

Jimma University

School of Graduate Studies

Jimma university Institute of Technology

Faculty of Civil and Environmental Engineering

Environmental Engineering Masters Program

Hydrogen gas production from agricultural waste using dark fermentation processes.

By : Surafel Yihune (MSc.)

A Thesis Submitted to Jimma University Jimma Institute of Technology  
Environmental Engineering Chair in Partial Fulfillment of the Requirements  
for the Degree of Masters of Science in Environmental Engineering

January, 2019

Jimma, Ethiopia

Jimma University

School of Graduate Studies

Jimma university Institute of Technology

Faculity of Civil and Enviromental Engineering

Enviromental Engineering Masters Program

Hydrogen gas production from agricultural waste using dark fermentation processes.

By: Surafel Yihune (MSc.)

A Thesis Submitted to Jimma University Jimma Institute of Technology  
Environmental Engineering Chair in Partial Fulfillment of the Requirements  
for the Degree of Masters of Science in Environmental Engineering

Advisor :- Dr. Dejene Beyene

Co-Advisor:- Mr. Wagari Mosisa (MSc.)

January, 2019

Jimma, Ethiopia

## DECLARATION

I Surafel Yihune do here by declare to the senate of Jimma University this thesis is entirely my original work and all other materials are appropriately acknowledged. This work has not been submitted and presented for any academic degree award at any other University.

Surafel Yihune

\_\_\_\_\_

\_\_\_\_\_

Student

Signature

Date

This thesis has been submitted for examination with my approval as a university supervisor.

Dr.Dejene Beyene

\_\_\_\_\_

\_\_\_\_\_

Main advisor

Signature

Date

Mr. Wagari .Mosisa

\_\_\_\_\_

\_\_\_\_\_

Co- advisor

Signature

Date

## APPROVAL PAGE

This thesis entitled “hydrogen gas production from agricultural waste using dark fermentation processes” has been approved by the following advisors, examiner, and coordinator and Director of post Graduate studies in partial fulfillment of the requirement of the degree of Master of Science in Environmental Engineering.

Submitted by:

<u>1. Surafel Yihune</u>	_____	_____
Student	signature	Date

**Approved by:**

<u>1. Dr.Dejene Beyene</u>	_____	_____
Main Advisor	signature	Date
<u>2. Mr.Wagari .Mosisa</u>	_____	_____
Co- advisor	Signature	Date
<u>3. Internal Examiner</u>	_____	_____
	Signature	Date
<u>4. External Examiner</u>	_____	_____
	Signature	Date
<u>5. EE Chair holder</u>	_____	_____
	Signature	Date

## ABSTRACT

*The global reserves of fossil fuels are depleting due to their increasing consumption. In addition the, environment is facing severe pollution problems due to gaseous emissions (NO<sub>x</sub>, SO<sub>x</sub>, CO, CO<sub>2</sub>, etc.) and waste generated from the production and use of fossil fuels. The objective of this study was to produce hydrogen gas from corn cobs using dark fermentation processes. Unlike fossil fuels hydrogen gas does not cause any CO<sub>2</sub>, CO, SO<sub>x</sub> and NO<sub>x</sub> emissions producing water as its only by-product when it burns reducing greenhouse effects. After corn cobs (samples) were collected in plastic bag reduce the sizes of corn cobs in pestle and dried at 105 °C for 4hr in oven to remove the moisture content and ground to the particle size of 2 mm. After that the sample was exploded by steam explosion pretreatment using 3.5MPa pressure steam in autoclave at 121°C for 15 min. Then the sample was hydrolyzed by adding 2% H<sub>2</sub>SO<sub>4</sub> and filtered under vacuum condition using activated carbon to remove hydrogen production inhibitors such as furfural, hydroxymethylfurfural (HMF), phenol etc. and stored at 4°C. After that fermentation was carried out by mixing the prepared sample to media ratio (10:1) and maintained its pH at 5.5, 6, 6.5, and 7 by digital pH meter and put it in incubator by adjusting at temperature of 25, 35, 55, and 70°C. Then after 72 hours the produced gas was characterized using gas chromatography. The corn cob used in this study was characterized by 9.2 % moisture content, 9% fixed carbon content, 2.4% ash content and 79.4% relatively high volatile matter content. It was employed dilute acid hydrolysis, because it is easy and productive process. The experiment was designed by Central Composite Design (CCD) with two factor temperature and pH at constant hydraulic retention time (HRT) was maintained at 25, 35, 55, 70°C, and at 5.5, 6, 6.5, and 7 respectively. The maximum yield of 3.18mole of H<sub>2</sub> /mole of hexose was observed at a temperature of 69.979°c and pH of 6.964. Significance of the process variables were analyzed using analysis of variance (ANOVA) and second order polynomial function was fitted to the experimental results. Thus, the influence of experimental variables, factors, and interaction effects on the response was investigated. Temperature and pH have a statistically significant effect on the yield with p-value<0.0001.*

**Key words:** *Corn cobs; Dark fermentation; Hydrogen gas; steam explosion pretreatment*

## **ACKNOWLEDGMENTS**

First of all I would like to say thanks to God for giving me strength and patience for the successful accomplishment of this study without His assistance every things is impossible.

Secondly I would like to express my gratitude to my advisors Dr. Dejene Beyene and Mr. Wagari. Mosisa (MSc) for their support and great advice to complete this thesis.

Next to this I would like to say thanks JU, JIT for sponsoring me and give the chance to learn MSc program and Environmental Engineering chair as well as my family from the bottom of my heart to help me to accomplish my thesis.

Finally I would like to say thanks to Department of Biology, water supply and Environmental Engineering laboratory assistant Suresa, Getnet, Hasen, and Merid for their help throughout the experimental work in making the setup of equipment and devices that I required for the processes and also I would like to say thanks to leather industry development institute to characterize my products.

Table of contents	page
ABSTRACT .....	i
ACKNOWLEDGMENTS .....	ii
LIST OF TABLES .....	vii
LIST OF FIGURES .....	viii
ACRONYMS .....	ix
<b>CHAPTER ONE.....</b>	<b>1</b>
<b>INTRODUCTION .....</b>	<b>1</b>
1.1 Background of the study.....	1
1.2 Statement of the problem.....	3
1.3 Objectives of the study .....	4
1.3.1 General objective.....	4
1.3.2 Specific objectives.....	4
1.4 Research questions/hypothesis .....	4
1.5 Significance of the study .....	4
1.6 Scope of the study .....	4
1.7 Limitation of the study .....	5
<b>CHAPTER TWO.....</b>	<b>6</b>
<b>LITERATURE REVIEW .....</b>	<b>6</b>
2.1 Sources of Energy .....	6
2.2 Hydrogen gas.....	7
2.3 Biohydrogen .....	8
2.4 Feed stocks for biohydrogen production .....	8
2.4.1 First generation feed stocks .....	8
2.4.2 Second generation feed stocks.....	9
2.4.3 Third generation feed stock.....	9
2.5 Choice of feed stocks for biohydrogen gas production .....	10
2.5.1 Overview of maize production in the world.....	10
2.5.2 Overview of maize production in Africa.....	10
2.5.3 Overview of maize production in Ethiopia.....	11
2.5.4 Corn cob as feed stocks for biohydrogen gas production.....	12
2.6 Hydrogen gas production steps .....	13
2. 6.1 Pretreatment.....	13
2. 6.1.1 Physical Pretreatments.....	14
2.6.1.1.1 Mechanical comminution .....	14
2.6.1.1.2 Pyrolysis and co-pyrolysis.....	14

2. 6.1.2 Chemical Pretreatments .....	14
2. 6.1.2.1 Acid hydrolysis.....	14
2. 6.1.2.2 Alkaline hydrolysis.....	14
2. 6.1.2.3 Ozonolysis .....	14
2. 6.1.2.4 Organosolvation .....	15
2. 6.1.2.5 Oxidative delignification .....	15
2. 6.1.3 Physicochemical Pretreatments .....	15
2. 6.1.3.1 Ammonia fiber explosion (AFEX).....	15
2. 6.1.3.2 Steam explosion.....	16
2. 6.1.3.3 CO <sub>2</sub> explosion.....	16
2. 6.1.4 Biological Pretreatments .....	16
2. 6. 2 Choice of steam-explosion over other pretreatment methods .....	17
2.6.3 Anaerobic digestion pathway for hydrogen gas production .....	17
2. 6.3.1 Hydrolysis.....	17
2. 6.3.2 Acidogenesis.....	17
2. 6.3.3 Acetogenesis.....	18
2. 6.3.4 Methanogenesis .....	18
2.7 Hydrogen production methods .....	19
2. 7.1 Electrochemical methods.....	20
2. 7.1.1 Electrolysis .....	20
2. 7.1.2 Photo electrolysis.....	20
2. 7.2 Thermochemical methods .....	20
2. 7.2.1 Partial oxidation.....	20
2. 7.2.2 Steam reforming of natural gas .....	20
2. 7.2.3 Coal gasification.....	21
2.7.3 Biological methods.....	21
2. 7.3.1 Direct bio photolysis.....	21
2. 7.3.2 Bio-photolysis-Indirect process.....	21
2. 7.3.3 Photo fermentation .....	22
2. 7.3.4 Dark fermentation.....	22
2.8 Choice of hydrogen production methods .....	23
2.9 Microorganisms for hydrogen production.....	23
2.10 Factors affecting DF pathway and H <sub>2</sub> yields .....	23
2. 10.1 pH and temperature .....	23
2. 10.2 Hydraulic retention time (HRT) .....	24
2. 10.3 Hydrogen and carbon dioxide partial pressure .....	24



2. 10.3.1 Hydrogen partial pressure.....	24
2. 10.3.2 Carbon dioxide partial pressure.....	25
2. 10.4 Organic acid concentration.....	25
2. 10.5 Inorganic elements.....	25
2. 10.5.1 Iron concentration.....	25
2. 10.5.2 C/N ratio.....	25
2. 10.6 Other factors.....	25
2.11 Uses of hydrogen gas.....	26
<b>CHAPTER THREE .....</b>	<b>27</b>
<b>MATERIALS AND METHODS.....</b>	<b>27</b>
3.1 Description of the study area.....	27
3.2 Materials and equipments used .....	27
3.3 Chemicals .....	27
3.4 Characterization of corncob .....	28
3.4 .1 Proximate analysis.....	28
3.4 .2 Moisture Content .....	28
3.4 .3 Volatile Matter Content.....	28
3.4 .4 Ash Content.....	29
3.4 .5 Fixed Carbon Content.....	29
3.5 Chemical composition of corncob.....	29
3.5.1 Determination of cellulose, hemicellulose, and lignin contents .....	29
3.6 Determination of hydrogen gas production .....	30
3.7 Experimental procedures .....	30
3.7.1 Sample Preparation.....	30
3. 7.2 Pretreatment of corn cobs .....	31
3. 7.2.1 Steam pretreatment .....	31
3. 7.2.1.1 Procedures in Steam Pretreatments .....	31
3. 7.3 Hydrolysis .....	32
3. 7.3.1 Procedures for Acid Hydrolysis .....	32
3.7.4 PH adjustment .....	33
3.7.4.1 Procedures in pH adjustment.....	33
3.7.5 Fermentation.....	34
3.7.5.1 Procedures in Media Preparation.....	34
3.7.6 Characterization by gas chromatography .....	36
3.8 Experimental Design .....	36
<b>CHAPTER FOUR.....</b>	<b>37</b>

<b>RESULTS AND DISCUSSION</b> .....	<b>37</b>
4.1 Characterization of corncob .....	37
4.1.1 Proximate analysis.....	37
4.1.2 Chemical composition analysis .....	38
4.2 Effect of temperature and pH on hydrogen yields.....	39
4.3 Development of regression model equation .....	42
4.4 Properties of H <sub>2</sub> produced from corn cobs .....	45
<b>CHAPTER FIVE</b> .....	<b>46</b>
<b>CONCLUSIONS AND RECOMMENDATIONS</b> .....	<b>46</b>
5.1 Conclusions .....	46
5.2 Recommendations .....	47
<b>REFERENCES</b> .....	<b>48</b>
<b>APPENDICES</b> .....	<b>60</b>

## LIST OF TABLES

Table 2. 1: production yield per hectare for top 10 producers worldwide.....	10
Table 2. 2: Maize production yield per hectare in 2014 in top ten African countries..	11
Table 2. 3: Percent maize area covered by organic fertilizers in selected regions of Ethiopia.....	12
Table 4. 1: The results of proximate analysis of the corn cob sample.....	37
Table 4. 2: The results of chemical composition of corn cob sample.....	38
Table 4. 3: The results of hydrogen yield at different pH and temperature.....	39
Table 4. 4: Design summary .....	40
Table 4. 5: Fit summary of quadratic model for corn cobs.....	40
Table 4. 6: Model summary statistics .....	41
Table 4. 7: Analysis of variance (ANOVA) for the quadratic model.....	41
Table 4. 8: Actual vs predicted .....	42

## LIST OF FIGURES

Figure 2. 1 Methods for pretreatment of cellulosic feedstock .....	13
Figure 2. 2 Anaerobic digestion pathway in DF for biohydrogen gas production .....	19
Figure 2. 3 Methods used for hydrogen production.....	19
Figure 3. 1 a) corn cob sample; b) prepared sample.....	31
Figure 3. 2 a) Autoclave ; b) soluble solution.....	32
Figure 3. 3 Mixture of soluble and activated carbon treated sample .....	33
Figure 3. 4 pH adjusted.....	34
Figure 3. 5 cultured media .....	35
Figure 3. 6 Incubator.....	35
Figure 4. 1 Nnormal plot of residuals.....	43
Figure 4. 2 response surface plots of effect of temperature and pH on the yield of H <sub>2</sub> gas at constant HRT .....	44
Figure 4. 3 counter plots of the effect of temperature and pH on the yield of H <sub>2</sub> gas at constant HRT .....	44
Figure 4.4 characterization of H <sub>2</sub> gas using gas chromatography.....	45

## ACRONYMS

AFEX	ammonia fiber explosion
ATP	Adenosine tray phosphate
BHP	Biochemical hydrogen potential
CCD	Central composite design
CD	Conductivity detector
CEL	Cellulose
DF	Dark fermentation
DOE	Department of Energy
EIA	Environmental impact assessment
FC	Fixed carbon content
GC	Gas chromatography
GF	Glass filter
GHG	Greenhouse gas
HCEL	Hemicellulose
HMF	Hydroxymethaylfurfural
HPLC	High performance liquid chromatography
HPR	Hydrogen production rate
HRT	Hydraulic Retention Time
JIT	Jimma institute of technology
JU	Jimma university
LHV	Lower heating value
MS	Moisture content
POME	Palm oil mill effluent
RSM	Response surface methodology
VFAs	Volatile Fatty Acids

# CHAPTER ONE

## INTRODUCTION

### 1.1 Background of the study

The year 1860 brought about global industrial revolution with the invention of the steam engine and energy sources in form of fossil fuels such as wood, coal, oil and natural gas in replacement of natural human energy sources (Veziroglu and Sahin, 2008). Energy revolves around all aspects of life and plays a huge role in our daily lives; hence sustainable energy supply and environmental friendly are two crucial issues for the sustainable development of global prosperity. The demand for energy is still increasing due to overall growth in the world population as well as the growing demand for improvement in standard of living especially in developing countries; this has made the development of an efficient and sustainable energy system an imperative for sustainable socioeconomic development (Barbir and Veziroglu, 1990).

The greatest energy challenge of the 21<sup>st</sup> century is to develop sustainable energy resources which will meet future demands. Currently, fossil fuels are the primary energy sources used to satisfy the global energy demand. Following the oil crisis in 1973, the need for developing alternative energy sources was initiated to reduce the dependency on fossil fuels and assist with mitigating environmental problems due to the usage of fossil fuels. Changes in global environmental conditions due to greenhouse gases (GHG), especially increasing CO<sub>2</sub> levels, can be linked to increasing use of fossil fuels (Bockris, 2002; Das and Veziroglu, 2001).

The energy crisis and environmental degradation are currently two vital issues for global sustainable development. It is now accepted that the dependence on fossil fuels (over 80% of energy consumption) contributes not only to climate change and global warming, but also to a rapid exhaustion of natural energy sources (Ni *et al.*, 2006).

The global reserves of fossil fuels are depleting due to their increasing consumption. In addition the, environment is facing severe pollution problems due to gaseous emissions (NO<sub>x</sub>, SO<sub>x</sub>, CO, CO<sub>2</sub>, etc.) and waste generated from the production and use of fossil fuels. The scientific community has widely accepted the fact that the increasing CO<sub>2</sub> level has impacted global warming phenomena, which is threatening the entire Earth's ecosystem (Ciais *et al.*, 2013). Therefore, a current need is to explore and invest in

alternative ways to harness the energy from the clean renewable sources that are carbon neutral and can reduce the global CO<sub>2</sub> emissions at the same time. In this context, hydrogen gas (H<sub>2</sub>) could represent a promising alternative energy carrier due to its social, economic, and environmental credentials (Kotay & Das, 2008). The net energy content of the H<sub>2</sub> per unit mass is higher than other conventional fuels. The lower heating value (LHV) of hydrogen varies between 2.4-2.8 KJ/g and is 4 times higher than that of methane, gasoline and coal respectively (Marbán & Valdés-Solís, 2007). Hydrogen is a promising alternative energy carrier and is also considered to be a clean energy. It only produces water when combusted with oxygen and has an energy content 2.75 times higher than hydrocarbon fuels (Benemann, 1996; Momirlan *et al.*, 2002).

Unlike fossil, fuels hydrogen does not cause any CO<sub>2</sub>, CO, SO<sub>x</sub> and NO<sub>x</sub> emissions producing water as its only by-product when it burns reducing greenhouse effects considerably. Hydrogen is considered to be a major energy carrier of the future and can directly be used in fuel cells for electricity generation (Gonzalez *et al.*, 2011).

Current energy policies address the use of renewable energy sources (i.e. wind, solar, hydraulic, geothermal, and biomass) in order to reduce greenhouse gas emissions, as well as to increase energy security. In this context, biomasses offer a huge potential for the production of biofuels, and their use could be beneficial to reduce the world's dependency on oils and reduce the global emissions of greenhouse gases (Naik *et al.*, 2010). Biofuels, referred to as liquid (bioethanol, vegetable oil, and biodiesel) or gaseous (biogas, bio syngas and biohydrogen) fuels, that are predominantly produced from biomass, can be categorized into three generations (1<sup>st</sup>, 2<sup>nd</sup>, and 3<sup>rd</sup> generations) according to the origin of the biomasses used (Dragone *et al.*, 2010).

