Biogenic Hydrogen Production from Potato Peel, Cabbage Waste and Application on Model Fuel Cell Car

By

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170908

Session 2016-18

Supervised by

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MASTERS of SCIENCE in

ENERGY SYSTEMS ENGINEERING

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THESIS ACCEPTANCE CERTIFICATE

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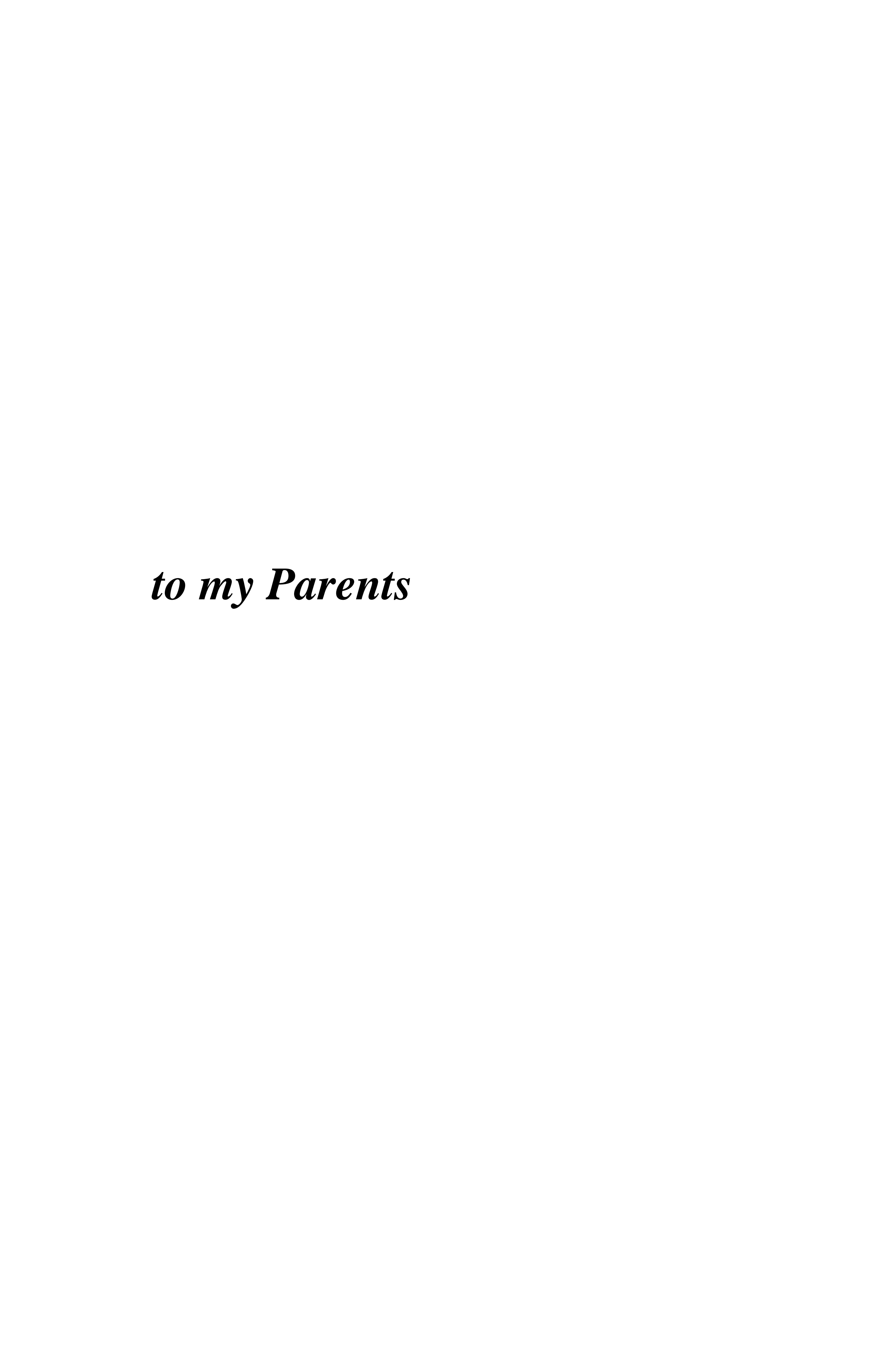

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Abstract

The effects of bacterial stress enrichment combined with different feed to inoculum (F/I) ratios on the bio-hydrogen yield from potato peel and cabbage waste were studied in various volatile solid (VS) concentrations using batch anaerobic digesters under mesophilic conditions in three stages. From results of the preliminary study, it was concluded that inoculum treated with heat shock at 95°C had the longest lag time of 48 hours, but it was most successful at suppressing the methanogens giving the least methane production out of all the 4 pre-treatment methods employed. Heat shock at 35°C produced most amount of hydrogen which was 158 times more than the control reactor. Heat shock at 35 and 95°C and aeration for 24 hours were selected to proceed to the next phase of experimentation which was to study the effects of feed to inoculum ratio at VS concentrations ranging from 0.35 to 0.76. In the repeated batch experimentation, 95P20 gave the highest specific biogas yield of 87.74 ml H_2 per gram of VS added followed by aCP20 which stood at 61.57 ml H_2/gVS added and 35P20 which yielded 56.86 ml H_2/gVS added. Chemical characterization revealed the high removal efficiencies in terms of volatile solids, chemical oxygen demand and total organic carbon for the above mentioned reactors. This study concluded that hydrogen production is feasible from mixed microflora if the suitable method of microbial enrichment is paired with the appropriate F/I ratio since different microbes give different response to the pre-treatment method and organic loading in the fermentative digester.

Keywords: Biogenic hydrogen production, mixed micro flora, inoculum pre-treatment, VS ratio.

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List of Journal/Conference Papers

1. **R. Manssor**, U. Jamil, Qamar, M.A., R. Liaquat. "Biogenic Hydrogen Production from Potato Peel by Mesophilic Dark Fermentation". **International Conference on Empowering Nation through Sciences, 2019.** (Presented)
2. U. Jamil, **R. Mansoor**, B. Batool, R. Liaquat. "Photocatalytic Degradation of Organic Dyes by Semiconducting Metal Sulphide Nanoparticles of Zinc and Copper Synthesized Through Single Source Precursor". **International Conference on Energy Materials and Nano-technology, 2018.** (Presented)
3. Qamar, M.A., U. Jamil, **R. Mansoor**, R. Liaquat. "Techno-Spatial Assessment of Agro-Forestry Waste to Bioenergy in Punjab, Pakistan". **Pakistan Journal of Agricultural Sciences, 2019**.
4. Qamar, M.A., U. Jamil, **R. Mansoor**, R. Liaquat, S. Azam. "Techno-Spatial Assessment of Waste Cooking Oil for Biodiesel Production in Pakistan". **Biomass and Waste Valorization.**

List of Abbreviations

Adenosine tri Phosphate	ATP
Anaerobic Digestion	AD
Association of Official Analytical Chemists	AOAC
Bromoethanesulfonate	BES
Carbon to Nitrogen	C/N
Chemical Oxygen Demand	COD
Coenzyme A	CoA
Energy-dispersive X-ray spectroscopy	EDS
Feed to Inoculum	F/I
Ferradoxin	Fd
Food Waste	FW
Gas Chromatography	GC
High Performance Liquid Chromatography	HPLC
Hydrogen Consuming Bacteria	HCB
Hydrogen Producing Bacteria	HPB
Low Heating Value	LHV
Moisture Content	MC
Nicotineamide Adenine Dinucleotide Hydrogenase	NADH
Proton Exchange Membrane	PEM
Pyruvate Ferradoxin Oxydoreductase	PFOR
Pyruvate Formate Lyase	PFL
Scanning Electron Microscopy	SEM
Total Organic Carbon	TOC
Total Solids	TS
Volatile Fatty Acids	VFAs
Volatile Solids	VS
Volatile Suspended Solids	VSS
Waste Activated Sludge	WAS

Chapter 1: Introduction

The current energy electricity generation mix of Pakistan is highly skewed towards thermal power plants, mostly operated on imported fuel oil. In addition to the carbon emissions, such dependence imposes a huge burden on country's economy and makes our electricity sector vulnerable to the fluctuations in international oil market prices. To overcome the supply-demand gap, the use of indigenous energy sources should be encouraged with a special focus on renewable energy deployment. This will lead to enhanced energy security of the country.

Hydrogen has broadened the horizon for ideal fuel in the future because upon its consumption no greenhouse gases and other potentially harmful by-products are released in the environment; moreover, it falls under the category of renewable fuels [1, 2]. Water is the main by-product of hydrogen when it is used as fuel, this water can either be discarded or reused to produce hydrogen again [3]. However, it does not occur in nature like fossil fuels, solar and wind energies, but has to be contrived just like electricity hence called the secondary form of energy [4]. Hydrogen combustion produces more energy on a mass basis than any other fuel. Its low heating value (LHV) is 2.4 times higher than that of methane, 2.8 time higher than gasoline and 4 times that of coal [5].

Due to the above mentioned properties of hydrogen, many energy scientists and economists see a great potential in hydrogen as a fuel, in shaping energy economics of the future [4]. This is supported by the fact that energy yield of hydrogen is 122 kJ/g or 61,000 Btu/lb, a significantly high value, which is 2.75 times greater than hydrocarbon fuels [6]. Science enthusiasts have demonstrated a number of model vehicles powered by hydrogen. These prototypes have paved way for the development of future hydrogen economy specific to its utility as a mobile fuel source [7]. The conversion to the renewable hydrogen economy from the existing fossil fuel-based economy requires a step-by-step process. Initial steps include careful designing of future energy scenarios which would sprout from the production and utilizations of hydrogen at industrial scale. In order for the hydrogen economy framework to thrive, current infrastructure of its production and utilization should be sustainable and competent with the fossil fuel economy [8].

Hydrogen is not only a clean fuel in terms of its conversion by-products to energy (which is only water), it is also quite versatile which means it can yield thermal energy in combustion engines and turbines through thermochemical reaction processes. Electrical energy can be obtained directly when hydrogen is subjected to electrochemical reaction with well- engineered fuel cells [8]. H_2 can be used either as the fuel for direct combustion in an internal combustion engine or as the fuel for a fuel cell [9]. These developments have sparked the interests of noticeable car corporations who were interested in the elimination of CO, HC and NOx from vehicular emissions, without compromising the automobile capability, fuel consumption and mileage [10].

Hydrogen is not only used as a fuel but is also a product in various industrial processes [6] Fertilizer and petroleum industries are the major users of H_2, utilizing respectively 50% and 37% of the total hydrogen produced on a commercial scale [9]. Food industry makes use of hydrogen gas in the hydrogenation of fats and oils, it is also extensively used for the production of chemicals, manufacture of electronic devices and processing of steel [6]. Hydrogen is used for desulfurization and re-formulation of gasoline in refineries [6] that is why the last five years have seen an annual increase in the production of hydrogen by 6%, because now a days refineries require this gas to conform the fuels to strict quality [9]. Another use of hydrogen is in the main engines of the space shuttles and rockets as a liquid fuel. Another very charming use of hydrogen is in the prospective fuel cell cars which though in the experimental stages, are now developed to a considerable extent in many countries [8].

As an “industrial gas,” hydrogen is already a big global business with strong fundamentals [11]. As per 2007, the yearly production of H_2 was about 0.1 Gton [5]. The hydrogen generation market is expected to grow to $154.74 billion USD in 2022 [11]. Contemporary process of hydrogen production to be used at industrial scale depends upon fossil fuel sources as shown in figure 1. It is evident from the figure that 96% hydrogen is produced from fossil fuels and about 4% is produced by using electricity that is also created through fossil fuels [12].

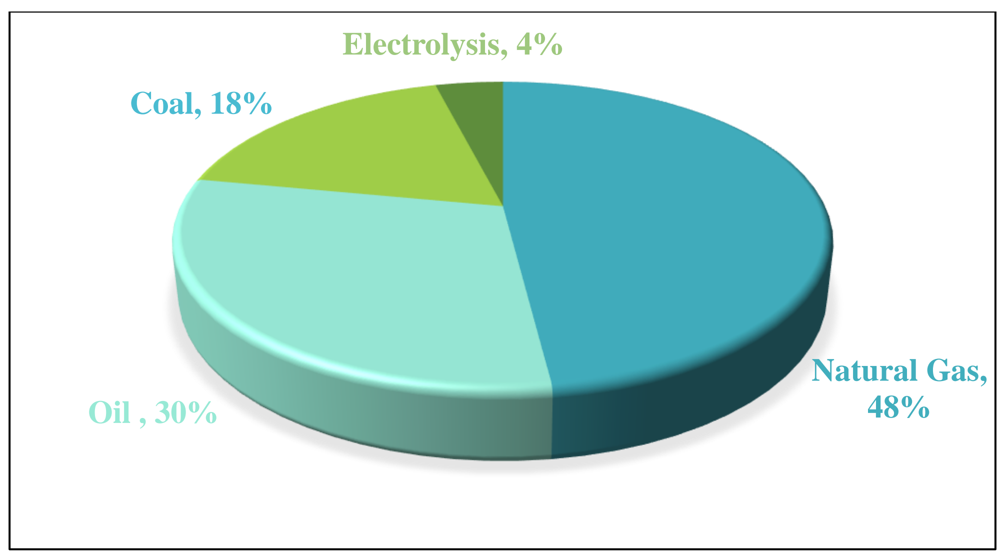


Figure 1.1: Feedstock used in current global hydrogen production [12].

1.1 Problem statement

Use of organic substrates to produce hydrogen through biological fermentation process seems like a plausible contribution to clean energy but it should be noted that this technology is still in its research phase. Reason being low hydrogen yields [13] [14]. While at the lab scale, the results are consistent and reportable, a lot of work has to be done in order to scale up this process of acidogenic fermentation for commercial hydrogen production. For this purpose, many parameters need to be streamlined; among them economical and easy availability of large amounts of anaerobic hydrogen producing biomass is of main concern [15, 14].

1.2 Scope of the study

This study aims to enhance hydrogen production by acidogenic digestion of vegetable wastes which is not only a renewable means of hydrogen production, it will also contribute to the reduction of food wastes. Furthermore, emphasis is laid on the importance of hydrogen as an efficient fuel by demonstrating its practical application.

1.3 Objectives of the study

I. To determine the influence of bacterial stress enrichment on anaerobic hydrogen-producing microorganisms.

II. To investigate the potential use of vegetable waste and pre-treated inoculum from first stage of H_2 production by dark fermentation.

III. To optimize the most suitable feed to inoculum ratio with locally available substrate and inocula.

IV. Demonstrate the operation of a model fuel cell car on biohydrogen.

1.4 Summary

Form the past few decades there is an increase in harnessing energy from renewable resources to meet the energy demands because conventional sources of energy have proven to be the culprits of environmental deterioration. Focus is also being given on novel energy means which are more efficient and less polluting. Hydrogen is one such example which is such an energy carrier that it can be produced by multiple methods and has an array of applications including fuel cells. This research has important applications in the field of waste to energy as it can enhance the hydrogen production which can be used for practical energy applications. This chapter presents the aims and objectives of this thesis.

Chapter 2: Literature Review

2.1 Hydrogen sources and production methods

Hydrogen sources and its production methods are diversifying. They range from conventional fossil fuels to zero carbon nuclear energy and also environment friendly renewable feedstocks like solar, wind and biomass. Figure 2.1 summarizes these methods and their sources.

Fossil Fuels		Nuclear Energy		Renewable Energy		
Natural Gas	Coal	Thermochemical Water Splitting	High Temperature Electrolysis	Solar Photo voltaic	Wind	Biomass
Steam Reforming	Gasification			Electrolysis		Gasification/ light fermentation/ dark fermentation
End use: Hydrogen energy						

Figure 2.1: Selected hydrogen production methods [16].

2.1.1. Hydrogen production from fossil fuels

2.1.1.1 **Steam reforming**

This method has been used for many years and is the most desirable from industrial point of view. In simple terms, steam reforming of hydrocarbons is a two-step process: in the first step, steam is used to split hydrocarbons usually natural gas [16] where CO and H_2 are produced [17] and the second step, called water gas shift [16] in which a catalyst converts the CO and water to hydrogen and CO_2. This is

followed by purification of hydrogen gas. Large reformers are capable of reaching more than 80% yield with this technology [17].

2.1.1.2 **Gasification**

Another technology which is favoured for large scale hydrogen production is gasification. In this process, a carbon source is subjected to high temperature (1200-1400 K) and moderate pressures (5-10 bar). Gasification yields a number of gaseous products including hydrogen [16].

2.1.2. Hydrogen production from nuclear energy

2.1.2.1 **Thermochemical water splitting**

Nuclear energy has been put into use for producing hydrogen by thermochemical water-splitting cycles. Temperature conditions of 500°C or higher are required which are achieved using nuclear reactors [17, 18].

2.1.2.2 **High Temperature Electrolysis**

Basic principle of this mechanism is to split water to obtain hydrogen using electricity produced from nuclear sources. Major drawback is the overall efficiency of this process which is limited by the efficiency of nuclear power plant (around 33% with recent reactors). Although electricity-to-hydrogen conversion efficiency itself can be as high as 80% under pressure [17, 19].

2.1.3. Hydrogen production from renewable energy

2.1.3.1 **Electrolysis**

This is a desirable process from environment point of view as the hydrogen produced is pure and free from carbon and sulphur impurities. But its energy needs contribute to more cost than other processes which utilize fossil [17, 20]. Nevertheless, electrolysis is still seen a viable process because of its small size and small scale applications [17].

2.1.3.2 **Solar photovoltaic**

In this technology, solar PV is used to produce electricity which is then directed to split water. Presently, this lies towards expensive side of the spectrum, but with the advancement in solar PV, cost of solar panels is expected to go down [17, 19].

2.1.3.3 **Wind**

Wind energy for hydrogen poses the con of high cost and optimization of wind turbines and electrolyser storage systems. But on the plus side, this option is highly

favourable among renewable resources because of zero carbon footprint especially for distributed systems. The cost factor is expected to decrease in future [17, 19].

2.1.3.4 **Biomass gasification**

Biomass is a carbon rich source, although its carbon density is not as high as fossil fuels. It can be served as a feed stock in the gasification process to yield syn-gas which is a mixture of CO, H_2, CH_4 and CO_2. Operational conditions for biomass gasification are slightly different from coal Gasification; pre-treatment of biomass feedstock is also required [21].

2.2 Hydrogen production routes by biological processes

Biological ways of hydrogen production pertain to the processes which occur in metabolic cycles of living organisms and are used to produce hydrogen by natural means. Algae and some species of bacteria produce hydrogen as a by-product in their natural food cycles. Their catabolic processes can be honed to increase the yield of hydrogen or specifically called biogenic hydrogen.

Key bio-processes for hydrogen gas creation can be categorized in three types (also shown in Figure 2.2):

i. Biological breakdown of water molecules in the presence of light by algae and cyanobacteria.
ii. Dark fermentation of organic matter when anaerobic digestion is at its acidogenic phase.
iii. Photo-fermentative process followed by dark fermentation in a two stage process. [6]

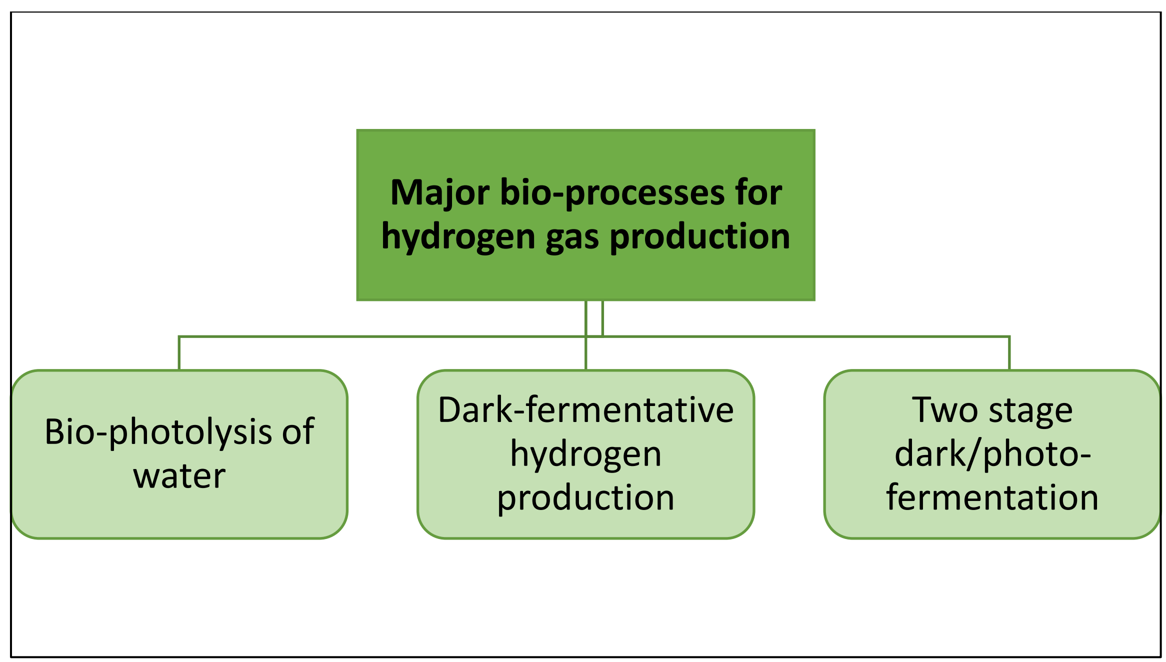


Figure 2.2: Major categories of bioprocesses involved in hydrogen production [6].

2.2.1. Bio-photolysis of water by algae and cyanobacteria

Higher plants use sunlight to reduce carbon dioxide and generate biomass. Similar photosynthetic process is found in micro algae (both eukaryotic and prokaryotic) and cyanobacteria but it reduces protons to produce hydrogen molecules. Special enzymes called hydrogenases perform this process which are absent in higher plants [22].

2.2.2. Dark fermentative hydrogen production during acidogenic phase of anaerobic digestion

A lot of attention has been given to the hydrogen production by photosynthetic organisms than to fermentative hydrogen production. Fermentative hydrogen production can prove to be industrially favourable due to:

- Fermentative microorganisms are capable of producing hydrogen at a fast pace.
- This process of fermentative hydrogen evolution is unremitting and proceeds constantly from organic substrates.
- They can have growth rate good for supply of microorganisms to the production system.

For the purpose of commercial hydrogen production by biological means, fermentative production is more feasible than photochemical emission by microorganisms.

Fermentative hydrogen evolution can be tailored to get good yield by enhancing electron transfer naturally done by hydrogenase enzyme. An external source of electron like supply of zero valent iron can serve this purpose [23].

2.2.3. Two stage dark and photo-fermentative production of hydrogen

Since both light dependent and light independent fermentative process for the evolution of hydrogen have their own limitations, this set-up aims to enhance H_2 yield by combining these two processes together. In this combination, organic substrate is first degraded by dark fermentative bacteria until anaerobic process reaches the stage where this cannot be proceeded further due to the accumulation of acid by-products. In the second phase, photosynthetic bacteria make use of light to degrade organic acids into more H_2, hence completely digesting glucose substrate into hydrogen and carbon dioxide. In this way, light energy demand of photosynthetic bacteria is also reduced [24].

