BIOGRANULATION DEVELOPMENT DURING TREATMENT OF REAL TEXTILE WASTEWATER

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In Loving Memory of My Godmother, Reginamary (1955-2014)

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ABSTRACT

Over the past decades, effluent discharge from textile industries to neighbouring water bodies can cause severe water pollution if discharged untreated. There are quite a number of treatment systems have been invented to treat textile wastewater, but each treatment system had its own limitations. The most common limitations are low efficiency, inapplicability to treat a wide variety of dyes, production of secondary waste and high cost. Currently, biological treatment using biogranules had showed promising results in treating textile wastewater. However, most studies are being done using synthetic wastewater at lab scale reactor. This study looks at the development and application of biogranules in treating the actual textile wastewater using pilot-scale reactor. A pilot-scale Sequential Batch Reactor (SBR) biogranular system with working volume of 70 L was developed and operated according to SBR's sequence for 24-hr cycle, which includes sequential anaerobic and aerobic reaction phases. Wastewater from two textile mills were used as feed, while sewage and pineapple wastewater were used as co-substrate. After operating the system for 60 days, 30% of the sludge transformed into biogranules and increased to 67% at the end of the study. The biogranules developed in the reactor have sizes ranging from 0.2 mm to 9.5 mm with a mean settling velocity of 28 ± 7 m/hr and Sludge Volume Index (SVI) of 73.9 mL/g. At the end of the development period, the system yields 92% removal of Chemical Oxygen Demand (COD), however color removal fluctuated throughout the development period in the range of 50 to 70%. The efficiency of biogranules in treating textile wastewater was evaluated using lab scale bioreactor with total working volume of 3L. The system was able to achieve 55% of average color removal and 88% of average COD removal at 7%v/v pineapple wastewater concentration during the treatment period. The experiment on treatment of raw textile wastewater without any added nutrient proved the importance of co-substrate in dye degradation. Conversely, the addition of external dye degrader microbes in the reactor had slightly improved color removal. The system obtained 59% of mean color removal and 81% of mean COD removal at 5%v/v pineapple wastewater concentration upon addition of dye degrader microbes into the bioreactor. Microorganisms under genus *Pseudomonas*, *Klebsiella* and *Enterococcus* were identified within the mature biogranules which are considered in the literature as dye degrader microbes. Although the biogranules development is much faster in lab-scale reactor under controlled environment, the findings indicate the feasibility of developing biogranules in a bigger scale reactor using actual textile wastewater and other high-strength biodegradable wastewater as co-substrate.

