Anaerobic Digestion of Terrestrial Weeds: Effect of Pre-Treatment and Co-Digestion on Biogas Production

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by

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CERTIFICATE

This is to certify that the thesis titled **'Anaerobic Digestion of Terrestrial Weeds: Effect of Pre-Treatment and Co-Digestion on Biogas Production'** submitted by **Biswanath Saha (Registration No. 166154003) to Indian Institute of Technology Guwahati,** for the award of degree **of Doctor of Philosophy (School of Agro and Rural Technology)** is recorded of bonafide research work carried out under our supervision and guidance. The thesis work is our estimation has reached the requisite standard fulfilling the degree of Doctor of Philosophy. The work is comprehensive, complete and fit for evaluation and it has not been submitted elsewhere for any degree.

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STATEMENT

I do hereby declare that the work contained in the thesis is original and has been done by me under the supervision of Prof. Ajay Kalamdhad (Supervisor-1) and Dr. Meena Khwairakpam (Supervisor-2).

The work reported herein has not submitted to any other Institute for any degree or diploma. Whenever I have used any material (concepts, ideas, text, expressions, data, graphs, diagrams, theoretical analysis, results, etc.) from other sources, I have given due credit by citing them in the text of the thesis and giving their details in the references.

June, 2021 Biswanath Saha

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ABSTRACT

Parthenium hysterophorus, *Lantana camara* and *Ageratum conyzoides* are considered to be among the world's top ten worst terrestrial weeds due to their reproduction potential. They can grow within a few weeks and cover entire agricultural land (*Parthenium hysterophorus*), forest land (*Lantana camara*), and native vegetation (*Ageratum conyzoides*). *Parthenium hysterophorus*, *Lantana camara*, and *Ageratum conyzoides* are difficult to manage as they can re-grow miraculously even after they are completely eradicated. The availability of *Parthenium hysterophorus*, *Lantana camara*, and *Ageratum conyzoides* in abundance makes attractive feedstock through anaerobic digestion. India is a fast-growing country with a population of 1.21 billion people in 2011 with a growth rate of 17.64% from 2001 to 2011 (15th Indian Census 2011) next census will be taken in 2021. The energy demand of the country is increasing rapidly due to the increase in population and industrialization. Non-renewable conventional energy sources like coal, oil, and natural gas, etc., play a crucial role in maintaining the major energy demands of the country. India depends totally on oil imports to meet energy demand. It is reported that about 200 million rupees were spent on imports to meet the two-third of energy demand in 2000. India consumes 40.34 million tons of diesel, i.e., 43.2% of total consumption in 2000-2001 and it is noted that the annual energy demand of India will increases from 0.58 to 4.02% between 2017 to 2026. According to the literature, the energy source is on the verge of extinction. The world's oil reserves are estimated to get depleted by 2050. It is expected that total oil reserves in India will only last up to 6 years. Biogas production from *Parthenium hysterophorus*, *Lantana camara*, and *Ageratum conyzoides* can effectively manage terrestrial weeds as well as mitigate environmental pollution caused by fossil fuels.

In phase I, the BMP study of *Parthenium hysterophorus*, F/M ratio 2 shows the ideal combination followed by 2.5 and 1.5 respectively. In the BMP study of *Lantana camara*, the highest methane production was obtained from the F/M ratio of 1.5 followed by ratios 2 and 2.5 respectively. *Ageratum conyzoides*, BMP assay revealed that, F/M ratio 2 acquired maximum biogas production from the anaerobic digestion of *A. conyzoides* biomass with cow dung as the microorganism source.

During phase II, the various thermal pretreatment modes (hot air oven, microwave, autoclave, and hot water bath) were applied where temperature 60 to 220°C and time (20 to 120 mins) study were conducted. In *Parthenium hysterophorus*, hot air oven showed the highest efficiency in the form of soluble chemical oxygen demand (sCOD) and volatile fatty acid (VFA), for *Lantana camara*, and *Ageratum conyzoides* autoclave pretreatment was found to be more efficient, followed by hot water bath, hot air oven and microwave pretreatments. During electrohydrolysis pretreatment, voltage (10 to 60 Voltage) and time (10 to 60 mins) were conducted. In Electrohydrolysis Pretreatment, *Parthenium hysterophorus* at 20V for 40 mins shows the highest sCOD and VFA, and for *Lantana camara* and *Ageratum conyzoides* at 30V for 20 mins shows the highest sCOD and VFA. During phase II, the various thermal pretreatment modes (hot air oven, microwave, autoclave and hot water bath) were applied where temperature 60 to 220 ˚C and time (20 to 120 mins) study were observed.

Phase III, the co-digestion of *Parthenium hysterophorus* was performed not only with cow dung as inoculum but also with other organic wastes (i.e., food waste). In co-digestion during the BMP assay, a mixing ratio of 1.5 (165 mL CH_4 g^{-1} VS on the 17th day) was observed. In co-digestion (food waste) of BMP assay, mixing ratio 1.5 (211 mL CH_4 g^{-1} VS on 14th day) for *Lantana camara*. In co-digestion of the BMP mixing ratio of 2 (205 mL CH_4 g^{-1} VS on 13th day) for Ageratum conyzoides are observed to be ideal.

In phase IV, the continuous scale, anaerobic digester was operated for the time span of 50 days for the best F/M ratio and pretreatment technique obtained from BMP assay. Average biogas production for untreated *P. hysterophorus* was 3270 mL, *L. camara* was 3010 mL, and for *A. conyzoides was* 3150 mL, for pretreated *P. hysterophorus* is 6454 mL; *L. camara is* 6219 mL, and *A. conyzoides* is 6982 mL, and for co digestion of *P. hysterophorus* is 5984 mL; *L. camara* is 5218 mL and for *A. conyzoides* is 5765 mL.

In Phase V, Various bacteria were identified in microbial study. Euryarchaeota, Bacteroidetes and Proteobacteria were majorly observe in this study.

Keywords: *Parthenium hysterophorus; Lantana camara; Ageratum conyzoides;* Anaerobic digestion; Biochemical methane potential (BMP); F/M ratio; Pretreatment; Co-digestion; Continuous reactor; Microbial study.

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LIST OF ABBREVIATIONS

AD	Anaerobic Digestion
P.hysterophorous	*Parthenium hysterophorous*
A.conyzoydes	*Ageratum conyzoides*
L.camara	*Lantana camara*
BMP	Biochemical Methane Potential
F/M	Food / Microorganism
sCOD	Soluble chemical oxygen
VFA	Volatile fatty acid
VS	Volatile solid
FESEM	Field Emission Scanning Electron Microscope
FTIR	Fourier Transform Infrared
XRD	X- Ray Diffractogram

CHAPTER 1

INTRODUCTION

This chapter consists of a brief discussion about the origin, spread, and problems of terrestrial weeds and their management. The overview, objective and the need of the study have also been included in this chapter.

1.1. Overview

A wild plant growing where it is not required and is competing with the cultivated plant is termed as a weed, and they are invasive by nature. A dynamic system involves the interaction between weeds, crops, humans, and the environment. According to different definitions of weed - a plant growing where it is not wanted a plant out of place, a plant growing where it is desired that something else growth (Georgia, 1916), those plants with harmful or objectionable habits. Therefore, it can be summarized that weeds are those uninvited plants, which are grown in undesirable places and periods, causing competition for cultivated crops and economic loss. Invasive plant species not only change the dynamics of species composition and biodiversity but also hamper the system productivity and efficiency in invaded regions; besides rapidly colonizing areas replacing the native vegetation, it is also known to cause several human health problems, environmental degradation including a threat to tourism activities (Kumar et al., 2013). The characteristics that make a plant weed are- (1) long seed life in the soil, (2) quick emergence, (3) rapid early growth, (4) no special environmental requirements for germination, (5) ability to survive and prosper under disturbed condition (Holm, 1977). The weed classes based on habitats are aquatic weeds which are grown on water, terrestrial weeds present on land and epiphytic or parasitic weeds those habitats on other plant species. The origin and evolution of weeds in different places have various geographical and ecological evidence. The majority of weeds species fall under angiosperms. In the terrestrial ecosystem, there are multiple weeds present all over the globe, which are very much problematic and are of great concern.

Among all the biotic components associated with economic loss in the agricultural sector, weeds are considered the most devastating. Terrestrial weeds, which are financial and ecological dangers in India and many different parts of the world, are *Parthenium hysterophorus, Lantana camara, Ageratum conyzoides, Galinsoga purviflora, Saccharum spontaneum, Argemone Mexicana etc.* In India, exotic weeds, especially *Parthenium hysterophorus* in urban areas, *Lantana camara* in forestlands, and *Ageratum conyzoides* in croplands, have assumed the proportion of noxious biological pollutants. *Parthenium hysterophorus,* (Fig 1.1) an outsider weed accepted to have been brought into India as a contaminant with PL 480 food grains imported from the USA two decades back. Currently, developing wild in numerous parts of India and world because of its wide adaptability, drought tolerance, and high seed generate on capacity and so on add to its quick presentation around the world, making agriculture and health dangers. Agriculturists are worried about terrestrial weeds influencing food and grain crops since the plant causes allergic contact dermatitis in people. A plant can produce 10,000 to 15,000 viable seeds, and these seeds can scatter and germinate to cover vast regions (Gunaseelan, 1987).

Fig. 1.1. *Parthenium hysterophorus*

The name *Lantana* is derived from the Latin Word Lento means to bend. The group *Lantana* as described by Linnaeus in 1753, consists of seven species, one species is from Ethiopia and the rest from South America. The *Lantana* genus is spread in approximately 50 countries, where several species are cultivated under hundreds of cultivar names. *Lantana camara* (Fig. 1.2), commonly known as wild or red sage, is one of the ten most noxious weeds globally, which is the most widespread species of this group. The species name *camara*, is probably adopted from the West Indian, an informal name for the common species (Ghisalberti, 2000; Sharma and Dawra, 1988). It has high regeneration potential and sexual reproduction throughout the year, which favors to their rapid growth in the area of their penetration. Due to its bushy nature and quick growth pattern *Lantana, camara* grows very dense on the floor of forest and plantation areas. Wildfire in many forest affluent parts of India and the world is also caused due to *Lantana* invasion (Sharma et al., 1988).

Fig. 1.2. *Lantana camara*

Ageratum conyzoides has been ranked as 19th worst weed of the world (Kohli et al., 2006), which grows in the tropical and subtropical regions; it appears like an instantly, branched, slim, busy, fragrant herb, which grows at the height of 1m, 4-10 cm long and 1-5 cm width (Fig. 1.3). Pleasant white hairs cover the whole stem and leaves. Flower color ranges from purple to white with fruits, consisting of lightweight seeds that can spread quickly. *Ageratum conyzoides* is also called goat weed (English), Visamustih (Sanskrit), Visadodi (Hindi), Uchunti (Bengali), Appa (Malayalam), Pumpillu (Tamil), and Nayitulasi (Kannada). The English name derives from the plant's curious smell, likened in Australia to a male goat (Palmer et al., 2019; Holm et al., 1977). The harvest of staple vegetation like rice, wheat, corn product has been reduced due to the aggressive conquering of *Ageratum conyzoides*. Due to the boom of the noxious weed inside the cultivated lands, the maintenance cost of the cropland extended (Kohli et al., 2006).

Fig. 1.3. *Ageratum conyzoides*

Various elimination and preventive measures are carried out to manage different terrestrial weeds. One of the primary techniques adopted in India to eradicate weed is manual hand weeding. However, it is labor-intensive and time-consuming at a large scale, and the biomass produced is disposed of as waste. Open disposal also facilitates seed germination and re-growth of the weed at the place of disposal. Anaerobic digestion is one way to manage this noxious weeds. It is also the sustainable conversion of energy, where exhaust of fossil fuel is one of the critical issues. There are very few literatures available related to the anaerobic digestion of terrestrial weeds. Anaerobic digestion is the process of biogas production, where particulate organic matter dissolved insoluble forms with the assistance of robust, mixed culture microbial communities in the absent oxygen, aiding in the conversion process of waste to energy (Abbasi et at., 1990). The substrates which contain high moisture or semi-solid are preferred for anaerobic digestion (Kondusamy and Kalamdhad, 2014). An increase of VFA up to a specific limit can lead to a drop in pH, inhibiting the growth of microorganisms, and high ammonia concentration is toxic for anaerobic bacteria (Koyama et al., 2014). Acidic pH can be maintained neutral by the addition of inoculums (Bhattacharya and Kumar, 2010). *Parthenium* can be done alternative feedstock for anaerobic digestion to produced methane (Gunaseelan, 1996). *Parthenium* has the potential to produce alcoholic biofuels after pretreatment (Singh et al., 2014). *Parthenium* content 23% lignin of its volatile solid so pretreatment is necessary to increase the biogas production (Gunaseelan, 1996). Pretreatment makes the substrate easier to microorganism for degrading, and it enhances the biogas production (Barua and Kalamdhad, 2016). Inoculum is the source of nutrients that boosts the enzyme activity leading to higher substrate degradation and biogas production. (Zhang et al., 2011). Manure of lives stock holds a high content of nitrogen like chicken manure (1.03%), fresh goat manure (1.01%), dairy manure (0.35%) and swine manure (0.24%) (Zhang et al., 2013). Therefore, when inoculums used along, there are chances of ammonia toxicity in anaerobic digestion. (Sawatdeenarunat, 2015). Appropriate inoculum was mixed with substrate together to maintain a proper C/N ratio. Maximum biogas can be obtained by maintaining a proper F/M ratio. Overall, dairy manure showed the best result (Dhamodharan et al., 2015).

There not much work has been done in anaerobic digestion of *Parthenium hysterophorous* and other weeds like *A.conyzoides*, *L.camara*. Limited works of literature were found on anaerobic digestion of terrestrial weeds, where also lacks significant study. Some studies were carried out on biogas production from terrestrial weeds on small scale (Barua and Kalamdhad, 2016). Studies showed that the initial characteristics of the noxious weeds had high moisture content and pH near to neutral making the weeds a potential candidate for biogas production. However, studies of the weeds on large scale biogas production has not been done. So more research is a necessity for anaerobic digestion of *Parthenium hysterophorous*, *L.camara* and *A.conyzyoides*.

1.2. Thesis organization

Chapter 1 consists of a brief introduction about the origin, spread and the problems associated with terrestrial weeds (*Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides*) its management through AD. This chapter also includes the objective, need of the study and scope for present work.

Chapter 2 deals with detailed literature reviews on anaerobic digestion, terrestrial weeds (Parthanium hysteroporus, Lantana camara and Ageratum conyzoides) inoculum. AD, pretreatment and co-digestion studies.

Chapter 3 deals with the knowledge gap, objective of the study, need for research, and scope of the present work.

Chapter 4 **is** about the materials and methods, the experimental procedure to determine parameters considering for anaerobic digestion, pretreatment technic and anaerobic co-digestion studies.

Chapter 5 deals with result and discussion of biochemical methane potential (BMP) of Parthanium *hysterophorus*, *Lantana camara and Ageratum conyzoides.*

Chapter 6 deals with result and discussion thermal pretreatment, electrohydrolysis pretreatment of *Parthanium hysterophorus, Lantana camara and Ageratum conyzoides.*

Chapter 7 deals with result and discussion of anaerobic co-digestion of *Parthanium hysterophorus, Lantana camara and Ageratum conyzoides.*

Chapter 8 focuses on the operation of two stages continuous reactor, the result, and discussion of the continuous reactor where terrestrial weeds were fed every day.

Chapter 9 focuses on identify bacterial commodities in the anaerobic digestion process.

Chapter 10 is all about the overall conclusion of the thesis and the scope for future work.

CHAPTER 2

LITERATURE REVIEW

This chapter deal with the literatures on terrestrial weeds, weed control and management practice in field problem, the problem associated with weeds**,** anaerobic digestion, inoculum, anaerobic co digestion and pre-treatments.

2.1. Weed

Crop-weed competition is one of the key factors for low harvest of agriculture product. Such kind of competition reduces the grain yield up to the extent of 32 % (Singh et al. 2007). *Parthenium* (*Parthenium hysterophorus* L.), *Lantana camara* and *Ageratum conyzoides* are in the list of the world's top twenty worst weeds, which are considered noxious terrestrial weeds because of its allelopathic effect. The allelopathy chemicals inhibit the germination, growth and metabolism of many species, including crops, weeds and vegetables. Allelopathy is an important mechanism of plant interference by the addition of plant-produced phytotoxins to the environment. Many of the phototoxic substances suspected of causing germination and growth inhibition have been identified from plant tissues and soil; these substances are termed allelochemicals. Allelochemistry, the production and release of toxic chemicals produced by one species that affect a receiving susceptible species, has been the subject of diverse degrees of scientific enquiry. Recent advances in plant biology have permitted the revamp of allelochemistry as a biologically and ecologically sound explanation for plant invasion and plant-plant communication in the rhizosphere. Recent progress has been made in understanding the biochemical and molecular changes induced by allelochemicals in susceptible plant species and the complex mechanisms used by allelochemical-resistant plants to defend against this toxic insult. Organizations engaged in invasion research defined invasive species as a species that is not native to the ecosystem under consideration whose introduction causes or is likely to cause economic, environment or human health (National Invasive Species Council, 2001). Species, subspecies or lower taxon, introduced outside its natural past or present distribution; includes any part, gametes, seeds, eggs, or propagates of such species that might survive and subsequently reproduce (Convention on Biological Diversity, 1992).

Animals, plants or other organisms introduced by man into places out of their natural range of distribution, where they established and disperse, negatively impact the local ecosystem and species (International Union for Conservation of Nature, 2000). An alien species whose introduction does or is likely to cause economic and environmental harm and harmful to animals and humans (Invasive Species Advisory Council, 2001).

2.1.1. *Parthenium hysterophorus*

Parthenium hysterophorus is considered one of the worst weeds in the world (Belgeri et al., 2015). The plant belongs to the plant family *Asteraceae*, commonly known as congress grass. Native to southern United States, Mexico and Central and South America, it has been accidentally introduced into several countries (Fig. 2.1) and has become a serious agricultural and rangeland weed in Australia, Asia, Africa and the Pacific Islands. In almost every part of India, this weed is prevailing. Wide adaptability, photo- and thermo-insensitivity, drought tolerance, intense competition and allelopathy, high seed production ability, the longevity of seeds in soil seed banks, and small and light seeds that are capable of long-distance travel via wind, water, birds, vehicles, farm machinery and other animal traffic, contribute to its rapid introduction worldwide, cutting across national boundaries and climate barriers. Due to its high fecundity, a single plant can produce 10,000 to 15,000 viable seeds, and these seeds can disperse and germinate to cover large areas. The allelopathic effect reduces crop production drastically. The biodiversity was also threatened by aggressive dominance by this noxious weed. Many allergic respiratory problems, contact dermatitis, mutagenicity in human and livestock are reported due to this weed (Patel, 2011). The allelopathic effect of this weed is evident in various experiments carried out over time. *Parthenium* weed can interfere with the growth of neighboring seedlings even at very early stages of growth (Belgeri et al., 2015). *Parthenium* extracts contain allelopathic effects, which could affect the seed germination and elongation of Onion and Bean demonstrated by an experiment carried out to evaluate the impact *of P. hysterophorus* on germination and elongation of Onion *(Allium cepa)* and Bean *(Phaseolus vulgaris)* (Demissie et al., 2013). A study carried out on the effect of *Parthenium* root extract on the germination and shoot growth of Maize and Barley it was reported that as the concentration of root extract increases, there is a decreasing trend in germination and shoot growth (Rashid et al., 2008). On germination, shoot length, root length, seed vigor, tolerance index, root length, shoot length, fresh and dry weight of bean seedlings are inhibited by the *Parthenium hysterophorus* leaf, stem and flower extracts (Tahseen et al., 2015). The aqueous extracts of root and shoot of *Parthenium* in higher concentration reduced Glycine max's germination percentage and growth (soyabean). This crop's productivity is adversely threatened by the *Parthenium* weed invasion (Netsere et al., 2011). *Parthenium hysterophorus* is a weed of worldwide impact (Navie et al., 1996); it presents over 20 countries in Africa, Asia and Oceania (Dhileepan and Strathie, 2009). On the African continent, *P.hysterophorus* arise from South Africa (Wood, 1897; Hilliard, 1977). Therefore, proper management with an integrated approach is a significant concern to control this invasive weed species.

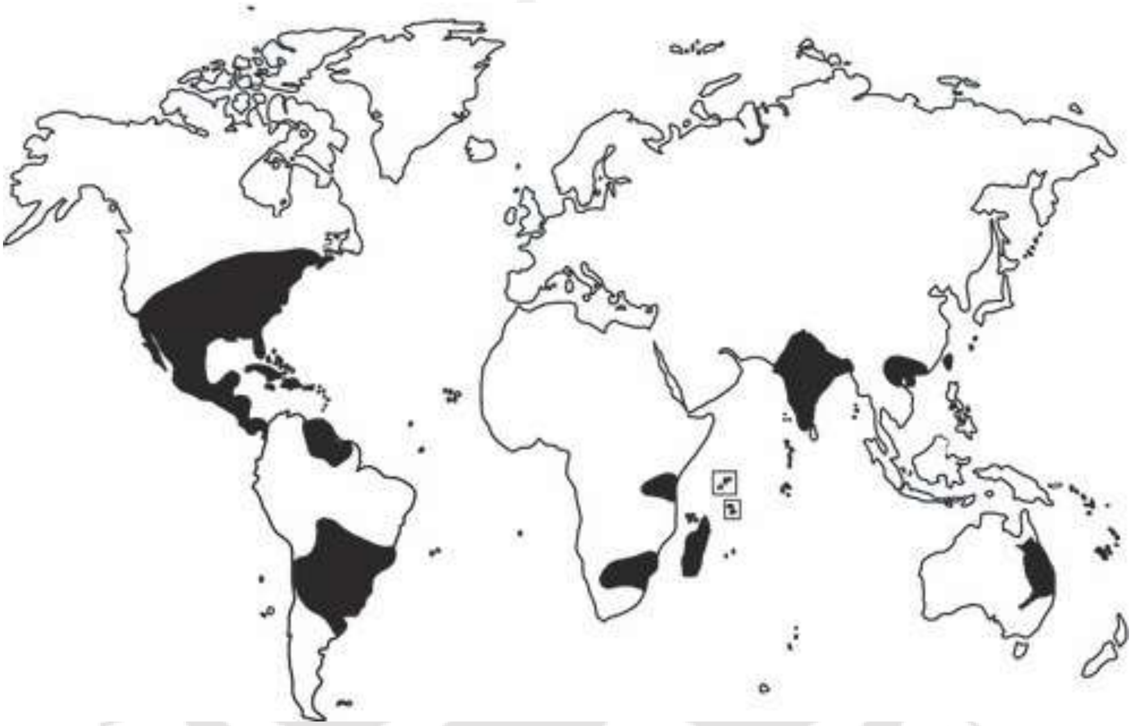


Fig. 2.1. Black colour area represent the distribution of *P. hysterophorus* (Adkins and Navie, 2006)

2.1.2 *Lantana camara*

Lantana camara was one of the ten worst weeds of the world belonging to the family Verbenaceae. *Lantana* forms huge dense thickets on the floor of forest and plantation areas due to its scrambling growth patterns and bushy nature, which hampers the forest's normal function and the growth and development of other underlying vegetation (Iyenger 1933, Singh et al., 1988). A native of Central and South America is an evergreen aromatic shrub (Raghubanshi et al., 2005). In India, it was introduced during 1809–1810 as an ornamental plant in Calcutta's gardens (Kohli et al., 2006). Now all over India, this weed is commonly found. More than 13.2 million ha pasture land and other areas in India had been invaded by *Lantana* (Singh et al., 1996). This weed has replaced *Quercus leucotrichphora* and *Pinus roxburghii* forests in Kumaun hills (U.P.) (Bhatt et al., 1994); invaded the Teak plantations in Tamil Nadu (Clarson & Sudha, 1997); covered Western Ghats (South India) (Muniappan and Viraktamath, 1993) and heartwater region of Garhwal (U.P.) (Rajwar, 1998). The cost of *Lantana* management is US$ 70 per hectare and is harmful to herbivores (Singh et al., 1996). The soil of *Lantana* invaded and non-invaded when analyzed edaphic factors such as soil pH, total Nitrogen, soil organic carbon, phosphorus, and potassium content positively influenced Lantana's growth helped in the further invasion process. *Lantana* invasion can not only significantly improve the soil nutrient level but also positively increase the chances of its further invasion with more copious plant attributes (Mandal et al., 2014). Due to fitness homeostasis, phenotypic plasticity, widespread geographical range, modes of reproduction, and ultimately none and the most potent, the phenomenon of allelopathy *Lantana* is highly invasive (Sharma et al., 2005). Some other allelochemicals identified in *Lantana camara* were cytotoxic in nature found in the leaves are lantadene A and lantadene B (Ma et al., 2004). In forests, due to allelopathy, *Lantana* density increases, due to which species richness declines (Day et al., 2003). Allelochemicals present in *Lantana* hampers the vigours of native plants of a particular region and ultimately results into low productivity (Sharma et al., 1988, Sharma and Sharma, 1989). Wildfire in many forest rich parts of India is also caused due to *Lantana* invasion (Raghubanshi et al., 2005). The stem, leaf and fruit leachates of *Lantana* inhibit seed germination and seedling growth of some terrestrial plants (Quan et al., 2009.). Due to high regeneration, potential, and sexual reproduction throughout the year favors their rapid colonization in the area of their penetration (Batish et al., 2004). The management of *Lantana* expansion in the forest and cultivable lands is a big challenge for the scientific community and policymakers (Suthar et al., 2013). The allelochemicals present in *Lantana camara* inhibit the process of seed and spore germination evident from the research on Seeds imbibed in aqueous extracts of leaf, stem and root of *Lantana camara* (Arpana Mishra, 2015). Many species of *Lantana* are native to Africa and America and had covered many of the neighboring countries (Day et al., 2003).

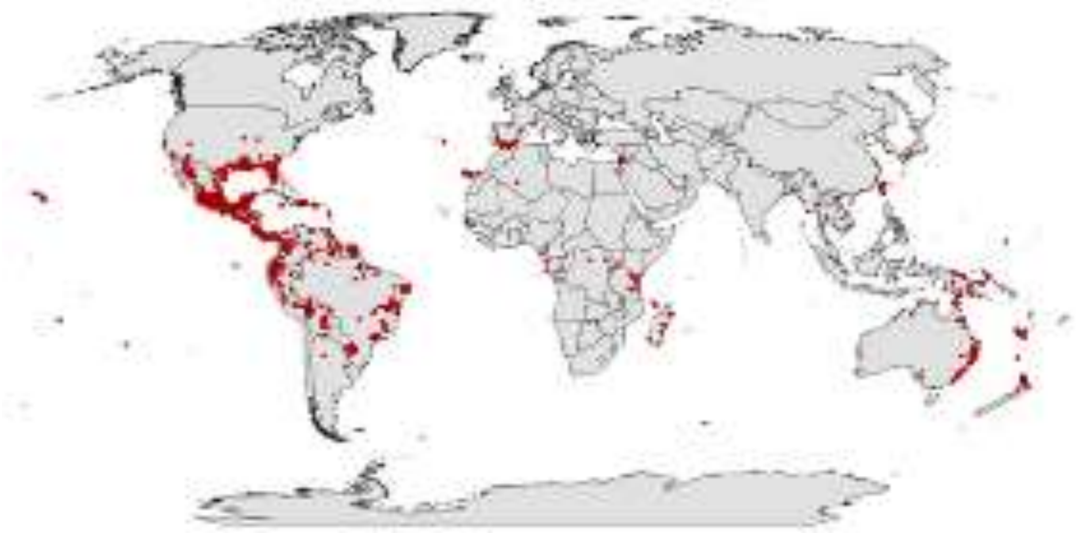


Fig. 2. 2. Red dots show existence records of *L. camara* (The current global spreading of *L. camara* taken from the Global Biodiversity Information Facility 2007)

2.1.3. *Ageratum conyzoides*

Ageratum conyzoides belongs to the plant family Asteraceae and is commonly known as billy goat weed or goat weed. It is widely distributed in the tropical and subtropical areas of the world. *Ageratum conyzoides,* a native of Central America and the Caribbean, were now found worldwide (Xuan et al., 2004) commonly in cultivated areas, pastureland, and road sides interfering with native plants. The plant is also found as a weed of over 36 crops (including plantations) in 46 different countries and has been ranked as 19th of the world's worst weeds (Holm et al., 1977). *Ageratum conyzoides*, a native of Central America and the Caribbean, were now found worldwide (Xuan et al., 2004). The special feature of *Asteraceae* plant family is that the flowers massed together in a head, a special kind of inflorescence having advantage to attract pollinators for cross-pollination that helps in increase in population in a short period. In addition, the parachute's effective seed dispersal mechanism like a special organ developed on the fruit, helps in the seeds' long-distance dispersal. The large seed-producing capacity helps this weed for rapid colonization and thus, it invades quickly. It makes a large number (8000–10000/plant) of fruits (achene) that have a pappus. Air, water and animals quickly disseminate these. The seeds are photoblastic and remain viable for one year (Kohli et al., 2006). These morphological characteristics are an advantage for easy dispersion of this plant species that leads to rapid invasion. Absence of natural predators and high reproductive potential attributes to a successful invasion. Coming to the morphology and physical characteristics, Goat weed is a common tropical annual herbaceous weed. It is an erect, softly yearly hairy plant, which grows up to a height of 2.5 feet. Oppositely, arranged leaves are ovate to lancelike, coarsely rounded, and have a toothed margin. Numerous pale blue or whitish flower heads are 6 mm across, often forming dense domed to flat-topped clusters in leaf axils or end of branches. It flowers most of the year (Batish, 2008; Batish et al., 2009 a & b). The stem is often red and has long white hairs. The weak aromatic unpleasant-smelling leaves are also covered with fine hair. The dark seeds have scales and ends in a needle-like shape. In alternative medicine, Goat weed is used against epilepsy and wounds, also used as an insect repellent (Okunade, 2002). The yield of staple crops like rice, wheat, corn, etc., has been diminished due to the invasion of *Ageratum.* When it invades rangeland areas, it out-competes for native grasses causing scarcity of fodder (Kohli et al., 2006). Due to hindrance in-field practices like plowing, croplands' maintenance cost is increased by the growth of these weeds (Kohli et al., 2006). In the lower Shivalik range of the Himalayas, due to infestation of cultivated lands, weed largely led to leaving farmers their agricultural fields (Kohli et al., 1996). A wide range of chemical compounds, including alkaloids, flavonoids, chromenes, benzofurans and terpenoids, have been isolated from this species. Extracts and metabolites from this plant have been found to possess pharmacological and insecticidal activities (Okunade, 2001). Both the volatile oil and the aqueous extract of the *A. conyzoides* have been shown to have allelopathic effects on several cultivated crops. Through volatilizing, leaching, and residue decomposion into the environment many kinds of allelochemicals are released by A conyzoides. The saturated aqueous solution of the isolated and purified precocene I and II have been reported to have a significant inhibitory effect on the seedling growth of radish, tomato and ryegrass (Kong et al. 2004). Its allelopathic potential varied with growth stages and environmental conditions. It releases more volatile allelochemicals (ageratochromene and its derivatives, monoterpenes, sesquiterpenes and flavones) under adverse conditions (Kong et al., 2004).

2.2. The problem associated with weeds

Parthenium competition increased with increased its dry mass up to 48% and relative competition index up to 52%. Consistent increases in the uptake of N (up to 81%), P (up to 70%) and K (up to 69%) were also recorded; competition period of *Parthenium* weed competition period is 35 decreased of grain yield and produced an index of maize (Safdar at al., 2016). Over the competitive displacement, it can cause up to 90% degeneration in the herbaceous constituent of natural plant communities where this weed invades (Mahadevappa, 1997). As the growth of *Parthenium* increased NPK-uptake was also increased, so this weed-crop competition with agriculture crops, it was observed that uptake of *Parthenium* during the different competition period at the range of 2.7–18.4, 0.2–2.4 and 2.3–17.7 N, P and K kg ha^{-1}, respectively at its different competition periods (Safdar at al., 2016*).* *Ageratum conyzoids* species had been informed to be one of the most overriding weeds of upland crops throughout South-east Asia (Kato Noguchi, 2001). *Lantana camara* is a significant problem in agricultural zones in most states in India as it forms dense thickets, spread openly, and disturbs equally flora and fauna. (Priyanka and Joshi, 2013). *Lantana camara* had been verified that it damaged the growth of various crops like wheat (Triticum aestivum), corn (Zea mays) and soybean (Glycine max) (Sharma et al., 1997). *Lantana camara* inhabits the land at the disbursement of several other plants (Hussain at al. 2015). Research gaps occur for humid forest zones, an over-all combination of primary forest and secondary vegetation scattered with covers cleared for cultivation or other non-forest practices (Bruijnzeel, 2004; Giambelluca, 2002). Although not much research had been done yet, more research is required to convert these notorious weeds into valuable products.

2.2.1 Weeds control and management practice in the field

Numerous preventions and elimination strategies such as cultural, mechanical, biological, chemical etc were adopted for weed control. Different cultural practices such as mulching, using transplants, tillage, drip irrigation, rapid cleanup after harvest etc., reduces weed numbers but cannot eliminate the weed. Stale seedbed techniques are to prepare the soil for planting and bring weed seeds to the surface, allows weeds to germinate and kills weeds with light tillage. However, after this method also, there will be a residual amount of weed biomass. Cover crops may reduce weed emergence by 75 -90%, but still, there is a remnant of weeds. Weeds tend to infest crops having a similar life cycle. Changing cultural practices related to the cultivation process, use of fertilizers and herbicide application may lead to management of weed but to eradicate weeds ultimately is beyond possible. Physical and mechanical practices also used like mourning, cultivation, soil solarization, Hand weeding, flaming. However, these processes have a cost-benefit impact. There are various adverse effects of all these processes. Cultivation is a better practice for perennial and biennial control than annual weed control. Some of the methods require month-long treatment processes. The different drawbacks of cultivation processes are that it exposes the bare ground, leads to soil compaction, and requires expensive equipment. It cannot be processed in a wet condition. Flaming is another process to knock down weed seedlings before planting, and just before crop germination, flaming is used to kill weed seedlings. Nevertheless, all of these processes lead to the generation of weed biomass, ultimately going to the waste stream. There is no proper method for the disposal of these weeds. Biological control is also practiced at various times. California's most pervasive weed, yellow starthistle, have been reported to be controlled by six species of overseas insects (Balciunas et al., 1999). As conventional weed management practices are unsuccessful for the weed *lythrum salicaria*, a biological control by boring root weevil *Hylobius transversovittatus* (highly host specific to the target weed have been approved in 1992 (Blossey et al., 1994). Though biological control attempts have been made on *L. camara* longer than on any other weed, the plant is still not under adequate control. As it is a hybrid species consisting of many phenotypes, originating from two or more *Lantana* species in tropical America and that it grows in a wide range of climatic areas that influence the biocontrol of this plant (Day et. al., 2000). However, weed biocontrol can cause a severe problem due to the agent's attack on non-target plant species. Therefore, it is essential to protect non-target species. Host specificity is required before approving the release of agents. There is various evidence in the history of unforeseen interaction of an agent with non-target plants. The chemical methods used to kill weeds using various weedicides have various environmental consequences. The soil fertility is lost due to repeated application of chemicals and using these chemicals in agricultural fields, the trances of these chemicals enter the food chain. The results of hand weeding are significantly better, but as it is time-consuming and laborious to humans, it cannot be recommended at a large scale (Hussain et al., 2008). The application of herbicides is expected today and is used on a wide scale. Nevertheless, the concern is directed towards its impact on non-target organisms and the ultimate fate of these chemicals on our environment. Even finding small traces of these elements on our background is a significant threat. The residual of these elements in the entire ecosystem leads to unsustainable conditions, especially the aquatic system, where the drainage flows from areas treated with herbicides. The prevention of water contamination is the utmost concern for humankind and the whole ecosystem. There are various data regarding the toxicity of weedicides to fish are available (Mullison, 1970).

2.2.2 Vermicomposting, an alternative viable option

The experiments regarding vermicomposting of various terrestrial weeds are carried out at different times. Many of these weeds result in the preparation of good quality compost inferred by other researchers. The experiments were conducted to obtain compost from some toxic weeds by using vermicomposting and conventional methods. The weeds used in the experiment were congress grass *(Parthenium hysterophorus*), water hyacinth (*Eichhornia crassipes*) and bhang *(Cannabis sativa Linn.).* Complete six sets of experiments were setup by using the above materials. The results show a high increase in nitrogen, potassium, phosphorus and a high decrease in organic carbon, C/N, and C/P ratio in the experiment with *Eisenia fetida* (Chauhan, 2010). There some work has been done on Vermicomposting of terrestrial weeds reported in Table 2.1.

Table 2.1. Work done on vermicomposting and composting of different terrestrial weeds

Author	**Work done**
Biradra et al., 2001	Vermicomposting of *Pathenium hystrophorous*
Sivakumar et.al, 2009	*Parthenium* compost and neem compost
Kishor et al., 2010	Observe the Parthenium compost application.
Chauhan et al., 2010	Compost of some toxic weeds by vermicomposting and conventional methods
Yadav et al., 2011	Vermicomposting of *Parthenium hysterophorus*
Reddy et al., 2012	Application of *Parthenium hysterophorus* vermicompost
Suthar et al., 2013	Vermicomposting of *Lantana camara*
Anbalagan et al., 2012	Vermicomposting of *Parthenium hysterophorus*
Rajiv et al., 2013	Toxicity of the terrestrial weeds
Suthar et al., 2013	Vermicomposting of *Lantana camara*

Rajiv et at., 2013	Germination Index was determined with *Lantana camara* mediated Vermi compost.
Sivaraj et al., 2013	Composting and vermicomposting of *Parthenium hysterophorus*
Hussain et al. 2015	Compositional or biochemical study *Parthenium hysterophorus* vermicompost
Mistry et al., 2015	Vermicomposting of various terrestrial weeds

Vermicomposting with *Eisenia fetida* of *Parthenium hysterophorus* mixed with cow dung in different ratios (25, 50 and 75%) in an 18 weeks' experiment shows that in all the treatments, a decrease in pH and C: N ratio, but increase in electrical conductivity (EC), N total, P available, Ca total, K total and heavy metals was recorded. The cocoon's production and growth rate was maximum in 100% cow dung. The results indicated that *Parthenium* could be a raw material for vermicomposting if mix with cow dung in appropriate quantity (Yadav et al., 2011). The macronutrients (N, P and K) and micronutrients were increased in *Parthenium* mediated vermicompost and decreased in compost of *Parthenium* mediated compost. *Parthenium* can be utilized effectively as organic manure by composting and vermicomposting and thereby control this weed (Sivaraj et al., 2013). The leaves of Neem and *Parthenium* were composted in an experimental set-up with Earthworm and without worm The result shows that higher *Parthenium* concentration reduced the growth and reproduction of *Eisenia fetida* (Earthworm species). Among *Parthenium* compost and neem compost, significant difference was not observed. (Sivakumar et.al, 2009). In various experiments, *Lantana camara* has been used by researchers for the production of vermicompost. The germination index (GI) was between 45% and 83% in all vermicompost as indicated by the seed bioassay test (Suthar et al., 2013). Some weed species' suitability in vermicomposting production was evaluated in an experiment conducted in Bijapur, Karnataka. *Parthenium hysterophorus* was reported to be a superior substrate for vermicomposting as clitellate, and non-clitellate worm's biomass were higher per bed compared to other weeds (Biradar et al., 2001). The *Azotobacter* microflora reported to be more in *Parthenium hysterophorus* mediated vermicomposting. The yield of vermicompost is more, predicted due to presence of some important microbes that help the earthworms to act faster on the weeds (Nirmalnath et al.2005). Composting of uprooted *Parthenium* can be a good option to stop its invasion and inhibit the growth of the weed. The high component of essential elements in compost from *Parthenium* increases the crop yield (Kishor et al., 2010). It was observed that *Parthenium hysterophorus* mixed with other organic supplements imparts suitable Physico-chemical conditions for maximum worm production and large scale vermicompost production. Future research is required to explore the utilization of *Parthenium hysterophorus* in vermicompost production to enhance crop yield (Anbalagan et al., 2012). *Parthenium hysterophorus* can be used as an additive for the effective vermicomposting process of sludge. A maximum rate of 80% germination of tomato seeds was observed in *Parthenium* and sludge mediated vermin extract (Reddy et al., 2012). The combination of *Parthenium* and cow dung enhanced the compost's nutrient value and increased the germination of *Arachis hupogeae*. The toxicity of allelochemicals could be minimized through compost (Rajiv et al., 2013). When *Parthenium* mediated vermicompost was analyzed with vermicompost produced from another weed *Argemone Mexicana*, Nitrogen's higher concentration was seen in *Parthenium* vermicompost than other weed. Overall NPK value is also observed in higher concentrations in both the weeds (Mistry et al., 2015). Various physical, chemical and biochemical characteristics were tested; the vermicomposting seemed to be plant-friendly, giving the finest results after applied at concentrations of 1.5% in soil (w/w) (Hussain et al. 2015). In a similar way, the experiments can be carried out with other terrestrial weeds, which are major constituents of agricultural land and available in another land cover. The significance of vermicompost prepared by these terrestrial weeds can be analyzed and can be initiated on a large scale. There are so many terrestrial weeds and dominating invasive species. The compost prepared out of it can be used as organic fertilizer and soil conditioner.

Cultivation practices such as mulching, shifting cultivation, drip irrigation, rapid clean up after harvest, etc., help control the weed population but cannot eliminate the weed. The use of fertilizers and herbicide application may lead to weed management, but to eradicate weeds completely is beyond possible and costly. Flaming is another process in which cultivated land is flamed up to burn all seeds present in it; this practice increases environmental pollution and loss of biomass. Various experiments were carried out to properly utilize biomass (weeds); vermicomposting is one of them.

2.2.3. Anaerobic digestion a possible management option

Anaerobic digestion is another way to manage these noxious weeds and is also the sustainable conversion of energy, where fossil fuel depletion is one of the key issues. Little work has been done related to the anaerobic digestion of terrestrial weeds. Anaerobic digestion is the process of biogas production, where particulate organic matter dissolved insoluble forms with the assistance of robust, mixed culture microbial communities in the absence of oxygen; aiding in the conversion processaste to energy (Abbasi et at., 1990) . The substrates which contain high moisture or semi-solid are preferred for anaerobic digestion (Kondusamy and Kalamdhad, 2014). Increase of VFA can lead to a drop in pH inhibiting the growth of microorganisms, and high ammonia concentration is toxic for anaerobic bacteria (Koyama et al., 2014). The higher the COD recovery higher the biogas. Acidic pH can be maintained neutral by the addition of inoculums (Bhattacharya and Kumar, 2010). *Parthenium* can be done alternative feed stock for anaerobic digestion to produced methane (Gunaseelan, 1996). *Parthenium* could grow to produce alcoholic biofuels after pre-treatment (Singh et al., 2014). *Parthenium* contains 23% lignin of its volatile solid, so pre-treatment is necessary to increase the biogas production. (Gunaseelan, 1996). Pre-treatment makes the substrate easier to microorganism for degrading, and enhances biogas production (Barua and Kalamdhad, 2016). Inoculum is the source of nutrients that boosts the enzyme activity leading to higher substrate degradation and biogas production. (Zhang et al., 2011). Manure of lives stock holds a high contains of nitrogen like chicken manure (1.03%), fresh goat manure (1.01%), dairy manure (0.35%) and swine manure (0.24%) (Zhang et al., 2013). Therefore, when inoculums are used, there is chances of ammonia toxicity in anaerobic digestion. (Sawatdeenarunat, 2015). To maintain proper C/N ratio, inoculums are mixed with substrate it yield higher biogas production. Overall dairy manure shows, the best result (Dhamodharan et al., 2015). *L.camara* was practice for ethanol production suggested by (Sharma et al. 1988). There was not much work done in anaerobic digestion of *Parthenium hysterophorus* and other weeds like *A. conyzoides*. S. *spountaenium, L.camara.* Initial characterization shows this noxious weeds have the potential to produce biogas as there is high moisture contain and pH is nearest to 7. Soluble chemical oxygen demand is also high, which is beneficial for high methane production. (Barua and Kalamdhad, 2016). It has been studied on a small scale, but a large-scale study has not been done. All though on a smaller scale, it shows 59% methane production. More research is necessary for the digestion of *Parthenium hysterophorous, A.conyzoides, S.spountaenium,* and *L.camara* on a continuous and pilot scale.

2.3 Anaerobic digestion study

Anaerobic digestion was brought into the spotlight of scientific researches initially in the early 1970s after the first energy crisis stuck in at period. Afterward, it was accepted and took a significant growth to which it is now considered as a matured technology. The scientific community is looking at it as a solution to end our dependency on fossil fuels. Anaerobic digestion is a series of biological processes in which microorganisms decompose biodegradable materials in Oxygen's absence. One of the end products is biogas, which mainly consists of CO_2 and CH_4. Methane thus produced can be utilized as a source of renewable energy. The advantages of using anaerobic digestion of terrestrial weed biomass to generate biomethane are many (a) it can help capture and combust methane, thus minimizes threats posed by it towards climate change, (b) Reduce dependency on fossil fuels, (c) Generation of energy, (d) Conservation of biodiversity, (e) Fight against invasive weeds, (f) Protection of crops and crop yield enhancement. (Gupta et al., 2012).

Gliricidia maculata is a tree grown in India for green leaf manuring. The digestibility of Gliricidia leaves for biogas production was determined in 3 litre batch digesters at room temperature (32±3 °C). Results indicate a gas yield of 165–180 ml CH_4 g^{-1} VS added and a VS reduction of 37–39%. Determination of the N, P, K content of the digester influent and effluent slurries indicates that the anaerobically digested slurry of the *Gliricidia* leaves is better in quality than the fresh *Gliricidia* leaves as organic manure (Gunaseelan, 1988) *Eupatorium odoratum L.* is a prolific producer of biomass among the weeds introduced into India and it can be used for energy production. Since freshly harvested biomass contains inhibitors of microorganisms involved in methanogenesis, the effects of leaching and partial aerobic decomposition of the weed before anaerobic digestion were studied (1·0 m^3 pilot-scale batch fermenters) for biogas production. The pretreated waste produced about 70% more biogas. It also gave a higher count of cellulolytic and methanogenic bacteria than the untreated material (Jagadeesh et al., 1990). The waste banana stem has a high organic content (83%); with 15–20% (w/w) lignin and cellulose, which gives it a sheath-like texture. Banana stem slurries (BSS) at 2–16% total solids (TS) concentration were anaerobically digested under mesophilic (37–40°C) as well as thermophilic conditions (50–55°C) in batch culture. The final biogas yields, 267–271 l/kg TS fed, were observed with 2–4% TS slurries under mesophilic conditions. The biogas yields in the thermophilic range, 212–229 l/kg TS fed, were found with 2–8% TS slurries. However, thermophilic digestion rates were 2.4 times faster than mesophilic. Methane accounted for 59–79% of the total biogas. Methane yield was maximum at 2% TS BSS in both the temperature ranges. The process led to 45–50% reduction in organic solids and 40–55% reduction in COD. With 16% TS BSS inhibition resulted in 50–60% loss in biomethanation process efficiency (Kalia and Sonakya, 2000). *Pistia stratiotes*, an aquatic weed, was investigated as a substrate for biogas production in batch digestion. An inoculum was necessary to obtain biogas production from the weed. With *Pistia* only, production of carbon dioxide alone was high during the first five days of digestion but began to level off after that. With inoculated Pistia, a high rate of biogas production was sustained for nearly 10 days, and the average methane content was 58–68%.

The digesters charged with *Pistia* alone had significant concentrations of propionic, butyric, isobutyric, valeric, and isovaleric acids. These acids were not present in detectable concentrations in the digesters running with inoculated *Pistia*, except during the first 4 days of the digestion when propionic acid was formed. When inoculum was added to a digester, the latter's performance improved dramatically (Abbasi and Nippaney, 1991). High livestock density is always accompanied by the production of a surplus of animal manure, representing a considerable pollution threat for the environment in these areas. Avoiding over-fertilization is not only important for environmental protection reasons but also for economic reasons. Intensive animal production areas need proper manure management, aiming to export and redistribute the excess of nutrients from manure and optimize recycling. Anaerobic digestion of animal manure and slurries offers several benefits by improving their fertilizer qualities, reducing odors and pathogens and producing a renewable fuel –biogas. The EU policies concerning renewable energy systems (RES) have set forward a fixed goal of supplying 20% of the European energy demands from RES by the year 2020. A major part of renewable energy will originate from European farming and forestry. At least 25% of all bioenergy in the future can originate from biogas produced from wet organic materials such as animal manure, whole crop silages, wet food and feed wastes (Holm-Nielsen and Seadi, 2006). Biogas and methane yields of food and green wastes and their mixture were determined using batch anaerobic digesters at mesophilic (35 ± 2 °C) and thermophilic (50 ± 2 °C) temperatures. The mixture was composed of 50% food waste and 50% green waste, based on the volatile solids (VS) initially added to the reactors. The thermophilic digestion tests were performed with four different feed to inoculum (F/I) ratios (i.e., 1.6, 3.1, 4.0 and 5.0) and the mesophilic digestion was conducted at one F/I (3.1). The results showed that the F/I significantly affected the biogas production rate. At four F/Is tested, after 25 days of thermophilic digestion, the biogas yield was determined to be 778, 742, 784 and 396 mL/g VS for food waste, respectively; 631, 529, 524 and 407 mL/g VS for green waste, respectively; and 716, 613, 671 and 555 mL/g VS for the mixture, respectively. About 80% of the biogas production was obtained during the first 10 days of digestion. At the F/I of 3.1, the biogas and methane yields from mesophilic digestion of food waste, green waste and their mixture were lower than the yields obtained at thermophilic temperature. The biogas yields were 430, 372 and 358 mL/g VS, respectively, and the methane yields were 245, 206, and 185 mL/g VS, respectively (Liu et al., 2009). Grassland biomass is suitable in numerous ways for producing energy. It is well established as feedstock for biogas production. This review aims to summarize current knowledge on the suitability and sustainability of grassland biomass for anaerobic digestion. In the first section, grassland management for biogas feedstock and specifics of harvest, postharvest and digestion technology are described. Many factors influence methane yields from the grass. While the effects of some parameters such as grass species, cutting period, and management intensity can be regarded as well known, other parameters such as preservation and processing still need investigation. In the second section, economic aspects and environmental impacts are discussed. Profitability can be achieved depending on grass silage supply costs and the concept of anaerobic digestion and energy use. Grassland biomass for biogas production competes with other feedstock and other grassland use forms, particularly animal husbandry. In developed countries, growing milk and meat production is achieved with decreasing ruminant numbers, resulting in an increasing amount of surplus grassland with a remarkable bioenergy potential. In emerging and developing countries, a rapidly rising demand for milk and meat production increases grasslands' growing pressure. Their use for animal feed presumably will take priority overuse of bioenergy. Grasslands provide various essential environmental benefits such as carbon storage, habitat function, preservation of ground and surface water quality. When producing biogas from grassland, these benefits will remain or even grow, providing appropriate grassland management is implemented. In particular, greenhouse gas emissions can be considerably reduced (Prochnow et al., 2009). Kalia and Kanwar (1990) studied the feasibility of fermentation, particularly of *Ageratum conyzoides* biomass. However, no comprehensive study was carried out, and attempts were made to enhance the production with pre-treatment analysis and BMP tests. Apart from this, the literature on *Ageratum* biomass mostly emphasizes the medicinal benefits out of this plant extract.

Table 2.2. Literature survey on BMP for similar feedstock

Sl. No	Reference	Weed/Substrate	Content
1.	Nallathambi Gunaseelan (1988)	*Gliricidia* Leaves	3L batch digesters used at room temperature. Biogas yield obtained -165-180 ml CH_4/g VS. VS reduction 37-39%.
2.	Kalia and Kanwar (1990)	*Ageratum conyzoides* (Billy goat weed)	Major part of gas production was obtained up to 4th week of fermentation. Methane content of *Ageratum* mixtures 62-77%.
3.	Nallathambi – Gunaseelan (1995)	*Parthenium*	Effect of inoculum/substrate ratio. HCl and NaOH pre-treatment 152 ± 15 ml CH_4 /g VS.
4.	Okunade (2002)	*Ageratum conyzoides*	Potential use of *A.conyzoides* in pharmaceutics. Pharmacological and insecticidal activities.
5.	Wall et al., (2013)	*Grass silage*	A biomethane potential (BMP)

			assessment of grass silage yielded 107 m^3 CH_4 T^{-1}. Economic evaluation: 1.1% of grasslands in Ireland can generate 10% renewable energy in transport
6.	Dahunsi et al., (2017)	*Tithonia diversifolia (Wild Mexican Sunflower)*	Optimized for temperature, pH, retention time, TS & VS Biogas yields were 2249.24, 1519.15 and 1043.50. 10^{-3} m^{-3}/kg TS CH_4 composition is 65 ± 1 of biogas
7.	Barua and Kalamdhad (2017)	*Water hyacinth with cow dung*	Comparative study between untreated and pretreated aquatic biomass for biogas production. Ideal F/M ratio for maximum methane yield 1.5. Methane yield 193 ± 22 mL CH_4 /g VS.

2.4 Anaerobic digestion process and biochemical reaction

AD is a biological treatment process used for all kinds of biodegradable waste materials, which includes biomass. In anaerobic process, degradation and stabilisation of an organic material takes place under anaerobic conditions, which reduces natural methane emission from landfills and from open environment (Kelleher et al., 2002; Rutz and Janssen, 2007). AD processes is classified based on operating parameters such as operating temperature, reactor design, process continuity (batch versus continuous) and solid content (dry or wet). AD process operates at three different temperatures psychrophilic (-20 to +15 ^{o}C), mesophilic (20 to 45 ^{o}C) and thermophilic (41 to 121 ^{o}C) (Li et al., 2011). The end products of AD is biogas which includes mainly of methane (CH_4≈50-75%), carbon dioxide (CO_2≈25-50% by volume) and small trace gasses such as hydrogen sulphate (H_2S), hydrogen (H_2), Nitrogen (N_2), oxygen (O_2), carbon monoxide (CO) and water vapors. Fig. 2.3 shows the steps involved in anaerobic digestion process.

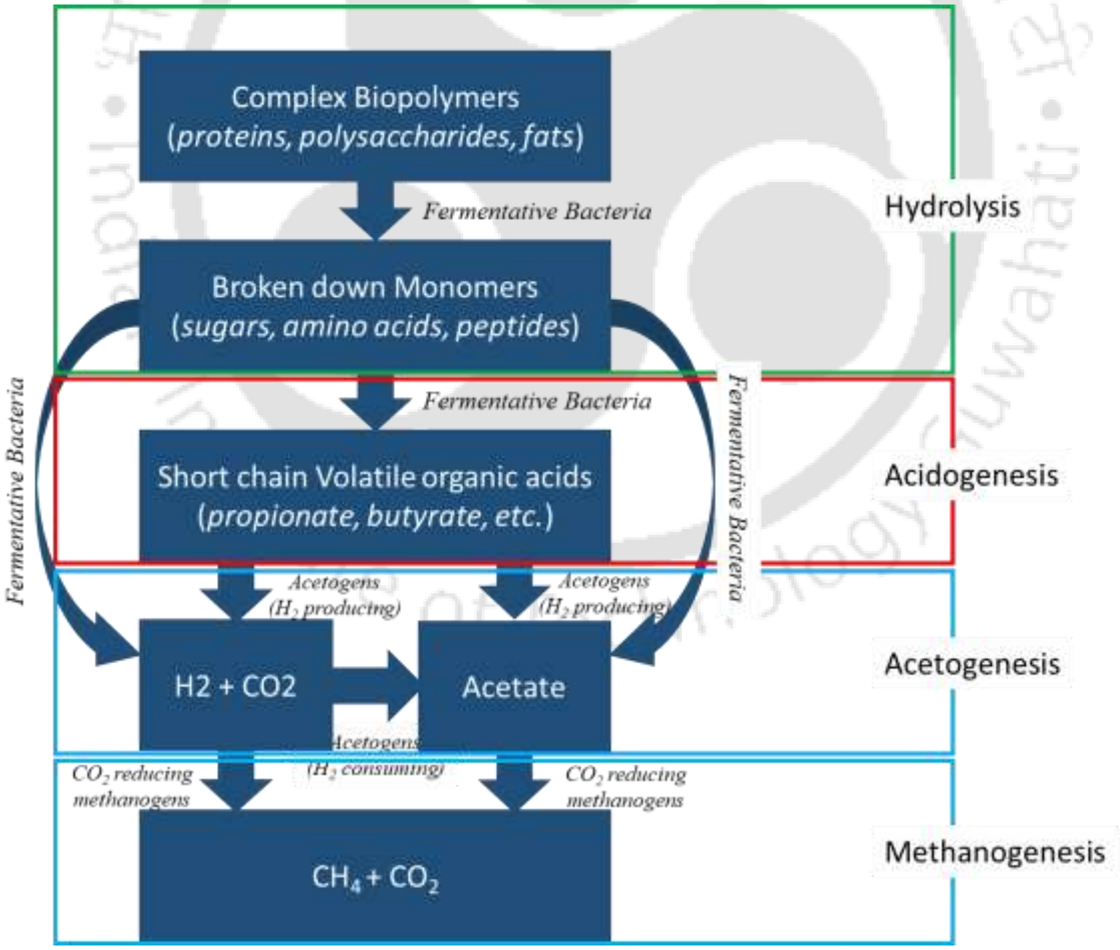


Fig. 2.3. Steps involved in Anaerobic Digestion process

2.4.1 Hydrolysis

AD process consists of four steps called hydrolysis, acidogenesis, acetogenesis (dehydrogenation) and methanation (Weiland, 2010). Hydrolysis is the first step in AD process, which consists of hydrolytic enzymes; this enzyme breaks down polymeric substances, i.e., lipids, polysaccharides, proteins etc., into more biodegradable organic compounds such as amino acids, long-chain fatty acids, simple sugars etc. (Yang et al., 2010).

In general, hydrolysis is a chemical reaction in which water breakdown occurs to form H^+ cations and OH^- anions. Hydrolysis is often used to break down larger polymers, often in the presence of an acidic catalyst. In anaerobic digestion, hydrolysis is the essential first step, as Biomass usually is comprised of very large organic polymers, which are otherwise unusable. These large polymers, namely proteins, fats, and carbohydrates, are broken down into smaller molecules such as amino acids, fatty acids, and simple sugars through hydrolysis. While some of the products of hydrolysis, including hydrogen and acetate, may be used by methanogens later in the anaerobic digestion process, the majority of the molecules, which are still relatively large, must be further broken down in the process of acidogenesis so that they may be used to generate methane.

$$\mathrm{nC_6H_{10}O_5 + nH_2O \xrightarrow{\text{hydrolysis}} nC_6H_{12}O_6} \tag{2.1}$$

2.4.2 Acidogenesis

Acidogenesis is the next step of anaerobic digestion in which acidogenic microorganisms further break down the biomass products after hydrolysis. These fermentative bacteria produce an acidic environment in the digestive tank while creating ammonia, H_2, CO_2, H_2S, shorter volatile fatty acids, carbonic acids, alcohols, as well as trace amounts of other byproducts. While acidogenic bacteria further breaks down the organic matter, it is still too large and unusable for the ultimate goal of methane production, so the biomass must next undergo the process of acetogenesis. In the acidogenesis stage, fermentative bacteria convert hydrolysed products into VFA and other minor products such as alcohol, aldehydes, and trace gases (CO_2, H_2, NH_3) by degrading organic matter up to pH of 4.

$$\mathrm{nC_6H_{12}O_6 \xrightarrow{\text{Acid formation bacteria}} 3nCH_3COOH} \tag{2.2}$$

2.4.3 Acetogenesis

In general, acetogenesis is the creation of acetate, a derivative of acetic acid, from carbon and energy sources by acetogens. These microorganisms catabolize many of the products created in acidogenesis into acetic acid, CO_2 and H_2. Acetogens break down the Biomass to a point to which Methanogens can utilize much of the remaining material to create methane as a Biofuel. The next step of AD is acetogenesis where acetogenic bacteria further converts VFA to acetate, carbon dioxide and hydrogen which act as direct substrate to methanogenesis.

$$CH_3COOH \xrightarrow{\text{Methane formation bacteria}} CH_4 + CO_2 \tag{2.3}$$

$$CO_2 + 4H_2 \xrightarrow{\text{Reduction}} CH_4 + 2H_2O \tag{2.4}$$

2.4.4 Methanogenesis

Methanogenesis constitutes the final stage of anaerobic digestion in which methanogens create methane from the final products of acetogenesis and some of the intermediate products from hydrolysis and acidogenesis. There are two general pathways involving acetic acid and carbon dioxide, the two main products of the first three steps of anaerobic digestion, to create methane in methanogenesis.

While CO_2 can be converted into methane and water through the reaction, the main mechanism to create methane in methanogenesis is the path involving acetic acid. This path creates methane and CO_2, the two main products of anaerobic digestion. This final step of AD is methanogenesis, where a methanogenic bacterium produces methane, carbon dioxide, and small trace gases.

Similarly, CO_2 is hydrolyzed to carbonic acid, which is reduced to methane.

$$CO_2 + H_2O \xrightarrow{\text{Hydrolysis}} H_3CO_3 \tag{2.5}$$

$$4H_2 + H_2CO_3 \xrightarrow{\text{Reduction}} CH_4 + 3H_2O \tag{2.6}$$

Equations (2.1 - 2.6) gives all the reactions undergoes in AD process (Kondusamy and Kalamdhad, 2014).

2.5 Inoculum

Biogas can be produced with almost all kinds of biological feedstock present. The largest resource of feedstock is represented by animal slurries of cattle and pig production units, poultry, fish, etc. (Holm-Nielsen et al., 2009). Miao et al., (2014) concluded that poultry litter from broilers mixed with an optimum proportion of cow dung was found to be a substrate with a high potential for biogas generation by AD. Masses et al. (2015) reported that cow dung is feasible in batch reactor at organic loading rate (OLR) of 9.0 and 12.0 g total chemical oxygen demand (TCOD)/kg inoculum d^{-1} with average specific methane yields of 154.0–116.0 mL CH_4 kg^{-1} VS fed during treatment cycle length of 21 days. Saha et. al., (2018) carried out anaerobic digestion of *Ageratum conyzoides* biomass using cow dung as inoculum and reported that *Ageratum conyzoide* mixed with cow dung produces 80% of the total biogas within the first 30 days. Veluchamy and Kalamdhad (2017) reported that Pulp and paper mill sludge anaerobically digested with cow dung in different mixing ratios produced the highest cumulative methane yield of 3.4 L in F/M ratio of 2.0 followed by 3.3 L and 2.9 L in F/M ratio of 1.5 and 2.5 respectively and concluded that cow dung act as the active inoculum that increased methane yield. Cow dung was the best inoculum (Dhamodharan and Kalamdhad, 2015) (Table 2.3).

Table 2.3. Initial characterization of different livestock inoculum. (Dhamodharan and Kalamdhad, 2015)

Parameters	**Livestock inoculum**				
	Goat dung	**Poultry dung**	**Cow dung**	**Rhinoceros dung**	**Piggery dung**
MC (%)	45.7 ± 0.6	78.42 ± 0.8	79.8 ± 2.3	80.29 ± 0.4	72.23 ± 1.6
TS (%)	55.1 ± 1.5	21.6 ± 0.9	20.19 ± 1.4	19.7 ± 1.2	26.7 ± 1.8
VS (%)	39.2 ± 0.9	16.2 ± 0.5	15.25 ± 1.1	15.62 ± 0.8	22.18 ± 1.3
pH	7.35–7.51	6.53–6.63	7.05–7.25	6.60–6.74	6.52–6.94
sCOD (g/L)	34.3 ± 4.7	22.5 ± 3.9	21.6 ± 4.8	13.5 ± 4.1	23.3 ± 3.6
TKN (g/L)	3.9 ± 0.1	3.0 ± 0.2	5.3 ± 0.6	3.2 ± 0.3	3.1 ± 0.5

2.6 Pre-treatment

Pre-treatment makes the substrate effortlessly degradable and reduced hydrolysis step before being introduced into the digester. The lignocellulosic complex is the challenging part for biogas production (Barua and Kalamdhad, 2016). To overcome these challenges, pre-treatment is necessary. Lignin present in terrestrial weeds is very high as compared to other feedstock, which reduced methane production. Cellulose is a portion of food for microbes; pre-treatment makes the cellulose readily available to microbes. In anaerobic digestion, hydrolysis is the rate-limiting (Khanal, 2008). Therefore, pre-treatment must be done to speed up the hydrolysis process and simultaneously increase the performance of the anaerobic digestion. Several researchers found, pre-treatment enhancing the digestibility through physical and chemical approaches for improving the biogas yield in a reduced time interval.

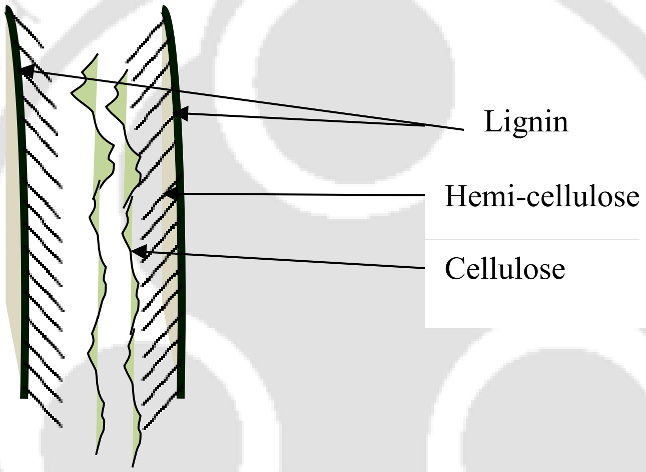
2.6.1 Substrate recalcitrance

Pre-treatment is an additional step applied before anaerobic digestion to improve the digestibility of the substrate. The substrates used for anaerobic digestion can be categorized as organic waste such as municipal solid waste, wastewater sludge, agricultural residues, and animal manure etc.; crop residues, which include rice straw, maize straw, banana stem etc. and non-conventional biomass like macroalgae, microalgae etc. (Karuppiah & Azariah, 2019). The AD process has been a well-developed technique to produce alternative and sustainable energy over the last two decades. However, improving methane yield is still heavily a challenging factor and studied as a research topic in academic and industrial areas (Atelge et. al., 2020). Among the four stages of anaerobic digestion, hydrolysis is the rate-limiting step for these feedstocks; therefore, it is necessary to overcome the recalcitrant nature and make them accessible to the microorganisms for effective biogas production (Patinvoh et. al., 2017).

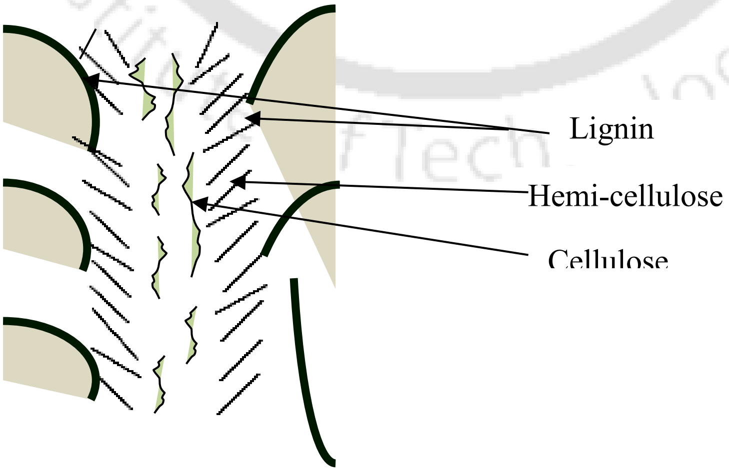
Lignocellulosic biomass is the most abundant renewable biological resource on earth, with a yearly production of 200 billion tons (Zhang, 2008). In the plant cell wall, lignocellulose is the main building block. It consists of a defensive inner structure, which has contributed to the hydrolytic stability and structural robustness of the plant cell walls and its resistance to microbial degradation (Lee et. al., 2014). The main motto of pre-treatment for the lignocellulosic substrate is to break down the lignin structure so that microorganisms get cellulose and hemicellulose easily for digestion. In addition, the crystallinity of cellulose is also gets decreased, and the porosity of the substrate is increased with pre-treatment. Various pretreatment method was applied to reduced the lignin content from the substrate (Table 2.4).

Keratin-rich wastes such as chicken feathers, wool, hair, nails, horns, hooves, and claws are produced worldwide by the poultry, wool, meat, and fish industries (Patinvoh et al., 2017). Keratin chains are tightly packed into a supercoiled polypeptide chain extensively cross-linked with disulfide bridges, hydrogen bonds and hydrophobic interactions, resulting in the mechanical stability of keratin and its recalcitrance to common proteolytic enzymes such as pepsin, trypsin and papain (Daroit et. al., 2009). Keratin is an insoluble structured protein which needs to be solubilized through pre-treatment prior to biogas production from keratin-rich substrates.

Complex proteins and carbohydrates are present in the cell wall of macroalgae. This complex structure makes them high chemical resistant and limits the accessibility of methanogenic organisms for digestion. Pre-treatment's primary function is to improve the liquefaction phase and release these biopolymers into the aqueous phase for such a substrate (Atelge et. al., 2020).



(a) Before Pre-treatment



(b) After Pre-treatment

Fig. 2.4. Images before and after pre-treatment of substrate

Table 2.4. Different type of pre-treatment approaches to recover energy (Barua and Kalamdhad, 2016)

Pre-treatment Method	Remarks
Acidic	Formation of inhibitory products, corrosive, expensive
Alkaline	Mild process condition, prolonged reaction time, high lignin solubility
Thermal	Short reaction time, high lignin solubility
Biological	Environment-friendly, long residence time, expensive

2.6.2 Pre-treatment methods

Depending on the physical and chemical characteristics of the substrate, the pre-treatment method should be chosen carefully. Generally, it is divided into three type's physical, chemical and biological pre-treatment. A successful pre-treatment should be able to: i) preserve the organic materials in biomass; ii) develop the progress of beneficial to hydrolysis; iii) avoid the formation of any toxic and/or inhibitory compounds; iv) environmental-friendly; and v) economically feasible (Panigrahi and Dubey, 2019).

2.6.3 Physical pre-treatment

No additional chemicals, enzymes or fungi were added with physical pre-treatment. Physical pre-treatment increases the surface area of the substrate, causing improved contact between microorganisms and substrate. In this section, the physical pre-treatment is classified as mechanical, thermal, ultrasonic, microwave irritation, and pulsed electric field pre-treatment (Atelge et al., 2020).

Table 2.5 Physical Pre-treatment

Substrate	Pre-treatment Method	Biogas Production and Methane yield	References
Microalgae biomass	Microwave (MW)	0.170–0.270 m^3 CH_4/kg VS	Passos at al., 2014

Waste activated sludge	Ultrasonic pre-treatment	Biogas overproduction of 31.43% (219.5 mL/g VS)	Lizama et. al., 2017
Sewage sludge	Hydrothermal	methane yield of 260 mL/g COD	Li et al., 2018

2.6.4 Chemical pre-treatment

Under different conditions, several types of acids and alkaline, ozonation, Fenton, and ionic liquids are used for chemical pre-treatment to improve the biodegradability of substrate. Acid pre-treatment involves sulfuric, nitric, hydrochloric acids to solubilize the hemicelluloses and make the cellulose exposed for enzymatic digestion. (Sahoo et al. 2018) studied *Zizania latifolia*, a wild rice grass, show that dilute acids pre-treatment is useful for this particular variety of grass for producing fermentable sugars for ethanol production compared to alkali pre-treatment. Alkali pre-treatment refers to applying alkaline solutions like NaOH, lime, ammonia to remove lignin and various uronic acid substitutions on hemicellulose that lower the accessibility of enzymes to the hemicellulose and cellulose.

Table 2.6. Chemical Pretreatment

Substrate	**Pre-treatment Method**	**Biogas Production and Methane yield**	**References**
Microalgae	Ozonation	The highest methane production 432.7 mL CH_4 g^{-1} VS	Cardeña et. al., 2017
Giant reed	Pre-treatment with 7–20% $Ca(OH)_2$	Improved methane yield by 1.4- fold	Jiang et. al., 2017
Corn Stalk	Pre-treatment with 12% of H_2O_2	It increased the final volume of biogas by 22%	Venturin et. al., 2018

2.6.5 Biological pre-treatment

Biological pre-treatment is a method in which organisms are involved directly or indirectly in an environmentally friendly approach. Biological pre-treatment requires significant less energy compared to physical and chemical treatment. It also does not require chemicals, hence very environment friendly. Among biological pre-treatment methods, fungal pre-treatment by white-rot fungi is the most effective for selective delignification (Alexandropoulou et al., 2017). However, long pre-treatment time is the only drawback of this pre-treatment method.

Table 2.7. Biological Pre-treatment

Substrate	**Pre-treatment Method**	**Biogas Production and Methane yield**	**References**
Miscanthus giganteus	Enzymatic pre-treatment	Biogas production yield of $421.5\ \mathrm{Ndm^3\ kg\ TS^{-1}}$	Michalska and Ledakowicz (2015)
Agropyron elongatum' BAMAR'	Fungi treatment	Biogas produced $398.07\ \mathrm{Ndm^3\ kg^{-1}\ VS^{-1}}$, which was 120% higher than the untreated sample.	Lalak et. al., (2016)
Corn stover	Microbial consortium BYND-9	Methane yield of 209.7 $\mathrm{mL_NCH_4}$ g−1 TS−1 after pre-treatment Untreated substrate had methane yield of 149.2 $\mathrm{mL_NCH_4}$ g−1 TS−1	Zhao et. al., (2019)

2.7 Co-digestion

Dhamodharan et al., (2014) reported that co-digestion of sludge with other organic substrates can be done to enhance the anaerobic digestion. Abueienien et. al., (2010) studied that co-digestion is the best way to reduce the formation of inhibitors or accumulation of toxic compounds. Co-digestion has many advantages, including increased methane production, improving nutrient balance in the digester and improved system economics.

2.7.1 Anaerobic co-digestion

In anaerobic digestion systems, the substrate is degraded based on factors like temperature, pH, C/N ratio, F/M ratio, etc. During mono-digestion of the substrates, there is a larger possibility that these factors are not balanced. This imbalance can lead to low biogas yield in the mono-digestion systems. To state expressly, the substrate might contain a more significant amount of Nitrogen, which leads to ammonia's formation (NH_3) and hence the inhibition of the normal degradation process takes place (Wagner et. al., 2012). Similarly, the carbon content found in organic matters like agricultural wastes may be very high, which is also not favorable for the digestion process. This happens because the microorganisms cannot survive more than food in the form of carbon. By calculating the C/N ratio, the combined effect of these elements is considered, and the overall impact on biogas production is studied. However, there is an optimal limit in which C/N ratio has to be maintained. Various literature works suggest that a lower and higher C/N rate can inhibit the digestion process. (Barua and Kalamdhad 2019; Yen and Brune, 2007). Barua and Kalamdhad 2019 reported that a C/N ratio in the range of 25 to 30 could exhibit good biogas production for lignocellulosic biomass rice straw and food waste. Co-digestion of two or more substrates can achieve this. In actual conditions, most factors like pH, temperature, etc., during anaerobic digestion are not under control inside the conventional reactors when a single substrate is digested. Hence, co-digestion can be performed to optimize these governing factors.

The area of research in the anaerobic co-digestion has witnessed great attention of the researchers. An exponential increase in the number of publications over the years has been observed. A search for the combined keyword "Anaerobic co-digestion” in top databases, namely Scopus, Web of Science, PubMed and springer, shows the growing trend of research in this field. Mata-Alvarez et al., 2014 in his work, evaluated all the papers published until 2013; 50% were published within two years.

