Biotechnological process for the treatment of fleshing from tannery industries for methane generation

Disposal of untreated wastes into land and water bodies from tanneries results in air and water pollution as well as emission of greenhouse gases like methane and carbon dioxide. The atmospheric concentration of methane is increasing at the rate of 1% per year and has more than doubled over the past two centuries[1]. This problem can be mitigated through adoption of eco-friendly waste-to-energy recycling technologies for treatment and processing of wastes before their disposal. Biomethanation, which is environment-friendly, is one of the most benevolent technologies as it leads to generation of energy from wastes, besides rendering them suitable for application as a rich source of organic manure. Biogas is relatively odour-free, and the biosolids residue after anaerobic digestion is rich in nutrients and finds application as a organic fertilizer in agriculture[2]. The environment is under increasing pressure from solid and liquid wastes emanating from industries, such as tanneries. Tannery waste disposal problem leads to environmental as well as social disharmony, making it a major industrial pollution faced by the country[3]. The solid wastes comprising sludge and fleshing are inevitable by-products of the leather manufacturing process and causes pollution. Yadav[4] has pointed out that the Jajmau tannery generates about 400 tonnes solid waste per day. The World Bank reported[5] that solid wastes can represent up to 70% of the wet weight of the original hides. Unless this is treated in some way prior to disposal, it poses odour as well as land pollution. Tannery fleshings, the major solid wastes emanating from the beam house of a tannery could be subjected to biomethanation. The fleshing from industries and bio-sludge from CETP (Common Effluent Treatment Plant) consists mainly of carbohydrates, lipids, proteins and inorganic materials. Microbes have the ability to transform these polymers into simple soluble molecules such as amino acids, fatty acids and simple sugars. Annapurna Raju *et al.*[6] have reported eco-friendly enzymatic dehairing using extracellular protease from a *Bacillus* sp. Biological liquefaction of limed fleshing and methane generation were conducted by Ravindranath[7]. Microbial liquefaction method could be safe for the recycling of organic substrates. It is one of the alternatives to conventional mechanical and chemical methods. Karmaraguru *et al.*[8] conducted hydrolysis of tannery fleshing using chicken intestine proteases. Muhammad Nauman Aftab *et al.*[9] have recorded the biodegradation of leather waste by *Bacillus subtilis*. According to Chandramouli[10], there are close to 3000 tanneries in India, of which 812 are situated in Tamil Nadu. In Dindigul district alone, there are 63 tanneries[11].

In Dindigul, the vegetable-tanning process is commonly used. Around 25,000–30,000 tonnes of hide is processed, which is 6–7% of the total quantity processed in India. Minimum effluent produced is 3000–4000 l per 100 kg of hide[12].

The objective of the present investigation is to accelerate the fleshing digestion process by inoculating with efficient proteolytic bacteria, after which it could be subjected to biomethanation.

Tannery sludge was collected from the CETP, Dindigul run by TALCO (Tamil Nadu Leather Development Corporation) and fleshing was obtained from the beam house of Shri Ramajayam Tanners, Dindigul.

The bacterial strains were isolated by enrichment culture technique in nutrient agar media. The isolates were purified by streak plate method and the purified bacterial isolates were then transferred to the nutrient agar slants and used for further studies. The primary screening was done by the hydrolysis of egg albumin, skimmed milk casein and gelatin. Among the 12 strains isolated from the enrichment culture technique, two were selected based on their proteolytic activity. Identification of selected isolates was based on morphological, biochemical and physiological characteristics. This work was also aimed at the analysis of physico-chemical properties of tannery fleshing and sludge, viz. total solids and moisture content[13], electrical conductivity[14], pH[15], COD, ash content, volatile matter, total Kjeldhal nitrogen[16], phosphorus[17], potassium[18] and total organic carbon[19]. Liquefaction of tannery fleshing was carried out by inoculating them with 5% proteolytic bacteria in log phase of growth as inoculum. The efficiency of liquefied tannery fleshing and sludge in combination with cow dung was studied for biogas production for a period of 30 days in batch digesters. Laboratory batch digestions were done in vials of 150 ml capacity and bottles of 3 l capacity. Slurry preparations were made by mixing liquefied fleshing and biological sludge (secondary sludge) in ratios of 1 : 1 (T_1), 1 : 2 (T_2), 2 : 1 (T_3), 3 : 1 (T_4) and 3 : 2 (T_5). A control digester (T_0) was maintained using cow dung and water in the ratio of 1 : 1. The conditions of digestion of slurry for the production of biogas are as follows: a set of vials was kept under open sunlight and another set of vials was kept under ambient temperature (30°C) using an incubator. The methane produced was estimated by gas chromatography (Nucon 5765) equipped with Flame Ionizing Detector and a column (2 m × 1/3 cm) packed with Porapak-N 80/10 mesh. The carrier gas was nitrogen[20] and oven temperature was 80°C. Biogas production was quantified every three days for a period of 30 days. The volume of gas produced in vials was measured by the downward displacement of water in an inverted burette and biogas production was represented as ml/h. Experiments were designed to compare methane production from raw and liquefied fleshing.

