Microbial degradation of animal hair and preparation of organic compost thereof

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Abstract

About 50 tons of hair waste is generated from the tanning industries on processing of every 1000 tons of hides/skins. The disposal of hair wastes results in many significant negative environmental impacts.Keratin has wide range of application in agricultural, cosmetic and pharmaceutical industries. The keratin rich hair waste is recalcitrant and its degradation is laggard. CSIR-CLRI developed a bioprocess technology for efficient degradation of hair using a novel bacterial strain, *Brevibacteriumluteolum* MTCC 5982 in liquid medium containing salts and animal hair. The process was standardized in flask level and scaled up to 500 L in 750 L fermentor. The resulting fermentation liquor was used for the manufacturing of organic compost. The process could be completed within a time period of 10 to 12 days as against the conventional composting period of 90 days. Pilot scale production of compost has been standardized and the efficacy of the product has been tested at field level. The compost could serve as an effective organic fertilizer and could increase the yield of paddy by 20±5% and horticultural crops of bhendi, chilli and amaranthus by 25±5%. The process provides viable technological solutions to address the issue of tannery hair waste disposal on one hand and ensure financial returns on the other hand. The bulk production of compost from hair wastes could reduce the import and consumption of chemical fertilizers. This is an exemplary model of circular economy as the waste of the leather industry serves as an organic fertilizer of the agricultural industry.

Keywords: Hair degradation, organic compost, keratinase, keratin hydrolysate, agricultural industry, tannery.

1. Introduction

Leather industry utilizes raw hides/skins, by-product of meat industry, as the raw material.About 6.6 million tons of raw hides from bovine animals and 0.8 million tons of skins from ovine animals are converted into leather world over every year. Hair wastes of about 56 kilotons is generated annually from the tanneries producing ovine skins (Food and Agriculture Organization of the United Nations, 2016; Thankaswamy et al., 2018). Poor management of hair wastes by dumping, burial and incinerationnot only pollute the soil, air and underground water resources, but also may cause different human infirmities (Jana et al., 2020). Keratins are recalcitrant, stable, water-insoluble structural fibrous proteins and its degradation is slow in nature. The poor degradability of keratin can be attributed to the stabilization of the cuticle of hair due to the high degree of cross linkages by disulphide and hydrogen bonds.

Conventional methods of hair degradation include the physico-chemical methods, which are energy intensive, economically not feasible and also results in the destruction of essential amino acids (Nnolim and Nwodo, 2020). Microbial and biotechnological approach could be a promising alternative for disposing or utilizing keratinous wastes using the enzyme, keratinase. Keratinases are non-specific serine type proteases that have many applications in several sectors viz., leather, textiles, detergents, cosmetics, prion degradations, in production of biodegradable films, glues and foils and agro-industrial waste degradation (Barman et al., 2017; Dada and Wakil, 2021). There have been many reports on degradation of animal hair and feather using various microorganisms, including bacteria such as *Bacillus licheniformis, Brevibacillus brevis, Stenotrophomonas maltophilia*andyeast strain, *Trichosporonloubieri* (Thankaswamy et al., 2018). However, the microorganisms required addition of nitrogen sources, such as yeast extract, peptone, soybean meal, ammonium ions and soy flour for the growth and production of

keratinase (Gafar et al., 2020)and the time required for degradation of hair was significantly high. Therefore, efficient degradation of hair in the shortest duration without addition of nitrogen source is essential to utilize the keratin towards production of valuable keratin hydrolysate for various applications.

Due to the growing demand for organic food, the requirement for bio-fertilizers has been increasing significantly. Previous reports on the preparation of organic fertiliser from keratinous waste is associated with alkaline treatment (Gousterova et al., 2003; Nustorova et al., 2006) or pre-treatment of the substrate by the methods such as chopping, cutting and grinding (Falco et al., 2019) and the time required for the preparation of the product was about 90 days.

