Anaerobic Digestion of Water Hyacinth: Effect of Pretreatment and Co-Digestion on Biogas Production

Thesis submitted in partial fulfilment of the requirement for the award of the degree of

DOCTOR OF PHILOSOPHY

by

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I dedicate this thesis to my father and my mother for their blessings, never ending support and constant encouragement.

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STATEMENT

I hereby declare that the work presented in this thesis is to the best of my knowledge an authentic record of investigations performed by me under the supervision of Dr. Ajay Kalamdhad except as acknowledged in the text.

The matter presented in this thesis has not been submitted by me for the award of any other degree in this or any other institute.

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CERTIFICATE

This is to certify that the thesis entitled *"***Anaerobic Digestion of Water Hyacinth: Effect of Pretreatment and Co-digestion on Biogas Production**" submitted by Visva Bharati Barua (Registration No. 146152012) to the Indian Institute of Technology Guwahati for the award of the degree of Doctor of Philosophy is a record of bonafide research work carried out by her under my guidance and supervision. The thesis work, in my opinion has reached the requisite standard fulfilling the requirement for the award of the degree of Doctor of Philosophy. The work embodied in this thesis has not been submitted earlier to any other institute for the award of any other degree or diploma to the best of my knowledge and belief.

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ABSTRACT

Water hyacinth is considered to be the world's worst aquatic due to its phenomenal reproduction potential. It can grow within a week and cover an entire freshwater body by forming thick dense mats. These thick dense mats hamper the aquatic ecosystem alongwith the health, livelihood and recreation of human beings. Water hyacinth is difficult to manage as it can re-grow miraculously even if it is completely eradicated. Presence of cellulose and its availability in abundance makes water hyacinth an attractive feedstock for biogas production through anaerobic digestion. Biogas production from water hyacinth can effectively manage the aquatic weed as well as mitigate environmental pollution which is caused by burning of fossil fuel. But the presence of lignin in water hyacinth makes hydrolysis the bottleneck of anaerobic digestion thereby delaying the hydrolysis phase and producing decreased amount of biogas. Therefore pretreatment of water hyacinth is essential for accelerated hydrolysis period and enhanced biogas production. In this study, thermal, electrohydrolysis and biological (microbial) pretreatment were investigated to enhance solubilisation of water hyacinth. Hot air oven pretreatment of water hyacinth at 90ºC for 1h demonstrated the highest solubilisation and biogas production when compared to the other pretreatment techniques. Even mono-digestion of water hyacinth produces lesser amount of biogas therefore co-digestion of water hyacinth is essential to balance the nutrients and dilute the toxic inhibitors. During the BMP assay F/M ratio 2 was observed to be ideal for untreated water hyacinth whereas for the pretreated water hyacinth F/M ratio 1.5 was observed to be ideal. Co-digestion of water hyacinth was tried not only with cow dung as inoculum but also with other organic wastes (i.e., food waste, *Hydrilla verticillata*, banana peels) with and without pretreatment. Pretreatment and anaerobic co-digestion of water hyacinth not only enhanced the quantity of biogas production but also the quality of the produced biogas by increasing the percentage of methane content. At last, a novel anaerobic digester was designed, fabricated and operated in continuous mode. Mixing and the separation of stages in a digester during anaerobic digestion demonstrated enhanced biodegradation efficiency of the feedstock. But two stage anaerobic digesters are difficult to operate and require huge space. In addition to that, continuous high intensity mixing minimises biogas production. Based on these criteria, a novel type of two stage anaerobic digester was designed. Performance of this novel anaerobic digester in continuous mode utilising water hyacinth as the feedstock was evaluated. Initially, untreated water hyacinth whole plant

was fed in the digester followed by hot air oven pretreated water hyacinth and water hyacinth co-digested with food waste. The optimal OLR for untreated and pretreated water hyacinth was observed to be 3.8 kg COD/m^3.d while for co-digested water hyacinth the optimal OLR was 6.7 kg COD/m^3.d; demonstrating an average COD removal of 72.5, 82 and 77% for untreated, pretreated and co-digested water hyacinth respectively. The novel anaerobic digester proved its immense prospective in treating water hyacinth in untreated, pretreated or co-digested form. The design of the novel anaerobic digester is proficient in minimising the cost, difficulty in operation and manages space constraint when compared to the traditional two stage anaerobic digesters with mixing operation. The performance of the novel anaerobic digester's in distribution of the microorganisms, improving the digestibility of the feedstock, removing COD thereby enhancing the overall anaerobic digestion process is worth mentioning.

Keywords: Water hyacinth; anaerobic digestion; lignocelluloses; pretreatment; co-digestion; biogas; F/M ratio

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LIST OF ABBREVIATIONS

BMP	Biochemical Methane Potential
F/M	Food/Microorganisms
I/S	Inoculum/Substrate
sCOD	Soluble chemical oxygen demand
COD	Chemical oxygen demand
VFA	Volatile fatty acid
VS	Volatile solids
TS	Total solids
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
XRD	X-Ray Diffractogram
GC	Gas Chromatography
OLR	Organic loading rate
HRT	Hydraulic retention time
SRT	Solid retention time
APHA	American Public Health Association
NREL	National Rennewable Energy Limited
ADF	Acid Detergent Fibre
NDF	Neutral Detergent Fibre
HEPA	High Efficiency Particulate Air

CHAPTER 1
INTRODUCTION

This chapter consists of a brief discussion about the origin, spread and the problems associated with water hyacinth and its management through anaerobic digestion. The essential parameters necessary for successful anaerobic digestion have been also mentioned in this chapter concisely. Finally, incorporating, the objective, need and the scope of the present study.

1.1 OVERVIEW

The Brazil inherent water hyacinth (scientifically know as *Eichhornia crassipes*) is a floating aquatic weed; which is very invasive in nature. Water hyacinth a member of the family Pontederiaceae; was formerly utilised as a decorative plant due to its eye-catching lavender hued flower and round, shiny leaves (Bhattacharya and Kumar, 2010). Water hyacinth has immense ability of hasty growth and spread. Due to its phenomenal reproduction capacity, the weed can envelop an entire aquatic body by forming thick compact mats (Forrest et al., 2010). Water hyacinth reproduces both sexually (by seeds) and vegetatively (by budding and stolon production) (Ruiz et al., 2008). Daughter plants develop from the stolons and doubles within 6-18 days. A single inflorescence of water hyacinth with 20 flowers reproduces up to 3000 seeds and the number of seeds produced per square meter of vegetation fluctuates from 400 to 3400 (Cronk and Fenessy, 2001; Perez et al., 2011). The seeds either germinate in a few days or remain quiescent for 15-20 years (Obeid and Tag el Seed, 1976). The seeds of water hyacinth usually sink and stay quiescent until the phase of trauma strikes (Cronk and Fenessy, 2001; Perez et al., 2011). Physical or mechanical elimination of water hyacinth from the freshwater bodies is futile. As even after absolute eradication of this noxious pest, it reoccurs miraculously, again spreading and enveloping the whole freshwater body within few days. Thereby, suggesting the existence of quiescent seeds underwater which germinate during the phase of trauma (Malik, 2007). Owing to its astonishing reproduction potential, it has made itself omnipresent throughout the world causing massive annoyance both to mankind and the aquatic ecosystem. Human beings are the root cause behind making this South American origin pest available throughout the six continents of the world including Africa, Asia, Australia, North America, South America and Europe (Tellez et al., 2008). Livelihood, navigation, recreation, irrigation and power generation are being hindered by

these thick compact mats in the aquatic body (Epstein, 1998; Mehra et al., 1999; Malik, 2007; Wang and Calderon, 2012) (Fig. 1.1). Underwater biodiversity is also seriously threatened due to thick water hyacinth mats. These thick, compact mats intimidate the survival of aquatic organisms and eradicate the native aquatic plant communities by modifying the habitat. Water quality is also degraded by water hyacinth mats as it blocks the passage of sunlight underwater. Consequently, oxygen level underwater immensely diminishes leading to the death of fishes and other underwater living organisms. Owing to low oxygen condition, thick water hyacinth mats endows appropriate breeding places for animal and human disease carrying vectors. Ultimately, leading to an increase in malaria, encephalitis, schistosomiasis (bilharzia), filariasis (caused by a parasitic nematode worm), river blindness and probably cholera threatening animal and human health (Malik, 2007; Singh and Kalamdhad, 2015). Increased rate of evapotranspiration due to water hyacinth is another issue of concern in regions where water is already limited as it leads to loss of water (Singh and Kalamdhad, 2015). Water hyacinth dislocates indigenous flora and fauna by modifying the habitat. Water hyacinth mats impedes water flow thereby increasing sedimentation ultimately causing flood and soil erosion. The financial system of the countries concerned is gravely influenced as the thick compact water hyacinth mats intimidates agricultural production by obstructing irrigation canals and drainage systems. Therefore, water hyacinth is considered to be the world's worst aquatic weed (Dirar and El-Amin, 1988).

Fig. 1.1. Thick compact water hyacinth mats hampers the aquatic ecosystem as well as the livelihood and recreational activities of human beings.

Still water hyacinth is being utilised in the elimination of heavy metals from polluted aquatic bodies through bioremediation (Ingole and Bhole, 2003; Malik, 2007). But after

absorbing heavy metals from waste water, the noxious weed is disposed off in the land due to the absence of engineered landfill. Thus, when the weed decays the absorbed heavy metals gets mixed with the soil and greenhouse gases are directly released into the atmosphere creating a great risk to pollution abatement (Wang and Calderon, 2012). Therefore, utilisation of water hyacinth for anaerobic digestion will provide an effective management of the weed. As it is a never ending source of cellulose which is easily and abundantly available worldwide. The non-stop consumption of fossil fuel makes it essential to consider a gradual shift towards a bio-based economy due to its diminishing fossil reserves and environmental pollution. Water hyacinth is a deadly weed in an aquatic environment but a potential feedstock for the generation of renewable biogas through anaerobic digestion. Anaerobic digestion of water hyacinth seems to be a sustainable alternative to generate eco-friendly renewable source of energy, the biogas and an effective way to manage water hyacinth. Anaerobic digestion of water hyacinth for methane production is a more efficient method for energy generation compared to other biological and thermo-chemical conversion processes, such as cellulosic ethanol (Deublin and Steinhauser, 2011).

1.2 BACKGROUND

Anaerobic digestion is the process of biogas production, where particulate organic matter is broken down into their simple soluble forms with the assistance of robust, mixed culture microbial communities in the absence of oxygen (Khanal, 2008); aiding in the conversion process of waste to energy. Benefit of anaerobic digestion process is the capture of methane emissions and utilising the methane for energy production; or else self putrefaction of biomass in landfills or other open environment would contribute to global warming and environmental pollution. The consortium of microorganisms performs synergistically to disintegrate complex compounds into their simple soluble forms and finally into methane. It is an effective, eco-friendly, low cost and low energy technology suitable for converting water hyacinth into energy rich biogas (O'Sullivan, 2010; Kondusamy and Kalamdhad, 2014). Methanogenesis is a multi-step phenomenon accomplished by synchronised action of an assortment of mesophilic bacterial community and consists of four stages i.e., hydrolysis, acidogenesis, acetogenesis and methanogenesis. The four stages are interconnected and so are the robust microbial communities engaged in the process; where the products of one phase act as the substrate for the next phase. The effectiveness of the process can be improved by upholding equilibrium between the hydrolysis, acidogenesis, acetogenesis and methanogenesis

stage. The acidogens develops rapidly and are less susceptible to environmental changes. The most favourable range of pH for the satisfactory performance of the acidogenic bacteria is 4.5 to 6.5. In case of rapid hydrolysis, build up of intermediate products is generally witnessed if acetogenesis is not rapid enough to ensure proficient consumption of the substrates to present them for methanogenesis stage, biogas production is reduced. The last stage of anaerobic digestion; methanogenesis is extremely vulnerable to upsets due to the instability between the acidogenic and methanogenic microorganisms participating in the anaerobic digestion process. Methanogenic bacteria are very sensitive to pH and the optimum pH for their operation is 6.5 to 7.5. Stabilisation of the process cannot occur without tangible contact of the microorganisms with the substrate.

The most effective way of ascertaining contact between the microorganisms and the substrate inside the anaerobic digester is through mixing. Mixing can be achieved either mechanically (mixer or stirrer) or manually. Incase of mechanical mixing the contents may be stirred continuously or intermittently. Better chemical oxygen demand (COD) removal efficiency and increased gas production can be achieved by intermittent mixing.The microorganisms directly take on the charge of the anaerobic digestion process by themselves; however, the operational conditions such as feedstock, inoculum, temperature, pH, essential trace nutrients and toxicants can play a chief responsibility in modifying reaction rates of the individual sub-processes. Various substrates degrade at varying time and produce different quantity of methane; which basically depends on the bio-degradability and methane potential, the available carbon and nutrients, and the moisture content of each substrate. Rigid solids, typically requires a prolonged period to assimilate than feedstocks that are easily soluble. Operational factors comprises of the quantity and type of substrate added to the anaerobic digester. The process also depends on sustaining the population of the microorganisms and organic loading rate in the anaerobic digesters, whether operating in a batch or continuous reactor. There are also differences in the operational factors and environmental conditions of the anaerobic digester. It is crucial to determine the total solids (TS) and volatile solids (VS) content of the substrate, the best retention time and to provide mixing. Mixing is also a significant feature in an anaerobic digestion process. The objective of mixing is to maintain the robust microorganisms in close contact with the substrate and nutrients. Mixing also shuns the development of a suspending layer, which can reduce the quantity of biogas percolating out of the slurry. Mixing will promote the disintegration of VS and boost biogas production. Environmental conditions consist of the temperature and pH of

the anaerobic digester, as well as concentration of volatile fatty acids (VFA), ammonia, salt, and cationic ions. A simple and efficient anaerobic digester for the anaerobic digestion of fibrous lignocellulosic substrates is essential as the use of fibrous feedstocks clogs the digester. Speece (1996) has reported that the environmental temperature has a significant effect on the anaerobic microbial systems, influencing the metabolic rate, ionisation equilibria, substrate solubility and bioavailability of nutrients. Higher temperature influences the activity of hydrogenotropic methanogens in the anaerobic process and enriches hydrogen producing bacteria and spore forming bacteria (Cecchi et al., 1989). Methanogenic bacteria have an inclination for neutral pH condition and accumulation of VFA can prevent biogas production; this occurs when disproportionate quantity of organic matter is added, a toxic compound is added, or there is an sudden change in temperature. Toxic compounds ceasing biogas production incorporates oxygen, antibiotics, cleaning chemicals, inorganic acids, alkali and alkaline earth salt toxicity, heavy metals, sulphides and ammonia. Another rationale for process failure might be due to the inequilibrium between the acetogenic bacteria and methanogenic bacteria. Generally, the rate of acid formation and methane production should be equivalent. When the methanogenic bacteria are not capable to sustain with the fermenting bacteria, the anaerobic digester becomes acidic; also known as “sour.” Careful amendment of the pH, temperatue, mixing, solids retention time (SRT), among several other operating parameters during anaerobic digestion process can improve methane production. Anaerobic digestion of water hyacinth also produces a nutrient rich fluid which can be used as a liquid fertilizer (Valo et al., 2004) while biogas can replace all the non-renewable sources of heat and power generation (Ferrer et al., 2010). But the presence of recalcitrant lignocellulose as a basic component in the cell wall of water hyacinth decreases the biodegradability of the feedstock ultimately making hydrolysis the rate limiting step of traditional anaerobic digestion process and reducing biogas production. The tough lignin network works as an adhesive firmly gripping cellulose and hemicellulose. The rigid network of lignin and hemicellulose interconnected by covalent bond creates robust natural barricade for the cellulose molecules with the enfolding surrounding, hence opening up the lignocellulosic complex is vital for enhanced biogas production (Noike et al., 1985).

Cellulose is a linear polysaccharide polymer strongly linked by ß-1, 4 glycosidic bonds whereas hemicellulose is a branched heterogenic polysaccharide of various pentoses, hexoses and acids. Lignin is a three dimensional amorphous phenolic polymer composed

of coniferyl, sinapyl and p-coumaryl alcohol alongwith hydroxyl, methoxyl and carbonyl functional groups (Zheng et al., 2014). Hydroxylic groups present in the cellulose chains interlink the hydrogen bonds with the other components. Cellulose, hemicellulose or lignin is bound together by hydrogen bond. Lignin binds to cellulose/hemicellulose by covalent bonding such as benzyl-ether, benzyl-ester, and phenyl-glycoside bonds, forming lignin–carbohydrate complexes (Kim et al., 2011). The phenylpropane (C9/C6C3) units in lignin are attached by C-C and ether (C-O-C) linkages. This three dimensional (3D) lignocellulosic matrix in plants acts as a shield to both abiotic and biotic aggression preventing easy degradation. Slow hydrolysis of water hyacinth is the main reason behind the successive inadequate acidogenesis and methanogenesis phase. Pretreatment of water hyacinth is necessary to ruptue the lignocellulosic complex of the noxious weed thereby releasing the sugars stored within the cellulosic fibres embedded in the hetero-matrix of plant cell wall. Once the lignocellulosic complex is torn open, it is easier for the microorganisms to access the cellulose leading to the acceleration of the hydrolysis phase. Conventionally, the whole lignocellulosic feedstock is dried, chopped, ground and fed into an anaerobic bioreactor to convert complex carbohydrates and organic matter into energy-rich biogas (Weiland, 2010). There are various number of other type of pretreatment studies stating the use of physical pretreatment, chemical pretreatment (Patil et al., 2011), ionic liquid pretreatment (Gao et al., 2013a), thermal pretreatment (Putra et al., 2014) and thermochemical pretreatment (Lin et al., 2015) for enhancing biogas generation from water hyacinth. Physical pretreatment i.e., cutting, chopping and drying of water hyacinth was mostly studied followed by chemical (acid/alkali) pretreatment. Among thermal pretreatment, microwave pretreatment is the most studied pretreatment technique.

Anaerobic co-digestion of water hyacinth with other organic wastes is also an efficient measure to enhance methane yield as it dilutes toxic intermediates, balances the nutrients and enhances the synergistic activities between the various organic substrates by providing a more stable environment inside the reactor. But a suitable mixing ratio or inoculum to substrate (I/S) ratio or food to microorganisms (F/M) ratio is necessary to ensure the stability of the overall anaerobic digestion process. Both I/S and F/M ratio are VS dependent, but the ratios are inverse of each other.Inappropriate mixing ratio may sometimes lead to inhibition due to the accumulation of intermediate products and may produce negligible or lesser quantity of biogas. Bio-chemical methane potential (BMP) tests are also useful in determining the potential ideal F/M ratio. BMP test acts as an

index for determining the potential of the substrate for biogas production (Chynoweth et al., 1993) and estimating the time required for complete degradation of the substrate.

In recent years, lignocellulosic materials have gained considerable importance owing to their renewability, recyclability and sustainability. Their physicochemical and biological characteristics make them a substrate of enormous industrial and biotechnological value to develop a range of value added products. Besides biogas production some potential applications include but not limited to the generation of biofuels (Bergier et al., 2012), composting (Singh and Kalamdhad, 2015), alternative sources for pulp and paper industry (Goswami and Saikia, 1994), animal feed and nanoparticle synthesis (Mochochoko et al., 2013). The problem with growing crops for biofuel is that they take up land that could be used for growing food. In a world with a population of around 7 billion and that is already short on food, there will necessarily be a tradeoff between food crop and biofuel feedstock. While composting, does not provide us with energy. Plant mediated nanoparticle synthesis has decreased rate of synthesis as plants produce low yield of proteins. Therefore, utilisation of water hyacinth for anaerobic digestion seems to be sustainable alternative as it produces biogas and digestate which can be used as fertilizer.

1.3 OBJECTIVE OF THE RESEARCH WORK

The main objective of the research work is anaerobic digestion of freshly pulverised whole water hyacinth plant. The purpose is to find the best strategy for enhancing biogas production while accelerating the process and its efficiency by optimising ideal F/M ratio performing different pretreatment techniques, co-digestion analysis, developing a novel anaerobic digester and its analysis in continuous mode. The scope of the thesis is limited to;

1. Initial characterisation of water hyacinth and BMP (1 L capacity) test for different food to microorganisms (F/M) ratio with cow dung as inoculum followed by batch study (20 L capacity) with the optimised F/M ratio.
2. Pretreatment studies i.e., thermal (hot air oven, microwave, autoclave, water bath), electrohydrolysis and microbial followed by BMP (1 L capacity) and batch studies (20 L capacity). Compositional analysis of the pretreated and untreated water hyacinth to examine the changes undergone in the rigid lignocellulosic complex.
3. Co-digestion study of water hyacinth with other organic substrates (food waste, hydrilla and banana peels) with and without pretreatment followed by BMP (1 L capacity) and batch studies (20 L capacity).

4. Design, fabrication and operation of lab scale continuous reactor (20 L) for anaerobic digestion of untreated water hyacinth, pretreated water hyacinth and co-digested water hyacinth.

1.4 NEED OF THE RESEARCH WORK

The ever-growing demands of energy and rapid consumption of non-renewable fossil fuel along with the need to control water hyacinth emphasise the significance of the need of a sustainable alternative energy source. Thus anaerobic digestion can be considered to be the unsurpassed route for transforming waste to bioenergy. Utilisation of renewable biogas as a source of energy possibly will replace fossil fuels, minimise the emission of greenhouse gases, trim down the depletion of resources, reduce the reliance on external sources of energy and cut down cost. This research work aims to explore the potential of anaerobic digestion of water hyacinth and compare the impact of different pretreatment and co-digestion techniques for enhanced methane production. The production of eco-friendly biogas from water hyacinth can be the solution, both to its management and production of renewable source of energy. But, the slightest presence of lignin, the recalcitrant compound in the water hyacinth's cell wall, limits the production of biogas making hydrolysis a rate limiting step in anaerobic digestion. Pretreatment aims at accelerating, the hydrolysis stage of anaerobic digestion as well as enhances the biogas yield. While anaerobic co-digestion works in synergism to balance the nutrients and dilute the toxic inhibitory products. Thus, maintaining the stability of the process. Even designing an easy to handle novel reactor with improved biogas production especially for the anaerobic digestion of fibrous lignocellulosic substrates is essential as the use of fibrous feedstocks clogs the digester.

1.5 SCOPE OF THE PRESENT RESEARCH WORK

The scope of the present research work is to enhance biogas production from freshly pulverised water hyacinth whole plant by optimising ideal pretreatment technique, F/M ratio and co-digestion techniques. Alongwith, the operation and feasibility study of anaerobic digestion of water hyacinth in a lab scale continuous digester. Initially characterisation of the water hyacinth collected from Amingaon industrial area and cow dung from Amingaon village near Indian Institute of Technology (IIT) Guwahati campus was performed. After the characteristics of the substrate and the inoculum were determined, BMP assay (1 L capacity) for untreated water hyacinth and the water hyacinth pretreated with various techniques at their ideal condition were conducted with cow dung in varying F/M ratio. Effect of different pretreatment techniques on the

hydrolysis of water hyacinth with respect to pH, soluble chemical oxygen demand (sCOD), VFA, VS and its characterisation using FESEM, FTIR, XRD was studied to determine the best pretreatment technique using fresh water hyacinth whole plant alongwith the variation in the composition of the substrate was analysed. Compositional analysis of the pretreated and untreated water hyacinth was performed to observe the changes undergone in the recalcitrant lignocellulosic complex. Meanwhile, co-digestion study of water hyacinth with other lignocellulosic and non-lignocellulosic substrates with and without pretreatment was also studied. Based on the above studies, batch reactor (20 L capacity) study was conducted with the optimised ideal F/M ratio, pretreatment and co-digestion technique. Finally, design, fabrication and operation of a novel lab scale continuous anaerobic reactor with the untreated, pretreated and co-digested water hyacinth for biogas production was performed. The organic loading rate (OLR) in the novel anaerobic digester was gradually increased until the drop of pH was observed. Thereby presenting the ideal conditions and treatment required for the anaerobic digestion of water hyacinth for enhanced biogas production.

1.6 THESIS ORGANISATION

The first chapter of the thesis consists of a brief discussion about the origin, spread and the problems associated with water hyacinth and its management through anaerobic digestion. The essential parameters necessary for successful anaerobic digestion have been also mentioned in this chapter concisely. Finally, incorporating, the objective, need and the scope of the present study. The organisation of the thesis has been incorporated in the flowchart (Fig. 1.2).

The second chapter covers the detailed literature review on the anaerobic digestion of water hyacinth. The use of various inoculum, pretreatment techniques, co-digestion, anaerobic digesters and their effect on biogas production and the stability of the process.

Various experimental studies were conducted to accomplish the objectives of the present research work. The research was conducted in different phases utilising water hyacinth as a feedstock and cow dung as an inoculum. The various experimental methodologies followed and the materials/equipments required for conducting the analyses has been incorporated in the third chapter.

The experiment starts with the initial characterisation of the water hyacinth. The fourth chapter deals with the effect of different pretreatment techniques i.e., thermal, electrohydrolysis and biological on the solubilisation of water hyacinth during anaerobic digestion in order to reduce the hydrolysis time period and enhance biogas generation.The

ideal conditions required to achieve the highest amount of solubilisation by each type of pretreatment was studied.

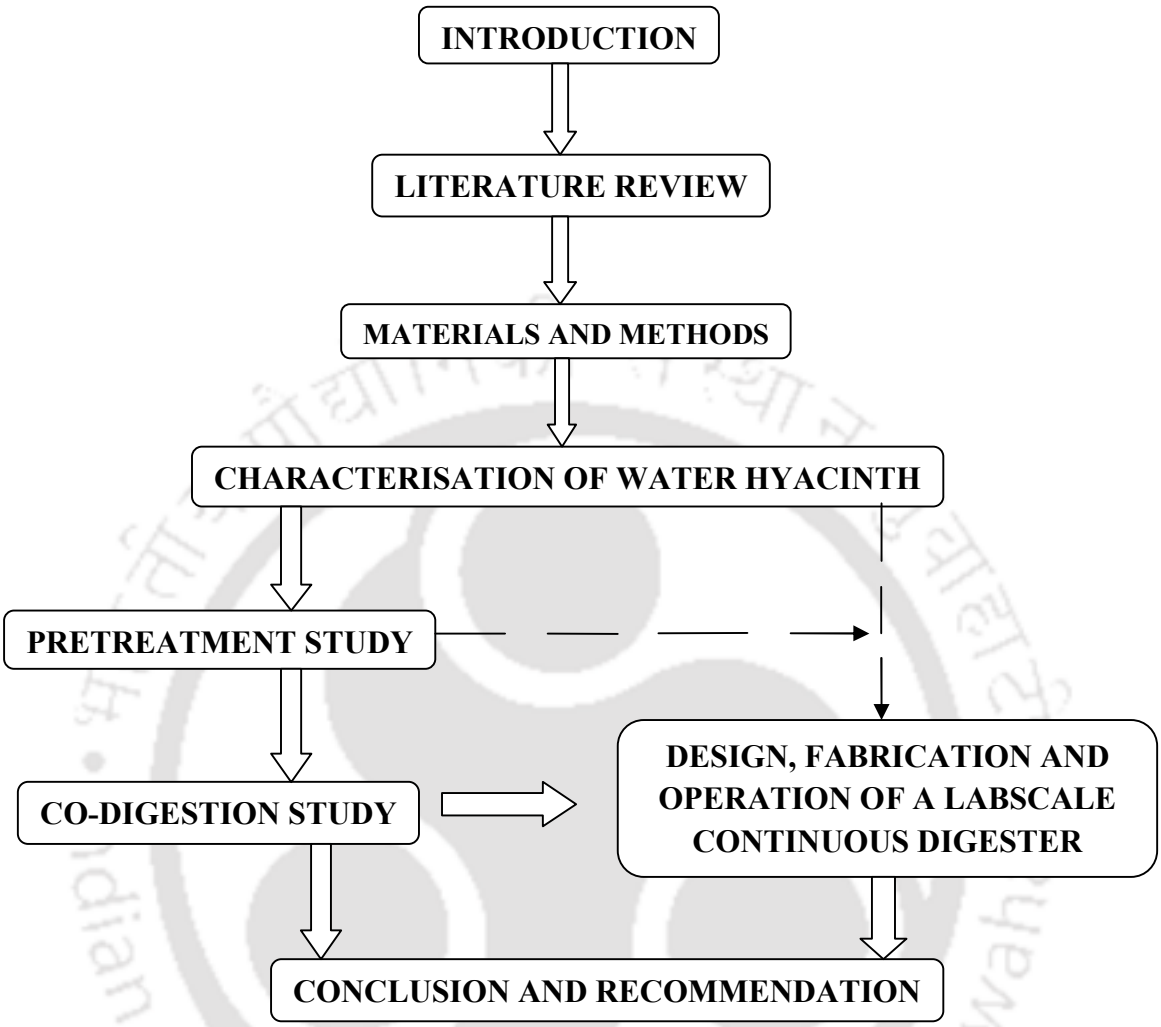


Fig. 1.2. Flowchart illustrating the organisation of the thesis

After the highest amount of solubilisation achieved by pretreatment was determined the BMP test of the substrate was conducted after pretreating the water hyacinth in the optimised ideal condition. The fifth chapter deals with the BMP test of water hyacinth before and after various kinds of pretreatment and their scaled up batch studies (20 L). Methane potential of water hyacinth was determined for various F/M ratios in order to optimise the ideal F/M ratio.

The sixth chapter deals with the effect of anaerobic co-digestion of water hyacinth with various organic wastes as a co-substrate with and without pretreatment and their scaled up batch studies. Methane potential of the various anaerobic co-digestion studies was determined for various F/M ratios in order to optimise the ideal F/M ratio.

The seventh chapter deals with the design and operation of a novel type of biogas digester in continuous mode for water hyacinth in three different ways. For, the first experiment freshly pulverised water hyacinth whole plant was used as a feedstock; then hot air oven pretreated water hyacinth was used followed by co-digestion of water hyacinth and food waste.

Finally the thesis ends with the conclusion attained from all the objectives and recommendation for future work.

CHAPTER 2
LITERATURE REVIEW

This chapter covers the detailed literature review on the anaerobic digestion of water hyacinth. The use of various pretreatment techniques, co-digestion, inoculum, anaerobic digesters in the previous available literatures and their effect on biogas production, stability of the process has been studied.

2.1 ANAEROBIC DIGESTION PROCESS AND BIOCHEMICAL REACTIONS

Anaerobic digestion is a multistep biological route based on a reduction process where a community of robust microorganisms executes in a stable, self-regulating steady-state in the absence of oxygen transforming complex organic matter into a mixture of methane, carbon dioxide and other gases. Biogas is a colourless gas that smoulders with clear blue flame similar to that of liquid petroleum gas. The biogas produced during anaerobic digestion is a mixture comprising mainly of methane ($CH_4 \approx$ 50-75%), carbon dioxide ($CO_2 \approx$ 25-50%) and small traces of hydrogen sulphide (H_2S), hydrogen (H_2), nitrogen (N_2), carbon monoxide (CO), oxygen (O_2), water vapor (H_2O) or other gases and vapors of various organic compounds. Methane formation in anaerobic digestion involves four different steps: hydrolysis, acidogenesis, acetogenesis and methanogenesis. Fig.2.1 represents the steps involved during the anaerobic digestion process. Microorganisms from two biological kingdoms, the Bacteria and the Archaea perform digestion of organic substrate in stern anaerobic conditions (Dugba and Zhang, 1999). The microorganisms that steer anaerobic digestion are basically hydrolytic bacteria, acid producers (acidogens and acetogens) and methane producers (methanogens). These groups of microorganisms vary physiologically, have altered growth rates and are vulnerable to operational conditions (Ruiz and Flotats, 2014). A diverse microbial community is required to ensure that the suitable exoenzymes and endoenzymes are available for degradation of the substrate. The relative profusion of microorganisms within an anaerobic digester often is more than 10^{16} cells per milliliter. This population of microorganisms comprises mainly of saccharolytic bacteria (approximately 10^5 cells/mL) and methane forming methanogenic bacteria (approximately 10^8 cells/mL) (Gerardi, 2003). Trace inorganic elements such as iron (Fe), managanese (Mn), nickel (Ni), cobalt (Co) and zinc (Zn) in desired concentration is necessary to activate the anaerobic digestion process. Performance of the anaerobic digester is also reliant on the microbial population within

the digester. Hence, it is necessary to sustain ample quantities of fermenting bacteria and methanogenic bacteria and supply the microorganisms with the essential nutrients.

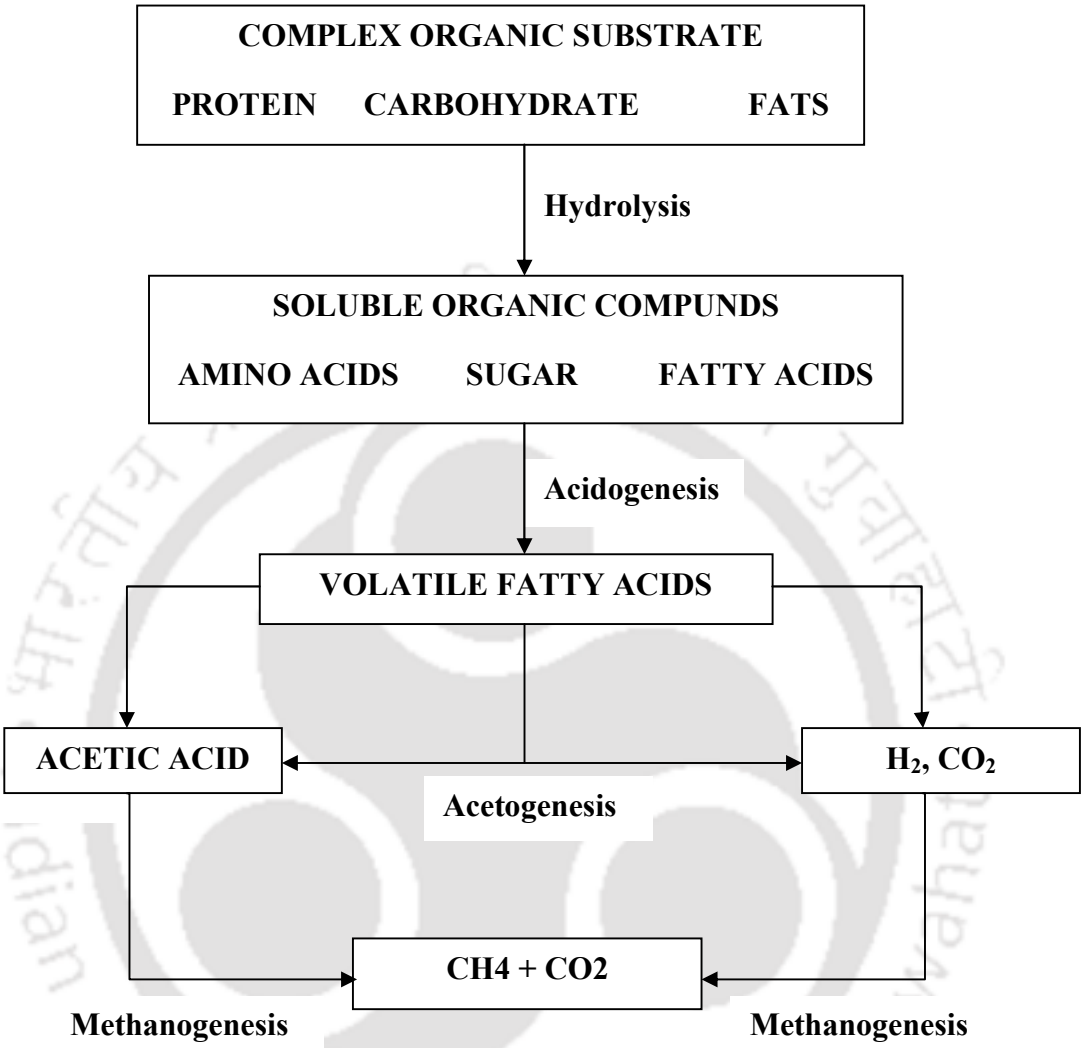


Fig. 2.1. Steps involved in anaerobic digestion

In general, hydrolysis is a chemical reaction where the disintegration of water (H_2O) molecule transpires to form H^+ cations and OH^- anions. Hydrolysis is the first step of anaerobic digestion engaging the enzyme-mediated conversion of complex organic compounds and higher molecular mass compounds i.e., lipids, polyssacharides, proteins, fats, nucleic acid into soluble organic compounds i.e. into monosaccharides, amino acids and other simple organic compounds. Hydrolysis is executed by stern anaerobes (i.e., bacterides, clostridia) and facultative bacteria (i.e., streptococci) (Christy et al., 2004). During hydrolysis, complex organic molecules are excessively huge to be directly devoured by microorganisms as food source. In order to hydrolyse complex organic molecules, microorganisms ooze out extracellular enzymes (cellulase, cellobiase, xylanase, amylase, protease, lipase), which solubilise the complex molecules into simpler

forms that the microorganisms can uptake and utilise as a source of energy and nutrition. For example, microorganisms ooze out enzymes that hydrolyse either sugar or protein. Microorganisms that hydrolyse sugars are called saccharolytic, while those that hydrolyse proteins are called proteolytic. Usually the conversion of cellulose and hemicellulose is more time-consuming than the conversion of protein. The chief set of cellulose degrading anaerobic microorganisms are *Bacterioides succinogenes, Clostridium lochhadii, Clostridium cellobioporus, Ruminococcus flavefaciens, Rumino coccusalbus, Butyrivibrio fibrosolvens, Clostridium thermocellum, Clostridium stercorarium* and *Micromonospora bispora.* The hemicellulose degrading chief bacteria found in the rumen are *Bacterioidesruminicola. B. fibrisolvens, R. flavenfaciens,* and *R.albus* (Palmisano and Barlaz, 1996). The rate of putrefaction during the hydrolysis phase depends immensely on the nature of the substrate. Hydrolysis is comparatively a sluggish route and it usually restricts the rate of the overall anaerobic digestion process. Biopolymers are converted into simple soluble monomers through enzymatic hydrolysis. Eq. (2.1) illustrates the hydrolysis reaction where organic substrate is hydrolysed into simple sugar, glucose. Hydrolysis of cellulose yield glucose, hemicellulose degradation results in monosaccharides such as xylose, glucose, galactose, arabinose and mannose (Lai et al., 2001).

$$nC_6H_{10}O_5 + n\,H_2O \xrightarrow{Hydrolysis} nC_6H_{12}O_6 \ldots. (2.1)$$

In general, during this phase, simple sugars, fatty acids and amino acids are converted into organic acids and alcohols. The second step of the anaerobic digestion process is acidogenesis or acidification, a process that consequences in the conversion of the hydrolysed products into simple molecules with a low molecular weight, like volatile fatty acids (VFA) (e.g. acetic acid, propionic acid and butyric acid), alcohols, aldehydes and gases like CO_2, H_2 and NH_3. *Streptococcus, Lactobacillus, Bacillus*, *Escherichia coli*, *Salmonella* assists in the conversion of hydrolysed products into VFA. Acidogenesis is influenced by an extremely different group of microorganisms, the mainstream of which are firmly anaerobic. Fortunately for these strict anaerobes, there are bacteria present that will utilise oxygen whenever it is accessible. For the subsistence of these bacteria, it is indispensable to eradicate oxygen that might be introduced into the digester. The acidogenic bacteria are competent of metabolising organic matter to a very low pH of 4. While in the third step of anaerobic digestion, acetogenesis, the products of the

acidogenic phase are devoured as substrates for the other microorganisms and converted into acetic acids, hydrogen, and CO_2 by acetogenic bacteria. The conversion from organic substrate to organic acids; drops the pH of the digester; this circumstance is constructive for acidogenic and acetogenic bacteria to flourish. The first three steps of anaerobic digestion are often grouped together as acid fermentation. Eq. (2.2) demonstrates the formation of volatile fatty acid.

$$nC_6H_{12}O_6 \xrightarrow{Acid\ forming\ bacteria} 3n\ CH_3COOH \quad .....(2.2)$$

Methanogenesis is the most significant step in the entire anaerobic digestion process. During methanogenesis, the final step of the anaerobic digestion process the products of the acid fermentation (mainly acetic acid) are transformed into methane and carbon dioxide by methanogenic bacteria under strict anaerobic condition. In each of the four sequential stages, the catabolic reactions described above develop together with anabolic activity. The energy liberated during the reactions is moderately used for the synthesis of anaerobic bacteria. As a result, a large fraction of the digested organic matter is converted into biogas (85-95%). These organic acids primarily acetic acid form the substrate for the third-stage. In the third stage, methane production takes place in two ways; either by break down of acetic acid molecules to generate carbon dioxide and methane or by reduction of carbon dioxide with hydrogen by hydrogenotrophic methanogens or by other bacterial species as illustrated in reactions (2.3), (2.4), (2.5) and (2.6) (Lin et al., 2009). Hydrogenotrophic methanogenesis is the most general metabolic pathway where CO_2 and H_2 are converted to methane. Besides H_2, most of the hydrogenotrophs can also utilise formate as the major electron donor. In the second type of methanogenesis, the aceticlastic methanogenesis, acetate is directly converted to methane. The carboxyl-group of the acetate is oxidized to CO_2 whereby the methyl-group is reduced to methane (Ferry, 1997). According to Archives of Environment Protection, the most common methanogenic bacteria are *Methanobacterium, Methanothermobacter, Methanobrevibacter, Methanosarcina,* and *Methanosaeta*.

$$CH_3COOH \xrightarrow{Methane\ forming\ bacteria} CH_4 + CO_2 \quad ..... (2.3)$$

$$CO_2 + 4H_2 \xrightarrow{Reduction} CH_4 + 2H_2O \quad ..... (2.4)$$

Similarly CO_2 can be hydrolysed to carbonic acid and to methane.

$$CO + H_2O \xrightarrow{Hydrolysis} H_3CO_3 \quad ..... (2.5)$$

$$4H_2 + H_2CO_3 \xrightarrow{Reduction} CH_4 + 3H_2O \quad ..... (2.6)$$

During hydrogenotrophic methanogenesis, CO_2 is reduced to methane by special coenzymes (methanofuran, tetrahydromethanoptein, coenzyme M) through the formyl, methylene and methyl levels. The key enzyme of this process is the methyl- coenzyme M reductase which reduces methyl-coenzyme M to methane whereby the oxidized coenzyme M forms a heterodisulfide complex M heterodissulfide complex with coenzyme B (Duin and Mckee, 2008). One group of acetotrophic methanogens, the *Methanosarcinaceae*, uses the acetate kinase phosphotransacetylase system for activating acetate to acetyl-coenzyme A. *Methanosaetaceae* the second group of acetate converts, adenosine monophosphate forming acetyl-coenzyme A synthatase for this reaction (Smith and Ingram-Smith, 2008). The H_2 consuming methanogens (*Methannospirillum hungatei*, *Methanoculles receptaculi*) grows comparatively faster than the aceticlastic methanogens (*Methanosarcina thermophila*). The maximum doubling time for hydrogenotrophic methanogens has been assessed to be 6 h when compared to leisurely growing (2.6 days) aceticlastic methanogens. H_2 utilising methanogens are more defiant to environmental changes than aceticlastic methanogens. Methanogenesis is the rate controlling segment of the anaerobic process (Davis and Cornwell, 1998). The CO_2 present in biogas not desirable. They are eliminated for optimum performance of biogas as fuel. CO_2 is eliminated by passing the gas into lime water which turns milky due to formation of calcium carbonate.

2.2 PARAMETERS INFLUENCING ANAEROBIC DIGESTION

A variety of parameters infuences the process of anaerobic digestion thereby ultimately producing biogas. The chief parameters influencing anaerobic digestion are discussed in detail in the paragraphs below.

2.2.1 pH

pH is a fundamental parameter influencing the performance of anaerobic digestion on biogas production. The pH in the anaerobic digester affects the growth of anaerobic methanogens. Methanogenesis occurs at a pH of 6.5-7.5. The distribution of ionised and non-ionised forms of sulfide and ammonia in the digester is also governed by the pH. At lower pH range, non-ionized sulfide (H_2S) predominates the system, whereas, at higher pH non-ionized ammonia (NH_3) dominates over NH_4^+ which inhibits the bacterial activity. Fluctuation in pH influences on H^+ concentration which has direct impact on bacterial growth development and consequently on anaerobic digestion process too. So, it is necessary to correct the unbalanced and low pH condition in the digester.

2.2.2 Temperature

Anaerobic microorganisms are highly susceptible to changes in the temperature. As a result the process of biogas production is strongly dependent on temperature. It is essential to maintain a stable temperature for the growth of anaerobic bacteria. Biogas production occurs at psychrophilic (< 20°C), mesophilic (20-40°C) and thermophilic (>40°C) temperature. Typically, mesophilic methanogenesis occurs at 20-45°C with an optimal temperature of 35°C and thermophilic methanogenesis at 50-65°C with an optimal temperature of 55°C (Grosser et al., 2017, Hagos et al., 2017). Decreased temperature might lead to an increase in VFA concentration which can influence the metabolic activity of the methanogens. Hence decrease in temperature can have radical aftermath on the overall process (Oliveira et al., 2014). While increased temperature leads to increased metabolic growth rate and higher ammonium toxicity. Rajagopal et al. (2013) reported that low temperature anaerobic digestion process is beneficial for the biodegradation of compounds with increased nitrogen content owing to minor concentration of free ammonia nitrogen. While Sung and Liu (2003), observed that thermophilic anaerobic digestion resulted in chronic inhibition of acetoclastic methanogens. They also observed that acclimation was extremely time-consuming for thermophilic bacteria due to ammonium toxicity. Thus, at thermophilic condition sharp decline in methanogens was observed when compared to mesophilic condition.

2.2.3 Nutrients and trace elements

Every microorganism-mediated route for organic waste treatment necessitates the need for nutrients and trace elements in proper concentration for their better growth. Nutrients and trace elements are indispensable constituent of a microbial cell. The balance of nutrients is vital for all biological processes. Nutrients and trace elements offer ideal physico-chemical condition for the growth and synthesis of microorganisms. Absence of essential nutrients and trace elements hampers the anaerobic biodegradability of the organic substrate. If a few nutrients are absent or less in supply, the process may become rate limiting. In some cases the lack of particular nutrients may reduce the stability of biological processes as the microorganisms are unable to grow and multiply at optimum rate.

2.2.4 Toxicity

The process of anaerobic digestion is sometimes inihibited by the toxic substances and by the byproducts of microbial metabolism. Substrates with high nitrogen content, produces excessive ammonia during anaerobic digestion process (Drennan and DiStefano,

2014, Akindele and Sartaj, 2018). Total ammonia nitrogen basically comprises of free ammonia nitrogen and NH_4^+. In an anaerobic system, under high pH and high temperature free ammonia nitrogen and NH_4^+ can be converted into each other. Free ammonia nitrogen is the most toxic type of total ammonia nitrogen. As free ammonia nitrogen can penetrate the bacterial cell membrane, leading to proton imbalances (Akindele and Sartaj, 2018). Inhibitory concentration of free ammonia nitrogen and total ammonia nitrogen are dependent on substrate, inoculum and environmental conditions.

While rapid acidification during anaerobic digestion occurs when the substrate is rapidly biodegradable. Proliferation of acid-producing bacteria inhibits the activity of methanogens, resulting in the accumulation of VFA (Yuan and Zhu, 2016). When the VFA consumption rate is lower than the rate of VFA production, inhibition of the overall process occurs due to decrease in pH.

2.3 WATER HYACINTH AS A SUBSTRATE FOR ANAEROBIC DIGESTION

Substrates with high moisture content or semi-solid organic matter are preferred for anaerobic digestion (Kondusamy and Kalamdhad, 2014; Koyama et al., 2014). Higher VFA can lead to drop in pH inhibiting the growth of microorganisms and higher ammonia concentration can be toxic for anaerobic bacteria thereby constraining methane production (Tada et al., 2005). Higher the sCOD higher is the biogas recovery. Water hyacinth has a moisture content of 85-95% (Nigam, 2002; Prasad et al., 2013) and a sCOD of 1600±320 mg/L (Prasad et al., 2013). Organic matter of water hyacinth determined as volatile solids constituted 72-85% of total solids, suggesting its potential in raw materials for bioconversion (Nigam, 2002; Prasad et al., 2013). Water hyacinth is considered to be a potential biomass source of cellulose and hemicellulose for conversion into useful biogas. Even the less amount of lignin (2-10%) when compared to cellulose (20-35%) and hemicellulose (25-33%) (Bolenz et al., 1990; Poddar et al., 1991; Gressel, 2008) present in water hyacinth can be dissolved by pretreating the substrate. Many previous studies have reported similar biogas yield from water hyacinth, in the range of 200-300 L biogas/kg VS and around 140-200 L methane kg/VS (Anand et al., 1991; Moorhead and Nordstedt, 1993; Kumar, 2005; Ferrer et al., 2010). O'Sullivan et al. (2010) have stated that the biogas yield from water hyacinth was in the range of 200-400 L biogas/kg VS. However, Vaidyanathan et al. (1985) reported a very high yield of 430 L methane/kg VS during batch digestions of water hyacinth. Anaerobic digestion of water hyacinth, giant reed and maize were explored for their potential for biogas

production by Shah et al. (2015). Water hyacinth was a successful substrate for mono-digestion which resulted in its highest biogas production rate. The cumulative biogas production during 30 days was highest for water hyacinth (25780 mL), followed by giant reed (18845 mL) and maize (15900 mL). Water hyacinth had the highest biogas generation rate of 1000 mL/day. The study revealed that utilization of water hyacinth as substrates for biogas production will overcome energy crisis in developing countries to a certain extend. Acidic pH of water hyacinth can be made neutral by addition of inoculum. Water hyacinth, the international pest, is available throughout the year, grows at a very rapid pace, holds high amount of moisture, low lignin, low VFA and ammonia, relatively high biodegradability, and is enriched with nutrients thereby proving it to be a phenomenal feedstock for the incessant production of bioenergy. Considering the unparalleled properties of water hyacinth and its worldwide existence, this invasive weed can be highly recommended for anaerobic digestion.

2.4 INOCULUM

Deshpande et al. (1979) stated that the use of water hyacinth and cow dung enhances volatile fatty acids in fermenting slurry because of its quick hydrolysis and fermentation. Dhahiyat et al. (1984) reported a biogas yield of 550 L/kg total solids when cow dung was used an inoculum to increase the digestibility of water hyacinth. Due to pre-fermentation in the ruminant's abdomen, cow dung has high nitrogen content and has been witnessed to be most apt for yielding higher amount biogas (Chonkor, 1983). El-Shinnawi et al. (1989) and Kumar (2005) observed when paralleled to manure alone, co-digestion of water hyacinth and manure upsurges the biogas yield specifying that the plant biomass subsidized more to the biogas production than the manure. The nutrient rich inoculum boosts the enzyme activity leading to higher substrate degradation and biogas production (Zhang et al., 2011; Mao et al., 2015). Fresh, easily flowing and additives free inoculum should be fed into the digester regularly for steady operation of the anaerobic digester. The most common inoculum used is cow dung followed by cattle dung, pig dung and poultry waste (Table 2.1 and 2.2). As, livestock wastes usually holds high content of nitrogen starting from chicken manure (1.03%), fresh goat dung (1.01%), dairy manure (0.35%) and swine manure (0.24%) (Zhang et al., 2013) so, inoculum when used alone for anaerobic digestion is prone to ammonical toxicity (Sawatdeenarunat et al., 2015). The mono-digestion of animal waste is not recommended as it can affect the digester stability leading to ammonia toxicity from the rapid degradation of organic nitrogen such as urea and protein (Abouelenien et al., 2014). Therefore a proper mixing

ratio should be maintained by digesting water hyacinth and inoculum together for producing biogas with higher methane yield.

Table 2.1. Initial characteristics of different livestock inoculum (Dhamodharan et al., 2015)

Parameters	Cow dung	Goat dung	Pig dung	Poultry dung
Moisture content (%)	79.8±2.3	45.7±0.6	72.23±1.6	78.42±0.8
Total solids (%)	20.19±1.4	55.1±1.5	26.7±1.8	21.6±0.9
Volatile solids (%)	15.25±1.1	39.2±0.9	22.18±1.3	16.2±0.5
pH	7.05-7.25	7.35-7.51	6.52-6.94	6.53-6.63
sCOD (g/L)	21.6±4.8	34.3±4.7	23.3±3.6	22.5±3.9
TKN (g/L)	5.3±0.6	3.9±0.1	3.1±0.5	3.0±0.2

BMP tests are also useful in determining the potential ideal feedstock to inoculum mixing ratio (Labatut et al., 2011). Determination of the ideal feedstock to inoculum mixing ratio is necessary to avoid process imbalance and enhance biogas generation. Improper feedstock to inoculum mixing ratio ratio may sometimes result in inhibition due to the stocked up intermediate products and may produce negligible or lesser quantity of biogas. Eiroa et al. (2012) observed that the increase in the waste/inoculum ratio led to process imbalance in the anaerobic reactor due to build up of volatile fatty acids (VFA). Raposo et al. (2009) also stated that the increase in feedstock to inoculum mixing ratio may lead to the accumulation of VFA during the process. Accumulation of VFA in higher feedstock to inoculum mixing ratio reveals the kinetic uncoupling between acid producing and acid consuming microorganisms. Co-digestion of the substrate and inoculum will also help to keep the pH neutral of the digester, when the substrate is already acidic in nature. Even pig slurry when used with dried powder of water hyacinth by Chuang et al. (2011) showed positive result. Besides cow dung and pig dung, poultry litter was also used for the co-digestion with water hyacinth. Patil et al. (2012) observed that poultry litter improved biogas yield nearly two times when compared to water hyacinth substrate without poultry litter. Ehiri et al. (2014) studied the possibility of producing biogas from a mixture of water hyacinth and fresh rumen residue. The maximum biogas production was observed on seventeenth day (16.4 mL). Patil et al. (2014) co-digested water hyacinth and sheep waste in the mixing ratio 4:12.01:83.90 (water hyacinth: sheep waste: water) demonstrating the highest biogas yield of 0.36 L/g

VS. The overall results demonstrated that co-digesting water hyacinth with sheep waste had significant enhancement on the biogas yield. Sukasem et al. (2017) reported water hyacinth and pig dung mixture to have illustrated better biogas yield than elephant dung and bat dung mixture. Inoculum is useful during anaerobic digestion of water hyacinth as it acts as a buffer and seeding material thereby improving biogas production.

Table 2.2. Different types of inoculum used for anaerobic digestion of water hyacinth

Reference	Inoculum Used	Remarks
Dirar and El Amin. (1988)	Digested domestic sewage sludge, sheep rumen liquor and sludge from an old digester working on water hyacinth.	Sludge from an old digester working on water hyacinth produced 552 L/kg TS, digested domestic sewage sludge produced 451 L/kg TS, sheep rumen liquor produced 401 L/kg TS, and the control produced 221 L/kg TS.
Chanakya et al. (1992)	Cattle dung	For fresh water hyacinth, a specific gas yield of 94.2 and 86.5 L/kg TS was observed for acidogenic and methanogenic phase respectively whereas for dry water hyacinth a specific gas yield of 61.6 and 85.8 L/kg TS was observed
Moorhead and Norstedt (1993)	Swine waste water and primary sludge waste water	Total biogas yield ranged from 0.20 to 0.28 L/g VS.
Singhal and Rai (2003)	Dairy cattle manure	Biogas production in 9-12 days was observed.
Ganesh et al. (2005)	Cow dung	About 22% higher quantity of biogas per unit feed than was obtained from equivalent mass (dry weight

		basis)
Verma et al. (2007)	Dairy cattle manure	Biogas production was quicker (8-12 days) in water hyacinth than in water chestnut (12-16 days).
Almoustapha et al. (2009)	Rumen liquor	The total volume of biogas produced after 65 days was 151.4m^3, i.e, 2.6m^3/ day. During summer and123.4m^3, that is, 1.48m^3/day during winter.
O'Sullivan et al. (2010)	Cattle manure	Biogas yield was found to be 292±43L/kg VS for water hyacinth.
Chuang et al. (2011)	Pig slurry	Total bioenergy of 885.2 GJ was produced from water hyacinth.
Patil et al. (2011)	Primary sludge and poultry waste	Cumulative biogas yield from water hyacinth and poultry wastewas 0.38 L/g VS and from water hyacinth and primary sludge was 0.345 L/g VS.
Patil et al. (2012)	Poultry Litter	Poultry liiter inoculum improved biogas yield nearly by 2 times when compared to water hyacinth without poultry liiter inoculum.
Mathew et al. (2014)	Cow dung	Biogas production was 552 L/kg VS.
Patil et al. (2014)	Sheep waste	Blending water hyacinth with sheep waste showed

	highest biogas yield of 0.36 L/gVS.

2.5 PRETREATMENT

Pretreatment is a significant process of converting recalcitrant lignocellulosic biomass to easily digestible biomass. Pretreatment is mostly performed to melt down lignin and break down cellulose crystallinity by enhancing enzyme accessibility thereby cutting down the hydrolysis period of anaerobic digestion and enhancing biogas production (Fig. 2.2). Hydrolysis of lignocelluloses i.e., the breakdown of large complex compounds into their simpler forms is a very time consuming process (Khanal, 2008). Pretreatment minimizes the surface area of lignin and segregates the cellulose making it easily accessible to the microorganisms (Jönsson and Martín, 2016). Pretreatment can be either physical, chemical, thermal or biological. Several studies have focused on enhancing the digestibility of water hyacinth through physical, chemical, thermal and thermo-chemical pretreatment approaches for increasing the biogas yield in a reduced time interval.

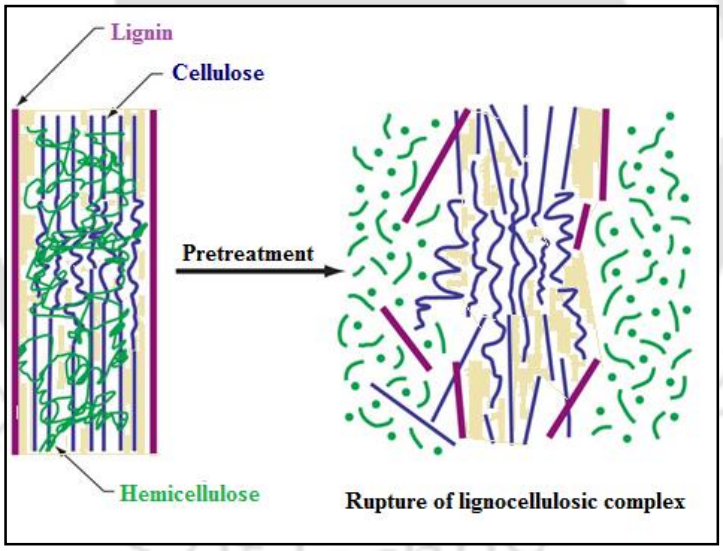


Fig. 2.2. Rupture of lignocellulosic complex due to pretreatment

2.5.1 Physical pretreatment

Chopping and drying is the most preferred approach of pretreatment. Moorhead and Nordstedt (1993) found that total biogas and methane production was highest for water hyacinth when chopped in 6.04 mm. Low biogas yields from dried water hyacinth have been reported previously by Singhal and Rai (2003) who reported a maximum of 28 L biogas/kg TS after a digestion period of 21 d and Verma et al. (2007) reported yields of approximately 24 L biogas/kg TS.

