Asian Journal of Water, Environment and Pollution, Vol. 12, No. 4 (2015), pp. 79–82.
DOI 10.3233/AJW-150020

Effective Utilization of Leather Waste for Cultivation of Bacteria

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Received March 9, 2015; revised and accepted October 5, 2015

Abstract: Environmental pollution is one of the major problems associated with rapid industrialization in developing countries. Tanneries generate huge amount of solid waste. Leather waste has been utilized for land filling, preparation of boards, soil fertilizer and animal feed. In the present study, solid leather waste was hydrolyzed with acid and alkali. The tanning agent was removed from the hydrolysate. The hydrolysate was analyzed for protein/amino acid content. The hydrolysate along with agar was used as solid media (Leather Hydrolysate Agar) for the cultivation of *Staphylococcus aureus* and *Escherichia coli*. The significant growth of bacteria on leather hydrolysate agar shows the possible use of leather waste hydrolysate in the preparation of microbiological media as well as supplement to bacteriological media.

Key words: Leather waste, hydrolysis, leather hydrolysate agar, bacteriological media.

Introduction

Tanning industry is one of the fastest growing industries in south and south eastern Asia. About four million metric tonnes of solid leather waste are produced globally every year and approximately 40–50% of the hides are lost to shavings and trimmings which is not utilized efficiently. Only 20% of hides are converted to leather products (Alexander et al., 1991). The production of much greater amount of leather waste has been a problem in many countries. Some of the waste may be saleable but the remainder must be disposed. There is an increased curb on land disposal due to their smell, increasing landfill costs, toxicity due to the quality and quantity of chromium content and their adverse effect on the surrounding land, water and the local flora and fauna. It was reported that a single tannery could cause pollution of groundwater around a radius of 7 to 8 km (Mondal et al., 2005). There is an increasing need for the effective utilization of these massive leather wastes. Now there is a growing interest in the effective utilization of the bio-waste primarily due to their valuable chemical composition. Leather wastes are considered as an excellent source of high valuable products such as collagen, gelatin and collagen hydrolysate. Collagen is used in drug delivery and tissue engineering (Pachence, 1996). Gelatin is primarily used as drug capsule and in other biomedical applications (Einersona, 2002).

Every organism in the universe requires nutrient for energy production, cell division and growth. These nutrients at the elementary level consists of carbon, hydrogen, oxygen, nitrogen, sulphur, phosphorus, calcium, magnesium, iron, manganese and some trace

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elements. Heterotrophic microorganisms require carbon and nitrogen to synthesize proteins and nucleic acids. Only a few genera of bacteria can make use of free molecular nitrogen present in air and others require nitrogen in the organic or inorganic form. Some of the microorganisms (proteolytic) can digest proteins to get free amino acids but most of them are not proteolytic. Peptones are the most widely used nitrogen source in microbiological media. In the present study, the leather waste was hydrolyzed to prepare leather hydrolysate agar for the cultivation of bacteria namely, *Staphylococcus aureus* and *Escherichia coli*.

Materials and Methods

Hydrolysis of Vegetable Tanned Leather Waste

5 g of moisture-free vegetable tanned leather waste was hydrolyzed with 50 ml of 6N hydrochloric acid (HCl) and 8N sulphuric acid (H_2SO_4) for 48 hrs at 110°C and with 6N Sodium hydroxide for 8 hrs at 80°C in vacuum. The hydrolysate was filtered through Whatman No. 1 filter paper. The hydrolysate was treated with equivalent amount of barium hydroxide solution to remove chloride ions and sulphate ions as barium chloride and barium sulphate respectively and filtered. Hydrolyzed samples were treated with lead acetate and zinc sulphate to precipitate the tannins.

Hydrolysis of Chrome Shavings

5 g of chrome shavings was hydrolyzed with 50 ml of 6N HCl and 8N H_2SO_4 at 98°C for 24 hrs. 50 g of chrome shavings was treated with 3.5 g of sodium hydroxide at 98°C for 6 hrs. The hydrolysate was filtered through Whatman No. 1 filter paper.

Analysis of Sample

The leather waste and hydrolysate were analyzed for moisture, ash content, nitrogen and protein content by TKN method, aminoacids by Ninhydrin method, chloride, sulphate, tannin (Vegetable tanned leather waste) and chromium content (chrome shavings).

Bacteria Culture Using Hydrolysate

Since the leather hydrolysate contains amino acids which are organic compounds containing carbon, hydrogen, oxygen, nitrogen and in certain cases sulphur also, the recovered hydrolysate was used as substitution for beef extract and peptone in Nutrient agar medium. An agar medium (leather hydrolysate agar) was prepared with hydrolysate solution (40 ml), sodium chloride (1.5 g), agar agar (2.0 g) was added to 100 ml distilled water and pH was adjusted to 7.5. The final volume was made to 1 L with distilled water and sterilized by autoclaving at 15 lbs pressure for 20 min. Cooled hydrolysate agar media was poured into petri dishes and allowed to solidify. The plates were streaked with the pure culture of *Staphylococcus aureus* and *Escherichia coli* incubated at 37˚C for 24 hrs. Nutrient agar media was used as positive control.

