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BIOREMEDIATION BY FREE AND IMMOBILIZED BACTERIA ISOLATED FROM TANNERY EFFLUENT

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ABSTRACT

The effect of free and immobilized cells of effluent tolerant bacteria isolated from the tannery effluents of Vaniyambadi to treat effluent water was studied. Two bacterial strains were isolated from tannery waste water and identified as *Pseudomonas putida* and *Bacillus cereus*. The level of turbidity and other Physico-chemical parameters found to decreases from high to moderate or optimum level in the effluent treated with free and immobilized cells of both isolated strains whereas, the pH was increased from 6.5 to 7.0. The percentage reduction of free CO_2, total alkalinity, hardness, dissolved oxygen, Nitrate and Nitrite was ranged from 45 to 75% when treated with *P. putida*, whereas they were ranged from 60 to 79% in the 15thday of the treatment. The level of BOD was reduced drastically after 15thday of exposure by the free cells of both bacteria. The maximum percentage of reduction (83%) was shown by immobilized *B. cereus.*The percent reduction of COD ranged from 16% to 65% in both the bacterial strains. The percentage of removal of Cr (VI) was 86 and 91% for free and immobilized cells respectively. In *B. cereus* treated sample, it reached 84% and 89% at the 15thday respectively. It is concluded that both *P. putida* and *B. cereus* investigated in this study are highly recommended for beneficial bioremediation applications for in-situ and off-site removal of pollutants.

KEYWORDS: Tannery Effluent, Immobilization, Vaniyambadi, Bioremediation

INTRODUCTION

India is naturally endowed with a large, domesticated animal population comprising of cattle, buffalo, sheep and goat. With the completion of the animal life cycle, the byproduct skin or hides of the animal had spawned the leather industry in India as in all parts of the world.Effluent – waste water discharged by tanneries is one of the major problems faced by the leather industry in India. The industry is very much aware of this problem and is doing its best to find out ways and means to overcome this problem of effluent treatment and disposal. A number of large tanneries have constructed their own individual effluent treatment plants (ETPs) at a heavy capital investment. Small Tanners with low investments could have their individual effluent treatment plants. Tannery industrial wastewater causesserious consequences for freshwater bodies and agricultural lands. The discharge of tannery effluents without proper treatments exhibited very high value for chromium, sulphide, and chloride, Total Dissolved Solids (TDS), Total suspended solids (TSS), Biological Oxygen Demand (BOD) and Chemical Oxygen Demand (COD) in the water bodies or land mass. The industry should attempt to treat its wastewater at the lowest cost that will yield a satisfactory effluent for the particular receiving stream, which may necessitate considerable study, research, and pilot investigations.

To prevent any health hazards caused by discharging wastewater to water streams, the wastewater must be treated before discharge. Such treatment should comply with the terms of the legislation defining the characteristics of the effluent discharging in water streams. The concept of planning and development should be based on the criteria to protect land, water resources, aquatic life in streams and rivers and marine life from pollution and to safeguard public health as a high priority.

Chromium is one of the most toxic heavy metals discharged into the environment through various industrial wastewaters, such as leather tanning, electroplating, paints, pigment production, steel manufacture. The effluents of these industries contain chromium at concentrations ranging from tenths to hundreds of milligrams per liter (Dermou*et al.*, 2005). The safe value in water for drinking purposes is 0.05 mg/l and recommended value for discharge is less than 5 mg/l (Directive EPA, USA, 2003; Debabrata*et al.*, 2006). High concentrations of chromium may pose an adverse impact onthe ecosystem and hence it would be desirable to remove chromium before subjecting the wastewater to biological treatment.

The conventional methods to detoxify and remove Cr (VI) from the environment involve chemical reduction followed by precipitation, ion exchange and absorption on coal, activated carbon, alum, kaolinite, and fly ash (Ohtake and Silver, 1994; Arundhati and Paul, 2005). However, biological treatments arouse great interest because of their lower impact on the environment. The processes by which microorganisms interact with toxic metals enabling their removal/and recovery are bioaccumulation, biosorption and enzymatic reduction (Srinath*et al*., 2002). Recent studies have shown that certain species of bacteria are capable of transforming Cr (VI), into the much less toxic and less mobile Cr (III) (Dermou*et al*., 2005; Camargo *et al*., 2005; Pal and Paul, 2005).

Poornima*et al.*,(2010) reported that the methods such as screening, flow equalization, primary sedimentation, chemical flocculation, aerobic activated sludge treatment, secondary sedimentation used for the waste treatment are very expensive and add to already present chemicals in the effluent. To overcome this problem biological methods could be tried which is cost-effective, simple and eco- friendly.

Bioremediation of tannery effluents is an environment-friendly, nontoxic and cost effective alternative technology. Recent developments in biotechnology now offer opportunities to modify organisms so that their basic biological processes are more efficient and can degrade more complex chemicals and higher volumes of waste materials. Bioremediation is addressed as one example of an environmental biotechnology.

Microbes in the environment play an important role in cycling and destroying them through bio-degradation. Microorganisms are very effective in pollution control, especially in effluent treatment (Srinivas*et al.*, 2011). The organisms which are naturally present in this effluent sample can withstand the adverse conditions (pH, turbidity, high BOD, COD, etc.) of it. Hence, these organisms (microbial consortia) have been isolated, identified and used for treating the same.