2.5.2 Chemical pretreatment

Chynoweth et al. (1982) employed two alkaline pretreatment: 5 and 50% NaOH/g VS for 72 h at 35ºC, 5% ammonium hydroxide/g VS for 30 days at 35ºC; one acid pretreatment with 5 and 10% acetic acid/g VS for 20 days at 75ºC and a steam pretreatment was carried out in a pressure bomb reactor at 690 kPa (164ºC) for 30 min. They observed that only 50% NaOH pretreatment was effective.

Patel et al. (1993) reported that water hyacinth when pretreated at pH 11 and 121ºC, solubility of water hyacinth increased thereby improving biogas production. They also reported that severe pretreatment conditions illustrated a negative effect, especially on methanogenic bacteria due to the formation of toxic compounds during pretreatment. Pang et al. (2008) observed that 6% NaOH-treated corn stover digested at the loading rate of 65 g/L achieved 48.5% more biogas production and 71% more bioenergy gain, as compared to the untreated corn stover.

Ofoefule et al. (2009) did a comparative study on the effect of different pretreatment methods on biogas yield from water hyacinth. The water hyacinth charged into metallic prototype digesters of 121 L capacity was pretreated in various ways i.e., dried and chopped alone, dried and treated with KOH, dried and combined with cow dung, while the fresh water hyacinth served as control. Water hyacinth dried and combined with cow dung showed highest cumulative biogas yield of 356.3 L/ TS. The mean biogas yield of fresh water hyacinth was 8.48±3.7 L/g TS whereas the mean biogas yield of dried and chopped alone, dried and treated with KOH and dried and combined with cow dung increased to 9.75±3.4, 9.51±5 and 11.8±2 L/g TS respectively.

Cheng et al. (2010) observed that the cogeneration of H_2 (51.7 mL H_2/g VS) and CH_4 (143.4 mL CH_4/g VS). He observed that when water hyacinth leaves are pretreated with 3 wt% NaOH and cellulose the energy conversion efficiency strikingly accelerates from 3.3-33.2%.

Patil et al. (2011) also conducted a comparative pretreatment study similar to Ofoefule et al. (2009). Patil et al. (2011) studied alkali pretreatment (NaOH) using poultry waste in combination with primary sludge instead of cow dung. The overall results revealed that water hyacinth with poultry waste and primary sludge had significant improvement on the biogas yield and treating water hyacinth with NaOH increased the biogas yield slightly. Alkaline pretreatment swells the substrate, increases the internal surface area of the substrate, disrupts the lignin layer and decreases the cellulose crystallinity thereby making

the carbohydrate more easily accessible. Chandra et al. (2012) stated the ranking of alkali efficacy as NaOH> KOH > $Mg(OH)_2$ and $Ca(OH)_2$.

Gao et al. (2013a) studied the effect of ionic liquid pretreatment of water hyacinth on the lignocellulosic composition, structure and biogas production. They observed that after pretreatment with [Bmim]Cl/ DMSO under 120ºC for 120 min, the cellulose content increased by 27.9%, lignin was removed by 49.2% and the biogas yield increased by 97.6% when compared to unpretreated water hyacinth.

2.5.3 Thermal pretreatment

Ferrer et al. (2010) studied the mesophilic and thermophilic anaerobic biodegradability of water hyacinth pretreated at 80ºC. It was observed that the thermal pretreatment enhanced the solubilization of water hyacinth from 4-12% after 30 min. As there was no significant effect observed on the methane yield, they concluded that higher methane production rate might be expected from thermophilic reactors working for short retention times.

The effect of hydrothermal pretreatment on biogas production from water hyacinth mixed with buffalo dung was reported by Putra et al. (2014). Maximum biogas production (7889 mL/day) was observed when hydrothermal pretreatment was carried for 60 min with a water hyacinth: buffalo dung ratio of 1:2. The optimum methane yield was 2856 mL/day. The study revealed that the ratio of water hyacinth to buffalo dung along with pretreatment has a noteworthy impact on biogas production.

Budiyono et al. (2015) investigated the use of microwave for the pretreatment of water hyacinth in a batch anaerobic digestion tank using cattle's rumen as an inoculum. Varying microwave power level of 240, 400, 560 and 800 W were used with varying microwave heating time of 5, 7 and 9 min respectively. A positive impact of microwave pretreatment was observed on the anaerobic biodegradability of fresh water hyacinth. The maximum biogas production of 75.1 mL biogas/g TS from fresh water hyacinth was obtained at 560 W for 7 min of microwave pretreatment whereas the unpretreated fresh water hyacinth produced 37.5 mL biogas/g TS. They concluded that almost all pretreated fresh water hyacinth produced more biogas than the unpretreated fresh water hyacinth.

2.5.4 Thermo-chemical pretreatment

Microwave pretreatment when compared to other thermal pretreatment methods illustrates higher rate of solubilisation, due to polarization of macromolecules (Toreci et al., 2009; Marin et al., 2010). Most of the studies depict the use of thermal and alkali pretreatment for treating the lignocellulosic weed, besides chopping and cutting. Thermal

and chemical pretreatment studies are factual not only for water hyacinth but also for a wide range of lignocellulosic biomass like wheat straw, barley, corn straw, sunflower stalks as these methods results in the recovery of useable lignocellulosic components in separate fractions. Jackowiak et al. (2011) optimised microwave pretreatment temperature at 150°C without any holding time for wheat straw solubilisation and anaerobic biodegradability. The cumulative methane production increased from 269.6±14.4 L_{CH4}/kg_{TVS} to 343.8±5.7 L_{CH4}/kg_{VS}. Sapci (2013) carried out a microwave pretreatment study of barley at 200°C for 15 mins and observed increase in cumulative biogas production from 406±13.9 mL biogas/g VS to 425.1±4.8 mL biogas/ gVS. Song et al. (2014) pretreated corn straw with 8% $Ca(OH)_2$ and an increase in methane yield of 206.6 mL CH_4/g VS was studied which was 1.05 folds greater than the untreated corn straw. Even the drop in lignin content from 7.5-5.4% was revealed.

Lin et al. (2015) studied microwave heated alkali pretreatment to improve enzymatic digestibility and H_2/CH_4 production from water hyacinth. SEM, XRD and FTIR revealed the decrease in lignin content and crystallinity index signifying the enhanced biodegradability of the lignocellulosic weed for methane production. They observed that sequentially fermentative hydrogen and methane yield from water hyacinth after microwave heated alkali pretreatment and enzymatic hydrolysis increased to 63.9 and 172.5 mL/g VS respectively.

Sukasem et al. (2017) observed that the lime treated fresh water hyacinth had highest values of BMP and methane concentration were at 59.3 and 63.6%, respectively than NaOH treated plants when kept at 37±2°C under anaerobic condition for 45 days. The COD removal was in range of 51-69%.

Monlau et al. (2012) investigated the use of seven types of thermo-chemical pretreatments on the structural features and anaerobic digestion of sunflower stalks. Pretreatment of sunflower stalks at 55°C with 4% NaOH for 24 h showed the highest methane production of 259±6 mL CH_4/g VS.

Hesami et al. (2015) conducted hydrothermal pretreatment of sunflower stalks at different temperature (140, 160, 180 and 200°C) for 30 and 60 min with/without addition of 1% sulfuric acid. The pretreatment of stalks with 50% (v/v) aqueous isopropanol containing 1% w/w (based on dry stalks) sulfuric acid at 160°C resulted in the highest lignin removal. Methane production yield of the pretreated substrate was improved by 45-124% compare to 124 mL CH_4/g VS obtained from the digestion of untreated stalks. In the best case, hydrothermal pretreatment at 180°C for 60 min and organosolv

pretreatment at 160°C for 30 min with 1% H_2SO_4 followed by 45 days anaerobic digestion resulted in 234 and 278 mL CH_4/g VS respectively.

Kaur and Phutela (2016) carried out a study to enhance paddy straw digestibility and biogas production through sodium hydroxide (NaOH) pretreatment. The paddy straw was pretreated with NaOH (2, 4, 6, 8 and 10%) by 24 h soaking followed by microwave irradiations (30 min, 720 W, 180°C). 4% NaOH, 30 min microwave was the optimum pretreatment condition which resulted in 65% reduction in lignin content, 88.7% reduction in silica content and 54.7% increase in biogas production.

Pretreatment of the biomass is the perfect response for aspects related to cellulose crystallinity, available surface area and lignin content which restricts the lignocellulosic biodegradability of the substrate (Hendriks and Zeeman, 2009). Dissolution of lignin occurs over a broad temperature range, due to the diverse thermal stabilities of the various oxygen functional groups present in its structure (Brebu and Vasile, 2010). In alkali pretreatment, the primary catalytic reagent (-OH group) liable for lignin dissolution is easily available commercially. The hydrolytic reactions cleave the lignin bonds giving lower molecular weight fragments (Thring, 1994). Thermal and alkali pretreatment breaks off the thin coating of lignin present not only in the water hyacinth cell wall but also in all lignocellulosic compounds, aiding in short retention time with elevated yield of biogas. These pretreatment processes are quite proficient when compared to other conventional methods as they are incredibly versatile in the dissolution of lignin. Strong acidic pretreatment may form inhibitory by-products whereas mild acid pretreatment dilute the lignin (Hendriks and Zeeman, 2001). As water hyacinth is acidic in nature so, alkali pretreatment is better when compared to acid pretreatment because alkali avoids the fall in pH increasing the proficiency of methanogenesis phase during anaerobic digestion. Also acidic pretreatment requires expensive acids and high cost of corrosive resistant equipment's.

2.5.5 Biological pretreatment

Biological pretreatment i.e., the use of microbial consortium and enzymes is an environment friendly route still it is not so widespread when compared to physico-chemical pretreatment as it is a time consuming procedure. Although there are many literatures available which state the utililisation of biological pretreatment on brewery spent grain (Cater et al., 2015), maize silage (Poszytek et al., 2016), pulp and paper mill sludge (Lin et al., 2017) pretreatment for anaerobic digestion of lignocellulosic waste material. Cater et al. (2015) demonstrated the utililisation of pure culture of bacteria and

Lin et al. (2017) utililised microbial consortium for pretreatment of lignocellulosic waste material. Microbial pretreatment when compared to enzymatic pretreatment exhibits much better result in anaerobic digestion process due to their higher functional diversity and tolerance to environmental factors i.e., temperature and pH (Shrestha et al., 2017).The microorganisms actively working during microbial pretreatment solubilize the lignocellulosic complex producing easily hydrolyzable oligomeric and monomeric carbohydrate compounds which are further altered by acidogenic and acetogenic microorganisms into VFA. The VFA i.e., acetic acid, butyric acid, propanoic acid generated during the microbial pretreatment of water hyacinth are useful for the generation of methane. Schroyen et al. (2014) studied the impact of pretreating corn stover with different enzymes (laccase, manganese peroxidase and versatile peroxidase) and different incubation time (0, 6 and 24 h). The enzyme laccase illustrated an increase in biomethane production by 25% after 24 h of incubation. Pretreatment with peroxidase increased biomethane production by 17% after 6 h of incubation. Hua et al. (2016) observed enhanced biogas yields by 74.7% when rotted silage maize straw was pretreated by microbial community. Ali et al. (2017) observed that pretreatment of sawdust for 10 days with a novel lignocellulosic degradation microbial consortium isolated from rotten sawdust led to significant reduction in cellulose, hemicellulose and lignin content by 37.5, 39.6 and 56.7%, respectively. In addition, the pretreatment enhanced cumulative biogas yield, which reached its maximum value of 312 L/kg VS after 28 days of ananerobic digestion (25.6% higher than the corresponding control). Moreover, the maximum significant cumulative methane yield was recorded after 28 days of anaerobic digestion of the pretreated sawdust (155.2 L/g VS), which represented 72.6% higher than the corresponding control. But biological (microbial and enzymatic) pretreatment of water hyacinth for the production of biogas has been rarely studied, may be because of its relatively time consuming process than the other pretreatment techniques. But, biological pretreatment even requires careful growth conditions as some of the carbohydrate fraction is consumed by the microorganisms (Agbor et al., 2011). The utilisation of bacterial strains for solubilising water hyacinth is inexpensive when compared to the direct utilisation of enzymes available in the market. Nevertheless, biological pretreatment does not generate any inhibitors (phenolic compounds, furfurals and hydroxymethylfurfural) during anaerobic digestion and is an eco-friendly process with low energy and chemical input. Microbial pretreatment is considered to be inexpensive when compared to other

physico-chemical pretreatment methods. Physical pretreatment methods require specialized equipments and machineries which lead to abundant energy consumption and chemical pretreatment methods involve acid, alkali which in turn creates harsh condition by generating inhibitory compounds (Mosier, 2005) that might hinder the anaerobic digestion process.

2.6 CO-DIGESTION

Anaerobic co-digestion of various raw materials may be a cost effective way to balance macro-and micronutrients and decrease formation of inhibitors or accumulation of toxic compounds thereby improving biogas production (Abouelenien et al., 2010). Anaerobic co-digestion is also beneficial in treating the wastes of a locality. Animal wastes are well-studied co-substrates for anaerobic co-digestion of water hyacinth for its high buffering capacity and high nitrogen content which could enhance the digestion efficiency and rich in micronutrients necessary for optimum bacterial growth. Animal wastes reduce the environmental impact while simultaneously enhancing energy production for their complementary properties.

2.6.1 Co-digestion of water hyacinth with animal wastes

Many studies were conducted on the co-digestion of water hyacinth and animal wastes (Dhahiyat et al., 1984; Madamwar et al., 1991; Patel et al., 1992). Chuang et al. (2011) reported that co-digestion of water hyacinth and pig slurry demonstrated maximum methane production rate of (29 mmol CH_4/L/d) at a concentration of 80 g/L. The study conducted by Raja and Lee (2012) revealed the possibility to produce biogas from a mixture of water hyacinth and cow dung. The results indicate that dried and chopped water hyacinth combined with cow dung had the highest cumulative biogas yield (64%) when compared with dried and chopped water hyacinth combined with wood charcoal (60%). Water hyacinth is a very good biogas producer which needs minimal pre-treatment to enhance the biogas yield. Ehiri et al. (2014) studied the possibility of producing biogas from a mixture of water hyacinth and fresh rumen residue. The maximum biogas production was observed on seventeenth day (16.4 mL). Patil et al. (2014) co-digested water hyacinth and sheep waste in the mixing ratio 4:12.01:83.90 (water hyacinth:sheep waste:water) demonstrating the highest biogas yield of 0.36 L/g VS with biogas composition 60.8% CH_4, 21.5% CO_2 and 17.6% others (H_2, N_2, H_2O and H_2S). The overall results demonstrated that co-digesting water hyacinth with sheep waste had significant enhancement on the biogas yield. Sukasem et al. (2017) co-digested water hyacinth with ping dung, elephant dung and bat dung in the ratio 1:1 at 37±2°C under

anaerobic condition for 45 days. They reported water hyacinth and pig dung mixture to have illustrated better biogas yield than elephant dung and bat dung mixture.

2.6.2 Co-digestion of water hyacinth with other organic co-substrates

But there are few studies conducted in the recent past where water hyacinth is utilised as co-substrate with some other raw material and animal waste is utilised as an inoculum. Shankar et al. (2013) reported effect of substrate concentration on biomethanation of water hyacinth. In this study the biomethanation was carried out for 60 days using a substrate concentration of 3-11% at mesophilic condition. Anaerobic co-digestion of water hyacinth with primary sludge, cow dung and poultry litter were evaluated. Maximum biogas production was observed with a biomass loading of 7%. In the present scenario, water hyacinth proves to be a promising renewable source of energy in the form of biogas. Okewale et al. (2016) reported that co-digestion of water hyacinth, elephant grass, cow dung and water gave the highest biogas yield of 2.3 L after 60 days of incubation with the highest methane content of 62%. Adakinin et al. (2017) co-digested morning glory (*Ipomoea aquatica*) with water hyacinth (*Eichhornia crassipes*) at five different morning glory:water hyacinth ratios of 100:0, 70:30, 50:50, 30:70 and 0:100 for 17 weeks. They did not observe any significant effect on biogas yield co-digestion, but morning glory:water hyacinth ratio 50:50 recorded the highest yield (0.29 dm^3/kg VS fed day). Tasnim et al. (2017) reported that anaerobic co-digestion of water hyacinth, cow dung and sewage sludge produced total biogas of 812 mL after 800 h with 65% CH_4, 14% CO_2 and 21% other gases.

Anaerobic co-digestion study of various other organic substrates also demonstrates positive synergism. Li et al. (2017) studied the methane potential of chicken manure and three co-substrates (chicken processing waste, *Miscanthus* and seagrass) in mono-digestion and co-digestion studies. However, the methane production rate/hydrolysis rates of mono digestion of chicken processing waste and co-digestion with chicken manure were above 2 times quicker under the inoculum to substrate (I/S) ratio of 6 than that at the I/S ratio of 2 and 4. *Miscanthus* co-digestion effect was influenced by its composition and seagrass showed high methane yield (11-34%). Wang et al. (2018) investigated the performance of cucumber residues, corn stover and pig manure co-digestion at different ratios. Results show that mixing cucumber residues with pig manure and corn stover significantly improved methane yields (1.27-3.46) times than the mono-feedstock.

2.7 ANAEROBIC REACTORS

Anaerobic reactors are basically an air-tight apparatus provided with all the favourable conditions to make it easier for the microorganisms to degrade the substrate in order to form biogas. There are two types of anaerobic reactors;

a) Single stage
b) Two stage

The anaerobic digestion process sometimes is designed in two stages based on the two main groups of microorganisms involved in anaerobic digestion: acidogenic and methanogenic to provide ideal environmental conditions for the different group of microorganisms and allow for better process control (Demirel and Yenigun, 2002). The objective of a two stage anaerobic digestion system is not only to reduce organic waste, but also to extract more net energy from the system (Thompson, 2008). In a single stage anaerobic digestion process, a variety of VFA (propionic, butyric and lactic, as well as alcohols and ketones), are formed during the breakdown of the organic substrates by acidogenic bacteria. Cooney et al. (2007) reported that, in a well operated process, VFA are mostly tranformed to acetic acid and hydrogen, which, in turn, are tranformed into methane. Usually, in terms of methane production and digestion stability two stage anaerobic digestion processes are preferable over conventional single stage anaerobic digestion process.

But Stover et al. (1984), observed promising performances in a single-stage anaerobic digestion of thin corn stillage (64.5 g COD/L; 32.2 g TS/L) in both suspended growth and fixed-film systems with a methane yield ranging from 0.22 to 0.33 m^3/kg $COD_{removed}$ that could replace 60% of the daily energy requirement of the bioethanol plant. Lanting and Gross (1985) reported 76% COD removal with 0.33 m^3 CH_4/kg TCOD removed from a pilot scale upflow anaerobic sludge blanket reactor. Voelklein et al. (2016) compared the single-stage and two-stage anaerobic digester at increased loading rates to assess the impact of an increasing loading rate on the two-stage digestion of food waste. De Gioannis et al. (2017) compared the energy recovery from one stage and two stage mesophilic anaerobic digestion of food waste. Zhang et al. (2017) developed a novel compact three-stage anaerobic digester was for high-efficiency anaerobic digestion of food waste and biogas production. By having three separate chambers, hydrolysis, acidogenesis and methanogenesis were separately optimised thereby improving the overall anaerobic digestion performance. Compared to traditional one stage and two stage anaerobic digesters, three-stage anaerobic digester had a 24-54% higher methane yield at

a high OLR of 10 g VS/L with a VS reduction rate of 83.5±1.3%. Even at a higher OLR, three-stage anaerobic digester illustrated a high buffering ability when the one stage and two-stage digesters had already soured and failed.

Shen et al. (2017) scaled up an integrated waste-to-energy system for producing pipeline quality biomethane from shake flasks to two stage digester in semi-continuous mode. Performance of thermophilic anaerobic digestion of sewage sludge amended with corn stover biochar and pine biochar was conducted. Both corn stover biochar and pine biochar promoted the substrate utilisation, methane productivity and process stability of anaerobic digestion process, while corn stover biochar showed superior potential. Corn stover biochar enhanced methane content in biogas and methane production rate by 25 and 37% respectively when compared to the control, with maximum methane content of 95% and methane yield of 0.34 L/g VS.

There are few previous literature reports available on the two stage anaerobic digestion of water hyacinth. Chynoweth et al. (1982) used stirred tank reactor (5 L) and upflow solid digester (10 L) for biogas production of water hyacinth (leaves and petioles) juice which exhibited 20% increase in feed conversion to methane. The juice was anaerobically digested in a two phase process (hydrolysis-acidogenesis and methanogenesis) configured with a 3.5 L glass hydrolysis reactor inoculated with 700 mL of sludge adapted from cow ruminal liquid and a 2~1 L upflow anaerobic sludge blanket reactor. The feed rate was fixed to reach 5 and 3 days retention time respectively for each reactor. Parameters like pH, alkalinity, VFA, COD, total nitrogen, carbon and phosphorus were analysed. Water hyacinth showed a stoichiometric methane yield of 0.57 L/g VS added.

Ilangovan et al. (1990) in an attempt to study the transport of heavy metals during the anaerobic digestion of water hyacinth shoot in a two phase upflow anaerobic sludge blanket reactor found 175.6 L/L.day of biogas in the first phase and 226 L/L.day of biogas in the second phase. The hydrolysis reactor volume was 3.5 L and upflow anaerobic sludge blanket reactor volume was 2.3 L.

Chanakya et al. (1992) undertook diphasic anaerobic digestion of water hyacinth by coupling a solid phase acidogenic system to an upflow packed bed methanogenic digester. Methane content for water hyacinth was observed to be 65% (highest) in methane phase in 33 days whereas on the same day in acid phase methane content was 6.3%. For dry water hyacinth, methane content was highest on 22nd day (78.5%) in methane phase and on the same day methane content in acid phase was 3.6%. They reported that the biomass bed may be used alone as a packed-bed digester to obtain high

biogas production rates. Specific gas yields between 146 L/kg TS were obtained for the substrates tested.

Kivaisi and Mtila (1998) established that for the overall conversion of the conversion of water hyacinth shoots and a mixture of the shoots with cowdung (7:3) into biogas can be achieved with a small reactor volume in the two phase rumen derived reactor process considering the short SRT and the comparatively high loading rate. The rumen reactor was serially connected to an upflow anaerobic sludge blanket type methanogenic reactor with a total working volume of 2.5 L. Under conditions similar to those of the rumen and loading rate in the range of 11.6±19.3 g VS/L.d in the rumen reactor, the degradation efficiencies were 38% for the shoots and 43% for the mixture. On the whole conversion of the substrate was maximum for the loading rate 15.4 g VS/L.d. At a loading rate of 15.4 VS/L.d, SRT of 90 h and a dilution rate of 0.5/h in the rumen reactor and connecting it to a methanogenic reactor of the upflow anaerobic sludge blanket type, 100% conversion efficiency of VFA into biogas with a methane content of 80% were reported. The average methane gas yield was observed to be 0.44 L/g VS digested.

Priya et al. (2018) conducted the biomethanation of water hyacinth in a two stage batch process unit consisting of an anaerobic leach bed reactor and an upflow anaerobic sludge blanket (20 L). Mechanically crushed water hyacinth biomass was loaded into the anaerobic leach bed reactor. The soluble organics released in the anaerobic leach bed reactor wass subsequently fed to the upflow anaerobic sludge blanket at a controlled rate where it was converted into biogas. In a typical batch operation, 4 kg (wet weight) of mechanically crushed water hyacinth biomass (0.049 g VS/g wet weight) was loaded into the reactor. The overflow from the upflow anaerobic sludge blanket was circulated to the anaerobic leach bed reactor. Once the digestion was over by 12 days, the digestate was removed, fresh water hyacinth was loaded into the anaerobic leach bed reactor and the cycle was continued. Co-digestion of water hyacinth with waste activated sludge and food waste revealed ~150 and ~400 mL biogas/g VS respectively against ~140 mL/g VS of water hyacinth alone.

Despite of their higher loading rates, improved process stability and flexibility, there are relatively few commercial two stage anaerobic digestion units. The added complexity and expense of building and operating commercial two stage systems have so far counteracted the yield and rate enhancements (Rapport et al., 2008). The theoretical higher biogas yields have also been questioned since the acidogenic phase separation prevents the hydrogen to methane pathway (Reith et al., 2003).

Anaerobic reactors which can be fed either in batch mode or in continuous mode.In a batch process, the substrate and the inoculum are added in desired quantity at the commencement of the process and the reactor is shut till the end of the anaerobic process. Batch digesters are the most preferred digester because of its simplicity, flexibility and high conversion per unit volume of pass. While in a continuous reactor, the raw materials are fed and the product is withdrawn in an uninterrupted manner. It is typically ideal for large scale production of free flowing materials. Greater uniformity of system parameters, such as temperature, mixing, chemical concentration and substrate concentration can be witnessed in a continuous reactor.

Batch reactors are also ideal for small scale experimental studies; therefore most of the literatures have cited the use of batch reactor of different volumes (Table 2.3) for dried and powdered form of water hyacinth. Digester size depends on the amount of organic material to be fed into the digester and the time required by that specific digester to break down the organic material. The largest batch digester used for anaerobic digestion of water hyacinth was by Vaidyanathan et al. (1985) which was of a volume of 165 L followed by Moorhead and Nordstedt (1993), who used four batch digester of 55 L. Jayaweera et al. (2007) used a six 3-barreled batch fed reactors with the innermost barrel (45 L) being used as the digester for the anaerobic digestion of water hyacinth. Gao et al. (2013) used 25 L batch digester and Srivastava et al. (1989) used 20 L digester. Most of the batch digesters were of the size 1-10 L (Vaidyanathan et al., 1985; Dirar and ElAmin, 1988; Srivastava et al., 1989; Madamwar et al., 1991; Chuang et al., 2011). Shiralipour and Smith (1984) found that in a 5 L batch digester water hyacinth shoots had a higher methane yield of 0.26-0.43 m^3/kg VS added than water hyacinth roots (0.13-0.23 m^3/kg VS added) . However, the study conducted by Vaidyanathan et al. (1985) and Chin and Goh (1978) reported a higher gas yield of 430 L methane/kg VS and 671 L biogas/kg VS, respectively, during the batch anaerobic digestion of water hyacinth.

Even batch digester of 25 mL was used for biogas production from water hyacinth by Gao et al. (2013). Nasir et al. (2015) states that as the digester size increases the amount of biogas production also increases which might be as a result of the fact that in bigger digesters the substrate was exposed to greater surface area for rapid multiplication of the methanogen for maximum utilization of the waste material for higher amount of gas production.

A few of the literatures has investigated the use of BMP test for the anaerobic digestion of water hyacinth. In order to determine the maximum quantity of biogas or

biomethane produced per gram of volatile solids present in the substrate, BMP tests are done. BMP assays, usually carried out in anaerobic batch digesters in bench scale, can be also used as an index to determine the anaerobic biodegradation potential of the substrate (Esposito et al., 2012). Paepatung et al. (2009) conducted a BMP test and found that the methane production potential from water hyacinth was appoximately 553 m^3 methane/ hectare.year, with an equivalent energy content of 0.16 kW or 440 L fuel/ hectare.year. BMP test done by O'Sullivan et al. (2010) revealed the methane production potential of water hyacinth to be 267 L/kg VS and methane yield to be 140 L/kg VS. VS reduction of 61% was observed. They found that the variability was low for water hyacinth. Houwanou et al. (2012) in order to evaluate the methanogen potential of water hyacinth and the effect of moisture tried four different combinations: 20 g water hyacinth with100 mL water, 20 g water hyacinth with 20 g faeces and 100 mL water, 20 g water hyacinth with 200 mL water and 20 g faeces with 100 mL water. They observed that 20 g water hyacinth with 20 g faeces and 100 mL water produced the highest amount of methane (500 mL) within 21 days and the sample 3 produced the lowest amount of methane (197 mL) within 21 days and thereafter it ceased. The reason for low methane production in 20 g water hyacinth with 200 mL water and 20 g faeces with 100 mL water was stated due to the presence of too much of water, which encouraged hydrolysis but hindered methanogenesis.

Wall et al. (2015) performed BMP assay of grass silage and rumen fluid for a ratio 2:1. Pellera and Gidarakos (2017) studied the effect of various lignocellulosic agro-industrial wastes after microwave pretreatment on biogas production when mixed with mesophillic anaerobic sludge. They tried various inoculum to substrate ratios for the various wastes. Substrate to inoculum ratio was 0.5 for winery waste and juice waste and 0.25 for cotton gin waste and olive pomace. O'Sullivan et al. (2010) observed that biochemical methane potential of cabomba, water hyacinth and salvinia to be 322±21, 292±43 and 52±55 L/kg VS respectively. Studies have been reported of enhanced biochemical methane potential of wheat straw after microwave pretreatment by 28% (Jackowiak et al., 2011). Even, Nges et al. (2016) observed 57% increase in biochemical methane potential of silver grass (*Miscanthus lutarioriparius*) after thermo-chemical pretreatment. Gao et al. (2013b) observed increase in methane production by 28.3, 11.1, 10 and 20.9% for water hyacinth, rice straw, mango leaves and spruce respectively after ionic liquid pretreatment. Wang et al. (2014) reported of enhanced solubilisation and methane production from goose grass (*Eleusine indica*) after hyperthermophillic

pretreatment. Lizasoain et al. (2016) stated that steam explosion pretreatment of reed biomass increased the specific methane yield by 89% than the untreated substrate. Similar observation was also reported by Gaur et al. (2017) after thermal pretreatment of duckweed and waste activated sludge when mixed in the appropriate proportion.

Table 2.3.Batch reactor configurations for anaerobic digestion of water hyacinth

Reference	Reactor Size	HRT	Temperature	Remarks
Shiralipour and Smith, (1984)	5 L	20 days	35ºC	Methane yield of 0.26-0.43 m^3/kg VS was observed from water hyacinth shoots
Vaidyanathan et al. (1985)	165 L	30 days	29±2ºC	228-327 L CH_4/kg dry mass was produced.
Dirar and El Amin, (1988)	2 L	90 days	37ºC	Total biogas yield of 552 L/kg TS was attained.
Mallik et al. (1990)	10 L	90 days	30-35ºC	Total biogas production of 179.9 L/kg TS was observed.
Singhal and Rai, (2003)	6 m^3	21 days	35±1ºC	Biogas production was observed in the range of 15400-21700 cc/kg of dry weight
Jayaweera et al. (2007)	45 L	27-30 days	30.3-31.3ºC	Cumulative biogas production of 14 L/kg of wet weight was attained.
Almoustapha et al. (2009)	six digesters measuring 5m^3	65 days	20-40ºC	Cumulative biogas production of 155

	each			m^3 was attained.
Paepatung et al. (2009)	120 mL	90 days	37ºC	0.35 L/g VS of methane yield was observed.
O'Sullivan et al. (2010)	200 mL	--	38ºC	150 L/kg VS of methane yield was observed.
Chuang et al. (1985)	125 mL	--	25, 35, 45, 55, and 65ºC.	Energy yield of 8.54 kg/g of dry biomass was reported.
Houwanou et al.(2012)	500 mL	--	25-28ºC	500 mL of biogas was achieved on the 21st day.
Gao et al. (2013)	25 mL	--	35ºC	Cumulative biogas production of 90 L/kg VS was attained from untreated water hyacinth.
Sudhakar et al. (2013)	20 L	35 days	26-35ºC	Average per day production of biogas was 0.326 L/day.
Mathew et al. (2014)	2 L	30-60 days	37±2ºC	Biogas yield of 552 L/kg VS was observed.

2.8 OUTCOME OF THE LITERATURE REVIEW

Anaerobic digestion is considered to be one of the most environment friendly alternatives for effective management of water hyacinth which assists in energy recovery too. This review analysed all the pretreatment techniques, co-digestion studies, inoculum and reactors used till date for anaerobic digestion of water hyacinth. Pretreatment of water

hyacinth is often claimed to hold a potential for higher methane recovery with lower retention time, but at present, it is difficult to assess which pretreatment technology holds the greatest potential for water hyacinth and its economic feasibility. Co-digestion of water hyacinth has been rarely conducted with other organic wastes besides animal wastes. Bio-chemical methane potential assays were rarely performed to determine the optimum F/M ratio. Optimising an ideal F/M ratio is essential for improving the overall anaerobic digestion process. And even almost all of the studies suggested the use of dried water hyacinth especially the leaf instead of the whole fresh plant. Studies can be conducted on fresh water hyacinth whole plant in a continuous multi-stage reactor for biogas production, as except a few, most of the literatures have stated the use of dried and powdered form of water hyacinth shoot in a batch reactor.

CHAPTER 3
MATERIALS AND METHODS

Various experimental studies were conducted to accomplish the objectives of the research work. The research was conducted in different phases utilising water hyacinth as a feedstock and cow dung as an inoculum. The detailed methodology is summarized below.

3.1 EXPERIMENTAL FLOWCHART

In order to accomplish the objectives, the research is proposed to be carried out in different phases as summarised in Fig. 3.1.

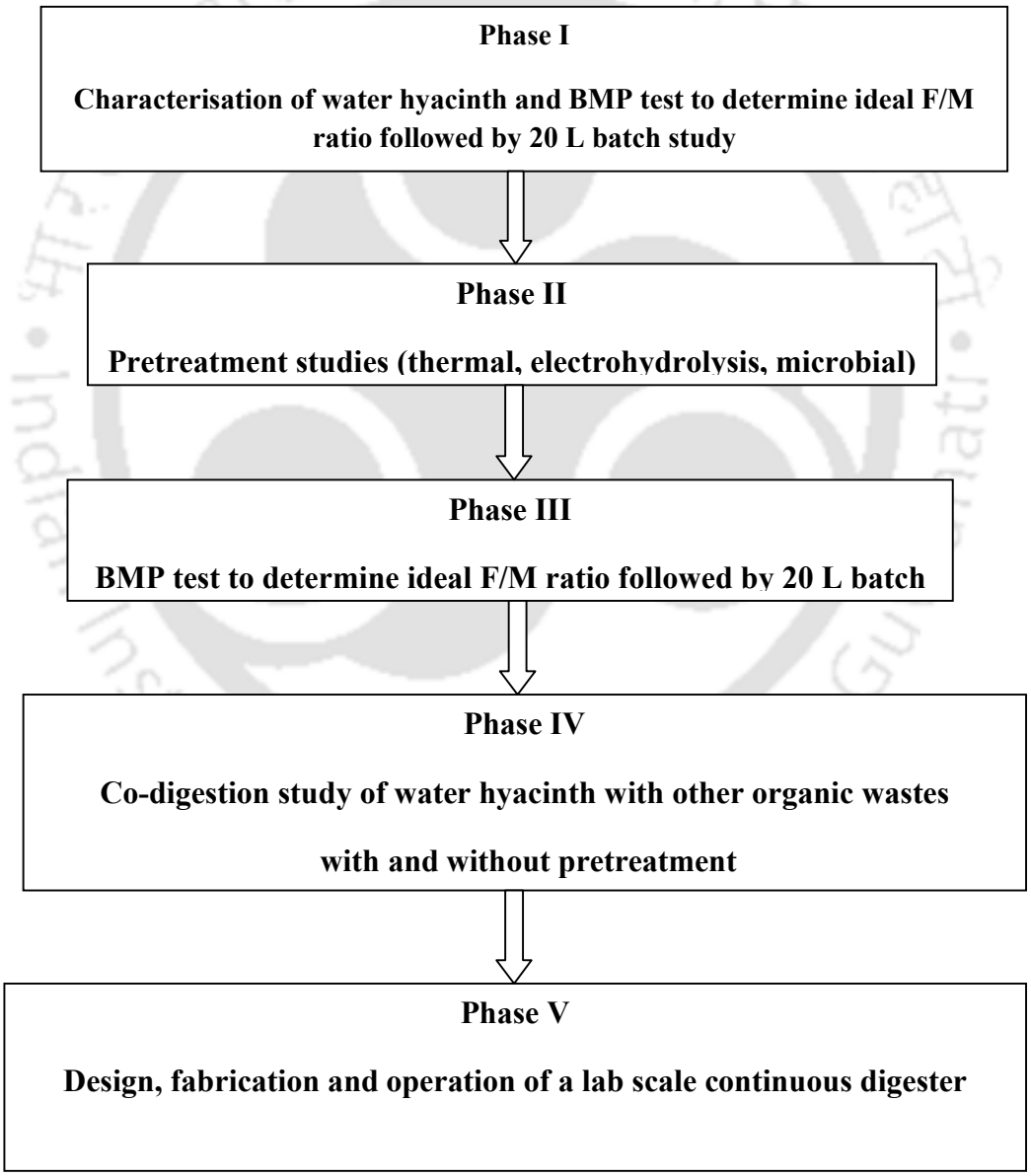


Fig. 3.1. Experimental flowchart

3.2 SUBSTRATE AND INOCULUM

Water hyacinth was collected from the Amingaon industrial area and cow dung was obtained from Amingaon village, located near the campus of Indian Institute of Technology Guwahati (IITG), India (Fig. 3.2). From the initial characterisation study, the average ratio of leaves, stem and roots of a full-grown fresh water hyacinth plant was found to be 13:69:45. Accordingly, the equal ratio of leaves, stem and root of water hyacinth was cut, macerated and utilised for each trial to minimise compositional variation.

Fig. 3.2. (a) Water hyacinth at the site of collection (b) chopped water hyacinth whole plant (c) pulverised water hyacinth and (d) cow dung

For the co-digestion study, *Hydrilla verticillata*, submerged aquatic weed, a co-substrate for co-digestion study, was collected from Deepor Beel, a Ramsar site in Guwahati, Assam, India. While, food waste and banana peels for the anaerobic co-digestion study was collected from the hostel mess of IITG, India. Even the collected food waste was segregated to minimise compositional variation and ground before being introduced into the anaerobic reactor.

3.3 INITIAL CHARACTERISATION STUDY

Initial characterisation study of a substrate is essential to determine whether the substrate is feasible for anaerobic digestion or not (Fig. 3.3). Once the characteristic of the substrate is determined it is easier to conduct experiments depending on the physico-chemical characteristics of the substrate. Moisture content, volatile solids (VS) and soluble chemical oxygen demand (sCOD) were analysed for water hyacinth according to standard methods (APHA, 2005). Moisture content is an analysis is used to determine the water content of a material by drying a sample to constant mass at a specified temperature. For analysing VS, the samples were initially oven dried at 105°C for 24 h and then ignited at 550°C for 2 h. Volatile fatty acid (VFA) was analysed using DiLalo and Albertson (1961) pH titration method. For sCOD and VFA, 5 g of blended mixture of the sample was taken and the volume was made up to 100 mL by distilled water. The sample was then kept in a horizontal shaker for 2 h at 150 rpm and filtered. The filtered sample was then directly used for analysis. Unfiltered sample was utilised to determine the pH of the sample. Dried sample was used to analyse lignin, cellulose and hemicellulose. Lignin, cellulose (NREL procedure, Updegraff, 1969) and hemicellulose analysis were also performed. 0.3 g (dry mass) of sample was utilised to examine the quantity of acid insoluble lignin by gravimetric method. The hydrolysate acquired subsequent to filtration of the cooled insoluble lignin was utilised to determine acid soluble lignin by UV spectrophotometer (205 nm). For analysing cellulose, acetic/nitric acid (3mL) was poured to the 0.5 g of sample and allowed to boil. The centrifuged supernatant was disposed after cooling. Anthrone reagent was poured in the diluted sample and boiled for 10 mins. Later the absorbance was determined at 630 nm. The residue was rinsed initially with distilled water followed by 67% H_2SO_4. Hemicellulose was determined by substracting acid detergent fibre (ADF) from neutral detergent fibre (NDF) (Goering and Van, 1975).

Fig. 3.3. Performing inital characterisation of water hyacinth

3.4 ANAEROBIC BATCH STUDY TO OPTIMISE F/M RATIO

BMP test was carried out in batch mode in 1L glass reagent bottles. The amount of cow dung and water hyacinth utilised was optimised on the basis of VS. F/M ratio of 0.5, 1.0, 1.5, 2.0 and 2.5 was studied. Two reactors as control were incorporated one with only cow dung and the other with only water hyacinth. Tests were conducted in triplicate for each F/M ratio and the control. F/M ratio is the amount of VS of water hyacinth divided by the amount of VS of cow dung. The batch reactors were fed with different amount of water hyacinth, cow dung and with essential macro and micro nutrients (phosphate buffer, ferric chloride, calcium chloride, magnesium sulphate, nickel chloride, cobalt nitrate). Cow dung was utilised as an inoculum to seed the anaerobic digester. The level was made up to 700 mL using distilled water. The headspace of each reactor was purged with pure nitrogen gas for 3 min to ensure an anaerobic environment. The reactor bottles were closed with airtight butyl rubber corks then it was connected to aspirator bottles having 1.5 N NaOH (Elliott and Mahmood, 2007). Biogas generation was measured daily by liquid displacement method (Fig. 3.4). 1.5 N NaOH is used instead of water so as to absorb the CO_2 produced (Walker et al., 2009) as CO_2 reacts with NaOH to form sodium carbonate. Thymol blue was added as an alkali indicator. The biogas produced in the reactor passes on to a bottle filled with NaOH, kept under pressure. When a gas bubble entered the NaOH containing bottle, NaOH is pushed out of the bottle into a beaker. The volume of NaOH displaced signifies the amount of methane produced. The displaced NaOH is measured using a measuring cylinder (Veeken and Hamelers, 1999).

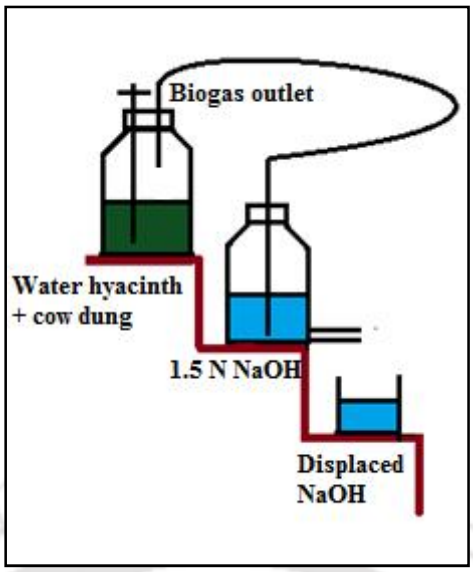


Fig. 3.4. Diagrammatic representation of the batch set up

(a)

(b)

Fig. 3.5. Pictorial representation of (a) 1L BMP set up and (b) 20L batch set up

Biogas composition was examined by gas chromatography (GC) (Thermo Ultra GC) through thermal conductivity detector (TCD) equipped with a PorapakQ column of 182.88 cm length and 2.1 mm i.d. The injector, oven and detector temperatures were set at 150, 60 and 200ºC. Argon was utilised as a carrier gas.Once the 1 L BMP study was over, the batch study was scaled up to 20 L to test the operational conditions during large scale and to decrease the unevenness due to heterogeneous physico-chemical characteristics of water hyacinth. All the essential parameters such as temperature, pH, stirring intensity, physical and chemical characteristics of substrates were monitored properly. Once the ideal F/M ratio is determined, the 1L BMP study is scaled up to 20L batch study with the intention of verifying the operational conditions and variations to be encountered during scaled up process. Both 1L BMP and 20L batch experiments were conducted until biogas generation stabilised. Fig. 3.5 is a representation of 1 L BMP set up and 20 L anaerobic batch experimental setup.

3.5 PRETREATMENT TECHNIQUES

In order to determine the most efficient pretreatment technique for biogas production from water hyacinth, three different types of pretreatment studies were conducted. The three different type of pretreatment techniques investigated are thermal pretreatment, electrohydrolyis pretreatment and biological pretreatment. Depending on the increase in biogas production and reduced hydrolysis period, the most efficient pretreatment technique for biogas production from water hyacinth was determined.

3.5.1 Thermal pretreatment

For temperature study, the prepared samples were kept at different temperature for a particular time. While for time study, the samples were kept at different time at the optimized temperature inside hot air oven, microwave, autoclave and hot water bath. A control sample was kept without giving any kind of thermal pretreatment. Parameters i.e., VFA and sCOD were studied to determine the optimum condition. In order to dissolve the lignin present in the lignocellulosic cell wall of water hyacinth, heat was transferred to the noxious water hyacinth by all the three fundamental modes of heat transfer: conduction, convection and radiation.

- *Hot air oven*

To study the effect of hot air oven pretreatment on the hydrolysis of water hyacinth, pretreatment temperature and time was decided based on previous literatures available (Rafique et al., 2010; Ariunbaatar et al., 2014a). The sealed conical flasks containing the freshly chopped and ground water hyacinth whole plant were heated at 80, 90, 100, 110

and 120ºC for 2 h. To study the effect of hot air oven time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 30, 60, 90, 120 and 150 mins.

❖ *Microwave*

To study the effect of microwave pretreatment on the hydrolysis of water hyacinth, the sealed conical flasks containing the chopped and ground fresh water hyacinth whole plant were heated at 160, 180, 200 and 220ºC for 10 mins. To investigate the effect of microwave time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 5, 10 and 15 mins. On the basis of preliminary study and previous literatures available, pretreatment temperature and time were chosen (Liu et al., 2010; Gabhane et al., 2011; Sapci, 2013; Lin et al., 2015).

❖ *Autoclave*

To study the effect of autoclave pretreatment on the hydrolysis of water hyacinth, the sealed conical flasks containing the chopped and ground fresh water hyacinth whole plant were heated at 80, 90, 100, 110 and 120ºC for 20 mins. To investigate the effect of autoclave time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 20, 40, 60 and 80 mins. Pretreatment temperature and time were selected based on previous literatures available (Sun and Cheng, 2005; Gabhane et al., 2011; Menardo et al., 2012; Toquero and Bolado 2014, Bolado-Rodríguez et al., 2016).

❖ *Hot water bath*

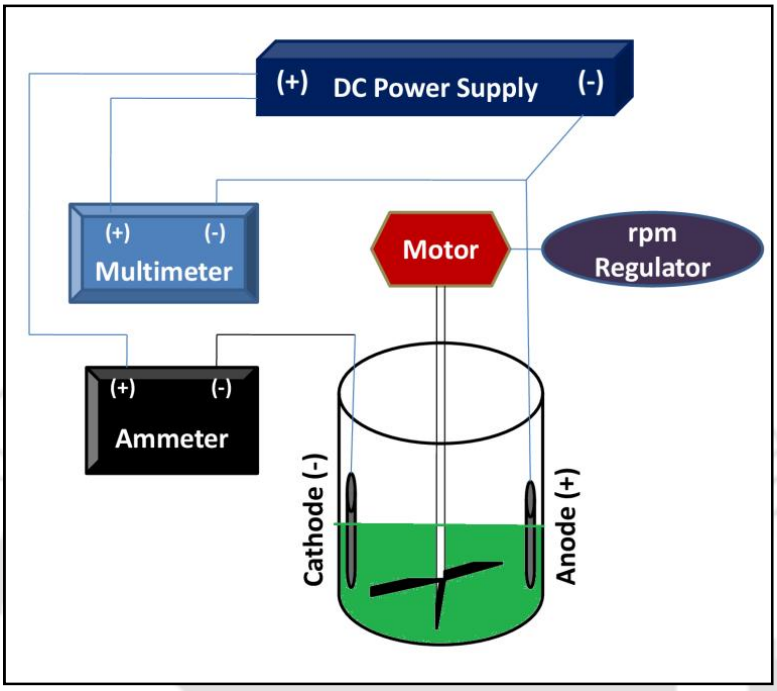
To study the effect of hot water bath pretreatment on the hydrolysis of water hyacinth, pretreatment time and temperature were selected based on previous literatures available (Li et al., 2007; Cho et al., 2013). The sealed conical flasks containing the freshly chopped and ground water hyacinth whole plant were heated at 70, 80, 90 and 100ºC for 30 mins. To investigate the effect of hot water bath time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 30, 60, 90 and 120 mins.

3.5.2 Electrohydrolysis pretreatment

The electrohydrolysis set up consisted of a 2 L plastic feed tank, DC power supplier, two graphite electrodes, flash mixer, ammeter, multimeter and tachometer (Fig. 3.6). The feed tank was half filled with the freshly ground water hyacinth whole plant. DC was passed through the sample with the help of the graphite electrodes which were half immersed in the substrate. The electrodes were placed at a distance of 10 cm. To keep the

substrate in suspension, it was continuously stirred at 300 rpm with the help of an insulated flash mixer. Tachometer was utilized to keep an eye on the speed of the flash mixer. Ammeter was used to measure the current while multimeter was used to check the voltage in the circuit.

(a)



(b)

Fig. 3.6. (a) Diagrammatic representation and (b) experimental set up of electrohydrolysis pretreatment

For voltage study, each sample of water hyacinth was exposed to different voltage. The voltage considered for this study are 10, 15, 20, 25 and 30 V (Zhen et al., 2014; Gharibi et al., 2013; Yuan et al., 2011). A sample was kept as control without providing

electrohydrolysis pretreatment. All samples were exposed to a time period of 30 min. The water hyacinth sample pretreated at 30 V was discarded due to excessive foaming. For time study, each sample was exposed to different time interval (20, 40, 60, 80 and 100 min) at the optimised voltage (Gharibi et al., 2013; Yuan et al., 2011). The sample pretreated at 100 min was discarded due to excessive foam formation. A sample was kept as control without giving any treatment. Optimum voltage and time exposure was selected based on sCOD and VFA.

3.5.3 Biological pretreatment

The aim of this microbial pretreatment study was to optimise the ideal bacterial strain for enhancing the hydrolysis of water hyacinth during anaerobic digestion (Fig. 3.7).

Fig. 3.7. Performing serial dilution and streaking during microbial pretreatment

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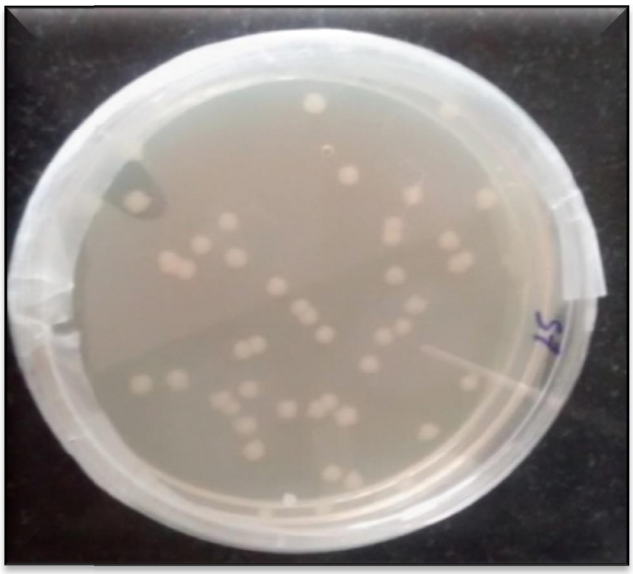
(a)



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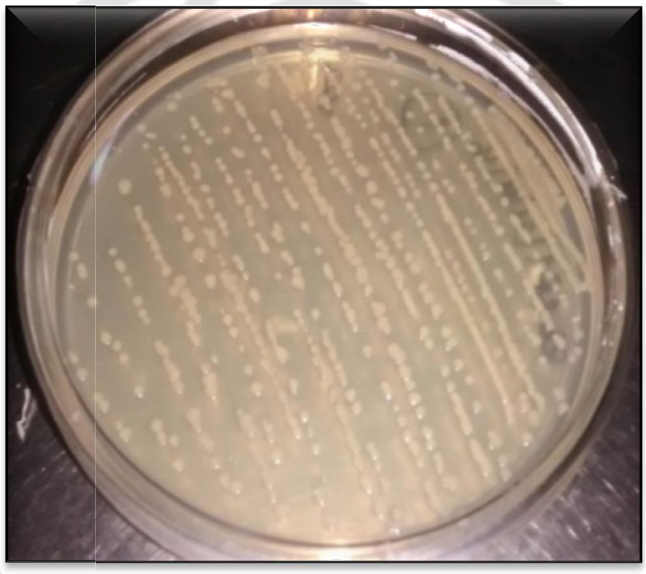


(c)

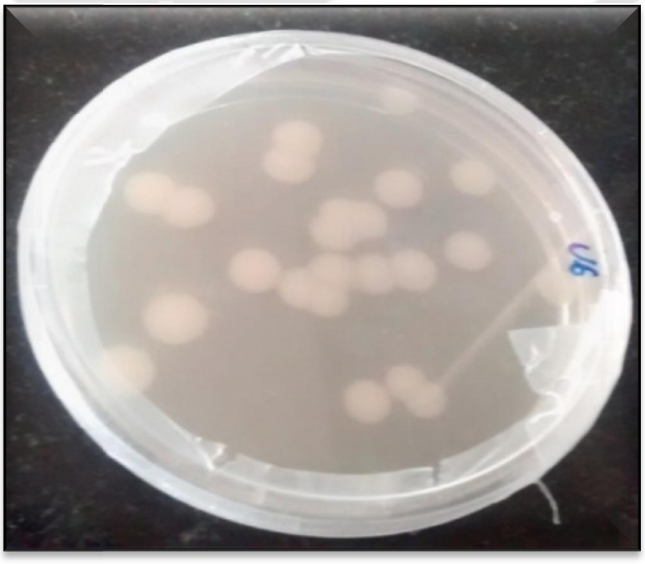


Fig. 3.8. Bacterial isolates of (a) silverfish, (b) millipede and (c) soil

Bacterial strain isolated from soil (*Bordetella muralis VKVVG5*), silver fish (*Citrobacter werkmanii VKVVG4*) and millipede (*Paenibacillus sp. VKVVG1*) was cultivated in autoclaved carboxymethyl cellulose (CMC) media for 2 days at 37ºC, 120 rpm (Fig. 3.8). The bacterial culture was ready for inoculation after 2 days of cultivation. The freshly pulverised water hyacinth mixed with minimal salt media (MSM) was inoculated with different dosage of bacterial culture (10^8, 10^9 and 10^{10} CFU/mL). After inoculation, the substrate was kept inside a shaking incubator at 37ºC, 120 rpm. A control sample (only pulverised water hyacinth) was set aside exclusive of microbial pretreatment. Bacterial culture was conducted according to Azizi-Shotorkhoft et al. (2016). Sample analysis was performed on every second day. Analytical study of sCOD and VFA were performed to determine the optimum condition required for the solubilisation of water hyacinth during microbial pretreatment.

3.5.4 Compositional analysis and characterisation

National renewable energy laboratory (NREL) (Templeton and Ehrman, 1995; Ehrman, 1996) and Updegraff (1969) method was handy in determining the composition of lignin and cellulose respectively. 0.3 g (dry weight) of sample was used to determine acid insoluble lignin content by gravimetric method. Acid soluble lignin was determined by UV spectrophotometer at 205 nm with the hydrolysate, obtained after filtration of the cooled insoluble lignin. For cellulose analysis, acetic/nitric acid (3mL) was added to 0.5 g of sample and boiled. After cooling, the centrifuged supernatant was discarded. To the diluted sample, anthrone reagent was added and boiled for 10 mins. Finally the absorbance was measured at 630 nm. The residue was washed with distilled water followed by 67% H_2SO_4. Goering and Van (1975) method helped in determining hemicelluloses by finding the difference between neutral detergent fibre (NDF) and acid detergent fibre (ADF).

Double-coated gold samples were utilized to make conductive samples for imaging, to put off degradation and for charge build up. FESEM (Zeiss, Sigma) micrographs of water hyacinth before and after pretreatment were taken at an accelerating voltage of 20 kV and an energy resolution of 130 eV. The X-ray diffractograms were recorded with an XRD diffractometer (Bruker, D-8 Advance) from 5-35ºC of diffraction angle (2θ) at a scanning speed of 5º/min. FTIR (PerkinElmer Spectrum 2) was employed to identify the chemical changes before and after the application of thermal pretreatment. 1 mg of dried water hyacinth 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. FTIR spectrum was recorded from 4000 to 400 cm^{-1} with 16 scans at a resolution of 4 cm^{-1}

3.6 ANAEROBIC CO-DIGESTION

Anaerobic co-digestion of water hyacinth was conducted with food waste, *hydrilla verticillata* and banana peels with varying range of mixing ratios to improve the operational stability of the overall anaerobic digestion process.

3.6.1 Co-digestion of water hyacinth and food waste

Two sets of BMP test were performed for the anaerobic co-digestion of water hyacinth with food waste. Set I consisted of the untreated water hyacinth and food waste while set II consisted of pretreated water hyacinth and food waste. In set II, water hyacinth was pretreated inside hot air oven at 90ºC for 1h before being introduced for the BMP test. The test was carried out for the mixing ratios 1.0, 1.5, 2.0 and 2.5 in triplicate. The food waste mainly composed of rice, lentil and vegetables.The quantity of water hyacinth, food waste and cow dung utilised was determined on the basis of volatile solids (VS) (Table 3.1).

Table 3.1. Quantity of water hyacinth, food waste and cow dung used for the various mixing ratios on the basis of VS

Mixing ratio	Water Hyacinth (g)	Food waste (g)	Cow dung (g)
Control 1	---	--	50
Control 2	50	--	--
1.0	47	16	50
1.5	36	12	50
2.0	30	10	50
2.5	26	9	50

Two reactors were supplied; one with only cow dung and another with only water hyacinth which were control 1 and control 2 respectively. Tests were conducted in 1 L glass batch reactors and were supplied with essential macro and micro nutrients besides the substrate and the inoculum. The total volume of each glass batch reactors was 700 mL. The reactors were sealed with rubber corks and connected to aspirator bottles containing 1.5 N NaOH (Elliott and Mahmood, 2007). To uphold anaerobic state,

nitrogen gas was flushed inside the reactor for 3 min. The experiment was performed for 50 days for both set I and set II.

3.6.2 Co-digestion of water hyacinth and hydrilla

A lab scale experiment was conducted in anaerobic glass batch reactors of 1 L. Batch anaerobic co-digestion of water hyacinth with hydrilla were performed simultaneously in two sets. Set I comprised of the untreated water hyacinth and hydrilla whereas set II comprised of pretreated water hyacinth and hydrilla. Prior to the batch test, water hyacinth and hydrilla was pretreated within hot air oven at 90ºC for 1h in set II. The test was performed for the mixing ratios: 1.0, 1.5, 2.0 and 2.5. The amount of water hyacinth, hydrilla and cow dung to be utilised in each reactor was determined on the basis of volatile solids (VS) (Table 3.2).