Hydrogen (H<sub>2</sub>) has been identified as a potential source of energy because of its high energy content per unit mass (William, 2004). However, most of the H<sub>2</sub> produced is from natural gas and when the demand for H<sub>2</sub> increases, the costs of natural gas, and the processes involved in H<sub>2</sub> production from this source is expected to rise. Moreover, the use of natural gas for H<sub>2</sub> production leads to increasing GHG emissions and consequently, using biomass rather than natural gas to produce H<sub>2</sub> aids in attenuating this problem.

The most important criteria for raw material selection for biohydrogen production are its availability, carbohydrate content, fermentability, and cost (Kapdan and Kargi 2006). It is a fact that biomass is the most abundant biopolymer on Earth and an alternative resource to fossil fuels.

Hydrogen can be produced using biological, chemical, and physical processes. The biological hydrogen production process has gained more interest than chemical and physical processes because it is a sustainable process that consumes less energy. Biological hydrogen production can be divided into two types these are phototrophic process and a dark fermentation process. Dark fermentation has advantages over the phototrophic process in terms of its ability to continuously produce hydrogen from a variety of feed stocks without an external input of energy (Chen *et al.*, 2006).

## **1.2 Statement of the problem**

Energy is an important part to sustain human life; almost all of the energy needed is derived from the conversion of fossil energy sources, such as for power generation, industrial, and transportation equipment that use fossil fuels as a source of energy. Fossil fuels are non-renewable energy source and also have seriously negative impacts on the environment.

The global reserves of fossil fuels are depleting due to their increasing consumption. In addition, environment is facing severe pollution problems due to the emission of gaseous pollutants (NO<sub>x</sub>, SO<sub>x</sub>, CO, CO<sub>2</sub>, etc.) and waste generated from the production and use of fossil fuels. The scientific community has widely accepted the fact that the increasing atmospheric CO<sub>2</sub> levels have impacted a global warming phenomenon, which is threatening the entire Earth's ecosystem. Therefore; a current need is to search an alternative ways to harness energy from the clean renewable sources that are carbon neutral and can reduce the global CO<sub>2</sub> emissions at the same time.

Ethiopia imports its entire petroleum fuel required, and the demand for petroleum fuel is rising rapidly due to a growing economy and expanding infrastructure. In our country, there is huge potential of biomass that can be used as feedstock for bio hydrogen gas production. Ethiopia is one of the biggest African countries in producing corn. Corn cobs are agricultural residue of corn that can be used as source of heat energy in rural areas of the country. This residue has cellulose that can be used for H<sub>2</sub> production beside its use as fuel for direct combustion, in this context substitution of imported petroleum fuels by hydrogen gas produced from agricultural wastes has its own economic importance due to its high efficiency, low pollution and renewable properties.



### **1.3 Objectives of the study**

#### **1.3.1 General objective**

The general objective of this study is to produce hydrogen gas from corn cobs using dark fermentation processes.

#### **1.3.2 Specific objectives**

- ✓ To analyze physicochemical properties of corn cobs.
- ✓ To estimate the amount of hydrogen gas production from a unit weight of corn cobs.
- ✓ To determine the optimum pH and temperature for hydrogen gas production from corn cobs.
- ✓ To characterize the properties of H<sub>2</sub> produced from corn cobs.

### **1.4 Research questions/hypothesis**

- 1 What are the physicochemical properties of corn cobs?
- 2 How much hydrogen gas can be produced from a unit mass of corn cobs?
- 3 What is the optimum pH and temperature to produce H<sub>2</sub> from corn cobs?
- 4 What are the characteristics of H<sub>2</sub> produced from corn cobs?

### **1.5 Significance of the study**

All energy sources have an impact on the environment. Concerns about the greenhouse effect and global warming, air pollution, and energy security have led to increasing interest and more development in renewable energy sources such as bio-fuel, solar, wind, geothermal, and hydrogen. This study investigates energy generation from renewable resources rather than fossil fuels due to limited future availability of petroleum and increased environmental impacts. Hence hydrogen gas production from cheap and easily available agricultural waste is a new alternative to replace fossil fuels because hydrogen gas does not cause any CO<sub>2</sub>, CO, SO<sub>x</sub> and NO<sub>x</sub> emissions producing water as its only by-product when it burns reducing greenhouse effects.

### **1.6 Scope of the study**

This study was focused on the generation of H<sub>2</sub> from agricultural waste (corn cobs). In addition, the analysis of hydrogen gas production from corn cobs starting from pretreatment up to dark fermentation process was conducted at laboratory scale in department of Biology in JU.

### **1.7 Limitation of the study**

The limitation of this study was difficult to characterize hydrogen gas produced from corn cobs using a gas chromatography instrument because of the sensitivity of instruments, very expensive.

Lack of gas chromatography instruments and lack of skilled man powers to characterize the hydrogen gas produced using gas chromatography.

The gas chromatography instrument experts are not interesting to see how gas chromatography instrument works because of the expensive and sensitivity of instruments.

## CHAPTER TWO

### LITERATURE REVIEW

#### 2.1 Sources of Energy

The global energy requirement is mostly met by fossil fuels which are the primary energy source, and the sources include petroleum, coal, bitumen, natural gas and tar sand (Das and Veziroglu, 2001). The world is presently faced with the challenges of providing sufficient energy with limited fossil fuel resources, potential climate change induced by greenhouse gas emissions and insecurity by nuclear energy incompetence and the storage of radioactive materials (Barbir and Veziroglu, 1990).

Fossil fuel utilization can result to serious environmental and health problems as a result of carbon emission (Levin *et al.*, 2004). Fossil fuel combustion causes the release of greenhouse gases, soot, and ash, tar droplets, and other organic compounds into the atmosphere which can lead to air pollution; oil spills and leakages can also occur during extraction, transportation, and storage resulting into environmental pollution. Greenhouse gases emission has been reported to cause increase in the atmospheric temperature, a situation known as global warming and other environmental problems such as acid rain, ozone depletion, eutrophication, climate change and serious health implications (Smith *et al.*, 2009; Hook and Tang, 2013). The effect of global warming ranges from increase in sea level, climate change, drought, floods, strong winds, and wildfires (Barbir and Veziroglu, 1990).

Globally, it has been estimated that about three million deaths are recorded annually due to air pollution (WHO, 2008) and poor air quality from fossil fuel combustion (Pimentel *et al.*, 2007).

Renewable energy production has become a global priority as a result of limited fossil fuel resources, the alarming rate of environmental pollution and global warming. Renewable energy sources includes biomass, hydropower, wind, solar (thermal and photovoltaic), and marine (Ramage *et al.*, 1996). The distribution of the total renewable energy consumption in the world includes biomass – 46%, hydroelectric 45%, geothermal- 6%, wind-2%, and solar- 1% (Dermibas, 2008).

## 2.2 Hydrogen gas

Hydrogen is one of the abundant elements in the universe; it is an odorless, colourless, tasteless, and non-poisonous gas (Dermibas, 2008). Hydrogen can be generated from renewable energy sources such as biomass, hydropower, solar energy using photovoltaic for direct conversion, thermal energy and wind power (Miranda, 2004). Hydrogen has a great potential as an energy source with a low carbon emission and represents a cleaner and more sustainable energy system (Veziroglu, 1975). Hydrogen is storable and transferable with high heat energy per mass unit and its sources are globally distributed. Hydrogen possesses properties that make it an ideal fuel and compatible with energy technologies such as fuel cells, engines and combustion turbines (Carglar and Ozmen, 2000). Hydrogen is considered as one of the promising fuel of the future because of its high energy efficiency, low pollution and renewable properties (Hohlein *et al.*, 2000; Das and Veziroglu, 2001). Over the last two decades, hydrogen has gained global attention as an environmental friendly renewable energy source (Koroneos *et al.*, 2005).

Most developed countries around the world have recognized the importance of the hydrogen economy and many researches are currently focusing on its implementation as an alternative energy source to improve energy security, economic development and environmental protection (Turner, 2004). According to the European Commission, hydrogen economy will help to provide a clean, safe, and sustainable energy supply (European Commission, 2003). The United States Department of Energy (DOE) projected that energy from hydrogen will contribute approximately 6-10% of the total energy market by 2025 (DOE, 2004).

Hydrogen as energy source has numerous benefits as a universal energy carrier; it is non-toxic since water is the only product when converted to energy (Midilli *et al.*, 2005). Hydrogen can be produced from a wide range of energy sources although hydrogen is mostly generated from fossil fuels using electrochemical, thermochemical, photo catalytic and photo electrochemical processes (Momirlan and Veziroglu, 2002). Steam reforming which is presently a commercial process of producing hydrogen is cheap; the process can lead to carbon emission during the hydrocarbon conversion (Rifkin, 2002).

### 2.3 Biohydrogen

Biohydrogen is defined as hydrogen produced biologically (mostly by bacteria) from waste organic materials. It includes hydrogen produced from renewable resources such as water, organic wastes or biomass either biologically or photo-biologically by photosynthesis and dark fermentation process (Benemann, 1998). Biohydrogen has several benefits of low energy requirements and low cost of operation compared to photo-electrochemical and thermochemical processes (Dermibas, 2008). Biological hydrogen production has become more attractive due to its ability to utilize renewable energy resources and its production at ambient temperature and pressure (Sinha and Pandey, 2011). Biological hydrogen production technologies include a wide range of process to generate hydrogen. These are direct bio photolysis, indirect bio photolysis, photo fermentation and dark fermentation (Dermibas, 2008). All these approaches are based on the ability of microorganisms to use protons ( $H^+$ ) as an electron sink for two electron equivalents:



### 2.4 Feed stocks for biohydrogen production

Biomass is a general term which is called as organic material that is produced via photosynthesis by green plants including algae, trees, and crops (McKendry 2002). Utilization of biomass as feedstock for hydrogen production is not only cost-effective but also environmentally friendly option, because the processes are carbon neutral (have net zero  $CO_2$  emission) due to the fact that  $CO_2$  is fixed in the atmosphere by plants during photosynthesis. Besides, agricultural crops (sugar and oilseed crops) and their waste by-products, lignocellulosic products such as wood and wood waste, aquatic plants like algae and water weeds, industrial or municipal solid wastes, and animal wastes are accepted as biomass sources (Nath and Das 2003; Caputo *et al.* 2005; Ni *et al.* 2006). A wide variety of feedstock's and wastes that are rich in carbohydrate content have the potential to produce hydrogen using dark fermentation (Azbar and Levin, 2012).

#### 2.4.1 First generation feed stocks

First-generation biomasses are often edible agricultural crops which are grown for food and animal feeds (Sims *et al.* 2008; Lee and Lavoie 2013). A number of studies have been reported in the literature for biohydrogen production from first-generation biomasses, especially from starchy and sugar-rich biomasses due to easy fermentability attribute of these feed stocks by anaerobic organisms. Even though higher hydrogen yields are

obtained from first generation biomasses, the biggest obstacle when using these sources as feedstock is the utilization of arable land to produce energy crops instead of food production. This will lead both severe food shortages and overmuch usage of water and fertilizers (Dragone *et al.* 2010). For this reason, nowadays, biohydrogen production studies have been shifted from first-generation biomass to second-generation biomass.

#### **2.4.2 Second generation feed stocks**

Lignocellulosic biomasses including agricultural and forestry wastes and non-edible crop residues, as well as industrial and municipal organic wastes, wastes from food processing, and industrial effluents, constitute second-generation biomasses (Cheng *et al.* 2011; Singh *et al.* 2011). solid organic wastes such as carbohydrate-rich wastes (apples, carrots, Jerusalem artichoke roots, maize flour, oats, potatoes, and wheat flour), protein- rich wastes (soybean milk cake, chicken meat, cow manure with straw, fish residues, and meat waste from restaurants), agro- industrial wastes (including food waste from restaurants, rapeseed oil cakes, sunflower oil cakes, grape marc, fruit peels – orange peels and banana peels – and maize cob), agricultural residues (Jerusalem artichoke leaves and stalks, giant reed stalks and leaves, maize stalks, rice straw, and sorghum stalks) (Guo *et al.*,2014 ), palm oil mill effluent (POME) (AlShorgani *et al.*,2014 ), distillery wastewater (Sridevi *et al.*,2014 ), and waste papers (Ntaikou *et al.*,2009 ) are second generation biomasses that were used as substrate for biohydrogen production.

#### **2.4.3 Third generation feed stock**

Algae are third-generation biomasses and they have been in use as feedstock for biohydrogen production due to their rich carbohydrate content. They are unicellular or multicellular organisms which can be classified as prokaryotic, like cyanobacteria (blue-green algae), or eukaryotic such as green algae, red algae, and brown algae.

Algae can store carbon in the form of starch, cellulose, and lipids. The carbohydrate source in algae is mainly starch which is deposited in the cytoplasm and cellulose in the cell wall. The carbohydrate storage type can be different according to the types of algae. For instance, cyanobacteria have glycogen, green algae and red algae have starch, and brown algae have  $\beta$ -glucans as a storage carbohydrate (Mollers *et al.*, 2014). The carbohydrate content of algae can vary between 30.7 and 48.2 % (Batista *et al.*, 2014; Yun *et al.*, 2014; Liu and Wang 2014; Nayak *et al.*, 2014). Algae are common biomass in bioethanol and biodiesel production.

## 2.5 Choice of feed stocks for biohydrogen gas production

The disadvantage of using the 1<sup>st</sup> generation biofuels is that they require significant amount of fossil fuels and fertilizers for their own cultivation (IEA, 2008). On the other hand, the production of 1<sup>st</sup> generation biofuels needs the use of arable land and thus competes with food consumption, increasing price and lowering availability, thus raising the “food vs fuel” dilemma (Chen and Khanna, 2012). Lignocelluloses are preferred over other available biomass sources because of global availability and lower cost of these feed stocks. Appreciable levels of H<sub>2</sub> production have been obtained from lignocellulosic feedstock (Cheng *et al.*, 2011; Sparling *et al.*, 2006). The high water use, the large areas and the high initial costs needed for their cultivation remain the major disadvantages that limit their commercialization (Azapagic and Stichnothe, 2011).

### 2.5.1 Overview of maize production in the world

Among the top 10 producers of maize worldwide, there is a large variation in production yield between the different areas: while yield is approximately 10.7 tons/hectare/year in the USA, yield is only 2.75 and 3.30 tons/hectare/year in India and Mexico respectively (Table 2.1).

Table 2. 1: production yield per hectare for top 10 producers worldwide (FAO, 2016).

Country	Million tons	Production yield per hectare (tons/hectare/year)
USA	361	10.7
China	216	6
Brazil	60	5.2
Argentina	33	6.6
Ukraine	28	6.2
India	23	2.7
Mexico	23	3.3
Indonesia	19	4.9
South Africa	14.9	4.5
Romania	11.9	4.8

### 2.5.2 Overview of maize production in Africa

The situation is even more dramatic in Africa: while the yield was still reasonable in countries like Egypt (7.73 tons/hectare/year), South Africa (4.54 tons/hectare/ year) and

Ethiopia (3.42 tons/hectare/year) the majority of countries have yields of less than 2 tons/hectare/year (35 of the 51 African countries producing maize) and even less than 1 ton/ hectare/year (15/51 African countries, such as Zimbabwe, South Sudan and Gambia) in 2014 (Table 2.2) .

Table 2.2: Maize production yield per hectare in 2014 in top ten African countries (FAO, 2016)

Country	Million tons	Production yield per hectare (tons/hectare/year)
South Africa	14.9	4.5
Nigeria	10.8	1.8
Ethiopia	7.2	3.4
Tanzania	6.7	1.6
Egypt	5.8	7.7
Malawi	3.9	2.3
Kenya	3.5	1.7
Zambia	3.3	2.8
Uganda	2.8	2.5
Ghana	1.7	1.7

Corn cobs are desirable as a sustainable feedstock because they represent about 12 percent of corn stover remaining on the field, their removal has negligible impact on soil carbon and they have limited nutrient value to the soil (Roberts, 2009).

### 2.5.3 Overview of maize production in Ethiopia

Maize production expanded rapidly and transformed production systems in Africa as a popular and widely cultivated food crop since its introduction to the continent around 1500 A.D. (McCann, 2005). Maize arrived in Ethiopia slightly later, around the late 17th century (Huffnagel ,1961), and was mainly grown as a subsistence crop in the mid-altitudes (1500– 2000 m above sea level) in southern, south-central, and southwestern parts of the country. The production system in the 1960s and for the first quarter of 1970s was truly subsistence, the yields barely exceeding 1 metric ton (MT)/ha. The rate of growth for area declined following the great drought of 1974, and while there was expansion in the 1980s, the average annual yield was volatile and rarely exceeded 1.5



MT/ha. Maize production and its status in determining food security in the country received a major focus in the mid-1980s, particularly spurred by the 1984 devastating drought and the famine that followed. The wide adaptability of the crop and the potential to produce more calories and food per area of land cultivated than all major cereals grown in Ethiopia were important factors in considering maize as part of the national food security strategy, including its inclusion under the government-led intensive agricultural extension program(Huffnagel ,1961).

Table 2. 3: Percent maize area covered by organic fertilizers in selected regions of Ethiopia (2004-13).

Region	year									
	2004	2005	2006	2007	2008	2009	2010	2011	2012	2013
Tigray	74	-	65	59	66	60	56	55	46	48
Amhara	37	31	32	29	32	27	24	28	25	20
Oromia	24	19	22	23	25	17	21	23	19	19
BSG	26	24	28	26	27	25	24	20	20	18
SNNP	18	13	13	13	16	14	9	11	11	8

Source: CSA ([www.csa.gov.et](http://www.csa.gov.et))

#### 2.5.4 Corn cob as feed stocks for biohydrogen gas production

Agricultural residues and food waste are the most abundant, cheapest, and most readily available organic waste to be biologically transformed; they include straw, stover, peelings, cobs, stalks, bagasse, and other lignocellulosic residues (Mtui, 2008). Agro residues such as leaves, grass and crop stalks are abundant in the world with approximately annual global yield of 220 billion tons (Khamtib *et al.*, 2011). They are the cheapest and most readily available organic waste to be biologically transformed and to varying degrees can be converted biologically in anaerobic digestion processes to biohydrogen because of their biodegradability (Saratale *et al.*, 2008).

