EXPERIMENTAL INVESTIGATION OF DARK FERMENTATION OF POPLAR LEAVES FOR BIOHYDROGEN PRODUCTION

by

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An oral defense of this thesis took place on August 10, 2023 in front of the following examining committee:

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The above committee determined that the thesis is acceptable in form and content and that a satisfactory knowledge of the field covered by the thesis was demonstrated by the candidate during an oral examination. A signed copy of the Certificate of Approval is available from the School of Graduate and Postdoctoral Studies.

ABSTRACT

Biohydrogen, a sustainable and environmentally friendly energy alternative, is pivotal in transitioning to a more energy-efficient future. Thesis research explores the variations of H_2 hydrogen production potential of poplar leaves as a substrate for biohydrogen production via dark fermentation, an underexplored area. Through a series of 58 experimental trials, insightful findings were obtained about the hydrogen production process. The Gompertz function is used to model the experimental trial results which represent cumulative hydrogen production rate. The hydrogen production rate varies between 0.14 to 2.73 ml/h. Moreover, variations were observed in the maximum hydrogen production per gram of substrate, between 0.02 ml/g to 0.46 ml/g. The ideal maximum hydrogen using the experimental data. production rate was estimated at approximately 0.2 ml/h, with an optimal time constant of about 1 hour. A comprehensive analysis of influential parameters was conducted using Design-Expert statistical software, identifying biomass quantity as a critical determinant of hydrogen production. The research also identified optimal operational conditions for maximizing hydrogen production: an acid concentration of 10%, a biomass quantity of 2.009 grams, an initial pH of 7.65, a temperature of 39.9 °C, and a mixing ratio of 325.66 rpm. These conditions were projected to produce a maximum hydrogen production of 0.76 mL/g. The results suggest that a biochemical reactor designed in this study effectively reduced the salinity of water and chemical oxygen demand (COD) of biomass by approximately 51% and 75%, respectively.

Keywords: dark fermentation; poplar leaves; biochemical reactors; hydrogen production.

AUTHOR'S DECLARATION

I hereby declare that this thesis consists of original work of which I have authored. This is a true copy of the thesis, including any required final revisions, as accepted by my examiners.

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STATEMENT OF CONTRIBUTIONS

I certify that I am the sole author of this thesis and that no part of this thesis has been published or submitted for publication. I have used standard referencing practices to acknowledge ideas, research techniques, or other materials that belong to others. Furthermore, I certify that I am the sole source of the creative works and inventive knowledge described in this thesis.

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LIST OF ABBREVIATIONS AND SYMBOLS

Abbreviations

ATP	Adenosine Triphosphate
AFBR	Anaerobic Fluidized Bed Reactor
BBD	Box-Behnken Design
BES	Bioelectrochemical System
COD	Chemical Oxygen Demand
CSTR	Continuous Stirred Tank Reactor
DF	Dark Fermentation
DMC	Defined Mixed Cultures
DRI	Direct Reduction of Iron
EFTA	European Free Trade Association
EGSB	Expanded Granular Sludge Bed
HER	Hydrogen Evolution Reaction
HRT	High Retention Time
HTE	High-Temperature Electrolysis
HPR	Hydrogen Production Rate
HPY	Hydrogen Production Yield
IEA	International Energy Agency
LKAB	Luossavaara-Kiirunavaara Aktiebolag
LCFA	Long-Chain Fatty Acids
MR	Membrane Reactors
MDC	Microbial Desalination
MEC	Microbial Electrolysis Cell
PBR	Packed Bed Bioreactor
PSA	Pressure Swing Adsorption
PEM	Proton Exchange Membrane
PEMFC	Proton Exchange Membrane Fuel Cell
RE	Removal Efficiency
RSM	Response Surface Methodology

SR Steam Reforming

SMR Steam Methane Reforming

SI Sulfur-Iodine

SSAB Svenskt Stål AB

TDI Toluene Diisocyanate

TCOD Total Chemical Oxygen Demand

TRS Total Reducing Sugar

UASB Up-Flow Anaerobic Sludge Blanket Reactor

UK United Kingdom

VFAs Volatile Fatty Acids

VS Volatile Solids

WGS Water-Gas Shift

Symbols

C Conductivity (mS/m)

H_{max} Maximum Value of Hydrogen Production (mL)

R_{max} Maximum Hydrogen Production Rate (mL/h)

R^2 Root Mean Square

λ Lag Phase (h)

Greek Letters

λ Lambda

Chapter 1. INTRODUCTION

1.1 Energy and Environmental Issues

The planet has never suffered so much throughout its entire existence. Despite its many advantages, globalization has created many obstacles to humanity and the planet, such as environmental, social, economic, and political. Globalization, emphasizing free trade and international connectivity, has enhanced economic growth, increased living standards, and brought diverse cultures closer together than ever before. However, it has also created some significant challenges. Environmental degradation is considered one of the most notable challenges due to globalization. The sharp increase in energy demand can be attributed to the rising level of industrialization and consumption fueled by rapid economic expansion. Figure 1.1 indicates the upward trend in energy consumption between 1800 and 2021, and it is expected to continue in the future.

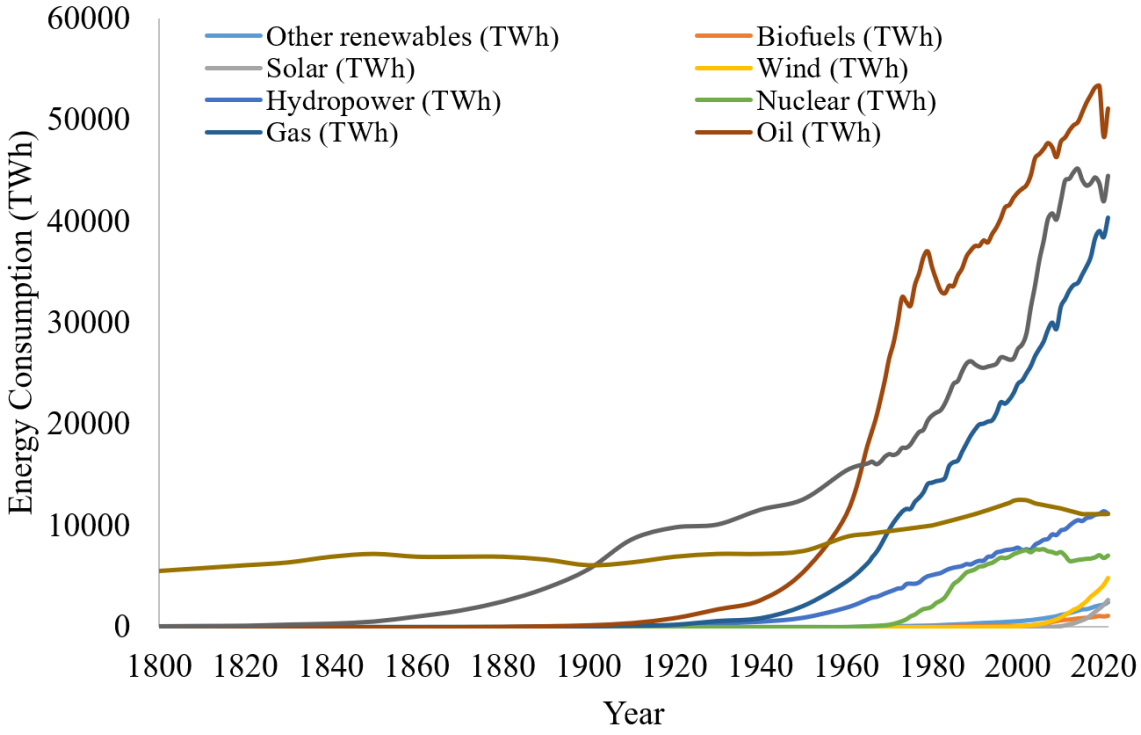


Figure 1.1 Energy consumption by source between 1800-2020 (data from [1]])

Likewise, renewable sources increased during that period, and fossil fuels increased between 1800-2020. The Earth has severe environmental issues because of anthropogenic pollution related to the use of gas and oil as well as coal products in the atmosphere. Figure 1.1 also shows the apparent increase between 1800-2020 in the use of fossil fuels [2]. The

consumption of non-renewable energy sources leads to increased carbon emissions, air pollution, and environmental damage. The extraction and usage of fossil fuels have accelerated cli mate change while posing a threat to biodiversity and causing health issues. Global warming is the process of an increase in the average temperature of the Earth caused by emissions from burning fossil fuels, particularly carbon dioxide and, indirectly, water vapor. Aside from that, the extraction process frequently causes environmental harm, such as habitat destruction and oil spills.

According to the United Nations, climate change is a worldwide emergency that crosses borders. This problem necessitates international cooperation and coordinates solutions at all levels [3].

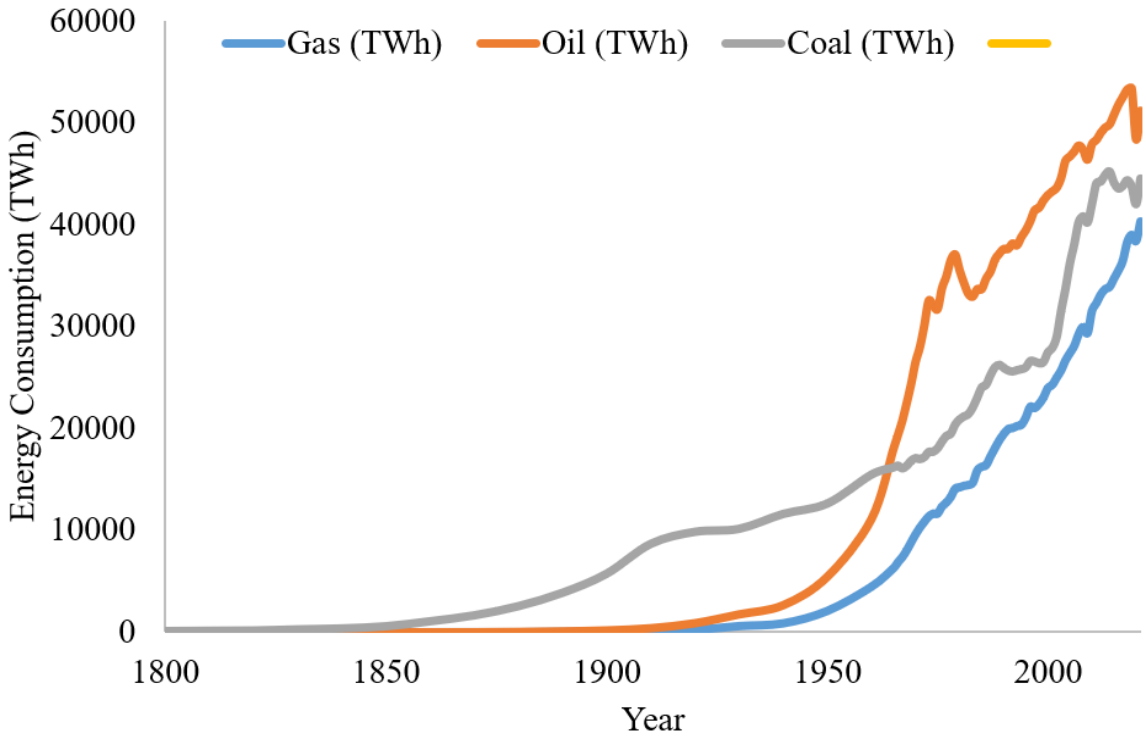


Figure 1.2 Global primary energy consumption by source (data from [4])

For this purpose, world leaders at the United Nations Climate Change Conference (COP21) in Paris reached a critical agreement to combat climate change and its harmful effects. The Agreement established long-term objectives for all nations. The decisions taken were:

- Progressively decreasing global greenhouse gas emissions to limit the increase in global temperature to 2 °C in this century while pursuing efforts to limit the increase to 1.5 °C.
- Evaluating the commitments of nations every five years.
- Providing developing nations funding for climate change mitigation, resilience building, and adaptation to climate impacts.

The Agreement urges countries to strengthen their commitments over time. It also establishes a mechanism for developed nations to aid developing nations in their climate mitigation and adaptation efforts and a framework for the transparent monitoring and reporting of countries' climate objectives.

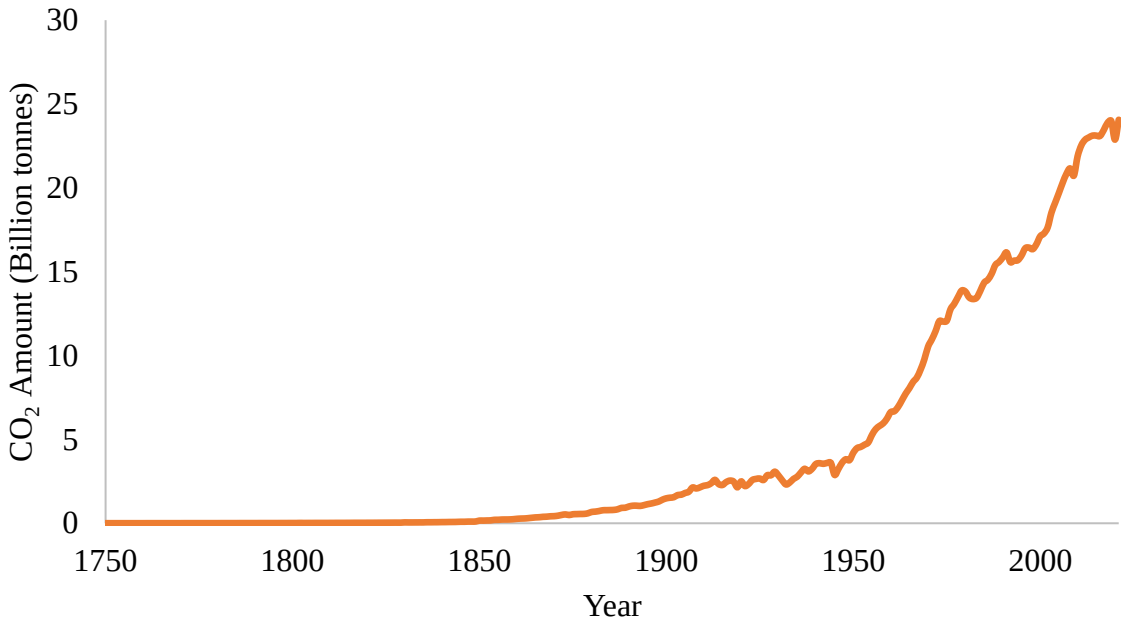


Figure 1.3 Annual CO_2 Emissions in the world (data from [1])

Figure 1.3 shows that the total amount of carbon dioxide has increased significantly throughout the years. This growing trend required a global response, which led to the incorporation of Net Zero Targets in the Paris Agreement. Net zero means reducing greenhouse gas emissions to near zero, with any remaining emissions being reabsorbed by the atmosphere, oceans, and forests [5]. According to research, to reduce the most severe consequences of climate change and maintain a livable Earth, it is crucial to restrict the rise in global temperature to a maximum of 1.5°C above pre-industrial levels. The Earth's

average temperature has witnessed an approximate increase of 1.1°C compared to the late 1800s, and the emission levels are continuously increasing. To limit global warming to 1.5°C, as the Paris Agreement requires, emissions must be reduced by 45 percent by 2030 and reach net zero by 2050 [5]. One of humanity's biggest challenges is the mission to move towards a net-zero world. This goal necessitates an extensive renovation of production, consumption, and transportation systems. The energy sector is responsible for approximately 75% of the present greenhouse gas emissions and plays a crucial role in addressing the most severe consequences of climate change [6]. Replacing carbon-emitting coal, gas, and oil energy sources with wind and solar power would substantially reduce carbon emissions. All being considered, the demand for renewable and sustainable energy sources has grown significantly recently.

A global movement has begun to switch to renewable energy sources in response to these urgent problems. These are renewable natural energy sources that do not become depleted when used. They consist of wind, solar, geothermal, hydro, and bioenergy. By using these resources to produce electricity, the environmental effect is considerably reduced, and principles of sustainable growth are followed. Moreover, these sources improve national power grid security, reliability, and resilience. However, there are challenges associated with the transition to renewable energy. Among the obstacles that must be surmounted are the variability of solar and wind power, the impact of hydropower on local ecosystems, and the present high costs of emerging technologies. However, these obstacles are gradually being overcome with ongoing research and innovation. Renewable energy is unquestionably the future of the global energy industry. Humanity should pave the way for a healthier, greener, and more prosperous world by prioritizing the transition to renewable energy.

1.2 A Powerful Alternative: Hydrogen

As the adverse effects of climate change become more apparent, the demand for sustainable, renewable energy solutions intensifies. The ambitious Net Zero Targets established by the Paris Agreement necessitate a global transition from traditional fossil fuels [3]. Hydrogen stands out in this search for viable alternatives due to its abundance and potential. Hydrogen presents an attractive case as a significant energy vector [7]. The rise of interest in hydrogen as an energy carrier is attributable to its unique properties. It

can effectively store and transport energy, and when combined with oxygen in a fuel cell, it produces electricity while emitting only water and heat. Furthermore, the superior nature of hydrogen over other fuels can be attributed to its unique specific energy. Specific energy is the quantity stored per unit mass, megajoules per kilogram (MJ/kg). It is crucial for applications in which weight is essential, such as transportation and mobile energy storage. While hydrogen has substantial advantages regarding specific energy, its complete functionality as a fuel source depends on addressing the issues associated with its volumetric energy density and developing effective storage and transportation systems.

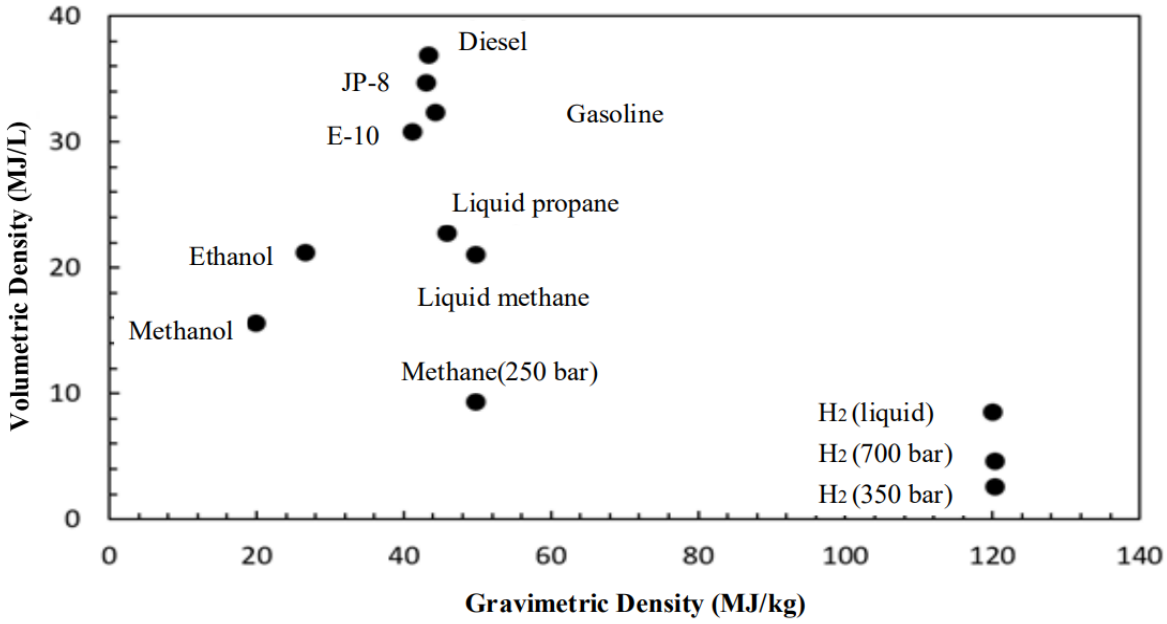


Figure 1.4 Comparison of specific energy for different fuels (data from [8])

As seen in Figure 1.4, hydrogen has a specific energy of approximately 33.33 kWh/kg (120 MJ/kg), making it one of the most powerful energy carriers by mass. This is far better compared to traditional fuels, such as gasoline, which has a specific energy of approximately 12.8 kWh/kg (46.4 MJ/kg), and coal, which has a specific energy of approximately 8 kWh/kg (28.8 MJ/kg)[9]. Even compared to alternative fuels such as biofuels or batteries, hydrogen remains significantly superior. The specific energy of lithium-ion batteries, which are extensively used in electric vehicles, is approximately 0.25 kWh/kg (0.90 MJ/kg), significantly less than that of hydrogen [10]. Hydrogen has great potential for use in industries like aviation and transportation because it has a high specific energy and is lightweight. When used in a fuel cell, it is much more efficient than in internal

combustion engines, which leads to better overall energy utilization. It is noteworthy that although hydrogen possesses a high specific energy, its energy density per unit volume is relatively low owing to its low ambient temperature density. This poses a significant challenge in terms of storage and transportation. As a result, various technologies are being developed to ensure this resource's efficient storage and transportation, including high-pressure tanks, cryogenic liquid storage, and metal hydrides. As a result of governments' net-zero emission goals, their significance and upward trend have accelerated. According to the International Energy Agency (IEA), hydrogen is a crucial piece of the puzzle for achieving net zero emissions by 2050 [11].

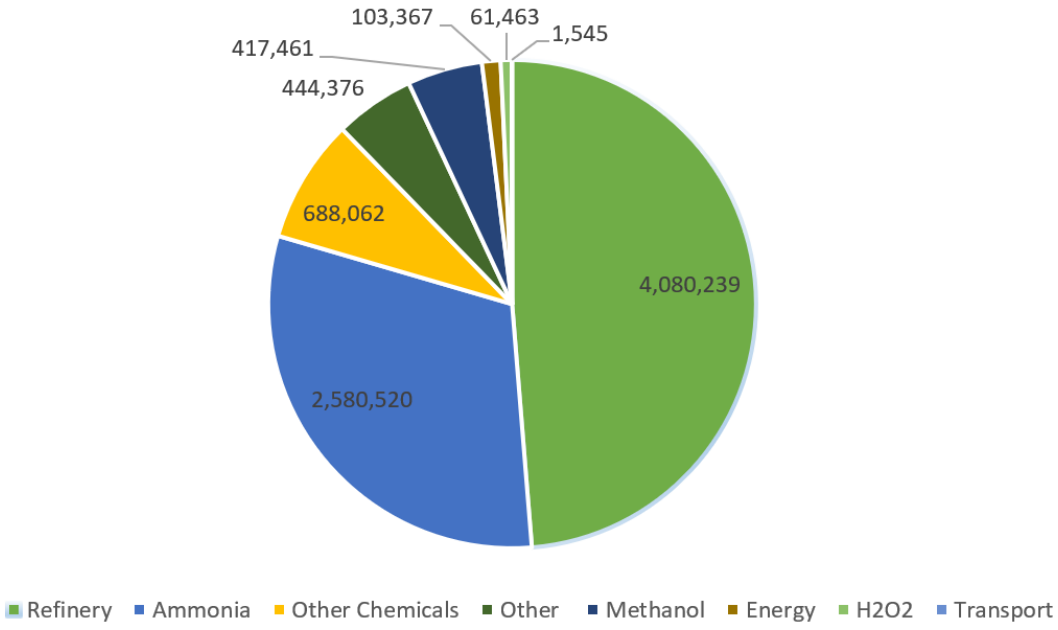


Figure 1.5 Total demand for hydrogen in 2019 by application

Hydrogen is a versatile element that finds application in various industries. These include chemical sand steel production, power generation, manufacturing, space exploration, and transportation. As seen in Figure 1.5, the estimated total demand for hydrogen in the analyzed countries, the EU, EFTA, and UK, in 2019 is 8.4 Mt [12]. The refinery industry accounts for 49% of total hydrogen consumption, while the ammonia industry accounts for 31%. These two industries accounted for 80% of the total hydrogen consumption. About 13% is consumed by the chemical industry, of which 5% is used to produce methanol. On

the other hand, emerging hydrogen applications, such as the transportation industry, represented a negligible portion of the market in 2019 (0.02%).

1.3 Uses of Hydrogen

Because of its adaptability, hydrogen is used in a wide variety of applications across a wide range of industries. Its versatility is demonstrated by the fact that it can be applied to numerous industries. The following outlines a selection of industries where hydrogen finds common and meaningful utilization.

1.3.1 Refining industry

Refineries mainly use hydrogen for hydrocracking and hydrotreating processes, including hydrodesulfurization. As regulations require ever-decreasing fuel sulfur levels, more desulphurization is needed to meet these objectives, driving the sector's hydrogen consumption [13]. The significance of hydrocracking is growing due to the increasing global demand for distillates, including aviation fuel, kerosene, high-quality lubricating oils, and diesel. While some refining processes can generate hydrogen, most refineries consume more than they produce. The precise estimation of hydrogen demand in a refinery is difficult due to its reliance on various factors, such as specific processes and the composition of output products. Consequently, it is challenging to precisely estimate the required amount of hydrogen solely based on production volume [14]. On the other hand, the total hydrogen demand from the oil refining and petrochemical industries is projected to be 4.1 Mt in 2019. This is based on the data provided in Figure 4, refinery hydrogen production capacities, and information on their capacity utilization.

1.3.2 The chemical industry

Ammonia manufacturers are one of the biggest consumers of hydrogen in the chemical industry. Ammonia is utilized to produce fertilizers and nitric oxide, which serves as a precursor to nitric acid. Ammonia is also used to produce sodium carbonate (soda ash), explosives, hydrogen cyanide, and synthetic fabrics, among other products. Eurostat data shows that the total demand for hydrogen by the ammonia industry in 2019 is estimated to be 2.6 Mt. Even though the ammonia industry exceeds other applications, it is not the only chemical industry consumer of hydrogen. Other chemicals whose production requires

hydrogen input include methanol, cyclohexane, aniline, caprolactam, hydrogen Peroxide, oxo alcohols C8, oxo alcohols c4, toluene diisocyanate (TDI), hexamethylenediamine.

1.3.3 Steel Manufacturing and Metal Processing

The steel industry is responsible for a large portion of global CO_2 emissions, accounting for approximately 7 to 9 percent [15]. Fortunately, recent technological advancements have made it feasible to significantly reduce these emissions by utilizing hydrogen-based steel production methods. The Direct Reduction of Iron (DRI) process is one such method, which replaces carbon with hydrogen, resulting in water instead of CO_2 as a byproduct [16]. An example of this transition in the actual world is the Swedish initiative HYBRIT, a joint venture between SSAB, LKAB, and Vattenfall. They aim to replace coking coal in the steel production process with hydrogen. According to their plan, they intend to manufacture one ton of fossil-free steel by 2026 and gradually increase production to 2.5 million tons annually by 2045 [17]. In metal processing, hydrogen is commonly used in heat treatment due to its low density, high thermal conductivity, and nonreactive properties. It serves as a reducing agent that prevents oxidation and preserves the metal's properties. An actual world example is the use of hydrogen in the copper annealing process. Copper producers such as Aurubis employ hydrogen atmospheres in continuous annealing lines to prevent copper oxidation and preserve its intended mechanical properties [18].

1.3.4 Transportation Industry

Hydrogen is becoming popular for cleaner and more eco-friendly fuel options in the transportation industry. Vehicles using hydrogen fuel cells create electricity through the reaction of hydrogen with oxygen, producing only water vapor as an emission. As of 2020, over 72,000 of these vehicles have been manufactured globally, according to the IEA [19]. Bus fleets are an example of the implementation of this technology in the real world. As of 2022, the Chinese city of Foshan operates one of the world's largest fleets of hydrogen buses, with more than 1,000 hydrogen fuel cell buses in operation. Moreover, major automobile manufacturers like Toyota and Hyundai have put hydrogen fuel cell vehicles on the market. As of 2021, Toyota has produced and sold over 10,000 Mira worldwide. Hyundai has also presented the NEXO Fuel Cell SUV, which has a single-charge range of up to 380 miles [20].

1.3.5 Space Exploration

Hydrogen has been used for centuries as a fuel in space exploration due to its exceptional energy density per unit mass. NASA has employed hydrogen gas to transport astronauts and freight to outer space. NASA has gained significant expertise in the secure and efficient handling of hydrogen through various missions, including Centaur, Apollo, and the space shuttle. [21]. Hydrogen will continue to be innovatively stored, measured, processed, and used in accordance with the current focus on human missions to the moon and, eventually, Mars. Beyond its usage as a rocket propellant, hydrogen can be produced locally from soil or water to provide crew members with oxygen to breathe [21].

Hydrogen is gaining popularity as a versatile and eco-friendly substitute in various industries. It is now widely used in energy production, space exploration, and transportation due to its ability to offer cleaner and more efficient options. Hydrogen's role in the energy system will become more significant as technology advances. This clean operation perfectly fits the Paris Agreement's Net Zero Emissions objectives.

1.4 Hydrogen Production Methods

Burning fossil fuels releases greenhouse gases that pose a significant danger to the environment and contribute to climate change. Furthermore, economies reliant on traditional fuel imports are vulnerable to the escalating prices of these fuels caused by rising energy demands. It is crucial to adopt carbon-free and renewable energy sources to address climate change over the long term and decrease dependence on imported oil [22]. Hydrogen can be obtained using different energy sources such as biomass, solar, wind, nuclear, and natural gas. When renewable sources are used to produce hydrogen, these processes provide a viable way to cut carbon emissions.

On the other hand, the most widely used techniques for producing hydrogen currently rely on fossil fuels, such as coal gasification and steam methane reforming (SMR). In these processes, steam and hydrocarbons react, producing hydrogen and carbon dioxide as a byproduct. Despite being efficient and affordable, they also play a significant role in producing greenhouse gas emissions. That is why renewable-based production methods promise a sustainable future while fossil fuel-based techniques dominate production, primarily considering the cost. Although hydrogen is not as abundant in nature as fossil

fuels, it can be used as fuel for fuel cells or in internal combustion engines. When used, it only emits water as a byproduct. This characteristic of hydrogen can promote energy security, diversify the energy supply, and minimize greenhouse gas emissions, leading to a more sustainable and resilient future.

