Université de Lomé

DRP-CCDRM

Universität Rostock Traditio et Innovatio

INTERNATIONAL MASTER PROGRAM

IN RENEWABLE ENERGY AND GREEN HYDROGEN

SPECIALITY: BIOENERGY/BIOFUEL AND GREEN HYDROGEN TECHNOLOGY

MASTER THESIS

Subject/Topic:

BIOHYDROGEN PRODUCTION FROM PINEAPPLE PEELS WASTE BY DARK FERMENTATION

2021-2023

Presented on the 27th September 2023

by:

Abu Bakarr BANGURA

Composition of the jury:

Jury president: Amivi TETE-BENISSAN, Professor Universite de lome – Togo

Examiner/judge: Komi AGBOKA, Associate professor, Universite de lome – Togo

Major Supervisor: Damgou MANIKONGNINE, Associate Professor, Universite de lome - Togo

Co-Supervisor: Satyanarayana NARRA, Professor, University of Roatock - Germany

SPONSORED BY THE

WASCAL West African Science Service Centre on Climate Change and Adapted Land Use

Federal Ministry of Education and Research

DEDICATION

This work is dedicated to my Father and Mother, especially my Wife, for her patience and support throughout this journey and the entire Bangura family.

ACKNOWLEDGEMENT

I will first of all like to express my sincere thanks and appreciation to the Almighty Allah for giving me strength, wisdom, and understanding throughout my academic pursuit.

I want to express my profound gratitude to the German Ministry of Education (BMBF) and WASCAL for scholarship awarded to me and also the presidents of both universities, Professor MAMADOU Saidou at Abmoumini University, Niger, and Professor KOKOROKO Dodzi Komla at the University of Lomé, Togo for their warm reception and for making the program very educative, especially to the president of the University of Lomé, Togo for his tireless effort in ensuring that the program went all.

A big thank you is also going to the director of WASCAL, Professor RABANI Adamou at Abmoumini University, Niamey for his unwavering support in ensuring the first and second semesters went all and for his kind pieces of advice in reshaping our mentality and preparing us for the future ahead.

My sincere thanks and appreciation go to the WASCAL team in Togo starting from the Director, Associate Professor AGBOKA Komi, his Deputy Associate Professor BEGEDOU Komi, and also the coordinator of GSP, Dr. MANI KONGNINE Damgou, and scientific coordinator, Dr. IDRISSOU Mouhamed for their warm reception and the effort they put in making this program a success.

I also want to express my deepest gratitude and appreciation to my Supervisor, Professor AZOUMA Yaovi Ouézou at the University of Lomé, Togo for his guidance and for making sure that I followed the right path in the earlier development of the work. However, you are gone but will never be forgotten. I pray that God grants you paradise.

I would like to extend my appreciation to my Co-supervisor, Professor NARA Satyanarayana at the University of Rostock for his mentorship and guidance in achieving success in this work.

Many thanks go to Dr. Ing. SPRAFKE Jan at the Bioenergy laboratory at the University of Rostock for his immense support and supervision in making this work a reality and to the entire team in the laboratory staff who has contributed in one way or the other throughout this thesis.

Finally, I would like to express my sincere thanks and appreciation to my mentor Dr. ANTWI Edward for his direction and advice throughout this work and to all my colleagues in the IMP-EGH program, especially ANGBÉ kassé Jean Hugues and DJANGBAJOA Gbièté.

DECLARATION OF AUTHOR

I Abu Bakarr BANGURA, hereby declare that this research project is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at any institution.

_____

ABSTRACT

The lack of proper management of pineapple peel waste has been an environmental and health challenge in developing countries such as Togo. Pineapple peel wastes could be a promising feedstock in the generation of bioenergy such as biohydrogen and biogas which has the potential to be used for cooking, transport, and electricity generation. This study assessed the feasibility of theoretically producing biohydrogen from pineapple peel waste through dark fermentation. A biogas test was also conducted from which a 53.0% methane production from the biogas was assumed to theoretically calculate the biohydrogen production potential. This process offers the best solution for properly managing pineapple peel waste, reducing the environmental and health impacts of releasing greenhouse gases (GHG) into the atmosphere and accelerating the energy transition.

The ultimate analysis of the pineapple peel sample was conducted using an Optic digital microscope (LIBS Analyser) VHX-7000 and the results show carbon 44.4%, hydrogen 9.40%, and oxygen 40.9%. These results were then used to theoretically calculate the biohydrogen production potential. The proximate analysis was conducted to determine the moisture content, total solids, and volatile solids in the pineapple peel sample. The fiber analysis test was also done for cellulose, hemicellulose, and lignin contents using Fibretherm. The biogas test was conducted in bottles using the pineapple peel sample. The loading was such that 5 g of the sample, 200 g of inoculum, and 100 g of water were added into the bottles and then placed in a water bath at a mesophilic temperature of 38^0C for 21 days.

The results obtained from the theoretical biohydrogen production was 3.5 moles and the biogas test was 493.14 mLg^{-1}_{VS}. The estimated theoretical hydrogen production potential from the 53.0% methane yield in the biogas assuming 90% conversion efficiency was 1045.84 mLg^{-1}_{VS}.

KEYWORDS: Biohydrogen; Dark fermentation; Pineapple peels; Energy transition; Greenhouse Effect

RÉSUMÉ

L'absence de gestion appropriée des déchets de peau d'ananas a constitue un défi environnemental et sanitaire dans les pays en développement tels que le Togo. Les déchets de peau d'ananas pourraient être une matière première prometteuse pour la production de bioénergie telle que le biohydrogène et le biogaz qui peuvent être utilisés pour la cuisson, le transport et la production d'électricité. Cette étude vise à évaluer la faisabilité de la production théorique de biohydrogène à partir de déchets de peau d'ananas par fermentation obscure. Un test de biogaz a également été réalisé, à partir duquel une supposition de 53,0 % de méthane a permis de calculer théoriquement le potentiel de biohydrogène. Ce processus offre la meilleure solution pour gérer correctement les déchets de peau d'ananas. Ceei pourrait réduire les impacts environnementaux et sanitaires associés à la libération de gaz à effet de serre (GES) dans l'atmosphère et accélérer la transition énergétique.

L'analyse finale de l'échantillon de peau d'ananas a été réalisée à l'aide d'un microscope numérique Optic (analyseur LIBS) VHX-7000 et les résultats indiquent 44,4% de carbone, 9,4% d'hydrogène et 40,9% d'oxygène. Ces résultats ont ensuite été utilisés pour calculer théoriquement le potentiel de production de biohydrogène. L'analyse proximale a été réalisée pour déterminer la teneur en humidité, les solides totaux, et les solides volatils de l'échantillon d'écorce d'ananas. Le test d'analyse des fibres a également été effectué pour déterminer la teneur en cellulose, en hémicellulose et en lignine à l'aide de Fibretherm. Le test de biogaz a été réalisé dans des bouteilles en utilisant l'échantillon de peau d'ananas. Le chargement était tel que 5 g de l'échantillon, 200 g d'inoculum et 100 g d'eau ont été ajoutés dans les bouteilles, puis placés dans un bain-marie à une température mésophile de 380 °C pendant 21 jours.

Les résultats obtenus pour la production théorique de biohydrogène étaient de 3,5 moles et le test de biogaz était de 493,14 mLg^{-1}_{VS}. Le potentiel théorique de production d'hydrogène estimé à partir du rendement de 53,0 % de méthane dans le biogaz, en supposant une efficacité de conversion de 90 %, était de 1045.84 mLg^{-1}_{VS}.

MOTS-CLÉS: Biohydrogène; Fermentation obscure; Épluchures d'ananas; Transition énergétique; Gaz effet de serre

ACRONYMS AND ABBREVIATIONS

ADF : Acid Detergent Fibre

ADL : Acid Detergent Fibre

ATP : Adenosine Triphosphate

AOAC : Association of Official Agricultural Chemists

C/N : Carbon to nitrogen ratio

DFE : Dark Fermentation Effluent

FAO : Food and Agricultural organization

FW : Food Waste

GHGs : Greenhouse Gases

MEC : Microbial Electrolysis Cell

MFC : Microbial Fuel Cell

NDF : Neutral Detergent Fibre

PNSB : Purple non-sulfur Bacteria

SMR : Steam Methane Reforming

WASCAL : West African Science Service Center on Climate Change and Adapted Land Use

WGS : Water Gas Shift Reaction

LIST OF TABLES

LIST OF FIGURES

TABLE OF CONTENTS

INTRODUCTION

Background of Study

There is a high demand for energy due to the increase in economic and population growth rates (Amekan et al., 2018). According to International Energy Outlook 2013 and 2018 reports, this demand is expected to increase further. It was estimated that between 2010 and 2040, world energy consumption will increase from 524 to 820 quadrillion Btu which is about 56% (Jemilatu et al., 2020). However, the rapid depletion of fossil fuels coupled with the recent increase in oil and natural gas prices and the environmental pollution caused by the release of greenhouse gases (GHG) has led to the search for alternative energy sources (Ventura et al., 2021). There is a growing interest in renewable energy sources in recent times due to their ability to minimize fossil fuel dependence and the associated environmental impacts. Hydrogen appears to be the most promising clean energy carrier in the future for generating electricity in fuel cells and as gaseous biofuel used in the transport sector (Saidi et al., 2018). The energy content of hydrogen is 2.75 times higher than hydrocarbon fuel and the combustion product of hydrogen with oxygen is only water and therefore considered environmentally friendly. Hydrogen production methods can be biological, chemical, or physical processes (Reungsang & Sreela-or, 2013). The majority of molecular hydrogen is mainly produced from fossil fuels. According to a report, it is stated that about 71.27% of hydrogen is produced from natural gas (NG), 27.27% from coal, 0.7% from petroleum, and the remaining 0.7% from the electrolysis of water (Osman et al., 2020). The thermochemical processes used to produce hydrogen are steam gasification, thermal decomposition, catalytic oxidation, auto-thermal reforming, and pyrolysis. However, the production of hydrogen from fossil fuel is not renewable as it involves the release of greenhouse gases (GHGs) into the atmosphere and is therefore not considered carbon neutral. Hydrogen production from biomass using biological pathways not only help reduce the dependence on fossil fuels but is also sustainable and eco-friendly (Chandrasekhar et al., 2015). Other advantages of biohydrogen production include operation under mild conditions (at ambient temperature and pressure), cost-effectiveness, and the potential utilization of renewable resources (biomass) and also various types of wastewater with a high content of carbohydrates and organic acid (Tiang et al., 2020).

The pineapple (*Ananas cosmos L.)* is considered one of the most important fruits around the globe and it is taking the lead in the Bromeliaceae family of edible members. It is extensively grown in tropical and subtropical regions. It has the potential of growing up to a height of about

75 to 150cm with a spread of 90 to 120cm (Zainuddin et al., 2014). Pineapple is viewed as a rich source of vitamins, antioxidants, fibers, and minerals. It is reported that the global production of pineapple in 2018 was approximately 27.92 million metric tons (Pereira et al., 2021), which mostly comes from countries like Costa Rica, Brazil, Philippines, Thailand, Indonesia, India, Nigeria, China, Mexico and Columbia (Dahunsi, 2019). The top three producers of pineapple around the globe are Costa Rica, the Philippines, and Brazil (Pereira et al., 2021). According to (FAO) crop database report, Costa Rica is the leading producer of pineapple with an estimated amount of 3 million tons (Eixenberger et al., 2022).

In Africa, Nigeria is the leading producer of pineapple and the yearly production is estimated at a share of about 1.41 million tons. Togo produced about 44,391 tons of pineapples during the 2021-2022 season and 33,737 tons from this volume were grown organically (https://www.togofirst.com/en/agriculture/2111-10979-togo-produced-over-40-000-t-of-pineapples-in-2021 last accessed on 20/07/2023). From these huge quantities of production, there is every possibility for the generation of a large amount of pineapple peel waste during the processing of the fruit, which is most of the time deposited in the area of production, market, and even in open landfills.

It is estimated that the total weight of pineapple accounts for about 50% of waste and the key components are pineapple peel (29–40%) and core (9–10%) among others (Eixenberger et al., 2022). The pineapple peel is reported to have a content of 16% lignin, 35% cellulose, 19.7% hemicellulose, 75 – 80% moisture, 4.7% total ash, 0.46% total fat, 23.71% total crude fibre, and 0.33% total proteins. It is made up of 27.08% total carbohydrate, 26.096 mg/kg potassium, 1.9 mg/kg magnesium, and 298.184 mg/kg zinc (Cahyari et al., 2018). Pineapple peel waste can be used either as fertilizer or burnt in open landfills releasing greenhouse gases that have the potential of causing global warming, and environmental and health challenges. Fermentation is a process by which biohydrogen is produced from organic materials using fermentative bacteria. In this light, the fermentation process is divided into dark and photo fermentation. Dark fermentation is carried out by dark fermentative bacteria while photo fermentation is by photosynthetic bacteria (Junghare et al., 2012). The merits of the dark fermentation process are simple operating procedures, low energy requirements, a higher stable rate of biohydrogen production by the utilization of a wide range of waste substrates as feedstock, and a better economic process. This process is however limited by the insufficient utilization of substrate giving rise to low hydrogen yield (Saratale et al., 2019). The use of pineapple peel waste to produce biohydrogen and biogas will provide energy in sectors like

clean cooking, transport, and electricity generation but also will help in the reduction of greenhouse gas emissions (GHGs).

Problem statement

It is reported that food waste (FW) accounts for 15-63% of total municipal solid waste around the globe and is regarded as one of the most challenging and abundant organic solid wastes (Yun et al., 2018). The issue of lack of proper pineapple waste management is still a challenge in Togo. The large quantities of pineapple production have a high tendency of generating pineapple peel waste during processing which most of the time ends up in open landfills, production sites, or being incinerated. This has the potential to generate greenhouse gases (GHG) such as carbon dioxide and methane which are eventually released into the atmosphere causing global warming, climate change, and environmental and health challenges. The Valorisation of this waste would not only contribute to proper waste management disposals but also helps to produce a value-added product such as biohydrogen and biogas (Abdullah & Mat, 2008).

Research questions

I. What is the effective way of utilizing pineapple peel waste to improve its waste management?

II. Does pineapple peel waste have the potential for biohydrogen and biogas production?

Research hypotheses

By performing dark fermentation using pineapple peels waste, it is believed that the problem of waste management and the associated environmental impact will be minimized and this process will also lead to the production of biohydrogen and biogas as alternative sources of energy to fossil fuels in mitigating climate change and accelerate the energy transition.

Research Objective

The main goal of this work is to use a dark fermentation approach to produce biohydrogen and test for biogas potential using pineapple peel waste. The specific objectives are:

I. To examine the characterization of solid pineapple peels waste

II. To theoretically determine biohydrogen production potential from pineapple peel waste

III. To perform a test for biogas production potential using pineapple peels waste

IV. To estimate the biohydrogen production potential from methane in the biogas

CHAPTER 1: LITERATURE REVIEW

1.1. Hydrogen

The production of hydrogen started in 1761 by Robert Boyle when he reacted iron filings and dilute acids. Hydrogen was later identified by Henry Cavendish in 1776 as a unique substance. Antoine Lavoisier was also able to produce hydrogen from iron in 1783 and named it the material hydrogen. In 1839, a British scientist named Sir William Robert Grove developed the first hydrogen-powered fuel cell. Through the construction of a cathode, anode, ceramic membrane, and mixed acid conductive medium, he was able to produce the flow of electric current. This discovery gives rise to the invention of the hydrogen-powered fuel cells of today (Rivkin et al.,2015).

Hydrogen is found to be the lightest, simplest, and most abundant element in the universe accounting for approximately 75% of all matter consisting of one proton and one electron. It is considered the tenth most abundant element in the earth's crust which is usually found in combination with other elements. The atomic weight of hydrogen is 1.00795 atomic mass units, usually approximated as 1.008 atomic mass units. The three isotopes of hydrogen are protium, deuterium, and tritium. It is mainly found in combined states such as water and organic compounds (Pareek et al., 2020; Dawood et al., 2019; Baykara, 2018). Notwithstanding the abundance of hydrogen, obtaining hydrogen in elemental form is very cumbersome. The pathways that can be used to obtain it are through renewable-assisted water splitting, thermochemical conversion of fossil fuels, and biological processes (Abdin et al., 2019). Hydrogen is also termed an energy carrier as it will play a key role in the energy transition. Biohydrogen production will supply global sustainable clean energy. It is a promising alternative to fossil fuels due to its potential of eliminating if not all, but most of the negative effects of the use of fossil fuels (Show et al.,2011).

1.2. Properties of Hydrogen

Hydrogen is a colorless, odorless, and flammable gas with zero-emission or emission-free which offers a great opportunity to be used instead of fossil fuel and also its high energy content of 122MJ/kg and less impact on the environment. Hydrogen has a density lower than that of air density and a gravimetric energy density of about seven times higher than that of fossil fuel density. At a higher heating value, the energy content of hydrogen is 141.8MJ/kg at 298k, and at a lower heating valve is 120MJ/kg which is found to be higher than most fuels (an example

is gasoline with a heating value of 44MJ/kg at 289k). However, the energy density by volume of liquid hydrogen is approximately less than hydrocarbon fuel by a factor of four (i.e., the density of 8 MJ/l whereas gasoline has a density of 32 MJ/l). Hydrogen gas has good energy by weight but rather a poor energy density by volume compared to hydrocarbons and hence a large storage tank is required (Tarhan and Çil, 2021; Vincent and Bessarabov, 2017; Liu et al., 2017).

