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RESEARCH ARTICLE

MICROCOSM ANALYSIS OF UNTREATED TEXTILE EFFLUENT FOR COD REDUCTION BY AUTOCHTHONOUS BACTERIA

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Abstract

Textile industry is one of the most important sectors which contributes to Rajasthan's economy and at the same generates huge volumes of waste water characterized by intense colour, high pH, BOD, COD. Keeping in view the above cited fact, an attempt has been made to characterize untreated textile waste water collected in accordance with standard procedures from a local dyeing unit based at Sanganer, Jaipur; under the parameters pH, temperature, colour, dissolved oxygen, biochemical oxygen demand, chemical oxygen demand, nitrate, phosphate, sulphate, alkalinity, oil and grease, hardness, chloride, TSS, TDS and to check whether the parameters were in compliance with standards or not.. The second objective of our study was to monitor COD reduction by microcosm analysis at an interval of 5 days till 20 days by indigenous bacteria and the strain is biochemically characterized as *Bacillus sp*. The strain was inoculated in Nutrient Broth (NB) to study growth curve. COD reduction was carried out by microcosm analysis in which the inoculum concentration was varied from 0.1 % to 1 % v/v. 1% inoculum concentration was found to be most promising in terms of COD reduction and COD was reduced to 88.5%.
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Keywords: COD, microcosm, physico -chemical parameters, textile effluent.

Introduction

Cleaner production is the need of an hour for sustainable development. Textile industry in particular becomes one of the culprits to be considered in terms of damage it causes to the environment. The strategic approach for cleaner production has gained momemtum in recent years for environmental protection and preservation of ecological resources from excessive depletion and, has shown its ability to decrease environmental pollution, preserve natural resources (Li et al., 2011). The current problem faced with industrial waste waters is absence of treatment prior to its being released into environment .Industrial effluents urban runoff, direct disposal of untreated textile effluent into adjoining water bodies specially the drains, fertilizers and animal wastes constitute the major reservoir of contaminants (Jaishree and Khan, 2014). Excessive usage of enormous volumes of water and chemically different types of synthetic dyes contributes to water pollution caused by textile industry. The major constituents of textile based effluents are several types of chemicals such as dyes, dispersants, leveling agents, acids and alkali (Olukanni et al., 2006) that are discharged into water bodies without treatment which increases the chemical oxygen demand (COD) and biochemical oxygen demand (BOD), alters the pH and gives the water bodies (rivers) intense colourations. Coloured wastewater from textile industries is considered to be the most polluting in almost all industrial sectors (Andleeb et al., 2010).

Sanganer textile industry which is one of the largest handloom manufacturing zone from western India covers more than 154 block printing units. It is because of the toxic nature of effluents generated by textile cluster, it has gained much attention (Talware et al., 2008**)**. Effluent from these industries is being discharged without treatment into agricultural fields and used for irrigational purposes and pollutes both ground water and soil due to the percolation of some water soluble pollutants (Pande et al., 2009).

COD being one the major pollution indicator reflects the quality of water both in terms of its use for irrigational purposes and release into adjoining surface waters. The fact has been established that untreated textile waste water has very high COD(Sharma et al., 2013; Goyal et al., 2013; Paul et al.,2012;Agarry and Ajani, 2011; Varma and Sharma, 2011; Rao and Prasad, 2010). Sanganer being one of the major textile clusters of Rajasthan is deprived of an Effluent Treatment Plant (ETP), the waste water generated from textile and dyeing units is directly introduced into the adjoining surface water and primarily is used for irrigational purposes.
Studies pertaining to reduction in COD by indigenous micro flora have been well established both nationally and internationally. (Sharma et al., 2010) studied the effect of indigenous bacteria on simulated textile effluent in terms of COD reduction and dye degradation. (Fakhruddin et al., 2010) studied the effect of algae and aquatic macrophytes on COD reduction of textile effluent collected from post discharge of equalization tanks and witnessed a decrease of 69% in COD. (Musa and Ahmad,2010) studied the effect of indigenous bacteria on waste water of pineapple industry in terms of reduction in COD by microcosm analysis.
Keeping in view, the above cited facts, our study is aimed to characterize the untreated textile effluent and to isolate and identify a potential bacterial strain the effect of which would be studied to minimize the level of COD through microcosm analysis.