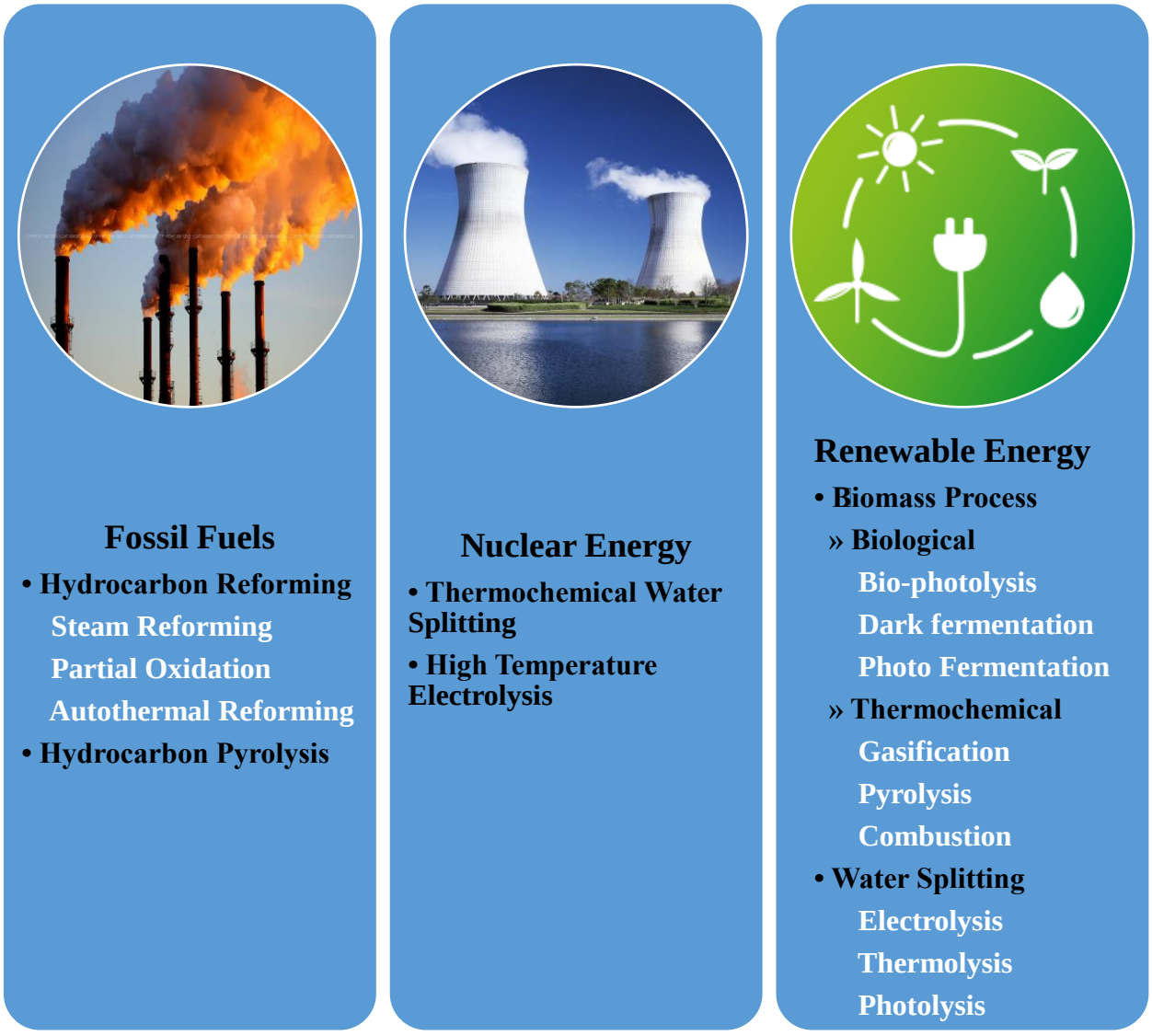


Figure 1.6 Hydrogen Production Methods

Figure 1.6 shows the hydrogen production methods. The most common method of hydrogen production in the present industrial world is the manufacture of hydrogen from fossil fuels, primarily using techniques like hydrocarbon reforming and hydrocarbon pyrolysis. This dominance is partly because of these methods' existing infrastructure, low cost, and high efficiency. Hydrocarbon reforming, particularly steam methane reforming

(SMR), uses natural gas and steam in a high-temperature and high-pressure reaction to produce hydrogen and carbon dioxide. In simple terms, the steam reforming process includes a catalytic conversion of the hydrocarbon and steam to hydrogen and carbon oxides. It consists of the three main processes of reforming or synthesis gas (syngas) generation, water-gas shift (WGS), and methanation or gas purification. Methane, natural gas, and other gases containing methane are examples of raw materials. With this technique, big reformers (e.g., 100,000 tons per year) can achieve more than 80% yields [23] However, as seen in the following equations, the process produces carbon dioxide ga, which is not desired as a byproduct. The reactions are shown below,

$$\text{Reformer}: C_nH_m + H_2O \rightarrow nCO + \left(n + \frac{1}{2}m\right)H_2 \quad (1.1)$$

$$\text{WGS}: CO + H_2O \rightarrow CO_2 + H_2 \quad (1.2)$$

Currently, it is the most popular and affordable technique for producing hydrogen. In steam reforming, natural gas is first purified of any impurities before being combined with steam and fed across a reformer that is heated from the outside. A reformer reaction produces carbon dioxide and hydrogen. Then, the produced gases are sent to the catalytic water gas shift reactor (WGS) to produce syngas. After that, the hydrogen gas is purified from the syngas. Hydrocarbon pyrolysis is the second primary process of producing hydrogen from fossil fuels, also known as thermal cracking. Pyrolysis is the chemical breakdown of organic compounds through heat application. During the pyrolysis process, a hydrocarbon, such as methane, undergoes thermal decomposition at temperatures between 300-800 degrees Celsius in an oxygen-free environment. In the absence of oxygen, pyrolysis breaks down organic materials to produce bio-oil, hydrogen/syngas, and solid biochar products [24]. Pyrolysis is a method of hydrogen production that does not generate carbon dioxide, making it a more sustainable option. Unlike other methods like steam reforming or partial oxidation, it has the potential to be even more environmentally friendly if the heat input comes from a carbon-free source. The most significant advantage of this process is that All of the carbon in the methane is trapped in solid form during methane pyrolysis instead of being released as carbon dioxide. The pyrolysis of the methane reaction can be seen in Equation 1.3.

$$C_nH_m \rightarrow nC_{(s)} + \frac{1}{2}m\,H_2 \quad (1.3)$$

On the other hand, although solid carbon seems like an advantage, it is also a significant challenge since dealing with solid carbon byproducts, if not managed properly, may lead to catalyst poisoning and reaction inhibition. However, if this byproduct is appropriately used or stored, pyrolysis might become critical in low-carbon hydrogen production.

1.4.1 H_2 production from nuclear energy

Using nuclear energy to produce hydrogen is both innovative and promising. With global energy demands rising and concerns about climate change, nuclear power is seen as a potential key player in a sustainable, low-carbon energy future. One of the benefits of using nuclear energy to produce hydrogen is its ability to produce large amounts of hydrogen consistently and reliably. Nuclear energy has a high energy density and a consistent power output regardless of weather or time of day. This is particularly important for techniques that require much energy, like high-temperature electrolysis and thermochemical water splitting. Applying thermochemical water splitting cycles represents a highly effective technological approach for hydrogen production through nuclear energy utilization. The process involves utilizing the heat generated by nuclear reactors as the only energy source to drive a series of chemical reactions that result in the decomposition of water. Given that all chemical reagents utilized in the process can be fully recycled, the sole resource consumption is water, with the only by-products being H_2 and O_2. Several thermochemical cycles are currently under investigation for water splitting, among which the sulfur-iodine (SI) cycle is deemed the most promising for commercialization due to its proximity to application, as illustrated by the following reactions.

$$H_2SO_4 \rightarrow SO_2 + H_2O + 0.5\,O_2 \quad (1.4)$$

$$I_2 + SO_2 + 2H_2O \rightarrow 2HI + H_2SO_4 \quad (1.5)$$

$$2HI \rightarrow I_2 + H_2 \quad (1.6)$$

The initial stage involves the breakdown of sulfuric acid into oxygen, sulfur dioxide, and steam at a high temperature of 850°C, as represented by Equation 1.4. During the second step, commonly called the Bunsen reaction (Equation 1.5), the chemical compound iodine

undergoes a reaction with sulfur dioxide and steam at a significantly lower temperature of 120°C. This reaction ultimately results in the formation of hydrogen iodide and sulfuric acid. The liquid phase containing hydrogen iodide and sulfuric acid is subjected to separation, purification, and concentration. The sulfuric acid that has been acquired is subjected to a recycling process. At the same time, the hydrogen iodide is decomposed at a temperature of approximately 450°C to generate hydrogen and iodine, which are subsequently recycled [25]. As a result, hydrogen is produced utilizing water and nuclear energy in high-temperature thermochemical water-splitting cycles with almost no emissions of greenhouse gases. The second method of producing hydrogen from nuclear energy is high-temperature electrolysis. High-temperature electrolysis (HTE), commonly referred to as steam electrolysis, uses the heat produced by nuclear reactions to boost the electrolysis process' effectiveness. Due to the heat energy input, electrical energy demand is reduced at high temperatures, increasing total efficiency [26]. High-temperature electrolysis operates at higher temperatures, ranging from 500 to 900 °C, than low-temperature water electrolysis. High-temperature electrolysis can electrochemically divide steam into H_2 and O_2 using electricity and heat at high temperatures (often in the 700-900 °C), even though it has not yet been commercialized [27]. At low temperatures (80 °C), the electricity required for HTE is approximately 35% less than that of traditional electrolysis since the necessary electricity decreases with rising temperature. That is why HTE has the potential to produce large-scale hydrogen with nearly zero greenhouse gas emissions when combined with nuclear power plants [28].

1.4.2 H_2 production from renewable energy

Hydrocarbons are the primary source for producing H_2, but it is crucial to promote renewable technologies. When renewables are used, the greenhouse gases associated with hydrogen production are significantly reduced. Numerous processes exist for H_2 production from renewable resources, and a brief overview of some biomass-based technologies and water-splitting methods is provided here. Using biomass to produce hydrogen is an eco-friendly and renewable method of converting various forms of organic matter into useful energy sources. The processes to convert biomass can be divided into thermochemical and biological methods. Using biomass to produce hydrogen is an eco-friendly and renewable way to transform various organic materials into useful energy

sources. Thermochemical processes, such as gasification, pyrolysis, and combustion, use heat as the primary catalyst to convert biomass into various chemical forms by producing hydrogen-rich syngas or hydrogen directly. These mechanisms have the advantage of being able to produce substantial quantities of hydrogen from a variety of biomass types. However, these processes' need for high temperatures makes energy efficiency a crucial issue [29].

The gasification of biomass is one of the most effective thermochemical processes. It involves controlled partial oxidation of biomass to generate a gas mixture rich in hydrogen and carbon monoxide, also known as syngas. The process typically operates at temperatures above 700°C and under varying pressures. The carbon monoxide reacts with water via a water-gas shift reaction to generate carbon dioxide and additional hydrogen. Hydrogen can be extracted from this gas stream using absorbers or membranes. The simplified version of gasification reactions can be seen in Equations 7-8. Equation 1.7 is based on glucose or cellulose for simplicity. However, the actual biomass is more complicated than Equation 1.7.

$$C_6H_6O_6 + O_2 + H_2O \rightarrow CO + CO_2 + H_2 + \text{other species} \quad (1.7)$$

$$CO + H_2O \rightarrow CO_2 + H_2 + \text{heat} \quad (1.8)$$

It offers the benefit of handling various types of biomasses, including agricultural residues and energy crops [30] However, biomass gasification has two main issues: a low carbon conversion rate and the need to treat tar afterward [31]. The tar has a high energy content, makes up 5–15% of the total gas products, and impacts gasification and energy conversion efficiency. However, it also severely clogs pipelines and valves after cooling, which is problematic for using syngas in the future [32]. Thus, tar cracking is a significant obstacle to improving syngas quality. Hence, several efficient catalysts and novel gasification methods have been developed to solve the above issues and increase H_2 output, concentration, and efficiency.

Biomass pyrolysis is a process that uses heat to break down biomass into liquid oils, solid charcoal, and gaseous compounds. This is done by heating the biomass at a 650-800 K temperature range and a pressure range of 0.1-0.5 MPa [22]. The process occurs without

oxygen, except in cases where partial combustion generates the necessary thermal energy. Hydrocarbon gases like methane can be converted into hydrogen through steam reformation and the WGS reaction. The CO is transformed into CO_2 and H_2, and the hydrogen is purified through the PSA (Pressure Swing Adsorption) process for maximum production. The steps of biomass pyrolysis are given in the following equations.

$$\text{Pyrolysis of Biomass} \rightarrow H_2 + CO + CO_2 + \text{hydrocarbon gases} + \text{tar} + \text{char} \quad (1.9)$$

$$C_nH_m + nH_2O \rightarrow nCO + \left(n + \frac{1}{2}m\right)H_2 \quad (1.10)$$

$$CO + H_2O \rightarrow CO_2 + H_2 \quad (1.11)$$

Factors such as feedstock type, catalyst type, temperature, and residence time influence the hydrogen produced from biomass pyrolysis [33] However, the negative characteristics of bio-oil, such as its poor heating value and significant instability at high temperatures, make biomass pyrolysis technology challenging to commercialize. Various methods, reactors, and catalysts have been developed to tackle the issues involved in biomass pyrolysis and catalytic pyrolysis [34].

One innovative and eco-friendly method of generating hydrogen is through biological conversion. This involves utilizing yeast, bacteria, algae, and their inherent biochemical processes to produce hydrogen. Over the past few years, research in biological hydrogen generation has grown significantly due to a greater focus on sustainable development and waste reduction. Most biological processes run at room temperature and pressure, requiring less energy. Additionally, they make use of renewable energy sources, which are limitless, and they support waste recycling by using a variety of wastes as feedstock. The three main processes in the biological conversion landscape are biophotolysis, photo fermentation, and dark fermentation. Biohydrogen production includes two different processes. The first process uses water for photolysis to produce hydrogen directly from bacteria or algae enzymes called hydrogenase or nitrogenase. The second and third processes involve using biomass to convert carbohydrate-containing materials to organic acids, which are then processed using biotechnologies to produce hydrogen gas [35]. Biophotolysis is the process by which microalgae or cyanobacteria use light energy to divide water into hydrogen and oxygen. This process replicates natural photosynthesis and can be a renewable and carbon-

free method for hydrogen production. Nonetheless, it encounters technical obstacles such as inefficiency and scaling difficulties [36]. Equation 1.12 represents the reaction happening in the biophotolysis.

$$2H_2O + \text{light energy} \rightarrow 2H_2 + O_2 \quad (1.12)$$

Fermentation involves microorganisms transforming organic materials into various products, such as alcohol, acetone, and hydrogen. This can occur with or without oxygen. These processes are environmentally friendly since they allow for repurposing waste products, making them an ideal route for bio-hydrogen production. It also helps to manage waste while generating affordable energy, making it a doubly beneficial solution. The second method of producing hydrogen from biomass is photo fermentation. It is accomplished in nitrogen-deficient conditions using solar energy and organic acids. Due to the presence of nitrogenase, certain photosynthetic microbes can convert organic acids into nitrogen [36] The mechanism of the photo fermentation with the acetic acid substrate can be seen in Equation 1.13.

$$CH_3COOH + 2H_2O + \text{light energy} \rightarrow 4H_2 + 2CO_2 \quad (1.13)$$

Although exposure to light generally produces better hydrogen, several obstacles prevent this method from being as effective as dark fermentation. These obstacles include the low efficiency of solar energy conversion, the need for large anaerobic photobioreactors, and the limited availability of organic acids [22].

The second method of producing hydrogen from biomass is dark fermentation. Anaerobic bacteria decompose organic substrates such as carbohydrates and glycerol without light to produce hydrogen as a byproduct. It is the simplest, most cost-effective, and environmentally friendly method for producing hydrogen using anaerobic microorganisms [37]. To initiate the process, the biomass feedstock is subjected to pretreatment to facilitate the substrate breakdown by bacteria. The pretreatment can be accomplished using physical, chemical, or biological methods. After pretreatment, the biomass is transferred to a bioreactor where bacteria digest the organic matter. Optimization of process parameters is essential for maximizing hydrogen production during dark fermentation. The fermentation

process and hydrogen yield are substantially influenced by pH, temperature, hydraulic retention time, and substrate concentration.

Equations 1.14-1.15 show the dark fermentation reaction when the substrates are carbohydrates. As seen in the equations, byproducts are acetic and butyric acids.

$$C_6H_{12}O_6 + 2H_2O \rightarrow 2CH_3COOH + 2CO_2 + 4H_2 \quad (1.14)$$

$$C_6H_{12}O_6 + 2H_2O \rightarrow CH_3CH_2CH_2COOH + 2H_2 + 2CO_2 \quad (1.15)$$

Dark fermentation has several advantages, making it an attractive option for hydrogen production. Although the most preferred substrate for dark fermentation is glucose, which is relatively expensive and difficult to obtain in large quantities, the method utilizes a variety of feedstocks such as lignocellulosic biomass, carbohydrate materials like industrial effluent, sugar-containing crop residues and municipal solid waste [38]. Additionally, this method does not rely on light, making it suitable for implementation in various environments, unlike the photo fermentation method. Among the biological methods, dark fermentation is the most effective way of producing hydrogen since it can produce it faster than the other biological methods. In Table 1.1, a comparison of different methods for producing biological H_2 is presented. This work uses the bioreactor volume required to run a 5 kW PEM fuel cell. It shows that dark fermentation has a higher synthesis rate than other methods, indicating its potential for commercialization.

Table 1.1: The hydrogen production rate and the bioreactor volume required for 5 kW PEMFC (adapted from [22])

Biohydrogen System	H_2 Synthesis Rate (mmol H_2/h)	Bioreactor Volume (m^3)
Biophotolysis	0.355	337
Dark fermentation	8.2-121	1-14.75
Photo fermentation	0.16	747

On the other hand, the relatively low hydrogen yield associated with fermentation is one of the dark fermentation obstacles. Producing other metabolic byproducts, such as acetic acid and butyric acid, limits the process's efficiency. However, dark fermentation remains the more viable option compared to biological methods.

Renewable hydrogen production includes water-splitting, separating water H_2O into hydrogen and oxygen. Electrolysis, thermolysis, and photolysis are the three primary techniques utilized for this procedure. Electrolysis, a widely recognized and effective technique, can be utilized. Due to the highly endothermic nature of the process, a considerable amount of energy is required. This energy can be supplied by electrical current from renewable sources. When powered by renewable sources, electrolysis emits zero greenhouse gases. The reaction results in hydrogen production at the cathode and oxygen at the anode can be seen through Equations 1.16 and 1.18.

$$\text{Anode: } 2H_2O \rightarrow 4H^+ + O_2 + 4e^- \tag{1.16}$$

$$\text{Cathode: } 2H^+ + 2e \rightarrow H_2 \tag{1.17}$$

$$\text{Total reaction: } 2H_2O \rightarrow 2H_2 + O_2 \tag{1.18}$$

Even though electrolysis has some benefits, it also has some problems. The process uses a lot of energy, which makes it more expensive than other ways to make hydrogen, especially when non-renewable energy sources are used. The effectiveness of electrolysis is also affected by the composition of the electricity grid, and if fossil fuels are used, the environmental advantages are significantly reduced [39]. Thermolysis, a less famous but promising technique for splitting water, uses heat to separate water into hydrogen and oxygen. The procedure typically needs high temperatures (over 2000°C) produced by nuclear reactors or concentrated solar electricity. The main benefit of thermolysis is the potential for excellent efficiency, particularly when heat sources with high temperatures are easily accessible. However, the stringent conditions necessary for the reaction to proceed present substantial technical difficulties with thermolysis [40]. Photolysis, also called photoelectrochemical water splitting, is a process that uses light energy to split water into hydrogen and oxygen. This is done by absorbing photons through a semiconductor material, which generates electrons and holes that participate in reactions to produce hydrogen and oxygen. The great advantage of photolysis is its ability to use sunlight directly, providing a sustainable and renewable method of hydrogen production.

1.5 Motivation

In recent years, environmental concerns like global warming and rising temperatures have prompted the search for alternative methods. Governments worldwide have shown a growing interest in finding more secure, environmentally friendly, and sustainable procedures to address these issues and prevent further harm to the planet. The Paris Agreement in 2016 further emphasized the need for action. Hydrogen, the most abundant element in the universe, has the potential to change the way people make and use energy. It can serve as a clean, flexible, and renewable tool to combat climate change and energy security. However, despite its potential, several limitations and challenges still need to be addressed, particularly in production. Although there are many methods to produce hydrogen, the dark fermentation process is promising for biohydrogen production since it is a sustainable and efficient method for producing hydrogen from organic waste. It can use many substrates, generate high production rates, and have no waste. This innovative solution contributes to reducing greenhouse gas emissions and supports global efforts to mitigate climate change.

This thesis aims to contribute to the current literature on creating biohydrogen using poplar leaves through the dark fermentation process. While the dark fermentation process is widely known for producing biohydrogen, there still needs to be more research on poplar leaves in this field. This thesis examines the hydrogen production from the dark fermentation process by optimizing its parameters experimentally. Then, an integrated bioreactor was designed for dark fermentation to enhance its efficiency and expand its applications for multipurpose such as waste treatment, water desalination, and hydrogen production.

1.6 Objectives

This study aims to investigate hydrogen production using dark fermentation as an alternative method for producing biohydrogen by utilizing poplar biomass. This thesis consists of three stages: the experimental setup of the dark fermentation process, optimization of dark fermentation, and building a new bioreactor for dark fermentation from poplar mass. The thesis has specific goals, which are:

• Designing, developing, and setting up an experimental setup with poplar leaves, a hydrogen sensor, and a new bioreactor for multipurpose hydrogen production.

• Testing and optimizing different parameters such as pH, temperature, mixing ratio, and amount of bacteria culture for the dark fermentation process.

• Developing a kinetic model for poplar mass in the dark fermentation process.

• Identifying the most efficient dark fermentation bioreactor for scaling up.

• Designing a bioreactor to produce hydrogen from poplar mass according to the findings.

• Installation of the designed bioreactor and testing it for different parameters.

•Studying the effects of various parameters on hydrogen production for the dark fermentation process.

1.7 Novelties

Recently, studies on hydrogen production using microorganisms have focused on reproducible organic biomass utilization. Despite being often utilized in laboratory studies, simple carbohydrate-rich substrates like sucrose, glucose, and starch hydrolysates are not commercially viable for usage on a large scale. Renewable resources are often required for large-scale H_2 production, especially lignocellulose or starch-based wastes. In more recent research, complex feedstock has gained significant attention, such as agricultural residues, industrial wastes, livestock waste, energy crops, and organic fractions of municipal waste. Energy crops have been utilized to generate H_2 sustainably among these biomass resources. They are mainly composed of lignocellulosic compounds and are easily accessible, biodegradable, non-toxic, environmentally friendly, economical, and renewable resources of first and second-generation biofuels. Moreover, lignocellulosic energy crops may produce more in less ideal conditions with low risk and need fewer fertilizer supplies than agricultural-based energy crops. Growing energy crops in marginal areas may also help with carbon capture, land rehabilitation, reducing soil erosion, and advancing sustainable development. Consequently, cultivating lignocellulosic energy crops on degraded lands can be a promising strategy for obtaining sustainable biomass. To better understand the potential for bioenergy systems, their commitment to renewable energy goals, and their sustainability practices, it is necessary to assess the energy crop biomass generated in

marginal or degraded lands. In this context, the novelty of this study lies in utilizing poplar leaves as an energy crop for H_2 production with commercial septic tank bacteria using the dark fermentation process. Moreover, pure bacterial cultures, mixed cultures, and anaerobic activated sludge are used as a microorganism source in the dark fermentation process. However, studies on using commercially available and cheap bacterial species in the dark fermentation process were missing. Namely, the availability and cost of bacterial species were the two most important variables as they directly affected the dark fermentation H_2 production performance. To our knowledge, H_2 production from poplar leaves using commercial and cost-effective bacterial species (used for septic tank treatment) was studied for the first time in this study. In addition, the impacts of initial pH, acid concentration, bacteria amount, mixing ratio, temperature, and biomass amount were considered comprehensively to improve the applicability of the dark fermentation system. Moreover, after optimization of the dark fermentation process, the novel three-compartment bio-membrane reactor was designed for the first time in the literature for simultaneous energy production and H_2 production using the fermentative approach in anode cell, salty water desalination in a desalination cell, and H_2 production using electrolysis in cathode cell.

CHAPTER 2. LITERATURE REVIEW

This section presents dark fermentation hydrogen production status through a comprehensive literature review. The dark fermentation process, its working principle, and its applications are briefly described. Secondly, the bioreactor configurations used in dark fermentation are investigated. Finally, a summary of the experimental and theoretical research on dark fermentation hydrogen production is presented.

2.1 Dark Fermentation Process

Dark fermentation has emerged as a critical technology for hydrogen production from renewable resources, including crop residues, livestock waste, and food waste. It produces hydrogen gas through the anaerobic breakdown of organic substrates by various microorganisms. It is an indirect biotechnological process involving using carbohydrates, proteins, and lipids as substrates by various bacterial genera, including Clostridium and Enterobacter [41]. This process produces hydrogen (H_2), carbon dioxide (CO_2), and organic acids through the acidogenic pathway [41]. The substrate for dark fermentation can vary based on the application and final product desired. Typical substrates include lignocellulosic biomass, starchy materials, organic detritus, and industrial byproducts [42]. An extensively studied substrate for dark fermentation is lignocellulosic biomass, such as agricultural residues (e.g., corn stover and wheat straw) and energy crops (e.g., switchgrass). These substrates consist of cellulose, hemicellulose, and lignin, which microbial enzymes can hydrolyze into fermentable carbohydrates. In addition to corn, rice, and cassava, other common substrates for dark fermentation include starchy materials[43]. Starch can be converted enzymatically to glucose, a carbon source for hydrogen-producing microorganisms [43]. As substrates for dark fermentation, organic wastes such as food refuse, agricultural waste and sewage sludge can be utilized [44]. These waste materials contain complex organic compounds that fermentative bacteria can break into simpler compounds, including volatile fatty acids, which can then be converted to hydrogen. Industrial residues, such as glycerol from biodiesel production or molasses from sugar production, have been examined as potential substrates for dark fermentation [45]. These byproducts can provide a readily accessible carbon source for hydrogen production. The substrate for dark fermentation can include lignocellulosic biomass, starchy materials,

organic waste, and industrial byproducts, depending on the application and availability of resources.

The dark fermentation method is widely regarded as a feasible and practical approach for generating biohydrogen, owing to its capacity to utilize organic waste and achieve high hydrogen production rates [46]. It produces biohydrogen without the need for any light energy input. Moreover, this process also produces byproducts such as fatty acids and solvents, which can be used for further combination with other processes that generate more bioenergy [47]. Although dark fermentation offers benefits such as efficient degradation of organic waste and higher hydrogen production rates, it faces several limitations. The production of byproducts causes a significant limitation during the fermentation process. These byproducts reduce hydrogen production and impede bacterial activity [48]. Also, the process may encounter restrictions owing to light limitations when compared to photo fermentation, as noted by Han et al. 2016 [49]. When investigating the potential of dark fermentation for biohydrogen production, these limitations and obstacles must be considered.

On the other hand, another crucial step in dark fermentation is determining process operating conditions. Dark fermentation is a complex system in which environmental factors and bioreactor operation conditions such as temperature, pH, H_2 partial pressure, and substrate-to-inoculum ratio control the metabolic pathways of hydrogen-producing microorganisms [49]. Optimizing these parameters is essential for maximizing the efficiency of biohydrogen production while providing optimal conditions for microbial growth and metabolism. The optimal pH and temperature are crucial in determining the most effective metabolic pathways for hydrogen production and inhibiting the simultaneous hydrogen consumption process [50] For example, pH influences the yield of hydrogen production in mixed cultures, the byproduct spectrum, and the structure of microbial communities[50]. Optimal H_2 production appears to occur at a pH of 5-6 for food waste, while crop residues and animal manure should have a neutral pH [49]. According to a study by Li et al., the optimal pH range for converting corn straw into hydrogen is between 7 and 7.5 [51]. However, this range depends on the substrate and microbial strain used. Similarly, temperature is a crucial factor affecting biohydrogen production and

microbes' metabolism in mixed cultures. Determining the optimal temperature for hydrogen fermentation is challenging due to the variability of operating conditions and the complexity of waste. That is why it is unreliable to use literature data for this purpose [51]. However, most fermentative hydrogen production research has been conducted at mesophilic temperatures (35 ºC) [52]. The study by Li et al. shows that 73 of the 101 case studies about fermentation were conducted at mesophilic temperatures. On the other hand, crop residues are known for producing higher yields at thermophilic temperatures ranging from 50 ºC to 70 ºC. This is due to the more efficient hydrolysis of lignocellulosic compounds. [52]. For example, the highest hydrogen production from the grass was found as 16 mL H_2 per gram VS at 70 ºC, using a heat-treated inoculum from a dairy farm digester [53]. Likewise, thermophilic temperatures are primarily preferred in the dark fermentation of food waste [50]. However, different observations were reported in the literature. In the fermentation of potato chip manufacturing waste, the optimal temperature for Monascus ruber fermentation under dark conditions was determined to be 30°C [54]. These variations in the temperature may be attributed to the origin of the inoculum, the amount of readily biodegradable compounds, and the operating conditions. Another parameter that affects biohydrogen production is the H_2 partial pressure. Numerous studies have demonstrated that hydrogen partial pressure is a limiting factor in the fermentation of organic waste.

$$\mathrm{n-LCFA \rightarrow (n-2)LCFA + 2\ Acetate + 2H_2}\ \ \Delta G^{º} = \ +48\ \mathrm{kJ\ mol^{-1}} \qquad (2.1)$$

$$\mathrm{CH_3COOH + 2H_2O \rightarrow 4H_2 + CO_2}\ \ \Delta G^{º} = \ +104.6\ \mathrm{kJ\ mol^{-1}} \qquad (2.2)$$

The conversion of reduced components like long-chain fatty acids (LCFA) into volatile fatty acids (VFAs), coupled with hydrogen production, results from low biohydrogen concentrations in the medium [55]. This is due to the thermodynamically unfavorable nature of these reactions, as seen in Equation 2.1. LCFA degradation requires an extremely low hydrogen partial pressure because the thermodynamics of fatty acids degradation through the b-oxidation pathway are unfavorable, as indicated by the positive Gibbs energy [55]. Equation 2.2 shows hydrogen can also be produced from the acetate's breakdown. The thermodynamic favorability of this conversion is limited at moderate temperatures, which results in a high reaction sensitivity to the quantity of biohydrogen available [52]. Additionally, a reverse reaction known as homoacetogenesis can negatively affect

bioreactor performance by causing the buildup of acetate in the medium [52]. Agitation is the most common method to lower the partial pressure of H_2 in the medium, particularly in highly concentrated bioprocesses handling organic waste. According to a study conducted by Chou et al., increasing the stirring speed from 20 to 100 rpm during the conversion of brewery grain resulted in a significant increase in biohydrogen output from 1.8 mL/L reactor to 6.1 mL/L reactor [56]. To achieve the best results for biohydrogen synthesis during dark fermentation, it is crucial to carefully regulate important factors like pH, temperature, and hydrogen partial pressure. This is because these variables significantly impact the activities of the microorganisms involved.

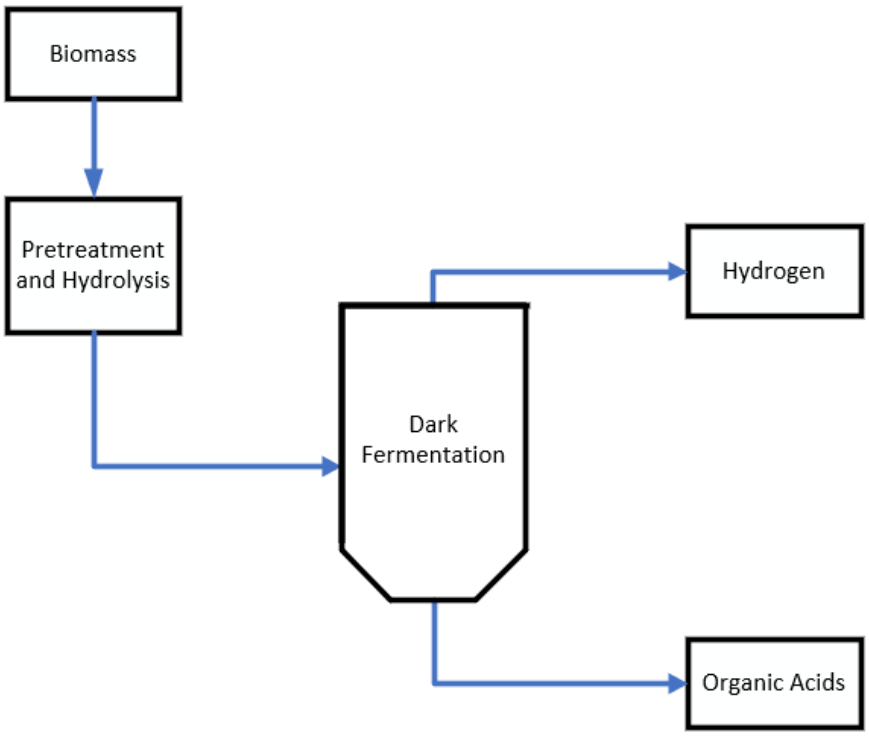


Figure 2.1 Dark fermentation steps

The process diagram of dark fermentation is depicted in Figure 2.1. Dark fermentation involves selecting a substrate, pretreatment, inoculation, fermentation, and product recovery. Various materials, including agricultural and organic byproducts and food residue, are used during dark fermentation. The selection of the most appropriate substrate is critical for the success of the process, considering factors such as availability, cost, and

desired final products [57]. Agricultural residues like rice straw, corn stover, and sugarcane bagasse are renewable and abundant substrates that can effectively reduce waste and produce sustainable energy [58]. Similarly, food waste is another source used for dark fermentation, providing an additional advantage by diverting organic waste from landfills while utilizing its energy potential [50]. Industrial byproducts such as glycerol from biodiesel production and distillery wastewater also have significant potential as substrates, contributing value to industrial processes and aiding in waste management [59]. Although lignocellulosic materials require pretreatment, they offer enormous potential for biohydrogen production. Substrates like wood chips and switchgrass can release fermentable sugars, which can be converted into biohydrogen through microbial action after breaking down their complex structures [60].

Pretreatment is the second crucial phase of the overall process. This process includes substrate and inoculum treatment. Substrate pretreatment is necessary mainly when dealing with lignocellulosic substrates like straw, corn stalks, and other agricultural residues. It aims to break down the complicated structure of lignocellulose so that the polysaccharides (cellulose and hemicellulose) can be used for further biological conversion. Physical, chemical, and biological pretreatment are the three methods available for substrate pretreatment. On the other hand, inoculum pretreatment is vital when using mixed microbial communities. Mixed communities include H_2-consuming microorganisms such as hydrogenotrophic methanogens, homoacetogens, lactic acid bacteria, propionate-producing bacteria, and sulfate reducers [61]. Mainly, hydrogenotrophic methanogens use most of the H_2 produced within all H_2 consumers, and their presence in mixed microflora dramatically decreases the amount of H_2 produced [62]. The idea behind the inoculum pretreatment is to allow the sporulation of the H_2-producing bacteria while suppressing the growth of H_2-consuming microorganisms. This process is carried out with heat, chemical, or mechanical methods that destroy just the organisms that use hydrogen without harming the ones that produce it. It not only makes more H_2 overall, but it also makes the microbial population more stable and predictable.

