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Effects of Dye Toxicity on Bacterial growth and its Kinetics

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Azo dyes are widely used in textile industries for dying purpose. However, most of these dyes are frequently released in water without any treatment process which causes water pollution and toxic effects of marine organism. In present study, we investigate the toxic effects of azo dye with different concentration and interpret the kinetics associated with dye toxicity.

Keywords : Azo dyes, bacterial decolorization, Monod equation, *Enterobacter cloacae*, Optical density.

INTRODUCTION

Synthetic dyes are extensively used for different purpose such as in textile, paper, leather, food, cosmetics and pharmaceutical industries for dyeing and printing purpose (Zollinger, 2003). It is estimated that more than 7 x 10^5 metric tons of dyestuff are produced worldwide annually (Robinson *et al.,* 2001). In the textile industry, among all the known classes of chromogenic groups of dyes, azo dyes are frequently used due to their favorable characteristics of bright color, stability, ease of application, water soluble and low energy consumption (Othman *et al.,* 2011). Azo dyes are characterized by the presence of Nitrogen-Nitrogen (-N=N-) double bonds (Pandey *et al.,* 2007). Unfortunately, most of these dyes are escaped from conventional wastewater treatment processes and persist in the environment. Discharge of these color effluents into aqueous eco-system results into reduced dissolve oxygen concentration, reduced sunlight penetration in water bodies, reduced photosynthesis, deteriorates the water quality and cause toxic effects on aquatic flora and fauna (Kao *et al.,* 2003). Therefore, the treatment of industrial effluents containing dyestuff becomes necessary prior to their final discharge to the environment.

Kinetic characteristics of azo dye decolorization by *E.cloacae* are determined using Congo red as the model azo dye substrate. The effect of substrate concentration on the rate of bacterial decolorization which associated with bacterial growth was investigated. Along with substrate concentration, the toxicity of dyes and their intermediate products also play an important role in decolorization process.

MATERIALS & METHODS

Bacterial growth at different substrate concentration : To study the effect of microbial growth on decolorization performance of microbial culture, a loopful of isolated microbial culture with best decolorization potential were incubated under anaerobic condition in Minimal salt medium (pH 7) containing Congo red (0.1 to 1 g/l) for 96 hours at static as well as shaking condition (150 rpm) at 34ºC. Samples (2ml) were withdrawn at different time intervals; centrifuge at 8000 x g for 10 min. to separate the bacterial cell mass and clear supernatant was used to measured decolorization, while bacterial biomass is used for growth kinetics. Decolorization of dyes was monitored by measuring the change in absorbance of cultured supernatants at the maximum absorption wavelength (λ_{max}) of the respective dyes in UV-visible spectrophotometer. The samples were diluted to less than 0.8 absorbance units. The absorbance reading obtained in UV-visible spectra were used to know the residual dye concentration in samples from the calibration curve for absorbance versus dye concentration to obtain by plotting the corresponding maximum absorbance at different concentrations of dyes. Abiotic control (without microorganism) was always included. The decolorization efficiency was calculated as-

$$\text{Percent decolorization} = \frac{A_0 - A}{A_0} \times 100 \quad .....(1)$$

The bacterial pellet were collected and suspended in sterilized distilled water and again centrifuge at 5,000rpm for 10minutes. The supernatant were discarded and the pellets were again suspended in sterilized distilled water.

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The samples collected at different intervals of time were used for spectrophotometric analysis of cell growth at 600nm of wavelength. *E. cloacae* cells with an initial $O.D._{600}$

RESULT AND DISCUSSION

It is well known that substrate concentration affect both the enzymatic activity and microbial growth. Hence, the effects of glucose on bacterial growth were tested in batch experiments. The growth of bacteria increase with time and O.D. reached nearly 0.9 at 24 hours of incubation in exponential phase. After 24 hours, there was not much more increase in O.D. was observed and stationary phase were reached as shown in Fig.1.

