BIOHYDROGEN PRODUCTION FROM MOLASSES AND ITS APPLICATION FOR BIOMETHANOL SYNTHESIS BY INTEGRATING REVERSE WATER GAS SHIFT (RWGS) AND HYDROGENATION REACTION

By

MISS Kuntima KREKKEITSAKUL

A Thesis Submitted in Partial Fulfillment of the Requirements

for Doctor of Philosophy ENERGY ENGINEERING

Department of MECHANICAL ENGINEERING

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การผลิตไบโอไฮโดรเจนจากกากน้ำตาลและการประยุกต์ใช้สำหรับการสังเคราะห์ไบโอเมทานอลโดยการบูรณาการปฏิกิริยารีเวิร์สวอเตอร์แก๊สชิฟต์ (RWGS) และปฏิกิริยาไฮโดรจีเนชัน

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By	MISS Kuntima KREKKEITSAKUL
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Commercial methanol derived from petroleum base is known as a value chemical in fuel, chemical and solvent, but it has impacted to environment in CO_2 generation. Biomethanol is an option to substitute commercial methanol. Molasses is an abundant source for Biomethanol. The fermentation of molasses was studied by optimizing of molasses concentration of 20, 30, or 40 g/L with the addition of 0, 0.01, or 0.1 g/L of trace elements (TEs) ($NiCl_2$ and $FeSO_4.7H_2O$). the most profitable condition was 31.36 L of biohydrogen (0.97 H_2/CO_2 ratio) derived from a 30 g/L molasses solution with adding 0.01 g/L of TEs. The feed flow rate of 60 g/hr of varying H_2/CO_2 ratios of 50/50%(v/v), 60/40%(v/v), and 70/30%(v/v) were studied in CO_2 conversion via methanol synthesis (MS) and reverse water gas shift (RWGS) reaction. MS was investigated by ranging temperatures of 170, 200, and 230 °C with a $Cu/ZnO/Al_2O_3$ catalyst and 40 barg. The maximum methanol product rate and the maximum H_2/CO_2 were 13.15, 17.81, and 14.15 g/hr at 70/30%(v/v), respectively. The optimum methanol purity was 200 °C and 62.9%(wt). RWGS was investigated by increasing temperatures from 150 to 550 °C at atm pressure with the same catalyst and constant feed. The higher temperature promoted CO generation until almost remain unchanged at 21 to 23% at 500 to 550 °C. There are 2 possible pathways to produce methanol. Pathway 1 Direct methanol synthesis (DMS) (1^{st} MS + 2^{nd} MS) represented to 1^{st} methanol synthesis (MS) and following with 2^{nd} methanol synthesis (MS) and Pathway 2 indirect methanol synthesis (IMS) (1^{st} RWGS + 2^{nd} MS) represented 1^{st} reverse water gas shift (RWGS) reaction and following with 2^{nd} methanol synthesis (MS). The same optimal H_2/CO_2 ratio at 60/40 % (v/v) or 1.49/1 (mole ratio), methanol production rates of 1.04 (0.033) and 1.01 (0.032) g/min (mol/min), methanol purities of 75.91%(wt) and 97.98%(wt), and CO_2 consumptions of 27.32% and 57.25%, respectively. Comparing Operating expenditure for 1 kg methanol by biohydrogen experiment and theory were 4.4349 and 4.0912 USD comparing with biogas 0.3446 USD based on commercial methanol price 0.449 USD/kg.

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Chapter 1

Introduction

1.1 Motivation of Research

Considering global warming and increasing in the environmental issue are the most importance of awareness on using conventional fuel-based petroleum source which is the major factor in releasing Carbon Dioxide to the global environment. The studies on the alternative fuel resource has been become incentive. Whatever the innovation or technique is aware of helping to reduce the CO_2 is all important. Biofuel is an alternative source and is advocated to save CO_2 from fuel using. In the present, common petroleum fuels include natural gas, gasoline and diesel had been substituted by biofuels, such as biogas, ethanol and biodiesel respectively over the past three decades. Biofuels not only supports to neutral carbon but also strongly supports the agricultural sector and bio-based industries (Kazamia & Smith, 2014). Biofuels are renewable and can act as replacement for petroleum-based fuel but the incoming of the advantages of EV vehicle system such as battery storage, electric consumption and reduce many maintenance engine parts are being fascinated for electric vehicle in competing internal combustion vehicle. Considering the vehicles, which causes disruption effects on the transformation of the biofuel era to the carbon neutral era. The domino effect is on ethanol and biodiesel production systems and has a chain effect on the agricultural raw materials that are sources of these biofuel chains. Molasses are obtained from the sugar industry and commonly used as a raw material for fertilizer production, animal feed ingredients, and biogas production for use in electricity generation and it is the major raw material of the bioethanol production. Bioethanol was produced for blending in different ratios in gasoline depending on the policy of each country. Electric vehicles have currently gradually dominated gasoline vehicles, and using molasses for ethanol production has gradually decreased. Moreover, global molasses price sourced from Tridge global market has been continuously decreasing since 2011 (over 1 USD/kg) till now (0.10

USD/kg) in each year. Because the price of molasses has been reducing until to acceptable cost, it becomes a significant bio-renewable source for producing biomethane (contained in biogas) and biohydrogen (Detman et al., 2017). Both cases generate CO_2 in the gas phase: biogas contains methane (CH_4), carbon dioxide (CO_2), and a small amount of hydrogen sulfide (H_2S), while biohydrogen comprises only hydrogen (H_2) and CO_2. Directly using biogas and biohydrogen leads to CO_2 emission to the atmosphere. Biogas case (CH_2 and CO_2), it is only CH_4 used in combustion for heat and electricity. Biohydrogen case, (H_2 and CO_2) the H_2 separation is required to obtain pure hydrogen. The hydrogen supplies to the market contained lots of applications such as petroleum refining, glass purification, semiconductor manufacturing, aerospace applications, fertilizer production, welding. Annealing and heat-treating metals, pharmaceuticals, as a coolant in power plant generators and for hydrogenation of unsaturated fatty acids in vegetable oil. However, H_2 produced from bio sources may be not compete with commercial hydrogen obtained from petroleum source.

Consequently, transforming CO_2 in both biogas and biohydrogen process to valuable product such as biomethanol is interested. Methanol is crucial for chemical and fuel industries and acts as a solvent, thinner, and a reactant in biodiesel and petrochemicals. Thus, methanol is forecasted in the methanol market to grow from 110 million metric tons in 2018 to 220 in 2030. Commercial methanol (black/gray methanol) had been traditionally produced from steam reforming of coal or natural gas. However, the last decade the market is focused on bio/green methanol, which has been widely studied to substitute conventional methanol. Consequently, the methanol in the market is divided into two groups: non-renewable normally obtained from petroleum source and bio/green methanol developed from bio source. The methanol projection by the Council on Energy, Environment and Water and International Energy Agency advised that the cost of green methanol will be gradually decreased due to its competition with gray/black methanol in 2030.

As the concerning of the carbon neutral policy, the green/biomethanol is much required for the substitution commercial methanol market. The first option: transforming biogas to biomethanol, biogas was studied for a raw material in

biomethanol production. Biogas is transformed to biomethanol in two step reactions under catalysts and high temperature in reforming process and high pressure in methanol synthesis. The advantage of using biogas as raw material caused it is well known and almost similar to commercialize technology. But the disadvantage is the process required H_2S separation process to obtain refined biogas and consumed much energy caused high temperature about 600 ℃ to supply for the reforming biogas (CH_4 and CO_2) to syngas (CO, CO_2 and H_2) before converting syngas to methanol. As a result, it is still unacceptable for the commercial cost. The other option is converting biohydrogen to biomethanol, the advantage is the raw gas contains only H_2 and CO_2 which it is not required separation process and the gases can be converted to biomethanol by direct and indirect process. However, the H_2 and CO_2 ratio in biohydrogen gas is normally lower level which is not match to the methanol synthesis reaction which is required H_2/CO_2 around 3 and H_2/CO about 2 for methanol synthesis by CO_2 hydrogenation and CO hydrogenation respectively (Chinchen et al., 1987).

Hence, Enterobacter aerogenes were studied to digest molasses in hydrogen production by: the first aim of this study is to find a way of addition of some metal oxide family such as CaO, MgO and KOH to reduce CO_2 generation by adsorb CO_2 in form of metal carbonate or delay generating carbon dioxide during biohydrogen production and to obtain higher ratio of carbon dioxide/hydrogen. The addition of trace elements containing NiCl and $FeCl_2$ was also studied for promoting biohydrogen production. The optimum ratio of CO_2/H_2 is important to further study of using this relative CO_2/H_2 for transforming both C O_2 and H_2 in biomethanol synthesis. The second aim of this study is to find an optimum ratio of CO_2/H_2 and appropriate technique for transforming biohydrogen to biomethanol. The comparison of direct and indirect methanol synthesis as two pathways for using the gas mixture of CO_2 and H_2 for biomethanol: Pathway 1, Direct methanol synthesis by hydrogenation on CO_2 and Pathway 2, Indirect methanol synthesis by reverse water gas shift of CO_2 and H_2 for syngas then hydrogenation on mixture of syngas containing CO and CO_2. Biomethanol obtained from biohydrogen by passing the appropriate route would be the guidance for future developing along with BCG (Bio

Circular Green) to sustainable development in the substitution of commercial methanol produced from petroleum sources. In Thailand, methanol is 100 percent imported for using in sectors such as biodiesel, solvent and thinner, and raw material in petrochemical and fuels.

1.2 Objectives of Research

1. To minimize CO_2 generation in biohydrogen production by using *Enterobacter Aerogenes* (E.A.) on molasses digestion.

2. To optimize the condition for bio-methanol synthesis from integrated with the RWGS and Hydrogenation.

1.3 Scope of the study

1. This study investigate the optimum condition of biohydrogen production under *Enterobacter Aerogenes* (E.A.) by vary molasses feed stock (10-40 g/L) and 3 substances as: MgO, KOH and CaO in range of 1-5 g/L.

2. The data of biohydrogen production from lab scale will be used for scale up to10 liter of bioreactor.

3. The gas product (CO_2 and H_2) from fermentation process will apply to biomethanol synthesis via RWGS in fixed-bed reactor (1 Lt/D) by using $Cu/ZnO/Al_2O_3$ Catalyst.

1.4 Definitions

1. **Biohydrogen** is one type of other biofuels like bioethanol, biodiesel, and bio-oil etc. Hydrogen can be generated by both chemical and biological method. Therefore, a method from which hydrogen is produced biologically (by using microorganisms) in a bioreactor will be termed as biohydrogen.

2. **Biomethanol** is simply methanol produced from biomass and other nonfossil sources.

3. **Reverse Water Gas Shift (RWGS)** was discovered in the 19th century as a method to produce water from carbon dioxide and hydrogen, with carbon monoxide as a side product

Chapter 2

Theory and Literature review

This chapter will be described the theoretical for biohydrogen production obtained from digesting molasses using Enterobacter Aerogenes and biomethanol synthesis by gathering data from various researches.

2.1 Background

Nowadays, the most topic for fuel and energy is focused on developing from clean and green sources. The first substance mentioned of clean and sustainable fuel is hydrogen (H_2). H_2 is realized as a kind of everlasting fuel. However, commercial H_2 obtained from reforming of coal and petroleum source such as methane. The process releases greenhouse gases, carbon dioxide (CO_2) and others, which impacted to the environmental global. However, Hydrogen application as a fuel in combustion generates energy and only water as solely by-product. The last, in term of clean and green energy, hydrogen can be clean and green because it is derived from water electrolysis and fermentation of bio-resources respectively.

Commercial production of hydrogen gas is obtained through steam methane reforming of natural gas, a process widely used commercially. This represents 95 percent of the total amount of hydrogen gas used in worldwide. The highlight of this technology is that it is a highly efficient process and has a low cost. However, the production of hydrogen gas from steam reforming and gasification process has limitations in that the raw materials used need to be carbon. During the production of hydrogen gas using these methods, Carbon dioxide gas will be generated within the process as shown in Equations 3 and 6, which means it creates greenhouse gases for the environment.

Steam Methane Reforming Process:

Step 1 $CH_4 + H_2O \longrightarrow CO + 3H_2$ (Eq. 1)

Step 2 $CO + H_2O \longrightarrow CO_2 + H_2$ (Eq. 2)

Overall step $CH_4 + 2H_2O \longrightarrow CO_2 + 4H_2$ (Eq. 3)

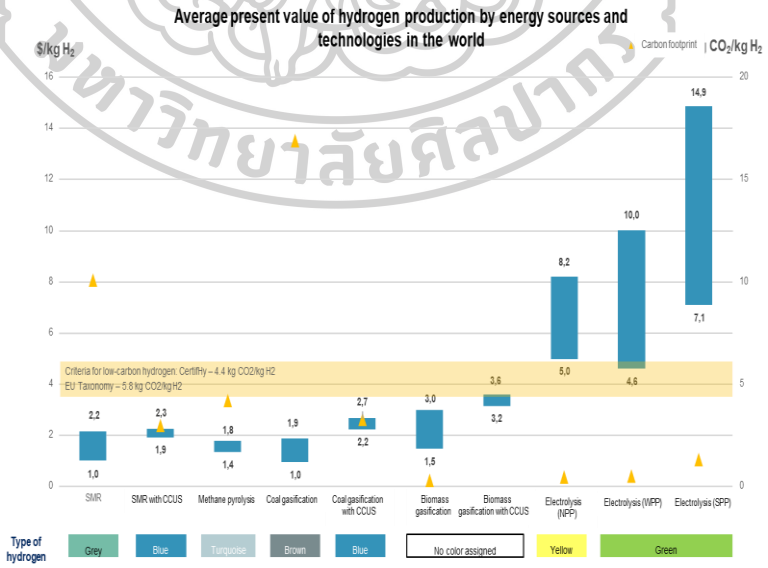
Coal Gasification Process:

Step 1 $C + H_2O \longrightarrow CO + H_2$ (Eq. 4)

Step 2 $CO + H_2O \longrightarrow CO_2 + H_2$ (Eq. 5)

Overall step $C + 2H_2O \longrightarrow CO_2 + 2H_2$ (Eq. 6)

The reforming and gasification process are method that releases carbon dioxide and is classified as hydrogen production with an environmental impact as gray hydrogen. Currently, there is a trend of wanting to reduce the impact of the grey hydrogen production process that reaches to carbon neutrality. Therefore, this issue has been discussed and hastened to develop for green hydrogen. The production of hydrogen through electrolysis which is classified as green hydrogen production because it releases only hydrogen and oxygen. A summary of Hydrogen process including price and CO_2 released is figured by (Parkinson et al., 2019) as shown in Figure 1.



Source: Parkinson, B. *et al* (2019)

Figure 1. The price and CO_2 emission of Hydrogen process classified in color

Figure 1 showed that the electrolysis process is classified as green hydrogen, but the production costs are highest in the range of $7.1 to $14.9 per kilogram of hydrogen. Therefore, the development of research to reduce the cost of this process is very necessary. The advantage of the hydrogen gas production process from water electrolysis is more than 99.99 percent purity. The electrochemical reaction from water electrolysis that occurs at each electrode depends on the acidity and baseness of the water solution used as a raw material for hydrogen gas production. The water electrolysis that occurs at each electrode can be shown as Equation 7 - Equation 9 in acid condition and Equation 10 – Equation 12 in alkaline condition.

Electrolysis Reaction in Acid Condition:

Cathode Reaction: $H^+ + e^- \longrightarrow H_2$ (Eq. 7)

Anode Reaction: $H_2O \longrightarrow H^+ + \frac{1}{2} O_2 + e^-$ (Eq. 8)

Overall Reaction: $H_2O \longrightarrow H_2 + \frac{1}{2} O_2$ (Eq. 9)

Electrolysis Reaction in Alkali Condition:

Cathode Reaction: $2H_2O + 2e^- \longrightarrow H_2 + 2OH^-$ (Eq. 10)

Anode Reaction: $2OH^- \longrightarrow H_2O + \frac{1}{2} O_2 + 2e^-$ (Eq. 11)

Overall Reaction: $H_2O \longrightarrow H_2 + \frac{1}{2} O_2$ (Eq. 12)

Another way, biohydrogen derived from bio-resources by fermentation process which was required small number of energies. A number of countries including South East Asian countries and South America have abundant of biomass from agricultural sources. Biomass such as sugar crane can be converted to green fuel and hydrogen. Molasses is a residue from sugar processing. 40 to 60 kg of Molasses is generated from one ton of sugar cane. It is composed of a value constitution of glucose, sucrose, and fructose(Detman et al., 2017). Dark fermentation of molasses generates only hydrogen and carbon dioxide. The theoretical maximum hydrogen yield from molasses can be calculated based on the stoichiometry of the fermentation process. For simplification, it can assume that the sugars in molasses (e.g., sucrose, glucose, and fructose) are

converted to hydrogen gas (H_2) through a process known as dark fermentation, which can be represented by the following simplified as Equation 13.

$$C_6H_{12}O_6 \text{ (glucose)} \longrightarrow 2\ CO_2 + 2\ H_2 \qquad \text{(Eq. 13)}$$

This Equation 13 shows that for every mole of glucose, two moles of hydrogen gas are produced. Molasses contains a mixture of sugars, so the actual yield will depend on the sugar composition of the molasses. The theorical H_2/CO_2 obtained equal to 1.

Although, the biohydrogen process by the fermentation generates H_2 and CO_2, the application CO_2, which is a by-product, can be solved by converting to biochemical such as biomethanol (Sarp et al., 2021). The global market of methanol is forecasted showing the opportunity of the increasing demand as shown in Figure 2.

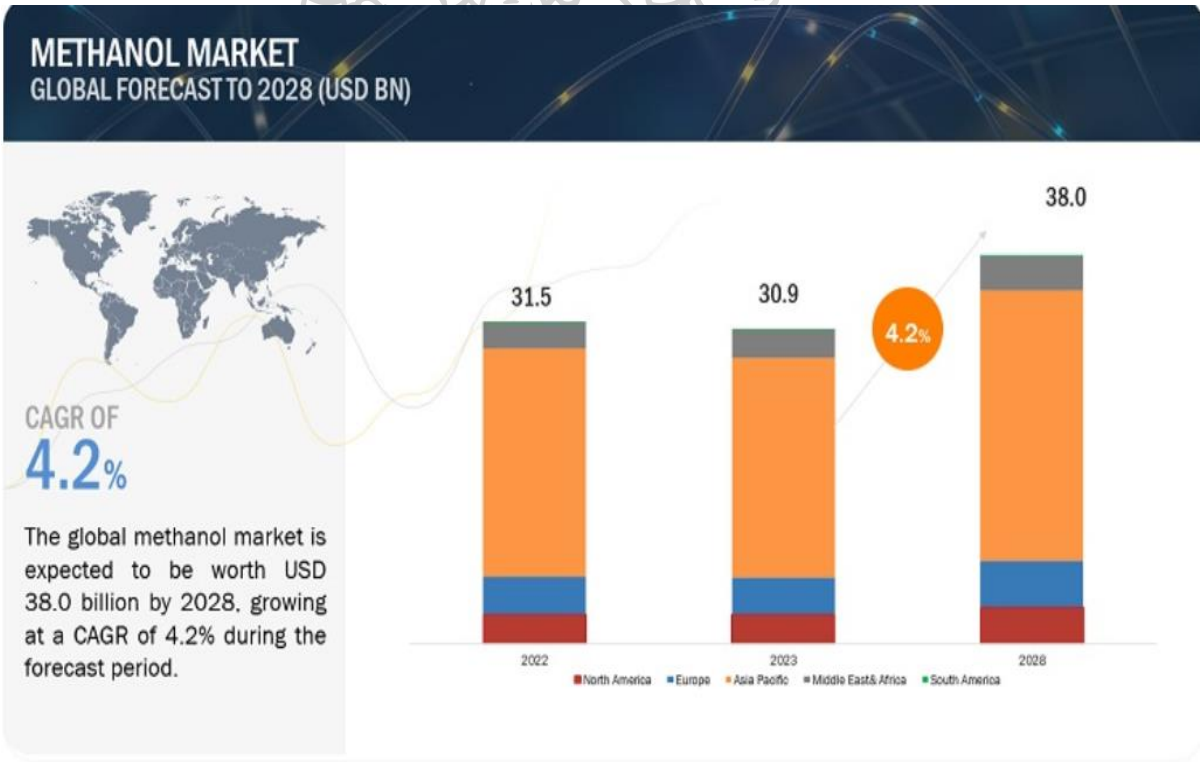
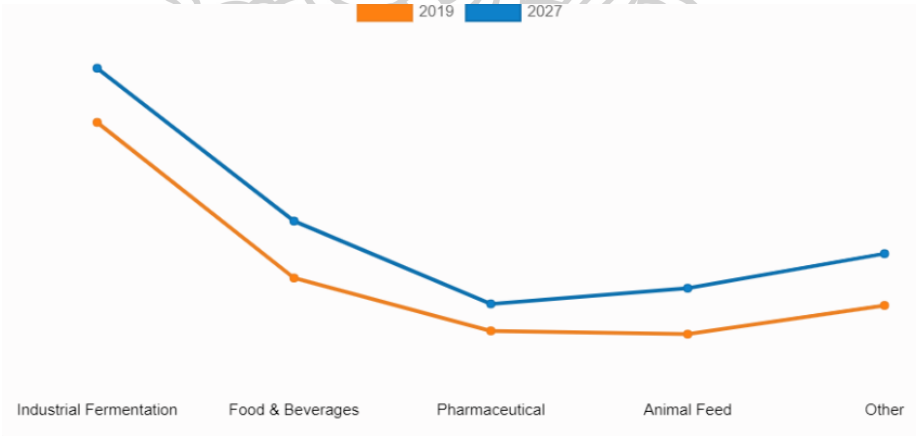


Figure 2. The global methanol forecast from 2022 to 2028.

The scope in this thesis literature review is contained of biohydrogen research and biomethanol synthesis.

2.2 Biohydrogen Research

Number of raw materials in the sugar group have been researched as raw material in biohydrogen. Glucose and fructose are hugely researched on biohydrogen production, they are products obtained from sugar processing, but they have a high price and so unprofitable in competition in hydrogen market. The other one, crude glycerol had been interested in the last decade because it is obtained from the biodiesel manufacturing industry. As a result, it has been large volume and cheap, but at the present, raw glycerol has been developed for a raw material in the oleochemical industry, thus causing the reduction in crude glycerol market and its price is shifted to high. Molasses become interested because 1) the cheapest in price comparing with glucose, fructose or even glycerol and abundant source obtained from sugar processing. Ratio of molasses application are widely used in industrial fermentation, food and beverages, pharmaceuticals, animal feeds, and others as shown in Figure 3.



Source: https://www.marketresearchfuture.com/reports/molasses-market-7007

Figure 3. Molasses consumption in various sector

The Global sugar balance has been collected in statistical view since 2011 and it has been continually recorded for 2020. The data showed that the production is over the domestic sugar consumption which was consumed from 162 M ton (2011) to 172 M ton (2020). The forecast showed the molasses production will be

adequate for the domestic consumption and had over demand which can be supply for other applications in the future demand. Based on the Figure 4, It was increased around 10 percent in ten years, and it is forecasted demanding in domestic sugar consumption around 179 M ton (2030).

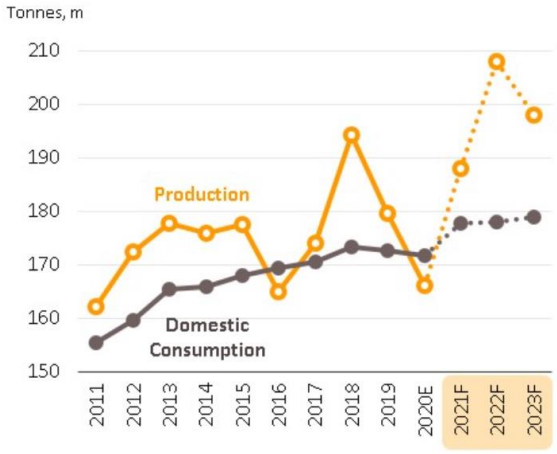
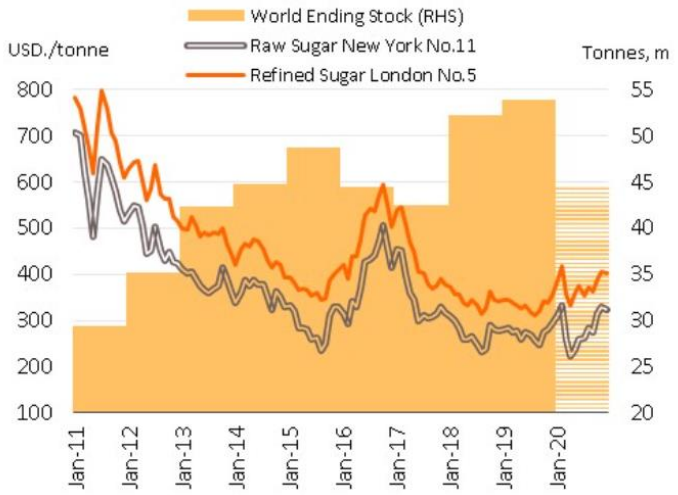


Figure 4. Global sugar balance

Source: USDA, Krungsri Research

Molasses is appropriate bio-source for biohydrogen because it is relatively inexpensive. Its statistical price is reducing in each year from 700-800 USD/ton (2011) and stable around 300-400 USD/ton (2020) as shown in Figure 5. An example price of Molasses has ranged in the year 2011 to 2020.



Source: OCSB, Bloomberg, USDA, Krungsri Research

Figure 5. World molasses price stock

Because molasses is a by-product from sugar-processing, the molasses comprises of contaminates, total organic content (TOC) and salts shown in Table 1 (Jamir et al., 2021). These are valuable components for growing of bacteria, which supported to biohydrogen production.

