UNIVERSITI PUTRA MALAYSIA

BIODEGRADATION OF OIL PALM EMPTY FRUIT BUNCHES IN LIQUID FERMENTATION USING MIXED MICROORGANISMS FROM PALM OIL MILL EFFLUENT

NOR FADILAH ABD. HALIM

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By

NOR FADILAH ABD. HALIM

Thesis Submitted to the School of Graduates Studies, Universiti Putra Malaysia, In Fulfilment of the Requirements for the Degree of Masters

April 2008

DEDICATION TO;

MY HUSBAND, JAIYA ABU

AND

MY LOVELY KIDS,

UMAR ABDUL AZZEZ & UMAIRAH NUR NUHA

AND MY PARENTS,

ABD. HALIM PEAI & FATIMAH LAHADI

FOR YOUR ENDLESS LOVE

Abstract of thesis presented to the Senate of Universiti Putra Malaysia in fulfillment of the requirements for the degree of Master of Science

BIODEGRADATION OF OIL PALM EMPTY FRUIT BUNCHES IN LIQUID FERMENTATION USING MIXED MICROORGANISMS FROM PALM OIL MILL EFFLUENT

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NOR FADILAH BINTI ABD. HALIM

April 2008

Chairman : Tey Beng Ti, PhD

Faculty : Engineering

The degradation of oil palm empty fruit bunch (EFB) in liquid fermentation using microbial complex obtained from palm oil mill effluent (POME) under non-sterile condition was investigated in the present study. A control study using carboxymethylcellulose (CMC) as substrate to examine the cellulose degradation ability of the microbial complex from POME. The effects of the operating parameters of fermentation; size of inoculum, aeration rate, pH and substrates concentration on the degradation of EFB and CMC were studied. The effectiveness of mixed microorganisms from POME on the biodegradation of pure cellulose EFB was also determined in this study. The degradation was performed at agitation speed of 150 rpm in a 10 liter bioreactor under various above mentioned process parameters for a 10 day period. The evolution of dry matter (DM) and Chemical oxygen demand of solids (COD_s) were used to measure the performance of the non-sterile liquid fermentation process on the solid degradation of EFB and CMC. An optimized biodegradation process of EFB and CMC by mixed microbes from POME was performed at the end of this study. The selection of the parameters for the optimized biodegradation process was based on those that provided the best degradation during the optimization study.

The study of the effect of inoculum size on the biodegradation of CMC showed that 300 mL/L is the optimal condition for both DM and CODs reduction. The DM and CODs reduction of EFB was slightly increased (< 7%) as the inoculum size increase to 450 mL/L but the biodegradation rate of CMC was reduced by more than 50% (in term of CODs reduction). Hence 300 mL/L was chosed as optimal inoculum size for the study of the optimized biodegradation of EFB and CMC.

The effect of aeration rate on the reduction of DM and CODs was different. The highest DM reduction was achieved at 1.0 vvm, while the highest CODs reduction was achieve at 0.5 vvm for both EFB and CMC biodegradation study. As a result aeration rate of 0.5 vvm was selected as the optimal condition for further study.

The effect of pH on the reduction of DM and CODs was somewhat different. Generally lower pH give a better DM reduction for both EFB and CMC biodegradation studies. However, the highest CODs reduction was achieved at pH 4.5 and 5.0 for the biodegradation study of EFB and CMC, respectively. The pH 5.0 was selected for the optimized study as the highest specific reduction rates of both DM and CODs were achieved at this pH value.

The reduction of DM and CODs was different when changing substrate concentration for the biodegradation studies of EFB and CMC. The highest DM reduction was achieved at the substrate concentration of 10 g/L for both EFB and CMC. The highest CODs reduction was achieved at substrate concentration of 20 and 25 g/L for the biodegradation studies of EFB and CMC, respectively. The substrate concentration of 15 g/L was selected for optimized biodegradation study due to the highest specific rates of reduction for both DM and CODs were achieved of this value of substrate concentration.

