PRODUCTION OF POLYHYDROXYBUTYRATE DURING THE TREATMENT OF PALM OIL MILL EFFLUENT

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Dedicated with love and gratitude to Bonda, Allahyarham Ayahanda, Isteri (Rohana), Anakanda (Zafirah Amni, Zahidah Amni, Zahirah Amni, Muhd Zakwan, Muhd Zul Aiman Adib, Muhamad Zul Iqbal Hafiz), Kekanda and Adinda

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ABSTRACT

Palm oil mill effluent (POME) is one of the most problematic waste, generated through out the year from the palm oil mill industry. The excessive production of this effluent without proper control results in serious environmental problems. However POME can be a cheap carbon source to produce bioplastic, like polyhydroxyalkanoate (PHA) or polyhydroxybutyrate (PHB) in the activated POME treatment system. In this study the sludge in anaerobic pond A1, anaerobic pond A2 and facultative pond F1 in the treatment plant at PPNJ, Kahang palm oil mill, were used as substrates to produce maximum amount of PHB. By using a 10 liters of laboratory reactor, the existing bacteria propagated in growth phase and the optimum formation of PHB in the bacterial cell was studied during accumulation phase under feast and famine process. The range of volatile fatty acid (VFA) was 11.43 mg/l to 577.22 mg/L for the scope of this study. Analytical measurement and PHB quantification were conducted by using gas chromatography. The highest production of PHB was found at anaerobic pond A1 (0.281 mt per day process and 0.0003 mt/mt fresh fruit bunch), at concentration of 0.11 g/L.

ABSTRAK

Efluen kilang minyak sawit (POME) yang dikeluarkan semasa pemprosesan buah sawit kini masih memberi masalah kepada industri sawit. Pengeluaran yang berlebihan jika tidak dikawal boleh mengakibatkan masalah besar terhadap alam sekitar. Namun begitu POME telah dikenalpasti boleh digunakan sebagai punca karbon yang murah untuk pengeluaran bioplastik, seperti polihidroksialkanoit (PHA) dan polihidroksibutirat (PHB). Dalam kajian ini enapcemar dari kolam anaerobik A1, kolam anaerobik A2 dan kolam fakultatif F1, semasa proses rawatan efluen di kilang Sawit PPNJ Kahang, digunakan sebagai substrat untuk menghasilkan jumlah PHB yang maksima. Dengan menggunakan bioreaktor makmal berkapasiti 10 liter, bakteria sedia ada di kolam dibiakkan melalui fasa pertumbuhan, dan pembentukan PHB secara optima di dalam sel bakteria telah dikaji semasa fasa penumpukan di dalam proses '*feast and famine'*. Julat asid lemak tak menentu (VFA) di dapati dari 11.43 mg/L hingga 577.22 mg/L. Jumlah PHB terhasil di analisa dengan menggunakan gas kromatografi. Pengeluaran PHB boleh dihasilkan dengan kuantiti maksima dari kolam anaerobik A1 (0.281 metrik tan setiap hari buah sawit diproses dan 0.0003 metrik ton bagi setiap metrik ton buah sawit diproses), pada kepekatan PHB 0.11 g/L.

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LIST OF ABBREVIATIONS

AN	-	Ammonical Nitrogen (mmol/l or mg/l)
CDW	-	cell dry weight (g/l)
COD	-	chemical oxygen demand c-mmol/l or mg/l)
CPO	-	crude palm oil
DO	-	dissolved oxygen (Oxygen Saturated) (mg/l)
DOE	-	Department of Environment
EFB	-	Empty fruit bunch
GC	-	Gas Chromatography
HRT	-	Hydraulic retention time
MPOB	-	Malaysian Palm Oil Board
Mt	-	Metric tone
PHA	-	Polyhydroxyalkanoates
PHB	-	Poly-β- hydroxybutyrate
POME	-	Palm oil mill effluent
SBR	-	Sequencing batch reactor
SS	-	Suspended Solid
Temp	-	Temperature (º C)
VFA	-	Volatile Fatty Acid

LIST OF SYMBOLS

CO_2	-	carbon dioxide (mmol/l or mg/l)
HAc	-	acetic acid (mg/l)
HBt	-	butyric acid (mg/l)
HPr	-	propionic acid (mg/l)
NO_3	-	nitrate (mg/l or mmol/l)
O_2	-	oxygen (mg/l or mmol/l)
PO_4	-	phosphate (mg/l or mmol/l)

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Biomaterials are natural products that are widely used in biotechnological applications. They are biodegradable, environmental friendly and do not cause toxic effects.

Bioplastics are polymers and are considered to be a special type of bio-material. Micro-organisms generate a source of bioplastics and biopolymers from renewable sources. Although it is currently more expensive than plastic derived from petrochemicals, bacteria have proved to help yield bioplastics with comparable properties. The most widely used microbial bioplastics are polyhydroxyalkanoates (PHA), polyhydroxybutyrate (PHB) and other different monomers and derivatives.