Table 1. General composition of molasses

Composition	Range %
Sucrose	29-40
Water	17-25
Glucose	4-14
Ash	7-15
Potassium	4-50.83
Calcium	0.8-15
Magnesium	1-14
Sodium	0.09-9
Protein	0.5-4.5
Sulphates	2.24-9.91
Amino acids	0.3-1.5
Non-nitrogenous acids	1.5-8
Wax, sterols and phosphatides	0.1-1
Biotin	0.1-2 ppm, 0.36 mg/kg
Riboflavin	1-6 ppm, 1.8 mg/kg

Nandi reviewed biohydrogen production from various sugar sources such as glucose, fructose, glycerol, and molasses. Four types of microorganisms can digest sugar sources and obtained biohydrogen depending on appropriate of these microorganisms and categories such as anaerobes, aerobes, facultative anaerobes, and photosynthetic. The products are contained H_2, CO_2, and organic compounds (Nandi & Sengupta, 1998). A research group (Özgür et al., 2010) used *Rhodobacter capsulatus* and *Rhodopseudomonas palustris* in digestion of molasses for

biohydrogen synthesis by working in photo-fermentation in batch by adding NH_4^+. The maximum hydrogen was improved when absence of NH_4^+ yield 4.2 mol of H_2 / mol sucrose to 13.7 mol H_2/mol sucrose in dark fermentation comparing with theoretical yield 24 mol of H_2/mol of sucrose. A research by (Cappelletti et al., 2012) worked on biohydrogen studying four types of *hyperthermophilic thermotoga* spp. (*T.neapolitana, T.maritima, T.naphtophila, petrophila*) in various sources: glucose, molasses, and cheese whey. They found that Thermotoga species were suitable in H_2 production with both molasses and cheese whey as substrates. The highest H_2/substrate yielded were similar for 2.95 and 2.50 mol H_2/mol monosaccharide on molasses and cheese whey respectively. The investigated hydrogen production by using bacteria from sludge called ethanol-type fermentation was done by (Wang et al., 2013). The work was performed in using molasses as a substrate in continuous stirred tank reactor (CSTR) by varying HRT rates from 4 to 10 hr. The result showed that the highest hydrogen production obtained in 5 hr. of HRT was 12.27 mmol/L/hr. An investigation of biohydrogen by using sugar beet molasses in different strains of purple non-sulfur bacteria composing *Rhodobacter capsulatus* DSM1710, *Rhodobacter capsulatus* YO_3, *Rhodobacter sphaeroides* O.U.001, and *Rhodopseudomonas palustris* DSM127 and different initial sucrose concentrations were studied by (Sagir et al., 2017). The experiments performed in a single-stage photo fermentative biohydrogen production and the result show that *Rp. Palustris* yielded maximum hydrogen productivity 0.55 mmol/L/hr. An immobilized technique was investigated to fix mesophilic bacteria on granular activated carbon by feeding an anaerobic sludge for biohydrogen production which molasses were combined with varying pH in the range of 5.5 to 7.5. The result showed that the optimal H_2 production 759 ppm in rate of 3.63 mL/hr. at pH 5.5. The data resulted that the granular activated carbon enhancing the biohydrogen production by stabilizing the pH as a carrier material (Zuhar et al., 2018). The study of the effect of adding Ginkgo biloba leaf (GL) in molasses on H_2 production by using *E.*

harbinense. It was found that sugarcane molasses (SM) yielded a maximum of 1.58 mol-H_2/mol-hexose and when using corn steep liquor (CSL) and SM hexose, H_2 were produced 2.31 g/L, 2.28 g/L and 10 g/L, respectively. H_2 yield was improved by 28.03% by (Li et al., 2020).

As previous researches, a large variety of bacteria were investigated to decompose molasses for hydrogen production. However, all four groups of bacteria had limitations for further scaling up. Aerobic bacteria were not suitable because they did not have a path to produce hydrogen when the system is contaminated with oxygen. In case of photo fermentation, it is burdensome for electricity consumption to supply the light all time of operation at the industrial level. In addition, it is difficult to protect the light dead zone in the large reactor. Thermophilic bacteria are also in the same situation for requiring heat and controlling temperature of the reactor to increase the rate of decomposition. Therefore, it is realized about the limitations, facultative bacteria are interesting. There was a research by (Jitrwung & Yargeau, 2011) that *Enterobacter aerogenes* is suitable in digestion of sugar digestion such as glycerol. They reported that *Enterobacter aerogenes* grown well in aerobic and decomposed glycerol completely in anaerobic conditions giving hydrogen. They also optimized and scaled up from 100 ml serum bottles to 3.6-liter bioreactor by using the optimized condition contained 18.5 g/L crude glycerol (15 g/l pure glycerol) controlled speed at 500 rpm and pH started at 6.4. This research group also performed the experiment in continuous feed rate 0.44 ml/min with 33% recycle of media solution in seven days and the result obtained in slightly lower in biohydrogen production (H_2/mol glycerol) of 0.86 compared with 0.96 in batch mode and 0.84 in 100 ml serum bottles reported in (Jitrwung & Yargeau, 2015). The continuous biohydrogen production from molasses were tested by anaerobic fermentation with a pilot-scale bioreactor system by (Ren et al., 2006). The study was performed over 200 days under the organic loading rates (OLR) of 3.11–85.57 kg COD/m^3 reactor/d (COD:

chemical oxygen demand) with molasses as the substrate. The biogas was mainly composed of CO_2 and H_2 with composition of H_2 ranging from 40% to 52%. Hydrogen production was obtained maximum rate of 5.57 m^3 H_2/m^3 reactor/d. In addition, they suggested that the by-products from the production of hydrogen from the digestion of molasses consists of hydrogen, carbon dioxide, and different organic substances contained various organic acids and ethanol depending on the metabolism pathway as shown in Figure 6 (Ren et al., 2006).

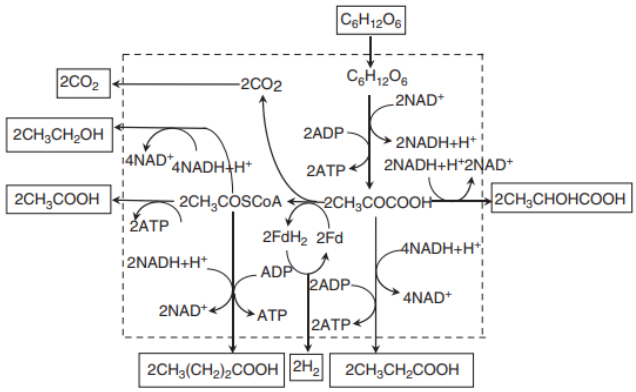


Figure 6. Glucose metabolic pathway

In this thesis, hydrogen production by *Enterobacter aerogenes* using molasses is an interesting biotechnological process that leverages the metabolic capabilities of this bacterium to produce hydrogen gas (H_2) from organic substrates like molasses. *Enterobacter aerogenes* is a facultative anaerobe, meaning it can grow in both aerobic (with oxygen) and anaerobic (without oxygen) conditions, so that it is not too difficult in maintain bioreactor with absolutely without oxygen which makes it suitable for hydrogen production.

The factors are considered in an overview of the biohydrogen process section:

1. Selection of *Enterobacter aerogenes* which have hydrogen-producing ability.

2. Preparation of Molasses: Molasses is a byproduct of the sugar refining process and contains sugars such as sucrose, glucose, and fructose, which can serve as a carbon source for bacterial growth and hydrogen production. Molasses should be prepared in media solution which contained mineral salts called and additives to promote *Enterobacter aerogenes* growth and hydrogen production pathway. The molasses in

media solution was sterilized before use to remove any contaminants that could interfere with the fermentation process.

3. Fermentation: Inoculate the selected strain of *Enterobacter aerogenes* into a bioreactor containing the sterilized molasses solution. The bacteria will consume the sugars in molasses through a process known as anaerobic fermentation. During this process, they will produce hydrogen gas along with other metabolic by-products.

4. Controlled Environment: Maintain the bioreactor in a controlled environment to optimize hydrogen production. Factors such as temperature, pH, and agitation must be carefully controlled to promote bacterial growth and hydrogen production.

5. Harvesting Hydrogen: As the bacteria ferment the sugars, they will release hydrogen gas. This gas can be collected and harvested from the bioreactor. Various methods can be used to capture the hydrogen gas, such as gas bags and containers.

6. Productivity Optimization: Researchers often work on optimizing the process by adjusting parameters like the initial molasses concentration, pH levels, and temperature to maximize hydrogen production. In this thesis, the concentration of molasses, salts and trace elements were varied and studied to obtain the optimum condition.

7. By-product Utilization: Besides hydrogen gas, the fermentation process may produce other byproducts like organic acids. Strategies can be employed to utilize or further process these byproducts to improve overall process efficiency. But in this thesis, the by-products will not be measured and analyzed, It will consider only biohydrogen gas for applying to biomethanol purpose.

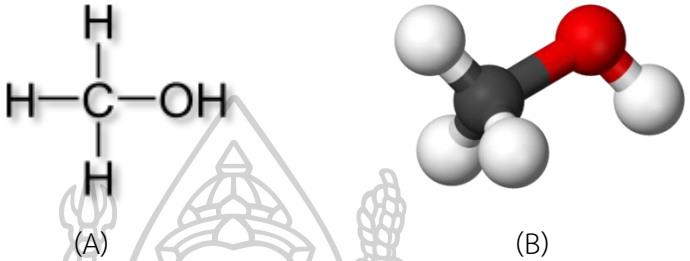
8. Monitoring and Analysis: Continuously monitor the progress of the fermentation process, including gas composition, hydrogen production rates and bacterial growth, without the concentration of metabolic byproducts.

9. Scale-up: Once a successful in bottle is developed, it can be scaled up for bioreactor with the optimum condition.

Biohydrogen produced through this process can be used as a clean and renewable raw material source for biomethanol synthesis purpose as described in section 2.3

2.3 Biomethanol Synthesis

Methanol is the first alcohol contained one carbon atom. It is a liquid alcohol known as methyl alcohol. The molecular formula of methanol is CH_3OH with molecular weight (MW) 32.042 kg/kmol, Methanol has a molecular structure shown in Figure 7.



(A) (B)

Figure 7. (A) Methanol Lewis Structure (B) Methanol-3D Structure

The worldwide methanol applications are fuel and chemical purposes: As alternative fuels such blending in gasoline (high octane value), synthesizing biodiesel and chemicals such as a solvent and chemical reactant for producing formaldehyde, acetic, MTBE and DME which summarized methanol derivatives, end uses and sector in Figure 8.

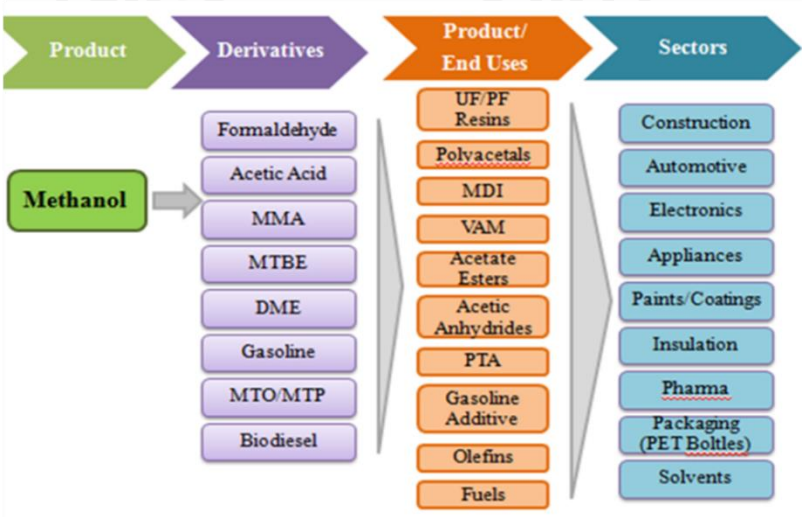


Figure 8. Methanol applications in terms of derivatives, product end uses and sectors

The reaction steps of methanol synthesis using methane contaminated with carbon dioxide as raw material are described in 2 steps: 1) Reforming of steam methane (SMR) (Equation 14) and dry methane (DMR) (Equation 15) and 2) hydrogenation on CO (Equation 16) and hydrogenation on CO_2 (Equation 17). Side reactions are contained water-gas shift reaction (WGS) (Equation 18) and reverse water gas shift reaction (RWGS) (Equation 19) (Peter et al., 2012).

Reforming Reactions:

SMR: $CH_4 + 2H_2O \longrightarrow CO + 3H_2$ ΔH_{298}= 206.0 kJ/mol (Eq. 14)

DMR: $CH_4 + CO_2 \longrightarrow 2CO + 2H_2$ ΔH_{298K}= 74.7 kJ/mol (Eq. 15)

Hydrogenations:

CO hydrogenation: $CO + 2H_2 \longrightarrow CH_3OH$ ΔH_{298}= -90.55 kJ/mol (Eq.16)

CO_2 hydrogenation: $CO_2 + 3H_2 \longrightarrow CH_3OH + H_2O$ ΔH_{298K}=-49.43 kJ/mol (Eq.17)

Side reactions:

WGS: $CO + H_2O \longrightarrow CO_2 + H_2$ ΔH_{298}= -41.12 kJ/mol (Eq.18)

RWGS: $CO_2 + H_2 \longrightarrow CO + H_2O$ ΔH_{298K}=41.12 kJ/mol (Eq.19)

Commercial methanol is normally composed of two steps; firstly, steam reforming of natural gas or coal by generating syngas containing a mixture of hydrogen (H_2), carbon monoxide (CO) and carbon dioxide (CO_2) then secondly the syngas is reacted over a Cu-based catalyst under conditions of low pressures and temperatures in range of 40-100 bar and 150-300 °C.

The purity and rate of methanol production is depended on ratio of H_2, CO and CO_2 in the syngas as known that the formation of methanol is followed by CO and CO_2 hydrogenation (Equation 16) and (Equation 17). If CO concentration overs the CO_2 concentration, the reaction prefers following CO hydrogenation obtaining lower

contaminated water and high purity of methanol. In case of CO_2 concentration overs the CO concentration, the reaction favors CO_2 hydrogenation obtaining high amount of water resulting in low purity of methanol. In addition, water is formed by CO_2 hydrogenation can be reacted with CO following WGS reaction and yielding CO_2 and H_2 by following Equation 18. There are three reaction equations proceed for methanol synthesis. The theoretically stoichiometric number (SN) for methanol synthesis is written in Equation 20 (Sheldon, 2017).

$$SN = \frac{[H_2]-[CO_2]}{[CO]+[CO_2]} = 2.0 \qquad \text{Eq. 20}$$

Methanol synthesis reactions by both CO and CO_2 hydrogenation are exothermic and generates heat, thereby the process favored at low temperatures to the detriment of the reaction rate. CO_2 hydrogenation releases lower energy than CO hydrogenation as minus 49.43 kJ/mol and minus 90.55 kJ/mol respectively. The commercial methanol process requires the proper catalyst and usually operate under high pressures to reach a reasonable industrial conversion rate (Manenti et al., 2011). Normally, syngas containing 59-80 % of hydrogen, 15-32 % of carbon monoxide and 2-8% of carbon dioxide. A research on the ratios of H_2/CO were done in ranging of 0.5 to 2 by (Roberts et al., 1999) and they found that the suitable of H_2/ CO ratio is approximately 2/1 which provides higher yield of methanol than H_2/CO ratio equal 0.5 as well as the hydrogenation of CO_2 with has the appropriate molar ratio of H_2/CO_2 about 3. The proper ratio of syngas is important cause it can increase methanol yield and can decrease activation energy. This suggestion is described by the research of Q. San et al. They studied about the combination of CO and CO_2 in gas reactant for methanol synthesis, the result is found that if the reactant contains both CO and CO_2 in some appropriate ratio of CO/CO_2 hydrogenation will promote to increase rate of methanol synthesis. It seems that mixed appropriate CO/CO_2 is better methanol synthesis than used only CO or CO_2 (Roberts et al., 1999), (Liu et al., 2003).

The first methanol mechanism (MM1):

MM1-1 CO hydrogenation as a main reaction for methanol synthesis, the reactions are following Equation 16 and 17. In this point, methanol is mainly synthesized via hydrogenation on CO that is an exothermic reaction and followed with the second reaction by reverse water gas shift reaction, RWGS as Equation 19 which is endothermic reaction. According to this mechanism, WGS proceeds in reverse direction result in reducing CO_2 concentration and obtaining more CO and enhancing the methanol synthesis by CO hydrogenation.

MM1-2 If the feed is free of CO, the methanol productivity is very low / because of RWGS will be occurred by converting CO_2 with H_2 to generate CO. So, CO_2 is required for both reactions: CO_2 Hydrogenation (Equation 17) and RWGS (Equation 19). If RWGS is faster than methanol synthesis reaction, the methanol production rate will be suffered. Water is produced from both reactions which are RWGS and CO_2 hydrogenation, high level of water in catalyst pore can harmful to catalyst. Subsequently, methanol productivity is decreased.

The second methanol mechanism (MM2):

MM2-1 CO_2 hydrogenation as a main reaction for methanol synthesis, the reactions are following methanol synthesis in predominantly via hydrogenation of CO_2 and proceeds side reaction of WGS in forward direction to convert CO to CO_2 and H_2 for boosting the eventual methanol productivity. Furthermore, absence of CO_2, can appear a reaction called Boudouard reaction (Equation 21). This reaction takes place on heterogeneous surfaces with involving carbon deposition resulting to catalyst deactivation.

Boudouard reaction: $2CO_{(g)} \longrightarrow CO_{2\,(g)} + C_{(s)}$ ΔH_{298K}=-172.0 kJ/mol (Eq. 21)

MM2-2 CO_2 hydrogenation, If CO hydrogenation is taken in CO_2 in syngas feed condition, possible reaction represented by CO hydrogenation (Equation 16) and when CO_2 occurred hydrogenation following Equation 17 resulting water in the product. In this case, CO is required by both reactions (CO hydrogenation and WGS), WGS is faster under this condition and proceeds in forward direction because CO is abundant and has some water in system. Additional WGS reaction can generate H_2 and CO_2 which is a reactant for CO_2 hydrogenation methanol synthesis and water would prevent carbon deposition.

2.4 OPEX evaluation of biomethanol obtained from biohydrogen

In this section, OPEX_BHM represented the biomethanol obtained from the optimized condition of transforming biohydrogen obtained from molasses and OPEX_BGM represented the biomethanol obtained from the biogas obtained from molasses which supported data by Jitrwung et. al., 2022. In term of Operating Expenditure (OPEX_BHM) composed 1) raw materials 2) Catalyst 3) Electricity consumption. The OPEX_BHM are compared with OPEX_BGM and the two processes are pictured as Figure 9.

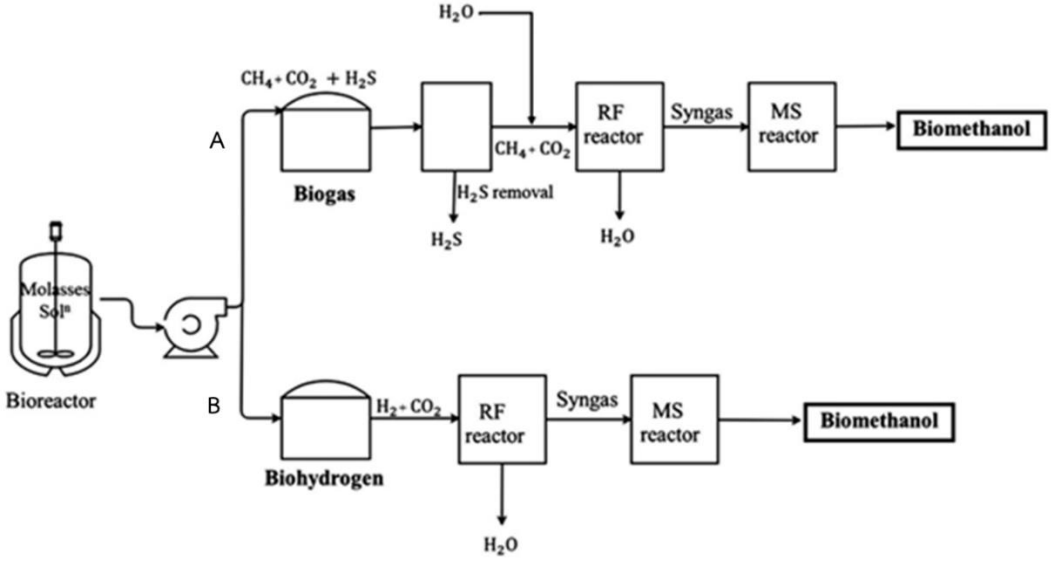


Figure 9. (A) Pathway of Molasses to biogas and biomethanol (B) Pathway of Molasses to biohydrogen and biomethanol

The advantages of transforming molasses passing biogas and biohydrogen pathway to produce biomethanol would not only substitute bio/green methanol but also promote molasses usage and reduce CO_2 from bioresource applications.

The first pathway (A) involves the application of molasses for biogas. The data about this process pathway were reviewed. Generally, biogas had been produced from fermented organic residues but is mostly in small and medium scales. Molasses is an abundant source for large scales of biogas production. Biogas contained approximately 55% (v/v) CH_4, 43% (v/v) CO_2, 2% (v/v) water, and a small amount of H_2S (Janke et al., 2015), (Suwanasri et al., 2015), (Chaiprasert, 2011). The transformation of molasses to biomethanol requires a four-step process as shown in Figure 9. Pathway A: (1) Biogas Production (BG), Molasses was diluted by water and fermented under anaerobic condition to obtain crude biogas. (2) Biogas Refinery (BGR), wherein crude biogas was refined by removing H_2S by bio-scrubber (Nishimura & Yoda, 1997) or Fe-EDTA solution. (3) Biogas Reforming (BGF), wherein CH_4 and CO_2 were reacted by steam reforming (Equation 14) and dry reforming (Equation 15) and to obtain syngas under Metal/Al_2O_3 catalyst under 600°C to 900°C in the atmosphere (Zhao et al., 2020). However, the optimized condition of biogas reforming was used followed by Jitrwung R. et al., 2022. (4) Methanol synthesis (MS), wherein H_2 in syngas was hydrogenated on CO/CO_2 by (Equation 16)/(Equation 17) and both reactions occurred under Cu/ZnO/Al_2O_3 catalyst (Sun et al., 1999); the optimized condition of CO hydrogenation was 170°C and 40 barg. After molasses passed through four steps, it was then transformed into crude biomethanol containing over 96% (v/v) methanol and contaminated 3% (v/v) of water, 0.02% (v/v) ethanol, and a small number of impurities.

The second pathway (B) involves an innovation route, namely passing biohydrogen route, which had been researched by using biosugar sources, such as glucose, fructose, molasses, and glycerol. Molasses was extensively investigated due to its wide use and abundance. Moreover, molasses is the cheapest source among other sugar sources. Producing biohydrogen is close to the biogas fermentation

because it is produced from biosugar sources by bacterial digestion [51] and [52]. Biohydrogen obtained from molasses comprised only hydrogen (H_2) and carbon dioxide (CO_2). The ratio of H_2/CO_2 was produced on the basis of bacterial strains and conditions; thus, in the use of Enterobacter aerogenes, the ratio of H_2/CO_2 was 1 following the metabolic pathway of glucose (Ren et al., 2006). Enterobacter aerogenes was utilized in biohydrogen fermentation because its temperature of 30°C is similar to equatorial room temperature and facultative condition. H_2/CO_2 ratio of biohydrogen was in the range of 0.5–1.0 (Jitrwung & Yargeau, 2015). Only three steps were required to convert molasses to biomethanol as shown in Figure 9.

Pathway B: (1) Biohydrogen Fermentation (BHF), wherein molasses was diluted by nutrient solution and then fermented under facultative condition obtaining crude biohydrogen. (2) Biohydrogen Conversion, wherein CO_2 and H_2 were reacted by reverse water gas shift (3) to obtain syngas under $Cu/ZnO/Al_2O_3$ catalyst under 500°C and atmospheric pressure (Daza & Kuhn, 2016); a side reaction of CO and H_2O and reversed CO and H_2 was observed and referred to as water gas shift (WGS) (Equation 18) (Wang et al., 2017). (3) MS (Methanol synthesis), wherein H_2 was hydrogenated into CO by (Equation 16) or hydrogenated into CO_2 by (Equation 17), demonstrating the occurrence of both reactions under $Cu/ZnO/Al_2O_3$ catalyst at 170°C and 40 barg. Biomethanol was only three steps when passing biohydrogen pathway. This article aims to provide a comparison using molasses for biomethanol. The comparison involved the four and three steps of molasses to biomethanol by biogas pathway and biomethanol by biohydrogen route to obtain the engineering view and cost.

Chapter 3

Research Methodology

The experimental method in this thesis is divided into 3 parts: biohydrogen, biomethanol, and preliminary operating expenditure (OPEX) calculation.

3.1 Biohydrogen Section

3.1.1 Enterobacter Aerogenes (TISTR 1540) obtained from TISTR

3.1.2 Raw materials and Nutrients

3.1.2.1 Hi-Molasses obtained from M-Molasses

3.1.2.2 Nutrient Broth obtained from HIMEDIA

3.1.2.3 Nutrient Agar obtained from HIMEDIA

3.1.3 Chemicals for media solution

3.1.3.1 Dipotassium phosphate (KH_2PO_4) obtained from KEMAUS

3.1.3.2 Ammonium sulfate ($(NH_4)_2SO_4$) obtained from KEMAUS

3.1.3.3 Magnesium sulfate heptahydrate ($MgSO_4{\cdot}7H_2O$) obtained from QReC

3.1.3.4 Calcium Chloride ($CaCl_2$) obtained from KEMAUS

3.1.3.5 Sodium Molybdate ($Na_2MoO_4{\cdot}2H_2O$) obtained from KEMAUS

3.1.3.6 Sodium Chloride (NaCl) obtained from KEMAUS

3.1.3.7 Sodium hydroxide (NaOH) obtained from KEMAUS

3.1.3.8 Tryptone Type-1 obtained from HIMEDIA

3.1.3.9 Peptone obtained from HIMEDIA

3.1.3.10 Yeast Extract Powder obtained from HIMEDIA

3.1.3.11 Beef Extract B Powder obtained from HIMEDIA

3.1.4. Additional Salts

3.1.4.1 Calcium Oxide (CaO) obtained from KEMAUS

3.1.4.2 Magnesium Oxide (MgO) obtained from KEMAUS

3.1.4.3 Potassium hydroxide (KOH) obtained from KEMAUS

3.1.4.4 Nickle Chloride hexahydrate ($NiCl_2{\cdot}6H_2O$) obtained from KEMAUS

3.1.4.5 Ferrous sulfate heptahydrate ($FeSO_4{\cdot}7H_2O$) obtained from KEMAUS

3.1.5. Sparking gas

3.1.5.1 N_2 gas in 99.999 % purity obtained from Thai Special Gas Co., Ltd.

3.1.5.2 7.5% O_2 in N_2 obtained from Thai Special Gas Co., Ltd.

3.1.6 Equipment

3.1.6.1 Weight Balance 4 positions from METTLER TOLEDO model ME204

3.1.6.2 Laboratory Laminar Air Flow Cabinet HL Series from HI-LAB

3.1.6.3 UV-VIS Spectrophotometer model UV-5100, VIS-5100 from METASH

3.1.6.4 Bioreactor from Marubishi (Thailand) model BEM type MDFT-N-5L with MCI 6C

3.1.2.6 Shaking Incubator model SI-100R from Santa Technology

3.1.2.7 Gas Chromatography model 7890B from Agilent Technologies

3.1.2.8 Pipette model FINNPIPETTE from Thermos scientific

3.1.2.9 Autoclave model MaXterile 60 from DAIHAN Scientific

3.1.7 Microorganism and inoculums preparation

3.1.7.1 Inoculums preparation

3.1.7.1.1 Prepare the culture in solid nutrient. To expand cells and preserve bacteria for use, bacterial cells were collected in pure culture storage bottles. 28 grams of solid nutrient was weighed in 1000 milliliters of distilled water and placed in an autoclave working in Laminar fume hood to preserve in sterilization, then poured the solid nutrient into a Petri dish inside the sterile cabinet, waited until the culture medium are cool and covered the edge of the culture dish with parafilm then placed in a hot bag and stored in the refrigerator.

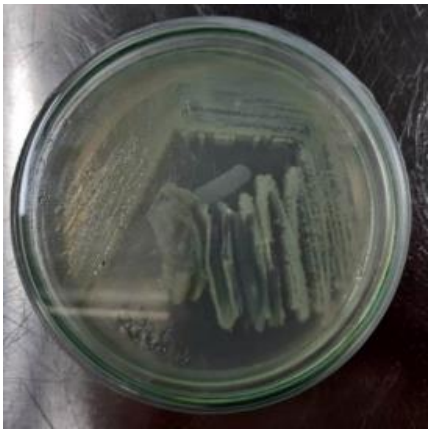


Figure 10. ***Enterobacter Aerogenes*** **growth with solid nutrient in petri dishes**

3.1.7.1.2 Prepare the culture. Taking cell culture from the petri dishes and dried in the sterile cabinet. Use a needle prepared aseptic by swirling on the fire until it got hot. Dip the needle into the pure *Enterobacter Aerogenes* bottle and took the needle out and swept the needle on the Petri dish prepared above, then covered the lid, incubated at 37 °C for 24 hr and stored in the refrigerator.

