# Microbial decolorization of tannery waste water

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### Abstract

One of the important parameters to be removed in tannery wastewater is color which is generally dark brown. Color removal by the mixed culture (MC) received from secondary basin of the tannery waste water plant, salt tolerant microorganisms (STM), mixed culture with sorbent (MCS) and salt tolerant microorganisms with sorbent (STMS) were performed in SBR. It was clear that the salt tolerant microorganisms have better ability to remove the color using MC, MCS, STM and STMS by the range of 54%, 84%, 78.6% and 94% respectively.

Addition of wheat bran sorbent in SBR effectively increased the removal efficiency. The average color removal efficiency using MCS and STMS was increased by 25 - 30% and 6 - 15% respectively. This enhanced capacity in color removal was because of the sorption of dye molecules on the surface of wheat bran sorbent. The results confirmed that both biodegradation and adsorption took place in the reactor. Adding wheat bran as sorbent was an efficient method for permitting uninterrupted working conditions of bio sorption method in the presence of chromium denoted by high color removal efficiencies.

**Keywords**: De colorization, Salt tolerant microorganisms, SBR and Sorbent.

## Introduction

Color is the most prime indicator of pollution in waste water; release of huge colored effluent is aesthetically displeasing and might damage the receiving water bodies by impeding penetration of light. Every year 10 lakhs tons of man-made dyes are generated around the world; 10-15% are discharged into the environment causing great loss to the environment. Tannery industry effluent is ranked as the most polluted water of the entire industrial sector in terms of quantity and composition<sup>1,2</sup>. It is basically dark brown in color and has a high amount of organic components differing based on the chemicals utilized<sup>3</sup> which causes environmental pollution<sup>4</sup>. It creates ecological imbalance to the receiving water body along with organic loading.

In tanneries, synthetic dyes are preferred than natural dyes due to their less cost, easier production, variety of shades and fastness coloring. Huge volumes of dyes used in tanneries are predominantly xenobiotic and in few cases mutagenic and carcinogenic in nature<sup>5,6</sup> cause allergic effects.<sup>7,8</sup>

Traditionally both physical and chemical treatment technologies are used to treat the synthetic dyes present in the tannery waste water which include coagulation, ozonation<sup>9</sup>, precipitation, adsorption by activated charcoal, ultra filtration, nanofiltration<sup>10</sup>, electrochemical oxidation and electro coagulation<sup>11,12</sup>. But industries are not adopting these treatment processes because of their high cost, less efficiency and ineffectiveness of various dyes. Hence developing an efficient treatment technology is the need of the hour.

However, *in situ* degradation is a newer technology under the biodegradation methods. In this method, treatment of wastewater is being carried out by the microbes isolated at the site of pollution <sup>13, 14</sup>. As these microbes were generated at the dye infected effluent of own industry, microbes can perfectly match for existing conditions. Therefore, these microbes can be effectively utilized to create wastewater treatment system mixed with dyes.

In Indian CETP's, mainly ASP and up flow anaerobic sludge blanket processes are employed to treat tannery waste water biologically. The SBR is an efficient ASP utilized for the treatment of hyper saline wastewater<sup>15</sup>. This biological treatment method is easy to exploit, simple function, requires less space and is having high flexibility in procedures<sup>16</sup>. The aim of this research is to identify and evaluate the performance of microbes similar to the natural microbes of secondary basin treating the tannery wastewater and to study the feasibility for reducing tannery colored waste water by sequential batch reactor. Thus, the microbial decolorization by SBR processes using salt tolerant microorganisms has been employed to combat the pollution of colored industrial liquid discharges from the tanning industry.

## **Material and Methods**

All the chemicals used in this work were of the best quality and grade available. Double distilled water was used throughout the experimental work. Tannery effluent was received from Ranipet Tannery Effluent Treatment Co. Ltd., Walajah, Tamil nadu, India and it was stored at 5°C. The wastewater was analyzed in the laboratory for various parameters including color as per Standard Methods of Analysis<sup>17</sup>. The seed sludge for the batch degradation study and for continuous degradation in sequential batch reactor was brought from the secondary basin treating the tannery wastewater. It was also stored at 5°C in a freezer and used for study.

*Pseudomonas aeruginosa, Bacillus flexus, Exiguobacterium homiense* and *Styphylococcus aureus* were obtained from Microbial Type Culture Collection Centre (MTCC),

Chandigarh and they were utilized for color removal. Senthilkumar et al<sup>18</sup> used these salt tolerant organisms for the treatment of tannery effluent in a batch reactor and found that they were efficient for the treatment process. Pure frozen microbes of bacterial strains were inoculated on 5 ml nutrient media at aseptic environment.

For an incubation of the broth, an arbitrary shaker (Remi, India) was used by maintaining the revolutionary speed of 150 rpm and temp of about 37°C for 1 day. Uniformed concentrated suspension was obtained at well-grown conditions used for the experiment as a source of incubation.

