

BIOHYDROGEN GENERATION FROM PALM OIL MILL EFFLUENT USING
AnSBR–MEC HYBRID SYSTEM

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ABSTRACT

The global dependency towards fossil fuels as an energy source has escalated resulting in rapid depletion of oil reservoir and global warming. The oil palm industry, the largest agricultural sector in the country, is expected to generate approximately 70–110 million tonnes of solid and liquid wastes by the year 2020. The large quantities of liquid waste, or palm oil mill effluent (POME), is highly polluting due to its high content of biological and chemical oxygen demand. Since POME is rich in substrates such as carbohydrates and fatty acids, this study investigated the application of bacteria from POME anaerobic sludge for hydrogen gas (H₂) production in a hybrid system of anaerobic sequencing batch reactor (AnSBR) and microbial electrolysis cell (MEC). Five pre-treatment methods (heat-shock, acid, alkaline, chemical, and aeration) were tested for selecting H₂-producers from POME anaerobic sludge. Out of the five methods, heat-shock showed the highest H₂ production. Several factors influencing H₂ production was determined using one-factor-at-a-time (OFAT) method. The results showed highest H₂ production at initial pH 6.0, incubation temperature of 55.0°C, and 10.0% (v/v) inoculum size. Further study on the optimisation of H₂ production using Box–Behnken Design (BBD) showed that all factors significantly contributed towards H₂ production. The combination of initial pH (6.4), incubation temperature (58.0°C), and inoculum size (8.0% v/v) enhanced the H₂ production up to 2.57-fold higher (239.0 mL) compared to before optimisation. Subsequently, these optimised conditions were applied in a 1.2 L AnSBR together with two factors: hydraulic retention time (HRT) and organic loading rate (OLR). The results showed that 12 h HRT and 36 g/L/d OLR were the best condition with maximum H₂ production recorded at >2.80 L H₂/L POME and almost 65% COD removal achieved in 16 cycles of operation. Single chamber MEC was then fed with the AnSBR treated POME. Voltage of 1.0 V was applied to operate the MEC with cumulative H₂ production of 3.63 ± 0.16 L H₂/L POME over a duration of seven days. The maximum Coulombic efficiency, C_E (83.9 ± 3.3%), maximum H₂ recovery, r_{cat} (99.1 ± 4.7%), and total energy recovery, η_{E+S} (103.9 ± 8.9%) were also recorded. To determine the microbial communities of the hybrid system, genomic bacterial DNA from AnSBR sludge and both bioanode of MFC and MEC were extracted and subjected to 16S rRNA gene amplicon sequencing. In the top 10 phyla of the three samples, phyla *Firmicutes* are predominant for AnSBR sludge while phyla *Proteobacteria* were dominant for both MFC and MEC bioanode, representing approximately 40% and 80% of the total gene fragment, respectively. Predictive functional metagenome profiles based on 16S rRNA marker genes showed that the most abundant metabolic functions in the three samples were related to central metabolisms and substrate utilisation, with “energy metabolism”, “carbohydrate metabolism”, and “amino acid metabolism” were the top three abundant pathways. Findings from this study showed effluent from POME AnSBR contains highly valuable volatile fatty acids (VFAs) that could be recycled and maximised to produce H₂ in MEC. This study showed high potential of turning highly polluting POME as source to generate clean and renewable H₂ energy.

