BIOGRANULES CONTAINING PHOTOSYNTHETIC BACTERIA FOR CARBON DIOXIDE REDUCTION IN PALM OIL MILL EFFLUENT TREATMENT

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Dedicated to

My beloved wife, mother and father, two brothers and sister,

Who taught me to trust in Allah, believe in hard work,

Whose affection, love, encouragement and prays of day and night make me able to achieve such success,

My supervisor, lecturers, technicians and friends,

Thank you for encouraging, helping and supporting me all the way…

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ABSTRACT

Presently global warming is the most highlighted subjects in environmental issues which is related to greenhouse gases (GHG) emissions especially carbon dioxide (CO_2). In Malaysia, one of the major sources of GHG is from industrial wastewater treatment such as ponding system to treat palm oil mill effluent (POME) where the accumulation of these gases will contribute to the greenhouse effect causing global warming. Since photosynthetic process offers the most effective and natural way of sequestering CO_2, biogranules containing photosynthetic microorganisms were developed in a sequencing batch reactor (SBR) system using POME. A mixed sludge consists of sludge taken from a local sewage treatment oxidation pond, palm oil mill facultative pond treatment system and POME was used as seed sludge. Intermittent supply of light with intensity at 3600 lux was provided for 100 days with an organic loading rate (OLR) of 2.75 kg COD/m^3/day, hydraulic retention time (HRT) of 4 hours and superficial air velocity of 2.07 cm/s. The developed biogranules had shown potential in retaining high accumulation of biomass concentration in the reactor (10.5 g/L), good settleability (43.5-102.9 m/h) and improvement in size from 0.5 to 2.0 mm as well as high physical strength at integrity coefficient (IC) of 2 %. The initial structure of sludge changed from dispersed loose shaped into denser, compact and more stable structure with sludge volume index (SVI) maintained between 10.30 to 14.80 mL/g SS leading to a good solid-liquid separation compared to conventional activated sludge. Also, the chemical oxygen demand (COD), nitrogen (N) and phosphorus (P) removal of 26 %, 21 % and 62 % were achieved during the development of the biogranules. The pigment analysis indicated the presence of the bacteriochlorophyll *a* implying the presence of purple photosynthetic bacteria. Molecular identification of the bacteria showed the presence of *Enterobacter cloacae, Bacillus cereus, Lysinibacillus sp.* which possess photosynthetic pigments. For CO_2 reduction using the biogranules, approximately 18 to 21 % of CO_2 removal was achieved due to possible formation of calcite were observed with FESEM-EDX. The biogranules had achieved a CO_2 biofixation rate at approximately 0.234 g/L/day in a week while using the regression analysis; the maximum CO_2 biofixation rate in a year was estimated at 1.733 g/L/day.

