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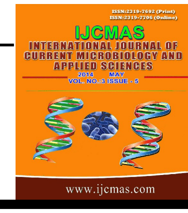

Original Research Article

Microbial Bioremediation of Heavy Metals From Textile Industry Dye Effluents using Isolated Bacterial Strains

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A B S T R A C T

Keywords

Textile dye effluent, Heavy metals, Microbial bioremediation

Today water pollution is one of the major global threats. Untreated industrial effluents discharged in to the environment pose a serious problem to the aquatic living organism and human beings also. Among pollution causing industries, textile industry achieve a major attention by environmentalists due to expenditure of large volume of water, utilization of dyes and chemicals during various processes of textiles manufacturing. Textile effluents contain carcinogenic aromatic amines, dyes, organic and inorganic materials. Almost all the traditional physicochemical methods do not provide effective solutions for the elimination of metals from textile industry effluents. But microbial bioremediation is a effective approach for elimination of heavy metals like Cadmium, Lead and Zinc from textile industry dye effluents. In this study four heavy metal resistance bacterial strains namely *Escherichia coli*, *Salmonella typhi*, *Bacillus licheniformis* and *Pseudomonas fluorescence* isolated from textile industry effluents were used for the bioremediation of heavy metals from the textile dye effluents. All the four bacterial strains effectively remove the heavy metals from the effluents.

Introduction

The textile industry is one of the industries that generate a high volume of waste water. Approximately 10, 000 different dyes and pigments are manufactured worldwide with a total annual market of more than 7×10^5 metric tones per year (Ahluwalia and Goyal, 2007). In India textile is one of the oldest establishments with nearly 1569 large cotton textiles industries, which gives employment to nearly thirty million people (Garg et al., 2012). India is the second largest exporter of dyestuffs, where ~ 80,000 tons of dyes and pigments are produced annually (Saraswathi and Balakumar, 2012). There are over 100, 000 verities of dyes such as acidic, basic, reactive azo dyes etc available in the market (Daneshwar et al., 2007). The textile dye effluent consists of heavy metals such as Cadmium, Lead and Zinc either in free ionic metals or complex metals, (Hill et al., 1993). This textile dye effluents cause serious environmental problems by absorbing light in receiving water bodies like streams, rivers and lakes etc and ultimately interfering with aquatic biological processes (Moore and Ramamoorthy, 1984). The water containing textile effluent used for irrigation contains heavy metals like Cd, Pb and Zn, which accumulate in various parts of plans that result in various clinical problems in animals as well as human beings including hepatic and renal system damages, mental retardation and degradation of basal ganglia of brain (FEPA 1991; Emongor et al., 2005). Environmental production agency, USA has determined that lead is a probable human carcinogen (APHA, 1992). Zinc is less toxic than compared with Cadmium and Lead.

There are many conventional Physico-chemical methods like Precipitation, Ion exchange, Electro-winning, Electro-coagulation, Cementation and Reverse osmosis are available for the removal of heavy metals from the industrial effluents. But all these methods are highly expensive and need skilled technicians. Hence bioremediation particularly microbial bioremediation is proved as an effective eco-friendly affordable technology for the removal of heavy metals from the textile dye effluents. In this investigation four bacterial strains namely *Escherichia coli, Salmonella typhi, Bacillus licheniformis* and *Pseudomonas fluorescence* isolated from textile dye effluent collected from Komarapalayam, Tamilnadu, India were used to remove the heavy metals such as cadmium, lead and zinc from textile industry dye effluents.

Materials and Methods

Textile dye effluent collection sites

Komarapalayam is an important textile town situated on the bank of Cauvery river (Ganges of South India) in Namakkal District, Tamilnadu, India. Komarapalayam lies between 11° 20'' and 11° 30'' northern latitude and between 77° 40'' and 77° 50'' eastern longitude. It is located about 405 km from Chennai. (Fig. 1)

Sample Collection

Samples were randomly collected from different areas of Komarapalayam (S_1, S_2, S_3, S_4, & S_5). The samples were collected in polyethylene bottles previously washed with 8M HNO3 and distilled water. The total volume of the bottle was filled completely and a cap was locked enough, so that no air space can be remained inside the bottles. The collected samples were shifted to the laboratory as soon as possible for the analysis of various physicochemical parameters. Some parameters like temperature, pH and colour were recorded at the sampling spot itself by adopted the method recommended by APHA (1992). The collected samples were preserved for further analysis.

