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Isolation and characterization of dye degrading bacteria from textile effluent

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Abstract

Textile industry is considered as one of the largest generators of Toxic chemical waste water in India. Dyes released by the textile industries pose a threat to environmental safety. The present study was an attempt for the assessment of different Physico-Chemical parameters such as pH, Temperature, Electrical Conductivity (EC), Total solids (TS), Total Dissolved solids (TDS), Total Suspended Solids (TSS), Dissolved Oxygen (DO), Biochemical Oxygen Demand (BOD), Total Alkalinity, Total Hardness, Calcium Hardness, Magnesium Hardness and Chloride. Biological decolorization is an alternative and efficient tool for environmental management. Therefore, the textile dye effluent degradation was herculean task because the textile effluents contain complex of chemicals, highly toxic compounds and heavy metals. The experiment was carried out to degrade the dye effluent by using bacterial isolates from textile dye effluent. From the textile dye effluent there are four different bacteria isolated such as *Bacillus cereus, Bacillus subtilis, Escherichia coli, Staphylococcus aureus* these bacterial strains have capability to degrade the Textile dye effluent as well as to decolourize the dye efficiently in Textile Effluent.

Keywords: Textile dye, Effluent, Decolourization, Bioremediation and Bacteria

1. Introduction

The textile industry plays an important role in the world economy as well as in our daily life, but at the same time, it consumes large quantities of water and generates large amounts of waste water. The chemical reagents used in the textile sector are diverse in chemical composition ranging from inorganic to organic molecules (Subhatra *et al*. 2013). The release of a wide range of compounds from industries is creating disturbance to the ecosystem causing climatic changes, reduction of water levels in the ground as well as oceans, melting ice caps, global warming, ozone layer depletion due to photochemical oxidation, etc. (Varsha *et al*. 2011). Physical and chemical methods (adsorption, chemical transformation, incineration, photo-catalysis, ozonation etc.) are not suitable for the removal of recalcitrant dyestuffs, because of high cost, low efficiency and in-applicability to a wide variety of dyes (Dawker *et al*. 2008).

The wastewater generated from the textile, dye and dyestuff industries is a complex mixture of various organics, like chlorinated compounds, pigments, dyes and inorganic compounds. Dyes usually have a synthetic origin and complex aromatic molecular structures which make them more stable and more difficult to biodegrade. The textile industry utilizes about 10000 different dyes and pigments. The worldwide annual production of dyes is over 7x105 tons. The dyestuff usage has been increased day by day because of tremendous increase of industrialization and man's urge for color. It is reported that approximately 15% of the dyestuffs are lost in the industrial effluents during the manufacturing and processing operations. The effluents of these industries are highly colored and the disposal of these wastes into receiving waters causes damage to the environment. The presence of dye compounds in the effluent, even at low concentrations (1-5 ppm) is highly visible and toxic to the biotic life.

In the biological methods, the microbes such as bacteria, fungi and algae are being used for the wastewater treatment, which could be a viable option as low-cost and eco-friendly technology. There are various microorganisms found in the contaminated environment, have potential to decolorize and even completely mineralize many dyes from the wastewater efficiently under certain environmental conditions. Have been reported by various researchers. Several bacterial strains that can aerobically decolourize dyes have been isolated during the past few years. Many of these strains require organic carbon sources, as they cannot utilize dye as growth substrate. Biodegradation using microorganisms are gaining importance as it is cost effective, environmental friendly and produces less sludge (Bella Devassy Tony *et al*. 2009).

2. Materials and Methods

2.1 Sample collection

The dye effluent was collected from a dyeing industry in Arakkonam region, Vellore district, Tamil Nadu, India. The effluent samples was collected in plastic can the can was rinsed in tap water and distilled water and it was refrigerated at 4°C and used without preliminary treatment.