ABSTRAK

Sejak beberapa dekad yang lalu, pelepasan air sisa daripada industri tekstil ke badan air berdekatan sungai telah menyumbang kepada pencemaran air yang kritikal jika dilepaskan tanpa dirawat. Terdapat beberapa sistem rawatan yang telah dicipta untuk merawat air sisa tekstil, tetapi setiap sistem rawatan mempunyai had tersendiri. Had yang biasa adalah kecekapan rendah, ketidakupayaan untuk merawat pelbagai jenis pewarna, penghasilan sisa sekunder dan kos yang tinggi. Rawatan menggunakan biogranul telah menunjukkan keputusan yang memuaskan dalam merawat air sisa tekstil. Walau bagaimanapun, kebanyakan kajian telah dilakukan dengan menggunakan air sisa sintetik dalam reaktor berskala kecil. Dalam kajian ini, keupayaan menghasil dan menggunakan enapcemar granul bagi mengolah airsisa tekstil dalam satu reaktor berskala loji-pandu dikaji. Satu sistem reaktor berkelompok berjujukan (SBR) biogranular berskala loji-pandu berisipadu 70 L telah dikendalikan mengikut urutan SBR untuk kitaran 24 jam, dengan fasa anaerobik dan aerobik beroperasi secara berselang-seli. Air sisa daripada dua kilang tekstil telah digunakan, manakala kumbahan dan air sisa nenas digunakan sebagai ko-substrat. Setelah beroperasi selama 60 hari, 30% daripada enapcemar telah berubah menjadi biogranul dan telah meningkat kepada 67% di akhir kajian. Biogranul yang terbentuk di dalam reaktor mempunyai saiz diantara 0.2 mm hingga 9.5 mm dengan halaju enapan purata 28 ± 7 m/hr dan mempunyai index isipadu enapcemar (SVI) serendah 73.9 mL/g. Pada akhir kajian ini, 92% penyingkiran COD telah dicapai oleh sistem, tetapi penyingkiran warna berubah-ubah di sepanjang tempoh pembentukan granul dalam lingkungan 50 hingga 70%. Kecekapan biogranul dalam merawat air sisa tekstil dinilai menggunakan reaktor berskala kecil berisipadu 3L. Sistem ini telah berjaya untuk mencapai 55% penyingkiran warna dan 88% penyingkiran COD pada kepekatan 7%v/v airsisa nenas sepanjang tempoh rawatan. Eksperimen menggunakan rawatan airsisa tekstil mentah tanpa sebarang penambahan nutrien membuktikan kepentingan ko-substrat dalam degradasi pewarna. Sebaliknya, penambahan mikrob penyahwarna dalam reaktor telah mempertingkatkan penyingkiran warna dalam kadar yang rendah. Sistem ini telah berjaya mencapai 59% penyingkiran warna dan 81% penyingkiran COD pada kepekatan 5%v/v airsisa nenas dengan penambahan mikrob penyahwarna. Mikroorganisma daripada genus *Pseudomonas*, *Klebsiella* dan *Enterococcus* telah dikenalpasti dalam biogranul matang dan mengikut rujukan merupakan mikrob penyahwarna. Walaupun pembentukan biogranul adalah lebih cepat dalam reaktor berskala kecil dan dalam persekitaran terkawal, hasil kajian menunjukkan keupayaan pembentukan biogranul dalam reaktor skala yang lebih besar menggunakan airsisa tekstil sebenar dan air sisa mudah biorosot kekuatan tinggi lain sebagai ko-substrat.

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LIST OF ABBREVIATIONS

16S rRNA	-	16 subunit ribosomal ribonucleic acid
ADMI	-	American Dye Manufacturing Index
AESB	-	American & Effird (M) Sdn. Bhd.
COD	-	Chemical oxygen demand (C-mmoL or mg/L or g/L)
DNA	-	Deoxyribonucleic acid
DO	-	Dissolved oxygen (mg/L)
EPS	-	Extracellular polymeric substances
F/M	-	Food-microrganisms ratio
FESEM-EDX	-	Field-emission scanning electron microscope coupled with Energy Dispersive X-ray Analysis
FT-IR	-	Fourier Transformed Infrared Spectroscopy
HRT	-	Hydraulic retention time (h or day)
IC	-	Integrity coefficient (%)
MIDA	-	Malaysian Industrial Department Authority
MLSS	-	Mixed liquor suspended solid (mg/L or g/L)
MLVSS	-	Mixed liquor volatile suspended solid (mg/L or g/L)
N/COD	-	Nitrogen/Chemical oxygen demand
NGS	-	Next Generation Sequencing
OLR	-	Organic loading rate (mg/L·day or kg/m3·day)
ORP	-	Oxidation reduction potential
OUR	-	Oxygen uptake rate (mg/L.h)
P/COD	-	Phosphorus/Chemical oxygen demand
PC	-	Pineapple concentration(%v/v)
RG	-	Residual granules (mg)
RTISB	-	Ramatex Textile Industrial Sdn. Bhd.
SAV	-	Superficial air velocity (cm/s or m/hr)
SBR	-	Sequencing batch reactor

SG	-	Settled granules (mg)
SRT	-	Sludge retention time (day)
SV	-	Settling velocity (cm/s or m/hr)
SVI	-	Sludge volume index (mL/g)
TN	-	Total nitrogen (mg/L)
TP	-	Total phosphorus (mg/L)
TSS	-	Total suspended solid (mg/L or g/L)
UASB	-	Up-flow anaerobic sludge blanket
UV-Vis	-	Ultraviolet visible spectroscopy
VER	-	Volumetric exchange rate