1.3. Storage of Hydrogen

The storage and transport of hydrogen are still a challenge. Because hydrogen is the lightest molecule, it has a very low density, and 1kg of hydrogen gas occupies over $11m^3$ at room temperature and atmospheric pressure. Hence the storage density must be increased for an economically viable hydrogen storage system. However, there are a lot of methods used to store hydrogen at increased density, and all these methods required some energy input in forms like work, heat, or even in some cases, hydrogen-binding materials (Andersson and Gronkvist, 2019). Large-scale storage plays a significant role in the hydrogen economy. The reason for storing hydrogen energy is simple to be safe and efficient which offers the opportunity to be used anywhere and at any time (Zhang et al., 2016). For any hydrogen-powered system, it is fundamental to develop hydrogen storage technologies. Conventional storage technologies store hydrogen as compressed gas and cryogenic liquid, while underground storage is mostly preferred for large-scale applications (Yue et al., 2021).

1.4. Transportation of Hydrogen

The transportation of hydrogen energy is a significant domain for any successful hydrogen economy. The important factors that can affect the choice of mode of hydrogen transportation are the application, the density of demand, and the distance from the production site to the points of delivery (Dagdougui et al., 2018). Transportation of hydrogen can either be by truck, pipeline, rail, and ship making use of the storage techniques mentioned above. Compression is the general transport method used for trucks while liquefaction has also gained increasing recognition for utilization at distances of 1000 km. Pipelines also play a very key role in the transportation of huge quantities of compressed hydrogen gas over a long distance for domestic use (i.e., transmission) as well as distribution to various locations of use in a network (i.e., distribution) (Bruce et al., 2018).

1.5. Applications of Hydrogen

Hydrogen has various applications as industrial feedstock, electricity generation, transport, and heating systems. Industrial application mostly involves the use of hydrogen as a refining material, a reactant in the production of ammonia as a fertilizer, and the treatment of metals as well as food. In the transport sector, hydrogen is mainly used as a fuel in automobiles, and marine vessels and as a propellant in aerospace. It can also be used in the petroleum industry as a reactant in processing petroleum and petrochemical production and fuel cells to generate electricity by an electrochemical reaction process. Hydrogen can play a great role in heating homes and metallurgical processes (Abdalla et al., 2018).

1.6. Colors of Hydrogen

The colors of hydrogen and their source of production are given in the table below

Table 1: Classification of hydrogen based on colors and sources of production (Adapted from Talapko et al. 2023).

Colors of Hydrogen	Sources of production
White	Natural source (thermochemical process)
Green	Renewable energy
Blue	Methane with carbon Capture
Pink	Nuclear energy
Gray	Steam methane reforming
Turquoise	Methane pyrolysis
Brown	Gasification of lignite Coal
Black	Gasification of bituminous coal
Yellow	Powered electricity mixed- grid
Orange	Natural source (from oxidized ion)

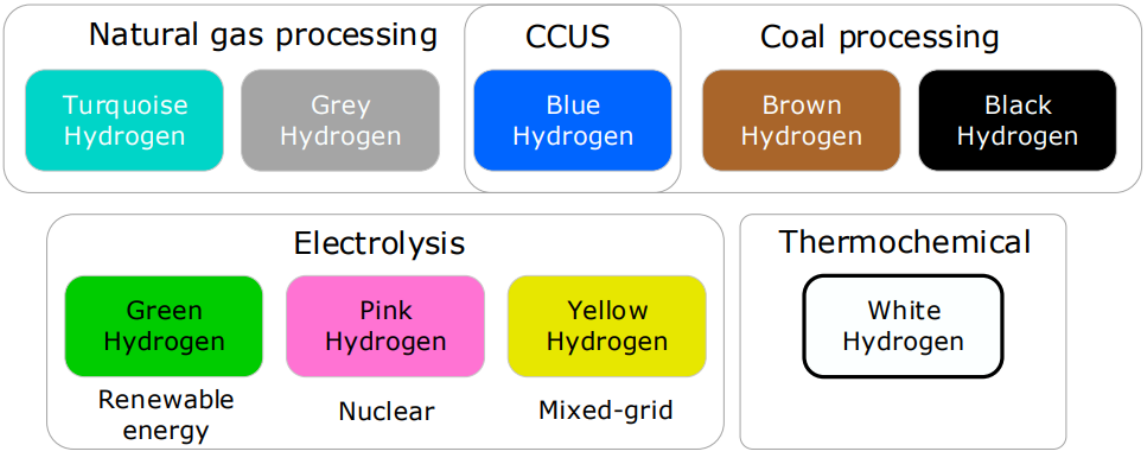


Figure 1: *Hydrogen colored-based classification (Panić et al., 2022)*

1.7. Hydrogen in the Energy Transition

The global energy demand is currently based on fossil fuel reserves of which its depletion is in evidence. The by-product of the utilization of fossil fuels is causing serious pollution problems in the world. The increasing amount of greenhouse gases (GHGs) in the atmosphere as a result of the use of fossil fuels has the potential of causing global warming and subsequent climate change (Anish Ghimire, 2016). Energy systems must transition towards technologies that can reduce the amount of greenhouse emissions and tackle the big problem of climate change. A potential player in this energy transition is hydrogen which can be applied in different sectors from industry to transport (Noussan et al., 2020). It is environmentally friendly due to its zero-emission and water as the only by-product (Akhlaghi and Najafpour-Darzi, 2020). Presently the production of hydrogen commercially accounts for 95% form methane steam reforming, the gasification of coal, and the electrolysis of water (Yang and Wang, 2017; Balachandar et al., 2019). Hydrogen will play seven main roles in the decarbonizing major sector of the economy. These roles included; (i) Enabling large-scale, efficient renewable energy integration (ii) Distributing energy across sectors and regions (iii) Acting as a buffer to increase system resilience (iv) Decarbonize transport (v) Decarbonize industry energy use (vi) Serve as feedstock using captured carbon (vii) Help decarbonize building heating (Hydrogen council, 2017).

1.8. Pathways to Biohydrogen Production

Biohydrogen is simply defined as the hydrogen obtained by microbial metabolism through various biological pathways. Microbes that can produce hydrogen from both cultivation and

any other form of organic waste materials include algae, bacteria, and archaea. When compared to the traditional way of producing hydrogen, its production mostly happens in mild conditions with less consumption of energy and impacts on the environment (Yin and Wang, 2022). The pathways to biohydrogen production mainly consist of bio-photolysis, fermentation, and biological electrolysis. They also can be classified as light-dependent and light-independent processes (Yin and Wang, 2022). Figure 2 gives the different biohydrogen production pathways

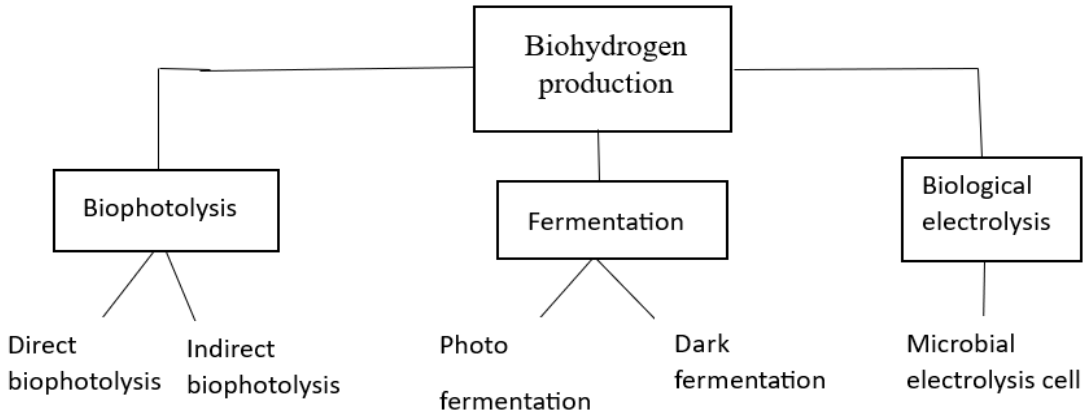


Figure 2: *Various pathways for biohydrogen production with energy source* ***(****Adapted from Yin and Wang, 2022****)***

1.8.1. Bio-photolysis

This is water-splitting photosynthesis which involves the production of hydrogen from water and sunlight using oxygenic photosynthetic microorganisms like cyanobacteria and green microalgae (Osman et al., 2020)**.** Bio-photolysis hydrogen production can be divided into direct bio-photolysis and indirect bio-photolysis**.**

1.8.1.1. Direct Bio-photolysis

This process involves the use of light energy by photoautotrophic organisms to convert water molecules into hydrogen with the catalytic activity of the hydrogenase enzymes under anaerobic conditions (Akhlaghi and Najafpour-Darzi, 2020). The overall reactions are given as follows:

$$H_2O \xrightarrow{\text{ligh}} 1/2O_2 + 2H^+ + 2e^- \quad (1.1)$$

$$2H^+ + 2e^- \xrightarrow{\text{Hydrogenase}} H_2 \quad (1.2)$$

The two groups of organisms that can carry out the production of hydrogen by this process are cyanobacteria and green algae. Green algae can produce hydrogen using sunlight energy under

anaerobic conditions or under dark conditions, hydrogen uptake can occur through the Carbon dioxide-fixation process (Kim and Kim, 2011). Algae uses the process of photosynthesis to split water molecules into hydrogen ions (H^+) and oxygen. The hydrogen (H^+) produced is then converted by hydrogenase enzymes into hydrogen gas. Other green algae like Scenedesmus obliquus, Chlorococcum littorale, Platymonas subcordiformis, and Chlorella fusca have been observed to carry out hydrogenase activity (Pareek et al., 2020, Fakhimi & Tavakoli, 2019). Figure 3 illustrates the process of direct bio-photolysis.

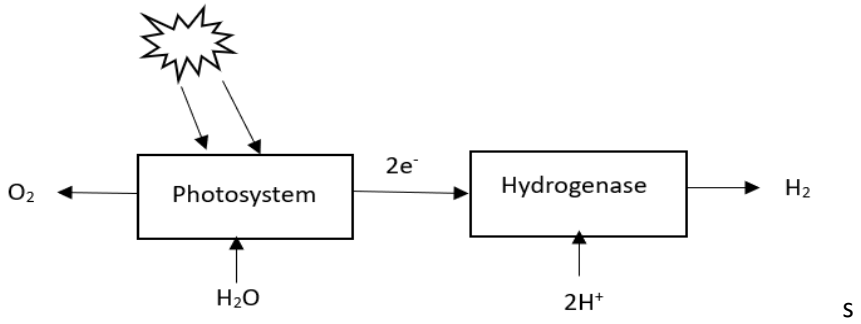


***Figure 3**:Direct bio-photolysis process (adapted from Abdallaa et al., 2018)*

The key problem of this process is the sensitivity of the hydrogenase enzyme to oxygen and the supporting pathway for reductant generation (Pareek et al., 2020).

1.8.1.2. Indirect Bio-photolysis

This process of producing hydrogen consists of a two-step photosynthetic conversion of light energy to carbohydrates which is a form of chemical energy. The first stage involves the use of light energy to produce oxygen (O_2) and carbohydrates. In the second stage, under anaerobic conditions, carbohydrate is converted into carbon dioxide (CO_2) and hydrogen(H_2) with light energy (Osman et al., 2020). The equations are given below

$$6CO_2 + 12H_2O + light\ energy \rightarrow C_6H_{12}O_6 + 6O_2 \quad (1.3)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2O + 2CH_3COOH + 2CO_2 \quad (1.4)$$

$$2CH_3COOH + 4H_2O + light\ energy \rightarrow 8H_2 + 4CO_2 \quad (1.5)$$

In this process, the hydrogenase pathway is used by green algae to produce hydrogen through the process of photosynthesis using sunlight energy under the deprivation of sulfur. When there is sulfur deprivation, large quantities of internal protein and starch are consumed by microbial cells. Subsequently, this catabolic conversion would indirectly maintain hydrogen production

(Show et al., 2019). This process solves the problem of the sensitivity of hydrogenase to oxygen.

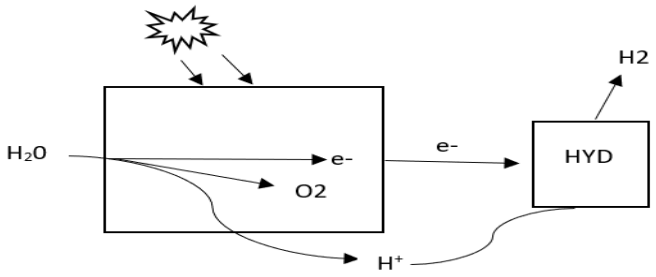


Figure 4: *Impeded photosynthesis under sulfur deprivation causing net oxygen consumption by cell respiration in anaerobic indirect Photolysis (adapted from Show et al., 2019)*

1.8.2. Biological Electrolysis

This involves the use of bio-electrochemical systems such as microbial fuel cells (MFC) and microbial electrolysis cells (MEC) to produce hydrogen from a variety of organic substrates. In an MFC, there is the oxidation of organics in an anode chamber by microbes which results in the release of protons, electrons, and carbon dioxide (CO_2). The released electrons are then migrated from the anode to the cathode through an external circuit, and the transfer of protons from the anode chamber to the cathode chamber takes place in the liquid phase. In the cathode chamber, there is a consumption of electrons from the cathode producing water (H_2O) along with hydrogen ion (H^+). This process has attracted lots of intention due to its ability to produce electric current from organics which offers a good potential simultaneously for the treatment of waste and the generation of electricity. However, the electricity produced by this method was found to be of low economic and environmental value which cannot compete with other sources of energy and hence MEC was developed (Logan et al., 2006; Yin and Wanga, 2022).

MEC is also referred to as a bio-catalyzed electrolysis cell or electro-fermentation. It is made up of two electrodes, a cathode, and an anode, which have the possibility of being placed in the same single chamber (single-chamber MEC) or two separate chambers (two-chamber MEC). There is a similarity in the reactions at the anode chamber with an MFC while the difference is in the cathode chamber in which hydrogen production replaces oxygen consumption. This technology required an external source of power to supplement the additional voltage needed for the electrolysis of water. The lack of oxygen in the cathode simplifies its design and speeds up the anaerobic growth of microbes in the anode chamber (Osman et al., 2020; Yin and Wang, 2022).

For the production of hydrogen, MEC requires a small external potential of more than 0.110V. The usage of batteries as an external power source is mostly considered, but also power generated from renewable solar, wind, MFCs, and waste heat can be utilized. The equation below shows the production of hydrogen from acetate (Cheng and Logan, 2007; Chandrasekhar et al., 2015).

Anode: $CH_3COOH + 2H_2O \rightarrow 2CO_2 + 8e^- + 8H^+$ (1.6)

Cathode: $8e^- + 8H^+ \rightarrow 4H_2$ (1.7)

Overall: $CH_3COOH + 2H_2O \rightarrow 2CO_2 + 4H_2$ (1.8)

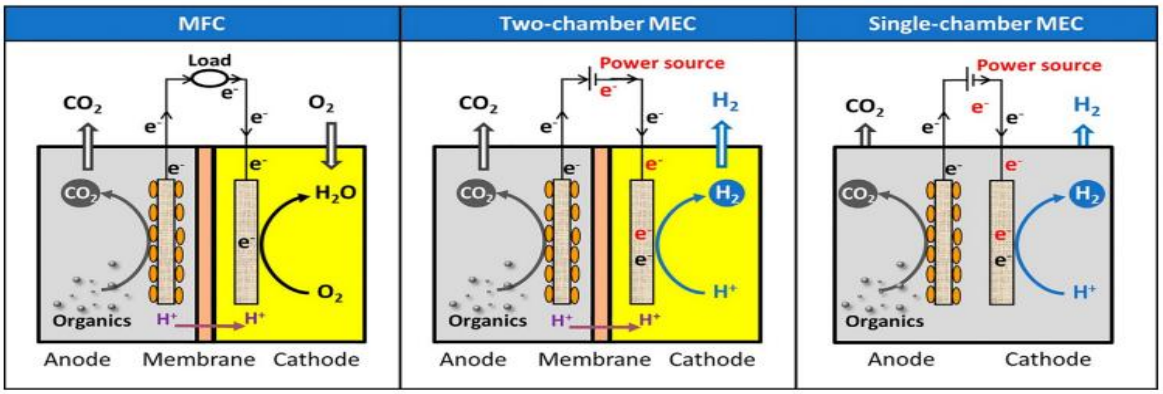


Figure 5: *Schematic diagram of a typical microbial fuel cell (MFC) and a microbial electrolysis cell (MEC) (Yin and Wanga, 2022).*

1.8.3. Fermentation

This is a method of generating energy that involves an endogenous electron acceptor from the oxidation of materials containing organic waste using a variety of microorganisms. The products of a fermentation process mainly depend on the type of catalyst used (isolated enzyme or microorganism producer), the organic substrate coupled with other process parameters. The nature of the fermentation process may either be aerobic or anaerobic (Osman et al., 2020). The two methods of biohydrogen production from fermentation are dark fermentation and photo-fermentation.

1.8.3.1. Dark Fermentation

This process involves the production of hydrogen from waste biomass in the absence of sunlight by utilizing microbial resources (Sarangi and Nanda, 2020). It is referred to as an anaerobic process, in which there is the decomposition of organic material usually carbohydrates like glucose by bacteria to carbon dioxide, hydrogen, and low-weight organic

acids. The hydrolysis of higher carbohydrates such as hemicelluloses, cellulose, starch, or molasses produces hexoses or pentoses (Sołowski, 2018).

