View metadata, citation and similar papers at core.ac.uk
brought to you by CORE
provided by Repository@USM

ACCUMULATION AND EXTRACTION OF POLYHYDROXYALKANOATE FROM AEROBIC GRANULES TREATING PALM OIL MILL EFFLUENT

GOBI KANADASAN

UNIVERSITI SAINS MALAYSIA
2015

ACCUMULATION AND EXTRACTION OF POLYHYDROXYALKANOATE FROM AEROBIC GRANULES TREATING PALM OIL MILL EFFLUENT

by

GOBI KANADASAN

Thesis submitted in fulfillment of the requirements

for the degree of

Doctor of Philosophy

December 2015

ACKNOWLEDGEMENT

I would like to express my deepest gratitude towards The Great Almighty Ganesha, who grants me the knowledge, strength and determination to accomplish Doctorate research work. My parents, Madam Vijaya Kothandaraman and Mr. Kanadasan Pachiappan who bestowed me with love needs special mention for me able to complete this research work. Their prayers, encouragement, patience and most importantly understanding the process of completing Ph.D has motivated and driven me throughout my studies.

Assoc. Prof. Dr.Vel Murugan Vadivelu as my main supervisor has guided, motivated and inspired me throughout this period. His stupendous ideas and vision have been the reason for this research to be successfully completed in time and with encouraging results. My appreciation also goes to Dean, Prof. Dr. Azlina bt. Harun @ Kamaruddin, deputy deans, and office staffs for their continuous support and help rendered throughout my studies.

I am very much indebted to MyBrain15 program for providing financial assistance in the form of scholarship. Besides that, the grant provided by Universiti Sains Malaysia (1001/PJKIMIA/814172) has helped me while doing this research. To all the technicians, especially Mr. Shamsul, Mr. Arif, Mr. Mohd Roqib, Mr. Muhamad Ismail, Madam Yusnadia and Madam Latifah who assisted me greatly while doing this research needs a special mention. Besides that, my colleagues, G.Sivarajah Ganesan, T.Vjayan, Lim Jing Xiang, and Tan Teong Ee who helped while conducting this research are gratefully acknowledged. Last but not the least, undergraduate students, Khesvina, Vasanthan, and Pavithra who worked in this project are acknowledged.

Gobi Kanadasan

December 2015

TABLE OF CONTENTS

CHAPTER 3 – MATERIALS AND METHODS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

ADF	Aerobic dynamic feeding
AR	Aeration rate
ASR	Activated sludge reactor
ATP	Adenosine triphosphate
BOD	Biodegradable organic content
$CDCl_3$	Deuterated chloroform
CDW	Cell dry weight
$CHCl_3$	Chloroform
COD	Chemical oxygen demand
DNA	Deoxyribonucleic acid
DO	Dissolved oxygen
DSLR	Digital single-lens reflex
EPS	Extracellular polymeric substances
F/M	Food to microorganism
FTIR	Fourier transform infrared spectroscopy
GC	Gas chromatography
GC-MS	Gas chromatography mass spectrometer
GPC	Gel permeation chromatography
H/D	Height to diameter ratio
HB	Hydroxybutyrate
HHx	Hydroxyhexanoate
HRT	Hydraulic retention time
HV	Hydroxyvalerate
IAAB	Integrated anaerobic-aerobic bioreactor
LB-EPS	Loosely bound EPS
MCL-PHA	Medium chain length PHA
MLSS	Mixed liquor suspended solids
MLVSS	Mixed liquor volatile suspended solids
Na_2SO_4	Sodium sulfate
NaCl	Sodium chloride
NADH	Nicotinamide adenine dinucleotide hydrogenase