2.2 Physico-Chemical Analysis of Textile Effluent

The collected effluent samples have been analyzed to determine its Physico-chemical parameters. The various parameters viz., pH, Electrical Conductivity (EC), Color, Odor, Total solids (TS), Total dissolved solid (TDS), Total suspended solids (TSS), Total Alkalinity, Biochemical oxygen demand (BOD), Dissolved Oxygen (DO), Total Hardness, Chlorides, Ca Hardness and Mg Hardness were analyzed as described in the standard methods for the examination of water and wastewater as per Indian standards and standard methods for water and effluents analysis

2.3 Isolation of Dye Degrading Bacterial Isolates from Dye Effluent

The bacterial isolates present in the textile dye effluent were isolated by Serial dilution (Pour plate) technique. In this method, 1 ml of sample was thoroughly mixed with 99 ml of sterile distilled water, and then it was serially diluted by following standard procedure up to concentration of 10^{-6}. Then, 1 ml of serially diluted samples from each concentration of samples were transferred to sterile petriplates and evenly distributed throughout the plates and sterile unsolidified Nutrient agar was poured and it was allowed to solidify. The Nutrient agar plates were incubated at 37°C for 24 hours. After incubation, the bacterial colonies were isolated and purified from the plates.

2.4 Identification by Biochemical Characterization

The isolated microorganisms after preliminary isolation and identification were further identified by conventional microbiological and biochemical techniques as described in Bergey's Manual of Determinative Bacteriology. The different biochemical tests like oxidase production, catalase production, lactose utilization, glucose utilization, saccharose utilization, citrate utilization, indole production, Methyl red, Voges-proskauer etc. were performed using KB003 Hi25TMEnterobacteriaceae Identification Kit procured from Himedia, India.

2.5 Dye decolourization experiments

Dye decolourization experiments were carried out in 250 ml Erlenmeyer flasks for textile effluent sample. The flask containing 100ml of nutrient broth with 10 ml of filtered textile dye effluent. The pH was adjusted to7. Then the flasks were Autoclaved at 121°C at 15 lbs pressure for 15 minutes. After the autoclaved process been over kept under the UV-rays for sterilization by using laminar air flow chamber the sample was cooled for a while then inoculate about 5ml of bacterial isolates to the autoclaved flask. The autoclave flasks were kept in the mechanical shaker and incubated at 37ºC for 4 days of observation. The samples were drawn at every 24 hours intervals for observation. Finally the decolourization was assessed by measuring absorbance at 510 nm of the supernatant with the help of spectrophotometer at wavelength maxima (ƛm) of respective dye.

2.6 Decolourization Assay

Decolourization assay was measured in the terms of percentage decolourization using spectrophotometer. The percentage decolourization was calculated from the following formula

$$\text{Decolourization (\%)} = \frac{\text{Initial} - \text{final}}{\text{Initial}} \text{ x } 100$$

3. Result and discussion

3.1 physico-chemical analysis of textile effluent

Table 1 shows results of the detailed analysis carried out on the effluent obtained from textile processing industry. The pH value was slightly alkaline. The temperature of the effluent was high. The textile effluent have high levels of Total solids (TS), total suspended solids (TSS) and total dissolved solids (TDS) it may be attributed to use of salts during dyeing process. The concentration of DO must be range between 8 to 10 mg/l but it exceeding the permissible limits so adversely affects aquatic life. The BOD were 210 from the result it indicates that the effluent are high in recalcitrant and hardly degradable compounds. Hardness of water mainly depends upon the amount of calcium and magnesium salts or both. High chloride concentration are harmful for metallic pipes as well as agriculture crops chloride in excess (>250 mg/l) imparts a salty taste to water and may subject to laxative effects the result indicates maximum chloride content of 719. Results of alkalinity levels are high more alkalinity associated with increase in the presence of bicarbonates and carbonates from effluent further increased alkalinity might be due to more CO_2 release to the environment.