3. 1. 7. 1.3 Prepare 5 grams per liter of NaCl in leavening liquid solution in 1000 milliliters of distilled water and placed in an autoclave for sterilization. Waited until the culture medium were cooled and covered the edge of the culture dish with parafilm. Placed in a hot bag and stored in the refrigerator.

3. 1. 7. 1.4 Prepare liquid nutrient and inoculums in leavening agent by taking 1 NA petri dish, using a needle to remove 1 loop of culture, heated the needle until got hot. Removed the culture from the petri dish and placed it in the liquid medium, then incubated at 37 °C for 18 hr.

Figure 11. Prepare liquid nutrient in leavening agent

3.1.7.2 Preparation of culture media in 100 ml bottles

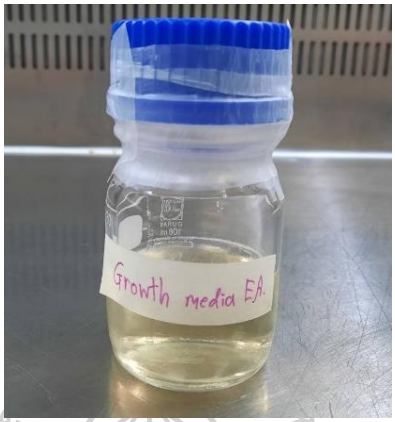


Figure 12. Prepare media for bacteria growth

Before experiments, bacteria were incubated in growth media. The Nutrients were composed of beef extract (1.0 g/L), yeast extracts (2.0 g/L), peptone (5.0 g/L), and NaCl (5.0 g/L) and dissolve in deionized water at 24 hr incubation.

3.1.7.2.1 Culture media for molasses concentration experiments in section 4.1.2.2

Figure 13. Culture media in 100 ml serum bottles

Put 5, 10, 20, 30, 40, and 50 grams of molasses in each 1000 ml of volumetric bottles and added 1 g of $(NH_4)2SO_4$), 5.5 grams of KH_2PO_4, 5 g of tryptone, 5 g of Yeast Extract, 0.25 g of $MgSO_4 \cdot 7H_2O$, 0.12 g Na_2MoO_4 $2H_2O$ and 0.020 g of $CaCl_2$ $2H_2O$ into a 1000 ml volumetric flask and dissolved with deionized water, stirred until the solution is homogeneous, adjusted pH to 7 with 4 mol sodium hydroxide solution and adjusted

the volume to 1000 ml. Take 50 ml. of the culture media solution and put into 100 ml. serum bottles and sparked with 7.5% O_2 in N_2 gas in 1 min to obtain anerobic condition. Subsequently, the serum bottles were sealed with rubber stoppers and aluminum caps and placed in autoclave for sterilization. Waited until the culture medium serum bottles cooled and placed them inside the sterile cabinet.

3.1.7.2.2 Culture media for salts concentration experiments in section 4.1.2.2

Prepare the same as section 3.1.7.2.1 and added 1, 2, 3, 4 and 5 grams of CaO, MgO and KOH in each 1000 ml. of volumetric bottles.

Figure 14. Culture media with salt in 100 ml serum bottles

3.1.7.2.3 Culture media for molasses concentration experiments (scale up testing) in section 4.1.3.1

Put 100, 150 and 200 g of molasses in each 5,000 ml in 10-L bioreactor and added 20 g of $(NH_4)_2SO_4$), 20 g of KH_2PO_4, 20 g Na_2HPO_4, 1.0 g of $MgSO_4 \cdot 7H_2O$, 5 g of Yeast Extract and 25 g of tryptone into the 10-L bioreactor and dissolved with deionized water, stirred until the solution is homogeneous, adjusted pH to 7 with 4 mol sodium hydroxide solution and adjusted the volume to 5 Liter and sparked with N_2 gas in 10-15 minutes to obtain anerobic condition. Subsequently the bioreactor was plugged with clamps and stoppers then placed in autoclave for sterilization. Waited until the culture medium in the bioreactor cooled and placed the bioreactor inside the sterile cabinet and installed equipment with the bioreactor then connected to the bioreactor station for the operation.

3.1.7.2.4 Culture media for study effect of Trace elements ($NiCl_2$ and $FeSO_4$) in molasses concentrations experiments in section 4.1.3.2

Prepare the same as section 3.1.7.2.3 and added 0 and 0.05 g of Trace elements (NiCl and $FeSO_4$) in each 5 L of culture media placed in 10-L bioreactor.

3.1.7.2.5 Culture media for optimization of salts ($NiCl_2$ and $FeSO_4$) in molasses concentrations experiments in section 4.1.3.3

Prepare the same as section 3.1.7.2.3 and added 0, 0.05, 0.25 and 0.50 g of Trace elements ($NiCl_2$ and $FeSO_4$) in each 5 L of culture media placed in 10-L bioreactor.

3.1.7.2.6 Culture media for continuous experiments in section 4.1.4

1) Prepare the same as section 3.1.7.2.3 by using 150 g of molasse and added 0.05 g of Trace elements ($NiCl_2$ and $FeSO_4$) in 5 L of culture media placed in 10-L bioreactor and used this operated in batch mode.

2) prepare the same as 1) but dividing in 1-L volumetric bottle for 5 bottles and used this operated in continuous mode.

3.1.8 Biohydrogen production experiments

3.1.8.1 Biohydrogen production experiments in 100 ml serum bottles

Inoculums were taken from the inoculum bottles which were previously slightly over pressurized with a gas mixture of 7.5% oxygen in nitrogen. The 10% of inoculum volume was transferred to 50 ml of molasses-containing media placed in a 100-ml serum bottle, referred to as the experiment bottles. The transfer was done using an aseptic syringe following the Hungate technique (Miller & Wolin, 1974). The experiment bottles were then placed in the incubator shaker at 30 °C and 150 rpm until hydrogen production ceased. The fermentation was carried out for three days in which hydrogen gas production was monitored daily. Molasses to biohydrogen experiment in serum bottles as shown in Figure 15.

Figure 15. Biohydrogen production in serum bottles and gas measured by a syringe

3.1.8.2 Biohydrogen production experiments in 10-L bioreactor.

3.1.8.2.1 Inoculums preparation

Enterobacter Aerogenes (E.A.) TISTR 1540 were cultivated as shown in Figure 16. E.A is diluted in 5 ml. of Nutrient broth (NB) and cultivated at 30 ℃ shaking speed at 150 rpm for 24 hr. and then transferred the E.A into growth medium (1 Liter of Growth medium comprised of 1 g beef extract, 2 g yeast extract, 5g peptone and 5g NaCl and stirred in homogeneous with DI water) placed for incubating at 30 ℃ at 150 rpm for 18 hr.

Figure 16. Enterobacter Aerogenes (E.A) cultivation for bioreactor experiments

3.1.8.2.2 Biohydrogen production experiments in bioreactor: batch mode

The 1,000 ml inoculum bottles obtained from 3.1.8.2.1 were flushed with 7.5% O_2 in N_2 to obtain semi anerobic conditions. The entire content of the inoculum bottle (500 mL of solution) was then fed to the bioreactor containing 5,000 ml of MMSM/molasses solution, using a peristaltic pump while replacing the liquid with an equivalent volume of the same gas mixture (7.5% O_2). A 10-L in model MDFT-10L bioreactor from Marubishi (working volume of 5.0 L) was used in batch and continuous mode of operation at a temperature 30 °C. In batch mode, mixing speed was set to 150 rpm and biohydrogen production was kept in gas bag and monitored off-line until production ceased. In addition to the previously mentioned parameters, monitoring of dissolved oxygen (using a pO_2 sensor probe, oxygen InPro 6850i, obtained from Mettler Toledo) and gas production as well as control, using peristaltic pumps, over pH using 10% sodium hydroxide, feed 1 L of fresh molasses solution by one time a day after drawn 1 L of liquid in bioreactor out.

The 10-L in model MDFT-10L bioreactor from Marubishi for producing biohydrogen gas shown in Figure 17.

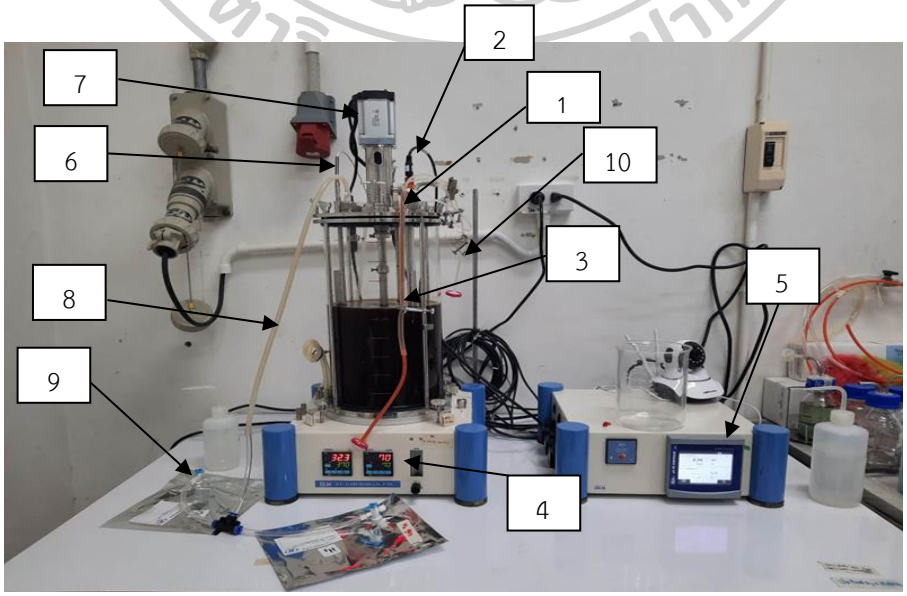


Figure 17. Ten Liter of bioreactor in model MDFT-10L bioreactor from Marubishi

The components of 10-L bioreactor are as follow;

1. Channel for adding inoculum and culture media
2. Channel for pH measurement
3. Channel for cell culture measurement
4. Temperature control unit
5. pH control unit
6. Channel for Temperature probe
7. Agitator and speed control
8. Biohydrogen tube installed to the channel
9. Biohydrogen gas bag
10. Air/N_2 supply channel

In the bioreactor, creating and maintaining anaerobic conditions when using *Enterobacter aerogenes* in a bioreactor is crucial for certain biotechnological processes, such as hydrogen production through anaerobic fermentation. Here are the steps and methods which can use to control anaerobic conditions in a bioreactor:

1. Select an Appropriate Bioreactor: Choose a bioreactor system that allows for precise control over environmental conditions. Common choices for anaerobic processes include stirred-tank reactors and anaerobic chambers or gloveboxes.

2. Purge with Inert Gas: Before inoculating the bioreactor with Enterobacter aerogenes, purge the reactor vessel and headspace with an inert gas such as nitrogen (N_2) or carbon dioxide (CO_2). This displaces oxygen from the system.

3. Monitor Oxygen Levels: Install oxygen sensors within the bioreactor to continuously monitor and control oxygen levels. These sensors can be linked to control systems that adjust the flow of the inert gas to maintain low oxygen concentrations.

4. Sealing: Ensure the bioreactor is properly sealed to prevent the ingress of air. Use high-quality seals and gaskets to maintain anaerobic conditions.

5. Sparging Gas: Depending on the design of bioreactor, it may introduce the inert gas through sparging. Sparging involves bubbling the inert gas through the culture medium to maintain anaerobic conditions.

6. pH Control: Maintain the pH of the culture medium within the desired range, as changes in pH can affect bacterial metabolism. Use pH sensors and controllers to regulate the pH with appropriate acid or base additions.

7.Temperature Control: Maintain the temperature at the optimal range for Enterobacter aerogenes growth and hydrogen production. This usually involves using a temperature-controlled jacket or heating/cooling coils within the bioreactor.

8. Agitation: Properly mix the culture medium to ensure uniform growth and prevent the accumulation of oxygen-rich zones. Stirring or agitation should be gentle enough to avoid excessive oxygen introduction.

9. Gas Tight Ports: Ensure that all ports, including sampling ports and feed ports, are gas-tight to prevent the ingress of oxygen during sampling or addition of nutrients.

10. Continuous Monitoring and Adjustment: Continuously monitor the conditions within the bioreactor and make adjustments as necessary to maintain anaerobic conditions throughout the fermentation process.

11. Sterilization: Prior to inoculation, sterilize the culture medium and the bioreactor components to eliminate any potential contaminants.

Remember that achieving and maintaining strict anaerobic conditions can be challenging, and careful attention to detail is essential. The specific conditions and equipment required may vary depending on the scale of the bioreactor and the nature of the anaerobic process. Regularly monitor and validate anaerobic conditions to ensure the success of the biotechnological process involving Enterobacter aerogenes under anaerobic conditions.

In this perform bioreactor experiments, it is allowed oxygen contaminated not over than 3 %, the nitrogen gas was filtered before using for flushing through the bioreactor to avoid the contaminants for 10 minutes. The gas that comes out of the machine in the biogas storage line and analyzed with Gas Chromatography to determine the percentage of oxygen gas. In this experiment, the initial oxygen before inoculation will be controlled to be between 1 and 3 percentage. Then take the liquid food leavening agent and added it through the leavening opening. Close the lid tightly and open the stirring blade. The rotation speed is 70 rpm. The temperature control is

turned on at 37 °C. When the microorganisms grow, the gas obtained will be stored in a gas storage bag and taken for further analysis.

3.1.8.2 Product sampling and analyze

3.1.8.2.1 Cell growth were measured by optical density at 600 nm. The liquid was sampled by channel 3.

3.1.8.2.2 Gas was collected in gas bag as shown in Figure 18 and then analyze gas composition by Gas Chromatography.

Figure 18. Gas collection bag (Aluminum type)

3.1.8.2.3 The gas composition was analyzed by gas chromatography model 7850B from Agilent as shown in Figure 19.

Figure 19. Gas chromatography model 7890B Agilent

Table 2. The condition for gas measurement with GC Agilent model 7890B

Inlet	
Oven Temperature	250 ℃
Split ratio	100:1
Column	
PoraPLOT Q-HT(FID)	0 ℃ to 290 ℃ 25 m × 320 μm × 10 μm.
MolSieve 13X (TCD)	0 ℃ to 400 ℃ : 10 ft. 1/8 2 mm. 45/60 SS
Porapak Q (TCD)	0 ℃ to 250 ℃ 6 ft. 1/8 2 mm80/100 SS
Oven Temperature Program	
Initial Temperature	60 ℃ hold 6 min
1st rate	20 ℃ min to 80 ℃
2nd rate	30 ℃ /min to 190 ℃, hold 5 min
Detector	
FID	350 ℃
TCD	250 ℃
Carrier gas	
Type	He

3.2 Biomethanol Section

3.2.1 Comparing crude biohydrogen gas with simulation synthetic gas in RWGS experiment.

3.2.1.1 Raw materials

3.2.1.1 The commercial catalyst Cu/ZnO/Al_2O_3 supplied by Xi'an Sunward Aeromat Co. Ltd., China.

3.2.1.2 Mixed gas of 15% H_2 in N_2 (Reduce gas) supplied by Thai special gas CO., Ltd.

3.2.1.3 The synthetic biohydrogen gas which is the same composition with 3.2.1.1.3 (A) was supplied by Thai special gas CO.,Ltd.

3.2.1.2 Equipment

3.2.1.1 The 50 g RWGS reactor obtained from Owner Food Machinery CO.,Ltd. as shown in Figure 20.

3.2.1.2 Aluminum gas bag model New devex gas sampling bag, 10-L Capacity obtained from P.T.KHRUEANG MUE VIT COMPANY LIMITED.

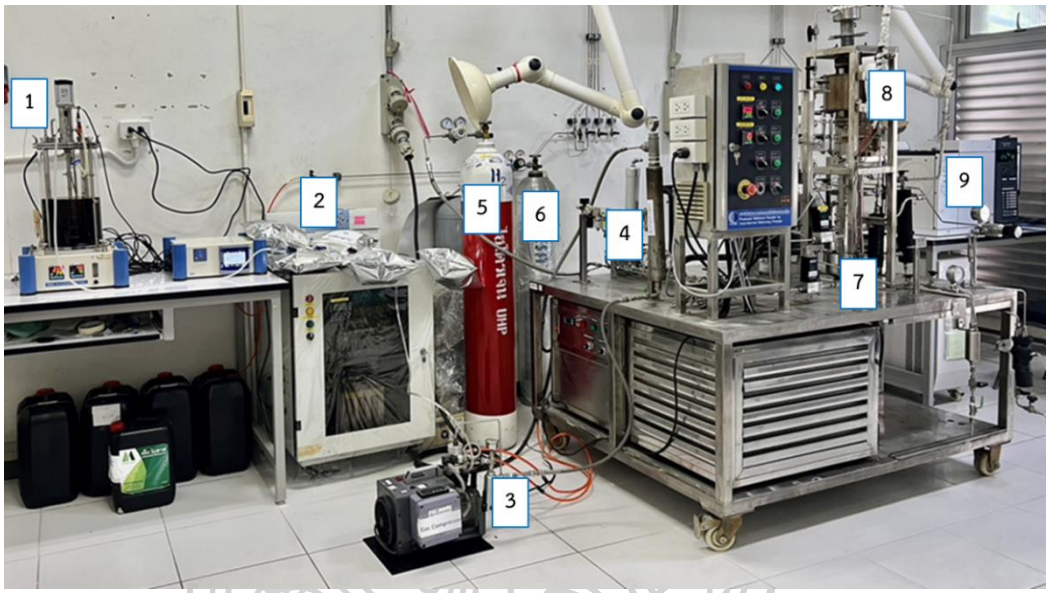


Figure 20. Schematic of biohydrogen to RWGS in Laboratory scale of TISTR

1. Bioreactor
2. Collected crude biohydrogen gas bag
3. Gas compressor
4. High pressure gas column
5. Reduce gas (15% H_2 in N_2)
6. Synthetic biohydrogen gas
7. Mass Flow Controller
8. 50 mg of Catalyst RWGS reactor (RX)
9. On line Gas Chromatography

3.2.1.3 Method

3.2.1.3.1 An amount of 50 g of the catalyst was placed in RWGS Reactor (RX).

3.2.1.3.2 The catalyst was activated under condition of 50 ml feeding 15% H_2 in N_2 at temperature 230 °C and pressure of 3 barg for 18 hr.

3.2.1.3.3 (A) The 50 ml of crude biohydrogen (H_2, CO_2, O_2 and N_2) obtained from biohydrogen CSTR experiments (section 4.1.4) which it collected by compressing in a high-pressure tank for feeding as a raw biohydrogen for testing biomethanol synthesis in section 4.2.1.

(B) The synthetic biohydrogen gas which is the same composition in 3.2.1.3.3 (A) was supplied by Thai special gas CO.,Ltd.

3.2.1.3.4 The synthetic biohydrogen gas (B) was operated under feeding 50 ml/min controlled by Mass flow controller in the operation of RWGS which was controlled at 500 ℃ and pressure of atmosphere for 6 hr. The gas out from the RWGS reactor was collected in a gas bag in every two hours and measured by the GC with the same condition in section 3.1.8.2.3.

3.2.1.3.5 After 6 hr of the RWGS operation, the synthetic biohydrogen gas was substituted by 50 ml/min of the crude biohydrogen (A) under the same condition for 6 hr. The gas out from the RWGS reactor was collected and measured by the GC with the same condition in section 3.1.8.2.3.

3.2.1.3.6 Cool down the reaction, after the operation was finished, the mixed 10% H_2 in N_2 gas was fed and set the reduction of temperature to 230 ℃ under atmosphere. When the temperature is cooled down to 230 ℃, the pressure was adjusted to 3 barg for 3 hr to maintain the activity of the catalyst, then set temperature to 35 ℃ and released pressure to the atmospheric pressure.

3.2.2 Two-step biomethanol synthesis

3.2.2.1 Raw materials

3.2.2.1.1 The commercial catalyst $Cu/ZnO/Al_2O_3$ supplied by Xi'an Sunward Aeromat Co. Ltd., China.

3.2.2.1.2 Mixed gas of 15% H_2 in N_2 (Reduce gas) supplied by Thai special gas.

3.2.2.1.3 Mixed gas of 50%H_2 in CO_2 supplied by Thai special gas.

3.2.2.1.4 99.99 % H_2 supplied by Thai special gas.

3.2.2.1.5 99.99 % CO_2 supplied by Thai special gas.

3.2.2.1.6 The synthetic biohydrogen gas which is the same composition with 3.2.1.3.3 (A) was supplied by Thai special gas.

3.2.2.2 Equipment

3.2.2.2.1 The 5 kg of two stages biomethanol reactors obtained from Owner Food Machinery CO.,Ltd and patented by TISTR as shown in Figure 21.

3.2.2.2.1.1 Reactor 1 (RX1) and Reactor 2 (RX2) are identical fixed bed reactors has capacity of 5 kg. of catalyst type $Cu/ZnO/Al_2O_3$. The RX1 and RX2 are the fixed bed reactors which inside diameter of 16 cm and length of 30 cm made from 304 stainless. The reactor temperature was controlled by temperature program in ranging of 30 to 600 °C. The RX1 and RX2 are used for biomethanol synthesis experiments.

3.2.2.2.1.2 Mass Flow controllers were used to control all gases feeding to the reactors: MFBH represented Mass Flow of Biohydrogen, MFH represented Mass Flow of Hydrogen, MFC represented Mass Flow of Carbon dioxide, MFN represented Mass Flow of Nitrogen, and MFSG represented Mass Flow of Syngas.

3.2.2.2.1.3 Other equipment: CS1 (Cool Separator 1) was used to separate liquid product out of gas product from the RX1. CS2 (Cool Separator 2) was used to separate liquid product out of gas product from the RX2. LPT (Low Pressure Tank) was used to receive gas product from CS1. A Compressor was used to build up low-pressure gas obtained from the RWGS reaction to high-pressure gas and then storing in HPT

(High Pressure Tank). A chiller supplied cool water for CS1 and CS2 to maintain well gas -liquid separation.

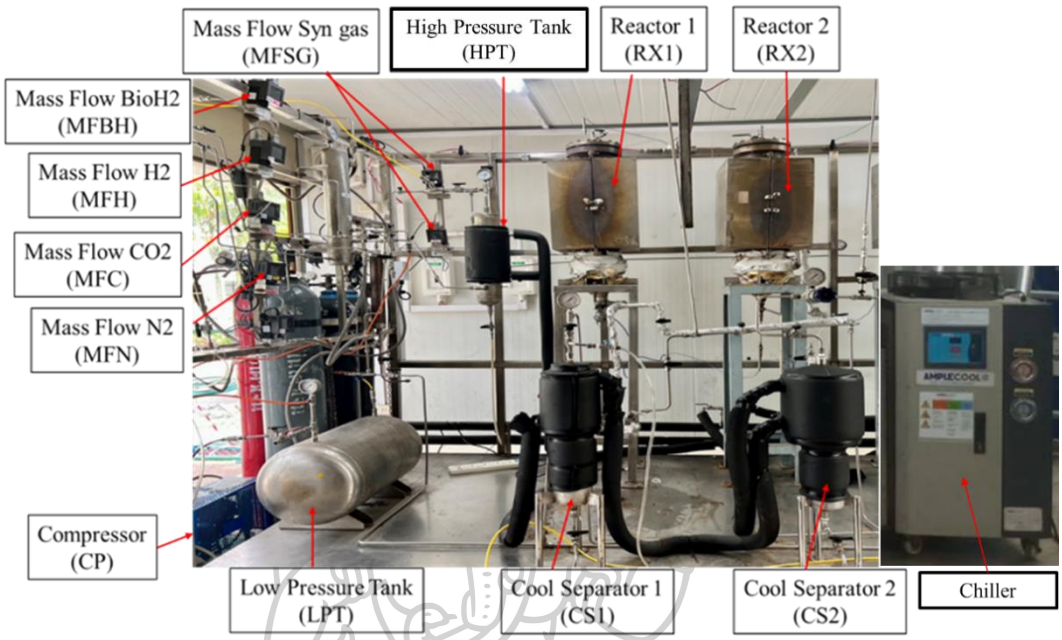


Figure 21. A Two-step biomethanol synthesis process in semi-pilot scale fixed-bed reactor under TISTR technology

3.2.2.2.2 Aluminum gas bag model New devex gas sampling bag, 10-L Capacity obtained from P.T.KHRUEANG MUE VIT COMPANY LIMITED.

3.2.2.2.3 The composition of gas feed and gas product were measured by a gas analyzer (GA), MRU model, Vario luxx as shown in Figure 22. The GA can measure gas compositions (H_2, CO, CO_2, O_2, N_2 and CH_4) in % by volume and summation of each gas was in 100% by volume.

Figure 22. A gas analyzer (GA), MRU model, Vario luxx

3.2.2.3 Method

3.2.2.3.1 An amount of 5 kg of the catalyst was placed in RX1 and RX2.

3.2.2.3.2 The catalyst was activated under condition of 5 L/min feeding 15% H_2 in N_2 at temperature 230 ℃ and pressure of 3 barg for 18 hr.

3.2.2.3.3 The catalyst in two reactors were activated and ready for the RWGS reaction and methanol synthesis (MS) for the experiment in section 4.2.1 to 4.2.3

3.2.2.3.4 The reactions were performed:

Single-step reaction by using only RX2 for Methanol Synthesis experiment (Section 4.2.1)

The 1 L of synthetic biohydrogen was adjusted by varying feed gas ratios by using MFBH, MFH and MFC until obtaining H_2/CO_2 50/50, 60/40 and 70/30 measured by GA. Each ratio was experimented by feeding to LP1 then compressed by CP for high pressure gas and stored in HPT. The gas in HPT was controlled the feed by MFSG to the RX2 for biomethanol synthesis under 170 ℃ and 40 barg. Liquid and gas were separated in CS2, and the gas were passed out all times. Every six hours, the gas was measured and analyzed by GA and the liquid was take out and weighed.

Single-step reaction by using only RX1 for RWGS experiment (Section 4.2.2)

The 5 L of synthetic biohydrogen was adjusted by varying feed gas ratios by using MFBH, MFH and MFC until obtaining H_2/CO_2 50/50, 60/40 and 70/30 measured by GA. Each ratio was experimented by feeding to RX1. The gas feed was controlled by MFSG and the reaction in RX1 was controlled under varying temperature from 150 to 550 ℃ by step up 50 ℃ and pressure at atmosphere. Liquid and gas were separated in CS1, and the gas were passed out all

times. Every two hours, the gas was measured and analyzed by GA and the liquid was take out and weighed.

Two-step methanol synthesis: Combined reactions by using RX1 and RX2 for the optimized methanol synthesis experiment (Section 4.2.3)

The two-step biomethanol synthesis (TSBS) process was designed to optimize biomethanol synthesis. The experiment, the transformation of biohydrogen into biomethanol via two pathways were studied:

Pathway 1—Direct Methanol Synthesis (DMS) + Direct Methanol Synthesis (DMS) or (MS1+MS2) as shown in Figure 23.

