BIOGRANULAR SLUDGE FOR RUBBER PROCESSING WASTEWATER IN A SEQUENCING BATCH REACTOR

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To my beloved mother and father

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ABSTRACT

Rubber is one of the major agro-based industrial sectors that contributes to the development of the country. Malaysia is one of the leading producers of natural rubber in the world. The rubber industry consumes large volume of water, uses chemicals and other utilities, and produces an enormous amount of wastes and effluent. As rubber effluent contributes to highly polluted wastewater, the need to find an efficient and practical approach to preserve the environment is essential. Thus, this study aims to investigate the applicability of aerobic granular sludge technology in treating rubber processing wastewater using a laboratory scale sequencing batch reactor (SBR) system. Aerobic granular sludge was developed in a 1.6 L working volume of column reactor that operated with 3 hours of cycle time for 90 days. The reactor had a volumetric exchange ratio of 30% and the superficial upflow air velocity was set at 1.2 cm/s. The dissolved oxygen in the reactor was within the range of 6.0-7.5 mg/L. The SBR system was run at organic loading rate of 0.8-3.3 kg COD/m^3 day and COD/N/P ratio was 100/19/8. Results showed that aerobic granules formed had an average diameter of 1.4 to 2.8 mm with settling velocity of 57.8 ± 3.5 m/h and sludge volume index (SVI) of 46.0 mL/g. These properties caused a significant increase in biomass concentration from 3.8 to 10.1 g/L, which was observed to be beneficial for the performance of the reactor system. The scanning electron microscope (SEM) examinations revealed that aerobic granular sludge consisted of non-filamentous cocci-shaped bacteria, tightly linked to one another to form a compact structure. The performances of aerobic granules that formed at three different cycle times of 3, 6 and 12 hours of SBR operation were studied. The highest cycle time favours the highest removal performances in removing organic and nutrients. 96.9% COD removal was achieved when the reactor was operated at high cycle time of 12 hours, while around 60.0% and 65.9% removal efficiencies were recorded for total nitrogen and total phosphorus in the granular SBR system for rubber processing wastewater treatment. The metagenome analysis was used to discover the microbial community that accumulated in aerobic granular sludge, which was potential in the granulation and biodegradation process. The abundance of COD degrading, denitrifying and polyphosphate bacteria such as *Pseudomonas*, *Agrobacterium* and *Thauera* bacteria were high in aerobic granules. Those bacteria had both capability in producing extracellular polymeric substances (EPS) and degrading waste. The characteristics of EPS of aerobic granular sludge were determined. Proteins (PN) were more dominant than polysaccharides (PS) in the EPS of aerobic granular sludge. The excitation-emission matrix (EEM) results also indicated the importance of aromatic protein-like substances, particularly tryptophan in maintaining the stable structure of granular sludge. Despite the different cycle times, aerobic granular sludge formation was successfully achieved for the treatment of high strength wastewater such as rubber effluent.