Two controls were run; cow dung without any co-substrate was used as control 1 and only hydrilla without any co-substrate and inoculum was used as control 2. All the mixing ratios for both sets were supplied with essential macro (phosphate buffer) and micro nutrients (ferric chloride, calcium chloride, magnesium sulphate, nickel chloride, cobalt nitrate) along with the co-substrate and the inoculum according to the mixing ratio. Triplicate reactors were run for each mixing ratio in both the sets to ensure reliable results. The total working volume of each reactor was 700 mL. The headspace of each reactor was purged with pure nitrogen gas for 3 min to ensure an anaerobic environment. Then the reactors were sealed with airtight butyl rubber corks and attached to aspirator bottles containing 1.5 N NaOH. Each reactor was mixed manually twice a day. The experiment was performed for 45 days for both set I and set II.

Table 3.2. Actual amount of water hyacinth, hydrilla and cow dung used for the various mixing ratios on the basis of VS

Mixing ratio	Water hyacinth (g)	Hydrilla (g)	Cow dung (g)
Control 1	---	---	50
Control 2	---	50	---
1.0	47	60	50
1.5	36	47	50
2.0	30	39	50
2.5	26	34	50

3.6.3 Co-digestion of water hyacinth and banana peels

Two sets of BMP test were performed for the anaerobic co-digestion of water hyacinth with banana peels. Set I consisted of the untreated water hyacinth and banana peels while set II consisted of pretreated water hyacinth and food banana peels. In set II, water hyacinth was pretreated inside hot air oven at 90ºC for 1h before being introduced for the BMP test. The test was carried out for the mixing ratios 1.0, 1.5, 2.0 and 2.5 in triplicate. The quantity of water hyacinth, banana peels and cow dung utilised was determined on the basis of volatile solids (VS) (Table 3.3). Two reactors were supplied; one with only cow dung and another with only water hyacinth which were control 1 and control 2 respectively. Tests were conducted in 1 L glass batch reactors and were supplied with essential macro and micro nutrients besides the substrate and the inoculum. The total volume of each glass batch reactors was 700 mL. The reactors were sealed with rubber corks and connected to aspirator bottles containing 1.5 N NaOH (Elliott and Mahmood, 2007). To uphold anaerobic state, nitrogen gas was flushed inside the reactor for 3 min. The experiment was performed for 50 days for both set I and set II.

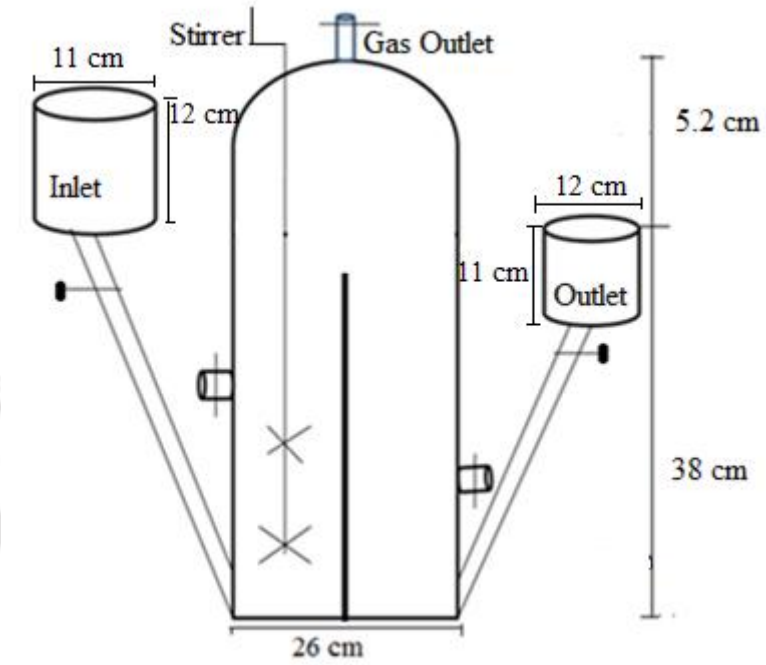
Table 3.3.Quantity of water hyacinth, banana peels and cow dung used for the various mixing ratios on the basis of VS

Mixing ratio	Water hyacinth (g)	Banana peels (g)	Cow dung (g)
Control 1	---	--	50
Control 2	50	--	--
1.0	47	187	50
1.5	36	146	50
2.0	30	121	50
2.5	26	104	50

3.7 CONTINUOUS DIGESTER

A novel two stage biogas digester was constructed with stainless steel (Fig. 3.9). The cylindrical shaped digester has a volume of 20 L. Unlike other two stage biogas digesters, which comprise of two different reactor vessels for hydrolysis, acidogenesis and methanogenesis; here a single reactor was separated in between solving the problem of enormous space requirement. Even a four blade agitator impeller was provided in the first part to impart axial (up and down) mixing. Axial flow impellers are useful in mixing

solid-liquid suspension as it prevents the solid particles from settling at the bottom of the tank.



(a)

(b)

Fig. 3.9. (a) Schematic and (b) pictorial representation of the novel anaerobic digester

The novel digester was provided with an inlet, outlet, gas outlet and two sample collection ports.The gas outlet was connected to an aspirator bottle containing 1.5 N NaOH in order to measure daily biogas production by liquid displacement method. The gas bubble entering the aspirator bottle filled with NaOH, shoves out NaOH solution into a beaker due to the formation of excess pressure. The amount of NaOH shoved out of the aspirator bottle determines the amount of biogas produced which is quantified by a measuring cylinder.

This two stage novel biogas reactor was run in continuous mode for three different experiments for 70 days each. Initially, the reactor was fed with only freshly pulverised water hyacinth followed by hot air oven pretreated water hyacinth at 90°C for 1h for the next 70 days and finally water hyacinth mixed with food waste was fed for the last 70 days. Prior to continuous feeding of the digester with water hyacinth, 5 kg of cow dung slurry was fed in the digester and kept as it is for 40 days. These 40 days was actually the acclimatisation period. After 40 days, feeding of the digester started at a very low OLR which was gradually increased until the pH of the digester fell. The digester was operated at room temperature.

3.7.1 Mass balance

The mass balance of the continuous anaerobic digestion process using utreated, pretreated and co-digested water hyacinth were calculated based on the obtained experimental data of COD (Fig.3.10). Mass balance was evaluated by measuring the total mass entering the anaerobic digester and the corresponding mass that was converted into biogas and the remaining sludge. Methane generated was represented in terms of COD and sludge generation was 10% of influent COD.

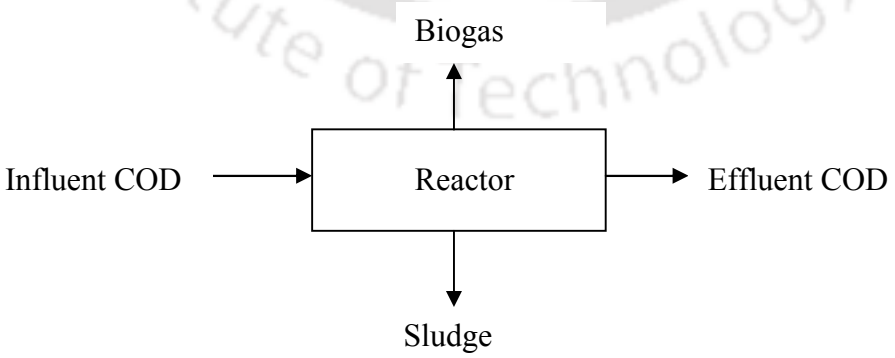


Fig. 3.10. Illustrating the overall mass balance of the process.

3.8 SAMPLE ANALYSIS

Analysing the samples is beneficial in order to determine the changes undergone in the substrate during the experiments due to microbial activity. Various experimental studies were carried out during the study to accomplish the stipulated objectives.

VS was measured. Initial weight of the crucible was taken as W_1 g. Weigh (10±0.1g) of sample in crucible and kept it in hot air oven for 24 h at 105ºC. After 24 h of heating, the crucible is weighed W_2 g after cooling. Then the crucibles are placed inside a muffle furnace operating at a temperature of 550°C for 2 h. After 2 h crucible was taken out off the muffle furnace and kept in desiccator for ½ h for cooling and then final weight of crucible with sample was taken as W_3 g. VS content of the samples were calculated according to eq. 3.1;

$$\text{VS}\ (\%) = \frac{(W2-W3)}{W2-W1} * 100 \quad \text{..... (3.1)}$$

For sCOD analysis, 5 g of blended mixture of the sample was taken and the volume was made up to 100 mL by distilled water. The sample was then kept in a horizontal shaker for 2 h at 150 rpm and filtered. Then it was filtered using Whatman filter paper and directly used for analysis. The supernatant of samples were taken and analysed for sCOD using closed reflux method where, 1.5 mL of $K_2Cr_2O_7$, 2.5 mL of sample and 3.5 mL of COD acid were added. The COD vial was tightly closed to avoid any leak and mixed. Then the COD vials were digested in a COD digester for 2 h at 150°C and cooled down to room temperature. Then to the cooled samples 2-3 drops of ferroin indicator were added and titrated against ferrous ammonium sulphate (FAS) until the color of the sample changes from green to wine red. Eq. 3.2 was utilised for determining the sCOD of the samples;

$$\text{sCOD}\ \left(\frac{\text{mg}}{\text{L}}\right) = \frac{\{(A-B)\times \text{molarity of FAS}\times \text{D.F}\times 8000\}}{\text{Volume of the sample}} \quad \text{..... (3.2)}$$

where,

A is the mL of FAS used for blank

B is the mL of FAS used for sample

D.F is the dilution factor

8000 is the milliequivalent weight of O_2 x 1000 mL/L

VFA was analysed by titration method on basis of pH (DiLallo and Albertson, 1961). VFA, 5 g of blended mixture of the sample was taken and the volume was made up to 100 mL by distilled water. The sample was then kept in a horizontal shaker for 2 h at 150

rpm and filtered. Then it was filtered using Whatman filter paper and directly used for analysis. Initially the pH of the 50 mL of the filtered sample was measured. Then the pH of the sample was made 3.3-3.5 using 0.05N H_2SO_4. The 50 mL filtered sample was then allowed to boil for 3 mins. After cooling, the pH of the sample is adjusted to 4 and the amount of 0.05N NaOH consumed for making the pH 7 is measured.

$$\text{VFA}\left(\frac{\text{mg}}{\text{L}}\right) = \frac{2500 \times \text{mL of 0.05N NaOH consumed} \times \text{Dilution factor}}{\text{Volume of the sample (mL)}} \quad \ldots\ldots (3.3)$$

If VFA was observed to be greater than 180 mg/L, then it was multiplied by 1.5.

3.9 INSTRUMENTAL ANALYSIS

3.9.1FESEM

FESEM is utilised to observe extremely minute topographic details on the surface of the sample. Electrons are liberated from a field emission source and hastened in a high electrical field gradient. Inside the high vacuum column, the primary electrons are focussed and redirected by electronic lenses to produce a constricted scan beam that bombards the object. As a result secondary electrons are liberated from each spot on the object. The angle and velocity of these secondary electrons relates to the surface configuration of the entity. A detector takes hold of the secondary electrons and produces an electronic signal. This signal is amplified and transformed to a video scan illustration that can be detected on a screen or to a digital image that can be saved and processed further. Double-coated gold samples were utilised to make conductive samples for imaging, to put off degradation and for charge build up. FESEM (Zeiss, Sigma) micrographs of water hyacinth before and after pretreatment were taken at an accelerating voltage of 20 kV and an energy resolution of 130 eV.

3.9.2 XRD

The analysis of XRD is based on constructive interference of monochromatic X-rays and a crystalline sample. The X-rays generated by a cathode ray tube are strained to generate monochromatic radiation, collimated to concentrate and deviated in the direction of the sample. When Bragg's Law ($n\lambda=2d \sin \theta$) is satisfied, interaction of the incident rays with the sample generates constructive interference and a diffracted ray. This law relates the wavelength of electromagnetic radiation to the diffraction angle and the lattice spacing in a crystalline sample.The distinctive X-ray diffraction archetype generated in a XRD analysis endows with an exclusive "fingerprint" of the crystals available in the sample. When standard reference prototypes and dimensions are inferred, this fingerprint

permits recognition of the crystalline form. When the 2D diffraction pattern is recorded, it displays concentric loops of scattering peaks analogous to the different spacings in the crystal lattice. Sharp peaks are observed if a sample is crystalline in nature otherwise peaks are absent in amorphous samples.

XRD is a rapid analytical technique primarily used for phase identification of a crystalline material and can provide information on unit cell dimensions. The analysed material is finely ground, homogenised and average bulk composition is determined. The X-ray diffractograms were recorded with an XRD diffractometer (Bruker, D-8 Advance) from 5ºC to 35ºC of diffraction angle (2θ) at a scanning speed of 5º/min.

3.9.3 FTIR

FTIR endows with quantitative and qualitative analysis of organic and inorganic samples. FTIR aids in recognising chemical bonds in a molecule by generating an infrared absorption spectrum. The spectra generate a silhouette of the sample, a distinctive molecular fingerprint that can be utilised to monitor and examine samples of different components. FTIR is an effective investigative mechanism for recognising functional groups and characterising covalent bonding information.

FTIR (PerkinElmer Spectrum 2) was employed to identify the chemical changes before and after the application of thermal pretreatment. 1 mg of dried water hyacinth 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. FTIR spectrum was recorded from 4000 to 400 cm^{-1} with 16 scans at a resolution of 4 cm^{-1}.

3.9.4 GC

GC is a frequently utilised investigative mechanism for detection and quantification of compounds in a mixture. The sample solution introduced into the GC enters a gas stream which passes on the sample into a separation tube identified as the column. Chemically inert gas is utilised as a carrier gas. The various constituents available in the gaseous sample are separated inside the column. The detector determines the amount of the components that leaves the column. To estimate a sample with an unknown concentration, a standard sample with known concentration is injected into the GC. The standard sample peak retention time and area are compared to the test sample to estimate the concentration.

Biogas composition was examined by GC through thermal conductivity detector (TCD) equipped with a PorapakQ column of 182.88 cm length and 2.1 mm i.d. The

injector, oven and detector temperatures were set at 150, 60 and 200ºC. Argon was utilised as a carrier gas.

3.9.5 UV- visible spectrophotometer

The operational principle of a UV-visible spectrophotometer is reasonably simple. A beam of light from a visible or UV light source is separated by a prism or diffraction grating into its component wavelength. Each monochromatic (single wavelength) beam in turn is split into two equal intensity beams by a half-mirrored device. The sample beam, passes through a small transparent glass or quartz cuvette filled with a solution of the compound being studied in a transparent solvent. The other beam, the reference beam, passes through a similar cuvette filled with only the solvent. In other words, UV-visible spectroscopy is the measurement of the attenuation of a beam of light after it passes through a sample solution or after reflection from sample surface.

3.9.6 Laminar air flow

A laminar air flow chamber upholds a unidirectional flow of high efficiency particulate air (HEPA) filtered air over the working region and shields the working environment from dust and other air-born particulates. Air is drawn through a HEPA filter and blown very smoothly, laminar flow in the route of the user. Microbial pretreatment of water hyacinth was strictly performed inside laminar air flow chamber in order to avoid contamination. Laminar air flow chamber has a UV-C germicidal lamp which is generally switched on for 15 mins to disinfect the chamber and contents meticulously before usage to avoid contamination of samples.

3.9.7 Shaking incubator

To endow with most favourable condition for cell growth, agitation or shaking is essential to incorporate oxygen and evenly distribute nutrients throughout the culture media. Shaking incubator was very useful for growing bacterial cultures during microbial pretreatment. The temperature and the speed of rotation of the shaking incubator can be controlled in order to facilitate the microorganisms with ideal condition for growth.

3.10 KINETIC STUDY

Batch degradation can be modeled using Gompertz equation to obtain parameters determining methanogenesis performance. This model was initially used to portray the growth of bacteria in batch mode (Lay et al., 1996). Thus, modified Gompertz equation (3.4) was employed to the monitored cumulative methane production assuming methane production is a function of bacterial growth to establish the highest methane production potential,

$$Y = M \times exp\left\{-exp\left[\frac{R_m \times e}{M}(\lambda - 1) + 1\right]\right\}. \ldots\ldots\ldots \quad (3.4)$$

Where Y represents the cumulative methane production (mL) at time t (d), M is the maximum methane production potential (mL CH_4), Rm is the maximum methane production rate (mL CH_4 d^{-1}), λ is the lag phase time (d) and e is constant equal to 2.71. The three parameters M, R_m and λ were estimated by curve-fitting using Matlab R2015b by minimising the residual amount of squared inaccuracy among the experimental data and the modeled curve.

3.11 INSTRUMENTS REQUIRED

Various instruments were utilised during the research work in order to analyse the samples and accomplish the aims and objectives of the present study. The various instruments that were utilised during the study and their brand names have been incorporated in Table 3.4.

Table 3.4. Various instruments and their brand name that were utilised during the study

Intruments	Brand
pH meter	Systronics
Weighing balance	Wesnar
Hot air oven	Ikon Instruments
Muffle furnace	LabTech
Horizontal shaker	Optics Technology
COD digester	Hach DRB200
Hot water bath	LabTech
Autoclave	Equitron
Microwave	Samsung
Mixer and grinder	Bajaj
UV- visible spectrophotometer	Lambda 45
Laminar air flow	Ikon Instruments
Shaking incubator	Orbitek
FESEM	Zeiss
XRD	Bruker

FTIR	PerkinElmer
GC	Ultra GC T100

CHAPTER 4
PRETREATMENT STUDY

This chapter deals with the effect of different pretreatment techniques i.e., thermal, electrohydrolysis and biological on the solubilisation of water hyacinth during anaerobic digestion in order to reduce the hydrolysis time period and enhance biogas generation.

4.1 INITIAL CHARACTERISATION

The characterisation study of water hyacinth incorporated in Table 4.1 illustrates that the substrate has a good amount of moisture, sCOD, VS and cellulose thereby suggesting its feasibility as a feedstock for anaerobic digestion. Although the pH is in acidic range, addition of cow dung will maintain the stability of the anaerobic digester. The presence of lignin illustrates the necessity of pretreatment of water hyacinth prior to anaerobic digestion to accelerate the hydrolysis period and enhance biogas production.

Table 4.1. Initial characteristics of water hyacinth

Parameters	Water Hyacinth
pH	5.5±2
Moisture Content (%)	90±5
sCOD (mg/L)	1600±50
VFA (mg/L)	750±10
VS (%)	65±5
Acid Soluble Lignin (%)	7.5±3
Acid Insoluble Lignin (%)	12±2.5
Cellulose (%)	32±5
Hemicellulose (%)	24±1.5

4.2 THERMAL PRETREATMENT

The present study investigated the effect of four different thermal pretreatment techniques i.e., hot air oven, microwave, autoclave and hot water bath on the hydrolysis of water hyacinth.

In order to soften the lignin, heat was transferred to the noxious weed by all the three fundamental modes of heat transfer: conduction, convection and radiation. In, hot air oven pretreatment, mode of heat transfer takes place initially by convection followed by conduction. Dry heat is uniformly circulated throughout the chamber with the help of a

fan. The dry heat is absorbed initially by the outer layer and then it gradually passes towards the centre, layer by layer, thereby rupturing the cell wall of water hyacinth. The passage of dry heat through air to the glassware containing the substrate is convection. Then, from the glassware to the substrate is conduction. In hot water bath, heat transfer occurs through convection initially followed by conduction just like hot air oven. But, the medium of heat transfer is hot water instead of hot air. Also, the hot water is in direct contact with the base of the glassware containing the substrate but the hot air entirely surrounds the glassware from all the direction in the hot air oven. The hot water set at specific temperature for a specific time period passes on the heat to the lignocellulosic biomass to be pretreated. While in an autoclave the moist heat solubilises the cellulose and helps in melting down the lignin (Tampio et al., 2014). Thermal pretreatment of water hyacinth in a microwave is induced by electromagnetic radiation. Due to which, polar molecules in the biomass, is provoked to rotate as they have a partial positive charge at one end and a partial negative charge at the other. As rotating molecules strive to align themselves with the alternating electric field of the microwaves, they push other molecules to put them into motion, thus dispersing thermal energy (Toreci et al., 2009). Microwave radiation is strong enough to break hydrogen bonds present in the weed's cell wall (Kaatze, 1995).

Thermal pretreatment study was conducted in two stages to determine the optimum conditions required for solubilising water hyacinth. They are as follows:

- Temperature study
- Time study

4.2.1 Hot air oven pretreatment

To determine the optimum condition parameters i.e., VFA and sCOD were studied for optimizing both temperature and time required for the hydrolysis of water hyacinth. For temperature study, the prepared samples were kept at different temperature for a particular time. A control sample was kept without giving any kind of thermal pretreatment. The sealed conical flasks containing the freshly ground water hyacinth whole plant were heated at 80, 90, 100, 110 and 120ºC for 2 h. To study the effect of hot air oven time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 30, 60, 90, 120 and 150 mins. From Fig. 4.1a and 4.1b, it can be observed that with the increase in VFA there was an increase in the sCOD. VFA and sCOD increased both hand in hand upto 90ºC and then it decreased. The decrease in sCOD at higher temperature i.e., after 90ºC is due to the loss of VFA through

vaporisation. At 90ºC, chemical bonds in the cell wall disrupted totally releasing the organic compounds (Appels et al., 2010). As, the sample at 90ºC showed the highest sCOD, therefore samples were kept inside hot air oven for time study; for various time period at the optimised temperature. For time study (Fig. 4.1b), the sample kept for 1 h inside hot air oven, showed the highest increase in sCOD. The rise and fall in sCOD with the passage of time, is directly related to the rise and fall of VFA. It was clearly observed that the sample pretreated at 90ºC for 1 h showed a hike of 55.5% sCOD i.e., 2.3 times increase in sCOD when compared to the control. Higher content of sCOD signifies higher biogas recovery cutting down the hydrolysis period (Junoh et al., 2015). Hot air oven pretreatment at 90ºC for 1 h disrupted the chemical bonds of the complex lignocellulosic structure thereby releasing extracellular and intracellular biopolymers into the soluble phase. Ariunbataar et al. (2014a) stated that higher solubilisation can be achieved at temperature below 100ºC but longer treatment duration is required. Thus time lesser than 1 h was too less for complete solubilisation and time higher than 1 h resulted in the loss of VFA. Appels et al. (2010) also observed that sludge solubilisation increased very significantly at 90ºC for 60 mins.

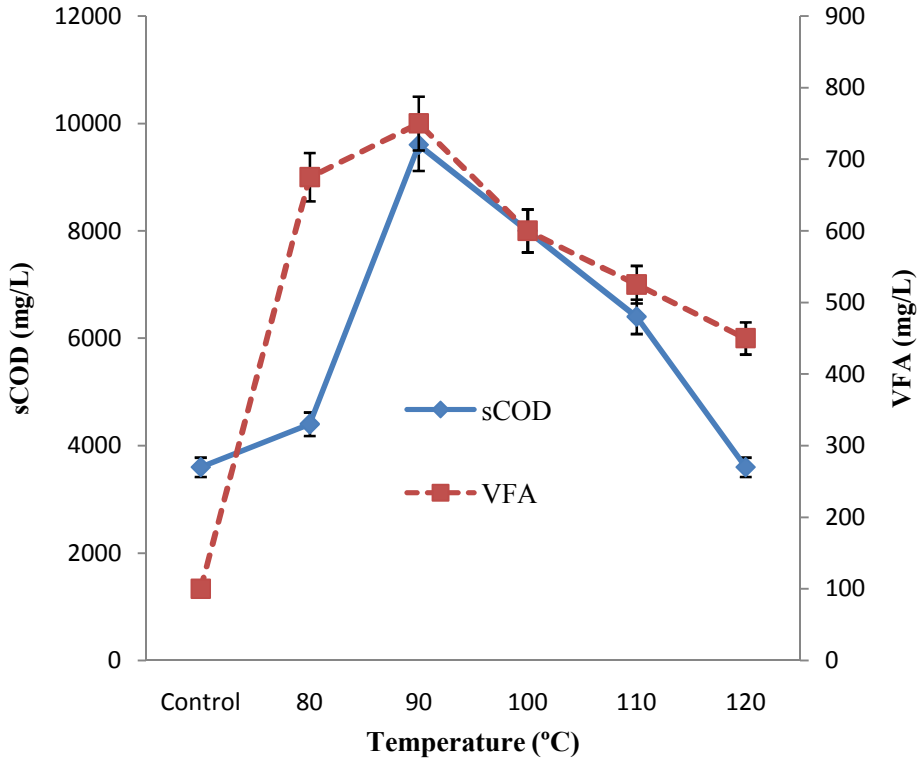


Fig. 4.1a. Effect of temperature on the VFA and sCOD of the different samples kept inside hot air oven for 2 h

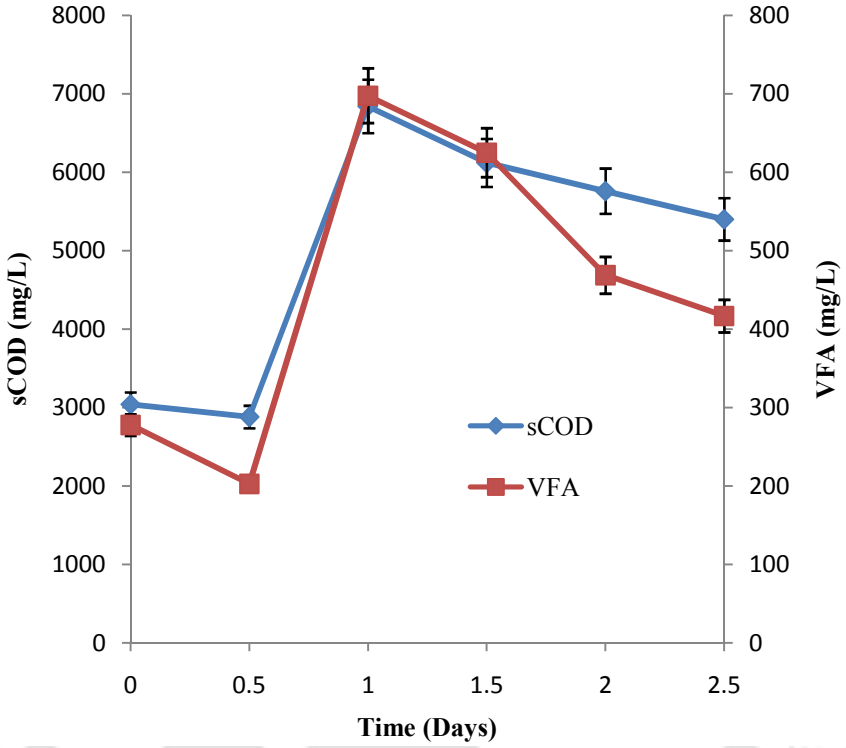


Fig. 4.1b. Effect of time on the VFA and sCOD of the different samples kept inside hot air oven at 90ºC

4.2.2 Microwave pretreatment

To study the effect of microwave pretreatment on the hydrolysis of water hyacinth, the sealed conical flasks containing the chopped and ground fresh water hyacinth whole plant were heated at 160, 180, 200 and 220ºC for 10 mins. To investigate the effect of microwave time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 5, 10 and 15 mins.

For microwave pretreatment temperature study (Fig. 4.2a), it was observed that, with the increase in temperature the VFA decreases but sCOD increases. This may be due to increased solubilisation than the loss of volatile compounds during pretreatment. The sCOD increased with the hike in temperature and it declined at 220ºC after showing the highest amount of sCOD at 200ºC. The chemical reaction between reducing sugars and amino acids in addition to the low water activity at 220ºC turned the sample dry and brown in colour thus, forming refractory compounds like melanoidins (Hendriks and Zeeman, 2009; Carrère et al., 2010; Ariunbataar et al., 2014b). From the time study (Fig 4.2b), 10 mins was optimised, as the boost in sCOD with the augment in VFA upto 10 minutes was witnessed. Lin et al. (2015) optimised 190ºC for 10 mins as they studied the effect of temperature at 150, 190, 210 and 230ºC and the effect of time for 5, 10, 20 and 30 mins respectively. It was analysed that the sample pretreated at 200ºC for 10 minutes

showed a hike of 44.2% sCOD i.e., 1.44 times increase in sCOD when compared to the control.

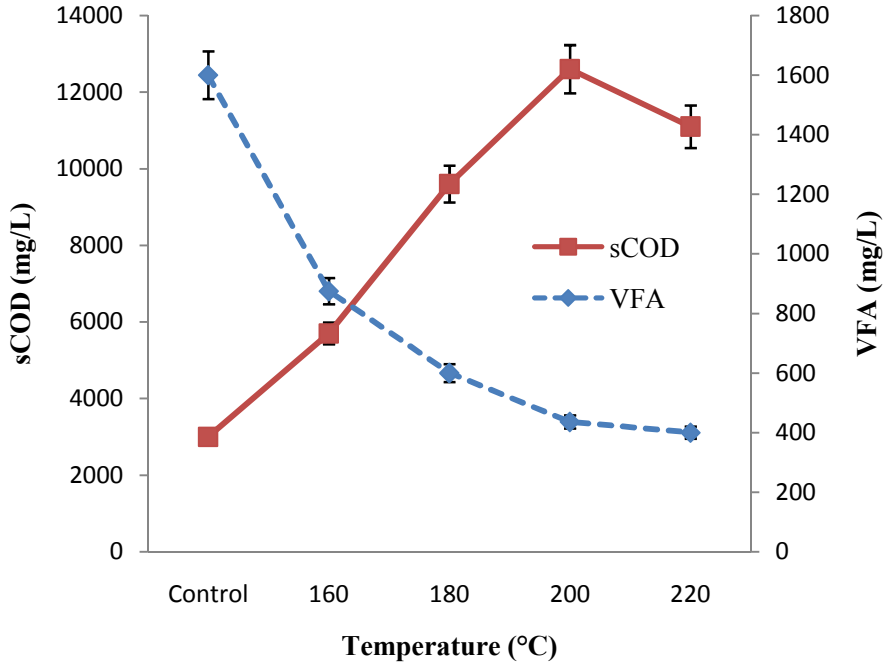


Fig. 4.2a. Effect of temperature on the VFA and sCOD of the different samples kept inside microwave for 10 mins

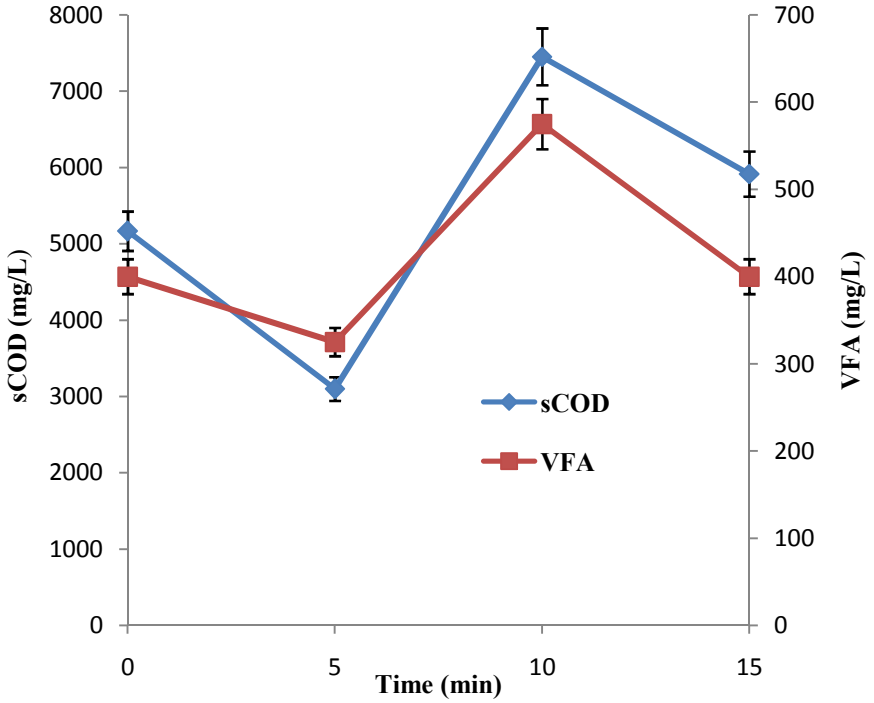


Fig. 4.2b. Effect of time on the VFA and sCOD of the different samples kept inside microwave at 200ºC

4.2.3 Autoclave pretreatment

To study the effect of autoclave pretreatment on the hydrolysis of water hyacinth, the sealed conical flasks containing the chopped and ground fresh water hyacinth whole plant were heated at 80, 90, 100, 110 and 120ºC for 20 mins.

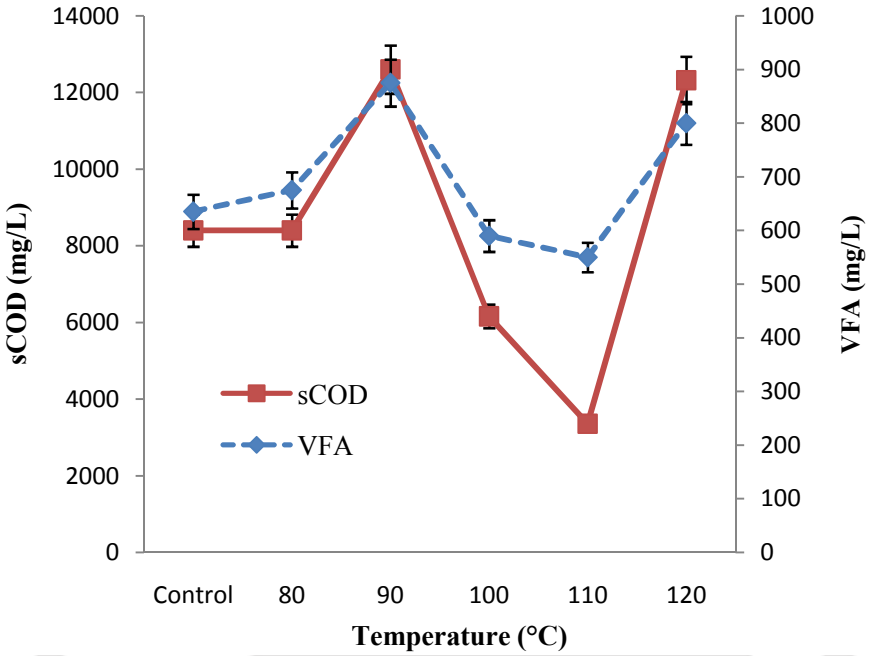


Fig. 4.3a. Effect of temperature on the VFA and sCOD of the different samples kept inside autoclave for 20 mins

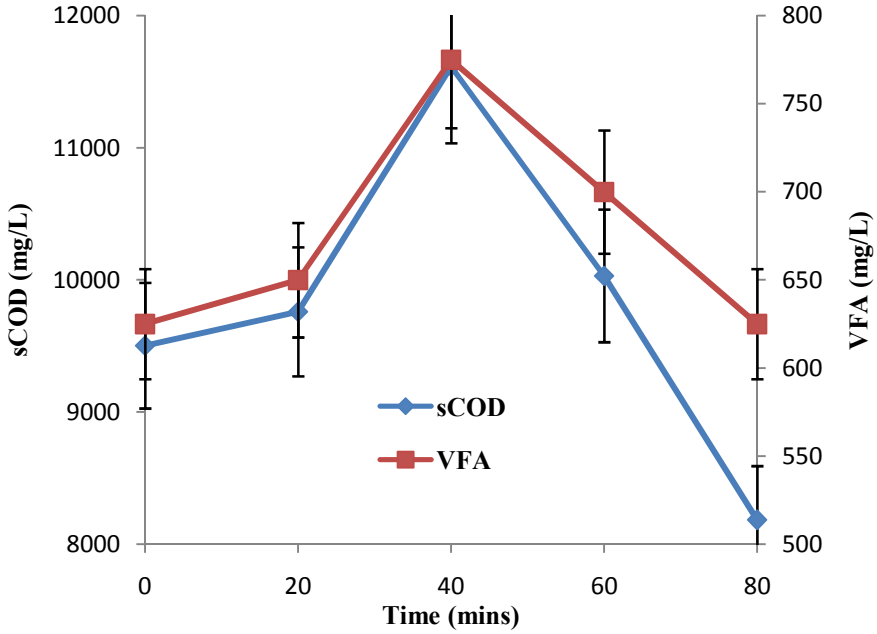


Fig. 4.3b. Effect of time on the VFA and sCOD of the different samples kept inside autoclave at 90ºC

To investigate the effect of autoclave time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 20, 40, 60 and 80 mins. From autoclave pretreatment temperature study (Fig. 4.3a) the increase in sCOD with the increase in VFA and the decline in sCOD with the decline in VFA was evident. However two peaks at two different temperature i.e., 90 and 120ºC were apparent. As the sample pretreated at 90ºC shows sCOD of 12600 mg/L which is more than the sCOD of 12320 mg/L obtained at 120ºC, so 90ºC was considered as the optimised temperature. Menardo et al. (2012) also observed similar results for wheat and barley straw. For time study (Fig 4.3b), the sample kept for 40 mins inside autoclave, showed the highest increase in sCOD. The hike and fall in sCOD with the passage of time is directly related to the rise and drop of VFA. It was found that the sample pretreated at 90ºC for 40 mins showed a hike of 22.2% sCOD i.e., 1.22 times increase in sCOD when compared to the control.

4.2.4 Hot water bath pretreatment

The sealed conical flasks containing the freshly chopped and ground water hyacinth whole plant were heated at 70, 80, 90 and 100ºC for 30 mins. To investigate the effect of hot water bath time on the hydrolysis of water hyacinth, the sealed conical flasks were heated at the optimised temperature for 30, 60, 90 and 120 mins.

From Fig. 4.4a and 4.4b, it is quite obvious that ascend and descend in sCOD with the passage of temperature and time is directly related to the rise and fall of VFA.

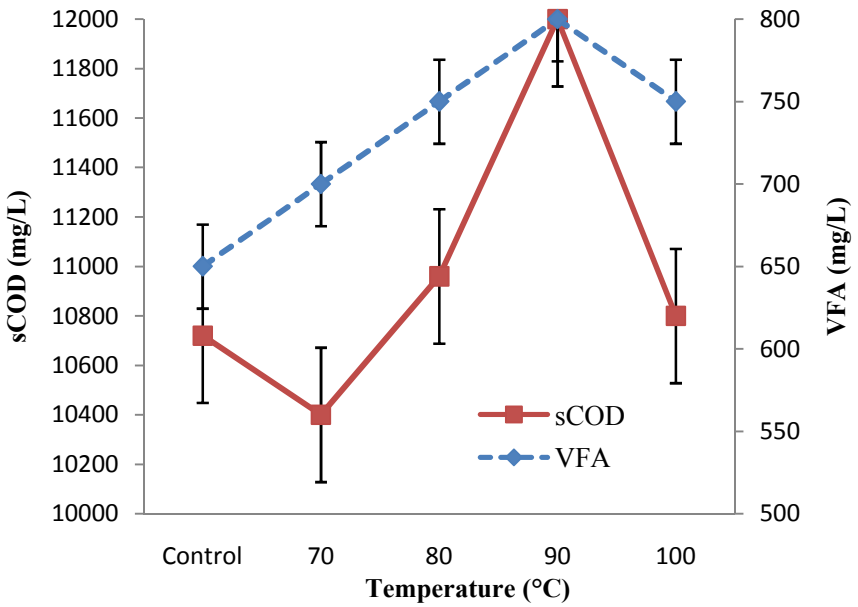


Fig 4.4a. Effect of temperature on the VFA and sCOD of the different samples kept inside hot water bath for 30 minutes

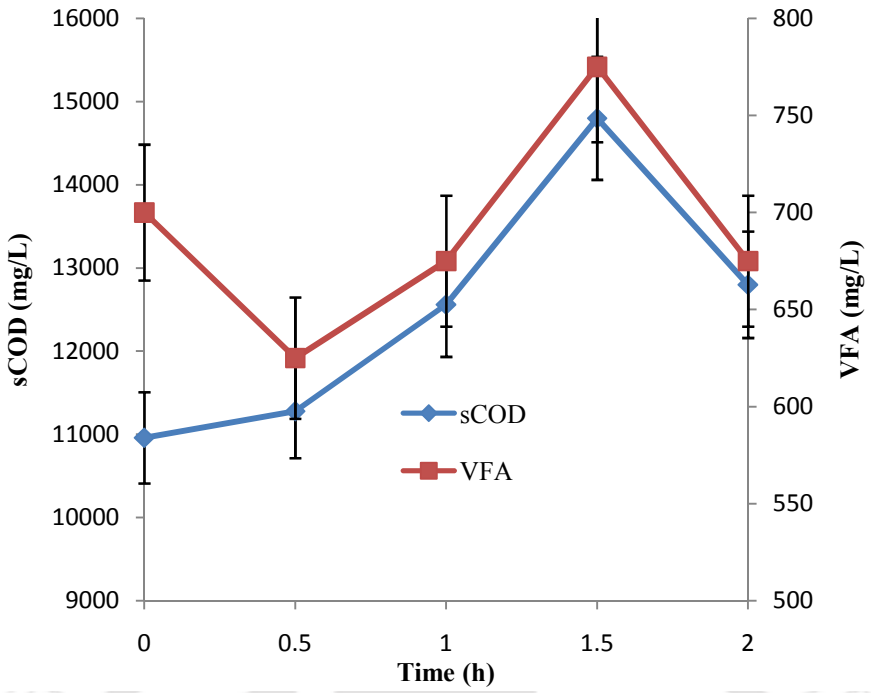


Fig. 4.4b. Effect of time on the VFA and sCOD of the different samples kept inside hot water bath at 90ºC

The decline in sCOD at higher temperature i.e., 100ºC is due to the loss of VFA through vaporisation. The sample at 90ºC confirmed the highest amount of sCOD (Fig. 4.4a). Therefore, samples were kept inside hot water bath for time study; for various time period at the optimised temperature. For time study (Fig. 4.4b), the sample kept for 1.5 h inside hot water bath, showed the utmost boost in sCOD. It was clearly observed that the sample pretreated at 90ºC for 1.5 h showed a hike of 35% sCOD. Thi et al. (2017) optimised hot water bath pretreatment at 100ºC for 1 h as they investigated the effect of temperature at 70, 80 and 100ºC for duration of 30, 60, 90 and 120 min.

4.2.5 Compositional Analysis

Compositional analysis was done to examine the changes in the composition of the thermally pretreated water hyacinth (Table 4.2). Hot air oven pretreated sample confirms the highest percentage of soluble lignin than the other thermal pretreatment techniques, suggesting enhanced delignification. The effectiveness of heat treatment in solubilisation of the lignin was indicated by the increase in the percentage of soluble lignin in the pretreated substrate when compared to the untreated water hyacinth. This suggests the presence of more soluble lignin, thus making the cellulose more easily accessible for the microbes as the lignin has softened. The augment in the percentage of acid insoluble lignin for the thermally pretreated samples than the control may be due to

repolymerization reaction. During thermal pretreatment, lignin is eradicated merely upto a certain limit; however because of dissolution and depolymerisation/repolymerisation of lignin reaction it is redistributed on the fibre surfaces again (Li et al., 2007; Kumar et al., 2009a). The quantity of lignin solubilisation is related to its degradation during pretreatment (Kamdem et al., 2015). Decrease in the percentage of cellulose content of thermally pretreated water hyacinth than untreated water hyacinth is much desired, as it depicts higher solubilisation of the thermally pretreated water hyacinth. Highest reduction in cellulose content was observed in hot air oven pretreated water hyacinth. Focussing on the fact that, cellulose is broken down into their simpler forms thereby enhancing solubilisation (Kumar et al., 2009a). Reduction in cellulose was also observed by previous available literatures (Diaz et al., 2015). Cellulose is a long chain of sugar molecules whereas hemicellulose is a short chain of sugar molecules. During thermal pretreatment, the cellulose long chain might have cleaved at any indefinite point of the chain into glucose fragments of different sizes; i.e., a short chain. This may be the reason behind the increase in hemicelluloses in hot air oven pretreated and other thermally pretreated water hyacinth.

Table 4.2.Changes in the composition of thermally pretreated and untreated water hyacinth whole plant

Pretreatment Technique	Acid Soluble Lignin (%)	Acid Insoluble Lignin (%)	Cellulose (%)	Hemicellulose (%)
Untreated	1.77	6.33	32.84	24.7
Hot air oven	2.62	7.08	19.58	70.5
Microwave	2.08	7.33	24.74	30.4
Hot water bath	1.84	9.05	29.26	26.3
Autoclave	1.58	8.35	29.26	27.8

4.2.6 Characterisation

4.2.6.1 FESEM

FESEM was used to investigate the morphological changes in the thermally pretreated water hyacinth. A rigid compact structure of the untreated water hyacinth was observed with

slight folds for untreated water hyacinth (Fig. 4.5a), a totally deconstructed lignocellulosic matrix for hot air oven pretreated water hyacinth (Fig. 4.5b), rough scaly destructured matrix depicting torn outer layer for microwave pretreated water hyacinth, rough surface with cracks and few hollows for hot water bath pretreated water hyacinth and loosely textured slightly split surface for autoclave pretreated water hyacinth were observed. Thereby, bestowing us an idea of delignification; leading to increased bioaccesibility of cellulose in the hot air oven pretreated water hyacinth followed by microwave, hot water bath and autoclave pretreatment.

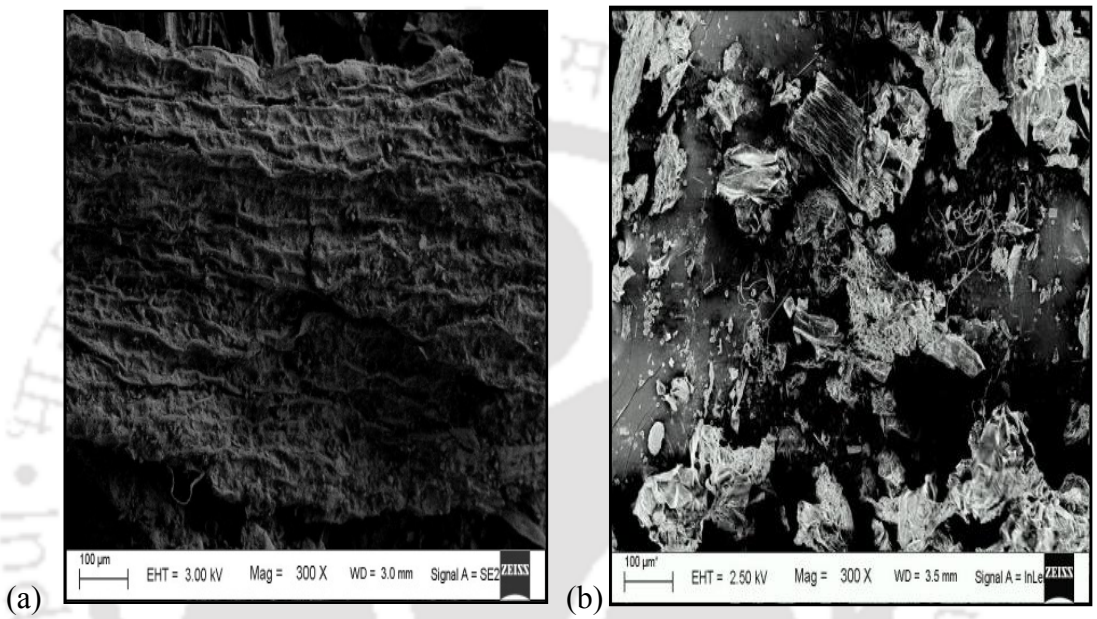


Fig. 4.5. FESEM images of (a) untreated and (b) hot air oven pretreated water hyacinth

4.2.6.2 FTIR

FTIR study was done to examine the chemical transformation in water hyacinth after pretreatment (Fig. 4.6). Fan et al. (2012) stated that ‘‘FTIR provides accurate analysis of constituent natural fibers i.e., cellulose, hemicellulose and lignin.” The change in band intensity after thermal pretreatment at 3333.3 and 2921.22 cm^{-1} signifies the disruption of OH stretch of lignin and CH stretch of cellulose (Wu et al., 2011) present in the water hyacinth cell wall. Moreover, the reduced peak intensity at 1622.64 cm^{-1}, portrays the breakage of C=O stretch in lignin. The absorption peaks at 1000 cm^{-1} corresponds to the C-O, C=C, C-C-O bond of cellulose, hemicellulose and lignin. Whereas the absorption peaks at 3333.3, 2921.22 and 1622.64 cm^{-1} corresponds to lignin only (Xu et al., 2013). The peak at 3333.3 cm^{-1} is attributed to the OH stretch of lignin, 2921.22 cm^{-1} represents the CH stretch of lignin, 1622.64 represents the C=C stretch of the aromatic ring and CH deformation respectively. Significant drop of band intensity observed at 1027.17 cm^{-1} and 1319.28 cm^{-1}

concludes about the C-O, C=C, C-C-O and C-H deformation in cellulose, hemicellulose and lignin due to thermal pretreatment (Kumar et al., 2009b; Li et al., 2010). Change in the peak intentsity of FTIR indicates some transformation in the sample composition while the broadening of peak indicates the occurrence of weaker intra and intermolecular hydrogen bonding and lower crystallinity (Goshadrou et al., 2011). Thus, providing us a clear picture of the modification undergone in the water hyacinth cell wall due to thermal pretreatment.

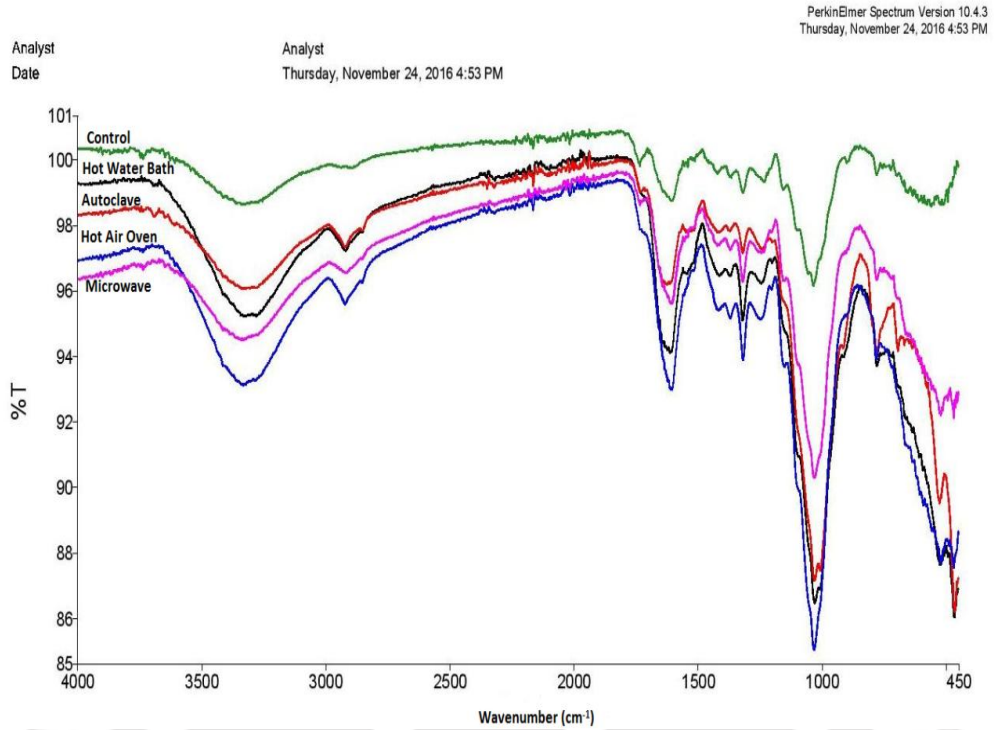


Fig. 4.6. FTIR spectra of untreated and thermally pretreated water hyacinth

4.2.6.3 XRD

XRD presents us straight information related to the crystallinity and amorphous nature of the untreated and thermally pretreated sample. Sharp peak in the untreated sample and the autoclave pretreated sample were observed whereas for hot air oven, hot water bath and microwave pretreated water hyacinth no sharp peak were seen (Fig. 4.7). Dissapeared cellulose crystallinity peaks in pretreated sugarcane bagasse was also reported by Chen et al. (2011). The diffraction from ideal crystalline substances is characterized by well defined Bragg peaks in X-ray diffraction. The presence of crystalline substances have long range order i.e., sharp peaks whereas amorphous substances do not possess this long range order so sharp peaks are missing (Karimi and Taherzadeh, 2016). Thus it is evident that thermal pretreatment is helpful in reducing the cellulose crystallinity of water hyacinth.

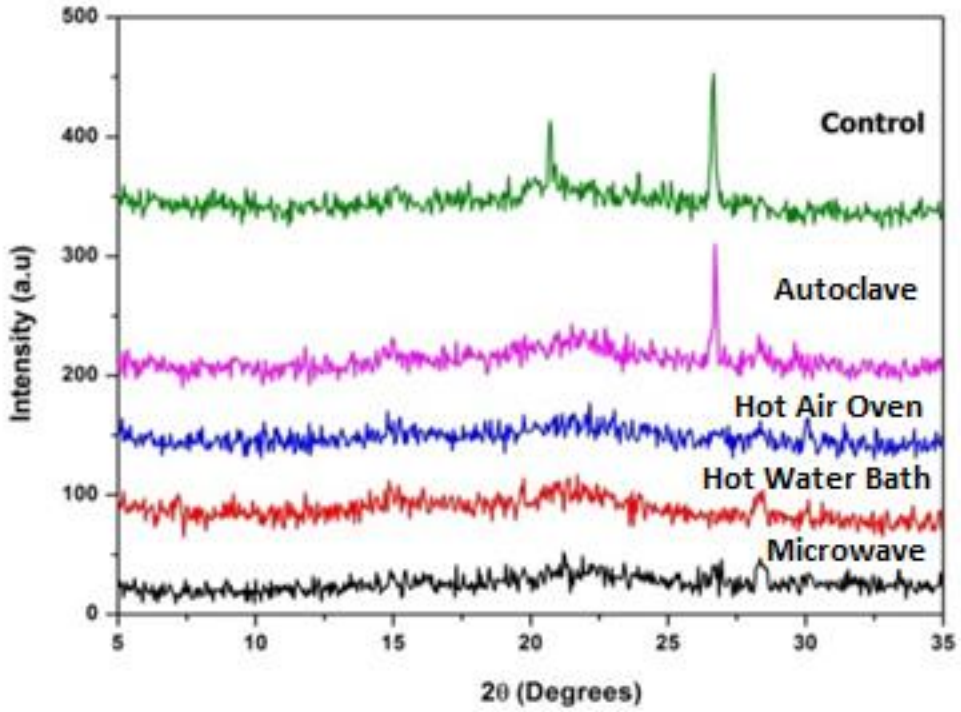


Fig. 4.7. XRD spectra of untreated and thermally pretreated water hyacinth

4.3 ELECTROHYDROLYSIS PRETREATMENT

The principle of electrohydrolysis pretreatment depends on ohmic heating, electrophoresis and electro-osmosis thereby unlocking the lignocellulosic complex i.e., making hydrolysis easier and faster (Mahmoud et al., 2010; Ibeid et al., 2013; Zhen et al., 2014). The process where thermal energy is generated by passing electric current through organic materials is known as Ohmic heating (Vicente et al., 2006; Sastry and Barach, 2000; Knirsch et al., 2010). The organic substrate acts as an electrical resistor. Electrical resistance aids uniform and rapid heating of mixture with high solid fraction (Varghese et al., 2014). The shifting of ions relative to a static phase is known as electrophoresis (Mahmoud, 2010). In electrophoresis, when electric current is passed through the substrate, the molecules in the mixture, passes through the medium at altered rate, depending on its electrical charge and molecular size. The motion of solid particles suspended in a liquid, under the influence of an electrical field is known as electro-osmosis (Mahmoud, 2010). Direct current (DC) is used for electrohydrolysis pretreatment of water hyacinth because in electrolysis the ionisation of the electrolyte occurs i.e., the cation and anion travels towards the opposite electrodes (Nandi, 2013). The polarity of the electrodes keeps switching places on the application of alternating current (AC), resulting in no ionisation i.e., the ions will not be attracted towards any of the electrode.

Electrohydrolysis pretreatment study was conducted in two stages to determine the optimum conditions required for solubilising water hyacinth. They are as follows:

- Voltage study
- Time study

4.3.1 Variation of Current and Resistance with time at different applied voltages

It was observed that at the constant applied voltage, current increases (Fig. 4.8a) and resistance decreases (Fig. 4.8b) gradually with the increase in time of exposure.

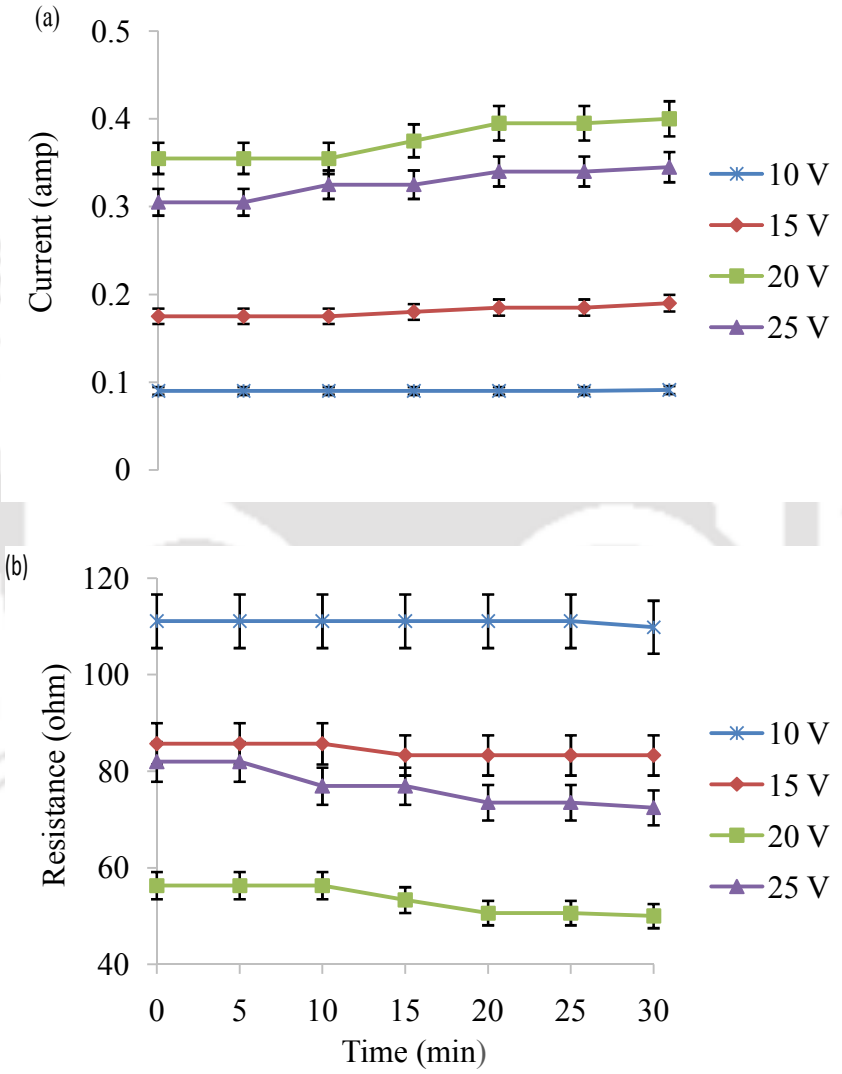


Fig. 4.8. Variation of (a) current and (b) resistance with time at different applied voltage (V)

The increase in current is due to increased conductivity caused by solubilisation of particulate organic matter induced by the application of current. And the decrease in resistance is due to the hydrolysis of polymers into simple soluble monomers.

