ISOLATION AND CHARACTERIZATION OF HYDROGEN PRODUCING BACTERIA FROM PALM OIL MILL EFFLUENT (POME)

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DEDICATION

I would like to dedicate this thesis to special people in my life:

To my mother and father who always give me energy and without their encouragement and support I could not achieve this degree.

To Ali my amazing husband for her wonderful love, patience and sacrifice during this difficult stage of our life.

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ABSTRACT

Hydrogen gas and its usage in electricity generation and transportation is attracting wider interests nowadays. This is because it possesses a high-energy yields (122 kJ g^{-1}), it is a renewable energy source, and does not contribute to the greenhouse effect. In Malaysia, 15.2 million tons of wastewater is generated annually by the palm oil industry. The wastewater is known as Palm Oil Mill Effluent (POME). This study is focused on the isolation and characterization of hydrogen-producing bacteria from POME. Samples were taken from the sludge and raw waste of palm oil processing effluent. Bacterial isolation was performed to isolate facultative anaerobes and strict anaerobes. The isolated colonies of bacteria were identified and characterized by microbiological analysis and biochemical tests (catalase, indole, nitrate reduction, urea, TSI and citrate test). From TSI test, it was found that one facultative anaerobe (out of 13 colonies altogether) produced gas; and all strict anaerobes produced gas (8 colonies altogether). The gas-producing colonies were then cultivated at 37°C for 24 hours in a synthetic media simulating starch wastewater for screening of hydrogen production. Hydrogen gas production was then analyzed using RGA (Residual Gas Analyzer). Results show that the facultative anaerobe colony did not produce hydrogen gas by starch fermentation; and four colonies obtained via strict anaerobe isolation show hydrogen production. These colonies can be the subject of future studies to optimize hydrogen production from waste materials.

ABSTRAK

Gas hidrogen dan penggunaannya dalam penjanaan elektrik dan pengangkutan menarik lebih luas kepentingan pada masa kini. Ini adalah kerana ia mempunyai hasil yang tinggi tenaga (122 kJ g-1), ia adalah satu sumber tenaga boleh diperbaharui, dan tidak menyumbang kepada kesan rumah hijau. Di Malaysia, 15200000 tan air sisa yang dijana setiap tahun oleh industri minyak sawit. Air sisa yang dikenali sebagai Kilang Minyak Sawit Efluen (POME). Kajian ini memberi tumpuan kepada pengasingan dan pencirian bakteria menghasilkan hidrogen dari POME. Sampel telah diambil daripada enapcemar dan sisa mentah pemprosesan minyak sawit efluen. Pengasingan bakteria telah dilakukan untuk mengasingkan fakultatif anaerobes dan anaerobes ketat. Jajahan terpencil bakteria telah dikenal pasti dan dicirikan oleh analisis mikrobiologi dan ujian biokimia (katalase, indole, pengurangan nitrat, urea, TSI dan ujian sitrat). Dari ujian TSI, ia telah mendapati bahawa satu fakultatif anaerobe (daripada 13 jajahan sama sekali) yang dihasilkan gas dan semua anaerobes ketat yang dihasilkan gas (8 jajahan sama sekali). Negara-negara jajahan gas menghasilkan kemudian ditanam pada 37 ° C selama 24 jam dalam media sintetik simulasi air sisa kanji untuk pemeriksaan pengeluaran hidrogen. Pengeluaran gas Hidrogen telah dianalisis menggunakan RGA (Gas Analyzer Residual). Keputusan menunjukkan bahawa koloni fakultatif anaerobe tidak menghasilkan gas hidrogen oleh penapaian kanji; dan empat jajahan yang diperolehi melalui pengasingan anaerobe ketat menunjukkan pengeluaran hidrogen. Tanah jajahan ini boleh menjadi subjek kajian masa depan untuk mengoptimumkan pengeluaran hidrogen daripada bahan-bahan buangan.

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LIST OF ABBREVIATIONS AND SYMBOLS