Dark fermentation pathways include Hydrolysis, Acidogenesis, and acetogenesis. Hydrolysis involves the breaking of organic substrate into smaller components which are subsequently converted into volatile fatty acids (VFAs), ethanol, Carbon dioxide (CO_2), and hydrogen (H_2) by acidogenic bacteria. These fermentation products are later converted by Acetogenic bacteria into acetic acid, carbon dioxide, and hydrogen (Ofomatah et al., 2021).

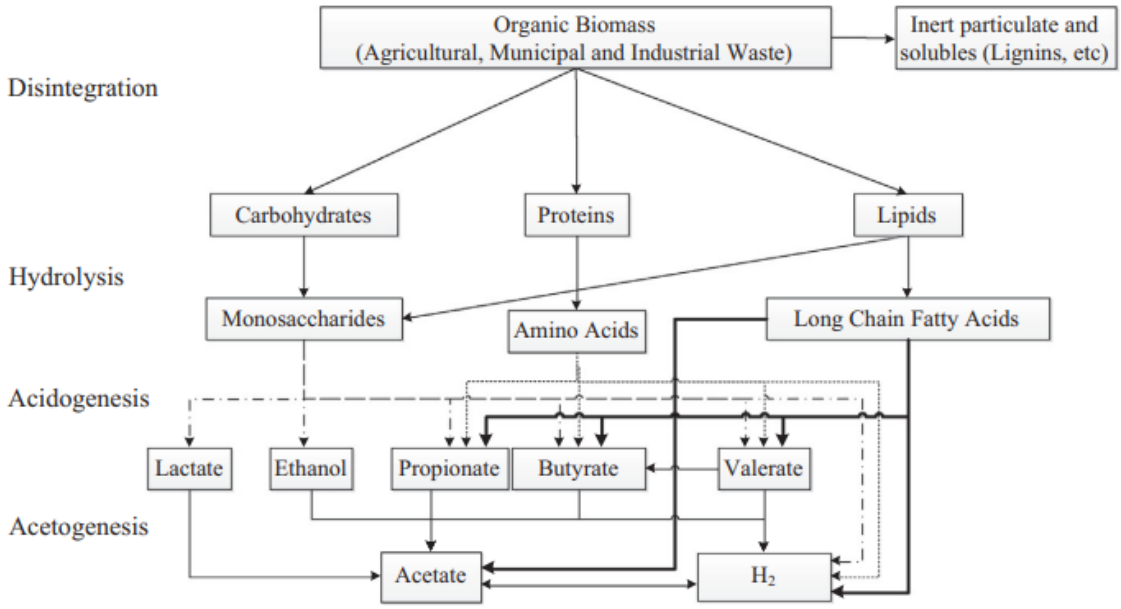


Figure 6: *Biodegradation steps and microbiological pathways involved in the fermentative breakdown of waste biomass (Ghimire et al., 2015)*

The production of molecular hydrogen (H2) occurs during the process of the disposition of the excess electrons under the activity of hydrogenase enzymes. Protons (H^+) can behave as electron acceptors under anaerobic conditions to neutralize the generated electrons through the oxidation of organic substrates leading to the production of hydrogen (H2) (Ghimire, 2015).

The formation of Molecular hydrogen follows mainly two pathways in the presence of specific coenzymes, i.e., either by formic acid decomposition route or by the re-oxidization of nicotinamide adenine dinucleotide (NADH) route (D.-J. Lee et al., 2011).

$$NADH + H^+ + 2Fd^{2+} \rightarrow 2H^+ + NAD^+ + 2Fd^+ \quad (1.9)$$

$$2Fd^+ + 2H^+ hydrogenese \rightarrow 2Fd^{2+} + H_2 \quad (1.10)$$

Glucose is converted into pyruvate which is linked with the conversion of NADH from NAD^+ by anaerobic glycolysis. This is shown in equation …

$$C_6H_{12}O_6 + 2NAD^+ \rightarrow 2CH_3COCOOH + 2NADH + 2H^+ \quad (1.11)$$

The re-oxidization of NADH by some specific microorganisms under acidogenic conditions in the presence of ferredoxin oxidoreductase and hydrogenase leads to the generation of hydrogen (D.-J. Lee et al., 2011).

Dark fermentation (DF) is viewed as one of the most promising and practical approaches for the production of hydrogen due to its fast conversion efficiency (Ventura et al., 2021). When compared to photo-fermentation, it is inexpensive, does not require sunlight or illumination, has less maintenance, and has small bioreactor types (Sarangi and Nanda, 2020).

The process of dark fermentation needs fermentative microorganisms, organic substrates, and an anaerobic environment. Dark fermentative bacteria may include strict anaerobes such as Clostridium and Desulfovibrio, species or facultative anaerobes such as Enterobacter, Escherichia coli, Bacillus, Citrobacter, and Klebsiella species. No external energy source is required by the fermentative bacteria except for the work of the reactor. Dark fermentation has the potential to use a different variety of substrates (agricultural, forestry, pulp/paper, and food industries waste) and low consumption of energy hence it is economically feasible and has a great role in the production of hydrogen and waste reduction. The production of hydrogen also generates dark fermentative effluent (DFE) like volatile fatty acids (acetic acid, butyric acid, lactic acid, etc.) that can be utilized as a feedstock for photo-fermentation (Morsy, 2017; Das and Basak, 2020; Ding et al., 2009). These VFAs are toxic to the hydrogen producers with the potential of inhibiting metabolic activity by deactivating enzymes for solvent production. This will in turn hinder substrate utilization and microbial growth (Anuar et al., 2020). The main route for producing hydrogen is the acetate–butyrate fermentation pathway of which theoretically, 4 mol of hydrogen can be produced when acetate is the main fermentation product and 2 mol when butyrate is generated, as shown in equations (1.9) and (1.10) (Anuar et al., 2020).

$$C_6H_{12}O_6 + 2H_2O \rightarrow 4H_2 + 2CH_3COOH + 2CO_2 \quad (1.12)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3CH_2CH_2COOH + 2CO_2 \quad (1.13)$$

In dark fermentation, hydrogen production is influenced by the following factors: type and substrate concentration, type of microorganisms, pH, inoculum age, temperature, metabolic pathway, pure culture, or mixed culture of bacteria (Das and Basak, 2020).

1.8.3.2. Dark Fermentative Microorganisms

Microorganisms such as bacteria are capable of breaking down organic matter to form hydrogen in fermentation-based systems. These hydrogen-producing microorganisms can occur in nature as single strains or mixtures of various species (Łukajtis et al.,2018).

Dark fermentative microorganisms are mainly divided into facultative anaerobic bacteria, obligate anaerobic bacteria, and thermophiles.

1.8.3.2.1. Facultative Anaerobic Bacteria

The production of ATP by facultative anaerobes takes place in the presence of oxygen through the process of anaerobic respiration and in the absence of oxygen, ATP is produced by anaerobic fermentation. Examples of common facultative anaerobes are Enterobacter sp. Which can produce hydrogen under anaerobic conditions. Members that are part of the group Enterobacteriaceae have many properties that favor the production of hydrogen due to their easy handling in a reactor during anaerobic hydrogen production (Balachandar et al.,2013). Furthermore, the high partial pressure generated in the reactor does not have an effect on the fermentation yield. The group members of Enterobacteriaceae include Arsenophonus, Branneria, Buchnera, Budvicia, Buttiauxella, Cedecea, Citrobacter, Cronobacter, Dickeya, Edwardsiella, Enterobacter, Erwinia, Escherichia, Ewingella, Hafnia, Klebsiella, Kluyvera, Coserella, Leclercia, Leminorella, Moellerella, Morganella, Obesumbacterium, Pantoea, Pectobacterium, Photorhabdus, Plesiomonas, Pragia, Proteus, Providencia, Rahnella, Raoutella, Salmonella, Samsonia, Serratia, Shigella, Sodalis, Tatumella, Thorsellia, Trabulsiella, Wiglesworhtia, Xenorhabdus, Yersinia, and Yokenella (Łukajtis et al.,2018).

According to Balachandar et al. (2013), two types of Enterobacteriaceae strains have been studied extensively which are Enterobacter aerogenes E.82005 and Enterobacter cloacae IIT-BT 08. It was observed that under anaerobic batch cultivation, E. aerogenes E.82005 produced 1.0 mol H2/mol glucose at a rate of 21 mmol/liter/h.

1.8.3.2.2. Obligate Anaerobic Bacteria

In this type of microorganism, a strict anaerobic condition is needed. These anaerobes are Clostridia, Metylotrophs, Methanogenic bacteria, Archaea, and Rumen bacteria (Łukajtis et al., 2018). To date, plenty of research has been carried out using obligate bacteria for the production of hydrogen due to their ability to make use of various types of carbohydrates, in addition to different varieties of wastewater. They also have the potential to produce a higher rate of hydrogen production. The most widely used obligate anaerobes are clostridia species and the production of hydrogen mainly occurs during the exponential growth phase. (Balachandar et al.,2013). There is a metabolic shift in the direction of liquid organic compound production mainly as volatile fatty acids (VFAs) in the stationary phase. The following species: C. butyricum, C. beijerinckii, C. welchii, C. thermolacticum, C. thermocellum, C. paraputrificum C. pasteurianum, C. beijerincki, Clostridium scatologenes, C. acetobutyricum, and C. Bifermentants (Łukajtis et al.,2018).

1.8.3.2.3. Thermophiles

These groups of microorganisms are mostly considered obligate anaerobes mainly found in different geothermally heated parts of the earth like hot springs and deep-sea hydrothermal vents. Examples of thermophiles are the strain of Caldicellulosiruptor, Thermoanaerobacterium, Thermoanaerobacter, and Thermotoga. At the moment there is an extensive study available on five different species that belong to the genus Caldicellulosiuptor. They show unusual behavior in their ability to degrade cellulose at elevated temperatures (up to 78^{O}C). Products like ethanol, lactate, and acetate are the major end metabolites. Notwithstanding the higher production of hydrogen, ethanol, and lactate formation are rather low (Balachandar et al.,2013).

Figure 7 shows the dark fermentative hydrogen pathways using strict anaerobes and facultative anaerobes metabolic pathways.

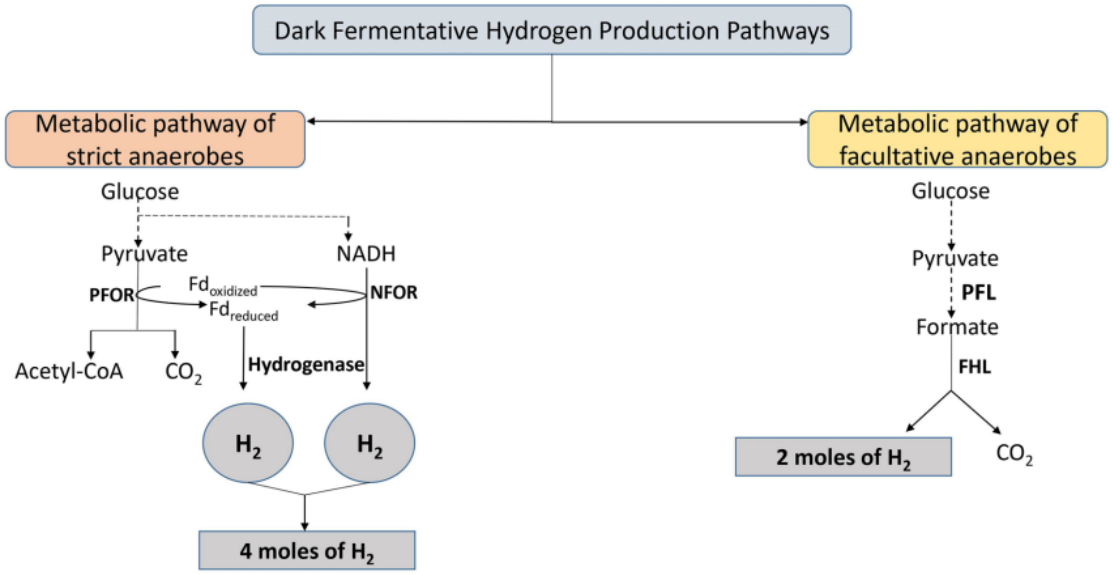


Figure 7: *Metabolic pathway of strict and facultative anaerobes in dark fermentative hydrogen production (Jayachandran et al., 2022).*

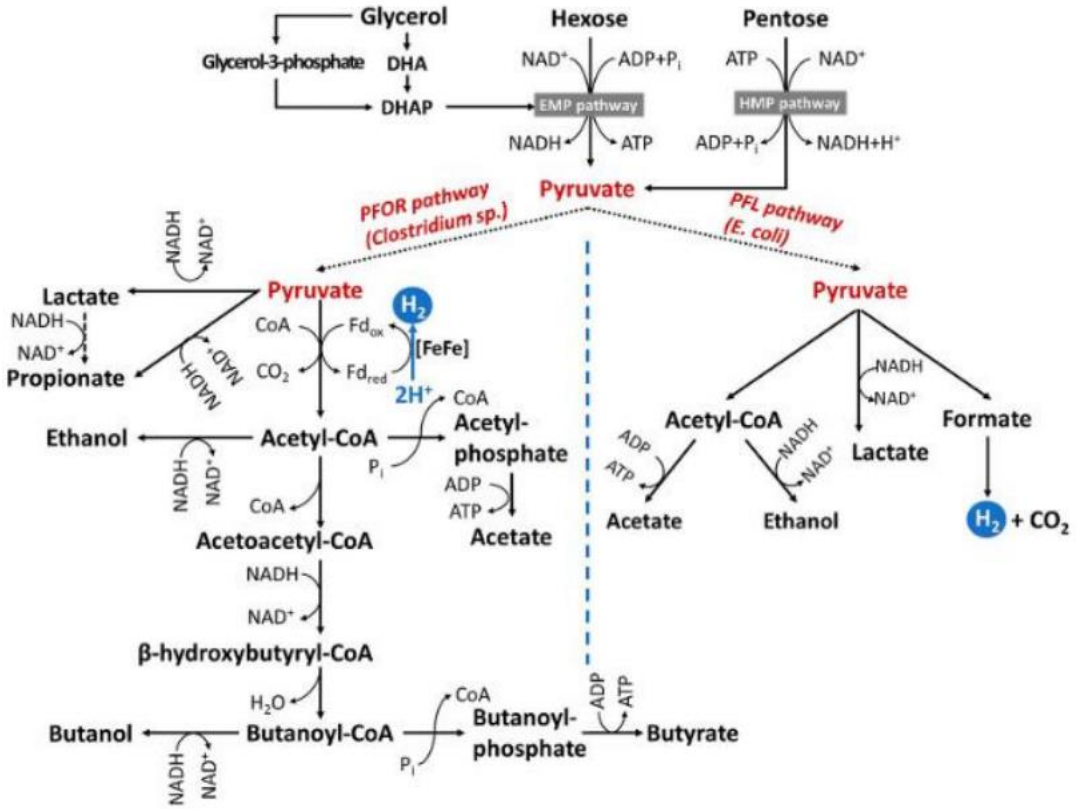


Figure 8: *Metabolic pathways in dark fermentation for biohydrogen production (Yin, Y., & Wang, J. 2022).*

1.8.3.3. Photo-fermentation

This is the process of conversion of organic substrates into hydrogen and carbon dioxide using photoheterotrophic bacteria under light and anoxygenic conditions. The suitable microorganisms that are usually used in this process are purple non-sulfur bacteria (PNSB) due to their ability to produce hydrogen with high yields from a different organic substrate. The most utilized organic substrates used are fermentation acids like acetate, lactate, propionate, and succinate; aromatic acids include cinnamate and benzoate; alcohols such as ethanol and propanol and sugars such as glucose. Anaerobic bacteria strains such as Rhodobacter, Rhodobium, Rhodopseudomonas, and Rhodospirillum are mostly used for photo-fermentation (Rai and Singh, 2016; Ventura et al., 2016; Ventura et al., 2021).

