Journal of Environmental and Applied Bioresearch
Published online 01 October 2014 (http://www.scienceresearchlibrary.com)

ISSN: 2319-8745
Vol. 02, No. 02, pp. 36-40

Research Article **Open Access**

Aerobic digestion of tannery soaking wastewater and reuse of recovered salt

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Received: 25 August 2014 / Accepted: 20 September 2014

Abstract

Synthetic wastewater with characteristics matching to the tannery soak liquor was treated using *Bacillus circulans*. The removal of COD and TKN was 90.48% and 91.12% respectively. IR spectra indicated the presence of conserved peptides. Significant level of bacterial growth and removal of COD and TKN indicated that the amount of conserved peptide was insignificant. Soak liquor was treated using *Bacillus circulans* and the removal in COD and TKN was 89.5% and 92.1% respectively. The treated soak liquor was clarified and treated using biocide. The salt recovered from the clarified liquor was reused for skin preservation and the preservation efficacy was found to as good as the efficacy of the fresh salt.

Keywords: Saline wastewater, *Bacillus circulans*, Biological treatment, soaking and Tannery

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Introduction

Many industrial processes generate saline wastewater. The waste stream from seasoning in pickled plums, traditional presented food contains about 15% of Nacl (Motoki kubo et al., 2001). In food processing industry, saline waste stream is generated by the use of braine solution and solid sodium chloride for obtaining the finished product (Lefebvre and Moletta, 2006). The reject from olive oil mills contains large quantity of salt (Vitolo et al., 1999). The source of salinity from fish processing industry is primarily the sea water discharged during unloading of fish. Fisheries also generate wastewater rich in proteinic nitrogen, organic matter and salt (Antileo et al., 1997). The process of refining of crude oil generates wastewater called production water. Production water contains broad range of salt as much as three times of sea water (Diaz et al., 2002). In leather manufacturing process, common salt is used for preservation of skins and pickling. About 15% to 40% (on the weight of the raw hides and skins) is used for preservation. Salt used for preservation is released along with the wastewater during soaking and the disposal of saline soaking wastewater is associated with significant environmental impacts. Per ton of raw skin or hide about 7 m^3 to 9 m^3 of wastewater containing 85 kg of chloride is released during soaking (Ludvik, 2000). During pickling about 100 kg of sodium chloride is used for every ton of skins. Pickling wastewater contains about 40 g of sodium chloride per litre (Rao J et al., 2003).

There are two important issues in the treatment of saline and hypersaline wastewater. Firstly, the pollutants mainly organic load needs to be treated or degraded. Secondly, the dissolved solids mainly the salts need to be segregated from the wastewater. Only when the organic pollutants are degraded and the salt load is removed or curtailed the wastewater can be discharged or reused.

Salinity in wastewater poses many problems in treatment especially in biological treatment. Hypersaline effluents are often recalcitrant to biological treatment (Lefebvre and Moletta, 2006). Effect of inorganic salts such as sodium chloride and sodium sulfate on the efficiency of activated sludge process was examined (Wang et al., 2005). Saline and hypersaline wastewater can be treated by physico-chemical techniques such as evaporation, coagulation, flocculation, ion exchange and membrane techniques. Solar evaporation is one of the low cost techniques followed for the evaporation of saline waste streams in leather industry particularly in India (Lefebvre O et al., 2005). This system is simple and cost-effective. However evaporation requires large area and the salt recovered is not suitable for reuse as it is contaminated with impurities and halophilic bacteria. Multiple effect evaporation (MEE) could be very effective and competitive with other modern desalination techniques such as reverse osmosis (Morin, 1993). In MEE water is boiled in a sequence of vessels. Due to boiling of wastewater, significant degree of disinfection of halophilic bacteria can be expected. However the organic contaminants would still be present with the salt. Hence the salt recovered can not be suitable for reuse. Membrane techniques are suitable for salt removal. But all the membrane techniques require effective pretreatment of saline wastewater to remove the pollution load in terms of BOD and COD significantly. Ultrafilteration (UF) can be used for the removal of suspended solids and colloidal COD in saline effluents (Ana Maria Brites Abres and Maria Novberta de Pinno, 2000). Treatment of saline wastewater containing from sea food processing had been treated using ultrafilteration and the treated wastewater had been reused (Albonso and Borquez, 2002). Salt removal can be achieved by membrane techniques such as reverse osmosis (RO) (Cassano R et al., 2001) and electrodialysis (Rao et al., 1989). High quality feed is required for membrane treatment techniques particularly for RO. Membrane is susceptible to fouling by the colloidal and organic matter in the feed stream. UF is a suitable pretreatment system for RO (Vitolo et al., 1999). Because of the high operating cost associated with RO and the requirement of pretreatment, RO is not economically viable for wastewater treatment.

