# Studies on the effect of supplements for improving the kinetics of anaerobic digestion

A Thesis presented by

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## CERTIFICATE

This is to certify that the project entitled " Studies on the effect of supplements for improving the kinetics of anaerobic digestion" is hereby approved a creditable study in the area of Chemical Engineering carried out satisfactorily by KAMAL KHASTAGIR (Class Roll Number: 002010302009 , University Roll Number: M4CHE22009 , University Registration Number: 131182 of 2015-2016) to warrant its acceptance as a partial prerequisite for the award for the degree of Master of Chemical Engineering from Jadavpur University, Kolkata. It is understood by this approval the undersigned do not necessarily endorse or approve any statement made, opinion expressed or conclusion drawn therein, but approve the project only for the purpose for which it is submitted. The contents embodied in this thesis have not been submitted to any other University for the award of any degree or diploma.

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## DECLARATION OF ORIGINALITY

## AND

## COMPLIANCE OF ACADEMIC ETHICS

I hereby declare that this thesis contains literature survey and original research work by the undersigned candidate, as a part of his Master of Chemical Engineering studies. All information in this document have been obtained and presented in accordance with academic rules and ethical conduct. I also declare that as required by this rules and conduct, I have fully cited and referenced all materials and results that are not original to this work.

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*Dedicated to my mother*

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# Nomenclature

AD: Anaerobic Digestion

B: Volume of methane produced under normal conditions of temperature and pressure per kilogram of substrate used ($m^3$ $CH_4$ STP/kilogram of dry matter)

$B^0$: Volume of methane produced under normal conditions of temperature and pressure per kilogram of substrate added at infinite retention time ($m^3$ $CH_4$ STP/kilogram dry matter)

HRT: Hydraulic retention time (days)

$\theta$ or T: Hydraulic retention time (In kinetic model) (days)

$\mu$: Specific microbial growth rate (per day)

$\mu_{max}$: Maximum specific growth rate (per day)

S: Substrate concentration in the effluent ($kg/m^3$)

$S_o$: Feed substrate concentration ($kg/m^3$) Volumetric substrate utilization rate ($kg/m^3$/day)

D: Dilution rate

VFA: Volatile fatty acids

K: Dimensionless kinetic parameter

Y: Cell yield coefficient (kg VSS/kg of the substrate)

X: Concentration of biomass (kg $VSS/m^3$)

TS: Total solid

GHG: Green House Gases

# Abstract

It is a very well-known fact that in recent times non-renewable energy-producing industries are in a state of crisis. The increased exploitation of these fossil fuels increases the likelihood of causing environmental pollution, which affects both plant and animal life. In this context, the development and application of technology for producing energy from renewable sources such as wind, sun, biomass, and waste appears promising for the advancement of sustainable or green energy.

Anaerobic digestion (AD) in biogas facilities has grown in popularity over the last 20 years due to its ability to produce sustainable energy from a wide range of organic materials.

Anaerobic digestion (AD) converts biomass into biogas. The nutrient status of the AD substrate, on the other hand, frequently influences how well it performs. In this study, a few substrate supplements were chosen to enhance the biogas production; MgO, $FeCl_3$,Pectin, $FeSO_4$, and silica gel were used as supplements, chosen based on literature review [1]. A kinetic study of the process was used to assess the potential impact of the supplements on the AD process. MgO, $FeCl_3$ were found to improve the ultimate methane yield and kinetic parameters in all three digesters with different total solid concentrations. Finally, the final batch of experiments was run with the appropriate amounts of all three supplements, and the process produced better results.

# Chapter 1: Introduction

## 1.1 Introduction

Global warming and climate change are major environmental concerns today, both nationally and internationally, and are attracting a lot of public attention. The primary cause of this issue is the rise in atmospheric greenhouse gas concentration brought on by culpable human activity. Agriculture expansion (especially livestock husbandry and rice cultivation), industrial activities, the exploitation and use of fossil fuels, and waste production and management (landfill and animal waste) [2] are some of the human activities that contribute to the atmospheric concentration of greenhouse gases (GHGs). Carbon dioxide, methane, nitrous oxide, and fluorinated gases like sulphur hexafluoride and hydrofluorocarbon are the most significant greenhouse gases produced by such activities. Such gases have a significant impact on the earth's climate and contribute to global warming.

$CO_2$ gas is currently receiving more public attention than other GHGs in the context of global warming. Other gases, such as methane gas, should also be taken into account. When specific microorganisms break down organic materials in anaerobic environments, methane is produced. Natural wetlands, rice paddies, landfills, termite colonies, riverbeds, and lakes [3] are among the main biological sources of methane. Methane is a significant GHG with a potential for 25 times more global warming than $CO_2$. More than 60% of the methane released globally is attributed to human activity, according to estimates. 16 percent of all global GHG emissions come from methane emissions. Therefore, methane is a significant GHG that requires careful consideration due to its role in global warming. When microorganisms break down organic materials in an oxygen-free environment, they produce biogas, a clean, environmentally friendly, and renewable energy source. Anaerobic degradation, which produces biogas either under controlled conditions in built-in biogas plants or the natural environment (AD). Among the places where biogas naturally form are swamps, marshes, river beds, and the rumen of herbivorous animals [4]. Both under natural and controlled conditions, the same microbiological processes are accomplished.

## 1.2 Renewable Energy

The energy that comes from regenerative energy sources found in the natural world is referred to as renewable energy. It is a sustainable substitute for non-renewable energy. As a result of the ongoing depletion of fossil fuels and the environmental risks posed by future development, the direction of development is gradually shifting toward sustainability,

improved sociability, and environmental responsibility, which emphasizes the need for renewable energy sources. An ideal renewable energy source should be locally available, cheap and can be easily used and managed by the indigenous communities. Anaerobic digestion is one such technology that offers the possibility of decentralized approaches to the supply of modern energy services using resources such as; cow dung, human waste and agricultural residues to produce energy[5].

The idea of a decentralized renewable energy system has been acknowledged as a solution to meet energy needs in both the domestic and agro-industrial sectors. Planners and decision-makers have been forced to look for alternative sources due to the depletion of natural resources and the accelerated demand for conventional energy. Renewable energy sources are currently gaining importance even though commercial energy sources like coal, oil, and natural gas are used to a great extent. Sustainable development relies on renewable energy as a fundamental component. Such sources, which produce far less pollution than fossil fuels, can provide us with the energy we need indefinitely. The benefits of renewable energy sources are well known, including their ability to increase market diversity for energy supplies, secure long-term sustainable energy sources, reduce local and global atmospheric emissions, and open up new job opportunities with potential for local manufacturing.

## 1.3 Anaerobic Digestion and Biogas

Anaerobic digestion for energy production was first applied as a technology in England in 1896 when biogas from sewage sludge digestion was used to fuel street lamps [5]. Like many other renewable energy methods, popularity of anaerobic digestion was diminished with the rise of the dependence of petroleum. But the world is currently moving from petroleum-based to a bio-based global economy, in this instance, biological wastes, which is usually seen as low-valued materials, are now being transformed from high volume waste disposal environmental problems to constituting natural resources for the production of eco-friendly and sustainable fuels[6]. Achieving a sustainable energy system needs reforms in not just economic, social and policy aspects, but also in all technical aspects, which represents one of the most crucial future investments for anaerobic digestion systems. Anaerobic digestion (AD) depends on efficient conversion of organic mass into a flammable product known as biogas, with methane ($CH_4$) as its main combustible constituent.

Biogas contains about 55-65 % of methane, 35- 44 % of carbon dioxide and traces of other gases, such as Hydrogen Sulphide, Nitrogen and Ammonia. Biogas, in its raw form, that is

without any purification can be used as clean cooking fuel like LPG, lighting, heating and other applications. It can be used in diesel engines to substitute diesel up to 80% and up to 100% replacement of diesel by using 100% Biogas Engines[7]. Further, Biogas can be purified and upgraded up to 98% purity of methane content to make it suitable to be used as a green and clean fuel for transportation or filling in cylinders at high pressure of 250 bar or so and called as Compressed Bio-Gas (CBG)[7]. This AD process , which is the main method of biogas production is heavily dependent upon the mutual and syntrophic interaction of a consortium of microorganisms to break down the complex organic matter[8].

## 1.4 Biomass as a source of energy

Nitric oxides, carbon monoxide, carbon dioxide, and other air pollutants are primarily produced by all conventional petroleum-based fuels. The researchers are investigating suitable renewable sources of energy as fuel for sustainability and pollution-free characteristics with natural ecology due to the limited availability of fossil fuels. Biomass is one of these natural resources from which biofuels are made using chemical and biological procedures.

Biomass recycles carbon from the air, reducing the need to add more fossil carbon to the atmosphere and reducing the need for fossil fuels. In biomass, sunlight provides the energy needed to form chemical bonds. Proteins, lipids, and carbohydrates are produced by green plants using the solar energy that they absorb to form energetic chemical bonds. This chemical energy is the fuel that can be used to generate heat, electricity, or fuels for vehicles. Because plant life renews and grows every year, the origin of biomass thus constitutes the most significant element of the carbon-dioxide loop. Biomass can therefore be regarded as a renewable energy source. The primary sources of biomass are wood, sawdust, agricultural wastes such as rice husk, bagasse, groundnut shells, coffee husk, straws, coconut shells, coconut busk, jute sticks, etc., aquatic and marine biomass such as algae, water hyacinth, aquatic weeds and plants, sea grass beds, kelp, coral reef, etc., and wastes such as municipal solid waste, municipal sewage.

## 1.5 Biofuel production processes:

Fresh biomass, when compared to traditional fossil fuels, has the following relatively poorer qualities:

- Compared to fossil fuels, they have a very low thermal content.

- They have a high moisture content, which prevents their ready combustion and results in a significant loss of energy during combustion.
- Since they typically have a low bulk density, handling, storing, and burning them requires the use of relatively large equipment.
- The physical form is frequently not uniform, which causes problems with transportation and feeding to end-use equipment.

Before being used as fuel, improving the biomass's relatively poor characteristics is the primary goal of conversion. The process of converting biomass typically involves lowering the material's water content, which simultaneously raises its thermal value and ensures its preservation. There are numerous methods for converting biomass into energy-efficient biofuels.

**1.6 Present and future biogas potential in India**:

The Indian government sees biogas technology as a tool in a larger effort to promote rural development and as a means of reducing rural poverty. The Indian Renewable Energy Development Agency (IREDA), which is part of the Ministry of Non-Conventional Energy Sources, promotes alternative energy sources (MNES). The government created the National Project on Biogas Development to spread awareness of biogas technology specifically, and several non-governmental organizations have been actively involved in putting the program into action. The Khadi and Village Industries Commission (KVIC), which promotes rural development through opportunities for small-scale income generation, also engages in actively spreading awareness.

The development and expansion of installed capacity through non-traditional sources of electricity generation are given top priority by the Indian government. The Government of India has a separate Ministry that is solely dedicated to this crucial aspect of power production. In India, there has been a national program for home biogas plants since 1982. The two main types of plants in use are fixed dome type and floating dome type. The Indian government has subsidized the cost of installation for the biogas program by 20 to 40 percent. Additionally, it offers capacity building through official and contractor training, information dissemination, user training for plants, and research and development sponsorship[9].

Biogas plants are a reliable source of decentralized Renewable Energy for heating, and cooking as well as generating electricity/ power generation and thermal energy application alternatives in our country. The Ministry of New and Renewable Energy (MNES) promotes

setting up biogas plants by implementing Central Sector Schemes under Off-Grid/distributed and decentralized Renewable Power. There are currently two ongoing schemes in this field which are known as New National Biogas and Organic Manure Programme (NNBOMP), for Biogas Plant size ranging from $1m^3$ to 25 $m^3$ per day and Biogas Power Generation (Off-grid) and Thermal energy application Programme (BPGTP), for setting up biogas plants in the size range of 30 $m^3$ to 2500 $m^3$ per day, for corresponding power generation capacity range of 3 kW to 250 kW from biogas or raw biogas for thermal energy /cooling applications[7].

## 1.7 Aim and scope of the thesis

Everyday organic waste such as vegetable waste alone is not capable of producing much biogas. By supplying deficient nutrients or by lessening the effects of toxins in feed, supplements are added to improve the AD process' performance. Mineral additives and biological enzymes are the most common supplements for the AD process.

The Chen-Hashimoto kinetic model is used in this thesis to evaluate kinetic parameters. Mathematical modelling was used to gain a better understanding of the anaerobic co-digestion of vegetable waste and cow dung inoculum. Models of the biochemical process are required for the creation, operation, and performance prediction of the bioreactor that conducts the anaerobic digestion process. Three different substrate concentrations were given six different supplements, and their impact on the kinetic parameters was recorded.

To produce biogas through the anaerobic digestion of vegetable waste, this thesis explores few topics, including kinetics, optimization, and the impact of supplements.