Lignocellulosic biomass, of which two thirds are carbohydrate polymers of cellulose and hemicellulose is the most abundant raw material (Ren *et al.*, 2009).

Leftover biomass from corn harvestings, such as corn cobs and corn stover, are currently waste materials but could offer an affordable lignocellulosic biomass source. In the past, corn cob and corn stover residues were left behind on corn fields to maintain soil quality (Jansen and Lübberstedt, 2012a). However, a report from U.S. Department of Agriculture (2013), indicates that soil quality would not decrease if the cobs/stovers are removed.

Corn cobs contain 32.3%-45.6% cellulose, 39.8% hemicelluloses-mostly pentosan, and 6.7%- 13.9% lignin (Zych, 2008). Cellulose is a linear polymer of cellobiose (glucose-glucose dimer) and upon hydrolysis yields free glucose molecules. Hemicellulose, on the other hand, consists mainly of xylose, arabinose, galactose, glucose, and mannose which are easily fermentable (Hamelinck *et al.*, 2005).

## 2.6 Hydrogen gas production steps.

### 2. 6.1 Pretreatment

The goal of the pretreatment is to improve the hydrolysis yield and hydrogen production. Pretreatment methods can be divided into different categories: physical (milling and grinding), physicochemical (steam explosion, hydrothermolysis, and wet oxidation, etc.), chemical (alkali, dilute acid, and oxidizing agents), biological, or a combination of them (sun *et al.*, 2002, Yang *et al.*, 2008, Kumar *et al.*, 2009). Among these methods, physicochemical and chemical pretreatments are frequently applied for enhancing hydrogen fermentation (Fan *et al.*, 2006, Pan *et al.*, 2010, Cao *et al.*, 2009, Ren *et al.*, 2010).

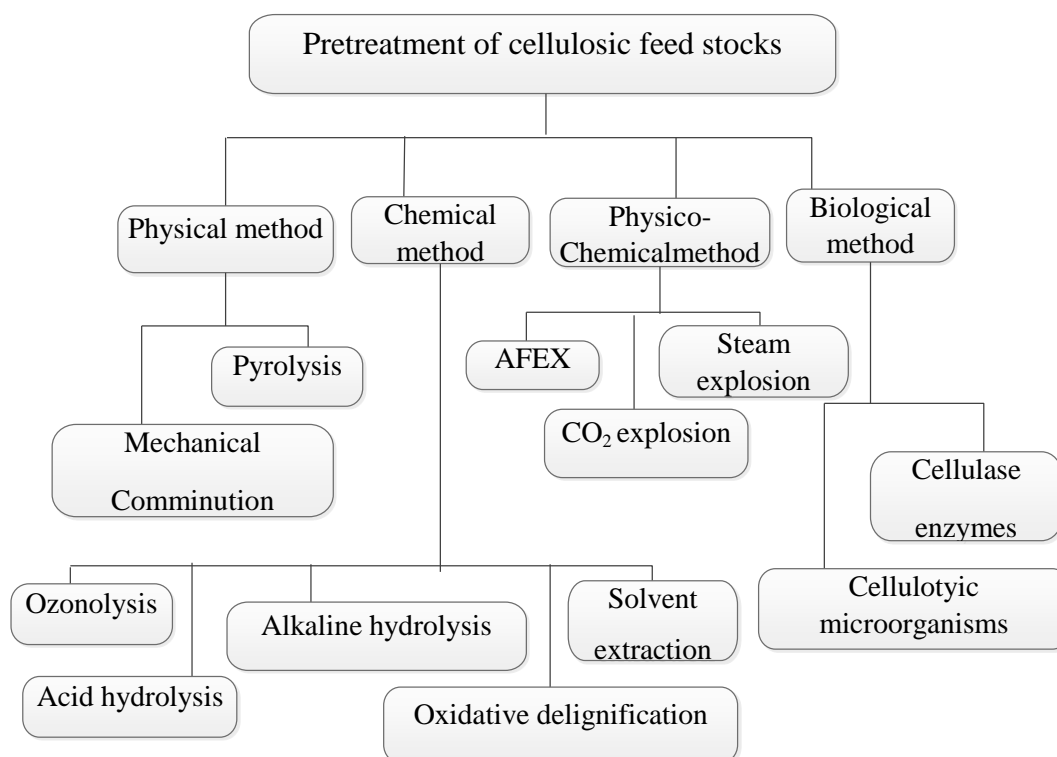


Figure 2. 1 Methods for pretreatment of cellulosic feedstock (Ganesh *et al.*, 2008).

## **2. 6.1.1 Physical Pretreatments**

### **2.6.1.1.1 Mechanical comminution**

Mechanical methods such as grinding, milling, and chipping convert the biomass into a fine powder, which increase the surface area of cellulose facilitating its consumption (Monlau *et al.*, 2013a). However, this process is not cost effective as it requires too much energy especially for lignocellulosic wastes with high moisture contents (Yu *et al.*, 2006).

### **2.6.1.1.2 Pyrolysis and co-pyrolysis**

Raw (organic) material is heated and degasified in vacuum at a pressure of 0.1–0.5 MPa to a temperature of 500–900 °C (Ni *et al.*, 2006). The process takes place in the absence of oxygen as well as air, and therefore the formation of dioxins can be almost ruled out.

The reaction can be generally described by the following equation (2) (Ni *et al.*, 2006).



## **2. 6.1.2 Chemical Pretreatments**

### **2. 6.1.2.1 Acid hydrolysis**

Acid pretreatment includes both weak and strong acid treatments. The weak acid treatment involves treatment with a dilute acids such as phosphoric, acetic, hydrochloric, and sulfuric acids at temperature > 150 °C. The temperature selection depends on both the acid concentration (0.5-3.0% v/v) and retention times (Baboukani *et al.*, 2012).

### **2. 6.1.2.2 Alkaline hydrolysis**

The major effect of alkaline pretreatment is the removal of lignin from biomass by improving the reactivity of the remaining polysaccharides, and decrystallisation of cellulose. In addition, alkali pretreatments remove acetyl and the various uronic acid substitutions on hemicellulose that lower the accessibility of the enzyme to the hemicellulose and cellulose surface (Chang *et al.*, 2000). Depending on the severity it also removes substantial amounts of hemicellulose. It is reported that the alkaline hydrolysis mechanism is based on saponification of intermolecular ester bonds crosslinking xylan hemicelluloses and other components such as lignin (Sun *et al.*, 2002).

### **2. 6.1.2.3 Ozonolysis**

Ozonolysis pretreatment by ozone is very effective in degrading lignin in a short period of time. It can be performed in high (e.g. 35 percent) or low (e.g. 1 percent) substrate consistency. Since ozone reacts with lignin in the gaseous phase, it was reported that ozone pretreatment conducted with high substrate consistency was more effective than with low consistency (Neely, 1984). Neither high temperatures nor pressures are involved

in the Ozonolysis pretreatment process and inhibitory residues are not generated. Thus, the process is exceedingly well suited for scaling-up or scaling-down with design simplicity. However, the main drawback of ozonolysis is the large amount of ozone required that makes this process economically undesirable (Alvira *et al.*, 2010).

#### **2. 6.1.2.4 Organosolvation**

Organosolvation is a pretreatment process utilizing organic or aqueous solvent mixtures to solubilize lignin and possibly a part of hemicellulose that results in easier access to cellulose for enzymatic hydrolysis (Pan *et al.*, 2006). A number of solvents can be used including methanol, ethanol, acetone, ethylene glycol, and tetra hydro furfuryl alcohol. Acid catalysts (e.g., HCl, H<sub>2</sub>SO<sub>4</sub>, oxalic or salicylic) have been studied in order to lower the reaction temperature and obtain high yields of xylose. One advantage of organosolv pretreatment is that this process produces two relatively pure byproducts, high -quality reactive lignin and an aqueous hemicellulose stream. However, the organosolv pretreatment process is relatively more expensive than steam explosion and the commercial feasibility highly depends upon complete recovery of the organic solvents (Duff & Murray, 1996).

#### **2. 6.1.2.5 Oxidative delignification**

Lignin biodegradation could be catalyzed by the peroxidase enzyme with the presence of H<sub>2</sub>O<sub>2</sub> (Azzam, 1989). About 50% of the lignin and most of the hemicellulose were solubilized by 2% H<sub>2</sub>O<sub>2</sub> at 30 °C within 8 h, and 95% efficiency of glucose production from cellulose was achieved in the subsequent saccharification by cellulase at 45 °C for 24 h(Azzam, 1989).

#### **2. 6.1.3 Physicochemical Pretreatments**

##### **2. 6.1.3.1 Ammonia fiber explosion (AFEX)**

In the AFEX process, biomass is treated with liquid ammonia at high temperature and pressure. After a few seconds, pressure is swiftly reduced. A typical AFEX process is carried out with 1-2 kg ammonia/kg dry biomass at 90 °C during 30 min. It reduces the lignin content and removes some hemicellulose while decrystallising cellulose. The cost of ammonia and especially of ammonia recovery drives the cost of the pretreatment (Holtzapple *et al.*, 1991, Holtzapple *et al.*, 1994), although ammonia is easily recovered due to its volatility, but like SO<sub>2</sub> shipping of NH<sub>3</sub> will be costly due to safety reasons. In a recent conceptual design for AFEX treatment of lignocellulose, the pretreatment is carried

out in a series of packed beds that are equipped with steam stripping of ammonia, which allows for recovery of 90% of ammonia (Campbell *et al.*, 2012).

### **2. 6.1.3.2 Steam explosion**

The steam-explosion process is carried out at high temperatures (ranging from 190 to 234 °C) and pressures (up to 3.3 MPa) with short residence times (less than 10-15 min), after which the vessel is depressurized and cooled (Boussaid *et al.*, 1999; Taherzadeh and Karimi, 2008). During steam treatment, the biomass is exploded, which disrupts the hemicellulosic components and solubilizes approximately 80-100% of the hemicellulose in the biomass (Grethlein and Converse, 1991).

### **2. 6.1.3. 3 CO<sub>2</sub> explosion**

This method is similar to steam and ammonia fiber explosion; high pressure CO<sub>2</sub> is injected into the batch reactor and then liberated by an explosive decompression. It is believed that CO<sub>2</sub> reacts to carbonic acid (H<sub>2</sub>CO<sub>3</sub>, carbon dioxide in water), thereby improving the hydrolysis rate. Yields of CO<sub>2</sub> explosion are in general lower than those obtained with steam or ammonia explosion (Sun *et al.*, 2002).

### **2. 6.1.4 Biological Pretreatments**

Biological pretreatment employs wood degrading microorganisms, including white, brown, and soft rot fungi, and bacteria to modify the chemical composition and/or structure of the lignocellulosic biomass so that the modified biomass is more amenable to enzyme digestion. Most biological pretreatment so far has focused on the degradation of lignin in lignocellulosic biomass. However, degradation of lignin usually accompanies the loss of cellulose and hemicellulose. In order to reduce and eliminate the sugar loss during biological pretreatment, the microbial strains should have low cellulase activity. White rot fungi are the most widely studied for biological pretreatment since they can degrade lignin more effectively and more specifically. Biological pretreatment appears to be a promising technique and has very clear advantages, including no chemical requirement, low energy input, mild environmental conditions, and an environmentally friendly working manner. However, biological pretreatment is very slow (taking from weeks to a year) and requires careful control of growth conditions and a large amount of space to carry out. In addition, most lignolytic microorganisms solubilize or consume not only lignin but also hemicellulose and cellulose (Mosier *et al.*, 2005).

### **2. 6. 2 Choice of steam-explosion over other pretreatment methods**

Steam explosion is the most commonly used method for the pretreatment of lignocellulosic materials (McMillan, 1994). In this method, biomass is treated with high-pressure saturated steam, and then the pressure is suddenly reduced, which makes the materials undergo an explosive decompression. Steam explosion is typically initiated at a temperature of 160-260 °C (corresponding pressure, 0.69-4.83 MPa) for several seconds to a few minutes before the material is exposed to atmospheric pressure (Sun and Cheng , 2002)

The process causes hemicellulose degradation and lignin transformation due to high temperature, thus increasing the potential of cellulose hydrolysis.

Hemicellulose is thought to be hydrolyzed by acetic and other acids released during steam-explosion pretreatment. Grous et al. reported that 90% efficiency of enzymatic hydrolysis was achieved in 24 h for poplar chips pretreated by steam explosion, compared to only 15% hydrolysis of untreated chips (Grous *et al.*, 1986).

### **2.6.3 Anaerobic digestion pathway for hydrogen gas production**

Dark fermentation is the conversion of organic substrates to bio-H<sub>2</sub> through a series of biochemical reactions by anaerobic bacteria in the absence of light .In comparison to other bio-H<sub>2</sub> production methods, dark fermentation is a promising technology (Levin et al., 2004). Dark fermentation is an intermediate step in the anaerobic digestion process and involves multiple series of oxidation and reduction reactions (Pavlostathis and Giraldo, 1991). Anaerobic digestion involves 4 major steps and the first two phases are very vital for biohydrogen production: hydrolysis; acidogenesis; acetogenesis; and Methanogenesis.

#### **2. 6.3.1 Hydrolysis**

Hydrolysis is the first step in which complex organic polymers are broken down into simpler compounds. This process is catalyzed by an array of extracellular hydrolytic enzymes such as cellulase, amylase, and protease. The end products of this phase are used up by the bacteria for metabolism (Gavrilescu, 2002).

#### **2. 6 .3.2 Acidogenesis**

Acidogenesis the second phase in which the soluble monomers are used as source of carbon and energy by the fermentative hydrogen producing bacteria to produce volatile fatty acids, alcohols and biogas (Reith *et al.*, 2003).

### 2. 6.3.3 Acetogenesis

Acetogenesis phase is characterized by the reduction of end products of Acidogenesis such as aromatic compounds, volatile fatty acids (VFAs) and alcohols which are converted to acetic acid and hydrogen. Acetate, butyrate, and propionate are the major intermediate products in this phase. The HAc produced by homoacetogenesis includes two types: one type grows autotrophically using H<sub>2</sub> and CO/CO<sub>2</sub> and the other heterotrophically by producing HAc from organic compounds (Ryan et al., 2008). Thus, acetogenesis and acidogenesis are the two steps in anaerobic digestion during which H<sub>2</sub> is produced. Acetic acid as end product of the fermentation can result to a theoretical production of 4 mol of hydrogen per mole of hexose which is equivalent to 498 ml H<sub>2</sub> per gram of hexose (0°C, 1atm) for acetic fermentation; while in butyrate pathway a lower molar hydrogen yield of 2 mol of hydrogen per mole of hexose is observed which is equivalent to 249 ml H<sub>2</sub> per gram of hexose (0°C, 1atm) (Hawkes *et al.*, 2007). However, the accumulation of acetate during the fermentation does not imply higher biohydrogen production since many microbial species can convert hydrogen and carbon dioxide to acetate (Equ.3) (Antonopoulos *et al.*, 2008).



### 2. 6.3.4 Methanogenesis

Methanogenesis is the final stage of anaerobic digestion where methane is the end product. Methane is primarily produced from H<sub>2</sub> and CO<sub>2</sub> (hydrogenotrophic methanogens) or from HAC (acetoclastic methanogens). The methane-producing bacteria belong to the Archaea class of microorganisms that are sensitive to oxygen and live in a syntrophic relationship with acetogens. Methanogens are affected by pH, high levels of VFAs produced during acidogenesis and acetogenesis, and the amount of H<sub>2</sub> produced (Zeikus, 1977).

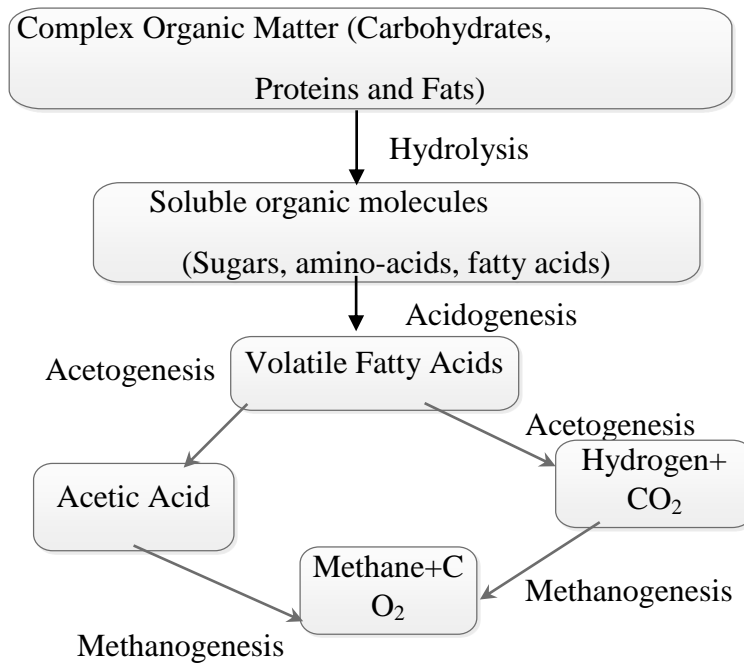


Figure 2. 2 Anaerobic digestion pathway in DF for biohydrogen gas production

### 2.7 Hydrogen production methods

Hydrogen produced from natural gas accounts for approximately 80% of the total hydrogen production while production from fossil fuels accounts for over 90% of the commercial hydrogen production (Rosen and Scott, 1998). These industrial processes are energy intensive as they operate at very high temperatures and pressures. Other methods to produce hydrogen include the following (Hallenbeck and Benemann, 2002).

