IDENTIFICATION AND POTENTIAL APPLICATION OF BACTERIAL POPULATION IN BIOGRANULE FOR THE TREATMENT OF TEXTILE WASTEWATER

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A dissertation submitted in partial fulfillment

of the Requirements for the award of the degree of

Master of Science (Biotechnology)

Faculty of Biosciences and Bioengineering

Universiti Teknologi Malaysia

FEBRUARY 2013

To my beloved mother and father

ACKNOWLEDGEMENT

In preparing this thesis, I was in contact with many people, researchers, academicians, and practitioners. They have contributed towards my understanding and thoughts. In particular, I wish to express my sincere appreciation to my main thesis supervisor, Dr. Adibah Yahya, for encouragement, guidance, critics and friendship. Without her continued support and interest, this thesis would not have been the same as presented here.

I am also indebted to my dear father and mother to support me in all aspects of my life.

My classmate postgraduate students at laboratory should also be recognised for sharing their experience with me. My sincere appreciation also extends to all my friends and others who have provided assistance at various occasions. Their views and tips were useful indeed. Unfortunately, it is not possible to list all of them in this limited space.

ABSTRACT

Identification of bacterial population from biogranules for remediating industrial wastewater has been carried out. In this study, molecular method of metagenomic was applied utilizing the 16S rRNA gene fragment to determine the diversity of bacterial population. The genomic DNA was directly extracted from biogranules. The 16S rRNA gene was amplified by PCR and universal primers. Transformation was done into JM 109 competent cell by heat-shock method for selecting the positive clones and gaining high number of cloned vectors. In order to recover the individual positive clones, the plasmid was extracted, for confirming the cloning from plasmid gel electrophoresis method was conducted. Then, reconfirmation by restriction enzyme was performed by EcoR1, after cutting the vector and the gene was separated at the band of 3000 bp for vector and 1500 bp for gene. Finally, 12 positive colonies were observed from extraction of plasmid. The positive colonies were sent for commercial sequencing, and the sequences obtained were then analyzed using bioinformatics tools in order to identify the bacterial genus and species as well as to locate them in the phylogenetic tree. Three main bacterial phylogroups including: Firmicutes, Spirochaetes, and proteobacteria as well as different species of bacterial were finally identified. In the second phase of this study, assessing of the biogranules performance in textile wastewater was accomplished. The parameters such as Ammonical-Nitrogen, Colour (ADMI), COD removal and pH have been analyzed.

ABSTRAK

Pengenalpastian populasi bakteria dari biogranul untuk rawatan air sisa industri telah dijalankan. Dalam kajian ini, kaedah molekul metagenomik telah digunakan, di mana gen 16S rRNA bakteria akan dikenalpasti untuk penentuan kepelbagaian populasi bakteria yang terkandung dalam biogranul. Genomik DNA diekstrak keluar daripada biogranul secara lansung, di mana kuantiti gen 16S rRNA yang terkandung dalamnya telah diamplikasikan oleh kaedah PCR berserta dengan primer universal. Gen 16S rRNA yang telah diamplikasikan akan dimasukkan ke dalam kompeten sel JM 109 melalui kaedah Transformasi dengan bantuan kejutan haba. Vector *pGEM*®-*T* yang berjaya diintegrasikan dengan gen 16S rRNA akan dimasukkan ke dalam kompeten sel, di mana pengenalpastian klon positif ini akan dijalankan menerusi kaedah saringan biru-putih. Dalam usaha untuk mendapatkan kembali gen 16S rRNA yang asli, plasmid atau vector *pGEM*®-*T* telah diekstrak daripada klon positif. Kaedah elektroforesis gel telah dijalankan untuk mengesahkan plasmid telah berjaya diekstrak keluar. Kemudian, pengesahan akan dilakukan sekali lagi oleh enzim sekatan EcoR1, di mana enzim ini akan memisahkan vektor *pGEM*®-*T* dan gen 16S rRNA. Akhirnya, sejumlah 12 plasmid telah diekstrak keluar daripada 12 klon positif dan dihantar untuk penjujukan komersial. Urutan jujukan yang diperolehi telah dianalisis dengan menggunakan perisian bioinformatik untuk mengenal pasti genus dan spesies bakteria-bakteria tersebut dan justeru itu melokasikan mereka di satu pokok filogenetik induk. Semua 12 bakteria yang dikenalpasti didapati merupakan ahli dalam tiga kumpulan-phylo utama bakteria iaitu: Firmicutes, Spirochaetes, dan proteobacteria. Dalam fasa kedua kajian ini, biogranul telah diapplikasikan untuk rawatan air sisa tekstil. Prestasi dan kecekepan biogranul dalam rawatan air sisa tekstil telah dinilai menerusi parameter seperti ammonia-nitrogen, Warna (ADMI), penyingkiran COD dan pH.

