

BIO-DEGRADATION AND DE-COLORIZATION OF REACTIVE ORANGE ME2RL DYE BY MIXED BACTERIAL CULTURE ISOLATED FROM MUNICIPAL WASTEWATER

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ABSTRACT: Toxic pollutant materials which is created by reactive dye is a worldwide problem due to increasing demand of these dyes as coloring agents in the most of industries such as textile, paint, ink, food, cosmetics, pharmaceutical, leather, paper, photographic and aquaculture. A Reactive Orange ME2RL dye which is used in textile industries causes serious environmental and health problems and shows lethal effects like genotoxicity, mutagenicity and carcinogenicity. This study examines the bio-degradation and de-colorization of Reactive Orange ME2RL dye by mixed bacterial culture isolated from municipal wastewater. A Reactive Orange ME2RL of concentration 150ppm has to be degraded by using mixed culture was found upto 83% with pH 7 and time 72 hrs with the help of co-substrate Glucose (0.1% w/v), Yeast extract (0.3% w/v). Biodegradation method showed satisfactory result that is efficient and cost effective for treatment of dyes de-colorization can be achieved by employing a mixed culture

Keywords: Pollution, Azo dye, Biodegradation, Effluents, Mixed culture

I. INTRODUCTION

Make use of dyes is behind the times as before as 2600 B.C. in China where colors were used in many industries like textiles., but textile industries one of the many industries add a multitude of chemical products like organic surfactants, dispersants, alkalis, solvent, acids, salts and residual dyes which causes water pollution [1]. Many several technologies such as physical and chemical like adsorption, ion exchange, and membrane filtration, ozonation, photooxidation and reverse osmosis is using by these industries for treatment of industrial effluents which are released from these industries but these effluent treatment methods are expensive and generate a secondary disposal problem after it. But as a cost point of view they are very costly and producing massive chemical sludge and imperfect removal of dyes [2]. Due to these demerits, bioremediation can play a major role and to be the best alternative of these physico-chemical methods because bioremediation processes are environment friendly and can formulate complete mineralization of organic pollutants like dyes at low cost. In the textile dyeing industry a huge amount of fresh water consumes for dyeing process and discharge equally large amount of colored effluent. In worldwide annually 70,0000 tones dyes are manufactured [3] in which 60% are azo dyes [4] from which 10 to 15% dyes are released in the water bodies and generate severe aesthetic

problems as a waste water which disturbing the photosynthetic reaction due to light penetration [5, 6]. Synthetic or azo dyes contain aromatic rings that is linked together by azo (-N=N-) bond and show the major group of dyes used in these textile industries [7].

At very low concentration of reactive dyes such as 1 mg L⁻¹ is visible in wastewater [8]. Bio-degradation and de-colorization of the effluents which containing the synthetic azo dyes can be degraded with the help of powerful microbes (like bacteria, fungi, algae, yeast) because they have the ability to treat various poisonous and xenobiotic azo dyes [9-11]. Among these microorganisms, bacteria are one of the most efficient players which play a major role for various bioremediation processes like bio-degradation and de-colorization of effluents containing of azo dyes. Wide range of studies has been concluded that bacteria have the potential of decolorizing a range of structurally different azo dyes [12-14]. De-colorization of azo dyes by bacterial that is containing two steps procedure be based on of a sequential anaerobic and aerobic process together with breakdown of -N=N- bond firstly followed by degradation of the dye. Decreasing of an azo dye by an anaerobic process is found to be favorable but it produces aromatic amines which are very toxic, carcinogenic and mutagenic to environment [15] and create dangerous problem.

An aerobic degradation and de-colorization of azo dyes can be found in very few reports [16-18]. Synthetic dyes wastewater effluents are containing high levels of alkalinity and salinity, hence, the microbial consortium which is able to sustain the rigorous conditions will be suitable for biodegradation. In this study we have focused on the de-colorization capability of a mixed culture to decolorize monoazo dye Reactive Orange ME2RL in static culture conditions.