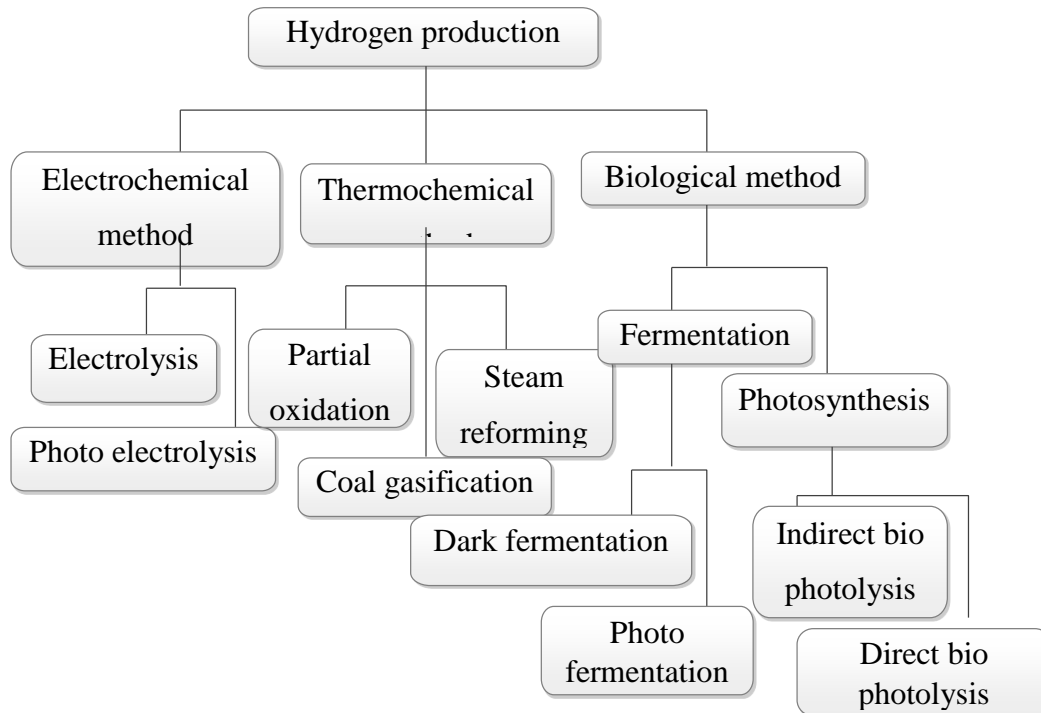


Figure 2. 3 Methods used for hydrogen production (Ganesh *et al.*, 2008).



## 2. 7.1 Electrochemical methods

### 2. 7.1.1 Electrolysis

In the case of water electrolysis, the reaction is mediated in a strong ionic solution in order to split water into hydrogen and oxygen gases. Electrolysis is a process in which a direct current passing through two electrodes in a water solution results in the cracking of the chemical bond of water into hydrogen and oxygen in (Equ.4).



The hydrogen action  $\text{H}^+$  reacts at the cathode, resulting in the creation of hydrogen, which is collected and later stored.  $\text{H}^+$  the negative electrode (anode), oxygen is created. This method produces extremely pure  $\text{H}_2$  a large amount of electric city is used.

### 2. 7.1.2 Photo electrolysis

Hydrogen production, exhibiting promising efficiency and costs, although it is still in the phase of experimental development (Holladay *et al.*, 2009; Turner *et al.*, 2009; Turner *et al.*, 2007; Huang, 2009). Currently, it is the least expensive and most effective method of hydrogen production from renewable resources.

Many current methods for producing large amounts of hydrogen for industrial uses utilize fossil fuels as their source of energy. The following processes are used to produce hydrogen from fossil fuels include (Rosen and Scott, 1998).

## 2. 7.2 Thermochemical methods

### 2. 7.2.1 Partial oxidation

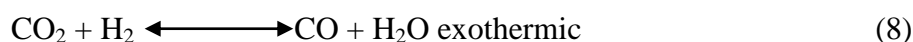
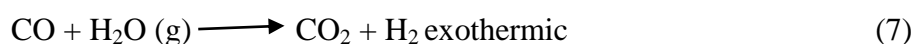
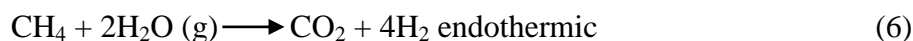
Partial oxidation of hydrocarbons involves the heating of the hydrocarbon in a low oxygen environment to create a hydrogen rich gas.

### 2. 7.2.2 Steam reforming of natural gas

Steam reforming is currently one of the most wide spread and at the same time least expensive processes of hydrogen production, through which more than 90 % of the hydrogen used is produced (Palmová and Schöngut, 2004). Its advantage comes from the high efficiency of its operation and low operational and production costs. The most frequently used raw materials are natural gas and lighter hydrocarbons. The process requires an external source of heat, which is added into the process by the direct combustion of part of the natural gas. The process has two stages. In the first stage, hydrocarbon raw material is fed into steam (500–900 °C, 0.3–2.5 MPa) in a tube reactor filled with a catalyst on the basis of nickel oxide (or Ni + MgO, Pt, Rh) (Wang *et al.*, 2004; Song *et al.*, 2007). The catalytic process requires a desulfurized initial raw material.

During its reaction, syngas ( $H_2 + CO$ ) is produced along with a lower proportion of  $CO_2$  (reactions 5, 6).

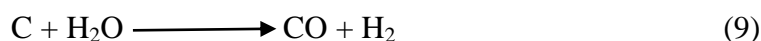
In the second stage, the cooled gas is led into the converters, where carbon monoxide is converted by means of steam into carbon dioxide (5).



The nascent carbonic gas is removed by a reversible exothermic reaction (8) usually implemented in two stages. In the first, so-called high temperature stage, the temperature of the products is raised to almost  $500^\circ C$ , which has the result of lowering the balanced yield of  $CO_2$  and  $H_2$ . The products are then cooled to approximately  $360^\circ C$  and are led to the low-temperature converter filled with a highly active copper catalyst (the second stage), where the concentration of  $CO$  is lowered to 0.2– 0.3 vol. % at low temperatures of  $180\text{--}230^\circ C$  (Holladay *et al.*, 2009).

### 2. 7.2.3 Coal gasification

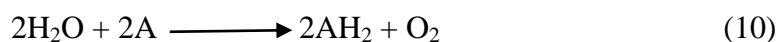
Is the heating and pressurizing of coal and water to create hydrogen and carbon monoxide according to the equation (equation 8).



## 2.7.3 Biological methods

### 2. 7.3.1 Direct bio photolysis

The action of light on a biological system that results in the dissociation of a substrate, usually water, to produce hydrogen is referred to as bio photolysis.

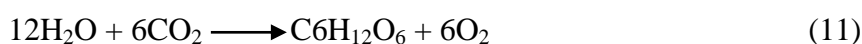


Where, A is an electron acceptor. For the purpose of employing these photosynthetic electrons for the reduction of protons to hydrogen by the action of a bacterial hydrogenase, the acceptor must have an oxidation-reduction potential near the potential of the hydrogen electrode and in its reduced state serve as a substrate for the hydrogenase. In this reaction oxygen produced by the photosynthesis strongly inhibits the hydrogen production (Benemann *et al.*, 1973)

### 2. 7.3.2 Bio-photolysis-Indirect process

The most credible processes for future applied research and development are those which couple separate stages of microalgal photosynthesis and fermentations (indirect bio

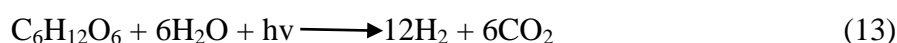
photolysis'). These involve fixation of CO<sub>2</sub> into storage carbohydrates (e.g. starch in green algae, glycogen in cyanobacteria) followed by their conversion to H<sub>2</sub> by the reversible hydrogenase, both in dark and possibly light-driven anaerobic metabolic processes (Beneman, 1997). In indirect bio photolysis, the problem of sensitivity of the H<sub>2</sub> evolving process to O<sub>2</sub> is usually circumvented by separating O<sub>2</sub> and H<sub>2</sub> (Beneman, 2000). In a typical indirect bio photolysis hydrogen is produced as follows:



### 2. 7.3.3 Photo fermentation

This is another light-dependent method in which 90 % of product gas is hydrogen and the process releases no hydrogen sulphide or carbon monoxide. Here, photo heterotrophs (eg, purple bacteria) convert organic acids in the presence of sunlight into H<sub>2</sub>, CO<sub>2</sub> and carbon compounds. The main enzymes utilized by these bacteria are the nitrogenases that require nitrogen-scarce conditions for hydrogen production. Disadvantages of this process include the use of costly bio-reactors, dependence on ATP-consuming nitrogenases and lack of efficiency of light-harvesting antennae (Mathews and Wang, 2009).

These photo-heterotrophic bacteria have been found suitable to convert light energy into H<sub>2</sub> using organic wastes as substrate in batch processes, continuous cultures (Shin *et al.*, 2004) or immobilized whole cell system using different solid matrices like agar gel and polyurethane foam. The overall reaction of hydrogen production is as follows:



### 2. 7.3.4 Dark fermentation

Dark fermentation is the main light-independent process for biohydrogen production. In this method, anaerobic bacteria consume sugars to produce H<sub>2</sub>, CO<sub>2</sub>, and organic acids. It is considered the most favorable process since hydrogen is produced at a higher rate and at low cost (Show *et al.*, 2012). The process can be carried out in simple reactors, requires no light energy and can be used on a wide range of substrates at non-aseptic conditions (Hallenbeck *et al.*, 2012; Wang and Wan, 2008; Valdez-Vazquez *et al.*, 2005).

Dark fermentative H<sub>2</sub> production is preferred for bio-fuel production because of its high HPR (Levin *et al.*, 2004). The maximum possible H<sub>2</sub> yield per mole of glucose is 4 mol corresponding to only 33% of the substrate conversion. However, in practice, attaining this theoretical maximum yield is not possible.



## 2.8 Choice of hydrogen production methods

Hydrogen can either be used in internal combustion engines or in fuel cells for energy generation (Brar and Sarma, 2013). Currently, most of the hydrogen produced is generated using steam reforming of oil and gas (Armor, 1999) or coal gasification (Stiegel and Ramezan, 2006) which are fossil-fuel reliant, unsustainable, incur high production costs, and cause environmental pollution. Hydrogen can also be produced from renewable sources (especially wastes). While utilizing these wastes to produce valuable products (such as energy recovery), issues associated with waste treatment and land pollution caused by disposal to landfills, can be simultaneously resolved (Duff and Murray, 1996; Panagiotopoulos *et al.*, 2009; Saratale *et al.*, 2008). Thus, biological hydrogen (biohydrogen) production from waste is fast-gaining significant global attention. For these reasons, biohydrogen has been described as the key energy carrier for the future (Kapdan and Kargi, 2006).

## 2.9 Microorganisms for hydrogen production

The use of microorganisms is gaining widespread attention as a cost-efficient way to produce hydrogen (Kotay and Das, 2008). Both pure and mixed cultures have been studied for biohydrogen production. *Clostridium butyricum*, *C. acetobutyricum*, *C. Saccharoperbutylacetonicum* and *C.pasteurianum* have been investigated as pure cultures with high efficiency for hydrogen production (Hawkes *et al.*, 2002; Pattra *et al.*, 2008). Mixed cultures from natural environments such as soil, animal waste, and anaerobic sludge have also been used to produce energy. They are easier to use, simpler to operate and can act on a wide range of substrates in contrast to using pure cultures (Li and Chen, 2007).

## 2.10 Factors affecting DF pathway and H<sub>2</sub> yields

DF via mixed cultures is a complex system where environmental factors and bioreactor operation conditions such as temperature, pH and H<sub>2</sub> partial pressure regulate metabolic pathways of hydrogen producing microorganisms (Guo *et al.*, 2010; Li and Fang, 2007a; Liu *et al.*, 2006; Wang and Wan, 2009).

### 2. 10.1 pH and temperature

The operational pH and temperature are the most crucial parameters that determine the optimum metabolic pathways of hydrogen synthesis as well as the inhibition of the

hydrogen consuming processes which may occur simultaneously (Hu *et al.*, 2005; Khanal *et al.*, 2003). An acidic operational pH (below 6) mainly inhibits the methanogenic activity under both mesophilic and thermophilic conditions, but the inhibition of hydrogen consuming homoacetogenic activity can only be achieved under thermophilic conditions at the initial pH of 5.5 (Luo *et al.*, 2011). Thus, the control of the process pH and temperature plays an important role in achieving high biohydrogen conversion rates by minimizing the activity of the hydrogen consumers.

Luo *et al.* (2011) found acetate as a major metabolic product when the operational pH was 7, while butyrate dominated at an initial pH 5.5 in the BHP tests carried under mesophilic (37 °C) conditions using an acid pre-treated inoculum. Luo *et al.* (2011) further reported the inhibition of homoacetogenesis can be achieved at pH 5.5 and thermophilic temperatures (55 °C). In a recent study of the DF of cheese whey from mozzarella production at different pH ranges (5.5-7.7) and a temperature of 39 °C, De Gioannis *et al.* (2014) reported pH 6 as the optimal pH and acetate levels were higher in all the tests except at pH 6.5 where butyrate and propionate levels exceeded those of acetate.

The optimum pH for organic food waste varies from 4.5 to 7, for lignocellulosic waste it varies from 6.5-7, whereas a neutral pH is optimal for animal manure (Guo *et al.*, 2010).

## **2. 10.2 Hydraulic retention time (HRT)**

The HRT can affect substrate hydrolysis and thus the production of intermediates and products, thus affecting fermentative H<sub>2</sub> production. Besides hydrolysis, the HRT can also be used as control parameter of the methanogenic activity. Some studies have demonstrated the effect of the HRT on the biohydrogen production in DF processes (dos Reis and Silva, 2011; Kim *et al.*, 2006; Liu *et al.*, 2008; Pakarinen *et al.*, 2011). The different growth rates of hydrogen producers and consumers make it possible to use the HRT as a controlling parameter to inhibit the activity of H<sub>2</sub> consumers in the DF. It has been reported that low HRTs favor hydrogen production as the methanogens are washed out, and hydrogen production increases as the HRT decreases (Kim *et al.*, 2006; Liu *et al.*, 2008; Oh *et al.*, 2004; Pakarinen *et al.*, 2011).

## **2. 10.3 Hydrogen and carbon dioxide partial pressure**

### **2. 10.3.1 Hydrogen partial pressure**

The hydrogen concentration in the liquid phase, related to hydrogen partial pressure, is one of the key factors affecting the hydrogen production (Hawkes *et al.*, 2002).

The partial pressure of H<sub>2</sub> is an extremely important factor especially for continuous H<sub>2</sub> synthesis (Hawkes *et al.*, 2007).

### **2. 10.3.2 Carbon dioxide partial pressure**

In case of carbon dioxide, high CO<sub>2</sub> concentration can favor the production of fumarate or succinate, which contributes to consume electrons, and therefore decrease hydrogen production (Tanisho *et al.*, 1998). It has been reported that the removal of CO<sub>2</sub> can improve the hydrogen production in dark fermentation (Tanisho *et al.*, 1998). After CO<sub>2</sub> was removed, the hydrogen production was doubled. Furthermore, when removing the CO<sub>2</sub> from the liquid with sparging of argon gas and hydrogen gas, they also found, compared to hydrogen partial pressure, the CO<sub>2</sub> partial pressure had higher inhibition effect to the dark fermentation process.

### **2. 10.4 Organic acid concentration**

It has been reported that high concentration of the organic acids result in a collapse of the pH gradient across the membrane and cause the total inhibition of all metabolic functions in the cell (Jones and Woods 1986). It has been claimed that both the total acetate or butyrate acid concentration and the undissociated form of these acids can inhibit the dark hydrogen fermentation process (Jones and Woods 1986; Van Ginkel and Logan 2005; van Niel *et al.*, 2003).

### **2. 10.5 Inorganic elements**

Recent research indicates elements such as iron and nitrogen, and compounds such as carbonate and phosphate can affect the hydrogen production in dark fermentation Process.

#### **2. 10.5.1 Iron concentration**

Iron is very important as an electron carrier and may affect the metabolic pathway of the hydrogen producing bacteria particularly in the oxidation of pyruvate to acetyl-CoA, carbohydrate and hydrogen (Dabrock *et al.*, 1992; Lee *et al.*, 2001).

#### **2. 10.5.2 C/N ratio**

The carbon/nitrogen (C/N) ratio is also important for dark fermentation process stability (Tanisho *et al.*, 1998). It has been reported that proper C/N ratio can increase the hydrogen production in mesophilic hydrogen fermentation from sewage sludge (Lin and Lay 2004).

### **2. 10.6 Other factors**

Other factors influencing H<sub>2</sub> fermentation include byproducts formed during fermentation. End-product inhibition occurs at high partial pressure of H<sub>2</sub> levels and high

VFAs concentrations leading to the development of a pH gradient across microbial membranes causes inhibition of many populations (Hegarty and Gerd, 1999).

### **2.11 Uses of hydrogen gas**

The combustion of hydrogen only produces water vapor, which is a non-greenhouse gas. It does not cause environmental and atmosphere pollution (Armor 2005) and also the combustion of hydrogen in automobiles is 50% more efficient than gasoline. Hydrogen battery is deemed as future supply for automobiles (Reith *et al.*, 2003a). Hydrogen gas has a high energy yield of 122 kJ/g, and this yield is 2.75-fold greater than that from hydrocarbon fuels on mass level (Ramachandran and Menon 1998) and the conversion efficiency of hydrogen to electricity could be doubled using fuel cell instead of gas turbine (Reith *et al.*, 2003a). In addition to this hydrogen can be easily stored as a metal hydride (Dong *et al.*, 2007).

# CHAPTER THREE

## MATERIALS AND METHODS

### 3.1 Description of the study area

The study was carried out in the Oromia regional state, Jimma zone, located at about 346 km south west of Addis Abeba. This zone lies between latitude 7°15'N and 8°45'S and longitudes 36°00'E and 37°40'E. The elevation of the zone ranges from 880 to 3360 m.a.s.l. The area experiences an annual average rain fall of 100 mm for 8 to 10 months. The main rain season extends from May to September and the small rain season takes place from February to April. The temperature of the area varies between 8 to 28°C with an annual average of 20°C. It has sub-humid, warm to hot climate the sample was collected in Asendabo, which is one of the major corn production area of Jimma zone.

### 3.2 Materials and equipments used

Corn cobs, were collected from Asendabo agricultural land in Jimma zone, Plastic bags were used to collect and transport samples to the laboratory. Pestle used to reduce the size of the sample, 2mm size mesh sieve was used to separate sized corn cobs, digital Balances (model-Sartorius with 0.01 mg sensitivity, and model EP214C) was used to weigh samples and media, crucible used to put the sample in oven and furnace. Incubator , Vertical Autoclave , pH- Meter , Ovens- Loading model 100 -800 were used to ferment ,used to heat the sample with high pressure steam to promote hemicellulose hydrolysis ,measure the pH of hydrolyzates before fermentation and to dry the sample respectively. Centrifuge used to separate the soluble liquid from non-soluble part, conical flasks were used to hold sample and additives for pretreatment, hydrolysis, and fermentation, gas chromatography (Varian CP4900) was used to characterize H<sub>2</sub> gas.

