Potential of lignocellulosic material as Biofilm Carriers in Anaerobic Digestion Technology

Atoofa Zainab

NUST-MS Energy Systems Engineering- 00000119282

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Supervisor

Dr. Rabia Liaquat

U.S. Pakistan Center for Advanced Studies in Energy Systems (USPCAS-E)

National University of Sciences and Technology (NUST)

H-12, Islamabad 44000, Pakistan

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THESIS ACCEPTANCE CERTIFICATE

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Date: _____

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Supervisor: _____

Dr. Rabia Liaquat
USPCAS-E
NUST, Islamabad

GEC member # 1: _____

Dr. Naseem Iqbal
USPCAS-E
NUST, Islamabad

GEC member # 2: _____

Dr. Bilal Sajid
USPCAS-E
NUST, Islamabad

GEC member # 3: _____

Dr. Zeshan
IESE
NUST, Islamabad

HoD _____

Dr. Zuhair S Khan
USPCAS-E
NUST, Islamabad

Principal/ Dean _____

Dr. Zuhair S Khan
USPCAS-E
NUST, Islamabad

Abstract

Anaerobic digestion (AD) technology has become a promise manner of choice because of its application in sustainable management of industrial and agricultural wastes and high value bioenergy generation in the form of Biomethane. Various strategies have been adopted to improve the efficiency of this process and one of them is to immobilize the microbial consortia on low cost porous carrier materials to create Biofilms. This thesis investigated the technique of using low cost lignocellulosic materials like luffa sponge, coconut coir and wood chips as support materials for bacterial cell immobilization with potential benefits of improved degradation of organic matter, high biogas yield and reactor stability. Anaerobic batch mode bioreactors comprising of carrier materials for biofilms and control bioreactor were run in parallel at mesophilic temperature (35^0C) for 55 days. A combination of various techniques like chemical characterization of substrate, electron microscopy and kinetic models were opted for this study to understand the phenomena of biofilm formation and its impact on gas yield. The results depicted that introduction of porous, fibrous support can play an important role in microbial retention. In current study luffa sponge reactor provided the best performance in terms of gas yield and reactor efficiency. Furthermore, results of Scanning electron microscopy (SEM) confirmed the main cellular morphologies of methanogenic bacteria in Luffa sponge. Among the Kinetic Models logistic growth and modified Gompertz model provided the best fit with the experimental gas yield. Hence selection of immobilization support is a key design feature to achieve the high microbial density within AD reactors and should be incorporated in future design framework for anaerobic fix bed reactors. Furthermore, a part of this thesis also comprised of policy study of bio digestion technology using TIS approach to understand its diffusion dynamics and barriers that affected the widespread adoption of this renewable energy technology in Pakistan.

Keywords: Anaerobic digestion, biofilm carriers, Kinetic Model, Scanning Electron Microscopy (SEM)

Table of Contents

List of Figures

List of Tables

List of Abbreviations

LS	Luffa Sponge
CHF	Coconut Husk fiber
WC	Wood Chip
SEM	Scanning Electron Microscopy
VS	Volatile Solids
TS	Total Solids
TCOD	Total chemical oxygen demand
GM	Gompertz model
LF	Logistic Function Model
TF	Transference Function Model
VFA	Volatile Fatty acids
TOC	Total organic carbon

List of Journal/Conference Papers

1. "Investigation of lignocellulosic material as biofilm carriers for optimization of Anaerobic digestion" Journal of Waste and Biomass Valorization Publisher Springer (Impact Factor: 1.37) (First Author), Status (Submitted) *

2. "Analysis on diffusion of Bio-digestion Technology in Pakistan using Technological Innovation System (TIS) Approach" Journal of Renewable Energy Publisher Elsevier (Impact factor: 4.357) (First Author), Status (Submitted)

*Attached in Annexure-I

Chapter # 1

Introduction

One of the most indispensable need of today's civilization is Energy. It plays a central role in global prosperity. With rapid increase in world population especially in non-OECD countries, the demand for energy has also increased. To meet this demand the most convenient and affordable energy resources are exploited that are the resources derived from fossil fuels. Coal, oil and natural gas are still the dominant source of providing energy for all sectors as shown in Fig 1.1 [1]. Being dependent on these conventional energy sources resulted in drastic consequences like global warming, climate change, environmental deterioration and associated health issues. To overcome these problems, a resurgence interest has been developed from the past few decades in harnessing energy from renewable resources to meet the energy demand and to reduce the environmental degradation.

Renewable energy and reduction of greenhouse gases are now on top priority of agenda worldwide. Globally there has been keen focus on increasing its share in world energy mix. Among the clean energy resources Bioenergy has attained the special attention as it covers 10% of global energy demand. Energy provided by biomass is in two ways through traditional means and advanced conversion technologies including biofuels [2].

Traditional biomass like fuelwood charcoal are used for cooking and heating purposes especially in developing countries whereas biomass when converted through advanced technologies into gas and liquid fuels are known as biofuels [3]. Under the category of biofuel, Anaerobic digestion is one of the promising bioenergy technology and has potential provide various benefits like waste stabilization, lower energy requirements and possible energy recovery in the form of biogas.

Being a sustainable technology, this process undergoes degradation of organic matter under oxygen free environment by the activity of microbial consortia to produce biogas. Different organic wastes like municipal waste, agricultural residues, waste by-products from sugar and food industry are utilized as substrates for energy recovery. Anaerobic digestion can produce up to 60% of methane and 35% of carbon dioxide. This technology not only provide Methane as renewable energy carrier but also have an additional benefit of reducing the volume of waste material and provision of digested material as final product that can be further used in improving the crop productivity in farming [4].

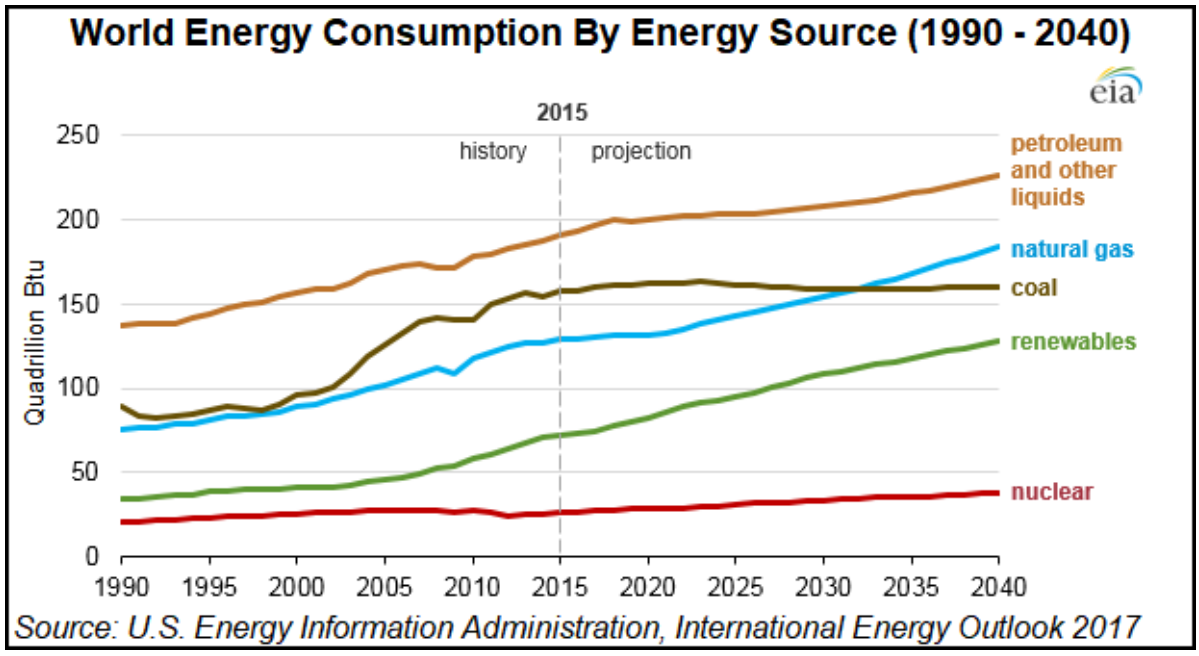

Figure 1.1: Global energy consumption by energy source

1.1 Problem Statement

Anaerobic Bioreactors are employed at industrial and lab scale for generation of methane through biochemical conversion. However, these advanced reactors are still hindered by few limitations which has thwarted its adoption on a larger scale. One of the most recurring issue that affected the operational function of anaerobic reactors is the slow growth of microorganisms and their frequent washout during shorter hydraulic retention time [5]. So, to maintain the microbial population in the bioreactor different type of strategies like anaerobic granules and usage of biofilm carriers were adopted. In anaerobic granule microbial communities are presented in the form granules with a size between 0.2 to 5mm. Diverse physiological types of microorganisms lived in close vicinity of each other resulting in high methanogenic activity [6].

Another strategy to overcome this limitation is the use suitable carrier materials that offer surface for methanogenic bacteria immobilization. By means of immobilization the methanogenic microbial population would be effectively retained in the reactor contributing to the stable methane production. This phenomenon of immobilization on support material is called biofilm formation which is the base of this undertaken research [4].

1.2 Aim and Objectives of Study

The aim of this study is to develop an understanding of anaerobic digestion process leading to biogas production using support materials for the retention of methanogenic archaea. Current study investigated the efficiency of natural materials like luffa sponge, coconut husk, wood chips as biofilm carriers in anaerobic digestion of spent wash. All these materials are cost effective and easily available with low environmental impact. It is the specific area of research which lack exploration especially usage of biofilm carriers for biomethane production. Following objectives were focused on to attain above mentioned aim;

- Study of anaerobic digestion process in batch experimental assay using lignocellulosic biomass as potential candidates for support material
- Study the impact of microbial biomass adhesion on methane production
- Determination of Biokinetic parameters for gas yield by using kinetic models.

1.3 Thesis Organization

This thesis is organized in five Chapters. Chapter 1 covers the Introduction portion of thesis. This Chapter presents the research problem statement and outlines the research objectives Chapter 2 documents the comprehensive review of the literature on the anaerobic digestion and biofilm support materials. Chapter 3 presents the Relevant Methodologies Available. Chapter 4 is about the Experimental set up and characterization techniques. Chapter 5 presents the Experimentation and kinetic modelling results and Discussion. conclusion and recommendations are present at the end of the Thesis. In the last chapter detailed information of the research work which was undertaken in Energy Policy Lab in Arizona State University is provided.

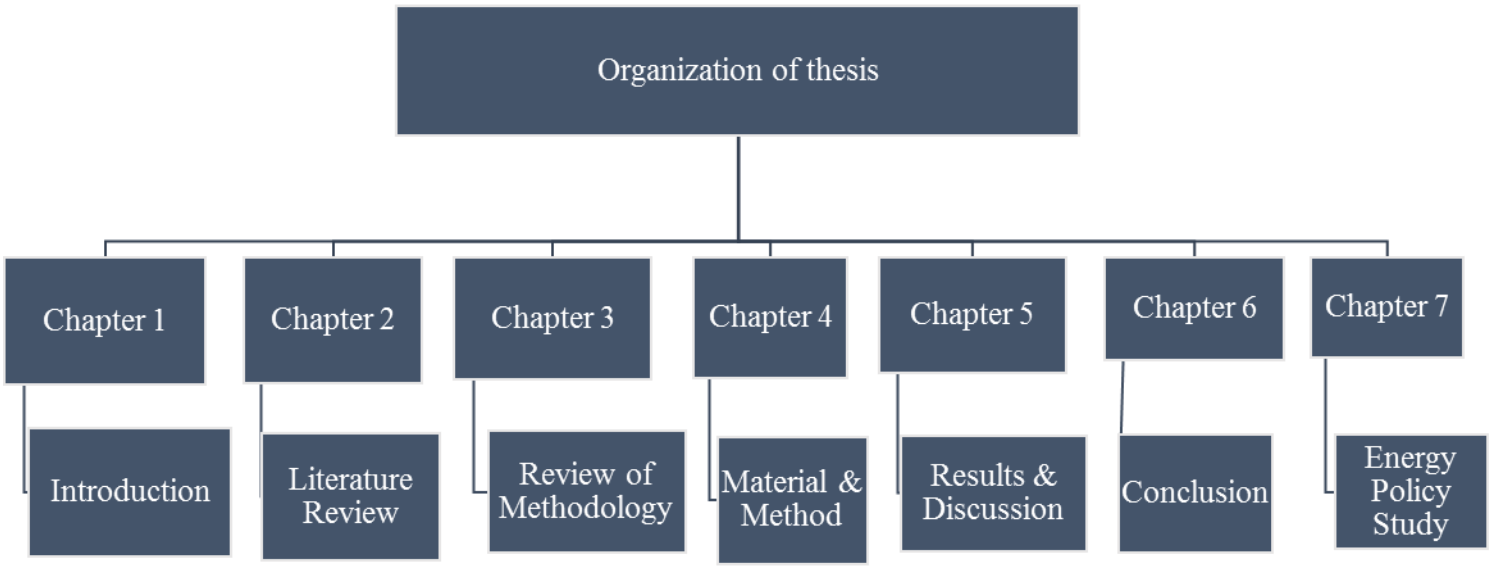

Figure 1.2: Thesis structure layout

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Summary

Energy plays a central role in global prosperity but being dependent on conventional energy sources resulted in drastic consequences like global warming, climate change, environmental deterioration and associated health issues. From the past few decades there is an increase interest in harnessing energy from renewable energy resources to meet the energy demand and to reduce the environmental degradation. Anaerobic digestion is one of the promising bioenergy technology and has potential provide various benefits like waste stabilization, lower energy requirements and possible energy recovery in the form of Methane.

Being a sustainable technology, this process undergoes degradation of organic matter under anaerobic condition by the activity microbial consortia and results in the formation of biogas. Waste sources from urban, industrial, agricultural sector are anaerobically processed to produce biogas but so far treating the high strength industrial waste water is the most practical target of this technology. On the other hand, various factors like slow growth of microorganisms, washout of microbial biomass with effluent, slow startup affects the efficiency of anaerobic bioreactors treating wastewater. So, to maintain the microbial population in the bioreactor different type of strategies like anaerobic granules and usage of biofilm carriers were adopted. Application of Biofilm carrier is another significant achievement in which microorganisms are attached to inert or natural support materials to form biofilm on the surface of support materials which ultimately results in viable, stable population of microbial consortia. This research has important applications in the field of waste to energy as it can enhance the methane production, and hence greatly improve the efficiency of anaerobic bioreactors This chapter presents the aims and objectives and thesis structure outline.

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Chapter # 2

Literature Review

2.1 Microbiology of Anaerobic digestion

Anaerobic digestion is the complex biochemical process which in the presence of strictly anaerobic conditions convert the degradable organic material in to carbon dioxide and methane [1]. This degradation and transformation is carried out by specialized consortium of anaerobic microorganisms which eventually results in energy recovery as methane production and formation of bio slurry to be used as natural fertilizer for crop productivity [2]. The science behind AD process is quite complicated because it involves key microbiological pathways and it is best comprehended if it is breakdown in 4 stages i.e. hydrolysis, acidogenesis, acetogenesis and methanogenesis [3]. These phases are interlinked with the product of one stage serve as the substrate for the bacteria of next stage [4].

2.1.1 Hydrolysis

Hydrolysis is the first step which involves the degradation of complex organic polymers like proteins, fats, lipids and carbohydrates into simple soluble organic compounds like amino acids and sugar and fatty acids. Both facultative and anaerobic bacteria exist and lived in symbiotic relationship with each other. The role of facultative bacteria is to consume dissolved oxygen from the water and make the environment favorable for anaerobic microorganisms by creating oxygen-reduction potential. This step is facilitated by hydrolytic enzymes like proteases, cellulases, lipases that breakdowns the proteins, fats and cellulose respectively. However, the time taken for polymer degradation depends on polymer type for example cellulose degradation take many days as compare to carbohydrates that's why hydrolysis period for digestion of crop residues is longer than low solid substrate [5][6].

2.1.2 Acidogenesis

Acidogenesis or acid-forming stage is second stage that is mediated by acid formers microorganisms (obligate anaerobic bacteria) to metabolize the products of hydrolysis into gaseous by products (carbon dioxide and hydrogen), alcohol and intermediate compounds (higher organic acids e.g. acetic acid, propionic acid and butyric acid) [7]. These volatile fatty acids reduce the pH of digestor because of their accumulation. Hydrogen concentration in this phase effects the type of final product for example acetate is produced at low partial pressure (<10-4 atm) and ethanol, organic acid is produced at high partial pressure (>10-4 atm) [5].

2.1.3 Acetogenesis

Acetogenesis is the third phase in which acetate bacteria (secondary fermenters) transform the undigested products of acidogenesis into acetates, carbon dioxide and hydrogen (methanogenic substrates) [8]. Methane production efficiency can be depicted from this stage because 70% of methane is produced due to reduction of acetates. Approximately 25% acetates and 11% of hydrogen is formed in this phase [9].

2.1.4 Methanogenesis:

Methanogenesis is the last and critical stage in the whole anaerobic digestion process that finally produces methane and carbon dioxide by the activity of methanogenic bacteria. Two groups of bacteria are involved in this stage. First group consists of *Methanosaeta* and *Methanosarcina* which are responsible for75% methane production as they transform acetic acid to methane and carbon dioxide. Second group comprises of hydrogen utilizing methanogens because they used hydrogen as an electron giver to reduce carbon dioxide to methane. Different species like *Methanococcus vanniellli, M. formicium, Methanobacterium omelianski* belongs to this category [8].