Optimized biodegradation studies of both EFB and CMC were conducted at the inoculum size of 300 mL/L, aeration rate of 0.5 vvm, pH of 5 and substrate concentration of 15 g/L. The DM and CODs reduction for EFB were respectively 70% and 30% higher than that of CMC. The microbial analysis revealed that more fungus and yeast population were found during the middle stage of EFB biodegradation compared to that of CMC. Bacteria population has dominated the fermentation medium of CMC. This may explained the higher level of biodegradation that has been achieved in the EFB experiment. Yeast and fungus has a better ability to degrade the complex structure of biomaterial such as lignin, hemicellulose and cellulose found in the EFB.

The degradation of EFB can be expeditiously carried out to achieve about 57% DM reduction under fermentation conditions of inoculum size of 300 mL/L mixed culture microorganisms, aeration rate of 1.0 vvm, pH 6.0 and substrate concentration of 15 g/L. The highest DM reduction of CMC, 31.11% was achieved under 150 mL/L of inoculum size, 0.5 vvm aeration rate, pH 6.0 and substrate concentration of 15 g/L. In conclusion, the biodegradation of EFB and CMC was affected differently by the parameters being studied due to the presence of different type of microbes in the middle stage of biodegradation process.

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

BIODEGRADASI TANDAN KOSONG KELAPA SAWIT MENGGUNAKAN KOMPLEK MIKROORGANISMA DARI EFLUEN KILANG KELAPA SAWIT DENGAN KAEDAH FERMENTASI DALAM KEADAAN CECAIR

Oleh

NOR FADILAH BINTI ABD. HALIM

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Fakulti : Kejuruteraan

Biodegradasi tandan kosong kelapa sawit (EFB) dengan kaedah fermentasi dalam keadaan cecair tanpa steril oleh komplek mikroorganisma dari efluen kilang kelapa sawit (POME) dikaji dalam penyelidikan ini. Satu penyelidikan kawalan menggunakan selulosa metil karboksil (CMC) sebagai substrat dijalankan untuk mengkaji kemampuan kompleks mikroorganisma daripada POME untuk membiodegradasikan selulosa. Kesan operasi parameter fermenter seperti saiz inokulum, kadar pengudaraan, pH dan kepekatan substrat terhadap biodegradasi EFB dan CMC telah dikaji. Keberkesanan penggunaan komplek mikrob daripada POME terhadap biodegradasi EFB dan CMC turut juga dikaji. Proses biodegradasi ini dijalankan dengan bantuan agitasi pada kelajuan 150 rpm didalam bioreaktor berkapasiti 10 liter dengan proses parameter yang telah dinyatakan di atas selama 10 hari. Evolusi terhadap bahan kering (DM) dan keperluan oksigen kimia pepejal (COD_s) digunapakai untuk mengukur keberkesanan fermentasi dalam keadaan cecair

tanpa steril terhadap proses biodegradasi pepejal bagi EFB dan CMC. Proses biodegradasi dalam keadaan yang optima untuk EFB dan CMC turut dikaji di akhir penyelidikan ini. Pemilihan parameter untuk proses optima adalah berdasarkan kepada kadar biodegradasi yang terbaik yang diperolehi semasa pengoptimuman proses.

Kajian terhadap kesan saiz inokulum terhadap biodegradasi EFB dan CMC menunjukkan inokulum bersaiz 300 mL/L adalah merupakan keadaan paling optima bagi pengurangan DM dan CODs. Pengurangan DM dan CODs bagi EFB mengalami sedikit kenaikan (kurang dari 7%) apabila saiz inokulum ditambah kepada 450 mL/L tetapi bagi CMC, kadar biodegradasinya berkurangan sebanyak lebih dari 50% (dalam bentuk pengurangan CODs). Oleh yang demikian, saiz inokulum sebanyak 300 mL/L telah dipilih sebagai saiz inokulum yang optima untuk kajian optimasi bagi biodegradasi EFB dan CMC.

Kesan oleh kadar pengudaraan adalah berbeza terhadap pengurangan DM dan CODs. Pengurangan DM tertinggi dicapai pada 1.0 vvm, sementara pengurangan CODs tertinggi dicapai pada 0.5 vvm bagi biodegradasi EFB dan CMC. Oleh itu, kadar pengudaraan sebanyak 0.5 vvm dipilih sebagai keadaan optima bagi kajian optimasi biodegradasi selanjutnya.

