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Optimization of Acid Blue 74 Biodegradation by the Newly Isolated *Pseudomonas* sp

S. Illanjiam[1], D. Arunachalam Kantha[2] and D. Ragunathan[3]

[1]Department of Microbiology Hindustan College of Arts and Science, Chennai, India.
[2]Departmrnt of Inter Disciplinary Research, SRM University, Chennai, India.
[3]Department of Biotechnology, Prathyusha Institute of Technology and Management, Chennai, India.

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Dyes are widely found in the textile, printing, food, pharmaceutical, leather, cosmetics and many other industries. The release of these compounds into the environment is undesirable, not only because of their color, but also because many azo dyes and their breakdown products are toxic and/or mutagenic to life. Azo dyes are characterized by one or more azo groups (R1-N = N-R2) linking substituted aromatic structures. Several microorganism are able to transform Azo dyes to non colored product or even mineralize them completely under certain environmental conditions. The present study involves the study about the biodegradation of Acid blue 74 and the optimization of the degrading process.

Key words: Acid Blue 74 dye, *Pseudomonas* sp., Biodegradation.

Dyes are widely found in the textile, printing, food, pharmaceutical, leather, cosmetics and many other industries (Raffi *et al*., 1990). Almost 106 tonnes of dyes are produced annually around the world, of which azo dyes represent about 70% by weight. Azo dyes are characterized by one or more azo groups (R1-N = N-R2) linking substituted aromatic structures. The release of these compounds into the environment is undesirable, not only because of their color, but also because many azo dyes and their breakdown products are toxic and/or mutagenic to life (Shore 1996; Chung *et al*. 1992).

The disposal of dye waste waters poses one of industry's major problems, because such effluents contain a number on contaminants including acid or caustic, dissolved solids, toxic compounds and color. Of all these, color is the first contaminant to be recognized because it is visible to human eye (Gordon Mckay and Andrew., 1980). The high concentrations of dyes causes many waterborne diseases hence degradation becomes necessary (Anliker, R.1979).

However, many of the degradation technologies are cost-prohibitive and therefore are not viable options for treating large waste streams. Compared with chemical/physical methods, biological processes have received much interest because of their cost effectiveness, lower sludge production and environmental friendliness (Banat *et al*., 1996; Stolz 2001).

Detoxification of the pollutants may result in either complete mineralization of the pollutant or partial decomposition associated with the loss of undesirable biological activities. Basically, degradation means loss of properties. This loss of property can occur because of changes in the assemblies of molecules which form the compound or because of breaking of molecules or both. Therefore, it is easy to understand that several

* To whom all correspondence should be addressed.
Mob.: +91-9884062445
E-mail: raghu_drn@yahoo.co.in

situations have to be distinguished to talk in terms of degradation (Miller, R., Smyth, W.F., 2001).

Azo dyes are synthetic organic colorants and are characterizes by the presence of one or more azo groups (-N).They are the largest and most versatile class of dyes. Azo dyes are of the following types: 1)Monoazo dyes R - N = N – R1, 2) Diazo dyes R – N = N – R1 – N = N – R2, and 3) Polyazo dyes. Azo dyes are generally considered to be xenobiotic compounds which are rather recalcitrant against biodegradative processes in conventional sewage treatment systems. The recalcitrance of the azo dyes to biological degradative processes result in severe contamination of the rivers and ground water in those areas of the world with a high concentration of dyeing industries

Several microorganisms are able to transform Azo dyes to non colored product or even mineralize them completely under certain environmental conditions (Richardson, 1983). In many bacteria that catalyse the degradation of xenobiotics, the genes responsible for this ability are located in extra chromosomal elements, the so-called degradative or catabolic plasmids. The Azo dye degradation may occur by the aerobic reductive metabolism of Azo dys which requires specific enzymes (aerobic azo reductase) to Cleave the Azo bond and Mineralization of intermediates. The enzyme involved in this process is Azo reductase, which catalyzes the cleavage of the Azo bond to produce aromatic amines (Brown, 1981; Kulla, 1981).

Effective and economic treatment of a diversity of effluents containing azo dyes by biological methods is widely done with the help of microorganisms. The ability to degrade azo dyes has been shown to exist in *Aeromonas, Pseudomonas* sp, *Bacillus, Rhodococcus, Shigella* and certain fungal and algal species (Dubin & Wright, 1975; Gingell, and Walker, 1971).

The present study involves the study about the biodegradation of Acid blue 74, (**Synonym:** Indigo Carmine;C.I. 73015;
Chemical Name: 5,5'-Indigosulfonic Acid, disodium salt; 2-(1,3-Dihydro-3-oxo-5-sulfo2H-indol-2-ylidene-2,3-dihydro-3-oxo-1H-indole-5-sulfonic acid; **Chemical Formula:** $C_{16}H_8N_2Na_2O_8S_2$; (Chudgar. 1993), an Azo dye, by the *Pseudomonas* sp. and the optimization of the degrading process.

MATERIALS AND METHODS

Screening and isolation of organisms

The azo dye (Acid Blue 74) degrading bacteria were isolated from an activated studge system utilized to treat wastewater from a dyeing factory in Tirupur, Tamilnadu. The medium used was Nutrient agar with 0.1% Acid Blue 74.

