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Bioremediation of textile industrial effluent using mixed culture of *Escherichia coli* and *Salmonella typhi* immobilized on agar-agar in a Bioreactor

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KEYWORDS

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A B S T R A C T

The physicochemical characterization of effluent generated from Textile Industry, Kothur Industrial Area, Mahaboob Nagar, Telangana, India was carried out. The effluent was analyzed for Biochemical Oxygen Demand (BOD), Dissolved Solid (DS), Colour, Intensity and heavy metals prior to treatment. *Escherichia coli* and *Salmonella typhi* found to have degradative capacity was immobilized on agar-agar, which was transferred into bioreactor fitted with air sparger. The effluent was treated in the bioreactor. The result showed that the effluent was highly polluted using stated parameters. The immobilized cells significantly reduced COD to 225mg/I, BOD to 32mg/l, TS< 300mg/I that are upper limit for disposal into surface water. The result indicates overall % reduction in COD, BOD, nitrate, sulphate, phosphates as 80%, 96%, 66%,67%,62% respectively. Heavy metals were also biosorptioned. It was concluded that immobilized cells represent a promising application in the bioremediation of textile industrial effluent and possible reusability of the cells for its commercial application can be achieved.

Introduction

Rapid industrialization is considered as a sign of development for developing and underdeveloped countries, but unfortunately most of the industries in these countries do not have proper waste treatment facilities (Upadhyay, 2002), thereby posing environmental threat.

In textile production, opportunities exist for the release into the ecosystem of potentially hazardous compounds at various stages of the operation. These pollutants are produced in an effort to improve human standard of living and fashion, but ironically, their unplanned intrusion into the environment can reverse the same standard of living impacting negatively on the environment. Textile effluents can seep into aquifer and pollute the underground water or where it is discharged without proper treatment into water bodies (Ezeronye *et al.*, 2005).

The dyes present in textile effluent impart persistent colour to the receiving streams and interfere with photosynthesis of phytoplankton (Cunningham and Siago, 2001). Biotechnology is providing environmentally acceptable methods of modifying or destroying chemical waste so that they are no longer toxic to the environment. Biological decolorization has been employed under either aerobic or anaerobic environment. This usually involves tolerating bacteria or other microbes that can be genetically engineered to provide strains with better contaminant degrading potential than their natural counterparts (John, 1971; Alken and Irvine, 1989; Hardman *et al.,* 1993; Fernando *et al.,* 1994).

Most of the physical and chemical methods employ, which in spite of cost, do not always ensure that the contaminants are completely removed (Hardman *et al.,* 1993). Bioremediation is the most desirable approach for cleaning up many environmental pollutants. It is a pollution control technology that uses biological systems to catalyze the degradation or transformation of various toxic chemicals to less harmful forms (Ashoka *et al.,* 2002). Most studies on the metabolism of organic contaminants have been performed with bacteria especially in the context of bioremediation (Glazer, 1997).

Colour is the first contaminant to be recognized in the dyeing effluents and has to be removed before discharging into the water stream. Aesthetic merit, gas solubility and water transparencies are affected by the presence of dyes even in small amount. The removal of colour from wastewater has been rated to be relatively more important than the removal of soluble colourless organic substances, which usually contribute the major fraction of biochemical oxygen demand (Rajamohan and Karthikeyan, 2004).

An awareness of environmental problems and potential hazards caused by industrial wastewater has prompted many countries to limit the discharge of polluting effluents into receiving waters (Ezeronye and Okerentugba, 1999; Okeretugba and Ezeronye, 2003; Ezeronye and Ugbogu, 2004; Ezeronye and Ubalua, 2005).Textile manufacturing yields a large quantity of black and highly toxic waste water that contain high concentration of chromium, phenolics, suspended solid, and high concentration of biochemical oxygen and chemical oxygen demands as well as sulphide azo and diazo compounds (FEPA, 1990). The biological treatment methods are attractive due to their cost effectiveness, diverse metabolic pathways and versatility of microorganisms (Banat *et al.,* 1996; Singh *et al.,* 2004; Mendez-Paz *et al.,* 2005; Pandey *et al.,* 2007).

Immobilized cells have been used and studied extensively for the production of useful chemical (Ohta *et al.,* 1994; Chang and Chou, 2002), the treatment of wastewaters (Chen *et al.,* 2000, Wang *et al.,* 2000), and the bioremediation of contamination not only simplifies separation and recovery of the immobilized bacteria and the binding agent, but it also makes the application reusable, which reduces the overall cost. Immobilized materials, furthermore, have comparatively longer operating lifetimes due to an enhanced stability of the macromolecules or cells and consequently, protection from adverse conditions (Diaz *et al.,* 2001).

