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by

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Abstract

Solid hair waste is generated by the leather industry as a by-product of the leather manufacturing process. Keratin, the main structural constituent of hair proteins, is highly resistant to degradation and their disposal is of environmental concern. The aim of this study was to develop conditions favorable for the degradation of bovine hair in a composting environment as an environmentally friendly option for the management of solid tannery hair waste. The thermophilic optimum temperature, 40 – 50°C, moisture content 55%, pH 7.0 and a carbon to nitrogen ratio of 35:1 were found to be favorable to sustain metabolic functions of thermophilic microbial flora, responsible for degrading keratins. The biodegradation and structural transformation of the substrate was assessed using scanning electron microscopy. The results show that under these conditions the bovine hair lost most of its integral structural stability and that the cuticular components were more resistant to degradation. The compost stability as evaluated by monitoring the degree of humification and carbon to nitrogen ratio indicated that the final product achieved reasonable stability by attaining 73% degree of humification, 26% humification rate and carbon to nitrogen ratio of 29:1. Hence the composting technology used in this study has potential application in the leather industry for the production of an economically viable product.

Resumen

Residuos sólidos de pelo son generados por la industria del cuero como un subproducto del proceso de fabricación. Queratina, el componente estructural principal de las proteínas del pelo, es altamente resistente a la degradación y su eliminación es de preocupación ambiental. El objetivo de este estudio fue establecer condiciones favorables para la degradación del pelo bovino en un ambiente de compostaje como una opción ecológica para la gestión de los residuos sólidos de pelo de la curtiembre. La temperatura termofílica óptima, 40 a 50°C, contenido de humedad del 55%, pH 7.0 y una relación de carbono a nitrógeno de 35:1 es encontrada como favorable para mantener las funciones metabólicas de la flora microbiana termofílica, responsable de la degradación de la queratina. La biodegradación y la transformación estructural del sustrato fueron evaluadas mediante microscopía electrónica de barrido. Los resultados muestran que bajo estas condiciones el pelo bovino pierde la mayor parte de su estabilidad estructural integral y que los componentes cuticulares son los más resistentes a la degradación. La estabilidad de compost como se evaluó mediante el control del grado de humificación y la relación de carbono a nitrógeno indicó que el producto final alcanzó una estabilidad razonable por la obtención de un grado de humificación del 73%, una tasa de humificación del 26% y una relación de carbono a nitrógeno de 29:1. De ahí que la tecnología de compostaje utilizado en este estudio tiene aplicación potencial en la industria del cuero para la producción de un producto económicamente viable.

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Introduction

The conventional method of hair-removal through the use of lime and sulfide during the industrial leather manufacturing process has always been associated with the pollution of the environment. Subsequently, in recent years, environmental legislation has become increasingly stringent leading to the development of alternative hair-save unhairing methods with hair recovery.[1, 2] Hair-save unhairing has enabled a number of tanneries to realise environmental benefits such as reduced levels of COD (40 – 60%), BOD (50%), sulfide and suspended solids (50 – 60%), respectively.[3,4] However, in the process of hair-save unhairing approximately 5% and 10% of dry hair per tonne of raw bovine and ovine is recovered, respectively. The recovered hair and other tannery solid waste are commonly disposed either via dumping on land or engineered landfill. [4-6] Although dumping or sanitary landfilling may be considered quick solutions, but due to their extremely rigid structures the biodegradation of hair under natural environmental conditions is inefficiently slow, causing environmental concerns. [7] Currently more emphasis is placed on environmental protection through efficient waste management systems which favour recovery and recycling of biodegradable organic waste to maximise their potential economic value.[8,9]

It is believed that the most potential application of the recovered tannery hair waste could be as a nutrient source of slow release nitrogen for agricultural purposes.[5, 10, 11] It has also been suggested that due to the large volume of hair recovered from the hair-save unhairing processes composting would be the most viable treatment option.[11] Although composting has been widely accepted as an environmentally-friendly alternative treatment of recycling of biodegradable organic solid waste,[12] currently there is little information available relating to the biotechnological processing of bovine hair waste in a composting environment for the production of a sanitised and stable product for use in agriculture.

