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# Environmental Treatment of Dyes: Methyl Orange Decolorization Using Hog Plum Peel and Mix-Bacterial Strains

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***Abstract :*** *The discharge of azo dye in to environment is of great alarmed due to color, toxicity, mutagenicity and carcinogenicity of the dye. Considerable attention has been given in evaluating the capability of bioadsorbent & a mix bacterial strain in decolorization and degradation of azo dye. Hog plum peel decolorizes methyl orange dye about 92% at $P^H$ 6.0, initial dye concentration 20 mg/L, adsorbent dose 0.5 g/100ml & 60 min contact time. Bacterial strains (E.coli & Enterobacter) showed a strong ability to decolorize methyl orange, azo dye. Aerobic conditions with pH 8.0, initial dye concentration 500 ppm and 37$^0$C were considered to be the optimum decolorizing conditions under static state and decolorize about 96% of dye. High decolorization level and simplistic conditions show the potential for this bioadsorbent &bacterial strain to be used in the physical & biological treatment of dyeing mill effluents.*

***Keywords:*** *Carcinogen, Decolorization, E.coli, Enterobacter, Hog plum peel.*

## I. INTRODUCTION

In the modern world, the environmental pollution has been recognized as one of the major problems. The increasing demand for water and declining supply has made the treatment and recycle of industrial effluents an attractive option. Color is one of the most important environmental pollution problems in water courses, although some of this color is usually present. Some dyed effluents are related with the manufacture and use of dyestuff. Azo dyes, the major chemical class of dyes with the maximum variety of colors, have been used widely for textile, dyeing and paper painting. These dyes cannot be easily degraded. Several combinations of treatment methods have been developed so far for efficiently process textile wastewater; decolorization being among the main targets to achieve. They are environmental friendly techniques since they produce no solid wastes. [1] Adsorption techniques have newly gained a considerable significance due to their efficiency in the removal of pollutants too stable for conventional methods.

Decolorization of wastewater by this process is influenced by many factors such as sorbent surface area, particle size, contact time, temperature, pH and presence of salts, surfactants and metals. It must be emphasized, however, that sorption processes simply transfers pollutants from one phase to another and therefore habitually generate sludge that must be disposed of, or regenerated, by some other process. [2]

Biological treatment is a more natural wastewater treatment process than other wastewater treatment methods. Microorganisms feed on the complex materials present in the wastewater and turn them into simpler substances, preparing the water for further treatment. Biodegradation is the chemical dissolution of materials by bacteria or other biological means. Although often conflated, biodegradable is distinct in meaning from compostable. While biodegradable simply means to be consumed by microorganisms and return to compounds found in nature, "compostable" makes the specific demand that the object break down in a compost pile. The term is often used in relation to ecology, waste management, biomedicine and the natural environment (bioremediation) and is now commonly associated with environmentally friendly products that are capable of decomposing back into natural elements. [3]

## II. MATERIALS & METHODS

### 2.1 Preparation of bioadsorbent & bacterial culture

The peel of **Hog Plum** (Aamra) used in this experiment was collected from local area of Dhaka City. The peel was washed several times with distilled water to remove the surface adhered dust and unwanted contaminants and then boiled in distilled water to remove coloring constituents with changing the water until the water became colorless. Then it was dried in an air oven at 90±5 $^O$C until almost all the moisture evaporated and peel became crispy. It was ground by a blender and treated with dilute acetic acid &conc. hydrochloric acid to compare the removal efficiency of both treated Hog Plum peel.

Bacterial culture medium was made by adding specific amount of nutrient agar into distilled water. Next the solution was autoclaved at 121ºC for 30 minutes and cooled down up to 60 ºC. The solution was put

on a petridish for solidification. Finally bacterial culture medium was inoculated & kept in incubator at 37ºC for bacterial growth. The culture medium was preserved in refrigerator for further use.

### 2.2 Preparation of stock solution

Methyl Orange (azo dye) supplied by MERK (Germany) were used & A 1000 ppm stock solution was prepared by dissolving 1000 mg of it in 1000 ml of distilled water and the required solutions are prepared by suitably dilution of it. The structure of this dye is shown in Fig.1

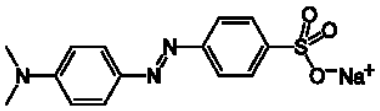

Figure 1: Structure of methyl orange

### 2.3 Decolorization by bioadsorbent: Adsorption Studies

The adsorption experiments were carried out on batch process. The batch processes were carried out in 250 ml conical flasks by mixing pre-weighted amount of adsorbent and 100 ml of dye solution of desired concentration. The flasks were kept in a shaker at 450 rpm oscillation at room temperature 25±2 $^{O}$C for predetermined time interval at a constant temperature.