Physicochemical characterization of textile effluents

Characterization of the effluent for various parameters such as chemical oxygen demand (COD), biochemical oxygen demand (BOD), total dissolved solids (TDS) and heavy metals (Cd, Zn & Pb) were analyzed by using the method of APHA (1998).

Isolation and identification of Bacteria

The bacteria present in the textile dye effluents were isolated through serial dilutions method recommended by Harley Prescott (2002). The isolated bacterial strains were cultured in nutrient agar medium (Hi media Laboratory, Mumbai). This isolated microorganisms were subjected to various biochemical procedures for its characterization.

Inoculum preparation

The suspension of 2 days old cultures of bacteria were used for the degradation of heavy metals from the textile dye effluents. They were prepared in saline solution (0.85% sodium chloride). A loopful of above bacterial cultures were inoculated into 50 ml of saline and incubated at 37°C for 3 hours.

Removal of heavy metals from textile dye effluents

Degradation of heavy metals experiments were carried out in 250 ml of separate flasks containing 100 ml of textile dye effluents collected from study sites S1, S2, S3, S4 and S5. The pH was adjusted to 7±0.2 using Nacl and H_2SO_4. Then, the flasks were autoclaved at 121°C for 15 minutes. The autoclaved flasks were inoculated with 5ml of bacterial inoculum of each isolates. The flasks were kept in mechanical shaker and incubated at 37°C for 5 days. 10 ml of the dye solution was filtered and centrifuged at 5000 rpm for 20 minutes. The heavy metal content was assessed by measuring supernatant with the help of Atomic Absorption Spectroscopy.

Heavy metal analysis

2 ml of treated dye effluent was taken in a boiling tube and was digested using 10 ml of triple acid solution (HNO_3, H_2SO_4 and $HClO_4$ in 9:2:1 proportion respectively) till the effluent becomes colorless. The digested sample was filtered using whatmann number 1 filter paper for two times made upto 50 ml and subjected for heavy metal assay using Atomic Absorption Spectroscopy (Mac: SL 176-Double beam Spectrophotometer) as per the standard method recommended by APHA, 1998. Three replication were maintained for each treatment. The percentage of degradation was calculated from the following equation,

$$\% \text{ Degradation} = \frac{\text{Initial amount} - \text{Final amount}}{\text{Initial amount}} \times 100$$

Statistical analysis

Data was statistically analyzed at 0.05 by one-way ANOVA using Microsoft excel.

Results and Discussion

Physico-chemical characterization of textile dye effluents

The textile dye effluents collected from different sites of Komarapalayam town indicates high level of pollution compared with standards of NEQS, (2000). The different samples were in orange, red, blue, dark green and dark black in colour. Almost all the sample having pungent fishy oder (Table 1). In the PH values of the effluents, there was a lot of variation which differ from 4.8 to 9.3 which indicates the acidic and baseic nature of the effluents. The BOD and COD values also very high in all the textile dye effluent samples. The heavy metals such as cadmium, lead and zinc content in the textile dye effluent of all the samples were very high. Hence all the textile dye effluent samples collected from study area indicated high level of pollution.

Isolation and characterization of bacterial strains from textile dye effluent

From the textile dye effluents collected from S1, S2, S3, S4 and S5 sites, the bacterial strains namely *Escherichia coli, Salmonella typhi, Bacillus licheniformis* and *Pseudomonas fluorescence* were isolated. The biochemical characterization of bacterial strains was given in Table. 2. This isolated microbial strains were cultured and developed bacterial colonies were used for the bioremediation of heavy metals from textile dye effluents.