The formation of hydrogen occurs when molecular nitrogen is reduced in the presence of nitrogenase and also reduced protons to molecular hydrogen (Łukajtis et al., 2018). The enzymes hydrogenase and nitrogenase in PNS bacteria play a key role in the photo-fermentation production of hydrogen. The enzyme nitrogenase is mainly responsible for the production of molecular hydrogen under anaerobic conditions. There is a fixation of nitrogen into ammonia by nitrogenase and during nitrogen absence, nitrogenase makes use of the reductants along with ATP to produce hydrogen ($2H^+$ $2e^-$ + 4 ATP→ H2 + 4ADP + Pi). For the effective photo-fermentative production of hydrogen, a sufficient ATP supply is highly required (Mishra et al., 2019). The major factors that affect the production of hydrogen by photo-fermentation are microbial species, inoculum age, light intensity, pH substrate, and operating temperature (Yin and Wanga, 2022). An example of hydrogen production from acetate is given:

$$CH_3COOH + 2H_2O + light\ energy \rightarrow 4H_2 + 2CO_2 \quad (1.14)$$

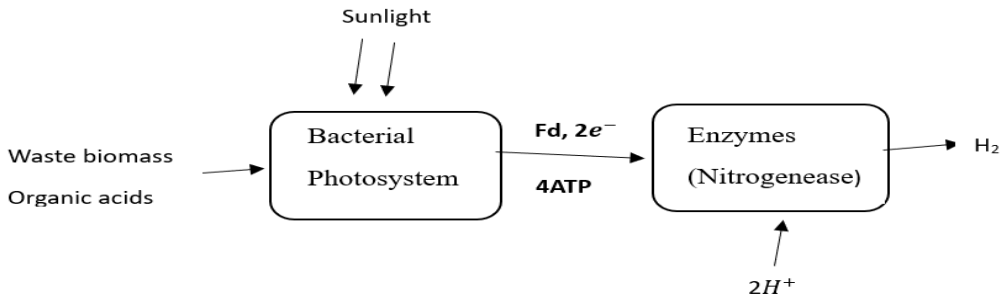


Figure 9: *Schematic diagram of the photo-fermentation process for biohydrogen formation (Adapted from Sarangi and Nanda, 2020)*

1.9. Factors Affecting the Biohydrogen Dark Fermentation Process

The factors affecting the production of hydrogen by fermentation are discussed below;

1.9.1. Temperature

Temperature is one of the key factors affecting the fermentation process since it can alter both the microbial use of the substrate and its specific growth rate, the production of hydrogen, and the formation of the metabolic product (Chandrasekhar et al., 2015). The different temperatures at which dark fermentation reactions are carried out are psychrophile (0–25 °C), mesophilic (25–45 °C), thermophilic (45–65 °C), extreme thermophilic (65–80 °C), and hyper-thermophilic (above 80 °C) (Levin et al., 2004; Łukajtis et al., 2018). It is stated by Akhlaghi and Najafpour-Darzi (2020) that each microorganism strain and the nature of the substrate have a specific optimum temperature for growth. The selection of optimum temperature largely depends on the type of bacteria used for both pure and mixed cultures during fermentation. The activity of specific enzymes during the fermentation process is dependent upon their optimal temperature value. A higher or lower temperature other than the optimal temperature will decrease the activity of the enzymes. Hence, the fermentation optimum temperature largely depends on the type of bacteria and the kind of substrate utilized. However, it is believed that the use of thermophilic and extreme thermophilic temperatures works better with the substrates undergoing hydrolysis during fermentation. Higher temperatures cause an increase in the enzyme's activity responsible for hydrolysis (Łukajtis et al., 2018). A study conducted by Sattar et al. (2016) shows the effect of temperature on biohydrogen production by batch fermentation in anaerobic bioreactors with ranges of temperatures between 37^0C and 55^0C using rice straw, rice husk, rice waste, and rice bran as substrates. It was observed that there was an increase in hydrogen production in rice bran, rice straw, and rice husk apart from rice waste as temperature increased. The maximum hydrogen yield observed with rice bran was 23.51% under thermophilic conditions (Sattar et al., 2016).

1.9.2. Hydraulic Retention Time (HRT)

This is a significant reactor control parameter that has a key influence on the rate of hydrogen production and the reactor's operational behavior (Sivagurunathan, 2015). Hydraulic retention time (HRT) is a measure of the average length of time the substrate remains in the bioreactor and is utilized by microorganisms. The maximization of the yield of hydrogen production by fermentation requires an optimal HRT to minimize the formation of unwanted metabolites such as ethanol and organic acid (Akhlaghi and Najafpour-Darzi, 2020). The optimal HRT value is

dependent on the kind and substrate concentration and more so on its biodegradability, support material for the case of an immobilized system, and the kind of bioreactors used. The rate of hydrogen production increases for a certain range of optimal HRT values and decreases when this optimal range is exceeded with an increase in HRT. (Łukajtis et al., 2018; Akhlaghi and Najafpour-Darzi, 2020). To obtain satisfactory H2 yields, the optimum HRTs for a variety of substrates were between 8h and 14 h (Chandrasekhar et al., 2015). Studies conducted show that longer HRTs allow enough time for the microorganism to sufficiently metabolize the substrate. The production of VFAs can be reduced with an increase in HRT due to the acidogenic-solventogenic transition stage (Strazzera et al., 2018; Sekoai et al., 2021). According to Sivagurunathan et al. (2015), hydrogen-producing bacteria prefer relatively short hydraulic retention times.

1.9.3. Hydrogen Partial Pressure

This is also a key factor in the synthesis of biological hydrogen. The production of hydrogen by dark fermentation using anaerobic bacteria is determined by the metabolic route and end products (Junghare et al., 2012). The production of hydrogen by dark fermentation is greatly influenced by the hydrogen partial pressure inside the biohydrogen reactor. Low partial pressure in the headspace of the reactors gives rise to a mass transfer of hydrogen from the liquid phase to the gas phase. In the fermentation process, the hydrogenase is involved in reversible oxidation and reduction of ferredoxin. Therefore, an increase in the concentration of hydrogen in the liquid phase causes a less favorable oxidation of the ferredoxin and the reduction of ferredoxin occurs which reduces the production of hydrogen (Ghimire et al., 2015).

Additionally, an increase in hydrogen concentration will shift the metabolic pathways toward the formation of inhibitory reactions like lactate, ethanol, acetone, and butanol (Sekoai et al., 2021). It is therefore very important to remove hydrogen as it is formed to maintain a constant production rate of hydrogen. The use of sparging external gases such as carbon dioxide (CO2), nitrogen (N2), and argon (Ar) or circulating gas mixtures is employed in controlling the partial pressure of hydrogen. However, the use of sparging gases has the potential of diluting the hydrogen produced and therefore a downstream purification process is required to obtain hydrogen (Akhlaghi and Najafpour-Darzi, 2020). Balachandar et al. (2013) show some optimal partial pressure of hydrogen depending on the temperature that is used such as 50 kPa at 60C, 20 kPa at 70C, and 2 kPa at 98C.

1.9.4. pH

The hydrogen ion concentration is a key factor that plays a great role in dark fermentation processes. The pH values affect the yield of hydrogen production, and the microorganism's metabolic pathways such as the hydrogenase activity coupled with their morphology, and cell structure. Enzymes taking part in the metabolic processes of bacteria are only active at certain pH ranges which are their optimal pH values. Therefore, controlling the pH and maintaining it at a constant optimal value is very paramount during the fermentation process due to the formation of organic acids which can lower the pH of the medium and gives rise to the inhabitation of the hydrogen-producing bacteria. The optimal pH is dependent on the type of substrate (Łukajtis et al., 2018; Ziara et al., 2018). According to Guo et al. (2010), the optimal pH value for hydrogen production for food waste is pH 5.0-6.0 while that for crop residues and animal manure is recommended at a neutral pH value. It is reported by Ghimire et al. (2015), that in the dark fermentation of sucrose, the optimal pH range for the production of biohydrogen varies from pH 4.5 to 9. It was observed that Low pH values (below 5) could cause the inhibition of the hydrogenase activity leading to the termination of hydrogen production (Bao et al., 2013). Pason et al. (2020) were able to conduct a study of the production of hydrogen utilizing initial pH values ranging from 5.0 to 8.0 from cassava pulp. The optimum condition for hydrogen production was observed at pH 7.0 with a hydrogen yield of 230.12ml.

1.9.5. Nutrient

For the growth and activity of bacteria, fermentative microorganisms need nutrients. The needed nutrients for enzymatic activities and the growth of biomass in the fermentation processes are nitrogen, phosphate, metal ions, and other micronutrients which pose a great effect on the production of hydrogen (Ghimire et al., 2015). However, the presence of an excess amount of nitrogen has the potential to not only affect the microorganism's pH used for hydrogen production but also inhibit the nitrogenase activity. Furthermore, an increase in the concentration of nitrogen can lead to ammonification which is not desirable for hydrogen production. Therefore, an optimal C/N ratio is needed for the growth of microbes and to improve hydrogen yield. Phosphorus present in the form of Adenosine triphosphate plays an important role in the generation of energy in the bacterial cell. When the concentration of phosphorus is increased there is excessive production of VFAs which can severely reduce the yield of hydrogen. The addition of suitable metal ions in the fermentation process plays a key role in the activation of the enzymes and coenzymes involved in microbial metabolism and cellular transport which are also important for the growth of cells. There is a specific role for

each of the metal ions during cell metabolism and any distortion can affect that role (Chandrasekhar et al., 2015; Wang et al., 2009; Lin and Shei, 2008).

The effect of the C/N ratio on the production of hydrogen was conducted by Saidi et al. (2018) from fruits, fish wastes, and vegetable wastes using anaerobic co-digestion. At a C/N ratio of 12, higher production of hydrogen was obtained at around 132mmol/L.

1.9.6. Organic Loading Rate (OLR)

This shows the quantity of substrate that is fed into the reactor a day and per unit working volume (Strazzera et al., 2018). It is a key operational parameter that affects the performance of dark fermentation due to the fact that the availability of substrate for the microbial community is determined by this parameter. It is generally stated that a higher organic loading rate is achieved either by shortening the hydraulic retention time or increasing the concentration of the substrate or shortening the HRT and increasing the substrate concentration (García-Depraect et al., 2020). The efficiency of biohydrogen production could be enhanced by the higher substrate concentrations and is a high possibility for substrate or product inhabitation occurring if the substrate/organic material exceeds the threshold loading rate. Furthermore, there is variation in the optimal substrate concentration with the substrate and inoculum and therefore no universal optimal substrate concentration for the fermentation process (Sivagurunathan et al., 2015; Lin et al., 2012). The determination of the optimal OLR must be carried out for each specific type of feedstock (García-Depraect et al., 2020). The optimal ORL of a bioreactor is dependent upon the following factors such as substrate loading rate, substrate type, and concentration, pH conditions, temperature, and the type of reactor. During the metabolization of the substrate by biohydrogen-producing microorganisms, the pH decreases as a result of the formation of VFAs which inhibit the substrate conversion efficiency. A very high organic loading decreases the hydrogen yield and is therefore not favorable (Arimi et al., 2015).

1.9.7. Volatile Fatty Acids (VFAs)

The fermentation process of producing biohydrogen is accompanied by the production of different types of liquid-state metabolites such as alcohol and VFAs etc., their high presence and concentration have an impact on the production and yield of hydrogen (Salem et al., 2018). These metabolic end products harm the yield of hydrogen. The dominant metabolites produce during fermentative hydrogen production are ethanol, ethanol, acetic acid, butyric acid, and propionic acid. Increasing the concentrations of these metabolites leads to an increase in the

ionic strength medium which will provoke cellular lysis. Furthermore, there is a possibility of permeation of the cell membrane of hydrogen-producing bacteria by protons when metabolites concentrations increase which will disrupt the physiological balance in the cell (Balachandar et al., 2013). Restoring the physiological balance in the cell requires the use of maintenance energy. Redirection of this maintenance energy will compromise the growth of bacteria and subsequently the production of hydrogen significantly (Balachandar et al., 2013).

1.10. Technical Challenges in Biological Hydrogen Production Pathways

Table 2: *Biological Pathways for Hydrogen Production and Technical Limitations (Adapted from* Chandrasekhar et al., 2015)

Type of bioprocess	Technical challenges
Dark fermentation	➢ Low Hydrogen yield ➢ Low substrate conversion efficiency ➢ Thermodynamic limitation ➢ Separation of hydrogen production from hydrogen and carbon dioxide is needed
Photo-fermentation	➢ An external source of light is needed ➢ Low hydrogen yield ➢ Low light conversion efficiency
Direct bio-photolysis	➢ Oxygen generation caused by the activity of PS II ➢ Low hydrogen yield ➢ Low light conversion efficiency
Indirect bio-photolysis	➢ Requirement of an external light source ➢ The hydrogen production rate is low
MEC	➢ Requirement of an external light source ➢ A catalyst is required for the electrode ➢ The need for external voltage

1.11. Biogas production from pineapple peels waste

Biogas from pineapple waste is mainly made of methane, carbon dioxide, and other gases like hydrogen sulfide, oxygen, nitrogen, and water. There is a variation in the percentage of methane present in the biogas between 55 % and 80 % depending on the process and the type of organic matter. The conversion of pineapple peel waste to methane and carbon dioxide occurs in four stages: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, and therefore requires different groups of microorganisms. In the hydrolysis stage, the organic substrate is converted into smaller components which are subsequently converted into volatile fatty acids (VFAs), ethanol, carbon dioxide, and hydrogen by acidogenic bacteria. These fermentation products are later converted by Acetogenic bacteria into acetic acid, carbon dioxide and hydrogen. Finally, methanogenic bacteria use hydrogen and acetate to produce methane and carbon dioxide (Ofomatah et al., 2021).

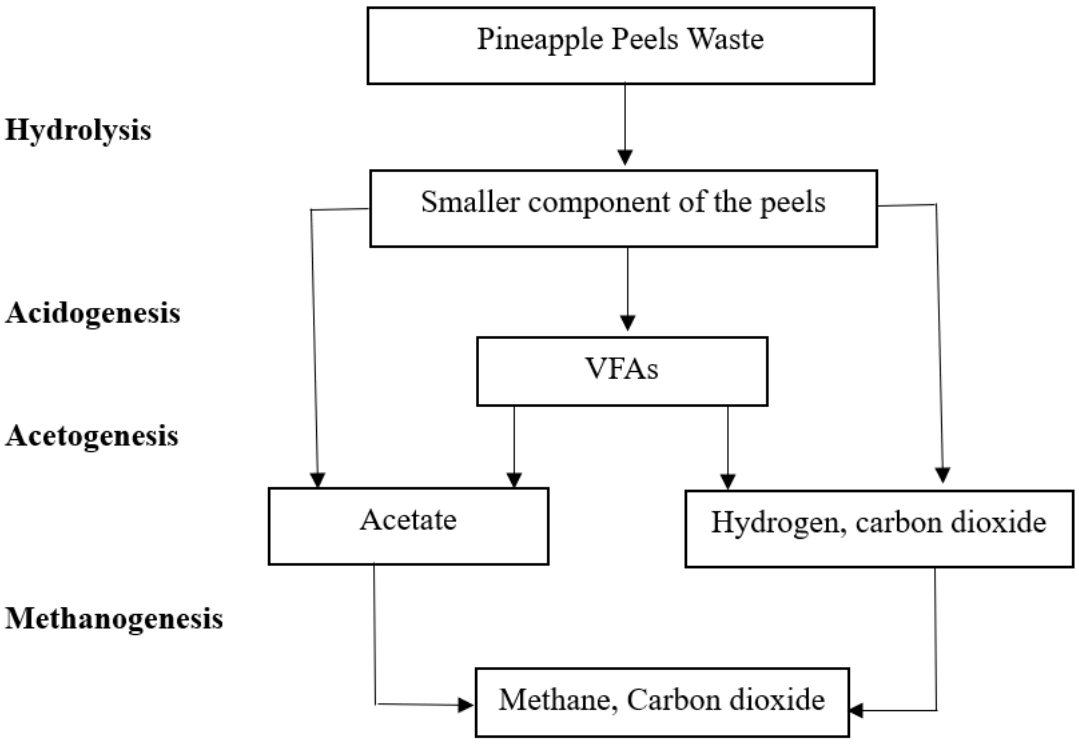


Figure 10: *Flow chart of biogas production from pineapple peels (adapted form Ghimire et al., 2015)*

Chulalaksananuku et al. (2012) conducted a biogas production from pineapple peels waste for batch process with a maximum biogas production at 94 liters/ kg of COD removal at HRT of 20 days with a methane content of 48% at a C/N ratio of 20. While the fed-batch process with

a maximum biogas production at 65.96 liters/ kg of COD removal at HRT of 20 days at an organic loading rate of 1 kg/m3 /day, with a methane concentration of 32.96%.

Some literature focused on the use of pineapple wastes to produce biogas such as biomethane, biohydrogen, and biomethane (a mixture of biomethane and biogas). The research was conducted by (Aili Hamzah et al., 2021) using a two-stage anaerobic reactor system using pineapple peels and the maximum methane yield was 174.6 mL CH4/g COD with 66.1% methane content. Another study using the same two-stage reactors was also used to produce biogas from the co-digestion of pineapple waste mixture and swine manure. The produced biogas contained 65% methane and on the application of heat pre-treatment to the swine manure before the process, there was an increment of methane content from 64.82% to 70.91%. Azevedo et al. (2021) investigated the co-digestion of pineapple peel and pig slurry. It is reported that the co-digestion of pineapple peel and pig slurry enhanced the synergetic effects between methane production, C/N ratio, and process efficiency. This synergic effect is responsible for anaerobic digestion improvement.

Table 3: Information on Other Research for the Production of Methane from Biogas Using Pineapple Peels (Aili Hamzah et al., 2021)

Substrate	**Co-substrate**	**Operating conditions**	**Pre-treatment**	**Methane yield**
Pineapple peels	-	T= 37 ^{0}C; STD= VDI 4630	Hydrogen peroxide and sulfuric acid pre-treatment	70
Pineapple peels	-	T = 37^{0}C; pH=7; Agitation =150 rpm	No pre-treatment	66.10
Pineapple peels	Cow manure and novel microbial consortia	T = 37 ◦C; pH = 7	No pre-treatment	56.61

Notes: T= Temperature; STD = Standard; VDI = Verein Deutscher Ingenieure standard.

CHAPTER 2: MATERIALS AND METHODS

2.1. Study Area

This research work was focused on the utilization of pineapple peel waste from Togo. The cultivation of pineapple fruits occurs in two agro-ecological areas which are the Maritime area which includes the whole region and the south of the plateau region and the Plateau area which entails the north-west of the plateau region. Two major varieties of pineapple are cultivated in Togo and these are the Smooth Cayenne and the Brazza (or Sugarloaf). The Smooth Cayenne contains a firm yellow flesh and a tangy taste, and it is preferred in organic agriculture, export, and processing. The Brazza variety is white in flesh, sweet, and juicy. Its usage is in local and regional markets (European Commission, 2020).

2.2 Materials

The test material for this process was pineapple peel wastes from pineapple fruits in Togo.

Other laboratory materials that were used for this process are Crucible, Laboratory binder oven, Desiccator, Electronic balance, Muffle furnace, and incinerator.

