator in the diffraction beam. The X-Ray unit was operated at 25ºC (50 kV and 100 mA) with CuKα radiation (λ=1.542 A^{O}). The θ-2θ method was used to collect the diffraction spectra. The sample was scanned over the angular range (2θ) from 5 to 60, with a step size of 0.03 and a step time of 0.5 s.

(iii) FETEM Analysis

Frieze dried bio solids were pulverized, dispersed in acetone, and then subjected to ultrasound vibration (Vibra-Cell Model VC 505, Sonics, USA) for 30 min to disperse the sample homogeneously. Then one drop of the suspension was drop-casted on the carbon-coated copper (TEM) grid using a micropipette. After being well dried under ambient conditions, the grid was mounted on the TEM specimen holder for examination. A transmission electron microscope (TEM) was carried out on JEOL 2100 FETEM operating at 200 kV to determine the spatial features of the bio-precipitate. Samples were investigated thoroughly by TEM selected-area electron diffraction (SAED), high resolution (HRTEM). A series of Fast Fourier Transform (FFT) patterns calculated from the HRTEM images of the sample was to identify their crystal structures. ImageJ and Gatan Digital Micrograph software (version 3.4) was used to determine the d-spacing from the selected area electron diffraction (SAED) pattern and HRTEM image, respectively.

TH-2881_146104040

4.6.5. Microbial Community Structure and Population Dynamics

Metagenomic study of raw paper mill sludge and bio sludge of batch reactors (SN3, SA3, and SAN3 on the 40th day) was performed, to identify microbial community and predicted the role of the mixed bacterial population present in complex polybacterial population. Metagenomics analyses of microbial community were performed on V3–V4 variant regions of the 16S rRNA genes and whole genomic. After completion of the performance study, bio sludge was collected in a plastic bottle. The bottle was filled up with N_2 gas (to avoid interference of oxygen) before sample collection. It was sealed with a teflon tap, immediately after sample collection with an ice gel pack, which was then shipped to Genombio Lab Pune, Maharashtra, India, for metagenomic sequencing. Genomic DNA was extracted using a DNA extraction kit as per the manufacturer's protocol.

(i) T-RFLP

T-RFLP analysis is a rapid, convenient, sensitive, high-throughput, and highly reproducible method in microbial ecology studies and thus has been often used for analyzing large numbers of samples in a study. In this method, T-RF patterns are generated by amplifying DNA fragments from bacterial assemblage using PCR with one or two fluorescently labeled primers, followed by digestion with restriction enzymes. 'Fingerprints' produced by T-RF patterns are used to assess the diversity and dynamics of microbial communities.

Furthermore, the community patterns generated by T-RFLP are quite consistent with those generated by other DNA fingerprinting techniques, e.g. the more laborious DGGE (Moeseneder et al., 1999) and ribosomal intergenic spacer analysis (RISA) (Hartmann et al., 2005). The other advantage of T-RFLP is that it offers the possibility of comparing data with available databases, e.g. the Ribosomal Database Project and Phylogenetic Assignment Tool (PAT; http://trflp.limnology.wisc.edu/), which is rarely possible with DGGE.

The primary goal of the t-RFLP analysis is to test the environmental samples using 16S rDNA as well as 18S rDNA amplification from community and restriction enzyme digestion (TaqI, HaeIII) and to perform T-RF analysis (presence/absence of peaks and area of peaks) on the determination of bacterial species richness, diversity index,

TH-2881_146104040

community structure and subsequent comparison of profiles between environmental samples.

T-RF numbers have been widely used as indicators of microbial species richness in current environmental microbial studies using the T-RFLP technique (Denaro et al., 2005). However, some results indicated that this estimation would be affected largely by the restriction enzyme used. Although this was suggested by previous studies (Bernbom et al., 2006; Hartmann et al., 2005) using multi digestions, most of them indicated that the use of different enzymes always generated the same trend of species richness or diversity index when comparisons were made among different environmental samples.

Furthermore, high variability in peak height and area was also observed in environmental studies (Dunbar et al., 2001; Osborn et al., 2000). These problems have limited the utility of T-RFLP as a quantitative method, as well as the utility of T-RFs in the calculation of diversity indexes. Our results highlighted the other biases produced by different restriction enzyme digestions. The variability of species richness and diversity index among enzyme digestions may be related to the fact that the same T-RF can be generated by different species of bacteria (Blackwood et al., 2003). The bacterial species that produced a particular T-RF after one restriction enzyme digestion may generate more than one T-RF after another enzyme digestion, and vice versa. Therefore, the peaks or 'genotypes' would have different species compositions in different enzyme digestions. The number and area of T-RFs varied depending on different species compositions. Consequently, the species richness and the diversity index changed. Furthermore, the variation between observed and theoretical T-RFs would depend on the restriction enzyme selected (Kaplan and Kitts, 2004). The 'precision' of enzyme digestion affected the definition of T-RFs and consequently the determination of the number and area of T-RFs.

(ii) DNA Isolation from Samples

A hundred milligram of the sample was weighed and processed as per the manufacturer's instruction (Qiagen DNeasy kit). Total DNA was eluted in 40.0 µL of Nuclease-free water. Quality assessment of DNA was done by agarose gel electrophoresis.

TH-2881_146104040

(iii) PCR Amplification of the 16S gene

The bacterial 16S rRNA gene was amplified by PCR using the primer set 8F: 5'-AGAGTTTGATCATGGCTCAG-3'and 1492R: 5'-GGCTACCTTGCCACGACTTC-3'(Lane, 1991). The 8F primer was labeled at the 5' end with HEX fluorescent dye. The PCR mixture was 0.5 µL of each primer (10 mM), 5 µL of the PCR buffer, 1 µL of dNTP (2.5 mM), 0.5 µL of Taqpolymerase (2.5 U/ µL), and double-distilled water for final reaction volume of 50 µL. PCR was performed at 95°C for 5 min; 30 cycles of 95° C for 1 min, 55°C for 1 min and 72°C for 1 min; and 72°C for 10 min. PCR products were checked on 1% agarose gels.

(iv) Restriction Digestion and Desalting of Digested Products

Following PCR, 5 µL of PCR products were digested with 0•5 U of Fast Digest TaqI restriction enzymes (NewEnglandBiolabs) for 5 min at 65°C in total 20 µL reaction volume. Digestion was Digests were separated on 3% agarose gel in 1X TBE buffer containing ethidium bromide and visualized under UV light. 10 µL of the digested DNA was desalted using the following procedure: 2.5 µL of 125mM EDTA, 2.5 µL of 3M Sodium Acetate pH 5.2, and 0.2 µL 50mM $MgCl_2$ were added to 10 µL of digested DNA. Further 50 µL of ice-cold ethanol was added into the tubes and mixed well. Tubes were then centrifuged at 12000 RPM for 15 minutes at 6°C. The supernatant was removed carefully. Pellet was then washed with 50 µL of 70% Ethanol with centrifugation at 12000 RPM for 10 minutes at 6°C temperature. Pellet was then dried at 37 °C for 30 minutes.

(v) Sample Preparation and Loading

Hi-Di Formamide (9.7 µL) from Applied Biosystems was added to the dried pellet. Each sample was also added with 0.3 µL GenScan 500 (-250) Liz Internal Size Standard. This mixture was denatured at 95°C for 3 minutes and immediately chilled on ice before loading. The samples were then subjected to electrophoresis on the 3130 Genetic Analyzer using the FA_36_POP7™ run module and G5 dye set.

(vi) GeneMapper Data Analysis

GeneMapper software-based analysis was performed for fragment analysis after completion of the capillary electrophoresis. Output from automated sequencers is in the form of an electropherogram, with peaks representing fluorescently labeled T-RFs detected over time about the size standard. The duration and intensity of the fluorescent

TH-2881_146104040

signal from T-RFs are reflected in the area and height of each peak detected, respectively. Software specific to each sequencing unit collects data from each run. The ABI 3730 capillary sequencer operates, GeneMapper v3.5 (Applied Biosystems), which performs the functions of both GeneScan and Genotyper. Either data collection program provides researchers with several algorithms for sizing sample fragments by comparing their mobility with that of the size standard. Once data are processed and fragment lengths assigned, the dataset is typically imported into a spreadsheet program, such as Microsoft Excel (Microsoft Corp., Redmond, WA). In the spreadsheet, sample identifiers can be added and presence/absence (1, 0) matrices developed. Other manipulations, such as matrix inversions, can also be performed. The T-RFs for each sample run should be closely examined and the entire run evaluated for the average number of T-RFs detected per sample and the number of T-RFs contained in the various size classes.

(vii) T-RFLP Analysis using TRFLP MiCA PAT+

During analysis peak, size and height were considered for analysis for the peak size threshold was set at 60. Fragments with sizes less than 60 bp and greater than 500 bp were not considered. Whereas, Peak height threshold was set at 70. Fragments with peak heights less than 100 were not considered. A separate file was generated for each sample TRFs in .csv format to upload to the online tool TRFLP MiCA PAT+. The necessary inputs were filled in the software like, Forward primer (Labelled) sequence, reverse primer sequence, and Restriction enzyme used for digestion of 16s fragments. The "Greengenes" database was selected for comparison of analyzed data with the data available in the database. The files retrieved from the toll are attached and provided separately.

A list of instruments and equipment used in the present investigation is given in table 4.6.

Table 4.6. Instruments and equipment used in the present investigation

SN	Instruments	Model Manufacturer	Purpose/Parameter tested
1	Digital PH meter	Oraion 3vstar, ThermoScientific	pH
2	Hot air oven	ICT	Thermoalkali pretreatment, MLSS, OM, OC, Lignin, Hemicellulose
3	Muffle Furnace	ICT	MLSS, MLVSS, Ash content
4	Autoclave	Equitron, AAG, 172	Thermoalkali pretreatment

TH-2881_146104040

5	Weighing Balance	SL-234, Denver Instrument	Weight
6	Refrigerator	MRC Scientific Instruments	Sample preservation
7	Lyophilizer	Labconco	Process of sublimation/lyophilisation (-105^{o} C)
8	Peristaltic Pump	Miclin India Limited	Feeding the wastewater
9	Centrifuge	Remi CM-8Plus	Solid separation from Sample
10	Soxhlet Apparatus	Kjheldal Distillation unit	VFA estimation
11	Centrifuge	Remi CM-8Plus	Solid separation from the sample
12	COD digester	DRB 200, HACH	COD
13	BOD Incubator	ICT, India	BOD
14	Nephelo Turbidity Meter	Digital Nephlo-132 Systronics	Sulfate
15	UV Visible Spectrophotometer	Varian, Cary 50 Bio	Nitrate
16	AAS	Varian, Spectra AA, 55B	Arsenic
17	Spectrophotometer	Visiscan	COD, TRS, Nitrite, and Cellulose
18	CHNS Analyzer	CHNS Analyzer (EuroEA3000 Elemental Analyser)	CHNS
19	Surface Area Analyser	Surface area analysis	Autosorb-IQ MP
20	TGA Analyser	NETZSCH STA 449 F3	Structural rigidity and thermal stability
21	FESEM Analyser	ZEISS, Sigma 300, Gemini	Visualize very small topographic details on the surface of the solid sample
22	EDX Analyser	ZEISS, Sigma, Oxford	For quantitative identification of elements
23	XRD Analyser	Rigaku TTRAX III	Indicates the compound formed in the precipitation
24	FETEM Analyser	JEOL, JEM-2100 Plus, Japan	For identification of morphological and structural characteristics of precipitate
25	Digital Ultrasonicator	Ultrasonic Cleaner (Lab companion)	Ultrasonication for FETEM analysis
26	FTIR Analyser	PerkinElmer Spectrum Version	For FTIR analysis

TH-2881_146104040

CHAPTER 5

RESULTS AND DISCUSSION

The discussion on experimental results is made as per the following sequence of aim and scope of the study.

The discussion on experimental results is made as per the following sequence:

1. Effect of thermoalkali (here pretreatment) methods using various alkalis on the hydrolysis, and change in physicochemical properties of rice straw.
2. Initial characteristics of thermoalkali treated rice straw slurry.
3. Collection, characterization, and acclimatization of a suitable biosludge to use as inoculum for bioreactors.
4. Use of rice straw slurry as a carbon source in biological reduction of oxyanions in batch mode.
5. Characterization of bio solids formed in the bioreactors using microscopic analysis.
6. Identification of microbial communities present in the bioreactors.
7. Performance evaluation of attached growth packed bed continuous reactor on oxyanions reduction.

5.1. Effect of thermoalkali (here pretreatment) methods using various alkalis on the hydrolysis, and change in physicochemical properties of rice straw.

This study aims to evaluate the effect of thermoalkali pretreatment techniques on hydrolysis and compositional characterization of the rice straw by varying the condition (time and temperature) and concentration of reacting agents ($Ca(OH)_2$, NaOH, ChOH) in a hot air oven and autoclave. The initial cellulose, hemicellulose, lignin, moisture, and ash content of rice straw was 39.4±2.2 %, 28.9±1.3 %, 12±1.5 %, 8±1.4 %, and 13±1.5% respectively. The organic matter and organic carbon percentage of rice straw were 77±2.8 % and 45±2.5 % respectively. Rice straw is a lignocellulosic compound; cellulose, hemicellulose, and lignin are the main components that can be converted from one form to another form of carbon. Literature (Cheng et al., 2011; Zhao et al., 2010)

TH-2881_146104040

suggests that rice straw can be utilized to produce various types of valuable products such as hydrogen and methane.

5.1.1. Effect of Temperature on Hydrolysis of Rice Straw

The effect of thermoalkali pretreatment on the hydrolysis of rice straw was investigated in terms of COD, TRS, and VFA production. The effect of thermoalkali pretreatment on the physicochemical properties of rice straw was evaluated in terms of quantification of lignocellulosic content and microscopic analysis.

The effect of temperature at a fixed time (20 min) on rice straw hydrolysis in a hot air oven is shown in figure 5.1.1 and given in table 5.1.1. TRS and VFA production were increased with the increase in temperature up to 85^{o}C. After 20 minutes of exposure time and 85^{o}C, the TRS and VFA were 7±0.6 %, and 1.4±0.23% respectively. At the higher temperature (95^{o}C) TRS and VFA production were 6±0.8 %, and 1.2± 0.1 % respectively which were less than that of at 85^{o}C.

Several other researchers have reported the temperature between 70^{o}C and 100^{o}C is the optimum time to obtain the maximum TRS production (Chin et al., 2018; Sukri et al., 2014). When exposed to a higher temperature, hemicellulose content in the products of rice straw increases (Sukri et al., 2014). Burning of the hemicellulose at higher temperatures might be the cause of the reduction in TRS production. This finding is agreeable with (Chin et al., 2018) in their findings, which mentioned that an increment in temperature will cause an increment in hemicellulose content; and overheating at high temperatures of biomass burns the biomass surface. This result also was reported by (Chin et al., 2018) wherein their findings, that the temperature did not significantly influence TRS recovery. In the present study, the optimum temperature was found to 85^{o}C for obtaning the highest TRS, and VFA production. The COD production was also increased with the increase in temperature up to 85^{o}C. However, COD content has no significant affect even after increasing the temperature (more than 85^{o}C). At the 85^{o}C, COD production was 12.7±0.5 %, while at the 95^{o}C it was 12.9±1 %.

TH-2881_146104040

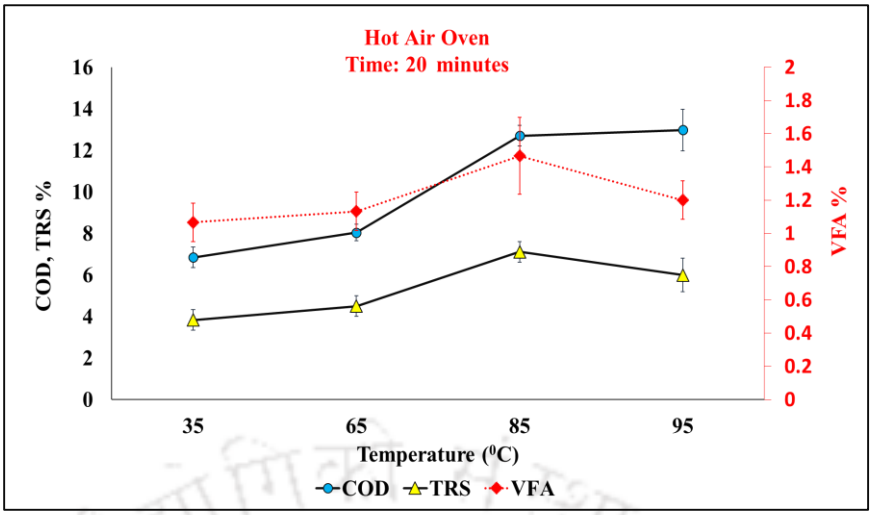


Figure 5.1.1. Effect of temperature on hydrolysis of rice straw in hot air oven

5.1.2. Effect of Exposure Time on Hydrolysis of Rice Straw

An initial increase in reaction time gave an increase in the concentration of COD, TRS, and VFA. At the exposure time of 240 min (at 85°C) using the hot air oven, the maximum COD, TRS, and VFA % were found to be 18.7±2.8 %, 10.4 ±1.5 %, and 1.45±0.15% respectively (shown in figure 5.1.2 (a) and given in table 5.1.1). However, increasing the reaction time for longer than 240 minutes gave a negative effect on the recovery of TRS and VFA. Initial, increment in TRS was mainly due to hydrolysis of hemicellulose in the form of xylose, but the further increment in the exposure time leads to the transformation of xylose into furfural (or hydroxymethyl furfural, HMF) compounds. The presence of furfural and other inhibitors hinders the hydrolysis process to produce fermentable sugar (Chin et al., 2018). As well as overheating at high temperature or for a longer duration leads to the loss of volatile fatty acids, and reduction TRS contents as shown in figure 5.1.2 (a) (hot air oven pretreatment), but the COD content has no significant effect even after increasing the time (more than 240 min). The effect of time on the hydrolysis of rice straw is given in table 5.1.1. Similarly, figure 5.1.2 (b) represents the effect of exposure time on the hydrolysis of rice straw in an autoclave. At the exposure time of 20 min (at 121°C), maximum COD, TRS, and VFA % were found to be 15.3±2 %, 8.6 ±1 %, and 1.2 ±0.8 % respectively (shown in figure 5.1.3 (b) and given in table 5.1.1). Thus, the optimum condition achieved for a hot air oven is 85°C and 240 min, whereas for an autoclave is 121°C and 20 min.

TH-2881_146104040

Table 5.1.1 Effect of thermoalkali pretreatment on the hydrolysis of rice straw

Case	Time (min)	Mass of Rice Straw	COD (mg/L)	COD %	TRS (mg/L)	TRS %	VFA (mg/L)	VFA%	Weight Loss %
Hot air Oven Pretreatment									
35ºC	20	1 gm/100 ml	688±54	6.8±0.5	380±50	3.8±0.5	106±17	1.06±0.17	-
65 ºC			804±46	8.04±0.4	455±55	4.5±0.5	113±11	1.13±0.11	-
85 ºC			1276±56	12.7±0.5	717±58	7.1±0.5	146±23	1.46±0.23	-
95 ºC			1296±145	12.9±1.4	645±85	6±0.8	120±11	1.2±0.11	-
Hot air Oven Pretreatment									
85 ºC	20	1 gm/100 ml	916±50	9.16±0.5	510±122	5.1±1.2	806±11	0.86±0.11	-
	60		1430±304	14.3±3	800±143	8±1.4	1135±145	1.13±0.14	-
	120		1810±221	18.1±2.2	1070±175	10.7±1.7	1340±55	1.34±0.05	-
	240		1870±282	18.7±2.8	1045±154	10.4±1.5	1458±157	1.45±0.15	-
	260		1850±187	18.5±1.8	740±183	7.4±1.8	1145±115	1.1±0.11	-
Autoclave Pretreatment									
121 ºC	5	1 gm/100 ml	852±55	8.52±0.5	475±55	4.7±0.5	106±8	1.06±0.08	-
	10		917±34	9.17±3	510±58	5.10±0.5	115±15	1.1±0.15	-
	20		1537±210	15.3±2.1	860±30	8.60±0.3	126±18	1.26±0.18	-
Hot air Oven Pretreatment (85ºC, 240 min)									
-	0 %	1 gm/100 ml	1876±109	18.7±1	910±90	9.1±0.9	168±20	1.68±0.2	20± 2.4
$Ca(OH)_2$ (w/w)	2.5 %		3430±167	34.3±1.6	2245±180	22±1.8	180±12	1.8± 0.12	35± 4
NaOH (w/w)	1%		4453±180	44±1.8	2700±310	27±3.1	340±15	3.4±0.15	47± 3.2

TH-2881_146104040

ChOH (v/v)	0.4 %		3945±245	39±2.4	2014±254	20±2.5	190± 15	1.9±0.15	44± 2.1
Autoclave Pretreatment (121ºC, 20 min)									
-	0 %	1 gm/100 ml	1564±134	15.3±1.3	790±80	7.9 ± 0.08	120±10	1.2± 0.1	18± 3.7
$Ca(OH)_2$ (w/w)	2.5 %		2781±244	27.7±2.4	1768±306	17±3.0	140±16	1.4±0.16	30±3.4
NaOH (w/w)	1%		3516±145	35±1.4	2235±128	22 ±1.2	316±24	3.16±0.24	40± 2.5
ChOH (v/v)	0.4 %		3436±202	34±2.0	1876±204	18 ±2.0	150±20	1.5±0.2	38±3
Condition at (150°C, 1 h, autoclave), (Chang et al., 2011)									
Acid	0.9 %	300 g/L	-	10-27	-	1-20	-	0.42-2.5	-
Condition at (35-42ºC, 5-15 min, ultra sonication), (Kumari and Singh, 2020)									
Petha Wastewater	-	-	-	-	-	17-20	-	-	
Condition at (90ºC, 24 h, stirrer), (Hou et al., 2012)									
Ionic Liquid (w/w) (Cholinium amino acids)	5-50%	-	-	-	-	-	-	-	29-48.4
Condition at (720 W, 180°C, 30 min; microwave), (Kaur and Phutela, 2016)									
NaOH (w/w)	0-10%	1 g/L	-	-	-	39-56	-	-	7.9-56.7
Condition at (121°C, 2 h; autoclave), (Zhao et al., 2010)									

TH-2881_146104040

Acid (Acetic –Propionic)	0.75 mol/L	1 gm/10 ml	-	-	-	-	-	-	18-23

Increment in pretreatment time or temperature has different effect on parameters (COD, TRS and VFA) and it has an individual effect on each and every carbonic compounds. At initial stage, at low temperature and less exposure time, formation of sugar units (xylose, glucose, arabinose and galactose) takes place due to hydrolysis of hemicellulose and cellulose. As much as time and temperature rises that much of sugar production takes place, but reaching to a certain level (time; 240 min and temperature; 85^{o}C), reduction in the sugar contents occurs at high temperature and longer exposure time. High temperature or longer duration of exposure leads to the losses of volatile fatty acids and reduction in TRS contents. But at the same time, high temperature and longer duration of exposure, leads to production of new phenolic groups produced by the lignin degradation. At the condition (high temperature and long-time), two processes are happening together. (i). Loss of VFA and further degradation of already produced sugars units (xylose, glucose, arabinose and galactose) to the (HMF; 5-hydroxymethylfurfural, furfural, and acids). Xylose is thermally instable compounds and predominant sugar than the glucose, so it might have been further degraded. Due to this reason, reduction in TRS was observed. High temperatures promotes xylose depletion, and induce the solubilization of lignin. And reduction VFA content was may be due to the losses of some of organic compounds by evaporation. (ii) Production of new phenolic groups (aromatic compounds, ferulic acid, vanillic, and syringic acids) is the result of lignin solubilization (Conesa et al., 2016; Li et al., 2012). Due to this reason the increment in COD content was observed even at high temperature and longer duration of exposure. However, COD is due to solubilisation of all carbonic compounds present in hydrolysate, but TRS and VFA are mainly due to reducing sugar and acid compounds respectively. Due to this reason there is no significant change in COD was observed, even after increasing the temperature (from 85^{o}C to 95^{o}C) and time (from 240 min to 260 min). COD has shown different trend as compare to TRS and VFA content. This phenomenon can be explained by other studies.

TH-2881_146104040

Li et al. (2012) conducted study on microwave-assisted pretreatment of bamboo under various times (10–120 min) and temperatures (90 and 109°C). They have clearly, mentioned that an increase of time from 10-60 min (at both temperature 90 and 109°C) leads the increment in solubilisation rate (%) and lignin fraction (%) and further increment in time (60 to 120 min) did not induce a further enhancement of the solubilisation rate (%) and lignin fraction (%) after reaching a certain maximum level. Lignin yield (phenolic content) was found to be higher at high temperature 109°C then 90°C. Xylose content was high at low temperature 90°C than 109°C. Prolongation of the time from 10 to 60 min at 109°C, resulted in a noticeable decrease of the xylose content. They have indicated that the glycosidic bonds between the sugar units is the backbone of the bound hemicelluloses were more sensitive to the severity of microwave heating than the ether linkages between lignin and arabinose. Structural investigation of the extracted lignin indicated that there was an increase of the phenolic hydroxyl content in the lignin, due to the cleavage of the linkages between lignin units (mainly β-O-4 linkages).

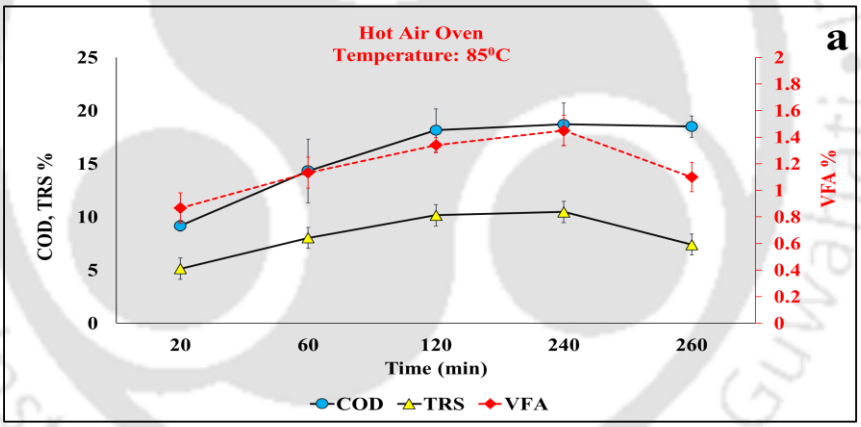


Figure 5.1.2 (a). Effect of exposure time on hydrolysis of rice straw in hot air oven

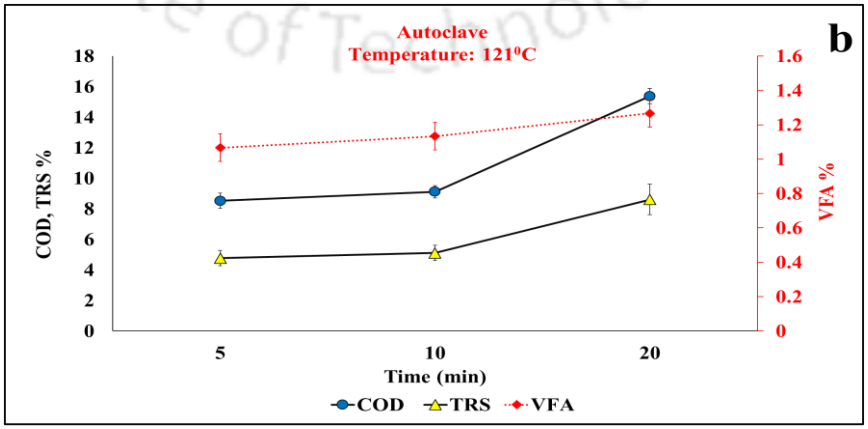


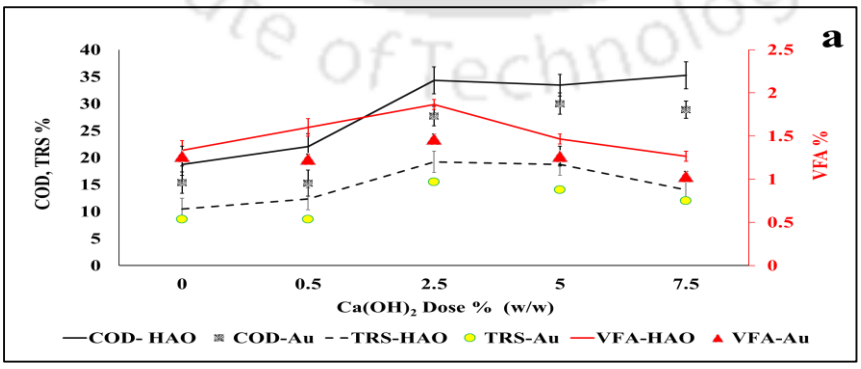
Figure 5.1.2 (b). Effect of exposure time on hydrolysis of rice straw in autoclave

TH-2881_146104040

5.1.3. Effect of Alkali Dose on Hydrolysis of Rice Straw

The effect of alkali ($Ca(OH)_2$, NaOH, and ChOH) dose on hydrolysis of rice straw using the hot air oven and the autoclave is shown in figure 5.1.3 (a, b and c, respectively). Hydrolysis of rice straw was found to be increased with an increase in the dose of alkali reagents. In all the cases, the concentration of TRS and VFA production was increased with increasing dose, but COD production was found to be almost constant beyond certain dosing. The increasing trend of TRS might be due to the lignin and hemicellulose removal with the increased alkali dose. A study on the bioethanol production from switchgrass and coastal bermudagrass (Keshwani and Cheng, 2009) has promulgated that with an increase in NaOH concentration and pretreatment time the rate of hydrolysis can be enhanced. However, the production of TRS and VFA started to drop when concentrations of alkali exceeded 2.5% (w/w) $Ca(OH)_2$, 1% (w/w) NaOH, and 0.4% (v/v) ChOH. VFA was mainly composed of n-butyric and acetic acids. Little declination in VFA was observed along with increased higher dosing of alkali, which was possibly due to dilution of VFA compounds by the additional COD released during high concentration of alkaline dosage (Janke et al., 2016).

This fact was explained by (Janke et al., 2016), who assessed the effect of NaOH concentration on pretreatment of sugarcane filter cake to produce VFA from it and observed a little reduction in VFA with increased dosing of NaOH. Over alkalinity due to bases can remove and/or reduce the concentration of acidic components, e.g. acetic and tannic acid, as well as the volatile compounds, such as furfural and phenols, are stripped by boiling, which also leads to the loss of glucose (10%) and xylose (4%) (Kumar et al., 2009).

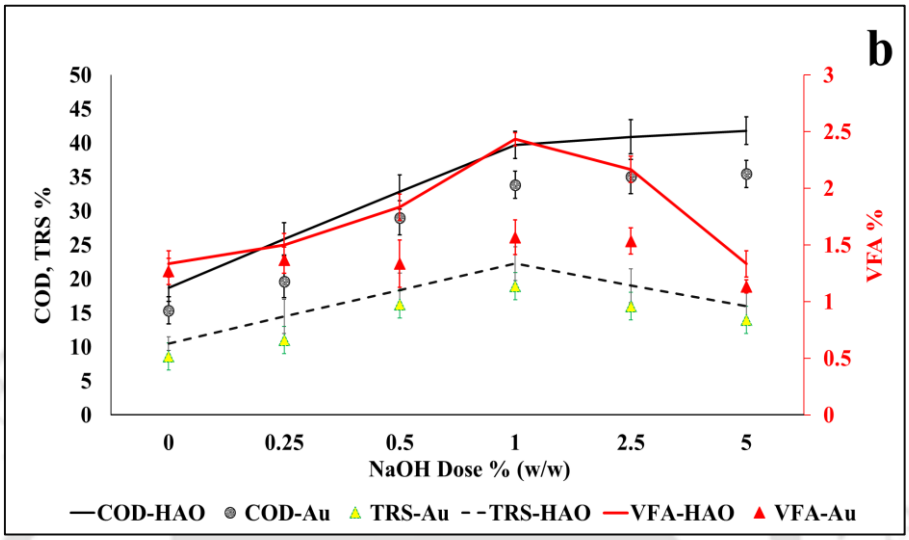


[HAU; Hot air Oven, Au; Autoclave]

Figure 5.1.3 (a). Effect of $Ca(OH)_2$ dose on hydrolysis of rice straw in hot air oven (at 85°C, 240 min) and autoclave (at 121°C, 20 min)

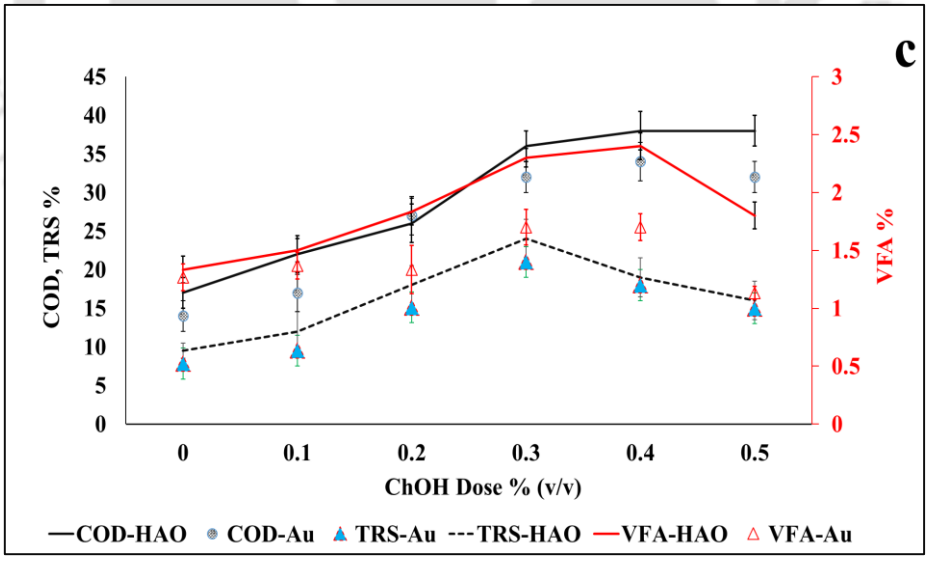
TH-2881_146104040

Malik et al. (2020) performed a study on the effect of different dosages of fenton (Fe: H_2O_2; 1:5 to 1:50) pretreatment on yard waste, they have reported TRS concentration increases with increasing the fenton dose (Fe: H_2O_2) from 1:5 to 1:20. The maximum TRS concentration of 135 mg/ gm biomass was observed at a ratio of 1:20, beyond that there has been observed a decline in the reducing sugar with increasing ratios.



[HAU; Hot air Oven, Au; Autoclave]

Figure 5.1.3 (b). Effect of NaOH dose on hydrolysis of rice straw in hot air oven (at 85°C, 240 min) and autoclave (at 121°C, 20 min)



[HAU; Hot air Oven, Au; Autoclave]

Figure 5.1.3 (c). Effect of ChOH dose on hydrolysis of rice straw in hot air oven (at 85°C, 240 min) and autoclave (at 121°C, 20 min)

With 2.5% (w/w) $Ca(OH)_2$ the COD, TRS and VFA percentage in the hot air oven (at 85°C, 240 min) was 34.3±1.6, 22±1.8 % and 1.8±0.6 % respectively, whereas in

TH-2881_146104040

autoclave pretreatment (at 121^{o}C, 20 min) was 27.7±2.4%, 17±3.0 % and 1.4±0.5% respectively as shown in figure 5.1.3 (a) and table 5.1.1. With 1% (w/w) NaOH the COD, TRS and VFA percentage in the hot air oven (at 85^{o}C, 240 min) was 44±1.8 %, 27±3.1%, and 3.4±0.15% respectively, whereas in autoclave pretreatment (at 121^{o}C, 20 min) was 35±1.4%, 22 ±1.2%, and 3±0.02% respectively as shown in figure 5.1.3 (b) and table 5.1.1. With 0.4% (v/v) ChOH the COD, TRS and VFA percentage in the hot air oven pretreatment (at 85^{o}C, 240 min) was 39±2.4%, 20±2.5% and 1.9±0.1 % respectively, whereas in autoclave pretreatment (at 121^{o}C, 20 min) with 0.4 % (v/v) ChOH was 34±2.0%, 18±2.0 % and 1.5±0.2 % respectively as shown in figure 5.1.3 (c) and table 5.1.1. Kumari and Singh (2020) reported a 17-20 % reducing sugar after providing ultrasonic assisted petha wastewater pretreatment of rice straw. Chang et al. (2011) reported COD of 27 %, TRS of 20 %, and VFA of 2.5 % using acidic pretreatment of rice straw (at 150°C, 1 h). Kaur and Phutela (2016) have reported 39-56 % TRS using alkaline pretreatment of rice straw (at 720 W, 180°C).

From the observed results, COD, TRS and VFA percentage in the hot air oven (at 85^{o}C, 240 min) in the absence of alkali (0 % (w/w)) was 1.22, 1.15, 1.4 times, higher than the autoclave (at 121^{o}C, 20 min). COD, TRS and VFA percentage in the hot air oven (at 85^{o}C, 240 min) with 2.5 % (w/w) $Ca(OH)_2$ was 1.23, 1.29, 1.28 times higher than the autoclave (at 121^{o}C, 20 min). COD, TRS and VFA percentage in the hot air oven (at 85^{o}C, 240 min) with 0.4% (v/v) ChOH 1.15, 1.11, 1.58 times higher than the autoclave (at 121^{o}C, 20 min).

5.1.4. Effect of Thermoalkali Pretreatment on Weight Loss Percentage

Weight loss % after the thermoalkali pretreatment was 20± 2.4, 35± 4, 47± 3.2, 44± 2.1 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (w/w) ChOH respectively in hot air oven [at 85^{o}C, 240 min] as given in table 5.1.1. Similarly, weight loss % after the thermoalkali pretreatment was 18±3.7, 30±3.4, 40± 2.5, 38±3 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (w/w) ChOH respectively in autoclave [at 121^{o}C, 20 min] (table 5.1.1). Weight loss % is attributed to the solubilization of the polysaccharides. Hou et al. (2012) reported 29-48.4 % weight loss using ionic liquid pretreatment (5-50% w/w; at 90^{o}C, 24 h). Zhao et al. (2010) reported 18-23 % weight loss, after acid pretreatment (acetic-propionic 0.75 mol/L; 121°C for 2 h).

TH-2881_146104040

5.1.5. Effect of Thermoalkali Pretreatment on Lignocellulosic Composition

The initial cellulose, hemicellulose, and lignin of raw rice straw were 39.4±0.22, 28.9±0.3, and 13.6±0.3% (table 5.1.5). Effect of thermoalkali pretreatment on lignocellulosic composition in autoclave [figure 5.1.5 (a) and hot air oven (figure 5.1.5 (b)] at the optimized conditions are shown.

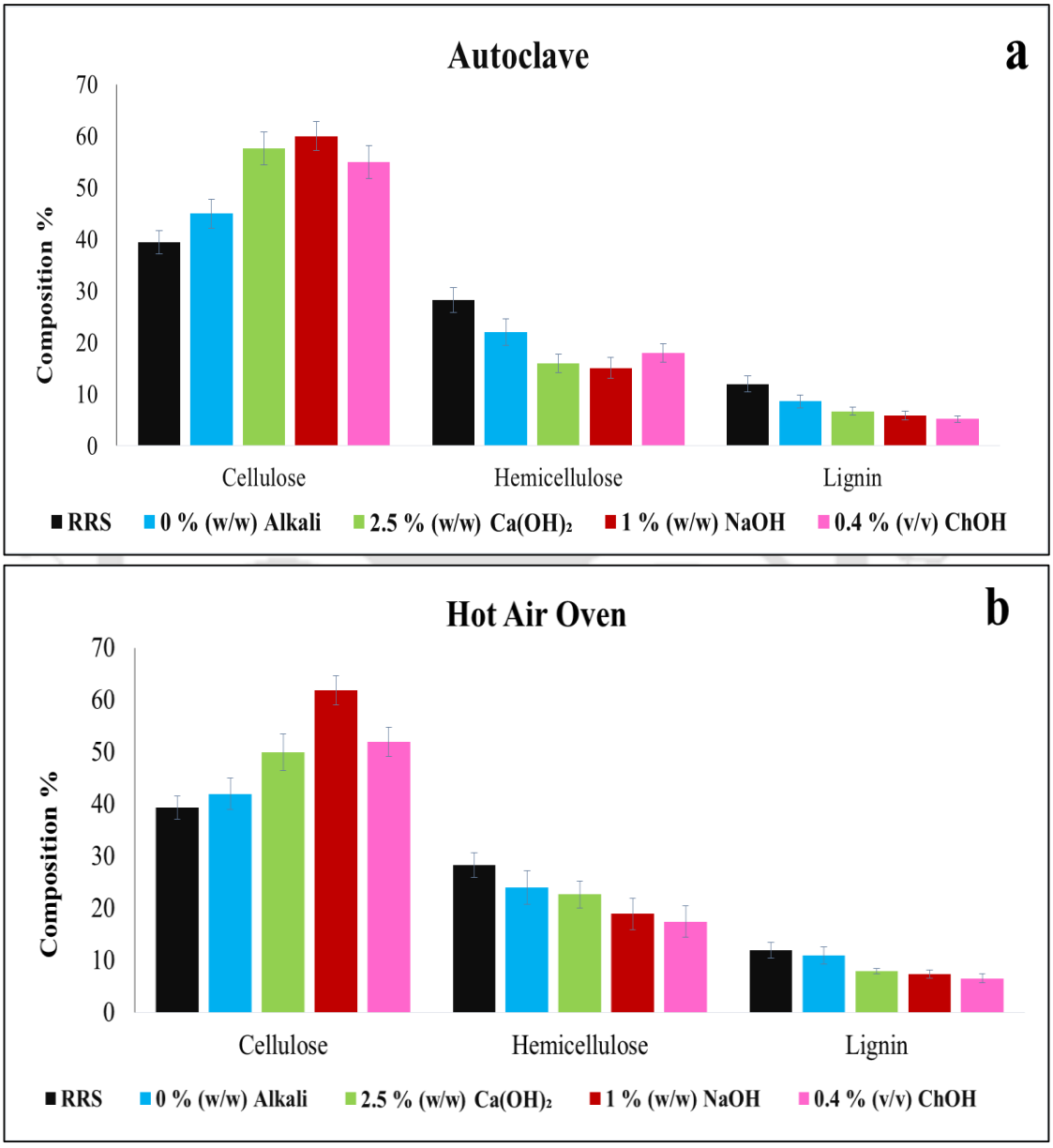


Figure 5.1.5. Effect of pretreatment on lignocellulosic composition in (a) autoclave (at 121°C, 20 min) (b) hot air oven (at 85°C, 240 min)

Cellulose content after the thermoalkali pretreatment was 53.1±0.33, 57.6±0.27, 67.5±0.53, 55±3.2% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4%

TH-2881_146104040

(w/w) ChOH respectively in hot air oven [at 85^{o}C, 240 min]. Similarly, cellulose content after the thermoalkali pretreatment was 44.7±0.13, 53.1±0.51, 61.9±0.33, 52 ±2.8% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (w/w) ChOH respectively in autoclave [at 121^{o}C, 20 min]. Increment in cellulose content is attributed to the solubilization of the polysaccharides.

