UNIVERSITI PUTRA MALAYSIA

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Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia in Fulfilment of the Requirements for the Degree of Master of Science

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Chairman: Prof. Mohd. Ali Hassan, PhD

Faculty: Faculty of Biotechnology and Biomolecular Sciences

Dark fermentation is light independent bioconversion of organic materials by anaerobes to biohydrogen (bioH_2), carbon dioxide and organic acids. Despite the successes achieved in biohydrogen production from simple sugars and carbohydrate rich wastewater, there are still limitations in the practical application of this process to complex substrate such as palm oil mill effluent (POME). In industrial application, the potential of readily available inoculums such as methane digester sludge has to be evaluated for biohydrogen production. Understanding the main chemical constituents either carbohydrates, protein or oil in POME that renders biohydrogen production will provide insight on the potential energy recovery in the form of hydrogen from POME. Continuous fermentation will be a step forward to determine the stability of biohydrogen production system from POME.

This thesis specifically investigates the potential of producing biohydrogen via continuous fermentation in a completely stirred tank reactor (CSTR) system using POME as feedstock under thermophilic (55°C) condition. Two different cells systems were evaluated separately; using suspended cells and immobilised cells entrapped in polyorganosiloxane polymer. Sludge from methane digester for treatment of POME was initially tested for its suitability as biohydrogen-producing inoculum under batch fermentation. Sludge pre-treated with hydrochloric acid at pH 3 for 24 hr and later pH reinstated to working pH of 5.5 was compared with the untreated sludge in batch experiments using synthetic medium-containing (5 g/L) glucose. Acid pre-treatment was part of attempt to suppress growth of hydrogen-consuming methanogens. The untreated sludge exhibited high specific hydrogen production rate (HPR) of 460 mL/g VSS/d as compared to the acid-treated sludge (135 mL/g VSS/d). Controlling the pH of fermentation at 5.5 suffices in eliminating methane generation and renders biohydrogen production without the requirement to pre-treat the suspended mixed cultures. The untreated sludge was then used for subsequent studies related to suspended cells system.

Another batch fermentation study was conducted to determine the fate of carbohydrate and oil that are presence in POME using model substrates; sucrose and crude palm oil. The kinetic profile indicated that mainly sucrose was metabolised by the mixed cultures with biohydrogen yield of 2.5 mol H_2/mol hexose$_{\text{-added}}$. Hence for subsequent POME fermentation, the hydrogen yield was monitored solely on unit volume of hydrogen produced per gram of carbohydrate consumed.

Continuous fermentative biohydrogen production was carried out over a period of 60 to 150 days for evaluating the system stability. The suspended cells system showed better performance than the polydimethysiloxane–immobilised cells system with biohydrogen yield of 1.72 and 1.58 mol H_2/mol POME-hexose, respectively at hydraulic retention time (HRT) of 4 days. The corresponding hydrogen production rate (HPR) was 2.6 ± 0.9 NL H_2/L POME/d with biohydrogen constituted up to 52% of the total biogas. The denaturing gradient gel electrophoresis (DGGE) profile of effluent samples showed increasing strong band of dominant hydrogen-producing bacteria species phylogenetically related to *Clostridaceae* at reduced HRTs, which also explained wash-out of these bacteria from the bioreactor. However, only 13% of the total organic contents of POME was utilised in the fermentation with potential energy yield of 2.4 MJ/kg COD consumed. These have render POME unfavourable as feedstock for recovering more biohydrogen energy under current operating conditions.

Abstrak tesis yang dikemukakan kepada Senat Universiti Putra Malaysia sebagai memenuhi keperluan untuk ijazah Master Sains

PENGHASILAN BIOHIDROGEN DARIPADA EFLUEN KILANG KELAPA SAWIT DALAM KEADAAN TERMOFILIK MENGGUNAKAN KULTUR CAMPURAN TERAMPAI DAN DISEKATGERAK