°C	Degree Centigrade Celsius
µL	Microlitere
ASBR	Anaerobic Sequencing Batch Reactor
BOD	Biochemical Oxygen Demand
CFC	Chloro Fluor Carbon
CH_4	Methane
CO	Carbon monoxide
CO_2	Carbon dioxide
COD	Chemical Oxygen Demand
CPO	Crude Palm Oil
DGGE	Denaturing Gradient Gel Electrophoresis
DOE	Department Of Environment
EFB	Empty Fruit Bunch
FFB	Fresh Fruit Bunch
g. cell/L	Gram. Cell/Liter
GHG	Green House Gas
gr	Gram
H	Hour
H_2/mol	Hydrogen/molarity
H_2O	Dihydrogen oxide(water)
H_2O_2	Hydrogen proxide
H_2S	Treated sugarcane samples with nitric acid
HRT	Hydrolic Retention Time
Kg	Kilogram

mg	Milligram
mg/L	Milligram/Liter
mg/L	Milligram/Liter
min	minute
ml	Milliliter
mm	Millimetre
mmol	Millimol
Mmol/gr	Millimole/ gram
mol/mol	Molarity/molarity
Mol\L	Moll/litre
NA	Nutrient Agar
NB	Nutrient Broth
O_2	Oxygen
PCR	Polymerase Chain Reaction
POME	Palm Oil Mill Effluent
RGA	Residue Gas Analysis
RT	Room Temperature
sec	second
SEM	Scanning Electron Microscopy
TSB	Triptic Soy Broth
TSI	Triple Sugar Iron

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CHAPTER 1

INTRODUCTION

1.1 Background of Study

Nowadays, through the rapid growth of world energy consumption, the focus of carbon and sustainable neutral energy sources has attracted more attention for future needs. One of the most important alternatives in existing petroleum-based fuels is biofuels that can be used as transportation fuels. It is capable to advance sustainability and decrease the greenhouse gas emissions by little change to current technologies. Organic materials such as oilseeds, starch, cellulose and animal fats can be used as biofuel sources and are divided into gaseous or liquid biofuels [1].

Over the past few decades, the hydrogen gas and its usage in electricity generation and transportation has attracted more attention because it possesses high-energy yields (122 $kJ.g^{-1}$), it is a renewable energy source, and does not contribute to the green house effect. Moreover, it easy to achieved, it means that it can be produced by various methods, through fermentation of biomass using microorganism, coal gasification, reforming of hydrocarbons, photochemical process, electrolysis, and biological routes. In order to generating the hydrogen, a wide variety of methods are presented in biological systems that comprises photo fermentations, direct bio photolysis, indirect bio photolysis, and dark fermentations. Among them the light fermentation has been considered by many investigators because of its economic viability and high profit [2].

Acidogenic waste treatment process produces biohydrogen in nature where acid forming bacteria yields hydrogen, organic acid compound and carbon dioxide [3]. Dark fermentation or light driven photosynthesis can produce biohydrogen [4,5], and compared to photosynthetic routes, it is achieved by dark fermentation of organic waste materials [6]. Dark fermentation has many advantages such as no light energy required, high rate of cell growth, no oxygen limitation problems and it is able to work on low capital cost [6,7,8].

Nowadays, many researches focus on the possibility of hydrogen production from different industries by applying wastewater treatment strategy with the organic wastes [9]. For instance, 15.2 million tons of wastewater is generated annually by the palm oil industry in Malaysia, which is known as Palm Oil Mill Effluent (POME) with high lignocellulose and cellulose material. To degrade the organic substances, it is very time consuming. The previous studies have reported on utilizing the POME sludge as an inoculum, and has reported a promising level of hydrogen production [10].

Palm oil is actually one of the most multipurpose crops in the tropical countries such as Indonesia and Malaysia. For processing 1 tonne of fresh fruit bunches (FFB), approximately 1.5 m^3 water are used, and about half of this would be considered as POME. Because of its high chemical and biological oxygen demands, it is a great threat for nature. The incomplete and raw treated POME contains high content of degradable organic materials. Therefore, due to oxygen depletion, this causes serious pollution of waterways. At present, in Malaysia 265 active palm oil mills exist with annual Crude Palm Oil (CPO) production capacity of 13 million tones. To treat POME, several techniques have been proposed, such as flotation, crop irrigation, ultra filtration adsorption, and various biodegradation processes [11].

Palm oil industry generate residues or wastes in two forms. The first one is liquid waste, mainly Palm Oil Mill Effluent (POME), which is highly polluting at an average of 50,000 mg/L chemical oxygen demand (COD) and 25,000 mg/L biochemical oxygen demand (BOD). The second waste comprises of Empty Fruit Bunch (EFB), trunks, shell, and fronds in the solid form. The cheapest technologies, open digester pond and tanks or lagoon systems are used to treat the POME. POME should be treated first before it is disposed to appropriate places based on the rules of wastewater disposal amendment of the Department of Environment (DOE) Malaysia. In general, the operations of these systems require wide spaces that uncontrollably releases GHG particularly CH_4 and CO_2 to the atmosphere [12].