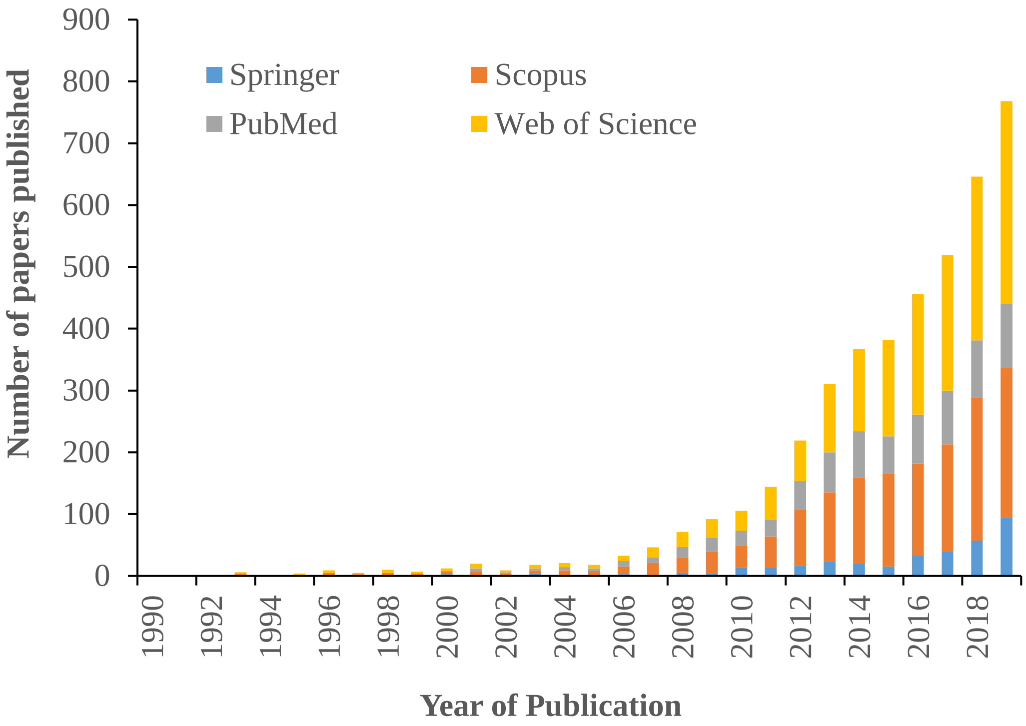


Fig. 2.6. Trend of research in the field of anaerobic co-digestion

Table. 2.8. Different types of substrates and inoculum used in anaerobic co-digestion process

Reference	**Substrate and Inoculum**	**Remarks**
Kabouris et al., 2009.	Mixture of municipal primary sludge (PS), thickened waste activated sludge (TWAS) and fat, oil, and grease (FOG).	Addition of a huge amount of FOG fraction (48% of the total VS load) to a PS + TWAS mix, resulted in 2.95 times increase in methane yield at 35^{o}C and 2.6 times increase in methane yield when operated at 52^{o}C.
Carrere et al., 2012.	Waste-activated sludge was co-digestion with fatty wastewater obtained from restaurants was studied	The co-digestion of waste activated sludge with highly concentrated fatty waste water (40% in volume, 49% in VS and 73% in COD, equivalent to 7 g L^{-1} of lipids), resulted in specific methane production of 362 mL CH_4 g^{-1}

		whereas waste-activated sludge alone produced only 116mL CH4 g^{-1} VS.
Miao et al., 2014.	Food: swine manure Inoculum: Taihu blue algae Inoculum substrate ratios 0.5 to 3.0	Co digestion of blue algae with swine manure led to the highest methane (CH_4) production of 212.7 mL g^{-1} VS at inoculum to substrate ratio of 2.0. While digestion of blue algae inoculated with granular sludge brought out the optimized CH_4 production of 73.5mL g^{-1} VS at ISR 3.0.
Jensen et al., 2014.	Food: sewage sludge and crude glycerol Inoculum:microbial community	The methane potential of crude glycerol varied from 370 mL CH_4 g^{-1} VS to 483 mL CH_4 g^{-1} VS for different samples tested
Toumi et aL., 2015.	Co-digestion of dairy wastewater (DW) and cattle manure (CM)	High concentrations of VFA produced from dairy wastes acidification allow the growth of *Methanosarcina* species. Growth in species results in reduction of VS by 88.6%. Biogas production of 0.87 L g^{-1} VS removed was obtained for the C/N ratio of 24.7 at hydraulic retention time (HRT) of 20 days.
Barua et al., 2018.	Co-digestion of water hyacinth and cooked food waste with and without pre-treatment.	The methane generation for co-digestion of water hyacinth and food waste without pre-treatment was 4328 ± 12 mL, in mixing ratio of 2 whereas for pre-treatment methane generation was 5017 ± 15 mL, in mixing ratio of 1.5.

2.8 Outcome of the literature

Anaerobic digestion is considered one of the most environmentally friendly techniques, which converts biodegradable substances into biogas in anaerobic conditions: this review analysis digestion and co-digestion studies, different inoculums used in AD process. Even though AD process seems to be useful and cost effective, it has some shortcomings. AD process suffers high HRT. It needs a minimum of 30 to 40 days to achieve treatment efficiency. The interaction between microorganisms results in low methane production and accumulation of toxic intermediates. Anaerobic co-digestion is one of the best measures to improve methane yield as it dilutes toxic intermediates and balances nutrients.

CHAPTER 3

OBJECTIVES OF THE STUDY

This chapter deal with the knowledge gap, objective of the study, need for research, and scope of the present work

3.1 Knowledge gap

- There is no systemic study on the anaerobic digestion of the terrestrial weed. There is no clear-cut idea about biochemical methane potential (BMP) test determining the optimized food/microorganisms (F/M) ratio for AD of Terrestrial weeds.
- No literature is available regarding pretreatment, comparative study of various thermal pretreatment techniques, especially for terrestrial weed.
- No literature review on pretreatment applied on terrestrial weed.
- Electrohydrolysis pretreatment is rarely studied. No BMP test or batch study were conducted for thermal, electrohydrolysis for terrestrial weeds.
- Continuous reactor was not performed.
- No microbial study for different stages of anaerobic digestion.

3.1.1 Need for research

The study's goal is to explore the potential of anaerobic digestion of terrestrial weeds (*P.hysterophorus, L.camara, A.conyzoides*) and its effect on different pretreatment and co-digestion techniques for enhanced methane production. The production of renewable biogas from terrestrial weeds might be the key solution for its control and production of a renewable energy source. However, the high lignin content in terrestrial weeds became the barrier, which reduces and slows down biogas production, making hydrolysis a rate-limiting step in anaerobic digestion. To overcome the challenges, pretreatment was applied to improving the hydrolysis stage of anaerobic digestion. It enhances the biogas yield; it lowers the hydraulic retention time (HRT). An increase in population results in an increase in demand for energy, food productivity and tends to increase in waste production. To achieve productivity, effective utilization of land resources should occur; weeds play a crucial role in decreasing productivity. The best way to utilize weeds is to use them as a feedstock for biogas production through AD or vermicomposting. AD can be considered the unsurpassed route for transforming waste into energy. Utilization of renewable biogas as an energy source not only replace fossil fuels but also minimize air pollution and increase productivity. This study aims to explore the potential of anaerobic digestion and co-digestion techniques for enhancing methane production using *Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides.* The production of biogas can be the solution for producing renewable energy and increasing agricultural productivity.

3.2 Objectives of the study

The main objective is the anaerobic digestion of freshly chopped terrestrial weeds. The purpose is to find the appropriate Food to microorganism (F/M) ratio for enhancing biogas production while accelerating the process and its efficiency by performing different pretreatment methodologies.

Phase 1: Characterization of weeds and BMP test to determine best F/M ratio-carried out to explore the potential of anaerobic digestion of terrestrial weeds (*P.hysterophorus, L.camara, A.conyzoides*)

Phase2: To enhance biogas yield by improving the hydrolysis through different pretreatment techniques.

Phase 3: To explore the potential of co-digestion techniques for enhancing methane production.

Phase 4: Operation of two-stage continuous reactor (design by Barua and Kalamdhad., 2019).

Phase 5: Microbial Study (Identify bacterial for different time intervals).

3.3 Scope of the present work

The present study's scope is to enhance biogas production from freshly collected *Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides* by AD and co-digestion. The initial characterisation of collected *Parthanium hysteroporus, Lantana camara, Ageratum conyzoides,* cow dung and food waste were performed. The BMP (1L capacity) test was performed for different F/M ratios of *Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides* with cow dung inoculum, to determine optimum F/M ratio by AD and co-digestion techniques. Food waste was used as co-substrate in co-digestion. Effect of digestion and co-digestion on hydrolysis of *Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides* with respect to pH, sCOD, VFA, VS was studied. The scope also includes a comparative study of the efficiencies of batch reactors of digestion and co-digestion of *Parthanium hysteroporus*, *Lantana camara* and *Ageratum conyzoides* terms of potential in methane production followed by continuous reactor.

CHAPTER 4

MATERIALS AND METHODS

This chapter deal with material and methods of collection of substrate and inoculum, initial characterization of terrestrial weeds, Biochemical methane potential (BMP) study, continuous reactor, microbial study and instrument used. Detailed experimental procedure for the test performed is explained in this chapter.

4.1 Collection of substrate and inoculum

P. hysterophorus, L. camara and A. conyzoides were collected from the campus of Indian Institute of Technology, Guwahati (IITG) and fresh cow dung was collected from a nearby village, Amingaon. Characterizations of terrestrial weeds and cow dung were done (Table 4.1). The complete plant was extracted from the soil, cut into the average size of 1 cm, and chopped correctly. Sharma et al., 1988 stated that smaller particle size enhanced methane production. Larger particle size reduced the methane production, as they were challenging for microbes to degrade. Pulverization was required to boost methane production, and at the same time, it reduced the volume of the reactor. (Moorhead and Nordstedt, 1993; Gollakota and Meher, 1988). Various amounts of cow dung were mixed with terrestrial weeds based on volatile solids (VS).

Table 4.1. Initial characteristics of the cowdung

Parameter	Cow dung
pH	7.10± 0.3
sCOD (mg/L)	2453 ± 145
TS (%)	18.98 ± 0.2
VS (%)	81 ± 0.7
VFA (%)	360 ± 4.0

MC (%)	80.45 ± 3.0
TKN	3.6 ± 0.06
TOC	41 ± 3.0

4.1.1 Characterization and compositional analysis

To determine the amount of insoluble lignin present in the substrate, the national renewable energy laboratory (NREL) method was followed. For cellulose analysis, 0.5 g sample was taken, and 3mL of acetic or nitric acid was added and boiled and allowed to cool. Once the sample was cooled, the centrifuged supernatant was separated. Anthrone reagent was added to the diluted sample and allowed to boil for 10 mins. Lastly, at 630 nm, and measured the absorbance. After that, the residue was washed using distilled after which it was washed with 67% of H_2SO_4. Hemicellulose was determined by finding the difference between neutral detergent fiber (NDF) and acid detergent fiber (ADF) as reported by Goering and Van (1975).

4.2 Experimental design

In order to achieve the objectives, the research was carried out in five different phases, as summarized in Fig. 4.1. In phase I, a characterization and biochemical methane potential (BMP) study was performed. Phase II, due to high lignin contents, various pretreatment technique were carried out. Phase III Co-digestion of terrestrial weeds with food waste was carried out to minimize the toxic substance and at the same time, enhance the methane production. Phase IV Operation of continuous two-stage reactor design, daily feeding was done. Phase V microbial study was done, where bacteria were identified at a different time interval.

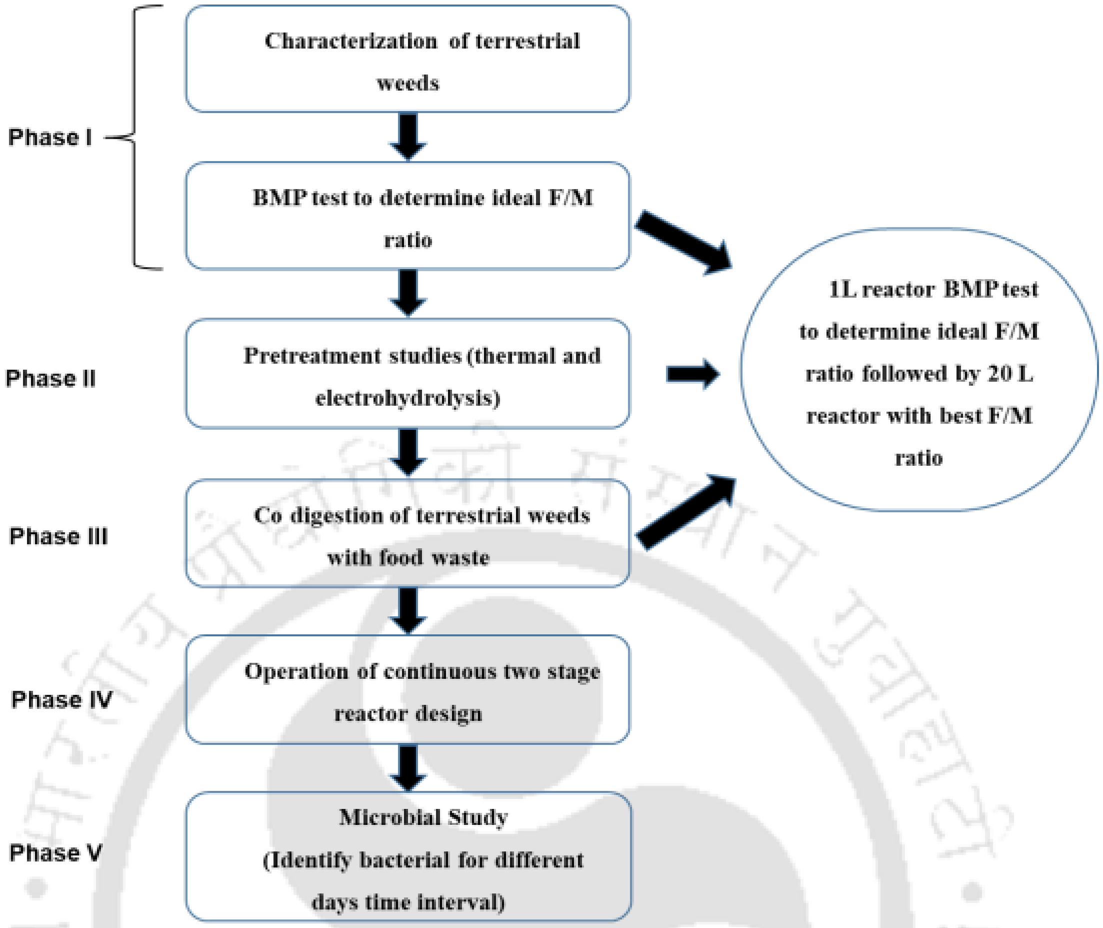


Fig 4.1. Experimental design phases

4.3 Phase 1: Anaerobic digestion

4.3.1 Initial characterization of terrestrial weeds

The sample was kept in a horizontal shaker for 2 h at 150 rpm, and then the sample was filtered. After filtration, the sample was analyzed for different parameters at an interval of every seven days, i.e., volatile solids (VS), total solids (TS), moisture content (MS), and soluble chemical oxygen demand (sCOD). APHA, 2005 technique was used for the analysis of VS and sCOD. Volatile fatty acid (VFA) was examined directly through the titration method (DiLallo and Albertson, 1961). For the preparation of sCOD and VFA, 5 g of the sample was mixed uniformly, and the volume was made up to 100 mL with distilled water. The sample was kept in a horizontal shaker for 2 h at 150 rpm, and then the sample was filtered. After filtration, the sample was analyzed (table. 4.2). Total kjeldahl nitrogen (TKN) were analyzed for terrestrial weeds according to standard methods (APHA, 2005).

Table 4.2. Initial characteristics of the weeds

Initial Characterization	*A. Conyzoides*	*L. camara*	*P. hysterophorus*
BOD(mg/L)	630 ± 190	750 ± 172	890 ± 155
COD(mg/L)	5000± 203	5552 ± 210	6108 ± 185
pH	6.08 ± 0.3	6.73 ± 0.5	6.75 ± 0.5
EC(ms/cm)	5.3 ± 0.2	5.1± 185	5.32 ± 0.5
Moisture %	75.63 ± 3.0	75.83 ± 3.0	73.77 ± 2.0
% Volatile Solids	71.6 ± 5	73.3 ± 5.0	83.13 ± 3.0
Ash	28.4 ± 0.7	27.1 ± 0.2	19.6± 0.4
% Total organic carbon	41.53 ± 4.0	44.32 ± 3.0	45.33 ± 3.0
TKN	1.43 ± 0.05	1.6 ± 0.07	1.67 ± 0.05
C:N ratio	29.04 ± 0.8	27.7 ± 1.0	25.05 ± 2.0

4.3.2. Morphological study

To determine the morphological changes during the digestion of *L. camara*, FESEM (Zeiss, Sigma), FTIR and XRD (Rigaku TTRAX III) were analyzed. A double coating of gold was used to prepare the conductive sample for FESEM to build up the charge. FESEM provided information directly on the surface of the substrate. Parameters maintained during FESEM analysis were 20 kV and 130 eV. X-ray diffraction (XRD) is a unique technique to identify the crystallinity of a compound. The X-ray diffractograms were recorded from 5 °C to 60 °C of a diffraction angle (2θ) at a scanning speed of 4°/min with an XRD diffractometer (Barua et al., 2017).

4.3.3 Biochemical methane potential (BMP) study

The significance of BMP study is to determine the methane production under different optimum conditions (Hussan and Dubey., 2015). The BMP was arranged as reported by Owen et al., (1979). 1 L of reagent bottle was used as a reactor to run the BMP study. The quantities of cow dung and substrate (weed) were estimated based on volatile solids (VS) calculation. F/M ratios of 1.0, 1.5, 2.0 and 2.5 were examined as shown in the table. 4.3. One control was studied with 50 g of cow dung kept in a 1L batch reactor. A Triplicate study was done for every F/M ratio, including control. Essential macro and micronutrients [$FeCl_3$ (40mg/L), ZnC_2 (0.5mg/L), $CaCl_2$ (50mg/L), $MgSO_4$ (400mg/L), $CoCl_2$ (10mg/L),

$NiCl_2$ (0.5mg/L) and phosphate buffer (80mg/L)] (Dinsdale et al., 2000) were fed along with cow dung and substrate to enhance the microbial activity inside the batch reactor. Important parameters inside the reactor, such as pH and stirring intensity, were required to be maintained (Browne and Murphy, 2013). The volume of the batch reactor was adjusted up to 700 mL with distilled water. Rubber corks were used to close the mouth of reactor bottles. Nitrogen gas was passed inside the reactor to maintain the anaerobic condition, and the pipe was connected to aspirator bottles containing NaOH of 1.5 N (Elliott and Mahmood, 2007). Glass beaker was placed at the bottom of the aspirator bottle to collect the NaOH (fig 4.2, 4.3 and 4.4). The experiment was run for 50 days.

Table 4.3. Quantity of substrate (terrestrial weeds) and Inoculum (cow dung) used for different F/M ratios

F/M ratio	*Parthenium hysterophorus (g)*	*Lantana camara* (g)	*Ageratum conyzydes* (g)	Cow dung (g)
Control 1	----	---	---	50
Control 2	50	50	50	---
0.5	31.01	---	---	50
1.0	54.59	43.39	28.42	50
1.5	81.88	65.08	42.64	50
2.0	109.19	86.78	56.84	50
2.5	136.48	108.48	71.06	50
3	186.06	---	---	50

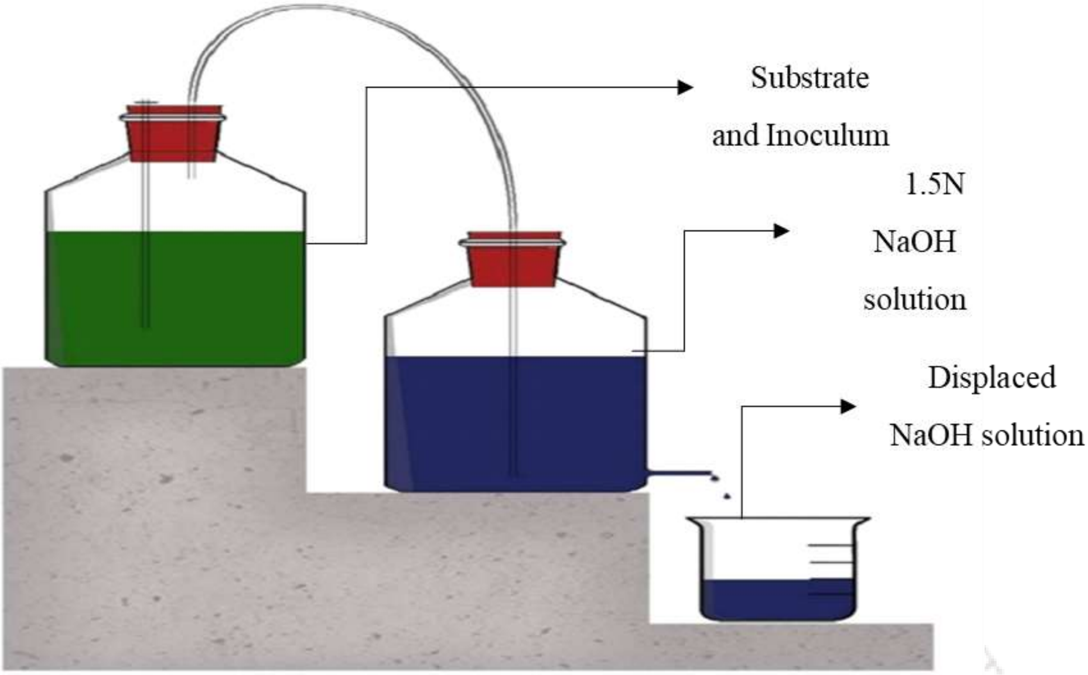


Fig 4.2. Pictorial representation of the batch set up

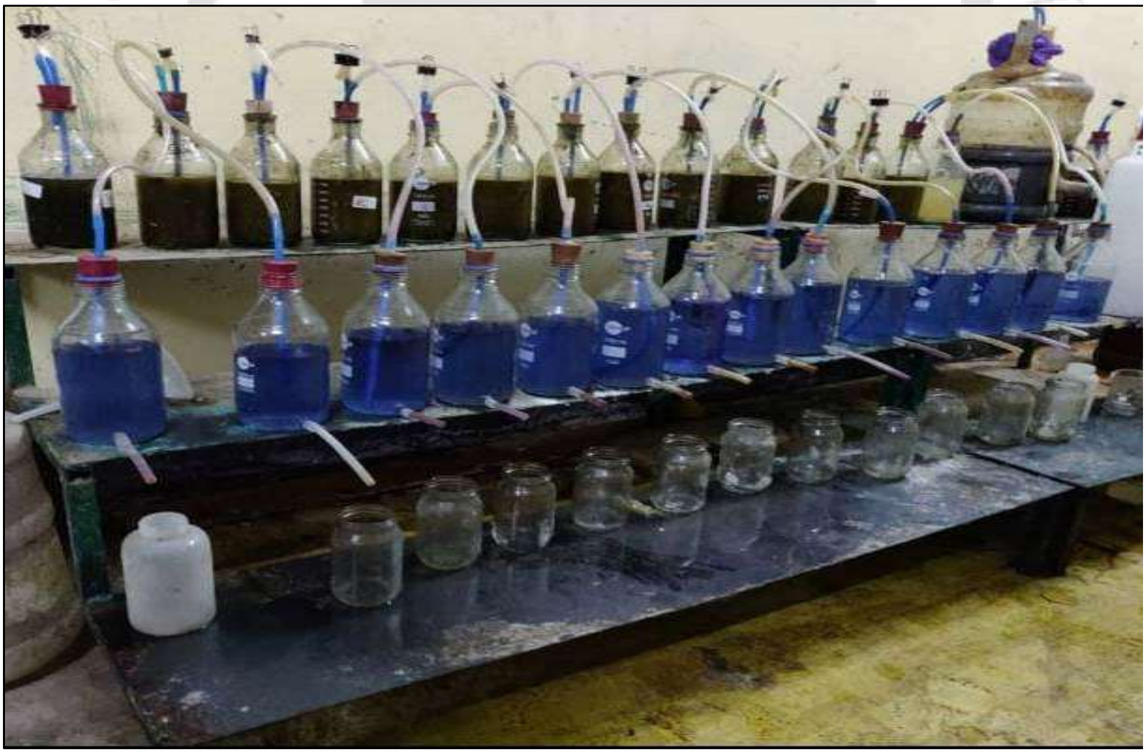


Fig. 4.3. Experimental setup of 1L BMP set up

Fig. 4.4. Experimental setup of 20L batch set up

4.4 Phase II: Pretreatment studies

Initially, the whole plant was chopped, ground uniformly and used for every trial. The effect of temperature was studied by preparing different sets of samples keeping the time constant. Similarly, the effect of time was studied by observing the sample for a different time interval at an optimized temperature.

4.4.1 Pretreatment by hot air oven

In hot air oven pretreatment (fig 4.5), different temperatures and times were considered to inspect according to earlier studies reported by (Rafique et al., 2010; Baura and Kalamdhad, 2016). The whole terrestrial weeds plant was taken off the soil and cut into small pieces. The freshly ground terrestrial weed was heated for 1 h at different temperatures 80, 90, 100, 110 and 120 ˚C using a closed beaker system. Similarly, various time studies have been done with optimized temperatures for 30, 60, 90, 120 and 150 mins to optimize the time.

Fig. 4.5. Hot air oven

4.4.2 Pretreatment by microwave

In microwave pretreatment (fig 4.6), freshly ground terrestrial weeds of 50 g was taken in a closed glass beaker and exposed to the microwave at 160, 180, 200 and 220 ˚C for 10 mins. After optimizing the temperature, the sample was kept for different time intervals, 5, 10 and 15 mins at an optimized temperature. As reported in literature, the temperature and time were selected (Sapci, 2013; Lin et al., 2015).

Fig. 4.6. Microwave

4.4.3 Pretreatment by autoclave pretreatment

The effect of autoclave pretreatment (fig 4.7) was examined by using 50 g of freshly ground *terrestrial* weeds in a glass beaker at different temperatures of 80, 90, 100, 110 and 120˚C for 20 mins and optimize temperature was set. The effect of autoclave time on the hydrolysis of terrestrial weeds, was observed by using the sample in a closed glass beaker by heating at the optimised temperature for 20, 40, 60 and 80 mins. Temperature and time of pretreatment were chosen on the basis of reported literature available (Menardo et al., 2012; Toquero and Bolado, 2014; Bolado-Rodríguez et al., 2016).

Fig. 4.7. Autoclave

4.4.4 Pretreatment by hot water bath

The effect of the hot water bath (fig. 4.8) was studied as reported in the literature (Li et al., 2007; Cho et al., 2013). Here temperature and time were the parameters for observation of the effect of hot water pretreatment. The closed beaker having fresh ground terrestrial weeds, the whole plant was heated at 70, 80, 90 and 100˚C for 30 mins. To observe the effect of hot water bath time on the hydrolysis of terrestrial weeds at optimized temperature, the sample was kept in the closed glass beaker for 30, 60, 90 and 120 mins.

Fig. 4.8. Hot water bath

4.4.5 Electrohydrolysis pretreatment

Whole weeds were cut into pieces of the average of 1 cm mixed with distilled water and grind it, and then 500 gm of *P. hysterophorus* 500 mL of distilled water was prepared in a large beaker to observe the effect of each voltage. After optimizing the voltage, the sample was kept for different time intervals to optimize the time.

In electrohydrolysis pretreatment (Fig 4.9 and 4.10), ohmic heating, electrophoresis and electro-osmosis play a key role. When electricity passes into the material, heat is generated, known as ohmic heat (Knirsch et al., 2010). In electrophoresis, dispersion of particles of the fluid under the influence of the uniform electric field. Ionic particles separate results from alterations in their velocity (*v*), which is the product of the molecule's versatility (*m*) and the field quality (*E*) (V=mE) (Fritsch and Krause., 2003). Electro osmosis is a flow of liquid due to the inducing of the applied potential. Direct current (DC) was provided on the substrate for pretreatment, due to electrolysis ionization take, leading to produced electrolyte i.e., the movement of cation and anions displaced into opposite electrodes (Nandi, 2013).

Fig 4.9. Electrohydrolysis Set up

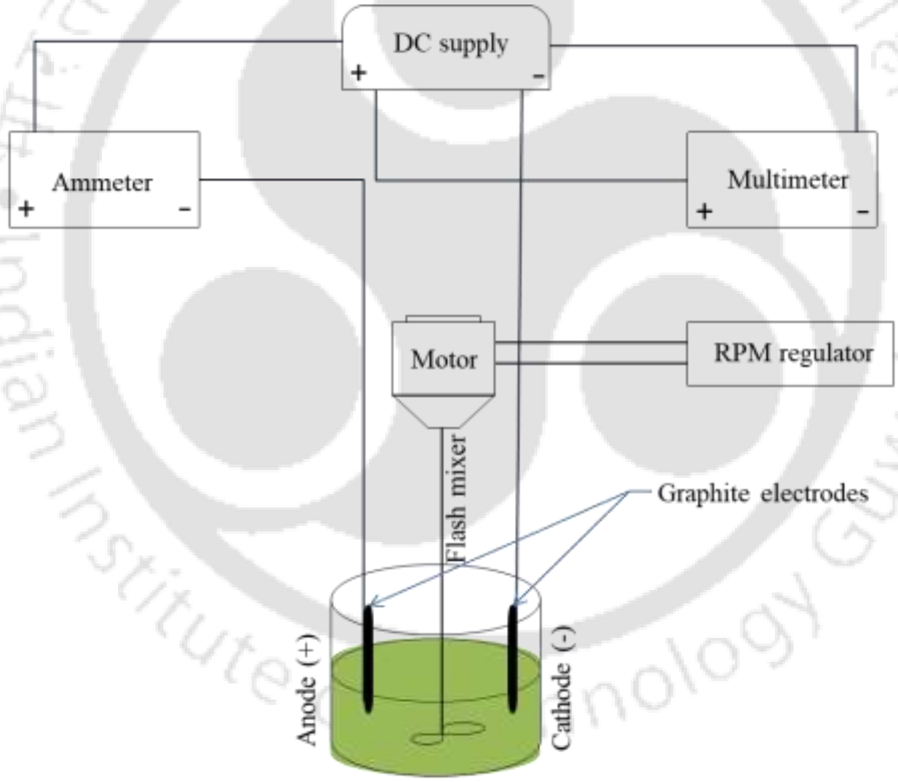


Fig 4.10. Pictorial representation electrohydrolysis Set up

4.5 Phase III: Anaerobic co-digestion

4.5.1. Anaerobic co-digestion of terrestrial weeds using food waste as co-substrate and cow dung as inoculum

Food waste, which is collected from different hostels was used as co-substrate in anaerobic co-digestion. BMP assay for anaerobic co-digestion of *Parthenium hysterophorus* with food waste, *Lantana camara* with food waste and *Ageratum conyzoides* with food waste using cow dung as inoculum was conducted and test was carried out for the mixing ratios of 1.0, 1.5, 2.0, and 2.5 in triplicates. *Patrhenium hysterophorus*, *Lantana camara*, *Ageratum conyzoides and* cow dung are used as controls. The quantity of *Pathenium hysterophorus*, *Lantana camara*, *Ageratum conyzoides*, food waste and cow dung are determined on basis of VS present in it (Table. 4.3). The experiment is performed for 7 weeks.

Table. 4.4. Quantity of *terrestrial weeds (Parthenium hysterophorus*, *Lantana camara, Ageratum conyzoides)*, food waste and cow dung

Mixing ratio	*Parthenium hysterophorus (g)*	*Lantana camara* (g)	*Ageratum conyzoides* (g)	Cow dung (g)	Food waste (g)
Control 1	----	---	---	50	----
Control 2	50	50	50	5	----
1.0	27.30	21.69	14.47	50	18.68
1.5	40.95	32.54	21.32	50	28.02
2.0	54.59	43.39	28.42	50	37.36
2.5	68.24	54.24	35.53	50	46.70

4.6 Phase IV: Continuous digester

Continuous reactor study is essential for the field and industrial application. A two-stage biogas digester made up of steel is used as a continuous digester for anaerobic digestion. The cylindrical-shaped digester has a volume of 20 L. The agitator assembly consists of 4 blades; the assembly is set up in a continuous digester to improve its efficiency by achieving proper mixing. Fig. 4.11and 4.12 shows the continuous reactor.

The digester was provided with an inlet, outlet for substrate and inoculum feeding and removing; it consists of a gas outlet and sample collection ports. The gas outlet is connected to the aspirator bottle, which is filled with NaOH solution. The gas produced is calculated by the displacement method.

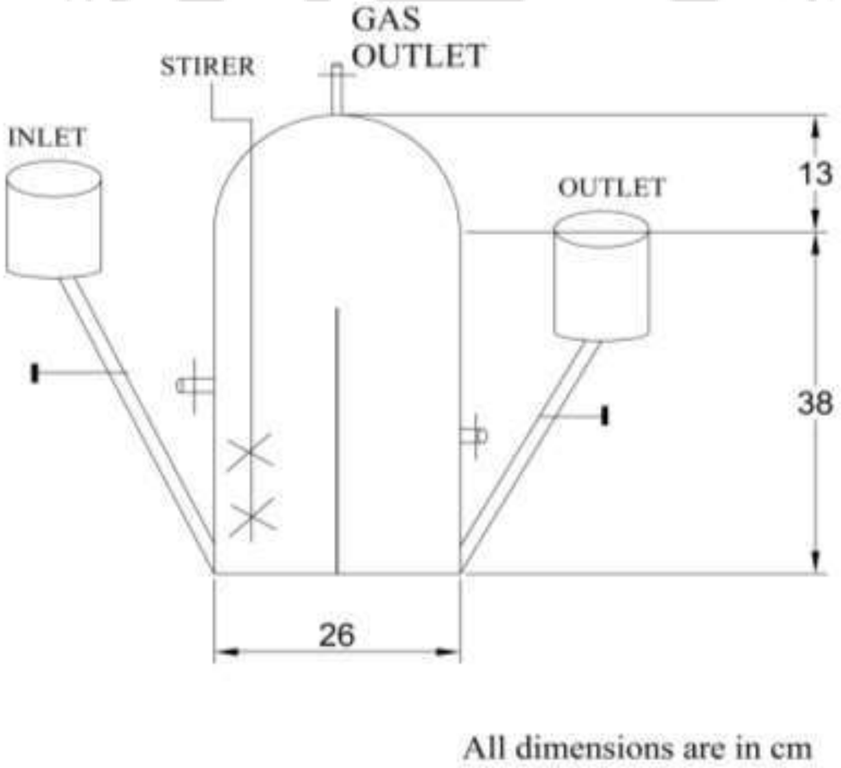


Fig. 4.11. Graphical representation of the continuous reactor

Fig. 4.12. Experimental setup of the continuous reactor

4.7 Phase V: Microbial study

The study aims to identify the various microbes in the reactor.

4.7.1. Metagenomic DNA isolation, qualitative and quantitative analysis

The metagenomic DNA was isolated from the received anaerobic reactor samples by a commercially available soil Kit (Nucleospin Soil). The qualities of the isolated metagenomics DNA sample were quantified using NanoDrop.

4.7.2. Preparation of 2 X 300 miseq library

The amplicon libraries were prepared using Nextera XT Index Kit (Illumina inc.) as per the 16S Metagenomics Sequencing (Table 4.4) Library preparation protocol (Part # 15044223 Rev. B). Primers for the amplification of the bacterial 16S V3-V4 region were designed and synthesized at Eurofins Genomics Lab. Amplification of the 16s gene was carried out. 3μl of PCR product was resolved on 1.2% Agarose gel at 120V for approximately 60 mins or until the samples reached 3/4th of the gel.

Table. 4.5. Primers used in the present study

16S rRNA F	GCCTACGGGNGGCWGCAG
16S rRNA R	ACTACHVGGGTATCTAATCC

The QC passed amplicons with the Illumina adaptor were amplified using i5 and i7 primers that add multiplexing index sequences and standard adapters required for cluster generation (P5 and P7) as per the standard Illumina protocol. The amplicon libraries were purified by AMPure XP beads and quantified using Qubit Fluorometer.

4.7.3. Quantity and quality check (qc) of library on agilent 4200 tape station

The amplified libraries were analyzed on 4200 Tape Station system (Agilent Technologies) using D1000 Screen tape as per manufacturer instructions.

4.7.4. Cluster generation and sequencing

After obtaining the mean peak sizes from Tape Station profile, libraries were loaded onto MiSeq at appropriate concentration (10-20pM) for cluster generation and sequencing. Paired-End sequencing allows the template fragments to be sequenced in both the forward and reverse directions on MiSeq. The kit reagents were used in binding of samples to complementary adapter oligoes on paired-end flow cell. The adapters were designed to allow selective cleavage of the forward strands after re-synthesis of the reverse strand during sequencing. The copied reverse strand was then used to sequence from the opposite end of the fragment.

4.7.5. Bioinformatics analysis

QIIME is comprehensive software comprising tools and algorithms such as FastTree for heuristic-based maximum-likelihood phylogeny inference (Price MN et al., 2010), the RDP classifier the assignment of taxonomic data using a naïve Bayesian classifier (Wang et al., 2007) and others fig 4.13. This allows QIIME, which continues to undergo development, to easily and relatively adapt novel standalone tools, and thus improve in step with advances in the field of microbial community ecology.

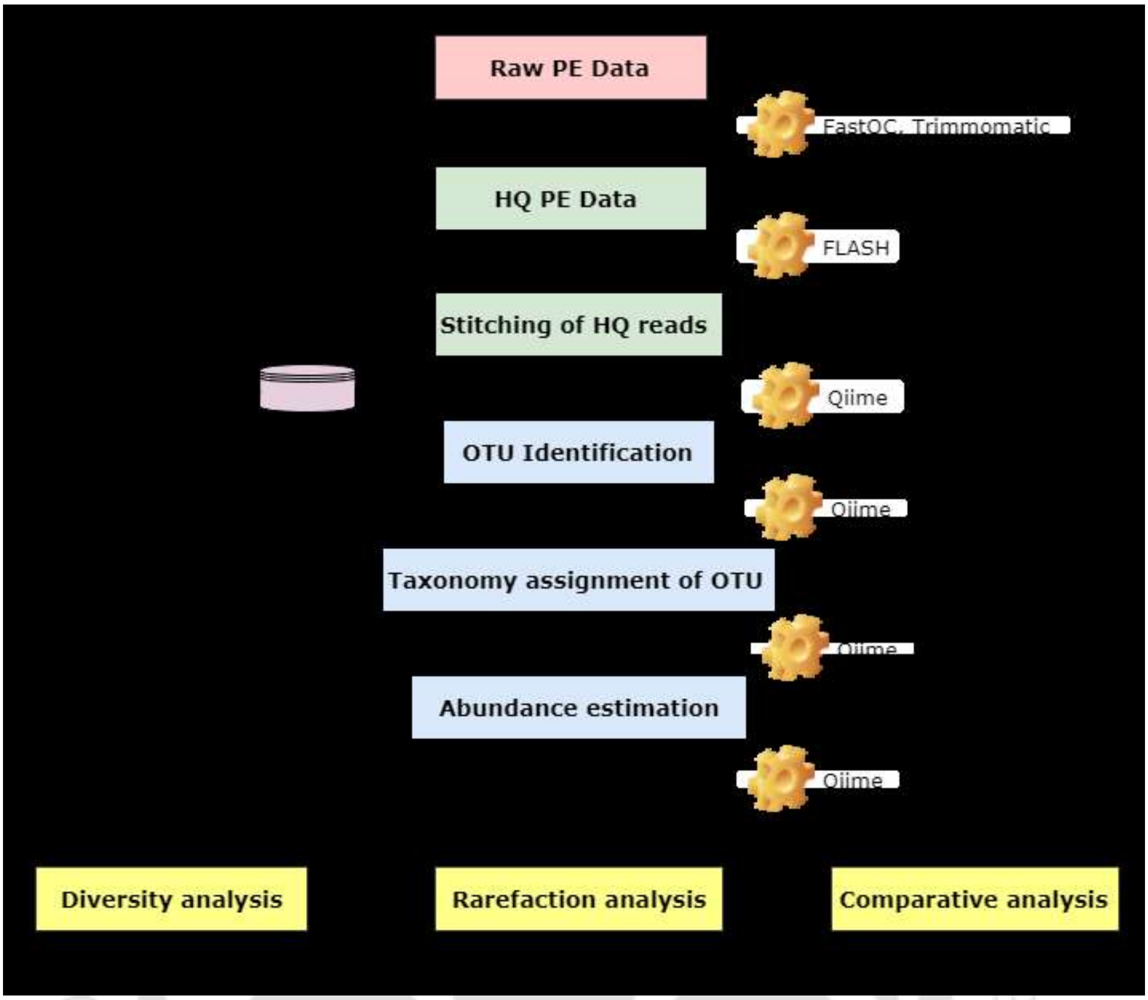


Fig. 4.13. Bioinformatics workflow

4.8 Kinetic study

Degradation of the substrate can be determined by using the Gompertz equation. The primary growth of bacteria was defined by this method (Lay et al., 1996). Later on, a modified Gompertz equation was successfully used to check the cumulative methane production, assuming methane production as a function of bacterial progress to find the highest methane production potential.

$$Y = M \times \exp\{-\exp[(R_m \times e / M)(\lambda - 1) + 1]\} \qquad (4.1)$$

Where the cumulative methane production (mL) is indicated by Y at time t (d), Highest methane production (mL CH_4) was denoted as M., highest methane production rate (mL CH_4 d^{-1}) was represented by R_m, the lag phase time (d) is represented by λ and e is constantly equal to 2.71. M, $R_,$ and λ are the three parameters whose values were calculated by curve-fitting using Matlab R2016b to minimize the remaining amount of squared incorrectness between the investigational data and the demonstrated curve.

4.9 Process parameter analysis

Due to microbial activity, several parameters undergo changes. In order to find out changes, a parameter such as pH, MC, VS, TS, VFA, sCOD etc. was analyzed. For measurement of pH, ground sample and deionized water were mixed in the ratio (1:10) and mechanically shaken for 2h in a conical flask to ensure homogeneity of the sample. After 2h, the pH of the filtered sample was measured using a portable pH meter. For evaluation of moisture content (MC), samples were oven-dried at 105 ^{0}C for 24 h, and percentage loss in moisture was evaluated thereafter as per Eq. (4.2).

$$\text{Moisture content}(\%) = \frac{\text{Initial weight} - \text{Final weight}}{\text{Initial weight}} \text{ X } 100 \tag{4.2}$$

Volatile solid (VS) in a sample was evaluated after measurement of moisture content in that sample. The oven-dried sample at 105ºC after 24 h was kept in a muffle furnace at 550ºC for 2 h. The VS of the sample was measured as the percentage loss in weight of the oven-dried sample using Eq. (4.3).

$$\text{Volatile solids (VS)}\% = \frac{(\text{W1} - \text{W2})}{\text{W1}} * 100 \tag{4.3}$$

where, W1 is the weight of oven-dried sample at 105ºC for 24h and W2 is the weight of the sample after being dried in a muffle furnace at 550 ºC for 2h.

For volatile fatty acids (VFA) evaluation, the ground sample was homogenized in a mechanical shaker for 2 h with de-ionized water in the ratio of (1:10) in a 250 mL conical flask. The filtered solution was diluted and distilled for 2-3 min at pH < 3.0 using sulphuric acid (H_2SO_4). After the distillation process, pH of the sample was raised to 4.0 using sodium hydroxide (NaOH). The volume of NaOH then required to raise the pH to 7 was noted, and VFA was evaluated using Eq. (4.4).

$$\text{VFA (mg/L)} = \frac{(\text{ml of 0.05 N NAOH consumed}) * \text{Dilution factor}}{\text{mL of the sample used}} \tag{4.4}$$

Chemical oxygen demand was evaluated using the closed reflux method (Standard Method 5220 C). Ground sample was homogenized in a mechanical shaker for 2h with de-ionized water in the ratio of (1:10). The sample was diluted, and 2.5mL of the sample was mixed with 1.5 mL of potassium dichromate ($K_2Cr_2O_7$) and 3.5 mL of COD acid in a COD vial. The samples were then digested in COD digester for 2 hours at 150°C. Using ferroin indicator, the sample was titrated against ferrous ammonium sulphate (FAS) until the colour of the sample changed to wine red, and sCOD was evaluated using Eq. (4.5).

$$\mathrm{sCOD}\left(\frac{\mathrm{mg}}{\mathrm{L}}\right) = \frac{\{(\mathrm{A}-\mathrm{B}) * \text{molarity of FAS} * \mathrm{D.F} * 8000\}}{\text{Volume of sample}} \tag{4.5}$$

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 and 8000 is milliequivalents weight of O_2*1000 mL/L.

4.10 Energy assessment

Energy changes were calculated to identify the efficiency of the hot air oven. In this study, methane is the final product considered as energy. Operation energy was taken as EU (J/g VS).

$$\mathrm{E_U} = (\mathrm{P.Et})/\mathrm{n} \tag{4.6}$$

Where P is the power of autoclave pretreatment applied, n signifies the volatile solids fraction of *P. hysterophorus* (g-VS), and Et is the time exposure time (s) (Passos et al., 2013). Methane production after pretreatment is signified as specific energy EQ (J/g VS).

$$\mathrm{E_Q}(\mathrm{Q_{raw}}.\S)/1000 \tag{4.7}$$

Where, § is considered as methane lower heating value (net calorific value) (35.80kJ/L CH_4) and Qraw (mL CH_4/g VS) reflect the potential methane yield represented as methane produced per gram of VS added (Kuglarz et al., 2013).

4.11 Gas chromatography (GC)

Biogas composition was examined by gas chromatography (GC) (Thermo Ultra GC) through thermal conductivity detector (TCD) Equipped with a porapa Q column maintained at 150 °C injection temperature, 60 °C oven temperature and 200 °C detector temperature.

Argon gas was used as a carrier gas in gas chromatography. Table 4.5 shows the company and model of GC and other instrument used

Table 4.6. Instruments required

Parameter analysis	Instrument	Make and model
Sieving	Sieve	Unique drawing & survey emporium (Mod 45H8U2)
Moisture content	Hot air oven Class-II high accuracy	Bionics scientific
pH	pH system 361or pH meter check	Systronics (Mod PED007)
TKN	TKN analyzer (Distillation)	Kelplus, Pelican Industries (Chennai)
Volatile solids	Muffle furnace	International commercial traders (Mod WAS59)
COD	COD digester	HACH (Mod DRB200)
VFA	HPLC	Shimadzu (Mod PBWE9811)
Biogas	GC	Chromatograph and instrument company (Mod CVDS7761)
Morphological study	Field Emission Scanning Electron (FESEM) Microscope	FEI Company (Netherland) (FEI Quanta 200 F SEM)
Morphological study	XRD	PA Nalytical Netherlands. (Model: X'Pert PRO)
Pretreatment and steralization	Autoclave	Berg
Pretreatment and drying	Hot air oven	Bionics scientific
Pretretment	Microwave	Philips
Pretreatment	Hot water bath	Frontline Electronics and Machinery
Pretreatment		
Weighing	Electronic balance	Wensar
Grinding	Grinder	Philips

CHAPTER 5

BIOCHEMICAL METHANE POTENTIAL (BMP) TRIAL OF TERRESTRIAL WEEDS (*Parthenium hysterophorus, Lantana camara* and *Ageratum conyzoides*)

This chapter explores the biochemical methane potential (BMP) study of *Parthenium hysterophorus*, *Lantana camara* and *Ageratum conyzoides* and optimizes the appropriate food to microorganism (F/M) ratio.

5.1 Phase I- Biochemical methane potential (BMP) trial

This study's main objective was to convert *P. hysterophorous*, *L. camara* and *A. conyzoides* into biogas and evaluate the best food to microorganism (F/M) ratio to find the appropriate combination between substrate and inoculum. Concentration and composition present in the substrate play significant roles in anaerobic digestion (Zhoe et al., 2011). Therefore, to improve methane production, the F/M ratio study is required. The biochemical methane potential is a crucial parameter for evaluation, economic and control issues for the complete processing of the anaerobic digestion process (Eiroa et al., 2012). 1L of reagent bottle was used as an anaerobic reactor. Cow dung was used as an inoculum source. Cow dung was the most efficient inoculum as compared to other cattle dungs (Dhamodharan et al., 2015). Two control studies had been done; where control 1 was cow dung, and control 2 were substrate (weeds), control 2 was quite less quantity. Therefore, it was not shown in the graph. Only cow dung (control 1) was mention in the graph. Similarly, in F/M ratios 0.5 and 3 were observed significantly less quantity in *P. hysterophorus*, therefore. 0.5 and 3 rates were not taken in *L. camara* and *A. conyzoides*. Different F/M ratios were investigated from 0.5 to ratio 3. After identification, the appropriate F/M ratio, 20L volume capacity batch reactor was operated in which the working space was 15.5L. This study indicated that biomass could produce methane, which can be utilized for various purposes. The exiperement is carried out in 6 different F/M ratio 0.5, 1, 1.5, 2, 2.5 and 3 (Table 4.2).

5.1.1 *Parthenium hysterophorus*

5.1.2 Biogas production

The rate of methane production is proportional to organic substrate breakdown (Esposito et al., 2012). There are various factors like inoculum, VS, VFA, sCOD, pH and temperature that affect methane production. Biogas yield was measured daily; it was measured by the liquid displacement method by the amount of NaOH displaced. Ratio 2 shows higher methane production up to 140 ± 5 mL CH_4/g VS in 28 days, followed by ratio 1.5 (99 mL); the highest cumulative biogas is observed in ratio 2 with 3319 mL, followed by ratio 2.5 (2903 mL) shown in Fig 5.1. Among all, the F/M ratio of 0.5 and 3 shows the lowest amount of biogas production, 2400 mL and 2527 mL, respectively, as shown in Fig 5.2. Biogas production increased from 0.5 to 2; then it started to decrease because of an imbalance in the F/M ratio. For *P. hysterophorous* the best order of F/M ratio is 2 >2.5 >1.5 >1> 3 >0.5. Methane production was observed to increase from F/M ratio 0.5 to 2; after that, it decreased at ratios 2.5 and 3. Initially, it takes time to produce biogas because of the presence of lignocellulose in the substrate. Inoculum concentration is higher compared to the substrate in the lower F/M ratio. As a result, biogas production is less in the lower ratio. Similarly, in the higher ratios, the substrate concentration is higher than the inoculum, which may increase the inhibitor like ammonia and nitrate, so moral degradation does not occur inside the reactor. Microorganisms require a balanced nutrient ratio for their efficient growth. Barua and Kalamdhad, 2017 observed a similar result. F/M ratio 2 was observed in the BMP study to show the highest potential compared to other ratios. After conducting 1000 mL of BMP study, the Batch reactor of 20L was studied with 15.5 L of working volume with ideal F/M ratio as observed in the BMP. *P. hysterophorus* and cow dung was mixed according to ratio 2. NaOH was used to measure biogas as was done in BMP. Kinetics was also found in 15.5 L of the batch reactor. 1.80 kg *P. hysterophorus* was taken, and 700 gm of cow dung was mixed and fed in the reactor. The 15.5 L of the batch reactor showed an increment of biogas yield compared to 1 L batch reactor; the increase was by 91.18%. With the increased volume of the reactor, biogas production was increased over 35time; it shows that *P. hysterophorus* had the potential to produce biogas on a larger scale. Microbial activity increased with an increase in the volume of the reactor, so proper digestion takes place. Soluble COD and volatile solid were also increased in 15.5L of batch scale reactor compared to the 1L batch reactor. Different biogas yields are shown in Fig

5.1, 5.2, 5.3 and 5.4 for reactors 1L and 15.5L, respectively. It can be concluded that *P. hysterophorus* can utilize for biogas production on a larger scale.

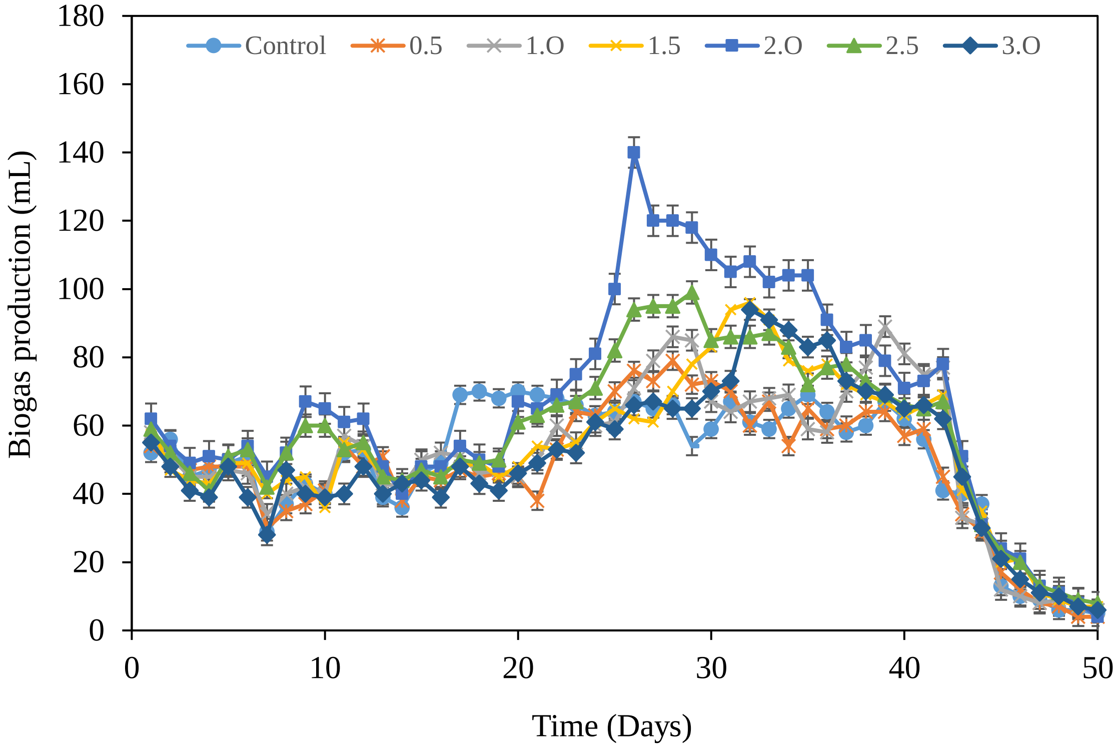


Fig. 5.1. Daily biogas production

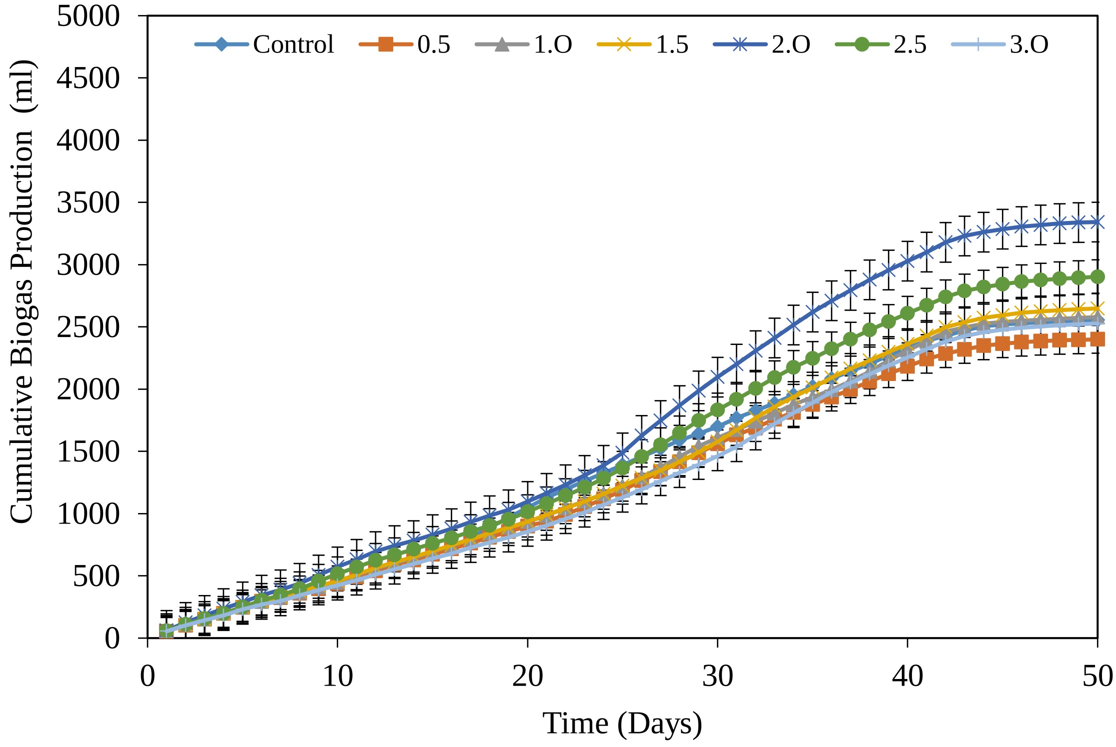


Fig. 5.2. Cumulative biogas production

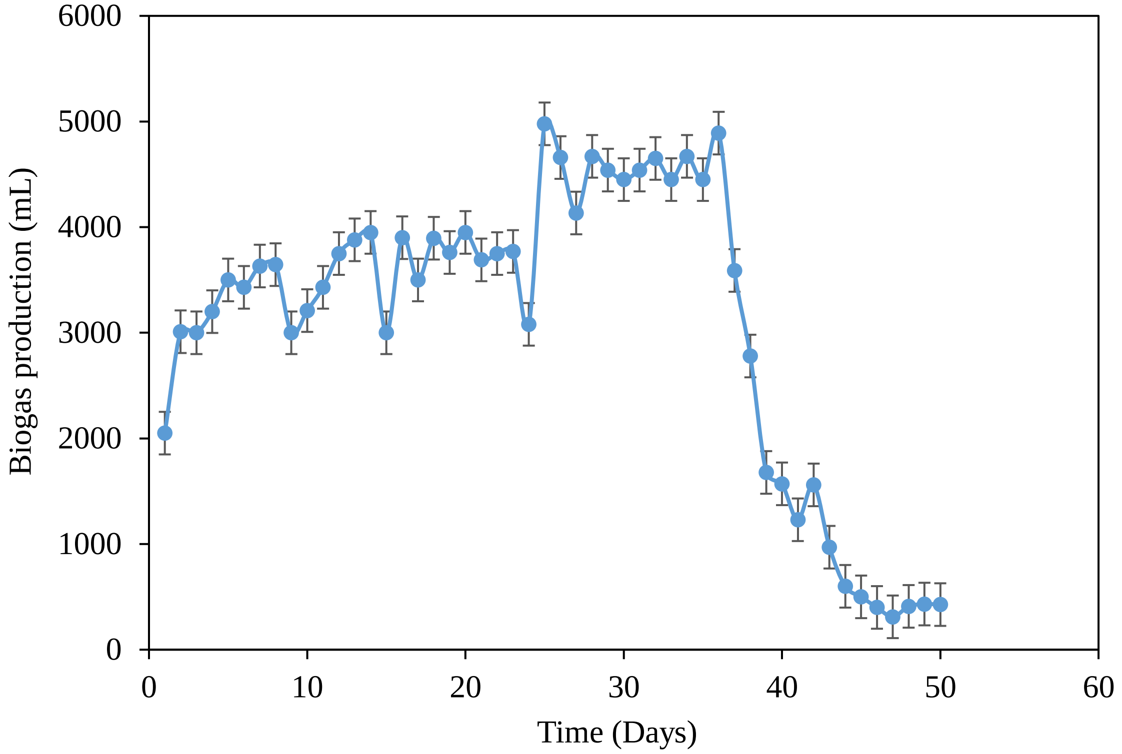


Fig. 5.3. Biogas production in 15.5L reactor

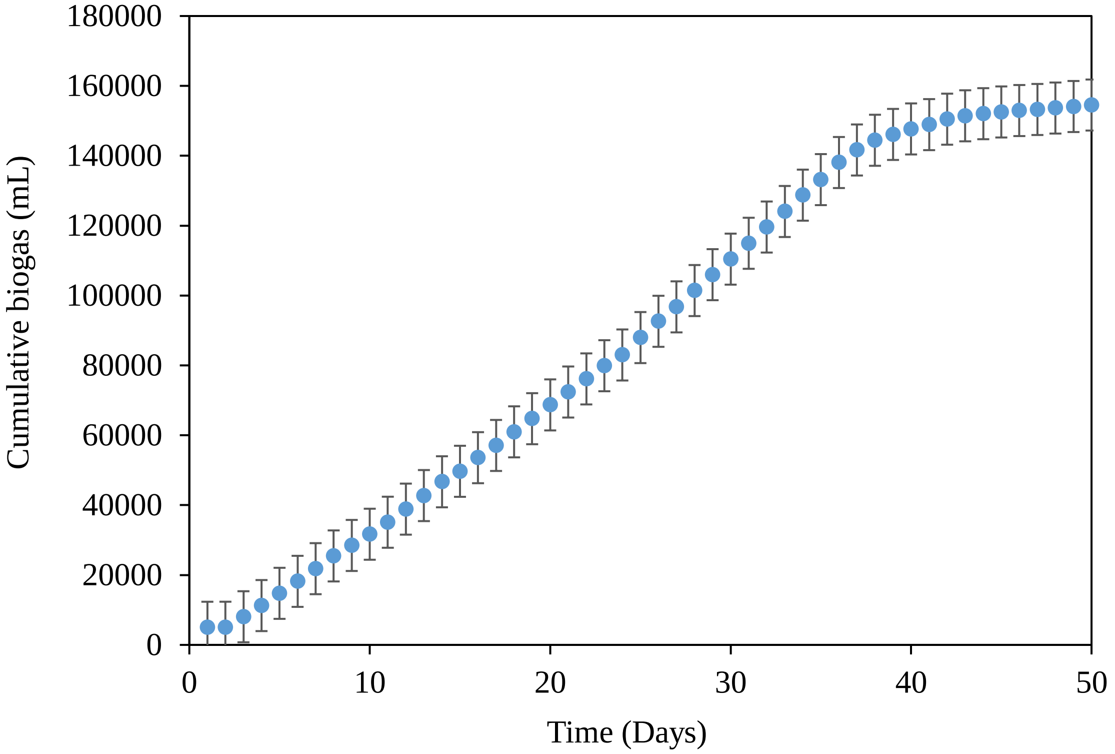


Fig. 5.4. Cumulative biogas production in 15.5L of reactor

5.1.3 Volatile solid reduction

In anaerobic digestion, the higher the VS reduction, the higher is the degradation and, at the same time, the higher the probabilities of biogas production (Dhamodharan et al.,2015). In these BMP studies, the highest VS reduction is observed in ratio 2 (about 28.40%), followed by ratio 2.5 with 26.03% (Fig. 5.5), and cumulative biogas production shows 3319 mL and 2903 mL, respectively. Volatile solid reduction increases from 0.5 to 2. The ratio greater than two (>2) started decreasing since degradation increased with increasing the ratio up to 2. Biogas yield was also less in lower ratio and higher than ratio 2. The reduction order followed by *P.hysterophorous* is 2 >2.5 >1.5> 1 >3 >0.5. In this study, it can be correlated that the higher the VS reduction higher is the production of biogas (Fig 5.1 and 5.2).

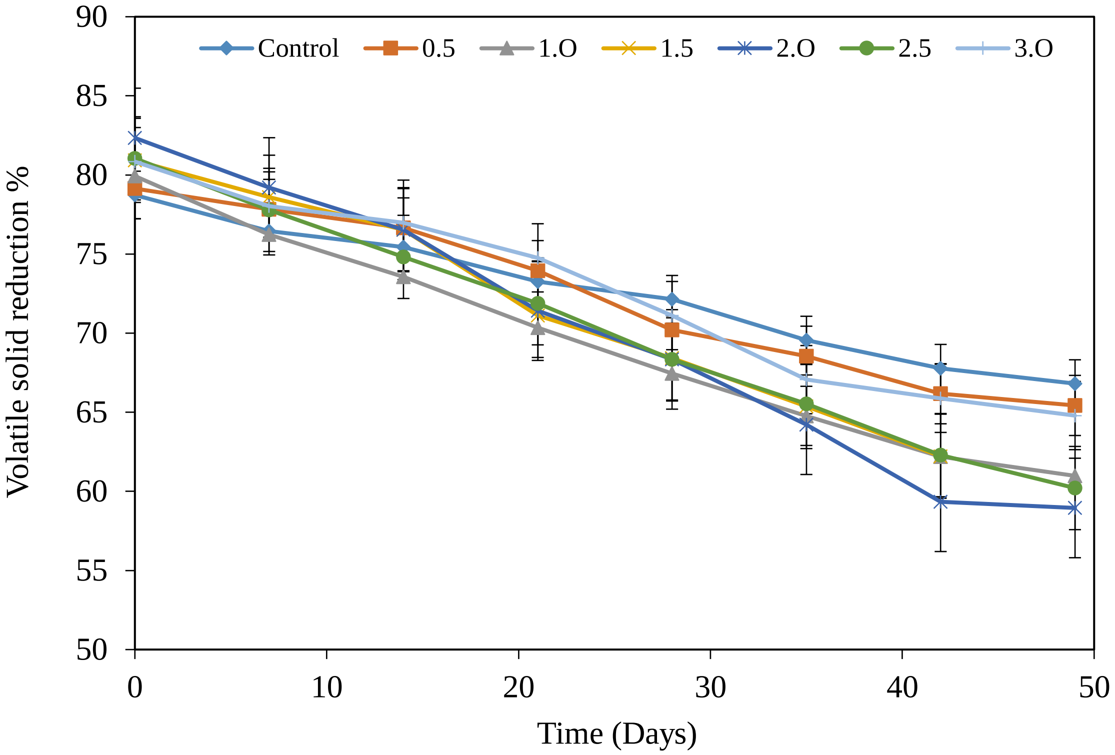


Fig. 5.5. Volatile solid reduction

5.1.4 Effect of soluble chemical oxygen demand (sCOD)

The complex molecule is converted into simpler molecules with the help of bacteria, which leads to increased sCOD. The amount of hydrolysis and solubilization can correlate with sCOD removal efficiency (Yang et al., 2011). In the present BMP study, the highest sCOD was obtained as 14780 mg/L in 28 days, and it got reduced to 5720, which is about

61.29% in F/M of ratio 2. For sCOD, the reduction order followed by *P. hysterophorousis* is 2>2.5>1.5>1>3>0.5. The sCOD increased up to 28 days, and later on, it decreased because of the acids produced by the microbial activity. Similar results were found by Moukazis et al., 2017. With an increase in the sCOD, methane production is also increased (Passos et al., 2015; Barua and Kalamdhad, 2016). The highest methane production was shown in 28 days in ratio 2 (Fig. 5.6). Soluble COD can be correlated with methane production. As the sCOD increased, VFA also increased. Barua and Kalamdhad, 2016 in water hyacinth substrate, found similar results.

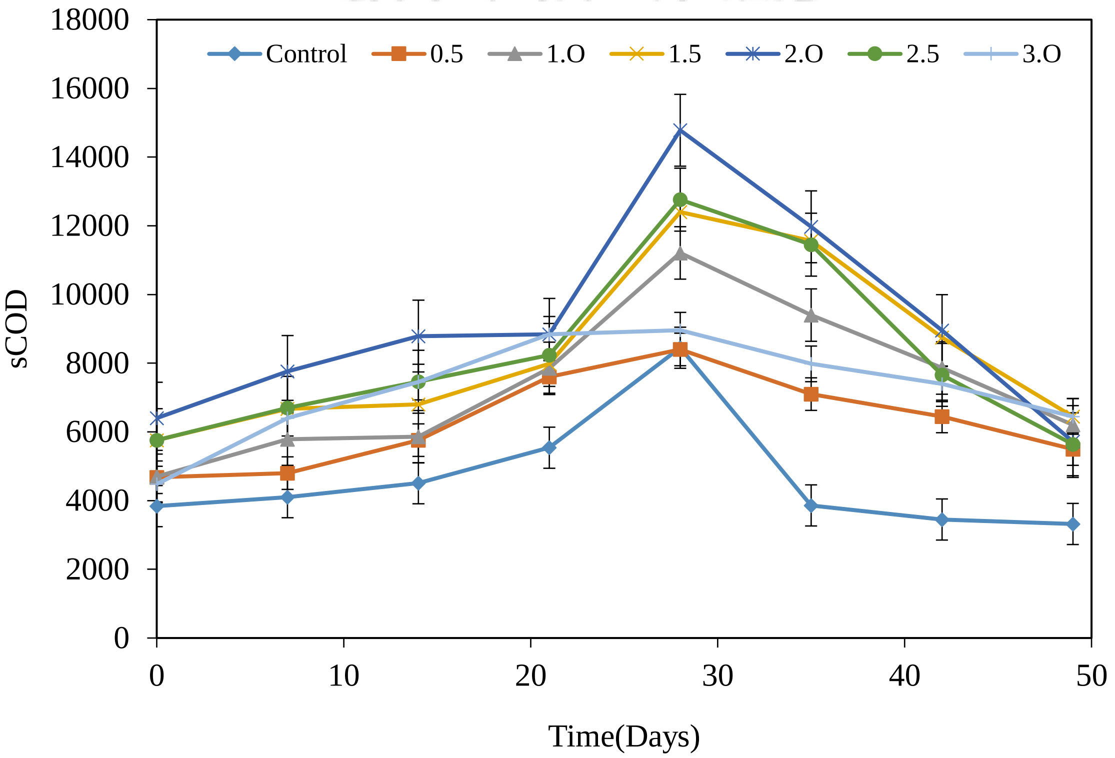


Fig. 5.6. Variation of sCOD

5.1.5 Volatile fatty acid (VFA)

As the digestion started, sugar was converted into acid, which leads to the rise of VFA. The highest amount for VFA concentration was found in F/M ratio 2 in 28 days, followed by the 1.5 ratios. 674 ±5 mg/L of VFA was obtained from ratio 2 followed by F/M ratio 1.5 around 665±7 (Fig. 5.7). Methane production gets hampered once the VFA concentration crosses 13000 mg/L (Vieitez and Ghosh, 1999). It was observed that VFA increased from 28 to 35 days in ratios 0.5 to 3; after that, it started decreasing. The same trend was found in all the ratios, which are due to the beginning of the methanogenic phases. Barua and

Kalamdhad, 2017 in water hyacinth substrate, observed same outcomes. During anaerobic digestion, the amount of VFA, acetic acid should come under 2000 mg/g (Yadvika et al., 2004). Methanogenic bacterial activity gets affected when the pH value drops to 6.0-6.5 (Speece, 1983); (Sallis and Uyanik, 2003; Lem et al., 2009;). It was observed above pH 5 that more than 75% of biogas production is achieved (Jain and Mattiasson., 1988). pH was started to decrease after three days, which was maintained between 6.5 to 7.5 using sodium bicarbonate (Owen et al.,1979). Production of acid leads to a drop in pH, which affects microbes' activity (Ahring, 1995). Therefore, if the reactor's VFA production passes beyond the limit, it inhabits the microbial activity, which leads to the effect acetogenesis stage in the anaerobic digestion process.

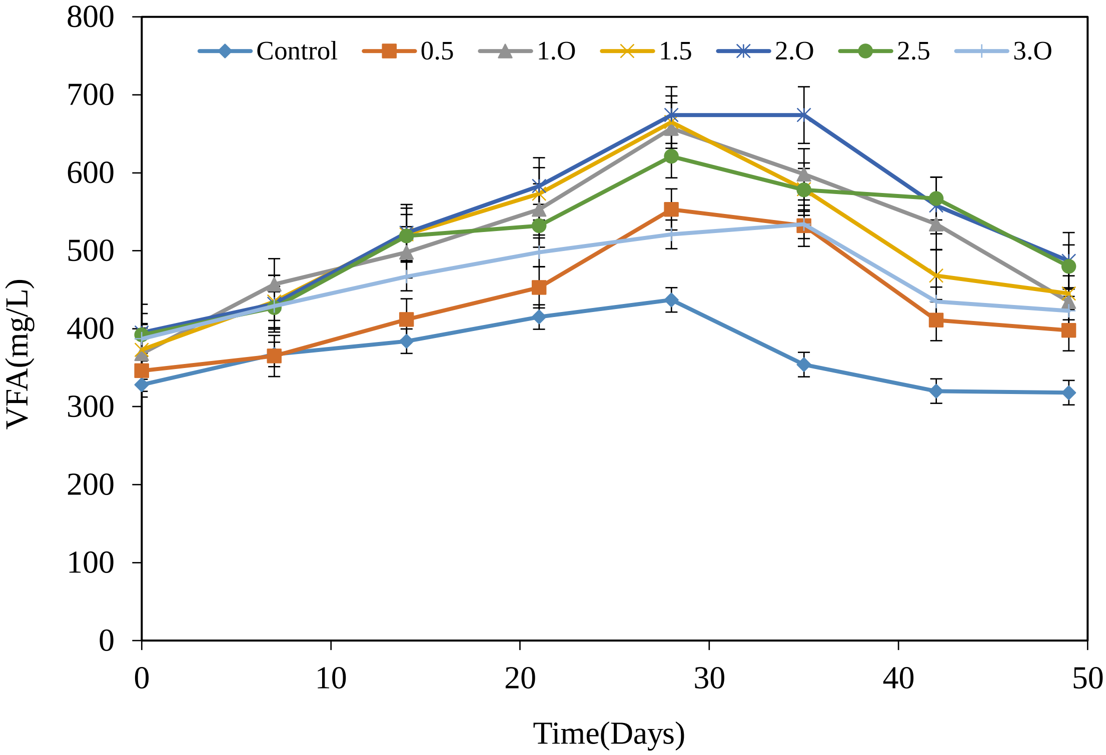


Fig. 5.7. Volatile Fatty acid

5.1.6 Kinetic study

The kinetics F/M ratios were determined by the Gompertz equation (Lee et al., 2013), where cumulative methane production value for *P.hysterophorous* was fitted for 50 days. The kinetic parameter was shown in table 5.1. The higher the value of M higher is methane production, the higher the VS reduction (Veluchamy and Kalamdhad, 2016). The value M was calculated for the best F/M ratio, which was found in rate 2 (4.0000 L CH_4) followed by ratio 2.5 (3.9219 L CH_4). In ratio, 2 there were sufficient microorganisms present; as the ratio increased, the solid content also increased, which made the hydrolysis difficult. Due to higher TS content, maximum methane production can be correlated with the mass transfer problem (Raposo et al., 2012; Xu et al., 2014). The R^2 value of the entire ratio was beyond 0.90. Barua and Kalamdhad, 2017 obtained similar kind of results. This kinetic study specifies that *P. hysterophorus* had the potential to produced biogas.

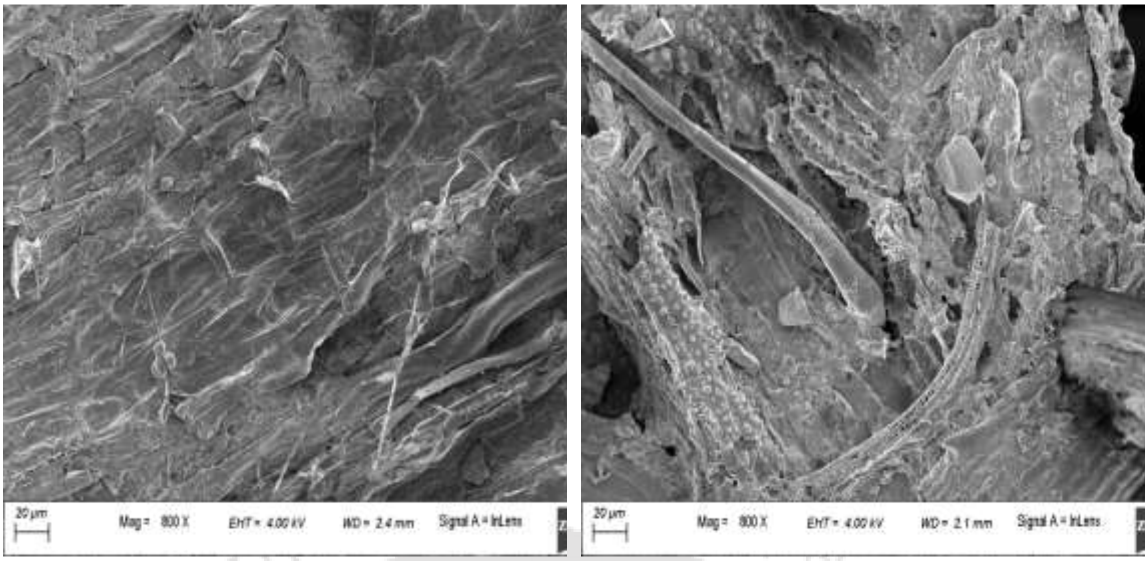
Table 5.1 Kinetic Value for *P. hysterophorous* with different F/M ratio

F/M ratio	**M(LCH_4)**	**R_m(CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
0.5	3.4511	0.0543	0.0010	0.987	2.400
1	3.8334	0.0577	0.0010	0.989	2.579
1.5	3.8334	0.0581	0.0010	0.987	2.648
2	4.0000	0.0686	0.0013	0.989	3.319
2.5	3.9219	0.0640	0.0010	0.982	2.903
3	3.7488	0.0564	0.0010	0.982	2.527

5.1.7 Morphological analysis

To recognize the changes of the internal structure of the *P. hysterophorous* FESEM and XRD was performed. Fig 5.8a 1st day sample, the rigid, smooth structure was observed that provide information of complex lignin structure in the cell wall. On 21st day, the sample (Fig 5.8b) minor free arrangement of the cell wall was observed as it was a tiny uncluttered because of microbial activity.

