ANAEROBIC DIGESTION OF CRUDE GLYCEROL FOR BIOHYDROGEN PRODUCTION

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To my dearest late father

Ahmad Bin Salim

May Allah Blessed You to Jannah

Special dedication to

My mother Che Kalthom Hassan

my father in-law Mr Haron Omar, and my mother in-law Rokiah Din

And specifically my beloved and lovely wife

Roslindawati Haron.

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ABSTRACT

Biodiesel has become one of the main interests for the diesel replacement because it is renewable, and can be produced from various sources. However, the rapid acceleration of biodiesel production consequently contributes towards the mass amount of generation of biodiesel by-product; the waste crude glycerol (CG) which consisted high amount of impurities including the heavy metal that may lead to environmental issues. This has raised the concern and also the interest for the researchers and industries to look for the best solution in managing the excessive crude glycerol. This is included to convert the CG into valuable products, chemically or biologically. Anaerobic digestion process for biohydrogen production using biodiesel waste or crude glycerol as sole substrate seems to have a great potential to be explored. Thus, this study used batch anaerobic fermentation approached using biodiesel wastewater sludge containing indigenous mixed bacteria to produce biohydrogen using CG as the main substrate, and to assess the performance of biohydrogen production under different pH, temperature, incubation time, and initial glycerol concentration. The experiments conducted with the biodiesel wastewater sludge and CG sample were collected from Vance Bioenergy Sdn. Bhd. and Carotino Sdn. Bhd, respectively. The results of the preliminary study using both pure and crude glycerol showed that the mixed culture favours crude glycerol than pure glycerol. There was no methane gas generated throughout this study based on the gas chromatography analysis. Thus, the crude glycerol is further used in this study. Results on the effect of various parameters showed that the mixed culture has successfully converted and utilized the CG up to 99% with hydrogen yield (HY) of 1.05 mol H_2/mol glycerol utilized with 10 g/l concentration of CG at 48 hours with 37°C. The optimization study showed that, the most significant parameter that influenced the high HY were the pH and temperature. The result of HY at 1.0 – 1.26 mol H_2/mol glycerol could be achieved under the pH, incubation time, and temperature range were within 7.0 – 9.0, 30 - 55 hours, and 31 – 38°C respectively.

ABSTRAK

Biodiesel telah menjadi salah satu kepentingan utama dalam menggantikan diesel kerana ia boleh diperbaharui dan boleh dihasilkan daripada pelbagai sumber. Walaubagaimanapun, pengeluaran biodiesel yang terlalu giat akhirnya menyumbang kepada penjanaan besar-besaran produk sampingan biodiesel; gliserol mentah (CG) yang mengandungi bendasing yang tinggi termasuk logam berat yang boleh menyebabkan isu-isu alam sekitar. Ia menimbulkan kebimbangan dan juga minat bagi penyelidik dan pemain industry untuk mencari penyelesaian terbaik di dalam menangani lebihan gliserol mentah tersebut. Ini termasuklah menukarkan CG kepada produk bernilai, secara kimia atau biologi. Proses penguraian anaerobik untuk pengeluaran biohydrogen menggunakan sisa biodiesel atau gliserol mentah sebagai substrat tunggal dilihat mempunyai potensi yang besar untuk diterokai. Oleh itu, kajian ini menggunakan pendekatan fermentasi anaearobik kelompok untuk menghasilkan biohidrogen menggunakan enap cemar air sisa loji biodiesel mengandungi bacteria campuran asli untuk menghasilkan biohidrogen menggunakan CG sebagai substrat utama dan juga menilai prestasi pengeluaran biohidrogen di bawah keadaan pH, suhu, masa pengeraman dan kepekatan awal CG yang berbeza-beza. Eksperimen dijalankan dengan masing-masing sampel enap cemar air sisa biodiesel dan CG diambil dari Vance Bioenergy Sdn. Bhd. dan Carotino Sdn. Bhd. Keputusan mengikut kajian awal yang menggunakan gliserol tulen dan gliserol mentah menunjukkan bahawa kultur campuran lebih sesuai menggunakan gliserol mentah berbanding gliserol tulen. Tiada gas metana dihasilkan sepanjang kajian ini berdasarkan kepada analisis kromatografi gas. Maka, gliserol mentah terus digunakan selanjutnya di dalam kajian ini. Keputusan daripada kesan pelbagai parameter menunjukkan kultur campuran telah berjaya menukarkan dan menggunakan CG sehingga 99% dengan hasil hydrogen 1.05 mol mol H_2/mol gliserol digunakan dengan kepekatan 10 g/l pada 48 jam pengeraman. Kajian pengoptimuman menunjukkan, parameter paling signifikan mempengaruhi HY yang tinggi adalah pH dan suhu. Hasil HY pada 1.0 – 1.26 mol H_2/mol gliserol dapat dicapai dengan masing-masing julat pH, masa pengeraman, dan suhu pada 7.0 – 9.0, 30 – 55 jam, dan 31 - 38°C.