In biological treatment of saline wastewater, high concentration of salt can hamper the microbial activity. Moreover there is a possibility of moderate acclimation of activated sludge to high salinity. Because of the problems, the efficiency of the aerobic biological treatment may decline. Employment of halophilic bacteria in the aerobic treatment of saline wastewater is appropriate. Addition of *Eurohaline halobacter* strain was found to enhance the efficiency of activated sludge process (Kargi and Dincer, 1998). COD removal to the tune of 95% could be attained using the activated sludge enriched with *Halobacter halobium* in the treatment of wastewater from pickling industry (Kargi et al., 2000). Two salt tolerant bacteria viz. *staphylococcus* sp. and *Bacillus cereus* were used for the treatment of wastewater from pickled plum, which contained as much as 15% of Nacl. By the biological treatment with these two organisms, respectively 30% and 35% of COD reduction was attained (Kubo et al., 2001).

In this present work the efficiency of *Bacillus circulans* in the treatment of saline wastewater that contained protein was investigated. On the basis of the results obtained from the studies using synthetic wastewater, saline wastewater from tannery was treated using the microorganism.

2. MATERIALS AND METHODS

2.1 Micro-organism

The strain of *Bacillus circulans* (MTCC ACC NO. 879) used in this present work was obtained from Institute of Microbial Technology, Chandigar, India.

2.2. Media ingredients

All the media ingredients and chemicals used were obtained from Hi Media.

2.3. Preparation of synthetic wastewater

Initial investigations were carried out using synthetic wastewater prepared akin to the composition of soaking wastewater from tannery. Four samples of soaking wastewater (combined wastewater of soaking I and soaking II) were collected from four different tanneries at Erode, India. They were analyzed for TKN and chloride to estimate the amount of protein primarily albumin and common salt present in the wastewater. It was found that the range of TKN and chloride were 220 to 300 mg/L and 28000 to 35000 mg/L. Synthetic wastewater was prepared to obtain a composition similar to the above with the following composition.

Egg albumin	10 gm
Common salt	45 gm
Distilled water (sterilized) to make up 1 L	

To the above, micronutrients such KH_2PO_4, $CaCl_2$ and $MNCl_2$ were added to a tune of 2 gm, 0.5 gm and 0.4 gm respectively.

2.4. Bioreactor

Initial investigations using synthetic wastewater was carried out in bioreactor of working volume of 2 L supplied by M/s Applikon, Holland. Air supply was provided to maintain dO_2. Samples were drawn using sampling tube provided with filters.

2.5. Preparation of samples for analysis

Samples from bioreactor experiment and soak liquor treatment were centrifuged at 10,000 rpm for 20 min to segregate the bacterial cells. This was carried out to eliminate the possible contribution of TKN and COD by the cells and interference of the cells in IR spectra. To validate the preparatory procedure, nutrient broth was prepared and with 10 ml/L of *Bacillus circulans* inoculum and incubated for 24 hours. The absorption of the broth at 610 nm was found out. Then the broth was centrifuged at 10,000 rpm for 20 min. The absorption was measured at 610 nm. It was found that the absorption of nutrient broth before and after centrifugation were almost equal. This is evident that the centrifugation at 10,000 rpm for 20 min could remove the bacterial cells effectively. Samples prepared as described were taken for the estimation of TKN and COD.

2.6. Total Kjeldhal Nitrogen (TKN)

TKN was estimated following the standard procedure as reported by American Public Health Association (APHA, 1989).

2.7. Chemical Oxygen Demand (COD)

COD was estimated following the analytical procedure as reported by American Public Health Association (APHA, 1989).

Table 1 Removal of COD and TKN in synthetic saline wastewater

Time (hours)	COD (mg/L)	COD removal (%)	TKN (mg/L)	TKN removal (%)
0	28220	0	310	0
3	28200	0.07	306	1.29
6	27860	2.25	294	5.16
9	27121	4.85	260	16.13
12	23488	17.59	221	28.71
15	17280	39.37	198	36.13
18	14388	49.52	179	42.23
21	12210	57.16	160	48.39
24	10264	63.99	139	55.16
27	8012	71.89	114	63.23
30	5824	79.57	89	71.29
33	3812	86.63	68	78.07
36	2982	89.53	43	86.13
39	2842	90.02	40	87.10
42	2720	90.46	36	88.39
45	2692	90.56	32	89.68
48	2687	90.48	28	90.96

2.8. Viable plate count method

The bacterial population was assessed by viable plate count procedure (Ronald M Atlas, 1997)

2.9. Preservation experiments

Experiments of skin preservation were carried out using fresh salt and recovered. Four freshly flayed Indian goatskins were taken and washed. The skins were cut along the backbone to get two halves. The left halves were applied with fresh salt. The purity of fresh salt was found to be 93.7%. For one kg of skin 400 g of salt was applied. Similarly the right halves were applied with recovered salt. In this case also 400 g of salt was used for 1 kg of skin. The skins were kept for 32 days.

