EFFECT OF INOCULUM TREATMENT METHODS ON BIOHYDROGEN PRODUCTION FROM FOOD WASTE BY THE PROCESS OF DARK FERMENTATION

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A thesis submitted in partial fulfillment of the requirements for the

Degree of

Bachelors of Engineering

In

Environmental Engineering

Institute of Environmental Sciences and Engineering (IESE)

School of Civil and Environmental Engineering (SCEE)

National University of Sciences and Technology (NUST)

2018

It is certified that the contents and forms of the thesis entitled

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Acknowledgement

We have no words to express our profound sense of gratitude and innumerable thanks to Almighty Allah, the compassionate and merciful, the only creator and Lord of this universe, the entire source of knowledge and wisdom, who bestowed on us his blessings and gave us the ability to complete the present studies successfully.

We present our sincere gratitude to our respectable and knowledgeable supervisor Dr. Zeshan from Institute of Environmental Sciences and Engineering, for his potential interest, skillful guidance, constructive criticism, and ever encouraging attitude throughout the course of research work.

We also wish to pay our heartiest gratitude to Mr. Basharat for his all kinds of support in the Chemistry Lab of Institute of Environmental Sciences and Engineering. Throughout the course of this study, he helped us with the chemicals and procedures during experimentation.

We owe our heartfelt gratitude to Sir Aamir Khan for his moral and technical support, encouragement, guidance and ever welcoming gratitude towards us. He was also very helpful with the equipment and procedures in the Wastewater Lab of Institute of Environmental Sciences and Engineering throughout the experimentation phase of this study.

Many thanks to our good friends, especially Syed Muhammad Mehdi, Muhammad Hassan Rasheed and Muhammad Taha Amerjee who were extremely co-operative and helpful during some unforeseen circumstances.

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List of Abbreviations

Abbreviation	Description
GHG	Green House Gas
CO_2	Carbon Dioxide
HRT	Hydraulic Retention Time
MEA	Monoethanolamine
Kg	Kilogram
EDTA	Ethylene diamine tetraaceticacid
Mo	Molybdenum
Fe	Iron
VFA's	Volatile Fatty Acids
NH4-N	Ammonium Nitrogen
T	Temperature
mg	milligram
L	liter
ml	milliliter
µm	micrometer
mm	millimeter
V	Volt
m	meter
TS	Total Solids
VS	Volatile Solids
OLR	Organic Loading Rate
C/N ratio	Carbon Nitrogen Ratio
g	Gram
mg/l	Milligram per Liter
%	Percentage
^0C	Degree Celsius
d	Day

Abstract

In September 2015, United Nations Development Program (UNDP) gave seventeen Sustainable Development Goals (SDGs) to transform the world. SDG 07 aims to ensure access to affordable and sustainable energy for all while SDG 12 aims to ensure the application of responsible consumption and production patterns on every level. Keeping these goals in view, the present project was designed to address the issues faced during the production of biohydrogen from food waste by dark fermentation. Biohydrogen is a highly efficient, clean, low cost and eco-friendly source of energy with many applications such as electricity generation and ammonia & aldehyde synthesis. It can be produced by photo, dark and combined fermentation but the most economically feasible method is dark fermentation. Dark fermentation is an anaerobic process in which hydrogen is produced by the growth of anaerobic bacteria in the absence of light source on carbohydrate rich substrate which in this project was food waste. Major drawback of dark fermentation was found to be the low hydrogen yield produced from the process and the most prominent cause was the consumption of biohydrogen by hydrogen consumers such as methanogens. Through literature review, it was found that in order to increase the hydrogen yield, suppression of hydrogen consumers was required by inoculum treatment, moreover, hydrogen purification was required in order to separate biohydrogen from other gases in the mixture. In this project, a comparison was done between the efficiency and cost effectiveness of four most common inoculum treatment methods (heat, acid, electric current and chloroform treatment) for biohydrogen production from dark fermentation. Moreover, bio hydrogen was purified by using Mono-Ethanol Amine (MEA) and the effectiveness of MEA for hydrogen purification was also found out. Cost of each method was estimated on both lab as well as industrial scales from dark fermentation. As a result of experimentation and cost estimation, Chloroform treatment was found to be the most efficient as well as economical method as it gave the highest percentage of hydrogen at a very reasonable cost.

Keywords: SDGs, Biohydrogen, Dark Fermentation, Anaerobic Process, Methanogens, Mono-Ethanol Amine, Chloroform treatment

Chapter 1

Introduction

1.1 Background of the Study

According to Germanwatch COP23, Pakistan is currently the 7th most affected country from climate change and the main reason for climate change is GHG in general and CO_2 in particular. There is a dire need in the world to shift towards renewable energy sources to tackle the current prevailing issue of climate change due to the immense fossil fuel burning.

The burning of fossil fuels in these amounts is not only disturbing the geologic balance of earth but also is responsible for increased air pollution which is causing fatal diseases such as lung cancer, asthma etc.

Pakistan has huge potential for renewable energy resources in terms of hydel and solar energy, but the only problem with these methods is that they have very high capital cost and low pay back period and use large areas to be deployed and require high cost maintenance and expertise to run and maintain.

According to Population Pyramid 2018, the ever growing population of Pakistan puts a huge hindrance when it comes to requirement of large areas for power production because according to a study Pakistan's population by 2100 will become 364 million and we will need area to support this population.

Pakistan being a low income country produces highest amount of solid wastes out of which a huge percentage of the solid waste is organic waste. Here we have a huge potential for energy by converting this waste to resource. Previously, energy has been extracted from food waste by production of biogas through anaerobic digestion of the waste but the problem with that process is that it has a very long HRT of 25-30 days (Khanal, 2008). The product is methane which itself is a GHG and when burned produces CO_2 which is the key role player in the current prevailing issue of climate change.

Another less used method for the digestion of this waste yet remains less explored due to unawareness probably and that is the production of bio hydrogen. The name bio hydrogen only states that it is produced through biological process elsewise it's the same molecular

hydrogen. This method is sustainable as compared to the previous obsolete method as it has a lower HRT which is of 2.9 days and produces hydrogen which when burned only produces heat and water (Venegas, 2015). Also that the energy content of hydrogen is almost 3 times that of methane and hence it is highly energy efficient.

This method can help manage landfills and can increase the life of the landfills due to its low HRT and significantly reducing the volume of the organic waste which we are producing that is 2.13kg/capita/day of our solid waste (Pharino, 2017). Employing this method on a large scale will help us reduce GHG emissions, reduce volume of solid waste, high energy content fuel and claim renewable energy which is cheap and has high energy content

1.2 Problem Statement

This study is based on development of a lab scale series of anaerobic digesters for the production of bio hydrogen through the anaerobic digestion of food waste and to check the increase in the efficiency of inoculum of producing bio hydrogen when it is subjected to four different treatment methods and purification of gas mixture to increase percentage of hydrogen.

1.3 Objectives of the Study

- Comparison of Hydrogen Production by Using Different Inoculum Treatment Methods in Terms of Efficiency
- Estimating cost of inoculum treatment for industrial scale
- Effectiveness of MEA for Hydrogen Purification

1.4 Scope of the study

- The study was conducted on lab scale
- Substrate was collected from cafeterias and markets
- Digested cow dung from a biogas plant was used as inoculum
- A series of reactor bottles were used as digesters of volume 300 ml
- MEA of 30% concentration was used for the purification of gas mixture

Chapter 2

Literature Review

In this chapter some basic facts and figures are discussed about biogas which will help develop the base for understanding the results which will be discussed in the upcoming chapters.

2.1 Background

Roughly 1.3 billion tons of food waste is produced annually. Losses occur in all stages of food supply chain. In low income countries, most loss occurs during production, while in developed countries much of the food wasted at the consumption stage (Gustavsson, 2011).

Generation of food waste has both economic and environmental impacts. According to Germanwatch COP23, Food worth of millions is lost in the name of waste and once food waste is disposed of in landfills and open dumping sites, anaerobic conditions are created once it is covered and CH_4 and CO_2 are produced which are highly potent GHG, causing the global climate change of which Pakistan is the 7^{th} most affected.

The problem can be catered by the controlled digestion of this food to produce biogas and used for energy but there is a problem with this method that it has become obsolete and the gas produced is methane and produces CO_2 once it is burned which is a GHG hence a more economic, efficient and cleaner method is required to manage this problem to handle the huge amounts of waste that is being produced.

2.2 Origin of food waste

Food waste is produced along the supply chain from harvesting to consumption of food waste. In case of third world countries like Pakistan most of the food waste is generated in the consumption stage of the food. Because it has become an ill habit of us and has taken more of the shape of culture of ours to waste food.

Huge masses of food is wasted in the waste bins every day in the commercial and as well as residential areas.

Pakistan does not have any engineering landfills except from that of Lahore therefore we are more confined towards open dumping which is not only covering the area rapidly but is also causing the spreading of diseases through vectors like mosquitoes which spread illnesses like

dengue and malaria. Also in case of famine the poor maybe attracted towards it as a source of food.

The food that is received in the dumping site is not in consumable form so it need to be digested to a smaller volume so that it covers a relatively lower volume, hence increasing the life of the dumping site.