Hemicellulose content after the thermoalkali pretreatment was 24.4±0.6, 19.1±0.7, 15.1±0.2, 18±1.8% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (w/w) ChOH respectively [85^{o}C, 240 min]. Similarly, hemicellulose content after the thermoalkali pretreatment was 26.4±0.2, 22.7±0.4, 18.4±0.5, 17±2% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min]. Lignin content after the hot air oven pretreatment was 9±0.8, 5.6±0.12, 4.8±0.24, 5.2±0.6 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 85^{o}C, 240 min]. Similarly, lignin content after the autoclave pretreatment was 10±0.5, 6.4±0.16, 5.1±0.43, 6.6±0.8 % in 0 % (w/w) alkali, 2.5% (w/w) Ca(OH)2, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min].

Kumar et al. (2016) reported an increment in cellulose content (from 40.0±1.8 to 46.0±0.9), a reduction in hemicellulose content (28.0±2.0 to 24.6±1.3), and a reduction in lignin content (9.0±0.8 to 3.8±0.5) after ionic liquid pretreatment (at 60 °C, 12 h). In this study also, a reduction in hemicellulose and lignin content and an increment in cellulose content were observed. As cellulose microfibrils formed by elementary fibrils which are cemented by a protective layer of hemicellulose and lignin in a complex manner, thus hemicellulose and lignin layers may have a greater chance of being hit by the effect of pretreatment, which leads to solubilization of hemicellulose and lignin in form of simple monomers. The increase in the cellulose content after pretreatment may be due to the deformation of the biomass and post-modifications of the crystalline cellulose, as evident from the XRD analysis (section 5.1.9); this might have increased the overall cellulose availability (Kumar et al., 2016). Alkali pretreatment primarily removes hemicellulose or lignin from lignocellulose biomass. It leads to the destruction of the lignin structure produces amorphous cellulose, and increases the surface area of fibers/ cellulose, which are more accessible and digestible than the original forms and more susceptible to the fermentation process (Zhu et al., 2006)

TH-2881_146104040

Table 5.1.5. Effect of thermoalkali pretreatment on the compositional and elemental analysis of rice straw

-	-		Component Analysis %			Elemental Analysis %				Cr Index %	Biodegradability Fraction	Hydrolysis Rate %	Residual Char %
-	-	Mass of Rice Straw	Cellulose	Hemicellulose	Lignin	C	H	N	S				
-	RRS	-	39.4±0.22	28.3±0.3	13±0.3	48.3	5.6	-	54.23	33.7	0.46		38
Pretreatment agents name	Alkali Dose		Hot air Oven Pretreatment (85°C, 240 min)										
-	0 %	1 gm/100 ml	53.1±0.33	24.4±0.6	9±0.8	51.02	5.9	-	46.04	52.7	0.57	12	25.9
$Ca(OH)_2$ (w/w)	2.5 %		57.6±0.27	19.1±0.7	5.6±0.12	39	6.7	-	41.32	55.1	0.67	23.4	21.7
NaOH (w/w)	1%		67.5±0.53	15.1±0.2	4.8±0.24	77.2	4.6	-	18.02	59.3	0.69	26	22.9
ChOH (v/v)	0.4 %		55±3.2	18±1.8	5.2±0.6	65.9	5.1	-	28.87	53.2	0.68	23.2	24.2
-	-		Autoclave Pretreatment (121°C, 20 min)										
-	0 %	1 gm/100 ml	44.7±0.13	26.4±0.2	10±0.5	52.5	6.1	-	53.18	51.1	0.55	9.5	30.8
$Ca(OH)_2$ (w/w)	2.5 %		53.1±0.51	22.7±0.4	6.4±0.16	39.5	5.7	-	42.98	50.5	0.68	18	23.8
NaOH (w/w)	1%		61.9±0.33	18.4±0.5	5.1±0.43	69.3	4.2	-	26.46	56.4	0.69	20	21.9
ChOH (v/v)	0.4 %		52 ±2.8	17 ±2	6.6 ±0.8	61.2	5.0	-	33.72	49.5	0.67	19	30.86
Condition at (60 °C, 12 h; microwave), (Kumar et al., 2016)													
-	Raw Rice Straw	2.5-4.75 gm/100 ml	40.0±1.8	28.0±2.0	9.0±0.8	36.6	-	-	-	46.4	-	-	32.26
Ionic Liquid NADES	5-50%		46.0±0.9	24.6±1.3	3.8±0.5	39.2	-	-	-	44.3	-	-	31.67

TH-2881_146104040

Condition at (121 °C, 2 h; autoclave), (Zhao et al., 2010)													
Acetic-propionic Acid	0.75 mol/L	1 gm/10 ml	-	-	-	-	-	-	-	-	-	8-21	-

TH-2881_146104040

5.1.6. Effect of Thermoalkali Pretreatment on Biodegradability Fraction

Biodegradability fraction in raw rice straw was found to be 0.46 (table 5.1.5) Biodegradability fraction after the hot air oven pretreatment was 0.57, 0.67, 0.69, 0.68 in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 85^{o}C, 240 min]. Similarly, biodegradability fraction after the autoclave pretreatment was 0.55, 0.68, 0.69, 0.67 in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min]. This increment in biodegradability fraction is confirming that rice straw has become more biodegradable. Biodegradability fractions of chicken manure, dairy manure, and sawdust were reported to be 0.55, 0.48, and 0.20 respectively (Zhang and Wang, 2014).

5.1.7. Effect of Thermoalkali Pretreatment on Hydrolysis Rate Percentage

Figure figure 5.1.7, represents the hydrolysis rate % after the hot air oven pretreatment [at 85^{o}C, 240 min] was 12, 23.2, 26.8, 23.4 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively (table 5.1.5). Similarly, hydrolysis rate % after the autoclave pretreatment [at 121^{o}C, 20 min] was 9.5, 18.4, 20.4, 19.4% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively (figure 5.1.7 and table 5.1.5). This increment in hydrolysis rate % is confirming that rice straw has been solubilized in simple monomers, which can become a direct source of food for microorganisms, as well as the solid portion of rice straw can be easily digestible or consumable during the anaerobic digestion or fermentation process.

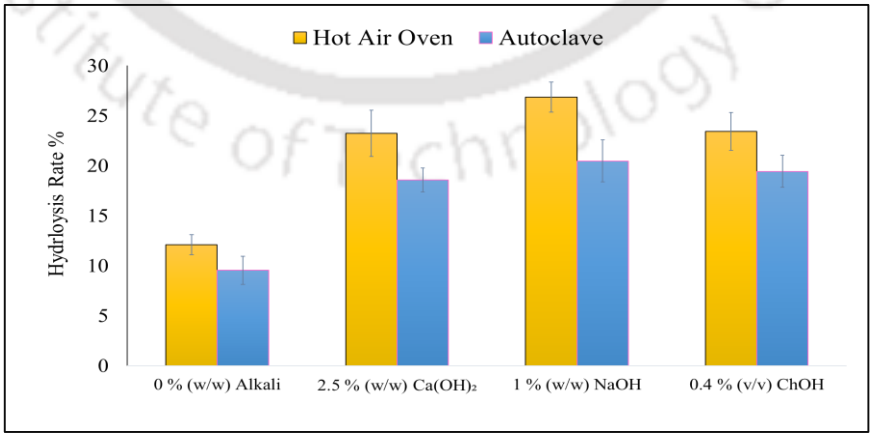


Figure 5.1.7. Effect of pretreatment on hydrolysis rate % in an autoclave (at 121^{o}C, 20 min) and hot air oven (at 85^{o}C, 240 min)

TH-2881_146104040

Hydrolysis rate % after the hot air oven pretreatment (at 85^{o}C, 240 min) was 1.26, 1.25, 1.29, 1.20 times higher than autoclave pretreatment (at 121^{o}C, 20 min) in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively. Zhao et al. (2010) reported an 8-21 % hydrolysis rate of rice straw using acidic pretreatment (0.75 mol/L acetic-propionic acids, at 121°C for 2 h).

5.1.8. Effect of Thermoalkali Pretreatment on CHNS Percentage

The C and H % in raw rice straw were found to be 48.3 and 5.6 % (table 5.1.5). C and H % after the hot air oven pretreatment was 51.0; 5.9 %, 39.0; 6.7%, 77.29;4.6, 65.99; 5.1 in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 85^{o}C, 240 min] . Similarly, C and H% after the autoclave pretreatment was 52.5; 5.1%, 39.5; 5.7%, 69.3; 4.2%, 61.2;5.0 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min] as given in table 5.1.5. Increment in C% indicates the alteration of cellulosic structure. Kumar et al. (2016) reported increment in C % (from 36.6 to 39.2) after ionic liquid pretreatment (at 60 °C, 12 h).

5.1.9. Effect of Thermoalkali Pretreatment on Crystallinity Index

To investigate the crystallinity changes on rice straw under different pretreatment conditions, X-Ray diffraction (XRD) was performed and the results are shown in figure 5.1.9. The initial cellulose, hemicellulose, and lignin of raw rice straw were 39.4±2.2 %, 28.9±1.3 %, 12±1.5 %, 8±1.4 %, and 13±1.5% respectively. As we have observed in table 5.1.5 and discussed in section 5.1.5, the increment in cellulose and reduction and hemicellulose and lignin content was observed, which is responsible for the changes in the crystallinity index of rice straw. The increase in the cellulose content after pretreatment may be due to the deformation of the biomass and post-modifications of the crystalline cellulose. The crystallinity index of raw rice straw before the pretreatment is 33.7 %. Crystallinity index after the hot air oven pretreatment were 52.7, 55.1, 59.3, 53.2 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 85^{o}C, 240 min]. Similarly, crystallinity index after the autoclave pretreatment were 51.1, 50.5, 56.4, 49.5% in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min] as given table 5.1.5. This change may be attributed to the exposure of the crystalline portion of the rice straw to the effects of alkali reagents.

TH-2881_146104040

Alkaline pretreatment has been widely used and proved to be an effective method of enhancing methane production of lignocellulosic substrates with the advantages of high efficiency in removing hemicellulose and lignin, providing extra buffering for the anaerobic digestion (Kang et al., 2018). Alkali pretreatment provides delignification by breaking the ester bonds between lignin and xylan (Tarkow and FEIST, 1969), and splitting the ester and C-C bonds of lignin molecules (ferulic acid) increasing porosity (Kim et al., 2013). Kang et al. (2018) conducted on lignocellulosic waste (Pennisetum Hybrid) using 2% NaOH (at 35 °C), they reported an increment in Cr I (from 35.7 to 44.9%) due to the removal of hemicellulose and lignin. They have also reported a 21 % increment in methane yield and explained the reason. i) The removal of hemicellulose and lignin, making cellulose easily accessible and promoting anaerobic fermentation. ii) The organic acids and sugars produced in the alkaline pretreatment were easily used substrates for methanogenesis. Xie et al. (2011) observed that the methane yield of grass silage increased by 38.9% after being pretreated with 5% NaOH at 100 °C. Michalska et al. (2015) reported that 5% NaOH treated energy grass achieved more than 60% volume of biogas. Kumar et al. (2016) reported a reduction in crystallinity index (from 46.4 to 44.3 %) after ionic liquid pretreatment (at 60 °C). Hideno et al. (2009) also reported an increment in crystallinity index (from 51.9 to 64.1 %) of rice straw from using hot compressed water treatment (180^{o}C) and they have reported a 67.5% increment in total sugar yield during the enzymatic hydrolysis. Hsu et al. (2010) reported crystallinity index (from 57 to 58-65%) was found to be increased after dilute acid pretreatment of rice straw. A maximum sugar yield of 83% was achieved when the rice straw was pretreated with 1% (w/w) sulfuric acid with a reaction time of 1–5 min at 160-180 °C, followed by enzymatic hydrolysis. Pretreatment can alter cellulosic crystalline areas and enlarge the pore ratio and inner surface areas; which are beneficial to later anaerobic digestion (Kumar et al., 2016). Increment in the crystallinity index of cellulose content of the rice straw may have been due to the removal of amorphous components, such as hemicellulose and lignin, during the pretreatment process, which leads to a relative increase in the proportion of crystalline cellulose (Kumar et al., 2016). Based on my observation and the previous reports its can be said that incement in crystallinity index would be benifical for enhancing the anaerobic digestion process of rice straw.

TH-2881_146104040

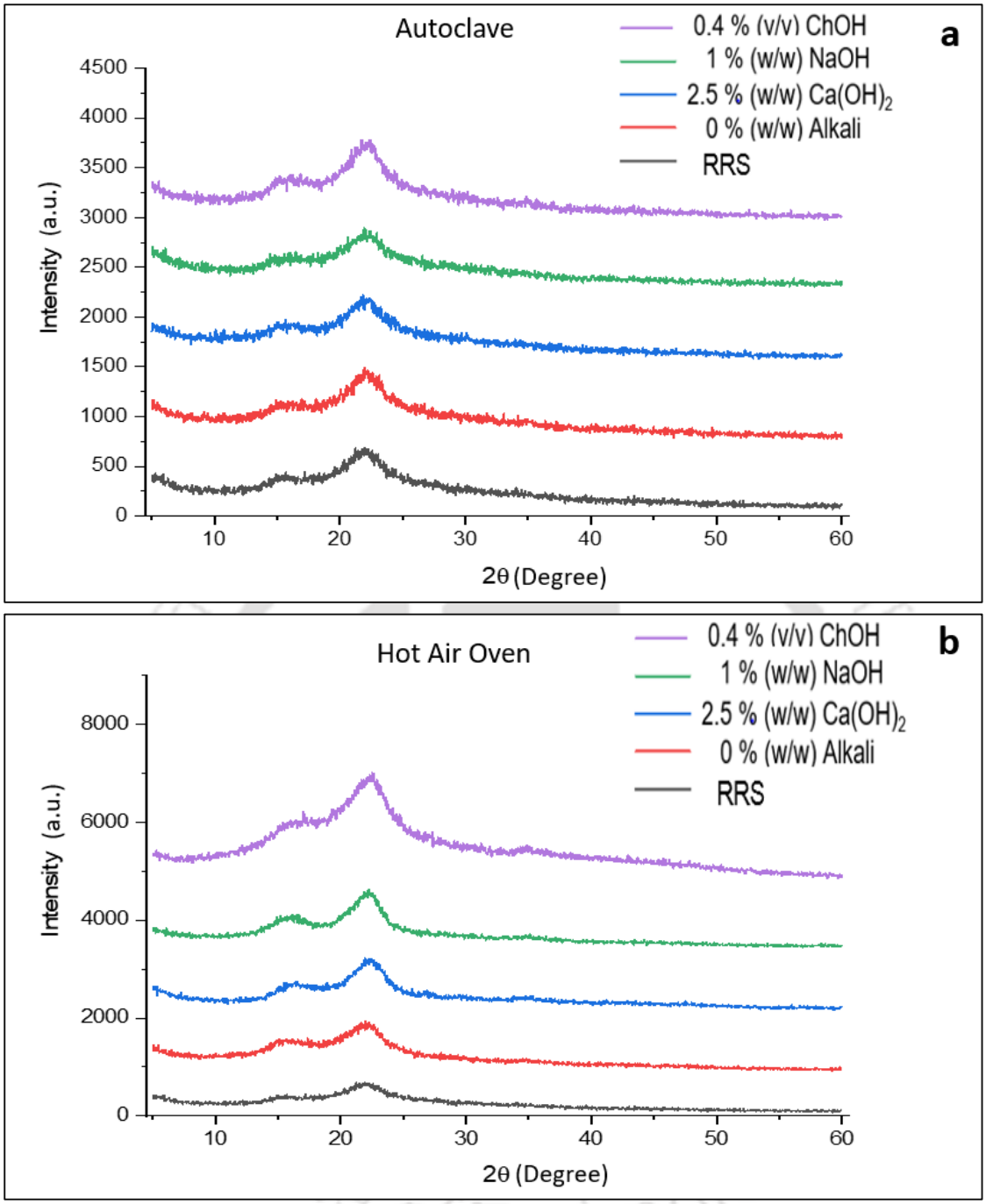


Figure 5.1.9. XRD analysis of rice straw in (a) autoclave (at 121°C, 20 min) (b) hot air oven (at 85°C, 240 min)

5.1.10. Effect of Thermoalkali Pretreatment on Thermogravimetric Properties

To observe the effect of thermoalkali pretreatment on thermogravimetric properties of rice straw, TGA analyses were also performed. The thermogravimetric properties of rice straw at different thermoalkali pretreatment conditions are shown in figure 5.1.10 (i) in an autoclave (at 121°C, 20 min) and figure 5.1.10 (ii) in a hot air oven (at 85°C,

TH-2881_146104040

240 min) that can be divided into different stages: In stage one, the initial weight loss step occurred at 30–120 °C due to the evaporation of water absorbed. At the second/third stage degradation of hemicelluloses occurs (250–300 °C) followed by cellulose (300–350°C) and finally lignin (300–500 °C) with a bell transition shape (Mohtar et al., 2017; Zhang et al., 2014). Residual char of raw rice straw was 38%. Residual char after the autoclave pretreatment was 30.80, 23.8, 21.9, 30.83 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 121^{o}C, 20 min] is shown in figure 5.1.11 (i) and given in table 5.1.5. Similarly, residual char after the hot air oven pretreatment was 25.9, 21.7, 22.9, 24.2 % in 0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 % (w/w) NaOH, 0.4% (v/v) ChOH respectively [at 85^{o}C, 240 min] is shown in figure 5.1.11 (ii) and given in table 5.1.5. Kumar et al. (2016) reported a reduction in residual char % (from 46.4 to 44.3) after ionic liquid pretreatment of rice straw (at 60 °C, 12 h). The total weight loss percentage of pretreated biomass was comparatively higher than the raw rice straw, as the pretreated biomass becomes more susceptible to degradation at the elevated temperature thereby leading to a higher weight loss of material (Kumar et al., 2016).

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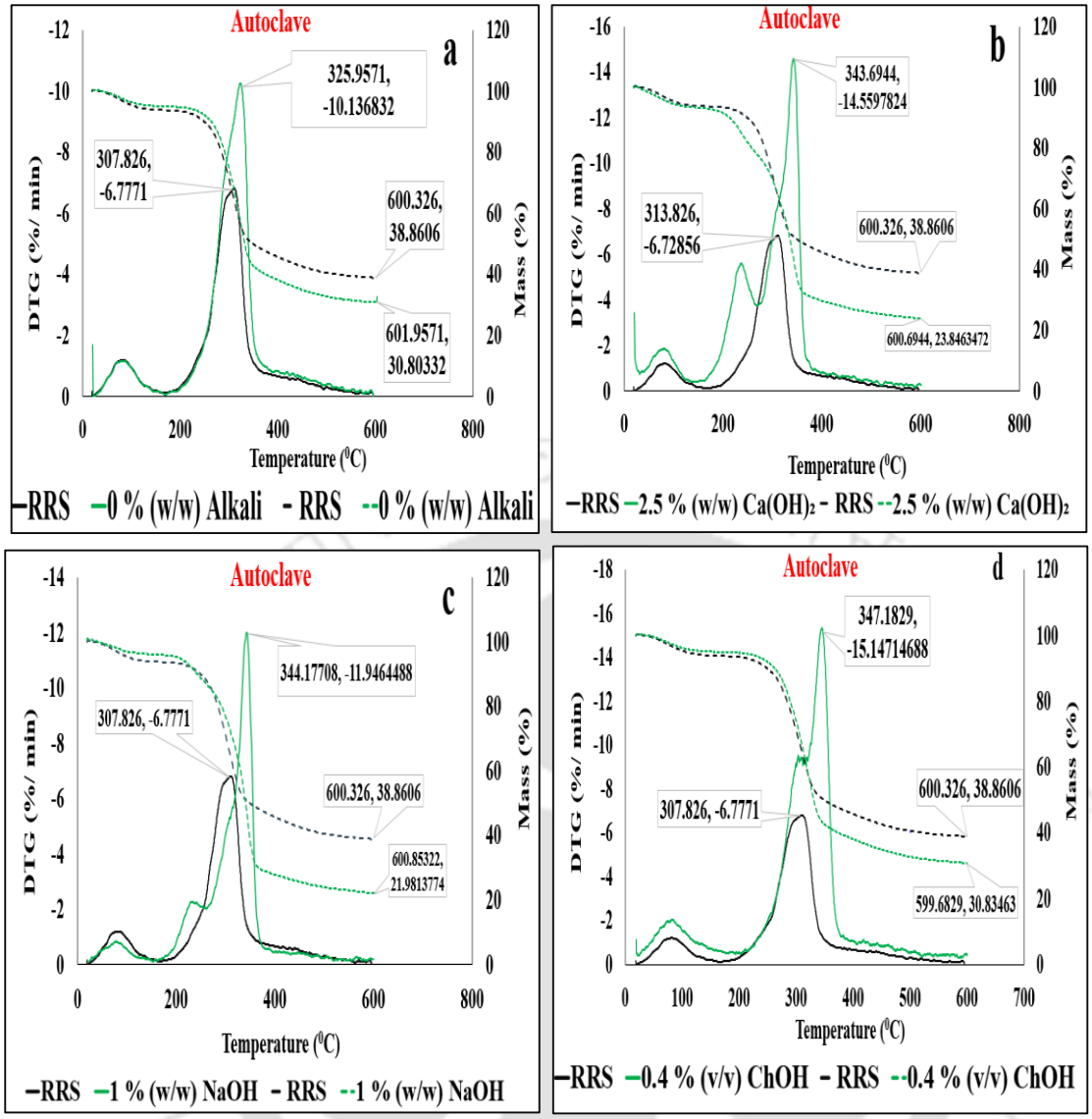

Figure 5.1.10 (i). TGA analysis of rice straw in autoclave (at 121°C, 20 min)

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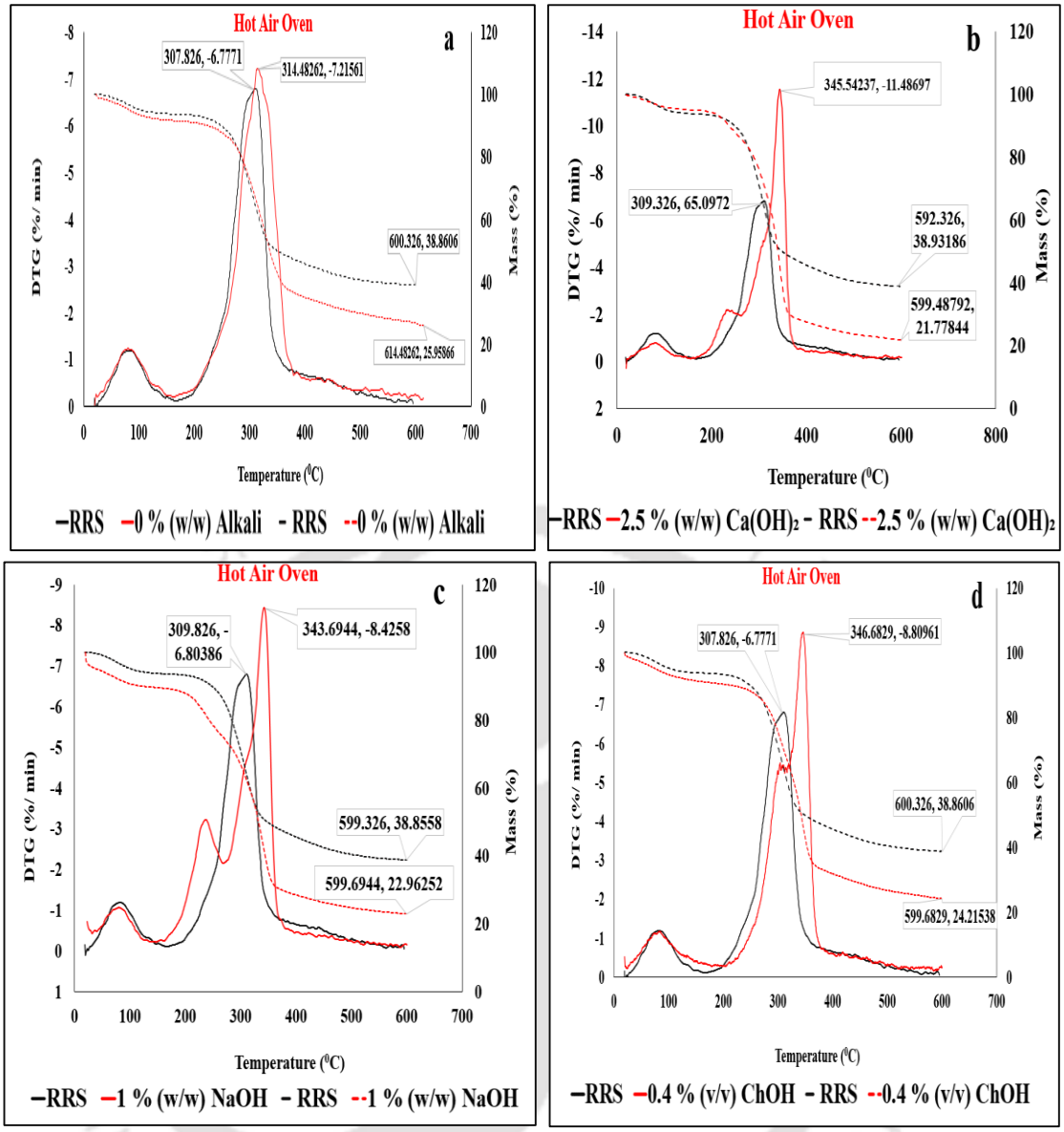

Figure 5.1.10 (ii). TGA analysis of rice straw in hot air oven (at 85°C, 240 min)

5.1.11. Effect of Thermoalkali Pretreatment on Morphological Properties

Field emission scanning electron microscope (FESEM) analyses were performed to observe the morphological changes after thermoalkali pretreatment on the surface of rice straw as shown in figure 5.1.11. Untreated biomass (raw rice straw) exhibited a rigid and high-ordered packed surface covered by a silica layer, while the pretreated biomass shows remarkable changes in the surface structure and porosity. Thermoalkali pretreated biomass has a completely destructed surface matrix with hollows and cracks. It became more abrupt and rough, and presented a series of irregular holes and folds after thermoalkali pretreatment which was possibly due to the breakdown of the bond

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between lignocellulosic biomass, separation of interconnected fibers, and also due to the opening of the silica layer from the surrounding, thereby increased accessible surface area for the bacterial decomposition (Soest, 2006).

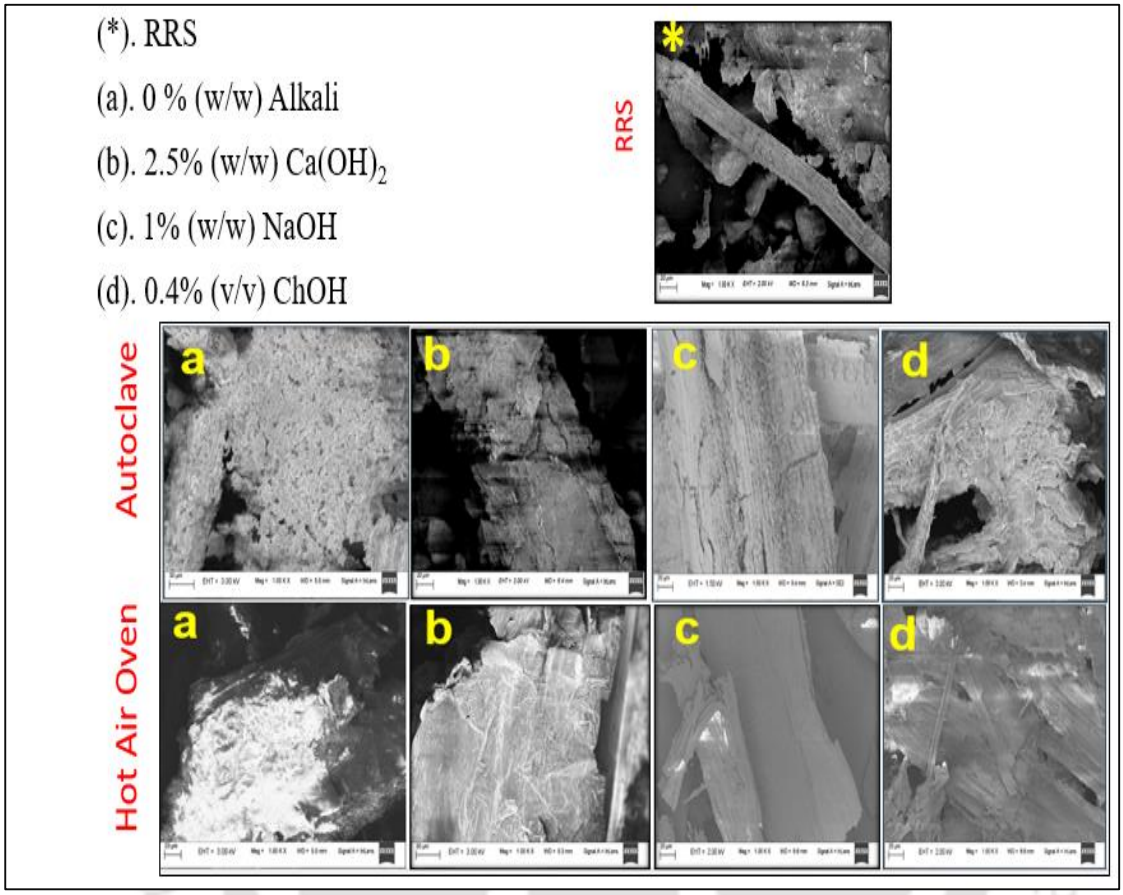

Figure 5.1.11. FESEM images of rice straw in autoclave (at 121^{o}C, 20 min) and hot air oven (at 85^{o}C, 240 min)

5.1.12. Effect of Thermoalkali Pretreatment on Band Position

FTIR was performed to identify the effect of thermoalkali pretreatment effect on the chemical/structural properties of rice straw under different thermoalkali pretreatment conditions in an autoclave and hot air oven as shown in figure 5.1.12 (a and b respectively). The band position between 1745 and 1732 cm^{-1}, are assigned for the carbonyl (C=O) stretching. Alterations in this band position are attributed to aromatic skeletal vibrations in lignin structures. Band position at 1732 cm^{-1} is attributed to the cross-linking of the lignin chain with branched hemicellulose. Disappearance of these bands after thermoalkali pretreatment (0 % (w/w) alkali, 2.5% (w/w) $Ca(OH)_2$, 1 %

TH-2881_146104040

(w/w) NaOH, 0.4% (v/v) ChOH), are attributed to removal of integrated lignin from the network of lignocellulose structure (Liu and Wyman, 2004).

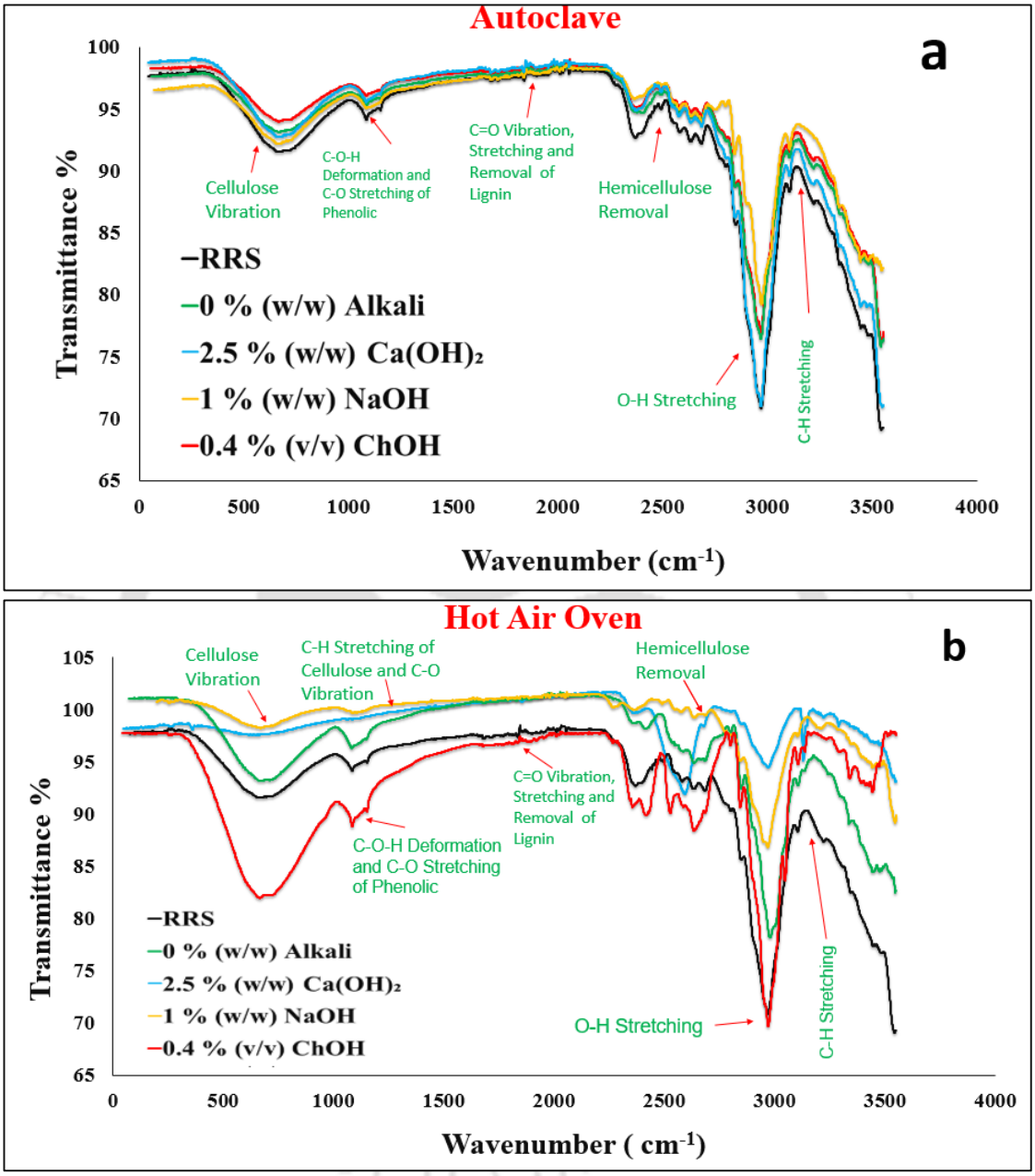

Figure 5.1.12. FTIR analysis of rice straw in (a) autoclave (at 121°C, 20 min) (b) hot air oven (at 85°C, 240 min)

The band position from 1638 cm $^{-1}$ to 1606 cm $^{-1}$ (assigned to an aromatic stretch) and 1512 cm^{-1} (aromatic C-O stretching) became weak and disappeared after thermoalkali pretreatment, suggesting delignification occurred from the surface as well as integrated lignin removal from the cellulosic structure. Band position at 1464 cm^{-1} (C–H deformations in lignin), 1416 cm^{-1} (attributed for non sterified uronic acid or phenolic

TH-2881_146104040

rings), 1244 cm^{-1} (assigned to b-ether bonds in lignin and between lignin and carbohydrates), and 835 cm^{-1} (C–H out of plane vibration in lignin) (Alonso-Simón et al., 2011; Harrison et al., 2013; Liu et al., 2009) all diminished in the FT-IR spectrum from alkali pretreated rice straw.

The present study demonstrates that the **hot air oven pretreatment was more effective than the autoclave pretreatment in all cases based on the hydrolyzate and compositional characteristics of rice straw**. Based on the hydrolysis of rice straw during the pretreatment, the optimized temperature and time were 85^{o}C, 240 min in the case hot air oven, and 121^{o}C, 20 min in the case of the autoclave. Extraction of soluble components and disintegration of lignocellulose can be enhanced by providing a higher temperature for a longer exposure time and a high dose of reacting agents. The optimum doses of alkali were 2.5% (w/w) $Ca(OH)_2$, 1% (w/w) NaOH, and 0.4% (v/v) ChOH for both of the cases in a hot air oven and autoclave in terms of COD, TRS, and VFA production. The maximum COD, TRS, VFA %, and hydrolysis rate % in the hot air oven treatment (at 85^{o}C corresponding to 240 min) with 1% (w/w) NaOH was 44±1.8 %, 27±3.1%, 3.4±0.15%, and 26.8 % respectively. The effectiveness of reacting agents was in the sequence of NaOH > ChOH > $Ca(OH)_2$. Production of COD, TRS, and VFA from rice straw hydrolyzate and alteration in various parameters (lignocellulosic compounds, CHNS %, residual char %, increment in Cr Index %, biodegradability fraction, and hydrolysis rate %) is suggesting that rice straw hydrolyzate, as well as amorphous rice straw both of them, has enough potential to be used as a carbon source for the microorganism. Extraction of COD, TRS, and VFA from the rice straw is suggesting that rice straw can be hydrolyzed into simple monomers by providing thermoalkali treatment, thereby it can be used as a carbon source for the microorganism. The solid portion of rice straw has become more fragile and amorphous which leads to easy digestion, because of the improvement in biodegradability fraction and alteration in lignocellulosic components. Furthermore, to assess the effect of pretreatment, XRD, FTIR, FESEM, TGA, and CHNS analyses were also performed, which also confirms that the rice straw has become easily digestible and consumable for the microorganism.

TH-2881_146104040

5.2. Initial characteristics of thermoalkali treated rice straw slurry.

The initial characterization of rice straw slurry (hydrolyzate and compositional characteristics) has been analyzed. Here also an alteration in various parameters as explained in the above section confirms that this rice straw slurry can be used as a carbon source for the microorganism during the oxyanions removal.

5.2.1. Rice Straw Hydrolyzate Characteristics

Rice straw hydrolyzate characteristics are given in table 5.2.1. COD production was 2.8±0.4 g/L, 8.4±0.76 g/L, 18.4±0.54 g/L, 20±0.85 g/L in RS-WTA, RS-$Ca(OH)_2$, RS-NaOH and RS-ChOH respectively. TRS production was 0.6±0.06 g/L, 4.1±0.65 g/L, 12±0.76 g/L, 11±0.64 g/L in RS-WTA, RS-$Ca(OH)_2$, RS-NaOH, and RS-ChOH respectively. VFA production was 0.26±0.05 g/L, 1.9±0.53g/L, 3.2±0.74g/L, 6.3±0.8 g/L. Results were compared with other studies (Chang et al., 2011; Sen et al., 2016; Tawfik et al., 2019).

5.2.2. Rice Straw Compositional Characteristics

Rice straw compositional characteristics are given in table 5.2.2, which represents the increment in cellulose %, reduction in hemicellulose %, and lignin % observed in pretreated rice straw. Figure 5.2.2 (i) represents (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-$Ca(OH)_2$. Figure 5.2.2 (ii) represents (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-NaOH. Figure 5.2.2 (iii) represents (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-ChOH. Figure 5.2.2 (iv) represents FESEM Images of (a) RS-WTA (b) RS-$Ca(OH)_2$ (c) RS-NaOH (d) RS-ChOH. It represents the changes in the morphology of rice straw after the pretreatment due to the effect of alkali. Increment in surface area, increment in Cr I, reduction in residual char % and alteration in C % and H % (given in table 5.2.2), and alteration in the band position attributed to structural and compositional changes may be due to the effect of thermoalkali pretreatment.