Although a number of workers described microbial degradation of textile effluent, limited literature is available on bioremediation of textile effluent using

immobilized bacterial cells. In the present investigation mixed culture of *Escherichia coli* and *Salmonella typhi*was immobilized on agar- agar in a bioreactor. The sample was analyzed for the initial level of BOD, COD, SS, DS, TS, Colour and some selected heavy metals.

Materials and Methods

Source of sample: The dye effluent was collected from Textile Industry, Kothur Industrial Area, Mahaboob Nagar, Telangana, India.

The effluent dye collected was analyzed in triplicates in the laboratory for BOD, COD, Nitrates, Phosphates Sulphates, Turbidity, Total Solids, Colour, and selected heavy metals were determined using standard method (APHA, 1989).

Determination of colour intensity: The colour intensity of the sample was determined with the aid of lovibond comparator by matching the colour of the sample with standard (APHA, 1989; Ademoroti, 1996).

Determination of Total Solid (TS): A clean dish of suitable size was dried at 102ºC-105ºC in an oven to a constant weight.100-250ml of thoroughly mixed effluent sample was accurately pipette into a dish, weighed and evaporated to dryness on a steam bath. The residue was dried in an oven for about 1hour at 103ºC–105ºC and re-weighed after cooling to room temperature. The cooling was done until the weight of the dish plus residue was constant to within 0.05mg. The Weight of the dish was subtracted to obtain the weight of the total solids (APHA, 1989; Ademoroti, 1996).

Determination of Suspended Solids (SS): 100 ml of effluent sample was withdrawn into a conical flask with a pipette. It was filtered in Gooch Fitted with glass fiber filter paper which has been pre-dried at 103–105ºC. The glass fiber was carefully removed from the Gooch and dried to a constant weight at 103–105ºC and the weight subtracted from the weight of the filter paper to obtain the weight of the suspended solids (APHA, 1989; Ademoroti, 1996).

Determination of Dissolved Solids (DS): Dissolved solids were obtained by difference between total solids and suspended solids (APHA, 1989; Ademoroti, 1996).

Determination of Chemical Oxygen Demand (COD): The untreated sample of the effluent was first analyzed for COD immediately after collection. The biological treated sample was also analyzed for COD. 10 ml of the sample was measured into the flask.1ml of 20% M/V mercuric sulphate solution was added and swirled to mix. 5ml of 0.020833M potassium dichromate was added.15 ml of 1%m/v silver sulphate was added boiled gently for 2hours.Two drops of ferroin indicator was added to the contents in the flask and the residual dichromate was titrated with standardized ferrous ammonium sulphate as reported in (APHA, 1989; ASTM, 2002; Asamudo, 2005).

Determination of Biological Oxygen Demand (BOD5): The untreated sample of the effluent was first analyzed for BOD5 immediately after collection. The biologically treated sample was also analyzed for BOD5 as earlier reported (APHA, 1989; Asamudo, 2005).

Nitrate, Sulphate and Phosphates levels were determined according to APHA 1995.Atomic absorption spectrophotometer (ASS) was used to determine the heavy

metals in the sample using standard protocols as described by (Hayat *et al.*, 2002). While Nitrate, Phosphate and Sulphates were determined according to standard method of (APHA, 1995).

Bioremediation process

Isolation and characterization of bacterial strains from textile dye effluent

From the textile dye effluents collected from sample site, the bacterial strains namely *Escherichia coli* and *Salmonella typhi* were isolated. The biochemical characterization of the bacterial strains was given in Table 2. These isolated microbial strains were cultured and developed bacterial colonies were used for the bioremediation of textile dye effluents.

Mixed culture of *Escherichia coli* and *Salmonella typhi* that mostly occurred during microbial isolation were selected as described by (Fawole and Oso, 2001), were grown in slant culture before use.

Immobilization of cells: Agar Solution and Inoculi were prepared separately. 50 ml of each of the inoculi was prepared and incubated for 24 hours. A solution containing 3% bacteriological agar (100ml) in a 250ml Erlenmeyer flask was sterilized and cooled to 40–45ºC. The inoculi with OD600nm= 0.1 which correspond to 109 CFU/ml was mixed with the prepared agar plates and solidification occurred after 10minutes.