Once the substrate and inoculum have been pretreated, they are infused with a specially chosen microbial culture that contains microorganisms capable of producing hydrogen.

The fermentation process provides a conducive environment for the growth and metabolism of microorganisms. This is achieved through the maintenance of anaerobic conditions and the provision of optimal temperature and pH levels. These conditions work together to create an ideal environment for microorganisms to thrive and perform their metabolic processes. After fermentation, biohydrogen can be extracted and recovered using separation techniques such as membrane filtration, gas stripping, and pressure swing adsorption [63]. These techniques allow the separation of biohydrogen gas from the fermentation broth, enabling its purification and subsequent use as a renewable energy source.

Table 2.1 Substrate pretreatment and its effect on yield

Feedstock	**Pretreatment Method**	**H_2 Yield**	**Change (%)**	**References**
Grass	Acid-heat (1 g grass with 20 ml HCl 4%w/v, boiled for 30 min)	Unpretreated: 4.38 ml g^{-1} dry grass Pre-treated: 72.2 ml g^{-1} dry grass	+1548%	[48]
Grass	Alkaline heat (1 g grass with 20 ml HCl 0.5%w/v, boiled for 30 min	Unpretreated: 4.38 ml g^{-1} dry grass Pretreated:19.25 ml g^{-1} dry grass	+339%	[64]
Cornstalk	Biological treatment (15 days)	Unpretreated: 20 ml g^{-1} VS Pretreated: 176 ml g^{-1} VS	+780%	[65]
Cornstalk	Biological pre-treatment (fungi, six days)	Unpretreated:28.8 ml g^{-1} VS Pretreated: 53.2 ml g^{-1} VS	+109 %	[66]
Vinegar residues	Acid (HCl, pH 1, 10 ml g^{-1} TS, 99 ºC, 30 min)	Unpretreated:23.8 ml g^{-1} VS Pretreated: 55.4 ml g^{-1} VS	+123 %	[64]
Vinegar residues	Alkaline (NaOH, pH 12, 24 h)	Unpretreated:23.8 ml g^{-1} VS Pretreated: 47.3 ml g^{-1} VS	+132 %	[64]
Vinegar residues	Heat (boiling, 30 min)	Unpretreated:23.8 ml g^{-1} VS Pretreated: 47.3 ml g^{-1} VS	+98 %	[65]

Poplar Mass	Viscozyme L	Unpretreated: 15.04 mL/g Pretreated: 44.92 mL/g	+198 %	[48]
Wheat straw	HCl-Microwave	Unpretreated: 0.5 mL/g-TVS Pretreated: 44.92 mL/g 68.1 mL/g-TVS	+13520%	[48]

Table 2.1 provides information on the feedstock, the specific pretreatment method used, the resulting hydrogen yield, the percentage change in yield compared to the untreated substrate, and the corresponding references. It highlights the impact of pre-treatment methods on various substrates. It is evident from the table that treating the substrates leads to a considerable rise in hydrogen yield.

Table 2.2 Inoculum pretreatment and its effect on yield

Inoculum	**Pretreatment**	**H_2 Yield**	**Change (%)**	**References**
Anaerobic digested sludge	Substrate: Glucose Temperature: 65, 80, 95 ºC Time: 30 min	Unpretreated: 0.43 mol mol^{-1} glucose Pretreated: 2.30 mol mol^{-1} glucose	+434.9%	[67]
Activated sludge	Substrate: Glucose Temperature: 65, 80, 95 ºC Time: 30 min	Unpretreated: 0.26 mol mol^{-1} glucose Pretreated: 1.64 mol mol^{-1} glucose	+530.8%	[67]
Sludge	Substrate: Glucose Temperature: 100 ºC Time: 30 min	Unpretreated: 0.2 mol mol^{-1} glucose Pretreated: 0.41 mol mol^{-1} glucose	+105.0%	[48]
Sludge from slaughterhouse	Substrate: Sucrose Temperature: 90 ºC Time: 10 min	Unpretreated: 0.2 mol mol^{-1} sucrose Pretreated: 0.41 mol mol^{-1} sucrose	+100.0%	[68]

Sludge from swine wastewater	Substrate: Sucrose Temperature: 90 ºC Time: 10 min	Unpretreated: 0.7 mol mol^{-1} sucrose Pretreated: 0.7 mol mol^{-1} sucrose	0%	[68]
Sludge from swine wastewater	Substrate: Sucrose Acid: 1.0 N HCl pH: 3.0 (24 h)	Unpretreated: 0.7 mol mol^{-1} sucrose Pretreated: 1 mol mol^{-1} sucrose	+42.9%	[68]

Table 2.2 provides information on the inoculum, the specific pretreatment method used, the resulting hydrogen yield, the percentage change compared to the untreated substrate, and the corresponding references. It highlights the impact of pretreatment methods on inoculum and its effect on hydrogen yield. It is observed that treating the inoculum resulted in a significant increase in yield.

Upon conducting a thorough analysis of the literature, it has been noted that sugarcane, corn, rice, wheat, and soybean are commonly used lignocellulosic materials for dark fermentation to produce hydrogen. Nevertheless, there needs to be more research conducted on implementing poplar leaves in this context. Poplar leaves and other fallen dry leaves are commonly disposed of in landfills. In this process, it takes several months for these leaves to decompose naturally or be incinerated, releasing polluted gases into the atmosphere. Consequently, this emission of harmful gases can harm the environment. Using new cellulosic biomasses for dark fermentation is a viable and novel strategy for producing hydrogen and other byproducts. The following section presents a literature review regarding the research on the dark fermentation of poplar mass.

Ramprakash et al. investigated the use of garden wastes by using Escherichia coli to produce hydrogen [69]. The garden waste comprised grass, fallen dry leaves from plants and trees, and small bushes. Fallen dry leaves make up more than 80 percent of garden waste, and they analyzed the fallen dry leaves from a lawn at Chulalongkorn University in Bangkok, Thailand. The hydrolysis of dry garden waste by enzymes and acid produced 89 and 74 mL of H_2/g, respectively. Maximum hydrogen production had been reached after fermentation using hydrolysate from acid and enzyme treatment, with a 2.7-fold increase in yield compared to wastes that had not been treated. 2% sulfuric acid and 2% Viscozyme

L pretreatments were optimal conditions for maximizing hydrogen production. The results demonstrate the viability of using garden wastes for hydrogen production after proper treatment.

Another study by Cui et al (2000). explores the use of various pretreatment techniques to produce biohydrogen from poplar leaves with the help of anaerobic mixed bacteria at a temperature of 35 ºC. It is the first report on hydrogen production from poplar leaves using anaerobic mixed microbes for pretreatment. The study analyzed the effects of acid (HCl), alkaline (NaOH), and enzyme (Viscozyme L) pretreatments on the saccharification of poplar leaves. Comparisons were made between the effects of acid and enzymatic pretreatment on hydrogen production and their respective degradation efficiencies for the total reducing sugar (TRS) and metabolites. The results showed that the maximum cumulative hydrogen yield of 44.92 mL/g-dry poplar leaves was obtained from substrate pretreated with 2% Viscozyme L, which was approximately three times higher than that of the untreated substrate and approximately one-third higher than that of substrate pretreated with 4% HCl. The findings suggest that enzymatic pretreatment is more efficient for increasing the hydrogen yield from poplar leaves than acid pretreatment.

A comparative study by Patel et al. [70] examines the use of poplar biomass to produce hydrogen. Their research showed bacterial H_2 production could be enhanced using well-defined mixed cultures (DMC) under non-sterile continuous culture conditions. They obtained poplar wood chips from a local market in India and dried them to a constant weight for this study. For pretreatment, 100 g of biomass was suspended in 600 mL of H_2SO_4 solution (0.5%) at a solid-to-liquid ratio of 1:6 and then autoclaved for 1 hour at 121 ºC. The separated biomass was then filtered, washed with water to remove dissolved biomass byproducts, and dried in an oven [70]. They treated 1.0 g of biomass with 18 FPU/g of Celluclast 1.5 L and 15 IU/g of b-glucosidase. They incubate the mixture for 48 hours at 45 ºC with 150 rpm stirring. 250 mL of poplar biomass hydrolysate with 20 g/L of total sugar concentration was inoculated with 10 mg of protein/mL of pure culture or DMC (microbe protein to food ratio 1:2000 mg/L) in 300 mL non-sterile reagent vessels for batch culture H_2 production. Their research showed a consistent production of 2.83 mol/mol of hexose in H_2 yield over forty days. The study has concluded that enhancing

bacterial H_2 production is possible through well-defined mixed cultures (DMC) in non-sterile continuous culture conditions.

Yang et al. [71] investigated the effect of co-fermentation on biohydrogen production [71]. For this purpose, the fallen leaves, including poplar leaves and sewage sludge, were used as substrates at various mixing ratios. The results of the experiments showed that the best ratio for mixing sludge and leaves was 20:80 (volatile solids (VS) base), and the co-fermentation process worked best when this ratio was used. At a mixed ratio of 20:80, the amount of biohydrogen produced was found to be 37.8 mL/g-VS compared to the mono-fermentation of sludge (10.3 mL/g-VS) or the leaves (30.5 mL/g-VS), which was higher.

Research has shown that using poplar biomass for dark fermentation is a sustainable and effective way to produce biohydrogen. This renewable resource can be grown sustainably, aligning with the goals of a circular economy. Additionally, dark fermentation can assist in reducing organic waste and promoting environmental sustainability. Nonetheless, more research and optimization are required to enhance the process efficiency and make it a commercially viable option.

2.2 Bioreactors used in dark fermentation

Among the known biological processes, dark fermentation has the most potential for practical applications, such as treating organic waste [72]. The technology faces cost, efficiency, and reliability challenges, especially for large-scale commercialization applications. Nonetheless, improvements in bioreactor configurations could significantly improve hydrogen yield and production rates, paving the way for viable commercial applications. Bioreactors play an integral role in the dark fermentation process by providing a controlled environment that facilitates efficient hydrogen production by microorganisms. The design and configuration of a bioreactor are critical as they ensure the regulation of key factors such as temperature, pH levels, and nutrient availability. Additionally, bioreactors assist with contamination control, mass transfer, and the integration of other processes. Their significance in optimizing hydrogen production is indispensable. The continuous stirred tank reactor (CSTR) is the most widely used bioreactor system, not only for H_2 production but also for various other biochemical processes; however, other types of reactors, such as the packed bed bioreactor (PBR), fixed bed reactor, membrane

bioreactor (MBR), up-flow anaerobic sludge blanket reactor (UASB), expanded granular sludge bed (EGSB). Anaerobic fluidized bed reactors (AFBR) are also utilized for hydrogen production.

a) CSTR (Continuous Stirred Tank Reactor)

The Continuous Stirred Tank Reactor (CSTR) is a commonly used method for producing hydrogen continuously. It involves mixing and suspending hydrogen-producing microbes in the reactor liquor, allowing for good substrate-microbe contact and mass transfer. However, the CSTR has limitations in maintaining high levels of fermentative biomass due to its rapidly mixed operating pattern, which may result in biomass washout at short hydraulic retention times (HRT) [73]. As a result, hydrogen production rates are considerably restricted. On the other hand, they provide better mixing and uniform conditions, which facilitate the control of process parameters such as pH, temperature, and substrate concentration. However, CSTRs have a lower hydrogen yield and production rate than other reactors [74]. The highest hydrogen production rate for CSTR was reported as 1.12 L/h/L during the fermentation of sucrose with a mixed hydrogen-producing culture [74]. Table 2.3 shows the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using CSTRs.

Table 2.3 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using CSTR.

HRT (hr)	HPR (L/L-d)	HPY	Substrate	References
4	16.32	1.02 mol H_2 /mol hexose	Rice Straw	[75]
3	17.5	1.28 mol H_2 /mol hexose	Sugarcane syrup	[72]
4	10	0.069 mol H_2 /mol T-sugar	Rice Straw Hydrolysate	[74]
6	11.6	2.14 mol H_2 /mol hexose	Galactose	[76]

b) Upflow anaerobic sludge blanket (UASB)

Upflow anaerobic sludge blanket (UASB) bioreactors are well-known for wastewater treatment and organic waste conversion. It is renowned for its capacity to manage a wide variety of organic waste streams at high loading rates [77]. The fundamental concept of a UASB reactor is that wastewater is fed into the reactor's bottom and flows upward through

a bed of granular sludge. The sludge contains a collection of microorganisms that ferment the organic matter in the wastewater to generate hydrogen gas and other byproducts. For hydrogen production, UASB reactors have several advantages over other bioreactors. Initially, UASB reactors can operate at high loading rates, enabling them to produce a large amount of hydrogen from small volumes of organic waste [72]. In addition, the operation and maintenance of UASB reactors are relatively simple. Lastly, UASB reactors can produce hydrogen from various organic wastes [74]. On the other hand, UASB reactors can become clogged with suspended particles. Table 2.4 compares the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using UASBs.

Table 2.4 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using UASB.

HRT(h)	HPR (L/L-d)	HPY	Substrate	References
2	52.4	0.73 mol H_2 /mol hexose	Sugarcane juice	[72]
3	32.7	1.95 mol H_2 /mol galactose	Galactose	[78]
2	56.8	2.25 mol H_2 /mol galactose	Galactose	[74]
2	10.78	1.4 mol H_2 /mol glucose	Glucose	[79]

c) Packed Bed Reactor (PBR)

PBR is operated with bio support materials packed inside for the growth and formation of biofilm by H2-producing microorganisms. Numerous materials have been utilized as bio-support materials, including glass crystals, expanded clay (EC), perlite, activated carbon, ceramic, coconut coir, synthetic polymers, and plastics [74]. For superior biofilm formation, support materials must be inert and have a high specific surface area, rough surface, and high porosity. A PBR system can accomplish high conversion rates due to its capacity to retain high biomass concentrations within the reactor [80]. However, the mixture regime in a PBR is compared to a CSTR, resulting in a low mass transfer and product yield to the substrate. PBRs have several advantages over other reactor designs for hydrogen production from dark fermentation. Firstly, PBRs have a high ratio of surface area to volume, allowing for efficient mass transfer between the reactants and catalysts [80]. PBRs are also relatively simple to operate and maintain. Furthermore, PBRs can be scaled up to produce substantial amounts of hydrogen. However, PBRs can be challenging

to clean, accumulating contaminants that can harm the catalyst [74]. Table 2.5 compares the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using PBRs.

Table 2.5 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using PBR.

HRT(h)	HPR (L/L-d)	HPY	Substrate	References
-	8.9	2.0 mol H_2 /mol sucrose	Sucrose	[74]
12	1.117	2.4 mol H_2 /mol total carbohydrates	Sugarcane Vinasse	[78]
2	65	2.6 mol H_2 /mol hexose	Hexose	[81]
2	65.6	2.6 mol H_2 /mol hexose	Hexose	[82]

d) Anaerobic Fluidized Bed Reactor (AFBR)

Anaerobic fluidized bed reactors (AFBRs) are a type of bioreactor that holds great promise in hydrogen production through dark fermentation. They offer several advantages over other bioreactors, including high biomass retention, efficient blending, and scalability[74]. The retention of a significant amount of biomass by AFBRs enables the maintenance of a high concentration of hydrogen-producing bacteria. The fluidized bed environment thoroughly mixes the biomass, preventing the formation of inhibitory hotspots. Additionally, these systems can be scaled up for large-scale hydrogen production. Operating AFBRs can be filled with inert particles such as sand or ceramic beads [83]. The wastewater is pumped to the reactor, and a rising gas flow fluidizes the particles. This process ensures efficient mixing, a high mass transfer rate, and improved conversion efficiency [74]. Anaerobic bacteria in the effluent attach to the particles and decompose organic matter into hydrogen and other byproducts. The hydrogen gas produced rises to the top of the AFBR and is collected. However, before AFBRs can be widely adopted for hydrogen production, challenges such as high costs and the need for pretreatment to remove effluent contaminants must be addressed. Nevertheless, AFBRs hold great potential for sustainable hydrogen production with further research and development. Table 2.6 compares the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using anaerobic fluidized bed reactors.

Table 2.6 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using AFBR.

HRT(h)	HPR (L/L-d)	HPY	Substrate	References
0.25	7.7	1.7 mol H_2 /mol glucose	Glucose	[74]
1	56.64	4.34 mmol H_2 /g glucose	Glucose	[74]
2	1.28	2.29 mol H_2 /mol glucose	Glucose	[84]
3-0.125	40.8	1.7 mol H_2 /mol hexose	Hexose	[85]

e) Membrane Bioreactors (MBRs)

Membrane bioreactors (MBRs) separate a biological system's solid and liquid phases using a semipermeable membrane. This permits continuous operation and high biomass retention while enhancing process performance. MBRs have been utilized for numerous purposes, including wastewater treatment, biogas, and hydrogen production. Furthermore, they effectively produce hydrogen from various organic substrates, such as food waste, agricultural waste, and wastewater[74]. MBRs help improve dark fermentation processes in several ways. They can eliminate suspended solids from the bioreactor, preventing clogging and enhancing efficiency. Moreover, they can retain bacteria, increasing hydrogen yield [86]. At the same time, MBRs can regulate the bioreactor's pH, boosting the bacteria's performance [87]. However, these reactors also have some drawbacks, such as high capital and operating costs, membrane fouling, and the need for membrane replacement. Despite these limitations, MBRs are a promising technology for hydrogen production. As technology advances, capital and operating costs are expected to decrease, and new membrane materials that resist fouling are being developed. These developments will make them a more cost-effective and efficient alternative for hydrogen production. Table 2.7 compares the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using MBRs.

2.2.6 Expanded Granular Sludge Bed Reactor (EGSBR)

EGSB reactors are a variant of UASB reactors distinguished by a high up-flow velocity, which usually occurs by effluent recycling [74].

Table 2.7 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using MBRs.

HRT(h)	HPR (L/L-d)	HPY	Substrate	References
9	5.8	1.19 mol H_2 /mol glucose	Glucose	[74]
14	10.7	111.1 mL H_2 / g VS	Glucose	[88]
3	54.07	3.22 mol H_2 /mol glucose	Glucose	[72]
3	60.5	2.39 mol H_2 /mol hexose	Glucose	[72]

The granular sludge is preserved in suspension by the upward flow of wastewater, which provides microorganisms with oxygen and nutrients[72]. EGSB reactors have been utilized to produce hydrogen from various organic substrates, such as food waste, agricultural waste, and wastewater. EGSB reactors for hydrogen production have several advantages over other varieties of anaerobic bioreactors. They have a large ratio of surface area to volume, facilitating the efficient transfer of oxygen and nutrients to the microbes[73]. Second, they have a high sludge retention time, which allows a large population of hydrogenotrophic microbes to proliferate. They are also relatively simple to operate and maintain. However, EGSB reactors have some drawbacks. For example, they are more costly than other anaerobic bioreactor varieties. They are also susceptible to clogging and fouling [73]. They can also produce a high sulfide concentration, which is toxic to microorganisms and humans. Table 2.8 indicates the hydrogen production rate (HPR) and yield (HPY) for different feedstocks treated using EGSBRs.

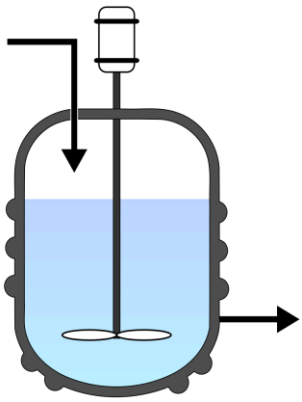
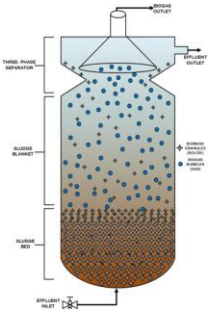
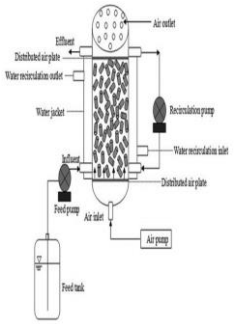
Table 2.8 Comparison of the hydrogen production rate (HPR) and yield (HPY) for different feedstocks using EGSBRs.

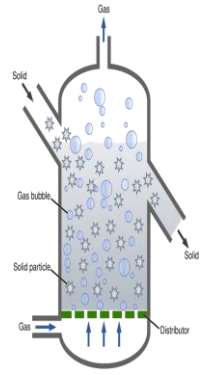
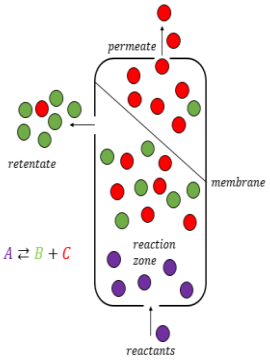
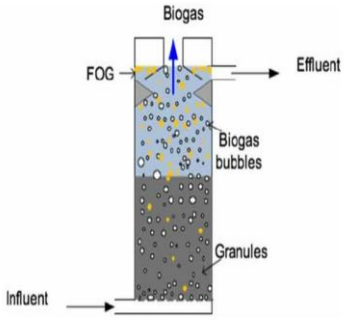
HRT(h)	HPR (L/L-d)	HPY	Substrate	References
10	4.1	1.6 mol H_2 /mol glucose	Glucose	[74]
1-6	0.71	3.47 L/ g VS	Molasses	[74]
2	52.4	-	Sugarcane Juice	[89]
3	60.5	2.39 mol H_2 /mol hexose	Glucose	[89]

The design of the bioreactor plays a crucial role in biohydrogen production. Different bioreactors have been used for biohydrogen production, each with advantages and

disadvantages. The choice of bioreactor depends on the microorganism and substrate used. Table 2.9 summarizes the advantages and drawbacks of each reactor.

Table 2.9 Reactors used in dark fermentation, their advantages and disadvantages

Reactor Type	Advantages	Disadvantages
Continuous Stirred Tank Reactor (CSTR)  Adapted from [90]	a. Better mixing, which provides uniform conditions throughout the reactor b. Easier control of process parameters, such as pH, temperature, and substrate concentration.	a. Lower hydrogen yield and productivity compared to other reactor types. b. Higher substrate and product inhibition risk due to the uniform concentration throughout the reactor.
Upflow Anaerobic Sludge Blanket Reactor  Adapted from [91]	a. High biomass retention and high organic loading rates b. Efficient gas-liquid-solid separation, reducing the need for post-treatment.	a. Susceptible to washout of biomass during high hydraulic loading rates b. Difficulty in maintaining the stability of the granular sludge bed.
Packed Bed Reactor  Adapted from [91]	a. High cell density and biomass retention, leading to improved hydrogen production rates b. Reduced risk of washout during high hydraulic loading rates	a. Potential clogging of the packed bed, leading to increased pressure drop and reduced efficiency b. Difficulty in controlling pH and substrate concentration due to limited mixing

Anaerobic Fluidized Bed Reactor (AFBR)  Adapted from [92]	a. High operational flexibility and ability to handle a wide range of substrate concentrations. b. Better control of reaction time and substrate degradation, improving hydrogen yield and production	a. Requires intermittent operation, which can lead to variations in hydrogen production rates. b. Sludge settling and separation can be challenging, requiring additional efforts in process control and reactor design.
Membrane Bioreactors  Adapted from [93]	a. High biomass retention and excellent effluent quality b. Reduced risk of washout during high hydraulic loading rates	a. Membrane fouling, which can lead to increased operational costs and reduced efficiency b. Higher capital and operating costs compared to other reactor types
Expanded Granular Sludge Bed Reactor  Adapted from [94]	a. Improved mass transfer rates and higher organic loading rates compared to UASB reactors b. Efficient gas-liquid-solid separation due to the three-phase separator design	a. Challenges in maintaining the stability and expansion of the granular sludge bed b. The risk of biomass washout during high hydraulic loading rates

Bioreactors can also be combined to achieve specific goals. Combined bioreactors may improve bioreactor systems' performance, productivity, or adaptability. One of those kinds of reactors is a hybrid two-stage system. In the first stage of a hybrid two-stage system, the

substrate is converted into hydrogen and organic acids in a conventional reactor. In the second stage, additional gaseous energy in the form of methane or hydrogen is extracted [74]. To optimize gas production, a different reactor is used for the second stage under varying operating conditions, such as a higher pH and longer HRT [95]. An attempt to increase the overall energy extraction in the second stage involves photofermentation or fuel cells [73]. It can be done by extracting additional hydrogen from the metabolites of dark fermentation.

Additionally, by integrating multiple processes, such as hydrogen and methane production, a single hybrid approach can maximize energy recovery from effluent [96].In a study by Nath et al., a hybrid two-stage photofermentation system was investigated, in which the second stage was fed with hydrogen-producing reactor effluent. The reported hydrogen yields were lower than anticipated, but combining dark and photofermentation in the hybrid system increased the overall yield [97]. In the second stage, a photobioreactor converted acetate to hydrogen and carbon dioxide. The combination of dark and photofermentation could approach the theoretical maximal production of 12 mol of hydrogen per mol of glucose equivalent. Theoretically, photofermentation can convert all organics into hydrogen, as an ATP-dependent nitrogenase drives hydrogen production, and the required ATP is produced using solar energy [97].

Multistage bioreactors are a specific type of bioreactor system used for hydrogen production. They incorporate multiple stages or compartments to optimize metabolic pathways and increase hydrogen yield. These bioreactors are designed to provide specific conditions to promote hydrogen production at each stage. In Figure 2.1, the process of a four-stage system is depicted. In the first stage, the visible light is utilized by blue-green algae through a direct photolysis re. In contrast, while photo-synthetic microorganisms utilize the unfiltered infrared light in the second stage photo-fermentative reactor [73]. The effluent from the second stage of photo-fermentation and the biomass feedstocks are introduced into the third stage of dark fermentation, where microorganisms convert the substrate into hydrogen and organic acids [74]. As the effluent is enriched with organic acids, the photo-fermentative process no longer requires an external supply of organic acids. The fourth stage is the use of a MEC to produce hydrogen. The MEC uses the organic

acids from dark fermentation under a light-independent process. It thus can be operated during the night or in low light conditions.

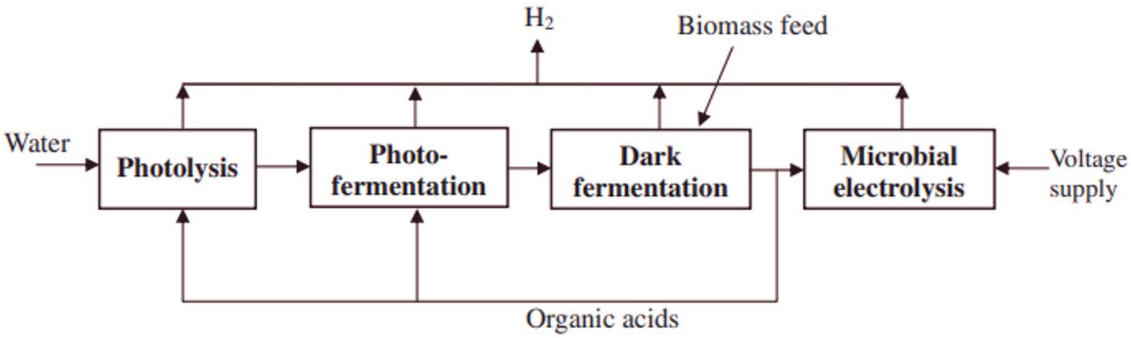


Figure 2.2 Multistage bioreactor system (Adapted from [98])

MEC (Microbial electrolysis cells) are often incorporated into multistage bioreactor systems as one of the compartments to improve hydrogen production efficiency, Figure 2.3. The MEC stage often follows the anaerobic fermentation stages, such as dark fermentation or photo fermentation. Using a coupled dark fermentation (DF) and MEC system, the waste produced by the DF reactor is utilized by the MEC. This allows the MEC to utilize the leftover organic matter that would have otherwise gone to waste. Additionally, this process lowers the pH of the MEC feed, which can enhance the efficiency of the hydrogen production process[99]. A MEC system is analogous to a microbial fuel cell (MFC) system, apart from its covered cathode and external voltage [100]. Electrochemically active microorganisms dominate at the anode of MECs and convert organic compounds to protons, CO_2, and electrons[101] Electrons produced by these microorganisms are transferred through a power line to the anode and then to the cathode, where hydrogen is produced. Ion-selective membranes, often called anion exchange and cation exchange membranes, block the reaction between oxygen and hydrogen produced. MEC application in fermentation enhances substrate degradation, leading to hydrogen production. Briefly, electroactive microorganisms, also known as exoelectrogens, act as biocatalysts in MEC by oxidizing organic matter in wastewater. During this process, they release electrons (e^-) and protons (H+), which are then transferred to the cathode. When combined with electron acceptors (such as potassium ferricyanide or phosphate buffer), a minor electrical input (>0.114 V) can produce hydrogen [102]. MEC requires less energy input (0.6-1.0 kWh/m^3 H2) for hydrogen production than water electrolysis (4.5-50.6

kWh/m^3 H_2). Numerous operational parameters (type of wastewater and its pH, microorganisms, conductivity, etc.) and design factors (applied potential, electrode spacing, electrode materials, reactor configuration, etc.) significantly affect hydrogen production in the MEC [102].

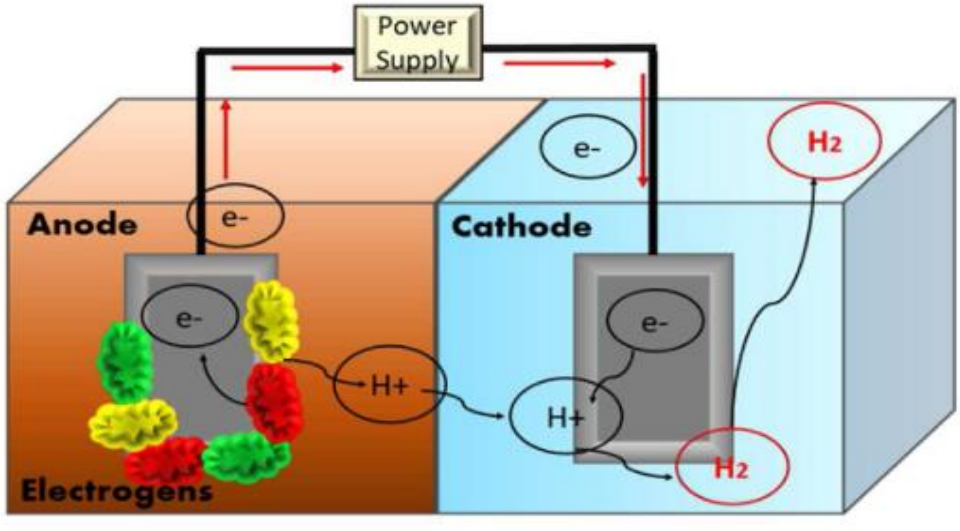


Figure 2.3 Illustration of microbial electrolysis cell (MEC) (adapted from [103])

Numerous studies have shown that connecting DF and MEC can substantially increase hydrogen production. A 2017 study by Marone et al. compared the hydrogen production yield using a single-stage dark fermentation, a single-stage MEC, and a combined dark fermentation and MEC. The integration of DF and MEC significantly increases hydrogen production efficiency from food waste by up to 50% [103].

A study by Dhar et al. (2015) investigated the use of sugar beet juice in hydrogen production through a combination of dark fermentation and microbial electrolysis cells. Their findings showed that the maximum hydrogen yield from dark fermentation was 13 % of initial COD when the substrate-to-inoculum ratio was 2 or 4. The MEC produced up to an additional 12 % of TCOD, resulting in 25 % of TCOD from sugar beet juice. The authors believe the integrated process is a promising method for producing hydrogen from sugar beet juice.

Another bioelectrochemical system like MEC is the microbial desalination cell (MDC), Figure 2.4. A microbial desalination cell (MDC) is a form of bioelectrochemical system

(BES) that desalinates water by utilizing the metabolism of electrochemically active bacteria (EABs) [104]. Three chambers form the MDC: the anode chamber, the middle chamber, and the cathode chamber. The anode chamber contains EABs, which generate electrons using the organic matter in the effluent. The electrons are then conveyed to the cathode chamber, which converts protons into hydrogen gas by producing electricity [104]. The middle chamber contains a semi-permeable membrane that allows ions but not water to pass by. It generates a concentration gradient, which drives the desalinated water from the anode chamber to the cathode chamber.