1.2 Statement of Problem

Currently, fossil fuels are the basis of the global energy requirements which lead to the foreseeable depletion of limited fossil energy resources. Because of the production of pollutants like NO_X, SO_X, CO_X, C_XH_X, ash, soot, and the droplets of tars, the use of fossil fuels causes the change of global climate. Furthermore, based on the growth of urbanization and industrialization, environmental pollution is a very important issue to be tackled. Therefore, the search for clean energy alternatives to satisfy growing energy demand is crucial [13].

Environment become increasingly unhealthy and polluted for living organisms since the global industrial revolution that include deforestation, release the pollutants into lands, rivers and air. Greenhouse gases (GHG) contains some dangerous combinations such as nitrous oxide, carbon dioxide (CO_2), chlorofluorocarbon (CFC), carbon monoxide (CO) and methane (CH_4) that trap a majority of the thermal radiation emitted from the earth's surface and have strong electromagnetic absorption capacity. These cause negative effects to the world such as global warming, depletion of ozone layer and significant raise of ocean level [12].

On the other hand, fossil fuels hydrogen that are from fuel cells or burned directly is a clean energy with zero carbon emissions. One of the main methods of producing hydrogen is the steam reforming of methane that leads to the release of large amounts of greenhouse gases. In addition, in spite of the wide adoption of hydrogen and accounts for around 2% of world consumption of energy as a fuel, it is still limited by several challenges [14,15,16].

These challenges can be overcome by the production of hydrogen from plant or waste material in a biological process [17,18,19]. Therefore, many studies have been done on the investigation of new sustainable energy sources to substitute fossil fuels. In conclusion, hydrogen is a viable alternative fuel and "energy carrier" of future due to the cleanness with no CO_2 emissions and its ease of use in electricity generation [20]. The procedure of the hydrogen production and its application has been shown in Figure 1.1.

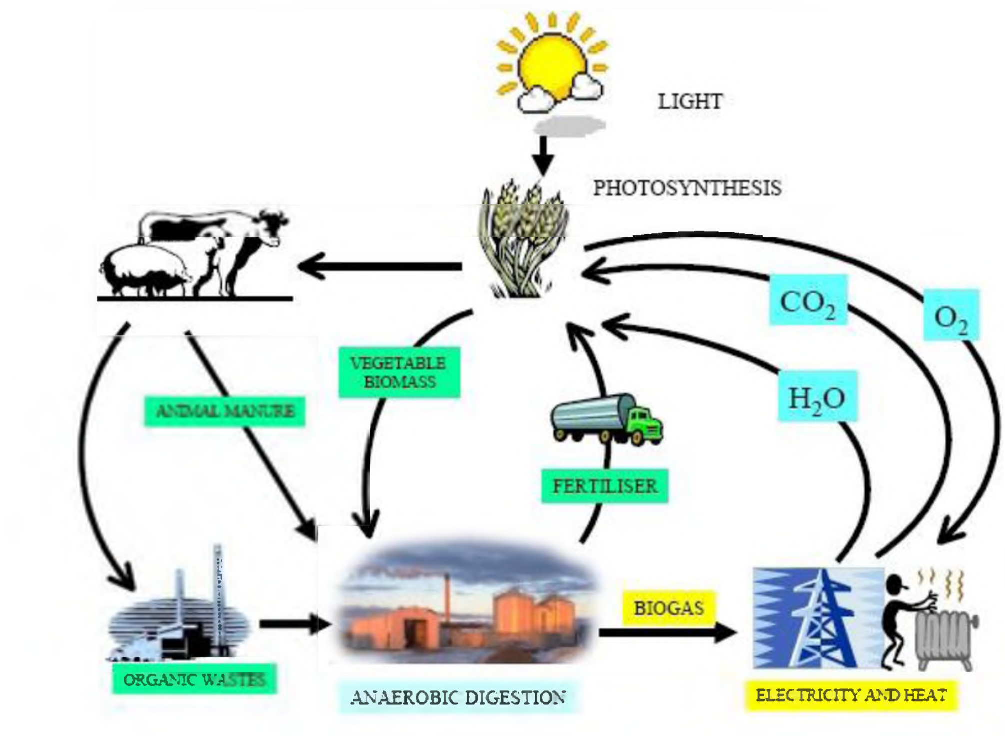

Figure 1.1 Hydrogen production and its applications (http://teenbiotechchallenge.ucdavis.edu)

1.3 Significance of Study

Hydrogen is one of the most powerful and clean and energy carriers that can be converted to electricity by using a fuel cell, which in the developed countries is modified as a main energy carrier [21].

This study aims to generate hydrogen as a final product by evaluating the possibilities of the bacteria as a biofuel producer. Additionally, biogas can be made in a huge amount by identifying the bacteria and culture them to use in industrial units.

