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Optimization of Magenta Dye Decolorization Different Parameters by *Citrobacter* sp.

Shankara S[1*], Vijayakumar M. H[2], Kotresha D[3], Gaddad S. M[4]

[1]Department of Microbiology, Government College for Women, Chintamani-563 125, Karnataka, India

[2]Department of Biochemistry, Gulbarga University, Gulbarga-585106, Karnataka, India

[3]Department of Microbiology, East West group of Institution, no. 63, Anjananagar, Vishwaneedam post, Bangaluru-560091,Karnataka, India

[4]Department of Microbiology, Gulbarga University, Gulbarga-565 106, Karnataka, India

Abstract: ***A thermo-alkalophilic bacterium Citrobacter sp. isolated from textile mill effluent sample as a decolouring microorganism. The maximum 500 mg L^{-1} Magenta (Basic Red-9) dye decolorization was takes place in 48 h and completely decolorizes the dye Magenta up to a maximum concentration 1000 ppm, however the time taken was 104 h. The optimum physical parameters such as temperature 40°C, pH 8.0 and 2.5% (w/v) of nitrogen source were required for the decolorization of Magenta dye by Citrobacter sp.Effect of seven different metal ions on decolorization of Magenta tested, in which most toxic was Cadmium and less toxic was Lead at 0.05 mM concentration. The curing study indicates that decolorization was takes place by enzyme which was plasmid mediated. The degraded dye metabolites are analyzed by TLC and diazotization, carbylamines test for individual metabolite indicates biotransformation of Magenta into aromatic amine and non-toxic aromatic metabolites. These results suggest that the isolated organism Citrobacter sp.as a useful tool to treat waste water containing reactive dyes at static condition.***

Keywords: *Citrobacter* sp., Decolorization, Magenta, Metabolite.

1. Introduction

Textile dyeing effluents containing recalcitrant dyes are polluting waters due to their color and by the formation of toxic or carcinogenic intermediates, such as aromatic amines from azo dyes[1, 2]. Water plays a vital and essential role in our ecosystem. This natural resource is becoming scarce, makingits availability a major social and economic concern. Use of a large variety of synthetic dyes in textile industries has raiseda hazardous environmental alert. About 17-20% of freshwater pollution is caused by textile effluents. These effluentsare recalcitrant to biodegradation and cause acute toxicity to the receiving water bodies, as these comprised of varioustypes of toxic dyes, which are difficult to remove[3].The conventional treatment systems based on physical or chemical treatment does not remove the color and dye compound concentration[4].

The decolorization of the dye takes place either by adsorption on the microbial biomass or biodegradation by the cells and bioremediation takes place by anaerobic and/or aerobic process. Biological methods are currently viewed as specific, less energy intensive, effective, environmentally safe and they result in partial or complete bioconversion of organic pollutants to stable and nontoxic end products[4, 5]. The products of intermediate metabolism during the decolorization process, such as aromatic amines, can be degraded by the hydroxylase and oxygenase produced by bacteria [5, 6].In this study, a thermo-alkalophilic bacterium *Citrobacter* sp. capable of decolorizing Magenta and the effects of various physical and chemical parameters on dye decolorization were investigated.

2. Materials and Methods

Dye decolorization studies

Decolorizing activity is expressed in terms of percentage of decolorization of the dye [7].

$$D = [(A_0-A_1)/A_0] \times 100$$

Where, D, % of decolorization; A_0, initial absorbance; A_1, final absorbance

The effect of Dye concentration, temperature, pH and Nitrogen source on dye decolorization

The 250 mL Erlenmeyer flasks having 100 mL of MM medium containing 100 ppm of dye were inoculated with selected bacterium. The flasks were incubated at various temperatures ranging from 20-60°C and the effect of pH on dye decolorization was studied with various pH values ranging from 5-9. The effect of Nitrogen source and dye on dye decolorization was studied at various concentrations ranging from 1-4% (w/v) and 200-1200 ppm, respectively. Uninoculated controls were prepared in parallel in all experiments.