ABSTRAK

Kebergantungan global terhadap bahan api fosil sebagai sumber tenaga telah meningkat menyebabkan pengurangan simpanan minyak dan pemanasan global. Industri kelapa sawit, sektor pertanian terbesar di negara ini, dijangka menghasilkan kira-kira 70–110 juta tan sisa pepejal dan cecair menjelang tahun 2020. Sisa cecair ini, dikenali juga sebagai efluen sisa minyak sawit (POME), dalam jumlah besar dianggap sangat mencemarkan kerana kandungan permintaan oksigen biologikal dan kimia yang tinggi. Oleh kerana POME kaya dengan substrat seperti karbohidrat dan asid lemak, kajian ini dijalankan untuk menyasat penggunaan bakteria dari enapcemar anaerobik POME bagi menghasilkan gas hidrogen (H_2) di dalam sistem hibrid yang menggabungkan reaktor kelompok urutan anaerobik (AnSBR) dan sel elektrolisis mikrob (MEC). Lima kaedah pra-rawatan (kejutan haba, asid, alkali, kimia, dan pengudaraan) telah diuji bagi memilih kaedah terbaik. Daripada lima kaedah tersebut, kejutan haba menunjukkan penghasilan H_2 tertinggi. Seterusnya, beberapa faktor yang mempengaruhi penghasilan H_2 ditentukan menggunakan kaedah satu-faktor-pada-satu-masa (OFAT). Keputusan menunjukkan penghasilan H_2 tertinggi adalah pada pH permulaan 6.0, suhu eraman $55.0^\circ C$, dan 10.0% (v/v) saiz inokulum. Kajian lanjut bagi mengoptimum penghasilan H_2 menggunakan Reka Bentuk Box–Behnken (BBD) menunjukkan bahawa semua faktor menyumbang secara signifikan terhadap penghasilan H_2 . Kombinasi pH permulaan 6.4, suhu eraman $58.0^\circ C$, dan saiz inokulum 8.0% v/v meningkatkan penghasilan H_2 2.57 kali ganda lebih tinggi (239.0 mL) berbanding sebelum pengoptimuman. Seterusnya, keadaan yang dioptimumkan ini dikaji secara meluas dalam AnSBR 1.2 L dengan dua faktor: masa tahanan hidraulik (HRT) dan kadar muatan organik (OLR). Keputusan menunjukkan 12 jam HRT dan 36 g/L/d OLR adalah keadaan terbaik dengan penghasilan maksima H_2 dicatatkan pada $>2.80 L H_2/L POME$ dan penyingkiran COD hampir 65% dicapai semasa 16 kitaran operasi. Sel tunggal MEC kemudiannya disalurkan dengan POME terawat AnSBR. Voltan 1.0 V telah digunakan bagi operasi MEC memberikan jumlah kumulatif penghasilan H_2 $3.63 \pm 0.16 L H_2/L POME$ sepanjang tempoh tujuh hari. Kecekapan Coulomb maksimum, C_E ($83.9 \pm 3.3\%$), pungutan H_2 maksimum, r_{cat} ($99.1 \pm 4.7\%$), dan jumlah pungutan tenaga, η_{E+S} ($103.9 \pm 8.9\%$) juga direkodkan. Bagi menentukan komuniti mikrob sistem hibrid, genom DNA bakteria dari enapcemar AnSBR dan kedua-dua bioanod MFC dan MEC telah diekstrak dan dilakukan jujukan gen amplikon 16S rRNA. Di antara 10 filum teratas daripada ketiga-tiga sampel, filum *Firmicutes* adalah dominan di dalam enapcemar AnSBR, sementara filum *Proteobacteria* dominan untuk kedua-dua bioanod MFC dan MEC, masing-masing mewakili sekitar 40% dan 80% dari keseluruhan serpihan gen. Fungsi ramalan profil metagenom berdasarkan gen penanda 16S rRNA menunjukkan fungsi metabolisme yang paling banyak dalam ketiga-tiga sampel tersebut adalah berkaitan dengan metabolisme pusat dan penggunaan substrat, dengan “metabolisme tenaga”, “metabolisme karbohidrat”, dan “metabolisme asid amino” sebagai tiga kumpulan besar yang teratas. Hasil kajian ini menunjukkan efluen POME yang dikeluarkan dari AnSBR mengandungi asid organik yang sangat berharga dan boleh dikitar semula serta dimaksimumkan untuk menghasilkan H_2 dalam MEC. Kajian ini menunjukkan potensi yang tinggi untuk penukaran POME yang sangat mencemar sebagai sumber tenaga H_2 yang bersih dan boleh diperbaharui.