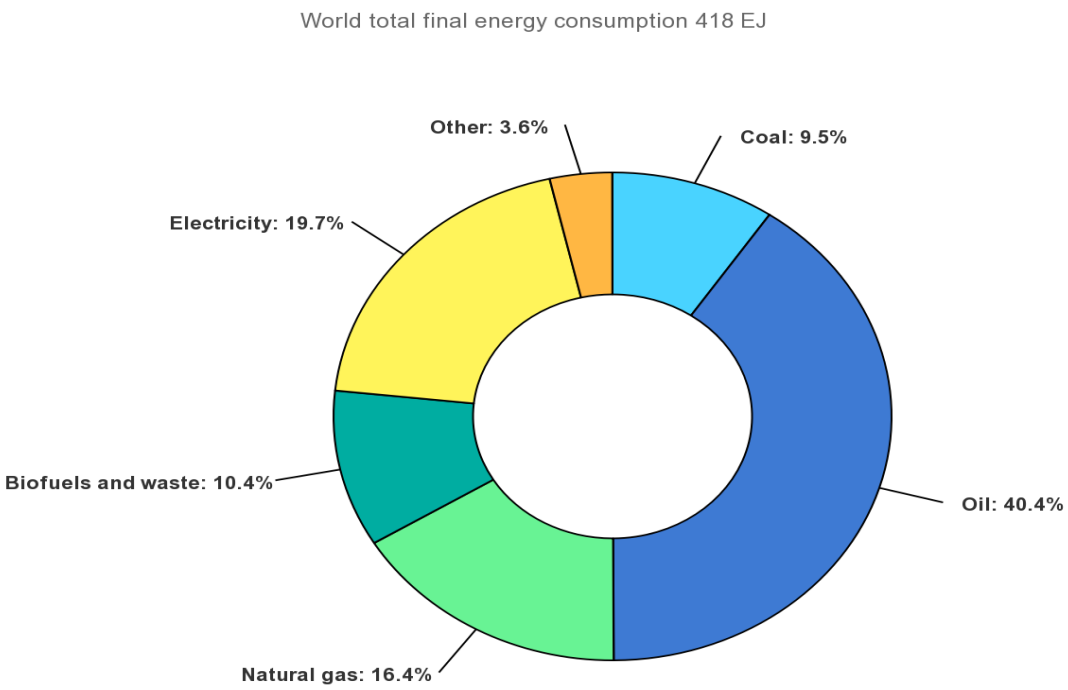



Figure 1.1: world total final consumption by source, 2019 ***[10]***

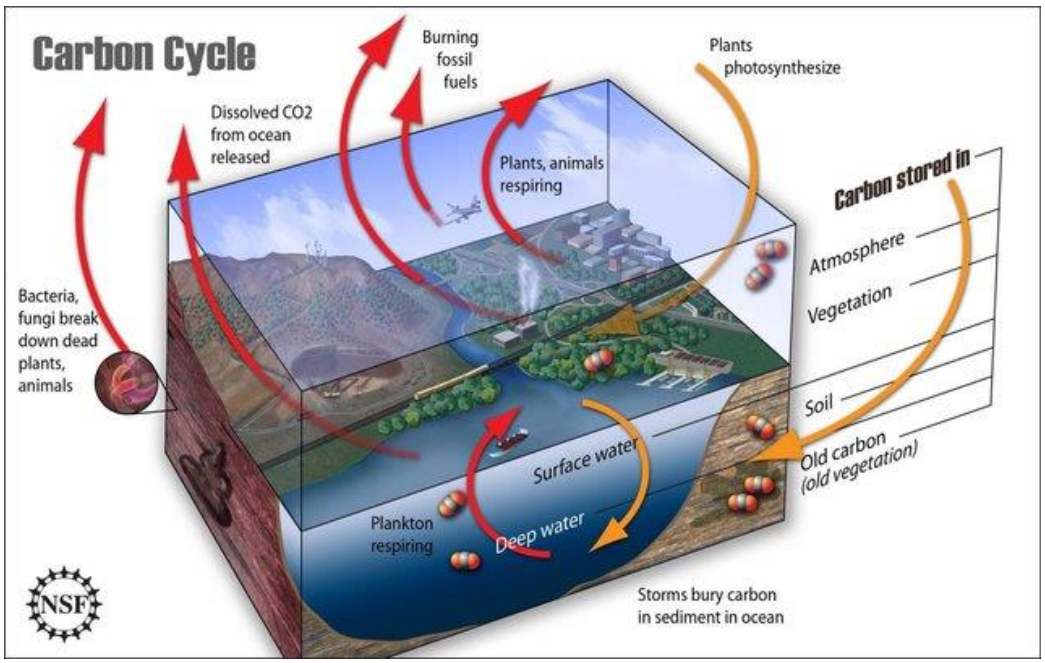



**Figure 1.2: Carbon cycle Loop in nature**

# **Chapter 2:** Literature Review

## 2.1 Use of supplementation in anaerobic digestion

This review focuses on some of the research done in Anaerobic Digestion where mixing traces of supplements improved the performance of the process.

- ✓ **Use of Carbon-conducting material supplements**

Different research works have reported that the use of Carbon-conducting materials, with the main focus on biochar, has improved the performance of Anaerobic digestion [11]. By providing an alternate path for electron transport, the use of these materials accelerates the rate of substrate degradation.

Biochar is a carbon-rich by-product of high-temperature (300-1000°C) biomass combustion, either with low levels of oxygen (gasification) or in the absence of oxygen entirely(pyrolysis) [12]. This material is known to have adsorption properties and special features as soil amendments that promote plant growth by retaining soil nutrients, increasing soil-water retention capacity, and improving cation exchange capacity. As it stands, various feedstocks are used to produce biochar, including organic wastes from agriculture and forestry, as well as urban wastes such as sewage sludge. However, the feedstock used and the processing conditions have a significant impact on its properties (temperature, atmosphere, and heating rate) [13]. The impact of biochar on soils has been studied for several years, and different benefits that may be related to its chemical and physical characteristics have been reported. Only recently have researchers begun to investigate its use in anaerobic digestion systems, taking into account its potential to act as a cheap adsorbent of inhibitory compounds and reporting pertinent results regarding the improvement in digestion performance that was not solely attributable to the removal of toxic compounds[14]. Several authors have reported a rise in methane yields[15]. Due to its ability to adsorb toxic substances, boost buffering capacity, and assist in the immobilization of anaerobic microflora, the addition of biochar to anaerobic digestion systems is suggested to reduce inhibitory stages and favor stability of the process[16].

**Table 1.1: Effect of Biochar on different substrates**

| Source of Substrate | substrate | Key findings | References |
|---|---|---|---|
| Fruitwood | Glucose (4–8 g/L) | At a glucose concentration of 4 g/L, biochar shortened the methanogenic lag phase and increased the maximum methane production rate by 86.6 percent, but only by 5.2 percent at the highest glucose concentration (8 g/L). | [15] |
| Fruitwood | Food wastes (4–10 g Dry Weight/L) | The methanogenic lag phase was shortened by biochar, and the maximum methane production rate increased by 123 percent. | [17] |
| Almond shell | Swine manure | yield of methane increased by 39%. Protein degradation was accelerated by the addition of biochar. | [18] |
| Fruitwood | Glucose N: 0.26–7 g/L | When ammonium and acids were acting as double inhibitors during anaerobic digestion, biochar accelerated the start of mechanisation. a 38 percent increase in the maximum methane production rate | [15] |
| corn stover | Sewage sludge | Digesters that had been amended with biochar produced biomethane that was nearly pipeline-quality (>90% CH4 and 5 ppb H2S). Additionally, the addition of biochar improved alkalinity and lessened ammonia inhibition. a 27.6% increase in the maximum methane production rate | [19] |
| Clean forestry wood residue | Organic fraction of municipal solid waste (Solid state fermentation) | Adding biochar increased methane yield by about 5%. | [20] |
| Vineyard pruning | Orange peels | yield of methane increased by 56%. A syntrophic metabolism between eubacterial and archaeal populations was created as a result of the biochar addition, which favoured the electro-active microorganism consortia. | [21] |

- ✓ **Use of different Additives composed of MgO, $MgCl_2$, $FeCl_2$, $FeCl_3$, and Cellulase**

In a particular study, different Additives composed of MgO, $MgCl_2$, $FeCl_2$, $FeCl_3$, and Cellulase were mixed with mixed organic waste composed of Food Waste, Swine sludge, and activated sludge[1]. The organic substrate was put through a series of BMP tests with various additives that were thought to be able to lessen the inhibition brought on by too much ammonia and sulfide in mixed. Food waste contains significant amounts of lignin and cellulose, so cellulase was used.

For added additives, the BMP values differed. MgCl2 or MgO treatment increased the amount of biogas produced by the reactor by 15% compared to the control. The batch reactors treated with $FeCl_2$ or $FeCl_3$ also showed higher BMP values than the control; with the addition of FeCl3, the reactor produced more biogas. Thus, $FeCl_3$ was selected for the present study. According to microbial community analysis, various additives may encourage the growth of various microorganism groups. For instance, the addition of MgO and FeCl3 to the mixture increased the activity of bacteria that produce hydrogen, such as Syntrophomonadaceae and Clostridium; Therefore, by easily converting the hydrogen produced by these bacteria to methane, biogas production could be increased. Methanosaeta concilii growth was seen to be encouraged by cellulase, which increased the production of biogas. Finally, a mixture of MgO, FeCl3, and cellulose was applied. The reactors treated with the combined additive showed a higher cumulative methane yield than the control; the BMP value and biodegradation efficiency of reactors were higher than those with the commercial products. Consequently, the combined additive can be applied in practice to encourage the production of biogas. In this study, we showed that anaerobic biogas production can be increased by simply supplementing the native substrates with less toxic ones. Therefore, more studies should be done to determine the inhibitory effects of substrate on AD and to create supplements to substrate to counteract the inhibitory effect.

- ✓ **Use of Ammonium Acetate and Betaine Supplements in AD under High salinity conditions**

High salinity often causes inhibition and can even lead to failure in anaerobic digestion. In a research Ammonium acetate and Betaine supplements were used as repair factors and their effect on the microorganisms was explored under high salinity conditions. High-salt environment caused severe inhibition to digestion. Performance was enhanced by the addition of ammonium acetate, especially later in the digestion process (400-720 hours). In a high-salt environment, betaine can significantly increase methane production, acidification hysteresis, and system stability during the pre-digestion period (0–400 hours). Methanogen activity and

methanogenic capacity are significantly increased when both repair factors are combined. Methane concentration increased by about 30%, the cumulative yield of methane increased by about 3.7 times, and stable methane concentration was reached about 100 hours earlier. While betaine can improve the stability of the intracellular environment even in harsh circumstances, ammonium acetate serves as an exogenous buffer [22].

- ✓ **Use of Nutrient supplementation in Biogas production**

One particular study showed the value of adding nutrients to a mixture of maize silage and cattle slurry during the aging process [23].

- The nutrient source was a typical cattle feed supplement which consisted of crude fiber, crude oils & fats, organic phosphorus, magnesium, crude proteins, and crude ash.
- Good biogas yield, high pH, low VFA concentrations, and an improvement in the VFA/TA ratio were all indicators of improved performance.
- The specific biogas yield increased as the amount of cattle feed supplement was increased, reaching a maximum of 894 L/kg VS from 543 L/kg VS in the control variant.
- One crucial finding was that adding nutrients to biogas did not increase its methane content, which ranged from 52 to 54 percent ($p > 0.05$).
- However, it was determined that an ideal supplement dose is essential for a successful AD and low process costs. Thus, adding 0.5 percent of fresh mass' worth of nutrients significantly increased biogas production.

There are other studies which also reveal that combination of Fe, Mn, Mo and Se enhances methane production[24].

## 2.2 Kinetic Modelling of biogas production and optimization

German biochemists Michaelis and Menten developed the theoretical framework for modelling the kinetics of bacterial growth. Their model explains how the enzyme activity changes depending on the amount of substrate present. Since microbial growth is also an autocatalytic reaction, this dependence can be applied to it [25]. When investigating the growth of bacterial cultures and the parallelism to the Michaelis-Menten theory in 1940, Monod discovered the non-linear relationship between a specific growth rate and a constrained substrate concentration. Monod created the following for bacterial growth:

$\mu = \mu_{max} (S/K_s + S)$

This model states that, until bacterial saturation is attained, the specific growth rate increases rapidly for low substrate concentration and slowly for high substrate concentration. The maximum specific growth rate, or $\mu_{max}$, is at this limit. The substrate concentration at 50% of the maximum specific growth rate ($\mu_{max}/2$) is the Monod-constant $K_s$.

The Monod model is accurate for pure cultures and simple substrates[26]. It was determined that the degradation of municipal wastes as a complex substrate cannot be described by the Monod kinetic. In addition, the Monod model omits the lag phase[27]. This led to the development of numerous modifications.

The Moser model explains growth with characteristics other than exponential growth. To incorporate the effects of microorganisms' adoption of stationary processes through mutation, Moser (1958) upgraded the Monod model with a parameter n (typically n> 1). The specific growth rate matches the Monod model for n=1 [28].

