AZO DYES DECOLORIZATION BY BACTERIA ORIGINATED FROM TEXTILE WASTEWATER

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To my beloved mother Tuba Husssain,

my beloved wife Dlxwaz Mohammed and all my loved ones

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ABSTRACT

This study investigated the ability of previously isolated bacteria *Escherichia* sp., *Acinetobacter* sp., *Enterobacter* sp. and *Klebsiella* sp. in decolorizing Acid Orange 7 and Reactive Black 5. Screening results indicated the decolorization of Acid Orange 7 under facultative anaerobic condition was faster in comparison to Reactive Black 5. No decolorization of Acid Orange 7 and Reactive Black 5 was observed under aerobic conditions in the tested strains. Due to the reason that two of the strains (*Klebsiella* sp. and *Escherichia* sp.) were more potent decolorizers and these strains were able to remove Acid Orange 7 faster than Reactive Black 5, they were selected for further characterization on decolorization of Acid Orange 7. The effects of temperature, pH and concentration of nitrogen $(NH_4)_2SO_4$ on Acid Orange 7 decolorization were studied. For both of the strains, the optimum pH, temperature and $(NH_4)_2SO_4$ concentration were found to be at pH7, 37°C and 0.5g/L respectively. At pH 7, 93% and 75% of Acid Orange 7 was decolorized by *Klebsiella* sp. and *Escherichia* sp., respectively. No decolorization was detected at pH 4 or 10. At optimum temperature (37°C), *Klebsiella* sp. and *Escherichia* sp. were found to be able to decolorize 84% and 68% of Acid Orange 7, respectively. The decolorization percentage was detected to be highest in both strains (*Klebsiella* sp., 83% and *Escherichia* sp., 68%) when the cells were incubated in medium contained 0.5g/l of $(NH_4)_2SO_4$. Inducibility studies indicated Acid Orange 7 did not show an inducing effect on decolorization in *Klebsiella* sp. However, Acid Orange 7 was found to have a positive inducing effect on decolourization in *Esherichia* sp. It was observed that the *Esherichia* sp., which was pre-exposed to Acid Orange 7 displayed a higher percentage of decolorization compared with non pre-exposed cells.

ABSTRAK

Kajian ini menyiasat kebolehan bakteria yang telah dipencilkan sebelum ini iaitu *Escherichia* sp., *Acinetobacter* sp. , *Enterobacter* sp., dan *Klebsiella* sp., dalam penyahwarnaan ‘Acid Orange 7’ dan ‘Reactive Black 5’. Keputusan penyaringan menunjukkan penyahwarnaan oleh ‘Acid Orange 7 dengan kaedah anaerob fakultatif lebih cepat berbanding ‘Reactive Black 5’. Tiada penyahwarnaan berlaku oleh stren tersebut dalam ‘Acid Orange 7’ dan ‘Reactive Black 5’ dengan keadaan aerobik. Berdasarkan keputusan di mana dua strain (*Klebsiella* sp. dan *Escherichia* sp.) penyahwarnaannya lebih kuat dan strain ini berupaya untuk menyahwarna ‘Acid Orange 7’lebih cepat berbanding dengan ‘Reactive Black 5’, strain ini telah dipilih untuk pencirian seterusnya dalam penyahwarnaan ‘Acid Orange 7’. Kesan suhu, pH dan kepekatan nitrogen $(NH_4)_2SO_4$ ke atas penyahwarnaan ‘Acid Orange 7’ juga telah dikaji. Untuk kedua-dua strain, masing-masing menunjukkan pH yang optimum, suhu and kepekatan nitrogen $(NH_4)_2SO_4$ menunjukkan ialah 7, 37^0C dan 0.5 g/l. Pada pH 7,sebanyak 93% dan 75% ‘Acid Orange 7’ telah dinyahwarna oleh *Klebsiella* sp. dan *Escherichia* sp. Tiada penyahwarnaan berlaku pada pH 4 atau pH 10. Pada suhu yang optimum (37^0C), *Klebsiella* sp. dan *Escherichia* sp. masing-masing menunjukkan penyahwarnaan sebanyak 84% dan 68% oleh ‘Acid Orange 7’. Peratusan penyahwarnaan paling tinggi telah dikesan dari (*Klebsiella* sp., 83% dan *Escherichia* sp., 68%) apabila sel tersebut diinkubasi didalam medium yang mengandungi 0.5 g/l $(NH_4)_2SO_4$. Kajian pendorongan menunjukkan ‘Acid Orange 7’ tidak bertindak balas atas kesan dorongan dalam penyahwarnaan oleh *Klebsiella* sp. Walau bagaimanapun, ‘Acid Orange 7’ telah menunjukkan kesan dorongan yang positif dalam penyahwarnaan yang dilakukan oleh *Escherichia* sp. *Escherichia* sp.yang terdedah kepada ‘Acid Orange 7’ menunjukkan peratusan yang lebih tinggi berbanding sel yang tidak terdedah.