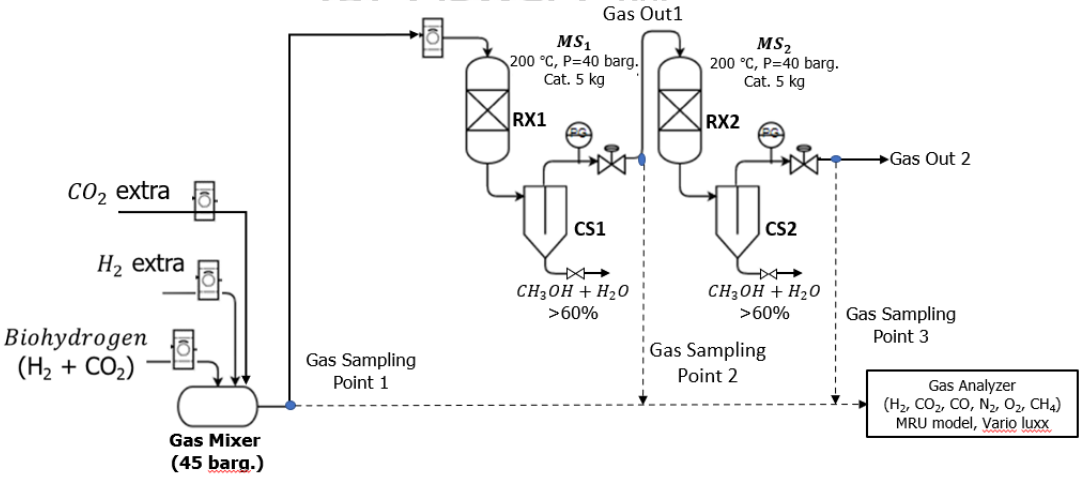
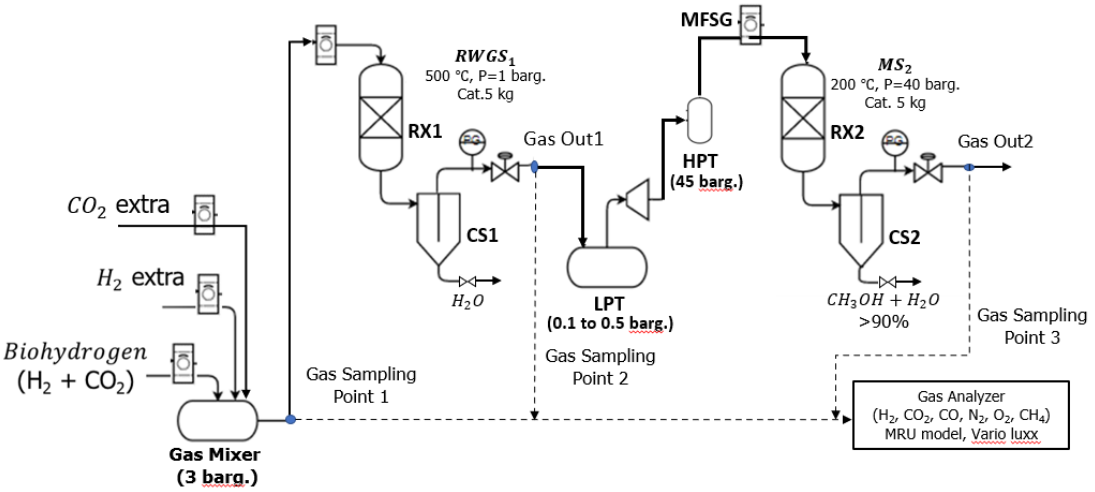


Figure 23. Process flow diagram of H_2/CO_2 experiment via pathway 1: MS1 and MS2 in fixed-bed reactor

Pathway 1: The process diagram of DMS+DMS, Methanol synthesis reaction step 1 (MS1) and methanol synthesis reaction step 2 (MS2), was shown in Figure 23. The experiment was started by feeding gas comprised of synthetic biohydrogen (H_2 + CO_2) by MFBH and adjusted gas composition by CO_2 or H_2 by MFC or MFH, then all gases were blended in a gas mixer until obtaining the following volume ratios of H_2/CO_2: 50/50%(v/v), 60/40%(v/v), and 70/30%(v/v). The gas was fed by MFSG to reactor 1 (RX1) controlled at 200 °C and 40 barg. After the reaction, hot fluid flowed out of the first reactor and cooled down in (CS1), Gas flowed out from the top of CS1 and was fed continuously into reactor 2 (RX2) controlled at 200 °C and 40 barg. for methanol

synthesis. After the reaction occurred in RX2, the fluid was cooled in CS2. the gas was extracted. Every 6 hours, Take the liquid sample (biomethanol) from CS1 by opening the bottom valve connected to CS1 and the liquid sample (biomethanol) from CS2 by opening the bottom valve connected to CS2. The compositions of gases from RX1 and RX2 were measured with a gas analyzer MRU which connected with three positions as shown in Figure 23.

Pathway 2— In Direct Methanol Synthesis (IMS) comprised of RWGS + Direct Methanol Synthesis (DMS) or (RWGS1+MS2) as shown in Figure 24.



Pathway 2 : Biohydrogen combined with extra H_2 then RWGS1 and following with MS2

Figure 24. Process flow diagram of H_2/CO_2 experiment via pathway 2: RWGS1 and MS2 in fixed-bed reactor

Pathway 2: The process diagram of IMS+DMS, the reverse water–gas shift reaction (RWGS) and methanol synthesis reaction (MS) were carried out as shown in Figure 24. The experiment was started by set up pressure 3 barg for all feeding gas comprised of synthetic biohydrogen (H_2 + CO_2) by MFBH and adjusted gas composition by CO_2 or H_2 by MFC or MFH, then all gases were homogeneous in a gas mixer until obtaining the following volume ratios of H_2/CO_2: 50/50%(v/v), 60/40%(v/v), and 70/30%(v/v). The gas was sent by MFSG to reactor 1 (RX1) controlled at 500 °C and pressure at atmosphere. After the reaction, hot fluid flowed out of the first reactor and cooled down in (CS1), Gas flowed out from the top of CS1 and was collected in (LPT) which the pressure gas was controlled the minimum pressure at 0.1 barg and the maximum pressure at 0.5 barg. A compressor (CP) started to compress the gas from

LPT to HPT when the gas pressure in the LPT reached the maximum pressure and stopped when the minimum pressure was reached. The gas was collected in the HPT until reaching over 45 barg and prepared to be fed continuously with MFSG into reactor 2 (RX2) maintained 170 °C and 40 barg by a gas back pressure regulator. After the reaction in RX2, the fluid was cooled in CS2. The gas was passed out continually. Every 6 hours, Take the liquid sample (water) from CS1 by opening the bottom valve connected to CS1 and the liquid sample (biomethanol) from CS2 by opening the bottom valve connected to CS2. The compositions of gases from RX1 and RX2 were measured with a gas analyzer MRU which connected with three positions as shown in Figure 24.

3.2.2.3.5 The end of experiment (Cool down the reaction), after the operation was finished, the mixed 10% H_2 in N_2 gas was fed and set the reduction of temperature of RX1 and RX2 to 230 °C under atmosphere. When the temperature is cooled down to 230 °C, the pressure was adjusted to 3 barg for 3 hr to maintain the activity of the catalyst, then set temperature to 35 °C and released pressure to the atmospheric pressure.

3.3. The preliminary OPEX calculation and comparison (section 4.3)

Case 1: $OPEX_{BG}$ represented pathway of Molasses to biogas and biomethanol which the most data obtained from the researches of Jitrwung et.al., 2022.

Case 2: $OPEX_{BH}$ represented pathway of Molasses to biohydrogen and biomethanol (data obtained from the optimized condition of section 4.2.3) in this thesis.

The OPEX was reported in term of USD/kg biomethanol, which the comparison was performed in three major costs contained:

1. Cost of Raw materials (Molasses, nutrients and chemical & substance addition)
2. Cost of Catalysts
3. Cost of Electricity

Chapter 4

Result and Discussion

4.1 Biohydrogen production experiments

4.1.1 *Enterobacter aerogenes* growth curve

Growth Curve of *Enterobacter aerogenes* (TISTR 1540) *obtained from TISTR* under 30 °C, 120 rpm, aerobic condition shown in Figure 25. and detailed data in Appendix A1 Condition: NB, 10% Inoculum, Growth media 18 hr., VB/VC = 0.5

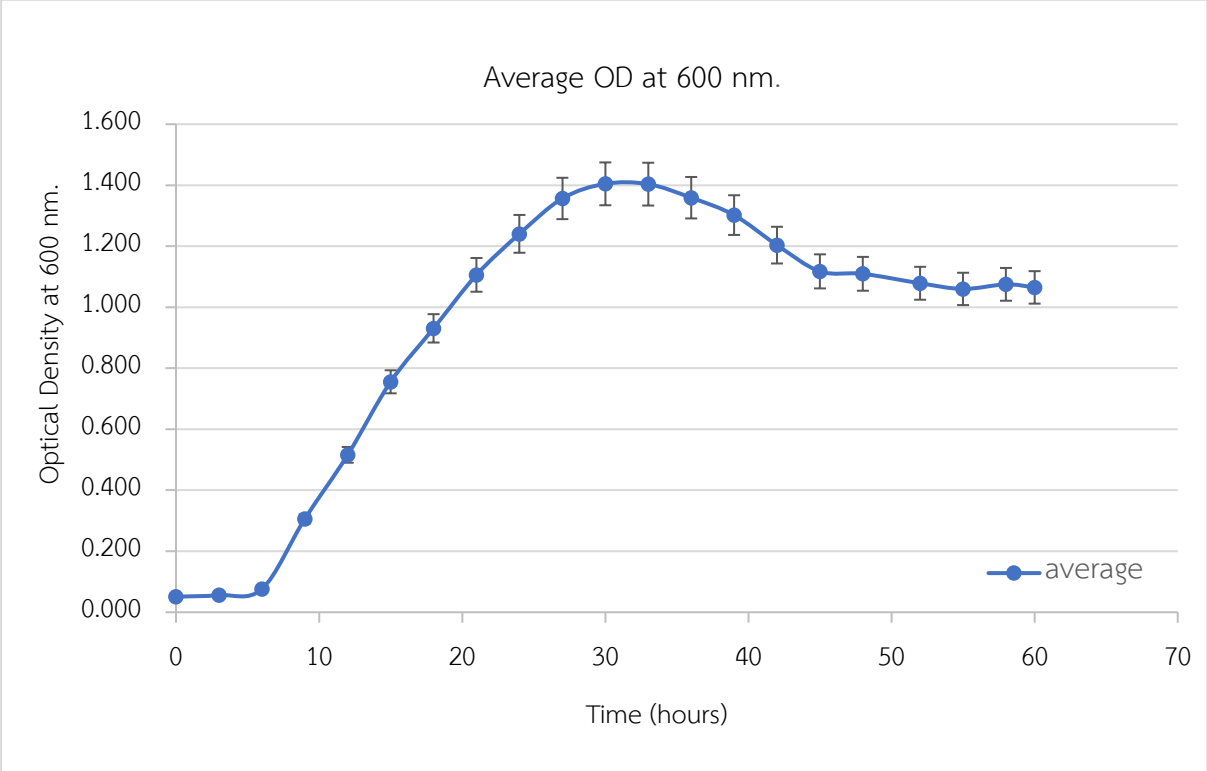


Figure 25. Growth Curve of *Enterobacter aerogenes* under 30 °C, 120 rpm and aerobic condition

Enterobacter aerogenes (E.A.) is a facultative anaerobic bacterium that typically follows a predictable growth curve when cultured in a laboratory setting. Enterobacter aerogenes is tested in growing under aerobic condition under 30 °C and 120 rpm. This growth curve consists of four distinct phases: lag phase, exponential (log) phase, stationary phase and death phase. It is found that E.A. stayed in lag phase in 6 hr, grew

up to log phase in 6 to 30 hr, then reached to stationary phase and obtained maximum Optical density at 600 nm. around 1.4 in 30 hr and keep and dropped down after 36 hr called death phase as shown in Figure 25.

Lag Phase: In this initial phase, the bacteria are adapting to their environment and preparing for growth. There is little to no increase in cell numbers, as they are synthesizing essential enzymes and acclimating to the culture conditions.

Exponential (Log) Phase: During this phase, Enterobacter aerogenes experiences rapid and exponential growth. The cells multiply at their maximum rate, dividing and doubling in number with each generation. This phase is characterized by a steep upward slope on a growth curve graph.

Stationary Phase: As the available nutrients become limited and waste products accumulate, the growth rate of Enterobacter aerogenes begins to slow down. In the stationary phase, cell division and death rates reach an equilibrium, resulting in a relatively constant population size. This phase reflects the bacterial population's stability in a closed environment.

Death Phase: In the final phase, the death rate exceeds the rate of cell division. Factors such as nutrient depletion and the buildup of toxic metabolites lead to a decline in the bacterial population. The growth curve shows a downward slope as the bacteria eventually die off.

Understanding the growth curve of Enterobacter aerogenes is essential for various applications including hydrogen production. Hydrogen production by Enterobacter aerogenes using molasses is an interesting biotechnological process that leverages the metabolic capabilities of this bacterium to produce hydrogen gas (H_2) from organic substrates like molasses. Enterobacter aerogenes is a facultative anaerobe, meaning it can grow in both aerobic (with oxygen) and anaerobic (without oxygen) conditions, which makes it suitable for hydrogen production.

Hydrogen produced through this process can be used as a clean and renewable energy source for various applications, including fuel cells and as a feedstock for chemical processes. Additionally, using molasses as a feedstock makes this process more sustainable and environmentally friendly by utilizing a waste product from the sugar industry.

The overview of the process which is crucial to hydrogen production by molasses using *Enterobacter aerogenes*:

4.1.1.1. Selection of *Enterobacter aerogenes*: Start by selecting a strain of Enterobacter aerogenes that is capable of efficient hydrogen production. In this thesis research, *Enterobacter aerogenes* (TISTR 1540) obtained from TISTR organization which is suited for their hydrogen-producing ability. This strain is belonging under patent of TISTR which the researcher is working in this organization.

4.1.1.2. Preparation of Molasses: Molasses used in this thesis research obtained from Sugar company. It is a byproduct of the sugar refining process and contains sugars such as sucrose, glucose, and fructose, which can serve as a carbon source for bacterial growth and hydrogen production. Molasses is prepared and sterilized before use for the fermentation process. The molasses concentration is dilution in media solution (see in section 3.1) to obtain 5, 10, 20, 30, 40, 50 percent by weight for testing hydrogen production.

4.1.1.3. Fermentation: The fermentation is operated in 100 ml. serum bottles and scaling up to 10 L. bioreactor. Inoculate the selected strain of Enterobacter aerogenes into both type of the reactors containing the sterilized molasses solution. The bacteria will consume the sugars in molasses through a process known as anaerobic fermentation. During this process, they will produce hydrogen gas along with other metabolic byproducts.

4.1.1.4. Controlled Environment: Maintain the bioreactor in a controlled environment to optimize hydrogen production. Factors are maintained: temperature at 30 ℃, initial pH around 6.5 to7.0, and agitation speed at 70 rpm for bottles and bioreactor. The conditions must be carefully controlled to promote bacterial growth and hydrogen production.

4.1.1.5. Harvesting Hydrogen: As the Enterobacter aerogenes ferment the sugars, they will release biohydrogen gas containing H_2, CO_2, O_2 and N_2. This gas can be collected and harvested from the bottles and bioreactor. Aluminum gas bag is used to store the biohydrogen gas.

4.1.1.6. Productivity Optimization: The optimum condition is decided by the ratio of H_2/CO_2, amount of biohydrogen gas, and the ratio of biohydrogen to molasses.

The works are on optimizing the process by adjusting parameters: 1) the initial molasses concentration, 2) salts addition such as CaO, MgO and KOH, 3) addition of trace elements such as NiCl and $FeCl_2$ to maximize biohydrogen gas production, H_2/CO_2 and Biohydrogen produced /molasses reactant.

4.1.1.7. Byproduct utilization: Besides biohydrogen gas, the fermentation process may produce other byproducts like organic acids. Strategies can be employed to utilize or further process these byproducts to improve overall process efficiency. However, in this research the measurement of liquid by-products is not measured.

4.1.1.8. Monitoring and analysis: Continuously monitor the progress of the fermentation process, including volume of gas, gas composition (%), gas production rates in gas (ml) /molasses (g), bacterial growth in OD at 600 nm. and pH. This data is critical for process control and optimization.

4.1.1.9. Scale-up: Once a successful serum bottle-scale process is developed, it can be scaled up for bioreactor and further calculate for OPEX in case of developing industrial applications.

Creating and maintaining anaerobic conditions when using Enterobacter aerogenes in a bioreactor is crucial for certain biotechnological processes, such as hydrogen production through anaerobic fermentation. Remember that achieving and maintaining strict anaerobic conditions can be challenging, and careful attention to detail is essential. The specific conditions and equipment required may vary depending on the scale of the bioreactor and the nature of the anaerobic process. Regularly monitor and validate anaerobic conditions to ensure the success of the biotechnological process involving Enterobacter aerogenes under anaerobic conditions.

4.1.2 Biohydrogen production experiment in serum bottles

At the stationary phase, the 100 ml inoculum bottles were flushed with 7.5% oxygen in nitrogen gas mixture (7.5% O_2) to obtain semi anerobic conditions and obtained a slightly over pressure in the bottles. Then over pressurized gas was drawn out the same as removing 5.0 ml inoculum to be transferred to 50 ml of the

MMSM/molasses solution placed in a 100 ml serum bottle (referred section 3.1.8.1 the experiment bottles). The experiment bottles were then placed in the incubator shaker controlled temperature at 30 ℃ and shaking speed 120 rpm until biohydrogen production finished.

4.1.2.1 Effect of Molasses concentration.

The concentration of molasses in anaerobic fermentation can have a significant effect on hydrogen (H_2) production. Molasses is a common carbon source in fermentation processes, and its concentration can influence the metabolic activity of microorganisms, which in turn impacts H_2 production. Therefore, the variation of molasses concentrations 5, 10, 20, 30, 40 and 50 g/L were tested for biohydrogen production (ml), gas composition produced (ml), and also H_2/CO_2 ratio and gas (ml)/molasses (g) are calculated. The effect of molasses concentrations was shown in Table 3. and detailed data in Appendix A2.

Table 3 Biohydrogen production by varying molasses concentration

Molasses Conc.	**Gas generation (ml.)**				**Total gas**	**Gas composition (ml)**			**Average**		**Gas /molasses**
(g/l)	12 hr.	24 hr.	36 hr.	48 hr.	(ml.)	H_2	CO_2	N_2	H_2/CO_2	% N_2	(ml/g)
5	31.1	3.6	0.0	0.0	34.6	14.7	11.5	22.3	1.33	45.97	138.6
10	46.8	8.9	0.0	0.0	55.7	23.2	21.7	33.1	1.48	42.45	111.4
20	66.8	16.8	0.0	0.0	83.6	35.8	45.4	35.8	0.77	30.63	83.6
30	77.5	60.0	32.5	0.0	170.0	73.4	117.3	47.4	0.62	19.91	113.3
40	77.9	37.5	53.6	0.0	168.9	84.0	128.9	23.6	0.65	9.98	84.5
50	81.8	73.2	45.4	0.0	200.4	88.0	154.9	37.5	0.57	13.38	80.1

The experiment showed that yielded of increasing gas volume (ml.) of 34.6 55.7, 83.6, 170.0, 168.9 and 200.4 related to adding molasses concentration 5, 10, 20, 30, 40 and 50% by weight respectively. Accordingly, both H_2 and CO_2 are increasing related to the amount of molasses adding. However, it is found that the tendency of H_2/CO_2 is decreasing when increasing molasses concentration except for 30 g/l of molasses addition as shown in Figure 26.

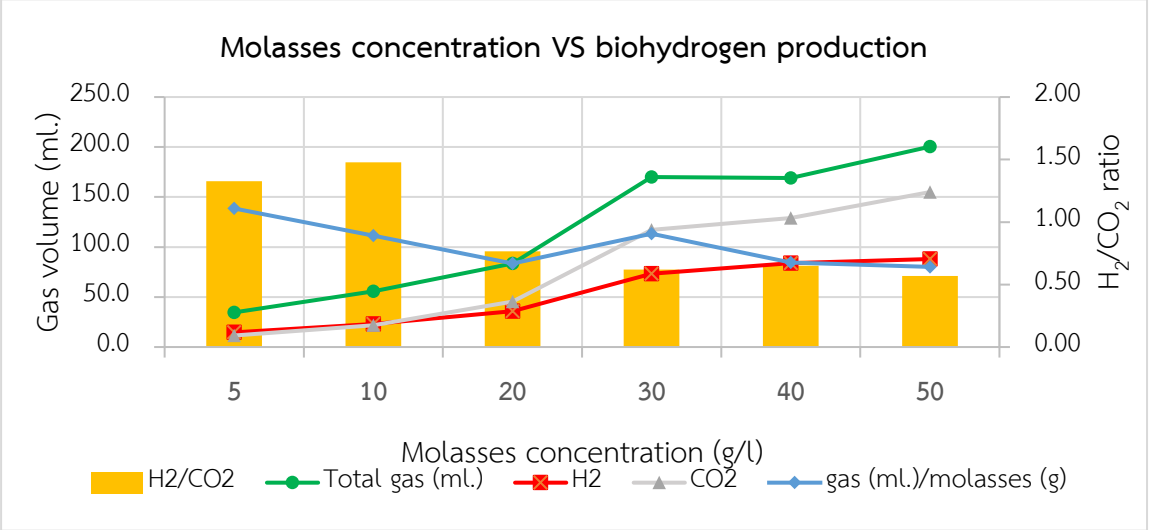


Figure 26. Biohydrogen production effected by varying 5, 10, 20, 30, 40 and 50 g/l Molasses concentration of 50 ml in 100 ml serum bottles

To determine the optimum molasses concentration for this experiment biohydrogen (H_2) production process. There are four conditions which are considered: Total gas production, H_2/CO_2 ratio, H_2 yield and Economic yield in term of gas/molasses (ml/min).

1. Total Gas Production: Consider the total gas production as well. In this experiment, even though the maximum total gas 200.4 ml obtained from 50 g/l molasses concentration, H_2/CO_2 ratio 0.57 and gas/molasses (80.1 ml/g) compared with 40 g/l molasses concentration yielded total gas 168.9 ml, H_2/CO_2 ratio 0.65 and gas/molasses (84.5 ml/g). It means that the 40 g/l molasses concentration resulted in higher value comparing to 50 g/l molasses concentration.

2. H_2/CO_2 Ratio: The H_2/CO_2 ratio is an important indicator of the efficiency of H_2 production. A higher ratio indicates a greater proportion of H_2 relative to CO_2, which is generally desirable. In this case, the 40 g/l molasses concentration resulted in a slightly higher H_2/CO_2 ratio (0.65) compared to H_2/CO_2 (0.62) derived from 30 g/l molasses concentration. This suggests that 40 g/l molasses concentration may be slightly more favorable in term of H_2 purity.

3. Hydrogen Yield: Biohydrogen yield, expressed as ml of H_2. The highest H_2 amount indicates that the H_2 metabolic pathway preferred. Comparing 40 g/l of molasses concentration yielded 84 ml of H_2 which is higher than 73.4 ml of H_2 derived from 30 g/l of molasses concentration.

4. Economic and Practical Considerations: in term of volume of gas yield per gram of molasses, provides insight into the efficiency of substrate utilization. A higher biohydrogen yield indicates that more H_2 is produced per unit of molasses consumed. In this case, 30 g/l molasses concentration resulted in a higher gas yield (113 ml/g) compared to 40 g/l (84 ml/g). This suggests that 30 g/l molasses concentration is more efficient in converting molasses into H_2.

Given the data obtained from the experiment, it appears that 30 g/l molasses concentration may be more favorable in terms of biohydrogen yield and total gas production, while 40 g/l molasses concentration slightly outperforms in terms of H_2/CO_2 ratio. The choice between the two concentrations depends on the specific goals and priorities. In this case the H_2/CO_2 ratio is much important because the H_2/CO_2 ratio should be close to the H_2/CO_2 for methanol synthesis approach which is required H_2/CO_2 ratio 3 as referenced in Equation 2-17. Thereby the prioritize of H_2/CO_2 is primary concern and 40 g/l molasses would be the optimum condition for the next step. However, 30 g/l molasses concentration did not absolutely neglect. Ultimately, the decision should be based on a combination of these factors and aligned with thesis specific objectives for H_2 production which fitted to biomethanol synthesis. It's also a

good practice to conduct additional experiments and optimizations to confirm the findings and ensure consistency in the results.

4.1.2.2 Effect of salts (CaO, MgO and KOH)

Calcium oxide (CaO), magnesium oxide (MgO), and potassium hydroxide (KOH) are chemicals that can influence hydrogen (H_2) production and the H_2/CO_2 ratio in anaerobic fermentation processes, depending on how they are used and their concentrations. Here's how each of these chemicals can potentially affect the process:

1. Calcium Oxide (CaO): CaO, also known as quicklime, is an alkaline compound. When added to a fermentation system, it can raise the pH of the medium. pH control is essential in anaerobic fermentation, as it can affect the metabolic pathways of microorganisms. In some cases, a moderate increase in pH within the optimal range for the microorganism can enhance its metabolic activity and potentially lead to increase H_2 production. However, excessive alkalinity can be detrimental and inhibit fermentation, so careful control of pH is crucial.
2. Magnesium Oxide (MgO): MgO is another alkaline compound that can raise the pH of the fermentation medium. Similar to CaO, it can influence the metabolic activity of microorganisms. Like CaO, the effect of MgO on H_2 production and the H_2/CO_2 ratio depends on the specific microorganism, the fermentation conditions, and the pH range in which the microorganism is most active. As with CaO, it's important to avoid excessive alkalinity.
3. Potassium Hydroxide (KOH): KOH is a strong base that can be used to adjust and control the pH of the fermentation medium. It can be used to increase pH if necessary. Similar to CaO and MgO, KOH can influence microbial activity and metabolic pathways by affecting pH. The impact on H_2 production and the H_2/CO_2 ratio will depend on the specific conditions and

microorganisms. Precise pH control using KOH can be beneficial for optimizing H_2 production.

In summary, the effect of CaO, MgO, and KOH on H_2 production and the H_2/CO_2 ratio in anaerobic fermentation is related to their ability to control pH. The specific impact will vary depending on the microorganisms used and the conditions of the fermentation process. Proper pH control and monitoring are critical when using these chemicals to ensure that the fermentation conditions are optimized for H_2 production. To determine the most suitable conditions for applying CaO, MgO and KOH, the experiments are performed by maintain 40 g/l molasses concentration and varying these three salts in 1, 2, 3, 4, and 5 g/l to observe biohydrogen production (ml), gas composition produced (ml), also H_2/CO_2 ratio and gas (ml)/molasses (g) are calculated and monitored. The comparison of Cao, MgO and KOH concentrations effected to biohydrogen production shown in Figure 27 - 29 and detailed data in Appendix A3, A4, and A5 respectively.

