DECOLOURISATION OF REACTIVE BLACK 5 USING LYSINIBACILLUS FUSIFORMIS ZB 2

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DECOLOURISATION OF REACTIVE BLACK 5 USING *Lysinibacillus fusiformis* ZB 2

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All praise to ALLAH the ALMIGHTY for His bless and guidance that had helped me in completing this project…

And also to my beloved family especially my father (Yousif Ahmed), my mother (Saadyah Rahman), and my wife (Banan Mohammed)

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ABSTRACT

The textile industries cause various problems related to the textile wastewater effluent, including that of discharge and treatment of dyes. The majority of the commonly used dyestuffs which accounted for nearly 50% of the dyes are azo dyes. In this research, the effects of three parameters including pH, temperature, and dye concentration were tested to optimise the decolourisation of azo dye Reactive Black 5 (RB 5) using pure culture of *Lysinibacilus fusiformis* ZB 2 in a sequential facultative anaerobic-aerobic condition. Glucose and yeast extract were used in the medium as co-substrates. The results achieved indicated that Reactive Black 5 decolourisation was most effective at pH 9, 37°C, and 100 ppm RB 5. Optimised decolourisation of 95.5% was achieved at 24 hours of the treatment process. In addition, the COD removal was 33.2% after 24 hours of incubation under the aerobic condition. In conclusion, *Lysinibacillus fusiformis* ZB 2 showed significant potential in decolourising the RB 5 and could be used as a candidate for biological treatment of coloured textile wastewater.

ABSTRAK

Industri tekstil menyebabkan pelbagai masalah yang berkaitan dengan effluen air sisa buangan tekstil, temasuk pelepasan dan rawatan pewarna. Hampir 50% bahan pewarna yang digunakan adalah pewarna azo. Dalam kajian ini, kesan tiga parameter iaitu pH, suhu dan kepekatan pewarna telah diuji bagi mengoptimumkan proses penyahwarnaan oleh pewarna azo "Reactive Black 5" (RB 5) menggunakan kultur tulen *Lysinibacillus fusiformis* ZB 2 dalam satu urutan keadaan fakultatif anaerobik-aerobik. Ekstrak glukosa dan yis digunakan dalam medium sebagai ko-substrat. Keputusan menunjukkan bahawa penyahwarnaan RB 5 adalah paling berkesan pada pH 9, 37 °C dan 100 ppm. Penyahwarnaan optimum pada 95.5% dicapai selepas 24 jam proses rawatan. Di samping itu, penyingkiran COD adalah 32.3% selepas tempoh pengeraman di bawah keadaan aerobik selama 24 jam. Sebagai kesimpulan, *Lysinibacillus fusiformis* ZB 2 menunjukkan potensi yang penting dalam penyahwarnaan RB 5 dan boleh digunapakai sebagai bahan dalam rawatan biologi bagi sisa air warna tekstil.

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LIST OF SYMBOLS AND ABBREVIATIONS

A_{597}	-	Absorbance at 597nm
A_{600}	-	Absorbance at 600 nm
COD	-	Chemical Oxygen Demand
EMIM-$MeSO_3$	-	1-ethyl-3-methylimidazolium methanesulfonate
et al.	-	and friends
g/L	-	Gram per liter
IL	-	Ionic liquid
M	-	Molarity
mg/mL	-	Milligram per milliliter
MW	-	Molecular Weight
NB	-	Nutrient Broth
Nm	-	Nanometer
ppm	-	Part per million
RB5	-	Reactive Black 5
rpm	-	Revolutions per minute
v/v	-	Volume per volume
w/v	-	Weight per volume
µL	-	Microlitre

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CHAPTER 1

INTRODUCTION

1.1 Background Study

Some decades ago, pollution of water has become a major problem to the human society. Approximately 280,000 tonnes of various dye types have been discharged to the environment yearly (Jin *et al*., 2007). The main problems and concerns of wastewater containing detergents, bleaches, organic material, and azo dyes are the pollution of hazardous compounds, for example biocides and toxic heavy metals such as Zn, Cu, Pb and Fe (Jadhav *et al*., 2010). Moreover, textile wastewater is a complex mixture of colorants (pigments and dyes) and different organic compounds. It also contains high concentrations of heavy metals, total dissolved solids and has higher chemical as well as biological oxygen demand. So, textile wastewater is chemically much complex in nature (Sharma *et al.*, 2007).