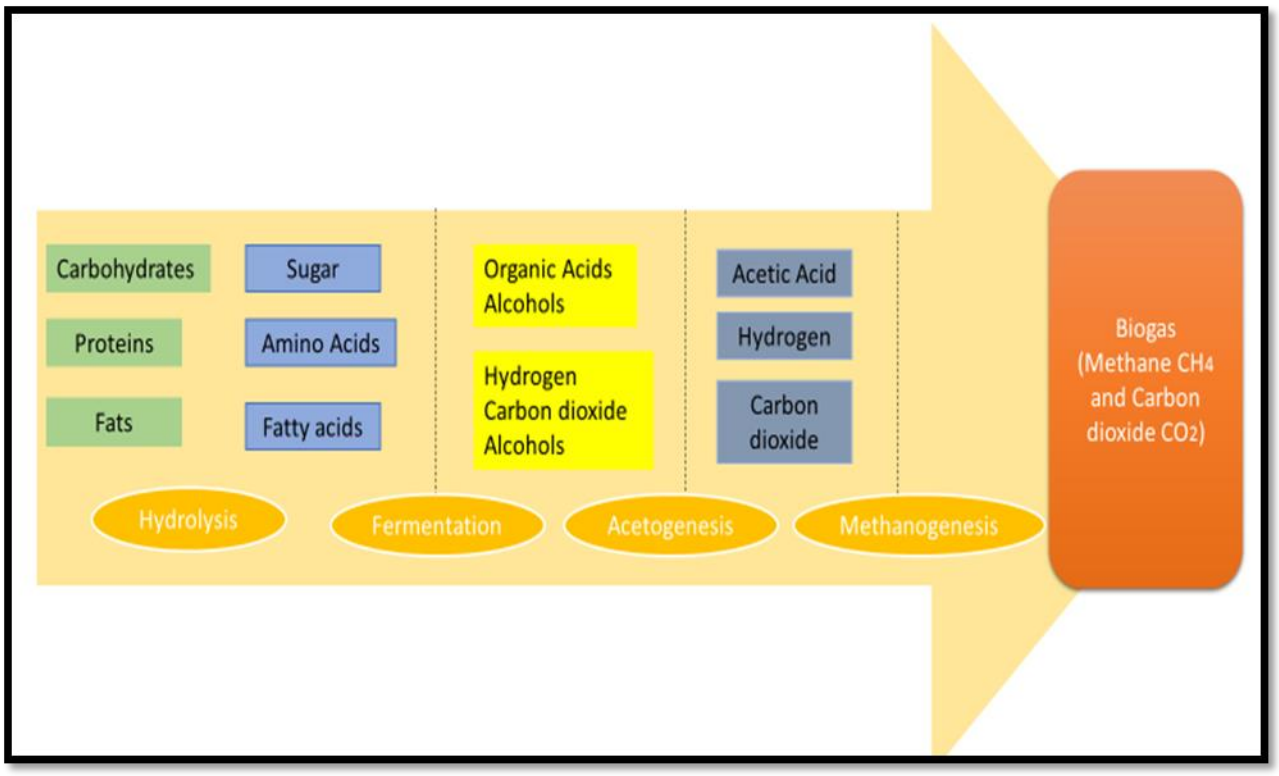

Figure 2.1: Overview of main conversion processes in Anaerobic Digestion [10]

2.2 Factors Affecting the Anaerobic Digestion

There are multiple important parameters that need to be controlled for optimization of anaerobic digestion process. These parameters should be in optimum ranges to ensure the maximum gas production and process stability. These parameters are briefly described below as stated in different literatures.

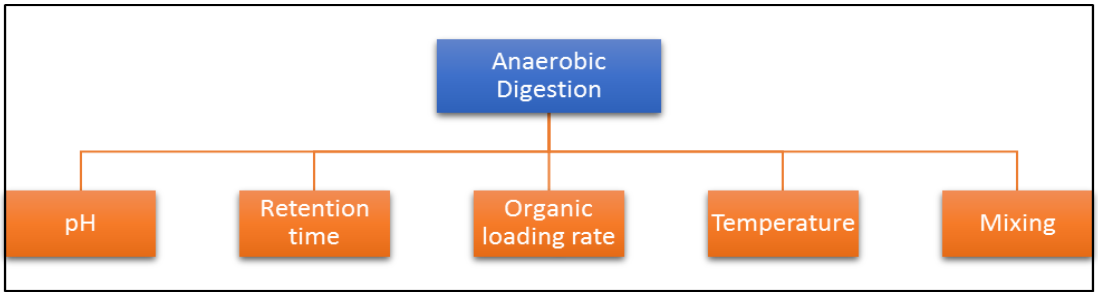

Figure 2.2: Anaerobic Digestion process parameters

2.2.1 pH

pH is a key parameter that influences the microorganisms' growth during anaerobic digestion. Methanogenic bacteria are very sensitive to pH variations and function optimally within the range of 6.8-7.4 as shown in Table 1. pH value outside of this range restrains the anaerobic digestion process by reducing the methane generation [11].

2.2.2 Retention time

This parameter refers to average amount of time the organic substrate resides inside the AD reactor. Shorter retention is more favorable as it corresponds to process efficiency and reduces the capital cost. Generally, digestion of lignocellulose waste takes longer retention time as compared to low solids waste/ wastewater [12].

2.2.3 Temperature

Temperature is another most important factor that effects the performance of anaerobic digestors as it plays the vital role in survival of microbial consortia. Generally anaerobic consortia are active at two temperature ranges i.e. 25-40°C (Mesophilic temperature) and 40-60°C (thermophilic temperature). The thermophilic temperature speeds up the biochemical processes, causing high production of methane but thermophilic bacteria cannot tolerate small changes in environment, also it is an energy intensive process cannot be favorable to employed at commercial level. Comparatively mesophilic temperature is ideal for anaerobic digestion as it more stable and does not require energy input [13].

2.2.4 Organic Loading rate

Organic loading rate signifies the biological conversion capacity of an anaerobic fermentation process. It is defined as a amount of organic matter that is daily fed per m3 of volume of digestor. High OLR can lead to VFAs accumulation causing system failure whereas low OLR corresponds to inefficient system. OLR is a vital operational parameter in continuous mode anaerobic digestion [14].

2.2.5 Agitation/Mixing:

Anaerobic digestion also relies on mixing/agitation to evenly distribute the temperature, nutrients and to increase the contact time between the microbial consortia and substrate material. Mixing can be done by means of propellers (mechanical mixing) or pumps (hydraulic mixing) [15].

Table 2.1: Optimum ranges of anaerobic process parameters [16]

Parameter	**Unit**	**Optimum range**
Temperature	°C	32-37 (Mesophilic) 50-60 (Thermophilic)
pH	-	6.8-7.4
Oxidation Reduction Potential	mV	-520 to -530
C: N ratio	-	25:1
Hydraulic Retention Time	Days	12-18
Volatile fatty acids	mg/L	50-500
Alkalinity	mg/L	1300-3000
Organic Loading rate	kg VS m-3d-1	0.8-2.0

2.3 Methane Forming Bacteria

Methanogens/ Methanogenic bacteria are present in those habitats that contain high concentration of degradable organic matter. They are free living, strictly anaerobe survive in the absence of oxygen. They have peculiar characteristics which are not present in other prokaryotic or eukaryotic cell like 1) their cell wall is rigid in nature 2) produce methane when undergoes material degradation 3) Factor f430, f420 and nickel containing specialized coenzymes [17].

2.3.1 Coenzymes and Factor f430 and f420

Only methanogenic bacteria have these unique coenzymes and factors that are instilled in enzymes to function more efficiently. Coenzyme M convert carbon dioxide to

methane and nickel containing coenzymes f420 and f430 are known as hydrogen carriers in methanogenic bacteria [17].

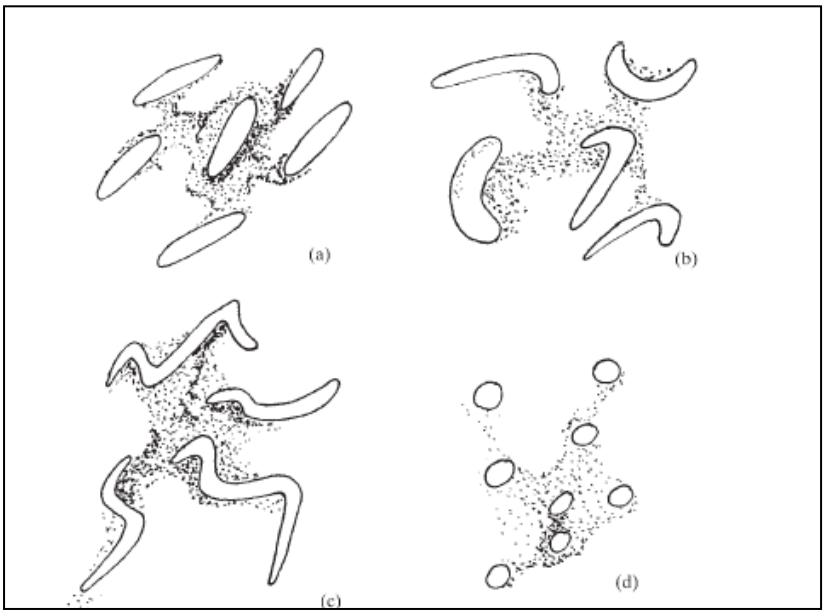

Figure 2.3: Classification of methanogenic bacteria based on morphology (a) rod (b) curved rod (c) spiral (d) spherical

2.4 Principal Groups of Methanogenic Bacteria

Methanogenic bacteria have three major groups that is (1) Hydrogenotrophic Methanogen (2) Acetotrophic methanogens (3) Methylotrophic Methanogens. All these groups have their specific function that collectively create optimize environment for methane production.

Group 1 Hydrogenotrophic Methanogens

This group uses hydrogen as an electron acceptor to produce methane from carbon dioxide as shown in equation 2.1. As carbon dioxide reduced to methane the partial pressure of hydrogen in anaerobic reactor got reduced. AD process stability is largely dependent on hydrogen partial pressure therefore these methanogens must be fully functional for maintaining process efficiency [18].

$$CO_2 + 4H_2 \rightarrow CH_4 + 2H_2O \qquad (2.1)$$

Group 2 Acetotrophic methanogens

The working mechanism of this group is to breakdown acetate as a substrate into carbon dioxide and methane. Acetate is the most important substrate as more than 70% methane comes from it conversion. Their reproduction rate is slower as compared to hydrogenotrophic methanogens and for their activity the partial pressure of hydrogen should be lower. Carbon monoxide is also reduced by this group to form methane [17].

$$4CH_3COOH \rightarrow 4CO_2 + 2H_2 \quad (2.2)$$

$$4CO + 2H_2O \rightarrow CH_4 + 3CO_2 \quad (2.3)$$

Group 3 Methylotrophic Methanogens

These bacteria use substrates that have methyl group for example methanol (CH3OH) and methylamines [(CH3)3-N] to produce methane [17].

$$3CH_3OH + 6H \rightarrow 3CH_4 + 3H_2O \quad (2.4)$$

$$4(CH_3)_3 - N + 6H_{2O} \rightarrow 9CH_4 + 3CO_2 + 4NH_3 \quad (2.5)$$

2.5 Biofilm Formation

Biofilms are dense assemblages of microbial cells, adhesive to support surfaces and enclosed in an extracellular polymer matrix (EPS) and works as cooperative microbial consortium [19]. EPS is normally composed of proteins, nucleic acids and polysaccharides and its purpose is to provide protection from shock loads and toxicity [20].

2.6 Events of Biofilm Production

Biofilm Formation involves the following stages as shown in fig 4 [21];

1. Organic macromolecules are transported and adsorbed on the surface
2. Transfer of bacteria to the surface by means of chemotaxis or twitching motility.
3. Adhesion of microorganisms on surface by means of weak Vander wall forces
4. Growth of biofilm resulted from bacterial growth and EPS production
5. Biofilm Detachment

In most cases Biofilm is known as a nuisance as it affects the scenic view of lakes, fountains but also it causes the biofouling, a typical problem faced by food industry. Biofilm are also considered as a source of corrosion of medical devices like heart valves, dialysis catheters etc. [19]. But it does not have only negative effects as it can be of useful application in remediation of wastewater by degrading the contaminants and to retain the microbial consortia inside the Anaerobic bioreactors [22].

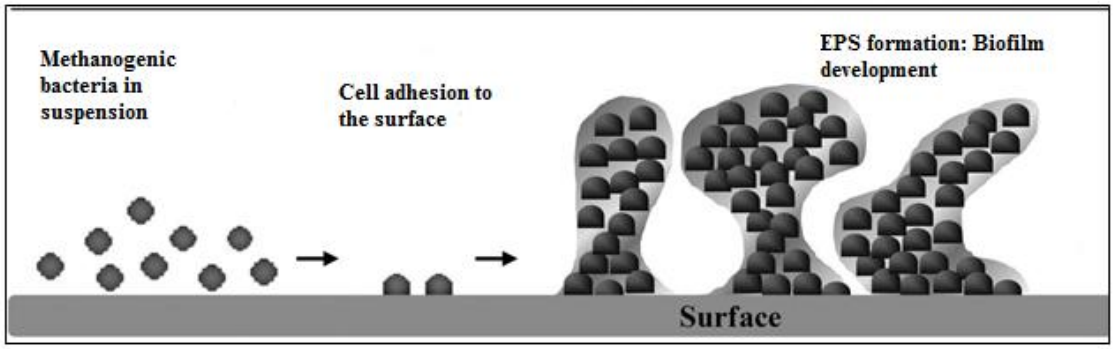

Figure 2.4: Biofilm formation mechanism

2.7 Role of Biofilm in Anaerobic Digestion

Methanogenic biofilms are used to increase the performance efficiency of anaerobic digestion (AD) reactors by keeping the microorganisms attached to inert or natural support materials to form biofilm which ultimately results in viable, stable population of microbial consortia inside the reactors [23]. Biofilm carriers addresses the limiting factors of AD reactors like slow start up, short duration of biogas production, sensitivity to high organic loading and low mass transfer between substrate and microbial population [24]. By operating reactor at shorter retention time microbial washout occurs, to prevent this various support materials are used to immobilized microorganisms to generate surface biofilm. The advantages of biofilm are that it retains bacteria for longer period eventually resulting in higher microbial density and higher methane production [25].

2.8 Physio-chemical Properties governing initial bacterial adhesion

Biofilm formation is very much dependent on the type of the support material selected. There are various properties that affects the degree of microbial adhesion to the substrate which are as follows;

Porosity and Coarse surface

Highly porous and roughness of substrate is of great importance for a stable biofilm formation. Porous surfaces create hydrodynamically quiescent environment thus preventing the chances of immobilized cells detachment. Surface roughness at nanoscale and microscale improves the microbial adhesion to support materials as it offers more surface area for attachment [26]. Porous surfaces also lead to reduce start up period of anaerobic reactors [27].

Hydrophobicity

Hydrophobic surfaces enhance microbial attachment to the surface in aqueous environment by displacing water molecules from microbe surface interface. Syntrophic and methanogenic bacteria have somewhat hydrophobic surfaces, so they will have a good adhesion with hydrophobic surfaces as compare to hydrophilic surfaces [28].

Surface charge

Surface charge also plays the important role in initiating the bacterial adhesion. Microbial surface has a negative charge and when it approaches the carrier of similar charge a strong repulsive electrostatic force is created which must be overcome to promote adhesion. carrier material with positive charge is considered favorable as it creates the less repulsive force and more efficiency [29].

2.9 Anaerobic fixed film reactors

It is a type of anaerobic bioreactor that comprised of packing media with the purpose of providing large surface area for bacterial growth. When influent comes in contact with the media, anaerobic microbes make themselves attached to the packing material thus creating biofilm. These reactors can be run at shorter hydraulic retention times as bacteria are already adapted to the support media and already active in converting organic matter to methane [30]. These reactors belonged from the category of the

advanced reactors like fluidized bed, UASB and up flow anaerobic filters [31]. Comparatively, these bioreactors have more advantages to the conventional units as they can work efficiently at higher organic loads and effectively cope with toxic inputs and organic shock loads. The main reason is the effective retention of the microbial biomass inside the reactor [32].

Summary

Methanogenesis in anaerobic digestion process is the most sensitive stage because of the slow growth of methanogenic bacteria. These bacteria can be efficiently retained in the reactor by making their immobilization possible on porous fibrous support materials. In this way the chances of microbial washout from the reactor can be prevented and high density can contribute to the improved methane yield. The support materials responsible for biofilm formation should be low cost, of coarse texture and economical in nature. Using microbial adhesion, the anaerobic reactors can be operated at shorter hydraulic retention time with the potential benefit of providing high methane yield.

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Chapter # 3

Review of Methodology

This chapter includes an overview of the experimentation, characterization and testing methods and techniques used or followed in this research work.

3.1 Biofilm Characterization

The Characterization of Biofilm can be performed by Microscopy as well as Molecular Techniques. Details of which are given below

3.1.1 Microscopy Techniques

Microscopy techniques for Biofilm characterization involves the use of Scanning electron microscopy (SEM) and Transmission Electron Microscopy (TEM). SEM is useful to study and identify the morphological structure of bacteria. Prior to microscopic analysis the microbial surface must be conductive for secondary electrons to pass through. In this regard proper sample protocol must be followed including chemical fixation, dehydration and coating/sputtering by conductive Material. Dehydration can be done by using critical point dryer or lyophilization [1].

Transmission Electron Microscopy (TEM) generates different type of image based on the principle of electron beam passes through the bacterial specimen and create the shadow image. This shadow like image is formed on fluorescent screen and then it is photographed [2]. For TEM analysis Bacterial specimen need to be negatively stained [1].

3.1.2 Molecular Techniques

Molecular Techniques are useful to characterize the microbial populations by identify their genetic makeup. 16s RNA analysis, denaturing gradient gel electrophoresis (DGGE), Fluorescence in situ Hybridization (FISH) technique comes under this

category. Various researchers have combined these techniques with microscopy to get information regarding morphology, function and genetic structure of biofilms [3].

3.2 Substrate Characterization

Substrate Characterization mainly includes the chemical analysis of organic substrate. Parameter like volatile fatty acids is characterized either by High Performance Liquid Chromatography (HPLC) or Gas Chromatography using FID detector. Both equipment are very helpful in quantifying the individual short chain fatty acids but before analysis sample preparation is required [4][5]. Apart from these methods, volatile fatty acids can also be determine using titration procedure, but it has one major drawback that it cannot quantify the individual acid rather it tells the quantity of total volatile fatty acids. Nordmann method is generally used as titration procedure [7].

For the determination of total organic carbon (TOC), two types of methods are used loss on ignition method which is the simplest and cheapest way of determination. Second method to quantify organic carbon is by TOC analyzer [7]. Chemical oxygen Demand (COD) is either determined by close reflux method or open reflux method. Both methods required the use of reagents and block digestor for reflux of COD vials. [8]

3.3 Gas Characterization

Biogas composition that is methane and carbon dioxide content is measured by Gas Chromatograph (GC) using TCD detector. It provides the accurate composition of biogas. Biogas analyzer can also be used for this purpose and as compared to GC it is portable in nature. Volume measurement can be done using water displacement method or plunger displacement method involving the use of glass syringes [9] [10].

