PRELIMINARY ANALYSIS OF TANNERY EFFLUENT TREATMENT USING MICROBIAL CONSORTIA

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ABSTRACT

Background: *Microbes are effective in control of pollution due to effluent. It has remarkable power to degrade and utilize complex organic substances and changing it to less toxic and simpler compounds.*
Objective: T*he present work is done to analyze the effectiveness of the microbes that occur naturally in the tannery effluent in its possible biological treatment.*
Methods: *Tannery samples were serial diluted and spread plated on the nutrient agar media. From that colonies were identified using standard protocols. The effluent treatment was carried out with the microbial consortia (10^5 cells/ml equal proportion of all the three isolates) of various effluent concentrations (ranging from 50% to 1.565%) by observing O.D and pH values for 12 days. The physio-chemical parameters were carried out with 6.25% diluted effluent of the 10^{th} day, which was the optimum one.*
Results: *The isolated organisms were identified as Staphylococcus aureus, Thiocapsa roseopersicina and Enterococcus fecalis. The microbial consortia decreases the pH of effluent from high (8.3) towards neutral (7.17) and changing the turbidity from 0.88 to 0.56 (O.D) for 50% concentration of effluent. The Physio-Chemical parameters of the treatment was found to be within the limit of Indian effluent water standard.*
Conclusion: *The present work reveals that the microbial consortia could be used effectively in treating the tannery effluent and could be recommended after checking for its biosafety.*

Key words: *Bioremediation; Microbial consortia; Tannery effluent*

INTRODUCTION

Leather industry is one of the major industries, which are involved in the economic development of the country [1]. The effluent from this industry, pose a major source of pollution among all other industries both in volume and composition[2]. The effluents usually contains high tannins, suspended solids, and dissolved solids, BOD, COD and some inorganic compounds such as chlorides, sulphides, sulphates, sodium and some toxic heavy metals, like chromium that affect the environment and their treatment is also very difficult[3,4]. There are numerous physical and chemical methods such as screening, flow equalization, primary sedimentation, chemical flocculation, aerobic activated sludge treatment, secondary sedimentation have been employed for the waste treatment[5]. These methods in spite of being effective, it is 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. Microorganisms are very effective in pollution control, especially in effluent treatment[6]. We have tried with treatment of effluent sample from Sembattu, Tiruchirappalli, Tamil Nadu, since this is the first work done to analyse the microbial flora and also on biological treatment of this effluent. Organism which is naturally present in this effluent sample can withstand the adverse conditions (pH, turbiditiy, high BOD, COD, etc) of it. Hence these organisms (microbial consortia) has been isolated, identified and used for treating the same[7].

MATERIALS AND METHODS

Tannery effluent sample was collected in large bottles from Sembattu, Tiruchirappalli, Tamil Nadu, India and transported to the lab immediately.

Isolation and identification of bacterial isolates:

Samples collected were serially diluted and 0.1ml of sample was plated by

spread plate method on nutrient agar at 30°C for 12hrs incubation. Then individual colonies were isolated. Individual colonies were plated in nutrient agar, and morphological characteristics and special level identification were done using standard protocol[8].

Effluent treatment:

The collected samples were diluted (50%, 25%, 12.5%, 6.25%, 3.125% and 1.565% - using sterile distilled water) and inoculated with microbial consortia [equal proportion of (1 x 10^5 cells/ml) *Staphylococcus aureus, Thiocapsa roseopersicina* and *Enterococcus fecalis*]. The above treatment was incubated for 12 days at 30°C and pH and O.D were noted periodically up to 12^{th} day. Physio-chemical parameters were observed on the 10^{th} day since; there is no significant change in O.D and pH. The lowest concentration (6.25%) was further considered for the above analysis, since at higher concentration color change is less and at lower concentration there is no remarkable change. Physio-chemical parameters such as turbidity, Electrical Conductivity (EC), Total Dissolved Solids (TDS), colour, odour, nitrite, nitrate, NH_3 alkalinity, free CO_2 ,Dissolved O_2, total phosphate both organic inorganic, Na, K, Ca, Mg, Fe, Cl ,F, SO_4, PO_4, and also BOD, COD were done as per the standard protocols [9].

RESULTS

The results of the physical (Colony colur, appearance, shape, motility and gram staining) and biochemical characteristics (MRVP test, citrate, catalase, TSI-Butt/Slant, Indole and urease) were depicted in table 1. It showed that, the isolated colonies were identified as *Staphylococcus aureus, Thiocapsa roseopersicina* and *Enterococcus fecalis.* The effect of the microbial consortia on the OD and pH of the various concentration of treatments, were represented in table 2. It revealed that, the microbial consortia decreases the turbidity from 0.88 to 0.56 (O.D) and changing the pH of effluent from high (8.3) towards neutral (7.17) for 50% concentration of effluent and similar results have been obtained for the other lower concentrations. The results of the physical (Appearance, colour, odour, turbidity, TDS and EC) and Chemical parameters (pH, alkalinity, BOD, COD, various metal and element concentrations) were shown in table 3. It showed that the treatments were within the permissible limits of Indian effluent water standard.