The aim of the present study is to degrade the animal hair waste efficiently without any pre-treatment of the raw material using a novel bacterial strain, *Brevibacteriumluteolum*MTCC 5982 and to prepare organic compost with the resulting keratin hydrolysate in a short duration of time. The preparation of organic compost was made by mixing appropriate volumes of the keratin hydrolysate with agro industrial residues and soil. The process was scaled up at pilot level. Throughout the composting process, the physico-chemical properties of the composting material were assessed at regular intervals. The effect of organic compost on growth and yield of various agricultural crops such as paddy (*Oryza sativa*), amaranthus (*Amaranthus viridis*), chilly (*Capsicum annuum*)and bhendi (*Abelmoschus esculentus*) was evaluated in field trials at different districts of Tamilnadu, India.

2. Materials and methods

2.1. Materials

Hair waste was obtained from Dindigul leather cluster, Tamilnadu, India, where wet-salted goatskins are processed into tanned leathers. Hair waste was cleaned by washing with water and air dried. All the chemicals used in the present study were of AR grade and purchased from Hi-Media Limited and S.D. Fine Chemicals Limited, Mumbai, India. Wheat bran and saw dust were purchased from local market, Chennai, India. Soil and dried leaves were collected from the CSIR- CLRI grounds, Chennai, India.

2.2. Degradation of animal hair

The keratinolytic organism, *Brevibacteriumluteolum* MTCC 5982 isolated from the hair dump yard of Dindigul Tannery, Tamil Nadu was used for the degradation of hair(Thankaswamy et al., 2018). The strain was maintained on nutrient agar slants at 4 ˚C. The organism was grown on nutrient broth for 24 h at 30 ˚C and used as preinoculum. Hair degradation was initially carried out in 250 ml Erlenmeyer flasks containing 50 ml of basal salt medium containing 0.3 g/L of K_2HPO_4, 0.4 g/L of KH_2PO_4, 0.5 g/L of NaCl and 40 g/L of goat hair using 1.0 % (v/v) preinoculum for 72 h at 30 ˚C. The process was scaled up in 75 L fermentor (Scigenics, India) with working volume of 50 L containing the basal salt medium and 4 % of goat hair. The medium was sterilized *in-situ* at 121 ˚C for 20 min and inoculated with 10 % (v/v) of preinoculum grown in nutrient broth for 24 h. The agitation speed and aeration were set at 200 rpm and 2.0 vvm, respectively and fermentation was carried out at 30 ˚C for 96 h. Samples were withdrawn at 6 h intervals and analysed for the keratinase activity and degree of hydrolysis. The parameters like pH and dissolved oxygen in the fermentation broth were monitored throughout the process. Further, scale-up of hair degradation was carried out in 750 L fermentor (Scigenics, India) with working volume of 500 L and all other conditions remained the same. The fermentation broth (keratin hydrolysate) was collected and used for compost preparation.

2.3. Enzyme assay and degree of hydrolysis

The samples collected from the fermentation culture mediumwere centrifuged at 8000 rpm for 10 min at 4 ˚C and the keratinase activity was determined using keratin azure as substrate (Thankaswamy et al., 2018). One unit (U) of keratinase activity was defined as the amount of enzyme required to bring about an increase in absorbance of 0.01 at 595 nm (Cai et al., 2008). The degree of hydrolysis (DH) was determined using TCA (Hoyle and Meritt, 1994). To 1 mL of culture supernatant, 1 mL of 20% TCA was added and centrifuged at 10000 rpm for 10 min at 4 ˚C to collect 10% TCA soluble fraction. Total nitrogen present in 10% TCA soluble fraction was estimated by Elemental CHNS analyser. The degree of hydrolysis was calculated using the formula,

$$\text{DH } \% = \frac{\text{Total soluble nitrogen content in 10\% TCA X 100}}{\text{Total nitrogen content}}$$