Food waste has high carbohydrate content and easily hydrolysable in nature, exhibiting higher H_2 production

2.3 Anaerobic Digestion Process

It is a universally used process to produce biogas around the globe. The process takes place from the stomach of humans to the landfills provided we are able to create the conditions for it. The anaerobic process has four steps in which in the first two steps which are Hydrolysis and Acidogenesis, produces hydrogen and in the last two steps of the anaerobic digestion, Acetogenesis and Methanogenesis, the hydrogen is consumed by methanogens and acidogens for the production of methane. The four steps of anaerobic digestion are displayed in figure 2.1.

Biohydrogen, in this process, is produced by inhibiting the last two steps by maintaining pH, temperature, treating inoculum and providing high organic loading rate which inhibit the activity of the hydrogen consumers.

The pH is kept at 5.5 which tends to be optimum for the production of biohydrogen and also no amount of methane is produced at this pH because the activity of methanogens is inhibited at this pH.

At HRT of 2.9 days, Temperature 55 ^{o}C and pH 5.5, biohydrogen produced from food waste had a yield of 147,300 L/kg and a productivity of 51,324 L/kg/d (Venegas, 2015).

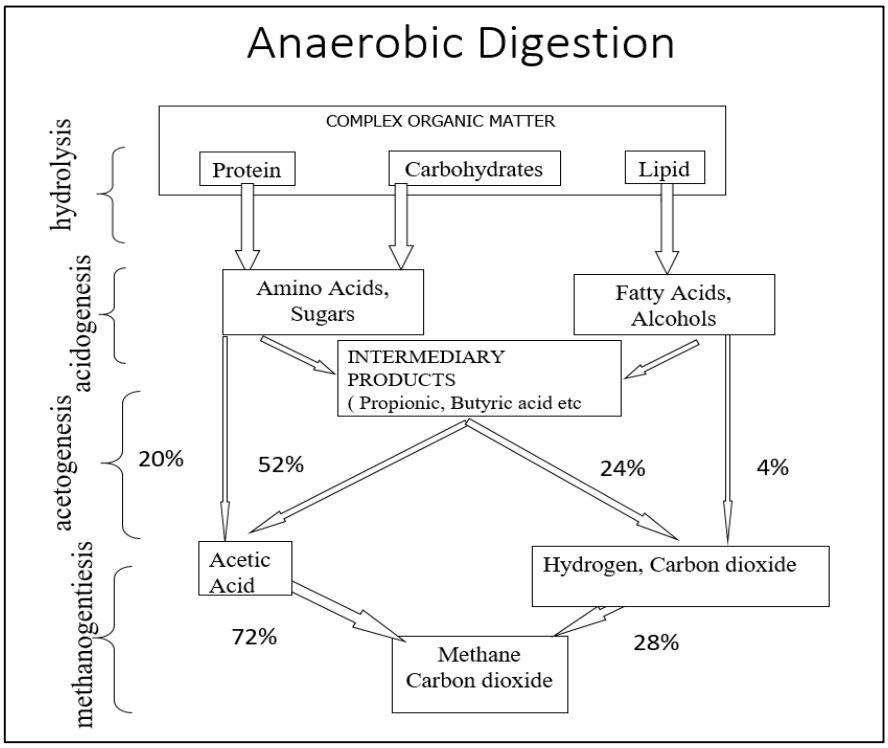


Figure 2.1 The four steps of Anaerobic Digestion

2.4 Methods of Hydrogen Production

There are three methods through which biohydrogen can be produced under anaerobic digestion and they are:

- Photo fermentation
- Dark fermentation
- Combined fermentation

A trade of analysis of all three processes was done under the project and is shown in table 2.1.

Table 2.1 Comparison of three Fermentation Processes

Dark Fermentation	**Photo Fermentation**	**Combined/Two-stage Process**
Advantages • Hydrogen evolution rate (HER) is higher than other processes • Production of carbon rich metabolites and CO_2 occurs which can be removed or separated from H_2, sequentially stored in biomass or converted to other substances, such as CH_4 • No light source required • Cost effective	**Advantages** • Natural sunlight and biomass is used • Natural photo-synthetic bacteria are used for productive activity • Higher yield of H_2 than dark fermentation • Good for large-scale purposes	**Advantages** • Both light and dark fermentation bacteria are used • Faster hydrogen formation • Higher hydrogen productivities • Lesser Volatile Fatty Acids (VFAs) formation than dark fermentation • Optimal D/L ratio results in balanced VFA formation and fermentation rates by the dark and light fermentations and the highest yield of hydrogen • In sequential dark and light fermentations, each step takes at least 5 days on the average yielding 10 days of total fermentation time. However, combined dark–light fermentation takes about 6 days

Disadvantages • Low yield of H_2 per substrate consumed due to metabolic fundamentals • Low efficiency of the process	**Disadvantages** • Complex nutritional requirements (EDTA, Mo, Fe) of the Rhodobacter species • Continuous light source required • Expensive for large-scale purposes • Strict control requirements for environmental conditions: ➢ T = 30–35°C ➢ pH = 6.8–7.5 ➢ light intensity = 4-6 Klux • Inhibitions caused by high concentrations of VFAs (>2500 mg L^{-1}) and NH4-N (>50 mg L^{-1})	**Disadvantages** • Inhibitions caused by high concentrations of VFAs and NH_4-N • Severe control of physico-chemical conditions • The medium used to grow bacterial species is dark fermentation's effluent, it needs complex nutritional added

On this basis, a problem Statement was developed to increase the efficiency of hydrogen production through different methods of inoculum treatment by the process of dark fermentation (because it had a low area requirement and was cost effective). The low yield problem was addressed as the key objective by the suppression of hydrogen consumers by the treatment of inoculum by four different methods.

2.4.1 Dark Fermentation

It is a biological process in which non-phototrophic fermentative bacteria which are basically heterotrophs undergo anaerobic digestion and use carbon rich sources as energy to produce hydrogen.

2.4.1.1 Microbiology of Dark Fermentation

The bacteria which are responsible for dark fermentation are enteric bacteria and clostridia species.

- **Enteric bacteria**

E. coli and *Enterobacter* are the commonly studied types of enteric bacteria in biohydrogen production through dark fermentation. These microbes are rod-shaped, gram-negative and facultative anaerobes when it comes to their biological characteristics. These bacteria are less sensitive to oxygen and are able to recover following accidental air exposure (Nath and Das 2004).

- **Clostridia**

Clostridia species are commonly obligate anaerobes and have rod like shape with round or pointed ends in case of few species. Rods shape can be straight or can be slightly curved with 0.5–2 μm in diameter and up to 30 μm in length. Out of all the most important characteristic of clostridia is its ability to form endospore. Endospore is a structure developed by a few microbial species when the environmental conditions become unfavorable (e.g., high temperature, desiccation, carbon or nitrogen deficiency, and chemical toxicity). When favorable conditions return, the spores germinate and become vegetative cells (Doyle 1989).

Mostly hydrogen is produced when pyruvate is anaerobically digested when organic substance are catabolized under certain conditions and presence of certain microbial colonies.

Simple sugars such as glucose are converted to pyruvate when it is subjected to glycolysis. The pyruvate is broken down to acetyl coenzyme, from which adenosine triphosphate (ATP) is produced and hence hydrogen is produced from the products, formate or reduced ferredoxin, which is dependent on the types of microbial groups present in the system.

The enteric bacteria derive hydrogen from formate, whereas obligate anaerobes (e.g., clostridia) derive hydrogen from ferredoxin. The various enzyme systems involved in breakdown of pyruvate are presented in Equation 2.1 and Equation 2.2 (Hallenbeck 2004).

(a) Pyruvate: formate lyase (PFL) Eq. (2.1)

Pyruvate + CoA → acetyl-CoA + formate

(b) Pyruvate: ferredoxin (flavodoxin) oxidoreductase (PFOR) Eq. (2.2)

Pyruvate + CoA + 2Fe (ox) → acetyl-CoA + CO_2 + 2Fe (red)

Figure 2.2 shows the glycolysis of sugar which in turn is responsible for the formation of hydrogen