Curing

The *Citrobacter* sp. was pre-cultured in BH medium with shaking for 18 h. the pre-culture was inoculated into 5 ml of fresh BH medium containing 0.5 mL curing agent (Acridine Orange 1%) and incubated for 24 h at 37 °C. After 24 h the culture was transferred to fresh BH medium containing 0.1 g L^{-1} of dye, Simultaneously transferred to fresh BH medium containing 0.5 mL of 1% Acridine orange, the process was repeated every 24 h up to 5 d. The culture was properly diluted with saline and 0.1 mL of diluted culture was placed on Nutrient agar. The inoculated plate was incubated at 37 °C for 24 h. Each colony appearing on the agar plate was tested for its ability to decolorize the Magenta. The colony

which did not decolorize the dye is considered as a cured colony. The Curing frequency was calculated by colony counts of the initial one, i.e., the difference between the initial colony counts, (*i*) and the final colony count (*f*) of the sample. The curing frequency was calculated by following formula.

Curing frequency (%) = Colonies (*i*) – Final (*f*) × 100/ Colonies (*i*)

Extraction of Metabolites

The complete decolorized medium of Magenta was centrifuged at 10,000 rpm for 15 min. The pH was adjusted to 7 and 200 mL of the supernatant was extracted twice with 500 mL diethyl ether. The remaining aqueous layer was acidified to pH 2 by 1N HCl and extracted twice with diethyl ether (500 mL). The acidic and basic extracted fractions were pooled and evaporated to very small quantity.

Thin layer Chromatography (TLC)

The glass plate of 200×100×2 mm (length × breadth × thickness) coated with 40% (w/v) aqueous slurry of Silica gel G with binder were used for carrying out TLC, 10 µl of extracted fractions were loaded on Silica gel, dried and the solvent system used were propanol : water : acetic acid (90 : 9 : 1 v/v) for developing the TLC plate. The dye and products on chromatogram was observed by exposing to long wavelength UV-Light (365 nm) and the metabolites were partially identified by Diazotization and Carbylamine test.

3. Results and Discussion

Effect of Dye Concentration, Temperature, pH and Nitrogen source on dye decolorization

Effect of dye concentration (Fig. 1) on the decolorization ability of the bacterium was studied by inoculation of the bacterium to BH medium supplemented with different concentrations of Magenta (100-1000 ppm). When the effect of different initial dye concentrations of Magenta on decolorization was observed using 100, 200 300, 400, 500, 600, 700, 800, 900 and 1000 ppm and the required times to reach a maximum decolorization extent were 24, 32, 40, 48, 48, 60, 72, 72, 84, and 104 h, respectively. These results indicate that as the initial dye concentration increases the time required for complete decolorization also increases and dye concentration above 1000 ppm was not completely decolorized even after extended incubation periods. It was reported that dye decolorization can be strongly inhibited when a high concentration dyestuff was used to examine the poisonous effect of the dye on the degrading microorganisms [8, 9].

One of the most important parameters for dye decolorization is temperature. In order to determine the optimum temperature for dye decolorization using Magenta (100 ppm) a temperature range of 20-50°C was examined. Fig. 2 shows that the complete dye decolorization increased with increase in temperature from 20-40°C and decreases with increase in temperature above the 40°C,which might be due to the loss of cell viability or deactivation of the enzymes responsible for decolorization [10, 11]. The 100% dye decolorization was observed at 40°C within 20 h and temperature between 35–45°C was optimum for the removal of Magenta.

The results on the effect of various hydrogen ion concentrations on decolourization of Magenta by *Citrobacter* sp. are represented in Fig. 3. The maximum decolourization was found to be between pH 7.0-8.5 within 24-30 h respectively. These results indicated that *Citrobacter* sp. is capable of decolorizing Magenta (100 ppm) within a wide range of pH and organism was completely decolourizes 100 ppm of the dye within the pH range from 6.0 to 10.0. Below pH 6.0 and above pH 10.0 complete decolourization of the Magenta (100 ppm) by *Citrobacter* sp. was not observed, however optimum pH was found to be 8.0 for decolourization of Magenta where decolourization occurred within 20 hours of incubation. The pH tolerance of decolorizing bacteria is quite important because reactive azo dyes bind to cotton fibers by addition or substitution mechanisms under alkaline conditions and at high temperatures [12]. These results indicate that the bacterium *Citrobacter* sp. was alkalophilic in nature.