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CHAPTER 1

INTRODUCTION

1.1 Preamble

Textile industry is a rapid growing industry and an important contributor to the economic growth of numerous Asian countries namely Bangladesh, Cambodia, India, China and Malaysia. Because of the cheap market price, Asian countries are ahead in textile industry as compared to African, European and South American countries (Tang, 2014). Textile industry in Malaysia begun to emerge in the early 1970s as export oriented industrialisation initiative. Over the years, growth of the textiles market has eventually contributed towards the economy of the country. Presently, there are more than 6800 workers working in this field and the industry yields RM13.2billion, placing it as the ten largest export earner in year 2015 (MIDA, 2017).

Nevertheless, the rapid growth of the industry in Malaysia has caused serious environmental problems. The textile industry uses large amount of water in the production and releases wastewater with high load of pollutants. There is a large number of published reports (McMullan *et al.,* 2001; Pearce *et al.,* 2003; Hai *et al.,* 2007; Lotito *et al.,* 2011; Pang and Abdullah, 2013; Chung, 2016) that describe the environmental impact of textile wastewater. Generally, untreated textile wastewater is highly coloured, contains high suspended solid and non-biodegradable organics and released at elevated temperature with variable pH ranging from 5 to 10 (Pushkar and Mungray, 2016). Moreover, poor fixation of dyes on textile fibers results in direct discharge of concentrated dyes into wastewater (Carmen and Daniela, 2012). These highly coloured and non-biodegradable nature of the effluents pose potential threat to human health and environment.

In Malaysia, the discharge of the industrial effluents is regulated through Environmental Quality (Industrial Effluents) Regulations 2009 (Department of Environment of Malaysia, 2015) and the factory management is responsible in ensuring the quality of treated wastewater comply with the legislative requirement. There have been a number of methods used in treating textile industrial effluent. Presently, the main methods in textile wastewater treatment involve physical and chemical processes. However, physico-chemical treatment systems are not favoured for treating textile effluent because of high selectivity towards certain dyes, considerable high capital and operational costs and generation of sludge (Neoh *et al.,* 2016; Holkar *et al.,* 2016). Conversely, most of the dyes could not be treated by conventional biological wastewater treatment processes and will remain in the environment because of their high stability to light, temperature, water, detergents, chemicals, soap and other parameters such as bleach and perspiration (Rajaguru *et al.,* 2000; Ali, 2010).

In the past decades, studies have shown that textile wastewater can be treated by the sequential combination of anaerobic and aerobic processes (Farabegoli *et al.,* 2010; Khouni *et al.,* 2012; Franca *et al.,* 2015; Mata *et al.,* 2015). The anaerobic process is able to decolorize the wastewater, while the following aerobic process further degrades the organics and also removes the amines that are produced during the anaerobic process (Haroun and Idris, 2009). These processes have been carried out using either separate anaerobic and aerobic tanks or in a single sequential batch reactor (SBR) with biomass either in the form of suspended flocs, biofilms or biogranules.

Biogranules has a compact structure formed by agglomeration of microorganisms known as granulation under specific environmental pressures. Biogranules have emerged as powerful medium in treating wide range of organic compound due to the co-existence of comprehensive microorganisms with diverse characteristics. Apart from this, excellent settling ability along with higher biomass retained in the reactor leads to competency of granulation technology.

A great deal of previous research into granulation has focused on the factors involved in granulation mechanism such as organic loading rate, substrate composition, seed sludge, settling time and hydrodynamic shear force (Tay *et al.*, 2005b; Chen *et al.*, 2007; Adav *et al.*, 2008b; Gao *et al.*, 2011; Bindhu and Madhu, 2013; Dahalan *et al.*, 2015). These theories of granulation mechanism provide a useful account of how to further improve the efficiency of biogranular treatment system in treating complex wastewater.