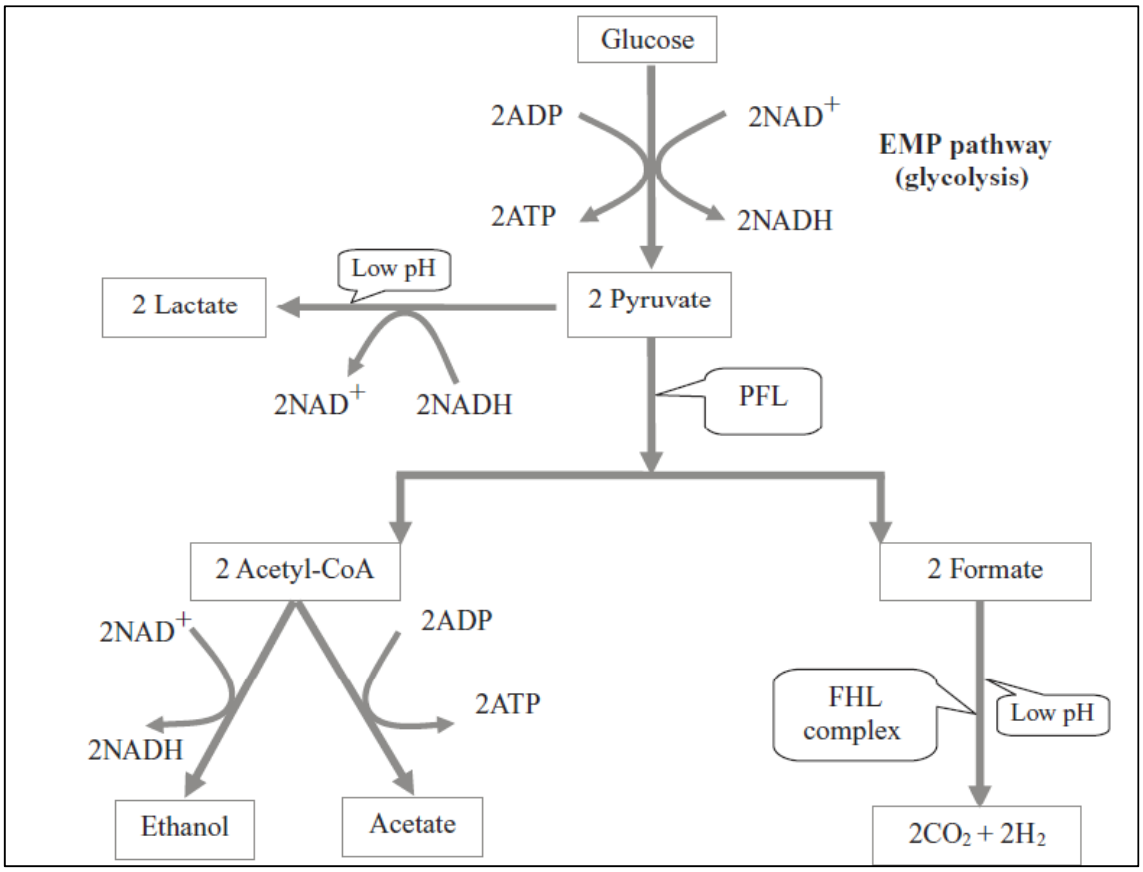


Figure 2.2 Glycolysis of Sugar for the Production of Hydrogen

2.5 Inoculum Used for Hydrogen Production

The inoculum used was digested cow dung from a biogas plant. The selection for the inoculum was done on the basis of the established fact that cow dung is a very rich microbial media and has long been used for the anaerobic digestion for the production of biogas hence it is very microbial rich.

2.5.1 Pretreatment of Inoculum

Digested cow dung was filtered through a 2 mm sieve. Then, the filtrate (inoculum) was stored in incubator under anaerobic conditions at 37°C for at least 14 days to consume the organic matter that is already present in the inoculum. After daily removal of biogas, the inoculum was enriched with microbes and most of the organic matter was degraded.

2.5.2 Inoculum Treatment Methods for Suppression of Hydrogen Consumers

The hydrogen consumers are quite sensitive and can survive at a lower range of pH and temperature while the hydrogen producers can survive in a rather wider range of temperature and pH.

The reason is that the hydrogen producers are gram positive microbes having thick peptidoglycan layer as compared to hydrogen consumers which are gram negative and have a relatively thinner peptidoglycan layer and are easily ruptured through these adverse conditions. In case of hydrogen producers these microbes form endospores when unfavorable conditions are present and germinate when the conditions are favorable according to the metabolic structure and realm. For this project, inoculum was treated with four different methods, which were:

2.5.2.1 Electric Current Treatment

In this method a low voltage of 3-4.5 V, with the help of electrodes, was induced into the inoculum for 11 minutes for the suppression of the hydrogen consumers.

The use of low-voltage electric current has been successfully used to suppress the growth of hydrogen consumers (Roychowdhury 2000).

This basically happens because the hydrogen producing bacteria have a thicker cell wall and once subjected to a lower voltage, the formed endospores are able to survive whereas the hydrogen consuming bacteria have their cell walls ruptured and are inhibited from consuming hydrogen.

2.5.2.2 Heat treatment

In this method, the inoculum was placed in an oven at 90°C for 20 minutes. Heat treatment is often used to enrich cultures with hydrogen producing microbes. Heat treatment inhibits activity of hydrogen consuming microorganisms and is responsible for selective survival of microbes which are hydrogen producing bacteria.

Many of the hydrogen producing bacteria (e.g., *Clostridium* and *Bacillus* species) form endospores, which are “survival structures” developed by these organisms when unfavorable environmental conditions are encountered. When favorable conditions return, the spores germinate and become vegetative cells (Doyle 1989).

2.5.2.3 Acid/Base treatment

The endospores formed are not only resistant towards heat but have high affinity to tackle higher variations of pH whereas hydrogen consuming bacteria have lower ability to cater for varying pH and hence are permanently inhibited.

In this case the seed inoculum is treated by adding 1-2N solution of HCL to bring down its pH to 3 or by the addition of 1-2N solution of NaOH to keep the pH at 10 (Khanal, 2008).

This completely inhibits the activity of hydrogen consumers and the media is left with enriched hydrogen producers which are basically enteric and clostridia species

2.5.2.4 Chemical treatment

Chloroform, of 0.05% of inoculum, is introduced in the inoculum and kept for 17 hours (before use) for the suppression of hydrogen consumers.

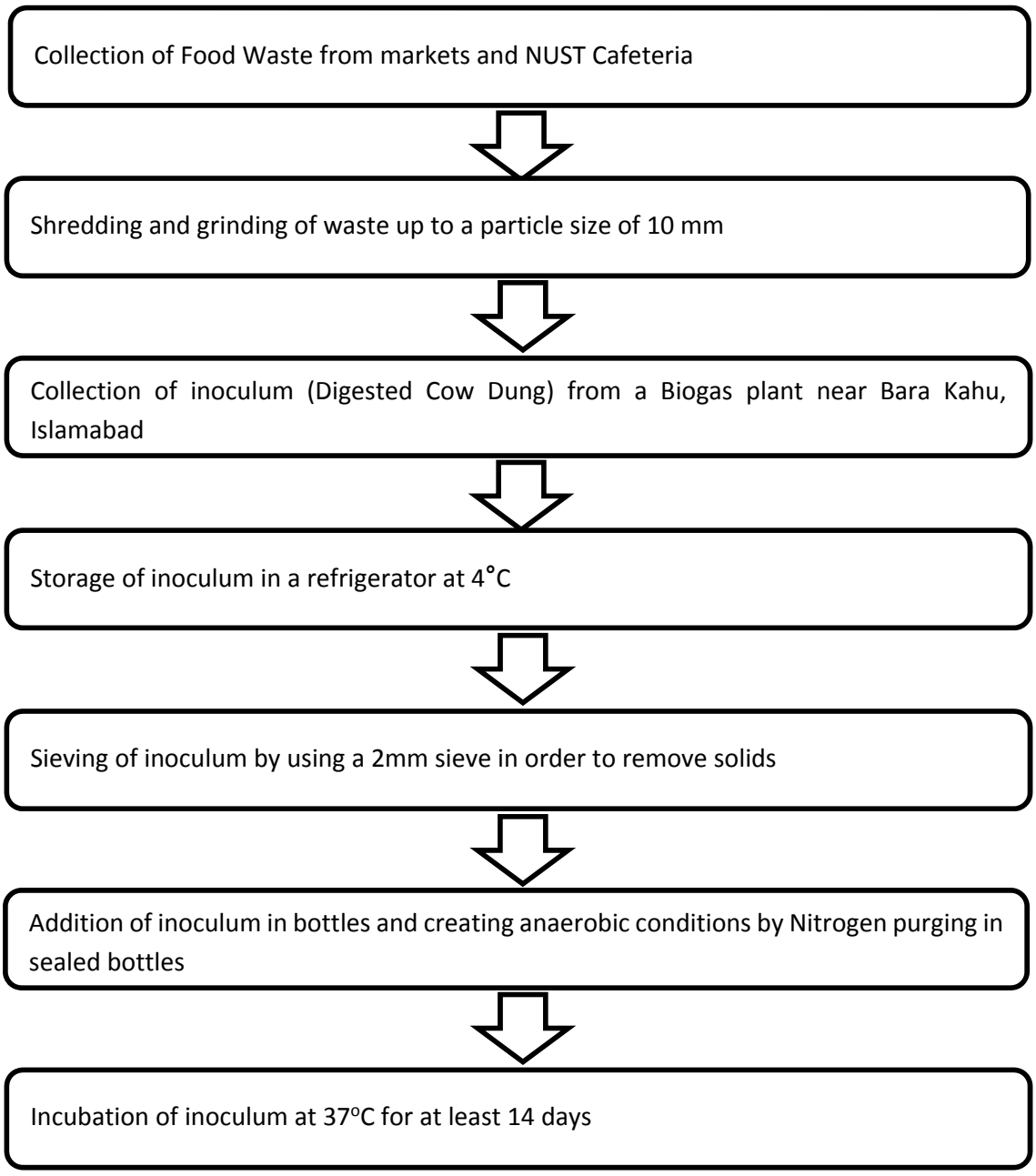
2.5.2.5 Kinetic control

The specific growth rate of hydrogen producing bacteria and their yield coefficients higher as that if we compare them with hydrogen consumers. Therefore, we can simply understand that a shorter HRT in a CSTR slow-growing hydrogen consumers such as methanogens could be washed out from the system and selective growth of hydrogen-producing bacteria could be achieved. In a bio kinetic study, Chen et al. (2001) reported maximum specific growth rate of 0.172 h−1 for sucrose utilizing hydrogen producing cultures.