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LIST OF ABBREVIATIONS

2-LFD	–	2-level factorial design
ADMI	–	American Dye Manufacturers Institute
AIM	–	Agensi Inovasi Malaysia
AnSBR	–	Anaerobic sequencing batch reactor
APHA	–	American Public Health Association
ATP	–	Adenosine triphosphate
BBD	–	Box–Behnken Design
BES	–	Bio-electrochemical system
BLAST	–	Basic Local Alignment Search Tool
BOD	–	Biochemical oxygen demand
bp	–	Base pair
CCD	–	Central Composite Design
CCV	–	Closed circuit voltage
CPO	–	Crude palm oil
COD	–	Chemical oxygen demand
DAD	–	Diode array detector
DC	–	Decanter cake
DNA	–	Deoxyribonucleic acid
DNS	–	Dinitrosalicylic acid
DO	–	Dissolved oxygen
DOE	–	Department of Environment
EIA	–	Energy Information Administration
EFB	–	Empty fruit bunch
EPS	–	Extracellular polymeric substances
EUMCCI	–	EU-Malaysia Chamber of Commerce and Industry
FIB–SEM	–	Focused Ion Beam–Scanning Electron Microscopy
FELCRA	–	Federal Land Consolidation and Rehabilitation Authority
FELDA	–	Federal Land Development Authority
FFB	–	Fresh fruit bunch
FTIR	–	Fourier Transform Infra-red

GC	–	Gas Chromatography
GDP	–	Gross domestic product
GHG	–	Greenhouse gaseous
HPLC	–	High-Performance Liquid Chromatography
HRT	–	Hydraulic retention time
ID	–	Internal diameter
IQR	–	Interquartile range
MEC	–	Microbial electrolysis cell
MF	–	Mesocarp fibre
MFC	–	Microbial fuel cell
MLSS	–	Mixed liquor suspended solids
MLVSS	–	Mixed liquor volatile suspended solids
MPOB	–	Malaysian Palm Oil Board
MPOC	–	Malaysian Palm Oil Council
Mtoe	–	Million tonnes of equivalent
NADH	–	Nicotinamide adenine dinucleotide
NAD(P)H	–	Nicotinamide adenine dinucleotide phosphate
NGO	–	Non-governmental organisation
NKEA	–	National Key Economic Areas
OCV	–	Open circuit voltage
OD	–	Optical density
OFAT	–	One-factor-at-a-time
OLR	–	Organic loading rate
OPF	–	Oil palm frond
OPT	–	Oil palm trunk
PCR	–	Polymerase chain reaction
PEM	–	Proton exchange membrane
PHA	–	Polyhydroxyalkanoate
PKC	–	Palm kernel cake
PKS	–	Palm kernel shell
POME	–	Palm oil mill effluent
r_{cat}	–	H ₂ recovery
R_{in}	–	Internal resistance

RSM	–	Response surface methodology
rRNA	–	Ribosomal RNA
SDR	–	Substrate degradation rate
TCD	–	Thermal conductivity detector
TSS	–	Total suspended solids
USA	–	United States of America
USD	–	US Dollar
UV-Vis	–	Ultraviolet-visible
VFA	–	Volatile fatty acid

LIST OF SYMBOLS

A	–	Ampere
A	–	Surface area
C_E	–	Coulombic efficiency
E_{ap}	–	Applied voltage
F	–	Faraday's constant
g	–	Gravitational force
g/L	–	Gram per litre
H	–	Cumulative H ₂ production
I	–	Current
K	–	Kelvin
M	–	Molarity
ml/min	–	Millilitre per minute
mM	–	Millimolar
m	–	Median
mA/m^2	–	Current density
max_p	–	Maximum point
min_p	–	Minimum point
mW/m^2	–	Power density
N_{cycle}	–	Number of cycles per day
P	–	Maximum H ₂ production, power, pressure
Q	–	Charge (Coulomb)
Q_1	–	Lower quartile
Q_3	–	Upper quartile
R	–	External resistance, constant Ideal Gas Law
R_m	–	Maximum H ₂ production rate
rpm	–	Rotation per minute
S	–	Conductivity
V	–	Voltage
v_r	–	Volume of liquid in the reactor
$\% v/v$	–	percentage of volume over volume