The biodegradation potential of *E. cloacae* was tested in batch experiments by using a single substrate (glucose) to verify the decolorization capacity of this strain. A set of the decolorization experiments were initially conducted with 2g/l of glucose solution in minimal salt medium supplemented with varying concentration of dye Congo red from 0.1 g/l to 0.5 g/l. The high concentrations of dye (above 0.3g/l) were used to analyze the effect of dye toxicity on E. cloacae cell growth over period of time. In another set of experiments, glucose concentrations were varies from 0.8 g/l to 3.2 g/l while keeping the dye concentration remains constant (0.2g/l). The samples collected at different intervals of time were used for spectrophotometric analysis of cell growth at 600nm of wavelength. *E. cloacae* cells with an initial $O.D._{600}$ of approximately 0.2 (for 0.1 g/l dye solution) and 0.1(for 0.5g/l dye solution) were in a lag phase for the first 9 hours. Figure 1 represents the cell growth with respect to time frame. This is followed by the exponential phase when cells at $O.D._{600}$ increased to almost 1.0 between 9 to 22 hours. The final phase of the growth was a stationary phase where cells growth remains constant over period of time. Similar types of observation were also reported by Jablonski *et al.* (2013) for degradation kinetics of resorcinol by *Enterobacter cloacae* isolate. However, the decolorization was not clearly observed in exponential phase up to 22 hours but it become prominent after this phase. Based on the growth curve Figure 1 of *Enterobacter cloacae*, the Monod kinetics model was prepared. Monod kinetics is an empirical model that can fit in our experimental model for Congo red decolorization by using the glucose as a substrate. The trends of bacterial growth which follow the Monod equation is represented by the following equation:

$$\mu = \frac{1}{x} \cdot \frac{dX}{dt} = \frac{\mu_m S}{K_s + S}$$, where μ= specific growth rate (h^{-1}),

μ_{max} = maximum specific growth rate (h^{-1}), K_s is half saturation constant, S is initial substrate concentration, X is microbial cell concentration where $X=X_O\ e^{\mu t}$. The kinetic parameters such as maximum specific growth rate (μ_m), Monod constant (K_s) were determined from a double reciprocal plot of specific growth rate against various concentrations of glucose. The relationship between specific growth rate (μ) and initial substrate concentration (S) is presented at Fig. 2. Regression analysis is used to find the best fit for a straight line on a plot of 1/ μ versus 1/S to determine the values of μ_{max} and K_s (Fig.3). The μ value increases with increase in glucose concentration to its maximum 2.8 g/l and then decreased with further increase (3.2g/l) in glucose concentration. Similar results were also observed by Gnanapragasam *et al.* (2011), where decolorization of reactive dyes decreases with increase in starch wastewater concentration up to certain extent and then decrease with increase in starch concentration. In that case, μ_{max} and K_s were found to be in the range of 0.037-0.094 and 213.4-985.6 respectively, while in our study, the specific growth rate (μ_{max}, h^{-1}) and substrate half saturation constant (K_S, g/l) was observed to be 0.504 and 0.433 respectively Fig.2. Lin *et al.* (2008) have found μ_{max} and K_S value were 1.8 day^{-1} and 300mg/l respectively for decolorization of Reactive red 22 by *Pseudomonas luteola* in a biological activated carbon process. Gopinath *et al.* (2009) also reported that specific growth rate of *Bacillus* sp. increases with initial concentration of Congo red till 0.2415 h^{-1} and beyond which the rate was found to decrease. In that experiment, Monod and Haldane model gave better correlation coefficient compared to other models. Since, the Monod equation is widely applicable for growth kinetics and therefore this kinetics is equally fit in present study. The equation gives an idea for utilization of substrate during concurrent bacterial growth or decay using specific growth rate and specific substrate consumption rate Kapdan *et al.,* (2003).

Effects of dye on growth inhibition : Although the inhibitory effects of azo dyes is widely known and acceptable, but the type and the level of inhibition on microbial metabolism have less investigated. The reports of previous available article have showed that the kinetic constant decreases as dye concentration increases. In present study, the effects of dye toxicity with increase in concentration on microbial metabolism and the rate of color removal was analyzed. Based on the effect of dye toxicity, inhibition model was formulated and maximum substrate utilization rate was calculated in dye decolorization experiment. The kinetic models are usually depends on the nature of dye, concentration and are expressed in term of the types of inhibition *e.g.* competitive inhibition, non-competitive inhibition and uncompetitive inhibition (Nelson and Cox, 2008). In order to determine the type of inhibition, the plot between 1/S versus 1/μ was analyzed. The reciprocal fits (Lineweaver-Burk plot) of inhibition (1/S versus 1/μ) showed that azo dye Congo red caused non-competitive inhibition on microbial growth rate. The slopes and the intercepts indicated the type of inhibition which was found from the Lineweaver-Burk plots at increasing dye concentrations. In this study, different concentration of dye (0.1, 0.2, 0.3, and 0.4 g/l) was taken at constant substrate (glucose) concentration of 2g/l. The results are represented in Fig. 4. It was observed that bacterial growth decreased with increase in dye concentration. Decline in growth rate may be due to the poisonous effect of the dye on bacteria, obstruction of active sites of azoreductase enzyme by complex dye structure or insufficient product of biomass for decolorization at higher concentration of dye (Shah *et al.,* 2012). Similar trend in inhibition- kinetics was also found by Punj *et al.* (2004) on decolorization and inhibition of Reactive Black 5 and Direct Brown 2 in anaerobic mixed culture using glucose