Materials and Methods

In this chapter, the methodology for bio hydrogen production from food waste by dark fermentation will be discussed in detail along with the operational parameters and other analysis of substrate, inoculum, digester and biogas.

3.1 Flow Chart of the Experimental Work



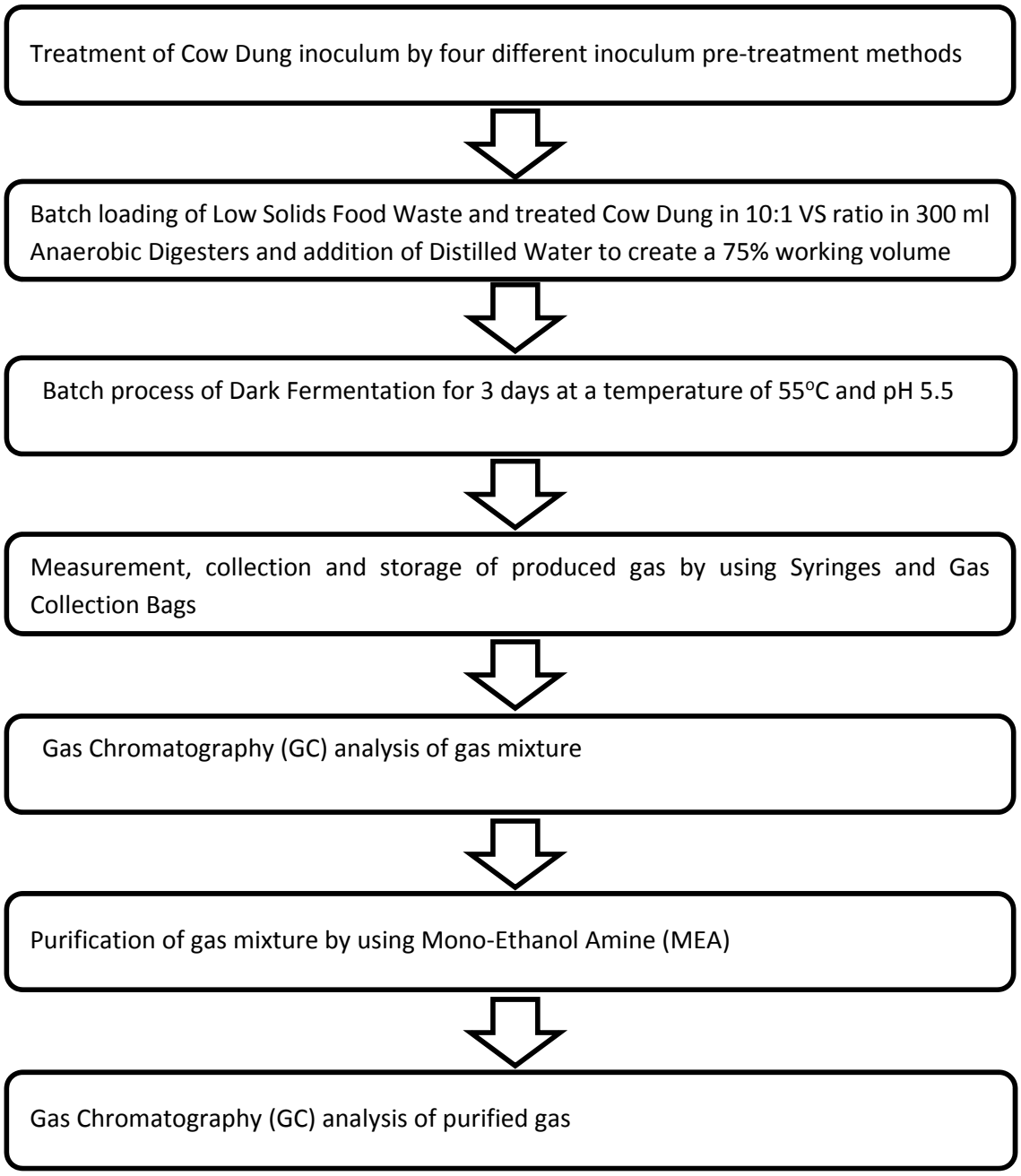


Figure 3.1 Flow Chart of Experimental Study

3.2 Substrate Collection and Preparation

The food waste used during the experimentation was collected from NUST Concordia 1 and the markets in Islamabad. Literature review suggested that organic waste is required for more efficient anaerobic digestion because organic waste is biodegradable and can be converted into carbon dioxide, methane, hydrogen etc. Organic waste is nearly 65% of the total waste generated in Pakistan. Organic waste can be easily found in Municipal Solid Waste in various forms.

3.2.1 Advantages of using Food Waste

Food waste was selected for this study because food waste is 50-85% of the organic waste generated in Pakistan. Because of its easy and abundant availability, food waste proved to be a very low-cost source of raw material needed for the experimentation. Now-a-days industries obtain Hydrogen by breaking down natural gas by providing high pressure steam generated at temperature as high as 1100°C. Using food waste for hydrogen production not only eliminates the high temperature and pressure requirement but also significantly decreases the cost of hydrogen production.

One of the advantages of dark fermentation is that complex organic substrates such as carbohydrate rich wastes can be used during this process. Food waste was selected for the study because it is a carbohydrate rich waste which makes it suitable for dark fermentation.

As it has already been mentioned that food waste constitutes a huge portion of organic waste generated in Pakistan, which in turn, is a huge portion of the total waste itself. Therefore, using food waste for hydrogen production is a way of recycling of a major portion of municipal organic solid waste and using it for the generation of a very efficient as well as environment friendly energy source i.e. Hydrogen.

Recycling of about 50-85% of the municipal organic solid waste and using it for energy generation, hence, causes a significant decrease in the quantity of solid waste that needs to be managed. This not only reduces the work load of solid waste managers but also increases the lifespan of the existing landfills.

3.2.2 Composition of Food Waste

Table 3.1 shows the components which have been used as well as their respective percentages during the preparation of food waste mixture.

Table 3.1 Composition of Substrate

Components of Substrate	**Percentage in total substrate**
Tomato	7
Potato peels	19
Radish	19
Onion	13
Bell Pepper	13
Cabbage	7
Lentils	22

The literature review suggested that the optimum Carbon to Nitrogen Ratio for the substrate to be used for anaerobic digestion was 20 and the optimum VS was found to be 60 g/L i.e. 13.5 g for a 300 ml digester. Therefore, the substrate i.e. food waste was prepared by using components whose C/N ratios were either slightly above or below 20. The percentages of substrate components to be used during the preparation of substrate were calculated on the basis of their C/N ratios.

From the literature review, the C/N values for vegetable wastes were found to be usually less than 20, therefore, vegetable wastes were used in greater proportion than that of lentils whose C/N value was found to be quite higher than 20. From the C/N value and percentage of each waste component, its C/N contribution in the substrate was calculated and the average of those C/N contributions of all components was found to be 20. Similarly, VS contribution of each waste component was calculated on the basis of its percentage in the substrate and the sum of those contributions was found to be 13.5 g for a 300 ml digester.

Table 3.2 summarizes the above description about the calculation of percentages of waste components on the basis of their C/N ratios

Table 3.2 Calculation of Percentages of Substrate Components

Components	C/N value	VS contribution per 300 ml bottle (g)	C/N contribution per bottle
Tomato	12	1.0	6.86
Potato peels	25	2.5	35.71
Radish	19	2.5	27.14
Onion	15	1.75	15.00
Bell pepper	15	1.75	15.00
Cabbage	12	1.0	6.86
Lentils	29	3.0	25.71

Therefore, the substrate was prepared in such a way that it had a C/N value of 20 approximately while its total VS was 13.5 g for a 300 ml digester, exactly as the literature review suggested.

3.2.3 Shredding and Mixing of Food Waste

Literature review suggested that the particle size of substrate should be kept less than or equal to 10 mm for easy and efficient biodegradation. Food waste components were therefore shredded up to a particle size of 10 mm or less by using knives and graters. The purpose of shredding was to make the substrate easily degradable. After shredding, the waste components were added in the proportions given above and they were mixed thoroughly to create a homogeneous food waste mixture.

Figures 3.2 and 3.3 show the process of shredding and mixing of food waste.

Figure 3.2 Mixing of Substrate Components

Figure 3.3 Food Waste Mixture

3.3 Inoculum Collection and Preparation

Inoculum is a microbe rich media injected in the substrate to initiate the activity of micro-organisms which are responsible for causing anaerobic digestion. In simple words, inoculum is the substance added to begin the process of biodegradation of substrate.

Different types of inoculum are used for anaerobic digestion e.g., swine wastewater, sewage sludge, animal manure, cow dung etc.

3.3.1 Collection and Storage of Cow Dung

For this study, digested cow dung was used as inoculum. Digested cow dung was collected from a biogas plant near Bara Kahu, Islamabad. It was found as the effluent of the biogas plant. It was collected from the effluent pipe in a bottle and stored in a refrigerator at 4^{o}C temporarily to keep the microbes alive but relatively inactive.

3.3.2 Sieving of Inoculum

Cow dung was sieved by using a 2 mm sieve. The purpose of sieving was to remove the solids and obtain inoculum in the form of a low-solids filtrate. Large sized solid particles reduce the efficiency of anaerobic digestion. Figure 3.4 depicts the process of sieving of inoculum.