2.3 Dark fermentation (Microbial fermentation)

Out of all the methods mentioned above, dark fermentation checks most of the boxes for the production of ideal fuel [25]. Although, still in the research phase, but mechanism of this process are better understood by the researchers (amongst all the processes) who are working on various aspects to refine it [26]. Dark fermentation is a complex process which, in the presence of strictly anaerobic conditions, covert the degradable organic material into a mixture of gases called biogas. The biogas mainly consists of hydrogen, methane and carbon dioxide [27]. This degradation and transformation is carried out by specialized consortium of anaerobic microorganisms which eventually results in energy recovery as biogas production and formation of bio-slurry to be used as natural fertilizer for crop productivity [28]. The science behind anaerobic fermentation process is quite complicated because it involves key microbiological pathways and it is best comprehended if it is broken down into different stages [29]. These stages are interlinked as the product of one stage serves as substrate for the bacteria of next stage [30].

2.3.1. Biochemical process of dark fermentation (overview of metabolism in dark fermentation)

Many anaerobic microorganisms utilize hydrogen as the main substrate in their metabolism. Hydrogen molecules are rich in energy as the oxidation of H_2 molecules

produces energy which is utilized by such microorganisms are capable of utilizing the electrons from hydrogen oxidation to produce energy [26]. Figure 2.3 shows the degradation of a simple substrate into hydrogen by means of fermentative bacteria.

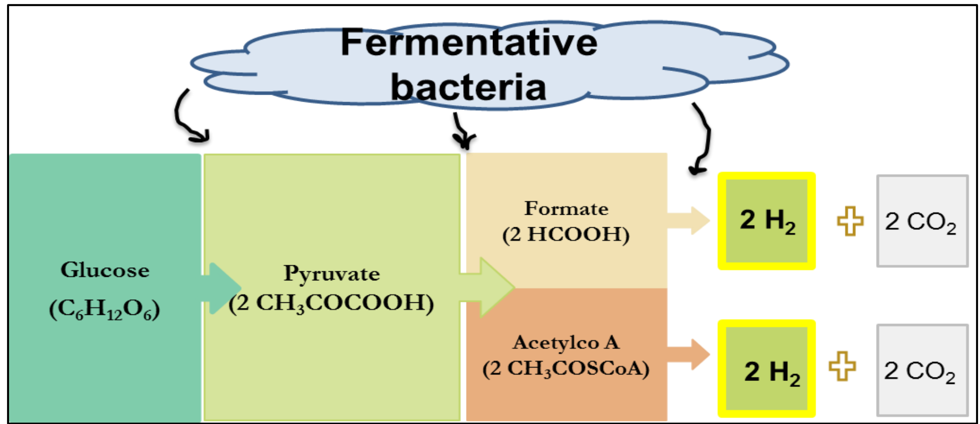


Figure 2.3: Simple model explaining the steps of dark fermentation.

Metabolic processes of organisms split molecular hydrogen into protons and electrons in a reversible reaction. When there is no external acceptor, these electrons become in excess. Hydrogen metabolism is majorly regulated by the enzymes called hydrogenases. There exist two main types of hydrogenases enzymes; differentiated on the basis of phylogenetics and their active sites. One of which is [FeFe]–hydrogenase (Iron iron-hydrogenase) and other one is called [NiFe]–hydrogenase (Nickle iron-hydrogenases). They serve as catalysts in the reversible reaction of proton oxidation:

$2H^+ + 2e^- \leftrightarrow H_2$……………………………….(1)

In the above chemical equation, [FeFe]–hydrogenases (which are typically oxygen sensitive) help the reaction move in forward direction while [NiFe]–hydrogenases, play major role in the oxidation of molecular hydrogen [31, 7].

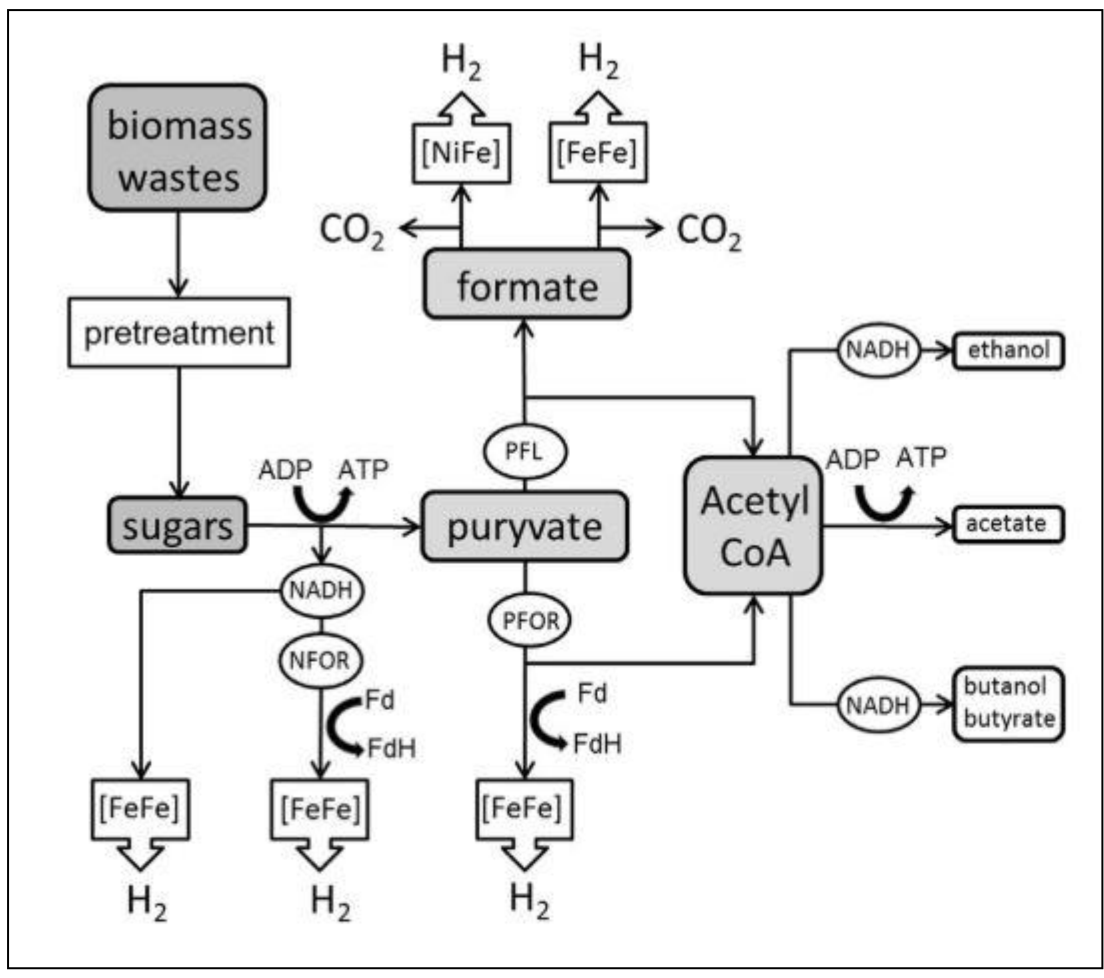


Figure 2.4: Typical metabolic pathways for conversion of substrate to hydrogen during dark fermentation [26].

Figure 2.4 illustrates the well understood metabolic process of hydrogen production from a simple substrate like glucose. In the first step called glycolysis, pyruvate is produced from glucose which is the key intermediate in this process along with the reduction of nicotinamide adenine dinucleotide (NADH). Pyruvate ferredoxin oxidoreductase (PFOR) catalyses the conversion of pyruvate into acetyl-CoA and CO_2 in anaerobic environment. Ferredoxin (Fd) is also reduced in this reaction which further reduces [FeFe]–hydrogenases. This enzyme, as mentioned above, yields H_2 by proton reduction. Pyruvate to acetyl-CoA conversion is also possible through another pathway and pyruvate formate lyase (PFL) serves as the catalyst. Formate is another product of this reaction besides acetyl-CoA. Formate conversion results into H_2 and CO_2 in the presence of either of hydrogenase enzymes. The last step of fermentation is accompanied by the formation of value added fermentation products like ethyl alcohol, butyl alcohol, butyric acid, acetic acid or acetone. These products are resulted from the

conversion of acetyl-CoA. Furthermore, NADH is oxidized and/or ATP is formed. [32, 33].

Glycolytic fermentations can follow many metabolic pathways. Difference lies in the final products which are formed. Following types of hydrogen fermentations have been distinguished:

- Butyrate, butanol fermentation: This particular type of fermentation yields out butyric acid, hydrogen, butanol, CO_2 and acetic acid as major products. In addition, various other substances like acetone, 2-propanol, ethyl alcohol, lactic acid, acetoin are also accounted as the products. Clostridium bacteria are the main cradle of butyrate butanol fermentation [26, 33].
- Mixed acid fermentation (acetic acid and formic acid fermentation): This fermentation pathway harbors formic acid, acetic acid, ethyl alcohol, hydrogen, CO_2, lactic acid and succinic acid, glycerol, acetoin, and 2,3-butanediol as the end products. Enterobacter and Bacillus bacteria are the dominant species. [26, 33].

The stoichiometric, theoretically maximal, amount of molecular hydrogen per mole of glucose, according to Eq. (2), equals 12 mol [33]:

$$C_6H_{12}O_6 + 6H_2O \rightarrow 6CO_2 + 12H_2 \qquad (2)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + 2CH_3COOH + 4H_2 \qquad (3)$$

$$C_6H_{12}O_6 + 6H_2O \rightarrow 2CO_2 + CH_3CH_2CH_2COOH + 2H_2 \qquad (4)$$

Practically, the process yields lesser moles of molecular hydrogen because in reality, the end result yields a blend of different chemical products, which depresses the hydrogen yield to 1–2.5 mol of H_2 per mole of glucose. When acetic acid is formed, moles of hydrogen are cut down from twelve to four (Eq. (3)). Creation of butyric acid as the by-product extracts only 2 moles of hydrogen from 1 mole of glucose (Eq. (4)). In order for hydrogen production to be economically viable from biomass, 60-80% of the energy trapped in substrate should be harnessed and converted to hydrogen [6]. Further economic efficiency can be achieved by isolating the by-products from anaerobic fermentation broth and finding some commercial use for them. Amount of hydrogen gas production through fermentation process depends upon many factors. It is indeed a task to optimize the contributing factors and design a good

performing process. For this reason, plentiful studies have been undergone to optimize of conditions of dark fermentation for the purpose of achieving the yield in the vicinity a theoretical maximum [26].

2.4 Key factors affecting the efficiency of bio-hydrogen production

It is crucial meticulous environmental conditions are maintained in the bio-hydrogen fermenter during gas production to keep hydrogen-consuming bacteria at bay. Hydrogen producing bacteria thrive under favorable growth conditions whereas other bacteria in the mixed culture like bacteria responsible for producing solvents and methane gas (also called hydrogen consuming bacteria) could be subdued [34, 35]. Major physio-chemical factors which affect anaerobic fermentative process are given in figure 2.5.

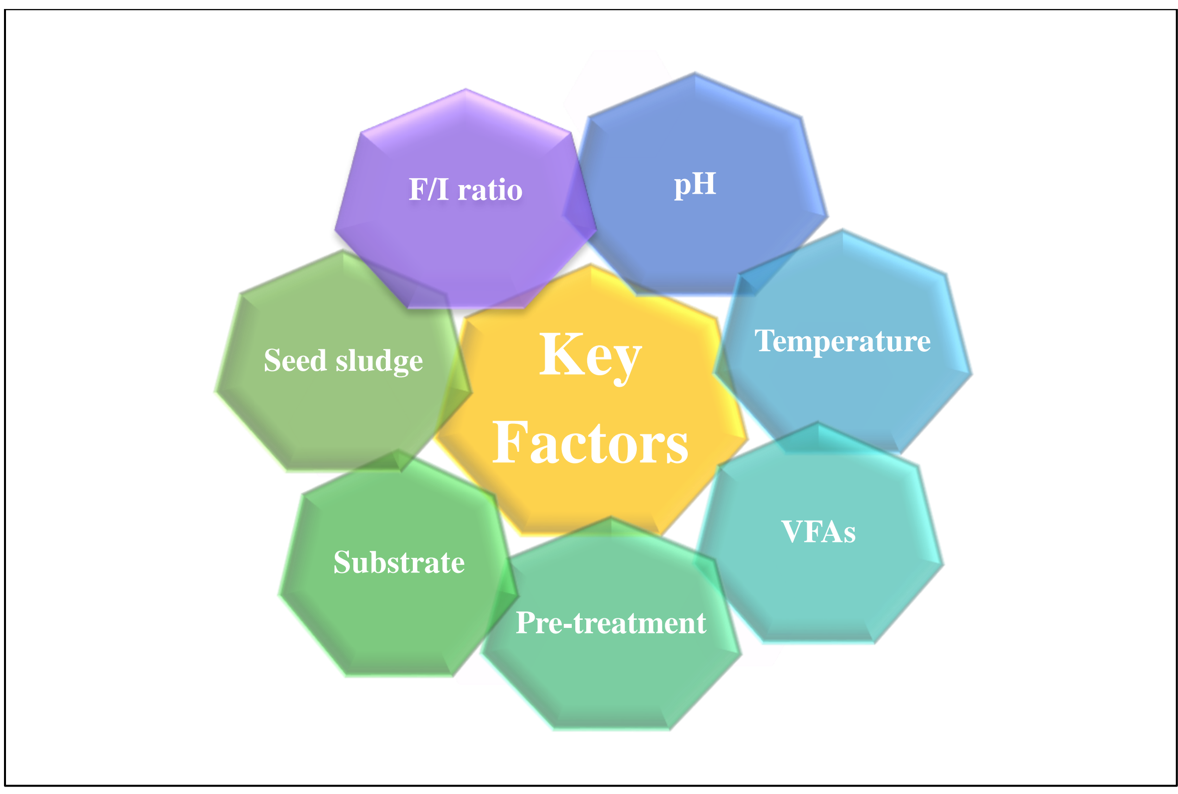


Figure 2.5: Key factors affecting the efficiency of bio-hydrogen production.

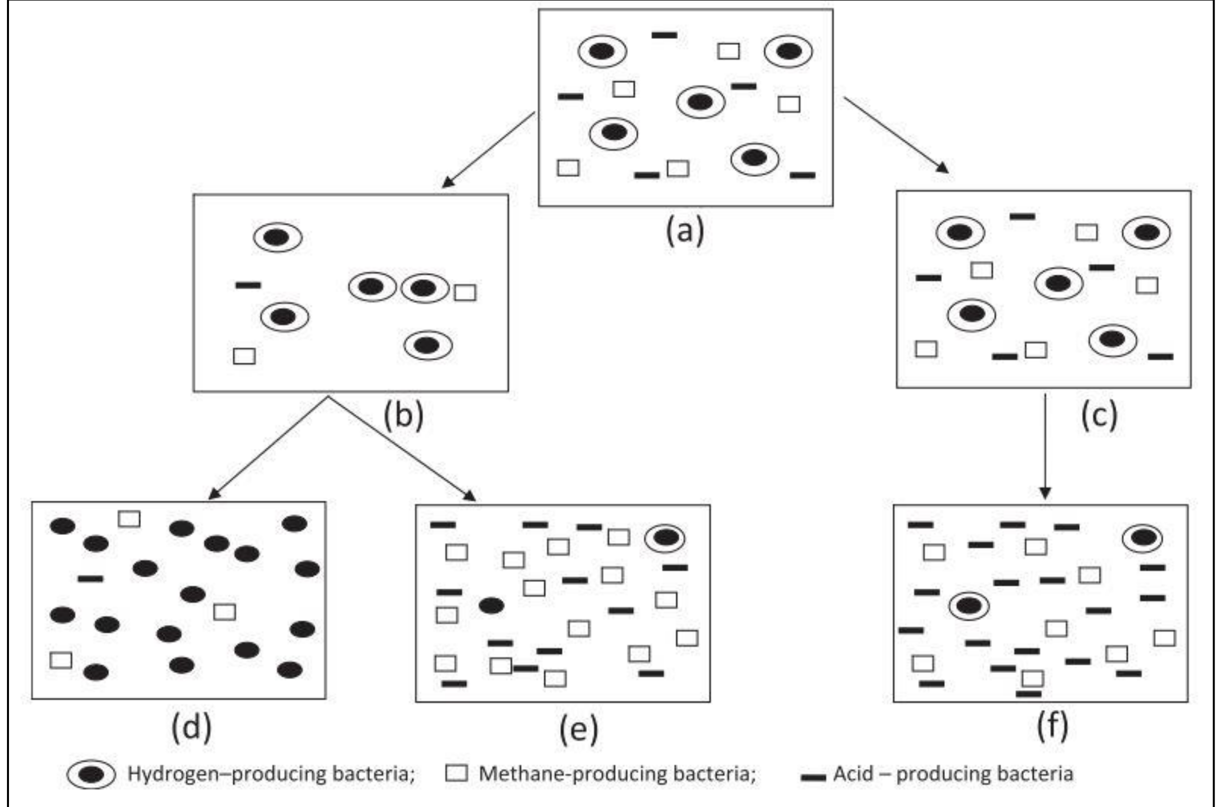


Figure 2.6: Mixed culture producing fermentative hydrogen and the factors like pre-treatment, pH and temperature affecting this process: (a) Untreated mixed culture, (b) Pre-treated mixed culture, (c) Mixed culture without pre-treatment (d) Mixed culture at pH 5.0-6.

2.4.1. pH

Metabolic pathways and enzyme activity of microbes producing hydrogen is heavily influenced by pH especially if the substrate is food waste or food processing waste [36, 37]. Methanogens (hydrogen consuming bacteria) should be oppressed in order to get good hydrogen yield. These are energetic in a slender array of 6.3 to 7.8, so pH adjustment is important to limit methanogens. [38]. Fig. 2.6e highlights the importance of pH adjustment by illustrating results of a study in which methane and acid-producing bacteria remained dominant in fermentation even after inoculum pre-treatment because pH was in the range of 6.3 to 7.8. The population of hydrogen-producing bacteria declined drastically if the temperature was not appropriate for these organisms to thrive [34]. Many researchers have concluded that the most suitable pH range for HPB growth lies between 5.0-6.0 [39, 40, 41, 42, 43] as indicated in Fig. 2.6d. Nevertheless, there are no records of studies which report biohydrogen evolution at pH less than 4.0 or more than 8.0. At pH lower than 4.0, ATP generated by bacteria

is used to maintain cell structure disrupted by acidic conditions rather than hydrogen production [44, 45].

2.4.2. Temperature

Anaerobic fermentation of food wastes and food processing wastes is heavily affected by temperature. At industrial level, mesophilic temperature is preferred because it is cost effective and easy to maintain. That is why, most researchers have focused on mesophilic fermentation at lab scale. The range for this lies between 30 to 37 ^{o}C, which can be achieved at lower energy and even at ambient temperatures when weather is hot for the direct conversion of waste to bio-hydrogen. However, thermophilic temperature has been reported to generate higher biohydrogen yield but is not economical [46].

2.4.3. Volatile fatty acids (VFAs)

Certain organic acids like butyric acid, acetic acid, propionic acid and lactic acid are amongst the main by-products of anaerobic digestion [47]. A group of researches devoted their study to monitor fermentative hydrogen production and the impact of produced VFAs. They concluded that acetic acid and butyric acid production goes hand in hand with hydrogen production. On the other hand, no hydrogen could be detected if lactic acid and propionic acid were found to be the by-products [48]. These results are justifiable because bacteria which are mainly responsible for food spoilage at room temperature are lactic acid bacteria; these organisms are also the cause of hydrogen fermentation failure [46, 49].

2.4.4. Pre-treatment

It is a known fact that mixed culture has a variety of bacterial species, so in order for hydrogen producing bacteria to thrive in mixed culture fermentative environment, hydrogen-consuming bacteria has to be eliminated. Fortunately, populations of hydrogen-producing bacteria are not much affected under severe environmental conditions such as high temperature, chemical or pH shock, resulting in the sprouting of bacterial spores [50]. Figure 2.6 illustrates the variations of microbial community in mixed culture inoculum. This type of inoculum contains native bacterial species like hydrogen-producing bacteria encapsulated with spores (which can sustain harsh living environments), methane-producing bacteria as well as organic acids-producing bacteria (Figure 2.6 a). Due to the presence of plethora of microbes, mixed culture has to undergo certain pre-treatments like high temperature exposure, introduction of

selectively harmful chemicals or pH jolt to enhance the development of hydrogen-producing bacteria and abolition of hydrogen-consuming bacteria [35]. Figure 2.6 b shows that when pretreatment is done, methane and acidproducing bacterial communities decline due to unfavorable conditions while the hydrogen-producing bacteria sprout from the protective spores and start their growth. Clostridium sp., and Caloramator australicus are the known hydrogen producers, the dominance of which has been confirmed in pre-treated mixed cultures by various scholars undertaking research on food waste as a substrate for bio hydrogen production [51, 52]. On the other hand, without pretreatment of the mixed culture, the amount of CH_4 and solvent producing bacteria increased which suppressed the germination of hydrogen-producing bacteria so they remained covered by the protective spores (Figure 2.6 c,f).

As testified, [53, 54] acid, base, heat-shock, aeration, freezing and thawing, chloroform, sodium 2-bromoethanesulfonate (BES) or 2-bromoethanesulfonic acid and iodopropane are commonly used pretreatment approaches to enrich hydrogen-producing bacteria in seed sludge.

2.4.5. Seed sludge

Naturally occurring substances like soil, waste water sludge and compost etc offer habitats for bacteria capable of producing hydrogen [55, 53, 54, 56]. Acidogenic process to produce hydrogen can be seeded by these inocula freely available in nature. Many researches have analyzed the effects of mixed microflore of bacteria from anaerobic sludge, municipal sewage sludge, compost and soil on fermentative hydrogen production [57].

The use of mixed cultures is more feasible than using pure cultures because of ease of availability, wider choices to select from, simple operation and easy control [57]. There is however, one major concern which is the consumption of hydrogen molecules produced in a fermentative hydrogen production process using mixed cultures, owing to the presence of hydrogen consuming bacteria. In order to curb this issue, mixed cultures are subjected to pre-treatment under harsh conditions, so that hydrogen-producing bacteria would have a better chance of survival than some hydrogen-consuming bacteria. That being said, in order for fermentative hydrogen production system to produce considerable hydrogen, the inoculum can be pretreated by some techniques to suppress as much hydrogen-consuming bacterial activity as possible

while still preserving the productivity of the hydrogen-producing bacteria [55]. The ultimate goal is highest hydrogen yield.

2.4.6. Type of substrate

Fermentative hydrogen production is a well-studied topic and different scientists have demonstrated the use of different substrates for this process. Glucose, sucrose and starch had been the substrates of choice for fermentative hydrogen production [58]. But in recent years, a new approach has surfaced to use organic wastes as substrate for hydrogen production. It is to be noted that most of the studies on fermentative hydrogen production were conducted in batch mode [6].

It seems to be a believable concept that increasing substrate concentration could increase the ability of hydrogen-producing bacteria to produce hydrogen as more substrate means high nutrient content for bacteria get nourishment from. But it was demonstrated that substrate concentrations have optimum level for achieving a successful hydrogen producing fermentation process; at much higher levels substrate can choke the system [59, 60]. To quote the single exact value or even range of optimum substrate concentration is not possible because results of each study vary from the other. It is because mixed cultures have extremely complex dynamics which change with even the slightest change of parameters. The researches have variations in terms of inoculum and substrate concentration range studied [58].