Oleh

ISNAZUNITA ISMAIL

Pengerusi: **Prof. Mohd. Ali Hassan, PhD**

Fakulti: **Fakulti Bioteknologi dan Sains Biomolekul**

Fermentasi anaerobik tanpa cahaya merupakan proses pengubahan bahan organik kepada penghasilan biohidrogen ($bioH_2$), karbon dioksida, dan asid organik. Walaupun pelbagai kejayaan diperolehi dalam penghasilan biohidrogen daripada gula ringkas dan air sisa kaya karbohidrat, masih banyak lagi kekurangan dalam mengaplikasikan proses ini ke atas substrat kompleks seperti efluen kilang kelapa sawit (POME). Di dalam aplikasi industri, potensi penggunaan enapcemar sediaada seperti enapcemar daripada pencerna gas metana perlu ditentuukur keberkesanannya dalam menghasilkan biohidrogen. Kefahaman tentang kandungan kimia utama di dalam POME samada karbohidrat, protein atau minyak yang membantu penghasilan biohidrogen akan memberi maklumat mengenai potensi perolehan tenaga dalam bentuk hidrogen di dalam POME. Fermentasi mod selanjar adalah salah satu kaedah yang digunakan dalam mengenalpasti kestabilan proses penghasilan biohidrogen daripada POME.

Tesis ini secara spesifik mengkaji potensi penghasilan biohidrogen melalui fermentasi selanjar menggunakan tangki pengaduk selanjar dalam keadaan termofilik (55°C) dan POME sebagai substrat. Dua sistem sel berbeza dinilai berasingan menggunakan sel terampai dan sel disekatgerak dalam polimer poliorganosiloksina. Kesesuaian enapcemar pencerna gas metana sebagai inokulum dinilai terlebih dahulu dalam ujikaji kelompok. Enapcemar dirawat terlebih dahulu semalaman dengan asid hidroklorik sehingga pH 3.0 sebelum dikembalikan ke pH 5.5 bertujuan untuk menyahaktifkan bakteria penghasil gas metana dibanding dengan enapcemar tidak terawat dalam ujikaji kelompok menggunakan kultur media sintetik mengandungi 5 g/L glukosa. Enapcemar tidak dirawat menunjukkan kadar penghasilan biohidrogen spesifik (SHPR) yang tinggi sebanyak 460 mL/g VSS/hari berbanding dengan enapcemar dirawat dengan asid (135 mL/ g VSS/hari). Pengawalan pH fermentasi pada pH 5.5 berkesan untuk menghalang penghasilan gas metana malah turut menghasilkan gas biohidrogen tanpa keperluan untuk merawat enapcemar atau sel terampai terlebih dahulu. Enapcemar ini digunakan untuk kajian selanjutnya.

Fermentasi sesekelompok juga dijalankan untuk mengenalpasti kesan akhir ke atas karbohidrat dan minyak yang terkandung di dalam POME dengan menggunakan sukrosa dan minyak kelapa sawit mentah sebagai model substrat. Profil kinetik menjelaskan bahawa hanya sukrosa dicerna oleh enapcemar dengan penghasilan biohidrogen bersamaan nisbah molar biohidrogen ke atas molar heksosa yang digunakan iaitu 2.5. Maka fermentasi selanjar selanjutnya menggunakan unit nisbah molar ini sebagai unit penentu ukuran fermentasi POME.

Fermentasi selanjar bagi penghasilan biohidrogen dijalankan dalam jangkamasa 60 ke 150 hari untuk mengenal pasti kestabilan proses. Sistem sel terampai adalah lebih baik berbanding sel tersekatgerak polidimetilsiloksina dengan nilai keterhasilan biohidrogen 1.72 dan 1.58 mol H_2/mol POME-heksosa, masing-masing pada had ketahanan masa 4 hari. Ini juga merujuk kepada kadar penghasilan biohidrogen sebanyak 2.6 ± 0.9 NL H_2/L POME/hari dengan biohidrogen merupakan 52% daripada jumlah biogas terhasil. Profil elektroforesis gel kecerunan denaturasi (DGGE) menunjukkan peningkatan sampel efluen mengandungi jalur terang terdiri daripada bakteria penghasil biohidrogen berkait rapat dengan filogenetik *Clostridaceae* pada HRT rendah dan secara tidak langsung menjelaskan pengaliran keluar bakteria daripada dalam tangki pengaduk. Walau bagaimanapun, hanya 13% daripada sejumlah kandungan organik dalam POME yang dicerna dalam keaadaan operasi semasa dengan potensi tenaga terhasil sebanyak 2.4 MJ/kg COD-digunakan. Ini menjadikan POME kurang sesuai sebagai bahan mentah untuk memperoleh lebih tenaga dalam bentuk biohidrogen.

