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MICROBIAL DECOLORIZATION OF DYE EFFLUENT

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Abstract - *The inlet and outlet of two water samples were taken from a dyeing factory (input to the dyeing unit and output from effluent treatment plant) in Sukkaliyur, Karur, Tamil Nadu, India. The water samples were analyzed according to standard water testing methods and the results of both the samples were compared with CPHEEO (Central Public Health and Environmental Engineering Organization) standards. The two samples were considered as not potable with respect to tested water parameters. The outlet of the dye effluent treatment unit was used for microbial decolorization. In this study, bacterial strains namely Bacillus subtitles , Pseudomonas aeruginosa and fungal strains namely Aspergillus flavus, Candida albicans were used for decolorization of azo dyes(azo dyes account for majority of textile dyestuff). Bacterial and fungal strains were isolated from the dye effluent itself. Decolorization of the textile dye effluent was analaysed by varying parameters like pH, temperature and dye concentration. The most suitable value of the above parameters was reported for maximum decolorization. The variation of other water parameters like Biological oxygen demand, Chemical oxygen demand and Nitrogen content were studied at the reported optimum level of basic parameters (pH, Temperature, Concentration) . The results were in favour of future reliability of textile industry on biodegradation of dye effluents. Among fungal strains Aspergillus flavus showed maximum decolorization of 82 % and among bacterial strains Bacillus subtitles showed maximum decolorization of 84%. This method was established to be the cheapest and most eco-friendly method of treatment of dye effluent.*

Key Words: Decolorization, Bacterial, Fungal isolates, Effluent.

1.INTRODUCTION

Textile industries are the major source of dye effluent. Approximately 10,000 different dyes are used industrially and about 0.7 million tons of synthetic dyes are produced annually, worldwide. The textile industry annually discharges 30,000 to 150,000 tons of dyes in water bodies causing pollution. Textile finishing generates a large amount of waste water containing dyes and represents one of the largest causes of water pollution, as 10 – 15 % of the dyes are lost in the effluent during the dyeing process. Dye imparts colour and is thus visually identifiable in water. Colour causes hindrance in light penetration which subsequently inhibits photosynthesis. This causes depletion of dissolved oxygen and deterioration of water quality thus adding toxicity to water and aquatic life. Dyes also have adverse impact on Total Organic carbon (TOC), Biological Oxygen Demand (BOD), Chemical Oxygen Demand (COD) of water bodies. Eventually, there is increase in organic load which leads to eutrophic condition in water bodies. Degradation of dyes is not easy due to its complicated aromatic structure and stability to sunlight, oxidizing agents and microorganisms. Most azo dyes are toxic, carcinogenic and mutagenic. When these compounds enter into human body, it causes hypertension, renal damage, and cramps. Therefore, treatment of dye effluent is important. Physico – chemical methods are not only costly but also have limited applicability and produce secondary sludge. Biological processes have been receiving increasing interest due to their low cost, effectiveness and environmental friendly nature. In the present investigation, two bacterial and fungal strains were isolated and identified from dye effluent in Sukkaliyur, Tamil Nadu, India and used for decolorization of the same.

2.Materials and methods

2.1.Sample collection

The raw dye effluent was collected from a dyeing factory in Sukkaliyur, Karur, Tamil Nadu, India by using sterile sample bottles and transported to the laboratory within 24 hrs of collection. The sample was stored at low temperature for further analysis.

2.2.Water parameters

Turbidity and Total dissolved solids (TDS) were measured by turbidity meter and gravimetric method respectively. Electrical conductivity was measured using an electrical conductometer and pH using a pH meter. Phenolphthalein alkalinity (PA), Total alkalinity (TA), hardness, chloride, Chemical Oxygen Demand (COD), Biological Oxygen Demand (BOD) and Dissolved oxygen (DO) were measured by titration methods. The amount of Sodium, Potassium, Iron, Manganese, free ammonia, Nitrate, Sulphate and phosphate was measured by using spectrophotometer method. Fluoride was measured by visual comparison method.

2.3.Enumeration of total microbes in dye effluent

Total microbial count in the dye effluent sample serially diluted (10^{-1} to 10^{-9}). One mL from the dilution 10^{-3} was plated in nutrient agar plates using spread plate method and incubated at 37°C for 24 hrs. Potato Dextrose Agar (PDA) for fungal counts incubated at 28°C for 48 hrs. Colonies on the plates were measured by using colony counter.

