

## MICROBIAL DECOLOURIZATION OF TEXTILE DYE EFFLUENT COLLECTED FROM PALLIPALAYAM USING INDIGENOUS BACTERIAL ISOLATES

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### ABSTRACT

Pollution load from the dye effluent and proved as able dye decolorizers. But there is a need to conduct studies in The textile industry occupies an important place in the national economy and many such industrie are located in the southern parts of Tamilnaqdu state, providing livelihood to millions of peoples. But this industry requires enormous amount of water for its processing ,which generates large amount of its effluents which directly released in to the environment without pretreatment. So this effluents polluted the water bodies and soil also acts as potential threat to human health. So far many physicochemical traditional methods are available, but these methods are quite expensive and need skilled technicians. Hence there is a need to develop alternative affordable ecofriendly methods to overcome this issue. In connection of this concept in this investigation attempts have been to use potent bacterial strains isolated from textile dye effluent for decolourization and degradation of dyes. The results obtained from the physicochemical analysis indicates the polluted nature of the effluent. This effluent is treated with bacterial isolates namely Pseudomonas and Salmonella species. The results obtained from this study indicates that the above bacteria significantly reduced the colour and depth to know the exact molecular mechanism behind this concept.

**Keywords:** Textile Dye Effluent, Microbial decolourization, Pallipalayam

## 1. INTRODUCTION

The water pollution is a major problem in the global context. It has been suggested that it is the leading cause of deaths and diseases worldwide (Pink and Daniel, 2006; West and Larry, 2006). Hence due to this problem developing countries like India continue to struggle. In recent years due to industrialization and urbanization in India water problem has become gigantic (Mukesh, 2012). The textile industry occupies an important place in the economy of the India and other developing countries. But this textile processing consumes enormous quantity of water in its dyeing, finishing and manufacturing process. These effluents are mostly discharged in to nearby rivers, lakes, ponds etc. after minimal pretreatment with a high amount of pollutants

Azo dyes are the widely used dyes in industry . (Sun and Demirer , 2003). Approximately 10000 azo dyes are currently manufactured and it is estimated that at least 15 % of these

are released into the environment (Donlon et al., 1997). The chromos phores of azo dyes consist of heavy metals like chromium, copper and cobalt etc. Which are suspected to be mutagenic and carcinogenic. (Alaguprathana and Poonkothai , 2015).

There are several methods have been used in the treatment of textile effluents to achieve decolorization and the removal of pollutants. These physic chemical methods like filtration, specific coagulation, use of activated carbon and chemical flocculation are not effective and also quite expensive (Noor Fatima et al., 2015). But bio treatment offers a cheaper and Ecofriendly alternative for decolorization and removal of pollutants from the textile effluent. At present days, this microbial bioremediation of textile dye effluents using isolated microbes meet out the interest in ecologically friendly remediation techniques for textile dye effluents because of increased awareness of environmental issues throughout the world.

Efforts has been made to isolate bacterial cultures capable of degrading azo dyes started in the 1970s with reports of a bacillus Subtilis (Horitsu et al., 1977), followed by numerous

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bacteria (Yu et al., 2001).Chang et al., (2004) . However many studies have been conducted microbial bioremediation textile dye effluents, but these studies mainly concentrated on the industrial areas only. But these studies on urban areas like Pallipalayam in Tamilnadu were very meagre. So in this dissertation attempts has been made to Isolation , characterization and screening of bacterial isolates for microbial degradation of textile dye effluents collected from Pallipalayam, urban area of Tamilnadu.

## 2.MATERIALS AND METHODS

### Sample collection and preservation

The textile dye effluents were collected from two dyeing units in Pallipalayam region by adopted standard effluent collection method recommended by APHA (1995). These samples were transported to laboratory in plastic bottles and refrigerated at 4°C and used without any preliminary treatment. (Fig.1).