As the applied voltage increased, the slope of the current versus time increased and the slope of the resistance versus time curve decreased. When the sample pretreated at 10 V

showed an increase of just 0.091 A and a decrease of 1.1 Ω, the sample pretreated at 20 V showed an increase of 0.345 A and a decrease of 6.3 Ω. This is due to the presence of more available energy for rapid solubilisation of organic matter at higher voltages. Solubilisation of organic matter will reflect as an increase in sCOD. Wang et al. (2005) mentioned that sCOD can be readily utilized for producing methane during anaerobic digestion.

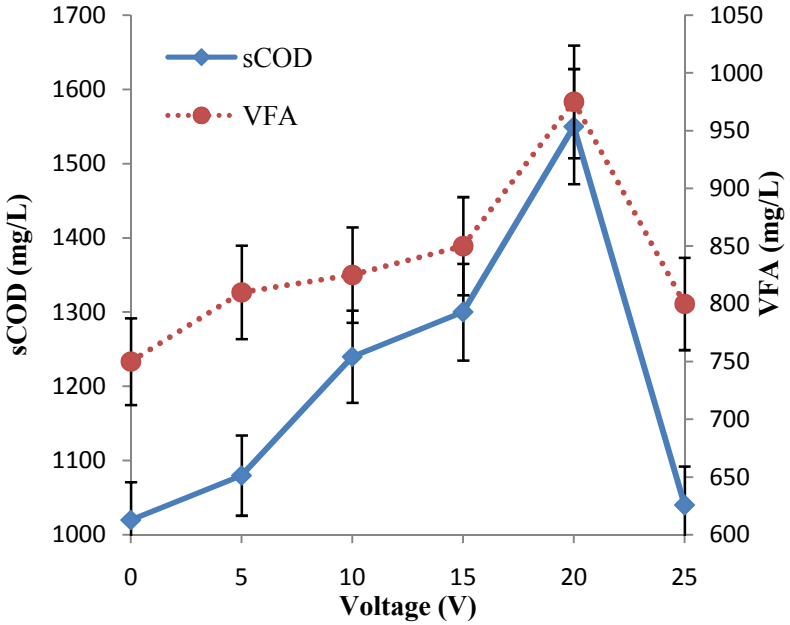


Fig. 4.9. Variation of sCOD and VFA with applied Voltage (V)

4.3.2 Effect on sCOD and VFA with voltage

sCOD and VFA (Fig. 4.9) showed an increasing trend with increase in applied voltage. While the control had a sCOD of 1010 mg/L, the sample pretreated at 20 V improved 1.53 times to become 1550 mg/L. sCOD decreased when the applied DC voltage was further increased to 25V, it can be inferred that the optimum voltage yielding the highest sCOD was 20 V for electrohydrolysis pretreatment of water hyacinth using graphite electrodes. The sample pretreated at 30 V was discarded due to excessive foaming. Zhen et al. (2014) also obtained an increase of 1.5 times sCOD in waste activated sludge for the sample pretreated at 20 V employed when compared to the sample pretreated at 5 V. Increase in VFA at 20V due to release of electrons, supplied by the DC current was sufficient to neutralise the huge amount of protons released from the substrate. Further increase in voltage showed decrease in VFA, as the optimum voltage yielding the highest VFA was 20V.

4.3.3 Effect on sCOD and VFA with time

The sCOD and VFA increases upto 60 min, then falls at 80 min (Fig. 4.10). sCOD improved 1.4 times i.e., 42.9% than the control for the exposure of 60 min at 20 V due to the

breakdown of particulate organic matter into their simpler forms. Li and Noike (1992) reported the increase in VFA concentration with the increase in time of exposure and optimum condition was selected based on high VFA content. Thus, 60 min at 20 V is optimised to pretreat particulate organic matter.

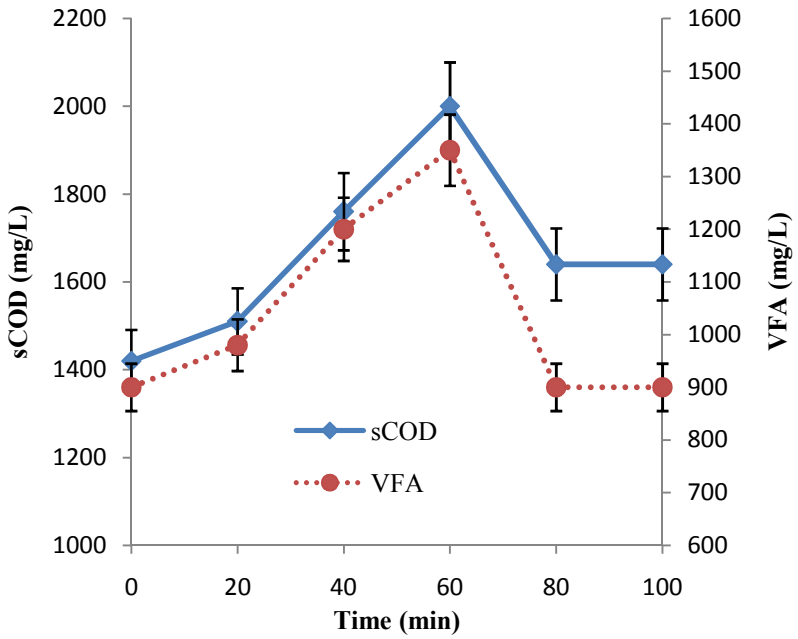


Fig. 4.10. Variation of sCOD and VFA with time (min)

4.3.4 Compositional Analysis

Changes in the electrohydrolysis pretreated water hyacinth at 20V for varied time period was studied (Table 4.3). Enhanced delignification was indicated by the increase in the percentage of soluble lignin in the pretreated substrate when compared to the untreated water hyacinth. Pretreated sample at 20 V for 60 min substantiates the utmost increase in the percentage of soluble lignin and the utmost decrease in the percentage of insoluble lignin. This signifies the existence of extra soluble lignin. Kamdem et al. (2015) stated that during pretreatment, the percentage of lignin solubilised is proportional to the biodegradability of the substrate. The softened lignin makes it easier for the microorganisms to access the cellulose. As for cellulose, a decrease was observed and for hemicelluloses an increase was observed in the pretreated sample. Electrohydrolysis pretreatment must have cut the long chain cellulose at some indistinct point. Thereby escalating the percentage of short string glucose fragments i.e., hemicellulose in electrohyrolysis pretreated water hyacinth. Thus, drop in the percentage of cellulose in electrohydrolysis pretreated water hyacinth than the untreated water hyacinth is much desired. As it depicted, higher solubilisation of the pretreated water hyacinth. Highest reduction in cellulose and highest increase in hemicellulose was observed in the sample pretreated at 20 V for 60 min. Focussing on the fact that, cellulose is broken down into their

simpler forms thereby enhancing solubilisation (Kumar et al., 2009a). Similar results were also obtained by previous available literatures (Diaz et al., 2015). Thus, the melting of lignin and breaking down of cellulose into short chains signifies the effectiveness of electrohydrolysis pretreatment in enhancing the biogas production of water hyacinth.

Table 4.3. Compositional changes in the electrohydrolysis pretreated water hyacinth at 20V for varied time period

Voltage (V)	Time (min)	Acid Soluble Lignin (%)	Acid Insoluble Lignin (%)	Cellulose (%)	Hemicellulose (%)
---	---	2.8±0.3	12.1±0.6	48.5±0.4	37.6±0.7
20	20	3.1±0.2	12.1±0.3	43.6±1.2	40.1±0.7
20	40	3.5±0.7	11.9±0.3	34.1±0.2	43.3±0.3
20	60	3.8±0.5	9.2±0.2	28.2±0.3	62.6±0.2
20	80	3.6±0.8	10.7±0.7	31.1±0.4	56.1±1.1

4.3.5 Characterisation

4.3.5.1 FESEM

FESEM analysis for electrohydrolysis pretreated and untreated water hyacinth was investigated for identifying the morphological changes (Fig. 4.11). Untreated water hyacinth showed a firm dense arrangement whereas electrohydrolysis pretreated water hyacinth illustrated a rough flaking destructured matrix i.e., a tattered external coating with fissures and hollows. Thus, providing an idea of enhanced bioaccesibility of cellulose in the electrohydrolysis pretreated water hyacinth due to delignification.

4.3.5.2 FTIR

To observe the chemical changes undergone in water hyacinth before and after electrohydrolysis pretreatment FTIR study was done (Fig. 4.12). Both the sample showed peaks at the same position but the pretreated sample showed broadening of peaks and reduced peak intensity. Remarkable fall in peak intensity was detected at 1027.17, 1319.28 and 1622.64 cm^{-1} depicting about the C-O, C=C, C-C-O, C-H deformation and rupture of C=O stretch in cellulose, hemicellulose and lignin due to electrrohydrolysis pretreatment (Kumar et al., 2009b; Li et al., 2010). In the pretreated sample, the change in band intensities at 3333.3 cm^{-1} corresponding to OH stretch signifies the rupture of cellulose hydrogen bonds and 2921.22 cm^{-1} corresponds to decrease in CH stretch due to the splitting of methyl/methylene groups of cellulose (Wu et al., 2011; He et al., 2017). Thus, reduced peak

intensity and broad peaks denoted delignification and reduced crystallinity of water hyacinth due to electrohydrolysis pretreatment.

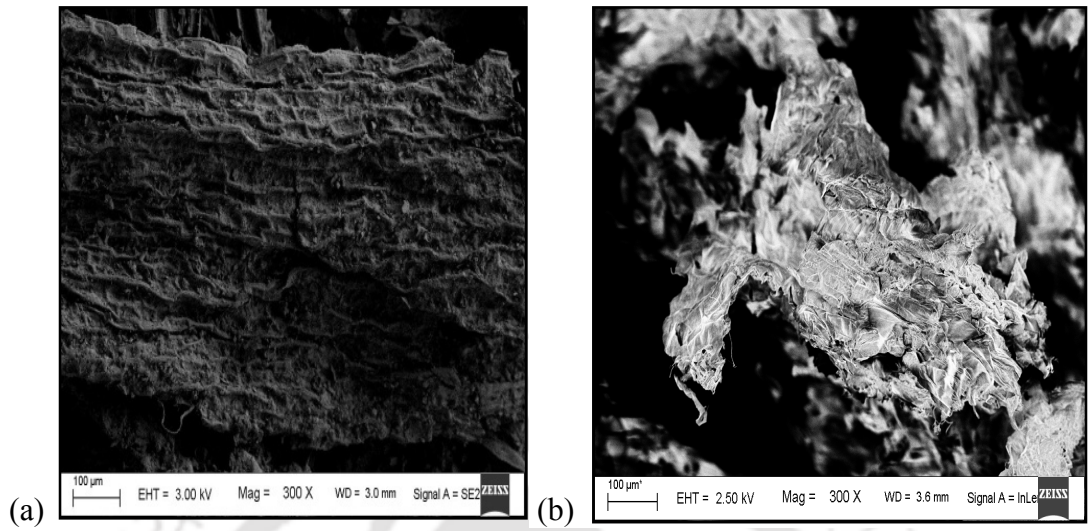


Fig. 4.11. FESEM images of (a) untreated and (b) electrohydrolysis pretreated water hyacinth

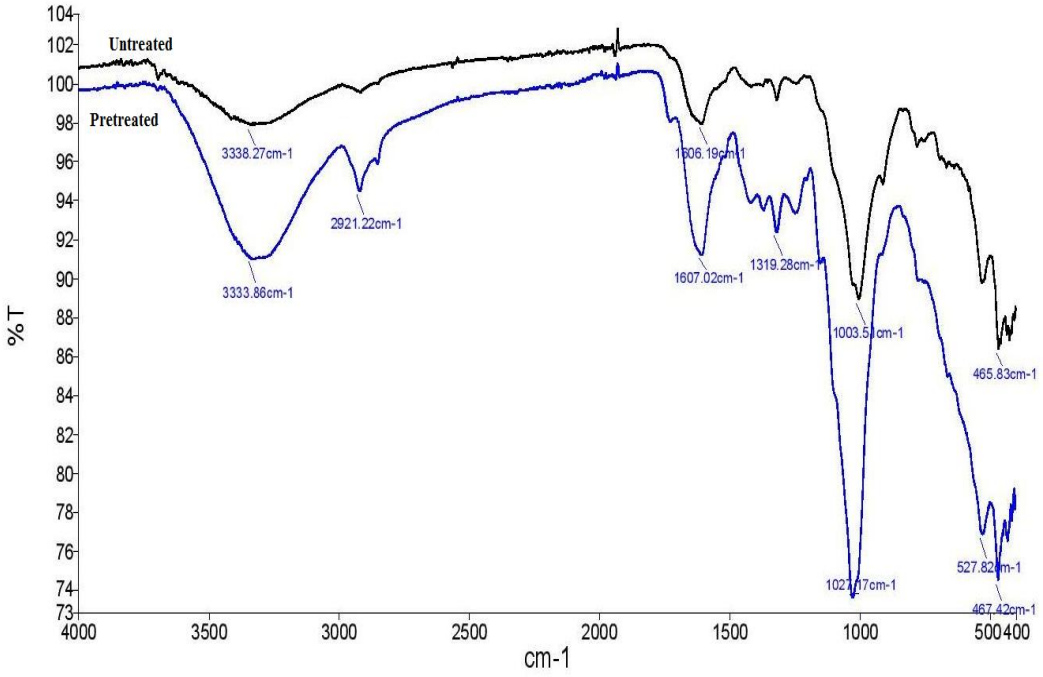


Fig. 4.12. FTIR spectra of untreated and electrohydrolysis pretreated water hyacinth

4.3.5.3 XRD

XRD bestows information related to the crystallinity or the amorphous nature of the sample. Karimi and Taherzadeh (2016) stated the presence of sharp peaks or long range order in crystalline substances and the absence of long range order or sharp peaks in amorphous substances. Similarly, sharp peak in the untreated sample was observed whereas no sharp

peaks were witnessed for electrohydrolysis pretreated water hyacinth. Electrohydrolysis pretreatment ruptured the cell wall of water hyacinth making it amorphous in nature, thus no peaks are observed (Fig. 4.13). Chen et al. (2011) also observed the absence of cellulose crystallinity peaks in pretreated sugarcane bagasse. The diffraction from crystalline sample is illustrated by distinct Bragg peaks in X-ray diffraction. Thus, electrohydrolysis pretreatment is supportive in dropping the cellulose crystallinity of water hyacinth.

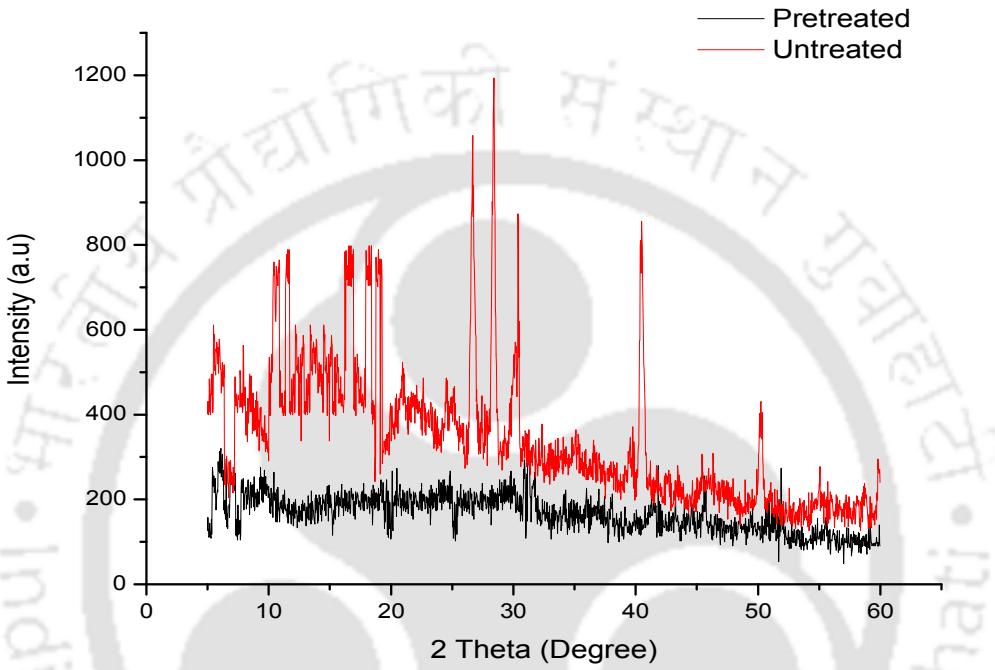


Fig. 4.13. XRD spectra of untreated and thermally pretreated water hyacinth

4.4 BIOLOGICAL PRETREATMENT

Biological (microbial and enzymatic) pretreatment of water hyacinth for the production of biogas has been rarely studied, may be because of its relatively time consuming process than the other pretreatment techniques. The utilisation of bacterial strains for solubilising water hyacinth is inexpensive when compared to the direct utilisation of enzymes available in the market. Nevertheless, biological pretreatment does not generate any inhibitors (phenolic compounds, furfurals and hydroxymethylfurfural) during anaerobic digestion and is an eco-friendly process with low energy and chemical input. Microbial pretreatment is considered to be inexpensive when compared to other physicochemical pretreatment methods. Physical pretreatment methods require specialized equipments and machineries which lead to abundant energy consumption and chemical pretreatment methods involve acid, alkali which in turn creates harsh condition by generating inhibitory compounds (Mosier, 2005) that might hinder the anaerobic digestion process. While microbial pretreatment uses metabolites of

microorganisms present in nature for rupturing the sturdy lignocellulosic structure thereby enhancing biogas generation. Microorganisms are capable of continuously disintegrating the complex organic matter during the different phases of growth (Aydin, 2016). Microbial pretreatment technique involves inoculation of bacterial, fungal or a mixed consortium in the lignocellulosic substrate to degrade cellulose or hemicellulose or lignin (Zhong et al., 2016). Microorganisms i.e., brown, white and soft rot fungi (Nkemka et al., 2015, Su et al., 2016) and bacteria are mostly employed for microbial pretreatment to attack the lignocellulosic material by secreting their enzymes. Factors influencing biological pretreatment are biomass composition, nature of microorganism, incubation time and temperature. Profuse cellulolytic and hemicellulolytic microorganisms are present in nature which can be employed for effective water hyacinth pretreatment in order to enhance biogas generation.

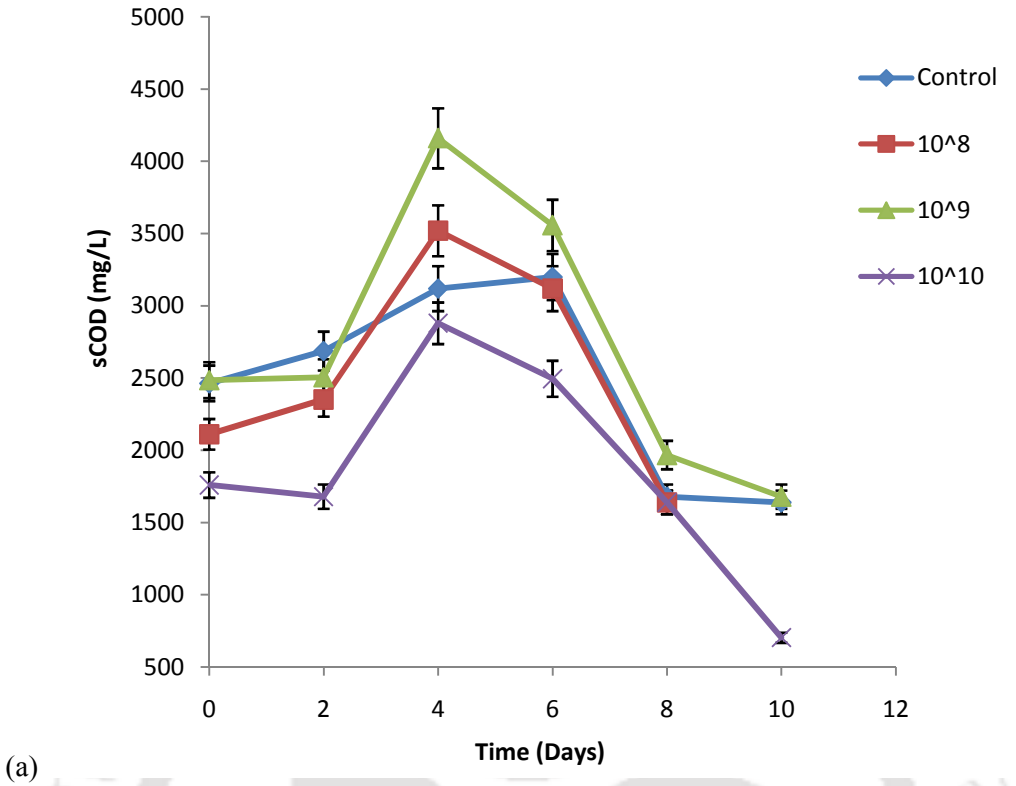
This study involves the utilisation of cellulolytic bacteria which synthesises potent cellulolytic enzymes to solubilise water hyacinth during the hydrolysis phase of anaerobic digestion. Therefore, the present study was conducted to optimise the most effective bacterial strain isolated from soil and both of the insects' gut for biological pretreatment of water hyacinth.

Biological pretreatment study was conducted in two stages to determine the optimum conditions required for solubilising water hyacinth. They are as follows:

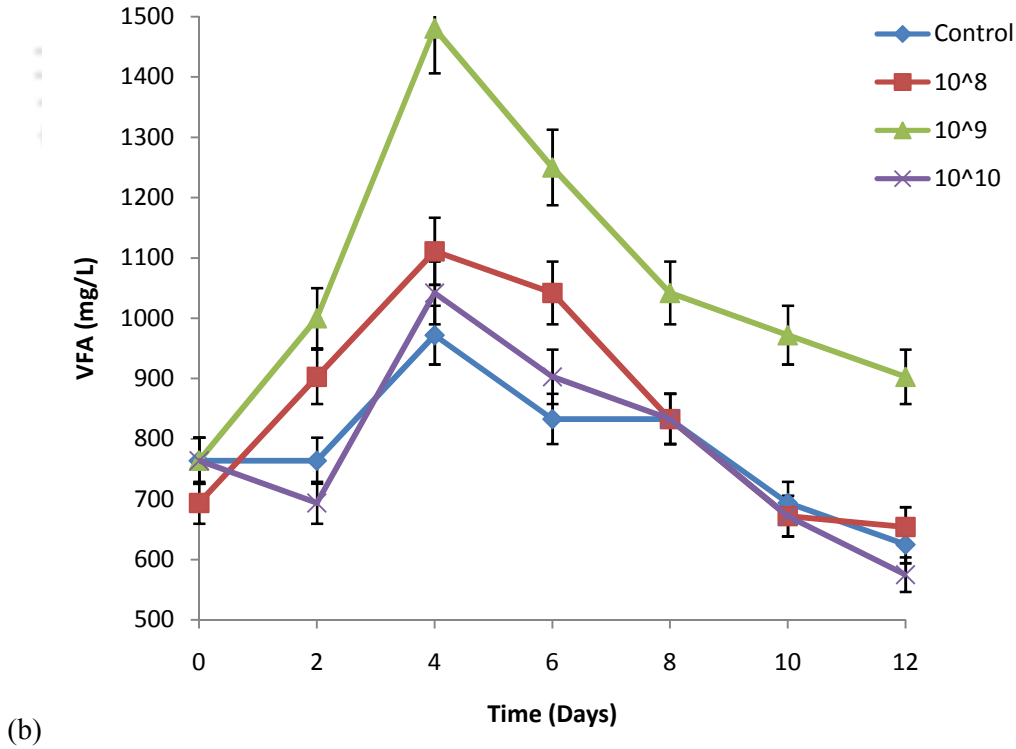
- Dosage study
- Time study

4.4.1 Effect of microbial (bacterial) pretreatment on solubilisation of the water hyacinth

In order to rupture the lignocellulosic cell wall and to improve the solubilisation of the substrate, isolated microorganisms were employed to pretreat water hyacinth. With respect to the solubilisation process of water hyacinth an appropriate selection of bacterial strain/ culture is necessary. Therefore to determine the ideal isolated bacterial culture providing the highest solubilisation of water hyacinth, sCOD and the VFA of the samples were analysed at various dosage and time. Fig. 4.14a, 4.14b, 4.15a, 4.15b, 4.16a and 4.16b depicts similar trends of increase in sCOD and VFA concentration. sCOD and VFA increased hand in hand as the number of days increased and after reaching the utmost peak, it starts decreasing. The increase in VFA led to the increase in sCOD. For water hyacinth, pretreatment with silverfish illustrated the highest sCOD (solubilisation) in 4 days followed by millipede and soil (6 days) at a dosage of 10^9 CFU/mL.

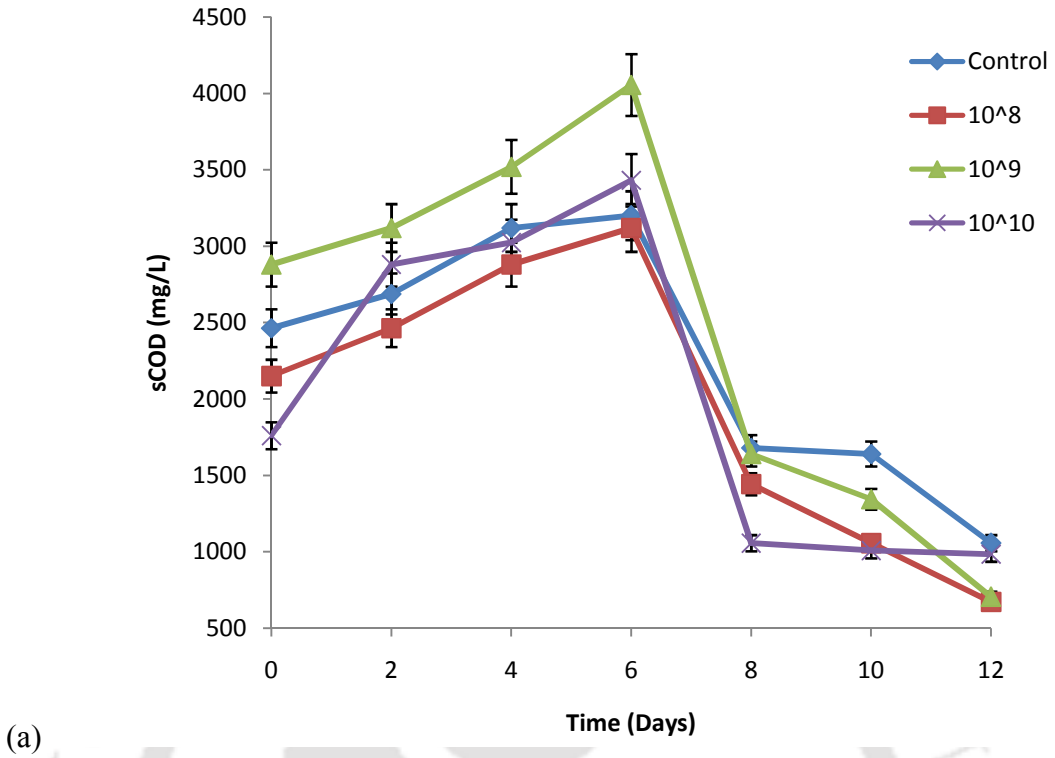


(a)

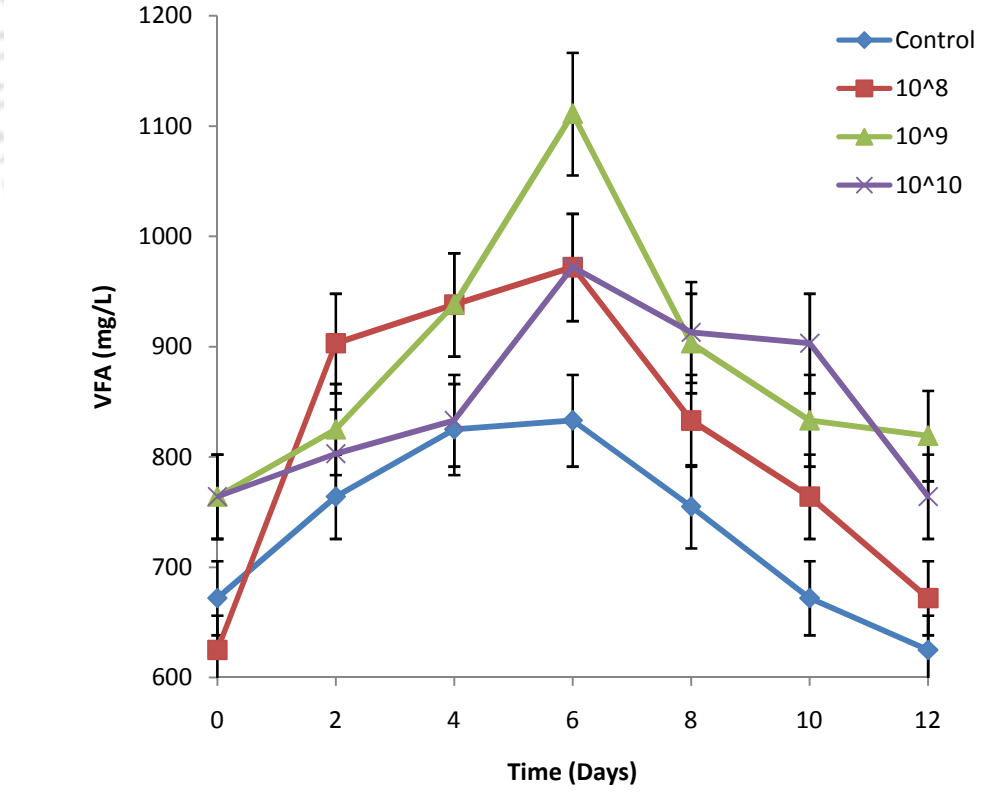


(b)

Fig. 4.14. Changes in (a) sCOD and (b) VFA concentration during microbial pretreatment of water hyacinth with *Citrobacter werkmanii VKVVG4*

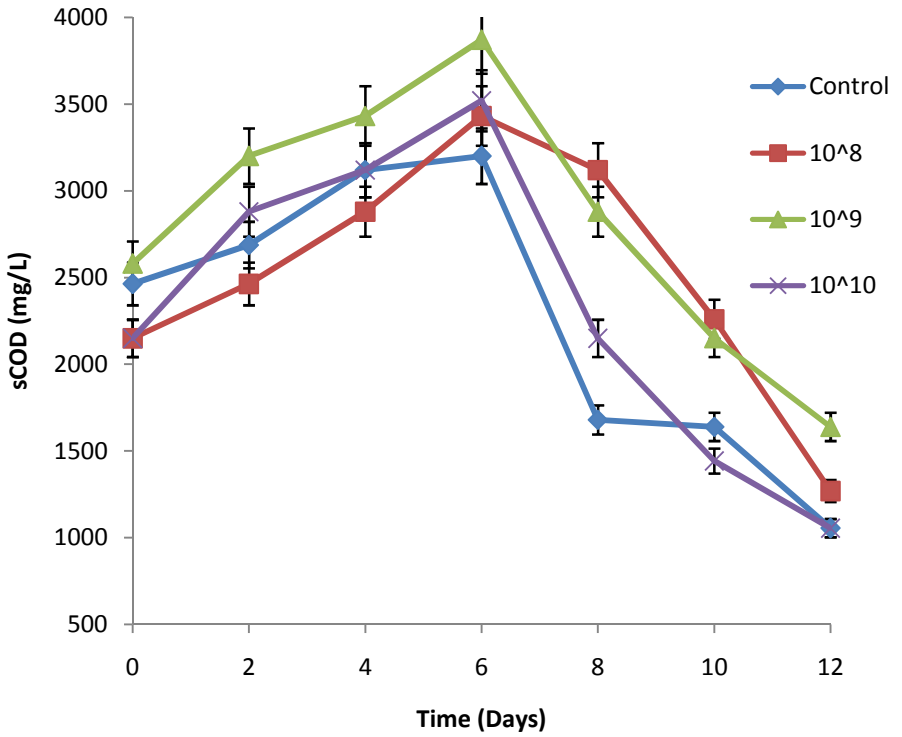


(a)

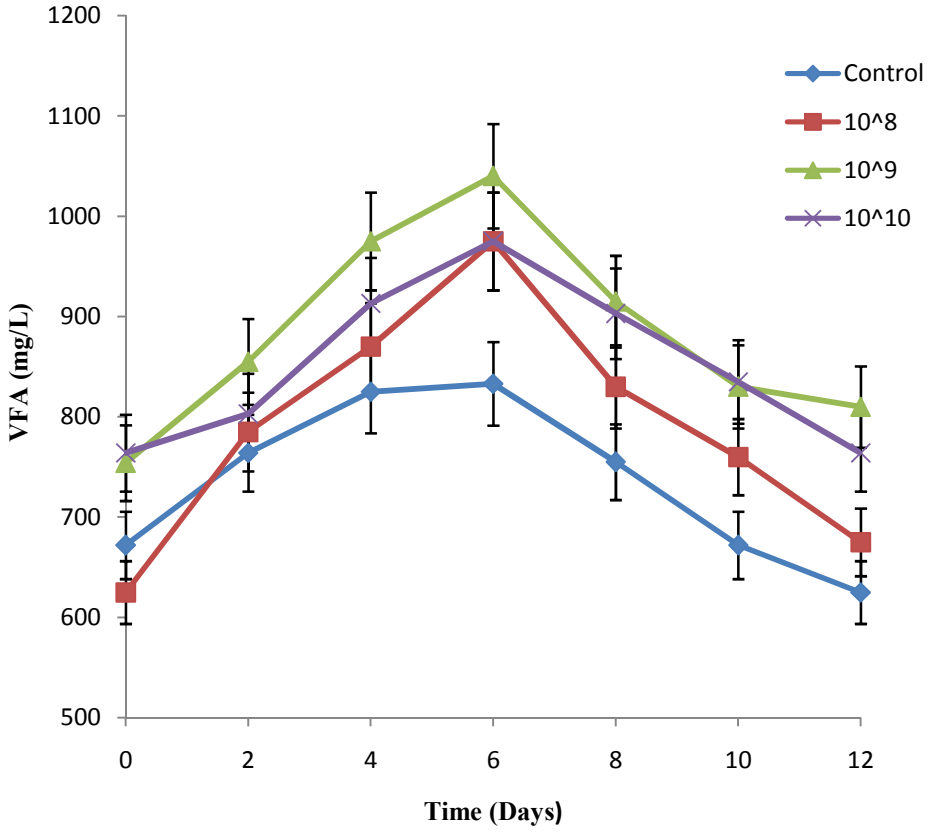


(b)

Fig. 4.15. Changes in (a) sCOD and (b) VFA concentration during microbial pretreatment of water hyacinth with *Paenibacillus sp.VKVVG1*



(a)



(b)

Fig. 4.16**.** Changes in (a) sCOD and (b) VFA concentration during microbial pretreatment of water hyacinth with *Bordetella muralis VKVVG5*

Although the same dose of inoculum showed the highest solubilisation for all of the three different bacterial strains still the degree of solubilisation and the time required for solubilisation differed. Silverfish illustrated the highest solubilisation of 1.33 times or 33.33% followed by millipede (1.26 times or 26.75%) and soil (1.21 times or 20.94%). The release of extracellular and intracellular biopolymers into the soluble phase is a fundamental factor to assess the proficiency of the pretreatment (Mahdy et al., 2016). Initially, the sCOD and VFA for water hyacinth inoculated with 10^9 CFU/mL of SFa2 was observed to be 2496 mg/L and 764 mg/L respectively. As the time required for solubilising is one of the key administering feature in bacterial pretreatment (Kavitha et al., 2017), the release of soluble organic matter amplifies with an augment in pretreatment time. On the 2nd day of pretreatment, sCOD and VFA enhanced to 2506 mg/L and 1000 mg/L, indicating that the rise in sCOD and VFA may be attributable to exopolymeric matrix discharge rather than the availability of the intracellular polymers. More increment in the pretreatment time to 4 days displayed a radical increase in sCOD and VFA to 4160 mg/L and 1480 mg/L respectively. This radical increase in sCOD designates the secretion of cellulase enzyme by the silverfish bacteria. Firstly, because the generated compounds are the substrates, as a result the recalcitrant lignocellulosic cell wall complex ruptured resulting in the discharge of extracellular and intracellular polymers into the soluble phase. From the 6th day, both VFA and sCOD started declining. Yuan et al. (2014) demonstrated rapid increase in the concentration of VFA and sCOD during 4 days of microbial pretreatment of corn stalk.

High sCOD indicates that huge amount of soluble organic matter are present for anaerobic digestion and the formed VFA performs a significant responsibility in accelerating the hydrolysis of lignocellulosic material (Yu et al., 2004). The mode of action of the microbial pretreatment can be hypothesised by two credible mechanisms. First of all, the bacteria might have attacked the sturdy outer layer by dissolving/ softening down the lignin resilience thereby increasing the permeability of the water hyacinth.

Followed by the second mechanism of the bacterial attack in the inner layer of the water hyacinth and finally leading to the hydrolysis of the cellulose and hemicellulose. The bacteria initially starts consuming the easily available soluble organic matter; once the soluble organic matter is over, the bacteria starts secreting exoenzymes to solubilise the particulate organic matter thereby increasing the quantity of soluble organic matter and the bacterial population, leading to the increase in sCOD. After few days, as the availability of food decreases, the bacteria starts consuming each other, leading to the decrease in sCOD. The outcome designates that the conversion of complex organic matter to soluble organic matter can be

time consuming, and the optimum pretreatment time and dosage must be equivalent to that, during which sCOD and the VFA accomplishes the highest peak value. The increased level of VFA and sCOD are a proof of the high hydrolytic and cellulolytic activity of the isolated bacteria especially silverfish. Thus the optimal pretreatment dosage and time is 10^9 CFU/mL of silverfish in 4 days for water hyacinth. This optimal pretreatment dosage and time makes sure that the exhaustion of soluble organic matter during microbial pretreatment is reduced and that the soluble organic matter is available for biogas generation.

4.4.2 Effect of microbial pretreatment on the compositional analysis of water hyacinth

The main objective of the microbial pretreatment is to rupture the lignocellulosic complex of water hyacinth so that a higher amount of easily degradable organic carbon fraction is bio-accessible. Improved biodegradability of water hyacinth can lead to increased biogas production in a short duration as the recalcitrant lignin softens making it easier for the microorganisms to feed on the depolymerised cellulose. Therefore compositional analysis was carried out to study the modification in the lignocellulosic composition of the water hyacinth before and after microbial pretreatment (Table 4.4).

Lignin is a complex, aromatic heteropolymer in which its main structural constituents, phenylpropanoid aryl-C_3 units, are connected by a diverse range of C-O and C-C bonds. Cellulose is a polysaccharide, composed of a linear string of D-glucose molecules connected by β-(1, 4)-glycosidic bonds. Hemicellulose is an easily hydrolysable branched heterogeneous polymer of pentose (xylose, arabinose), hexose (mannose, glucose and galactose) and acetylated sugars. Enhanced delignification in the pretreated substrate when compared to the untreated was evident as the percentage of soluble lignin increased. Silverfish pretreated sample substantiates the utmost increase in the percentage of soluble lignin (3.23±0.8%) followed by millipede (2.93±0.1%) and soil (2.82±0.4%) pretreated when compared to the untreated substrate (2.77±0.3%). The presence of more soluble lignin signifies the softening of the sturdy lignin. The softened lignin makes the cellulose easily bio-accessible. Repolymerization reaction of the lignin is the main reason behind the slight increase in the percentage of acid insoluble lignin in the pretreated samples (Li et al., 2007). Reduction in the composition of cellulose in pretreated water hyacinth than the untreated is advantageous as it represents enhanced solubilisation. The long string cellulose must have split at some indefinite point by the microorganisms during pretreatment leading to the decline in cellulose. In this manner the percentage of short string glucose fragments (hemicellulose) in the microbial pretreated water hyacinth increased. Highest reduction in cellulose was observed in silverfish (30.89±0.2%) followed by millipede (32.63±0.3%) and

soil (33.87±0.8%) respectively. Conversely, silverfish pretreated water hyacinth illustrated the increase in the availability of hemicellulose (43.06±0.7%) than the untreated water hyacinth (27.7±0.2%). Microbial pretreatment helped in the modification of the chemical composition and physical configuration of the lignocellulosic water hyacinth. These chemical and physical modifications team up to improve the biodegradability of the water hyacinth and enhance biogas generation. Thus the compositional analysis indicates the availability of the utmost amount of readily degradable soluble organics in the silverfiah pretreated water hyacinth.

Table 4.4. Compositional changes in water hyacinth due to microbial pretreatment

Microbial consortium	Acid soluble lignin (%)	Acid insoluble lignin (%)	Cellulose (%)	Hemicellulose (%)
---	2.77±0.3	7.93±0.5	36.84±0.8	27.7±0.2
Silverfish	3.23±0.8	8.34±0.3	30.89±0.2	43.06±0.7
Millipede	2.93±0.1	8.92±0.6	32.63±0.3	36.02±0.8
Soil	2.82±0.4	9.05±0.9	33.87±0.8	30.62±0.9

4.4.3 Characterisation

4.4.3.1 FESEM

FESEM analysis for microbial pretreated and untreated water hyacinth was investigated for identifying the morphological changes (Fig. 4.17). Untreated water hyacinth showed a firm dense arrangement whereas microbial pretreated water hyacinth illustrated a rough flaking destructured matrix i.e., a tattered external coating with fissures and hollows. Thereby, demonstrating, an idea of improved bioaccesibility of cellulose in the microbial pretreated water hyacinth due to delignification.

4.3.3.2 FTIR

To observe the chemical changes undergone in water hyacinth before and after microbial pretreatment FTIR study was done (Fig. 4.18). Both the sample showed peaks at the same position but the pretreated sample showed broadening of peaks and reduced peak intensity. Remarkable fall in peak intensity was detected at 1027.17 cm^{-1}, 1319.28 cm^{-1} and 1622.64 cm^{-1}depicting about the C-O, C=C, C-C-O, C-H deformation and rupture of C=O stretch in cellulose, hemicellulose and lignin due to microbial pretreatment (Kumar et al., 2009b; Li et al., 2010). In the pretreated sample, the change in band intensities at 3333.3 cm^{-1} corresponding to OH stretch signifies the rupture of cellulose hydrogen bonds and

2921.22 cm^{-1} corresponds to decrease in CH stretch due to the splitting of methyl/methylene groups of cellulose (Wu et al., 2011; He et al., 2017). Thus, reduced peak intensity and broad peaks denoted delignification and reduced crystallinity of water hyacinth due to microbial pretreatment.

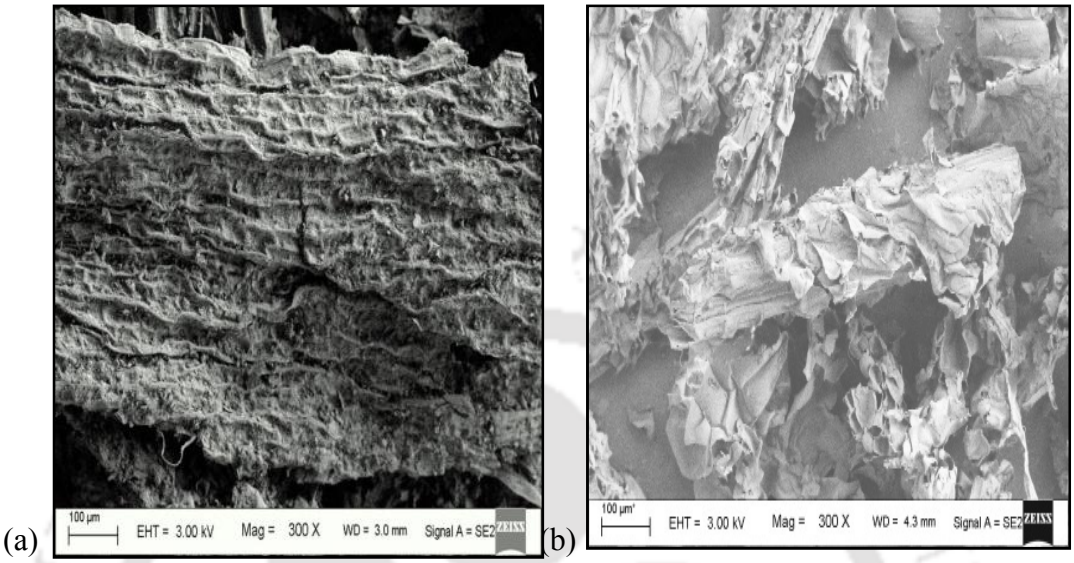


Fig. 4.17. FESEM images of (a) untreated and (b) microbial pretreated water hyacinth

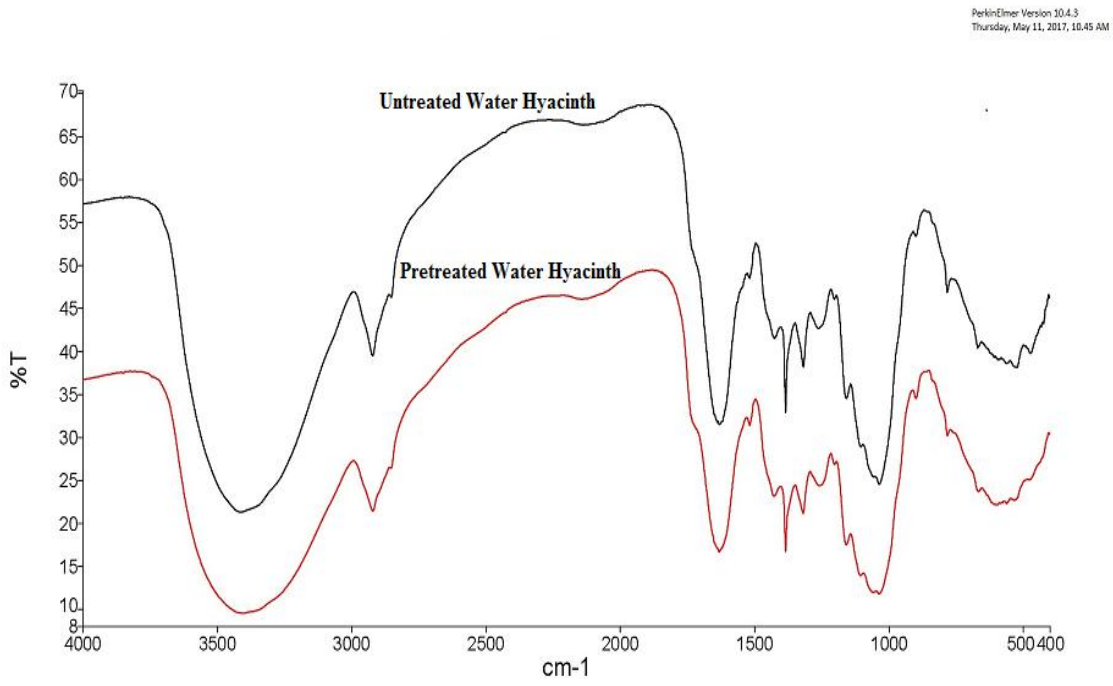


Fig. 4.18. FTIR spectra of untreated and microbial pretreated water hyacinth

4.4.3.3 XRD

XRD bestows information related to the crystallinity or the amorphous nature of the sample. Karimi and Taherzadeh (2016) stated the presence of sharp peaks or long range order

in crystalline substances and the absence of long range order or sharp peaks in amorphous substances. Similarly, sharp peak in the untreated sample was observed whereas no sharp peaks were witnessed for microbial pretreated water hyacinth (Fig. 4.19). Microbial pretreatment ruptured the cell wall of water hyacinth making it amorphous in nature, thus no peaks are observed. Chen et al. (2011) also observed the absence of cellulose crystallinity peaks in pretreated sugarcane bagasse. The diffraction from crystalline sample is illustrated by distinct Bragg peaks in X-ray diffraction. Thus, microbial pretreatment is supportive in dropping the cellulose crystallinity of water hyacinth.

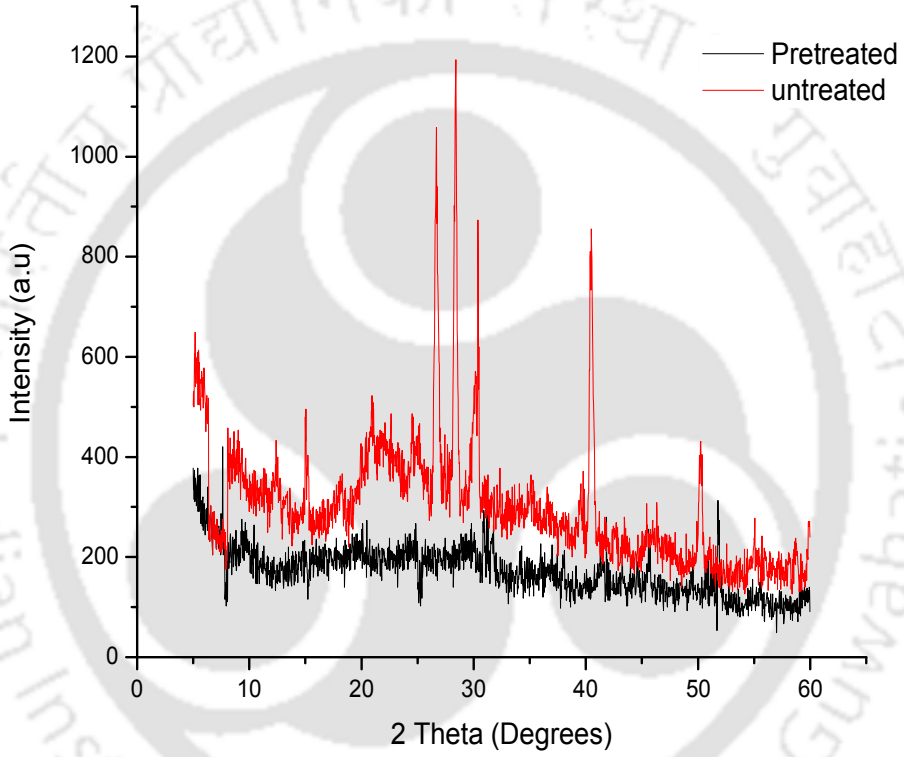


Fig. 4.19. XRD spectra of untreated and microbial pretreated water hyacinth

4.5 COMPARATIVE ANALYSIS

From the conducted pretreatment study, it was observed that thermal pretreatment of water hyacinth demonstrates the highest solubilisation in a reduced time period when compared to biological and electrohydrolysis pretreatment (Table 4.5). Among all the four thermal pretreatment techniques (i.e., hot air oven, microwave, autoclave and hot water bath), hot air oven illustrated the highest solubilisation of 55.5% at 90ºC for 1h. It is important to note that although the ideal temperature for hot air oven, autoclave and hot water bath was same (i.e., 90ºC) still the amount of solubilisation differs. This can be attributed to the medium of heat transfer. In hot air oven, the medium of heat transfer is hot air instead of hot water. Hot water

is in direct contact with the base of the glassware containing the substrate but the hot air entirely surrounds the glassware from all the direction in the hot air oven. Also, the energy transfer efficiency of each thermal pretreatment technique is different. Determining the ideal pretreatment technique showing the highest solubilisation is essential during anaerobic digestion in order to enhance biogas production and cut down the hydrolysis period thereby improving the overall process.

Table 4.5. Comparative analysis of the pretreatment study

Pretreatment	Ideal condition	% Solubilisation
Hot air oven	90ºC for 1h	55.5
Microwave	200ºC for 10 mins	44.2
Autoclave	90ºC for 40 mins	22.2
Hot water bath	90ºC for 1.5 h	35
Electrohydrolysis	20V for 60 mins	42.9
Citrobacter werkmanii VKVVG4	10^9 CFU/mL for 4 days	33.3
Paenibacillus sp. VKVVG1	10^9 CFU/mL for 6 days	26.75
Bordetella muralis VKVVG5	10^9 CFU/mL for 6 days	20.94

CHAPTER 5

BIOCHEMICAL METHANE POTENTIAL (BMP) STUDY

This chapter deals with the biochemical methane potential of water hyacinth before and after various kind of pretreatment and their scaled up batch studies (20 L). Methane potential of water hyacinth was determined for various F/M ratios in order to optimise the ideal F/M ratio.

5.1 BMP STUDY

BMP tests are carried out in anaerobic batch digesters in bench scale for verifying the anaerobic biodegradation likelihood of the substrate (Esposito et al., 2012). It is an indicator for determining the potential of the substrate for biogas production (Chynoweth et al., 1993) and estimating the time required for complete degradation of the substrate. BMP tests are also useful in determining the potential ideal F/M ratio (Labatut et al., 2011). Determination of the ideal F/M ratio is necessary to avoid process imbalance and enhance biogas generation. Improper F/M ratio may sometimes result in inhibition due to the stocked up intermediate products and may produce negligible or lesser quantity of biogas. Eiroa et al. (2012) observed that the increase in the waste/inoculum ratio led to process imbalance in the anaerobic reactor due to build up of volatile fatty acids (VFA). Raposo et al. (2009) also stated the increase in F/M ratio may lead to the accumulation of VFA during the process. Accumulation of VFA in higher F/M ratio reveals the kinetic uncoupling between acid producing and acid consuming microorganisms.

5.1.1 BMP study of untreated water hyacinth

5.1.1.1 Biogas/Methane production

The rate of biomethanation is directly proportional to the biodegradability of the substrate (Esposito et al., 2012). It was observed that the untreated water hyacinth (F/M ratio 2) produced the highest amount of biogas (143±14 mL CH_4/g VS) on the 32nd day (Fig. 5.1). From Fig. 5.2, it is obvious that F/M ratio 2 demonstrated the highest cumulative methane production for untreated water hyacinth. The lag phase observed during the BMP test of untreated water hyacinth is due to the existence of the rigid lignocellulosic network. Lignin acts as a barrier for biogas production because the microorganisms find it difficult to degrade the recalcitrant outer layer and access the cellulose.

5.1.1.2 VS

A decreasing trend of volatile solids with the passage of time was observed. Although there is a decline in the pattern of volatile solids of the untreated water hyacinth (Fig. 4.3) but

it is not as steep. Higher the VS reduction, higher is the biogas production (Dhamodharan et al., 2015). F/M ratio 2 in the untreated water hyacinth showed the highest VS reduction of 33%. The microbial activity was restricted in the untreated water hyacinth leading to very less VS reduction.

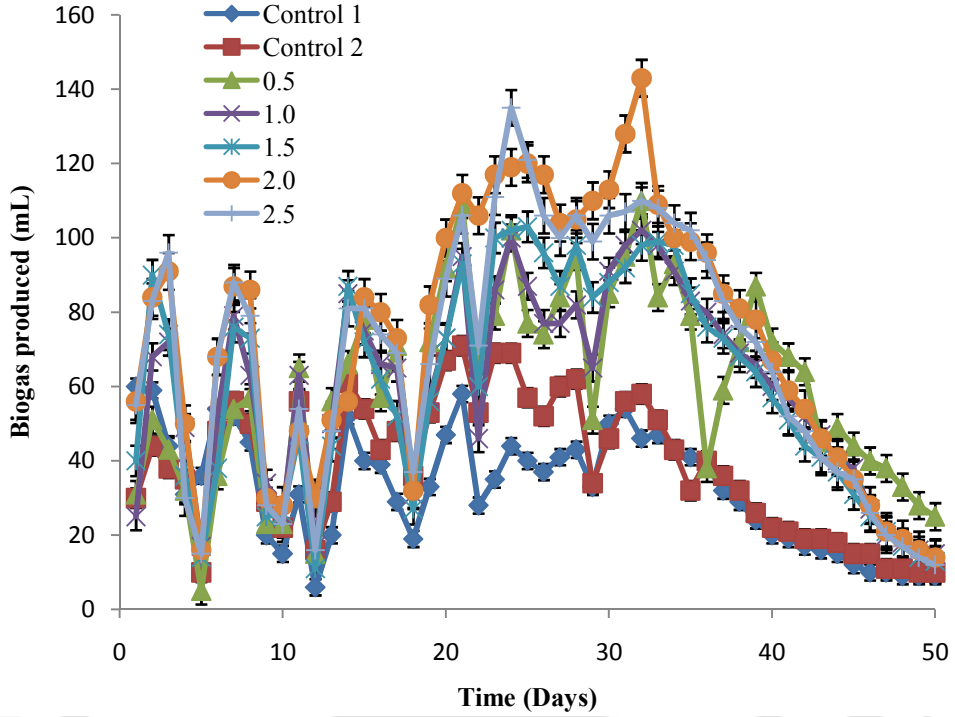


Fig. 5.1. Biogas production per day in untreated water hyacinth

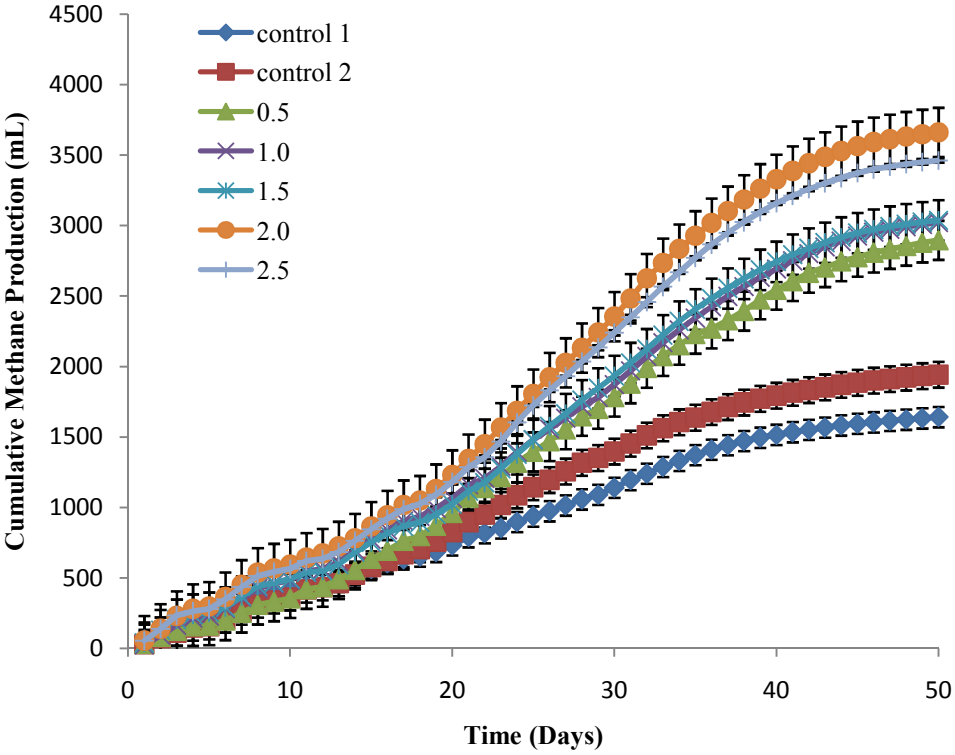


Fig. 5.2. Cumulative methane production in untreated water hyacinth

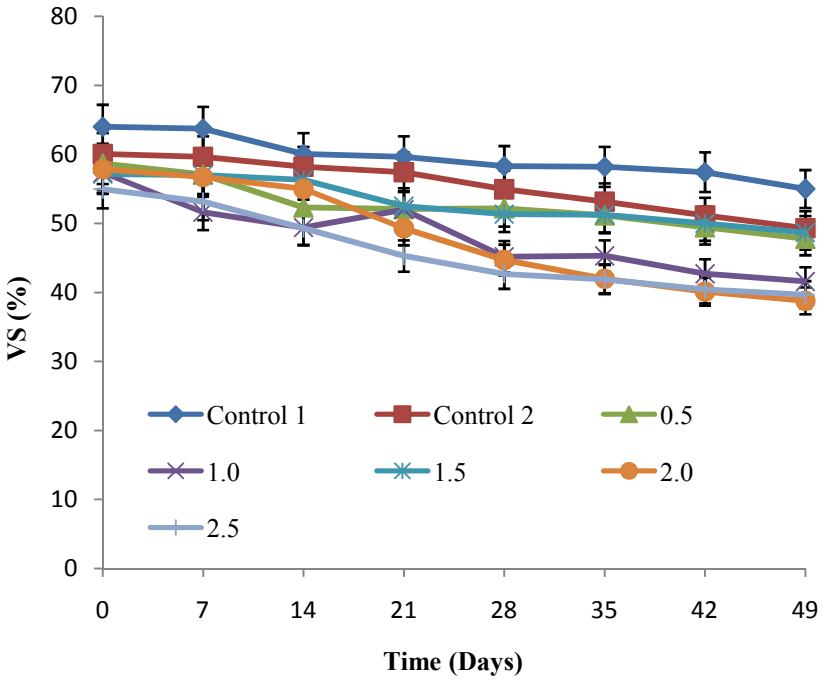


Fig. 5.3. VS reduction in untreated water hyacinth

5.1.1.1.3 sCOD and VFA

sCOD for the F/M ratios of untreated water hyacinth showed no specific trend (Fig. 5.4). This might be due to the presence of more organic matter in particulate form which is yet to be solubilised in the untreated water hyacinth. For untreated water hyacinth F/M ratio 2 showed the highest sCOD (6,321±23 mg/L) on the 28th day.