Kesan oleh pH terhadap pengurangan DM dan CODs adalah sedikit berbeza. Secara amnya, pH yang rendah memberikan pengurangan CODs yang lebih baik bagi biodegradasi EFB dan CMC. Oleh yang demikian, pH 5.0 telah dipilih untuk kajian optimasi berdasarkan kadar pengurangan spesifik bagi DM dan CODs yang tertinggi telah dicapai pada pH ini.

Kesan oleh kepekatan substrat adalah berbeza terhadap pengurangan DM dan CODs bagi kajian biodegradasi EFB dan CMC. Pengurangan DM tertinggi dicapai pada kepekatan substrat sebanyak 10 g/L bagi EFB dan CMC.

Pengurangan CODs tertinggi dicapai pada kepekatan substrat 20 dan 25 g/L bagi kajian biodegradasi EFB dan CMC. Kepekatan substrat sebanyak 15 g/L dipilih untuk kajian optimasi biodegradasi bagi EFB dan CMC berdasarkan kadar pengurangan spesifik bagi DM dan CODs yang tertinggi telah dicapai pada kepekatan substrat ini.

Kajian biodegradasi optimal bagi EFB dan CMC dijalankan dengan inokulum bersaiz 300 mL/L, kadar pengudaraan sebanyak 0.5 vvm, pH 5 dan kepekatan substrat sebanyak 15 g/L. Pengurangan DM dan CODs bagi EFB adalah 70% dan 30% lebih tinggi berbanding CMC. Analisa populasi mikrob menunjukkan lebih banyak populasi kulat dan yis dikesan semasa peringkat pertengahan proses biodegradasi EFB berbanding dengan proses biodegradasi CMC. Media formulasi bagi CMC telah didominasikan oleh populasi bakteria. Ini telah menerangkan mengapa aras biodegradasi yang tertinggi telah dicapai dalam EFB berbanding dengan CMC. Yis dan kulat mempunyai kemampuan untuk mengdegradasikan struktur biomaterial kompleks seperti lignin, hemi selulosa dan selulosa yang terdapat di dalam EFB.

Degradasi EFB boleh dilakukan degan pantas untuk mencapai penguranga DM sebanyak 57% dibawah keadaan fermentasi dengan saiz inokulum 300 mL/L kulturan mikroorganisma campuran,kadar pengudaraan 1.0 vvm, pH 6.0 dan kepekatan substrak 15 g/L. Pengurangan DM tertinggi, 31.11% telah tercapai dibawah keadaan 150 mL/L saiz inokulum, kadar pengudaraan 0.5 vvm, pH 6.0 dan kepekatan substrak 15 g/L. Secara kesimpulan, kesaan pproses parameter ke atas biodegradasi EFP dan CMC dipengaruhi adalah berlainan disebabkan oleh kehadiran jenis mikrob yang berlainan semasa peringkat pertengahan proses biodegradasi.

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I certify that an Examination Committee met on 22nd April 2008 to conduct the final examination of Nor Fadilah Binti Abd. Halim on her Master of Science entitled “Biodegradation of oil pam empty fruit bunches in liquid fermentation using mixed microbes from palm oil mill effluent” in accordance with Universiti Pertanian Malaysia (Higher Degree) Act 1980 and Universiti Pertanian Malaysia (Higher Degree) Regulations 1981. The Committee recommends that the candidate be awarded the Master of Science.

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Date: 11 September 2008

DECLARATION

I declare that the thesis is my original work except for quotations and citations which have been duly acknowledged. I also declare that it has not been previously, and is not concurrently, submitted for any other degree at UPM or other institutions.