Summary

This chapter presents a review of methods that have been used by various researchers during their study of anaerobic digestion. For the analysis of biofilms on carrier materials diverse nature of techniques are adopted like microscopic analysis using SEM, TEM and molecular analysis including FISH and 16s RNA analysis. Parameters for substrate characterization can be analyzed using simple titration procedures or highly advanced equipment but the former ones are more cost effective. For example, to quantify the volatile organic acids Nordmaan method is adopted by performing titration but to identify the different types of fatty acids HPLC equipment is the preferred one. Gas characterization can also be done by water displacement method, plunger displacement method or using highly advanced equipment known as Gas chromatograph (GC).

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Chapter # 4

Experimentation & Kinetic Modeling

4.1 Collection of Materials

4.1.1 Biofilm Support Materials

In this Study three kind of natural waste materials loofa sponge (luffa cylindrica), coconut husk fiber (Coir) and wood chips were applied as support media for biofilm formation. All these materials are considered as low-cost materials and easily available. All these materials are selected because of their porous microstructure suitable for retention of microorganisms. Prior to their use they were cut down to average size of 2-3 inches and were rinsed with distilled water to remove attached impurities, air dried and then were used in BMP setup.

Table 4.1: Properties of lignocellulosic support materials [6]

Biofilm Support Material	Cellulose	Hemicellulose	Lignin	Shape
Luffa Sponge	63%	20.88%	11.69%	cylindrical
Coconut husk Fiber	24.70%	12.26%	40.10%	cylindrical
Wood Chip	42%	27%	28.3%	chip

4.1.2 Substrate and Inoculum

The Spent wash used as substrate was obtained from a Sugar industry (Noon Sugar Mills, Bhalwal), transported to Biofuel Laboratory of USPCAS-E and was stored in airtight plastic bottles at 4^{o}C, whereas inoculum was acquired from an active mesophilic bio digester treating cow manure to produce biogas. The inoculum was stored in airtight 5L plastic bottle with anaerobic headspace for degradation of easily degradable organic matter still present in the inoculum.

Table 4.2: Physiochemical characteristics of substrate and inoculum used

Parameter	Spent wash (Substrate)	Inoculum
pH	8.12	6.6
Total Solids (%)	4.37	9.37
Volatile Solids (%TS)	33.33	60.7
Moisture Content (%)	95.62	90.6
TCOD (mg/L)	50,752	-
VS/TS	7.62	6.47

4.2 Experimental set-up

The Biochemical methane potential (BMP) assay was adopted from [1]. Batch type fermentation test were carried out in 300 mL glass bottles and were sealed airtight with silicon stoppers and scotch tape. The packing and working volume were determined to be as 180 ml and 250 ml. The packing volume (volume to be occupied by carrier materials) was determined by marking a horizontal line over the glass, previously filled by 180 ml water. Then bottles were filled with water up to the 250 ml which represents the working volume. Working volume was consisted of immobilization material, substrate and inoculum volume. Dried and Empty bottles were filled with carrier materials (luffa Sponge, coconut coir, wood chips) up to packing volume of 180mL. After that substrate and inoculum were added in the ratio of 2:1 i.e. (40ml:20ml). Since these carrier materials made the different packing tendencies and working volume was already fixed so additional water was added to make it up to 250ml. In the end pH was checked and adjusted to 7-7.5 by adding some drops of HCL 10 M solution, after that the bottles were purged with pure Nitrogen gas (5 minutes each) to maintain anaerobic conditions and sealed tight with silicon stopper. For the collection of Biogas, the bottles were inserted with gas tight syringes of 20mL. Incubation of bottles was carried out at mesophilic conditions (35oC) which is the optimal temperature for the growth of mesophilic bacteria. For the comparative analysis control reactor was also run in parallel with out the biofilm carrier. All the bottles were shacked manually once a day. experiments were performed in triplicate to improved accuracy.

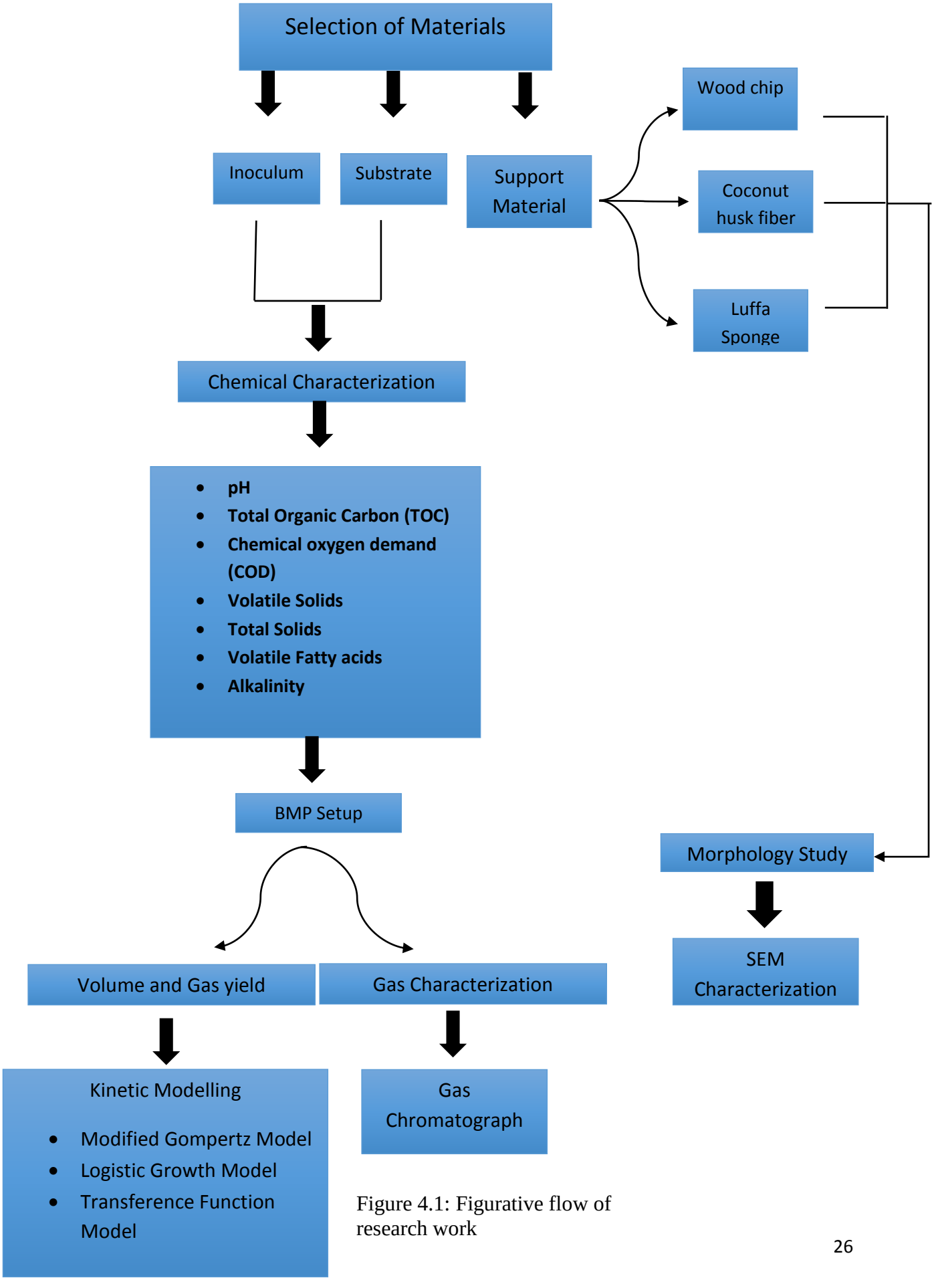

Figure 4.1: Figurative flow of research work

4.3 Parameters and Methods Employed in Chemical Analysis

4.3.1 pH

pH of samples was analyzed using Digital Multiparameter (Hanna HI 9829). Before analyzing the pH the Multiparameter was first calibrated with standard Buffer Solution of pH 7 (neutral), 10 (alkaline) and 4 (acidic). After Calibration, sample to be analyzed is collected in a beaker and pH electrode is placed in a sample. The reading is noted down when the multiparameter provide stable reading. After that electrode is washed with distilled water, capped and placed in the kit.

4.3.2 Chemical Oxygen Demand

Chemical Oxygen Demand test was performed according to the Standard Method for the examination of Water and Wastewater [2]. Close reflux method was adopted for COD test. Prior to test, three reagents i.e. Potassium dichromate ($K_2Cr_2O_7$) digestion reagent, Sulfuric Acid Reagent, and Ferrous Ammonium Sulfate titrant reagent were prepared. The method of these reagents preparation is mentioned below;

Potassium dichromate ($K_2Cr_2O_7$) digestion reagent: Primary standard grade $K_2Cr_2O_7$ of 2.45g was dried in a drying oven for 2 hours at 150^0C and later dissolved in 250mL distilled water. After that 83.5 mL of H_2SO_4 acid and 16.65 g of $HgSO_4$ was added and dissolved by means of continuous stirring. In the end the solution is diluted to 500ml.

Sulfuric Acid Reagent: 5.5g of Silver Sulfate $AgSO_4$ was added in 1L of $H2SO_4$ acid and was continuously stirred for 1 day until it was completely dissolved in the acid.

Ferrous Ammonium Sulfate Titrant Reagent: 39.2 g of Ferrous Ammonium Sulfate was dissolved in distilled water after this 20mL of H_2SO_4 acid was added and diluted to 1000mL. Molarity of this solution was calculated by taking distilled water as a blank sample which is mixed with digestion reagent and Ferroin Indicator and titrated against FAS reagent.

Molarity of FAS Solution= [Volume of $K_2Cr_2O_7$ (mL) X 0.1] / Volume of FAS (mL)

Procedure:

COD vials were washed with 20% H_2SO_4 acid to remove all contaminants. 2.5mL and 1.5mL of sample and K_2Cr_2O7 digestion reagent was placed in vial respectively. Then 3.5mL of H_2SO_4 acid was slowly added which resulted in the formation of two layers acids layer and digestion solution layer. Vials were tightly capped and were inverted several times to get a complete homogenous mixture. These tubes were placed and refluxed in COD Digester for 2 hours at 150^0C. After 2 hours vials were cooled down at room temperature. The solution is then placed in beaker and 2 drops of Ferroin Indicator was added. The solution turned into reddish color after that the beaker was placed on magnetic stirrer and solution was titrated against the 0.9M FAS. Titration was stopped when reddish brown color was changed into bluish green color. The amount of FAS volume used was noted down and same procedure was repeated with blank sample containing distilled water. The formula used for COD calculation and Removal efficiency is as following

COD (mg/L) = [(A-B) *M*8000]/Volume of Sample

Whereas A=volume of FAS solution used for blank sample (mL)

B= volume of FAS Solution used for sample (mL)

M= Molarity of FAS solution

COD removal Efficiency (%)= [(COD influent-COD effluent)/COD influent] *100

4.3.3 Total Volatile Fatty Acids (FOS) and Alkalinity (TAC)

Total VFAs, Alkalinity and FOS/TAC ratio was determined according to the Nordmann Method [3]. Prior to the determination 0.1N Sulfuric acid solution was prepared to perform titration. 20mL of sample was placed in a beaker on magnetic stirrer and its pH was measured. After this it is titrated slowly with 0.1N Sulfuric acid solution until it reached the pH 5. Volume used is recorded which was later employed in total alkalinity formula. To measure the total volatile fatty acids the solution is further titrated with 0.1N Sulfuric acid until pH 4.3 is obtained. Again, the added volume of FAS is recorded to be used in total volatile fatty acids formula.

Total Volatile Fatty acids (FOS mg/L) = (20/A*B*1.66-0.15) *500

Total Alkalinity or CaCO3 (mg/L) = 20/A *C*250

Where A= volume of sample used (mL)

B= Volume of 0.1N Sulfuric acid used to titrate sample from pH 5 to pH 4.4

C= Volume of 0.1N Sulfuric acid used to titrate sample from starting pH to pH 5

4.3.4 Total Organic Carbon

Total Organic Carbon in samples was measured by Loss of ignition method [4]. In this method 10 g of sample was dried in drying oven for 12 hours at 103°C. when sample was completely dried it was further ignited in box furnace at 550°C for organic carbon estimation. Organic Carbon content constitute of all the carbon that is emitted between the temperature of 103°C and 550°. After igniting the sample, the weight of organic matter was divided by factor of 1.8 to obtain the value of total organic carbon.

TOC (g/L) =Volatile Solids/1.8

4.3.5 Total Solids (TS)

Total Solids was performed according to Standard Method for the examination of Water and Wastewater [2]. In this method empty china dish was washed and dried in an oven (Memmert Universal oven UF160) for 1 hour at 103°C. After drying they were cooled down and weight was recorded. 10g of sample was placed in empty in china dish and again weight was recorded. China dish with sample was placed in drying oven at 103°C for 6 hours until the sample was completely dried then it's temperature was lowered down at room temperature and final weight was recorded. The recorded readings were employed in following formula for TS calculation.

Total Solids (mg/L) = [(A-B) *1000]/sample volume (mL)

A= weight of china dish and dried residue (mg)

B= Weight of China dish (mg)

For Total Solids percent removal following formula was used [5]

Percent Removal= Influent-Effluent/Influent *100

4.3.6 Volatile Solids (VS)

Volatile Solids test was performed according to Standard Method for the examination of Water and Wastewater [2]. Samples which were previously dried for total solids at 103°C were placed in Box furnace and ignited at 550°C for 2 hours. The residue which was left behind in china dish was cooled at room temperature and weighed. The recorded reading was used in following formula

Volatile Solids (mg/L) = (A-B) *1000/sample volume (mL)

A= weight of china dish and residue before ignition (mg)

B= weight of china dish and residue after ignition (mg)

Volatile Solids Removal Efficiency % [6] = Volatile Solids concentration (influent)-Volatile Solids concentration (effluent)/Volatile Solids concentration (influent) × 100%

4.3.7 Gas Composition Analysis

Methane proportion in biogas was analyzed by using Gas Chromatograph (GC 2010 plus, Shimadzu) with a thermal conductivity detector (TCD) equipped with Molecular sieve 5A PLOT (Porous layer open tubular) column. Biogas samples (4mL) for composition analysis were injected in duplicate into GC autosampler. Initially the column temperature was set as 35^0C for 2 minutes, then it was increased as 10^0C per minute and finally increased to 150^0C for 5 minutes. Helium and Nitrogen was used as the carrier gases.

4.4 Carrier Material and Biofilm Characterization

Microbial cell immobilization on carrier materials was visualized using Scanning Electron Microscopy (SEM) (Model: Vega3, Tescan). Support materials before and after immobilization were scanned. Protocol for sample preparation was taken from study [7]. First samples were fixed in glutaraldehyde (2.5% in 0.1M phosphate buffer, pH was 7.4). Then samples were dehydrated through an ethanol gradient (50,70,80,90 and 100%) followed by lyophilization. After freeze drying the support materials were sputtered with Gold using a SEM coating system.

4.5 Kinetic Modelling

The cumulative Biogas yield obtained from experiment was fitted with various kinetic models to understand the kinetics of biogas production. Biokinetics parameters were estimated by Non-Linear regression approach using IBM SPSS Statistics Software 24. The simulated and experimental data was plotted on Origin Pro8 Software to produce graphs.

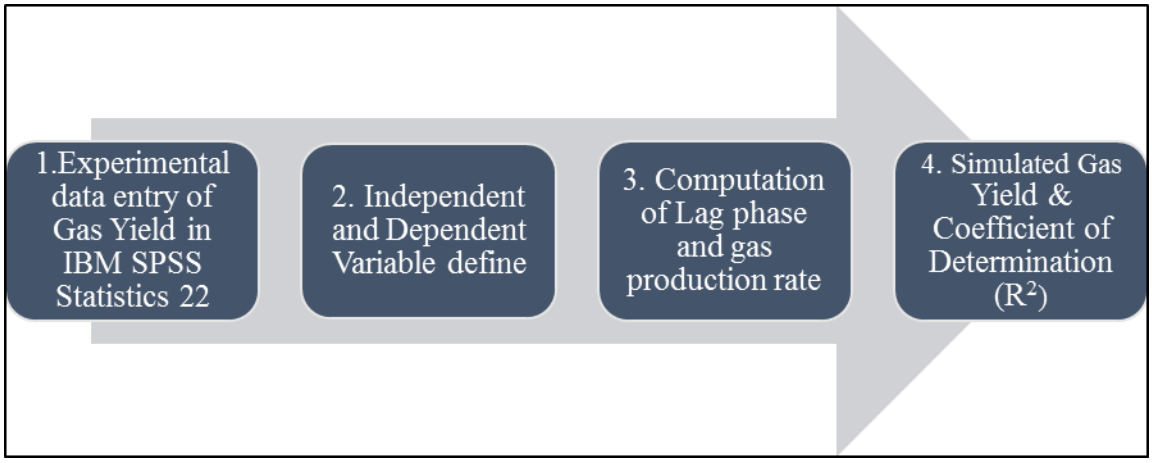

Figure 4.2: Steps involved in kinetic modelling

Summary

This chapter involves the details for the development of BMP experimental setup and methods to analyze the chemical parameters. Chemical parameters like COD, Total solids, volatile solids, volatile fatty acids were incorporated in study for substrate characterization. Microscopy technique SEM was undertaken to identify the morphology of biofilms. Kinetic modelling was done to fit the experimental data with the growth sigmoidal models which are Modified Gompertz model, logistic function model and transference function model.

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Chapter # 5

Results & Discussion

5.1 Substrate Characterization and Process Stability

Characterization of substrate before and after the digestion period was performed to verify the anaerobic bioreactors stability. Total six operational parameters were tested in this study and their results are as following;

5.1.1 pH

pH is the most significant parameter in anaerobic digestion as it is an indicator of reactor performance. The pH profile of control and biofilm reactors has been shown in figure 5.1. It is quite evident from the graph that pH for all reactors after the digestion ranged between 7 and 7.8. For methanogenic bacteria the optimum pH value is ranged between 6.5-7.5. Growth of microbes is affected when pH is drop below this range [1].