2.3 Sample collection

The pineapple fruits were bought from one of the Togo markets, the Hanoukopé fruit market. The pineapple fruits were washed and then peeled off and the peels were later taken to the physics laboratory at the University of Lomé for drying.

Figure 11: *Pineapple fruit collection site in Lomé, Togo*

2.4 Sample Preparation and Pre-treatment

The sample preparation was carried out at the physics department where the pineapple peels were sun-dried for seven days. The dried pineapple peels were ground in a motor and pistol into a small size and stored in a rubber bottle for one month before transportation to Germany for further analysis. In Germany, no further pre-treatment was carried out. The ground pineapple peel sample was analyzed at the technical lab for waste management and Bioenergy, Department of Waste and Resource Management, Faculty of Agriculture and Environmental Sciences at the University of Rostock.

Figure 12a: Drying of pineapple peels

Figure 12b: ground pineapple peels sample

Figure 12a & b: *Sample Preparation and Pretreatment*

2.5 Characterization of solid pineapple peels waste

The dried pineapple peel sample was characterized for ultimate, proximate, and fiber analysis.

2.5.1 Ultimate analysis

The dried ground pineapple peel sample was analyzed for the various elemental composition that is present in the sample such as carbon, hydrogen, nitrogen, oxygen, and sulfur by using an Optic digital microscope (LIBS Analyser) VHX-7000. The laser-induced breakdown spectroscope (LIBS Analyzer) is a type of analyzer that make use of the light emission analysis approach. The analyzer produces a short pulse laser with a high density of energy projected at the sample's surface, thereby converting a small piece of the sample to plasma. The plasma becomes atomized and excited; light is emitted when the part exposed to the laser returns to the ground state. This emitted light is then transferred through fiber optics and enters the spectrometer through the silt to analyze the elements contained in the sample.

Figure 13: *Elemental analysis of pineapple peels*

2.5.2 Proximate analysis

This was done to determine certain properties of the pineapple peels such as moisture content, ash content, volatile matter, and total solids.

2.5.2.1 Determination of Moisture Content

The determination of the moisture content involves the drying of the sample to obtain a constant weight at 105^0C (Zainuddin et al 2014). The moisture content is then calculated as the loss in weight of the dried sample. The crucibles were weighed using a weighing balance and their weight was recorded as (W1). Then the dried ground pineapple peel sample was introduced into the crucibles and later weighed and recorded as (W2). The crucible containing the sample was later placed inside an oven and dried at 105^0C for 4hrs and then cooled in a desiccator for 30min and weighed and values were recorded as (W3). This procedure was conducted in triplicate using the same sample and the moisture content was calculated on a dry basis using the equations below: (Nielsen, 2010). The average mean and the standard error were determined from the three samples.

Weight of empty crucible = W1(g)

Weight of empty crucible + sample = W2 (g)

Weight of empty crucible + sample after drying =W3 (g)

Mass of water in the sample = W2-W3(g)

Mass of dry sample = W3-W1(g)

$$\%MC = \frac{weight\ of\ water\ in\ the\ sample}{weight\ of\ dry\ sample} \times 100 \quad (2.1)$$

$$\%\boldsymbol{MC} = \frac{\boldsymbol{(W2-W3)}}{\boldsymbol{(W3-W1)}}\boldsymbol{X100} \quad \textbf{(2.2)}$$

***Figure 14**: Weighing of the sample*

2.5.2.2 Determination of Ash Content

The ash content was determined by incineration of the total mass of the sample after drying in a muffle furnace at 550^{0}C for 2hrs and was then cooled in a desiccator for 30min. The weighing was carried out immediately after the sample was removed from the desiccator to avoid moisture content in the ash samples. The weighing process was done in triplicate and values were recorded. The average mean was calculated from the three samples and the standard error.

The ash content was calculated on a dry basis as thus: (Nielsen, 2010)

Weigh to empty crucible = W1(g)

Weigh to empty crucible + dry Sample = W2 (g)

Weigh to empty crucible + Ash after incineration = W3 (g)

Mass of Ash = W3-W1(g)

Mass of dry sample = W2-W1 (g)

$$\%Ash = \frac{Weight\ of\ ash}{weight\ of\ dry\ sample} \times 100 \quad (2.2)$$

$$\%\boldsymbol{Ash} = \frac{\boldsymbol{(W3-W1)}}{\boldsymbol{(W2-W1)}} \times \boldsymbol{100} \quad \textbf{(2.3)}$$

An ash analysis was also done to determine the elemental composition of the ash content. This was done by putting a small portion of the sample into a thimble and pressing it using a pellet press to form a small pellet which was later placed in an Optic digital microscope (LIBS Analyzer) VHX-7000 for analysis.

Figure 15a: pellet press

Figure 15b: analysis of ash sample

Figure 15a & b: *Ash analysis experimental setup*

2.5.2.3 Determination of Total Solids

It is the amount of solid that is present in the sample after the loss of water molecules. It also refers to the quantity of the material residue left in the crucible after evaporation of the sample and its subsequent drying in a laboratory oven at 105°C for a period of one hour. The equation (2.4) can be used to calculate the percentage of total solids (Kelly Orhorhoro, 2017). Three samples were used and the average mean was calculated from these three samples with the standard deviation.

$$\%\boldsymbol{Total\ Solids} = \frac{\boldsymbol{(W2-W1)}}{\boldsymbol{(W3-W1)}} \times \boldsymbol{100} \quad \textbf{(2.4)}$$

Where:

Weight of crucible = W1(g)

Weight of crucible + dried sample = W2(g)

Weight of crucible + wet sample = W3(g)

2.5.2.4 Determination of volatile solids

The volatile solid is the solid remaining after the dried sample was weighed in a crucible and incinerated in a muffle furnace at 550°C for 2hrs. The crucible was then allowed to cool by placing it into a desiccator. After the cooling process, the sample was weighed and this was repeated three times. The equation below was used to calculate the percentage of volatile solids (Kelly Orhorhoro, 2017). Three samples were used, the average mean was calculated, and the standard deviation was also determined.

$$\%\boldsymbol{Volatile\ Solids} = \frac{(\boldsymbol{W2}-\boldsymbol{W4})}{(\boldsymbol{W2}-\boldsymbol{W1})} \times \mathbf{100} \quad \textbf{(2.5)}$$

Where:

W4(g) = weight of crucible + weight of sample after incineration

2.6 Compositional analysis (Fibre Analysis)

Fibretherm was used in this process to determine cellulose, hemicellulose, and lignin content. It involves a fully automated digestion and filtration of the pineapple peel sample. This was done using two analytical methods: neutral-detergent fibre (NDF) and acid-detergent fibre (ADF). NDF analyzed the total fiber in the samples, that is, the residue that remains after treatment of the biomass with the neutral detergent solution while ADF after treatment with an acid detergent solution was oxidized by Cetyl trimethylammonium bromide in H2SO4 solution. The mass difference between the pineapple peels samples digested with acid detergent (72% H2SO4) followed by oxidation of buffered solution of acetic acid together with potassium permanganate was taken as the lignin content (ADL) (Kabenge et al., 2018)

Hemicellulose = Neutral Detergent Fiber (NDF) – Acid Detergent Fiber (ADF)

Cellulose = Acid Detergent Fiber (ADF) – Acid Detergent Lignin (ADL)

Lignin = Acid Detergent Lignin (ADL)

Procedures to Determine NDF, ADF, and ADL

The empty weight of the fibre bags was determined and 1g of the dried sample was accurately weighed and introduced in a beaker, 300-400ml of acetone was added into the beaker for 5mins to remove the fat and was later transferred to another beaker for drying. The dried sample was then placed into the fibre bags. A glass spacer is carefully inserted into the fibre bags and both together are placed in the sample carousel. This was then introduced in the Fibretherm to wash

it with NDF solution (Amylase). The spacer was removed from each fibre bag and was then placed in the crucible rolled up and dried for approximately 24h at 105°C. This was allowed to cool down by placing it in a desiccator and the mass of NDF was determined.

To determine the mass of ADF the weighed fibre bags were hung in a sample carousel. The sample carousel with the fibre bags was placed in a beaker and covered at room temperature with 72% sulfuric acid. The sample was later introduced in the Fibretherm to be washed with ADF solution (40g of N-Acetyl-N, N, N- trimethylammonium bromide + 2L of sulfuric acid) and was left for two days. The fibre bags were then removed and dried in a muffle oven at 550^0C for 6hrs and then placed in a desiccator for cooling and the mass of ADF was calculated.

A similar procedure was used to determine the ADL immediately after the ADF determination.

$$\text{NDF} = \frac{(M4-M1)-(M5-(M6-M3)}{((M2-M1)\times TSmd)\times 100\times 100} \quad \textbf{(2.6)}$$

$$\text{ADF} = \frac{(M4-M1)-(M5-(M6-M3)}{((M2-M1)\times TSmd)\times 100\times 100} \quad \textbf{(2.7)}$$

$$\text{ADL} = = \frac{(M7-M1)-(M5-(M6-M3)}{((M2-M1)\times TSmd)\times 100\times 100} \quad \textbf{(2.8)}$$

Where: NDF = Share of neutral detergent fibre %TS, ADF = Share of acid detergent fibre %TS

ADL = Share of acid detergent lignin %TS M1 = Mass of the empty dried fibre bag (g)

M2 = Mass of the dried fibre bag with sample (g)

M3 = Mass of the empty crucible of the blank reading (g)

M4 = Mass of the crucible + fibrebag + sample after drying (g)

M5 = Mass of the crucible + fibre bag + sample after calcination (g)

M6 = Mass of the crucible + fibre bag after calcination of the blank reading (g)

M7 = Mass of the ADL-crucible + fibre bag after drying (g)

TSmd = Total solids of the dried and milled sample

2.7 Theoretical determination of biohydrogen production potential from pineapple peel waste

The masses obtained from the ultimate analysis of carbon, hydrogen, and oxygen were used to theoretically calculate the biohydrogen production potential from pineapple peel waste.

The equation of dark fermentation of biomass is given as:

α Biomass + βH2O → γAcetic acid + δPropionic acid + εButyric acid + ζValeric acid + θHexanoic acid + κH2 + λCO2 + μMicrobial biomass + πOthers (e.g., ethanol)

where: α, β, γ, δ, ε, ζ, θ, κ, λ, + π are molar coefficients (Talapko et al. 2023).

However, the acetate pathway of biohydrogen production was considered in this work and the equation is given as:

$$\alpha \boldsymbol{C_x H_y O_z} + \beta \boldsymbol{H_2 O} \rightarrow \gamma \boldsymbol{CH_3 COOH} + \lambda \boldsymbol{CO_2} + \kappa \boldsymbol{H_2} \quad \textbf{(2.9)}$$

Where x, y, and z are the moles of carbon, hydrogen, and oxygen. The number of moles of Carbon, Hydrogen, and Oxygen were determined by dividing their weight percentages by their molar masses. This was in turn divided by the smallest mole ratio. This is shown below

$$\mathrm{n} = \frac{\mathbf{m}}{\mathbf{M}} \quad (2.10)$$

where: n= number of moles, m = mass, M = molar mass

The number of moles was substituted into the equation above for x, y, and z and the entire equation was then balanced to determine the number of moles of biohydrogen that will be produced. This number of moles was then converted to milliliters of biohydrogen using the formula below:

$$1mole \rightarrow 22.4l \qquad 1l \rightarrow 1000ml$$

2.8 To test for biogas production potential using the pineapple peels waste

The biogas test was conducted on the pineapple peel sample to determine the potential biogas yield from the substrate under standard conditions. The inoculum used was anaerobic sludge from a biogas plant in Germany. The masses of the bottles were initially weighed and recorded. The inoculum was properly stirred to maintain homogeneity before introducing 200g into the bottles. 5g of the weighed sample and 100g of water were also added into the same bottles and this was done in triplicate. The bottles were covered with a magnetic stirrer to filter particles and prevent foaming. The methanogenic bacteria were activated by swirling the bottles thoroughly. A Gas measurement module with a lithium battery was introduced on top of the bottle's filter to measure the biogas production potential. The sample bottles were then placed in a water bath at a mesophilic temperature of 38^0C for 21 days.

A blank and cellulose standard test was also prepared using the above procedure except that in the blank no inoculum source and pineapple sample were used and for the cellulose standard test, 5g of cellulose was added without adding the pineapple peel sample.

***a**. sample bottles*

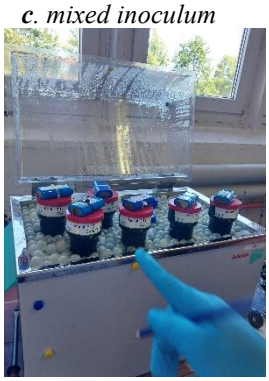
***b**. mixing of inoculum*

***c**. mixed inoculum*

***d.** measuring of inoculum*

e. Addition of water

f. putting lid and stirrer



e. samples in water bath

h. starting of test

***Figure 16**: Biogas experimental procedures*

2.9 Estimation of the biohydrogen produced from the biogas test

This was done by using the cumulative biogas yield from the pineapple peels sample and estimating the biohydrogen yield. The volume of methane was calculated from the biogas yield and the volume of hydrogen was later estimated from the volume of the methane using steam methane reforming and water gas-shift reactions as shown below;

SMR: $\boldsymbol{CH_{4(g)} + H_2O(l) \rightarrow CO_{(g)} + 3H_{2(g)} \quad \Delta H_{298} = +206.20 kJ/mol}$ **(2.11)**

WGS: $\boldsymbol{CO(g) + H_2O(l) \rightarrow H_{2(g)} + CO_2(g) \quad \Delta H_{298} = -41.20 kJ/mol}$ **(2.12)**

Overall reaction: $\boldsymbol{CH_{4(g)} + 2H_2O(l) \rightarrow 4H_{2(g)} + CO_2(g)\ \Delta H_{298} = +165 kJ/mol}$ **(2.13)**

A 53% methane production from the biogas and 90% theoretical conversion efficiency were assumed.

The volume of methane = Percentage of methane in the biogas x Cumulative biogas yield

$1 mole = 22.4 l$ $\qquad\qquad$ $1 l = 1000 ml$

CHAPTER 3: RESULTS AND DISCUSSIONS

3.1 RESULTS

Characterization of solid pineapple peels waste

The characterization was carried out by doing ultimate, proximate, ash, and fiber analysis of the pineapple peel sample.

3.1.1 Ultimate analysis

Table 4: Ultimate Analysis of Pineapple Peels (in %wt.)

Elements	C	H	O	N	S
Values (%wt.)	44.40	9.40	40.90	-	-

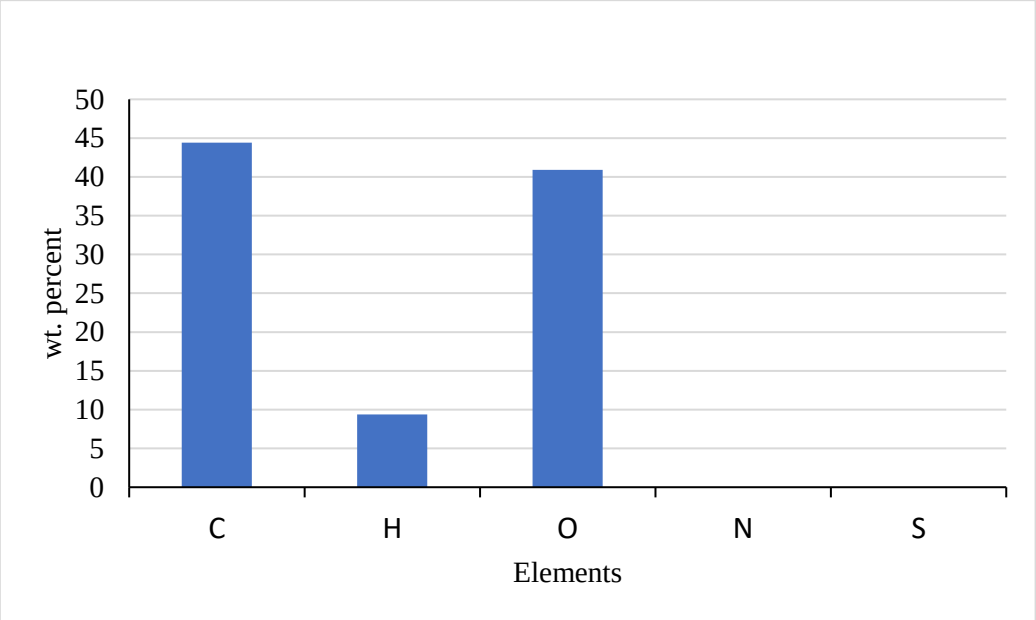


***Figure 17**: Ultimate analysis of pineapple peels*

3.1.2 Proximate analysis

Table 5: Proximate Analysis of Pineapple peels (on a dry basis)

Items determined	% Composition
1. Moisture content	9.07 ± 0.02
2. Total solids	91.67 ± 0.02
3. Volatile solids	94.70 ± 0.02
4. Ash content	5.30 ± 0.02
5. Dry matter content	98.46

$* mean \pm standard\ deviation$

3.1.3 Ash analysis

Table 6: Ash Analysis of the pineapple Peel Sample (in mol%)

Elements	K	O	Ca	Si	Mg	Na	C	S
Values(%mol)	16.23	11.27	4.87	2.93	1.33	0.30	0.50	0.31