Materials and methods

Chemicals

All the chemicals used in the study were of analytical grade and procured from Hi Media.

Study Area

Sanganer, being one of the major textile clusters of Rajasthan is famous for its ethnic hues like bandhej and popularly known as Sanganeri prints. Textile sector utilizes different types of dyes and hence generates significantly large volumes of coloured effluent.

Sampling Site

Untreated textile waste water samples (Figure 1b) were collected in pre sterilised plastic cans from dye house society, Sanganer, Jaipur (Figure 1a) transported and analyzed in accordance with standard procedures (APHA, 1998).

Figure 1 a: Study area; Dye house society, Sanganer, Jaipur

Figure 1b: Sampling Site; Untreated textile waste water collected from dye house society

Effluent analysis

Untreated textile effluent samples(figure 1c) from site 1b were collected in triplicates in pre cleaned plastic cans according to Grab Methodology (APHA, 1998) and were transported and stored at 4ºC in accordance with standard procedures(APHA, 1998).

Figure 1 c: Untreated textile effluent samples

The parameters conducted at site were pH, colour and temperature. Other parameters like Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), acidity, alkalinity, hardness, chloride, nitrate, phosphate, sulphate, oil and grease, free $C0_{2,}$ TDS and were analysed with their minimum retention time (APHA, 1998). For bacteriological analysis, the samples (Figure 1 c) were collected in pre sterilized screw- capped BOD bottles which were rinsed with dilute acid and effluent prior to sampling.

Isolation and biochemical characterization of indigenous bacteria

For bacteriological analysis, the sample was serially diluted upto dilution 10^{-10} and plated onto general purpose media(Nutrient Agar)with the following composition (gm/l): peptone-5, meat extract-1, yeast extract-2, Nacl-5, agar-15, pH-7). Following plating, the plates were incubated at 37ºC for 48 hours (Rao and Prasad, 2011). Mixed culture at all dilutions revealed the diversity of bacterial populations adapted to harsh conditions of textile effluent. It was then, from a mixed culture, the strain which was predominant at all dilutions was chosen for further investigations.

COD reduction by selected strain

COD determines the amount of oxygen required for the chemical oxidation of organic matter and presence of non biodegradable matter .It is also an important pollution indicator which reflects the chemical quality of effluent. In the present study, bioefficacy of an autochthonous bacterial strain has been accounted.

Inoculum preparation

A loopful of pure culture of strain TexA was inoculated into Nutrient Broth (NB) with the following composition (gm/l): peptone-5, meat extract-1, yeast extract-2, Nacl-5, ,pH-7) and incubated as described earlier. Growth kinetics of the strain was studied till a desired $O.D._{660}= 0.6$ was attained (Suizhou et al., 2006) .Different inoculums were preapared by varying the concentration from 0.1%, 0.5% and 1%.

Microcosm analysis

Microcosm analysis of untreated textile effluent was carried out in accordance with protocol devised by Musa and Ahmad, 2010. The effluent sample was equally distributed (400 ml each) into a set of 3 erlenmeyer flasks (1000ml capacity) and labeled as I, II and III A separate flask containing only the effluent sample was maintained as negative or abiotic control .In flask I, 0.1 % (v/v) inoculums was added, to flask II, 0.5 % (v/v) inoculum was added and to flask III, 1 % (v/v) inoculums was added whereas flask IV was devoid of inoculum. All the flasks were labeled as

Tex A 0.1%(I)
Tex A 0.5%(II)
Tex A 1%(III)
Tex Ct (IV)

Bioefficacy of selected strain in terms of COD reduction

The simulated effluent samples were analyzed for COD reduction by dichromate reflux method at an interval of 5 days till a period of 20 days for all inoculum concentrations (0.1 %, 0.5 % and 1%) of Tex A bacterial strain and compared with negative control . The decrease in COD was expressed in terms of percent reduction by the formula:

$$\%\ \text{COD reduction} = \frac{\text{Initial COD} - \text{Final COD}}{\text{Initial COD}} \times 100$$

Where, Initial COD was the COD calculated prior to bacterial treatment and final COD was calculated on each respective day (0 day, 5th day, 10th day, 15th day and 20th day).

Results and Discussion

Effluent analysis

The findings of physicochemical characterization of untreated textile effluent are represented in Table 1.

