IDENTIFICATIONS OF LOCALLY ISOLATED REACTIVE BLACK 5 DECOLORIZING BACTERIA

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To my beloved mother, father and my dearest brother Dr. Hewa

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ABSTRACT

Seven bacterial strains were successfully isolated from textile wastewater. They were screened for their ability to degrade azo dyes including Reactive Black 5 and Acid Orange 7. The screening showed that the decolourization of Reactive Black 5 was best performed under facultative anaerobic conditions with color removal ranging from 80% - 93% for the three bacterial strains (L1, L6 and L7) at 37°C after 96 hours incubation. However, no decolorization of Acid Orange 7 was observed in all of the isolated bacteria under facultative anaerobic condition. The L1, L6 and L7 were further tested for decolorization of Reactive Black 5 and Acid Orange 7 under aerobic conditions. No decolourization was observed in all sets of experiments. Inducibility studies showed Reactive Black 5 did not show an inducing effect on decolourization process. Two parameters (pH and temperature) were optimized for decolorization. The strains were incubated in medium with pH 4, 7 or 10 and it was found that the optimum pH for decolourization was 7 with 49%, 63% and 70% of decolourization by bacterial strains of L1, L6 and L7, respectively, after 48 hours of incubation. When the strains were incubated at 30°C, 37°C and 55°C, it was found that the optimum temperature was 37°C, which the highest decolourization percentage in the tested strains ranged from 53% - 71% after 48 hours of incubation. The 16S ribosomal ribonucleic acid (16S rRNA) sequences analysis revealed that strains L1 and L6 belonged to genus *Bacillus* with 99% identity to 16S rRNA gene of *Bacillus thuringiensis*. Strain L7 was found belong to genus *Escherichia* with 99% identity to 16S rRNA gene of *Escherichia coli* strain SCDC-1.

ABSTRAK

Tujuh strain bakteria telah berjaya dipencil daripada air sisa tekstil. Semua strain bakteria ini disaring untuk menguji keupayaan mereka untuk mengurai pewarna azo seperti Reactive Black 5 dan Asid Orange 7. Keputusan eksperimen menunjukkan pelunturan warna Reactive Black 5 adalah terbaik dalam keadaan fakultatif anaerobik, dengan penyingkiran warna antara 80% - 93% untuk tiga strain bakteria (L1, L6 dan L7), pada suhu 37 ° C selepas 96 jam inkubasi. Walau bagaimanapun, tiada sebarang penyahwarnaan Asid Orange 7 diperhatikan bagi semua strain bakteria dalam keadaan fakultatif anaerobik. Bakteria strain L1, L6 dan L7 turut diuji untuk penyahwarnaan Reactive Black 5 dan Asid Orange 7 dalam keadaan aerobik. Namun tiada sebarang penyahwarnaan direkodkan dalam semua set eksperimen. Kajian Indusibiliti menyatakan Reaktif Hitam 5 tidak memberi sebarang kesan yang mendorong kepada proses pelunturan warna. Dua parameter (pH dan suhu) telah dioptimumkan untuk memperoleh keputusan penyahwarnaan yang lebih baik. Ketiga-tiga bakteria strain dikulturkan dalam medium dengan pH 4, 7 dan 10. Selepas 48 jam inkubasi, keputusan eksperimen mempamerkan pH optimum untuk penyembunyian warna adalah 7, dengan peratus penyahwarnaan sebanyak 49%, 63% dan 70% oleh strain bakteria L1, L6 dan L7. Apabila ketiga-tiga bakteria strain dikulturkan pada suhu 30 ° C, 37 ° C dan 55 ° C, keputusan menunjukkan suhu optimum bagi L1, L6 dan L7 adalah 37 ° C, di mana kadar pelunturan warna tertinggi yang tercatat dari 53%- 71% selepas 48 jam inkubasi. Analisis 16S ribosomal asid ribonukleik (16S rRNA) menunjukkan strain L1 dan L6 adalah genus *Bacillus*, dengan 99% persamaan kepada gen 16S rRNA *Bacillus thuringiensis*. Manakala strain L7 adalah genus *Escherichia*, dengan persamaan 99% kepada gen 16S rRNA *Escherichia coli* strain SCDC-1.

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LIST OF ABBREVIATIONS

%	-	Percent
°C	-	Degree Centigrade Celsius
λ	-	Wavelength
A	-	Absorbance
AO7	-	Acid orange 7
ADMI	-	American Dye Manufacturers Institute
BLAST	-	Basic local alignment search tool
BOD	-	Biological oxygen demand
A595	-	Absorbance at 595nm
C.I	-	Colour Index
CaCl2	-	Calcium chloride
CDM	-	Chemically defined medium
COD	-	Chemical oxygen demand
DNA	-	Deoxyribonucleic acid
EDTA	-	Ethylene diamine tetra acetic acid
g	-	Gram
H	-	Hour
HCl	-	Hydrochloric acid
Kb	-	Kilo base
K_2HPO_4	-	Potassium dichromate
KH_2PO_4	-	Potassium dihydrogen phosphate
$MgSO_4.7H_2O$	-	Magnesium sulphate heptahydrate
MEGA4	-	Molecular evolutionary genetics analysis software version 4
mg	-	miligram
Nm	-	Nanometer
µ L	-	Microlitre
mg/Ml	-	Miligram per mililiter
mg/L	-	Miligram per Liter
NCBI	-	National Center for Biotechnology Information
$(NH_4)_2SO_4$	-	Ammonium sulphate
NaCl	-	Sodium chloride
NAD	-	Nicotinamide adenine dinucleotide
NAD^+	-	Nicotinamide adenine dinucleotide(oxidized)
NADH	-	Nicotinamide adenine dinucleotide(reduced)
NADP	-	Nicotinamide adenine dinucleotide phosphate