### 3.3 Chemicals

Dilute Sulfuric Acid (H<sub>2</sub>SO<sub>4</sub>) used for hydrolysis and pH adjustment , Sodium Hydroxide (NaOH) used to adjust the pH of soluble cellulose and hemicellulose before fermentation,

Glucose,peptone,yeastextract,MgSO<sub>4</sub>.7H<sub>2</sub>O,K<sub>2</sub>HPO<sub>4</sub>,KH<sub>2</sub>PO<sub>4</sub>,urea(NH<sub>2</sub>.CO.NH<sub>2</sub>),

HCl.H<sub>2</sub>O, anaerobic digested sludge were used for media preparation for fermentation and activated carbon was used for the removal of hydrogen gas production inhibitors.



### 3.4 Characterization of corncob

Experiments were conducted to determine the moisture content, fixed carbon content, ash content, and volatile matter content of air-dried corn cob samples ground to particle size below 2.0 mm.

#### 3.4 .1 Proximate analysis

The proximate analysis gives moisture content (MC), volatile matter content, the fixed carbon content, the ash content (the inorganic residue remaining after combustion of the sample).

#### 3.4 .2 Moisture Content

Samples were weighed in clean preheated moisture crucible of known weight by using sensitive balance. The sample and crucible were kept in an oven 105°C for an hour. The crucible was covered and transferred to desiccators, and weighed after reaching room temperature. The crucible was heated in the oven for another two hours and was re-weighed.

This was repeated until constant weight was obtained. The loss of weight was calculated as percent of weight and expressed as moisture content.

$$\text{Moisture content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (15)$$

Where:  $W_1$  = Initial weight

$W_2$  = weight after drying

#### 3.4 .3 Volatile Matter Content

A crucible was weighed empty, and then samples were put in it. The sample and the crucible were placed in furnace for 30 min at 600 °C. The crucible was removed from furnace and placed in a desiccators to cool, then was reweighed. The process was repeated until constant weight was obtained.

$$\text{Volatile content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (16)$$

Where:

$W_1$  = Original weight of the sample

$W_2$  = Weight of sample after cooling

#### 3.4.4 Ash Content

A crucible was weighed empty, and then samples were put in it. The sample and the crucible were placed in a furnace for 2 hours at 550°C. The crucible was removed from furnace and placed in a desiccators to cool, then was reweighed.

$$\text{Ash content (\%)} = \frac{W_1 - W_2}{W_1} \times 100 \quad (17)$$

Where:

$W_1$  = Original weight of the sample

$W_2$  = Weight of sample after cooling

#### 3.4.5 Fixed Carbon Content

This is the residue left after the moisture, volatile and ash is given up. It is deduced by subtracting from 100, the percentage of moisture, volatile matter, and ash content. The fixed carbon content (FC) is given as

$$\text{FC} = 100 - (\% \text{ moisture} + \% \text{ volatile matter} + \% \text{ ash}) \quad (18)$$

### 3.5 Chemical composition of corncob

#### 3.5.1 Determination of cellulose, hemicellulose, and lignin contents

Cellulose (CEL), hemicelluloses (H-CEL) and lignin were measured using a strong acid hydrolysis method adapted from Effland *et al.* (1977). Samples (200 mg) were first hydrolyzed with 12 M H<sub>2</sub>SO<sub>4</sub> acid for 2 hr at room temperature, then diluted to reach a final acid concentration of 1.5 M and kept at 100°C for 3 hr. The insoluble residue was separated from the supernatant by filtration on fibre glass filter (GF/F, WHATMAN). This insoluble residue was washed with 50 mL of deionized water and then placed in a crucible. The crucible and the paper fibre glass were dried at 100°C during 24 h to determine by weighting the amount of lignin. After centrifugation of the sample in 2 mL Eppendorf® tubes, followed by filtration at 0.2 μm (Nylon membrane), 800 μL of supernatant were transferred to a vial prior to the analysis by high-pressure liquid chromatography (HPLC). Structural carbohydrates (i.e. glucose, xylose, arabinose, glucuronic and galacturonic acids) were measured by High Performance Liquid Chromatography (HPLC) coupled to refractometric detection (Waters R410).

Cellulose and hemicelluloses contents were estimated as follows (equation 19 and 20):

$$\text{Cellulose (\% VS)} = \frac{\text{Glucose (\% VS)}}{1.11} \quad (19)$$

$$\text{Hemicelluloses (\% VS)} = \left[ \frac{\text{Xylose (\% VS)} + \text{Arabinose (\% VS)}}{1.13} \right] \quad (20)$$

$$\text{Lignin (\% VS)} = 100 - [\text{cellulose (\% VS)} + \text{hemicelluloses (\% VS)}] \quad (21)$$

Where:

1.11 is the ratio of the molecular weights of glucose to glucan (180/162) and 1.13 is the ratio of the molecular weights of xylose and arabinose to xylan (150/132).

### 3.6 Determination of hydrogen gas production

The hydrogen yield (mol H<sub>2</sub>/mol glucose) was calculated using Equ. (22). The substrate degradation rate (%) was calculated by dividing the amount of glucose consumed after hydrogen production process by the amount of initial glucose added in the system:

$$\text{Hydrogen yield} \left( \frac{\text{Mole of hydrogen}}{\text{Mole of hexoses}} \right) = \frac{\text{cumulative hydrogen production (mol)}}{\text{amount of glucose consumed (mol)}} \quad (22)$$

### 3.7 Experimental procedures

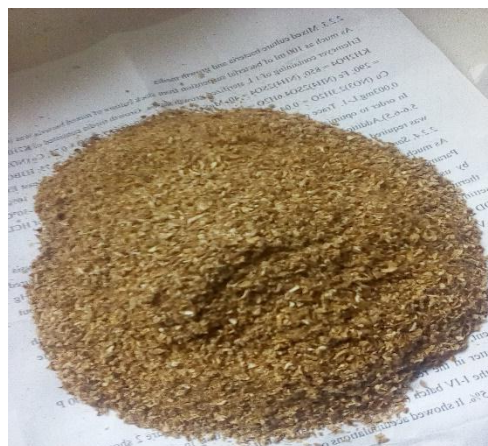
The followings basic steps were used for the production of hydrogen gas. These steps were: Sample collection, a pre- treatment phase to make corn cobs easier to hydrolysis process, hydrolysis to break down the molecules of cellulose and hemicelluloses into simple sugar, fermentation of the resulting (sugar) solution and finally optimize temperature and pH of H<sub>2</sub> gas produced in RSM software.

#### 3.7.1 Sample Preparation

5 kg of corn cobs were collected in plastic bags from Asendabo agricultural lands, Jimma zone and take to the laboratory. Then the sample was dried in oven to remove the moisture content of corn cobs <10% at 100°C and 4 hours Before using in subsequent experiments, the dried corn cobs were crushed to fine size. A pestle was used to reduce the corn cobs into small sizes of particle. These particles were then sieved to 2mm in diameter by mesh sieve.



a)



b)

Figure 3. 1 a) corn cob sample; b) prepared sample

### **3. 7.2 Pretreatment of corn cobs**

#### **3. 7.2.1 Steam pretreatment**

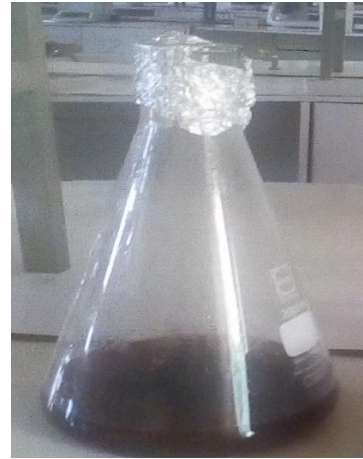
Steam explosion is the most commonly used method for pretreatment of lignocellulosic materials. The purpose of pretreatment is to remove lignin, reduce cellulose crystallinity, and increases the porosity of the materials (Prasad, 2003). Pretreatment must meet the following requirements: improve the formation of sugar, avoid the degradation or loss of carbohydrate, avoid the formation of by-product inhibitors, and must be cost effective.

##### **3. 7.2.1.1 Procedures in Steam Pretreatments**

100g of corn cobs powder (sample) was soaked with 1000 mL distilled water in 2000 mL conical flasks by capped with the help of aluminum foil for 12 hours periods. After that the sample was rapidly heated at 121°C by 3.5 MPa pressure steam without addition of any chemicals in autoclave for 15 mints to promote hemicellulose hydrolysis. Then the sample in autoclave was allowed to cool and the soluble portion was separated from the non-soluble portion .After that the soluble solution was placed in another conical flask and the non-soluble portion was hydrolyzed in the next steps.



a)



b)

Figure 3. 2 a) Autoclave; b) soluble solution

### 3. 7.3 Hydrolysis

The cellulose molecules are composed of long chains of glucose molecules. In the hydrolysis process, these chains are broken down, before it is fermented for hydrogen gas production.

#### 3. 7.3.1 Procedures for Acid Hydrolysis

2% (v/v) diluted sulfuric acid was added to the non-soluble component obtained from pretreatment steps in the order of experimental design. After hydrolysis, the solid part was separated from the liquid in the hydrolyzate by vacuum filtration (to remove the non-fermentable lignin portion) and put in another flask.

The filtered hydrolysate was treated with activated carbon to remove furans (furfural and 5- hydroxymethyl furfural (HMF)), phenols, and acetic acid. After that removal of fermentation inhibitors were accomplished by adding 10 g of activated carbon with a 1 L of hydrolyzed liquor with a contact time of 2 to 3 h and the activated carbon treated liquor was filtered through a 0.45  $\mu\text{m}$  filter under vacuum conditions and the solution was stored at 4 °C. Then the soluble component mixed with the previously filter solution from the pretreatment Step for the next procedure.

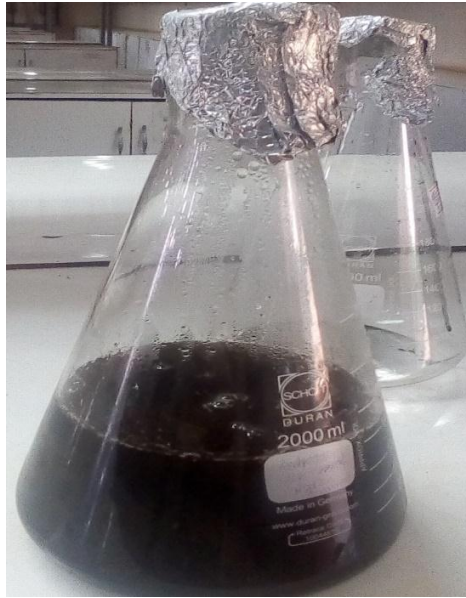


Figure 3. 3 Mixture of soluble and activated carbon treated sample

### **3.7.4 PH adjustment**

Before addition of any micro-organism to the above prepared samples, pH of these samples has to be adjusted. Otherwise the micro-organism will die in hyper acidic or basic state. A pH of around 5.5 -7 is maintained.

#### **3.7.4.1 Procedures in pH adjustment**

First the pH meter was calibrated by using buffer solution and the hydrolyzate solution is acidic, so it needs highly basic solution to bring the pH in the range of 5.5-7. Then Sodium hydroxide solution was added drop wise to the other flask with constant stirring until the pH reaches to a range of 5.5-7. If suppose the pH goes beyond 5.5-7, concentrated sulfuric or hydrochloric acid was added drop wise to maintain the pH in the range.



Figure 3. 4 pH adjusted for the sample

### 3.7.5 Fermentation

Anaerobic digested sludge was collected from water treatment plant in Boye area around Jimma town .This was preheated at 70 °C for 30 mints prior to use in order to eliminate methanogens and to select spore forming acidogenic bacteria. The preheated treated anaerobic digested sludge was cultivated to produce 200 ml media containing glucose (12g), peptone (2g), yeast extract (0.06g), Urea (0.1g),  $MgSO_4 \cdot 7H_2O$  (0.05g),  $K_2HPO_4$  (0.2g),  $KH_2PO_4$  (0.2g) and  $HCl \cdot H_2O$  (0.02g ) and Distilled water ( 200 ml ).Argon gas was passed through the cultivation media before incubation to remove oxygen in the adjusted sample and the cultivation flasks were closed with gas-tight rubber stoppers.

#### 3.7.5.1 Procedures in Media Preparation

The mixture glucose (12g), peptone (2g), yeast extract (0.06g), Urea ( 0.1g),  $MgSO_4 \cdot 7H_2O$  (0.05g),  $K_2HPO_4$  (0.2g),  $KH_2PO_4$  (0.2g) and  $HCl \cdot H_2O$  (0.02g) was added to the 200 ml distilled water in a 250 ml conical flask. Then 2g of anaerobic digested sludge was add in solution prepared from the above chemicals and finally the conical flasks were covered properly with aluminum foil.





Figure 3. 5: cultured media

### **The Procedure for Fermentation**

The prepared sample and media were mixed in to 16 different 250 ml flasks. Each flask contains 100 ml prepared sample and 10 ml media with the ratio of 10 % (1% media with 10% sample). Then, it placed in incubator at a temperature of 25 °C, 35 °C, 55 °C, 70 °C and pH of 5.5, 6, 6.5 ,7, for 72 hrs.



Figure 3. 6: Incubating adjusted sample



### **3.7.6 Characterization by gas chromatography**

After 72 hrs the gas collected from the fermented sample was analyzed by gas chromatography (GC).

### **3.8 Experimental Design**

Design expert® 11 software experimental method was used to determine the effect of two operating variables in hydrogen gas production from corn cobs. These were, pH, temperature at constant HRT. The response variable was Hydrogen gas yield. Significance of the result was set from analysis of variance (ANOVA).

## CHAPTER FOUR

### RESULTS AND DISCUSSION

In this section the study discussed proximate analysis and chemical composition of the sample, the amounts of hydrogen gas produced per unit weight of the sample, effect of temperature, pH in hydrogen yield and finally the properties of hydrogen gas produced.

#### 4.1 Characterization of corncob

##### 4.1.1 Proximate analysis

The proximity analysis of corn cobs was as shown below in table 4.1

Table 4. 1: The results of proximate analysis of the corn cob sample

Physical composition	Weight percentage (%wt. dry basis)
Moisture	9.2
Volatile matter content	79.4
Ash	2.4
Fixed carbon content	9

According to Alabama, (2012) moisture content, volatile content, ash content, and fixed carbon content was 8.72, 80.72, 2.96, and 7.60 respectively. However in this study the results are not exactly in accordance with (Alabama, 2012) report. The difference in these value might be happened due to a number of reasons such as the sources of corn cobs, species varieties used and handling conditions. Moisture content is a measure of the amount of water present in the corn cob. As the moisture content increases in the sample of corn cob, affects the product quality, and needs more heat for vaporization of moisture. Moisture content beyond 20% would create difficulties to poor combustion conditions within the gasification system and will immediately inhibit the composition of the material at the same time increasing. However this value (9.2%) moisture content is desirable for gasification to take place.

The corn cob used in this study was characterized by relatively high volatile matter content (79.4%).The volatile matter contents in corn cobs are usually high due to the organic nature of corn cobs, which indicates the corn cobs potential to create huge amounts of inorganic vapors when used as feedstock in gasification process. The higher

the volatile content of the biomass, the better its combustion and gasification rate because of the biomass yield up on carbonization.

Biomass ash content greater than 6% is not desirable for gasification because of the formation of agglomeration, fouling, sintering, and slagging, which leads to process efficiency reduction. However in this study the ash content (2.4%) is less than 6% which indicates the corn cob is desirable for gasification.

Fixed carbon content of corn cob indicates the amounts of carbon present after volatile matters are driven off. In this study the fixed carbon content of corn cob was 9% which is enough to allude for the formation of chart during gasification. The volatile and fixed carbon contents of biomass are related to the yields and composition of solid, liquid, and gaseous product formed during gasification.

#### 4.1.2 Chemical composition analysis

The chemical composition of corn cobs was analyzed as shown below in table 4.2

Table 4. 2: The results of chemical composition of corn cob sample

Chemical composition	Weight percentage (w/w %)
Cellulose	44.8
Hemicellulose	38.9
Lignin	16.3

Zych, (2008) made chemical composition of corn cobs analysis range from 32.3%-45.6% cellulose, 39.8% hemicelluloses-mostly pentosan, and 6.7%-13.9% lignin. Monlau ,(2013a) also reported that the composition of different lignocellulosic compounds like wheat straw and bran, rice straw, barley straw, maize bran and stover, and poplar wood with cellulose, hemicellulose, and lignin compositions ranging between 32% - 45%, 18% -37%, and 3% - 26%, respectively. The results of the study agree with values reported by these scholars. In this study corn cob contains high contents of cellulose and low contents of lignin which make it more advantageous for hydrogen gas production. The lower the lignin content the easier hydrolysis condition, and decrease formation of toxic chemicals such as, aromatic, polyaromatic, phenolic and aldehydic that inhibit hydrogen gas production.

## 4.2 Effect of temperature and pH on hydrogen yields

In this study, the hydrogen gas production yields from corn cob powders as a substrate at different temperature and pH was investigated and there results are shown below in Table 4.3.

Table 4. 3: The results of hydrogen yield at different pH and temperature

Std	Run	Factor 1 A:temperature	Factor 2 B:pH	Response H <sub>2</sub>
1	12	55	7	2.12
2	16	70	7	3.31
3	11	55	6.5	1.56
4	9	55	5.5	0.87
5	14	70	6	2.73
6	6	35	6	0.67
7	8	35	7	1.23
8	15	70	6.5	2.92
9	4	25	7	0.86
10	2	25	6	0.43
11	3	25	6.5	0.71
12	1	25	5.5	0.23
13	7	35	6.5	0.81
14	5	35	5.5	0.51
15	13	70	5.5	2.41
16	10	55	6	1.34

According to Guo , (2010) the optimum pH for organic food waste varies from 4.5 to 7, for lignocellulosic waste it varies from 6.5-7, whereas a neutral pH is optimal for animal manure Valdez-vazquez et al.,(2005) reported that higher H<sub>2</sub> yields was obtained at thermophilic fermentation than in the mesophilic temperature range. Also acetic acid was a dominant by-product in thermophilic digestion, whereas butyrate was formed in a higher proportion during mesophilic digestion. In this study maximum 3.18 mole of H<sub>2</sub> /mole of hexose yield was observed at 69.979°C and 6.964 temperature and pH respectively, which agree with Guo, (2010), pH 6.5- 7 and Valdez-vazquez ,(2005) at

thermophilic fermentation than mesophilic temperature . Corn cobs powder used as a substrate for H<sub>2</sub> production in anaerobic digested sludge inoculum at different pH and temperature, but the amounts of H<sub>2</sub> yield was higher at pH of 6.5 and 7 in the current study at different temperature, which increases H<sub>2</sub> yield as temperature increases from (25°C, 35°C) and (55°C, 70°C) proportionally. H<sub>2</sub> yield increases with temperature proportionally which indicates the better hydrolysis of cellulosic feed stocks was observed as temperature increases.