TH-2881_146104040

Table 5.2.1. Rice straw hydrolyzate characteristics

Parameter	-	This Study RS-WTA	This Study RS-$Ca(OH)_2$	This Study RS-NaOH	This Study RS-ChOH	(Tawfik et al., 2019)	(Chang et al., 2011)		(Sen et al., 2016)
Pretreatment Condition	Temp. /Time	-	Heated at 85ºC for 240 min			Heated at 90ºC for 60 min	Autoclaved at 150ºC for 60 min		Autoclaved at 121ºC for 60 min
Pretreatment Agent Name	Unit	-	$Ca(OH)_2$	NaOH	ChOH	NH_4OH	H_2SO_4	HNO_3	HCl
Pretreatment Dose	-	-	2.5 % (w/w)	1 % (w/w)	0.4 % (v/v)	0.3 % (w/w)	0.9 % (w/w)	0.9 % (w/w)	0.6M
Rice Straw Dose	g/L	100	100	100	100	25	300	300	100
COD	g/L	2.8±0.4	8.4±0.76	18.4±0.54	20±0.85	1.3	69.4	83.9	-
TRS	g/L	0.6±0.06	4.1±0.65	12±0.76	11±0.64	1	36.7	64.4	38-45
VFA	g/L	0.26±0.05	1.9±0.53	3.2±0.74	6.3±0.8	-	-	-	-
COD Production	%	2.8	8.1	18	20	5.3	23	27.6	-
TRS Production	%	0.6	4.1	12	11	4	12	21.4	38-45
VFA Production	%	0.26	1.9	3.2	6.3	-	-	-	-

TH-2881_146104040

Table 5.2.2. Rice straw compositional characteristics

Case		-	RRS	RS-WTA	RS-$Ca(OH)_2$	RS-NaOH	RS-ChOH
Dose of Prepretreatments Agents		-	-	-	2.5 % (w/w)	1 % (w/w)	0.4 % (v/v)
Compositional Analysis	Cellulose	%	39±0.8	40±2.5	57±2	55±2.5	58±3.2
	Hemicellulose	%	25 ±2	24±0.8	19±1.6	18±1.4	15±2.3
	Lignin	%	13.2±1.5	12±0.2	10±0.43	7.4±1.8	8±0.68
Biodegradability Fraction		%	0.45	0.49	0.55	0.62	0.59
Surface Area Analysis		m^2/gm	1.9	-	5.5	14.8	11.8
Elemental Analysis	C	%	48.3	-	40.72	68.92	72.61
	H	%	5.6	-	6.10	4.82	5.01
	N	%	-	-	-	-	-
	S	%	-	-	-	-	-
XRD Analysis	Cr Index	%	33.3	37.7	41.4	42.9	56
TGA Analysis	Residual Char	%	37.9	35.0	30.8	26.6	24.3

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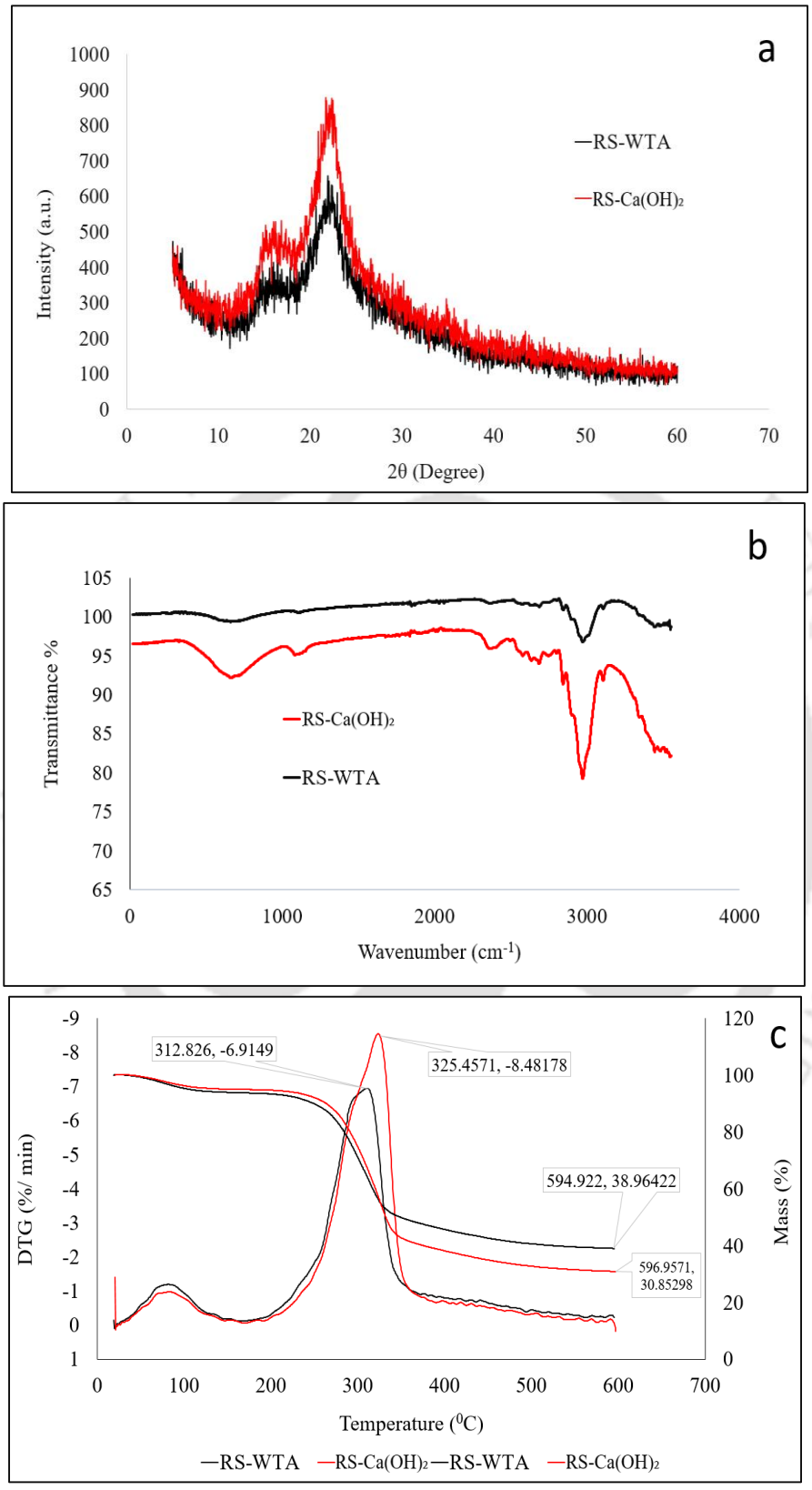

Figure 5.2.2 (i). (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-Ca(OH)$_2$

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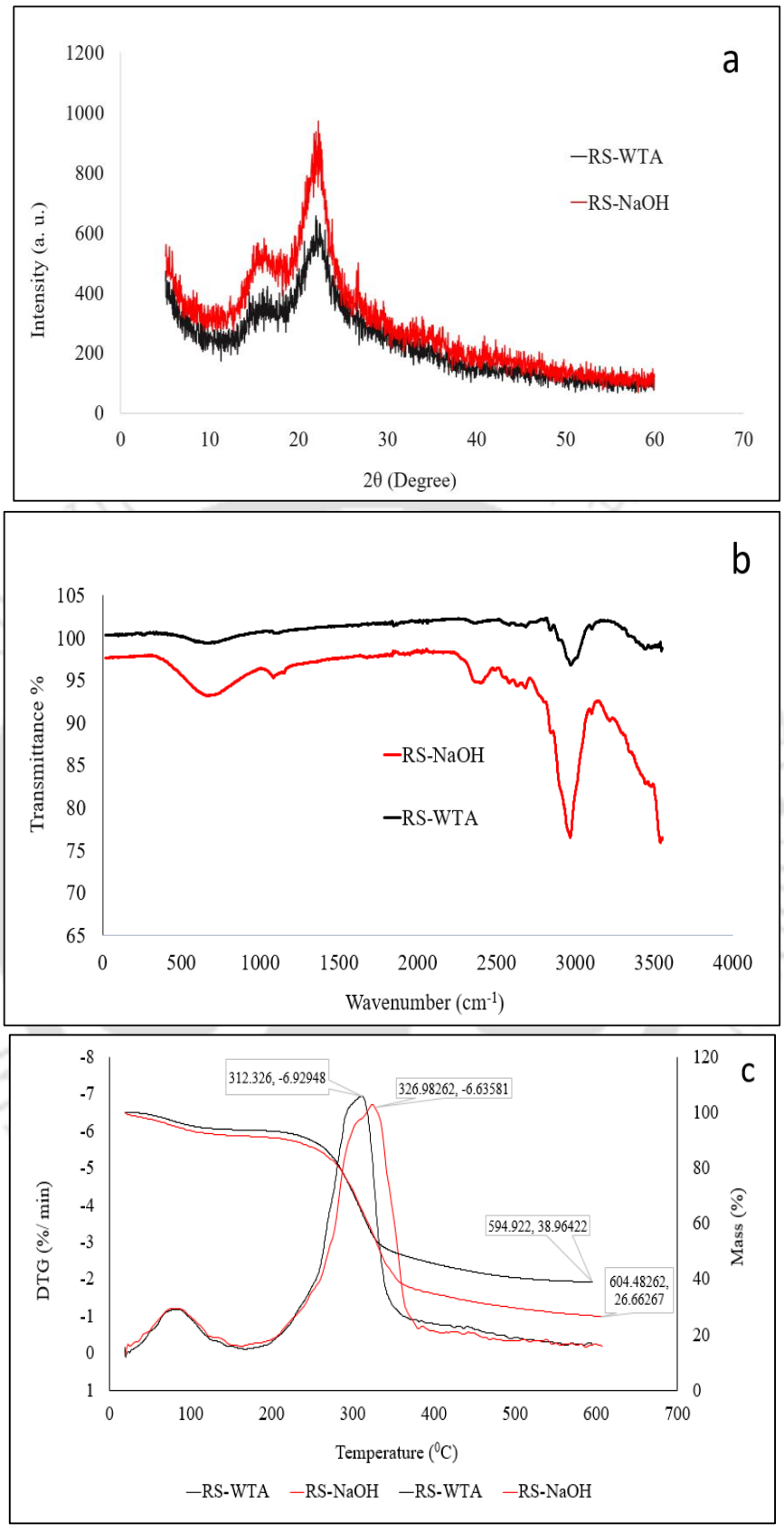

Figure 5.2.2 (ii). (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-NaOH

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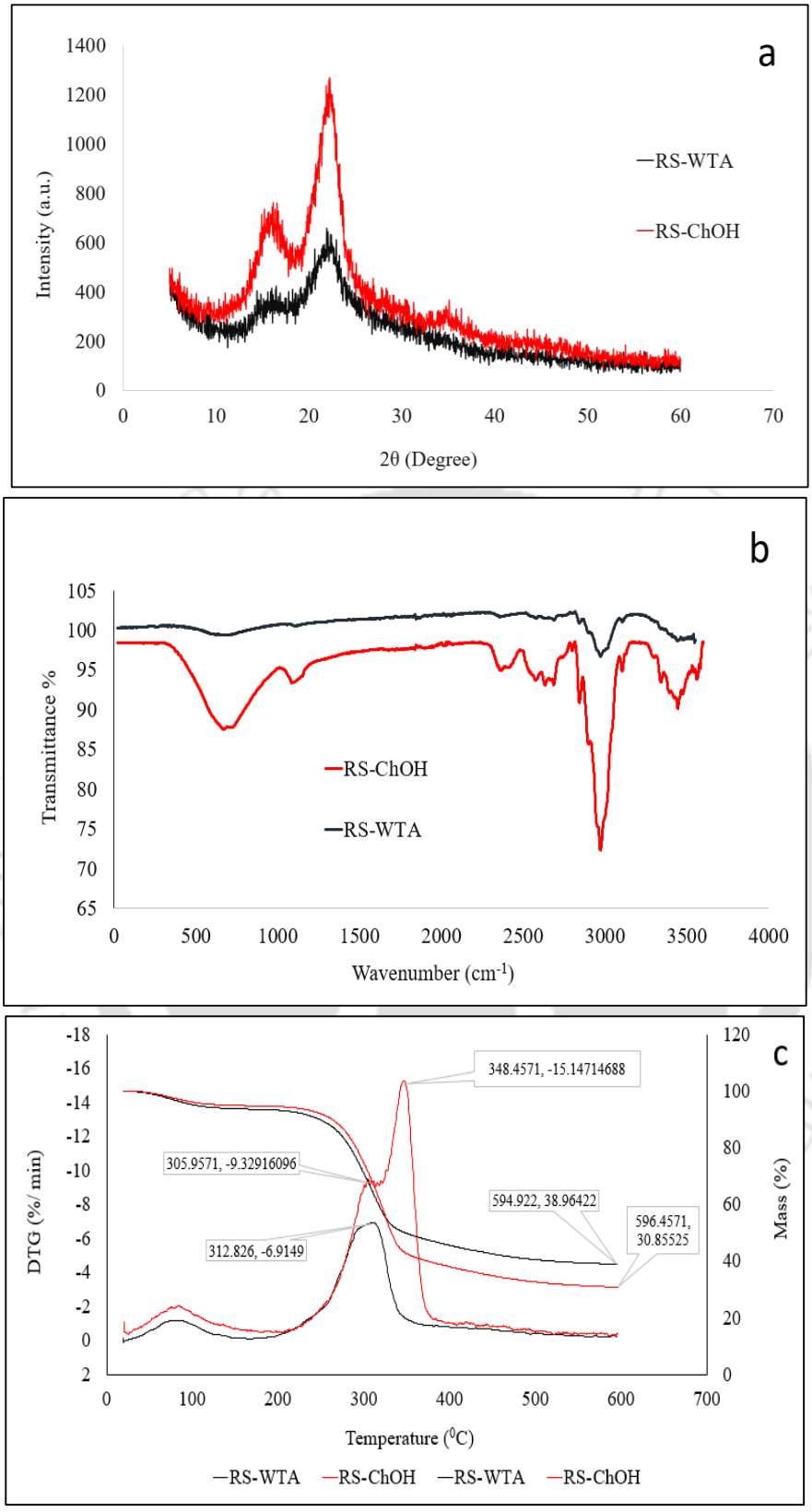

Figure 5.2.2 (iii). (a) XRD (b) FTIR (c) TGA analysis of RS-WTA and RS-ChOH

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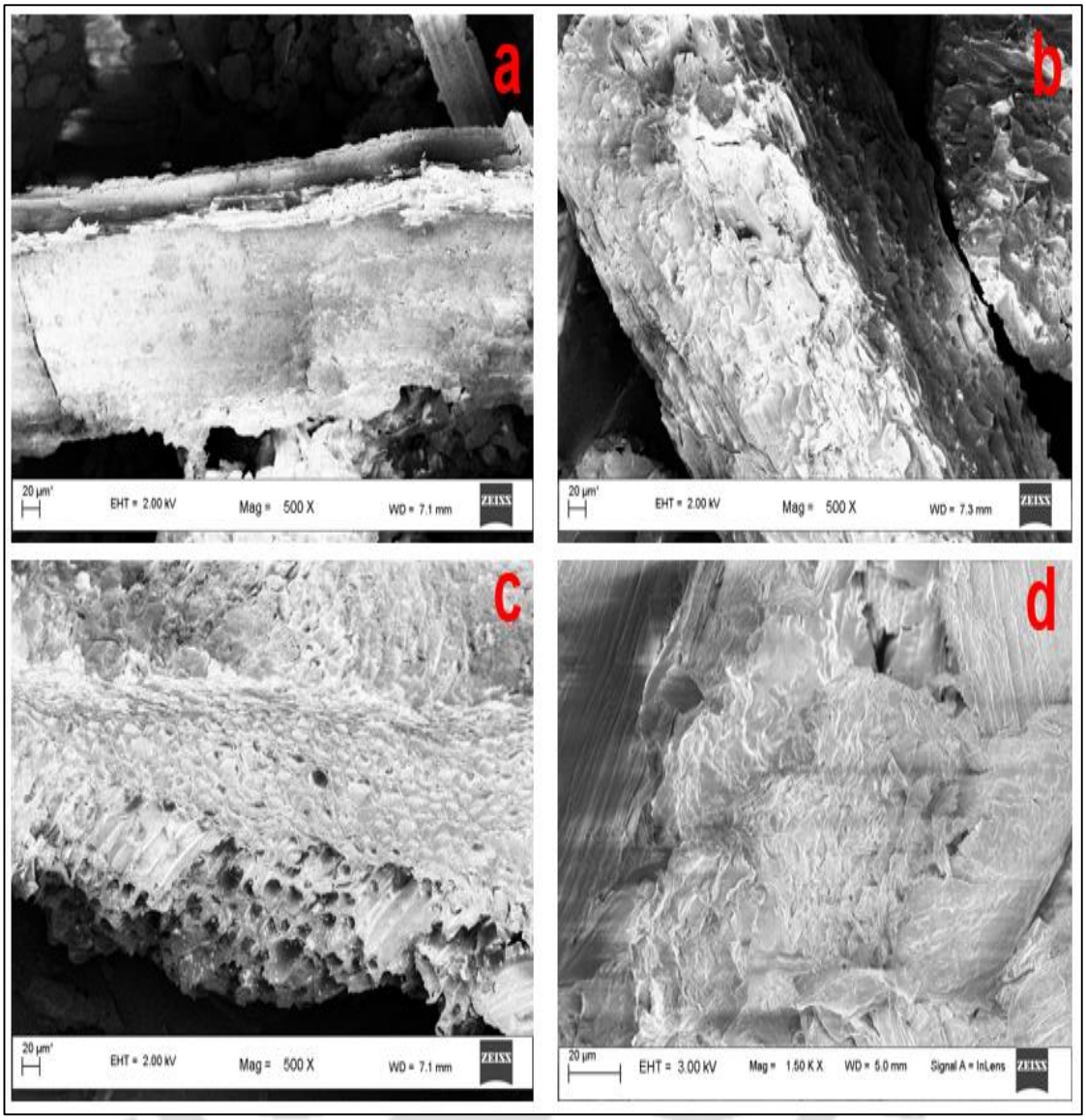

Figure 5.2.2 (iv). FESEM images of (a) RS-WTA (b) RS-$Ca(OH)_2$ (c) RS-NaOH (d) RS-ChOH

TH-2881_146104040

5.3. Collection, characterization, and acclimatization of a suitable biosludge to use as inoculum for bioreactors.

This study aims to know the initial characteristics of paper mill sludge, and also to acclimatize the bacterial consortia in presence of sulfate, nitrate, and arsenic-containing environment.

5.3.1. Initial Characteristics of Paper Mill Sludge

Table 5.3.1, shows the initial characteristics of paper mill sludge. The pH of the sludge was in the range of 6.6-6.8. The MLSS and MLVSS concentration were 12154-15514 mg/L and 8860-11690 mg/L respectively. COD, BOD, TRS and VFA were 37518-41253, 10696-15456, 13164-16114 and 3291-6445 mg/kg respectively. The presence of sulfate, sulfide, nitrate, nitrite and arsenic was found to be 12340-15469 mg/kg, 1245-2256 mg/kg, and 3291-4383 mg/kg, and 1562-1611 mg/kg and bdl respectively.

Table 5.3.1 Characteristics of paper mill sludge

Parameter	Concentration	Unit
pH	6.6-6.8	-
MLSS	12154-15514	mg/L
MLVSS	8860-11690	mg/L
MLVSS/MLSS	0.72-0.76	-
COD	37518-41253	mg/kg
BOD	10696-15456	mg/kg
BOD: COD	0.28-0.37	-
TRS	13164-16114	mg/kg
VFA	3291-6445	mg/kg
Sulfate	12340-15469	mg/kg
Sulfide	1245-2256	mg/kg

TH-2881_146104040

Nitrate	3291-4383	mg/kg
Nitrite	1562-1611	mg/kg
Arsenic	bdl	-

5.3.2. Performance of Reactor AC During Acclimatization

Paper mill sludge was acclimatized for 40 days before being used as inoculum at the room temperature of 30 ± 5°C. The acclimatized bacterial culture was used as inoculum for all further experiments. In the 1st cycle, 43.7 % COD, 41.3 % SO_4^{2-}, 80.9 % NO_3^-, and 76.8 % arsenic were removed. In the 2nd cycle, 66.5 % COD, 66.3 % SO_4^{2-}, 81.4 % NO_3^-, and 89.7 % arsenic were removed. After the 3rd cycle, 90 % COD, 77 % SO_4^{2-}, 88 % NO_3^-, and 86 % arsenic were removed. In the 4th cycle the removal efficiency of the above-mentioned parameters was not improved (which was 90.4 % COD, 83 % SO_4^{2-}, 90 % NO_3^-, 87 % arsenic). During the last two phases of the acclimatization period, the removal of COD, sulfate, nitrate, and arsenic remained almost the same. The acclimatized bacterial culture was used as inoculum for all further experiments. The performance of reactor AC during the acclimatization is shown in figure 5.3.1.

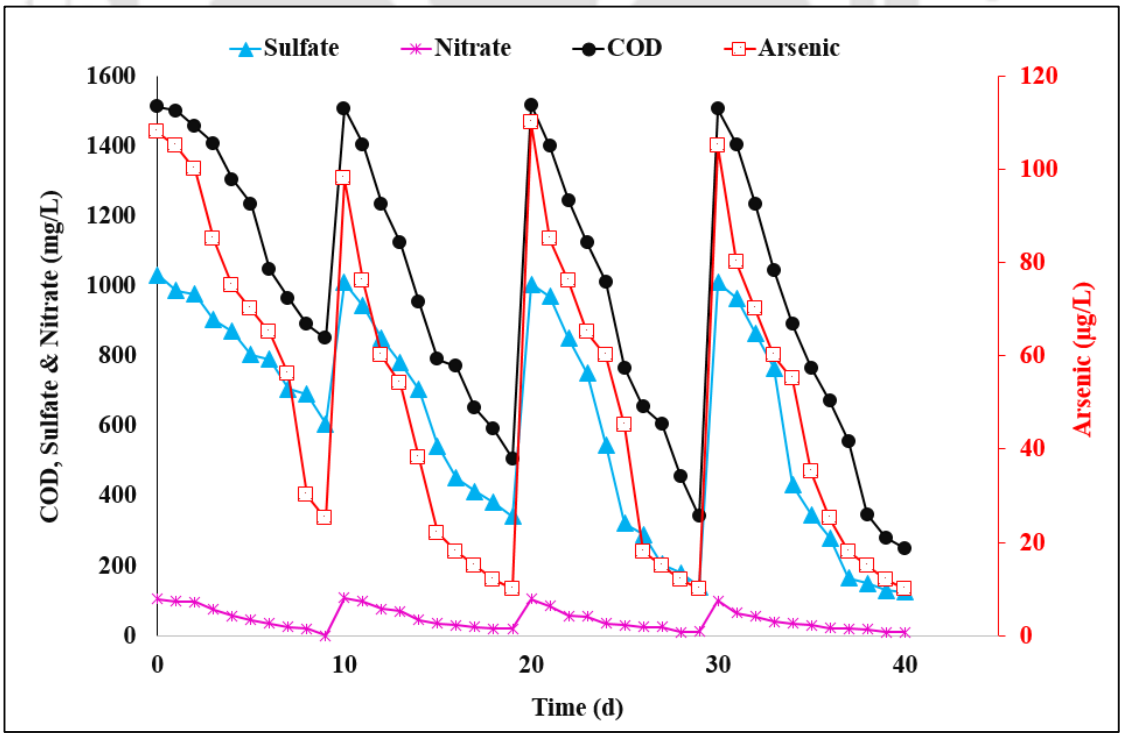

Figure 5.3.1. Performance of reactor AC during the acclimatization

TH-2881_146104040

5.4. Use of rice straw slurry as a carbon source in biological reduction of oxyanions in batch mode.

5.4.1. Performance Evaluation of Batch Reactors R1, R2, R3, and R4

Figure 5.4.1, represents the profile of (a) COD (b) BOD (c) VFA and pH (d) sulfate (e) and sulfide in batch reactors (R1, R2, R3, and R4).

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.1 (a)], BOD [figure 5.4.1 (b)] and VFA [figure 5.4.1 (c)] concentration were 877± 74, 176±12, 70±15 mg/L respectively in reactor R1; 740±80, 210±40, 85±25 mg/L respectively in R2; 690± 60, 198±30, 90±32 mg/L respectively in R3; and 910±105, 240±45, 105±38 mg/L respectively in R4 at 0^{th} day. Due to the anaerobic digestion process of lignocellulosic compounds of rice straw, the increment in COD, BOD, and VFA was observed.

At 10^{th} days, the COD [figure 5.4.1 (a)], BOD [figure 5.4.1 (b)] and VFA [figure 5.4.1 (c)] concentration were reached to 1590±216, 876±103, 650±70 mg/L in R1; 1955±126, 1173±125, 754±85 mg/L in R2; 2786±140, 1672±156, 1034±190 mg/L in R3; and 3456±250, 1978±180, 1245±150 mg/L in R4. The maximum accumulation of COD, BOD, and VFA was in reactor R4 at 10 days, then reactor R3> R2 > and R1. As acidic pH creates the inhibitory effect on acids formation thereby lower production of COD, BOD and VFA was observed as compared to neutral and alkaline pH. These results are consistent with the literature (Xu et al., 2021), they studied on hydrolysis behavior of lignocellulose compound (Corn Stover) and observed higher COD accumulation in the trend of pH 8 > pH 7 > pH 6 and pH 5 in the fermentation process. It was also observed that the reactor R1 and R2 (acidic pH) took a long time (25 days) to reach the maximum level of COD production and gave lesser production as compared to R3 (neutral pH) and R4 (alkaline pH). Reactor R3 and R4 took 15 and 10 days respectively to reach their highest level of COD. Under the acidic conditions (reactor R1 and R2), a poor degradation rate of lignocellulosic compounds of rice straw was observed in contrast to reactor R3 and R4. It may be because acidic conditions create the inhibition of microbial hydrolysis and suppress the activity of fermentative bacteria whereas alkaline pH promotes the hydrolysis process and VFA accumulation (Karthikeyan et al., 2016; Van Ginkel et al., 2005; Wang et al., 2016; Xu et al., 2021).

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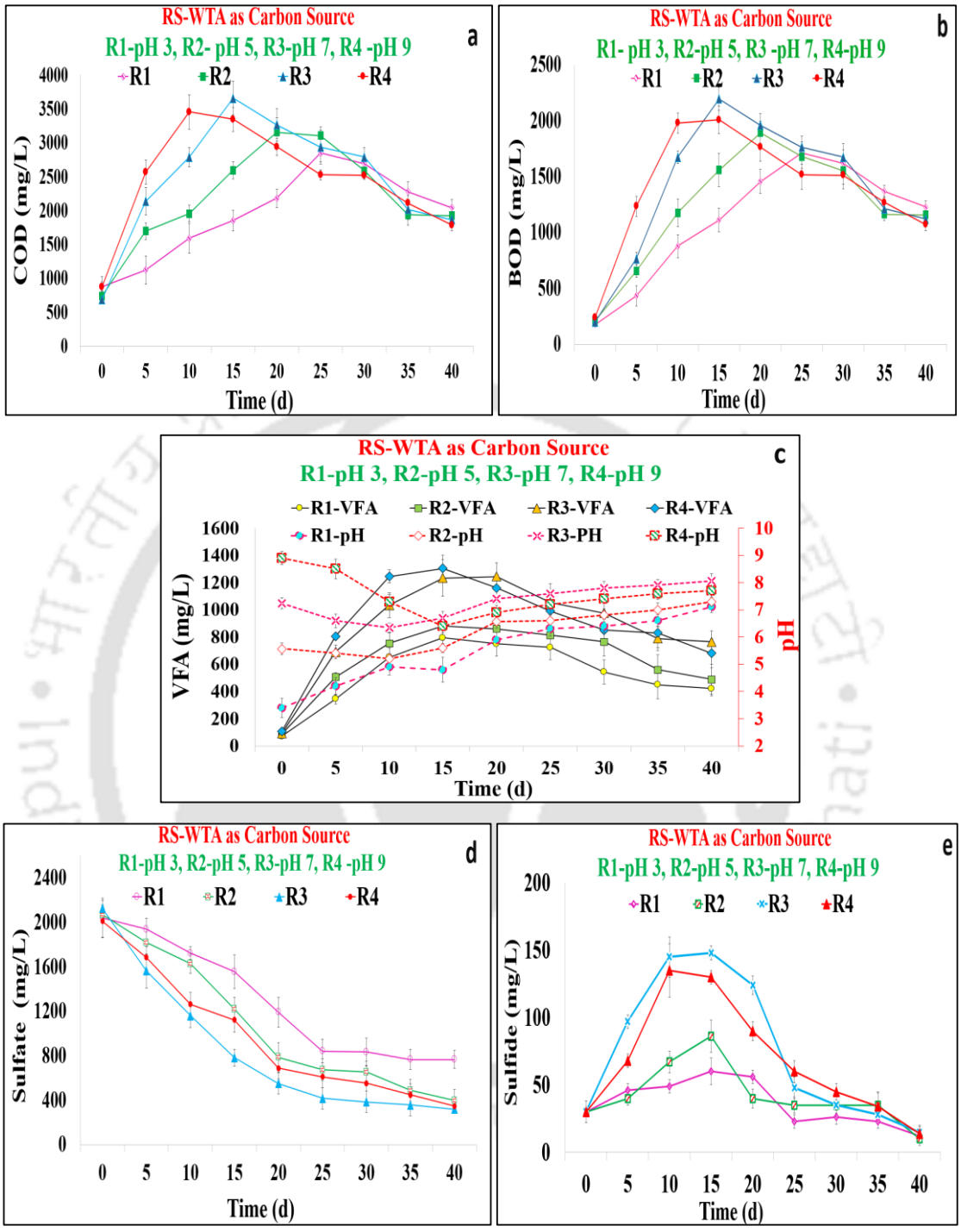

Figure 5.4.1. Profile of: (a) COD (b) BOD (c) VFA and pH (d) sulfate (e) and sulfide in batch reactors (R1, R2, R3, and R4)

Initial pH is responsible for affecting the anaerobic digestion of lignocellulosic compounds of rice straw as well as sulfate removal efficiency. There may be two types of microorganisms active in this biological system first viz fermentative bacteria and

TH-2881_146104040

second is sulfate-reducing bacteria (Wang et al., 2016). The initial pH can affect the hydrolysis and metabolic pathways of the bacteria.

The bacteria which are responsible for lignocellulose degradation are mainly facultative anaerobic bacteria, which are widely active in various anaerobic and microaerobic degradation processes, and play a role in macromolecular hydrolysis and organic acid production (Xu et al., 2021). A study conducted by (Van Ginkel et al., 2005), they have reported that the suppression effect takes place at pH=5 because of the impact on dissociated acid content. Karthikeyan et al. (2016) reported that the VFA content within the traditional continuous stirred tank reactor increased by 35% at pH = 9 relative to that at pH = 6 in the processes of kitchen waste hydrolysis and acidification. During the intermediate phase of the study dropdown in pH was observed that may be due to the formation of volatile fatty acids. The intermediate pH was 4.8±0.45, 5.6±0.16, 6.7±0.12, 6.4±0.24 on the 15^{th} day and the final pH was 7.1 ±0.2, 7.3±0.18, 8±0.26, 7.7±0.3 in reactors R1, R2, R3, and R4 respectively [figure 5.4.1 (c)]. The increment in the pH may be due to buffer capacity developed by CO_2 release and the presence of bicarbonate (HCO_3^-) (López González et al., 2013).

Sulfate Removal and Sulfide Production

With the initial sulfate concentration of 2040±180 mg/L, 2069±70 mg/L, 2127±71 mg/L, and 2012±146 mg/L, the overall sulfate removal efficiency was 62%, 80%, 85%, and 82% in the reactors R1, R2, R3, and R4 respectively within 40 days [figure 5.4.1 (d)]. Final sulfate concentration was 765±80 mg/L, 395± 98 mg/L, 318± 28 mg/L, 346±40 mg/L in R1, R2, R3, and R4 respectively [figure 5.4.1 (d)]. In terms of sulfate removal efficiencies, the sequence of reactors is R3 (pH 7) > R4 (pH 9) > R2 (pH 5) > R1 (pH 3), as it is well known that neutral pH favors sulfate removal. Initial pH was found to be responsible for suppressing the activity of sulfate-reducing microorganisms thus sulfate removal efficiency was affected. Results are consistent with the literature (Najib et al., 2017), they have studied the effect of pH (6, 7, and 8) on sulfate removal with initial concentration (1000-4000 mg/L), and reported the optimum pH of 7.19. Similarly, Meulepas et al. (2009) reported the optimum pH of 7.5 at various pH conditions (6, 6.5, 7, 7.5, 8, and 8.5) for the sulfate removal process. Sulfate reducing bacteria is generally suitable for growth in neutral conditions at the pH of 6–8 and is sensitive to pH changes (Widdel, 1988).

TH-2881_146104040

The maximum sulfide concentration was 60 ±10 (15^{th} d), 86 ±12 (15^{th} d), 145± 15 (10^{th} d), 135± 20 mg/L (10^{th} d) in reactors R1, R2, R3, and R4 respectively [figure 5.4.1 (d)]. Sulfide formation was found to be higher in reactor R3 than in R4>R2> and R1. The concentration of sulfide was gradually enhanced with the sulfate reduction. As much as sulfate removal takes place much sulfide formation takes place thereby it produces alkalinity during the sulfate removal process, this also may be the reason for pH increment.

TH-2881_146104040

5.4.2. Performance Evaluation of Batch Reactors R-Ca(OH)$_2$, R-NaOH, and R-ChOH

Figure 5.4.2, represents the profile of (a) COD (b) BOD (c) VFA and pH (d), sulfate (e), and sulfide in batch reactors (R-Ca(OH)$_2$, R-NaOH, and R-ChOH).

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.2 (a)], BOD [figure 5.4.2 (b)] and VFA [figure 5.4.2 (c)] concentration were 1886±356 mg/L, 842±50 mg/L, and 471±80 mg/L respectively in the reactor R-Ca(OH)$_2$; 4120±534 mg/L, 2408±256 mg/L, and 1030±135 mg/L respectively in reactor R-NaOH; 5234±486 mg/L, 2976 ±231 mg/L, and 1308 ±120 mg/L respectively in reactor R-ChOH.

The maximum accumulation of COD [figure 5.4.2 (a)], BOD [figure 5.4.2 (b)], and VFA [figure 5.4.2 (c)] was observed in reactor RS-NaOH, i. e. 11233±456 mg/L, 6534±350 mg/L, and 3865±245 mg/L respectively on the 10th day. The concentration of these parameters was 4.03, 3.9, and 3.72 times higher than reactor R3 which was run using RS-WTA (without thermoalkali treated rice straw) on the 10th day. Furthermore, it was 1.46, 1.79, and 1.52 times higher than reactor R-Ca(OH)$_2$ and 1.25, 1.15, and 1.23 times higher than reactor R-ChOH at 10th days. An increase in the concentrations of these parameters could have been due to rapid hydrolysis of the lignocellulose of thermoalkali-treated rice straw by microorganisms. Breakdown of the cellulose, hemicellulose, and lignin in rice straw after thermoalkali pretreatment would have made it more vulnerable to hydrolysis by bacterial mass (Harrison et al., 2013). Reactor R-NaOH took only 10 days to reach the highest level of COD, BOD, and VFA accumulation, whereas reactor R3, R-Ca(OH)$_2$, and R-ChOH took 15 days to reach the highest level. From this observation, it can be concluded that R-NaOH was found to be more effective for giving the maximum accumulation of COD, BOD, and VFA with a faster rate of hydrolysis. The concentration of these parameters was sharply increased in thermoalkali treated rice straw (R-Ca(OH)$_2$/R-NaOH/R-ChOH) as compared to without thermoalkali treated (RS-WTA).

Alkaline pretreatment is responsible for changes in chemical composition, chemical structure, and physical characteristics of the lignocellulosic compounds (Hendriks and Zeeman, 2009). During the alkali pretreatment following reactions takes place such as

TH-2881_146104040

dissolution; 'peeling' of end-groups, hydrolysis, degradation, and decomposition of dissolved polysaccharides. Alkali pretreatment can also cause solubilization, redistribution, and condensation of lignin and modifications in the crystalline state of the cellulose, solvation, and saponification attributed to higher biodegradability, which makes the component more accessible for bacteria (Hendriks and Zeeman, 2009). During the intermediate days of operation, VFA content was increased, thus a simultaneous dropdown in pH was observed [figure 5.4.2 (c)]. Final pH was 8.1±0.23, 8.3±0.28, 8.5±0.18 in reactors R-$Ca(OH)_2$, R-NaOH, R-ChOH respectively [figure 5.4.2 (c)]. The final pH values increased due to OH^- addition during pretreatment, buffer capacity developed by CO_2 release, and the presence of buffer capacity in the form of bicarbonate (HCO_3^{2-}) (López González et al., 2013). The VFA initially increases then decreases which is contrary to the pH curve. A similar trend of VFA and pH was observed in a study (Song et al., 2014) on the anaerobic digestion of corn straw.

Sulfate Removal and Sulfide Production

The batch reactors R-$Ca(OH)_2$, R-NaOH, and R-ChOH, with an initial sulfate concentration of 2035±150 mg/L, 2014±25 mg/L, 2067±130 mg/L, showed sulfate removal efficiency of 90%, 94%, and 92% respectively with the final sulfate concentration of 210±94 mg/L, 138±30 mg/L, and 164±40 mg/L which is well below the permissible limit of water quality standards [figure 5.4.2 (d)]. The sulfate removal rate was 117, 144, and 141 mg/L/d, within 10 days in the reactors R-$Ca(OH)_2$, R-NaOH, and R-ChOH respectively. The sulfate removal rate in RS-NaOH was 1.5 times higher than the reactor R3 (which was run using RS-WTA, without thermoalkali pretreated rice straw), 1.23 and 1.04 times than the R-$Ca(OH)_2$, and R-ChOH, it was enhanced due to the effect of thermoalkali pretreatment, possibly due to the availability and production of more carbon source present in the reactor. Sulfate removal was found to be highest in the sequence of R-NaOH > R-ChOH > R-$Ca(OH)_2$. Initial sulfide concentration was 30±5 mg/L, 30±8 mg/L, 38±6 mg/L in reactor R-$Ca(OH)_2$, R-NaOH, and R-ChOH respectively [figure 5.4.2 (d)]. As much as sulfate reduction was started that much sulfide formation was observed. The maximum concentration of sulfide was 127 ±18 mg/L, 135± 10 mg/L, 145± 12 mg/L in reactors R-$Ca(OH)_2$, R-NaOH, and R-ChOH respectively on the 10^{th} day [figure 5.4.2 (d)].

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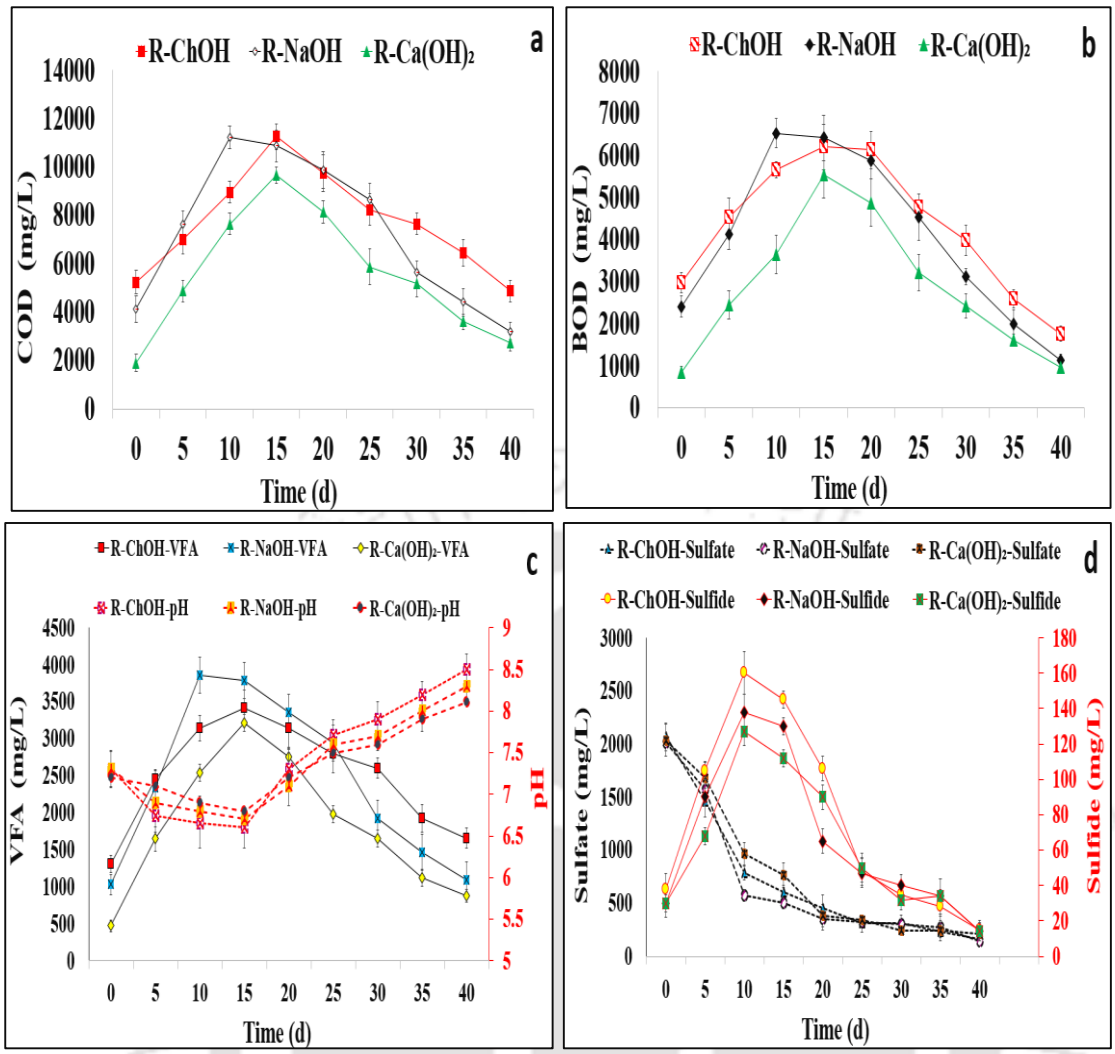

Figure 5.4.2. Profile of: (a) COD (b) BOD (c) VFA and pH (d), sulfate (e) and sulfide in batch reactors (R-Ca(OH)$_2$, R-NaOH, and R-ChOH)

Present studies 5.4.1 and 5.4.2, demonstrates that the rice straw was found to be useful for treating sulfate and nitrate-rich wastewater. It can be concluded that thermoalkali pretreatment was found to be beneficial for enhancing the soluble compounds or electron donors produced by the decomposition of lignocellulose. It provides more amount of electron donors within lesser time as well as improves the sulfate removal efficiency. Due to the effect of pretreatment, alkali and water can penetrate through the cell wall/structure of biomass, hydrates cellulose, and dissolve hemicellulose and lignin. Consequently, the release of organic acids/monomers from hemicellulosic groups facilitates the alteration of lignocellulosic structure, producing a highly reactive cellulose fiber that can be further easily hydrolyzed by the bacteria without inhabitation during anaerobic digestion. Enhancement in sulfate removal processes was observed (in

TH-2881_146104040

reactors R-Ca(OH)$_2$, R-NaOH, and R-ChOH) due to the generation of more electron donors from thermoalkali pretreated rice straw.

TH-2881_146104040

5.4.3. Performance Evaluation of Batch Reactors S1, S2, and S3

Figure 5.4.3 represents the profile of (a) COD (b) BOD (c) VFA and pH (d) sulfate (e) and sulfide in batch reactors (S1, S2, and S3).

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.3 (a)], BOD [figure 5.4.3 (b)] and VFA [figure 5.4.3 (c)] concentration were 4660±350 mg/L, 1608±345 mg/L, and 1034±264 mg/L respectively in the reactor S1; 4056 ±326 mg/L, 1456±370 mg/L, and 1235±286 mg/L respectively in the reactor S2; 4208± 456 mg/L, 1560±430 mg/L, and 1124 ±105 mg/L respectively in the reactor S3.

With time due to anaerobic digestion of the lignocellulosic compounds of rice straw the increment in COD, BOD and VFA was observed. The maximum produced COD was 10852±540 mg/L, 9976 ±456 mg/L, and 9320±554 mg/L in the reactors S1, S2, and S3 respectively on the 15th day. The BOD was 10852±540 mg/L, 9976 ±456 mg/L, 9320±554 mg/L, and VFA was 4393±254 mg/L, 4121±256 mg/L, 3765±159 mg/L in the reactors S1, S2, and S3 respectively at the 10th day. After reaching its peak level (at 10-15 days) reduction in COD, BOD and VFA were observed. An intermediate dropdown in pH was observed that may be due to the formation of volatile fatty acids [figure 5.4.3 (c)]. Final pH was observed i. e. 7.9 ±0.2, 8.0±0.25, and 8.3±0.3 in reactors S1, S2, and S3 respectively on the 40th day.

Sulfate Removal and Sulfide Production

The batch reactors S1, S2, and S3, with an initial sulfate concentration of 1040±50 mg/L, 2008±105 mg/L, and 3018±130 mg/L, showed the removal efficiency of 95 %, 94 %, and 92 % respectively with the final concentration of 48 ±18 mg/L, 102± 30 mg/L, 234± 35 mg/L, which is well below the permissible limit of water quality standards [figure 5.4.3 (d)]. The sulfate removal rate was 91, 135, and 147 mg/L/d, within 10 days in reactors S1, S2, and S3 respectively. The initial sulfide concentration was found to be 35±6 mg/L, 47±8 mg/L, and 50±8 mg/L in the reactors S1, S2, and S3 respectively [figure 5.4.3 (d)]. As much as sulfate reduction was started that much sulfide formation was observed, reaching its maximum level, 96 ±6 mg/L, 165 ±18 mg/L, 198± 12 mg/L in reactors S1, S2, and S3 respectively on the 15th day. The present

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study demonstrates that the rice straw was found to use for treating sulfate-rich wastewater.