Colour

The presence of even small amount of dye in water (10–20 mg/l) is highly visible, Color is the first contaminant to be recognized in the dyeing effluents and has to be removed before discharging into the water stream (Mohan and Karthikeyan, 2004). In the present study, colour of effluent was dark blue in colour which may be attributed to excessive use of blue dyes. Different reports pertaining to different colouration of raw textile effluent are available. Blackish green effluent has been reported by Sharma et al, 2013. Untreated textile effluent with brownish black colour has been investigated by Desai and Kore, 2011.

Temperature

It is the most important physiological parameter which not only governs the aquatic life but is also dependent upon the different operations of textile industry. In this study, it was found to be 26ºC. Higher temperature (41ºC) for untreated textile effluent has been reported.(Rao and Prasad, 2010)

pH

Geology of catchments areas as well as agricultural runoff influences pH of effluent (Goyal et al., 2013). pH of untreated textile effluent was found to be 12 and it may be attributed to excessive usage of bleaching agents, surfactants, sodium hydroxide (Paul et al., 2012).pH value in the range from 5.4 to 10.8 has been reported (Goyal et al., 2013) whereas similar pH value i.e 12 for untreated textile effluent has been reported (Sharma et al., 2013).

Dissolved Oxygen (DO)

DO of an effluent sample reflect the extent the pollution and hence it is an important pollution indicator. In the present study, DO of untreated textile effluent was found to be 3.4 mg/l. Nil DO of raw textile effluent has been reported(Sharma et al., 2013; Paul et al.,2012). Treated textile effluent has also witnessed nil DO (Garg and Kaushik, 2008).

Biochemical Oxygen Demand (BOD)

It is measured as the concentration of organic matter present in any water The amount of decomposable organic matter present in an effluent sample is directly linked with amount of oxygen required for decomposition and hence high BOD(Savin and Butnaru, 2008). In the present study, amount of BOD was found to be 278mg/l. A higher BOD value (485.5mg/l) of untreated textile effluent has been reported.(Rao and Prasad, 2010); 350 mg/l (Sharma and Varma, 2011) Values of BOD for treated textile effluent similar (275mg/l)to our findings have been reported.(Sharma et al, 2013).

Chemical Oxygen Demand (COD)

High COD levels imply toxic condition and the presence of biologically resistant organic substances. In the present study, COD of untreated textile effluent was found to be 670mg/l. The range of COD 455-1349mg/l for untreated textile effluent is well reported(Paul et al, 2012).Very high range of COD for untreated textile waste water(810-856.6 mg/l) has also been reported(Sharma et al., 2013); 1170-3998mg/l (Goyal et al.,2013).(Fakhruddin et al., 2010) studied the effect of aquatic macrophytes and algae on COD reduction of textile effluent; COD of untreated textile effluent was found to be 720mg/l and after treatment it reduced to 300-391.6mg/l. (Imtiazuddin et al., 2012) studied COD of untreated textile effluent generated by textile industries of Karachi, Pakistan and reported it to be in the range 115.66-705.25mg/l.

Alkalinity

It is measured as buffering capacity of water also it indicates the ability of water to neutralize acids from effluents. The higher values of alkalinity are associated with increase in the presence of bicarbonates and carbonates from effluents and use of different types of synthetic dyes. In the present study, value of alkalinity for untreated textile effluent was 446mg/l. Alkalinity of untreated textile effluent has found to be in the range 319.2-380 mg/l(Sharma et al, 2013); 280-500mg/l(Paul et al, 2012).

Hardness

Hardness of any water sample is the combined effect of the calcium and magnesium concentrations, both expressed as calcium carbonate, in mg/L Water is conventionally classified as hard or soft from the following classification 50 – 100 mg/l(Soft), 100 –250 mg/l (Moderately hard) 250 – 350 mg/l(Hard),> 350 mg/l(Excessive hard)(Mohabansi et al., 2011)In the present investigation, hardness content of untreated textile effluent is 336mg/l.In similar studies, hardness content of untreated textile effluent was 470- 1050mg/l(Paul et al ,2012); low hardness values 297.6-322mg/l of untreated textile effluent have also been reported (Sharma et al, 2013).