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LIST OF ABBREVIATIONS

1,2-PDO	-	1,2-Propanediol
1,3-PDO	-	1,3-Propanediol
AD	-	Anaerobic Digestion
ANOVA	-	Analysis of Variance
ASBR	-	Anaerobic sequencing batch reactors
ATP	-	Adenosine Triphosphate
CG	-	Crude Glycerol
COD	-	Chemical Oxygen Demand
DoE	-	Design of Experiment
DF	-	Degree of Freedom
DHA	-	Dihydroxyacetone
DHAP	-	Dihydroxyacetone phosphate
DHAK	-	Dihydroxyacetone kinase
FAME	-	Fatty acid methyl esther
FFA	-	Free fatty acid
FHL	-	Formate hydrogen lyase
g/L	-	gram per liter
GC-FID	-	Gas chromatography – flame ionization detector
GC-TCD	-	Gas chromatography – thermal conductive detector
HPLC	-	High performance liquid chromatography
HY	-	Hydrogen Yield
kJ/g	-	kilojoule per gram
MONG	-	Matter Organic Non-Glycerin
MS	-	Mean Square
mL	-	Mililiter

mmol/L/h	-	millimole per liter per hour
OD	-	Optical Density
PG	-	Pure Glycerol
rpm	-	Rotation per minute
RSM	-	Response Surface Methodology
SS	-	Sum of Square
sp	-	Species
WCO	-	Waste cooking oil
VFA	-	volatile fatty acid

LIST OF SYMBOLS

%	-	Percent
atm	-	atmosphere
°C	-	degree Celcius
K	-	Kelvin
e^-	-	Electron
n	-	Mol of gas
P	-	Pressure of gas
V	-	Volume of gas
R	-	Gas constant
T	-	Temperature

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The surge of biodiesel production around the globe has significantly shown United State (US) and Europe as its major producer (Park, 2008, Kotrba, 2015). Figure 1.1 displays the production of biomass-based biodiesel in the US alone with an amount of 200 million gallons (757 million liters) in 2014 (Kotrba, 2015). Kotrba (2015) also highlighted that Germany alone has produced approximately 507 million gallons (1919 million liters) of biodiesel. As predicted by Deloitte (2015), the biodiesel amount is forecasted to increase to 1900 barrels in the year 2020.

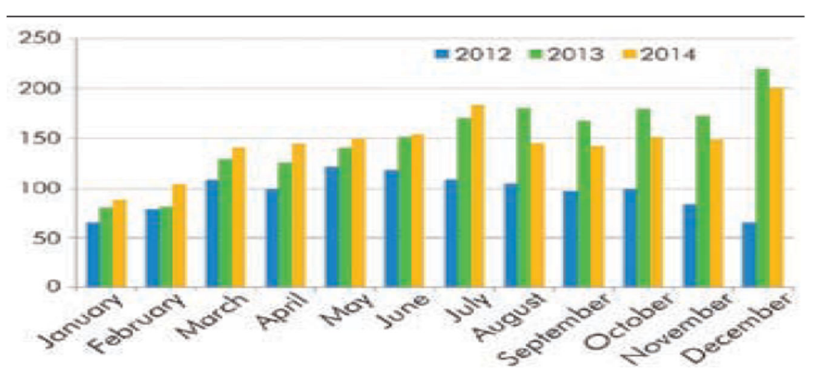

Figure 1.1 Biodiesel production 2012 – 2014 (million gallons) (Kotrba, 2015)

