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A Comparative Study on Sulphate Reduction from Tannery Effluent by Biosorbent with Microbes and Isolation and Molecular Characterization of Microbes Involved in Reduction

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The paper deals with a comparative study on sulphate reduction from Tannery effluent by bio sorbent with microbes & Isolation and Molecular Characterization of Microbes involved in reduction. Four different plant material such as bark, seed, leaf and root of plants were screened and used for sulphate reduction Among this, flower of *Bongaim Villea Spectalrilus* gives much Sulphate reduction than the other sorbent the efficiency is 28% in a time interval of 120 hours but in the time interval 36hours microbial reduction there is a rapid reduction of sulphate is seen. The molecular identification is made and microbe is identified with 16S r RNA as *Aneurinibacillus aneurinilyticus* and submitted on GenBank.

Key words: Sulphate Reduction, Tannery effluent, Microbes and Biosorbent.

Tanning is an ancient craft in India and has been practiced for many centuries as an industrial operation at the village level. With the progress of time, however, it has acquired the status of a mature industry playing an important role in the country's economy. The industry flourishes in conditions of natural advantage offered by large cattle population, which supplies raw material (hides and skins) amounting for 15% of cattle, 46% of buffalo, 17% of goat and 4% of sheep of the world. The tanning industry is scattered unevenly in the country and it exists in the large, medium, small and cottage sections with about 150 large, 600 medium and over 1,00,000 cottage units. The medium sector alone contributes about 60-70% of the total production and 20% of the exports of leather and leather goods. Leather is one among the major foreign exchange earners for India, with the emphasis on exporting more of quality finished leather and leather products to meet international demand, many of the leather manufacturing units have diversified their activities and considerable investment has been made on sophisticated equipment, thereby replacing the manual operations by mechanization in the production of

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leather industry is about Rs.500-600 Crores, out of which Rs.300 Crores are earned by the export of semi-tanned leathers, semi-finished leathers, leather goods and footwear. Although tanning industry has been in existence for a long time, the problem of environmental pollution received serious consideration only in recent years. The pollution from large number of tanneries in the country has caused considerable damage of water sources and affected drinking water supply and irrigation. It is realized that the untreated waste waters when allowed to stagnate as is being done in most cases, give rise to odour nuisance, uprightly appearance creating ground and surface water pollution. The industry is generally associated with noxious smell arising from its raw materials, solids, liquid and gaseous wastes emanating from the industry adopting media methods of processing, with the increase in number of tanning units and the lack of application of suitable methods of disposal of the wastes, the problem of environmental pollution is likely, to aggregate further in coming years with a severe impact on the environment, necessitating early measures for the control of pollution from tanneries. Tanneries are attractive because of its foreign exchange potential. Tannery is one of the major water consuming industries. There are about 1000 tanneries in India, of which 568 are located in TamilNadu (Paul Bhaskar 1992). The chemicals used in tanning process are sodium carbonate, sodium bicarbonate, sodium chloride, chromium sulphate, sulphide, fat liquors, oil and dyes. For every 100 kgs of skins tanned 3200 litres of fresh water is used (Apparae and karthikeyan 1990). The effluents discharge from the tanneries contains high values of pH, EC, chlorides, sulphides, sulphate, carbonate, chromium, BOD, COD, oils and dyes. The values are much higher than the tolerance limits for industrial effluents discharged into inland surface or into public sewers as prescribed by BIS standard (sastry 1981). Out of the 568 tanneries in TamilNadu, 68 are located in Dindigul district. All these tanneries releases 500 lakhs litres of polluted water per day and contaminate ground water to a radius of 6 kms. Due to continued discharge of the effluent, the irrigation water also has become highly sorbent. The irrigation of the lands with sorbent water has fo a long period of time has reduced the soil fertility and there is a considerable loss in the yield of crops (Rajagopalan and Davies 1967). In order to abate pollution from the tanneries a common effluent treatment plant has been set up in Dindigul by the government of TamilNadu in association with Dindigul Tanner's association. The treatment processes involved are physical, chemical and biological. The physical processes include screening, sedimentation, flotation and filtration, chemical processes include precipitation, coagulation and disinfection and biological processes involved are anaerobic lagoon and aerated lagoons. Even after the establishment of common effluent treatment plant, the treated effluent is not suitable for normal agricultural purposes of surface irrigation, since the treated effluent contains high values of pH, EC, total dissolved solids, salts and COD. A number of more advanced treatment processes such as chemical coagulation and filtration, chemical oxidation, ion exchange, electro dialysis, adsorption, reverse osmosis and air stripping were used for the improvement of liquid effluents. These methods are costly and the treatment efficiency is also low adsorption is a cost effective technique for the removal of salts from the effluents. Some of the locally available biosorbents such as bark, leaf, seeds and roots of trees and plants and agricultural residues have the capacity to sorb the sulphate from the effluents. Hence a comparative study of sorbent reduction as well as microbial reduction is carried out for reducing sulphate form the effluent, and molecular characterization of microbes using sulphate reduction is also studied.