In the XRD spectra (Fig 5.9), a sharp peak was found in the 1-day sample. However, no such peaks were observed after the 21-day sample, which indicates that the crystalline substrate converted into amorphous. Cellulose is a tough crystalline group defiant to hydrolysis, present in the lignocellulose cell wall of *P. hysterophorous*. Thus, crystalline cellulose takes a long time to degrade, which increases the hydrolysis period in anaerobic digestion.



(a) (b)

Fig. 5.8. FESEM images of (a) sample on 1st day (b) Sample on 21st day

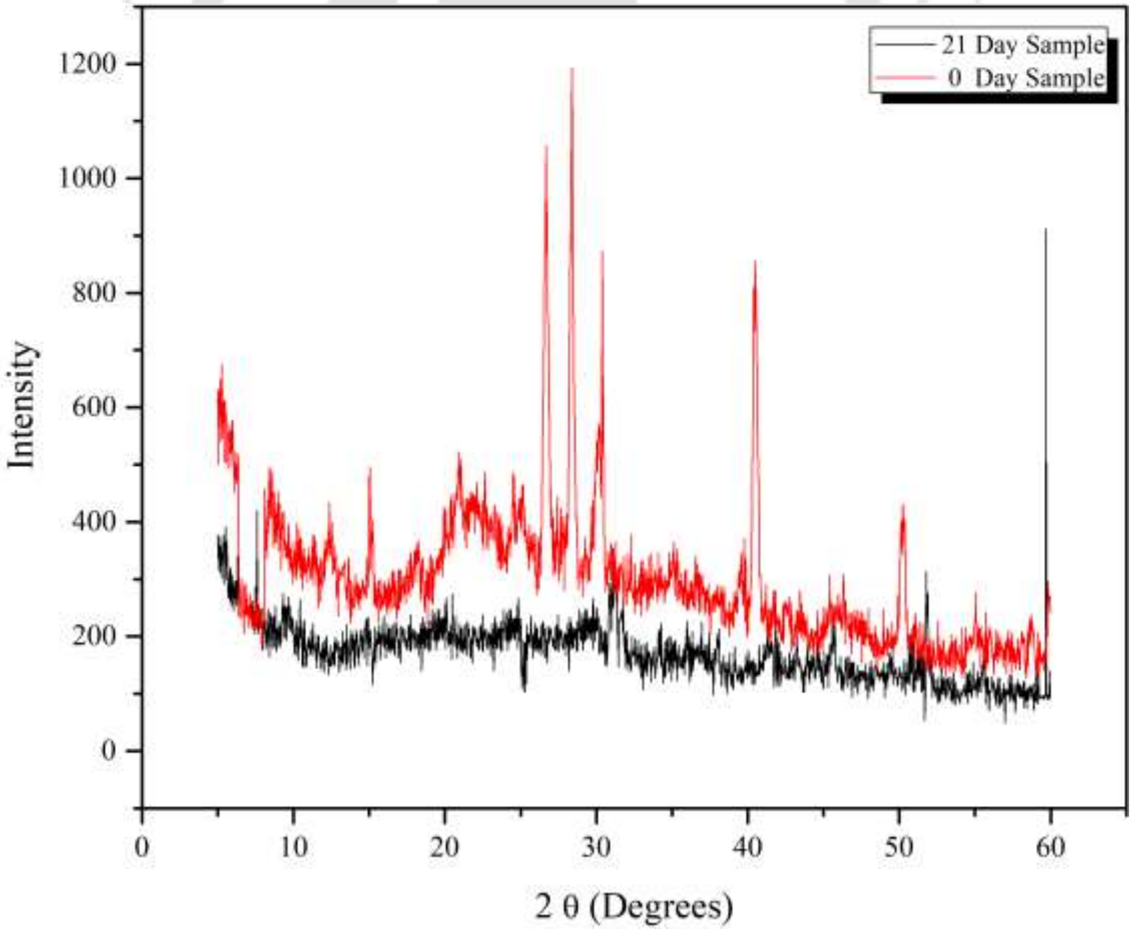


Fig 5.9. XRD Study

5.2 *Lantana camara*

5.2.1 Biogas production

The BMP trial was prepared with various F/M ratios, i.e., 1, 1.5, 2 and 2.5 for 50 days, and the ambient temperature fluctuated between 30 °C and 40 °C. The BMP study observed that (F/M) ratio 1.5 showed the maximum methane production followed by ratios 2 and 2.5, as shown in fig. 5.10. In 25 days, it showed the highest methane production (195.5 ± 8 mL CH_4/g VS). The amount of biodegradation of substrate was directly related to the production of methane (Esposito et al., 2012). Generally, the hydrolysis stages are time-consuming, and substances' degradation occurs very slowly (Gurung et al., 2012). In F/M ratio 1.5, the hydrolysis period was less as compared to other ratios. Methane production is dependent on the rate of biodegradation of a substance (Barua and Kalamdhad, 2017). Cumulative methane production in F/M ratio 1.5 was found out to be highest (4801.5 mL) followed by 2 and 2.5, respectively, as shown in Fig 5.11. Methane production tendency rose from F/M ratio 1 to 1.5; after that, it started reducing from F/M ratio 2. Lignocellulose present in a plant cell is a substrate source, so initially, it takes time to produce methane. In F/M ratio 1, the substrate concentration was less compared to the inoculum. Similarly, in F/M ratio 2, the substrate concentration was higher than the inoculum, which may be the reason for the increased production of the inhibitors like ammonia and nitrate, which resist the production of methane and affect microbial activity. The methane and cumulative methane production of all F/M ratios were relatively higher than the control (50 g cow dung). After conducting a 1L of BMP study, the batch reactor of 20 L capacity was performed with a working volume of 15.5 L with an ideal F/M ratio 1.5. The highest methane yield was observed on the 19th day (2650 ± 18 mL CH_4/g VS) (Fig.5.12), and in (Fig 5.13), cumulative methane production was 42574 mL in 20L of the batch reactor, which is quite valuable.

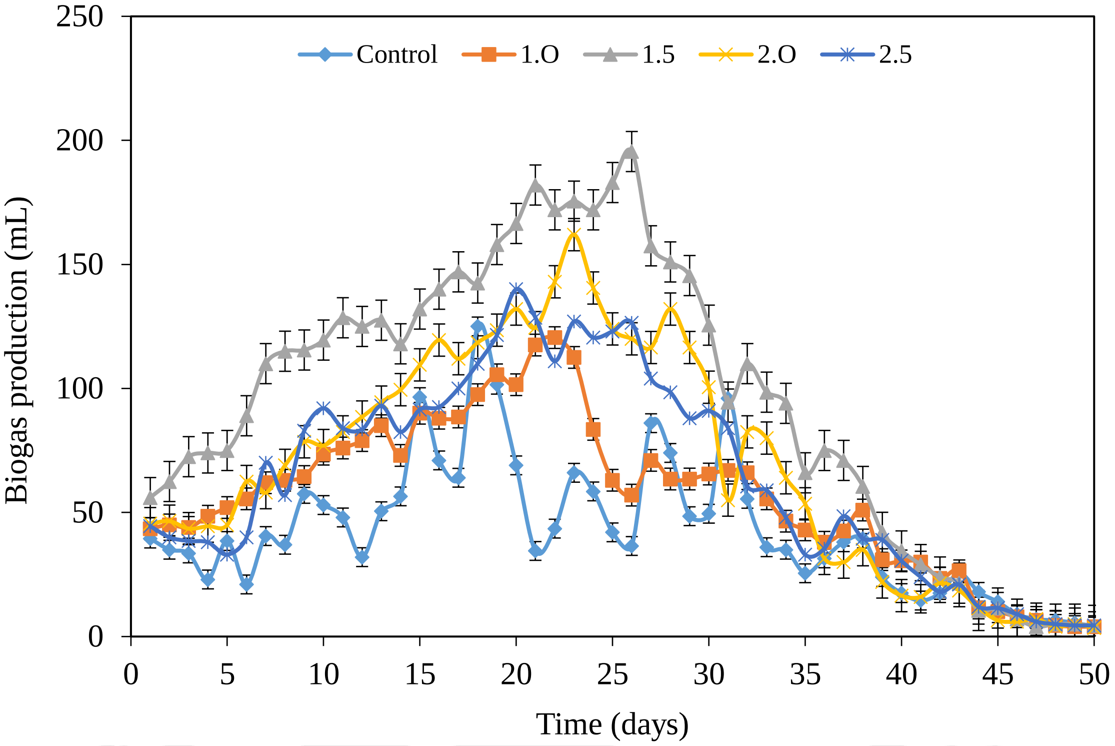


Fig. 5.10. Daily biogas production

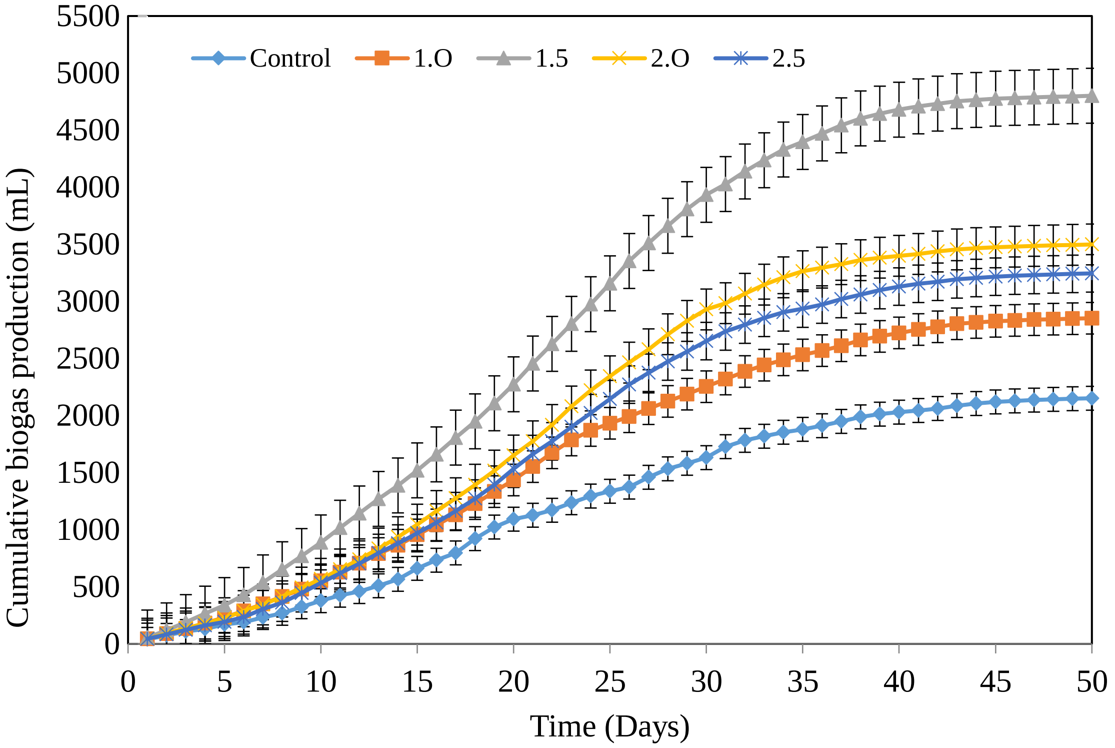


Fig 5.11. Cumulative biogas production

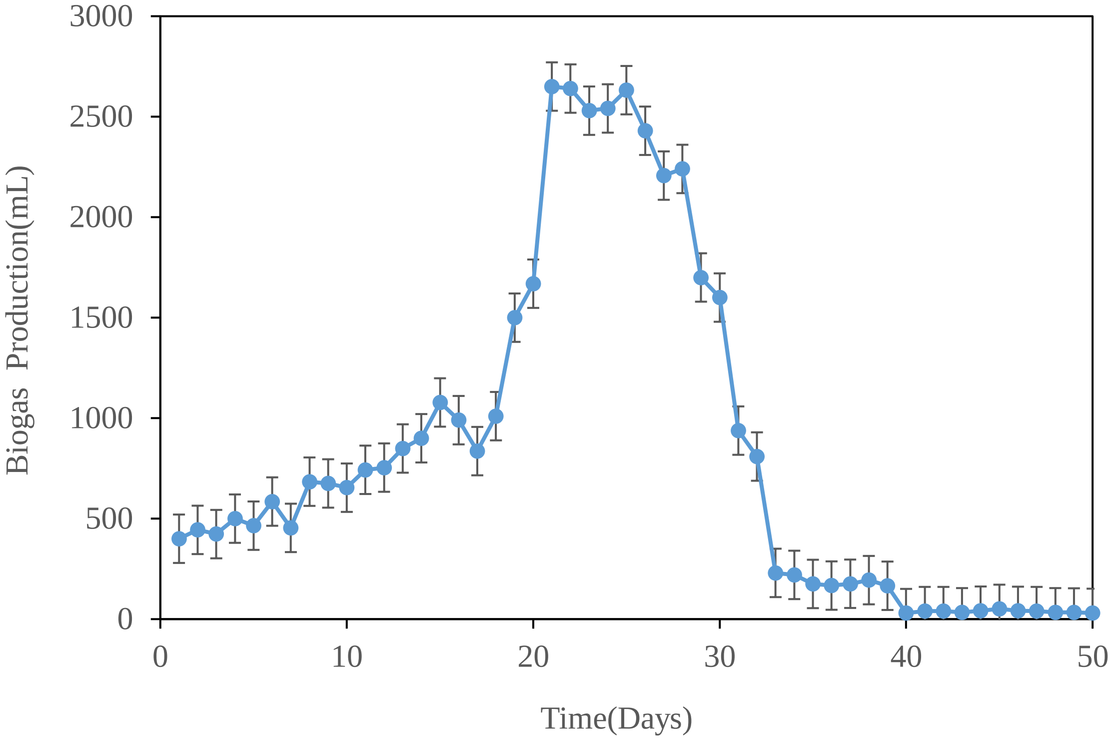


Fig. 5.12. Daily biogas production

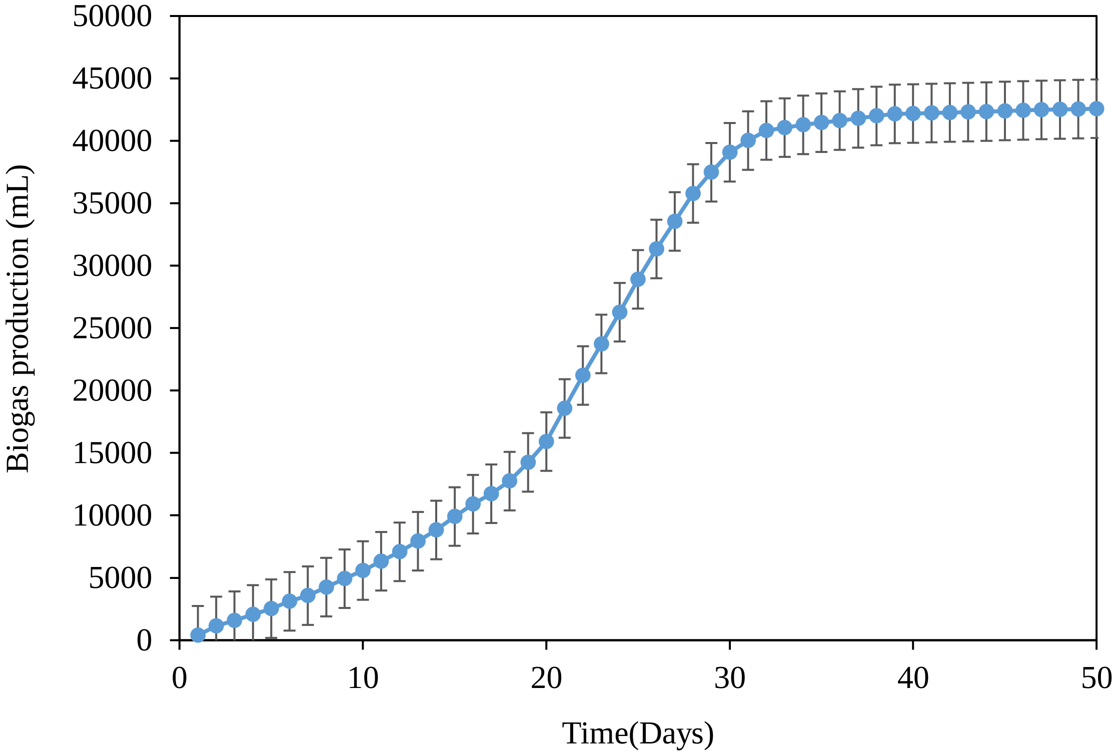


Fig. 5.13. Cumulative biogas production

5.2.2 Effect of volatile solids reduction

The degradation pattern of volatile solids (VS) was observed in Fig 5.14. Reduction of VS can be directly correlated to methane production (Dhamodharan et al., 2015). Although there is a decline in the pattern of volatile solids at F/M ratio 1.5, the highest VS reduction was found (49.63%), followed by F/M ratio 2 (46.47%) and F/M ratio 2.5 (45.54%), respectively. The order of the F/M ratio during VS reduction of *L.camara* follows 1.5 >2 >2.5> 1. Evidently, the higher the VS reduction, the higher the methane and cumulative methane production, as shown in Fig 5.10 and 5.11. VS reduction is directly correlated to methane yield. VS reduction is also dependent on microbial activity. Microbes require balanced nutrition for their growth; hence, a proper ratio between inoculum and substrate is necessary to develop the microbial activity. In control, relatively less VS reduction was found as compared to all other F/M ratios (32.58%).

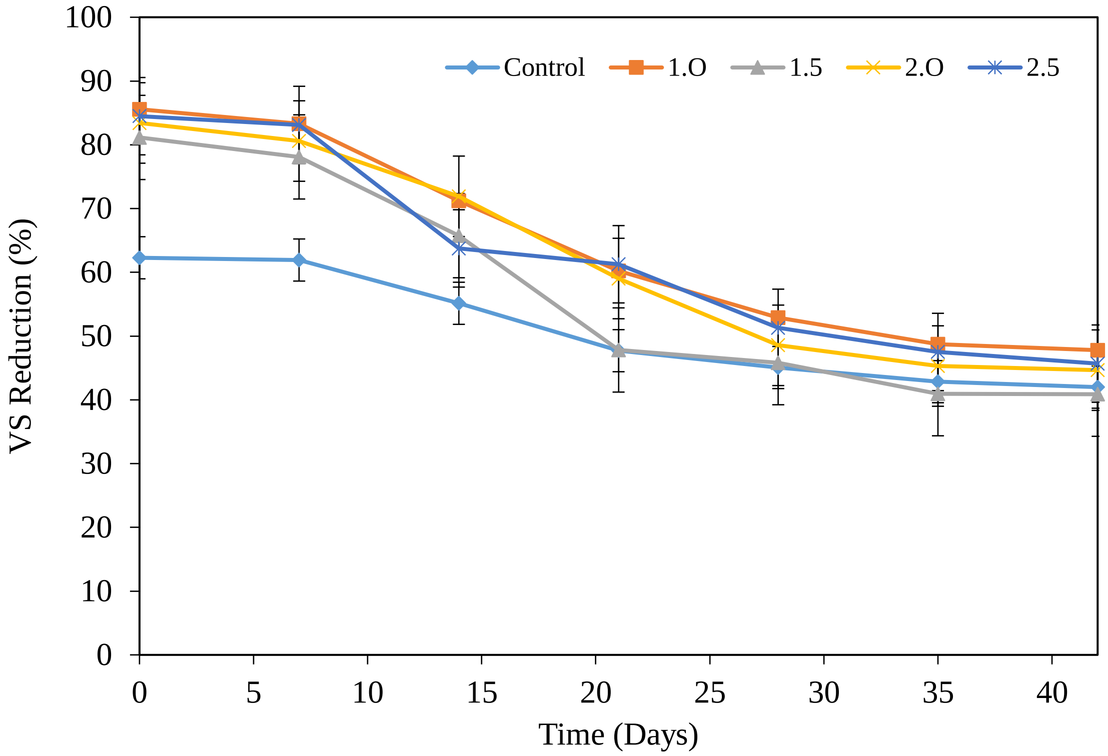


Fig. 5.14. Volatile solid reduction

5.2.3 Effect of volatile fatty acids

When organic molecules started to break down, volatile fatty acid production (VFA) started. VFA is produced due to sugar convert into VFA and lastly into biogas. VFA increased with an increase in the number of days until the 21st day, and then VFA started to decrease progressively. The action of the acidogenic led to the production of VFA up to the 14th day. After 14th-day, the methanogenesis stage might start, which leads to the decline of VFA production (Barua et al., 2019). With the end of the hydrolysis period, the production of VFA started, which then was converted to acetic acid and finally producing carbon dioxide and methane as products. The maximum amount of VFA production was found in F/M ratio 1.5 and 2 (715 ±10 mg/L) followed by ratios 2.5 and 1, respectively, as shown in fig 15. The reduction of VFA after the 14st day may be due to the start of the methanogenesis phase. The increase and decrease of VFA may affect the pH of the reactor. So, in order to maintain the methanogenic activity, pH in the reactor should be maintained. The decrease in pH from 6.0-6.5 may affect bacterial activity (Sallis and Uyanik, 2003; Lem et al., 2009). More than 75% of methane can be achieved when the pH is above 5. (Jain and Mattiasson., 1988). The methanogens grow best in the optimum pH range of 6.6-7.6 (Rittmann and McCarty, 2001). Methane production is reduced when VFA is more than 13000 mg/L (Viéitez and Ghosh, 1999). During VFA production, pH fluctuated inside the reactor, which was maintained using sodium bicarbonate. (Owen et al., 1979; (Veluchamy and Kalamdhad, 2016).

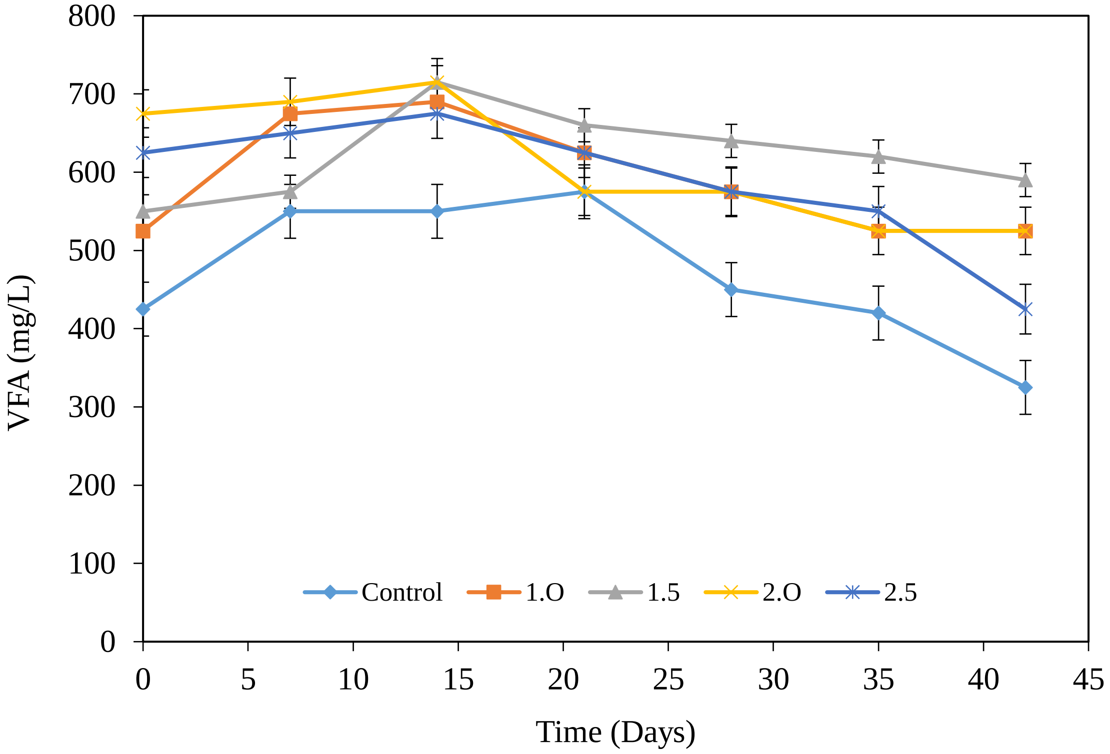


Fig. 5.15. Volatile fatty acids

5.2.4 Effect of soluble chemical oxygen demand (sCOD)

In Fig. 5.16, sCOD increased from 0th day to 21st day for all F/M ratios and then it dropped after the 21st day. The amount of sample hydrolyzed can be related to the amount of organic matter degraded (Yang et al., 2011). The increase in sCOD up to the 21st day and its decrease thereafter might be related to the production of toxic substances during the growth of the microbial community, which may had hampered the degradation process, such as the production of ammonia and sulphite acting as inhibitors in the long chain of organic matter, which might not degrade further. Sulphite ion is toxic to microorganisms (Stefanie et al., 1994). Maximum score found in F/M ratio 1.5 (8000 mg/L). Order of second followed by *L. camara* is 1.5>2>2.5>1. In ratio 1, the amount of substrate is less as compared to inoculum and in a higher ratio more than 1.5 inoculum amount was more, which might hamper the degradation process. Therefore, 1.5 is the appropriate ratio. The increase in sCOD leads to increased methane production, VFA, and vice-versa (Barua and Kalamdhad, 2016).

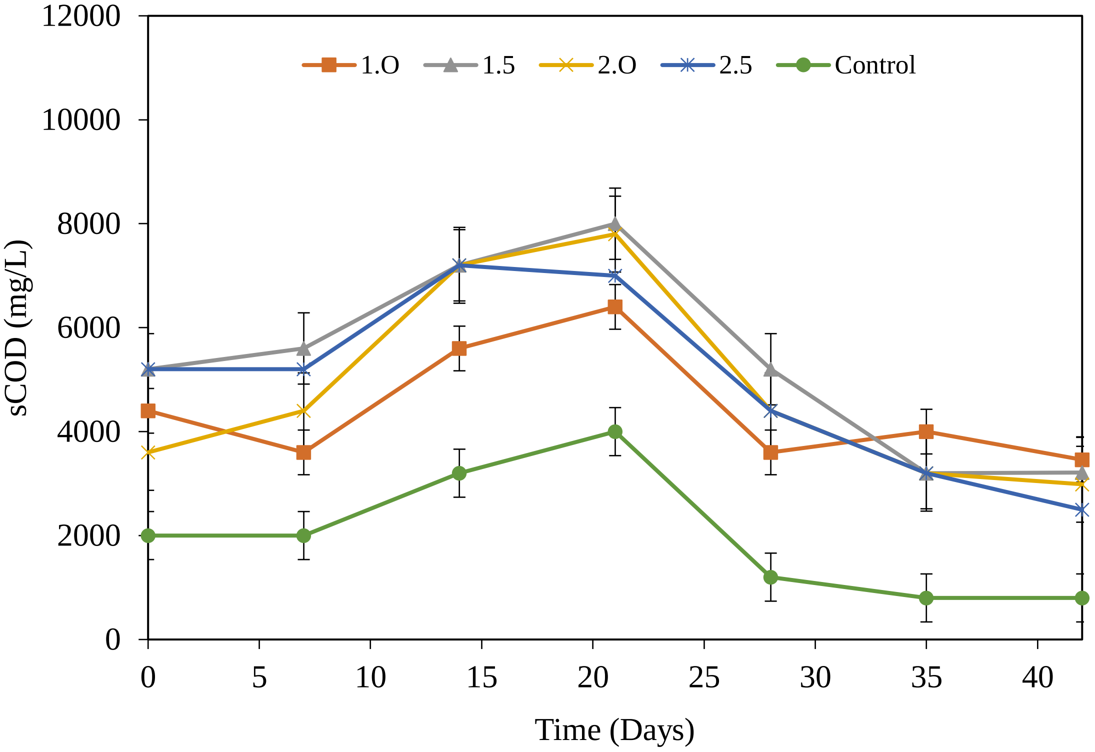
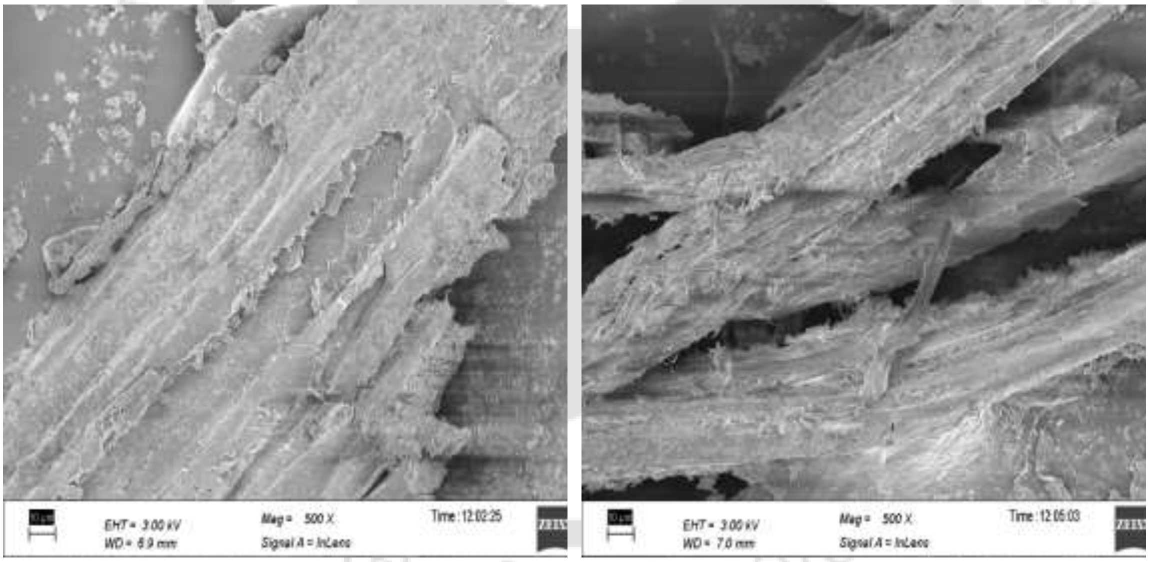


Fig. 5.16. Variation of sCOD

5.2.5. Morphological study

To recognize the internal changes of *L. camara* during its digestion, spectroscopy analysis was performed. Fig. 5.17a denotes the 0-day FESEM, which appeared to be tough, hard and smooth as the outer part of the plant cell wall is covered with strongly bound lignin cover. On the 21st day, the sample (fig 5.17b) structure was fragmented into pieces, which may be because of large particles being degraded into simpler compounds. Time taken to break down the complex molecule was 21 days.

Crystallinity and amorphous nature of the substrate can have determined by XRD analysis. Sharp peaks were observed at the 0th-day sample, which represented the crystalline nature of the substrate. After 21 days, the peak was reduced as compared to 21st-day sample, as shown in fig 5.18. Cellulose is a harsh crystalline nature present in the cell wall of the *L. camara, which* is responsible for slowing down of the hydrolysis period. Microbial activity also slows down when the sample is highly crystalline.



(a) (b)

Fig. 5.8. FESEM images of (a) sample on 0th day (b) Sample on 21st day

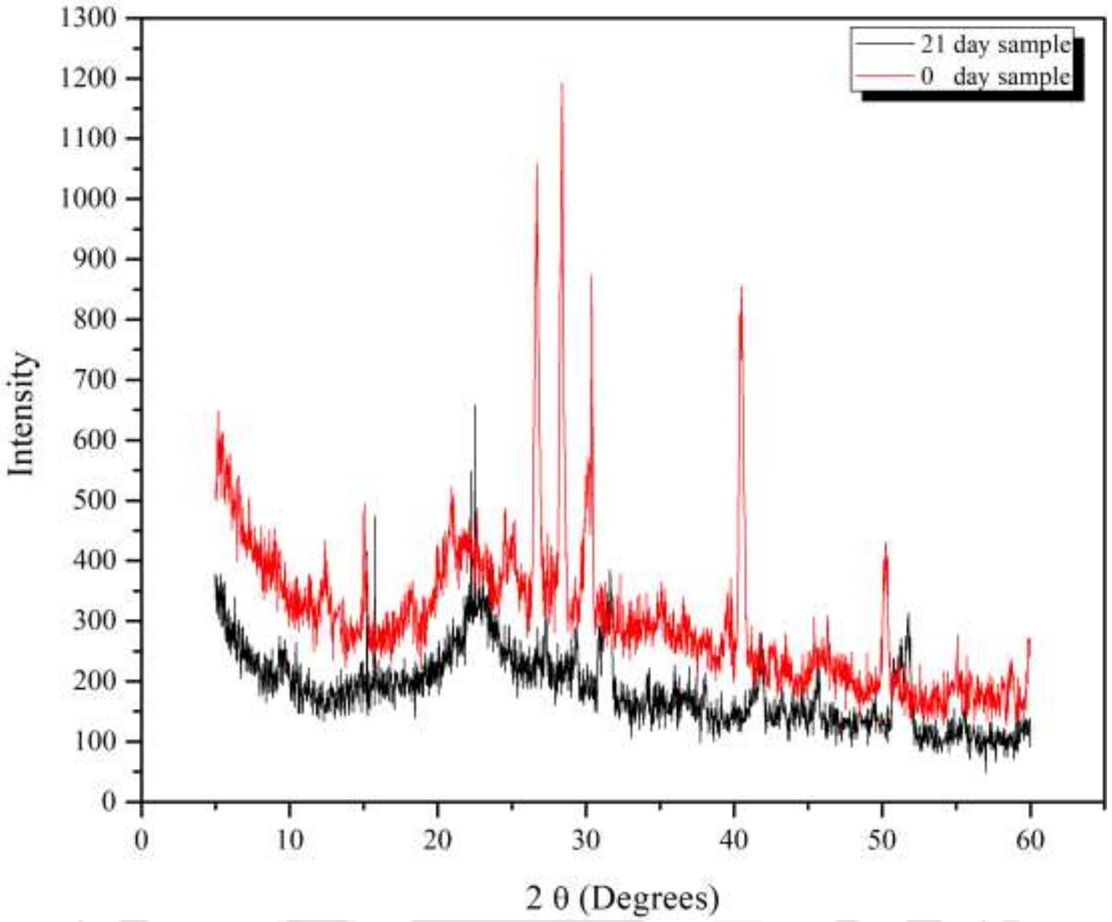


Fig 5.9. XRD Study

5.2.6. Kinetic study

A kinetic study can determine the efficiency of the ideal F/M ratio. The cumulative methane production values were fitted to the Gompertz equation curve for 50 days (Lee et al., 2013). In table 5.2, kinetic values were provided for all the F/M ratios. Values of M can be related to VS reduction (Veluchamy and Kalamdhad, 2016). Maximum M value was found equal in F/M ratios 1.5, 2 and 2.5 (4.0000 L CH_4) followed by ratio 1 and control, but experimental value for F/M ratio 1.5 showed the highest methane yield. In all F/M ratios, R^2 values were above 0.90 that specified the efficiency of methane production.

Table 5.2. Kinetic Value for *L. camara* with different F/M ratio.

F/M ratio	**M(LCH_4)**	**R_m(L CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
Control	3.6773	0.0556	0.0010	0.903	2.151
1	3.8634	0.0954	0.0010	0.909	3.365
1.5	4.0000	0.1000	0.0010	0.970	4.177
2	4.0000	0.1000	0.0010	0.945	4.994
2.5	3.9000	0.0974	0.0010	0.942	3.184

5.3 *Ageratum conyzoides*

5.3.1 Biogas production

The BMP test was done with various F/M ratios, i.e., 1, 1.5, 2 and 2.5 for 50 days and the ambient temperature varied between 30°C to 38° C. It was observed that in *A. conyzoides* (F/M), ratio 2 showed the maximum biogas production followed by ratios 1.5 and 2. In 25 days, it showed the highest methane production around (205 ± 10 mL CH_4/g VS) (Fig 5.19). The rate of biodegradation of substrate was directly related to methane production (Esposito et al., 2012). In F/M ratio 2, the hydrolysis period was less as compared to other ratios. The hydrolysis period is dependent on the biodegradation of a substance. (Barua and Kalamdhad, 2017). It was observed in Fig 5.20 that ratio 2 showed the highest cumulative production 4994 mL followed by 1.5 and 2 respectively. Biogas production trend was increased from 1 to 2 after that it started decreasing at ratio 2.5. As the source of the substrate was lignocellulose (plant), initially it took time to produce biogas. In a lower F/M ratio, the inoculum concentration was higher compared to the substrate. Similarly, in the higher ratios, the substrate concentration was higher than the inoculum, which may increase the inhibitor like ammonia and nitrate that hampered the biogas production. As a result, biogas production was less in a lower ratio. The biogas and cumulative biogas production of all the F/M ratios were fairly higher than the control (50 g cow dung). After conducting 1L of BMP study, 20 L capacity of the batch reactor was performed with a working volume of 15.5 L with ideal F/M ratio 2. Highest biogas yield was observed in 21st day (2450 ± 15 mL CH_4/g VS), and cumulative biogas was found to be 41059 mL in 20L of the batch reactor (Fig. 5.21 and 5.22) which was quite beneficial.

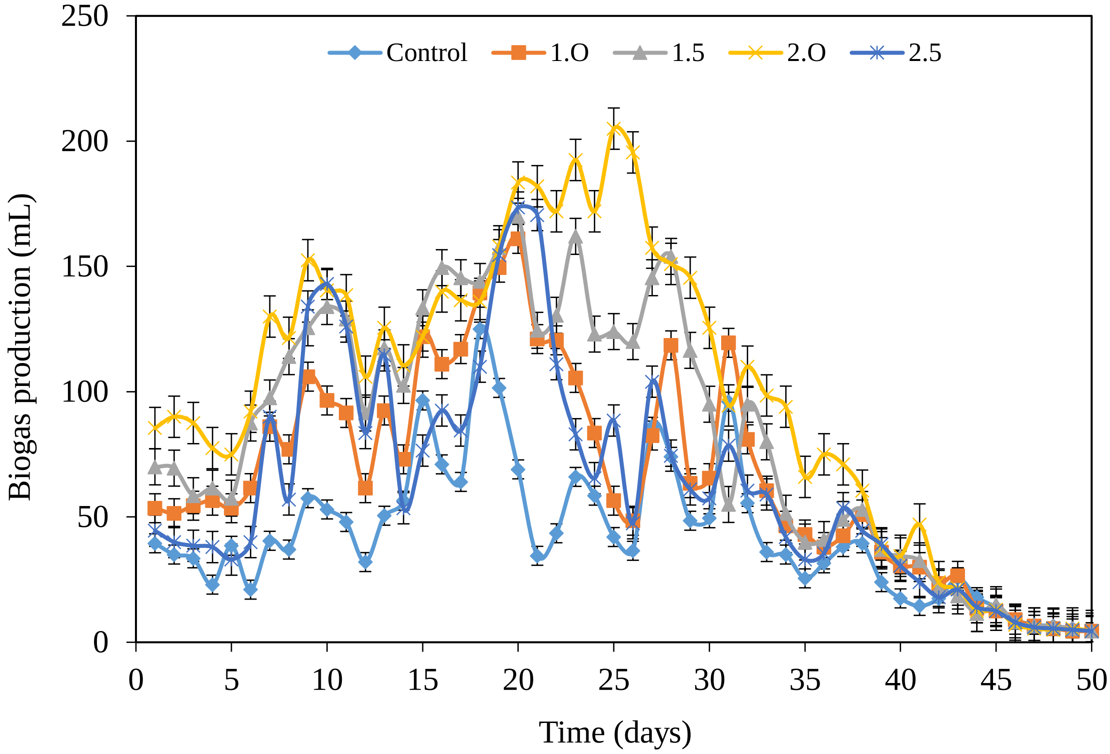


Fig. 5.19. Daily biogas Production

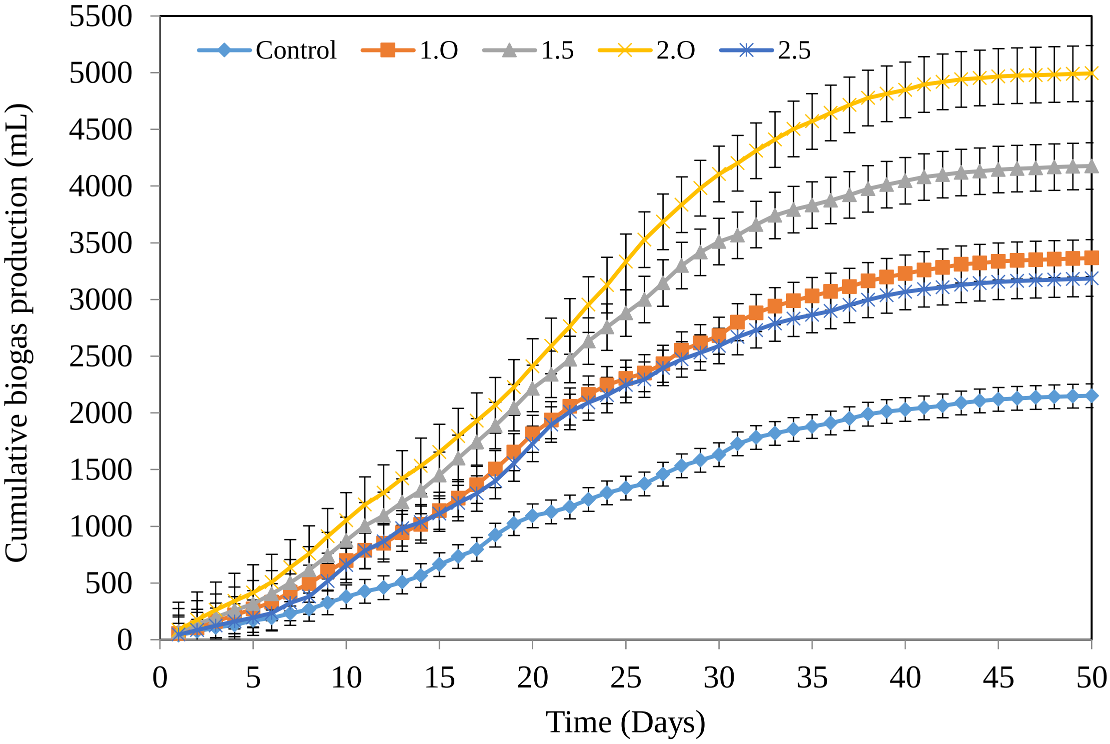


Fig. 5.20. Cumulative biogas Production

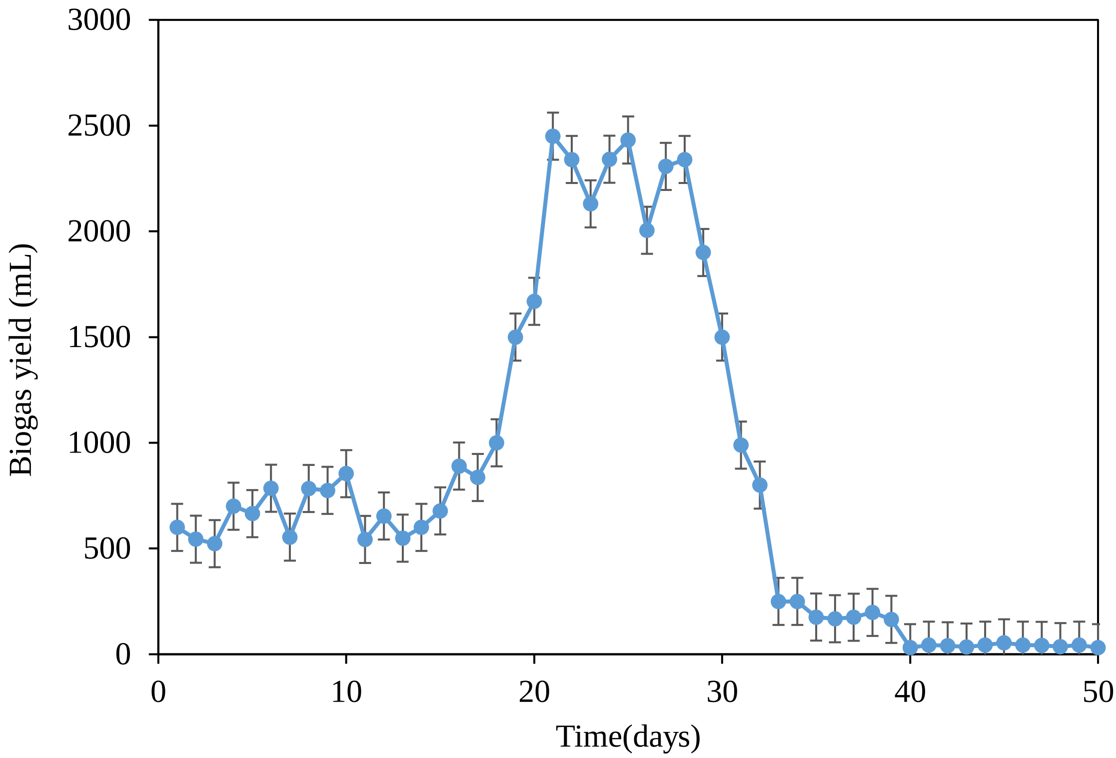


Fig. 5.21. Daily biogas production

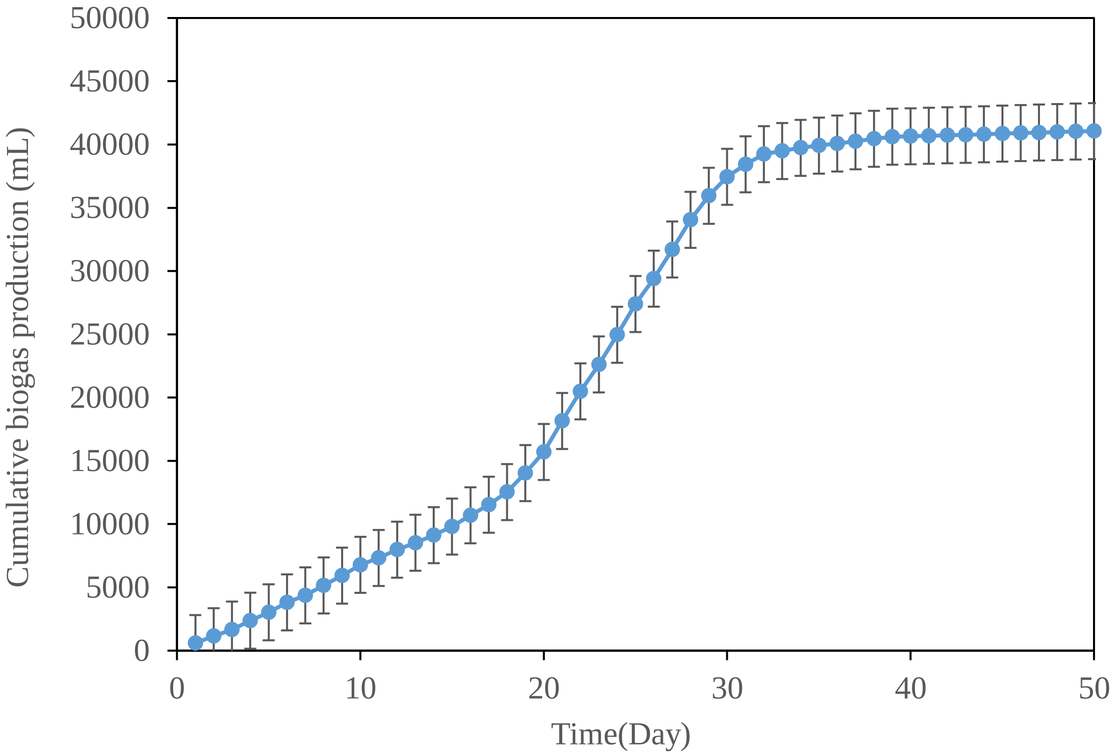


Fig. 5.22. Cumulative biogas production

5.3.2 Effect of volatile solids

In Fig 5.23, a reduction pattern of VS was observed. Dhamodharan et al., 2015 reported that higher the VS reduction, the higher is the probability of biogas production. The highest VS reduction was found at F/M ratio 2 (45.30%) followed by 2.5(43.03%) and 1.5 (42.92%) respectively (Fig 5.23). The reduction trend followed for *A. conyzoides* as 2 >2.5 >1.5> 1. As the VS, the reduction was higher at F/M ratio 2, the biogas and cumulative biogas production were also higher. VS reduction was directly correlated with methane yield. The microbial activity can reduce VS. A balanced combination between inoculum and substrate is required to improve the microbial activity. In control (cow dung), the VS reduction was found to be 41.23%, which was comparatively less than that of all the F/M ratios.

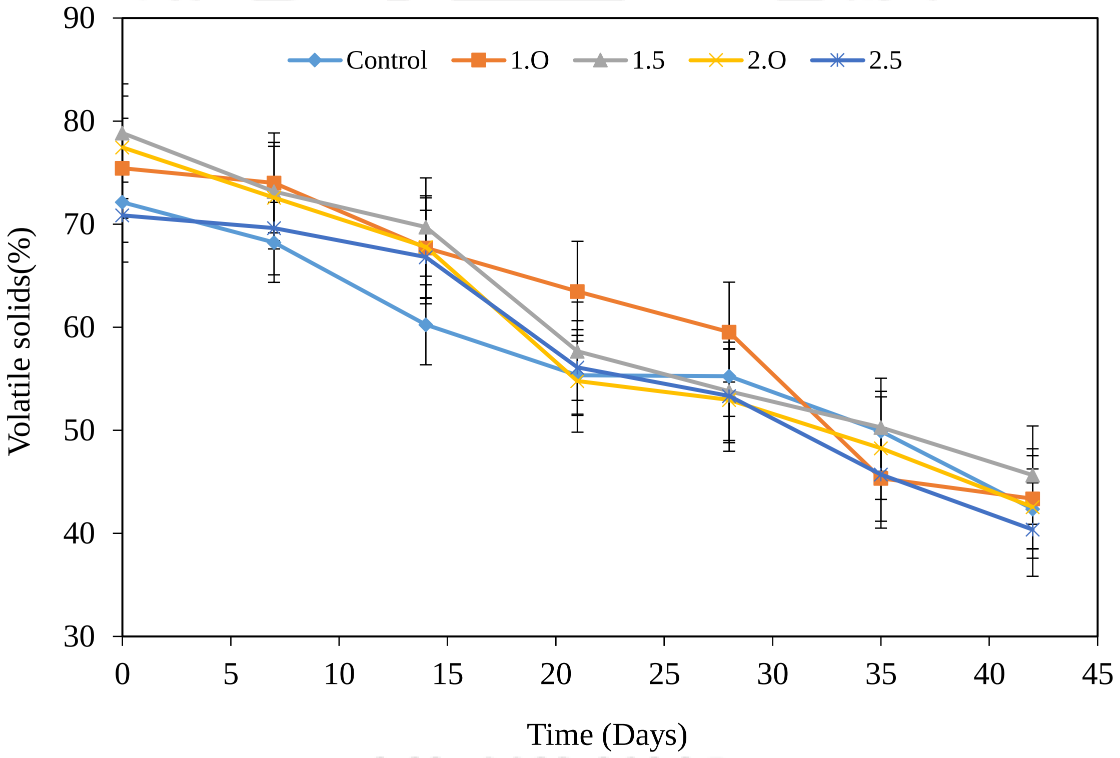


Fig. 5.23 Volatile solid

5.3.3 Effect of volatile fatty acids

As the complex molecule started to break down, the VFA began to be produced. VFA continuously increased for 21 days, and then it gradually decreased. An increase in the VFA was due to sugar being converted to acid, and finally, the products were carbon dioxide and methane. Acidogen's activity led to the production of VFA on the 21st day; after that, methanogens came to play their function, as they were sensitive to acidic conditions. The highest amount of VFA production was found corresponding to F/M ratio 2 (625 ± 25) followed by ratio 2.5 and 1.5, respectively, as shown in Fig. 5.24.VFA declined after 21 days due to the beginning of the methane production phase. Increasing and decreasing VFA may affect the pH of the reactor, as the pH of the reactor is a crucial factor to maintain the methanogenic activity. Methanogenic bacterial activity was affected due to a decrease in pH from 6.0-6.5 (Sallis and Uyanik, 2003; Lem et al., 2009; Speece, 1983). Above pH 5, more than 75% of biogas was produced (Jain and Mattiasson., 1988). pH was maintained between 6.5 and 7.5 using sodium bicarbonate (Owen et al.,1979).

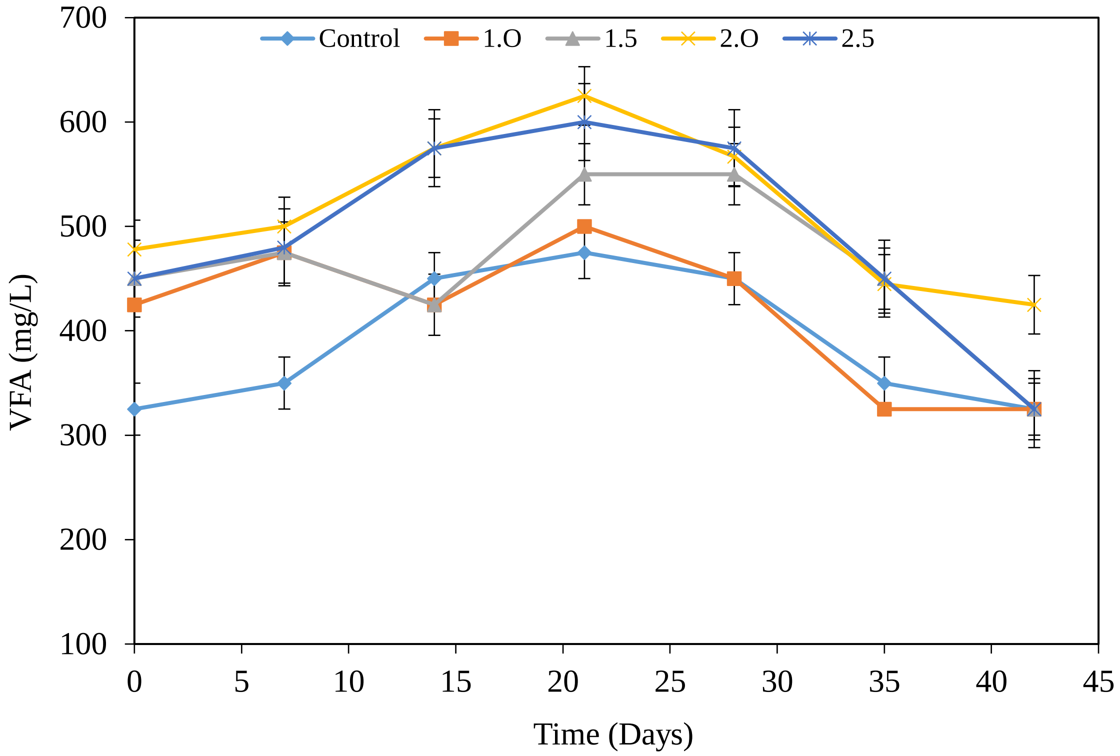


Fig. 5.24. Volatile fatty acids

5.3.4 Effect of soluble chemical oxygen demand

It was clearly observed in fig 5.25 that sCOD increased from 0 to 21 days in all the F/M ratios after which it started decreasing. Hydrolysis and solubilization can be correlated with the amount of organic matter degraded (Yang et al., 2011). The sCOD increased up to 21 days, and later on, it decreased, which may be due to the production of toxic substances during the growth of microbial numbers, which may hamper the degradation process. Other parameters like ammonia and nitrate also act as inhabitors so that a long chain of organic matter might not degrade further. F/M ratio 2 (8000 mg/L) showed the highest solubilization as compared to other ratios. The declining order of sCOD followed by *A. conyzoides* was 2>2.5>1.5>1. The increase in sCOD simultaneously increased methane production (Barua and Kalamdhad, 2016).

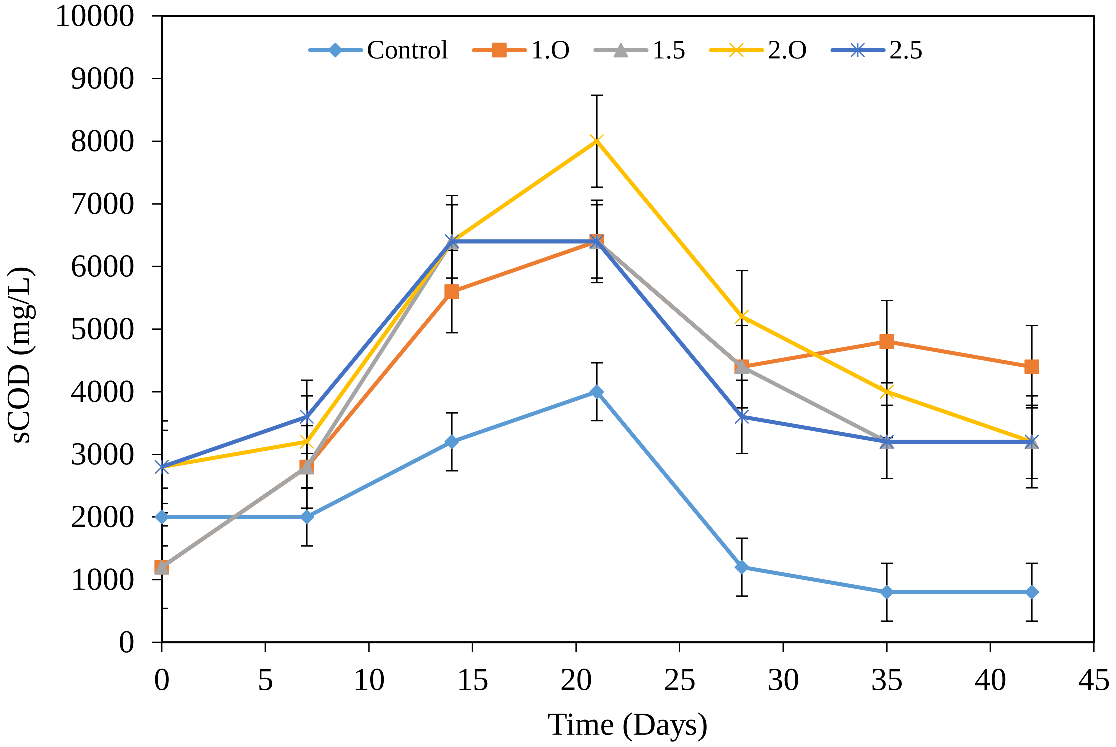
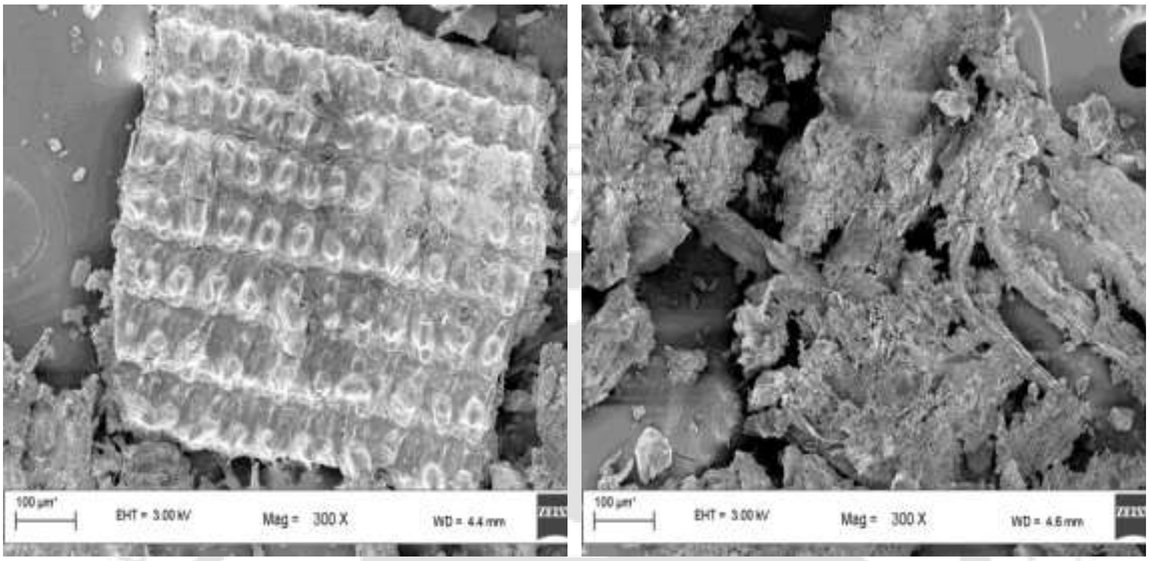


Fig. 5.25. Variation in sCOD

5.3.5 Morphological study

To identify the internal structure of *A. conyzoides*, a spectroscopy study was performed. Fig. 5.26a represented the 0-day FESEM, which was found to be rigid, inflexible and smooth. It might be strongly bound of lignin in the cell wall, as shown in Fig. 5.26a. The strong bond of lignin present in the outer part of the cell wall was responsible for appearing hard rocky structure. Barua and Kalamdhad, 2017 observed a similar type of result. On the 21st day, the sample (Fig.5.26b) represented a broken structure, which was free from the arrangement of the cell wall. It was also observed to be tiny and uncluttered because of microbial activity. Therefore, it required 21 days to break down the complex lignin structure completely.

To identify the crystalline and amorphous nature of the substrate, XRD analysis was done. In the 0-day sample, sharp peaks were observed, which showed the crystalline nature of the substrate. However, peaks were reduced in the sample after 21 days Fig. 27. Cellulose is a tough crystalline group which increased the hydrolysis period. It was difficult for the microbes to degrade the crystalline substance. The high crystallinity of cellulose reduced the production of biogas. Father pretreatment of this noxious weed might reduce the crystalline.



(a) (b)

Fig. 5.8. FESEM images of (a) sample on 0th day (b) Sample on 21st day

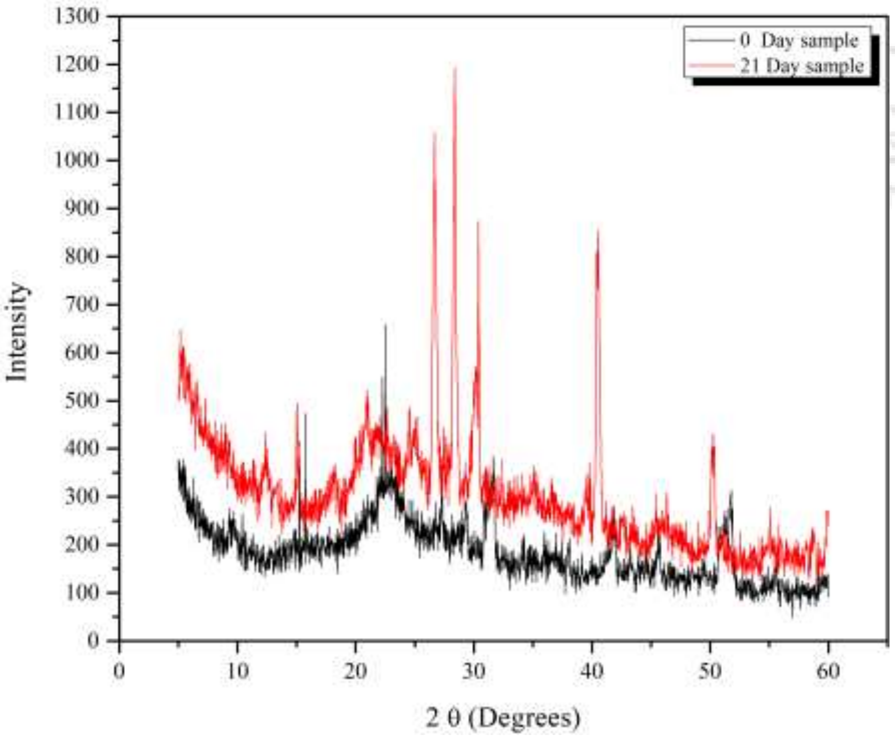


Fig 5.9. XRD Study

5.3.6. Kinetic study

To determine the efficiency of the ideal F/M ratios of both, the cumulative methane yield values 0f 50th days were fitted to the Gompertz equation curve (Lee et al., 2013). Kinetic parameters of *A. conyzoides* were shown in table 5.3 for all the F/M ratios. M value can be correlated with VS reduction (Veluchamy and Kalamdhad, 2016). It showed a higher M value in ratio 2 (4.0000 L CH_4) followed by ratios 2.5 and 1.5 (both having 3.9000 L CH_4). In all F/M ratios, the R^2 value was above 0.90 that indicated the efficiency of methane production.

Table 5.3 Kinetic Value for *A. conyzoides* with different F/M ratios

F/M ratio	**M(LCH_4)**	**R_m(L CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
Control	3.5773	0.0566	0.0010	0.913	2.151
1	3.8634	0.0954	0.0010	0.929	3.365
1.5	3.9000	0.0981	0.0010	0.917	4.177
2	4.0000	0.1000	0.0010	0.969	4.994
2.5	3.9000	0.0974	0.0010	0.952	3.184

5.4 Summary for Phase I

BMP study shows that the terrestrial weeds were the potential feedstock for the production of biogas through anaerobic digestion. This is an eco-friendly and cost-effective way to manage the weed. During anaerobic digestion of BMP assay, F/M ratio 2 (140 mL CH_4 g^{-1} VS on 26th day) for *Parthenium hysterophorus*, F/M ratio 1.5 (191 mL CH_4 g^{-1} VS on 26th day) for *Lantana camara* and F/M ratio 2 (178 mL CH_4 g^{-1} VS on 25th day) for *Ageratum conyzoides* were observed to be ideal. The BMP confirmatory test showed the biogas production potential and quite a viable quantity of methane production in a 20L batch scale reactor, which opens the field for pilot-scale study. These noxious weeds, creating havoc in agricultural areas worldwide, can be controlled and utilized to create cooking fuels for their households, which will prove to be a cost-effective approach in this weeds management. Further, pretreatment is required for these lignocellulose weeds to extract maximum methane and reduce the hydrolysis period.

CHAPTER 6

EFFECT OF THERMAL AND ELECTROHYDROLYSIS PRE-TREATMENT ON TERRESTRIAL WEEDS (*Parthenium hysterophorus, Lantana camara* and *Ageratum conyzoides*)

This chapter explores various thermal pre-treatment (hot air oven, microwave, autoclave and hot water bath) and electrohydrolysis pre-treatment. Thermal and electrohydrolysis pre-treatment shows the impact on the solubilisation and volatile fatty acid (VFA) of terrestrial weeds during anaerobic digestion to reduce the hydrolysis time and enhance biogas generation.

6.1. Phase II - Thermal pre-treatment

The study investigated the effect of various thermal pre-treatment on *P. hysterophorus, L. camara and A. conyzoides*. Due to lignin's presence, high cellulose crystallinity, which delimited available area, declines the biodegradability of terrestrial weeds and makes hydrolysis step tough in anaerobic digestion, which is also responsible for increasing the hydrolysis time and hampers the production of biogas. Hence, to speed up the hydrolysis phase and maximize methane production, pre-treatment is required. Pre-treatment is the disruption of the lignocellulose complex (Barua and Kalamdhad, 2016). To break down the lignin bond of these harmful weeds, all three heat modes were applied: conduction, convection and radiation. The heat transfer mode took place in a hot air oven at first by convection than by conduction, consistently circulating dry heat throughout the space with a fan's help. Initially, the outer cover is heated, then it steadily passed towards the center, and it ruptured the lignin bond containing the cell wall of *P. hysterophorus* (Barua and Kalamdhad, 2016).

Similarly, during hot water bath treatment, heat transfer occurred through convection initially followed by conduction here, the heat was passed through hot water. The moist heat was applied to soften the lignin and solubilize the cellulose in autoclave pre-treatment (Tampio et al., 2014). In microwave pre-treatment, radio wave was passed through the substrate, which led to a break down of lignin in the weed cell wall (Kaatze, 1995), when then vibrated the water molecule inside the sample which lead to break down of the hydrogen bond inside the sample. During autoclave and hot water bath pre-treatment, moist heat was passed in the form of hot vapour, here the mode of heat transfer taking place was convection, after which when the heat passed through the sample, conduction took place inside the sample.

6.1.1. *Parthenium hysterophorus*

6.1.1.1. Hot air oven pre-treatment

During hot air oven pre-treatment, it was observed that both VFA and sCOD increased up to 110˚C, after which they decreased, as shown in Fig. 6.1. After 120˚C, VFA started decreasing, which was due to the vaporization of the volatile compound. Cell wall started to break down at 110˚C, making organic compounds free, making it easy for bacteria to degrade them within a short period. As maximum sCOD was observed at 110˚C, samples were kept for different time intervals inside the hot air oven at the optimized temperature to investigate the optimum time. Fig. 6.2 showed that sCOD and VFA increased with time up to 90 mins after which it started decreasing. Fig.s. 6.1 and 6.2 showed that the increase and decrease of sCOD were directly related to the increase and decrease of VFA (Barua and Kalamdhad, 2016). During hot air oven pre-treatment, it was observed that sCOD increased up to 51.56 %, i.e., 2.06 times increase in sCOD as compared to the control. Higher biogas recovery could be achieved if the sCOD were higher and reduced the hydrolysis phase (Junoh et al., 2015). Complex lignocellulose structure was broken down after hot air oven pre-treatment at 110˚C for 90 mins, which released organic matter in the plant cell's soluble form. In hot air oven pre-treatment, dry heat was passed to the sample's surface where penetration power was very less compared to moist heat. Therefore, temperature 110˚C for 90 mins met the sufficient heat requirement to achieve maximum sCOD. Although temperature required above 100°C, biogas production also was higher compared to other weeds. Wang et al. (1997) described that lignocellulosic bonds did not disrupt at low-temperature pre-treatments (less the 100°C). Low-temperature pre-treatments close to 100˚C had been suggested as a pre-digestion phase to boost the biological activity of some hydrolytic bacteria (Nielsen et al., 2004).

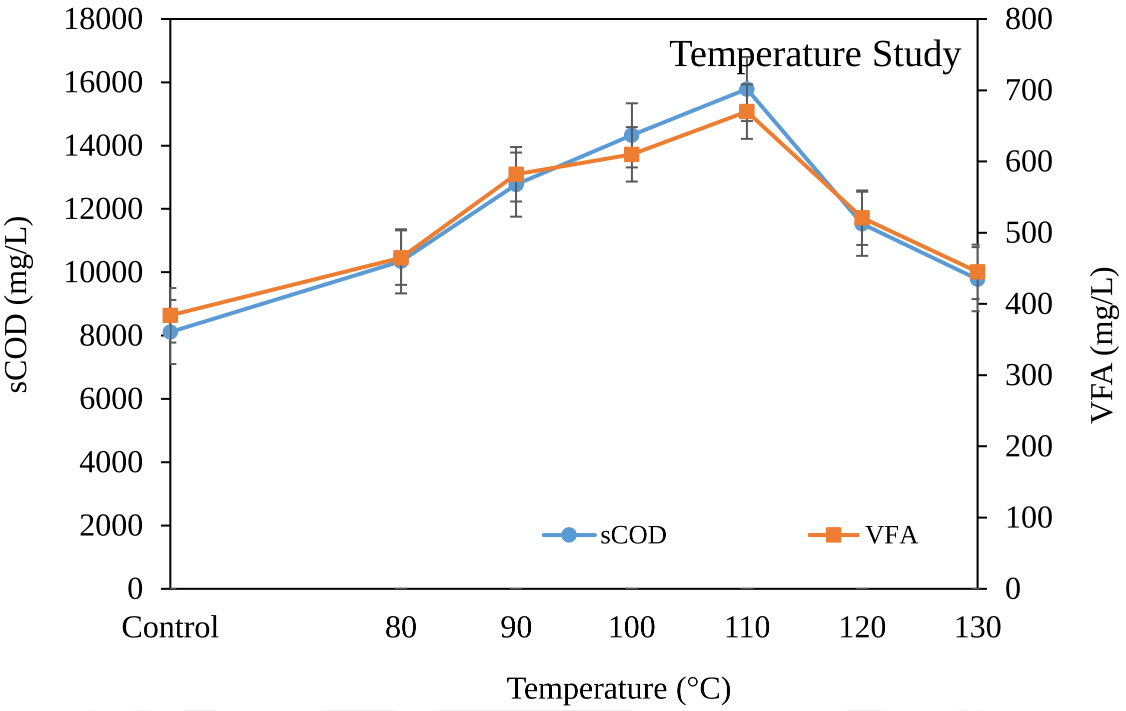


Fig. 6.1. Effect of temperature pre-treatment on the VFA and sCOD for different samples set inside the hot air oven for 2 h

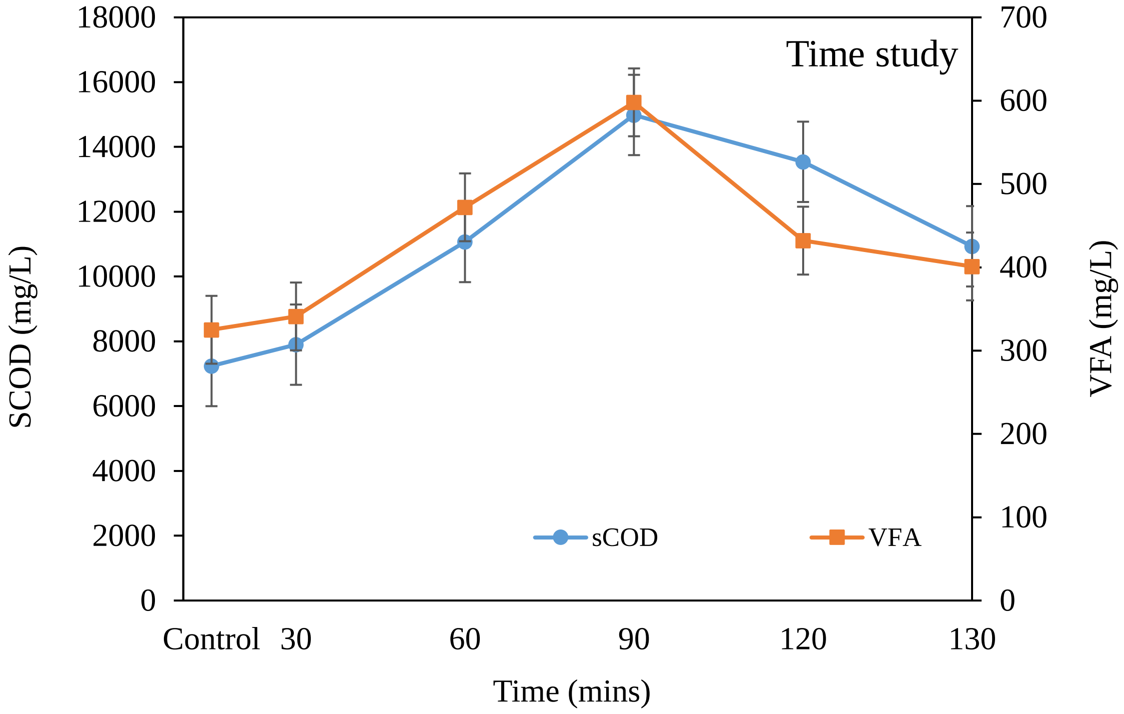


Fig. 6.2. Effect of time on the VFA and sCOD of the different samples set inside the hot air oven at optimized temperature (110 °C)

6.1.1.2. Microwave pre-treatment

Microwave pre-treatment was done to improve the sCOD. It was observed that with the increase in VFA, there was a increase in both microwave and hot air oven pretreatment studies. However, when compared with the control the increasing observed in hot air oven study much higher then the microwave pretreatment (Fig. 6.3 and 6.4). This happened because microwave high penetration power leads to denaturing of the organic matter. VFA and sCOD both started to decline after 180˚C. This was due to the fact that high-temperature chemical reaction took place between reducing sugar and the amino acid at the same time water content was also reduced, so at 220 ˚C the sample turned into a brown colour, which leads to the formation of inhibitory compounds like melanoidins (Hendriks and Zeeman, 2009; Carrère et al., 2010; Barua and Kalamdhad, 2016). In microwave pre-treatment, radio waves were used for heating, responsible for raising the heat transfer efficiency. At very high temperatures, sugar and enzymes were affected and denatured, which stopped the rise of further sCOD. During time study, 15 mins were found to be optimum, as shown in Fig. 6.4. Maximum sCOD of around 20.55% was obtained at 180˚C for 15 mins, i.e., 1.25 times increased from the very less control.