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LIST OF ABBRIVATIONS

ADMI	-	American Dye Manufacturing Index
Ag_2SO_4	-	Silver Sulfate
AFBR	-	Anaerobic Fluidized Bed Reactor
APHA	-	American Public Health Association
BAS	-	Biofilm Airlift Suspention
$BLAST_n$	-	Basic Local Alignment Search Tool
BOD	-	Biological Oxygen Demand
COD	-	Chemical Oxygen Demand
CSTR	-	Continuous Stirred Tank Reactor
DNA	-	Deoxyribonucleic Acid
DO	-	Dissolved Oxygen
EDTA	-	Ethylene diamine tetraacetic acid
EPS	-	Extracellular Polymeric Substances
EtBr	-	Ethidium Bromide
GES	-	Groundwater Environmental Services
$HgSO_4$	-	Mercury (II) Sulfate
HRT	-	Hydraulic Retention Time
NPDES	-	National Pollutant Discharge Elimination Systems

N	-	Nitrogen
P	-	Phosphorus
PBS	-	Phosphate Buffer Saline
PCR	-	Polymerase Chain Reaction
POME	-	Palm Oil Mill Effluent
POWTS	-	Publicly-owend Treatment Works
OLR	-	Organic Loading Dye
RNA	-	Ribonucleic Acid
SBAR	-	Sequencing Batch Airlift Reactor
SBR	-	Sequencing Batch Reactor
SDS	-	Sodium Dodecyl Sulphate
SOUR	-	Specific Oxygen Uptake Rate
TAE	-	Tris-acetate-EDTA
TS	-	Total Solids
UASB	-	Upflow Anaerobic Sludge Blanket
UV	-	Ultraviolet
VSS	-	Volatile Suspended Solids

LIST OF SYMBOLS

Ag_2So_4	-	Silver Sulphate
Ca^{2+}	-	Calcium
Co_2	-	Carbone Dioxide
ddH_2O	-	Double Sterile Water
Fe^{2+}	-	Ferrous
Fe^{3+}	-	Ferric
H/D	-	Column High to Diameter Ratio
H_2SO_4	-	Acid Sulphuric
$HgSO_4$	-	Mercuric Acid
$K_2Cr_2O_7$	-	Potassium Dichromate
Mg^{2+}	-	Magnesium

APPENDIX

CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Treatment of industrial wastewater is continuously complicate by various factors including the presence of mix pollutant which is commonly recalcitrant to natural biodegradation. In addition, the rates of organic loading in the wastewater frequently give direct influence to the efficiency of the treatment system. Biological treatment has became a major part of the whole wastewater treatment system where indigenous microbial community in the wastewater is directly used in the form of suspended cell or in activated sludge system. However biological system is frequently complicate by inconsistent performance of the microbes (Weber *et al.*, 2007).

Biogranulation technology developed for wastewater treatment is becoming a more constructive technology in many countries. Granulation is a developing technology in wastewater treatment which involves the application of whole bacteria cells in mixed cultures.

Biogranules represent a relatively new form of cell immobilization developed for biological wastewater treatment. Biogranulation involves cell-to-cell interactions that include biological, physical and chemical phenomena. There are two types of biogranulation namely aerobic and anaerobic granulation (Liu *et al.*, 2010).

Aerobic granulation may be initiated by microbial self-adhesion. Aerobic granulation has been reported in Sequencing batch Reactor (SBR) and has been used in treating high strength wastewater (organics, nitrogen and phosphorous and toxic substances). Upflow anaerobic sludge blanket (UASB) reactors have used for treating industrial wastewater in anaerobic granulation technology. However, there are some limitations in using UASB reactors such as long start-up period and a relatively high operation temperature. Then, anaerobic granulation is also highly sensitive to hydraulic and organic load variations, and it is unsuitable for the removal of nutrients (nitrogen (N) and phosphorus (P)) and treatment of low-strength organic wastewater (Schmidt and Ahring, 1996).