II. MATERIALS AND METHODS

2.1 Dyes and Chemicals

The Reactive Orange ME2RL textile dye that is used mostly for dyeing purpose was selected for this study was obtained from textile industries, Ankleshwar, Gujarat.

2.2 Medium

The media which was used Bushnell and Haas medium (BHM) (HiMedia) having the following composition in gl⁻¹: Magnesium sulfate (MgSO₄), 0.20; Ammonium nitrate (NH₄NO₃), 1.00; Ferric chloride (FeCl₃), 0.05; Monopotassium phosphate (KH₂PO₄), 1.00; Calcium chloride (CaCl₂), 0.02; Dipotassium phosphate (K₂HPO₄),

1.00; supplemented with cofactors Glucose (0.1% w/v), Yeast extract (0.3% w/v) and 150 mg/l Reactive Orange ME2RL was used for enrichment of organisms in the water sample ,pH after sterilization 7.3±0.1.

2.3 De-colorization Assay

Take 100ml Bushnell and Haas Broth (BHB) in a 250ml Erlenmeyer flasks along with glucose (0.1%w/v), Yeast Extract (0.3%w/v) and 150 mg/l of ROME2RL was inoculated with mixed cultures (4% w/v) and incubated at 35°C in static conditions. The percentage of de-colorization was found out at 0, 12, 24, 36, 48, 60 and 72 h after incubation. Culture aliquots which were taken after certain time intervals and these were centrifuged at 15*103 X g for 15 mins. The supernatant filtrates were examined with a range of wavelength (λmax=490 nm) in UV/Visible Spectrophotometer (Specord). De-colorization reaction of dyes was resolute by checking the decrease in absorbance at the maximum wavelength of dye by spectrophotometer. A BHM media supplemented with ROME2RL dye was used as control without the mixed culture. De-colorization rate was calculated as the following,

Dye Decolonization assay

$$\% \text{ Decolorization} = \left(\frac{Y - Y_0}{Y} \right) \times 100$$

Where,

Y : Initial absorbance

Y₀ : Observed absorbance

III. RESULTS AND DISCUSSION

3.1 Effects of time on dye decolorization

UV/visible spectral study of ROME2RL (λmax = 490 nm) dye was performed and results by this analysis showed degradation and decolourization of the dye in the visible region gradually increased with increasing incubation time. It demonstrates that the indigenous compound (ROME2RL dye) provided strong evidence of the decolorization method with time (Fig. 1).

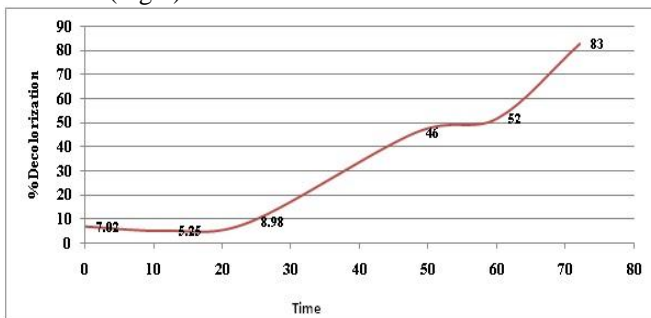


Fig. 1: Effects of time on dye decolorization

3.2 Effects of pH on decolorization

It was observed by this study that the effects of pH on the decolorization of ROME2RL over a range of pH (3.0 – 8.0) for 72 hrs cultivation. The influence of pH factor on decolorization of the reactive dye is demonstrated in Fig. 2.

For removal ROME2RL, the best color removal was achieved at pH 7 with 83%. The most favorable pH for decolorization of color is often between 6.0 - 10.0 [4]. Hence, in many investigations, pH was maintained at pH 6. Furthermore, as continue increasing of pH from 5.0 to 6.0, the percentage decolorization of ROME2RL was raised from 52% to 70%. However, decolorization has decreased at lower pH (3.0 – 4.0) and also increased at pH 7.0. However, percentage of decolorization decreased at lower pH (3.0 – 4.0) and also at higher pH 7.0. The efficiency of decolorization of Methyl orange dye by Lactobacillus case strain TISTR 1500 was found to maximum degrade at pH 6.