ABSTRAK

Getah merupakan salah satu sektor utama industri berasaskan agro yang menyumbang kepada pembangunan negara. Malaysia merupakan salah satu pengeluar utama bagi getah asli di dunia. Industri getah menggunakan air, bahan kimia dan utiliti lain dalam jumlah yang banyak, dan menghasilkan jumlah sisa dan efluen yang tinggi. Oleh kerana efluen getah menghasilkan air sisa yang sangat tercemar, keperluan untuk mencari pendekatan yang efisien dan praktikal bagi memelihara alam sekitar adalah penting. Oleh itu, kajian ini bertujuan mengkaji keterterapan teknologi enap cemar berbutir aerobik dalam merawat air sisa pemprosesan getah menggunakan sistem reaktor kelompok berjujukan (SBR) berskala makmal. Enap cemar berbutir aerobik dihasilkan di dalam reaktor turus berisipadu 1.6 L yang dioperasikan pada masa kitaran 3 jam selama 90 hari. Reaktor mempunyai nisbah pertukaran isipadu sebanyak 30% dan halaju aliran udara ditetapkan pada 1.2 cm/s. Kandungan oksigen terlarut dalam reaktor adalah dalam julat 6.0-7.5 mg/L. Sistem SBR telah dijalankan pada kadar beban organik 0.8-3.3 kg COD/m^3 hari dan nisbah COD/N/P adalah 100/19/8. Hasil kajian menunjukkan granul aerobik yang terbentuk mempunyai saiz purata diameter 1.4 hingga 2.8 mm dengan halaju pengenapan 57.8 $\pm$ 3.5 m/h dan indeks isipadu enap cemar (SVI) sebanyak 46.0 mL/g. Sifat ini menyebabkan peningkatan ketara dalam kepekatan biojisim dari 3.8 ke 10.1 g/L dan dilihat memberi manfaat kepada prestasi sistem reaktor. Pemeriksaan mikroskop elektron pengimbas (SEM) mendedahkan enap cemar berbutir aerobik mengandungi bakteria tidak berfilamen berbentuk kokus dan berpaut rapat antara satu sama lain bagi membentuk struktur yang padat. Prestasi granul aerobik yang terbentuk pada tiga masa kitaran yang berbeza selama 3, 6 dan 12 jam di dalam operasi SBR telah dikaji. Masa kitaran tinggi menggalakkan kecekapan penyingkiran yang tertinggi di dalam menyingkirkan bahan organik dan nutrien. Penyingkiran COD sebanyak 96.9% dicapai apabila reaktor dikendalikan pada masa kitaran yang tinggi selama 12 jam, manakala kecekapan penyingkiran sekitar 60.0% dan 65.9% telah dicatat bagi nitrogen jumlah dan fosforus jumlah dalam sistem butiran SBR semasa rawatan air sisa pemprosesan getah. Analisis metagenom digunakan untuk mengenal pasti komuniti mikrob yang terkumpul dalam enap cemar berbutir aerobik yang berpotensi dalam proses pembutiran dan proses biodegradasi. Jumlah bakteria yang terlibat dalam penguraian COD, nitrogen dan fosforus seperti bakteria *Pseudomonas*, *Agrobacterium* dan *Thauera* adalah tinggi di dalam enap cemar berbutir aerobik. Bakteria tersebut berupaya menghasilkan bahan polimer ekstraselular (EPS) dan menguraikan sisa. Ciri EPS enap cemar berbutir aerobik telah dikaji. Protein (PN) lebih dominan daripada polisakarida (PS) dalam EPS enap cemar berbutir aerobik. Keputusan pengujaan pelepasan matriks (EEM) juga menunjukkan kepentingan bahan seperti sebatian protein aromatik, terutamanya triptofan untuk mengekalkan struktur yang stabil bagi enap cemar berbutir. Walaupun masa kitaran yang berbeza, pembentukan enap cemar berbutir aerobik telah berjaya dicapai untuk rawatan air sisa berkekuatan tinggi seperti efluen getah.

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LIST OF ABBREVIATIONS

AGS	-	Aerobic granular sludge
AN	-	Ammoniacal nitrogen
AnSBR	-	Anaerobic sequencing batch reactor
AOB	-	Ammonia oxidizing bacteria
APHA	-	American Public Health Association
BAS	-	Batch activated sludge
BOD	-	Biochemical oxygen demand
CLSM	-	Confocal laser scanning microscopy
COD	-	Chemical oxygen demand
DGGE	-	Denaturing gradient gel electrophoresis
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen
EGSB	-	Expanded granular sludge bed
EM	-	Effective microorganism
EPS	-	Extracellular polymeric substances
EQA	-	Environmental Quality Act
FESEM	-	Field emission scanning electron microscope
FISH	-	Fluorescence *in situ* hybridization
GAO	-	Glycogen accumulating organism
HRT	-	Hydraulic retention time
IC	-	Integrity coefficient
LB-EPS	-	Loosely bound EPS
MG-RAST	-	Meta genome rapid annotation using subsystem technology
MLSS	-	Mixed liquor suspended solids

MLVSS	-	Mixed liquor volatile suspended solid
OLR	-	Organic loading rate
ORP	-	Oxidation-reduction potential
PAH	-	Polycyclic aromatic hydrocarbon
PAO	-	Phosphate accumulating organism
PHA	-	Poly-β-hydroxyalcanoates
PHB	-	Poly-3-hydroxybutyrate
PLC	-	Programmable logic controller
PN	-	Protein
PS	-	Polysaccharide
RG	-	Residual granules
RNA	-	Ribonucleic acid
RRIM	-	Rubber Research Institute Malaysia
SBR	-	Sequencing batch reactor
SEM	-	Scanning electron microscopy
SG	-	Settled granules
SMA	-	Specific methanogen activity
SMR	-	Standard Malaysian Rubber
SRT	-	Solids / Sludge retention time
SS	-	Suspended solids
SVI	-	Sludge volume index
TB-EPS	-	Tightly bound EPS
TDS	-	Total dissolved solid
TGGE	-	Temperature gradient gel electrophoresis
TKN	-	Total Kjeldahl nitrogen
TN	-	Total nitrogen
TP	-	Total phosphorus
TS	-	Total solid
TSS	-	Total suspended solids
UAFP	-	Upflow anaerobic filter process
UASB	-	Upflow anaerobic sludge blanket
UTM	-	Universiti Teknologi Malaysia
UV	-	Ultraviolet
VER	-	Volumetric exchange ratio