Fig.1a. Textile Industry Dye Effluent Collected from Pallipalayam



Fig.1b. Isolation and identification of bacterial strains from textile dye effluent sample



Fig.1b. Isolation and identification of bacterial strains from textile dye effluent sample



Fig.1c. Decolourization of textile dye effluent using *Pseudomonas aeruginosa* (sample-I)



Fig1d.. Decolourization of textile dye effluent using *Salmonella typhi* (sample-II)



Fig.1d. Decolourization of textile dye effluent using *Salmonella typhi* (sample-II)

### Analysis of physico-chemical parameters of textile dye effluent

The Physicochemical parameters of textile industry effluents like temperature, colour, odour, Ph, dissolved oxygen, BOD, COD, TDS were measured by adopting standard methods recommended by APHA (1995)

### Isolation and identification of dye decolourizing bacterial strains from textile dye effluent

Pour plate technique was used for the isolation of dye decolourizing bacteria (Harley prescott, 2002). Well grown bacterial colonies were picked and further purified by streaking. The isolated strains were maintained on Nutrient agar slants and stored at 4°C.

### Identification of the bacterial strains

Identification of the bacterial strains were carried out by the routine bacteriological methods like the colony morphology, observed the bacteria under the microscope, may be categorized according to the shape of their cells. These shapes include bacilli (rod-shaped) bacteria cells and cocci (spherical-shaped) bacteria cells by microbiology manual (Harley prescott, 2002). At the same time Gram staining test was employed to identify the different types of bacteria.

### Inoculum preparation for the screening of bacteria for decolourization of effluent

The suspension of 2 days old cultures of bacteria were used to investigate their abilities to decolourize dyes. They were prepared in saline solution (0.85% sodium chloride). A loop full of above bacterial cultures were inoculated into 50 ml of saline and incubated at 37°C for 3 hours (Harley prescott, 2002).

### Dye decolourization experiments

Dye decolourization experiments were carried out by adopted the method of Asad et al., (2007). Decolourization was assessed by measuring absorbance of the supernatant with the help of UV spectrophotometer at 430 nm.

### Decolourization assay

The percentage decolourization was calculated from the following equation,

$$\% \text{ Decolourization} = \frac{\text{Initial OD} - \text{Final OD}}{\text{Initial OD}} \times 100$$

## 3. RESULTS

The physico-chemical analysis of the textile dye effluent collected from various parts of Pallipalem revealed that the samples are orange and dark green in colour with unpleasant odour and having high temperature and chemical oxygen demand then compared with NEQS (2000) standards. Totally the physico-chemical analysis of the above textile dye effluents clearly indicates the toxic/pollutant nature. (Table.1 and Fig.1,2). There is a need to treat these effluents before released into the environment.

Table.1 Physico-Chemical Characterization of Textile Dye Effluent.

S. NO	NAME OF THE PARAMETERS	NAME OF THE DYE EFFLUENT SAMPLES		NEQS*
		SAMPLE -I	SAMPLE -II	
1.	Temperature ( C)	35 C	45 C	40 C
2.	pH	6.0	8.2	6-9
3.	Colour	Orange	Dark green	Colourless
4.	Odour	Unpleasan	green	Odourless
5.	Total dissolved solid (mg/l)	t	Unpleasan	3500
6.		1186	t	150
7.	Total Suspended Solids (mg/l)	120	1398	-
8.		103	165	-
9.	Dissolved oxygen (mg/l)	268	111	
10.	Dissolved carbon di oxide (mg/l)	750	224	156-400
	Chemical oxygen demand (mg/l)		857	80-250
	Biological oxygen demand (mg/l)	146	178	

\*NEQS= National Environmental Quality Standards (2000)

The screening of microbial population of the collected samples led to the isolation of two morphologically distinct bacterial strains (Fig.2). These isolated bacterial strains are cultured and identified as *Pseudomonas aeruginosa* and *Salmonella typhi* (Table.2). The above bacterial isolates are used for the decolourization of textile dye effluents.