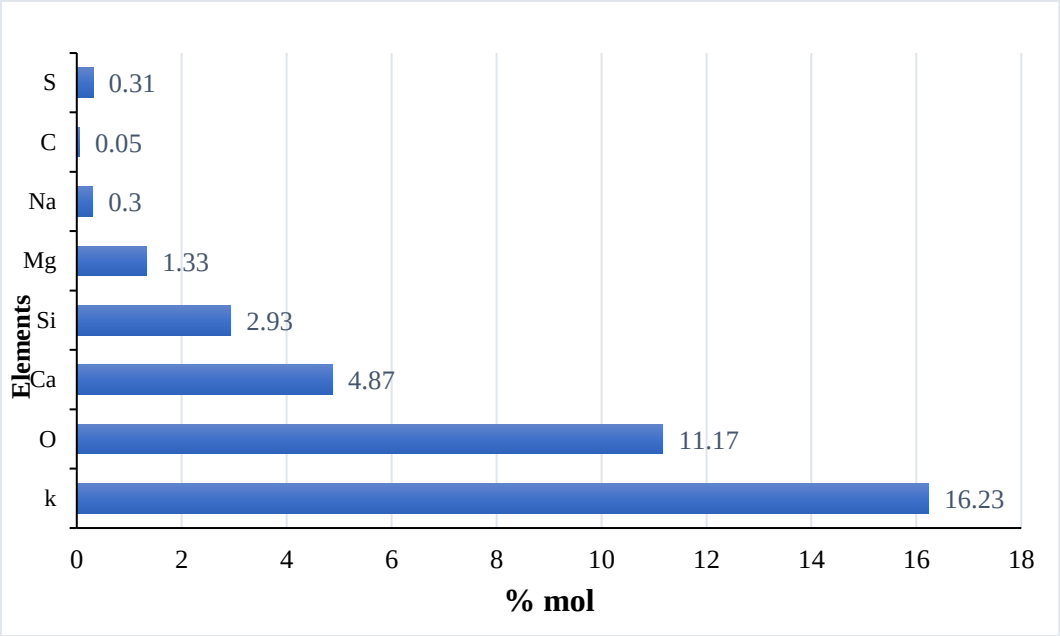


***Figure 18**: Ash analysis of pineapple peels sample*

3.1.4 Fibre analysis

Table 7: Fibre Analysis of Pineapple Peels

Items determined	% Composition
1. Cellulose	8.50
2. Hemicellulose	16.92
3. lignin	2.52

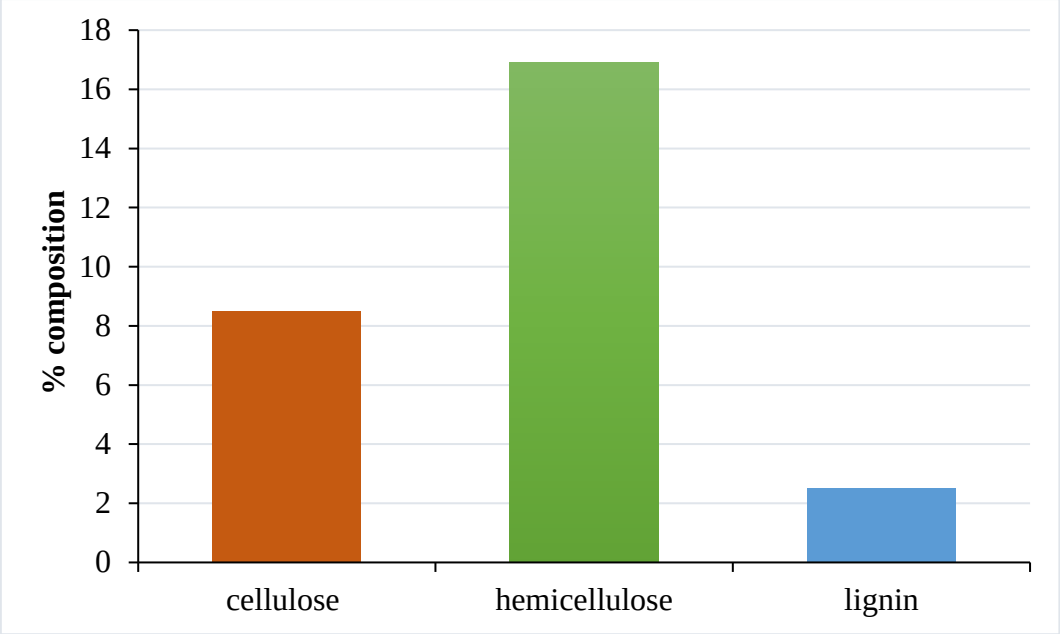


***Figure 19**: Percentage composition of cellulose, hemicellulose, and lignin in sample*

3.1.5 Theoretical determination of biohydrogen production potential from pineapple peels wastes

Ultimate analysis values of carbon, hydrogen, and oxygen are 44.4%, 9.4%, and 40.9% respectively.

Convert the values to grams and divide by their molar masses to determine the number of moles

Determination of the number of moles of carbon, hydrogen, and oxygen

No. moles of Carbon	No. of moles of Hydrogen	No. of moles of Oxygen
$\frac{44.4g}{12g/mol}$	$\frac{9.4g}{1g/mol}$	$\frac{40.9g}{16g/mol}$
3.7 mol	9.4 mol	2.6 mol

Divide by the smallest mole ratio

$\frac{3.7}{2.6}$	$\frac{9.4}{2.6}$	$\frac{2.6}{2.6}$
1.4	3.6	1

Multiply by a whole number to remove the decimals

(1.4	3.6	1) x 2
3 mol	7 mol	2 mol

Hence, the theoretical pineapple substrate formula is given as $C_3H_7O_2$

Using the substrate formula and balancing it according to the dark fermentation reaction

$$C_3H_7O_2 + 2H_2O \rightarrow CH_3COOH + CO_2 + 3.5H_2 \quad (3.1)$$

From the equation above, the theoretical biohydrogen production is 3.5 moles

In terms of volume is calculated as:

$1mole \rightarrow 22.4\ l$ $\qquad$ $1l \rightarrow 1000ml$

$3.5mole \rightarrow x$ $\qquad$ $78.4l \rightarrow x$

$x = 22.4 \times 3.5 = 78.4\ l$ $\qquad$ $x = 78.4 \times 1000 = 78400ml$

Hence, the theoretical volume of biohydrogen production is 78.4 l or 78400 mml.

3.1.6. Test for biogas production potential using the pineapple peels waste

Table 8: Biogas Production Potential Test (**mLg^{-1}vs**)

Time (days)	Blank sample	Cellulose sample	Pineapple peels sample
7	62.67	687.77	390.95
14	102.15	731.95	455.81
21	128.58	742.27	493.14

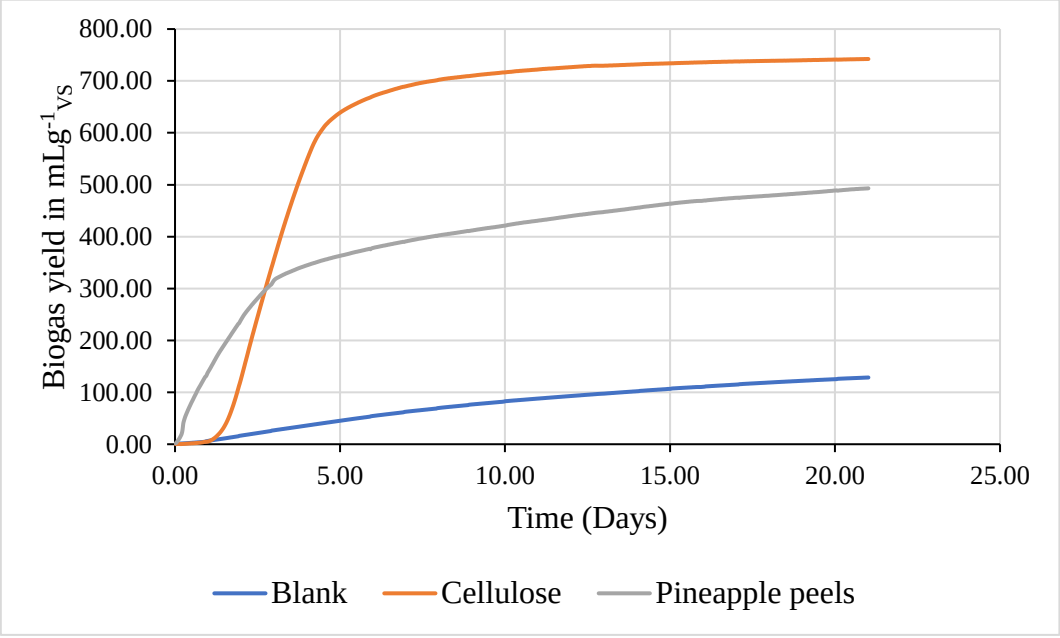


Figure 20: *Cumulative biogas production from pineapple peels sample, blank and cellulose standard*

3.1.7 Determination of Hydrogen production potential from the biogas yield

Table 9: Theoretical Biohydrogen Production from the Biogas Yield

Theoretical yield	Conversion efficiency
0.214 mole	90%
1045.84 mLg^{-1}_{VS}	

3.2 DISCUSSION

3.2.1 Ultimate analysis

Table 4 shows the ultimate analysis of pineapple peel waste in weight percent on a dry basis. The value of carbon is 44.40% which is very close to a similar report by (Mansor et al., 2018) for pineapple leaves and stems with values of 43.49 % and 41.08 % respectively.

Oxygen shows a value of 40.90 % which is similar to the result reported by (Tsai et al., 2022) for pineapple peels (48.25%). This value is slightly lower than pineapple leaf (59.26 %), stem (57.31 %), and roots (75.72%) (Mansor et al., 2018).

The value of hydrogen is 9.40% which is slightly greater than the values published by (Ozyuguran et al., 2018) for black sesame residue (6.79%), apple pulp (6.70%), and also by Banerjee et al. (2019) for pineapple peels with a value of 5.70%.

However, the value of Nitrogen and Sulfur was not detected in this analysis rather potassium, silicon, and zinc were detected with 3.60%, 0.90%, and 0.008% respectively. When the ash content was analyzed, it was observed that there was little amount of sulfur (0.31 mol %) and no nitrogen detected. The lack of nitrogen in the sample might be due to the low protein content in pineapple peels.

3.2.2 Proximate analysis

Table 5 gives the proximate analysis of the pineapple peel sample on a dry basis. It shows high total solids, volatile solids, and dry matter content while a low moisture and ash content was also observed in the sample.

The moisture content of the sample is $9.07 \pm 0.02\%$ which is similar to results published by (Zainuddin et al., 2014) for Josapine pineapple leaves (9.42 ± 0.02 %), (Mansor et al. 2018) for stems (9.103%) and (Azevedo et al., 2021) for pineapple

peels (13.0%), while (Owoeye et al. 2022) reported a value for pineapple peels ($5.10 \pm 0.70\%$) which is slightly lower than the value in this report. This might be due to the difference in the variety of pineapple fruit and the region of cultivation.

The proximate analysis value of total solids $is\ 91.67 \pm 0.02\%$ which constitute the percentage of volatile matter, ash, and dry matter content present in the pineapple peel sample. This represents the solid remaining after the loss of water molecules in the sample. This value is however higher than a similar study of pineapple peels with a total solid of 72.8% (Ofomatah et al., 2021).

The volatile solids show a value of $94.70 \pm 0.02\%$ which is in consonant with studies conducted for pineapple peels at 93.60% (Paepatung et al., 2009). This value is seen as higher than a similar study of pineapple peels with a volatile solid of 73.23 ± 0.71% (Tsai et al., 2022).

The ash content is $5.30 \pm 0.02\%$ which is similar to results obtained for pineapple peels (5.05±0.10) (Pereira et al., 2021) and (3.78±0.05 %) (Owoeye et al., 2022). However, another study revealed a high ash content for pineapple peels (8.28 ± 0.36 %) (Tsai et al., 2022).

The dry matter content of the pineapple peel sample is 98.46% constituting the mass percent of the organic matter and the ash content of the sample. This value is close to a similar study of pineapple peels ($94.04 \pm 0.06\%$) (Banerjee et al., 2019).

3.2.3 Ash analysis

Table 6 gives the result for the analysis of the ash content of pineapple peels. The ash content shows minerals such as potassium, oxygen, calcium, silicon, magnesium, sodium, carbon, and sulfur with zero moles of hydrogen and nitrogen. Potassium shows the highest number of moles at 16.23 mol and sodium with the lowest value of 0.30 mol. However, a trace of sulfur (0.31) was found in the ash which was not present in the ultimate analysis of the sample. This shows that during the sample's combustion, sulfur could oxidize after the volatile matter was expelled from the sample.

3.2.4 Fibre analysis

Table 7 shows the composition of the fiber analysis of pineapple peel waste. From the results, the highest composition present in the sample is hemicellulose followed by cellulose with 16.92% and 8.50 % respectively. Lignin shows the lowest composition of 2.51%.

The cellulose content of 8.50% was found to be very close to similar reports for pineapple peels (7.86%) (Mibulo et al., 2023), (10.90%) (Azevedo et al., 2021). However, this value is lower than other studies done for pineapple peels ($20.9 \pm 0.6\%$) (Banerjee et al., 2019), skin (14.0%), crown (29.6%) and pulp (14.3%) (Casabar et al., 2019).

Hemicellulose content is 16.92% which is in consonant with a similar report for pineapple peels (16.03%) (Mibulo et al., 2023), and slightly lower than pineapple skin (20.20%), crown (23.2%), and pulp (22.2%) (Casabar et al., 2019). This value is however higher than a report by Mathew et al. 2015 for pineapple leaves (13.6 ± 0.82%) and stems (8.26 ± 2.10%).

The Lignin content of 2.51% is in consonant with reports for orange peels (2.65±0.70%) (Buxoo & Jeetah, 2020) and pineapple pulp (2.30%) (Casabar et al., 2019). In another report, this value is seen slightly lower than pineapple stem (5.42 ± 0.97%) (Mathew et al., 2015), crown (4.50%) (Casabar et al., 2019), and peels (7.10%) (Azevedo et al., 2021). Mibulo et al. (2023) reported a value for pineapple peels (1.99%) and (Paepatung et al., 2009) a value (1.37%) for pineapple peel which is lower than the value in this report.

3.2.5 Theoretical determination of biohydrogen production potential from pineapple peels wastes

The result of the theoretical biohydrogen produced from the pineapple peels waste was obtained by using the fermentation equation below;

$$\alpha C_x H_y O_z + \beta H_2 O \rightarrow \gamma CH_3 COOH + \lambda CO_2 + \kappa H_2 \quad (3.2)$$

From the values of the ultimate analysis, carbon, hydrogen, and oxygen produce 3 moles, 7 moles, and 2 moles respectively. Substituting these values into equation (3.2) gives equation (3.3) when the acetate pathway is considered.

$$C_3 H_7 O_2 + 2H_2 O \rightarrow CH_3 COOH + CO_2 + 3.5 H_2 \quad (3.3)$$

From equation (3.3), it was observed that 1 mole of the substrate produced 3.5 moles of biohydrogen. This is however close to the theoretical value reported in the literature when glucose is used as the main substrate with a maximum hydrogen yield of 4 moles as shown in equation (3.4) (Liu et al., 2020; Talapko et al., 2023)

$$C_6 H_{12} O_6 + 2H_2 O \rightarrow 2CH_3 COOH + 2CO_2 + 4H_2 \quad (3.4)$$

This pathway is selected since it has been proven to have the highest hydrogen production potential compared to the butyric pathway and other metabolites (Liu et al., 2020).

In terms of volume, the theoretical volume of biohydrogen produced is $78.4\ l$ or 78400 ml.

3.2.6 Test for biogas production potential using the pineapple peels waste

Table 8 shows the result of the biogas production potential test for blank, cellulose standard, and pineapple peel samples in terms of volatile solids for a period of 21 days. It is seen that all samples produce biogas. The production was relatively low in the first 7 days and increased for the reaming 21 days. It was observed that the production of biogas from the blank sample is the lowest with values ranging from 62.67 - 128.58 mLg^{-1}_{VS} than the cellulose standard and pineapple peels sample with values ranging from 689.77 - 742.27 mLg^{-1}_{VS} and 390.95 - 493.14 mLg^{-1}_{TVS} respectively. It was also observed that the production of biogas from the cellulose standard remains almost constant for the first 7 days while there was a steep increase in biogas production from the pineapple peels sample throughout the 21 days with a cumulative production rate of 493.14 mLg^{-1}_{VS} which is significantly higher than value for pineapple peel (31.70 ± 1.60 mLg^{-1}vs) (Muenmee & Prasertboonyai, 2021). This high volume of biogas produced from the pineapple peel sample could be a result of the high %TS and %VS in the sample (Kelly Orhorhoro, 2017).

3.2.7 Determination of biohydrogen production potential from the biogas yield

Table 9 shows the theoretical biohydrogen yield from methane in the biogas. Naturally, biogas from the anaerobic digestion process consists of a mixture of mainly (50–75%) methane (CH4), (19–34%) carbon dioxide (CO2), and trace amounts of other gases (Hamzah et al., 2022). In this light, a 53% methane from the biogas yield was assumed at 90% theoretical conversion efficiency. The number of moles produced is 0.214 moles and in terms of volume is 1045.84 mLg^{-1}_{VS}.