VFA	-	Volatile fatty acid
VSS	-	Volatile suspended solid
WWTPs	-	Wastewater treatment plants
16s rRNA	-	16 sequencing ribosomal ribonucleic acid
3D-EEM	-	Three-dimensional excitation-emission matrix

LIST OF SYMBOLS

Al^{3+}	-	aluminium
Ca^{2+}	-	calcium
d_p	-	diameter of a particle
Fe (II)	-	ferum
H/D	-	column height to diameter ratio
HOCl	-	hypochlorous acid
H_2	-	hydrogen
M	-	biomass concentration
M_w	-	molecular weight
Mg^{2+}	-	magnesium
$NaHCO_3$	-	sodium bicarbonate
N/COD	-	nitrogen to organic ratio
NH_3-N	-	ammonia nitrogen
NH_4^+-N	-	ammonium
N-NO_2^-	-	nitrite
N-NO_3^-	-	nitrate
Na^+	-	natrium / sodium
N_2	-	nitrogen gas
N_C	-	number of cycles per day
O_2	-	oxygen
P	-	phosphorus
P/COD	-	phosphorus to chemical oxygen demand ratio
P-PO_4^{3-}	-	phosphate
Q_e	-	effluent flow rate
Q_I	-	influent flow rate

SS_0	-	total amount of granular sludge
SS_t	-	amount of sludge solids in supernatant after t min
t_A	-	aerobic time
t_C	-	cycle time
t_D	-	decant time
t_F	-	filling time
t_I	-	idle time
t_R	-	reaction time
t_S	-	settling time
V_d	-	manually discharge mixture volume
V_e	-	effluent volume of the SBR operating cycle
V_F	-	filled volume
V_{MIN}	-	minimum volume
V_r	-	working volume of reactor
V_s	-	settling velocity of a particle
V_T	-	total volume
X_d	-	biomass concentration of manually discharged
X_e	-	mixed liquor volatile suspended solid in effluent
X_r	-	mixed liquor volatile suspended solid in reactor
X_{vss}	-	volatile solid concentration in reactor
μ	-	viscosity of a solution
ρ_p	-	density of a particle
ρ	-	density of a solution
θ	-	solid retention time

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Rubber is one of the main agro-based industrial sectors that play an important role in Malaysia's economy. Presently, Malaysia is the third largest producer of natural rubber in the world (Rois Anwar *et al*., 2013). Malaysia's rubber plantations are spread out across both Peninsular and East Malaysia. Based on the report by Department of Statistics (2015), the total planted rubber area was 1,078,630 ha during the year of 2015 and is expected to increase in the subsequent year. The smallholding sectors contributed 92.0% of total area hectare while the estate sectors contributed the remaining 8.0%. Malaysia produced 720,996 tonnes of natural rubber in the year 2015, marginally up 7.3% from 668,613 tonnes produced in the previous year (Malaysian Rubber Board, 2015). Nonetheless, the contribution of natural rubber industry to national exports was RM 6.10 billion.

Natural rubber, an elastic hydrocarbon polymer, originally derived from a milky colloidal suspension or latex of *Hevea brasiliensis*. The processing of raw natural rubber can be divided into two types of processes; the production of latex concentrate and the production of Standard Malaysian Rubber (SMR) (Sulaiman *et* *al*., 2010). SMR is the current bulk of Malaysian rubber produced in the form of technically specified crumb rubber. Large quantities of effluent were produced from the processing of raw natural rubber since it required huge amount of water for its operation. The effluent typically contains a small amount of uncoagulated latex, serum with substantial quantities of proteins, carbohydrates, sugars, lipids, carotenoids, as well as inorganic and organic salts and also includes washings water from the various processing stages (Mohammadi *et al*., 2010). These substances are characterized of a high concentration of ammonia, BOD, COD, nitrate, phosphorus and total solids. If high level of nitrogen and ammonia is discharged to water bodies, it could contributes to undesirable eutrophication and lead to death of some aquatic organisms living in the water.

