Comparison between the biodecolorization of Reactive Dye by Single & Mix-Bacterial Strains

Jannatul Ferdous Rumky, Hafizur Rahman, M.Ali Hossain

Abstract— In the present study, an attempt was made to examine the potential of different bacterial strains (E.coli, Enterobacter & mix-bacterial strain) for decolorization of Sunfix Red (reactive dye), in static system . The effect of initial dye concentration, pH and static-rotating conditions were studied to measure the optimal conditions of degradation. Here, the bacterial mediums used in this study were E.coli, Enterobacter and mix-bacterial of them. Out of these, maxmimum decolorization emergerd out to be the most potent decolorizer among all. These bacterial strains showed higher decolorization in static medium in comparison with rotating condition. The optimum pH for decolorization of sunfix red by E.coli, Enterobacter & mix-bacterial strains were 8, 9, and 9. The optimum dye concentrations for these ones were 700,500 & 700 ppm. The optimum temperature was 37°C and the strain would decolorize 90-97% within 48 hours under the optimum conditions and the initial dye concentration was 300 ppm. The results showed that, the mix-bacterial strain has more good potential than single bacterial strain in removal of reactives dye from wastewater.

Index Terms— Bacterial strain, concentration, decolorization, E.coli, Enterobacter, pH, static, sunfix red.

1 Introduction

Textile is the most significant sector of Bangladesh's economy. Textile industry uses large amount of water in its manufacture processes and highly polluted and toxic waste waters are discharged into sewers and drains without any kind of treatment. The textile dyeing industries of Bangladesh generate large amount of effluents, sewage sludge and solid waste materials everyday which are being directly discharged into the neighboring channel, rural fields, irrigation channels, surface water and these finally enter in to rivers. Textile and dyeing industrial effluents may cause variation of the physical, chemical, and biological properties of aquatic environment by continuous change in temperature, odor, noise, turbidity etc that is harmful to public health, livestock, wildlife, fish, and other biodiversity. The presence of dyes in surface and subsurface water is making them not only aesthetically ojectionable but also causes many water borne diseases. [1]

Although several physical-chemical methods have been used to eliminate the colored effluents in wastewater, they are generally expensive, produce large amounts of sludge. More often these conventional modes of treatment lead to the formation of some harmful side products. Interest is therefore now focused on the microbial biodegradation of dyes as a better alternative. Some microorganisms, including bacteria, fungi and algae, can degrade or absorb a wide range of dyes .The biological mode of treatment of dye bath effluents offers distinct advantages over the conventional modes of treatment. [2]

This method is more economical and leads to less accumulation of relatively harmless sludge. Most importantly, biological treatment of dye bath effluents is ecofriendly. It causes mineralion of dyes to simpler inorganic compounds which are not lethal to life forms. In view of these problems the most potent bacterial culture was selected in this study for maximum decolorization of Sunfix Red (reactive dye) [3], being selected as model azo dye.

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2 Materials & Methods

2.1 Stock Solution Preparation

Sunfix red supplied by MERK (Germany) was used & 1000 ppm stock solution was prepared by dissolving 1000 mg of it in 1000 ml of distilled water and the required solutions were prepared by suitably dilution of it.

2.2 Culture Medium Preparation

Bacterial culture medium was made by adding specific amount of nutrient agar into distilled water. Next the solution was autoclaved at 121°C for 30 minutes and cooled down up to 60°C. The solution was put on a petridish for solidification. Finally bacterial culture medium was inoculated & kept them in incubator at 37°C for bacterial growth. The culture medium was preserved in refrigerator for further use.

2.3 Growth Curve Study

5ml bacterial suspension of each inoculum was inoculated in 250 mL nutrient broth flasks containing 300 mg/L dye solution incubated at 37□C to study the growth curve of E.coli, Enterobacter & mix-bacterial strains. Growth curve studied in nutrient broth with or without dyes to compare the death phase of culture medium. Lack of glucose inhibited the decolorizing activity of single & mix bacterial (E.coli & Enterobacter) strain.

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2.4 Decolorization by Mix-Bacterial Strains

The bacterial cultures were transferred to fresh nutrient medium containing sunfix red (300 mg/l) and were incubated at 37°C, under static condition for 3 days. Between this 3 day period, aliquots (5ml) of the culture media were withdrawn, centrifuged at 10,000 rpm for 10 minutes at room temperature to separate the bacterial cell mass. The supernatant was used for analysis of decolonization and all the experiments were repeated.