10 grams of solid sample from the waste water treatment system was taken and mixed with 100ml of sterile distilled water. The soil suspension was mixed thoroughly on a rotary shaker for 30 minutes and was shaken gently before use. 1 ml of the sample was mixed with 9 ml of sterile distilled water in a test tube and serially transferred to 5 test tubes each containing 9 ml of sterile distilled water.

The sample from the last three dilutions (10^{-5}, 10^{-6}, 10^{-7}) of 1 ml was pipetted out into the petriplates. 0.1% of Acid Blue 74 was added to the plate and 20 ml of sterile melted agar was measured with sterile measuring cylinder and was poured into the plates, containing the dye and sample. The dominant organisms capable of degrading the dyes were isolated after incubation at 37°C.

Identification and characterization of isolated bacteria

The isolated organisms were subjected to gram staining, motility test, catalase test, oxidase test, and biochemical tests by the standard microbiological methods.

Growth and Maintenance of bacteria

The culture media used for the cultivation and maintenance of the isolated bacteria had the following chemical composition: Dextrose-20g,Peptone 10.0 g, Beef extract 10.0 g, Agar 15.0 g, Sodium Chloride 5.0 g in 1000 ml of Distilled water. The pH of the medium was 7.2 and the incubation temperature is 37°C.

Azo dye degradation

Mineral Salt Medium (MS Medium) used for the degradation studies throughout the experiment had the following composition: K_2HPO_4 1.0 g.,$(NH_4)SO_4$ 5.0g, NaCl 1.0g.,$MgSO_4.7H_2O$ 0.5g., $FeSO_4.7H_2O$ 0.1 g., ZnSO4.$7H_2O$ 0.1g.,$CuSO_4.5H_2O$ 0.1g., $MnSO_4.7H_2O$ 0.1g., in 1000 ml distilled water. The pH of the medium was 7.2.

Dye Stock Solutions

0.2% of Acid Blue 74 stock solutions were

prepared (2 mg/ml).100 ml of distilled water taken and sterilized in a conical flask. 2g of dye powder weighed and added to it under sterile conditions. Then the dye solution was filter sterilized using 0.22 µm Millipore membrane filters and stored in a sterile container. It was shaken well each time before use so that they are not settled.Working Concentration of the Dyes from the stock solutions of dyes for25 ml of MS medium 25 ppm = 0.125ml, 50 ppm = 0.250 ml, 100 ppm = 0.500 ml.

Assay

Dyes were scanned spectrophotometrically in the wave length range of 400 – 800 nm to find out the wavelength showing maximum absorbance. It was found out to be 420 nm for ACID BLUE 74. Degradation of the dyes were followed by taking absorbance in the respective wavelengths throughout the experiment.

4 ml of the samples from each experiment flask were taken separately after inoculation at 0 day and were subjected to centrifugation. The supernatant constituting the dyes were taken and its absorbance value read spectrophotometrically at 420nm (ACIDBLUE74).

The extent of color removal in the culture medium was assessed through the decrease in dye absorbance of the supernatant for the subsequent days (1st day, 2nd day 3rd day & 4th day).

Estimation of percentage of degradation

For analysis of degradation, the 0day absorbance value and final day absorbance (4th day) were obtained after inoculation. The percentage of degradation was calculated using the formula,

$$\text{Percentage of degradation} = \frac{\text{Initial absorbance-Final absorbance}}{\text{Initial absorbance}} \times 100$$

Optimization of Dye degradation

Effect of Various Carbon sources on Dye degradation

The effect of following Carbon sources on the growth and dye degradation by *Pseudomonas* sp. was studied: Glucose, Mannitol, Maltose, Mannose, Sucrose and Xylose.

Effect of Various nitrogen sources on Dye degradation

The effect of following Nitrogen sources on the growth and dye degradation by *Pseudomonas* sp. were studied: Tryptone, Peptone, Yeast extract and Beef extract

Effect of Temperature on Dye degradation

The effect of following temperatures on the growth and dye degradation by *Pseudomonas* sp. was studied: 25°C, 30°C, 35°C, 40°C and 45°C.

Effect of pH on dye Degradation

The effect of following pH on the growth and dye degradation by *Pseudomonas* sp. was studied: 5.5, 6.0, 6.5, 7.0 and 7.5.

RESULTS

Identification and characterization of Bacteria

Growth was observed on both nutrient agar (basal medium) and Pseudomonas agar (selective media). The colonies on both the plates were more or less similar and were 1-2mm in diameter, circular, pale white, convex, buttery, translucent, TSI, indole, VP negative, and citrate positive. The biochemical results were tabulated in Table 1. From the above characteristics, the

Table 1. Biochemical results for the isolated Bacteria

S. No.	Charecteristics	Result
1	Morphology(Microscopic)	Rod
2	Colony Morphology	Smooth round cology
3	Staining	Negative
4	Spore formation	Negative
5	Aerobic	Positive
6	Growth at 5Degree C	Positive
	10 Degree C	Positive
	30Degree C	Positive
	40Degree C	Positive
	50 Degree C	Negative
7	Pigment	Positive
8	Catalase	Positive
9	Oxidase	Positive
10	Indole	Positive
11	MR	Positive
12	VP	Negative
13	Citrate	Negative
14	O/F	Oxidative
15	Gelatin	Negative
16	Coagulase	Negative
17	Nitrite	Positive
18	TSI	Negative
Result	Identified Organism	*Pseudomonas* sp.