A number of treatment methods of raw natural rubber factory effluents especially biological treatment processes have been developed and implemented to meet regulatory standards before being discharged into the waterways. For example, anaerobic-cum-facultative lagoon system, anaerobic-cum-aerated lagoon system, aerated lagoon system and oxidation ditch system have been developed for the treatment of rubber wastewater (Sulaiman *et al*., 2010; Xin *et al*., 2013). However, the main drawbacks of these systems include large land area requirement, high energy consumption for the aerators, longer effluent treatment period, odour problems and high operating and maintenance costs. As a result, these circumstances lead to frequent non-compliance to the legal discharge limits (Mohammadi *et al*., 2010).

Research into aerobic granular sludge technology applications using sequencing batch reactor (SBR) system fed with various organic substrates including industrial wastewaters has been extensively reported by previous researchers. This technology offers a small footprint compared to conventional activated sludge process due to the elimination of clarifier. Activated sludge can be operated to form aerobic granules instead of microbial flocs. Aerobic granules form through self-immobilization of microorganisms and can be regarded as a special case of biofilm forming phenomenon (Liu and Tay, 2002; Yang *et al*., 2004). These granules are larger (1-4 mm as compared to sludge flocs, which are 0.1-0.3 mm diameter) and have the advantages of regular and compact structure, good settling properties, high biomass retention and strong ability to withstand high-strength wastewater and shock loadings (Morgenroth *et al*., 1997; Beun *et al*., 1999; Tay *et al*., 2001a; Yang *et al*., 2003; Liu and Tay, 2004; de Kreuk *et al*., 2007a; Adav *et al*., 2008a; Liu *et al*., 2009; Gao *et al*., 2011a; Khan *et al*., 2013; Seow *et al*., 2016).

For bacteria to form aerobic granules, the contributions of physical, chemical and biological conditions to the granulation process should be collectively considered. A number of factors such as the type of substrate, the loading rate, aeration intensity, and the hydraulic retention time have been previously reported to have influence on the sludge granulation process (Liu and Tay, 2002; Liu and Tay, 2004). However, several other issues regarding aerobic granulation in real industrial wastewater such as the mechanism, crucial operating factors and evolution of the microbial community remain to be addressed.

1.2 Problem Statement

Aerobic granular sludge is an ideal option for biological treatment applications, particularly for municipal wastewater. Nevertheless, its potential in treating other types of wastewater is limited. The real world sees various types of high strength industrial wastewaters being generated daily from different sources. In Malaysia, the biggest sources of industrial water pollution are mainly come from food and beverage producers, chemical based industries, textiles, paper, palm oil and rubber processing industries (Usa, 2007; Iyagba *et al*., 2008). The wastewater produced from rubber processing industry poses environmental concerns and may adversely affect public health. This is because rubber processing effluent has high chemical oxygen demand (COD) and biochemical oxygen demand (BOD) concentrations which may reach up to 14000 mg/L and 7000 mg/L, respectively

(Mohammadi *et al*., 2010; Rois Anwar *et al*., 2013). The treated rubber processing effluent must comply with the discharge limits as outlined in the Third Schedule Regulation 12 (2) Environmental Quality (Prescribed Premises) (Raw Natural Rubber) (Amendment) Regulations 1980 (Federal Subsidiary Legislation, 1974). To meet this restriction, effective treatment system has to be developed and built in place.

The application of aerobic granulation technology in the treatment of real industrial wastewaters is still relatively under-researched. Different characteristics of industrial wastewater will be act differently in an attempt to develop the biogranules. Using real wastewater is more challenging due to inconsistency of the concentration of compound in the wastewater. So, the development of aerobic granules using rubber processing wastewater is still a question to be answered.

1.3 Aim and Objectives of the Study

The overall aim of this study is to investigate the feasibility and applicability of aerobic granulation technology in treating wastewater from rubber industry using a sequencing batch reactor system. This can be achieved by the following specific objectives:

i. To develop, characterize and study the performance of aerobic granular sludge for rubber processing wastewater in a sequencing batch reactor system.

ii. To investigate the effect of cycle time with variation of reaction (aeration) mode on the characteristics of aerobic granular sludge and the reactor removal performances.

iii. To analyze the microbial diversity of aerobic granular sludge using molecular investigations via metagenome sequencing analysis. The basic understanding of evolutionary shift of microbial population during aerobic granulation process and the role of selected bacterial species in the formation of mature granules is proposed.

iv. To determine the characteristics of extracellular polymeric substances (EPS) of aerobic granular sludge and specific components analysis of EPS such as polysaccharides, proteins and carbohydrates using three-dimensional excitation-emission matrix (3D-EEM) technology.