2.4. Preparation of compost

Compost was prepared in triplicates at lab-scale by mixing the fermentation liquor (5 L of hair hydrolysate) and optimized ratio of 5 kg of composting material (soil – 2.0 kg; pulverized dry leaves – 1.5 kg; saw dust – 1.0 kg and wheat bran – 0.5 kg). The mixture was transferred to the trays and kept at ambient temperature (30 ± 2 ˚C) for 10-12 days. The moisture content was maintained at 55 ± 2% throughout the process by sprinkling water. Samples were collected from the trays at 24 h intervals to determine the pH, temperature, moisture content, total nitrogen, organic carbon and CFU/g. The moisture content was determined with a moisture analyser based on weight loss at 120 ˚C, ending with a constant weight (Moisture analyzer, Radwag MA 50.R). The pH measurements were carried out with aqueous suspensions of the compost samples (1:6,

w/v, compost/water ratio) using a pH electrode (Cyberscan pH Tutor Meter). Quantitative estimation of culturable aerobic bacteria was determined by inoculating 0.1 ml of different tenfold serial dilutions of compost samples on the nutrient agar plates and incubated at 30 ˚C for 72 h. The number of organisms were expressed as colony forming units (CFU) per gram dry weight of compost. After 12 days of composting, the compost was analysed for its pH, electrical conductivity (mS/cm), total nitrogen (%), phosphate P_2O_5 (%), potassium as K_2O (%), organic carbon (%) and C/N ratio at M/s. National Agro Foundation (NAF) Ltd., Tharamani, Chennai. The prepared compost was evaluated at lab scale (10-15 %) on the growth and yield of chilly plant.

The compost preparation was scaled up to 500 kg level. The keratin hydrolysate of 500 L was mixed with 500 kg of composting material comprising of soil (200 kg), pulverized dry leaves (150 kg), saw dust (100 kg) and wheat bran (50 kg) in 10 batches (50 kg/batch) using ribbon blender mixer (Thirumalai Industries, India). The resulting mixture was transferred to composting pits with following dimensions of 5 X 2.5 X 1.5 m (length X width X height) (Fig. 1) and mixed well at 12 h interval from top to bottom. The moisture content was maintained at 55 ± 2 % by sprinkling water. Temperature of the composting material was measured regularly using a glass thermometer. Samples were collected at 24 h interval and analysed for elemental analysis, pH and CFU/g. The process was continued until a target C/N ratio of less than 20 was attained. Thereafter, the compost was collected and dried. The characteristics of compost were evaluated by NAF for field application studies.

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Fig. 1. Preparation of organic compost at pilot level (500 kg)

2.5. Field application studies

Field application studies were conducted by NAF at Illedu village, Kanchipuram District for paddy crop, bhendi and amaranthus and at Ramanathapuram District for chilly crop using the prepared compost. Randomized block design (RBD) was used for the application of compost ranging from 250 kg to 1000 kg / acre. The compost was sprinkled in the field before transplantation of seedlings. The growth and yield of the crop was assessed and compared with the yield of crops that were grown without compost.

3. Results and discussion

3.1. Degradation of animal hair

The *Brevibacteriumluteolum* MTCC 5982 degraded the animal hair in shake flasks at 72 h with production of 98.85 U/mL of keratinase and 80% degree of hair hydrolysis

(Thankaswamy et al., 2018). The keratinase produced by the bacterium hydrolysed the complex supramolecular organization of keratin. The degradation of hair is primarily due to the cleavageof –S–S– bond in cystine present in keratin to form cysteine by the keratinase enzyme (Fig. 2) (Paul et al., 2016). The disulfide bond in cystine is primarily responsible for the structural integrity of hair.