Table 1. Identification characteristics of bacterial isolates

Sl. No.	Characteristic features	Isolate 1	Isolate 2	Isolate 3
1.	Colony colour	Yellow (golden yellow – endo pigment)	Rose to Milky white	Dull White
2.	Colony Appearance	Circular pin head, convex with entire margin	Rose to milky white colonies	Smooth dull white colonies
3.	GramStaining	+	-	+
4.	Shape	cocci	spherical tetrad	coccus
5.	Motility	+	-	+
6.	Oxidase	-	-	-
7.	Methyl Red	+	-	+
8.	VogesProskauer	-	-	-
9.	Citrate	-	-	+
10.	Catalase	+	+	+
11.	TSI-Butt/Slant	A/K	A/K	A/K
12.	Indole	-	-	-
13.	Urease	+	+	-
14.	Confirmatory test	β hemolytic on blood agar	Growth at anaerobic condition (Glucose+ thiosulphate media)	Growth at 50°C
15.	Species Name	***Staphylococcus aureus***	***Thiocapsa. roseopersicina***	***Enterococcus fecalis***

Table 2. OD/pH values of the different treatment concentrations of the tannery effluent

Time (Days)	O.D/pH					
	50%	25%	12.5%	6.25%	3.125%	1.5625%
0	0.88/8.30	0.80/8.10	0.77/7.90	0.70/7.71	0.65/7.69	0.55/7.50
1	0.84/8.21	0.79/8.09	0.75/7.87	0.67/7.69	0.63/7.60	0.53/7.58
2	0.82/8.15	0.77/8.01	0.74/7.78	0.65/7.65	0.61/7.57	0.55/7.56
3	0.81/8.00	0.75/7.97	0.71/7.70	0.63/7.53	0.59/7.49	0.56/7.45
4	0.77/7.95	0.70/7.83	0.69/7.67	0.59/7.53	0.57/7.45	0.50/7.44
5	0.77/7.93	0.67/7.70	0.67/7.61	0.57/7.47	0.55/7.41	0.49/7.39
6	0.74/7.80	0.65/7.61	0.65/7.59	0.57/7.45	0.51/7.39	0.47/7.34
7	0.68/7.77	0.63/7.52	0.62/7.47	0.45/7.39	0.49/7.33	0.46/7.28
8	0.66/7.62	0.62/7.45	0.59/7.39	0.43/7.36	0.47/7.29	0.44/7.28
9	0.62/7.55	0.59/7.38	0.55/7.36	0.42/7.32	0.45/7.27	0.41/7.19
10	0.60/7.45	0.55/7.21	0.49/7.30	0.42/7.26	0.43/7.22	0.39/7.10
11	0.55/7.25	0.54/7.17	0.48/7.19	0.41/7.17	0.41/7.19	0.37/6.99
12	0.56/7.17	0.54/7.11	0.46/7.14	0.40/7.10	0.39/7.09	0.36/6.80

Table 3. Physical and chemical properties of control and treated

Physical Properties	**Control Tannery (6.25%) on 12th day**	**Treated tannery (6.25%) on 12th day**	**Acceptable limits**	**Permissible limit in the absence of alternate source**
1. Appearance	Turbid	Turbid	-	_
2. Color (pt.co-scale)	Slightly White	Slightly white	5	15
3. Odor	None	None	Agreeable	Agreeable
4. Turbidity NT Units	12	10	1	5
5. Total dissolved Solids mg/L	7565	6469	500	2000
6. EC Micro mho/cm	10507	9241	-	-
Chemical Properties				
7. Ph	6.27	6.95	6.5-8.5	6.5-8.5
8 . Ph. Alkalinity as $CaCo_{3\ mg/L}$	0	0	-	-
9. Total Alkalinity. as $CaCo_{3\ mg/L}$	2000	1140	200	600
10. Total Hardness as $CaCo_{3\ mg/L}$	2720	2160	200	600
11. Calcium as Ca mg/L	584	328	75	200
12. Magnesium as Mg mg/L	302	202	30	100
13. Sodium as Na mg/L	896	566	_	_
14. Potassium as K mg/L	242	134	_	_
15. Iron as Fe mg/L	0.47	0.37	0.3	0.3
16. Manganese mg/L	0	0	0.1	0.3
17. Free Ammonia as $NH_{3\ mg/L}$	0.06	0.08	0.5	0.5
18. Nitrite as $NO_{2\ mg/L}$	0.08	0.03	-	-
19. Nitrate as $NO_{3\ mg/L}$	27	38	45	45
20. Chloride as Cl mg/L	2550	2150	250	1000
21. Fluoride as F mg/L	1.8	1.5	1.0	1.5
22. Sulphate as $SO_{4\ mg/L}$	102	78	200	400
23. Phosphate as $PO_{4\ mg/L}$	0.06	0.05	-	-
24. Tidys Test 4 hrs.as $O_{2\ mg/L}$	1.1	1.0	-	-
COD	890	686	-	-
BOD	248	124		

DISCUSSION

Water pollution is a major pollution that affects the environment much and one of main source of this pollution is industrial effluent[6]. Tannery waste is a major hazard that affects the drinking water and hence it should be treated effectively before release[10]. In this work we have tried a biological treatment to treat tannery effluent since it ecofriendly [11]. The microbial consortia of the collected Tannery showed the presence of *Staphylococcus aureus, Thiocapsa roseopersicina* and *Enterococcus fecalis*[12]. In the present study the counts of the *S. aureus* was found dominantly rather than the other organisms this might be due to the occurrence of normal microbial flora of the animal dermal and epidermal layers[13]. Similarly, Srinivas Gidhamaari[6] also reported the presence of *S. aureus* from the effluent sample of NRM tannery, Tiruchirappalli, Tamil Nadu, India. From the present findings, the level of turbidity (O.D), pH and Physio-chemical parameters found to decreases from high to moderate or optimum level with various concentrations of the effluent treatment. It may be due to the concurrent process of absorption and metabolism properties of the microbial consortia[14], includes the heavy metal tolerance by permeability barrier, intra- and extra-cellular sequestration, active transport efflux pumps, enzymatic methods and also reduction in the sensitivity of targeted cellular organelles to metal ions[15]. From the above work it is evident that the flora which is isolated from the Sembattu, Tiruchirappalli, Tamil Nadu, India, could be used for biological treatment after analyzing their biosafety, feasibility and efficacy levels.

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