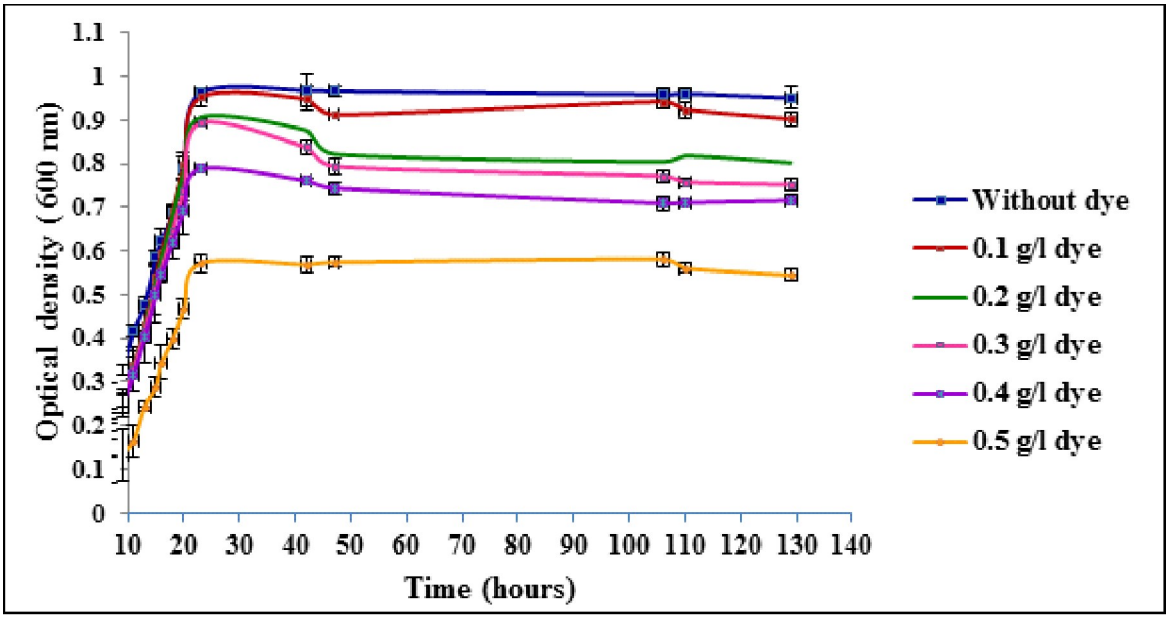

Figure: 1. Bacterial growth curve with time frame

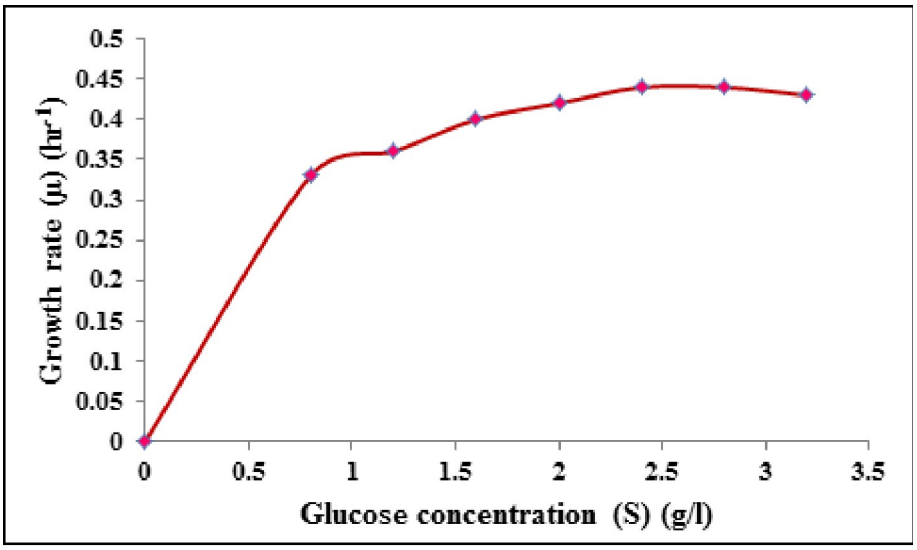

Figure: 2. Bacterial specific growth rate curve

(3000mg/l COD). From the above results, the value of inhibitory constant indicated that the inhibition effect is noted with increases in dye concentration.