Chloride

It forms one of the major inorganic anions in waste water. The amount of chloride in any water sample is directly proportional to amount of mineral content. The presence of chloride in textile effluents is mainly attributed to the presence of bleaching agents used in different unit operations of textile industries. In the present investigation, chloride content was found to be 469 mg/l. A higher range (845.1-896.9 mg/l) of chloride content in untreated textile waste water has been reported.(Sharma *et al,* 2013); 950-2750mg/l (Paul et al, 2012).

Nitrate

Ammonium nitrogen contributes significantly to high nitrate concentrations are frequently encountered in textile wastewater and lead to eutrophication of surface water bodies. The level of nitrate of untreated textile effluent was found to be 13.7mg/l. very high nitrate concentration (22.1-26.3mg/l) of untreated textile waste water has been reported(Sharma et al, 2013) whereas a trend with lower nitrate values (3.719-7.991mg/l)have also been reported(Goyal et al, 2013).

Phosphate

Textile effluent samples are characterized by high phosphate content presumably because of inorganic and organic matter present both in dissolved and particulate forms. In the present study, phosphate content of untreated textile effluent was found to be 13.9mg/l. in similar studies, the phosphate content of untreated textile waste water was found to be in the range 1.39-10.45mg/l(Paul et al, 2012); phosphate content in the range 22.1-26.3 mg/l for untreated textile effluent has also been reported(Sharma et al, 2013); 7.437-8.897mg/l of phosphate content was reported by (Goyal et al,2013).

Sulphate

It is one of the major inorganic ions found in arid and semi arid regions. In industrial effluents, it owes its origin to biological oxidation of sulfur compounds. In the present study, sulphate content of untreated textile effluent was 289mg/l. In a similar study, sulphate content of untreated textile effluent was found to be in range 440-912mg/l (Paul et al, 2012); (Varma and Sharma, 2011) observed the value of sulphate for untreated textile effluent as 348mg/l.

Oil and Grease

In industrial effluents, oil and grease involves low molecular weight hydrocarbons, esters, oil, fats, waxes and detergents. In the present study, oil and grease content of untreated textile effluent was found to be 7.9 mg/l. Very high content of oil and grease (60mg/l) has been reported in a similar study conducted by(Varma and Sharma, 2011)

Total Dissolved Solids(TDS)

It determines the salinity of a water body, the common salts found in dissolved state are carbonates, bicarbonates, chlorides, sulphates, phosphates, and nitrates of calcium, magnesium, sodium, potassium, iron, and manganese, etc (Lokhande et al., 2011) (from my paper) In the present study , TDS of untreated textile effluent was found out to be 598 mg/l.in similar investigations, TDS of untreated textile effluent was found to be 3270.6-4276.3mg/l (Sharma et al., 2013); 3260-17,700mg/l (Goyal et al, 2013);2264-5752mg/l(Paul et al, 2012) ; 2352mg/l (Varma and Sharma, 2011).

Total Suspended Solids (TDS)

Solids which are present in suspended form and are attributed to the presence of different dyestuffs used in textile industry. In the present investigation, value of TSS of untreated textile effluent was 352mg/l. Reports on similar studies on untreated textile effluent are well cited (Goyal et al., 2013) observed TSS of untreated textile effluent in the range 124-930mg/l. (Sharma et al., 2013) reported TSS in the range 920.6-925.3mg/l. Low values of TSS 270mg/l then our findings have also been reported (Varma and Sharma, 2011).

Isolation, identification and biochemical characterization of indigenous bacteria

Autochthonous microbial diversity of any effluent system is of primary importance in terms of execution of *in situ* bioremediation approach. Untreated textile effluent was serially diluted and the bacterial isolate which was predominant at all dilutions was chosen for further studies. Preliminary biochemical investigations revealed the isolate Tex A as *Bacillus sp.*;. The growth curve of the isolate is represented in figure 2.

Table 1. Physicochemical characterization of untreated textile effluent

Properties	Units	Textile effluent	Standards [A B]
Colour	-	Dark blue	NA
Temperature	-	26ºC	NA
pH	-	12	6-9[A]
DO	mg/l	2.1	NA
BOD	mg/l	278	30[A]
COD	mg/l	670	250[A]
Alkalinity	mg/l	446	NA
Hardness	mg/l	332	NA
Chloride	mg/l	469	500[A]
Nitrate	mg/l	13.7	NA
Phosphate	mg/l	13.9	NA
Sulphate	mg/l	289	1000[B]
Oil and grease	mg/l	7.9	10[B]
TDS	mg/l	598	2000[A]
TSS	mg/l	352	200[B]

