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Original Article

STUDY ON DECOLORIZATION OF DYE STUFF (AZO DYE–CONGO RED) BY USING BACTERIAL CONSORTIA

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ABSTRACT

Objective: The present study is focused on the screening, isolation of effective bacterial microorganisms for the decolorization of textile effluent and evaluation of the performance of the process also optimization of parameters for enhanced decolorization.

Methods: The isolation was carried out by serial dilution method and the decolorization was carried out in the batch reactor.

Results: The effective bacteria isolated from the textile were *Proteus mirabilis, Staphylococcus aureus, Bacillus subtilus, Bacillus funiculus and Pseudomonas fluorescens* have been characterized by biochemical assay also with Scanning Electron Microscopy (SEM).

Conclusion: The decolorization process using bacterial consortia for various dye yields 95% decolorization. The process was simple, cost-effective and eco-friendly. The parameters such as temperature, pH, nutrients, time had the greater potential for the effective growth of microorganisms were optimized and can be applied to the diverse textile treatment applications.

Keywords: Congo red dye, Bacterial consortia, Decolorization, Degradation.

INTRODUCTION

Application of bio remediation in wastewater treatment is a prominent technology, which was successfully used to treat various organic effluents and dye effluents. Waste water from textile effluent contains the variety of pollutants. The textile effluents contain high dissolved and suspended solids, organic compounds, and have high pH [1]. The major pollutant presented in the effluent was dye stuff and organics. The textile and dyeing industries have occupied a major position in the industrial sector of Coimbatore, Erode district. Textile industry consumes a large amount of water thereby generate a large amount of industrial effluent which are discharging daily into our environment. With respect to the quantity and composition, the textile wastewater is the most polluted sources among all industrial sectors [2].

The environment can be saved from its hazardous effects by reprocessing and reuse of the reclaimed water so that the dependency on groundwater can be reduced to a great extent. The environmental pollution created by these industries becomes an issue and enforcement of rules and action initiated by Tamil Nadu Pollution Control Board, almost all the industries should have Effluent Treatment Plant (ETP) or the member of Common Effluent Treatment Plant (CETP). Due to the adverse effects of the dye containing effluent, the textile effluent is to be treated and discharged according to Water Act, 1974.

There is the variety of physiochemical methods available for treating textile effluent, but the major disadvantage of these methods was high capital investment and the generation of secondary effluent. By contrast, biological process was cheaper than the others. Operating costs for biological processes were also less than chemical treatment methods. Azo dyes constituted the largest and versatile class of dyes used in the textile industries. Hence biological decolorization occurs efficiently under low aerobic to anaerobic conditions [3–5]. The biodegradation of dye by different types of microbial cultures in pure and form were performed which was able to decolorize the dye. As an alternative approach mixed cultures of the bacteria were used to metabolize the Congo red dye. Using of diverse bacterial organisms has more advantages over pure culture due to its stability in metabolic activities. This study was focused to construct the consortium and to compare the bacterial decolorization capacity of the strains for azo dye.

MATERIALS AND METHODS

Collection of effluent sample

The effluent samples were collected from the Common Effluent Treatment plant (CETP) located at Kanchipuram district, Tamil Nadu. The effluent samples used for the isolation of microbial strains and treatment trials.

Dye stuff and chemicals

All the chemicals, inorganic salts for the media preparation were purchased from Thermo Fisher Pvt. Ltd., in Mumbai and dye was purchased from Sigma Aldrich Company in Chennai city.

Microorganism and growth condition

The dye decolorizing bacteria were isolated from the secondary clarifier underflow sludge adopting the serial dilution method. The strain was grown and maintained on nutrient agar slant at 4ºC. Inoculums were prepared by suspending the spores from slant. The bacterial cells were grown in 250 ml Erlenmeyer flasks containing 100 ml medium containing glucose (2.0 mg/l), yeast extract (0.2 mg/l), peptone (0.5 mg/l), supplemented with 500 mg/l of dye for adapting dye degradation in further studies. The cell suspensions were aseptically transferred to experimental flasks for studies. The degradation studies were carried out in mineral salt medium (MSM) containing KH_2PO_4 (1.6 g/l), Na_2HPO_4 (0.6 g/l), $(NH_4)_2SO_4$ (1.0 g/l), NaCl (0.5 g/l), $MgSO_4.7H_2O$ (0.1 g/l), $CaCl_2.2H_2O$ (0.1g/l), $(NH_4)_2SO_4$ (0.1g/l) respectively under agitation condition. The culture was acclimatized for a period of one month to grow in MSM containing dye as the carbon source up to a concentration of 1000 mg/l. Individual strains of the microbial consortium were isolated by plating on Potato Dextrose Agar (PDA) consisting of the enrichment media supplemented with 500 mg/l of dye [6].