Immobilised cells have been used extensively in various industrial and scientific endeavours since they have been reported to be very effective in bioremediation. Freely suspended microbial biomass has disadvantages that include small particle size and low mechanical strength (Katiyar and Katiyar, 1997). Immobilised cells appear to be of greater potential in controlling particle size, better capable of regeneration, easy separation of biomass and effluent and re – circulation, high biomass loading, minimal clogging and reduced depletion of a nutrient source. It has also been reported that immobilized cells have found to be most effective in designing small and large – scale bioreactor for effluent degradation (James, 2002).

Immobilized microbial cells are used in organic synthesis, clinical and chemical analysis, food industries, medicine, and environmental applications as well (Chibata and Tosa, 1981). The expansion of biotechnology and the expected developments has encouraged effects to immobilize enzymes and cells for applied purpose (Bickerstaff, 1987). For a particular application, it is necessary to find an immobilization procedure that would be simple and inexpensive. Immobilization of the biomass within a suitable matrix provides a physical support for cells, ideal size, mechanical strength, rigidity and porous characteristics of the biological material (Bucke, 1983; Trujillo *et al*., 1995).

Based on the available literature, it is evident that bioremediation of tannery effluent of Vaniyambadi region using immobilized bacteria is scanty and hence a preliminary attempt has been made to investigate the effect of free and immobilized cells of effluent tolerant bacteria to biodegrade certain important physical and chemical parameters including BOD, COD and chromium.

MATERIALS AND METHODS

Collection of Effluent Samples

The tannery effluent wastewater sample was collected from a commercial tannery at Vaniyambadi, India. The sample collected in pre-cleaned container was brought to the laboratory with due care and stored at 4°C to arrest any biological activity.

Analysis of Physico-Chemical Parameters

From this stock, required amount of samples was taken to analyze physico-chemical parameters such as pH, free CO_2, total alkalinity, total hardness, dissolved oxygen, Biological oxygen demand (BOD), Chemical oxygen demand (COD), nitrates and nitrites following standard methods outlined by APHA (1989). Colour removal was studied by optical density and visual appearance.

Isolation and Screening of Effluent Tolerant Bacterial Strains

Effluent tolerant bacteria were isolated from tannery effluents obtained from Vaniyambadi region, Vellore District. For the isolation and enumeration of bacteria, the samplewas serially diluted and plated on Luria–Bertani (LB) agar (tryptone: 10 g l^{-1}; yeast extract: 5 g l^{-1}; NaCl: 10 g l^{-1}; glucose: 0.1 g l^{-1}) adjusted at normal pH value of 7.0 (Charurvedi, 2011). Plates were incubated at 30^{o}C in the dark and read after 48 hrs. Subsequently, four isolates were selected according to their morphological shapes for further studies.

Morphological and Biochemical Characterization of Isolated Bacteria

The isolated bacteria were subjected to Gram's staining technique followed by the various biochemical tests such as Mae Conkey agar test, Iodole test, Methyl red test, Voges Proskauer test, Citrate utilization test, Starch hydrolysis test, Urea hydrolysis test, Nitrate reduction test, Hydrogen sulfide production test, Cytochrome oxidase test, and finally Catalase test were done for the identification of bacteria (Cappuccino and Sherman, 1999).

Immobilization of Bacteria

The cells in the exponentially growing culture were harvested and harvested cells were then homogenized. The homogenate and distilled water were measuredas the ratio of 20:80 into a 250 ml conical flask and mixed properly to ensure homogeneity. After settling for he supernatant (concentrated cells) was collected and stored at 20°C. Exactly 3.063

g of sodium alginate was weighed into the concentrated cells. The mixture was subsequently dropped through a sterilized syringe and a needle into a flask containing sterilized 70 ml of 0.12 M $CaCl_2$. Gel formation was achieved at room temperature as soon as the sodium alginate drops come in direct contact with the calcium solution. Complete precipitation formed spherical beads of diameter 3 -4 mm. The beads are allowed to fully harden at 1-2 hours. The beads were washed with fresh calcium cross linking solution (Shide*et al* 2004).

Bioremediation of Tannery Effluent Using Bacterial Inoculum and Immobilized Bacteria

Approximately 10 ml of fresh *Pseudomonasputida* and *Bacilluscereus* were transferred to experimental flasks containing 100 ml of sterilized tannery effluent. They were kept in an orbit shaker (200 rpm) at 30 ± 2°C. The experiment was conducted in batch cultures in duplicates for a total period of 15 days under laboratory conditions. Since the effluent was dark in colour and the growth of *P. putida* and *B. cereus* was very slow in the effluent, a long duration was provided to get a culture of exponential growth.

Physico-chemical parameters were observed on 5^{th},10^{th} and 15^{th} day of the experimental period. Physico-chemical parameters such as turbidity, pH, total alkalinity, total hardness, colour, odour, turbidity, nitrite, nitrate, free CO_2, Dissolved O_2 and also BOD and COD were done as per the standard protocols to check the degradation process by the free and immobilized bacteria.

Chromium Remediation by Individual cells and Immobilized cells of Selected Bacteria

The batch adsorption experiments were carried out to determine the reduction of Cr (VI) by immobilized *P. putida* strain S1 and *B. cereus* strain S2. 2 mg cell dry weight were transferred to experimental flasks containing 100 ml of sterilized tannery effluent.Incubated at 30 ± 2°C with orbital shaking (200 rpm) and the samples were taken from each flask on 5^{th}, 10^{th} and 15^{th}day of the experimental period.