In this study, 21 various sorbents were screened for color removal from tannery wastewater using Plackett-Burman design. The sorbents used were agro wastes and algae. Their levels are indicated in table 1 and the design of research is shown in table 2. The selected sorbent was used in the SBR.

Experiments were carried out in a batch reactor to select a sorbent for the reduction of color in the tannery effluents based on Plackett-Burman design and the results are shown in table 3. From the Pareto chart (Fig. 1), it was found that the sorbent, wheat bran was effective to remove the color in tannery wastewater. Hence it was utilized in the SBR.

	Variable	Levels (g/L)					
Sorbent Code	Sorbents	Low (-1)	High (+1)				
А	Enteromorpha prolifera	1	10				
В	Hydrilla verticillata	1	10				
С	Hypnea valentiae	1	10				
D	Tamarind seed	1	10				
E	Wheat bran	1	10				
F	Rice husk	1	10				
G	Paddy straw	1	10				
Н	Hardwood sawdust	1	10				
Ι	Plant leaves	1	10				
J	Turbinaria ornata	1	10				
Κ	Pressmud	1	10				
L	Turninaria Conoides	1	10				
М	Sugarcane bagasse	1	10				
Ν	Coconut shell	1	10				
0	Bamboo waste	1	10				
Р	Grain sorghum	1	10				
Q	Tamarin wood	1	10				
R	Sargassum tenerrimum	1	10				
S	Chlorella bulgaris	1	10				
Т	Synechocystis sp.	1	10				
U	Scenedesmus obliquus	1	10				

Table 1Screening of sorbents



Fig. 1: Pareto chart for screening of sorbents

Run No.	A	В	C	D	E	F	G	Η	Ι	J	K	L	M	Ν	0	Р	Q	R	S	Т	U
1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1
2	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1
3	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1
4	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1
5	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1
6	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1
7	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1
8	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1
9	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1
10	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1
11	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1
12	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1
13	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1
14	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1
15	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1
16	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1
17	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1
18	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1
19	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1	-1
20	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1
21	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1
22	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1	-1	-1	1	1	-1	1	-1	1	1
23	-1	1	1	-1	-1	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1
24	1	1	-1	1	-1	1	1	1	1	1	-1	-1	-1	-1	1	-1	1	-1	-1	1	1

 Table 2

 Plackett –Burman design for screening sorbents

Table 3
Selection of sorbent based on Plackett - Burman design

Run Number	% Color removal	Run Number	% Color removal
1	61	13	44.3
2	46.3	14	43.5
3	40.1	15	55.2
4	51.2	16	56.9
5	46.5	17	41.2
6	50.8	18	44.3
7	52.1	19	52.3
8	48.7	20	57.8
9	61.2	21	44.6
10	49.6	22	55.6
11	47.5	23	48.3
12	38.2	24	51.2

**Experimental setup:** Two laboratory-scale plexiglass reactors of 10 L volume were used for the degradation study. Peristaltic pump was used for the filling and withdrawal of waste water by tubes. Fine air bubble diffuser was used for oxygen supply. The operating conditions were maintained based on our previous work<sup>19</sup>. Figure 2 shows the schematic diagram of the experimental procedure.

The reactions were carried out for 50 days by changing the initial substrate concentration as 25%, 50%, 75% and 100%

for the total concentration of 6240 mg COD/L. 24 hr cycle was followed for the entire treatment in which 1 h for filling, 20 hr for reaction, 2 hr for settling, 3/4 hr for withdrawal and 1/4 hr for idle were maintained. Different OLR were followed, during startup OLR was 2 kg COD/m<sup>3</sup>day for first 15 days and then raised to 2.5 kg COD/m<sup>3</sup> from the day of 16-31, further OLR was increased to 3.3 kg COD/m<sup>3</sup> from the day of 32-40 and finally 5 kg COD/m<sup>3</sup> till the experiments were over.

Consequently, the HRTs in the reactors were reduced by 5, 4, 3 and 2 days. Color reduction was performed by the method recommended by Bajpai et al.<sup>20</sup> In this method, sample was centrifuged at 10,000 rev/min for 30 min and the pH was maintained by 7.6. The absorbance was analysed at 465 nm and transformed into color units.

#### **Results and Discussion**

Color of the leather industrial effluent was generally dark brown. The color reduction depends on the microbes used. Color removal by the mixed culture (MC), salt tolerant microorganisms (STM), mixed culture with sorbent (MCS) and salt tolerant microorganisms with sorbent (STMS) were performed in SBR. The results obtained are shown in figs. 3-6. The figures show the inability of salt tolerant microorganisms to remove the color without the addition of sorbent. According to the data obtained, color reduction levels were improved by addition of wheat bran to SBR.