e	–	Euler number
Δ	–	Differences
Ω	–	Ohm
e_E	–	Electrical energy input
e_S	–	Substrate energy input
η_E	–	Electrical energy recovery
η_{E+S}	–	Total energy recovery
η_S	–	Substrate energy recovery
λ	–	Lag-phase time

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CHAPTER 1

INTRODUCTION

1.1 Research Background

The production of palm oil has introduced vast interest globally due to its advantages, especially in dealing with other competing oils, such as sunflower, olives and vegetable oils that have low-cost production, the capability to yield high amount and trans-fatty acid-free. The demand and value for palm oil has increased drastically because of its multi-purpose benefits in both food and non-food industries (Abdullah *et al.*, 2013; Hansen *et al.*, 2012). It was reported that the total production of palm oil alone has accounted for 40.0% which makes it the leading exploited vegetable oil in the world (Hansen *et al.*, 2015; Oosterveer *et al.*, 2015).

Malaysia's oil palm industry has grown over the years and, Malaysia is well-known as the second-biggest producer of palm oil in the world, accounting for 33.0% of the global oils and fats production in 2017 (Kushairi *et al.*, 2017; Tan and Lim, 2019). Nowadays, Malaysia remained a significant player in the palm oil industry with its export market accounts for 33.6% of the global palm oil trade, which is approximately 16.56 million tonnes in 2017 compared to the previous year of 3.2% increment (Kushairi *et al.*, 2017; MPOC, 2017; Tan and Lim, 2019). Malaysia, Indonesia, and Thailand accounted for more than 80.0% of the worldwide distribution of palm oil production annually (Amiruddin *et al.*, 2005; Awalludin *et al.*, 2015). The industry served as a crucial backbone to the economy of the country and improved the living standards of its communities considerably (Yusoff and Hansen, 2007). This situation supported by the national gross output, which the oil palm industry in Malaysia has contributed over RM 53 billion, coming as fourth largest sources of national income since 2014, making it is an enormous contributor to the economic

growth (AIM, 2015). In 2017 alone, Malaysia has recorded more than 16 million tonnes of palm oil products which accounted for approximately 34% of global export in palm oil trade (MPOC, 2017).

Since the rapid development of the palm oil industry in Malaysia, the risks of pollution produced by the industry have intensified. The risks discovered primarily in surface water pollution are due to either insufficient or inappropriate disposal of solid and liquid waste. These justified further evaluation of the potential contribution to improving the sector's environmental performance in Malaysia by the cleaner palm oil industry. Besides, life cycle assessments of palm oil waste treatment have been implemented to evaluate the potential use of palm oil residues (Hansen *et al.*, 2012). The amount of palm oil mill effluent (POME) also produced increases in proportion to the world demand for edible oil (Ahmed *et al.*, 2015; Bala *et al.*, 2015). The extraction of CPO involves the boiling of fresh fruit bunch (FFB), which requires a significantly huge amount of water during the process. It was estimated that each tonne of CPO produced required 5.0–7.5 tonnes of water, with more than 50.0% of this water ends up as POME (Wu *et al.*, 2009; Yacob *et al.*, 2006).