Hexavalent chromium reduction was determined from the difference between before and after treatment.The samples were analyzed for hexavalent chromium using Atomic Absorption Spectroscopy (US EPA Method 7195, 1986).

After the first cycle (5 days), the alginate beads containing encapsulated cells were filtered, washed and used in a second cycle for reduction of Cr (VI) at concentration 120 mgl^{-1}. Three repeated batch cycles were performed.

RESULTS

Isolation of Bacterial Strains from Effluent

From the present investigation, a total of four isolates of bacterial flora were obtained from the effluent sample. Among the four isolates two dominate isolates were characterized and identified by using standard morphological, physiological and biochemical tests.

Biochemical Tests

Biochemical characteristics of isolated bacteria were shown in Table 1. Among the two strains (S1 and S2), S1 showed a positive test of Mac Conkey agar, citrate utilization, Hydrogen sulfide production, Cytochrome oxidase, catalase, ammonia production, IAA, phosphate solubilization and dextrose tests, whereasS2showed the positive test Methyl red, Voges Proskauer, Citrate utilization, Starch hydrolysis, Urea hydrolysis, Nitrate reduction, Catalase, Dextroseand Mannitol tests.

On the basis of these features, bacterial strains S1 and S2 were identified as *Pseudomonas putida and Bacillus cereus*respectively**.**

Bioremediation of Tannery Effluent Sample

The results of the changes in physical properties of the effluent sample by both free and immobilized cells of *P. putida* and *B. cereus* are given in the Table 2 and 3 respectively. The results of the changes in chemical properties of the effluent sample by both free and immobilized cells of *P. putida* and *B. cereus* are given in the Table 4 and 5 respectively.

Colour

The colour of the untreated effluent was blackish in colour with offensive odour. But when exposed to free and immobilized cells of both *P. putida* and *B.cereus*, effluent was slightly brownish to white in colour, but the odour was similar to that of the untreated. Both *P. putida* and *B.cereus* significantly reduced the colour from the effluent in both free and immobilized conditions (Figure1). More than 75% removal of colour was recorded in all the treatment in the incubation period of 15 days; though the immobilized cells performed marginally well over free cells (More than 80%).

pH

The pH was recorded in the effluent initially 6.5 and it came to 7.0 in free *P. putida* cells treated effluent whereas it was 7.2 in immobilized cells' treated effluent (Table 4). A similar trend was observed in the effluent treated with both free and immobilized cells of *B. cereus*. (Table 5).

FreeCO_2

The initial free CO_2 was recorded 33 mg/L and it was reduced slightly from 5th day onwards. The maximum of 16 and 17 mg/L reduction was observed in sample treated with the free cells of *P.putida* and *B.cereus* respectively on 15th day. In the effluent sample treated with immobilized cells of both bacteria, the reduction of free CO_2 was maximum 8 and 7 mg/L, respectively on the 15th day of the experiment (Table 4 and 5). After 15th day, there was nearly 51 and 75 % reduction of free CO_2 observed in the sample 1 exposed to free cells and immobilized cells of *P. putida* (Figure 1) whereas, maximum reduction of about 48 and 79 % was shown by *B. cereus* (Figure2).

Total Alkalinity

The alkalinity was recorded 1890 mg/L initially. It was brought down from the 5th onwards (Table 4 and 5). The maximum (52 and 68) percentage reduction was observed in 15th day in effluent treated with free and immobilized cells of *P. putida* respectively (Figure.1). But the maximum reduction (52 and 76%) was observed in the sample treated with free and immobilized cells of *B. cereus* respectively (Figure. 2).

Total Hardness

The total hardness in the control was recorded 2550 mg/L. It was reduced from 2100 mg/L on the 5thday to 980 mg/L on the 15th day of treatment by free cells of *P. putida* whereas maximum reduction of 760 mg/L of hardness was observed by immobilized cells. There was no much variation in the reduction of hardness between the free and immobilized cells of both species of bacteria (Table 4 and 5). The percentage reduction was nearly 70% than control (Figure 1 and 2).

DO

The dissolved oxygen level was increased from 1.3 mg/L in control to 2.2 mg/L in the sample treated with both free and immobilized cells of *P. putida* (Table 4). The DO level was increased from 1.3 to 2.3 mg/L on the 15th day in immobilized *B. cereus* inoculated effluent (Table 5). The 69 and 76 % reduction was observed on 15th day when compared to control with immobilized cells of both bacteria (Figure 1 and 2).

Nitrate and Nitrite

The Nitrate and Nitrite were recorded initially 95 and 50 mg/L respectively. (Table 4 and 5). On 15th day it was reduced to the maximum of 45 and 60% by immobilized cells of *P. putida* respectively, but immobilized cells of *B. cereus* showed maximum reduction 52 and 64 %, respectively (Figure 1 and 2).

Reduction of BOD and COD by Bacterial Cells

The optimum concentration of sodium alginate which offers stability and maximum activity to the immobilized cell bead was studied by determining the percent reduction of BOD and COD. Maximum reduction of BOD and COD in both the species of bacteria was observed at 4% sodium alginate level (Table 6).