Contois (1959) used both the cell concentration and substrate dependence to determine the precise growth rate. Thus, even though the lag phase is ignored, the effects of inhibition and inoculum are directly included[29]. This model produces good results for both continuous and discontinuous processes, but it has very limited ability to model dynamic processes[30]. The cell concentration, which depends on the degree of substrate degradation, is included via the relationship between substrate concentration and initial substrate concentration in a model modified by Chen & Hashimoto (1978) from Contois (1959).

To investigate the effects of temperature and waste concentration on model parameter values, Hashimoto et al.[31]–[33] propose an unstructured non-segregated model based on kinetic equations like Monod or Contois (μ and K). On the other hand, Hill [34], [35] proposed an unstructured segregated kinetic model that included two different types of biomass and had six equations and ten parameters. Based on the Hashimoto model, other authors[36]–[38] have modeled the anaerobic digestion process.

The total methane output from solid waste substrates was optimized, and it was discovered that the temperature does not affect the initial concentrations of either bacteria or biodegradable volatile solids, which maximizes the total methane output. However, temperature affects the ideal hydraulic residence time and the initial concentration of

volatile fatty acids. To maximize methane output, it was suggested that flow rates should be modified based on temperature[39].

It optimized the amount of biogas produced from vinasse in terms of total solid content [40]. This study employed a batch system and an anaerobic digester on a laboratory scale at room temperature. The results showed that vinasse: water ratio of 1:2 (TS 9.31010.014 percent) had the greatest COD removal (23.58010.532 percent), while vinasse: water ratio of 1:3 (TS 7.015+0.007 percent) produced the most total biogas (37.409 mL/g COD). Overloading in the digester was caused by variables with TS higher than 7.015+0.007 percent, while instability in the decomposition process was caused by variables with TS lower than that.

## 2.3 Enhancement of Biogas production:

A critical review of the literature reveals that there are several ways to improve the production of biogas in the field, some of which are listed below:

- Inorganic additives:

Metal cations could be added to maintain a higher concentration of bacteria in the digester because they make bacteria more dense and able to aggregate on their own[41]. It was discovered that the plant with the higher heavy metal content (Cr, Cu, Ni, and Zn) produced more $CH_4$ than the control[42]. $FeSO_4$ (50 mM) and $FeCl_3$ (70 mM) concentrations of iron salts have been found to increase gas production rate[43], [44]. Due to the activity of Ni-dependent metalloenzymes involved in biogas production, nickel ions (2.5 and 5 ppm) enhanced biogas by up to 54%[45]. Certain adsorbents are also reported to improve gas production for example a maximum enhancement of over 150% with higher $CH_4$ content (65% $CH_4$) on the addition of 10g/L, commercial pectin was obtained [46]. Commercial charcoal Darco G-60, according to [47], increased biogas in batch and semi-continuous fermenters by 17.5% and 34.7%, respectively. Additionally, batch digesters found that locally produced wood charcoal (16 percent improvement in biogas) was just as good as commercial charcoal. On anaerobic digestion of water hyacinth-cattle dung, a trend of improved gas production with high $CH_4$ content and lower effluent BOD and COD was observed with increasing doses of various adsorbents (gelatine, polyvinyl alcohol, powdered activated charcoal, pectin, kaolin, silica gel, aluminium powder, and tale powder) [48]. On adding 4g/L silica gel with a 72.8 percent $CH_4$ content as opposed to the control (62 percent), a two-fold increase in gas production was seen. The process became more stable as the amount of silica gel was

increased, indicating that volatile acids were consumed more quickly when there was an adsorbent present [49]. Ca and Mg salts were used as energy boosters to increase $CH_4$ production and prevent foaming [50]. Eosin Blue dye at a concentration of 0.1 M was found to improve anaerobic digestion of manure by 25–35% [51].

- Gas enhancement via slurry/slurry filtrate recycling (that has been digested):

Reintroducing the washed-away microbes into the reactor through the recirculation of digested slurry has been shown to slightly increase gas production, thereby increasing the microbial population. To reduce water consumption and increase the production of biogas, the recycling of the digested slurry and filtrate gas has also been tried. Simply recycling the digested slurry in 1 $m^3$ plug flow type pilot plants can produce 60–65 percent more biogas[52]. The issue of underfed biogas plants may be resolved, and higher gas production during the winter months may be maintained, by recycling digested slurry along with fresh dung. No additional issues, such as substrate precipitation, an increase in acidity or alkalinity, or ammonia toxicity, could be encountered.

- Temperature:

The process of producing biogas is significantly impacted by the temperature inside the digester. However, anaerobes are most active in the mesophilic and thermophilic temperature range[53]. A two-step anaerobic treatment of cattle dung is proposed [54] that is,

1) acidogenic fermentation at higher temperature(55°-82°C), and

2) separation of the liquid and solid components and low-temperature treatment of the liquid manure (5°-20°C).

Effective treatment of cattle dung at low temperatures was found to require long-term adaptation of active psychrophilic microbial communities. There is, in fact, a published review paper that discusses bio-methanation under psychrophilic conditions[55].

- pH:

The growth of microbes during anaerobic fermentation is greatly influenced by pH. By feeding the digester at the ideal loading rate, the pH can be maintained between 6.8 and 7.2. The pH of the digester's contents is impacted by the amount of $CO_2$ and volatile fatty acids produced during the anaerobic process. VFA concentration, in particular acetic acid concentration, should be less than 2000 mg/L for anaerobic fermentation to proceed normally.

Above pH 5.0, CH4 production was found to be more than 75% efficient by Jain and Mattiasson (1998) [56].

- Pre-treatment:

To increase the methane yield during the anaerobic digestion process, feedstocks occasionally need to be pre-treated. The complex organic structure is reduced during pre-treatment into simpler molecules that are then more amenable to microbial degradation. Pre-treatment could be done in any of the following ways:

1) Pre-treating the feedstock with alkali or acid
2) Pre-digestion of fresh substrate
3) Thermochemical pre-treatment
4) Ultrasonic pre-treatment
5) Ensilage of feed.

Dar and Tandon (1987) found that adding alkali-treated (1 percent NaOH for 7 days) plant residues to cattle dung improved microbial digestibility by 31–42% and nearly doubled the production of biogas [57]. Fresh cattle slurry was pre-digested in a system for 1-2 days at 30-35°C, which increased the production of acetate. This slurry was then used as a feedstock for anaerobic digesters, which increased the production of biogas by 17–19% and the $CH_4$ content from 68–75% to 75–86% [58]. According to Patel's (1993) research, water hyacinth's bio methanation was improved by thermochemical pre-treatment when it was treated at pH 11.0 and 121°C, which produced the best results.

Due to large global alcohol production, millions of tonnes of solid and liquid waste is discharged annually, so the potential for waste-to-energy conversion can make anaerobic digestion an attractive treatment option for the waste streams of distilleries and breweries

But these waste streams are lignocellulosic, containing high fractions of lignin and crystalline cellulose, which suggests pre-treatments before anaerobic digestion can significantly enhance the biogas yield and organic mass degradation[59].

- Particle size:

To avoid clogging the digester and making it difficult for microbes to carry out their digestion, the feedstock should not be too large. On the other hand, similar particles would have a large surface area for absorbing the substrate, which would increase microbial activity and, as a result, increase gas production. Sharma et al. (1988) found that out of five particle sizes (0.088, 0.40, 1.0,6.0, and 3.0mm), The greatest amount of biogas was produced from raw materials with particle sizes between 0.088 and 0.40 mm [60].

- C: N ratio:

To operate a plant efficiently, it is necessary to maintain the feedstock's proper composition so that the CN ratio in the feed stays within the desired range. In anaerobic digestion, carbon is typically found to be used by microorganisms 25–30 times more quickly than nitrogen. Microbes, therefore, require a 20–30:1 ratio of carbon to nitrogen, with the majority of the carbon being easily degradable to meet the requirement [61]. To achieve the desired CN ratio of 30:1, waste materials low in carbon can be combined with materials high in nitrogen[62]. According to the research done by Kinani and Laura (1971), the addition of 200ml of urine nearly doubled the biogas production from 0.5 kg of cow thing to 17.2-31.5 L. Utilizing waste materials that have been exposed to urine is especially beneficial during the winter when gas production is typically low [63].

- Agitation:

It is necessary to stir the contents of the digester to ensure close contact between the substrate and the microorganisms, which ultimately leads to a better digestion process. Agitation of digester contents can be accomplished in a variety of ways, such as daily slurry feeding rather than periodic feeding to achieve the desired mixing effect.

- Organic loading Rate (OLR):

Loading rate has a significant impact on gas production rate. With a decrease in loading rate, methane yield was found to rise [64]. Another study conducted in Pennsylvania on a 100 $m^3$ biogas plant (working on manure) found that increasing OLR from 346 kg per day to 1030 kg per day increased gas yield from 67 to 202 $m^3$ per day. Based on pilot plant studies (1 $m^3$ capacity maximum gas yield was observed for a loading rate of 24 kg dung/$m^3$ in digester per day although the percent reduction of VS was only 2/3 of that with a low loading rate.

- Hydraulic retention time (HRT):

The HRT measures how long on average the input slurry stays in the digester before it exits. HRT ranges from 30 to 50 days in tropical nations like India, but it can reach 100 days in countries with a colder climate. Desai and Madamwar (1994) observed maximum gas production of 2.2 I/day($CH_4$ 62%) at an HRT of 10 days having a loading rate of 6 g TS/L while treating a maximum of cattle dung, poultry waste, and cheese whey in the ration of 2:1:3 [61]. Baserja (1984) observed that at a TS concentration of 7% the duration of digestion could

be reduced to 10 days without compromising the stability of the process, but the optimum period was 16-20 days [65].

- Solid concentration:

The amount of fermentable material of feed in a unit volume of slurry is defined as solid concentration. Ordinarily 7-9% solids concentration is best suited[17]. The biogas yield increased reaching 0.46 $m^3$/ ($m^3$ day) at 37°C and 0.68 $m^3$/ ($m^3$ day) at 55° C respectively. Baserja (1984) reported that the process was unstable below a total solid level of 7 % (of manure) while a level of 10% caused an overloading of the fermenter.

# **Chapter 3:** Process Description

### 3.1 Anaerobic digestion process

Organic material is transformed into biogas and digested without the presence of oxygen during the multi-step biological process known as anaerobic digestion. When there is no oxygen present and anaerobic microorganisms are present, organic material is biodegraded anaerobically. AD results from several metabolic interactions between various microorganism groups. Before the uptake of organic compounds, viable bacteria must first be killed and lysed using anaerobic decomposition of viable, biological solids (such as waste-activated sludge, algae, etc.). Lipids, proteins, and carbohydrates found in the sludge are biologically broken down by extra-cellular enzymes like lipase, protease, and cellulase to produce small, soluble products that can cross bacterial cell membranes and go through various intracellular metabolic processes. There, they are transformed into intermediary substances such as fatty acids, amino acids, acetic acids, sugars, and hydrogen. These intermediate substances are then transformed into biogas, which is primarily made up of methane ($CH_4$) and carbon dioxide ($CO_2$)[66].

Hydrolysis/liquefaction, acidogenesis, acetogenesis, and methanogenesis are the four stages that it goes through. Cellulose, hemicellulose, pectin (carbohydrate), and lignin (noncarbohydrate) polymers are the main substrates of lignocellulose-degrading enzymes. They are abundant in the primary cell wall and dietary fibers of most fruits and vegetables [67].