In general, raw POME is an acid brownish colloidal suspension consisting of high polluting properties with an average level of chemical oxygen demand (COD, 50,000 mg/L), biochemical oxygen demand (BOD, 25,000 mg/L), total solids (40,500 mg/L), suspended solids (18,000 mg/L), and oil and grease (6,000 mg/L) (Ahmad *et al.*, 2003), which were produced from the steriliser condensate, separator sludge, and hydrocyclone waster (Wu *et al.*, 2010). It also contained cellulosic material, fat, oil, and grease (Iskandar *et al.*, 2018; Rupani *et al.*, 2010). The excessive level of COD and BOD in POME could severely harm the aquatic life forms in rivers and water stream (Borja *et al.*, 1996; Loh *et al.*, 2013; Neoh *et al.*, 2013). Moreover, the intense colour of POME would prevent sunlight from penetrating the water and thus threatening photosynthetic organisms in the water (Azreen *et al.*, 2017; Okwute and Isu, 2007). Although the effluent is non-toxic as no chemicals are added during the extraction process of CPO from FFB, it generates polluted wastewater that not only impacts the aquatic niche but also causes odour issues for the local community. Hence, the palm oil mill industry is increasingly requiring sustainable treatment of POME to

minimise pollution. With over 453 palm oil mills across the country, producing POME in large amount every day (MPOB, 2018), the discharge of POME into the rivers present a potential threat to the ecosystem and environment.

Malaysia is also blessed with fossil fuels which make them well-known for the producers of petroleum oil and natural gas. The primary energy sources in Malaysia, besides oil and gas, come from coal and a small percentage of renewable energy sources such as hydroelectricity, biomass and solar energy (Oh *et al.*, 2018; Shaikh *et al.*, 2017). Like many other developing countries, development and economic growth continue to impact the nation's increasing demand for energy consumption. With the anticipated need for future energy to develop at an annual pace of 5.0–8.0% over the next two decades, energy security is becoming a serious problem as fossil fuels are non-renewable energies and will deplete eventually soon (Bhattacharya *et al.*, 2016; Ong *et al.*, 2011). Hence, energy-related policy measures have been a top priority to ensure energy security and sustainability in the country (Bong *et al.*, 2017; Yatim *et al.*, 2016).

The excessive, sole dependency on fossil fuels in thriving the nation has resulted in a severe global climate change, apart from possible depletion of oil reservoir from the global perspective point (Guo *et al.*, 2010; Shafiee and Topal, 2009; Singh and Singh, 2012). With the subject of Montreal Protocol 1972 and Kyoto Protocol 1997, the Malaysian Government recognised the use of green technology as a long-term and practical alternative towards climate change and energy security (Mekhilef *et al.*, 2014; Shi *et al.*, 2013). Furthermore, the Malaysian Government also actively engaged in research and development (R&D) by using multiple renewable energy sources including biomass wastes, hydropower, solar power, and wind energy to sustain its energy demand and consumption (Kardooni *et al.*, 2016; Shekarchian *et al.*, 2011).

Hydrogen gas (H₂) can be classified as an ideal, green and sustainable energy carrier. Compared to any hydrocarbon fuel, it contains the highest gravimetric energy density. It can be seen as an alternative source of renewable energy to fossil fuel since the combustion of H₂ generates no emissions of greenhouse gases (GHG) (Azwar *et*

al., 2014; Chong *et al.*, 2009; Rezania *et al.*, 2017). H₂ has been suggested as the ultimate transport gas for vehicles and boats due to its non-polluting properties and offers the use of highly efficient fuel cells to transform chemical energy into electricity (Forsberg, 2007). This energy is high in demand for various applications of numerous sectors, such as the transportation industry, as a coolant in power plants and rocket propulsion (Goyal *et al.*, 2006).