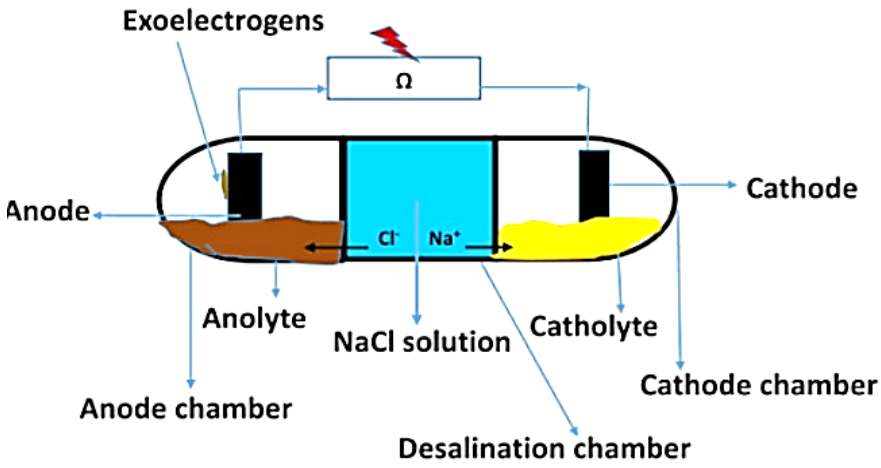


Figure 2.4 Illustration of microbial desalination cell (MDC) (adopted from [104])

A 2019 study by Xu and colleagues suggested a new system combining microbial desalination and dark fermentation to produce hydrogen from saline wastewater [105]. The innovative system uses the MDC to desalinate wastewater and the DF to generate hydrogen from the organic matter present in the wastewater. According to the study, this integrated system produced hydrogen at a 1.22 mL H_2/g COD rate, substantially exceeding the rates produced by MDC or DF alone [105]. Notably, the MDC desalinated the wastewater to a concentration of 1000 ppm, which is sufficient for most hydrogen production applications. In addition, the integrated system removed up to 90 percent of COD from the wastewater, resulting in a highly effective and environmentally beneficial option.

Luo et al. (2011) created a microbial electrolysis and desalination cell that generates hydrogen gas and desalinates saline water [106]. The anode chamber is filled with saline solution and inoculated with bacteria capable of oxidizing organic matter and producing

electrons. The authors of the study discovered that the MEDC could produce 1.5 m^3/m^3 d (1.6 mL/h) of hydrogen gas and remove 98.8% of the salt ions from a saline solution when operated at 0.8 V applied voltage, 0.2 M buffer concentration, and 10 mL/min flow rate [106].

In Table 2.10, the comparison of MDC and MEC is shown. MDCs and MECs are bioelectrochemical systems that generate electricity using microbial fuel cells. However, their products, applications, and advantages/disadvantages are distinct. MDCs are better for desalination, whereas MECs are more beneficial for hydrogen production. Ultimately, the optimal option for a given application will depend on its requirements.

Table 2.10 The comparison of MDC and MEC

Feature	MDC	MEC
Operation Principle	It uses microbial fuel cells to generate electricity, which is then used to drive the desalination process.	It uses microbial fuel cells to generate electricity, which is then used to produce hydrogen gas.
Components	Anode, cathode, ion exchange membranes, and saline water	Anode, cathode, ion exchange membranes, saline water, and an external power supply
Products	Desalinated water, electricity	Hydrogen gas, electricity
Applications	Desalination, wastewater treatment, power generation	Hydrogen production, wastewater treatment, heavy metal removal
Advantages	Low-cost, environmentally friendly, scalable	High hydrogen production rate, versatile
Disadvantages	Low desalination rate, limited power output	Requires an external power supply, potential for hydrogen embrittlement

2.3 Gaps in Literature

More research is needed to fully understand the potential of poplar leaves in dark fermentation for hydrogen production. While the initial results showed promise, additional research is required before considering commercial applications. Optimization of the process is crucial to improving its efficiency. Various bioreactor configurations are available for dark fermentation, each with advantages and disadvantages. An integrated bioreactor design and optimization can help increase hydrogen production while

minimizing the disadvantages of the reactors. Bioelectrochemical cells, such as microbial electrolysis cells (MECs), offer many advantages for hydrogen production. MECs are more energy-efficient than conventional electrolysis because they decompose organic waste with the metabolic activities of microorganisms, generating electricity. MECs are also sustainable as they can use various organic waste streams, including municipal wastewater, agricultural refuse, and industrial waste, to generate hydrogen while mitigating waste management issues. That is why more importance should be given to integrated processes.

2.4 Contributions

This research project has two main objectives. The study's first aim is to optimize the dark fermentation system using poplar leaves. The second objective is to create a multi-stage bioreactor system that enhances the efficiency of the single-stage dark fermentation process. For this purpose, an integrated bioreactor system is designed to simultaneously enable waste treatment, bioenergy production, electricity generation, and water desalination.

Chapter 3. EXPERIMENTAL APPARATUS and PROCEDURE

This section discusses the experimental studies conducted to produce hydrogen from poplar biomass. Initially, the experimental setup and procedure are thoroughly described. The types of equipment used in the study are detailed. Methods for measuring hydrogen are described, and errors are analyzed.

3.1 Experimental Setup

This section discusses the experimental setup of the system. The dark fermentation setup includes hydrogen sensor, a computer system for data acquisition, a heating and mixer, Arduino Uno, poplar leaves, blender, bacteria culture, and acid-based chemicals.

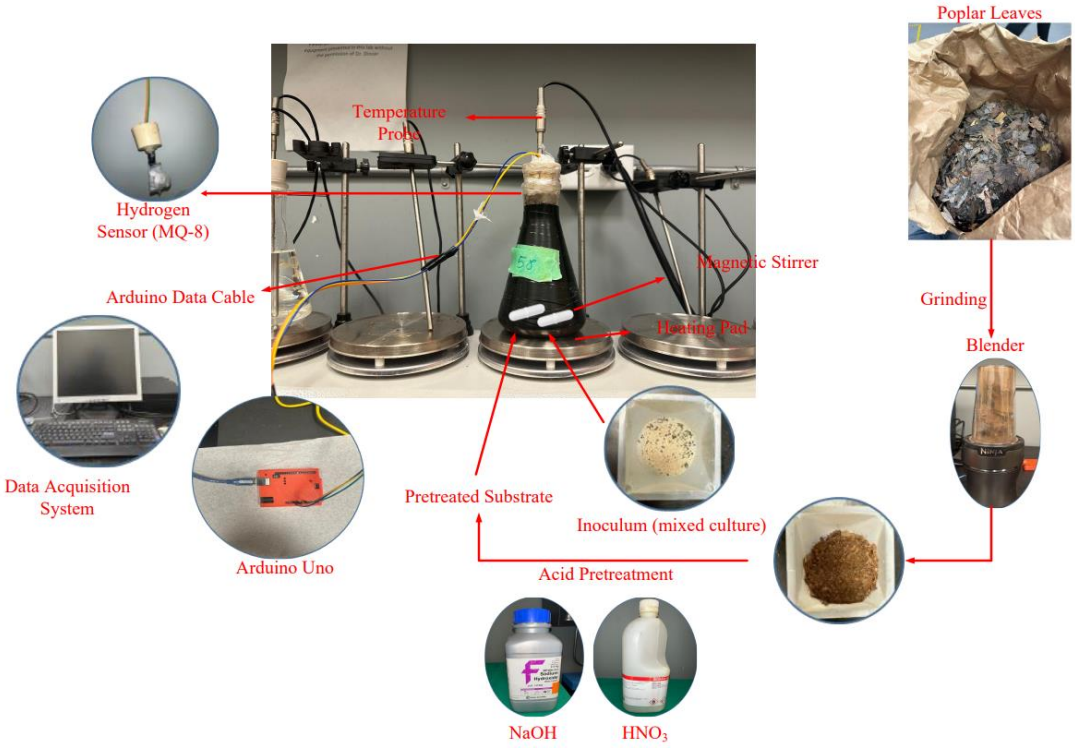


Figure 3.1 Experimental Setup

As seen in Figure 3.1, experiments were conducted using 250 ml Erlenmeyer flasks. Before initiating the system, all the Erlenmeyers were covered with black tape to prevent exposure to light. Two holes for Erlenmeyers were opened for each Erlenmeyer flask: one for a temperature probe to monitor the batch systems' temperature levels and the other connected to an Arduino Uno system used for hydrogen measurement.

All the experiments were performed on the magnetic heating stirrer to provide heat and mixing in the Erlenmeyer flasks. Magnetic stirrers were placed inside the flasks before closing them for the dark fermentation process. The system was initiated then, and the temperature was arranged to the desired level for each experiment. The rpm values are also adjusted accordingly on the magnetic heating stirrer. A computer stored the hydrogen data as ppm (parts per million). Each experiment lasted 12 hours. For biomass preparation, the dried poplar mass was supplied by Viona Consulting Inc. The dried biomass was ground and crushed in a blender. After the biomass preparation, the ground poplar leaves were transferred to a hood for acid pretreatment. Acid solutions were prepared using a 70% HNO_3 solution to achieve the desired acid concentration. For each trial, the required acid solutions are prepared by using 100 ml (v/v) solutions. The desired acid solutions were %2, %6, and %10. The biomass was mixed with the acid solution and subjected to heat treatment at 60 ºC. Each pretreatment process was conducted at 250 rpm to ensure consistent mixing and better treatment. For this purpose, magnetic stirrers were used. The heater was turned off one hour later, and the system was cooled down. When the system reached 25 ºC, the pH was adjusted according to the experimental setup. First, the system's pH was measured using a pH meter, and then, the required pH was obtained. To reach the desired pH level, HNO_3 and NaOH pellets were used. Once the system achieves the desired pH, a measured amount of the mixed bacteria culture is added to the Erlenmeyer flask. Viona Consulting supplied the mixed culture used in the study. It is a commercial septic tank treatment powder. It includes a mixture of Clostridium, Bacteroides, Ruminococcus, Butyribacterium, Propionibacterium, Eubacterium, and Lactobacillus bacteria cultures. After this step, the flask was inserted with a stopper, and the required temperature and mixing ratio were set on the magnetic heating stirrer. Subsequently, the computer started recording the system's ppm values using the Arduino Uno interface program called Coolterm. After 12 hours had passed, the system was shut down. These steps were repeated for each fifty-eight runs.

3.2 Reactor Design

The primary objective of this study is to conceptualize and develop a novel reactor model that harnesses the dark fermentation process as its primary operative method. At the same time, the ambition is to integrate the advantageous features of high-profile

bioelectrochemical systems. This research aims to develop an innovative reactor design that relies primarily on dark fermentation. The endeavor also aims to integrate the defining characteristics of the renowned biochemical systems. The design principles of microbial electrolysis and desalination cells have been adopted as foundational frameworks. Literature shows many examples in which micro-electrolysis cells have been utilized to increase the substrate's conversion rate, resulting in a higher hydrogen production rate. In most of these applications, volatile fatty acids, a byproduct of dark fermentation, are further processed in Microbial Electrolysis Cells (MECs) to produce hydrogen. In these processes, exoelectrogen bacteria are used since they enable electron transport. Microbial Electrolysis Cells (MECs) utilize exoelectrogenic microbes due to their unique ability to "excrete" electrons. Essentially, these microorganisms can directly transfer electrons produced by their metabolic processes to a solid-state electrode. These microorganisms can directly transfer electrons produced by their metabolic processes to a solid-state electrode. Consequently, using exoelectrogenic bacteria in MECs enables the conversion of organic waste into usable energy (such as electricity or hydrogen gas) by improving the system's overall efficiency and sustainability.

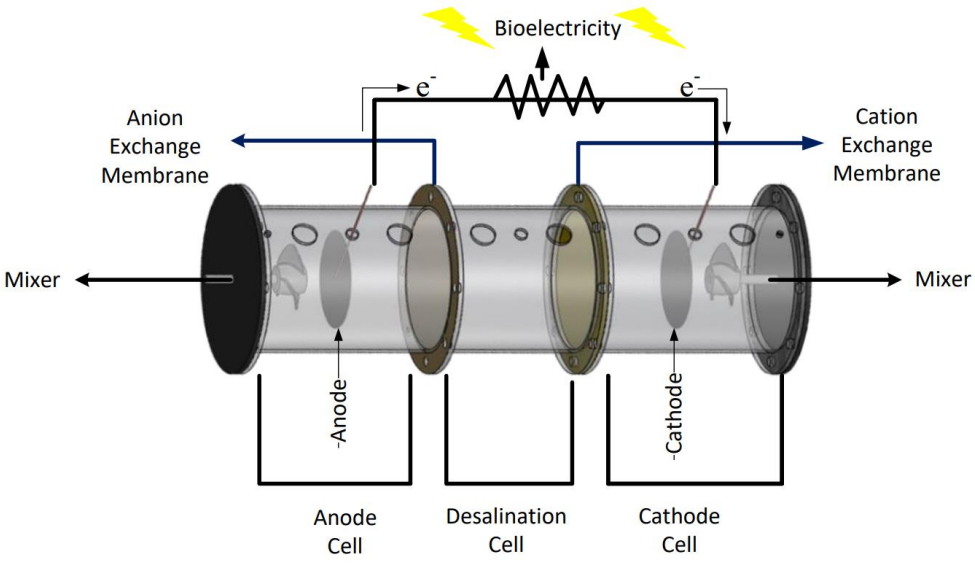


Figure 3.2 Reactor Configuration

This design aims to evaluate the potential of dark fermentation of poplar leaves in a biochemical system. The amount of the released electrons during the dark fermentation process can vary depending on factors such as substrate, microbial community, and

environmental factors. However, electron transport is critical to generating electricity and desalination. In the dark fermentation process, a significant amount of substrate electrons goes to volatile fatty acids (60-70%), H_2 electrons (<20 %), and utilization-associated products, respectively [107]. As seen in Figure 3.2, the reactor consists of three chambers. The anode cell is filled with biomass and sewage sludge, which includes exoelectrogenic and dark fermentation bacteria. These bacteria consume organic matter in the waste and generate electrons, which are then transferred to the anode. Simultaneously, the free electrons in the anode cell travel through the external circuit to the cathode, resulting in an electric current. This process generates an ion gradient in the desalination chamber. The cations of the NaCl in the desalination cell are drawn to the cathode, and the anions are drawn to the anode. At the same time, the cathode cell, which is filled with 30 % KOH solution, produces hydrogen simultaneously. The reactor aims to produce hydrogen from two chambers simultaneously while generating hydrogen, generating electricity, and desalinating water. For this purpose, three-chambered reactors are designed using plexiglass material. Membranes International Inc. supplies the ion exchange membranes. The reason for selecting KOH solution in the cathode comes from being a solid electrolyte that fully dissociates in water.

$$\text{Anode: } CH_3COO^- \ 4H_2O \rightarrow 2HCO3^- + 9H^+ + 8e^- \quad (3.1)$$

$$\text{Cathode : } H_2O + e^- \rightarrow H_2 + OH^- \quad (3.2)$$

To produce hydrogen in the cathode cell, an external current should be given to the cathode cell of the reactor through a power supply. In the cathode compartment, the process of proton reduction to produce hydrogen gas is not thermodynamically favorable under typical circumstances. The reaction carried out in the cathode cell is seen in Equation 3.2. It is known as the hydrogen evolution reaction (HER). Hence, an additional potential is required to facilitate this reaction, and the external supply provides it. The externally provided electric current enhances the energy level of electrons, allowing them to overcome the energy barrier required for HER. Adding this external current facilitates the reaction process, which leads to hydrogen gas generation. Without the additional force, the reaction would have been unable to proceed. The exact amount of electrical current required can fluctuate depending on various factors, such as the pH level of the surrounding

environment and the specific elements utilized within the cell. Furthermore, it is compulsory to maintain an equilibrium between the necessity for hydrogen generation and the system's overall energy efficiency [108]. Excessive reliance on externally sourced energy can diminish the efficiency of the reactor as an energy generation system despite the possibility of increased hydrogen production.

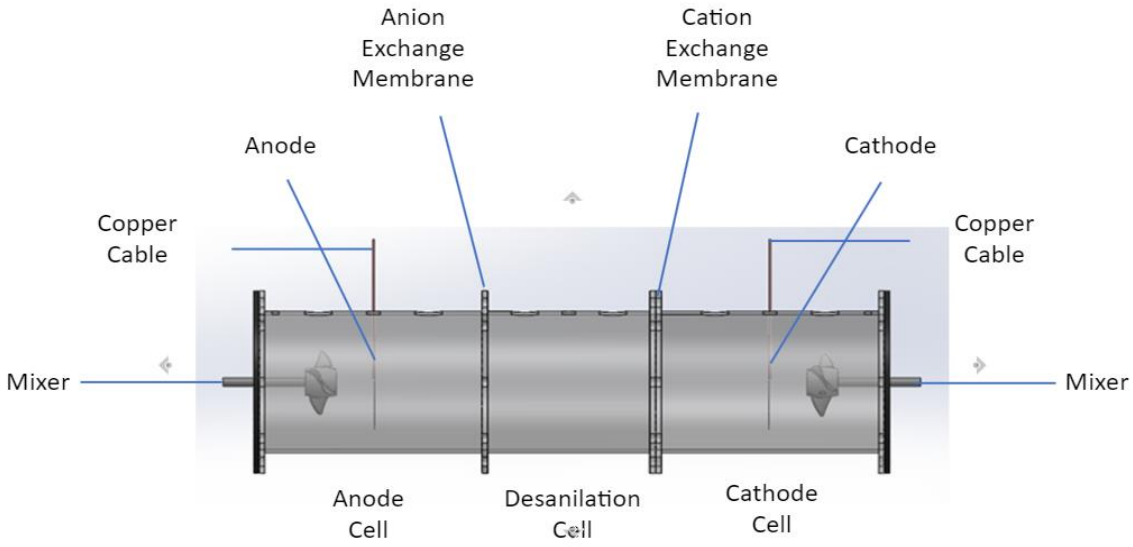


Figure 3.3 Side view drawing of the designed reactor

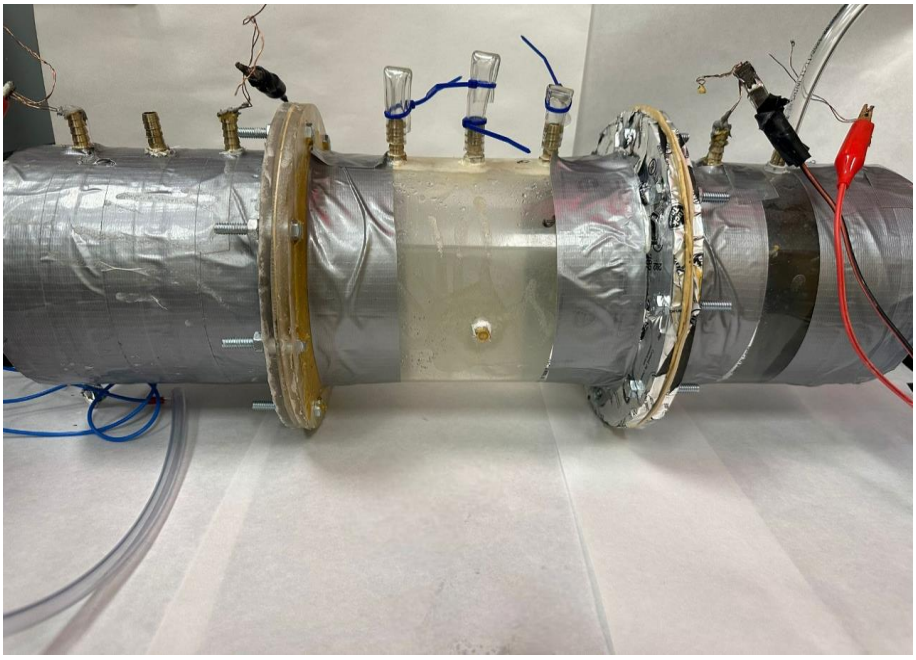


Figure 3.4 Front view of the designed reactor

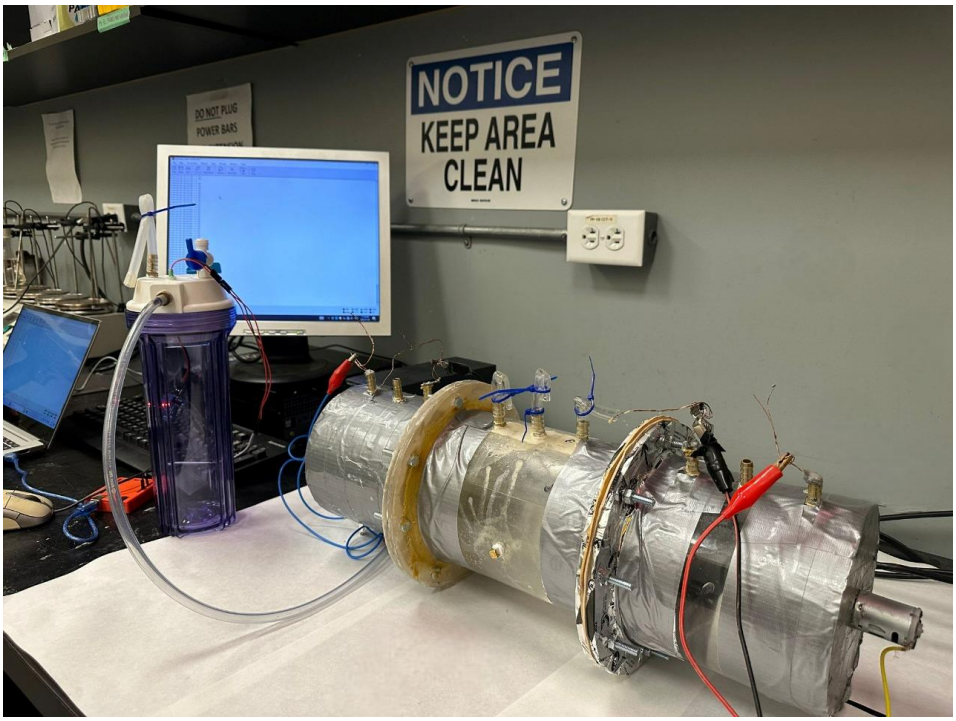


Figure 3.5 Side view of the designed reactor while collecting the data

3.3 Devices Used

During our experiment, we processed poplar leaves using a 600-watt Ninja Blender. To measure the hydrogen concentration, we employed MQ-8 hydrogen sensors. This sensor was selected for its affordability and user-friendly operation. Connections were established between the sensors and an Arduino Uno board to collect and monitor the data. Additionally, 500-ml Erlenmeyer flasks were used in our experimental setup, and we ensured that everything was properly sealed with parafilm to prevent potential hydrogen leaks from the stopper. The reactor was built using plexiglass material and stuck with a strong waterproof adhesive. Black tapes were used to cover the reactor to prevent light exposure. This study employed a Keithley 2400 Source Meter to provide the required external potential for hydrogen production in the cathode cell. Chemical Oxygen Demand (COD) was measured using a Hach DR900 Colorimeter. A Watson-Marlow Bredel 520 Series Peristaltic Pump was chosen to feed the fluid within the reactor.

3.4 Chemicals and Reagents

This research also utilized sewage sludge sourced from a local municipal wastewater facility, Harmony Creek Wastewater, except for commercial culture. The study employed

membranes from Membranes International Inc., including strong base anion exchange membranes and cation exchange materials made from gel polystyrene. 70% (w/w) HNO3 was used to adjust the pretreated mixture's acidity. NaOH pellets were added to increase the pH level as needed. Viona Consulting provided the dark fermentation culture. The septic tank treatment powder contained several bacterial species, including Clostridium, Bacteroides, Ruminococcus, Butyribacterium, Propionibacterium, Eubacterium, and Lactobacillus. This diverse culture was used to optimize poplar leaves optimization then mixed with the sewage sludge to initiate the anode chamber of the designed reactor. The central chamber was filled with a solution containing 35 grams per liter of sodium chloride (NaCl) for desalination. The electrodes utilized in the biochemical reactor for the cathode and anode compartments were built using graphite plates. Any alterations in the electrode potential were monitored by employing a graphite reference electrode positioned within the anode chamber. The selection of this material aligns with current trends in studies, which prefer the use of graphite due to its excellent electrical conductivity and high resistance to corrosion.

3.5 Hydrogen Measurements

During the experiments, a simple and low-cost MQ-8 hydrogen sensor was used to measure the concentration of hydrogen. Using the resistance change, the sensor measures the hydrogen concentration as part per million (ppm) within a controlled volume. The sensor utilizes a semiconductor sensing layer made of SnO_2, which has low conductivity when exposed to air, but its conductivity increases with hydrogen gas concentration. The detection range of the sensor is from 100 ppm to 10000 ppm. To take the measurements from the sensor, an Arduino Uno board was connected to the sensor using a 5V power input, an analog input, and a grounding input. The sensor was allowed to stabilize for 15 minutes before each trial to achieve steady-state readings. It was then placed inside the Erlenmeyer flask to obtain steady readings from the experimental setup. For this study, the sensor was calibrated with a known amount of hydrogen concentration before the experiments started, and the results were validated. In the present investigation, systematic error quantification stems from the equipment specifications used to measure each operating parameter.

3.5 Experimental Procedure

Figure 3.6 illustrates the experimental algorithm for the dark fermentation of poplar leaves and the reactor setup. As depicted in Figure 3.6a, the process commences with the grinding of poplar leaves using a blender. Subsequently, the feedstock is pretreated with HNO_3 for an hour at 60°C. This step is succeeded by pH adjustment of the pretreated sludge using NaOH pellets and HNO_3, in accordance with the pH determined from experimental trials. Following this stage, inoculation is performed using a bacterial culture, preparing the system for the subsequent steps. The system is then left for 12 hours to facilitate hydrogen production. Similarly, as seen in Figure 3.6b, the reactor setup initiates with the cutting and preparation of chambers. The constructed reactor undergoes testing to identify and seal any potential leaks. This is succeeded by the installation of the reactor, including the connection of data acquisition for hydrogen measurements.

3.6 Uncertainty Analysis

This study conducted a series of experimental trials to investigate the performance of the dark fermentation process with poplar leaves under different operating conditions. It is crucial to quantify the associated experimental uncertainties to understand the observed variations in results with different operating parameters. The types of experimental uncertainties are systematic errors and random errors. Systematic errors are inaccuracies caused by apparatus malfunctions or faults in the experimental design. On the other hand, random errors cause fluctuations in the results of repeated studies due to uncontrollable variables such as environmental conditions and data recording errors. In this study, the conducted experimental data set was determined by Design-Expert software. By the nature of the program, this software repeats certain experimental conditions to enhance confidence in the results. As demonstrated in Table 5.1, some experiments share identical parameters. For example, runs 2, 10, 23, 24, 36, 41, 42, 50, and 57, as well as Runs 4 and 8, Runs 6 and 18, and Runs 17 and 29, all had the same experimental parameters. That is why it can be used to estimate experimental error and improve the experiment's precision. When these experiments are repeated, the hydrogen production amount varies by the third digit after the decimal point. That is why the standard deviation of the repeated series is neglected. The software provided a standard deviation for all the data sets with a standard deviation

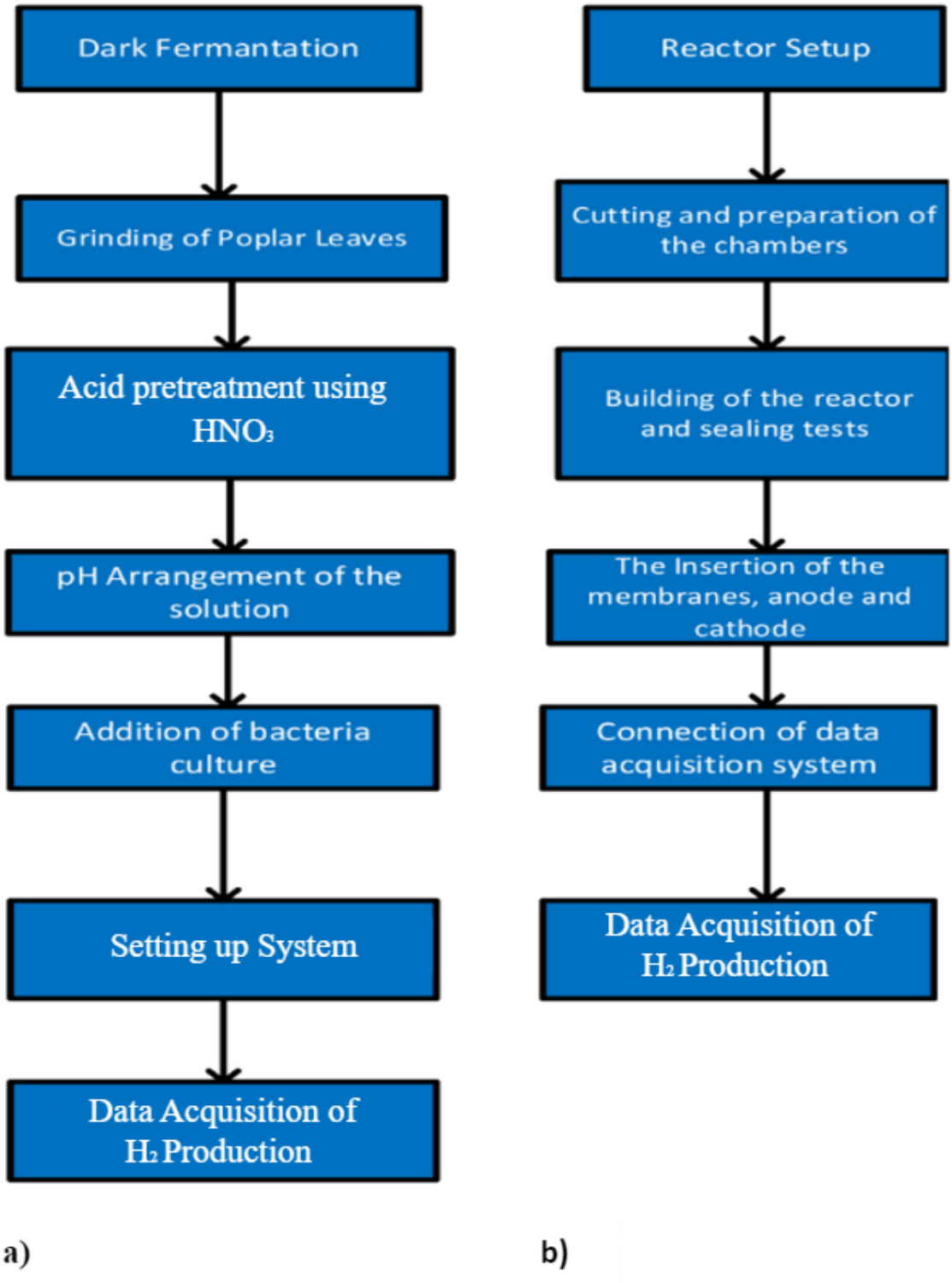


Figure 3.6 Algorithm of the experimental setup; a) Dark fermentation experimental procedure, b) Reactor setup experimental procedure

of 0.014 and a mean of 0.1617. The accuracy of the acid concentration measuring burette was ±0.15%. The biomass and inoculum amounts were measured with a precision of ±0.01 g using a lab-scale balance. The initial pH was measured with a precision of ±%1 pH units using a standard pH meter. The temperature was recorded with an accuracy of ±%5 using a magnetic heating stirrer thermometer. The motor's agitation speed was accurate to within ± % 0.1 rpm. The error propagation formula was utilized to obtain an overall assessment of systematic errors in the experiment. Given the equipment used, the total systematic error for the experimental setup is approximately 0.0053. The total uncertainty in the experimental results is approximately ±0.01495 by evaluating the equipment inaccuracies and the variation in repeated runs.

Chapter 4. BACKGROUND and ANALYSIS

This section provides an overview of the background information and the analyses conducted during the study. The first section of this chapter analyzes the optimization of dark fermentation. The second part covers the analysis of the designed reactor.

4.1 Dark Fermentation

Dark fermentation is a biological process where anaerobic microorganisms break down the organic matter without light. These anaerobic bacteria yield several volatile fatty acids and hydrogen. Since dark fermentation is a biological process involving bacteria, the bacterial growth curve shows the process. Investigation of the bacterial growth curve of dark fermentation is essential for a better understanding of the optimization and management of the process. The bacterial growth curve represents the different stages of growth that a bacterial population undergoes when introduced to a new environment with enough nutrition. Typically, this curve has four distinct phases: lag, exponential (log), stationary, and death (decline) phase. Figure 4.1 shows the bacterial growth curve. The duration of each stage depends on many parameters, such as the microbial strain, pH, temperature, and mixing ratio.