In biofilm reactors using luffa as a carrier material lead to pH conditions (pH 7.3) better suited for methane productivity. The pH of control reactor was in the permissible range 6.8-8.5[2]. As comparison to control reactor, pH of all biofilm carrier reactors in effluent was in stable range that is 7.3 and 7.5, clearly indicating the good neutralizing capacity of bed materials which eliminated any need of pH control. [3]

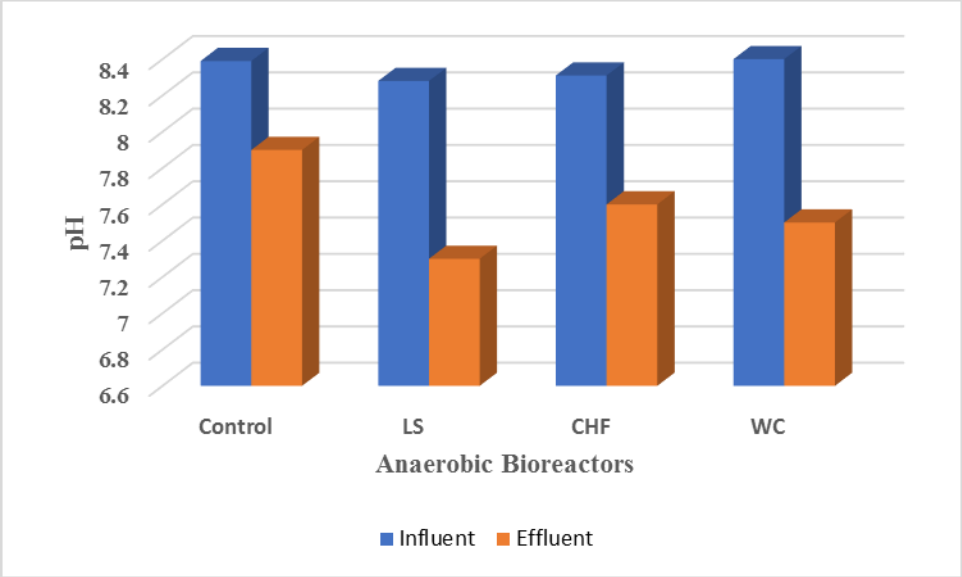

Figure 5.1: pH profile of influent and effluent of BMP assay with retention time of 55 days

5.1.2 Chemical Oxygen Demand (COD) and Removal efficiency

In this test chemically oxidizable organic matter was measured to examine the COD concentration removed during anaerobic process. Figure 5.2 represent the COD of Influent substrate fed to the reactor and was calculated as 51 g/L clearly depicting the higher concentration of carbonaceous organic matter. The effluent concentration of COD was highest in control reactor i.e. 24.98 g/L and lowest in LS and CHF reactor i.e. 5.94 g/L and 11.4g/L respectively. COD removal is a key factor to determine the efficiency of waste stream anaerobic treatment [4]. In terms of COD removal efficiency, the highest reduction was achieved in LS reactor that is 88% and low removal efficiency was 51% and 57.3% in the case of Control and WC reactor respectively.

In LS reactor high substrate (COD) removal efficiency can be attributed to the formation of biofilms that retain the higher concentration of microorganisms within the reactor and results in increased cellular activity and favored the transportation of substrate within the biofilm [5]. This finding is also supported by another study in which 92.8% COD removal efficiency was achieved by using luffa fiber as biofilm carrier in the biological treatment of domestic wastewater [6].

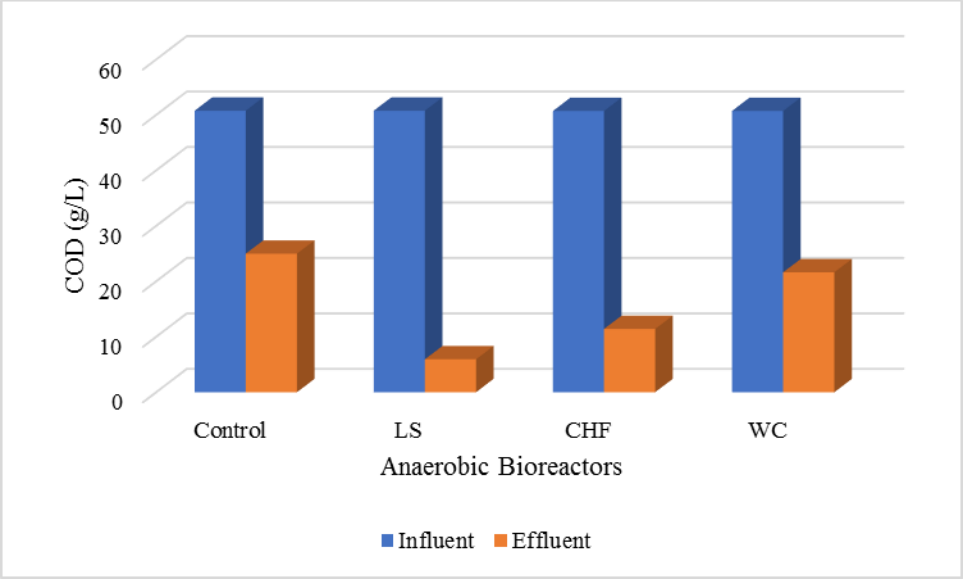

Figure 5.2: COD characterization of influent and effluent of BMP assay with retention time of 55 days

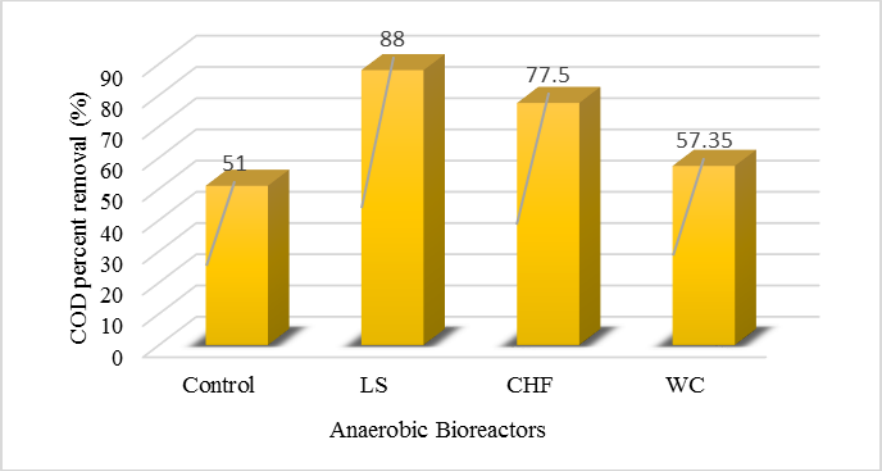

Figure 5.3: COD removal efficiency of control and biofilm reactors

5.1.3 Total Solids test and Removal Efficiency

Total Solids (TS) test was undertaken for determination of solids destruction in control and biofilm reactors. TS content in reactor comprised of total suspended solids and settleable solids [7]. The influent concentration of total solids was 53.8 g/L. Among the effluents, the lowest concentration was found in LS reactor that is 7.2 g/L and highest concentration was 19.3 g/L in WS reactor. In terms of removal efficiency, the reduction was quite significant in LS reactor (86.6 %) lowest was in WC reactor (64.1) as shown in figure 5.4. High solids reduction efficiency in LS reactor can be attributed to the adequate population of methanogenic bacteria immobilized on luffa fiber that results in improved metabolic activity. This collaboration of microbial population in biofilm had efficiently hydrolyzed the organic matter into volatile fatty acids that will eventually lead to improved production of methane as a final product [8]. The result obtained has been consistent with another study that had used the Activated Carbon fiber as biofilm carrier and achieved the high TS removal efficiency as compared to blank reactor [9]. Moreover, when TS percentage is increased the water content declined that eventually affect the activity of microorganisms. [10]

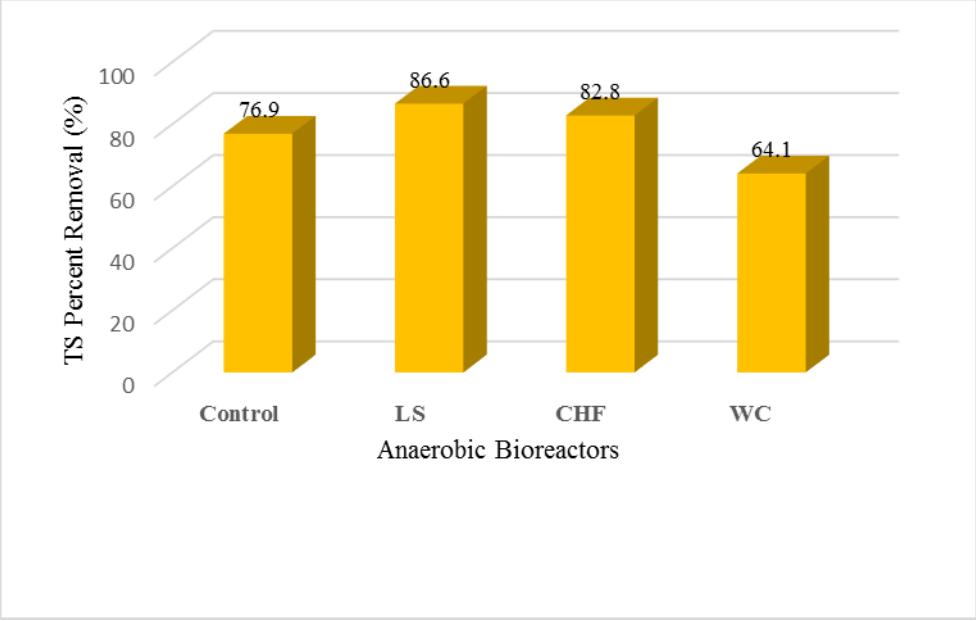

Figure 5.4: Total solid percent removal of control and biofilm reactors

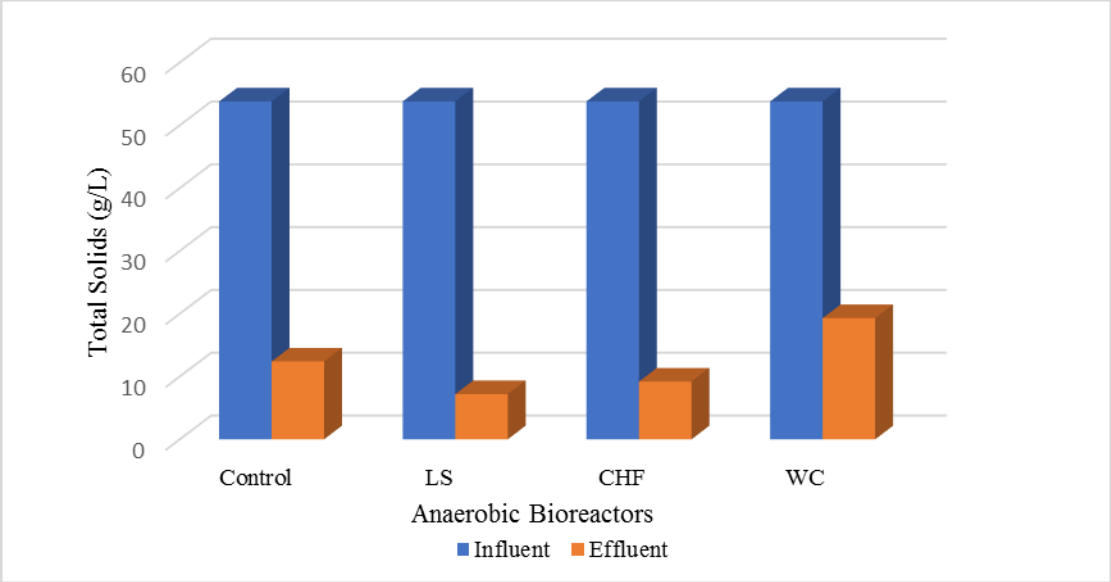

Figure 5.5: Total solids content of influent and effluent with retention time of 55 days

5.1.4 VOAs/TAC Ratio

The volatile organic acids/alkalinity ratio is an indicator of anaerobic digestion process stability and efficiency. The process favorably operates when this ratio lies below the range of 0.3-0.4 [11]. From figure 5.6 we have observed that ratio for all reactors was low than 0.3 except WC reactor which has ratio of 1.64. It has also been reported that restriction of methanogenic activity occurred when proportion of vfa/alkalinity ratio surpassed the ratio of 0.80 [12]. Similarly, total volatile fatty acids concentration has been reported to be low for a stable anaerobic digestion process [13]. Result of this study was in line with previously mentioned literature study and showed the lowest concentration of total volatile organic acids in terms of acetate i.e. 4490 mg/L and 3494 mg/L in LS and CHF reactor. This lower concentration can be attributed to the fact that total volatile fatty acids were rapidly metabolized indicating the adequate activity of methanogenic biofilm [14].

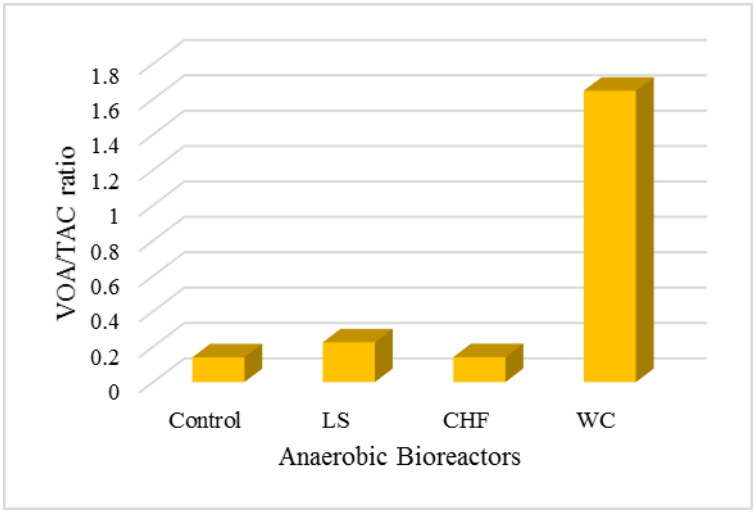

Figure 5.6: VOA/TAC profile of control and biofilm reactors

5.1.5 Volatile Solids Content and Removal Efficiency

Volatile solid test is performed to indicate the organic matter concentration in wastewater [15]. Volatile solids removal is one of the important pollution index that corresponded to the formation of biomethane when it undergoes effective degradation [9]. Total amount of VS fed to all reactors was 32.4 g/L and lowest concentration was measured 2.7g/L in effluent of LS reactor and highest one was measured as 8.3g/L in WS reactor. Similarly, VS reduction has further supported this result and highest VS removal was measured to be 82.6% in LS reactor and lowest was 64.1% in Woodchip reactor (fig 5.8). These results clearly demonstrate that biofilm support reactors of luffa and coconut husk fiber performed better in comparison to control and wood chip reactor. Performance enhancement of these reactors is consistent with the previously reported results that already specified the fast attachment and growth of microbial population over the surface of support materials and efficiently bring about the effective degradation of organic matter in biofilm support reactors [16] [17].

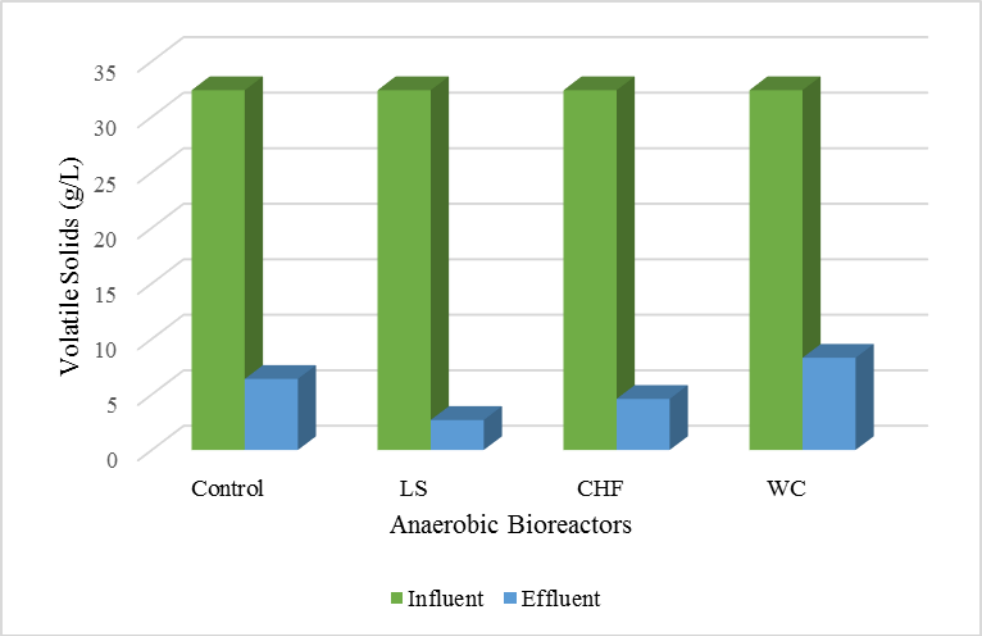

Figure 5.7: Volatile solids content of influent and effluent with retention time of 55 days

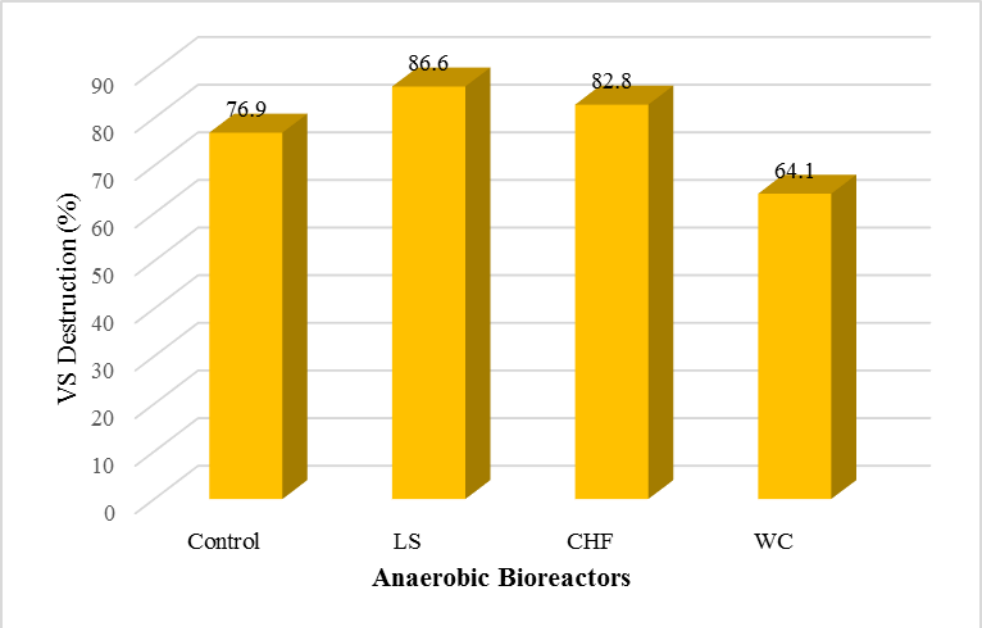

Figure 5.8: VS removal efficiency of control and biofilm reactors

5.1.6 Total Organic Carbon (TOC)

The total organic carbon test was performed to measure the organic carbon concentration in anaerobic bioreactors. According to literature the function of the biofilm is to oxidize the organic compounds in wastewater including organic carbon that will lead to toc concentrations to reduce over time [18]. However, another study has reported the consumption of organic carbon by means of anabolic pathway. In anabolic conversion the organic carbon played an important role in formation of biofilm matrix and cellular growth [19]. In the present study the organic carbon concentration has been decreased over time and the lowest concentration was found in effluent of LS reactor i.e. 1.5g/L. This implied the biofilm was properly developed on luffa fiber by means of anabolic conversion of organic carbon thereby reducing its concentration and in addition had also contributed to biogas production through catabolism.