Even though H₂ can be produced directly from fossil fuels, however, the dependency towards it will be unlikely to be a future energy source. Thus, microbial H₂ production was introduced to divert the attention of fossil fuels dependency. A wide diversity of potential substrates, wastes, and biomass are present in abundance to make a significant impact on achieving energy demands if they are efficiently converted to H₂ by fermentation (Hallenbeck, 2011; Hallenbeck and Ghosh, 2009; Hallenbeck *et al.*, 2012; Kumar *et al.*, 2018). Thus, the production of H₂ by utilising these wastes and biomass have achieved new milestones in this area of research, including biophotolysis and electrohydrogenesis using microbial electrolysis cell (MEC). Recently, a study of biophotolysis was conducted by Kossalbayev *et al.* (2020) in order to determine promising H₂-producers among cyanobacteria and understand the mechanisms along with the conditions for increasing H₂ production. As for fermentation, the application of bioreactor in the study is a must in order to obtain higher volumetric and yield of H₂ with the utilisation of wastes and biomass as substrates. Recent studies including photo-bioreactor (Gao *et al.*, 2017; Sun *et al.*, 2016), membrane bioreactor (Buitrón *et al.*, 2019; Ramírez-Morales *et al.*, 2019), anaerobic sequencing batch reactor (AnSBR) (Intanoo *et al.*, 2019; Maaroff *et al.*, 2019), continuous stirred tank reactor (CSTR) (El-Qelish *et al.*, 2019; Srirugsa *et al.*, 2019), and upflow anaerobic sludge blanket bioreactor (UASB) (Jiraprasertwong *et al.*, 2019; Mahmud *et al.*, 2019). Even though MEC is still novel research in this H₂ production, researchers are enthusiastically putting up their efforts in realising this prospect for the future application. Presently, most of the studies were focusing on biocathode development (Jafary *et al.*, 2019) and reactor design improvement (Jwa *et al.*, 2019) for higher production of H₂.

1.2 Problem Statement

Although Malaysia's palm oil industry has been a success story for its economic growth for decades, it still troubled with the environmental issue, especially the amount of POME being produced (Bala *et al.*, 2015; Mumtaz *et al.*, 2010). As a result, more than 400 palm oil mills across the country, which results in the increase of POME discharge into the water bodies consequently are polluting the environment. In fact, in the year of 2014 alone, POME was generated approximately 64 million tonnes (EUMCCI, 2017; Loh, 2017; Loh *et al.*, 2013). Nevertheless, there are almost 90% of these palm oil mills in Malaysia treating POME by applying the low-cost ponding system treatment. Besides the disadvantage of the large area required for the ponding system to be established, the escaping GHG to the atmosphere is another serious environmental issue risen from this treatment system (Choong *et al.*, 2018). This whole scenario directs to a demanding sustainable treatment of POME, which is to avoid any lengthy consequences. Several reports remarked that the discharge of POME during the palm oil mill processing in Malaysia was recognised as a significant contribution to pollution in the country's rivers (Wu *et al.*, 2009; Ahmed *et al.*, 2015). Therefore, POME is considered highly polluting and could achieve more than 100 times polluted than domestic sewage in terms of BOD and COD limit, which exceed the value of 25,000 mg/L and 50,000 mg/L, respectively (Mumtaz *et al.*, 2010). Besides, the high level of oil and grease together with the suspended solids could clog fish gills, thus potentially harming the aquatic life (Hameed *et al.*, 2003; Jameel and Olanrewaju, 2011). The palm oil mill industry is now facing an uphill challenge to meet the requirement of increasingly stringent environmental standards (Wu *et al.*, 2009; Cheng *et al.*, 2010). Since then, researchers were highly emphasised and driven towards solving this issue by introducing several mechanisms and innovation, such as membrane bioreactors, anaerobic digestion, and physicochemical treatment (Lee *et al.*, 2019) which can successfully treat POME up to the acceptance level for discharge.