Figure 3.4 Sieving of Cow Dung

3.3.3 Nitrogen Purging and Sealing of Inoculum bottles

After sieving, the inoculum was added into 500 ml bottles. The bottles were not filled completely with inoculum, instead, some volume was left empty for the collection of biogas produced during inoculum preparation.

The bottles were then closed by rubber septum and sealed by applying 20 mm crimp seals. Nitrogen purging was performed on the bottles in order to create anaerobic conditions inside them.

During nitrogen purging, a hypodermic needle was used to connect a nitrogen cylinder to the inoculum bottle. When the Nitrogen supply from the cylinder was turned on by using a regulator, the Nitrogen gas started entering the inoculum bottle through the needle. Another hypodermic needle, inserted in the bottle through another opening in the rubber septum was used to make a way for air to exit from the bottle. This process was carried out on each inoculum bottle for 4-5 minutes. Figure 3.5 displays Nitrogen cylinder used during Nitrogen purging.

Figure 3.5 Nitrogen Cylinder

Hence, Nitrogen purging created an inert atmosphere with a low dew point. Since, Nitrogen was clean and dry, therefore, it replaced an undesirable atmosphere inside the bottles with an inert, dry and anaerobic atmosphere with no air or gas inside the bottles.

3.3.4 Incubation of Inoculum

Inoculum bottles were placed in an incubator at 37^{o}C temperature for at least 14 days. Biogas produced in the bottles was measured and discharged on daily basis. Initial observations showed that biogas production was higher in the beginning but with the passage of days, the volume of gas produced in a day decreased.

3.4 Inoculum Treatment

Anaerobic digestion is a four step process consisting of Hydrolysis, Acidogenesis, Acetogenesis and Methanogenesis. During the first two steps, hydrogen is produced which is quickly consumed by hydrogen consumers such as methanogens for the production of methane in the last two steps of the process. Therefore, in order to obtain bio hydrogen as a product, the last two steps of anaerobic digestion need to be inhibited, which in turn, requires the suppression of growth and activity of methanogens.

Literature review suggested that activity and growth of methanogens can be stopped by Inoculum Treatment. Inoculum treatment is a process under which inoculum is subjected to such conditions under which acidogens can survive but methanogens cannot survive.

The inoculum treatment methods used during this study were: Electric Current Treatment, Acid Treatment, Heat Treatment and Chemical Treatment

3.4.1 Electric Current Treatment

During the electric current treatment, a low voltage of 3.5 volts was provided to the inoculum for 11 minutes as it was the most efficient way of providing current treatment suggested by the literature review.

- **Electrodes**
  - Copper electrode, Iron electrode.
- **Electrode Dimensions**
  - 40 mm × 4.5 mm × 152.4 mm

3.4.2 Acid Treatment

During acid treatment, 1 N solution of Hydrochloric Acid (HCl) was added to the inoculum and as a result pH of the inoculum was reduced and set at 3.

- **Preparation of 1 N HCl solution**
  - Molecular weight of HCl = 36.48 g/mol
  - Density of HCl = 1.19 g/ml
  - Volume required for 1 L HCl solution = 36.48/1.16 = 30.66 ml
  - Percentage of weight = 35%
  - Volume required for 1 L and 1 N HCl solution = 30.66/0.35 = 87.59 ml
  - Volume required for 100 ml of 1 N HCl solution = 8.759 ml
  - According to calculations, 50 ml of 1 N HCl solution was prepared by adding 4.38 ml of HCl in 50 ml of distilled water.
- **Instruments Used**
  - Beaker, Pipette, pH meter
- **HCl Required**
  - 10 ml HCl per 500 ml of inoculum
- **Materials Required**
  - Hydrochloric Acid (HCl), Distilled Water

3.4.3 Heat Treatment

During heat treatment, inoculum was heated at a temperature of 90°C for a duration of 20 minutes. Providing this temperature kills methanogens but acidogens survive these conditions.

- **Instruments Used**
  - Oven
- **Power Consumption of Oven**
  - 2400 Watts/hour

3.4.4 Chemical Treatment

During chemical treatment, chloroform which was 0.05% of the volume of inoculum was added to the inoculum by using a micro-pipette.

- **Volume of Inoculum**
  - 500 ml
- **Volume of Chloroform**
  - 0.05% of 500 ml = 0.25 ml

Therefore, 0.25 ml of chloroform was added to 500 ml inoculum and inoculum was then kept undisturbed for 17 hours.

3.5 Characterization of Food Waste and Inoculum

For characterization of food waste and inoculum, four parameters were considered. The procedures of measurement are as given below.

3.5.1 Moisture Content

A sample of substrate and inoculum was weighed and subsequently heated to allow for the release of moisture. Following this, the sample was cooled in the desiccator before re-weighing. Moisture content was calculated by the difference in wet and dry weight.

$$Moisture\ Content\ (\%) = \frac{\text{Wet weight } - \text{Dry weight}}{\text{wet weight}} \times 100$$

3.5.2 Total Solids (TS)

Total Solids (TS) content was calculated by subtracting the moisture content from total (100%).

Total Solids (%) = 100 – *Moisture Content (%)*

3.5.3 Volatile Solids (VS)

Same sample of substrate and inoculum was placed in a muffle furnace for 30 minutes at 550˚C VS was calculated by the difference between weight of sample before and after ignition.

$$Volatile\ Solids\ (\%\ of\ TS) = \frac{\text{Dry weight } -\ \text{weight after ignition}}{\text{dry weight}} \times 100$$

3.5.4 Total Organic Carbon (TOC)

It was not a parameter found by any experimental method. Instead, it was calculated by the formula given in the table below.

$$Total\ Organic\ Carbon\ (\%\ of\ TS) = \frac{VS\ (\%\ of\ TS)}{1.724}$$

Figure 3.6 and 3.7 shows sample of food waste and inoculum used for characterization.

Figure 3.6 Substrate Sample for Characterization

Figure 3.7 Inoculum Sample for Characterization

3.6 Experimental Setup

As shown in the figure 3.8, anaerobic digesters of 300 ml containing a mixture of substrate, inoculum and distilled water whose pH was maintained at 5.5 by adding buffer solution, were sealed, subjected to nitrogen purging and placed in a water bath for 3-day dark fermentation process. A temperature of 550C was maintained by using a heating rod and thermostat during this period. The gas produced during this period was extracted and measured by using a syringe and collected in gas collection bags on regular basis. Collected gas was then analyzed

by Gas Chromatography (GC) analysis in order to find out the percentage of bio hydrogen in the biogas.

Bio hydrogen was then purified and isolated by using Mono-Ethanol Amine (MEA). For isolation of bio hydrogen, biogas was added from gas collection bag into a bottle containing 30% MEA which was already sealed by applying Silicon and a clean, dry and inert atmosphere had been already created inside the bottle by performing nitrogen purging. The bottle was then placed on a hot plate at 300C for 2 hours. After this process, the purified and isolated bio hydrogen was collected in the gas collection bag and analyzed by Gas Chromatography (GC) again to find out the increase in the percentage of hydrogen in the gas, which was a measure of the effectiveness of MEA for purification and isolation of bio hydrogen.

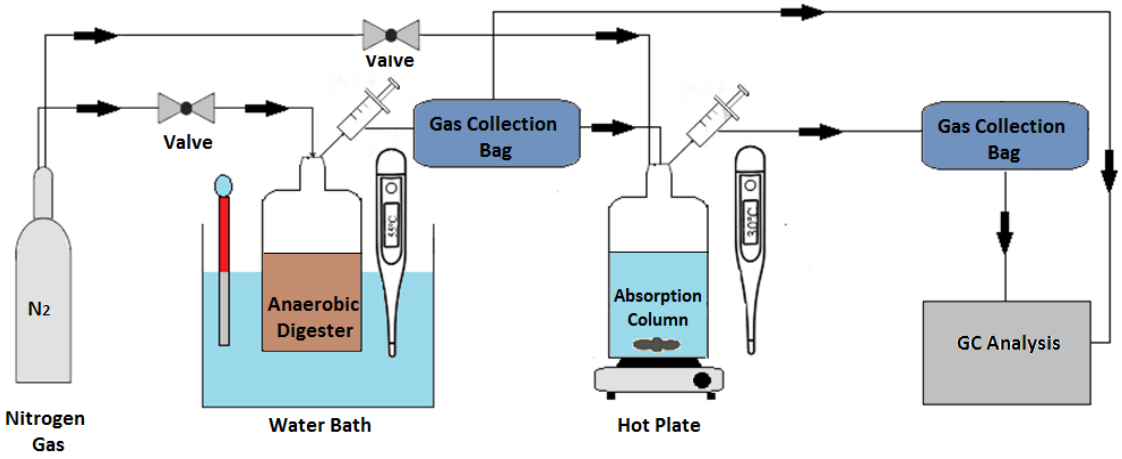


Figure3.8 Experimental Setup of Dark Fermentation

3.7 Operating Conditions for Dark Fermentation

The table 3.3 shows the operating conditions of dark fermentation.