Some substrates do not yield commendable results when used directly in dark fermentation owing to their complex structures; therefore, they require additional pre-treatments which breakdown their structures and they can be easily used by hydrogen-producing bacteria [61]. Waste activated sludge (WAS) which is sourced from wastewater treatment plants has high organic matter content and thus is a potential substrate for hydrogen production. After appropriate pre-treatments such as ultrasonication, acidification, freezing and thawing, sterilization, methanogenic inhibitor and microwave, the ability of hydrogen-producing bacteria to produce hydrogen from it can be improved [62, 63].

Other than the ones mentioned above, Food waste and food processing wastes are also being seen as probable candidates for biohydrogen production. Food waste mixture has different combinations of carbohydrate, fat, protein, cellulose and hemicellulose. This variation in nutrient composition has a profound impact on bio-hydrogen yield

which is not clearly understood by biochemical means [34]. Out of all the nutrients, carbohydrate has been reported to be the most suitable feedstock for biohydrogen production although other components in food waste such as fat, protein and cellulose can also be used as substrate [64, 43]. Lipids and proteins have complex biochemical structures and are difficult to degrade so cannot yield much hydrogen in theory [64].

2.4.7. Feed to inoculum (F/I) ratio

When setting up a batch digester, the regular practice is the addition of inoculum and substrate in calculated quantities. This parameter is called the feed to inoculum ratio and symbolized as F/I of the digester. F/I of a reactor is measured either as the amount of feedstock volatile solids (VS) added per the amount of inoculum VS or per the amount of inoculum volatile suspended solids (VSS) [65]. F/I of any anaerobic process is an important entity as its value can increase or decrease the bio-gas yiled. Guangqing Liu et al, 2009 demonstrated that biogas yields after 25 day digestion time was influenced by the F/I ratio: the higher the F/I ratios the lower biogas yield. This inverse relation was due to low methanogenic activity and/or the number of methanogens, in the digesters, that could result in the accumulation of the volatile fatty acids (VFA) produced during the acidogenic step [65]. High concentrations of volatile fatty acids could cause inhibition to methanogenesis [66].

2.5 Application of hydrogen as a fuel in mobile assemblies

Internal combustion engines rule the vehicles and stationary distributed energy applications but they have also proved to be environment polluters. In contrast, fuel cells are emerging as an attractive technology for electricity applications. It is also likely that in future portable electric power equipment will demand more efficient source of power than current battery technology [67].

In very basic term, design of fuel cell encompasses two electrodes (anode and cathode) separated by an electrolyte. Many types of fuel cells have been developed with main difference in the electrolyte (solid, liquid or membrane) and operating temperature. These different types serve for various energy applications ranging from small to large scale, stationary and mobile. Hydrogen (or a hydrogen-containing fuel) is introduced at the anode and air is fed to the cathode. Catalysts speed up the electrochemical reactions at the electrodes. The electrolyte serves as the medium for the transportation

of ions from anode to while the excess electrons flow through an external circuit to provide electrical power [67].

Hybrid and electric cars have already marked their place in the automobile industry. The fuel cell running on hydrogen is one step further in the race to manufacture environment friendly and energy efficient vehicles. It eliminates emissions on the tank-to-wheel path, the fuel (hydrogen) can be produced from many sources, and it provides very high average efficiencies. Fuel cell system can be easily integrated into cars just like the internal combustion engines. Fuel cell technology for vehicles is gradually becoming more user friendly and compact as compared to its earlier versions. HydroGen3 is a hydrogen fuel cell vehicle used for testing. It has been sized in such a way that it requires same amount of space and same mounts to be fixed as the internal combustion engine propulsion module. This assembly can be easily and cost effectively retrofitted in existing vehicles paving way for the mass manufacture without making massive changes in the current car manufacturing platforms. Different vehicle sizes can be accommodated due to ease of scalability of the fuel cell system. One example is the fuel cell system that was developed for the GM HydroGen3, and then was adapted to a small vehicle, the Suzuki MR Wagon FCV, using a shorter fuel cell stack with lesser number of cells. Later, it was adapted to a GMT800 truck by doubling the stack and some other components [68].

2.6 Summary

Hydrogen production by biological routes is an active research field and utilization of waste as a raw material gives an added advantage of waste to energy conversion. Dark fermentation is one such biological process which encompasses waste utilization and robust means of hydrogen production. This is a complex process which, in the presence of strictly anaerobic conditions, covert the degradable organic material into a mixture of gases called biogas. The biogas mainly consists of hydrogen, methane and carbon dioxide. This degradation and transformation is carried out by specialized consortium of anaerobic microorganisms which eventually results in energy recovery as biogas production and formation of bio-slurry to be used as natural fertilizer for crop productivity. Many factors govern the success of this process including pH, temperature, fermentative microflora, pre-treatment method, feed to inoculum ratio, hydrogen partial pressure and type of substrate. All these parameters should be taken

into consideration in order to get enhanced hydrogen yield capable of being used in practical scenarios like fuel cells.

Chapter 3: Methodology

Schematic diagram of the research process is shown in figure 3.1. Experimental work began with the selection, characterization, and pre-treatment of the precursor materials followed by hydrogen production potential set-up experiment in batch mode. Methodology is visually presented in figure 3.1 with dotted arrows indicating the methods of performed in each step.

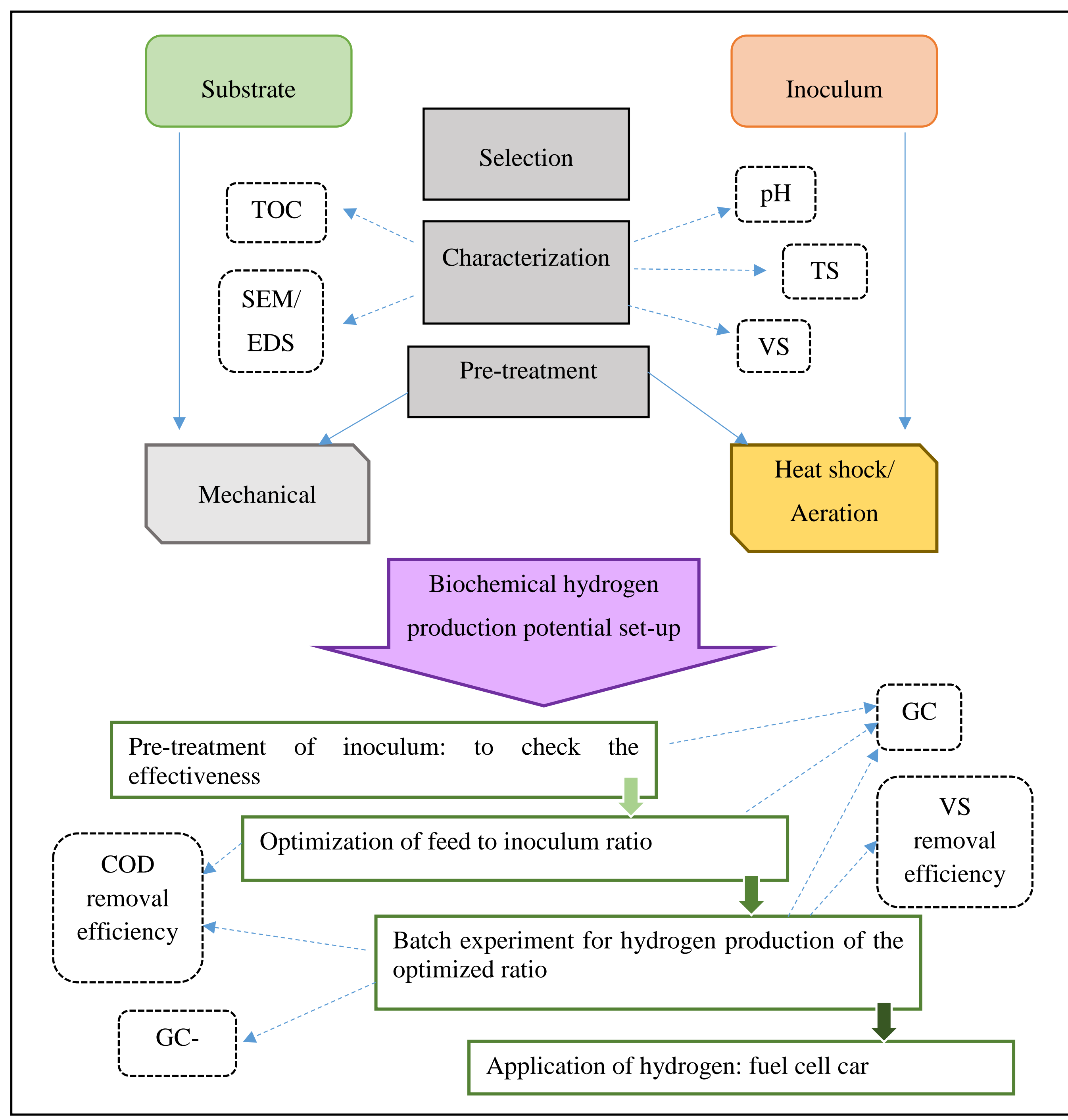


Figure 3.1: Scheme of experimental work

3.1 Substrate and inocula selection

3.1.1. Substrate

As mentioned in the previous section, most suitable substrates for anaerobic digestion particularly to get hydrogen out of this process are the organic materials rich in starch, which is readily hydrolysed to simple carbohydrates, is particularly convenient. Naturally occurring, renewable sources of sugars are starch as well as cellulose and hemi- cellulose, present in plants mostly as polymers [26, 33]. For these reasons, the substrates selected for this research were potato peel and waste cabbage leaves obtained from house hold kitchen waste.

Figure 3.2: Selected substrates: Cabbage leaves (left) and potato peel (right)

3.1.2. Inoculum

Inoculum used in this study was the digestate of digester undergoing anaerobic co digestion of dairy waste and various food wastes. It was obtained from a biogas digester situated in Fateh Jang; digesting animal manure and vegetable waste. The digestate was stored in the lab in wide mouthed bottles at 4°C.

Figure 3.3: Inoculum used in the study

3.2 Substrate and inoculum characterization

3.2.1. Nutritional values of the selected substrates

3.2.1.1 **Determination of Carbohydrates**

High Performance Liquid Chromatography (HPLC) is used to determine the content of various saccharides in food. Sample preparation includes solid-liquid extraction of solid food samples [69].

3.2.1.2 **Determination of Proteins**

Kjeldahl method for the determination of proteins is the internally practiced routine in food and other organic samples. Depending upon the sample size, macro and micro Kjeldahl methods have been established. In simple words, this process is a digestion and titration process. Sulfuric acid and a catalyst digest the sample. Most of organic nitrogen is reduced to ammonium sulphate, which is distilled in the presence of NaOH, to release ammonia in gaseous form. The distillate is collected into boric acid solution, and the borate anions formed are titrated with standardized HCl acid solution. The milliequivalents of acid required for titration are used to calculate the nitrogen content in the sample [70].

3.2.1.3 **Determination of Fats**

Fats require both qualitative as well as quantitative determination for their sound analysis. The Gas Chromatography can serve this purpose. Accuracy and reliability of fat analysis depends upon the capability of instrument to detect and

measure all fatty acids in a sample. It is for this reason that very long, highly polar capillary GC columns are recommended to maximize the resolution of as many fatty acid isomers as possible [71].

3.2.1.4 **Determination of Fibres**

To determine dietary fibres in an organic sample, the fibre has to be defined according to selected analytical method. Therefore, this is a complex procedure. Methods for the determination of dietary fibre may be divided into three categories: non-enzymatic-gravimetric, enzymatic gravimetric, and enzymatic-chemical methods. Enzymatic-chemical methods include enzymatic-colourimetric and enzymatic-chromatographic (GLC/ HPLC) methods. Enzymatic-gravimetric methods, Association of Official Analytical Chemists (AOAC) method and enzymatic-chemical method are the most popular ones these days [72].

3.2.2. Proximate analysis of substrate and inoculum

3.2.2.1 **Total solids (TS)**

Total solids test was performed according to standard methods for the examination of water and waste water [citation missing]. In this method, empty china dishes were washed and dried in an oven (Shel Lab SMO10 HP-2) at 103^0C. After drying, they were cooled down in a desiccator to ensure minimum contact with atmospheric moisture and then their weight was recorded. 10 g of food waste sample and 10 ml of inoculum sample was placed in individual china dishes and again weight was recorded. China dishes along with samples were placed in drying oven at 103^0C for about 5 hours until the samples had completely dried (figure 3.4). Then, they were allowed to cool at room temperature in the desiccator and again their weight was recorded. The noted readings were employed in the following formula for TS calculation.

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

Where,

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

B = weight of china dish (mg)

For total solids percent removal, following formula was used

Percent removal = influent – effluent/influent * 100

3.2.2.2 **Volatile solids (VS)**

Volatile solids test was performed according to the standard methods for the examination of water and waste water [citation missing]. Samples which were previously dried for TS at 103^0C were placed in a muffle furnace and ignited at 550^0C for 2 hours (figure 3.5). The residue which was left behind in a china dish was cooled at room temperature before being weighed. Recorded reading was used in the following formula

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

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

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

Volatile solids removal efficiency % = volatile solids concentration (influent) – volatile solids concentration (effluent)/ volatile solids concentration (influent) * 100

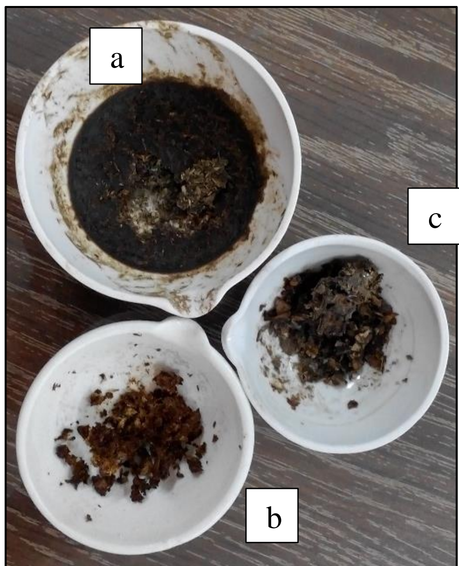


Figure 3.4: Samples inoculum (a), cabbage (b) and potato (c) after drying at 105oC in the drying oven.

Figure 3.5: Samples inoculum (left), cabbage (centre) and potato (right) after ignition at 550oC in the muffle furnace.

3.2.2.3 **Total Organic Carbon (TOC)**

Total Organic Carbon in samples was measured by Loss of ignition method [73]. In this method, 10 g of sample was dried in drying oven for 24 hours at 103°C. When the sample was completely dried it was further ignited in a box furnace at 550^0C for organic carbon estimation. Organic carbon content constitutes all the carbon that is emitted between the temperature of 103^0C and 550^0C. After igniting the sample, weight of the organic matter was divided by a factor of 1.8 to obtain the value of total organic carbon.

TOC (g/L) = Volatile Solids/1.8

3.2.3. Ultimate analysis of substrates

Elemental analysis of the wastes and inoculum (shown in Table 3) was performed using SEM/EDS (Oxford instruments, model XM5061) available in USPCAS-E. Sample preparation involved drying the sample in oven at 100°C for 3 hours, crushing it into fine particles and again drying to completely remove the moisture. As the samples were organic in nature, they were also sputter coated with a 15 nm thick layer of gold to increase their conductivity (2 shots of 30 seconds each to ensure complete sample coating). For elemental detection, angle of detector was kept 45° to the sample and voltage was set at 20 kV.

3.2.4. Chemical Oxygen Demand (COD)

Chemical Oxygen Demand test was performed according to the standard methods for the examination of water and waste water [citation missing]. Close reflux method was adopted for this particular test. Prior to test, three reagents, i.e. Potassium dichromate ($K_2Cr_2O_7$) digestion reagent, Sulfuric Acid Reagent (SAR), and Ferrous

Ammonium Sulfate titrant reagent (FAS) were prepared. Preparation method of these reagents is mentioned below:

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

Sulfuric Acid Reagent (SAR): 5.5 g of silver sulfate ($AgSO_4$) was added in 1 litre of sulfuric acid and was continuously stirred for one day until it was completely dissolved in the acid.

Ferrous Ammonium Sulfate titrant reagent (FAS): 39.2 g of ferrous ammonium sulfate was dissolved in distilled water. After this, 20 ml of sulfuric acid was added and the solution was diluted to 1000ml. molarity of this solution was calculated by taking distilled water as a blank sample which is mixed with digestion reagent and Ferroin indicator and titrated against FAS reagent.

Molarity of FAS Solution = [Volume of $K_2Cr_2O_7$ (ml) * 0.1] / volume of FAS (ml)

Procedure:

COD vials were washed with 20% H_2SO_4 acid to remove all contaminants. 2.5 ml of sample and 1.5 ml of $K_2Cr_2O_7$ digestion reagent were pipetted out in a vial. Then 3.5 ml sulfuric acid was slowly added which resulted in the formation of 2 layers: acid layer and digestion solution layer. Vials were tightly capped and were inverted several times to get a homogenous solution. Prepared vials were placed and refluxed in COD digester (Thermoretaker CR 3200) available in biofuel lab for 2 hours at 150^0 C. After 2 hours, the vials were cooled down to room temperature. The contents were then poured in a small beaker and 2 drops of diluted ferroin indicator were added. The solution changed its colour to red, after that the beaker was placed on a magnetic stirrer and the solution was titrated against 0.9M FAS. Change of colour from reddish brown into bluish green signalled the completion of reaction and at that point, titration was stopped. The volume of FAS consumed was noted from the burette. The formula used for COD calculation and removal efficiency is as follows:

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

Where,

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

B = volume of FAS solution used for sample (ml)

M = Molarity of FAS solution

COD removal efficiency (%) = [(COD influent – COD effluent)/COD influent] * 100

3.3 Gas Composition analysis

Biogas was collected in syringes, gas volume was measured by the plunger displacement method. Hydrogen content present in the biogas was analysed by comparing sample with pure hydrogen standard using gas chromatograph Shimadzu 2010 plus equipped with 30m long column of RT-MS5A (TCD). Detector temperature was 200^oC and the rate was 35^oC/minute. Nitrogen was used as a carrier gas at the flow rate of 1.76 mL/min and 98.8 kPa. Biogas samples were injected manually using a syringe of 60ml.

3.4 Volatile Fatty Acids (VFA) concentration analysis

The Shimadzu GCMS-QP2020 NX gas chromatograph-mass spectrometer was used equipped with SH-Rxi-5Sil Ms column to determine the composition of VFAs in the fermentation broth. Length and diameter of the column were 30m and 0.25mm respectively. Fermentation broth was first filtered and the filtrate was dissolved in GC grade di-chloro methane (DCM) to extract all the organic compounds from the aqueous fermentation broth. The mixture was allowed to settle down for 24 hours so for solvent extraction to take place. Once the clear layers were formed, lower layer of DCM was isolated by means of a syringe.

The resulting sample solution was ten times diluted with a solvent and filled in auto sampler vial and injected in GC/MS in split less mode to identify the VFAs. Helium was used as the carrier gas with initial set temperature of oven at 35^oC and was kept there for 0.1 min, then raised to 240^oC with the ramp of 10^oC/min and held at this temperature for 10 minutes. Post-run temperature at the interface was 280^oC for 3 minutes. The ionization temperature was 230^oC and the quadrupole mass detector temperature was 150^oC. 1 µL sample volume was injected for GC analysis and the run time for each analysis required 28 minutes to complete.

3.5 Substrate and inoculum pre-treatment

3.5.1. Pre-treatment of Substrate

The selected vegetable waste was obtained from household kitchens, particle size was reduced in the food processor and stored in the freezer in USPCAS-E Biofuel Lab at temperature below 0^{0}C until further use.

Figure 3.6: Pre-treated substrates: cabbage (left) and potato (right).

3.5.2. Pre-treatment of Inoculum

Inoculum was subjected to various pre-treatment methods (briefly tabulated in Table 3.1) to enrich the hydrogen producing bacteria and suppress methane producing bacteria.

Table 3.1: Inoculum pre-treatment methods and conditions

Sr. no.	Type of pre-treatment	Conditions	
1.	**Heat shock**	35^{0}C	For 20 minutes
		65^{0}C	
		95^{0}C	
2.	**Aeration**	For 24 hours	

i. **Heat shock:** Heat-shock pre-treatment has been most widely used in literature [56, 64, 74, 62, 75, 76, 77] for enrichment of hydrogen producing bacteria like

Clostridium. Heat-shock is popular because high temperature destroys methanogenic bacteria while does not damage hydrogen producing microflora as it can form heat resistant spores. The temperature condition of the heat-shock pre-treatment in literature has ranged from 80 to 121^0C, and exposure time between 15 and 120 minutes. Repeated heat-shock pre-treatment [78] and two-stage cultivation heat-shock pre-treatment [56] were also reported using sucrose as medium [14].

Known volume of inoculum was placed in the shaking incubator in 250ml Schott bottles. There were three sets of bottles and each set was treated at a different temperature of 35^0C, 65^0C and 95^0C for 20 minutes; cooled, and then stored at 4^0C until further experimentation.

ii. **Aeration:** when a mixed culture is subjected to fermentative hydrogen production in the form of inoculum, one of the many technologies used to rule out HCB while preserving HPB is Aeration. [79, 80]. Aeration pre-treatment is effective on the principle that methanogens which are main HCBs are of strict anaerobic nature, the introduction of oxygen in the form of air can prove inhibiting and toxic, thereby threatening the survival of obligatory anaerobes [81, 80]. At O_2 concentrations starting from 0.1 mg/L, Deublein and Steinhauser [82] stated that methanogenic activities are suppressed as a result of increase in oxidation-reduction potential [14, 83, 80]. As such, the aim of aeration is to inhibit any HCB (complete anaerobes) to eventually obtain a higher H_2 yield [81]. Aeration may also hinder development of obligatory anaerobic HPB from the genus Clostridium until the setting is purified completely from oxygen again [83, 80].
For this study, known volume of inoculum was placed in 250 ml Schott bottles. Air was bubbled through each sample by using aquarium pumps for 24 hours. The bottles were stored at 4^0C until further experimentation.