This paper represents preliminary steps aimed at developing suitable parameters for the biodegradation of hair in a composting environment for the tanning industry. For that purpose, small scale composting of bovine hair was carried out at the University of Northampton (UK) under controlled conditions as described below.

Materials and Methods

Materials

Bovine hair-waste was recovered from wet salted bovine hides (Institute for Creative Leather Technologies, University of Northampton) by shaving using electric clippers. Fresh soil (sieved through a 2 mm sieve), dry leaves and wood chips/saw dust were used as source of micro-organisms and bulking agents. Dry leaves were shredded manually to particle size range 0 – 10 mm to provide porosity. Stainless steel drums (Rowan Electronica, Italy) of a 2 litres capacity, fitted with temperature control panel were used to simulate an in-vessel composting process. All the reagents used were of analytical grade obtained from Sigma Aldrich, UK. All analyses were carried out in duplicate.

Methods

Composting Process

The composting feedstock comprised of bovine hair (0.025 kg), dry leaves (0.3 kg), woodchips/saw dust (0.875 kg, 1:1), and fresh soil (0.3 kg). The composting process was carried out in duplicate in a 2 litres capacity stainless steel drums for 120 days under controlled temperature and moisture conditions. The initial proportion of carbon to nitrogen was determined and moisture adjusted to 55%. During the composting process the moisture content was monitored and maintained at 55% ± 2% by adding water on the feedstock when necessary, and the temperature controlled at the range of 40°C–50°C. The composting drums were occasionally turned to allow mixing and distribution of moisture and oxygen within the compost feedstock. Sampling was periodically carried out for chemical analyses, pH measurement, moisture content, and for the evaluation of the physical structural changes of the substrate.

Carbon and Nitrogen

Organic matter of the compost was determined by weight loss on ignition of oven dried (105°C) samples in a muffle furnace at 560°C overnight, followed by cooling in a desiccator to a constant weight. The percentage organic carbon was calculated from the organic matter as described by Haug (1980).[13] Percentage nitrogen was determined by the standard Total Kjeldhal Nitrogen (TKN) method.

pH, Moisture, and Temperature

pH was monitored weekly on a suspension of compost sample in deionised water (1:5 w/v), agitated for 30 minutes on a rotary shaker and allowed to stand for 10 minutes as described by Allen (1989).[14] Moisture content was determined gravimetrically by weight loss on drying a measured mass of fresh compost sample overnight in an oven at 105°C, and cooling in a desiccator to a constant weight. Temperature was monitored daily by inserting a thermometer into the compost feedstock through the drum sampling pot.

Scanning Electron Microscopy (SEM)

Microscopy examination of structural changes of hair during composting was performed using scanning electron microscopy (Hitachi S-3000N, Japan). Hair samples were washed with phosphate buffer, pH 7.5, fixed in 1% v/v glutaraldehyde and stained in 1% v/v osmium tetroxide according to Wagner and Bailey (1999).[15] The stained samples were dehydrated in 70% v/v and 96% v/v alcohol and mounted on aluminium stubs using adhesive carbon. Samples were coated with gold and examined using SEM.

Progressive stability level of the composting process was estimated by determining the degree of humification (DH) and humification rate (HR). This was carried out by extraction of the total extractable carbon (TEC) from the products, and fractionation of the extractable humic-like acid fractions (HA) from fulvic-like acid (FA) and non-humic fractions (NH): 2 g of ground oven dried (105 °C) compost samples taken at different stages of the composting process were extracted at 65°C under nitrogen with 100 ml solution containing 0.1 M NaOH and 0.1 M $Na_4P_2O_7$ for 48 hrs.[16] The samples were centrifuged at 5000 rpm for 15 minutes and the supernatant filtered. This represented the total extract (TE). The total extractable carbon (TEC) was determined from the total extract by the dichromate acid method of Walkley-Black, as described by Allison (1965).[17] Humic-like acid (HA) and fulvic-like acid (FA) was separated by repeated precipitations after acidification of 25 ml of the total extract with 6 M hydrochloric acid to pH 1.0 and allowed to stand overnight before centrifuging at 5000 rpm. Degree of humification (DH) and Humification rate (HR) were calculated according to the following Equations.[18]