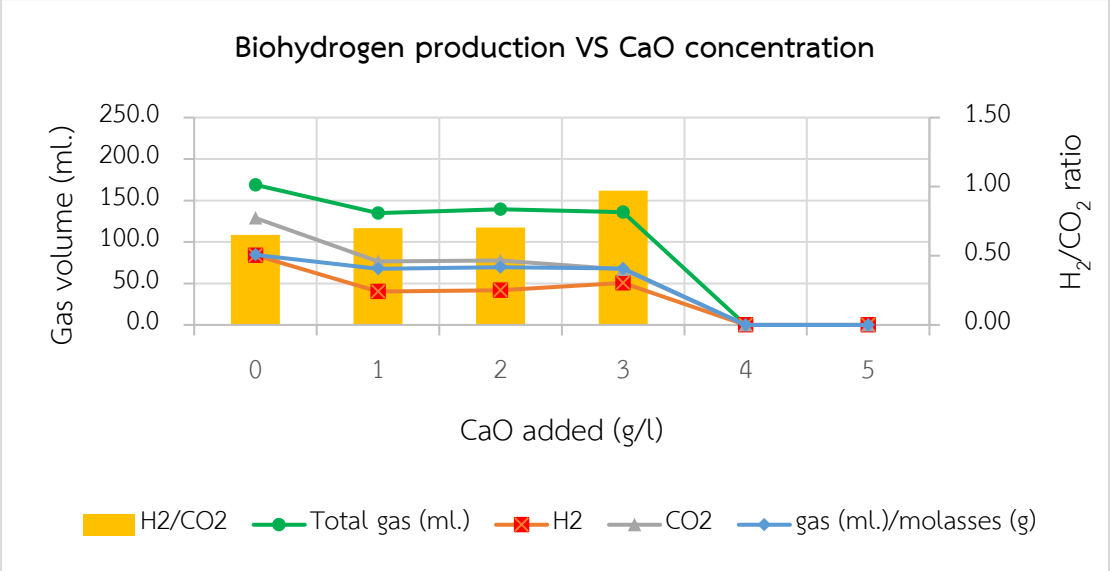


Figure 27. Biohydrogen production effected by varying 0, 1, 2, 3, 4 and 5 g/l CaO in 40 g/l Molasses concentration of 50 ml media solution in 100 ml serum bottles

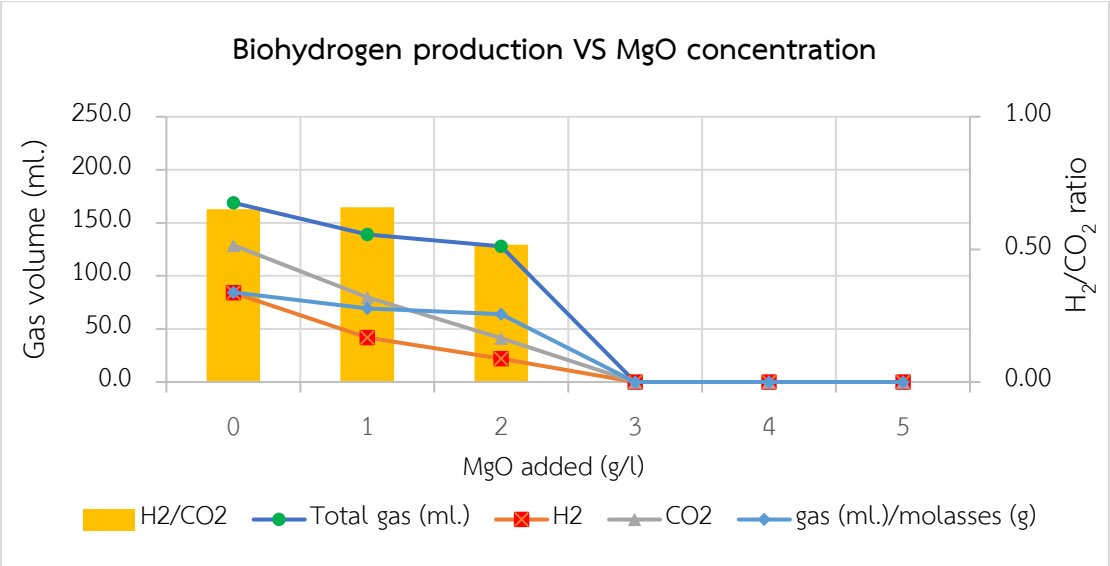


Figure 28. Biohydrogen production effected by varying 0, 1, 2, 3, 4 and 5 g/l MgO in 40 g/l Molasses concentration of 50 ml media solution in 100 ml serum bottles

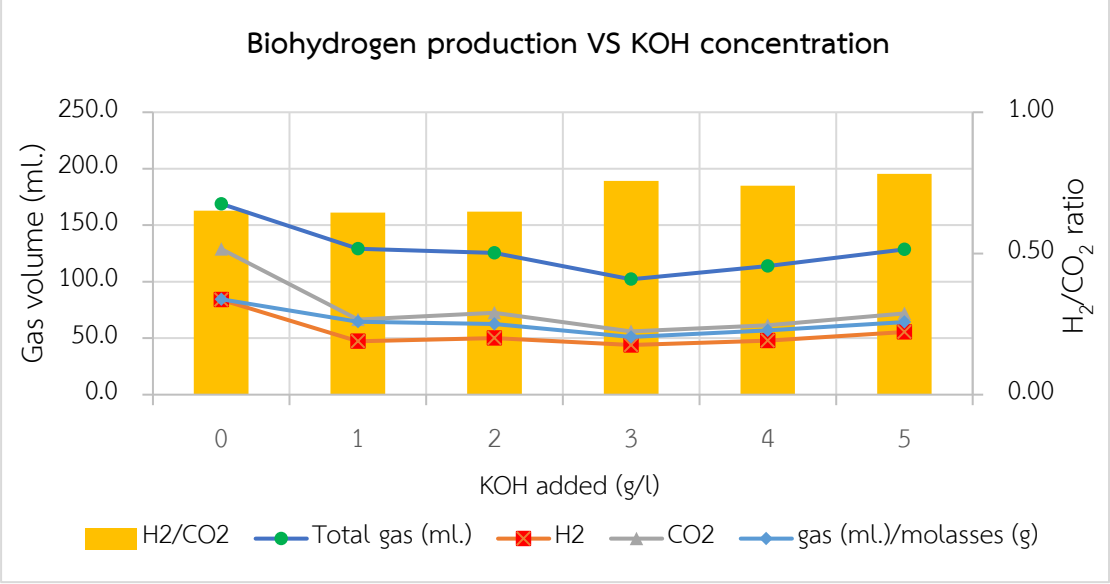


Figure 29. Biohydrogen production effected by varying 0, 1, 2, 3, 4 and 5 g/l KOH in 40 g/l Molasses concentration of 50 ml media solution in 100 ml serum bottles

The result showed adding MgO and CaO over 2 and 3 g/l terminated biohydrogen production as shown in Figure 28 and 27 respectively, but adding KOH 1 to 3 g/l effected to decreasing biohydrogen production then added KOH over 3 g/l turned up for biohydrogen production but it is still lower than no add KOH. The comparison of adding CaO, MgO and KOH effected to H_2/CO_2 ratio shown in Table 4 and Figure 30 detailed data in Appendix A6. MgO did not show the tendency effect to H_2/CO_2. The result showed H_2/CO_2 0.66 and 0.52 when added 1 and 2 g/l MgO respectively. However, the addition CaO increased H_2/CO_2 from 0.65 to 0.70, 0.71 and 0.97 when added 1, 2 and 3 g/l CaO according with KOH increased H_2/CO_2 from 0.65 to 0.64, 0.65, 0.76, 0.74 and 0.78 when added 1, 2, 3, 4 and 5 g/l KOH respectively. The addition of appropriate level of Cao and KOH promoted increasing H_2/CO_2, but it caused negative effect to reducing biogas production which it resulting to decreasing gas/molasses in both salts.

Table 4. Comparison of biohydrogen production effected by varying salts: CaO, MgO and KOH

Salts added	Gas generation (ml.)			H_2/CO_2			gas (ml)/molasses (g)		
(g/l)	CaO	MgO	KOH	CaO	MgO	KOH	CaO	MgO	KOH
0	236.5	236.5	236.5	0.65	0.65	0.65	84.5	84.5	84.5
1	189.0	194.5	181.0	0.70	0.66	0.64	67.5	69.5	64.6
2	195.0	179.0	175.5	0.71	0.52	0.65	69.6	63.9	62.7
3	190.5	0.0	143.0	0.97	-	0.76	68.0	0.0	51.1
4	0.0	0.0	159.5	-	-	0.74	0.0	0.0	57.0
5	0.0	0.0	180.0	-	-	0.78	0.0	0.0	64.3

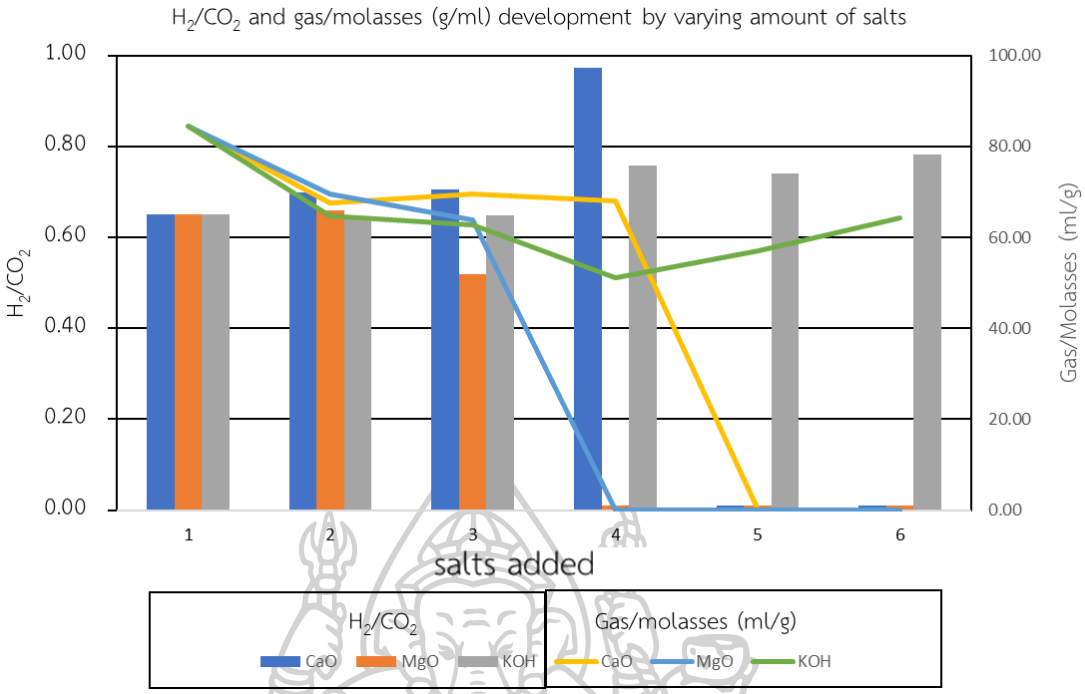


Figure 30. H_2/CO_2 and gas/molasses (g/ml) changed by varying 0, 1, 2, 3, 4 and 5 g/l salts (CaO, MgO and KOH) in 40 g/l Molasses concentration of 50 ml media solution in 100 ml serum bottles

In this thesis, the addition of calcium oxide (CaO), magnesium oxide (MgO), and potassium hydroxide (KOH) is to capture carbon dioxide (CO_2) in the form of calcium carbonate ($CaCO_3$), magnesium carbonate ($MgCO_3$), and potassium carbonate (K_2CO_3). It can be a viable approach to enhance the H_2/CO_2 ratio in anaerobic fermentation processes. This approach is commonly used to reduce CO_2 levels in biohydrogen production and other applications. It can be described as following:

1. Alkaline Conditions: When CaO, MgO, or KOH is added to the fermentation medium, they raise the pH, creating alkaline conditions. In an alkaline environment, CO_2 can react with the hydroxide ions (OH^-) present to form carbonate ions ($(CO_3)^{2-}$) and bicarbonate ions (HCO_3^-)

The carbonate formation was appeared because the carbonate ions (CO_3^{2-}) can further react with calcium (Ca^{2+}), magnesium (Mg^{2+}), or potassium (K^+) ions to form insoluble carbonate salts, such as $CaCO_3$, $MgCO_3$, or K_2CO_3. These salts were precipitate out of

the solution as solid particles and the liquid can be observed more turbidity. This turbidity effected to all liquid phase and interrupted to cell growth measurement by optical density (OD.)

2. CO_2 Capture: The precipitation of these carbonate salts effectively captures CO_2 from the fermentation medium in a solid form, removing it from the gas phase. This reduces the concentration of CO_2 in the gas phase and increases the H_2/CO_2 ratio.

3. H_2 Production: With lower CO_2 concentrations in the gas phase, the microorganisms involved in H_2 production may shift their metabolic pathways toward increased H_2 production, as they are less inhibited by high CO_2 levels.

4. Alkali Tolerance: It can see that overdose of CaO over 3 g/l and MgO over 2 g/l resulted in the inhibited to biohydrogen generation by E.A. that are not tolerant to the increasing of alkaline conditions.

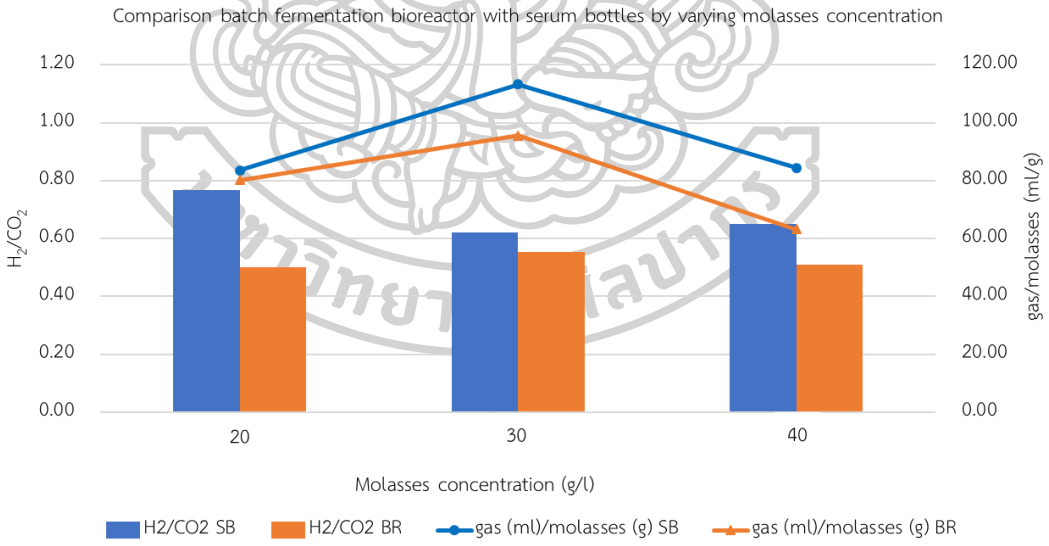
Overall, CaO have a positive effect on the E.A. conditions. it is possible to capture CO_2 as carbonate salts while boosting the H_2/CO_2 ratio in anaerobic fermentation processes, but it requires careful control and monitoring to achieve the desired results. While this approach can be effective in enhancing the H_2/CO_2 ratio when applying CaO and KOH, it's essential to carefully optimize the concentrations of these additives. Cao increased H_2/CO_2 ratio to 0.97 because 1) CaO is an effective pH adjuster and can raise the pH of the fermentation medium. 2) CaO may create a more favorable environment for this type of E.A. 3) The alkalinity provided by CaO can act as a buffer, helping to maintain a stable pH throughout the fermentation process. 4) Calcium ions (Ca^{2+}) released from CaO can also have some beneficial effects on microbial metabolism. 5) Calcium is an essential nutrient for many microorganisms and can influence enzyme activity and cell membrane stability. However, the lack of a similar effect with KOH and MgO may be due to differences in the ions introduced to the specific requirements of the E.A.

Although, the addition of appropriate level of Cao and KOH promoted increasing H_2/CO_2, it caused negative effect to reducing biogas production which it resulting to decreasing gas/molasses in both salts. In addition, the precipitation of solid particles which is observed by more turbidity. This turbidity effected to all liquid phase and interrupted to the reduction of cell growth and hard to measurement by optical density (OD.). As reasons, Salts such as CaO, MgO and KOH are not suitable for the biohydrogen improving.

4.1.3 Biohydrogen production experiment in batch fermentation

4.1.3.1 Scale up testing

The scale up was tested by using condition obtained from a 100 ml serum bottle to a 10 L bioreactor. The comparison of 20, 30, 40 g/l of Molasses concentrations were operated in serum bottle and bioreactor. The results of the scale up study are shown in Figure 31 detailed data in Appendix A7.



* SB = Serum Bottle, BR = Bioreactor

Figure 31. Comparison batch fermentation bioreactor with serum bottles by varying molasses concentration 20, 30 and 40 g/l of 50 ml and 5 L media solution in 100 ml serum bottles and 5 L bioreactor respectively

Figure 31 compares the H_2 (ml)/Molasses (g) change by varying 20, 30 and 40 g/l Molasses concentration obtained in 10 l bioreactor operated in batch mode to the one obtained at the small scale (100 ml bottles). Resulted showed that, due to scale-up effects, the gas (ml)/Molasses (g) of 10 l decreased from 83.57 to 80.10, 113.33 to 95.60 and 84.46 to 63.25 when applying 20, 30 and 40 g/l Molasses concentration comparing with 100 ml serum bottles. Although the gas (ml)/Molasses (g) decreased in all molasses concentration, but it decreased in the same pattern which it showed the maximum gas (ml)/Molasses (g) at loading 30 g/l Molasses concentration. The H_2/CO_2 ratios are compared by varying 20, 30 and 40 g/l Molasses concentration obtained in 10 l bioreactor operated in batch mode to the small scale (100 ml bottles). The result showed that, due to scale-up effects, the H_2/CO_2 of 10 l decreased from 0.77 to 0.50, 0.62 to 0.55 and 0.65 to 0.51 when applying 20, 30 and 40 g/l Molasses concentration comparing with 100 ml serum bottles. All conditions showed the reduction of H_2/CO_2 when scaled-up, because O_2 was contaminated around 1.2 to 1.5 % in all case of batch fermentation in bioreactor. The reasons are described as following:

1. Small-scale experiments in serum bottles have a higher surface area-to-volume ratio compared to large bioreactors. This increased surface area can facilitate faster equilibration with the external atmosphere, potentially allowing for more efficient removal of any residual oxygen during the initial purging step. In contrast, larger bioreactors may have a slower rate of equilibration, making it more challenging to eliminate traces of oxygen.
2. As mentioned earlier, scale can influence H_2 production. Smaller-scale serum bottle experiments may have a higher surface area-to-volume ratio, potentially leading to more efficient gas exchange and different microenvironmental conditions compared to larger bioreactors.
3. Dissolved Oxygen Sources: The source of dissolved oxygen may differ between the two setups. In a 10-liter bioreactor, there may be larger volumes of air or gas trapped within the system, which can contain residual

oxygen. In contrast, smaller serum bottles may have less trapped air or gas, reducing the potential for oxygen ingress.

4. Gas Transfer: The efficiency of gas transfer (e.g., gas sparging, agitation, and mixing) in the bioreactor can impact the rate of H_2 production. Inadequate gas transfer can lead to lower H_2 yields in the bioreactor.
5. Metabolic Differences: This excess Oxygen in the bioreactor promoted the growth of Enterobacter aerogenes as type of facultative bacteria over H_2 metabolic pathway.

This oxygen contaminated may be corrected by increasing the duration and rigor of inert gas purging during the setup of the bioreactor to ensure the removal of oxygen from all areas.

4.1.3.2 Effect of addition trace elements ($NiCl_2$ and $FeSO_4.7H_2O$)

Since the trace elements (TE) were added in very small quantities in the range of 0 to 0.1 g/L, the test was carried out in a 10 L bioreactor system at 50% working volume in order to clearly observe the results and reduce the amount of scale errors from small size obtained from the serum bottles. The trace element modification test was carried out after testing the variation of molasse concentration in batch fermentation in Section 4.1.3.1 by varying the molasses concentration to levels of 20, 30, and 40 g/l by each concentration was added with 0.01 g/l trace elements consisting of $NiCl_2$ and $FeSO_4$. The results are shown in Figure 32 to Figure 34.

Figure 32 shows that when using higher molasses concentrations of 20, 30, and 40 g/l, cell growth decreased slightly, respectively, as measured by the Optical Density (O.D.) value. 1.1, 1.08 and 0.92, but when 0.01 g/l trace element was added it helped to increase the number of cell growth to 1.1, 1.42 and 1.98 respectively. The increase in the number of cells had the effect of promoting the increase of a higher hydrogen production rate which the results are shown in term of H_2/CO_2 ratios. It was found that using molasses concentrations of 20 and 30 g/l, when adding 0.01 g/l trace element,

H_2/CO_2 increased from 0.50 to 0.99 and 0.55 to 0.96, but when the molasses concentration was as high as 40 g/l, the H_2/CO_2 ratio decreased from 0.51 to 0.45. The decrease of H_2/CO_2 at higher molasses concentrations resulted because E.A. uses nutrients to increase cell proliferation rather than the path of hydrogen production, namely the generation of more carbon dioxide. This can be seen from Figure 33 showing the increase in gas when adding TE by increasing the molasses concentration at 20, 30 and 40 g/l. Biohydrogen gas produced was 8.01 to 8.24, 14.34 to 16.52 and 12.65 to 26.92 liters, respectively. It was also found that the H_2/CO_2 ratio increased even more when TE was added. But when the molasses concentration was increased to 40 g/l when TE was added, it was found that the increase in % CO_2 gas was greater than % CO_2 obtained from 20 and 30 g/l of molasses concentration resulting in the decrease of H_2/CO_2 ratio from 0.51 to 0.45. By the way the increase in the ratio of H_2 and CO_2 in various conditions changed when the molasses concentration was increased from 20, 30, and 40 g/l and the addition of TE can be shown in Figure 34. H_2 (%) increased from 16.17 to 25.07 and 24.00 to 31.37 and remained constant when the molasses concentration was 40 g/l, H_2 (%) was 25.16 and 25.05, but CO_2 (%) decreased from 32.35 to 25.27 and 43.37 to 32.79. When increasing the molasses concentration to 40 g/l, CO_2 increased from 49.49 to 55.07 %. (Detailed data in Appendix A8)

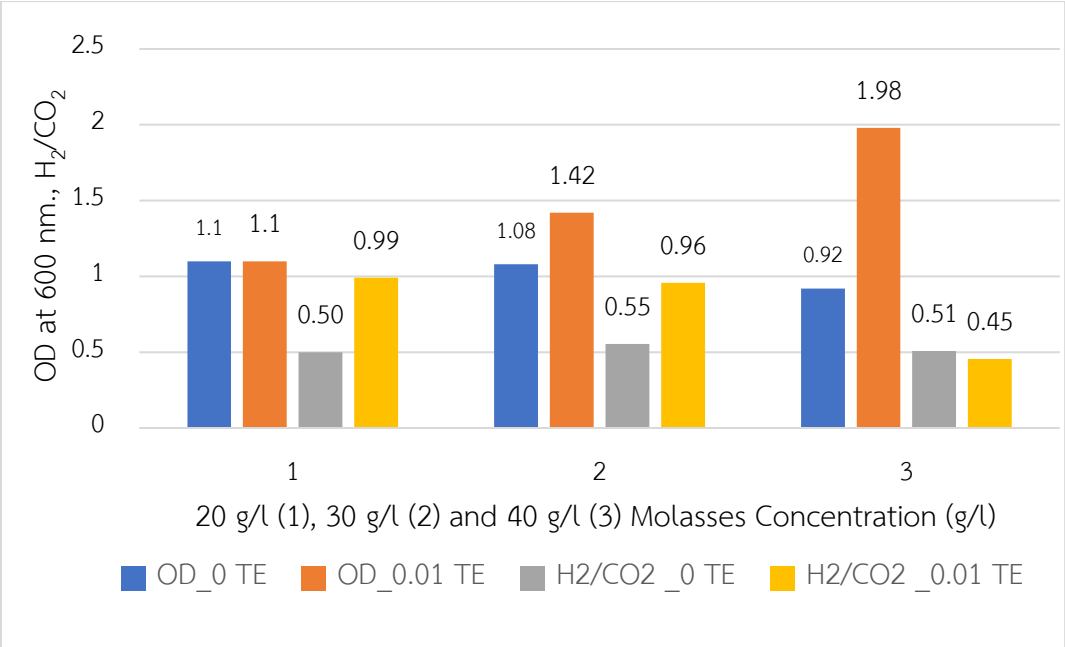


Figure 32. Comparison of Cell E.A. growth at OD 600 nm. and H_2/CO_2 when varying 20, 30 and 40 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$

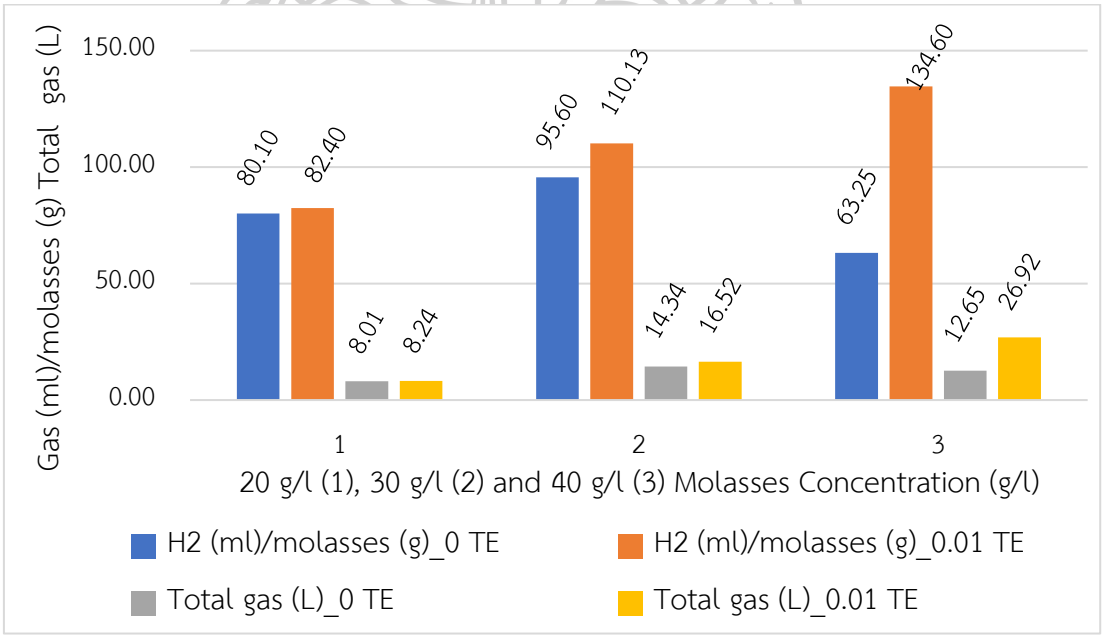


Figure 33. Comparison of Total gas production (L) and gas (ml) /molasses (g) when varying 20, 30 and 40 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$

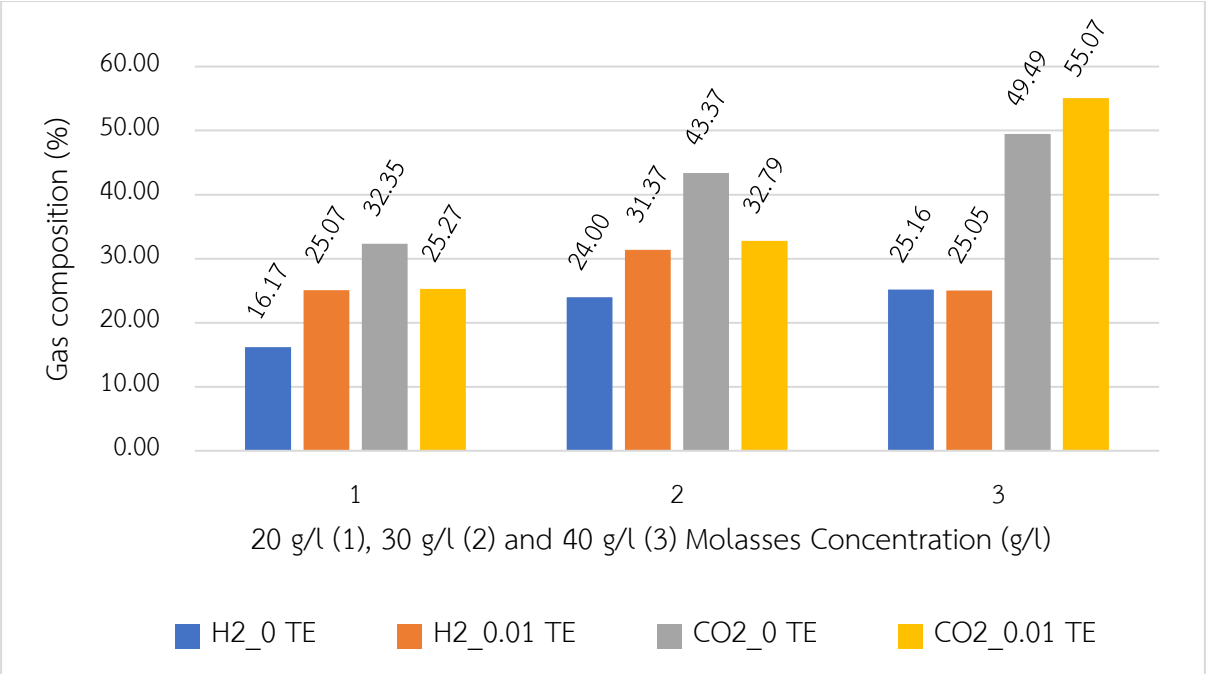


Figure 34. Comparison of gas composition (%) of H_2 and CO_2 when varying 20, 30 and 40 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$

The trace elements such as NiCl and $FeSO_4$ effected to increase the ratio of H_2 to CO_2 can be described:

Trace elements like nickel chloride ($NiCl_2$) and ferrous sulfate ($FeSO_4$) can have a significant impact on the metabolic pathways of microorganisms involved in anaerobic fermentation, including *Enterobacter aerogenes*. These elements can influence the production of hydrogen gas (H_2) and carbon dioxide (CO_2) by affecting the activity of certain enzymes and metabolic pathways. Here's how they can potentially increase the H_2/CO_2 ratio:

Hydrogenase Enzyme Activation: Nickel (Ni) is an essential cofactor for certain hydrogenase enzymes. Hydrogenase enzymes are involved in the production and consumption of hydrogen gas. The presence of Ni ions, typically supplied as $NiCl_2$, can activate hydrogenase enzymes and enhance their activity. This increased hydrogenase

activity can lead to higher hydrogen production and, consequently, an increased H_2/CO_2 ratio.