3.6 Experimental Set-up (bioreactor configuration)

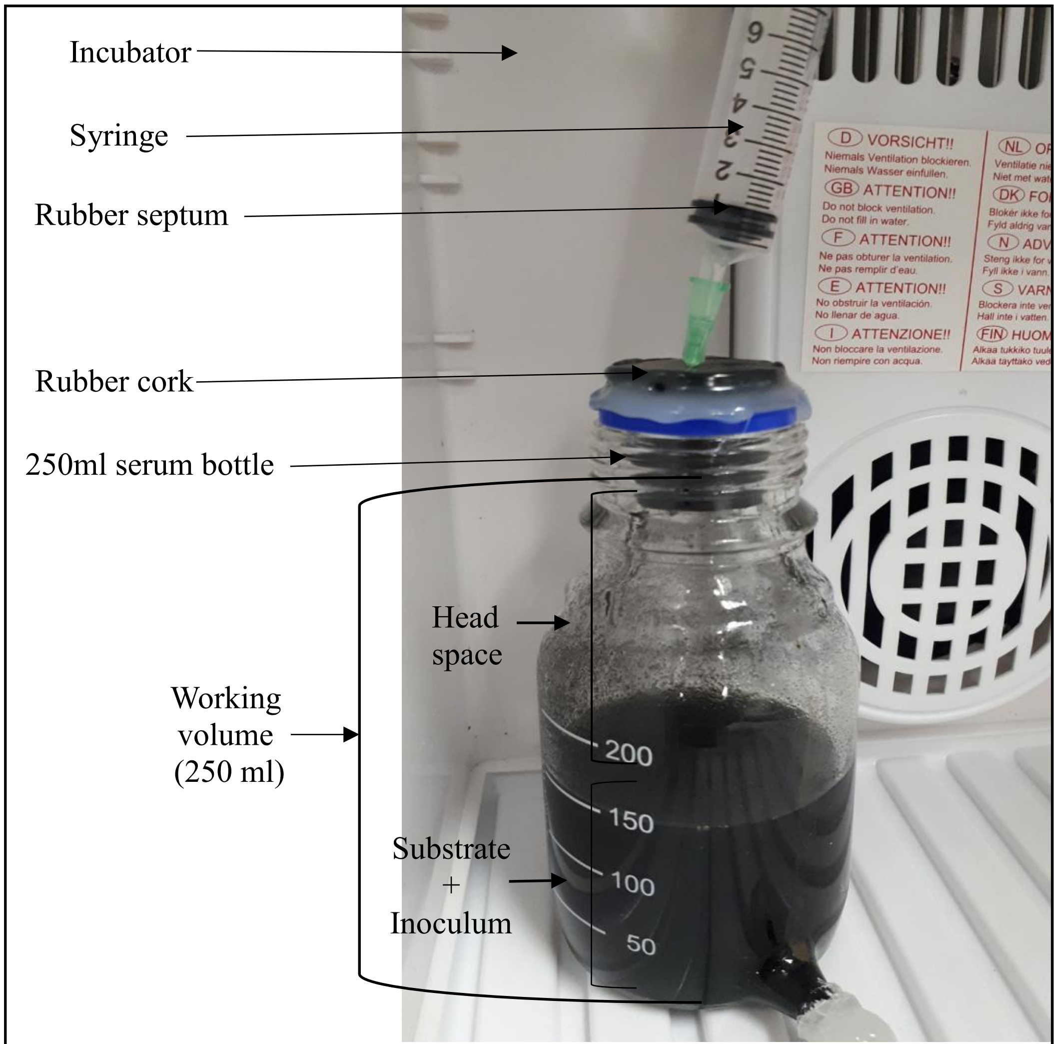


Figure 3.7: Lab scale batch type digester set-up

Batch type fermentation tests were carried out in the lab (configuration shown in figure 3.7). Digester hardware consisted of 250ml Schott bottle, tightly fitted with a cork and sealed with silicone sealant to ensure that no air could pass through. These bottles were filled with substrate and inoculum according to the determined working volume and head space as shown in figure 9. Since the activity of hydrogen-consuming methanogens is inhibited at low pHs [33, 84, 85], the pH was adjusted to 5.5$\pm$0.5 by adding 1 molar H_2SO_4 solution. After adding feed and inoculum, the head space of bottles was flushed with nitrogen gas for 4-5 minutes to eliminate all the oxygen and create anaerobic environment. After that the bottles were properly sealed with the cork and sealant. One or 2 syringes were inserted through the cork for collection and

monitoring of bio gas produced. The prepared digesters were placed in the incubator in mesophilic conditions i.e. 36^0C for fermentation.

Three sets of experiments:

1. To check the effectiveness of pre-treatment
2. To measure the concentration of bio-hydrogen produced by varying feed to inoculum ratio
3. To replicate the best results obtained in experimental set-up 2 and fuel the hydrogen fuel cell car.

3.6.1. First set of reactors: To check the effectiveness of inoculum pre-treatment methods

Two types of pre-treatment methods were selected i.e. heat shock and aeration because of their simplicity and cost effectiveness and non-use of strong chemicals as opposed to other pre-treatment methods. This experimental set up consisted of 6 digesters in duplicate. Only inoculum was added because the purpose was to investigate the effectiveness of each pre-treatment method. One of the digesters was selected as control in which untreated and unbuffered inoculum was added. Working volume was 150ml which corresponded to 0.12g VS content (per 150ml of inoculum) while the head space was left to be 100 ml. Details of each digester are given in Table 3.2.

Table 3.2: Details of digesters in 1st experimental set-up

<table>
<tr><th colspan="2">Type of pre-treatment</th><th>Sample ID</th><th>pH</th></tr>
<tr><td colspan="2">No pre-treatment</td><td>CONTROL</td><td>8</td></tr>
<tr><td colspan="2">No pre-treatment</td><td>UNTREATED</td><td>5</td></tr>
<tr><td rowspan="3">Heat shock</td><td>35°C</td><td>35</td><td>5</td></tr>
<tr><td>65°C</td><td>65</td><td>5</td></tr>
<tr><td>95°C</td><td>95</td><td>5</td></tr>
<tr><td colspan="2">Aeration</td><td>Aeration</td><td>5</td></tr>
</table>

3.6.2. Second set of reactors: To check the amount of bio-hydrogen produced by varying feed to inoculum ratio

A total of eighteen digesters were set up: half of them were heat treated while half were treated with air. Feed to inoculum ratio was adjusted, details are given in Table 3.3. Inoculum amount was kept constant in all the digesters (same as in the first experimental setup: section 3.4.1). Only substrate was varied according to grams of volatile solids.

Table 3.3: Details of digesters in 2nd experimental set-up

Sr. No.	Pre-treatment	Digester ID	F/I ratio (gVS)	Mass of potato	Mass of cabbage	pH	
				g	g	initial	adjusted
1	Aeration	**a P (20)**	0.32/0.12	20	0	7.64	5.80
2		**a P (30)**	0.48/0.12	30	0	7.17	5.87
3		**a P (40)**	0.64/0.12	40	0	6.89	6.09
4		**a C (20)**	0.23/0.12	0	20	7.50	5.93
5		**a C (30)**	0.35/0.12	0	30	7.33	5.76
6		**a C (40)**	0.46/0.12	0	40	6.77	5.84
7		**a CP (20)**	0.33/0.12	10	10	7.20	5.95
8		**a CP (30)**	0.41/0.12	15	15	6.94	6.07
9		**a CP (40)**	0.55/0.12	20	20	6.99	5.49
10	Heat shock	**h P (20)**	0.32/0.12	20	0	7.39	5.81
11		**h P (30)**	0.48/0.12	30	0	7.16	5.89
12		**h P (40)**	0.64/0.12	40	0	7.29	5.85
13		**h C (20)**	0.23/0.12	0	20	7.14	6.02
14		**h C (30)**	0.35/0.12	0	30	7.01	5.76
15		**h C (40)**	0.46/0.12	0	40	7.09	5.05
16		**h CP (20)**	0.33/0.12	10	10	7.55	5.95
17		**h CP (30)**	0.41/0.12	15	15	7.35	5.79
18		**h CP (40)**	0.55/0.12	20	20	6.79	6.04

3.6.3. Third set of reactors: To replicate the best results obtained in experimental set-up 2 and fuel the hydrogen fuel cell car.

Reactors which showed better performance in the second phase were replicated in the third phase, details are given in Table 3.4.

Table 3.4: Details of digesters in 3rd experimental set-up

Sr. No.	Pre-treatment	Digester ID	F/I ratio (gVS)	Mass of potato	Mass of cabbage	pH	
				g	g	initial	adjusted
1	Aeration	**a Control**	0/0.12	0	0	7.35	5.47
2		**a P (30)**	0.48/0.12	30	0	8.13	5.77
3		**a C (20)**	0.23/0.12	0	20	7.66	5.80
4		**a C (40)**	0.46/0.12	0	40	7.45	5.98
5		**a CP (20)**	0.33/0.12	10	10	7.51	5.90
6	Heat shock	**35 Control**	0/0.12	0	0	7.52	5.72
7		**35 P (20)**	0.32/0.12	20	0	7.38	5.96
8		**35 CP (40)**	0.55/0.12	20	20	7.03	5.91
9		**95 Control**	0/0.12	0	0	7.41	5.83
10		**95 P (20)**	0.32/0.12	20	0	7.42	5.93
11		**95 CP (40)**	0.55/0.12	20	20	7.56	5.61

3.6.4. Fuel the model hydrogen fuel cell car with bio-hydrogen

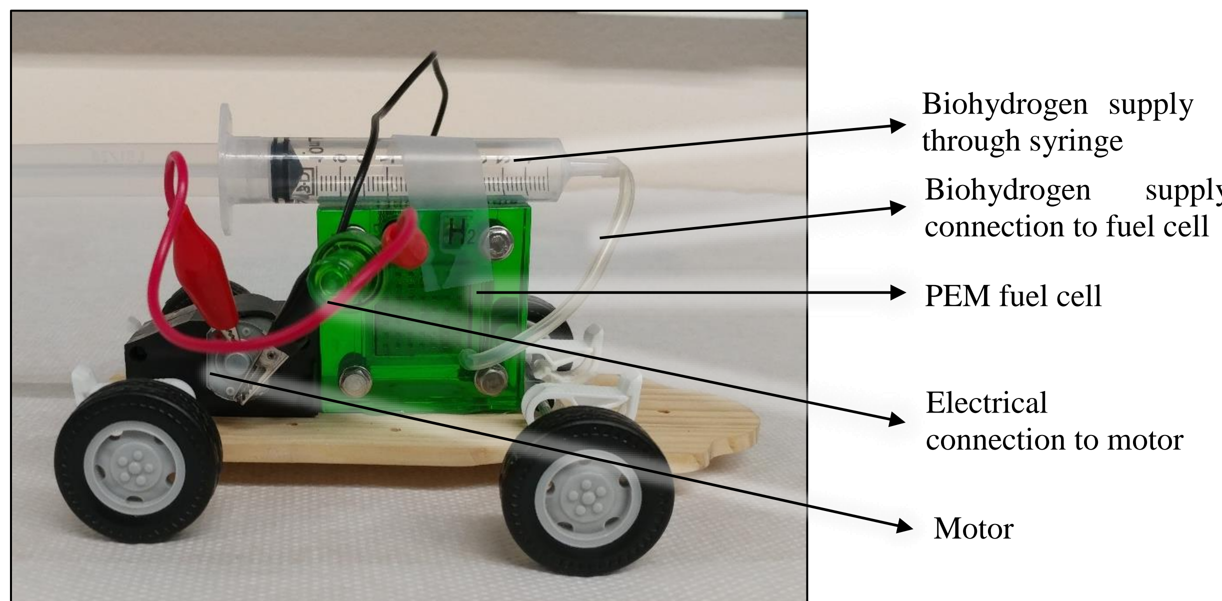


Figure 3.8: Model fuel cell car for practical application of bio-hydrogen

A fuel cell is a device that uses chemicals to produce electricity very much like a battery. But unlike a battery, fuel cell consumes the reacting chemicals and not the electrodes meaning that it can be used continuously for a longer period of time as long as the chemicals it uses are available. There are many different types of fuel cells which use different types of chemicals. The fuel cell used in this particular study was a reversible Proton Exchange Membrane (PEM) fuel cell. It converts hydrogen and oxygen into electricity and water and vice versa. This particular fuel cell is called PEM fuel cell because it uses a membrane to separate hydrogen side of the cell from oxygen side. A thin foil made of special polymer serves this purpose and is located in the MEA or membrane electrode assembly at the center of the cell. In addition to PEM, MEA contains two electrodes on either side of PEM. The electrode on hydrogen side is called anode and the one on oxygen side is called cathode. On the anode, electrically neutral hydrogen molecules split into electrons and hydrogen ions with the help of a catalyst. The positively charges hydrogen ions migrate through the polymer membrane towards the negatively charged cathode, while the electrons travel through the circuit with an electrical load (for example, the motor) from the anode to the cathode. The hydrogen ions or protons, pass through PEM into cathode side of the cell. Electrons are conducted by the metal plates through external circuit also reach this side of the cell. On cathode, protons react with oxygen molecules and electrons to form water and

complete the electrochemical reaction. Chemically, following reactions occur at the electrodes of fuel cell:

Anode: $2 H_2 \rightarrow 4 H^+ + 4 e^-$

Cathode: $O_2 + 4 H^+ + 4 e^- \rightarrow 2 H_2O$

Net reaction: $2 H_2 + O_2 \rightarrow 2 H_2O$

Procedure: Once the car had been assembled (figure 3.8), electric current was supplied by the batteries to split water into oxygen and hydrogen by the process of electrolysis. As soon as the collection tanks were filled with the respective gases, the battery was disconnected. To run this car on bio-hydrogen, hydrogen tank of the kit was replaced by the syringe filled with biogenic hydrogen obtained from the fermentative bioreactor and observations were recorded in a video.

3.7 Summary

This chapters illustrates the methodological framework adopted to carry out the research. Selection of pre-cursor material and their characteristics are mentioned. Techniques and equipment which are used in characterization and measurements of pH, total solids, volatile solids, elemental analysis, and gas composition are described in detail. All together three sets of anaerobic batch scale reactors were set up to evaluate the inoculum pre-treatment method and optimum F/I ratio for hydrogen production. Working mechanism of a model PEM fuel car used in this study is also elucidated.

Chapter 4: Results and Discussion

4.1 Compositional analysis

Food waste (FW) is an excellent source of organic matter which acts as a medium for bacterial growth, for this reason, many scientists consider it as an appropriate feedstock for anaerobic digestion (AD) [86]. Anaerobic digestion is the biological degradation of organic matter into respective products [87]. AD is a multi-step process which starts with hydrolysis of high molecular materials and granular organic substrates (e.g., lipids and carbohydrates, protein) into organic molecules which are smaller in molecular weight and easily soluble (e.g., lipids and carbohydrates, protein) by fermentative bacteria. Fermentative bacteria release hydrolytic enzymes which require their adsorption on the surface of solid substrate, making hydrolysis the rate limiting step of AD [88] [89]. Next step is the degradation of small molecular materials and granular organic substrates which are the products of hydrolysis into volatile fatty acids or VFAs (e.g., acetate, propionate and butyrate) accompanied by the creation of by-products (e.g., NH_3, CO_2 and H_2S). products of the second step serve as the substrate for third step in AD where they are further digested into acetate, H_2,CO_2 and so on [90]. The bacteria's ability to produce hydrogen is influenced by the nature of carbon source [91]. So the substrate used should have a good nutrient balance for optimal microbial growth.

Table 4.1: Nutritional values of potato and cabbage.

Principle	Nutritional value (per 100g)	
	Potato	Cabbage
Carbohydrate	17.49 g	5.8 g
Protein	2.05 g	1.3 g
Total fat	0.10 g	0.1 g
Dietary fiber	2.1 mg	2.50 mg

It can be seen from table 4.1 that the selected substrates have good amount of carbohydrates and lesser proteins, this combination is most suitable for microbial degradation and negligible amount of fats which are difficult to degrade.

4.2 Proximate analysis

Characterization of substrate was carried out to verify the fermentative bioreactors stability. Proximate analysis refers to the quantitative analysis of certain compounds present in the sample. A total of five parameters were analyzed and their results for substrate and inoculum are given in table 4.2.

Table 4.2: Proximate analysis of inoculum and substrate.

Sample	Moisture Content (MC)	Total Solids (TS)	Volatile Solids (VS)	Total Organic Carbon (TOC)	Chemical Oxygen Demand (COD)
	%	%	%	g/kg	g/L
Inoculum	87.64	12.35	10.30	5.85	7.9
Potato peel	82.54	17.45	16.05	8.92	210
Cabbage	87.61	12.38	11.62	6.45	85.3

Due to the high moisture contents as indicated in table 4.2, potato peel and cabbage waste are easily biodegradable substrates. Water is required by bacteria in their metabolic processes. TS and VS concentrations range between 10% to 17%, indicating that majority of the solid content is organic matter [37].

4.3 Ultimate analysis

Ultimate analysis is the quantitative analysis of elements present in the sample. In an anaerobic fermentative process, main elements of concern are carbon and nitrogen which determine the C/N ratio. Anaerobic hydrogen fermentation needs carbohydrate rich organic wastes as its substrate [64]. It was known that carbohydrate and protein were the main nutrition released from the bio-sludge used for hydrogen production [75]. It is important to know that how much amount of carbon and nitrogen is present in substrate and inoculum in order to perceive their digestability. Carbon, nitrogen and oxygen percentages of the samples along with their C/N ratios are given in table 4.3.

Table 4.3: Proximate analysis of substrates and inoculum.

Sample	Carbon %	Nitrogen %	Oxygen %	C/N
Cabbage	57.57	10.63	27.62	5.415804
Potato	39.66	29.25	25.90	1.355897
Inoculum	45.88	17.52	28.11	2.618721

4.4 Reactors performance (hydrogen production)

4.4.1. First set of reactors: Effect of pre-treated inoculum

This experiment aimed to screen out the best pre-treatment method applied for enriching hydrogen producing inocula. As mentioned in the section 2.5.4, various pre-treatment methods have been employed by different researchers to eliminate the methanogens from mixed cultures and till date varied results have been obtained. A study utilized waste activated sludge, subjected it to five different pre-treatment methods and found acid pre-treatment to be the most effective [79]. Another group of scientists pre-treated the digested sludge in five different ways and found heat treatment to be the most effective one [55]. Other researchers found aeration [92, 56] while some proclaimed base treatment [93, 75] to be the most effective.

Results obtained from the first array of bio reactors are shown in table 4.4. All reactors had inoculum only. Control reactor produced highest percentage of methane as methanogens were most active in this culture; consequently, hydrogen production had been minimal. Untreated inoculum showed results in the same trend. Heat treated inoculum at 35°C produced most amount of hydrogen, second came the aerated inoculum. Heat treated inoculum at 95°C also produced significant amount of hydrogen and was most successful at diminishing methanogens since corresponding methane production was minimum for this reactor amongst all the other reactors. The reason might be very high temperature successfully killed the methanogens but also damaged hydrogen producing species from the sludge. Heat treated inoculum at 65°C did not show any results worth mentioning. The effect of bacterial stress enrichment method was in the order: heat shock at 35°C > aeration > heat shock at 95°C > heat shock at 65°C. So the pre-treatment methods: heat shock at 35°C, heat shock at 95°C and aeration were selected for the second experimental set up.

Table 4.4: Maximum hydrogen and corresponding methane production from different pre-treatment methods.

Type of pre-treatment		Digester ID	pH	Maximum hydrogen yield (%)	Corresponding methane yield (%)	Lag Time (days)
No pre-treatment		CONTROL	8	0.17	58.27	5
No pre-treatment		Untreated	5	0.19	54.84	8
Heat shock	35°C	35	5	29.74	6.35	7
	65°C	65	5	0.04	6.61	6
	95°C	95	5	13.64	0.57	6
Aeration		Aeration	5	20.01	1.141	4

4.4.2. Second set of reactors: Optimization of feed to inoculum ratio

Anaerobic digestion of potato peel and cabbage waste for hydrogen production was performed in serum bottles under various volatile solids (VS) concentrations and mixing ratios of two substrates (0:100–100:0, VS basis). Inoculum was pre-treated with the three previously selected pre-treatment methods. A total of 27 digesters were set up. One third of which were cultivated with aerated inoculum, half of the remaining were cultivated with heat treated inoculum at 35^0C while heat treated inoculum at 95^0C was introduced in remaining one third of the total 27 digesters. VS content of the inoculum was kept 2.1. Feed to inoculum ratio was varied by introducing the feed at different VS concentrations (details given in table 3.4). This phase aimed to choose F/I ratios which offered stable hydrogen production combined with the inoculum pre-treatment. Figures 4.1, 4.2 and 4.3 illustrate the trends of hydrogen production from different combination ratios in the increasing order of VS concentrations for their respective inoculum pre-treatment methods.

Figure 4.1 explains the hydrogen content of all the combination ratios over a period of 22 days inoculated with aerated mixed culture. Hydrogen gas was detected in all the combination ratios. The potential of hydrogen production increased as VS concentration increased up to 0.6 which was present in the digester aP30, giving the highest yield of 67.47% on the very first day. However, potential of hydrogen production decreased as VS concentration increased further, which might be due to

product inhibition by H_2 and VFAs [94] [60]. The digesters with low VS concentrations like aP20, aC30 and aCP30 showed uneven trends of hydrogen production indicating that VS concentration was unsuitable for hydrogen producing bacteria. Digesters aC20 and aCP20 showed fairly stable production although VS content in these was low (0.35 and 0.45). The reason might be balanced carbohydrate/protein ratio for these ratios [95]. Lag time was also less than 24 hours for the digesters (except three) as the desired gas was detected the next day. Combination ratios aC20, aC40, aCP20 and aP30 produced hydrogen in a stable manner, so they were chosen for the next phase of this study.

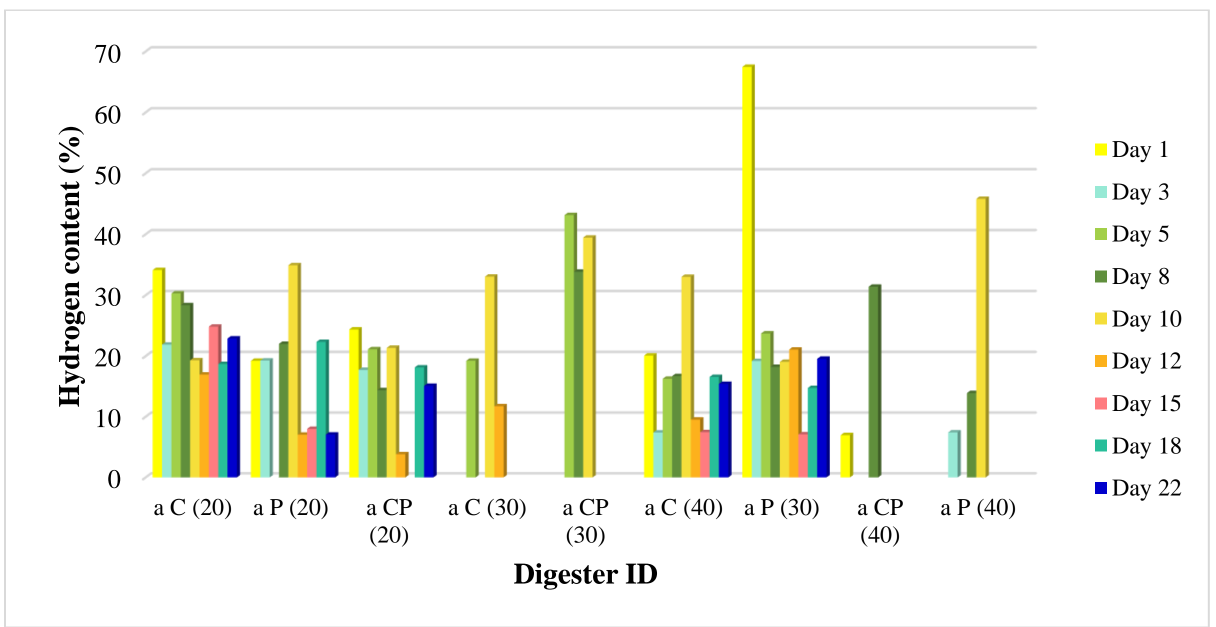


Figure 4.1: Hydrogen production from different combinations of F/I. Inoculum pre-treated by aeration method.

