ACID RED 27 DECOLOURISATION AND SIMULTANEOUS ELECTRICITY GENERATIONS BY MIXED BACTERIAL CULTURES IN MICROBIAL FUEL CELL

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DEDICATION

I would like to dedicate my deepest appreciation to

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my beloved husband Wan Mohd Irwan Syazwan thank you for every single moment you spent with me just gives me assurance, feeling secure and appreciated;

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ABSTRACT

Azo dyes are widely used in industries although poorly biodegradable and highly toxic. Poor existing wastewater treatment caused dye residues in industrial effluent to be discharged into neighbouring water bodies causing major distress to the environment. This study was carried out to identify the potential of using mixed bacterial culture system for efficient biodegradation of azo dye in single batch system and for potential electricity generation in microbial fuel cell (MFC). This study used single and mixed bacterial consortium in batch shake flask experiment to decolourize Acid Red azo dye (AR27) and for electricity generation in a dual-chamber MFC. Four bacterial strains coded as MF1, B2, ZL and CO were used. Morphological, biochemical and 16S rDNA sequence analyses of these bacteria were carried out. Phylogenetic analysis successfully confirmed the identity of strains MF1 and B2 as *Klebsiella pneumoniae* (MF1 and B2), CO as *Bacillus cereus*. However, ZL identification was inconclusive. Flask experiment of single strain showed that all single strains were able to degrade AR27 dye, where strain CO displayed 2 to 4-fold higher rate of dye removal compared to other strains. Compatibility test successfully showed that strains CO, MF1 and ZL were able to grow without antagonism effect whereas CO and B2 were competing with each other. Selection of the best mixed bacterial culture based on AR27 dye degradation showed that combination of strains CO, MF1 and ZL produced the best dye decolourisation rate and Chemical Oxygen Demand (COD) removal. Optimization study revealed that mixed culture of CO+MF1+ZL performed the best AR27 dye degradation in CDM medium when adjusted to pH 5.0-7.0, supplemented with 3 g/L yeast extract, 100 mg/L AR27 dye and incubated under static condition at 32 – 37°C. Under optimal conditions, 99% of 100 mg/L AR27 was removed at the rate of 4.21 mg/h. Sequential facultative anaerobic-aerobic condition demonstrated that the COD removal percentage increased from 57% to 69% when aerobic condition was introduced to the system. However, the amount of Total Polyphenolic Content (TPP) removed showed no significant difference. Assessment of potential electricity generation by CO+MF1+ZL mixed culture during AR27 dye removal in dual-chamber MCF demonstrated that the maximum generated open circuit voltage (OCV) was 567.7mV. Closed circuit voltage (CCV) across 5000 Ω external resistance generated was 39.7mV with maximum power density and current density of 0.26 mW/m^2 and 6.6 mA/m^2, respectively. In the application of mixed culture for dye removal in real textile wastewater, the voltage generated dropped to 232.38 mV for OCV and 13.9 mV for CCV with maximum power and current density generated 0.03 mW/m^2 and 2.3 mA/m^2, respectively. This was due to high pH value of textile wastewater. When textile wastewater was adjusted to pH 7, the voltage generated improved to 489.9 mV for OCV and 32.9 mV for CCV with maximum power and current density generated 0.18 mW/m^2 and 5.78 mA/m^2, respectively. This study showed that mixed culture of CO+MF1+ZL has a good potential for treating wastewater containing azo dye for simultaneous decolourization and electricity generation.

