Pilot-Scale Composting And Biodegradability Of Bovine Hair

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Abstract: The aim of this study was to develop process factors favourable for composting as an environmental-friendly and sustainable management for solid tannery hair waste. The experimental process conditions developed to sustain thermophilic microbial flora, responsible for degrading highly resistant organic materials, were: temperature 49°C, moisture 55% RH, pH 7 and a carbon to nitrogen ratio of 35:1. The biodegradation and structural modification of the substrate was assessed using scanning electron microscope. The results show that under these experimental conditions hair was highly degraded. The biodegradation was observed to be selective starting with the most vulnerable components, i.e. (1) cell membrane (2) endocuticle (3) medulla, and (4) the cytoplasmic material and fibrillar components of the cortex, with the cuticle being most resistant. The degradation mechanism was identified as both biochemical and mechanical. The gradual decrease in carbon and nitrogen ratio indicated progressive stabilisation of the compost.
**Key words:** composting; bovine hair; tannery wastes; biodegradation; microscopy

1. Introduction

Environmental concerns and legislation have led to alternative unhairing methods that aim to eliminate the dissolution of hair and its discharge into wastewater.[1,2] Although this has lowered chemical and biological oxygen demand in tannery effluents, the accumulation of recovered hair poses disposal problems[1]. In many countries, there is increasing demand to reduce the volume of commercial and industrial wastes going to landfill, while encouraging alternative treatments with the objective of maximising the economic value of resources through reuse, recycling and energy recovery.[3]

In the global leather industry it is believed that the most popular use of the recovered hair waste would be as a nutrient source, of slow-release nitrogen, for agricultural purposes.[4] However, it is generally agreed that hair, wool and feathers are organic substrates that are resistant to degradation, mainly due to their chemical characteristics, thus making their treatments challenging.[5] Composting is widely accepted as an environmental friendly alternative treatment of recycling of organic solid wastes. It is a microbial-mediated process in which the biodegradable organic substrate is broken down into simpler compounds in the presence of oxygen producing a stabilised and sanitised product that is beneficial to plants.[6] The key factors to the success of the process are those that will promote and sustain microbial growth, i.e. environmental and nutritional factors.[6,7] Currently, there is very little information available on the practicalities of composting of hair waste, especially process parameters and biodegrading behaviour in terms of structural modification during microbial decomposition.

Keratin (α-keratin), the crystalline protein constituent of hair, is well known for its stability and resistance to enzymatic digestion due to its densely packed structure characterised by numerous disulfide bonds[8]. Mammalian hair share three common histological features: (a) the cuticle, (b) cortex, and (c) medulla (present only in coarse keratin fibres)[8,9] (Fig. 1).

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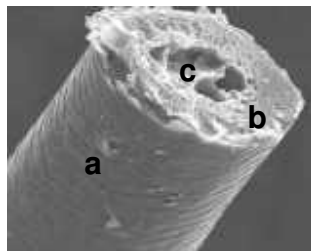
Fig. 1: SEM micrograph of hair shaft: (a) cuticle, (b) cortex,
and (c) medulla, (magnification: x600, 5.0kV).

The susceptibility of hair to degrading agents is dependent on these structural components and their differences in the proportion of disulfide links.[9,10] A cross section of the cuticle (Fig. 2) reveals lamellar components consisting of the outer sulfur-rich bands known as the exocuticle and the inner regions of low sulfur proteins called the endocuticle[11].
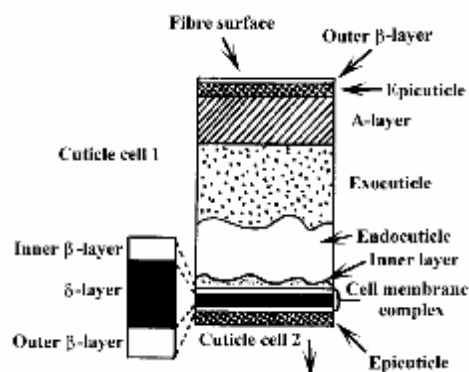
Fig. 2: An illustration of a cross section of the cuticle.[11]

The sheet-like cuticle cells are separated from each other by the cell membrane complex (CMC) which also provides adhesion to the cortex. The CMC consists of a less cross-linked δ-layer and the lipid-rich β-layers.[11,12] The cortex consists of elongated macrofibrils making up the bulk of the hair and is responsible for its mechanical properties. Between the macrofibrils are the melanin granules and cytoplasmic remnants. There is a variable packing in its two cells designated as para and orthocortex: paracortex is more densely cross-linked than the orthocortex. The cortical cells in turn, surround the vacuolated medulla cells which contains no sulfur proteins.[8,9,12]

In spite of its high resistance some microorganisms, i.e. bacteria, fungi and actinomycetes, are known to possess the ability to breakdown keratinous substrates.[5,13] However, the breakdown mechanism is not well known.[5] It is assumed that the unhairing processes with hair recovery occurs through the detachment of the hair at the follicle, leaving the integrity of the hair shaft intact.[10,14] This suggests that any biodegradation process will target the cuticle since the more vulnerable components are far less accessible for attack.