The average color removal efficiencies using MCS and STMS were increased by 25 - 30% and 6 - 15% respectively after adding wheat bran to SBR. This color removal increase was because of the sorption of dye molecules to the surface of wheat bran sorbent. The results showed that both biodegradation and adsorption take place in the reaction. The maximum color removal using MC, MCS, STM and STMS is 54%, 84%, 78.6% and 94% respectively.

Table 4 presents the maximum percentage of color removal for different initial substrate concentrations and at different HRTs. It also shows the corresponding OLR with respect to various HRTs. From the table it was observed that color removal efficiency decreased by increasing OLR. High reduction efficiencies were obtained in relatively shorter HRT for low effluent concentrations.



Fig. 2: Major Phases of the SBR Operational Cycle





Table 4Effect of OLR and HRT on Color Removal

Substrate Concentration	HRT, d	OLR, KgCOD/m <sup>3</sup> .d	% Color removal						
mg COD/L		0	MC	MCS	STM	STMS			
1560	5	0.312	54	84	78.6	94			
	4	0.39	45	81	76	91			
	3	0.52	39	76	74.6	87			
	2	0.78	36	69	66	78			
3120	5	0.644	51	78	63	88			
	4	0.805	48	75	60	85			
	3	1.07	43	69	55	80			
	2	1.61	33	61	41	71			
4680	5	0.936	46	69	60	82			
	4	1.17	43	65	56	79			
	3	1.56	38	60	52	74			
	2	2.34	31	53	37	65			
6240	5	1.308	40	60	53	78			
	4	1.635	37	56	50	75			
	3	2.18	33	53	45	71			
	2	3.27	24	45	32	61			

Figure 7 indicates the correlation between the OLR and the color reduction capacity for entire performance of the reactor. At the initial conditions, reduction level was found to be high (over 90 %) at low OLR. In the figure, the different percentage color reduction was obtained for the same OLR. It was because of various initial substrate concentrations maintained in the reactor.

Effect of HRT on color removal is shown in fig. 8. It was noticed from the figure that at high HRT, color removal obtained was to be high irrespective of microorganisms and inlet substrate concentration. When HRT was reduced, the percentage color removal also reduced, but the significant removal level occured when the HRT was reduced from 3 days to 2 days. The optimum HRT level was found to be 3 days for effective COD reduction for both less and high concentration.

Modeling for microbial decolorization of tannery wastewater in SBR: Conventional experimental methods were time consuming one for predicting the plant operational parameters. In this experiment, artificial neural network (ANN) modeling approach was used to get an idea of a real SBR. The radial basis function network was trained to predict the performance of SBR. The criterion used to evaluate the efficiency of the reactor was to reduce the color present in the tannery wastewater. The neural network was trained with the inlet substrate concentration, HRT and effluent ranges of the reactor.

The input layer consists of organic matter present in the influent and the output layer contains color reduction. 75% of data points were used to train the neural network and 25% were used to test the applicability of ANN at various hydraulic retention times of 5, 4, 3 and 2 days and for various inflow substrate concentrations of 1560, 3120, 4680 and 6240 mg COD/L.

For various intermediate days of operation, the experimental data were compared with the predicted neural network data. The capacity of the network was analyzed on the basis of an overall absolute error and root mean square error (RMSE) specified by the difference in the desired and actual outputs. A comparison between experimental and the ANN predicted values was depicted in fig. 9 to 12. From the figures, it was found that artificial neural network model based parameters match perfectly with the experimental values at different operating conditions of the reactor.

The use of neural network for the prediction of the effectiveness of SBR has been found to be valid and robust neglecting the purpose of the complex mathematical and computations incorporated in the modeling of the SBR performance.<sup>21</sup>







Salt tolerant microbes namely Pseudomonas aeruginosa, Bacillus flexus. Exiguobacterium homiense and Styphylococcus aureus were used in the sequential batch reactor for the color removal of tannery waste water. In continuous degradation, incorporating mixed microbes were received from the secondary sludge of tanneries at lower OLR (<0.312 kg COD/m<sup>3</sup>d); it was known that a maximum color removal was 54% for the initial concentration of 1560 mg COD/L. At higher OLR, greater than  $3.27 \text{ kg COD/m}^3 \text{d}$ , a maximum degradation of color were found to be 24%. Salt tolerant microorganisms have better ability to remove the color using mixed culture, mixed culture with sorbent, salt tolerant microorganisms and salt tolerant microorganisms with sorbent by the range of 54%, 84%, 78.6% and 94% respectively. The efficiencies of color reduction were increased significantly by the addition of wheat bran to SBR.

The average color removal efficiencies using mixed culture with sorbent and salt tolerant microorganisms with sorbent were increased by 25 - 30% and 6 - 15% respectively after adding wheat bran to SBR. This color removal was increased because of the sorption of dye molecules on the surface of wheat bran sorbent.

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