1.4 Objectives of study

The objectives of this study are as follows:

1. To perform isolation of bacteria from waste of palm oil.

2. To conduct bacterial characterization (microbiology and biochemical tests).

3. To perform hydrogen production assay.

1.5 Scope of study

The goal of the current study is to produce hydrogen from the residue of palm oil (POME). Residue of palm oil is contained strict anaerobic and facultative anaerobic bacteria, which are responsible for the manufacture of hydrogen. To obtain single colonies the bacteria must be isolated and identified, after that biochemical and microbial tests direction of characterization of bacteria. Finally, isolated bacteria from fermentation media will be investigated in order to estimate the biogasses. The amount of biogasses will be assayed by RGA (residue gas analysis).

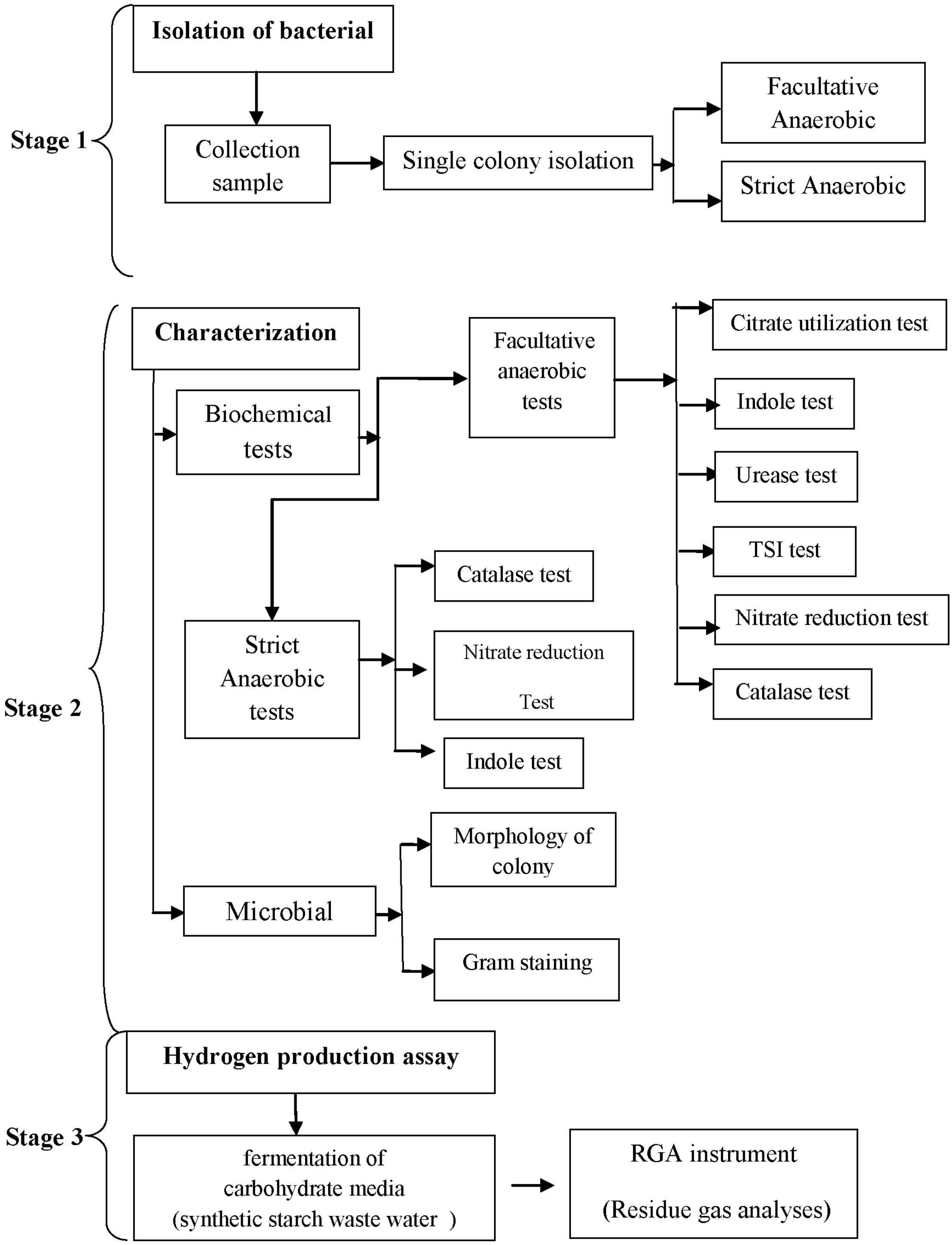

Figure 1.2 Research methodology flow

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