NaOCl	Sodium hypochlorite
NaOH	Sodium hydroxide
NCBI	National Centre for Biotechnology Information
NMR	Nuclear magnetic resonance
OLR	Organic loading rate
OsO_4	Osmium tetroxide
P3(HB-*co*-HHx)	Poly-3-hydroxybutyrate-*co*-hydroxyhexanoate
P3(HB-*co*-HV)	Poly-3-hydroxybutyrate-*co*-hydroxyvalerate
P4HB	Poly-4-hydroxybutyrate
PCR	Polymerase chain reaction
PHA	Polyhydroxyalkanoate
PHB	Polyhydroxybutyrate
PHV	Polyhydroxyvalerate
POME	Palm oil mill effluent
SBR	Sequencing batch reactor
SCL-PHA	Short chain length PHA
SDS	Sodium dodecyl sulfate
SEM	Scanning electron microscope
SOUR	Specific oxygen uptake rate
$SOUR_{max}$	Maximum specific oxygen uptake rate
SRT	Solid retention time
SVI	Sludge volume index
TB-EPS	Tightly bound EPS
TEM	Transmission electron microscopy
UAF	Upflow anaerobic filtration
UASB	Upflow anaerobic sludge blanket reactor
UASFF	Upflow anaerobic sludge fixed film
UFF	Upflow Fixed Film
VER	Volume exchange ratio
VFA	Volatile fatty acids

LIST OF SYMBOLS

16S rRNA	16S ribosomal ribonucleic acid
A	Weight of sample and filter paper
B	Weight of empty filter paper
C	Weight of sample which is equivalent to A
C	Capacity ratio of the samples occupying the equipment.
C_2	Acetic acid
C_3	Propionic acid
C_4	Butyric acid
C_5	Pentanoic acid / Valeric acid
C_6	Hexanoic acid
C_{14}	Tetradecanoic acid
C_o	Concentration of the initial oxygen level
CO_2	Carbon dioxide
C_s	Concentration of oxygen at equilibrium
C_t	Concentration of oxygen at time t
D	Weight of the filter paper after drying at 550 °C.
K_I	Inhibition constant
K_La	Oxygen diffusion rate
K_s	Half saturation constant
M_n	Number average molecule
M_w	Weight average molecule
M_w/M_n	Polydispersity index
O_2	Oxygen
PhaC	Polymerase
PhaR	Depolymerase
PhbA	β-ketothiolase
PhbB	Acetoacetyl-CoA reductase
pKa	Acid Dissociation constant
$Power_{equipment}$	Maximum power required by the equipment
q	Bacterial activity
$-q_{Ac}$	Acetic acid utilization rate

$-q_{But}$	Butyric acid utilization rate
q_{max}	Maximum bacterial activity
$-q_{Pr}$	Propionic acid utilization rate
R1	Batch SBR 1
R2	Batch SBR 2
R3	Batch SBR 3
R4	Batch SBR 4
S_I	Concentration of inhibitory compound
T_m	Melting temperature
V_f	Final VFA concentrations
V_i	Initial VFA concentrations
$V_{s/min}$	Minimum settling velocity
$Y_{PHA/CDW}$	PHA content
$Y_{PHA/COD}$	PHA yield

PENUMPUKAN DAN PENYARIAN POLIHIDROKSIALKANOAT DARIPADA BUTIRAN AEROBIK YANG MERAWAT AIR SISA KILANG KELAPA SAWIT

ABSTRAK

Polihidroksialkanoat (PHA) adalah polimer biodegradasi yang telah menarik minat di kalangan penyelidik akhir-akhir ini. Selaras dengan itu, sintesis PHA dalam mikroorganisma merawat air sisa kilang minyak sawit (POME) mempunyai prospek yang menarik. Butiran aerobik yang dihasilkan dengan menggunakan POME belum diterokai untuk sintesis PHA sehingga kini. POME (dicerna secara acidogenik) untuk kajian ini dikumpul dari tasik rawatan anaerobik di kilang kelapa sawit. Hasil kajian ini menunjukkan bahawa butiran aerobik telah berjaya dihasilkan dengan POME yang dicerna secara acidogenik dalam 9 hari dan pada masa yang sama, 85 % suapan COD berjaya disingkirkan secara purata. Butiran aerobik mempunyai purata indeks isipadu enapcemar (SVI) 48 mL/g. Manakala, kandungan substrat polimerik sel (EPS) dalam butiran aerobik menunjukkan kandungan protin adalah lebih tinggi berbanding karbohidrat. Selain itu, analisa 16S rRNA menunjukkan genera *Bacillus* dan *Bordetella* mendominasi kumpulan bakteria dalam butiran aerobik tersebut. Analisa berikutnya mendedahkan bahawa butiran aerobik dapat menumpuk PHA disamping merawat POME. Purata kandungan PHA adalah 0.68 g PHA/g CDW dan PHA yang terkumpul adalah poli-3-hidroksibutirat-*co*-hidroksivalerat (P3(HB-*co*-HV)). Kajian yang seterusnya mendedahkan bahawa masa kitaran 6 jam dan strategi penyuapan aerobik dinamik (ADF) menumpuk kandungan PHA yang tertinggi (0.651 g PHA/g CDW) berbanding strategi yang lain. Sementara itu, kajian penyarian PHA mendedahkan bahawa kaedah serakan natrium hipoklorit-kloroform adalah pilihan yang terbaik berbanding dengan tiga kaedah yang lain. Penyingkiran hampir kesemua