Bioremoval of heavy metals-cadmium, lead and zinc from textile dye effluents
Biodegradation of Cadmium

In all the samples the Cadmium content was very high than compared with NEQS, (2000) standards. But after the inoculation of textile dye effluent samples with isolated bacterial strains there was a significant decrease in Cadmium content in all the samples. Same time *Bacillus licheniformis* showed maximum heavy metal removal ability (98.34%) than compared with other bacterial strains. *Salmonella typhi* showed maximum of 92.4%, *Pseudomonas fluorescence* showed maximum of 94.8% and *Escherichia coli* showed maximum of 92.06% of Cadmium removal ability from the textile dye effluents (Table. 3)

Biodegradation of Lead

In all the textile dye effluent samples collected from various study areas namely S1, S2, S3, S4 and S5, the heavy metal Lead concentration was very high. After the inoculation of effluent samples using above isolated bacterial strains there was a significant decrease in the Lead content from all the samples. *Bacillus licheniformis* showed maximum of 89.45% heavy metal removal ability, *Salmonella typhi* showed maximum of 92.15%, *Pseudomonas fluorescence* showed maximum of 94.32% and *Escherichia coli* showed maximum of 86.73% of Lead removal ability from all the above samples.

Biodegradation of zinc

The above bacterial strains were inoculated with textile dye effluent for the period of five days. In all the samples there was a significant decrease in the heavy metal contents. *Bacillus licheniformis* bacterial strains removes maximum of 96.14% of Zn from the treated samples. *Salmonella typhi* effectively removed the Zn from all samples. But maximum 91.78% of bioremoval was observed. *Pseudomonas fluorescence* and *Escherichia coli* also effectively removed the heavy metal Zinc from all the textile dye effluent samples. But maximum metal removal of 96.14 % was observed with *Pseudomonas fluorescence*, using *Escherichia coli* maximum metal removal of 93.27% was observed (Table. 5). In figure. 2 the percent degradation of heavy metals from textile dye effluent using bacterial strains were given.

Table.1 Physico-chemical analysis of the textile effluent samples

Parameters	collection sites					*NEQS Limit
	S1	S2	S3	S4	S5	
Colour	Orange	Red	blue	Dark green	Dark black	Colour less
Odour	Pungent	Fishy	Pungent	Fishy	Fishy	Odour less
TDS (mgl-1)	1213.00	3849.67	3286.00	2457.00	1836.00	3500
PH (mgl-1)	7.00	4.8	9.3	6.2	7.9	6-9
BOD (mgl-1)	243.00	1842.00	553.00	480.00	270.00	80-250
COD (mgl-1)	1088.00	2080.00	1728.00	1532.00	873.00	156-400
Cadmium (mgl-1)	24.51	8.25	23.14	32.68	9.12	0.1
Lead (mgl-1)	11.12	23.41	18.22	14.55	32.10	0.5
Zinc (mgl-1)	17.45	36.13	22.41	20.23	13.03	5

**NEQS – National Environmental Quality Standard (2000).*

Table.2 Biochemical characterization of isolated bacterial stains from textile dye effluents **(n=5)*

Test	Colony 1	Colony 2	Colony 3	Colony 4
Gram staining	Gram negative, straight rods	Gram negative, straight rods	Gram Positive, rods	Gram Positive, rods
Motility	Motile	Motile	Motile	Motile
Catalase	+	+	+	+
Oxidase	-	-	-	+
Nutrient agar	Circular, smooth and colour less	Circular, moist, smooth, translucent	Circular, Entire and transparent	Circular, Entire and transparent
Glucose	+	+	-	-
Lactose	+	+	-	-
Sucrose	+	+	-	-
Manitol	+	+	-	-
Indole	+	-	+	+
Methyl red test	+	+	+	+
Voges Proskauar test	-	-	+	-
Citrate	-	+	+	-
Urease	-	-	-	-
TSI	-	+	+	+
Strains	*Escherichia coli*	*Salmonella typhi*	*Bacillus lichenifomis*	*Pseudomonas fluorescens*