$$DH\ (\%) = \frac{(HA + FA)}{TEC} \times 100$$

$$HR\ (\%) = \frac{(HA + FA)}{C_{org}} \times 100$$

Where C_{org} = organic carbon

Statistical Analysis

All the data were analysed using SPSS 17.0 and the results expressed as mean of duplicate samples.

RESULTS AND DISCUSSION

pH, Moisture and Temperature

Composting is a microbial-mediated process in which the decomposition of the organic constituent of the material into simpler compounds and a sufficiently stable product is undertaken under aerobic conditions.[12,19] Among the basic requirements for the microbial community for successful composting are favourable environmental conditions dependent on a continual supply of adequate moisture, oxygen, pH and temperature control. [20,21,22] In this study the initial pH of the feedstock was 6.3, which is regarded as within the optimum level tolerable to microbial growth, particularly bacteria which need a pH range of 6.0 – 7.5.[13,20,23] Figure 1 shows the general trend of the average pH monitored in the replicate compost mixtures during the composting period. The process was characterised by a gradual increase in pH to 6.8 after 2 weeks, followed by a buffering at about pH 7.0. A further increase to an average pH 7.7 was recorded in both compost mixtures after 20 weeks of composting. The rise in pH was indicative of a proteolytic process with a possible volatilisation of ammonia during the decomposition of the compost organic substrates.[20,24,25] The pH gradually dropped to a final average of 6.7 ± 0.4 at the termination of composting process. The decrease in pH towards the end of the process may be attributed to the increased buffering capacity caused by humus formation.[26]

The importance of moisture is to enable the micro-organisms to perform their metabolic and physiological functions efficiently as well as influencing both structural and thermal properties of the materials. Although the moisture content (wet weight basis) varies with feedstocks, the recommended optimum for composting ranges from 55 – 65%.[13,20,21] Figure 2 represents the moisture profile during the 120 days of composting. The objective of controlling the moisture content was to maintain optimum conditions suitable for microbial cellular metabolism. The initial average moisture content of the feedstock was 55%. Through process control and monitoring the moisture content was generally maintained within the optimum range of 55% ± 2 for the entire period of composting.

Temperature is perhaps the most important parameter that controls microbial metabolism in a composting environment.[21,23,27] Microbial decomposition of highly resistant biodegradable organic substrates is considered to be most favoured at thermophilic temperature range of 45 – 55°C.[20,21,28] Keratin is the main structural protein of bovine hair. Keratin is known for its insolubility and resistance to proteolytic degradation due to the tightly packed structure, stabilised by covalent cross-linking of disulfide bonds, hydrophobic interactions and hydrogen bonds.[29,30] However, despite their resistance to degradation some thermophilic bacteria such as Bacillus species are known to possess the capability of degrading keratins.[7,31,32] In order to influence the proliferation of such groups of thermophilic bacteria and sustain their optimum metabolic activities the composting process was carried out at a controlled temperature range of 40 – 50°C, with an average temperature maintained at 49 ± 2°C during the active composting process. The experimental conditions allowed the ease of monitoring and simulation of an optimised full-scale process. Towards the termination of the experimental composting process, the controlled temperature regime included constant pasteurisation at about 60 °C for 5 days, a regulatory recommendation for the destruction of potential pathogens.[33]