The initial level of BOD (348 mg/L) was reduced drastically to 110 and 90 mg/L after 15th day of exposure by the free cells of *P. putida* and *B. cereus* respectively. The maximum percentage of reduction (83%) was observed in the sample treated with immobilized *B. cereus* against free cells (73%) (Figure 3).

Likewise, COD (860 mg/L) was also remarkably reduced, and it varied from 720 to 440 mg^{-1} after the 15th day of exposure by the free cells of *P. putida* and from 735 to 320 mg^{-1} by *B. cereus*. Immobilized cells of both species showed a maximum reduction of 64 and 65%, respectively (Figure 4).

In general, the percent reduction of BOD varied from 27% to 83% by both the bacterial strains. In the case of COD, the percent reductions ranged from 16% to 65% by both the bacterial strains (Table 7).

Chromium Remediation by Individual cells and Immobilized cells of Selected Bacteria

The results of chromium removal from treated effluent water are shown in Table 8. The data shows chromium removal efficiency by both free and immobilized cells of *P. putida* and *B. cereus*. The ability of chromate-resistant bacterial *P.putida* for reduction of Cr (VI) at 435 mg/L using free and immobilized cell using calcium alginate were estimated as described before in the materials and methods section. The removal percentage of Cr (VI) ion increased with increasing the time, which reach to the maximum reduction after 10 days, the reduction is 86 and 91% for free and immobilized cells respectively as shown in Figure 5.

The Cr (VI) reduction was observed with free and immobilized cell suspensions of *B.cereus* which reached to 84 and 89% on the 15th day respectively.

The reduction of Cr (VI) was high with immobilized cell comparing with the free cell, also using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which make it more economical.

Table 1:Biochemical Characteristics of Isolated Bacteria

Sl No	Characteristics	Isolate 1	Isolate 2
Morphology			
1	Gram Reaction	-	+
2	Cell shape	Rod	Rod
Biochemical Tests			
1	Mac Conkey agar test	+	-
2	Indole test	-	-
3	Methyl red test	-	+
4	Voges Proskauer test	-	+
5	Citrate utilization test	+	+
6	Starch hydrolysis test	-	+
7	Urea hydrolysis test	-	+
8	Nitrate reduction test	-	+
9	Hydrogen sulfide production test	+	-
10	Cytochrome oxidase test	+	-
11	Catalase test	+	+
12	Ammonia production	+	-
13	IAA	+	-
14	Phosphate solubilization	+	-
15	Dextrose	+	+
16	Mannitol	-	+
Species name		***Pseudomonasputida***	***Bacilluscereus***

Table 2: Effect of Free and Immobilized Cells of *Pseudomonasputida*on Physical Parameters of Untreated and Treated Tannery Effluent Sample

Parameters	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		Free cells	Immobilized cells		Free cells	Immobilized cells		Free cells	Immobilized cells
Appearance	Turbid	Turbid	Turbid	Turbid	Turbid	Slightly clear	Turbid	Slightly clear	Clear
Color	Dark Brown	Dark Brown	Light yellow	Dark Brown	Light brown	Slightly White	Dark Brown	Slightly White	Slightly White
OD at 620 nm	1	0.87	0.81		0.61	0.60		0.34	0.21
Odor	None	None	None	None	None	None	Agreeable	Agreeable	Agreeable
Turbidity NT Units	12	10	10	11	9	8	10	7	6

Table 3: Effect of free and Immobilized cells of *Bacilluscereus*on Physical Parameters of Untreated and Treated Tannery Effluent Sample

Parameters	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		Free cells	Immobilized cells		Free cells	Immobilized cells		Free cells	Immobilized cells
Appearance	Turbid	Turbid	Turbid	Turbid	Turbid	Slightly clear	Turbid	clear	Clear
Color	Dark Brown	Dark Brown	Light yellow	Dark Brown	Light brown	Slightly White	Dark Brown	Slightly White	Slightly White
OD at 620 nm	1	0.80	0.6		0.54	0.4		0.32	0.2
Odor	None	None	None	None	None	None	Agreeable	Agreeable	Agreeable
Turbidity NT Units	12	10	10	10	9	8	10	6	5

Table 4: Effect of free and Immobilized cells of *Pseudomonasputida*on Chemical Parameters of Untreated and Treated Tannery Effluent Sample

Parameters	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		Free cells	Immobilized cells		Free cells	Immobilized cells		Free cells	Immobilized cells
pH	6.5	6.6	6.7	6.5	6.8	6.9	6.5	7.0	7.2
Free CO2 (mg/L)	33	28	22	33	20	17	30	16	8
Total Alkalinity (mg/L)	1890	1510	1110	1870	1100	800	1700	900	600
Total Hardness (mg/L)	2550	2100	1800	2520	1810	1110	2400	980	760
DO (mg/L)	1.3	1.4	1.4	1.3	1.9	1.9	1.4	2.2	2.2
Nitrate (mg/L)	95	85	75	87	70	60	80	62	52
Nitrite (mg/L)	50	40	30	48	35	25	45	30	20

Table5.Effect of Free and Immobilized Cells of *Bacilluscereus*on Chemical Parameters of Untreated and Treated Tannery Effluent Sample