MATERIAL AND METHODS

Collection of *Tannery* Effluent

The tannery effluent was collected from Dindigul and transported to the laboratory in polythene cane. For the present study spot or great sampling technique was adopted (Rainwater and Thatcher, 1963).The bottles for sample preservation were thoroughly cleaned by rinsing with 8ml HNo_3, followed by repeat washing with distilled water. The parameters like temperature & colour was determined during the period of analysis the water sample was preserved as per preservation technique outlined in the standard methods (APHA, 1990).

Plant as a Sorbent

The various sources of plants such as Barks, Leaves, Stems, Flowers and Roots were dried and powdered. It was treated with 40% formaldehyde and neutralized by acid-base test.200mg of Sorbents were treated with 200ml tannery effluent and then incubated in water bath shaker at 50°C. (Control- Without Sorbents)The sulphate reduction was carried out with the time interval 30 minutes, 60 minutes and 120 minutes.

Microbial Reduction

Isolation and enrichment of Culture

5-15% High salt concentration broth was prepared, 1ml of Tannery effluent sample was inoculated with the broth and Incubated at 30°C for 48 hours.25% of High salt concentration was prepared, a loop full of culture from the enrichment broth was treated on the plants and Incubated at 37°C for 48 hours. The bacterial strains isolated from tannery effluent were identified up to generic level by employing the standard morphological and biochemical characteristics described in Bergey's manual of systemic bacteriology (Holt et al., 1994).

Molecular identification

Isolation of genomic DNA

Bacterial isolates were sub cultured in Luria Bertani broth and genomic DNA was isolated by employing lysozyme, SDS and phenol-chloroform method followed by Wawer and Muyzer 1995.

PCR amplification, cloning and sequencing of 16S rRNA genes

16S rRNA gene of the bacterial isolates were amplified with genomic DNA isolates as template and forward and reserve primer in the following composition and amplification cycle. Each reaction mixture contained 2µl of template DNA (100ng), 0.5µM of two primers, and 25µl of Enzyme Master Mix (Bioron). The PCR program consisted of a intial denaturation step at 94°C for 5min, followed by 30 cycles of DNA denaturation at 92 C for 30 sec, primer annealing at 50 C for 1 min, and primer extension at 72 C for 2 min was carried out in Thermal Cycler (Thermo Hybaid) . After the last cycle, a final extension at 72 C for 20 min was added. The PCR products were purified by QIA quick PCR purification kit as described by the manufacturer and cloned using QIAGEN PCR cloning plus kit as described by the manufacturer. Clones were selected and isolated plasmids with insert were sequenced with M13 Sequencing Primer using AB I Biosystems automated sequencer.

Database Searching and Deposition of Gene in GENBANK

Nucleotide database was searched with the sequences obtained with NCBI BLAST (Blastn) tool (http://www.ncbi.nlm.nih.gov/BLAST) (Altschul *et al.,* 1997).The sequences were submitted in GenBank (NCBI) using BankIt sequence submission software. http://www.ncbi.nlm.nih.gov/GenBank submission).