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LIST OF ABBREVIATIONS

ASBR	-	Anaerobic Sequencing Batch Reactor
BOD_5	-	Biological Oxygen Demand 5 days
CSTR	-	Continuous Stirred Tank Reactor
COD	-	Chemical Oxygen Demand
EFB	-	Empty Fruit Bunches
EtOH	-	Ethanol
FFB	-	Fresh Fruit Bunches
GC-FID	-	Gas Chromatography – Flame Ionisation Detector
GC-TCD	-	Gas Chromatography – Thermal Conductivity Detector
GC-FPD	-	Gas Chromatography – Flame Photometric Detector
$GWP_{(100\ years)}$	-	Global Warming Potential over 100 years time horizon
HAc	-	Acetic acid
HPr	-	Propionic acid
HBu	-	Butyric acid
i-HBu	-	Iso-butyric acid
HVa	-	Valeric acid
i-HVa	-	Iso-valeric acid
$bioH_2$	-	Biohydrogen
HCO_3^-	-	Hydrogen carbonate ion
H_2S	-	Hydrogen sulphide
HRT	-	Hydraulic Retention Time
HY	-	Hydrogen Yield (mol H_2/mol substrate)
HPR	-	Hydrogen Production Rate (mol H_2/L/d)
LCA	-	Life Cycle Assessment
LCI	-	Life Cycle Inventory
LCFAs	-	Long Chain Fatty Acids
LULUCF	-	Land Use, Land Use Change and Forestry
NH_3	-	Ammonia
NL/ NmL	-	Normalised volume (liter or milliliters) of gas to standard temperature (237 K) and absolute pressure of 760 mm Hg.
VFAs	-	Volatile Fatty Acids
O&G	-	Oil and Grease
O_2	-	Oxygen
OHPA	-	Obligate Hydrogen-Producing Acetogens
PCR-DGGE	-	Polymerase Chain Reaction – Denaturing Gradient Gel Electrophoresis

POME	-	Palm Oil Mill Effluent
SMP	-	Soluble Microbial Product
SRT	-	Solid Retention Time
SHPRT	-	Specific Hydrogen Production Rate (mol H_2/g VSS/d)
TN	-	Total Nitrogen
TKN	-	Total Kjedhal Nitrogen
TS	-	Total Solids
TSS	-	Total Suspended Solids
VSS	-	Volatile Suspended Solids

CHAPTER 1

INTRODUCTION

Malaysia is currently the world largest exporter of palm oil (Fig. 1.1) and produces about 41% of the world supply of palm oil in 2008; second in ranking after Indonesia. Among other main exporting countries are Papua New Guinea, Colombia, Singapore, Cote d'lvoire and Hong Kong. With a projected export volume of 17 million tonnes (Palm Oil HQ, 2010) and crude palm oil prices around MYR2,430 to 2,550 per tonne (i.e based on one month CPO prices traded in May 2010), the crude palm oil export earnings may slightly dwindle between MYR41.3 to 43.4 billion in 2010.