Palm oil mill effluent (POME) is a liquid waste, generated during the processing of fresh fruit bunches to produce crude palm oil and palm kernel. POME is non-toxic in nature but must be treated to acceptable quality before it can be discharged into the water course. The biological treatment of POME comprises both

anaerobic and aerobic treatment. POME can serve as a cheap substrate for commercial scale production of PHB/PHA as it is available in huge quantities from 406 mills present in Malaysia as listed in Table 2.1.

Fresh POME or mixed raw effluent consists of 95 to 96% water, 0.6 to 0.7% oil, 4 to 5% total solids and 2 to 4% of suspended solids and without proper treatment it contributes to a major source of water pollution in Malaysia.

POME has been reported to be suitable for PHA production (Hassan *et al*., 1997, 2002; Md Din *et al*., 2004, 2006, Mumtaz *et al*., 2010). Fresh POME is usually present in the complex form containing mainly triacyglycerides, diacylglycerides, monoacylglycerides and some other derivatives like fatty acids (Alias and Tan, 2005).These form cannot be directly utilized by PHA producing bacterial species for PHA synthesis. Hydrolysis and acetogenesis processes in anaerobic treatment have been proposed to reduce the POME characteristics and also converting undissolved compound of POME to simpler short–chain VFAs (Volatile Fatty Acids) compounds like acetic, butyric, and propionic acids. The VFAs will be utilized by PHA- producer for PHA production (Lee and Yu, 1997). Hassan *et al*., (1997, 2002) found that 7 g/l of organic acids could be obtained from POME and can be utilized for PHA production with pure culture. The high organic acids contained in POME are fit to be used as carbon source to produce PHA/PHB.

PPNJ Kahang Palm Oil Mill is owned by Pertubuhan Peladang Negeri Johor. Its processing capacity of 60 mt fresh fruit bunch (FFB) per hour generating POME as a waste is average at about 800mt per day. The POME has to be treated according to the Department of Environment's Regulations. In the mill, POME is treated in two anaerobic ponds, three aerobic ponds, followed by extended aeration plant for polishing before final discharge to river as shown in Figure 1.1.

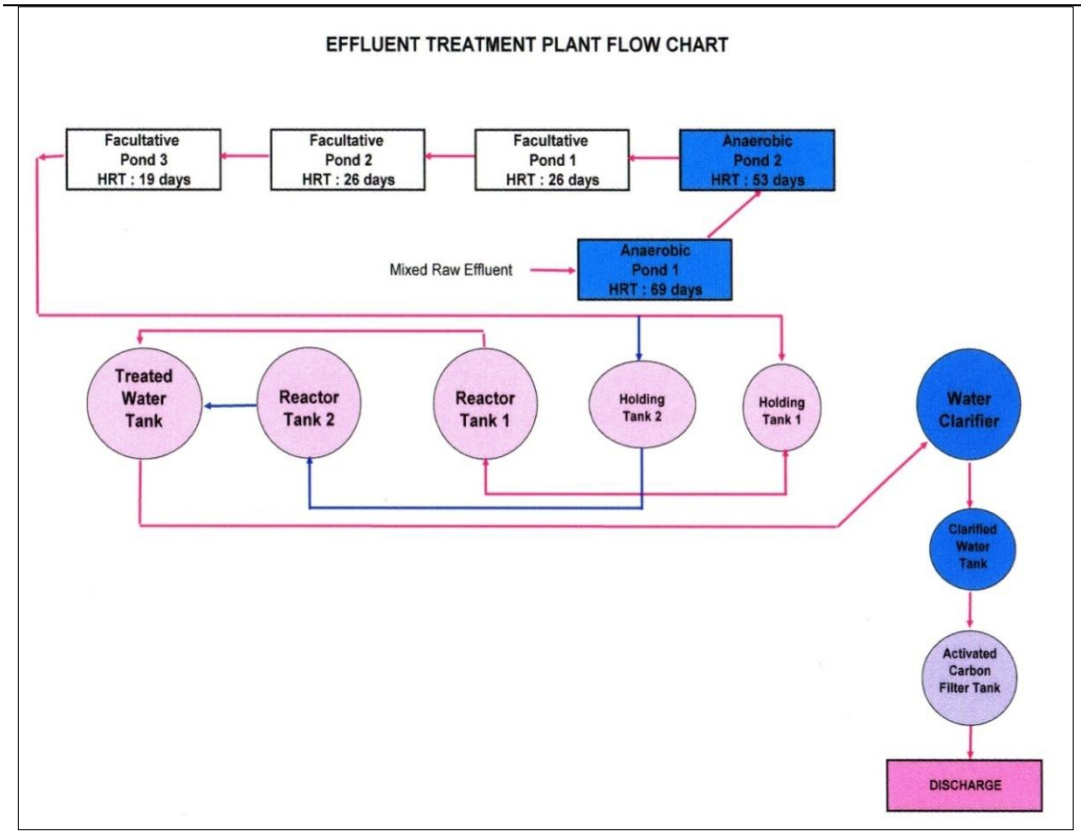

Figure 1.1 Effluent treatment system at PPNJ Kahang Palm Oil Mill

Considering that about 300,000 mt of fresh fruit bunch is processed a year, as budgeted for every year, PPNJ Kahang Palm oil Mill has approximately 210,000 mt of POME per year that could be used as a cheap carbon source in producing PHB/PHA.