RESULTS AND DISCUSSION

The physico-chemical characteristic of industrial effluent was presented in table. The pH value of effluent was 8.04 which was slightly exceeded the recommended value. The electrical conductivity was 12,500 micro mhos/cm. This value was higher than the permissible limit. The total dissolved solid in the effluent was 17,070 mg/l. This value was also higher than that of BIS recommended value (2100mg/l). The value of total hardness was comparatively higher in *tannery* effluent. Out of three cations tested (Na, K, Ca) the sodium contains 1275 mg/l and followed by potassium (40mg/l) and calcium (390mg/l). The chloride content was higher (12,682 mg/l) in the tannery effluent. Sulphate level in tannery effluent was very high (390mg/l) with sodium adsorption ratio (223.68mg/l) because of the presence of high amount of Na, Ca, Mg.

The table 2 and graph 1 reveals that the reduction of the sulphate by using sorbents *Bongaim Villea Spectalrilus* gives much Sulphate reduction than the other sorbent.

The Table 3 reveals that the hours increase the rate of Sulphate is decreases.

The experimental result shows that the Microbial Reduction is more efficient than the Sorbent Reduction. As for as Sorbent Reduction is concern the reduction of sulphate is very minimum in the interval of time but in the case of microbial reduction there is a rapid reduction of sulphate is seen.

Biochemical characterization

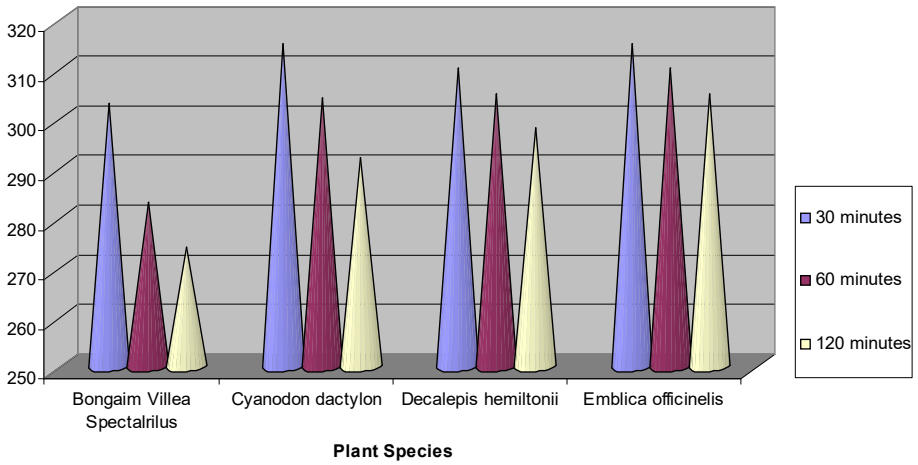
Colonies were observed on High Salt

agar plates. The isolated colony was sub cultured in sterile nutrient medium and inoculated on sterile 25% Salt agar plates. Colonies observed on Salt Agar plates were rod shape Bacteria and were biochemically characterized.