The general outline of biodiesel production scheme indicating significant processes is shown in Figure 1.2. More importantly, the figure provides an overview of the significant part for crude glycerol generation. In biodiesel production, vegetable oils (corn, cottonseed, crambe, peanut, rapeseed, soybean, sunflower, palm oil, coconut and animal fats (lard and tallow) are the most main commercial ingredients used (Ma and Hanna, 1999; Van Gerpen, 2005; Marchetti *et al,* 2007; Park, 2008; Borugadda and Goud, 2012; Bergmann *et al.*, 2013). However, these commercial feedstocks are expensive and some are eligible feed (Yaakob *et al.*, 2013). Thus, further advancement in biodiesel production then explored the potential of using used-cooking oil as an alternative to highly priced animal fats and fresh vegetable oils (Siles *et al*, 2010;Wan Omar and Amin, 2011; Zhang *et al*., 2003). In general, biodiesel is produced via transesterification process (Figure 1.3), that generates its by-product—crude glycerol (CG). Overall, approximately 10 kg of CG was generated from out of 100 kilogram biodiesel produced from most of the biodiesel plants (Sarma 2013; Santibanez, 2011; Chi *et al*., 2007). In Malaysia, the abundantly generated CG waste is classified as waste under Schedule S181 of the Malaysia Environmental Regulation, and sealed in drums prior to disposal at landfills (Sakinah, 2011). This scheduled disposal is costly for the collection to ensure the crude glycerol is transferred away from the biodiesel plants for disposal and incineration process (Ayoub and Abdullah, 2012)

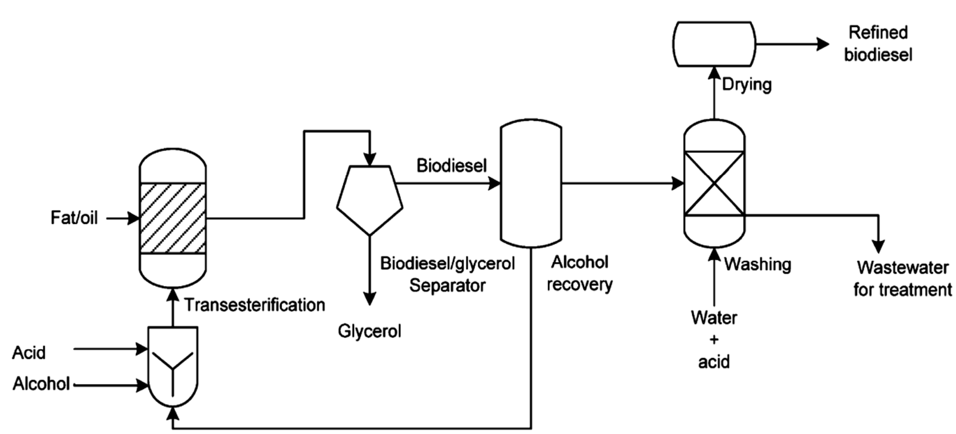

Figure 1.2 Process flow of biodiesel production via conventional transesterification process (Daud *et al*., 2014)

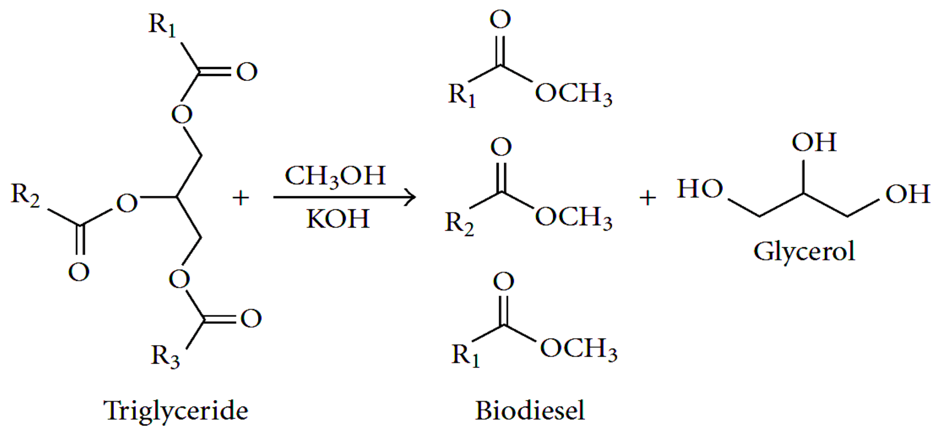

Figure 1.3 Transesterification process produce glycerol as main by-products via base catalytic reaction with methanol (Kolesárová *et al*., 2011; da Silva *et al*., 2009)

CG contains heavy metals and other contaminants (residues from transesterification process). Methanol, soap, oils, salts and solid organic materials were among contaminants reported in CG (Kumar *et al*., 2015). The impurities were complied with those reported by Yang *et al*. (2012) except they added catalysts, non-glycerol organic matter, and water impurities in the list of CG contaminants. These contaminants if not treated properly can harm the environment and thus will be costly to treat (Fernando *et al*., 2007). Some treatment process will be affected by the contaminants, as in Sulaiman *et al*. (2009) and Yang *et al*. (2012), who reported CG with a high concentration of NaCl can cause the biological treatment process to be uneffective. A common practice to destroy CG presently is by incineration, but this method leads to the production of primary green house gases like nitrogen oxide and carbon dioxide (CO_2) (Gholami *et al*., 2014; Markov *et al.*, 2011). Thus, an urgent requirement for sound management of CG-containing wastewater along with the potential of CG for biogas generation has generates interest on anaerobic treatment process (Siles *et al.,* 2010, Viana, 2012 and Hutnan *et al,*2013).