The maximum hydrogen yield (3.18 mole of H<sub>2</sub>/mole of hexose) was obtained at a temperature of 69.979°C and pH 6.964.

The resulting data, (Table 4.3), were analyzed using Design expert® 11 software to determine the effects of temperature and pH in hydrogen yield. The dependent variable used as a response parameter was hydrogen yield and temperature and pH were the independent variables.

Table 4. 4: Design summary

Study type	Response surface
Initial point	Central composite design
Center point	8
Design model	Quadratic polynomial
Run	16
Blocks	No

Table 4. 5: Fit summary of quadratic model for corn cobs

Source	Sequential p-value	Lack of Fit p-value	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	
Linear	< 0.0001		0.9092	0.8812	
2FI	0.5171		0.9051	0.8539	
<b>Quadratic</b>	<b>0.0002</b>		<b>0.9796</b>	<b>0.9619</b>	<b>Suggested</b>
Cubic	0.0798		0.9900	0.9691	
Quartic	0.2237		0.9945	0.9172	Aliased

The “pre R- squared” of 0.9619 is as close to the “Adj R- square” of the 0.9796 in less than 0.2 difference as one might expect. The difference between Adj R-Squared and Pred R-Squared is 0.0177 (i.e. they are reasonably close to each other). This indicated a close

fit of the model to the actual response data. “Adeq precition” measures the signal to disturbance ratio due to random error.

Table 4. 6: Model summary statistics

Source	Std. Dev.	R <sup>2</sup>	Adjusted R <sup>2</sup>	Predicted R <sup>2</sup>	PRESS	
Linear	0.2947	0.9213	0.9092	0.8812	1.70	
2FI	0.3012	0.9241	0.9051	0.8539	2.10	
<b>Quadratic</b>	<b>0.1396</b>	<b>0.9864</b>	<b>0.9796</b>	<b>0.9619</b>	<b>0.5468</b>	<b>Suggested</b>
Cubic	0.0978	0.9960	0.9900	0.9691	0.4430	
Quartic	0.0726	0.9989	0.9945	0.9172	1.19	Aliased

The predicted R-squared of 0.9619 is nearly close to the adjusted R-squared of 0.9796 as one might normally expect. This may indicate a small block effect or a possible problem with the model and/or data.

To determine whether a quadratic model is significant or not, it was necessary to preform analysis of variance (ANOVA).

Table 4. 7: Analysis of variance (ANOVA) for the quadratic model

Source	Sum of Squares	df	Mean Square	F-value	p-value	Remark
<b>Model</b>	14.15	5	2.83	145.37	< 0.0001	Significant
A-tem	11.62	1	11.62	596.46	< 0.0001	
B-pH	1.63	1	1.63	83.85	< 0.0001	
AB	0.0404	1	0.0404	2.08	0.1803	
A <sup>2</sup>	0.8855	1	0.8855	45.47	< 0.0001	
B <sup>2</sup>	0.0086	1	0.0086	0.4394	0.5224	
<b>Residual</b>	0.1947	10	0.0195			
<b>Cor Total</b>	14.35	15				

F-value is a test for comparing model variance with residual (error) variance. If the variances are close to the same, the ratio will be close to one and it is less likely that any of the factors have a significant effect on the response. It is calculated by Model Mean Square divided by Residual Mean Square. Here the Model F-value of 145.37 implies the model is significant. There is only a 0.01% chance that a "Model F-Value" this large

could occur due to personal error or disturbance. Probability values and/ or "Prob > F" values less than 0.0500 indicate model terms are significant. In this case A (temperature) and B (pH) are significant model terms. And there is no interaction between the two factors. Values greater than 0.1000 indicate the model terms are not significant.

### 4.3 Development of regression model equation

The application of RSM gives an empirical relationship between the response function and the independent variables. The mathematical relationships between the response, temperature (A) and pH (B) in terms of coded factors can be determined by Design Expert software. The model equation that correlates the response ( $H_2$ ) to the hydrolysis process variables in terms of coded factor for corn cob is shown by equation (4.1)

$$H_2 = 1.10 + 1.10 A + 0.4297 B + 0.0869 AB + 0.5864 A^2 + 0.0520 B^2 \quad (23)$$

From the equation (22) Hydrogen yield per unit weights of corn cob 28.274 ml of  $H_2$  /g of corn cobs or 0.227 mole of  $H_2$  /mole of corn cobs powder was obtained.

Table 4. 8: Actual vs predicted

Run Order	Actual Value	Predicted Value
1	0.2300	0.2941
2	0.4300	0.4764
3	0.7100	0.7049
4	0.8600	0.9797
5	0.5100	0.3382
6	0.6700	0.5462
7	0.8100	0.8005
8	1.23	1.10
9	0.8700	1.12
10	1.34	1.38
11	1.56	1.69
12	2.12	2.04
13	2.41	2.32
14	2.73	2.62
15	2.92	2.96
16	3.31	3.35

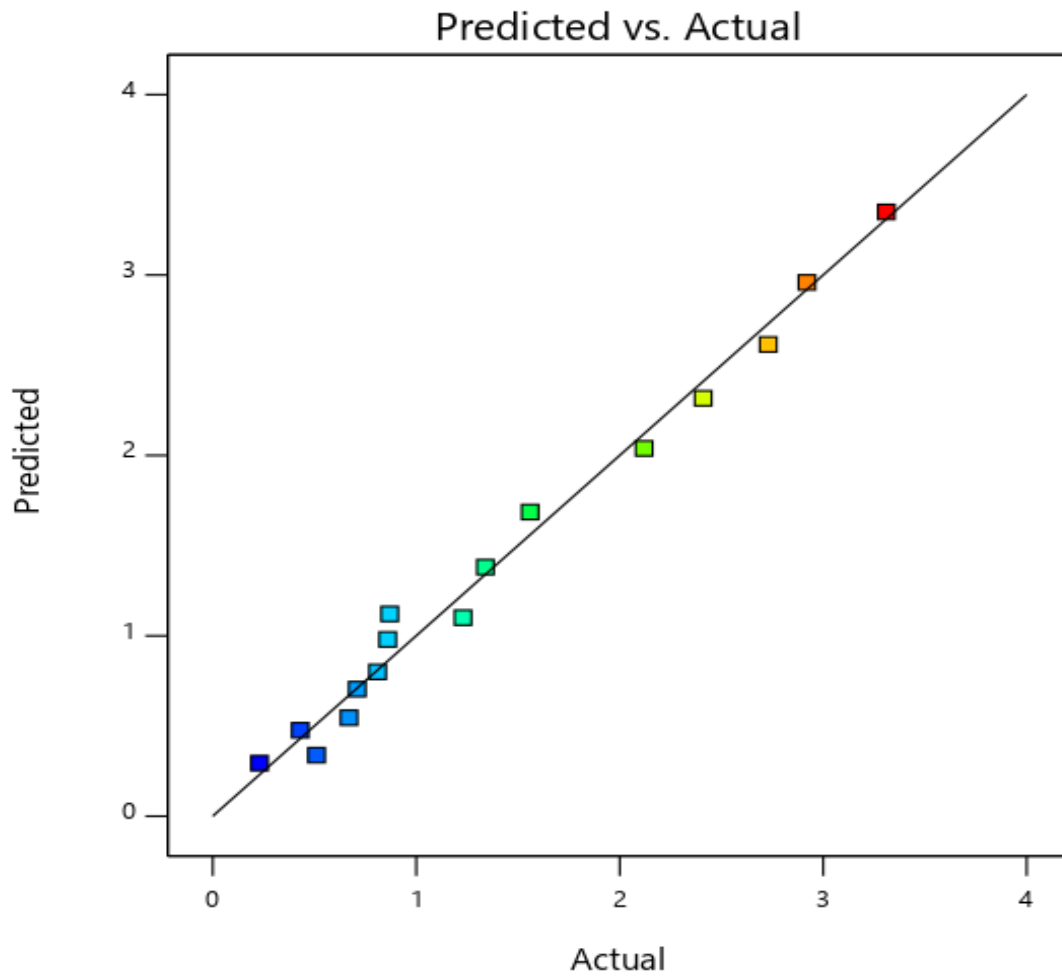


Figure 4. 1 Nnormal plot of residuals

From the plot as shown above, the normal probability plot indicates the residuals following by the normal % probability distribution, in the case of this experimental data the points in the plots shows fitted to the straight line in the figure, this shows that the quadratic polynomial model satisfies the assumptions analysis of variance (ANOVA) i.e. the error distribution is approximately normal.



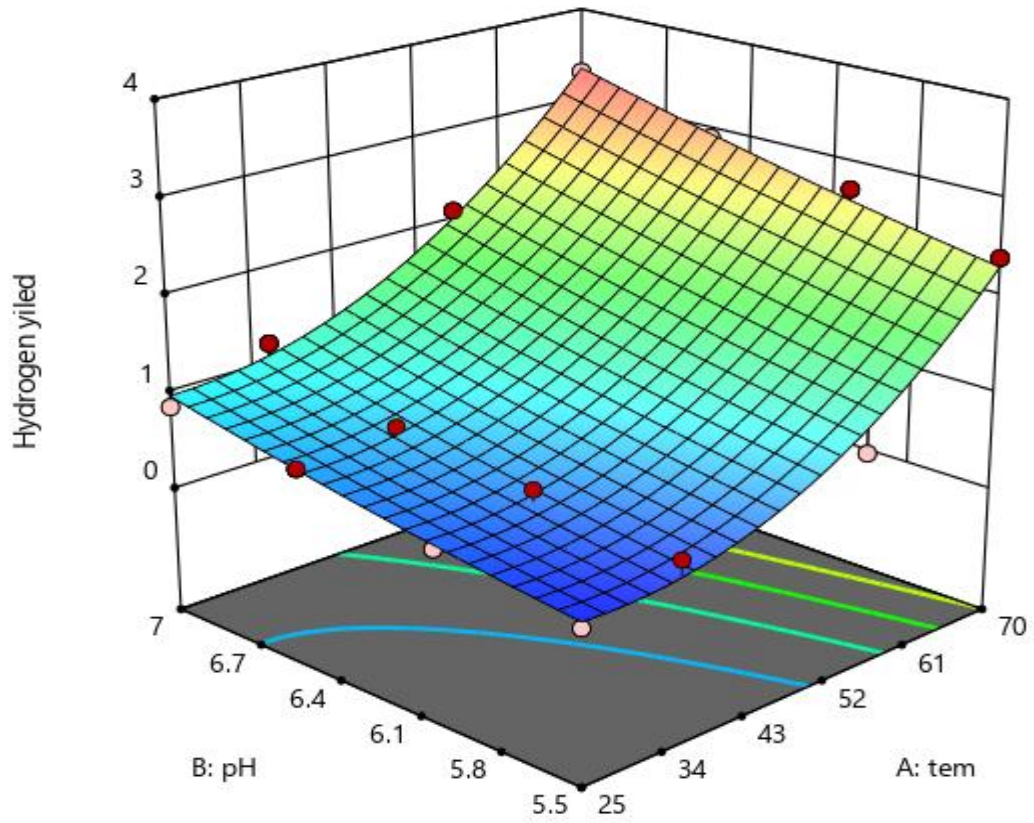


Figure 4. 2 response surface plots of effect of temperature and pH on the yield of H<sub>2</sub> gas at constant HRT

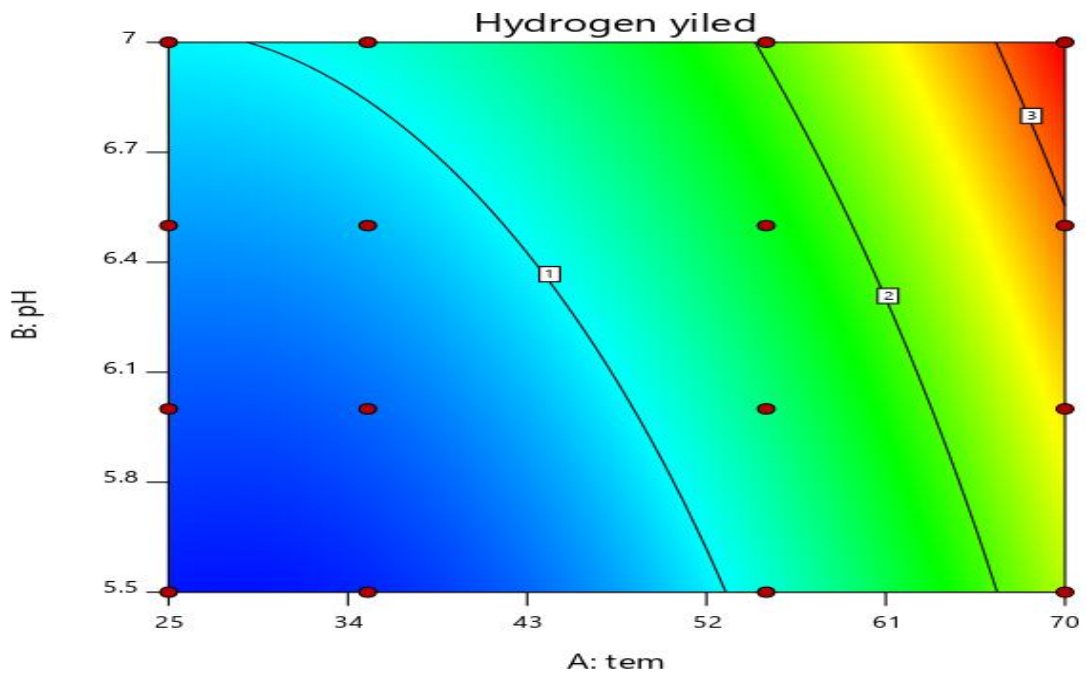


Figure 4. 3 counter plots of the effect of temperature and pH on the yield of H<sub>2</sub> gas at constant HRT

Three-dimensional surface and 2D contour plots were obtained by plotting the response (yield of H<sub>2</sub> gas) on the Z axis against any two variables while keeping the other variable at zero level. These plots are created to analyze the change in the response surface. The response optimized value for the production of H<sub>2</sub> gas was based on the two process variables described on the response surface plot. The effect of the independent variables and their mutual interaction on the yield of H<sub>2</sub> gas can be seen in Figures (4.2, 4.3) above.

#### 4.4 Properties of H<sub>2</sub> produced from corn cobs

Hydrogen gas produced from corn cobs as a substrate after fermentation was odorless, colorless, very light, and flammable. It has an atomic number of 1 and an atomic weight of 1.00794. The density of H<sub>2</sub> gas 0.00523 lb/ft<sup>3</sup>, has a specific gravity of 0.0696 and is thus approximately 7% the density of air. From gas chromatography characterization results H<sub>2</sub>, CH<sub>3</sub>COOH and CO<sub>2</sub> as shown below in figure 4.4. This assures that the product obtained from corn cob was hydrogen gas due to the confirmation as shown in the figure below.

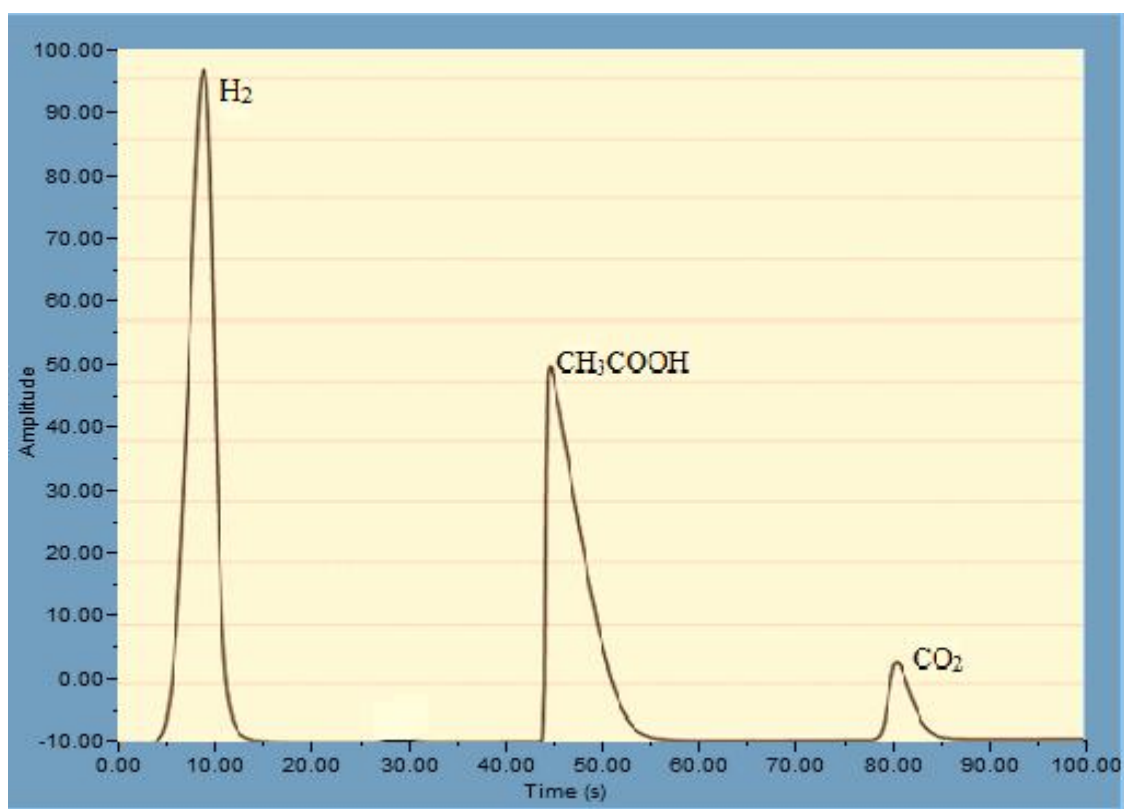


Figure 4.4 Characterization of H<sub>2</sub> gas by gas chromatography

## CHAPTER FIVE

### CONCLUSIONS AND RECOMMENDATIONS

#### 5.1 Conclusions

Corn cob is promising lignocellulosic feed stocks for H<sub>2</sub> gas production. It is the most abundant by-product generated from corn. Biohydrogen production from such lignocellulosic material was carried out in three main stages such as pretreatment, hydrolysis, and fermentation. The conversion of corncob to H<sub>2</sub> was carried out with steam explosion pretreatment, dilute acid hydrolysis and fermentation steps. In this study the proximate analysis (moisture content, volatile matter content, ash content and fixed carbon content), chemical composition of corn cobs, effect of temperature and pH in the yield of H<sub>2</sub> gas and finally the properties of H<sub>2</sub> gas produced from corn cobs was investigated and optimized using response surface methodology. Corn cobs characterized by moisture content (9.2%), volatile matter content (79.4%), ash content (2.4%) and fixed carbon content (9%). The chemical composition of corn cobs in this study contains cellulose (44.8%), hemicellulose (38.9%) and lignin (16.3%). Based on analysis of variance (ANOVA) the temperature and pH has significant effect on the yield of H<sub>2</sub> gas. As the result of RSM optimization at temperature of 69.979°C and pH 6.964 high yield 3.31 mole of H<sub>2</sub>/mole of hexose was obtained. All points were located near to the central point of the design. Based on this study, it is evident that the chosen method of optimization was efficient, and reliable.