Results and Discussion

Vegetable tanned leather shavings and trimmings contain 40.7% protein and 35% tannin content. Chrome shavings contain 16.54% TKN and 2.6% chromium content (Table 1). The tannins were completely hydrolyzed with HCl and H_2SO_4 and do not precipitate with lead acetate or zinc sulphate, whereas the tannins were not hydrolyzed completely with NaOH and form precipitate (lead tannate) with lead acetate. Trace amount of chloride, sulphate and lead was present in hydrolysate treated with HCl, H_2SO_4 and NaOH (Table 2). HCl hydrolysate contains more amino acid content (455 µg/ml) than H_2SO_4 (195 µg/ml) and NaOH (185

Table 1: Analysis of different leather waste

(%)	*Vegetable tanned leather waste*	*Chrome shavings*
pH	-	3.5
Moisture	22.75	13.35
Protein	40.72	-
TKN	7.39	16.54
Ash	1.0	13.42
chromium	-	2.6
Tannin	35	-

Table 2: Analysis of leather hydrolysate

	HCl hydrolysate	*H_2SO_4 hydrolysate*	*NaOH hydrolysate*
Protein content (%)	5.95	4.37	5.5
TKN (%)	1.176	0.7	0.294
Amino acid content (µg/ml)	455	195	185
Sulphate ions	Absent	Present	-
Chloride ions	Present	Absent	-
Tanin	Absent	Absent	Trace
Lead	-	-	Present

µg/ml) representing the protein content of 5.95%, 4.37% and 5.5% respectively. The major aminoacids present in the hydrolysate include glycine, hydroxyproline, proline, alanine, arginine, glutamic acid, serine and valine.

Complete hydrolysis of chrome shavings and vegetable tanned leather waste cannot be carried out with alkali agents. This may be due to the hydrophobic nature of non-polar and alkaline amino acids in alkaline condition, covalent cross-linking of alkaline amino acids with hydroxyl amino acids and cross-linking of chromium(III) complex (Changdao Mu et al., 2003). Figures 1 and 2 show the *Staphylococcus aureus* and *Escherichia coli* colonies developed in the Petri plates with media containing the hydrolysate. *E. Coli* grows faster than *S. aureus*. The growth of *S. aureus* on leather hydrolysate agar is considerably less when compared to their growth on nutrient agar. This may be due to the lower concentration of leather hydrolysate in the medium. The results show that the hydrolysate can be used as a carbon and nitrogen source in microbiological media. This hydrolyzed media is very economic due to the abundant quantity of waste available in everyday production process. Further it increases the profitability to the media production industries due to the cost-effective raw material and economically feasible production process. This waste management process reduces the pollution caused by leather industry to a greater extent.

It is well known that the extent of microbial growth is influenced by kinds of substrate available and the environmental conditions in which they are growing. Of the various constituents of microbial media, peptones are one of the most expensive and are obtained from plants (Parrado et al., 1993), dairy proteins such as casein (Reissbrodt et al., 1995) or whey (Lund et al., 1992). Recently peptones from fish waste and meat are used in microbiological media (Green et al., 1977; Vázquez et al., 2004). Taskin and Kurbanoglu (2011) proposed the use of chicken feather as substrate for cultivation of *Bacillus subtilis, E. coli* and *L. delbrueckii*. Hydrolysates of cod viscera was proved to be as an alternative to commonly used complex nitrogen sources for the type strains of the lactic acid bacteria (Aspmo et al., 2005; Horn et al., 2005; Beaulieu et al., 2009). 0.5% solution of the lyophilized shrimp head and hull digest heated at 121 °C for 15 min supported excellent growth of fungi and good growth of bacteria (Stephens et al., 1976). Fish protein hydrolysates were used as nitrogen source for the production of extracellular lipase (Ghorbel et al., 2005). The collagen hydrolysate is used in cosmetics, food additive (Langmaier et al., 2001, 2002; Morimura et al., 2002), treatment of osteoarthritis and osteoporosis (Moskowitz, 2000). The leather waste can be hydrolyzed to recycle aminoacids and peptides for use in feeds and fertilizers (Ohtsuka, 1973; Taylor et al., 1992, 1993). The leather hydrolysate can be used as a basal medium for the culture of auxotrophic bacteria or as supplementary material for various microbiological media.

Conclusion

Solid leather wastes were hydrolyzed to produce leather hydrolysate agar. Acid hydrolysis proves promising than alkaline hydrolysis. Tanning agent free-collagen

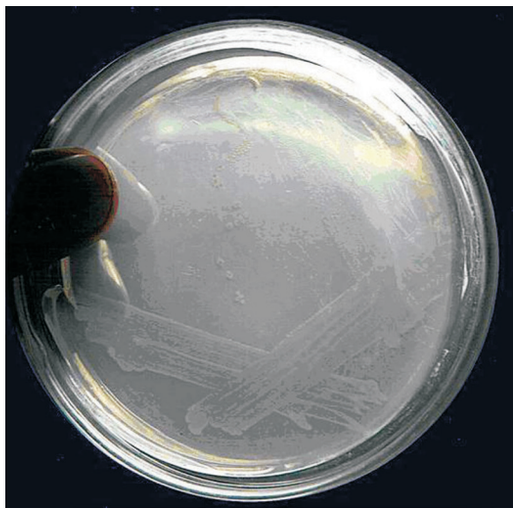

Figure 1: Growth of *Staphylococcus aureus* on leather hydrolysate agar medium after 24 h.

Figure 2: Growth of *Escherichia coli* on leather hydrolysate agar medium after 24 h.

hydrolysate was checked for the growth of bacteria without any supplements. *Staphylococcus aureus* and *Escherichia coli* showed considerable growth on 4% leather hydrolysate agar. Higher concentration of the collagen hydrolysate may provide excellent growth of microorganisms. This study shows the potential use of leather waste in the production of microbiological media and as a supplemental protein source to complex media.

Acknowledgement

Authors thank the management of VIT University, Vellore and the Director, CLRI for providing facilities to carry out the work.

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