Table 2. Acid blue degradation

S. No.	Concentration	% Degradation *Pseudomonas* sp
1.	25	**94.4**
2.	50	91.8
3.	100	89

isolated strain was identified as *Pseudomonas* species.

Effect of Carbon source on Acid blue 74 degradation

The effect of various carbon sources on the biodegradation of Acid Blue 74 is given in Table 3 and Fig. 1.

Table 3. Effect of carbon sources on degradation

S. No	Conc. of Dye (PPM)	Sources of Carbohydrate	% Degradation *Pseudomonas* sp
1.	25	Glucose	96.2
2.	25	Mannitol	92.5
3.	25	Maltose	85.2
4.	25	Mannose	64
5.	25	Sucrose	62.8
6.	25	Xylose	60

Table 4. Effect of Nitrogen sources on degradation

S. No	Conc. of Dye (PPM)	Sources of Nitrogen	% Degradation *Pseudomonas* sp
1.	25	Tryptone	68
2.	25	Peptone	64.3
3.	25	Yeast extract	59
4.	25	Beef extract	38

Effect of nitrogen sources on Acid Blue 74 degradation

The effect of various Nitrogen sources on the biodegradation of Acid Blue 74 is given in Table 4 and Fig. 2.

Effect of various Temperature on Acid Blue 74 degradation

The effect of various temperatures on the biodegradation potential of Pseudomonas sp on Acid Blue 74 is given in Table 5.

Effect of various pH on Acid Blue 74 degradation

The effect of various pH on the biodegradation potential of Pseudomonas sp on Acid Blue 74 is given in Table 6.

Table 5. Effect of temperature on dye degradation

S.No.	Temperature	% Degradation
1	25°C	86.8
2	30°C	88.9
3	35°C	95.1
4	40°C	91.3
5	45°C	89.7

Table 6. Effect of pH on dye degradation

S.No.	pH	% Degradation
1	5.5	85.2
2	6.0	86
3	6.5	88.2
4	7.0	91.6
5	7.5	89.9

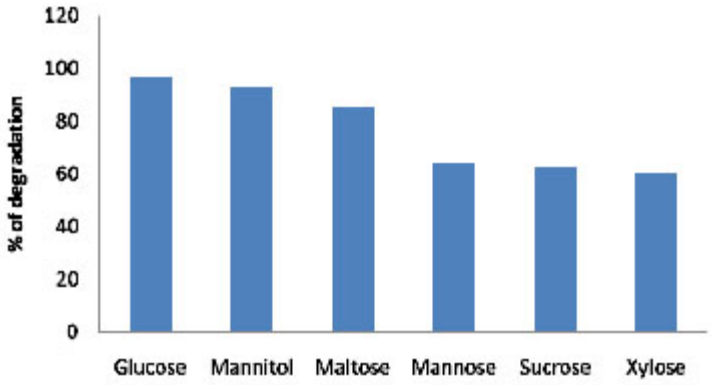

Fig. 1. Effect of carbon sources on dye degradation

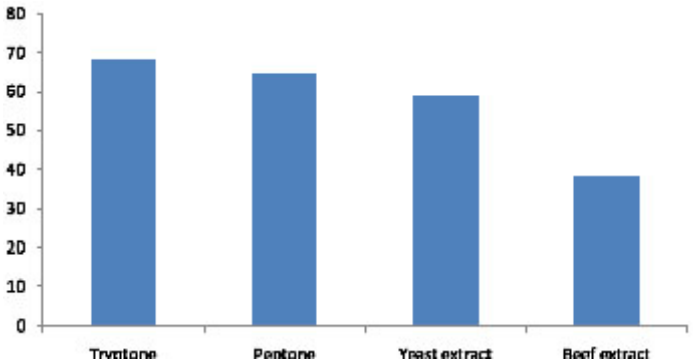

Fig. 2. Effect of Nitrogen sources on dye degradation

DISCUSSION

The isolate was identified as *Pseudomonas* sp. the maximum dye degradation was seen with 25ppm (94.4%) followed by 50 ppm(91.8%) and 100 ppm (89%), indicating that the decrease in biodegradation with increase in dye concentration . Of the various carbon sources Glucose (96.2%) and Mannitol (92.5%) were found to give best results. Among the nitrogen sources Tryptone (68%) and Peptone (64.3%) were shown to give better results when compared to other nitrogen sources.

CONCLUSION

The isolate was Pseudomonas sp and the best degradation of Acid Blue 74 was achieved with 25 ppm concentration of the dye and glucose found to be the best carbon source and trypton was the best nitrogen sources. The optimum temperature was 35°C and the pH was 7.0.

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