Anaerobic biological treatment of the tannery effluent using *Paracoccus pantotrophus* FMR19 (JX012237) isolated from rumen fluid

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Abstract

Tanning industry is generating considerable quantity of toxic chemicals and heavy metals which are hazardous to the environment. Major problems are due to the complex nature of chemicals and heavy metal chromium. Even though there are many conventional technologies available for the treatment of the tannery effluent, still the problems are not being overwhelmed by the technologies because of the disadvantages such as huge amount of sludge generation, high cost etc. Therefore, the biological treatment of industrial effluents is gaining importance because of its advantages such as low sludge generation, low cost, sludge recycling etc. There are two different methods of biological treatment: 1) aerobic and 2) anaerobic. The anaerobic method of treatment is most favorable than the aerobic method; because in this method, the complex organic molecules can be degraded by the anaerobic type of microorganisms and also the energy can be recovered in the form of methane.

Rumen microbes are a kind of anaerobic bio-reactor, it can able to convert the complex polysaccharides such as cellulose. This property is being exploited in the present study to degrade the complex organic molecules from the tannery effluents. Therefore, the present study was designed to use the anaerobic rumen bacteria Paracoccus pantotrophus FMR19 (JX012237) which was isolated from the goat rumen for the degradation of tannery wastewater in the anaerobic conditions. The wastewater was characterized for Chemical oxygen Demand (COD), Total dissolved solids (TDS), chloride content, total hardness (TH), sulphate, etc before and after the treatment. It was observed that the Paracoccus pantotrophus FMR19 (JX012237) reduced the TDS, TH, sulphate, chloride and Chemical Oxygen Demand (COD) within 72 h. The degradation of the components in the effluent was confirmed by the UV-Visible spectrophotometer and High performance liquid chromatography (HPLC) analysis.

Key Words*: Tannery Effluent treatment; Anaerobic biodegradation; Rumen micro flora; Paracoccus pantotrophus FMR 19 (JX012237).*

1. Introduction

Every human society is it rural or urban, disposes different kinds of byproducts and waste products into biosphere in large qualities. The industrial effluents are the major pollutants that pollute not only the water bodies but also the entire biosphere. Leather industries contribute to be one of the major industrial pollution facing the country and there are about 2165 tannery industries in India. The quantity of effluent released from the tanneries is about 30-35 l/kg of leather produced. There waste products serious consequences of pollution on fresh water streams and lands for agriculture [Moore, 1953].

The tanning industry was designated as "Red Category" due to the high pollution contributed by its solid and liquid wastes. It generates wastes that frequently accumulate in the environment [Taylor *et al*, 1987]. The leather processing and manufacturing involves a variety of aggressive chemicals and also consumer large quantity of waste water. The water treatment system of the industry was found to in effective both in performance and in economics [Sztajer and Zboinska, 1988].

The tannery effluent wastes ranked as high pollutants among all other industrial wastes [Eye and Lawerence, 1971]. The tannery waste water contains vegetables tannins high amount of proteins that excretes BOD, Chlorides, trivalent chromium, nitrogen, phosphorus, sulphate and sulphides are the inorganic constituents present. The presence of color, oil and turbidity of tannery makes nor useful for domestic purpose.

The receiving streams of water slowly increase in chlorides and hardness contents, the presence of ammonia, sulphates, vegetable tanning agents, synthetic tanning agents and chromium in the water streams cause pollution and are high toxic to the aquatic life. High salinity (1–10% w/v) of tannery wastewater makes it difficult to be treated by conventional biological treatment [Senthilkumar Sivaprakasam *et al*., 2008].

The physicochemical characteristics and the impact of tannery effluent and water streams are analyzed and found that the population of phytoplankton and zooplankton decreased both in tannery effluent discharge point and downstream of Kalingarayan canal [Saravana babu *et al*, 1990]. Only the treated effluent is safe for the disposal it to the environment.

The rumen is a special digestive vessel, within which the digestion of cellulose and other plant polysaccharides occurs, through microbial activity. Microbes produced in the reticulo-rumen are also digested in the small intestine. Fermentation continues in the large intestine in the same way as in the reticulo-rumen [Schwarz, 2001]. Superior biological efficiency has been claimed for the goat [Fitzhugh, 1981] and its superior digestive efficient has been mostly associated with utilization of plant structural polymer [Gihad, 1976; Devendra, 1978] and with a larger digestive capacity compared to sheep [Houston, 1978].