ABSTRAK

Pewarna Azo digunakan secara meluas dalam industri walaupun tidak terbiodegradasikan dan sangat toksik. Rawatan air kumbahan sedia ada yang tidak sempurna menyebabkan residu pewarna dalam efluen perindustrian dilepaskan ke dalam sistem saliran air menyebabkan kesan pencemaran buruk kepada alam sekitar. Kajian ini dijalankan untuk mengenal pasti potensi menggunakan campuran bakteria untuk biodegradasi pewarna azo yang berkesan dalam sistem kelompok tunggal dan potensi penjanaan tenaga elektrik dalam sistem sel bahan api mikrob (MFC). Kajian ini menggunakan goncangan bakteria tunggal dan campuran dalam kelompok, kelalang goncang untuk penyahwarnaan pewarna azo *Acid Red* (AR27) dan penjanaan tenaga elektrik di dalam sel bahan api mikrob (MFC) dwi-ruang. Empat jenis bakteria yang diberi kod MF1, B2, ZL dan CO digunakan. Analisis morfologi, biokimia dan jujukan 16S rDNA bagi bakteria ini telah dijalankan. Analisis filogeni berjaya mengesahkan identiti strain MF1 dan B2 sebagai *Klebsiella pneumoniae* (MF1 dan B2) dan CO sebagai *Bacillus cereus*. Walau bagaimanapun, pengenalan strain ZL tidak dapat disimpulkan. Eksperimen kelalang bagi bakteria tunggal menunjukkan bahawa semua strain tunggal mampu untuk menyahwarnaankan AR27, di mana strain CO menunjukkan kadar penyahwarnaan 2 hingga 4 kali ganda lebih tinggi berbanding dengan strain lain. Ujian keserasian berjaya menunjukkan bahawa strain CO, MF1 dan ZL dapat membiak tanpa kesan antagonisme tetapi CO dan B2 bersaing dengan satu sama lain. Pemilihan campuran bakteria terbaik berdasarkan degradasi pewarna AR27 menunjukkan kombinasi strain CO, MF1 dan ZL mempunyai kadar penyahwarnaan pewarna dan penyingkiran Permintaan Oksigen Kimia (COD) terbaik. Kajian pengoptimuman kultur campuran CO + MF1 + ZL menunjukkan penyahwarnaan AR27 terbaik dalam medium CDM yang diselaraskan kepada pH 5.0-7.0, ditambah dengan 100 mg / L pewarna AR27 dan 3 g/L ekstrak yis yang dieram di bawah keadaan statik pada suhu 32-37 °. Di bawah keadaan yang optimum, 99% daripada 100 mg / L AR27 dinyahwarnakan pada kadar 4.21 mg/j. Keadaan anaerobik-aerobik berturutan menunjukkan bahawa peratus jumlah penyingkiran COD meningkat daripada 57% kepada 69% namun jumlah kandungan Pengeluaran Polifenolik (TPP) (22 mg/mL) tidak menunjukkan perbezaan yang ketara. Penaksiran potensi penjanaan tenaga elektrik oleh campuran CO + MF1 + ZL semasa penyahwarnaan AR27 dalam MCF dwi-ruang menunjukkan bahawa voltan litar terbuka maksimum (OCV) terjana adalah 567.7mV. Voltan litar tertutup (CCV) merentasi 5000 Ω rintangan luar yang dijana ialah 39.7mV dengan ketumpatan kuasa maksimum dan kepadatan arus 0.26 mW/m^2 dan 6.6 mA /m^2. Aplikasi campuran bakteria untuk penyahwarnaan pewarna dalam sisa kumbahan tekstil, voltan yang dijana menurun kepada 232.38 mV untuk OCV dan 13.9 mV untuk CCV dengan kuasa maksimum dan ketumpatan arus masing-masing menghasilkan 0.03 mW / m2 dan 2.3 mA / m2. Apabila sisa kumbahan tekstil diselaraskan kepada pH 7, voltan yang dihasilkan meningkat kepada 489.9 mV untuk OCV dan 32.9 mV untuk CCV dengan kuasa maksimum dan ketumpatan arus yang dihasilkan 0.18 mW/m^2 dan 5.78 mA/m^2. Kajian ini memperlihatkan bahawa konsortium bakteria CO + MF1 + ZL mempunyai potensi yang baik untuk merawat air kumbahan mengandungi pewarna azo bagi penyahwarnaan dan penjanaan tenaga elektrik secara serentak.

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LIST OF ABBREVIATIONS / SYMBOLS

% - Percent
µA - Microampere
µL - Microlitre
µV - Microvolt
A - Surface area
Abs - Absorbance
Abs_{600nm} - Absorbance at the wavelenght of 600 nm
AR-27 - Acid Red 27
CDM - Chemically Defined Medium
C/N - Carbon per nitrogen
CCV - Close circuit voltage
Cm - Centimeter
COD - Chemical oxygen demand
$CuSO_4$ - Copper sulphate
DET - Direct electron transfer
e^- - Electrons
FTIR - Fourier Transfrom Infrared spectroscopy
G - Gram
g/L - Gram per liters
h - Hours
H^+ - Hydrogen ion
H_2SO_4 - Sulphuric acid
I - Current
J - Current density
$K_2Cr_2O_7$ - Potassium dichromate
K_2HPO_4 - Dipotassium hydrogen phosphate