**From all the 5 study sites.*

Fig. 1 Textile dye effluent collection sites

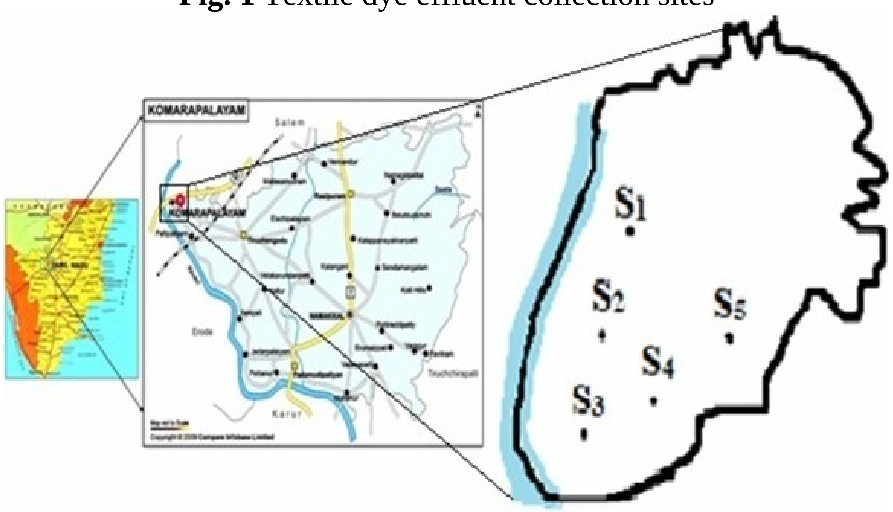


Table.3 Biodegradation of heavy metal Cadmium from textile dye effluent using isolated bacterial strains *(p > 0.01) (n=6)*

Bacterial Isolates	S1			S2			S3			S4			S5		
	I	F	%	I	F	%	I	F	%	I	F	%	I	F	%
Bacillus lichenifomis	**10.11**	**1.34**	**86.74**	**23.34**	**3.51**	**84.96**	**17.11**	**2.30**	**86.55**	**14.53**	**0.24**	**98.34**	**31.50**	**21.36**	**32.19**
Salmonella typhi	**10.11**	**2.54**	**74.87**	**23.34**	**17.10**	**26.73**	**17.11**	**1.03**	**92.40**	**14.53**	**2.45**	**83.13**	**31.50**	**13.01**	**58.69**
Pseudomonas fluorescens	**10.11**	**1.18**	**88.32**	**23.34**	**1.21**	**94.81**	**17.11**	**5.12**	**70.07**	**14.53**	**5.40**	**62.83**	**31.50**	**4.33**	**86.25**
Escherichia coli	**10.11**	**2.14**	**78.83**	**23.34**	**13.30**	**43.01**	**17.11**	**15.23**	**10.98**	**14.53**	**8.31**	**42.80**	**31.50**	**2.50**	**92.06**
t- Value	**25.65**			**3.81**			**3.47**			**5.93**			**4.88**		

I=Initial, F=Final, S=sample

Table.4 Biodegradation of heavy metal Lead from textile dye effluent using isolated bacterial strains *(p > 0.01) (n=6).*

Bacterial Isolates	S1			S2			S3			S4			S5		
	I	F	%	I	F	%	I	F	%	I	F	%	I	F	%
Bacillus lichenifomis	**24.32**	**2.51**	**89.67**	**7.32**	**1.03**	**85.92**	**23.13**	**3.53**	**76.09**	**32.64**	**3.43**	**89.49**	**8.32**	**0.43**	**94.83**
Salmonella typhi	**24.32**	**4.16**	**82.89**	**7.32**	**0.51**	**92.75**	**23.13**	**5.00**	**78.38**	**32.64**	**15.11**	**53.70**	**8.32**	**1.24**	**85.09**
Pseudomonas fluorescens	**24.32**	**1.38**	**94.32**	**7.32**	**2.43**	**66.80**	**23.13**	**16.43**	**28.96**	**32.64**	**14.43**	**55.79**	**8.32**	**2.30**	**72.35**
Escherichia coli	**24.32**	**4.53**	**81.37**	**7.32**	**1.41**	**80.73**	**23.13**	**10.51**	**54.56**	**32.64**	**4.33**	**86.73**	**8.32**	**1.40**	**83.17**
t- Value	**28.84**			**-1.70**			**4.86**			**7.39**			**18.20**		