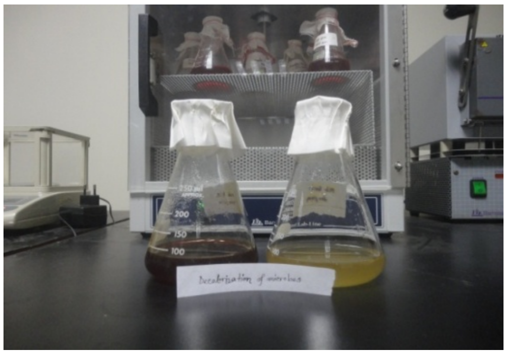

Fig 1: Sunfix Red decolorization by bacterial strains

2.5 Effect of p^H, Initial Dye Concentraion

In an attempt to study the effect of static and shaking (100 rpm) condition, the selected, most potent decolorizing bacterial culture, was cultivated for 72 hrs in nutrient broth and amended separately with 300 mg/l of sunfix red. This inspection was performed for the detection of decolorizing activity of bacteria & find out that static medium was more useful than rotating condition. So, static condition was selected for further experiments.

To determine the effect of pH on decolorization, the fully-grown culture was inoculated in conical flasks containing 100 ml nutrient broth of varying pH (6-9) and was amended with 300 mg/l of sunfix red. The pH values were adjusted using 1N NaOH and 1M HCl. The result of this study can be concluded that mix-bacteria (E.coli & Enterobacter) could be used effectively for the removal of sunfix red from aqueous solution. The adsorption capacity was found to be high at higher pH range. For concentration difference study, media was amended with the dye sunfix red at a concentration of 100, 300, 500, 700 and 900 mg/l separately to study the effect of increasing dye concentration on percentage dye decolorization & finally calculated the results.

The dye concentrations were measured with a UV/VIS spectrophotometer [4] at regular intervals during the decolorization process. The concentration of reactive dye was detected spectrophotometrically by reading the culture supernatant after a certain period of time and calculates the % of decolorization by using below equation 1.

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100 \% \ldots (1)$$

3. Results & Discussion

3.1 Calibration curve of Sunfix Red

The Absorbance vs. Concentration of Sunfix Red dye solution is presented in figure - 2

From the above plot, it is seen that the absorbance vs. concentration curve is a straight line passing through the origin. The correlation coefficient (R2) of the line is 0.999. The equation of the line is the calibration equation and is used to determine the unknown concentrations of the solutions from absorbance.

$$\text{Abs.} = 0.003 \times \text{Conc} \ldots\ldots (2)$$

From the plot, the calibration equation is

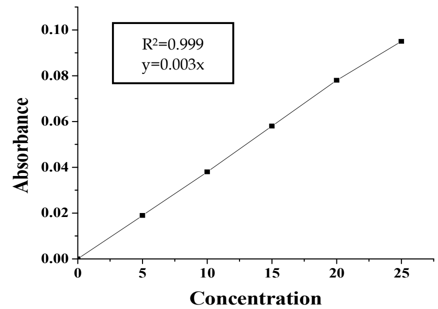

Fig 2: Calibration Curve of Sunfix Red

3.2 Growth Curve Study

From the above graphs, for E.coli bacteria, the death phase started after 65 hrs in presence of dye in comparison with non-dye solution started death phase after 47 hrs. For Enterobacter bacteria, the death phase started after 63 hrs in presence of dye in comparison with 55 hrs of nutrient broth without dye solution. For mix-bacterial strains, the death phase started after 64 hrs in presence of dye in comparison with 50 hrs of nutrient broth without dye solution.

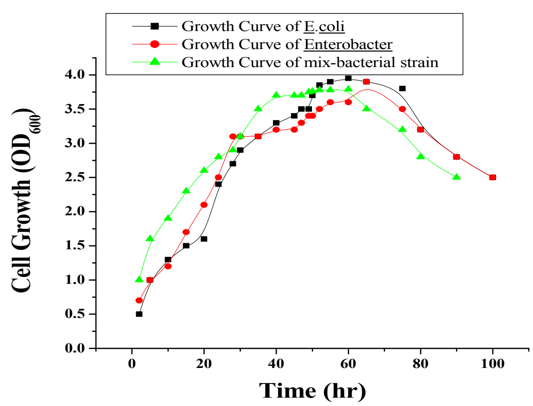

Fig 3: Growth Curve Study (Bacterial culture with dye solution, dye conc. 300 ppm, aliquots of bacterial culture 5ml, time 100 hrs)

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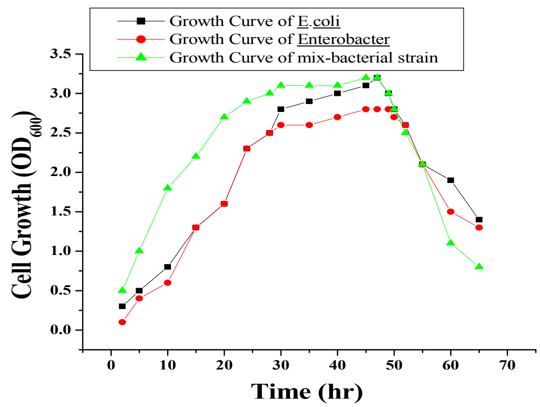

Fig 4: Growth Curve Study (Bacterial culture without dye solution, dye conc. 300 ppm, aliquots of bacterial culture 5ml, time 70 hrs)