Tannery solid wastes (biological sludge and fleshing) were first analysed for their physico-chemical composition (Table 1). Twelve strains were isolated from the enrichment culture technique using cow dung and tannery fleshing. Two isolates (I and II) which showed higher proteolytic ability were selected for further studies based on casein hydrolysis (Figure 1) and

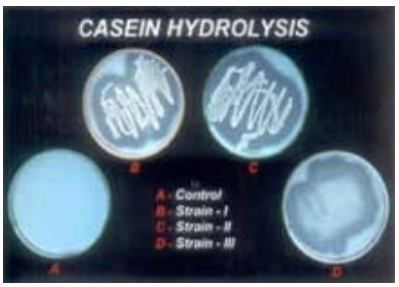

Figure 1. Casein hydrolysis by proteolytic bacterial isolates.

Table 1. Physico-chemical characteristics of the feed stock materials

Parameter	Tannery biosludge	Fleshing
Total solids (%)	46.5	33.3
Moisture (%)	53.5	66.7
Volatile matter (%)	78.0	82.0
Total organic carbon (%)	15.60	32.2
Total Kjeldhal nitrogen (%)	3.80	3.27
Phosphorus (%)	0.09	0.26
Potassium (%)	0.40	0.52
C : N ratio	4 : 10	8.5 : 1
pH	8.0	8.2
EC (ds/m)	1.75	1.06
Appearance (colour)	Grey to brown	–
Odour	Fishy	–
Chromium (mg/kg)	2.8	–
COD (mg/kg)	2485	–

Table 2. Morphological, cultural and biochemical characteristics of bacterial isolates

Characteristics	Isolate I	Isolate II
Vegetative cells	Short rod	Straight rod
Gram staining	–ve	+ve
Spore staining	No spore	No spore
Acid fast staining	Not acid fast	Not acid fast
Motility	+ve	+ve
Catalase test	+ve	+ve
Oxygen relationship	Aerobic	Aerobic
Indole test	–ve	–ve
Casein hydrolysis	Hydrolysed skimmed milk	Hydrolysed skimmed milk
Gelatin hydrolysis	+ve	+ve
Voges–Proskaur test	–ve	–ve
Methyl red test	–ve	–ve
Optimum temperature for growth (°C)	37–40	45–50
Fluorescence	+ve	–ve

Identification: The above characteristics indicate that isolate I is *Pseudomonas fluorescence* and isolate II is *Bacillus subtilis*, according to the *Bergey's Manual of Determinative Bacteriology*[21].

Table 3. Comparative gas production of raw fleshing and liquefied fleshing

	Raw fleshing		Liquefied fleshing	
Treatment	Biogas production (ml/h)	Methane production (%)	Biogas production (ml/h)	Methane production (%)
Control (T_0)	31.0	62	32.0	63
1 : 1 (T_1)	21.0	44	63.0	45
1 : 2 (T_2)	24.0	53	71.0	55
2 : 1 (T_3)	23.0	46	69.0	47
3 : 1 (T_4)	27.0	55	81.0	59
3 : 2 (T_5)	33.0	58	102.0	64

free amino acid liberation during hydrolysis.

These isolates were characterized based on their morphological, cultural and biochemical properties (Table 2). The bacterial isolates I and II were identified as *Pseudomonas fluorescence* and *Bacillus subtilis* respectively, based on *Bergey's Manual of Determinative Bacteriology*[21]. Results of the screening tests showed that the tannery fleshing was completely digested by the isolated proteolytic bacterial strains effectively. Zerdani *et al.*[22] have studied digestion of solid tannery wastes by strains of *Bacillus* sp. isolated from compost. In the second phase, investigations were carried out using liquefied fleshing mixed with biological sludge (secondary sludge), cow dung and water for biogas production. For solid organic wastes, the common pre-treatment method

is hydrolysis, which liquefies the substrate in a bioreactor before using it as feedstock for biomethanation[23]. It was observed that the cumulative gas production and methane content (maximum 64%) was in T_5, which is almost similar to control (T_0) in terms of methane content (63%; Table 3). Further investigations were carried out and the results indicate that the biologically liquefied tannery fleshing could be effectively used for production of methane at a higher rate, compared to raw fleshing. Liquefied fleshing produced threefold the amount of gas compared to raw fleshing, and methane content was recorded as 64%. Ratna Chakraborthy[24] had carried out similar studies on the ecofriendly solid waste management in the leather industry.

Proteolytic bacteria can treat tannery solid waste (fleshing) biologically; this microbial enzymatic hydrolysis could be a safe method of recycling these organic materials, thus enhancing the rate of biodegradability. The isolates tested were better adapted to liquefaction of tannery fleshing, which could be effectively used for higher rate of biomethanation. After biomethanation, the digested slurry obtained could act as good organic manure. Thorstensen and Madhur Shah[25] have made a technical and economical evaluation which indicates that tannery sludge has definite value as a fertilizer based on its nutrient content. This relatively simple biological treatment of leather waste may provide a practical and economical solution.

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