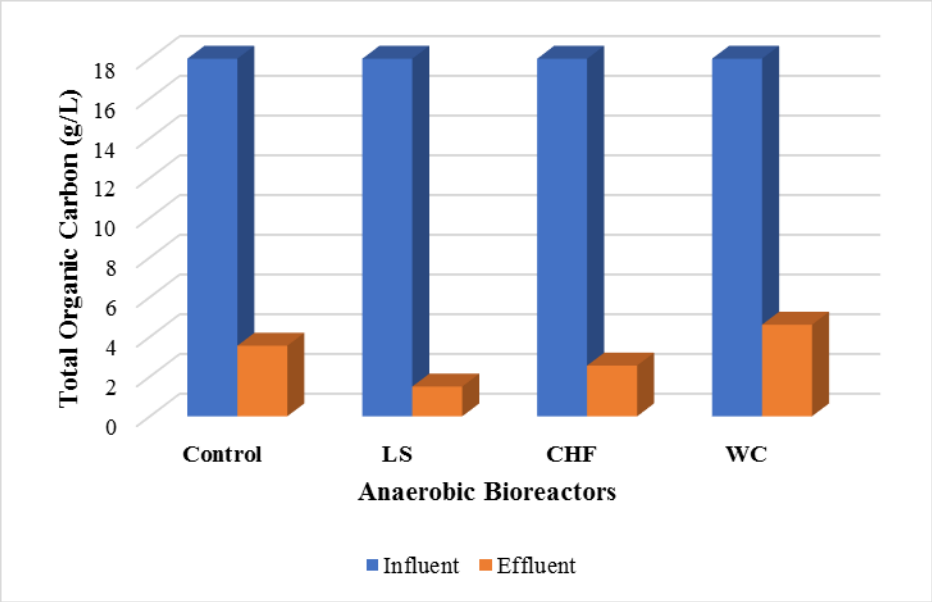

Figure 5.9: TOC content of influent and effluent of control and biofilm reactors

5.2 Reactors performance in terms of Biogas yield and Composition

Biogas composition is a very significant parameter in the monitoring of an anaerobic process. Results of previous studies also supported the fact that by adding the suitable porous and fibrous materials could lead to the improvement in methane production [20]. Results in figure 5.10 showed that bioreactor comprising of (LS) and (CHF) had relatively high methane content as compared to bioreactor containing (WC) and control bioreactor. In the LS and CHF reactor the level of methane concentration lied between the range of 60-77% and 40-60% respectively. Maximum recorded methane content for LS reactor is 77.7% at day 21 after that concentration slightly plummeted and remained stable between 60-74%. Increased methane content can be supported by dense methanogenic consortia in LS as Luffa fiber was more than 90% porous and became a favorable place for microbial biomass to adhere and actively playing their role in methane production. Comparatively VS content of LS bioreactor was also low after digestion as shown in figure 7. This further supported the result of increase methane content because microorganisms were efficiently playing their role in degradation and conversion of organic dry matter into methane. Although no significant microbial density was present on CHF but diversity of microbial morphotypes (rod and coccus shaped) might be the reason for improved methane content after LS reactor. In the WC bioreactor steadily increase in methane concentration was measured till day 25 after that the concentration got reduced and varied between range of 23-29%. This low concentration might be due to the poor microbial biomass retention on woodchip and few isolated microbial cells did not contribute well in the improvement of methane production. However, in the case of WC reactor by increasing the incubation time then it might be possibility that more microbial cells will be attached to form the mature biofilm and could lead to better performance. In the period of first 30 days the methane concentration of control reactor was low as compared to the carrier materials reactor, however after this period control reactor started showing better performance than woodchip bioreactor and it is quite evident from the trend of figure 5.10. that there is higher methane content in control reactor than wood chip reactor. In the case of LS reactor fast startup was also observed but other three reactors were unable to provide that performance. Similar result was presented in previous study in which loofa Sponge

showed the highest methane yield and was considered as the suitable carrier material when tested for acetic acid as sole organic substrate [21].

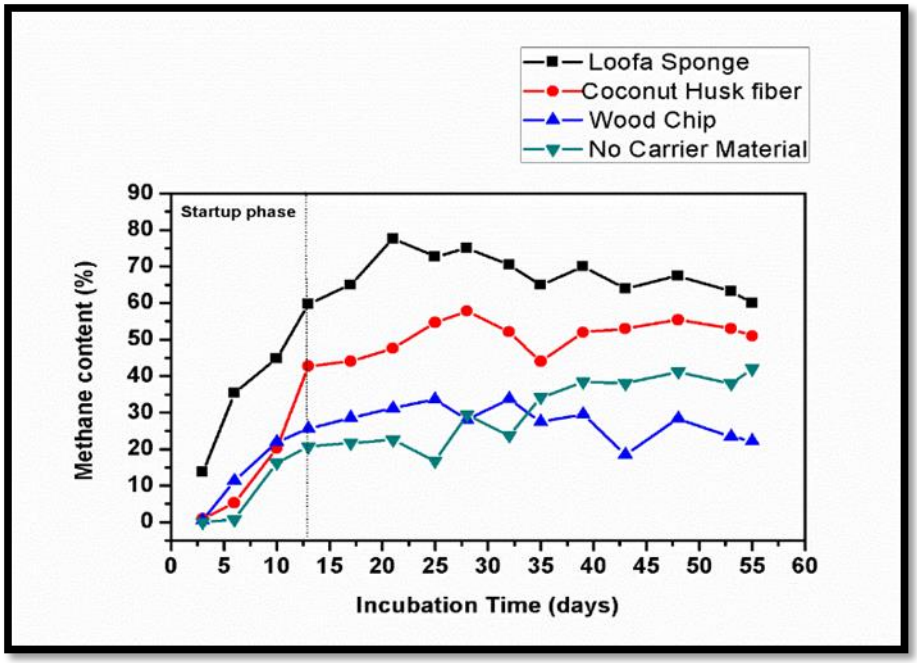

Figure 5.10: Methane content profile of control and biofilm reactors

5.2.1 Biogas yield

Biogas yield is a typical parameter to monitor the process efficiency. It is defined as amount of biogas/biomethane produced for a given amount of organic substrate removed because of anaerobic bacteria activity [22]. Figure 5.12 illustrated the accumulated methane yield of control and biofilm support reactors. The highest cumulative yield was obtained from LS reactor that is 196.3 mL g-1VS and lowest yield was 34 mL g-1VS obtained from control reactor. This study results showed that the provision of bed materials for microbial retention enhanced the performance of anaerobic bioreactors. The improvement of performance could be explained by rapid immobilization of methanogenic bacteria on support surfaces that undergoes the efficient degradation of organic material contributing to high biogas yield [23].

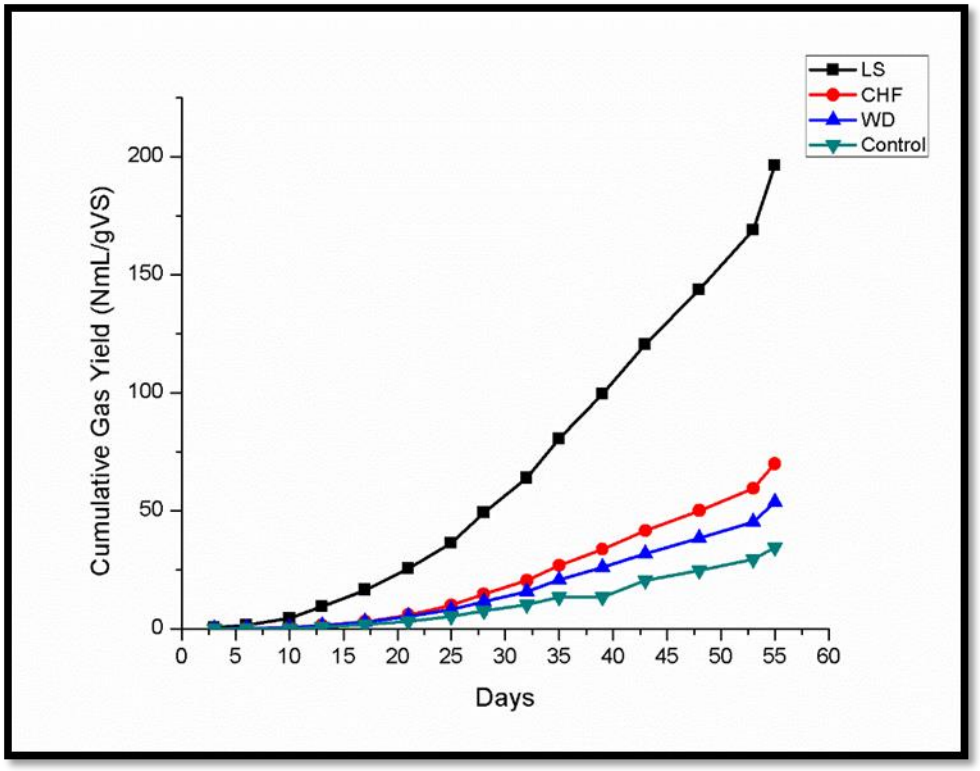

Figure 5.11: Cumulative Biogas yield of control and biofilm reactors

5.3 Biofilm Characterization

Characterization can be performed by various microscopy techniques like Epifluorescence Microscopy, Transmission electron microscopy (TEM) and Scanning Electron Microscopy (SEM). Biofilm characterization was performed by SEM to understand the phenomena of immobilization of microbial biomass on carrier materials. SEM micrographs of before and after the biofilm formation in luffa sponge, coconut husk fiber(coir) and wood chips are given below. SEM micrographs without microbial cells are shown in figure 5.12(a), (b)and(c). It could be observed that microscopic ridges, micro cracks, pores are present in these support materials. Macro porosity, fibrous structure and uneven surface of loofa sponge was also reported in previous study [24]. Irregular surface of wood chips and coconut husk fiber can also be seen from figure 5.12(b) and 5.12(c). These uneven surfaces could be the ideal places for the Methanogens for colonization [25].

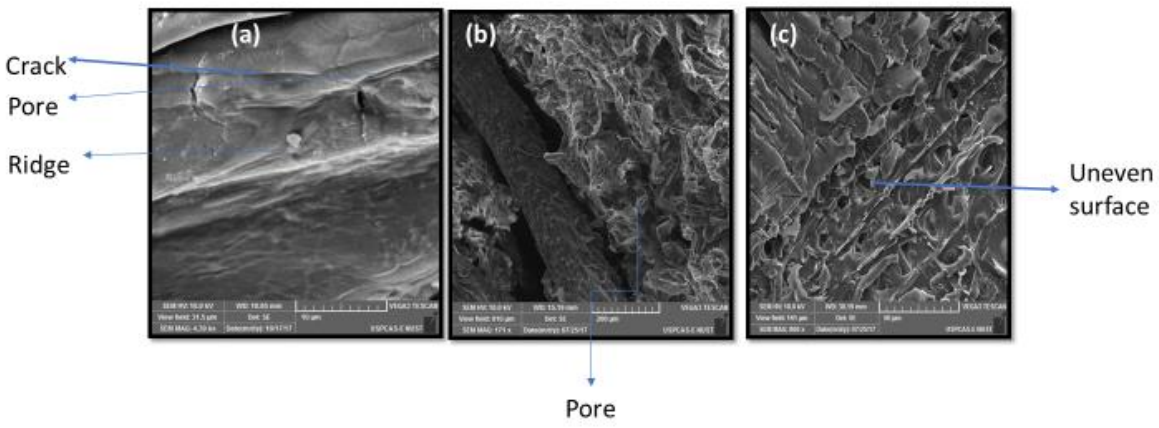

Figure 5.12: SEM micrographs of (a) LS (b) CHF and (c) WC before biofilm formation

Fig 5.13 (a), (b) revealed the biofilm after microbial immobilization on Loofa sponge. High microbial density can be seen on this support material and had mainly occupied the uneven ravine structure. These microbial cells are primarily attached with each other by means of extracellular polymer matrix as shown in fig 5.13(b). Methanogens are better immobilized and retained when biofilm formation occurred [26]. Cellular morphology on LS was coccus shaped that closely resembled the Methanobactins. Figure 5.14 (a) and (b) demonstrated the microbial biomass on coconut husk fiber. Although the density of microorganisms was not predominant but multi morphotypes like rod-shaped and coccus shaped can be identified and can be correspond to genus *Methanobacterium* and *Methanosarcina* respectively. Individual cocci shaped bacteria were found on support surface of wood chip and few were joined by polymer matrix, but rest of the area was not occupied as shown in (figure 5.15 a and b). So, these micrographs revealed the immature biofilm on WD. It might be the possibility that composition of WD was not favorable for the adherence if anaerobic microorganisms.

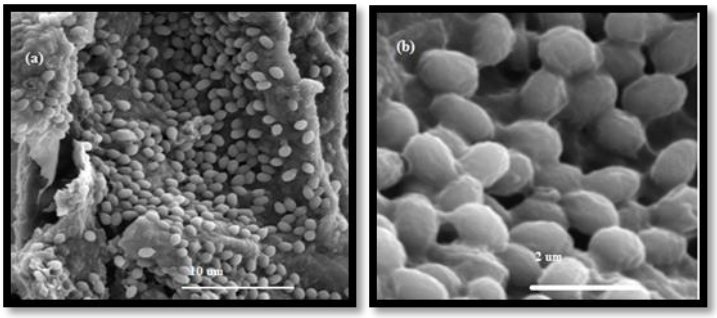

Figure 5.13: Biofilm formation on LS (a)10um (b)5um

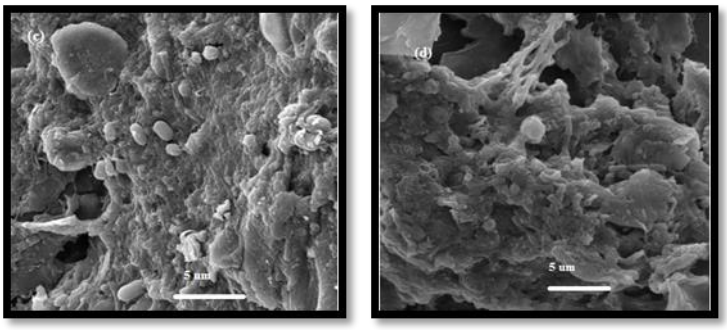

Figure 5.14: Microbial Immobilization on CHF (a)10um (b) 5um

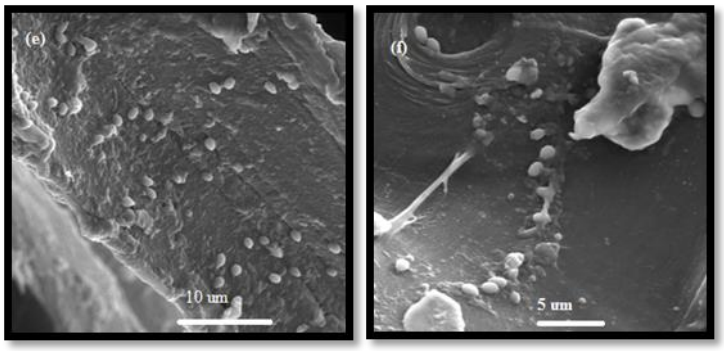

Figure 5.15: Microbial immobilization on WC (a 5um (b)5um

5.4 Kinetic Modelling

Table 1 summarizes the results of kinetic modelling using three kinetic models that is Modified Gompertz Model, Logistic Function Model and Transference function model. These three models predicted the biokinetic parameters lag phase (), methane production potential and biogas production rate (Rm) by using the accumulated biogas yield values obtained from the batch experimental assay.

Gompertz model and logistics function model were used as they linked the growth of methanogenic bacteria with methane production using the sigmoidal function [27]. Transference function also correlates the function of microbial activity with the gas production [28]. The empirical equations used in these models are as following;

Modified Gompertz Model

$$P = Pmax \times exp\{-exp[\frac{Rmax \times e}{P\,max}(\lambda - t) + 1]\}$$

Logistic Function Model

$$P = \frac{Pmax}{1 + exp[\frac{4 \times Rmax(\lambda - t)}{Pmax} + 2]}$$

Transference Function Model

$$P = Pmax \times \left(1 - exp\left(-\frac{Rmax \times (t - \lambda)}{A}\right)\right)$$

Here, Pmax is maximum potential biogas yield at time (mL/gVS)

P= Cumulative biogas yield (mL/g VS)

Rmax= biogas production rate (mL/gVS-d)

ʎ= lag phase time (days)

t= duration of BMP assay

e= exponential (2.71)

Table 5.1: Biokinetic parameters and coefficient of determination calculated using GM, LF and TF model

Kinetic Models	Reactors	Rm mL/g VS-d	Lag Phase (λ)	R2	Predicted Gas Yield	Experimental Gas Yield	% difference
Modified Gompertz model (GM)	LS	5	18	0.975	196.3	166.6	16.3
	CHF	2.24	22	0.989	69.94	59.68	15.8
	WD	1.68	24	0.978	53.65	44.5	18.55
	Control	1	26	0.979	34.60	29.44	16.2
Logistic Function Model (LF)	LS	5.9	18	0.989	196.3	174.04	12%
	CHF	2.29	23	0.989	69.94	62.25	11.6
	WD	1.7	24	0.989	53.65	47.62	11.9
	Control	1.1	26	0.988	34.60	30.77	11.9
Transference Function Model (TF)	LS	9	5	0.823	196.3	134.98	37.02
	CHF	1.7	10	0.798	69.94	46.61	40
	WD	1.3	10	0.803	53.65	35.95	39.5
	Control	0.8	11	0.806	34.60	23.2	39.4

From the table 5.1 it is quite evident that by comparing the three models, logistic function and modified Gompertz model provided the best fit with the experimental data. The coefficient of determination (R2) in both models were 0.989 and 0.978 respectively in all four reactors. The curves obtained by plotting these empirical models were shown in figure 14. The highest biogas production rate was given by LS reactor i.e. 5 mL/g VS-d, 6 mL/g VS-d, 9 mL/g VS-d by GM, LF and TF model respectively. Lowest production rate was found in control reactor. In addition, lag phase time was also determined to determine the effect of using biofilm carriers on gas yield. Lowest lag phase was found in LS reactor i.e. 19 and 21 days given by GM and LF model

respectively. The reduced lag phase can be attributed to the fact that methanogenic archaea acclimatized quickly to the support material and contributed to efficient methane production after immobilization. Result of this improved performance by using biofilm carrier was consistent with previous studies that had used various synthetic and biological support for immobilization to enhance hydrogen production [29] [30].