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LIST OF SYMBOLS

µm	-	Micro milliliter
°C	-	Celcius
G	-	Gram
L	-	Liter
mL	-	Milliliter
g/L	-	Gram per liter
Mg/L	-	Milligram per liter
nm	-	Nanometer
rpm	-	Rotation per minute
v/v	-	Volume per volume
w/v	-	Weight per volume
h	-	Hour

LIST OF ABBREVIATIONS

AO 7	-	Acid Orange 7
RB5	-	Reactive Black 5
NaOH	-	Sodium Chloride
HCl	-	Hydrochloric acid
K_2HPO_4	-	Dipotassium phosphate
KH_2PO_4	-	Mono potassium phosphate
$MgSO_4$ $7H_2O$	-	Magnesium Sulphate Heptahydrate
$CaCl_2$	-	calcium chloride
$(NH_4)_2$ SO_4	-	Ammonium sulphate
OD	-	Optical density
UV	-	Ultraviolet

CHAPTER 1

INTRODUCTION

1.1 Introduction

Water pollution is a global environmental problem. It occurs when harmful compounds, which include organic substances and heavy metals, are discharged from the industries into water bodies without proper treatments (Joshni *et al*., 2011). Among the pollutants, artificial dyes are one of them. The uses of artificial dyes in the textile industries have increased due to their ease and it is cheap to synthesis, high stability to temperature and has more variety in color compared with natural dyes (Ganem *et al.,* 2011)

Azo dyes constitute the largest class of synthetic dyes, which are aromatic compounds that contain one or more –N=N- groups (Pandy *et al.,* 2007). In dyes industries, it is recorded that 80% of total amount of dyes, which are produced annually, are composed of azo dye (Tripathi *et al*., 2011). It is found that azo dyes are toxic, highly colored and can cause water contamination (Zolliger, 1991; Ganghua *et al.,* 2011).

Azo dyes are considered to be organic pollutant (Jonstrup *et al.*, 2011). During the dyeing process, a significant amount of azo dyes cannot bind with the fiber (Forgacs *et al.*, 2004). Subsequently they affect the aquatic life. In addition, these dyes were found to have mutagenic and carcinogenic effect on human body and other organisms (Robinson *et al*., 2001). Therefore, the suitable treatment to remove this dye is very important.

Several physical-chemical methods such as photo catalytic degradation, filtration, and activated carbon could be used to eliminate the color from the waste water. The problems with these methods are that these methods caused the formation of some harmful side products, producing a lot of sludge and generally expensive (Tripathi *et al*., 2011). The use of microbes to biodegrade azo dye is a better alternative because this method is cheaper and less accumulation of sludge. Many of microorganisms have been found to have the ability to remove color. These included bacteria, fungi and yeasts (Robinson *et al.*, 2001).

1.3 Objectives of the study

Three objectives of this study were:

1. To screen the ability of the selected bacteria on decolorization of azo dyes.
2. To optimize the decolorization process in the selected bacteria’s whole cells, specifically in pH, temperature and concentration of nitrogen source.
3. To determine whether azo dye (substrate) can induce the decolorization process in the tested bacteria.

1.2 Scope of the study

This research focused on biological decolorization of azo dyes Acid Orange 7 and Reactive Black 5 using individual culture of bacteria (*Escherichia* sp., *Acinetobacter* sp. *Enterobacter* sp. and *Klebsiella* sp.), in order to obtain the best candidate that was able to remove color. The effect of three parameters (temperature, pH and nitrogen source $(NH_4)_2$ SO_4 concentration) on decolourization were investigated. Inducibility studies were carried out to determine if the azo dye (substrate) is the inducing agent for decolorization process in the tested bacteria.

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