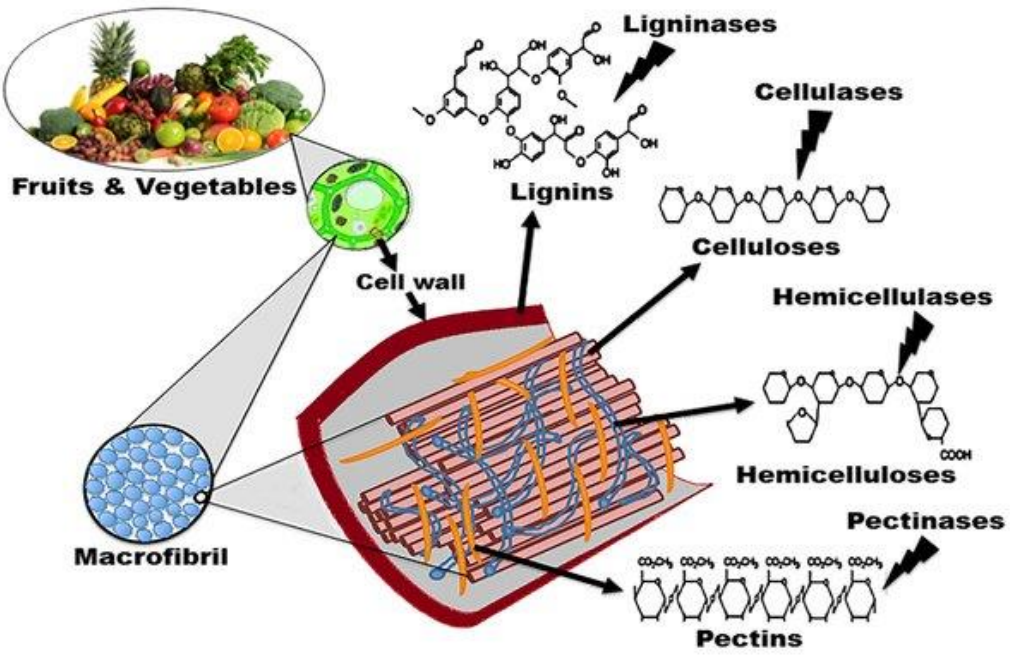
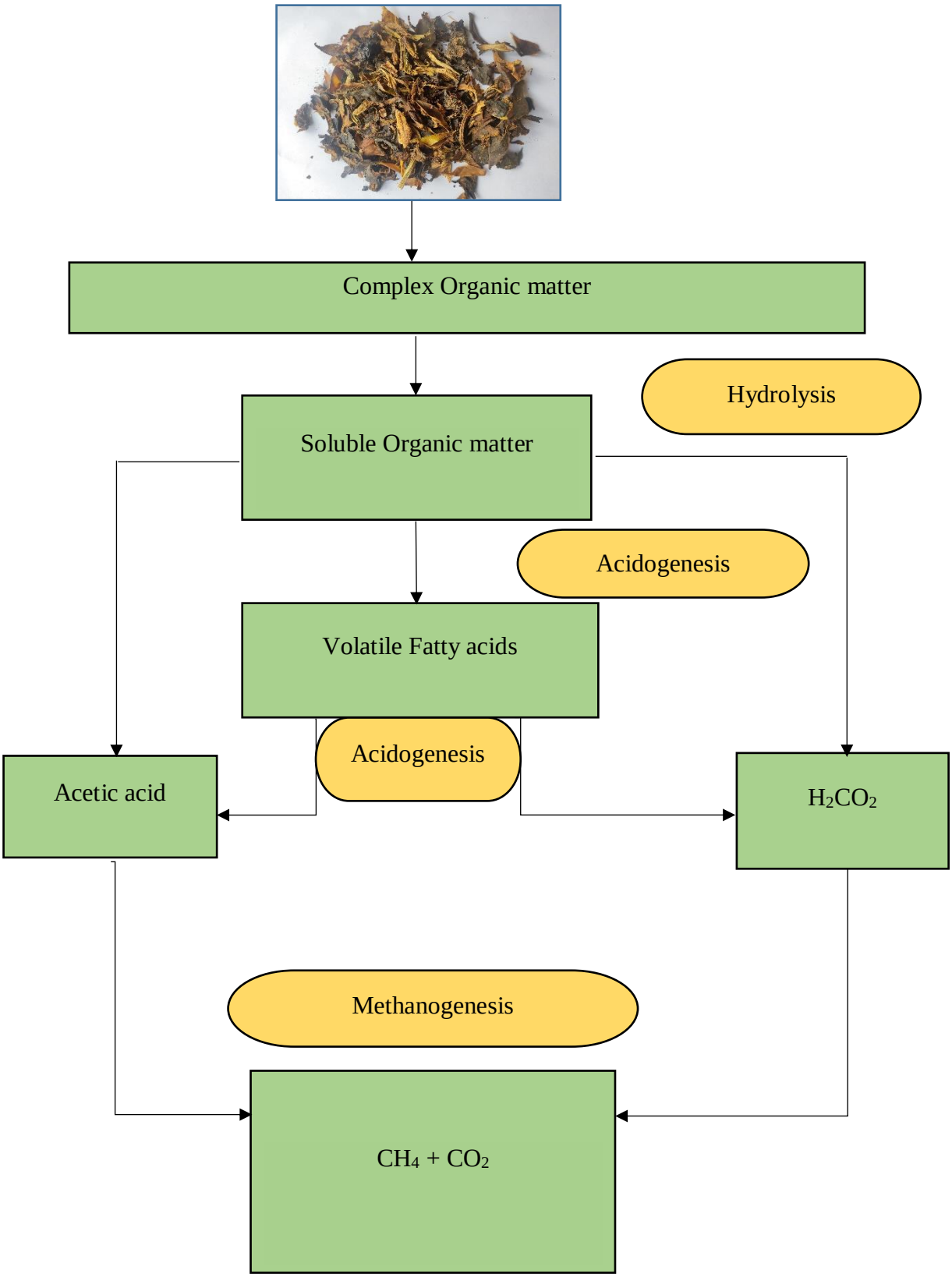



Figure 2.1: Schematic illustration of lignocellulosic substrates (cellulose, hemicellulose, pectin, and lignin) in fruits and vegetables***[67]***

Table 2.1: Flow diagram of the anaerobic digestion process

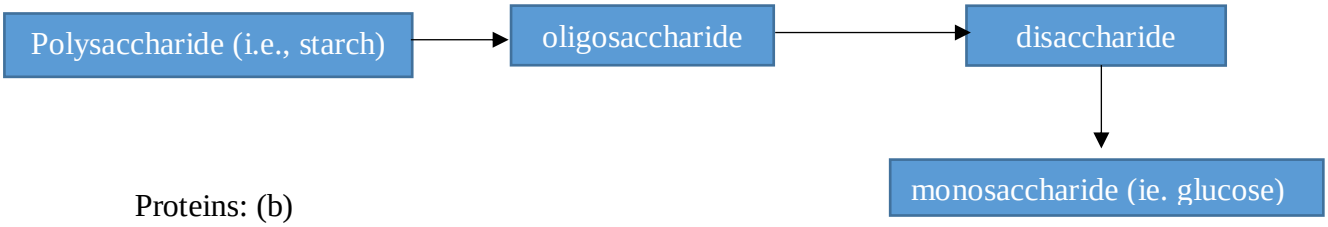


- Hydrolysis/Liquefaction:

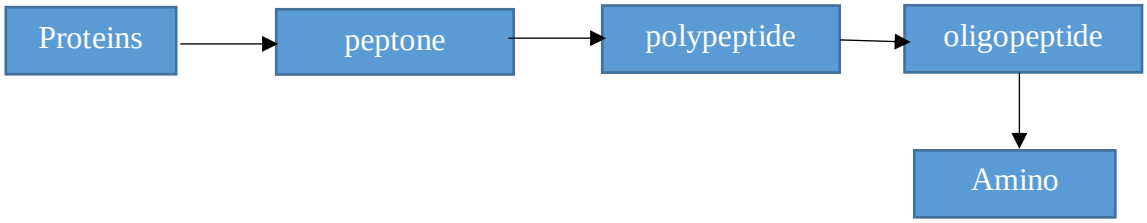
  Since fermentative microorganisms cannot directly utilize polymers, this step is crucial for the AD process. The substrate is made available for the subsequent conversion process by hydrolysis. For insoluble complex organic matter to pass through the membrane of the microbial cell, it is disassembled into its parts in this step [68]. Hydrolytic enzymes carry out the hydrolysis process. Fermentative bacteria transform complex organic matter that is insoluble, like cellulose, into soluble molecules, like sugars, amino acids, and fatty acids, in the first stage of hydrolysis. Proteases secreted by proteolytic microbes convert proteins into amino acids, whereas celluloses and/or xylanases produced by cellulitic and xylanolytic microbes hydrolyze cellulose and xylose (both complex carbohydrates) into glucose and xylem, respectively. Lipids (fats and oils) are finally transformed into long-chain fatty acids and glycerol by lipases, which are produced by lipolytic microbes[69]. In high organic waste, the hydrolytic activity is crucial and could become a rate-limiting factor. Some industrial processes use chemical reagents to enhance hydrolysis to get around this restriction. It has been discovered that adding chemicals to the first step can speed up the digestion process and increase methane hydrolysis reactions.

  The hydrolysis of lipids, proteins, and carbohydrates is illustrated by reaction pathways (a), (b), and (c) [70].

  Carbohydrates: (a)




  Proteins: (b)




  Lipids: (c)


- Acidogenesis (fermentation):

Fermentation involves converting sugars, amino acids, and fatty acids into hydrogen, acetate, carbon dioxide, VFAs like propionic, butyric, and acetic acid, ketones, alcohols, and lactic acid using facultative and anaerobic bacteria. Even though a straightforward substrate like glucose can be fermented, the diverse bacterial community still produces a variety of products. The conversion of glucose to acetate, ethanol, and propionate, respectively, is shown in equations d, e, and f.

$C_6 H_{12}O_6 + 2 H_2O \rightarrow 2CH_3COOH + 2 CO_2 + 4 H_2$ (d)

$C_6H_{12}O_6 + 2 CH_3CH_2OH + 2CO_2$ (e)

$C_6H_{12}O_6 + 2H_2 \rightarrow 2 CH_3CH_2COOH + 2H_2O$ (f)

Acetate, hydrogen, and carbon dioxide are the most readily available substrates for methanogenic microbes in an equilibrium system, but a sizeable portion of the organic matter—roughly 30%—is converted to short chain fatty acids or alcohols [71]. In this stage, biodegradable organic material is eliminated. Ammonia and hydrogen sulfite, which are by-products of the fermentation of amino acids, can inhibit AD.

- Acetogenesis:

It is the process by which certain fermentation products, like VFAs with more than two carbon atoms, alcohols, and aromatic fatty acids, are transformed into acetate and hydrogen by obligate hydrogen-producing bacteria[72]. Acetogenic bacteria, also known as acid formers, transform the first-phase products into simple organic acids, carbon dioxide, and hydrogen in this stage. Acetic acid, propionic acid, butyric acid, and ethanol are the main acids produced. Numerous different microbes, such as, are responsible for the products created during acetogenesis. *Syntrophomonos wolfei*, a butyrate decomposer, and *Syntrophobacter wolinil*, a propionate decomposer. Clostridium spp., *Peptococcus Antrobus*, Lactobacillus, and Actinomyces are additional acid formers (www.biogasworks.com. microbes in AD). Homoacetogenic bacteria produce acetate from CO and H while hydrogen-producing acetogenic bacteria produce acetate, hydrogen, and carbon dioxide from volatile fatty acids and alcohol. However, bacteria that produce

acetogenic hydrogen are what produce the majority of the acetate. Below is a diagram of an acetogenesis reaction:

$C_6H_{12}0_6 \rightarrow 2C_2H_5OH + 2CO_2$

- Methanogenesis:

Since no single species can break down all the available substrates, the AD system needs a variety of methane-forming bacteria. *Methanobacterium*, *Methanobacillus*, *Methanococcus*, and *Methanosarcina* are among the methanogenic bacteria. Acetate and $H_2/CO_2$ consumers are two additional categories for categorizing methanogenesis. As consumers of acetate and $H_2/CO_2$, methanosarcina spp. and methanothrix spp. (also known as methanosacta) are thought to be significant in AD. The production of methane from acetate accounts for roughly 70% of its total production[73], with the remaining 30% coming from the reduction of $CO_2$ by $H_2$ and other electron donors. Methanogenesis is divided into two main types [74] based on the type of substrate used by the methanogens:

1. Hydrogenotrophic methanogenesis. $H_2$ and $CO_2$ are converted into methane according to the following reaction:

$CO_2 + 4H_2 \rightarrow CH_4+2H_20$

2. Acitotrophic or acetoclastic methanogenesis. Methane is formed from the conversion of acetate through the following reaction.

$CH_3COOH\rightarrow CH_4+ CO_2$

## 3.2 Important operating parameters in the AD process

The AD process depends critically on how quickly the microorganisms grow. To increase microbial activity and the system's anaerobic degradation efficiency, the digester's operating parameters must be kept under control. The section after this one discusses some of these variables.:

- Waste composition/ Volatile solids (VS):

An organic biodegradable, combustible, or inert fraction may be present in the waste treated by AD. Kitchen waste, food waste, and garden waste are all included in the biodegradable organic fraction. The slowly deteriorating lignocellulosic organic matter, which includes coarser wood, paper, and cardboard, is included in the combustible fraction. These lignocellulosic organic materials are better suited for waste-to-energy plants because they do not easily degrade under anaerobic conditions. The inert fraction also includes metal, glass, sand, and other materials. The ideal solution would be to remove, recycle, or

landfill this portion. Before digestion, the inert fraction should be removed to reduce equipment wear and increase digester volume. The total solids minus the ash content, as produced by the complete combustion of the feed waste, are used to calculate the volatile solids (VS) in organic wastes. The biodegradable volatile solids (BVS) fraction and the refractory volatile solids make up the volatile solids (RVS). It has been observed that understanding the BVS fraction to substrate aids in a more accurate estimation of the rate of organic loading, C/N ratio, and biodegradability of biogas waste. The RVS in organic matter is made up of lignin, a complex organic substance that anaerobic bacteria find difficult to break down. The best wastes for AD treatment are those with high VS and low non-biodegradable matter or RVS.