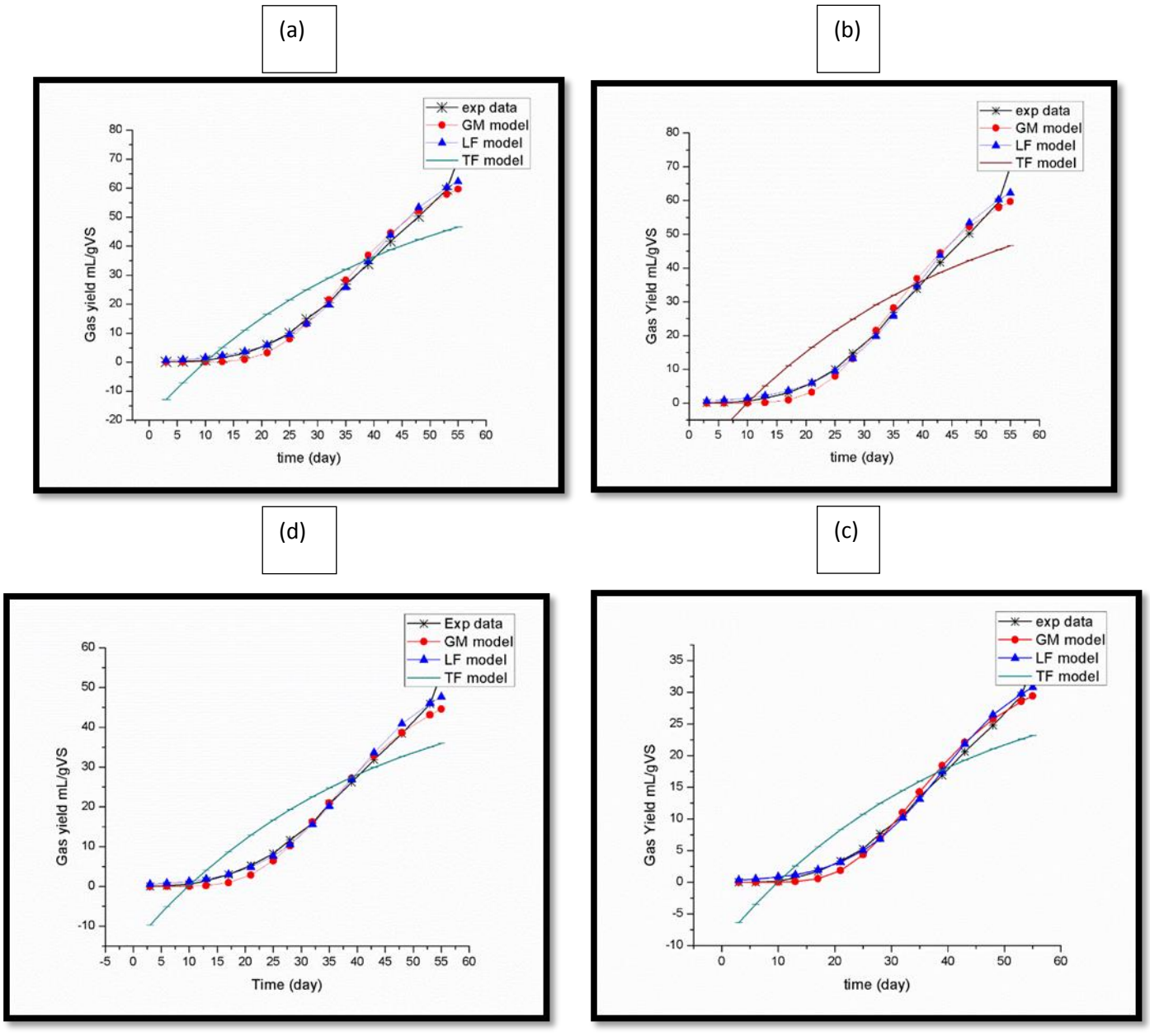

Figure 5.16: Comparison of predicted and actual biogas yield using GM, LF and TF model (a) LS (b) CHF (c) WC (d) control

Summary

This chapter presents the results obtained during the study and explains them with reference to existing literature. Results of chemical characterization revealed that best performance was provided by LS reactors in terms of highest COD removal efficiency (88%) and volatile solids removal efficiency (86.6%). Total organic carbon was also found to be 1.5 g/L in the effluent of LS reactor as biofilm was properly developed on luffa fiber by means of anabolic conversion of organic carbon thereby reducing its concentration. Methane content in biogas was measured to evaluate the performance of biofilm carriers in anaerobic reactors. Bioreactor comprising of LS and CHF had relatively high methane content as compared to bioreactor containing WC and control bioreactor. In the LS and CHF reactor the level of methane concentration lied between the range of 60-77% and 40-60% respectively. Increased methane content can be explained by rapid immobilization of methanogenic bacteria on support surface of LS that undergoes the efficient degradation of organic material contributing to high concentration of methane. Microscopic characterization revealed that high microbial density was present in loofah sponge due to its porous structure. Diverse microbial flora like rod shaped and coccus closely resembled to genus *Methanobacterium* and *Methanosarcina* were detected on Coconut husk fiber however on wood chips no significant abundance of microbial biomass was identified. Results of Kinetic Model determined that Logistics Function model reproduce the closest cumulative biogas yield as a function of retention time followed by modified Gompertz model.

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Chapter # 6

Conclusions & Recommendations

The following conclusions and recommendations were extracted from the current study compiled in this thesis.

6.1 Conclusion

In this study the utilization of natural waste materials like Luffa sponge, Coconut husk fiber and wood chip was reported as support material for microbial immobilization in the anaerobic digestion of sugar industry effluent spent wash. From the results it can be inferred that performance of bioreactor containing Luffa sponge was superior to other bioreactors in terms of methane concentration and biogas production. Chemical characterization revealed that highest removal efficiency in terms of total solids, volatile solids, chemical oxygen demand, organic carbon was in the case of LS reactor. Microscopic characterization revealed that high microbial density was present in luffa sponge due to its porous structure. The results of gas characterization indicated high methane content between the range (60-77%) in LS bioreactor followed by CHF bioreactor (40-60%). Diverse microbial flora like rod shaped and coccus closely resembled to genus *Methanobacterium* and *Methanosarcina* were detected on Coconut husk fiber however on wood chips no significant abundance of microbial biomass was identified. This study concluded that improvement in methane production by adding the low cost natural waste materials as biofilm carriers can be achieved which lead to overall efficiency of anaerobic bioreactor. Results of Kinetic Model determined the reduced lag phase by using the luffa sponge as an immobilization material.

6.2 Future Recommendations

Further study is required for detailed phylogenetic study of microbial biomass by using molecular techniques like FISH and DGGE. The support material that has provided the best result must be employed in anaerobic fix bed reactor and it should be run at shorter hydraulic retention time for its applicability.

Chapter # 7

Energy Policy Lab

This chapter comprises the detailed information of the research work which was undertaken in Energy Policy Lab in Arizona State University. A systematic approach known as Technological Innovation System Approach was adopted to analyze the Bio Digestion Technology in Pakistan. The purpose of this study was to understand the diffusion dynamics of this technology in Pakistan by analyzing its functional and structural components using TIS approach.

7.1 Introduction

Human demand for energy is continuously exceeding with the rapid urbanization, economic growth and modernization. To meet the requirements of energy demand world is heavily relying on the conventional sources such as oil, gas and coal but their high depletion rate and continuous utilization is resulting in strong repercussions like increased fuel prices and various environmental problems [1][2]. In the recent era these issues were taken into high consideration thus all around the world new policies and market mechanisms for e.g. renewable energy portfolio standards, net metering, installation rebates for alternative energy systems were formulated to increase the share of renewable energy resources and technologies in global energy mix. This will ensure the bright energy future for humanity with negligible environmental impacts [3][4]. The increased interest in renewable energy technologies is also because of it is taking as an effective way to curtail the greenhouse gases (GHG) emissions. In this regard many countries have signed the Kyoto Protocol to mitigate the impact of Global Warming [5]. Among the clean energy technologies, Biogas technology has the high potential of mitigating climate change thus it can profit more from the clean development mechanism established under Kyoto Protocol [6]. In the context of Pakistan, bio digestion technology has high untapped potential, but its widespread adoption has been

affected by the presence of many blockage mechanisms therefore unable to create the well-functioning market of this technology [7].

The exploration of this technology was started by Directorate General, New and Renewable Energy resources (DGNRER), Government of Pakistan in year 1974 and commissioned 4137 plants of capacity between 5-15 cubic meter by 1987 all over the country. This biogas scheme was implemented in three phases; in the first phase 100 units were established with 100% subsidy and technical support granted by Government of Pakistan. During the second phase the remaining units were installed but government cut down the subsidy from 100% to 50% thus imposed the fifty percent cost on the beneficiary. In the third phase, government decided to withdraw the 50% subsidy but continued with the provision of technical support. Eventually this scheme did not progress after the second phase because of this withdrawal of subsidy [8]. After the failure of this project partnership was founded between some semi-governmental organization and private Nongovernmental organization, hence plants based on small household designs (3-5 cubic meter) were installed for the promotion of biogas technology [9]. Floating drum design is the most commonly used technology for biogas plants. In the past Chinese fixed dome design was implemented on pilot basis but did not provide satisfactory performance due to gas leakage from the cracks in the dome component of the digester and resulted in low gas pressure [8]. Another design known as fixed dome technology originally created in Nepal was imported to Pakistan under the umbrella of Pakistan Domestic Biogas Programme (PDBP) to overcome the cost issue. This technology does not require much maintenance as compare to floating drum technology and was cost effective because it involved the use of locally available materials for the construction of dome [10].

In Spite the efforts of private sector in implementation of bio digestion technology, the diffusion of this technology was not accelerated as barriers at various levels are present. In the past studies general recommendations for the elimination of barriers was provided but in the context of renewable energy technologies. In Pakistan no specific literature has been present that comprised the systematic study of bio-digestion technology for the effective removal of blockage mechanisms. So, the purpose of current study is to

understand the diffusion of this technology in Pakistan through the systematic framework of Innovation system analysis approach as shown in figure 7.1. The key objectives of this study are as following

- To identify the all the relevant stakeholders in Bio-digestion technology in Pakistan using the structural component of TIS approach.
- Identification and categorization of barriers that affected the widespread adoption of this technology.
- Determination of functional events and assessment of their intensity level using the functional approach of TIS analysis.
- Provision of policy insights for the further strengthening of functions from the perspective of innovation system approach.

The Structure of this study is as follows: In the first section the background of Bio digestion technology in Pakistan was provided. Second section composed of brief description of the TIS approach adopted for this study. In section third results were presented and discussed and in last section policy insights were provided and study was concluded.

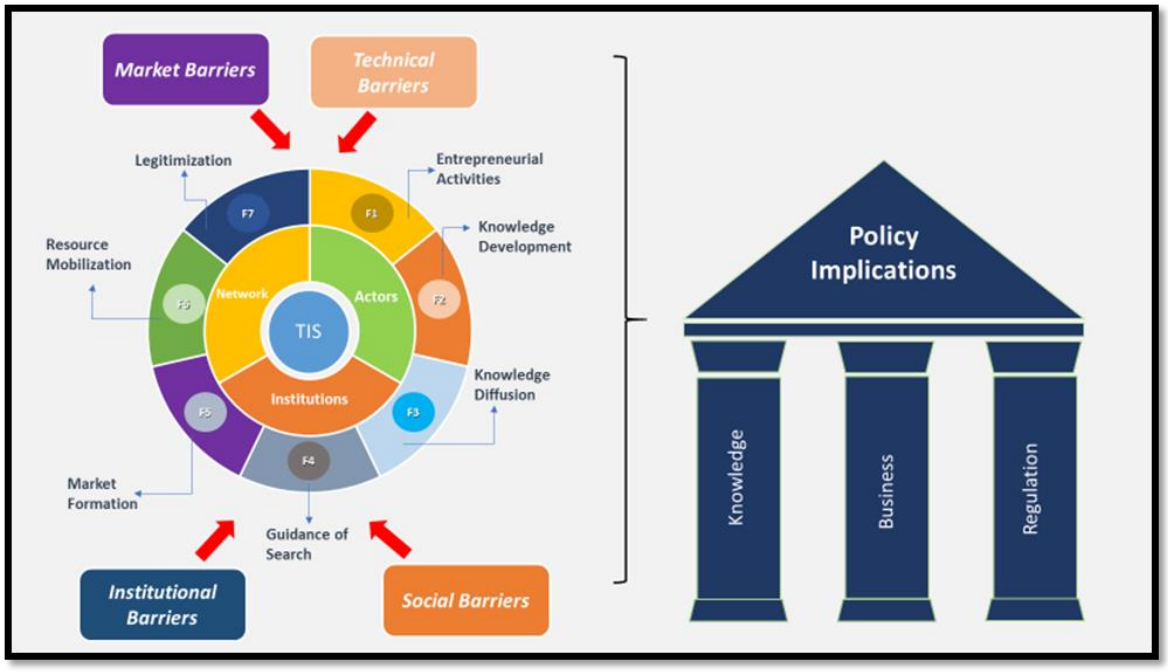

Figure 17.1: Framework of TIS of bio digestion technology in Pakistan

7.2 Technological Innovation System Approach

Innovation system is considered as a process in which technical possibilities are matched with market opportunities involving multiple interactions and types of learning [11]. National innovation System takes a broader perspective and defined as ‘’elements and relationships which interacts in the production, diffusion and use of new and economically useful knowledge…and are either located within or rooted inside the borders of a nations state” [12]. Whereas technological innovation systems approach is usually focused on institutions and networks of agents performing their role in generation, diffusion and utilization of a specific technology that fits best with their interest in technological change [13]. Technological innovation System are less complex as they involved relative small number of agents and only small number of institutions are aligned with the needs of the new technology [14].

Innovation Structural elements of innovation system [15]

1. Knowledge and Technologies

2. Actors and Networks

3. Institutions

Actors include heterogeneous agents like organizations (e.g. users, producers, input suppliers, universities, government agencies) and individuals (e.g. entrepreneurs, scientists etc.). These agents interact through processes of communication exchange, cooperation, competition and characterized by specific learning processes, beliefs, goals and organizational structure and behaviors. In innovation there is a systematic interaction (market and non-market relations) among the different actors for generation and exchange of knowledge [15].

Networks are the channels that transfer both implicit and explicit knowledge [16]. The interactions between actors are shaped by institutions which can be formal or informal in nature. Formal institutions are rules, laws, standards and informal institutions are cultural aspects, traditions, norms, practices etc. these institutions govern the actions and interactions of actors within the innovation system. [15] [17] [18]

There is a technological innovation system for each technology and that each innovation system is unique in its ability to develop and diffuse a new technology. However, the growth of a TIS is claimed to be related to the interaction dynamics between the System Functions. Fulfillment of more systems functions will lead to better performance of innovation system thus providing better chances for a successful development, diffusion and implementation of renewable energy technologies [19].

There are seven system functions

1. Entrepreneurial activities
2. Knowledge development
3. Knowledge exchange
4. Guidance of search
5. Market Formation
6. Resource mobilization
7. creation of legitimacy

Various authors have used this approach to analyze the innovation system of bio-digestion technology in their respective countries. In India diffusion scale of large scale biogas plants was analyzed by incorporating the aspect of spatial dimension into the TIS literature and focused on the international functions of the TIS and their role in technology transfer and barrier removal [6]. Different programs and projects on the promotion of renewable energy technologies implemented by public and private sector of Bangladesh were scrutinized by combining the approach of innovation system and appropriate technology. This approach was used to analyze the alignment of three important building blocks of TIS in the diffusion in biogas technology especially at the grassroot level [17]. In Rwanda the functional dynamics of bio-digestion technology was explored by identifying the important activities and processes performed by the key players in the historical perspective [20]. With the help of this methodology functional strength and weaknesses were identified and then policy interventions were provided for the strengthening and shaping of innovation system.

Table 7.1: Functions in innovation system [14] [19][21]

F1 entrepreneurial activities	**An innovation system function in which entrepreneurs take actions "to turn the potential of new knowledge development, networks and markets into concrete action to generate and take advantage of business opportunities"**
F2 knowledge development	This function incorporates all activities where R&D and technological learning takes place. It encompasses "learning by searching" and ''learning by doing"
F3 Knowledge diffusion	This function involves the exchange of information by means of network among vital actors (for e.g. R&D, competitors, government)
F4 Guidance of the search	Guidance of search encompasses all activities in innovation system "that can positively affect the visibility and clarity of specific wants among technology users." This function help actors to mobilize resources by beckoning opportunities.
F5 Market formation	The function of market formation entails activities that create protected spaces for new technologies. Government can also initiate the market formation activity for sustainable technologies by implementing favorable tax regime.
F6 Resource mobilization	The function resource mobilization consists of all activities which mobilize human, financial and natural resources for the development, diffusion and use of new technology
F7 creation of legitimacy	The activities in this function consists of the advocacy efforts from actors for technology acceptance because it may lack the support required especially from the key actor i.e. government

7.3 Technological Innovation System of Bio digestion in Pakistan

We have studied the specific TIS of bio-digestion within the national boundary of Pakistan. In the first step all the relevant actors involved in this innovation system were identified as they are the main building blocks that contribute to development, support and diffusion of bio-digestion Technology in Pakistan (Table 7.2). We have enlisted actors from all the sectors like Private, Public, production, knowledge, financial and consumers. In the public sector Alternative energy development board (AEDB) and Pakistan Council of Renewable energy and Technologies (PCRET) are the central actors. PCRET comes under the jurisdiction of Ministry of Science and Technology, Pakistan and started working in 2001. The main functions of PCRET is to perform the research and development activities pertaining to the Biogas technology, distribution of training material, provision of technical assistance especially to other non-governmental organizations that are involved in the installation of domestic biogas plants [22]. Alternative Energy Development Board (AEDB) also comes under the jurisdiction of Federal government of Pakistan and was established in 2003 for the sole purpose of development and promotion of renewable energy technologies in Pakistan [23].