The aim of this study is to determine the pond and the maximum amount of PHB that can be produced from the entire system of effluent treatment plant at PPNJ Kahang Palm oil mill. The amount of PHB produced will be calculated base on one tonne of FFB processed, as well as per day of FFB processed at PPNJ Kahang Palm oil mill.

1.2 Problem Statement

The problem statements in this study are:

i. PPNJ Kahang Palm Oil Mill with processing capacity of 60 mt FFB/hour, discharges 48 mt POME/hour or 960 mt of POME/day to anaerobic and facultative ponds for treatment. The POME, which is rich in organic carbon, could be used as a cheap carbon source to produce biodegradable plastic (PHA/PHB). This could be useful in converting palm oil mill waste to wealth.

ii. Where and how maximum PHB can be produced from the actual treatment system, need to be studied. In PPNJ Kahang palm oil mill, there are two anaerobic ponds (no 1 and 2) followed by three aerobic ponds at different range of BOD, pH and VFA concentrations. The maximum quantities of PHB to be produced per day and per metric tonne of FFB processed could be calculated and identified.

iii. Anaerobic bacteria from the existing anaerobic pond are possible to be used as PHB producer, without the used of any additional inocula. As stated in previous studies, bacteria have to be properly conditioned under feast and famine to produce higher amounts of PHB.

1.3 Objectives of the Study

The objectives of this study are:-

a. To determine the pond which produces maximum amount of PHB (in metric tonne PHB per metric tonne of fresh fruit bunch processed) in the actual POME treatment system at PPNJ Kahang Palm Oil Mill.

b. To quantify the optimum concentration of PHB produced in the actual POME treatment system in percentage on sample and in g/L.
c. To determine the quantity of PHB that can be produced per day, from the processing of 60 mt FFB/hour (PPNJ Kahang Palm Oil Mill), and the maximum PHB that can be produced from one tonne of fresh fruit bunch processed , where POME is the sole carbon source.

1.4 Scope of the Study

The scopes of this study are as follows:

i. Samples for this study were taken at the potential ponds along the POME treatment system at PPNJ Kahang Palm Oil Mill, both anaerobic and aerobic treatment, for maximum recovery of PHB.
ii. 10 liters laboratory scale reactor was used and operated based on sequential batch reactor (SBR) system. Aeration time needed was studied and set for feast and famine condition to get optimum recovery of PHB at every point sample.
iii. Analytical measurement and PHB quantifications were conducted at UTM laboratory according to the standard methods used for the examination of water and waste water (APHA, 2000).

1.5 Significance of the Study

Three important aspects which will be beneficial in achieving the objectives of this study are:

i) Investigation of abundance POME, which could be useful in converting it to harmless and beneficial substrates (biodegradable plastic). This study will convert oil palm industry waste to wealth.

ii) PHB extraction from POME will contribute to palm oil industry in diversifying their non-food uses at a low cost of production (by using available free carbon source from the estimated 70 million tonne available POME per year).

iii) POME is a major cource of water pollution in Malaysia. From the total amount of fresh fruit bunch (FFB) processed at palm oil mills, 80 percent of POME is generated. Thus, this study provides an alternative mean to reduce the pollution load due to the separation of solid biomas and carbon uptake to produce PHB.

1.6 Thesis Layout

The remaining part of the thesis is organized into four chapters as follow:

i. Chapter 2 contains literature review related to the topics discussed in this study. This chapter explains the existence of POME during the processing of fresh fruit bunch to produce crude palm oil and palm kernel, and the characteristic of POME to serve as substrate and to extract PHA/ PHB. Description of PHA/PHB as biodegradable plastic generated by the microorganism from waste

water (as carbon source), and the potential of PHA/PHB as a revenue for palm oil industry are also discussed in this chapter.

ii. Chapter 3 presents the implementation of the study in terms of detailed theory and methodology, including processes and equipment.

iii. Chapter 4 examines the results of the study at every point of sample collection along the POME treatment system. Specifically, this chapter discusses the inoculation technique, statistical data analysis of the obtained data and PHB quantification.

iv. Chapter 5 presents the overall finding, achievements and conclusions of the study. The conclusions are drawn from the experience gained throughout this study. The significance of the findings and achievements that could be used for considerations of future work is also discussed.

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