• Alkalinity:

Alkalinity refers to a digester's ability to neutralize or buffer acids. It is achieved with the aid of several substances, but the digester's carbonate, bicarbonate, and hydroxide content best describe it [75]. Since the $CO_2$-bicarbonate system is primarily in charge of regulating alkalinity at the neutral pH at which anaerobic digesters function, bicarbonate alkalinity is of utmost significance [76]. For bacteria that produce methane, bicarbonate serves as the primary source of carbon. Alkalinity is important for pH regulation and improves digester stability. At a specific pH, the majority of the alkalinity is present as bicarbonate, which is in equilibrium with $CO_2$ [77]. Another source of alkalinity in AD is the breakdown of organic nitrogen-containing compounds. These substances include proteins and amino acids. When they degrade, amino groups are released, which react with oxygen to produce ammonium bicarbonate, an alkaline product. According to Speece et al, 1996, 2008, The metabolism of the microorganisms in AD can produce more alkalinity. When organic compounds degrade, cations are released, resulting in this type of alkalinity [78].

- pH level:

Different groups of microorganisms involved in AD have different pH requirements. While methanogenic bacteria need a pH of at least 6.2 to function, acidogenic bacteria can thrive in environments with pH levels above 5. Acidic conditions can inhibit anaerobic bacteria's growth because they are sensitive to the digester's acid concentration, especially the methanogens. According to research, the ideal pH range for AD during digestion is between 5.5 and 8.5. The two processes of acidification and methanogenesis require different pH ranges for the best process control. In a batch reactor, acetogenesis proceeds quickly and the pH value is influenced by the digestate's retention time. When large

amounts of organic acids accumulate due to acetogenesis, the pH can fall below 5. The pH is the best predictor of digester instability after gas production[79]. The pH will initially drop as acetogenesis takes place on organic matter, but methanogens quickly consume those acids, restoring pH to normal and stabilizing digester performance. Due to their sensitivity to acidic environments, methanogens are inhibited by excessive acid production. By adding lime or recycled filtrate obtained from residue treatment, pH reduction can be controlled.

• Sulphate:

If the sulphite concentration is higher than 200 mg/L, the biological process that converts sulphate to sulphide in the AD system may be upset. Some inhibitory substances, such as LCFA and phthalate esters, may have an equal impact on all significant microbial groups in the digester. Some microbial species may specifically suffer from the effects of others.

• Temperature:

The main environmental factor influencing performance is temperature. It has an impact on the kinetics and thermodynamics of biological processes as well as the physical and physicochemical properties of the compounds present in the digester. The mesophilic and thermophilic ranges are primarily the two temperature ranges that offer the best conditions for digestion and the production of methane.:

1. Where mesophilic are the predominant microorganism present, mesophilic digestion occurs best between 30°C-38°C or at ambient temperatures between 20°C and 45°C.

2. When thermophilic microorganisms are the predominant ones present, thermophilic digestion occurs best between 49 and 57 degrees Celsius or at elevated temperatures up to 70 degrees Celsius.

However, for the majority of AD processes, the mesophilic temperature range is preferred. The two main causes of this are that such low process temperatures are challenging to maintain, especially during the summer, and that AD carried out by psychrophilic bacteria is slower than that carried out by mesophilic and thermophilic bacteria. Although biogas productivity is the highest, thermophilic bacteria are known to be extremely sensitive to disturbances, necessitating expensive process monitoring and control[80].

- Carbon to Nitrogen ratio (C/N):

The C/N ratio serves as a representation of the relationship between the amounts of carbon and nitrogen present in the feedstock. It is a crucial process parameter because a low ratio can inhibit ammonia production while a high ratio will result in a deficiency. Co-digestion of various waste streams can be used to adjust the ratio to fall within the desirable range (25–30); the ideal C/N ratio for anaerobic digesters is between 20 and 30. Low gas production is caused by a high C/N ratio, which indicates that methanogens are quickly consuming the nitrogen. Contrarily, a lower C/N ratio results in ammonia buildup and pH levels higher than 8.5 are toxic to methanogenic bacteria. By combining materials with high and low C/N ratios, such as organic solid wastes combined with animal manure and sewage, the optimal C/N ratio of the digester materials can be achieved.

- Nutrient:

Specific growth conditions apply to bacteria that produce methane. It is known that certain metals, such as iron, nickel, cobalt, and molybdenum, are necessary for methane production and healthy growth. Trace metals are crucial for promoting methanogenic activity. As supplemental elements in media, selenium, molybdenum, manganese, aluminium, and boron have been suggested [81]. Iron, cobalt, nickel, and zinc are each recommended at 0.002, 0.004, 0.003, and 0.02 mg/g of acetate produced, respectively. It should be noted that biological systems rarely require nickel and that methanogenic bacteria are the only organisms that do. Anaerobic digesters can function more efficiently if they are supplemented with a metal ion solution.

- Total solids content (TS) /organic loading rate (OLR):

Low solids (LS) AD systems have less than 10% total solids (TS), medium solids (MS) are between 15% and 20%, and high solids (HS) processes range from 22% to 40%. Reactor volume decreases in proportion to an increase in TS in the reactor. The organic matter flowing into the digester at any given time is known as the organic loading rate or OLR. OLR values typically range from 0.5 to 3 kg VS/$m^2$/d [79]. OLR is also described as a measurement of the AD system's capacity for biological conversion. Low biogas yield occurs when the system is fed above its sustainable OLR because the digester slurry builds up with inhibitory substances like fatty acids. In this situation, the system's feeding rate must be slowed down.

- Retention (or residence) time:

Solids retention time is also known as SRT and hydraulic retention time is known as HRT. HRT is the amount of time the feed's fluid component is kept in the digester. The term "SRT" refers to the amount of time that bacteria (solids) have been present in the reactor. With different technologies, process temperatures, and waste compositions, different retention times are needed for the AD reaction to being completed. Waste treated in a mesophilic digester is retained for 10 to 40 days. In digesters run in the thermophilic range, shorter retention times are necessary. The retention time for a high solids reactor operating in the thermophilic range is 14 days. Retention times for anaerobic digestion range between 14 and 30 days. SRT should be longer than 12 days to prevent microbial washout due to the relatively long generation time of methanogens [77]. A short retention period will result in greater biogas production per volume but less organic matter degradation. A balance needs to be struck to achieve the desired operational conditions, even though a short retention time is preferred to reduce the digester volume.

# **Chapter 4:** Experimental Setup & Procedure

## 4.1 Collection and preparation of sample:

Mixed vegetable waste, mostly leaves and peels of various vegetables like cauliflower, Beans, cabbage, green chili, gourd, spinach, pumpkin, etc., was collected from a local market in Jadavpur, south 24 Parganas. The mixed waste was shredded into pieces that ranged in size from 2.5 to 3.5 cm.. then the mixed sample was kept inside a hot air oven for three days to minimize the moisture content.

After three days the dried sample was applied to a Willey mill to attain a particular size. The smaller pieces of the waste were allowed to pass through IS sieves of 7 mm size. The fine sample was stored in air-tight containers kept inside a desiccator to avoid atmospheric moisture absorption [82].

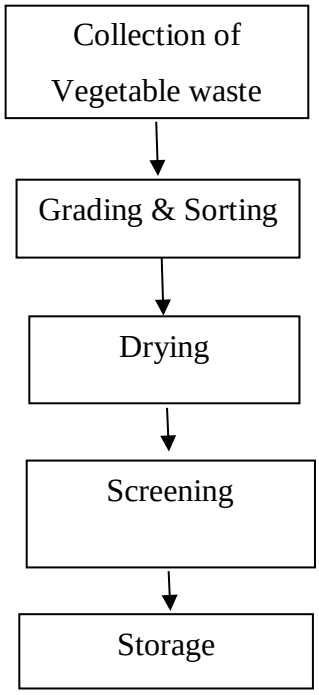



**Figure 3.1: Sample preparation flow diagram**

## 4.2 Inoculum preparation

We need an inoculum rich in bacteria, particularly methanogens that will break down the biomass more quickly, to begin the anaerobic digestion process. Cow dung is frequently regarded as an inoculum suitable for the start-up of anaerobic digesters because it contains a diverse microbial community that can easily adapt to changing operational conditions. Fresh cow dung was gathered from a cowshed In Barasat, north 24 Parganas. For one day, the gathered cow dung was dried in the sun. Cow dung was then finely ground in a ball

mill and further screened through IS sieves of a size of 1 mm before being stored in airtight containers and kept at 4°C until further use.

## 4.3 Preparation of the Substrate solution

According to the literature review, 7-10% solid concentration is best suited for anaerobic digestion. Three concentrations were picked up to study the process such that two of them fall below and above the range (6.5% & 10.5%) and one falls within the range (8%).

So, for this research, three different percentages of total solid concentration were chosen 6.5%,8%, and 10%. A 2% solution of the inoculum was prepared using the dried cow dung. The inoculum was stirred well to form a consistent slurry. A magnetic stirrer at 500 rpm for 30 minutes was used for this purpose.

**Table 3.1: composition of the material in each digester.**

| Digester | Concentration (weight/volume) | Vegetable waste (g TS) | Inoculum(ml) | Water (ml) |
|---|---|---|---|---|
| A | 6.5% | 24.4 | 10 | 365 |
| B | 8% | 30 | 10 | 365 |
| C | 10.5% | 39.4 | 10 | 365 |

## 4.4 Analysis of the substrate

- Determination of moisture:

In a silica crucible, 5g of finely powdered, air-dried samples are weighed. The crucible's surface area ought to be chosen so that the sample weight per square centimeter doesn't go over 0.3g. In an air oven, the exposed sample is heated for about an hour from 105°C to 110°C. The sample is then taken out of the oven, cooled over calcium chloride or sulfuric acid in a desiccator, and weighed. The sample is heated continuously at the designated temperature until its weight reaches a constant value. After that, the moisture content is calculated by converting the weight loss to a percentage.

- Determination of volatile matter:

A silica crucible is filled with 1g of the final powdered, air-dried sample. By lightly tapping the crucible on the table, the sample is spread out into an even layer. The muffle furnace's door is properly closed after the crucible has been moved inside and is kept at a temperature of 925° C. The sample is kept in the furnace for precisely 7 minutes before the crucible is removed and placed on a cold iron plate to ensure quick cooling.

The crucible is moved to a desiccator while it is still warm, and once it has cooled, it is weighed. The final result is reported as volatile matter less moisture after the weight loss is calculated to a percentage based on the sample that was taken.

- ❖ Determination of ash:

A silica crucible containing about 1 g of the finely powdered, air-dried sample weight is set inside a muffle furnace at room temperature. For 30 minutes, the sample is heated to between 400°C and 450°C, and this temperature is held for 30 minutes. The incineration is then finished by beating at a temperature of 775+/-25° C for an hour.

The crucible with the residue is then finished heating, placed on a cold iron plate for quick cooling, and while it's still warm, put in a desiccator. Once it's cold, it's weighed. Before inserting the crucible into the desiccator, it is preferable to cover it with a lid to prevent light ash from being blown away by the desiccator's rush of air when it is opened. The incineration should be repeated if incomplete combustion is suspected until the weight is constant. On the sample that was taken to determine the ash content, the residue that was left over after heating is calculated as a percentage.

- ❖ Determination of fixed carbon:

It is determined by subtracting from 100 the total moisture content, volatile matter, and ash content.

- ❖ Determination of C:H: N:

The combustion process used by the analyzer reduces substances into simple compounds so they can be measured. An automated process is used for organic elemental analysis. the homogenization of product gases, reduction and combustion, separation, and detection. The 2400 Series Il offers improved combustion features under user-directed microprocessor control and combustion conditions of temperature, available oxygen (or pyrolysis gas in the case of Oxygen Mode), and time, and to further optimize the combustion step for the broadest range of samples. The integrated solid-state pressure transducer in the 2400 Series II ensures constant pressure that is unaffected by changes in

flow rate, barometric pressure, or altitude. Direct connection to a personal computer or LIMS system for data storage and analysis is possible using an industry-standard RS-232C port. As a result, it provides the weight percentage of the components or the component to be measured.

## 4.5 Digester feed and supplements

The ground vegetable waste stored in air-tight containers was used as feed for the growth of microorganisms in this experiment.

In the first batch of the experiment, three digesters were used having concentrations of 6.5%, 8%, and 10.5% TS.

The second batch of the experiment was run using 5 different types of supplements which were selected based on the literature review. In this set total of 15 digesters were used to study the kinetics of the anaerobic process. Those supplements are :

- MgO
- FeCl3
- FeSO4
- Pectin
- Silica gel

The supplements and the percentage of dosing were selected based on the literature review.

**Table 3.2: Supplements used and their doses**

| Supplement | Dose |
|---|---|
| MgO | 647 mg/L |
| $FeCl_3$ | 70 µM |
| $FeSO_4$ | 50mM |
| Pectin | 10 g/L |
| Silica gel | 4 g/L |

## 4.6. Experimental Layout

Every experiment has been carried out in glass 0.5 L batch digesters. A set of a total of 18 digesters was used. Three of the digesters had no supplements.

The working volume of the digester was 375 ml. 125 ml volume of the digester was left as headspace.

Since temperature changes have a negative impact on biogas production, a mesophilic temperature range of 33–34°C was maintained throughout the experiment. To maintain the temperature, a B.O.D. incubator was used for this purpose. The mesophilic temperature range was selected because mesophilic bacteria can withstand temperature changes of up to 3°C without significantly reducing their ability to produce methane. To prevent methanogenesis from being significantly inhibited, pH was attempted to be maintained between 6 and 8.