This research paper aims to contribute to the knowledge of the degradation mechanism of hair and represents the first phase aimed at developing standardised processing parameters for composting hair. These were classified into three groups: (1) nutritional, (2) environmental, i.e. pH, temperature and moisture, and (3) operational.

2. Experimental

2.1 Materials and Methods

Hair samples: intact hair was obtained from wet salted bovine hides (British School of Leather Technology, The University of Northampton, UK), washed with deionised water and air dried. Micro-organism: fresh soil samples (sieved through a 2 mm sieve) were used as the source of micro-organisms. Pilot-composting: experimental drums (British School of Leather Technology, The University of Northampton, UK), of 2 L capacity were used for composting.

2.2 Laboratory Biodegradation Study

Duplicate samples of intact hair, 0.2 g, were incubated with 30 g of fresh soil in sterile Petri dishes at 40°C for 0, 10, 15, 20, 25, and 30 days with regular inspection to ensure that the moist conditions were maintained. At the end of each incubation period, the hair samples were removed and prepared for microscopy (SEM).

2.3 Pilot-Composting

Feedstock, 1.5 kg, composed of sawdust/wood chips, dry leaves, fresh soil and hair were loaded into two 2 L capacity experimental drums. Composting was done for 120 days under controlled conditions to simulate an in-vessel composting process. The drums were periodically agitated to allow mixing and distribution of moisture within the matrix. Representative samples were taken for chemical analysis, moisture content and pH. The progress of the structural modification of hair was assessed using the scanning electron microscope.

3. Measurements

3.1 Scanning electron microscopy (SEM): hair samples were washed with phosphate buffer, pH 7.5, fixed in 1%v/v glutaraldehyde and stained with 0.1%v/v osmium tetroxide, according to Wagner and Bailey (1999).[15] Stained samples were dehydrated in 70% and 96% alcohol and mounted on aluminium stubs. Samples were gold coated and examined using scanning electron microscope (Hitachi S-3000N, Japan).

3.2 Temperature: the feedstock temperature was monitored by inserting a thermometer into the feedstock.

3.3 pH: measurement was performed on a suspension of 10 g of sample in deionised water (1:3 w/v) on a SevenMulti electrochemical pH meter (Mettler-Toledo Ltd, UK) as described by Stewart *et al*. (1974).[16]

3.4 Moisture: measurement was done by drying fresh compost 10 g in an oven at 105°C overnight, and then cooled in a desiccator to a constant weight.

3.5 Nitrogen: nitrogen was determined by TKN method for Total Nitrogen as described by Vogel (1961).[17]

3.6 Organic carbon: carbon was estimated from weight loss on-ignition of oven dried samples after igniting at 560°C and cooling in a desiccator to a constant weight. Carbon was estimated from the equation below according to Haug, (1980).[7] % Carbon = Organic matter x 100/1.8

## 4. Results and Discussion

4.1 Biodegradation study

From the SEM analysis evidence of degradation was observed after 15 days (Fig. 3). In most cases the cuticle appeared to have been attacked from its underlying adhering layer. There was further evidence that there was a weakening and separation between the cuticle/cuticle layers with subsequent flaking (Fig. 3 & 4). It was clear that the pathway of microbial intrusion into the hair shaft was through the less cross-linked regions. This first region was identified as the δ-layer of the cell membrane, followed by a progressive attack on the underlying endocuticle region (Fig. 2) subsequently causing delamination of the cuticle (Fig. 4). These regions are believed to have been composed of proteinaceous material with low disulfide cross-links[18] thus providing vulnerable links. Once the cuticle delaminated, the rest of the remaining β-layer of the cell membrane was degraded exposing the underlying cortical cells (Fig. 4 & 5). Within the cortical cells there was evidence of selective degradation of the more vulnerable components i.e. the cytoplasmic remnants and less cross-linked cells orthocortex [12]as observed by the splitting separation (Fig. 4 & 5). In some cases the structure appeared to collapse indicating the medulla and the microfibrils of the cortical cells have been degraded. The characteristic degradation of the non-keratinised and the less keratinised regions indicated both general proteolysis and keratinolysis.