Even though those technologies have been proven successful to deal with POME, the enormous abundance of POME produced by the industry have triggered the related stakeholders and researchers to explore other usages of POME, by means converting it into valuable products such as bioenergy, biofuels, and bioplastics, not

only focused on treating and disposing of it later on. Hence, the utilisation of POME as a substrate have paved the way towards sustainable, clean production of H₂. Various studies have embarked remarkable achievements of producing H₂ via biological methods in various technologies and applications since the early 2000s (Chan *et al.*, 2010; Chong *et al.*, 2009a; Ismail *et al.*, 2010; Mohammadi *et al.*, 2017; Vijayaraghavan and Ahmad, 2006). Although rapid signs of progress and achievements were obtained, studies have reported that the H₂ yield was far from convincing when it comes to up-scale the production and its practicality (Balachandar *et al.*, 2020; Piemonte *et al.*, 2014; Ren *et al.*, 2006; Tapia-Venegas *et al.*, 2015; Vatsala *et al.*, 2008). The questionable problem lies within the substrate. In general, the substrates being used are only partially hydrolysed to produce H₂, which leave the by-products as wastes (Das and Veziroglu, 2008; Kumar *et al.*, 2018). This would be a wasteful resource if the substrate could not be maximised to deliver the acceptable production yield of H₂, particularly those valuable by-products with volatile fatty acids (VFAs) that could be converted and used as backbones of alcohols, aldehydes, ketones, esters, and olefins, not only for H₂ production (Boboescu *et al.*, 2016; Kumar *et al.*, 2018; Lee *et al.*, 2014) but could also for the renewable bio-refinery industry (Eggeman and Verser, 2005, Singhania *et al.*, 2013).

Since the production of H₂ from the non-renewable sources were hampered with the uncertainty of global oil prices, the depletion of oil reservoirs soon, and the environmental issues including the rise of global warming and climate change (Sharma *et al.*, 2020; Sheth and Babu, 2010), the researchers are currently intensified their efforts in exploring the renewable sources as substrates for green, clean, and sustainable production of H₂. Apparently, the microbial H₂ production can be divided into three mechanisms which are biophotolysis, fermentation, and electrohydrogenesis (Mishra *et al.*, 2019). However, each of these mechanisms hampered with difficulties: low light conversion efficiency causing low yields of H₂ for biophotolysis (Kosourov *et al.*, 2002; Rahman *et al.*, 2016), low conversion of substrates from wastes and biomass with only 15% of energy to be converted to H₂ from fermentation (Logan, 2004; Hallenback and Ghosh, 2009; Rahman *et al.*, 2016; Zainal *et al.*, 2018). As for MEC, substrates with less complexity are suitable to be used (Kadier *et al.*, 2014; Sleutels *et al.*, 2011) and its reactor design practicality especially the external voltage

and energy losses during MEC process (Kadier *et al.*, 2016; Mishra *et al.*, 2019) have been the main hurdle for its progressing forward into a real application. Even though fermentation still not capable to achieve a higher yield of H₂, but, compared to other mechanisms, it is the only one that can manage to convert a variety of substrates with fewer difficulties. Hence, several strategies have been suggested to overcome the difficulties such as developing two-stage mechanisms or systems derived from the fermentation by-products such as organic acids which later could be reused and recycled (Adessi *et al.*, 2012; Hallenbeck, 2011; Hallenbeck *et al.*, 2012; Kumar *et al.*, 2018).

The introduction of the hybrid system for H₂ production has established a breakthrough among researchers due to the low yield of H₂ obtained via biological methods. This hybrid system, also known as an integrated system involving the two-stage process (Boboescu *et al.*, 2016; Hallenbeck and Ghosh, 2009), which: i) the fermentation of the substrate to H₂ and organic acids takes place in a process that has been optimised using one or several of the advanced technologies in the first-stage and followed by ii) the additional H₂ is extracted from the effluent of the first-stage reactor, happens in the second-stage. This not only could overcome the bottle-necks facing for H₂ production but can be a bonus towards the use of wastewaters as substrates which allow the technology to become economically feasible by combining bioenergy production with wastewater treatment (Boboescu *et al.*, 2016). Thus, this could be the answer for the oil palm industry in handling the current issue it is facing on treating POME discharge.