In recent years, most researchers focus on application of CG and pure glycerol for the production of value-added products (1,3-propanediol, citric acid, erythritol, polyhydroxyalkanoates, phytase, fumaric acid, fungal protein and butanol, butanol,) using mixed and pure cultures of microorganisms (bacteria and fungus) as summarized in Table 1.1

Table 1.1 : Application of CG and PG for value added product

Value added product	Glycerol type	References
Hydrogen	Crude glycerol	(Sarma *et al.*, 2013)
Hydrogen	Crude glycerol	(Ghosh *et al.*, 2012)
Methane	Crude glycerol	(Siles López *et al.*, 2009)
Methane	Crude glycerol	(Hutnan *et al.*, 2013)
Ethanol	Pure glycerol	(Legendre *et al.*, 2009)
Dihydroxyacetone	Crude glycerol	(Liu *et al.*, 2013)
Poly 3-hydroxybutyrate and bioethanol	Crude glycerol	(Shah *et al.*, 2014)
Biogas	Crude glycerol	(Nuchdang and Phalakornkule, 2012)
Biohydrogen	Crude glycerol	(Costa *et al.*, 2011)
Butanol	Crude glycerol	(Gallardo *et al.*, 2014)
1,3-propanediol and gellan	Crude glycerol	(Raghunandan, 2013)
n-butanol	Crude glycerol	(Khanna *et al.*, 2013)
Docosahexaenoic acid	Crude glycerol	(Chi *et al.*, 2007)
Lipid	Crude glycerol	(Liang *et al.*, 2010)
1,3-propanediol and ethanol	Crude glycerol	(Rossi *et al.*, 2012)
Glyceric acid and hydrogen	Crude glycerol	(Kondamudi *et al.*, 2012)
Omega-3 and carotenoids	Pure glycerol	(Adarsha Gupta *et al.*, 2013)
Hydrogen, ethanol, and diols	Crude glycerol	(Wu *et al.*, 2011)
Succinic acid	Crude glycerol	(Vlysidis *et al.*, 2011)

A quite similar scenario was also developed for biodegradation of banana peel wastewater using CG or pure glycerol (PG) as co-substrate under buffered (0.1 $NaHCO_3$) and unbuffered fermentation to observe the potential of biomethane production (Housagul *et al.*, 2014). From the analysis, they found that addition of buffer in both mixtures helps in improving the content, production potential, lag phase period, and yield of methane. They also found that glycerol concentration can influence the rate and yield of the methane produced.

According to Ma *et al*. (2008) and Wohlgemut (2011), using CG as co-substrate in anaerobic digestion gives the advantage to some other wastes. This is because, CG is degradable, contains a high level of COD, and can be stored for long periods before being used in biogas production. Nevertheless, co-digestion has weaknesses too; in terms of cost, as well as the risks and process itself. According to Astals *et al*. (2011), transporting CG or other co-substrates to the anaerobic plant can be costly. The transporting act can also lead to the risk of spreading poisonous substances. In terms of quality, co-digestion can lower the digestate quality, change the digestion behaviour, and need a proper design so the ratio between the main- and co-substrate will not affect the digestive medium.

Despite a number of studies on anaerobic co-digestion of CG and digestion of CG using pure cultures, there have been few studies on using CG as sole substrate by mixed cultures. Hutnan *et al*. (2013) for example, investigates the use of CG as the main substrate by mixed cultures from biodiesel sludge as biomethane producer. They used two types of CG in their study: acidulated CG (80% of glycerol) and untreated CG (55% of glycerol). The process using acidulated CG, was enhanced by the addition of washing water (or biodiesel wastewater). Their findings showed no significant difference between both process; they obtained about 0.328L/g chemical oxygen demand (COD) for non-acidulated CG and 0.345L/g COD for acidulated CG. They also proved that the use of CG and mixed cultures in anaerobic digestion was promising in producing biogas.

Another study by Nuchdang and Phalakornkule (2012) on the used of CG as the single substrate has been studied in a continuous digester. However, studies on using raw CG with locally isolated indigenous microorganisms in biohydrogen production is still at infancy state. Thus, this study was performed by applying batch anaerobic digestion process to transform the biodiesel waste by-product or crude glycerol to biohydrogen using locally isolated mixed microorganism.