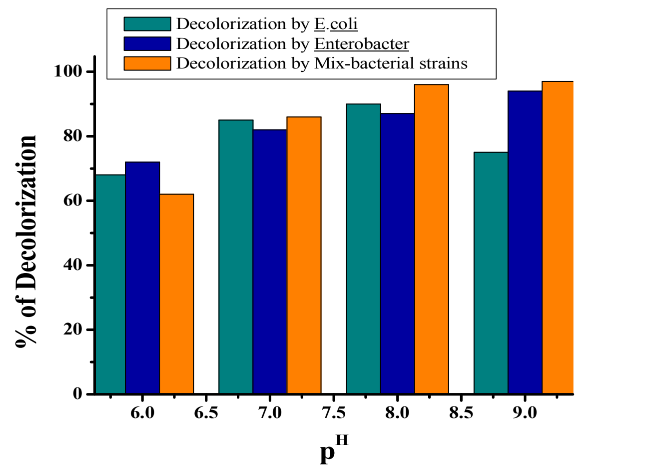

Fig 5: Decolorization at different pH (dye conc. 300 ppm, aliquots of bacterial culture 5ml/100ml, time 72 hrs)

Two opinions have been argued for many years: one deems that dyes are not a carbon source since the anaerobic bacteria obtain energy from the glucose instead of the dyes and glucose can enhance the decolorizing performance of biological systems. [5] While another deems that glucose can inhibit the decolorizing activity. The variability may be due to the different microbial characteristics involved. Our results showed that a certain concentration of carbon source was necessary for all bacterial culture medium.

3.3 Effect of pH & initial dye concentration

The best results were achieved at pH 8.0, 9.0, 9.0 for sunfix red by E.coli, Enterobacter & mix-bacterial strain bacteria with 90%, 94% & 97 % decolorization in 72 h. This could be due to the fact that the optimum pH for the growth of E.coli was neutral or slightly alkaline. The rate of color removal was much lower at strongly acidic medium. pH has a major effect on the efficiency of dye decolorization, and the optimal pH for color removal is often between 6.0 and 10.0. The pH tolerance of decolorizing bacteria is quite important because of binding to different fibers by addition or substitution mechanisms depend on acid-alkaline conditions. The fact that E.coli, Enterobacter, mix-bacterial strains might decolorize reactive dyes in a relatively wide range of pH, make them suitable for practical bio-treatment of dyeing mill effluents.

For concentration difference study, media was amended with the dye sunfix red at a concentration of 100, 300, 500, 700 and 900 mg/l separately to study the effect of increasing dye concentration on percentage dye decolorization & finally calculated the results. Here, 700mg/l, 500 mg/l & 700mg/l showed the best end results for E.coli, Enterobacter & mix-bacterial strain.

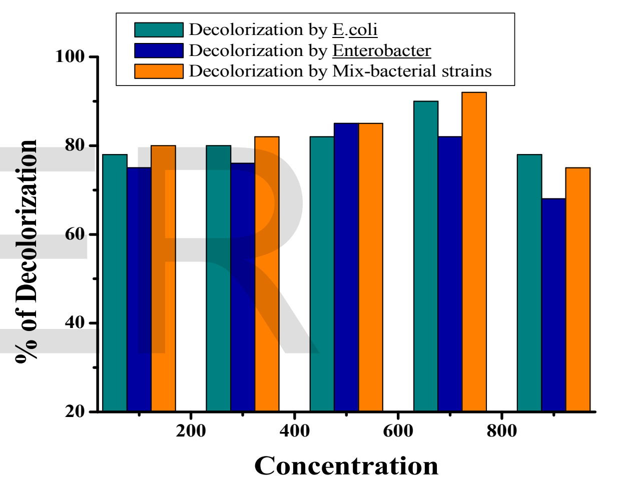

Fig 6: Decolorization at different conc. (pH 7.0, aliquots of bacterial culture 5ml/100ml, time 72 hrs)

3.4 Evaluation between the results

TABLE 1

pH range	Decolorization by E.coli	Decolorization by Enterobacter	Decolorization by Mix-actreial strain (E.coli & Enterobacter	Most efficient result
6	68	72	74	**97% removal by mix-bacterial strain at pH 9**
7	85	82	85	
8	90	87	95	
9	75	94	97	

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TABLE 2

Initial Conc. of dye	Decolorization by E.coli	Decolorization by Enterobacter	Decolorization by Mix-actreial strain (E.coli & Enterobacter	Most efficient result
100	78	75	81	**93% removal by mix-bacterial strain at dye conc.700 ppm**
300	81	76	83	
500	82	85	85	
700	91	82	93	
900	78	68	75	

4 Conclusion

In this study work, sunfix red was decolorized and degraded by using biological degradation. This clearly indicates that decolorization was due to degradation of dyes into intermediate products. In biological degradation, E.coli, Enterobacter and mix-bacterial strain degraded 90%, 94% & 97% from sunfix red solution. So that, the ability of the bacterial strains to tolerate, decolorizes and degrade dyes at high concentration & pH gives it an advantage for treatment of textile industry wastewater.

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