The digesters inoculated with mixed cultures pre-treated by heat shock at 35^0C and 95^0C showed similar trend in hydrogen production (figures 4.2 and 4.3 respectively). This means that heat shock destroyed the methanogenic activity and affected spore germination of hydrogen producing bacteria in the same manner for both the applied temperatures. Highest hydrogen yield was obtained for combination ratio C40 for both the pre-treatments. One stark difference was observed in the lag time. Inoculum pre-treated at 95°C took longer to initiate biogas production than both the other pre-treatments. The reason for this may be the high temperatures of 95°C were harsher for hydrogen producing bacteria that the desired microflora took longer time to adapt when introduced to the favorable conditions. But at the same time, it suppressed the activity of methanogens more successfully than any other method employed. For the

heat shock pre-treated inoculum, combination ratios P20 and CP40 were selected to proceed to the next phase of experimentation because their hydrogen production trends were more stable than rest of the combination ratios.

The results of this research suggested that chemical nature of seed inoculum and its pre-treatment method could obviously favour different anaerobic spore-forming hydrogen producing species in the sludge [64]. That is why the digesters inoculated with heated and aerated sludge show maximum productions with different F/I configurations. Furthermore, since acids are produced simultaneously with hydrogen gas [60], the substrate concentration should not be too high to shock load the system.

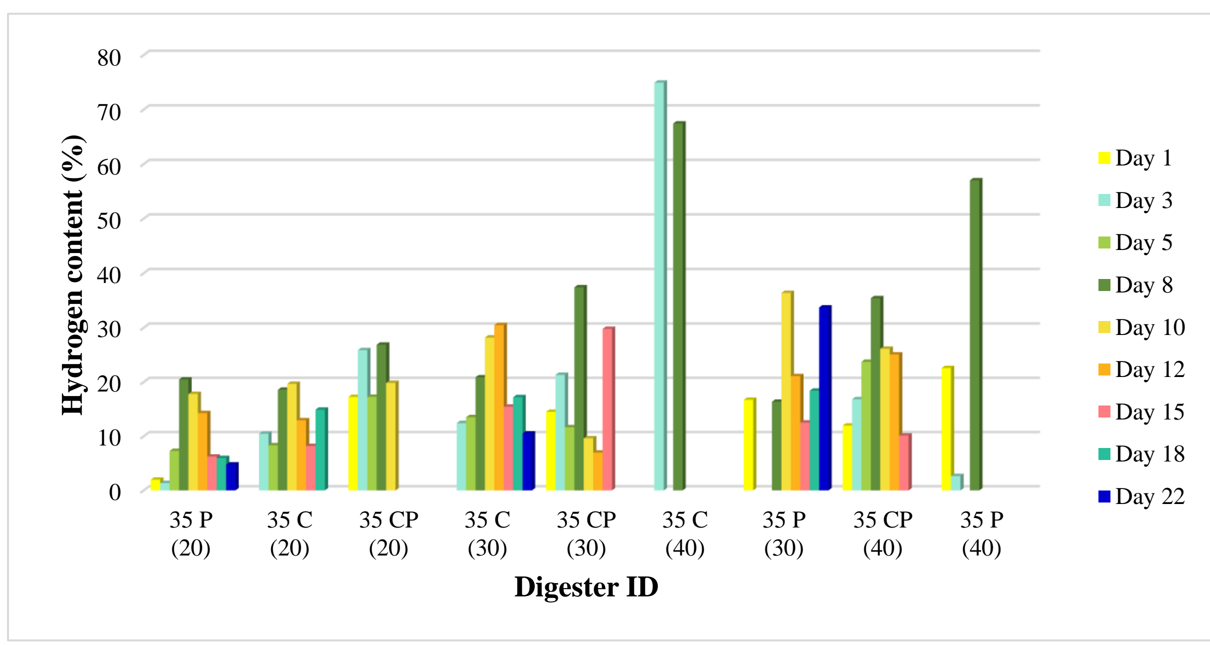


Figure 4.2: Hydrogen production from different combinations of F/I. Inoculum pre-treated by heat shock at 35°C.

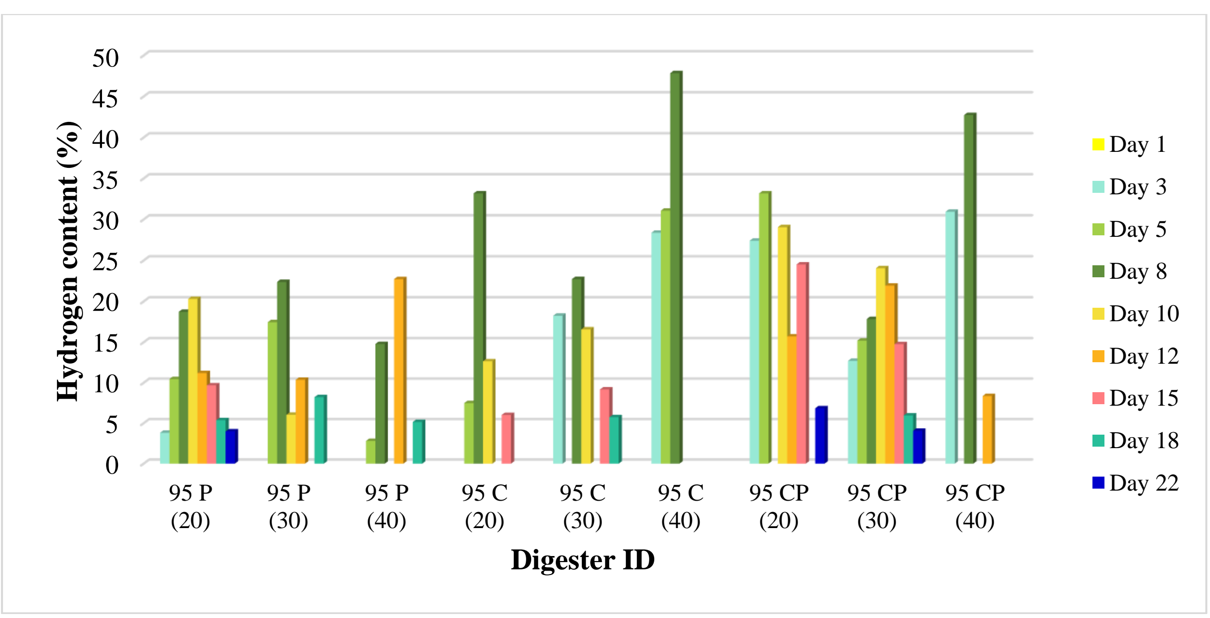


Figure 4.3: Hydrogen production from different combinations of F/I. Inoculum pre-treated by heat shock at 95°C.

4.4.3. Third set of reactors: Repeated batch experimentation and demonstration of hydrogen fuel cell car.

This phase was basically the repeated batch experiment in which those substrate combination ratios were selected which produced best results in the second phase of experimental study combined with suitable inoculum pre-treatment methods. To confirm that there was no background hydrogen production resulting from degradation of the organic matter in the inoculum, three blank batch bottles (one for each pre-treatment) were incubated. These digesters were investigated in detail as various parameters were studied including pH, VS, COD, TOC and VFAs in addition to their hydrogen content.

4.4.3.1 **Incubation time**

This fermentation process was observed for 9 days because after this period, hydrogen production in most reactors started to diminish indicating the end of fermentative process. This decrease in hydrogen concentration is because next phase of anaerobic digestion called methanogenesis starts in which hydrogen produced starts to get consumed by methanogenic bacteria. VFA accumulation in the fermentation broth and increase in hydrogen partial pressure are two of the many reasons for triggering methanogenesis. Termination of hydrogen producing phase beginning of methanogenesis can be prolonged up till a certain amount of time. This is supported

by some studies in which the same process terminates within a week [95, 38]. Even more reported hydrogen production to be lasted for only a few hours [93, 92, 81, 77, 76]. One study, however reported fermentative hydrogen production to be lasted for 14 days, but in that experiment, a specialized up-flow anaerobic sludge blanket reactor was used [74].

In this study, fermentative hydrogen production phase was prolonged by decreasing the partial pressure of hydrogen as the syringes were degassed daily.

4.4.3.2 **Biogas Volume**

Biogas volume was measured daily by plunger displacement method over the period of 9 days. Among the control reactors, only inoculum treated at 35^0C showed biogas production. The digesters inoculated with different feed to inoculum ratios showed considerable biogas production indicating that introduction of feed boosts the anaerobic bacterial activity. Observation and record of biogas production is important because hydrogen is one of the many gases present in the biogas and the percentage of this hydrogen should dominate all the other gases for a feasible fermentative process. The volume measured for every digester for the incubation time of 9 days is shown in figure 4.4.

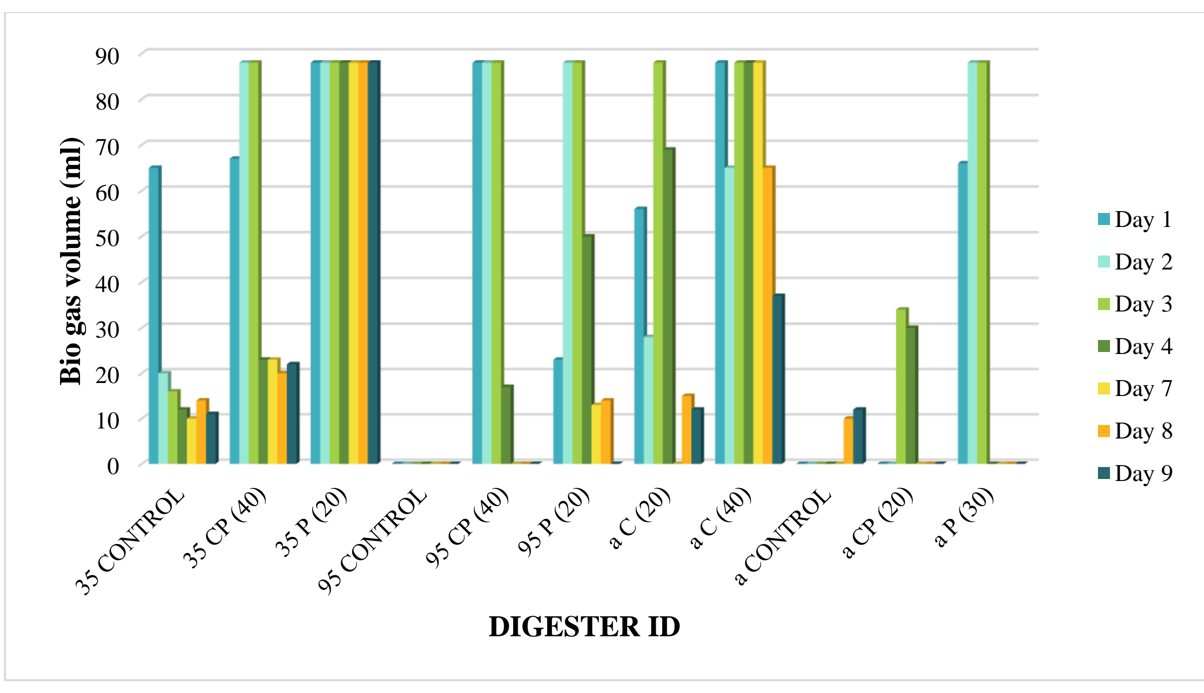


Figure 4.4: Daily record of biogas volume from all the digesters.

4.4.3.3 **Analysis of bio-hydrogen content in the biogas**

Biogas produced from different combinations of potato peel and cabbage waste was analysed daily (except on weekends) by GC (Shimadzu 2010 plus) for the determination of hydrogen content.

Figures 4.5, 4.6 and 4.7 show the development of hydrogen production while the pre-treated inoculum sludge consumed substrates. Bio-hydrogen increased with the progress of fermentation and reached a maximum of 83% on 3rd day of fermentation for the heat-treated sludge at 35^0C and combination ratio CP40; 79% after 4 days for the heat-treated sludge at 95^0C and combination ratio P20, and 60% on day 4 for the aerated one with combination ratio C40. Afterwards all of them remained nearly unchanged before declining with time

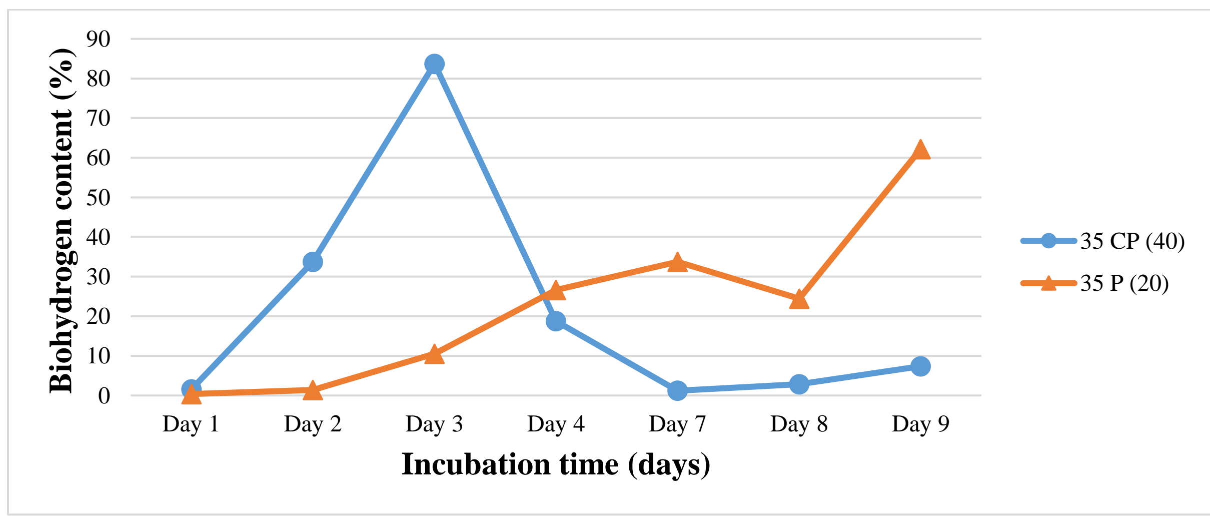


Figure 4.5: Hydrogen content profile of reactors inoculated by heat treated mixed culture at 35°C.

Trend of hydrogen production for the heat pre-treated inoculum at 35^0C is shown in the figure 4.5. CP40 (VS concentration 0.67) showed better results in this case than P20 (VS concentration 0.44) both in terms of high yield and lag time, so bacterial microflora which dominated this inoculum was better adapted with higher VS concentration .

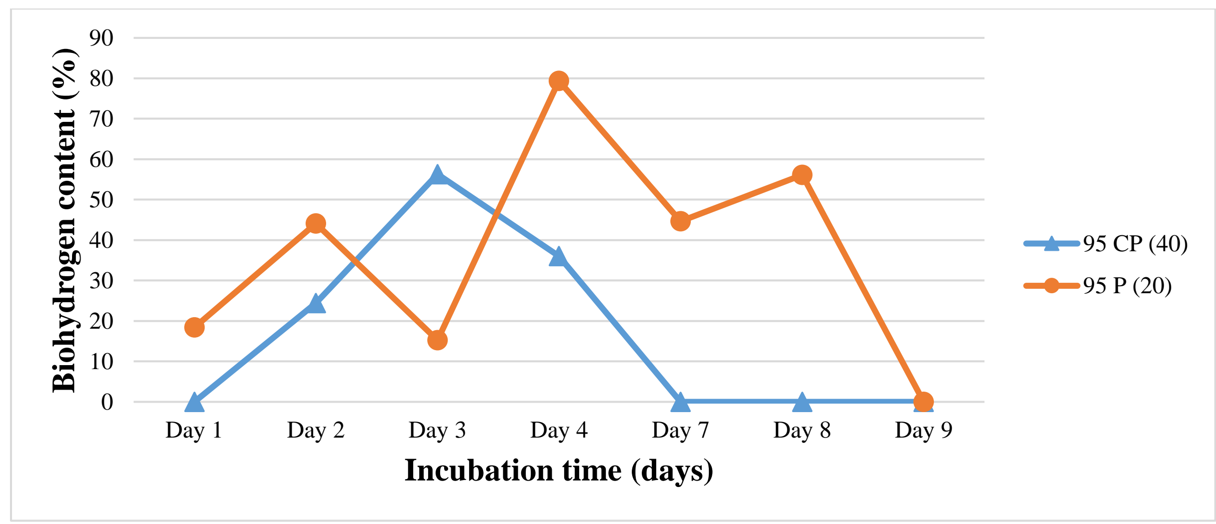


Figure 4.6: Hydrogen content profile of reactors inoculated by heat treated mixed culture at 95°C.

Development of hydrogen production trend for heat shocked inoculum at 95°C (figure 4.6) is more consistent than the one shown by heat shocked inoculum at 35°C which means that high temperature ranging up to 95^{0}C brought out better performance of the hydrogen producing microflora and suppressed the methane producing bacteria.

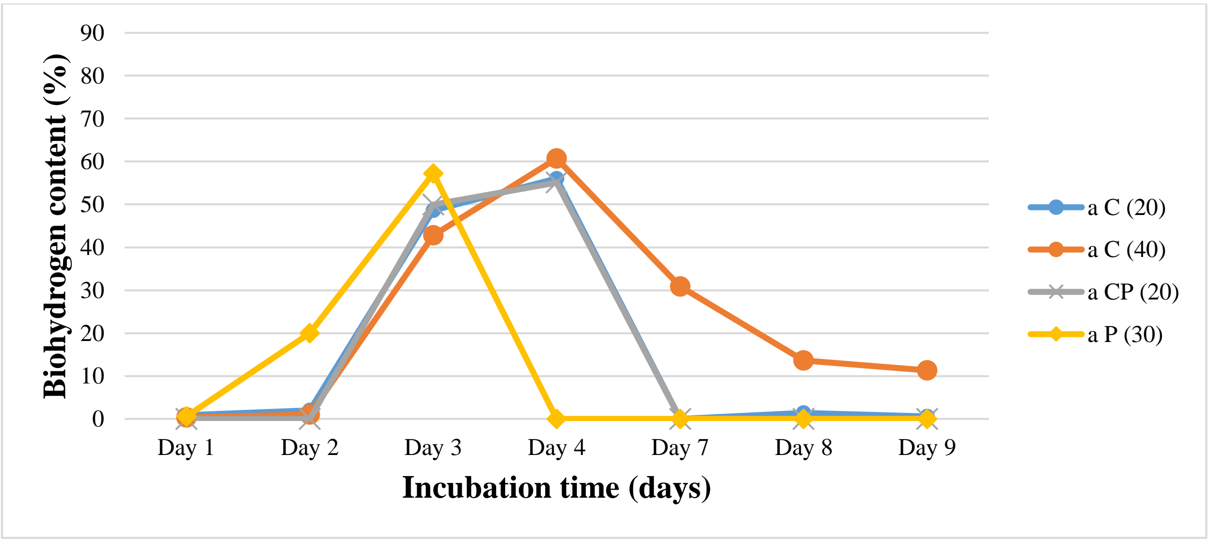


Figure 4.7: Hydrogen content profile of reactors inoculated by aerated mixed culture.

Aeration method of inoculum pre-treatment shown in figure 4.7 gave fairly consistent hydrogen production but yielded least amount amongst the three selected methods. Lag time was two days for all the digesters except P30 (VS 0.60) which was one day. The highest production was shown in C40 (VS 0.58) so this ratio was most suitable for aerated inocula.

Biogas produced in the process of anaerobic digestion is a mixture of many gases mainly hydrogen, methane and carbon dioxide. Success of inoculum pre-treatment depends upon how much content of hydrogen is present in the produced biogas. For this purpose, the percentage of biogas produced and the percentage of bio hydrogen within that produced bio gas was analyzed and compared on daily basis for each digester.

Figure 4.8 shows the amount of hydrogen produced in the corresponding biogas volume over entire incubation period for 35°C heat shock pre-treatment. Extremely low hydrogen was detected in the biogas produced by the control reactor suggesting that nutrients may be necessary to germinate the spores in the inoculum [60]. In 35CP40, hydrogen content increased with the increase in biogas content and vice versa. It shows that the culture had more active hydrogen producing bacteria than methanogens. Digester 35P40 gave a very good biogas yield whereas hydrogen yield in that biogas increased with time, showing that hydrogen producing bacteria slowly adapted to their environment and methanogens were very active throughout the incubation period.

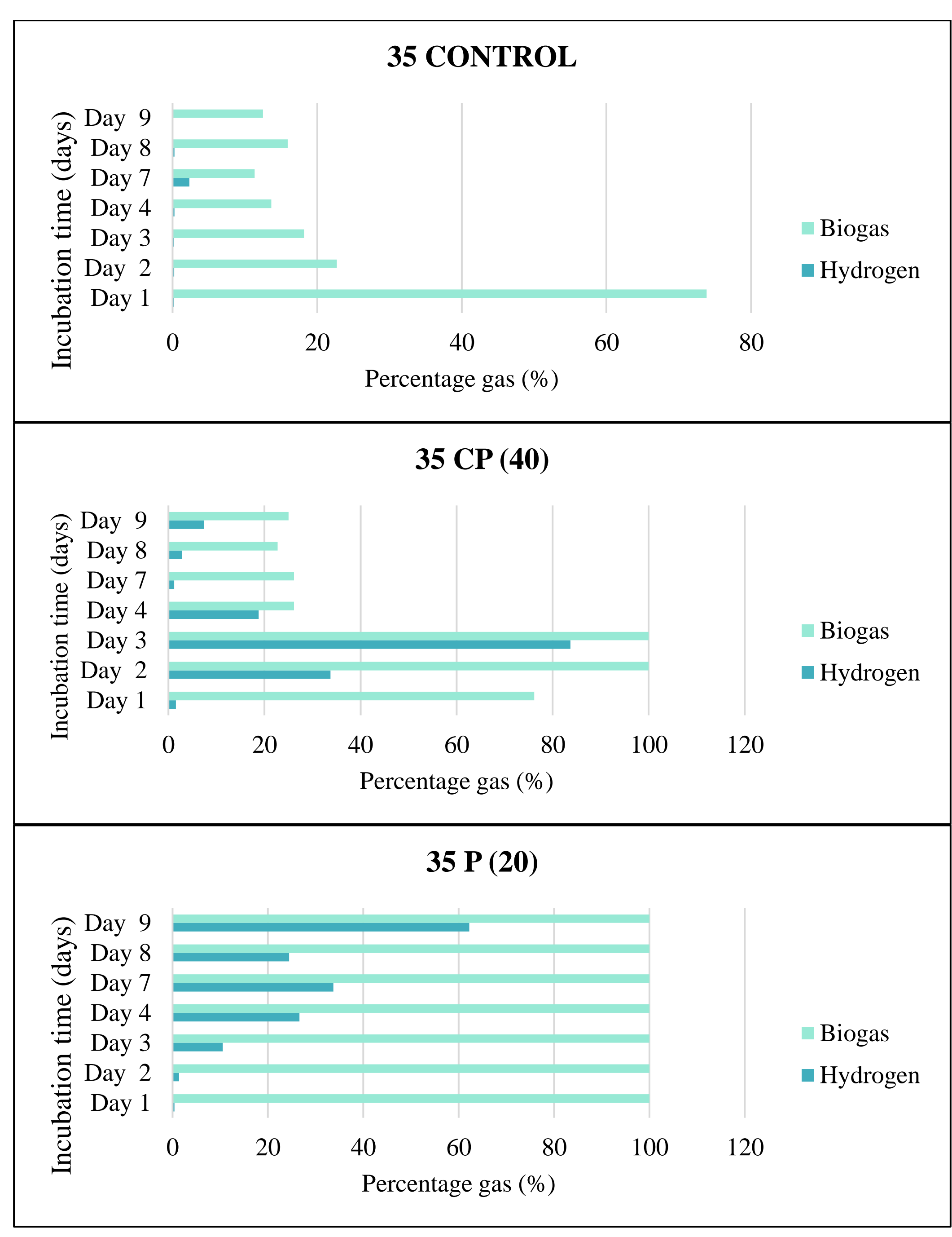


Figure 4.8: Comparison of hydrogen percentage in the corresponding biogas yield for heat treated mixed culture at 35°C.