Decolorization experiment

The ability of the microbial consortium to degrade Congo red was measured under batch conditions. All biodegradation experiments using the microbial consortium were carried out in 250 ml Erlenmeyer flask containing 100 ml of MSM with dye concentration varying from 100 to 1000 mg/l [7, 8]. The flasks are incubated in Shaker incubator at 35 °C samples were withdrawn at regular time interval, centrifuged and analyzed for residual concentration by

using [9–11] the supernatant. Absorbance of the supernatant withdrawn at different time intervals was measured at the maximum wavelength for the dye (530 nm) in the visible region on a Schimadzu UV-Visible spectro photometer (UV 1800). The percentage of decolorization was calculated from the difference between initial and final values of absorbance [12].

$$\text{Percentage Degradation} = \left\{\frac{A_o - A_f}{A_o}\right\} \times 100$$

Where, A_o be the initial dye concentration and A_f be the residual dye concentration.

Design and optimization of decolorization by bacterial consortium

Each bacterial isolates was given a number and the 10 combination of 5 isolates were performed for the study. Optimization of variables for dye degradation by the consortium was carried out [13-14]. The variables of initial concentration, temperature, pH and time were optimized. The biomass concentration was estimated using the dry weight method. 5 ml samples of culture broth were withdrawn centrifuged at 5000 rpm for 15 min in plastic centrifuge tubes. The supernatant was used for subsequent dye concentration estimation [15, 16].

RESULTS AND DISCUSSION

Consortium formulation

The dye degrading strains were isolated based on their degrading ability by enrichment technique. 10 best degrading strains shown in fig. 1 were selected and used in different combination for the development of consortium. The percentage decolorization/ degradation by different consortia were shown in table 1. A consortium containing B2, B3, B5, B6 and B10 strains showed 96.5±2% decolorization/degradation was designated as CB7. Further studies were carried out using CB7 bacterial consortium.

Morphological analysis

The strains of the consortium CB7 was used in extensive studies and were identified on the basis of morphological characteristics and 16S rDNA sequences. They initially analyzed at National Center for Biotechnology Information (NCBI) server was (B3) *Proteus mirabilis,* (B2) *Staphylococcus aureus,* (B5) *Bacillus subtilus,* (B6) *Bacillus funiculus,* (B10) *Pseudomonas fluorescens***.** Characterization of the members revealed that consortium consisted of *Proteus mirabilis* (1.5×10^5CFU/ml), *Staphylococcus aureus* (1.2×10^5 CFU/ml), *Bacillus subtilus* (3.0×10^4 CFU/ml), *Bacillus funiculus* (2.5×10^3 CFU/ml) and *Pseudomonas fluorescens* (1.0×10^4CFU/ml).

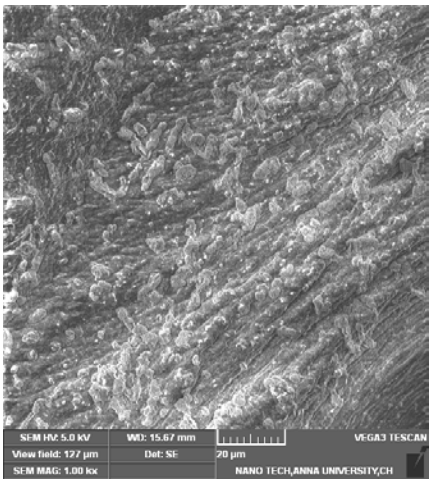

Fig. 1: Scanning electron microscopic image of bacterial consortium (CB7)

Table 1: Congo red degradation by bacterial consortia

Run	Consortium	Isolates	Percentage decolorization (%)
1	CB1	B1, B3, B4, B6, B7	86.4±2
2	CB2	B1, B2, B3, B6, B9	80.4±2
3	CB3	B1, B2, B7, B8, B10	82.4±2
4	CB4	B1, B3, B8, B9, B10	87.7±2
5	CB5	B1, B4, B6, B8, B9	77.5±2
6	CB6	B1, B3, B4, B5, B7	86.6±2
7	CB7	B2, B3, B5, B6, B10	96.5±2
8	CB8	B3, B4, B5, B6, B7	78.5±2
9	CB9	B4, B7, B8, B9, B10	75.5±2
10	CB10	B5, B6, B8, B9, B10	90.5±2

Optimization of physiochemical parameters

Effect of agitation

Decolorization performance by the bacterial consortium has greatly influenced by various process conditions. The enhanced decolorization of Congo red dye by bacterial consortium was observed under shaking conditions while only 80±5% for static condition. The percentage of decolorization was found to be higher in shaking conditions. Hence, the microaerophilic conditions were adopted to optimize pH, temperature and dye concentration for enhanced degradation studies.