To maintain anaerobic conditions inside the digesters, rubber corks and cement were used to seal the digesters. It was permitted to remain still for three days. For the processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, at least 3 days are needed. Following these processes, the gas volume was measured every 24 hours using the downward displacement of water method. To maintain anaerobic conditions inside the digesters, rubber corks and cement were used to seal the digesters. It was permitted to remain still for three days. For the processes of hydrolysis, acidogenesis, acetogenesis, and methanogenesis, at least 3 days are needed. Following these processes, the gas volume was measured every 24 hours using the downward displacement of water method.

**Figure 3.2 Digesters used in the experiment**

**Figure 3.3: Hot air oven**

**Figure 3.4: Apparatus to measure Biogas by downward displacement of water**

**Figure 3.5: Willey mill**

**Figure 3.6: Magnetic Stirrer**

**Figure 3.7: Incubator**

# **Chapter 5:** Kinetic Model

## 5.1 Chen- Hashimoto Kinetic Model

For effective reactor design where operating conditions, methane ($CH_4$) production, system stability, and effluent quality can be predicted or specified, an understanding of process mechanisms and kinetics is necessary. To gain a deeper understanding of the factors affecting the biochemical AD process, several models have been built.

Determining kinetic parameters becomes a complex task because it is difficult to quantify the number of active microorganisms inside a digester. Chen and Hashimoto proposed a kinetic model for the digestion of complex substrates over a wide range of retention times. Since this model is realistic, measuring the bacterial concentration periodically to determine the kinetic coefficients is not necessary.

The following equations describe how cell mass and substrate concentration change at different rates in a fully mixed continuous digester:

$$\frac{dX}{dt} = \mu X - \frac{X}{\theta}$$

$$\frac{dS}{dt} = -r + \frac{\mathrm{S0} - \mathrm{S}}{\theta}$$

Where,

X is the concentration of cell mass,

μ is the specific microbial growth rate,

$S_0$ the concentration of substrate in the influent,

θ the hydraulic retention time,

S the concentration of substrate in the effluent,

r is the volumetric substrate utilization rate.

The relationship between r and μ is defined by the following equation:

$$\mu = \frac{Yr}{X}$$

Where,

Y is the yield coefficient (cell mass/substrate mass) and is considered constant.

In the steady-state, dX/dt=0 and dS/dt=0, hence

$\mu = 1/\theta = D$

Where D is the dilution rate.

$$r = \frac{So - S}{\theta}$$

$$X = \frac{Y}{So - S}$$

Substituting these expressions in Contois' equation:

$\mu = \frac{\mu maxS}{ßX+S}$

Where,

$\mu_{max}$ is the maximum specific microbial growth rate

ß is a dimensionless kinetic parameter

$$\frac{S}{So} = \frac{K}{(\mu max\ \theta - 1 + K)}$$

Where K is a dimensionless kinetic parameter.

This equation demonstrates the relationship between influent substrate concentration and effluent substrate concentration.

The minimum retention time at which microorganism washout occurs is proportional to the maximum growth rate in numbers.

$\theta_{min} = \frac{1}{\mu max}$

Finding the rate-limiting substrate for the kinetic evaluation is the method used to study the kinetics of biogas production from complex substrates.

The biodegradable substrate concentration in the reactor is directly proportional to ($B_0$-B), and $B_0$ will be directly proportional to the biodegradable substrate loading if B denotes the volume (in liters) of methane produced under normal conditions of pressure and temperature per gram of substrate at the infinite retention time or for complete utilization of substrate.

Therefore,

$$\frac{Bo - B}{Bo} = \frac{K}{\mu max\ \theta - 1 + K}$$

$$\theta = \frac{1}{\mu max} + \frac{K}{\mu max}\frac{B}{Bo - B}$$

Thus, by first calculating the value of B0, the graph of θ versus B/($B_0$-B) produces a straight line with an intercept of 1/μmax and with a slope of K/μmax. To obtain the parameter $B_O$ one uses the following equation, which is easily derived:

$$B = Bo\ [1 - \frac{K}{\mu max\ \theta - 1 + K}]$$

Since B is the methane production per gram of added substrate, the volumetric methane production rate (δ) equals B multiplied by the loading rate:

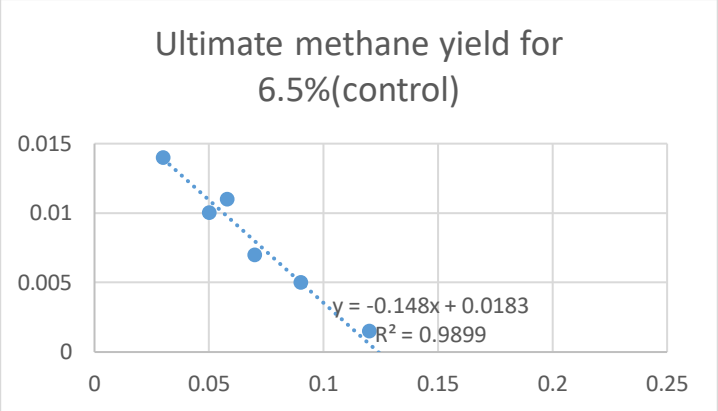
$$\delta = \frac{BSo}{\theta} = [\frac{BoSo}{\theta}]\ [1 - \frac{K}{\mu max\ \theta - 1 + K}]$$

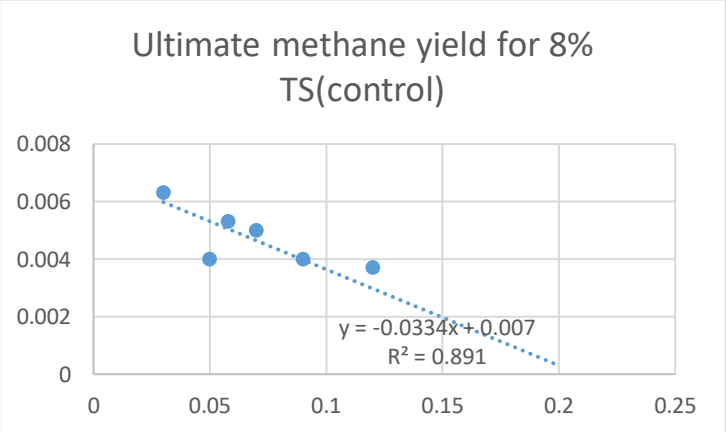
Where δ has the dimensions of volume methane per volume digester per unit time.

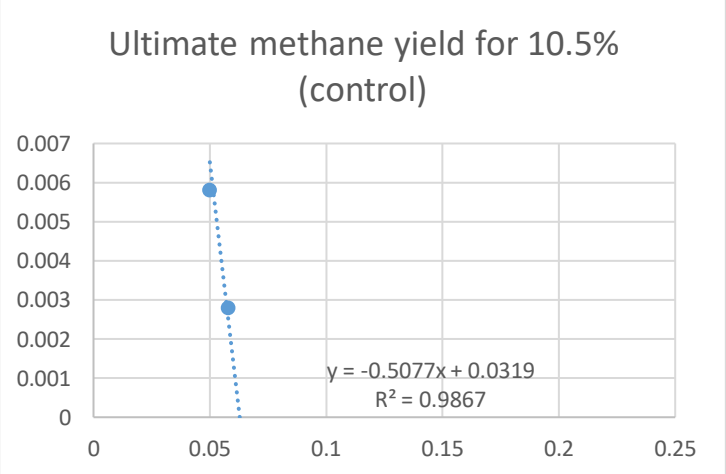
## 5.2. Evaluation of Ultimate methane yield:

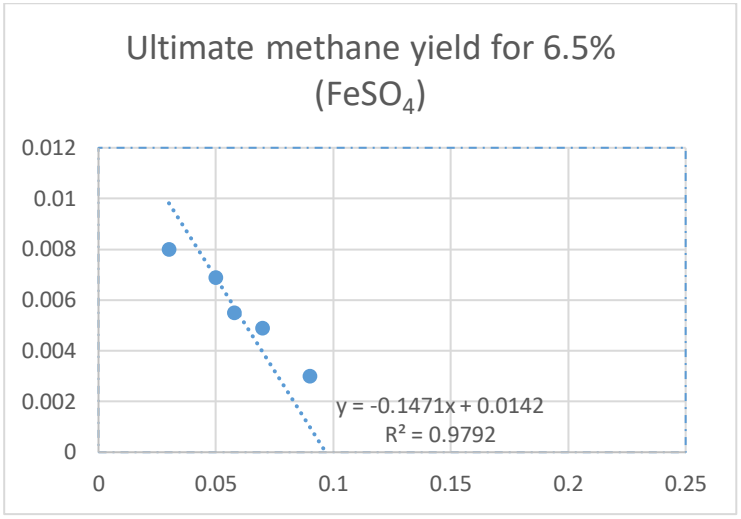
To obtain the ultimate methane yield ($B_0$), a graph of cumulative methane yield (B) is plotted against the inverse of retention time. The intercept of the graph gives the ultimate methane yield. Hence experimental per day methane yield was used to calculate the cumulative methane yield (B) and plotted against 1/T for the thirty-one digesters. From the graphs, the intercept of each line has been evaluated for ultimate methane yield ($B_0$).

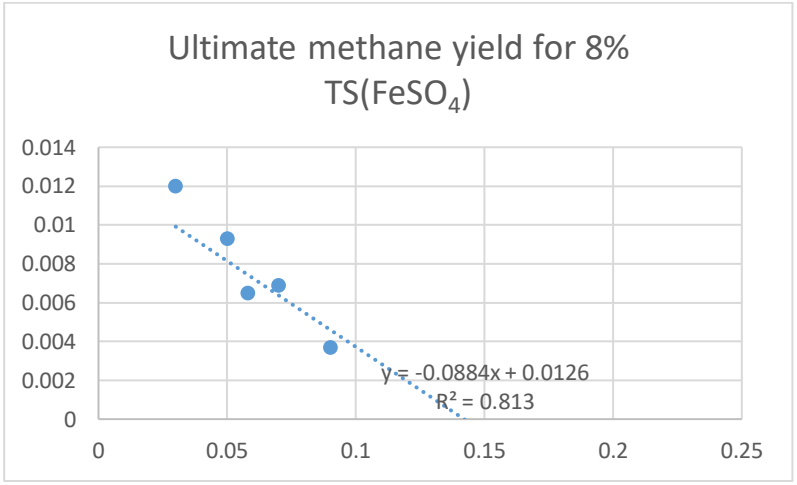
Following are the graphs for the evaluation of B°

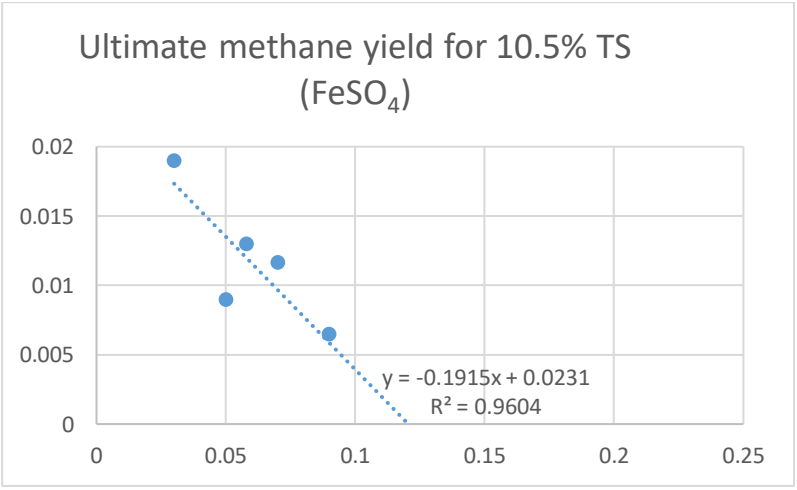

Ultimate methane yield for 6.5%(control)
0.015
0.01
0.005
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.148x + 0.0183
R² = 0.9899

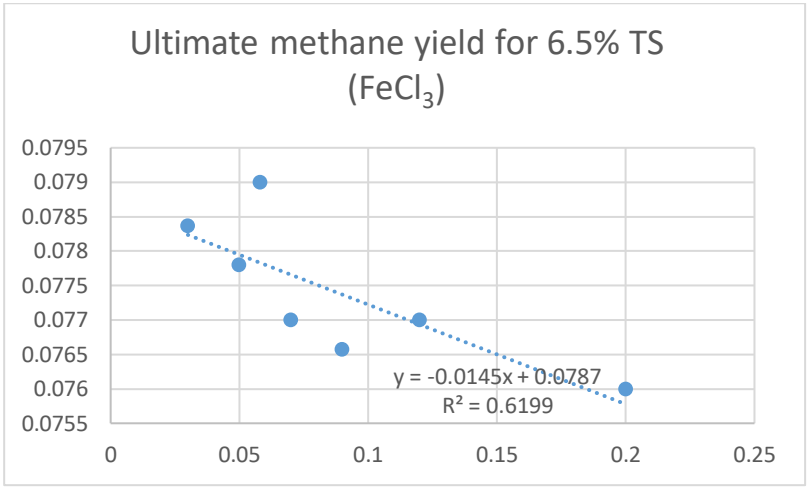

Ultimate methane yield for 8% TS(control)
0.008
0.006
0.004
0.002
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0334x + 0.007
R² = 0.891