Table 1: Physico-Chemical Analysis of Textile Dye Effluent sample

S. No.	Parameters	Textile Dye Effluent Sample
1	pH	9.8
2	Temperature	35°C
3	Electrical conductivity (µS/cm)	590
4	Colour	Greenish
5	Odour	Unpleasant
6	Total dissolved solids (mg/l)	18200
7	Total suspended solids (mg/l)	17600
8	Total Solids (mg/l)	35800
9	Biochemical Oxygen Demand (mg/l)	210
10	Dissolved oxygen (mg/l)	17
11	Total hardness (mg/l)	266
12	Chloride (mg/l)	719.7
13	Ca Hardness (mg/l)	35
14	Mg hardness (mg/l)	231
15	Phenolphthalein Alkalinity as $CaCo_3$ (mg/l)	70
15 a	Methyl Orange (or)Total Alkalinity as $CaCo_3$ (mg/l)	180

3.2 Identification and Characterization of bacteria isolated from textile dye effluent

The degradation of dye effluents used several physical, chemical and biological methods. The Physico-chemical methods are economic limited. On the other hands, the biological methods are more effective and low expensive of treatment and amenability to scale up easily are the merits of biological methods. The present study was focused on biodegradation of textile dye effluent by using bacteria isolated from textile dye effluent. Therefore, different bacterial isolates were isolate from dye effluent sample. From the four bacteria are effective against the effluent sample. Four different dye degrading bacteria have been identified by biochemical properties as *Bacillus cereus, Bacillus subtilis, Escherichia coli and Staphyloccus aureus* respectively. The characteristics of the identified bacterial isolates were furnished in Table 2.

Table 2: Biochemical characterization of bacterial isolates

S. No.	Characters	Bacillus subtilis	Bacillus cereus	Escherichia coli	Staphylococcus aureus
1	Gram staining	+	+	-	+
2	Shape	Rod	Rod	Rod	Cocci
3	Motility	_	+	+	
4	Catalase	+	+	+	+
5	Oxidase	-	+	-	-
6	Indole test	-	-	+	-
7	Methyl red	-	+	+	-
8	Voges proskauer	+	-	-	+
9	Citrate test	+	-	-	+
10	Starch test	+	+	+	+
11	Urease test	-	-	-	-
12	Triple sugar ion agar test	-	+	+	+

3.3 Decolourization of textile dye effluent by selected bacterial isolates

The decolourization of textile dye effluent by selected four bacterial isolates *bacillus subtilis*, *bacillus cereus*, *Escherichia coli*, *staphylococcus aureus* and bacterial isolates were studied. The decolourization process was observed by the decolourization assay. The dye degradation was calculated by percentage. Maximum decolourization was reported in the karthikeyan and anbu saravan *et al*., Rashid Mahmood *et al*.

Table 3: Decolourization of textile effluent by selected bacterial isolates

S. No.	Bacterial Isolates	Decolourization (%)
1	Bacillus subtilis	94.3
2	Bacillus cereus	93.4
3	Escherichia coli	90.1
4	Staphylococcus Aureus	87.3

3.4 Dye degradation efficiency of bacterial isolates

Table 4: Dye decolourization efficiency of bacterial isolates after 96 hours inoculation

S. No.	Days	Bacterial isolates	Combination of isolates	% dye effluent decolourization after 96 hours
1	96 hours	Bacillus subtilis	DD1+DD2+DD3+DD4/4	94.85
2	96 hours	Bacillus cereus	DD1+DD2+DD3+DD4/4	93.34
3	96 hours	Escherichia coli	DD1+DD2+DD3+DD4/4	90.05
4	96 hours	Staphylococcus Aureus	DD1+DD2+DD3+DD4/4	87.43

4. Conclusion

The physico-chemical results indicate that the effluents affect the ground water quality which lead to significant environmental and health risk to the rural communities. The results obtained from this study showed that the effluent from the textile processing industries was alkaline and had a high salt concentration. From this present study, it was concluded that the bacterial isolates like *bacillus subtilis*, *bacillus cereus*, *Escherichia coli,* and *staphylococcus aureus* were predominantly present in textile dye effluent and they were used as a good microbial source for the textile dye decolourization and waste water treatment in textile dye industries. The microorganism resulted in isolation of four bacterial isolates capable of degrading the dyes with some capability. In the four bacterial isolates *bacillus subtilis* is the efficient bacteria which to degrade the dye colour efficiently when compared to other bacterial isolates such as *bacillus cereus, Escherichia coli, staphylococcus aureus*, through this review paper, it is evident that bacteria can be employed as a vital biological tool for developing decentralized wastewater treatment systems for decolorization of dye effluents through biosorption or biodegradation.

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