Parameters	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		Free cells	Immobilized cells		Free cells	Immobilized cells		Free cells	Immobilized cells
pH	6.5	6.6	6.7	6.5	6.8	6.9	6.5	7.1	7.2
Free CO2 (mg/L)	33	28	20	33	21	17	30	17	7
Total Alkalinity (mg/L)	1890	1520	1020	1870	1150	750	1700	900	450
Total Hardness (mg/L)	2550	2200	1700	2520	1810	1010	2400	950	750
DO (mg/L)	1.3	1.4	1.4	1.3	1.9	1.9	1.4	2.3	2.3
Nitrate (mg/L)	95	80	70	87	70	60	80	55	45
Nitrite (mg/L)	50	42	32	48	36	26	45	28	18

Table 6: BOD (Mg/L) of Effluent Sample before (Control) and after Biodegradation (15 Days) Using Bacteria

Bacterial cells	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		P. putida	*B. cereus*		*P. putida*	*B. cereus*		*P. putida*	*B. cereus*
Free Cells	348	255	220	345	165	158	340	110	90
Immobilized cells	348	205	210	345	145	158	340	85	60

Table 7: COD (Mg/L) of Effluent Sample Before (Control) and after Biodegradation (15 Days) using Bacteria

Bacterial cells	5th Day			10th Day			15th Day		
	Control	Treated		Control	Treated		Control	Treated	
		P. putida	*B. cereus*		*P. putida*	*B. cereus*		*P. putida*	*B. cereus*
Free Cells	860	720	735	855	680	660	855	440	320
Immobilized cells	860	700	705	855	610	580	855	310	300

Table 8:Chromium (Mg/L) Remediation in Tannery Effluent Sample by free And Immobilized cells of *Pseudomonasputida*and *Bacilluscereus*

Group	Test organisms	Initial	5th Day	10th Day	15th Day
Individual cells	*P. putida*	435	170	110	60
	B. cereus	435	185	120	70
Immobilized cells	*P. putida*	435	98	65	40
	B. cereus	435	95	70	45

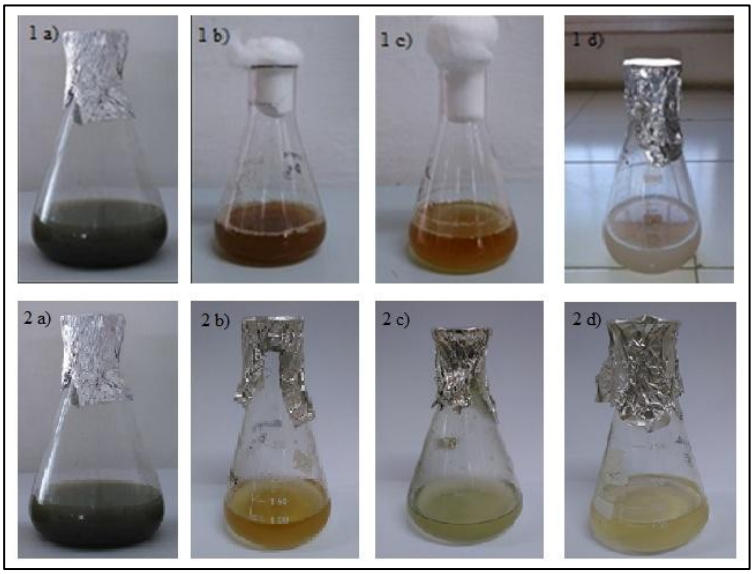

Figure 1: Change of Colour of the Effluent Sample when Exposed to Immobilized cells of *Pseudomonas Putida* and *Bacillus Cereus*

- a) and 2 a). Tannery effluent water sample 1 on 0 day (control)
- b) When exposed to immobilized cells of *P. putida* after 5 days
- c) When exposed to immobilized cells of *P. putida*after 10 days
- d) When exposed to immobilized cells of *P. putida*after 15 days
- b) When exposed to immobilized cells of *B. cereus* after 3 days
- c) When exposed to immobilized cells of *B. cereus* after 6 days
- d) When exposed to immobilized cells of *B. cereus* after 10 days

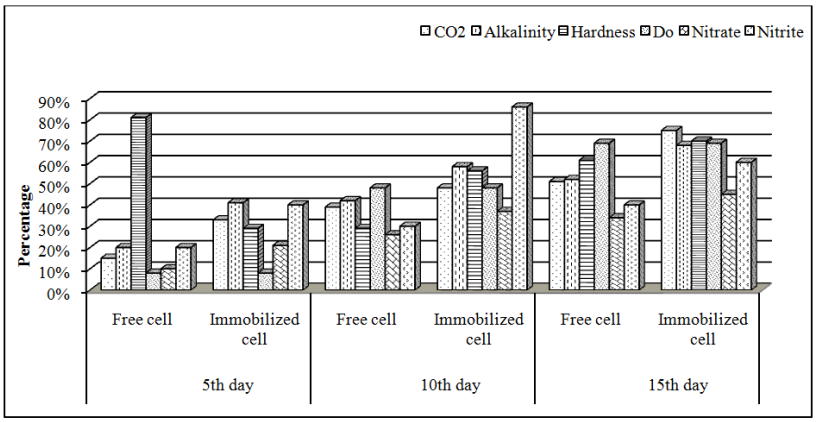

Figure 2: Percentage Reduction of Chemical Parameters of Effluent water Sample 1 Exposed to free and Immobilized cells of *Pseudomonas Putida*