The parameters such as pH, time of contact, adsorbent amount and dye concentration were varied during the different sets of batch experiments. At fixed time intervals, concentration of methyl orange was analyzed using a UV-VIS Spectrophotometer [4] at λmax = 470 nm. pH of the solution was adjusted before addition of adsorbent using 0.1 N HCl and 0.1 M NaOH. Amount of dye uptake, q (mg/g), was calculated using the following equation 1:

$$q = \frac{(C_i - C_f)V}{100.W} \dots\dots\dots\dots\dots (1)$$

Where

$C_i$ (mg/l) is the initial dye concentration, $C_f$ (mg/l) is the dye concentration after adsorption, W (g) is the amount of bioadsorbent and V (ml) is the volume of the solution.

### 2.4 Decolorization by mix-bacterial strains:

The bacterial cultures were transferred to fresh nutrient medium containing methyl orange (250 mg/l) and were incubated at 37ºC, 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.

## III. RESULTS & DISCUSSION

### 3.1 Isotherm Study:

The resulted model parameters of Langmuir [5] is plotted for different initial dye concentration with constant adsorbent showed a straight line loading in Fig.: 2

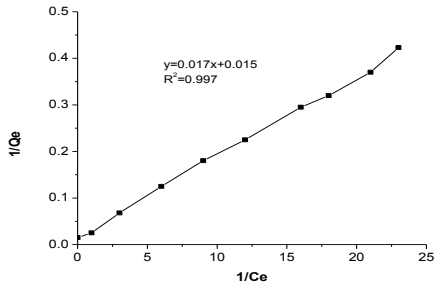


Figure 2: Langmuir isotherm model of hog plum peel

The $R^2$ value suggests that the Langmuir isotherm provides a good model of sorption. Finally, hog plum peel suggested as a perfect adsorbent for any type of adsorption.

### 3.2 Effect of adsorbent dose, contact time, initial dye conc. & pH :

The effects of adsorbent dose, contact time, initial de concentration & pH were studied and the results are showed in the below figures.

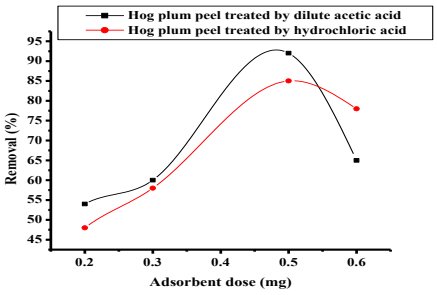


Figure 3: Effect of adsorbent dose (methyl orange removal by hog plum peel, dye conc.20mg/L, pH 6, agitation time 100 min)

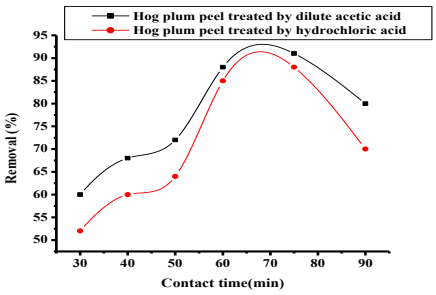


Figure 4: Effect of contact time (methyl orange removal by hog plum peel, dye conc. 20 mg/L, 0.5g hog plum peel/100ml and pH 6)

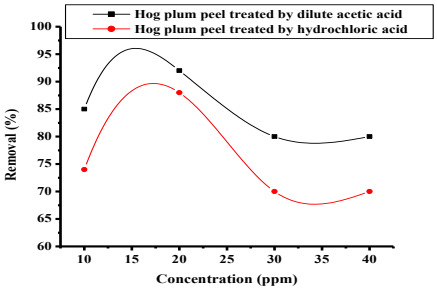


Figure 5: Effect of initial dye concentration (methyl orange removal by hog plum peel: 0.5g hog plum peel/100ml, time 100 min and pH 6)

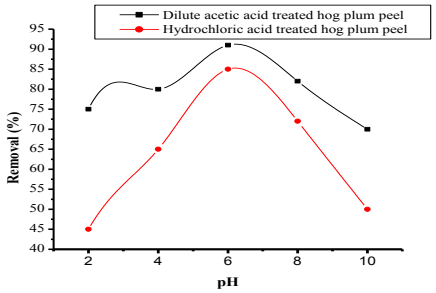


Figure 6: Effect of pH (methyl orange removal by hog plum peel, dye conc. 20mg/L, 0.5g hog plum peel/100ml, time 100min)

It is seen that a large number of vacant surface sites are available for the adsorption during the initial stage and with the passage of time, the remaining vacant surface sites are difficult to be occupied due to repulsive forces between the solute molecules on the solid phase and in the bulk liquid phase. So that, after a certain period of time, the adsorbent reached to its equilibrium condition and we find out the optimum condition.