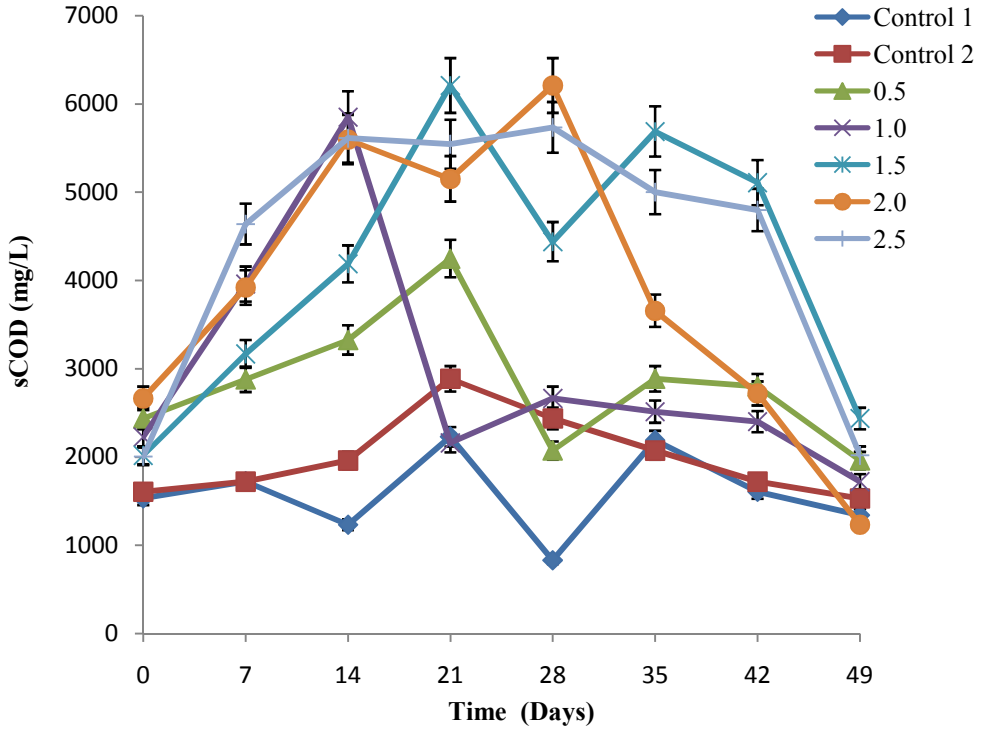


Fig. 5.4. Variation in sCOD in untreated water hyacinth

An increase in the VFA concentration was observed up to the 14th day and the gradual decline can be marked by the start of methanogenesis phase. Activity of the acidogens led to the production of VFA upto the 14th day, then methanogens came into function as they are sensitive to acidic conditions. Highest amount of VFA production for untreated water hyacinth was observed to be 1491±12 mg/L on the 14th day (Fig. 5.5).

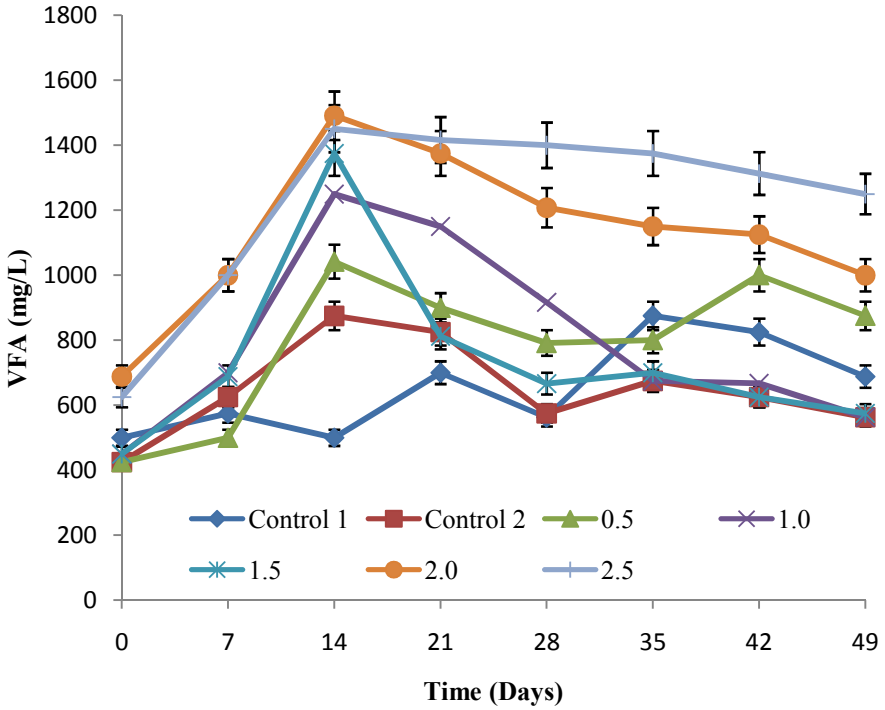


Fig. 5.5. Variation in VFA concentration in untreated water hyacinth

5.1.2 BMP study of hot air oven pretreated water hyacinth

5.1.2.1 Biogas production

For hot air oven pretreated water hyacinth, highest biogas production (193±22 mL CH_4/g VS) was observed on the 14th day itself for the F/M ratio1.5. Usually, the hydrolysis of slow degrading substances i.e., lignocellulosic compounds is very time consuming (Gurung et al., 2012) but hot air oven pretreatment of water hyacinth induced rise in methane production, in a short period. The shorter time period (14 days) taken by the pretreated substrate when compared to the untreated substrate (32 days) for attaining highest amount of biogas represents the ready to degrade phase inside the anaerobic reactor. Fast or slow hydrolysis relies on the existence of easily biodegradable substances. On the application of heat, the hydrogen bonds of the lignocellulosic complex were split apart. Thus converting, the crystalline cellulose to amorphous nature i.e., making the water hyacinth easily degradable for the methanogens to feed on.

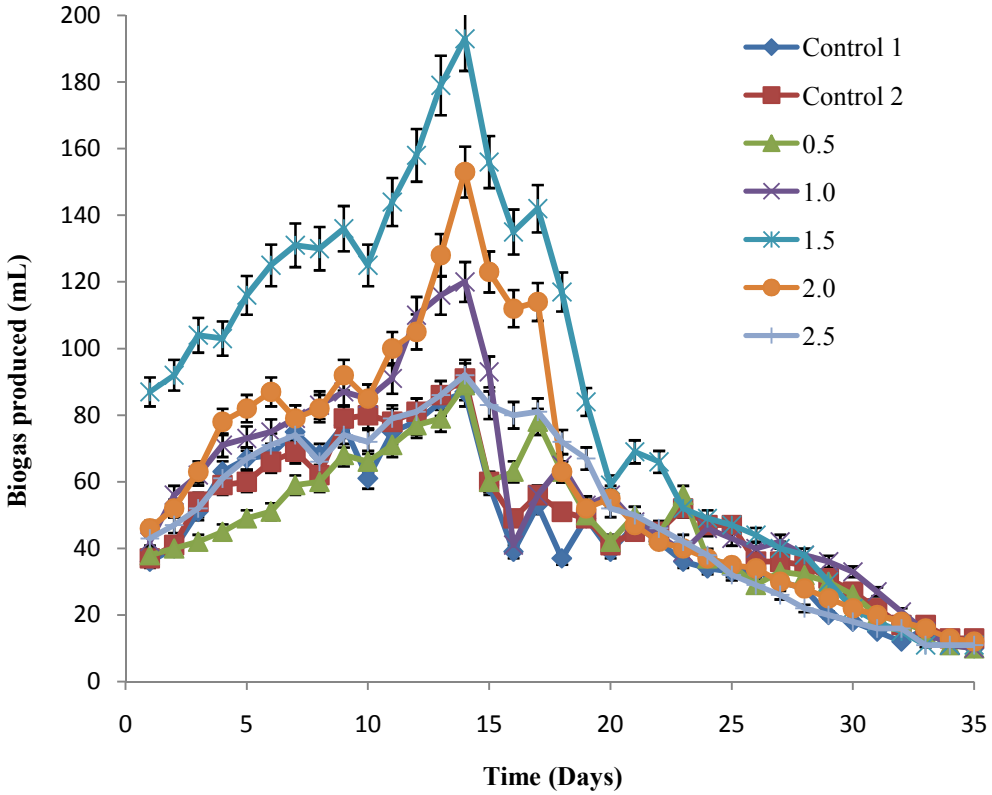


Fig. 5.6. Daily biogas production in hot air oven pretreated water hyacinth

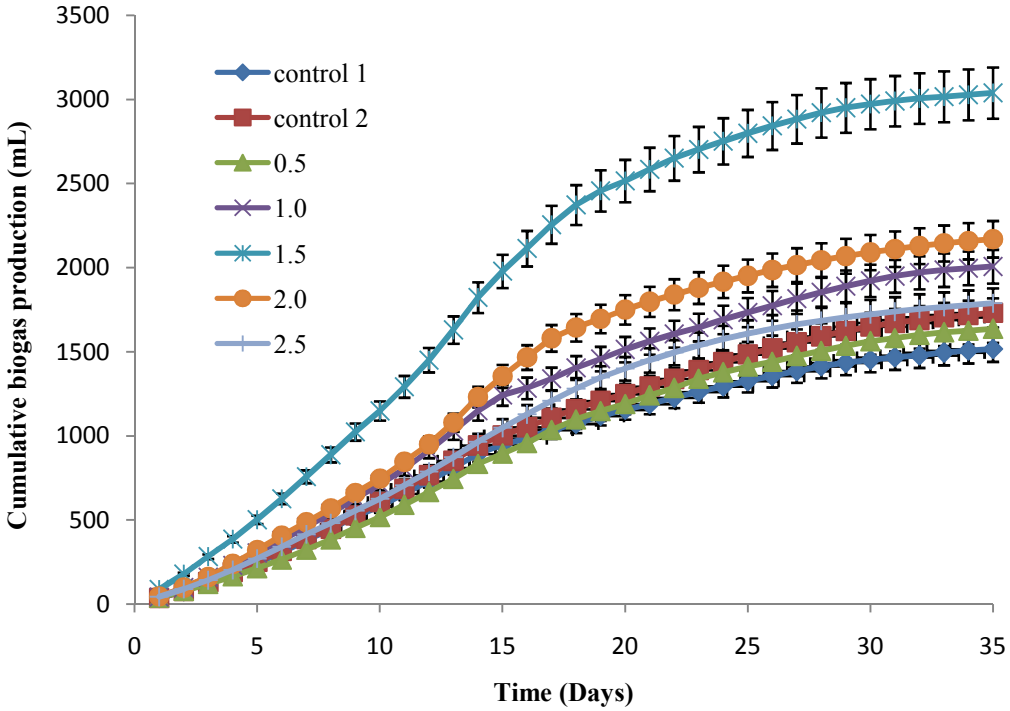


Fig. 5.7. Cumulative biogas production in hot air oven pretreated water hyacinth

From Fig. 5.6, it was evident that F/M ratio 1.5 shows the highest cumulative methane production for hot air oven pretreated water hyacinth when compared to the other ratios respectively. F/M ratio 1.5 of the pretreated water hyacinth showed a cumulative methane

production of 1,806±19 mL on the 14th day. On the 35th day, cumulative methane production of hot air oven pretreated water hyacinth was 3039±32 mL. Hot air oven pretreatment melted the lignin present in water hyacinth, making it easier for the microorganisms to degrade the substrate. Even an exact F/M ratio co-operated very well for easy solubilisation of the water hyacinth.

5.1.2.2 VS

Rapid VS reduction was observed in hot air oven pretreated water hyacinth. Higher the VS reduction, higher is the biogas production (Dhamodharan et al., 2015). F/M ratio 1.5 in the pretreated water hyacinth showed the highest VS reduction of 64% (Fig. 5.8). Heat pretreatment enabled the loosening of the rigid lignin, i.e., the high degree of polymerisation, the three dimensional (3D) network arrangements along with the unwavering inter-unit covalent bonds were dissolved down, making the substrate readily degradable. Hence, it can be stated that thermal pretreatment enhanced the biodegradability efficiency of the water hyacinth as the microorganisms in the cow dung were able to acclimatise with the substrate effortlessly.

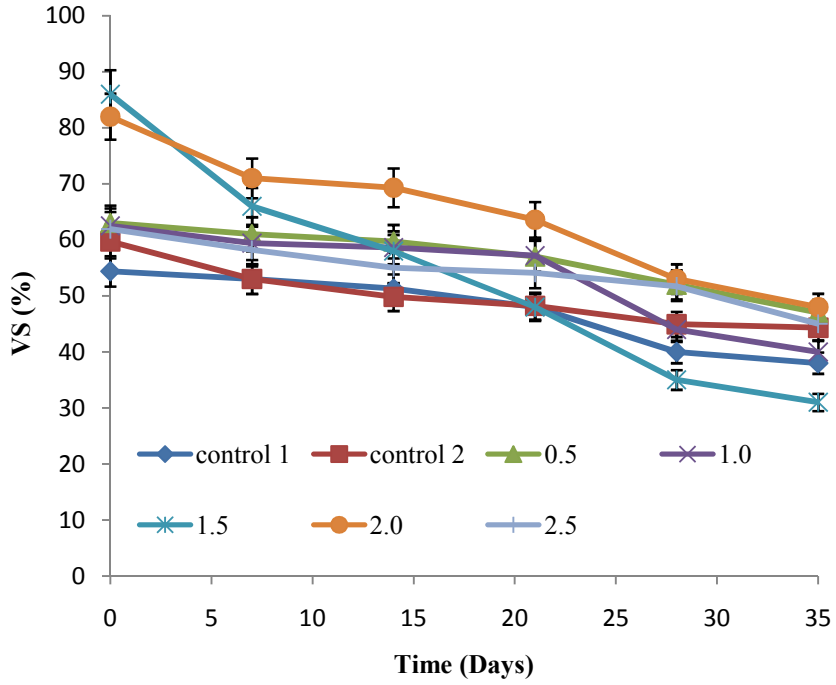


Fig. 5.8. VS reduction in hot air oven pretreated water hyacinth

5.1.2.3 sCOD and VFA

As thermal pretreatment enhanced the solubilisation process of the substrate therefore particulate COD was converted into sCOD. In the hot air oven pretreated water hyacinth, sCOD increased steadily upto the 14th day and then it decreased rapidly. F/M ratio 1.5 of hot

air oven pretreated water hyacinth showed the highest sCOD (6846±16 mg/L) on the 14th day (Fig. 5.9). The hot air oven pretreatment at 90°C for 1h; efficiently ruptured the intra and inter molecular hydrogen-bond present in the lignocellulosic complex; thus shaking up the consistency of the cellulose making it easily extractable. In simpler words, heat pretreatment helped in the conversion of the complex organic substances of the water hyacinth into their simple soluble form; leading to the increase in sCOD at a reduced period.

Thermal pretreatment of water hyacinth enabled each F/M ratio to synergistically degrade cellulose, i.e., convert sugars into VFA and finally into methane. Highest amount of VFA production for hot air oven pretreated water hyacinth was observed to be 1758±19 mg/L on the 14th day (Fig. 5.10). Hot air oven pretreatment caused slight increase in VFA production due to disintegration of complex organic molecules, releasing more soluble matter. VFA concentration more than 13000 mg/L is inhibitory for methane production (Viéitez and Ghosh, 1999). Reduced pH and kinetic disentanglement between the acid producers and consumers devastates the methanogenic activity (Ahring, 1995). The pH of the reactor may stoop down unless there is enough buffering capacity. Depending on the degree and duration of drop in pH, biogas production will absolutely cease. Nevertheless, the acetoclastic methanogens in the reactor were able to consume the acetate quickly, thereby yielding methane. Therefore VFA production in the reactors was within the limit and no process imbalance was witnessed due to VFA accumulation.

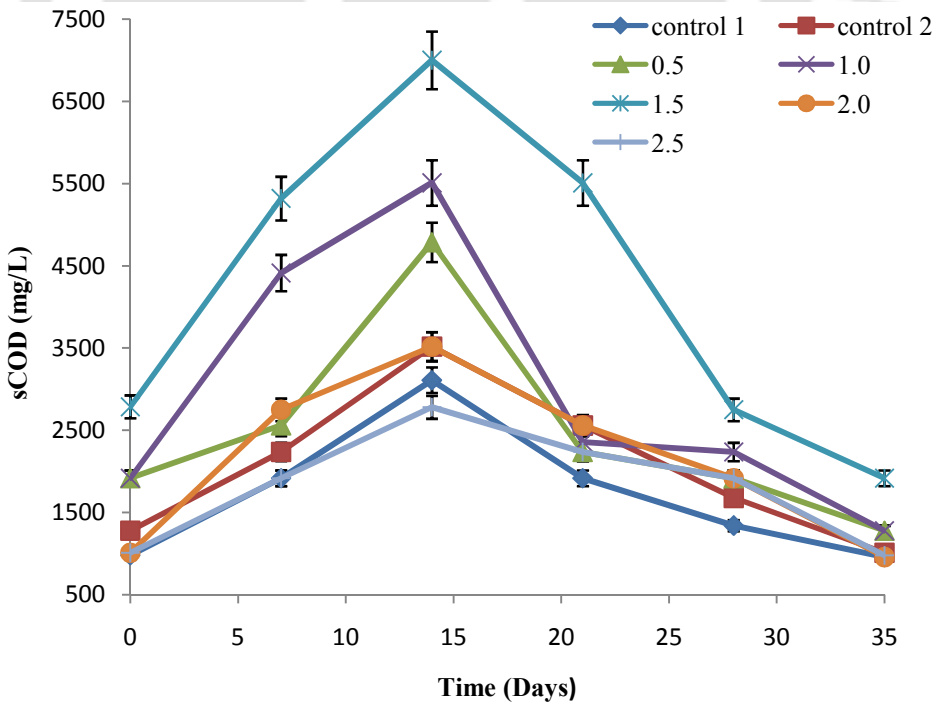


Fig. 5.9. Variation in sCOD in hot air oven pretreated water hyacinth

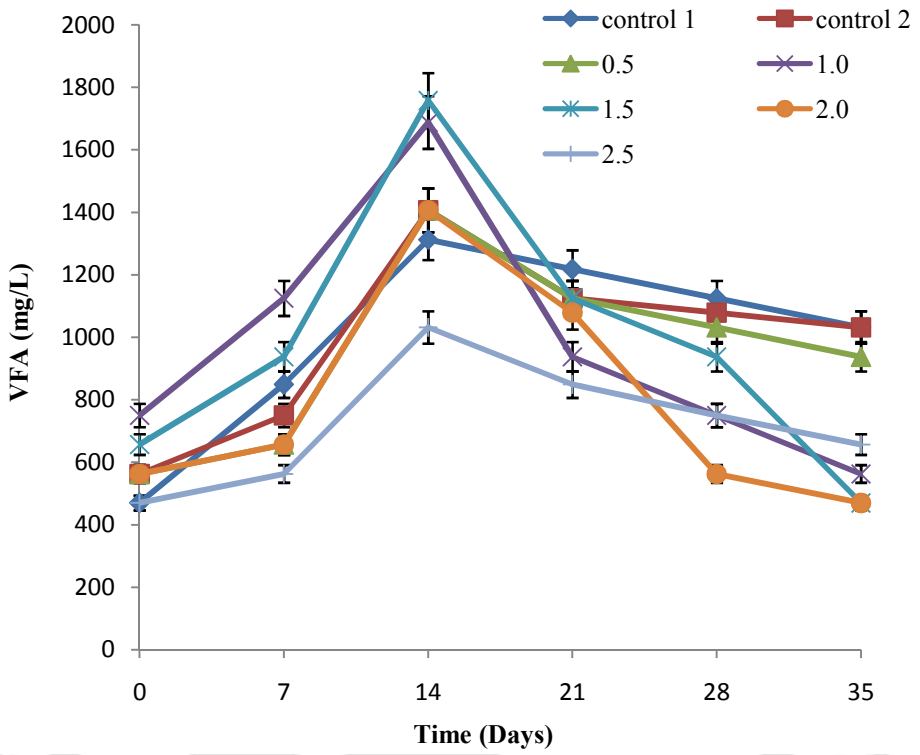


Fig. 5.10. VFA generation in hot air oven pretreated water hyacinth

5.1.3 BMP study of electrohydrolysis pretreated water hyacinth

5.1.3.1 Biogas production

The effect of various F/M ratios on biogas production for electrohydrolysis pretreated water hyacinth has been investigated. The daily production of biogas was higher in the pretreated water hyacinth than the untreated. Also with the increase in F/M ratio the biogas production increased upto F/M ratio 1.5 in pretreated water hyacinth. F/M ratio1.5 for the electrohydrolysis pretreated water hyacinth showed the highest biogas production (155±19 mL CH_4/g VS) on the 7th day itself (Fig. 5.11). This indicates that electrohydrolysis pretreatment dissolved the hydrogen bonds that was clutching the sturdy lignin and crystalline cellulose of the water hyacinth together; thereby reducing the lag phase. Hydrolysis of lignocellulosic compounds is usually very slow (Kelly and Dworjanyn, 2008; Weiland, 2010; Gurung et al., 2012) but electrohydrolysis pretreatment was supportive enough to cut short the time to 7 days. Higher biogas production is related to the easier biodegradability of the substrate. F/M ratio 1.5 showed the highest cumulative methane production for electrohydrolysis pretreated water hyacinth (Fig. 5.12). F/M ratio 1.5 showed a cumulative methane production of 808±16 mL for pretreated water hyacinth on the 7th day. F/M ratio played a major role in the hasty enhanced production of biogas. By 30th day,

cumulative methane production of electrohydrolysis pretreated water hyacinth was 2454±31 mL.

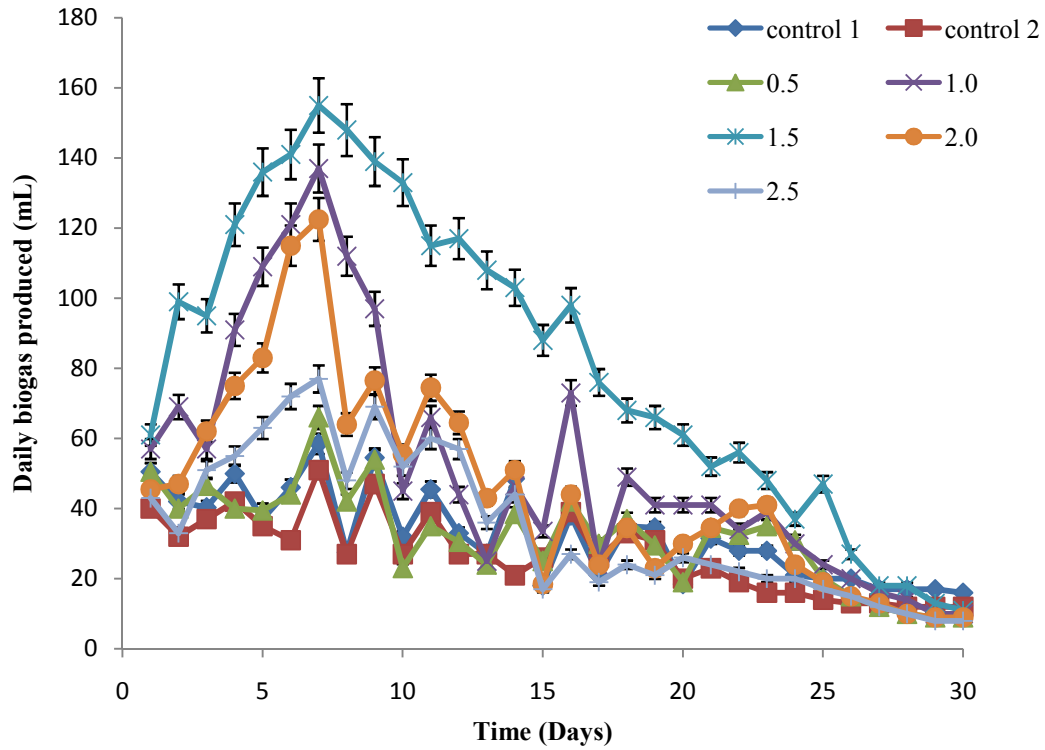


Fig. 5.11. Daily biogas production in electrohydrolysis pretreated water hyacinth

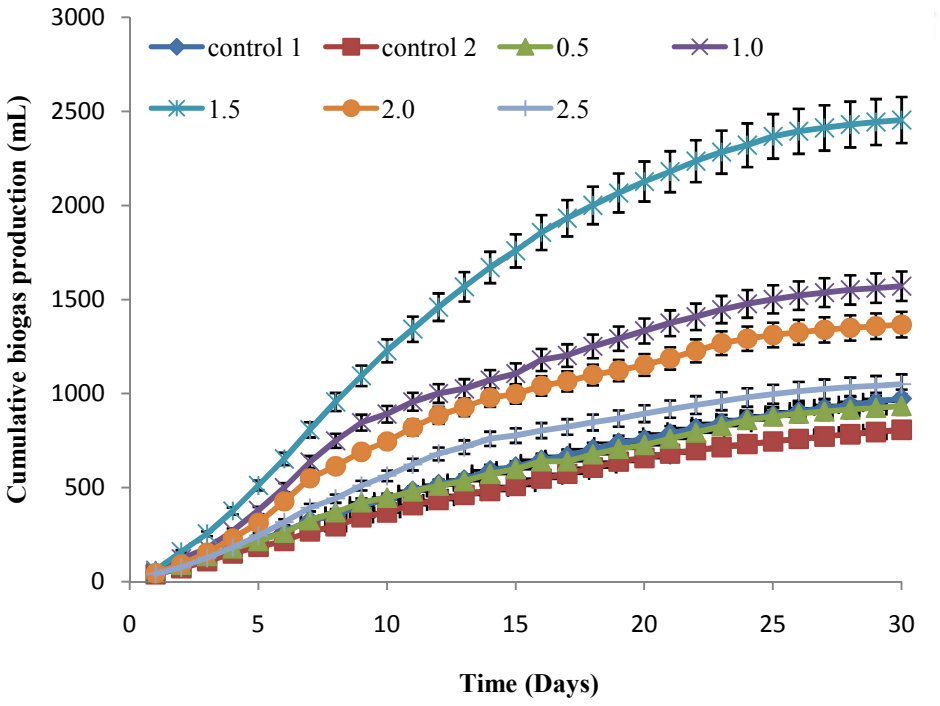


Fig. 5.12. Cumulative biogas production in electrohydrolysis pretreated water hyacinth

The availability of huge amount of solubilised organic fraction in the electrohydrolysis pretreated water hyacinth and acclimatized anaerobic microorganisms in the reactor i.e., the appropriate F/M ratio diminished the lag phase. In other words, the hydrolysis period in the pretreated substrate quickened up due to easy accessibility of solubilised matter by the microorganisms. The results demonstrated that the start-up time required for the digester to attain stability were different. It mostly depended on whether the substrate was pretreated or not and on the F/M ratio.

5.1.3.2 VS

Highest VS reduction of 50% was achieved in the F/M ratio 1.5 for the pretreated water hyacinth. The reduction in the VS of the electrohydrolysis pretreated water hyacinth was clearly evident (Fig. 5.13). Rapid VS reduction in the pretreated water hyacinth for the F/M ratio 1.5 was due to the easily accessible organics and a suitable F/M ratio. The microorganisms available in the cow dung were proficient enough to acclimatize effortlessly with the electrohydrolysis pretreated water hyacinth. Dhamodharan et al. (2015) stated that higher VS reduction leads to higher biogas production.

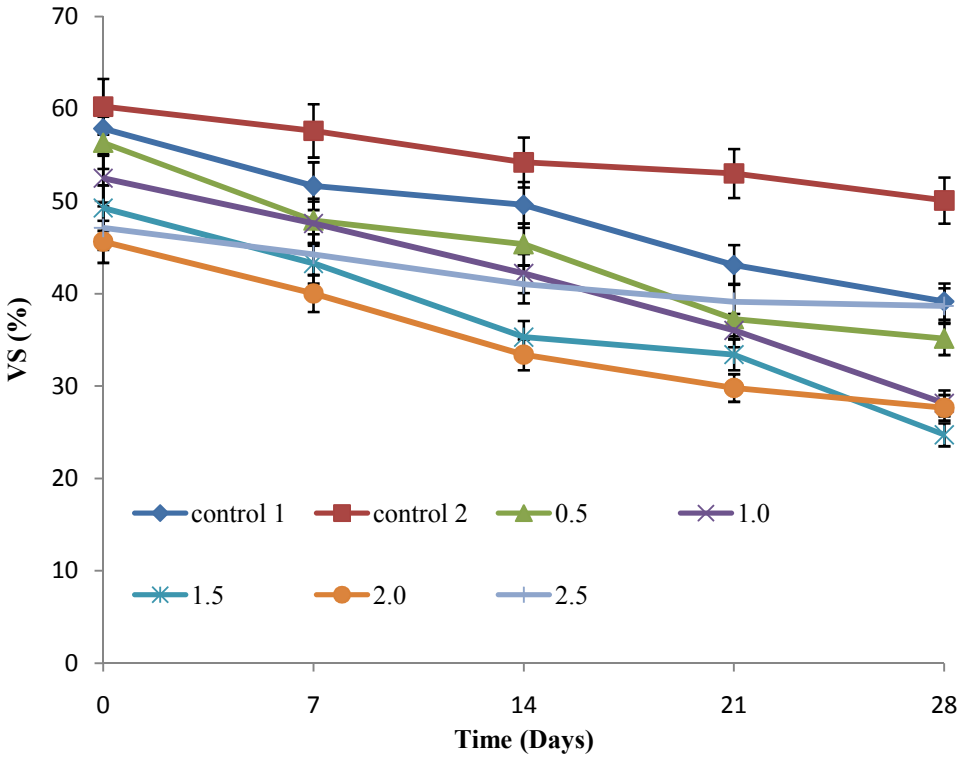


Fig. 5.13. VS reduction in electrohydrolysis pretreated water hyacinth

5.1.3.3 sCOD and VFA

Electrohydrolysis pretreatment augmented the solubilisation of the substrate thereby modifying the particulate COD into sCOD. In the electrohydrolysis pretreated water hyacinth, sCOD amplified steadily up to the 7th day and then it rapidly diminishes. On the 7th day, F/M ratio 1.5 of electrohydrolysis pretreated water hyacinth achieved the maximum sCOD (6440±23 mg/L) (Fig. 5.14). Solubilisation of the organic matter in the water hyacinth by the fermentative bacteria present in the anaerobic reactor led to the increase in sCOD upto the seventh day. After 7th day, sCOD reduces due to the acetogenic and methanogenic bacteria activity. Higher sCOD indicates higher biogas recovery. Thus, it can be stated that electrohydrolysis pretreatment solubilised the lignocellulosic water hyacinth providing higher sCOD values.

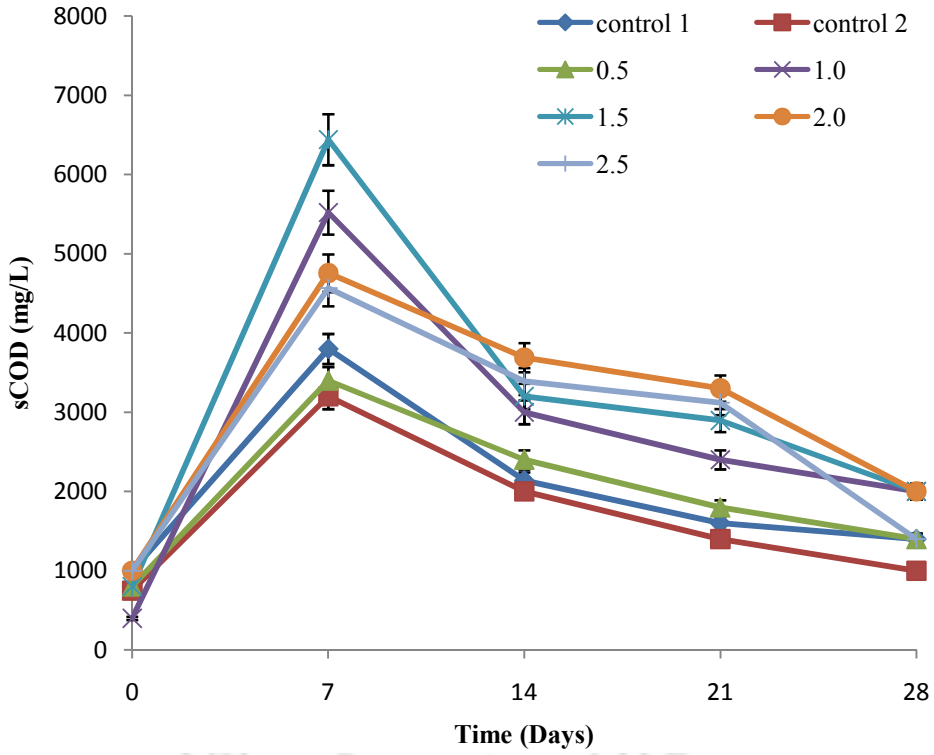


Fig. 5.14. sCOD variation in electrohydrolysis pretreated water hyacinth

Electrohydrolysis pretreated water hyacinth sharp peak was observed on the 7th day for all the F/M ratios. Each F/M ratio; executed synergistically to solubilise cellulose, transform monomers into VFA and ultimately into methane. Both untreated and electrohydrolysis pretreated water hyacinth showed a gradual increase and then decrease in VFA after attaining the utmost peak. The increase in the VFA signifies the acidogenesis phase and the gradual decrease marks the start of the methanogenesis phase. The acidogenic activity produced VFA up to the 7th day for the pretreated water hyacinth respectively. For electrohydrolysis pretreated water hyacinth highest amount of VFA

produced was 1520±13 mg/L on the 7th day respectively (Fig. 5.15). As the fermentative bacteria were more active in the initial days of the process, therefore VFA was increasing during the commencement of the process. As, acetogens and methanogens vigorously devoured the produced VFA therefore, decrease in VFA was witnessed. Rapid VFA degradation is proportional to the rate of methanogenesis. The kinetic disentanglement between the acid producers and consumers ruins the methanogenic activity (Ahring, 1995). The pH of the reactor may collapse due to inadequate buffering capacity and toxicity in microbes influencing the microbial growth due to nutrient deficiency. The acetoclastic methanogens proficiently consumed the acetate, so, no process imbalance was observed.

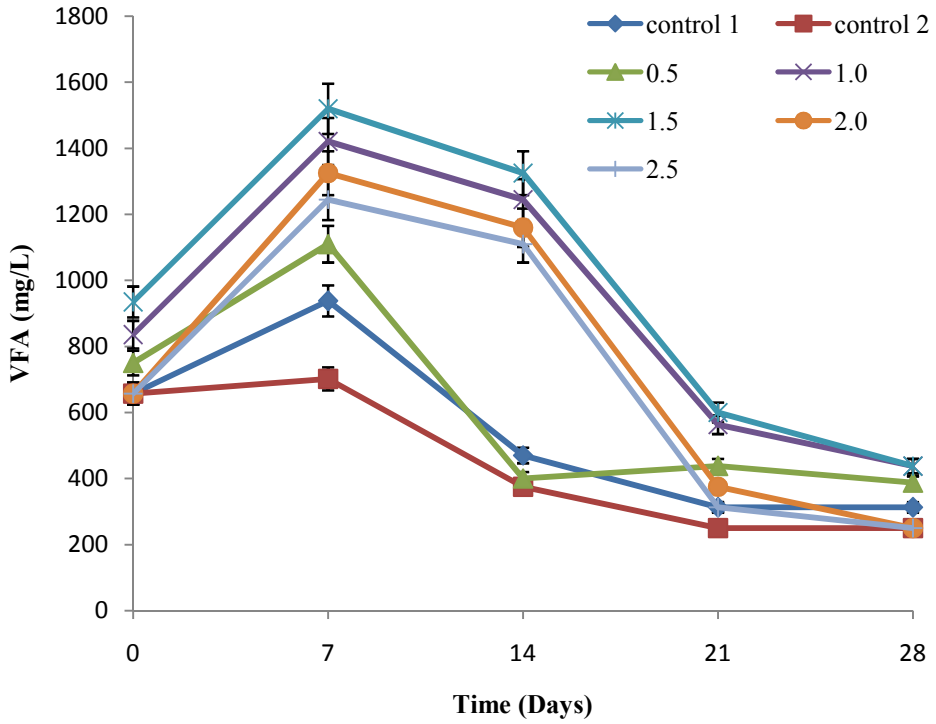


Fig. 5.15. VFA concentration variation in electrohydrolysis pretreated water hyacinth

5.1.4 BMP study of biological pretreated water hyacinth

5.1.4.1 Effect of microbial pretreatment and F/M ratio on Biogas/Methane production

Microbial pretreatment and F/M ratio has a great impact on the generation of biogas from water hyacinth. Daily biogas generation (Fig. 5.16) and cumulative biogas generation (Fig. 5.17) of microbial pretreated water hyacinth was examined during the anaerobic digestion process. Biogas generation is a significant factor for evaluating the performance of anaerobic digesters loaded with varying F/M ratios. No lag phase was observed during the anaerobic digestion of microbial pretreated water hyacinth.

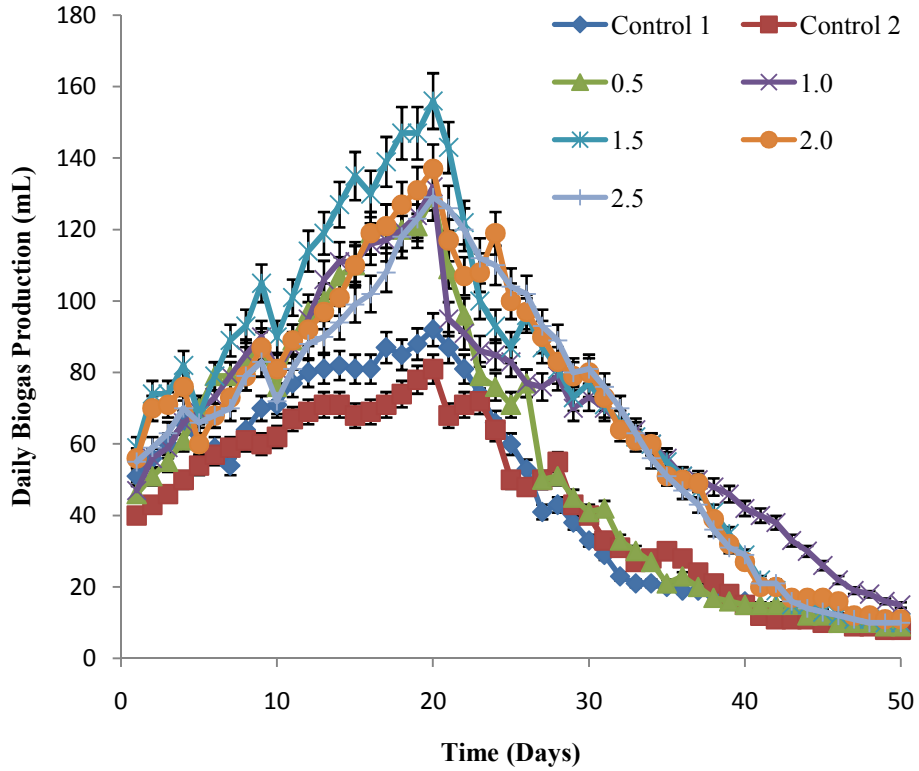


Fig. 5.16. Daily biogas production in microbial pretreated water hyacinth

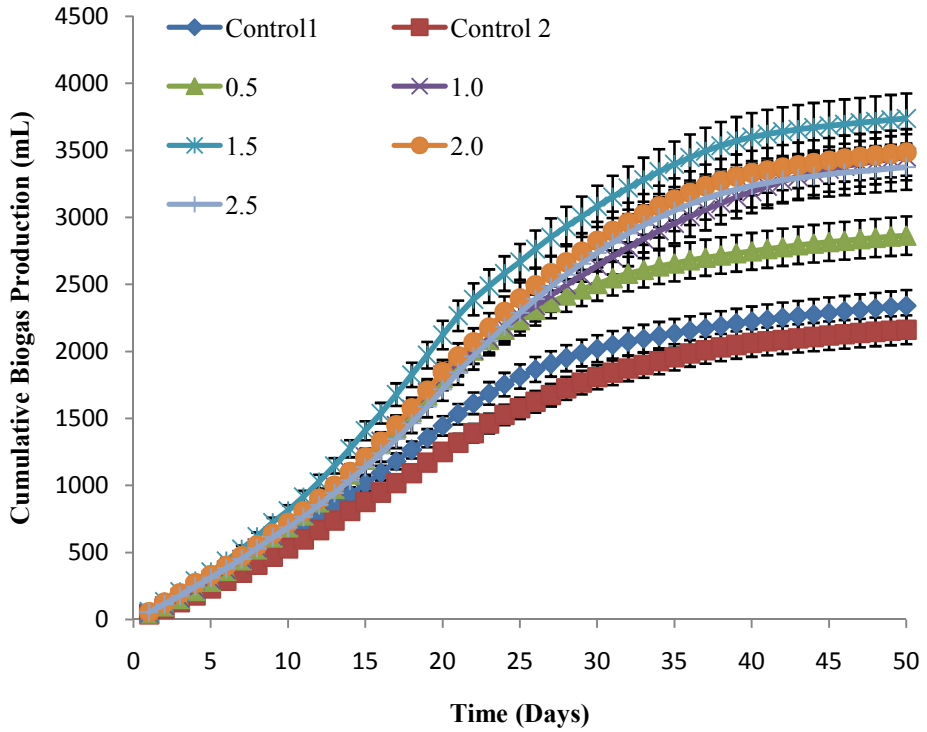


Fig. 5.17. Cumulative biogas production in microbial pretreated water hyacinth

Biogas generation initiated from the very first day itself for pretreated water hyacinth. For water hyacinth after microbial pretreatment, F/M ratio 1.5 demonstrated the

maximum daily biogas generation when compared to the other F/M ratios. The highest amount of biogas (156±11 mL CH_4/g VS) was generated by water hyacinth after microbial pretreatment on the 20th day. Further amplification in biogas generation persisted throughout the start-up and the steady phase. By the end of the 50 days, biogas generation stabilized and steady phase was achieved for each and every F/M ratio. F/M ratio 1.5 confirmed the highest cumulative methane production for microbial pretreated water hyacinth when compared to the other ratios respectively. Cumulative methane production of 3737±19 mL was achieved by F/M ratio 1.5 for the microbial pretreated water hyacinth by the end of 50 days. An appropriate F/M ratio acted synergistically for easier and faster hydrolysis of the water hyacinth when compared to the other ratios. The results lay emphasis on the easily bioaccessible soluble organic matter of the water hyacinth which was amenable to methanogenic bacteria after microbial pretreatment and the existence of highly resistant lignocellulosic cell wall of untreated water hyacinth which was hard for methanogens to degrade. The quantity of soluble organic matter to be digested anaerobically, increases after the microbial pretreatment. Kuijk et al. (2015) reported that an effective strain and ideal culture condition makes the pretreatment process efficient by reducing the pretreatment time and carbohydrate loss. Thus, *Citrobacter werkmanii* VKVVG4 pretreatment of water hyacinth with a dosage of 10^9 CFU/mL for 4 days assisted in breaking the chemical bonds of the rigid lignocellulosic cell wall. The reduced pretreatment time and the optimised strain (*Citrobacter werkmanii* VKVVG4) and culture condition required for microbial pretreatment of water hyacinth illustrates to have the same opinion with Kuijk et al. (2015). Thereby, microbial pretreatment aid in discharging the extracellular and intracellular biopolymers of the water hyacinth into the soluble aqueous phase. The discharge of soluble organic matter during microbial pretreatment assists in accelerating the biodegradability of the substrate or enhancing the biogas generation.

5.1.4.2 Effect of microbial pretreatment and F/M ratio on sCOD, VFA and VS

Weekly analysis of the samples, illustrates the variation undergone during biochemical methane potential assay of water hyacinth after microbial pretreatment and the importance of an ideal F/M ratio. sCOD of microbial pretreated water hyacinth increased with the passage of time for each and every F/M ratio. In microbial pretreated water hyacinth sCOD increased (Fig. 5.19) with the increase in VFA (Fig. 5.18). Both VFA and sCOD increased hand in hand and after attaining the utmost peak, it started decreasing.

Yu et al. (2004) stated that the increase in sCOD signifies the increased quantity of soluble organics that can be easily converted to methane.

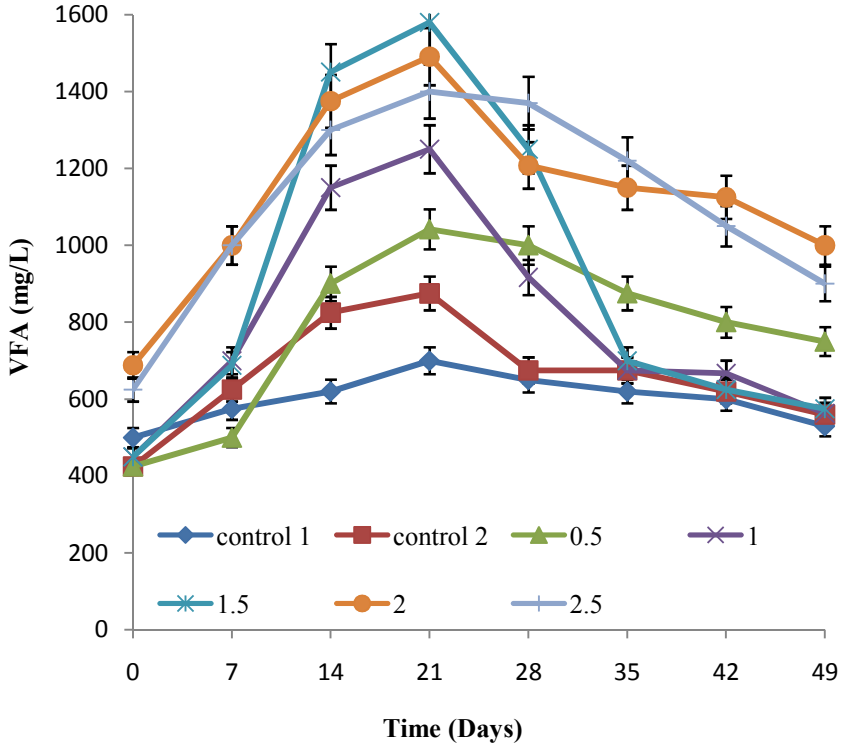


Fig. 5.18. Variation in VFA concentration in microbial pretreated water hyacinth

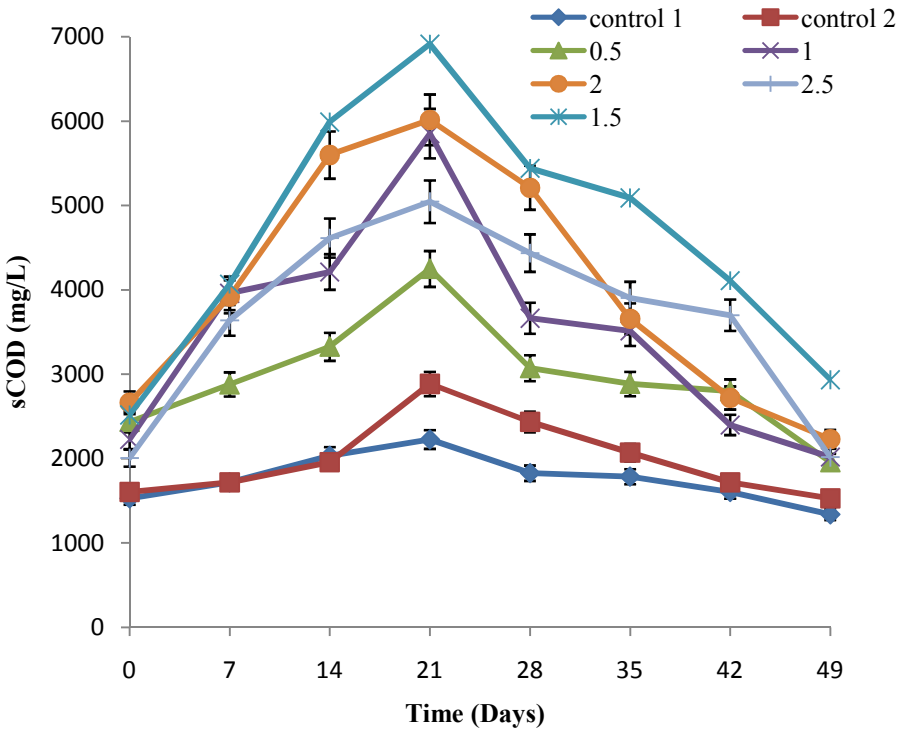


Fig. 5.19. sCOD variation in microbial pretreated water hyacinth

The exoenzymes secreted during the microbial pretreatment solubilised the particulate organic matter of water hyacinth, thereby leading to the increase in the availability of soluble organic matter and the microbial population.

VFA is produced when carbohydrates are broken down. VFA concentration was the highest on the 21st day for each and every F/M ratio of microbial pretreated water hyacinth. F/M ratio 1.5 of microbial pretreated water hyacinth achieved a VFA concentration of 1580±5 mg/L. An increase in the VFA concentration initially, was observed due to the activity of the acidogens and the steady fall in the concentration of VFA illustrates the beginning of methanogenic phase (Lin et al., 2017). As the methanogens are sensitive to acidic conditions so they come into action by the end of acidogenic phase. The produced VFA executes a significant duty in accelerating the hydrolysis of lignocellulosic water hyacinth. Acidification of the medium due to the accumulation of VFA collapses the anaerobic digestion process during the startup period as highly acidic condition inside the reactor inactivates the methanogens. But, VFA concentration was within range (less than 13000 mg/L) thus, no inhibition during biogas generation was witnessed. Maintaining neutral pH inside the anaerobic reactor is very essential as subtle biochemical equilibrium between the acidogens and the methanogens should be prevalent (Syaichhurozi, 2017). sCOD increased with the passage of time because the microbial pretreatment made the soluble organics of water hyacinth easily bioaccessible for the microorganisms. Similarly, increase in sCOD for every F/M ratio was witnessed on the 21st day but F/M ratio 1.5 of microbial pretreated water hyacinth achieved a highest sCOD of 6912±6 mg/L. After 21 days, sCOD starts decreasing as the soluble organics starts exhausting.

During the weekly analysis of VS, reduction in the VS of the samples with the passage of time was observed F/M ratio 1.5 in the microbial pretreated water hyacinth demonstrated a maximum VS reduction of 41.8% (Fig. 5.20). Higher VS reduction was observed in the microbial pretreated water hyacinth. Higher VS reduction is favourable as it signifies higher generation of biogas. In untreated water hyacinth the microbial activity was restricted as the availability of the soluble organics was limited when compared to the microbial pretreated water hyacinth demonstrating lesser reduction in VS. F/M ratio 1.5 in the microbial pretreated water hyacinth was demonstrated as the ideal ratio where methanogenic bacteria were able to flourish successfully.

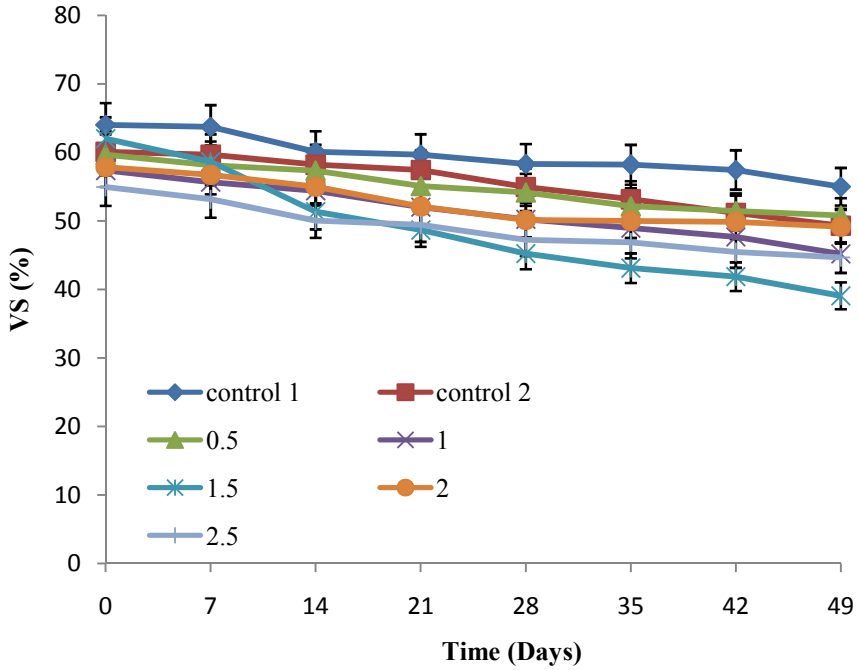


Fig. 5.20. VS reduction in microbial pretreated water hyacinth

➢ ***Kinetic study***

Modified Gompertz equation was used to establish the improvement in the efficiency of the ideal F/M ratios of both untreated and pretreated water hyacinth. The cumulative methane generation values were placed in Gompertz equation curve (Lee et al., 2013). Table 5.1 demonstrates the kinetic parameters of microbial pretreated and untreated water hyacinth. The kinetic parameters of the untreated and microbial pretreated water hyacinth utilized during BMP assay were determined where the value of M of the pretreated water hyacinth (4.2314 L CH_4) was confirmed to be greater than the untreated water hyacinth (4.1796 L CH_4). For both untreated and pretreated water hyacinth R^2 value exceeds 0.90, signifying that methane generation can be well simulated.

Table 5.1. Kinetics values of the optimised F/M ratio of water hyacinth used in BMP test

Pretreatment	F/M ratio	M (L CH_4)	Rmax (L CH_4 d^{-1})	λ (d)	Y (L CH_4)	No. of days
Thermal	1.5	4.4971	0.1000	0.0000	0.93	35
Electrohydrolysis	1.5	3.1813	0.1000	0.0000	0.95	30
Biological	1.5	4.2314	0.1000	0.0000	0.95	50
Untreated	2.0	4.1796	0.0772	0.0010	0.91	50

➢ ***Biogas composition***

The composition of biogas produced from water hyacinth with and without pretreatment has been incorporated in Table 5.2. It has been observed that quantity of methane (CH_4) has increased after pretreatment of water hyacinth. Higher the methane content in biogas; better is the quality of biogas. Highest content of methane (67.4±0.3%) was illustrated by thermally (hot air oven) pretreated water hyacinth followed by electrohydrolysis pretreatment (61.25±0.8%) and microbial pretreatment (59.99±0.3%). The untreated water hyacinth showed the least methane content of 57±0.2%. As the methane content of the hot air oven pretreated water hyacinth is appreciably high (67.4 ± 0.3 %), therefore the calorific value is 5,783 kcal/m^3. Thus it can be suggested that pretreatment of water hyacinth not only enhances biogas production but also improves the quality of biogas.

Table 5.2. Biogas composition of water hyacinth with and without pretreatment

Pretreatment	CH_4 (%)	CO_2 (%)	H_2 (%)	N_2 (%)
Untreated	57± 0.2	39±0.2	2±0.1	1±0.1
Thermal	67.4±0.3	24.88±0.6	4.97±0.8	1.59±0.2
Electrohydrolysis	61.25±0.8	28.15±0.1	8.35±0.2	1.85±0.3
Microbial	59.99±0.3	30.89±0.6	6.97±0.8	1.82±0.2

5.2 BATCH STUDY

Once the 1 L BMP study was over and the F/M ratio was optimised for both untreated and all the pretreated water hyacinth, the batch study was scaled up to 20 L to test the operational conditions during large scale and to decrease the unevenness due to heterogeneous physico-chemical characteristics of water hyacinth.

Fig. 5.21 and 5.22 illustrated the phenomenal increase in biogas production from water hyacinth after thermal (hot air oven) pretreatment. When compared to the untreated water hyacinth electrohydrolysis and microbial pretreatment also improved biogas production from water hyacinth. Lag phase was observed in untreated water hyacinth but all the pretreatment techniques were beneficial in cutting down the lag phase. Biogas production in the pretreated water hyacinth initiated from the very first day itself. It took 100 days for the biogas production to be stable for the hot air oven pretreated water hyacinth. While biogas production; stabilised in 80 days for both untreated and microbial pretreated water

hyacinth. Fastest stabilisation of biogas production was observed in electrohydrolysis pretreated water hyacinth. Each and every pretreatment was beneficial in enhancing biogas production from water hyacinth and in accelerating hydrolysis period.