CHAPTER 4: CONCLUSION AND PERSPECTIVES

4.1 Conclusions

From this work, it can be established that the production of biohydrogen from pineapple peel waste by dark fermentation is feasible which can help combat the issue of proper management of pineapple peel waste and the associated environmental and health challenges. The ultimate and proximate analysis conducted shows similar results to other feedstock used for biohydrogen production. The ultimate analysis of the pineapple peels shows little sulfur and no nitrogen contents which is an indication of a good feedstock for biohydrogen production that will not pollute the environment during consumption. The theoretical biohydrogen production potential was estimated at 3.5 moles and in terms of volume is $78400\ mL$. The biogas test conducted shows high biogas yield of 493.14 mlg^{-1}_{VS} after 21days which can be attributed to the fact that there are high total solids ($91.67 \pm 0.02\%$) and volatile solids ($94.70 \pm 0.02\%$) and low lignin content (2.51%) in the pineapple peels. The estimated biohydrogen production from the biogas test is 1045.84 mLg^{-1}_{VS}. Biogas and biohydrogen could be used as alternative sources of energy to fossil fuels in mitigating climate change and helping accelerate the energy transition. This research will provide valuable baseline information to relevant stakeholders on biohydrogen production from pineapple peels by dark fermentation as a possible way of managing its waste.

4.2 Perspectives

It has already been established that dark fermentation is one of the best options to properly manage pineapple peel waste and at the same time serves as a source of biohydrogen and biogas production. However, to make this energy easily accessible and affordable the following must be taken into consideration;

- Economic and technical feasibility studies should be carried out on the production chain starting from feedstock availability to the technologies involved in the production process to know whether it would be competitive with fossil fuel.
- An experimental approach is needed to be conducted on a large scale to ascertain the actual biohydrogen production potential from pineapple peels as this work only focused on theoretical biohydrogen production and biogas test from which an estimation of biohydrogen potential was made.

- Since the production of biogas from pineapple peels is very high on a lab scale, investigations should be conducted on the safety, utilization, storage, and optimization of the biogas yield on a commercial scale for cooking.
- A combination of both physical and chemical pretreatment could significantly increase the yield of biohydrogen and biogas. Since the hemicellulose percentage is the highest followed by cellulose and lignin, a combination will further reduce the hemicellulose, cellulose, and lignin contents.
- The implementation of waste education and pineapple waste management to foster a circular economy.
- Policies should the drafted by the government on the adoption of biohydrogen and biogas as a supplement to the energy mix of the country.

REFERENCES

1. Abdalla M. Abdallaa, Shahzad Hossaina, Ozzan B. Nisfindya, Atia T. Azadd, Mohamed Dawood, Abul K. Azad (2018). Hydrogen production, storage, transportation and key challenges with applications: A review. Energy Conversion and Management. https://doi.org/10.1016/j.enconman.2018.03.088

2. Abdullah, A., & Mat, H. (2008). CHARACTERISATION OF SOLID AND LIQUID PINEAPPLE WASTE. *Reaktor, 12*(1), 48. https://doi.org/10.14710/reaktor.12.1.48-52

3. Ahmed I. Osman, Tanmay J. Deka, Debendra C. Baruah, David W. Rooney (2020). Critical challenges in biohydrogen production processes from the organic feedstocks. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-020-00965-x

4. Ahmed H. Salem, Ruth Brunstermann, Thorsten Mietzel, Renatus Widmann (2018). Effect of pre-treatment and hydraulic retention time on biohydrogen production from organic wastes, International Journal of Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2018.01.114

5. Aili Hamzah, A. F., Hamzah, M. H., Che Man, H., Jamali, N. S., Siajam, S. I., & Ismail, M. H. (2021). Recent Updates on the Conversion of Pineapple Waste (Ananas comosus) to Value-Added Products, Future Perspectives and Challenges. *Agronomy*, *11*(11), 2221. https://doi.org/10.3390/agronomy11112221

6. Alka Pareek, Rekha Dom, Jyoti Gupta, Jyothi Chandran, Vivek Adepu, Pramod H. Borse (2020). Insights into renewable hydrogen energy: Recent advances and prospects. Materials Science for Energy Technologies. https://doi.org/10.1016/j.mset.2019.12.002

7. Amekan y., Wangi, D.S.A.P., Cahyanto, M.N., Sarto and Widada, J (2018). Effect of Different Inoculum Combination on Biohydrogen Production from Melon. Int. Journal of renewable energy development 101.

8. Anish Ghimire (2015). Dark fermentative biohydrogen production from organic waste and application of by-products in a biorefinery concept. Material chemistry. Université Paris-Est. English. NNT: 2015PESC1197f.

9. Anish Ghimire, Luigi Frunzo, Francesco Pirozzi, Eric Trably, Renaud Escudie, Piet N.L. Lens e, Giovanni Esposito (2015). A review on dark fermentative biohydrogen production from organic biomass: Process parameters and use of by-products. Applied Energy 44 (2015) 73–95. http://dx.doi.org/10.1016/j.apenergy

10. Anuar, A. N., Halim, M. H. A., Rosman, N. H., Othman, I., Harun, H., Basri, H. F., Ujang, Z., & van Loosdrecht, M. (2020). Microbial Identification and Extracellular Polymeric Substances Characterization of Aerobic Granules Developed in Treating Rubber Processing Wastewater. In Z. A. Zakaria, R. Boopathy, & J. R. Dib (Eds.), *Valorisation of Agro-industrial Residues – Volume I: Biological Approaches* (pp. 257–286). Springer International Publishing. https://doi.org/10.1007/978-3-030-39137-9_13

11. Asma Sattar, Chaudhry Arslan, Changying Ji, Saba Sattar, Muhammad Umair, Sumiyya Sattar, Muhammad Zia Bakht (2016). Quantification of temperature effect on batch production of bio-hydrogen from rice crop wastes in an anaerobic bioreactor. International Journal of Hydrogen Energy. http://dx.doi.org/10.1016/ j. ijhydene.2016.04.087

12. Azevedo, A., Gominho, J., & Duarte, E. (2021). Performance of Anaerobic Co-digestion of Pig Slurry with Pineapple (Ananas comosus) Bio-waste Residues. *Waste and Biomass Valorization*, *12*(1), 303–311. https://doi.org/10.1007/s12649-020-00959-w

13. Banerjee, S., Patti, A. F., Ranganathan, V., & Arora, A. (2019). Hemicellulose based biorefinery from pineapple peel waste: Xylan extraction and its conversion into xylooligosaccharides. *Food and Bioproducts Processing*, *117*, 38–50. https://doi.org/10.1016/j.fbp.2019.06.012

14. Buxoo, S., & Jeetah, P. (2020). Feasibility of producing biodegradable disposable paper cups from pineapple peels, orange peels, and Mauritian hemp leaves with beeswax coating. *SN Applied Sciences*, *2*(8), 1359. https://doi.org/10.1007/s42452-020-3164-7

15. Bruce S, Temminghoff M, Hayward J, Schmidt E, Munnings C, Palfreyman D, Hartley P (2018). National Hydrogen Roadmap. CSIRO, Australia.

16. C. Rivkin, R. Burgess, and W. Buttner (2015). Hydrogen Technologies Safety Guide. National Renewable Energy Laboratory. http://www.osti.gov/scitech.

17. Casabar, J. T., Unpaprom, Y., & Ramaraj, R. (2019). Fermentation of pineapple fruit peel wastes for bioethanol production. *Biomass Conversion and Biorefinery*, *9*(4), 761–765. https://doi.org/10.1007/s13399-019-00436-y

18. Cahyari, K., Putri, A. M., Oktaviani, E. D., Hidayat, M. A., & Norajsha, J. D. (2018). Biohydrogen Production from Pineapple Waste: Effect of Substrate Concentration and Acid

Pretreatment. *IOP Conference Series: Materials Science and Engineering*, *358*, 012001. https://doi.org/10.1088/1757-899X/358/1/012001

19. Cevahir Tarhan, Mehmet Ali Çil (2021). A study on hydrogen, the clean energy of the future: Hydrogen storage methods. Journal of Energy Storage. https://doi.org/10.1016/j.est.2021.102676

20. Chiu-Yue Lin, Chyi-How Lay, Biswarup Sen, Chen-Yeon Chu, Gopalakrishnan Kumar, Chin-Chao Chen, Jo-Shu Chang (2012). Fermentative hydrogen production from wastewaters: A review and prognosis. International journal of hydrogen energy 37 (2012) 15632-1564. doi: 10.1016/j.ijhydene.2012.02.072

21. Chulalaksananukul, S., Sinbuathong, N., & Chulalaksananukul, W. (2012). *Bioconversion of Pineapple Solid Waste under Anaerobic Conditions through Biogas Production*.

22. Dagdougui H, Sacile R, Bersani C, Ouammi A. Chapter 4 - Hydrogen storage and distribution: Implementation scenarios. In: Dagdougui H, Sacile R, Bersani C, Ouammi A, editors. Hydrogen infrastructure for energy applications. Academic Press; 2018, p. 37–52. http://dx.doi.org/10.1016/B978-0-12-812036-1.00004

23. Dahunsi, S. O. (2019). Liquefaction of pineapple peel: Pretreatment and process optimization. *Energy*, *185*, 1017–1031. https://doi.org/10.1016/j.energy.2019.07.123

24. David B. Levin, Lawrence Pitt. Murray Love (2004). Biohydrogen production: prospects and limitations to practical application. International Journal of Hydrogen Energy. doi:10.1016/S0360-3199(03)00094-6

25. DemiRbaş, A. (2003). Relationships Between Heating Value and Lignin, Fixed Carbon, and Volatile Material Contents of Shells from Biomass Products. *Energy Sources*, *25*(7), 629–635. https://doi.org/10.1080/00908310390212336

26. Dong-Hoon Kim and Mi-Sun Kim (2011). Hydrogenases for biological hydrogen production. Bioresource Technology. doi: 10.1016/j.biortech.2011.02.113

27. Duu-Jong Lee, Kuan-Yeow Show, Ay Su (2011). Dark fermentation on biohydrogen production: Pure culture. Bioresource Technology, 102 (2011) 8393–8402. doi: 10.1016/j.biortech.2011.03.041

28. Eixenberger, D., Carballo-Arce, A.-F., Vega-Baudrit, J.-R., Trimino-Vazquez, H., Villegas-Peñaranda, L. R., Stöbener, A., Aguilar, F., Mora-Villalobos, J.-A., Sandoval-Barrantes, M.,

Bubenheim, P., & Liese, A. (2022). Tropical agro-industrial biowaste revalorization through integrative biorefineries—review part II: Pineapple, sugarcane and banana by-products in Costa Rica. *Biomass Conversion and Biorefinery*. https://doi.org/10.1007/s13399-022-02721-9

29. Erol, M., Haykiri-Acma, H., & Küçükbayrak, S. (2010). Calorific value estimation of biomass from their proximate analyses data. *Renewable Energy*, *35*(1), 170–173. https://doi.org/10.1016/j.renene.2009.05.008

30. Fan Zhang, Pengcheng Zhao, Meng Niu, and Jon Maddy (2016). The survey of key technologies in hydrogen energy storage, International Journal of Hydrogen Energy. http://dx.doi.org/10.1016/j.ijhydene.2016.05.293

31. Fatthy Mohamed Morsy (2017). Synergistic dark and photo-fermentation continuous system for hydrogen production from molasses by Clostridium acetobutylicum ATCC 824 and Rhodobacter capsulatus DSM 1710. Journal of Photochemistry & Photobiology, B: Biology. Doi: 10.1016/j.jphotobiol.2017.02.011

32. Furat Dawood, Martin Anda, G.M. Shafiullah (2019). Hydrogen production for energy: An overview, International Journal of Hydrogen Energy, https://doi.org/10.1016/j.ijhydene.2019.12.059

33. Giuseppe Strazzera, Federico Battista, Natalia Herrero Garcia, Nicola Frison, David Bolzonella (2018). Volatile fatty acids production from food wastes for biorefinery platforms: A review. Journal of Environmental Management. https://doi.org/10.1016/j.jenvman.2018.08.039

34. Guang Yang and Jianlong Wang (2017). Enhanced Hydrogen Production from Sewage Sludge by Co-fermentation with Forestry Wastes *Energy Fuels* 2017, 31, 9, 9633–9641. https://doi.org/10.1021/acs.energyfuels.7b02135

35. Hamzah, A. F. A., Hamzah, M. H., Man, H. C., Jamali, N. S., Siajam, S. I., & Show, P. L. (2022). Biogas Production Through Mono- and Co-digestion of Pineapple Waste and Cow Dung at Different Substrate Ratios. *BioEnergy Research*. https://doi.org/10.1007/s12155-022-10478-2

36. Hydrogen Council (2017). How hydrogen empowers the energy transition. www.hydrogencouncil.com

37. https://wacomp.projects.ecowas.int/activities/national-level/togo/ last accessed on 09/03/2023. European Commission (2020). Pineapple value chain analysis in Togo. VCA4D.

38. https://www.togofirst.com/en/agriculture/2111-10979-togo-produced-over-40-000-t-of-pineapples-in-2021. (Last accessed on 20th July, 2023)

39. Immanuel Vincent and Dmitri Bessarabov (2017). Low-cost hydrogen production by anion exchange membrane electrolysis: A review. Renewable and Sustainable Energy Reviews. http://dx.doi.org/10.1016/j.rser.2017.05.258

40. Jemilatu, Omuwa Audu, Eka, Latiffah Nadia Dzulkarnain, Zaharah, Ibrahim, Norahim, Ibrahim& Mohd, Firdaus Abdul-Wahab (2020). Microbial Identification and Extracellular Polymeric Substances. DOI: 10.1007/978-3-030-39137-9-13.

41. Jey-R S. Ventura, Saul M. Rojas, Ruby Lynn G. Ventura, Fidel Rey P. Nayve Jr, Nacita B. Lantican (2021). Potential for biohydrogen production from organic wastes with focus on sequential dark- and photo-fermentation: the Philippine setting. Biomass Conversion and Biorefinery. https://doi.org/10.1007/s13399-021-01324-0

42. Jingping Liu, Xiongbo Duan, Zhipeng Yuan, Qi Liu and Qijun Tang (2017). Experimental study on the performance, combustion, and emission characteristics of a high compression ratio heavy-duty spark-ignition engine fuelled with liquefied methane gas and hydrogen blend, Applied Thermal Engineering. Doi: http://dx.doi.org/10.1016/ j. applthermaleng.2017.06.067

43. Joakim Anderson and Stefan Gronkvist (2019). Large-scale storage of hydrogen. International Journal of Hydrogen Energy. https://doi.org/10.10116/j.ijhydene.2019.o3.063

44. Junghare, M., Subudhi, S., & Lal, B. (2012). Improvement of hydrogen production under decreased partial pressure by newly isolated alkaline tolerant anaerobe, Clostridium butyricum TM-9A: Optimization of process parameters. *International Journal of Hydrogen Energy*, *37*(4), 3160–3168. https://doi.org/10.1016/j.ijhydene.2011.11.043

45. Kelly Orhorhoro, E. (2017). Experimental Determination of Effect of Total Solid (TS) and Volatile Solid (VS) on Biogas Yield. *American Journal of Modern Energy*, *3*(6), 131. https://doi.org/10.11648/j.ajme.20170306.13

46. Kuan-Yeow Show, Duu-Jong Lee, Zhen-Peng Zhang (2011). Production of Biohydrogen: Current Perspectives and Future Prospects. Biofuels. Elsevier (Pp 467-479). ISBN: 978-0-12-385099-7. DOI: 10.1016/B978-0-12-385099-7.00021-8

47. Kuppam Chandrasekhar, Yong-Jik Lee and Dong-Woo Lee (2015). Biohydrogen Production: Strategies to Improve Process Efficiency through Microbial Routes. Int. J. Mol. Sci. 2015, 16, 8266-8293. doi:10.3390/ijms16048266

48. Liu, Y., Min, J., Feng, X., He, Y., Liu, J., Wang, Y., He, J., Do, H., Sage, V., Yang, G., & Sun, Y. (2020). A Review of Biohydrogen Productions from Lignocellulosic Precursor via Dark Fermentation: Perspective on Hydrolysate Composition and Electron-Equivalent Balance. *Energies, 13*(10), 2451. https://doi.org/10.3390/en13102451

49. Logan, B. E., Hamelers, B., Rozendal, R. A., Schrorder, U., Keller, J., Freguia, S. (2006). Microbial fuel cells: Methodology and technology. Environmental Science and Technology, 40(17), 5181–5192

50. Mathew, S., Zakaria, Z. A., & Musa, N. F. (2015). Antioxidant property and chemical profile of pyroligneous acid from pineapple plant waste biomass. *Process Biochemistry*, *50*(11), 1985–1992. https://doi.org/10.1016/j.procbio.2015.07.007

51. Meiling Yue, Hugo Lambert, Elodie Pahon, Robin Roche, Samir Jemei, Daniel Hissel (2021). Hydrogen energy systems: A critical review of technologies, applications, trends, and challenges. Renewable and Sustainable Energy Reviews 146 (2021) 111180. https://doi.org/10.1016/j.rser.2021.111180

52. Mibulo, T., Nsubuga, D., Kabenge, I., Kiggundu, N., & Wydra, K. D. (2023). Comparative Study of Biogas Production from Jackfruit Waste, Banana Peels, and Pineapple Peels Co-Digested with Cow Dung. *Journal of Sustainable Bioenergy Systems*, *13*(01), 1–15. https://doi.org/10.4236/jsbs.2023.131001

53. Milton M. Arimi, Jan Knodel, Ambrose Kiprop, Saul S. Namango, Yongjun Zhang, Sven-Uwe Geißen (2015). Strategies for improvement of biohydrogen production from organic-rich wastewater: A review. Biomass and Bioenergy 75, (2015) 101-118. http://dx.doi.org/10.1016/j.biombioe.2015.02.011

54. Muenmee, S., & Prasertboonyai, K. (2021). Potential Biogas Production Generated by Mono- and Co-digestion of Food Waste and Fruit Waste (Durian Shell, Dragon Fruit, and Pineapple Peel) in Different Mixture Ratio under Anaerobic Conditions. *Environmental Research, Engineering and Management*, *77*(1), 25–35. https://doi.org/10.5755/j01.erem.77.1.25234

55. Neda Akhlaghi and Ghasem Najafpour-Darzi (2020). A comprehensive review on biological hydrogen production. International journal of hydrogen energy. https://doi.org/10.1016/j.ijhydene.2020.06.182.