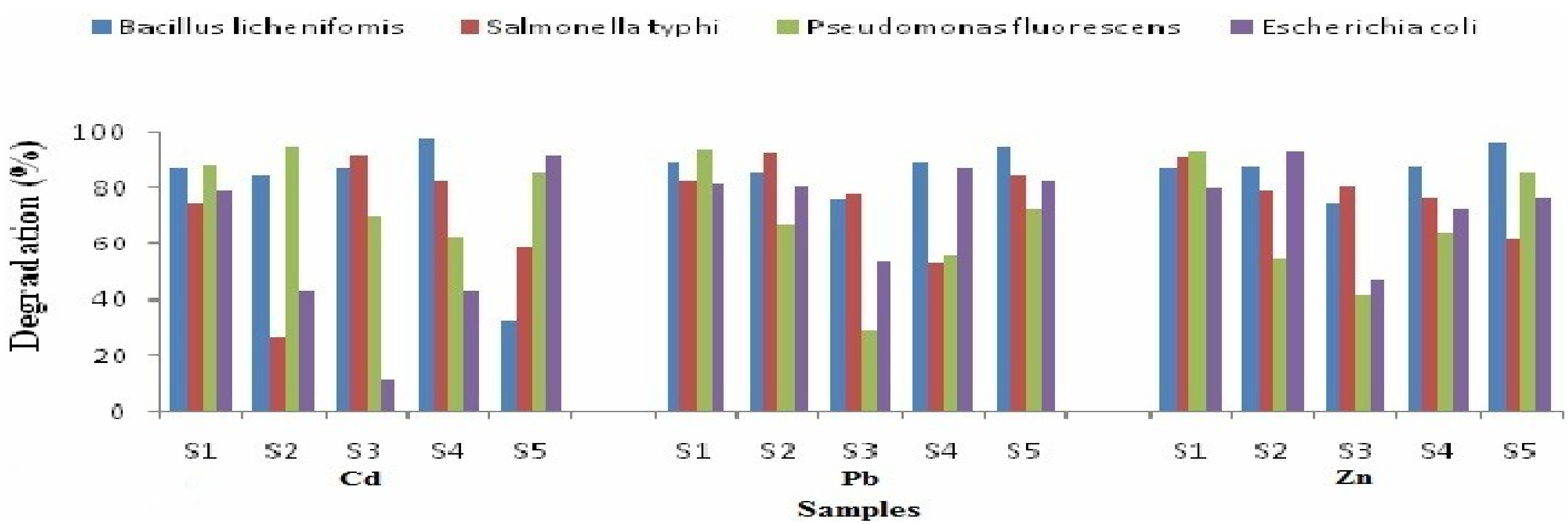
I=Initial, F=Final, S=Sample

Table.5 Biodegradation of heavy metal Zinc from textile dye effluent using isolated bacterial strains *(p > 0.01) (n=6)*

Bacterial Isolates	S1			S2			S3			S4			S5		
	I	F	%	I	F	%	I	F	%	I	F	%	I	F	%
Bacillus lichenifomis	16.32	2.16	86.76	34.50	4.34	87.42	21.32	5.31	75.09	20.01	2.51	87.45	11.42	0.44	96.14
Salmonella typhi	16.32	1.34	91.78	34.50	7.33	78.75	21.32	4.03	81.09	20.01	4.07	76.66	11.42	4.33	62.08
Pseudomonas fluorescens	16.32	1.06	93.50	34.50	15.43	55.27	21.32	12.35	42.07	20.01	7.33	63.36	11.42	1.54	86.51
Escherichia coli	16.32	3.21	80.33	34.50	2.32	93.27	21.32	11.21	47.42	20.01	5.54	72.31	11.42	2.64	76.88
t- Value	29.78			9.41			6.28			14.71			11.06		

I=Initial, F=Final,S=Sample

Fig.2 Microbial bioremediation of heavy metals from textile industry dye effluents using bacterial strains



Water pollution due to toxic heavy metals through textile dye effluent remains a serious environmental and public problem in developing countries. Strict environmental legislations on the discharge of textile dye effluents without pre treatment make it necessary to develop various innovative technologies for the removal of pollutants from the effluents. Therefore numerous approaches have been made for the development of technology for the treatment of effluents before released in to the environment. In this study the textile dye effluent was collected from various study sites of Komarapalayam, which indicated high pollution load.

The physico chemical characters of the textile dye effluent were given in Table 1. All the samples collected from the study area indicated high level of pollution. This study was correlated with previous studies on physic chemical characterization of textile dye effluents (Rajaganesh et al., 2014). Many studies indicated that a number of bacterial species were capable of removing metals from aqueous environment (Manisha et al., 2011). In this study four heavy metal resistant bacteria strains namely *Bacillus licheniformis, Salmonella typhi, Pseudomonas fluorescence* and *Escherichia coli* were isolated from the textile dye effluents collected from Komarapalayam, India.