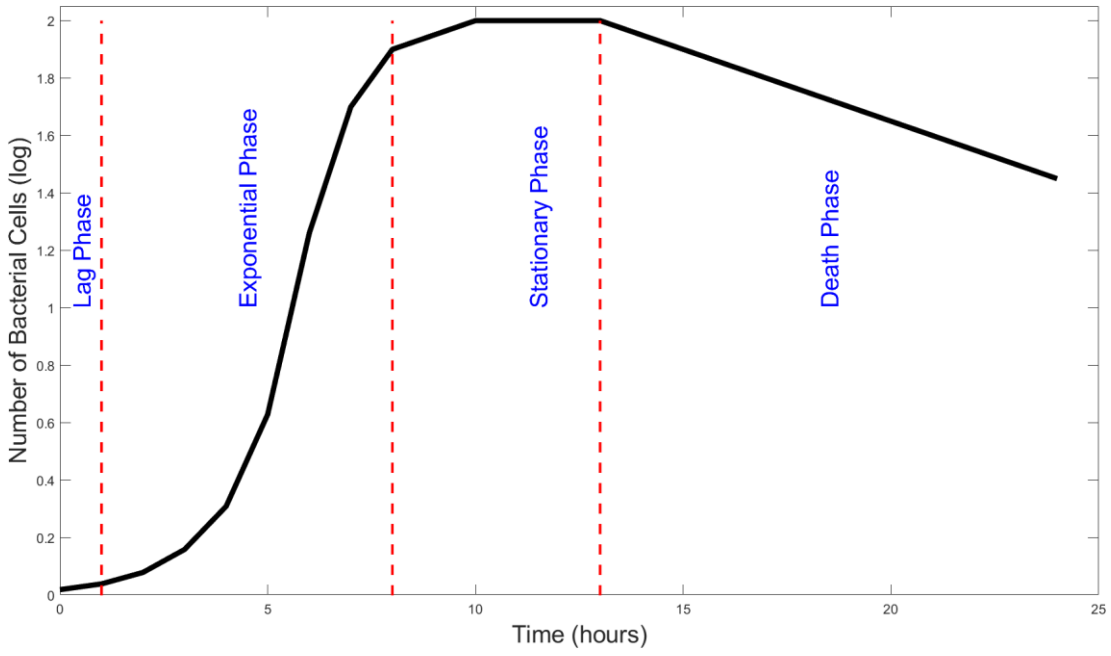


Figure 4.1 Growth phases of microorganisms in batch experiments

Lag Phase: The bacteria require time to adapt when introduced to their new nutrient-rich medium. In this phase, bacteria do not replicate but prepare themselves for reproduction. The cells are metabolically active and continually grow. Since they are not actively fermenting during this stage, volatile fatty acids and hydrogen production are negligible.

Exponential Phase: Bacterial cells go through the exponential or log phase following the lag phase. Once the bacteria have adapted, they will rapidly begin consuming the organic substrate, significantly increasing the production of VFAs and hydrogen. This phase represents the optimal period of operation for the anaerobic fermentation process, as it corresponds to the highest rates of substrate degradation and hydrogen production. Hydrogen production primarily occurs during this phase when bacteria metabolism and cell division peak.

Stationary Phase: After the exponential phase, the stationary phase occurs. During this phase, the bacterial growth rate stabilizes, mainly due to limiting nutrient conditions or the accumulation of metabolic end products. After stabilization, the production of VFAs and hydrogen levels will eventually plateau due to the depletion of available nutrients and the accumulation of waste. This leads to a decline in population growth previously observed during the exponential phase. However, hydrogen production can continue at this stage, although at a reduced rate.

Death Phase: In the final stage of bacterial growth, dying cells increase due to decreased nutrient availability and increased waste products. In this stage, cell death is higher than cell formation due to nutrient deficiency, physical conditions, or other cell injuries.

4.2 Application of Design-Expert for Experimental Design and Statistical Analysis

Design-Expert is a software application developed by Stat-Ease that focuses on applying statistical methods to the design and optimization of experiments. It is known for its user-friendly interface to implement response surface methodology, a combination of mathematical and statistical techniques for empirical model building. It also provides various tools that help create, refine, and visualize models. These tools aid in understanding the relationship between factors and response variables. Moreover, it employs a technique known as factorial design to simultaneously vary multiple factors, which provides insight into the individual effects of each factor and their interactions. This multifactor approach

provides a significantly more comprehensive image than the conventional method of modifying one factor at a time, which can neglect significant interactions between variables. In the context of this research, it is utilized to plan and analyze a series of experiments to optimize hydrogen production for the dark fermentation process. Design Expert employs strategies like Design of Experiments (DOE) to identify the parameter combinations that would be most useful for achieving desired goals. With a small number of experimental runs, DOE approaches seek to explore the parameter space and retrieve valuable data efficiently. It is founded on statistical principles that reduce experimental error and bias while assisting in identifying significant main effects and interactions between factors. This study focused on six critical operational parameters that impact the dark fermentation process: pH, temperature, mixing ratio, biomass amount, bacterial culture amount, and acid concentration for pretreatment. Combining these parameters into the Design-Expert software generated a design matrix. This design matrix determined the experimental conditions for 58 trials, Table A.1. Design-Expert does not randomly choose the 58 experimental conditions. Instead, it was designed to explore the parameter space diligently. This systematic approach maximizes the effectiveness of our experiments and reduces the total number of required trials, which is a significant time and resource savings. After conducting the experiments under the conditions specified by the software, the data was analyzed comprehensively using Design-Expert's robust analytic tools. This includes developing statistical models that reflect the relationships between the input parameters and the response, which enable us to comprehend and predict how parameter changes affect the fermentation process's output [109]. Statistical parameters such as R-squared, adjusted R-squared, and predicted R-squared values were employed to assess and modify these models to ensure a reliable and accurate prediction. Finally, the Design-Expert software assisted us in determining the optimal conditions for the dark fermentation procedure by identifying the optimum combination of parameters to maximize our output. In conclusion, Design-Expert played a crucial role in our research's experimental design, process optimization, and data analysis phases, enabling a more efficient and comprehensive investigation of dark fermentation.

4.3 Kinetic Modelling

Dark fermentation is affected by many parameters, including pH, temperature, bioreactor configuration, inoculum size and age, pretreatment conditions, substrate type hydraulic retention time, and hydrogen partial pressure. That is why optimization and modeling are essential to enhance hydrogen production. As seen in Figure 4.2, while the substrate is consumed during the fermentation, hydrogen, alcohols, and organic acids are simultaneously produced along with bacterial growth.

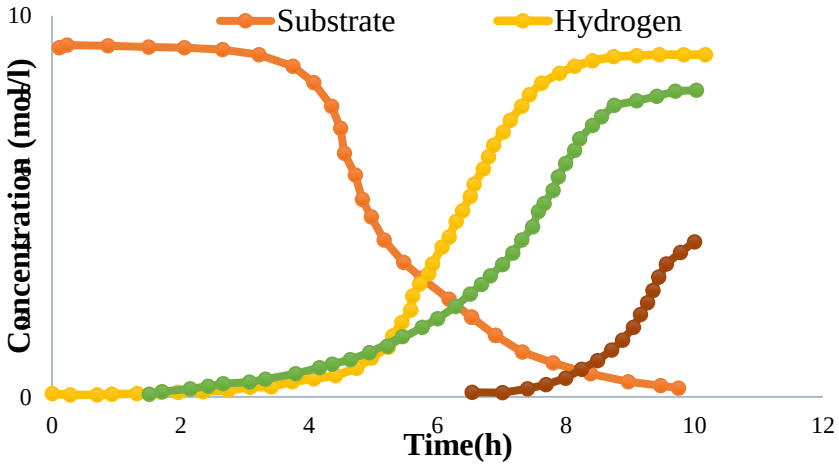


Figure 4.2 Substrate consumption and product generation in dark fermentation

Kinetic modeling has been applied in numerous disciplines, such as biology and engineering environmental processing, to understand the processes and variables in a better way. The kinetic models can be classified as either structured or unstructured. Structured models are more complex than unstructured models because of taking metabolic pathways into account. Structured kinetic models typically offer insights into microbial population changes by shedding light on morphological, chemical, and metabolic pathways. On the other hand, unstructured kinetic models are commonly used for their robustness and simplicity. Using kinetic models, it is possible to characterize the relationship between the parameters. Moreover, they offer valuable data for designing, operating, and analyzing microbial systems and explaining fermentation quantitatively. The quality of a model depends on its ability to correlate theoretical and experimental values with each other. If there is an inconsistency, the model is appropriate and needs to be modified. The widely used kinetic models in dark fermentation are Cone, Monod, Andrews, Richards, Arrhenius, Ratkowsky, Han-Levenspiel, Michaelis-Menten, modified Logistic, modified Gompertz,

and anaerobic digestion model No. 1. These kinetic models can forecast the production of hydrogen, biomass, alcohols, organic acids, substrate degradation, the concentration of inhibitors, pH and temperature effects on hydrogen production, and the relationship between biomass and product formation. Among the kinetic models, the Gompertz model has been widely preferred to study hydrogen production because of its robustness, accuracy, and ease of use. It can give some insight into the maximum growth rate and the time at which the maximum growth rate occurs.

$$H(t) = H_{max} \times \exp\left(-\exp\left[\left(\frac{R_{max} \times e}{H_{max}}\right) \times (\lambda - t) + 1\right]\right) \quad (4.1)$$

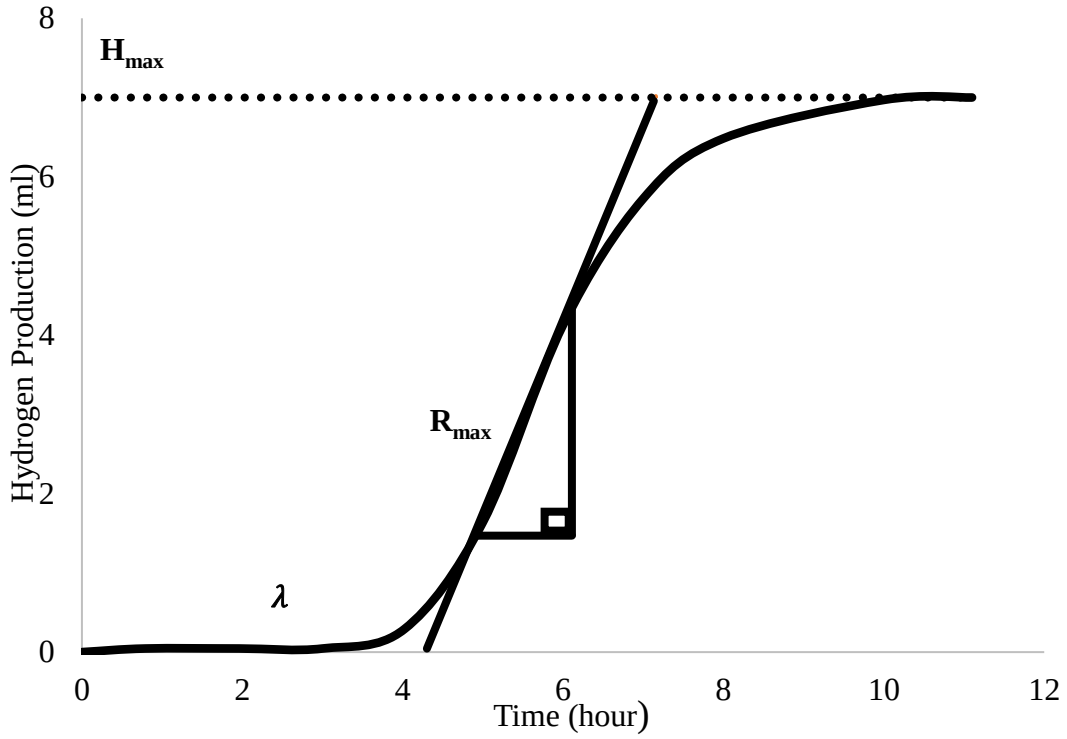


Figure 4.3 Gompertz function: visualization of key parameters [110]

Equation 4.1 represents the Gompertz equation, which models a microbial system's cumulative hydrogen production H(t). In this equation, H_{max} denotes the maximum value of hydrogen production achieved on the y-axis. The parameter R_{max} represents the maximum hydrogen production rate, indicating the highest rate at which hydrogen production occurs per unit of time. λ represents the duration of the lag phase, during which hydrogen production begins to increase significantly. The cumulative hydrogen production

with these critical parameters can be effectively analyzed and predicted by utilizing the Gompertz equation. MATLAB software was utilized to calculate the cumulative hydrogen amounts and make graphs.

4.4 Conductivity of Saline Water

The performance evaluation of a desalination cell in the reactor for salt removal can be effectively conducted by measuring the conductivity of water. Conductivity is a property that describes the capacity of an electrolyte to carry an electric charge. It is directly related to the concentration of ions present in a solution. In the context of the designed reactor, the salinity of the water being processed in the desalination chamber is determined by its conductivity measurement. At the beginning of the process, the saline water demonstrates increased conductivity because of the existence of dissolved salts. The reactor system functions by enabling the movement of ions from the desalination chamber to the anode and cathode chambers, reducing the salinity and conductivity of the water in the desalination chamber. Therefore, by monitoring the variations in water conductivity from the beginning to the end of the process, the desalination effectiveness of the reactor can be assessed. This offers a simple and direct approach to evaluating the efficiency of the reactor for removing salt. Hence, it is possible to quantitatively estimate the desalination degree by comparing the initial and final conductivity values. Equation 4.2 shows the formula used to calculate the salt removal efficiency (RE) [111].

$$\mathrm{RE}\ (\%) = \left(\frac{(\mathrm{C_i} - \mathrm{C_f})}{\mathrm{C_i}}\right) \times 100 \qquad (4.2)$$

In this equation, C_i refers to the initial conductivity of the saline solution before treatment, while C_f represents the final conductivity measured after the solution passes through the system.

4.5 Chemical Oxygen Demand (COD)

Chemical oxygen demand is a metric for assessing the concentration of organic molecules in water, especially wastewater. In simple terms, it serves as both an indirect measurement of pollution levels and a water quality metric. The term "demand" represents the quantity of oxygen that would be necessary to break down the organic molecules contained in the sample chemically. A high COD implies a high concentration of organic molecules in the

water. These organic substances are often byproducts of microbial metabolism in dark fermentation. This measurement can be significant in dark fermentation since it can provide insight into the potential for energy production.

$$\mathrm{COD}\ (\%) = \left(\frac{(\ \mathrm{COD_i} - \ \mathrm{COD_f})}{\mathrm{COD_i}}\right) \times\ 100 \qquad (4.3)$$

In equation 4.3, COD_i refers to the initial chemical oxygen demand of the reactor's anode part, and COD_f shows the final chemical oxygen demand of the anode part of the reactor.

CHAPTER 5. RESULTS AND DISCUSSION

This section presents the results of dark fermentation experiment trials and reactor design. The results of the experiments were discussed first and followed by reactor results. Several parameters were studied, including temperature, pH, biomass amount, inoculum amount, acid concentration, and mixing ratio, to demonstrate their effect on hydrogen production and system efficiency. Then, dark fermentation and reactor results were compared with literature data.

5.1 Dark Fermentation Results

This section provides valuable information about the hydrogen production rate. Gompertz function models the experimental data obtained by tested trials. The obtained data during the experiments is fitted with the function. The function fit, as seen in the following figures, helps us to identify cumulative hydrogen production, maximum hydrogen production rate (R_{max}) and the lag phase of the system which shows the time of the activation of the bacteria culture. For all the runs, R^2 values indicates a perfect fit for the experimental trials. The reason of that is explained in the discussion part. The Gompertz model was utilized to fit the data set given by the Design-Expert Table. Table 5.1 below shows the values of the parameters of the conducted experiments. When Table 5.1 root mean square coefficient (R^2) is examined, it is seen that the values are high for all the runs, which indicates that the Gompertz function is an excellent fit to the data. The maximum hydrogen production rate (H_m) ranges between 0.14 and 2.73 ml/h. This indicates that the maximum amount of hydrogen produced per hour varies with each run. The maximum hydrogen production rate per gram of substrate (R_{max}) ranges between 0.02 and 0.46 ml/g. Consequently, the hydrogen produced per gram of substrate also varies for each run. The time constant (lambda) range is between 0.31 h to 3.37 h. This means the time required to attain the maximum hydrogen production rate also varies depending on the run. The optimal amount of substrate to use for maximum hydrogen production is unclear from the data. It will be examined again using Design Expert. On the other hand, the optimal maximum hydrogen production rate is around 0.2 ml/h, and the optimal time constant is around 1 hour. The highest hydrogen production in a single run was 2.73 ml (Run 6), and the lowest amount in a single run was 0.14 ml (Run 34). The longest lag phase was 3.37 hours (Run 49), while