Effect of time on degradation

The time taken for complete degradation of dye was influenced by an initial concentration of the dye. The time taken for maximum degradation of dye for various concentrations was shown in fig. 2. The studies were carried out till complete degradation of dye at concentrations of 100 to 1000 mg/l at the interval of 200 mg/l. The dye was completely degraded in 40 h, 75 h, 98 h, 104 h, 130 h, and 160 h, respectively. At the time of 96 h maximum amounts of dye were degraded for all concentration. Hence further studies were carried out at 96 h for the optimization of dye concentration, pH, and temperature.

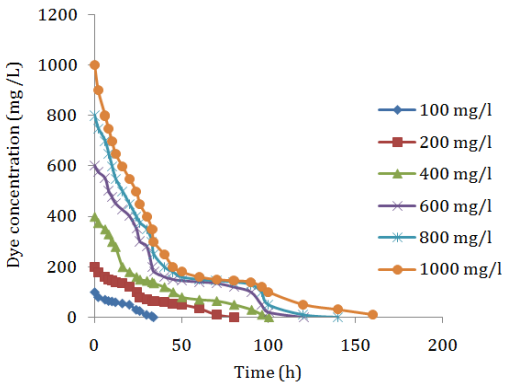

Fig. 2: Optimization of concentration and time on decolorization percentage

Effect of initial concentration on degradation

The effect of initial concentration on the degradation was studied from 100 to 1000 mg/l as shown in fig. 2. The degradation percentage of dye increased from 90.68% to 95.15% with increase in concentration of dye from 100 to 600 mg/l. Further increase in concentration beyond 700 mg/l percentage degradation decreased from 95.2 % to 85 % substantially resulted from intense substrate inhibition at high substrate concentration. Fig. 2 shows that the specific degradation rate of Congo red varied with the initial concentration of dye. At the higher concentration the dye inhibits the growth of microorganisms present in the bacterial consortium.

Effect of temperature on degradation

The degradation percentage of dye increased from 70 % to 95 % as the temperature increases from 20 to 37 °C. Further increase in the temperature decreased the percentage of degradation. This phenomenon might be due to inhibition of enzyme secretion at higher temperature [17].

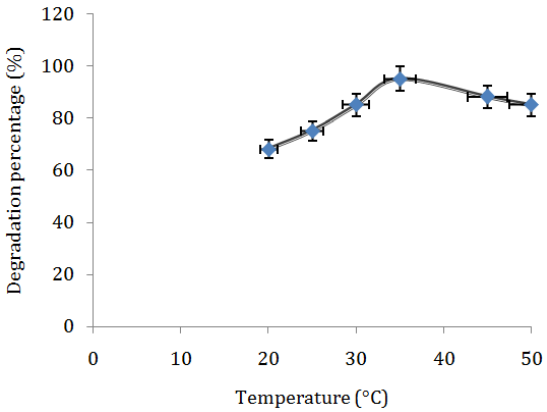

Fig. 3: Optimization of temperature on decolorization percentage

Effect of pH on degradation

The pH is directly associated with the overall biochemical processes and the growth of microorganisms also related to the transport of dye molecules across the cell membrane, which was considered as the limiting factor in decolorization performance.

The effect pH on degradation is shown in fig. 4. The dye degradation decreases as the medium pH deviates from its neutrality. The degradation percentage increases from 80.5% to 87.5% as the pH increases from 4 to 6 and reaches the maximum of 96.5% at pH 7.

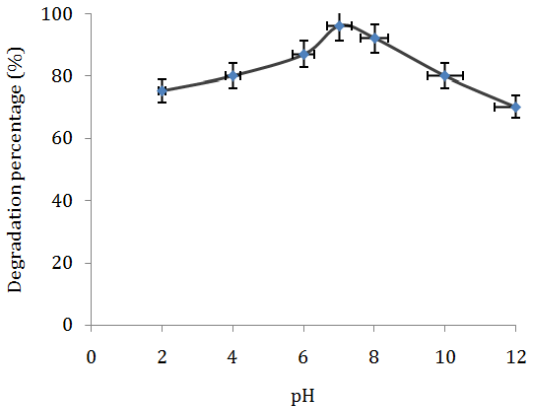

Fig. 4: Optimization of pH on decolorization percentage

Further increase in pH decreases the percentage degradation. The increase in pH from 7 significantly affects the biochemical reactions required for degradation by micro-organism [18].