Thus the effect of various concentrations (0.25–2.5 g L^{-1}) of the nitrogen source (peptone) was investigated and the results are represented in Fig.4.The decolorization of Magenta (100 ppm) along with organic nitrogen though *Citrobacter*sp. was observed. The presence of organic nitrogen source like peptone the rate of color reduction was increased with the concentration of organic nitrogen 0.25 g L^{-1} to 2.25 g L^{-1} and above 2.25 g L^{-1} it was constant. Praveenkumar and Bhat, (2012) have reported similar reports of increase in the efficiency of decolorization with increase in the organic nitrogen concentration and many report shows the maximum decolorization of dyes takes place in presence of nitrogen source [13, 14] and these investigations pointed nitrogen source was essential for decolorization of reactive dyes.

Effect of heavy metals

The effect of different heavy metals (0.025-1mM) on decolourization of Magenta (100 ppm) by *Citrobacter* sp. was investigated and the results are shown in Fig.5. Out of the seven metals tested cadmium was most toxic. With this metal the incubation time required for 100% decolourization sharply increased from 24 h at 0.025 mM to 32 h at 0.05 mM and further to 72 h at 0.5 mM and above 0.5 mM complete dye decolourization did not occur even at the extended periods. Similar observations were made with zinc, mercury and copper where complete decolourization of 100 ppm of Magenta occurred after 72 h incubation up to 0.5 mM of the respective metals and 100% decolourization could not be achieved above 0.5 mM. Contrarily, nickel, lead and cobalt, appeared to be comparatively less toxic and lead appeared to even stimulate and enhance decolourization process at its lower concentrations.

Curing

Curing of *Citrobacter* sp. was carried out to determine the location of genes responsible for decolourization. The percentage frequency of curing was found to be 24.42% on first day and it increased gradually to 90.83% at 5^{th} d (Fig.6). The plasmid was found to be lost during successive cycles and after 5^{th} d the dye decolorizing ability of the bacteria was completely lost. This result indicates that the genes for

due decolourization are present on plasmid andnot on bacterial genome.

Isolation and Identification of metabolites

The biodegradation products of Magenta were loaded on TLC plate and developed by Propanol: Water: Acetic acid (90: 9: 1 v/v) solvent system. The developed TLC plate has shown three spots under UV light observation with $R_f$values of 0.33, 0.46, 0.71 andthe λ_{max} values of the peaks were found at 260 nm, 320 nm and 280 nm respectively (Table 1). Further metabolites were characterised by chemical test such as Diazotization and Carbylamines tests. The spot with R_f values 0.33 and 0.46 are negative to diazotization and carbylamine tests and whereas R_f value 0.71 showed positive result for the diazotization and carbylamine tests. These results indicate that our isolated bacterium was potential for the degradation azo dyes and converted into aromatic compounds with and without amine group metabolites.

References

[1] Hao, O.J., Kim, H., Chiang, P.C. "Decolorization of wastewater". Cri. Rev. Environ. Sci. Technol. 30, 449–505,2000.
[2] Srikanlayanukul M, Wirut K, Takashi W, and Chartchai K. "Decolorization of orange-II by immobilized thermotolerant white rot fungus Coriolusversicolor RC3 in packed-bed bioreactor". Biotechnology, 7:280-286, 2008.
[3] Naik NM, Jagadeesh KS, Alagawadi AR."Microbialdecolorization of spentwash: a review". Indian J Microbiol48:41–48, 2008.
[4] Sarioglu, M., Bali, U., Bisgin, T. "The removal of C.I. Basic Red 46 in a mixed methanogenic anaerobic culture". Dyes Pig.74,223–229, 2007.
[5] Saratale, R.G., Saratale, D., Chang, J.S., Govindwar, S.P. "Bacterial decolorization and degradation of azo dyes: a review". J.Taiwan Inst. Chem. Eng.42, 138–157, 2011.
[6] Shah, M.P. "Microbial Degradation of Acid Blue Dye by Mixed Consortium". Int. J. Environ.Bioremed.Biodegrad.2, 125-132, 2014.
[7] Yatome C, Maiti KT, Bhattacharyya BC. "Degradation of crystal violet by *Nocardiacrallina"*. Application MicrobiolBiotechnol38:565-569, 1993.
[8] Khehra, M.S., Saini, H.S., Sharma, D.K., Chadha, B.S., Chimni, S.S. "Comparative studies on potential of consortium and constituent pure bacterial isolates to decolorize azo dyes". Water Res. 39, 5135–5141, 2005.
[9] Kalme, S.D., Parshetti, G.K., Jadhav, S.U., Govindwar, S.P. "Biodegradation of benzidine based dye Direct Blue-6 by *Pseudomonas desmolyticum*NCIM 2112". Biores.Technol. 98, 1405–1410, 2007.
[10] Panswad, T., Luangdilok, W. "Decolorization of reactive dyes with different molecular structures under different environmental conditions". Water Res. 34, 4177–4184, 2000.
[11] Cetin, D., Donmez, G."Decolorization of reactive dyes by mixed cultures isolated from textile effluent under anaerobic conditions". Enzyme Microb.Technol. 38, 926–930, 2006.
[12] Aksu, Z. "Reactive dye bioaccumulation by Saccharomyces cerevisiae". Process Biochem, 38, 1437–1444, 2003.
[13] Ponraj, M., Gokila, K., Zambare, V. "Bactarial decolorization of textile dye- orange 3R". Int. J. Advanced Biotech and Resea. (2), 168-177, 2011.
[14] Bhatt, N.S., Vagadiya, D.R., Junnarkar, N.S."Decolorization, degradation and azo-reductase study by bacterial transformation of reactive red HE8b". Int. J. Res. BioSci.1(1), 29-41, 2012.