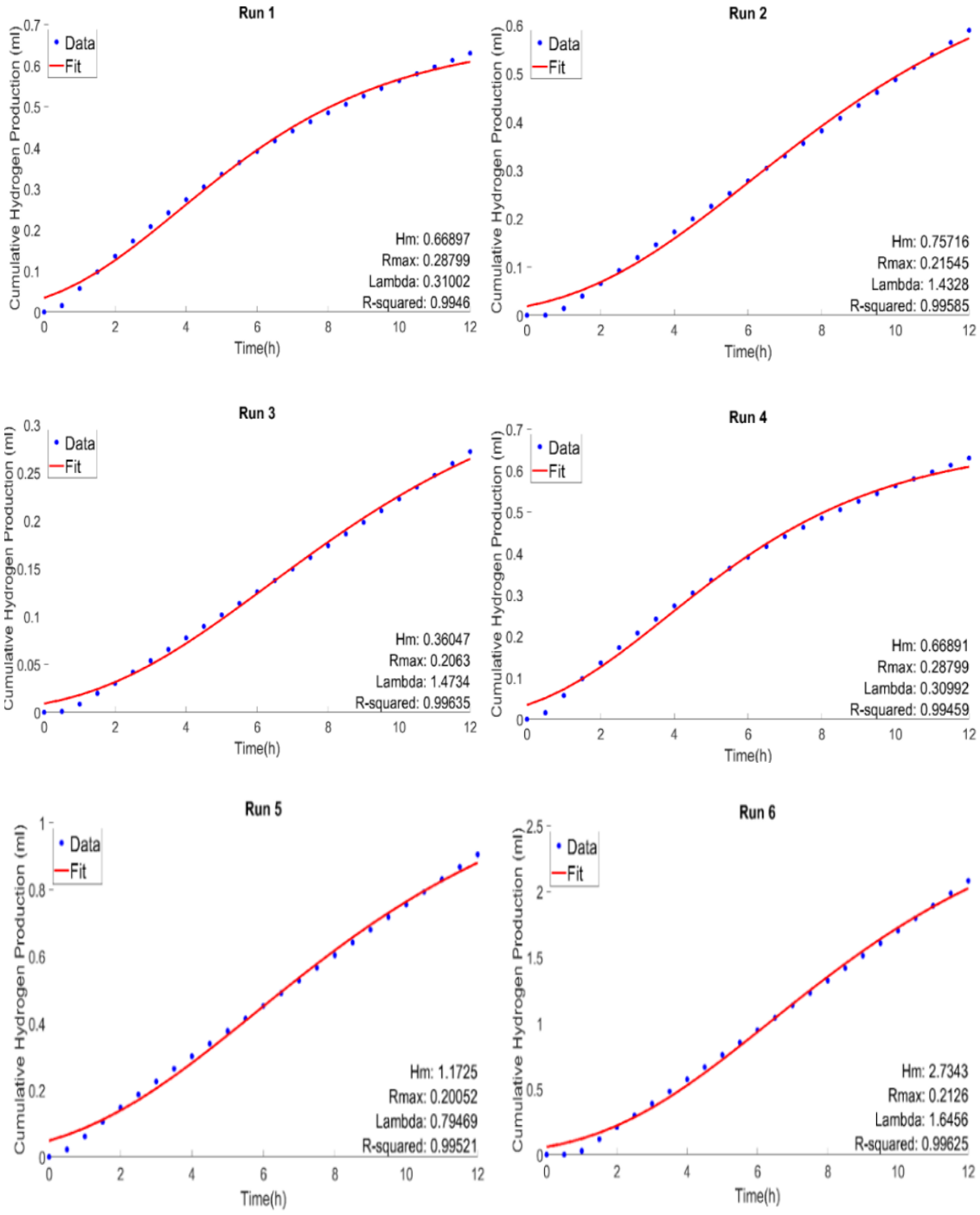


Figure 5.1 Experimental Results and Gompertz Fit between Trials 1-6

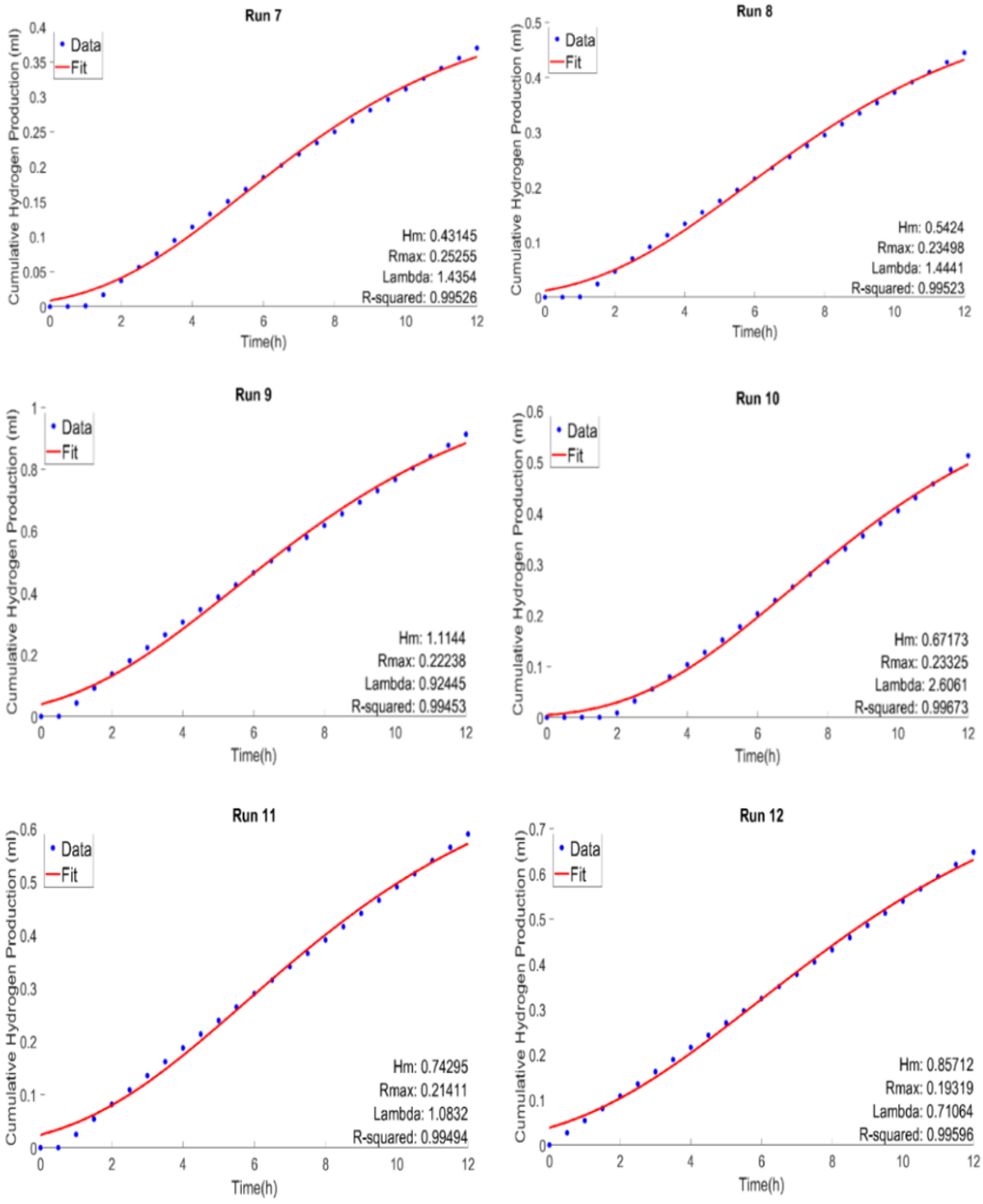


Figure 5.2 Experimental Results and Gompertz Fit between Trials 7-12

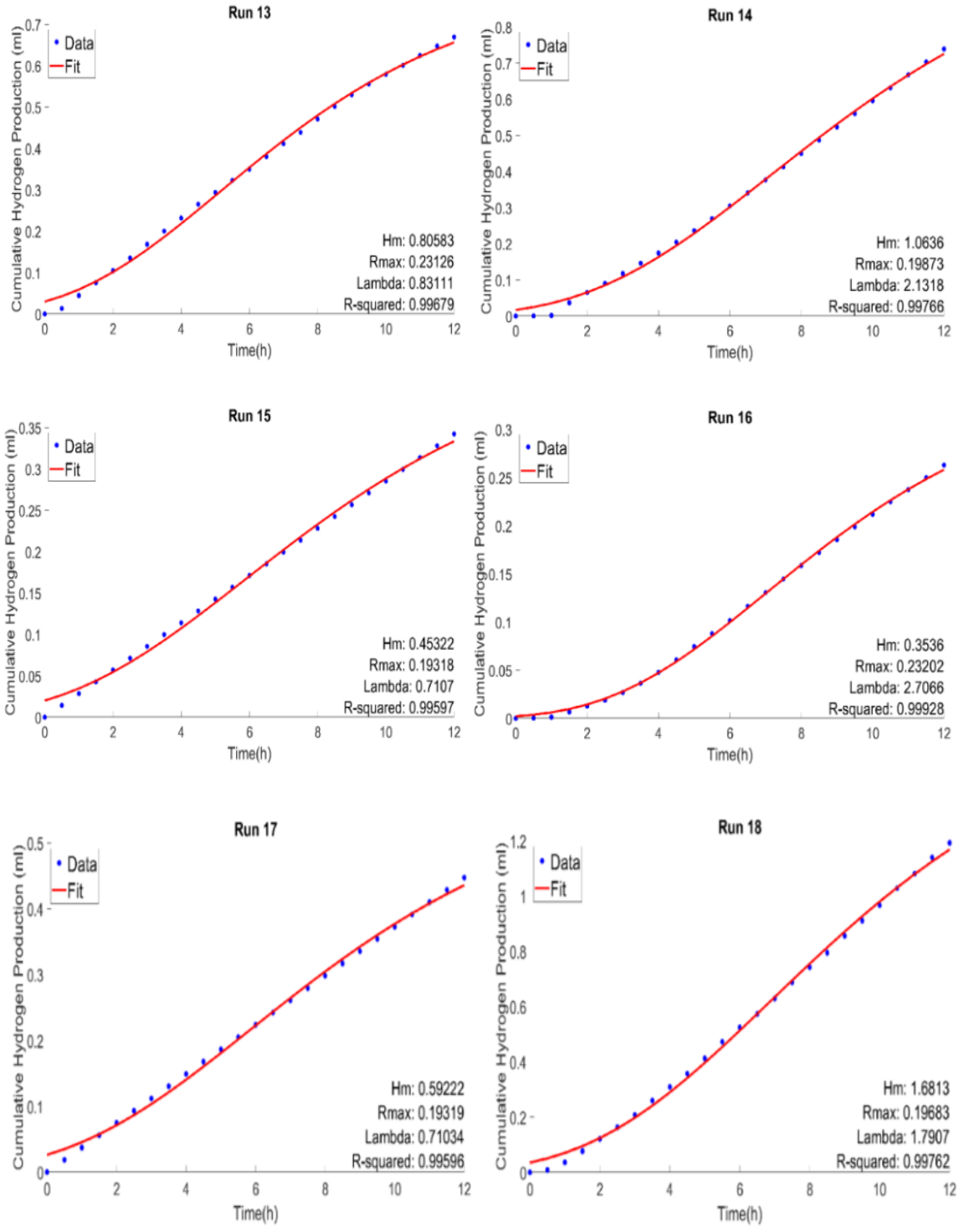


Figure 5.3 Experimental Results and Gompertz Fit between Trials 13-18

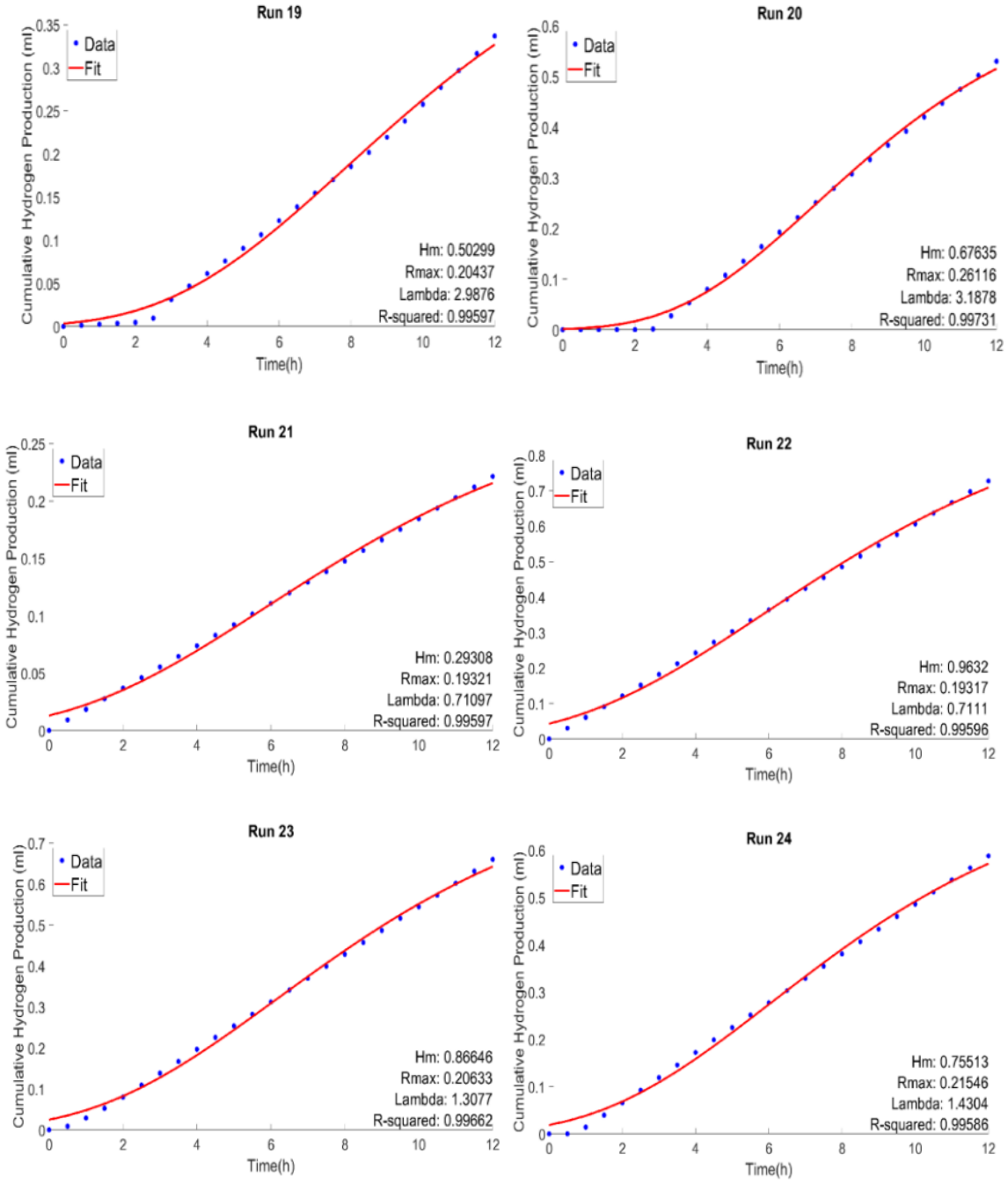


Figure 5.4 Experimental Results and Gompertz Fit between Trials 19-24

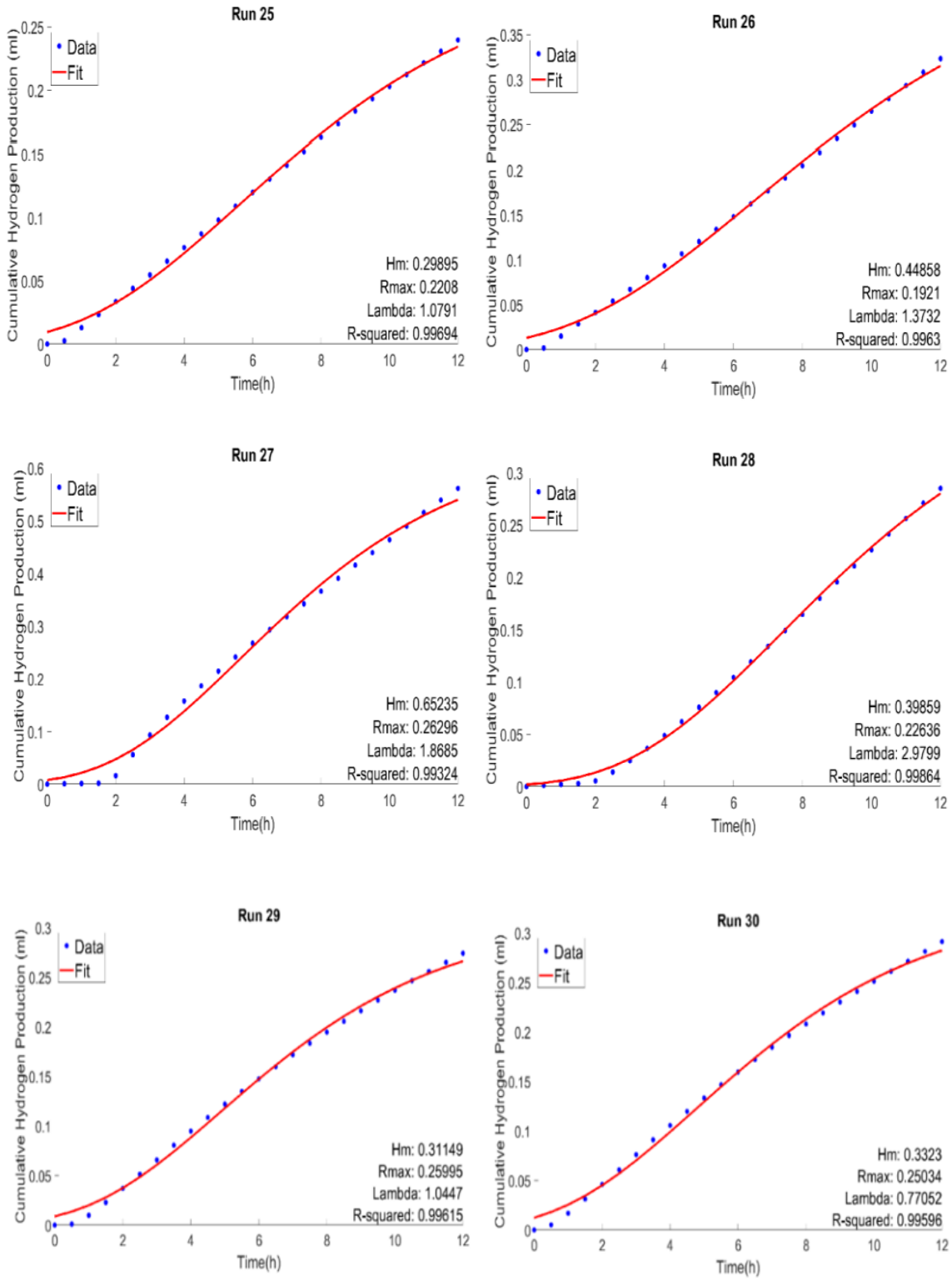


Figure 5.5 Experimental Results and Gompertz Fit between Trials 25-30

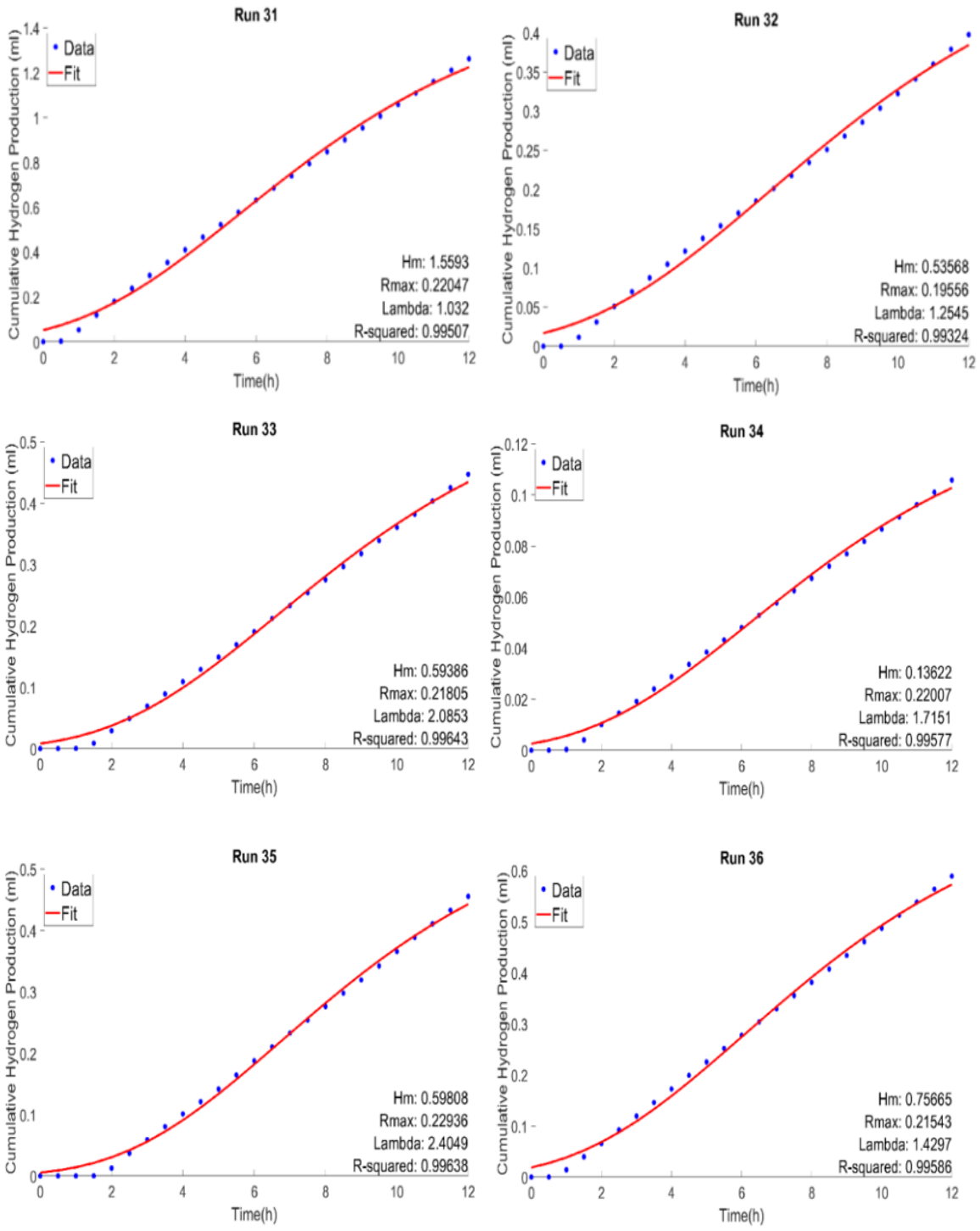


Figure 5.6 Experimental Results and Gompertz Fit between Trials 31-36

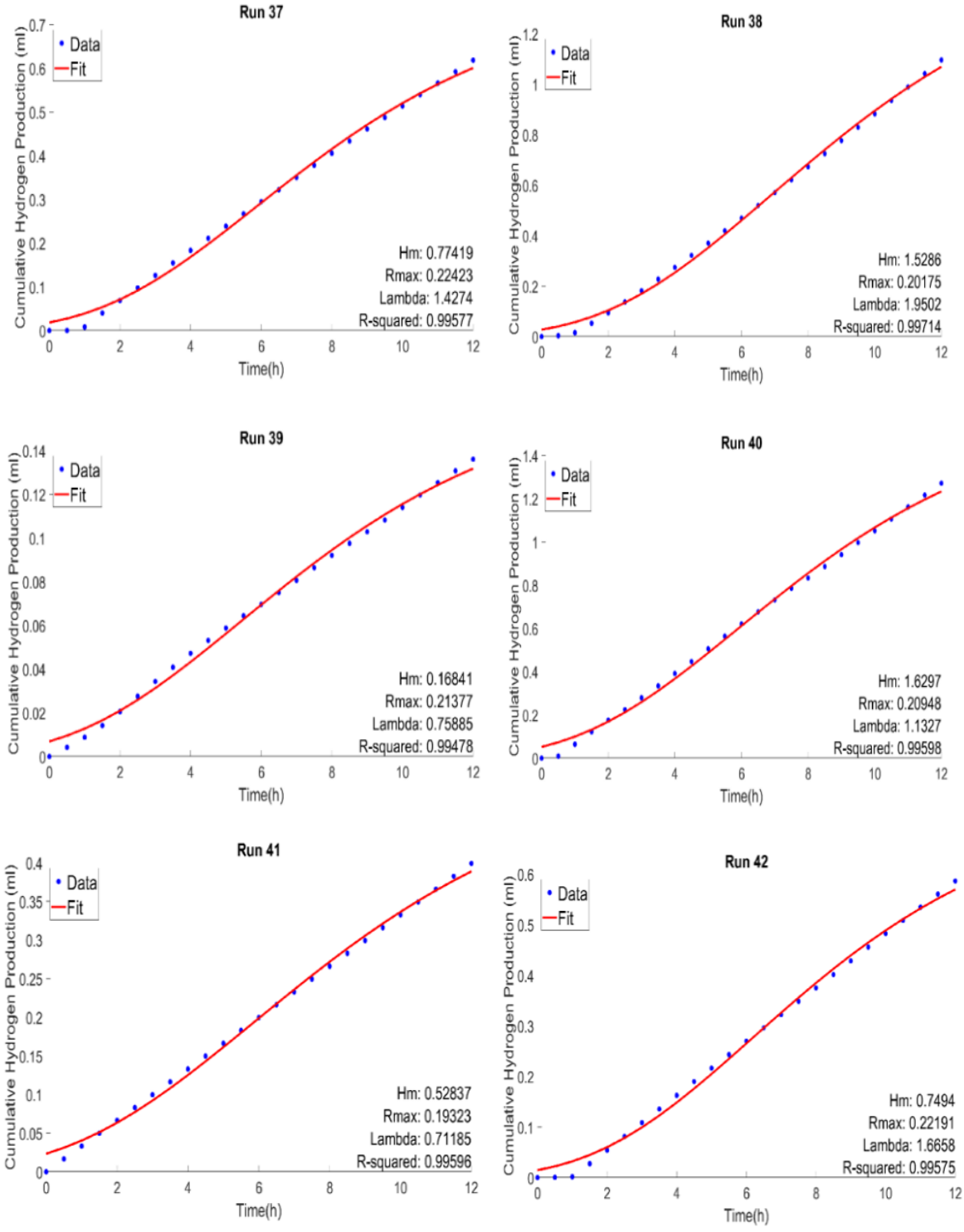


Figure 5.7 Experimental Results and Gompertz Fit between Trials 37-42

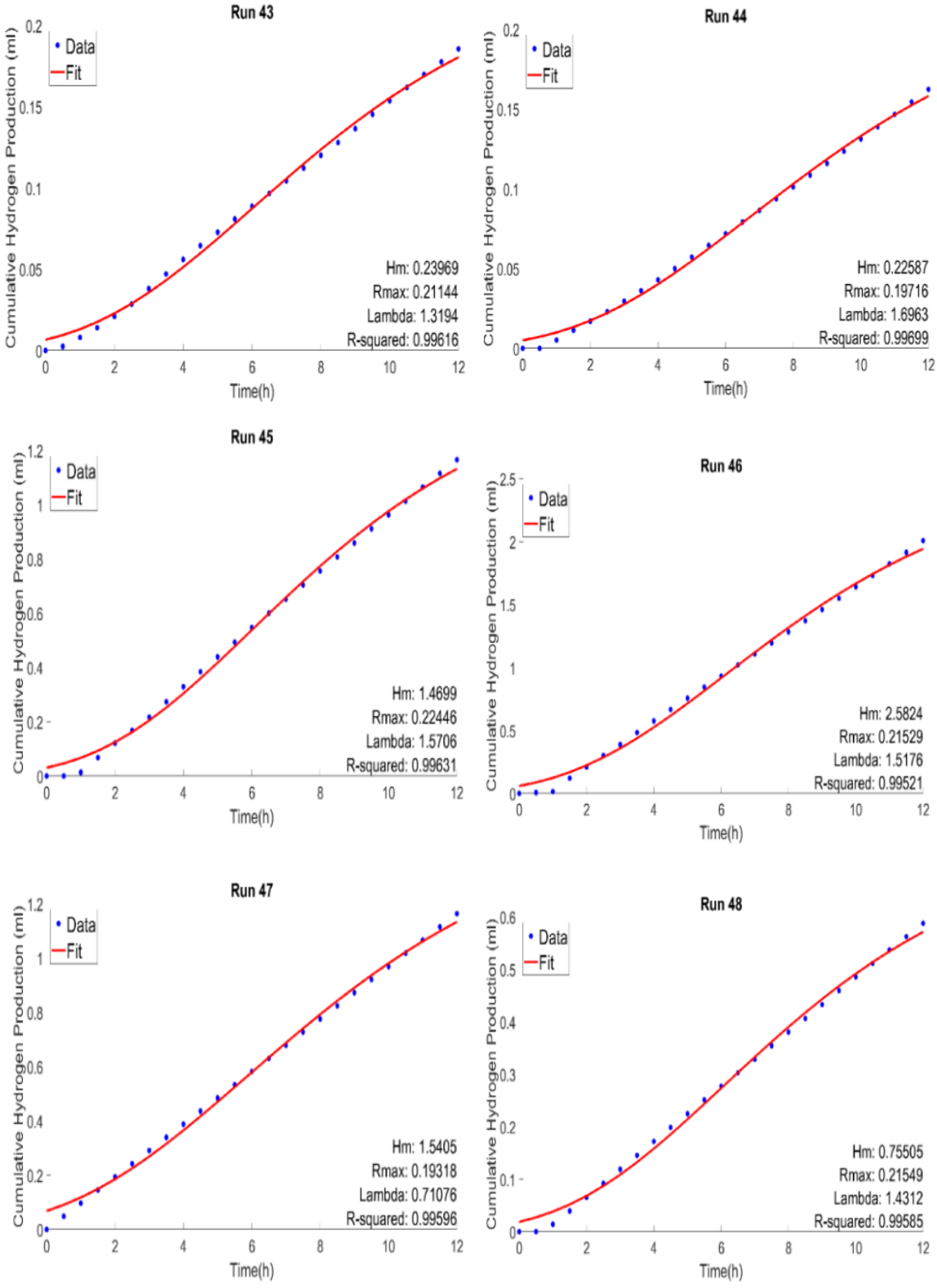


Figure 5.8 Experimental Results and Gompertz Fit between Trials 43-48

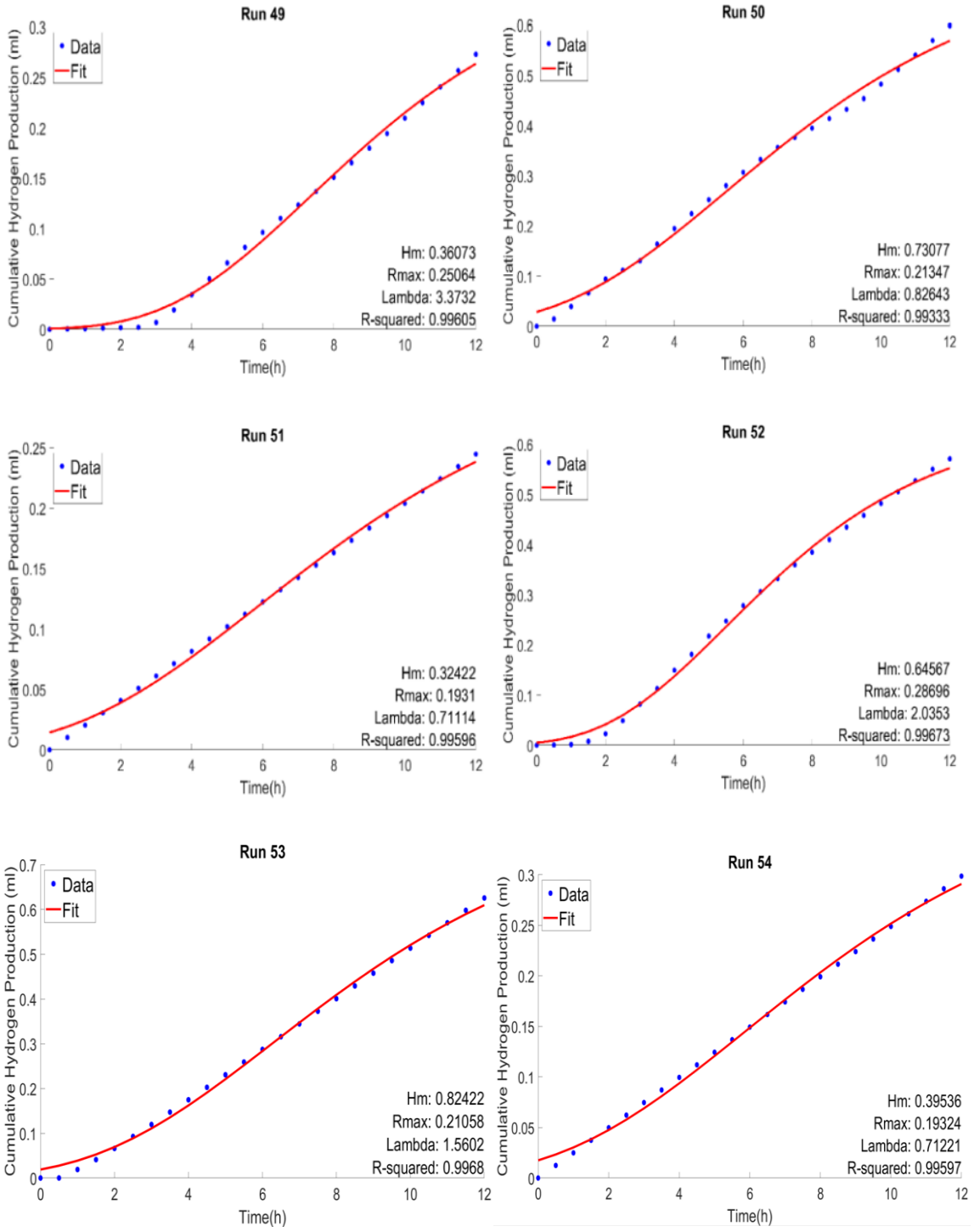


Figure 5.9 Experimental Results and Gompertz Fit between Trials 49-54

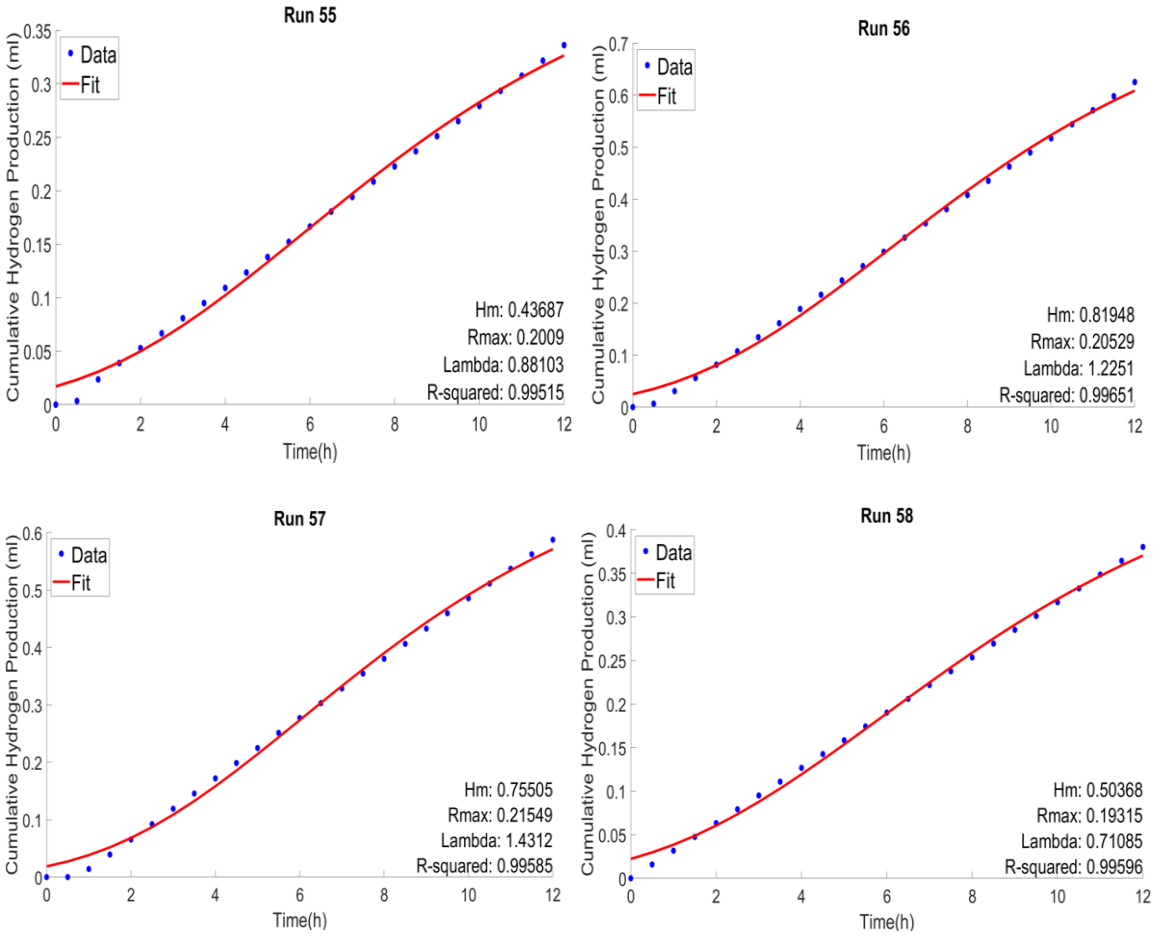


Figure 5.10 Experimental Results and Gompertz Fit between Trials 55-58

Table 5.1 Dark Fermentation Results

Runs	Hm (ml)	R_{max} (ml/h)	Lambda (h)	R^2	Substrate Amount	H_2 Production per g substrate (ml/g)
Run 1	0.67	0.29	0.31	0.995	6.00	0.11
Run 2	0.76	0.22	1.43	0.996	6.00	0.13
Run 3	0.36	0.21	1.47	0.996	10.00	0.04
Run 4	0.67	0.29	0.31	0.995	2.00	0.33
Run 5	1.17	0.20	0.79	0.995	10.00	0.12
Run 6	2.73	0.21	1.65	0.996	6.00	0.46
Run 7	0.43	0.25	1.44	0.995	6.00	0.07
Run 8	0.54	0.23	1.44	0.995	2.00	0.27
Run 9	1.11	0.22	0.92	0.995	10.00	0.11
Run 10	0.67	0.23	2.61	0.997	6.00	0.11

Run 11	0.74	0.21	1.08	0.995	6.00	0.12
Run 12	0.86	0.19	0.71	0.996	6.00	0.14
Run 13	0.81	0.23	0.83	0.997	2.00	0.40
Run 14	1.06	0.20	2.13	0.998	6.00	0.18
Run 15	0.45	0.19	0.71	0.996	6.00	0.08
Run 16	0.35	0.23	2.71	0.999	6.00	0.06
Run 17	0.59	0.19	0.71	0.996	2.00	0.30
Run 18	1.68	0.20	1.79	0.998	6.00	0.28
Run 19	0.50	0.20	2.99	0.996	10.00	0.05
Run 20	0.68	0.26	3.19	0.997	10.00	0.07
Run 21	0.29	0.19	0.71	0.996	6.00	0.05
Run 22	0.96	0.19	0.71	0.996	6.00	0.16
Run 23	0.87	0.21	1.31	0.997	6.00	0.14
Run 24	0.76	0.22	1.43	0.996	6.00	0.13
Run 25	0.30	0.22	1.08	0.997	6.00	0.05
Run 26	0.45	0.19	1.37	0.996	6.00	0.07
Run 27	0.65	0.26	1.87	0.993	2.00	0.33
Run 28	0.40	0.23	2.98	0.999	6.00	0.07
Run 29	0.31	0.26	1.04	0.996	2.00	0.16
Run 30	0.33	0.25	0.77	0.996	2.00	0.17
Run 31	1.56	0.22	1.03	0.995	6.00	0.26
Run 32	0.54	0.20	1.25	0.993	6.00	0.09
Run 33	0.59	0.22	2.09	0.996	6.00	0.10
Run 34	0.14	0.22	1.72	0.996	6.00	0.02
Run 35	0.60	0.23	2.40	0.996	10.00	0.06
Run36	0.76	0.22	1.43	0.996	6.00	0.13
Run37	0.77	0.22	1.43	0.996	10.00	0.08
Run 38	1.53	0.20	1.95	0.997	2.00	0.76
Run 39	0.17	0.21	0.76	0.995	10.00	0.02
Run 40	1.63	0.21	1.13	0.996	6.00	0.27
Run 41	0.53	0.19	0.71	0.996	6.00	0.09
Run 42	0.75	0.22	1.67	0.996	6.00	0.12
Run 43	0.24	0.21	1.32	0.996	2.00	0.12
Run 44	0.23	0.20	1.70	0.997	6.00	0.04
Run 45	1.47	0.22	1.57	0.996	6.00	0.24
Run 46	2.58	0.22	1.52	0.995	6.00	0.43
Run 47	1.54	0.19	0.71	0.996	10.00	0.15
Run 48	0.76	0.22	1.43	0.996	10.00	0.08

Run 49	0.36	0.25	3.37	0.996	6.00	0.06
Run 50	0.73	0.21	0.83	0.993	6.00	0.12
Run 51	0.32	0.19	0.71	0.996	2.00	0.16
Run 52	0.65	0.29	2.04	0.997	6.00	0.11
Run 53	0.82	0.21	1.56	0.997	2.00	0.41
Run 54	0.40	0.19	0.71	0.996	6.00	0.07
Run 55	0.44	0.20	0.88	0.995	10.00	0.04
Run 56	0.82	0.21	1.23	0.997	2.00	0.41
Run 57	0.76	0.22	1.43	0.996	6.00	0.13
Run 58	0.50	0.19	0.71	0.996	10.00	0.05
Sewage Sludge Run	4.06	0.28	0.71	0.095	24.00	0.17

the shortest lag phase was 0.31 hours (Run 1, 4). The highest hydrogen production efficiency per gram of substrate was 0.76 ml/g, achieved in Run 38, and the lowest was 0.02 ml/g (Runs 34 and 39). The highest maximum rate of hydrogen production was 0.29 ml/h, achieved in several runs (Run 1, 4, 27, 52), and the lowest was 0.19 ml/h, also observed in several runs. The study conducted on the dark fermentation of poplar leaves by Cui et al. found that the lag phase time was around 8.76 – 11.9 hours using acid pretreatment and mixed bacterial culture enriched from cereal [112]. Compared to their study, mixed commercial culture decreased the lag phase time to a significant extent. On the other hand, their result showed the maximum cumulative hydrogen yield of 44.92 mL/g-dry poplar leaves when the substrate was pretreated with 2% Viscozyme L. However, our study found the highest amount of hydrogen production as 0.76 g-dry poplar leaves. Although this result indicates that treatment of substate with Viscozyme L, a valuable tool to break hemicellulose, cellulose, and cell wall components to liberate proteins from cells, favors hydrogen production, it is more costly than acid treatment. This added cost could make the process less economically feasible for large-scale hydrogen production, especially compared to other pre-treatment methods such as acid treatment. Acid treatments can be less expensive and easier to handle on large scales. However, they might not be as efficient as enzymatic treatments like Viscozyme L in breaking down complex substrates. On the other hand, their acid pretreatment result is 33.45 g-dry poplar leaves. Compared with our result, the acid pretreatment resulted in 45 times higher hydrogen production than ours. Similarly, their R_m varies from 1.25 ml/h to 2.17, while ours varies

from 0.02 and 0.46 ml/g. From this point of view, our result is less than the compared study in many parameters except the shortness of the lag phase. After completing 58 experimental trials, the inoculum was changed from commercial culture to activated sewage sludge. It produced 4.06 ml hydrogen, and the lag phase was 0.71 hours. In this trial, the lag phase has initiated hydrogen production rapidly compared to commercial culture cultures. However, the hydrogen production per gram substrate yielded 0.17 ml/g. Although commercial bacteria culture produced more hydrogen than commercial one, the reactor was fed with sewage sludge since it also includes exoelectrogenic bacteria. In this way, electricity production was generated.

5.2 Optimization Result

The data being examined is a result generated by the well-known statistical software Design-Expert, which is highly regarded for its specialized methodology in experimental design. The software enables precise and systematic planning and implementation of experimental tests. Thus, it sheds light on the complex relationship between input variables and the resulting outcomes. In the present examination, the variables involve acid concentration, biomass amount, pH, temperature, mixing ratio, and microorganism amount. This software enables the analysis of the multi-dimensional interaction among these parameters, which contributes to the comprehension of the dynamics of the process. The comprehensive methodology employed in experimental design helps systematically assess the individual and interactive impacts of the factors on the outcome. Furthermore, it produces an in-depth statistical examination that helps formulate reliable and evidence-based conclusions. In the Design-Expert part of this study, a quadratic model was employed to clarify the underlying consequences of the data. The success of the quadratic model was assessed and compared with alternative models, including the cubic, linear, and mean models. Even though the cubic model produced a higher R^2 value of 0.9, indicating more consistency with the data, the quadratic model was chosen for analysis despite its lower R^2 value of 0.74. The primary factor affecting this decision was the quadratic model's ability to offer a more profound and simpler comprehension of the data for the dark fermentation process. This approach facilitated an extensive analysis of the interpretation between the experimental variables and results. This comprehensive assessment highlights the careful consideration of model suitability, considering not only conventional statistical

indicators like R^2 but also the interpretability and applicability within the framework. An Analysis of Variance (ANOVA) was performed to understand better the factors associated with the response variable. The statistical technique employed in this study is utilized to examine differences among multiple sources by dividing the overall variability of a dataset into two distinct components, which are random and systematic factors. Table 5.2 presents the statistical results obtained from the ANOVA. The (ANOVA) revealed that factors B (biomass amount) and E (mixing ratio) displayed a significant influence on the response variable, which is hydrogen production. The obtained p-values were found to be less than 0.05 for these variables. A p-value below 0.05 indicates statistical significance, suggesting that the observed results are highly unlikely to have emerged randomly. The values greater than 0.1000 indicate that the model terms are not significant. If there are many insignificant model terms (not counting those required to support hierarchy), model reduction may improve the model. In this context, it can be observed that the variables C (pH), D (temperature), and F (microorganism amount) did not exhibit a significant impact on the response variable, which is shown by their p-values above the limit of 0.05. The higher p-values indicate that the impact of these factors on the response variable may be related to random variations. On the other hand, A, B, E, and EF are significant model terms in this analysis. Additionally, the p-values associated with all squared terms exceed the limit of 0.05. This implies that the quadratic effects of each parameter are not statistically significant. In particular, the impact of B (biomass amount) is particularly significant and plays a role in hydrogen production, as indicated by its remarkably low p-value. Another statistical measure employed in the ANOVA is the model f-value, which determines the equality of means across multiple groups; it was calculated as 3.20. This value suggests that the model under consideration exhibits statistical significance. The probability of observing an f-value of this magnitude solely due to random variation is only 0.14%. On the other hand, the insignificance of the lack of fit f-value, which is 0.97, provides additional evidence that the selected model adequately fits the data. Moreover, there is a 55.24% probability that this degree's lack of fit f-value could arise entirely from random variations. Another parameter, adequate precision, is a measure of the signal-to-noise ratio.