1.4 Scope of the Study

This study involves the design and application of a laboratory-scale reactor that are based on the sequencing batch reactor mode which was set-up at Level 2, Block C07 of the Laboratory of Environmental Engineering, Universiti Teknologi Malaysia, Skudai, Johor. The development and microbial characterization of aerobic granular sludge including its physical, chemical and biological properties using domestic activated sludge as seeding and raw rubber processing wastewater as substrate were the main focus of the present study. In general, rubber processing wastewater is characterized as the high strength agro-based industrial wastewater. The raw rubber processing wastewater is taken from a nearby rubber factory plant located in Kota Tinggi, Johor. The granulation process was reported in terms of the morphology and settling ability of aerobic granular sludge developed in a SBR system. Reactor performances were observed based on the COD, total nitrogen and total phosphorus removal efficiencies during granulation and steady state conditions of the reactor system. The effects of cycle time on the characteristics and performance of aerobic granular sludge were investigated by providing different cycle time (3, 6 and 12 h). The evolution of microbial population and characterization of microbial community using novel molecular techniques which is metagenome sequencing analysis was investigated. The bacterial species present in both seed sludge and aerobic granular sludge are identified and the relationship between the bacterial community structure and aerobic granulation was suggested. In addition, the characteristics of extracellular polymeric substances (EPS) of aerobic granular sludge were also investigated.

1.5 Significance of the Study

Aerobic granular sludge is widely known to have several beneficial engineering properties i.e. strong and dense microstructure, ability to withstand high organic loading rates, stable and compact shape making it an increasingly popular choice for recent wastewater treatment system. Considering the advantages of aerobic granulation over the conventional activated sludge method, aerobic granular sludge system was proposed in this present study. Apparently, the use of aerobic granular sludge for the treatment of wastewater from rubber factory industry has not been carried out. Thus, the significant contribution of this study to the literature is as follows:

i. The study provides the design and technical input of a compact laboratory-scale reactor system fabricated specifically for the formation of aerobic granular sludge in treating rubber processing wastewater.

ii. The study provides the procedures for the formation of aerobic granular sludge cultivated with rubber processing wastewater and its physicochemical and biological characteristics, as well as organic and nutrient removal process from rubber processing wastewater.

iii. The study provides details in relation to the effect of cycle time on the formation and properties of cultivated granules. The findings would provide knowledge on suitable operating conditions for the development of the aerobic granular sludge, customized for treatment process of rubber processing wastewater.

iv. The study also provides input on the microbial community structure of the developed aerobic granules fed with rubber processing wastewater. The molecular investigations of aerobic granular sludge provides basic understanding in the microbial evolution of the granules to further strengthen aerobic granulation technique specifically designed for efficient and sustainable rubber wastewater treatments.

1.6 Organization of the Thesis

The thesis is organized into eight chapters. **Chapter 1** as an introductory part gives a problem background related to the wastewater generates from the rubber factory industry as well as the setbacks in the conventional rubber wastewater treatment system.

Chapter 2 presents an overview of relevant literature and covers basic principles of characteristics of rubber processing wastewater, the available treatment system applied for rubber processing wastewater, the fundamental concepts of aerobic granular sludge technology, granulation process in sequencing batch reactors (SBR), and mechanisms of aerobic granulation. The characterization of aerobic granular sludge and the relation to its microbial structure, chemical composition and physical properties is also described in this chapter. Several factors that affect aerobic granulation process and the applications of granular sludge in SBR system were briefly explained.

Chapter 3 provides the methodologies and research materials, including wastewater feed and sludge used throughout the study. The laboratory scale SBR operational conditions, the liquid and solid phase analytical methods, molecular analyses employed and the details of experimental procedures in the present study are also described in this chapter.

Chapter 4, 5 and **6** discuss the research findings that have been obtained in the present study. The findings are divided into three main chapters; (a) the development of aerobic granular sludge using rubber processing wastewater, (b) the effects of cycle time on the performances of aerobic granules in the SBR system and (c) microbial community of the developed aerobic granular sludge and the characteristics of extracellular polymeric substances (EPS) of aerobic granular sludge.

Lastly, the conclusions derived from this research are given in **Chapter 7**. This chapter also provides some recommendations for future research exploration in relation to the findings of this study.

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