ABSTRAK

Pada masa ini, pemanasan global adalah perkara yang paling diketengahkan dalam isu alam sekitar yang berkaitan dengan pembebasan gas rumah hijau (GHG) terutamanya gas karbon dioksida (CO_2). Di Malaysia, salah satu sumber utama GHG adalah berpunca daripada rawatan air sisa industri seperti sistem takungan untuk merawat efluen sisa minyak sawit (POME) di mana pengumpulan gas-gas ini akan menyumbang kepada kesan rumah hijau yang menyebabkan pemanasan global. Memandangkan proses fotosintesis menawarkan kaedah yang paling berkesan dan semula jadi mengurangkan CO_2, biogranul yang mengandungi mikroorganisma fotosintetik telah dibangunkan melalui sistem reaktor kelompok urutan (SBR) menggunakan POME. Campuran enapcemar yang terdiri daripada enapcemar dari kolam pengoksidaan rawatan kumbahan tempatan, sistem rawatan kolam fakultatif kilang minyak sawit, dan POME telah digunakan sebagai benih enapcemar. Bekalan pengcahayaan dengan keamatan pada 3600 lux telah diberikan selama 100 hari dengan kadar muatan organik (OLR) 2.75 kg COD/m^3/day, masa tahanan hidraulik (HRT) 4 jam dan halaju udara superfisial 2.07 cm/s. Biogranul yang terbentuk telah menunjukkan potensi dalam pengumpulan kepekatan biojisim yang tinggi dalam reaktor (10.5 g/L), kebolehenapan yang baik (43.5-102.9 m/h) serta penambahbaikan dalam saiz antara 0.5-2.0 mm dan kekuatan fizikal yang tinggi pada 2 % pekali integriti (IC). Struktur awal enapcemar telah berubah dari bentuk longgar tersebar kepada lebih tebal, padat dan stabil dengan indeks isipadu enapcemar (SVI) berkekalan antara 10.30-14.80 mL/g SS menuju ke arah pemisahan pepejal-cecair yang baik berbanding enapcemar teraktif konvensional. Penyingkiran permintaan oksigen kimia (COD), nitrogen (N) dan phosphorus (P) sebanyak 26 %, 21 % dan 62 % turut dicapai semasa pembentukkan biogranul. Analisis pigmen menunjukkan kehadiran *bacteriochlorophyll a* yang mejurus kepada kehadiran bakteria fotosintetik ungu. Pengencaman molekul bakteria menunjukkan kehadiran *Enterobacter cloacae, Bacillus cereus, Lysinibacillus sp*. yang mempunyai pigmen fotosintetik. Untuk penyingkiran CO_2 menggunakan biogranul, penyingkiran CO_2 mencapai hampir 18 hingga 21 % mungkin disebabkan oleh pembentukan kalsit sepertimana diperhatikan dengan FESEM-EDX. Akhir sekali, biogranul telah mencapai lebih kurang 0.234 g/L/hari kadar biofisasi CO_2 dalam seminggu menggunakan analisis regresi; kadar maksimum biofisasi CO_2 untuk tempoh setahun dianggarkan sebanyak 1.733 g/L/hari.

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LIST OF ABBREVIATIONS

16S rDNA	-	16S Ribosomal DNA
ANOVA	-	Analysis of Variance
APHA	-	American Public Health Association
ATP	-	Adenosine Triphosphate
BLAST	-	Basic Local Alignment Search Tool
BOD	-	Biological Oxygen Demand
C	-	Carbon
Ca	-	Calcium
$C_3H_5NaO_3$	-	Sodium Lactate
$C_4H_6O_5$	-	Malic Acid
$C_5H_9NO_4$	-	L-glutamic Acid
$C_6H_{12}O_6$	-	Glucose
$CaCl_2.2H_2O$	-	Calcium Chloride Dihydrate
CDM	-	Clean Development Mechanism
CFU	-	Colony Forming Units
CH_4	-	Methane
CMTR	-	Completely Mixed Tank Reactor
CNRS	-	French Centre for Scientific research
CO_2	-	Carbon Dioxide
CO_3^{2-}	-	Carbonate
COD	-	Chemical Oxygen Demand
CPO	-	Crude Palm Oil
DIC	-	Dissolved Inorganic Carbon
DLVO	-	Derjaguin-Landau-Verwey-Overbeek
DO	-	Dissolved Oxygen
DOE	-	Department of Environment
EDX	-	Dispersive X-Ray Analyser

EDTA	-	Ethylene Diamene Tetra Acetic Acid
EFB	-	Empty Fruit Bunch
EPS	-	Extracellular Polymeric Substances
FESEM	-	Field Emission Scanning Electron Microscope
GHG	-	Greenhouse Gases
GWP	-	Global Warming Potential
H/D	-	Height to Diameter
H^+	-	Protons
H_2	-	Hydrogen Gas
H_2CO_3	-	Carbonic Acid
H_2S	-	Hydrogen Sulphide
HCO_3^-	-	Bicarbonate
HRT	-	Hydraulic Retention Time
HS	-	Bisulfide
HSO_4	-	Hydrogen Sulphate
IC	-	Integrity Coefficient
IPPC	-	Intergovernmental Panel on Climate change
K_2HPO_4	-	Dipotassium Phosphate
KH_2PO_4	-	Monopotassium Phosphate
LD	-	Linear Dichroism
LHs	-	Light harvesting complexes
MF	-	Mesocarp Fibre
$MgCl_2$	-	Sodium Chloride
$MgSO_4.7H_2O$	-	Magnesium Sulfate Heptahydrate
MLSS	-	Mixed Liquor Suspended Solid
MLVSS	-	Mixed Liquor Volatile Suspended Solid
MPOB	-	Malaysian Palm Oil Board
MPOC	-	Malaysian Palm Oil Council
N	-	Nitrogen
N_2	-	Nitrogen
N_2O	-	Nitrous Oxide
NaCl	-	Sodium Chloride
NADPH	-	Nicotinamide Adenine Dinucleotide Phosphate
$NaHPO_4$	-	Sodium Phosphate