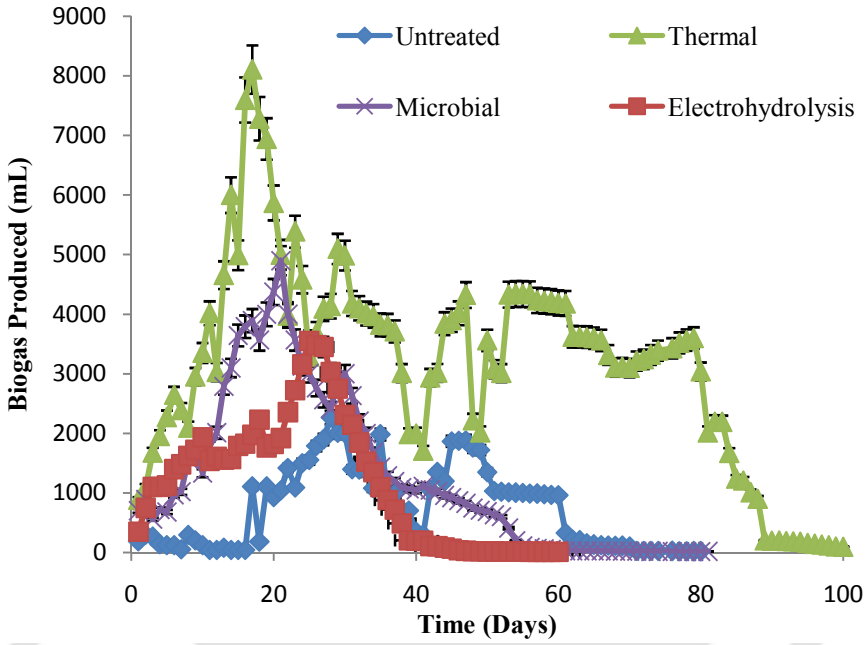


Fig. 5.21. Daily biogas production in water hyacinth after pretreatment in 20 L batch study

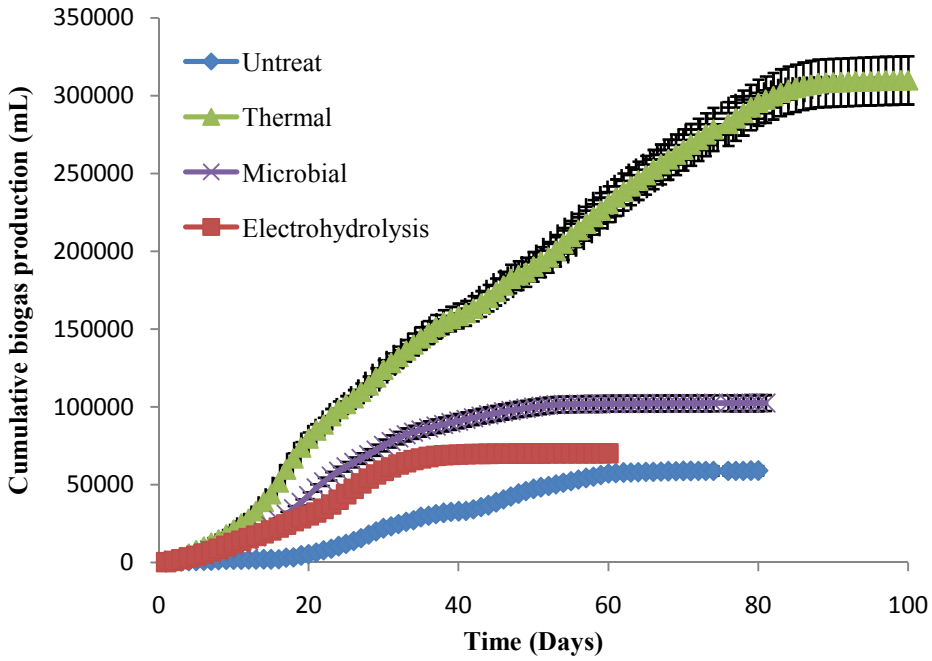


Fig. 5.22. Cumulative biogas production in water hyacinth after pretreatment in 20 L batch study

5.3 COMPARATIVE ANALYSIS

From the BMP study it was demonstrated that for untreated water hyacinth F/M ratio 2 produced the highest amount of biogas (143±14 mL CH_4/g VS) on the 32nd day. Thereby, suggesting, the need of pretreatment for anaerobic digestion of water hyacinth. Water hyacinth was pretreated with the necessary ideal condition required during hot air oven, electrohydrolysis and microbial pretreatment. It was observed that after pretreatment the highest biogas production was observed in the F/M ratio 1.5. Among all the three types of pretreatment techniques, remarkably improved biogas production at reduced time period by demonstrated by hot air oven pretreatment (Table 5.3). Alongwith the improvement in biogas production, the percentage of methane also increased after hot air oven pretreatment at 90ºC for 1h.

Table 5.3 Comparative analysis of the BMP study

Pretreatment	F/M ratio	Highest biogas production
Untreated	2	143±14 mL CH_4/g VS on the 32nd day
Thermal	1.5	193±22 mL CH_4/g VS on the 14th day
Electrohydrolysis	1.5	155±19 mL CH_4/g VS on the 7th day
Microbial	1.5	156±11 mL CH_4/g VS on the 20th day

CHAPTER 6
ANAEROBIC CO-DIGESTION STUDY

This chapter deals with the effect of anaerobic co-digestion of water hyacinth with various organic wastes as a co-substrate with and without pretreatment and their scaled up batch studies. Methane potential of the various anaerobic co-digestion studies was determined for various F/M ratios in order to optimise the ideal F/M ratio.

6.1 ANAEROBIC CO-DIGESTION STUDY OF WATER HYACINTH AND FOOD WASTE

Burning of fossil fuel, scarce energy supply, price hike in energy, environmental pollution, ever increasing rise in food waste and phenomenal reproduction potential of water hyacinth are few of the most important problems prevailing throughout the world. The alleviation of greenhouse gases emission and global warming stipulates the utilization of alternative green energy so as to diminish the dependence on fossil fuel. Water hyacinth "the world's worst aquatic weed" is a potential reservoir of cellulose. The presence of cellulose makes water hyacinth an attractive biomass for biogas generation due to its easy accessibility worldwide and its affinity to re-grow even during phase of trauma. While one-third of the food produced per year globally for consumption is thrown away, this sums upto about 1.3 billion tons per year (FAO, 2011). Food waste comprises the major portion of municipal solid waste. Anaerobic digestion of organic waste is a sustainable alternative for the generation of environment friendly biogas. Sometimes anaerobic digestion may involve lengthy hydrolysis period or less biogas generation due to the utilization of a solo substrate (Yamashiro et al., 2013) making it typically unfavourable for energy production. Anaerobic mono-digestion of food waste has comparatively higher biogas potential than water hyacinth due to its excessively high biodegradability. Food waste is easily hydrolysable as protein and carbohydrate degrading bacteria grows fast. But the problem associated is that the methanogenic bacteria takes considerable time to grow when compared to the acidogens (Koch et al., 2015). Thereby; inducing the build up of the metabolic intermediates; mainly volatile fatty acids (VFA). This further causes nutrients imbalance and accumulation of volatile fatty acids (VFA) inside the reactor during the process might lead to the failure of biogas generation. Zhang et al. (2013) reported digestion failure during mono-digestion of food waste. While; the presence of lignocellulosic compounds makes biodegradation of water hyacinth slightly difficult than food waste. Anaerobic co-digestion of organic wastes seems to be an

efficient measure as it balances the nutrients and enhances the synergistic activities between the various organic substrates by providing a more stable environment inside the reactor. There are a number of previous literature reports available that suggests improved biogas generation rate and methane yield when lignocellulosic waste was co-digested with food waste. Capson-Tojo et al. (2017) reported that co-digesting cardboard with food waste helped in enhancing the methane potential by 71-93%. Zhou et al. (2015) observed that co-digestion of food waste and corn stalk enhanced biomethane production by 22.48% and 41.55% than mono-digestion of food waste and corn stalk, respectively. Yong et al. (2015) also reported that co-digestion increased methane production yield by 39.5% and 149.7% when compared to mono-digestion of food waste and straw respectively. Brown and Li (2013) reported that co-digestion of yard waste with food waste improved the yield of methane. Similar results were also reported by Wan et al. (2013) when Chinese silver grass and food waste was co-digested. Thus co-digestion of organic substrates aids in diminishing the prospective noxious compounds existing in any of the co-substrates, regulating the moisture content and pH, providing buffering capacity to the mixture inside the anaerobic digester, enhancing the amount of easily biodegradable material, expanding the range of microorganisms participating in the process and improved biogas generation (Esposito et al., 2012). Anaerobic co-digestion process necessitates the utilization of a suitable mixing ratio to maintain steady condition within the reactor. An appropriate mixing ratio of various waste materials generates superior digestion performance by recuperating the content of the nutrients as well as by diminishing the negative effect of toxic compounds (Murto et al., 2004).

6.1.1 Characteristics of substrate and co-substrate

Anaerobic co-digestion process is influenced by the characteristics of substrate and co-substrate. The initial characteristics of the substrate and co-substrate are essential parameters to be examined which affect the anaerobic process stability and biogas production. The initial physico-chemical characteristics of the substrate and co-substrate used in this study were investigated and the results are incorporated in Table 6.1. The pH of water hyacinth and food waste was in the acidic range. Cow dung was utilised as an inoculum along with the substrate and co-substrate which helped in retaining the pH of the anaerobic digesters. Both the substrate and co-substrate has good amount of moisture content and sCOD; which are beneficial for the anaerobic digestion process. Food waste is readily degradable but water hyacinth takes sometime due to the presence of lignin.

The presence of lignin in water hyacinth can be dissolved down by hot air oven pretreatment.

Table.6.1.Initial characterisation study of the substrate and co-substrate

Parameter	Water hyacinth	Food waste
Moisture content (%)	90	70
pH	5.8	4.2
sCOD (mg/L)	1600	2000
VFA (mg/L)	750	1250

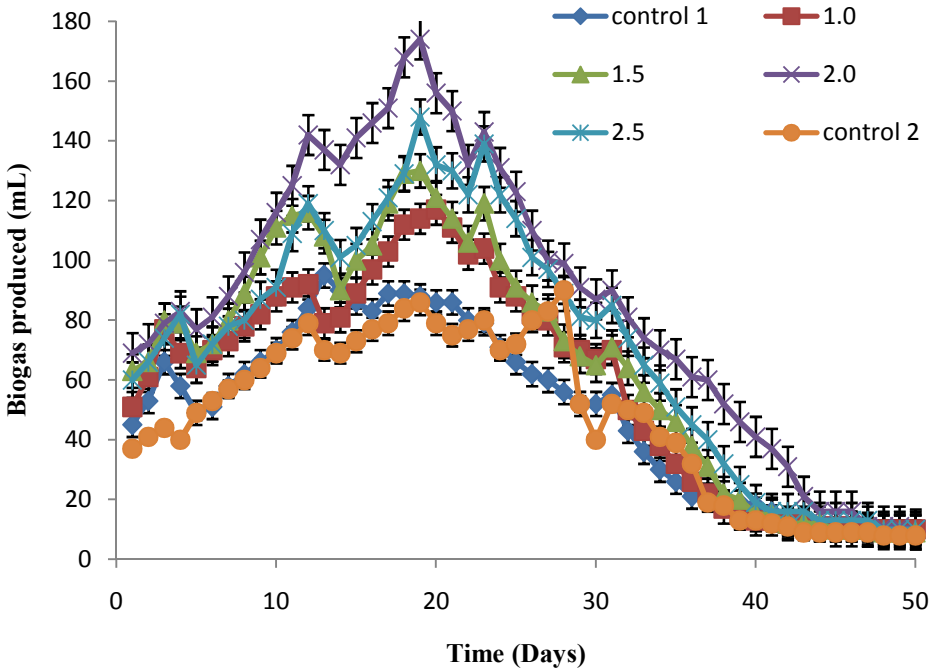
6.1.2 Effect of co-digestion and mixing ratios on biogas production

In order to study the effect of anaerobic co-digestion of water hyacinth and food waste two sets of experimental study was performed simultaneously with various mixing ratios. In set I water hyacinth was untreated and in set II water hyacinth was pretreated thermally. Food waste was not pretreated as it is easily degradable and non-lignocellulosic in nature.

Fig. 6.1a and 6.1b represents the daily biogas generation of various mixing ratios for both set I and set II respectively. Biogas generation commenced from the very first day itself for all the ratios but in varying proportion. The total biogas generation boosted steadily throughout the digestion period. Lesser biogas generation was observed in mono-digestion when compared to co-digestion because co-digestion and mixing ratio worked synergistically to balance the nutrients and to develop a versatile and robust microbial community. In set I, mixing ratio 2 demonstrated the highest biogas production of 174±6 mL CH_4/g VS on the 19th day. While in set II mixing ratio 1.5; demonstrated the highest biogas production of 206±10 mL CH_4/g VS on the 13th day itself. In both the sets, biogas generation increased with the increase in mixing ratio upto mixing ratio 2 in set I and upto mixing ratio 1.5 in set II.

Biogas generation decreased in set I for the mixing ratio 2.5 and in set II for the mixing ratio 2. Thermal pretreatment in set II for the mixing ratio 2 must have increased the amount of solubilisation of the substrate. This increased amount of solubilisation of the substrate inhibited the methanogenesis process by over-production of VFA. Therefore, the mixing ratio 2 in set II demonstrated lower biogas generation when compared to the mixing ratio 2 in set I. Syaichurrozi (2018) observed highest biogas

production of 113.92±6.90 mL CH_4/g VS on the 18th day from the co-digestion of *Salvinia molesta* and rice straw in the ratio 40:60.



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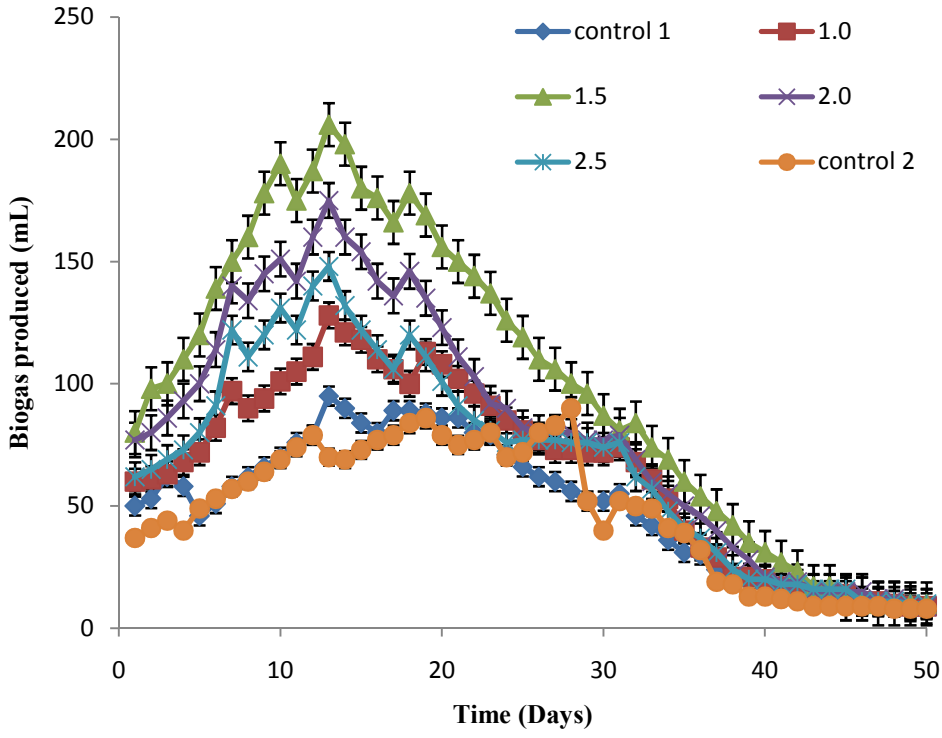

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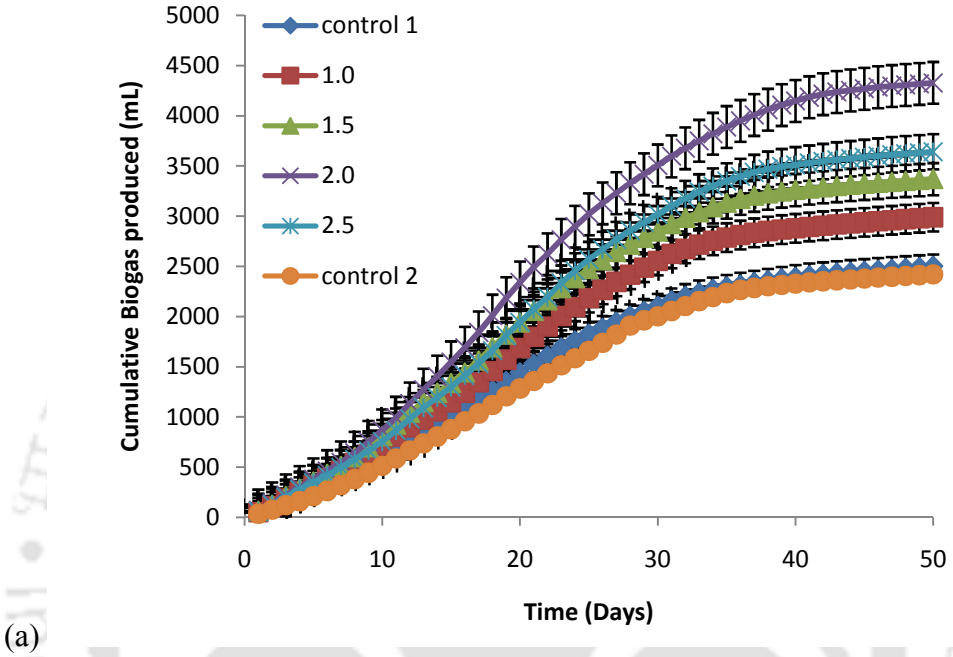
Fig. 6.1. Daily biogas generation for the various mixing ratios of (a) untreated water hyacinth and food waste and (b) pretreated water hyacinth and food waste

No extremely long duration of lag phase was witnessed in the cumulative biogas generation graphs for both set I (Fig. 6.2a) and set II (Fig. 6.2b) during anaerobic co-

digestion of water hyacinth and food waste. In both set I and set II continuous increase in biogas generation was observed throughout the start-up and the steady phase. Until generation of biogas reduced by the end of 50 days and steady phase was accomplished for each and every mixing ratio. Mixing ratio 1.5 in set II illustrated the highest cumulative biogas generation when compared to the other ratios of set II and all the ratios of set I respectively.



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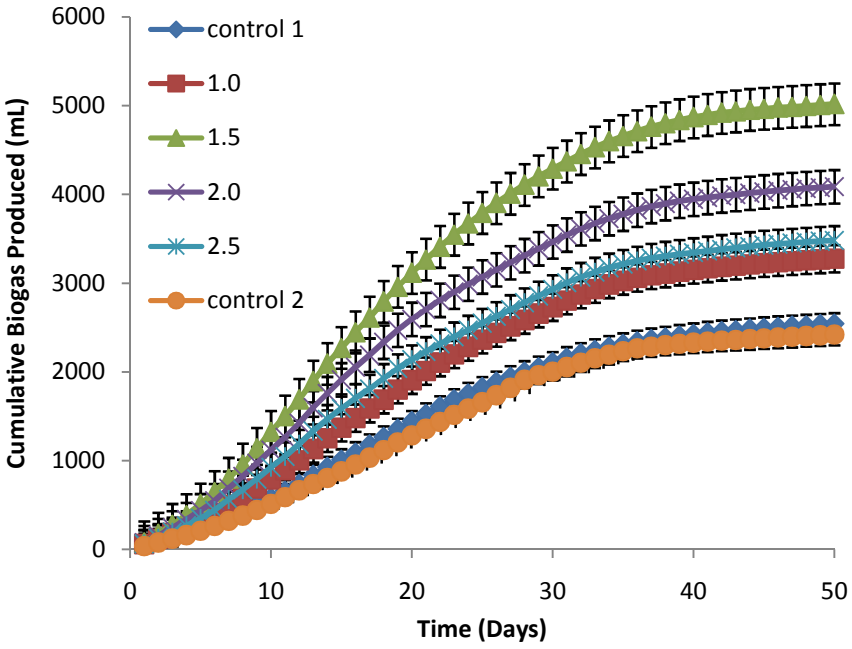


(b)

Fig. 6.2. Cumulative biogas generation for the various mixing ratios of (a) untreated water hyacinth and food waste co-digestion and (b) pretreated water hyacinth and food waste co-digestion

Cumulative biogas generation of 5017±15 mL was attained by the ratio 1.5 by the end of 50 days in set II. While mixing ratio 2 in set I exhibited the highest cumulative methane production of 4328±12 mL by the end of 50 days. Higher quantity of methane content in biogas indicates better quality of biogas. Tasnim et al. (2017) reported the presence of 65% CH_4, 14% CO_2 and 21% other gases in the anaerobic co-digestion of water hyacinth, cow dung and sewage sludge. Increased amount of methane content was observed for mixing ratio 1.5 in set II than the mixing ratio 2 in set I (Table 6.2). Thereby suggesting that pretreatment and co-digestion has a synergistic effect rather than co-digestion alone. An apt mixing ratio worked synergistically for enhanced biogas generation in both set I and II. The relatively faster degradation or reduced digestion period (13 days) in set II when compared to set I (20 days) was exhibited due to the effect of hot air oven pretreatment. As, the amount of soluble organic matter in water hyacinth to be digested anaerobically increased after hot air oven pretreatment in set II. Application of heat melts down lignin leading to easier and faster biodegradation thereby increasing biogas generation. Hot air oven pretreatment of water hyacinth in set II aided the methanogenic bacteria to easily access the soluble organic matter present in the lignocellulosic water hyacinth. While in set I, biogas generation was relatively lesser because the methanogenic bacteria found it difficult to degrade the recalcitrant lignocellulosic cell wall present in the untreated water hyacinth.

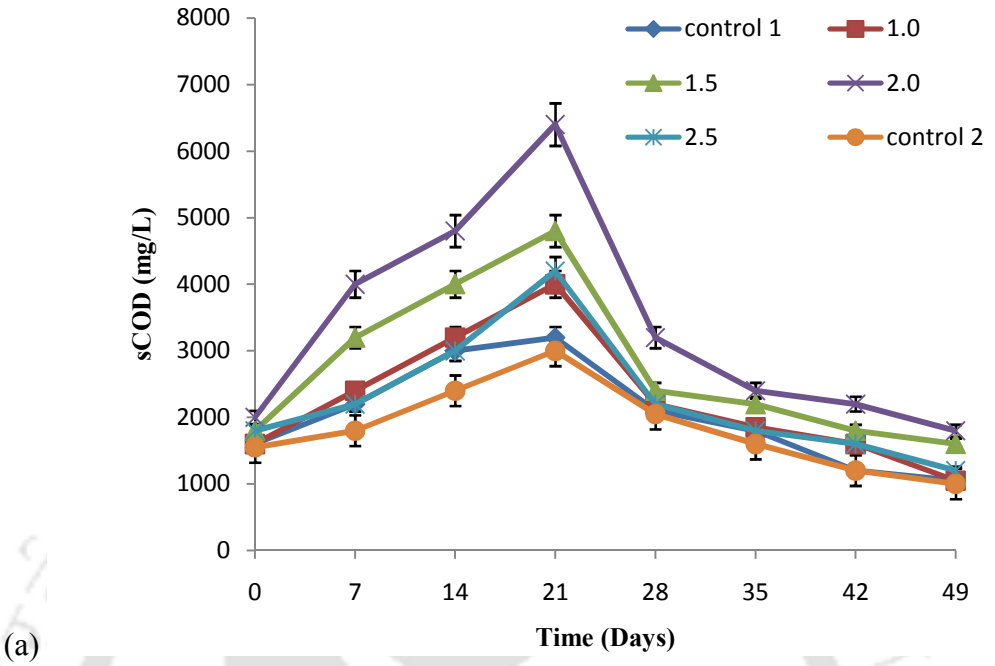
Table 6.2. Biogas composition of set I and II.

Compound	Set I (%)	Set II (%)
CH_4	59.45 ± 0.2	68.44 ± 0.5
CO_2	30.67 ± 0.5	22.98 ± 0.9
H_2	6.54 ± 0.3	8.79 ± 0.8
N_2	2.54 ± 0.4	----

6.1.3 Effect of co-digestion on sCOD, VFA and VS

sCOD, VFA and VS analysis were conducted on a weekly basis for all the ratios of both the sets. Weekly assessment of the samples reveals the changes undergone during BMP assay due to co-digestion of water hyacinth and food waste. Also the significance of

an ideal mixing ratio was emphasized. sCOD of both set I and II amplified with the passage of time for each and every ratio.



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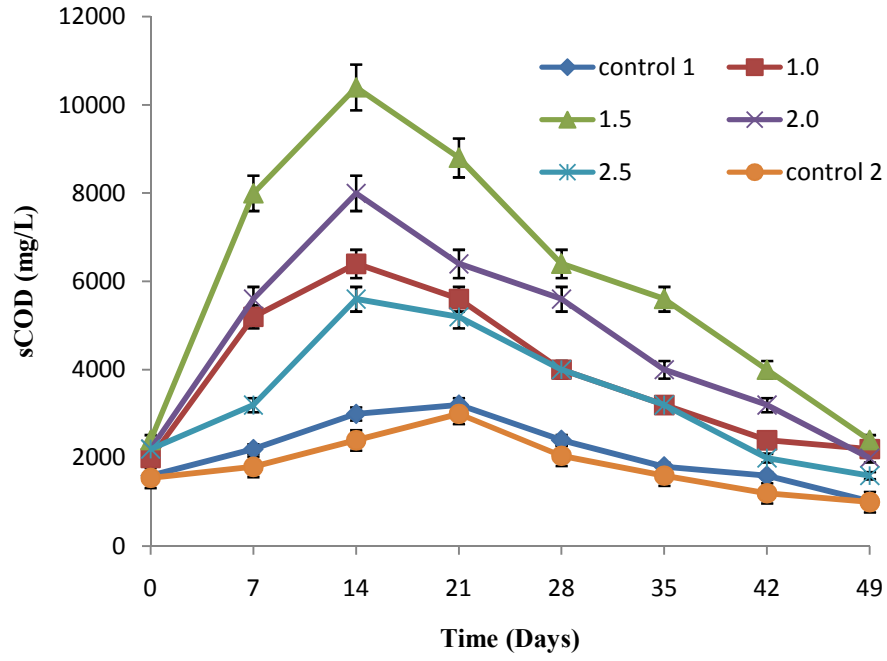

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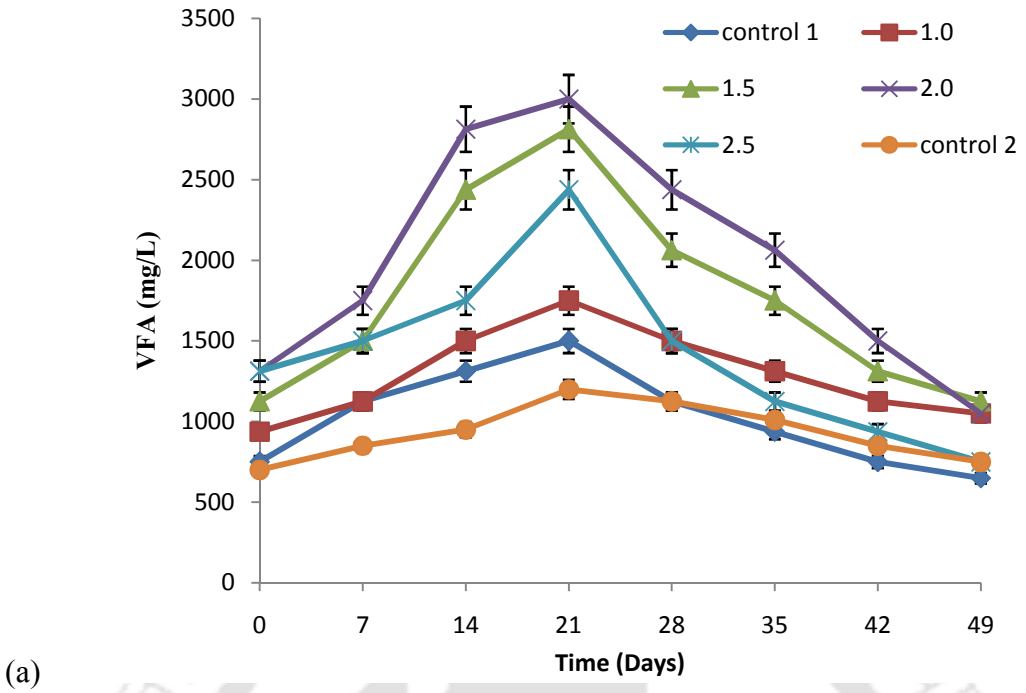
Fig. 6.3. Variation in sCOD for the various mixing ratios of (a) untreated water hyacinth and food waste co-digestion and (b) pretreated water hyacinth and food waste co-digestion

In both set I and II sCOD amplified (Fig.6.3a and 6.3b) as VFA amplified (Fig. 6.4a and 6.4b). Hydrolysis of carbohydrates generates VFA. The generated VFA performs a prominent function in hastening the hydrolysis phase during the anaerobic co-digestion process.



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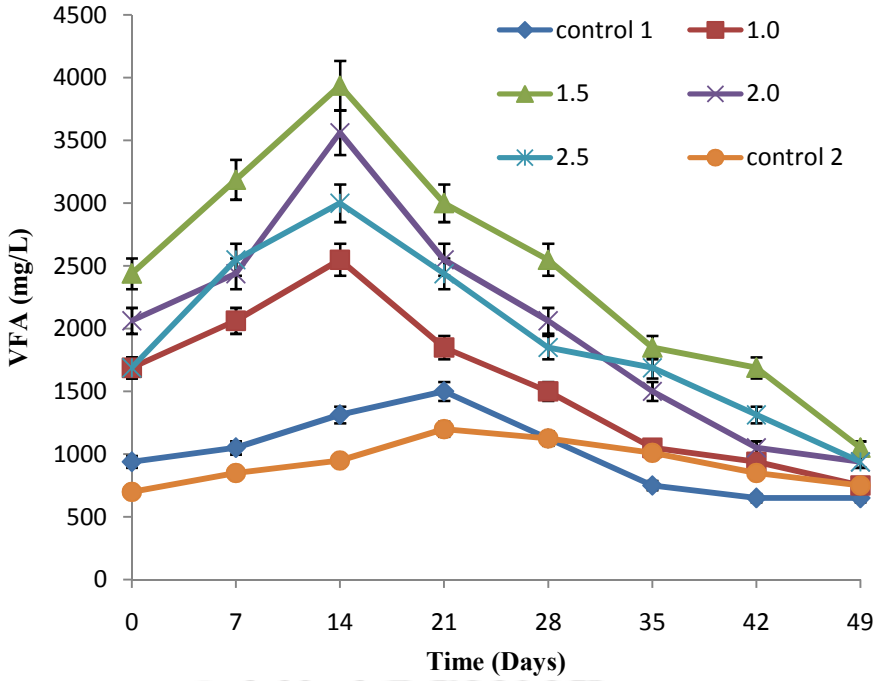
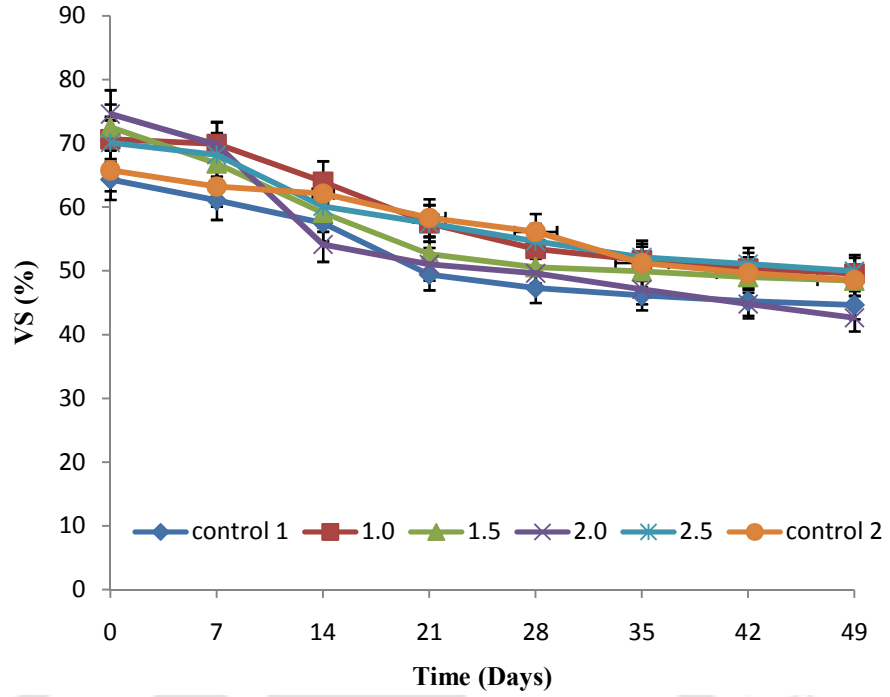


(b)

Fig. 6.4. Variation in VFA concentration for the various mixing ratios of (a) untreated water hyacinth and food waste co-digestion and (b) pretreated water hyacinth and food waste co-digestion

In both the sets amplification in VFA and sCOD was observed to occur concurrently and after achieving the maximum value, it started descending. Rise in sCOD indicates the amplified amount of simple soluble organic matter that can be effortlessly converted to biogas by the methanogenic bacteria (Yu et al., 2004).



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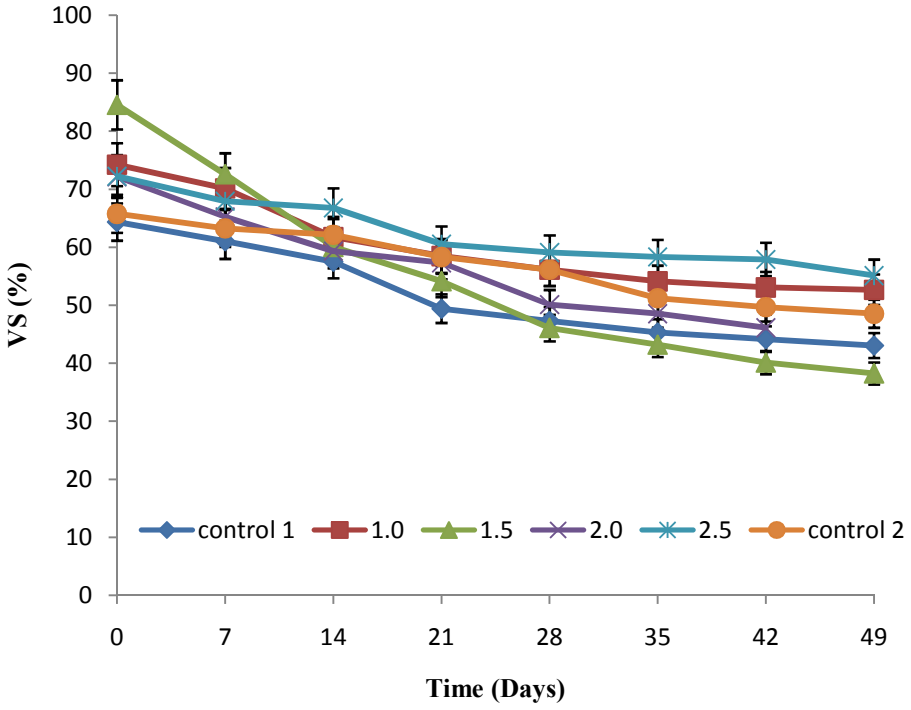


(b)

Fig. 6.5. Reduction in VS for the various mixing ratios of (a) untreated water hyacinth and food waste co-digestion and (b) pretreated water hyacinth and food waste co-digestion

In set I, both sCOD and VFA concentration was observed to be maximum on the 21st day for each and every ratio. While in set II sCOD and VFA concentration was observed to be maximum on the 14th day. Mixing ratio 2 in set I achieved a maximum VFA concentration of 3000±10 mg/L whereas in set II for the ratio 1.5 maximum VFA

concentration was observed to be 3938±12 mg/L. Initially, the activity of the acidogenic bacteria amplified the VFA concentration, and the commencement of the methanogenic phase led to the drop in VFA concentration (Lin et al., 2017). Although, higher concentration of VFA was observed in the co-digested samples rather than the mono-digested samples still higher stability was observed in the co-digestion system since the addition of water hyacinth enhanced the buffer capacity. Likewise, ratio 1.5 in set I, achieved a maximum sCOD of 6400±12 mg/L while ratio 2 in set II achieved a maximum sCOD of 10,400±10 mg/L. After 21 days and 14 days in set I and set II respectively, reduction in sCOD was witnessed because the simple soluble organic matter was exhausting due to the activity of the microorganisms. Yin et al. (2016) also observed maximum sCOD when mixed activated sludge and food waste pretreated with fungal mash was co-digested than mono-digestion of substrate with and without pretreatment.

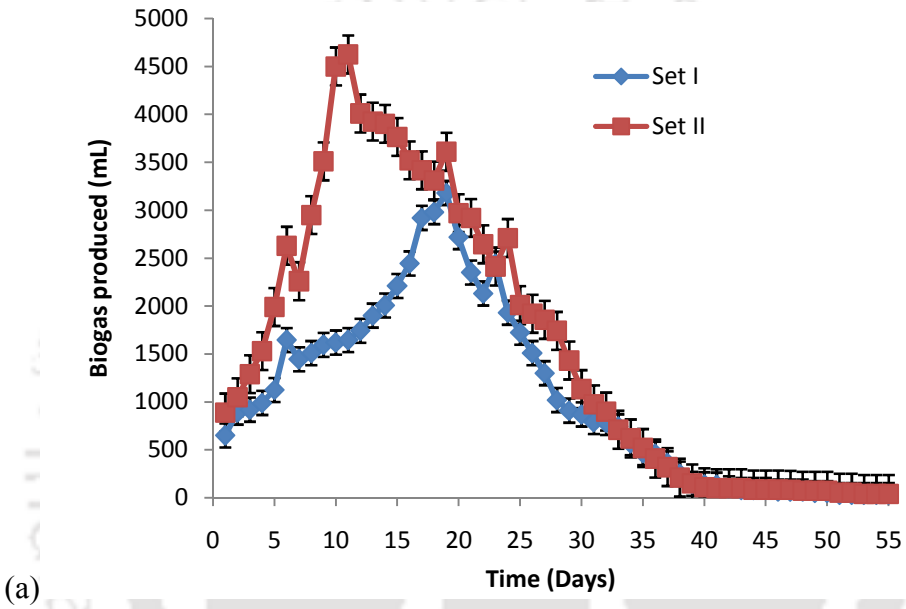
Decrease in VS was witnessed with the increase in time, during weekly analysis of the samples. Mixing ratio 2 in set I exhibited the highest decrease in VS of 43% (Fig. 6.5a) while the mixing ratio 1.5 in set II exhibited a highest decrease in VS of 55% (Fig. 6.5b). Higher VS reduction was observed in mixing ratio 1.5 in set II when compared to the mixing ratio 2 in set I. Anaerobic co-digestion of organic fraction of municipal solid waste with thermo-alkaline pretreated thickened waste activated sludge and H_2O_2 pretreated rice straw witnessed the highest VS reduction of 76.9% due to the synergism (Abudi et al., 2016). Decrease in VS is a positive effect of anaerobic co-digestion process as it indicates increase in the generation of biogas. In set I, bioaccessibility to the simple soluble organic matter was limited when compared to set II thereby exhibiting lesser decrease in VS as the microbial activity was restricted. Mixing ratio 1.5 in set II was observed to be the ideal mixing ratio because the methanogens were able to flourish generating increased quantity of biogas. The highest biogas generation was depicted by the mixing ratios showing maximum VS reduction and vice versa.

6.1.4 Kinetic study

To establish the effectiveness of the ideal mixing ratio and pretreatment on the anaerobic co-digestion of water hyacinth and food waste, the cumulative methane production values were fitted to Gompertz equation curve (Lee et al., 2013). Table 4 summarises the results of the kinetic study for both set I and II. The kinetic parameters of set I and II used in BMP assay were determined where M of set II (6.3921 L CH_4) was observed to be higher than set I (5.5190 L CH_4). Both set I and II have R^2 value above 0.9, indicating that methane production can be well simulated.

Table 6.3. Kinetics values of untreated and hot air oven pretreated water hyacinth used in BMP test.

Substrate	Mixing ratio	M (L CH_4)	R max (L CH_4 d^{-1})	λ (d)	R^2	Y (L CH_4)
Set I	2.0	5.5190	0.1000	0.0000	0.95	4.328
Set II	1.5	6.3921	0.0780	0.0000	0.93	5.017



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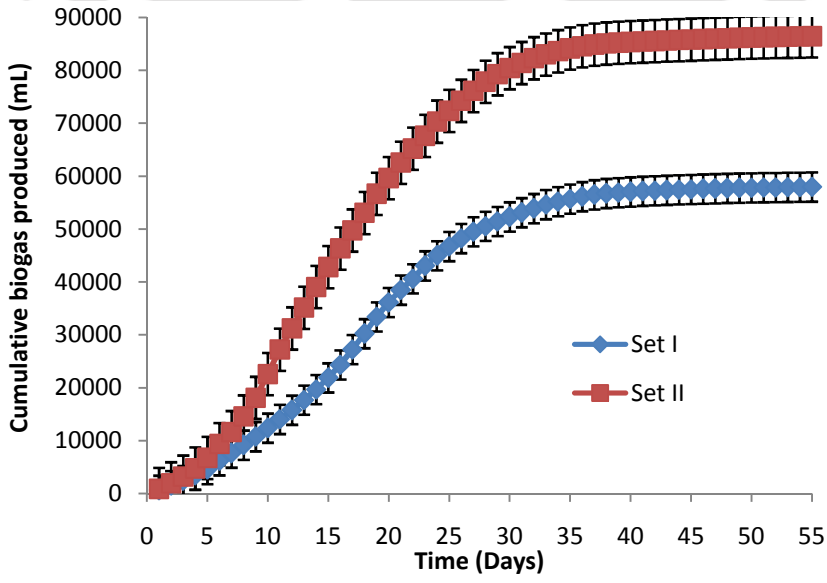


(b)

Fig. 6.6. (a) Daily biogas production and (b) cumulative biogas production of set I and II in 20 L anaerobic batch digester

6.1.5 Scaled up batch anaerobic digestion

The aforementioned methodical investigation demonstrated confirmatory results and disclosed the ideal F/M ratio for both set I and II; therefore the batch study was scaled up (20 L) with the purpose of verifying the operational conditions and variations to be encountered during scaled up process.

In both set I and II, biogas production commenced instantly and a sharp increase in the production of biogas was observed (Fig. 6.6a). Still enormous improvement in biogas production in set II was credibly apparent (Fig. 6.6b). Cumulative biogas production from set I was examined to be 57969±14 mL while a cumulative biogas production of 86454±18 mL was attained from set II in 55 days; which is approximately 1.5 times higher than set I. The maximum daily biogas production of 3180±8 mL was achieved on 19^{th} day for set I whereas in set II highest daily biogas production of 4625±12 mL on 11^{th} day was attained. The result clearly indicates the improvement in the production of biogas in set II. The dynamic deviation in the production of biogas observed in set II was due to the easy availability of excessive quantity of easily soluble organic compounds due to hot air oven pretreatment of water hyacinth. While in set I due to co-digestion of water hyacinth and food waste the nutrients were balanced and the positive synergism between the organic substrates led to the absence of lag phase but due to the absence of easily bio-available soluble organics biogas production was lesser. In short, the performance of the anaerobic bacteria was lethargic in set I when compared to set II due to the existence of recalcitrant lignin and crystalline cellulose in water hyacinth.

6.2 ANAEROBIC CO-DIGESTION STUDY OF WATER HYACINTH AND HYDRILLA

Water hyacinth and hydrilla (*Hydrilla verticillata*) are invasive aquatic weeds which envelopes an aquatic body by forming dense mats. Effective management of aquatic weeds i.e., water hyacinth and hydrilla is challenging due to its rapid growth and spread. Both water hyacinth and hydrilla damages the aquatic ecosystem, minimise the livelihood and recreational opportunities due to their phenomenal reproduction potential. Anaerobic digestion of these aquatic weeds seems to be a sustainable option in converting these aquatic biomasses into eco-friendly biogas; as these weeds are easily available in abundance and holds high moisture content. Anaerobic co-digestion of both the aquatic weeds seems to be a more efficient way to amplify the array of synergistic interaction of microorganisms alongwith the metabolic regulation of the system, maintain equilibrium between macro-and micronutrients and lessen the noxious inhibitors generating during

the process thereby improving biogas production. Additional inoculum is essential to activate the co-digestion process because animal wastes (cow dung) have high nitrogen, broad range of supplementary nutrients and enhanced microbial activity (Wu et al., 2010; Mu et al., 2017). During hydrolysis, biodegradation of carbon-rich feedstock produces volatile fatty acids (VFA) while biodegradation of nitrogen-rich feedstock produces either ammonium or ammonia. Over production of both VFA and ammonia perturbs the microbial activity inside the anaerobic digester due to kinetic disentanglement between the acid producers and acid consumers. Thus, cautious assortment of organic substrates and an appropriate mixing ratio is essential during anaerobic co-digestion for acetogenic and methanogenic bacteria to flourish in order to facilitate better digestion performance (Álvarez et al., 2010; Fonoll et al, 2015). Kalamaras and Kotsopoulos (2014) performed a set of experiments to evaluate the methane production yield from anaerobic co-digestion of maize, cardoon, milk thistle and sorghum with cattle manure. Results illustrated the predominance of cardoon silage co-digested with cattle manure over other biomass residues for methane production. Abouelenien et al. (2014) studied the co-digestion of cassava waste, coconut waste and coffee grounds with chicken manure and reported a maximum of 93% enhancement in methane production yield when compared to solo cattle manure digestion. Tasnim et al. (2017) reported that anaerobic co-digestion of water hyacinth, cow dung and sewage sludge produced total biogas of 812 mL after 800 h with 65% CH_4, 14% CO_2 and 21% other gases. Syaichhurozi (2018) observed highest biogas yield of 113.92±6.9 mL/g VS was obtained from anaerobic co-digestion of *Salvinia molesta* and rice straw for an ideal ratio of 40:60 demonstrating 60.58% CH_4, 38.69% CO_2 and 0.73% H_2. Ye et al. (2013) demonstrated that co-digestion of kitchen waste, pig manure and rice straw illustrated the highest biogas yield of 674.4 L/kg VS for the ideal ratio of 0.4:1.6:1 which was 71.67 and 10.4% higher than the mono-digestion of rice straw and pig manure respectively. Thus appropriate assortment of co-substrate and mixing ratio is crucial during anaerobic co-digestion to enhance the biodegradability and bioenergy recovery. But hydrolysis of these aquatic weeds (water hyacinth and hydrilla) maybe an obstacle during the anaerobic digestion process as it is lignocellulosic in nature; restricting the biodegradability of the feedstock and biogas yield. Pretreatment of these lignocellulosic feedstocks prior to anaerobic digestion helps in accelerating the hydrolysis period and at the same time improve biogas production. Gaur et al. (2017) observed that the maximum methane yield of 468 mL CH_4/g VS was achieved from the co-digestion of duckweed and waste activated sludge after thermal pretreatment for the ideal mixing ratio

50:20 where acclimatized anaerobic granular sludge was utilised as an inoculum. Thermal pretreatment of the lignocellulosic substrates constructively affected the chemical dynamics and biogas production during the anaerobic co-digestion process.

The present study is a comparative evaluation between; set I: anaerobic co-digestion of water hyacinth and hydrilla and set II: anaerobic co-digestion of water hyacinth and hydrilla after thermal pretreatment. Till date, no study has reported the comparative feasibility of the co-digestion of water hyacinth and hydrilla with and without thermal pretreatment. The aim of the novel study was to investigate the effect of various mixing ratios and pretreatment on the anaerobic co-digestion process.

6.2.1 Characteristics of the substrate and co-substrate

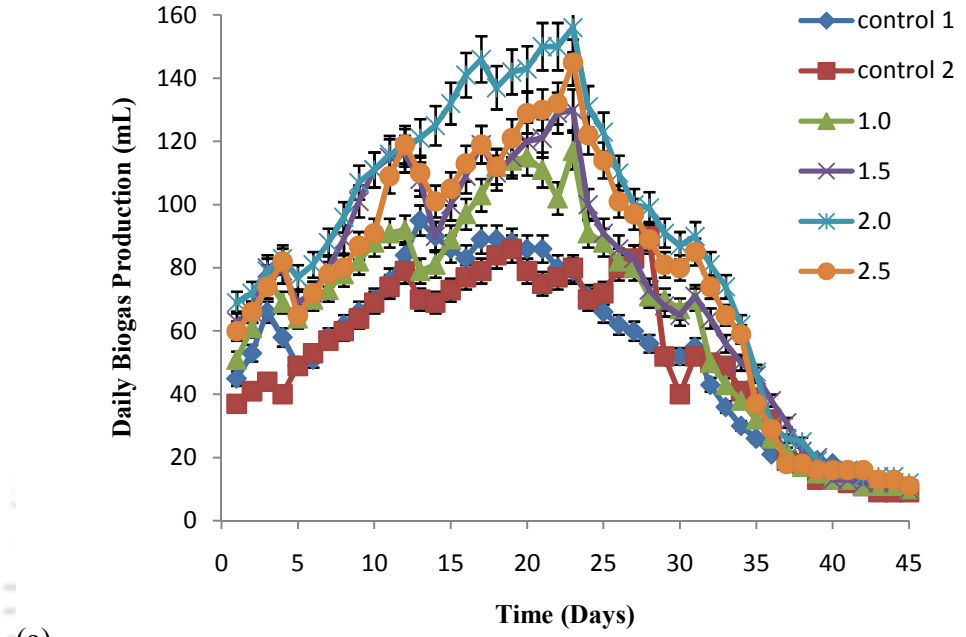
Substrate and co-substrate characteristics are essential parameters to be determined which influences the anaerobic co-digestion process. The initial characteristics of the substrate and co-substrate have a strong effect on the anaerobic process stability and biogas production. The initial physico-chemical characteristics of the substrate and co-substrate utilised in this study were investigated and the results are incorporated in Table 6.4. The pH of water hyacinth and hydrilla was in the acidic range. Cow dung was utilised as an inoculum along with the feedstock which helped in maintaining the pH of the anaerobic digesters. Both the substrate and co-substrate has good amount of moisture content and sCOD; which are beneficial for the anaerobic digestion process. The presence of lignin can be dissolved down by hot air oven pretreatment.

Table 6.4. Initial characteristics of the substrates and the co-substrate

Parameter	Water hyacinth	Hydrilla
Moisture content (%)	90±5	75±5
pH	5.8±2	4.2±1
sCOD (mg/L)	1650±50	2000±10
VFA (mg/L)	750±10	1250±10
Acid soluble lignin (%)	1.77±0.75	1.5±0.5
Acid insoluble lignin (%)	6.33±1	2±0.5
Cellulose (%)	32.84±5	27±2
Hemicellulose (%)	24.7±2	17±2

6.2.2 Effect of anaerobic co-digestion and mixing ratios on biogas production

Two sets of investigational experiments were executed simultaneously with diverse mixing ratios to study the effect of anaerobic co-digestion of water hyacinth and hydrilla. Set I comprised of untreated water hyacinth and hydrilla and set II comprised of hot air oven pretreated water hyacinth and hydrilla.



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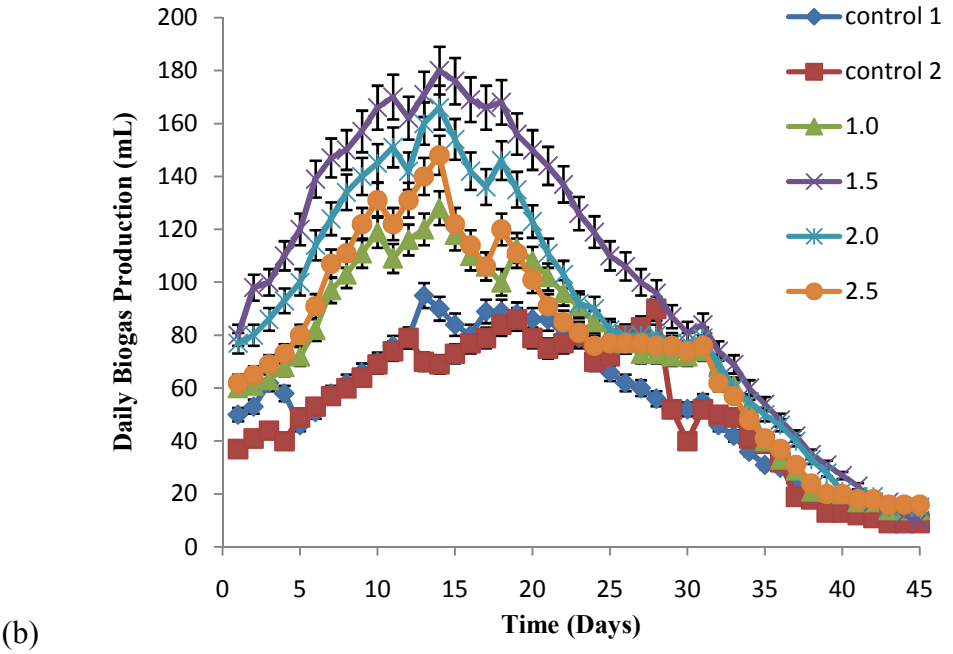

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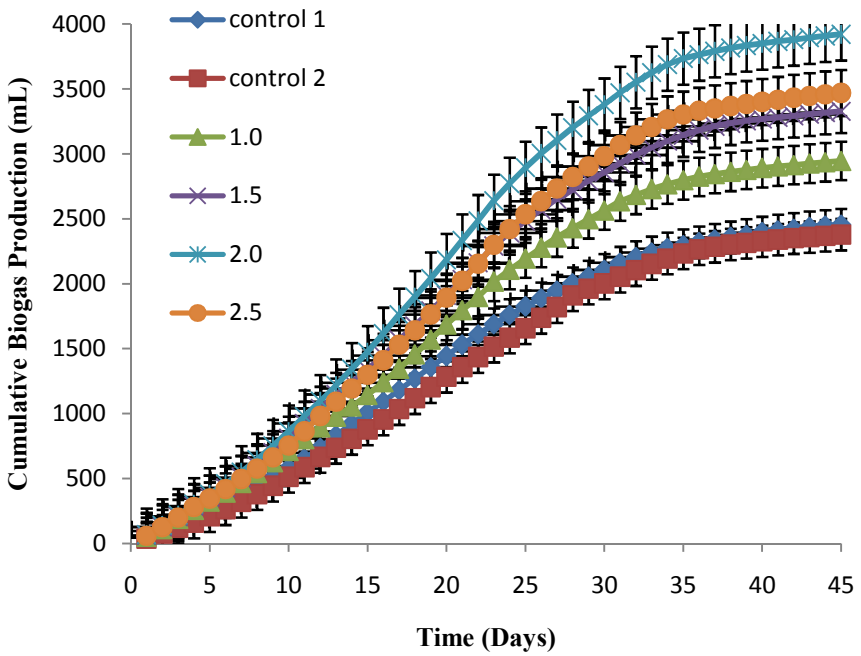
Fig. 6.7. Daily biogas generation for the various mixing ratios of (a) untreated water hyacinth and hydrilla and (b) pretreated water hyacinth and hydrilla



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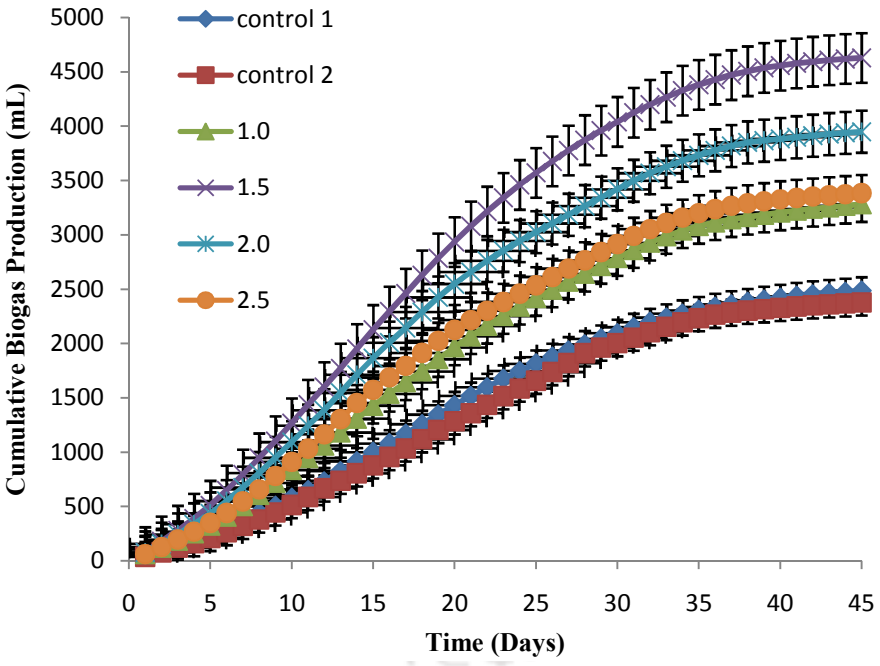


(b)

Fig. 6.8. Cumulative biogas generation for the various mixing ratios of (a) untreated water hyacinth and hydrilla co-digestion and (b) pretreated water hyacinth and hydrilla co-digestion

The profile of daily biogas production with diverse mixing ratios is incorporated in Fig. 6.7a and 6.7b for both set I and set II respectively. For all the mixing ratios, although the quantity fluctuated biogas production initiated from the very first day itself. The total biogas production improved progressively throughout the digestion time. Biogas

production during anaerobic mono-digestion was observed to be lesser when compared to anaerobic co-digestion. Wang et al. (2018) reported of significantly improved methane yields 1.27-3.46 times higher than mono-feedstock when cucumber residues with pig manure and corn stover was co-digested. Increased biogas production due to anaerobic co-digestion of carbon rich co-substrates were reported earlier (Herrmann et al., 2016; Li et al., 2016). This can be attributed to the synergism of the co-substrates and the inoculum alongwith the suitable mixing ratio that provided more balanced nutrients, increased buffer capacity and a more adaptable and robust microbial community.

In set I, the highest biogas production of 156±7 mL CH_4/g VS was exhibited on the 23rd day by the mixing ratio 2. While in set II, highest biogas production of 180±9 mL CH_4/g VS was exhibited on the 14th day itself by the mixing ratio 1.5. Syaichurrozi (2018) examined highest biogas production (113.92±6.90 mL CH_4/g VS) on the 18th day when of *Salvinia molesta* and rice straw was co-digested in the ratio 40:60.Biogas production was witnessed to be improving with the increase in mixing ratio upto mixing ratio 2 in set I and upto mixing ratio 1.5 in set II. Biogas production was observed to reduce for the mixing ratio 2.5 in set I and for the mixing ratio 2 in set II. Hot air oven pretreatment in set II for the mixing ratio 2 must have enhanced the quantity of solubilisation of the co-substrates i.e., water hyacinth and hydrilla. This enhanced quantity of solubilisation of the co-substrates hindered the methanogenesis step by excess production of VFA. Hence, the mixing ratio 2 in set II exhibited lesser biogas production than the mixing ratio 2 in set I.