[A]: CPCB (Paul *et al*, 2012) [B]: ISI 2490-1981 (Varma and Sharma, 2011) NA: Not available

Table 2. Biochemical characterization of Bacterial strain used for microcosm analysis

Character	+ve/-ve
Gram stain reaction	+
Agar slant characteristic	Abundant , opaque, white , waxy growth
Lactose fermentation	-
Dextrose Fermentation	Acid production
Sucrose Fermentation	Acid production
Indole production	-
Methyl red test	-
Voges-Prauskauer	+
Citrate utilisation	-
Catalase	+
Oxidase	-
Gelatin hydrolysis	+
Starch	+
Lipid hydrolysis	+

Figure 2: Growth curve of *Bacillus sp.*

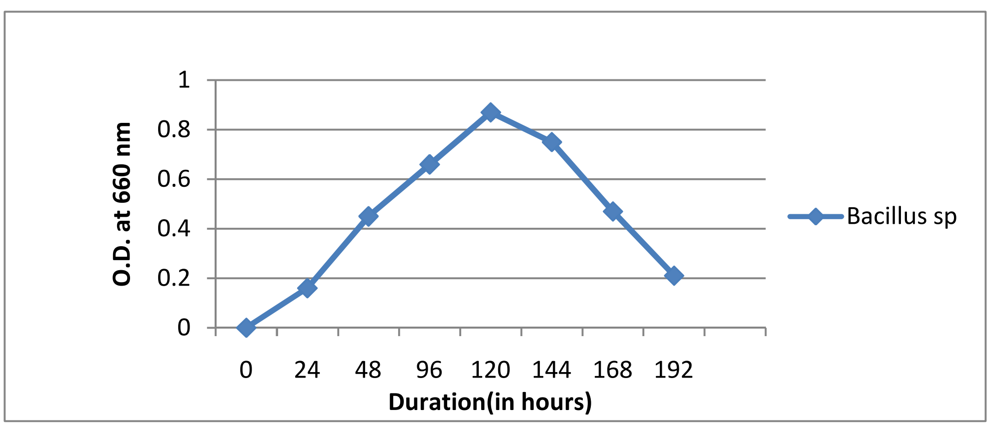
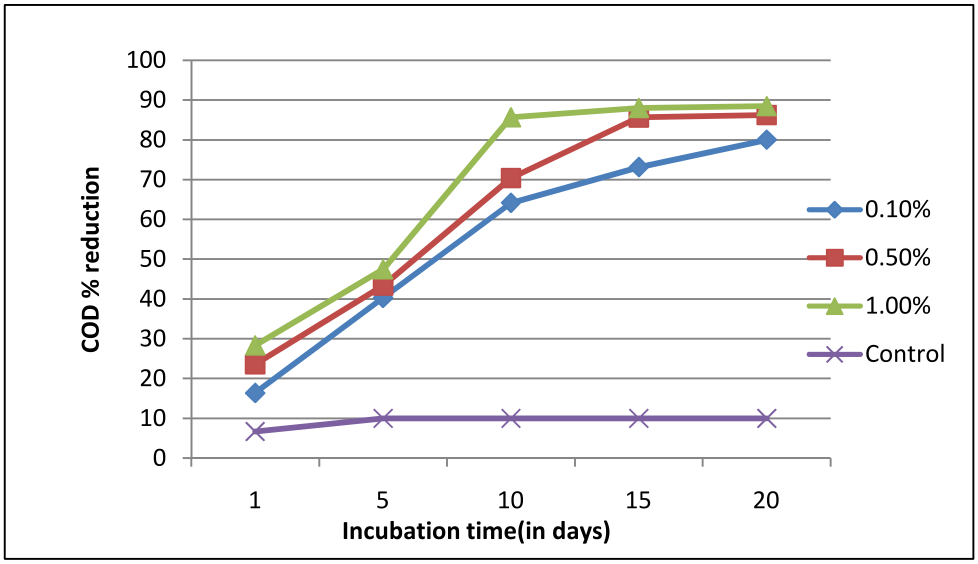