The biochemical characterizations of above isolated bacteria were given in Table 2. The textile dye effluents consist of heavy metal such as cadmium, lead and zinc. The concentration of Cd was ranged from 10.11 to 31.50 mg/litre, the lead concentration ranged from 7.32 to 32.64 mg/litre and the concentration of Zn was ranged from 11.42 to 34.50 mg/litre. The primary goal of this study is to remove the heavy metals or to decrease its content from the textile dye effluent samples. The cadmium content in the samples were decreased after the inoculation of isolated bacterial strains from 10.11 to 1.34, 23.34 to 3.51, 17.11 to 2.30, 14.53 to 0.24 and 31.54 to 21.36 mg/litre (Table. 3). This result did concur with previous study reported by Ashok et al (2010), which confirmed that different bacterial strains were used to remove heavy metals present in the aqueous environment. Hanjun et al., 2010 also reported the bioremoval of cadmium from aqueous environment using endophytic bacterium bacillus species (Hanjun et al., 2010).

The lead content present in the textile dye effluent was ranged from 7.32 to 32.64 mg/litre, but after the inoculation of above sample with mentioned bacterial strains, there was a significant decrease in the lead content from 24.32 to 2.51,7.32 to 1.03, 23.13 to 3.53, 32.64 to 3.43, 8.32 to 0.43 mg/litre (Table.4). The above studies are correlated with the investigations of Khosro et al (2011), which indicates the bioremediation of heavy metal lead from polluted sediment soils using Bacillus spp. The zinc content present in the textile dye effluent is given in Table 5. The zinc content ranged from 11.42 mg/litre to 34.5 mg/litre.

After the inoculation of the above sample with heavy metal resistant strains namely *Escherichia coli, Salmonella typhi, Bacillus licheniformis* and *Pseudomonas fluorescence* there was a significant decrease in Zn content from 16.32 to 2.16, 34.50 to 4.34, 21.32 to 5.31, 20.01 to 2.51, 11.42 to 0.44 mg/litre. The microbes play a vital role in the remediation of heavy metals and pollutants through biosorption. The main important mechanism by which metal ions bind to the cell surface include electrostatic interactions, vander waals forces, covalent bonding, redox interactions and extra cellular precipitation (Blencowe and Morby, 2003). The negatively charged groups (carboxyl, hydroxyl and phosphoryl) of the bacterial cell wall absorb metal ions (Wase and Foster, 1997). Cadmium and lead metal ions present in the textile dye effluent cause toxicity in receiving aquatic environment by interacting with nucleic acids, by binding to essential respiratory proteins (Vallee and Ulmer 1972) and by displacing Ca^{2+} and Zn^{2+} in protein (Markovac and Goldstein, 1998; Bouton et al., 2001). Zinc is involved in a wide variety of cellular processes. It is required for maintaining the structural stability of macromolecules and it serves as a co-factor for more than 300 enzymes (McCall et al., 2000). Howeres in excess it can inhibit the aerobic respiratory chain, have significant toxicity and act as a potent disrupter of biological system (Blanco, 2000). Zn^{2+}, Cd^{2+} and Pb^{2+} resistance in bacteria is mainly based on active efflux of metal ions to prevent toxic effects in the cell.

From the present study it is clear that heavy metal resistance bacterial strains effectively removed the heavy metals from textile dye effluents. In this study four microbial strains namely *Escherichia coli, Salmonella typhi, Bacillus licheniformis* and *Pseudomonas fluorescence* reduced maximum of 98.34% of cadmium, 94.83% of lead and 96.14% of zinc from the effluent samples. It may be concluded that microbes can tolerate against the heavy metals and they are armed with various resistance and catabolic potentials. This catabolic potential of microbes is enormous and is advantageous to mankind for a cleaner and healthiest environment through bioremediation. Howeres it is essential to conduct more studies on gene level to know the biosorption potential and heavy metal resistance ability of the microorganism. This study may be helpful to develop affordable Ecofriendly technology for the treatment of textile dye effluents before released in to the environment.

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