1.2 Problem Statement

Research on the application of CG as the main substrate in biogas production that has been done so far mostly used pure cultures (Abdeshahian *et al*., 2014; Chen *et al*., 2008; Chookaew *et al*., 2011; Chookaew *et al*., 2014) as producers. According to Elsharnouby *et al*. (2013), pure cultures offer more insights on metabolic shift and conditions that can promote high hydrogen yield and production rate. However, pure cultures are not resilient enough with the change of environment, thus need to be adapted first. In addition, pure culture must also be steriled enough to avoid contamination. Secondly, the most CG-related biogas production research was done using the CG as co-substrate instead of main substrate (Mata-Alvarez *et al*., 2014). Research by Mata-Alvarez *et al*. (2014) critically discussed the function of CG as a co-digestion substrate for treatment of other waste such as olive mill waste (OMW), animal manure (pig, cow, poultry, goat, horse, etc.) organic fraction-municipal solid waste (OFMSW), and cheese whey. Another work by Kullavanijaya and Thongduang (2012) also used anaerobic digestion to treat cassava wastewater with biodiesel waste as the supplement or co-substrate it to enhance the production of biogas and methane, as well as for the chemical oxygen demand (COD) removal.

Crude glycerol (CG) from biodiesel waste contain impurities that can harm environment and ecosystem if been disposed of without proper management. The existing approaches in handling this problem are by incineration but this approach generates greenhouse gases. In order to minimize the generation of the GHG production, a better and sustainable treatment of crude glycerol is vital for generation of future renewable energy, the biohydrogen. Since research on using crude glycerol as the sole carbon source for biohydrogen production still lacking, the urge for its potential is very promising. Even though the studies by Hutnan *et al*., 2009, 2011, and 2013 have claimed that there is potential in using crude glycerol as the main carbon source in their study, their main focus was specifically on biomethane production. Additionally, their seeding inoculums were originating from suspended sludge taken from municipal wastewater treatment plant which is totally not an adapted indigenous source of inoculums for crude glycerol fermentation. Meanwhile, the study by Sarma *et al.,* 2013 mentioned clearly that the inoculum used was a commercially known single culture of *Enterobacter aerogens* NRRL B-407 for the fermentation of their crude glycerol.

Thus, in this study, to differentiate between the studies by other researchers mentioned earlier, the anaerobic digestion process is performed totally using crude glycerol as the main source of carbon for the biohydrogen production using sludge originating from the biodiesel wastewater treatment plant, which potentially well adapted with the existing crude glycerol element.

1.3 Research Objectives

This work is proposed to utilize the waste crude glycerol in the anaerobic treatment process for the potential production of biohydrogen. The main objectives for this work are:

1. To apply batch anaerobic digestion system using the mixed culture of bacteria from biodiesel wastewater sludge to utilize crude glycerol for biohydrogen production.

2. To assess the biohydrogen production from the anaerobic process using local microorganisms under four operating parameters: pH, temperature, incubation time, and initial crude glycerol concentration.

1.4 Research Scopes

The scopes of this study involved:

1. The sampling of anaerobic sludge from the biodiesel wastewater treatment plant and crude glycerol from edible oil manufacturer in Pasir Gudang, Johor.
   i. Characterization of crude glycerol samples
   ii. Sludge samples preservation to maintain its microbiological content viability

2. Develop an anaerobic digestion for batch fermentation process of the crude glycerol
   i. Batch anaerobic system setup at 37°C, with initial pH of 7 and 48 hours incubation time.
   ii. Design of Experiment (DoE) for optimum operational parameters determination: pH, temperature, incubation period, and crude glycerol initial concentration

3. Monitoring the biohydrogen production and percentage of crude glycerol consumption

1.5 Research Significance

Crude glycerol cannot be disposed into the environment without any treatment, and the cost of such treatment could be prohibitive. Methanol, one of the impurities in crude glycerol, is known to be a toxic alcohol. It can affect soil microbial flora if crude glycerol is disposed of to the environment. It can contaminate ground waters too. The sodium or potassium hydroxide residue of the crude glycerol gives it elevated pH, may endanger biotic community if crude glycerol is directly disposed of without neutralization. Moreover, the action of indigenous microorganisms will release the offensive odor that pollutes the atmospheric environment (Sarma *et al*., 2013). Therefore, to treat crude glycerol before disposal is an economically inefficient and ineffective process for biodiesel industries (Nwachukwu, 2012).

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