NASA	-	National Aeronautics and Space Administration
NCBI	-	National Center for Biotechnology Information
NH_3-N	-	Ammonical Nitrogen
NH_4Cl	-	Ammonium Chloride
O_2	-	Oxygen
OLR	-	Organic Loading Rate
PO_4-P	-	Orthophosphate
P	-	Phosphorus
PCP	-	Pentachlorophenol
PCR	-	Polymerase Chain Reaction
PHA	-	Polyhydroxyalkanoate
PKS	-	Palm Kernel Shell
PNSB	-	Purple Non-Sulphur Bacteria
PNSBEM	-	Purple Non Sulphur Bacteria Enrichment Medium
POME	-	Palm Oil Mill Effluent
RCs	-	Photosynthetic Reaction Centers
Rubisco	-	Ribulose-1,5-bisphosphate carboxylase/oxygenase
S	-	Sulphur
SBR	-	Sequencing Batch Reactor
SEM	-	Scanning Electron Microscopy
SIRIM	-	Standards and Industrial Research Institute of Malaysia
SO_2	-	Sulphur Dioxide
SS	-	Suspended Solids
SVI	-	Sludge Volume Index
TAE	-	Tris-acetate-EDTA
TCE	-	Tetrachloroethylene
TDS	-	Total Dissolved Solid
TKN	-	Total Kjeldahl Nitrogen
TOC	-	Total Organic Carbon
TS	-	Total Solid
TSS	-	Total Suspended Solid
TVC	-	Total Viable Count
U.S.EPA	-	United States Environmental Protection Agency
UASB	-	Upflow Anaerobic Sludge Bed Reactor

UV	-	Ultraviolet
VCSEL	-	Vertical Cavity Surface Emitting Laser
VFAs	-	Volatile Fatty Acids
VSS	-	Volatile Suspended Solid
WMO	-	World Meteorological Organization’s
WRI	-	World Resources Institute
ZSV	-	Zone Settling Velocity

LIST OF SYMBOLS

v/v	-	Volume/Volume
w/v	-	Weight/Volume
ppm	-	Parts per Million
R_{CO_2}	-	CO_2 Biofixation Rate
C_c	-	Carbon content
P_{max}	-	Biomass Productivity
M_{CO_2}	-	Molar Mass of CO_2
M_c	-	Molar Mass of Carbon
R-Sq	-	Regression Coefficient
p-value	-	Significance Value
S	-	Standard Error

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CHAPTER 1

INTRODUCTION

1.1 Background of the Problem

The World Meteorological Organization's (WMO) Green House Gas Bulletin 2011 had reported that the amount of greenhouse gases (GHG) has increased since the pre-industrial time (Shun *et al*., 2012). Amongst all GHG, nearly 64 % of carbon dioxide (CO_2) gas contributes to climate change. From the year 1980 to 2011, the abundance of atmospheric CO_2 has increased globally averaging 1.7 CO_2 ppm per year (Hartmann *et al.*, 2013). Biological carbon sequestration using technologies such as controlled photosynthetic reactions may help to alleviate GHG problems, by carrying out reactions where CO_2 is transferred to the aqueous phase of the system (Jacob-Lopes *et al*., 2009). The photosynthetic bacteria signify as a promising tool for the development of various fields of biotechnology due to their capabilities to assimilate CO_2 gas, fix molecular nitrogen via photosynthetic metabolism and grow on different wastes (Paronyan and Gasparyan, 2009).

The CO_2 is naturally removed by plants from the atmosphere and replaces it with oxygen (O_2). Thus, it would tend to restore the CO_2 released by the burning of fossil fuels. However, the condition is complicated by the fact that plants themselves react to the amount of CO_2 in the atmosphere. Some plants appear to grow more rapidly in an atmosphere rich in CO_2, but this may not be true for all species. Furthermore, active deforestation in developing countries must be also accounted and monitored since the atmosphere could accumulate CO_2 gases leading to a higher global warming effect (Michaelowa and Michaelowa, 2015; Malhi *et al*., 2008; Campbell-Lendrum and Corvalán, 2007).