Table 5.2 Design Expert ANOVA Results

Source	Σ of Squares	df	Mean Square	f-value	p-value
Block	0.0172	1	0.0172	0	1
Model	0.8065	27	0.0299	3.2	0.0014
A-Acid concentration	0.0662	1	0.0662	7.08	0.0126
B-Biomass amount	0.3626	1	0.3626	38.82	< 0.0001
C-pH	0.0004	1	0.0004	0.04	0.8342
D-Temperature	0.0018	1	0.0018	0.19	0.6607
E-Mixing ratio	0.0451	1	0.0451	4.83	0.0362
F-Microorganism amount	0.0001	1	0.0001	0.01	0.9166
AB	0.005	1	0.005	0.53	0.4702
AC	0.019	1	0.019	2.04	0.1643
AD	0.0233	1	0.0233	2.49	0.1254
AE	0.0021	1	0.0021	0.22	0.6379
AF	0.0015	1	0.0015	0.16	0.6903
BC	0.0045	1	0.0045	0.48	0.4925
BD	0.0004	1	0.0004	0.05	0.8278
BE	0.0248	1	0.0248	2.66	0.114
BF	0.02	1	0.02	2.14	0.1541
CD	0.0098	1	0.0098	1.05	0.3141
CE	0.0091	1	0.0091	0.97	0.3314
CF	0.0039	1	0.0039	0.42	0.5229
DE	0.0055	1	0.0055	0.59	0.4486
DF	0.0072	1	0.0072	0.77	0.3872
EF	0.1104	1	0.1104	11.83	0.0018
A^2	0.0036	1	0.0036	0.38	0.5416
B^2	0.0156	1	0.0156	1.67	0.2059
C^2	0.0072	1	0.0072	0.77	0.3869
D^2	0.0143	1	0.0143	1.53	0.2256
E^2	0.0109	1	0.0109	1.16	0.2899
F^2	0.0002	1	0.0002	0.02	0.8859
Residual	0.2709	29	0.0093		
Lack of Fit	0.1947	21	0.0093	0.9746	0.5524
Pure Error	0.0761	8	0.0095		
Cor Total	1.09	57			
Std. Dev.	0.0966		**R^2**	0.7486	
Mean	0.1617		**Adjusted R^2**	0.5145	
C.V. %	59.76		**Adeq Precision**	9.7677	

A ratio above 4 is considered beneficial as it demonstrates a strong signal about the noise within the model, where the signal represents the impact of the variables, and the noise represents random errors or unexplained variances [113]. In our analysis, the adequate precision measure was determined as 9.768. This value significantly exceeds the recommended threshold of 4. When the ratio exceeds the value of 4, the model exhibits a signal of considerable strength, allowing it to move through the design space with high confidence. Briefly, the quadratic model has provided a comprehensive and valuable understanding of the various factors that influence the process of biohydrogen production in dark fermentation. The quantity of biomass and the mixing ratio have been identified as crucial factors in the process. Despite the statistical significance of these findings, it is compulsory to note that additional research and experimentation are required to support these results for a more detailed study. This methodology offers a viable path for future investigations focused on improving the efficiency of the dark fermentation process.

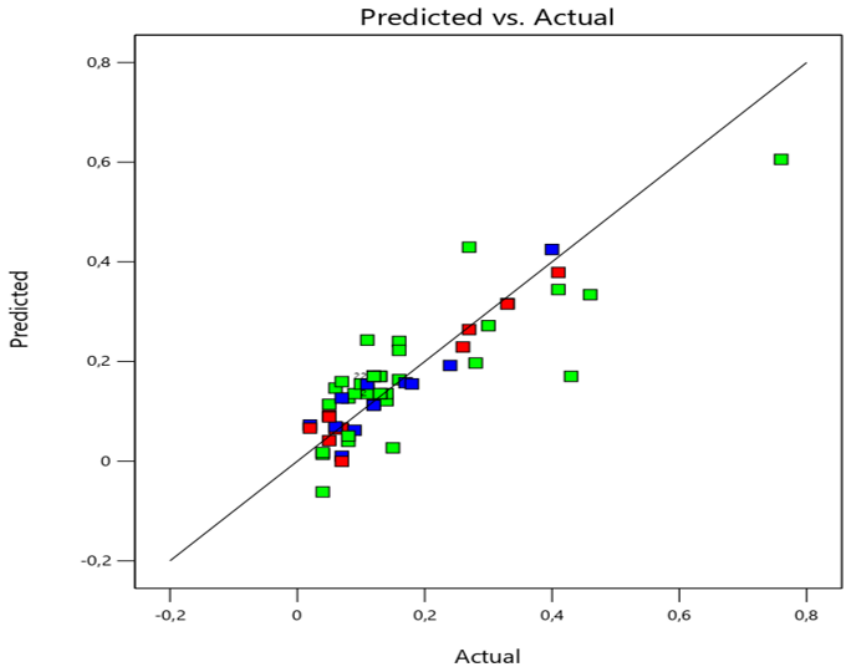


Figure 5.11 Predicted values in the model vs. actual values

Upon analyzing Figure 5.11, it can be seen that the model's predictions regarding hydrogen production exhibit a remarkable consistency with the observed values. Although there are a few outliers, the technique demonstrates a notable ability to handle and accurately predict experimental results. This confirms the model's robustness and reliability. The Design-

Expert software indicates the function of hydrogen production as in Equation 5.1, which is represented as a combination of variables and their interactions. The variables can be seen in Table 5.2.

$$\text{Cumulative hydrogen production}\left(\frac{\text{ml}}{\text{g}}\ \text{per substrate}\right) =$$

$$0.1530 + 0.0525\text{A} - 0.1229\text{B} + 0.0042\text{C} - 0.0087\text{D} + 0.0433\text{E} + 0.0021\text{F}$$
$$- 0.0250\text{AB} + 0.0487\text{AC} - 0.0381\text{AD} + 0.0162\text{AE} + 0.0137\text{AF} - 0.0237\text{BC}$$
$$- 0.0075\text{BD} - 0.0394\text{BE} + 0.0500\text{BF} + 0.0350\text{CD} - 0.0337\text{CE} - 0.0156\text{CF}$$
$$- 0.0263\text{DE} - 0.0300\text{DF} - 0.1175\text{EF} + 0.0179\text{A}^2 + 0.0375\text{B}^2 - 0.0254\text{C}^2$$
$$- 0.0359\text{D}^2 + 0.0312\text{E}^2 - 0.0042\text{F}^2 \qquad (5.1)$$

The given equation represents a polynomial regression model developed to predict hydrogen production based on multiple factors (A, B, C, D, E, F). The equation involves linear terms, interaction terms such as AB, AC, etc., and quadratic terms such as A^2, B^2, etc.). The coefficients specified in the linear terms (A to F) demonstrate the expected modification in hydrogen production when the corresponding factor increases by one unit while keeping all other factors constant. For example, the coefficient of variable A exhibits a positive value of 0.0525. This means that a unit increase in the variable value will result in a corresponding increase of 0.0525 units in hydrogen production if all other variables remain constant. The coefficients associated with the interaction terms represent the supplementary impact on hydrogen production when both factors change simultaneously. For example, the interaction term AB exhibits a negative value of -0.0250, which indicates that the combined influence of variables A and B is associated with a decrease in hydrogen production. The coefficients associated with the quadratic terms in this function represent the magnitude of the second derivative, which implies the rate at which the rate of change of hydrogen production is changing. Likewise, the coefficient of the A^2 variable is positive (0.0179), which shows an open relationship between A and hydrogen production. This implies the existence of an optimal value of A that maximizes hydrogen production.

The parameters for the Figures between 5.12 and 5.22 were chosen based on the p-values from Table 5.2. The significance of the variations was assessed, revealing that parameters A, B, E, and EF substantially influenced hydrogen production. Consequently, these parameters and their interactions were selected for a 3-parameters contour plot. This selection ensures that the significant factors influencing hydrogen production are appropriately represented and considered in the figures. Figure 5.12 demonstrates the effect of the acid concentration and biomass amount effect on H_2 Production. Theoretically, an increase in substrate amount should initially result in an elevation of hydrogen production during dark fermentation. Nevertheless, it is crucial to highlight that substrate inhibition may occur when substrate concentrations reach high amounts. Furthermore, when substrate concentrations are increased, the fermentation process may exhibit a favor for the synthesis of other metabolites, such as volatile fatty acids.

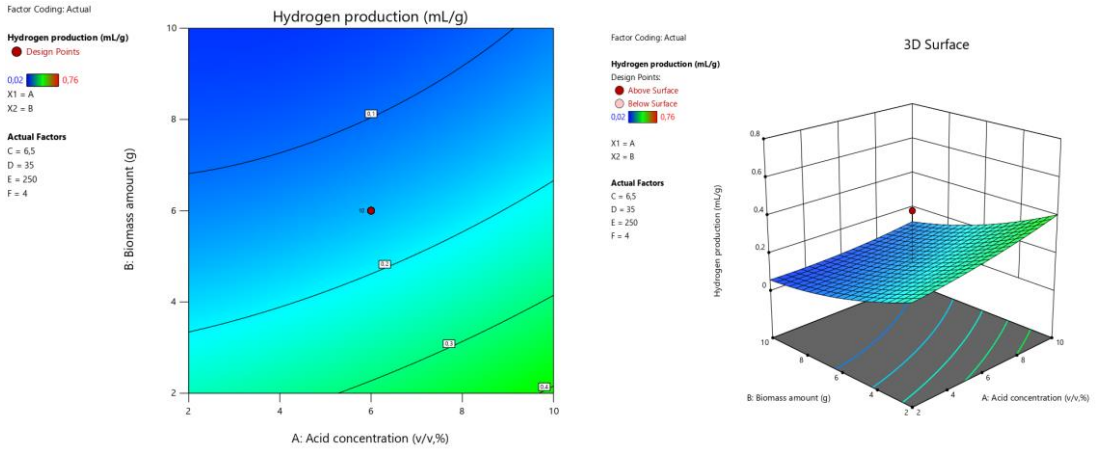


Figure 5.12 Acid concentration and biomass amount effect on H_2 production

Based on the observations illustrated in Figure 5.12, there is a relationship between the amount of biomass and acid concentration for hydrogen production. Increasing the acid concentration increases hydrogen production if the amount of biomass is less than 6 grams. The graph also demonstrates a positive correlation between biomass amount and the required acid concentration for pretreatment. For example, hydrogen production demonstrates a notable deficiency when utilizing a biomass amount of 10 grams and an acid concentration of approximately 2 2 v/v %. Similarly, even with a 10 v/v % acid concentration, higher biomass amounts do not significantly increase hydrogen production.

However, it is noteworthy that when the biomass quantities are less than 6 grams, the hydrogen production persists in its upward trend as the acid concentrations are higher.

Figure 5.13 shows the correlation between the acid concentrations and the pH value of the pretreated substrate. Although acid concentrations are essential parameters affecting hydrogen production, pH is a significant parameter in the graph. It shows that the inoculum resists pH changes and lives in various pH levels. However, if the acid concentration of HNO3 pretreatment is higher than 7.5 v/v %, the pH level of the solution starts to be crucial. For example, as seen in Figure 5.9, the highest amount of hydrogen was observed when pH was higher than 6 and the acid concentration was between 7.5 and 10 % v/v.

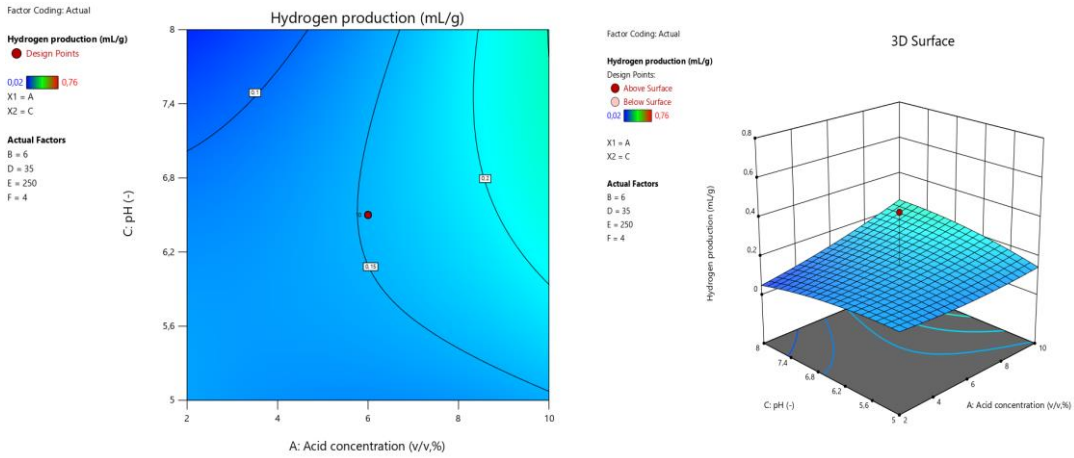


Figure 5.13 Acid concentration and pH effect on H_2 production

Figure 5.14 illustrates the correlation between the acid concentration and temperature regarding hydrogen production. The data reveals a significant pattern in which the highest hydrogen yield is achieved under specific conditions. Specifically, a temperature range of 30-37 degrees Celsius and an acid concentration exceeding 8 percent was the most favorable condition for achieving optimal hydrogen production. The data presentation offers valuable insight into the relationship between these two crucial variables in hydrogen production.

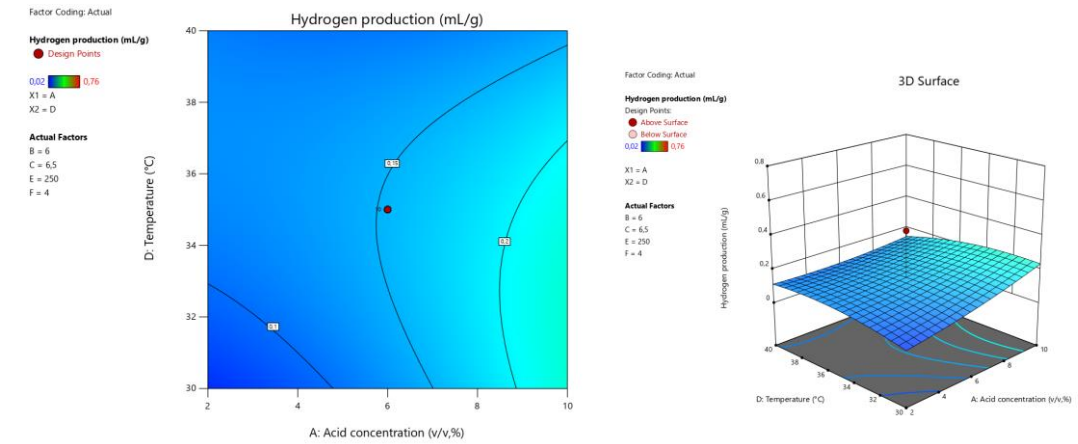


Figure 5.14 Acid concentration and temperature effect on H_2 production

Similarly, Figure 5.15 shows the interaction between two significant variables: acid concentrations and mixing ratio. These variables were prioritized due to their higher importance compared to less significant ones. The concave curves in the graph represent a higher range of hydrogen production, which varies between 0.15 ml/g and 0.3 ml/g. Hydrogen production demonstrates an upward trend as both parameters increase, ranging from 0.15 ml/g to 0.3 ml/g.

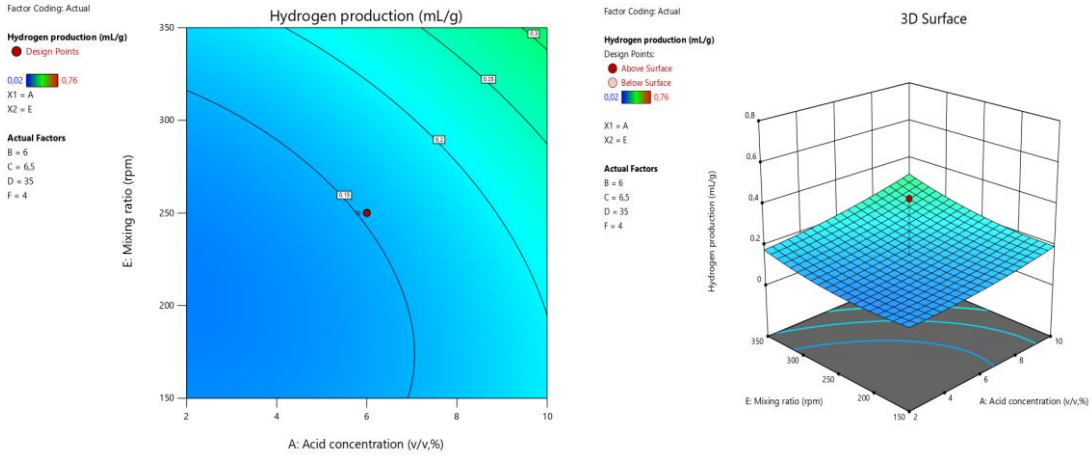


Figure 5.15 Acid concentration and mixing ratio effect on H_2 production

Another graph by Design-Expert shows the correlation between acid concentration and inoculum amount in Figure 5.15. Compared to other figures, this interaction shows stepper lines through the graph. It means that the dependent variable, hydrogen production, is more sensitive to changes in the variable represented by the acid concentration. The graph shows that hydrogen production increases by 0.1 ml/g per a 1 percent increase in the acid

concentration. For example, if the acid concentration of pretreatment is around 5 percent, the hydrogen production through the line is 0.15 m/g. However, when there is a 1 g increase in the microorganism amount, the change is almost negligible, up to 7.4 percent acid concentration.

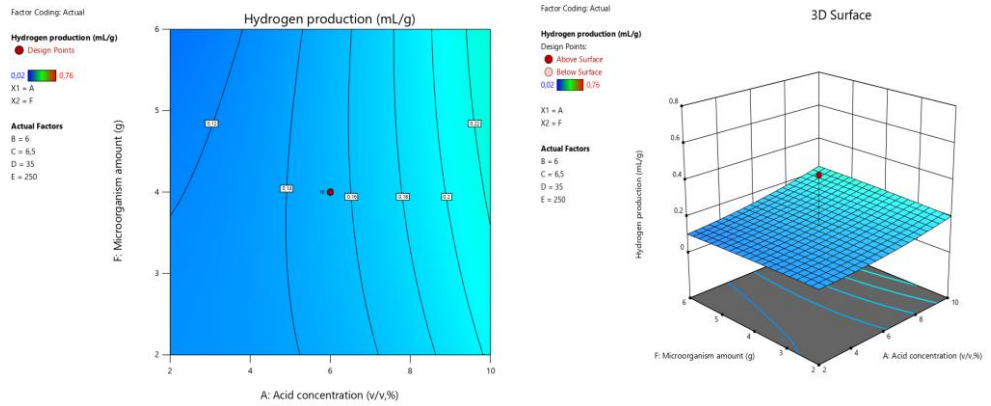


Figure 5.16 Acid concentration and microorganisms on H_2 production

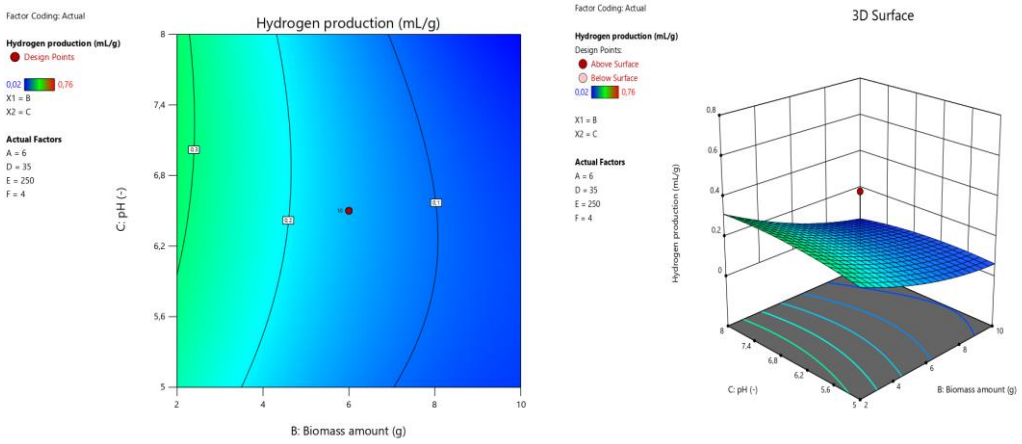


Figure 5.17 Biomass amount and pH effect on H_2 production

Figure 5.17 also shows the interaction of the most critical parameters, biomass amount, and pH. It is essential to highlight that biomass amount decides the hydrogen production significantly compared to other parameters. As the pH increases while keeping the biomass amount constant, the hydrogen production is almost the same for the y-axis.

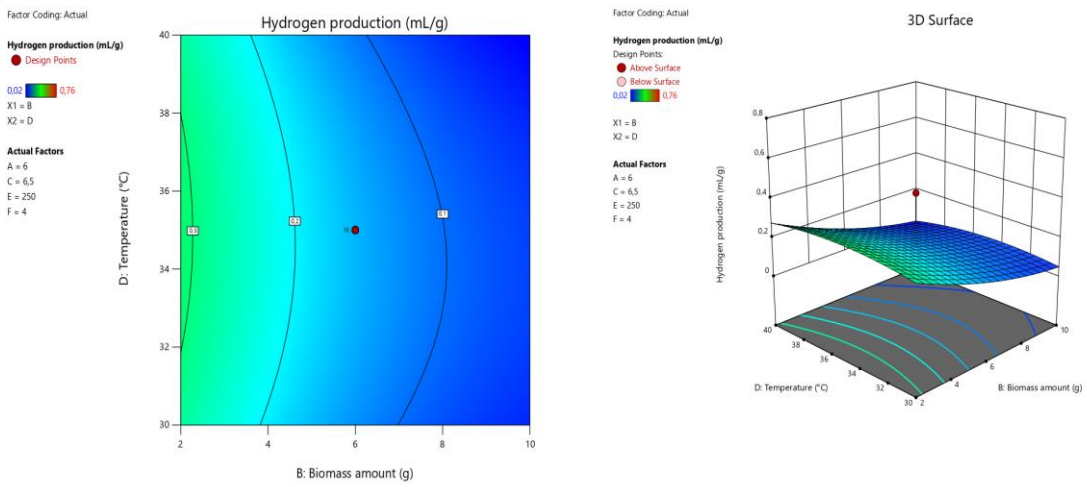


Figure 5.18 Biomass amount and ph effect on H_2 production

The relationship between temperature and biomass quantity in hydrogen production is illustrated in Figure 5.18. Upon examining the graph, a notable resemblance to Figure 5.15 becomes evident. This similarity is primarily attributed to the fact that, like the previous analysis, the quantity of biomass also emerges as a prominent factor in this case. Notably, the maximum production of hydrogen is attained at varying temperatures when the quantity of biomass remains below 5 grams. This highlights the complex interplay between these variables in determining the outcomes of hydrogen production.

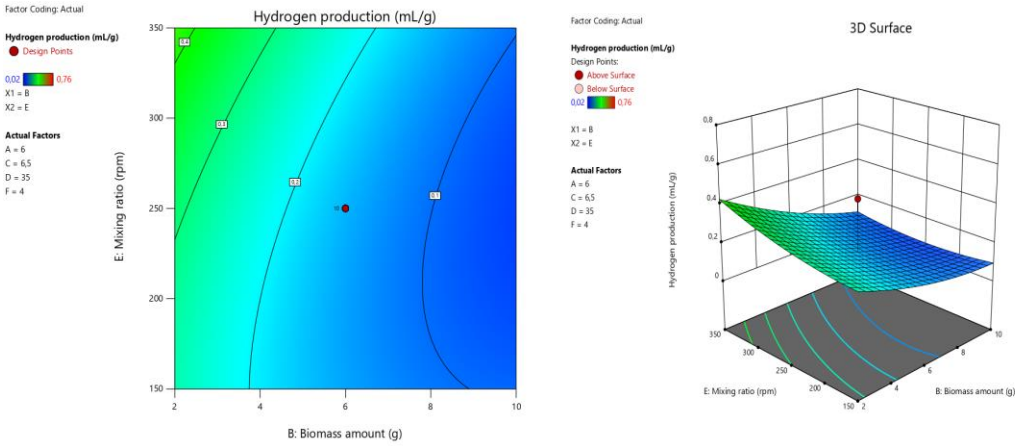


Figure 5.19 Biomass amount and mixing ratio effect on H_2 production

The correlation between the biomass amount and the mixing ratio is depicted in Figure 5.19. When biomass is less than 6 grams, the mixing ratio significantly affects hydrogen production. In this set, it can be observed that an increase in the mixing ratio leads to a

corresponding upward trend in hydrogen production. The correlation between these parameters suggests a more robust association as both variables are responsible for significant weight within the model. Nevertheless, when biomass quantities surpass 6 grams, the mixing ratio's influence on hydrogen production becomes almost insignificant. Significantly, hydrogen production peaks when the biomass quantity is 2 grams, and the mixing ratio is adjusted to 350 rpm.

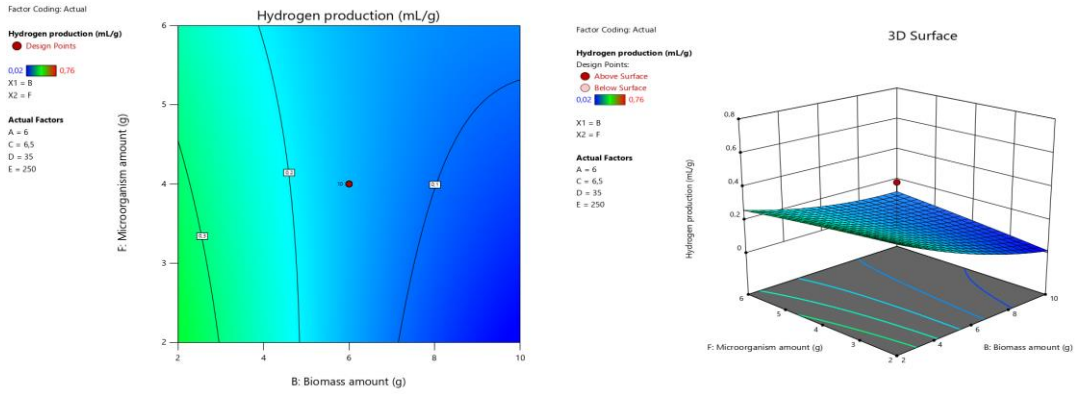


Figure 5.20 Biomass amount and microorganism effect on H_2 production

Figure 5.20, which was like various other graphs, including biomass quantity, highlights the impact of this factor on hydrogen generation. It has been observed that there is a positive correlation between the amount of biomass and the production of hydrogen up to a threshold of 4 grams. The impact of the inoculum amount on hydrogen production seems to exhibit a comparable trend. Upon initial examination, an increased quantity of inoculum would likely result in a corresponding increase in hydrogen production. However, carefully analyzing the graph demonstrates an inverse correlation between these two variables. Several potential factors could lead to this condition, which include nutrient competition, waste accumulation, or shifts in microbial population dynamics.

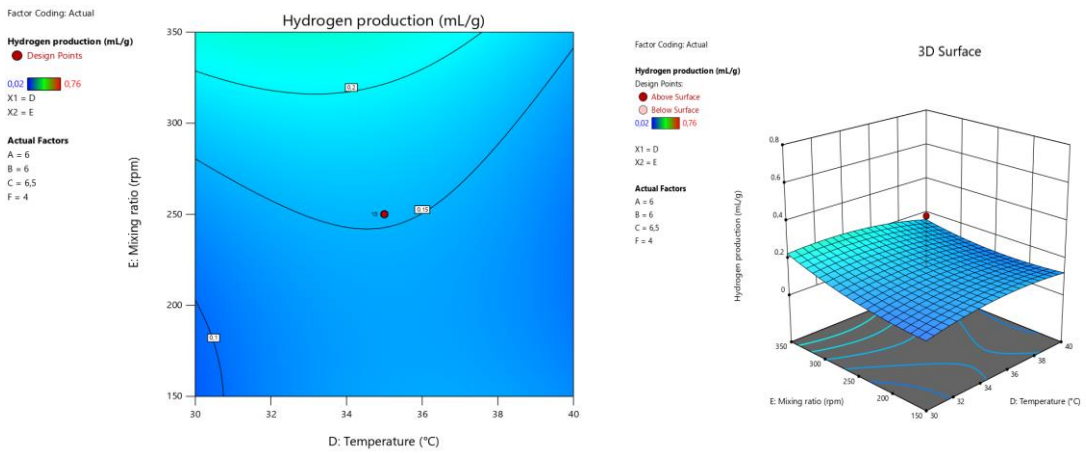


Figure 5.21 Temperature and mixing ratio effect on H_2 production

Figure 5.20 presents valuable insights regarding the overall impact of mixing ratio and temperature on hydrogen production. This observation illustrates that the mixing ratio is crucial for predicting hydrogen production, regardless of temperature variations. It emphasizes the significance of employing the mixing ratio in the model. Specifically, in the 30-37 ºC temperature range, hydrogen production peaks when the mixing ratio exceeds 300 rpm. The hydrogen production amount observed under these conditions is approximately 0.23 ml/g, implying that a higher mixing ratio and the specified temperature range have a positive impact.

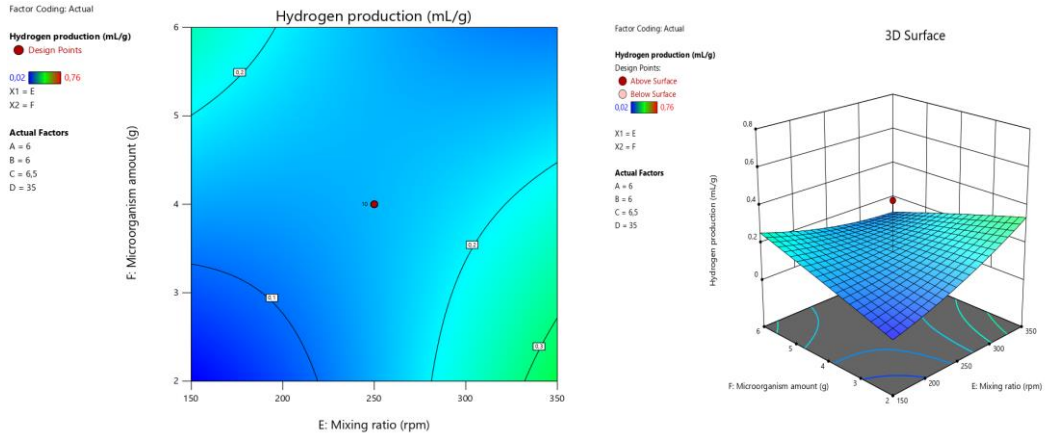


Figure 5.2 Mixing ratio and microorganism effect on H_2 production

The last graph, Figure 5.22, illustrates the correlation between two important variables: the mixing ratio and the microorganism concentration. The relationship between the variables in the contour graph displays two distinct peaks. These peaks' existence signifies both

variables' influence on hydrogen generation. A distinct peak is observed within the range of mixing ratios exceeding 280 rpm up to 350 rpm. The second peak is observed when the mixing ratio falls below 190 rpm, with an inoculum amount surpassing 5 grams. Significantly, the most significant amount of production, reaching 0.34 milliliters per gram of substrate, is accomplished on the right-hand side of the graph.

Utilizing design expert optimization software for dark fermentation is crucial in improving hydrogen generation efficiency. This tool's primary objective is to determine and measure the correlations between different operational parameters and the resulting outcome. By conducting design optimization, it is possible to determine the impact of various factors, such as acid concentration, biomass quantity, pH, temperature, mixing ratio, and microorganism concentration, on the output individually and in combination. By defining these interactions, the software assists in identifying the most advantageous configurations for each parameter that would end up in the maximum hydrogen production. This approach enables a more significant focus on optimizing processes, bringing about substantial costs and time savings. It eliminates the necessity for trial-and-error techniques. Moreover, the design expert tool's forecasting ability aids in foreseeing effects within specific parameters. This dramatically helps informed decision-making throughout the process of design and operation. The capacity to understand the impact of alterations in one or multiple variables on the overall fermentation process is also beneficial for diagnosing and implementing process control and optimization strategies. In general, using design expert optimization in dark fermentation can result in increased hydrogen yields, enhanced process efficiency, financial savings, and a more comprehensive comprehension of the dynamics of the

fermentation process. Additionally, it offers a systematic approach for conducting future investigations and advancements in biohydrogen production.