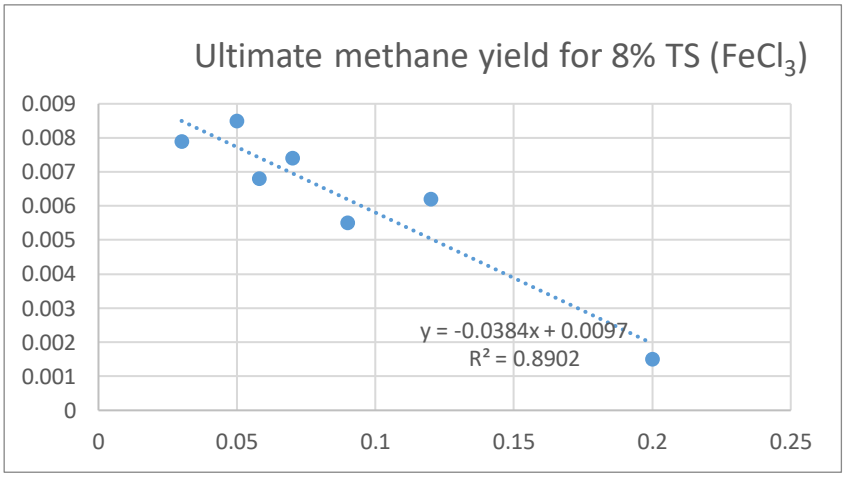

Ultimate methane yield for 10.5% (control)
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.5077x + 0.0319
R² = 0.9867

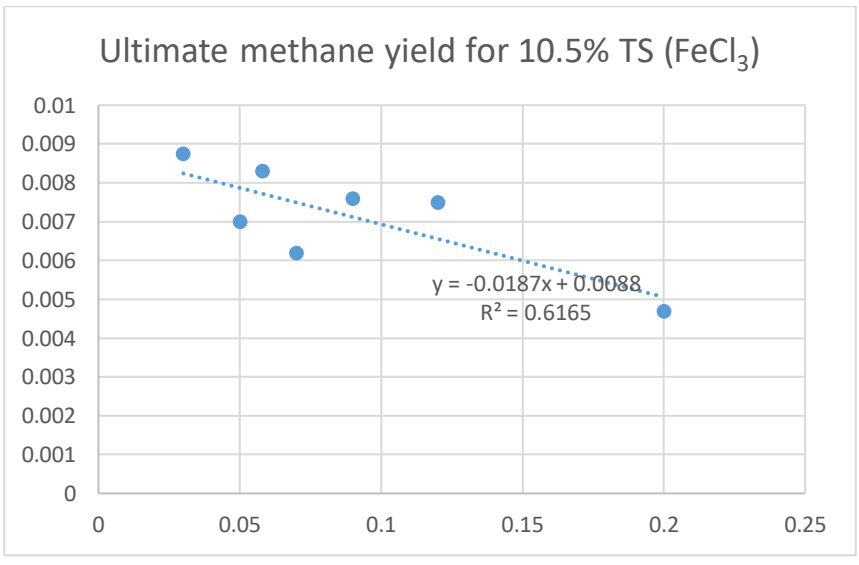

Ultimate methane yield for 6.5% ($FeSO_4$)
0.012
0.01
0.008
0.006
0.004
0.002
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.1471x + 0.0142
R² = 0.9792

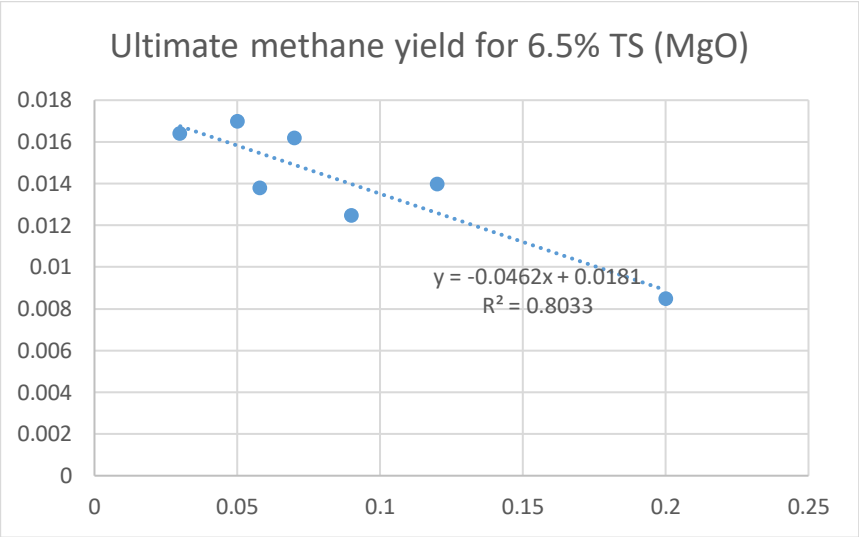

Ultimate methane yield for 8% TS($FeSO_4$)
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0884x + 0.0126
R² = 0.813

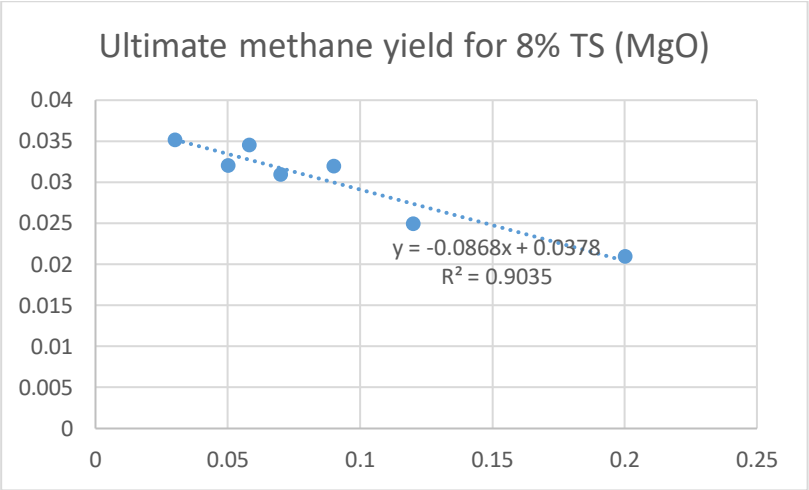

Ultimate methane yield for 10.5% TS ($FeSO_4$)
0.02
0.015
0.01
0.005
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.1915x + 0.0231
R² = 0.9604

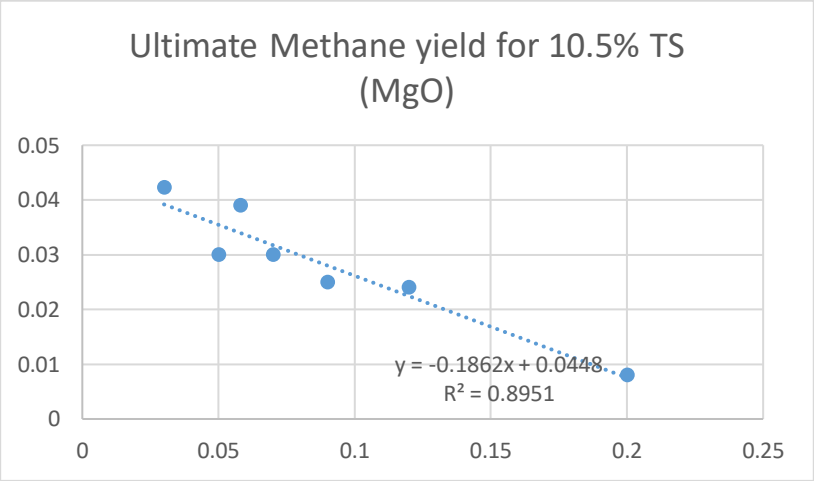

Ultimate methane yield for 6.5% TS ($FeCl_3$)
0.0795
0.079
0.0785
0.078
0.0775
0.077
0.0765
0.076
0.0755
0
0.05
0.1
0.15
0.2
0.25
y = -0.0145x + 0.0787
R² = 0.6199


Ultimate methane yield for 8% TS ($FeCl_3$)
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0384x + 0.0097
R² = 0.8902


Ultimate methane yield for 10.5% TS ($FeCl_3$)
0.01
0.009
0.008
0.007
0.006
0.005
0.004
0.003
0.002
0.001
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0187x + 0.0088
R² = 0.6165


Ultimate methane yield for 6.5% TS (MgO)
0.018
0.016
0.014
0.012
0.01
0.008
0.006
0.004
0.002
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0462x + 0.0181
R² = 0.8033


Ultimate methane yield for 8% TS (MgO)
0.04
0.035
0.03
0.025
0.02
0.015
0.01
0.005
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.0868x + 0.0378
R² = 0.9035


Ultimate Methane yield for 10.5% TS (MgO)
0.05
0.04
0.03
0.02
0.01
0
0
0.05
0.1
0.15
0.2
0.25
y = -0.1862x + 0.0448
R² = 0.8951

## 5.3. Evaluation of Kinetic parameters μ and K:

To evaluate specific growth rate ($\mu_{max}$) and kinetic constant (K) another set of a graph is needed in which retention time (T) is plotted against $B/(B_0-B)$.

Accordingly, μ =1/intercept and

K=slope/intercept.

Values of $B/(B_0-B)$ were evaluated using $B_O$ values and plotted Vs time for the 18 digesters. From the graphs, slope and intercept were found to calculate the corresponding μmax and K.

## 5.4. Evaluation of maximum volumetric methane production rate:

Using values of $B_0$, μ, and K maximum volumetric methane yield, $\delta_{max}$ have been evaluated and reported in chapter 6 section 6.4

# **Chapter 6:** Results and discussion

## 6.1 Result for the analysis of substrates:

The vegetable waste sample was analyzed for moisture content, total solids, volatile matter, ash, fixed carbon, and carbon-hydrogen-nitrogen ratio using the procedures explained in chapter 4, section 4.4. The results are tabulated in the following table

**Table 4.1: Analysis of the substrate**

| Parameters | Value |
|---|---|
| Moisture content (%) | 70.10 |
| Total solid (TS) percentage | 29.90 |
| C:H: N | 25:6:1 |
| Volatile matter (%) | 10.70 |
| Ash (%) | 4.26 |
| Fixed Carbon (%) | 0.91 |

## 6.2. Variation of ultimate biogas yield with change in total solids and types of supplementations:

Experiments were conducted in 21 digesters having different combinations of total solids and types of supplements for the production of biogas. A detailed procedure for the evaluation of ultimate methane yield using cumulative methane yield data has been illustrated in chapter 5, the Kinetic model.

Ultimate methane yields ($B_0$) have been evaluated in each case and tabulated in the following table

**Table 4.2: Values of $B_0$**

| | Ultimate methane yield (in cubic m/Kg DM) | | | |
|---|---|---|---|---|
| | 6.5% TS | 8% TS | 10.5% TS | average |
| control | 0.0183 | 0.007 | 0.0319 | 0.019067 |
| FeCl3 | 0.0787 | 0.0097 | 0.0088 | 0.0324 |
| FeSO4 | 0.0142 | 0.0126 | 0.0231 | 0.016633 |
| MgO | 0.0181 | 0.0378 | 0.0448 | 0.033567 |
| Pectin | 0.002 | 0.0005 | 0.0096 | 0.004033 |
| silica gel | 0.0073 | 0.0065 | 0.0052 | 0.006333 |

## 6.3 Variation of Kinetic parameters with change in total solids and Types of supplementations:

For all possible mixtures of total solids and supplement type, the two kinetic parameters i.e. maximum specific microbial growth rate, $\mu_{max}$, and saturation constant, K, have been established. Details of the procedure for evaluation of parameters using cumulative yields & maximum yields have been illustrated in chapter 5, Kinetic model, and are summarized in the following table

**Table 4.3: Values of the kinetic parameters**

| Type of supplement | TS % | $\mu_{max}$ | K |
|---|---|---|---|
| Control | 6.5 | 0.165 | 0.658 |
| | 8 | 0.156 | 0.560 |
| | 10.5 | 0.102 | 0.684 |
| $FeCl_3$ | 6.5 | 0.103 | 0.864 |
| | 8 | 0.125 | 0.782 |
| | 10.5 | 0.149 | 0.856 |
| $FeSO_4$ | 6.5 | 0.156 | 0.650 |
| | 8 | 0.102 | 0.632 |
| | 10.5 | 0.144 | 0.592 |
| Pectin | 6.5 | 0.103 | 0.812 |
| | 8 | 0.092 | 0.756 |
| | 10.5 | 0.125 | 0.856 |
| Silica gel | 6.5 | 0.116 | 0.763 |
| | 8 | 0.145 | 0.741 |
| | 10.5 | 0.101 | 0.652 |
| MgO | 6.5 | 0.145 | 0.874 |
| | 8 | 0.125 | 0.784 |
| | 10.5 | 0.160 | 0.774 |