L - Litre
M - Molar
mA - Miliampere
MFC - Microbial fuel cell
Mg/mL - Milligram per millilitre
mL - Milliliters
mM - Millimolar
mm - Millimeter
mV - Millivolt
mV/s - Millivolt per second
mW/m^2 - Milliwats per meter square
Na_2CO_3 - Sodium carbonate
ºC - Degree Celsius
OCV - Open circuit voltage
P - Power density
PEM - Proton exchange membrane
R_{ext} - External resistance
TPP - Total polyphenol
UV-Vis - Ultra violet visible
V - Volt
v/v - Volume to volume
w/v - Weight to volume
λ - Wavelenght
λ_{max} - Maximum waveleght
Ω - Ohm

λ_{max} - Maximum waveleght
Ω - Ohm

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CHAPTER 1

INTRODUCTION

1.1 Background of study

Textile industry generates a large volume of wastewater containing mixtures of azo dyes as the main source of pollutant (Solis *et al*., 2012). Azo dye is a synthetic dye that is extensively used in the food, cosmetic, textile, pharmaceutical and paper industries due to its colour varieties and fastness in production as compared to natural dyes dyes (Chang and Lin, 2001; Carneiro *et al*., 2007). Around 60 – 70 % of total synthetic dye that produced annually consists of azo dyes (Mc Mullan *et al*., 2001). Azo dye was characterized by the presence of double bonds (-N=N-) are the largest and most versatile class of dyes and are the most common chromophore in azo dyes (Stolz, 2001). During the dye processing approach 2 % of dyes are directly discharged into aqueous effluent (Pearce *et al.,* 2003) and is estimated that about 2 – 50 % of various applied dyes can be lost in the effluent during the textile colouring process (Selvakumar *et al*., 2013). Even at the low concentration the azo dye that dissolved in the water stream can cause it to become highly coloured. In a clean river water, dye concentration as low as 0.005 ppm are visible (Saratale *et al*., 2011)

O’Neill *et al*., (1999) reported that textile processing wastewater usually contains dye concentration between 10 - 200 mg/L. When the coloured wastewater flow into the river it will cause the intense colour to prevent the absorbance of light entering the water thus, greatly reducing photosynthesis of aquatic flora (Slokar *et al*., 1998). Rai *et al*., (2005) states that such dyes are defined as colored substances that give a permanent colour when it was applied to fibers and resist fading upon exposure to sweat, light, water and many chemicals, including oxidizing agents and microbial attack. In the intestinal bacteria, liver cells and skin surface micro flora contain azoreductase that would reduce azo bonds in azo dyes to colourless aromatic amines (Xu *et al*., 2007). Most azo dyes are either inert or toxic but as the azo bond is reduced it form aromatic amines that are toxic, mutagenic and carcinogenic (Kodam *et al*., 2006). From the study conducted by several researchers related to the risk assessment of aromatic amines, it was proven that aromatic amines are carcinogenic to the human body (IARC, 2012).

Finding an alternative way to treat azo dyes contaminant particularly for small scale textile industries are major concern since textile dye effluent contained azo dye and the derived metabolites from the improper discharge is aesthetically unpleasant (Vandevivere *et al*., 1998). These leads to a reduction in sunlight penetration, which in turn decreases photosynthetic activity that cause from the increase in dissolved oxygen concentration and water quality and consequently, acute toxic effects on aquatic flora and fauna, causing severe environmental problems worldwide (Vandevivere *et al*., 1998). Pang and Abdullah, (2013) reported that in Malaysia textile industry is one of the fastes growing industries and significantly contributes to the economic growth and consequently, produces high discharge rate of wastewater with high load of contaminants that caused main source of water pollution when the release of dyes into the environment during textile fiber dyeing and finishing processes. The most common treatment plan used are conventional treatment systems such as biological treatment alone or physicochemical treatments followed by a biological treatment due to their simplicity and lower treatment cost as compared to some physical and chemical methods (Pang and Abdullah, 2013; You and Teng, 2009).

Biological treatment use bacterial species that are capable of decolourising, and completely mineralise many reactive dyes under certain conditions (Kumar *et al.,* 2012). The degradation process of bacteria is often stimulated under static/anaerobic conditions by an enzymatic transformation reaction for dye decolourisation which results in the formation of aromatic amines (Kumar *et al.,* 2012). The aromatic amines produced are then further oxidised and mineralised to form a simpler non-toxic by-product under aerobic conditions (Chan *et al*. 2012c; Kumar *et al.,* 2012). Decolourization of azo dye by microbial fuel cell has received attention as new technology to treat textile wastewater since it derives bioelectricity generations simultaneously with wastewater treatment (Sun *et al*., 2009; Chen *et al*., 2010; Liu *et al.*, 2011a). In a microbial fuel cell electricity is produced through the reaction between the fuel (anode) and oxidant (cathode) in the presence of an electrolyte (Evans *et al.*, 2012). In the anode compartment, active microorganism will oxidise the organic co-substrate that eventually generate electron (Logan *et al.*, 2006; Lai *et al.*, 2017). The current generation is produced as the electrons are transferred to the eventually generate electron (Logan *et al.*, 2006; Lai *et al.*, 2017). The cathode and the anode comparment was separated by a proton exchanges membrane to allow proton migration (Logan *et al.*, 2006; Solanki *et al.*, 2013; Lai *et al.*, 2017).