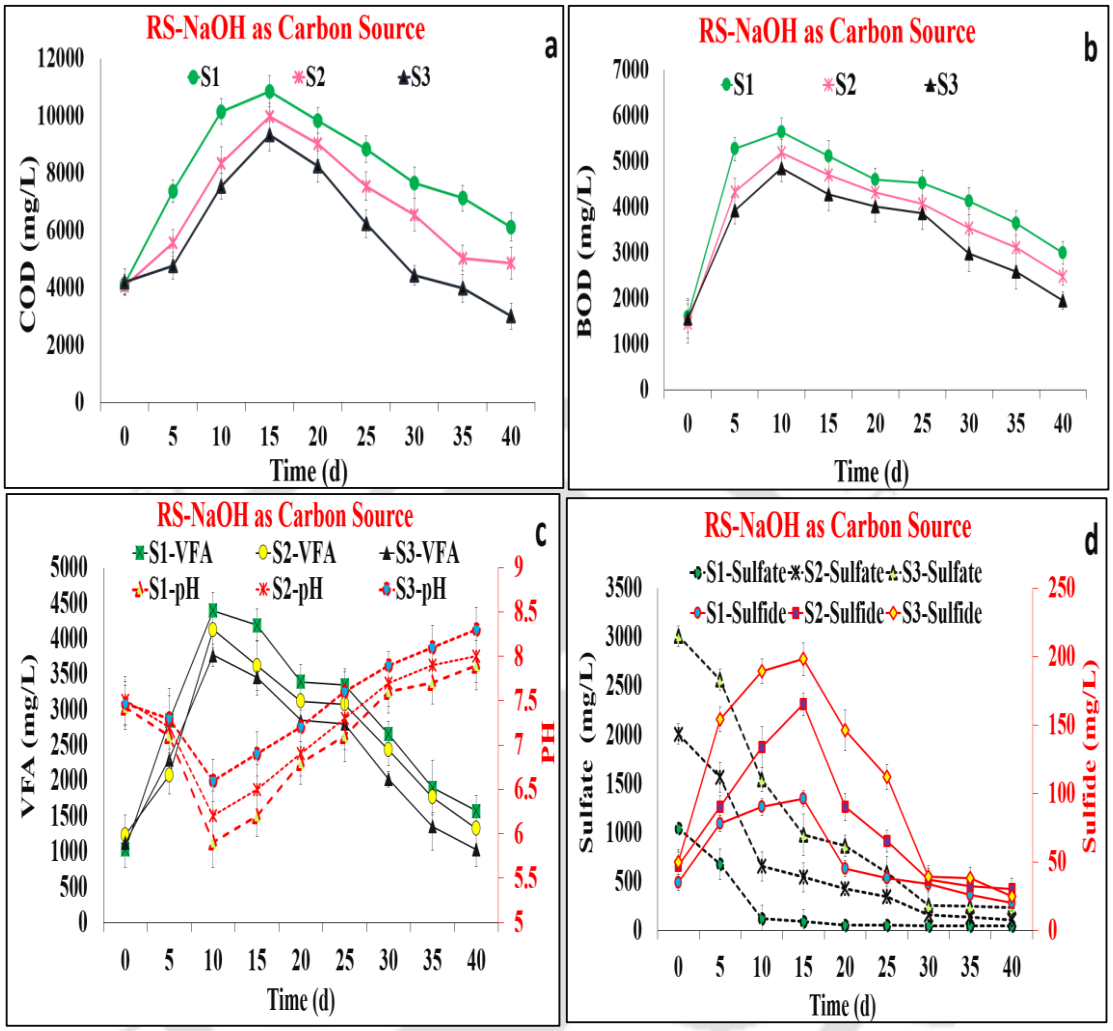

Figure 5.4.3. Profile of: (a) COD (b) BOD (c) VFA and pH (d) sulfate and sulfide in batch reactors (S1, S2, and S3)

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5.4.4. Performance Evaluation of Batch Reactors N1, N2, and N3

Figure 5.4.4, represents the profile of (a) COD (b) BOD (c) VFA and pH (d) nitrate (e) and nitrite in batch reactors (N1, N2, and N3).

COD, BOD, VFA and pH Profile

The COD [figure 5.4.4 (a)], BOD [figure 5.4.4 (b)] and VFA [figure 5.4.4 (c)] concentration were 4208±450 mg/L, 1690±250 mg/L, and 1070±180 mg/L respectively in reactor N1; 4015±543 mg/L, 1480±352 mg/L, and 1006±108 mg/L respectively in reactor N2; 4276±520 mg/L, 1558 ±218 mg/L, and 1290 ±118 mg/L in reactor N3.

At the intermediate phase, the increment in COD, BOD, and VFA was observed. After reaching its peak level reduction in COD, BOD and VFA were observed. Maximum produced COD was 9228±476 mg/L, 8433±687 mg/L, and 8124±456 mg/L from reactor N1, N2, and N3 respectively on the 10^{th} day. As well as intermediate dropdown in pH was observed that may be due to the formation of volatile fatty acids figure 5.4.4 (c). The final pH was 8.2±0.25, 8.4±0.20, and 8.6 ±0.3 in the reactors N1, N2, and N3 respectively.

Nitrate Removal and Nitrite Production

The batch reactors N1, N2, and N3, with an initial nitrate concentration of 502 ±90, 1067±145 mg/L, 2076±124 mg/L, showed the removal efficiency of 94, 96, and 97 % and final concentration of 30±5 mg/L, 40±8 mg/L, 45±10 mg/L which is well below the permissible limit of water quality standards [figure 5.4.4 (d)]. The nitrate removal rate was 28, 51, and 62 mg/L/d in reactors N1, N2, and N3 respectively, within 10 days. As much as nitrate reduction was started that much nitrite formation was observed, reaching its maximum level, 56 ±6 mg/L, 112 ±10 mg/L, 134± 12 mg/L in reactor N1, N2, and N3 respectively on the 5^{th} day.

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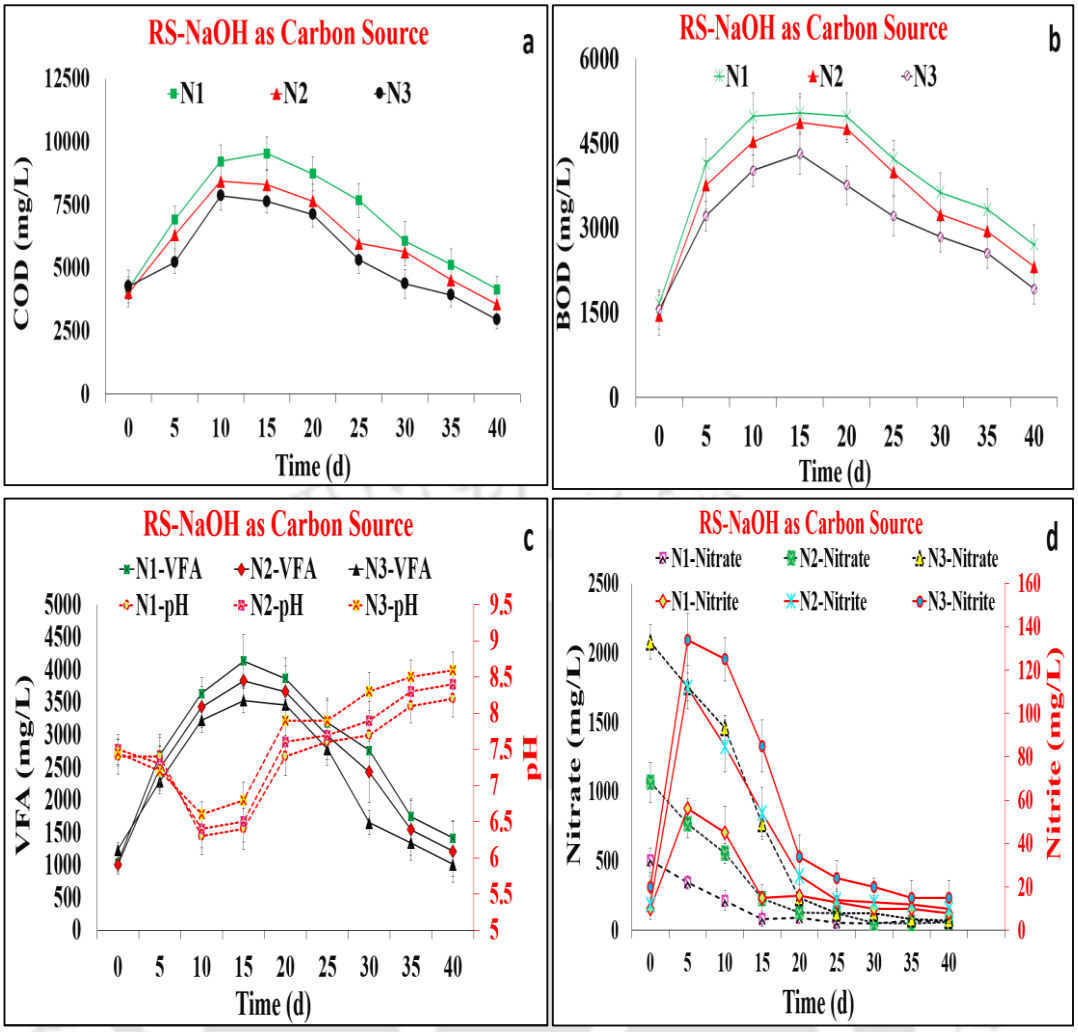

Figure 5.4.4. Profile of: (a) COD (b) BOD (c) VFA and pH (d) nitrate and nitrite in batch reactors (N1, N2, and N3)

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5.4.5. Performance Evaluation of Batch Reactors SN1, SN2, and SN3

Figure 5.4.5, represents the profile of (a) COD (b) BOD (c) VFA and pH (d) sulfate (e) sulfide (f) nitrate (g) nitrite in batch reactors (SN1, SN2, and SN3).

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.5 (a)], BOD [figure 5.4.5 (b)] and VFA [figure 5.4.5 (c)] concentration were 4156±550 mg/L, 1345±208 mg/L, 1006±230 mg/L in reactor SN1; 4225±345 mg/L, 1380±350 mg/L, 1235±120 mg/L in reactor SN2; 4034±435 mg/L, 1432±234 mg/L and 976±125 mg/L in reactor SN3. After reaching its peak level reduction in COD, BOD and VFA were observed. Maximum produced COD was 8654±856 mg/L, 8076±512 mg/L, and 7764±343 mg/L in the reactor SN1, SN2, and SN3 respectively on the 10^{th} day. A simultaneously dropdown in pH was observed that may be due to the formation of volatile fatty acids [figure 5.4.5 (c)]. Final pH was 8.3 ±0.22, 8.6 ±0.25, 8.8±0.26 in reactors SN1, SN2, and SN3 respectively [figure 5.4.5 (c)].

Sulfate Removal and Sulfide Production

In the batch reactors SN1, SN2, and SN3, the initial sulfate concentration was 2107±125 mg/L, 2065±120 mg/L, and 2042±145 mg/L respectively. The sulfate removal efficiency was 73, 67, and 58 % with the final concentration of 560±80, 675±50, 865± 90 mg/L in reactors SN1, SN2, and SN3 respectively [figure 5.4.5 (d)]. The sulfate removal rate was 66, 41, and 26 mg/L/d in reactors SN1, SN2, and SN3 respectively, within 10 days. The initial sulfide concentration was 25±6, 34±5, and 42±8 mg/L in reactors SN1, SN2, and SN3 respectively [figure 5.4.5 (e)]. As much as sulfate reduction was started that much sulfide formation was observed and reached its maximum level, 220 ±15 mg/L, 180± 12 mg/L, 146 ±10 mg/L in reactors SN1, SN2, and SN3 respectively on the 10^{th} day [figure 5.4.5 (e)].

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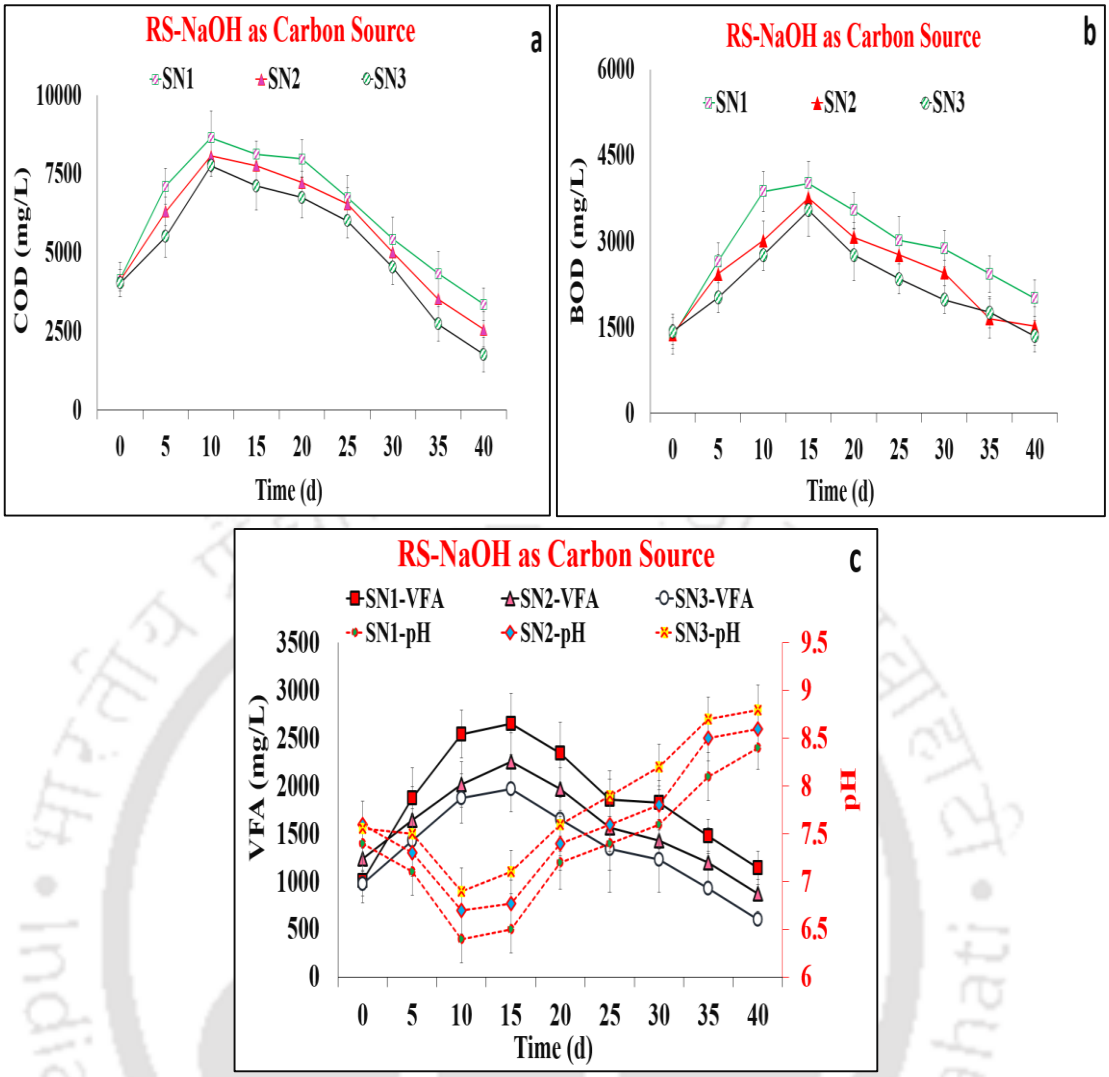

Figure 5.4.5. Profile of: (a) COD (b) BOD (c) VFA and pH in batch reactors (SN1, SN2, and SN3)

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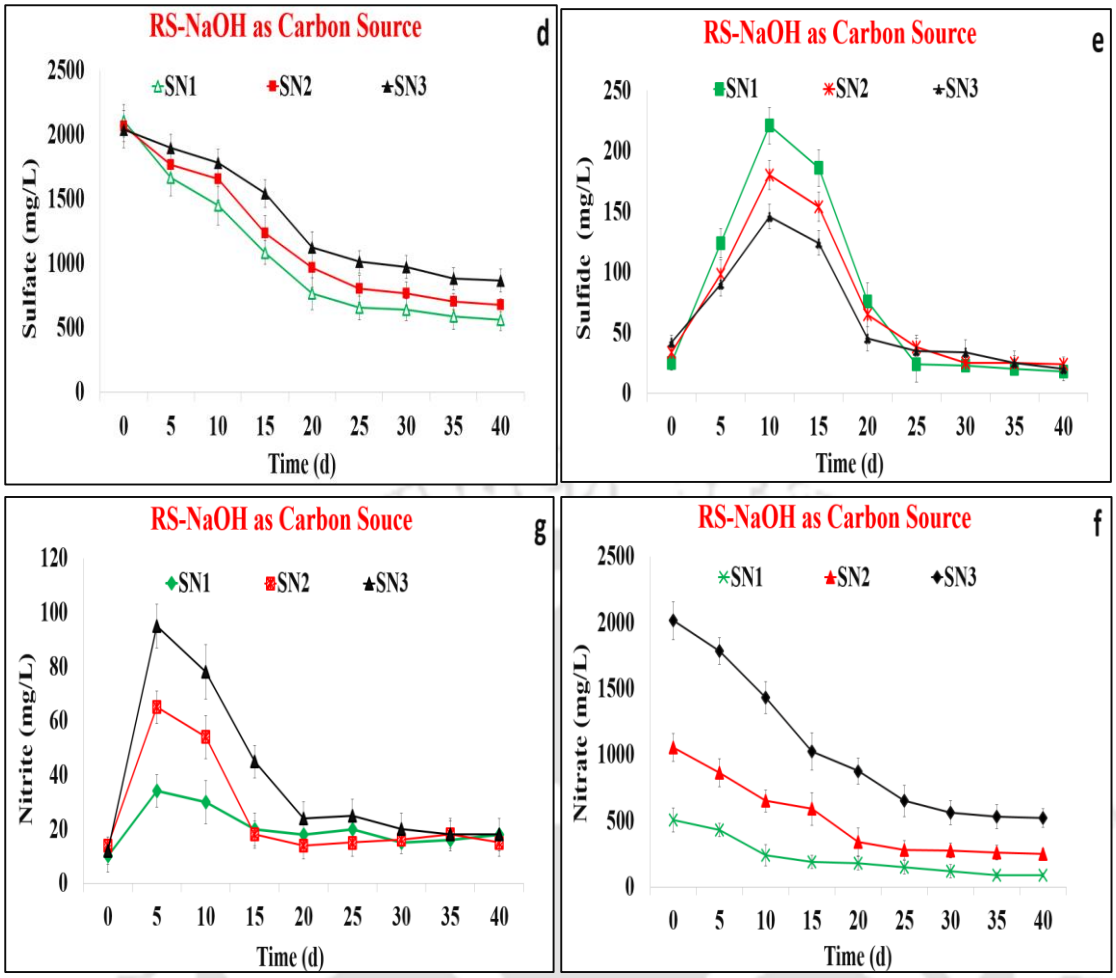

Figure 5.4.5. Profile of: (d) sulfate (e) sulfide (f) nitrate (g) nitrite in batch reactors (SN1, SN2, and SN3)

Nitrate Removal and Nitrite Production

In the batch reactors SN1, SN2, and SN3, the initial nitrate concentration was 505±90 mg/L, 1056±105 mg/L, and 2014±145 mg/L respectively. The nitrate removal efficiency was 82, 76, and 64 % with the final concentration of 90±15 mg/L, 250± 40 mg/L, and 520±70 mg/L in the batch reactors SN1, SN2, and SN3 respectively [figure 5.4.5 (f)]. The nitrate removal rate was 26, 41, and 58 mg/L/d in reactors SN1, SN2, and SN3 respectively, within 10th days. As much as nitrate reduction was started that much nitrite formation was observed, reaching its maximum level of 34±6, 65±6, and 95±8 mg/L in reactors SN1, SN2, and SN3 respectively on the 5th day [figure 5.4.5 (g)].

Nitrate removal rate increases with an increase in concentration. With the increment in nitrate concentration, sulfate removal rate and removal efficiency both were found to

TH-2881_146104040

be reduced due to competition between the electron donor. Increasing doses of nitrate concentration were found to be responsible for suppressing sulfide production, activity of sulfate reducing bacteria might have been affected. In multiple oxyanions system (sulfate and nitrate both) the removal rate and removal efficiency were lesser compared to the single oxyanion system. This may be due to the percentage flow of electron donors that may be distributed between the two electron acceptors.

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TH-2881_146104040

5.4.6. Performance Evaluation of Batch Reactors SA1, SA2, and SA3

Figure 5.4.6, represents the profile of (a) COD and arsenic (b) BOD (c) VFA and pH (d) sulfate and sulfide in batch reactors (SA1, SA2, and SA3)

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.6 (a)], BOD [figure 5.4.6 (b)] and VFA [figure 5.4.6 (c)] concentration were 4080±550 mg/L, 1880±240 mg/L, 1086±112 mg/L in reactor SA1; 4125±570 mg/L, 1654±350 mg/L, 976±40 mg/L in reactor SA2; 4234±640 mg/L, 1545 ±390 mg/L and 876±50 mg/L in reactor SA3. With time due to the anaerobic digestion of lignocellulosic compounds of rice straw the increment in COD, BOD and VFA was observed. After reaching its peak level reduction was observed. Maximum produced COD was 8134±467 mg/L 8321±370 mg/L and 7765±410 mg/L in the reactors SA1, SA2, and SA3 respectively on the 15th day [figure 5.4.6 (a)].

A simultaneously dropdown in pH was observed that may be attributed due to the formation of volatile fatty acids. An intermediate dropdown in pH was observed that maybe the due formation of volatile fatty acids during the hydrolysis process. The intermediate pH was 5.8 ±0.3, 5.7 ±0.25, 6.4 ±0.24 (at 15th day) in reactors SA1, SA2, and SA3 respectively [figure 5.4.6 (c)]. Following these days, an increment in the pH was observed, which was possibly due to the VFA consumption by SRB and other microorganisms and the alkalinity formation during the sulfate reduction process. Final pH was 7.9±0.2, 8.1 ±0.2, 8.3±0.24 in reactors SA1, SA2, and SA3 respectively [figure 5.4.6 (c)].

Sulfate Removal and Sulfide Production

In the batch reactors SA1, SA2, and SA3, the initial sulfate concentration was 2014±115 mg/L, 2134±106 mg/L, 2018±54 mg/L [figure 5.4.6 (d)]. The overall sulfate removal efficiency was 88, 91, and 92 % with the final concentration of 234±35 mg/L, 190 ±30 mg/L, and 170 ±15 mg/L in reactors SA1, SA2, and SA3 respectively, which is close to the permissible limit as per Indian standards (IS: 2296, 2015) [From figure 5.4.6 (d)]. The sulfate removal rate was 112, 115, and 123 mg/L/d in reactors SA1, SA2, and SA3 respectively, within 10 days. The maximum level of sulfide was 250 ±16 mg/L, 165±12 mg/L, and 90±10 mg/L in reactors SA1, SA2, and SA3 respectively on the 10th day [figure 5.4.6 (d)]. Increment in initial arsenic concentration did not affect the sulfate

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removal. Maximum available sulfide was observed in SA1 followed by SA2 than SA3. The concentration of sulfide was more at the initial 15 days of operation due to maximum sulfate removal taking place at this phase.

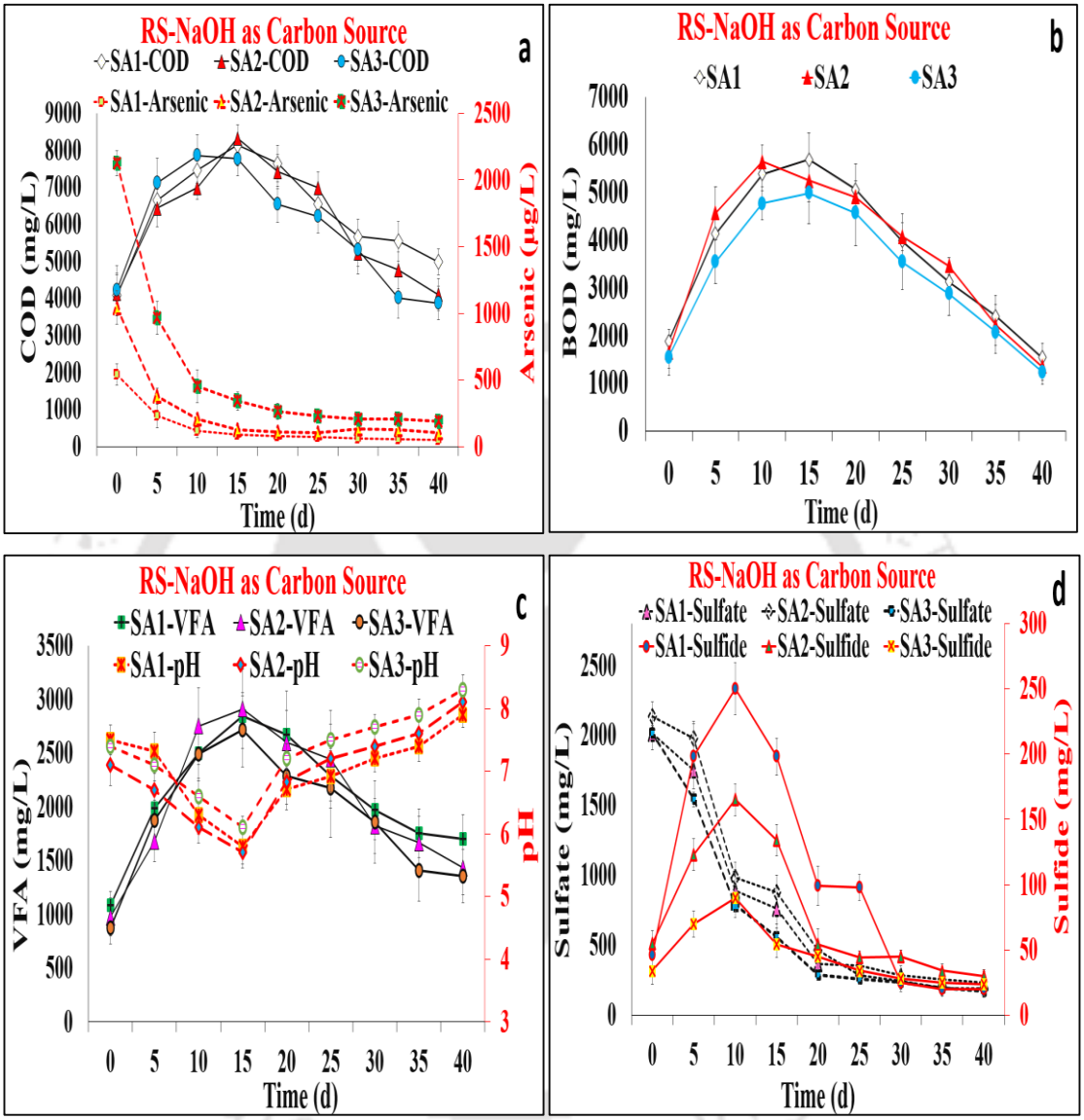

Figure 5.4.6. Profile of: (a) COD and arsenic (b) BOD (c) VFA and pH (d) sulfate and sulfide in batch reactors (SA1, SA2, and SA3)

Arsenic Removal

The initial arsenic concentration was 541±80 µg/L, 1042±124 µg/L, and 2123±95 µg/L in reactors SA1, SA2, and SA3 respectively [figure 5.4.6 (a)]. The overall arsenic removal efficiency was 90, 89, and 91 % with the final concentration of 50± 8 µg/L, 105 ±20 µg/L, 185± 28 µg/L in reactors SA1, SA2, and SA3 respectively, which is close to the permissible limit as per Indian standards (IS: 2296, 2015). The arsenic removal

TH-2881_146104040

rate was 42, 83, and 167 µg/L/d in reactors SA1, SA2, and SA3 respectively within 10 days. The present study demonstrates that the rice straw was found to be useful for treating sulfate and arsenic-rich wastewater.

TH-2881_146104040

5.4.7. Performance Evaluation of Batch Reactors SAN1, SAN2, and SAN3

Figure 5.4.7, represents the profile of (a) COD and arsenic (b) BOD (c) VFA and pH (d) sulfate and sulfide (e) nitrate and nitrite in batch reactors (SAN1, SAN2, and SAN3)

COD, BOD, VFA and pH Profile

The initial COD [figure 5.4.7 (a)], BOD [figure 5.4.7 (b)] and VFA [figure 5.4.7 (c)] concentration were 4324±345 mg/L, 1876±270 mg/L, 876±50 mg/L in reactors SAN1; 4086±450 mg/L, 1765±246 mg/L, 986±180 mg/L in reactor SAN2; 4245±380 mg/L, 1986±250 mg/L, 1078±70 mg/L in reactors SAN3. With time due to the anaerobic digestion of lignocellulosic compounds of rice straw the increment in COD, BOD and VFA was observed. After reaching its peak level reduction in COD, BOD and VFA were observed. Maximum produced COD was 9852±450 mg/L, 8865 ±405 mg/L, and 8047 ±502 mg/L in reactors SAN1, SAN2, and SAN3 respectively on the 15th day [figure 5.4.7 (a)].

The pH has an inverse trend with the VFA concentration. During the initial days of operation dropdown in pH was observed that may be attributed due to the formation of volatile fatty acids. The intermediate pH in the reactors was 6.8 ±0.2, 6.7 ±0.22, and 7.1 ±0.25 reactors SAN1, SAN2, and SAN3 respectively, on the 15th day [figure 5.4.7 (c)]. Following these days, an increment in the pH was observed, which was possibly due to the VFA consumption by SRB and other microorganisms and the alkalinity formation during the sulfate reduction process. Thus, the final pH again rose i. e. 8.1 ±0.20, 8.4±0.25, 8.6 ±0.24 in reactor SAN1, SAN2, and SAN3 at 40th day [figure 5.4.7 (c)]. Such a pH increase was also observed in previous studies (Cocos et al., 2002).

The increase of pH in the bioreactor may be associated with the dissolution of a surface-bound hydroxyl group from substrate materials in acidic conditions or from the alkalinity increase that resulted from the formation of carbonate species either through the dissolution of inorganic carbon from rice straw or through oxidation of readily available organic substrates by SRB. Additionally, as already reported by previous studies, the presence

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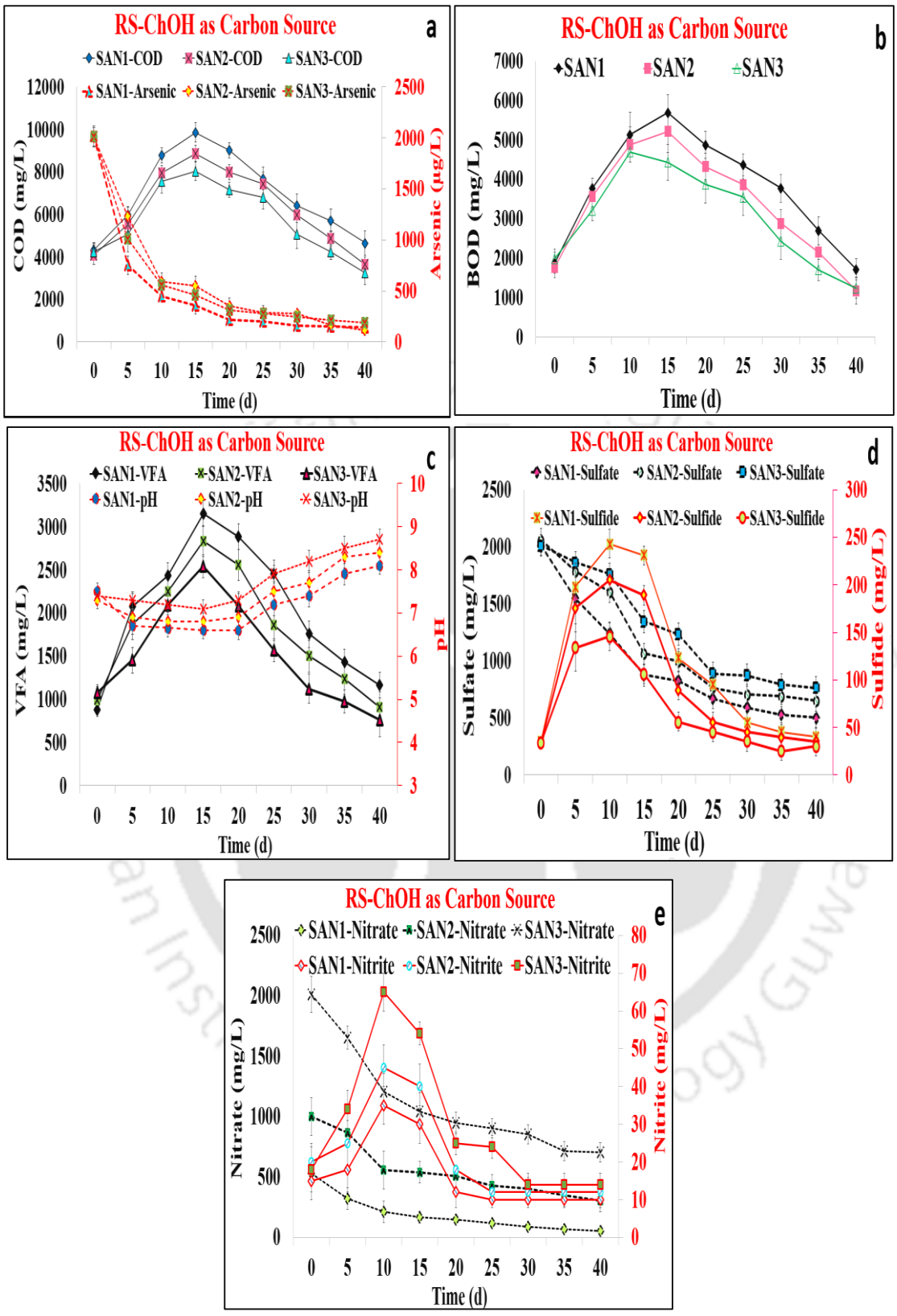

Figure 5.4.7. Profile of: (a) COD and arsenic (b) BOD (c) VFA and pH (d) sulfate and sulfide (e) nitrate and nitrite in batch reactors (SAN1, SAN2, and SAN3)

TH-2881_146104040

of ammonium originated from the nitrogen source/feed media, which was a commune substrate integrated with all cases tested in this study, and may have provided a pH-enhancing effect (Béchard et al., 1994; Cocos et al., 2002; Zagury et al., 2006).

Sulfate Removal and Sulfide Removal

The initial sulfate concentration was 2028±126 mg/L, 2175±154 mg/L, and 2046±50 mg/L in reactors SAN1, SAN2, and SAN3 respectively [figure 5.4.7 (d)]. The overall sulfate removal efficiency was 75, 68, and 62 % with the final sulfate concentration of 504±80 mg/L, 650±75 mg/L, and 765±95 mg/L in reactors SAN1, SAN2, and SAN3 respectively [figure 5.4.7 (d)]. The sulfate removal rate was 77, 45, and 25 mg/L/d in reactors SAN1, SAN2, and SAN3 respectively, within 10 days. Sulfate removal rate and efficiency both were reduced with an increment in nitrate concentration. The maximum level of sulfide was 243 ±15 mg/L, 205 ±10 mg/L, and 146± 15 mg/L in reactors SAN1, SAN2, and SAN3 respectively on the 10^{th} day [figure 5.4.7 (d)]. Sulfate removal rate and removal efficiency both were reduced with an increment in nitrate concentration.

Nitrate Removal and Nitrite Production

The initial nitrate concentration was 534±40 mg/L, 1005±130 mg/L, and 1030±180 mg/L in reactors SAN1, SAN2, and SAN3 respectively. The overall nitrate removal efficiency was 90, 70, and 65 % with the final nitrate concentration of 55±10 mg/L, 305±30 mg/L, and 704±80 mg/L in reactors SAN1, SAN2, and SAN3 respectively. The maximum level of nitrite was 32 ±5 mg/L, 45±8 mg/L, and 65±5 mg/L in reactors SAN1, SAN2, and SAN3 respectively on the 10^{th} day [figure 5.4.7 (e)]. The nitrate removal rate was 32, 44, and 81 mg/L/d in reactors SAN1, SAN2, and SAN3 respectively, within 10 days.

Arsenic Removal

The initial arsenic concentration was 2065±105 µg/L, 2004±122 µg/L, and 2074±134 µg/L in reactors SAN1, SAN2, and SAN3 respectively. The overall arsenic removal efficiency was 92, 94, and 90 % with the final arsenic concentration of 146±12 µg/L, 115±24 µg/L, and 190±20 µg/L in reactors SAN1, SAN2, and SAN3 respectively

TH-2881_146104040

[figure 5.4.7 (a)]. The arsenic removal rate was 156, 145, and 167 µg/L/d in reactor SAN1, SAN2, and SAN3 respectively, within 10 days.

The present study demonstrates rice straw could be efficiently used for treating sulfate, nitrate, and arsenic-containing wastewater under anaerobic digestion conditions.

TH-2881_146104040

5.4.8. Performance Evaluation of Batch Reactor S0N0

Initial COD, BOD, and VFA concentration of the batch reactor S0N0 was 4456±540 mg/L, 1650±350 mg/L, and 1045±150 mg/L. after 10 days of operation, COD, BOD, and VFA concentrations increased and reached 12343±565 mg/L, 6975±506 mg/L, and 4876±456 mg/L on the 10th day [figure 5.4.8 (a)], afterward reduction in the carbon sources was observed. At the intermediate days of operation [figure 5.4.8 (a)], (10-15 days) dropdown in pH (6.3±0.24) was observed which may be due to the formation and accumulation of volatile fatty acids due to the anaerobic digestion of lignocellulosic compounds of rice straw. The final pH was observed at 7.6 ±0.3 in reactor S0N0 [figure 5.4.8 (a)]. The initial sulfate and nitrate concentration was 80±15 mg/L and 33±8 mg/L respectively. The final sulfate and nitrate concentration was 40±10 mg/L and 20±5 mg/L respectively. The maximum sulfide and nitrite concentration was 45±10 mg/L and 24±8 mg/L respectively.

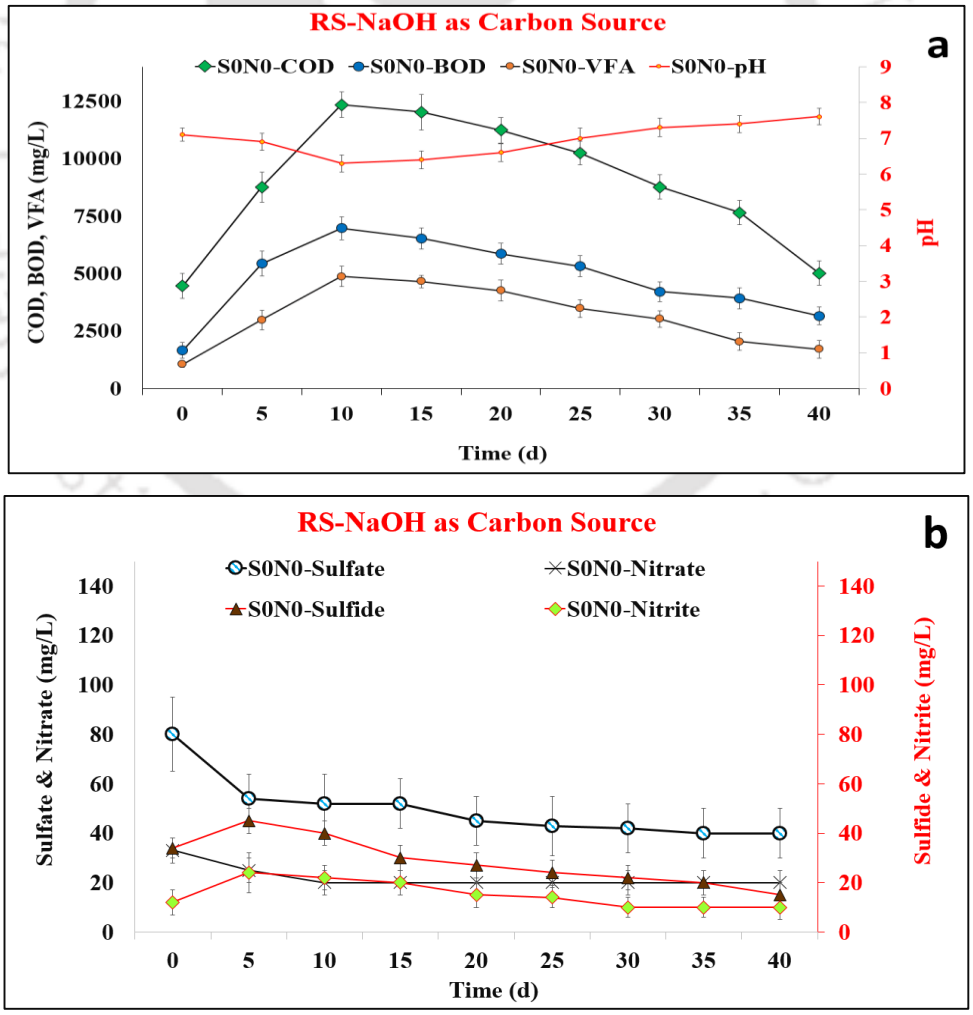

Figure 5.4.8. Profile of batch reactor S0N0

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5.4.9. Performance Evaluation of Batch Reactor CR

COD, Sulfate, Nitrate, and Arsenic Profile

The initial and final COD was 280 ± 25 mg/L and 110 ±20 mg/L. The initial sulfate, nitrate, and arsenic concentration was 2005±105 mg/L, 2004±90 mg/L, 1005±112 mg/L, and the final concentration was 1906±70 mg/L, 1954±90 mg/L, 965±70 µg/L respectively [figure (5.4.9)]. Due to the lack of carbon source (rice straw), there is no significant removal in oxyanions was observed. There was no significant change in pH was observed, which was from 7.4±0.4 to 7.5± 0.2.

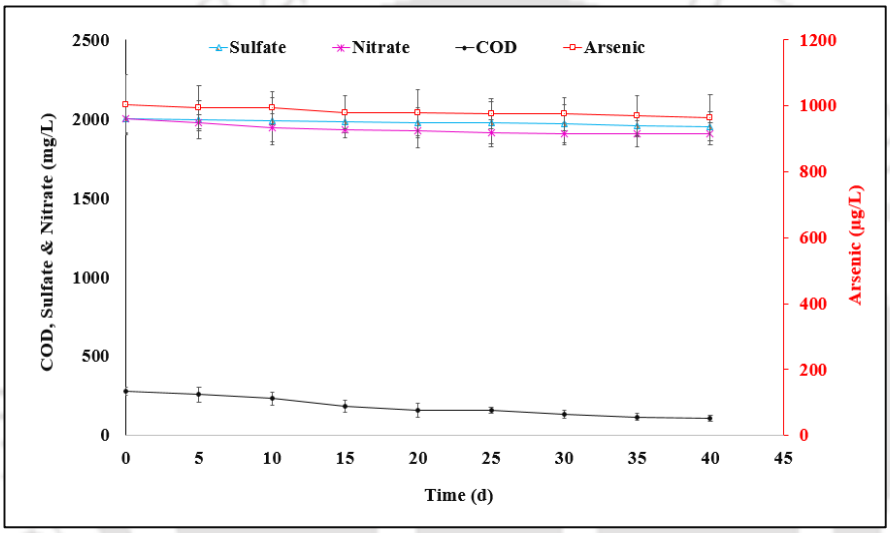

Figure 5.4.9. Profile of: COD, sulfate, nitrate, and arsenic in batch reactor CR

TH-2881_146104040

Performance evaluation of batch reactors for oxyanions removal using rice straw as a carbon source is shown in table 5.4.