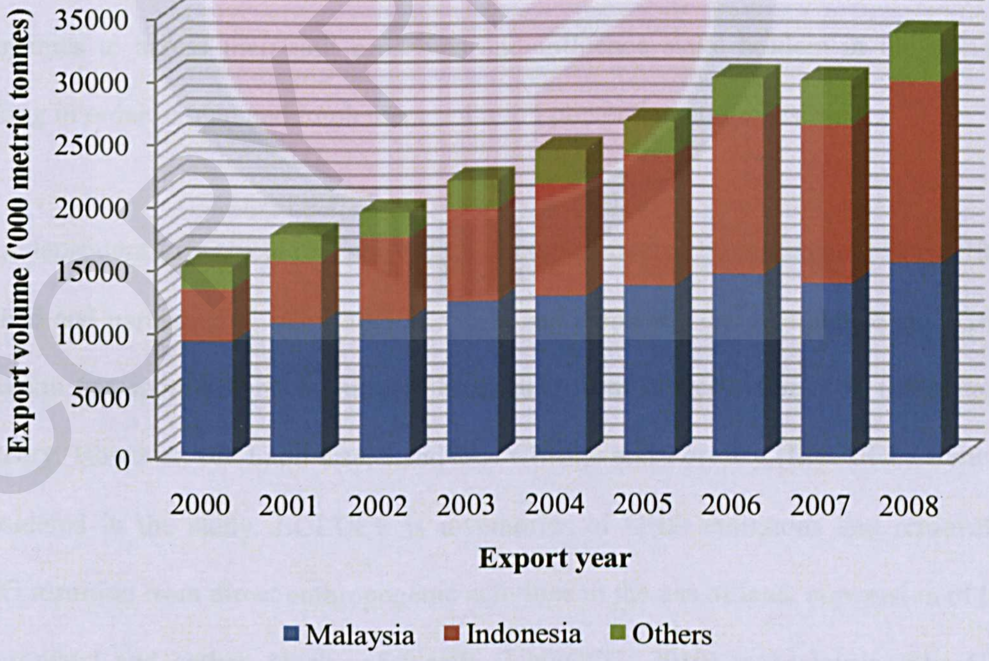

Figure 1.1: World Major Exporters of Palm Oil
(Source: MPOB, 2010)

The fresh fruit bunches (FFB) yield was about 85.71 million tonnes in 2009 which also reflected the concomitant volumes of palm oil mill effluent produced to approximately 57.42 million cubic metres. The potential energy recovery as methane is calculated to reach 1607.76 M cubic metres per year (EPU, 2010) which warrant serious concern on the long term environmental and social impact in tandem with uncontrolled methane emission to the atmosphere. The coined word herewith is "sustainability of oil palm industry". Plantation companies will no longer focus mainly on gaining the maximum yield of the trees but also have to consider the environmental issues related to the palm oil production; right from the seed production to the final product stage.

Palm oil industries are being proactive in curbing further pollution by adopting Environmental Quality System ISO 14000 and life cycle assessment in their operation. These self regulated environmental management tools will enable the plantation companies to assess their unit processes and influence stake holders in the decision making in order to plan and implement pollution prevention strategies.

An independent Life Cycle Inventory (LCI) study conducted by researchers from SIRIM Berhad and participating palm oil companies had identified the greenhouse gas (GHG) emission hot spots within the studied boundary; from nursery to mill in the oil palm industry. However, the Land Use, Land-Use Change and Forestry (LULUCF) were not considered in the study. LULUCF is inventories of GHG emissions and removal of GHG resulting from direct anthropogenic activities in the use of land, conversion of land to cropland and carbon stocks of forests (UNFCCC, 2010) in Malaysia. The GHG emission determined in the LCI study was in the range of 650 – 1,300 kg CO_2 per ton crude palm oil (CPO) depending on the mode of allocation and industry practice (Chen, 2008). The most prominent hot spot was the biogas emission from open ponds equivalent to 65% of the total GHG emission (Fig. 1.2).

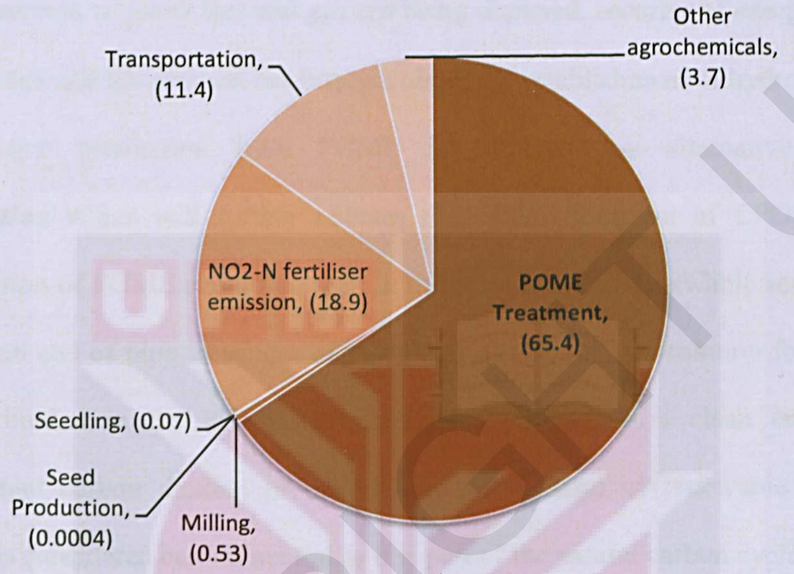

Figure 1.2: Distribution of Greenhouse Gases Emission in the Production of Crude Palm Oil
(Source: Ismail and Chen, 2009)
Values in parentheses (% GHG emission)

It was estimated that 30.3 kg methane per ton CPO were emitted during the inventory year. The GHG emission attributed from POME open ponding treatment was equivalent to 758 kg CO_2 per ton CPO, if the latest 2006 IPCC methodology was used for basis of calculations. The emission of 1 kg CH_4 is equivalent to the emission of 25 kg CO_2 ($GWP_{100years}$) (IPCC, 2010). Global Warming Potential (GWP) is a comparative index to carbon dioxide where it represents the combined effect of the greenhouse gas that remains in the atmosphere over a time horizon i.e over 100-year time frame.