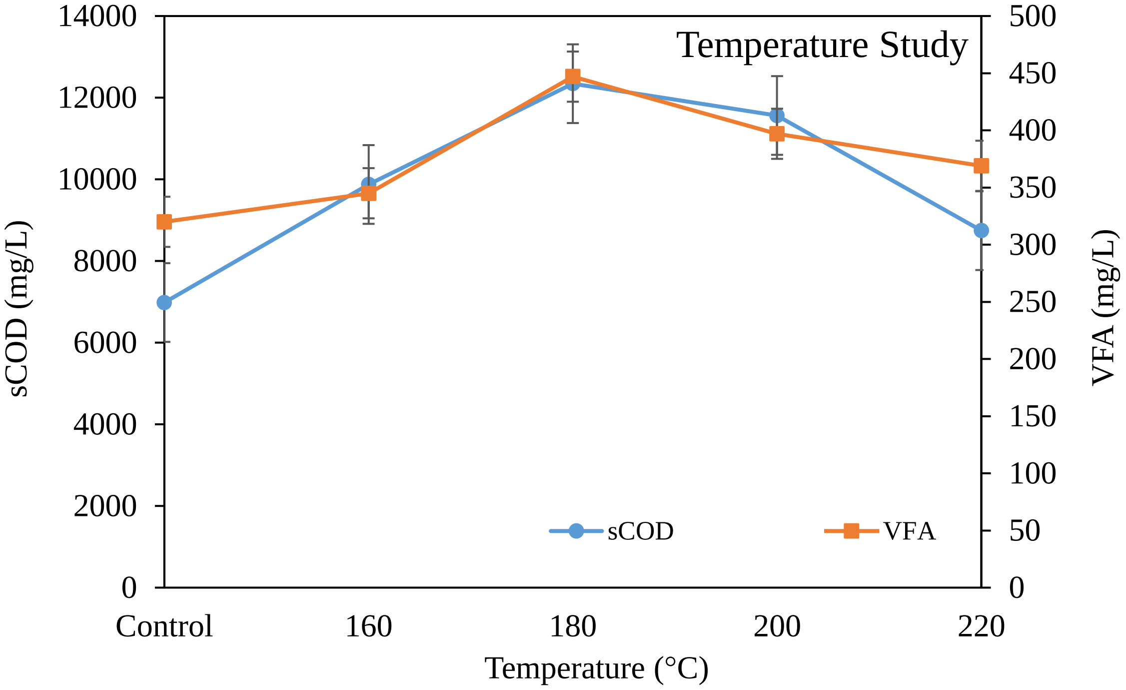


Fig. 6.3. Effect of temperature pre-treatment on the VFA and sCOD for different sample set inside microwave for 10 mins

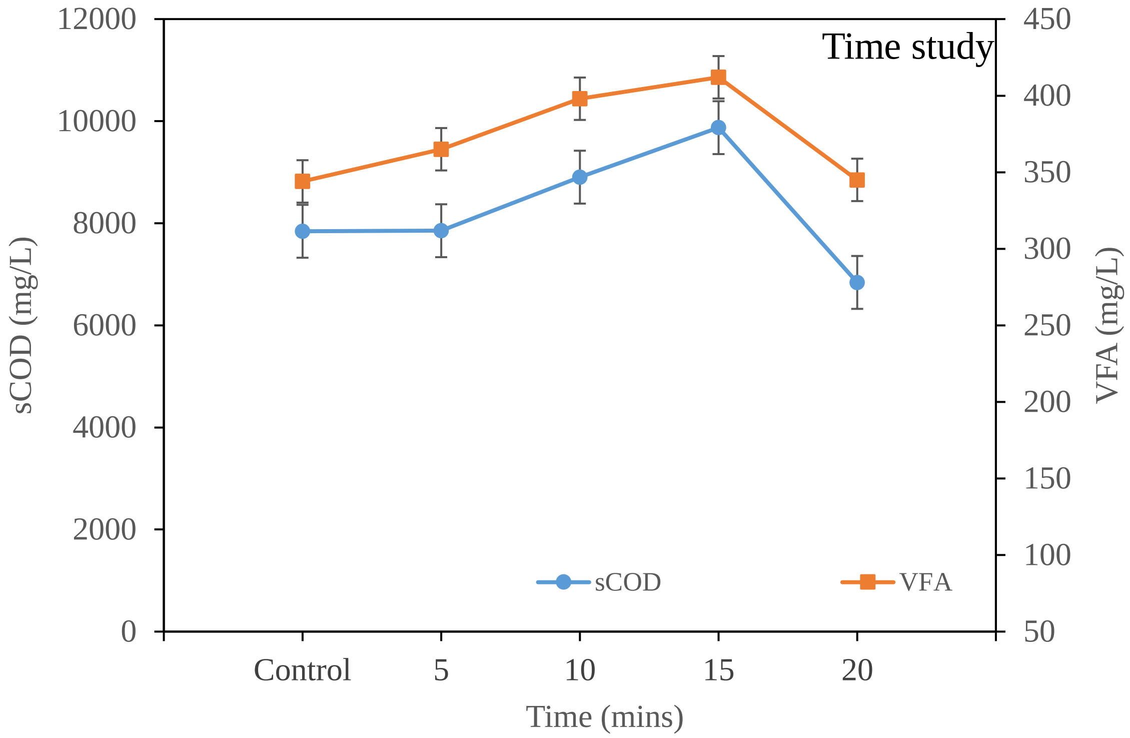


Fig. 6.4. Effect of time on the VFA and sCOD for different samples set inside the microwave at optimize temperature (180 °C)

6.1.1.3. Autoclave pre-treatment

In the autoclave pre-treatment study, it was observed that firstly, both sCOD and VFA increased up to 100 °C, after which it started reducing at 110˚C by lastly increasing slightly at 120˚C. During autoclave pre-treatment, two high points were found at 100˚C and 120˚C as shown in Fig. 4.14, but at 100˚C, sCOD reached up to 14210 mg/L, and at 120˚C it showed 13900 mg/L, so at 100˚C it showed greater sCOD recovery than at 120˚C, i.e., 100˚C was taken as an optimized temperature. Menardo et al. (2012) reported the same result for wheat and barley. In the time study, the sample was kept for different time intervals at the optimized temperature inside the autoclave, as shown in Fig. 6.5. The highest sCOD was obtained at 60 mins for 100˚C. It was clearly observed in Fig. 6.5 and 6.6 that VFA and sCOD were directly related to each other. In autoclave pre-treatment, moist heat was passed over the sample which had high penetration power as compared to dry heat, which led to denaturing few parts of sugar, and enzymes responsible for hydrolysis or further break down of organic molecules. It was found that at optimized temperature and time, the sCOD improved up to 40.85% i.e., 1.69 times that of control. During autoclave pre-treatment, the heat had an oxidative effect, which denatured the proteins present in the plant's cell, which is why maximum sCOD could not be achieved.

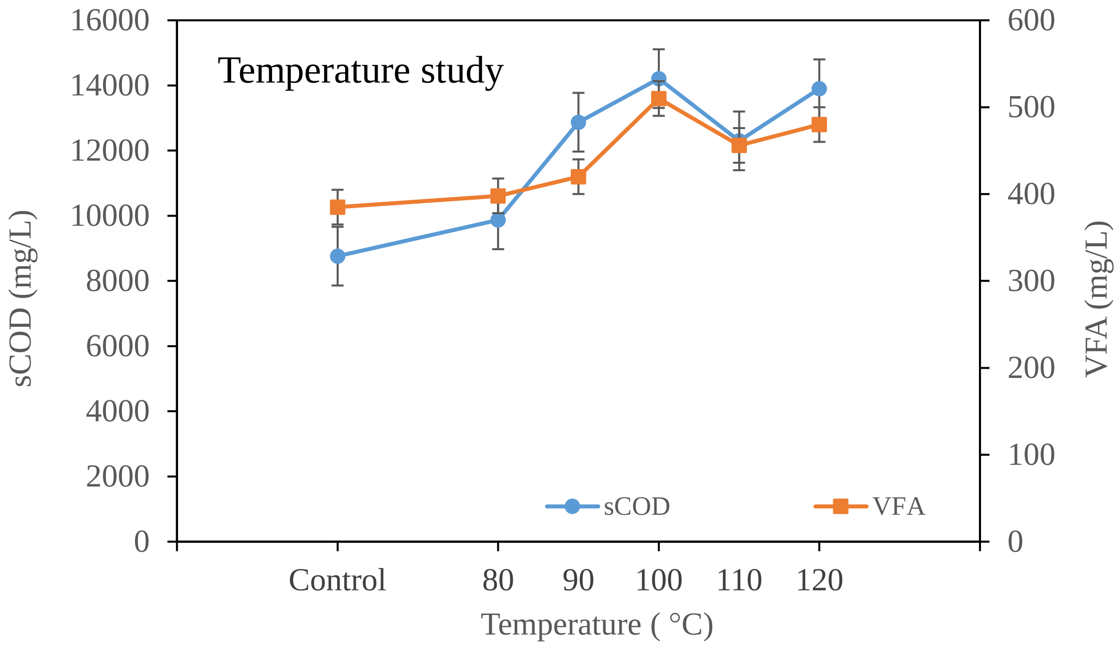


Fig. 6.5. Effect of temperature pre-treatment on the VFA and sCOD of the different samples set inside the autoclave for 20 mins

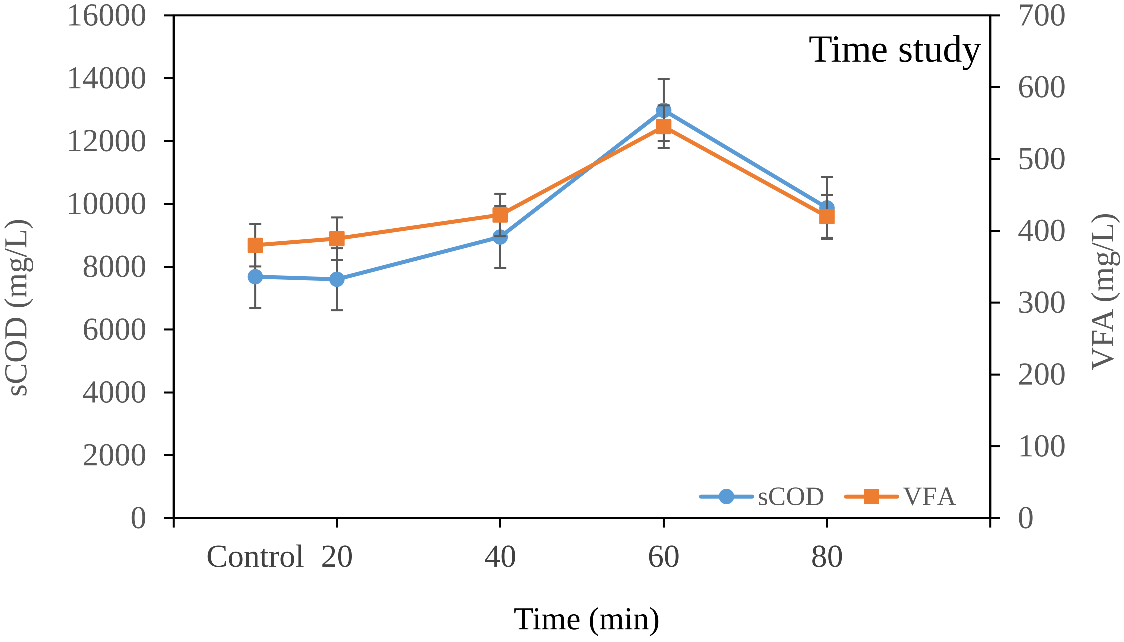


Fig. 6.6. Effect of time on the VFA and sCOD of the different samples set inside the autoclave at optimize temperature (100 °C)

6.1.1.4. Hot water bath pre-treatment

In hot water bath pre-treatment, as sCOD increased, VFA also increased up to 90˚C, but at 100˚C, it declined for both sCOD and VFA as shown in Fig. 6.7. The reduction of sCOD and VFA at 100˚C was due to the vaporization of VFA. The sample kept at 90˚C showed the highest amount of sCOD as compared to the sample held at 70, 80 and 100˚C. The sample was then kept inside the hot water bath at 90˚C for different time intervals to optimize time. In time study, it was observed that the increment of sCOD and VFA up to 90 mins after which it started declining as observed in Fig. 6.8. The sample pretreated at 90˚C for 90 mins showed the highest recovery of sCOD around 44.14%, which was increased by 1.79 times. Thi et al., (2017) observed that at 100˚C for 60 mins was the optimum condition as they examined the effect of temperature at 70, 80 and 100˚C for durations of 30, 60, 90 and 120 mins. The rise and fall of sCOD were directly related to the rise and fall of VFA as observed in fig. 5a, and b. During hot water bath pre-treatment, moist heat was applied to the sample, which vaporized the volatile fatty acids like propionic, butyric and valeric acid. This were the essential acids to regulate during the anaerobic digestion process and further converted into acetic acid, which was very important for methane production. (Gil et al., 2018).

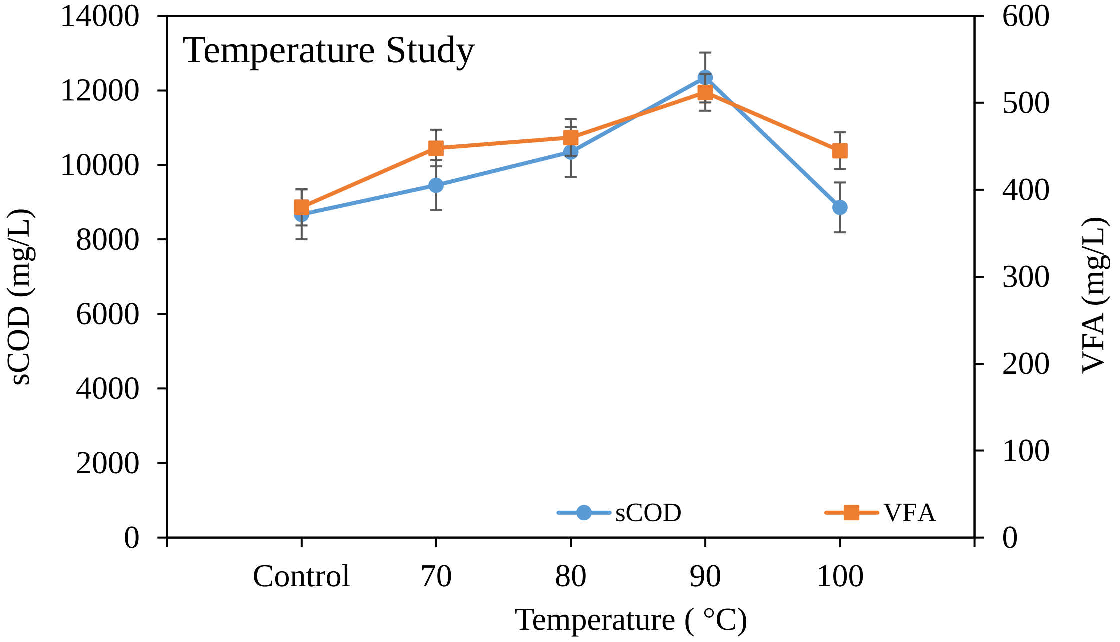


Fig. 6.7. Effect of temperature pre-treatment on the VFA and sCOD for different samples set inside the hot water bath for 30 mins

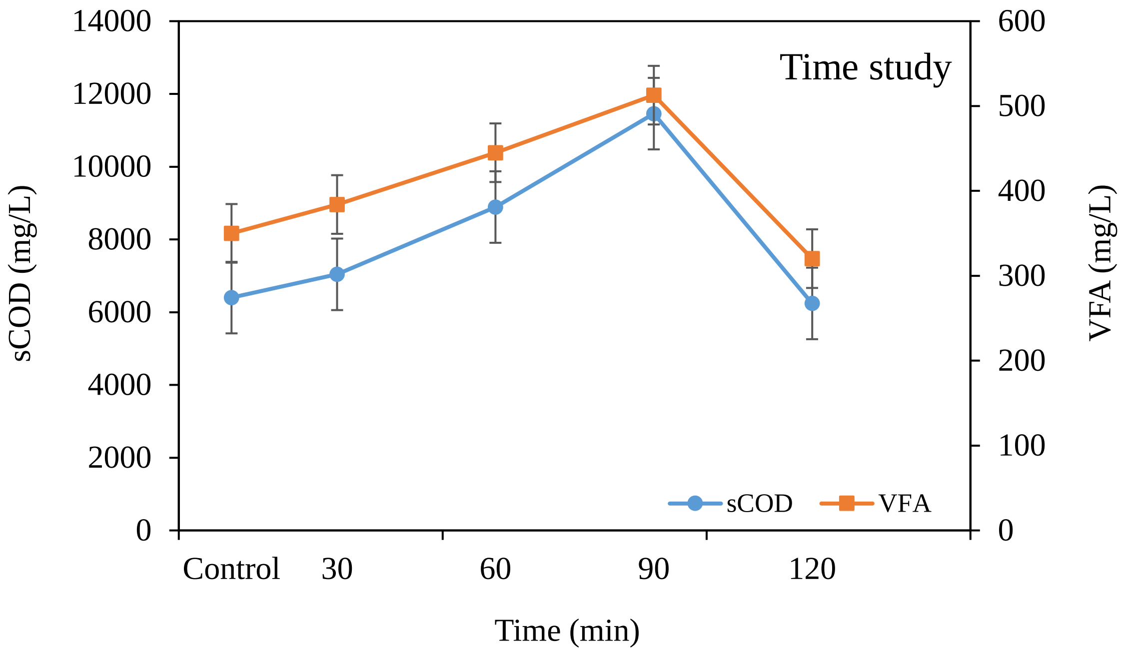


Fig. 6.8. Effect of time on the VFA and sCOD of the different samples kept inside hot water bath at (90°C)

6.1.1.5. Effect of thermal pre-treatment on the compositional analysis of *P. hysterophorus*

A compositional study was done to observe the variations in the compositions of (lignin, hemicellulose, cellulose) during thermally pretreated *P. hysterophorus*, as shown in table 6.1. The highest amount of soluble lignin recovery was obtained in hot air oven pre-treatment as compared to microwave, autoclave and hot water bath pre-treatments. The percentage of soluble lignin increased in all heating techniques as compared to the untreated *P. hysterophorus*. More soluble lignin made the cellulose more readily available for the microbes to degrade within a less period. Increment of cellulose observed after pre-treatment was due to a long chain of cellulose breaking down into small chains. During thermal pre-treatment, lignin bond became weaker, or depolymerization occurred, which was beneficial for the degradation (Kamdem et al., 2015). Kumar et al. (2009a) reported that the solubilization enhancement was due to the breaking down of cellulose into simpler forms. After pre-treatment, the decrease of lignin content was due to breaking down the ether and ester bonds of lignin and hemicellulose.

Table. 6.1. The variation was observed in the composition of thermally pretreated and untreated *P. hysterophorus* whole plant

Pretreatment technique	**Acid soluble lignin (%)**	**Acid insoluble lignin (%)**	**Cellulose (%)**	**Hemicellulose (%)**
Untreated	2.12±.43	5.93±.21	42.84±.56	29.1±.45
Hot air oven	3.62±.27	4.67±.43	46.58±.43	20.8.7±.71
Microwave	1.89±.39	4.33±.32	44.34±.21	25.2±.32
Hot water bath	2.54±.31	6.05±.11	43.16±.34	22.3±.45
Autoclave	2.58±.17	5.35±.08	42.93±.27	27.8±.32

6.1.1.6. FESEM study untreated and pretreated *P. hysterophorus*

Structural changes have been observed between untreated and pretreated in *P.hysterophorus.* In untreated *P.hysterophorus,* it was seen as a tight and smooth structure; therefore, after pre-treatment, it was observed that the substrate's surface was broken into pieces, which makes it easier for microbes to degrade and also reduce the complex hydrolysis phase. Availability of cellulose increased after hot air pre-treatment due to degradation. In microwave pre-treatment, it was observed that the complex structure form was not easy to degrade. In an autoclave, it was observed that very few parts were broken. In hot water bath pre-treatment, it broke down but not entirely as compared to hot air oven. Pre-treatment showed total deconstruction of lignocellulose matrix in a hot air oven compared to microwave, autoclave, and hot water Fig. 6.9, 6.10, 6.11, 6.12, and 6.13.

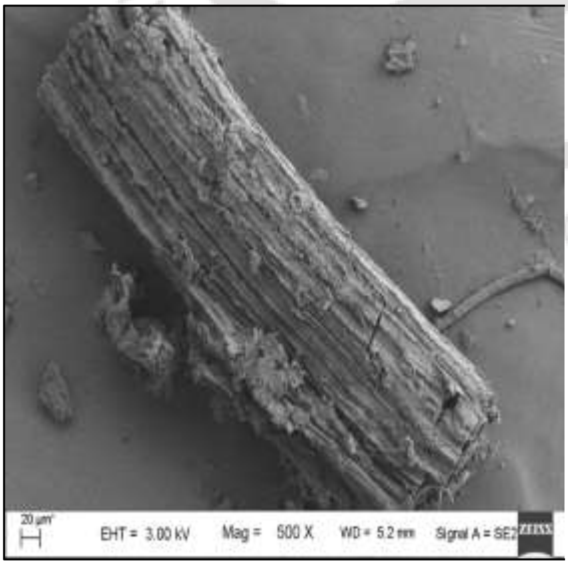


Fig. 6.9. FESEM image of Untreated *P.hysterophorus*

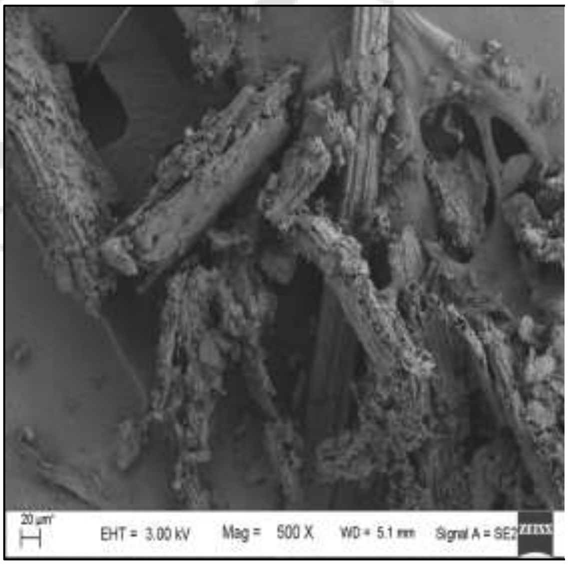


Fig. 6.10. FESEM image of hot air oven pre-treatment *P.hysterophorus*

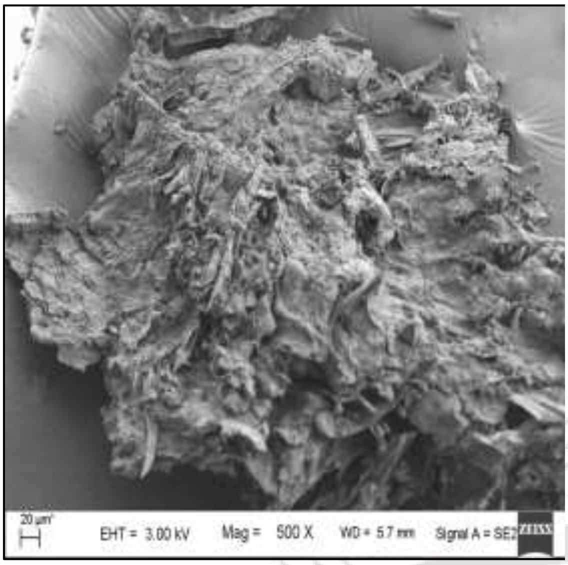


Fig. 6.11. FESEM image of microwave pre-treated *P. hysterophorus*

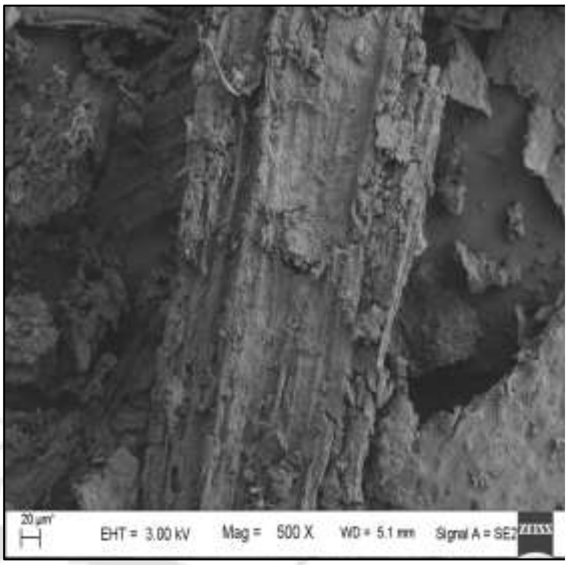


Fig. 6.12. FESEM image of autoclave pretreated of *P. hysterophorus*

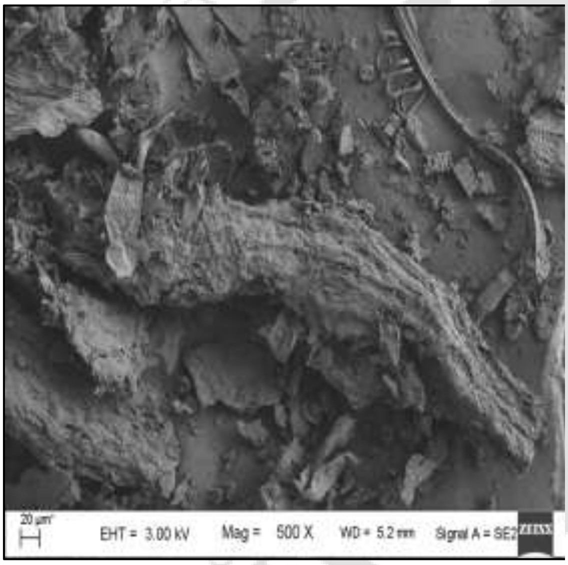


Fig. 6.13. FESEM image of hot water bath pretreated *P. hysterophorus*

6.1.1.7. XRD study untreated and pretreated *P. hysterophorus*

XRD study provided information on the crystallinity and amorphous nature of the sample. High and sharp peaks were found in the untreated sample, which signified the crystalline cellulose present in the sample. After the hot air oven pre-treatment, no such rise was observed as shown in Fig. 6.14. High peaks indicated the crystalline nature of the cellulose, which was very hard to degrade. After pre-treatment, it was converted into amorphous nature, which was easier for microbes to degrade. Chen et al., (2011) got similar results after pre-treatment of sugarcane bagasse. Therefore, thermal pre-treatment was found to be useful for decreasing the crystallinity of cellulose in *P. hysterophorus*.

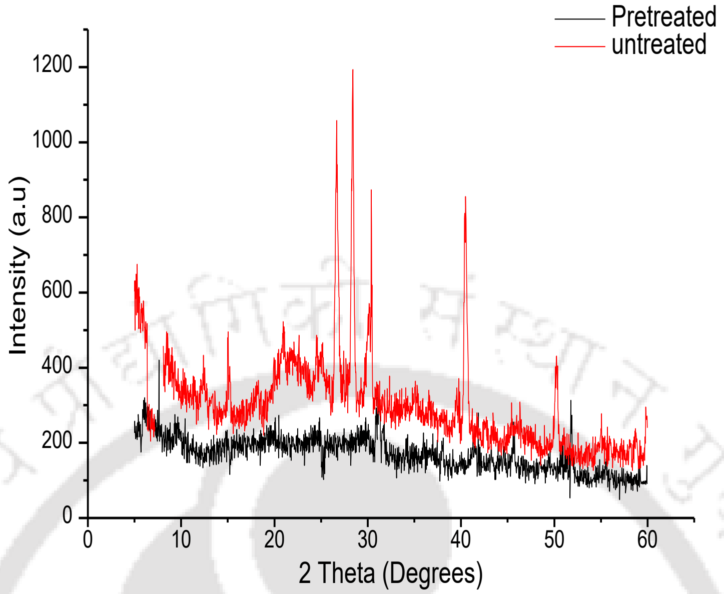


Fig. 6.14. XRD image of pretreated and untreated *P.hyterophorus*

6.1.1.8. FTIR study untreated and pretreated *P. hysterophorus*

Structural changes were observed in FTIR analysis after pre-treatment of *P. hysterophorus* as shown in Fig. 6.15. The band was observed at 3421.47cm^{-1}and 3428.93 cm^{-1} in untreated and pretreated, which signified the stretching of OH in cellulose (Lazzari et al., 2017. Reduction of the band from 1319.87 cm^{-1} to 1076.02 cm^{-1} gave a clear idea of deformation occurring in cellulose, hemicellulose and lignin after hot air oven thermal pre-treatment. (Kumar et al., 2009b; Li et al., 2010). After pre-treatment, broadening of wavelength was observed, which indicated that inter and intra hydrogen bond became weaker in lignin, cellulose, and hemicellulose. Changes in the band after pre-treatment weakened the hydrogen bond and reduced the crystallinity of *P. hysterophorus***.**

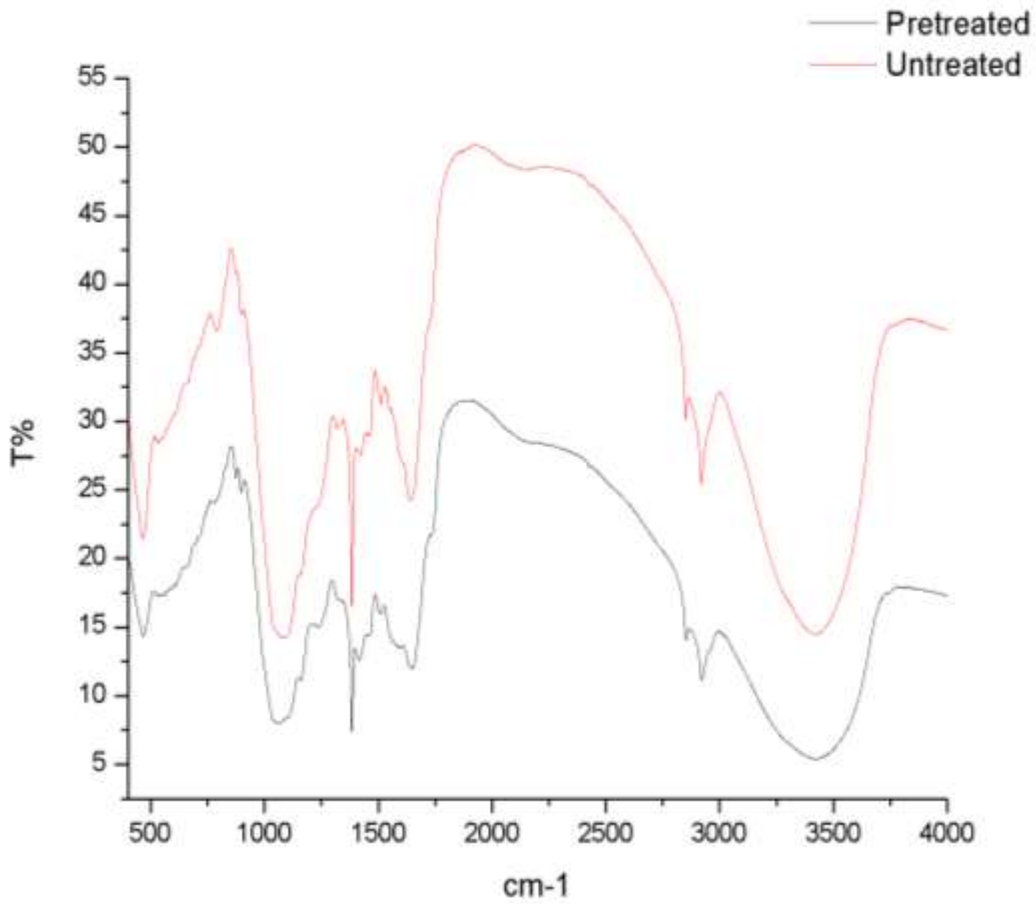


Fig. 6.15. FTIR image of pretreated and untreated *P.hyterophorus*

6.1.1.9. Biogas production of control, untreated and pretreated *P. hysterophorus*

Four different thermal pre-treatments were applied on *P.hysterophorus* (Hot air oven, micro oven, autoclave and hot water bath). Hot air oven showed *the* most efficient thermal pre-treatment among all in the form of increment of sCOD to 51.56% and VFA to 46.48%. BMP experimental set up was run for hot air oven pre-treatment (best ratio 2), untreated and two different controls. In control 1, only untreated *P.hysterophorus*, which is quite less, not to mention the graph and in control 2, only cow dung was applied. Due to high lignin content in *P.hysterophorus*, the degradation process is delayed leading to an increase in the hydrolysis period (Gurung et al., 2012). Cumulative biogas was found around 3148 mL CH_4 in 50 days in untreated (Fig.s. 6.16). A sharp increment of cumulative biogas production (3723 mL CH_4) was found in *P.hysterophorus* in 35 days after hot air oven pre-treatment, and within 30 days, cumulative methane production started to stabilize. After the hot air oven pre-treatment, lignin melted down in *P.hysterophorus*, which led to a decrease in the hydrolysis period. Wang et al. (1997) stated that pre-treatment at low temperatures helped increase the degradation rate in anaerobic digestion. Inhibitors may be produced at high-temperature pre-treatment, decreasing the efficiency of anaerobic digestion. (Laser et al., 2002; Nizami et al., 2009).

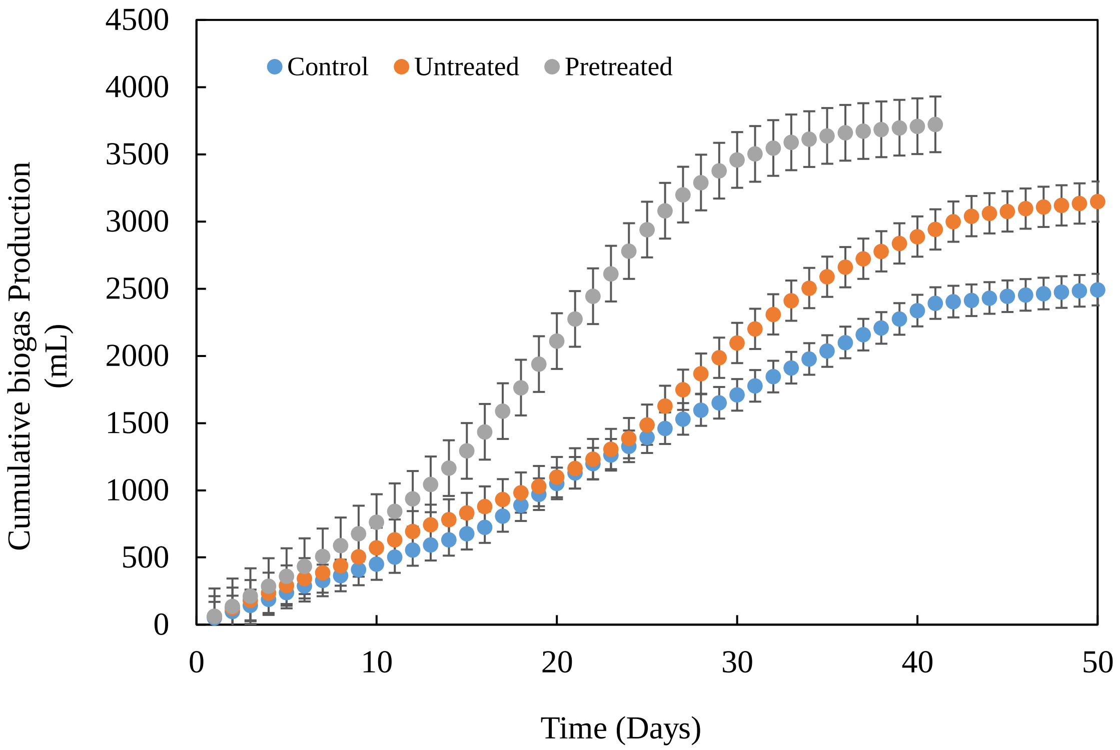


Fig. 6.16. Cumulative Biogas Production of control, untreated and pretreated *P. hysterophorus* (mL)

6.1.1.10. Kinetic study of control, untreated and pretreated *P. hysterophorus*

The efficiency of biogas production was determined by the Gompertz equation (Lee et al., 2013), where the value of cumulative methane production was fitted for 50 days. The kinetic parameter was given in Table 6.2. The higher the production of methane, the higher will be the M value (Veluchamy and Kalamdhad, 2016). The value M was calculated for control, untreated and hot air oven pre-treatment. The pretreated sample was found to be 4.0000 L of CH_4 followed by untreated (3.8334 L CH_4). Due to pre-treatment, there was sufficient cellulose available for microorganisms, which helped speed up the hydrolysis stage and enhance the biogas production.

Table. 6.2. Kinetic value for control, untreated and pretreated *P.hysterophorus*.

Substrate	**M(LCH_4)**	**R_m(L CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
Control	3.2966	0.0594	0.0010	0.987	2.493
Untreated	3.8334	0.727	0.0010	0.989	3.148
Pretreated	4,000	0.1000	0.007	0.997	4.239

6.1.1.11. Energy assessment of hot air oven pre-treatment *(P.hysterophorus)*

During the hot air oven, the specific energy utilized E_u is determined as per the eqn-6.1

$$Eu = \frac{P.t}{n} \tag{6.1}$$

Where P is considered as a power rating of the hot air oven (Watt), t signifies as exposure time, and n is the weight of the volatile solid fraction of 7.9 (g)

By substituting the value of P, t, and W. The value of E_u was found to be 3.2 kJ/g.

The specific energy available E_a due to the methane generated was determined as per this eqn-6.2.

$$E_Q = \frac{Q_{raw}.\S}{1000.\mathrm{n}} \tag{6.2}$$

Where Q is the maximum amount of the methane that was observed to produce on a daily basis, § was the calorific value of methane, and n is the weight of the volatile solids fraction that was added. By substituting the value of Q, § and n in Eqn 6.2, the E_a was 7.70kJ/g (VS).

6.1.2. *Lantana camara*

6.1.2.1. Effect of hot air oven pre-treatment on *L.camara*

During hot air oven pre-treatment, VFA and sCOD both showed an increasing trend up to 120˚C, but at 130˚C, a decrease in sCOD and VFA were observed (Fig. 6.17). After 120˚C VFA started to vaporize, which led to the loss of volatile fatty acids; thus, VFA and sCOD had both started to decline. Strong chemical bonds of lignin present in the cell wall of *L. camara* was broken down when the temperature was applied, which made the organic compound easily accessible for bacteria. At high temperatures more than 120˚C, organic compounds like proteins and enzymes denatured which were not further converted into VFA and sCOD. After hot air oven pre-treatment at 120˚C, an increment of 1.86 and 2 times rise in the sCOD, and VFA (38.39% and 33.33%) was observed compared to control. After the temperature was optimized, samples were kept for different time intervals inside the hot air oven at the optimized temperature to determine the optimum time. sCOD and VFA increased with time up to 60 mins after that it was started to decline (Fig. 6.18). The vaporization of VFA caused a decrease in sCOD. A similar result was observed by (Barua and Kalamdhad, 2016) during the water hyacinth's thermal pre-treatment. Nielsen et al., 2004 reported that a temperature less than 100˚C was not suitable to break the strong lignin bond present in the outer cell wall but can only boost the biological activity slightly. At 60 mins, sCOD and VFA increased to 37.38% and 33.33%, which were 1.60 and 1.5 times compared to the control. After the hot air oven pre-treatment, maximum sCOD and VFA was found for pre-treatment at 120˚C for 60 mins.

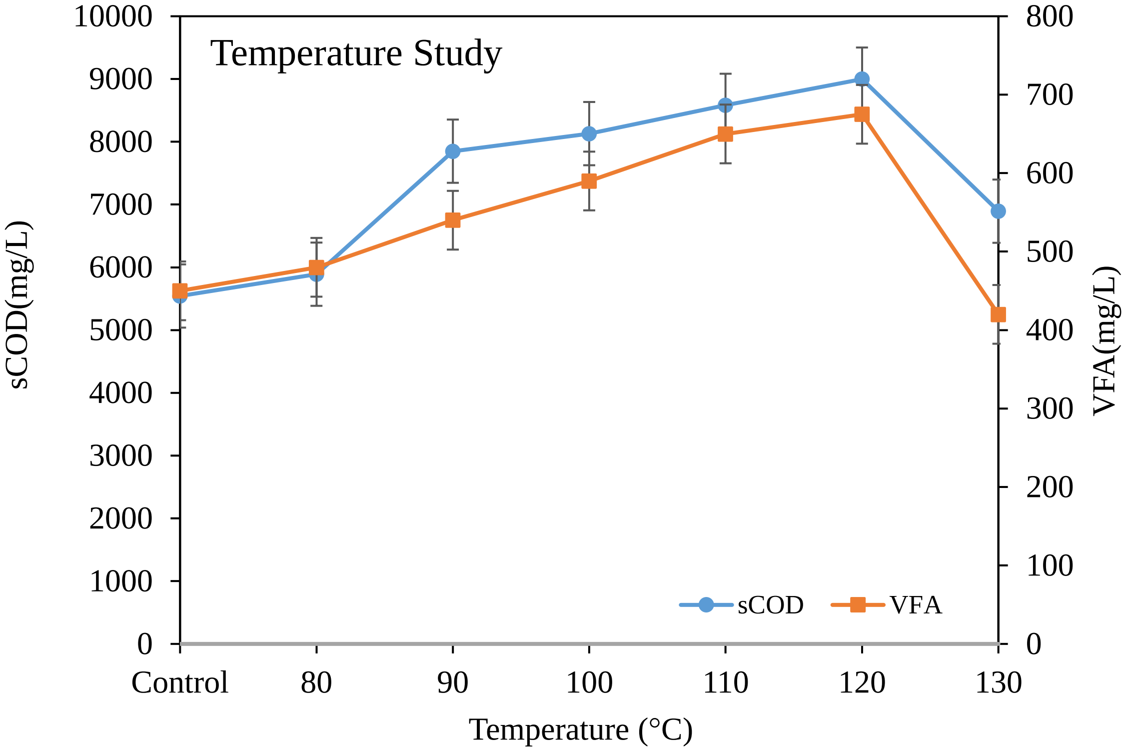


Fig. 6.17. Hot air oven pre-treatment temperature study

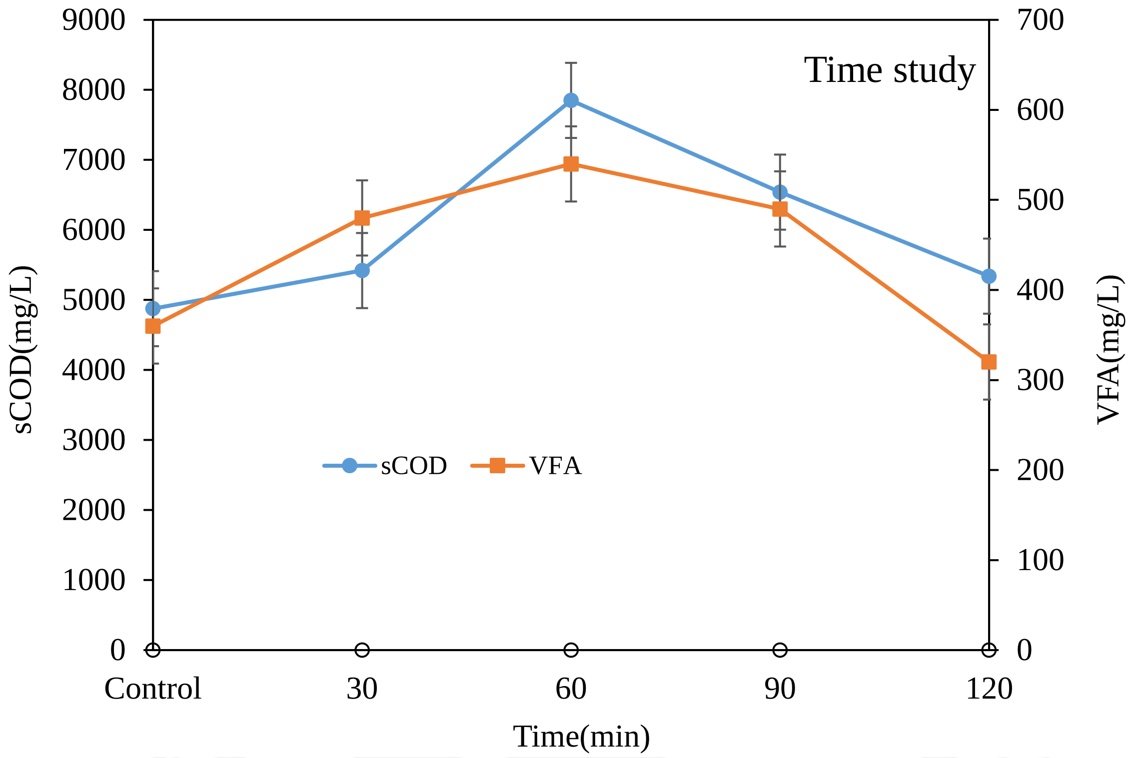


Fig. 6.18. Hot air oven pre-treatment time study

6.1.2.2. Effect of micro oven pre-treatment on *L.camara*

During micro oven pre-treatment, it was observed that sCOD and VFA raised up to 180˚C but at 200˚C it started to fall (Fig. 6.19). During the micro oven, a pre-treatment radio wave passes through the sample, which has high penetration power that leads to the denaturation of organic compounds. At high temperatures, VFA starts to vaporize. Simultaneously, it reduces the moisture content of the sample, which might be the reason for not so significant increment of sCOD and VFA with temperature compared to other thermal pre-treatments. After temperature optimization the increment in sCOD and VFA were found around 40.40 % and 32.5 %. This was 1.67 and 143 times as compared to the control. The samples were then retained at distinct time intervals inside the hot air oven at the optimized temperature to study the optimum time. In (Fig. 6.20) it was observed that sCOD and VFA rose with time up to 20 mins and then declined at 25 mins. Samples exposed to high temperatures for a long time lead to vaporization of the VFA, and at the same time color of the sample turned brown, which signified the presence of melanoidins after 20 mins at 200˚C. It occurs due to complex formation between reducing sugar and amino acids. They inhabit the acidogenesis and methanogenesis steps (Hendriks and

Zeeman, 2009; Carrère et al., 2010; Barua and Kalamdhad, 2016). During time study maximum sCOD and VFA were obtained around 43.51% and 50%, which was 1.77 and 2 times higher than the control. In micro oven pre-treatment, optimum temperature and time was found at 180˚C for 20 mins.

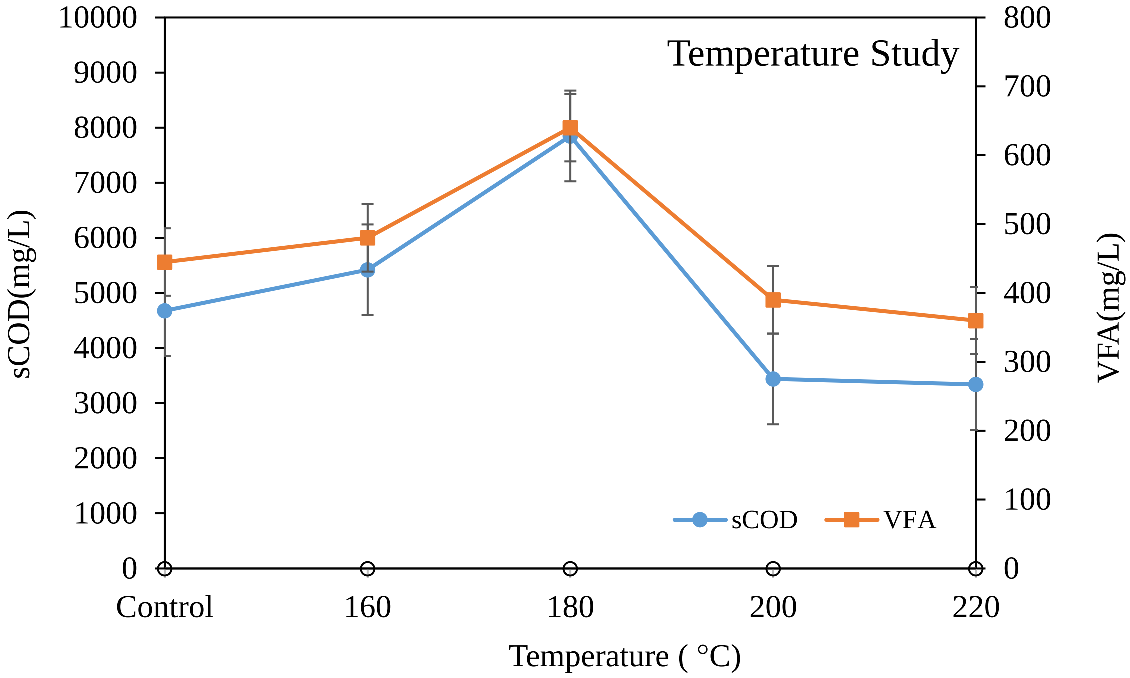


Fig. 6.19. Microwave pre-treatment temperature study

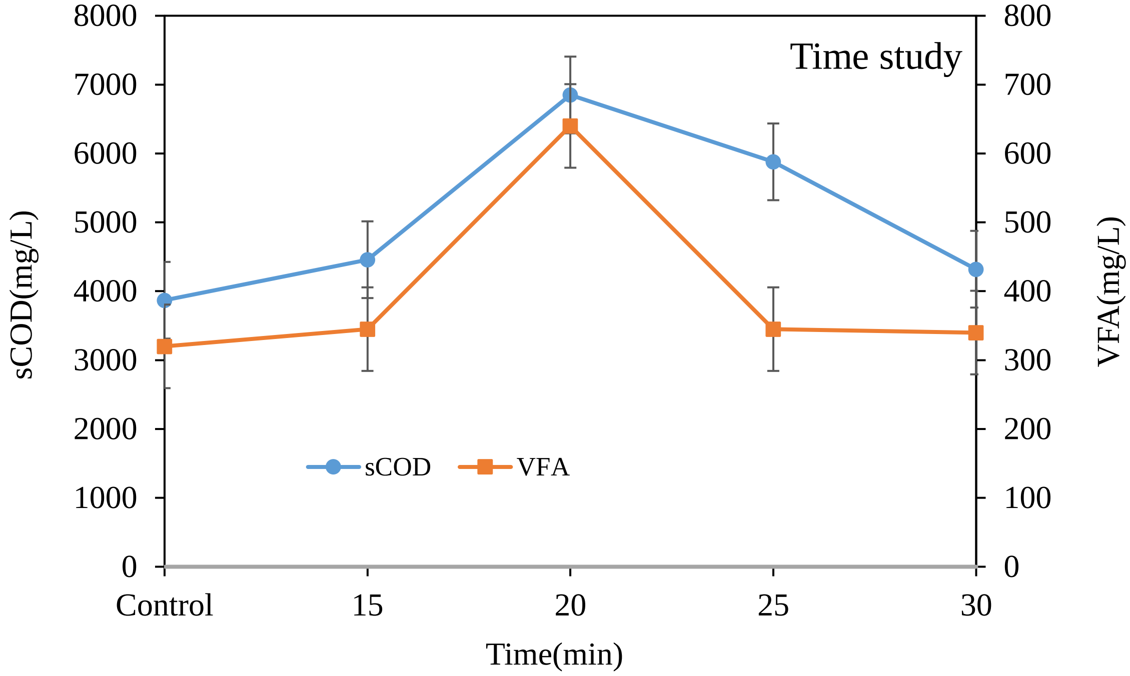


Fig. 6.20. Microwave pre-treatment time study

6.1.2.3. Effect of Autoclave pre-treatment on *L.camara*

During the autoclave pre-treatment, both sCOD and VFA raised to 110˚C. At 120˚C, VFA started to vaporize, which might be the reason for the decrease of sCOD (Fig. 6.21). Increment of sCOD and VFA was observed around 38.73% and 32.54%, which was 1.78 and 1.48 times as compared to the control. In autoclave, pre-treatment, moist heat was applied to the samples, which have high penetration power, and at the same time, it keeps the sample moist. High penetration power denatures the organic compounds like proteins and enzymes, which might be another reason for fall of sCOD and VFA at 100˚C. Similarly, the temperature was optimized by keeping the samples at different time intervals inside the autoclave at optimized temperature. As the time interval, increase sCOD and VFA both increased up to 80 mins then started to decrease (Fig. 6.22). After 40 mins VFA started to vaporize and denature the organic, leading to falling of sCOD and VFA at 60 mins, increment of sCOD and VFA was observed at 80 mins around 45.44% 56.75% which was 1.83 and 2.31 times than the control. Menardo et al., 2012 reported a similar type of results for ethanol production from barley and wheat. In autoclave pre-treatment, maximum sCOD and VFA was obtain at 110˚C for 80 mins.

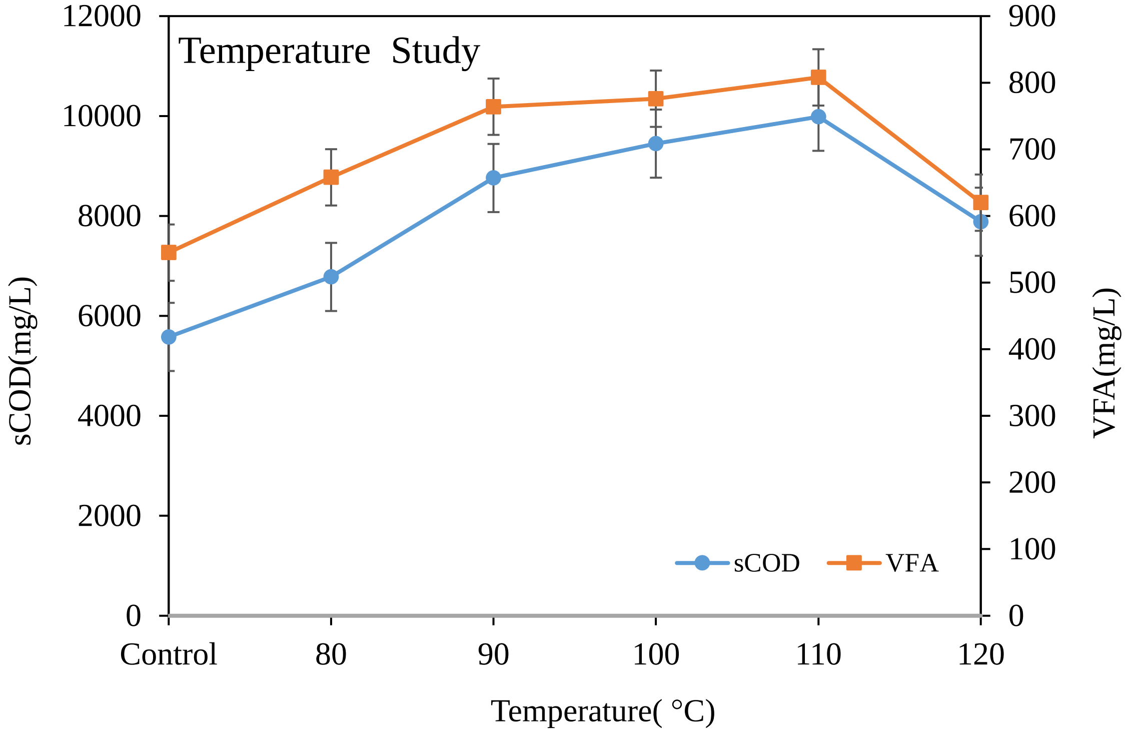


Fig. 6.21. Autoclave pre-treatment temperature study

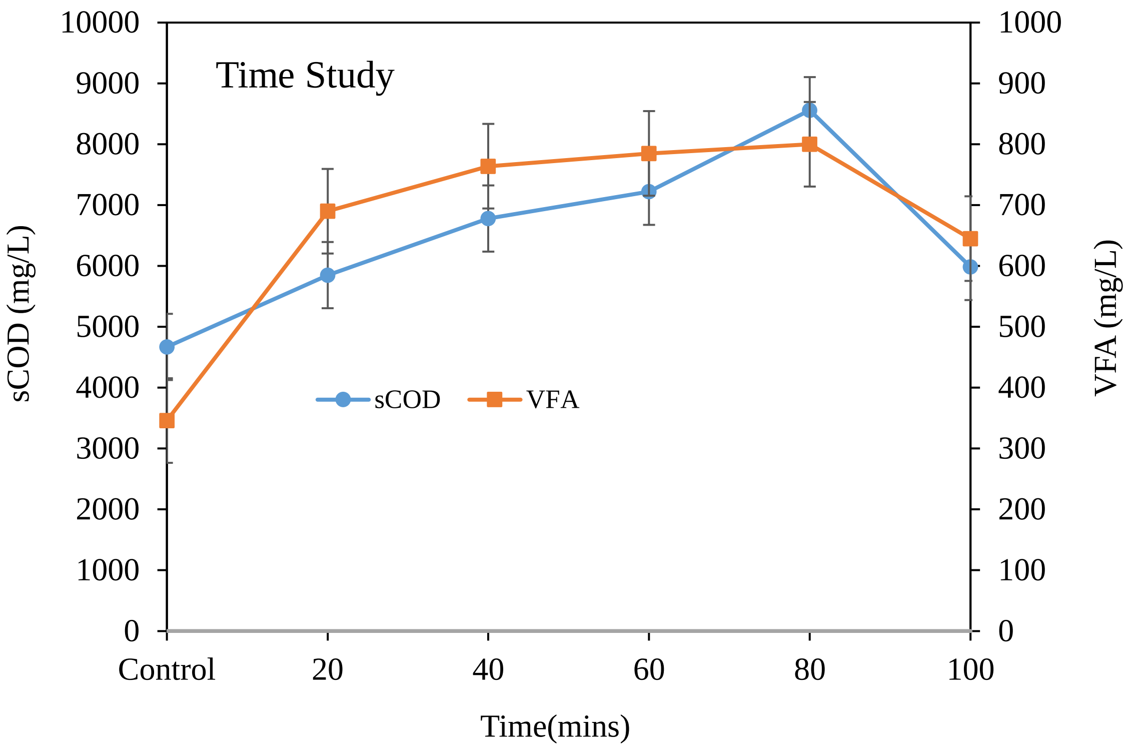


Fig. 6.22. Autoclave pre-treatment time study

6.1.2.4. Effect of hot water bath pre-treatment on *L.camara*

The effect of a hot water bath on the pretreated weed is shown in fig. 6.23 and 6.24. As the temperature raised, solubilization also increased, which can be seen as the increment of sCOD and VFA. In fig. 6.23 sCOD and VFA both incremented up to 80˚C; after that, it started to fall. After 80˚C volatile compound vaporized, that may affect the solubilization, which leads to reduction in the sCOD and VFA. When the high temperature was applied for the pre-treatment, the solubilization of the sample was reduced (Appels et al., 2010). In hot water bath pre-treatment, the highest sCOD and VFA were observed at 80˚C around 42.53 % and 39.62%, which was 1.74 and 1.65times compared to the control. After the optimum temperature was determined, the samples were kept at different time intervals inside the hot water bath at the optimized temperature. Solubilization increased with time (Fig. 6.24) up to120 mins, which showed an increment of sCOD and VFA. To increase the efficiency of solubilization rate, pre-treatment exposure time (at least 1 h) is required (Ariunbaatar et al., 2014). Maximum sCOD and VFA was obtained at 120 mins around 39.93% and 63.00% times, which was 1.66, and 2.32 times to the control. During the hot water bath pre-treatment study, optimum temperature and time were observed at 80˚C for 120 mins.

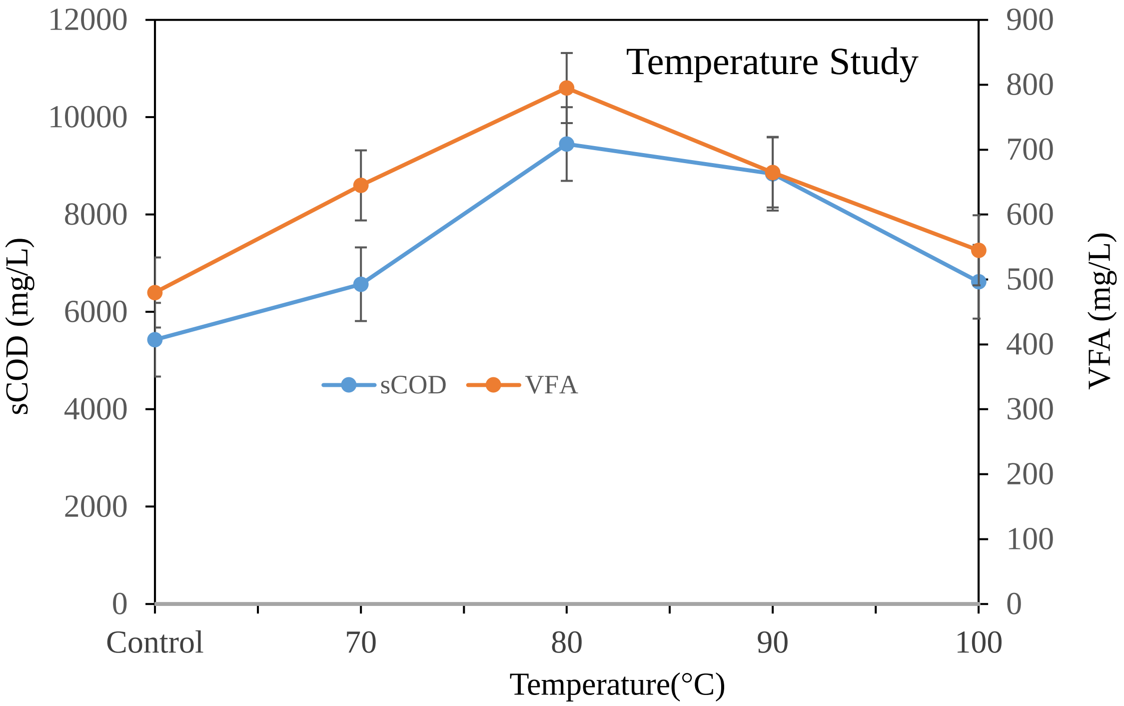


Fig. 6.23. Hot water bath temperature study

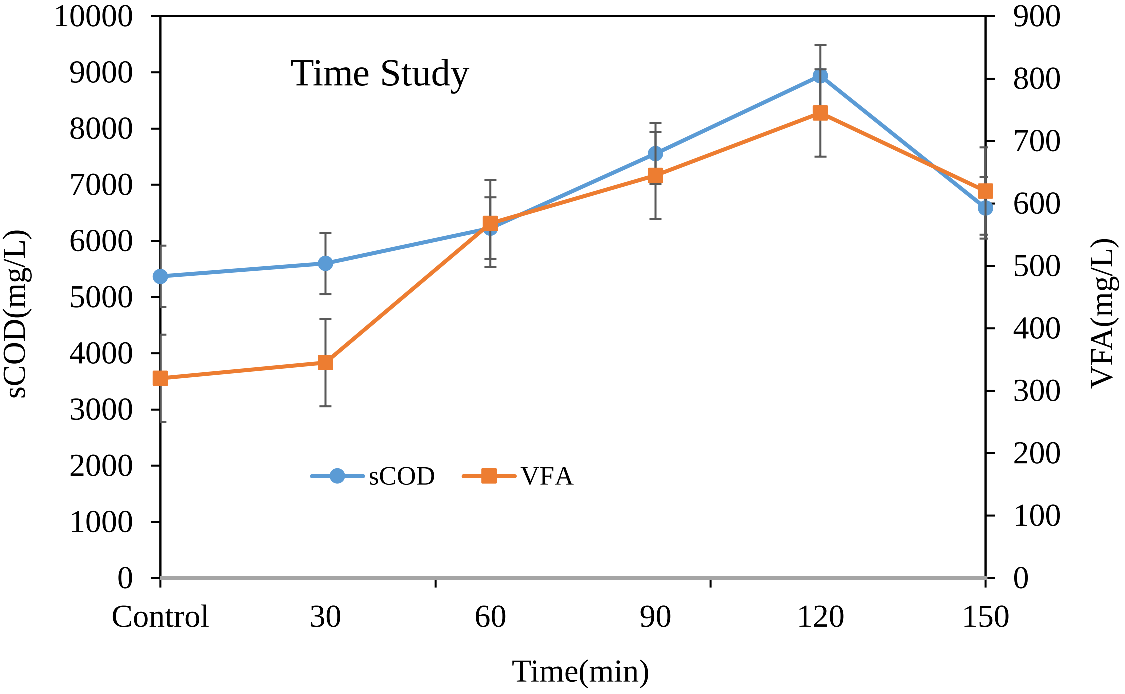


Fig. 6.24. Hot water bath time study

6.1.2.5. Compositional analysis of *L.camara*

During compositional analysis, changes of composition were observed in lignin, hemicellulose and cellulose, both pre and post different thermal pre-treatments. The thermal pre-treatment efficiency was confirmed in the form of a rise in acid-soluble lignin and cellulose compared to untreated/control *L. camara*. When the heat was applied to the sample, lignin started to melt, due to which acid-soluble lignin had increased. Table 6.3 found that autoclave pre-treatment exhibited the highest increment of acid-soluble lignin (51.93%) and cellulose (11.77%), which was 2.08 and 1.18 times than that of the control. On the other hand, hemicellulose had started to reduce. Increment of acid-soluble lignin is beneficial for fermentative microbes as it can be degraded within a short period. Hemicellulose is a short-chain biopolymer, which forms a bridge between lignin and cellulose, slightly decreasing after all the pre-treatment studies. Veluchamy et al., 2017, observed a similar result. The reduction of hemicellulose may be due to the reconstruction of its short chain to form cellulose. A significant increment of soluble acid lignin (P ¼ 0.0045) and cellulose (P ¼ 0.0088) was observed after analyzing the ANOVA's experimental data.

Table 6.3. The variation was observed in the composition of thermally pretreated and untreated *L.camara* whole plant

Pretreatment technique	Acid soluble lignin (%)	Acid insoluble lignin (%)	Cellulose (%)	Hemicellulose (%)
Untreated	1.24±.23	3.83±.47	38.22±.87	22.34±.13
Hot air oven	2.29±.24	3.21±.25	40.48±.43	22.34±.11
Microwave	1.89±.39	3.30±.32	39.39±.98	21.97±.63
Hot water bath	2.54±.31	3.68±.11	41.65±.55	23.23±.50
Autoclave	2.58±.17	3.25±.08	43.32±.34	21.41±.32

6.1.2.6. FESEM Study of untreated and pretreated *L.camara*

In fig. 6.5, the control can be seen like a rocky, rigid and inflexible structure, while after pre-treatment, changes were observed on the surface of the *L.camara* (Fig. 6.26). Due to thermal pre-treatment, the hard outer surface was broken down into pieces, which became more manageable for the microbes to degrade. The completely broken and long circular ring-like structures were observed after autoclave pre-treatment, which might be the cellulose chain.

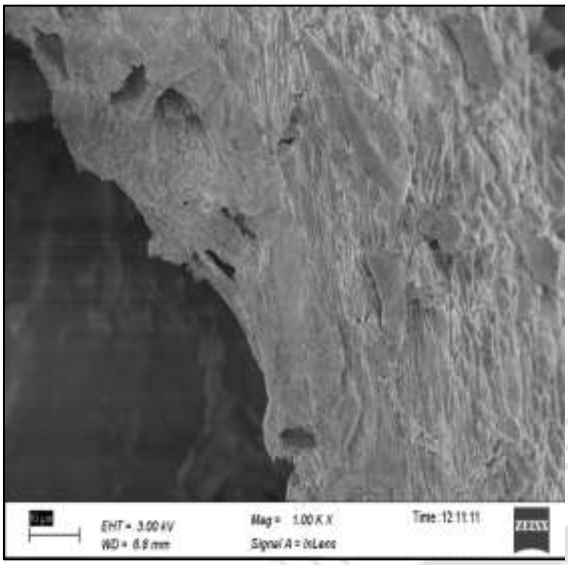


Fig. 6.25. FESEM image of untreated *L.camara*

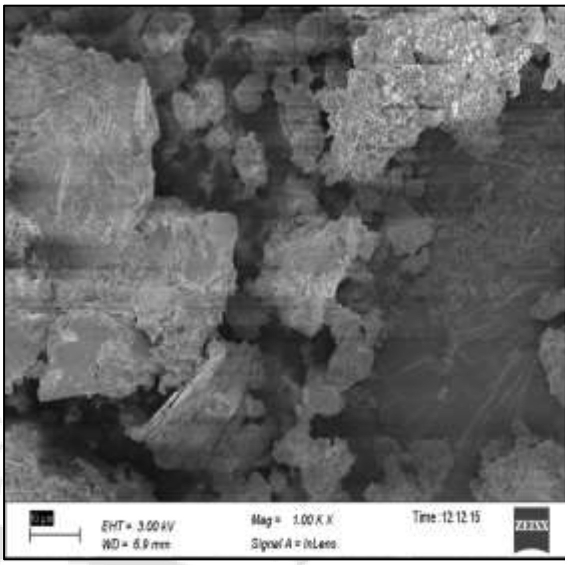


Fig. 6.26. FESEM image of after autoclave pre-treatment

6.1.2.7. XRD study of untreated and pretreated *L.camara*

XRD analysis was done to discern the crystalline or amorphous nature of the sample. In the untreated (control) sample, sharp peaks were observed while, after the autoclave pre-treatment, no such peak was observed (Fig. 6.27). High peaks were found in untreated (control) due to cellulose's crystalline nature, which is very hard to degrade. After autoclave pre-treatment, it was converted into an amorphous structure that became easier for microbes to degrade. Chen et al., (2011) reported similar results after pre-treatment of sugarcane bagasse. Therefore, thermal pre-treatment was found to be favorable for the conversion of crystalline cellulose to amorphous.

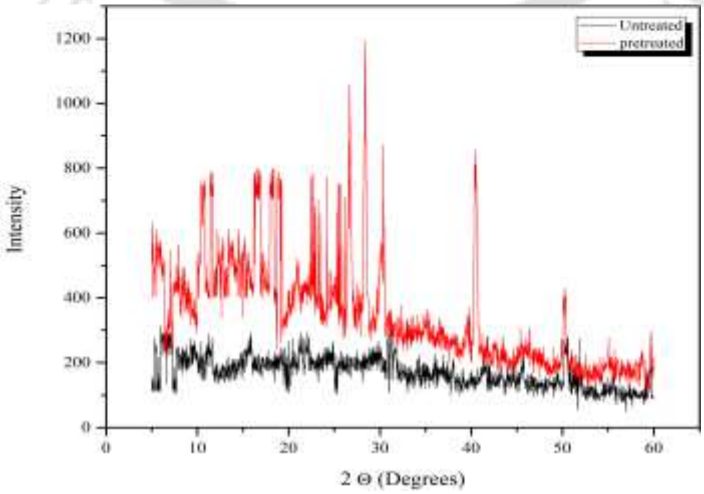


Fig. 6.27. XRD study of untreated and pretreated *L.camara*

6.1.2.8. FTIR study of untreated and pretreated *L.camara*

Prediction of lignin, cellulose and hemicellulose content in the plant sample can be identified in FTIR (Chen et al., 2010). In FTIR, it was seen that after autoclave pre-treatment, the band was broadened as compared to the untreated (control) (Fig. 6.28). This signifies the weakening of intra and intermolecular hydrogen bonds in cellulose, hemicellulose and lignin. All through the peak was found at a similar point, significant peak reduction was observed. Peaks corresponding to 1056.53 cm$^{-1,}$ 1256.45 cm^{-1} and 1678.22 cm^{-1} indicates C = O stretch in lignin, cellulose and hemicellulose (Li et al., 2010). After autoclave pre-treatment, a fall of the peak was observed at 3328.54 cm-1, which indicates the stretch of OH bond in lignin. Compaction of peak intensity and peak changes after pre-treatment represented the delignification and low crystallinity of *L. camara*.

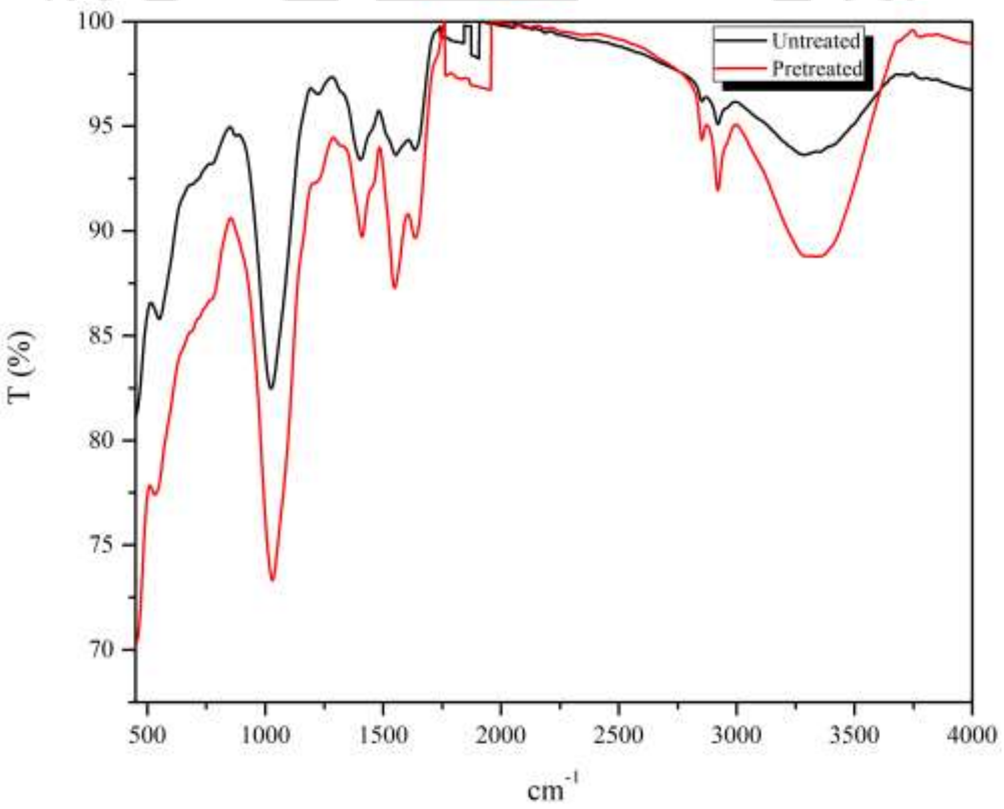


Fig. 6.28 FTIR study of untreated and pretreated *L.camara*

6.1.2.9. Biogas production of control, untreated and pretreated

After four different heating pre-treatment (hot air oven, micro oven, autoclave and hot water bath) was applied on *L. camara*, out of that, the autoclave was found to be most efficient in the form of increment of sCOD (45.44%) and VFA (56.75%). BMP set up was conducted for autoclave pretreated sample, untreated sample and two different controls. In control 1, only *L.camara* was taken, and in control 2, only cow dung was taken. High lignin content in *L.camara* delays the degradation process, which increases the hydrolysis period (Gurung et al., 2012). In untreated *L. camara*, cumulative biogas was found around 3565 mL CH_4/g VS in 50 days, and it required 40 days to stabilized the cumulative biogas production (Fig.6.22). After autoclave pre-treatment sharp increment of cumulative biogas production (2895 mL CH_4/g VS) was found in *L camara* in 35days and within 30 days' cumulative methane production started to stabilize. In (Fig. 6.29) a long hydrolysis period can be seen in the untreated sample. Low-temperature pre-treatment helps to increase the efficiency of the degradation process in anaerobic digestion (Wang et al. 1997). High-temperature pre-treatments may produce inhibitors, which decreases the efficiency of anaerobic digestion (Laser et al., 2002; Nizami et al., 2009).