For the digesters which contained heat treated inoculum at 95°C, the control reactor failed to produce any biogas, so no results could be obtained. This suggests that all the methanogens were successfully eliminated from this culture and due to the lack of substrate, no hydrogen could be produced. Biogas and corresponding hydrogen production profiles of 95CP40 and 95P30 are given in figure 4.8. For 95CP40, biogas production was constant for the 1st three days before it declined on the 4th day.

Hydrogen production increased with time and reached highest on the 4th day. 95P20 showed an increase in hydrogen concentration with time.

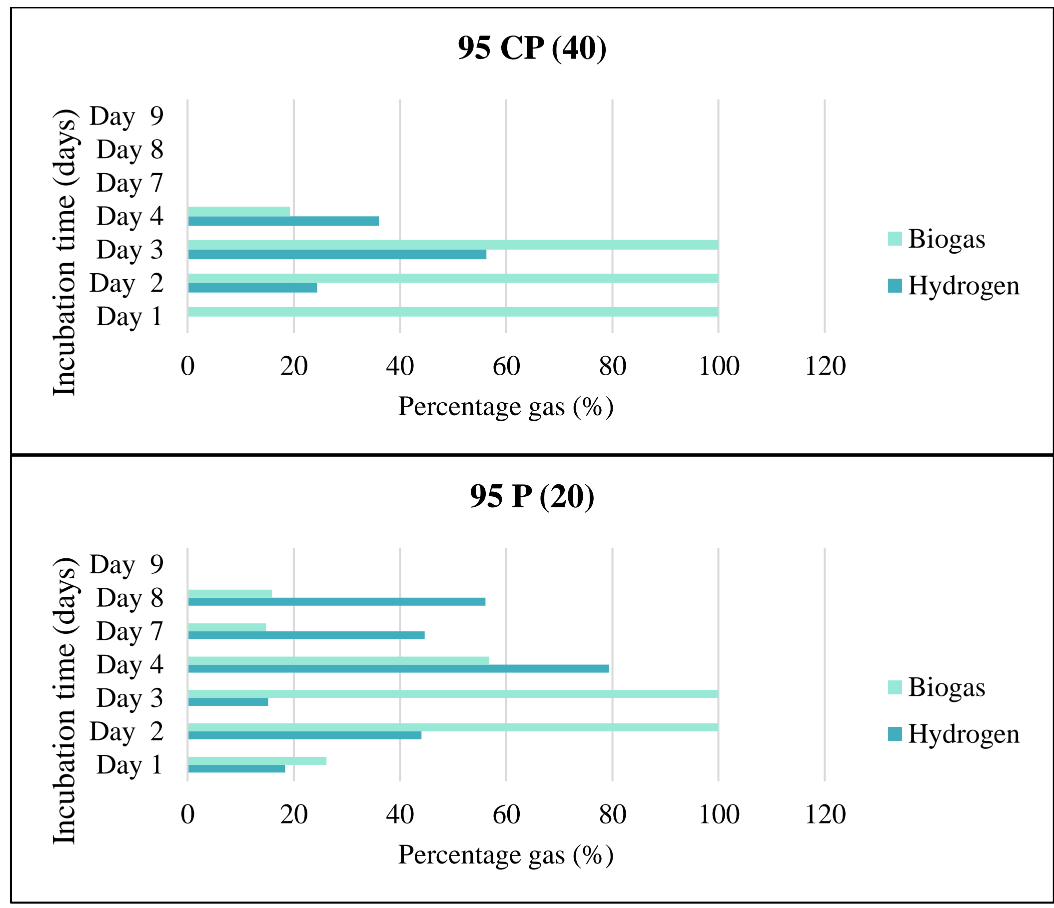
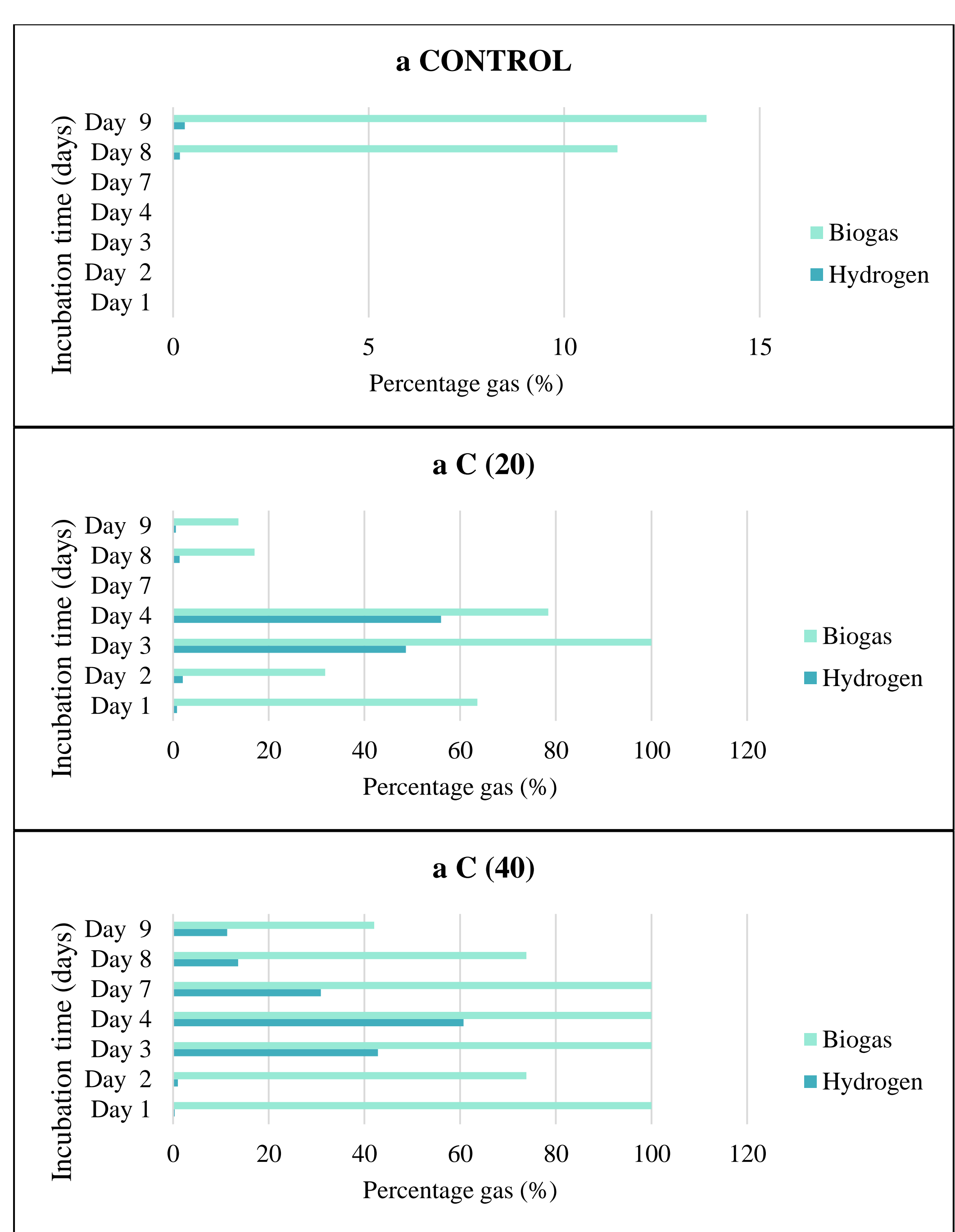


Figure 4.9: Comparison of hydrogen percentage in the corresponding biogas yield for heat treated mixed culture at 95°C.

As for the aeration method of inoculum pre-treatment (figure 4.10), the control digester had a lag time of 7 days before it started to produce biogas. The biogas had almost no amount of hydrogen. It shows that aeration pre-treatment was successful at keeping methanogens at bay for some time but not as good as heat shock at 95°C. Out of all the selected combination ratios, aCP20 produced the most amount of hydrogen but for two days only so the fermentative process was no consistent for this ratio. aC40 showed a fairly stable hydrogen production which increased with time before declining linearly.


a CONTROL
Incubation time (days)
Day 9
Day 8
Day 7
Day 4
Day 3
Day 2
Day 1
0
5
10
15
Percentage gas (%)
Biogas
Hydrogen
a C (20)
Incubation time (days)
Day 9
Day 8
Day 7
Day 4
Day 3
Day 2
Day 1
0
20
40
60
80
100
120
Percentage gas (%)
Biogas
Hydrogen
a C (40)
Incubation time (days)
Day 9
Day 8
Day 7
Day 4
Day 3
Day 2
Day 1
0
20
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60
80
100
120
Percentage gas (%)
Biogas
Hydrogen

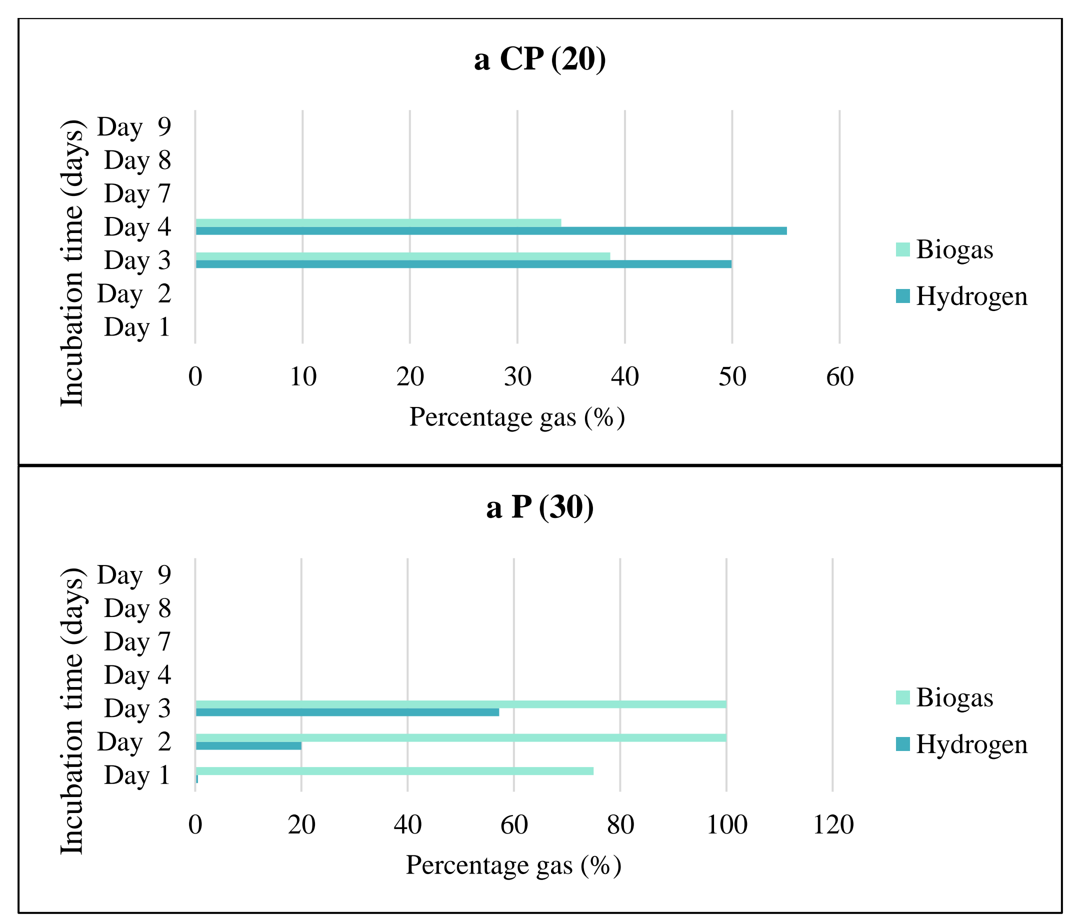

Figure 4.10: Comparison of hydrogen percentage in the corresponding biogas yield for heat treated mixed culture at 950C.

4.4.3.4 **pH**

The anaerobic bacteria's ability to produce hydrogen is also influenced by the pH and buffering capacity of the medium and culture conditions [64]. Figure 4.11 shows pH values of the digester before and after fermentation. pH is believed to be a key factor in successful operation of a fermentative bioprocess producing hydrogen because the activity of hydrogenase, an iron-containing enzyme inhibited by extremely low pH [91]. pH values of the digesters were adjusted in the range of 5-6 because hydrogen producing bacteria are more active in this range [60] as mentioned in section 2.5.1.

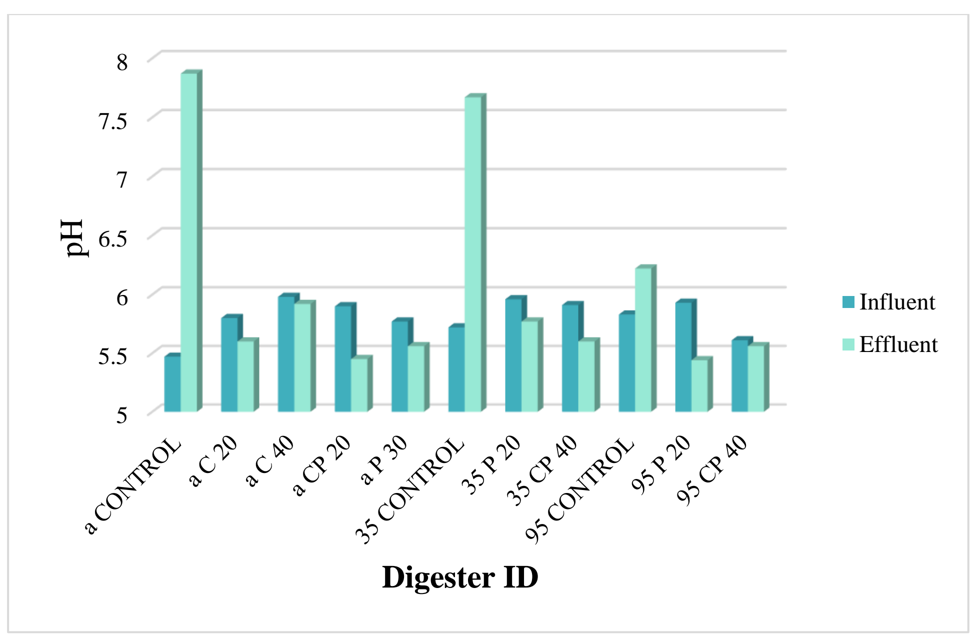

Figure 4.11: pH of all combination ratios and controls

Figure 4.11 shows that pH values of all the influent combination ratios are higher than their effluents. The lower final pH values indicate the presence of volatile fatty acids in the fermentation broth that are the end products of anaerobic fermentative process [26, 24]. On the other hand, all three of the control reactors show higher final values of pH showing process instability. This means that substrate introduction favors hydrogen producing bacterial growth in the anaerobic system which leads to hydrogen production. For a given seed material, the pH for hydrogen-producing microorganisms growth may vary depending on chemical nature of a substrate.

4.4.3.5 **Volatile Solids (VS) content and removal efficiency**

Volatile solid test is performed to indicate the organic matter concentration in the sample [96]. Volatile solids removal is one of the important pollution index that corresponds to the formation of biogas when it undergoes effective degradation [97]. Additionally, the VS degradation depends on the activity and adaptability of inoculum towards substrate in anaerobic system [98]. The amount of VS fed to the reactors ranged from 10 to 14 g/kg. Upon effluent VS analysis, the lowest VS content was found to be present in aC40 i.e. 1.35 g/kg and 35CP40 i.e. 1.6 g/kg although these reactors initially had one of the higher VS contents of 14.2 g/kg and 13 g/kg respectively. VS reduction has further supported this result and the highest VS removal was measured to be 87.9% in aC40 and 87.8% in 35CP40. In addition to this reactor, aCP20, aP30, and 95CP40 also showed VS removal efficiencies above 80%.

The VS reduction values show that anaerobic bacteria degraded the substrates to the maximum and maximum utilization of carbon content has been achieved which is responsible for bio-hydrogen production [98].

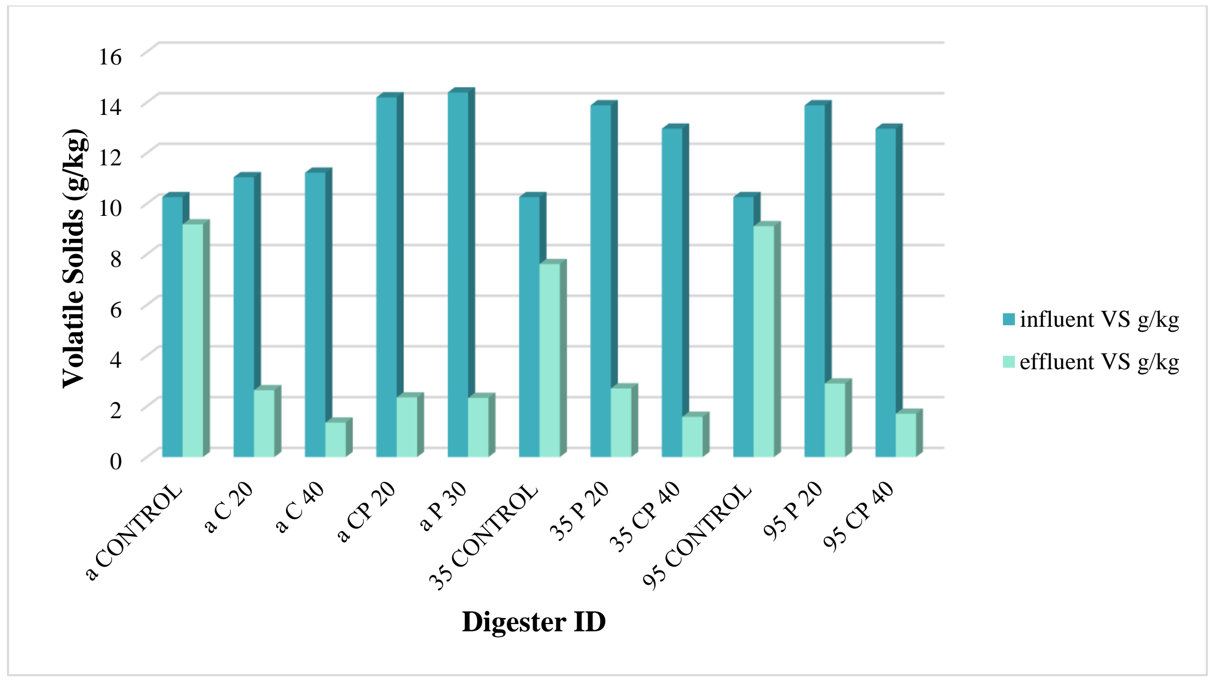

Figure 4.12: Volatile Solids (VS) characterization of influent and effluent of anaerobic fermentation essays with retention time of 9 days.

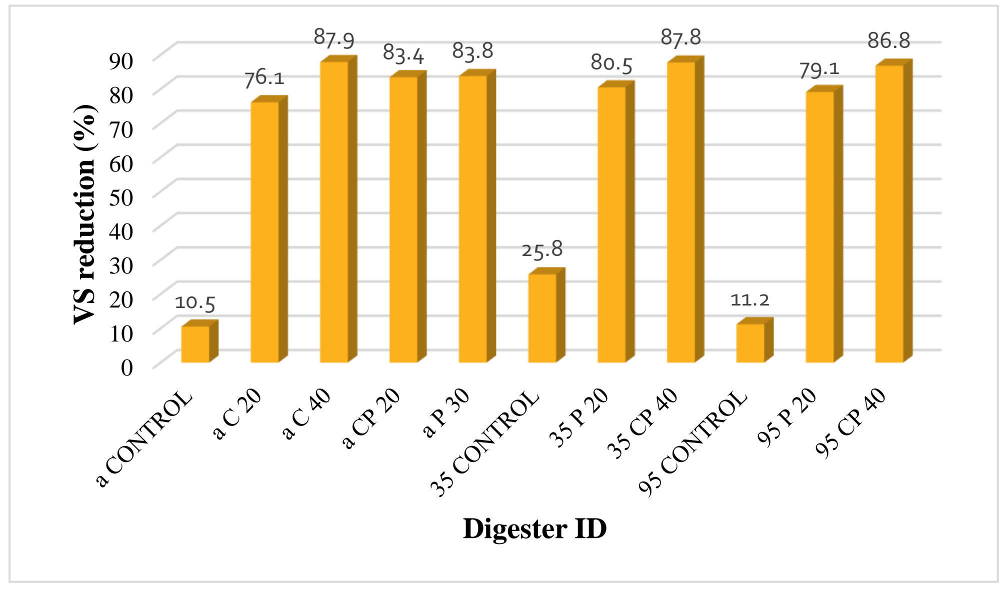

Figure 4.13: VS removal efficiency of control and all combination ratios reactors and inoculum pre-treatments.

4.4.3.6 **Total Organic Carbon (TOC)**

Total organic carbon test was performed to measure the organic carbon concentration in anaerobic bioreactors. The organic carbon present in the substrate is

utilized in the bacterial anabolic pathways and results in bacterial colony formation. This carbon is also utilized in catabolic pathways of bacteria and contributes to biogas production. Therefore, the decrease in TOC with time is an indication of stable anaerobic fermentative process. In the present study, TOC concentration had been decreased with time and the greatest difference between the influent and effluent TOC was found in aP30. Bioreactors aCP20, 35P20, 35CP40, 95P20 and 95CP40 also showed considerable decrease between their influent and effluent TOCs.

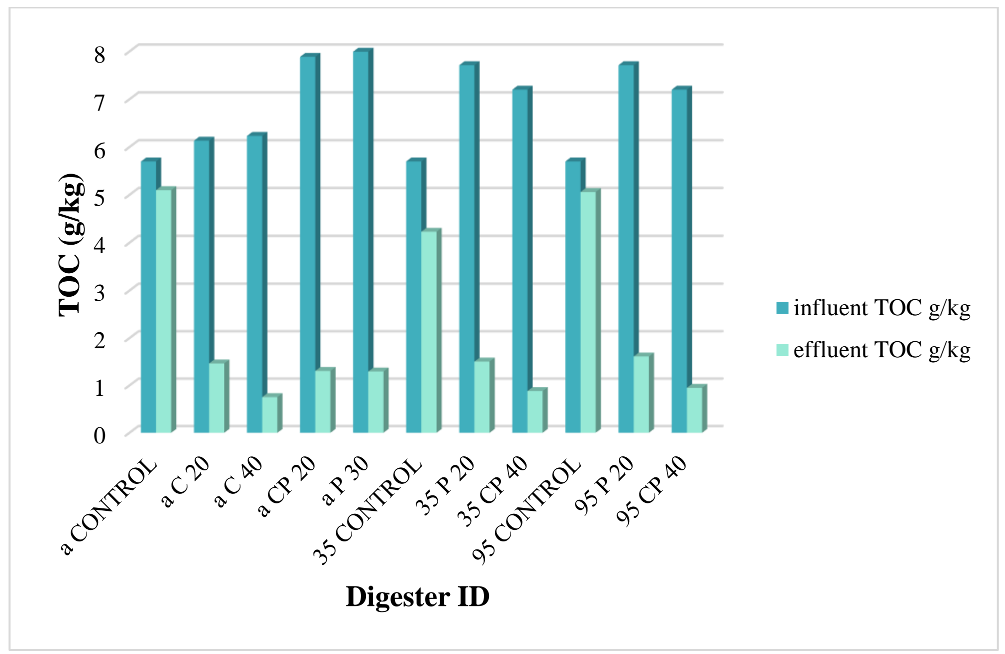

Figure 4.14: TOC content of influent and effluent of control and all combination ratios reactors and pre-treatments.