## 5.2 Recommendations

Based on the current investigation the following recommendations are forwarded:

- Further researches have to be carried out to increase the yield of H<sub>2</sub> gas from corn cobs by use other types of inoculum which are capable of converting 5- and 6-carbon sugar into H<sub>2</sub> gas.
- Optimization of temperature and pH variables was carried out in this study. But further optimization of the pretreatment, hydrolysis, and fermentation are recommended to maximize the yield of H<sub>2</sub> gas from corn cobs.
- In this study the same corn cob species used to produce hydrogen gas. But further researches have to be carried out to determine the effects of Variety of corn cob species used for the yield of H<sub>2</sub> gas.
- In this study hydrogen gas production from corn cobs was conducted at the same substrate concentration. But further researches have to be carried out at different substrate concentration to determine the effects of substrate concentration on hydrogen gas yields.

## REFERENCES

- Alabama, Auburn, (2012). Bio-oil Production through Fast Pyrolysis and Upgrading to “Green” Transportation Fuels.
- Al-Shorgani NKN, Tibin EM, Ali E et al, (2014). Biohydrogen production from agroindustrial wastes via *Clostridium saccharoperbutylacetonicum* N1-4 (ATCC 13564). *Clean Technol Environ*, 16:11–21
- Alvira, P., Tomas-Pejo, E., Ballesteros, M., Negro, M, (2010). Pretreatment technologies for an efficient bioethanol production process based on enzymatic hydrolysis. a review. *Bioresource technology*,101(13): 4851-4861.
- Antonopoulou, G., Hariklia, N., Skiadas, V., Angeloulous, K. and Gerasimos, L, (2008).
- Armor JN,(2005). Catalysis and the hydrogen economy. *Catalysis Letters* , 101(3-4):131-135.
- Armor, J. N,(1999). The multiple roles for catalysis in the production of H<sub>2</sub>. *Appl Catal A-Gen*,176: 159 – 176.
- Azapagic ,A. and Stichnothe, H(2011). Lifecycle sustainability assessment of biofuel .Clark (Eds.). Cambridge UK .R. Luque, J. Campelo, J, Handbook of biofuels production–processes and technologies, Wood head Publishing.
- Azbar, N., Levin, D.B, (2012). State of the art and progress in production of biohydrogen (e-book). Bentham Science Publishers. DOI: 10.2174/97816080522401120101
- Azzam, M,(1989). Pretreatment of cane bagasse with alkaline hydrogen peroxide for enzymatic hydrolysis of cellulose and ethanol fermentation. *J. EnViron. Sci. Health B*, 24 (4):421–433.
- Baboukani, B.S., Vossoughi, M., Alemzadeh, I,(2012). Optimization of dilute-acid pretreatment Conditions for enhancement sugar recovery and enzymatic hydrolysis of wheat straw. *Biosyst Eng*, 111(2): 166-174.
- Brar, S. K., Sarma, S. J,(2013). Biohydrogen production- A promising strategy for organic waste management. *Hydrol, Curr Res.*; S5: e002
- Barbir, F, Veziroglu, T.N. and Plass, H.J,(1990). Environmental damage due to fossil fuels use. *Int J Hydrogen Energy* ,10:739.
- Benemann, J,(1996). Hydrogen biotechnology: Progress and prospects. *Nat. Biotechnol*, 14: 1101-1103.

- Benemann, J. R., Berenson, J. A., Kaplan, N. O., Kamen, M. D, (1973 ).“Hydrogen evolution by chloroplast-ferredoxin-hydrogenase system”, Proc. Natl. Acad. Sci,70: 2317-2320.
- Benemann, J. R.( 1997) “Feasibility analysis of photo biological hydrogen production”, Int. J. Hydrogen Energy,22:979-987.
- Benemann, J. R,(2000).“Hydrogen production by microalgae”, J. Appl. Phycol, 12: 291-300.
- Benemann, J.R,(1998). The technology of biohydrogen. Plenum, New York.
- Bockris, J.O.M,(2002).The origin of ideas on a Hydrogen Economy and its solution to the decay of the environment. Int J Hydrogen Energy, 27(7-8): 731-740.
- Boussaid, A., Robinson, J., Cai, Y., Gregg, D.J., Saddler, J.N,(1999). Fermentability of the Hemicellulose-derived sugars from steam-exploded softwood (douglas fir).Biotechnol Bioeng,64(3): 284-289.
- Caglar, A. and Ozmen, H,(2000). Hydrogen as an attractive fuel in future. Energy Edu Sci Technol .6:1-18.
- Campbell, T.J., et al.,( 2012) A packed bed Ammonia Fiber Expansion reactor system for pretreatment of Agricultural residues at regional depots. Biofuels, 4(1): p. 23-34.
- Caputo AC, Palumbo M, Pelagagge PM et al, (2005). Economics of biomass energy utilization in combustion and gasifi cation plants: effects of logistic variables. Biomass Bioenerg ,28:35–51.
- Cao GL, Ren NQ, Wang AJ, (2009). Acid hydrolysis of corn Stover for biohydrogen production Using Thermoanaerobacterium thermosaccharolyticum W16. Int J Hydrogen Energy, 34(17):7182–7188
- Ciais, P., Sabine, C., Bala, G., Bopp, L., Brovkin, V., Canadell, J., Chhabra, a., DeFries, R., Galloway, J., Heimann, M., Jones, C., Quéré, C. Le, Myneni, R.B., Piao, S., Thornton, P., France, P.C., Willem, J., Friedlingstein, P., Munhoven, G., 2013.
- Chang, V.S. and M.T. Holtzapple, (2000).Fundamental factors affecting biomass enzymatic reactivity. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology,84-86: p. 5-37.
- Chen, X. and Khanna, M,( 2012). Food vs. Fuel: The Effect of Biofuel Policies, American Journal of Agricultural Economics, doi: 10.1093/ajae/aas039
- Cheng CL, Lo YC, Lee KS et al, (2011) .Biohydrogen production from Lignocellulosic

- Feedstock. *Bioresour Technol*, 102:8514–8523
- Chen, W.H.; Chen, S.Y.; Khanal, S.K.; Sung, S,(2006). Kinetic study of biological hydrogen production by anaerobic fermentation. *Int. J. Hydrog. Energy* ,31:2170–2178.
- Dabrock, B., Bahl, H. and Goltschal, G(1992). Parameters affecting solvent production by *Clostridium pasteurianum*. *Appl Environ Microbiol*, 58:1233-1239.
- Das, D., and Veziroglu, T.N, (2001). Hydrogen production by biological processes: a survey of literature. *International Journal of Hydrogen Energy*, 26: 13-28.
- De Gioannis, G., Friargiu, M., Massi, E., Muntoni, A., Poletini, A., Pomi, R., & Spiga, D,( 2014). Biohydrogen production from dark fermentation of cheese whey: Influence of pH. *International Journal of Hydrogen Energy*, 39(36): 20930–20941.
- Demirbas, A,( 2008). Biohydrogen generation from organic wastes. *Energy Sources Part A* ,30:475- 482.
- DOE,( 2014) . Annual Energy Report. United States Department of Energy. Washington DC,USA.\_<http://www.energy.gov/pdf>. Accessed 15 September.
- Dong GX, Wu BR, Zhu L, Du J,( 2007). Microstructure and electrochemical properties of low-temperature hydrogen storage alloy used in Ni/MH batteries. *Transactions of Nonferrous Metals Society of China*, 17:S941-S944.
- Dos Reis, C. M., & Silva, E. L,( 2011). Effect of upflow velocity and hydraulic retention time in anaerobic fluidized-bed reactors used for hydrogen production *Chemical Engineering Journal*, 172(1):28–36.
- Dragone D., Fernandes, B., Vicente A.A. and Teixeira, J.A, (2010). Third generation biofuels from microalgae. *Current Research, technology and Education Topics in Applied Microbiology and Microbial Biotechnology*, A. Mendez-Vilas (Ed).
- Duff S. J. B., Murray W. D,( 1996). Bioconversion of forest products industry waste cellulose to fuel ethanol: a Review. *Bioresour Technol.*,55:1–33
- Effland, M.J, (1977). Modified Procedure to Determine Acid-Insoluble Lignin in Wood and Pulp. *Tappi*, 60(10): 143-144.
- EIA (2011). Annual Energy Outlook- 2011 with Projections to 2035. Energy Information Administration. Washington DC, USA. <http://> Accessed 10 September 2018.

- European Commission,(2003) . Hydrogen and fuel cells: A vision for our future.  
European Commission, Directorate-General for Research, Energy and Transport,  
EUR 20719 EN ,[www.hfpeurope.org/uploads/59/hydrogen-vision-report\\_HLG\\_2003\\_en.pdf](http://www.hfpeurope.org/uploads/59/hydrogen-vision-report_HLG_2003_en.pdf) Accessed 20 September 2018.
- Fan YT, Zhang YH, Zhang SF, How HW, Ren BZ, (2006).efficient conversion of wheat straw Wastes into biohydrogen gas by cow dung compost. *Bioresour Technol* ,97:500–505
- Fan YT, Zhang GS, Guo XY, Xing Y, Fan MH,( 2006). Biohydrogen-production from beer lees Biomass by cow dung compost. *Biomass Bioenergy* ,30:493–496
- FAO,( 2016) .Available at <http://www.fao.org/faostat/en/#data> . 11 September 2018.
- Ganesh.D, Saratal ,Shing-Der,Chen,Yung-chung lo,( 2008). *Jornal of scientific and industrial Research* , 67:962-979.
- Garrote G, Dominguez H, Parajo JC, (1999). Hydrothermal processing of lignocellulosic materials. *Holzforschung*, 57:191-202.
- Gavrilescu, M, (2002). Engineering concerns and new developments in anaerob wastewater treatment. *Clean Technol Env Policy.*; 3:346-362.
- Grous, W. R.; Converse, A. O.; Grethlein, H. E,( 1986). Effect of steam explosion pretreatment on pore size and enzymatic hydrolysis of poplar. *Enzyme Microb. Technol.*, 8: 274–280.
- Guo, X. M., Trably, E., Latrille, E., Carrère, H., & Steyer, J.-P, (2010). Hydrogen production from agricultural waste by dark fermentation: A review. *Internationa Journal of Hydrogen Energy*, 35(19): 10660–10673.
- Guo XM, Trably E, Latrille E et al, (2014). Predictive and explicative models of fermentative hydrogen production from solid organic waste: role of butyrate and lactate pathways. *Int J Hydrog Energy*, 39:7476–7485.
- González LJR, Dávila IMMM, García YG, De la Garza JAR, Martínez JR,( 2011). Biohydrogen production from diary processing wastewater by anaerobic biofilm reactors. *African Journal of Biotechnology*, 10(27):5320-5326.
- Grethlein, H.E., Converse, A.O, (1991). Common aspects of acid pre hydrolysis and steam explosion for pretreating wood. *Bioresour Technol*, 36(1): 77-82.
- Hegarty, R.S., Gerd, R, (1999). Hydrogen production and transfer in the rumen. *Recent advances in animal nutrition in Australia*, 8 12: 37-44.



- Hallenbeck, P. C., Abo-Hashesh, M., Ghosh D, (2012). Strategies for improving biological hydrogen production. *Bioresour Technol*, 110: 1 – 9
- Hallenbeck, P.C. and Benemann, J.R,( 2002). Biological hydrogen production; fundamentals and limiting processes. *Int. J. Hydrogen Energy*, 27: 185-1193.
- Hawkes, F. R, Dinsdale R., Hawkes, D. L, (2002). Hussy I. Sustainable fermentative hydrogen production: challenges for process optimization. *Intl J of Hydrogen Energ*, 27:1339-1347
- Hawkes, F.R., Hussy, I., Kyazza, G., Dinsdale, R. and Hawkes, D.L, (2007). Continuous dark fermentative hydrogen production by mesophilic microflora: principles and progress. *Int J of Hydrogen Energy*, 32: 172 - 184
- Hamelinck, C.N., van Hooijdonk, G., Faaij, A.P.C, (2005). Future prospects for the production of ethanol from lingo-cellulosic biomass. *Biomass Bioenergy*, 28: 384 410
- Höhlein, B., von Andrian, S., Grube, T.H and Menzer, R, (2000). Critical assessment of power trains with fuel-cell systems and different fuels. *J Power Sources*, 86:243 249.
- Holladay, J.D., Hu, J., King, D.L. and Wang, Y, (2009). An overview of hydrogen production technologies, *Catalysis Today*, (139):244–260.
- Holtzapple, M.T., et al,( 1991). Ammonia Fiber Explosion (AFEX) pretreatment of lignocellulose. In *Symposium Papers - Energy from Biomass and Wastes*.
- Holtzapple, M.T., E.P. Ripley, and M. Nikolaou,( 1994). Saccharification, fermentation, and protein recovery from low-temperature AFEX-treated coastal bermudagrass. *Biotechnology and Bioengineering*, 44(9): p. 1122-1131.
- Hook, M. and Tang, X, (2013). Depletion of fossil fuels and anthropogenic climate change -A review. *Energy Policy*, 52: 797-809.
- Hu, Z.-H., Yu, H.-Q., & Zhu, R.-F,( 2005). Influence of particle size and pH on anaerobic degradation of cellulose by ruminal microbes. *International Biodeterioration & Biodegradation*, 55(3): 233–238.
- Huang, R,( 2009). Hydrogen production by photo electrolysis.  
<http://bhuang02.tripod.com/photoelectrolysis.htm>
- IEA, (2008).From 1st to second generation Biofuel Technologies: An Overview of Current Industry and RandD activities.  
[http://www.iea.org/papers/2008/2nd\\_Biofuel\\_Gen.pdf](http://www.iea.org/papers/2008/2nd_Biofuel_Gen.pdf).

- Jansen, C. & Lübberstedt, T, (2012). Turning Maize Cobs into a Valuable Feedstock. *BioEnergy Research*, 5: 20-31.
- Jones DT, Woods DR, (1986). Acetone-Butanol Fermentation Revisited. *Microbiological Reviews*, 50(4):484-524.
- Kapdan IK, Kargi F, (2006). Bio-hydrogen production from waste materials. *Enzym Microbiol Tech* , 38:569–582
- Kloeppe J, Rogerson S, (1991). The Hydrogen Economy. *Electronics World & Wireless World*, 97(1666):668-671.
- Khanal, S., Chen. WH., Li, L. and Sung, S, (2004). Biological hydrogen production: effects of pH and intermediate products. *International Journal of Hydrogen Energy*, 29:1123–1131.
- Khamtib, S., Plangklang, P. and Reungsang, A, (2011). Optimization of fermentative hydrogen production from hydrolysate of microwave assisted sulphuric acid pre-treated oil palm trunk by hot spring enriched culture. *Int J Hydrogen Energy*, 36: 14204-14216.
- Koroneos, C., Dompros, A., Roubas, G. and Moussiopoulos, N, (2005). Advantages of the use of hydrogen fuel as compared to kerosene. *Res Conser Recyc*, 44:99-113.
- Kim, J. K., Oh, B. R., Chun, Y. N., & Kim, S. W, (2006). Effects of temperature and hydraulic retention time on anaerobic digestion of food waste. *Journal of Bioscience and Bioengineering*, 102(4): 328–32.
- Kotay, S.M., Das, D, (2008). Biohydrogen as a renewable energy resource -Prospects and potentials. *Int. J. Hydrogen Energy*, 33: 258–263.
- Kumar P, Barrett DM, Delwiche MJ, Stroeve P, (2009) . Methods for pretreatment of lignocellulosic biomass for efficient hydrolysis and biofuel production. *Ind Eng Chem Res* , 48:3713–3729
- Lee RA, Lavoie JM, (2013) . From first- to third-generation biofuels: challenges of producing a commodity from a biomass of increasing complexity. *Anim Front*, 3:6–11
- Lee, Y.J., Miyahara, T. and Noike, T, (2001). Effect of iron concentration on hydrogen fermentation. *Bioresour Technol* , 80(3): 227-231.
- Levin, D.B., Pitt, L., Love, M, (2004). Biohydrogen production: prospects and limitations to practical application. *Int J Hydrogen Energy*, 29(2): 173-185.
- Li, D. M., Chen, H. Z, (2007). Biological hydrogen production from steam-exploded

- straw by simultaneous saccharification and fermentation. *Int J Hydrogen Energ*, 32: 1742 -1748
- Lin CY, Lay CH, (2004). Carbon/nitrogen-ratio effect on fermentative hydrogen production by mixed microflora. *International Journal of Hydrogen Energy*, 29(1):41-45.
- Liu, D., Zeng, R., & Angelidaki, I, (2008). Enrichment and adaptation of extremethermophilic (70°C) hydrogen producing bacteria to organic household solid waste by repeated batch cultivation. *International Journal of Hydrogen Energy*, 33(22): 6492–6497.
- Luo, G., Xie, L., Zou, Z., Wang, W., & Zhou, Q, (2010). Evaluation of pretreatment methods on mixed inoculum for both batch and continuous thermophilic biohydrogen production from cassava stillage. *Bioresource Technology*, 101(3): 959–64.
- Luo, G., Karakashev, D., Xie, L., Zhou, Q., & Angelidaki, (2011). Long-term effect of inoculum pretreatment on fermentative hydrogen production by repeated batch cultivations: homoacetogenesis and methanogenesis as competitors to hydrogen production. *Biotechnology and Bioengineering*, 108(8):1816–27.
- Marbán, G., Valdés-Solís, T, (2007). Towards the hydrogen economy? *Int. J. Hydrogen Energy* , 32: 1625–1637.
- Mathews, J., Wang, G, (2009). Metabolic pathway engineering for enhanced biohydrogen production. *Int J Hydrogen Energ*, 34: 7404 -7416
- McCann, J. C, (2005). *Maize and grace: Africa's encounter with a new world crop*. Cambridge: Harvard University Press, 1500–2000.
- McKendry P, (2002) .Energy production from biomass (part 1): overview of biomass. *Bioresour Technol*, 83:37–46.
- McMillan, J. D, (1994). Pretreatment of lignocellulosic biomass. In *Enzymatic Conversion of Biomass for Fuels Production*; Himmel, M. E., Baker, J. O., Overend, R. P., Eds.; American Chemical Society: Washington, DC; pp 292-324.
- Midilli, A., Ay, M., Dincer, I. and Rosen, M.A, (2005). On hydrogen and hydrogen energy strategies I: current status and needs. *Renewable and Sustainable Energy Reviews* , 9:255-271.
- Miranda, R, (2004). Hydrogen from lignocellulosic biomass via thermochemical processes. *Energy Edu Sci Technol*, 13:21-30.