CONCLUSION

The dye degrading bacterial consortium consisting of *Proteus mirabilis,* (B2) *Staphylococcus aureus,* (B5) *Bacillus subtilus,* (B6) *Bacillus funiculus,* (B10) *Pseudomonas fluorescens* isolated from secondary clarifier sludge has been developed and analysed for decolorization of Congo red dye under microaerophilic conditions. The individual bacterial cultures were able to decolorize the dye in mixing conditions. But as a consortium for the enhancement of decolorization rate, the optimization physiochemical parameter for decolorization has been carried out. The results of the decolorization study indicate that consortium was able to decolorize the selected dye with 95±2 % efficiency. The optimized temperature was found to be 35±2 °C with the initial concentration of 700 mg/l at the neutral pH of 7. Under aerobic conditions the maximum decolorization for 700 mg/l was achieved in 96 h.

CONFLICT OF INTERESTS

Declared None

REFERENCES

1. Mathias A Chia, Rilwan I Musa. Effect of indigo dye effluent on the growth, biomass production and phenotypic plasticity of Scenedesmus quadricauda (Chlorococcales). Biol Sci 2014;86:419–28.
2. Mir Tariq Ahmad, Manderia Sushil, Manderia Krishna. Influence of dye industrial effluent on physico chemical characteristics properties of soil at Bhairavgarh, Ujjain, MP, and India. Res J Environ Sci 2012;1:50–3.
3. Rajendra Ramasany, Hasab Abdelbagi Muhammad Ahmed, Sundaram S Karthik. Development of microbial consortium for the biodegradation and biodecolorization of textile effluents. J Urban Environ Eng 2012;6:36-41.
4. Cripps C, Bumpus JA, Aust SD. Biodegradation of azo and heterocyclic dyes by Phanerochaete chrysosporium. Appl Environ Microbiol 1990;56:1114-8.
5. Franciscon E, Grossman MJ, Paschoal JA, Reyes FG, Durrant LR. Decolorization and biodegradation of reactive sulfonated azo dyes by a newly isolated Brevibacterium sp. strain VN-1. Epub 2012;1:1801-8.
6. Sivasubramanian S, Karthick Raja Namasivayam S. Phenol degradation studies using microbial consortium isolated from environmental sources. J Environ Chem Eng 2015;3:243–52.
7. Haug W, Schmidt A, Nortemann B, Hempel DC, Stolz A, Knackmuss HJ. Mineralization of the sulphonated azo dye mordant yellow 3 by a 6-aminonaphthatene-2-sulphonate-degrading bacterium consortium. Appl Environ Microbiol 1991;57:3144-9.
8. Horitsu H, Takada M, Idaka E, Tomoyeda M, Ogawa T. Degradation of p-aminoazobenzene by Bacillus subtilis. Eur J Appl Microbiol 1977;4:217-24.
9. Idaka E, Ogawa Y. Degradation of azo compounds by Aeromonas hydrophila. J Soc Dyers Colourists 1978;94:91-4.
10. Knapp JS, Newby PS. The microbiological decolorization of an industrial effluent containing a diazo-linked chromophore. Water Res 1995;29:1807-9.
11. Marchant R, Nigam P, Banat IM. An unusual facultatively anaerobic fungus isolated from prolonged enrichment culture conditions. Mycol Res 1994;98:757-60.
12. McKay G. Waste colour removal from textile effluents. Am Dyest Rep 1979;68:29-36.
13. Nigam P, Singh D, Marchant R. An investigation of the biodegradation of textile dyes by aerobic and anaerobic microorganisms. In: Environmental Biotechnology: Principles and Applications. M. Moo-Young. Kluwer Academic; 1995. p. 278-92.

14. Ogawa T, Fuji I, Kawal K, Yatome C, Idaka E. Growth inhibition of Bacillus subtilis upon interaction between basic dyes and DNA. Bull Environ Contam Toxicol 1989;42:402-8.
15. Zimmermann T, Kulla HG, Leisinger T. Properties of purified orange IIazoreductase the enzyme initiating azo dye degradation by Pseudomonas KF46. Eur J Biochem 1982;129:197–203.
16. Wuhrmann K, Mechsner KI, Kappeler T. Investigation on rate determining factors in the microbial reduction of azo dyes. Eur J Appl Microbiol 1980;9:325-38.
17. Sathish S, Joshua Amarnath D. Experimental studies on decolorization of azo dye in textile effluent by using bio remediation technique. Int J ChemTech Res 2014;6:5114–7.
18. Harshad S Lade, Tatoba R Waghmode, Avinash A, Kadam, Sanjay P Govindwar. Enhanced biodegradation and detoxification of disperse azo dye Rubine GFL and textile industry effluent by defined fungal-bacterial consortium. Int Biodeterior Biodegrad 2012;72:94–107.