All most all the glucose produced by the breaking down of cellulose and hemicellulose is used by microbes in the rumen, and as such ruminants usually absorb little glucose from the small intestine [Hays, 2004]. The bacteria *Fibrobacter succinigenes, Bactroide succinogenes, Clostridium ochheadii, Bacillus licheniformis and Streptococcus* anaerobes are generally regarded as the predominate cellulolytic microbes in the rumen [Findlay, 1998].

In recent years many rumen microbes have been isolated and characterized by sequence analysis of 16S rRNA. Prokaryote diversity in the rumen of yak (Bos grunniens) and Jinna cattle (Bos Tauras) were estimated by 16S rDNA homology analysis [Dengdi*, et al*., 2005]. Some of the microbes have also been recommended as feed additive for improving the overall growth or production of animals [Mamen *et al*., 2010].

The enzymes produced by rumen bacteria may contribute to the breakdown of switch grass, a renewable biofuel energy source [Anonymous, 2011]. The *Pseudomonas aeruginosa, Bacillus, Penicillium, Aspergillus, Mucor and Fusarium species* were isolated from the rumen of cow, sheep and goat. These organisms are active in cellulose breakdown [Oyeleke *et al*., 2008]. The *Butrivibrio fibrisolvens, Streptococcus sp.,* and *Clostidium aminophilum* were isolated from cattle rumen fluid and they have better cellulase enzyme activity [Krushna Chandra Das, 2012].

In the present work the isolate from rumen fluid was used for biodegradation of tannery effluents and was found to be significantly effective in the removal of toxic effects of tannery effluent.

2. Materials and Methods

Rumen fluid was collected from the slaughter house and filtered through double layer muslin cloth. 1ml of filtrate was used for serial dilution, 100 µl of each dilution (10^{-6} to 10^{-9}) was cultured by spread plate using Hungate's medium [Hungate, 1969]. The tannery effluent was treated with the rumen microbial strains and degradation of organics in the effluent. The enriched microbial sources were screened and identified using 16s rRNA sequencing and better isolate were screened for the efficient removal of organics from the tannery effluent. The isolate was identified as *Paracoccus pantotrophus* and the sequence obtained in the rRNA sequencing was submitted to NCBI Gen Bank for the accession Number.

The isolated culture was inoculated into tannery effluent for the degradation of complex organic molecules. Physicochemical parameters were characterized for pH, Conductivity, Total Dissolved Solids, Total Hardness, Chemical Oxygen Demand, Calcium Hardness, Magnesium Hardness, Chloride and Sulphide. The degradation of the organic components in the treated effluent was confirmed by UV visible spectrophotometer and HPLC analysis.

3. Results and Discussion

The treatment of tannery effluent using rumen microbe *Paracoccus pantotrophus* in the anaerobic fermentation showed that the rumen microbes are efficient enough to degrade the organic pollutants in the tannery effluent. The COD was greatly reduced to 272 mg/L from 6240 mg/L (95.64% removal) (Fig. 2) at 12^{th} day of the anaerobic treatment. Around 95.72% of the TDS were removed upon the anaerobic treatment (Fig. 2). In addition, other pollutants in the effluents also efficiently removed by the anaerobic rumen microbial treatment (Table. 1). As a comparison to the anaerobic treatment by *Paracoccus pantotrophus*, the tannery effluent was subjected to the chemical treatment such as coagulation by aluminium hydroxide and polyelectrolyte (Table. 1). But it showed very less efficiency in the organic load removal. Also it has the main disadvantage that the chemical coagulation method generated huge quantity of sludge and it causes sludge disposal problem.