lapisan EPS dalam butiran aerobik dikenalpasti sebagai elemen utama dalam penyarian PHA daripada butiran aerobik. Hasil PHA yang diperoleh adalah 0.89 g PHA/g CDW dengan menggunakan kaedah serakan natrium hipoklorit-kloroform. Sebaliknya, kitaran pertumbuhan-perpecahan didapati mempengaruhi jumlah keupayaan penumpukan PHA. Butiran aerobik di antara 0.35 dan 0.5 mm menumpuk 0.68 g PHA/g CDW berbanding dengan 0.60 g PHA/g CDW dalam butiran aerobik lebih besar daripada 2 mm. Resapan substrat dan oksigen ke dalam butiran aerobik didapati sebagai penyebab kepada perbezaan ini. Peningkatan kepekatan substrat dari 0.91 hingga 3.64 kg COD/m^3.hari telah meningkatkan kandungan PHA daripada 0.66 ke 0.87 g PHA/g CDW. Manakala, peningkatan kadar pengudaraan dari 1 ke 4 L/min telah mempercepatkan penumpukan PHA maksimum, tanpa mempengaruhi jumlah kandungan PHA dalam butiran aerobik. Manakala, sisa minyak didapati melekat pada permukaan butiran aerobik dan seterusnya menghadkan peresapan substrat. Akibatnya, kandungan PHA telah menurun. Pemalar perencatan didapati bernilai 5.61 g/L sisa minyak. Hasil keseluruhan kajian ini menumpu ke arah penumpukan PHA dalam butiran aerobik.

ACCUMULATION AND EXTRACTION OF POLYHYDROXYALKANOATE FROM AEROBIC GRANULES TREATING PALM OIL MILL EFFLUENT

ABSTRACT

Polyhydroxyalkanoate (PHA) is a biodegradable polymer which has attracted a lot of interests among researchers lately. In tandem with that, PHA synthesis in microorganisms treating palm oil mill effluent (POME) is an interesting prospect. Aerobic granules developed using POME are totally unexplored till date for PHA synthesis. POME (acidogenically digested) used in this study was collected from an existing anaerobic pond of palm oil mill wastewater treatment plant. The findings of this study showed that aerobic granules were successfully developed using acidogenically digested POME within 9 days and 85 % of the influent COD was removed on average. The developed aerobic granules had an average sludge volume index (SVI) of 48 mL/g. The extracellular polymeric substance (EPS) in aerobic granules contained higher proportion of protein than carbohydrate. Besides that, 16S rRNA analysis indicated that *Bacillus* and *Bordetella* genera were the dominating group of bacteria in aerobic granules developed using acidogenically digested POME. The ensuing analysis reveals that aerobic granules were able to accumulate PHA during the treatment of acidogenically digested POME. The average PHA content was 0.68 g PHA/g CDW and the PHA accumulated was co-polymer of poly-3-hydroxybutyrate-*co*-hydroxyvalerate (P3(HB-*co*-HV)). The subsequent study reveals that at the cycle time of 6 hrs, the aerobic dynamic feeding (ADF) strategy accumulates the highest PHA (0.651 g PHA/g CDW) of all other strategies. Meanwhile, the PHA recovery study reveals that the sodium hypochlorite-chloroform dispersion method is the best choice compared to other three methods used. The key element in recovering PHA from aerobic granules found to be nearly complete removal of EPS layer in the