Several physico-chemical techniques such as photo catalytic filtration, degradation, and activated carbon can be used to remove the colour from the waste water. The disadvantages of these techniques include the formation of toxic sludges, which needed further disposal (Tripathi *et al*., 2011), high in operating cost (Prasade *et al*., 2010) and energy consuming (Hosseini *et al*., 2011). One of main benefits of using dye-eliminating microbes to decolourise azo dyes is that it is a low cost technique (Mohahmed, 2013). In addition, it minimises the toxic compounds contained in the wastewater effluent via mineralization (Forgacs *et al*., 2004). Furthermore, the use of microbes to biodegrade azo dye is a better alternative because this technique is environmental friendly and there is less accumulation of sludge. Many of the microorganisms have been found to have the capability to eliminate colour such as azo dye. These microbes include yeasts, fungi, and bacteria (Robinson *et al.*, 2001 and Mohammed, 2013). Therefore, many researches have been done on microorganisms which have the decolourisation ability such as *Enterobacter agglomerans* (Moutaouakkil *et al*., 2003), *Pseudomonas* KF46 (Zimmermann *et al*., 1982), *Micrococcus* strain (Olukanni *et al*., 2009), *Staphylococcus aureus* (Chen *et al*., 2005). Fungi also have the decolourisation ability, one such example is using *Issatchenkia occidentalis* which is used for decolourisation of methyl orange and orange II (Ramalho *et al*., 2004). In some researches, mixed bacterial culture is more preferable than the pure bacterial culture as it has higher co-metabolic activities within a microbial community. However, the ability of pure bacterial culture in treatment of azo dyes is much easier to be observed and studied in terms of its specific activity.

Dye toxicities vary because of the structure of azo dyes and are related to the types of intermediate used in their synthesis. After the azo bonds are cleaved, aromatic amine compounds are produced, which is more toxic and mobile compounds than the compounds of original azo dyes. (Hildenbrand *et al*., 1999).

Among all of different industrial wastewater, the textile industries use large quantities of water in its process and the wastewater is discarded into the environment. In view of the toxicity of untreated effluents, treatment of the wastewater is crucial before the effluents are discharged to our environments.

This study focused on the biological decolourisation and degradation of azo dye Reactive Black 5 (RB 5), due to its high solubility in aquatic environment, and not easily decolourised via traditional techniques for example, physical adsorption and flocculation-coagulation (Fahmi *et al*., 2010). Another reason is because Reactive Black 5 is also known as one of the most recalcitrant dyes (Elisangela *et al*., 2009).

1.2 Statement of Problem

Many microbes have been reported to degrade azo dye, and a number of bacteria tolerant towards higher temperature and fluctuations of pH (Maier *et. al*, 2004). Hence some factors such as temperature, pH, and concentration of dye were optimised to improve the decolourisation and degradation of RB 5 by *Lysinibacillus fusiformis* ZB 2. When suitable parameters have been identified, it is hoped that it can increase the bacterial activity responsible in the treatment of the dye and furthermore increase the decolourisation percentage.

1.3 Research Objectives

The two major objectives of this study were as follows:

1. To observe the determine decolourisation of RB 5 using *Lysinibacillus fusiformis* ZB 2.

2. To optimise the decolourisation of the *Lysinibacillus fusiformis* ZB 2 at different pH, temperature and concentration of the dye.

1.4 Scope of Research

This project was mainly focused on biodecolourisation and biodegradation of azo dyes Reactive Black 5 using individual culture of *Lysinibacillus fusiformis* ZB 2. This bacterium was being used for treatment of azo dye in a sequential facultative anaerobic and aerobic system. The effect of three parameters such as temperature, pH and concentration of dye on decolorisation were investigated to determine the best condition for colour removal.

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