Table: 1. Physico-chemical parameters for Tannery Effluent treated with *Paracoccus pantotrophu*

Treatment	pH	COD (mg/L)	TDS (mg/L)	Conductivity (mV)	Chloride (mg/L)	Sulphide (mg/L)	TH (mg/L)	Calcium Hardness (mg/L)	Magnesium Hardness (mg/L)
Raw	7.0	6240	4820	9740	2066	12	1100	500	600
Chemical Treatment (coagulation with Aluminium hydroxide and polyelectrolyte)	8.3	800	5040	9330	2100	BDL	1000	790	210
Treatment with *P.pantotrophus* Day 1	8.0	1584	3360	6550	1733	6	970	600	370
2	7.8	1408	3300	6400	1733	BDL	270	220	50
3	7.4	1360	3290	6300	1433	BDL	120	100	20
4	7.4	1280	2820	6000	1400	BDL	100	90	10
5	7.4	1280	2670	4670	1267	BDL	90	50	40
6	7.2	1232	1438	2390	1237	BDL	84	40	36
7	7.1	1088	667	1330	1233	BDL	81	32	49
8	7.1	832	394	753	1233	BDL	80	30	50
9	7.1	768	385	733	1233	BDL	60	28	32
10	7.1	752	364	360	1230	BDL	60	25	35
11	7.0	560	218	350	1100	BDL	50	23	27
12	6.5	272	206	310	1000	BDL	50	10	40

**(BDL-Below detectable limit)*

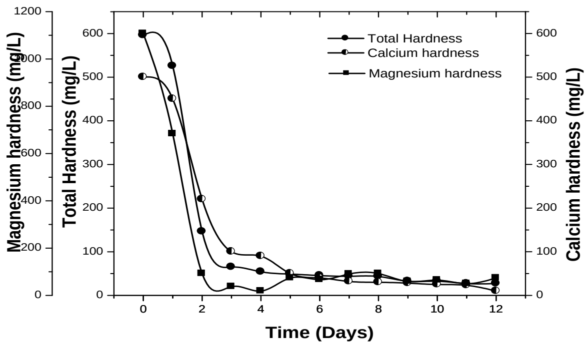


Fig. 1: Total hardness, Calcium Hardness and Magnesium Hardness in effluent treated with *Paracoccus pantotrophus.*

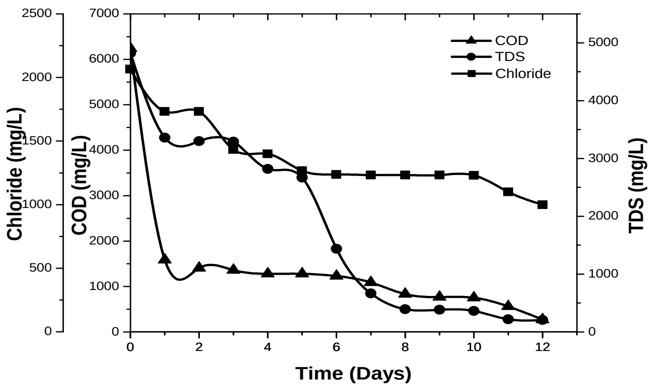


Fig. 2: Total Dissolved Solids, Chemical Oxygen Demand and Chloride reduction in effluent treated with *Paracoccus pantotrophus.*

3.1. UV-Visible spectrophotometer:

The UV-Visible spectrophotometric studies (Fig. 3) confirmed that the organic pollutants were removed efficiently by the *Paracoccus pantotrophus*. In the raw effluent, the major peak which is formed in the 280 nm was greatly reduced in the treated sample (Fig. 3). This indicates the removal of organic pollutants and the observation in UV-visible spectra corroborates with the COD removal.