Table 5.4. Performance evaluation of batch reactors for oxyanions removal using rice straw as carbon source

Reactor Name	Matrix Used	Initial Concentration			Removal Efficiency %		
-	-	Sulfate (mg/L)	Nitrate (mg/L)	Arsenic (µg/L)	Sulfate	Nitrate	Arsenic
R1	RS-WTA + Inoculum	2000	-	-	62	-	-
R2					80		
R3					85		
R4					82		
R-$Ca(OH)_2$	RS-$Ca(OH)_2$ + Inoculum	2000	-	-	89	-	-
R-NaOH	RS-NaOH + Inoculum				94		
R-ChOH	RS-ChOH + Inoculum				92		
S1	RS-NaOH + Inoculum	1000	-	-	95	-	-

TH-2881_146104040

S2		2000			94		
S3		3000			92		
N1	RS-NaOH + Inoculum	-	500	-	-	94	-
N2			1000			96	
N3			2000			97	
S0N0	RS-NaOH + Inoculum	-	-	-	-	-	-
SN1		2000	500		73	82	
SN2			1000		67	76	
SN3			2000		58	74	
SA1	RS-NaOH + Inoculum	2000	-	500	88	-	89
SA2				1000	91		90
SA3				2000	92		91
SAN1	RS-ChOH + Inoculum	2000	500	2000	75	90	92
SAN2			1000		68	70	94

TH-2881_146104040

SAN3			2000		62	65	90
CR	Inoculum	2000	2000	2000	4.7	2.3	3.5

TH-2881_146104040

5.5. Characterization of bio solids formed in the bioreactors

5.5.1. FESEM and EDX Analysis

Figure 5.5.1 (i) shows (a) FESEM and (b) EDX of the raw paper mill sludge containing the presence of elements like C, O, Si, Ca, Mg and S.

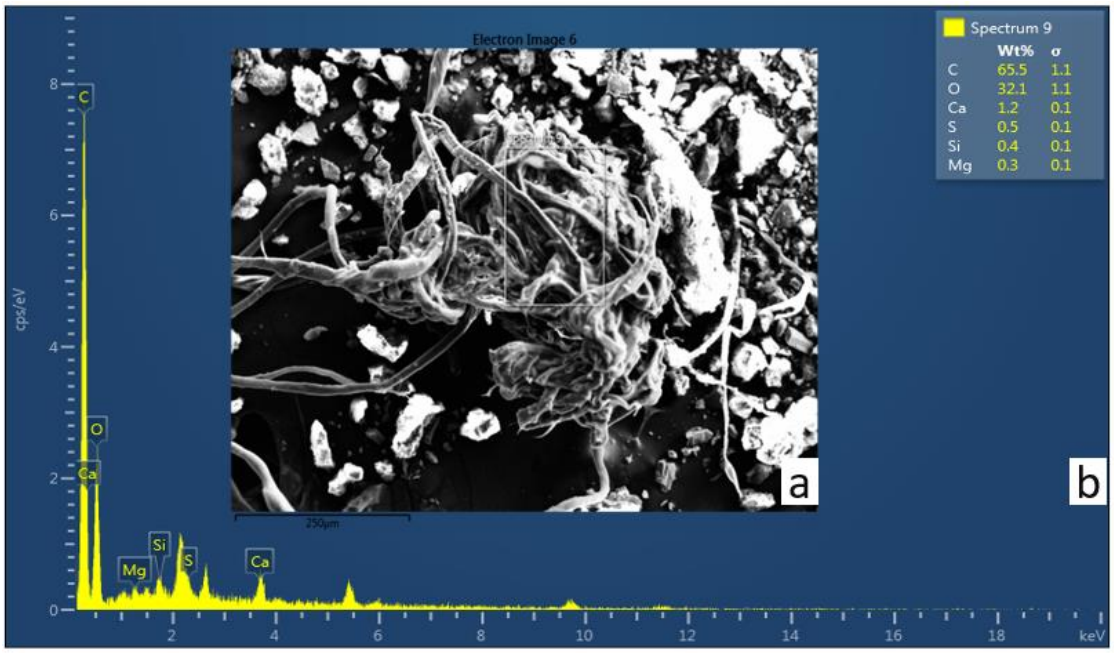

Figure 5.5.1 (i). (a) FESEM and (b) EDX of paper mill sludge

Figure 5.5.1 (ii) shows FESEM images of bio solids of batch reactors (a) R1 (b) R2 (c) R3 (d) R4 (e) R-$Ca(OH)_2$ (f) R-NaOH and (g) R-ChOH. Figure 5.5.1 (iii, iv, v, and vi), represents the FESEM and EDX images of the batch reactor (R7, R-$Ca(OH)_2$, R-NaOH, and R-ChOH respectively), which confirms the precipitation of elemental sulfur.

TH-2881_146104040

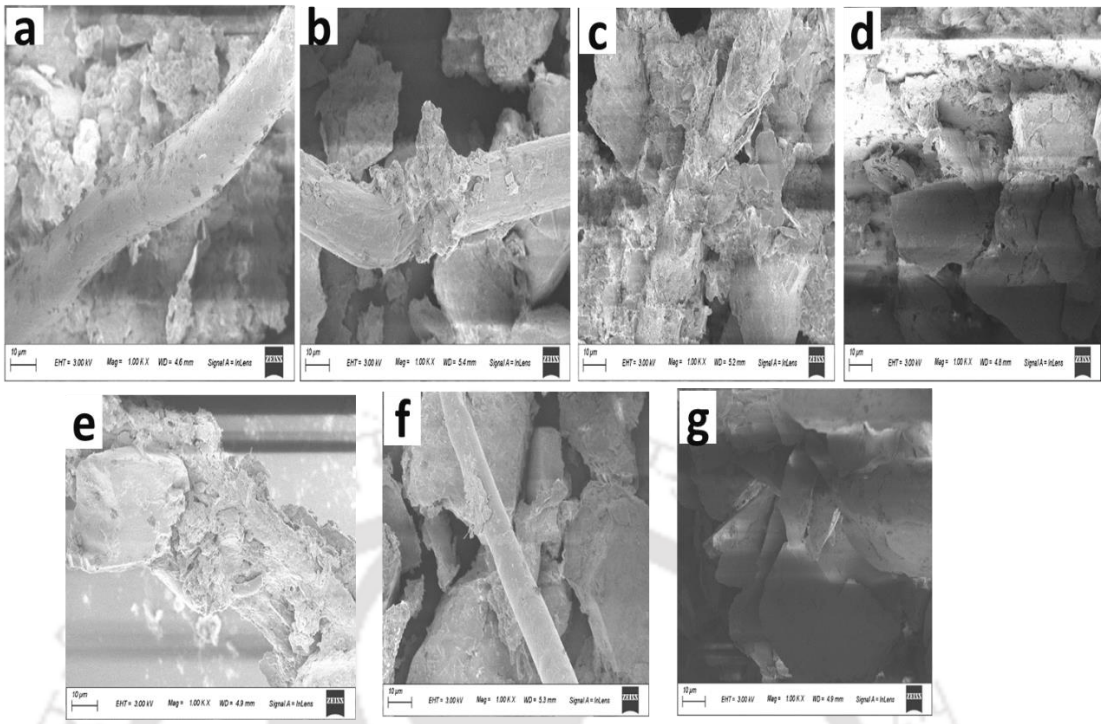

Figure 5.5.1 (ii). FESEM images of bio solids of batch reactors (a) R1 (b) R2 (c) R3 (d) R4 (e) R-$Ca(OH)_2$, (f) R-NaOH (g) R-ChOH

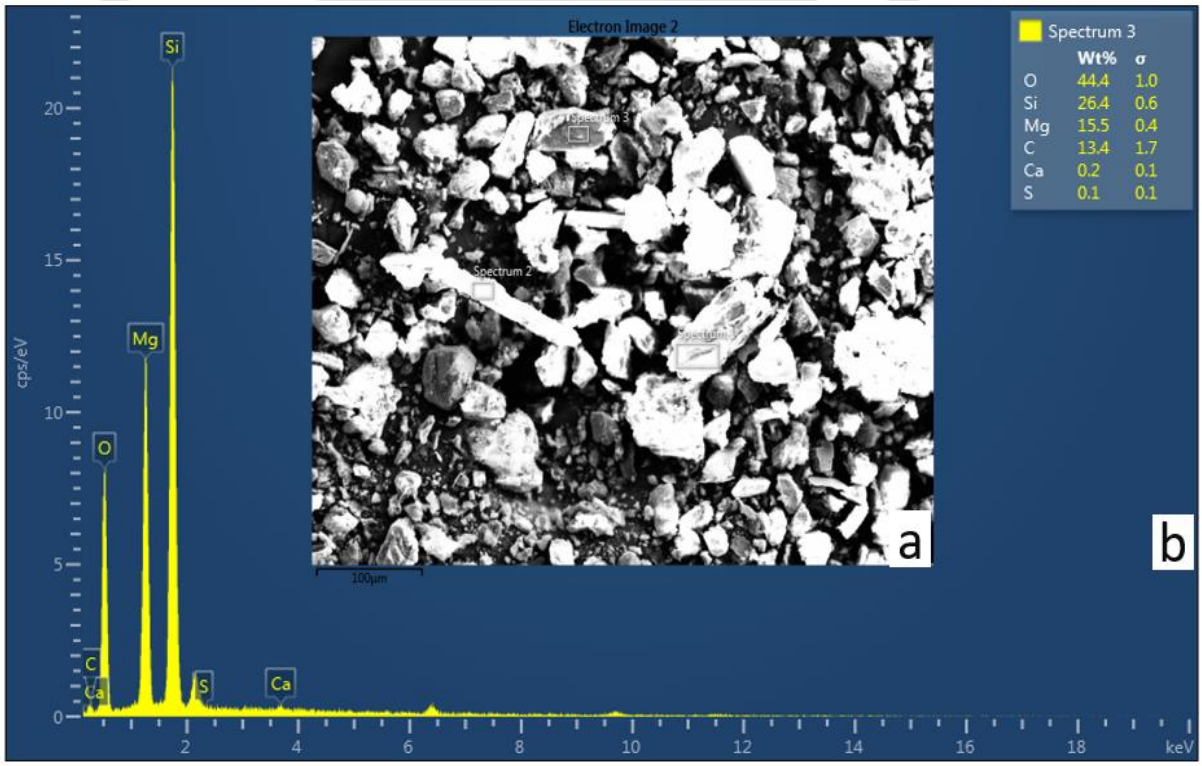

Figure 5.5.1 (iii). (a) FESEM (b) EDX of bio solids of batch reactor R7

TH-2881_146104040

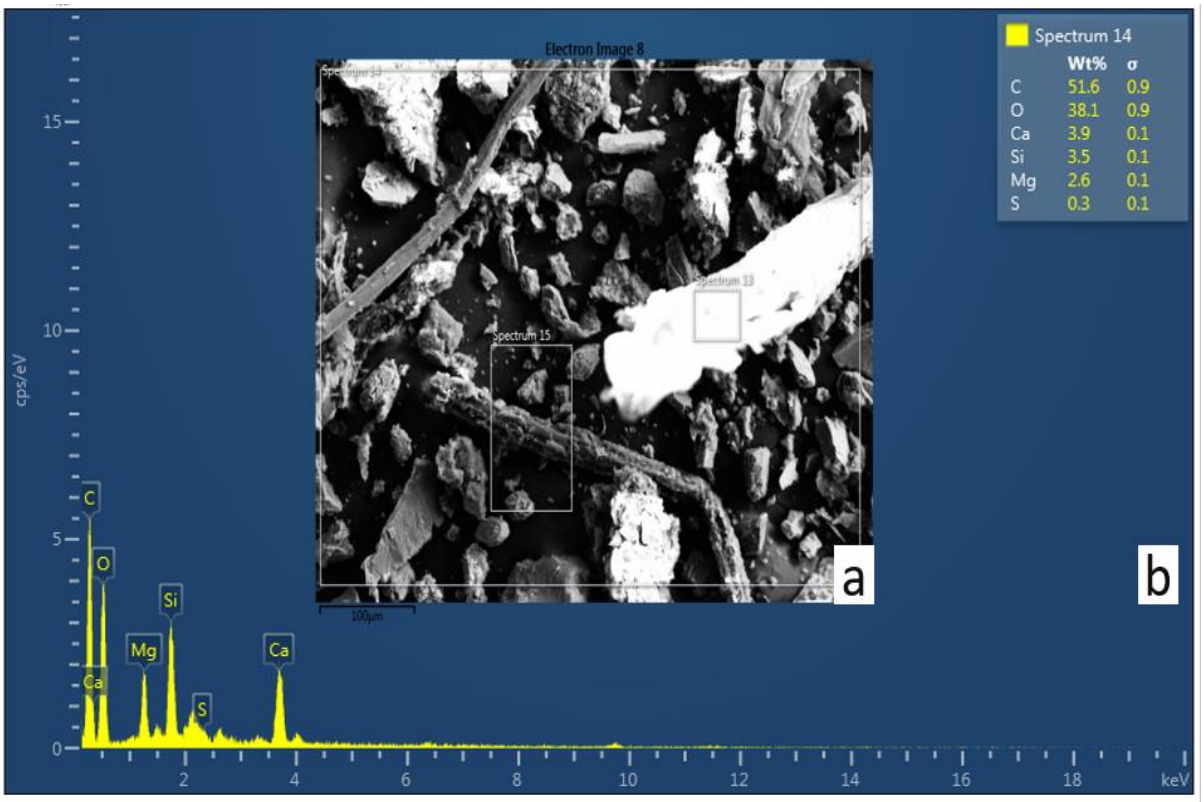

Figure 5.5.1 (iv). (a) FESEM (b) EDX of bio solids of reactor R-$Ca(OH)_2$

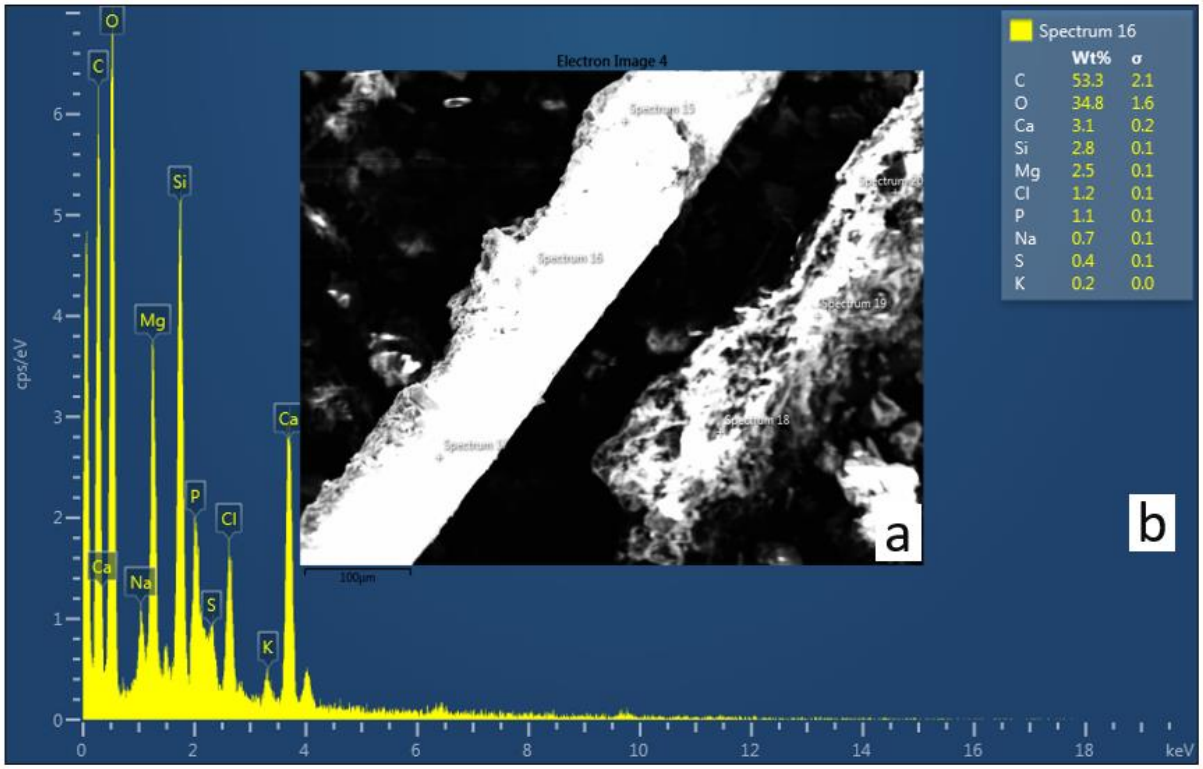

Figure 5.5.1 (v). (a) FESEM (b) EDX of bio solids of reactor R-NaOH

TH-2881_146104040

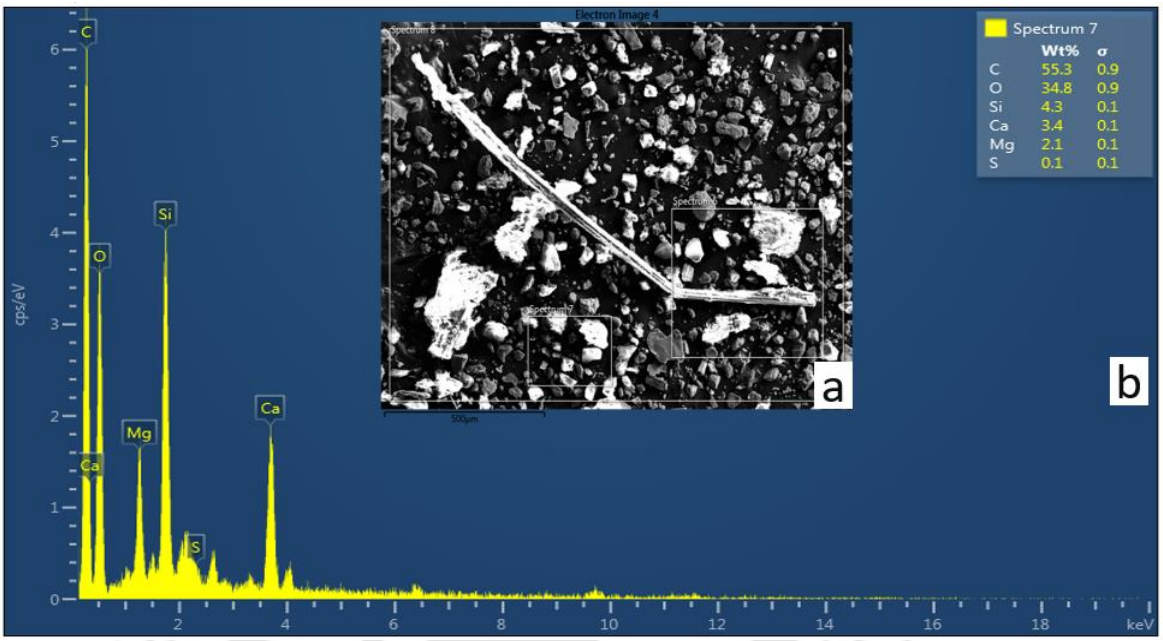

Figure 5.5.1 (vi). (a) FESEM (b) EDX of bio solids of reactor R-ChOH

Figure 5.5.1 (vii) shows the FESEM images of bio solids of batch reactors in different cases (a) S0N0, (b) S3, (c) N3, (d) SN3. Figure 5.5.1 (viii) shows the (a) FESEM (b) EDX images of bio solids of batch reactor S3. Figure 5.5.1 (ix) shows the (a) FESEM (b) EDX images of bio solids of batch reactor SN3.

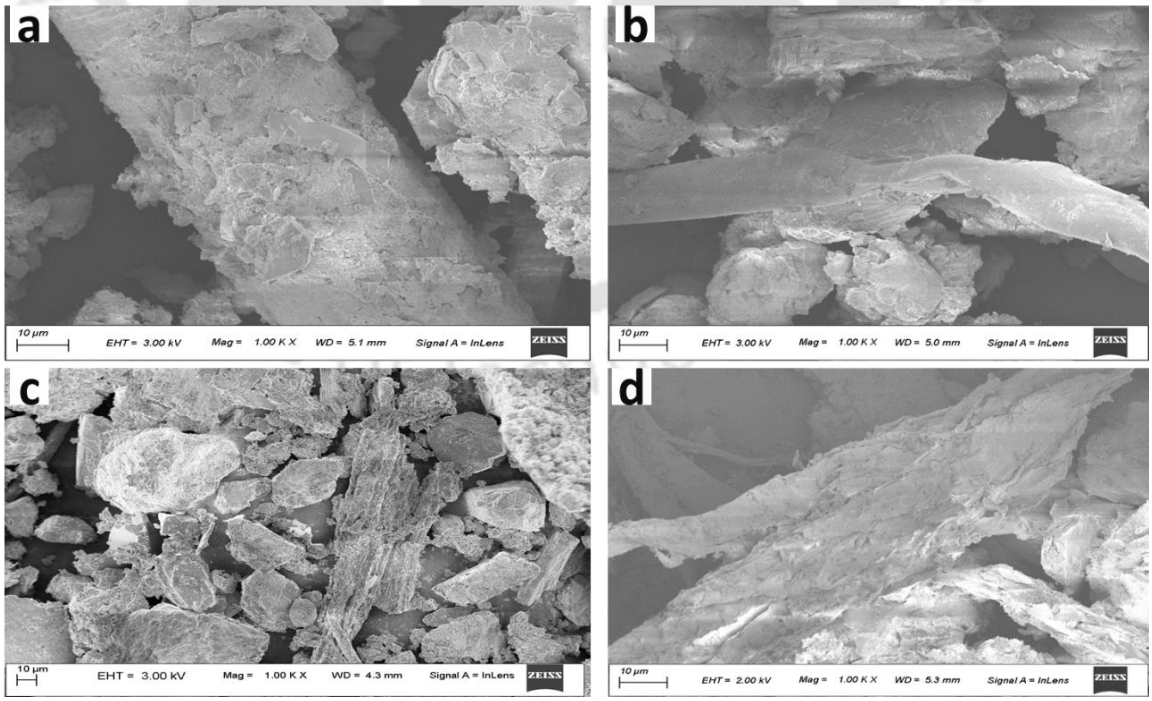

Figure 5.5.1 (vii). FESEM images of bio solids of batch reactors at different cases (a) S0N0, (b) S3, (c) N3, (d) SN3

TH-2881_146104040

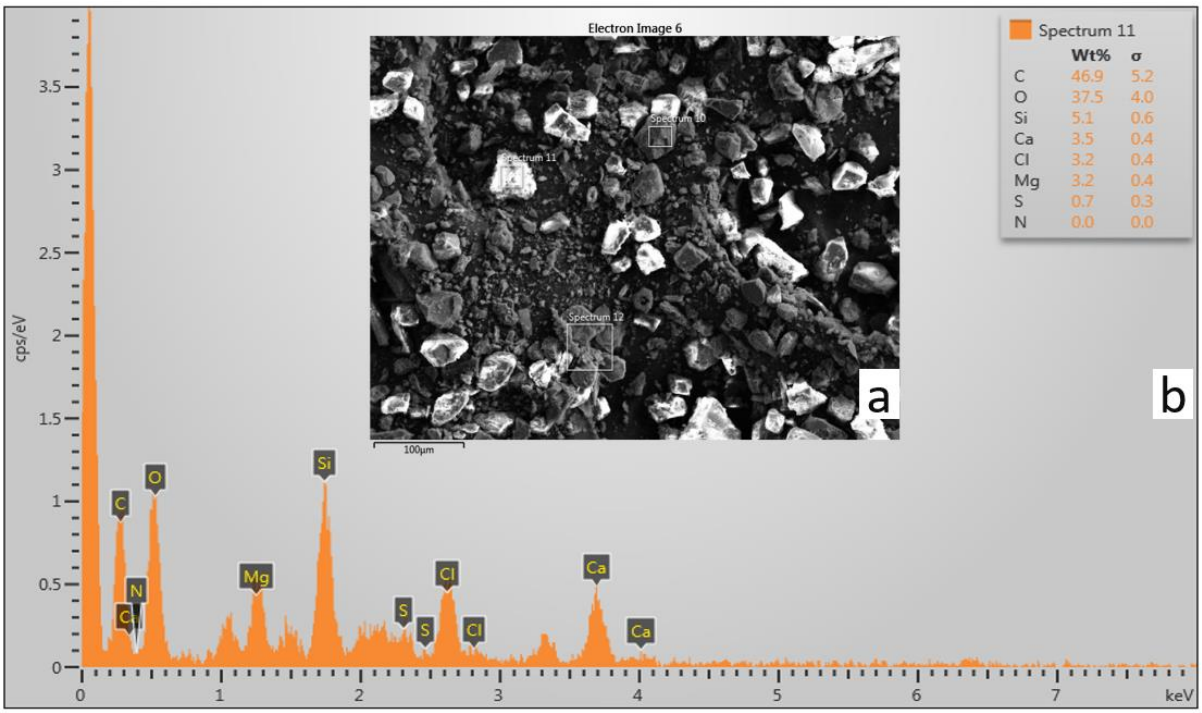

Figure 5.5.1 (viii). (a) FESEM (b) EDX images of bio solids of batch reactor S3

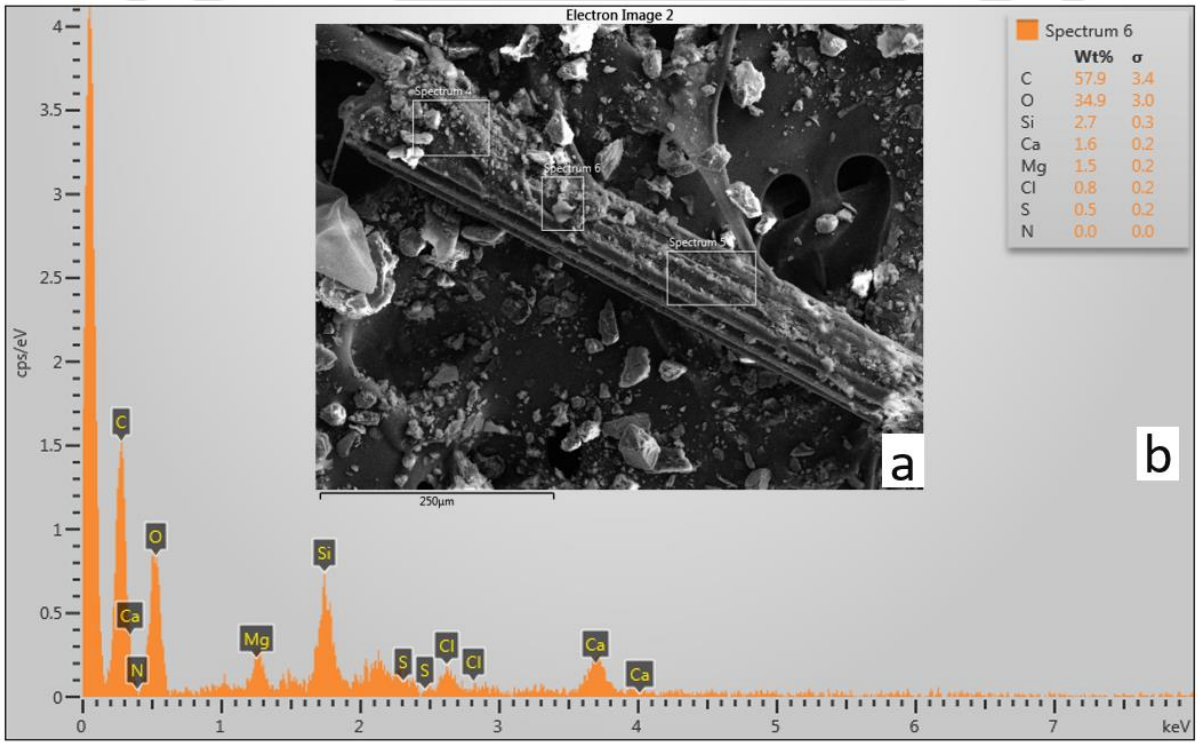

Figure 5.5.1 (ix). (a) FESEM (b) EDX images of bio solids of batch reactor SN3

TH-2881_146104040

Figure 5.5.1 (x) shows (a) FESEM (b) EDX (c) X-Ray mapping of bio solids (d) X-Ray mapping of element arsenic (e) X-Ray mapping of element sulfur of batch reactor SA3. FESEM, EDX analysis confirmed the elemental composition of the bio solids precipitated in batch reactor SA3. The precipitates were mainly composed of arsenic indicated arsenic precipitation in the form of arsenosulfides.

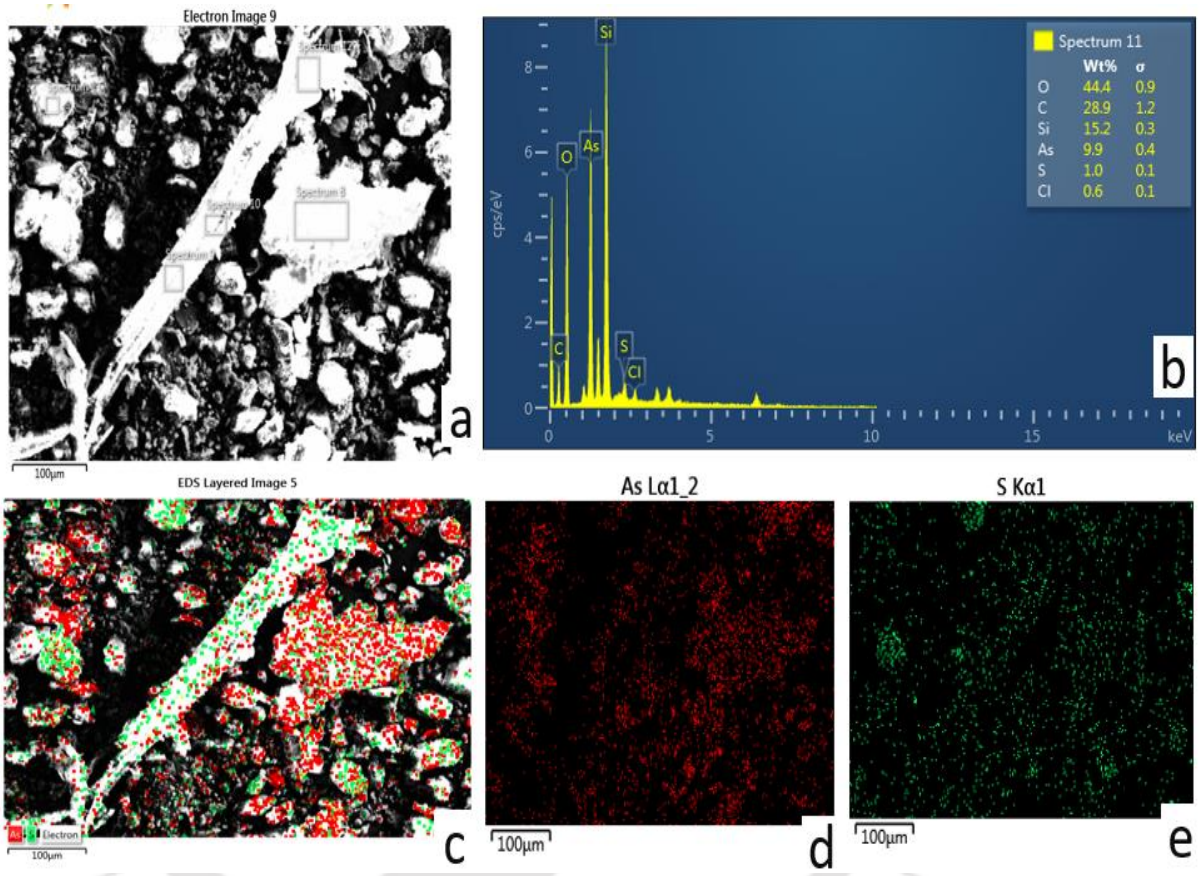

Figure 5.5.1 (x). (a) FESEM (b) EDX (c) X-Ray mapping of bio solids (d) X-Ray mapping of element arsenic (e) X-Ray mapping of element sulfur of batch reactor SA3

Figure 5.5.1 (xi) shows (a) FESEM (b) EDX of bio solids of reactor SAN3. Figure 5.5.1 (xii) shows (a) FESEM image (b) X-Ray elemental mapping micrograph of bio solids of reactor SAN3 (c) X-Ray mapping of element sulfur (d) X-Ray mapping of element arsenic (e) X-Ray mapping of element nitrogen. FESEM micrograph and EDX mapping confirmed the elemental composition of the bio solids precipitated in both batch reactors. It can be observed that the presence of elements such as sulfur, nitrogen, and arsenic in the bio solids obtained from the batch reactor. Peaks of other elements such as Ca, Mg, N, and Cl may be due to feed media, whereas C, O, and Si are due to the presence of rice straw components. The presence of elemental N may be because silica

TH-2881_146104040

reacts with nitrogen gas and forms silicon nitride (Si_3N_4) (Carlson, 1990). However, the precipitates were mainly composed of arsenic and sulfur indicating arsenic precipitation in the form of arsenosulfides. These results suggest that the mixed bacterial culture induced the precipitation of arsenic and sulfides which is responsible for the arsenic removal. FESEM/EDX results were further validated by XRD analysis.

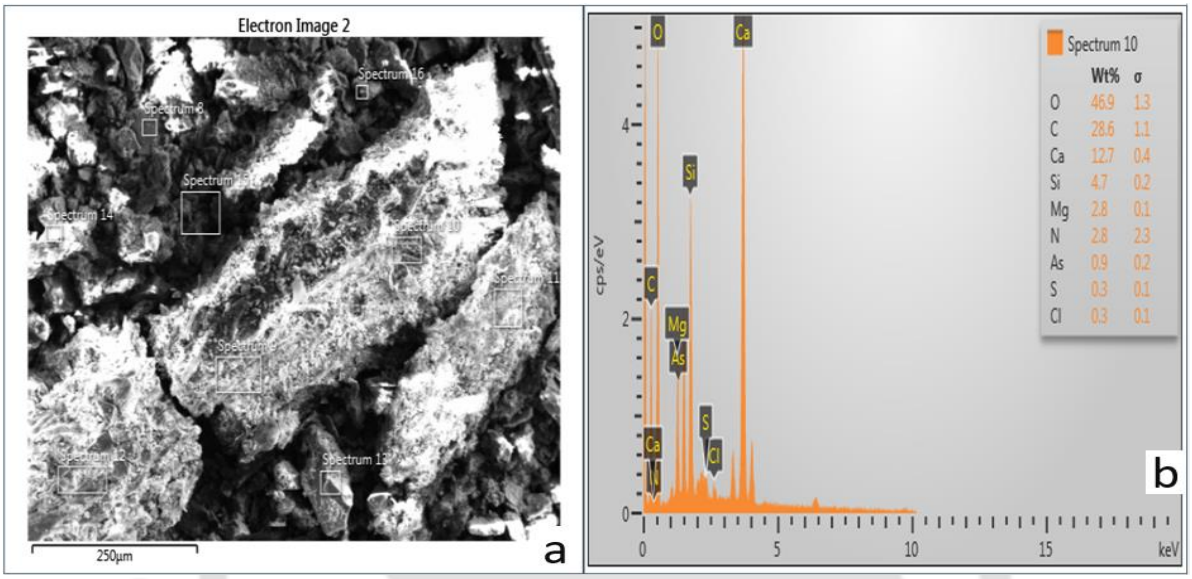

Figure 5.5.1 (xi). (a) FESEM (b) EDX of bio solids of batch reactor SAN3

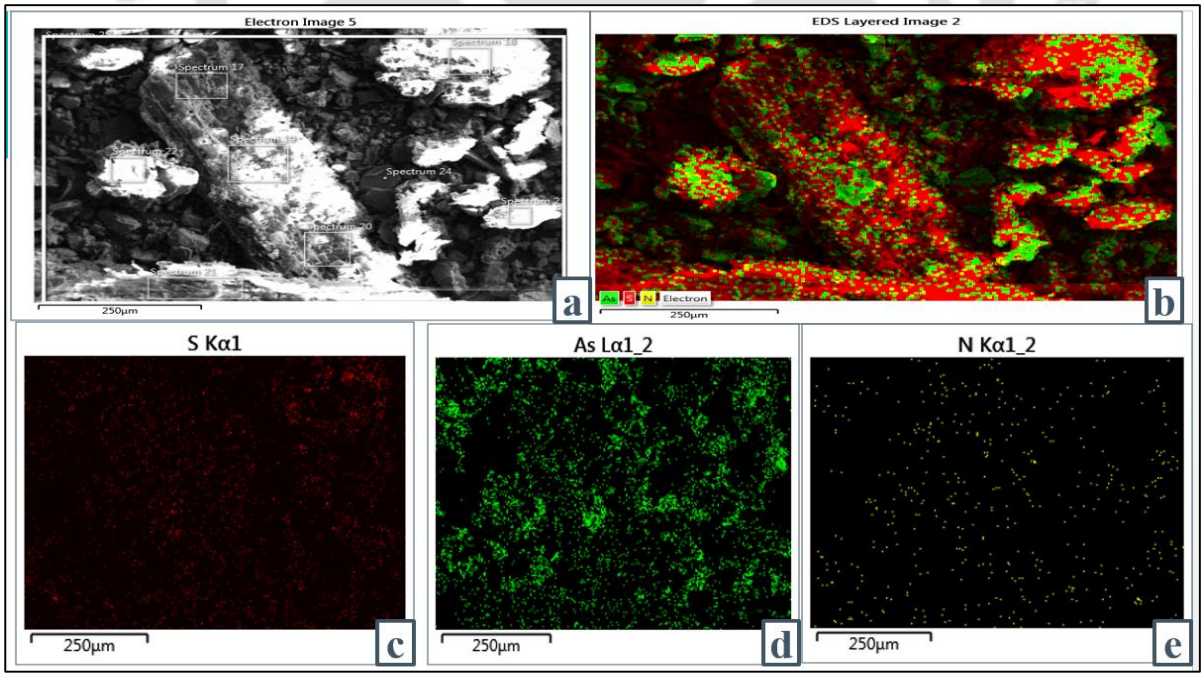

Figure 5.5.1 (xii). (a) FESEM image (b) X-Ray elemental mapping micrograph of bio solids of batch reactor SAN3 (c) X-Ray mapping of element sulfur (d) X-Ray mapping of element arsenic (e) X-Ray mapping of the element nitrogen

TH-2881_146104040

5.5.2. XRD Analysis

XRD analysis [Figure 5.5.2 (i)] of paper mill sludge depicted the existence of quartz (SiO_2; JCPDS # 01-085-0795), quartz (SiO_2; JCPDS# 01-085-0795), calcium silicate ($CaSiO_3$; JCPDS # 01-072-2284), magnesium aluminum oxide ($MgAl_2O_4$ JCPDS # 01-075-1799), magnesium hydrogen silicate ($Mg_7H_6Si_2O_{14}$; JCPDS#00-026-1216), magnesium oxide (MgO_2; JCPDS # 00-019-0771, aluminum oxide ($(Al2O_3)_{5.333}$: JCPDS # 01-073-1199), magnesium sulfate hydroxide (Mg_3 $(SO_4)_2$ $(OH)_2$; JCPDS # 00-039-0359).

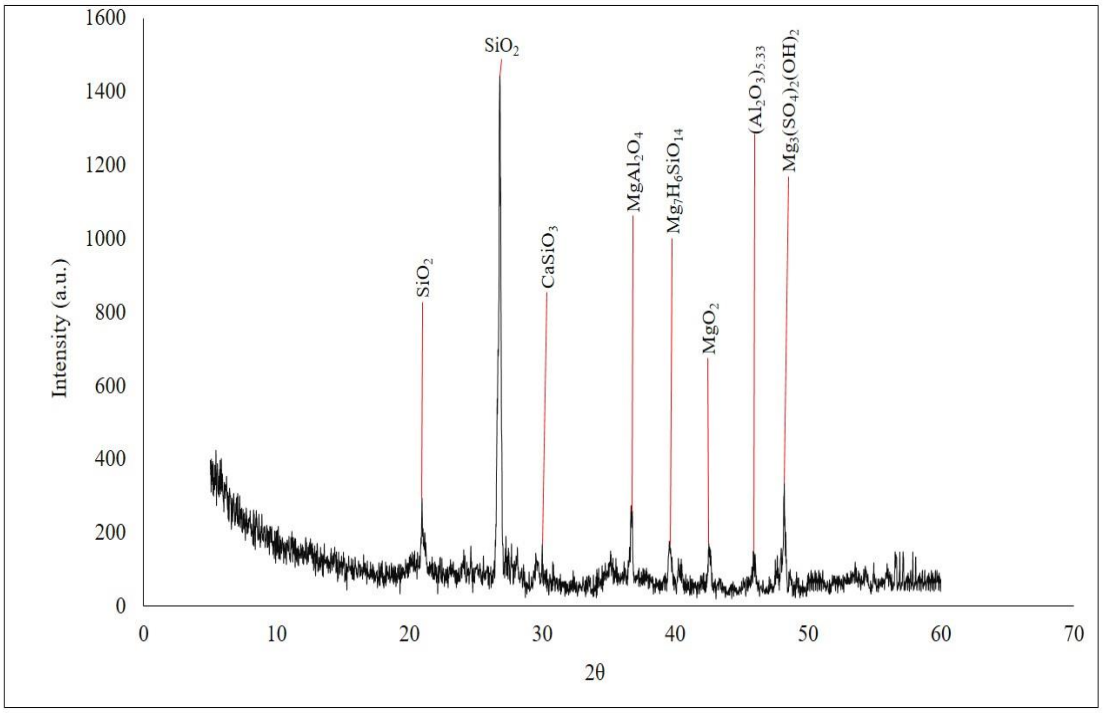

Figure 5.5.2 (i). XRD analysis of paper mill sludge

Figure 5.5.2 (ii), XRD analysis of bio solids of batch reactor SA3 depicted the existence of realgar (As-S; JCPDS#00-041-1494), orpiment (As_2S_3; JCPDS#00-003-0153), dimorphite-I (As_4S_3; JCPDS#00-026-0125), orpiment (As_2S_3; JCPDS#00-003-0153), orpiment (As_2S_3; JCPDS#00-019-0084), arsenic sulfide (As_2S_2; JCPDS#00-021-0805), arsenic sulfide (As_4S_3; JCPDS#01-072-0726), realgar (As-S; JCPDS#01-071-2434), as the solids deposited in the batch reactor.