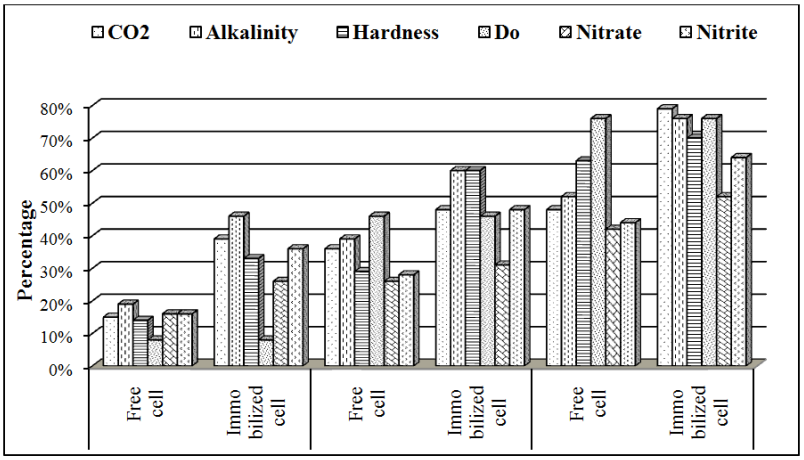

Figure 3:Percentage Reduction of Chemical Parameters of Effluent water Sample 1 Exposed to free and Immobilized cells of *Bacillus Cereus*

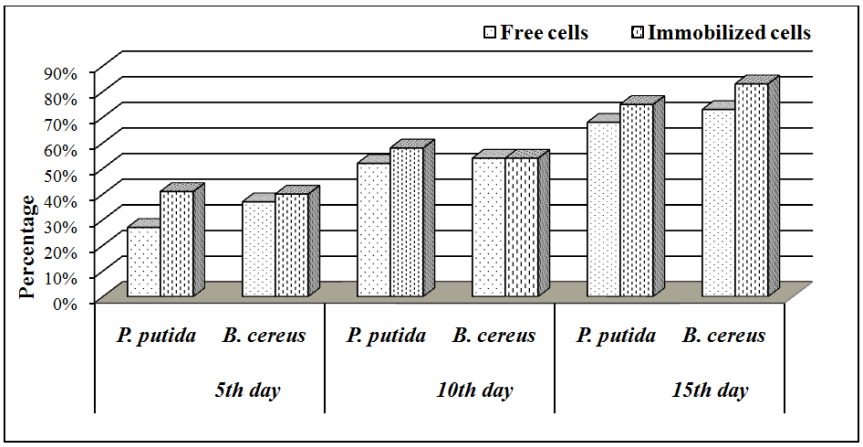

Figure 4:Percentage Reduction of BOD in Effluent Sample 1 before and after Treatment with free and Immobilized cells of *Pseudomonas Putida* and *Bacillus Cereus*

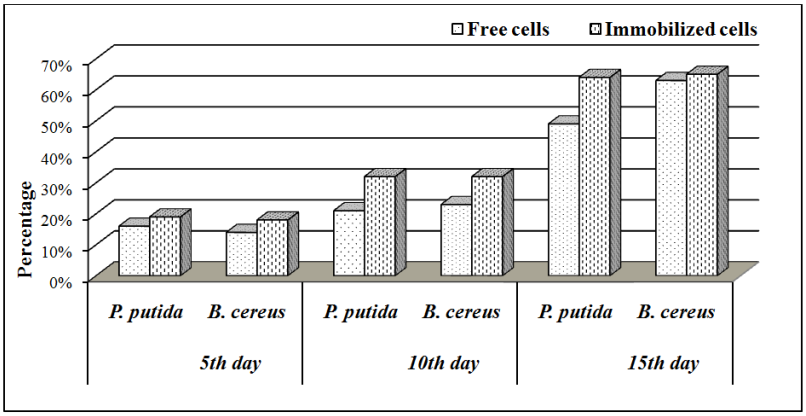

Figure 5: Percentage Reduction of COD in Effluent Sample before and after Treatment with free and Immobilized cells of *Pseudomonas Putida* and *Bacillus Cereus.*

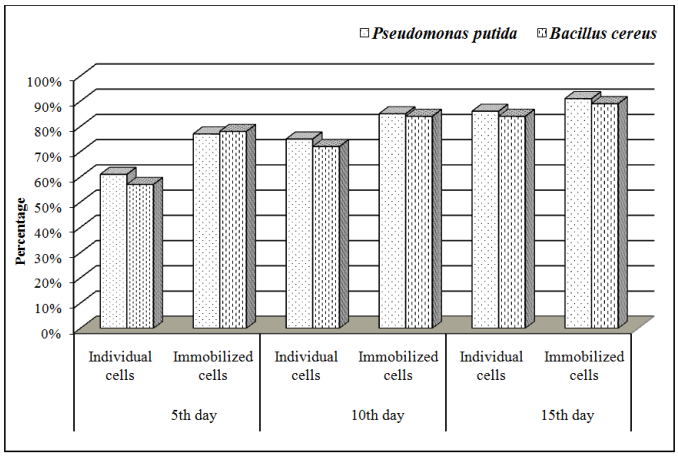

Figure 6:Percentage Reduction of Chromium in Tannery Effluent Sample 1 by free and Immobilized cells of *Pseudomonas Putida* and *Bacillus Cereus.*

DISCUSSIONS

Water pollution is a major pollution that affects the environment much and one of the main sources of this pollution is industrial effluent (Srinivas*et al.*, 2012). Tannery waste is a major hazard that affects the drinking water and hence it should be treated effectively before release (Alebel*et al.*, 2010).