NOR FADILAH ABD. HALIM

DATE: 18 August 2008

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LIST OF ABBREVIATIONS

EFB	Empty fruit bunches
CMC	Cellulosemethylcarboxyl
DM	Dry matter
POME	Palm oil mill effluent
COD_s	Chemical Oxygen Demand solids
COD_l	Chemical Oxygen Demand liquids
COD_t	Chemical Oxygen Demand total

CHAPTER I

INTRODUCTION

Malaysia is the largest producer of palm oil in the world. It supplies 144 million tones or about 24.1% of total world consumption of oils and fats in 2005 (Cheah and Choo, 2005). The production of crude palm oil (CPO) continued to increase for seven consecutive years reaching 15.0 million tones in 2005. The total palm oil planted area increased by 4.5% or 174,000 hectares to 4.0 million hectares in 2005 and about 136 palms are planted per hectares (Wahid, 2005). Palm oil is one of the main sources of foreign exchange for Malaysia. A palm oil mill produces palm oil and palm kernel oil as main products. However, CPO and its economic co-products – palm kernel cake; constitute only 10% of the crop, leaving the rest of the biomass to waste. The biomass includes the oil palm trunks (OPT) and fronds (OPF), kernel shell, empty fruit bunch (EFB), pressed fruit fibre (PFF) and palm oil mill effluent (POME).

As depicted in Figure 1.1, 23 million tones of fresh fruit bunches (FFB), 6 million tones of shell and 13 million tones of fibre are produced for the processing of every 100 million tones of FFB in a palm oil mill. EFB, stalk material after fruit stripping, is a major cellulosic waste of the palm oil industry. Like other ligninolytic agricultural waste materials, it has a fairly high cellulose content, with an average of 50% dry matter basis (Kume et al., 1990; Husin et al., 1985). This by-product is left unutilized and its disposal has become an environmental problem. The EFB discharge from 351 palm oil mills in Malaysia is estimated to be 30 millions tones (dry weight basis) per year (Business Time, 2003). Transforming this agricultural residue into useful products is an option worth considering that has impact on local economy and the reduction in pollution (Birch et.al., 1976).