2.4.Enumeration of dye degrading microbes

The dye effluent was serially diluted. One µL of sample from dilution 10^{-3} was plated on pre dried mineral salt agar and Potato Dextrose Agar (PDA) using spread plate technique. A filter paper saturated with sterile dye effluent sample was aseptically placed on the inside of inverted Petri dishes and culture plates were incubated for 4 days. Plates yielding 30 to 250 colonies were enumerated for bacterial isolates and 15 to 105 colonies for fungal isolates.

2.5.Isolation and Identification of bacterial sample

The bacterial samples were isolated from dye effluent samples, 0.1 dilution sample was inoculated on nutrient agar plates and incubated at 37°C for 24 hrs. The isolated cultures were identified by biochemical tests.

2.6.Isolation and Identification of fungus

Fungus was isolated from dye effluent sample. After serial dilution 0.1 mL of sample was inoculated on agar plates and kept for incubation at 24°C for 48 hrs. Then the colony appearance was noted. For identification of fungus, staining by Lactophenol Cotton Blue (LCB) was performed. Phenol kills fungi and lactic acid increases preservation chino.

2.7.Assay of decolorization activity

The fungal and bacterial strains were grown on PDA and nutrient agar plates and were streaked on plates containing dyes in media. Decolorization of the dye was visually observed for the extent of zone clearing on the plates. The percentage of dye decolorization by the cells was done using the modified method of Yatome et al.,(1991). Cultures were grown in 50 mL of broth overnight at 37°C and 80 rpm to an Organisation Development (OD) of 1.00 at 600 nm. The cultures were centrifuged at 10,000 rpm for 10 min and washed twice with sterile saline (0.85%) and resuspended in 10 mL of saline solution. 0.1 mL of the inoculum was added to the broth containing dye and incubated at 37°C, 85 – 110 rpm for 24 hrs. The supernatant was collected after centrifugation for absorbance measurement at respective wavelengths. This works for bacterial culture and for fungal culture the incubation temperature is 37°C and the time is 48 – 96 hrs. Percentage decolourization was calculated as follows:

% decolorization =

{(Intial OD – Final OD) * 100} ÷ Initial OD

2.8.Effect of pH on decolorization

Sterile nutrient broth of different pH 4,5,6,7 was inoculated with 10% inoculum and incubated at 37°C under static condition. The dye concentration was kept at 100 mg/L. All decolorization experiments were performed in all sets. % decolorization was measured as mentioned earlier.

2.9.Effect of temperature on dye decolorization

Sterile nutrient broth of pH 7 was inoculated with 10% inoculum and filter sterilized dye at 100 mg/L was added after sterilization. The broth was incubated at 28°C, 37°C and 46°C. %decolorization was mentioned as earlier.

2.10.Effect of dye concentration on dye decolorization

In order to examine the effect of initial dye concentration on decolorization under static condition 50 – 100 mg/L of the dye effluent was added to sterile nutrient broth inoculated with 10 % of the respective bacterial or fungal strain. It was incubated at 37°C. % decolorization was measured.

% decolorization =

(Initial absorbance–Final absrobance) * 100 ÷ Initial observance

2.11.Analysis of physico-chemical parameters of dye effluent sample

The dye effluent sample was used for various physico-chemical analysis viz., colour of the sample. Odour by direct smelling of the sample. Standard thermometer was used for temperature measurements. BOD, COD, Nitrogen content were also analysed.

2.12.Biological Oxygen Demand

Adjust the pH of water to neutrality by using 1N acid or 1N alkaline solution. Fill the water sample in 6 BOD bottles without bubbling. Add 1 mL of Allyl thiourea to each bottle. Determine dissolved oxygen content in 3 of the 6 BOD bottles by titration method. Take the mean of 3 readings (D1). Incubate the rest 3 bottles at 37°C in a BOD incubator for 3 days. Estimate the oxygen concentration in all 3 incubated samples. Take the mean of 3 readings (D2). Calculate the BOD of water in mg/l using the formula given below

BOD (mg/l) = D1 – D2

Where D1 = Initial dissolved oxygen in sample (mg/l)

D2 = Dissolved oxygen in water sample (mg/l) after 3 days of incubation

Here, instead of water, we use dye effluent.