TH-2881_146104040

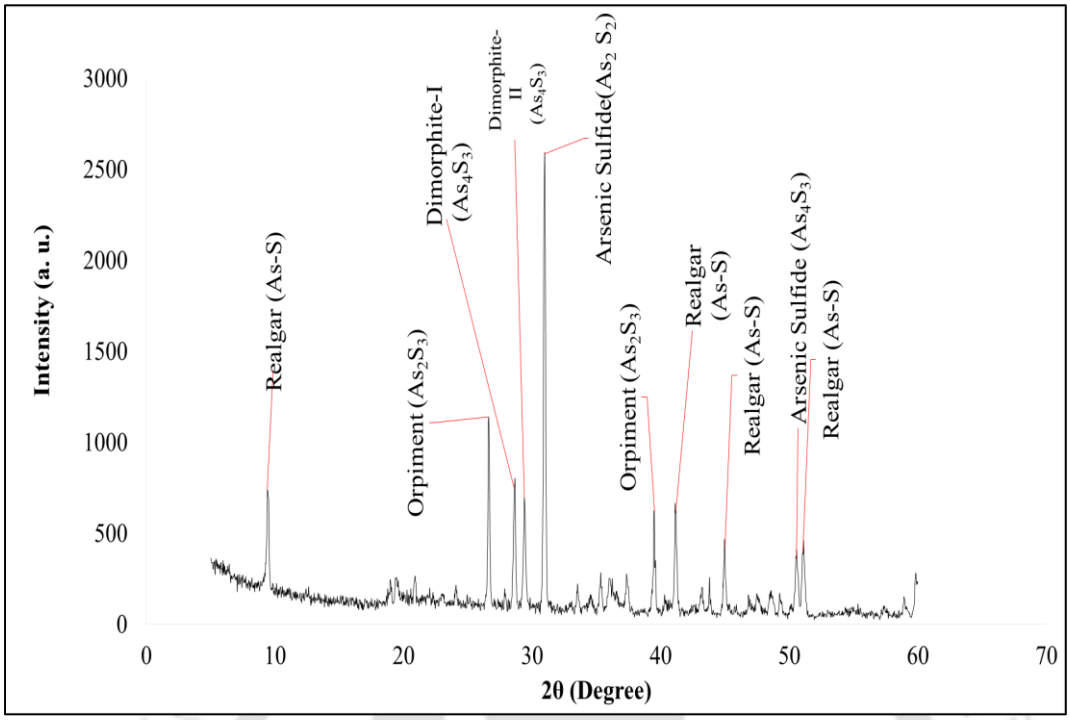

Figure 5.5.2 (ii). XRD analysis of bio solids of batch reactor SA3

Similarly, figure 5.5.2 (iii), XRD analysis of bio solids of reactor SAN3, depicted the existence of orpiment (As_2S_3; JCPDS# 00-001-0273), arsenic sulfide (As_2S_5; JCPDS #00-027-0029), dimorphite-I (As_4S_3; JCPDS #00-026-0125), arsenic sulfide (As_2S_5; JCPDS #00-027-0029), arsenic sulfide (As_4S_3; JCPDS #01-072-0726), dimorphite-I (As_4S_3; JCPDS #00-026-0125) as the solids deposited in the batch reactor.

XRD analysis was in agreement with FESEM/EDX and confirmed the precipitation of orpiment (As_2S_3) and realgar (AsS). TEM micrographs provided a more detailed morphology of the precipitates and revealed amorphous, nanocrystalline, and crystalline phases formed in the batch and continue reactor systems. The HRTEM revealed the ultrastructure of the precipitates and interplanar d-spacing values measured from FFT analysis confirmed the presence of mineral orpiment (As_2S_3) and arsenosulfides minerals. The possible arsenic removal mechanism was arsenosulfides precipitation in an anaerobic reducing environment, where arsenite (As (III)) and biogenic sulfide (S(II)) react to form arsenic sulfides, such as orpiment (Newman et al., 1998) and realgar (Battaglia-Brunet et al., 2012). The peak intensities showed that the compound was semi-crystalline, which was further supported by TEM analysis.

TH-2881_146104040

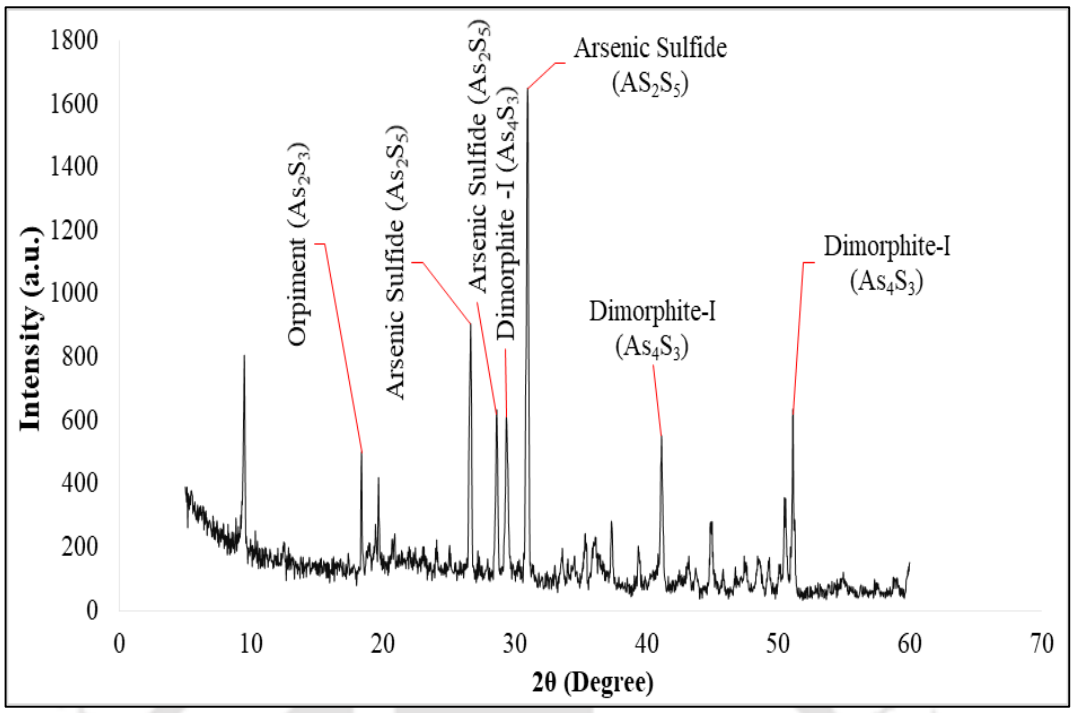

Figure 5.5.2 (iii). XRD analysis of bio solids of batch reactor SAN3

5.5.3. FETEM Analysis

Figure 5.5.3 (i), shows the FETEM analysis [(a,b) TEM images, (c) high-resolution transmission electron microscopy (HRTEM) (d) SAED Pattern (e) IFFT (f) Profile of IFFT] of bio solids of the batch reactor (SA3). TEM analysis showed the spherical structure of the precipitate formed in the reactor. The presence of a bacterial cell can be seen in the inset image. The TEM image, selected area electron diffraction (SAED) pattern, and high-resolution transmission electron microscopy (HRTEM) is depicted in figure 5.5.3 (i) (j, k, m, and n), which indicates amorphicity as well as crystallinity in the biogenic precipitates. The SAED pattern indicated the precipitated compound exhibiting both crystallinity, and amorphicity in nature, which was also observed in the XRD pattern. The d spacing values obtained from the FFT pattern showed distinctive clear lattice fringes of 0.299 nm [figure 5.5.3 (i) (l)] are in good agreement with calculated values (d =2.93 Aº, hlk plane 122 and sharp peak value 30.1°θ) from the XRD analysis, which corresponds to the compound arsenosulfide (As_4S_3; JCPDS #01-072-0726). Bujňáková et al. (2015) reported the d spacing value of arsenic sulfide nanoparticles i. e. 0.19 nm to 0.31 nm. The crystalline nature of the obtained precipitates suggests the stable nature of bio solids (de Matos et al., 2018), which ensures their safe disposal in a landfill.

TH-2881_146104040

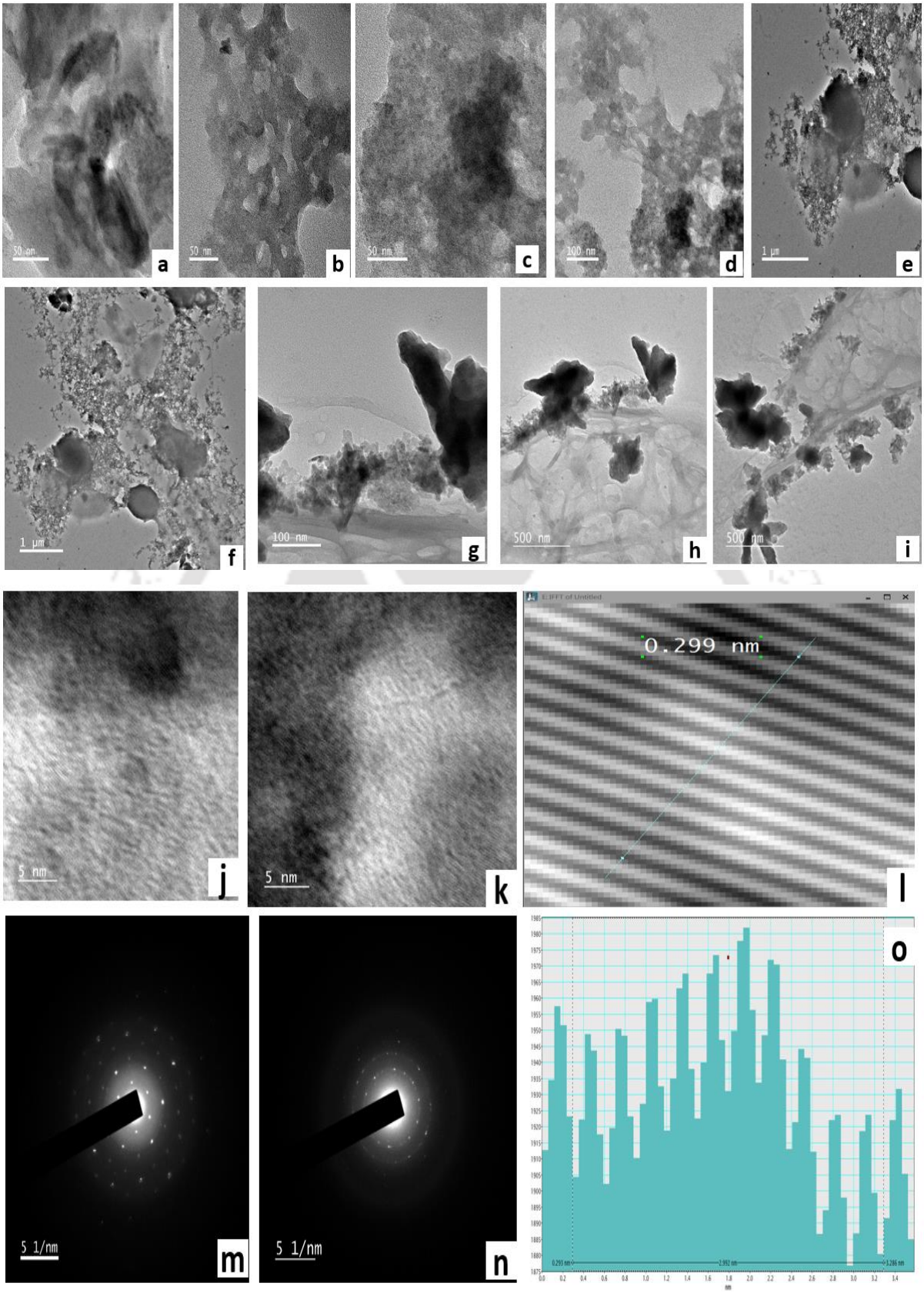

Figure 5.5.3 (i). FETEM analysis of bio solids of batch reactor SA3; (a-i) TEM images (j,k) HRTEM (l) IFFT (m, n) SAED Pattern (o) Profile of IFFT

TH-2881_146104040

Figure 5.5.3 (ii), shows the FETEM analysis of bio solids of reactor SAN3; (a-f) TEM images (g, h) HRTEM (i) SAED (j) IFFT Pattern (k) Profile of IFFT [(a,b) TEM images, (c) high resolution transmission electron microscopy (HRTEM) (d) SAED Pattern (e) IFFT (f) Profile of IFFT] of bio solids of the batch reactor (SAN3). TEM analysis showed the spherical structure of the precipitate formed in the reactor. The presence of a bacterial cell can be seen in the inset image.

The TEM image, selected area electron diffraction (SAED) pattern, and high-resolution transmission electron microscopy (HRTEM) is depicted in figure 5.5.3 (ii) (c), which indicates amorphicity as well as crystallinity in the biogenic precipitates. The SAED pattern indicated the precipitated compound exhibiting both crystallinity, and amorphicity in nature, which was also observed in the XRD pattern. The belt structure showed distinctive clear lattice fringes with a d-spacing of 0.32 nm [figure 5.5.3 (ii) (d)], which corresponds to the compound orpiment (As_2S_3; JCPDS # 00-001-0273) [corresponding to sharp peak value and hlk plane 27.9°θ (211)] in line with the TEM analysis. XRD results match with TEM results d spacing 0.32 nm. Kampf et al. (2011) reported the d spacing of orpiment (As_2S_3) i. e. 0.28 nm. Crystalline nature of the obtained precipitates suggests the stable nature of bio solids (de Matos et al., 2018), which ensures their safe disposal in a landfill.

Arsenosulfide (As_2S_3) is so insoluble that it is not toxic. Arsenosulfide is used as a tanning agent. It was formerly used with indigo dye for the production of pencil blue, which allowed dark blue hues to be added to fabric via pencil or brush. Arsenosulfide in amorphous form is used as a chalcogenide glass for infrared optics. It is transparent between 620 nm and 11 µm (Martin, 1983). The arsenic trisulfide glass is more resistant to oxidation than crystalline arsenic trisulfide, which minimizes toxicity concerns. It can be also used as an acousto-optic material. As_2S_3 has been investigated for the fabrication of photonic crystals with a full-photonic band-gap. As_2S_3 and As_4S_4 have been investigated as treatments for acute promyelocytic leukemia (APL). Arsenic pentasulfide (As_2S_5) is used as a pigment and as a light filter in thin sheets.

TH-2881_146104040

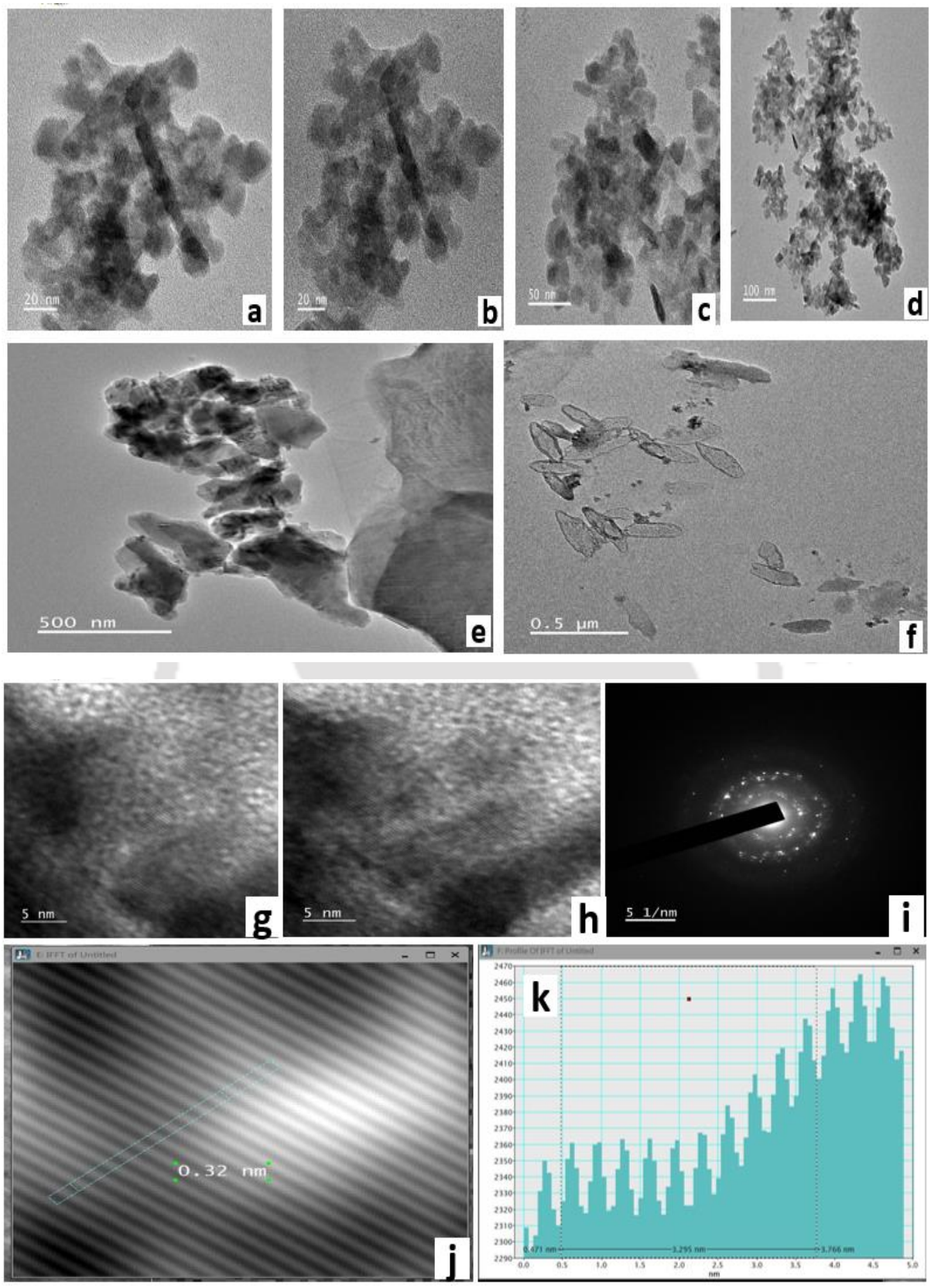

Figure 5.5.3 (ii). FETEM analysis of bio solids of batch reactor SAN3; (a-f) TEM images (g, h) HRTEM (i) SAED (j) IFFT Pattern (k) Profile of IFFT

TH-2881_146104040

5.6. Identification of microbial communities present in the bioreactors.

5.6.1. TRFLP Analysis

Figure 5.6.1 (i), shows Agarose gel electrophoresis of genomic DNA performed on 1% (w/v) gel, and figure 5.6.1 (ii), shows 16S PCR IMAGE: 2% (W/V) Agarose Gel electrophoresis. T-RFLP profiling is a graph called Electropherograms, which is an intensity plot representation of an electrophoresis experiment (gel or capillary). In Electropherograms the X-axis marks the sizes of the fragments (base pairs) while the Y-axis marks the fluorescence intensity in a fluorescent unit of each fragment. Thus, what appears on an electrophoresis gel as a band appears as a peak on the Electropherograms whose integral is its total fluorescence. In a T–RFLP profile each peak assumingly corresponds to one genetic variant in the original sample while its height corresponds to its relative abundance in the specific community. Often, several different bacteria in a population might give a single peak on the Electropherograms due to the presence of a restriction site for the particular restriction enzyme used in the experiment at the same position. T-RFs having a size of less than 40 bases were eliminated from the analysis as they might result from primer-dimers. The fluorescent signal threshold was set to 40 fluorescent units as per the standards to minimize the background signal and signals arising from ssDNA nonspecific amplicons/fragments.

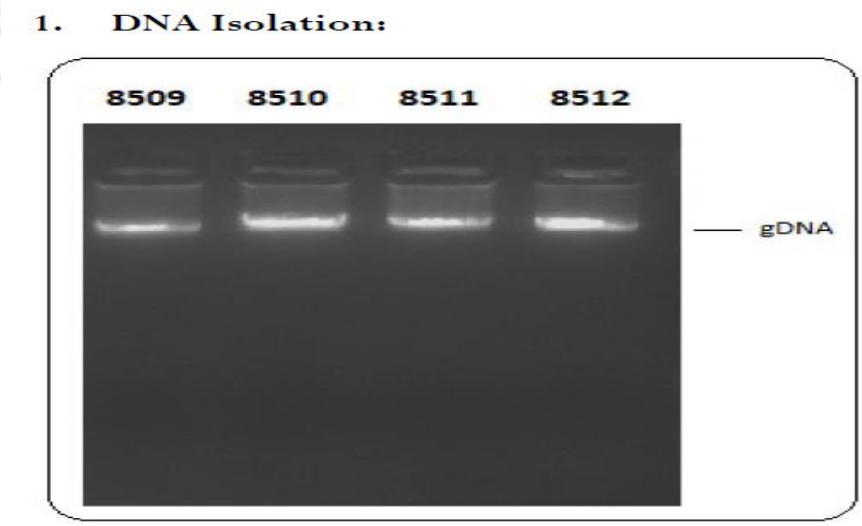

Figure 5.6.1 (i). Agarose gel electrophoresis of genomic DNA performed on 1% (w/v) gel, (8509-8512)

TH-2881_146104040

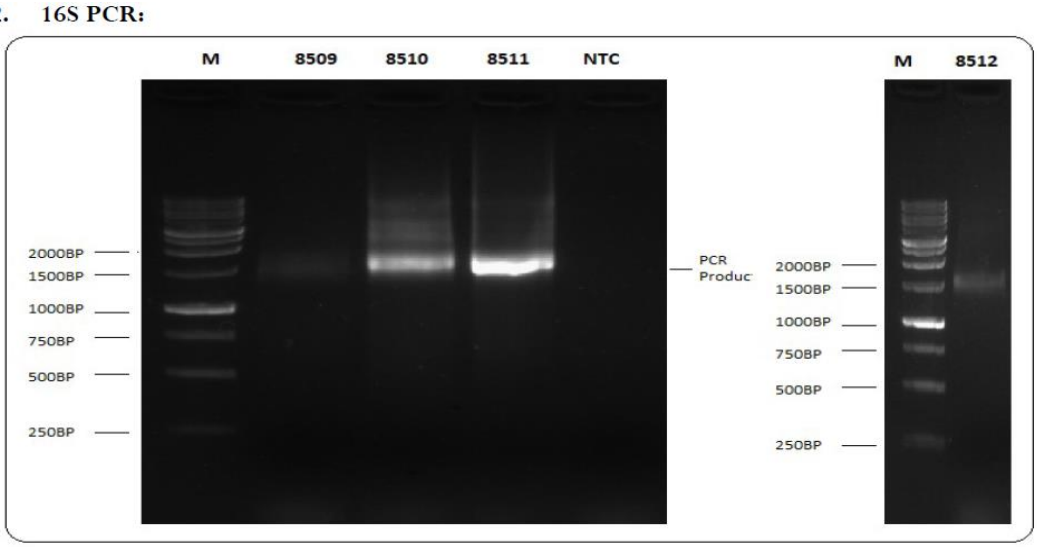

Figure 5.6.1 (ii). 16S PCR IMAGE: 2% (W/V) Agarose Gel electrophoresis: Lane M: 1kb DNA marker; Lane Well 8509-8512 sample PCR Products, NTC: Negative test control

Figure 5.6.1 (iii), shows the TRFLP analysis of the paper mill sludge which shows the presence of *Thiomicrospira crugena str., Riemerella anatipestifer str., Acetobacter sp. Str., Glucanacetobactor diazotrophicus, Pirellula sp. str. OJF26 k-Bacteria*.

Figure [5.6.1 (iv)], shows the TRFLP analysis of the batch reactor SN3 which shows the presence of *Marinobacter sp., Riemerella anatipestifer, Glucanobactor Oxydans, Acetobacter oeni (T), Streptococcus suis, Uncultured bacterium SN28*.

Acrobactor (Gevertz et al., 2000), *Bacteroids* (Plumb et al., 2001), *Psudonomous* (Chen et al., 2008), *Azoarcus* (Liu et al., 2016), *Clostridium* (Wang et al., 2015), *Thouera* (Liu et al., 2015), *Soehngenia* (Zhang et al., 2020), *Macellibacteroides* (Liu et al., 2018a), *Thiovirga* (Lan et al., 2019) were found in sulfate and nitrate removal bioreactors or environment. *Marinobacter sp.* has been in sulfate and nitrate reducing environments (An et al., 2017). *Riemerella anatipestifer* has ability to do the fermentation five basic sugars (dextrose, glucose, maltose, lactose and sucrose) (Sarker et al., 2017), *Glucanobactor Oxydans* has a role on lignocellulose fermentation and sugar monomer production (Yao et al., 2017). *Acetobacter oeni (T)* can produce / glucose and acetic acids by fermentation of cellulosic material (Bader et al., 2010). *Streptococcus suis* is a microorganism that uses nitrate as an alternative terminal electron acceptor (Philippot et al., 2002).

TH-2881_146104040

TRFLP analysis [figure 5.6.1 (v)] reveals, that reactor SA3 contains, *Rhodobacteraceae bacterium, Neisseria gonorrhoeae (T), Dyadobacter sp., Streptococcus suis, Uncultured bacterium, Uncultured soil bacterium. Thaurea* (Li et al., 2014), *Bacteroides* (Gnanaprakasam et al., 2017), *Arcobacter, Paludibactor* (Zhang et al., 2018), *Chlorobaculum* (Wan et al., 2019), *acholeplasma* (Angulo et al., 2003), *Chlostridium* (Alam and McPhedran, 2019), *Azoarcus* (Sperfeld et al., 2018), *Bacillus* (Bennett et al., 2001), *Desulfovibrio* (Macy et al., 2000), *Aromatoleum* (Wunderlich et al., 2012), *Pseudomona* (Bajaj et al., 2010), *Magnetospirillum* (Okpala and Voordouw, 2018) were found in either sulfate or sulfate and arsenic environment. *Rhodobacteraceae bacterium* and *Dyadobacter sp.* (Ranawat and Rawat, 2018), *Neisseria Gonorrhoeae (T)* (Schoen et al., 2014), *Streptococcus suis* (Anderson and Cook, 2004) have been reported in sulfate and arsenic removal bioreactors.

TRFLP analysis [figure 5.6.1 (vi)], reveals that the reactor SAN3 contains, *Thiomicrospira crunogena, Desulfarculus baarsii, Thermobaculum terrenum, Streptococcus suis, Pirellula sp., Uncultured bacterium. Psudonomous* (Chen et al., 2008), *Acrobactor* (Gevertz et al., 2000), *Paludibactor* (Blázquez et al., 2019), *Closriridium* (Wang et al., 2015), *Sphaerochaeta* (Liu et al., 2018c), *Spirochaeta* (Zhou et al., 2014), *Azoarcus* (Philipp and Schink, 1998), *Sedimentobacter* (Hiraishi, 2008), *Rikenella* (Rodríguez et al., 2015), *Anaerocella* (Song et al., 2020) were found in sulfate and nitrate removal process or reactor. *Thiomicrospira crunogena* and *Desulfarculus baarsii* were found in the sulfate reduction system (Gevertz et al., 2000; Sampaio et al., 2020). *Desulfarculus baarsii* was found in sulfate and high arsenic concentration environment (Li et al., 2014). *Streptococcus suis*, and *Pirellula sp.*, was found in nitrate reducing environment (Hamid, 2010; Zhang et al., 2009). *Thermobaculum terrenum* was found in sulfate, nitrate, and arsenic removal bioreactor (Botero et al., 2004).

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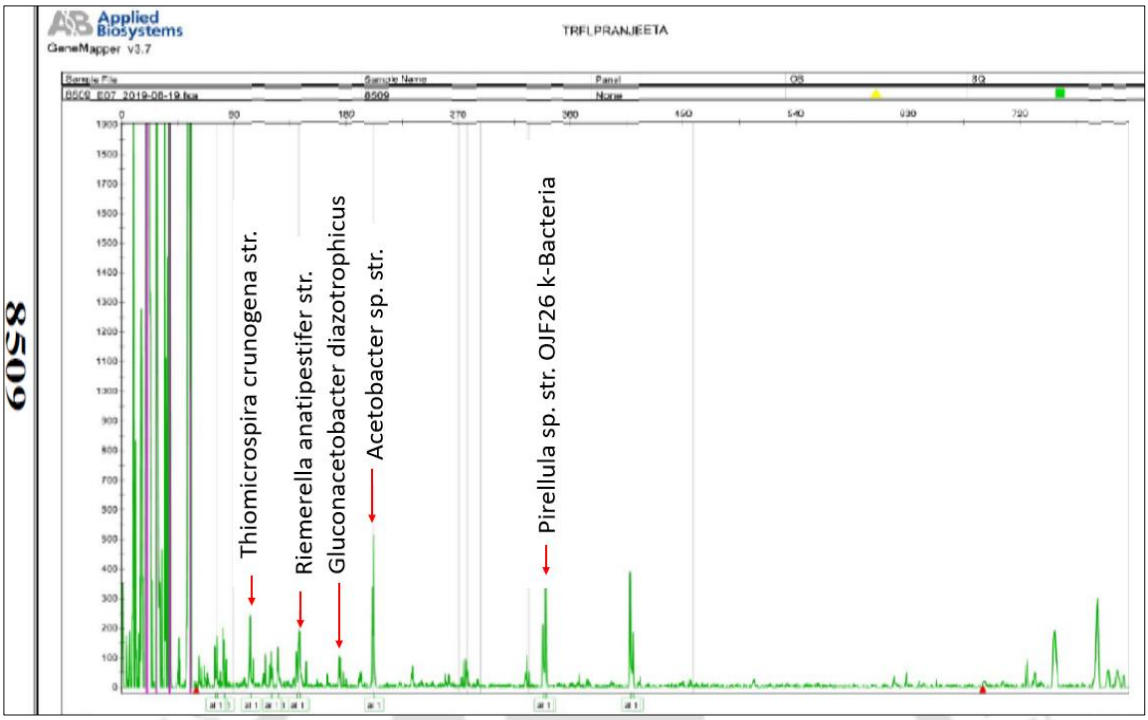

Figure 5.6.1 (iii). TRFLP analysis of paper mill sludge

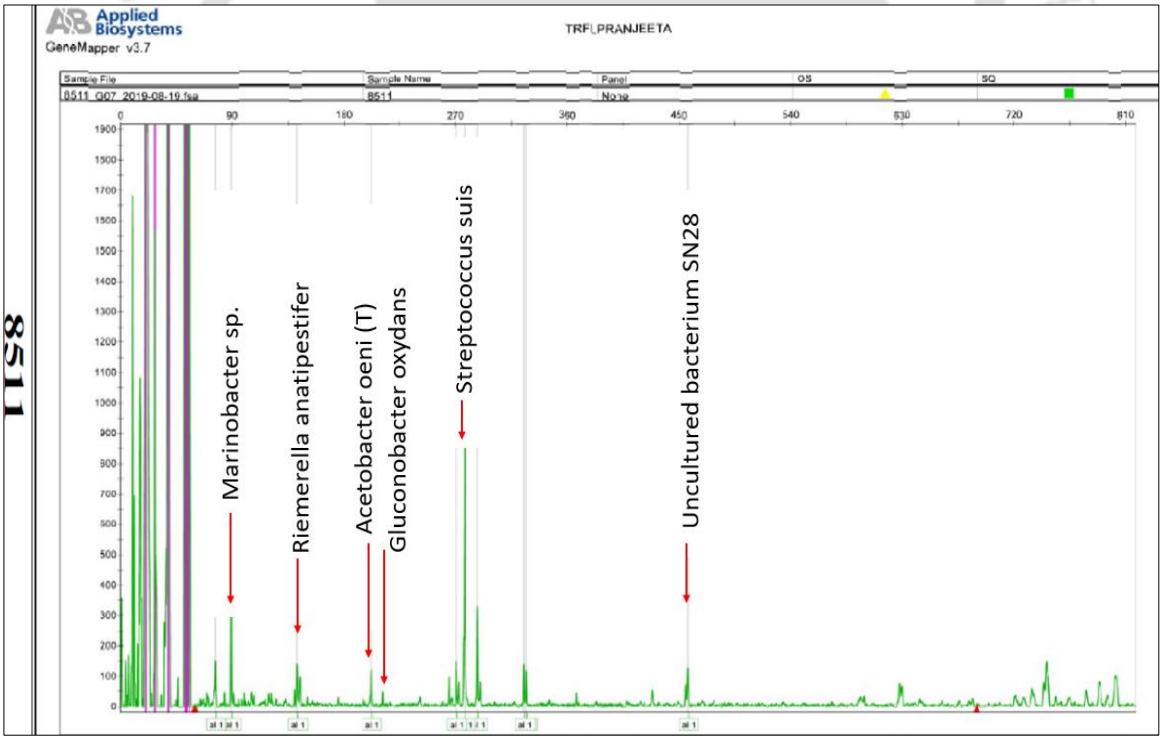

Figure 5.6.1 (iv). TRFLP analysis of bio sludge of batch reactor SN3

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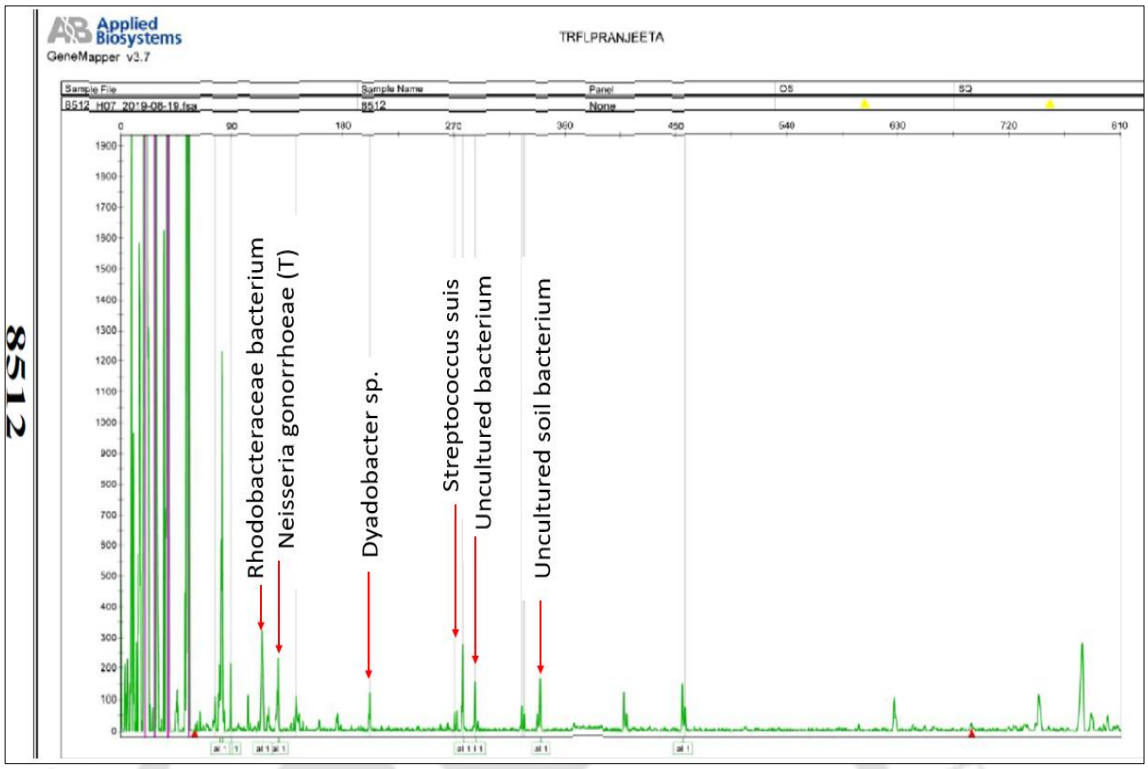

Figure 5.6.1 (v). TRFLP analysis of bio sludge of batch reactor SA3

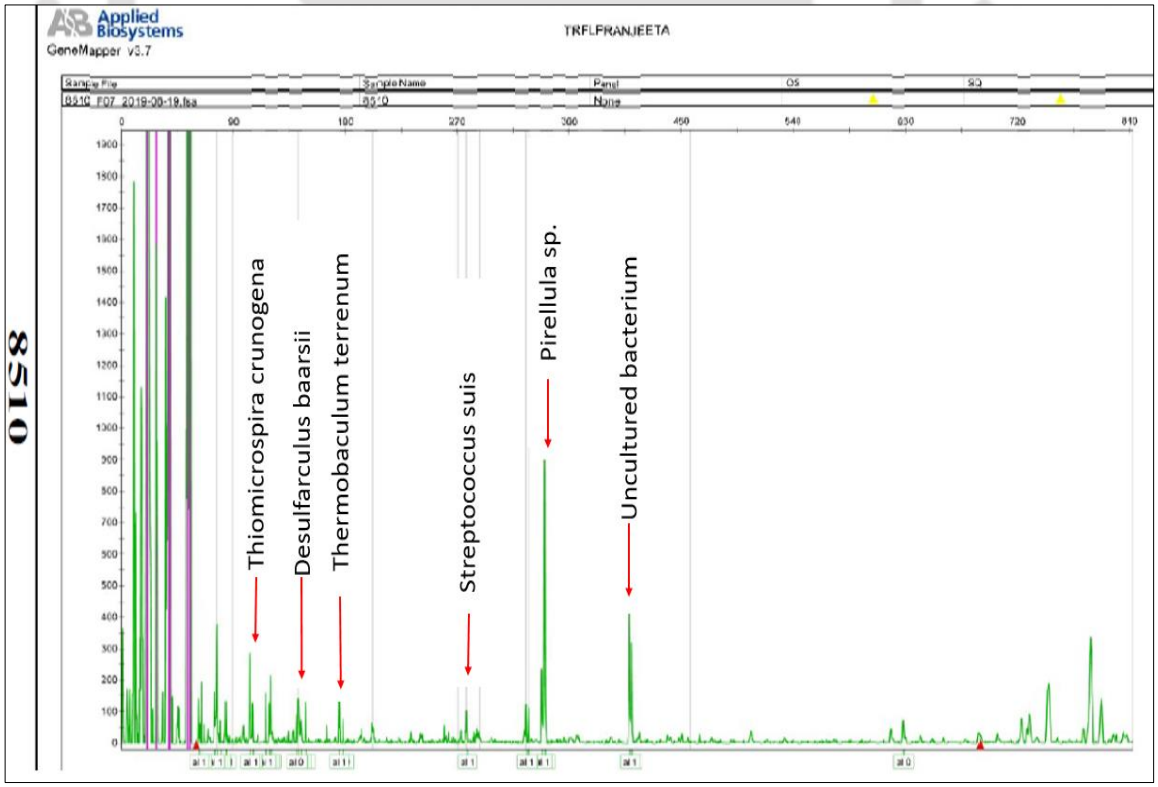

Figure 5.6.1 (vi). TRFLP analysis of bio sludge of batch reactor SAN3

TH-2881_146104040

5.6.2. 16S rRNA and Top Ten Genus

Figure 5.6.2 (i), represents the (a) 16S rRNA and (b) top ten genus of raw paper mill sludge. Figure 5.6.2 (i) a, shows that the paper mill sludge contains Ammonia oxidizers 28.1%, Chitin degraders 10.5%, Denitrifying 0.3%, Nitrite reducers 19.2%, Sulfate reducers 15.3%, Xylene degraders 2%, and Unknown 65.8%. From figure 5.6.2 (i) (b), the top 10 genus percentage present in paper mill sludge was i. e. *Acrobactor* 9 %, *Closriridium* 8 %, *Anaerorcus* 5 %, *Sedimentobactor* 4 %, *Azosprillum* 4 %, *Sulfurospirillum* 4 %, *Acholeplasma* 2 %, *Psudonomous* 1%, *Desulfovibrio* 1%, *Ilyobactor* 1%. Microorganism observed in paper mill sludge such as *Acetobacteraceae, Deferribacteraceae, Desulfobulbaceae, Desulfomicrobiaceae, Desulfovibrionaceae, Pseudomonadaceae, Rhizobiaceae, Spirochaetaceae, Victivallaceae, Selenomonadaceae, Bacteroidia, Deferribacteres, Nitrospira, Spirochaetia, Arcobacter, Betaproteobacteria, Chitinispirillia Bacteroides, Denitratisoma, Desulfitobacterium, Desulfobulbus, Desulfocurvus, Desulfomicrobium, Desulfosporosinus, Desulfotomaculum, Desulfovibrio, Opitutus, Gammaproteobacteria, Rhizobium, Spirochaeta, Thiovirga, Deferribacterales, Desulfobacterales, Desulfovibrionales, Desulfuromonadales, Nitrospirales, Planctomycetales, Spirochaetales, Selenomonadales, and Spirochaetales.* Moreover, *Desulfocurvus* (Spring et al., 2019), *Desulfomicrobiaceae* (Rojas et al., 2018), *Desulfovibrio* (Macy et al., 2000), and *Desulfobulbus* (Newman et al., 1997) strain is capable of reducing arsenate by using sulfide as electron donors. *Desulfosporosinus* play a major role in As (V) respiration in sulfidogeneic system, which is also capable of utilizing nitrate or arsenate as electron acceptor (Ramamoorthy et al., 2006). *Spirochaetia* (Prajapat et al., 2019), *Gammaproteobacteria*, and *Spirochaetia* (Li et al., 2017; Sampaio et al., 2020) were found in sulfate and nitrate reducing environment.