The granules are dense microbial consortia packed with different bacterial species and typically contain millions of organisms per gram of biomass. The bacteria perform different roles in degrading complex industrial wastes. Granules have a regular, dense, strong structure, and good settling properties. Compared to the conventional flock sludge, the biogranulation has an excellent settling property for enabling high biomass retention and dense microbial structure for withstanding high-strength organic wastewater and its shock loading (Zhu *et al.,* 2008).

In this study, metagenomic approach based on 16S rDNA fragment amplification has been used, employing universal primer sequences for identification of bacteria. Metagenomics approach can be used to address the challenges of studying prokaryotes in the environment that are, as yet, unculturable and represent even more than 99% of the organisms in some environments (Amann *et al.,* 1995). This approach has already opened new avenues of research by enabling unprecedented analyses of genome heterogeneity and evolution in environmental contexts and providing access to far more microbial diversity than that has been viewed in the petri dish. Approaches that are enriched for a portion of the microbial community or for a collection of Metagenomic clones will enhance the power of metagenomic analysis to address targeted questions in microbial ecology and to discover new biotechnological applications. To realize the full potential of metagenomics, however, a number of obstacles need to be overcome. Perhaps the most significant of these obstacles is the microbial complexity in most communities.

Another focus for improvement in metagenomics is the use of robust sampling and DNA extraction processes (Zeyaullah1 et al., 2009).

1.2 Statement of Problem

Identification of bacterial population in a wastewater treatment system is an important task as this enable determination of the treatment performance. A microbial interaction with pollutants in the wastewater varies according to the types of bacteria in the consortium population that determine how they interact with each other and with their environment. Due to this fact, identification of bacteria population would be useful. However, conventional method for bacterial identification using culture method would be highly time consuming, costly and laborious. In addition, it contributes to high error and bias (Abdullah *et al.*, 2011; Muda *et al.*, 2010).

An alternative method using molecular approach gives more accurate results and does not require culturing individual bacterial genus in the laboratory. Growth of different bacterial strains may require different growth condition and media but with the advance of polymerase chain reaction strategy; the highly conserved 16S rDNA region can be easily amplified from the genomic DNA of bacterial consortium, this enable identification of bacterial based on the total number of individual positive clone of amplified 16S rDNA obtained. Sequencing technology allows the use of bioinformatics tools to identify the genus and species of the bacteria.

Biogranules have been used for the treatment of various types of wastewater ranging for domestic and industrial wastes. However, microbial architecture in the biogranule was not identified in most cases. Therefore, the role of microbes incorporated as biogranules was not able to be predicted. Due to different microbial community, that needs adapting on different types of wastewater.

1.3 Objectives of the Study

The main objectives of this study were:

1) To identify mix bacterial population accommodating biogranules used for treating industrial wastewater.

2) To determine the potential application of biogranules for treating coloured wastewater obtained from textile industry. The potential of biogranules was measured based on the analysis of parameters that indicate quality of water such as chemical oxygen demand (COD), colour and nutrient content such as nitrogen, phosphate, aromatic compounds and etc.

3) To analyze the relationship between the bacterial population identified and their possibly role in textile wastewater treatment.

1.4 Significance of the Study

During the last twenty years, intensive research in the field of biological wastewater treatment and other applications has demonstrated that biofilms are often more efficient for water purification than suspended activated sludge (Lettinga, 1995). Today, the application of anaerobic and aerobic granular sludge in wastewater treatment is regarded as one of the most useful and promising biotechnologies. Through the application of granular sludge technology, the persistent problem associated with wastewater treatment process has been much reduced (Muda *et al.,* 2010). This shows that concentrating microbial population in the treatment system is able to improve the overall performance of the biological treatment. However, the consistency of biological function of the granular sludge is also determined by the type of microbes, particularly bacteria population in the granule sludge system (Barr *et al.,* 2010). Therefore, it is important to identify

bacterial population in the granular sludge as it will significantly affect the performance of industrial wastewater treatment system at certain period of time.

1.5 Scope of the Study

The potential application of biogranules for the treatment of the coloured wastewater was studied by using textile wastewater collected from a textile industry in Johor, Malaysia. The performance of biogranule used in the treatment was monitored in a batch shake flask treatment based on the reduction of Chemical Oxygen Demand (COD), colour, ammonical nitrogen and changes of pH. Bacterial population of the biogranules was also identified using molecular-based method. Genomic DNA was extracted from the biogranule to amplify the 16S rDNA fragment using universal forward and reverse primers. The PCR product was then cloned into pGem-T easy vector, prior to transformation into JM 109 competent cell. Plasmid preparation was carried out prior cloning PCR product for commercial sequencing.

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