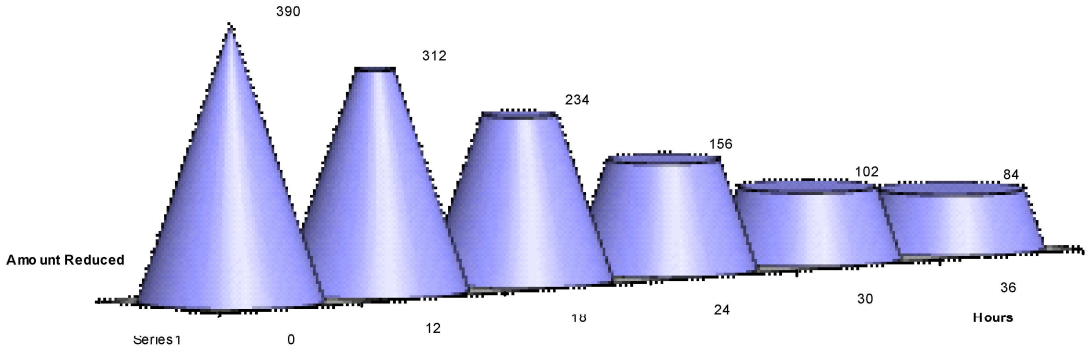
Molecular identification

A discrete band was observed in 1% Agarose gel on UV illumination after loading the Genomic DNA sample isolated. A discrete band having size of 1.5 Kb was observed in 1% Agarose gel on UV illumination after loading the PCR product amplified with 16S rRNA primers. The PCR product was purified and loaded in Agarose gel and band without any primer band was observed. The Sequence was obtained from automated sequencer (Genotypic Technology, Bangalore) as a text file and .abi file. The sequence obtained was searched in nucleotide database using BLAST software in NCBI server. BLAST results showed that the sequence was having similarity with 16S rRNA gene sequences of *Aneurinibacillus aneurinilyticus*.

The sequence was deposited as 16S rRNA partial sequence of in *Aneurinibacillus aneurinilyticus* GenBank using BankIt software and given a BankIt ID as 798801.The sequence



Graph 1. Reduction of Sulphate by Sorbents



Graph 2. Reduction of Sulphate by using microorganisms

Table 1. Physico-chemical characteristics of tannery effluent

S. No	Parameters	Values	BIS
1.	Colour	Dark Brown	-
2.	Smell	Unpleasant	-
3.	pH	8.04	6.5 to 8.5
4.	Temperature	33.3	-
5.	Electrical Conductivity (m.mhos)	12,500	-
6.	Total Solids (mg/l)	18,030	-
7.	Total Dissolved Solid (mg/l)	17,070	2100
8.	Total Suspended Solids	960	-
9.	Chloride (mg/l)	12,682	250
10.	Total Hardness (mg/l)	2,210	300
11.	Alkalinity (mg/l)	140	-
12.	Sodium (ppm)	1,275	-
13.	Potassium (ppm)	40	-
14.	Calcium (ppm)	390	75
15.	Magnesium (mg/l)	27	30
16.	Sulphate (mg/l)	390	150
17.	Sulphide (mg/l)	5.3	-
18.	Bicarbonate (mg/l)	5.6	-
19.	DO_2 (mg/l)	5.8	-
20.	DCO_2 (mg/l)	52.3	-
21.	Sodium Absorption Ration (mEg/l)	223.68	60
22.	Soluble Na%	13.16	-
23.	Biological Oxygen Demand (mg/l)	54	25
24.	Chemical Oxygen Demand (mg/l)	464.0	-
25.	Residual Sodium Chloride	75.5	-
26.	Water Quality Index	2348.75	-

Table 2. Reduction of Sulphate by Sorbents

S. No	Sorbents	Part Used	Sulphate Reduction (mg/l)		
			30 min	60 min	120 min
1.	*Bongaim Villea Spectalrilus*	Flower	303	283	274
2.	*Cyanodon dactylon*	Leaf	315	304	292
3.	*Decalepis hemiltonii*	Stem	310	305	298
4.	*Emblica officinelis*	stem	315	310	305

All the values are the average of the 10 individual observations

Table 3. Reduction of Sulphate by using microorganisms

S.No	Hours	Amount Reduced (mg/l)
1.	0	390
2.	12	312
3.	18	234
4.	24	156
5	30	102
6	36	84

was given an accession number (DQ513424). The isolate was also confirmed by molecular identification with 16S rRNA gene sequence

Cells were lysed by Lysozyme and proteins were precipitated by Proteinase K and SDS. The genomic DNA was extracted and precipitated and used as template DNA for PCR amplification. The PCR product amplified with 16S rRNA primers was having size of 1.5Kb

confirmed with molecular marker (ëDNA / *Hind*III).The amplified PCR product might contain remaining unreacted primers and enzymes that would interfere with sequencing reactions. The product was purified with Qiagen Purification kit and a distinct band was obtained without any non-specific bands. The purified product was sequenced with forward primer, which initiates the polymerization from 5'→3' with 3'→5' strand as a template.

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