In this dissertation 2 samples were taken to know the decolourization ability of two isolated bacterial strain for the period of 120 hours (Table.3,4 and Fig.3,4,5,6 and 7). There was maximum of 72.62 % of decolourization was achieved in sample-I for the incubation period of 120 hours by using *Pseudomonas aeruginosa*. But in sample-II the decolourization percentage is 85.38 % . At the same time *Salmonella typhi* bacterial isolate also screened for the

**Table.2** Biochemical Characterization Of Isolated Bacterial Strains From Textile Dye Effluent

S.NO	TEST	SAMPLE-I	SAMPLE-II
1.	Gram staining	Gram positive, rods	Gram negative straight rods
2.	Motility	Motile	Motile
3.	Catalase	+	+
4.	Oxidase	+	-
5.	Nutrient agar	Circular, Entire and translucent	Circular, moist smooth, translucent
6.	Glucose	-	+
7.	Lactose	-	+
8.	Sucrose	-	+
9.	Monital	+	+
10.	Indole	+	-
11.	Methyl red test	-	+
12.	Citrate	-	+
13.	Urease	-	-

**Table.3** Decolourization of textile dye effluent by using *Pseudomonas aeruginosa*

S.NO	SAMPLE	INCUBATION PERIOD	OD VALUE OF THE SAMPLE		% DECOLOURIZATION
			CONTROL	EXPRIMENTA L	
1.	SAMPLE-I	1 DAY (24 hours)	1.79	1.62	9.49
A		2 DAY (48 hours)	1.79	1.37	23.46
B		3 DAY (72 hours)	1.79	1.01	43.57
C		4 DAY (96 hours)	1.79	0.58	67.59
D		5 DAY (120 hours)	1.79	0.49	72.62
2.	SAMPLE -II	1 DAY (24 hours)	2.19	1.98	9.58
A		2 DAY (48 hours)	2.19	1.732	20.68
B		3 DAY (72 hours)	2.19	0.238	43.47
C		4 DAY (96 hours)	2.19	0.938	57.16
D		5 DAY (120 hours)	2.19	0.32	85.38

**Table.4**Decolourization of textile dye effluent by using *Salmonella typhi*.

S.NO	SAMPLE	INCUBATION PERIOD	OD VALUE OF THE SAMPLE		% DECOLOURIZATION
			CONTROL	EXPRIMENTAL	
1.	SAMPLE-I	1 DAY (24 hours)	1.79	1.437	19.72
A		2 DAY (48 hours)			
B		3 DAY (72 hours)	1.79	1.328	25.81
C		4 DAY (96 hours)			
D		5 DAY (120 hours)	1.79	0.936	47.70
	SAMPLE -II	1 DAY (24 hours)	1.79	0.485	72.90
E		2 DAY (48 hours)	1.79	0.39	78.21
2.		3 DAY (72 hours)			
A		4 DAY (96 hours)			
B		5 DAY (120 hours)	2.19	1.832	16.34
C		2.19	1.48	32.42	
D		2.19	1.03	52.96	
E		2.19	0.78	64.38	
			2.19	0.28	87.21

**Table.5**Decolourization Efficacy Of Isolated Indigenous Bacterial Isolates

S.NO	NAME OF SAMPLE	BACTERIAL STRAIN	% DECOLOURIZATION (DECOLOURIZATION EFFICACY)
1.	SAMPLE-I	a. <i>Pesudomonasaeruginosa</i>	72.62 %
		b. <i>Salmonella typhi</i>	78.21 %
2.	SAMPLE-II	a. <i>Pesudomonasaeruginosa</i>	85.38 %
		b. <i>Salmonellatyphi</i>	87.21 %

decolourization of textile dye effluent. Maximum of 78.21 % of decolourization was achieved in sample-I for the incubation period of 120 hours and 87.21 % of decolourization was achieved in sample-II by using *Salmonella typhi* bacterial isolate. However based on the above experimental results *Salmonella typhi* bacterial isolate is the potent decolourization agent then compared with *Pseudomonas aeruginosa* (Table.5).