Table 3. Microcosm analysis for COD reduction of untreated textile effluent

Bacillus sp	**Initial COD (mg/l)**	**1st day (mg/l)**	**5th day (mg/l)**	**10th day (mg/l)**	**15th day (mg/l)**	**20th day/Final COD (mg/l)**
0.1%*	670	560	400	240	180	134
0.5 %*	670	512	379.2	198	96	92
1.0%*	**670**	480	352	96	80	**77**
Control	670	656	631	624	613	603

*: Inoculum% (v/v)

Figure 4. Bio efficacy of *Bacillus sp.* with different inoculum % for COD reduction



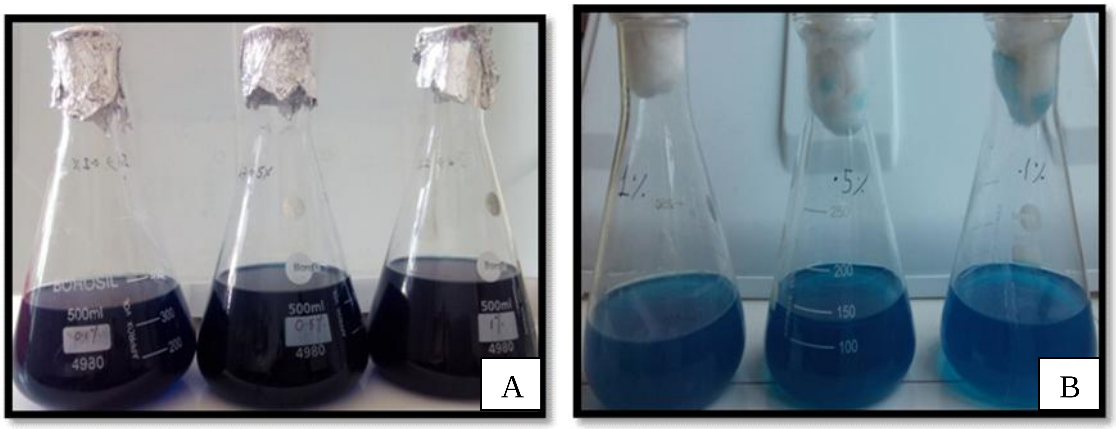
COD reduction

Microcosm analysis was carried out by standardizing the $O.D._{660}$ =0.6 of the isolate. Table 3 represents COD reduction by *Bacillus sp* inoculated at different concentrations (0.1%.0.5% and 1.0%) in effluent sample at an interval of 5 days with a final time of experiment being 20 days. Maximum reduction in COD was observed on 20th day with 1% (v/v) concentration of *Bacillus sp.*

The effect of bacterial consortium on COD reduction in dye contaminated soil sampled from vicinity of textile industry was studied by (Sharma et al, 2010). A similar study in which indigenous bacterial strains have been used for COD reduction has been investigated in effluent generated from pineapple industry has also been reported (Musa and Ahmad, 2010).

Figure 5 illustrates bio efficacy of Tex A strain in terms of COD reduction in untreated textile effluent and its comparative analysis with abiotic control. It is expressed as percent reduction which is calculated at regular intervals of 5 days. Microcosm analysis with an aim to reduce COD of textile effluent by bacteria and fungi isolated from textile dye contaminated soil has been reported. (Samuel and Ayobami, 2011) . Reduction in COD of textile effluent by establishing aerobic –anaerobic bioreactor has been well accounted (Kassa, 2007). It is appreciated that maximum COD reduction which is expressed in terms of COD reduction was found to maximum be at 20th day and correspond to 88.5 % which was with 1 % inoculum. Strikingly, it was found that COD reduction was related to number of cells, the possible mechanism of which may be utilization of organic matter by bacterial cells and colour removal by biosorption as represented in figure .

Figure 5: Microcosm analysis: COD at 0th day(A);COD at 20th day (B)



Conclusion

The present study which was aimed to access the level of pollution caused by untreated textile waste effluent revealed that almost all the physicochemical parameters studied were not in compliance with the standards prescribed by CPCB and Environment Protection Rules, 1986. For non-compliance of standards, we suggest the possible remedial measure in terms of establishment of an Effluent Treatment Plant (ETP).Another objective of our research was based on bio augmentative approach aimed to reduce COD of untreated textile effluent. The autochthonous bacterial strain exhibited a tremendous potential (88.5 %) to reduce COD by microcosm analysis. We hereby suggest that a consortium could be established and through simulated approach the effect of the same on other pollution indicators like BOD, suspended solids could be studied and the process could be s.

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