- Momirlan, M.; Veziroglu, T.N, (2002). Current status of hydrogen energy. *Renew. Sustain. Energy Rev*, 6:141–179.
- Monlau, F., Barakat, A., Trably, E., Dumas, C., Steyer, J., Carrère, H, (2013a). Lignocellulosic materials into biohydrogen and biomethane: Impact of structural features and pretreatment. *Critic Rev Environ Sci Technol*, 43: 260-322
- Mosier, Ce., Wyman, B.d., Dale, R.T., Elander, Y.Y., Lee, M. Holtzapple, and C.M., Ladisch, (2005). Features of promising technologies for pretreatment of Lingo-cellulosic Biomass. *Bio-resource Technology*, 96:0960-8524.
- Mtui, G.Y.S, (2008). Recent advances in pretreatment of lignocellulosic wastesb and production of value added products. *Afr J Biotechnol*, 8(8):1398-1415.
- Naik, S.N., Goud, V.V., Rout, P.K. and Dalai, A.K, (2010). Production of first and second generation biofuels: A comprehensive review. *Renewable and Sustainable Energy Reviews*, 14(2): 578-597.
- Nath K, Das D, (2003) . Hydrogen from biomass. *Curr Sci* , 85:265–271.
- Neely, W.C, (1984). Factors affecting the pretreatment of biomass with gaseous ozone: a review. *Bioresource Technology*, 83: 1-11.
- Ni, M., Leung, D.Y.C., Leung, M.K.H. and Sumathy, K, (2006). An overview of hydrogen production from biomass, *Fuel Processing Technology*, 87:461–472.
- Ntaikou I, Koutros E, Kornaros M, (2009) .Valorisation of wastepaper using the fibrolytic/hydrogen producing bacterium *Ruminococcus albus* . *Bioresour Technol*, 100:5928–5933.
- Oh, Y.-K., Kim, S. H., Kim, M.-S., & Park, S, (2004). Thermophilic biohydrogen production from glucose with trickling biofilter. *Biotechnology and Bioengineering*.;88(6):690–8.
- Pakarinen, O., Kaparaju, P., & Rintala, J, (2011). The effect of organic loading rate and retention time on hydrogen production from a methanogenic CSTR. *Bioresource Technology*,102(19):8952–7.
- Pan, X., Gilkes, N., Kadla, J., Pye, K., Saka, S., Gregg, D., Ehara, K., Xie, D., Lam, D., Saddler, J, (2006). Bioconversion of hybrid poplar to ethanol and co-products using an Organosolv fractionation process: optimization of process yields. *Biotechnology and Bioengineering*, 94(5):851-861.

- Panagiotopoulos, I. A., Bakker, R. R., Budde, M. A. W, de Vrije, T., Claassen, P. A. M., Koukios, E. G, ( 2009). Fermentative hydrogen production from pretreated biomass: a comparative study. *Bioresour Technol*, 100 (24): 6331 – 6338.
- Palmová, I. and Schöngut, J, (2004). Outlook of production and utilization of hydrogen, *Chemické listy*, 8: 205– 210.
- Park JH, Yoon JJ, Park HD et al, (2009). Feasibility of biohydrogen production from *Gelidium amansii* . *Int J Hydrog Energy*, 36:13997–14003
- Patra, S., Sangyoka, S., Boonmee, M., Reungsang, A, (2008). Biohydrogen production from the fermentation of sugarcane bagasse hydrolysate by *Clostridium butyricum*. *Int. J Hydrogen Energ*, 33: 5256 – 5265.
- Pavlostathis, S.G., Giraldo, G.E, (1991). Kinetics of anaerobic treatment: A critical review. *Cri Rev Env Contr*, 21(5-6): 411-490.
- Pimentel, D., Cooperstein, S., Randell, H., Filiberto, D, Sorrentino, S., Kaye, B., Nicklin, C., Y , J , B n, J , O’H n, J , H s, A nd W ns n, C Ec y nc s n,( 2007). population growth and environmental degradation. *Human Ecology* , 35: 653-668.
- Ramachandran R, Menon RK, (1998). An overview of industrial uses of hydrogen. *International .Journal of Hydrogen Energy*, 23(7):593-598.
- Reith, J.H., Wijffels, R.H. and Barten, H, (2003). Biomethane and biohydrogen; status and perspective of biological methane and hydrogen production. Dutch biological hydrogen foundation Wageningen.
- Ren NQ, Cao GL, Guo WQ, Wang AJ, Zhu YH ,( 2010)..Biological hydrogen production from corn stover by moderately thermophile *Thermoanaerobacterium thermosaccharolyticum* W16. *Int J Hydrogen Energy* , 35:2708–2712.
- Rifkin, J,( 2002.). *The hydrogen economy*. Jeremy P. Tarcher/Penguin, New York.
- Rosin, M. A. and Scott, D.S,( 1998). Comparative efficiency assessments for a range of hydrogen production processes. *Int. J. Hydrogen Energy.*; 23: 653-659.
- Saratale, G.D., Chen, S., Lo, Y.C., Saratale, R. and Chang,( 2008), J.Outlook of biohydrogen production from lignocellulosic feedstock using dark fermentation -a review. *J Sci Ind Res*, 67:962-979.
- Shin, H.S., Youn, J.H., Kim, S.H. “Hydrogen production from food waste in anaerobic mesophilic and thermophilic acidogenesis” *Int. J. Hydrogen Energy*. 2004; 29:1355-63.
- Show, K. Y, Lee, D. J, Tay, J. H., Lin C. Y., Chang, J. S,( 2012). Biohydrogen

- production. Current perspectives and the way forward. *Intl J Hydrogen Energy*, 37(20): 15616 – 15631.
- Sims R, Taylor M, Saddler J, Mabee W, (2008) .From first to second generation biofuel technologies. An overview of current industry and RD&D activities. International Energy Agency, France. [https://www.iea.org/publications/freepublications/publication/2nd\\_Biofuel\\_Gen.pdf](https://www.iea.org/publications/freepublications/publication/2nd_Biofuel_Gen.pdf) access date 13 october 2018.
- Singh A, Olsena SI, Nigamb PS ,( 2011). A viable technology to generate third-generation biofuel. *J Chem Technol Biotechnol*, 86:1349–1353.
- Smith, K.R., Jerrett, M., Anderson, H.R., Burnett, R.T., Stone, V., Derwent, R., Atkinson, R.W., Cohen, A., Shonkoff, S.B., Krewski, D., Pope, C.A., Thun, M.J. and Thurston, G, (2009). Public health benefits of strategies to reduce greenhouse-gas emissions: health implications of short-lived greenhouse pollutants. *Health and Climate Change*, 374: 2091-2103.
- Sparling, R., Levin, D.B., Islam, R., Cicek, N,( 2006). Hydrogen production by *Clostridium thermocellum* 27405 from cellulosic biomass substrates. *Int J Hydrogen Energy*, 31(11): 1496-1503.
- Sridevi K, Sivaraman E, Mullai P,( 2014) . Back propagation neural network modelling of Biodegradation.
- Stiegel, G. J., Ramezan, M,( 2006). Hydrogen from coal gasification: An economical pathway to a sustainable energy future. *Int J Coal Geol*, 65 (3-4): 173 – 190.
- Sun Y, Cheng J ,( 2002). Hydrolysis of lignocellulosic materials for ethanol production: a review. *Bioresour Technol*, 83:1–11.
- Taherzadeh, M.J., Karimi, K, (2008). Pretreatment of lignocellulosic wastes to improve ethanol and biogas production: A review. *Int J Mol Sci*, 9(9): 1621-1651.
- Tanisho S, Kuromoto M, Kadokura N,( 1998). Effect of CO<sub>2</sub> removal on hydrogen production by fermentation. *International Journal of Hydrogen Energy* ,23(7):559-563.
- Turner, J.A,( 2004). Sustainable hydrogen production. *Science* ,305: 972-974.
- Turner, J.A., Deutsch, T., Head, J. and Vallett, P, (2007.). Photoelectrochemical water

- systems for H<sub>2</sub> Review, U.S. Department of Energy, Washington, DC.  
[http://www.hydrogen.energy.gov/pdfs/review07/pd\\_10\\_turner.pdf](http://www.hydrogen.energy.gov/pdfs/review07/pd_10_turner.pdf), 1–20 access date 16 June 2017.
- Turner, J., Sverdrup, G., Mann, M.K., Maness, P.C., Kroposki, B., Ghirardi, M., Hans, R.J. and Blake, D, ( 2009). Renewable hydrogen production, *International Journal of Energy Research*, 32: 379–407.
- Sinha, P. and Pandey, A,( 2011). An evaluative report and challenges for fermentative biohydrogen production. *Int J Hydrogen Energy* ,36:7460-7468.
- Song, H., Zhang, L., Watson, R.B., Braden, D. and Ozkan, U.S,( 2007). Investigation of bio-ethanol steam reforming over cobalt-based catalysts, *Catalysis Today* ,(129):346–354.
- Valdez-Vazquez, I., Rios Leal, E., Esparza-Garcia, F., Cecchi, F., Poggi-Varaldo, H. M,( 2005). Semi-continuous solid substrate anaerobic reactors for H<sub>2</sub> production from organic waste: mesophilic versus thermophilic regime. *Int J Hydrog Energy* .:30 (13 -14): 1383 -1391.
- Valdez-vazquez, I., Riosleal, E., Esparzagarcia, F., Cecchi, F., & Poggivaraldo, H,( 2005).  
 Semi-continuous solid substrate anaerobic reactors for H<sub>2</sub> production from organic waste: Mesophilic versus thermophilic regime. *International Journal of Hydrogen Energy*, 30(13-14): 1383–1391.
- Van Ginkel S, Logan BE,( 2005). Inhibition of biohydrogen production by undissociated acetic and butyric acids. *Environmental Science & Technology* ,39(23):9351-9356.
- van Niel EWJ, Claassen PAM, Stams AJM,( 2003).. Substrate and product inhibition of hydrogen production by the extreme thermophile, *Caldicellulosiruptor saccharolyticus*. *Biotechnology and Bioengineering*, 81(3):255-262.
- Veziroglu, T,( 1975). *Hydrogen Energy, Part B*. Plenum, New York.
- Veziroglu, T.N. and Sahin, S,( 2008). 21st Century's hydrogen energy Conversion and Management , 49:1820-1831.
- Wang, Y., Chin, Y.H., Rozmiarek, R.T., Johnson, B.R., Gao, Y., Watson, J., Tonkovych, A.Y.L. and Vander, Wiel D.P,( 2004). Highly active and stable Rh/MgOAl<sub>2</sub>O<sub>3</sub> catalysts for methane steam reforming, *Catalysis Today*, 98:575–581.
- Wang, J., & Wan, W,( 2009). Factors influencing fermentative hydrogen production: A

- review. *International Journal of Hydrogen Energy*, 34(1): 799–811.
- Wang, J., Wan, W,( 2009). Kinetic models for fermentative hydrogen production: A review. *Int J Hydrogen Energ*, 34: 3313 – 3323.
- WHO (2008). Protecting Health from Climate Change. World Health Day. World Health Organization, Geneva, Switzerland. 2008.  
<http://www.who.int/globalchange/publications>. Accessed 12 May 2017.
- William, E.E,( 2004). The potential for a hydrogen energy economy. *R & D of Energy Technologies, ANNEX A- IV Hydrogen Energy*, pp. 207-214.
- Yang B, Wyman CE,( 2008) . Pretreatment: the key to unlocking low-cost cellulosic ethanol. *Biofuels Bioprod Bioref* , 2:26–40.
- Zeikus, J.G,( 1977). The biology of methanogenic bacteria. *Bacteriol Rev*, 41(2): 514–541.
- Zych, D, (2008.). The viability of corn cobs as a bioenergy feedstock. WestCentral Research and Outreach Center, University of Minnesota.



## APPENDICES

### Appendix A .Fit summary

Table A.1: Sequential Model Sum of Squares

Source	Sum of Squares	df	Mean Square	F-value	p-value	
Mean vs Total	32.23	1	32.23			
Linear vs Mean	13.22	2	6.61	76.10	< 0.0001	
2FI vs Linear	0.0404	1	0.0404	0.4454	0.5171	
<b>Quadratic vs 2FI</b>	<b>0.8941</b>	<b>2</b>	<b>0.4471</b>	<b>22.96</b>	<b>0.0002</b>	<b>Suggested</b>
Cubic vs Quadratic	0.1373	4	0.0343	3.59	0.0798	
Quartic vs Cubic	0.0416	3	0.0139	2.63	0.2237	Aliased
Residual	0.0158	3	0.0053			
Total	46.58	16	2.91			

### Appendix B. Laboratory work pictures

preparation of the collected samples sample



Fig.1: Corn cob sample



Fig.2: Pestle for corn cob crush



Fig.3:Grounded corn cobs



Fig.4: sieved corn cob



Fig .sample in crucible



Fig .6: furnace

### Pretreatment and hydrolysis steps of H<sub>2</sub> gas production

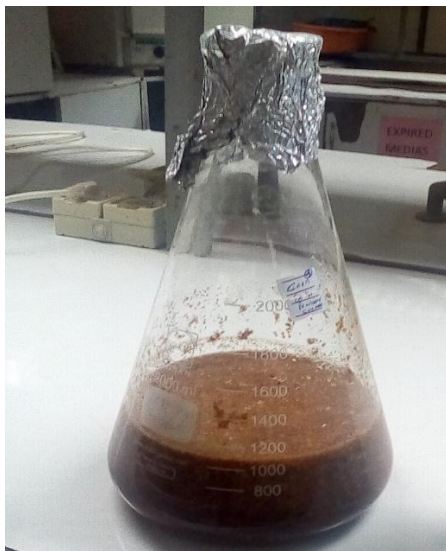


Fig .7: Soaked sample



Fig.8:Autoclaved sample





Fig .9:Autoclave



Fig .10: Activated carbon treated sample

Prepared media for fermentation and nutrients used for fermentation



Fig.11:Boye WTP

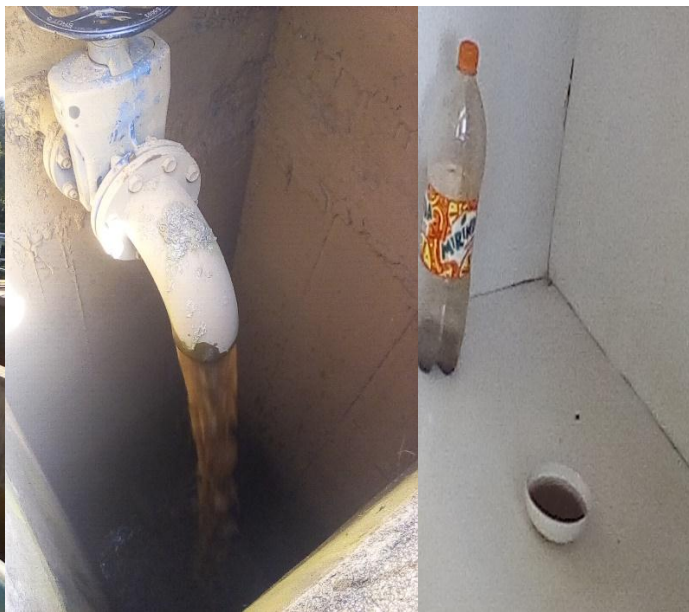


Fig.12: Anaerobic digested sludge



Fig.13: Reagents for media preparation



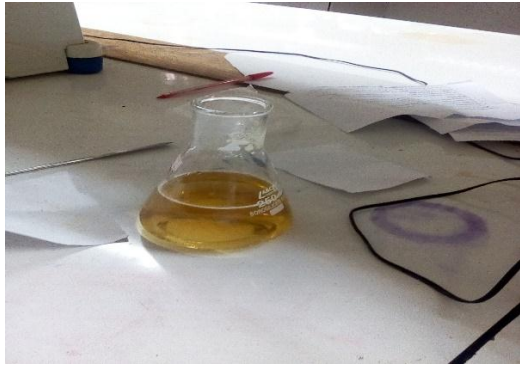
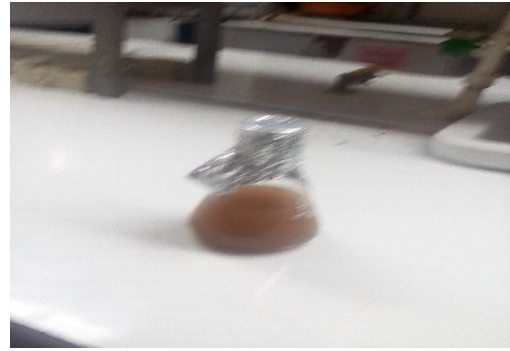


Fig .14: The prepared media



pH adjustments for fermentation by adding the media with sample in to 1:10 ratio



Fig .15: pH adjustment



Fig.16: pH adjusted sample

Fermentation in an incubator



Fig .17: incubator



Fig .18: Fermentation in incubator