In Malaysia, one of the major sources of GHG is from industrial wastewater treatment such as the ponding system to treat palm oil mill effluent (POME). Malaysia's palm oil industry had generated approximately 80 million dry tonnes of solid biomass per annum as the volume is expected to increase to 85-110 million dry tonnes by 2020 (Agensi Inovasi Malaysia, 2013). Generally, one tonne of crude palm oil production needs 5.0-7.5 tonnes of water from which 50 % are released in the form of POME. This POME contained a high organic content (COD = 50 g/L, BOD = 25 g/L) and substantial amounts of plant nutrient (MPOB, 2014; Abdul Rahman *et al*., 2013; Ahmad *et al.*, 2005; Singh *et al*., 1999).

A ponding system for POME conventionally treated in a series of aerobic and anaerobic ponds that required over 100 days of the treatment period. For instance, the anaerobic pond produces harmful and odorous gases such as sulphur dioxide (SO_2), methane (CH_4) and CO_2 (Daelman *et al.*, 2012; Olah *et al*., 2009; Alimahmoodi and Mulligan, 2008). The continuous production of CH_4 in the air will increase the threat of global warming, since CH_4 reacts with water to form CO_2 and water. These could cause the accumulation of CH_4 and CO_2 in the atmosphere which contributes to the greenhouse effect causing global warming synergism (WRI, 2014; Bandara *et al*., 2011). There is a lot of interest in reducing these GHG emissions commonly in anaerobic wastewater treatments (Martinez *et al*., 2013; Chotwattanasak and Puetpaiboon, 2011) with the latest study concentrating on the CO_2 utilization using algae, microalgae and photobioreactor (Nugroho *et al*., 2015; Nayak *et al*., 2013; Pankaj and Awasthi, 2013).

Biogranulation technology is a promising new technology in aerobic wastewater treatment system and has increasingly attracted interest in recent years due to its ability to overcome the limitations in conventional activated sludge system. This technology is a self-immobilization of microorganisms which can withstand high biomass retention, high strength wastewater and shock loadings as they are rich with microbial diversity. It also improves the characteristics of sludge whereby its granular form having a compact structure with good settling capability and simultaneous removal of organic matter, nitrogen and phosphorus (Aqeel *et al*., 2015; Wagner and Costa, 2015; Bassin *et al*., 2012; Wei *et al*., 2012).

Consequently, a special attention on how can the ecological balance between photosynthetic and respiratory activities in municipal wastewater be restored is needed. An improvement on the CO_2 uptake rate within the microbial community in the sludge has to be deliberate. To avoid mass emission, photosynthesis needs to be enhanced in the sludge community in order to minimize the release of CO_2. Various efforts by researchers was done on carbon recycling within the microsystem in order to realize photosynthesis in wastewater treatment (Kamarudin *et al*., 2015; Zeng *et al*., 2015; Masunaga *et al*., 2007; Ogbonna *et al*., 2000). Additionally, Malaysia is suitable for photosynthesis with its location within the tropical region and an average daily sunlight of 4.5 to 8 hours. Photosynthesis is usually known as the conversion of light energy to chemical energy that can be used by cells. All phototrophs absorb energy from light to reduce CO_2 to organic compounds. Phototrophic microorganisms are of commercial interest due to the fact that they perform photosynthesis (Fernandes *et al*., 2015).

Photosynthetic microorganism or bacteria plays an important role for the CO_2 sequestration within the microsystem of wastewater as it utilizes the CO_2 from the environment (Liu *et al.*, 2015; Nowak *et al.*, 2015; Bently and Melis, 2013; Farrelly *et al*., 2013). Microbial communities in aerobic granules have been shown to be highly distinct from activated sludge, even within a single reactor system. Recent studies emphasize the importance of understanding the functions of microbial communities (Egan *et al*., 2013; Rastogi and Sani, 2011; Zak *et al.*, 2011) as population diversity alone may not be adequate in determining the microbial characteristics.