The solidified agar block was cut into equal size cubes, then added to sterile 0.1ml phosphate buffer (pH 7.0), and kept in the refrigerator (1 hour) for curing. Phosphate buffer was decanted and the cubes were washed with sterile distilled water 3 to 4 times before used (Ellaiah *et al.*, 2005).

Two liters of the effluent supplemented with Minimum Basal medium in g/l: NaCl (0.8), $MgSO_4$. $7H_2O$ (0.001); KH_2PO_4 (2); $NaNO_3$ (2); $CaCl_2.2H_2O$ (0.5), $NaHPO_4.12H_2O$(2), sterilized in an autoclave at 121ºC for 15mins. 1000ml of the sterilized effluent was dispensed into 5liters bioreactor fitted with air sparger aseptically. Thirty grams each of the immobilized bacterial cells was added (Margesin and Schinner, 2005) Physicochemical Parameters were determined at every five days intervals to monitor the progress of bioremediation for 15days.

Results and Discussion

The table-1 represents the result obtained at the initial characterization of the sample of the effluent from Textile Industry, Kothur Industrial Area, Mahaboob Nagar, Telangana, India. The result indicated the mean values of COD, BOD, TS, SS, DS, Sulphate, Nitrate and Phosphate of 1150 ± 9.34 mg/l, 800 ± 5.67 mg/l, 4100 ± 29.32 mg/l, 1150 ± 17.73 mg/l, 3150 ± 4.18 mg/l, 6.35 ± 0.67 mg/l, 15.26 ± 2.48 mg/l and 2.98 ± 1.37 mg/l respectively. Heavy metals were also higher than the permissible limit. If this effluent is not treated before being discharged into the receiving river or soil, it can pose ecological threat. A high BOD and COD values show that the effluent have highly oxygen demanding waste (Kumar, 1989) which cause the depletion of DO which is a fundamental requirement for aquatic life. The highest value of COD gives valuable information about the pollution potential of the textile industrial effluents (Gupta *et al.,* 2003) (Table 1; Figure 1).

The colloidal and suspended impurities cause turbidity in the receiving streams and reduce the light penetration into water and ultimately decrease the photosynthesis (Sofianosheen *et al.,* 2000). Total solid

determined in this study was very high which has great implications in the biological and physical waste water treatment processes (Srivasta and Sinha 1996; Tobata et at., 2007; Ashish and Yogendra, 2009).

The dissolved minerals may increase salinity of the water and thus may render it unfit for irrigation or consumption. Toxic chemicals such as chromium and sulphites may destroy fishes and microorganisms responsible for self – purification of water. Impurities such as sulphites and nitrites can cause depletion of dissolve oxygen content of water. Water contaminated with metallic effluent can cause several health problems. Lead for instance, can interfere with enzyme activities and function of red blood cells. It can affect nerves and brain at low concentration (Ezeronye *et al.,* 2005). Heavy metals such as mercury, cadmium and chromium can bio accumulate and through the food chain to toxic levels in man (Howells, 1990).

The results obtained after treatment indicate a very good correlation with (Rajamohan and Karthikayan, 2004) who reported the reduction in the COD load of effluent below the upper limit of 25mg/l. The COD was reduced from 1150mg/l to 225mg/l after 15days of treatment. BOD (96%) and other physicochemical parameters such as TS (93%), SS (86%) DS (96.0%),. Nitrate, Sulphates and Phosphates were also reduced considerably. While some selected heavy metals were biosorphoned. Surprisingly, copper was completely disappeared after 15days of treatment (Table 1; Figure 1).

Table.1 Physicochemical parameters of raw & treated and overall percentage reduction of textile industrial effluent