### 3.3 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 $37^0$C to study the growth curve of 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 mix bacterial (E.coli & Enterobacter) strains.

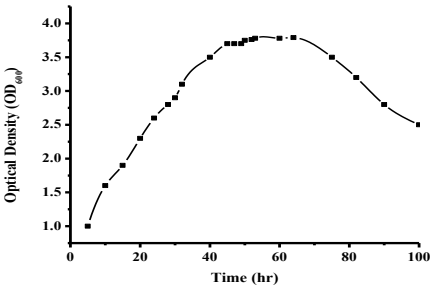


Figure 7: Growth curve (mix-bacterial strains, medium containing nutrient broth with dye)

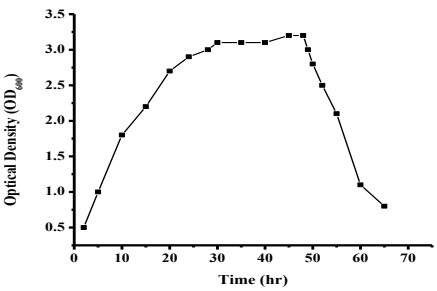


Figure 8: Growth curve (mix-bacterial strains, medium containing nutrient broth without dye)

For mix-bacterial strains, the death phase started after 68 hrs in presence of dye in comparison with 50 hrs of nutrient broth without dye solution. Availability of carbon source helps bacterial strains to live more in presence of dye in the solution.

**3.4 Effect of pH & initial concentration of dye on bacterial decolorization:**

To determine the effect of pH on decolorization, the culture was inoculated in conical flasks containing 100 ml nutrient broth of varying pH (6-9) and was amended with 300 mg/l of Methyl Orange. 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 Methyl Orange from aqueous solution. The adsorption capacity was found to be high at pH 9. Media was amended with the dye Methyl Orange at a concentration of 300, 500, 700 and 900 mg/l separately to study the effect of increasing dye concentration on percentage dye decolorization & finally 500mg/l showed the best end result.

By using below equation 2, we calculated the final decolorization rate after filtering the solution.

$$\% \text{ Decolorization} = \frac{\text{Initial absorbance value} - \text{final absorbance value}}{\text{Initial absorbance value}} \times 100\ \% \ldots\ldots\ldots (2)$$

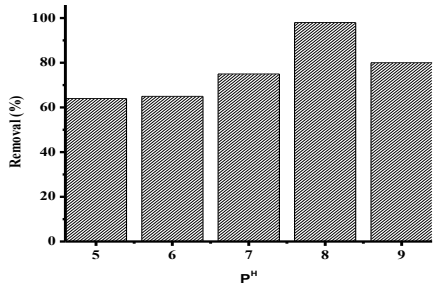


Figure 9: Effect of pH on the dye decolorization efficiency of mix bacteria (E.Coli & Enterobacter) on Methyl Orange was studied for 48 h in nutrient medium containing 300 mg/l dye at 37$^0$C and under static conditions.

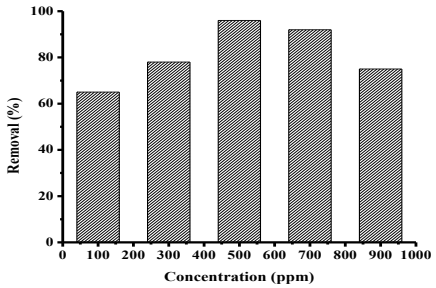


Figure 10: Effect of increasing dye concentration on the Percentage Decolorization of Methyl Orange by mix bacteria *(*E.Coli & Enterobacter*)* was studied for 48 h in nutrient medium containing different dye concentration, at 37ºC and pH 8.0 and under static conditions.

## IV. CONCLUSION

From the result of this study it can be concluded that hog plum peel and, mix bacterial strains (E.coli & Enterobacter) could be used effectively for the removal of methyl orange from aqueous solution. The adsorption capacity was found to be higher at pH 6, 20mg/l dye conc. within 60 min timing of physical adsorption. Beside that the removal percentage of dye was increased when the adsorbent was increased & finally 0.5g was the most favorable measurement. Finally the decolorization of methyl orange by mix bacterial strains showed excellent result at pH 8 & 300mg/l concentrated dye solution under static condition, so that, the ability of the adsorbent & strains to tolerate, decolorize and degrade dyes at high concentration.

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