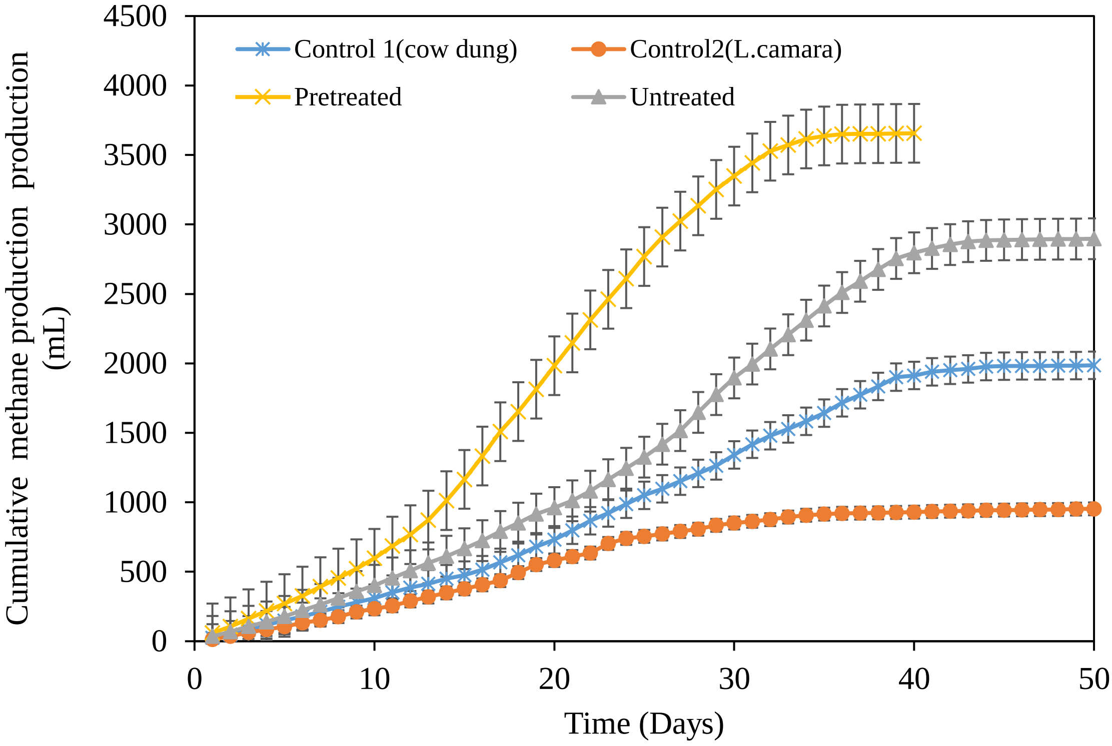


Fig. 6.29. Cumulative biogas production of *L.camara*

6.1.2.10. kinetics of control, untreated and pretreated *L.camara*

The cumulative biogas production values were fitted to the Gompertz equation curve for 50 days (Lee et al., 2013). In table 6.4, kinetic values were provided for treated and untreated *L. camara*. Values of M can be correlated with VS reduction (Veluchamy and Kalamdhad, 2016). Maximum M value was found in autoclave pre-treatment (4.0000 L

CH_4) followed by the untreated sample (3.6344 L CH_4) and control (cow dung) (3.2599 L CH_4). R^2 values were above 0.90 after pre-treatment that specified the efficiency of methane production.

Table 6.4 Kinetic value for control, untreated and pretreated *L.camara*

Substrate	**M(LCH_4)**	**R_m(L CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
Control	3.5435	0.0463	0.0010	0.943	2.493
Untreated	3.8654	0.679	0.0010	0.984	3.148
Pretreated	4.000	0.1000	0.007	0.992	4.239

6.1.2.11. Energy assessment of autoclave pre-treatment *(L. camara)*

During the autoclave pre-treatment, the specific energy utilized E_u is determined as per the eqn-6.3.

$$\mathrm{Eu} = \mathrm{P.t/n} \tag{6.3}$$

Where P in the process is the power rating of the autoclave (Watt), t is the exposure time, and n is the weight of the volatile solid fraction of 8.1 (g)

By substituting the value of P, t and W. The value of E_u was found to be 6.2 kJ/g (VS).

The specific energy available E_a due to the methane generated was determined as per this eqn-6.4.

$$E_Q = (Q_{raw}.\S)/1000.\mathrm{n} \tag{6.4}$$

Where Q is the maximum amount of the methane that was observed to produce daily, § is the calorific value of methane, and n is the weight of the volatile solids that were added. By substituting the value of Q_{raw}, § and n in Eqn 6.4, the E_a was 8.50kJ/g (VS).

6.1.3 *Ageratum conyzoides*

6.1.3.1. Effect of hot air oven pre-treatment on *A. conyzoides*

During hot air oven pre-treatment, VFA and sCOD both showed an increasing trend up to 110˚C, but at 120˚C, decreased sCOD and VFA were observed (Fig. 6.30). After 120˚C

VFA started to vaporize, which led to the loss of volatile fatty acids, VFA and sCOD had both started to decline. Strong chemical bonds of lignin present in the cell wall of *A. conyzoides* was broken down when the temperature was applied, which made the organic compound easily accessible for bacteria. At high temperatures of more than 110˚C, organic compounds like proteins and enzymes denatured were not further converted into VFA and sCOD. After the hot air oven pre-treatment at 110˚C, an increment of 1.86 and 2 times rise in the sCOD, and VFA (46.30% and 50%) was observed as compared to control. After the temperature was optimized, samples were kept for different time intervals inside the hot air oven at the optimized temperature to determine the optimum time. sCOD and VFA increased with time up to 90 mins after that it was started to decline (Fig. 6.31). The vaporization of VFA caused a decrease in sCOD. Barua and Kalamdhad, 2016 observed a similar result during the thermal pre-treatment of water hyacinth. Nielsen et al., 2004 reported that a temperature less than 100˚C was not suitable to break the strong lignin bond present in the outer cell wall but can only boost the biological activity slightly. At 90 mins, sCOD and VFA increased up to 48.14% and 47.69%, which were 1.92 and 1.91 times compared to the control. After the hot air oven pre-treatment, maximum sCOD and VFA were found pre-treatment at 110˚C for 90 mins.

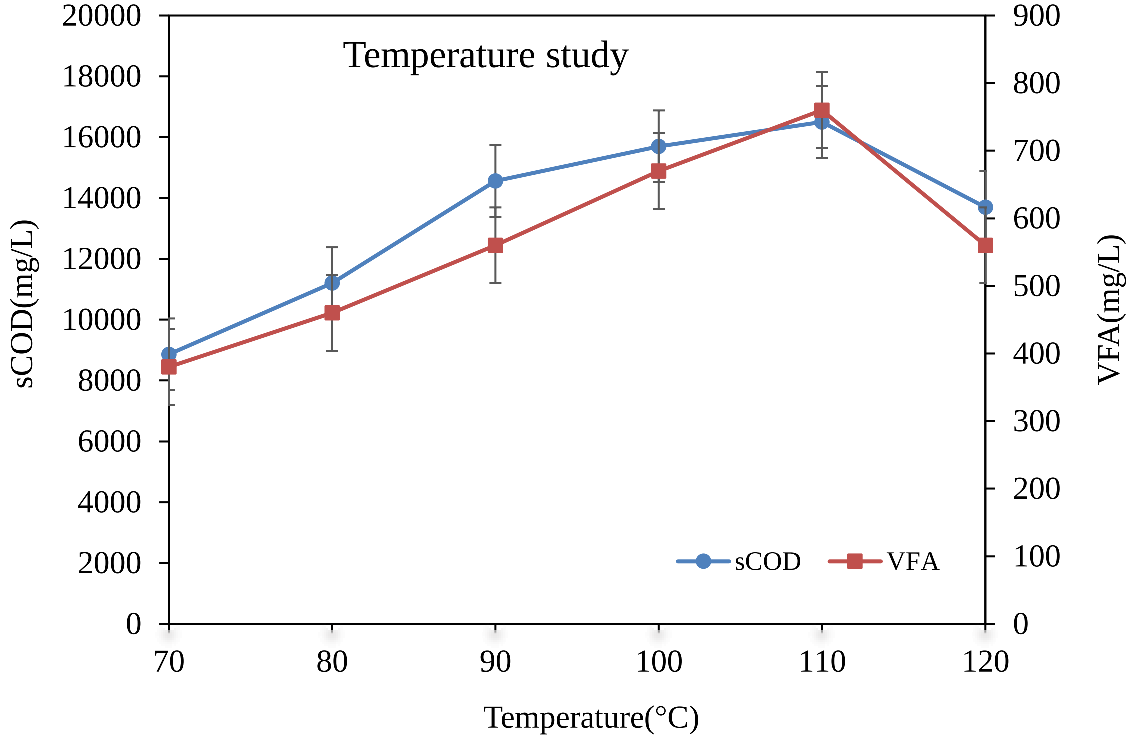


Fig.. 6.30. Hot air oven pre-treatment temperature study

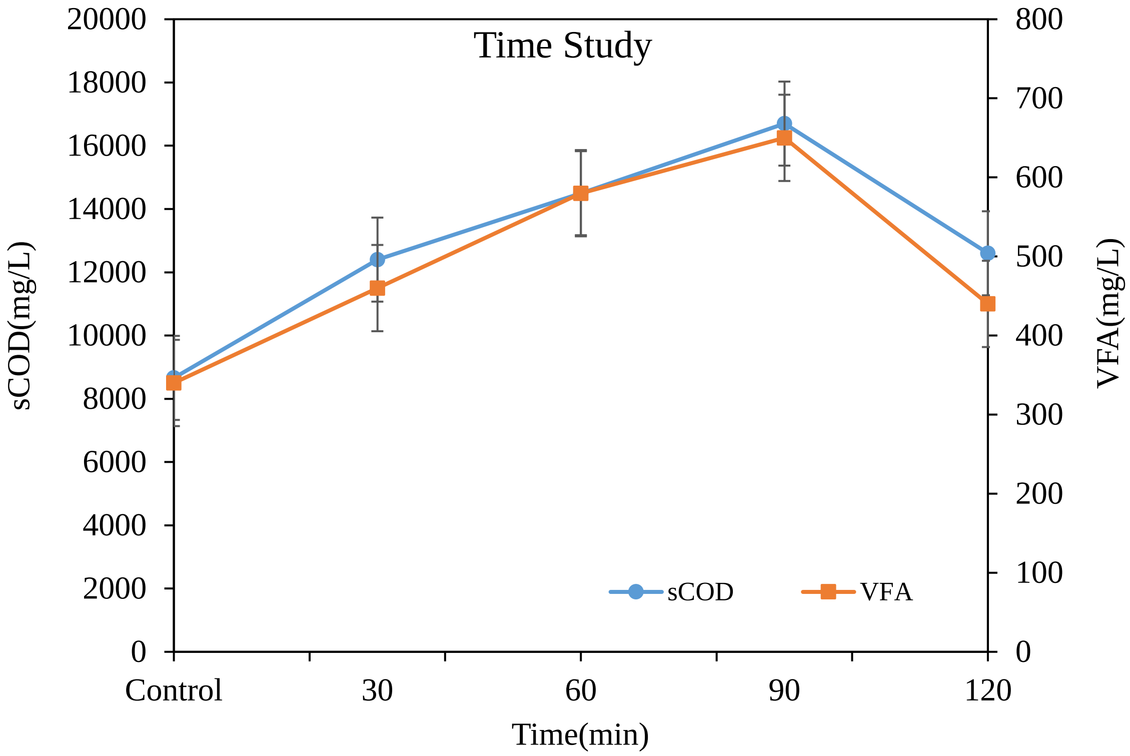


Fig. 6.31. Hot air oven time study

6.1.3.2. Effect of micro oven pre-treatment on *A. conyzoides*

During micro oven pre-treatment, it was observed that sCOD and VFA raised to 200˚C but at 220˚C it started to fall (Fig. 6.32). During micro oven, pre-treatment radio wave passes through the sample, which has high penetration power that leads to the denaturation of organic compounds. At high temperatures, VFA starts to vaporize, and at the same time, it reduces the moisture content of the sample, which might be the reason for not so significant increment of sCOD and VFA with temperature as compared to other thermal pre-treatments. After temperature optimization the increment in sCOD and VFA were found around 42.28% and 33.65%. This was 1.73 and 1.50 times as compared to the control. The samples were then kept at different time intervals inside the hot air oven at the optimized temperature to study the optimum time. In (fig. 6.33), sCOD and VFA rose with time up to 20 mins and then declined at 25 mins. Samples exposed to high temperatures for a long time lead to vaporization of the VFA, and at the same time color of the sample turned brown, which signified the presence of melanoidins after 25 mins at 200˚C. It occurs due to complex formation between reducing sugar and amino acids. They inhabit the acidogenesis and methanogenesis steps (Hendriks and Zeeman, 2009; Carrère et al., 2010;

Barua and Kalamdhad, 2016). During time study, maximum sCOD and VFA were obtained around 39.40% and 37.03%, which was 1.65 and 1.58 times higher than the control. In micro oven pre-treatment, optimum temperature and time was found at 200˚C for 20 mins.

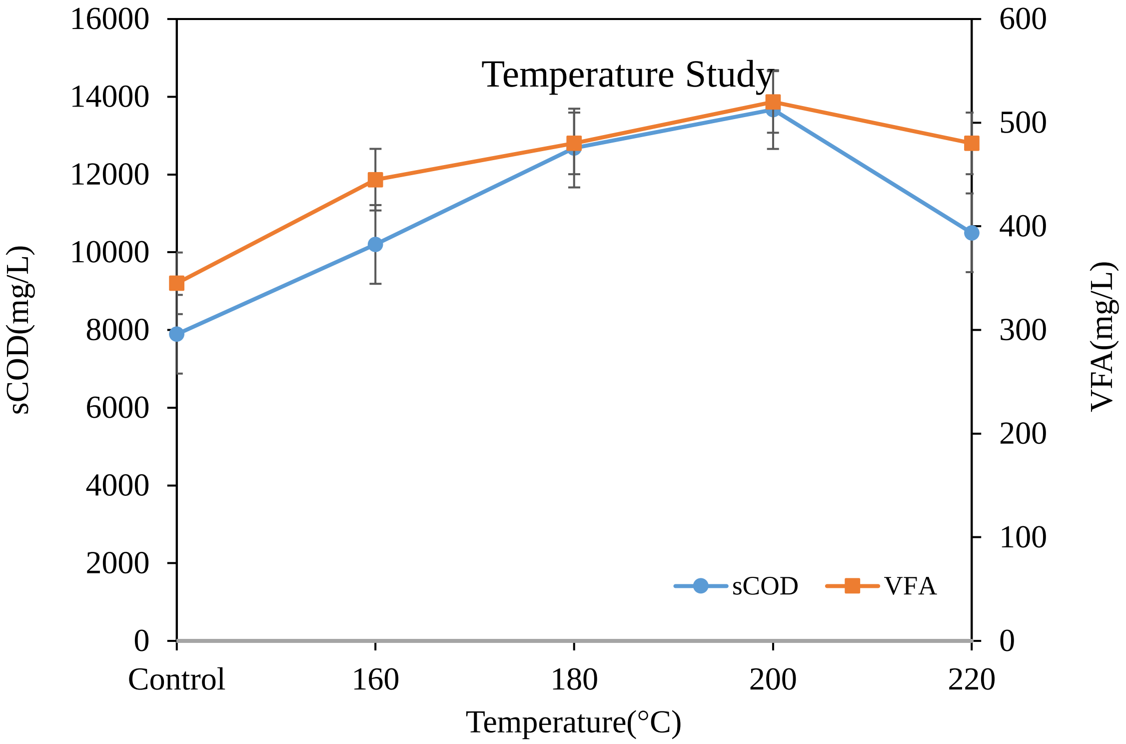


Fig. 6.32. Micro wave pre-treatment temperature study

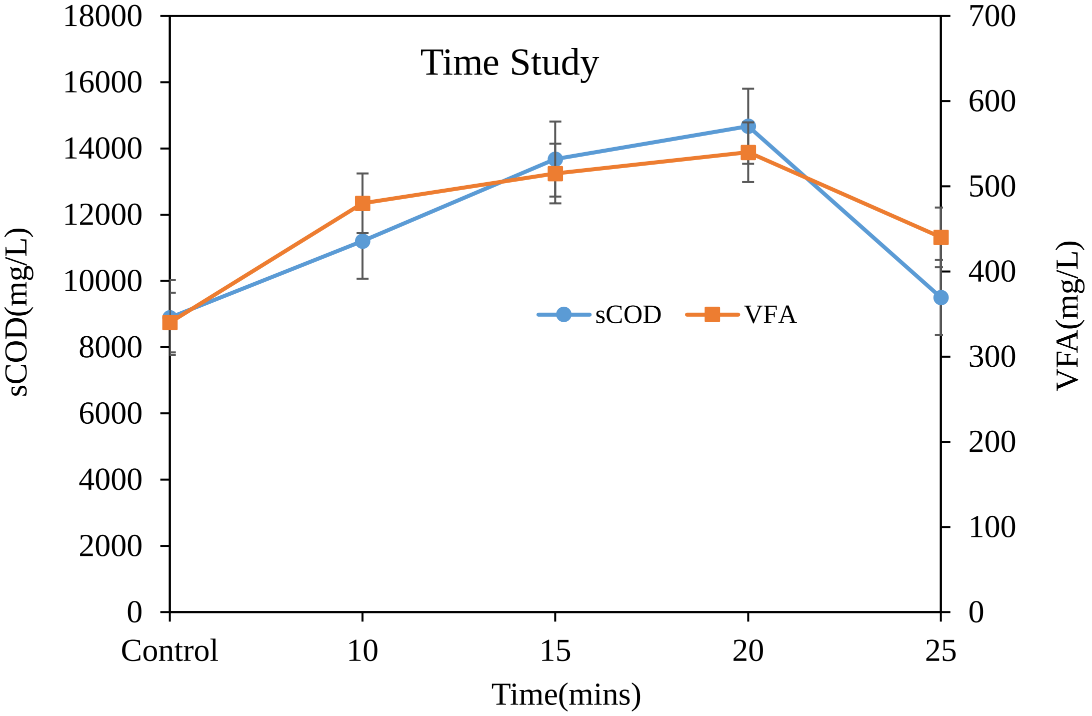


Fig. 6.33. Micro wave pre-treatment time study

6.1.3.3. Effect of autoclave pre-treatment on *A. conyzoides*

During the autoclave pre-treatment, both sCOD and VFA raised to 90˚C. At 100˚C, VFA started to vaporize, which might be the reason for the decrease of sCOD (Fig. 6.34). Increment of sCOD and VFA was observed around 56.85% and 56.81%, which was 2.31 and 2.31 times as compared to the control. In autoclave, pre-treatment, moist heat was applied to the samples, which have high penetration power, and at the same time, it keeps the sample moist. Due to high penetration power, it denatures the organic compounds like proteins and enzymes, which might be another reason for fall of sCOD and VFA at 100˚C. Similarly, the temperature was optimized by keeping the samples at different time intervals inside the autoclave at an optimized temperature. As the time interval, increase sCOD and VFA both increased up to 40 mins then started to decrease (Fig. 6.35). After 40 mins VFA started to vaporize and denature the organic, leading to the fall of sCOD and VFA at 60 mins. Increment of sCOD and VFA was observed at 40 mins around 55.33% and 54.59, which was 2.23 and 2.20 times than the control. Menardo et al., 2012 observed similar types of results on wheat and barley for ethanol production. In autoclave pre-treatment, maximum sCOD and VFA were obtaining at 90˚C for 40 mins.

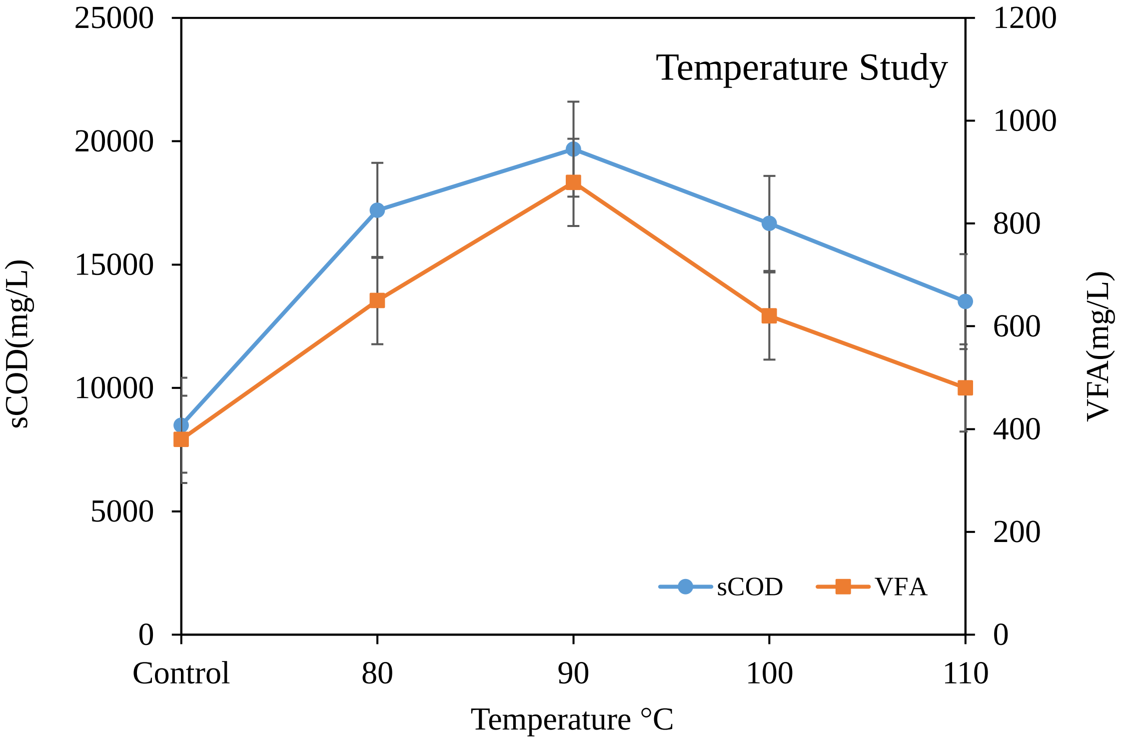


Fig. 6.34. Autoclave pre-treatment temperature study

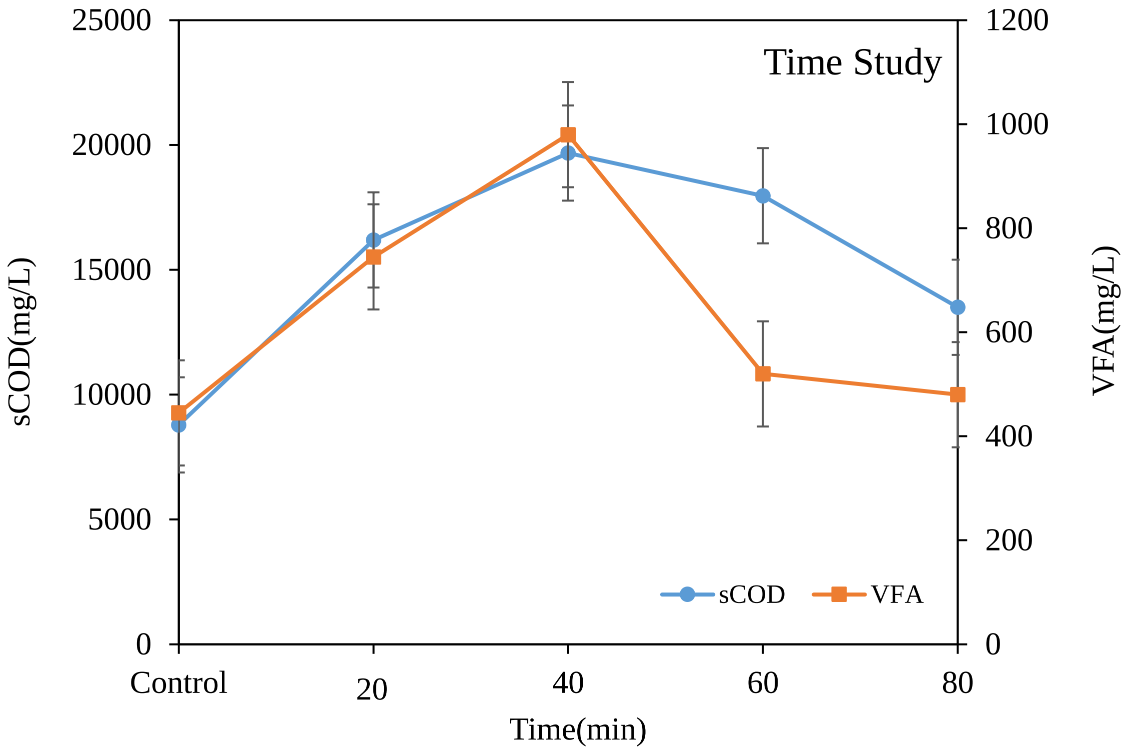


Fig. 6.35. Autoclave pre-treatment time study

6.1.3.4. Effect of hot water bath pre-treatment on *A. conyzoides*

The effect of hot water bath on the pretreated weed is shown in Fig. 6.36 and 6.37. As the temperature increased, solubilization also increased, which can be seen as the increment of sCOD and VFA. In Fig.6.36 sCOD and VFA both increased up to 90˚C; after that, it started to fall. After 90˚C volatile compound vaporized may affect the solubilization, leading to a reduction in the sCOD and VFA. When the high temperature was applied for the pre-treatment, the solubilization of the sample was reduced (Appels et al., 2010). In hot water bath pre-treatment, the highest sCOD and VFA were observed at 90˚C around 55.24% and 56.81%, which was 2.21 and 2.31times compared to the control. After the optimum temperature was determined, the samples were kept at different time intervals inside the hot water bath at the optimized temperature. Solubilization increased with time (Fig. 6.37) up to 90 mins, which showed in the form of increment of sCOD and VFA. To increase the efficiency of solubilization rate, high pre-treatment exposure time (at least 1 h) is required (Ariunbaatar et al. 2014). Maximum sCOD and VFA were obtained at 90 mins around 52.33% and 45.12% times, 2.09, and 1.82 times to the control. During the hot water bath pre-treatment study, optimum temperature and time were observed at 90˚C for 90 mins.

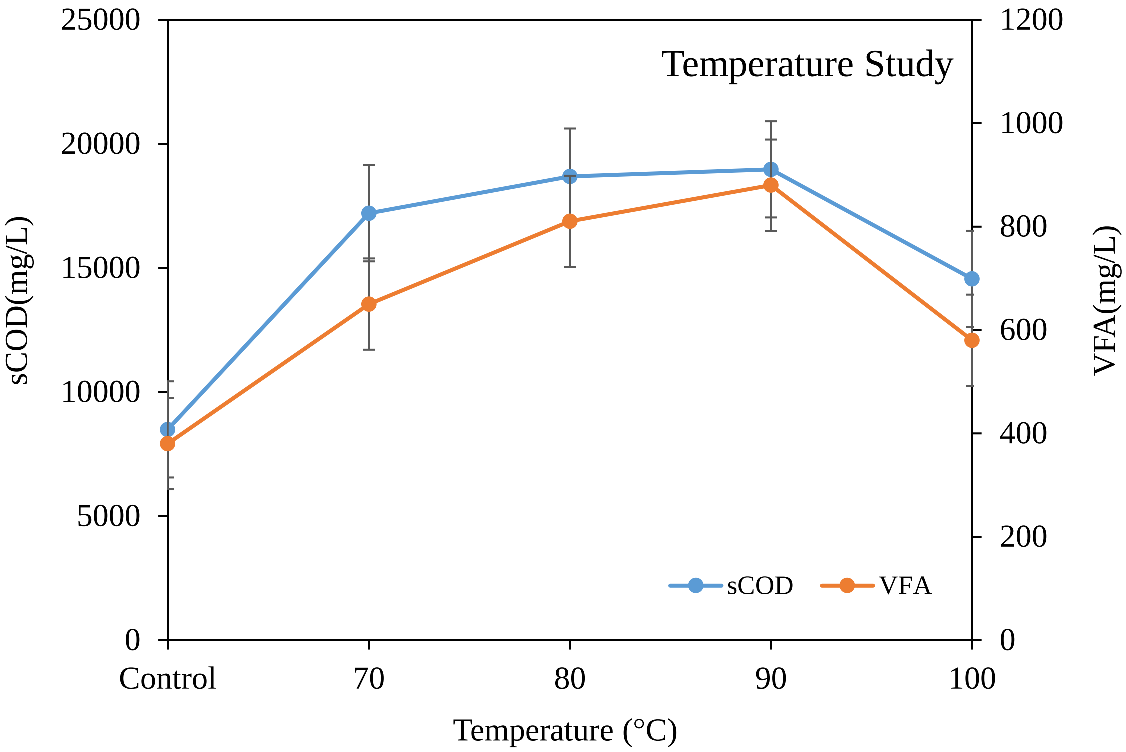


Fig. 6.36. Hot water bath Temperature study

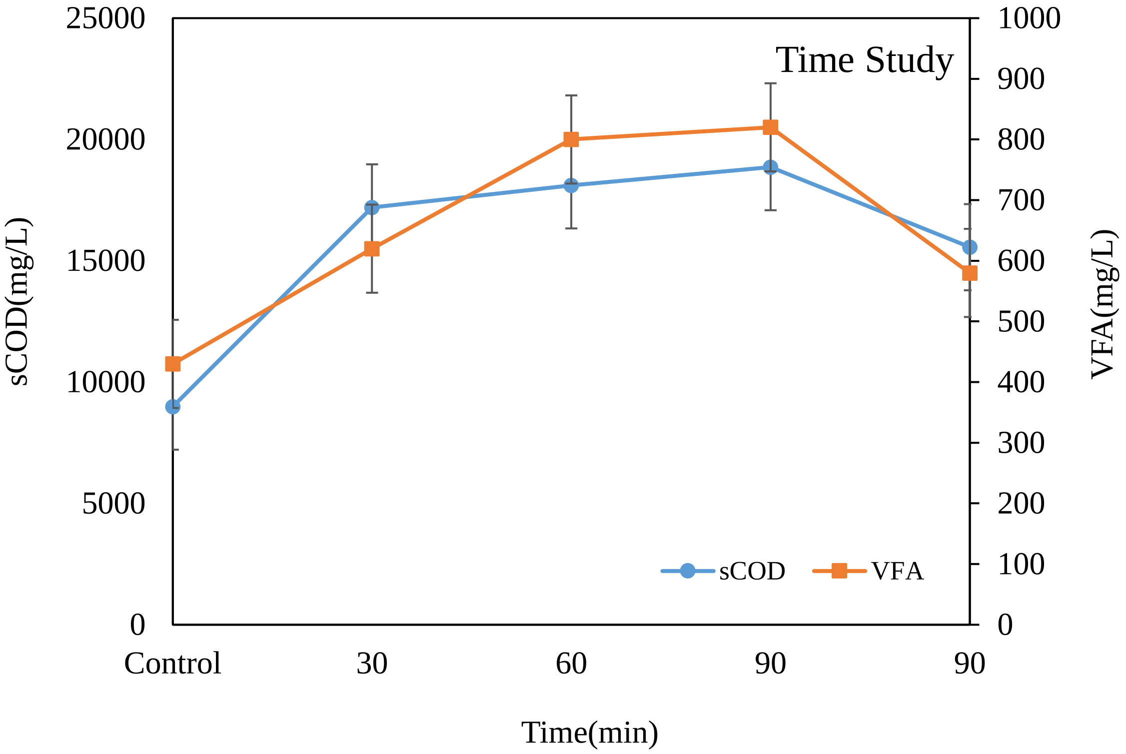


Fig. 6.37. Hot water bath time study

6.1.3.5. Compositional analysis of untreated and pretreated *A.conyzoides*

During compositional analysis, composition changes were observed in cellulose, hemicellulose and lignin before and after different thermal pre-treatments. The efficiency of thermal pre-treatment was confirmed in the form of a rise in soluble acid lignin and cellulose as compared to untreated/control *A. conyzoides*. When the heat was applied to the sample, lignin started to melt, due to which acid-soluble lignin had increased. Table 6.5 found that autoclave pre-treatment showed the highest increment of acid-soluble lignin (68.71%) and cellulose (15.65%), which was 3.19 and 1.18 times than that of the control. On the other hand, hemicellulose had started to reduce. Increment of soluble acid lignin is beneficial for fermentative microbes as it can be degraded within a short period. Hemicellulose is a short-chain biopolymer that forms a bridge between lignin and cellulose, slightly decreasing after all the pre-treatment studies. Veluchamy et al., 2017, observed a similar result. The reduction of hemicellulose may be due to the reconstruction of its short chain to form cellulose. A significant increment of acid-soluble lignin (P ¼ 0.0041) and cellulose (P ¼ 0.0082) was observed after analyzing the ANOVA's experimental data.

Table 6.5. The variation was observed in the composition of thermally pretreated and untreated *A.conyzoides* whole plant

Pre-treatment technique	**Acid soluble lignin (%)**	**Acid insoluble lignin (%)**	**Cellulose (%)**	**Hemicellulose (%)**
Untreated	1.12±.43	4.93±.21	37.22±.56	31.13±.15
Hot air oven	2.12±.27	3.14±.43	42.17±.43	24.56±.21
Microwave	2.89±.14	3.19±.32	42.12±.21	23.25±.13
Hot water bath	1.54±.11	3.15±.11	43.16±.34	22.13±.25
Autoclave	3.58±.18	3.01±.23	44.13±.27	22.01±.20

6.1.3.6. FESEM study of untreated and pretreated *A.conyzoides*

In Fig. 6.38, the control can be seen as rocky, rigid and tough structure while, after pre-treatment changes were observed on the surface of the *A. conyzoides* (Fig. 6.39). Due to thermal pre-treatment, the hard outer surface was broken down into pieces, which became more comfortable for the microbes to degrade. Completely broken and long circular ring-like structures were observed after autoclave pre-treatment, which might be the cellulose chain.

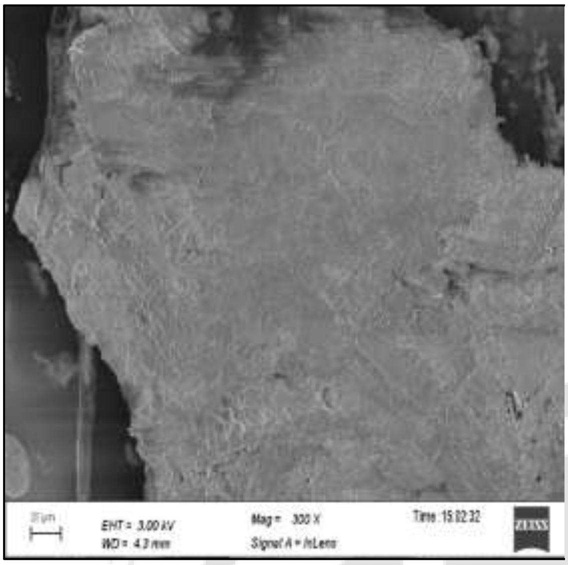


Fig. 6.38. Before pre-treatment

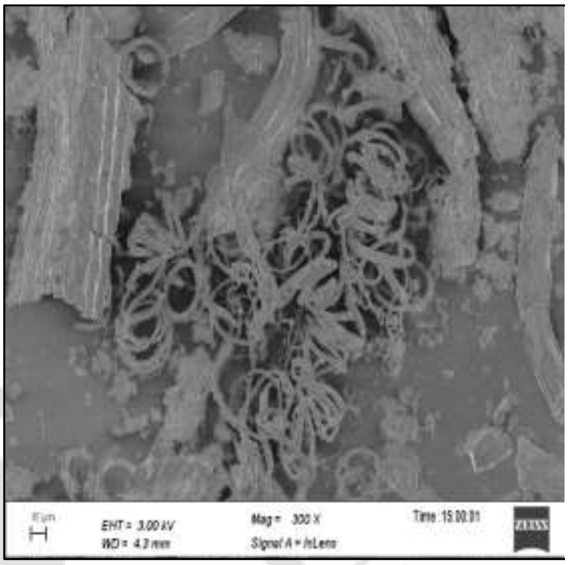


Fig. 6.39. After autoclave pre-treatment

6.1.3.7. XRD study of untreated and pretreated *A.conyzoides*

XRD analysis was done to identify the crystalline or amorphous nature of the sample. In the untreated (control) sample, sharp peaks were observed while, after the autoclave pre-treatment, no such peak was observed (Fig. 6.40). High peaks were found in untreated (control) due to cellulose's crystalline nature, which is very hard to degrade. After autoclave pre-treatment, it was converted into an amorphous structure that became easier for microbes to degrade. Chen et al., (2011) reported similar results after pre-treatment of sugarcane bagasse. Therefore, thermal pre-treatment was found to be favorable for the conversion of crystalline cellulose to amorphous.

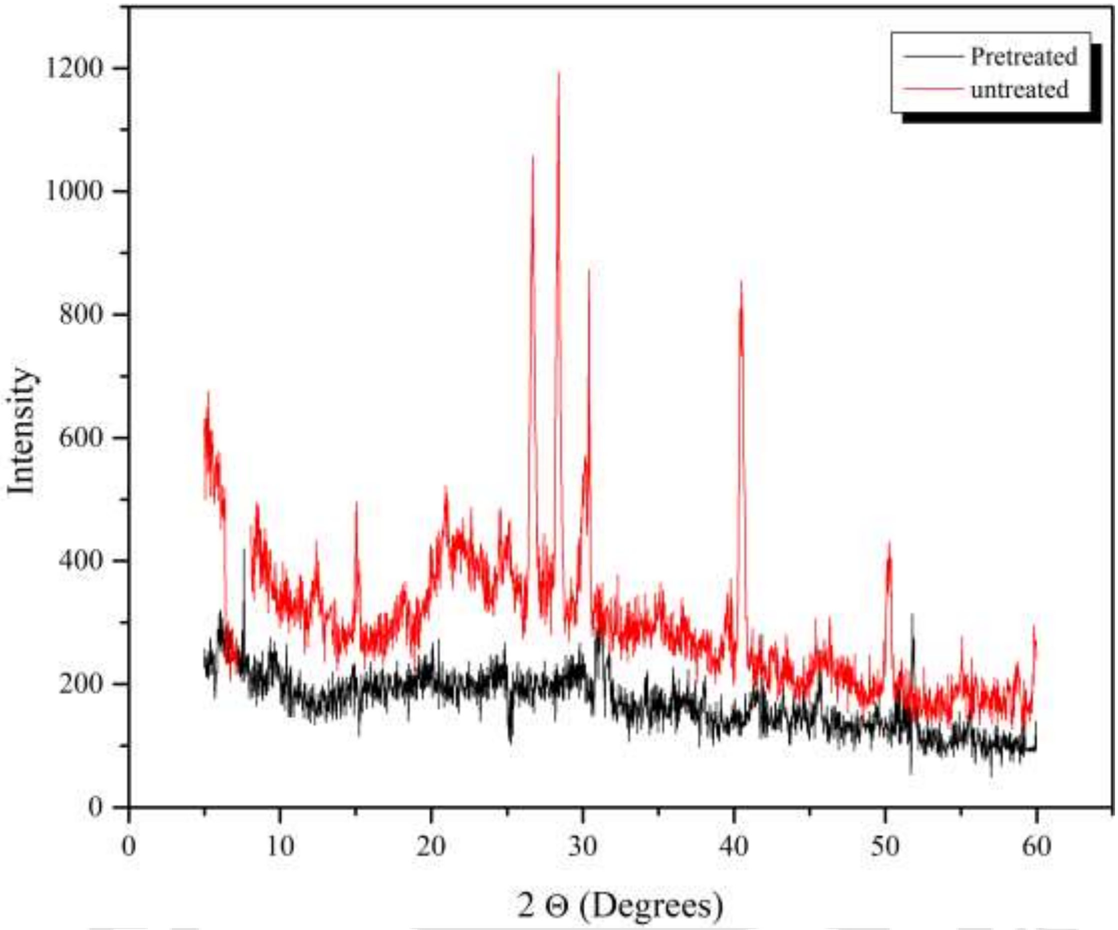


Fig. 6.40. XRD study of untreated and pretreated *A.conyzoides*

6.1.3.8. FTIR study of untreated and pretreated *A.conyzoides*

Prediction of lignin, cellulose and hemicellulose content in the plant sample can be identified in FTIR (Chen et al., (2010). In FTIR, it was seen that after autoclave pre-treatment, the band was broadened as compared to the untreated (control) (Fig. 6.41). This signifies the weakening of intra and intermolecular hydrogen bonds in cellulose, hemicellulose and lignin. All through the peak was found in similar points but a significant reduction in the peak was observed. Peaks corresponding to 1056.53 cm^{-1}, 1256.45 cm^{-1} and 1678.22 cm^{-1} indicates C = O stretch in cellulose, hemicellulose and lignin (Li et al., 2010). After autoclave pre-treatment, a fall of the peak was observed at 3328.54 cm-1 that indicates the stretch of OH bond in lignin. Compaction of peak intensity and changes of peak after pre-treatment represented the delignification and low crystallinity of *A. conyzoides*.

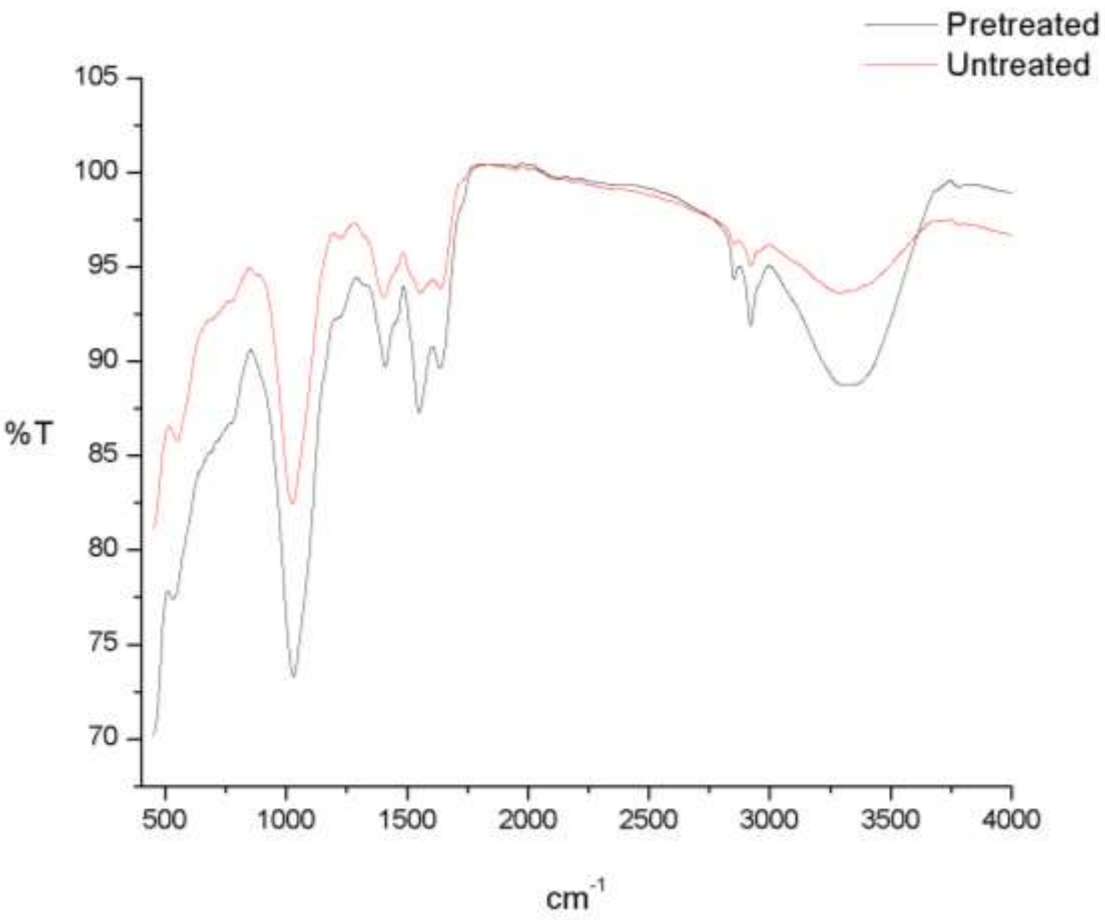


Fig. 6.41. FTIR study of Untreated and pretreated *A.conyzoides*

6.1.3.9. Biogas production of control, untreated and pretreated *A.conyzoides*

After four different heating pre-treatment (hot air oven, micro oven, autoclave and hot water bath) was applied on *A. conyzoides*, out of that, autoclave was found to be most efficient in the form of increment of sCOD (55.33%) and VFA (54.99%). BMP set up was conducted for autoclave pretreated sample (best ratio 2), untreated sample and two different controls. In control 1, only *A. conyzoides* was taken, and in control 2, only cow dung was taken. High lignin content in *A. conyzoides* delays the degradation process, which increases the hydrolysis period (Gurung et al., 2012). In untreated *A.conyzoides*, cumulative biogas was found around 3011 mL CH_4/g VS in 50 days, and it required 40 days to stabilized the cumulative biogas production. After autoclave pre-treatment sharp increment of cumulative biogas production (4053 mL CH_4/g VS) was found in *A.conyzoides* in 35days and within 30 days cumulative methane production started to stabilize. In (Fig. 6.42) a long hydrolysis period can be seen in the untreated sample. Low-temperature pre-treatment helps to increase the efficiency of the degradation process in anaerobic digestion (Wang et al. 1997).

High-temperature pre-treatments may produce inhibitors, which decreases the efficiency of anaerobic digestion (Laser et al., 2002; Nizami et al., 2009).

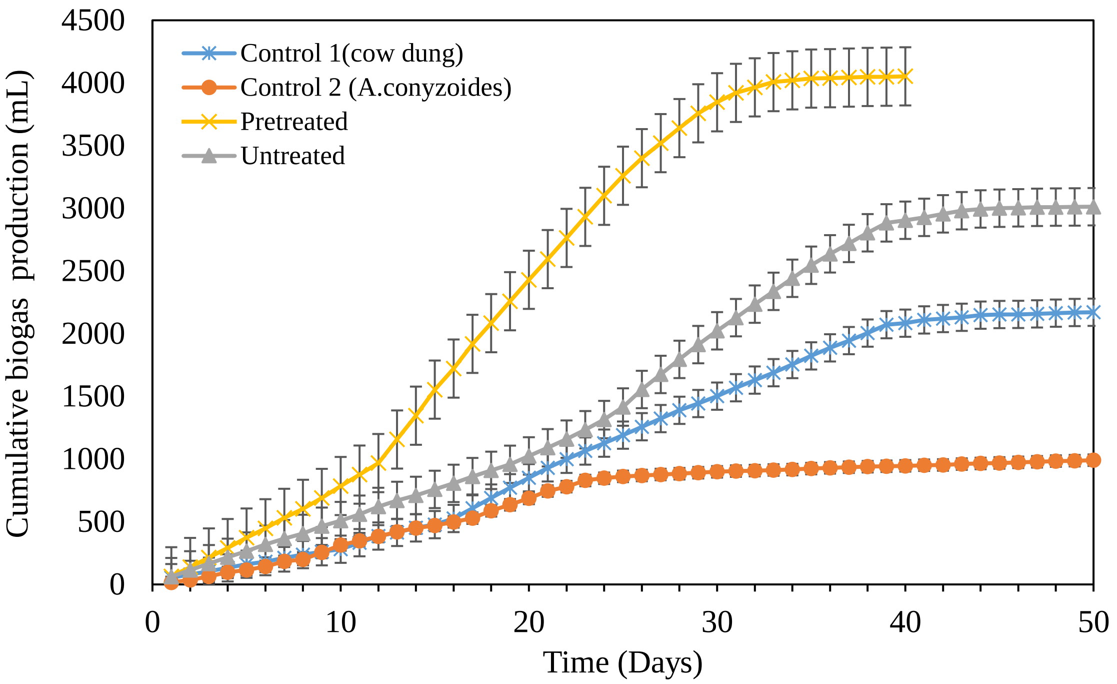


Fig. 6.42. Cumulative biogas production of *A.conyzoides*

6.1.3.10. Kinetics of control, untreated and pretreated *A.conyzoides*

The cumulative biogas production values were fitted to Gompertz equation curve for 50 days (Lee et al., 2013). In table 6.6, kinetic values were provided for control (cow dung), untreated and pretreated. *A. conyzoides.* Values of M can be correlated with VS reduction (Veluchamy and Kalamdhad, 2016). Maximum M value was found in autoclave pre-treatment (4.0000 L CH_4) followed by the untreated sample (3.6344 L CH_4) and control (cow dung) (3.2599 L CH_4). R^2 values were above 0.90 after pre-treatment that specified the efficiency of methane production.

Table 6.6. Kinetic value for control, untreated and pretreated *A. conyzoides*

Substrate	**M(LCH_4)**	**R_m(L CH_4 d^1)**	**λ(d)**	**R^2**	**Y(L CH_4)**
Control	3.2599	0.0594	0.0010	0.987	2.493
Untreated	3.6344	0.727	0.0010	0.989	3.148
Pretreated	4.0000	0.1000	0.007	0.996	4.239

6.1.3.11. Energy assessment of autoclave pre-treatment *(A.conyzoides)*

During the autoclave pre-treatment, the specific energy utilized E_u is determined as per the eqn-6.5.

$$\mathrm{Eu} = \mathrm{P}.\frac{\mathrm{t}}{\mathrm{n}} \tag{6.5}$$

Where P in the process is the power rating of the autoclave (Watt), t is the exposure time, and n is the weight of the volatile solid fraction of 7.3 (g)

By substituting the value of P, t, and W. The value of E_u was found to be 5.98 kJ/g (VS)

The specific energy available E_a due to the methane generated was determined as per this eqn-6.6.

$$E_Q = \frac{Q_{raw}.\S}{1000}.\mathrm{n} \tag{6.6}$$

Where Q_{raw} is the maximum amount of methane (best F/M ratio) that was observed to produce on a daily basis, § is the calorific value of methane, and n is the weight of the volatile solids that were added. By substituting the value of Q_{raw}, § and n in Eqn 6.6, the E_a was 9.85kJ/g (VS).

6.2. Phase II- Electrohydrolysis pre-treatment

This study's main objective was to observe the effect of electro hydrolysis pre-treatment on P. hysterophorus, L.camara and A.conyzoides, whereas direct current (DC) was supplied to treat this noxious weed. The main principle behind electrohydrolysis pre-treatment is ohmic heating, electrophoresis, and electro-osmosis, which are responsible for breaking down the blockage of the lignocellulose complex result, which reduced the time of hydrolysis (Mahmoud et al., 2010; Barua et al., 2017). When current passes through the substrate, ohmic heat was generated (Knirsch et al., 2010), helping to melt the lignin. In electrophoresis, the transfer of ions occurs in the static state (Mahmoud et al., 2010), where the electric energy is transferred through the substrate, leading to a break down of the chemical bonds in lignin. The first time electrohydrolysis pre-treatment was explored on freshly *P. hysterophorus*, *L.camara* and *A.conyzoides*. This pre-treatment's effectiveness was identified in the form of increment of soluble chemical oxygen demand, volatile fatty acid, methane production, and reduction of hydrolysis stage.

6.2.1. *Parthenium hysterophorus*

6.2.1.1. Change of current and resistance with time at different applied voltages

In Fig. 6.43a, b and 6.44, it was found that when applied voltage increased, current and resistance varied. Current remained constant at 10 V and 20 V at 0.08 A and 0.18 A, respectively. Resistance and current both started to fluctuate, as the voltage increased the production of organic matter like glucose, amino acids, and volatile fatty acids rise due to the increased current supply. As the current started fluctuating at 30 V, VFA started to vaporize, and sCOD started to decline. At 30 V, the number of flowing electrons increased, which lead to a rise in the temperature and increase conductivity that may denature the organic compound; as a result, VFA and sCOD started to decline.

As the time progressed, the current flow remained constant at 10 V and 20 V, but at 30 and 40 V, it started to rise. As the current fluctuated (Fig. 6.43b), resistance also varied, which caused the uneven flow of electron, that lead to the drop of VFA and sCOD. If the resistance varies the effectiveness of energy, distribution became no uniform all over the sample. Although 10 V and 20 V show a uniform flow of current and resistance, at 20 V, higher efficiency was observed in terms of sCOD and VFA. In fig 44-time study was observed, where sCOD increased in all the oltage up to time 40 mins except in 40V then it started to decline. After 40 mins, the VFA in the sample began to vaporize, which decreases sCOD. At the Voltage of 40 V, sCOD dropped at 40 mins then increased at 60 mins and fell again at 80 mins., it was happened due to uncontrolled flow of electrons and energy distribution that was also not uniform all over the sample. Therefore, a time of more than 40 mins and a voltage above 20 V is not efficient for pretreated *P. hysterophorous.* At the higher voltage and longer time, it was also challenging to control the uniform flow of current (Fig. 6.44).

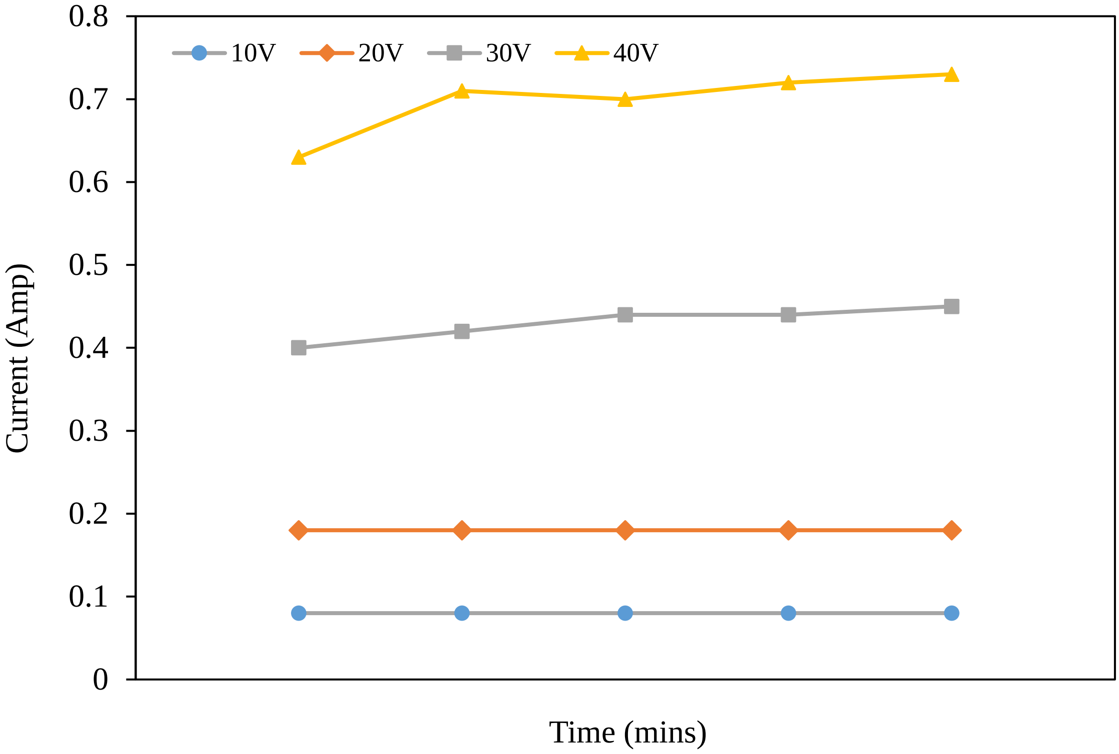


Fig. 6.43a. Change of current

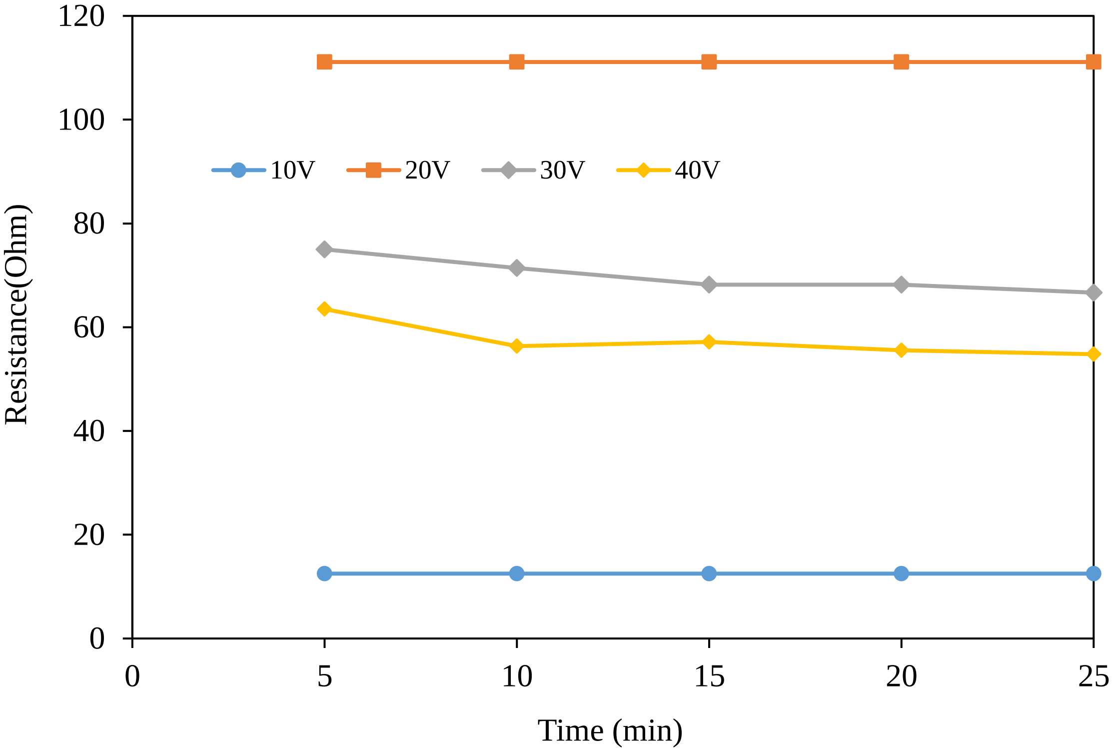


Fig. 6.43b. Change of resistance with time

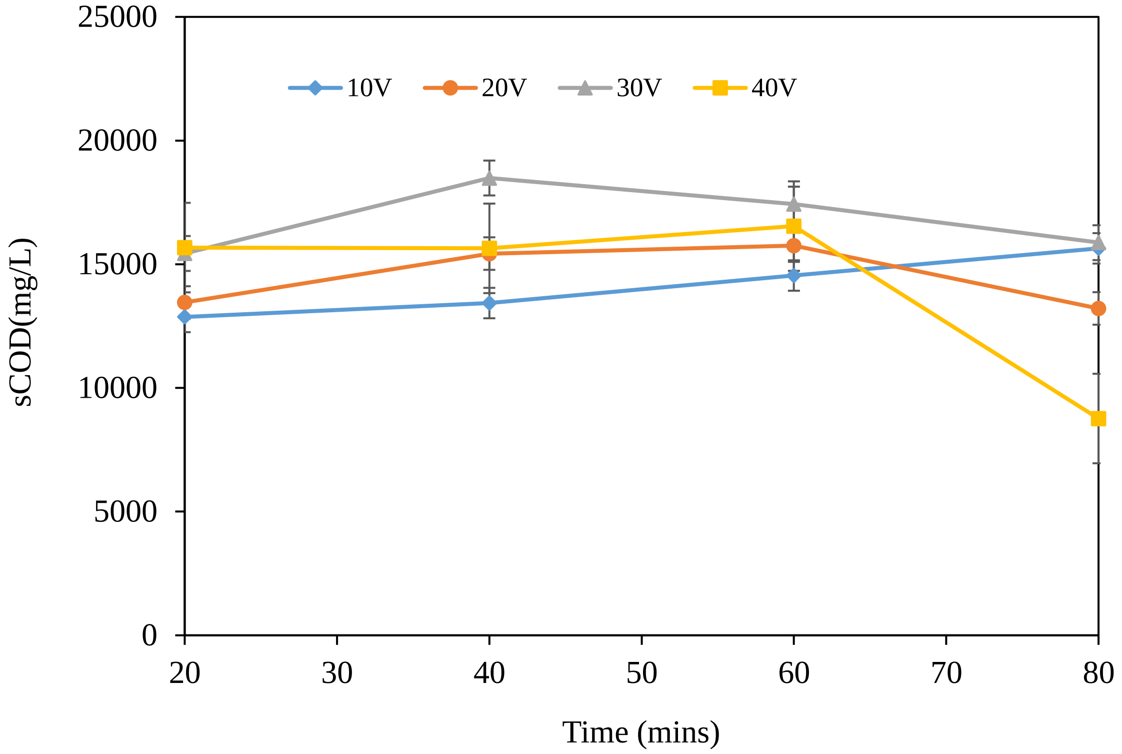


Fig. 6.44. Variation of sCOD with time

6.2.1.2. Effect on VFA and sCOD

In Fig. 6.45, it was observed that VFA and sCOD increased with 0 to 20 V applied voltage, and at 30V, it started to fall down. The efficiency of solubilization increased with voltage, which proves in the form of improvement of sCOD and VFA as compared to control. Still, at 30 V VFA and sCOD started to decline because, at high voltage, the number of electron passes through the sample increased, which lead to rises in ion, so the current was also rising in the sample measured by ammeter as a result decreasing of efficiency was observed (Fig. 6.45). The rise of electric current leads to increasing heat and produced a foam, making the VFA vaporization. When VFA started to vaporize, the sCOD was also started to decline (Zhen et al., 2014; Veluchamy et al., 2017). The highest increment of sCOD was observed at 20 V around (17600±21mg/g), which represented a 50.17% increment when compared with control. At 20 V, there might be adequate electric energy to break down complex organic compounds without denaturing the other organic compounds. Higher voltage more than 20V, some of the organic matter like protein, enzymes started to denature, that is not further converted into soluble sCOD and VFA, which might be another reason to fall of sCOD and VFA after 20 V. Therefore, 20V was the ideal voltage to pretreated *P. hysterophorus*, which shows 2.0 times increment of sCOD.

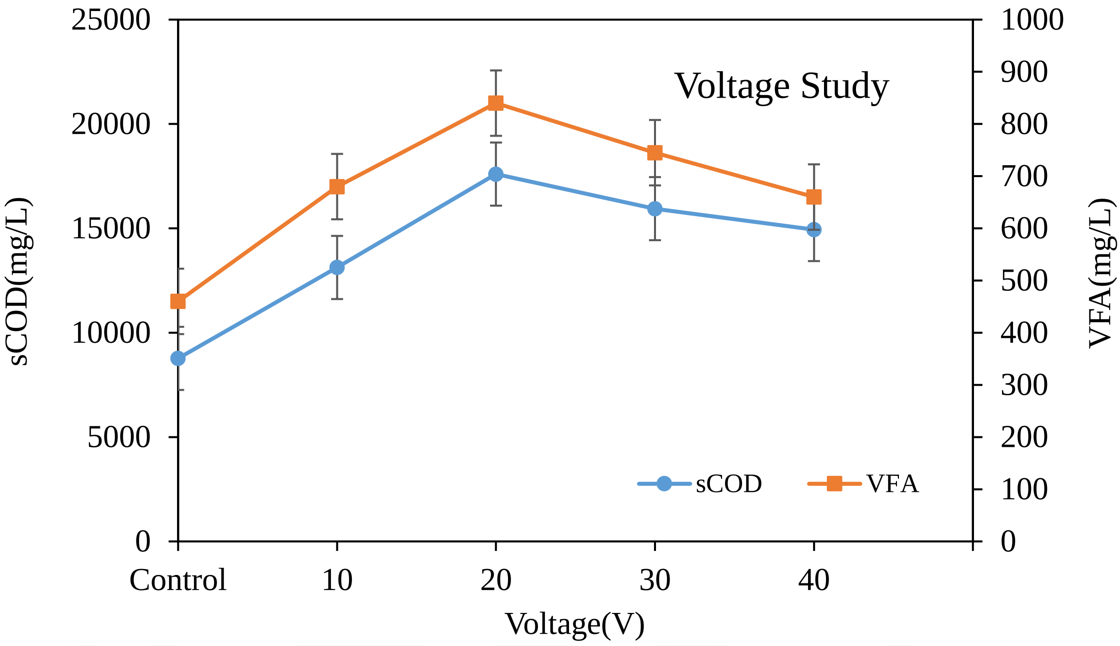


Fig. 6.45. Effect on VFA and sCOD

6.2.1.3. Effect on VFA and SCOD with time

During the time study, the sample was kept for a different time interval at an optimized voltage (20). It was clearly observed in the fig 6.46, that the VFA and sCOD both increased with time up to 40 mins. Then both started to decline. VFA and sCOD were found 780±8mg/L and 18550±11mg/L, respectively, which rises up to 1.44 and 2.30 times within 40 mins from control. Around 56.65% of sCOD increased at 40 mins in 20 V when it is compared to control. Due to the expose of the sample, for a longer duration at 20 V, heat is generated; as a result, VFA vaporized, which leads to a drop off the sCOD after 40 mins. An organic compound like protein, enzymes, a sugar, which is sensitive to heat, gets denatured when the sample was pretreated with electric energy for a longer duration, which is not further converted into VFA and sCOD. Therefore, 20 V for 40 mins was the ideal voltage and time to pretreat *P. hysterophorus*.

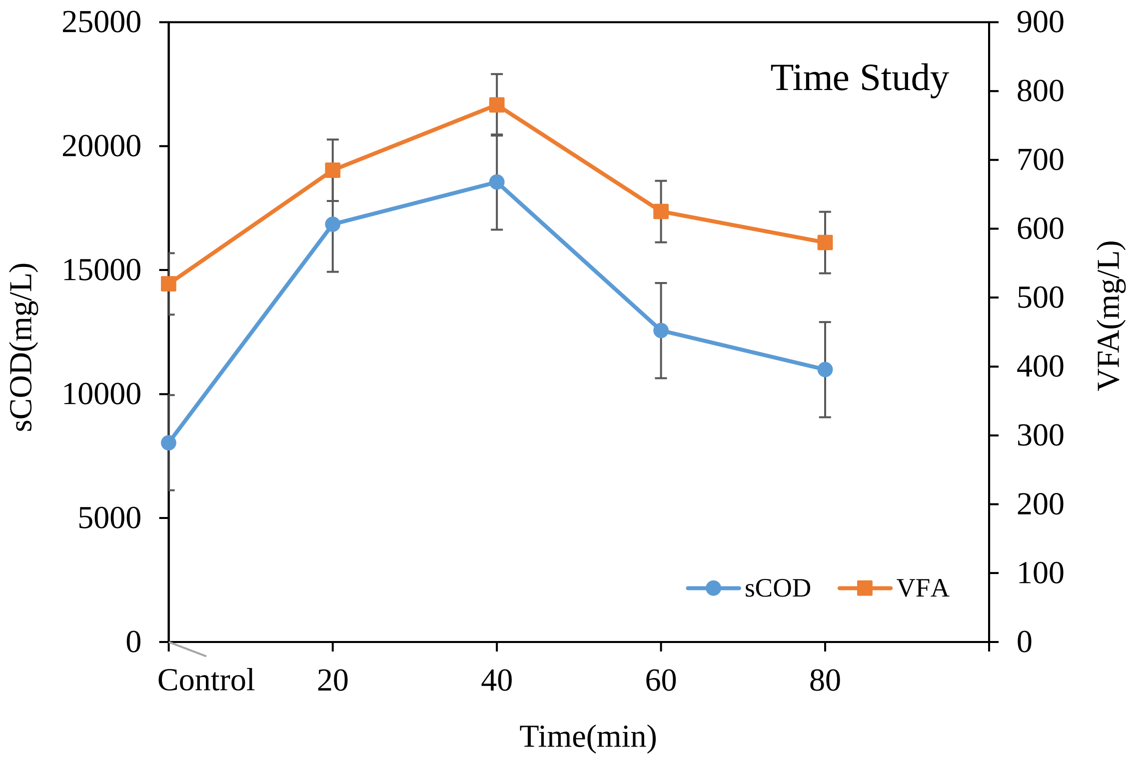


Fig. 6.46. Effect on VFA and sCOD with time

6.2.1.4. Effect of electrohydrolysis pre-treatment on lignin, cellulose, and hemicellulose

Several changes were found after electrohydrolysis pre-treatment at 20V for 40 mins. In table 6.7 it was shown that after pre-treatment, acid-soluble lignin increased and at the same time, cellulose also increased when it was compared to control. Increasing solubilization of lignin is directly related to the amount of substrate biodegradable (Kamdem et al. 2015). As the soluble lignin increased, it became easier for the fermentative bacteria to degrade the cellulose (Patel et al., 2015). Cellulose is a long chain during electrohydrolysis pre-treatment; it breaks down into small fragments, which might be the reason for increasing cellulose. Break down of cellulose into small units lead to increased solubilization (Kumar et al., 2009a). Fall of hemicellulose may convert it into the monomer of glucose and xylose. Therefore, softening of lignin from lignocellulose substrate after electrohydrolysis pre-treatment, reduced the time of hydrolysis phase, which is the first step of anaerobic digestion, and at the same time, it increased methane production during anaerobic digestion.

Table 6.7. The variation was observed in the composition lignin, cellulose and hemicellulose after and before pre-treatment of *P. hysterophorus*

Parameters	Time (min)	Voltage (V)	Acid insoluble lignin (%)	Acid soluble lignin (%)	Hemicellulose (%)	Cellulose (%)
Control	-	-	2.65 ± 0.29	4.19 ± 0.31	22.22 ± 0.55	32.75 ± 1.26
After Pretreatment	20	40	2.26 ± 0.28	4.81 ± 0.25	15.18 ± 0.29	15.18 ± 0.29

6.2.1.5. FESEM Study of untreated and pretreated *P.hysterophorus*

To recognize the structural changes during the electrohydrolysis pre-treatment, spectroscopy analysis was performed (Fig. 6.47) and it was observed that in untreated *P. hysterophorus*, which seems to be tough, hard and smooth, that is, strongly bonded lignin shelter the outer part of the plant cell. After pre-treatment (Fig. 6.48) some cracks and destructures were found on the surface of *P. hysterophorus* and some parts appear as broken into pieces, which indicate that after pre-treatment the surface of the lignin melt down and cellulose was readily available to bacteria. It can be concluded that after pre-treatment the bond present in the lignin become weaker and breakdown, which is easier for the microbes to degrade. A similar type of lignocellulose structure identified after applying different thermal pre-treatments (Gabhane et al., 2011).

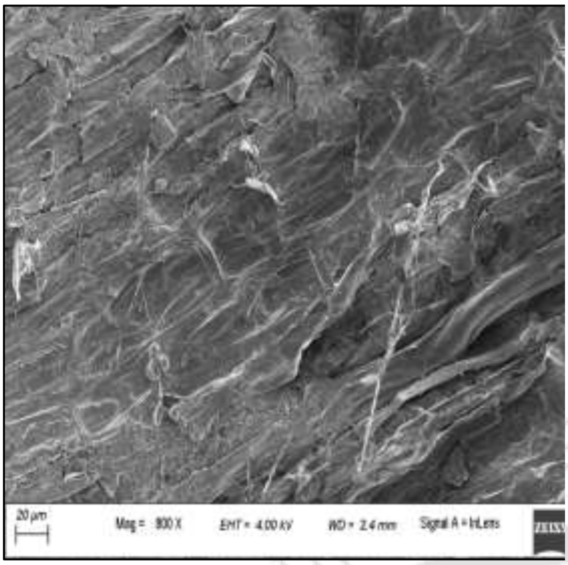


Fig. 6.47. Before pre-treatment

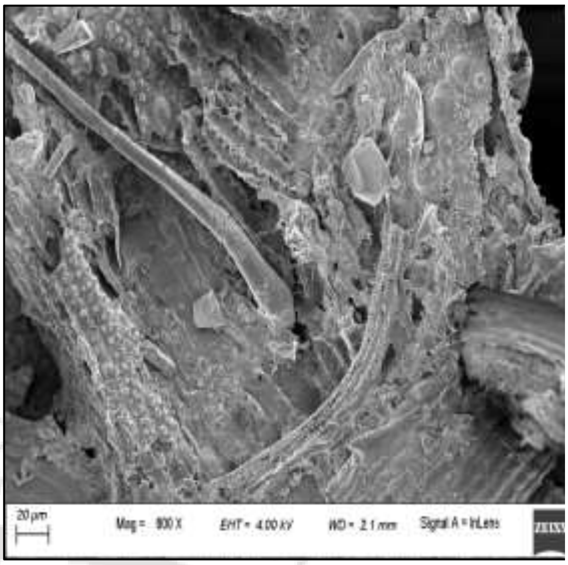


Fig. 6.48. After Electrohydrolysis Pre-treatment

6.2.1.6. XRD study of untreated and pretreated *P.hysterophorus*

XRD analysis signifies the crystallinity and amorphous nature of the substrate. Sharp peaks were observed in the untreated sample (Fig. 6.49), which represents the substrate's crystalline nature, but after pre-treatment, no such high peaks were found. High peaks were found in the untreated sample due to the crystalline cellulose, which is very hard to degrade for the microbes. The sharp peaks indicate the crystalline structure, and the absence of sharp peaks means amorphous substances (Karimi and Taherzadeh, 2016). Bragg peaks in X-ray diffraction was a well-defined characterizing substance for the diffraction from perfect crystalline. After pre-treatment, no such high peaks were found because of the conversion of crystalline cellulose into amorphous substances. Reduction of crystallinity was observed from 0.9856 to 0.5643 after electrohydrolysis pre-treatment. Yang et al. (2009) reported similar results.

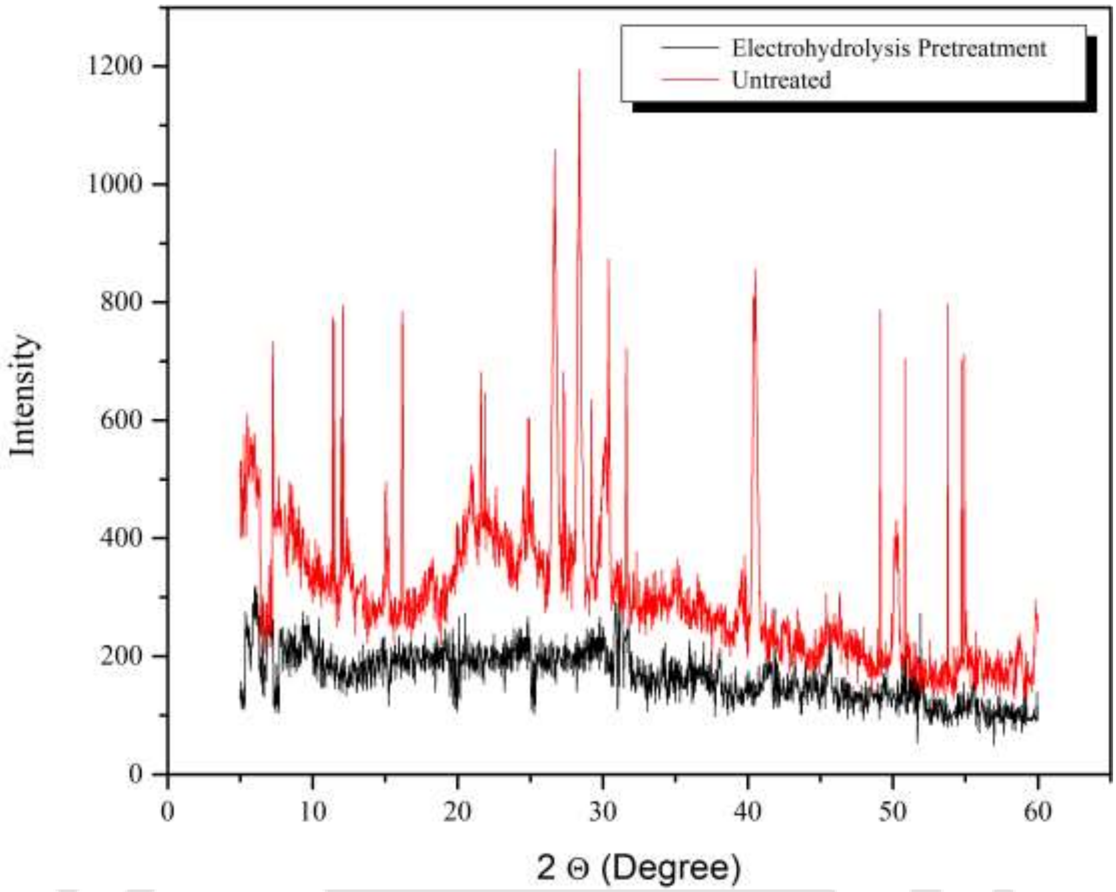


Fig. 6.49. XRD study of untreated and pretreated *P.hysterophorus*

6.2.1.7. FTIR study of untreated and pretreated *P.hysterophorus*

FTIR gives detailed analysis for a constituent of basic fibers, i.e., cellulose, hemicellulose, and lignin (Fan et al., 2012; Barua and Kalamdhad., 2016). During the FTIR study, it has been found that the band turns larger and bigger as compared to untreated one (Fig. 6.50) in treated sample, which signifies intra and intermolecular hydrogen bond became weaker after pre-treatment. In contrast, cellulose, hemicellulose, and lignin are bound together with a hydrogen bond. After electrohydrolysis pre-treatment, the peak was reduced at 3533.7, and 2721.22 cm^{-1} indicates stretch of OH in lignin and CH bond in cellulose (Wu et al., 2011). A drop of peak intensity at 1599.54 cm^{-1} indicates the C=O stretch in lignin. Reduction of band intensity detected at 1279.68 cm^{-1}, which specifies C-O, C=C, C-C-O, and C-H bond break down in cellulose, hemicellulose, and lignin (Barua and Kalamdhad., 2017). After observing major changes in peak, intensity it can conclude that the major bond that exists in lignin became weaker in *P. hysterophorus* after electrohydrolysis pre-treatment, and it will be easier for the microbes to degrade within a short period.