CONCLUSION

1. The microbial growth kinetic parameters such as maximum specific growth rate (μ_m), Monod constant (K_s) were determined from a double reciprocal plot of specific growth rate against various concentrations of glucose. The specific growth rate (μ_{max}, h^{-1}) and substrate half saturation constant (K_S, g/l) was found to be 0.504 and 0.433 respectively.
2. The toxicity of dye was also noticed in following experiments. The specific growth decreased with increase in dye concentration and a non-competitive type of inhibition was observed.

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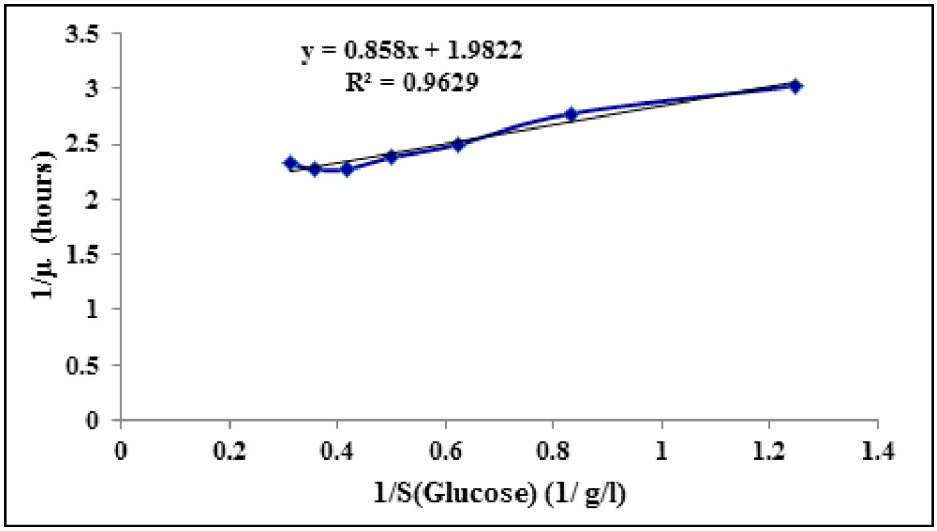

Figure: 3. The reciprocal fits (Lineweaver-Burk plot) of Bacterial growth curve

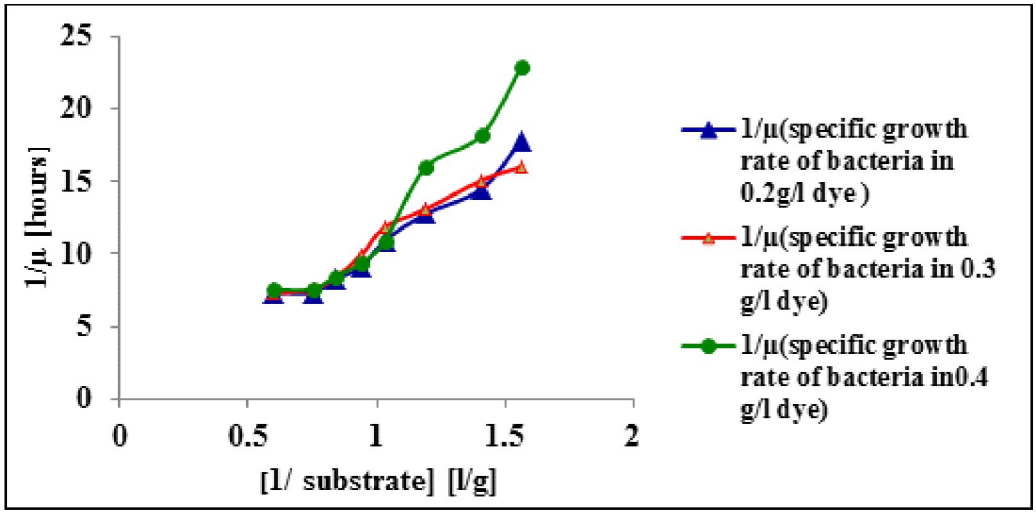

Figure: 4. Effects on dye on bacterial growth inhibition

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