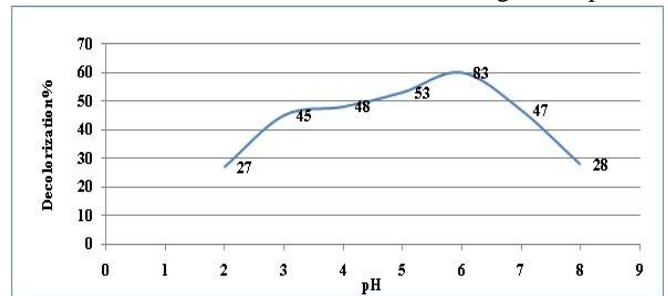


Fig. 2: Effects of pH on dye decolorization

3.3. Effects of temperature on decolorization

The effect of temperature on decolorization of ROME2RL by mixed culture during the study was observed for wide range of temperature from 30°C to 42°C. It was noticed in Fig. 3, the most favorable temperature for ROME2RL was at 37°C, with 83% color removal, A decline of dye decolorization efficiency at high temperature as a result of turn down in microbial activities that start to the inactivation of the microbial enzyme activities and loss the microbial cell viability.

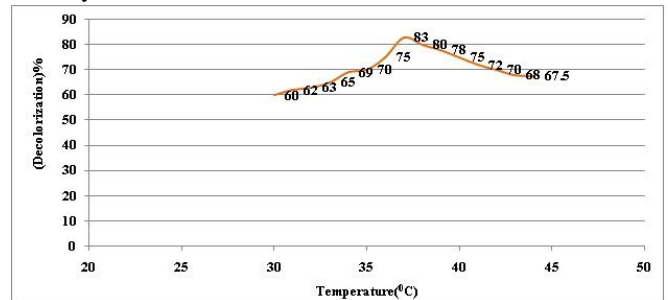


Fig.3: Effects of temperature on dye decolorization

3.4. Effects of dye concentration on dye decolorization

The proportion of decolorization of ROME2RL by mixed culture was examined with different initial reactive dye concentrations (10 mg/l-150 mg/l) which can be seen in Fig. 4. The mixed culture could effectively decolorize ROME2RL with removal percentage of 49% and 46% for 20 and 10 mg/l, respectively. Besides, it was noticed that decrease in percentage color removal of ROME2RL by mixed culture until only 27% removal, with increase in concentration condition. The reduction in decolorization percentage at high dye concentration was noted and it was likely to appear because the effects of high dye concentrations.

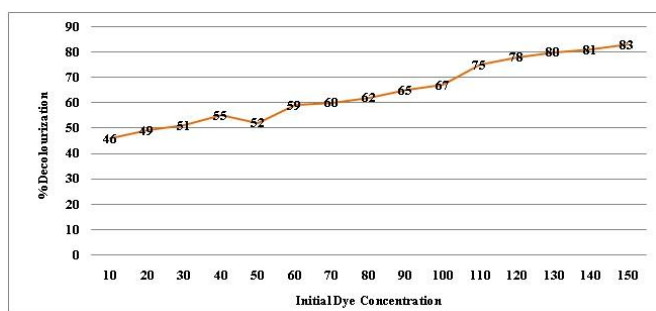


Fig.4:Effects of initial dye concentration on dye decolorization

IV. CONCLUSION

The mixed culture successfully brought about significant decolorization of Reactive Orange ME2RL in 72 hrs. In presence of glucose and yeast extract with simple minimal medium the micro-organism could effectively decolorized the dye (83%) in anaerobic conditions than aerobic conditions. The results shown that complete reduction of azo dyes within a feasible time will be possible with suitable nutritional and anaerobic conditions are maintained. Bacterial degradation may provide a promising economical and cleaner alternative to replace or supplement current treatment processes for the removal of very high concentrations of dyes in industrial wastewater effluents; we can conclude that the members of the consortium are potential candidate and would act as suitable agent for the treatment of environmental pollution caused by dyes industries.

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