In the private sector Rural Support Programs Network (RSPN) is the key actor that is involved in the implementation of this technology at the rural scale. This NGO started the biogas program in 2009 and successful in installing 5360 biogas plants of fixed dome design by the end of 2014. Training of the technical manpower, beneficiaries and gender mainstreaming was the key agenda of this program [24]. Pakistan Dairy Development Company (PDDC) has also undertaken the installation of biogas units to the rural groups and installed 450 units in the year 2009. Various construction companies like BETA-Pak, Reon Energy Solutions had provided their services in the construction of fixed dome plants. Their clients are mostly famers, entrepreneurs and other companies [24]. The consumers of this technology are basically farmers that belonged to the areas of Central Punjab of Pakistan. Basically, two groups are present as the end users of this technology. First group comprised of families that have cattle, house and land, second group consist of families that do not have land, so community bases biogas plants can be tried for this group [7].

Table 7.2: Overview of actors involved in TIS of bio digestion technology in Pakistan

Structural Dimensions Of Technological Innovation System of Bio digestion In Pakistan	**Key Actors Involved**	**Financing Infrastructure**	Pakistan Council of Renewable Energy Technologies (PCRET), RSPN, Microfinancing Institutions (MFI) like Pakistan Poverty Alleviation Fund(PPAF), ASASAH, Rural Community Development Society(RCDS), Human Appeal International (HAI-Pak), , First Women Bank, UNDP
		Knowledge Infrastructure	R&D organizations like Pakistan Council of Renewable Energy Technologies (PCRET), Rural Support Program Network (RSPN), NARC, PARC, PCSIR, Punjab agriculture department, AARI University of Agriculture Faisalabad, other Public and Private Universities
		Production Infrastructure	Biogas Construction Companies like Bioenergy Technology Application Pakistan (BETA-Pak), Pak Diary Development Company, Solar and Biogas Creative group, REON Energy Solutions, Nordtec Pakistan, Revgreen Pakistan, PDBP
		Public Infrastructure	Government of Pakistan (GOP), Alternative Energy Development Board (AEDB), PCRET, Ministry of Climate Change, Provincial Governments, livestock and dairy development department Punjab

		Private Sector	Nongovernmental Organizations like Rural Support Program Network, Win rock International, Initiative for rural and sustainable development (IRSD), Foundation of Integrated Development (FIDA), Green Circle Organization, Koshis, United Nations Development Program (UNDP)
		End User	Biogas Technology users especially from Rural Areas of Pakistan especially villagers, famers of Central Punjab and local Technicians
	Institutions		The Pakistan Climate Change Act, 2017 Policy for development of Renewable generation for Power Generation 2006

7.3.1 Major Barriers in the Diffusion of Bio Digestion Technology in Pakistan

The identified barriers from extensive literature survey are classified into the categories proposed by framework given by Study [25]. Identified barriers are organized into four categories which are Technical, Market and Economic, Social and Institutional Detail of these barriers are mentioned in the table below;

Table 7.3: Barriers in diffusion of Bio digestion technology in Pakistan

Barrier Category	**Code**	**Identified Barrier**	**Source**
Technical	T1	Limited number of trained personnel for the post monitoring and maintenance of the biogas plants at rural scale.	[22]

	T2	Lack of supervision during construction of plants	[26]
	T3	No extensive training programs are conducted to provide knowledge and skills to masons, biogas plant owners and staff of NGO's	[27]
	T4	Once technology is installed it is not further developed or adapted. For e.g. the floating drum plant design is most widely used but has high operating cost because it requires the replacement of gas holder.	
	T5	Construction Materials of Substandard quality are used in construction of digesters.	
	T6	Lack of awareness among users about the usage of Bio slurry (digestate of Biogas plant)	
Economic and Market	E1	High Capital Cost discourages the consumers to install the biogas plant. Private sector like RSPN only provides 10% subsidy to the consumers, therefore these end-users cannot pay heavy amount to cover 80-90% of the installation cost.	[24] [22] [28]
	E2	Consumers from Semi Urban areas still preferred to be connected to piped natural gas (conventional fuel) as they are promised by political parties so investment in this technology is hampered due to lack of awareness among the key actors (politicians and consumers) about the benefits of biogas technology	
	E3	Actors involved in Financial system like micro finance institutions does not take this technology as income generating activity hence appropriate credit scheme for investment in this technology is still	

		absent.	
Social	S1	Due to limited number of promotional activities at grassroot level technology adoption rate is not up the mark. Early initiatives taken by government and private sector were not successful in the longer run thus it did not leave the good impression consequently leading to low consumer confidence. Also, stories about bad experiences spread quicker among the people of rural areas and stays longer in their memory.	[22] [29]
Institutional	I1	lack of involvement of governmental bodies in monitoring activities	[22] [8] [30]
	I2	Absence of proper mechanism/platform where all relevant stakeholders can share their experiences and lessons that are learned from past projects and programs can be exchanged act as main hurdle in dissemination of technology	
	I3	Lack of government interest in creating a cohesive approach where all main actors can work together to create a real impact is still present	
	I4	Improper need assessment is performed by private actors as biogas plant size is not according the feedstock generated by single household	

7.3.2 Effect of TIS Functional events on the Barriers:

In this step determination of functional events were carried out (Table 4) in the context of technological innovation system of Biogas technology in Pakistan. These events are responsible for the removal of single or more than one barriers hence determining the intensity of each function. A fully functional TIS lead to the eradication of all barriers hence contribute to the successful diffusion of the renewable energy technology [25].

Table 7.4: Associated events with TIS functions in context of Pakistan

Functions	Associated Events	Sources
F1	Linkage between research institutes and technology beneficiaries through the platform of workshops. Formation of society containing members of biogas construction companies, for creating manuals for construction and after sales service	[31] [22]
F2	Training and Capacity building programs for Masons, Biogas Construction Companies, Research and Development activities focusing on the technical designs of plant, Initiatives by provincial government to improve technology access in other sectors for like in Agriculture. Joint projects undertaken by public and private actors for improvement in technology access	[24]
F3	Technology awareness among financers especially microfinance institutions increase the access of capital to the entrepreneurs. Dissemination of the technology basics technical know-how to the public by means of information campaigns, marketing, promotional activities. Coordination of research institutes and non-beneficiary farmers for demonstration of bio slurry research.	[7] [28]
F4	Sharing experiences of successful projects if any by governmental based agencies to make technology more reliable in the eyes of consumers, involvement of financial sector experts for the advocacy of Biogas Projects, initiatives taken by government and private NGO's for the registration of biogas projects under the umbrella of Clean development mechanism (CDM)	[31]
F5	Formation of quality control centers, norms and technical	[31]

	standards that will contribute to the reliability of the technology. Appropriate Policy framework to regulate the delivery of right amount of incentives to eradicate the market distortions and secure the chances of market development in future.	
F6	Development of loan scheme that improve the access to capital, introduction of capacity building programs in collaboration with international training expert to address the problem of poor technical skills among the consumers, masons and construction companies, for mobilization of resources establishment of networking and coordination forums. This mechanism will ensure wider participation of stake holders which can also address the technical inadequacies.	[27]
F7	Formation of National Biogas Steering Committee, regulatory framework at provincial and federal level	[31]

The events which are determined above are analyzed further by determining their impact in the removal of the barriers. Table 4.5 consists of matrix that linked the functional event with the barriers. The methodology of matrix formation was adopted from study [25]. Functions are listed in the column and barrier codes were listed in the rows. The cells in the matrix are filled with "H", "P" and "A" If all events are existed for the removal of barrier then that cell is coded with "H" and function will be termed as high strength function. If some of the events are present, then that cell is labelled as "P" and that function will be represented as partial/medium strength function and if no event type is present then that function is Absent in the Pakistan's TIS of bio-digestion technology.

Table 7.5: Matrix of TIS functions linked with barriers

Barriers / Functions	Technical						Market and Economic			Social	Institutional			
	T1	T2	T3	T4	T5	T6	M 1	M 2	M 3	S1	I1	I2	I3	I4
F1							P					A		
F2				P										
F3	P	A	P			P			A	P		A		
F4								A		P				
F5	P				A									
F6			P										A	
F7													A	

P= Partial/Medium strength Function

A= Function is Absent

From the matrix it is quite evident that none of the function can be termed as high strength function as all events relevant to each function were not present within this innovation system. However, some of the functions of partial/medium strength are still working like knowledge diffusion (F3), resource mobilization (F6), knowledge development (F2) and have their reasonable share in the dissemination of the technology. Organization like Rural Support Program Network (RSPN) under the umbrella of Pakistan domestic biogas program (PDBP) did contribute in raising awareness on this technology by conducting radio and tv campaigns, workshops, engaging rural people in meetings and distribution of promotional materials to biogas construction companies and masons. As a result, this program was quite successful by

creating biogas market in private sector. When it comes to resource mobilization capacity building programs were started by non-governmental organizations and again PDBP did some noticeable work in this regard. Financial resources on the other hand were not quite helpful in increasing the demand for this technology and it can be attributed to two main reasons that is lack of interest on the behalf of micro finance institutions (MFI) and market distortions caused by huge differences in subsidies offered by various organizations to their clients. MFI did not consider this technology as income generating activity therefore failed to create the appropriate loan scheme for the users in addition there is no policy framework at provincial or federal level that regulate the provision of incentives for the eradication of market distortion. So, this remain as the major barrier in the widespread adoption of bio digestion technology. In Pakistan a few activities were undertaken in the domain of research and development for the upscaling of bio digestion technology. R&D related to bio-slurry was started by PDBP, University of Agriculture Faisalabad, Ayub Agricultural Research Institute (AARI) and its findings were conveyed to beneficiary farmers, but these demonstrations were not executed at wider scale including non-beneficiary farmers because of the persistent ignorance of public sector in the development of rules and regulations that can linked these findings to the provincial agriculture department. In the same way sufficient R&D aspects were not explored in bringing improvements in biogas storage options especially related to fixed dome technology at medium scale plants.

Unfortunately, in Pakistan application of renewable energy technologies for the generation of power is held under more consideration than for thermal applications. Mainstreaming bio-digestion technology is almost negligible in 2006 policy for Renewable Energy Technology for power generation, so this factor is consequently affecting the widespread adoption and thus unable to play its role in the strengthening of legitimization function (F7). It also sheds light on the fact that there is an improper coordination and networking at planning and policy level because policy formulation was mainly done in isolation and lack of stakeholder's participation and networking was another shortcoming that does not compensate for appropriate policy instruments. A few conspicuous efforts were made by NGOs like RSPN to strengthen the institutionalization of this technology by making their program registered with Clean Development

Mechanism to earn carbon revenue but failed to cover the annual certification costs due to construction of limited number of plants. To create entrepreneurial activities, Pakistan Domestic Biogas Program (PDBP) trained local beneficiaries by providing them trainings about construction, finance management and masonry and made them self-sufficient in earning income by enabling them to create localized construction companies. However, a robust policy advocacy component is required to attract foreign investors in establishing commercial plants for waste to energy applications at wider scale.

7.4 Conclusion and Policy Recommendations

This study delivered the in-depth investigation of the diffusion of Bio-digestion technology innovation system in Pakistan by emphasizing on the structural and functional components of TIS. It has also determined the functional strength and weaknesses and finally concluded that technological innovation system for bio digestion technology in Pakistan is functional to a limited extent. The functional approach of TIS clearly indicated that there many blocking mechanisms which are not completely removed because of the absence of some functional events hence no single function is fully operational. This study has also identified the weaknesses and gaps within the TIS of Pakistan where systematic policy implications are required for the strengthening and efficient functioning of the innovation system. This study can also be act as the guideline for the assessment of TIS of other renewable energy technologies in Pakistan. To overcome the barriers, policy interventions in each function are provided in table 7.6 so that TIS can reach to its full potential.

Table 7.6: Policy Implications

	Functions	**Policy Implications**
F1	Entrepreneurial activities	Development of Biogas Branch Associations/society that will contain representatives the biogas construction companies. Association of these nature can act as a platform for entrepreneurs pertaining to promotion and marketing regulation. Furthermore, they can actively play their role in policy formulation and regulatory framework at provincial or national level.
F2	Knowledge development	High emphasis should be given to research and development activities. Knowledge development should focus on the following areas like technical designs of the plants, bio slurry management, improved efficiency of plant during winters and cost reduction.
F3	Knowledge diffusion	Required more innovative approaches in information dissemination especially at grass root level. Marketing campaigns should incorporate the demonstration of benefits to the non-beneficiaries as well. A focal knowledge facility should be created that contain all the relevant information for developing and running of biogas plants.
F4	Guidance of the search	Formulation of Biomass Energy Policy that addresses both biogas for thermal applications and waste to energy technology for electricity generating activities. Also, a proper platform, should be present for sharing of successful project experiences
F5	Market formation	Introduction of right amount of incentives for biogas sector development in combination with strong biogas policy framework. Also, robust quality standards pertaining to construction and design should be developed. Linkage with international investors to promote the waste to energy mega plants in Pakistan to setup success stories of technology
F6	Resource mobilization	Formation of manuals that contain all the necessary information of financing and experiences from other developing countries successful biogas schemes and sharing of these manuals with Microfinance institutions. Much focus should be given on capacity building and technical skills training programs for all relevant stakeholders. Bring the international funding agencies to invest under climate change or clean energy polices to set up commercial plants.
F7	Creation of legitimacy	Governmental bodies should play it role in releasing and sharing successful project experiences for the purpose of technology advocacy, sensitization of waste management authorities to strengthen the waste to energy technologies through legal bindings.

Summary

In Pakistan, renewable energy technologies have a huge potential to meet the rising energy demand of country's population. A major portion of the country's population reside in rural areas but suffers severe energy shortage for agricultural and domestic need. Bio-based technologies particularly bio digestion technology can play a significant role in the eradication of fuel energy shortage considering the socio-economic condition of rural communities. Despite some efforts by government and non-governmental organizations for the promotion of this technology, it is still suffering by limited functionality and low rate of diffusion. In this chapter we have analyzed the case of bio-digestion technology under the systematic framework of the technological innovation system approach. We have identified the barriers that effect the widespread diffusion of this technology followed by studying their impact on each of functional element of TIS approach and then determined the overall adequacy of the innovation system. Our analysis points to the two main root causes that is lack of cohesive coordination between key stakeholders and poor functionality in the field of legitimization, knowledge development and resource mobilization. Furthermore, policy implications within each function are also provided for the attainment of well-functioning innovation system for bio digestion technology in future.

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Annexure 1

Investigation of lignocellulosic material as biofilm carriers for the optimization of Anaerobic Digestion

Atoofa Zainab[a*], Rabia Liaquat[a], Saadia Meraj[a]

[a]US-Pakistan Centre For Advanced Studies in Energy (USPCAS-E), National University of Sciences and Technology(NUST), H-12 Campus, Islamabad 44000, Pakistan

[*]Corresponding Author

Email Address: atoofazainab@gmail.com

US-Pakistan Centre For Advanced Studies in Energy (USPCAS-E), National University of Sciences and Technology (NUST) Islamabad 44000, Pakistan

Abstract

Increased Methane productivity and organic matter removal efficiency in anaerobic digestion can be achieved by retaining microbial consortia inside the reactors using biofilm carriers. In this study the performance of anaerobic reactors was assessed using the low cost lignocellulosic materials loofah sponge, coconut coir and wood chips as biofilm carriers. Anaerobic batch mode bioreactors comprising of carrier materials for biofilms and control bioreactor were run in parallel at mesophilic temperature (35^0C). Methane content, biogas yield, pH, volatile solids (VS), chemical oxygen demand (COD), VFA/alkalinity ratio and total organic carbon (TOC) was measured before and after the experiment. Scanning electron microscopy (SEM) was used to identify the morphology of the methanogenic bacteria present in the biofilms. Maximum methane concentration and volatile solids removal efficiency was obtained from loofah sponge reactor i.e. 77.7% and 82.6% respectively. Furthermore, determination of biokinetic parameters like lag phase (λ) and production rate (R_m) was also determined using three kinetic models Modified Gompertz Model, logistics growth model and transference function model. Among the kinetic models' logistics growth model and modified Gompertz model provided the best fit with the experimental data providing the R^2 of 0.989 and 0.975 respectively.

Keywords: Anaerobic digestion, Biofilm carriers, Scanning Electron Microscopy (SEM), Volatile Solids (VS), Kinetic models

Annexure 1

1.Introduction

Energy plays a central role in global prosperity but being dependent on conventional energy sources resulted in drastic consequences like global warming, climate change, environmental deterioration and associated health issues. From the past few decades there is an increase interest in harnessing energy from renewable energy resources to meet the energy demand and to reduce the environmental degradation. Anaerobic digestion is one of the promising bioenergy technology and has potential provide various benefits like waste stabilization, lower energy requirements and possible energy recovery in the form of Methane [1].

Being a sustainable technology, this process undergoes degradation of organic matter under anaerobic condition by the activity microbial consortia and results in the formation of biogas. [2] Waste sources from urban, industrial, agricultural sector are anaerobically processed to produce biogas but so far treating the high strength industrial waste water is the most practical target of this technology [3]. On the other hand, various factors like slow growth of microorganisms, washout of microbial biomass with effluent, slow startup affects the efficiency of anaerobic bioreactors treating wastewater [4][5]. So, to maintain the microbial population in the bioreactor different type of strategies like anaerobic granules and usage of biofilm carriers were adopted. In anaerobic granule microbial communities are presented in the form granules with a size between 0.2 to 5mm. Diverse physiological types of microorganisms lived in close vicinity of each other resulting in high methanogenic activity [6]. Application of Biofilm carrier is another significant achievement in which microorganisms are attached to inert or natural support materials to form biofilm on the surface of support materials which ultimately results in viable, stable population of microbial consortia [4]. Properties like porosity, surface roughness, availability and cost effectiveness of support materials are considered in selection of biofilm carriers. Previous studies reported that materials with rough, surface with crevices and pores are favorable for entrapment and retention of microorganisms [7][8].