3. RESULTS AND DISCUSSION

Primary inoculum of *Bacillus circulans* was prepared in yeast – triptophone starch medium (Ronald M Atlas, 1997). After inoculating a loop-full of culture in the media, it was incubated for 24 hours. Synthetic wastewater of volume 2 L was taken and inoculated with 10 ml of inoculum.

The degradation experiment was carried out in bioreactor for 48 hours. Samples were taken at 3 hours interval and analyzed for TKN and COD (Table 1). Bacterial population of the samples was also enumerate. In 48 hours, 90.48% removal of COD and 91.12% removal of TKN were attained. The trend of bacterial growth was found to undergo acclimatization for first three hours and then the growth was exponential. After 36 hours, the growth started declining. The bacterial growth and removal of COD and TKN indicate that the strain could grow in saline condition and degrade the protein.

Table 2 Removal of pollution parameters (COD and TKN) in tannery soaking wastewater

Time (hours)	COD (mg/L)				Average COD removal (%)	TKN (mg/L)				Average TKN removal (%)
	Batch 1	Batch 2	Batch 3	Batch 4		Batch 1	Batch 2	Batch 3	Batch 4	
0	23140	23140	23140	23140	0	288	288	288	288	0
3	22820	22630	22310	22140	2.87	282	284	286	280	1.74
6	20140	20520	21480	20730	10.47	278	280	280	276	3.30
9	17450	18110	18600	17200	22.90	252	268	274	264	8.16
12	14920	15240	15100	14620	35.31	236	242	258	248	14.58
15	12110	13210	12450	12040	46.19	218	220	228	224	22.74
18	9890	10020	9940	9820	57.14	198	208	212	210	28.13
21	7630	8040	7820	7620	66.39	174	185	196	188	35.50
24	6710	7120	6920	6750	70.29	159	170	170	165	42.36
27	5820	6030	5940	5880	74.43	132	148	152	142	50.17
30	5090	5460	5200	5120	77.45	118	134	138	128	55.03
33	4720	5050	4850	4780	79.04	94	108	110	104	63.89
36	4220	4340	4500	4480	81.05	72	86	84	92	71.00
39	3900	3870	4010	3960	82.99	56	64	60	61	79.08
42	3320	3200	3380	3360	85.67	34	58	52	42	83.85
45	3190	3110	3160	3180	86.34	34	50	46	36	85.59
48	3136	3060	3120	3060	86.63	32	46	40	32	86.98

To understand better about the degradation pattern of protein samples were taken initially and after 48 hours IR spectra of the samples obtained (Fig. 2 and 3). It is evident from the IR spectra of

final sample that the synthetic wastewater has not undergone total degradation. In other words the protein in the synthetic wastewater has not been degraded completely into amino acids. Signal indicating the presence of peptide link demonstrates that still some peptide portions have been conserved. Taking into account the bacterial growth and substantial removal of COD and TKN, it can be holistically deduced that the amount of conserved peptides was insignificant. The presence of conserved peptides is responsible for peptide signal in the IR spectra.

Soak liquor of volume 8 L of a single batch of soaking from a commercial tanning unit was collected. Four batches of treatment were carried out with 2 L of soak liquor. Aerobic treatment was carried out in bioreactor for 48 hours after inoculating with 10 ml of inoculum. Samples were collected for every four hours and analyzed for TKN and COD (Table 2).

It was found that the removal of COD was 86.63% and removal of TKN was 86.98%. The treated soak liquor was then clarified by simple settling in a conical vessel. The clarified soak liquor should be free from bacteria so that the recovered salt would be free from halophilic bacteria. Therefore to the clarified liquor, Busan 30 L was added at the rate of 10 g per L. The clarified liquor was evaporated under sun similar to the open solar evaporation system (Lefebvre O et al., 2005).

Table 3 Preservation efficacy

Period	Moisture content of skin* (% w/w)		Microbial population* (CFU/mg of skin)	
	Control	Experiment	Control	Experiment
0 h	66.4	68.3	42×10^3	62×10^3
2 h	53.2	52.4	36×10^5	55×10^5
1 day	48.8	49.8	51×10^3	48×10^4
2 days	42.4	41.4	49×10^2	66×10^2
4 days	39.8	38.8	42×10^2	49×10^2
8 days	38.7	37.4	32×10^1	44×10^1
16 days	38.2	37.4	52×10^1	59×10^1
32 days	38.0	37.0	34×10^1	41×10^1

* Values are the average of four values of four samples

It was found that the moisture and purity of the recovered salt were 2.9% and 90.3% respectively. Salt should not contain significant level of organic load and should be free from halophilic bacteria. The recovered salt was also tested for bacterial population.