4.4.3.7 **Chemical Oxygen Demand (COD) and removal efficiency**

In this test, chemically oxidizable organic matter was measured to examine the COD concentration removed during anaerobic fermentative process. Figure 4.20 represents that COD of influent mixtures lied in the range of 18-47 g/L (not including the control reactors) clearly depicting the high concentration of carbonaceous organic matter. Effluent concentration of COD was lesser than corresponding influent COD in all the reactors indicating the occurrence of anaerobic digestion to some extent. More successful reactors are those in which there is larger difference between the effluent and influent COD values because COD removal is the key factor to determine the efficiency of waste stream anaerobic treatment [99]. In terms of COD removal efficiency, the highest reduction was achieved in aP30 i.e. 80.3% followed by aCP20 at 76% and then 95P20 at 74% (figure 4.15).

Highest COD removal efficiency in aP30 reactor can be attributed to the most suitable substrate to inoculum ratio which encouraged balanced bacterial growth without chocking the system.

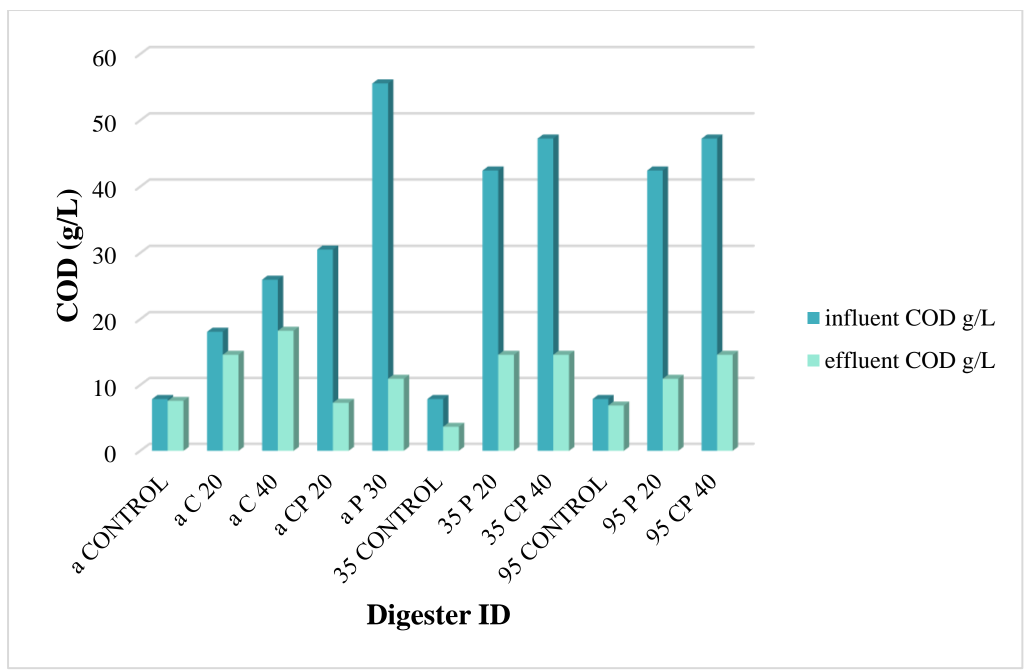

Figure 4.15: COD characterization of influent and effluent of anaerobic fermentation essays with retention time of 15 days.

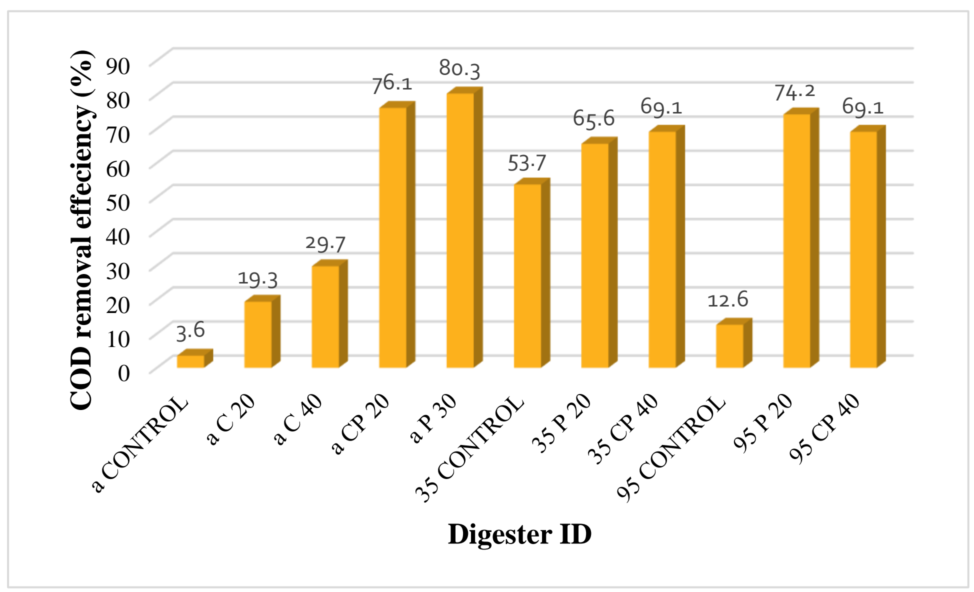

Figure 4.16: COD removal efficiency of control and all combination ratios reactors and pre-treatments.

Important parameters depicting the performance of all the combination ratios with their respective pre-treatment methods are tabulated in table 4.5. Highest specific hydrogen yield was obtained from P20 in which potato was the substrate, VS

concentration was at 0.44, and inoculum pre-treatment was heat shock at 95°C. These results suggest that for the particular type of inoculum used, the bacterial microflora which dominated the sludge after heat shock pre-treatment at 95°C was best adapted with fermentation of potato at the VS concentration of 0.44 out of all the pre-treatments and combination ratios tested. This may be due to differences in bacterial species in the inoculum as well as the buffering capacity of organic matter in each digester.

Table 4.5: Hydrogen yield obtained from all the digesters with all combination ratios

Type of inoculum pre-treatment	**Heat Shock 35°C**		**Heat Shock 95°C**		**Aeration**			
Digester ID	CP40	P20	CP40	P20	P30	C20	C40	CP20
VS content	0.67	0.44	0.67	0.44	0.6	0.35	0.58	0.45
Cumulative hydrogen yield (ml)	21.57	25.07	22.14	38.61	12.94	18.61	30.97	27.71
Specific hydrogen yield (ml/gVS)	32.19	56.86	33.04	87.74	21.57	53.16	53.40	61.57

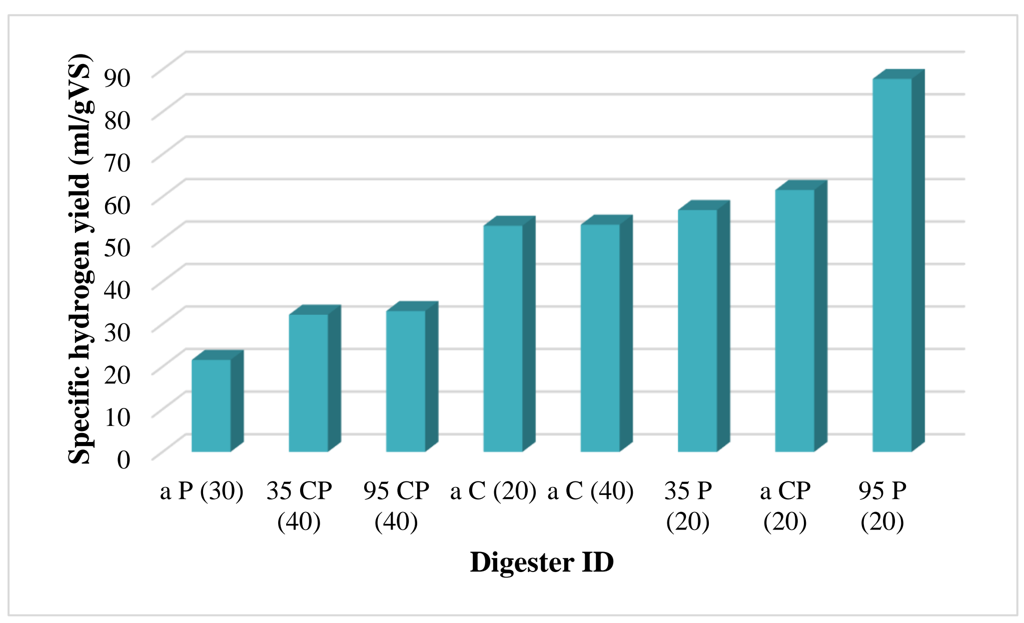

Figure 4.17: Comparison of specific hydrogen yield of all the combination ratios

4.4.4. Application of Hydrogen

Hydrogen gas produced by acidogenic fermentative process was connected to hydrogen side of the fuel cell. After connecting motor to the fuel cell, the car started to run off immediately on the ground. Observations are given in table 4.5.

Table 4.6: Observations recorded by conducting experiment on a model fuel cell car.

Source of hydrogen	OBSERVATIONS
Electrochemical water Splitting	A → B: 10 minutes, 150 feet
Dark fermentation	A → B: 6 minutes, 82 feet
Control reactor	A ✕ B: No movement

As reference, the car was first fueled by pure hydrogen according to standard operating procedure of the fuel cell car kit. Time was recorded for both the samples of hydrogen and results were compared. Hydrogen produced by electrochemical water splitting is extremely pure, therefore, gives good efficiency when compared to fermentative hydrogen. But at the same time the electrolysis of water an energy intensive process. Biohydrogen, on the other hand, is naturally produced by tailoring bacterial consortium and utilizing easily available organic matter. This biogenic gas can also be purified to get better results in fuel cell applications.

4.4.5. IV comparison of electro-chemically produced hydrogen with bio-genically produced hydrogen by Cyclic Voltammetry

In order to get quantitative results, current density measurements of the fuel cell stack were plotted in the form of IV curves. This experiment was conducted by doing

cyclic voltammetry (CV) in a three electrode system. This experiment was done first by fueling the cell stack by electrochemically produced hydrogen and recording the behavior. Same procedure was repeated but source of hydrogen was changed to biogenic. The plots obtained are shown in figure 4.23 whereas respective current densities are given in table 4.7.

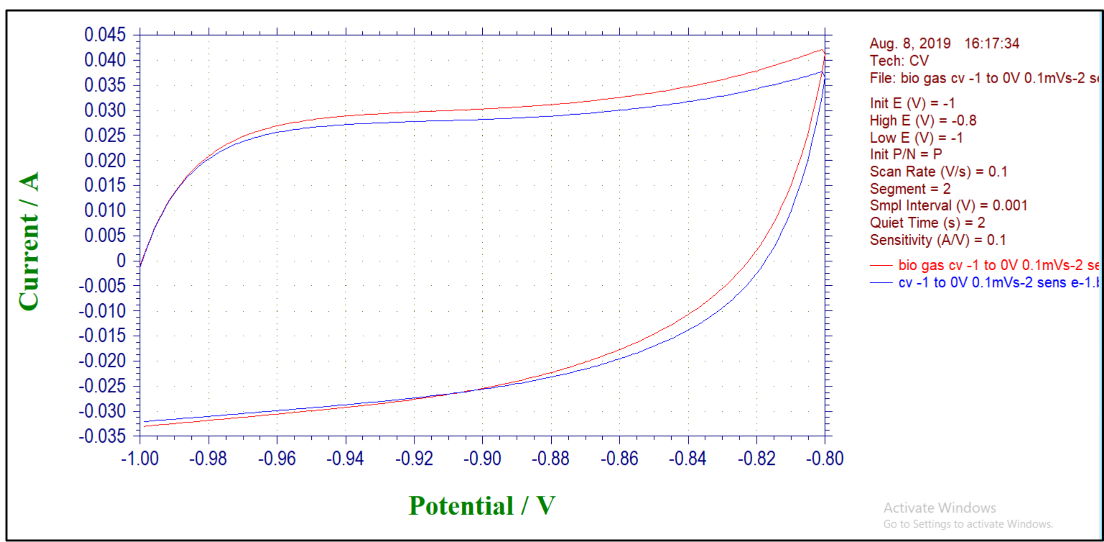

Figure 4.18: Cyclic Voltammetry comparison of electro-chemically produced hydrogen with bio-genically produced hydrogen.

Table 4.7: Comparison of current density tested in CV.

Source of hydrogen	**Current Density (mA/cm^2)**
Electrochemical	6.35
Biogenic	6.00

4.5 Summary

The influence of different inoculum pre-treatments methods and substrate combination ratios on hydrogen production was investigated. Best pre-treatment methods and substrate combination ratios were selected in the first and second phase of this study. Initial setup was designed to screen out the suitable inoculum pre-treatment method to suppress the methanogens and enhance hydrogen producing bacteria from mixed culture. Heat shock at 35 and 95 degree Celsius and aeration for 24 hours were selected to proceed to the next phase of experimentation which was to study the effects of feed to inoculum ratio at several VS concentrations. Results

showed that hydrogen is obtained for the first 9 days of incubation. From results of the preliminary study, it was concluded that heat shock at 95°C had the longest lag time of 48 hours, but it was most successful at suppressing the methanogens giving the least methane production out of all the 4 pre-treatment methods employed. Heat shock at 35^0C produced most amount of hydrogen which was 158 times more than the control reactor. In the next experimental phase which tested the most suitable F/I ratio in combination with the pre-treatment method, the ratios C20, C40, CP20, and P30 were selected for aeration and the ratios P20 and CP40 were selected for heat treatment at 35 and 95 degree Celsius. In the repeated batch experimentation, 95P20 gave the highest specific biogas yield of 87.74 ml H_2 per gram of VS added followed by aCP20 which stood at 61.57 ml H_2/gVS added and 35P20 which yielded 56.86 ml H_2/gVS added. Chemical characterization revealed the high removal efficiencies in terms of volatile solids, chemical oxygen demand and total organic carbon for the 95P20, aCP20 and 35P20 reactors. Hydrogen produced was used as a fuel in a toy fuel cell car.

Chapter 5: Conclusions and Recommendations

The following conclusion and recommendations were extracted from this study which has been compiled in the form of a thesis.

5.1 Conclusions

1. Mesophilic batch acidogenic hydrogen production was carried out from mixed microbial culture and potato peel and vegetable waste.
2. From results of the preliminary study, it was concluded that heat shock at 35°C most successful at enhancing hydrogen yield out of all the 4 pre-treatment methods employed, producing 158 times more hydrogen than the control reactor. While heat shock at 95°C had been most successful at oppressing the methanogens, producing 101.7 times less methane than the control reactor.
3. In the next experimental phase which tested the most suitable F/I ratio in combination with the pre-treatment method, the ratios C20, C40, CP20, and P30 were selected for aeration and the ratios P20 and CP40 were selected for heat treatment at 35 and 95 degree Celsius owing to their stable hydrogen production.
4. For the repeated batch experimentation, results showed that hydrogen is obtained for the first 9 days of incubation. 95P20 gave the highest specific biogas yield of 321730.98 ml H_2 per gram of VS added followed by aCP20 which stood at 62972.4 ml/gVS added and 35CP40 which yielded 61625.4 ml/gVS added.
5. Chemical characterization revealed the high removal efficiencies in terms of volatile solids, chemical oxygen demand and total organic carbon for the 95P20, aCP20 and 35CP40 reactors.
6. Model fuel cell car was connected to the produced biohydrogen and was successfully driven across the ground.
7. This study concluded that hydrogen production is feasible from mixed microflora if the suitable method of hydrogen producing microbial enrichment is paired with the appropriate F/I ratio since different microbes give different

response to the pre-treatment method and organic loading in the fermentative digester and is viable for practical applications like in fuel cells.

5.2 Recommendations

1. Further detailed study is required for detailed phylogenetic study of microbial biomass by using microbial techniques like PCR to get the clear picture of the microbial response towards the pre-treatment method and F/I ratio.
2. The data obtained from this study could be used as a basis for designing large scale anaerobic digesters for treatment of food and green wastes and their mixture.
3. Purification of hydrogen gas is another research area which is closely linked to fermentative hydrogen production because hydrogen produced in this way is not pure and further work is necessary for its purification to increase the market value of biohydrogen.
4. Hydrogen is a very light gas and much improvement is needed on fool proof methods of hydrogen storage when this gas is produced from the digester.
5. The fermentative slurry obtained at the end of acidogenic hydrogen production contains many useful alcohols and volatile fatty acids which can be recovered in the form of value added products.
6. Fermentative slurry can also be used further in the production of methane or as a fertilizer.

Chapter 6: Research work at ASU

Synthesis of Bismuth Vanadate as a Photocatalyst in Photoelectrochemical Cell (PEC)

6.1 Introduction and literature review

The photo electrochemical (PEC) approach combines two different processes: absorption of solar radiation and fuel generation by chemical processes into one gadget. There exist various forms of PEC devices in which these two technologies are integrated to a varying extents; ranging from electrolysis assisted by PV to pure PEC, and the advantages of various configurations also vary [100]. The technical and economic comparison of the pure PEC approach to PV coupled electrolysis is multifaceted, but has potential cost savings due to the reduction in the balance of expenses [101]. A recent viewpoint has also shown that PV coupled PEC devices are capable of exhibiting better performance than PV coupled electrolysers on efficiency basis [102].

For the upfront hydrogen generation from solar energy, PV coupled electrolysis is the method to consider; as it amalgamates the pros of two technologically sound technologies i.e., photovoltaics and electrolysers [101].

6.1.1. Bismuth vanadate as a photoanode

One of the materials that is making its way to be used as an efficient and state of the art photo anode in PEC systems is Bismuth vanadate ($BiVO_4$). Its properties like, a relatively narrow band gap ($E\,g$ = 2.4 eV), composition of fairly ample and non-toxic elements, and an over potential of around 1V is available for the water oxidation reaction account for a high-performing photo anode [103].

6.1.2. AIM/Scope: Improve $BiVO_4$ light harvesting capabilities to enhance water splitting in (PEC).

Up till now, synthesis of good quality $BiVO_4$ photoanodes is done by nanostructuring the material to compensate for the short carrier diffusion length including rare earth doping [104]. Rare earth doping can effectively inhibit the recombination of photo-generated electron-hole pairs.

Group members:

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- **Umesh Prasad,** PhD Candidate, Systems Engineering, Arizona State University.
- **Rida Mansoor**, MS Energy Systems Engineering, NUST.

6.2 Methodology

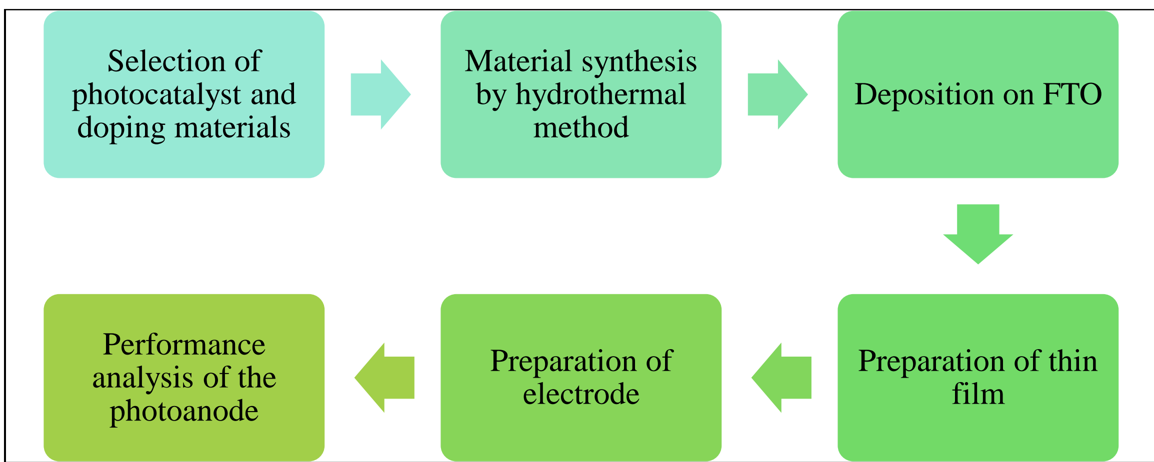

Figure 6.1: Basic bending process of the project.

6.2.1. Materials and Synthesis process:

Bismuth Vanadate ($BiVO_4$) was used as a base material for film deposition. It was synthesized using hydrothermal method by mixing 1mM $Bi(NO_3)_3$ and 3mM NH_4VO_3 in 10 ml (2 mol L^{-1}) HNO_3 solution. Scheme of the process is shown in figure 6.2.

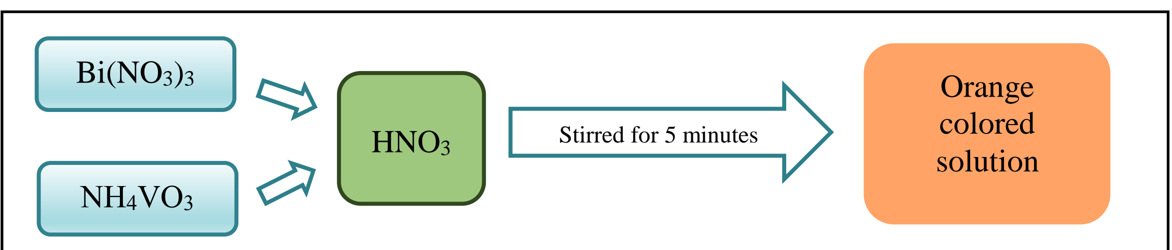

Figure 6.2: Hydrothermal process for bismuth vanadate synthesis.

6.2.2. Preparation of thin film: Dip Coating Method

Dip coating was done in a four step process shown in figure 5.3.