Thus, this research was conducted to develop a hybrid system using POME as the source of the substrate, for efficient production of H₂ as converting the abundance waste produced into bioenergy, simultaneously could solve treatment process which enormously hampered the industry itself. With the incorporation of anaerobic sequencing batch reactor (AnSBR) and microbial electrolysis cell (MEC) in a hybrid system, it would be an ideal for optimising the POME as a substrate for H₂ production, especially the effluent from AnSBR which contains highly valuable organic acids after first-stage wastewater treatment. Hence, the barrier of H₂ production would be overcome and could improve the current wastewater treatment process for POME at

the palm oil mills. This proposal could be a defining moment in the era of clean, renewable energy produced from the abundance of wastes available to overcome yield efficiencies, which hinders the larger-scale development of H₂ production.

1.3 Objectives of Research

The main objective of this research was to maximise the production of H₂, utilising raw POME as its substrate and simultaneously treat the undesirable characteristics of POME. The objectives were as follows:

- i. To compare the H₂ production and microbial composition between different pre-treatment methods for H₂-producers from POME anaerobic sludge
- ii. To optimise the H₂ production of raw POME using statistical approach under batch condition
- iii. To investigate the best initial start-up of bioanode development for the microbial fuel cell (MFC) before being used in MEC setup using heat-shock pre-treated POME anaerobic sludge as inoculum
- iv. To develop an AnSBR–MEC hybrid system for efficient H₂ production and wastewater treatment of raw POME under the optimised condition
- v. To monitor and identify microbial diversity in the AnSBR–MEC hybrid system using a metagenomics approach

1.4 Scope of Research

The scope for this research was divided into several parts which were as follows:

- i. This research would compare the H₂ production and microbial composition between different pre-treatment methods from POME anaerobic sludge. Methods of pre-treatment for the POME anaerobic sludge applied in this study were heat-shock, acid, alkaline, chemical, and aeration. This research would also perform wastewater and VFA analyses for each of the pre-treatment methods besides the H₂ production profile. Microbial composition/diversity for each of the pre-treatment method would be analysed using DNA cloning (Chapter 4)
- ii. For the optimisation using OFAT, three significant factors based on the reported literature: the initial pH of the medium, incubation temperature, and inoculum size using raw POME as a substrate and pre-treated POME anaerobic sludge as a source of microorganisms. Results from OFAT approach was used to identify the factor levels that can produce maximum H₂ production as an input in 2-Level Factorial Design (2-LFD). This was followed by Box–Behnken Design (BBD) which was used to determine their optimal values by analysing the influence and interactions of factors identified on the 2-LFD (Chapter 5)
- iii. The best initial start-up of bioanode development based on indirect enrichment approach via a dual-chambered MFC setup. Pre-treated POME anaerobic sludge was used as inoculum for the development of bioanode. To ensure the successful development of biofilm on the surface of bioanode, several factors were optimised in MFC – external resistance, electrode surface area, ionic concentration of salt bridge, anodic initial pH, and substrate concentration. The results from this research were converted

into electricity generation i.e. power generation, the current generation, polarisation curve, and internal resistance (Chapter 6)

- iv. The performance of AnSBR–MEC hybrid system was based on H₂ production and water quality parameter analyses. Other factors such as hydraulic retention time (HRT), organic loading rate (OLR), applied voltage (E_{ap}), and different types of POME applied were determined with the aim to produce a high yield of H₂ (Chapter 7)
- v. The microbial diversity of AnSBR–MEC hybrid system was analysed using metagenomics approach, choosing 16S gene amplicon sequencing as the platform analysis. Metabolic profiling was also determined in better knowledge and understanding, especially in H₂ production (Chapter 8)

1.5 Significance of Research

This research is anticipated to contribute to a significant milestone in solving the environmental issues related to the palm oil industry in Malaysia, especially POME and concomitantly introduce the H₂, which reflects as clean, sustainable energy for the future. By implementing the hybrid system technology in the palm oil industry, POME will be utilised to produce H₂ and facilitate the treatment of POME. Aforementioned, the H₂ will make our dependency on fossil fuels lesser and solve other issues related to the environment such as GHG and global warming.

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