2.13.Chemical oxygen Demand (COD)

Take three, 100 mL conical flasks and pour 50 mL of water sample in each. Simultaneously run distilled water blank standards (also in triplicate). Add 5 mL of potassium dichromate solution in each of the 6 flasks. Keep the flasks in water bath at 100°C for 1 hour. Allow the sample to cool for 10 minutes. Add 5 mL potassium iodide solution in each flask. Add 10 mL of dilute sulphuric acid in each flask. Titrate the contents of each flask with 0.1 M of sodium thiosulphate solution until the appearance of pale yellow colour. Add 1 mL of starch solution to each flask. Titrate again with 0.1 M sodium thiosulphate until the blue colour disappears completely. Find out the COD (mg/l) of water sample by using the following formula

COD of the sample = $8*C*(V_A-V_B)*100 \div S$

Where

C = Concentration of titrant (0.1 M)

V_B = Volume of titrant used for sample (mL)

V_A = Volume of titrant used for blank (mL)

S = Volume of sample taken (mL)

Table 1: Water parameters of inlet and outlet

1 – Output

Sample	Appearance	Odour	Turbidity	TDS
1	Blockish, Turbid	Unobjectionable	15	7885
2	Slightly brownish	Unobjectionable	4	3411

Sample	Total hardness (as $CaCO_3$) mg/L	Sodium (as Na) mg/L	Potassium (as K) mg/L	Calcium (as Ca) mg/L
1	3300	836	25	840
2	1250	336	18	320

Sample	Magnesium (as Mg) mg/L	Iron (as Fe) mg/L	Manganese (as Mn) mg/L	Free ammonia (as NH_3) mg/L
1	288	9.26	NIL	0.01
2	108	0.80	NIL	0.00

2- Input

Sample	Sulphate (as SO_4) mg/L	Phosphate (as PO_4)mg/L	Tidy's test (as O) mg/L	BOD mg/L
1	522	057	0.28	213
2	450	0.12	0.16	81

Sample	EC	pH	Alkalinity P ($CaCO_3$)	Alkalinity total (as $CaCO_3$) mg/L
1	11264	7.44	0	2500
2	4873	7.20	0	400

Sample	COD mg/L	D.O mg/L
1	700	2.6
2	198	3.9

2.14.Enumeration of total dye utilizing microbes in dye effluent samples

Table 2 : Total microbial count

S.No	Sample	Microbial counts (CFU g^{-1})
1.	Bacterial sample	1.98×10^3
2.	Fungal sample	0.76×10^3

2.15.Isolation and identification of bacterial and fungal sample

The bacterial isolates were identified based on biochemical characterization. The species of Bacillus, Pseudomonas were more dominantly isolated among bacteria and among fungal culture Aspergillus flavus, Candida albicans were isolated.

Table 3: Biochemical tests for isolates

S.No	Biochemical tests	Bacillus subtilis	Pseudomonas aeruginosa
1.	Indole test	-	-
2.	Methyl red test	-	-
3.	Voges-proskauer	-	-
4.	Citrate test	+	+
5.	Catalese test	+	+
6.	Oxidase test	-	+

2.16.Effect of pH on decolorization

Table 4 : Effect of pH variation on decolorization in %

S.No	Bacterial/Fungal strain	pH 4	pH 5	pH 6
1	Bacillus subtilis	42 %	56 %	63 %
2	Pseudomonas aeruginosa	25 %	30 %	41 %
3	Aspergillus flavus	76 %	83 %	74 %
4	Candida albicans	30 %	48 %	59 %

S.No	Bacterial/Fungal strain	pH 7	pH 8
1	Bacillus subtilis	84 %	77 %
2	Pseudomonas aeruginosa	52 %	43 %
3	Aspergillus flavus	67 %	52 %
4	Candida albicans	65 %	55 %

Effect of temperature on decolorization (%)

Table 5 : Effect of temperature variation on decolorization in %

SS.No	Bacterial /Fungal strain	28^{0}C	37^{0}C	46^{0}C
11	Bacillus subtilis	60 %	83 %	44 %
22	Aspergillus Pseudomonas aeruginosa	44 %	53 %	41 %
33	flavus	65 %	80 %	59 %
44	Candida albicans	52 %	68 %	49 %