Fe-S Cluster Formation: Iron (Fe) is involved in the formation of iron-sulfur (Fe-S) clusters, which are essential cofactors in various enzymes involved in anaerobic metabolism. These enzymes play roles in electron transfer reactions during fermentation processes. The availability of Fe, often supplied as $FeSO_4$, can influence the activity of these enzymes, potentially favoring hydrogen production.

Redox Balance: Both Ni and Fe can contribute to maintaining a favorable redox balance within the cells. This balance is essential for the functioning of metabolic pathways, including those involved in hydrogen production. When the redox balance is properly maintained, it can lead to a higher yield of hydrogen gas compared to other metabolic byproducts.

Enzyme Inhibition: In some cases, the absence or limitation of certain trace elements, such as Ni or Fe, can result in the inhibition of enzymes involved in competing metabolic pathways, which may produce other byproducts like organic acids or methane. By providing these trace elements, which can potentially shift the metabolic pathway towards hydrogen production and away from these byproducts.

It's worth noting that the effects of trace elements on hydrogen production can be strain-specific and may also depend on the specific metabolic capabilities of the microorganism being used. Therefore, the optimization of trace element concentrations, as well as other fermentation conditions, should be determined experimentally for the particular strain of Enterobacter aerogenes and the desired outcome (in this case, increasing the H_2/CO_2 ratio).

Overall, trace elements like Ni and Fe can play a crucial role in promoting hydrogen production during anaerobic fermentation, and their concentration should be carefully considered and optimized to achieve the desired metabolic outcomes.

4.1.3.3 Optimization of biohydrogen with varying molasses concentrations versus trace elements (TEs) in Batch mode.

Table 5. Biohydrogen experimental conditions: TEs = Trace Elements concentrations (0, 0.01, 0.05 and 0.10 g/L) Molasses concentrations (20, 30 and 40 g/L)

No.	Conditions	Total gas (L)			H_2/CO_2 ratio		
		1st batch	2nd batch	Ave.	1st batch	2nd batch	Ave.
1.	20 g/L Molasses Not added TEs	10.50	9.50	10.00	0.37	0.33	0.35
2.	20 g/L Molasses added 0.01 g/L TEs	10.00	11.00	10.50	1.01	1.05	1.03
3.	20 g/L Molasses added 0.05 g/L TEs	9.87	10.59	10.23	0.36	0.32	0.34
4.	20 g/L Molasses added 0.10 g/L TEs	9.20	9.82	9.51	0.29	0.25	0.27
5.	30 g/L Molasses Not added TEs	14.01	14.67	14.34	0.43	0.41	0.42
6.	30 g/L Molasses added 0.01 g/L TEs	31.00	31.72	31.36	0.99	0.95	0.97
7.	30 g/L Molasses added 0.05 g/L TEs	20.48	21.32	20.90	0.35	0.41	0.38
8.	30 g/L Molasses added 0.10 g/L TEs	11.50	9.38	10.44	0.32	0.3	0.31
9.	40 g/L Molasses Not added TEs	11.60	13.70	12.65	0.53	0.49	0.51
10.	40 g/L Molasses added 0.01 g/L TEs	26.70	27.14	26.92	0.51	0.49	0.50
11.	40 g/L Molasses added 0.05 g/L TEs	17.12	16.68	16.90	0.50	0.5	0.50
12.	40 g/L Molasses added 0.10 g/L TEs	6.30	7.46	6.88	0.52	0.48	0.50

The optimization of biohydrogen was studied by investigating the combination of two parameters consisting of the molasses concentration (MC) and trace elements (TEs) concentration. The set of experiments were performed by varying the molasses concentrations of 20, 30, and 40 g/L corresponding to low, medium, and high levels, respectively, and then combining them with the trace elements (TEs) composed of 2 salt sources ($NiCl_2$ and $FeSO_4.7H_2O$) at varying concentrations of 0, 0.01, 0.05, and 0.1 g/L as shown in Table 5. The experiments were set in 12 batches and double time as 24 experiments. The primary of the measurement were gas production and gas composition then converting to the ratios of H_2/CO_2.

The results show that the molasses concentration affected the biohydrogen generation the same as the previous experiment in section 4.1.3.1-2. It was found that

using 30 g/l of molasses resulted in the best biohydrogen generation compared with molasses concentrations of 20 and 40 g/l for every concentration of TEs added the same result obtained in section 4.1.3.2. The best concentration of molasses was obtained at 30 g/l when increase the TEs concentration in the system from 0, 0.01, 0.05, to 0.1 g/l demonstrated that 0.01 g/l of TEs enhanced both the maximum biohydrogen and H_2/CO_2 ratio. As shown in Table 5, the optimal combination of the molasses concentration with the TE concentration was a 30 g/l molasses concentration required a 0.01 g/l TEs concentration, which produced the maximum gas 31.36 L and resulted in the highest H_2/CO_2 mole ratio at 0.97. Adding a TEs concentration above this level (0.01 g/l) did not increase biohydrogen production at every molasses concentration because TEs concentrations above this level retarded hydrogenase activities, resulting in constant hydrogen production, but promoted cell growth resulting in increasing CO_2 concentration as a result the lower H_2/CO_2 ratios. The optimum of the combination of molasses concentration and appropriate amount TEs is not only enhanced biohydrogen development but also increased the ratio of H_2/CO_2 up to one related to the research of Hisham et al. that the role of the metal irons, such as Fe^{2+}/Fe^{3+} and Ni^{2+}, was found in facilitating the increase of hydrogen production during the dark fermentation (Alshiyab et al., 2008).

4.1.4. Biohydrogen production experiment in continuous mode.

The optimized conditions of the process, determined in the section 4.1.3.3, were used to test the stability of the continuous system. The system was duplicate run in two consecutive modes, batch and continuous. After 48 hr in batch mode, H_2/CO_2 ratio was reached over 75% or (0.75) and the liquid in bioreactor was drawn out 1 Liter before the fresh molasses solution containing media was fed in switching from batch mode to CSTR mode. The cell density increased to maximum in 72 hours, then it decreased and obtained stability after 120 hr. The stable of the CISTR in Fed-batch was maintained in four days (until 168 hr). Figure 35 presents the Cell growth in term of optical density measured at 600 nm., Figure 35B the gas volume generation, while Figure 35C presents the H_2/CO_2 ratio over time for the two modes of operation

(delimited by the vertical dashed lines on Figure 35). Detailed data in Appendix A9-A10.

During the first 24 hr of the batch operation, cell growth was rising with steeply slope (Figure 35) as abundance of nutrient and a few percent amount of oxygen. H_2/CO_2 ratio was lower than 75% (0.65) because E.A. cell used nutrient and oxygen which promoted by TEs in increasing cell number over hydrogen production by hydrogenase pathway. The 24 to 48 hr of the batch operation, cell growth was slightly grown and almost obtained maximum optical density. The gas volume was produced to maximum volume and H_2/CO_2 ratio was increased over 75% (0.75). This stage the batch mode was switched to CSTR mode by drawn 20% of liquid volume out (1 L) and feed the fresh molasses solution containing media the same amount of volume drew out. The transition state consumed 48 to 120 hr, in the 48 to 72 hr, E.A. adjusted themselves with the new environment of new feed, they tried to balance in building new cell growth company with hydrogenase to obtain biohydrogen gas. It can be seen from the cell growth with maximum OD. and dropping down of gas volume production from about 10,000 ml to 2,000 ml, but H_2/CO_2 was reached to maximum over 0.95. In the period of 72 to 120 hours, cells of E.A. were slightly decreased until maintaining stability in range 1.05 to 1.1. Volume of gas was produced around 2,000 ml and H_2/CO_2 ratio was slightly decreased to the range (0.94-0.96) and maintaining this range.

After 120 hr (the full CSTR mode), the reactor operation was maintained in CSTR mode and the cell growth was consistency in the range of 1.05-1.10 by OD at 600 nm., the gas volume was produced about 2,000 ml and obtained H_2/CO_2 ratio in range of 0.94 to 0.96.

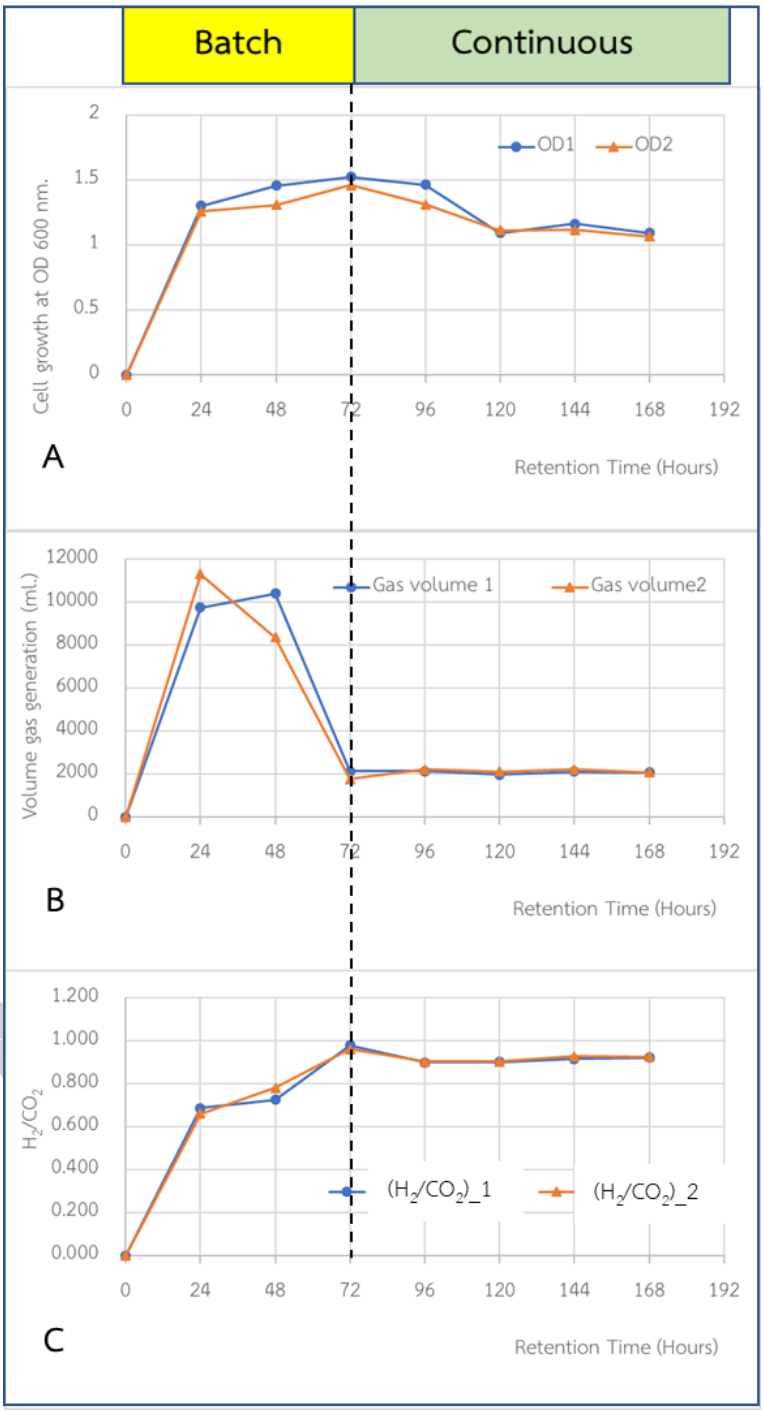


Figure 35. (A) Cell growth measured by optical density (OD.) at 600 nm., (B) Gas volume generation in Liter/day (24 hr), (C) H_2/CO_2 ratios with respect to time over the two modes of operation in batch and continuous and duplicates as shown in subscribe (1) and (2). With the optimized condition 30 g/L molasses concentration and added 0.01 g/L of Trace Elements contained $NiCl_2$ and $FeSO_4$ *Detailed data in Appendix A11.*

4.2 Biomethanol Section

4.2.1 Biohydrogen to syngas for biomethanol by RWGS

The biohydrogen gas which derived from the continuous biohydrogen experiment in section 4.14 were collected and compressed to a 2 L high pressure cylindrical container for supplying for the RWGS reaction. The objective is to compare the crude biohydrogen gas with the synthetic gas. The gas composition composed of H_2, CO_2, O_2 and N_2 with 38.59, 41.77, 3.41 and 16.23 % respectively (Detailed data in Appendix B1). The experiment was followed the method shown in section 3.2.1 The RWGS experiment used to perform the comparison crude biohydrogen with simulation synthetic gas in Table 6 (Detailed data in Appendix B2)

Table 6. Comparison of crude biohydrogen with synthetic biohydrogen by RWGS

	Gas composition (%)					Gas ratio			
	H_2%	CO_2%	O_2%	N_2%	CO%	H_2/CO_2	H_2/CO	CO/CO_2	$CO/(CO_2+N_2)$
1) Feed pure H_2/CO_2	50.0	50.0				1.00			
1) RWGS average	45.9	33.0	0.0	0.0	21.1	1.4	2.17	0.6	
2) Feed synthetic biohydrogen	38.5	41.5	3.5	16.5		0.93			
2) RWGS average	31.4	29.1	0.0	16.5	23.0	1.08	1.37	0.79	0.50
3) Feed crude biohydrogen	38.6	41.8	3.4	16.2		0.92			
3) RWGS average	31.2	29.6	0.0	16.3	23.0	1.05	1.36	0.78	0.50

The biohydrogen gas was compared with RWGS reaction (Equation 2-19) which is a capability in converting CO_2 by H_2 and obtaining CO and H_2O and yielded the mixed gas product H_2, CO and CO_2. The advantage of RWGS is the reaction which adjusted

the ratio of H_2/CO_2 and created H_2/CO, then it fitted to synthesis biomethanol following Equation 2-16. It can see that 1) Feed pure synthetic gas % (v/v) of H_2/CO_2 with 50/50 (without O_2 and N_2), 2) synthetic biohydrogen and 3) crude biohydrogen contaminated with 3.5 and 3.4 % $O_2$and 16.5 and 16.2% N_2 respectively effected to the increasing H_2/CO_2 from 1.0, 0.93, 0.92 to 1.4, 1.08 and 1.05 respectively and decreasing the H_2/CO 2.17, 1.37 and 1.36 respectively. To summarize synthetic biohydrogen gas is similar with crude biohydrogen, but the contaminant of % O_2 in Case (2 and 3) caused to create more CO_2 in the gas composition and H_2/CO_2 (2 and 3) lower than H_2/CO_2 (1), and also effected to reduce in H_2/CO from 2.1 to 1.3 which it will effect to methanol synthesis required stoichiometry of 2 as equation 2-16. In addition, this work was related that CO/CO_2 which showed around 0.6 to 0.7 for CO_2 conversion by [56]. Therefore, the synthetic pure gas without the contaminants of O_2 and N_2 will be used for the next experiments because biohydrogen process can be treated O_2 and N_2 before used as feed gas.

4.2.2. Single biomethanol reaction

4.2.2.1 Effect of H_2/CO_2 ratio on Direct CO_2 hydrogenation by adding extra H_2 in Methanol synthesis

This biohydrogen experiment yielded a gas composition with a maximum H_2/CO_2 ratio of approximately 1 (50/50%(v/v) H_2/CO_2). As of Equation (2-17), direct methanol synthesis normally requires a mole ratio of H_2/CO_2 of approximately three, therefore extra hydrogen was added to the system. To find out an optimum ratio of H_2/CO_2 that yields the maximum methanol, the effects of 3 different H_2/CO_2 ratios, 50/50, 60/40, and 70/30%(v/v), were studied. Direct CO_2 hydrogenation was performed at temperatures of 170, 200, and 230 °C with the TISTR optimum condition following section 3.2.2.1. (Constant pressure at 40 barg under 5 kg of $CuZnO/Al_2O_3$ at a total gas feed flow rate of 1 g/min (60 g/hr). Figure 4-12 showed that increasing the H_2/CO_2 ratio resulted in an increase in the methanol yield at every temperature applied: at 170 °C, the methanol yield was 9.91, 12.01, and 13.15 g/hr. at 200 °C, the yield was 16.24, 17.00, and 17.81 g/hr. and at 230 °C, the yield was 10.91, 13.01, and 14.15 g/ hr. The maximum methanol amount obtained by the optimum temperature at 200 °C.

However, there is no indication that a difference in the purity of the methanol product, which ranged from 61 to 63%, which the result was related with a report of CO_2 hydrogenation [57]. The direct CO_2 hydrogenation followed stoichiometry of Equation (17) yielded about 50% methanol percentage, but the methanol percentage obtained from this experiment over 50% because of the side reaction of CO_2 converted to CO. The side reaction involving the transformation of CO_2 into CO via RWGS appeared in parallel, as shown in Equation (19), which is related to [58]. The gas mixture containing an amount of CO in the reaction resulted in some CO hydrogenation that had a positive effect on the methanol yield following Equation (16) resulted to yield over 50%.

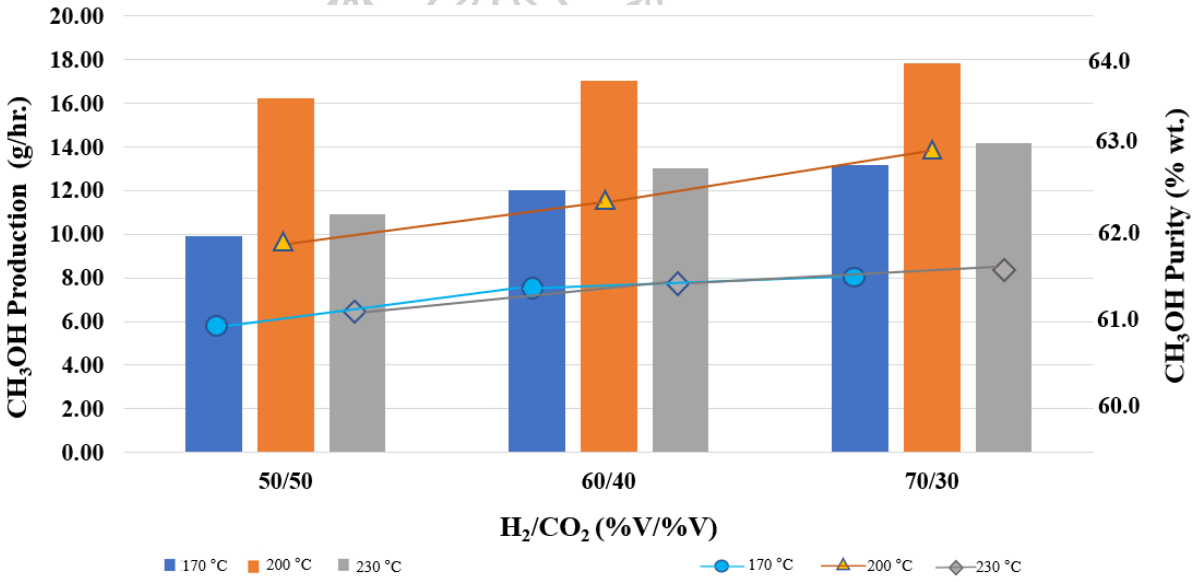


Figure 36. Direct methanol synthesis via CO_2 hydrogenation with varying H_2/CO_2 ratios at feed flow rate of 1 g/min. The bars represented methanol production (g/hr.) and the curves represented methanol purity in % by weight (Detailed data shown in Appendix B3)

4.2.2.2 Effect of H_2/CO_2 ratio on CO generation in RWGS with varying temperatures

It is clear result that Direct methanol synthesis via CO_2 hydrogenation gave low methanol production rate and contaminated with high amount of water, resulting in Figure 36. Therefore, indirect methanol synthesis via the transformation of CO_2 into CO by RWGS followed Equation (19) then CO hydrogenation followed Equation (16) may be a method to increase methanol purity. This was expected to produce a higher

methanol yield than direct methanol synthesis. Therefore, the optimum ratio of H_2/CO_2 would be the best for CO conversion via RWGS. As in the section 4.2.2.1, the biohydrogen yielded a H_2/CO_2 ratio of approximately one. Therefore, via RWGS, the H_2/CO_2 feed ratio which was a relationship with temperatures and the generated mixed gas product composed of H_2, CO_2, and CO was studied. The previous section demonstrated that adding H_2 to the biohydrogen increased the normal H_2/CO_2 % v/v or (mole ratio) of 50/50 (1/1) to 60/40(1.49/1) and 70/30(2.33/1). The three H_2/CO_2 ratios were studied in RWGS with $Cu/ZnO/Al_2O_3$ at temperatures ranging from 150 to 550 ℃ followed the method of section 3.2.1. The results in Figure 37 show that the change of % by vol. of H_2 and CO_2 (raw materials gas) for all ratios effected by increased temperature from 150 to 500 ℃, but CO_2 and H_2 in gas slowly declined when a temperature from 500 to 550 ℃ was applied. The CO generation increased from 150 to 500 ℃ and then slightly stabilized from 500 to 550 ℃. This phenomenon follows Le Châtelier's principle as the endothermic reaction; it was thermodynamically favored at higher temperatures until the CO and CO_2 contents were balanced in equilibrium in WGS and RWGS following the reverse reactions in Equations (2-18) and (2-19). In addition, increasing the H_2/CO_2 ratio improved the CO_2 conversion and favored the RWGS reaction related to [18]. The RWGS equilibrium in Equation (2-19) dominated over the WGS Equation (2-18) was clearly exhibited that % by vol. (mol) CO generation of approximately 21.20, 21.71, and 23.31 at 550 ℃ respectively. Under all conditions, CO generation increased according with applying higher temperature, but the final amounts of CO conversion differed in terms of minimum numbers in range 500 to 550 ℃. This phenomenon can be simplified by the mole of CO and CO_2 being balanced in the RWGS and WGS conditions [59]-[62]. In addition, the $Cu/ZnO/Al_2O_3$ catalyst is limited with the maximum temperature of approximately 550℃, which detailed by the specification of the catalyst company. Therefore, the optimum temperature for CO_2/H_2 in RWGS was 500 ℃, which was used in the RWGS reaction for the generation of the maximum CO and minimum CO_2 contents and for safety reasons.

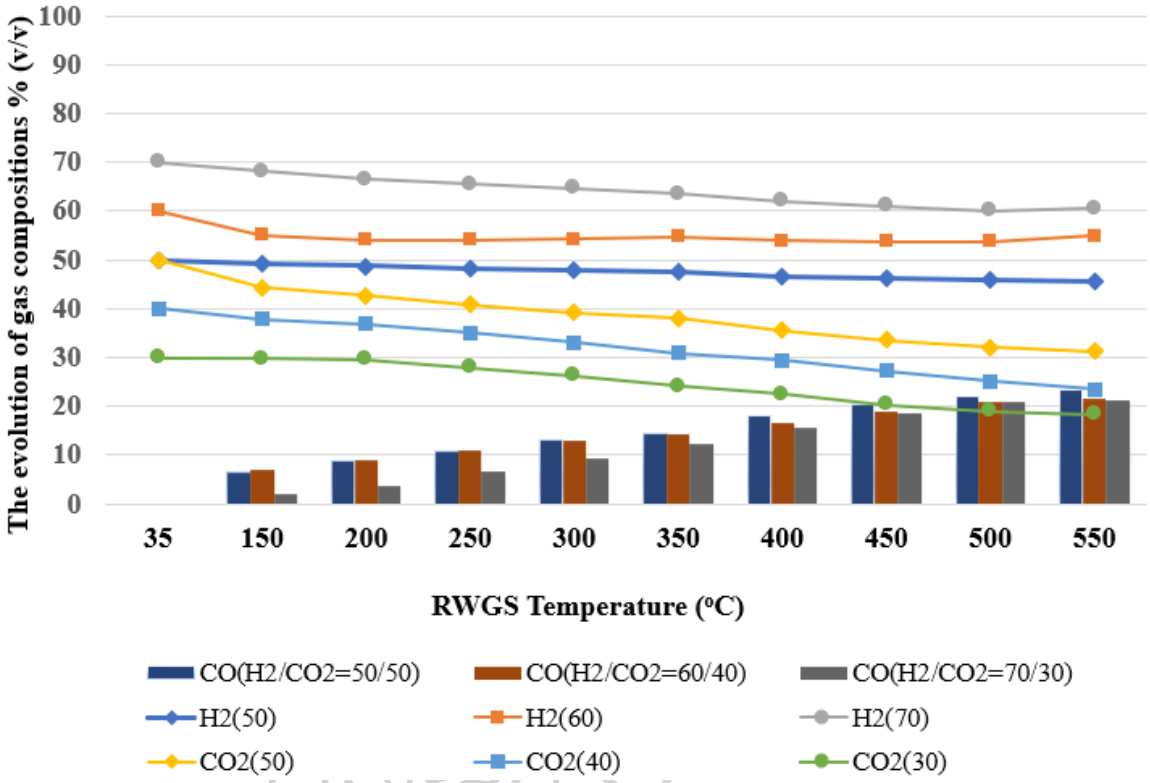


Figure 37. The changes in gas compositions with varying H_2/CO_2 ratios, and CO generation applying RWGS temperatures at feed flow rate of 5 g/min (Detailed data shown in Appendix B4)

4.2.2.3. The comparison of H_2/CO_2 ratios for methanol synthesis via two pathways: 1) MS and MS and 2) RWGS and MS

To Study the integrating method of biohydrogen and biomethanol processes in semi-pilot scale (1 Lt/D). The two-step biomethanol synthesis (TSBS) process was designed to optimize biomethanol synthesis. The experiment, the transformation of biohydrogen into biomethanol via two pathways were studied followed the method in Section 3.2.2.