Development of environmental technical know-how has increased in parallel with rapid development of palm oil industries. Palm oil mill effluent (POME) is now seen as untapped renewable energy reserve as it can be anaerobically converted to generate biogas and the enriched methane can be used as source of energy for power generation. As the reserves of fossil fuel and gas are being depleted, security of energy has become world issues and the demand has brought about the establishment of hydrogen economy. Biohydrogen production from POME is perceived as alternative to methane fermentation which will further reduces the carbon footprint of CPO. Hence, dark fermentation of POME should be seen in the perspective of renewable source of energy rather than end of pipe treatment of POME as it is not a total solution for reducing the organic burden in the POME. Primarily, biohydrogen is a clean energy and the concomitant carbon dioxide produced from metabolism of renewable resources e.g POME, is considered carbon neutral as it is part of the natural carbon cycle.

1.1 Research Objectives

The major hurdles to the practical application of biohydrogen production from complex wastewater are lack of understanding on the substrate conversion processes, the influence of the organic and inorganic constituents in the wastewater on the process, stability of biohydrogen production process when mixed microflora is used and the potential energy yield as compared to methane fermentation. This thesis is focused in establishing the know-how on generating biohydrogen from POME as knowledge gained by studying dark fermentation of carbohydrate–based substrate is insufficient to be applied for conversion of complex wastewater. Hence, POME was selected as model wastewater among other complex, agricultural wastewater due to the fact that first, it is the most abundance organic polluters in Malaysia; if not treated. Secondly, the high carbon footprint of CPO production in relation to direct emission of biogas from the open ponding system and lastly, the commitment of oil palm industries in tandem with national aspiration to reduce 40% of carbon intensity of Gross Domestic Product (GDP) by the year 2020 compared to 2005 levels.

Therefore, the objectives of this study are;

1. To determine the hydrogen-producing potential of mixed cultures obtained from methane digester treating palm oil mill effluent.
2. To investigate the potential of suspended sludge in generating biohydrogen from POME under thermophilic condition.
3. To evaluate the capacity of immobilised cells in generating biohydrogen under continuous culture.

1.2 Research Approach

This thesis addresses fundamental and applied aspects of relevant biohydrogen fermentation processes; focusing on hydrolysis and acidogenesis of soluble and particulate substrates. Issues related to fermentation of soluble components of the complex wastewater to generate biohydrogen are discussed and the application of synthetic wastewater with similar influent matrix to model the conversion process. Fig.

1.3 described the research approach diagrammatically and the research objectives have been approached stepwise as follows;

1.2.1 Evaluation on Pre-treated Sludge

The work mainly focused on requirement of treating the readily available sludge prior to seeding in the selected reactor system. The aim is to remove hydrogen-consuming methanogens and enriching thermophiles from the mixed population of microorganisms. Selection pressures were carried out by subjecting the mesophilic methane digester sludge to low pH and high temperature. Chapter 4 described the sludge activity test that was used to evaluate the potential of sludge under study in generating biohydrogen when fed with synthetic substrate.

1.2.2 Full Characterisation of POME and Substrate Selectivity Study

Understanding the components of the POME is important in order to evaluate the potential of POME in generating biohydrogen and also determine the energy yield from the biological conversion process. The main task is to identify which component among others in the POME that renders evolution of biohydrogen under low hydraulic retention time. Detailed methodology will be discussed in Chapter 5.