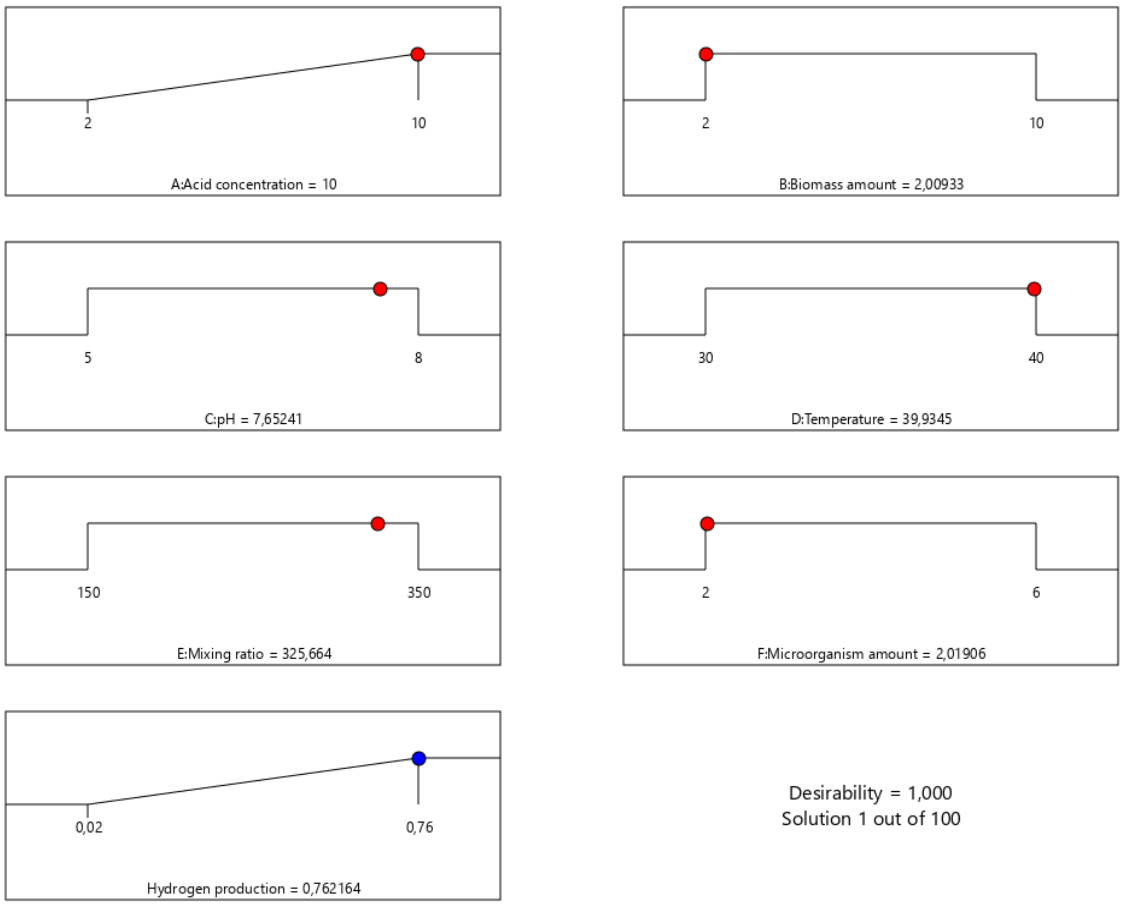


Figure 5. 23 Optimization of operational parameters for maximum hydrogen production

The primary objective of this study is to optimize the dark fermentation process. The main aim is to achieve a desired target through process optimization. To achieve this objective, the Box-Behnken Design (BBD) methodology was utilized to optimize the operational parameters, such as biomass amount, pH, mixing ratio, and microorganism amount. These parameters were varied within predetermined ranges, except the acid concentration, which remained fixed at its maximum value. This optimization procedure aims to maximize the cumulative hydrogen production, thereby improving the overall efficiency of the dark fermentation process. It is a specific design within the response surface methodology (RSM) framework employed in statistical experimental design. Its purpose is to develop a model, examine the response of interest, and identify an optimal response within a defined region of interest. The targeted operational parameters and response values are presented

in Figure 23 based on the BBD model. The recommended values of operational parameters were as follows: acid concentration: 10%, biomass amount: 2,009 g, initial pH: 7.65, temperature: 39.9 °C, and mixing ratio: 325.66 rpm for maximum hydrogen production of 0.76 mL/g. The combined effects of optimized operational parameters were also reported for the highest hydrogen production. The effect of acid concentration combined with other operational parameters on hydrogen production is presented in Figure 5.24.

The results revealed that the hydrogen production performance of the dark fermentation process decreased with the increasing biomass amount at constant acid concentrations (Figure 5.24a). For instance, the hydrogen production decreased from 0.7 mL/g to 0.2 mL/g with increasing biomass concentration from 2 to 10 g at the acid concentration of 10%. Similar results were observed for the other acid concentrations. The inadequacy of the fermentable sugars could explain the decrease in hydrogen production at high biomass amounts. Namely, the low hydrogen production obtained at high biomass concentrations shows that the acid utilized in the pre-treatment step cannot adequately decompose the biomass into valuable sugars. Therefore, increasing the biomass by keeping the acid concentration constant will not increase hydrogen production alone. On the contrary, as seen in the results, the remaining biomass, without being degraded into sugars, causes agglomeration in the environment, creating an unsuitable environment for microorganisms and causing a decrease in hydrogen production. Similarly, Figure 5.24b shows that hydrogen production increases as the pH of the solution's value increases while keeping the acid concentrations constant. For example, when acid concentration is 4%, going upward through the plot has increased hydrogen production from 0.55 ml/g to 0.74 ml/g. However, the highest response in the graph occurred when acid concentration was higher than 7%. The hydrogen production rate was placed in the 0.3-0.76 ml/g range.

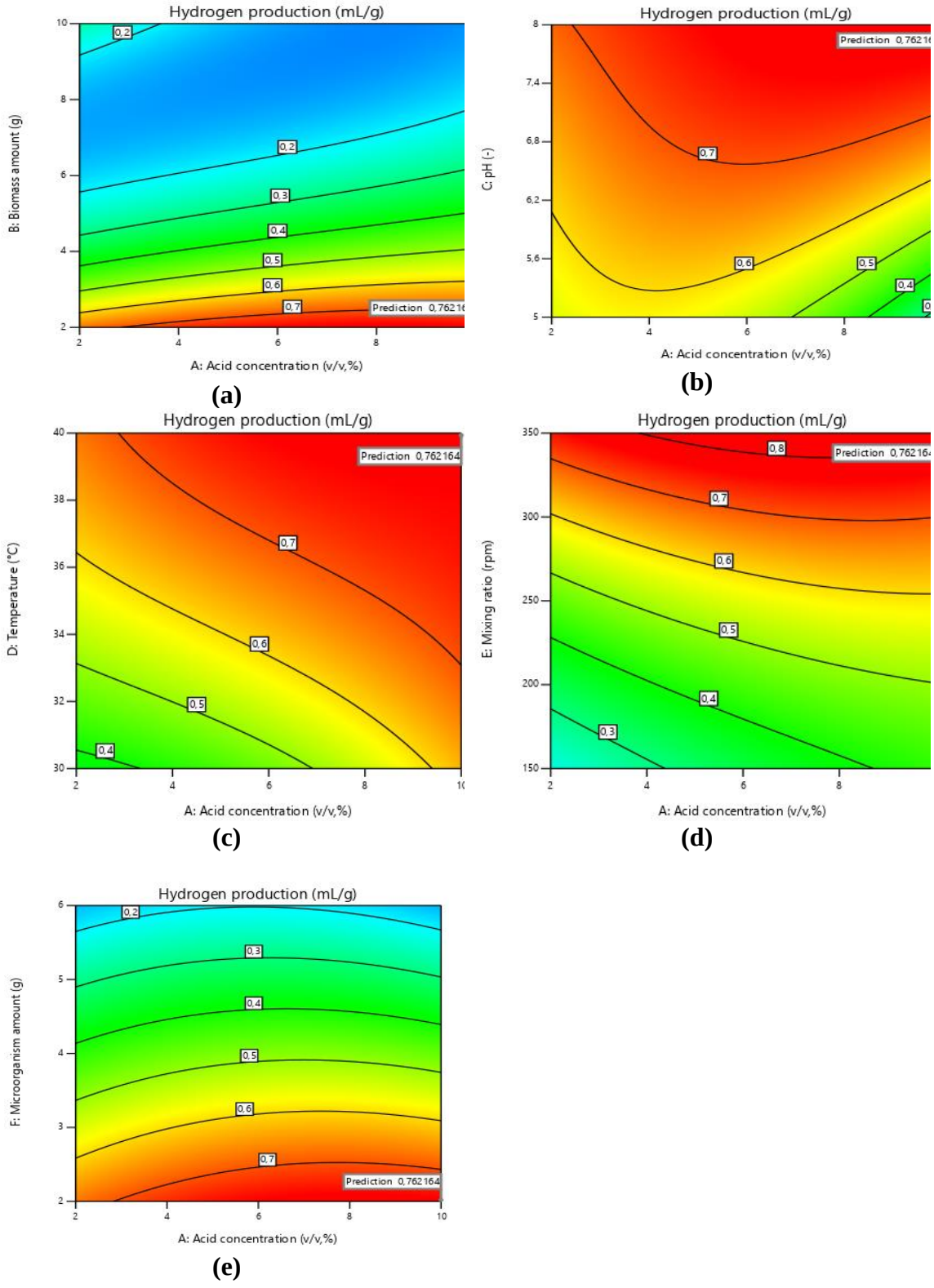


Figure 5. 24 Effect of acid concentration combined with (a) biomass amount, (b) initial pH, (c) temperature, (d) mixing ratio, and (e) microorganism amount for maximum hydrogen production.

Figure 5.24c shows the relationship between temperature, acid concentration, and hydrogen generation. As temperature and acidity rise, there is an apparent rise in hydrogen formation. When the temperature rises but the acid level stays constant, there is a noticeable increase in hydrogen generation, and vice versa.

The optimization of acid concentration and mixing ratio is the main topic of Figure 5.24d. A rise in hydrogen production is observed with an increase in the mixing ratio while the acid level stays constant. For instance, with a 4% pretreatment acid concentration, increasing the agitation rate boosts hydrogen production. This effect results from enhanced mass transfer and substrate interaction, which consequently promotes better treatment of biomass with higher concentrations at a particular mixing ratio. As the biomass is better treated with more significant concentrations at a specific mixing ratio, hydrogen generation also rises by releasing more valuable carbohydrates, which involve cellulose, lignin, and hemicellulose.

Figure 5.24e gives a more detailed explanation of how differences in the number of microorganisms affect hydrogen generation at various acid concentrations. When the number of microorganisms is decreased throughout a range of acid values, a substantial rise in hydrogen generation is observed. Notably, a rise in an organism's amount at a fixed acid concentration results in a substantial change in hydrogen production. However, regardless of the acid content, keeping a steady inoculum quantity has little effect on hydrogen generation. Competition for resources, waste buildup, or changes in the microbial population dynamics are among a few possibilities for this occurrence.

An optimization in software like Design-Expert has many possible solutions for desired parameters. The parameters desired to be optimized can be chosen among the parameters affecting the process. A simple cost analysis was conducted on the experimental runs for another possible optimization. The nitric acid cost per liter is $94 [114]. The cost of electricity per kWh (household) is 0.11 per kWh [115]. The cost of biomass was only calculated based on the labor since the poplar leaves are easily accessible in the gardens. The NaOH pellet per kg is $80 [116]. The septic tank bacteria cost $64 per kilogram culture [117]. When the cost of all experiments runs are considered, it is seen that the cost of acid

used in the pretreatment and the cost used in the magnetic heating stirrer are the most expensive part of the system. For example, the cost of run 38 can be seen in Table 5.1.

Table 5.3 Cost of Parameters for Run 38 (The Highest Hydrogen Production Run)

Acid Cost	Biomass Cost	pH Arrangement Cost	Temperature and Mixing Ratio Cost	Inoculum Cost
$ 0.94	$ 0.0003	$ 0.48	$ 1.58	$ 0.18

Since the cost of acid pretreatment and temperature and mixing ratio cost, another optimization can also be executed for further research. That is why one of the possibilities is to keep the cost of acid concentration and mixing ratio minimum and hydrogen production at the maximum value. Figure 5.25 shows the acid concentration and mixing ratio optimization graph. This configuration achieves the highest amount of hydrogen using 6 % acid concentration, 2 g biomass, 8 pH, 40 °C, and at 320 rpm. Design Expert provided a cheaper way of producing the same amount of hydrogen produced in the Run 38 , which is the most hydrogen-produced run.

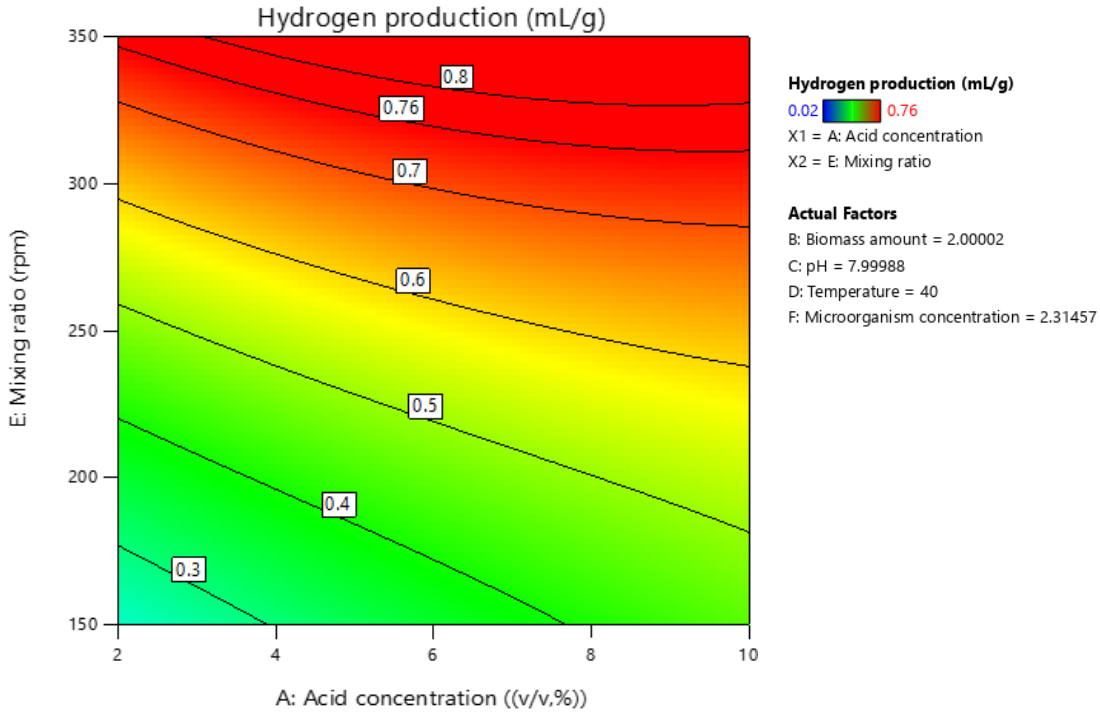


Figure 5. 25 Effect of acid concentration combined with mixing ratio.

For example, the biomass and inoculation amounts can be minimized in another optimization result. Figure 5.26 shows keeping biomass amount and inoculum amount minimum. That is why B and F were minimized, and H_2 production should have been

maximized. The highest hydrogen production amount, which is 0.76 mL/g hydrogen, was also achieved using 10 % acid concentration at 325 rpm and 39.93 °C by using 2-gram biomass and inoculum.

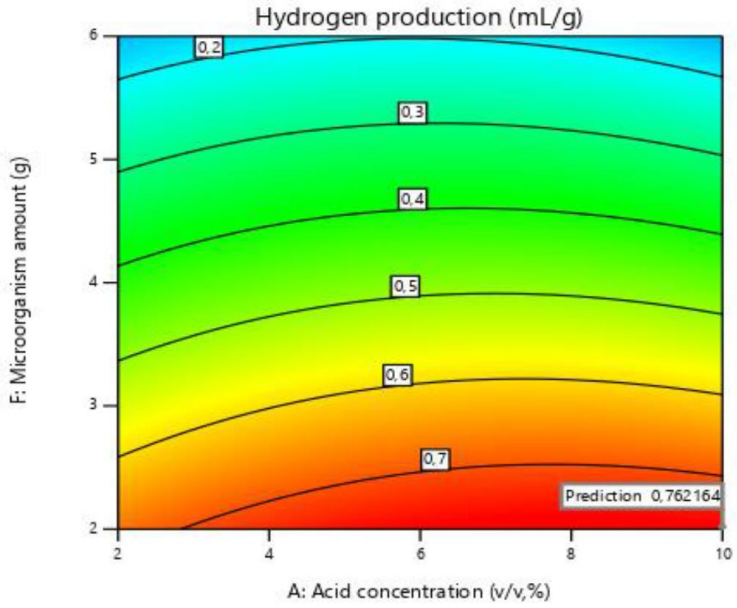


Figure 5. 26 Effect of biomass amount combined with inoculum amount

5.4 Reactor Results

The reactor under consideration operates based on the principles of biochemical reactors. The optimized output derived from the dark fermentation process is implemented into the anode compartment of the reactor. The optimal parameters for the dark fermentation process, as determined by Design-Expert, consist of an acid concentration of 10%, a biomass quantity of 2,009 grams, an initial pH value of 7.65, a temperature of 39.9°C, and a mixing speed of 326 rpm. Similarly, the minimum cost of the process was achieved under the conditions of using 6 % acid concentration, 2 g biomass, 8 pH, 40 °C, at 320 rpm, and using 2.3 g inoculum.

The initial set of experiments, consisting of 58 trials, was carried out using Erlenmeyer flasks with a volume capacity of 250 ml. We needed to scale up the processes to handle the 2650 ml capacity of the anode chamber. The substrate was also pretreated as part of the dark fermentation process using a 10 % acid concentration. The reactor's design specifications include chambers with a length of 15-16-15 cm and a radius of 15 cm.

After setting up the system, we launched the dark fermentation process for 12 hours. Nevertheless, according to the Gompertz-Fitz model in the Figures, more than 12 hours were needed to attain the stationary phase of the dark fermentation process, which also produces hydrogen. Therefore, the hydrogen yield may be lower than documented in the literature.

The energy production assessment in this biochemical system was conducted by employing a voltmeter, which yielded a value of 0.184 volts after 12 hours. The voltage constantly given to the cathode cell is usually within the 0.4 to 0.8 volts range [118]. Various voltages are provided to the cathode cell to increase hydrogen production at 15-minute intervals. As expected, a boost in voltage resulted in a significant rise in hydrogen production. As a result, we started applying a voltage of 4V, despite being aware that this value surpasses the typical values documented in the existing literature. 2 V, 3 V, 4V, and 5V were applied to cathode cell graphite material to identify this value. However, when 5V is applied to the cathode plate, deterioration starts with the material. The excess voltage damaged the material, and hydrogen production couldn't be achieved. That is why 4V was chosen as a threshold voltage and selected for the reactor's external voltage value.

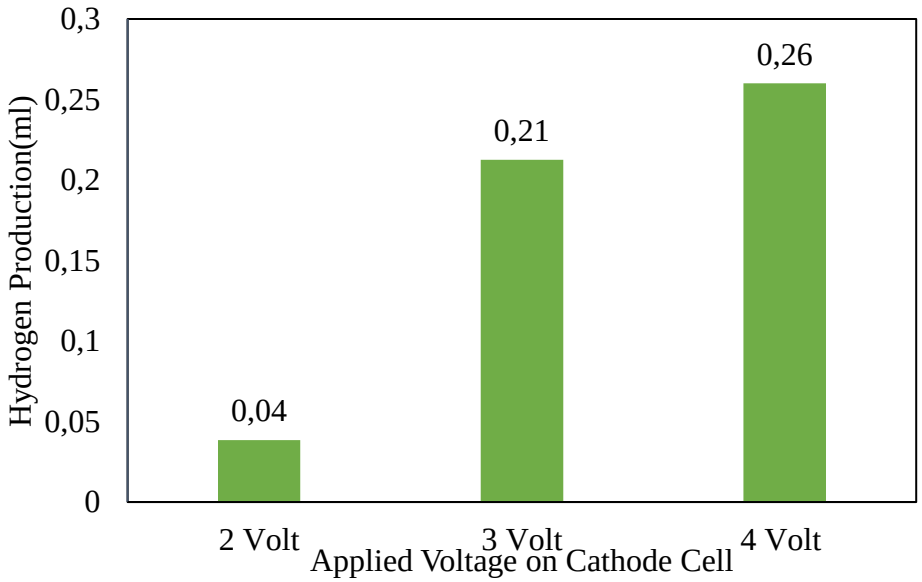


Figure 5. 27 Hydrogen production in cathode cell for different voltage values

Table 5.4 The Reactor Run Results

	Hm (ml)	**R_{max} (ml/h)**	**Lambda (h)**	**R^2**	**Substrate Amount**	**H_2 Production**
Reactor Run Anode	6.6	0.19	0.71	0.995	40	**0.1649 per g substrate (ml/g)**
Reactor Cathode Hydrogen Production						**15.8 ml**

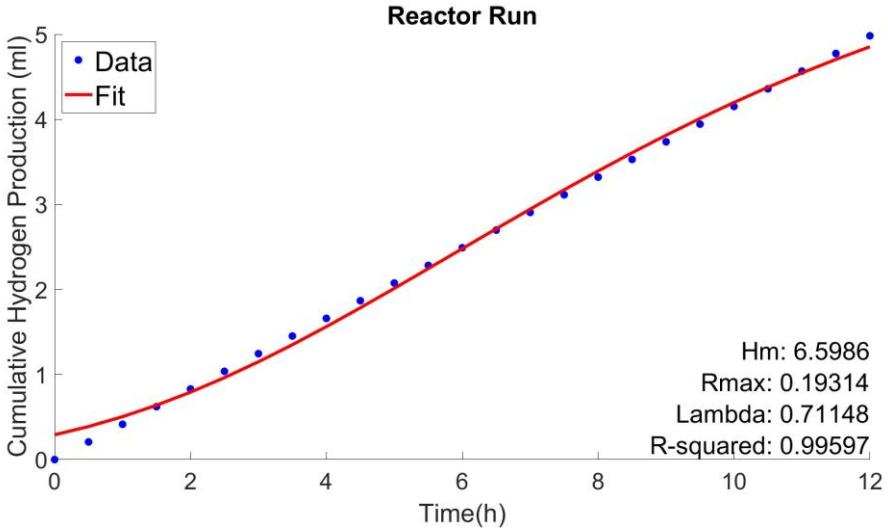


Figure 5.28 Hydrogen production in anode cell

The efficiency of the desalination cell was evaluated by analyzing changes in conductivity. The initial conductivity of the saline water was 7706 µS/cm. After 12 hours, the electrical conductivity decreased to 3778 µS/cm. It implies that the reactor effectively reduced the salinity by approximately 51%. In the same way, notable reductions were observed in the values of chemical oxygen demand within the anode cell. The initial amount of chemical oxygen demand was recorded as 1452.6 mg/l. Subsequently, the COD level decreased to 358.7 mg/l, demonstrating a valuable COD removal efficiency of approximately 75%. Extending the residence time to more than 12 hours makes it likely that even greater removal efficiencies could have been achieved. This claim is supported by a comprehensive review conducted by Kim et al. (2013), which reported salinity removal efficiencies ranging from 44% to 100% through various studies [108]. Therefore, the performance of our reactor is consistent with the findings stated in the existing literature.

Throughout the 12-hour operation, the reactor produced a total of 0.165 milliliters per gram of hydrogen in the anode cell, while the cathode cell yielded 15.8 milliliters. Nevertheless, these numerical values do not align with the anticipated outcomes derived from the optimized parameters. The experimental trials revealed a maximum hydrogen yield of 0.76 ml/g per gram of substrate, significantly surpassing the reactor's anode yield.

Various factors could account for this discrepancy. One potential explanation could be the occurrence of a reactor leakage, which leads to the loss of hydrogen due to the highly volatile nature of this gas. Furthermore, as the reactor operates, cations from the saline water migrate to the anode cell, disturbing the pH balance and stressing the bacterial culture, thus impacting hydrogen production. This movement can disrupt the pH balance and stress the bacterial culture, consequently affecting hydrogen production. This claim is supported by the observed decreased hydrogen production as pH levels decrease, as illustrated in Figure 5.24b. Additionally, the configuration of the reactor itself has the potential to impact its overall performance.

In conclusion, the most successful experiments conducted in our study on hydrogen production from poplar leaves (Run 38) employed a 10% acid concentration, a biomass quantity of 2 grams, an initial pH of 6.5, a temperature of 35°C, a mixing ratio of 350, and an inoculum quantity of 2 grams. The results of the optimization study suggest that the optimal conditions for achieving maximum hydrogen production are as follows: an acid concentration of 10%, a biomass quantity of 2 grams, a pH value of 7.65, a temperature of 40°C, and a mixing speed of 325 rpm. Although hydrogen production through acid treatment of poplar leaves has shown promising results, the obtained yields were lower than the highest values reported in the existing literature, specifically, 44.92 ml/g of dry poplar leaves [112]. Therefore, additional research and advancements are necessary to optimize the performance of the reactor.

CHAPTER 6. CONCLUSION AND RECOMMENDATIONS

Hydrogen is a critical player in the transition toward a more sustainable future. In the present context, biohydrogen emerges as a sustainable, promising, and environmentally friendly alternative. Poplar leaves are attracting considerable interest among the substrate due to their widespread availability and common usage; the poplar tree leaves present unique benefits. By employing the dark fermentation process, it is possible to convert these leaves into a viable and environmentally friendly energy source. This study presents a novel approach to generating renewable hydrogen. Despite the potential benefits of poplar leaves, research must be conducted due to the need for more existing literature. As part of this thesis, a particular biochemical reactor was created and built to handle poplar leaves. Furthermore, comprehensive investigations have been conducted through experimental and numerical approaches to gain a more profound understanding of this process. The primary objective of this study is to identify the most favorable conditions and potential obstacles to improving the biohydrogen production process from poplar leaves. By accomplishing this, we are progressing towards achieving a more sustainable and energy-efficient world.

6.1 Conclusions

In the context of a renewable-energy-driven future, hydrogen production stands out as a pivotal element. In this respect, biohydrogen generation has gained momentum because it is more current, effective, and environmentally friendly than traditional methods of producing hydrogen. In this regard, using poplar leaves to produce hydrogen using a dark fermentation method offers a promising option. This method not only yields bioenergy but also addresses waste management concerns, showcasing a dual benefit that aligns seamlessly with sustainable practices.

- The 58 experimental trials conducted in this study provide significant insights into the hydrogen production process. Based on the root mean square coefficient (R^2) values, the Gompertz function effectively fits the experimental data in all the runs. The maximum hydrogen production rate (H_m) exhibits significant variation, ranging from 0.14 ml/h to 2.73 ml/h.

- The maximum rate of hydrogen production per gram of substrate (R_{max}) showed variability across the runs, varying between the maximum hydrogen production rate per

gram of substrate also exhibits variability across the runs, oscillating between 0.02 ml/g and 0.46 ml/g.

•The most favorable maximum hydrogen production rate is approximately 0.2 ml/h, with an optimum time constant of approximately 1 hour.

• The highest observed hydrogen production in individual runs was 2.73 ml in Run 6. On the other hand, the lowest recorded hydrogen production was 0.14 ml, observed in Run 34. The duration of the lag phase exhibited significant variation, ranging from a short 0.31 hours in Run 1 and 4 to a considerably longer 3.37 hours in Run 49. Hydrogen production efficiency per gram of substrate ranged from 0.76 ml/g (Run 38) at its highest to 0.02 ml/g at its lowest (Runs 34 and 39). Finally, it was observed that the maximum rates of hydrogen production ranged from 0.29 ml/h to 0.19 ml/h. These values were consistently observed in multiple runs.

• A comprehensive and systematic analysis of different parameters affecting the dark fermentation process was carried out using the Design-Expert statistical software. As shown by p-values lower than 0.05, the model terms A, B, E, and EF emerged as significant, although the quadratic impacts of each parameter were negligible. Among the parameters, biomass amount (B) notably impacted hydrogen production, demonstrated by its significantly low p-value.

• The f-value and the lack of fit f-value indicated the statistical significance and adequacy of the chosen model. Furthermore, it was determined that the measure of precision achieved a value of 9.768, exceeding the recommended threshold of 4. This observation suggests a robust signal-to-noise ratio. Upon conducting a comprehensive assessment of the model's predictions compared to the observed values, it was determined that a notable level of consistency was observed. This outcome serves to validate the model's robustness and reliability.

•This study employed the Box-Behnken Design (BBD) methodology, a component of the response surface methodology (RSM) framework, to enhance the efficiency of the dark fermentation process for hydrogen production. The results obtained from the optimization procedure have indicated that the most favorable operational conditions are as follows: an acid concentration of 10%, a biomass quantity of 2.009 grams, an initial pH

of 7.65, a temperature of 39.9 °C, and a mixing ratio of 325.66 rpm. After implementing these parameters, the dark fermentation process was projected to produce a maximum hydrogen production of 0.76 mL/g.

- The efficiency of the desalination cell was assessed by analyzing the changes in conductivity. The initial conductivity of the saline water was 7706 μS/cm. After 12 hours, the conductivity decreased to 3778 μS/cm. This data showed that the reactor had reduced salinity by approximately 51%.

- Significant decreases were also observed in the anode cell's chemical oxygen demand values. The initial chemical oxygen demand (COD) level was recorded as 1452.6 milligrams per liter (mg/l), subsequently decreasing to 358.7 mg/l. This suggests a removal efficiency of approximately 75% for chemical oxygen demand (COD).

6.2 Recommendations

In this study, hydrogen production of poplar leaves via dark fermentation is investigated. The optimization of the experimental results and a novel reactor design is carried out to reach better amount of hydrogen production. Following recommendations is made for the future studies.

- Since the hydrogen production of poplar leaves using dark fermentation is low compared to the literature study, a well-defined mixed culture should be added to sewage sludge to improve hydrogen production. Moreover, the commercial bacteria used in the study can be mixed with sewage sludge to improve the hydrogen yield.

- Different types of pretreatments, except the applied acid pretreatment, can be executed for poplar leaves. The pretreatment of inoculum and substrate can also be utilized. Heat pretreatment, enzyme pretreatment, and their interactions can potentially increase hydrogen production.

- Instead of relying only on hydrogen sensors, measuring hydrogen production using gas chromatography can provide more confident and reliable results.

- The bacteria can be activated before inoculation. Allowing the bacteria culture to become active and adapt to the environment before the start of the experiment may lead to increased hydrogen production.

- Considering the observed discrepancies between expected and actual hydrogen yields, which may be attributable to a leak in the reactor, it is recommended that future research be conducted for a comprehensive investigation into the structural integrity of the reactor. Future reactor designs could be designed to prevent gas leaks better to ensure a higher hydrogen yield.

- The movement of positively charged ions from saltwater to the anode compartment has the potential to disturb the pH equilibrium and stress the bacterial culture, which may consequently affect hydrogen generation. Therefore, future research should prioritize exploring methods to effectively stabilize the reactor's pH levels or consider utilizing microorganisms that exhibit greater resilience towards fluctuations in pH.

Appendix

A.1 Experimental Data Set by Design Expert

Run no.	Acid concentration (v/v)	Biomass Amount (g)	Initial pH (-)	Temperature (°C)	Agitation Speed (rpm)	Inoculum Amount (g)
1	6	6	8	40	250	2
2	6	6	6.5	35	250	4
3	2	10	6.5	30	250	4
4	10	2	6.5	40	250	4
5	6	10	5	35	350	4
6	10	6	6.5	30	350	4
7	6	6	5	40	250	6
8	10	2	6.5	30	250	4
9	6	10	6.5	35	150	6
10	6	6	6.5	35	250	4
11	2	6	5	35	250	2
12	10	6	6.5	40	150	4
13	6	2	5	35	350	4
14	10	6	5	35	250	6
15	2	6	6.5	40	350	4
16	6	6	5	40	250	2
17	2	2	6.5	40	250	4
18	10	6	6.5	30	150	4
19	10	10	6.5	30	250	4
20	6	10	8	35	150	4
21	6	6	8	40	250	6
22	10	6	6.5	40	350	4
23	6	6	6.5	35	250	4
24	6	6	6.5	35	250	4
25	2	6	8	35	250	2
26	2	6	6.5	40	150	4
27	6	2	8	35	150	4
28	2	6	8	35	250	6
29	2	2	6.5	30	250	4
30	6	2	5	35	150	4
31	10	6	8	35	250	2
32	6	6	5	30	250	2
33	2	6	6.5	30	350	4
34	6	6	8	30	250	2
35	6	10	6.5	35	350	2
36	6	6	6.5	35	250	4
37	10	10	6.5	40	250	4
38	6	2	6.5	35	350	2
39	6	10	5	35	150	4

40	10	6	8	35	250	6
41	6	6	6.5	35	250	4
42	6	6	6.5	35	250	4
43	6	2	6.5	35	150	2
44	2	6	6.5	30	150	4
45	6	6	5	30	250	6
46	6	6	6.5	35	250	4
47	2	10	6.5	40	250	4
48	6	10	6.5	35	350	6
49	6	6	8	30	250	6
50	6	6	6.5	35	250	4
51	6	2	6.5	35	350	6
52	2	6	5	35	250	6
53	6	2	6.5	35	150	6
54	10	6	5	35	250	2
55	6	10	6.5	35	150	2
56	6	2	8	35	350	4
57	6	6	6.5	35	250	4
58	6	10	8	35	350	4

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