Pathway 1—Direct Methanol Synthesis (DMS) + Direct Methanol Synthesis (DMS) or (MS1+MS2) as shown in Figure 38.

Pathway 2— In Direct Methanol Synthesis (IMS) comprised of RWGS + Direct Methanol Synthesis (DMS) or (RWGS1+MS2) as shown in Figure 39.

Table 7. Comparison of Pathway 1 and 2 for Biohydrogen to Biomethanol

(Detailed data in Appendix B5 to B10)

	Pathway 1 MS1_MS2			Pathway 2 RWGS1_MS2		
H_2/CO_2	50/50	60/40	7/30	50/50	60/40	7/30
CH_3OH g/min (mol/min)	0.93 (0.029)	1.04 (0.033)	1.09 (0.034)	0.69 (0.022)	1.01 (0.032)	1.05 (0.033)
H_2O g/min (mol/min)	0.31 (0.017)	0.33 (0.018)	0.43 (0.024)	0.85 (0.047)	1.81 (0.001)	2.62 (0.146)
Total liquid g/min (mol/min)	1.24 (0.046)	1.37 (0.051)	1.52 (0.058)	1.54 (0.069)	1.54 (0.069)	3.67 (0.178)
CO_2 conversion (g/min)	1.18	1.27	1.62	1.84	2.67	3.24
CO_2 consumption (%)	24.71	27.32	36.00	38.61	57.25	72.02
CH_3OH concentration (%)	78.69	75.91	71.71	95.81	97.98	92.93

Pathway 1 (DMS+DMS) was conducted as follows: Two-step direct methanol synthesis was set up with 2 methanol synthesis reactors at a feed flow rate of 5 g/min, 200 °C, and 40 barg, data were detailed as shown in Appendix B5 to B7. H_2/CO_2 feed in unit % v/v (mol ratios) of 50/50 (1.00), 60/40 (1.50), and 70/30 (2.33) were followed the experiment method section 3.2.2. The first MS reactor yielded in methanol production rate of 0.43, 0.49, and 0.49 g/min. The feed was composed only CO_2 and H_2 then the reactions was followed direct CO_2 hydrogenation by equation (2.17) which formed mixture of methanol and water around 61.43%(wt), 62.03%(wt), and 63.64%(wt) respectively. The off gas was sent of the first reactor contained mixed $H_2/CO_2/CO$ %v/v/v or (mol/min) of 44.17/45.45/10.38 (0.085/0.083/0.018), 53.52/36.80/9.67 (0.120/0.078/0.020), and 64.93/24.71/10.36 (0.195/0.070/0.028), respectively. The clear evidence was that an increase in the H_2/CO_2 feed increased the %(wt) methanol purity and methanol production rate but generated quiet no difference in CO generation which is obtained from the side reaction by following Equation (2-19). The mixed gas product that was released from the first MS reactor passed to be the feed in the second MS reactor in a series. The results showed that

the series of continual H_2/CO_2 feed ratios of 50/50, 60/40, and 70/30 (%v) generated percentage of CO in the mixed gas and improved higher mole ratio of H_2/CO (4.70, 6.11, 6.92) than H_2/CO_2 (1.03, 1.54, 2.78) which reinforced the methanol synthesis via CO hydrogenation following Equation (2-16). As a result, the methanol concentration in the second step obtained a higher yield compared with the first step in which the methanol purity was 93.29%(wt), 94.16%(wt), and 80.39%(wt), also the methanol production rates were 0.50, 0.55, and 0.60 g/min. The mixed syngas contained $H_2/CO_2/CO$ %v/v/v or (mol/min) derived from the second reactor obtained 35.59/62.43/1.98 (0.050/0.082/0.003), 49.02/49.35/1.63 (0.080/0.077/0.003), and 63.94/31.64/4.41 (0.140/0.065/0.009), respectively. There was evidence that the mixed feed gas contained CO supported the selectivity of the CO hydrogenation (Equation 16) or CO_2 hydrogenation (Equation 17). The first effect was that a lower percentage of CO_2 in the feed gas increased both the methanol production rates and methanol purity (%), and the second effect was that a higher H_2/CO ratio in the feed gas composition promoted Equation 16 by competing with Equation 17. However, the H_2/CO_2 ratio was close to 3, the H_2/CO ratio was over 2 following stoichiometry, and the methanol synthesis favored CO_2 hydrogenation (Equation 17) over CO hydrogenation (Equation 16), as shown in the case of 70/30%(v/v) H_2/CO_2 feed in the second reactor.

Pathway 2 (IMS+DMS), which applied RWGS before MS, was conducted The first reaction with the RWGS reactor was operated at a feed flow rate of 5 g/min, 500 °C, and atm pressure with H_2/CO_2 feed ratios of 50/50 (1.00), 60/40 (1.50), and 70/30 (2.33). Then, it was connected with the second methanol synthesis reactor in series at 200 °C and 40 barg, The experiments were followed method in section 3.2.2 and the results are revealed in Appendix B8 to B10. The higher ratio of H_2/CO_2 fed in the RWGS displayed higher conversion by following Equation (2-19) which yielded 0% methanol concentration, only water derived of 0.82, 1.79, and 2.54 g/min, and mixed $H_2/CO_2/CO$ gas containing % (v/v/v) or (mol/min) 37.08/39.26/23.66 (0.065/0.067/0.039), 51.23/26.73/22.05 (0.095/0.046/0.036), and 62.68/17.70/19.62 (0.115/0.030/0.032), respectively. The RWGS transformed CO_2 to CO by RWGS Equation 19 equating with the WGS by Equation 18 which resulted to balance the ratio of CO_2 and CO. The equilibrium of RWGS and WGS showed a slightly reduction in the % CO (mol) generation as 23.66(0.039), 22.05(0.036) and 19.62(0.032); however, whereas it

improved H_2/CO ratios 1.73, 2.56, and 3.53 respectively which favored the methanol synthesis via CO hydrogenation following Equation 16. The mixed gas that was released from the first RWGS reactor was collected in the LPT tank, and the CP compressed the low-pressure gas to high pressure collected in HPT, then fed into the second MS reactor in series. The methanol concentration in the second step derived higher purity percentages of 95.81%(wt), 97.98%(wt), and 92.93%(wt) and methanol yields of 0.69, 1.01, and 1.05 g/min. The H_2/CO_2/CO mixed gas obtained from the second reactor contained % v/v/v or (mol/min) 18.24/64.12/17.64 (0.020/0.067/0.018), 32.01/61.15/6.84(0.025/0.045/0.005) and 57.54/40.10/2.36 (0.045/0.029/0.002), respectively. The results are the same as those of pathway 1, caused containing CO in the mixed feed resulted in influenced the selectivity of the CO hydrogenation (Equation 16) or CO_2 hydrogenation (Equation 17). The dominance effect was that a lower percentage of CO_2 in the feed gas promoted both the methanol yield and the methanol purity %, and a higher H_2/CO in the feed gas composition advocated the dominance of CO hydrogenation over CO_2 hydrogenation. However, the H_2/CO_2 mole ratio was close to 3 or over 3, and the reaction preferred CO_2 hydrogenation following Equation (2-17), resulting in a degrade in the methanol purity, as shown in the 70/30 feed H_2/CO_2 ratio case in the 2nd reactor.

Two-step methanol synthesis between Pathway 1-DMS (MS+MS) and Partway 2 – IMS (RWGS+MS) was clearly evidence that pathway 2 produced lower methanol production rates (g/hr) of 0.69, 1.01, and 1.05 than the 0.93, 1.04, and 1.09 obtained via pathway 1, but it provided higher average methanol purities %(wt) of 95.81, 97.98, and 92.93 than the purities derived via pathway 1 (78.69, 79.10, and 72.71). The IMS pathway (adding RWGS before MS) for biohydrogen (H_2 and CO_2) can promote the methanol more purity than the only DMS pathway because CO_2 was transformed to CO by RWGS reaction following equation (2-19) and limited by WGS reaction as Equation 18, The evolution of CO_2 to CO were relied on conditions (CO/CO_2 mole ratio, temperature and pressure). The CO_2 was converted by reacting with H_2 and CO was produced obtaining the optimum H_2/CO around to 2 as the stoichiometry followed Equation 16. This phenomenon was the experiment of H_2/CO_2 (60/40) % (v/v) showed the optimum condition of generating high purity of biomethanol (97.98%). Otherwise, the mole ratio of H_2 to CO over than 2 was derived from higher ratio of H_2/CO_2 in raw

gas feed as 70/30% (v/v) or 2.33/1 (mol/mol), this scene generated higher ratio over than 3 of both H_2/CO_2 (3.74) and H_2/CO (3.53). Aftermath, CO_2 hydrogenation (Equation (2-17) will compete with CO hydrogenation (Equation (2-16), the methanol was degraded by generating water mixed with methanol resulting to lower methanol purity as 92.93%. In conclusion, the IMS would be expected to provide a higher methanol yield than DMS.

4.3. The comparison of OPEX of biohydrogen to biomethanol with biogas to biomethanol

In this thesis, data of molasses to biogas and to biomethanol pathway was followed the researches from (Miller & Wolin, 1974), (Zhu et al., 2020), (Slotboom et al., 2020) and the detail and cost of biogas were obtained from two biogas companies in Thailand, namely RE Power Service Co., Ltd. and Tanachewasap Co., Ltd., Thailand. The scope of the biogas to biomethanol process can be summarized in Figure 38.

4.3.1. Biogas to Biomethanol (BGM)

The BGM process contains four-step process: 1) molasses to biogas, 2) biogas refinery, 3) refined biogas to syngas then biomethanol and 4) biomethanol refinery Jitrwung R., 2022.

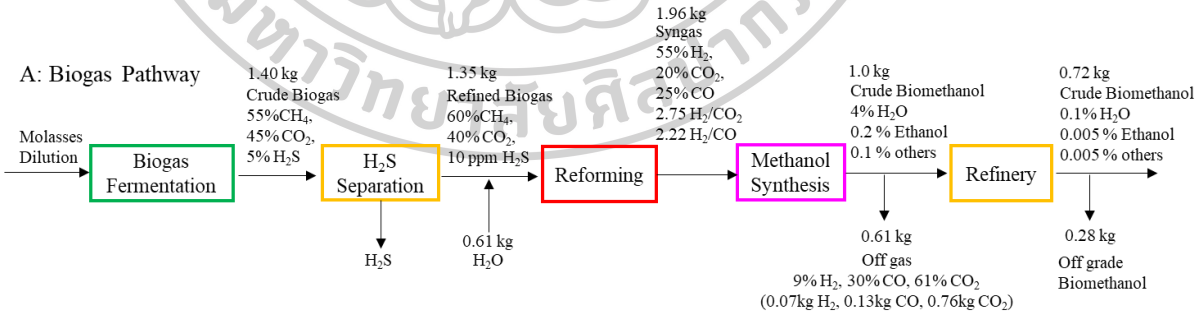


Figure 38. Box flow diagram of Molasses to Biomethanol by passing biogas direction

4.3.1.1 Molasses to crude biogas (CB)

The operating expenditure (OPEX) of crude biogas obtained by molasses fermentation in 10,000 m^3/day biogas plant (Tanachewasap Co., Ltd.,) was 3.5 THB/m^3 biogas compared with 2.0 THB/m^3 biogas produced from cassava residue in 100,000 m^3/day biogas plant (RE Power Service Co., Ltd). In this calculation, molasses was used as the raw material, therefore the higher price OPEX-CB was used at 3.50 THB/m^3 biogas (0.1 USD/m^3 biogas).

4.3.1.2 Biogas refinery (BGR)

Because the biogas produced from molasses contaminated with high concentration of hydrogen sulfide over 50,000 ppm, the two stages for H_2S removal was required from 50,000 ppm to 500 ppm then lesser than 10 ppm. The RPS biogas company provided that the OPEX (H_2S-Bio) cost was around 0.40 and 0.01 THB/m^3 biogas. As a result, the concentration of refined biogas remaining at 60% CH_4 and 40% CO_2 based on the total OPEX-BGR was 4.00 THB/m^3 biogas (0.114 USD/m^3 biogas).

4.3.1.3 Refined biogas to syngas (BS) and Syngas to biomethanol (SM)

The data obtained from Jitrwung et.al [56] provided that 1 kg of crude biomethanol refined and obtained 0.72 kg refined biomethanol and 0.28 kg off grade biomethanol, the OPEX-BMR (Biomethanol Refinery) was 0.60 THB/kg biomethanol (0.0171 USD/kg biomethanol).

4.3.2. Biohydrogen to Biomethanol (BHM)

The BHM process contains three-step process: 1) molasses to biogas, 2) refined biogas to syngas then biomethanol and 3) biomethanol refinery as shown in Figure 39.

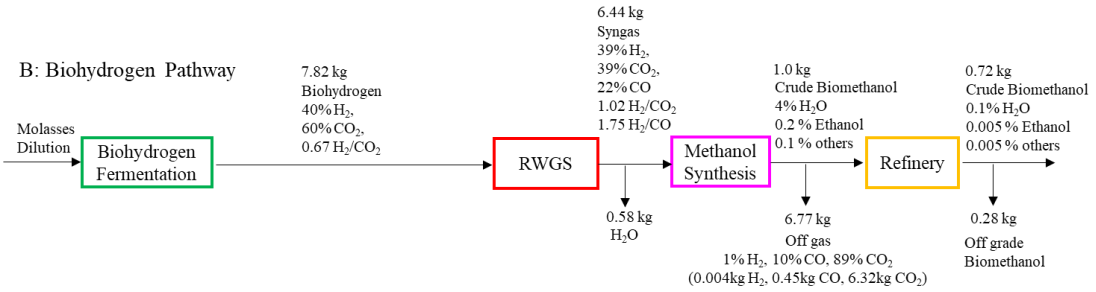
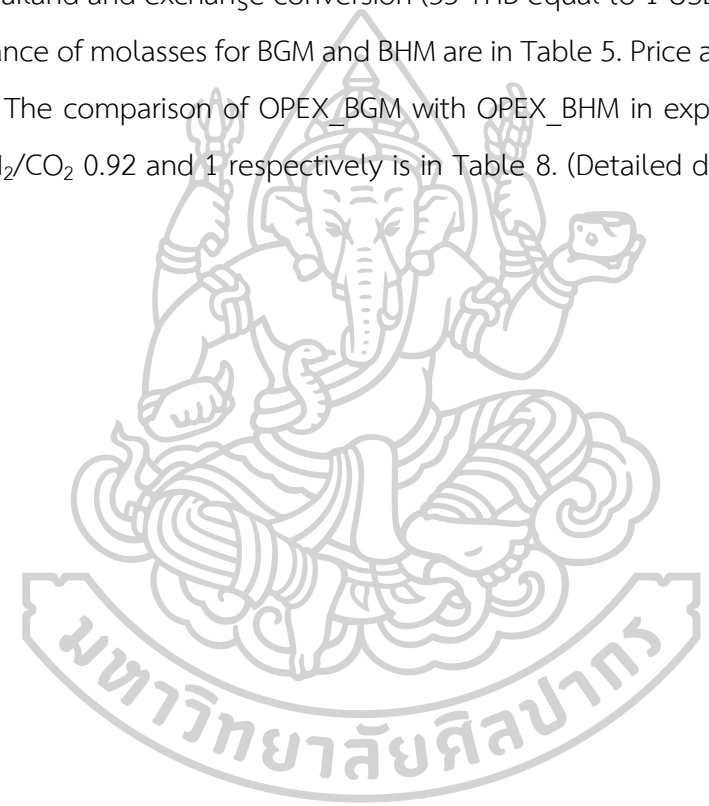


Figure 39. Box flow diagram of Molasses to Biomethanol by passing biohydrogen direction

The optimal condition of biohydrogen production was experimented with working volume 5 L in a 10 L batch reactor obtained from section 4.1 and biomethanol synthesis section 4.2 which was used to evaluate the OPEX of this research. The optimal condition yielded H_2/CO_2 around 0.92 and close to theoretical H_2/CO_2 (1), thereby this OPEX_BHM used H_2/CO_2 ratio 1 as of theoretical for the first calculation and then transform the factor of 1 to 0.92 and compared with OPEX_BGM for two cases (experimental and theory). The price of raw materials and energy are based on prices in Thailand and exchange conversion (35 THB equal to 1 USD). The material and energy balance of molasses for BGM and BHM are in Table 5. Price assumption is shown in Table 6. The comparison of OPEX_BGM with OPEX_BHM in experiment and theory based on H_2/CO_2 0.92 and 1 respectively is in Table 8. (Detailed data in Appendix C1-C2).



4.3.3 OPEX comparison

Table 8. Materials and energy balance of molasses to biomethanol by biogas and biohydrogen pathway

	Pathway A	**Pathway B**	
	Biogas	**Biohydroen**	
	CH_4/CO_2 (50/50)	Theory (H_2/CO_2)=1	Unit
	Reference [4,17,44]		
Molasses to Biogas/Biohydrogen			
Molasses	1.35	43.72	kg
Biogas/Biohydrogen to syngas			
Biogas feed	1.35	0.00	kg
Crude Biohydrogen feed	0.00	6.61	kg
Water for SMR of Biogas	0.61	0.00	kg
Syngas to Biomethanol			
Crude Methanol	1.00	1.00	kg
Energy Used	2.00	1.40	kW
Refinery Methanol			
Crude Methanol feed	1.00	1.00	kg
Refinery Methanol Product	0.72	0.72	kg
Off-grade Methanol	0.28	0.28	kg
Energy Used	0.20	0.20	kW
By products			
Crude Organic Substances	0.00	37.11	kg

Table 9. Price assumption (based on Thailand 35THB/USD) and catalyst duration

Assumption	Price	Unit
Molasses price	0.100	USD/kg
Biogas price	0.114	USD/kg
Crude Biohydrogen price	0.598	USD/kg
Water price	0.000	USD/kg
Electrical price	0.086	USD/kWh
Catalyst A (Ni/Al_2O_3)	18.571	USD/kg
Catalyst B ($Cu/ZnO/Al_2O_3$)	13.943	USD/kg
Catalyst A duration	3	Years
Catalyst B duration	3	Years
Catalyst A	0.0005	USD/kg
Catalyst B	0.0004	USD/kg
Methanol	0.449	USD/kg
Crude Organic substances	0.143	USD/kg

Table 10. Comparison of OPEX_BGM and OPEX_BHM

	Biogas Pathway CH_4/CO_2 (50/50) %	Biohydrogen Pathway		Unit
		Experiment H_2/CO_2 (0.92)	Theory H_2/CO_2 (1)	
Chemical Cost				
Biogas Price	0.1543	0.0000	0.0000	USD/kg MeOH
Crude Biohydrogen Price	0.0000	4.2965	3.9528	USD/kg MeOH
Water Price	0.0003	0.0000	0.0000	USD/kg MeOH
Total Chemical Cost	0.1545	4.2965	3.9528	USD/kg MeOH
Energy Cost				
Energy for Methanol Process	0.1714	0.1200	0.1200	USD/kg MeOH
Energy Refinery Process	0.0171	0.0171	0.0171	USD/kg MeOH
Total Energy Cost	0.1886	0.1371	0.1371	USD/kg MeOH
Catalyst Cost				
Catalyst A	0.0007	0.0005	0.0005	USD/kg MeOH
Catalyst B	0.0008	0.0008	0.0008	USD/kg MeOH
Total Catalyst Cost	0.0014	0.0013	0.0013	USD/kg MeOH
Total OPEX	**0.3446**	**4.4349**	**4.0912**	**USD/kg MeOH**
Product Sale				
Biomethanol Sale	0.4486	0.4486	0.4486	USD/kg MeOH
Organic Substances	0.0000	5.7624	5.3014	USD/kg MeOH
Total Sale Price	0.4486	6.2110	5.7500	USD/kg MeOH
Margin	0.1040	1.7761	1.6588	USD/kg MeOH
Margin Cap	30.19	40.04	40.55	%

The OPEX for 1 kg of crude methanol by passing biohydrogen pathway experiment and theory were 4.4349 and 4.0912 USD compared with that of passing biogas pathway 0.3446 USD based on commercial methanol price 0.449 USD/kg. The OPEX of biohydrogen pathway were more cost than that of the biogas route approximated 12.82 and 11.87 times, respectively. However, biohydrogen generated by-products such as valuable organic substances which was be valuable (which contains ethanol, acetic acid, propanoic acid, and pyruvic acid, as reported by Ren et al., (2006)), then adding this by products, which were produced by the biohydrogen pathway, the total sale prices per kg biomethanol produced were 6.2110 USD (Exp.) and 5.7500 USD (Theory) compared with only single biomethanol obtained from biogas route at 0.4486 USD/kg of produced biomethanol. The margin caps were evaluated around 30.19%, 39.66%, and 40.55% for biogas pathway, biohydrogen experiment, and theory route, respectively.

Chapter 5

Conclusion and Recommendations

In this study, Molasses was used as raw material for biohydrogen which was a supply gas for a valuable product such as biomethanol. The thesis comprised three sections: molasses to biohydrogen, biohydrogen to biomethanol and preliminary operating expenditure (OPEX). The biohydrogen experiment was performed to find out the optimization condition to obtain H_2/CO_2 close to 1, and the optimum condition obtained H_2/CO_2 around 0.92. The crucial investigation was found that an optimum of trace elements ($NiCL_2$ and $FeSO_4$) promoted biohydrogen production and increasing H_2/CO_2 ratio. The optimum condition comprised 30 g/l molasses concentration and added 0.01 g/l trace elements ($NiCl_2$ and $FeSO_4$) prepared by dilution in media solution referred in the media solution preparation in chapter3. The biohydrogen production was tested with continuous fed batch bioreactor to obtain crude biohydrogen by using the optimum condition, then the crude biohydrogen gas was collected by build-up the pressure with a compressor to store crude biohydrogen gas for used in comparison with synthetic biohydrogen gas. There is almost no difference in use crude biohydrogen and synthetic biohydrogen. To discover the optimum ratio of H_2/CO_2 which is the best for biomethanol synthesis, the H_2/CO_2 was varied ratio by adding extra hydrogen to increase H_2/CO_2 ratio close to methanol synthesis reaction such CO_2 hydrogenation followed Equation 2-17 and CO hydrogenation followed Equation 2-16 which required H_2/CO_2 around 3 and H_2/CO around 2 respectively. The biohydrogen contained H_2 and CO_2 for biomethanol production from Pathway 1— DMS (1^{st} MS + 2^{nd} MS)—and Pathway 2—IMS (1^{st} RWGS + 2^{nd} MS). Pathway 1 was studied to focus on direct methanol synthesis based on CO_2 hydrogenation which yielded a methanol production rate of 1.04 g/min (0.033 mol/min), methanol purity of 79.10% wt, and a CO_2 consumption of 27.32%. Pathway 2 was focused on CO hydrogenation by an innovative route that inserted indirect methanol synthesis for transforming CO2 to CO then followed CO hydrogenation. The clear evidence that the addition of RWGS before MS, it provided higher methanol production rate of 1.04 g/min (0.032 mol/min), higher methanol purities of 97.98%wt and obtaining more CO_2 consumption of 72.02% comparing with pathway 1. As a result, higher purity biomethanol would be required

small methanol refinery approaching that of commercial methanol (99.9%) and higher CO_2 consumption which would create an opportunity to apply this innovative data for the management of CO_2 calling CO_2 utilization.

The preliminary OPEX cost of biomethanol derived from molasses by biohydrogen pathway was compared with the previous research study of biomethanol obtained from molasses by biogas pathway. The optimal condition of biohydrogen production was yielded H_2/CO_2 around 0.92 and close to theoretical H_2/CO_2 (1). Therefore, the OPEX_BHM represented to use H2/CO2 around 1 from biohydrogen and the OPEX_BGM represented to use CH_4/CO_2 around 50/50 % around 1 from commercial biogas. The price of raw materials and energy are based on prices in Thailand and exchange conversion (35 THB equal to 1 USD). The material and energy balance of molasses for BHM and BGM are calculated and then linked to evaluate OPEX from both routes. The OPEX for 1 kg of crude methanol by passing biohydrogen pathway experiment and theory were 4.4349 and 4.0912 USD compared with that of passing biogas pathway 0.3446 USD based on commercial methanol price 0.449 USD/kg. The OPEX of biohydrogen pathway were more cost than that of the biogas route approximated 12.82 and 11.87 times, respectively. However, biohydrogen route can make a profit from by-products such as valuable organic substances which contains ethanol, acetic acid, propanoic acid, and pyruvic acid as reported by (Ren et al., 2006). When adding this value cost of by products, the biohydrogen pathway, the total sale prices per kg biomethanol produced were 6.2110 USD (Exp.) and 5.7500 USD (Theory) compared with only single biomethanol obtained from biogas route at 0.4486 USD/kg of produced biomethanol. The margin caps showed good opportunity around 30.19%, 39.66%, and 40.55% for biogas pathway, biohydrogen experiment, and theory route, respectively.