Microbial communities in aerobic granules have been shown to be highly distinct from activated sludge, even within a single reactor system. Recent studies imply the importance of gaining an understanding of the functions of microbial communities, as population diversity alone may not be adequate in determining the microbial characteristics. For that reason, this study focuses on the development of microbial granules containing photosynthetic bacteria that are able to minimize CO_2 emissions from wastewater treatment plant especially POME.

1.2 Statement of Problem

Most of the aerobic granulation studies that have been carried out mainly involve the sequencing batch reactor (SBR) designs and operations, physicochemical conditions, and biodegradation performances (Khalida *et al.*, 2013; Abdullah *et al*., 2013; Nor-Anuar *et al*., 2007). To date, a few studies on aerobic granulation have considered the global warming effects focusing on the important roles of microorganisms for the reduction of CO_2 concentration in simultaneous anaerobic-aerobic process. In order to accomplish such modification of the SBR operational parameters, suitable requirements for growth of microorganisms need to be first established. Salmiati *et al*. (2015) started to develop phototrophic aerobic granular sludge for CO_2 reduction and synthetic wastewater was used as substrates.

Therefore, this study was focused on the development of biogranules that were able to minimize CO_2 emissions from POME. The biogranules were developed in the SBR using high strength agricultural wastewater such as POME to investigate their physical properties and microbial diversity. This latest development highlighted the potential application of biogranules containing photosynthetic pigments for CO_2 reduction and CO_2 biofixation of high strength wastewater which will be useful for small footprint wastewater treatment process. Hence, this study was designed to investigate the performance of developed biogranules containing photosynthetic pigments for removal of CO_2 in POME

1.3 Objectives of Study

This study was embarked on the following objectives:

i. To develop and characterise biogranules containing photosynthetic bacteria in POME using sequencing batch reactor (SBR) system.
ii. To evaluate the microbial characterisation of the developed biogranules containing photosynthetic bacteria.

iii. To investigate the production, removal of CO_2 and the CO_2 biofixation rate efficiency of the developed biogranules.

1.4 Scope of Study

This study had involved the laboratory and batch scale experimental work in the development of biogranules containing photosynthetic bacteria as well as investigate its efficiency to reduce CO_2. The main instrument used to achieve biogranulation is the lab-scale SBR system alternating anoxic and aerobic conditions whereas photosynthetic condition was created by setting up the proper light intensity, pH and temperature. POME used as influent was autoclaved to get rid of indigenous bacteria that may interfere with the biogranulation process. Also, the seed sludge was a mixed of sludge collected from a local palm oil plant and domestic wastewater treatment plant as well as the POME. Throughout the study, the physiochemical characteristics of the biogranules containing photosynthetic bacteria were observed during the biogranulation process. For the application of biogranules in reducing CO_2, the production and removal of CO_2 in POME were monitored focusing on the CO_2 concentration released from liquid and gas form as well as the pH changes. Later on, the CO_2 biofixation rate was calculated using the equation from Tang *et al*. (2011) and its elemental information such as the carbon (C) content obtained through an Energy Dispersive X-Ray Analyser (EDX).

1.5 Significance of Research

This study will be a significant endeavour in promoting and introducing the utilization of photosynthetic bacteria within a biogranule formed as an alternative approach to current CO_2 mitigation strategies in minimizing the CO_2 emission from the atmosphere and POME. By implementing the biogranulation technology in the palm oil industry, the sludge production will be improved by developing into granules consist of compact, denser structure, and higher settleability lead to a better solid-liquid separation in the wastewater. Furthermore, for CO_2 sequestration, this biological treatment method will be beneficial than physical method with a very expensive process such as capturing, transporting and storing CO_2. Also, this study will provide some understanding of the CO_2 cycle exchanging between the atmosphere and water as well as effectiveness of CO_2 fixation using the biogranules for future work.

From this study, it appears to be necessary for developing future adaptation strategies and knowledge to manage GHG emissions from wastewater cycle and the vulnerability of climate assessment to interact with the adaptive responses that could address emission sources. In other words, the increase of the global warming effects has raised the challenge of finding sustainable technological approaches to stabilize CO_2 emissions in the atmosphere and counteract impinging interconnected issues such as desertification and loss of biodiversity. For that reason, the biological CO_2 mitigation through biological fixation is considered a promising and eco-sustainable method, mostly owing to its downstream benefits that can be exploited.

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