NADPH	-	Nicotinamide adenine dinucleotide phosphate(reduced)
NaOH	-	Sodium Hydroxide
NA	-	Nutrient Agar
NB	-	Nutrient Broth
PCR	-	Polymerase chain reaction
L	-	Liter
rDNA	-	Ribosomal DNA
RNA	-	Ribonucleic acid
RB5	-	Reactive Black 5
RBB	-	Remazol Black B
rpm	-	Rotation per minute
rRNA	-	Ribosomal RNA
TAE	-	Tris-acetate buffer
Tris	-	2-hydroxymethyl-2-methyl-1,3-propanediol
v/v	-	Volume per volume
w/v	-	Weight per volume
UV	-	Ultraviolet

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CHAPTER 1

INTRODUCTION

1.1 Background of the Study

Nowadays, one of the most critical global issues is environmental pollution. Due to the quick economic development, the developing countries such as Malaysia, China, India and some other countries are facing the same pollution problem. Environmental pollution, particularly in water pollution, has caused a number of negative impacts to the environment. These include harmful algal blooms, eutrophication and contamination of groundwater, which is the fresh water resources for drinking (Wu *et al.*, 2012).

Wastewater contains a variety of pollutants such as toxic organics, dissolved solids, heavy metals, and color. The present of color in the waste water gives a basic indication of water being contaminated by dyes such astriarylmethane, anthraquinone and azo dyes. Dyes are chemical materials consist of chromophores, delocalized electron system with conjugated double bonds and auxochromes. The intensity of the dyes color can be altered by electron drawing or donating substances (Christie, 2001). It is estimated that almost 10^9 kg of dyes are produced annually in the world, of which azo dyes represent about 70% by weight (Zollinger, 1987). Textile-processing wastewaters, typically with dye content in the range 10-200 mgL^{-1} (O'Neill *et al.*, 1999), are usually highly coloured and when discharged in open waters presents an aesthetic problem. As dyes are designed to be chemically and photolytically stable, they are highly persistent in natural environments. The release of dyes may therefore present an ecotoxic hazard and introduces the potential danger of bioaccumulation that may eventually affect man by transport through the food chain.

Amongst these dyes, azo dye is mostly used in the industries (Stolz 2001). In general, azo dyes are xenobiotic that are very rigid against bio-degradative processes (Stolz 2001). These compounds are characterized by aromatic rings linked to one or more azo groups. The metabolic pathway of azo dye may result in free aromatic amines productions, which have been shown to be carcinogenic and mutagenic to human (Banat *et al.*, 1996).

Currently, there are different methods of textile wastewater treatment including physical and chemical process such as adsorption, membrane technologies, oxidation, and coagulation/flocculation. The advantages of these methods are that they are able to remove a wide range of dyes and rapid processes (Pearce *et al.*, 2003). On the other hands, these methods are costly and the accumulation of concentrated sludge may cause removal problems as well (Kamilaki, 2000; Pearce *et al.*, 2003). Biological treatment methods in general are more efficient and environmentally friendly (Pearce *et al.*, 2003). It has been reported that microorganisms are able to degrade azo dye efficiently under anoxic conditions and the intermediate products (amines) could be detoxified under aerobic environment (Grekova-Vasileva *et al.*, 2009). Under anaerobic condition bacteria secreted azoredutase enzyme, which could reductively cleavage the azo bond, yielding aromatic amines (Bibi *et al.*, 2012). The aromatic compounds were then degraded under aerobic condition (Stolz, 2001), to produced catechol compound and eventually to CO_2 , water and ammonia (Van der Zee and Villaverde, 2005).

1.2 Problem Statement

The release of highly colored dye effluents has caused serious environmental damages. Therefore, color elimination in wastewater is one of the main issues in the textile industries. It has to be removed before discharging into receiving water body (Khadijah *et al.*, 2009). Azo dyes are considered as a toxic and undesirable substance in wastewater and their intermediate productsare considered as a main cause of mutagenecity and carcinogenicity to living organisms. Hence, isolation and characterization of color removing bacteria is one of the important aspects as more competent isolated strains may enhance the efficiency of biodegradation of dyes.

1.3 Objectives of the Study

1- To isolate color removing bacteria from textile waste water

2- To optimize the decolorization process in a whole cell system

3- To identify the isolated bacteria using 16S rRNA analysis

1.4 Scope of the Study

The bacteria were first isolated from textile wastewater using serial dilution and spread plate method. They were screened for their ability to remove color in synthetic medium. The color removing bacteria were further characterized and the optimized pH and temperature were determined. In addition, the possible role of the azo dye as inducing agent for decolorization was also investigated. Finally, the color removing bacteria were identified using 16S rRNA analysis.

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