Table 3.3 Operating Conditions for Dark Fermentation

Mode of Operation	Batch
Scale	Lab
Organic Loading Rate	60gVS/L
Inoculum-Substrate Ratio	0.1
pH	5.5
Temperature	55◦C
HRT	3 days

3.7.1 Batch Loading of Food Waste and Treated Inoculum

The bottles of 300 ml were used as anaerobic digesters. Mass of food waste required to provide 13.5g of VS was calculated and since Inoculum-Substrate Ratio (ISR) was to be kept equal to 0.1, therefore, mass of inoculum required to provide 1.35g of VS was also calculated. These quantities were calculated on the basis of VS values of substrate and inoculum found as results of characterization.

The calculated quantities of both inoculum and substrate were added in 300 ml digesters and distilled water was added in the digesters to completely cover the working volume i.e. 75% or 225ml of the digesters leaving the remaining volume empty for the collection of biogas produced during dark fermentation.

Out of four digesters used for testing each inoculum treatment methods, three digesters contained both inoculum as well as substrate and distilled water while one digester, known as blank digester, contained only untreated inoculum and distilled water and no substrate in it.

3.7.2 pH Control

As the literature review suggested, the optimum pH of 5.5 required for anaerobic digestion was set inside the anaerobic digesters by using buffer solution and measured by using pH meter.

3.7.3 Sealing and Nitrogen Purging of Digesters

After adding inoculum, substrate and distilled water, the bottles were closed by rubber septum and sealed by applying 20mm crimp seals. Nitrogen purging was then performed on them by the same process as described before. The purpose of nitrogen purging was to create anaerobic conditions and replace an undesirable atmosphere inside the bottles with an inert, dry and clean atmosphere.

3.7.4 Water Bath and Temperature Control

The anaerobic digesters were placed inside a water bath for 3 days and optimum temperature of 55^{o}C was maintained throughout this period by using a 1000 W heating rod with a thermostat (Venegas et. al., 2015). A relay and a temperature sensor i.e. thermocouple were used to maintain the temperature at 55^{o}C. The heating rod provided the specified temperature to water inside the bath and the water provided that temperature to digesters. Therefore, the purpose of water was to serve as a medium of heat transfer from the rod to

the digesters since the direct supply of heat to all the digesters was impossible. Figures 3.9 and 3.10 show the reactor setup used for dark fermentation.

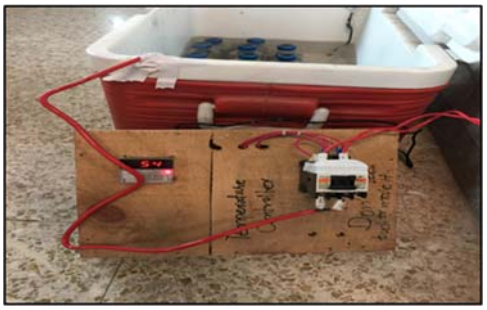


Figure 3.9 Temperature Controller

Figure 3.10 Water Bath and Heating Rod

3.7.5 Gas Extraction, Measurement and Collection

During dark fermentation, gas produced in the digesters was extracted and measured by using a syringe and the gas was then stored in gas collection bags. Figure 3.11 shows the glass syringe, used for extraction and for measuring the volume of gas, and gas collection bag for storing the produced gas.

Figure 3.11 Syringe and Gas Collection Bag

3.7.6 GC Analysis of Produced Gas

The gas produced by the process of dark fermentation was stored in gas collection bags and taken to Centre for Advanced Studies in Energy NUST (CASEN) for GC analysis. The purpose of GC analysis was to find out the percentage of hydrogen present in the gas mixture. Therefore, GC analysis was performed to observe the effect of different inoculum treatments on bio hydrogen production from food waste by dark fermentation.

3.8 Purification by Mono-Ethanol Amine (MEA)

GC analysis showed that the gas obtained from dark fermentation was a mixture which contained hydrogen, carbon dioxide and hydrogen sulfide as well as other gases in very small proportions.

The gas was treated by Mono-Ethanol Amine (MEA) for isolation and purification of Hydrogen. For purification, 30% MEA was added into a bottle. The bottle was sealed by applying silicon and nitrogen purging was performed in order to create an inert, clean and dry atmosphere inside the bottle. Gas was added from the gas collection bag into the bottle by using a pipe.

The bottle was then placed on a hot plate at 30°C for 2 hours. After purification, gas was again collected in a gas collection bag by using a pipe.

3.8.1 GC Analysis of Purified Gas

The gas treated by MEA was collected in gas collection bag and taken to CASEN for GC analysis. The purpose of GC analysis was to find out the percentage of hydrogen in the gas mixture after treatment by MEA. Therefore, GC analysis was performed to observe the effectiveness and efficiency of MEA for isolation of biohydrogen and removal of hazardous gases like carbon dioxide and hydrogen sulfide.

Figure 3.12 GC Apparatus

Chapter 4

Results and Discussion

4.1 Introduction

In this chapter the result obtained during the experimentation and the effect of different inoculum treatments on hydrogen production will be discussed. The data is shown in the form of tables and graphs followed by detailed discussions.

4.2 Waste Characterization

The waste (inoculum and food waste) were characterized by calculating the moisture content, total solids (TS), volatile solids (VS) and total organic carbon (TOC). The food waste was added in the reactor bottles with an organic loading rate of 60 gVS/L while the inoculum was 6 gVS/L. Table 4.1 shows the results of waste characterization of substrate (food waste) and inoculum (digested cow dung). Zhang et al. (2007) stated that the M.C. and VS of food waste was 79.5% and 84 % respectively, and average TS and VS of inoculum is 3.40% and 61.0%, respectively which is close to values given in table 4.1.The reason that the substrate M.C., given in table 4.1, is higher because the food waste mostly contained vegetable waste and vegetables have higher percentage of water in them. Zhang et al. (2007) also stated that the TOC of food waste is 46.78 % of TS while that of food waste used for our experiment is 49.44 % of TS which shows quite similarity. On the basis of the result of waste characterization, inoculum and food waste were added in the reactor bottles.

Table 4.1 Waste Characterization of Substrate and Inoculum

Parameters	**Unit**	**Substrate**	**Inoculum**
Moisture Content	%	87.36	95.09
Total Solids	%	12.64	4.90
Volatile Solids	% of TS	85.23	61.82
Total Organic Carbon	% of TS	49.44	35.86

4.3 Daily Biogas Production from Inoculum

After removing solids from inoculum by using a 2 mm sieve, it was placed in a 500 ml reactor bottle and sealed using rubber septum and bottle cap. Then, nitrogen purging (for 2-3 minutes) was done in order to remove air inside the reactor bottle. After that, the reactor bottle was placed in an incubator at 37°C to provide the microorganisms with optimum temperature in order to remove excess organic matter from the inoculum. The inoculum was placed in incubator until the biogas production was reduced.

The biogas was daily removed from the reactor bottle using glass syringe and its volume was measured daily. Figure 4.1 shows the daily biogas production from inoculum up to a period of 18 days. The total volume of biogas came out to be 2.23 L for 18 days. Agarry et al. (2012) carried a study to produce biogas from cow dung which came out to be a total of 3.8 L in 18 days which is more as compared to our result because of the difference in the size of reactor.

This procedure was performed to remove excess organic matter from inoculum so as to obtain a pure inoculum for dark fermentation process.

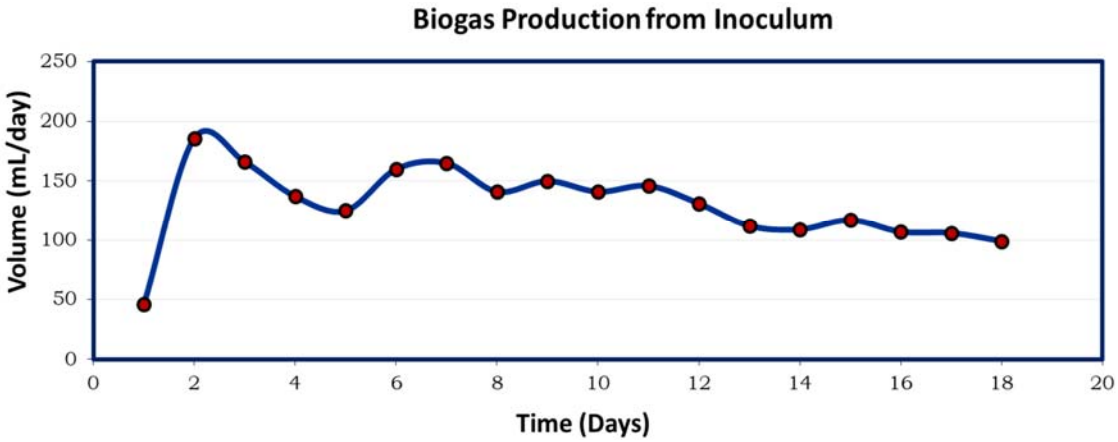


Figure 4.1 Daily Biogas Production from Inoculum

4.4 Gas Production from Different Inoculum Treatments

The prepared inoculum was then treated with four different inoculum treatments to check its effectiveness on efficiency (in terms of percentage) of hydrogen. Total of five different inoculum were prepared in which four of them were treated and one of them was untreated.