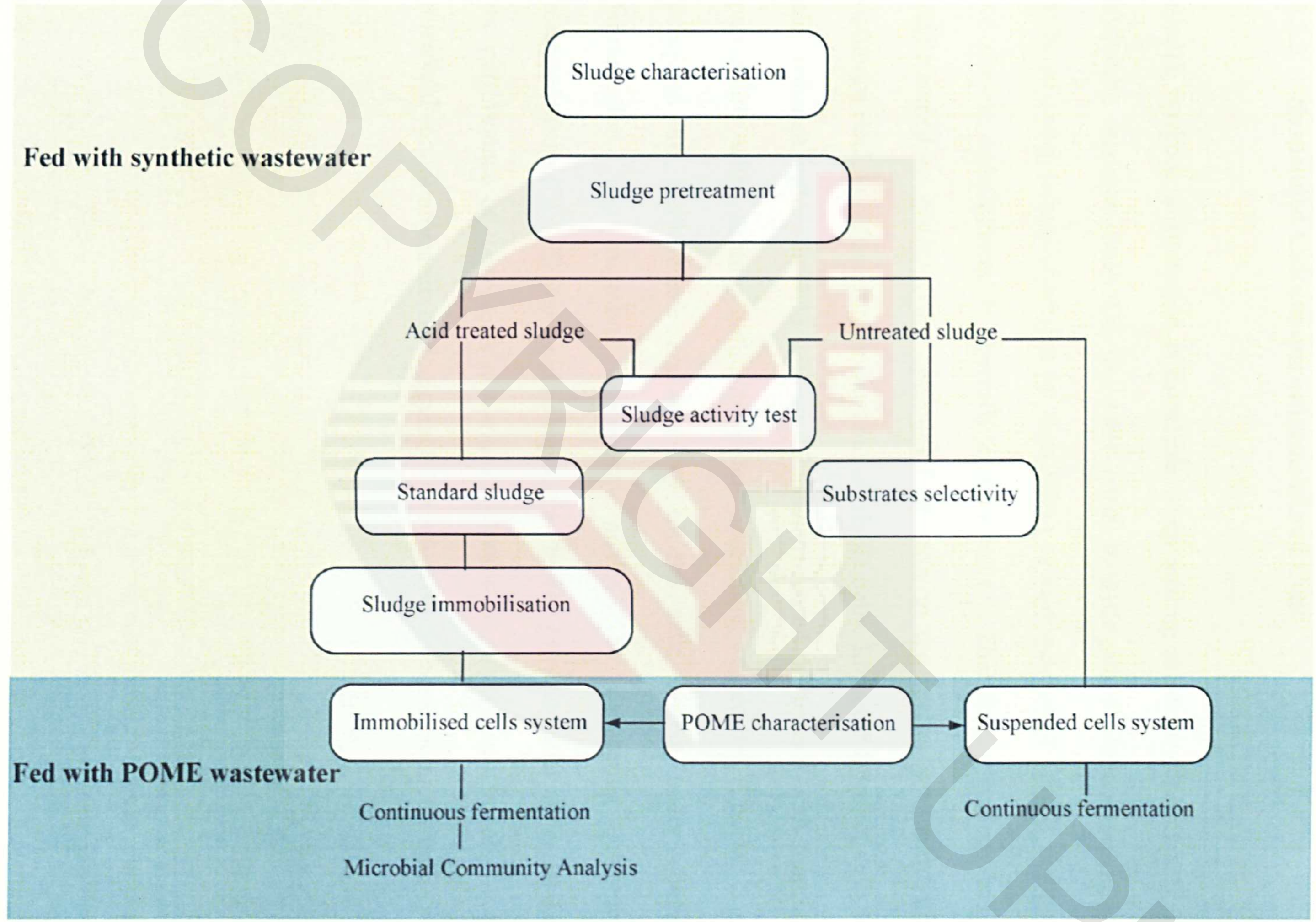

Figure 1.3: Research Approaches

1.2.3 Suspended Culture *versus* Immobilised Cells

Sludge washout phenomenon is inevitable when feeding substrate continuously at low hydraulic retention time. On the other hand, biohydrogen evolution rate is greatly affected under famine condition (at high HRT) as acidogenesis of soluble components i.e. soluble carbohydrates is generally a rapid process. Therefore, a strategy has to be implemented to maintain the optimum F/M ratio in the reactor by identifying the optimum HRT to reduce further washout of viable cells. Immobilisation of cells would also enhance hydrogen yield without the need to reseed the reactor with fresh sludge particularly when dealing with high solids–containing wastewater. Chapter 5 and 6 described the performance and stability of the cells system under continuous fermentation. The microbial community analysis by denaturing gradient gel electrophoresis (DGGE) was used as supporting tool to provide better understanding on the performance of the reactor at various hydraulic retention times in relation to the microbial community shifts of dominant species of hydrogen-producing bacteria.

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