56. Neda Fakhimi and Omid Tavakoli (2019). Improving hydrogen production using co-cultivation of bacteria with Chlamydomonas reinhardtii microalga. Materials Science for Energy Technologies. https://doi.org/10.1016/j.mset.2018.09.003

57. Nielsen, S. S. (2010). Determination of Moisture Content. In S. S. Nielsen (Ed.), *Food Analysis Laboratory Manual* (pp. 17–27). Springer US. https://doi.org/10.1007/978-1-4419-1463-7_3

58. Noussan. M; Raimondi. P.P; Scita. R; Hafner. M (2020). The Role of Green and Blue Hydrogen in the Energy Transition: A Technological and Geopolitical Perspective. Sustainability 2021, 13, 298. https://doi.org/10.3390/su13010298

59. Octavio García-Depraect, Roberto Castro-Munoz, Raúl Munoz, Eldon R. Rene, Elizabeth Leon-Becerril, Idania Valdez-Vazquez, Gopalakrishnan Kumar, Luis C. Reyes-Alvarado, Leonardo J. Martínez-Mendoza, Julian Carrillo-Reyesf, German Buitron (2020). A review on the factors influencing biohydrogen production from lactate: The key to unlocking enhanced dark fermentative processes. Bioresource Technology 324 (2021) 124595. https://doi.org/10.1016/j.biortech.2020.124595.

60. Ofomatah, A. C., Ugwu, K. E., & Ani, J. U. (2021). Biogas Production and Storage from Pig Dung Co-Digested with Pineapple Peel. *IOP Conference Series: Earth and Environmental Science*, *730*(1), 012004. https://doi.org/10.1088/1755-1315/730/1/012004

61. Owoeye, T. F., Akinlabu, D. K., Ajayi, O. O., Afolalu, S. A., Popoola, J. O., & Ajani, O. O. (2022). Phytochemical constituents and proximate analysis of dry pineapple peels. *IOP Conference Series: Earth and Environmental Science*, *993*(1), 012027. https://doi.org/10.1088/1755-1315/993/1/012027

62. Ozyuguran, A., Akturk, A., & Yaman, S. (2018). Optimal use of condensed parameters of ultimate analysis to predict the calorific value of biomass. *Fuel*, *214*, 640–646. https://doi.org/10.1016/j.fuel.2017.10.082

63. Paepatung, N., Nopharatana, A., & Songkasiri, W. (2009). *Bio-Methane Potential of Biological Solid Materials and Agricultural Wastes*.

64. Panić, I., Ćuculic, A. & Ćelić, J. (2022). Color-coded Hydrogen. Production and storage in maritime sector. Journal of Marine Sciences and Engineering, 10(12), 1995. https://doi.org/10.3390/jmse10121995.

65. Pankaj K. Rai and S.P. Singh (2016). Integrated dark- and photo-fermentation: Recent advances and provisions for improvement. International Journal of Hydrogen. http://dx.doi.org/10.1016/j.ijhydene.2016.08.084

66. Pason, P., Tachaapaikoon, C., Panichnumsin, P., Ketbot, P., Waeonukul, R., Kosugi, A., & Ratanakhanokchai, K. (2020). One-step biohydrogen production from cassava pulp using novel enrichment of anaerobic thermophilic bacteria community. *Biocatalysis and Agricultural Biotechnology*, *27*, 101658. https://doi.org/10.1016/j.bcab.2020.101658

67. Patrick T. Sekoai, Anish Ghimire, Obinna T. Ezeokoli, Subramanya Rao, Wing Y. Ngan, Olivier Habimana, Yuan Yao, Pu Yang, Aster Hei Yiu Fung, Kelvin O. Yoro, Michael O. Daramola, Chun-Hsiung Hung (2021). Valorization of volatile fatty acids from the dark fermentation waste Streams-A promising pathway for a biorefinery concept. Renewable and Sustainable Energy Reviews. https://doi.org/10.1016/j.rser.2021.110971

68. Pereira, P. H. F., Ornaghi, H. L., Arantes, V., & Cioffi, M. O. H. (2021). Effect of chemical treatment of pineapple crown fiber in the production, chemical composition, crystalline structure, thermal stability, and thermal degradation kinetic properties of cellulosic materials. *Carbohydrate Research*, *499*, 108227. https://doi.org/10.1016/j.carres.2020.108227

69. Pereira, P. H. F., Ornaghi, H. L., Arantes, V., & Cioffi, M. O. H. (2021). Effect of chemical treatment of pineapple crown fiber in the production, chemical composition, crystalline structure, thermal stability, and thermal degradation kinetic properties of cellulosic materials. *Carbohydrate Research*, *499*, 108227. https://doi.org/10.1016/j.carres.2020.108227

70. Periyasamy Sivagurunathan, Gopalakrishnan Kumar, Peter Bakonyi, Sang-Hyoun Kim, Takurou Kobayashi, Kai Qin Xu, Gabor Lakner, Gabor Toth, Nandor Nemestothy, Katalin Belafi-Bako (2015). A critical review on issues and overcoming strategies for the enhancement

71. Prakash K. Sarangi and Sonil Nanda (2020). Biohydrogen Production Through Dark Fermentation. Chem. Eng. Technol. 2020, 43, No. 4, 601–612. DOI: 10.1002/ceat.201900452

72. Puranjan Mishra, Santhana Krishnan, Supriyanka Rana, Lakhveer Singha, Mimi Sakinaha, Zularisam Ab Wahid (2019). Outlook of fermentative hydrogen production techniques: An

overview of dark, photo, and integrated dark-photo fermentative approach to biomass. Energy Strategy Reviews. https://doi.org/10.1016/j.esr.2019.01.001

of dark fermentative hydrogen production in continuous systems. International Journal of Hydrogen Energy. http://dx.doi.org/ 10.1016/j.ijhydene.2015.12.081

73. Rafał Łukajtis, Iwona Hołowacz, Karolina Kucharska, Marta Glinka, Piotr Rybarczyk, Andrzej Przyjazny, Marian Kamiński (2018). Hydrogen production from biomass using dark fermentation. Renewable and Sustainable Energy Reviews. https://doi.org/10.1016/j.rser.2018.04.043

74. Rami M.M. Ziara, Daniel N. Miller, Jeyamkondan Subbiah, Bruce I. Dvorak (2018). Lactate wastewater dark fermentation: The effect of temperature and initial pH on biohydrogen production and microbial community, International Journal of Hydrogen Energy. https://doi.org/10.1016/ j. ijhydene.2018.11.045

75. Reungsang, A., & Sreela-or, C. (2013). Bio-Hydrogen Production from Pineapple Waste Extract by Anaerobic Mixed Cultures. *Energies*, *6*(4), 2175–2190. https://doi.org/10.3390/en6042175

76. Ruby Lynn G. Ventura, Jey-R S. Ventura and Young-Sook Oh (2016). Investigation of the Photoheterotrophic Hydrogen Production of Rhodobacter sphaeroides KCTC 1434 using Volatile Fatty Acids under Argon and Nitrogen Headspace. American Journal of Environmental Sciences. DOI: 10.3844/ajessp.2016.358.369.

77. Saidi, R., Liebgott, P. P., Hamdi, M., Auria, R., & Bouallagui, H. (2018). Enhancement of fermentative hydrogen production by Thermotoga maritima through hyperthermophilic anaerobic co-digestion of fruit-vegetable and fish wastes. *International Journal of Hydrogen Energy*, *43*(52), 23168–23177. https://doi.org/10.1016/j.ijhydene.2018.10.208

78. Saratale, G. D., Saratale, R. G., Banu, J. R., & Chang, J.-S. (2019). Biohydrogen Production from Renewable Biomass Resources. In *Biohydrogen* (pp. 247–277). Elsevier. https://doi.org/10.1016/B978-0-444-64203-5.00010-1

79. Satya Ranjan Das and Nitai Basak (2020). Molecular biohydrogen production by dark and photo fermentation from wastes containing starch: recent advancement and future perspective. Bioprocess and Biosystems Engineering. https://doi.org/10.1007/s00449-020-02422-5

80. Sema Z. Baykara (2018). Hydrogen: A brief overview on its sources, production, and environmental impact, International Journal of Hydrogen Energy. https://doi.org/10.1016/j.ijhydene.2018.02.0

81. Seyedeh Azadeh Alavi-Borazjani, Luı´s Antonio da Cruz Tarelho &Maria Isabel Capela (2021). Parametric optimization of the dark fermentation process for enhanced biohydrogen production from the organic fraction of municipal solid waste using the Taguchi method. International Journal of hydrogen energy 46 (2021) 21372e21382. https://doi.org/10.1016/j.ijhydene.2021.04.017.

82. Shaoan Cheng and Bruce E. Logan (2007). Sustainable and efficient biohydrogen production via electrohydrogenesis. www.pnas.org/cgi/doi10.1073/pnas.0706379104

83. Show, K.-Y., Yan, Y.-G., & Lee, D.-J. (2019). Biohydrogen Production: Status and Perspectives. In *Biofuels: Alternative Feedstocks and Conversion Processes for the Production of Liquid and Gaseous Biofuels* (pp. 693–713). Elsevier. https://doi.org/10.1016/B978-0-12-816856-1.00028-2

84. Sołowski Gawel (2018). Biohydrogen Production - Sources and Methods: A Review. Int J Bioprocess Biotech: IJBBT-101. DOI: 10.20911/IJBBT-101.

85. Tiang, M. F., Fitri Hanipa, M. A., Abdul, P. M., Jahim, J. M. d., Mahmod, S. S., Takriff, M. S., Lay, C.-H., Reungsang, A., & Wu, S.-Y. (2020). Recent advanced biotechnological strategies to enhance photo-fermentative biohydrogen production by purple non-sulfur bacteria: An overview. *International Journal of Hydrogen Energy*, *45*(24), 13211–13230. https://doi.org/10.1016/j.ijhydene.2020.03.033

86. Tsai, W.-T., Ayestas, R., Tsai, C.-H., & Lin, Y.-Q. (2022). Preparation and Characterization of Porous Materials from Pineapple Peel at Elevated Pyrolysis Temperatures. *Materials*, *15*(13), 4686. https://doi.org/10.3390/ma15134686

87. Wang, B., Wan, W., & Wang, J. (2009). Effect of ammonia concentration on fermentative hydrogen production by mixed cultures. *Bioresource Technology*, *100*(3), 1211–1213. https://doi.org/10.1016/j.biortech.2008.08.018

88. Xin Mei Guo, Eric Trably, Eric Latrille, Helene Carrere, Jean-Philippe Steyer (2010). Hydrogen production from agricultural waste by dark fermentation: A review. International journal of hydrogen energy 35 (2010) 10660 e10673. Doi: 10.1016/j.ijhydene.2010.03.008

89. Yanan Yin and Jianlong Wang (2022). Production of biohydrogen. Biofuels and Biorefining. Elsevier (Pp 283-337). ISBN:978-0-12-824116-5. https://doi.org/10.1016/B978-0-12-824116-5.00002-7.

90. Yun, Y.-M., Lee, M.-K., Im, S.-W., Marone, A., Trably, E., Shin, S.-R., Kim, M.-G., Cho, S.-K., & Kim, D.-H. (2018). Biohydrogen production from food waste: Current status, limitations, and future perspectives. *Bioresource Technology*, *248*, 79–87. https://doi.org/10.1016/j.biortech.2017.06.107

91. Zainuddin, M. F., Shamsudin, R., Mokhtar, M. N., & Ismail, D. (2014). Physicochemical Properties of Pineapple Plant Waste Fibers from the Leaves and Stems of Different Varieties. *BioResources*, *9*(3), 5311–5324. https://doi.org/10.15376/biores.9.3.5311-5324

92. Zainul Abdin, Ali Zafaranloo, Ahmad Rafiee, Walter Merida, Wojciech Lipinski, Kaveh R. Khalilpour (2019). Hydrogen as an energy vector. Renewable and Sustainable Energy Reviews. https://doi.org/10.1016/j.rser.2019.109620

APPENDICES

Appendix A

Table 10: Ultimate analysis Results of the sample

Element analyzed	Wt. (%)
Carbon	44.4
oxygen	40.9
Hydrogen	9.4
potassium	3.6
Silicon	0.9
Zinc	0.008

***Figure 21a**: Focus image view*

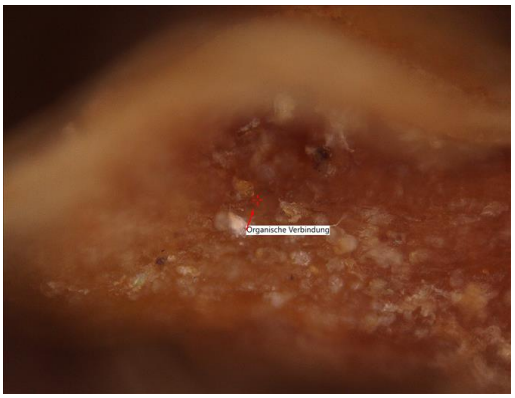


***Figure 21b**: Navigation image*

***Figure 21a&b:** Focus and navigation images of the pineapple peels during the analysis of the sample*

Appendix B

Table 10: Proximate analysis results of the sample (g)

Mass of empty crucible	Total mass of crucible + sample	Total mass after drying (105^0C)	Total mass after incineration (550^0C)
57.5887	72.7302	71.4757	58.3297
61.2273	75.7584	74.5498	61.9296
65.6857	79.9948	78.8016	66.3818

Appendix C

Table 11: Ash analysis results of the sample (mol%)

No.	Guess Material	K	O	Ca	Si	Mg	Na	H	C	N	S
1	Potassium compound	28.0%	62.6%	4.9%	2.6%	1.4%	0.5%	0.0%	0	0	0
2	Potassium compound	10.9%	38.6%	3.4%	3.2%	1.5%	0.2%	0.0%	0.027	0	0.395
3	Potassium compound	9.8%	52.3%	6.3%	3.0%	1.1%	0.2%	0.0%	0.068	0	0.205

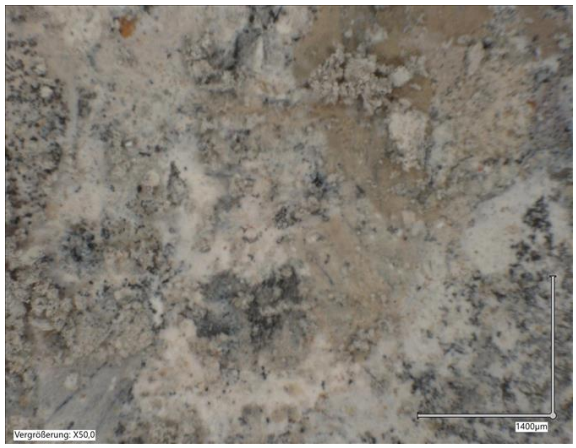


Image view of ash during ash analysis

Appendix D

Table 12: Fibre analysis results of the sample

TM in %FM	oTM in %FM	Ash in %FM	NDF	ADF	ADL
91.7	86.8	4.9	27.93	11.01	2.51

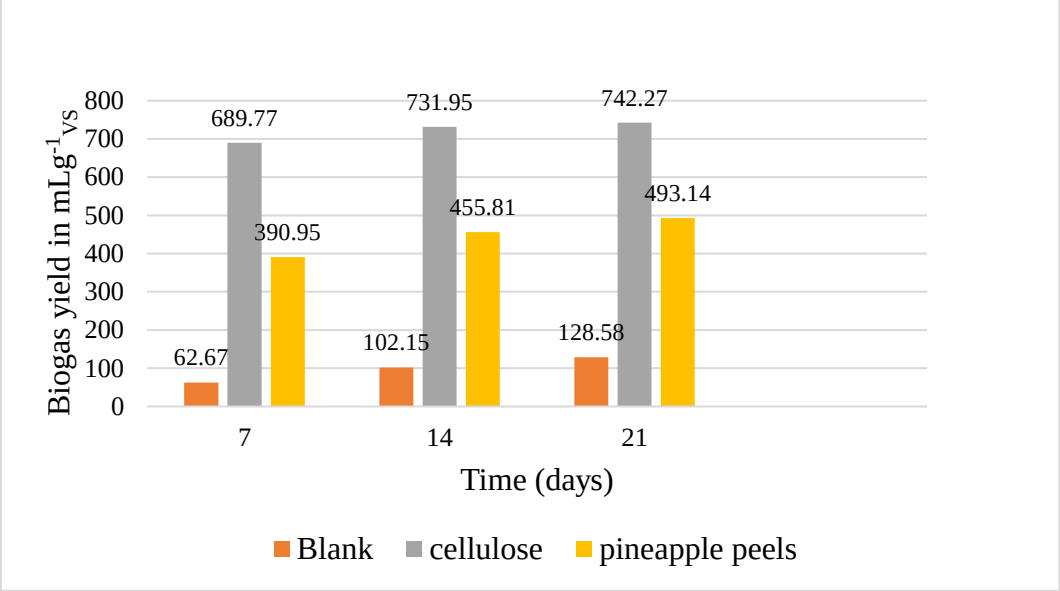


***Figure 22**: Cumulative biogas production from pineapple peels sample, blank and cellulose standard*