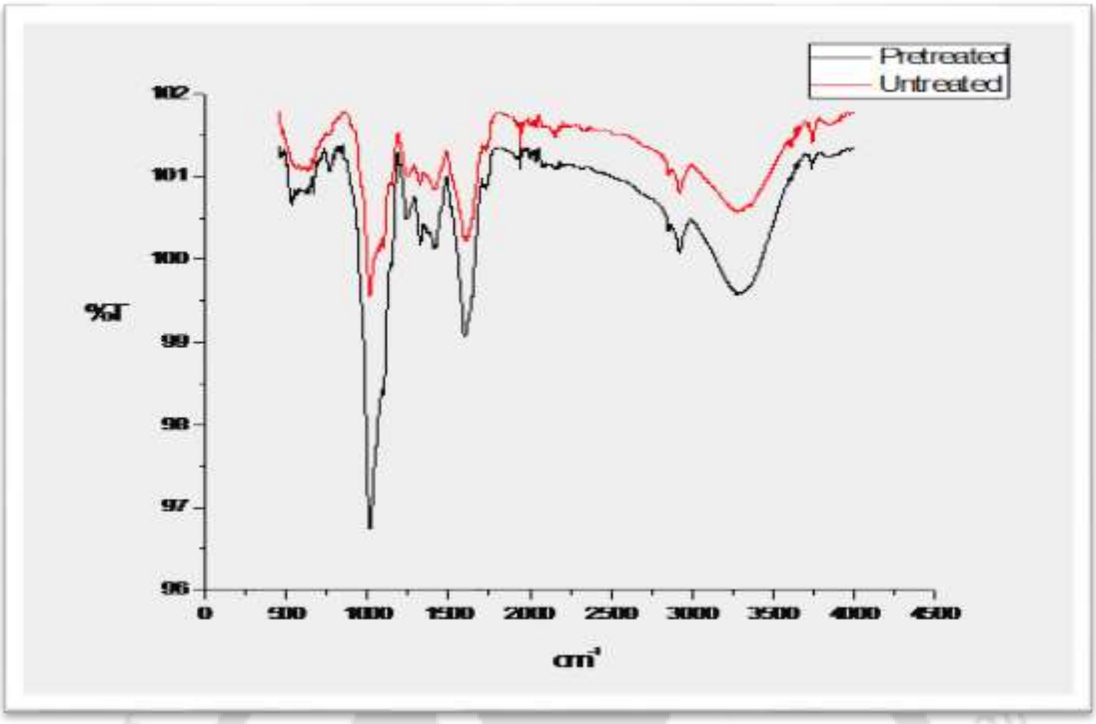


Fig. 6.50. FTIR of untreated and pretreated *P.hysterophorus*

6.2.1.8. Biogas production of control, pretreated and pretreated

The batch study was conducted after optimizing the voltage and time. After that biomethane potential trial with (best F/m ratio) was done for 50 days, where cumulative biogas production was observed 4242 ± 17 mL CH_4/g VS for pretreated and in untreated it was observed around 3063 ± 29 mL CH_4/g VS. After pre-treatment biogas production and cumulative biogas production rise when it is compared with untreated one. After electrohydrolysis pre-treatment, the hydrolysis stage was found to reduce, and a sharp increment of biogas production achieved as shown in the fig 6.51. Electrohydrolysis pre-treatment makes it easier for the microbes to break down the organic matter and easily accessible the cellulose present in the substrate; as a result, hydrolysis phases reduced and at the same time enhance the methane production was observed. Both the control represented quit less amount of biogas production as compared to the treated and untreated ones. In control, 1 only cow dung was studied, and in control 2, only *P. hysterophorus* was used. At 20 V for 40 mins show efficient increment of biogas production.

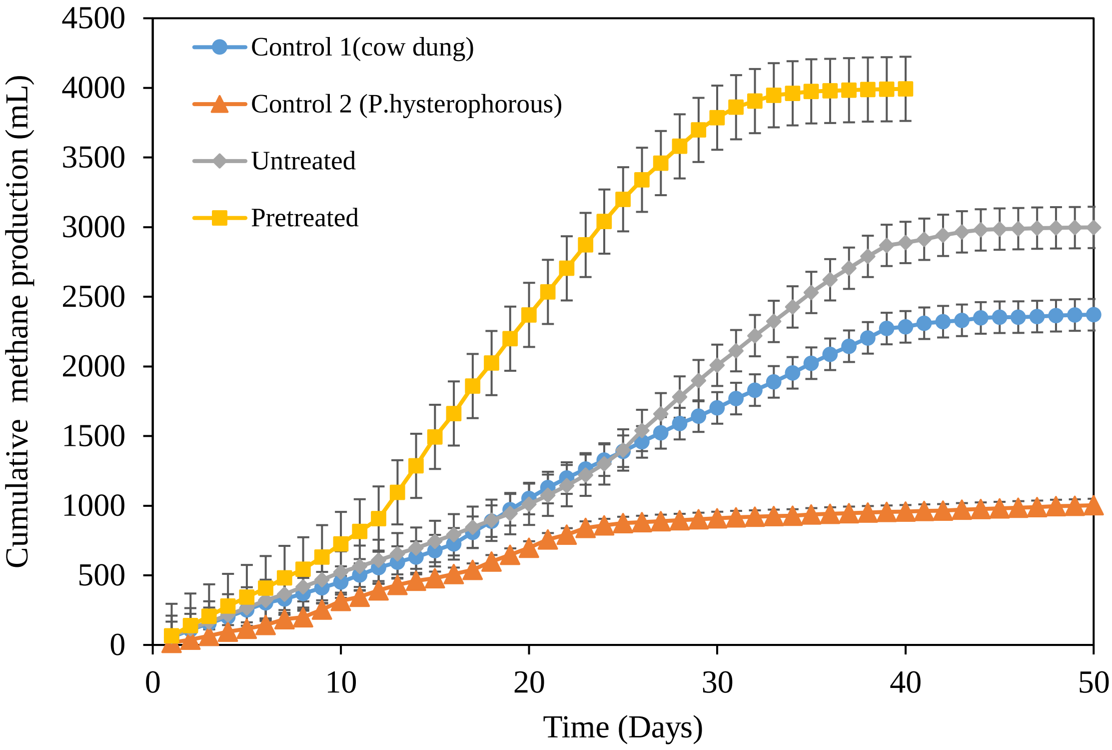


Fig. 6.51. Cumulative biogas production of *P.hysterophorus*

6.2.1.9. Energy assessment of electrohydrolysis pre-treatment *(P.hysterorhorus)*

The energy balance was done to determine the sustainability of the process. The E_C is the amount of the energy consumed in the electrohydrolysis pre-treatment process and E_G is the amount of the energy generated from the pretreated sample of *P.hysterophorus.*

The specific energy consumed E_C (J/ g VS) in the pre-treatment process was determined as per Eqn 6.7.

$$E_U = \frac{(P.Et)}{n} \tag{6.7}$$

Here, the P in the process is the product of the Voltage (V) and current (I) that was found to give the maximum daily methane yield, t is the exposure time, and n is the volatile solids' weight.

By substituting the values of V, I, t and n. The value of E_c was found to be 0.12 KJ/g (VS).

The specific energy generated E_G (J/ g VS) that pretreated *P.hysterophorus* was generating mainly due to methane and can be determined as per this eqn 6.8.

$$E_Q = \frac{(Q_{raw}.\S)}{1000.n} \tag{6.8}$$

Where, Q_{raw} is the taken as the maximum value of the daily methane production per gram of the VS added, H is the lower heating value (net calorific value) of methane. As the maximum value of methane was found in F/M ratio 2.0 in which 17.1 g VS of the pretreated sample was added.

§ = 35.80 KJ/ L

By substituting the values of Q_{raw}, and § in the eqn number 6.8, the value of E_G was found to be .33KJ/g (VS). Which is 2.53 times more than input energy.

6.2.2. *Lantana camara*

6.2.2.1. Current and resistance with time at different applied voltages

With the rise of applied voltage, current and resistance also started to fluctuate, which was clearly visible in Fig. 6.52. Current remain constant at 10, 20 and 30V, around 0.01, 0.15 and 0.44A respectively. At 40V, current and resistance both started to fluctuate; in high voltage, production of organic matter like glucose, amino acid, and volatile fatty acid rises, which may lead to increases in the current. As the voltage increases up to 40V, the current started to rise the number of electrons flow also increases, which may lead to increases in the temperature. The foam was produced at 40V, which was due to rises of excess temperature that may denature the organic compound, which was sensitive to heat; as a result, VFA and sCOD started to decline.

As time increased, the flow of current constant at 10, 20 and 30V, then at 40V rises of current was observed. When current started to fluctuate at 40V Fig. 6.52, resistance also varies; as a result, constant flow of electron cannot achieve, which affect the efficiency of pre-treatment that was seen in terms of a drop of VFA and sCOD. As the resistance varies, efficiency of energy distribution was not uniformly all over the sample. Although 10, 20 and 30V show the uniform flow of current and resistance, at 30V, a higher amount of sCOD and VFA were observed when compared to control. In fig., 6.53 a rise of sCOD was observed in all the voltage up to time 15 mins except at 40V then it started to fall. After 20 mins, the VFA in the sample began to vaporize; as a result, drop sCOD was observed. With evidence in fig 6.54 shows long time expose of voltage decreases the efficiency of pre-treatment. Therefore, a high voltage above 30V and longtime exposure of sample reduced the efficiency of pretreated on *L.camara* and at the same time, it was difficult to control the uniform flow of current.

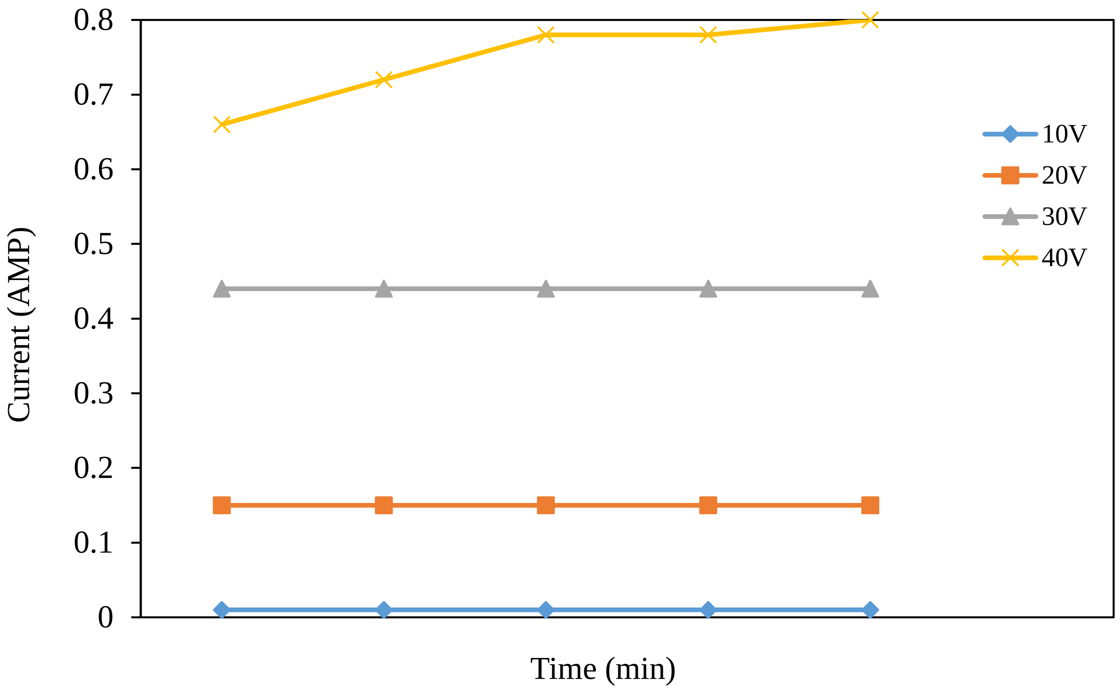


Fig. 6.52. Current study

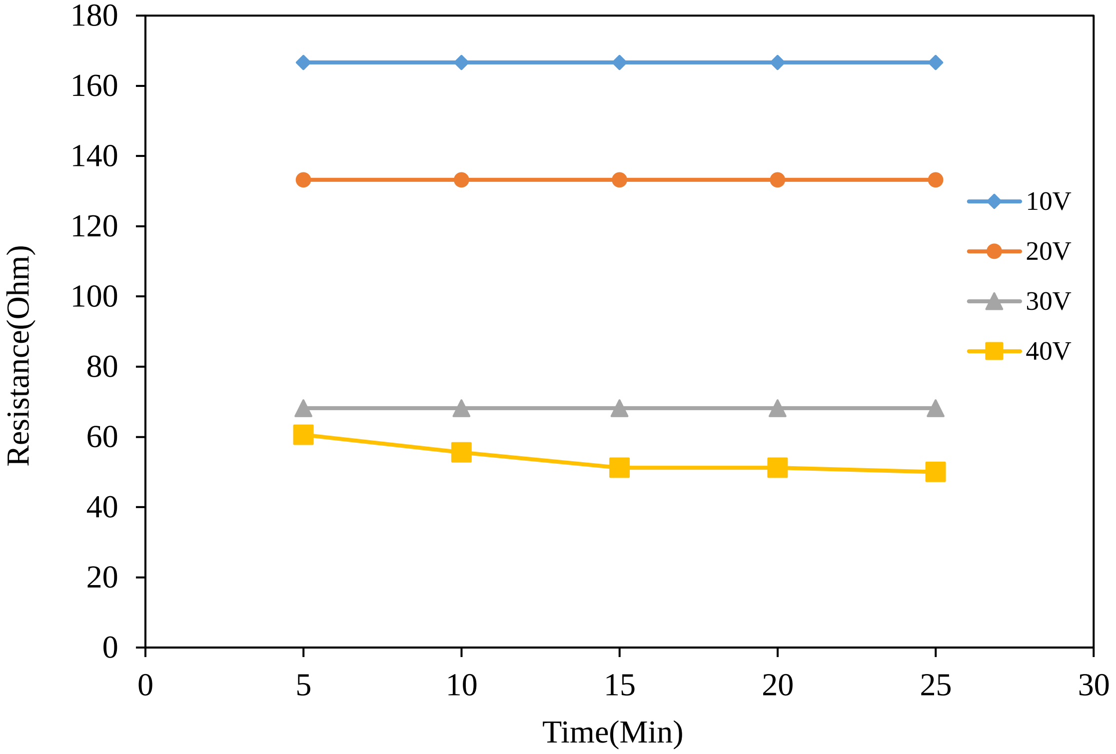


Fig. 6.53. Resistance variation

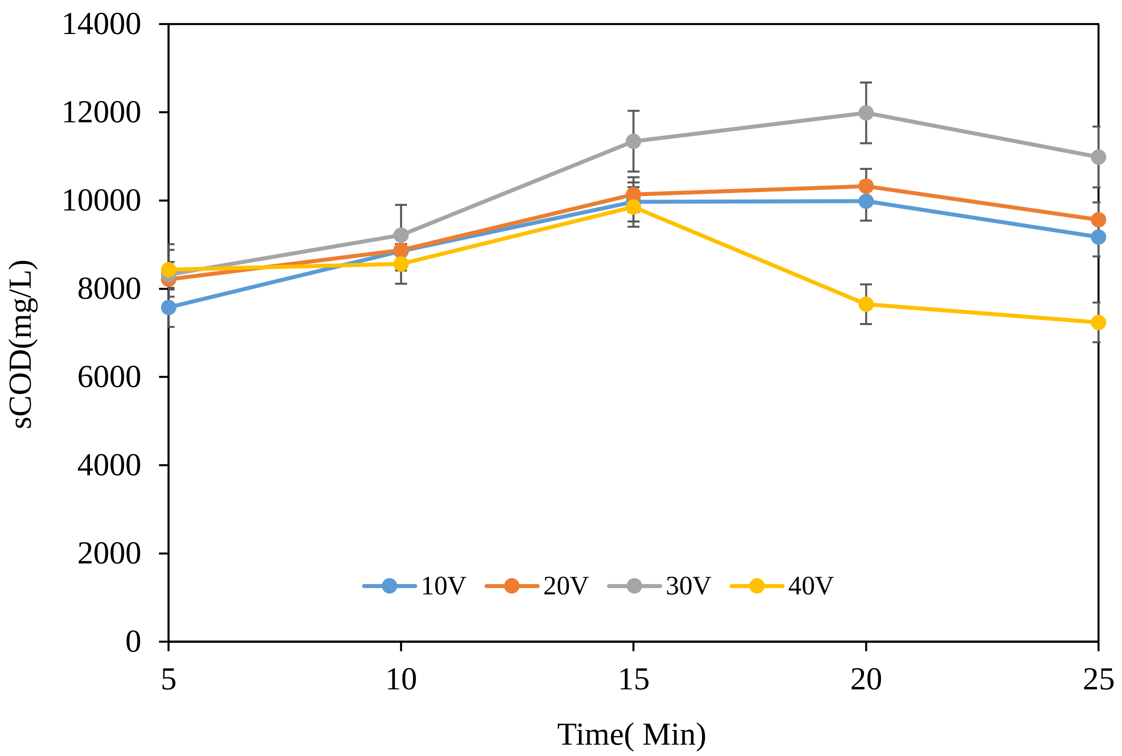


Fig. 6.54. sCOD and time study

6.2.2.2. Effect on VFA and sCOD

In Fig. 6.55, it was observed that VFA and sCOD increased with 0 to 30 V applied a voltage and at 30V, it started to fall down. The efficiency of solubilization increased with voltage, which shows the proof in the form of improvement of sCOD and VFA as compared to control but at 30 V VFA and sCOD started to decline because, at high voltage, the number of electron passes through the sample increased which lead to rises of ion, so the current was also rising in the sample measured by ammeter as a result decreasing of efficiency was observed (fig. 6.23). The rise of electric current leads to increased heat and produced a foam that makes the VFA vaporization. When VFA started to vaporize, the sCOD was also started to decline (Zhen et al., 2014; Veluchamy et al., 2017). The highest increment of sCOD was observed at 30 V around (11944±18mg/L), which represented 45.21% increment when it was compared with control. At 30V there might be adequate electric energy to break down complex organic compounds without denaturing the other organic compounds. Higher voltage more than 30V, might some of the organic matter like protein, enzymes started to denature, which is not further converted into soluble sCOD and VFA, which might be another reason to fall of sCOD and VFA after 30 V. Therefore, 30V was the ideal voltage to pretreated *L.camara*, which shows 1.82 times increment of sCOD.

In the time study, the sample was placed for a different time interval at an optimized voltage. In Fig. 6.56, it was observed that the VFA and sCOD both increased up to 20 mins, then both started to decline. VFA and sCOD were found 720±3mg/L and 11328±16mg/L, respectively, which rises to 1.89 and 1.73times within 20 mins from control. Around 42.24% of sCOD increased at 20 mins in 30voltage when it is compared to control. Due to the sample's exposure at 30V for a long time, heat is generated and foam was observed, leading to VFA vaporized and a drop of sCOD was observed at 40 mins. An organic compound like protein, enzymes, sugar might affect and denature due to a long time expose the sample to electrohydrolysis set up, which is not further converted into VFA and sCOD. Therefore, at 30V for 20 mins was the perfect voltage and time to pretreat *L.camara*.

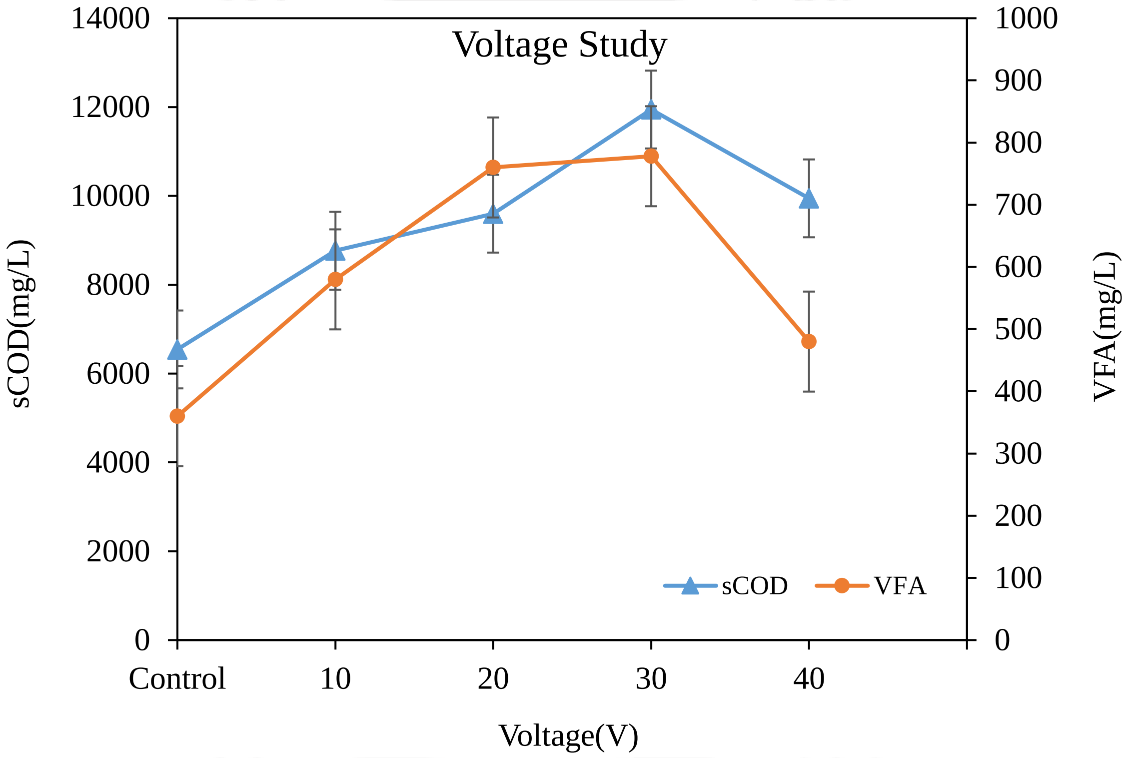


Fig. 6.54. Voltage sCOD and VFA study

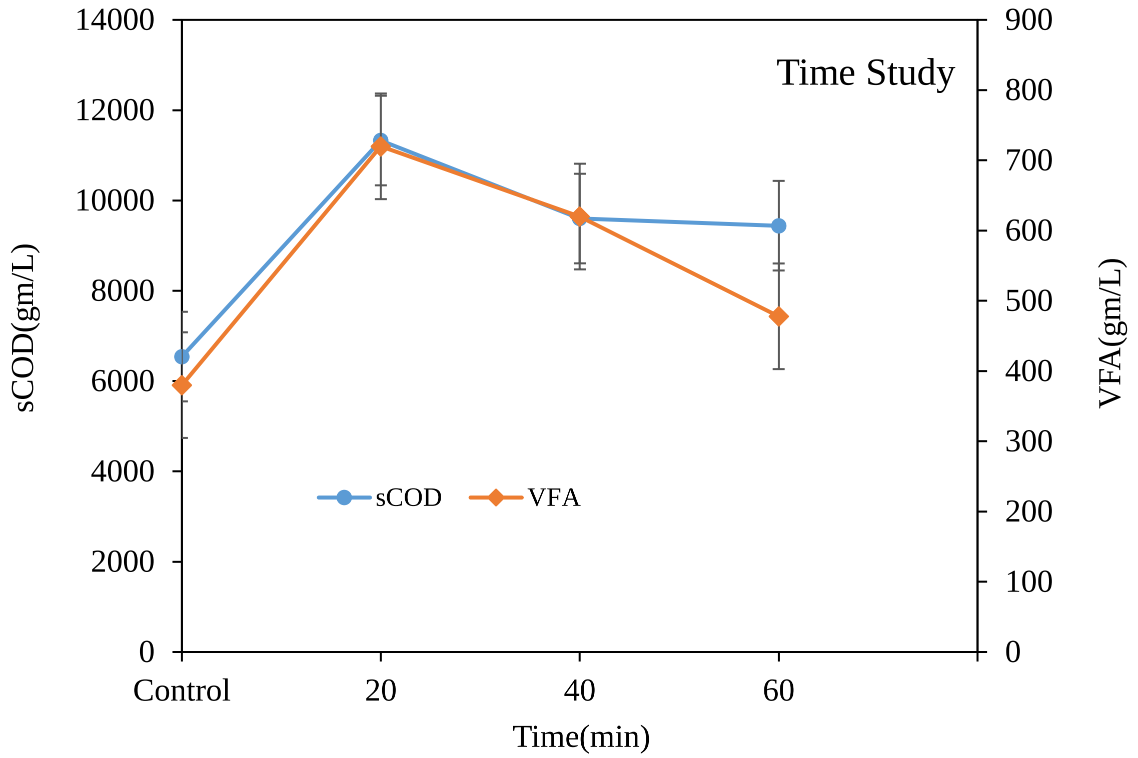


Fig. 6.56. Time, sCOD and VFA study

6.2.2.3. Effect of electrohydrolysis pre-treatment on lignin, cellulose and hemicellulose

After electrohydrolysis pre-treatment at 30V for 20 mins, different compositional changes was observed. In table 6.8 it was shown that after pre-treatment, acid-soluble lignin and cellulose increased up to 23.61% and 19.40%, which is 1.30 and 1.27 times, respectively. Acid-insoluble lignin and hemicellulose decreased around 34.89% and 15.58%, which is 1.53 and 1.24 times, respectively. The amount of biodegradable substrate is directly related to the increment of solubilization of lignin (Kamdem et al., 2015). As the soluble lignin increased, it became easily accessible for the fermentative bacteria to break down the cellulose (Patel et al., 2015). A long chain of cellulose breakdown into small pieces after electrohydrolysis pre-treatment, may be the reason for cellulose increases. Break down of a long chain of cellulose into a small unit, as a result, an increase of solubilization (Kumar et al., 2009a). Reduction of hemicellulose was observed; it might due to conversation into monomer of glucose and xylose. Therefore, weakening of lignin bond from lignocellulose substrate after electrohydrolysis pre-treatment might be the reason for reduced the period of hydrolysis phases, which is the fast step of anaerobic digestion, and at the same time, it enhances the methane production during anaerobic digestion.

Table 6.8. The variation was observed in the composition lignin, cellulose and hemicellulose after and before pre-treatment of *L.camara*

Parameters (V)	Voltage (V)	Time (min)	Acid insoluble lignin (%)	Acid soluble lignin (%)	Hemicellulose (%)	Cellulose (%)
Control	-	-	3.21± 0.39	3.04 ± 0.11	21.43 ± 0.42	34.85 ± 2.34
After Pretreatment	30	20	2.09± 0.67	3.98 ± 0.64	18.09 ± 0.14	43.24 ± 5.09

6.2.2.4. FESEM study of untreated and pretreated *Lantana camara*

To identify the physical changes after the electrohydrolysis pre-treatment, the sample was seen under FESEM. In fig..6.57 untreated *L.camara* appear like a tough, hard, and smooth surface, that is due to strongly lignin bond cover the outer part of the plant cell. In Fig. 6.58, after pre-treatment some crack appeared on the sample's surface, which signifies that strong bond of lignin break down and the outer surface became softer. It can easier for microbes to degrade the lignin within a short period, and cellulose easily accessible. Electrohydrolysis pre-treatment proved efficient under FESEM study in the form of softening the lignin. A similar type of lignocellulose structure was observed after different thermal pre-treatment (Gabhane et al., 2011); (Barua and Kalamdhad, 2017).

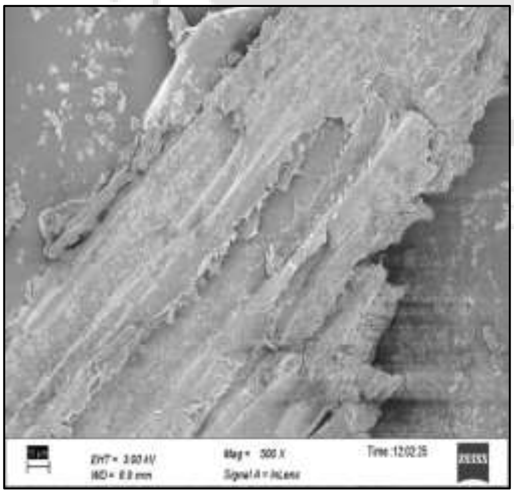


Fig. 6.57. FESEM image of untreated *L.camara*

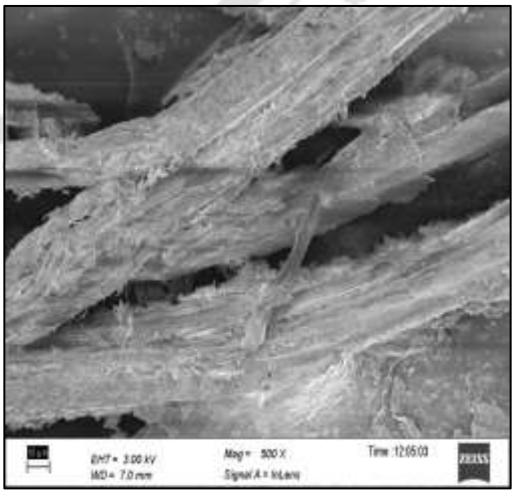


Fig. 6.58. FESEM image of after electrohydrohysis pre-treatment

6.2.2.5. XRD study of untreated and pretreated *Lantana camara*

The crystallinity of the substrate can be identified by XRD analysis. In fig 6.59 sharp peaks were found in the untreated sample, which signifies the substrate's crystalline nature. After the sample was exposed to the electrohydrolysis set up, no such peak was seen. Due to crystalline cellulose present in the *L.camara*, the high peak was observed in the untreated sample. The crystalline substrate was very hard to degrade, which lead to an increase in the length of the hydrolysis period. Karimi and Taherzadeh (2016) described that sharp peaks specify the crystalline structure, and for amorphous substances, there will not be any sharp peaks. X-ray diffraction characterizing of substance for the diffraction from perfect crystalline was well described by Bragg peaks. Absents of sharp after pre-treatment may due to crystalline cellulose transformed into amorphous. The crystallinity of the sample reduced after pre-treatment from 0.8640 to 0.6163. Yang et al. 2009 observed a similar result.

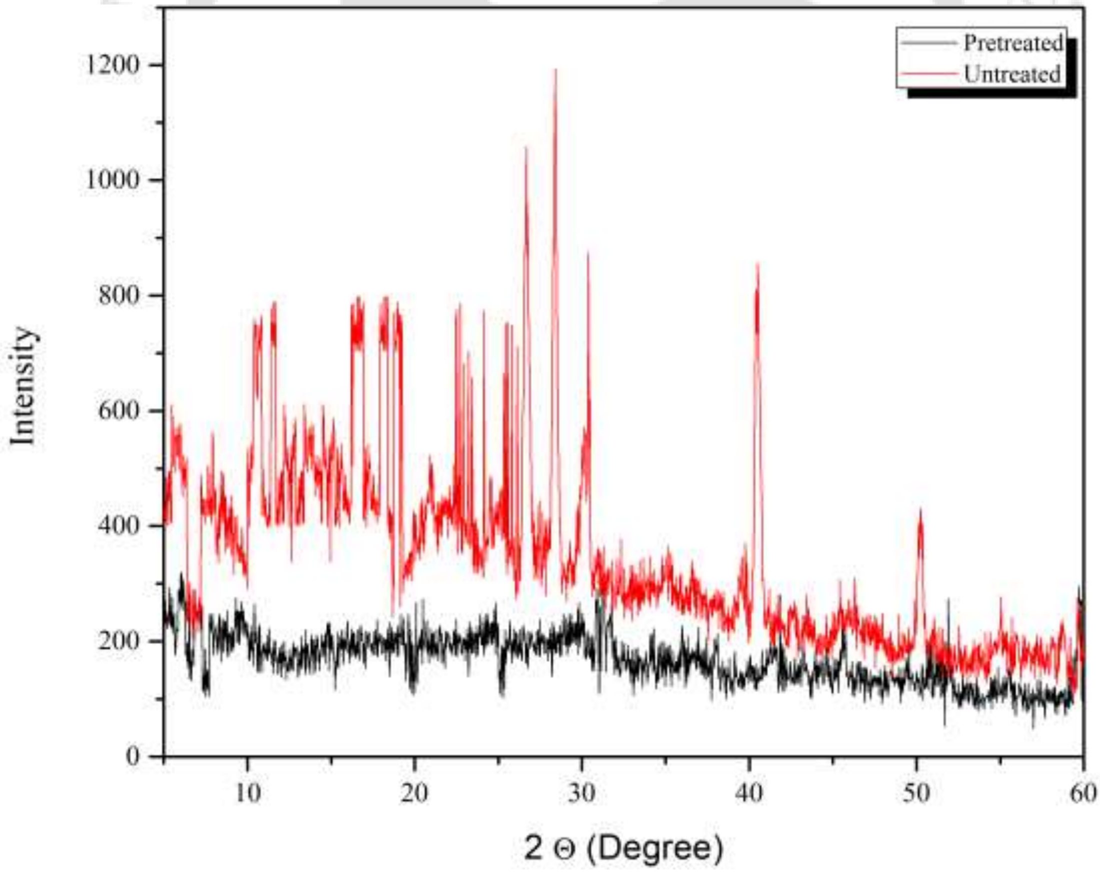


Fig. 6.59. XRD study of untreated and pretreated *L.camara*

6.2.2.6. FTIR study of untreated and pretreated *Lantana camara*

The constituent of fiber can be determined through FTIR analysis. i.e., cellulose, hemicellulose and lignin (Fan et al. 2012); (Barua and Kalamdhad, 2017). In fig. 6.60, the band appears brought after pre-treatment, which indicates intra and intermolecular hydrogen bond weaker. Although peak was observed at the same point remarkable fall of the peak was observed at 3490.1, and 2675.4 cm^{-1} indicates the expanse of O-H bond in lignin and C-H bond in cellulose (Wu et al., 2011). Reduction of peak intensity at 969.14 cm^{-1} specifies aromatic hydrogen bond deformation in lignin (Veluchamy, et al., 2017). Band intensity reduced at 1360.08 $cm^{-1,}$ which stated that C-O, C=C, C-C-O and C-H bond disruption in cellulose, hemicellulose and lignin (Barua et al., 2017). Significant changes of band intensity detected in the FTIR prove that after electrohydrolysis pre-treatment, the sample's outer surface became softer and more comfortable for microbes to degrade.

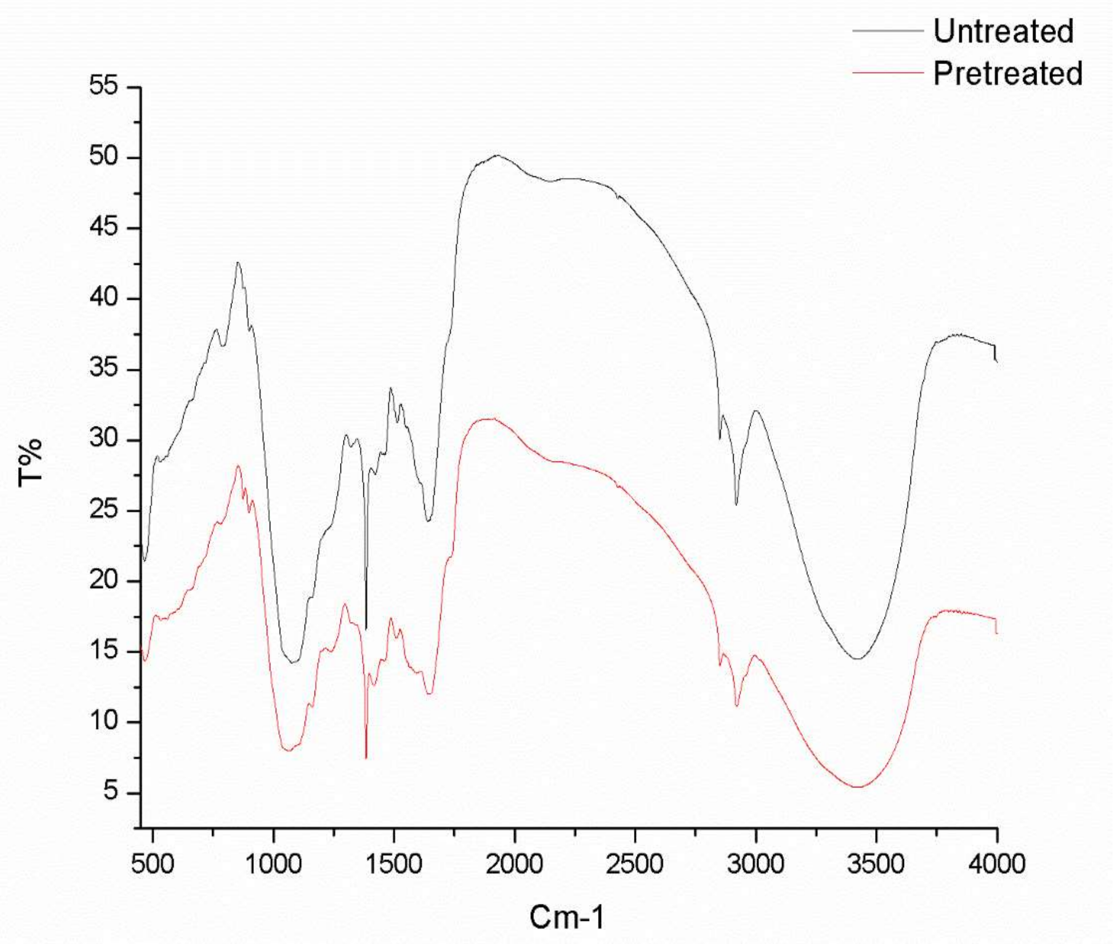


Fig. 6.60. FTIR study of untreated and pretreated *L.camara*

6.2.2.7. Biogas production of controls, untreated and pretreated

After optimized the voltage and time batch study was conducted for 50days (with best F/M ratio). Where cumulative biogas production was observed around 3842 ± 12 mL CH$_4$ in 40days for pretreated and in untreated, it was observed around 2950± 08 mL CH$_4$ in 50days. After pre-treatment, increment of biogas and cumulative biogas production was observed when it is compared with untreated, both the control show quit less amount of biogas production when it compares to untreated and pretreated *L.camara*. Due to lignin, content in *L.camara* increases the hydrolysis phases and decreases the performance of microbes (Gurung et al., 2012). In the pretreated sample, the straight increment of biogas production achieved and the reduction of hydrolysis period was observed, which is the time limiting stages of anaerobic digestion as shown in the fig 6.61. After electrohydrolysis pre-treatment lignin bond became softer that make it easier for microbes to degrade the substrate; that might be the reason for the reduction of hydrolysis phases and at the same time enhance the methane production. At 30V for 20 mins show efficient electrohydrolysis pre-treatment of increment of biogas production and reduced the hydrolysis period.

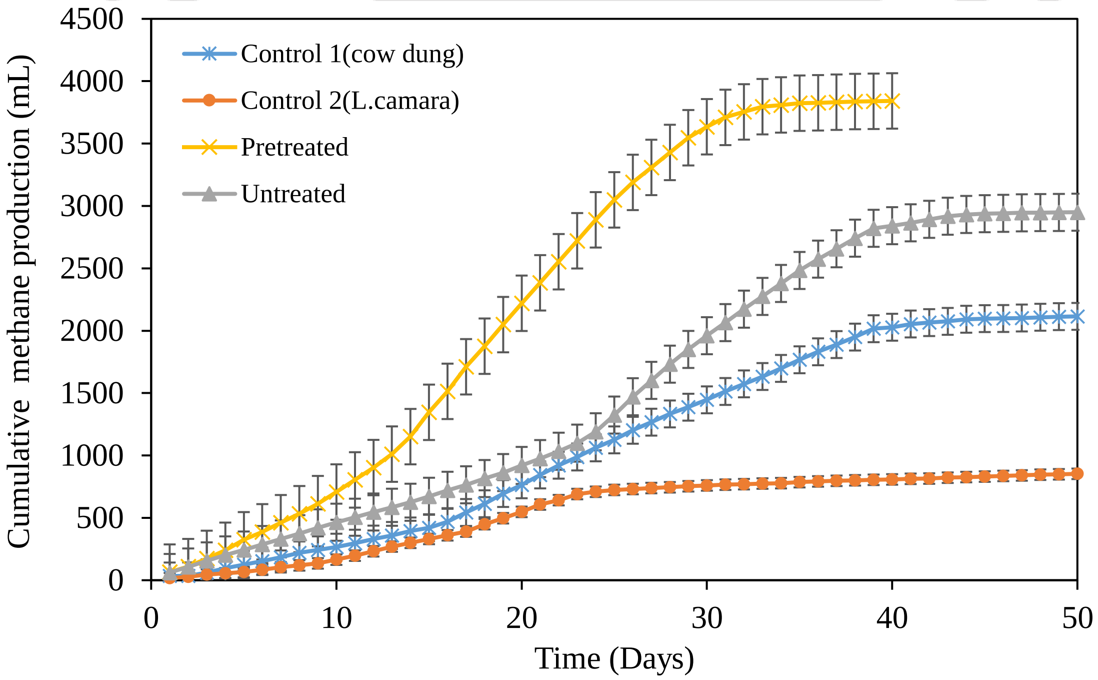


Fig. 6.61. Biogas production from control untreated and pretreated

6.2.2.8. Energy assessment of electro hydrolysis pre-treatment *(L. camara)*

The energy balance was done to determine the sustainability of the process. The E_C is the amount of the energy consumed in the electrohydrolysis pre-treatment process and E_G is the amount of the energy generated from the pretreated sample of *L.camara.*

For the pre-treatment process, the specific energy consumption E_C (J/ g VS) was estimated by using equation 6.9.

$$E_U = \frac{(P.Et)}{n} \qquad (6.9)$$

Here, the P in the process is the product of the voltage (V) and current (I) that was found to give the maximum daily methane yield, t is the exposure time, and n is the weight of the volatile solids.

By substituting the values of V, I, t and n. The value of E_c was found to be 0.18 KJ/g (VS).

The specific energy generated E_G (J/ g VS) that pretreated L.*camara* was generating mainly due to methane and can be determined as per this equation 6.10.

$$E_Q = \frac{(Q_{raw}.\S)}{1000.n} \qquad (6.10)$$

Where, Q_{raw} is the taken as the maximum value of the daily methane production per gram of the VS added, § is the lower heating value (net calorific value) of methane. As the maximum value of methane was found in F/M ratio 1.5 in which 15.7 g VS of the pretreated sample was added.

By substituting the values of Q_{raw}, and § in eqn number 6.10, the value of E_G was found to be .46KJ/g(VS).

For the process to be sustainable the ratio E_G/ E_c should be greater than 1, which was found to be true in our study. We can obtain 2.55 times more energy than the input energy.

6.2.3. *Ageratum conyzoides*

6.2.3.1. Change of current and resistance with time at different applied voltages

In Fig. 6.62a, it was detected when applied voltage rises, current and resistance also started fluctuating. Current remain constant at voltages 10 V, 20 V and 30 V as 0.01 A, 0.15A and 0.44A respectively. At 40V, current and resistance both started to fluctuate. In 40V, organic matter production like glucose, amino acid, and volatile fatty acid was triggered rose, which may have led to an increase in the current. As the voltage increased up to 40V, the current started to rise, which may be due to an increase in electron flow number. Due to the rise of electron flow, temperature also increased. Production of foam observed at 40V, which may due to the ohmic heat. With the excessive rise in temperature. Organic compounds that were sensitive to heat started to volatile; thus, VFA and sCOD started to decline (Barua et al., 2017; Velucharmy et al., 2017).

It was observed that the time progress, current remain constant at 10, 20 and 30V, but at 40V current started to fluctuate. When current started to change at 40V fig 6.62b, resistance also varies; thus, the constant flow of electron cannot achieve, which affects the efficiency of pre-treatment that proved in the form of a drop of VFA and sCOD. As the resistance varies, the efficiency of energy distribution was not uniformly all over the sample. . Although 10, 20 and 30V show the uniform flow of current and resistance, at 30V, higher amounts of sCOD and VFA were observed as compared to control. In the fig 63, rises of sCOD was observed in all the voltage up to time 15 mins except at 40V. After 20mins, the VFA in the sample started to vaporize, due to which a drop in sCOD was observed. With evidence in Fig., 7.25 shows long time exposure of voltage decreases the efficiency of pre-treatment. Therefore, high voltage above 30V and longtime expose of sample reduced the efficiency of pretreated on *A.conyzoides* and at the same time, it was problematic to control the uniform flow of current.

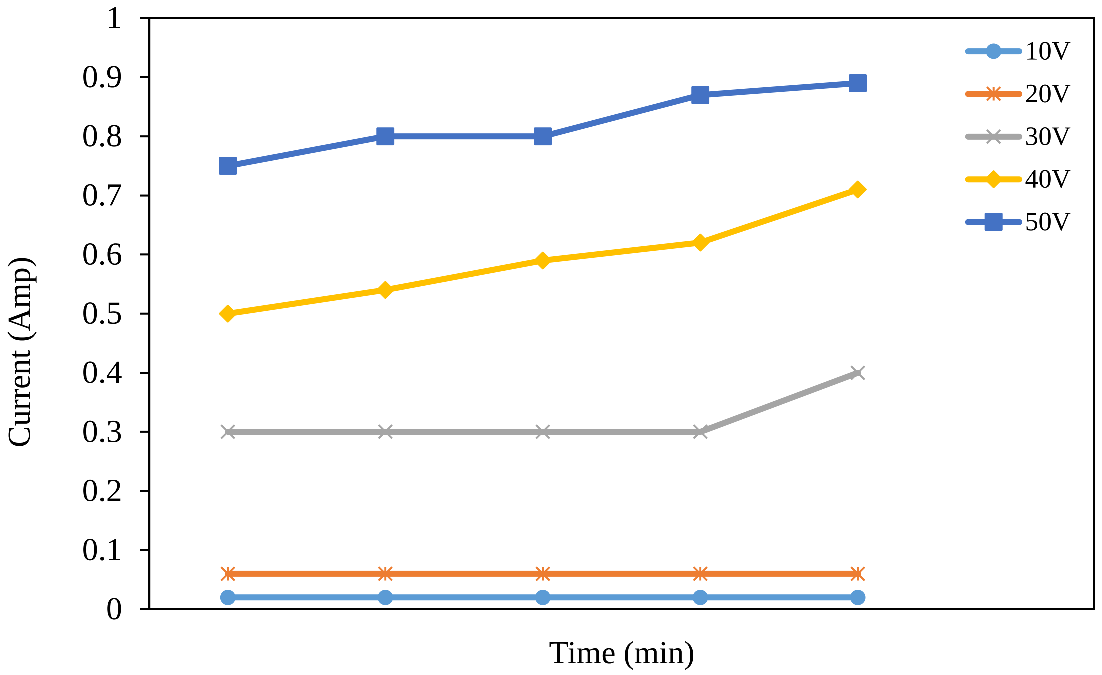


Fig. 6.62a. Change of current

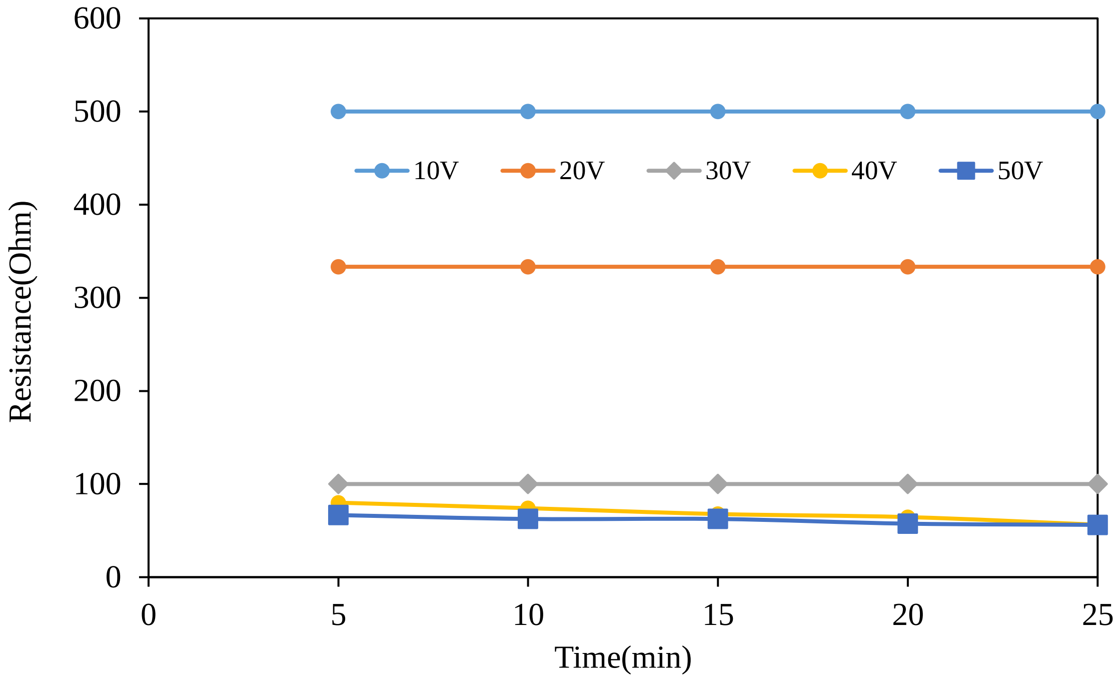


Fig. 6.62b. Resistance study

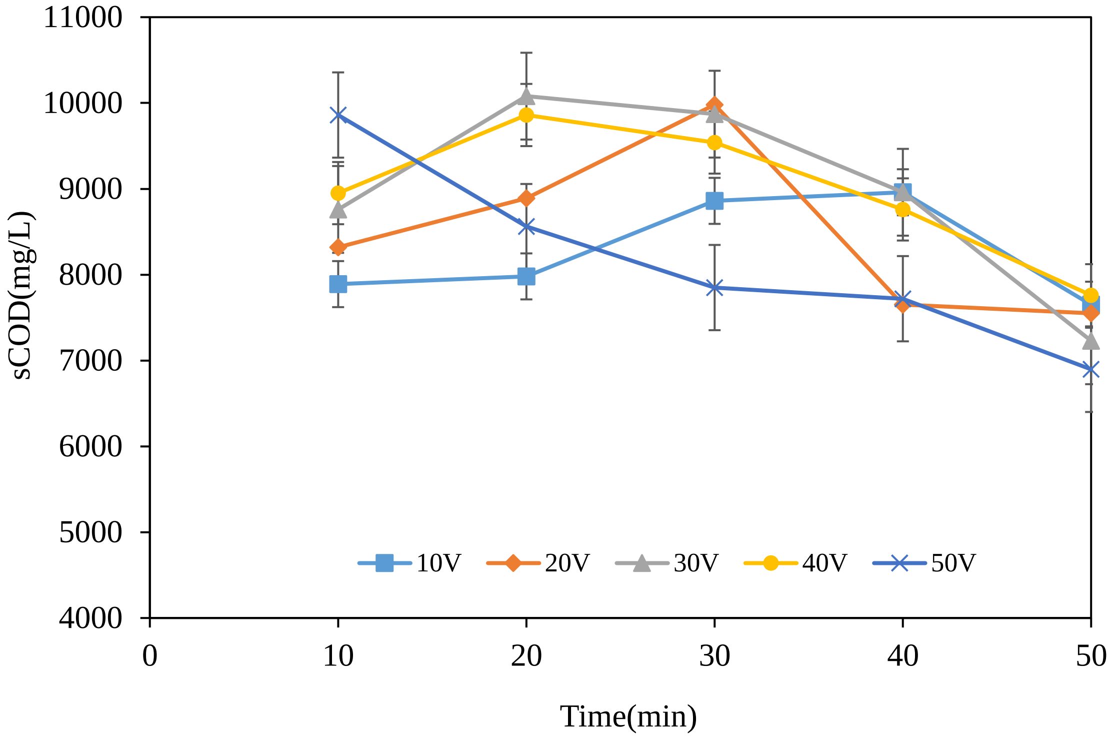


Fig. 6.63. sCOD changes with time

6.2.3.2. Effect on VFA and sCOD

Figure 6.64, it was observed that VFA and sCOD increased when 0 to 30 V was applied and at 40V (>30V) it started to decline. The degree of solubilization increased with voltage, which was proved in the form of an increase in sCOD and VFA as compared to control. However, at 40V, VFA and sCOD started to decline because, at high voltage, the number of an electron passing through the sample increased which led to rising of concentration of ion, ultimately rising the current as measured by ammeter; as a result, decrease in efficiency was observed (Fig. 1b). The rise of electric current leads to increasing heat and a foam that makes the VFA vaporization. When VFA started to vaporize, the sCOD was also started to decline (Zhen et al., 2014; Veluchamy et al., 2017). The highest increment of sCOD and VFA was observed at 30V around (10400 ± 27mg/L) and (820 ± 20 mg/L), representing 42.13% and 36.58% increment when compared with control. At 30 V, there might be adequate electric energy to break down complex organic compounds without denaturing the other organic compounds. Persuaded pre-treatment is accountable in the interruption of bonds in lignocellulosic weed and later improved the organic matter in additional soluble form. The number of electrons released at 30 V was suitable for balancing the substrate protons (Hþ). At higher voltage (>30 V), sCOD and VFA falls, which might be due to the extra electrons released by the DC current (Fernandes, 2009). Higher voltage more than 30V, extra electron rises the temperature, which might be another reason for the fall of sCOD and VFA after 30 V. At 30 V the proportion of soluble inert organic carbon concentration increased in the solution, which enhances the nutrients like N, P, and K (Reithmaier et al., 2017). Therefore, 30V was the appropriate voltage to pretreated *A.conyzoides*, which shows 1.94 and 1.57 times increment of sCOD and VFA respectively.

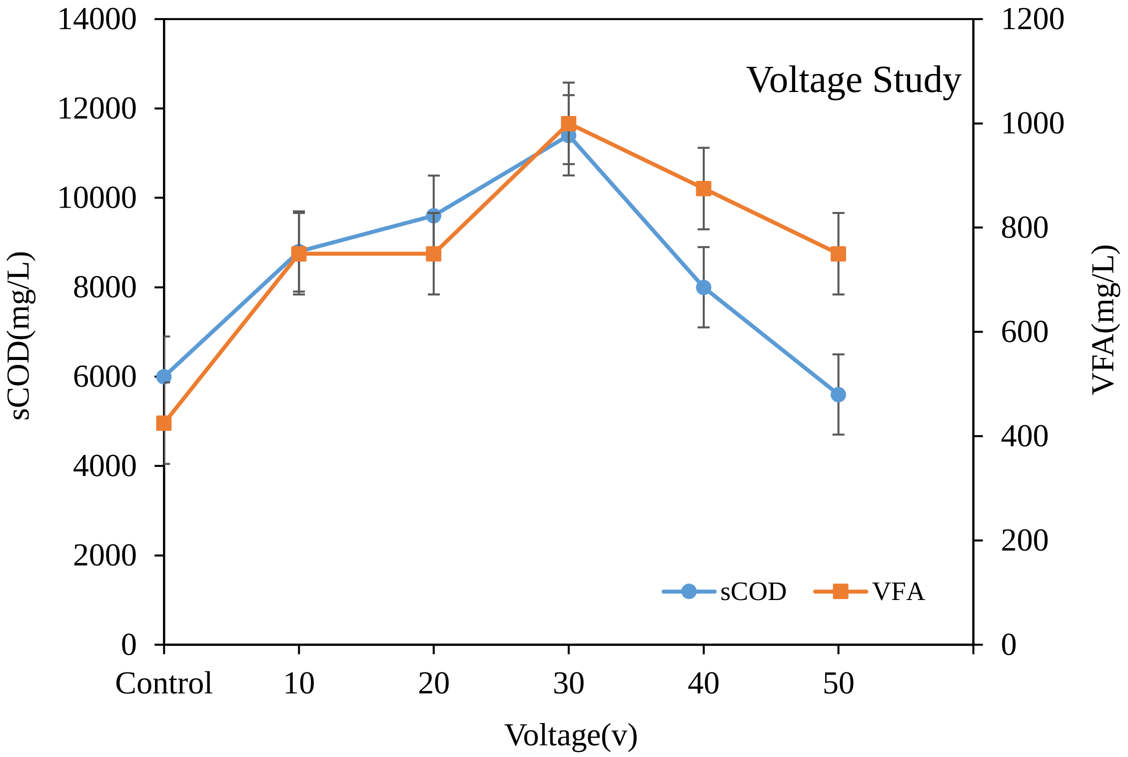


Fig. 6.64. Voltage study

6.2.3.3. Effect on VFA and sCOD with time

In the time study, the sample was placed for different time intervals at an optimized voltage. In Fig. 6.65, it was observed that VFA and sCOD increased up to 20 mins then both started to decline. At 20 mins contact time, VFA and sCOD were found as 750±5mg/L and 9600±48mg/L, respectively, which rose up to 2.30 and 1.37 times. Around 56.66% of sCOD increased at 20 mins in 30 volts when compared to control due to delignification, which makes cellulose compound easily available. Due to the sample's exposure at 30V for a long time, excessive ohmic heat is generated, and foam was observed, which led to VFA vaporization and drop of sCOD at 40 mins (Varghese et al., 2014). Organic compounds like protein, enzymes, and sugar might be affected and denatured due to the sample's long-time exposure to electrohydrolysis, which was not further converted sCOD. Therefore, 30V for 20 mins was found to be the best voltage and time for electrohydrolysis pre-treatment of *A.conyzoides*, which damage the lignin layer and permitted the soluble layer to come outside, with evidence increment of sCOD and VFA concentration (Eskicioglu et al., 2006).

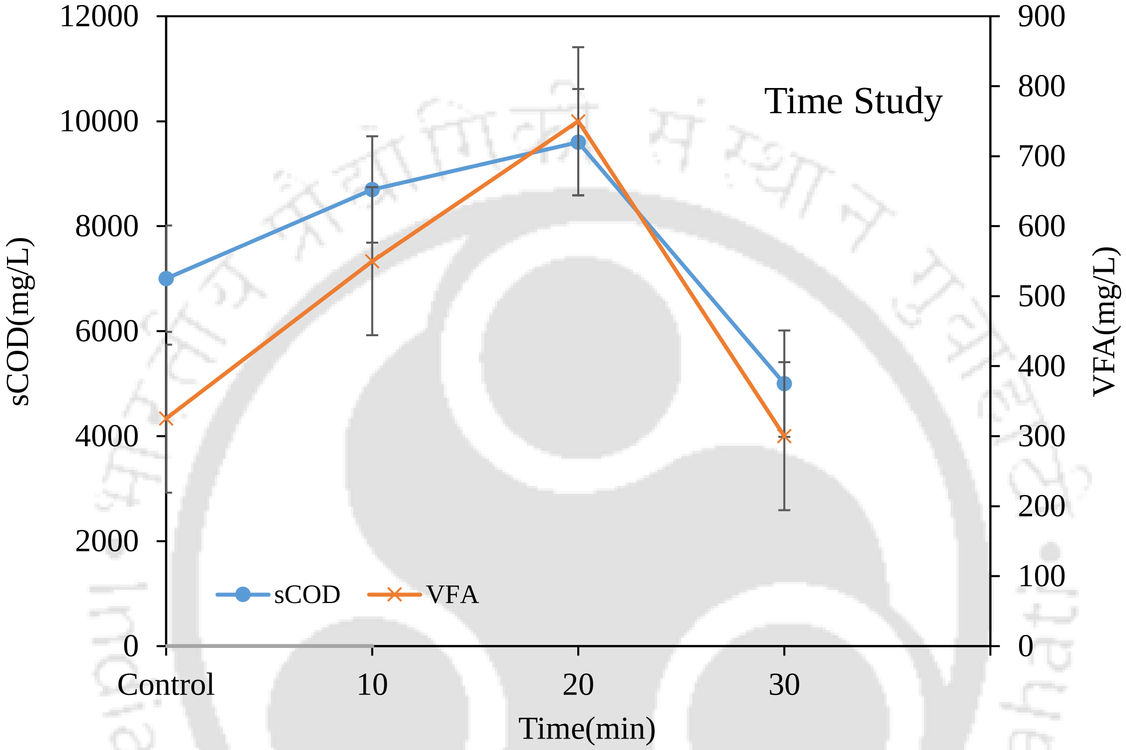


Fig. 6.65. Effect of sCOD and VFA with time

6.2.3.4. Effect of electrohydrolysis pre-treatment on lignin, cellulose and hemicellulose

After electrohydrolysis pre-treatment at 30V for 20 mins, different compositional changes were observed. As shown in table 6.9, acid soluble lignin and cellulose increased up to 38.92% and 2.68% which is 1.16 and 1.11 times respectively, when compared with control. Acid-insoluble lignin and hemicellulose decreased around 41.61% and 4.68%, which is 1.24 and 1.14 times, respectively, compared with control. The number of biodegradable substrates is directly related to the increment of solubilization of lignin (Kamdem et al., 2015). As the soluble lignin increased, it became easily accessible for the fermentative bacteria to break down the cellulose (Patel et al., 2015). A long chain of cellulose was broken down into smaller pieces after electrohydrolysis pre-treatment, which might be the reason for the increase of cellulose. Break down the long chain of cellulose into smaller units results in a rise in solubilization (Kumar et al., 2009a). Reduction of hemicellulose was detected due to the conversion of the same monomer into glucose and xylose. Elimination of lignin contains in lignocellulose weed increased the degradation rate; higher lignin content reduced the biogas production (Liew et al., 2012). Therefore, weakening lignin bond from lignocellulose substrate after electrohydrolysis pre-treatment might be the reason for the reduced hydrolysis phase, which is the first step of anaerobic digestion, and at the same time, it enhanced the methane production during anaerobic digestion. Thus, with confirmation electrohydrolysis pre-treatment has shown to be a very efficient pre-treatment method, which diminishing the hurdles and providing required effects (e.g. lignin elimination).

Table 6.9. The variation was observed in the composition lignin, cellulose and hemicellulose after and before pre-treatment of *A.conyzoides*

Parameters	**Voltage (V)**	**Time (min)**	**Acid insoluble lignin (%)**	**Acid soluble lignin (%)**	**Hemicellulose (%)**	**Cellulose (%)**
Control	-	-	2.78 ± 0.12	2.56 ± 0.78	24.34 ± 0.88	37.12 ± 1.34
After Pretreatment	30	20	2.23 ± 0.19	2.98 ± 0.34	21.33 ± 0.91	41.43 ± 4.34

6.2.3.5. FESEM of Untreated and pretreated *A.conyzoides*

To identify the physical changes after the electrohydrolysis pre-treatment, sample was observed under FESEM. As shown in Fig..66, untreated *A.conyzoides* appeared as tough, hard, and smooth-surfaced, which might be due to the strong lignin bond covering the plant cell's outer part. In fig 6.67, after pre-treatment, some cracks appeared on the sample's surface, which signifie break down of lignin bond allowing the outer surface to become softer. The circular ring like structure was captured under FESEM, which may be long chain of cellulose ring. It made it easier for microbes to degrade the lignin within a short period and cellulose easily accessible. Electrohydrolysis pre-treatment proved efficient under FESEM study in terms of softening the lignin. Similar type of lignocellulose structure was observed after different thermal pre-treatment (Gabhane et al., 2011; Barua and Kalamdhad, 2017).

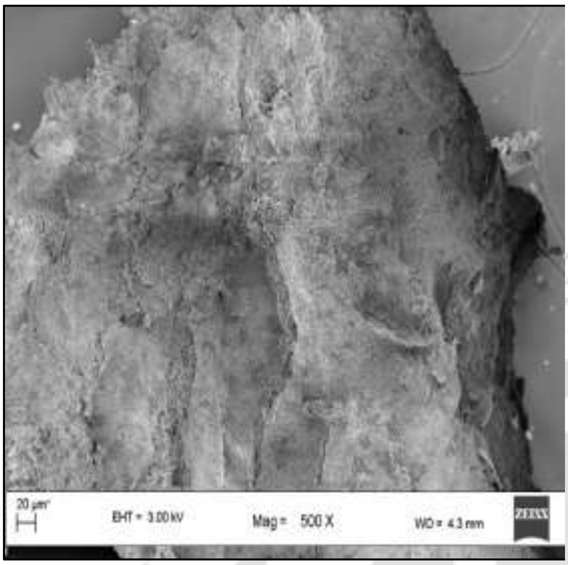


Fig. 6.66. FESEM image of untreated *A.conyzoides*

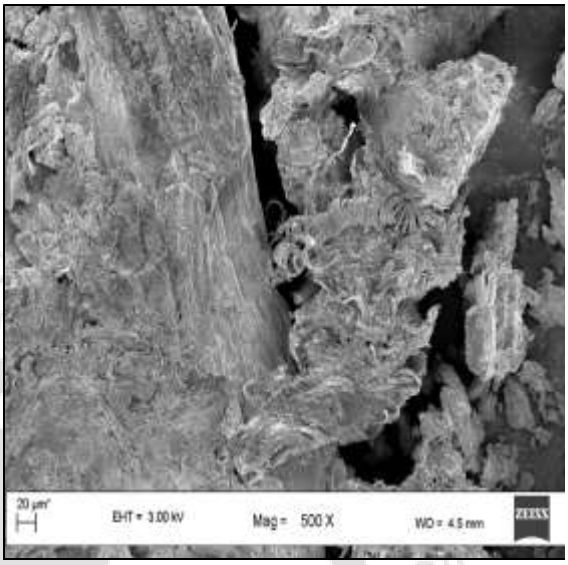


Fig. 6.67. FESEM image after Electrohydrolysis pre-treatment

6.2.3.6. XRD study of untreated and pretreated *A.conyzoides*

XRD analysis gives the idea of the crystallinity of the substrate. In fig 6.68, sharp peaks were found in untreated samples, signifying the substrate's crystalline nature. After the sample was exposed to the electrohydrolysis set up, no such peak was seen. Due to crystalline cellulose present in the *A.conyzoides*, the high peak appeared in the untreated sample. The crystalline substrate was very hard to degrade, which led to an increase in the hydrolysis period. Karimi and Taherzadeh (2016) described that sharp peaks specify the crystalline structure, and the nonappearance of sharp peaks means amorphous substances. Bragg peaks well told the X- ray diffraction characterizing of substance for the diffraction from perfect crystalline. Absents of sharp after pre-treatment may due to crystalline cellulose transformed into amorphous. The crystallinity of the sample reduced after pre-treatment from 0.7654 to 0.6163. Yang et al. 2009 observed a similar result.

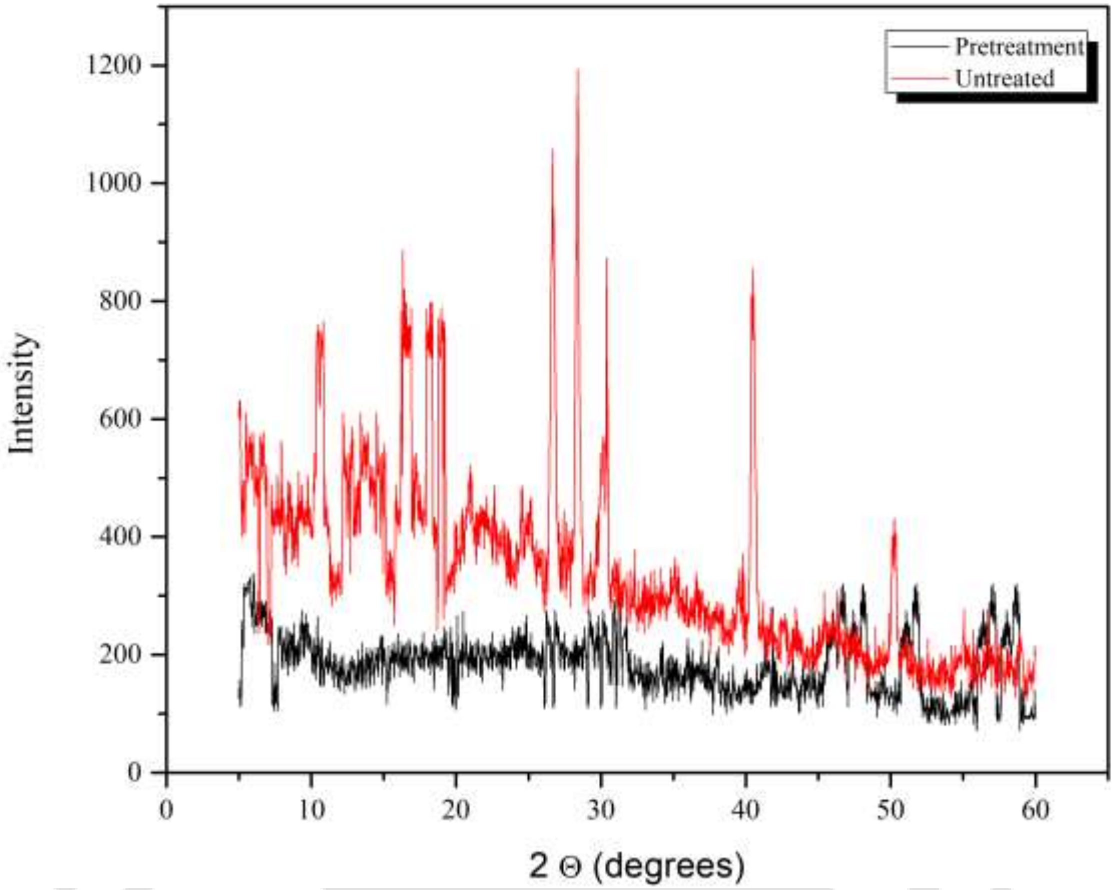


Fig. 6.68. XRD study of untreated and pretreated *A.conyzoides*

6.2.3.7. FTIR study of untreated and pretreated *A.conyzoides*

The constituent of fiber can be determined through FTIR analysis. i.e., cellulose, hemicellulose and lignin (Fan et al. 2012; Barua and Kalamdhad, 2017). In Figure 6.69, the band appears brought after pre-treatment, indicating intra and intermolecular hydrogen bonds weaker. Although peak appeared simultaneously, remarkable fall of peak seemed at 3350.8 and 2298.4 cm^{-1}, expand of OH bond in lignin and CH bond in cellulose (Wu et al., 2011). Reduction of peak intensity at 969.14 cm^{-1} specifies aromatic hydrogen bond deformation in lignin (Veluchamy, et al., 2017). Band intensity reduced at 1478.6 cm$^{-1,}$ which denotes C-O, C=C, C-C-O and C-H bond disruption in cellulose, hemicellulose and lignin (Barua et al., 2017). Significant changes of band intensity detected in the FTIR prove that after electrohydrolysis pre-treatment, the sample's outer surface became softer and easier to microbes to degrade.

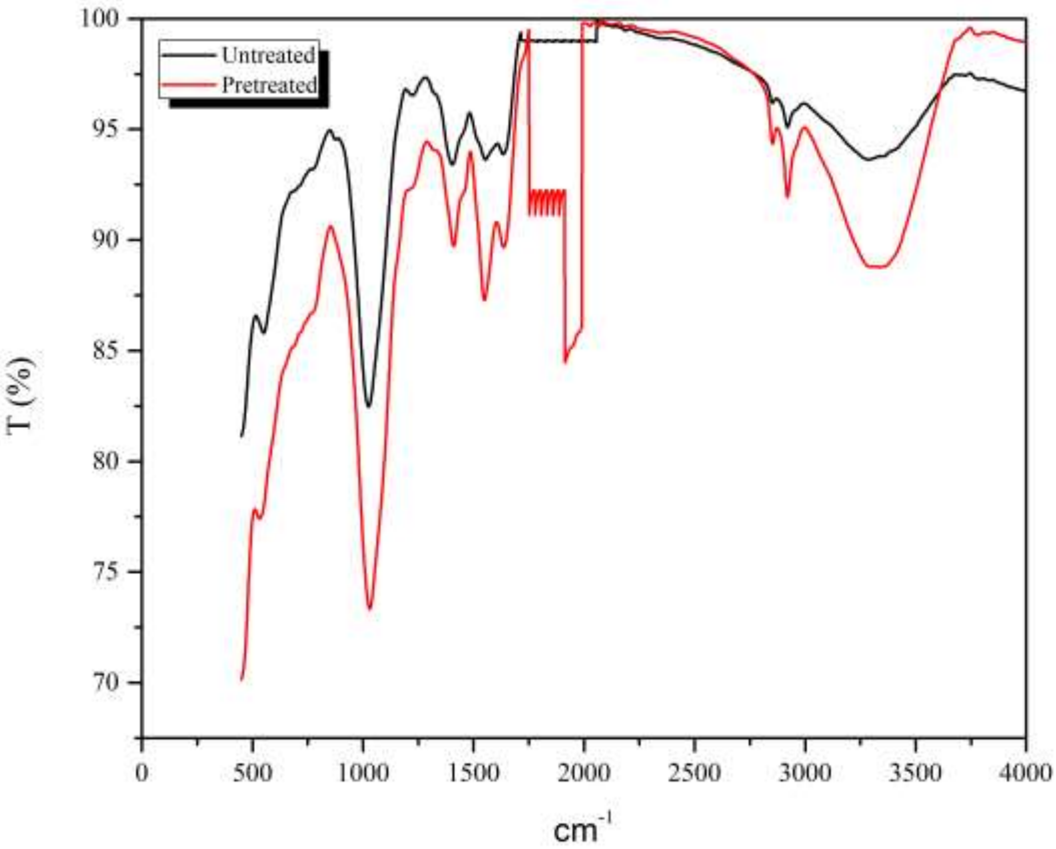


Fig. 6.69. FTIR study of untreated and pretreated *A.conyzoides*

6.2.3.8. Biogas production of control, untreated and pretreated *A.conyzoides*

After optimizing the voltage and time, the batch study was conducted with (best F/M ratio) for 50 days, where cumulative biogas production was observed around 3707 ± 30 mL CH_4 in 40 days pretreated and 2820± 25 mL CH_4 in 50 days for the untreated. A significant increment in biogas and cumulative biogas production was observed for the pretreated biomass compared to that for untreated. Both the controls showed a relatively less amount of biogas production compared to untreated and pretreated *A.conyzoides*. The lignin content in *A.conyzoides* increases the hydrolysis phases and decreases microbes' performance (Gurung et al., 2012). In the pretreated sample, the straight increment of biogas production was achieved simultaneously with a time reduction of the hydrolysis period. Hydrolysis is observed to be the time limiting stages of anaerobic digestion, as shown in fig 70. After electrohydrolysis pre-treatment, lignin bond became softer, making it easier for microbes to degrade the substrate, which might reduce hydrolysis phases and enhance methane production. At 30V for 20 mins showed the most efficient electrohydrolysis pre-treatment with the highest increment of biogas production and high hydrolysis period reduction.