It was found that the salt was free from bacterial colony. Recovered salt was reused for skin preservation. Control preservation was also carried out using fresh salt to comparatively assess the preservation efficacy of the recovered salt. The skins were kept in preservation under ambient conditions for 32 days. Skin samples were taken initially and after every 8 days. They were tested for bacterial growth and moisture content (Table 3).

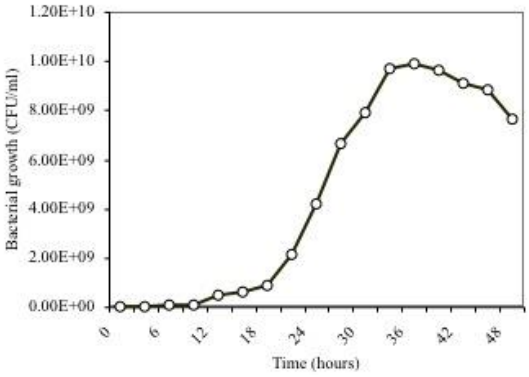

Figure. 1 Bacterial growth in synthetic wastewater

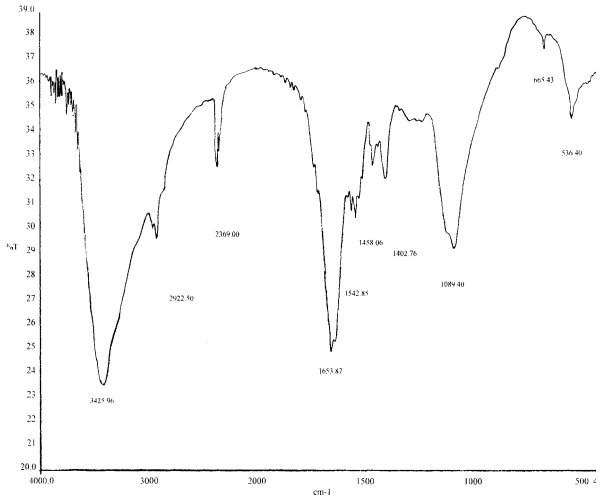

Figure. 2 IR spectrum of medium at 0 hours

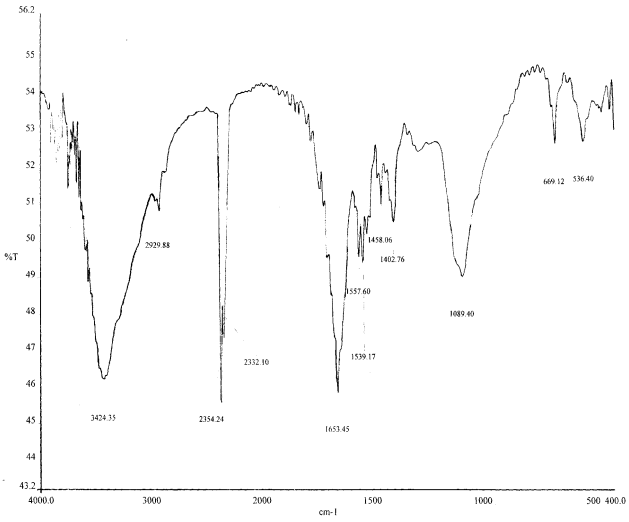

Figure. 3 IR spectrum of medium at 48 hours

Moisture content of the skins preserved by fresh salt was 38% in 32 days. The bacterial population was reduced from 42×10^3 to 34×10^3. In the case of experiment where recovered salt was used

for preservation, the moisture content of the skins in 32 days was 37% and the bacterial population was reduced from 62×10^3 to 41×10^3. Moisture content and bacterial population of the skins are the two important indicators of the efficacy of preservation. From the results it is evident that the recovered salt is as efficacious as the fresh salt in preserving the recovered salt.

5. Conclusion

The bacterial strain *Bacillus circulans* could grow under saline condition of 30 g of NaCl per liter. Protein present in the wastewater could be degraded by the strain. Despite considerable growth of the strain and significant level of COD and TKN removal, presence of peptide link as seen from the IR spectra indicated the presence of conserved peptides. However, it can be stated that the amount of conserved peptide could be insignificant. Biological treatment of soak liquor could remove COD and TKN to the extent of 89.5% and 92.1% respectively. The moisture and bacterial population of the skins preserved using the recovered salt were indicated that the preservation efficacy of the recovered salt was as good as the efficacy of the fresh salt. Therefore, it is possible to treat the soak liquor using *Bacillus circulans* and by the addition of biocide, it is also possible to reuse the recovered salt for preservation.

ACKNOWLEDGMENTS

It is acknowledged herewith that this project has been carried out under the "Network project – Zero Emission Research Initiative in Leather (NWP 044)" of Council of Scientific Industrial Research, India.

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