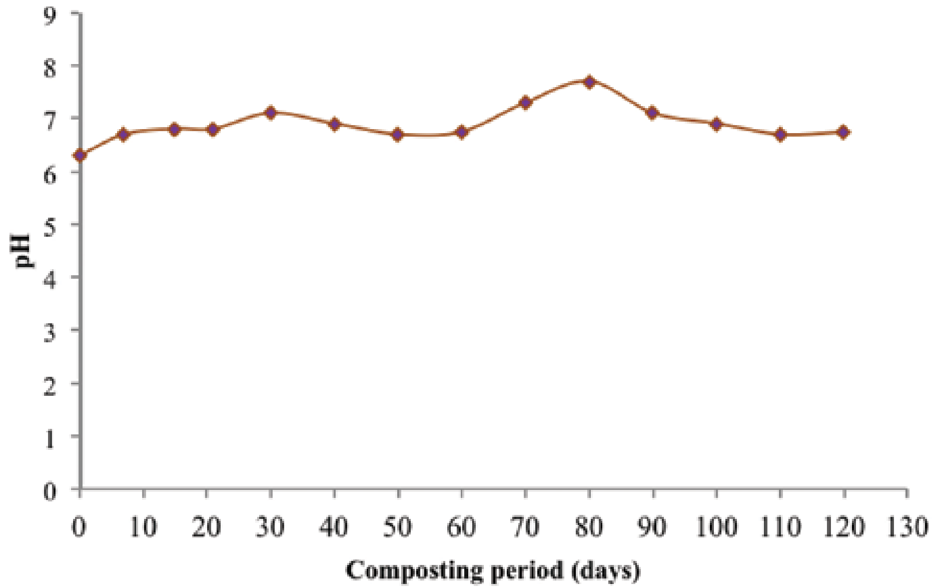

Figure 1. pH profile (7.0 ± 0.2) during controlled drum composting for 120 days.

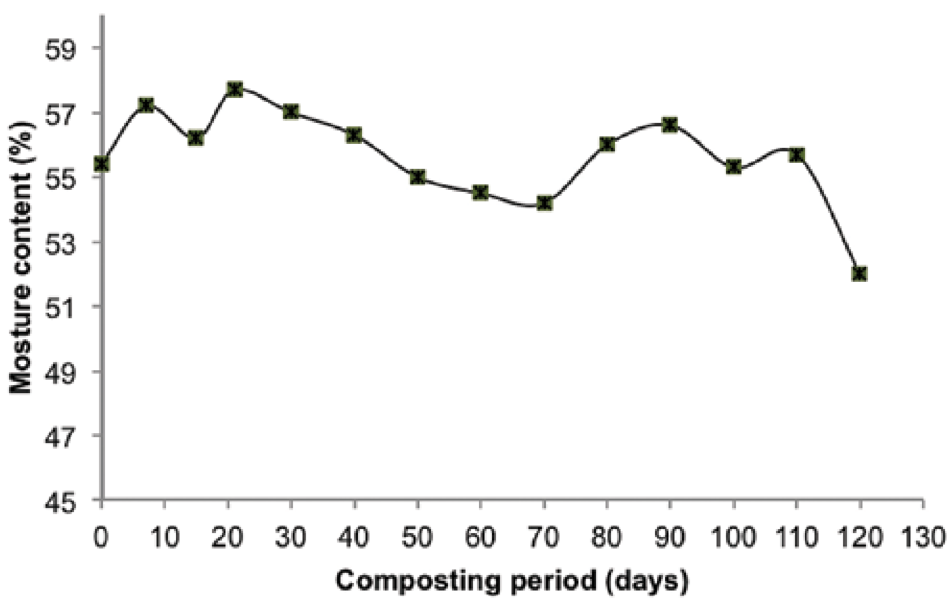

Figure 2. Profile of moisture content (55% ± 2) in a rotary controlled drum composting for 120 days.