Four different treatment methods were: Heat treatment, Acid Treatment, Electric Current Treatment and Chloroform Treatment. The reactor bottles of 300 ml were then filled with food waste, inoculum and distilled water (to fill up to the working volume i.e. 225 ml). For each inoculum treatment three replicates were made as well as for untreated inoculum. The experiment was performed by providing the optimum conditions for dark fermentation process, which continued for three days. Figure 4.2 shows the gas production using four different treated inoculum and untreated inoculum from the process of dark fermentation. It can be seen from Figure 4.2 that maximum amount of gas was produced in the reactor bottle in which untreated inoculum was added. This is because of the fact that untreated inoculum had both the hydrogen producing microorganisms (homoacetogens) and methane producing microorganisms (methanogens). Although, methane was not produced in the gas mixture because of the operating conditions, but it has a high percentage of carbon dioxide (CO_2) due to which the gas produced from untreated inoculum by dark fermentation was maximum.

The total volume of gas from untreated inoculum was 439 ± 30 ml. The minimum amount of gas was produced in the reactor bottle in which chloroform treated inoculum was added. This means that most of the hydrogen consumers were removed. The volume of the gas from chloroform treated was 261 ± 19.7 ml. Similarly, gas produced from acid treated, electric current treated and heat treated were 301 ± 24 ml, 377 ± 10 ml and 387 ± 28.2 ml respectively.

Osuagwu and Agamuthu (2014) carried out a study to check the effect of temperature on biohydrogen production. He found that the total volume of the gas produced was around 340 ml which is close to our result. Han et al. (2016) compared hydrogen production on batch and continuous scale and total volume of hydrogen in case of batch mode came out to be 250 ml.

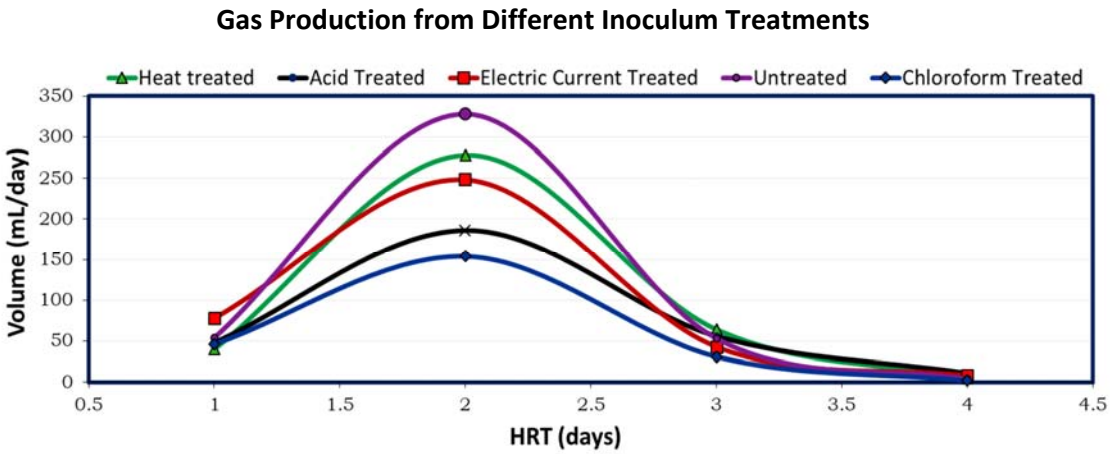


Figure 4.2 Daily Gas Production from Different Inoculum Treatments

4.5 Comparison of Hydrogen Production from Different Inoculum Treatments

The gas produced by dark fermentation of food waste using different treated inoculum were then stored in gas collection bags. The gas was then analyzed by means of gas chromatography. The result of gas chromatography was obtained in the form of peak of hydrogen.

4.5.1 Comparison of Hydrogen Peak from Different Inoculum Treatments

Figure 4.3 shows the peak of hydrogen from different inoculum treatments obtained as a result of performing gas chromatography. It can be clearly seen from the Figure 4.3 that the highest peak of hydrogen was obtained when chloroform treated inoculum was used in the dark fermentation of food waste. It means that the maximum percentage of hydrogen was obtained when chloroform treated inoculum was used.

The second highest peak was achieved when heat treated inoculum was used while the smallest peak was obtained when untreated inoculum was used. This clearly shows effect of different treatments on inoculum. The inoculum treatments increase the percentage of hydrogen by killing methanogens.

All of the treated inoculum have higher peak than untreated inoculum. So, in order to obtain a higher percentage of hydrogen, inoculum should first be treated.

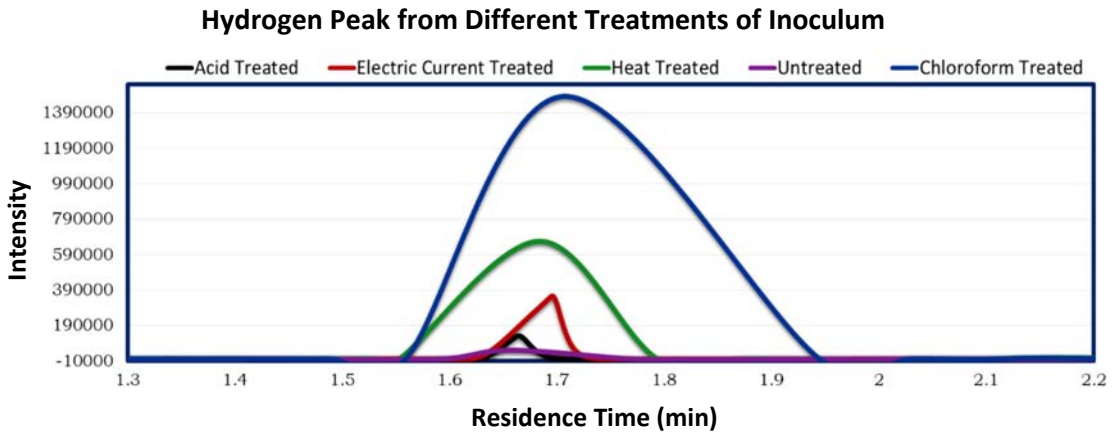


Figure 4.3 Comparison of Hydrogen Peak from Different Inoculum Treatments

4.5.2 Hydrogen Percentages from Different Inoculum Treatments

In order to obtain the percentage of hydrogen, the peak of pure hydrogen was attained and then compared with the peaks of hydrogen produced from different inoculum treatments. The percentages of hydrogen were calculated by comparing the area of pure hydrogen with each of the area of different treated inoculum and untreated inoculum. Figure 4.4 displays the percentage of hydrogen from different inoculum treatments.

Han and Shin (2004) produced biohydrogen from food waste by using untreated inoculum and the result they got for maximum hydrogen percentage was 5.1 % which is quite close to our result which shows 4.2 % of hydrogen when untreated inoculum was used. Hay et al. (2013) got a total of 23.6 % of hydrogen when treated by alkali. It gave a high percentage of hydrogen as compared to our result because of different substrate and inoculum.

It can be seen from figure 4.4 that hydrogen gas produced using chloroform treated inoculum has the highest percentage of hydrogen which is 29.26 % while the lowest percentage of hydrogen i.e. 4.2 % was produced when inoculum was untreated.

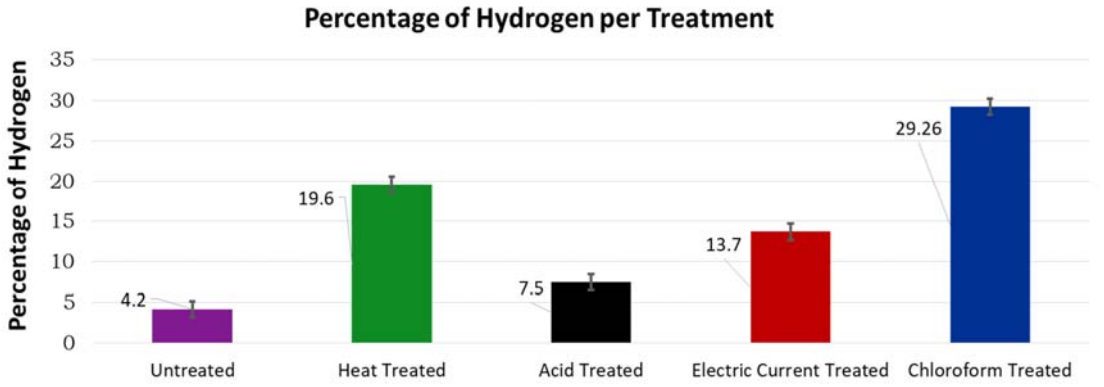


Figure 4.4 Hydrogen Percentages from Different Inoculum Treatments

4.5.3 Total Volume of Gas Vs Volume of Hydrogen

After calculating the percentage, the volume of hydrogen was calculated by multiplying the percentage of hydrogen with the total volume. For each treated inoculum and for untreated inoculum, volume of hydrogen was calculated and compared with its total volume of gas. From Figure 4.5, it can be seen that volume of hydrogen for Heat treated and Chloroform treated are almost the same but the total volume of gas from Heat treated is higher than the chloroform treated which means that in Heat treated there is more amount of CO_2 and H_sS as compared to that of Chloroform treated.