Results of Treatment

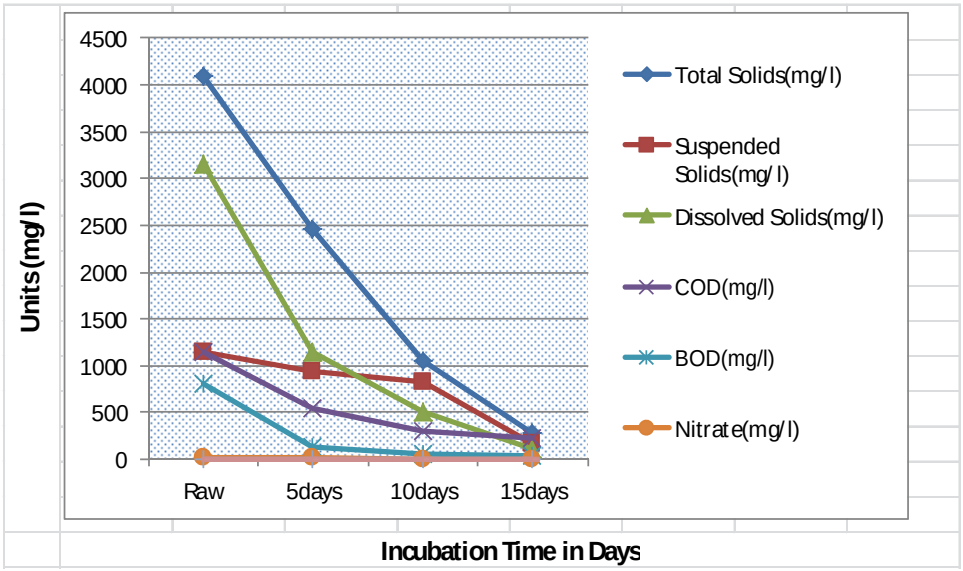
Parameters	Raw	5days	10days	15days	% of Reduction
pH	9.38	9.0	7.59	6.92	-
Colour	2.0	5.0	12.0	20	-
Total Solids(mg/l)	4100±29.32	2452±0.56	1050±0.82	290±0.11	93.0
Suspended Solids(mg/l)	1150±17.73	940±11.17	820±6.23	162±2.11	86.0
Dissolved Solids(mg/l)	3150±4.18	1145±5.72	510±5.52	115±3.45	96.0
COD(mg/l)	1150±9.34	545±1.87	295±1.54	225±1.09	80.0
BOD(mg/l)	800±5.67	125±9.26	56±0.93	32±0.11	96.0
Nitrate(mg/l)	15.26±2.48	12.49±2.02	9.13±2.47	5.18±0.53	66.0
Sulphate(mg/l)	6.25±0.67	4.43±0.78	3.18±0.48	2.02±1.28	67.0
Phosphate(mg/l)	2.98±1.37	2.48±0.33	1.84±0.27	1.12±0.47	62.0
Pb	0.11±0.02	0.09±0.02	0.06±0.022	0.04±0.059	-
Cu	1.096±0.069	0.71±0.23	0.48±0.121	0	-
Zn	0.302±0.06	0.14±0.04	0.03±0.028	0.027±0.114	-
Cr	0.065±0.00	0.046±0.048	0.03±0.219	0.03±0.218	-
Mn	1.06±0.034	0.33±0.060	0.02±0.110	0.02±0.212	-
Fe	8.53±0.327	5.20±0.16	2.25±0.17	0.41±0.124	-

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

Test	Colony 1	Colony 2
Gram staining	Gram negative, straight rods	Gram negative, straight rods
Motility	Motile	Motile
Catalase	+	+
Oxidase	-	-
Nutrient agar	Circular, smooth and colour less	Circular, moist, smooth and translucent
Glucose	+	+
Lactose	+	+
Sucrose	+	+
Manitol	+	+
Indole	+	-
Methyl red test	+	+
Voges proskauar test	-	-
Citrate	-	+
Urease	-	-
TSI	-	+
Strains	*Escherichia coli*	*Salmonella typhi*

Values are mean ± Standard error

Figure.1 Physicochemical Parameters of Raw and Treated of Textile Industrial Effluent



The result obtained from this present investigation showed that textile effluents are highly polluted in close agreement with the Randall and King (1980), (Kertell and Hill, 1982); (Sofianosheen and Khalil, 2000). The removal efficiency of the physicochemical parameters suggested the adoption of immobilized mixed culture of

Escherichia coli and *Salmonella typhi* for bioremediation of industrial effluent and this can also enhance ability to maintain the capacity of the isolates for bioremediation for extended periods of time and also make the application reusable.

Conclusion

In conclusion, we developed Immobilized microbiological preparation comprising a mixed culture of bacteria capable of removing toxic component of textile industrial effluent within few periods of time, under laboratory condition. It is clear from this research work that bioremediation of textile industrial effluents by bacteria is an effective method for treating textile effluent and can be a good substitute for conventional remediation processes. For improved commercial use of microorganisms, immobilization or pellet preparations in suitable carriers will prove economical for their use. However, if this can be developed in large scale it will be useful for the treatment of not only the textile effluent but industrial effluent of any kind.

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