Table 1: Characterization of Magenta dye degradation products

Dye	R_f	λ_{max}	Diazotization test	Carbylamine test
1) Magenta	0.21	557	-Ve	-Ve
2) Dye degraded products of Magenta				
a)	0.33	260	-Ve	-Ve
b)	0.46	320	-Ve	-Ve
c)	0.71	250	+ve	+ve

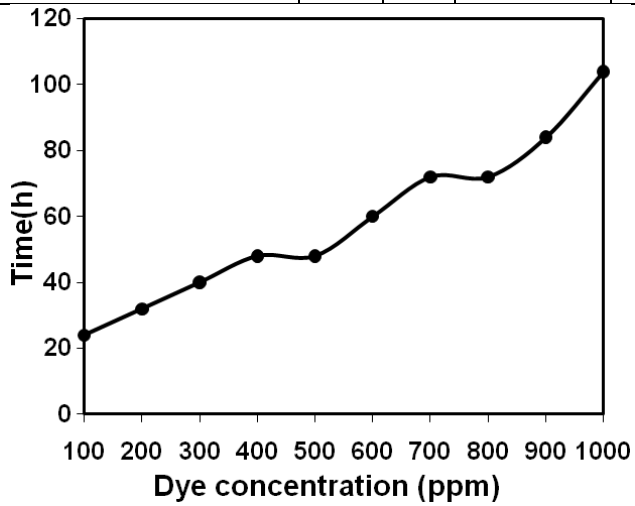

Figure 1: Effect of dye concentration on decolourisation of Magenta by *Citrobacte*sp.

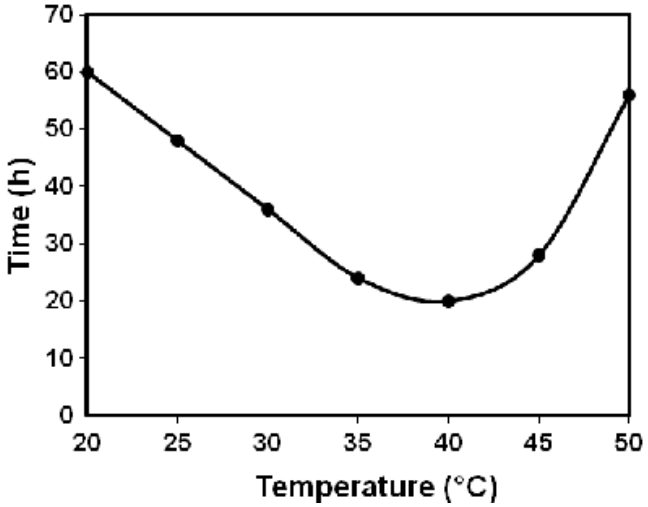

Figure 2: Effect of temperature on decolourisation of Magenta by *Citrobacter* sp.