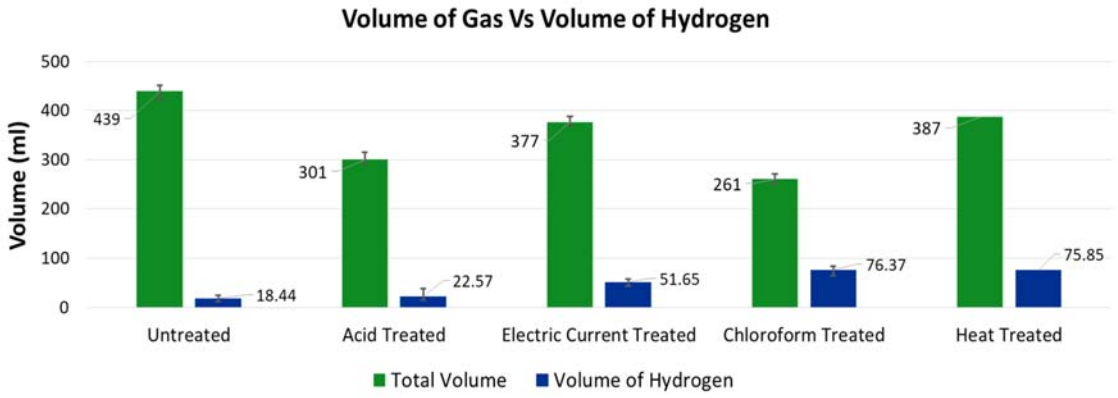


Figure 4.5 Total Volume of Gas Vs Volume of Hydrogen for Different Inoculum Treatments

4.6 Percentage of VS Removal from Different Inoculum Treatments

VS removal percentage shows how much of the substrate and inoculum have been consumed. VS of a mixture of substrate and inoculum was calculated before and after the experiment and then the percentage removal was calculated for each treated inoculum and for untreated inoculum. Figure 4.6 displays the VS removal percentage for different inoculum treatments compared to that of untreated inoculum. VS removal percentage has been the highest in case of untreated inoculum since it also produced the maximum amount of gas. However, this trend is not the same in case of treated inoculums. It shows a random trend.

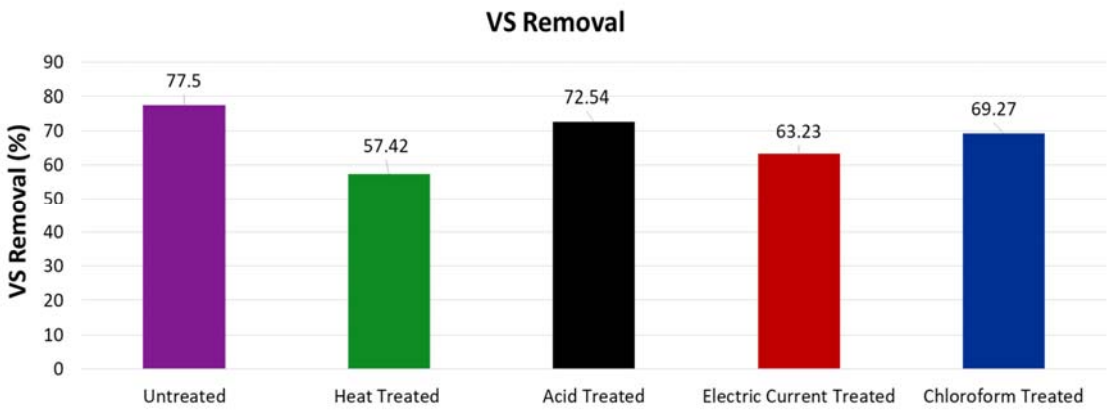


Figure 4.6 Percentage of VS Removal from Different Inoculum Treatments

4.7 Effect of Purification on Gas Mixture

The second objective of the project was to purify the gas mixture using Mono-Ethanol Amine (MEA) of 30% concentration. MEA absorbs CO_2 and H_2S that are present in the gas mixture. The reactor bottle was sealed using silicone and the gas was bubbled into MEA solution. After that, nitrogen purging was done in order to remove the air present inside the bottle. The reactor bottle was then placed on a hot plate at a temperature of 30˚C for two hours. The gas was then again collected in the gas bag and was analyzed by GC. Figure 4.7 shows the purification of gas produced from chloroform treated inoculum.

The percentage of hydrogen was increased from 29.26 % to 83.67 % which shows that most of the CO_2 and H_2S was absorbed by MEA.

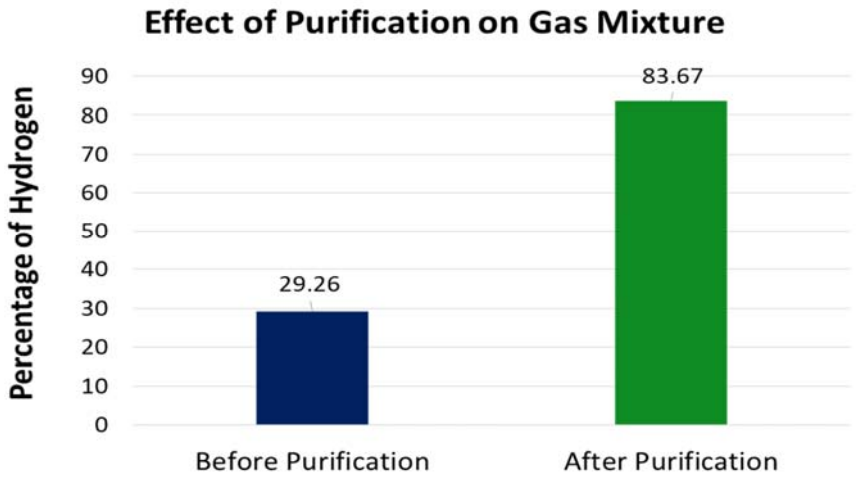


Figure 4.7 Purification of Gas Produced from Chloroform Treated Inoculum

4.8 Cost Estimation for Inoculum Treatments

The upgrading from lab scale reactor to industrial scale reactor and cost estimation of different inoculum treatments was carried out to comprehend which treatment is efficient considering the cost and percentage of hydrogen. The data is shown in the form of tables 4.2 and 4.3.

4.8.1 Upgrading from Lab Scale to Industrial Scale Reactor

The project was performed on lab scale and in order to estimate the cost an industrial scale reactor of $8m^3$ was considered. Table 4.2 discusses the parameters that were considered in the upgrading of reactor. The parameters include: size of the digester, working volume, volume of substrate and volume of inoculum.

Table 4.2 Design Parameters of Lab Scale Reactor and Industrial Scale Reactor

Design Parameters	Lab Scale	Industrial Scale
Size of Digester	300 ml	8 m^3
Working Volume	225 ml	6 m^3
Required Substrate	130 g	3466.67 kg
Density of Food waste	800 g/l	800 kg/m^3
Volume of Substrate	163 ml	4333 liters
Inoculum Required	45 g	1200 kg
Density of Cow Dung	1050 g/l	1050 kg/m^3
Volume of Inoculum	43 ml	1140 liters

4.8.2 Cost of Different Inoculum Treatment Methods at Lab and Industrial Scale

The cost of different inoculum treatment methods was calculated on lab scale and industrial scale considering the parameters given in table 4.2. The cost of different inoculum treatment methods were calculated according to the material used for treatment. Table 4.3 displays the cost of four different inoculum treatments at lab and industrial scale.

For Acid treatment, the cost of HCl and its total volume used was considered. For Heat treatment, the cost of power consumption of oven and burners was considered. Cost of chloroform was considered according to its volume used for Chloroform treatment. Lastly, for

Electric Current treatment, the cost of electrodes and the cost of power consumption was considered.

Table 4.3 Cost of Inoculum Treatments at Lab Scale and Industrial Scale

Treatment Method	**Cost at Lab Scale PKR**	**Cost at Industrial Scale PKR**	**Efficiency (% of Hydrogen)**
Acid Treatment	0.63	252	7.50
Heat Treatment	17.40	200	19.60
Chloroform Treatment	0.02	40	29.26
Electric Current Treatment	0.01	2.33	13.70

Chapter 5

Conclusions and Recommendations

5.1 Conclusions

Following conclusions were drawn from this study:

- Untreated inoculum produced the highest volume of gas (439 ml) during three-day dark fermentation process. Chloroform treated inoculum lead to the production of least volume of biogas (261 ml).
- As a result of Gas Chromatography (GC) analysis, Hydrogen peak was found to be the highest for chloroform treatment and lowest for untreated inoculum.
- Percentage of Hydrogen was found to be 29.26% for chloroform treated inoculum while it was found to be 4.2% for untreated inoculum. Volume of hydrogen was found to be the highest (76.37 ml) for chloroform treated inoculum while it was observed to be the lowest (18.44 ml) for untreated inoculum.
- Chloroform treatment was also found to be the most efficient as well as cost effective inoculum treatment method for bio hydrogen production from dark fermentation.
- After purification of bio hydrogen by using Mono-Ethanol Amine (MEA), percentage of hydrogen in the biogas increased from 29.26% to 83.67%. Mono-Ethanol Amine (MEA) was found to be very effective for purification and isolation of bio hydrogen and removal of hazardous gases like carbon dioxide and hydrogen sulfide.

5.2 Recommendations

5.2.1 Recommendations for field application

- Use a mixture of food waste and some other organic wastes as substrate for bio hydrogen production on field scale.

5.2.2 Recommendations for future study

- Use inoculum other than cow dung for dark fermentation.
- Conduct a study to investigate the hazardous aspects of chloroform treatment and Mono-Ethanol Amine and the strategies to counter those aspects.
- It is recommended to conduct a study based on continuous production of bio hydrogen by dark fermentation.

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