Fig. 3: SEM micrograph of hair shaft showing initial stages of surface degradation, (mag. x1.0K, 5.0kV).
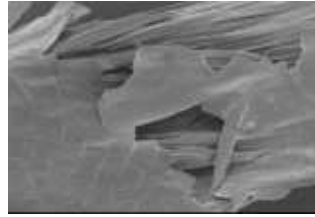
Fig. 4: SEM micrograph of hair shaft illustrating flaking cuticle and cortex undergoing degradation, (mag. x1.0K, 5.0kV).
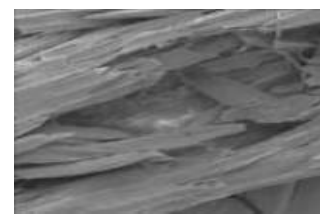
Fig. 5: SEM micrograph of hair shaft showing degraded medulla and microfibrils the cortex, (mag. x1.0K, 5.0kV).

4.2 Composting

Table I is a summary of the control parameters and changes in nitrogen and carbon on selected days during the composting period. The data are mean values from the replicate drum processes.

Tab. 1: Summary of the control parameters, nitrogen and carbon analysis.

Day	pH	Temperature (°C)	% Moisture	% Nitrogen	% Carbon	C/N
0	6.3	40	55	1.62	56.0	34.7
15	6.8	45	56	1.50	49.7	33.1
30	7.1	49	57	1.73	52.8	30.5
60	6.7	49	55	1.80	54.0	30.0
80	7.7	51	56	1.70	52.2	30.7
100	6.9	49	55	1.54	48.3	31.4
110	6.7	58	56	1.47	45.2	30.8
120	6.7	49	52	1.40	41.4	29.6

To sustain cellular functions of a biological system, nutrients are a prerequisite, especially carbon and nitrogen.[7] The initial balanced ratios of carbon to nitrogen recommended for composting, ranges from 20:1 to 35:1.[6,7] From the study the nutrients were available in the correct proportion sufficient to sustain microbial metabolism. There was fluctuation in the carbon nitrogen ratios which could be as a result of the metabolic processes. The decrease in carbon to nitrogen ratio has been observed in other composting studies and is attributed to the process of microbial transformation of nitrogen to ammonium and organic carbon to CO_2, generally referred to as mineralisation.[6,7,19] The pH was low in the initial the stages but gradually rose to pH 7.7. This rise may be due to enzymatic breakdown of the organic substrate releasing ammonia.[19] Measurements and monitoring were part of the operational parameters to ensure the pH did not rise above 9.0 which could lead to reduced microbial activity and possible loss of nutrients.[6,7] The temperature profiles observed were as a result of a controlled process, simulating the thermophilic phase (35-65°C) where there is a high microbial metabolism and decomposition of resistant organic substrates.[18] The highest temperature recorded was 58°C. This was a controlled sanitisation process as recommended in composting systems.[6,7] The average moisture content was 55% RH for the entire process. This was in agreement with the recommended range to maintain optimum metabolic activity.[6,7] Monitoring and adjustments of the moisture content to remain at the recommended optimum range was part of the operational parameter.

The choice of drums to carry out composting allowed a high degree of process control and monitoring. The process performance could be related to the physical changes on the substrate (Fig. 6-8). At the initial stages of the process, mechanical breakdown was observed (Fig. 6). The later stages were characterised by both

mechanical and biochemical degradation (Fig. 7). At the end of composting period, the hair structure had disintegrated but the cuticle remained as fragmented sheets (Fig. 8).
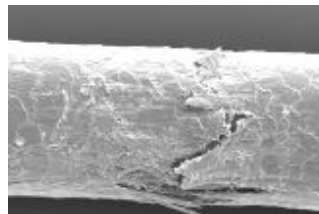
Fig. 6: SEM micrograph of hair shaft showing the mechanical damage after 15 days composting (mag. x800, 5.0kV).

Fig. 7: SEM micrograph of hair shaft showing mechanical and biochemical degradation after 90 days composting (mag. x1.0K, 5.0kV).
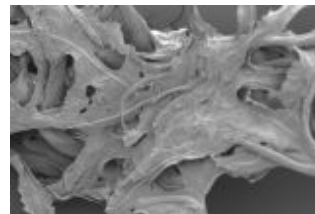
Fig. 8: SEM micrograph showing cuticular remnants of hair after 120 days composting (mag. x100, 5.0kV).

5. Conclusion

This research study has demonstrated that the disposal of tannery hair waste through composting can be achieved through optimisation of process factors favouring the biological system and incorporating operational parameters to ensure those conditions are maintained to sustain cellular functions. The in-vessel composting technology can fit within the existing tannery industrial set-up thus providing the industry with an environmental friendly treatment of hair waste with a potential economic value.

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