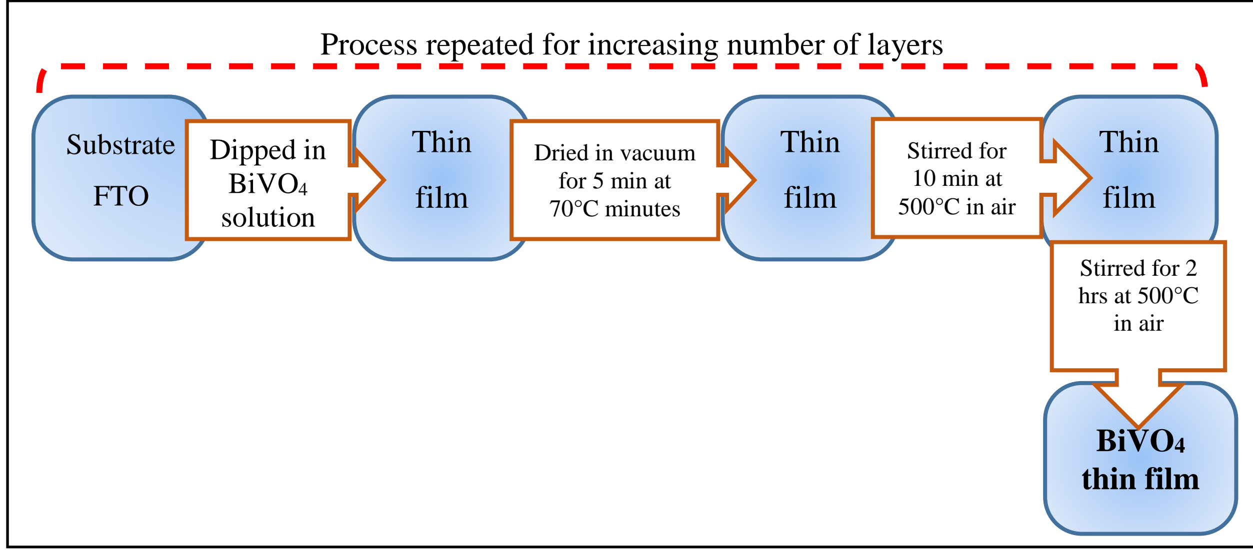

Figure 6.3: Dip coating method for thin film preparation.

Based on this dip coating method, four different types of films were prepared which varied on the basis of number of dip coatings and various doping materials. Details are given in table 5.1

Table 6.1:Types of electrodes prepared by dip coating.

No	Type of electrode	Acronyms
1	$BiVO_4$	BVO
2	BVO+(3%)W	BVO+W
3	L1-BVO, L2-BVO+W, L3-BVO	BWB
4	L1-BVO+W, L2-BVO, L3-BVO+W	WBW

6.2.3. Performance Analysis of prepared electrodes

Photocatalytic activity of each electrode was tested by cyclic voltammetery in a three electrode system. Picture of CV set-up is shown in the figure. Bio-electrochemical behaviour of the mixed consortia was evaluated employing cyclic voltammetry (CV) using electrochemical cell having platinum and graphite rod as working and counter electrodes, respectively against Ag–AgCl(S) reference electrode. Voltammograms were recorded by potentiostat–glavanostat system (Autolab, PGSTAT12, Ecochemie)

by applying a potential ramp at a scan rate of 30 mV/s over the range of −0.4 to +1.0 V to the working electrode. Plots were obtained which compared current density against voltage of the electrode.

Figure 6.4: Cyclic voltammetery set-up

6.3 Results and discussion

6.3.1. Photocurrent density of pristine $BiVO_4$

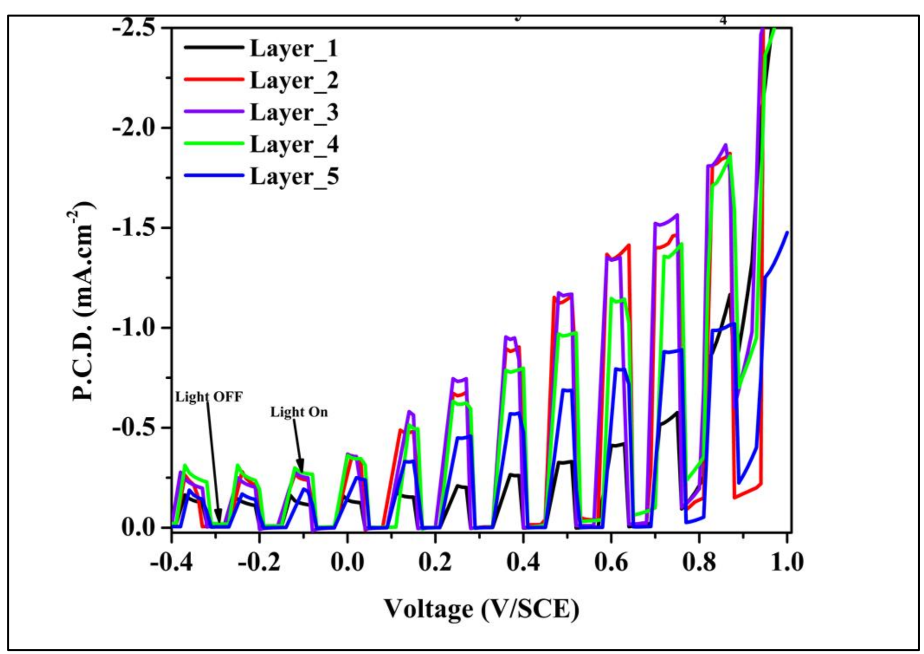

Figure 6.5: Photocurrent density of pristine BVO up to 5 layers

The graph (figure 5.3) shows the photocatalytic performance of pristine $BiVO_4$ tested up to 5 layers. Results depicted that electrode performance increased with the increase in number of coatings up to three layers after which it declined as the number of layers was increased further. Based on these results, electrodes prepared with other samples (BVO+W, BWB and WBW) were dip coated three times.

6.3.2. Photocurrent density of electrodes with and without N_2 treatment:

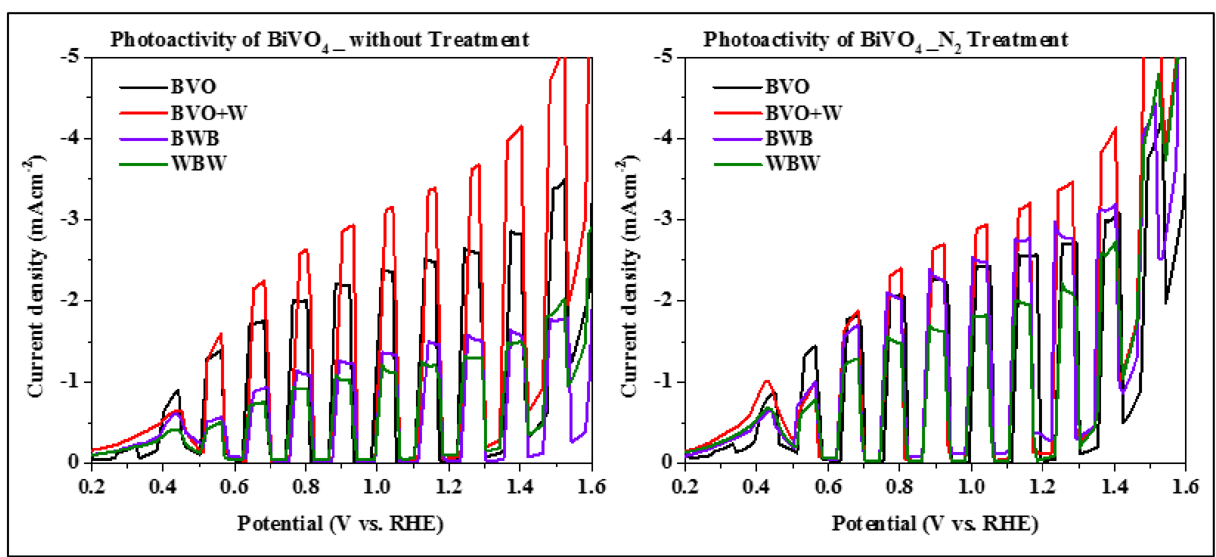

Figure 6.6: Photoelectrochemical performance of electrodes with and without N2 treatment

6.4 Conclusion

- Performance of $BiVO_4$ is optimum till 3 layers
- Application of N_2 decreases the performance since it replaces V by N elements.
- W doping is increasing adhesion with charge collector which is helping in higher mobility.
- However, W doping samples do not get affected by N_2 doping

6.5 Recommendations

- Application of co-catalyst to enhance the photocurrent
- Doping different element for higher performance
- Changing the nanostructures shape for higher absorption.

- Using different medium treatment to generate vacancy in $BiVO_4$ for higher performance.

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Appendix – Research Article

BIOGENIC HYDROGEN PRODUCTION FROM POTATO PEEL BY MESOPHILIC DARK FERMENTATION

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Abstract:

Enriching hydrogen producing bacteria from locally available inocula is being seen as one of the sustainable and green methods to produce hydrogen by dark fermentation. Hydrogen (H_2) shows really broad prospects in the process of clean fuel evolution so renewable means of its generation are crucial to combat global climate change. In this research, two pre-treatment methods, heat shock and aeration were conducted in mesophilic batch tests to evaluate the performance efficiency of hydrogen production. Anaerobically digested cow manure was used as inoculum and potato peel and cabbage waste were the selected substrates. Results of the experiments proved heat shock pre-treatment of inoculum at 35°C and initial pH of 6 to be the most effective one. Maximum hydrogen yield of 40 micro litres was achieved at the above mentioned pre-treatment conditions when only inoculum was used in the digester. This yield increased to 2049 micro litres when potato was introduced as substrate. Substrate to inoculum volatile solids (VS) ratio (henceforth VS ratio) was kept as 0.2:2. The study showed that hydrogen production from dark fermentation of organic waste like potato peel is feasible and the area is open to many researches to make this process more efficient.

Key words: Bio hydrogen, dark fermentation, green energy, organic waste, pre-treatment.

Introduction

Hydrogen has broadened the horizon for ideal fuel in the future because no greenhouse gases and other potentially harmful by-products are released in the environment upon its consumption; moreover, it falls under the category of renewable fuels [1] [2]. Water is the main by-product of hydrogen when it is used as fuel, this water can either be discarded or reused to produce hydrogen again [3]. However, it does not occur in nature like fossil fuels, solar and wind energies, but has to be contrived just like electricity hence called the secondary form of energy [4]. Hydrogen combustion produces more energy on a mass basis than any other fuel. Its low heating value (LHV) is 2.4 times higher than that of methane, 2.8 time

higher than gasoline and 4 times that of coal [5].

Hydrogen sources and its production methods are diversifying. They range from conventional fossil fuels to zero carbon nuclear energy and also environment friendly renewable feedstocks like solar, wind and biomass [17]**.** Out of all the methods mentioned above, dark fermentation checks most of the boxes for the production of ideal fuel [25]. Although, still in the research phase, but mechanism of this process are better understood by the researchers (amongst all the processes) who are working on various aspects to refine it [26]. Dark fermentation is a complex process which, in the presence of strictly anaerobic conditions, covert the degradable organic material into a mixture of gases called biogas. The biogas mainly consists of hydrogen, methane and carbon dioxide [27]. This degradation and transformation is carried out by specialized consortium of anaerobic microorganisms which eventually results in energy recovery as biogas production and formation of bio-slurry to be used as natural fertilizer for crop productivity [28]. The science behind anaerobic fermentation process is quite complicated because it involves key microbiological pathways and it is best comprehended if it is broken down into different stages [29]. These stages are interlinked as the product of one stage serves as substrate for the bacteria of next stage [30].

Materials and methods

Inoculum and substrates: The substrates selected for this research were potato peel and waste cabbage leaves obtained from house hold kitchen waste. Particle size was reduced in the food processor and stored in the freezer in USPCAS-E Biofuel Lab at temperature below $0^{\circ}C$ until further use. Inoculum was the digestate of digester undergoing anaerobic co digestion of dairy waste and various food wastes. It was obtained from a biogas digester situated in Fateh Jang; digesting animal manure and vegetable waste. The digestate was stored in the lab in wide mouthed bottles at $4^{\circ}C$. Proximate analysis of inoculum and food waste is given in table 1.

Table 1: Proximate analysis of inoculum and substrate

Sample	Moisture Content (MC)	Total Solids (TS)	Volatile Solids (VS)	Total Organic Carbon (TOC)	Chemical Oxygen Demand (COD)
	%	%	%	g/kg	g/L
Inoculum	87.64	12.35	10.30	5.85	7.9
Potato peel	82.54	17.45	16.05	8.92	210
Cabbage	87.61	12.38	11.62	6.45	85.3

Methods of analysis: MC, TS, VS, TOC and COD were analysed according to the standard methods [105]. Biogas was collected in syringes, gas volume was measured by the plunger displacement method. Hydrogen content present in the biogas was analysed by comparing sample with pure hydrogen standard using gas chromatograph Shimadzu 2010 plus equipped with 30m long column of RT-MS5A (TCD). Detector temperature was 200ºC and the rate was 35ºC/minute. Nitrogen was used as a carrier gas at the flow rate of 1.76 mL/min and 98.8 kPa. Biogas samples were injected manually using a syringe of 60ml.

Methods of microbial enrichment: For heat shock pre-treatment, inoculum bottles were kept in a water bath whereas vacuum aerator pumps were used for aeration process. Details are given in table 2.

Table 2: Inoculum pre-treatment methods and conditions

<table>
<tr><th>Sr. No.</th><th>Type of pre-treatment</th><th colspan="2">Conditions</th></tr>
<tr><td rowspan="3">1</td><td rowspan="3">Heat shock</td><td>35°C</td><td rowspan="3">20 minutes</td></tr>
<tr><td>65°C</td></tr>
<tr><td>95°C</td></tr>
<tr><td>2</td><td>Aeration</td><td>Bubbling</td><td>24 hours</td></tr>
</table>

Reactor configuration: Batch type fermentation tests were carried out in the lab. Digester hardware consisted of 250ml Schott bottle, tightly fitted with a cork and sealed with silicone sealant to ensure that no air could pass through. These bottles were filled with substrate and inoculum according to the determined working volume and head space as shown in figure 9. Since the activity of hydrogen-consuming methanogens is inhibited at low pHs [33][84][85], the pH was adjusted to 5.5$\pm$.5 by adding 1 molar H_2SO_4 solution. After adding feed and inoculum, the head space of bottles was flushed with nitrogen gas for 4-5 minutes to eliminate all the oxygen and create anaerobic environment. After that the bottles were properly sealed with the cork and sealant. One or 2 syringes were inserted through the cork for collection and monitoring of bio gas produced. The prepared digesters were placed in the incubator in mesophilic conditions i.e. 36°C for fermentation.

Table 3: Details of digesters in the experimental set-up

Sample ID	Substrate (g VS)		F/I ratio (gVS)	pH adjustment
	Cabbage	Potato		
CONTROL	-	-	0/2.1	-
UNTREATED	-	-	0/2.1	5.5
35 CP 40	0.15	0.2	0.35/2.1	6.04
35 P 20	-	0.2	0.2/2.1	5.81
a CP 20	0.76	0.14	0.9/2.1	5.95
a C 20	0.15	-	0.15/2.1	5.93
a C 40	0.3	-	0.3/2.1	5.84
a P 30	0.4	-	0.4/2.1	5.87

Results and discussion:

Figure 1 illustrates the effect of different pre-treatment methods on the cumulative hydrogen production in batch tests. The results showed that for all tests the hydrogen production process started from day 4 and ceased within 17 days. UNTREATED and CONTROL reactors produced the minimum amount of hydrogen because in these digesters, existing microflora was not subjected to any pre-treatment to suppress methanogens. As for the digesters inoculated with both seed sludge and substrate, there was considerable hydrogen production in all of them which reached a maximum value on day 10 for aC20, a C40, aP30 and aCP20 before declining with time. 35P20 and 35CP40 were the two highest producers of hydrogen indicating that heat shock method of microbial enrichment at 35°C proved to be more successful than

aeration. Both these digesters produced maximum hydrogen on 8th day, earlier than others. The amount of hydrogen remained fairly consistent till 12th day before it started to decline. Reason for good production can be attributed to the suitable volatile solids concentration combined with inoculum pre-treatment method provided favourable environment for the particular hydrogen producing strains in the used inoculum.

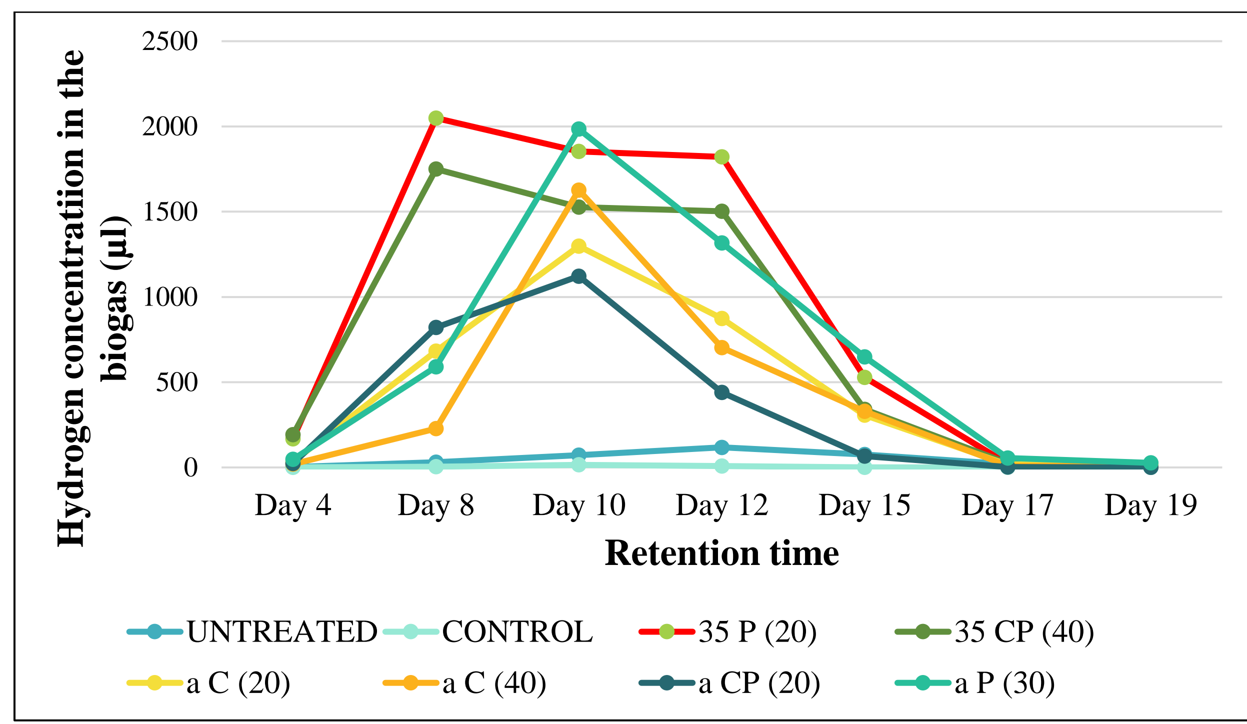

Figure 1: Hydrogen production from different combinations of F/I. Inoculum pre-treated by aeration and heat shock method

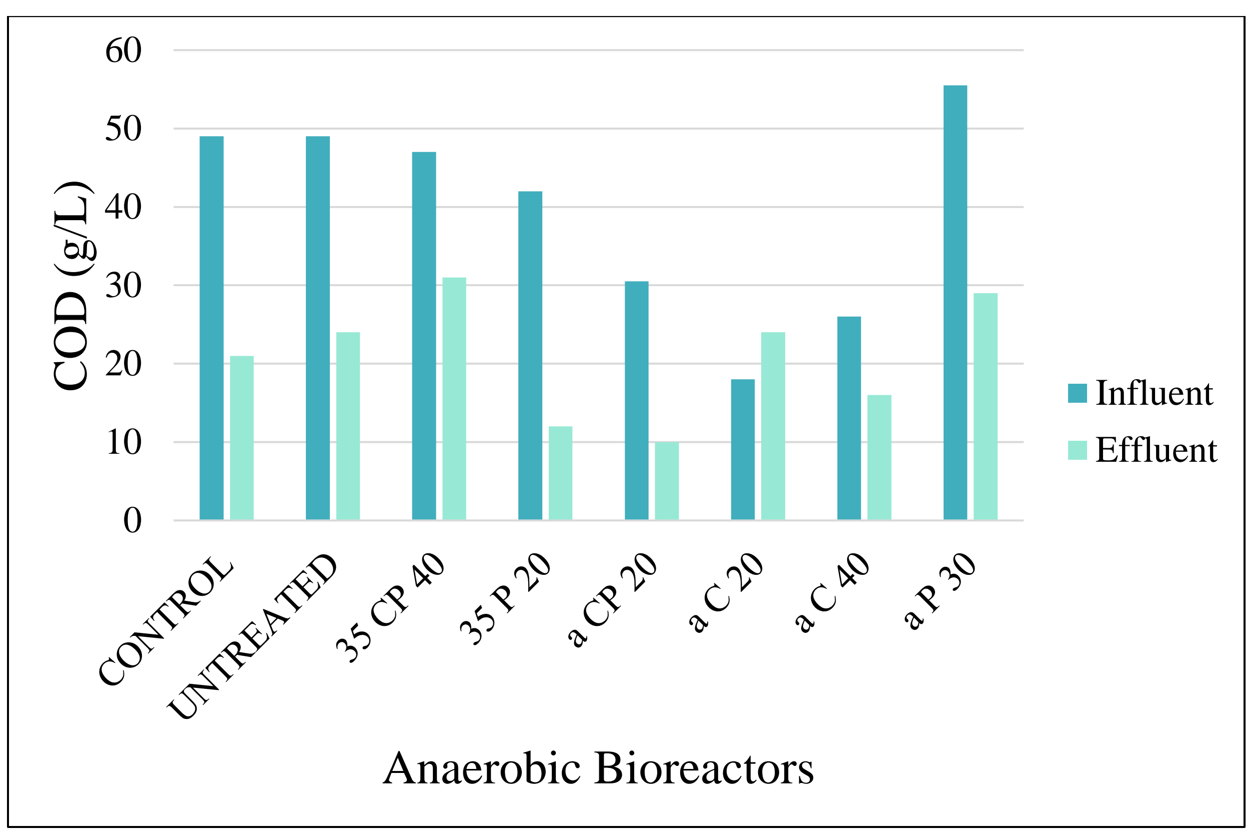

Figure 2: COD characterization of influent and effluent of digesters with retention time of 15 days

In this test, chemically oxidizable organic matter was measured to examine the COD concentration removed during anaerobic fermentative process. Figure 2 represents that COD of influent mixtures lied in the range of 18-47 g/L (not including the control reactors) clearly depicting the high concentration of carbonaceous organic matter. Effluent concentration of COD was lesser than corresponding influent COD in all the reactors indicating the occurrence of anaerobic digestion to some extent. More successful reactors are those in which there is larger difference between the effluent and influent COD values because COD removal is the key factor to determine the efficiency of waste stream anaerobic treatment [99]. Highest COD removal efficiency in aP30 reactor can be attributed to the most suitable substrate to inoculum ratio which encouraged balanced bacterial growth without chocking the system.

These results suggest that for the particular type of inoculum used, the bacterial microflora which dominated the sludge after heat shock pre-treatment at 35°C was best adapted with fermentation of potato at the VS concentration of 0.44 out of all the pre-treatments and combination ratios

tested. This may be due to differences in bacterial species in the inoculum as well as the buffering capacity of organic matter in each digester.

Conclusion

This study concluded that hydrogen production is feasible from mixed microflora if the suitable method of hydrogen producing microbial enrichment is paired with the appropriate F/I ratio since different microbes give different response to the pre-treatment method and organic loading in the fermentative digester. Heat shock pre-treatment at 35°C was found to be most viable for enhancing the hydrogen producing bacteria in the mixed culture inoculum.

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