Carbon and Nitrogen

Carbon and nitrogen are essential micronutrients needed by the micro-organisms to carry out their cellular processes during the oxidative decomposition process. In a composting process the proportion of carbon to nitrogen (C:N) is an important factor as it controls the rate of decomposition, and although it varies with feedstocks, the theoretical optimum range is between 20:1 and 35:1.[13,20,34] Table I shows the initial proportion of carbon to nitrogen (C:N) in the starting material and the changes that occurred as composting progressed. The initial carbon to nitrogen ratio of the feedstock mixture was about 35:1, which was within the optimum balance ratio required to sustain microbial metabolism. As composting progressed the general trend in the decline in the proportion of carbon to nitrogen available was experienced. This decline may be explained by the fact that during the microbial oxidative decomposition process the organic substrate is transformed into CO_2, H_2O and heat. The loss of organic carbon to CO_2 is referred to as mineralisation.[20,35-37] Similarly, gradual decreases in organic nitrogen represents mineralisation to NH_4^+ and possibly some losses due to ammonia volatilisation resulting from proteolysis.[12,27] The percentage organic carbon and nitrogen of the final product was 41 and 1.4, respectively, representing a C:N ratio of 29:1 at the termination of the composting experiment.

Humification Parameters

Humification parameters have been used to indicate the level of compost stability and maturity. The agronomical value of a compost increases when the organic material reaches a high level of humification.[38,39] The results of the humification parameters estimated as the Degree of Humification (DH) and Humification Rate (HR) are presented in Table II. The total alkali extracted carbon (TEC) increased gradually with composting time, ranging from 9.5% – 14.7% (w/w). This may be attributed to the increase in carbonaceous materials as composting progressed. [40] Similarly, the data shows that both DH (%) and HR (%) increased with time at different stages of composting as shown in Table II. The DH at the start of composting was 52.6%, increasing to 73% during the active composting period. At the same period the HR increased from 8.9% to 26%. These changes were a reflection of microbial utilisation of the organic materials. As composting progresses the percentage humification is expected to increase relative to the total dry mass.[41] The DH (72%) and HR (26%) values of the end-product were indicative of a reasonable stabilisation, comparable with previous studies.[40,42,43]

Physical Changes

Aerobic decomposition of substrates during composting is carried out by a complex mixture of microbial population. In the process the substrate may undergo physical and biological changes accomplished by enzymatic actions secreted by indigenous microbes which are specific to the substrate being composted.[12,13] Although degradation of keratin presents a problem due to the histological complexity and rigidity of the structure stabilised by disulfide cross-links, the microscopy results indicated a physical transformation of the structure of hair as evidence of degradation. The progressive physical changes that occurred during composting are illustrated in Figures 4 – 6. Figure 3 represent the image of the intact hair shaft before composting. The evidence from the microscopy images shows that the biodegradation of hair was characterised by erosion of the surface, mechanical splitting and fibrillation of the structure. It was also evident that the biodegradation of the different components of hair varied indicating that the highly keratinised components such as the cuticle and parts of the cortex were slowly degraded. At the conclusion of the composting experiment the hair structure had undergone major disintegration leaving the cuticular fragments which were more resistant to degradation (Figure 6). The apparent structural changes demonstrate utilisation of keratin by the indigenous micro-organisms under the controlled parameters of composting.

TABLE I
Changes in organic carbon and total nitrogen during drum composting process, (percentages are mean values based on oven dried samples at 105°C).

Composting day	Organic carbon (%)	Nitrogen (%)	C/N ratio
0	56	1.62	35
15	50	1.50	33
30	53	1.73	31
40	51	1.64	31
60	54	1.80	30
80	52	1.70	31
90	51	1.58	32
100	48	1.54	31
120	41	1.40	29

TABLE II
Humification parameters of compost samples at different stages of composting

Composting period (days)	TEC (%)	HA (%)	FA (%)	DH (%)	HR (%)
0	9.5	2.3	2.7	52.6	8.9
30	11.5	4.4	2.7	61.7	13.4
60	12.4	4.7	3.4	65.3	15.0
80	13.0	5.1	3.6	67.0	16.7
90	13.0	5.8	3.4	69.1	18.0
100	13.9	6.3	3.8	73.0	21.0
120	14.7	6.7	4.2	72.1	26.0

TEC = total extractable carbon; HA = humic acid; FA = fulvic acid; DH = degree of humification; HR = humification rate.