In this work, biotechnological treatment of tannery effluent was tried since the use of immobilized bacteria is ecofriendly (Central pollution control board, 2009). This study of the efficiency of immobilized bacteria in Tannery effluent treatment showed the presence of two different strains S1 and S2. The isolate 1 was found to be gram negative, rod shaped and showed negative results for spore staining while, Isolate - 2 was gram positive, cocci shaped and recorded positive results for spore staining. The colonies of Isolate -1 showed dirty white colour, whereas Isolate - 2 produced creamy white colonies. Similar findings were reported by Chaturvedi (2011). Poornima*et al.*, (2010) also described a Cr (VI) tolerant, gram-negative, non-spore forming, non-capsulated and motile bacteria. It is confirmed and supported by the earlier finding of Ramlake and Bhattachrjer (1992). They also suggested that the polluted habitats had found mostly *Pseudomonas putida* and *Bacilluscereus* because they have the ability to degrade various pollutants from water samples. In the present study, the count of the *B.cereus* was found dominantly rather than the count of*P. putida*. Srinivas Gidhamaari (2012) also reported the presence of these species from the effluent sample of NRM tannery, Tiruchirappalli, Tamil Nadu, India.

In the present investigation, immobilized bacteria were employed to treat the effluents collected from the tannery. For immobilization sodium alginate was used. Ramesh and Singh (1993) reported the immobilized bacteria having more efficiency to remove the suspended particles than free cells.

The pH of the tannery waste water from the study station did not meet the general standards recommended (Hugo Springer, 1994) for the discharge of wastewater into the inland surface water for irrigation purposes. Discharge of untreated effluents with such a pH into ponds, rivers or on lands for any purpose may be detrimental to soil fauna and aquatic biota such as zooplankton and fishes, since low pH level may affect the physiology of fish (Islam *et al.*, 2014).

The level of turbidity (O.D), pH and Physico-chemical parameters found to decreases from high to moderate or

optimum level in the effluent treated with free and immobilized cells of isolated strains. It may be due to the various factors such as concurrent process of absorption and metabolism properties of the microbial consortia (Prakash, 2001), heavy metal tolerance by permeability barrier, intra- and extra-cellular sequestration, active transport efflux pumps, enzymatic methods and also a reduction in the sensitivity of targeted cellular organelles to metal ions (Bruins*et al.*, 2000). From the above work, it is evident that the flora which was isolated from the tannery effluent of Vaniyambadi, Vellore, Tamil Nadu, India, could be used for biological treatment after analyzing their biosafety, feasibility and efficacy levels.

The colour of the untreated effluent was found to blackish in colour with offensive odour. A similar result was reported by Noorjahan (2014) for the untreated tannery effluent collected for a period of 6 months (May 2011 to October 2011). A large number of pollutants can impart colour, taste and odour to the receiving waters, thereby making them unaesthetic and unfit for domestic consumption (Jamal *et al.*, 2011). However, in the present study, treated effluent was slightly brownish to white colour, but the odour was similar to that of the untreated. Brown colour of the tannery effluent was also reported by Smrithi and Usha (2012). The colour of the effluent might be due to the presence of biodegradable and non-biodegradable high molecular weight organic compounds and high amount of inorganic chemicals like sodium and chromium used during the processing and the odour may be due to putrefaction of the organic residues from the processed skin and hides (Smrithi and Usha, 2012).

Both *P. putida* and *B.cereus* significantly reduced the colour from the effluent in both free and immobilized conditions. More than 75% removal of colour was recorded in all the treatment in the incubation period of 15 days; though the immobilized cells performed marginally well over free cells (more than 80%). Among the bacteria, *B.cereus* was more efficient in colour removal than *P.putida*. Hence the effluent was used in 100% without dilution. Similar observations were made in textile dyes by using various fungi. Kasinath*et al.*, (2003) reported that *Aspergillus niger* and *Trichoderma viride* proved to be efficient in decolourization of scarlet red up to 80%. Immobilized bacteria were found to decolourize the effluents effectively than free bacterial cells. The above observations were made mostly with different fungi and bacteria.

For the evaluation of the pollution load of industrial or domestic wastewaters, a measure of oxygen requirement of pollution matter has been developed as standard parameters, which is known as Biochemical Oxygen Demand (BOD). BOD is a measure of the content of organic substances in the waste water which are biologically degradable with consumption of oxygen usually indicated as 5-day biochemical oxygen demand (BOD). This is the amount of oxygen in milligrams per litre (mg O_2/l) that consumed by microorganisms in 5 days at 20^{o}C for oxidation of the biologically degradable substances contained in the water.

The BOD level recorded initially (348 mg/L) indicates high organic load surpassedthe legal limit of (5-200) mg/L of effluent discharge into inland surface waters (Hugo Springer, 1994). This result is in agreement with the result of Kulkarni (1992) who observed that the bacterial culture removed BOD almost completely in two weeks from the date of inoculation in the water samples.