1.2 Problem statement

Remarkably, most studies in granules have only been carried out in a lab scale reactor using synthetic wastewater. It is only in recent years, there has been an increasing interest in developing biogranules at pilot scale SBR reactor (Liu *et al.*, 2011; Verawaty *et al.*, 2012; Morales *et al.*, 2013; Pronk *et al.*, 2015; Santana *et al.*, 2016). However, far too little attention has been paid in cultivating biogranules with real industrial wastewater in pilot scale reactor. Much of the research up to now has been focus on domestic wastewater and synthetic wastewater observing significant differences between pilot- and lab-scale results (Pronk *et al.*, 2015; Gouveia *et al.*, 2015; Santana *et al.*, 2016). Environmental factors such as wastewater characteristics, influent substrate concentration and temperature could be precisely controlled at lab-scale as compared to pilot-scale. The main challenge faced by many experiments is the long start-up period in pilot scale reactor (Pronk *et al.*, 2015; Santana *et al.*, 2016). This indicates a need to understand the granulation process in pilot scale reactor using real wastewater in relation to the operating conditions, substrate composition and evolution of the microbial community.

Moreover, the development and characteristic of granules are known to be affected by substrate present in reactor. Various co-substrates contribute to diverse microbial community in granules which eventually influence the performance of granules (Feng *et al.*, 2012; Sirianuntapiboon and Chairattanawan, 2012). Most researchers investigating co-substrate have utilised pure substances such as glucose, ethanol and acetate but only a couple of researchers had introduced biodegradable

industry waste as co-substrate in treating textile wastewater namely sago wastewater, starch wastewater and noodle industry wastewater (Gnanapragasam *et al.,* 2011; Senthilkumar *et al.,* 2011; Sirianuntapiboon and Chairattanawan, 2012). Hence, it is needed to evaluate the feasibility of other readily degradable wastewater as co-substrate in the treatment of real textile wastewater.

Extensive research has been carried out regarding dye degradation by bacteria, yeast and fungi to develop a practical biologic method for dye waste (Kurade *et al.,* 2015; Adnan *et al.,* 2016; Neifar *et al.,* 2016; Sayahi *et al.,* 2016; Younes *et al.,* 2016). There are few studies that inoculate sludge together with consortium of dye degrader microbes during start-up period to enhance the granular system (Muda *et al.,* 2010; Ibrahim *et al.,* 2010; Kee *et al.,* 2015; Franciscon *et al.,* 2015). However, there have been no controlled studies which compare differences in treatment efficiency with and without external dye degrader in treating actual textile wastewater using biogranules.

The present study is regarded as the first study dedicated to the development of biogranules using actual textile wastewater in pilot scale reactor. The system utilizes the concept of sequential anaerobic and aerobic biological reactions for complete degradation of the wastewater. The factors influencing the competency of granular system in treating raw textile wastewater are addressed in this study. Additionally, sewage and pineapple wastewater were tested as potential co-substrate in development of granular system for textile wastewater and the influence of external dye degrader on treatment efficiency were explored.

1.3 Objectives of the study

This study aimed to ascertain how the complex characteristic of textile wastewater affect the granulation process and removal efficiency of organic matter.

The specific objectives of the experimental study are as follows:

i. To develop biogranules in treating actual textile wastewater in a pilot scale bioreactor with the use of sewage and pineapple wastewater as co-substrate.

ii. To assess the effect of pineapple wastewater (co-substrate) concentration (%v/v) on the performance of the biogranules.

iii. To evaluate the effect of dye degrader microbes on the performance of system in terms of color removal and COD removal.

iv. To characterize and compare the biogranules developed in the pilot-scale against those developed in lab scale reactor in terms of physical, chemical and biological characteristics.