## 6.4 Variation of maximum volumetric methane production rate with change in total solids and supplements:

The maximum volumetric methane yield, $\delta_{max}$ has been evaluated for all the 18 digesters using values of ultimate methane yield ($B_0$) and kinetic parameters. Details of the procedure have been illustrated in chapter 5, Kinetic model, and are tabulated in the following table

**Table 4.4: Values of $\delta_{max}$**

| Supplement | TS % | $\delta_{max}$ |
|---|---|---|
| Control | 6.5% | 0.0025 |
| $FeCl_3$ | | 0.0036 |
| $FeSO_4$ | | 0.0009 |
| MgO | | 0.0046 |
| Silica Gel | | 0.0019 |
| Pectin | | 0.0006 |
| Control | 8% | 0.0027 |
| $FeCl_3$ | | 0.0038 |
| $FeSO_4$ | | 0.0015 |
| MgO | | 0.0048 |
| Pectin | | 0.0020 |
| Silica Gel | | 0.0009 |
| Control | 10.5% | 0.0026 |
| $FeCl_3$ | | 0.0038 |
| $FeSO_4$ | | 0.0016 |
| MgO | | 0.0036 |
| Silica gel | | 0.0011 |
| Pectin | | 0.0006 |

## 6.5 Discussion of the results

Experiments were conducted in 18 digesters having different combinations of Solid concentration and supplements for the production of biogas. Detailed procedure for the evaluation of ultimate methane yield ($B_0$) using experimental cumulative methane yield data have been illustrated in chapter 5, kinetic model. Maximum specific microbial growth rate $\mu_{max}$, kinetic constant and maximum volumetric methane production rates ($\delta_{max}$) have also been evaluated. Here the values of these kinetic parameters have been plotted against the different supplements that have been used in this experiment. A visual representation of the values becomes helpful while drawing comparison between the effectiveness of different supplements.

For the comparison of the maximum specific microbial growth rate $\mu_{max}$ and kinetic constant K, 8% solid concentration of the 6 different supplement types is chosen as it lies between the two extreme solid concentration (6.5% and 10.5%) used in the experiment.

Below is the maximum specific microbial growth rate chart obtained from the table 6.3

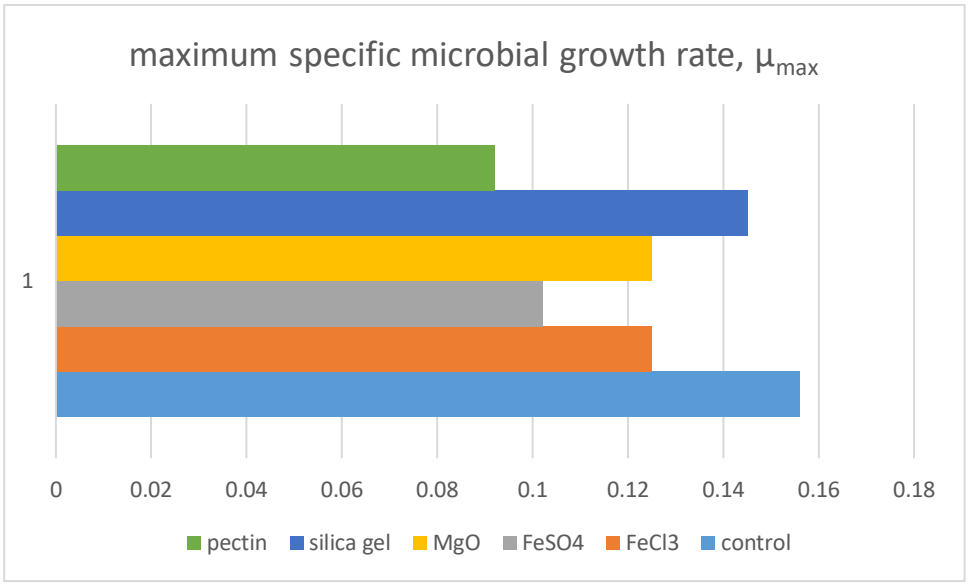



**Figure 4.1: Variations in $\mu_{max}$**

Below is the Kinetic constant K chart obtained from the table 6.3

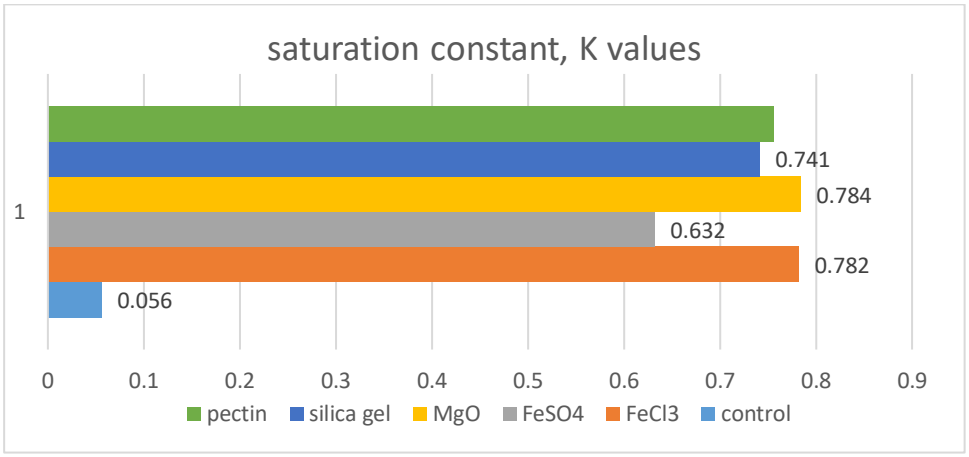



**Figure 4.2: variations in K**

Below is the Ultimate methane yields ($B_0$) chart obtained from the table 6.2.

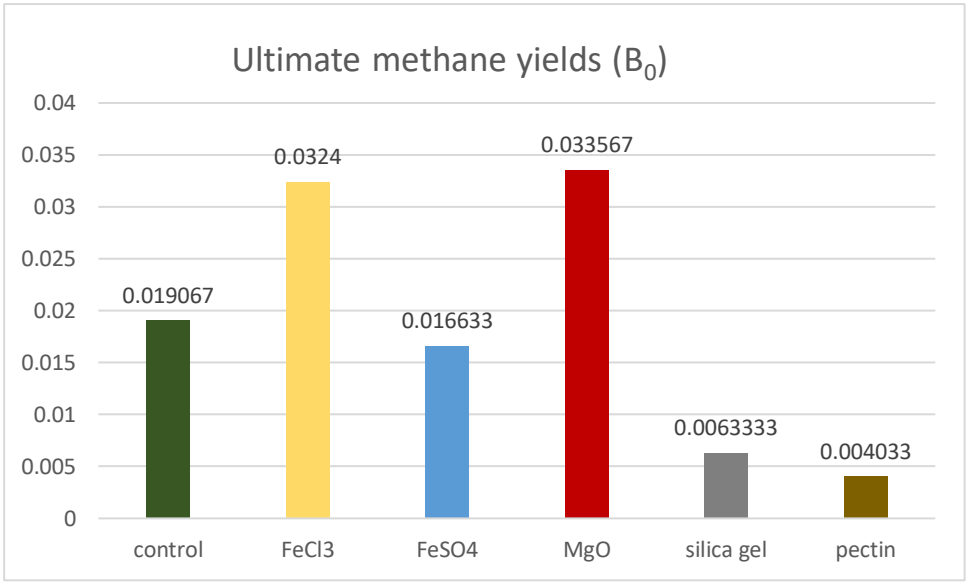



**Figure 4.3: variations in $B_0$**

**Table 4.5: variations in maximum volumetric methane production rate**

| maximum volumetric methane production rate | | | | |
|---|---|---|---|---|
| | 6.5% TS | 8% TS | 10.5% TS | Average |
| Control | 0.0025 | 0.0027 | 0.0026 | 0.0026 |
| FeCl3 | 0.0036 | 0.0038 | 0.0038 | 0.003733 |
| FeSO4 | 0.0009 | 0.0015 | 0.0016 | 0.001333 |
| MgO | 0.0046 | 0.0048 | 0.0036 | 0.004333 |
| silica gel | 0.0019 | 0.0009 | 0.0011 | 0.0013 |
| pectin | 0.0006 | 0.002 | 0.0006 | 0.001067 |

Below is the maximum volumetric methane production rate chart obtained from the above table.

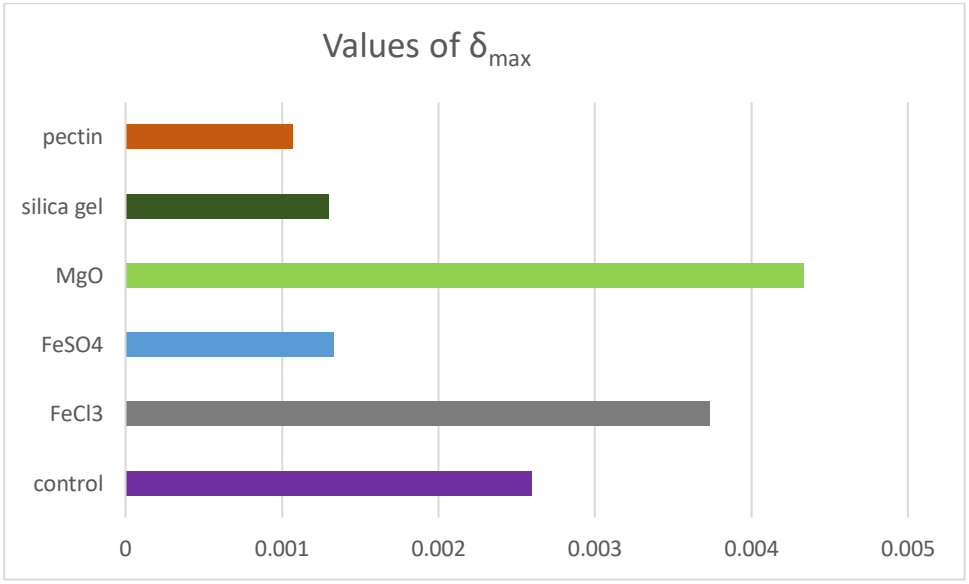



**Figure 4.4: Variations in $\delta_{max}$**

From the above charts, percentage calculations are done and it is found that the ultimate methane yield improved with the use of $FeCl_3$ and MgO, respectively 69.92% and 76.04%. This may have happened because of the ability of $FeCl_3$ to remove hemicelluloses and MgO has the potential to remove ammonia produced during the process. The addition of $FeSO_4$ causes a slight decrease in the $B_0$ value (-12%) and the maximum volumetric

methane production rate also suffered due to the addition of $FeSO_4$ (-48.71%). So $FeSO_4$ negatively impacted the AD by reducing the volume of produced biogas. Maximum volumetric methane production rate is improved both in the case of MgO (66.66%) and $FeCl_3$ (43.58%) and this again verifies their potential to enhance the biogas production.

Two other supplements which were used (Silica gel, Pectin) did not show promising results. Silica Gel caused 50% decrease in Maximum volumetric methane production rate and 66% decrease in ultimate methane yield. Pectin caused 58% decrease in Maximum volumetric methane production rate and 78% decrease in ultimate methane yield.

There are myriad number of optimization tools available which can be used to find out the exact dose of the most appropriate combination of supplement which will give the maximum methane yield. Although, much more time and a vast amount of data are required to invest in such meticulous calculations.

# **Chapter 7:** Conclusion and Future scope

### 7.1. Conclusion on the research performed:

It has been found in various studies that various additives may encourage the growth of various microorganism groups. For instance, the addition of MgO and $FeCl_3$ improved the activity of bacteria that produce hydrogen, such as *Syntrophomonadaceae* and *Clostridium*; as a result, the production of biogas could be easily increased by converting the hydrogen produced by these bacteria to methane. the production of biogas was increased as a result.

It was demonstrated in the current experiment how various supplements can impact the kinetic parameters. This study demonstrates that the production of anaerobic biogas can be increased by merely adding substrate supplements that can reduce the toxicity of substrates. Determining a substrate's inhibitory effects on AD and developing additional substrates to counteract the inhibitory effect should therefore be the focus of further research.

## 7.2. Future scope

The theory and technology behind biogas production are now well-developed and advanced, but the key to further research will be optimization. Additionally, more research comparing various substrates with various supplements should be done because there is a wide variety of supplements that can be experimented with.

Due to microorganisms' sensitivity to the environment, it is possible to optimize the appropriate factors that have an impact on efficiency. Additionally, by including supplements in the AD process, the substrate's ability to bind to the surface of the additives greatly improves digestion performance. Greenery biomass is regarded as a promising feedstock because it occurs naturally in the environment and produces no secondary pollution. However, the efficiency of green biomass is low.

It is advised that consideration be given to the proper dosages of various additives/supplements to enhance digestion process performance for the further development of biogas AD.

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