Chemical oxygen demand (COD) is a quantity of oxygen expressed in milligram consumed by the oxidisable matter contained in one liter of the sample. The test is performed by vigorous oxidation with chemicals and back-titrating the chemical consumed for oxidation. COD is a system of measuring the content of organic impurities with oxidizing agents. The COD level recorded initially (860 mg/L) exceeds the permissible COD level of (50-450) mg/L (Hugo Springer,

1994). This indicates that the effluent is unsuitable for the existence of the aquatic organisms due to the reduction in the dissolved content. The ability of the bacteria to reduce the pollution load in effluent water has been studied by different workers all over the world (Bruins, *et al.,* 2000; Shashirekha*et al.,* 2008; Ravishankar*et al.,* 2015). In the present study also, nearly 65 to 75% reduction of various parameters including BOD and COD were achieved by immobilized bacteria than that of free cells when treated with *P.putida* and *B.cereus*.

Though nitrate removal was also observed, the bacteria could not remove nitrate completely from the effluent. In general, a gradual reduction of nitrate level was noticed from 5thday onwards. On the15th day, the percentage removal of nitrate and nitrite was maximum in effluent with immobilized bacteria and minimum with free cells of both bacteria. Of the two bacteria, *B. cereus* was more efficient in removing nitrate than *P. putida*. It is supported by earlier report of Madhu and Pillai (1994) who reported nearly 60% of removal of nitrate and ammonical nitrogen from the fertilizer industry wastewater by using bacterium *P. putida.*

There was a gradual increase in dissolved oxygen (DO) content from 5thday onwards. On the 15th day, immobilized bacteria recorded maximum level of DO as compared to free cells. Increase DO level when treated with different bacteria with different effluents has already been reported (Srinivas*et al.,* 2012).

The alkalinity of water is its acid neutralizing capacity. It is the sum of all the bases. The alkalinity of natural water is due to the salt of carbonates, bicarbonates, borates silicates and phosphates along with hydroxyl ions in the Free State. However, the major portion of the alkalinity is due to hydroxides, carbonates and bicarbonates.

The composition of solids present in tannery effluent mainly depends upon the nature and quality of hides and skins processed in the tannery (Islam *et al.,* 2014). The level of average suspended solids was found to be higher in the untreated effluent water. The total hardness of untreated effluent was found to be higher (2550 mg/L) than the permissible limit of Central Pollution Control Board, India (2009), whereas for treated effluent, the values were 980 mg/L (Free cells of *P. putida*), 760 mg/L (Immobilized cells of *P. putida*), 950 mg/L (Free cells of *B. cereus*) and 750 mg/L (Immobilized cells of *B. cereus*). Similarly, in the study conducted by Noorjahan (2014), total hardness (1416-3850 mg/L) was observed for untreated effluent.

The percentage of removal of Cr (VI) ion increased with increasing the time, which reached to the maximum reduction after 15 days, the reduction was 86 and 91% for free and immobilized cells respectively.

Similarly, McLean *et al.,* (2000) with *Pseudomonas* strain CRB5 reported the reduction rate decreased during the first 24 hrs at Cr (VI) concentrations of 30 and 40 μgml^{-1}. Also,DeLeo*et al.,* (1994) reported a 99.7% reduction of 112.5 $\mu g\ ml^{-1}$ Cr (VI) by *P. fluorescence* LB300 within a period of 289 hrs. The *Pseudomonas* strain CRB5, however, showed a complete reduction of 20 µg ml-1 of chromate after 120 hrs Mclean *et al.,* (2001). However,Sikander*et al.,* (2007) showed that the rate of Cr (VI) reduction by *Ochrobactrumintermedium* strain SDCr-5 decrease with time irrespective of initial Cr (VI) concentration used.

The Cr (VI) reduction was observed with free and immobilized cell suspensions of *B. cereus* strain which reached at 84 and 90% of the 15th day respectively. The reduction of Cr (VI) was high with immobilized cell comparing with the free cells. Using the immobilized cell is preferable due to its capability for using several times with the same efficiency, which make it more economical. Similar work is done by Sikander*et al.,* (2007) showing the higher reduction with

premeabilized cell of *Ochrobactrum intermedium* strain SDCr-5. The results revealed the isolation and identification of isolates with potential for reduction of Cr (VI) to Cr (III). Results indicated that immobilized *B. cereus* could be efficiently used for reduction of Cr (VI).

CONCLUSIONS

From the result of physicochemical analysis of untreated and treated tannery effluents, it has been concluded that alkalinity, total hardness, nitrites, nitrates, BOD, COD, etc. are very high in untreated sample compared to the treated effluent. The parameters of untreated effluent in some instance, exceeded the standards prescribed by CPCB. Chromium (VI) oxide concentration also showed great variability. Such effluents should not be discharged into the nearby water body or soil without proper treatment. This study extensively investigated the effect of both free and immobilized conditions of *Pseudomonas putida*and *Bacillus cereus* in treating all these parameters. From the above discussion, it is clear that both bacteria can successfully be used not only for colour removal, but also to reduce the pollution load from the effluent. However, among the two bacteria, *B. cereus* showed significant reduction of the all the physico-chemical parameters analyzed, but the percentage reduction of chromium was slightly lesser when treated with *P. putida*. Both the immobilized bacteria have potential value for the removal of various chemicals including chromium. Therefore, the *P. putida* and *B. cereus* investigated in this study are highly recommended for beneficial bioremediation applications for in-situ and ex-situ removal of pollutants.

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