1.4 Scope of study

This study explores the development of biogranules in pilot scale bioreactor to treat real textile wastewater. The design and operation of the pilot scale bioreactor are based on Muda (2010). The reactor had a working volume of 70 L and was operated according to SBR's sequence for 24-hr cycle, which includes sequential anaerobic and aerobic reaction phases. Wastewater from two textile mills were used as feed, while sewage and pineapple wastewater were used as co-substrate. Along the granulation process, samples of granules were collected and examined in terms of the morphology and physical characteristics namely settling velocity, sludge volume index (SVI), mixed liquor suspended solids (MLSS), mixed liquor volatile suspended solids (MLVSS) and integrity coefficient. Reactor performances were determined based on the COD, color, total nitrogen and total phosphorus removal efficiencies during granulation and maturation phases of the granules. The investigation involved the effect of pineapple wastewater concentration (%v/v) on the organic and nutrient removal in raw textile wastewater by biogranules were performed using lab scale bioreactor as the pilot scale bioreactor was shutdown due to a technical problem. The lab scale bioreactor with 3L working volume were operated in similar operation

parameters to pilot scale bioreactor such as reaction time (anaerobic and aerobic time), volumetric exchange ratio (VER) and hydraulic retention time (HRT).

External dye degrader obtained from Microclear Sdn Bhd was employed for the experiment to assess the influence of dye degrader microbes on the color and COD removal. The degradation of dye was justified by characterizing the untreated and treated wastewater using ultraviolet visible spectroscopy (UV-Vis) together with fourier transformed infrared spectroscopy (FT-IR). Furthermore, field-emission scanning electron microscope coupled with energy dispersive X-ray analysis (FESEM-EDX) was used to inspect the microstructure and elemental composition of biogranule. The study also included analysis of bacterial population within biogranule via next generation sequencing (NGS) analysis. There was no addition of any chemical to control the pH as well as no nutrients were supplied for the growth of microbes throughout the study. In this study, the reactors were operated without controlling the parameters such as pH, temperature, DO, MLSS and MLVSS

1.5 Significance of study

Extensive research has shown that biological treatment has a good prospect in treating textile wastewater attributed to its economical and green technology (Sudha *et al.*, 2014; Parmar and Shukla, 2015; Mahmoud *et al.*, 2017). There is a growing body of literature that recognises the application of biogranular system in treating textile wastewater (Gnanapragasam *et al.*, 2010; Senthilkumar *et al.*, 2011; Baeta *et al.*, 2012; Talouizte *et al.*, 2013; Franca *et al.*, 2015; Mata *et al.*, 2015). However, majority of studies were conducted using synthetic wastewater, mixture of certain dye wastewater and raw textile wastewater at lab scale. Notably, biogranulation study treating actual textile wastewater in an SBR system at a bigger scale is apparently missing. The significance of this study is therefore listed as follows;

i. As the characteristics of the textile effluent is well known for its low biodegradability and high variation, many practical aspects of the treatment in

developing biogranules need to be explored. Hence, the present research explores, the development of biogranules in the pilot scale reactor using actual textile wastewater as prime substrate.

ii. This study provides new insights into usage of pineapple wastewater as co-substrate for granulation. Although co-substrates such as glucose and acetate are commonly used in lab-scale study to aid the biogranules development and treatment process, their applications in actual plant is costly unattractive. Therefore, it is required to consider the potentials of a readily degradable wastewater as a co-substrate in the treatment of real textile wastewater.

iii. The study also provides the effect of using different concentrations of pineapple wastewater in relation to dye degradation by the biogranules. The addition of pineapple wastewater excludes the addition of external nutrients and improve the degradation of textile wastewater which is recalcitrant in nature.

iv. The findings on bacterial population within biogranule presents basic understanding in the microbial evolution of the biogranules developed using textile wastewater to further enhance the treatment process.

1.6 Organization of thesis

The thesis is composed of five chapters. The first chapter deals with problems generated from the textile industry and the importance of developing biogranules using pilot-scale reactor using actual wastewater. The second chapter focuses on the literature review, mainly discusses on the textile waste, azo dye characteristics, dye degradation mechanisms, granulation process and pilot scale application. Then, the third chapter explains the methodology used for this study and provides the experimental work involved throughout the research. Chapter Four discusses the

findings of the research focusing on each objective. Finally, Chapter Five lays out the conclusions and proposes the recommendation for future research exploration.

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