Excessively lengthy period of hydrolysis phase was not observed in the cumulative biogas production profile in both set I (Fig. 6.8a) and set II (Fig. 6.8b) throughout the anaerobic co-digestion period of water hyacinth and hydrilla. Persistent improvement in biogas production was observed in both set I and set II throughout the initial and the steady phase. For each and every mixing ratio in both set I and II, production of biogas stabilised by the end of 45 days and steady phase was achieved. Mixing ratio 1.5 exhibited the highest cumulative biogas production than the other mixing ratios of set II and all the mixing ratios of set I respectively. The mixing ratio 1.5 by the completion of 45 days in set II attained a cumulative biogas production of 4630±10 mL.Whereas, the mixing ratio 2 in set I attained the highest cumulative biogas production of 3921±11 mL by the completion of 45 days. The mixing ratio usually varies based on the composition and digestibility of substrates or the co-substrates utilised. Yong et al. (2015) observed that the mixing ratio 5:1 was optimal when food waste and straw was co-digested. Jacob

and Banerjee (2016) reported that anaerobic co-digestion of potato waste and *Pistia stratiotes* (aquatic weed) in the ratio 1:1 enhanced methane yield by 76.5%. Mu et al. (2017) also reported 1:1 to be the optimal mixing ratio for co-digesting potato waste and cabbage waste. Higher amount of methane (CH_4) content in biogas signifies better quality of biogas. In set II, enhanced quantity of methane was detected for mixing ratio 1.5 when compared to the mixing ratio 2 in set I (Table 6.5). Thereby, illustrating that hot air oven pretreatment, anaerobic co-digestion and mixing ratio all together demonstrates a better synergistic effect relatively than co-digestion alone in both set I and II. The reasonably quicker degradation or reduced digestibility period of 9 days (14^{th} day) in set II than set I (23^{rd} day) was demonstrated as the quantity of soluble organic matter in water hyacinth and hydrilla to be digested anaerobically enhanced after hot air oven pretreatment. Application of thermal pretreatment to the lignocellulosic co-substrates dissolved down the lignin leading to easier and quicker biodigestibility, hence improving the biogas production. Thermal pretreatment of the lignocellulosic co-substrates in set II assisted the robust microorganisms to effortlessly avail the soluble organic matter present in the lignocellulosic water hyacinth and hydrilla. While in set I, biogas production was comparatively less significant as the methanogenic microorganisms found it complex to digest the headstrong lignocellulosic cell wall present in the untreated water hyacinth and hydrilla.

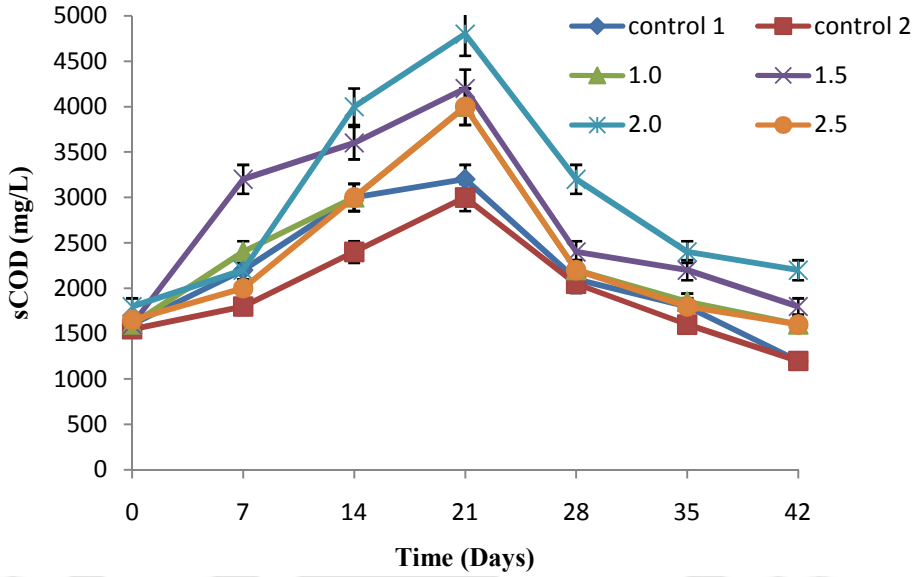
Table 6.5. Biogas composition of both set I and II.

Compound	Set I (%)	Set II (%)
CH_4	57. 87±0.3	63.54±0.2
CO_2	30.87±0.2	26.78±0.2
H_2	6.94 ±0.3	8.97±0.4
N_2	3.64 ±0.4	----

6.2.3 Effect of anaerobic co-digestion on sCOD, VFA and VS

Sample analysis for all the ratios of both the sets were performed on a weekly basis. The parameters analysed were sCOD, VFA and VS. The analysis of the samples on a weekly basis illustrates the alteration undergone and the significance of an ideal mixing ratio during the BMP test due to anaerobic co-digestion of water hyacinth and hydrilla. As time passed by, sCOD for both set I and II increased for each and every mixing ratio.

In both set I and II sCOD increased (Fig. 6.9a, 6.9b) as VFA increased (Fig. 6.10a, 6.10b).



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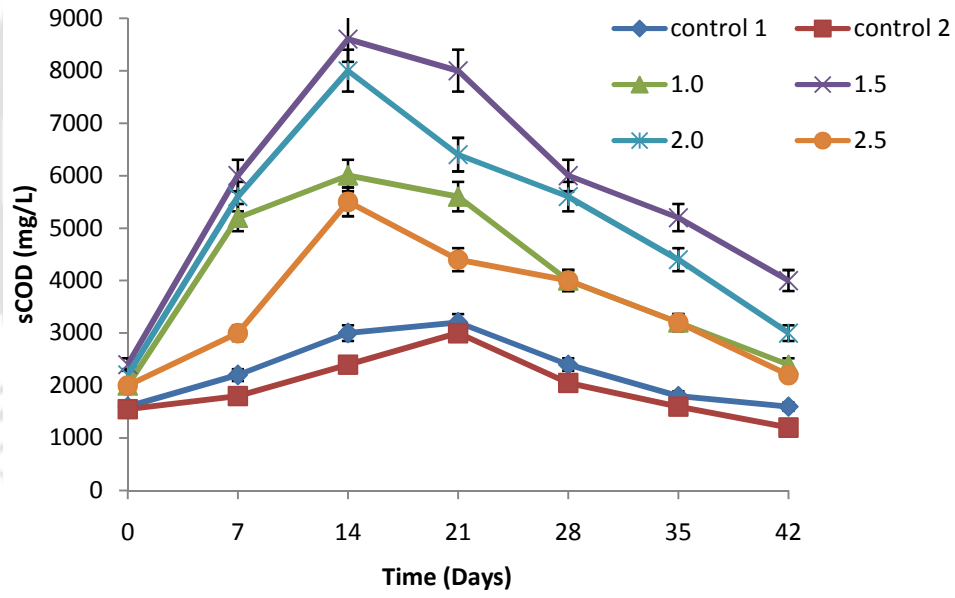


(b)

Fig. 6.9. Variation in sCOD for the various mixing ratios of (a) untreated water hyacinth and hydrilla co-digestion and (b) pretreated water hyacinth and hydrilla co-digestion

Carbohydrates when broken down produce VFA. Increase in VFA and sCOD was witnessed to take place simultaneously in both the sets and after attaining the highest value, it decreased gradually. Increased sCOD signifies the enhanced amount of simple soluble organic matter which can be readily transformed to biogas by the methanogenic microorganisms (Yu et al., 2004). Both sCOD and VFA concentration was witnessed to be highest on the 21st day for each and every mixing ratio in set I. Whereas in set II sCOD

and VFA concentration was witnessed to be highest on the 14th day. In set I mixing ratio 2 demonstrated a highest VFA concentration of 2400±12 mg/L whereas in set II for the ratio 1.5 highest VFA concentration of 3800±10 mg/L was demonstrated. At first, the acidogenic microorganisms activity increased the VFA concentration, and the beginning of the methanogenic stage directed to the fall of VFA concentration (Lin et al., 2017).

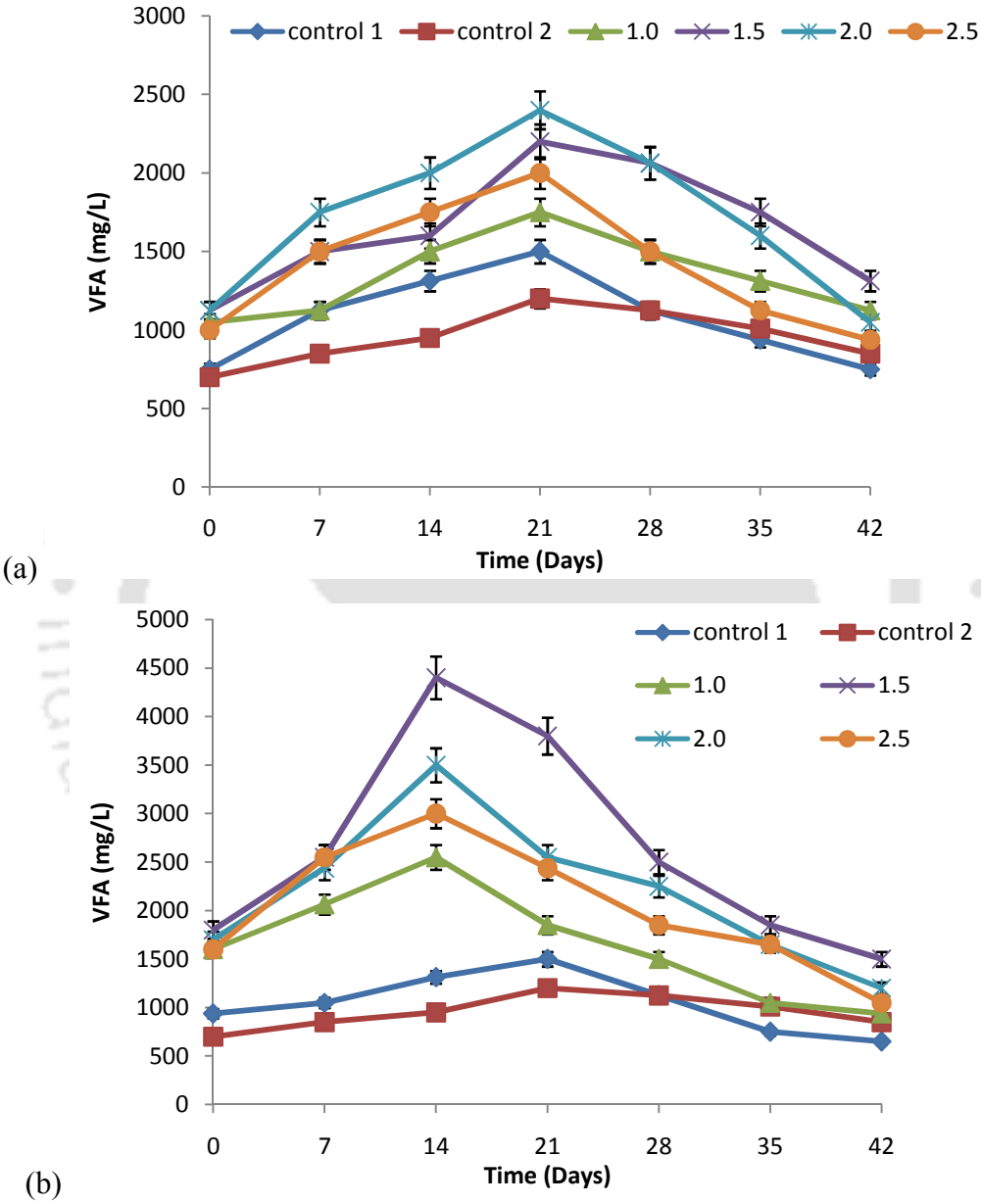


Fig. 6.10. Variation in VFA concentration for the various mixing ratios of (a) untreated water hyacinth and hydrilla co-digestion and (b) pretreated water hyacinth and hydrilla co-digestion

Although, elevated concentration of VFA was witnessed in the co-digested samples relatively than the mono-digested samples still elevated steadiness was detected in the co-digestion system as the addition of hydrilla improved the buffer capacity. Similarly, ratio

2 in set I, attained a highest sCOD of 4800±11 mg/L while ratio 1.5 in set II achieved a maximum sCOD of 8600±10 mg/L. Yin et al. (2016) also reported that when mixed activated sludge and food waste pretreated with fungal mash was co-digested than mono-digestion of substrate with and without pretreatment the sCOD increased. Decline in sCOD was observed after 21 days and 14 days in set I and set II respectively because the activity of the microorganisms was gradually exhausting the simple soluble organic matter.

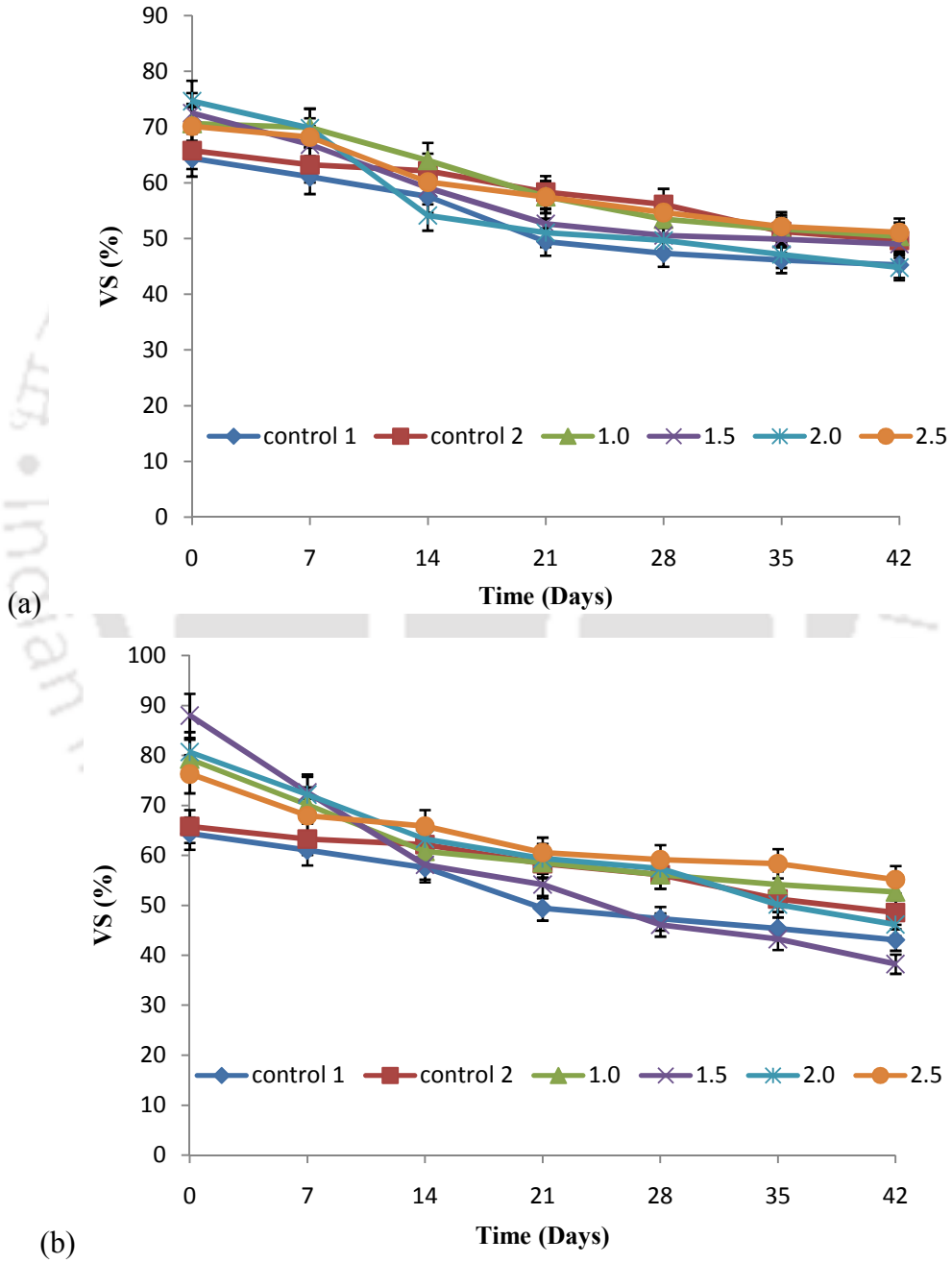


Fig. 6.11. Reduction in VS for the various mixing ratios of (a) untreated water hyacinth and hydrilla co-digestion and (b) pretreated water hyacinth and hydrilla co-digestion

As the number of days passed by, fall in VS was observed. In set I, mixing ratio 2 revealed the highest fall in VS by 40% (Fig. 6.11a) while the mixing ratio 1.5 in set revealed the highest fall in VS by 56% (Fig. 6.11b).

More decrease in VS was witnessed in mixing ratio 1.5 in set II than the mixing ratio 2 in set I. Abudi et al. (2016) reported the highest VS reduction of 76.9% in the co-digestion of organic fraction of municipal solid waste with thermo-alkaline pretreated thickened waste activated sludge and H_2O_2 pretreated rice straw. Fall in VS is a favourable outcome of the anaerobic co-digestion process as it denotes enhanced biogas production. The highest biogas production was portrayed by the mixing ratios illustrating highest VS reduction and vice versa. Availability to the simple soluble organic matter was restricted in set I due to the confined microbial activity than set II thus demonstrating minor reduction in VS. In set II, the mixing ratio 1.5 was identified to be the optimal mixing ratio as the methanogenic microorganisms flourished demonstrating improved biogas production.

6.2.4 Kinetic study

In order to ascertain the efficiency of the optimal mixing ratio and pretreatment on the co-digestion of water hyacinth and hydrilla, the cumulative biogas production values were fitted to Gompertz equation curve. Table 6.6 incorporates the results of the kinetic study for both set I and II. The kinetic parameters of set I and II used in BMP test were verified where M of set II (5.1521 L CH_4) was witnessed to be elevated than set I (4.5190 L CH_4). R^2 value above 0.91 in both set I and II, denotes that biogas production is well simulated.

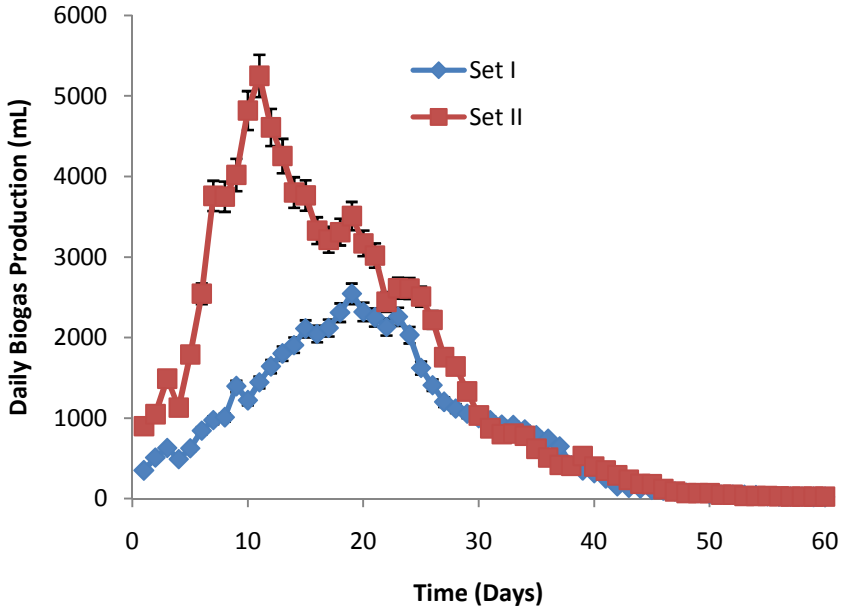
Table 6.6. Kinetics values of the optimal mixing ratio of set I and II

Set	Mixing ratio	M (L CH_4)	Rmax (L CH_4 d^{-1})	λ (d)	R^2	Y (L CH_4)
I	2.0	4.5190	0.1000	0.0000	0.92	3.921
II	1.5	5.1521	0.0780	0.0000	0.93	4.630

6.2.5 Scaled up 20 L batch study of water hyacinth and hydrilla

The aforesaid systematic examination illustrated positive results and divulged the ideal F/M ratio for both set I and II; therefore the anaerobic batch digester study was scaled up

to 20 L with the intention of corroborating the operational conditions and discrepancies to be encountered during the scaled up process.



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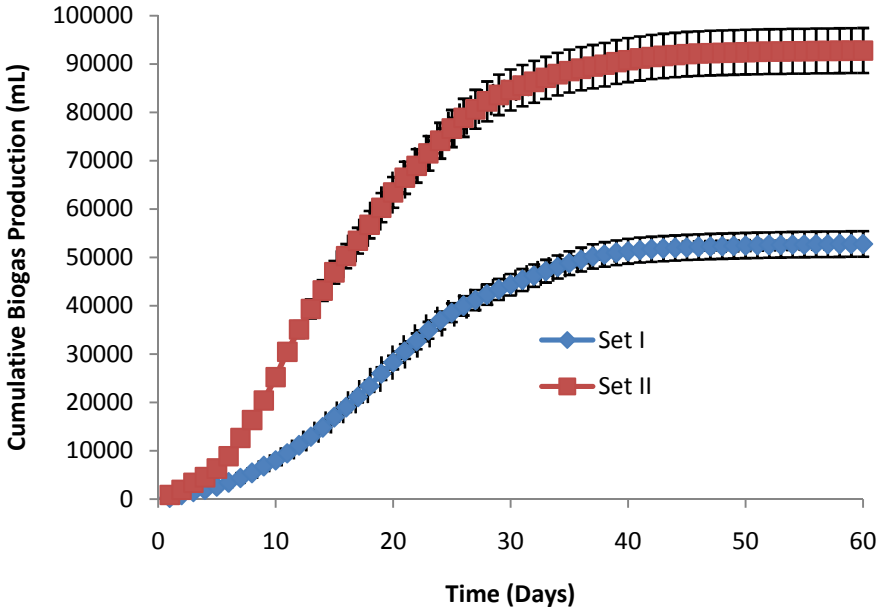


(b)

Fig. 6.12. Daily biogas production and (b) cumulative biogas production of set I and II in 20 L anaerobic batch digester

Biogas production initiated instantaneously in both set I and II and a sharp increase in the production of biogas was monitored (Fig. 6.12a). Cumulative biogas production from set I was observed to be 52805±15 mL while a cumulative biogas production of 92804±10 mL was observed from set II in 60 days; exhibiting an approximately 1.8 times more biogas production than set I (Fig. 6.12b). The highest daily biogas production of

2545±12mL was accomplished on 19th day for set I whereas in set II highest daily biogas production of 5250±10 mL on 10th day was accomplished.

The result evidently suggests the enhancement in the production of biogas in set II. The dynamic increase in the production of biogas exhbited in set II was due to the easy bioaccessibility of extreme amount of simple soluble organics due to hot air oven pretreatment of the lignocellulosic aquatic weeds. While in set I due to co-digestion of water hyacinth and hydrilla the nutrients were balanced and the positive synergism between the organic substrates led to the absence of lag phase but due to the deficiency of extra amount of easily bioaccessible simple soluble organics biogas production was comparatively lower. In short, the performance of the anaerobic bacteria was lethargic in set I when compared to set II due to the presence of recalcitrant lignin and crystalline cellulose in the lignocellulosic aquatic weeds

6.3 ANAEROBIC CO-DIGESTION STUDY OF WATER HYACINTH AND BANANA PEELS

Decline in fossil fuel and environmental pollution caused due to burning of fossil fuel is a major concern worldwide. It is of utmost necessary to reduce our dependency on fossil fuel and find a suitable eco-friendly option. Anaerobic digestion seems to be a sustainable alternative as it can transform organic wastes into biogas (bioenergy) in the absence of oxygen with the assistance of robust microorganisms thereby providing solution to both organic waste management and renewable bioenergy production. However, anaerobic digestion is an extremely sensitive process because the microorganisms involved in transforming organic wastes into biogas requires certain operational environmental conditions in order to flourish. The kind of substrate utilised is one of the major parameter influencing the efficiency of biogas production due to their chemical composition and biodegradability. Banana peels which are basically organic waste products may serve as a good feedstock for the production of biogas as they are rich in organic matter and readily biodegradable. Readily biodegradable substrates can also cause excess acidification. Odedina et al. (2017) observed that methane yield from ground banana peel and chopped banana peel to be 330.6 mL CH_4/g VS and 268.3 mL CH_4/g VS respectively. But during continuous digestion of banana peels, mesophilic single stage digester run at 20 day hydraulic retention time failed at 2% TS. Water hyacinth on the other hand is considered to be the world's most dangerous aquatic weed; disturbing the aquatic ecosystem and the livelihood or amusement activities of people. Water hyacinth and banana peels contain high moisture and easily available in abundance

throughout the world, therefore anaerobic digestion of both water hyacinth and banana peels seems to be a feasible option for the production of eco-friendly biogas. However, using feedstock as mono-substrates is not recommended due to nutritional imbalance and deficiency of varied range of microorganisms (Wang et al., 2017; Pellera and Gidarakos, 2017).Anaerobic co-digestion of two or more substrates is a feasible alternative to surmount the drawbacks of mono-digestion and perk up biogas production. Anaerobic mono-digestion of water hyacinth would be rate limiting as the hydrolysis of lignocellulosic cell wall will be very time consuming and restrain biogas production. Anaerobic co-digestion of water hyacinth with banana peels will be beneficial in diluting inhibitory substances, balance the nutrients, accelerating the hydrolysis process, maintain reactor equilibrium and improve biogas production because banana peels contain relatively high level of nutrients, while water hyacinth does not produce excess volatile fatty acid when compared to banana peels. Supplementary inoculum is fundamental to trigger the co-digestion process as animal wastes (cow dung) have high nitrogen, wide variety of nutrients and enhanced microbial activity (Wu et al., 2010; Mu et al., 2017). Kalamaras and Kotsopoulos (2014) performed a set of experiments to evaluate the methane production yield from anaerobic co-digestion of maize, cardoon, milk thistle and sorghum with cattle manure. Results illustrated the predominance of cardoon silage co-digested with cattle manure over other biomass residues for methane production. Syaichhurozi (2018) observed highest biogas yield of 113.92±6.9 mL/g VS was obtained from anaerobic co-digestion of *Salvinia molesta* and rice straw for an ideal ratio of 40:60. Pavi et al. (2017) observed increased methane yield from anaerobic co-digestion of organic fraction of municipal solid waste and fruit and vegetable waste when compared to the mono-digestion of each substrate. Ye et al. (2013) demonstrated that co-digestion of kitchen waste, pig manure and rice straw illustrated the highest biogas yield of 674.4 L/kg VS for the ideal ratio of 0.4:1.6:1 which was 71.6 and 10.4% higher than the mono-digestion of rice straw and pig manure respectively. Nowadays, pretreatment of lignocellulosic feedstock are also conducted before co-digestion to enhance biogas production. Pretreatment of lignocellulosic feedstock ruptures the lignocellulosic complex allowing more easy microbial access of soluble organics. Ramos-Suarez et al. (2014) studied the anaerobic co-digestion of *Scenedesmus* microalgal biomass and *Opuntia maxima* cladode. Feedstock composed of 75% *O. maxima* and 25% *Scenedesmus* (VS basis) showed the highest methane yield increasing 66.4% and 63.9% that of *Scenedesmus* and *O. maxima*, respectively. Zhang et al. (2017) observed 10.1% more

solid reduction in anaerobic co-digestion of food waste and waste activated sludge by biological co-pretreatment. Surra et al. (2018) observed significant increase (36.3%) in methane yield when co-digesting H_2O_2 pretreated maize cob waste with organic fraction of municipal solid waste. Alagoz et al. (2018) reported highest cumulative biogas production of 6351 mL when grape pomace and microwave pre-treated wastewater sludge was co-digested.

6.3.1 Characteristics of substrate and co-substrate

The initial physico-chemical characteristics of the substrate and co-substrate are essential factors to be examined as it influences the overall anaerobic process stability and biogas production. The initial physico-chemical characteristics study of the substrate and co-substrate utilised were investigated and the results are provided in Table 6.7. The pH of water hyacinth and banana peel was observed to be in the acidic range. Cow dung was used as an inoculum in conjunction with the feedstock which facilitated in upholding the pH of the anaerobic digesters. Both the substrate and co-substrate has high quantity of moisture content and sCOD; which are favourable for the anaerobic digestion process. The presence of lignin can be dissolved down by hot air oven pretreatment.

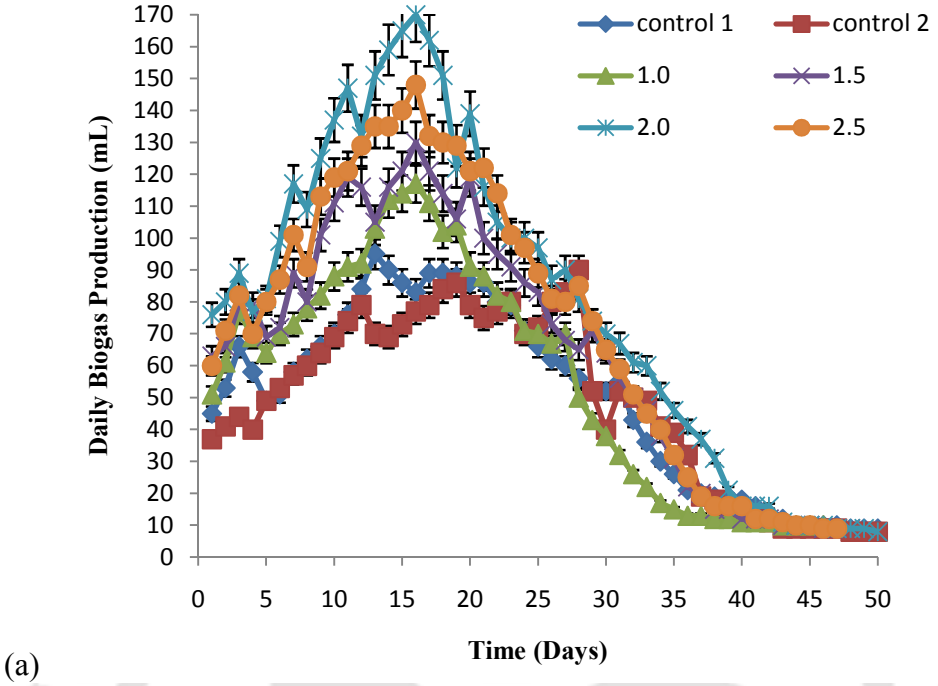
Table 6.7. Initial characterisation study of the substrate and the co-substrate.

Parameter	Water hyacinth	Banana peel
Moisture content (%)	90±5	80±2
pH	5.8±0.5	5.2±0.5
sCOD (mg/L)	1600±50	2150±30
VFA (mg/L)	750±50	1175±75
Acid soluble lignin (%)	1.77±0.75	0.52±0.5
Acid insoluble lignin (%)	6.33±1	0.75±0.5
Cellulose (%)	32.84±5	12.55±2
Hemicellulose (%)	24.7±2	9.24±1

6.3.2 Effect of anaerobic co-digestion and mixing ratios on biogas production

During the methane potential assay, the effect of anaerobic co-digestion of water hyacinth and banana peels was investigated by performing two sets of experimental study simultaneously with a range of mixing ratios (1, 1.5, 2 and 2.5). Water hyacinth was

untreated in set I and pretreated thermally inside hot air oven in set II. As banana peels are low in lignin content so it was not thermally pretreated.



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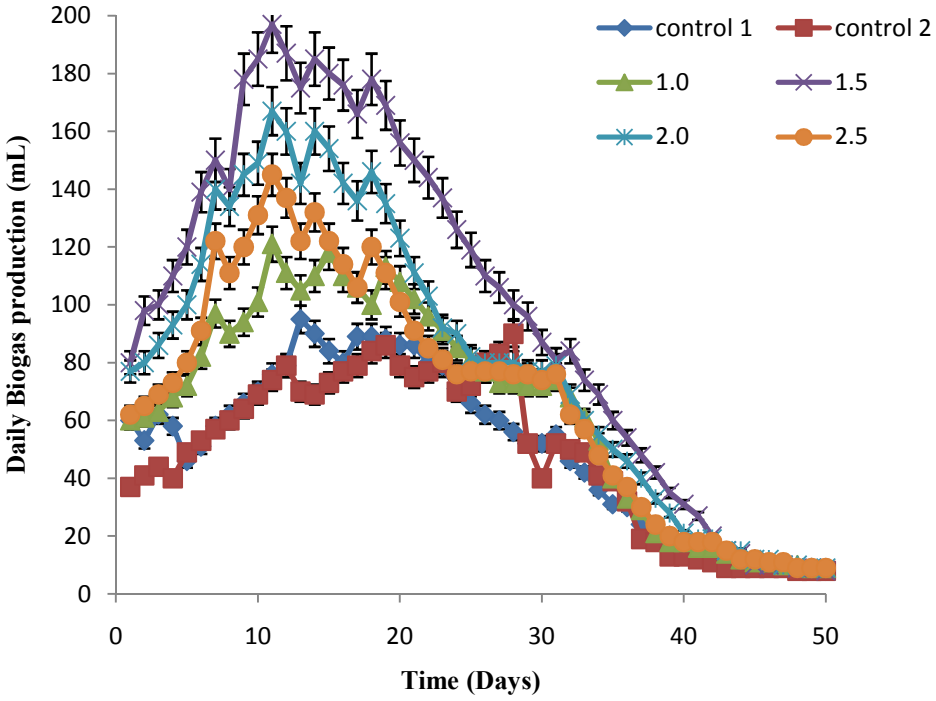
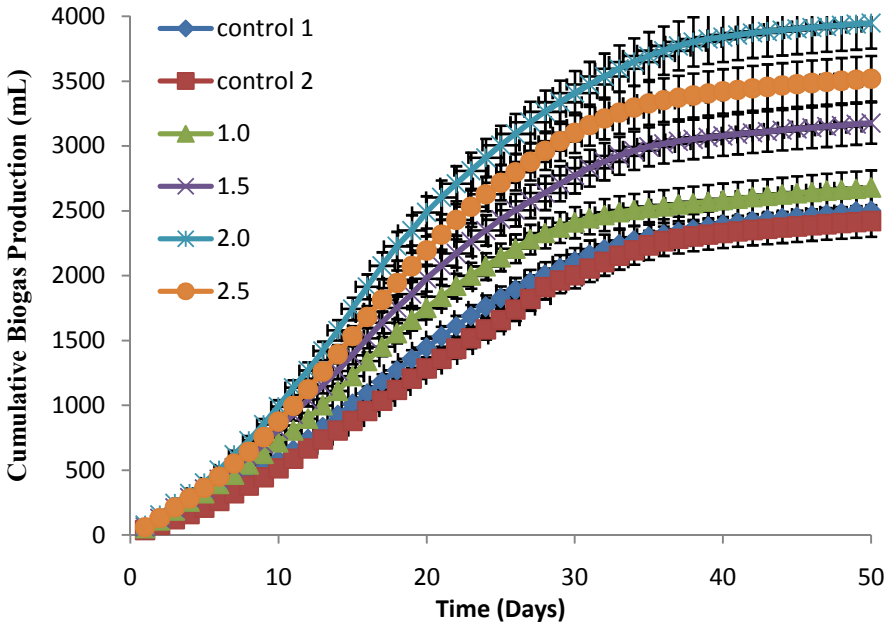


(b)

Fig. 6.13. Daily biogas generation for the various mixing ratios of (a) untreated water hyacinth and banana peel and (b) pretreated water hyacinth and banana peel

The graphs of Fig. 6.13a and 6.13b correspond to the daily biogas generation of a range of mixing ratios for both set I and set II respectively. Biogas production began immediately after setting up the experimental assay for all the mixing ratios but in varying quantity. The cumulative biogas production enhanced progressively throughout the anaerobic co-digestion

phase. Biogas production was observed to be less quantitatively during mono-digestion of substrate when compared to co-digestion since co-digestion and mixing ratio acts in synergism to balance the nutrients and to expand the existence of adaptable and dynamic microbial community.



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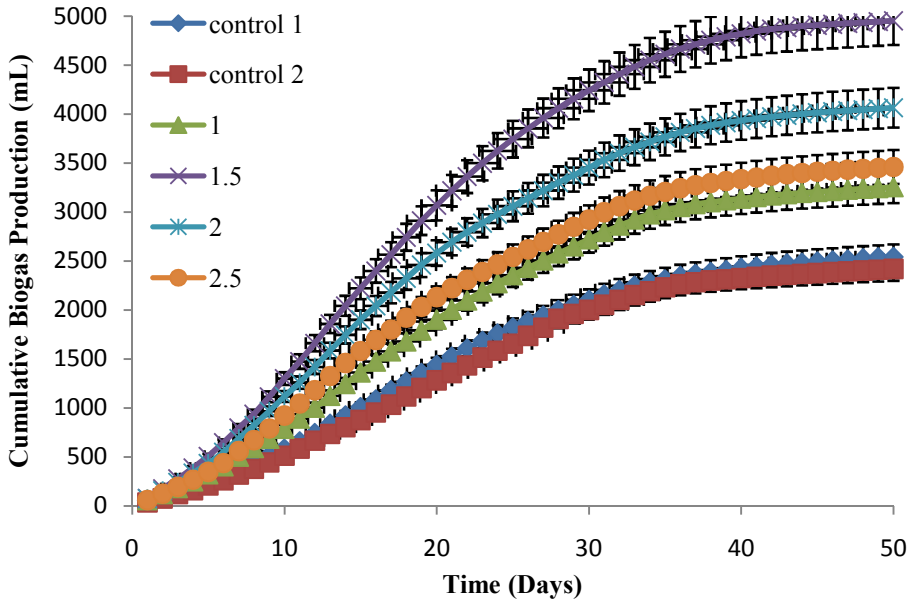


(b)

Fig. 6.14. Cumulative biogas production for the various mixing ratios of (a) untreated water hyacinth and banana peel and (b) pretreated water hyacinth and banana peel

Mixing ratio 2, in set I displayed the maximum biogas production of 170±10 mL CH_4/g VS on the 16th day. While, mixing ratio 1.5 in set II; displayed the maximum biogas production of 197±10 mL CH_4/g VS on the 11th day itself. In both set I and II, biogas

production improved as the mixing ratio increased upto 2 in set I and upto 1.5 in set II. In set I, biogas production reduced for the mixing ratio 2.5 and in set II for the mixing ratio 2.

Hot air oven pretreatment of water hyacinth in set II for the mixing ratio 2 must have amplified the quantity of easily available simple soluble organics of the substrate. This amplified quantity of easily available simple soluble organics of the substrate inhibited the methanogenic bacteria to flourish due to the accumulation of toxic intermediates (VFA). As a result, the mixing ratio 2 in set II exhibited lower biogas production when compared to the mixing ratio 2 of set I. Syaichurrozi (2018) observed highest biogas production of 113.92±6.90 mL CH_4/g VS on the 18th day from the co-digestion of *Salvinia molesta* and rice straw in the ratio 40:60. In Fig 2a and 2b cumulative biogas production graphs, extensively lengthy hydrolysis phase was not observed during anaerobic co-digestion of water hyacinth and banana peels in both set I and II. For both set I and II, constant quantitative improvement in biogas production was observed throughout the start-up and the steady stage. Until biogas production reduced by the end of 50 days and steady stage was attained for each and every mixing ratio during the anaerobic co-digestion of water hyacinth and banana peels. In set II mixing ratio 1.5 demonstrated the maximum cumulative biogas production when compared to the other mixing ratios of set II and all the ratios of set I respectively. Cumulative biogas generation of 4954±12 mL was attained by the ratio 1.5 by the end of 50 days in set II. While mixing ratio 2 in set I exhibited the highest cumulative methane production of 3948±12 mL by the end of 50 days.

Biogas with higher methane content suggests better quality of biogas. Existence of 65% CH_4, 14% CO_2 and 21% other gases during the anaerobic co-digestion of water hyacinth, cow dung and sewage sludge was reported by Tasnim et al. (2017). Amplified percentage of methane was detected for mixing ratio 1.5 in set II when compared to the mixing ratio 2 of set I (Table 3). This can be attributed to the synergistic effect of both hot air oven pretreatment and anaerobic co-digestion rather than anaerobic co-digestion alone. An appropriate mixing ratio assisted synergistically for enhanced biogas generation in both set I and II by letting the methanogenic bacteria flourish and balancing the nutrients. The illustration of comparatively quicker degradation or improved digestion time (11 days) in set II when compared to set I (16 days) may be attributed to the effect of hot air oven pretreatment. As, the quantity of simple soluble organic matter present in water hyacinth to be digested anaerobically amplified after hot air oven pretreatment in set II. On the application of heat, the lignin present in water hyacinth dissolved down leading to easier and faster bioaccessibility of the soluble organic compounds thereby improving biogas

production. Thermal pretreatment of water hyacinth assisted the microorganisms to easily access the soluble organic matter available in the lignocellulosic complex of water hyacinth in set II. While, biogas production was relatively low in set I as the microorganisms found it tough to break down the strong lignocellulosic network available in the untreated water hyacinth.

Table 6.8. Biogas composition of set I and set II

Compound	Set I (%)	Set II (%)
CH_4	57.65±0.2	65.65±0.5
CO_2	31. 87±0.5	25.3±0.9
H_2	7.35 ±0.3	8.67±0.8
N_2	2.45±0.4	----

6.3.3 Effect of co-digestion on sCOD, VFA and VS

Weekly analysis of the samples for sCOD, VFA and VS were performed for all the mixing ratios of both the sets thereby demonstrating the modification undergone during anaerobic co-digestion of water hyacinth and banana peels. Also the significance of an ideal mixing ratio was emphasised. As the time passed by, sCOD for both set I and II amplified for each and every ratio. In both set I and II, sCOD amplified (Fig. 6.15a and 6.15b) with the amplification of VFA (Fig. 6.16a, 6.16b). Hydrolysis of carbohydrates generates VFA. Increase in VFA and sCOD was observed to take place simultaneously in both the sets and after achieving the maximum value, it started to decrease. Increase in sCOD suggests the availability of increased quantity of soluble organic matter that can be readily transformed to biogas by the versatile methanogenic microorganims (Yu et al., 2004).

Both sCOD and VFA concentration was exhibited to be highest on the14th day in set I for each and every ratio. Whereas, sCOD and VFA concentration; was observed to be highest on the 7th day in set II. Mixing ratio 2 in set I achieved a highest VFA concentration of 3000±11 mg/L whereas in set II for the ratio 1.5 maximum VFA concentration was observed to be 3800±10 mg/L. In the very beginning, the acidogenic bacterial activity increased the VFA concentration, and the beginning of the methanogenic phase led to the fall of VFA concentration (Lin et al., 2017). Although, higher concentration of VFA was exhibited during co-digestion rather than the mono-digestion still superior stability was exhibited during the anaerobic co-digestion process as the addition of water hyacinth improved the buffer capacity. Similarly, in set I ratio 2, accomplished a highest sCOD of 6200±10 mg/L whereas

ratio 1.5 in set II accomplished a highest sCOD of 9400±10 mg/L. Decline in sCOD was observed after 14 days and 7 days in set I and set II respectively as the simple soluble organic matter was exhausting owing to the activity of the dynamic microorganisms in the digester. Highest sCOD was also reported by Yin et al. (2016) when mixed activated sludge and food waste pretreated with fungal mash was co-digested when compared to the mono-digestion of substrate with and without pretreatment.

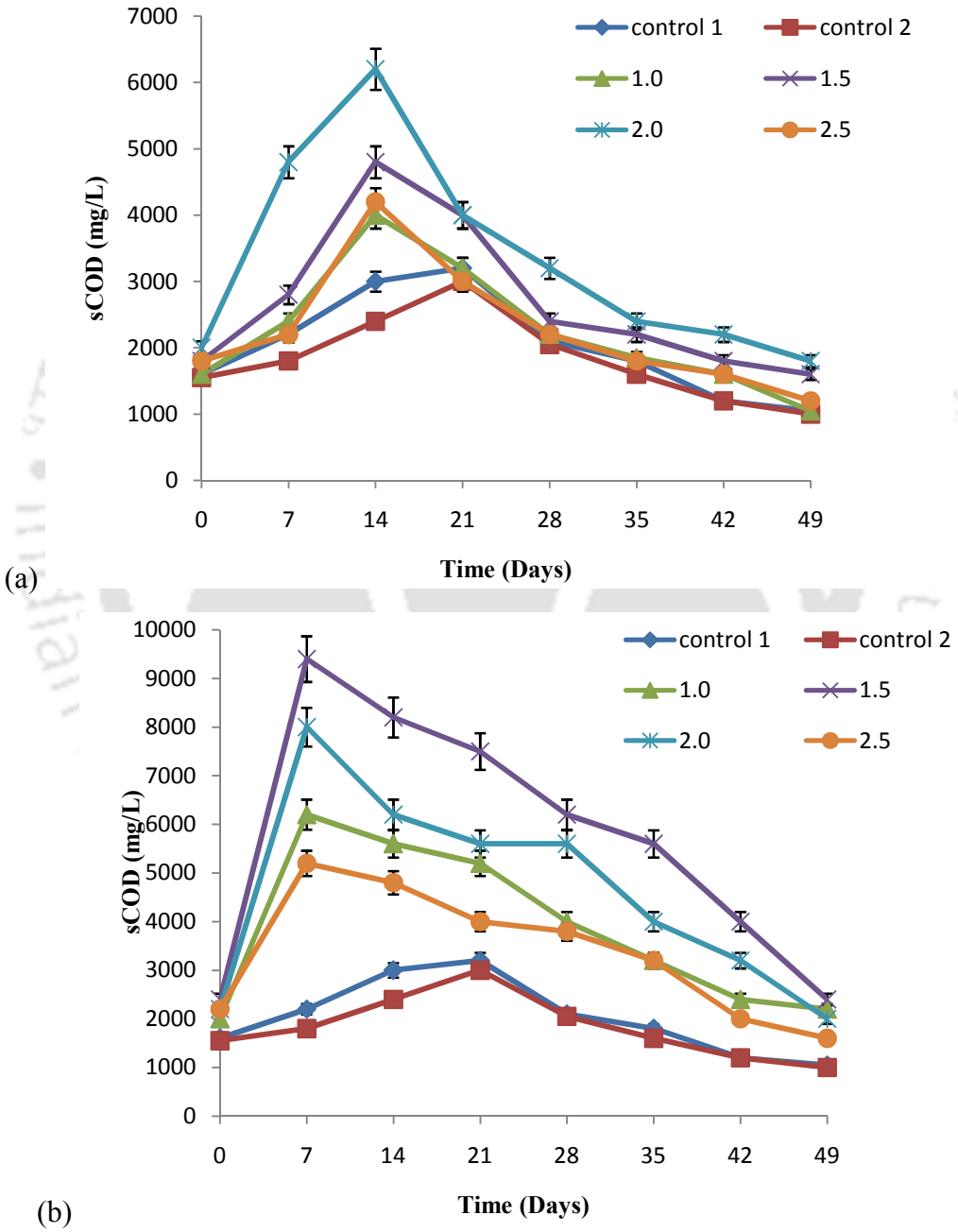
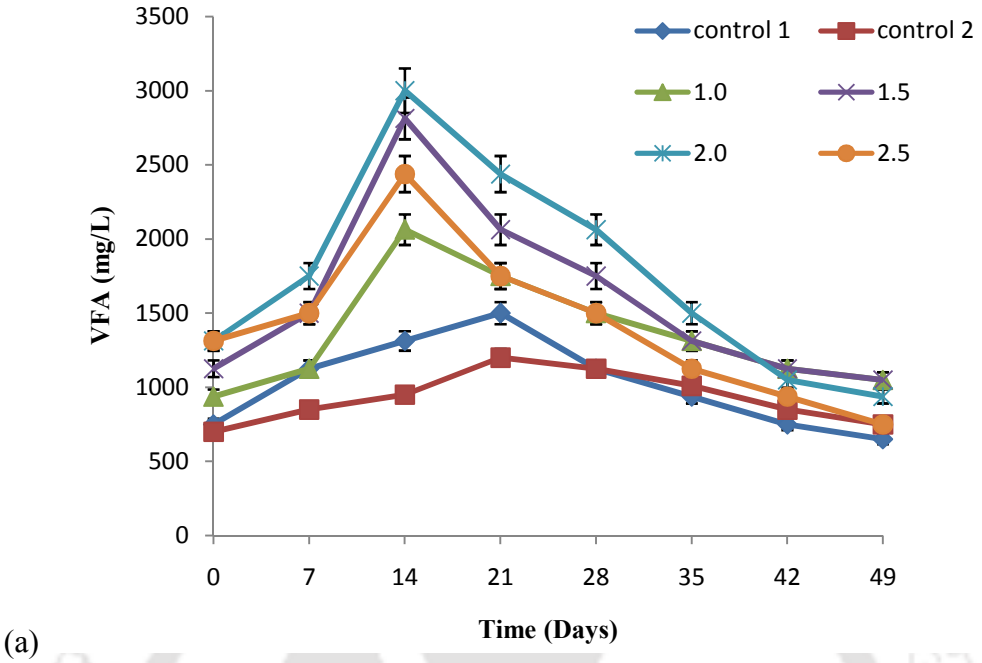


Fig. 6.15. Variation in sCOD for the various mixing ratios of (a) untreated water hyacinth and banana peel co-digestion and (b) pretreated water hyacinth and banana peel co-digestion



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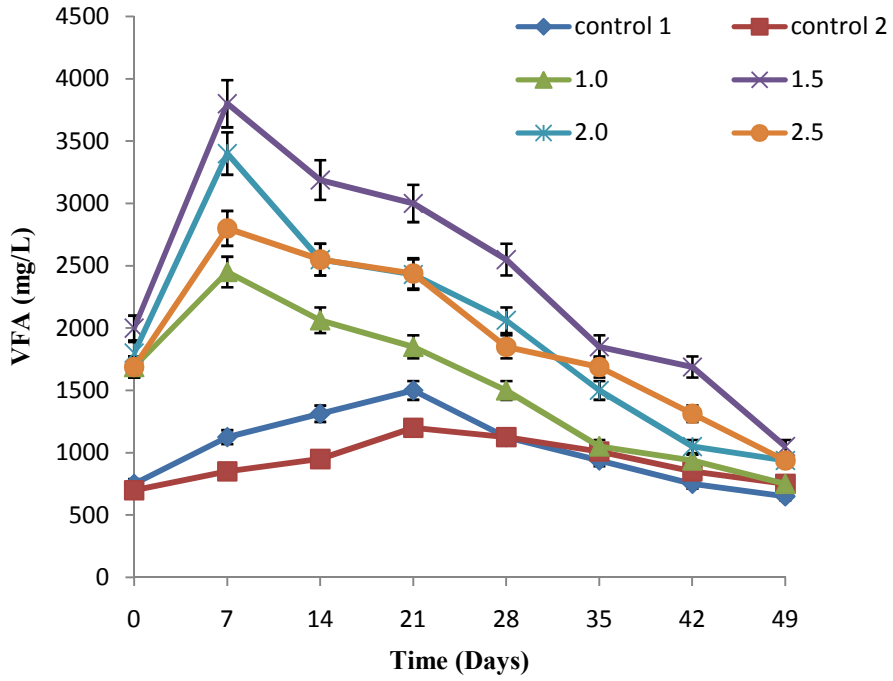
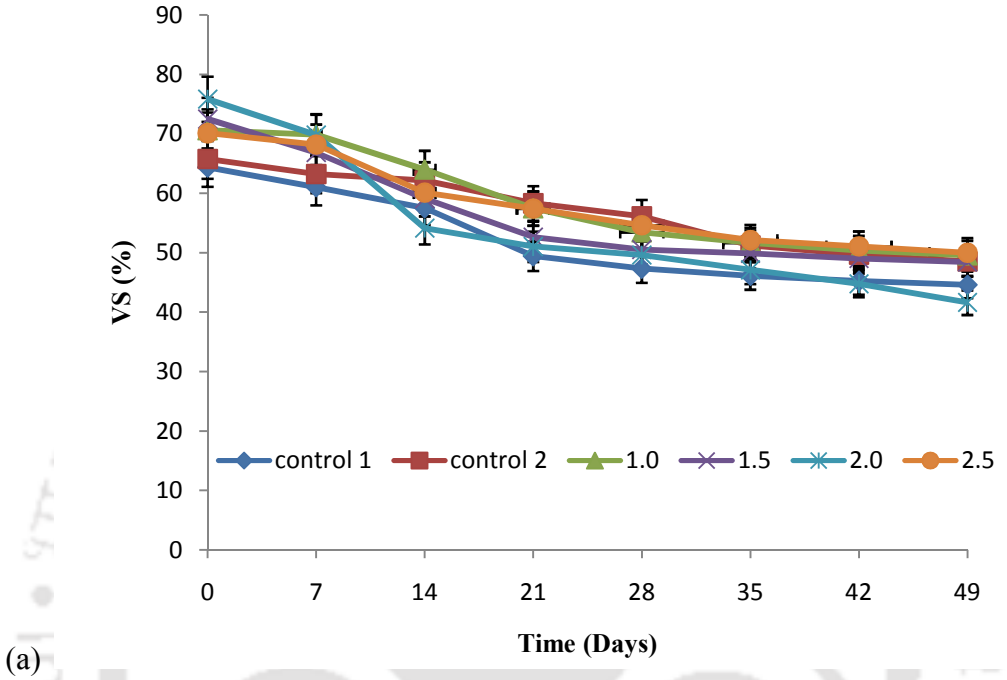


(b)

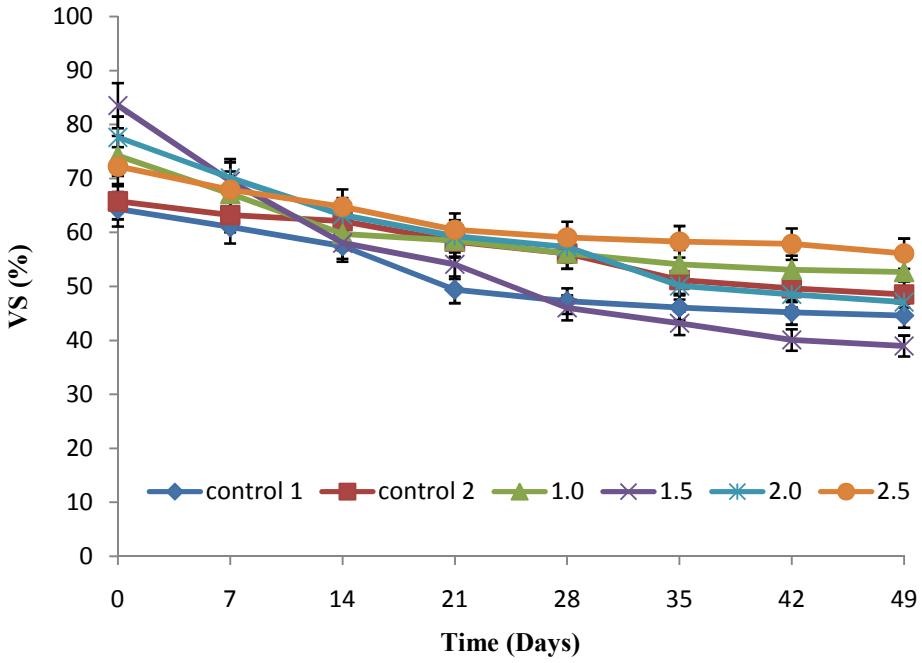
Fig. 6.16. Variation in VFA concentration for the various mixing ratios of (a) untreated water hyacinth and banana peel co-digestion and (b) pretreated water hyacinth and banana peel co-digestion

Decline in VS was illustrated with the increase in co-digestion period, during weekly testing of the co-digested samples. Highest decrease in VS of 45% (Fig. 6.17a) was displayed by the mixing ratio 2 in set I whereas in set II a highest decrease in VS of 53% was exhibited

by the mixing ratio 1.5 (Fig. 6.17b). Higher VS reduction comparatively was exhibited by the mixing ratio 1.5 in set II when compared to the mixing ratio 2 in set I. Abudi et al. (2016) reported the highest VS reduction of 76.9% due to the synergism during anaerobic co-digestion of organic fraction of municipal solid waste with thermo-alkaline pretreated thickened waste activated sludge and H_2O_2 pretreated rice straw.



(a)



(b)

Fig. 6.17. Reduction in VS for the various mixing ratios of (a) untreated water hyacinth and banana peel co-digestion and (b) pretreated water hyacinth and banana peel co-digestion

Reduction in VS is an affirmative consequence of anaerobic co-digestion process as it suggests the increase of biogas production. In set I, bioaccessibility to the simple soluble organic matter was restricted as the microbial activity was limited in comparison to set II thereby exhibiting lesser reduction in VS. Mixing ratio 1.5 in set II was illustrated to be the optimal mixing ratio as the methanogenic bacteria were capable of thriving well showcasing an improvement in the production of biogas. The highest biogas production was represented by the mixing ratios illustrating maximum VS reduction and vice versa.

6.3.4 Kinetic study

To establish the effectiveness of the ideal mixing ratio and pretreatment on the anaerobic co-digestion of water hyacinth and food waste, the cumulative methane production values were fitted to Gompertz equation curve (Lee et al., 2013). Table 6.9 summarises the results of the kinetic study for both set I and II. The kinetic parameters of set I and II used in BMP assay were determined where M of set II (6.3921 L CH_4) was observed to be higher than set I (5.5190 L CH_4). Both set I and II have R^2 value above 0.90, indicating that methane production can be well simulated.

Table 6.9. Kinetics values of untreated and hot air oven pretreated water hyacinth used in BMP test.