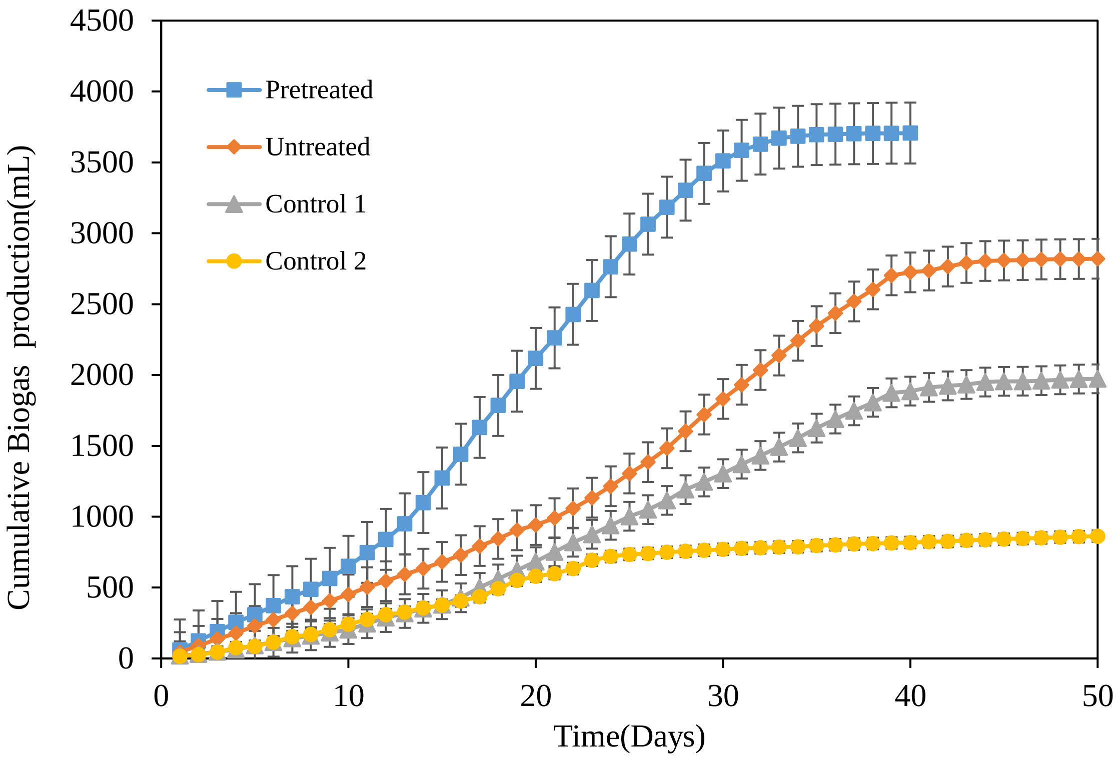


Fig. 6.70. Cumulative biogas production of *A.conyzoides*

6.2.3.9. Energy assessment of electrohydrolysis pre-treatment *(A.conyzoides)*

The energy balance was done to determine the sustainability of the process. The E_C is the amount of the energy consumed in the electrohydrolysis pre-treatment process and E_G is the amount of the energy generated from the pretreated sample of *A.conyzoides*

The specific energy consumed E_C (J/ g VS) in the pre-treatment process was determined as per Eqn 6.11.

$$E_U = \frac{(P.Et)}{n} \qquad (6.11)$$

Here, the P in the process is the Voltage (V) product and current (I) that was found to give the maximum daily methane yield, t is the exposure time, and n is the weight of the volatile solids.

By substituting the values of V, I, t and n. The value of E_c was found to be 0.15 KJ/g (VS).

The specific energy generated E_G (J/ g VS) that pretreated *A.conyzoides* was generating mainly due to methane can be determined as per this eqn 6.12.

$$E_Q = \frac{(Q_{raw}.\S)}{1000.n} \qquad (6.12)$$

Where, Q_{raw} is the taken as the maximum value of the daily methane production per gram of the VS added, §) is considered as a heating value (net calorific value) of methane. As the maximum value of methane was found in F/M ratio 2.0 in which 11.2 g VS of the pretreated sample was added.

By substituting the values of Q_{raw}, §) and n) in eqn number 2, the value of E_G was found to be .43KJ/g(VS), which is 2.86times more than the input energy.

6.3. Summary for Phase- II

Thermal and electrohydrolysis pre-treatment on *P.hysterophorus, L.camara and A.conyzoides* was found to be valuable to reduce the hydrolysis phase, and at the same time, it enhanced the biogas production. All four thermal pre-treatments showed a significant increase of sCOD, but pre-treatment with hot air oven was found out to be the most effective for *P.hysterophorus* with increment up to 51.56% of that of the untreated, at 110˚C for 90 mins and for *L.camara and A.conyzoides, the* autoclave was found to be more efficient succeeded by hot water bath, hot air oven and microwave pre-treatment. Autoclave pretreatment enhanced the solubilisation (sCOD) 45.44% in *L.camara* at 110 °C for 80 mins and 55.33% for *A.conyzoides* at 90° C for 40 mins. Electrohydrolysis pre-treatment seemed to be efficient in increasing the percentage of solubilisation of *P.hysterophorus* around 50.17% at 20V for 40mins, for *L.camara* 47.22% at 30V for 20 mins and *A.conyzoides*, 27.08%at 30V for 20 mins.

CHAPTER 7

EFFECT OF ANAEROBIC CO-DIGESTION ON TERRESTRIAL WEEDS (*Parthenium hysterophorus, Lantana camara* and *Ageratum conyzoides*)

This chapter dealt with co-digestion of terrestrial weeds (*Parthenium hysterophorus, Lantana camara and Ageratum conyzoides)* with food waste.

7.1. Phase III - Anaerobic co-digestion study

Biochemical methane potential test for co-digested *parthenium hysterophorus, Lantana camara and Ageratum conyzoides* was conducted results obtained are as follows. Food waste was used as a second substrate.

7.1.1. Anaerobic co-digestion of *Parthenium hysterophorus*

7.1.1.1. Biochemical methane potential (BMP) trial

Co-digestion of *Parthenium hysterophorus* and food waste were carried out in 1 litre batch reactor using different mixing ratios with cow dung as inoculum. Methane generation commenced from the first day itself for all mixing ratios in varying proportions. The total methane production boosted steadily throughout the digestion period. It was observed that for co-digested *Parthenium hysterophorus* for mixing ratio 1.5 produced the highest amount of methane 165 mL CH_4 g^{-1} VS on 17th day (fig 7.1). Fig 7.2 shows the highest cumulative methane production of 4165 mL CH_4 g^{-1} VS (mixing ratio1.5). The cumulative methane production followed order 1.5 > 2.0 > 2.5 > 1.0. Lesser methane generation was observed in mono digestion when compared with co-digestion (Table 7.1). BMP study for F/M ratio 1.5 was observed to show the highest methane potential compared to other ratios. After conducting 1000 mL of BMP study, the batch reactor of 20 L was studied with 14 L of working volume with ideal F/M ratio as observed in the BMP study. *P. hysterophorus,* food waste and cow dung were mixed according to ratio 1.5. Scale-up batch reactor experiments were conducted for 49 days. In the 14 L of the batch reactor, maximum biogas yield of 3840 mL CH_4 g^{-1} VS was obtained on 19th day and cumulative methane produced of 66150 mL was obtained on 49 days' period shown in fig 7.3 and 7.4. With increased the volume of the reactor methane production was increased, it shows that *P. hysterophorus* have the potential to produce biogas on a larger scale.

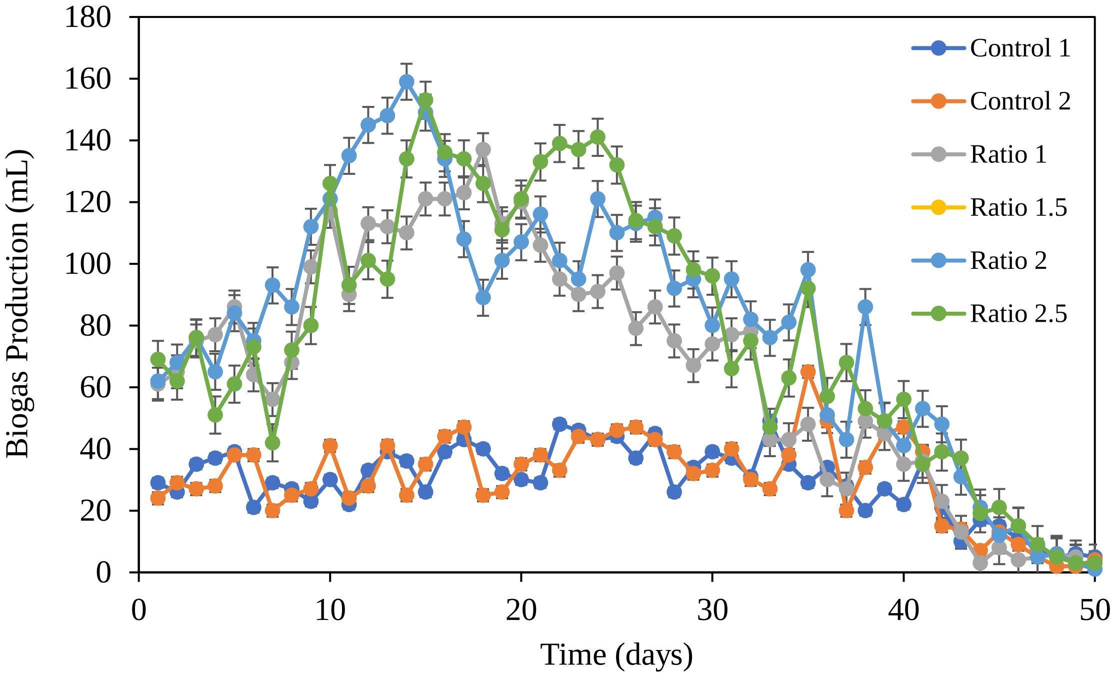


Fig. 7.1. Daily biogas production in co-digestion of *Parthenium hysterophorus* for different mixing ratios

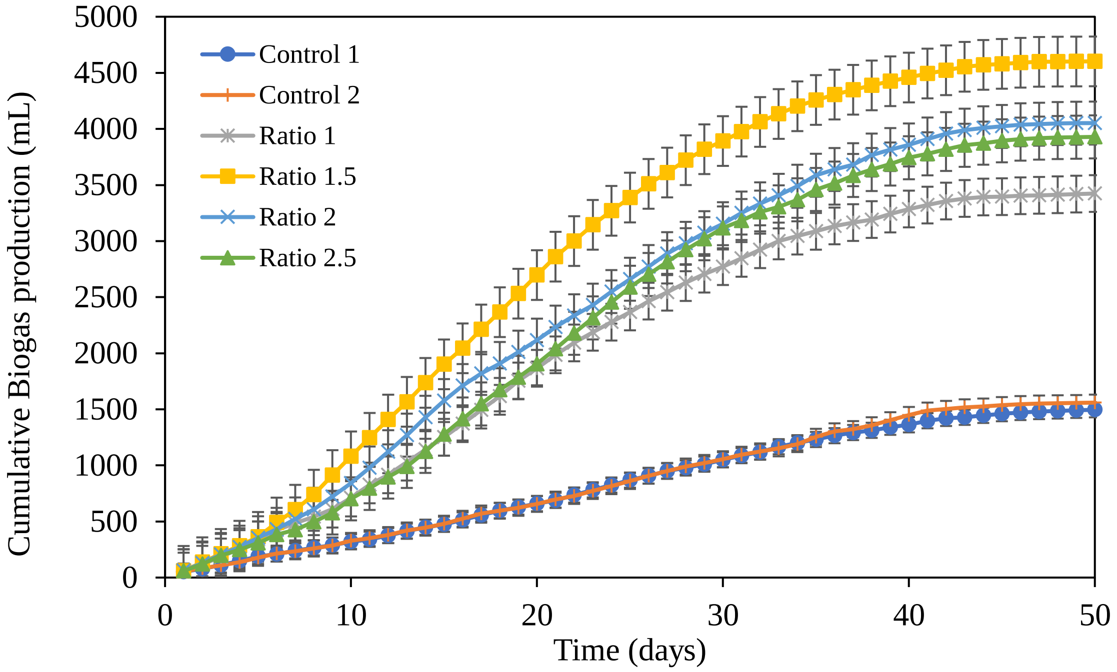


Fig. 7.2. Cumulative biogas production in co-digestion of *Parthenium hysterophorus* for different mixing ratios

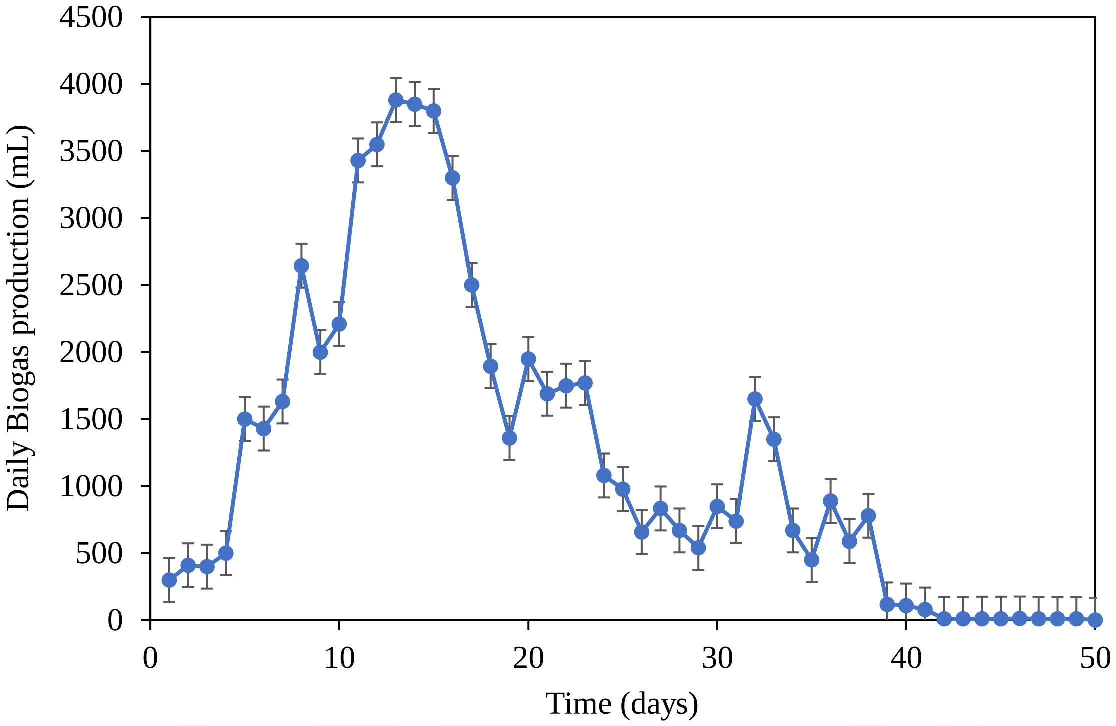


Fig. 7.3. Daily biogas production in 20 L reactor of *Parthenium hysterophorus* and food waste for mixing ratio 1.5

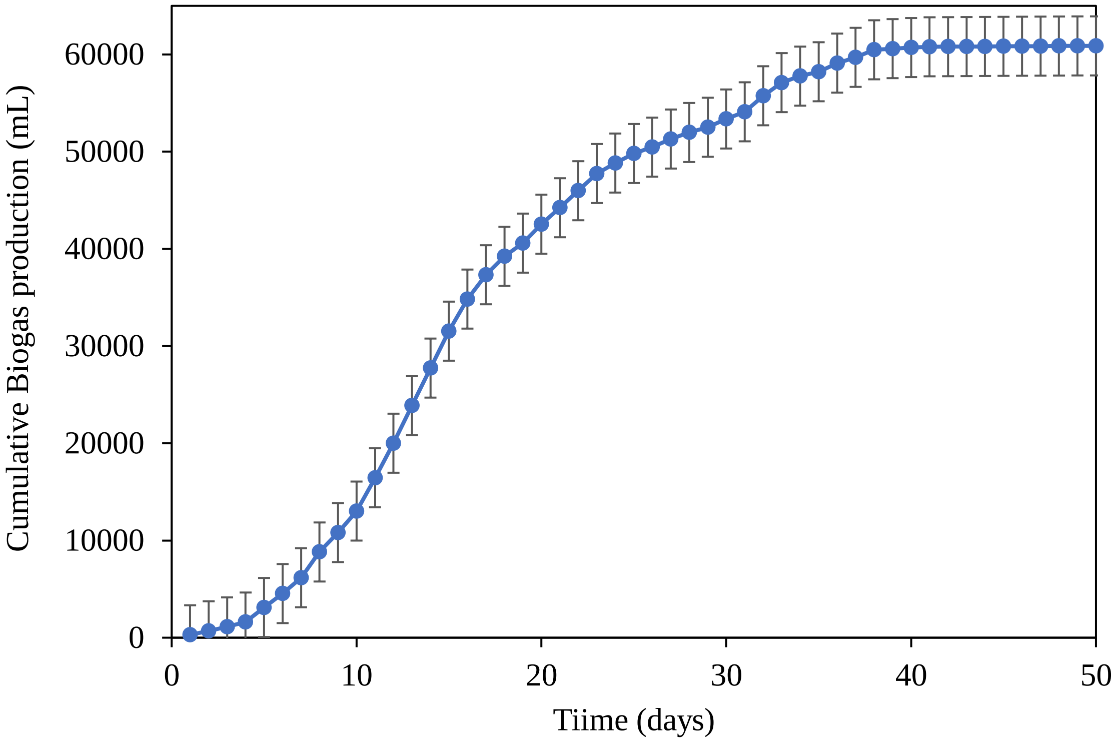


Fig. 7.4. Cumulative biogas production in 20 L reactor of *Parthenium hysterophorus* and food waste for mixing ratio 1.5

7.1.1.2. Volatile solids (VS) reduction

In co-digestion, less toxic substances were formed, resulting in more VS degradation (Barua et al., 2018). The VS reduction rate depends on the microbial activity of anaerobic co-digestion. A decreasing trend in VS is observed with respect to time. The mixing ratio of 1.5 shows the highest VS reduction of 40.03%. (fig.7.5). Dhamodharan et al. (2015) stated that higher VS reduction leads to higher biogas generation. The trend of VS degradation is 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2.

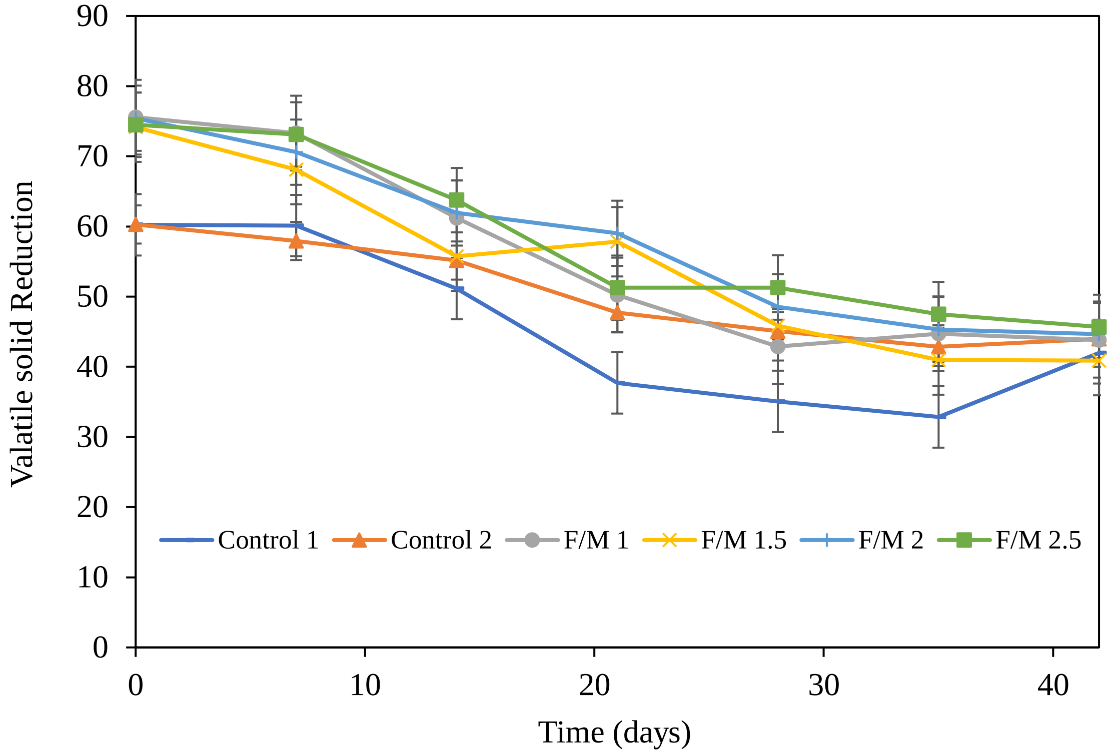


Fig. 7.5. Reduction of VS in co-digestion of *Parthenium hysterophorus* for different mixing ratios

7.1.1.3. Soluble chemical oxygen demand (sCOD) and volatile fatty acid (VFA) study

The sCOD depends upon the organic substance present in the anaerobic reactor. sCOD for the different mixing ratios of co-digestion was studied, the observations made were the sCOD started to increases from 0th day to 21st day and then started to decrease theirs after. The highest sCOD 14444 (mg/L) was observed on the 3rd week of anaerobic digestion for mixing ratio 1.5 (fig. 7.6) followed by mixing ratio 2.0 and mixing ratio 2.5. The decrease in sCOD is observed after 3rd week. The decrease in sCOD may be due to the accumulation of toxic substances in the anaerobic reactor.

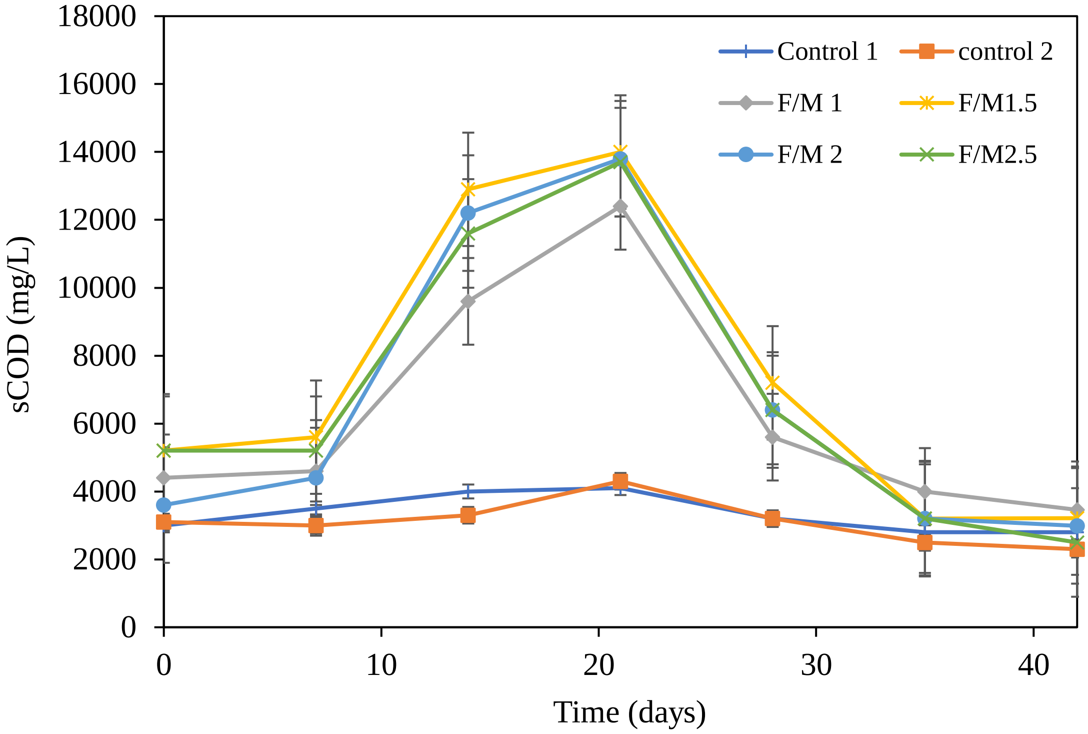


Fig. 7.6. Variations of sCOD in co-digestion of *Parthenium hysterophorus* for different mixing ratios

As the complex molecule started to break down, the VFA began to be produced. VFA continuously increased for 21 days, and then it gradually decreased. An increase in the VFA was due to sugar being converted to acid, and finally, the products were carbon dioxide and methane. Acidogen's activity led to the production of VFA to 21 days; after that, methanogens came to play their function, as they were sensitive to acidic conditions. The highest amount of VFA production was found corresponding to F/M ratio 1.5 (1897 mg/L) followed by ratios 2 and 2.5, respectively, as shown in Figure. 7.7.VFA declined after 21 days due to the beginning of the methane production phase.

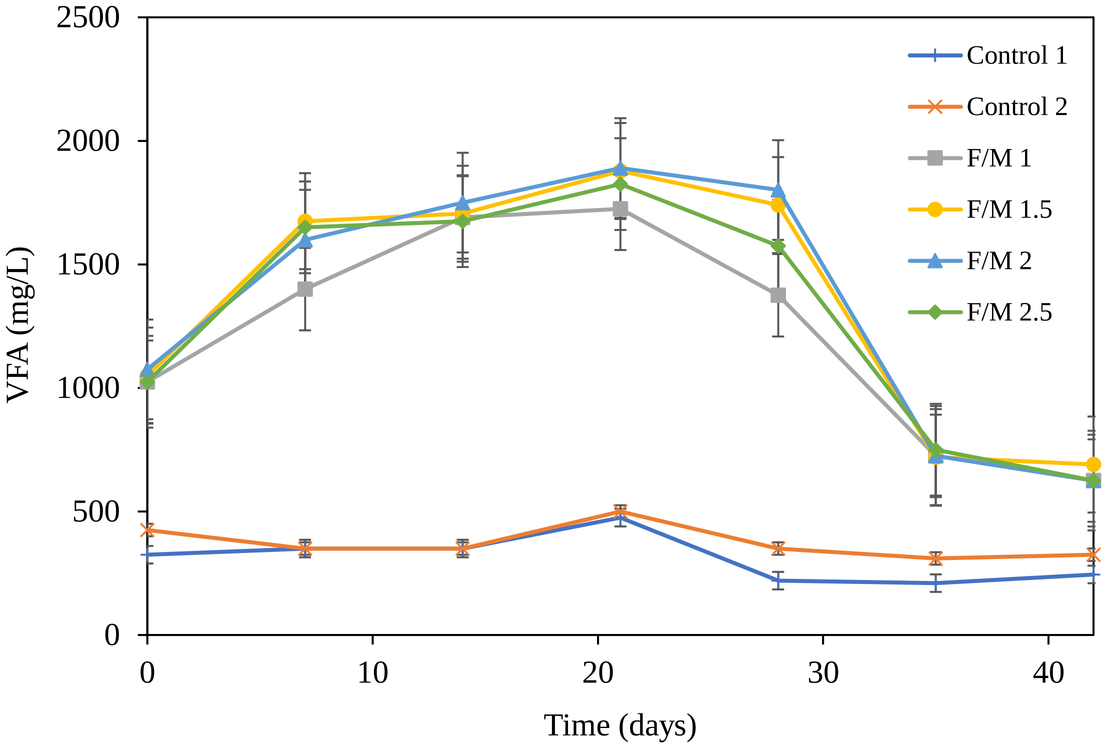


Fig. 7.7. Variations of VFA in co-digestion of *Parthenium hysterophorus* for different mixing ratio

7.1.2 Anaerobic codigestion of *Lantana camara*

7.1.2.1. Biochemical methane potential (BMP) trial

To study the effects of co-digestion of *Lantana camara* and food waste, experiments were carried out with different mixing ratios using cow dung as inoculum. Food waste is easily degradable and not lignocellulosic (Barua et al., 2018). It was observed that for co-digestion of *Lantana camara*, mixing ratio 1.5 produced the highest amount of methane 211 mL CH_4 g^{-1} VS on the 14th day (fig 7.8). The timespan of the hydrolysis process was reduced, resulting in early methane production.

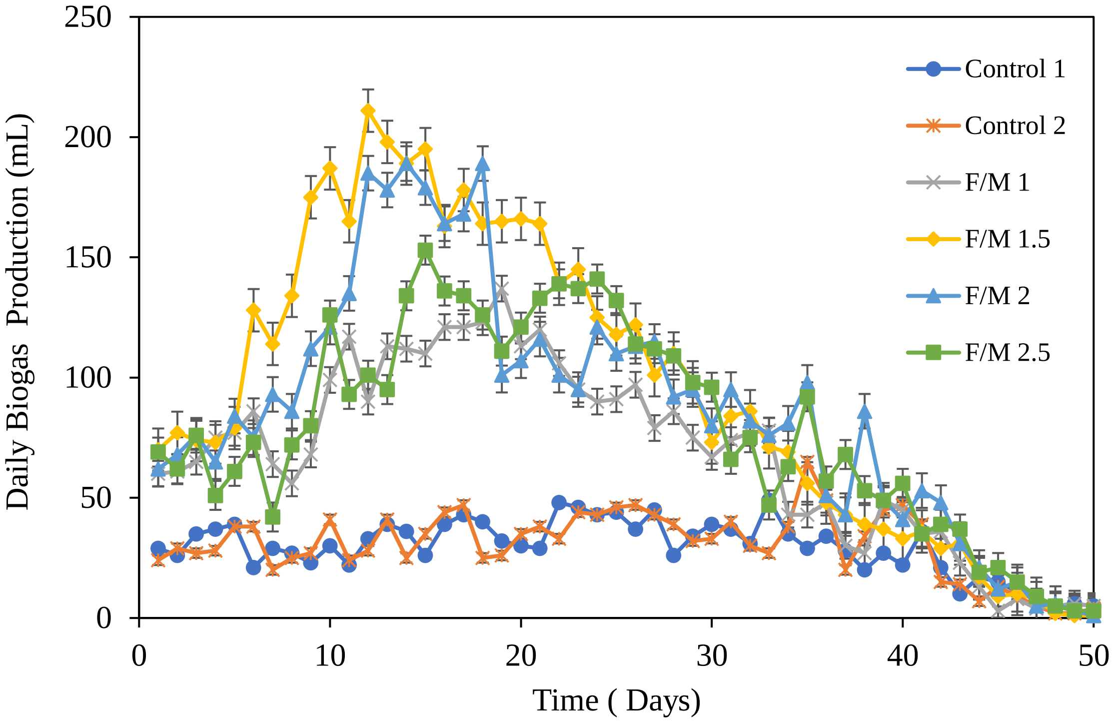


Fig. 7.8. Daily methane production in co-digestion of *Lantana camara* for different mixing ratios

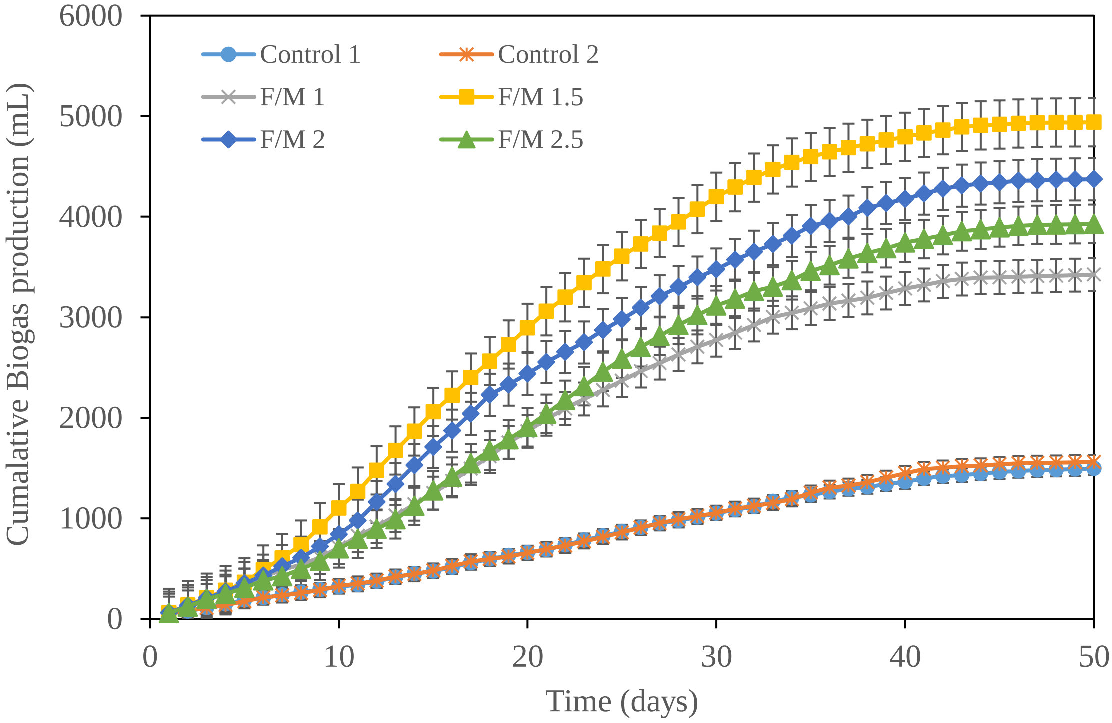


Fig. 7.9. Cumulative methane production in co-digestion of *Lantana camara* for different mixing ratios

In mono digestion, it took 26 days for maximum methane production. In the co-digestion hydrolysis, the phase is reduced due to the absence of lignin content in food waste; the microorganisms can easily degrade biomass resulting in earlier methane production (reduction of lag time). The cumulative methane production follows order 1.5 > 2.0 > 1.0 > 2.5 > 1.0. After conducting a 1 L of BMP study, the batch reactor of 20 L capacity was performed with a working volume of 14 L with an ideal F/M ratio of 1.5. The highest methane yield was observed on the 13th day (4374 mL CH_4 g^{-1} VS), and in (Fig.7.10 and 7.11), cumulative methane production was 83955 mL in 20 L of the batch reactor, which is quite valuable.

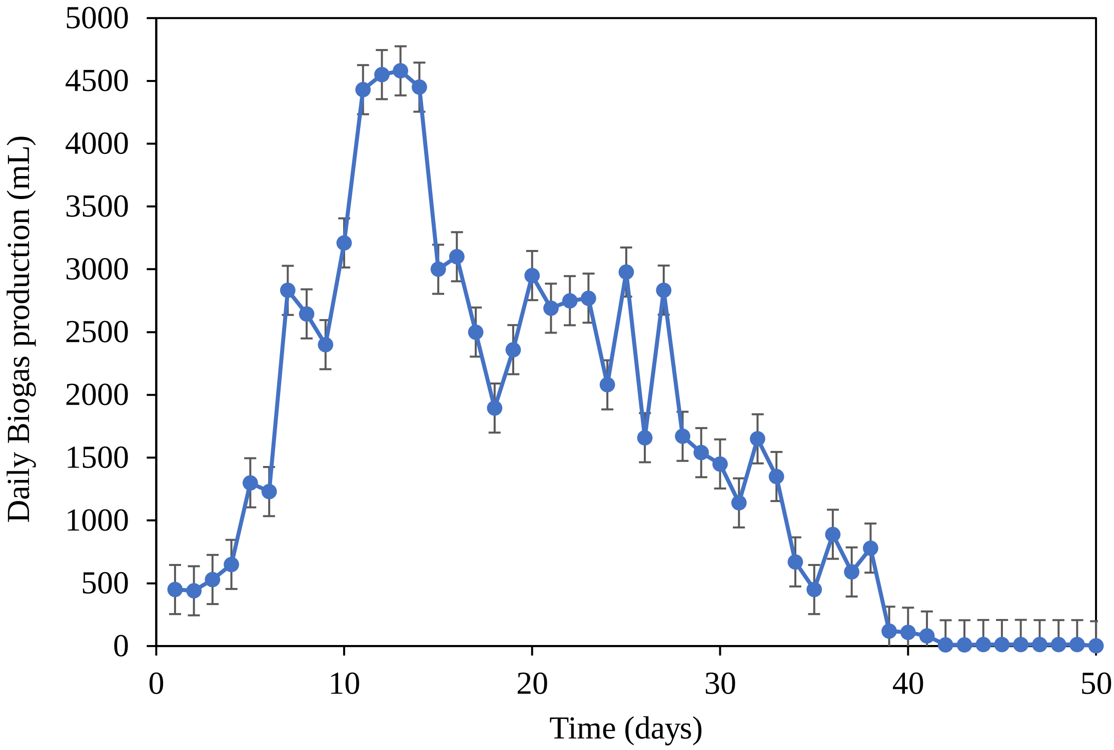


Fig. 7.10. Daily methane production in 20 L reactor using *Lantana camara* and food waste for mixing ratio 1.5

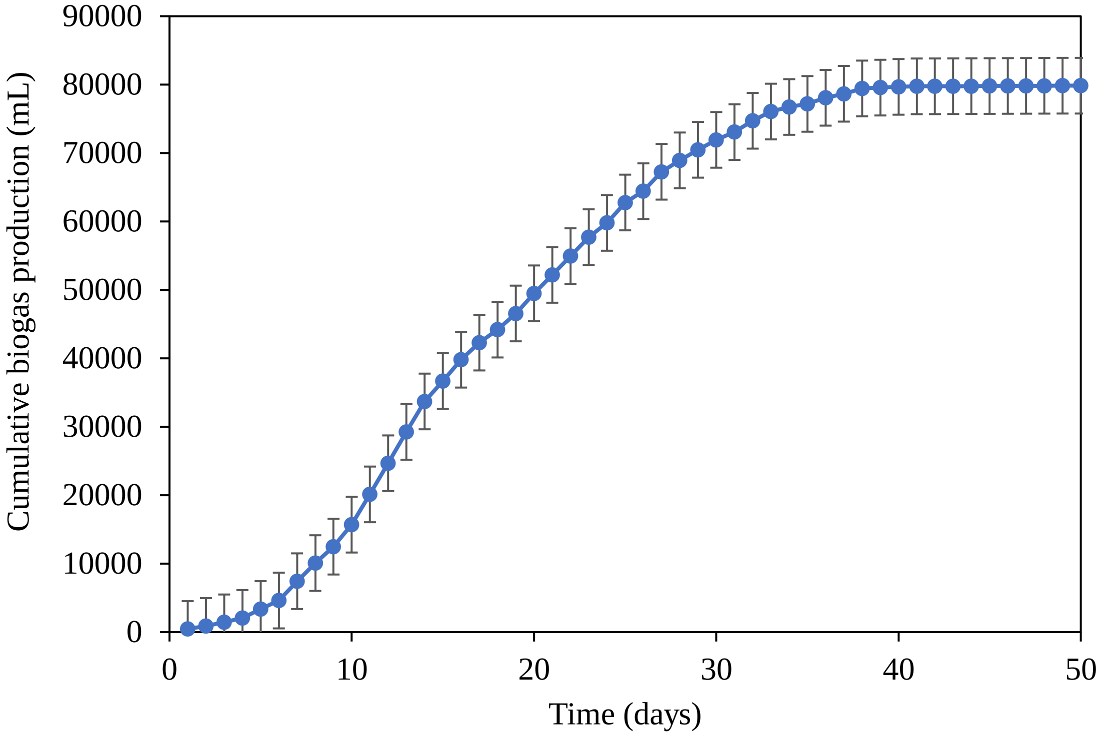


Fig. 7.11. Cumulative methane production in 20 L reactor using *Lantana camara* and food waste for mixing ratio 1.5

7.1.2.2. Volatile solid (VS) reduction

Different mixing ratios show varying VS reduction; the mixing ratio of 1.5 shows the highest VS reduction of 48.5% (fig 7.12). A decreasing trend in VS is observed with respect to time. Higher VS reduction leads to higher biogas generation. VS reduction in co-digestion is 13% greater than the VS reduction in mono digestion. The VS reduction rate depends on the microbial activity of anaerobic co-digestion. VS reduction follows the order 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2. All VS percentage reduced were greater than the control.

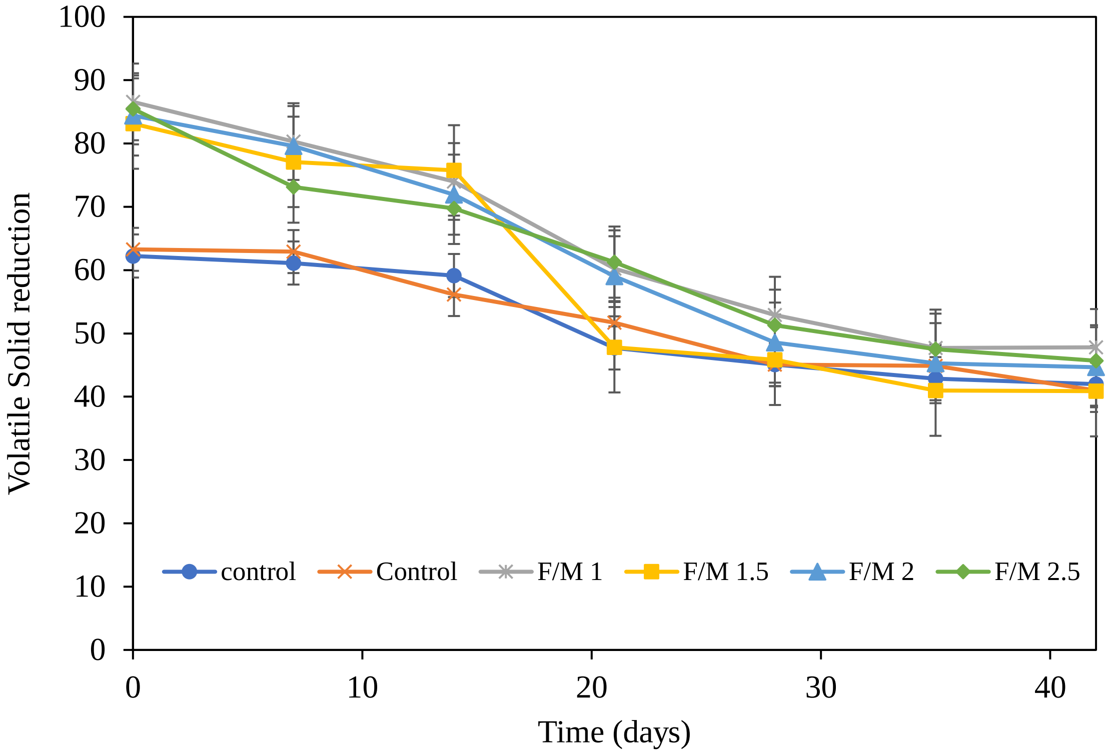


Fig. 7.12. Reduction of VS in co-digestion of *Lantana camara* for different mixing ratios

7.1.2.3 Soluble chemical oxygen demand (sCOD) and volatile fatty acid (VFA) study

An increase in sCOD signifies the enhanced amount of simple soluble organic matter, which can readily transfer to biogas (Barua et al., 2018). Higher the sCOD, there will be more organic substance present in the anaerobic reactor. sCOD for different mixing ratios of co-digestion, it was observed that there is an increase in sCOD up to 14th day and started to decline after that. The declination trend in the graph is due to the accumulation of toxic substances in the reactor. The highest sCOD 9032 (mg/L) was observed on the 2nd week of anaerobic digestion for F/M ratio 1.5 (fig 7.13). The sCOD follows the mixing ratios order 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2.

Increase and decrease of VFA may affect the pH of the reactor. So, in order to maintain the methanogenic activity, pH in the reactor should be maintained. The decrease in pH from 6.0-6.5 may affect bacterial activity (Sallis and Uyanik, 2003). More than 75% of methane can be achieved when the pH is above 5. (Jain and Mattiasson., 1988). The methanogens grow best in the optimum pH range of 6.6-7.6 (McCarty et al., 2001). The higher amount of VFA production results in a higher methane production rate (Barua et al., 2018). The steep increase in VFA was observed; it might be due to the presence of easily degradable food waste in the reactor. An increase in VFA concentration was observed up to 14th day, and gradually, the decline is started after 2nd week; decline in VFA is due to start of the methanogenic phase. The activity of the acidogenic leads to the production of VFA upto the 14th day. The highest VFA produced in co-digestion is 2692 mg/L on 14th day by mixing ratio 1.5 (fig 7.14). VFA follows the order 1.5 > 2.0 > 2.5 > 1.0 > control 1 > control 2.

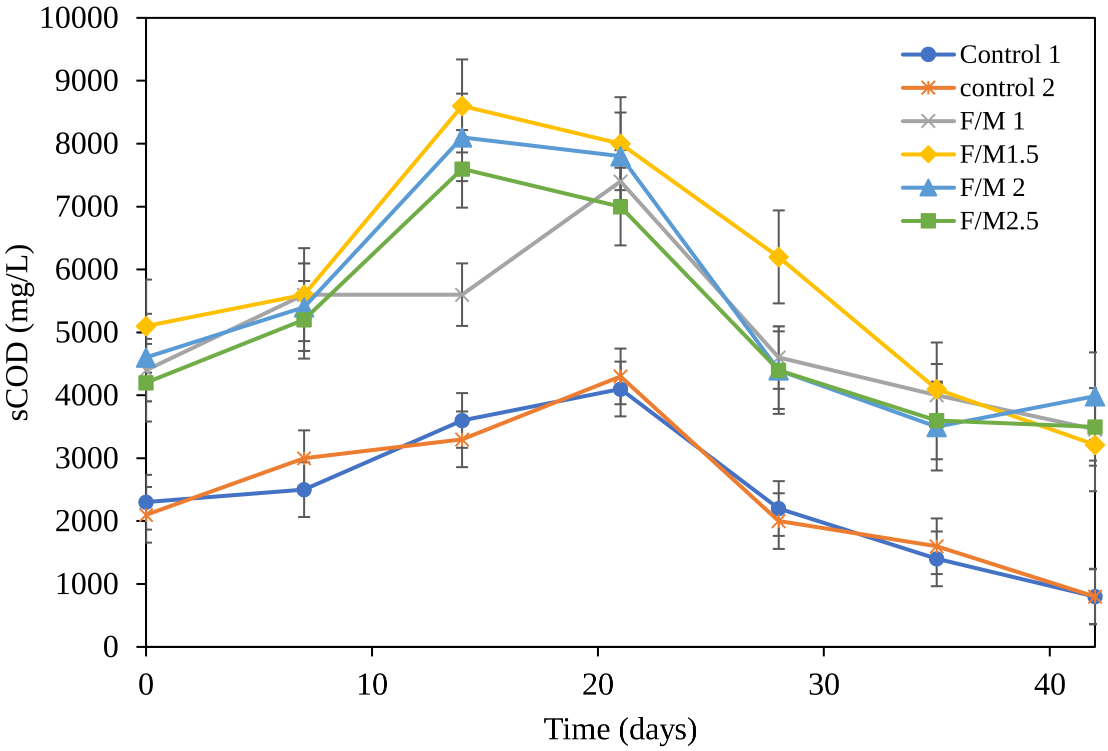


Fig. 7.13. Variations of sCOD in co-digestion of *Lantana camara* for different mixing ratios

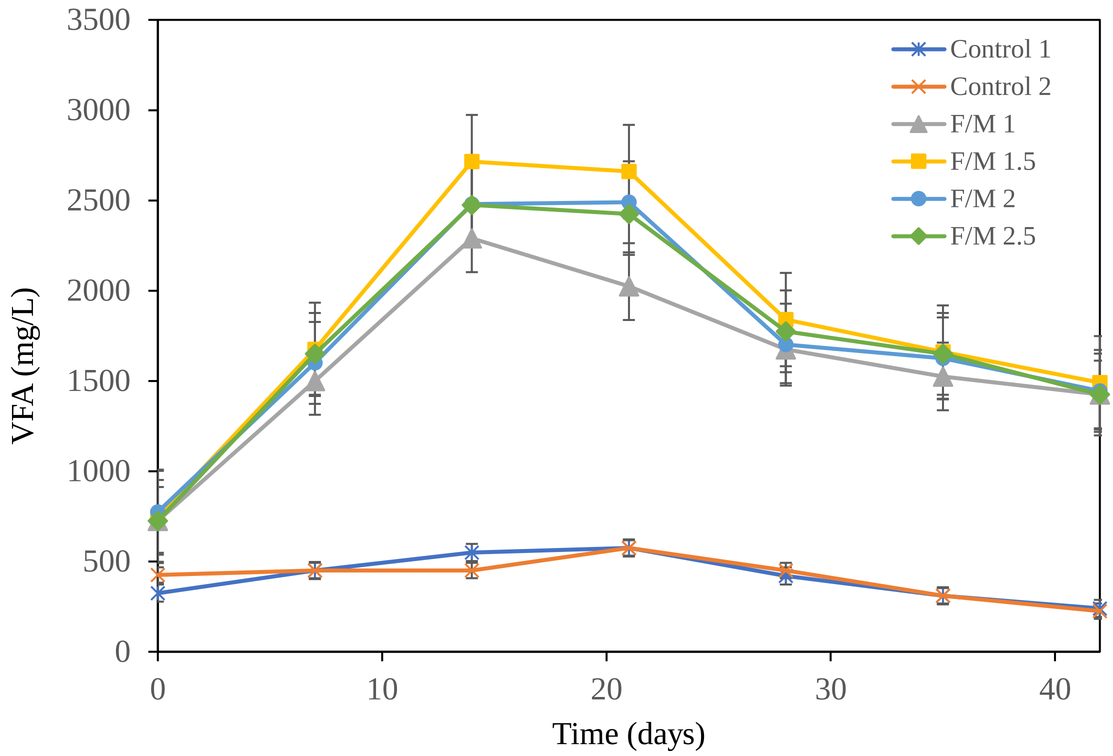


Fig. 7.14. Variations of VFA in co-digestion of *Lantana camara* for different mixing ratio

7.1.3. Anaerobic co-digestion of *Ageratum conyzoides*

7.1.3.1. Biochemical methane potential (BMP) Trial

To study the effects of co-digestion of *Ageratum Conyzoides* and food waste, experiments studies were carried out with different mixing ratios using cow dung as inoculum. The co-digestion of *Ageratum Conyzoides with food waste*, mixing ratio 2 produced the highest amount of methane 205 mL CH_4 g^{-1} VS on the 13th day (fig 7.15). The hydrolysis process's period was reduced, resulting in early methane production; it may be due to food waste was easily degradable and not lignocellulose in nature (Barua et al., 2018). In mono digestion, it took 25 days for maximum methane production. Fig 7.33 shows the highest cumulative methane production of 4875 mL CH_4 g^{-1} VS (mixing ratio 2). In co-digestion hydrolysis phase is reduced due to the absence of lignin content in food waste; the microorganisms can easily degrade biomass resulting in earlier methane production (reduction of lag time). The cumulative methane production follows order 2 > 1.5 > 2.5 > 1.0. After conducting a 1L of BMP study, the batch reactor of 20 L capacity was performed with a working volume of 14 L with ideal F/M ratio 2. Highest methane yield was observed on the 15th day (3450 mL CH_4 g^{-1} VS) and in (fig 7.17 and 7.18) cumulative methane production was 69996 mL in 20L of the batch reactor which is quite valuable.

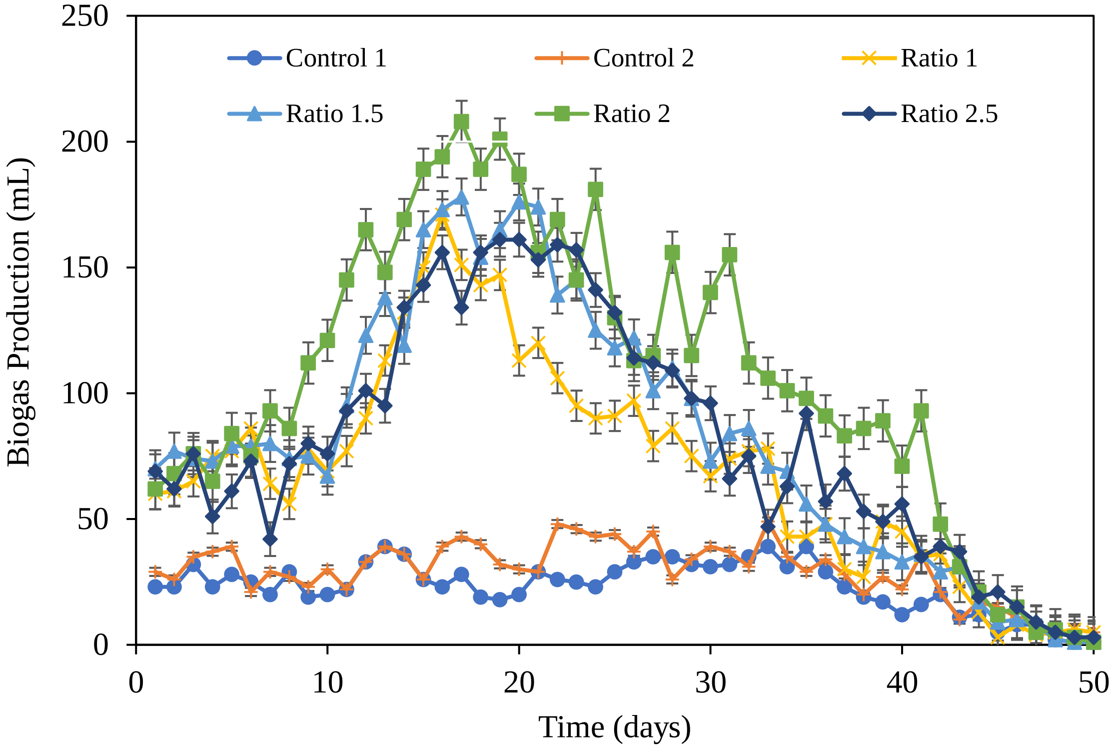


Fig. 7.15. Daily biogas production in co-digestion of *Ageratum Conyzoides* for different F/M ratios

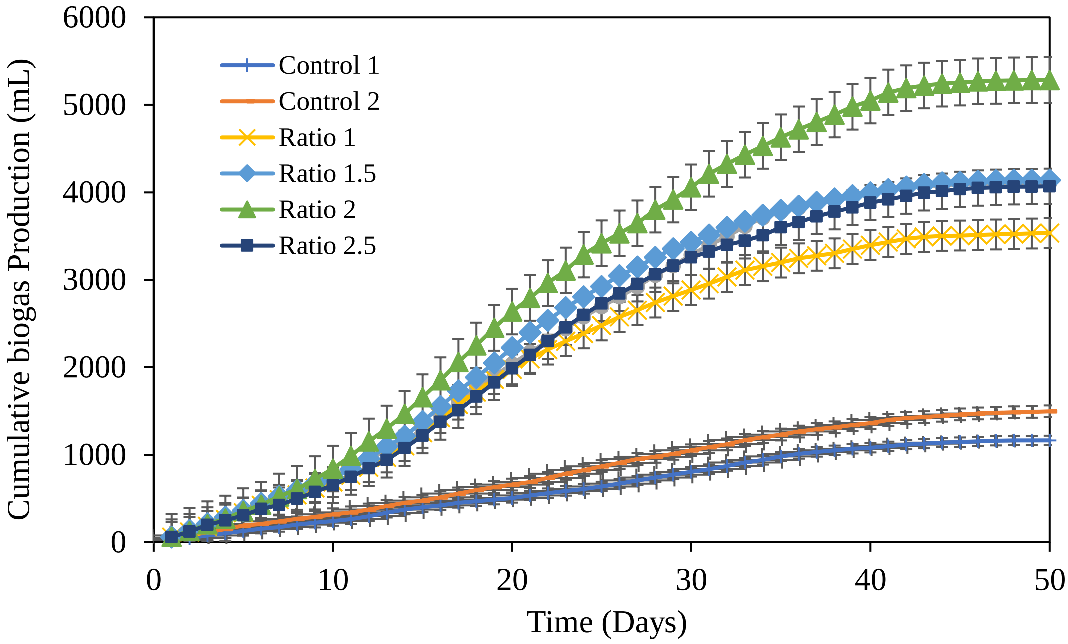


Fig. 7.16. Cumulative biogas production of co-digestion of *Ageratum Conyzoides* for different mixing ratios

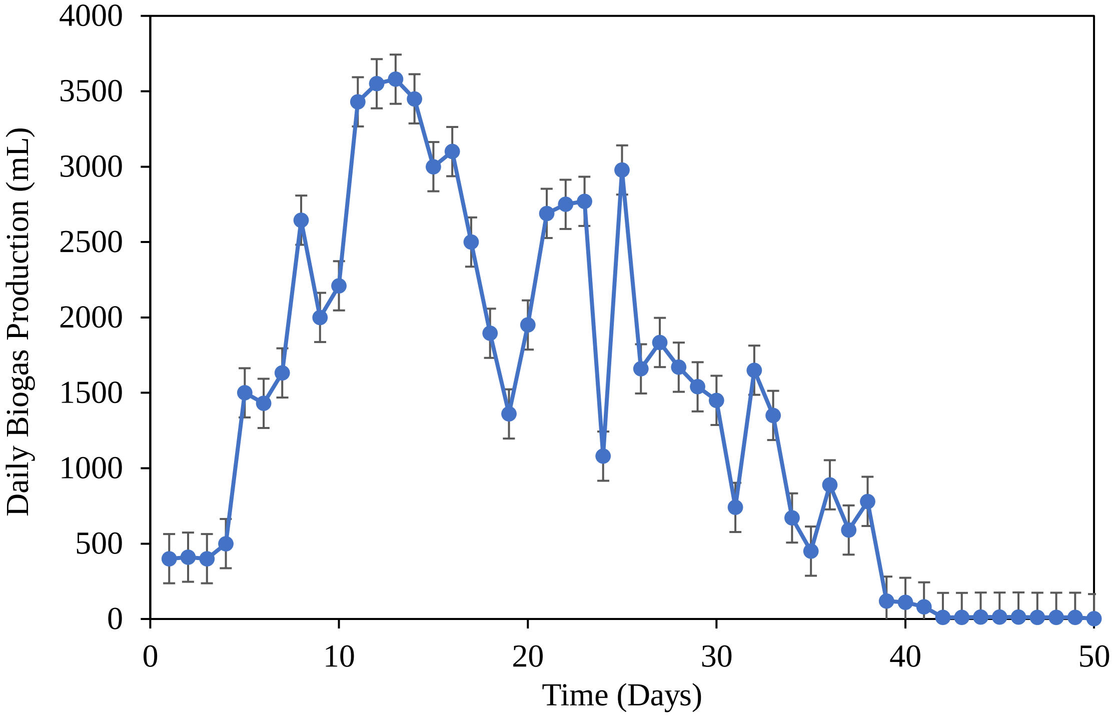


Fig. 7.17. Daily biogas production in 20 L anaerobic digester using *Ageratum Conyzoides* for F/M ratio 2

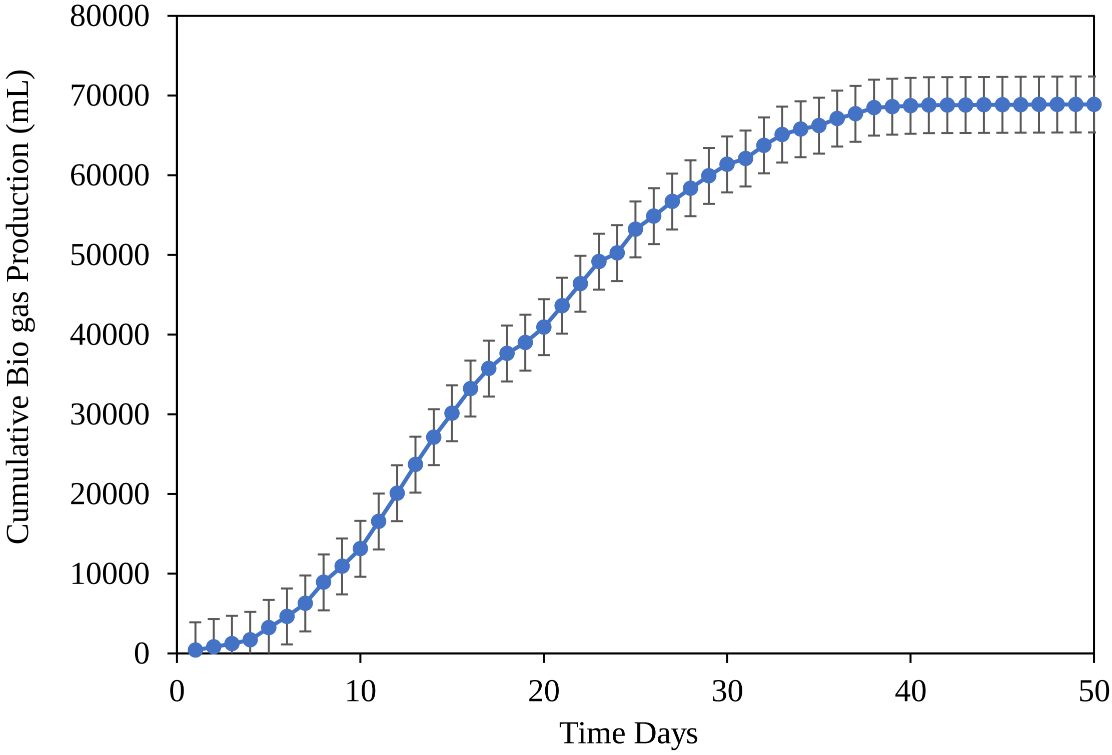


Fig. 7.18. Cumulative biogas production in AD of *Ageratum conyzoides* and food waste for mixing ratio 2

7.1.3.2 Volatile solids (VS) reduction

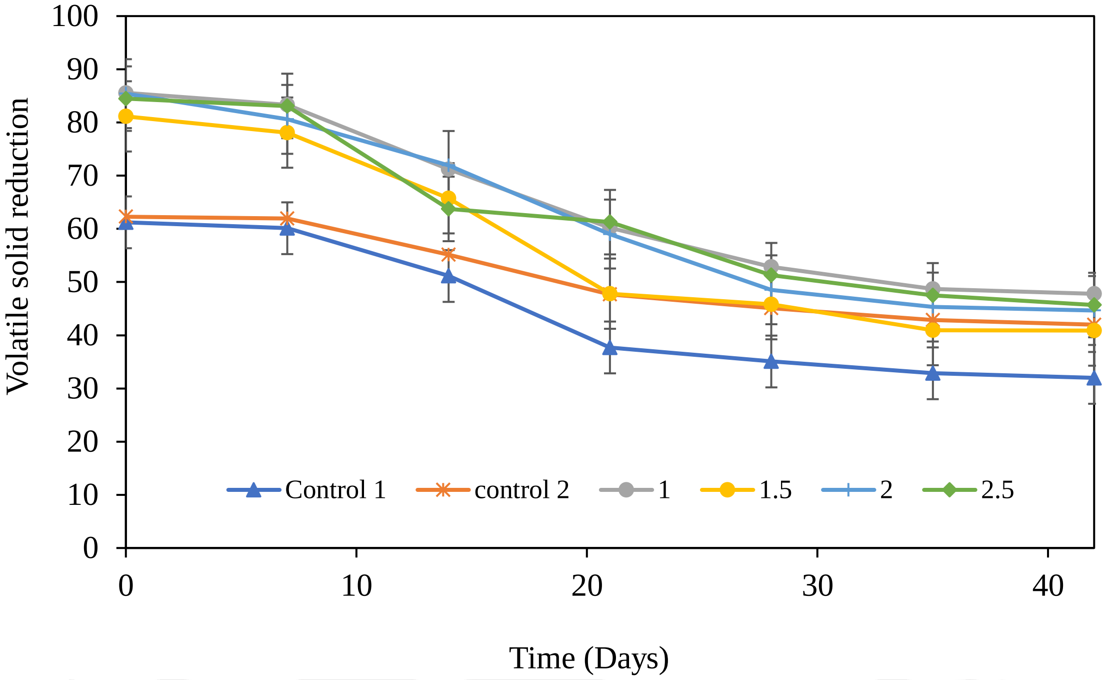


Fig. 7.19. Reduction of VS in co-digestion of *Ageratum Conyzoides* for different mixing ratios

Different mixing ratios show varying VS reduction; the mixing ratio of 2 shows the highest VS reduction of 39.3% (fig 7.19). A decreasing trend in VS is observed with respect to time. Dhamodharan et al. (2015) stated that higher VS reduction leads to higher biogas generation. The VS reduction rate depends on the microbial activity of anaerobic co-digestion. VS reduction follows the order 2 > 1.5 > 2.5 > 1.0. All VS percentage reduced are greater than control 2.

7.1.3.3 Soluble chemical oxygen demand (sCOD) and volatile fatty acid (VFA) study

Similarly, in *A. conyzoides*, it was observed that there were increases in sCOD up to 14th day in all the F/M ratios, which is due to the organic matter in the reactor started to break down and it started to decline after. After 14 days, the graph observed a declined trend that might be an accumulation of a toxic substance in the reactor. There was a fixed amount of substrate fed in the batch reactor after the 14th day; there might not be much organic matter present. The highest sCOD 7987 (mg/L) was observed on the 2nd week of anaerobic digestion for F/M ratio 2 (fig 7.20). The sCOD follows the mixing ratios order 2.0 > 1.5 > 2.5 > 1.0.

Initially days, VFA in all the BMP ratios were started increasing; after that, it started to fall. The rise and fall of VFA affect the pH of the reactor, which can hamper the methanogenic activity. To avoid such effect, pH in the reactor should be maintained. VFA production can directly co-related to methane production (Barua et al., 2018). In *A. conyzoides*, a steep increase in VFA was observed due to the presence of easily degradable food waste in the reactor. An increase in VFA concentration was observed up to the 21st day, and gradually, the decline is started after the 3rd week; the decline in VFA is due to the start of the methanogenic phase. The activity of the acidogens leads to the production of VFA up to the 21st day. The highest VFA produced in co-digestion is 1845 mg/L on the 21st day by mixing ratio 2 (fig. 7.21). VFA follows the order 2 > 1.5 > 2.5 > 1.0. VFA concentration decreased in high and low mixing ratios because of the high percentage of inoculum and substrate in that ratio.

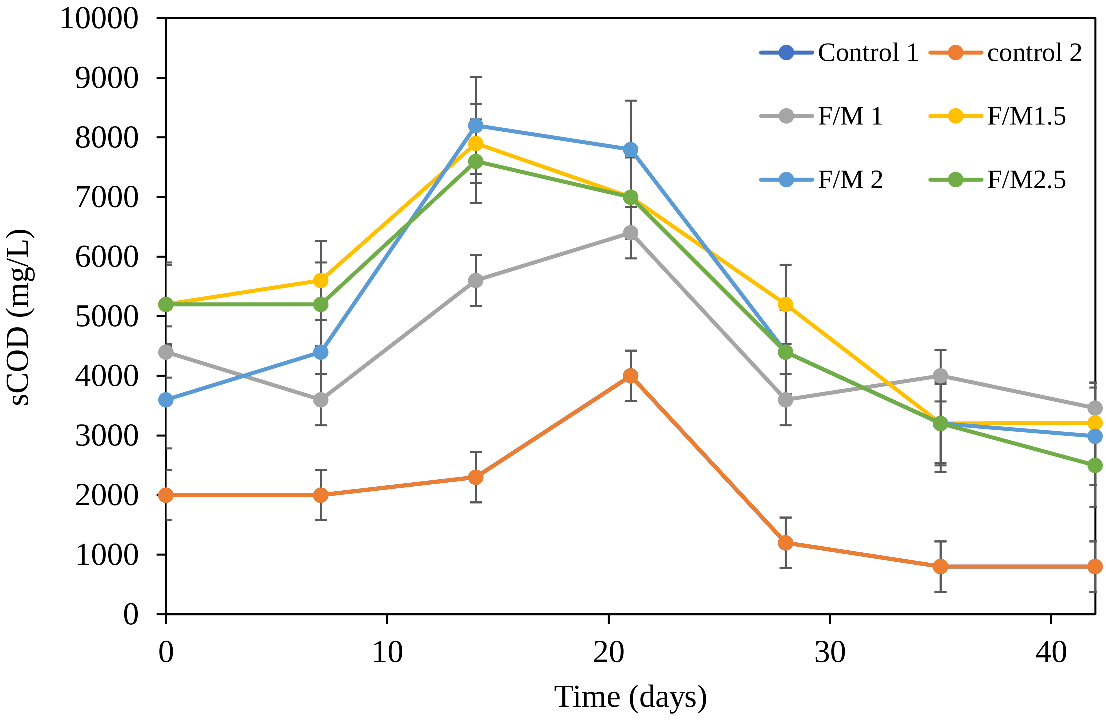


Fig. 7.20. Variations of sCOD in co-digestion of *Ageratum Conyzoides* for different mixing ratios

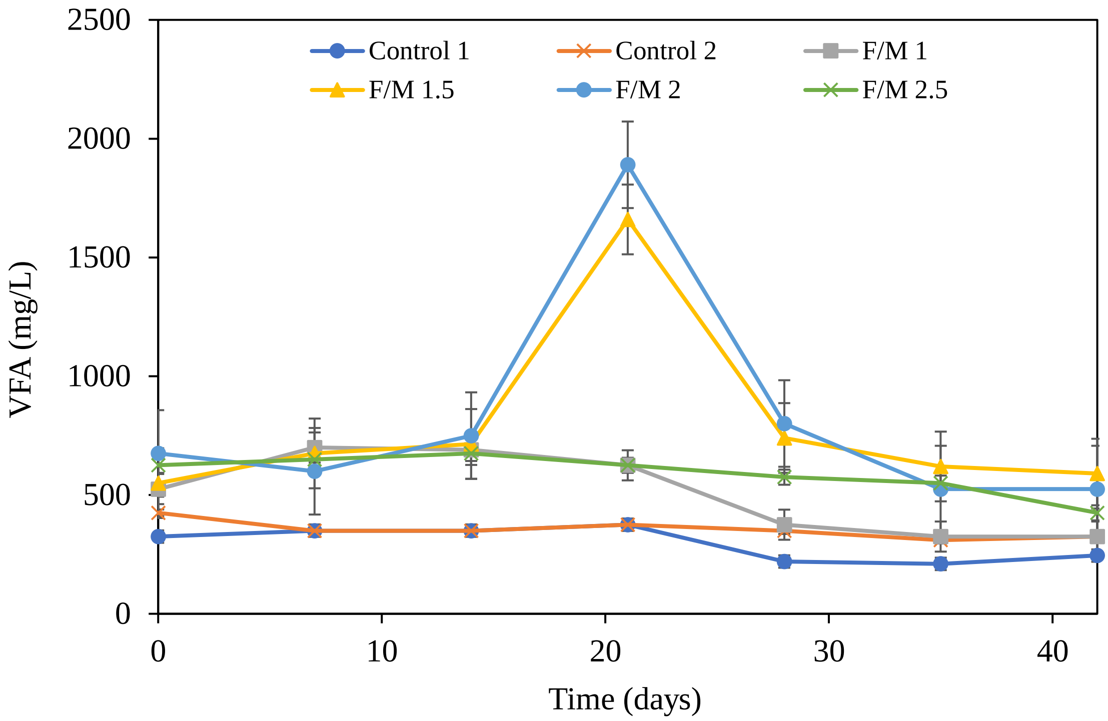


Fig. 7.21. Variations of VFA in co-digestion of Ageratum conyzoides for different mixing ratio

Table 7.1. Comparison of biogas production between mono digestion and co-digestion

Sl. no	Weed	Biogas Production(mL CH_4/g VS) (Mono digestion)	Biogas Production mL CH_4/g VS) (Co-digestion)
1.	*Parthenium hysterophorus*	140± 5 (28 days) F/M ratio (2)	165± 11 (17 days) F/M ratio (1.5)
2.	*Lantana Camara*	195.5 ± 8 (25 days) F/M ratio (1.5)	211± 13 (14^{t}days) F/M ratio (1.5)
3.	*Ageratum conyzoides*	205 ± 10 (25 days) F/M ratio (2)	205 ± 2 (13 days) F/M ratio (2)

7.2. Summary for Phase III

Anaerobic co-digestion of *Parthenium hysterophorus, Lantana camara, Ageratum Conyzoides* and food waste were studied using cow dung as an inoculum. In 1 litre batch BMP study it was observed that co-digestion of *Parthenium hysterophorus* and food waste produced 165 mL CH_4 g^{-1} VS on 17th day, co-digestion of *Lantana camara* and food waste produced 211 mL CH_4 g^{-1} VS on 14th day and co-digestion of *Ageratum Conyzoides* and food waste produced 205 mL CH_4 g^{-1} VS on 13th day. The lag phase was minimum in co-digestion resulting in the highest methane production in 14 days (*Lantana camara*) and 17 days for (*Parthenium hysterophorus)* and 13 days (*Ageratum Conyzoides)*. On the other hand, in mono digestion highest methane production was observed on the 26th day for *Parthenium hysterophorus* and *Lantana,* the 25th day for *Ageratum Conyzoides.*

CHAPTER 8

CONTINUOUS REACTOR

This chapter focuses on operation of two stages continuous reactor, where terrestrial weeds were fed every day.

8.1. Phase IV- 20L Scale continuous reactor

Finally, a novel anaerobic digester designed reactor (Barua and Kalamdhad, 2019), was operated. Mixing and the separation of stages in a digester during anaerobic digestion demonstrates enhanced biodegradation efficiency of the feedstock. However, two stage anaerobic digesters were very tough to operated and require vast space. In addition, continuous high intensity mixing minimizes biogas production. Based on substrate, anaerobic two stage anaerobic digester was designed. The goal was to performed anaerobic digester continuous reactor to utilizing terrestrial weed as the feedstock. Initially, it was used for water hyacinth whole plant was fed in the digester. This novel anaerobic digester also showed its great potential in treating terrestrial weeds in the form untreated, pretreated or co-digested substrate.

8.1.1. Biogas Production

The 20L scale two-phase continuous reactor was operated; usually, a two-stage biogas digester improves the biodegradation rate of fed, process stability and biogas recovery than the traditional single stage anaerobic digester (Maspolim et al., 2015). The anaerobic reactor was fed with 5kg of cow dung slurry and sealed for 40 days for acclimatization. After 40 days, feeding of the anaerobic digester started. Initially, the digester was fed with a small amount of organic loading rate (OLR) of 0.600kg COD/m3.d, and pH was checked. pH is a vital parameter influencing the anaerobic digester performance. The ideal pH range for the microorganisms to flourish inside anaerobic digester is 6.5–7.5. Optimization of the substrate was done based on pH. The change significantly influences the growth rate of microorganisms in pH. As the pH was stable for a few days, the OLR was increased. After sudden amount pH was drop below the range of 6.5-7.7. At that particular point, the last amount was considered as the optimized amount for the substrate.

Among terrestrial weeds, Untreated, pretreated and co-digestion of *Parthenium hysterophorus*, Lantana *camara*, *Ageratum* conyzoides were added separately, shows the positive results in terms of biogas generation. The anaerobic digester was operated at a specified OLR for 50 days. Shown in Table 8.1.

Table 8.1. Amount of substrate fed

OLR (KgCOD/m^{3}D)	**Untreated**	**Pretreated**	**Co-digestion**
P.hysterophorus	3.9	3.9	7.1
L.camara	4.1	4.1	6.9
A.conyzoides	3.6	3.6	6.6

The average biogas production for Untreated *P.hysterophorus* was 3270mL, *L.camara* was 3010mL, and for *A.conyzoides was* 3150mL. Average biogas production for pretreated *P.hysterophorus* was 6454mL; *L.camara was* 6219 mL and *A. conyzoides* was 6982mL.Average biogas production for co digestion of *P. hysterophorus* was 5984mL; *L.camara* was 5218 and for *A.conyzoides* was 5765mL shown in fig 8.1. 8.2 and 8.3. GC analysis was carried out which shows the biogas composition of CH_4 shown in Table 2.