Annexure 1

Commercially available synthetic materials like polyvinyl chloride (PVC), activated carbon fiber, glass fiber (ACF), Polypropylene (PP) were used as a carrier substrate for microbial attachment, of which activated carbon fiber and polyvinyl chloride favored the colonization of anaerobic microorganisms [5][9]. In addition, the use of Sisal fiber waste, biochar, natural zeolite, cedar charcoal as a natural material was successful in the adherence of anaerobic microbial consortia but also enhanced the methane production [7] [10] [11] [12]. Present study investigated the efficiency of natural materials like loofah sponge (LS), coconut husk fiber (CHF), wood chips (WC) as biofilm carriers in anaerobic digestion of spent wash. All these materials are cost effective and easily available with low environmental impact. Fibrous structure and macro porous structure was already reported in luffa sponge [13]. On the other hand, coconut husk fiber (coir) and wood chips were also reported as biofilm carriers in the anaerobic treatment of greywater and denitrification of waste water respectively [14] [15].

2. Materials and Methods

2.1. Biofilm Carrier Materials

In this Study three kind of natural waste materials loofah sponge (luffa cylindrica), coconut husk fiber (Coir) and wood chips were applied as support media for biofilm formation. All these materials are considered as low-cost materials and easily available. All these materials are selected because of their porous microstructure suitable for retention of microorganisms. Prior to their use they were cut down to average size of 2-3 inches and were rinsed with distilled water to remove attached impurities, air dried and then were used in anaerobic bioreactors.

2.2 Substrate and Inoculum

The Spent wash used as substrate was obtained from a Sugar industry (Noon Sugar Mills, Bhalwal), transported to Biofuel Laboratory of USPCAS-E and was stored in airtight plastic bottles at $4^{o}C$, whereas inoculum was acquired from an active bio digester treating cow manure to produce biogas. The inoculum was stored in airtight 5L plastic bottle with anaerobic headspace for degradation of easily degradable organic matter still present in the inoculum. Characterization of substrate and inoculum was performed by

means of pH, total Solids (TS), Volatile Solids (VS) and Chemical Oxygen Demand (COD).

Table 1: Physiochemical Characteristics of Substrate and Inoculum used

Parameter	Spent wash (Substrate)	Inoculum
pH	8.12	6.6
Total Solids (%)	4.37	9.37
Volatile Solids (%TS)	33.33	60.7
Moisture Content (%)	95.62	90.6
COD (mg/L)	50,752	-
VS/TS	7.62	6.47

2.3. Experimental set-up

The Biochemical methane potential (BMP) assay was adopted from [16]. Batch type fermentation test were carried out in 300 mL glass bottles and were sealed airtight with silicon stoppers and scotch tape. The packing and working volume were determined to be as 180 ml and 250 ml. The packing volume (volume to be occupied by carrier materials) was determined by marking a horizontal line over the glass, previously filled by 180 ml water. Then bottles were filled with water up to the 250 ml which represents the working volume. Working volume was consisted of immobilization material, substrate and inoculum volume. Dried and Empty bottles were filled with carrier materials (luffa Sponge, coconut coir, wood chips) up to packing volume of 180mL. After that substrate and inoculum were added in the ratio of 2:1 i.e. (40ml:20ml). Since these carrier materials made the different packing tendencies and working volume was already fixed so additional water was added to make it up to 250ml. In the end pH was checked and adjusted to 7-7.5 by adding some drops of HCl 10 M solution, after that the bottles were purged with pure Nitrogen gas (5 minutes each) to maintain anaerobic conditions and sealed tight with silicon stopper. For the collection of Biogas, the bottles

were inserted with gas tight syringes of 20mL. Incubation of bottles was carried out at mesophilic conditions (35ºC) which is the optimal temperature for the growth of mesophilic bacteria. For the comparative analysis control reactor was also run in parallel with out the biofilm carrier All the bottles were shacked manually once a day. All the experiments were performed in triplicate.

2.4 Chemical Analysis:

Volatile Solids(VS) and chemical oxygen demand (COD) was determined according to APHA standard methods [17]. pH of samples was measured using Digital Multiparameter (Hanna HI 9829). Total Volatile fatty acids (TVFA) were determined according to the Nordmann Method [18] and total organic carbon was evaluated using relation TOC=VS/1.8 [19]. Methane proportion in biogas was analyzed by using gas chromatograph (GC 2010 plus, Shimadzu) with a thermal conductivity detector (TCD) equipped with Molecular sieve 5A PLOT (Porous layer open tubular) column. Biogas samples (4mL) for composition analysis were injected in duplicate into GC autosampler. Initially the column temperature was set as 35^0C for 2 minutes, then it was increased as 10^0C per minute and finally increased to 150^0C for 5 minutes. Helium and Nitrogen was used as the carrier gases.

2.5 Scanning Electron Microscopy:

Microbial morphology on carrier materials was visualized using scanning electron microscopy (SEM) (Model: Vega3, Tescan). Support materials before and after immobilization were scanned. Protocol for sample preparation was taken from study [12]. First samples were fixed in glutaraldehyde (2.5% in 0.1M phosphate buffer, pH was 7.4). Then samples were dehydrated through an ethanol gradient (50,70,80,90 and 100%) followed by lyophilization. After freeze drying the support materials were sputtered with Gold using a SEM coating system.

2.6 Kinetic Modelling:

Using the cumulative biogas yield data from batch experiment, biokinetic parameters like lag phase (λ) and biogas production rate (Rm) were calculated by three different

kinetic models like Modified Gompertz Model, Logistic Function Model and Transference function model. Lag phase is a very significant factor to determine the efficiency of anaerobic digestion [20]. Gompertz model and logistics function model were used as they linked the growth of methanogenic bacteria with methane production using the sigmoidal function [21]. Transference function also correlates the function of microbial activity with the gas production [22]. The empirical equations used in these models are as following [23];

Modified Gompertz Model

$$P = Pmax \times exp\{-exp[\frac{Rmax \times e}{P\ max}(\lambda - t) + 1]\}$$

Logistic Function Model

$$P = \frac{Pmax}{1 + exp[\frac{4 \times Rmax(\lambda - t)}{Pmax} + 2]}$$

Transference Function Model

$$P = Pmax \times \left(1 - exp\left(-\frac{Rmax \times (t - \lambda)}{A}\right)\right)$$

Here, P_{max} is maximum potential biogas yield at time (mL/gVS), P is Cumulative biogas yield (mL/g VS), R_{max} is biogas production rate (mL/gVS-d), λ is lag phase time (days), t is duration of BMP assay and e is exponential (2.71). The parameters were estimated by nonlinear least-square regression method using IBM SPSS statistics 22 software. In addition, coefficient of determination (R^2) was also obtained to determine the correlation of experimental data to the simulated data of biogas yield.

Annexure 1

3. Results and Discussion

3.1 Reactor Performance

Table 2 shows that reactor containing luffa sponge was quite superior to coconut husk fiber and woodchip as biofilm carrier for anaerobic digestion of spent wash. The utilization of LS appears to have good impact on substrate degradation in terms of COD removal, VS removal and TOC. In LS reactor Maximum COD and VS removal was 88% and 86.6% and TOC was 1.5g/L respectively. High Removal efficiency can be attributed to the adequate population of methanogenic bacteria immobilized on luffa sponge that results in improved metabolic activity eventually causing effective degradation of organic matter [24]. This finding is also supported by another study in which 92.8% COD removal efficiency was achieved by using luffa fiber as biofilm carrier in the biological treatment of domestic wastewater [25]. The use of WC appeared to have minimal impact on organic matter degradation and the performance of this reactor was almost like the control reactor. pH is also the most significant parameter in anaerobic digestion as it is an indicator of reactor performance. For methanogenic bacteria the optimum pH value is ranged between 6.5-7.5. Growth of microbes is affected when pH is drop below this range [26]. Except WC reactor pH in all other reactors lied in optimum range. The volatile organic acids/alkalinity ratio is an indicator of anaerobic digestion process stability and efficiency. The process favorably operates when this ratio lies below the range of 0.3-0.4 [27]. The ratio for all reactors was low than 0.3 except WC reactor which has ratio of 1.64. It has also been reported that restriction of methanogenic activity occurred when proportion of vfa/alkalinity ratio surpassed the ratio of 0.80 [28].

Methane content in biogas was measured to evaluate the performance of biofilm carriers in anaerobic reactors. Results in Figure 1 showed that bioreactor comprising of LS and CHF had relatively high methane content as compared to bioreactor containing WC and control bioreactor. In the LS and CHF reactor the level of methane concentration lied between the range of 60-77% and 40-60% respectively. Maximum recorded methane content for LS reactor is 77.7% at day 21 after that concentration slightly plummeted and

remained stable between 60-74%. Increased methane content can be explained by rapid immobilization of methanogenic bacteria on support surface of LS that undergoes the efficient degradation of organic material contributing to high concentration of methane [27]. Similar result was presented in previous study in which loofa Sponge showed the highest methane concentration and was considered as the suitable carrier material when tested for acetic acid as sole organic substrate [28].

Table 2: Summary of Chemical parameters of anaerobic bioreactors after digestion

	Control	LS	CHF	WC
pH	**7.9**	**7.3**	**7.6**	**6.3**
VS removal (%)	**76.9**	**86.6**	**82.2**	**64.1**
COD removal (%)	**51**	**88**	**77.5**	**57.35**
TVFA/TAC	**0.13**	**0.2**	**0.14**	**1.6**
TOC (g/L)	**3.5**	**1.5**	**2.5**	**4.6**

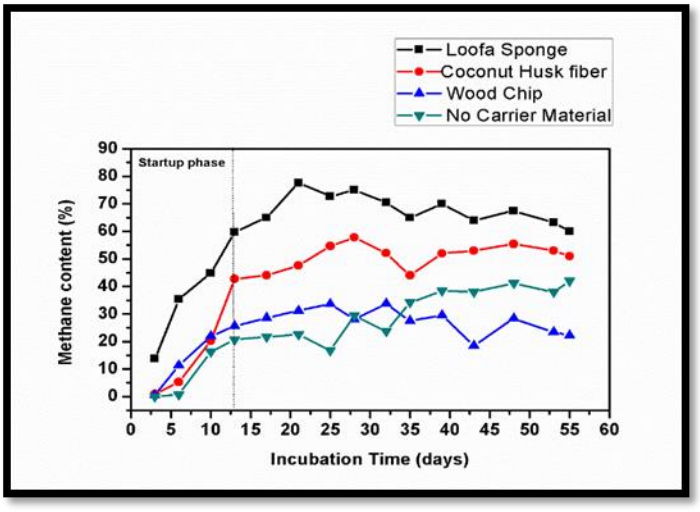

Figure 18 : Methane content profile of control and biofilm reactors

3.2 Microscopic Observations:

Figure 2 shows the SEM images of support materials before immobilization of methanogens. A seen from the figure microscopic ridges, micro cracks, pores are present in these support materials. Macro porosity, fibrous structure and uneven surface of loofa sponge was also reported in previous study [29]. Irregular surface of wood chips and coconut husk fiber can also be seen from figure 2(b) and 2(c). These uneven surfaces could be the ideal places for the Methanogens for colonization [14]. Figure 3 (a) and (b) revealed the biofilm after microbial immobilization on Loofa sponge. High microbial density can be seen on this support material and had mainly occupied the uneven ravine structure. These microbial cells are primarily attached with each other by means of extracellular polymer matrix as shown in figure 3(b). Methanogens are better immobilized and retained when biofilm formation occurred [30]. Cellular morphology on LS was coccus shaped that closely resembled the *Methanobactins.* Figure 3 (c) and (d) demonstrated the microbial biomass on coconut husk fiber. Although the density of microorganisms was not predominant but multi morphotypes like rod-shaped and coccus shaped can be identified and can be correspond to genus *Methanobacterium* and *Methanosarcina* respectively. Individual cocci shaped bacteria were found on support surface of wood chip and few were joined by polymer matrix, but rest of the area was not occupied as shown in figure 3 (e) and 3 (f).

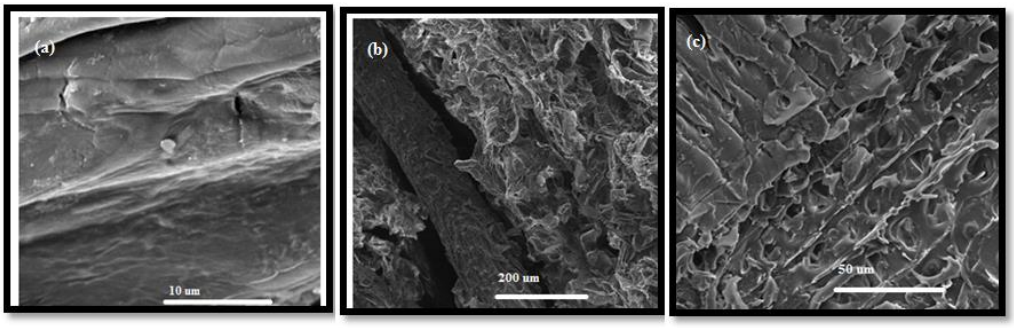

Figure 19: SEM photographs of support material before biofilm formation (a) Luffa Sponge (b) Coconut husk fiber (c) wood chips

Annexure 1

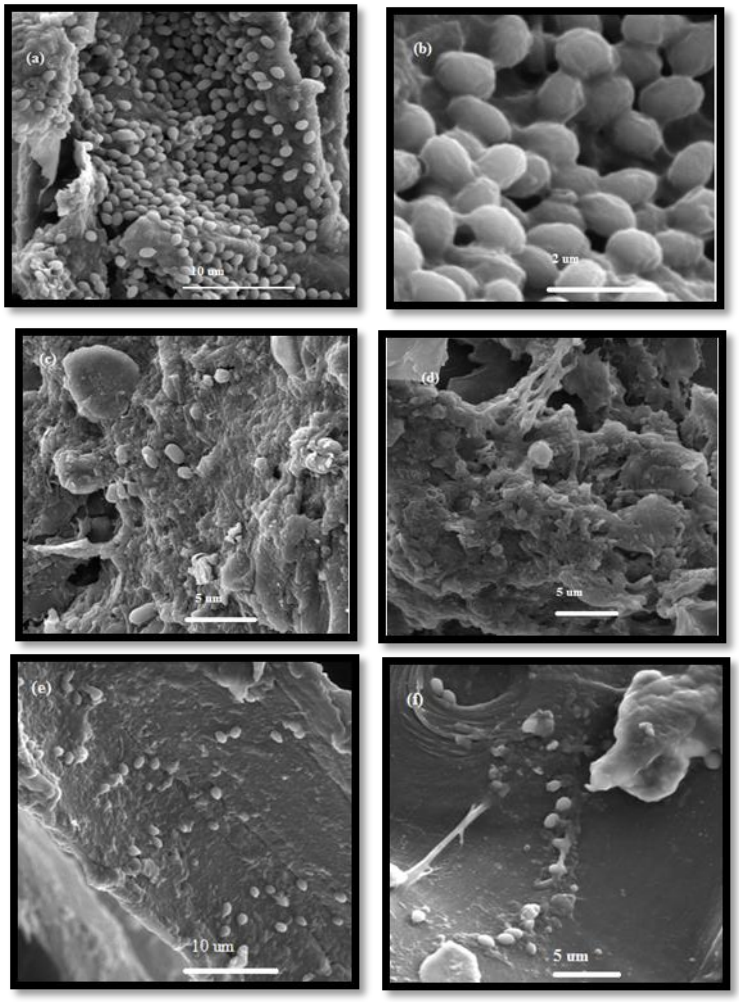

Figure 20: SEM photographs of microbial immobilization on support material (a), (b) Luffa sponge (c), (d) coconut husk fiber (e)(f) wood chips

3.3 Kinetic Modeling:

The results of all three models are presented in Table 3. From the table it is quite evident that by comparing the three models, logistic function and modified Gompertz model provided the best fit with the experimental data. The coefficient of determination (R^2) in both models were 0.989 and 0.978 respectively in all four reactors. The curves obtained by plotting these empirical models were shown in figure 14. The highest biogas production rate was given by LS reactor i.e. 5 mL/g VS-d, 6 mL/g VS-d, 9 mL/g VS-d by GM, LF and TF model respectively. Lowest production rate was found in control

reactor. In addition, lag phase time was also determined to determine the effect of using biofilm carriers on gas yield. Lowest lag phase was found in LS reactor i.e. 19 and 21 days given by GM and LF model respectively. The reduced lag phase can be attributed to the fact that methanogenic archaea acclimatized quickly to the support material and contributed to efficient methane production after immobilization. Result of this improved performance by using biofilm carrier was consistent with previous studies that had used various synthetic and biological support for immobilization to enhance hydrogen production [31] [32].

Table 3: Biokinetic Parameters and coefficient of determination evaluated using GM, LF and TF model

Kinetic Models	Reactors	R_m mL/g VS-d	Lag Phase (λ)	R^2	Predicted Gas Yield	Experimental Gas Yield	% difference
Modified Gompertz model (GM)	LS	5	18	0.975	196.3	166.6	16.3
	CHF	2.24	22	0.989	69.94	59.68	15.8
	WD	1.68	24	0.978	53.65	44.5	18.55
	Control	1	26	0.979	34.60	29.44	16.2
Logistic Function Model (LF)	LS	5.9	18	0.989	196.3	174.04	12%
	CHF	2.29	23	0.989	69.94	62.25	11.6
	WD	1.7	24	0.989	53.65	47.62	11.9
	Control	1.1	26	0.988	34.60	30.77	11.9
Transference Function Model (TF)	LS	9	5	0.823	196.3	134.98	37.02
	CHF	1.7	10	0.798	69.94	46.61	40
	WD	1.3	10	0.803	53.65	35.95	39.5
	Control	0.8	11	0.806	34.60	23.2	39.4

4.Conclusion

In this study the utilization of natural waste materials like Loofah sponge, Coconut husk fiber and wood chip was reported as support material for microbial immobilization in the anaerobic digestion of sugar industry effluent spent wash. From the results it can be inferred that performance of bioreactor containing Loofah sponge was superior to other bioreactors in terms of methane concentration and organic matter removal efficiency. Microscopic characterization revealed that high microbial density was present in loofah sponge due to its porous structure. Diverse microbial flora like rod shaped and coccus closely resembled to genus *Methanobacterium* and *Methanosarcina* were detected on Coconut husk fiber however on wood chips no significant abundance of microbial biomass was identified. Results of Kinetic Model determined that Logistics Function model reproduce the closest cumulative biogas yield as a function of retention time followed by modified Gompertz model.

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