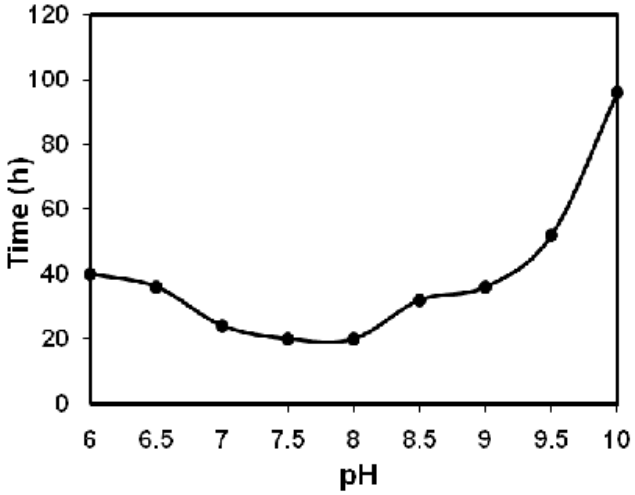

Figure 3: Effect of pH on decolourisation of Magenta by *Citrobacter* sp.

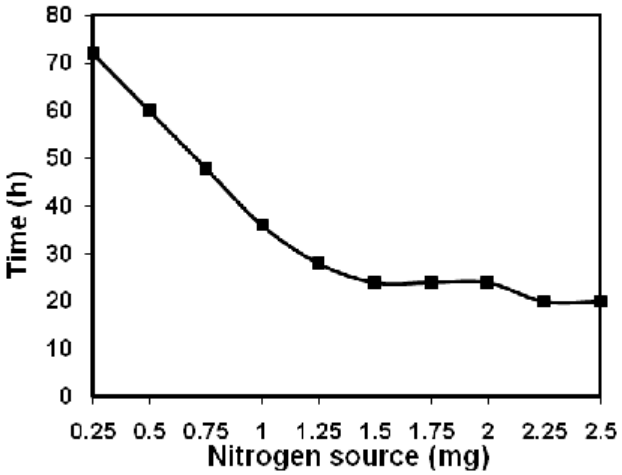

Figure 4: Effect of Nitrogen concentration on decolourisation of Magenta by *Citrobacter* sp.

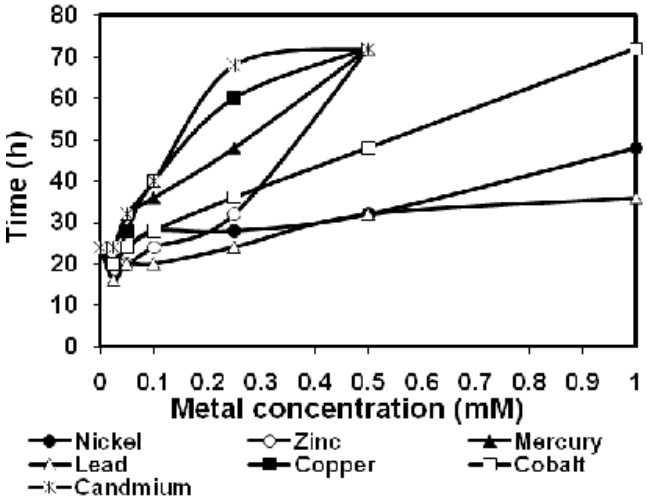

Figure 5: Effect of heavy metals on decolourisation of Magenta by *Citrobacter* sp.

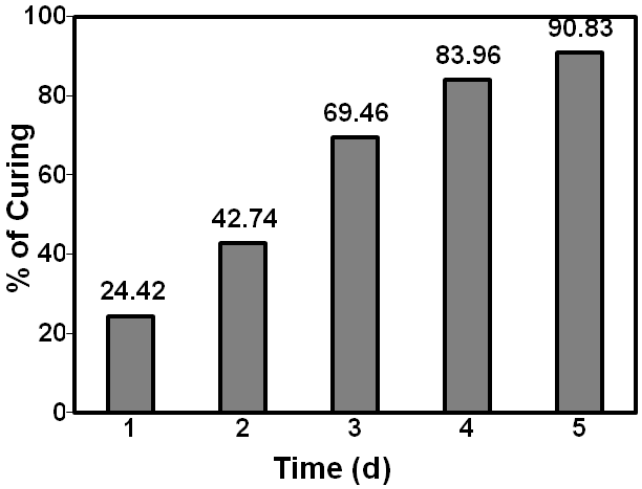

Figure 6: Curing of *Citrobacter*sp with acridine orange for the loss of Magenta decolourising activity