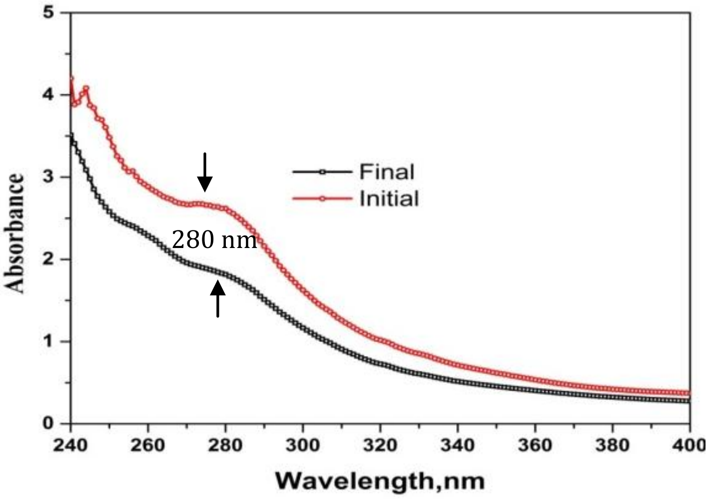
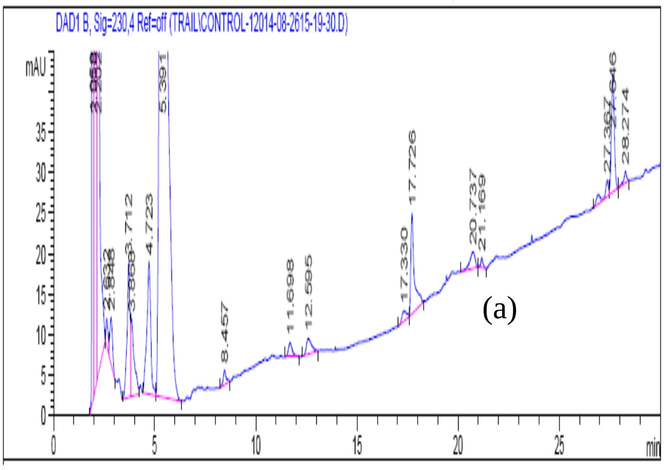


Fig. 3: UV visible spectra of both raw effluent and *Paracoccus pantotrophus* treated effluent.

3.2. HPLC analysis:

HPLC and Chromatographic condition: HPLC was carried out using Agilent 1260 series instrument and chromatographic separation performed using C18 reverse phase analytical column (5um, 250 X 4.6 mm, i.d.). The analysis was achieved by gradient elution using Water (A) and Acetonitrile (B) as the mobile phase and flow rate 1ml/min with a run time of 80 min. The photo diode array detector was set at 230nm and column was maintained at 30°C temperature.



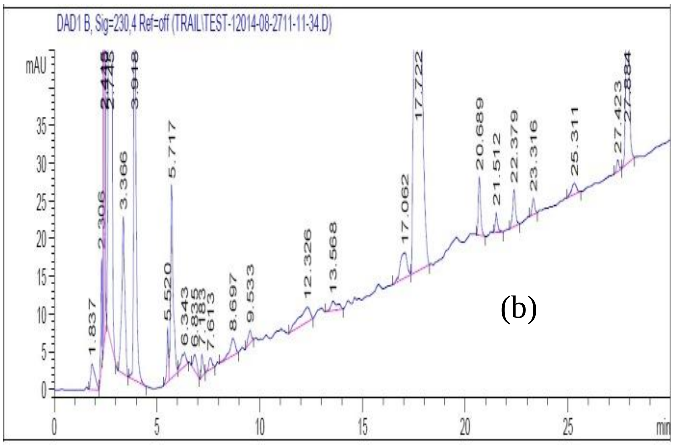


Fig. 4: HPLC analysis of (a) raw and (b) treated effluent

The organic pollutant removal in the tannery effluent by the *Paracoccus pantotrophus* was further confirmed by the HPLC analysis (Fig. 4). The HPLC indicated that the peak at 5.39 min was presented in the control and it was disappeared in the treated effluent and also the new peaks are formed in the effluent treated sample at the retention time of 2.30, 2.44, 2.74, 3.36, 3.91, 5.55, 5.71, 22.37, 23.31, 25.31 min. which are clearly separated when compared to control sample. This may be due to the conversion of complex organic molecules into simpler intermediates and other products.

4. Conclusions

In the current scenario, the tannery effluent treatment problem is considered as major issue since there are no suitable treatment technologies for their treatment. Even though there are many chemical and biological based technologies for the treatment of tannery effluent, still the industries couldn't able to achieve the efficiency due to the complicated organic components. The presented study may overcome the issue related to the difficulties in the removal of tannery effluent. Since the rumen microbes are considered as anaerobic bioreactor and efficient enough for the removal of complex organic compounds, this may be utilized for the treatment of tannery effluent containing various organic compounds.

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