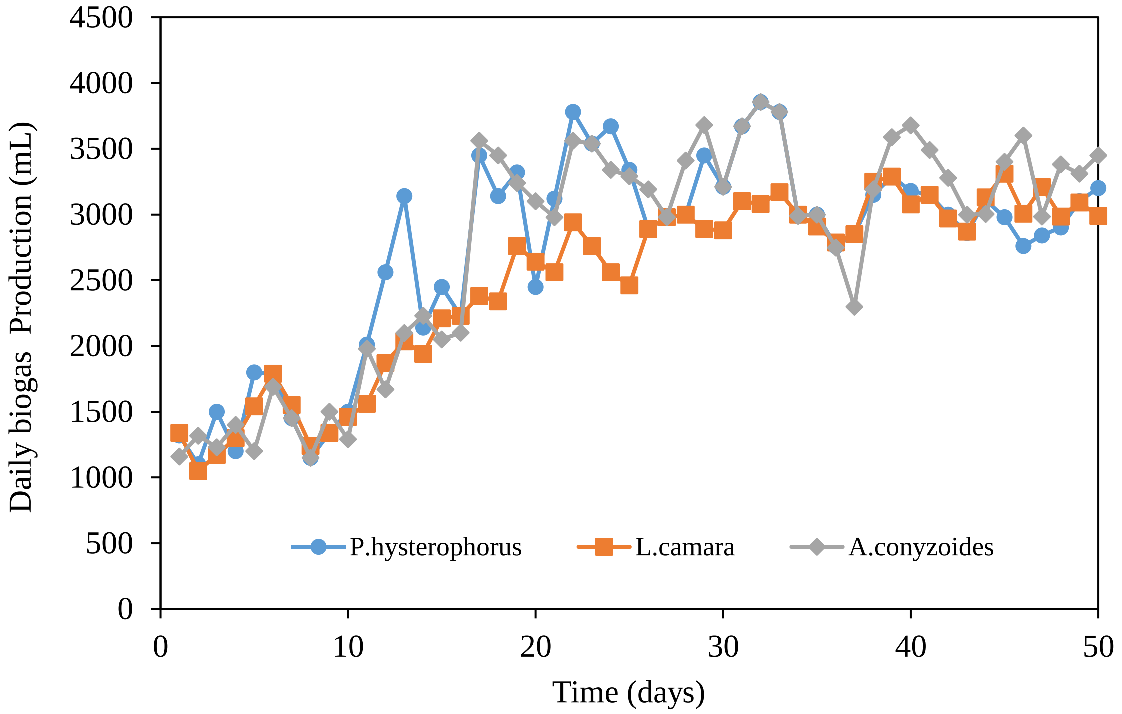


Fig. 8.1. Daily biogas production for untreated substrate

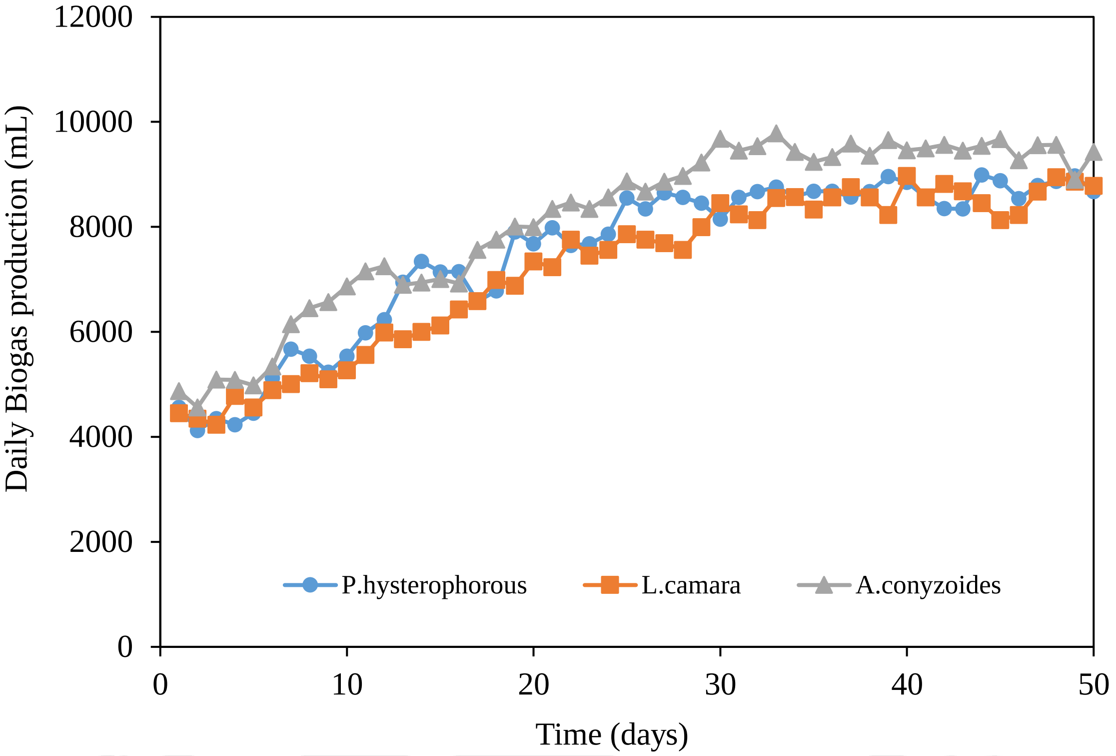


Fig. 8.2. Daily biogas production for pretreated substrate

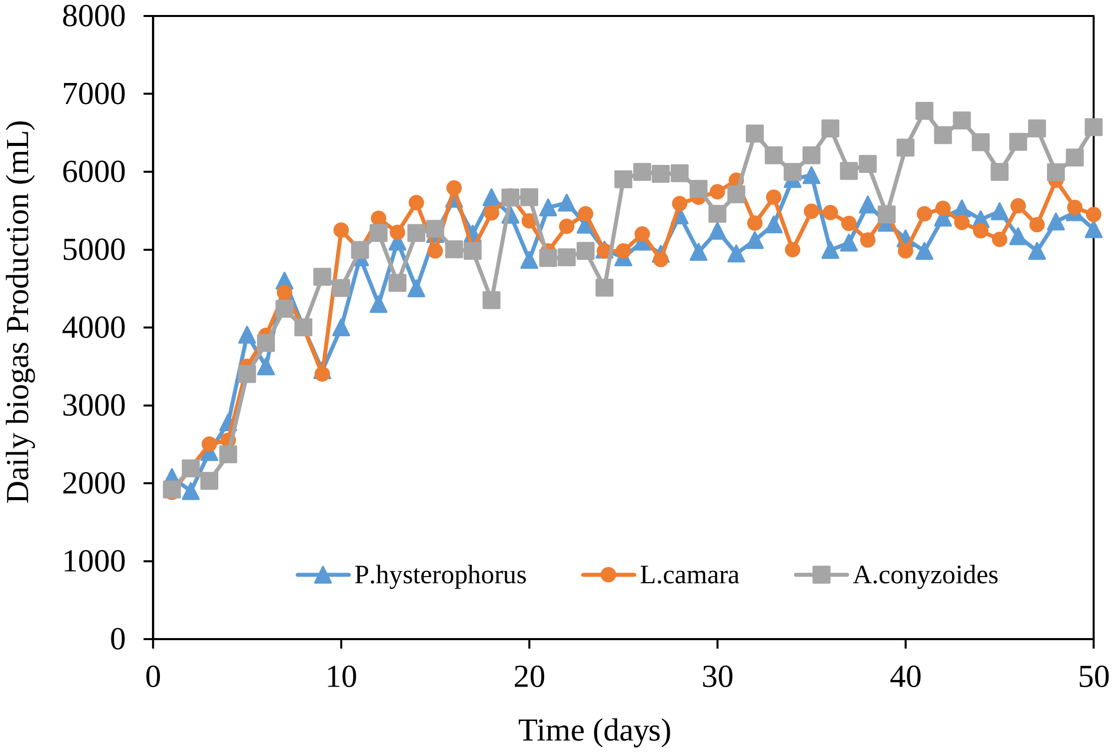


Fig. 8.3. Daily biogas production for co digestion substrate

Table 8.2. Composition of methane production

Gas Chomatography	Untreated (%)	Pretreated (%)	Co digestion (%)
P. hysterophorus	54.04	65.08	62.54
L.camara	51.79	63.60	59.58
A.conyzoides	57.38	68.02	66.29

8.1.2. Soluble chemical oxygen demand (sCOD) study

Fig 8.4, 8.5, 8.6, 8.7. 8.8 and 8.9 showed the sCOD profile of the continuous anaerobic digester. sCOD removal is an essential parameter determining the efficiency of the continuous anaerobic digestion reactor. Usually, after hydrolysis, cellulose and hemicellulose of the lignocellulosic feedstock are transferred into COD (generally simple solid sugars). Higher the sCOD removal, higher the stability of the process. In this study, the sCOD removal rate for untreated *P.hyserophorus*, *L.camara* and *A.conyzoides* is 45.6, 41.7 and 51.3%, respectively. In pretreated sCOD removal rate observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 75,6, 74.3 and 81.1% respectively. In co-digestion sCOD removal rate was observed for *P.hyserophorus*, *L.camara* and *A.conyzoides 6.89*, 66.2 and 71.8% respectively. Overall COD removal efficiency was relatively high in the novel anaerobic digester. Overall COD removal efficiency was relatively high in the novel anaerobic digester. This novel anaerobic digester was beneficial for its biochemical level because of its two stages (Table 8.3. shows the continuous reactor study). Also, the presence of proper in the digester helps the solid feedstock in suspension and homogenized the inlet feed with the robust microbial community.

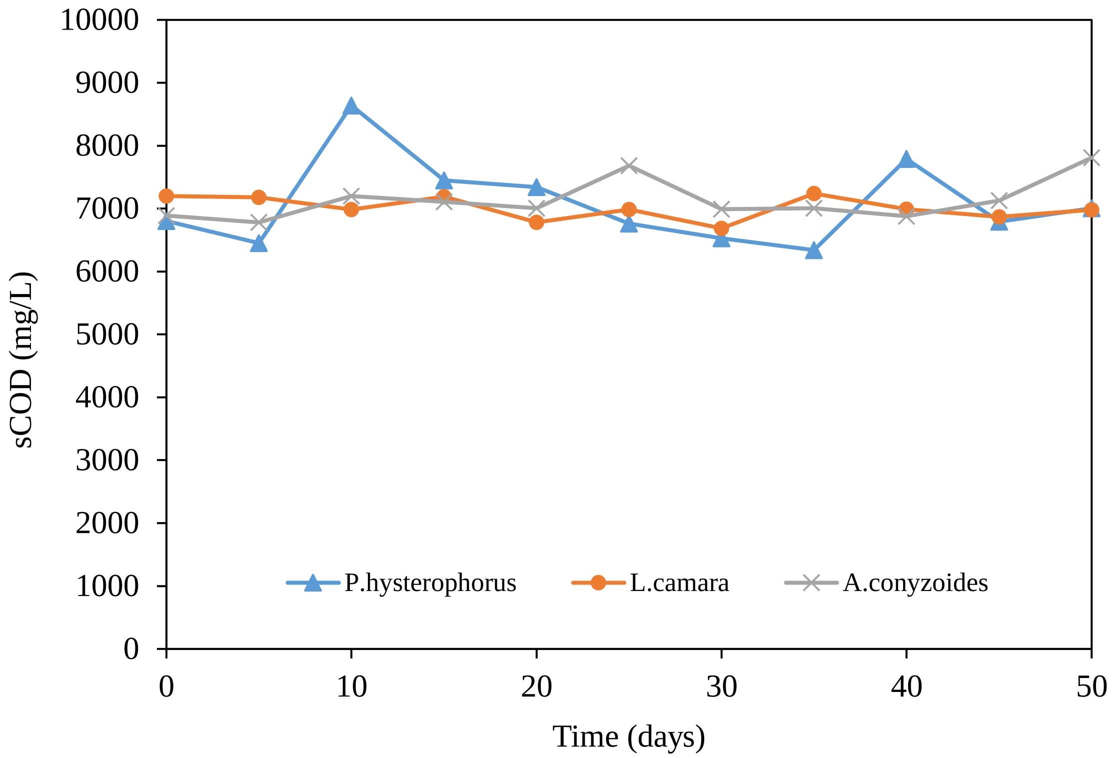


Fig. 8.4. sCOD Inlet for Untreated

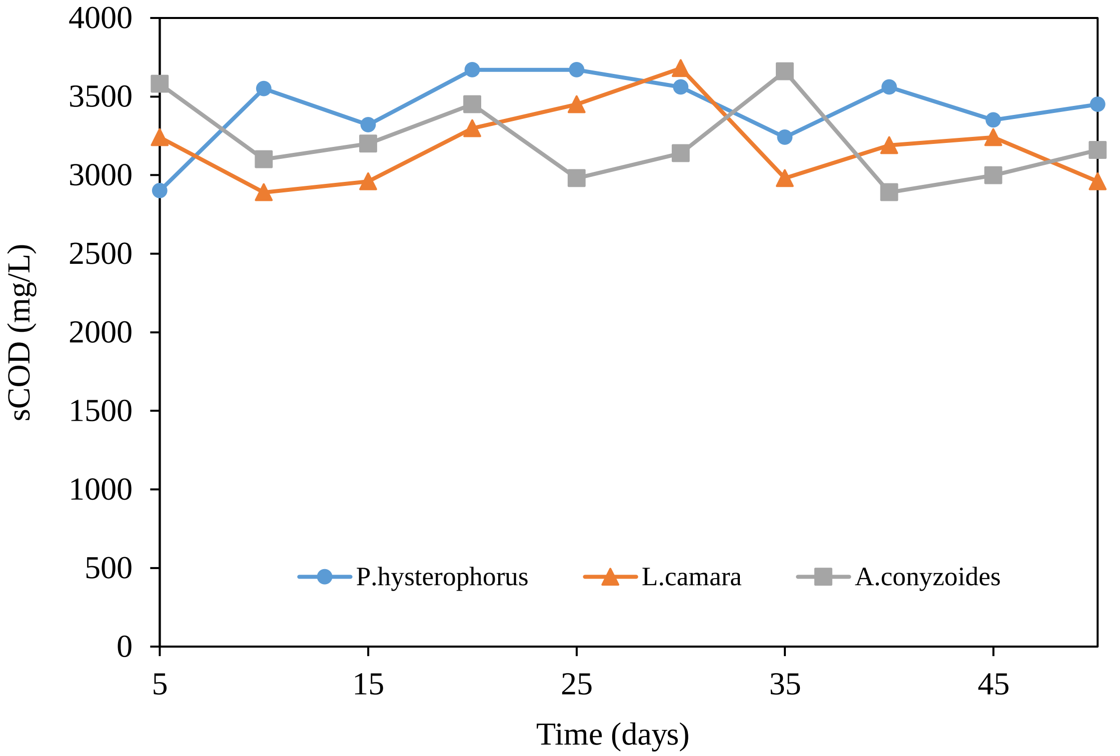


Fig. 8.5. sCOD outlet for Untreated

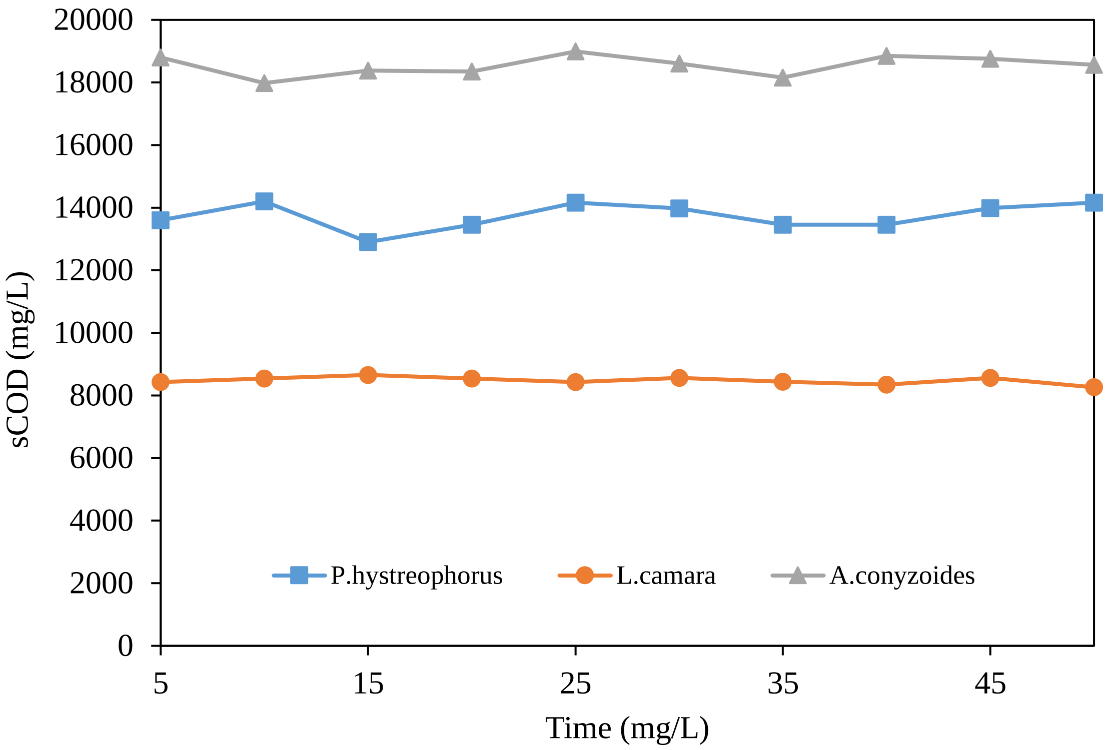


Fig. 8.6. sCOD inlet for pretreated

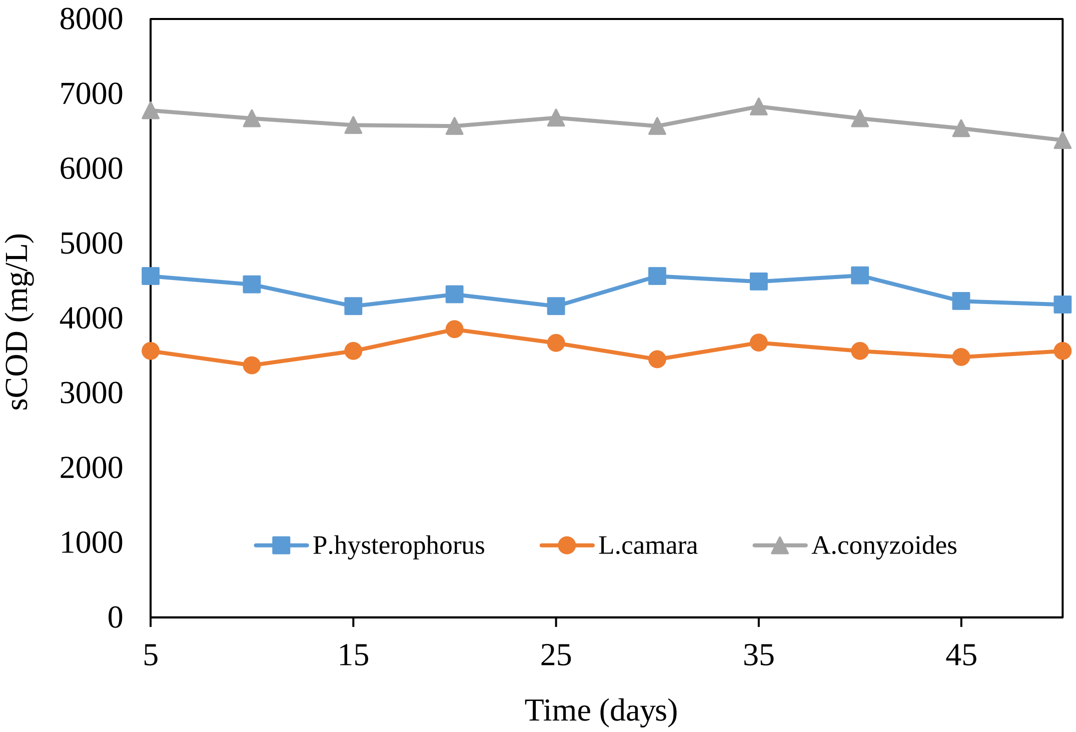


Fig. 8.7. sCOD outlet for Pretreated

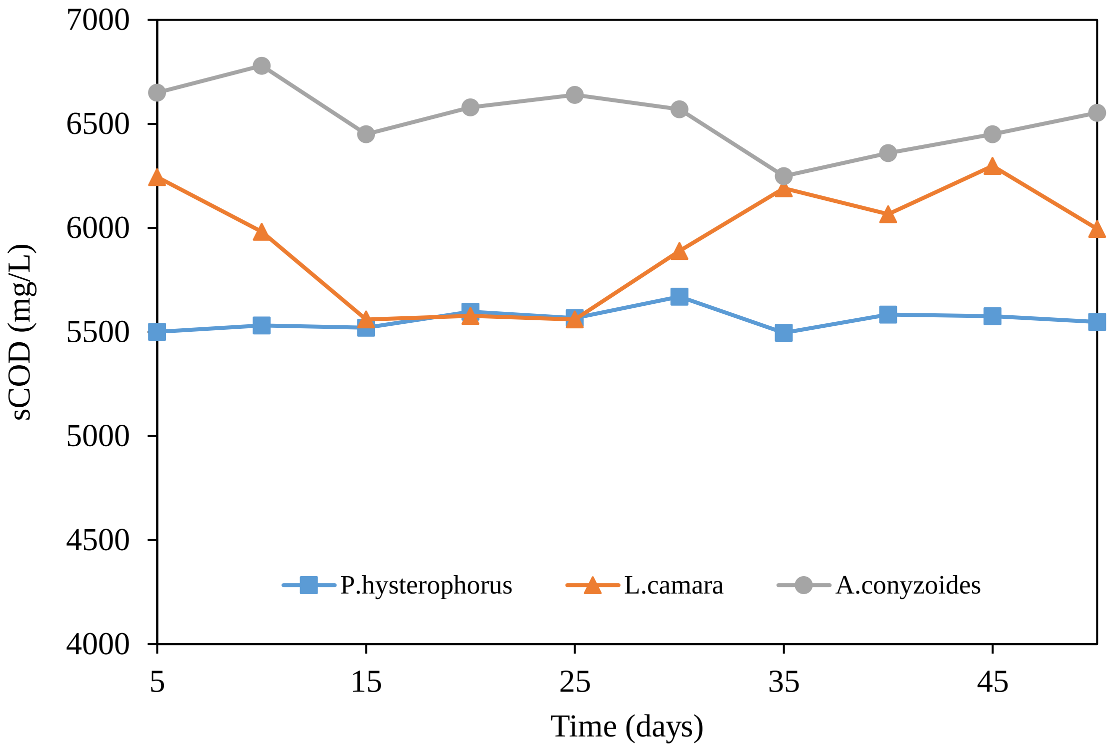


Fig. 8.8. sCOD inlet for Codigestion

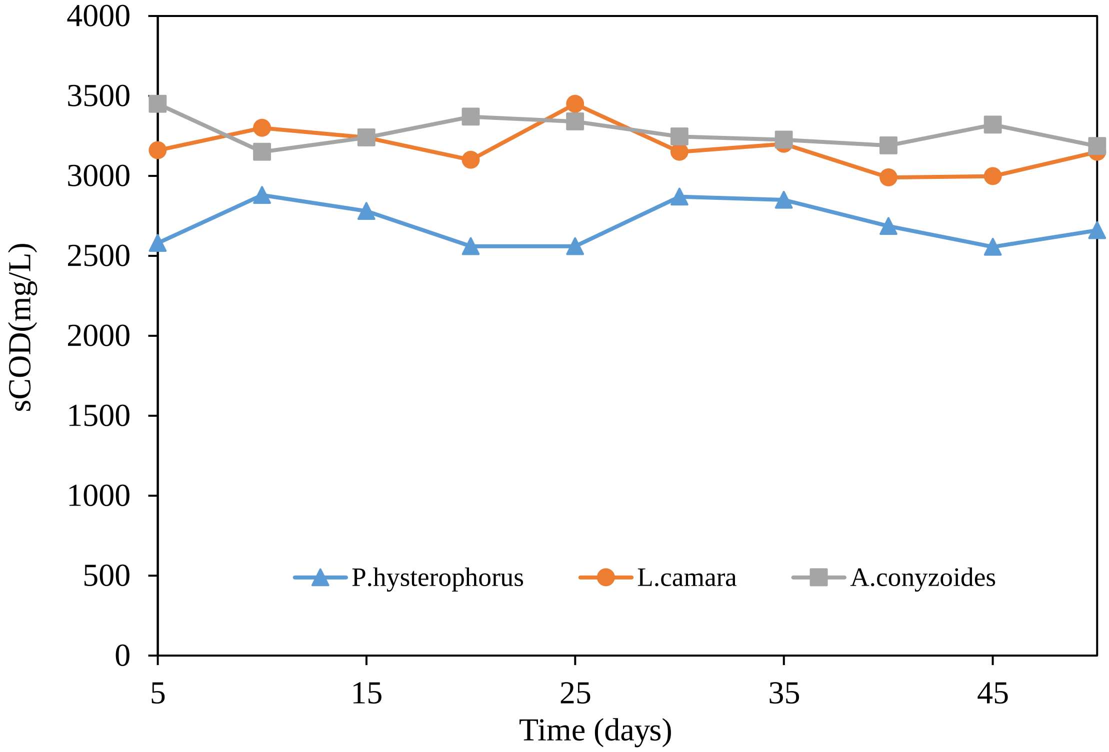


Fig. 8.9. sCOD outlet for co-digestion

8.1.3. Volatile fatty acid (VFA) study

VFA analysis was often utilized as an index process imbalance during anaerobic digestion. The inlet VFA was relatively stable throughout the continuous digestion process. The outlet VFA took a few days to stabilize as it was initially increased during the continuous reactor startup. VFA concentration, instead of hindering the biogas production, increased the biogas production as the VFA were degraded, illustrating that the higher the VFA production higher the biogas production. In this study, the VFA reduced from untreated *P.hyserophorus*, *L.camara*, and *A.conyzoides* is 21.4, 16.5 and 28.3%, respectively. In pretreated VFA reduction rate observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 35, 6, 32.7 and 40.3% respectively. In co-digestion, VFA reduction rate was observed for *P.hyserophorus*, *L.camara* and *A.conyzoides 29.8*, 28.2 and 30.4% respectively shown in fig.. 8.10, 8.11, 8.12, 8.13, 8.14 and 8.15.

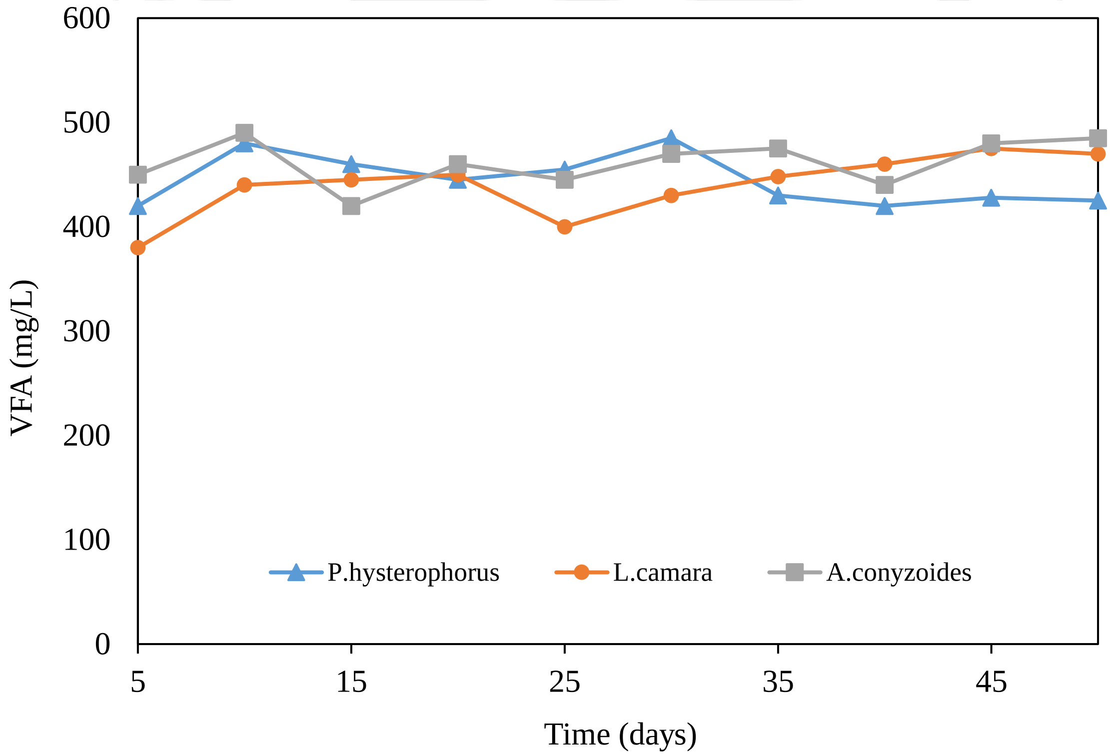


Fig. 8.10. VFA Inlet for Untreated

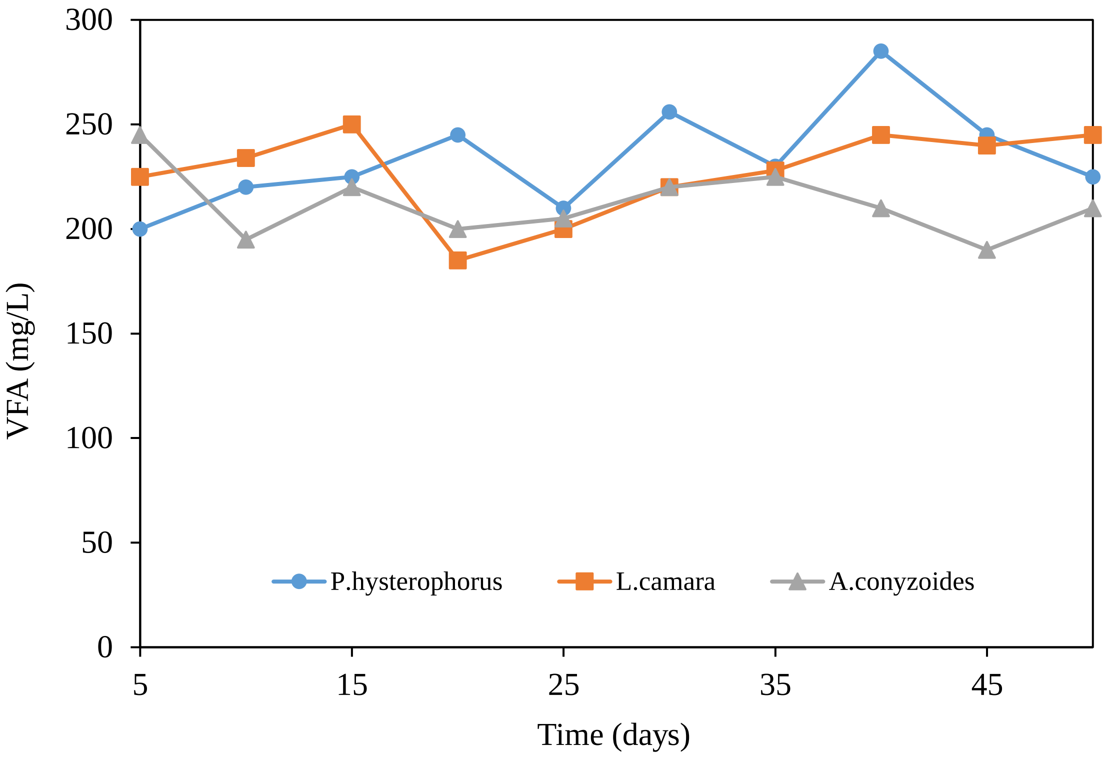


Fig. 8.11. VFA outlet for Untreated

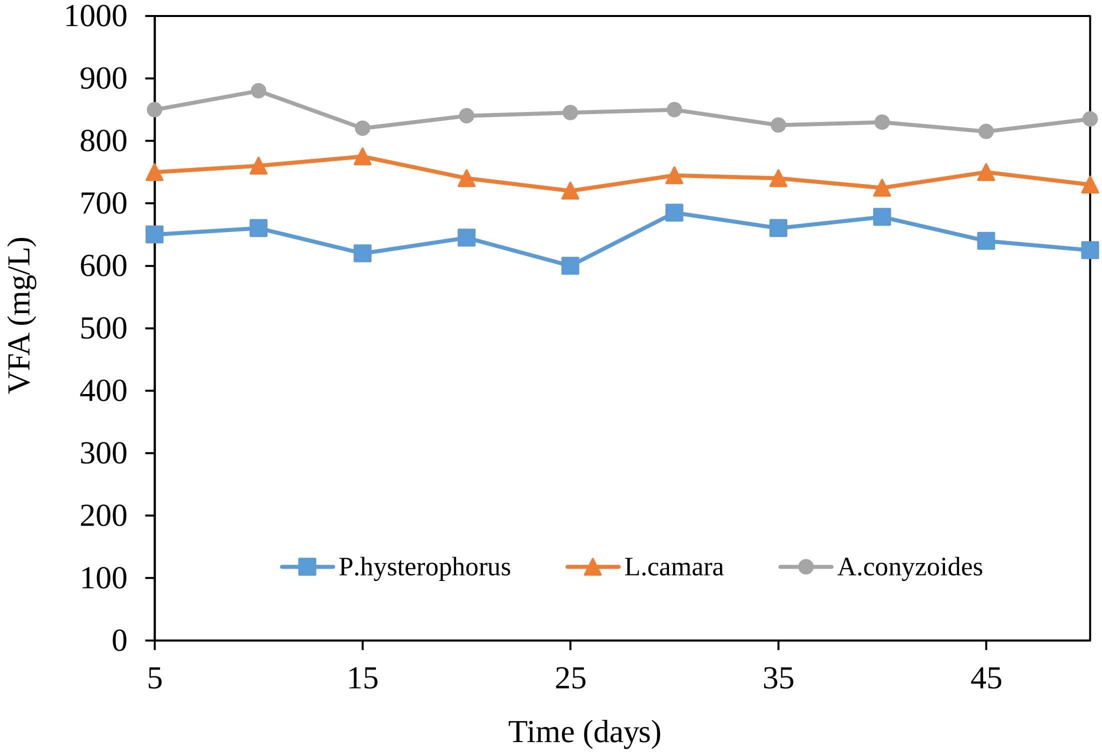


Fig. 8.12. VFA inlet for pretreated

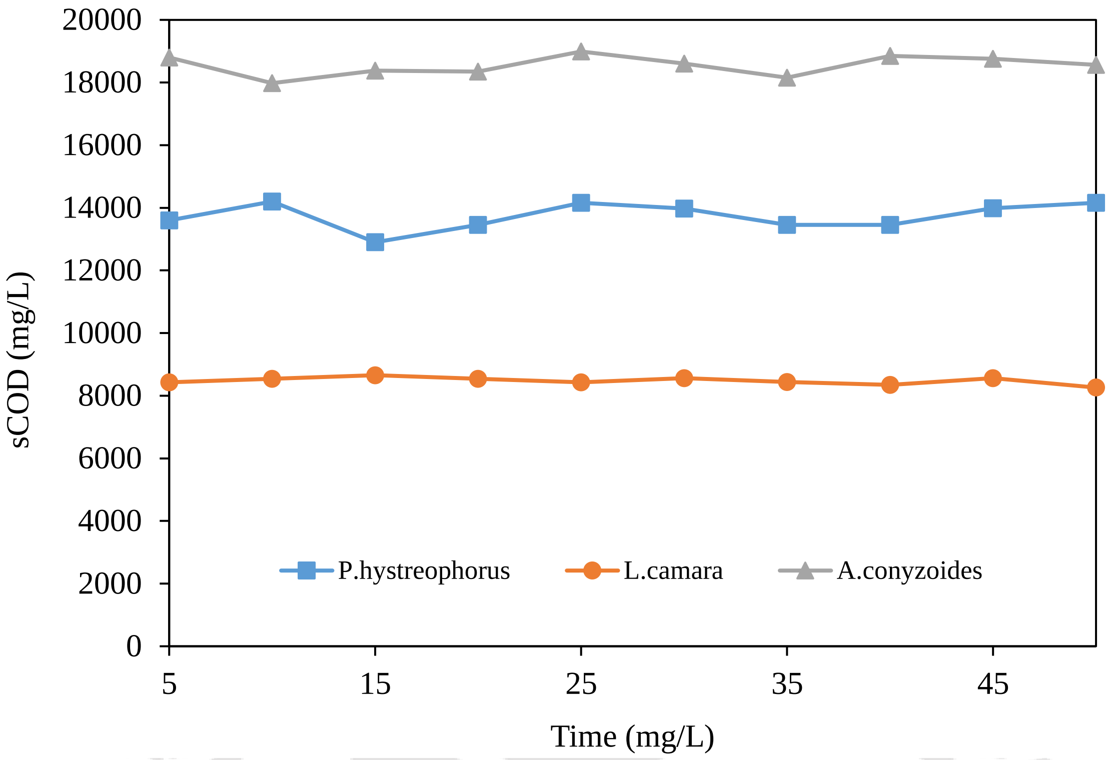


Fig. 8.13. VFA outlet for Pretreated

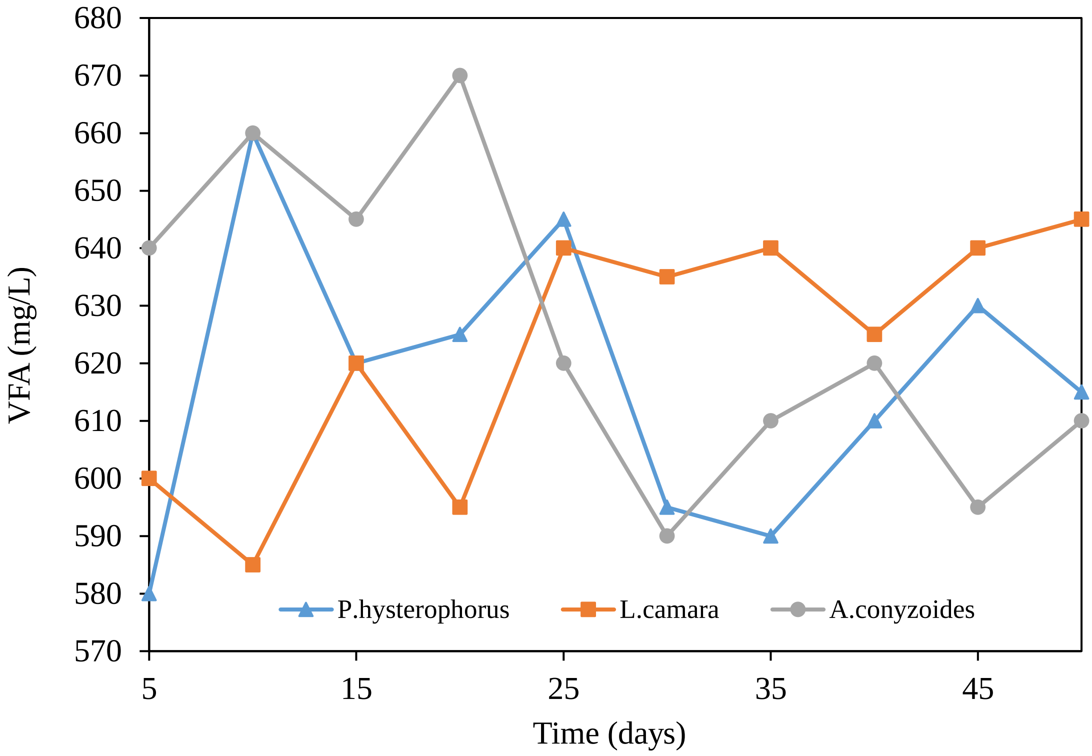


Fig. 8.14. VFA inlet for co-digestion

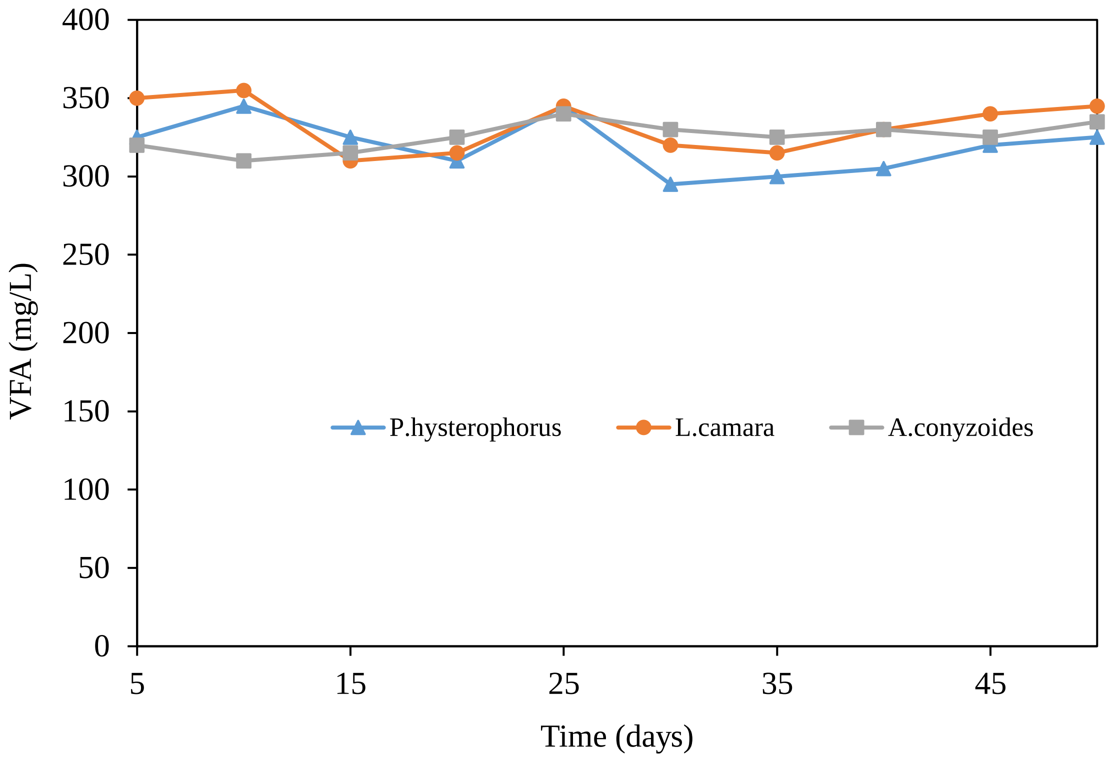


Fig. 8.15. VFA outlet for co-digestion

Table 8.3. Continous reactor study

Reference	Study/Substrate	Digester type	Result
Raynal et al. (1998)	Vegetable wastes	Two stage (2.5 L Sequential Batch Reactor and 10 L Fixed Film Packed Reactor)	87.5% COD removal
Roberts et al. (1999)	Sewage sludge	Two stage (Two CSTR of 5 and 10 L)	56% VS reduction
Rajeshwari et al. (2001)	Fruit and vegetable waste	Two stage (solid bed hydrolyser and UASB)	94% VS removal
Dinsdale et al. (2000)	Fruit/vegetable waste and waste activated sludge	Two stage (Two 5 L CSTR)	40% VS destruction and a system biogas yield of 0.37m^3 /kg VS
Bouallagui et al. (2004)	Fruit and vegetable waste	Two phase (Anaerobic Sequential Batch	96% COD removal

Continuous Reactor			
		Reactor of 1.5 and 5 L)	
Nizami and Murphy (2011)	Grass silage	Two stage (Sequentially Fed Leach Bed Reactor of 17 L and Upflow Anaerobic Sludge Blanket of 31.4 L)	11.8% increase in methane production and reduction in retention time from 42 days to 30 days
Siddiqui et al. (2011).	Food waste and sewage sludge	Two-phase (1.67 L and 5 L) provided with mechanical paddle mixer	VS removal (89%
Nasr et al. (2012)	Thin stillage	Both single and two stage	24% increase in biogas yield in the two stage process when compared to single stage anaerobic digestion
Ganesh et al. (2014)	Fruit and vegetable waste	Both single (15 L) and two (15 L and 6 L) stage. Mechanical mixers were provided in both single and two stage reactors.	97.5% VS reduction of for two stage while for single stage 82% VS removal
Fu et al. (2017)	Vinasse	Both single and two stage	Methane yield from two-stage anaerobic digestion was 10.8% higher than that of one-stage
Micolucci et al., 2018	Food waste	Both single (230 L) and two stage (200 L and 760 L; mechanical anchor agitator were provided for mixing)	Two stage removal efficiency 17% higher than single stage
Barua and Kalamdhad, (2019)	Water hyacinth (untreated, pretreated and co-digested)	Two stage (20 L Single digester with a phase separation and manual mixer)	Average COD removal of 72.5%, 82% and 77% for untreated, pretreated and co-

			digested water hyacinth respectively.
This Study	*Parthenium hysterophorous, Ageratum conyzoides,Lantana camara* (untreated, pretreated and co-digested)	Two stage (20 L Single digester with a phase separation and manual mixer)	The average COD removal rate for untreated *P.hyserophorus*, *L.camara* and *A.conyzoides* is 45.6, 41.7 and 51.3%, respectively. In pretreated sCOD removal rate observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 75.6, 74.3 and 81.1% respectively. In co-digestion sCOD removal rate was observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 68.9, 66.2 and 71.8% respectively.

8.2 Summary for phase IV

A two-stage continuous reactor was fed with different terrestrial weeds. Average biogas production for untreated *P.hysterophorus* was 3270mL, *L.camara* was 3010mL, and for *A.conyzoides was* 3150mL, for pretreated *P.hysterophorus* is 6454mL; *L.camara is* 6219 mL, and *A. conyzoides* is 6982mL, and for co digestion of *P. hysterophorus* is 5984mL; *L.camara* is 5218 and for *A.conyzoides* is 5765mL. The average COD removal rate for untreated *P.hyserophorus*, *L.camara* and *A.conyzoides* is 45.6, 41.7 and 51.3%, respectively. In pretreated sCOD removal rate observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 75.6, 74.3 and 81.1% respectively. In co-digestion sCOD removal rate was observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 68.9, 66.2 and 71.8% respectively. Average VFA reduced from untreated *P.hyserophorus*, *L.camara* and *A.conyzoides* are 21.4, 16.5 and 28.3%, respectively. In pretreated VFA reduction rate observed for *P.hyserophorus*, *L.camara* and *A.conyzoides* 35, 6, 32.7 and 40.3% respectively. In co-digestion, VFA reduction rate was observed for *P.hyserophorus*, *L.camara* and *A.conyzoides 29.8*, 28.2 and 30.4%, respectively.

CHAPTER 9

MICROBIAL STUDY

This chapter focuses on identify bacterial commodity in the anaerobic digestion process.

9.1. Phase V- Metagenomics study

The metagenomics DNA was isolated from the received anaerobic reactor samples by commercially available soil Kit (Nucleospin Soil). The qualities of the isolated metagenomic DNA sample were quantified using NanoDrop. The QC passed samples were processed for first amplicon generation followed by NGS library preparation using Nextera XT Index Kit (Illumina Inc.). The QC passed libraries were sequenced on illumina MiSeq platform using 2 x 300 bp chemistry.

9.1.1. Sample taken from the reactor and microbial study

Samples were taken from the batch reactor of *Parthenium hysterophorus* (F/M ratio 2). The first sample was taken after 7 days (Fig. 9.1), and then the second sample was taken after 14 days. The third and fourth sample was taken from 28 and 35 days. Sample name was given as stages (days) shown in Table 9.1.

Table 9.1. Sample taken

Stage 0	Stage 1 (S1)	Stage 2 (S2)	Stage 3 (S3)	Stage 4 (S4)
Cow dung (fresh)	7 days sample	14 days sample	28 days sample	35 days sample

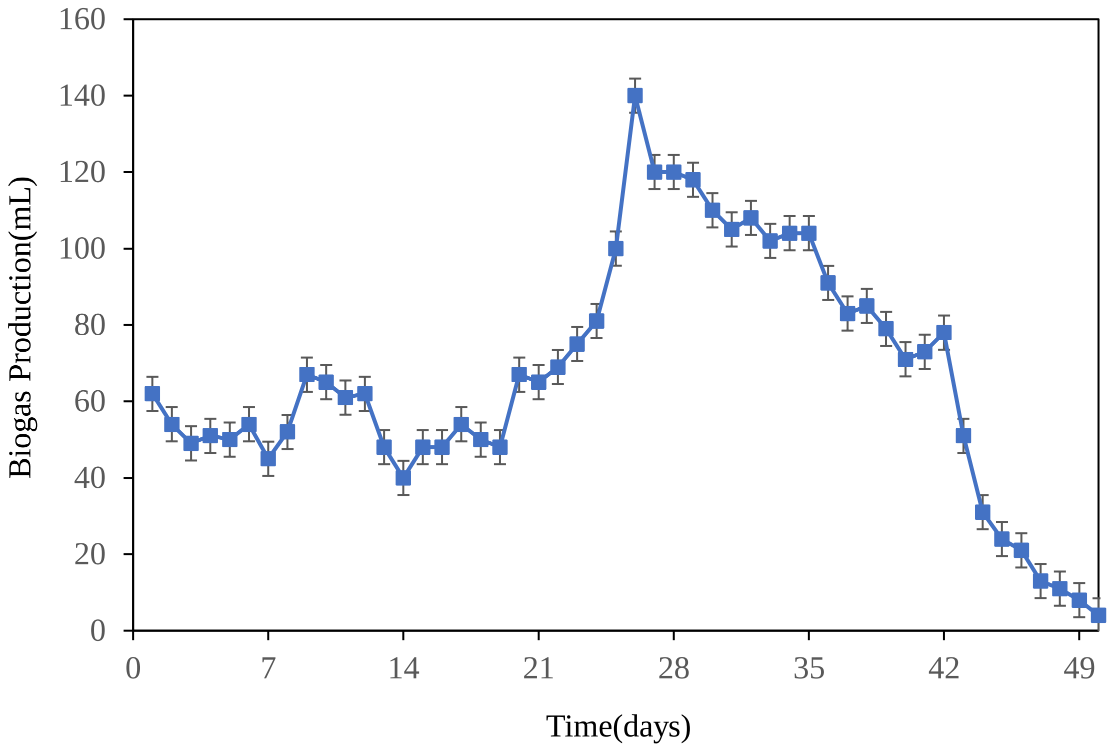


Fig. 9.1. Sampling time (days)

It was observed that Archaea bacteria play a significant role in biogas production (Table 9.2). Archaea mostly found in the stomach of the cows and buffalo. Archaea bacteria species can be co related with biogas production.increase.In Fig. 9.1and table 9.1, it was observed that biogas increase when Archaea are the major microorganisms in extreme environments (Kamekura, 1998), which are complex in the nutrient cycling and energy stream within the ecosystem (Bhattacharyya et al., 2015). Archaea are also considered a microbial source for novel bioactive, applied in medicine and the biotechnological industry (Roberts, 2005). Metagenomics results were shown in Fig. 9.2, 9.3, 9.4, 9.5 and 9.6.

Table 9.2. Most abundance microbes detected from an anaerobic reactor

Kingdom	**Phylum**	**Cow dung (%)**	**(7days) (%)**	**(14 days) (%)**	**(21 days) (%)**	**(28 days) (%)**
Archaea	Euryarchaeota	24.78	29.64	0.65	33.66	0.04
Bacteria	Bacteroidetes	18.75	19.94	44.01	20.93	23.93
Bacteria	Proteobacteria	18.66	9.13	16.85	14.55	45.88

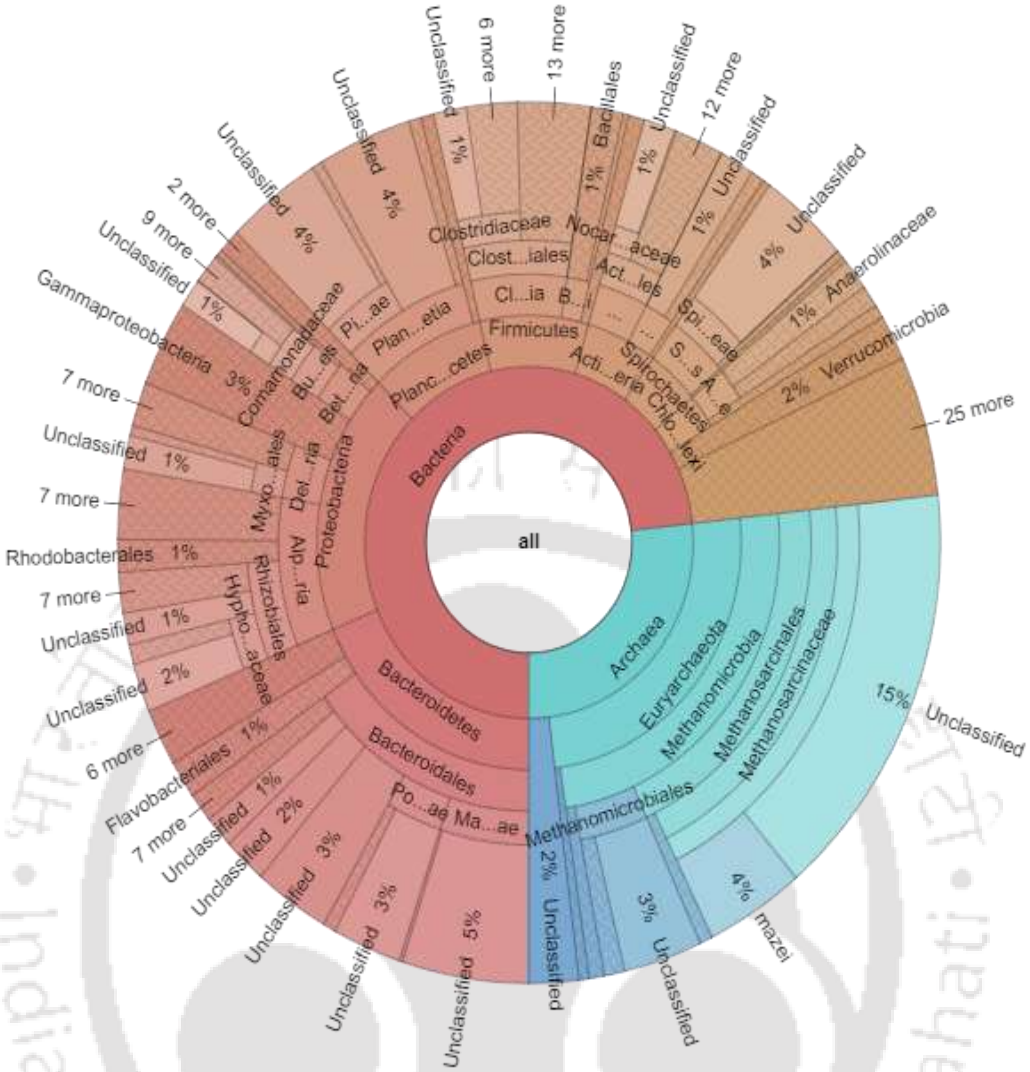


Fig. 9.2 Stage 0 (cow dung)

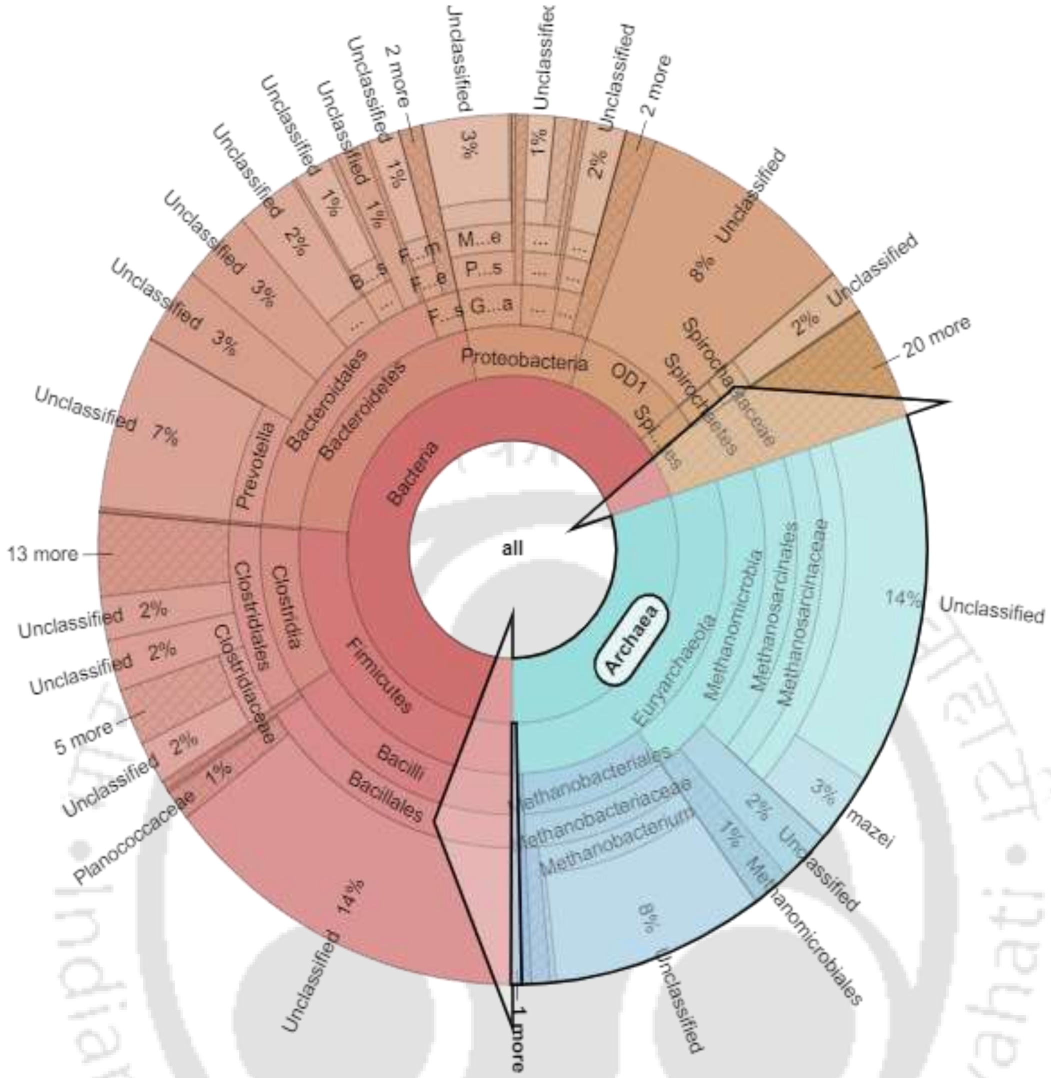


Fig. 9.3. Stage 1 (7 days)

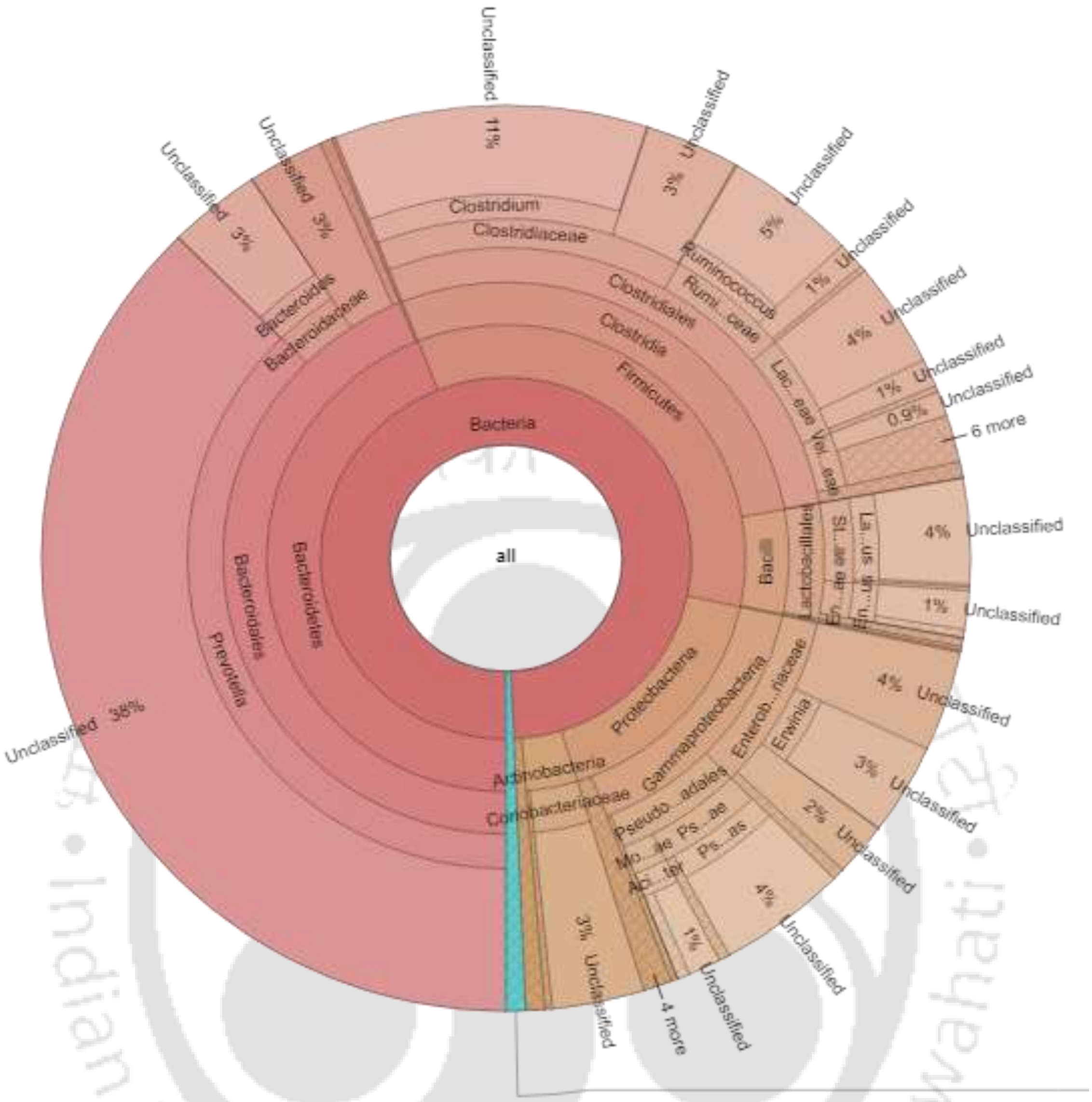


Fig. 9.4. Stage 2 (14 day)

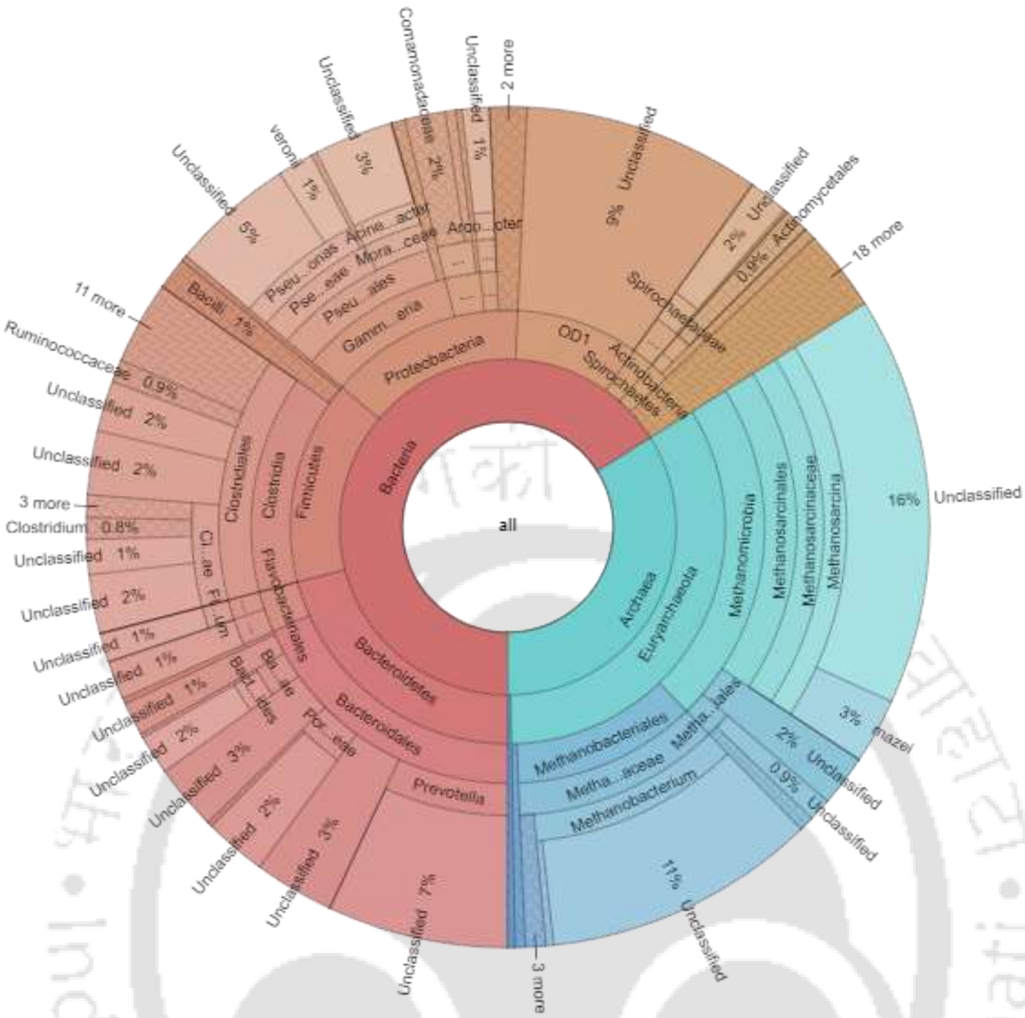


Fig. 9.5. Stage 3 (28 day)

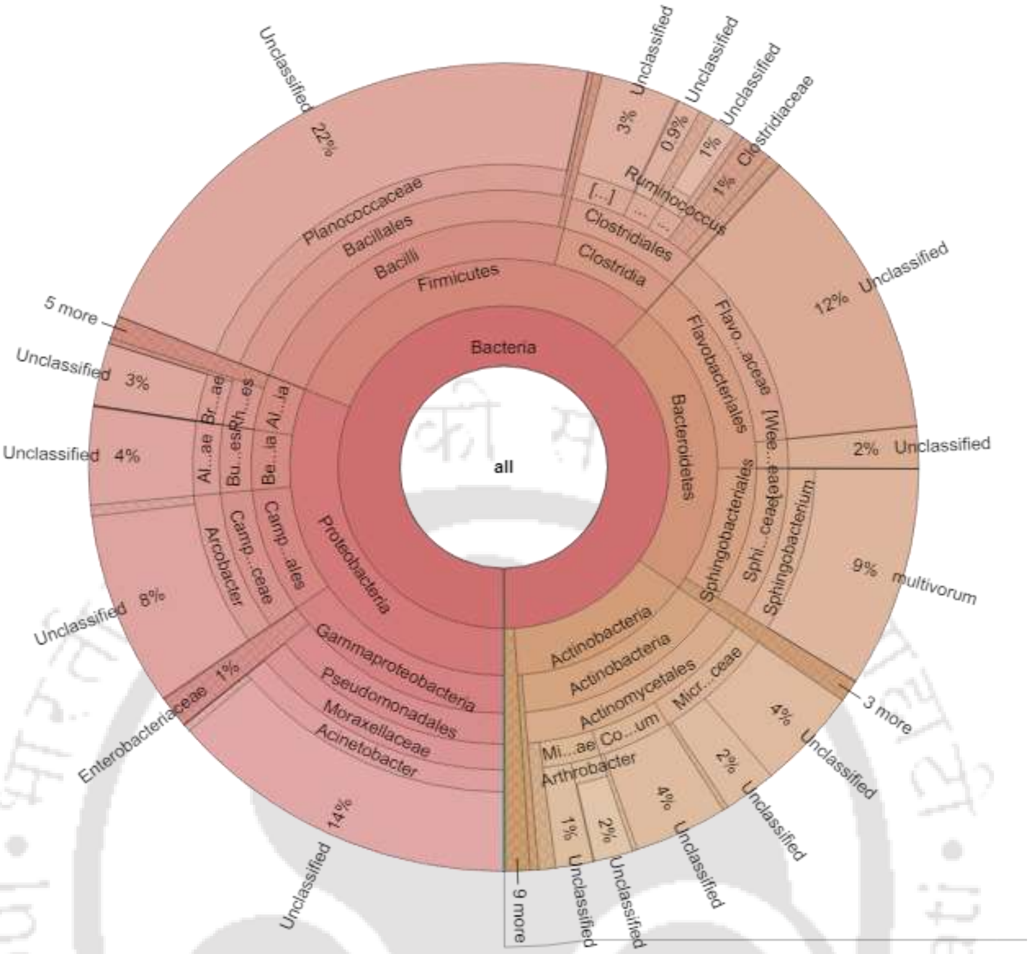


Fig. 9.6. Stage 4 (35 days)

9.2. Summary for Phase V

Their number of species were observed, but the most frequent phylum was Euryarchaeota, Bacteroidetes, and Proteobacteria. In phase V, methane production was depending on Archaea bacteria count. When Archaea bacteria count, were increased methane production also increased.

CHAPTER 10
CONCLUSION AND FUTURE SCOPE

This chapter dealt with overall conclusion of all the phases conducted with different batch study to optimized F/M ratio, different pretreatment technic, co-digestion and continuous reactor.

10.1. Overall Conclusion

Characterization of Parthenium hysterophorus, Lantana camara, and Ageratum Conyzoides, shows potential feedstock for biogas production. Initially, parameter pH was observed around the range of 6-7. It had a fair amount of moisture content, sCOD, VS, and VFA, which acts as a promising feedstock for anaerobic digestion. Terrestrial weeds had the potential biomass for the production of biogas through anaerobic digestion. In Phase I, BMP assay reveals that F/M ratio 2 shows the maximum biogas production for P.hysterophorous and 1.5 for *A.conyzoides* and *L.camara*. The highest methane production was shown in the F/M ratio 2 (140-±3 mL), Volatile Solid (VS) reduction was found about 28.40% in F/M ratio 2 followed by in the ratio 2.5 (26.03%). In a kinetics study, maximum methane production was found about 4.000 L. In the BMP study of *Lantana camara*, the highest methane production was obtained from the F/M ratio of 1.5 (195.5 ± 8 mL CH_4/g VS) and cumulative methane production from it was 4801.5mL followed by ratios 2 and 2.5 respectively. In control, methane production was relatively low as compared to all the F/M ratios (2151.5± 8 mL). 20 L capacity batch reactor was performed with a working volume of 15.5 L where substrate and cow dung were fed according to the best ratio found during biochemical methane potential trial (BMP). Maximum methane yield was observed on the 19th day (2650 ± 18 ml CH_4/g VS). Maximum volatile solids (VS) reduction was observed in the F/M ratio 1.5 (49.63%) followed by 2 and 2.5 respectively. The maximum amount of volatile fatty acid (VFA) was produced in F/M ratio 1.5 (715 ±10) and 2 (715 ± 15) followed by ratios 2.5 and 1 respectively. Maximum soluble chemical oxygen demand (sCOD) was found in F/M ratio 1.5 (8000 `mg/L). Morphological changes were captured in FESEM and XRD. In *Ageratum conyzoides*, BMP assay revealed that, F/M ratio 2 acquired maximum biogas production from the anaerobic digestion of *A. conyzoides* biomass with cow dung as the microorganism source. Highest methane production was achieved on 25th day around 205±10 mL CH4/g VS (volatile solid) and cumulative biogas production reach up to (4994 ± 25 mL). 80% of the total biogas produced was achieved within the first 30 days. The highest Volatile solid reduction was observed in F/M ratio 2 (45.30%) as compared to other ratios. Volatile fatty acid (VFA) and Soluble chemical oxygen demand (sCOD) also found to be highest in F/M ratio 2. In the kinetic study, the M value was found to be 4.0000 L CH4 in ratio 2. Due to lignin content in terrestrial weeds, further pretreatment was required for recovery more energy. In Phase II, pretreatment of terrestrial weeds accelerates the hydrolysis period, followed by enhancement of biogas production. In thermal pretreatment, hot air oven pretreatment shows bestfor *P.hysterophorous* and *A.conyzoides* and *L.camara* autoclave found to the best pretreatment based on VFA and sCOD. the thermal pretreatment of *Parthenium hysterophorus*, hot air oven showed the highest efficiency in the form of soluble chemical oxygen demand (sCOD) and volatile fatty acid (VFA). The application of thermal pretreatment increased the solubilization up to 51.5% and it was found in a hot air oven followed by a hot water bath, autoclave, and microwave. In biochemical methane potential study, cumulative methane production increased up to 25.73% when it was compared with untreated *Parthenium hysterophorus*. The highest efficiency was found after hot air oven pretreatment at 110 ˚C for 90 min. The impact of various thermal pretreatment techniques on the anaerobic digestion of *Lantana camara* viz., hot water bath, hot air oven, autoclave, and microwave was studied. Among them, the autoclave was found to be more efficient succeeded by hot water bath, hot air oven, and microwave pretreatment. Autoclave pretreatment enhanced the solubilization (sSOD) and an increment in volatile fatty acids (VFA) was observed i.e. 45.44% and 56.75% at 110°C for 80 min respectively, as compared to the control/untreated. Cumulative methane production after autoclave pretreatment had raised to 3656 mL CH4/g VS in 35 days from 2895 mL CH4/g VS in 50 days for the untreated sample. During thermal pretreatment reduced the hydrolysis period and at the same time increased biogas production as compared to the untreated *Ageratum conyzoides*. The effects of four different thermal pretreatment techniques i.e., hot air oven, microwave, autoclave, and hot water bath on the anaerobic digestion of *Ageratum conyzoides* were studied. Among them, autoclave pretreatment was found to be more efficient followed by hot water bath, hot air oven and microwave pretreatments. Autoclave pretreatment enhanced the solublisation. At the same time, increment in volatile fatty acids and soluble chemical oxygen demand was observed as 55.33% and 54.59% for autoclave at 90° C for 90 min respectively, as compared to the control/untreated. Cumulative methane production after autoclave pretreatment rose to 4053 mL CH4/g VS in 35 days from 3011 mL CH4/g VS in 50 days for the untreated sample.

In electrohydrolysis, pretreatment was also efficient for terrestrial weed in increments of sCOD, VFA, and biogas production. After electrolysis pretreatment, a drop of acid-insoluble lignin and hemicellulose were observed compared to control and rise of acid-soluble lignin and cellulose, respectively. Lignin content and weakening of the hydrogen bond increased the rate of degradation that leads to the reduction of the hydrolysis phases, which is the first phase of anaerobic digestion, and at the same time, it enhances biogas production. In energy assessment, specific energy shows output energy is more than the input energy. In Electrohydrolysis Pretreatment, at 20V for 40mins shows the highest sCOD and VFA around (17600±21mg/L and 780±24mg/L) respectively in *Parthenium hysterophorus*, which is 50.17% and 33.33%. Therefore, a biochemical methane potential trial was studied for 50days, where cumulative methane yield was observed 4242 ± 17 mL CH4/g VS after pretreatment and untreated it was observed around 3063 ± 29 mL CH4/g VS. During compositional analysis, soluble lignin and cellulose increased, whereas hemicellulose decreased. Morphological and structural changes were observed after electrohydrolysis pretreatment, in XRD high peak was reduced, the broken fragment was observed on the surface of the substrate during FESEM study and in FTIR study reduction of the peak was found in 3533.7 and 2721.22 cm^{-1} which specifies stretch of OH in lignin and CH bond of cellulose. Electrohydrolysis pretreatment proved to be efficient in the form of increasing the VFA and sCOD when it is compared with untreated *L.camara*. At 30V for 20min shows the highest sCOD and VFA around (720±3mg/L and 11328±16mg/L) which is 47.22% and 42.24% respectively. Therefore, biochemical methane potential studies were examined for 50days, where cumulative biogas production was observed 3842 ± 12 mL CH4 in 40days in pretreated *L.camara* whereas in untreated it was around 2950± 08 mL CH4 in 50days. In pretreated samples, soluble lignin and cellulose increased, whereas hemicellulose decreased. After electrohydrolysis pretreatment morphological and structural changes were observed, whereas in XRD high peak was reduced, during FESEM analysis broken fragment was observed on the surface of the substrate. After electrohydrolys pretreatment on *Ageratum conyzoides*, reduction of hydrolysis period and enhancement of biogas production was seen. This novel pretreatment also shows the efficiency in terms of increasing the volatile fatty acids (VFA) and soluble chemical oxygen demand (sCOD) as compared with untreated *A.conyzoides*/control. The highest sCOD and VFA have observed around (750±5mg/L and 9600±48mg/L) at 30V for 20min, which is 27.08% and 56.66% respectively. Therefore, biochemical methane potential set up was conducted for 50days, where cumulative biogas production was found to be 3707 ± 30 mL CH4 within 40days for pretreated and 2820± 25 mL CH_4 in 50 days for untreated. During the compositional study, soluble lignin and cellulose increased whereas hemicellulose decreased. Morphological and structural changes were captured under FESEM, XRD, and FTIR analysis after pretreatment, which proved the effectiveness of pretreatment. During FESEM, the ring-like structure was observed which might be a cellulose ring. In the XRD study, a reduction of the high peak was observed. In FTIR study, enlargement of peak after pretreatment signified the weakening of inter and intra hydrogen bond.

Furthermore, in Phase III co-digestion of *Parthenium hysterophorus*, *Lantana camara*, *Ageratum conyzoides*, and food waste were studied using Cow dung as an inoculum. A 1-liter batch BMP study observed that all three weeds with food waste co-digestion showed more efficiency than mono digestion. In phase IV, Finally, in a continuous reactor, a significant increase in biogas production was observed in pretreated biomass, opening up the field and industrial application and co-digestion compared to untreated biomass. In Phases V, a microbial community study was done, where the most frequent phylum was Euryarchaeota, Bacteroidetes, and Proteobacteria were observed. These noxious weeds, creating havoc in agricultural fields worldwide, can be controlled and utilized to create cooking fuels for their households and electricity generation, proving to be a cost-effective approach in this weed management.

10.2 Recommendations for future work

- Microbial pretreatment needs to apply to avoid energy consumption.
- Feasibility study of anaerobic co-digestion of terrestrial weeds with more than two organic wastes can be performed.
- Many other pretreatment techniques i.e., Ultra sonication, ionic liquid and ozonolysis pretreatment studies to reduce hydrolysis phase of terrestrial weeds.
- Implementation of industrial-scale biogas reactor for *Parthenium hysterophorus*, *Lantana camara and Ageratum Conyzoides.*
- Application of biogas for electricity production.

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