Commercial methanol can be replaced by biomethanol using molasses as an abundant raw material. Molasses can be digested under bacteria by biogas and biohydrogen pathway, syngas conversion, and Methanol synthesis which obtained high purity. The choices were dependent on the following presented conditions. 1) The biogas pathway was appropriate in the case of sailing single products as biomethanol. 2) The biohydrogen pathway was suitable for adding organic refinery to obtain income from valuable organic substances along with sailing biomethanol

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Appendix

Appendix A: Biohydrogen

Appendix A1: Enterobacter aerogenes growth data VS time (Section 4.1.1)

Condition: NB, 10% Inoculum, Growth media 18 H., V_B/V_C = 0.5

	Absorbances at OD600 nm.				pH			
Hours	1	2	Average	Deviation	1	2	Average	Deviation
0	0.050	0.052	0.051	0.0010	6.46	6.45	6.46	0.007
3	0.055	0.057	0.056	0.0010	6.36	6.46	6.41	0.071
6	0.070	0.082	0.076	0.0060	6.52	6.60	6.56	0.057
9	0.304	0.308	0.306	0.0020	6.57	6.60	6.59	0.021
12	0.512	0.520	0.516	0.0040	6.60	6.62	6.61	0.014
15	0.750	0.761	0.756	0.0055	6.67	6.68	6.68	0.007
18	0.926	0.936	0.931	0.0047	6.66	6.69	6.68	0.021
21	1.102	1.110	1.106	0.0040	6.65	6.70	6.68	0.035
24	1.240	1.241	1.241	0.0005	6.75	6.75	6.75	0.000
27	1.355	1.358	1.357	0.0015	6.81	6.82	6.82	0.007
30	1.404	1.405	1.405	0.0005	6.82	6.82	6.82	0.000
33	1.404	1.403	1.404	0.0005	6.82	6.82	6.82	0.000
36	1.368	1.350	1.359	0.0090	6.75	6.75	6.75	0.000
39	1.300	1.304	1.302	0.0020	6.71	6.72	6.72	0.007
42	1.202	1.205	1.204	0.0015	6.76	6.77	6.77	0.007
45	1.115	1.120	1.118	0.0025	6.72	6.73	6.73	0.007
48	1.110	1.109	1.110	0.0005	6.77	6.73	6.75	0.028
52	1.080	1.077	1.079	0.0015	6.75	6.74	6.75	0.007
55	1.055	1.065	1.060	0.0050	6.74	6.73	6.74	0.007
58	1.070	1.080	1.075	0.0050	6.72	6.75	6.74	0.021
60	1.066	1.064	1.065	0.0010	6.70	6.73	6.72	0.021

Appendix A2: Biohydrogen production by varying molasses concentration (Section 4.1.2.1)

Molasses (g/l)	Molasses (mol/l)	Total gas (ml)	H_2 (ml)	CO_2 (ml)	H_2/CO_2	gas / molasses (ml/g)	Bio-H_2 /molasses (ml/g)	H_2 (mol/l)	H_2 (mol)/Molasses (mol)
5	0.028	34.6	14.7	11.5	1.33	138.6	58.74	0.0117	0.422
10	0.056	55.7	23.2	21.7	1.48	111.4	46.40	0.0185	0.334
20	0.111	83.6	35.8	45.4	0.77	83.6	35.78	0.0286	0.257
30	0.167	170.0	73.4	117.3	0.62	113.3	48.91	0.0586	0.352
40	0.222	168.9	84.0	128.9	0.65	84.5	42.00	0.0671	0.302
50	0.278	200.4	88.0	154.9	0.57	80.1	35.21	0.0703	0.253

Appendix A3: Biohydrogen production by varying CaO concentration (Section 4.1.2.2)

Condition:

Molasses concentration	40	g/l
Molasses concentration	0.222	mol/l

Cao added (g/l)	Total gas (ml)	H_2 (ml)	CO_2 (ml)	H_2/CO_2	gas / molasses (ml/g)	Bio-H_2 /molasses (ml/g)	H_2 (mol/l)	H_2 /Molasses (mol/mol)
0	168.9	84.0	128.9	0.65	84.5	42.00	0.0671	0.302
1	135.0	40.1	76.6	0.70	67.5	20.07	0.0321	0.144
2	139.3	42.0	77.6	0.71	69.6	21.00	0.0335	0.151
3	136.1	50.9	67.1	0.97	68.0	25.43	0.0406	0.183
4	0.0	0.0	0.0	#DIV/0!	0.0	0.00	0.0000	0.000
5	0.0	0.0	0.0	#DIV/0!	0.0	0.00	0.0000	0.000

Appendix A4: Biohydrogen production by varying MgO concentration (Section 4.1.2.2)

Condition:

Molasses concentration 40 g/l

Molasses concentration 0.222 mol/l

MgO added (g/l)	Total gas (ml)	H_2 (ml)	CO_2 (ml)	H_2/CO_2	gas / molasses (ml/g)	Bio-H_2 /molasses (ml/g)	H_2 (mol/l)	H_2 /Molasses (mol/mol)
0	168.9	84.0	128.9	0.65	84.5	42.00	0.0671	0.302
1	138.9	41.8	79.8	0.66	69.5	20.90	0.0334	0.150
2	127.9	21.8	41.3	0.52	63.9	10.92	0.0174	0.078
3	0.0	0.0	0.0	#DIV/0!	0.0	0.00	0.0000	0.000
4	0.0	0.0	0.0	#DIV/0!	0.0	0.00	0.0000	0.000
5	0.0	0.0	0.0	#DIV/0!	0.0	0.00	0.0000	0.000

Appendix A5: Biohydrogen production by varying KOH concentration (Section 4.1.2.2)

Condition:

Molasses concentration 40 g/l

Molasses concentration 0.222 mol/l

KOH added (g/l)	Total gas (ml)	H_2 (ml)	CO_2 (ml)	H_2/CO_2	gas / molasses (ml/g)	Bio-H_2 /molasses (ml/g)	H_2 (mol/l)	H_2 /Molasses (mol/mol)
0	168.9	84.0	128.9	0.65	84.5	42.00	0.0671	0.302
1	129.3	47.3	66.5	0.64	64.6	23.63	0.0377	0.170
2	125.4	50.0	72.5	0.65	62.7	25.00	0.0399	0.180
3	102.1	44.0	56.0	0.76	51.1	21.99	0.0351	0.158
4	113.9	47.9	61.4	0.74	57.0	23.93	0.0382	0.172
5	128.6	55.4	71.8	0.78	64.3	27.69	0.0442	0.199

Appendix A6: Comparison of biohydrogen production effected by varying salts: CaO, MgO and KOH. (Section 4.1.2.2)

Condition:

Molasses concentration 40 g/l

Molasses concentration 0.222 mol/l

Salts added (g/l)	H_2/CO_2			Bio-H_2 (ml.)/molasses (g)			gas (ml.)/molasses (g)			H_2 (mol)/Molasses (mol)		
	CaO	MgO	KOH	CaO	MgO	KOH	CaO	MgO	KOH	CaO	MgO	KOH
0	0.65	0.65	0.65	42.00	42.00	42.00	84.46	84.46	84.5	0.302	0.302	0.302
1	0.70	0.66	0.64	20.07	20.90	23.63	67.50	69.46	64.6	0.144	0.150	0.170
2	0.71	0.52	0.65	21.00	10.92	25.00	69.64	63.93	62.7	0.151	0.078	0.180
3	0.97	0.01	0.76	25.43	0.00	21.99	68.04	0.00	51.1	0.183	0.000	0.158
4	0.01	0.01	0.74	0.00	0.00	23.93	0.00	0.00	57.0	0.000	0.000	0.172
5	0.01	0.01	0.78	0.00	0.00	27.69	0.00	0.00	64.3	0.000	0.000	0.199

Appendix A7: Comparison batch fermentation bioreactor with serum bottles by varying molasses concentration. (Section 4.1.3)

Condition:

Molasses concentration (g/l) 20 30 40

Molasses concentration (mol/l) 0.111 0.167 0.222

Molasses (g/l)	H_2/CO_2 SB	H_2/CO_2 BR	Gas (ml)/molasses (g) SB	Gas (ml)/molasses (g) BR
20	0.77	0.50	83.57	80.10
30	0.62	0.55	113.33	95.60
40	0.65	0.51	84.46	63.25

Molasses (g/l)	Gas composition in serum bottle (SB)				Gas composition in Bioreactor (BR)			
	H_2%	CO_2%	O_2%	N_2%	H_2%	CO_2%	O_2%	N_2%
20	30.1	39.3	0.0	30.6	16.2	32.3	1.5	50.0
30	30.7	49.4	0.0	19.9	24.0	43.4	1.3	31.5
40	35.5	54.5	0.0	10.0	25.2	49.5	1.2	24.1

Remark: SB = Serum Bottle BR = Bioreactor

Appendix A8: Effect of addition trace elements (NiCl2 and FeSO4) (Section 4.1.3.2)

Condition: varying 20, 30 and 40 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$.

Molasses (g/l)	OD 0 TE	OD (0.01) TE	H_2/CO_2 (0) TE	H_2/CO_2 (0.01) TE	Gas (ml)/molasses (g) 0 TE	Gas (ml)/molasses (g) (0.01) TE	Total gas (L) (0) TE	Total gas (L) 0.01 TE	$\%H_2$_0 TE	$\%H_2$_0.01 TE	$\%CO_2$_0 TE	$\%CO_2$ (0.01) TE
20	1.1	1.1	0.50	0.99	80.10	82.40	8.01	8.24	16.17	25.07	32.35	25.27
30	1.08	1.42	0.55	0.96	95.60	110.13	14.34	16.52	24.00	31.37	43.37	32.79
40	0.92	1.98	0.51	0.45	63.25	134.60	12.65	26.92	25.16	25.05	49.49	55.07

Appendix A9: Optimization of biohydrogen with varying molasses concentrations versus trace elements (TEs) in Batch mode

(Section 4.1.3.3)

Conditions: 30 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$.

Continuous Biohydrogen Run#1

30 g/l Molasses Solution loaded (L)	Solution in Bioreactor Draw out (L)	Hour	OD	pH	Gas volume (ml)	Gas/Molasses (ml/g/day)	Gas amount (ml)				Gas composition (%)				H_2/CO_2
							H_2	CO_2	O_2	N_2	H_2	CO_2	O_2	N_2	
5	0	0	0.000	6.68	0	0	0	0	0	0	0	0.23	7.81	91.96	0
0	0	24	1.300	5.69	9,750	65.00	3,036	4,425	293	1,996	31.14	45.38	3.01	20.47	0.69
0	0	48	1.454	5.24	10,400	79.60	3,966	5,459	153	823	38.13	52.49	1.47	7.91	0.73
1	1	72	1.524	4.87	2,120	70.67	899	918	72	231	42.39	43.32	3.41	10.88	0.98
1	1	96	1.461	4.20	2,110	70.33	779	866	84	382	36.92	41.02	3.97	18.09	0.90
1	1	120	1.089	5.12	1,950	65.00	745	826	60	319	38.19	42.38	3.07	16.36	0.90
1	1	144	1.160	4.70	2,100	70.00	790	862	72	377	37.60	41.05	3.41	17.94	0.92
1	1	168	1.092	4.59	2,060	68.67	787	853	68	352	38.21	41.42	3.28	17.09	0.92
Collected the gas after the steady					10,340	69.90	3,999	4,326	355	1,660	38.68	41.83	3.43	16.06	0.92

Appendix A10: Optimization of biohydrogen with varying molasses concentrations versus trace elements (TEs) in Batch mode (Section 4.1.3.3)

Conditions: 30 g/l Molasses concentration by adding 0.01 g/l of Trace Elements contained $NiCl_2$ and $FeSO_4$. Continuous Biohydrogen Run#2

30 g/l Molasses Solution loaded (L)	Solution in Bioreactor Draw out (L)	Hour	OD	pH	Gas volume ml	Gas/Molasses (ml/g/day)	Gas amount (ml)				Gas composition (%)				H_2/CO_2
							H_2	CO_2	O_2	N_2	H_2	CO_2	O_2	N_2	
5	0	0	0.000	6.70	0	0.00	0	0	0	0	0	0.21	10.07	89.72	0
0	0	24	1.255	5.54	11,300	75.33	3,423	5,535	332	2,010	32.29	48.98	0.94	17.79	0.66
0	0	48	1.307	5.16	8,350	55.67	3,393	4,350	78	529	40.63	52.09	0.94	6.34	0.78
1	1	72	1.460	5.14	1,750	58.33	735	764	61	190	42.00	43.68	3.49	10.83	0.96
1	1	96	1.311	4.06	2,200	73.33	821	909	73	396	37.31	41.33	3.34	18.02	0.90
1	1	98	1.112	4.03	2,100	70.00	784	868	80	368	37.33	41.32	3.81	17.54	0.90
1	1	120	1.113	3.77	2,200	73.33	839	905	70	386	38.15	41.14	3.18	17.53	0.93
1	1	168	1.064	4.59	2,060	68.67	790	853	64	352	38.37	41.42	3.12	17.09	0.93
Collected the gas after the steady					10,310	68.73	3,969	4,300	349	1,692	38.63	41.78	3.39	16.20	0.92

Appendix A11: Comparison of Continuous Biohydrogen Run#1 and Run#2

30 g/l Molasses Solution loaded (L)	Solution in Bioreactor Draw out (L)	Hour	OD1	OD2	Gas volume 1 ml	Gas volume2 ml	Gas/Molasses run#1 (ml/g/day)	Gas/Molasses run#2 (ml/g/day)	(H_2/CO_2) 1	(H_2/CO_2) 2	N_2 %	N_2 %
5	0	0	0.000	0.000	0	0	0	0.00	0	0	91.96	89.72
0	0	24	1.300	1.255	9,750	11,300	65.00	75.33	0.69	0.66	20.47	17.79
0	0	48	1.454	1.307	10,400	8,350	79.60	55.67	0.73	0.78	7.91	6.34
1	1	72	1.524	1.460	2,120	1,750	55.95	58.33	0.98	0.96	10.88	10.83
1	1	96	1.461	1.311	2,110	2,200	70.33	73.33	0.90	0.90	18.09	18.02
1	1	120	1.089	1.112	1,950	2,100	65.00	70.00	0.90	0.90	16.36	17.54
1	1	144	1.160	1.113	2,100	2,200	70.00	73.33	0.92	0.93	17.94	17.53
1	1	168	1.092	1.064	2,060	2,060	68.67	68.67	0.92	0.93	17.09	17.09
					30,490	29,960	67.79	67.81	0.92	0.92	16.07	16.20

Appendix B: Biomethanol

Appendix B1: Collected gas from continuous biohydrogen to use for comparison with synthetic gas in the RWGS reaction (Section 4.2)

Data from Appendix A9 and A10

Lab No.	Gas volume ml	Gas/Molasses (ml/g/day)	Gas amount (ml)				Gas composition (%)				H_2/CO_2
			H_2	CO_2	O_2	N_2	H_2	CO_2	O_2	N_2	
Run #1	10,340	70	3,999	4,326	355	1,660	38.68	41.83	3.43	16.06	0.92
Run #2	10,310	69	3,969	4,300	349	1,692	38.50	41.70	3.38	16.41	0.92
Average	**20,650**	**69**	**3,984**	**4,313**	**352**	**1,676**	**38.59**	**41.77**	**3.41**	**16.23**	**0.92**

Appendix B2: Comparison crude biohydrogen gas with synthetic biohydrogen gas in the RWGS reaction (Section 4.2)

Lab No.	Gas composition (%)					Gas ratio			
	H_2%	CO_2%	O_2%	N_2%	CO%	H_2/CO_2	H_2/CO	CO/CO_2	$CO/(CO_2+N_2)$
1) Feed pure H_2/CO_2	50.0	50.0				1.00			
1) RWGS reaction 1 hr.	45.7	33.3	0.0	0.0	21.0	1.37	2.18	0.63	
1) RWGS reaction 2 hr.	45.9	32.9	0.0	0.0	21.2	1.40	2.17	0.64	
1) RWGS reaction 3 hr.	46.0	32.9	0.0	0.0	21.1	1.40	2.18	0.64	
1) RWGS average	45.9	33.0	0.0	0.0	21.1	1.4	2.17	0.6	
2) Feed synthetic biohydrogen	38.5	41.5	3.5	16.5		0.93			
2) RWGS reaction 1 hr.	31.6	28.9	0.0	16.5	23.0	1.09	1.37	0.80	0.51
2) RWGS reaction 2 hr.	30.8	30.0	0.0	16.4	22.8	1.03	1.35	0.76	0.49
2) RWGS reaction 3 hr.	31.9	28.4	0.0	16.6	23.1	1.12	1.38	0.81	0.51
2) RWGS average	31.4	29.1	0.0	16.5	23.0	1.08	1.37	0.79	0.50
3) Feed crude biohydrogen	38.6	41.8	3.4	16.2		0.92			
3) RWGS reaction 1 hr.	31.3	29.4	0.0	16.3	23.0	1.06	1.36	0.78	0.50
3) RWGS reaction 2 hr.	30.9	29.9	0.0	16.3	22.9	1.03	1.35	0.76	0.50
3) RWGS reaction 3 hr.	31.3	29.5	0.0	16.3	23.0	1.06	1.36	0.78	0.50
3) RWGS average	31.2	29.6	0.0	16.3	23.0	1.05	1.36	0.78	0.50

Appendix B3: Effect of H_2/CO_2 ratio on Direct CO_2 hydrogenation by adding extra H_2 in Methanol synthesis (Section 4.2.2.1)

CH_3OH (g/hr.)									
	170 °C			200 °C			230 °C		
H_2/CO_2	1	2	Average	1	2	Average	1	2	Average
50/50	10.50	9.32	9.91	17.00	15.48	16.24	11.50	10.32	10.91
60/40	11.50	12.51	12.01	16.50	17.50	17.00	12.20	13.81	13.01
70/30	13.80	12.50	13.15	18.40	17.21	17.81	13.60	14.70	14.15

% Methanol Purity									
	170 °C			200 °C			230 °C		
H_2/CO_2	1	2	Average	1	2	Average	1	2	Average
50/50	61.5	60.5	61	61.7	62.1	61.9	61.1	61.3	61.2
60/40	61.1	61.5	61.3	62.2	62.4	62.3	61.2	61.6	61.4
70/30	61.2	61.8	61.5	62.8	63	62.9	61.3	61.9	61.6

Appendix B4: Effect of H_2/CO_2 ratio on CO generation in RWGS with varying temperatures

Temperature °C	The development of gas composition (%)					
	35	350	400	450	500	550
H_2	50.0	42.8	46.0	45.5	45.9	45.5
CO_2	50.0	42.8	35.5	34.1	32.1	31.2
CO	0.0	14.5	17.9	20.3	22.1	23.3
H_2	60.0	54.8	54.0	53.8	53.8	55.0
CO_2	40.0	30.9	29.5	27.2	25.2	23.4
CO	0.0	14.3	16.6	19.0	21.0	21.6
H_2	70.0	64.5	61.0	61.0	60.2	60.5
CO_2	30.0	25.2	22.4	20.3	19.0	18.3
CO	0.0	10.3	16.6	18.6	20.9	21.2

Appendix B5: Pathway 1: combining MS1 and MS2 at constant feed flow rate of 5 g/min and H_2/CO_2 ratio of 50/50 (%v/v).

MS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v (mol/min)	%v (mol/min)	%v (mol/min)	g/min	g/min	g/min	g/min (mol/min)
H_2	50(0.11)	44.17(0.085)	35.59(0.050)	0.23	0.17	0.1	0
CO_2	50(0.11)	45.45(0.083)	62.43(0.082)	4.77	3.63	3.59	0
CO	0	10.38(0.018)	1.98(0.003)	0	0.51	0.07	0
Total gas	100(0.22)	100(0.186)	100(0.134)	**5**	4.3	3.77	0
CH_3OH	0	61.7(0.013)	93.29(0.016)	0	0.43	0.5	**0.93(0.029)**
H_2O	0	38.3(0.015)	6.71(0.002)	0	0.27	0.04	**0.31(0.017)**
Total liquid	0	100(0.028)	100(0.018)	0	0.7	0.54	**1.24(0.046)**
CO_2 conversion (g/min)							**1.18**
CO_2 consumption (%)							**24.71**
CH_3OH concentration (%)					61.43	92.59	**78.69**
H_2/CO_2 (mol/mol)	**1**	**1.03**					
H_2/CO (mol/mol)		**4.7**					

Appendix B6: Pathway 1: combining MS1 and MS2 at constant feed flow rate of 5 g/min and H_2/CO_2 ratio of 60/40 (%v/v).

MS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v(mol/min)	%v(mol/min)	%v(mol/min)	g/min	g/min	g/min	g/min(mol/min)
H_2	60(0.170)	53.52(0.120)	49.02(0.080)	0.34	0.24	0.16	0
CO_2	40(0.106)	36.8(0.078)	49.35(0.077)	4.66	3.42	3.39	0
CO	0	9.67(0.020)	1.63(0.003)	0	0.55	0.07	0
Total gas	100(0.276)	100(0.217)	100(0.160)	**5**	4.21	3.63	0
CH_3OH	0	62.2(0.015)	94.16(0.017)	0	0.49	0.55	**1.04(0.033)**
H_2O	0	37.8(0.017)	5.84(0.002)	0	0.3	0.03	**0.33(0.018)**
Total liquid	0	100(0.032)	100(0.019)	0	0.79	0.58	**1.37(0.051)**
CO_2 conversion (g/min)							**1.27**
CO_2 consumption (%)							**27.32**
CH_3OH concentration (%)					62.03	94.83	**75.91**
H_2/CO_2 (mol/mol)	**1.5**	**1.54**					
H_2/CO (mol/mol)		**6.11**					

Appendix B7: Pathway 1: combining MS1 and MS2 at constant feed flow rate of 5 g/min and H_2/CO_2 ratio of 70/30 (%v/v).

MS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v(mol/min)	%v(mol/min)	%v(mol/min)	g/min	g/min	g/min	g/min(mol/min)
H_2	70(0.250)	64.93(0.195)	63.94(0.140)	0.5	0.39	0.28	0
CO_2	30(0.102)	24.71(0.070)	31.64(0.065)	4.5	3.06	2.88	0
CO	0	10.36(0.028)	4.41(0.009)	0	0.78	0.25	0
Total gas	100(0.352)	100(0.292)	100(0.214)	**5**	4.23	3.48	0
CH_3OH	0	63.3(0.015)	80.39(0.019)	0	0.49	0.6	**1.09(0.034)**
H_2O	0	36.7(0.016)	19.61(0.008)	0	0.28	0.15	**0.43(0.024)**
Total liquid	0	100(0.031)	100(0.027)	0	0.77	0.75	**1.52(0.058)**
CO_2 conversion (g/min)							**1.62**
CO_2 consumption (%)							**36.00**
CH_3OH concentration (%)					63.64	80.00	**71.71**
H_2/CO_2 (mol/mol)	**2.33**	**2.78**					
H_2/CO (mol/mol)		**6.92**					

Appendix B8: Pathway 2. combining RWGS and MS at constant feed flow rate 5 g/min and H_2/CO_2 ratio 50/50 (%v/v).

RWGS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v (mol/min)	%v (mol/min)	%v (mol/min)	g/min	g/min	g/min	g/min (mol/min)
H_2	50(0.11)	37.08(0.065)	18.24(0.020)	0.23	0.13	0.04	0
CO_2	50(0.11)	39.26(0.067)	64.12(0.067)	4.77	2.96	2.93	0
CO	0	23.66(0.039)	17.64(0.018)	0	1.09	0.49	0
Total gas	100(0.22)	100(0.171)	100(0.104)	5	4.18	3.46	0
CH_3OH	0	0	95.81(0.022)	0	0	0.69	**0.69(0.022)**
H_2O	0	100(0.046)	4.19(0.002)	0	0.82	0.03	**0.85(0.047)**
Total liquid	0	100(0.046)	100(0.024)	0	0.82	0.72	**1.54(0.069)**
CO_2 conversion (g/min)							**1.84**
CO_2 consumption (%)							**38.61**
CH_3OH concentration (%)					0	95.81	**95.81**
H_2/CO_2 (mol/mol)	**1**	**1**					
H_2/CO (mol/mol)		**1.73**					

Appendix B9: Pathway 2. combining RWGS and MS at constant feed flow rate 5 g/min and H_2/CO_2 ratio 60/40 (%v/v).

RWGS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v	%v	%v	g/min	g/min	g/min	g/min

	(mol/min)	(mol/min)	(mol/min)				(mol/min)
H_2	60(0.170)	51.23(0.095)	32.01(0.025)	0.34	0.19	0.05	0
CO_2	40(0.106)	26.73(0.046)	61.15(0.045)	4.66	2.01	1.99	0
CO	0	22.05(0.036)	6.84(0.005)	0	1.01	0.14	0
Total gas	100(0.276)	100(0.177)	100(0.075)	**5**	3.21	2.18	0
CH_3OH	0	0	97.98(0.032)	0	0	1.01	**1.01(0.032)**
H_2O	0	100(0.099)	2.02(0.001)	0	1.79	0.02	**1.81(0.001)**
Total liquid	0	100(0.099)	100(0.033)	0	1.79	1.03	**1.54(0.069)**
CO_2 conversion (g/min)							**2.67**
CO_2 consumption (%)							**57.25**
CH_3OH concentration (%)					0	97.98	**97.98**
H_2/CO_2 (mol/mol)	**1.49**	**2.03**					
H_2/CO (mol/mol)		**2.56**					

Appendix B10: Pathway 2: combining RWGS and MS at constant feed flow rate of 5 g/min and H_2/CO_2 ratio of 70/30 (%v/v).

RWGS1_MS2	IN1	Out1	Out2	IN1	Out1	Out2	Total
	%v (mol/min)	%v (mol/min)	%v (mol/min)	g/min	g/min	g/min	g/min (mol/min)

H_2	70(0.250)	62.68(0.115)	57.54(0.045)	0.5	0.23	0.09	0
CO_2	30(0.102)	17.7(0.030)	40.1(0.029)	4.5	1.33	1.26	0
CO	0	19.62(0.032)	2.36(0.002)	0	0.9	0.05	0
Total gas	100(0.352)	100(0.177)	100(0.075)	**5**	2.46	1.33	0
CH_3OH	0	0	92.93(0.033)	0	0	1.05	**1.05(0.033)**
H_2O	0	100(0.141)	7.07(0.004)	0	2.54	0.08	**2.62(0.146)**
Total liquid	0	100(0.141)	100(0.037)	0	2.54	1.13	**3.67(0.178)**
CO_2 conversion (g/min)							**3.24**
CO_2 consumption (%)							**72.02**
CH_3OH concentration (%)					0	92.93	**92.93**
H_2/CO_2 (mol/mol)	**2.33**	**3.74**					
H_2/CO (mol/mol)		**3.53**					

Appendix C: Preliminary operating expenditure (OPEX)

Appendix C1: Gas compositions by varying H_2/CO_2 feed ratios.

	Unit	Feed				RWGS			
Feed	**H_2/CO_2**	**40/60**	**50/50**	**60/40**	**70/30**	**40/60**	**50/50**	**60/40**	**70/30**
H_2/CO_2	Start	0.67	1.00	1.50	2.33	0.67	1.00	1.50	2.23
H_2/CO_2	Change	0.67	1.00	1.50	2.33	1.02	1.55	2.03	3.65

H_2/CO	Product					1.75	2.16	2.48	2.95
CO_2	%	60.00	50.00	40.00	30.00	38.47	30.54	26.04	17.00
H_2	%	40.00	50.00	60.00	70.00	39.28	47.47	52.96	62.00
CO	%	0.00	0.00	0.00	0.00	22.41	21.96	21.37	21.00
flow	kg/day	7.82	6.61	5.41	4.21	6.44	5.63	4.96	3.91
CO_2	kg/day	7.57	6.31	5.05	3.79	4.59	3.76	3.10	2.03
H_2	kg/day	0.24	0.30	0.36	0.42	0.23	0.27	0.30	0.36
CO	kg/day	0.00	0.00	0.00	0.00	1.63	1.60	1.55	1.53
H_2O	kg/day					0.58	0.58	0.60	0.58
CH_3OH	kg/day								

Appendix C2: Gas compositis and biomethanol produced by varying H_2/CO_2 feed ratios.

Feed	Unit	Feed				MS (Hydrogenation)				H_2/CO_2 (Change)			
	H_2/CO_2	40/60	50/50	60/40	70/30	40/60	50/50	60/40	70/30	40/60	50/50	60/40	70/30
H_2/CO_2	Start	0.67	1.00	1.50	2.33	0.67	1.00	1.50	2.33	0.67	1.00	1.50	2.33

H_2/CO_2	Change	0.67	1.00	1.50	2.33	0.01	0.26	0.76	1.45	0.01	0.26	0.76	1.45
H_2/CO	Product					0.10	2.46	5.48	14.95				
CO_2	%	60.00	50.00	40.00	30.00	88.63	73.00	52.70	39.30				
H_2	%	40.00	50.00	60.00	70.00	1.07	19.20	40.00	56.80				
CO	%	0.00	0.00	0.00	0.00	10.30	7.80	7.30	3.80				
flow	kg/day	7.82	6.61	5.41	4.21	6.77	5.46	3.62	2.49	Conversion (%)			
CO_2	kg/day	7.57	6.31	5.05	3.79	6.32	5.07	3.23	2.09	16.56	19.70	35.95	44.69
H_2	kg/day	0.24	0.30	0.36	0.42	0.00	0.06	0.12	0.15	98.49	78.88	67.59	65.74
CO	kg/day	0.00	0.00	0.00	0.00	0.45	0.33	0.27	0.25				
H_2O	kg/day												
CH_3OH	kg/day					1.00	1.16	1.54	1.59				