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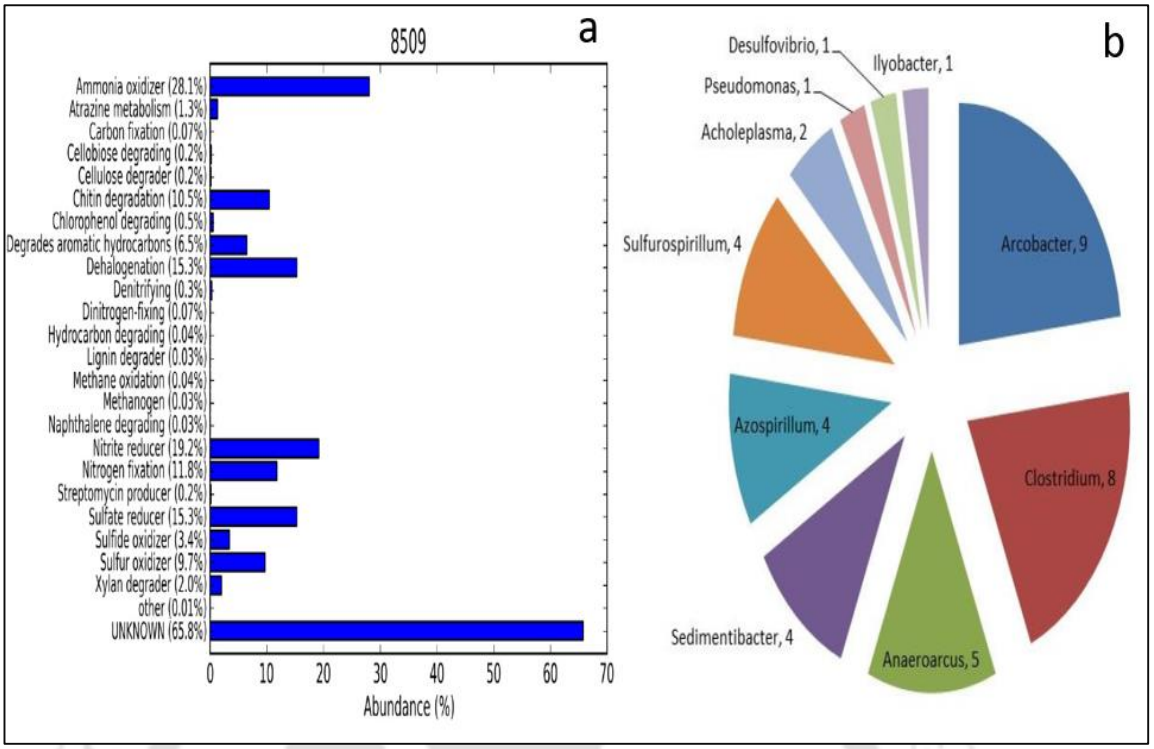

Figure 5.6.2 (i). (a) 16S rRNA (b) top ten genus of raw paper mill sludge

Figure 5.6.2 (ii), shows the (a) 16S rRNA and (b) top ten genus of bio sludge of batch reactor SN3. From figure [5.6.2 (ii) (a)], shows the presence of Ammonia oxidizers 51.1%, Chitin degraders 9.7%, Denitrifying 2.6%, Nitrite reducers 40.0%, Sulfate reducers 40.0%, Xylene degraders 11.3%, and Unknown 41.0%. From figure [5.6.2 (ii) (b)], it shows the presence of *Acrobactor* 29%, *Bacteroids* 11%, *Psudonomous* 6%, *Ruminofilibactor* 5%, *Azoarcus* 4%, *Clostridium* 3%, *Thouera* 3%, *Soehngenia* 1%, *Macellibacteroides* 1%, *Thiovirga* 1%.

Figure 5.6.2 (iii), shows the (a) 16S rRNA and (b) top ten genus of bio sludge of batch reactor SAN3. Figure 5.6.2 (iii) (a), it shows the presence of Ammonia oxidizers 45.2%, Chitin degraders 27.6%, Denitrifying 30.1%, Nitrite reducers 34.7%, Sulfate reducers 40.6%, Xylene degraders 6.9%, and Unknown 51.4%. Figure 5.6.2 (iii) (b), it shows the presence of *Psudonomous* 18%, *Acrobactor* 15%, *Paludibactor* 6%, *Closriridium* 3%, *Sphaerochaeta* 2%, *Spirochaeta* 1%, *Azoarcus* 1%, *Sedimentobacter* 1%, *Rikenella* 1%, *Anaerocella* 1%.

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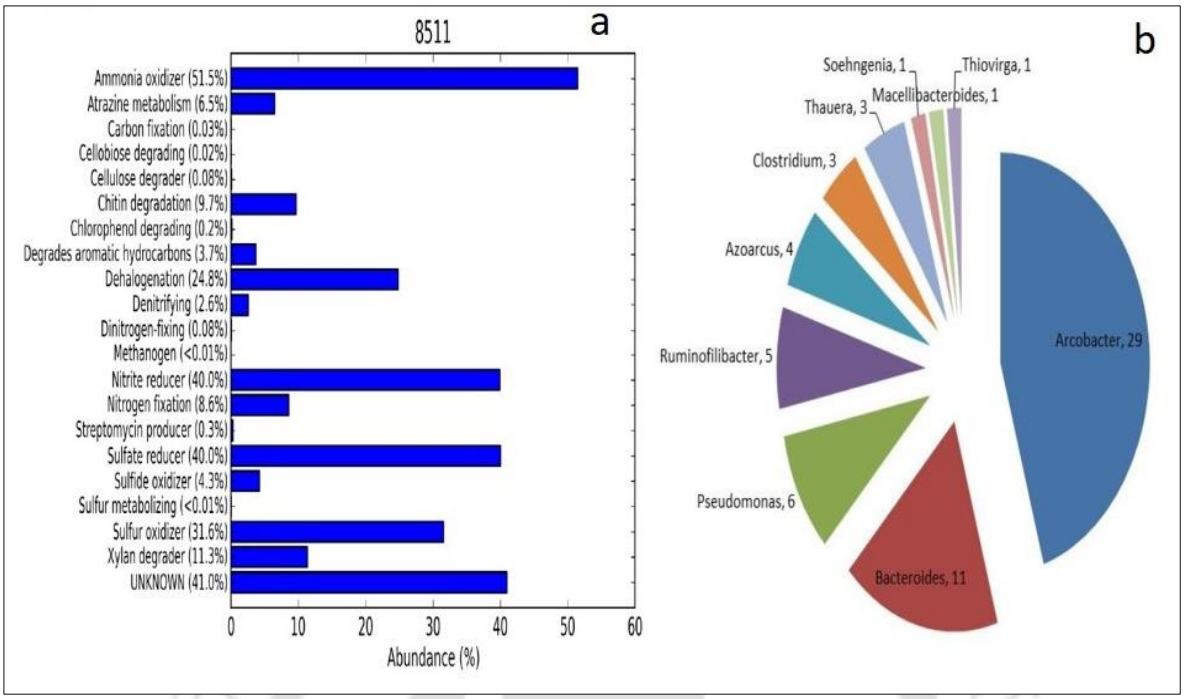

Figure 5.6.2 (ii). (a) 16S rRNA (b) top ten genus of batch reactor SN3

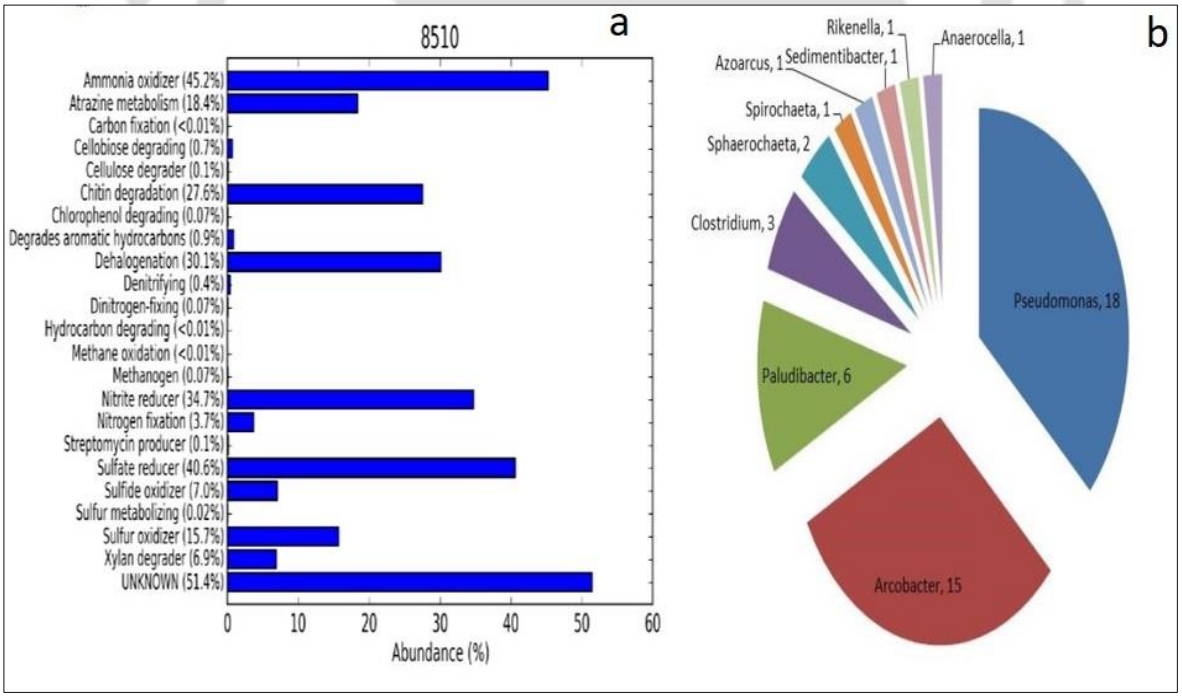

Figure 5.6.2 (iii). (a) 16S rRNA (b) top ten genus of batch reactor SAN3

TH-2881_146104040

5.6.3. Taxonomic Profiling

Taxonomic Profiling is shown in figure 5.6.3 (a), Genus distribution-1, reveals that reactor SA3 contains, *Thaurea*, *Bacteroides*, *Arcobacter*, *Chlorobaculum*, *Candidatus Cloacamonos*, *Acholeplasma*, *Chlostridium*, *Azoarcus*, *Bacillus*, *Desulfovibrio*, *Paludibactor*, *Aromatoleum*, *Psudomonas*, and *Magnetospirillum*. Figure 5.6.3 (b), Domain distribution-1, reveals that it contains *Bacteria*, *Archaea*, *Eukaryota*, *Unclassified sequences*, and *Viruses*.

Figure 5.6.3 (c), Domain distribution-2, reveals that it contains *Bacteria*, *Archaea*, *Eukaryota*, *Unclassified sequences*, and *Viruses*. Figure 5.6.3 (d), Genus distribution-2, reveals that it contains, *Thaurea*, *Bacteroides*, *Arcobacter*, *Chlorobaculum*, *Candidatus Cloacamonos*, *Acholeplasma*, *Chlostridium*, *Azoarcus*, *Bacillus*, *Desulfovibrio*, *Paludibactor*, *Aromatoleum*, *Psudomonas*, and *Parabacteroides*.

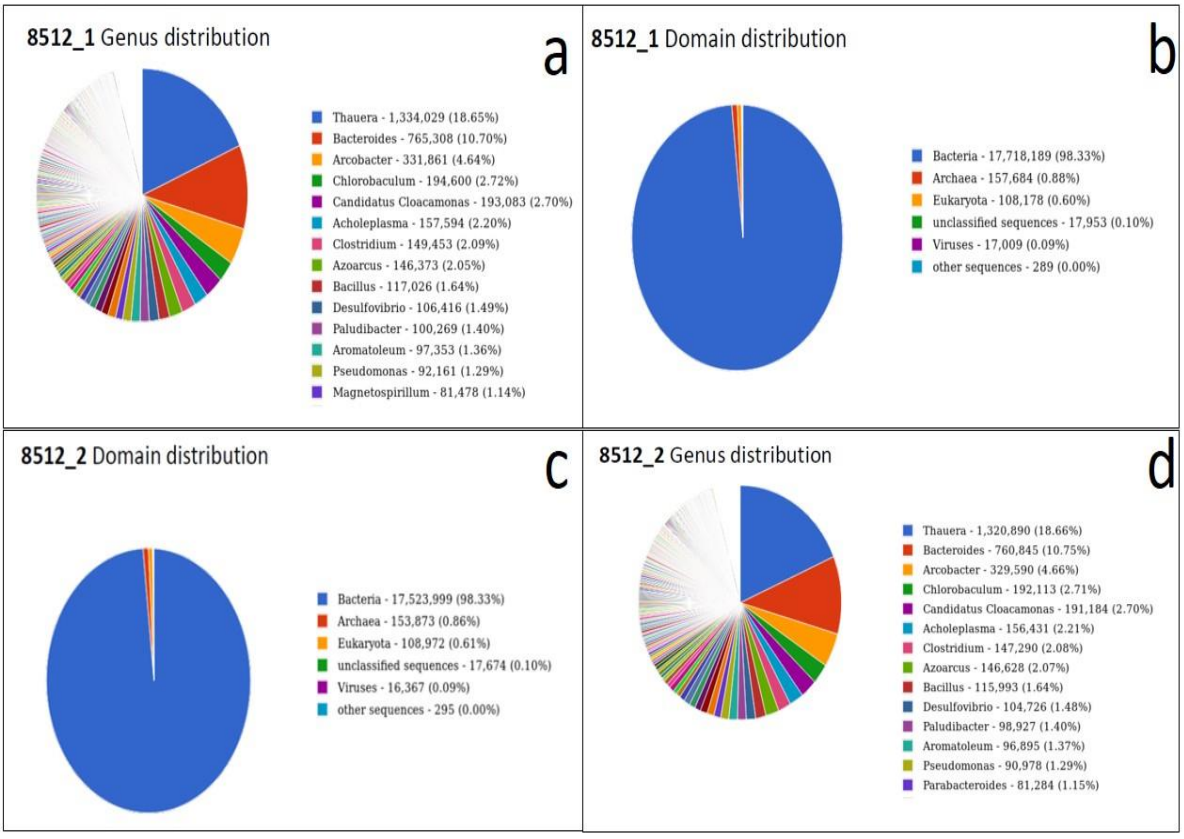

Figure 5.6.3. Taxonomic profiling of bio sludge of batch reactor SA3

TH-2881_146104040

5.7. Performance evaluation of the attached growth packed bed continuous reactor

The purpose of this study is to observe the effect of HRT, the effect of nitrate and arsenic concentration on PBCR performance using dextrose as a carbon source, and finally, the application of liquor of partially digested rice straw slurry (RS-NaOH) as the carbon source for the simultaneous the removal of oxyanions. Port profile sampling and characterization of bio solids through FESEM/EDX, XRD, and FETEM analysis to understand the mechanisms of oxyanion removal.

5.7.1. Effect of HRT

In the first phase of the study (phase 1), optimization of HRT was done using dextrose as a carbon source with the initial sulfate (1500 mg/L), nitrate (100 mg/L), and arsenic (1000 µg/L) concentration by varying the different HRTs. Figure 5.7.1 shows the effect of HRT on PBCR performance (a) effluent pollutant concentration and (b) removal efficiency. The removal efficiencies of COD, sulfate and arsenic were low during the start-up stage as shown in figure 5.7.1 (b). During the first 20 days (HRT 36 h) the average COD, sulfate, nitrate, and arsenic removal were 53 %, 60 %, 66, and 68% respectively. The low removal efficiency was due to the adaptation period of microorganisms in the bioreactor. During 20-30 d (HRT of 36 h), little increment in removal efficiencies was observed, in this phase average COD, sulfate, and arsenic removal were 63 %, 85, 81 %, and 78 % respectively.

When the HRT was reduced to 24 h (30-40 d), the COD, sulfate, nitrate, and arsenic removal were 69.9, 85, 85, and 86 % respectively. Further reduction in HRT 18 h (40-50 d), and reduction in removal efficiencies were observed. Average COD, sulfate, nitrate, and arsenic removal were 48, 55, 65, and 76 % respectively. Once the HRT was further reduced to 15 h (50-60 d), average COD, sulfate, and arsenic removal were further reduced i. e. 42, 52, 67, 71 % respectively. While the HRT was again increased to 24 h (60-70 d), reactor performance was improved steadily. The average COD, sulfate, nitrate, and arsenic removal were 69.4, 87, 88, and 90 %. The removal efficiency was satisfactory in the range at the HRT of 24 h as compared to other HRTs, thus it was considered an optimum HRT.

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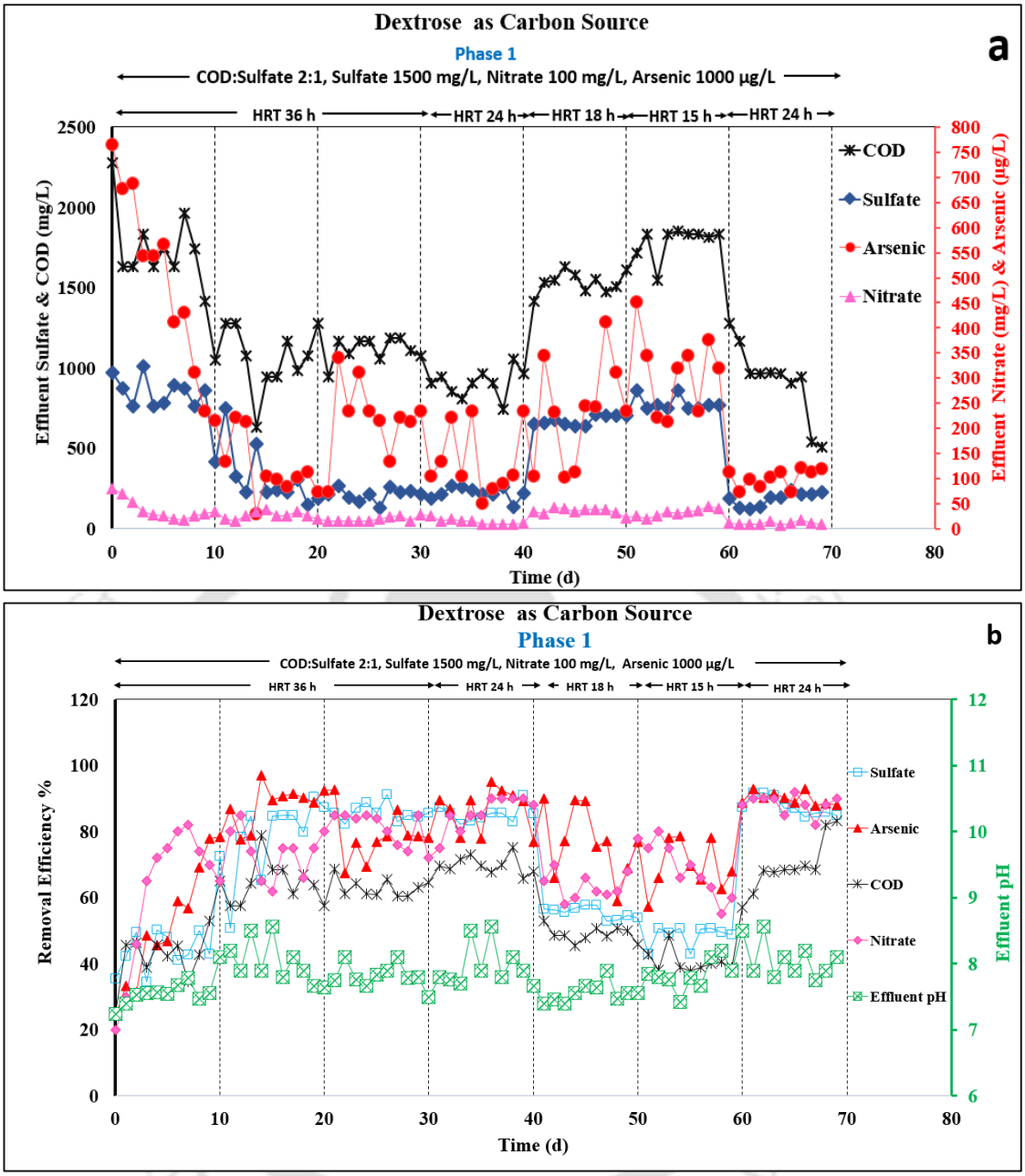

Figure 5.7.1. Effect of HRT on PBCR performance (a) Effluent pollutant concentration (b) Removal efficiency

5.7.2. Effect of Different Nitrate Concentration

In the second phase of the study (phase 2), the performance of the bioreactor with the addition of nitrate was monitored, with the initial sulfate (1500 mg/L), and arsenic (1000 µg/L) and at the HRT of 24 h. Figure 5.7.2, shows the effect of nitrate and arsenic concentration on PBCR performance (a) effluent pollutant concentration and (b) removal efficiency. From the 70 days onwards, with the addition of 100 mg/L nitrate, (70-84 d), the average COD, sulfate, nitrate, and arsenic removal were 66%, 81 %, 88,

TH-2881_146104040

and 86% respectively. From 85 days onwards, with the addition of 400 mg/L nitrate, (85-100 d), the average COD, sulfate, nitrate, and arsenic removal were 62%, 82 %, 89, and 87% respectively. From the 101 days onwards, with the addition of 600 mg/L nitrate, (101-115 d), the average COD, sulfate, nitrate, and arsenic removal were 68%, 80 %, 92%, and 90% respectively. Further increment in nitrate concentration 800 mg/L, (116-130 d), sulfate, and COD removal was gradually reduced but nitrate removal was steadily increased. However, arsenic removal was unaffected. At this phase average COD, sulfate, nitrate, and arsenic removal were 76%, 81 %, 94%, and 91% respectively. With further increment in nitrate concentration 1000 mg/L, phase (131-144 d), sulfate removal was decreased. The average COD, sulfate, nitrate, and arsenic removal were 83, 69, 90, and 91%, respectively. Moreover, as can be seen from [as shown in figure 5.7.2 (a and b)], with an increment in nitrate concentration, sulfate removal was decreased, but COD removal was improved. This indicated that there was an inhibition of sulfate reduction by nitrate as reported previously (Telang et al., 1997). Results indicate that nitrate inhibited the reduction of sulfate. This result was similar to the reported literature (Hubert and Voordouw, 2007; Telang et al., 1997). COD and sulfate removal was affected by the presence of nitrate. With the increment in nitrate concentration, sulfate removal was reduced, but arsenic removal was unaffected. This indicates that nitrate inhibited the reduction of sulfate. There are two possible mechanisms: competition for nutrients and inhibition of the sulfate reduction pathway. Firstly, nitrate addition stimulates resident heterotrophic nitrate-reducing bacteria which compete with SRB for the same carbon sources (Grigoryan et al., 2008). No strictly sequential reduction but a slight decrease in the sulfate removal due to the presence of nitrate was observed. Nitrate reduction is thermodynamically more exergonic than sulfate reduction, and nitrate inhibiting sulfate reduction might be due to electron donor competition when limited. Whereas other studies showed that inhibition of sulfate reduction by nitrate was attributed to the accumulation of denitrification intermediates, nitrite (Greene et al., 2003).

5.7.3. Effect of Different Arsenic Concentration

In phase 3 (from 145 d to 160 d), arsenic concentration was varied at the fixed sulfate (1500 mg/L) and nitrate (800 mg/L) concentration, with the arsenic concentration of 1000 µg/L, the average COD, sulfate, nitrate, and arsenic was 80, 84, 95 and 91%

TH-2881_146104040

respectively [as shown in figure 5.7.2 (b)]. With the arsenic concentration of 1500 µg/L (from 161-175 days), the average COD, sulfate, nitrate, and arsenic were 81, 85, 94, and 94% respectively. With the arsenic concentration of 2000 µg/L (from 176-190 days), the average COD, sulfate, nitrate, and arsenic were 81, 88, 96, and 93% respectively.

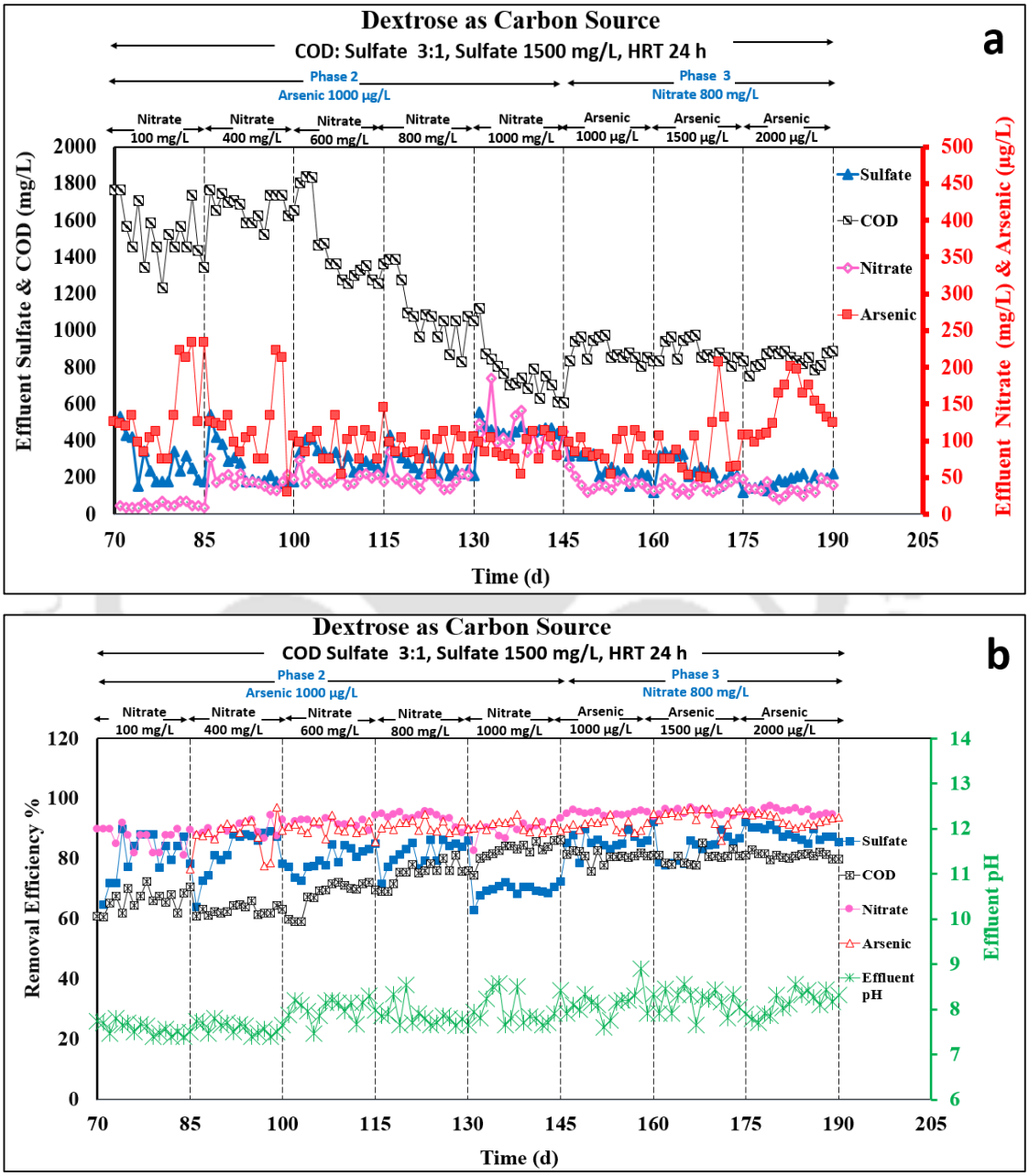

Figure 5.7.2. Effect of nitrate and arsenic concentration on PBCR performance (a) Effluent pollutant concentration (b) Removal efficiency

TH-2881_146104040

Increment in arsenic concentration had no significant effect on the performance of the reactor. A comparison between the performances of different bioreactors using various types of carbon sources for the removal of multipollutant is shown in table 5.7.

TH-2881_146104040

Table 5.7. Comparison between the performances of bioreactors using various types of carbon sources for the removal of multipollutant

Reactor Type	**Carbon Source**	**HRT (h)**	**Influent Pollutant Concentration (mg/L)**		**Removal %**	**Ratio**		**Reference**
						COD/Sulfate	Sulfate/Nitrate	
Expanded granular sludge bed	Lactate	18	COD Sulfate Nitrate	3000 1000 500	85 96 100	3	NR	(Xu et al., 2017)
Expanded granular sludge bed	Ethanol	15	COD Sulfate Nitrate	7200 3600 300-1200	66-93 66-93 90-99	2	1.33	(Liao et al., 2014)
Upflow anaerobic sludge bed reactor	Dextrose and Acetate	24	COD Sulfate Nitrate	265 165 30-70	95 80 99	1.05-2.4	1.4-2.23	(Wang et al., 2009)
Cylindrical-type anaerobic baffled reactor	Lactate	24	COD Sulfate Nitrate	1000 168 132-505	64-73 82-89 96-99	5.95	0.51	(Huang et al., 2015)
Upflow anaerobic sludge bed reactor	Lactate	24	COD Sulfate Nitrate Se	2000 1500 47 5-12	58-70 15 92 61-85	1.33	NR	(Tan et al., 2018)
Packed bed reactor	Dextrose	24	COD Sulfate Nitrate Arsenic	3000 1500 100 0.1	69.4 87 88 90	2	15	This Study
Packed bed reactor	Dextrose	24	COD Sulfate Nitrate Arsenic	4500 1500 100-1000 0.1-0.2	66-83 81-88 88-96 87-94	3	1.5-3.75	This Study

NR, Not Reported

TH-2881_146104040

5.7.4. Application of Liquor of Partially Digested Rice Straw Slurry (RS-NaOH) as Carbon Source

In phase 4 (from 191-230 days), figure 5.7.4, shows the application of liquor of partially digested rice straw slurry (RS-NaOH) as a carbon source for the removal of oxyanions in PBCR (a) effluent pollutant concentration (c) removal efficiency. Once the fermented liquor of rice straw (RS-NaOH) was applied as a carbon source the average of sulfate, nitrate and arsenic were 76, 85, and 93% respectively. COD and BOD removal were 78 and 91 % respectively.

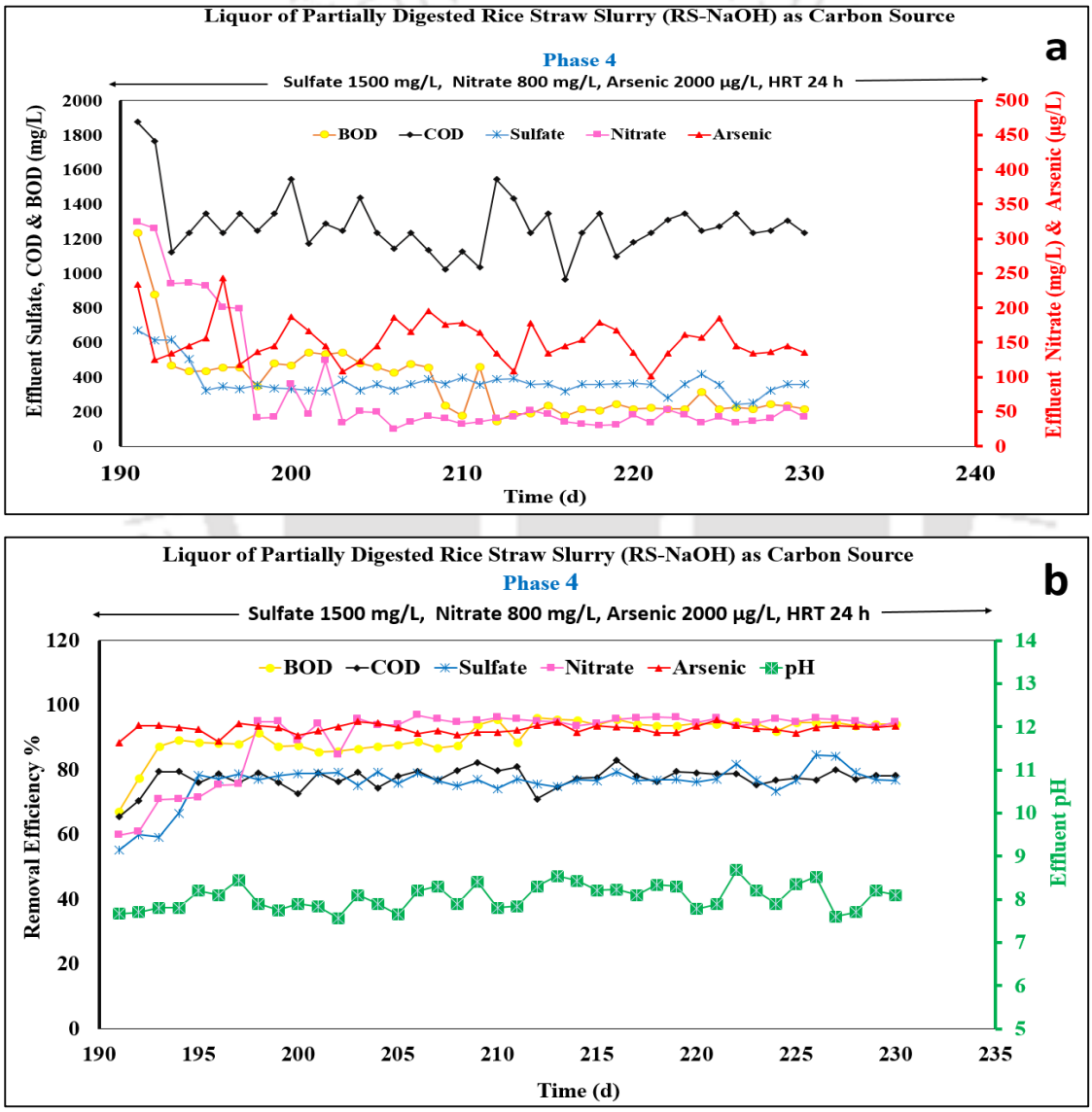

Figure 5.7.4. Application of liquor of partially digested rice straw slurry as a carbon source for the removal of oxyanions in PBCR (a) Effluent pollutant concentration (b) Removal efficiency

TH-2881_146104040

5.7.5. Port Profile Sampling

Profile sampling confirmed sequential utilization of nitrate, arsenic, and sulfate in a continuous reactor. Profile samples were taken during the day on 105, 140, 190, and 220 days, representing the concentration of various pollutants along the depth of the reactor as shown in figure 5.7.5 (i and ii). From the port profile of 105 days [figure 5.7.5 (a)], at the 4^{th} port COD, sulfate, nitrate, and arsenic removal were 40, 66, 51, and 76.6 % respectively. Final COD, sulfate, nitrate, and arsenic were 69, 69, 79, and 92% respectively. The final pH of treated water was 7.85. From the port profile of 140 days [figure 5.7.5 (b)], at the 4^{th} port COD, sulfate, nitrate, and arsenic removal were 37, 53, 90, and 77% respectively. Final COD, sulfate, nitrate, and arsenic were 74, 72, 90, and 92.5% respectively. The final pH of treated water was 7.68. Port profile of 190 days [figure 5.7.5 (c)], at 4^{th} port COD, sulfate, nitrate, and arsenic removal were 33, 65, 61, and 73 % respectively. Final COD, sulfate, nitrate, and arsenic were 80, 87, 86, and 94 % respectively. The final pH of treated water was 7.78. Port profile of 220 days [figure 5.7.5 (d)], shows the average sulfate, nitrate, and arsenic removal of 65, 63, and 75%, at the 4^{th} port. COD and BOD removal were 66, and 68%. Final sulfate, nitrate, and arsenic removal were 84, 94, and 93 %. COD and BOD removal were 79, and 95% Final pH of treated water was 7.74. Profile sampling data shows sequencing utilization of electron acceptor present in influent media following NO_3^-, As, and SO_4^{2-}. After nitrate reduction, sulfate and arsenic removal were observed in port 3 because of the formation of biogenic sulfides responsible for arsenic removal. The sequential utilization of electron acceptors in the system confirms the establishment of TEAP zones based on thermodynamics favourability.

TH-2881_146104040

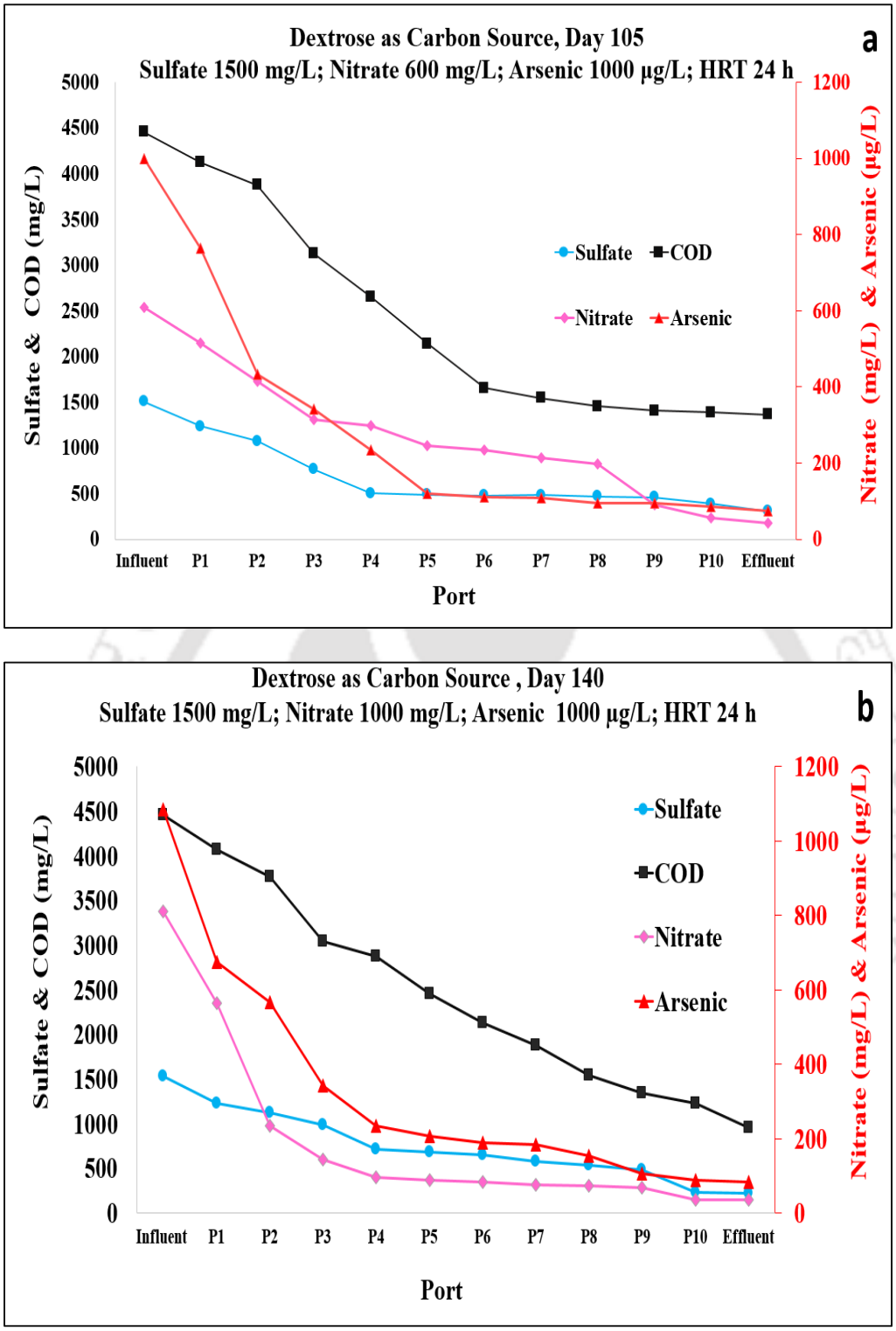

Figure 5.7.5 (i). Port profile of PBCR (a) at 105 d (b) at 140 d

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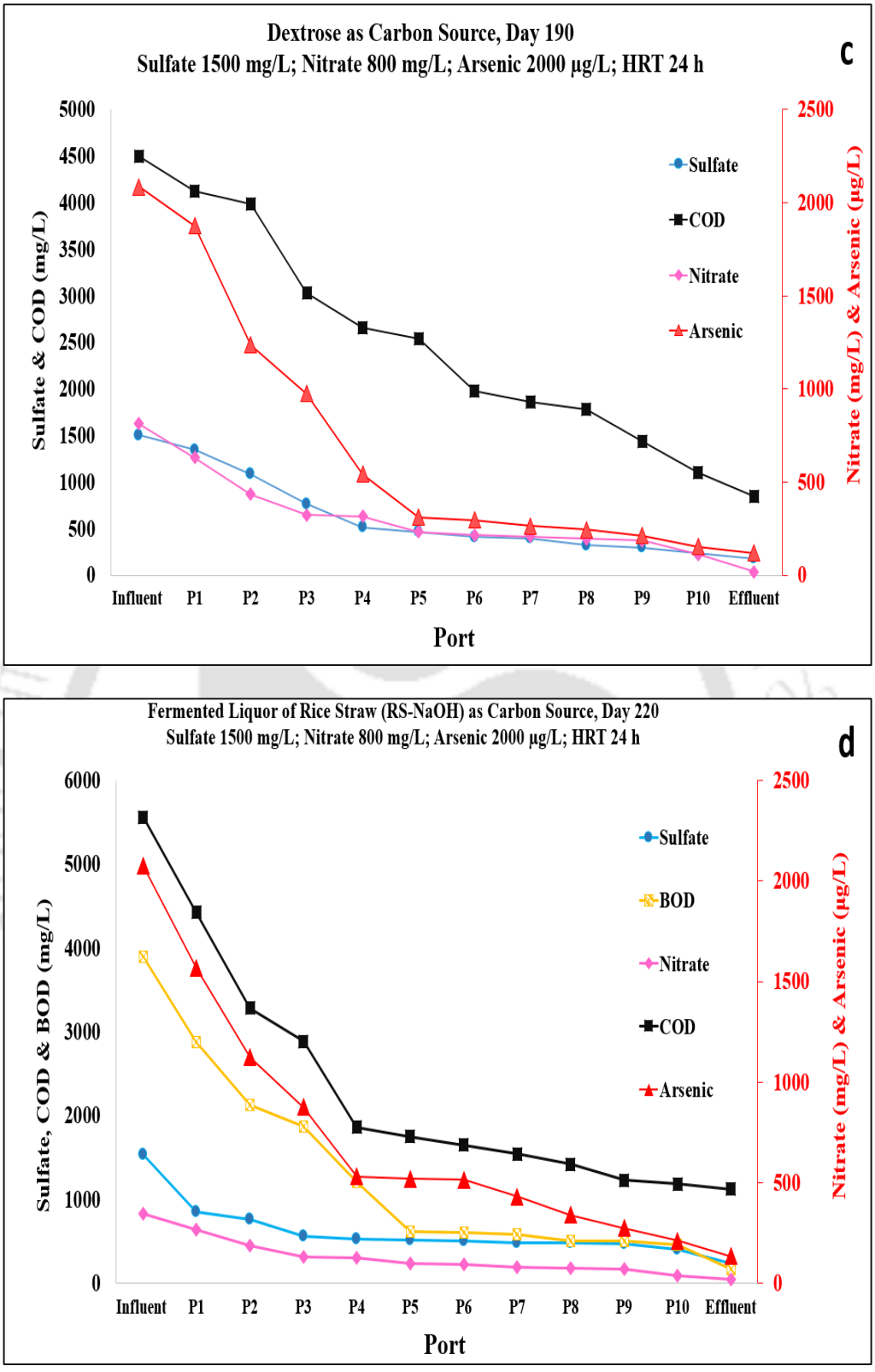

Figure 5.7.5 (ii). Port profile of PBCR (c) at 190 d (d) at 220 d

TH-2881_146104040

5.7.6. Microscopic Analysis

FESEM and EDX Analysis of Bio solids at 190 day

FESEM and EDX have been used as a tool for bio solids characterization and elucidation of probable mechanisms involved in arsenic removal. Figure 5.7.5 (i). (a) FESEM (b) X-Ray elemental mapping micrograph of bio solids of PBCR at 190 d (c) X-Ray mapping of element sulfur (d) X-Ray mapping of element arsenic. Figure 5.7.5 (ii), shows the EDX image of bio solids of PBCR at 190 d. It represents the presence of arsenic, sulfur other peaks of oxygen, carbon and calcium may be due to feeding media composition. Other peaks may be due to elements present in influent and trace mineral solution. It is most probable that the arsenic precipitated mainly in form of amorphous arsenosulfide (As_2S_3) which was further analyzed by finer mineral analysis like XRD and TEM. X-Ray elemental mapping of bio solids depicts the uniform distribution of arsenic and sulfur over the entire surface area which further supports the formation of arsenosulfides precipitates. The surface area of bio solids is rough and irregular with large area.