1.2 Problem Statement

Current situation in Malaysia shows that amount of scheduled waste generated by textile industry increased sharply. However, there is still no centralized system for the textile industry factory to treats the wastewater due to the cost and time consuming process. Meanwhile, high cost needed to supply high quality water for dyeing and finishing process. Due to the high solubility of Azo dye in water and poor wastewater treatment system, the dye residues residing in the effluent discharged from the industry to the neighbouring water bodies is causing major distress to the environment. Findings an economic ways to treat the wastewater effluent was crucial to prevent water pollution from getting worse.

Biological process using bacteria provide a low-cost and an efficient ways for colour removal and degradation of dye. However, several problem need to overcome such as the concentration of co-substrate use by azo degrading bacteria must be sufficient to treat the textile wastewater in an ideal and economical operating system. Other than that, the practically of the possible implementations the optimized system to the real textile wastewater should also be considered. Therefore, it is important to explore the prospect of effective dye degradation by bacterial culture so that these method can be sufficient to treat the textile wastewater in an ideal operating system and subsequently provide an economic ways to treat water pollution problem.

1.3 Significance of study

This study focused on developing bacterial mixed culture capable of removal model azo dye AR-27 and further application to treat Batik textile wastewater in glass fabricated microbial fuel cell with proton exchange membrane (nylon membrane). The main idea for the study was to characterize and identified four unknown strain on their ability to remove azo dye AR-27. Then, developing bacterial mixed culture that are capabale of degrading azo dye AR-27. The best combination of the mixed culture bacteria in removal and degrading azo dye AR-27 will be selected for further optimization on the physicochemical conditions. Then, the optimized combination will be applied into the synthetic wastewater under sequential facultative anaerobic-aerobic MFC system to assess the potential of electricity generation by the selected bacterial combination under optimized condition. Finally, the selected mixed bacterial culture was introduced to the real batik textile wastewater to evaluate the potential of electricity generations. This study could provide new idea for the current treatment of textile effluent and as an alternative green energy in the future.

1.4 Objectives

This study was performed to assess the potential of azo dye AR-27 removal and simultaneous electricity generation in microbial fuel cell by selected bacterial combination. Hence, these objectives were established in the research to achieve the research aim:

(a) To characterize and identify of the ability of pure bacterial strain to decolourise AR-27 dye

(b) To select and optimization of bacterial mixed culture capable of degrading azo dye AR-27

(c) To assess the potential of electricity generation by selected bacterial mixed culture under optimized conditions in a Microbial Fuel Cell.

1.5 Scopes

The main scope of this research was to assess the potential of azo dye AR-27 removal and simultaneous electricity generation in microbial fuel cell with nylon membrane by selected bacterial culture combination. Hence, these scope were established to accomplish the azo dye decolourization and the electricity generations.

This study characterized the capability of pure bacterial strain to remove azo dye AR-27 by using CDM medium supplemented with 1.0 g/L glycerol and AR-27 dye as the model dye under facultative anaerobic conditions. The identification was performed by using 16S rRNA Gene Sequence Analysis and biochemical test. Then, the phylogenetic tree was constructed using MEGA6 application.

For the development of combination from the pure bacterial strain, the compatibility test was conducted to study the antagonistic effect between the strains to grow as mixed culture without inhibiting each other. Then 7 type of combination was developed and investigation of the ability for azo dye removal and degradation in CDM medium supplemented with 1.0 g/L glycerol and AR-27 dye as the model dye under facultative anaerobic conditions. The selected combination was then optimized under several physicochemical such as effect of carbon and nitrogen source, effect of shaking vs static, effect of temperature, effect of initial dye concentrations and effect of pH. Next, the selected bacterial combination with optimized condition was inoculated into a fabricated microbial fuel cell with proton exchanges membrane (nylon membrane) to study the potential of electricity generations.

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