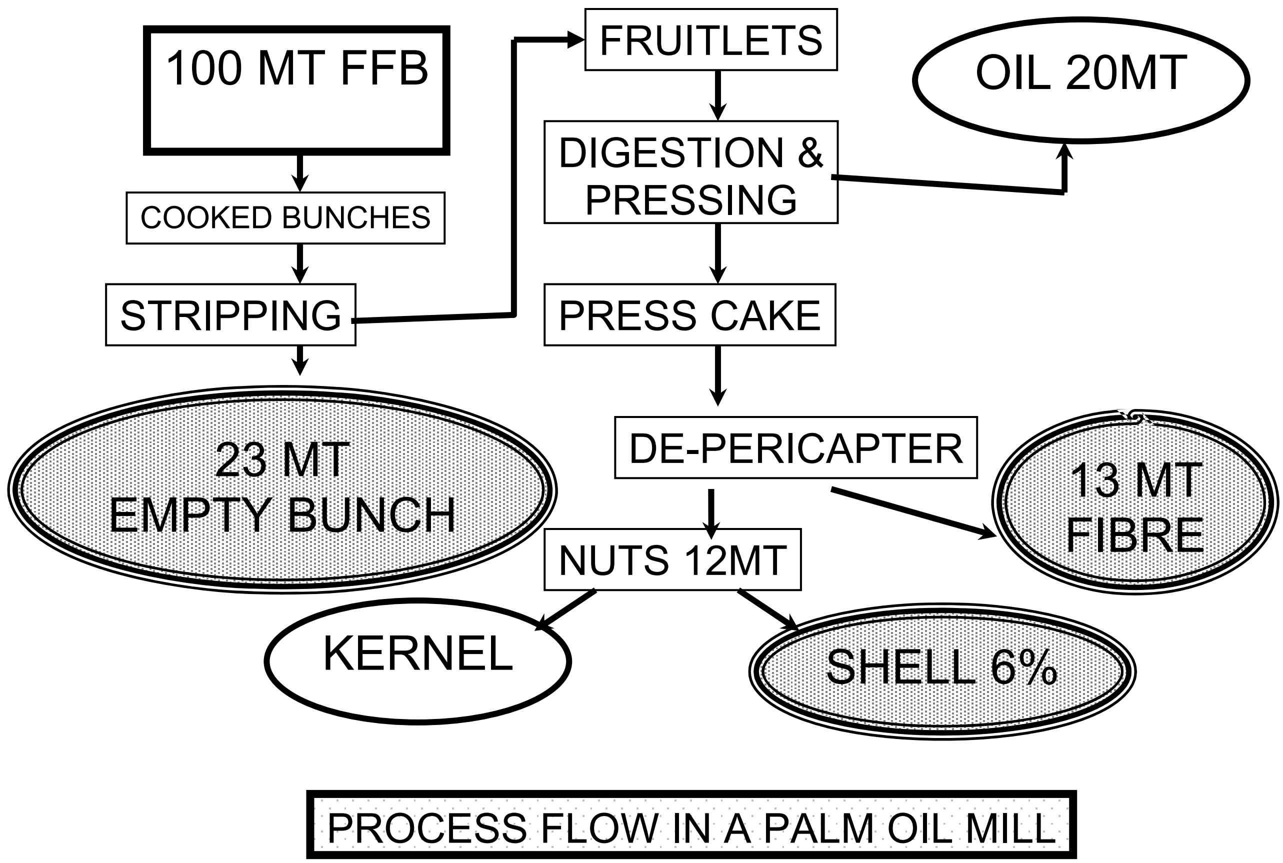

Figure 1.1: Ratio of oil palm by-products generated by the mills (Cheah and Choo, 2005)

The bulky nature of EFB causes a high land-fill disposal cost. The mills, therefore, burn the EFB down to ash which is used as potash fertilizer or distributed directly in the field as mulch. Particulates and gas (SO_2, CO_2, CO and NO_x) emitted from the furnaces sometimes cause air pollution to the nearby communities. Its results in public outcries, and hence is prohibited by the Environment Protection Act (1974). In abiding to the regulations, these residues are becoming expensive to dispose.

The understanding of the mechanism and kinetics of degradation of organic polymers such as polymeric agricultural waste will not only enable the technology of their processing to be improved but also making the use of polymeric materials to be rationalized. In most cases, degradation does not occur by depolymerization alone; it is accompanied by diverse reactions involving the primary cleavage of complex multi components structure. The process often used for the degradation of polymers occur under heterogeneous conditions, in which the limiting factors is not represented by chemical reactions but rather by mass and heat transfer.

Cellulose being the major component of EFB, is a very large molecule and completely insoluble material which requires exogenous hydrolytic enzymes for its degradation. No microbial cell can absorb molecular complexes like the size of a cellulose fiber. Cellulose breakdown under soil and compost environment is catalyzed by enzymes secreted by the soil microbe, which constitute a major fraction of the extracellular proteins.

1.1 Problem statement

The EFB discharge from 351 palm oil mills in Malaysia is estimated to be 30 millions tones (dry weight basis) per year. This huge volumes of organic waste, which are produced on a continuous basis, require effective removal procedures adapted to the nature of the by-product. EFB is mostly used as a mineral fertilizer substitute by direct application in the field or, in some cases, after incineration and or after composting. In fact, fresh EFB returns the mineral nutrients and organic matter to the soil and helps to maintain soil fertility (Loong *et al.,* 1987; Lim and Chan, 1989; Hornus and Nguimjen, 1992; Sadi *et al.,* 1992). The main constraints to EFB application are the high cost for storage and distribution and its pests attraction (Turner and Gillbanks, 1974; Hartley, 1980), but the tangible benefits to be accrued outweigh its practical disadvantage. The results from direct application are generally better vegetative growth, palm tree nutrition and yield (Chan and Goh, 1978; Lim and Pillai, 1979; Gurmit *et al.,* 1981; Loong *et al.,* 1987). The positive responses are attributed to its improvement in the soil moisture regime, soil structure, organic matter content and microbial activity, and reduction in soil erosion, nutrient losses and soil surface temperature (Chan *et al*., 1986; Hoong and Nadarajah, 1988).

In the past, EFB was incinerated in the mill as means to eliminate this waste as well as to provide energy for the boilers in fresh fruit bunches (FFB) sterilization. The bunch ash produced, contents about 30-40% of K_20. The ash is used as a