XRD Analysis of Bio solids at 190 day

From figure 5.7.5 (iii), XRD analysis of continuous reactor (at 190^{th} d), depicted existence of orpiment (As_2S_3; JCPDS # 01-074-0335), dimorphite-II (As_4S_3; JCPDS # 00-026-0126), orpiment (As_2S_3; JCPDS # 00-019-0084) as the solids deposited in the continuous reactor. The possible arsenic removal mechanism was arsenosulfide precipitation in anaerobic reducing conditions, where arsenite (III) and biogenic sulfide (S (II) react to form arsenic sulfides such as orpiment (Newman et al., 1997) and realgar (O'Day et al., 2004).

FETEM Analysis of Bio solids at 190 day

Transmission electron microscopy (TEM) provides a more detailed morphology of a solid while selected area electron diffraction (SAED) revealed amorphous, crystalline, and or nanocrystalline phases solid formed in the continuous reactor systems. Figure 5.7.5 (iv), shows the FETEM analysis of bio solids of PBCR at 190 d; (a-e) TEM images (f-h) HRTEM (i-l) IFFT (m) SAED Pattern (n) Profile of IFFT. The corresponding SAED pattern shows a diffused halo ring, which indicates a uniform amorphous

TH-2881_146104040

microstructure with crystalline features. The HRTEM revealed the ultrastructure of the precipitates confirming the presence of mineral orpiment (As_2S_3) and arsenosulphides. The interplanar d-spacing values were measured from IFFT of high-resolution images. The belt structure showed distinctive clear lattice fringes with a d-spacing of 0.27 nm [figure 5.7.5 (iv) (e)]. The d spacing values from the FFT pattern are in good agreement with calculated values (d =2.7 A^o, hkl plane 211, sharp peak value 27.9°θ) from the XRD analysis, which corresponds to the compound orpiment (As_2S_3; JCPDS # 01-074-0335).

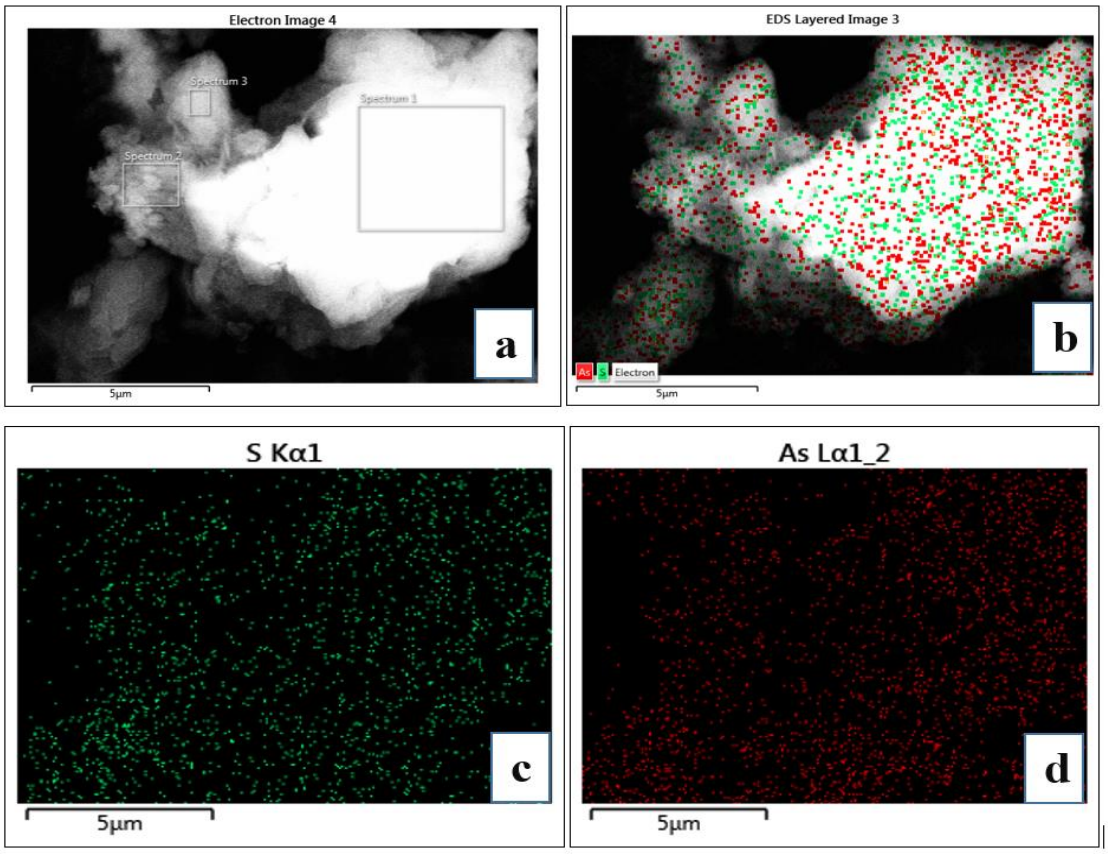

Figure 5.7.5 (i). (a) FESEM (b) X-Ray elemental mapping micrograph of bio solids of PBCR at 190 d (c) X-Ray mapping of element sulfur (d) X-Ray mapping of the element arsenic

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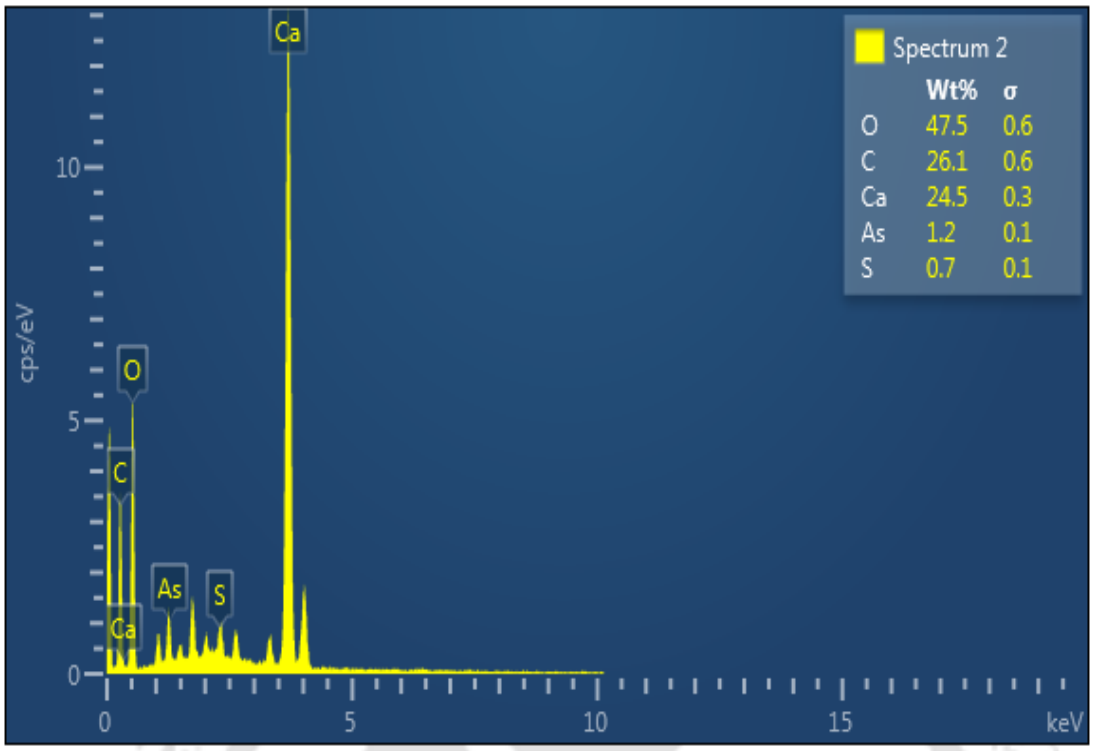

Figure 5.7.5 (ii). EDX image of bio solids of PBCR at 190 d

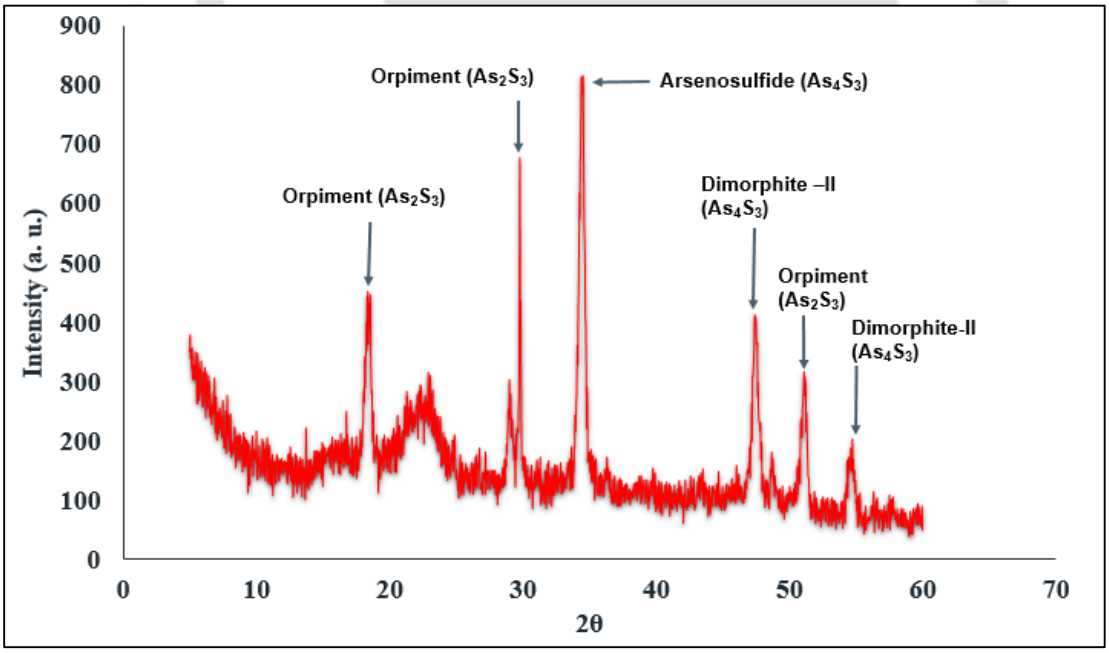

Figure 5.7.5 (iii). XRD spectra of bio solids of PBCR at 190 d

TH-2881_146104040

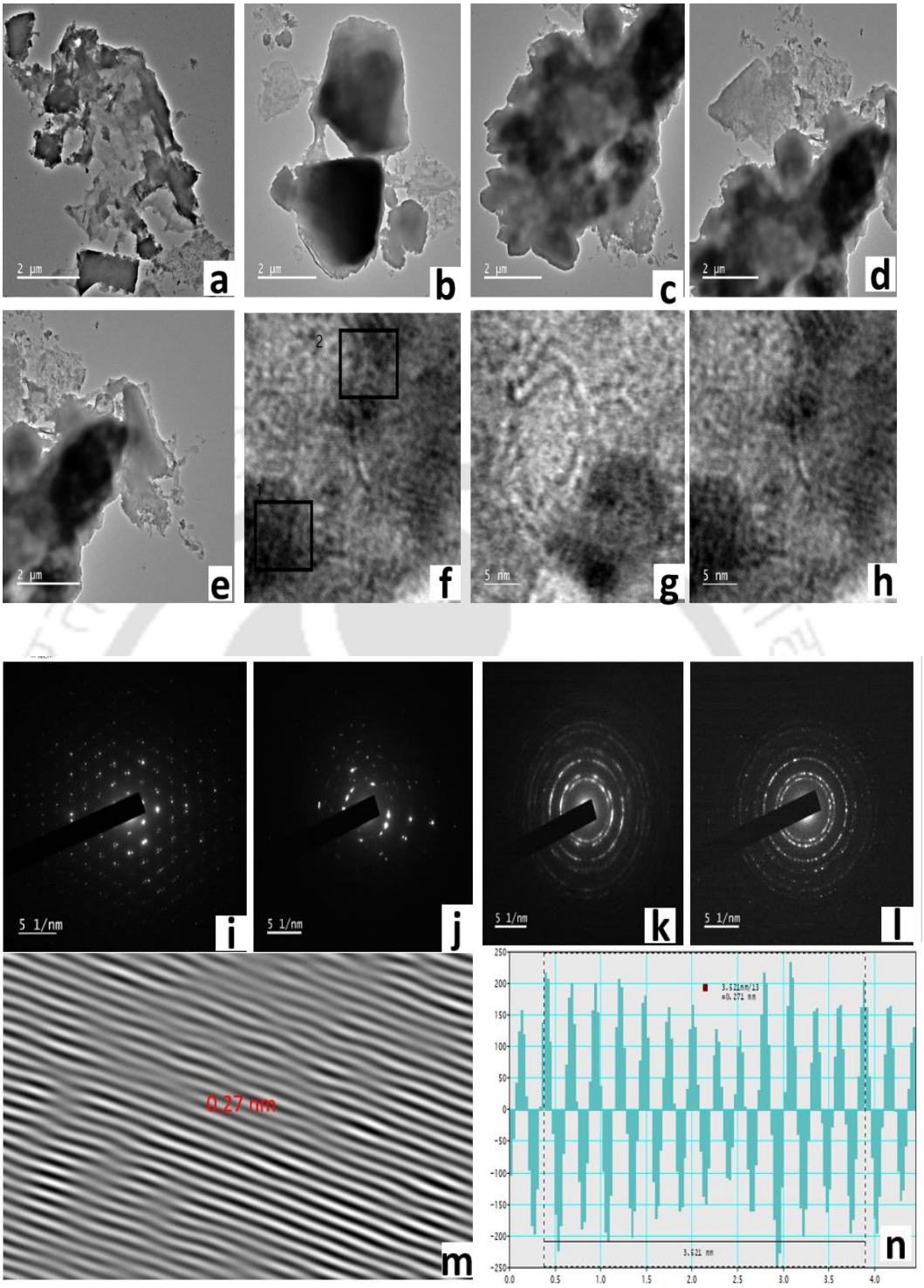

Figure 5.7.5 (iv). FETEM analysis of bio solids of PBCR at 190 d; (a-e) TEM images (f-h) HRTEM (i-l) IFFT (m) SAED Pattern (n) Profile of IFFT

FESEM and EDX Analysis of Bio solids at 220 day

Similarly (a) FESEM (b) EDX (c) X-Ray mapping of bio solids, X-Ray mapping of (d) element arsenic (e) element sulfur of PBCR (at 220th d) are shown in figure 5.7.5 (v),

TH-2881_146104040

represents the presence of arsenic, sulfur, other peaks such oxygen, carbon, silica, is may be due to rice straw and chlorine due to feed media composition.

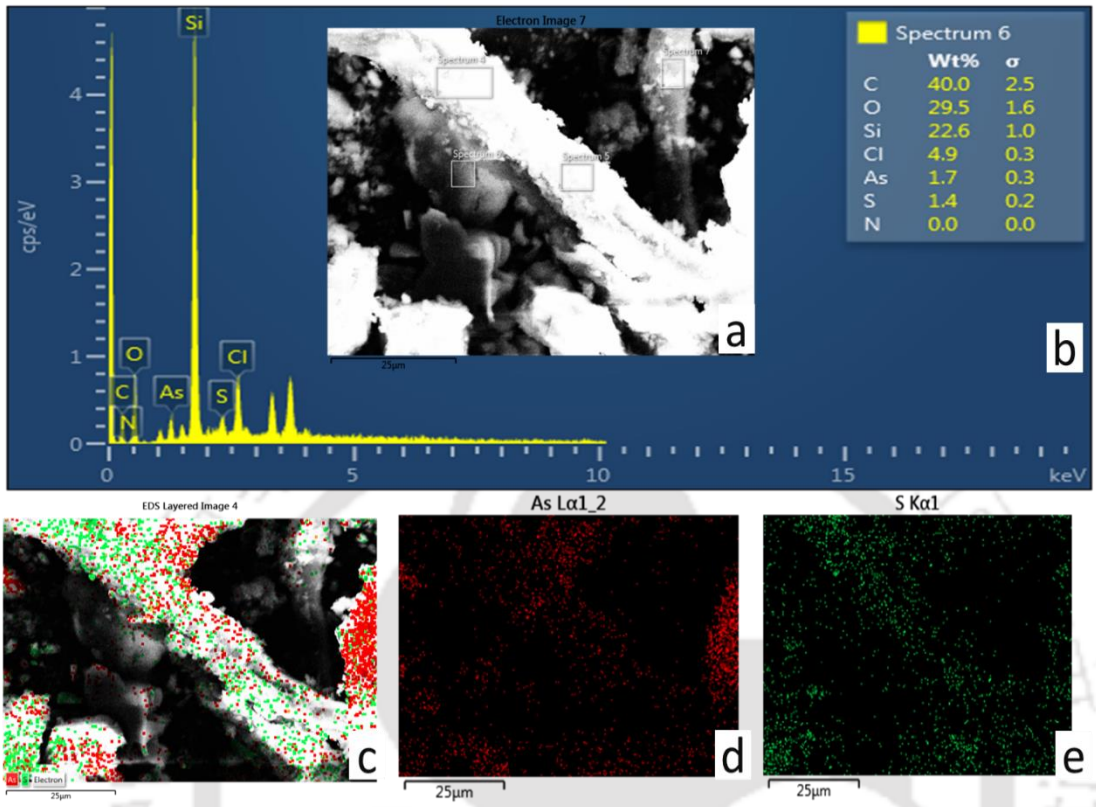

Figure 5.7.5 (v). (a) FESEM (b) EDX (c) X-Ray mapping of bio solids, and X-Ray mapping of (d) element arsenic (e) element sulfur of PBCR at 220 d

XRD Analysis of Bio solids at 220 day

Similarly, XRD spectra of bio solids of PBCR (220th d) are shown in figure 5.7.5 (vi), depicting the existence of arsenic sulfide (As_2S_5; JCPDS # 00-027-0029), arsenic sulfide (As_4S_4; JCPDS # 01-072-0686), orpiment (As_4S_4; JCPDS # 00-044-1419). XRD results confirm the arsenic and sulfur precipitation mechanism.

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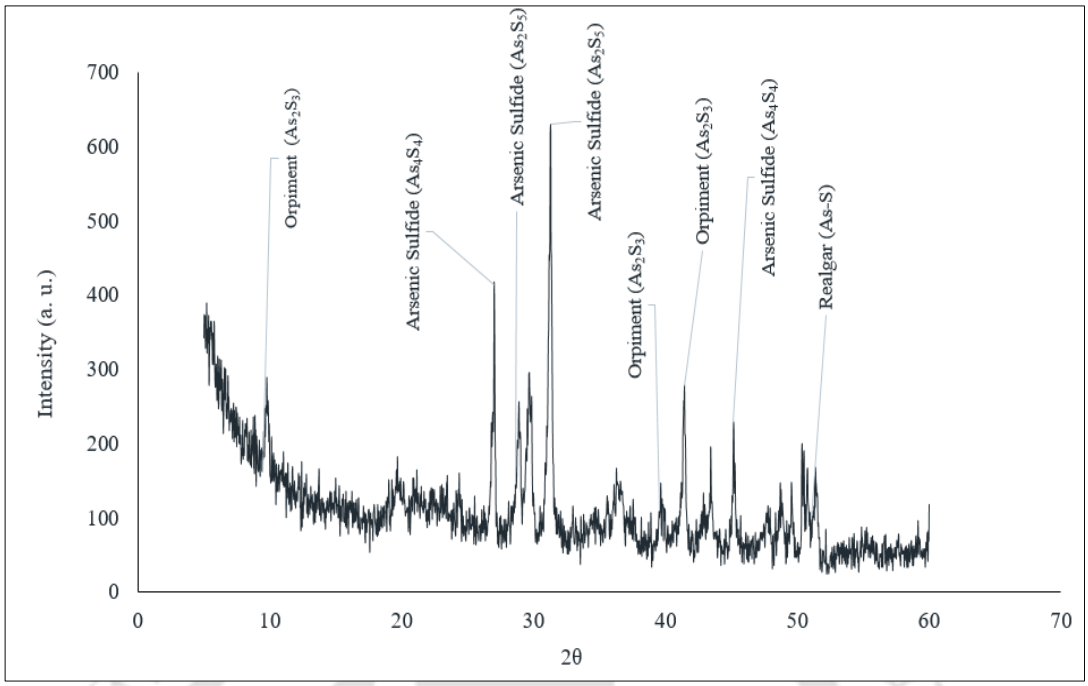

Figure 5.7.5 (vi). XRD spectra of bio solids of PBCR at 220 d

The present study demonstrates the simultaneous removal of sulfate (1500 mg/L), nitrate (100–1000 mg/L), and arsenic (1000-2000 µg/L) in a packed bed continuous reactor by biological process. Optimum HRT was found to be 24 h in phase 1. The average COD, sulfate, nitrate, and arsenic removal were 69.9, 87, 88, and 90 % at the HRT of 24 h. In phase 2 and 3, COD, sulfate, nitrate and arsenic removal were 66-83, 81-88%, 88-96%, 87-94 %, with the initial COD, sulfate, nitrate and arsenic concentration of 4500 mg/L, 1500 mg/L, 100-1000 mg/L, 1000-2000 µg/L respectively. With the increment in nitrate concentration sulfate and COD removal were gradually reduced but nitrate removal was steadily increased, however, arsenic removal was unaffected. An increment in arsenic concentration had no significant effect on the performance of the reactor. In phase 4, once the fermented liquor of rice straw (RS-NaOH) was applied as a carbon source the average of sulfate, nitrate and arsenic were 77, 91, and 93% respectively. COD and BOD removal were 78 and 91 % respectively. Thus it can be replaced by the synthetic carbon source. The characterization of bio solids confirmed the bio precipitation as As-S, As_2S_3, and As_4S_3 were the removal mechanisms of arsenic and sulfate. FETEM analysis suggested the formation of stable bio-sludge in the bioreactor, which is of stable nature, thus the sludge-handling problem can be expected to be low.

TH-2881_146104040

CHAPTER 6

CONCLUSION AND SCOPES OF FUTURE STUDIES

6.1 Conclusion

The main objective of this research work is to utilize the rice straw as a carbon source for simultaneous removal of sulfate, nitrate, and arsenic from the contaminated wastewater by a biological process in batch and continuous reactor. Mechanisms of sulfate, nitrate, and arsenic removal were investigated through their instrumental analysis and characterization.

Summary of salient results and conclusions made out of the present investigation are given below:

1. Present study demonstrates that the hot air oven pretreatment was more effective than the autoclave pretreatment in all cases based on the hydrolyzate and compositional characteristics of rice straw. Based on the hydrolysis of rice straw during the pretreatment, the optimized temperature and time were 85^{o}C and 240 min in the case of the hot air oven and 121^{o}C and 20 min in the case of the autoclave. The optimum doses of alkali were 2.5% (w/w) $Ca(OH)_2$, 1% (w/w) NaOH, and 0.4% (v/v) ChOH for both of the cases in a hot air oven and autoclave in terms of COD, TRS, and VFA production. The maximum COD, TRS, VFA %, and hydrolysis rate % in the hot air oven treatment (at 85^{o}C corresponding to 240 min) with 1% (w/w) NaOH was 44±1.8 %, 27±3.1%, 3±0.01%, and 26.8 % respectively. Effectiveness of reacting agents was in the sequence of NaOH > ChOH > $Ca(OH)_2$. Production of COD, TRS, and VFA from rice straw hydrolyzate and alteration in various parameters (lignocellulosic compounds, CHNS %, residual char %, increment in Cr Index %, biodegradability fraction, and hydrolysis rate %) is suggesting that rice straw hydrolyzate, as well as amorphous rice straw both of them, has enough potential to be used as a carbon source for the microorganism.

2. Based on batch studies it can be summarised rice straw can efficiently remove the oxyanions (single/multiple) from the wastewater containing sulfate (1000-3000 mg/L), nitrate (500-2000 mg/L), and arsenic (500-2000 µg/L) with the removal efficiency of 62-95% and 64-94 %, 89-94 % respectively within 40 days of HRT using biological process.

TH-2881_146104040

3. Performance evaluation of continuous reactor was investigated for simultaneous removal of sulfate, nitrate, and arsenic using the biological process in packed bed continuous reactor. This reactor can treat the wastewater with initial sulfate, nitrate, and arsenic concentration of 1500 mg/L, 100-1000 mg/L, and 1000-2000 µg/L leaving the final effluent concentration in the range of 150-524 mg/L, 20-140 mg/L, and 54-230 µg/L respectively. This reactor and the process adopted in this research work are suitable for industrial application and for treating wastewater containing sulfate, nitrate, and arsenic.

4. The characterization of bio solids from reactors SA3, SAN3, and PBCR confirmed the bio precipitation as As-S, As_2S_3, and As_4S_3 were the removal mechanisms of arsenic and sulfate. FETEM analysis suggested the formation of stable bio-sludge in the bioreactor, which is in stable nature, thus the sludge-handling problem can be expected to be low. The belt structure showed distinctive clear lattice fringes with a d-spacing of 0.299 nm, 0.32 nm, and 0.27 nm of bio solids from SA3, SAN3, and attached growth reactor (PBCR), respectively. The crystalline nature of the obtained precipitates suggests the stable nature of bio solids which ensures their safe disposal in a landfill.

5. Metagenomic studies (16S rRNA analysis, TRFLP analysis, and Taxonomic profiling) of the raw paper mill sludge and reactors SN3, SA3, and SAN3, confirm the presence of sulfate, nitrate, and arsenic removing microorganisms.

6.2. Scopes of Future Studies

The present study was confirmed to assess the ability and the potential application of rice straw as a carbon source for the removal of sulfate, nitrate, and arsenic in biological process in batch

To carry forward the research of the present investigation, the following suggestions are listed:

1. Fabrication of pilot plant scale bioreactor and its performance evaluation on simultaneous removal of targeted contaminants.

2. Studies on utilization of other agricultural waste material as a carbon source for the removal of targeted contaminates.

3. Studies on the introduction of other treatment/pretreatment technologies for the extraction of carbon sources from different agricultural waste materials.

TH-2881_146104040

VISIBLE RESEARCH OUTPUT

International / National Conferences

- Bhande, R., and Ghosh, P. K. Oxyanions removal by biological processes: A review, ICWEES, International Conference on Water, Environment, Energy and Society' held at AISECT University Bhopal, India, March 15 -18, 2016.

- Bhande, R., and Ghosh, P. K. Autoclave assisted thermal pre-treatment of Rice Straw, NEC, National Environmental Conferences held at IIT Bombay, India, January 31- February 1, 2020.

- Bhande, R., and Ghosh, P. K. Potential Application of Agri. Waste as a Carbon Source for removal of Sulfate & Co-Contaminates, WEES 2020, International Conference on Water, Energy and Environmental Sustainability, held at NIT Durgapur. *(Best Oral Presentation)*, January 13-15, 2020.

- Bhande R., and Ghosh, P.K. Anaerobic digestion of rice straw in presence of sulfate containing environment. CHEMBION, International Conference on Chemical, Bio & Environmental Engineering, held at NIT Jalandhar, February 13-14, 2020.

- Bhande R. "Restore our Earth". Online National seminar attended at IIM Sirmour. April 22, 2021.

Book Chapter

- Bhande, R., & Ghosh, P. K. (2018). Oxyanions removal by biological processes: A review. Water Quality Management, 37-54.

Manuscript on Preparation

- Bhande, R., & Ghosh, P. K. Study on the Effects of Thermoalkali Treatment on the Hydrolysis and Physicochemical Properties of Rice Straw.

TH-2881_146104040

- Bhande, R., & Ghosh, P. K. Studies on the Effect of the pH and Different Thermoalkali Treatment Methods on the Sulfate Removal and Hydrolysis of Rice Straw during Anaerobic Digestion Process in the Batch Reactor.

- Bhande, R., & Ghosh, P. K. Studies on the Removal of Sulfate and Nitrate using NaOH Pretretreated Rice Straw as a Carbon Source during Anaerobic Digestion Process in the Batch Reactor.

- Bhande, R., & Ghosh, P. K. Studies on the Simultaneous Removal of Sulfate and Arsenic using NaOH Pretretreated Rice Straw as a Carbon Source during Anaerobic Digestion Process in the Batch Reactor.

- Bhande, R., & Ghosh, P. K. Studies on the Simultaneous Removal of Sulfate, Nitrate and Arsenic using ChOH Pretreated Rice Straw as a Carbon Source during Anaerobic Digestion Process in the Batch Reactor.

- Bhande, R., & Ghosh, P. K. Performance Evaluation of Packed Bed Continuous Reactor for Simultaneous Removal of Sulfate, Nitrate, and Arsenic using Biological Process.

- Bhande, R., & Ghosh, P. K. Review on High-Cost and Low-Cost Carbon Sources for Oxyanions Removal in Biological Treatment Methods and Their Aspects.

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TH-2881_146104040

APPENDIX

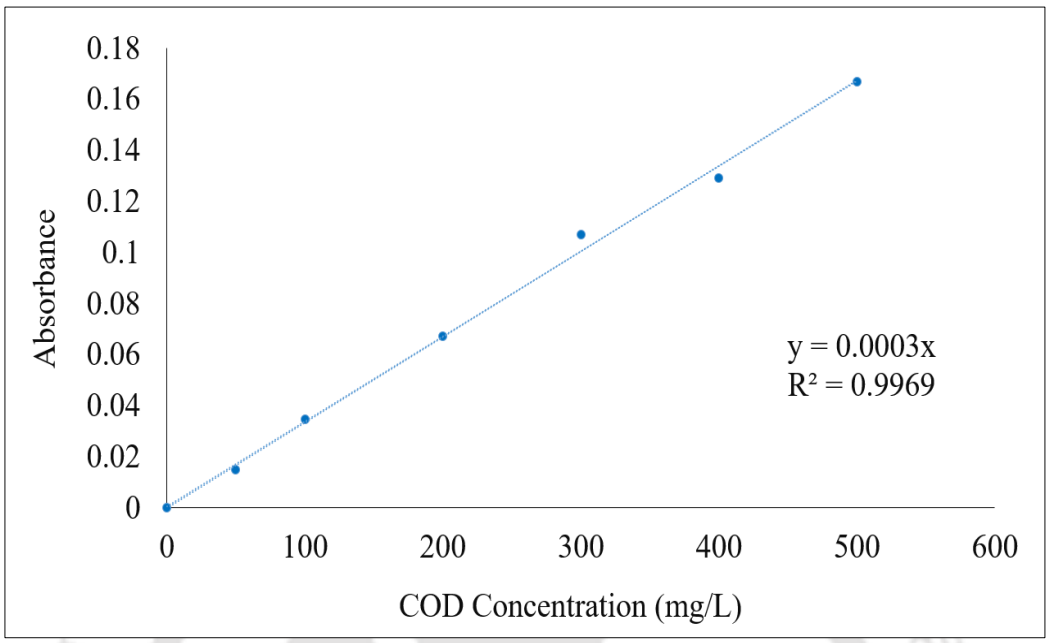

Figure A1. COD calibration curve

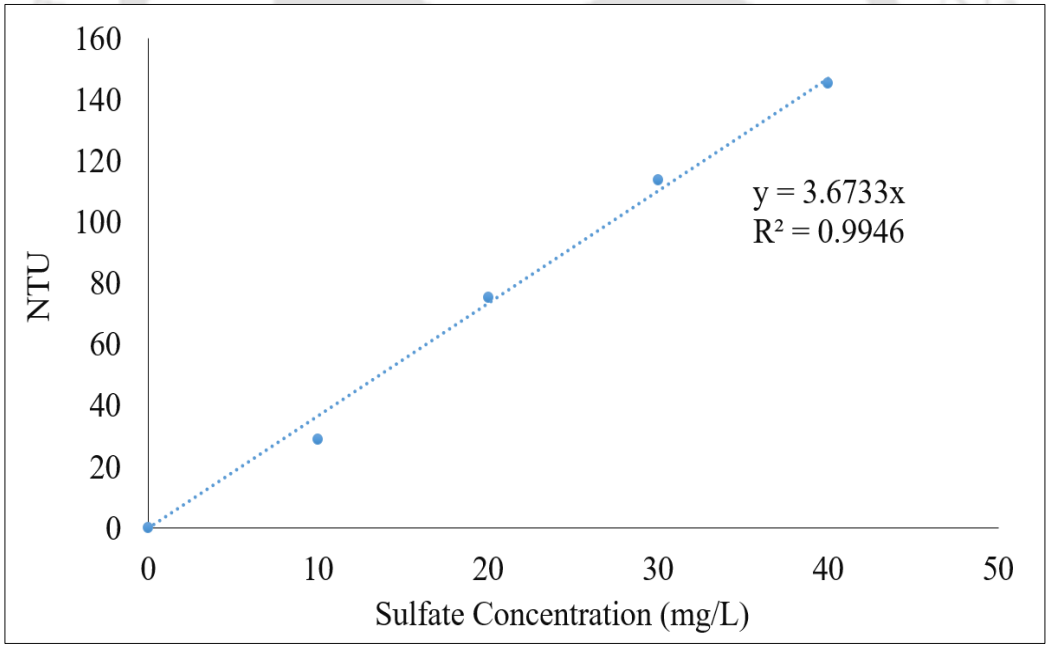

Figure A2. Sulfate calibration curve

TH-2881_146104040

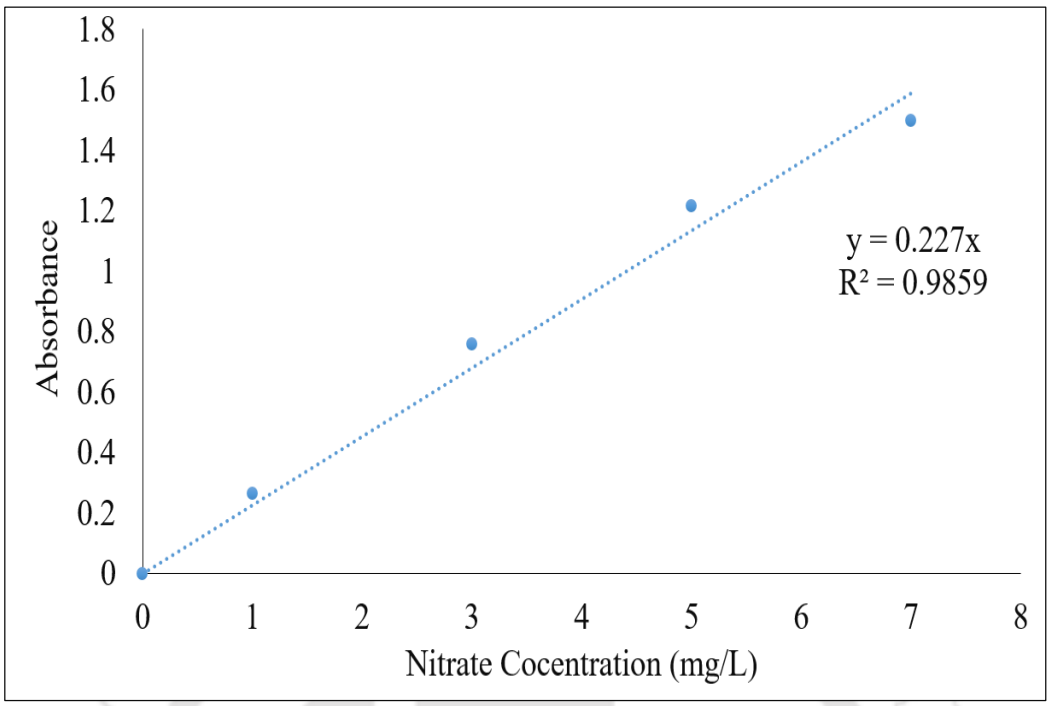

Figure A3. Nitrate calibration curve

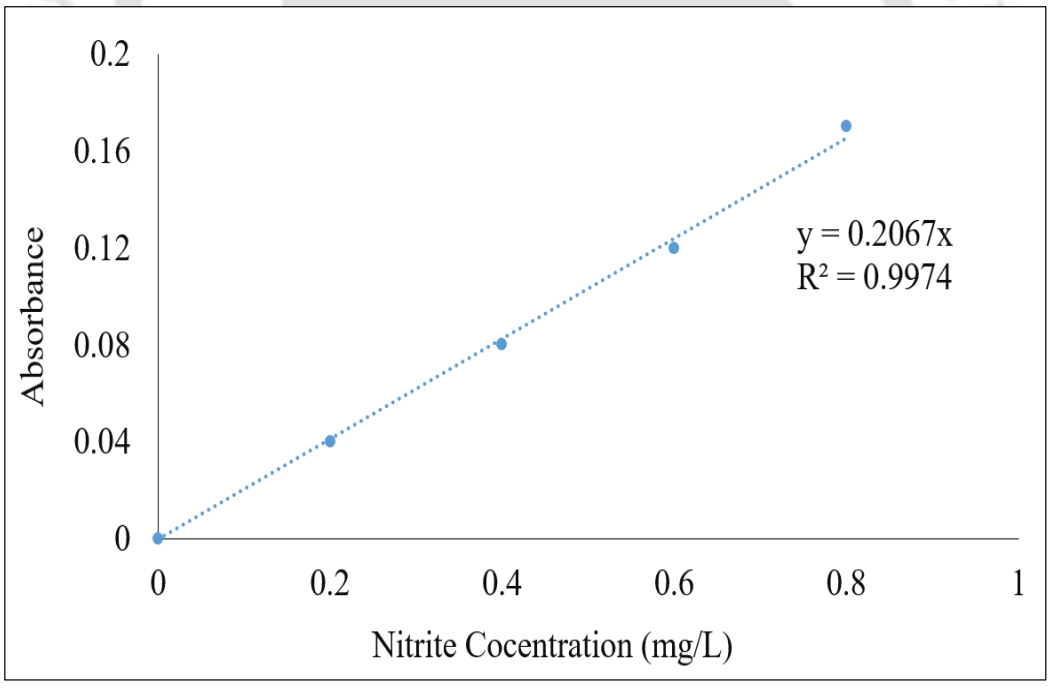

Figure A4. Nitrite calibration curve

TH-2881_146104040

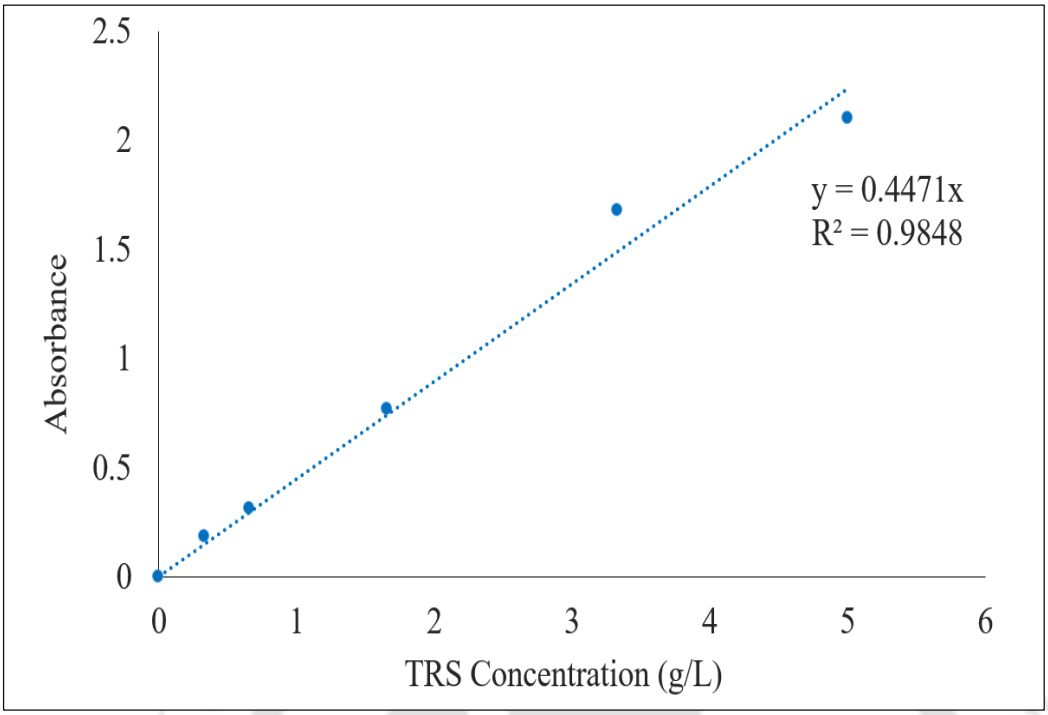

Figure A5. TRS calibration curve

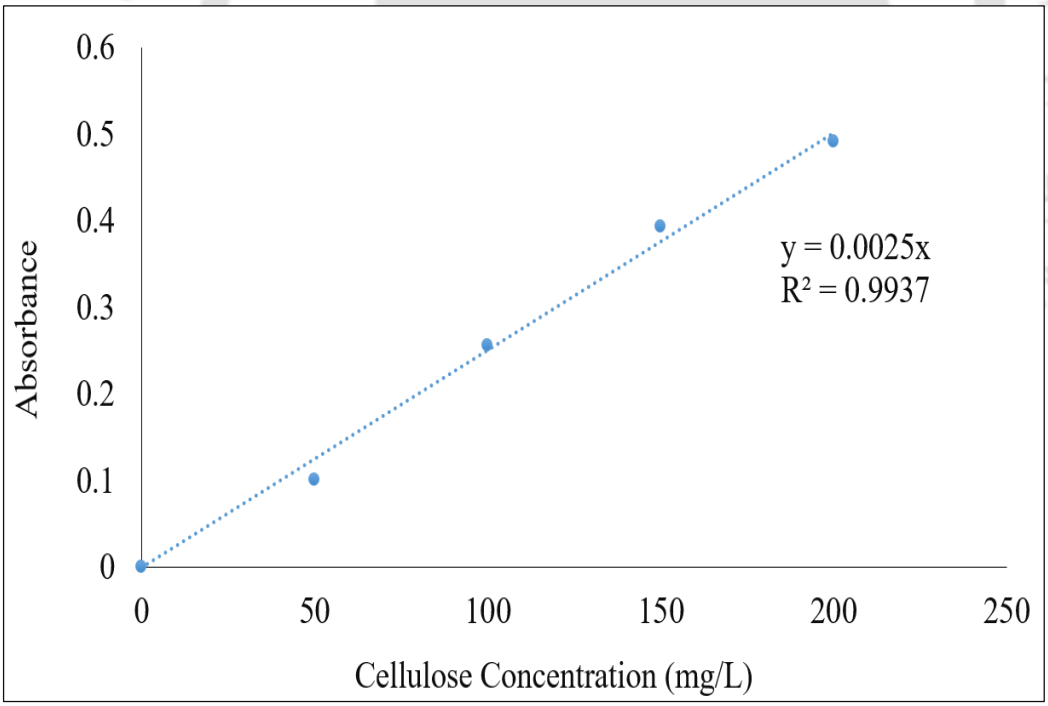

Figure A6. Cellulose calibration curve

TH-2881_146104040

TH-2881_146104040