Effect of dye concentration on decolorization :

Table 6 : Effect of dye concentration variation on decolorization in %

S.No	Bacterial /Fungal strain	50 mg/L	100 mg/L
1	Bacillus subtilis	83 %	78 %
2	Pseudomonas aeruginosa	54 %	43 %
3	Aspergillus flavus	82 %	78 %
4	Candida albicans	67 %	60 %

S.No	Bacterial /Fungal strain	150 mg/L	200 mg/L
1	Bacillus subtilis	70 %	60 %
2	Pseudomonas aeruginosa	39 %	28 %
3	Aspergillus flavus	72 %	61 %
4	Candida albicans	51 %	43 %

Percentage decolorization at optimum pH, Temperature, Concentration

Table 7 : Maximum decolorization at optimum pH,temperature,dye concentration

SS.No	Bacterial /Fungal strain	Maximum Decolorization
11	Bacillus subtilis	84 %
22	Pseudomonas aeruginosa	53 %
33	Aspergillus flavus	82 %
44	Candida albicans	67 %

Pysicochemical parameters BOD variation (mg/L)

Table 8 : Variation of BOD in mg/L

S.No	Bacterial /Fungal strain	IInitial	3 days	6 days
11	Bacillus subtilis	446	37	32
22	Pseudomonas aeruginosa	446	36	35
33	Aspergillus flavus	446	38	36
44	Candida albicans	446	39	37

Table 9 : Variation of COD in mg/L

SS.No	Bacterial /Fungal strain	IInitial	3 days	6 days
11	Bacillus subtilis	9920	540	510
22	Pseudomonas aeruginosa	9920	700	610
33	Aspergillus flavus	9920	610	513
44	Candida albicans	9920	650	588

SS.No	Bacterial /Fungal strain	9 Days	12 Days
11	Bacillus subtilis	25	20
22	Pseudomonas aeruginosa	31	27
33	Aspergillus flavus	27	23
44	Candida albicans	29	25

COD variation (mg/L)

SS.No	Bacterial /Fungal strain	9 Days	12 Days
11	*Bacillus subtilis*	315	230
22	*Pseudomonas aeruginosa*	570	480
33	*Aspergillus flavus*	330	247
44	*Candida albicans*	420	332

Nitrogen content (mg/Kg)

Table 10 : Variation of Nitrogen content in mg/Kg

SS.No	Bacterial /Fungal strain	IInitial	3 days	6 days
11	Bacillus subtilis	119.8	17.2	16.5
22	Pseudomonas aeruginosa	119.8	18.7	18.1
33	Aspergillus flavus	119.8	17.4	16.9
44	Candida albicans	119.8	18.1	17.2

SS.No	Bacterial /Fungal strain	9 Days	12 Days
11	Bacillus subtilis	14.1	13.3
22	Pseudomonas aeruginosa	17.4	16.8
33	Aspergillus flavus	14.4	13.8
44	Candida albicans	16.1	14.9

3.DISCUSSION

The tested water parameters were not potable as per CPHEEO standards. All the bacterial and fungal isolates showed maximum decolorization at pH 7 except for *Aspergillus flavus* which showed maximum decolorization at pH 5. All starins showed maximum decolorization at a temperature of 370C and at a dye concentration of 50 mg/L. Among bacterial strains, *Bacillus subtilis* showed maximum decolorization of 84 % and among fungal strains, *Aspergillus flavus* showed maximum decolorization of 82 %. BOD, COD and Nitrogen content varied constantly and at the end of 12 days their values matched the discharge standards given by Central pollution Control Board.

4.CONCLUSION

Bacterial decolorization of azo dyes is often initiated by cleavage of azo bonds by azoreductases which are followed by aerobic degradation of resulting amines. Fungal decolorization of azo dyes mainly occurs from lignin peroxidise activity under aerobic conditions. Some of the amines produced from these type of reaction are potentially carcinogenic. However, this is a report that shows formation of a product from microbial metabolism of dye. The use of microorganisms for removal of synthetic dyes from industrial effluents offers considerable advantages. This process is relatively inexpensive, running costs were low and the end products were completely mineralized with no toxicity. Microbial consortium has become a very good source for the textile industry in getting rid of effluent problem by biodegradation and decolorization.

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