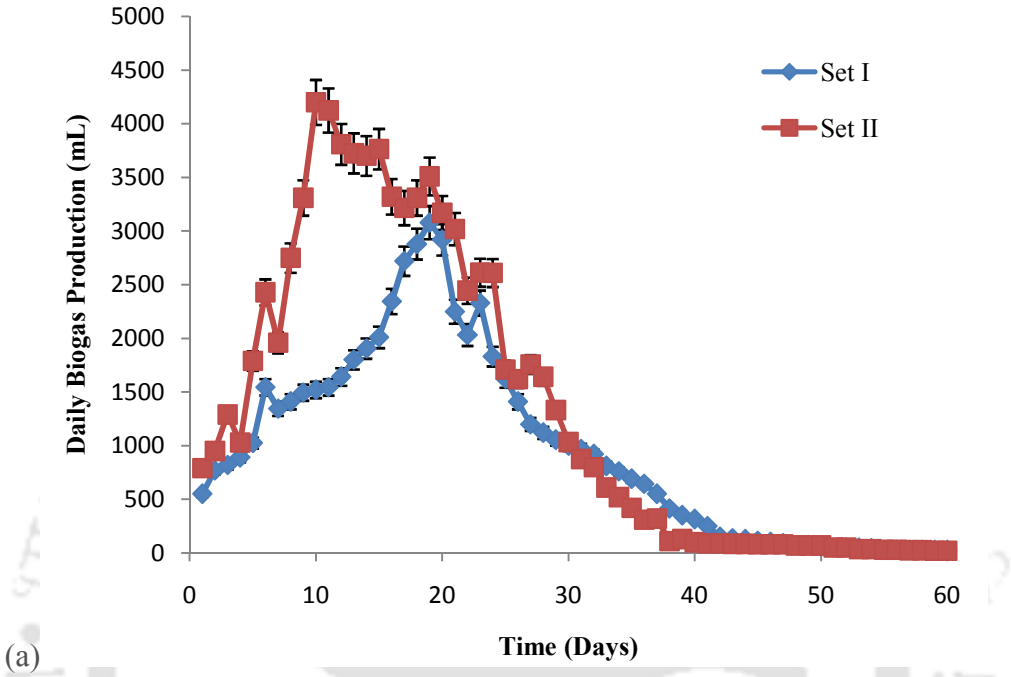
Substrate	Mixing ratio	M (L CH_4)	Rmax (L CH_4 d^{-1})	λ (d)	R^2	Y (L CH_4)
Set I	2.0	5.5190	0.1000	0.0000	0.95	4.328
Set II	1.5	6.3921	0.0780	0.0000	0.93	5.017

6.3.5 Scaled up batch anaerobic digestion

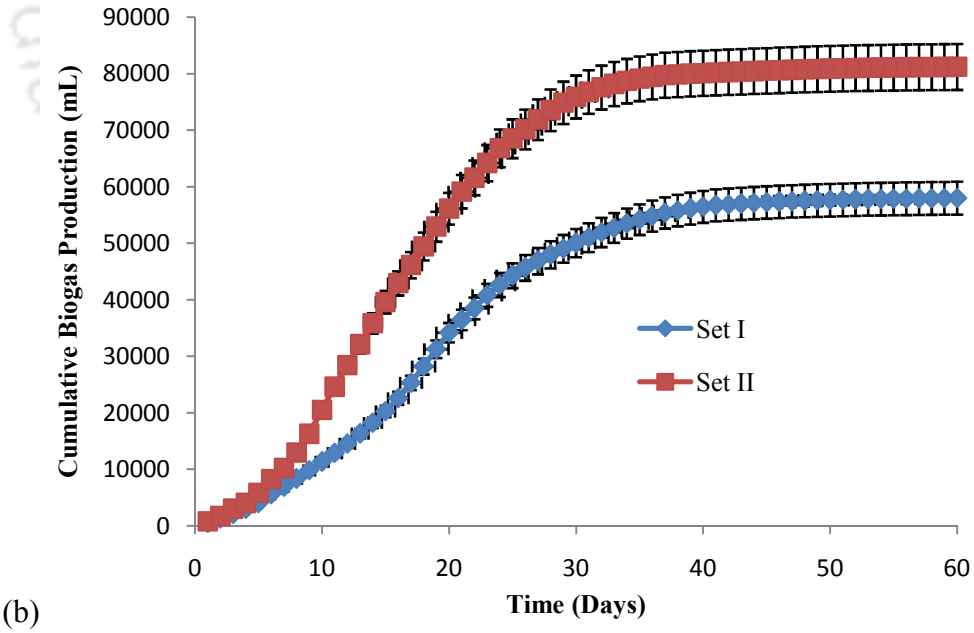
The aforementioned methodical investigation demonstrated confirmatory results and disclosed the ideal F/M ratio for both set I and II; therefore the batch study was scaled up (20 L) with the purpose of verifying the operational conditions and variations to be encountered during scaled up process.

In both set I and II, biogas production commenced instantly and a sharp increase in the production of biogas was observed (Fig. 6.18a). Still enormous improvement in biogas production in set II was credibly apparent (Fig. 6.18b). Cumulative biogas production from set I was examined to be 58000±14 mL while a cumulative biogas production of 81254±10 mL was attained from set II in 60 days; which is approximately 1.4 times higher than set I.

The maximum daily biogas production of 3080±12mL was achieved on 19th day for set I whereas in set II highest daily biogas production of 4220±15 mL on 10th day was attained. The result clearly indicates the improvement in the production of biogas in set II.



(a)



(b)

Fig. 6.18. (a) Daily biogas production and (b) cumulative biogas production of set I and II in 20 L anaerobic batch digester

The dynamic deviation in the production of biogas observed in set II was due to the easy availability of excessive quantity of easily soluble organic compounds due to hot air oven

pretreatment of water hyacinth. Thitilertdecha et al. (2008) reported that the peel of fruits demonstrate the low methane potential compared to their pulp due to the occurence of the cellulose and hemicellulose rigid network complex. Had the banana peels been pretreated the crystalline cellulose would be converted to its amorphous form, leading to more increase in biogas production in set II. While in set I due to co-digestion of water hyacinth and banana peels the nutrients were balanced and the positive synergism between the organic substrates led to the absence of lag phase but due to the absence of easily bio-available soluble organics biogas production was lesser. In short, the performance of the anaerobic bacteria was lethargic in set I when compared to set II due to the existence of recalcitrant lignin and crystalline cellulose in water hyacinth.

6.4 COMPARATIVE ANALYSIS

Water hyacinth was co-digested with food waste, *Hydrilla verticillata* and banana peels. There were two sets of experiments performed simultaneously for each co-digestion one set was without pretreatment and other set was co-digestion alongwith pretreatment. Readily biodegradable organic wastes i.e., food waste and banana peels were not pretreated whereas *Hydrilla verticillata* was pretreated alongwith water hyacinth as it is lignocellulosic in nature.

Table 6.10. Comparative analysis of co-digestion studies

Set	Parameters	Water hyacinth and Food waste	Water hyacinth and *Hydrilla verticillata*	Water hyacinth and Banana peels
I	Highest daily biogas production	174±6 mL CH_4/g VS on the 19th day	156±7 mL CH_4/g VS on the 23rd day	170±10 mL CH_4/g VS on the 16th day
	Cumulative biogas production	4328±12 mL	3921±11mL	3948±12 mL
	% CH_4	59.4±0.2	57. 8±0.3% CH_4	57.6±0.2% CH_4
II	Highest daily gas production	206±10 mL CH_4/g VS on the 13th day	180±9 mL CH_4/g VS on the 14th day	197±10 mL CH_4/g VS on the 11th day
	Cumulative biogas production	5017±15 mL	4630±10 mL	4954±12 mL
	% CH_4	68.44±0.5	63.54±0.2	65.65±0.5

The optimal mixing ratio was illustrated to be 2 where the substrate was untreated while the optimal mixing ratio was illustrated to be 1.5 where the substrate was pretreated. Pretreatment followed by co-digestion favoured in accelerating the hydrolysis period. While only co-digestion improved the biogas production when compared to mono-digestion by strikingly improving the quantity of soluble organics, balancing the nutrients, buffering toxic inhibitors. Still a steep increase in biogas production was missing when compared to pretreatment followed by co-digestion as pretreatment was beneficial in quickening the digestibility of the lignocellulosic substrate/co-substrates and the quality of biogas. More improvement in biogas production was observed where both the substrate and co-substrate were pretreated rather than the pretreatment of only substrate.

CHAPTER 7

DESIGN AND OPERATION OF A CONTINUOUS DIGESTER

This chapter deals with the design and operation of a novel type of biogas digester in continuous mode for water hyacinth in three different ways. For, the first experiment freshly pulverised water hyacinth whole plant was used as a feedstock; then hot air oven pretreated water hyacinth was used followed by co-digestion of water hyacinth and food waste.

7.1 DIGESTER DESIGN

The universal requirement of energy is steadily rising due to progress in standard of living, social and financial advancement. Burning of fossil fuel is the main reason behind increase in anthropogenic greenhouse gases worldwide finally contributing to environmental pollution and dilapidation of human health. The production of biogas as a renewable energy through anaerobic digestion is a sustainable alternative significantly leading to the alleviation of greenhouse gas emissions. Biogas is a valuable source of energy useful for electricity, heat production and as a fuel for transportation. The left over digestate after anaerobic digestion possess high amount of nutrients, which can be used as a natural fertilizer. A wide assortment of lignocellulosic organic wastes can be tranformed to biogas through anaerobic digestion. Water hyacinth is a renewable feedstock which is considered to be the world's worst aquatic weed. Water hyacinth the storehouse of cellulose is a nuisance for the aquatic ecosystem as well as for the human beings as it hampers the health, livelihood and recreational activities. Cellulosic water hyacinth seems to be an attractive feedstock to produce renewable biogas due to its potential to re-grow miraculously and comparatively easy availability worldwide. Cellulose is the most profuse biopolymer present in the world (Zhang and Lynd, 2005) and biogas production from water hyacinth through anaerobic digestion is a solution for the decrease in fossil fuel reserves, rise in energy demand and environmental pollution. However, a few noteworthy concerns such as reasonably lesser biogas production and process instability due to high solid content and decreased biodegradability of the lignocellulosic feedstock exists which can be improved by the use of an appropriate anaerobic digester. Anaerobic digester design is one of the chief characteristic in the techno-economic feasibility of biogas process development. Shape of the digester also plays an important role in enhancing biogas production. Oloko-Oba et al. (2018) observed that cylindrical shaped anaerobic digester provided the highest biogas production when compared to conical or cubical shaped anaerobic digester. A sophisticated anaerobic digester may be difficult to handle resulting in increased requirement of manpower with suitable expertise which is

difficult to find. Therefore a biogas digester that is simple, efficient and cost-effective in the production of biogas is the need of the hour. Usually a two stage biogas digester improves the rate of biodegradation of the feedstock, process stability and biogas recovery than a traditional single stage anaerobic digester (Maspolim et al., 2015; Schievano et al., 2014). There are various previous literature reports available stating the advantages of two stage anaerobic digestion of organic wastes (Dinsdale et al., 2000; Mata-Alvarez et al., 2000; Bouallagui et al., 2004). Bouallagui et al. (2004) reported that two stage anaerobic digestion of fruit and vegetable wastes demonstrated enhanced process stability, noteworthy biogas productivity and improved effluent quality. Nasr et al. (2012) conducted a comparative study between the single and two stage anaerobic digestion of thin stillage and observed 24% increase in biogas yield in the two stage process when compared to single stage anaerobic digestion. But the utilisation of two digester vessels in series makes it difficult to operate due to enhanced process configuration, increased pumping and control complexity. Also a two stage anaerobic digester requires enormous space. Karim et al. (2005) stated that at the micro-environment level, successful start-up and stability of anaerobic digesters are greatly influenced by the extent of microbial consortia and the feedstock contact and the synergism between methanogenic bacteria and their syntrophs; which is the chief purpose of mixing. Mixing of substrate inside the anaerobic digester is a key parameter to be considered because mixing enhances the presence of robust microorganisms and eliminates the end products of metabolism thereby improving the efficiency of microbial synthesis and biogas production. Mixing or stirring is necessary to avoid settling of the feedstock on the surface of the digester, which may hinder the biogas from escaping out. Absence of mixing may encourage temperature or concentration gradients (pH, feedstock and dissolved gases) inside the anaerobic digester that may affect the biological reaction, feedstock availability, stripping of dissolved gases, spatial distribution of microorganisms, process stability and biogas production. Lemmer et al. (2013) observed that mechanical mixing is a major consumer of electric energy in biogas plants contributing a large extent to the operational costs. Kaparaju et al. (2008) reported that nominal irregular mixing of liquid phase (10 min mixing prior to feeding) enabled a higher biogas production than continuous mixing. Kress et al. (2018) reported that reduced mixing time of renewable energy crops in a full-scale biogas digester did not hinder the nutrient distribution and biogas production. Impellers are useful for mixing or stirring the solid-liquid feedstock in the anaerobic digester. Depending on the type of impeller blade used in the digester, the mixing may be axial or radial. Axial impellers are beneficial for mixing solid suspension as axial impellers provides effective top to bottom

motion in tank disabling settling of the solids in the bottom of the digester. While radial impellers provide side to side mixing and provide more shear and less flow. Mixing and the separation of stages in a digester during anaerobic digestion demonstrates enhanced biodegradation efficiency of the feedstock. But two stage anaerobic digesters are difficult to operate and require huge space. Also, continuous high intensity mixing minimises biogas production. Based on these criteria, a novel type of two stage anaerobic digester was designed. The aim of this study was to evaluate the performance of this novel anaerobic digester in continuous mode utilising water hyacinth as the feedstock.

7.1.1 Activation of the novel anaerobic digester

Acclimatisation of the novel anaerobic digester is an indispensable step for providing suitable operational conditions for stable biogas production (Ganesh et al., 2014). The activation of the continuous anaerobic digester might be a lengthy phase when compared to batch anaerobic digester typically involving few weeks to months. In this study, the anaerobic digester was fed with 5 kg of cow dung slurry and sealed for 40 days for acclimatisation of the microorganisms inside the digester. After 40 days, feeding of the anaerobic digester started. Initially, the digester was fed with an organic loading rate (OLR) of 0.63 kg COD/m^3.d and pH was checked. As the pH was stable for a few days, the OLR was increased to 1.35 kg COD/m^3.d. The pH was stable until the OLR was increased to 4.5 kg COD/m^3.d. So, the OLR was fixed at 3.8 kg COD/m^3.d which was to be fed continuously for the next 70 days. Lee et al. (2004) observed that when OLR is increased after the optimal value, the stability of the digester deteriorates significantly leading to the abrupt reduction in pH, thereby provoking an abrupt increase in the VFA concentration, which may be toxic for methanogenic bacteria. Thereby, suggesting that the effluent COD is mostly composed of the unused VFA produced in the digester at higher OLR. Similarly, for co-digested water hyacinth and food waste the OLR was fixed at 6.7 kg COD/m^3.d as pH was stable until the OLR was increased to 8 kg COD/m^3.d. A gradual increase in OLR permits the methanogenic microorganisms to flourish and perk up the start-up of an anaerobic digester. Secure start-up of the anaerobic digester is usually conducted at a low OLR, which is steadily boosted with time, permitting proper acclimatisation of the microorganisms to avoid shock load due to sudden increase in COD loading. Biogas production initiated almost immediately once the feeding of the digester commenced. Biogas production was 2000, 6085 and 5200 mL on the very first day for untreated, pretreated and co-digested water hyacinth respectively. Biogas production was steadily increasing and was stable after 20, 9 and 12 days of digestion for untreated, pretreated and co-digested water hyacinth respectively. The chief purpose of start-

up was to acclimatise the inoculum with the feedstock. On the whole, biogas production started immediately and became stable quickly. This can be attributed to the well-acclimatised bacterial community in relative abundance that reduced the metabolic period (hydrolysis, acidogenesis, acetogenesis and methanogenesis) greatly. Wang et al. (2009) observed that the start-up process of continuous anaerobic digestion using lignocellulosic biomass as additive substrate for co-anaerobic digestion with swine manure was about 50 days. However, in this study the start-up process was relatively shorter for continuous biogas production of water hyacinth reducing the cost of biogas production which is favourable for industrial application.

7.1.2 Production of biogas

In this study, the performance of the novel anaerobic digester was investigated in a continuous mode for biogas production from water hyacinth in untreated, pretreated and co-digested form. Speedy start-up was witnessed in the novel anaerobic digester as biogas production started immediately after water hyacinth was fed in the digester suggesting that the inoculum (cow dung) commenced acclimatising to the environment of water hyacinth (Fig. 7.1). A linear increase in the biogas production was observed as the OLR was increased. Moreover, at OLR value higher than the optimal OLR, reduction in biogas production was observed, suggesting that the activity of methanogenic bacteria was not impaired up to OLR values of 3.8 kg COD/m^3.d for untreated, pretreated water hyacinth and 6.7 kg COD/m^3.d for co-digested water hyacinth; due to the appropriate stability and adequate buffering capacity provided in the experimental system. Subsequent to the initial start-up, an increase in mixing assisted in the mass transfer of nutrients and quickened the digestibility of the water hyacinth. Extremely vigorous mixing might hinder the survival of microorganisms as they are susceptible to high mixing intensity (Lindmark et al., 2014). The physical partition of hydrolysis and acidogenesis stages from acetogenesis and methanogenesis stages in the novel anaerobic digester defended the methanogenic bacteria from the difficulties associated with acidification. Monlau et al. (2013) stated that the operating condition in the first stage of a two-stage anaerobic digester favours fermentation of the feedstock and the accumulation of VFA whereas in the second stage, VFAs from the effluent of the first stage favours methane formation. However, it is worth mentioning that the biogas production was lesser for the untreated and co-digested water hyacinth when compared to the pretreated water hyacinth suggesting that pretreatment of water hyacinth significantly reduced the viscosity of the feedstock enabling more efficient mixing thereby enhancing biogas production (Mönch-Tegeder et al., 2014).

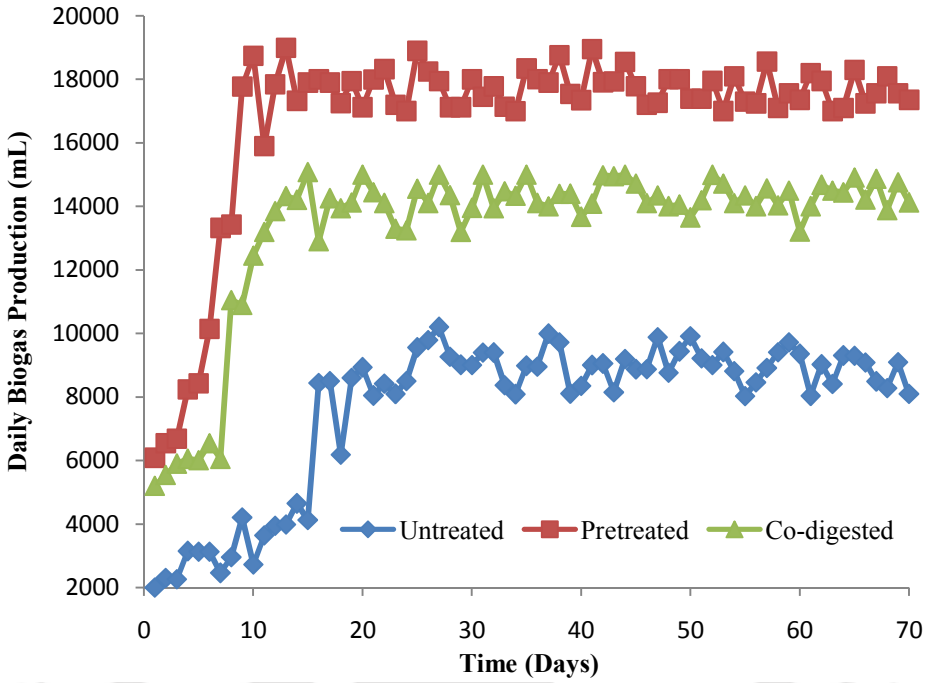


Fig. 7.1. Daily biogas production during continuous anaerobic digestion

Also at the time of feeding the pretreated water hyacinth the inoculum consisted of digested untreated water hyacinth alongwith the acclimatised cow dung providing a wide range of microorganisms participating in the metabolic process thereby improving biogas production. Gagliano et al. (2017) also observed that dissimilarity in the composition of the microorganisms during start-up strongly influences the overall performance of the anaerobic digestion process. Thus the variation in composition of the microorganisms in the anaerobic inoculum robustly influenced the overall performance of the continuous anaerobic digestion process of water hyacinth. The existence of well acclimatised anaerobic bacterial community in relative abundance during the start up positively influenced the conversion of soluble organic matter into biogas. The decreased biogas production from co-digested water hyacinth when compared to the pretreated water hyacinth might be attributed to the utilisation of food waste alongwith water hyacinth. As food waste is readily degradable as protein and carbohydrate degrading bacteria develops quickly thereby provoking the accumulation of VFA. In this study, water hyacinth was co-digested with water hyacinth therefore VFA accumulation was under control due to natural buffering action in the anaerobic co-digestion process and balanced nutrients leading to higher biogas production than untreated water hyacinth but lesser biogas production than the pretreated water hyacinth.

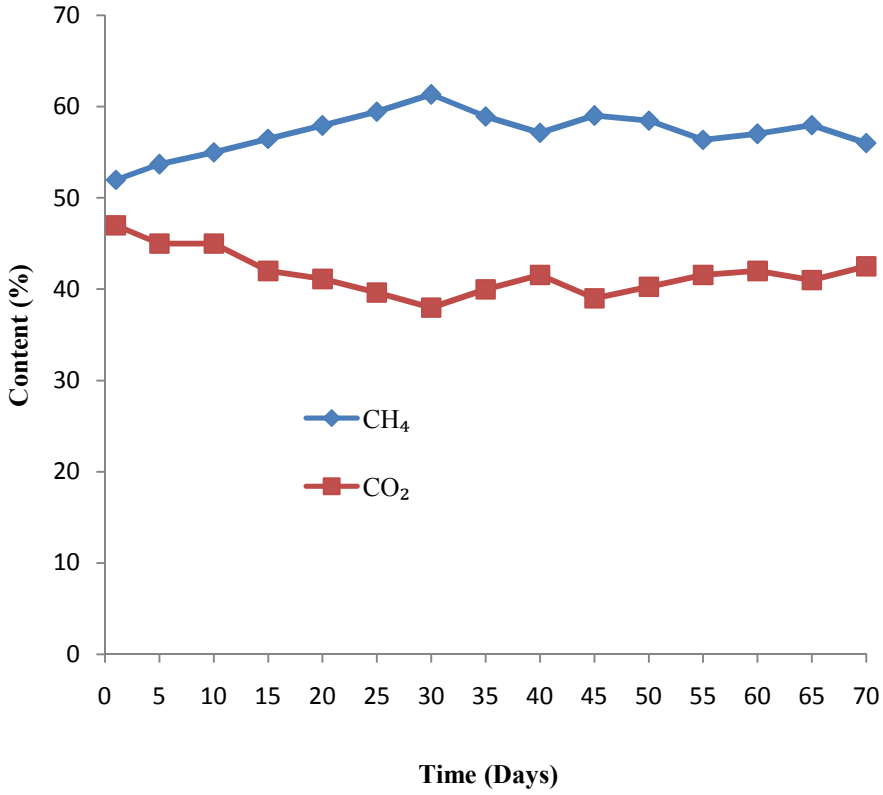


Fig. 7.2a. CH_4 and CO_2 content in biogas produced during continuous anaerobic digestion of untreated water hyacinth

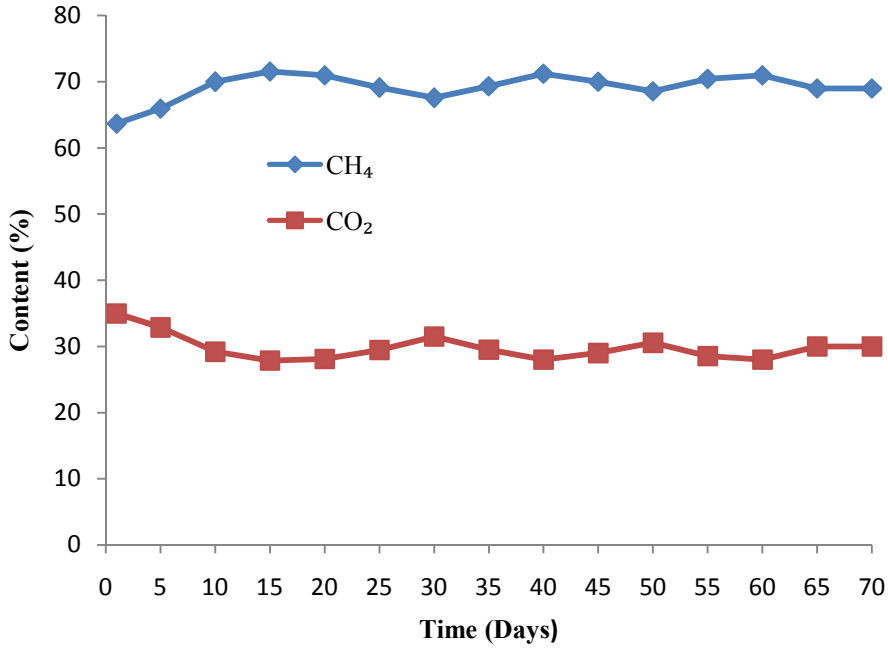


Fig. 7.2b. CH_4 and CO_2 content in biogas produced during continuous anaerobic digestion of pretreated water hyacinth

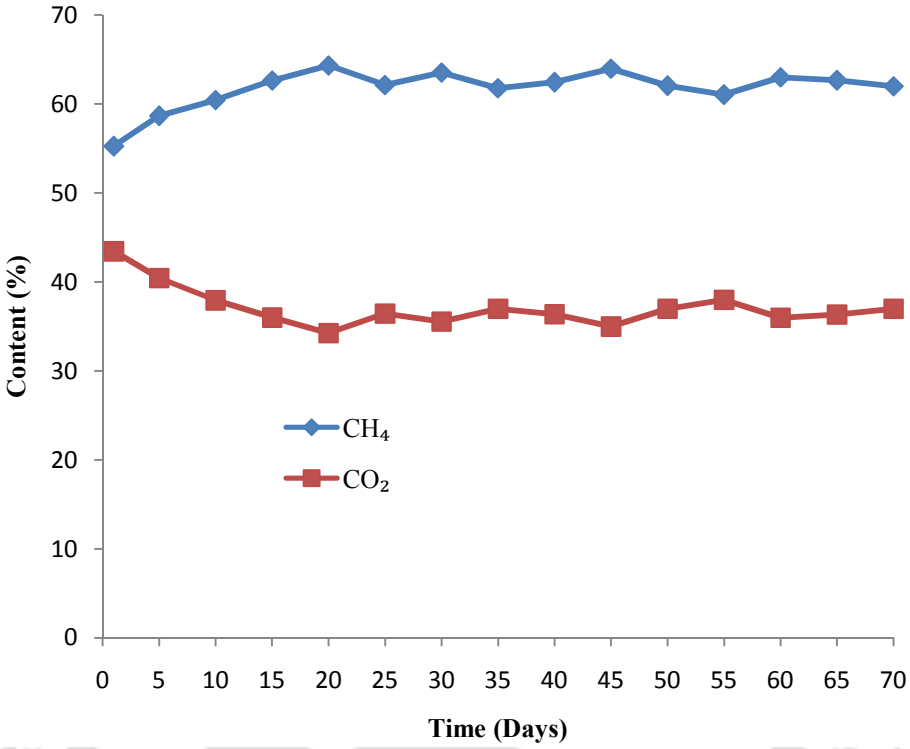


Fig. 7.2c. CH_4 and CO_2 content in biogas produced during continuous anaerobic digestion of water hyacinth co-digested with food waste

After the start-up, the biogas production increased with the increase in OLR until a point was achieved which lowered the biogas production. Base on this phenomenon, it is possible to fix the OLR of the feedstock in the novel anaerobic digester and control the biogas production. Intermittent mixing and the presence of two stage during the continuous anaerobic digestion process was beneficial as it helped in the growth of methanogens leading to better degradation of the complex lignocellulosic water hyacinth, alleviated process instability problems, produced a good amount of biogas, minimising the maintenance and energy requirement of the process. Methane (CH_4) and carbon dioxide (CO_2) are the two chief components of biogas. CH_4 content in biogas is the primary indicator of the quality of biogas. Generally, higher the CH_4 content in biogas better is the quality of biogas produced during anaerobic digestion. In this study, the CH_4 and CO_2 content of the produced biogas was quantified every fifth day throughout the continuous anaerobic digestion process (Fig. 7.2a, 7.2b and 7.2c). In the initial days CH_4 content was comparatively lower and it started increasing gradually, illustrating that the inoculum is familiarising itself to the environment of water hyacinth. CH_4 content was observed to be 51.9, 63.6 and 55.2% for untreated, pretreated and co-digested water hyacinth respectively on the very first day. After few days of initiation, the CH_4 content maintained a relatively stable value throughout the continuous

anaerobic digestion process. CH_4 content was demonstrated to be in between 57-61, 68-71and 60-63% for untreated, pretreated and co-digested water hyacinth respectively. Ong et al. (2002) reported 70% increase in release of biogas from the liquid digestate in intermittently mixed digesters during mixing periods thereby suggesting that the release of biogas is hampered when not mixed and mixing increases the mass transfer of the gas from the liquid phase.

7.1.3 COD, VFA and VS

COD removal is an essential parameter determining the efficiency of the continuous anaerobic digestion system in the novel anaerobic digester. Usually after hydrolysis, cellulose and hemicellulose of the lignocellulosic feedstock are transformed into COD (generally into simple soluble sugars). Higher the COD removal higher is the stability of the process. In this study, the performance of COD removal with different OLR was investigated throughout the continuous anaerobic digestion process for untreated, pretreated and co-digested water hyacinth (Fig. 7.3a, 7.3b and 7.3c). The rate of COD removal increased linearly with OLR up to an OLR value of 3.8 kg COD/m^3.d untreated, pretreated water hyacinth and 6.70 kg COD/m^3.d for co-digested water hyacinth. In Fig. 7.3a, 7.3b and 7.3c the inlet COD was relatively stable whereas the outlet COD was increasing initially then it was also stable after 25, 10 and 15 days for untreated, pretreated and co-digested water hyacinth respectively.

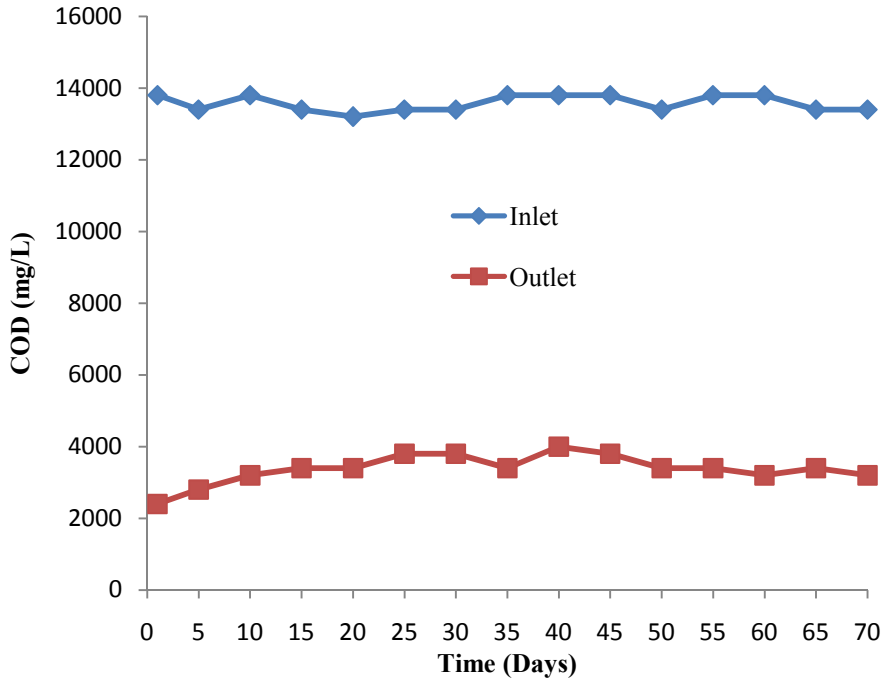


Fig. 7.3a. COD profile of untreated water hyacinth during continuous anaerobic digestion

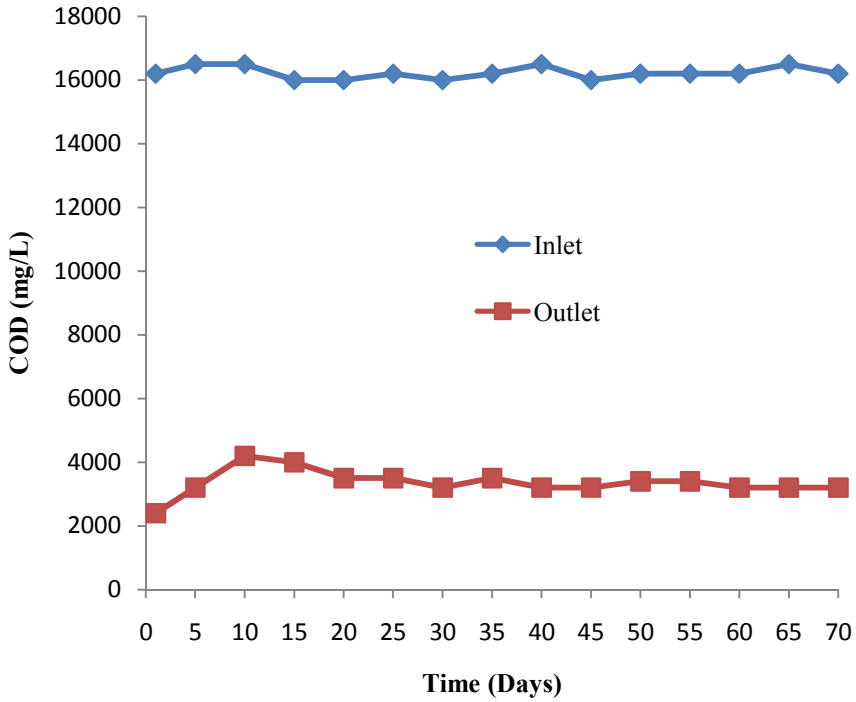


Fig. 7.3b. COD profile of pretreated water hyacinth during continuous anaerobic digestion

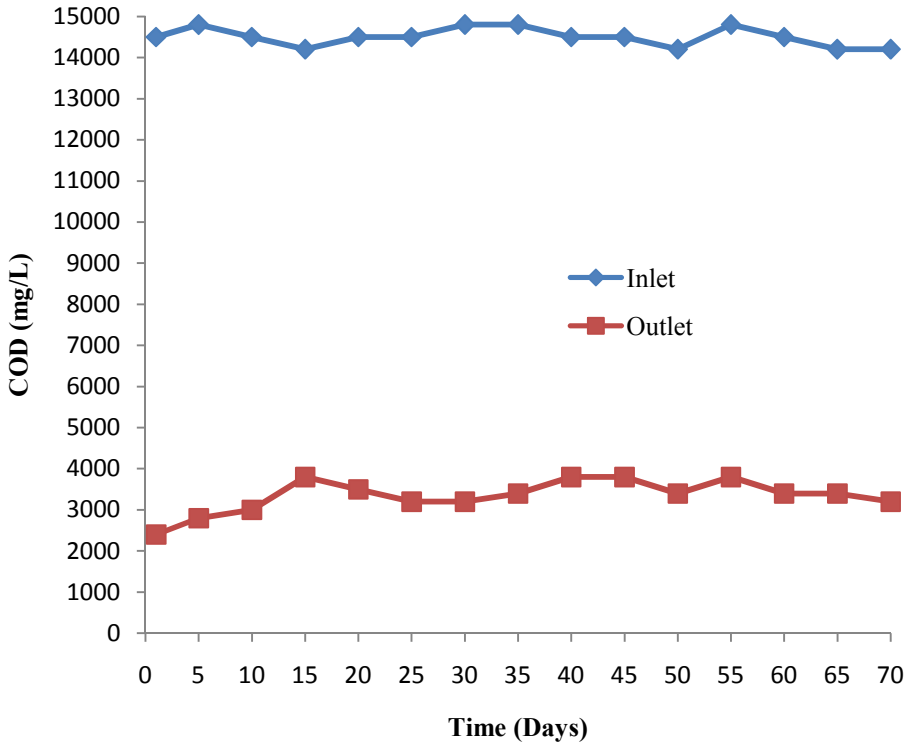


Fig. 7.3c. COD profile of water hyacinth co-digested with food waste during continuous anaerobic digestion

Highest percentage of average COD removal of 82% was observed in pretreated water hyacinth followed by 77% in co-digested water hyacinth and 72.5% in untreated water

hyacinth. Akobi et al. (2016) also reported COD removal of 75-82% in the two-stage anaerobic digestion process of poplar wood. The enhanced removal of COD can be attributed to two stage novel anaerobic digester. The presence of two stages inside the novel anaerobic digester was beneficial because at a biochemical level the environmental conditions in each digester stage the suitable microorganisms appropriate for the stage was prevalent. Also proper mixing kept the solid feedstock in suspension and homogenised the inlet feed with the robust microbial community present in the novel anaerobic digester.

VFA analysis is often utilised as an index process imbalance during anaerobic digestion (Stroot et al., 2001; Hoffman et al., 2008). The inlet VFA was relatively stable throughout the continuous anaerobic digestion process whereas the outlet VFA took a few days to stabilise as it was initially increasing for untreated, pretreated and co-digested water hyacinth (Fig. 7.4a, 7.4b and 7.4c). VFA concentration instead of hindering the biogas production increased the biogas production as the VFA were degraded, illustrating a relationship between high VFA production and high biogas production. Vavilin and Angelidaki (2005) observed that dispersal of VFA during higher mixing intensities possibly decreases the activity of the methanogenic bacteria as the organisation of the methanogenic zones is obstructed. Thus, intermittent mixing and the two stage of the novel anaerobic digester were beneficial for VFA degradation.

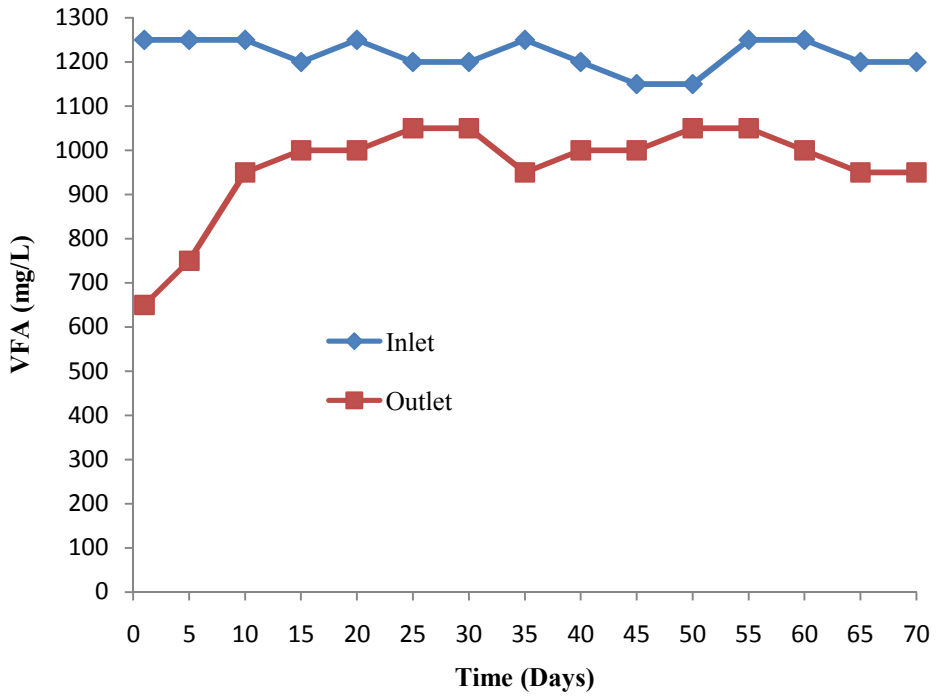


Fig. 7.4a. VFA concentration profile of untreated water hyacinth during continuous anaerobic digestion

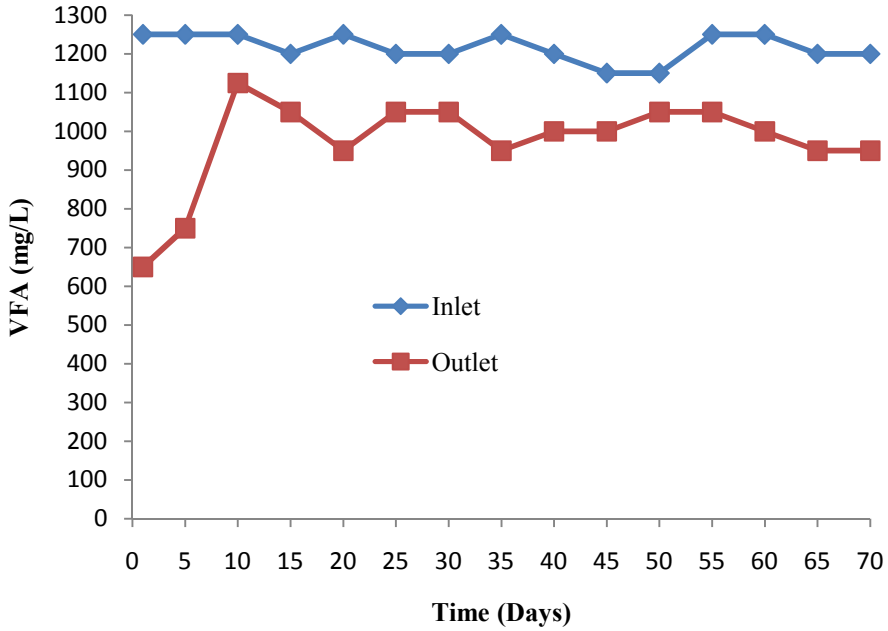


Fig. 7.4b. VFA concentration profile of pretreated water hyacinth during continuous anaerobic digestion

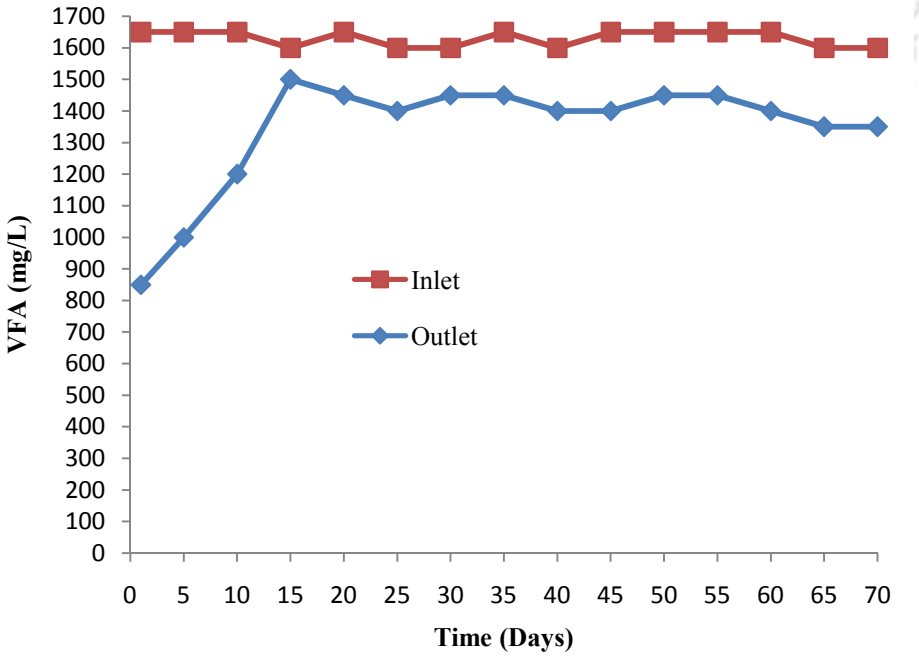


Fig. 7.4c. VFA concentration profile of water hyacinth co-digested with food waste during continuous anaerobic digestion

7.1.4 COD balance

The parameter COD was beneficial to establish a mass balance for the utilization of various organic compounds inside the novel anaerobic digester. A COD balance, was executed using the experimental data attained from the anaerobic digestion of untreated, pretreated and co-digested water hyacinth in the novel anaerobic digester. The percentage of COD distribution for untreated, pretreated and co-digested water hyacinth is illustrated in Fig. 7.5. Particulate COD fraction accumulated inside the novel anaerobic digester which was not hydrolyzed is identified as "remaining". When pretreated water hyacinth was run in the continuous novel anaerobic digester 0.56% balance of COD was observed. Pretreated water hyacinth demonstrated the best result followed by co-digested and untreated water hyacinth. Increased percentage of COD remaining can be attributed to the particulate organic matter which was not solubilised and was accumulated inside the reactor as a result of previous operational period. Accumulation of particulate organic matter affects the hydrolytic and methanogenic activity leading to lower percentage of biogas production comparatively. Hence, the utilization of the COD balance appears to be a reliable tool to detect the organic compounds accumulated inside the novel anaerobic digester.

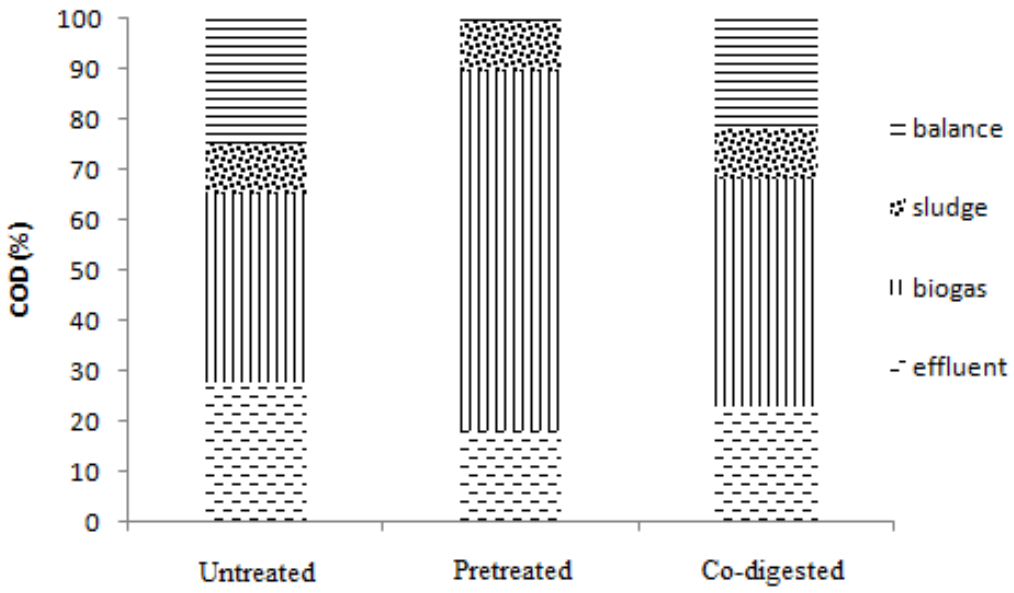


Fig. 7.5. COD balance for untreated, pretreated and co-digested water hyacinth.

7.2 COMPARATIVE ANALYSIS

The novel anaerobic digester proved its great potential in treating water hyacinth in any form; be it untreated, pretreated or co-digested. The design of the novel anaerobic digester is efficient in reducing the cost, complexity of operation and space requirement when compared

to the traditional two stage anaerobic digesters with mixing operation. The performance of the novel anaerobic digester's performance in distribution of the microorganisms, improving the digestibility of the feedstock, reducing the volatile solids thereby enhancing the overall anaerobic digestion process is worth mentioning. The optimal OLR for untreated and pretreated water hyacinth was observed to be 3.8 kg COD/m^3.d whereas for co-digested water hyacinth the optimal OLR was 6.7 kg COD/m^3.d; illustrating an average COD removal of 72.5, 82 and 77% for untreated, pretreated and co-digested water hyacinth respectively (Table 7.1). The novel anaerobic digester can be recommended for treating other organic wastes/ feedstocks as well.

Table 7.1. Comparative analysis of the efficiency of the novel anaerobic digester when fed with water hyacinth in various forms

Water hyacinth	OLR (kg COD/m^3.d)	COD removal (%)
Untreated	3.8	72.5
Pretreated	3.8	82
Co-digested	6.7	77

CHAPTER 8
CONCLUSION AND RECOMENDATION

This chapter deals with the overall conclusion attained after accomplishing all the objectives of the research work.

8.1 CONCLUSION

Initial characterisation of water hyacinth demonstrates itself to be a potential feedstock for the production of biogas through anaerobic digestion as it has a good amount of moisture content, sCOD and cellulose. Water hyacinth is the storehouse of cellulose and cellulose is the main component responsible for the production of biogas through anaerobic digestion. The presence of the hard outer layer lignin which guards the cellulose delays the hydrolysis of water hyacinth. Therefore to speed up the hydrolysis period and enhance the production of biogas; pretreatment of water hyacinth is necessary.

Thermal, electrohydrolysis and microbial pretreatment techniques were performed to breakdown the rigid lignocellulosic network of water hyacinth. In thermal pretreatment, highest solubilisation of water hyacinth was achieved by hot air oven (55.5%), followed by microwave (44.2%), hot water bath (35%) and autoclave (22.2%). The optimal condition for the solubilisation of water hyacinth achieved by hot air oven pretreatment is 90ºC for 1h. Electrohydrolysis pretreatment of water hyacinth at 20V for 60 min exhibited improved solubilisation (42.9%).Biological pretreatment with novel isolated microbial pure culture was utilised to pretreat water hyacinth to enhance its solubilisation. Lignocellulose degrading bacterial strains isolated from soil (*Bordetella muralis VKVVG5*), the gut of silverfish (*Citrobacter werkmanii VKVVG4*) and millipede (*Paenibacillus sp. VKVVG1*) were employed to optimise the ideal bacterial strain illustrating accelerated hydrolysis of water hyacinth *Citrobacter werkmanii VKVVG4* pretreatment of water hyacinth with an optimum dosage of 10^9 CFU/mL and time of 4 days helped in achieving the highest solubilisation of 33.3%. The comparative analysis of all the three pretreatment techniques suggests that hot air oven pretreatment at 90ºC for 1h is the most efficient pretreatment technique as it demonstrates the highest solubilisation of water hyacinth comparatively.

After pretreatment of water hyacinth the improvement in biogas production was analysed alongwith the ideal F/M ratio. BMP test was conducted in order to check it's prospective for producing biogas. BMP was examined for both untreated as well as pretreated water hyacinth whole plant to determine the ideal F/M ratio. Determination of

the ideal F/M ratio is necessary to avoid process imbalance and enhance biogas generation. BMP test revealed that F/M ratio 2 of the untreated water hyacinth, showed the highest methane yield of 143±14 mL CH_4/g VS on the 32nd day whereas for hot air oven pretreated water hyacinth F/M ratio 1.5 showed the highest methane yield of 193±22 mL CH_4/g VS on the 14th day itself. For electrohydrolysis pretreated water hyacinth, F/M ratio 1.5 achieved the highest methane yield of 155±22 mL on the 7th day itself. For microbial pretreatment of water hyacinth by *Citrobacter werkmanii VKVVG4* the F/M ratio 1.5 showed the highest methane yield of 156±19 mL CH_4/g VS on the 20th day. Although the BMP test of water hyacinth after electrohydrolysis pretreatment illustrated the highest biogas production on the 7th day but when the process was scaled up to 20 L batch study the highest biogas production was witnessed on 28th day. This suggests that electrohydrolysis pretreatment was not feasible for solubilising an increased amount of water hyacinth which was easily solubilised when performing pretreatment for a lower amount of water hyacinth. The 20 L batch study of hot air oven pretreated water hyacinth demonstrated cumulative biogas production by the end of the process to be 310045±27 mL whereas for untreated water hyacinth it was observed to be 59021±19 mL. Augment in cumulative biogas production by 5.25 folds in hot air oven pretreated substrate than the untreated feedstock illustrates the remarkably significant effect of hot air oven pretreatment on the chemical kinetics and solubility of organic compounds within the anaerobic digester. Even hot air oven pretreated water hyacinth demonstrated better quality of biogas when compared to untreated water hyacinth. 67.4, 61.2, 59.9 and 57% of methane was observed in hot air oven pretreated, electrohydrolysis pretreated, microbial pretreated water hyacinth and untreated water hyacinth repectively.

After pretreatment study and methane potential analysis of water hyacinth anaerobic co-digestion study of water hyacinth was conducted with other organic wsstes/feedstocks with and without pretreatment. As there are many literature reports available on the anaerobic co-digestion of water hyacinth and various animal wastes so in the present study water hyacinth (substrate) and cow dung (inoculum) was kept constant while the co-substrate was changed (food waste, *hydrilla verticillata* and banana peels). During anaerobic co-digestion of water hyacinth and food waste, mixing ratio 2 demonstrated the highest biogas production of 174±6 mL CH_4/g VS on the 19th day in set I while in set II, mixing ratio 1.5 demonstrated the highest biogas production of 206±10 mL CH_4/g VS on the 13th day itself. During anaerobic co-digestion of water hyacinth and *hydrilla verticillata,* in set I, the highest biogas production of 156±7 mL CH_4/g VS was exhibited

on the 23rd day by the mixing ratio 2.While in set II, highest biogas production of 180±9 mL CH_4/g VS was exhibited on the 14th day itself by the mixing ratio 1.5. Anaerobic co-digestion of water hyacinth and banana peels illustrated for mixing ratio 2, the maximum biogas production of 170±10 mL CH_4/g VS on the 16th day in set I. While, mixing ratio 1.5 in set II; displayed the maximum biogas production of 197±10 mL CH_4/g VS on the 11th day itself. It was observed that water hyacinth co-digestion with food waste and banana peels displayed enhanced biogas production when compared to the anaerobic co-digestion of water hyacinth and *hydrilla verticillata* as food waste and banana peels are readily biodegradable. While pretreatment followed by ananerobic co-digestion was beneficial in accelerating the hydrolysis period of anaerobic digestion and increasing the percentage of methane content.

Finally a novel anaerobic digester was designed, fabricated and operated. Mixing and the separation of stages in a digester during anaerobic digestion demonstrated enhanced biodegradation efficiency of the feedstock. But two stage anaerobic digesters are difficult to operate and require huge space. Also, continuous high intensity mixing minimises biogas production. Based on these criteria, a novel type of two stage anaerobic digester was designed. The aim of this study was to evaluate the performance of this novel anaerobic digester in continuous mode utilising water hyacinth as the feedstock. Initially, untreated water hyacinth whole plant was fed in the digester followed by hot air oven pretreated water hyacinth and water hyacinth co-digested with food waste. The optimal OLR for untreated and pretreated water hyacinth was observed to be 3.8 kg COD/m^3.d whereas for co-digested water hyacinth the optimal OLR was 6.7 kg COD/m^3.d; illustrating an average COD removal of 72.5, 82 and 77% for untreated, pretreated and co-digested water hyacinth respectively. The novel anaerobic digester proved its great potential in treating water hyacinth in any form; be it untreated, pretreated or co-digested. The design of the novel anaerobic digester is efficient in reducing the cost, complexity of operation and space requirement when compared to the traditional two stage anaerobic digesters with mixing operation. The performance of the novel anaerobic digester's in distribution of the microorganisms, improving the digestibility of the feedstock, removing COD thereby enhancing the overall anaerobic digestion process is worth mentioning.

8.2 RECOMMENDATIONS FOR FUTURE WORK

- Various other type of pretreatment techniques i.e., ultrasonication, ionic liquid, ozonolysis and fungal pretreatment can be studied for enhancing the digestibility of water hyacinth.

- Feasibility study of anaerobic co-digestion of water hyacinth with more than two organic wastes can be performed.
- Operation of the novel anaerobic digester with various other lignocellulosic and non-lignocellulosic organic feedstocks.
- Biogas production from water hyacinth in an industrial scale and its application.

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- **Barua, V.B**., Goud, V.V., Kalamdhad, A.S., 2018. Microbial Pretreatment of Water Hyacinth for Enhanced Hydrolysis followed by Biogas Production. *Renewable Energy* 126, 21-29.
- **Barua, V.B**., Kalamdhad, A.S., 2018. Anaerobic Biodegradability Test of Water Hyacinth after Microbial Pretreatment to Optimise the Ideal F/M ratio. *Fuel* 217, 91-97.
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- Barua, V.B., Rathore, V., Kalamdhad, A.S., 2018. Anaerobic co-digestion of water hyacinth and food waste with and without pretreatment. *Process Safety and Environmental Protection* (*Under review*)
- Barua, V.B., Rathore, V., Kalamdhad, A.S., 2018. Effect of anaerobic co-digestion of aquatic weeds with and without pretreatment on biogas production. *Biomass & Bioenergy* (*Under review*)
- Barua, V.B., Raju V.W., Lippold, S., Kalamdhad, A.S., 2018. Biochemical Methane Potential Assay of Water Hyacinth after Electrohydrolysis Pretreatment to Optimize the Ideal F/M ratio. *Environmental Technology & Innovation* (*With Editor)*
- Barua, V.B., Kalamdhad, A.S., 2018. Biogas production from water hyacinth in a novel anaerobic digester: A continuous study. *Process Safety and Environmental Protection* (*Under review)*

- Barua, V.B., Rathore, V. and Kalamdhad, A.S., 2018. Anaerobic co-digestion of water hyacinth and banana peels with and without thermal pretreatment. *Renewable Energy* (*Under review*)

NATIONAL/INTERNATIONAL CONFERENCES

- **Barua, V.B**., Kalamdhad A.S., 2018. Effect of Electrohydrolysis Pretreatment on Anaerobic digestion of Water Hyacinth. *Research Conclave, 9-11 March,* IIT Guwahati, India**. (Best Oral Presentation Award)**
- **Barua, V.B**., Kalamdhad A.S., 2018. Microbial Pretreatment of water hyacinth followed by biogas production. *RECYCLE, International Conference on Waste Management, 22-24 February,* IIT Guwahati, India.
- **Barua, V.B**., Kalamdhad A.S., 2017. Anaerobic digestion of water hyacinth with and without pretreatment: A comparative evaluation. *Bioprocessing India, 9-11 December,* IIT Guwahati, India.
- **Barua, V.B**., Kalamdhad A.S., 2017. Pre-requisite of thermal pretreatment for accelerating hydrolysis and biogas production from water hyacinth. *Symposium on Recent Advancements in Environmental Research, 5 June,* IIT Guwahati, India. **(Best Oral Presentation Award, Runner Up)**
- **Barua, V.B**., Kalamdhad A.S., 2017. Optimization of the most efficient thermal pretreatment technique for enhanced biogas production from water hyacinth. *International Conference on Integrated solid waste management practices in developing countries, 11-12 April,* NEERI (CSIR), Nagpur, India.
- **Barua, V.B**., Kalamdhad A.S., 2017. Thermal Pretreatment of Water Hyacinth (Eichhornia crassipes) for Augmenting Biogas Production. *Reflux, 24-26 March,* IIT Guwahati, India**.**
- **Barua, V.B**., Kalamdhad A.S., 2017. Pretreatment for Enhancing Biogas Production from Water Hyacinth. *Research Conclave,*16-19 March, IIT Guwahati, India. (**Best Poster Presentation Award**)
- **Barua, V.B**., Kalamdhad A.S., 2016. Effect of Microwave Pretreatment on the Hydrolysis of Water Hyacinth. *National Conference on Recent Advancements in Environmental Research (RAER),* 4-5 June, IIT Guwahati, India.
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- **Barua, V.B**., Kalamdhad A.S., 2015. Water Hyacinth: A Potential Biomass for Biogas Production. *National Conference on Challenges in Environmental Research (NCOCER),* 4-6 June, IIT Guwahati, India.

ACHIEVEMENTS

- **Outstanding Contribution** in **Reviewing** by **Bioresource Technology (Elsevier),** October 2017.
- **Only Indian** to receive the **ISWA-SWIS 2016 full scholarship** to attend winter school at the **University of Texas at Arlington, USA.**