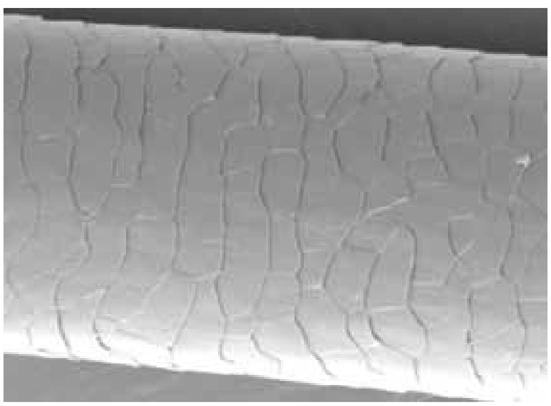

Figure 3. SEM image of an intact hair shaft (mag. x1.0K, 5.0kV).

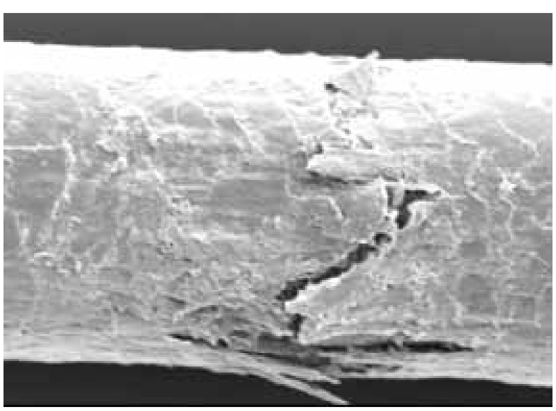

Figure 4. SEM image of hair shaft illustrating damage following 15 days composting (mag. x1.0K, 5.0kV).

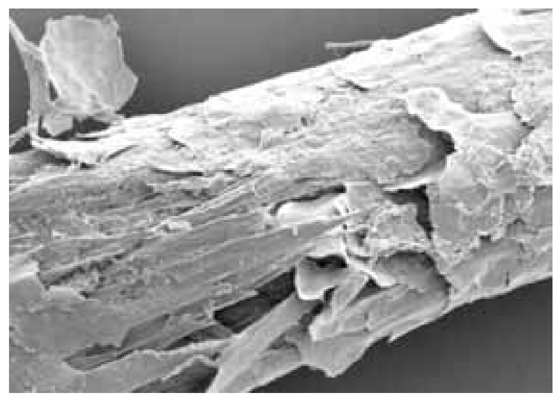

Figure 5. SEM image of a hair shaft illustrating damage after 90 days of composting (mag. x1.0K, 5.0kV).

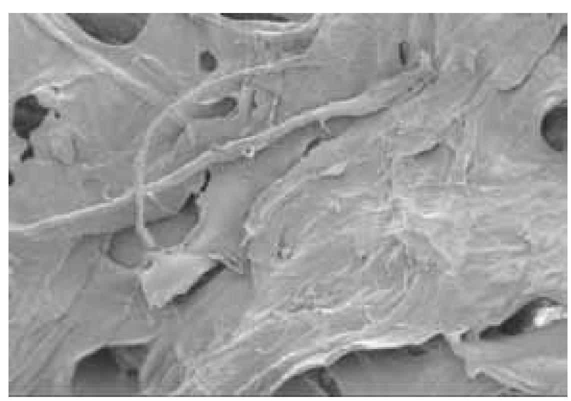

Figure 6. SEM image of cuticular remnants of degraded hair after 120 days composting (mag. x1.0K, 5.0kV).

Composting Benefits

The potential key benefits include:

- Overall reduction of up to 50% of the volume and disposal of tannery solid hair waste
- Production of valuable soil amendment for sustainable agriculture
- Reduction of the COD and BOD of the tannery waste streams
- Less capital and operating costs
- Can integrate other tannery biodegradable solid wastes

Conclusions

This study has demonstrated the appropriate practical process parameters for the successful decomposition of hair in a composting environment and provides the fundamental basis towards the establishment of an environmentally friendly technology for the management of bovine hair waste for the leather industry. The composting trial demonstrates the technology can fit within the existing tannery facilities, offering the industry the opportunity of designing an overall waste management plan that is environmentally acceptable and with a potential commercial value.

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