#### 4.DISCUSSION

The degradation of environment is due to the discharge of highly polluted waste water from the textile industry (Bhagirath and Reddy, 2000). Millions of these untreated effluents from textile industry which directly mixed in to the rivers and lakes and alters the  $p^H$ , BOD, COD and colour of water resources (Alaguprathana and Poonkothai, 2015). In this dissertation textile dye effluent were collected from industrial areas of Pallipalayam town of Tamilnadu.

The textile dye effluent collected from two sites of Pallipalayam town found reasonably heavy load of pollutants such as high Temperature, TSS, COD etc. compared with that of recommends NEQS(2000) standards. These studies are similar to the studies of (Naeem et al.,2010). These effluent are rich in dyes particularly azo dyes. Which are xenobiotic in nature and also very difficult to remove from the effluent. Many physical and chemical technologies so far available for the degradation of these effluents, but these methods do not achieve total decolourization and also too expensive. At the same time the traditional biological waste water treatment methods also have low removal efficiencies (Robinson et al.,2001). Hence in recent years, considerable interest has been emerged in the screening of indigenous bacterial strains for dye biodegradation. In this investigation the isolated bacterial strains from the textile dye effluents have been used for the decolourization of textile effluent. In this study two bacterial strains namely *Pseudomonas aeruginosa* and *Salmonella typhi* were isolated. The consortium of the above indigenous bacterial isolates was used for the decolourization of textile dye effluent. In both the textile dye effluent samples (sample I &II) decolourization proceeded gradually even upto 5<sup>th</sup> day in effluent inoculated with bacteria. At the same time control showed no decolourization which confirmed that the decolourization is due to the metabolic activities of the introduced microbes and not due to abiotic factors. When each isolated bacterial strain is tested individually they showed remarkable decolourization potential. Where as there is a slight difference in the decolourization ability of 2 different bacterial strains. This may be due to the presence of a specific mutant gene and further study is needed to confirm this mechanism. These results contradicted the observations of previous investigators who suggested a synergistic role of other bacterial species in decolourization (Leena and Selvaraj, 2008; Knapp and Newby,1994). The main important mechanism involved in the textile dye decolourization by bacterial strains was the presence of an NADH dependent azoreductase enzyme present in the above bacterial isolates. The role of azoreductase enzyme in the decolourization of textile dye effluent was already demonstrated (Maier et al.,2004). The other enzymes present in the bacterial strains which are responsible for decolourization are peroxidases (Goszczyński et al.,1994) and laccases (Abdulla et al.,2000). The microbes

utilized carbon, nitrogen and sulphate found in effluent medium for their nutrition. Our experimental results correlated with the work of previous investigators (Willetts and Ansholt,2000., Vander zee et al., 2001). Hence our study may be useful for the development of more potent indigenous bacteria for the bioremediation of coloured effluents.

#### 5. CONCLUSION

Tamilnadu state having many textile towns like Pallipalayam. These towns are having many textile dye industries and releases its effluents directly into the nearby water bodies, which is a potential threat to the living organisms including human beings. Many physico chemical methods for the treatment of effluent was not effective, more expensive and produce large amount of sludge. Hence microbial bioremediation in an effective alternative technology for the treatment of these effluents. In this microbial bioremediation also all the bacterial strain do not have the dye degradation ability and some strains not able to survive in the areas contaminated with textile dye effluent. So there is a need to isolate the bacterial strains from textile dye effluent and develop effective consortium of the above bacterial isolates for the biodegradation of textile dye effluents. This study may fulfill the above need where isolated bacterial strains were used for the decolourization of textile dye effluents. There is a need to conduct more studies on identification and development of effective microbial consortium for the decolourization of dye effluent in lesser time period.

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