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Fig. 2. Structural and molecular breakdown of keratin by keratinase

The process of hair degradation was scaled up in 75 L fermentor using the optimized production medium. A maximum keratinase activity of 100 ± 2 U/mL and 81.5 % degree of hydrolysis was obtained at 66 h of fermentation (Fig. 3a). The hair degradation was further scaled up to 750 L fermentor with a working volume of 500 L under the optimum conditions. The keratinase productivity was 1.50 and 1.65 U/ml/h (at 66 h and 60 h of fermentation) using 75 L and 750 L fermentors, respectively. The degree of hydrolysis increased constantly with increase in keratinase production and a maximum of 82% hydrolysis was attained in 750 L fermentor. The profile of dissolved oxygen and pH of the fermentor results are shown in Fig.

3b.During fermentation, the pH increased gradually from 7.0 to 8.4 and 8.9 in 750 and 75 L fermentor, respectively. The tendency towards alkalization of the medium was due to the hydrolysis of hair and accumulation of NH_4^+ ions and the change in pH was an indicator for degradation of keratin (Maruthi et al., 2011).

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Fig. 3. Scale up of hair degradation at 50 L and 500 L level in fermentor. (a) Degree of hydrolysis and keratinase production and (b) Profile of pH and dissolved oxygen during degradation.

Complete solubilization of hair within72 h indicated that the organism utilizes hair as the sole source of carbon and nitrogen with maximum production of keratinase (Williams et al., 1990). The degree of hydrolysis was 82% and the presence of amino acids in the keratin hydrolysate indicated that the integrity of keratin was completely lost in 72 h (Thankaswamy et al., 2018). Thus, the keratin hydrolysate (fermentation broth) could be used as nitrogen source for the preparation of compost.

3.2. Preparation of compost

3.2.1. Physico-chemical properties and microbiological analysis

The changes in pH, temperature, moisture content, total nitrogen, organic carbon and bacterial count during composting process are shown in Table 1. Temperature measurements throughout the composting process provided an adequate real-time indication ofmaintenance of ideal conditions that support microbial degradation (Said-Pullicino et al., 2007). The temperature increased rapidly from 33 to 45±2˚C on second day of the process and turning of the composting materials at 12 h intervals enhanced the aeration and decreased the temperature. The increase in temperature could be due to the utilization of organic matter by the bacterial population as reported by Liu et al. (2011). The temperature gradually increased to 48±2˚C on the 8th day and thereafter decreased to 34±2 ˚C at the end of composting process. The initial pH of the composting matrix was pH 7.6±0.2 and gradually increased to pH of 8.2 as the degradation progressed and remained at 7.4±0.2 on the 14th day. The moisture content was adjusted to 55 ± 2% throughout the process, since microbial activity is limited at lower moisture levels. Moisture loss during the composting process could be considered as an index of decomposition rate because the heat generation leads to vaporization (Liao et al., 1997). Similar trend was observed at pilot level production of compost. The population of total culturable aerobic bacteria was initially $18 \pm 4 \times 10^3$ CFU/g and increased to $205 \pm 10 \times 10^3$ CFU/g at the end of composting.

3.2.2. Characteristics of compost

The compost prepared at 5, 50 and 500 kg levels were analysed at National Agro Foundation (NAF)for elemental analysis and compared with the acceptable limits of city compost. The results are shown in Table 2. Initially, the organic carbon was found to be 45.12 % and 46.52 % in 5 kg and 500 kg level of compost, respectively. It decreased to 23.18 % and 24.80% as the degradation progressed. During the composting process, carbon dioxide was

mainly emitted because of the degradation of complex organic molecules (Guo et al., 2012). The total nitrogen content was 1.22% and 1.29 % in 5 kg and 500 kg level of compost, respectively at the end of composting process. The loss of nitrogen through the formation of ammonia resulted in decrease of total nitrogen contentduring the initial 4 days of composting. The initial C/N ratio of 38.23 and39.42was decreased to 19.23and 19.75at the end of composting (12th day) at 5 and 500 kg level, respectively. The C/N ratio represents the forms of organic carbon and nitrogen in the composting material that are more susceptible to biological degradation and the decrease in ratio indicated the stability of composting process (Said-Pullicino et al., 2007).

3.3. Field application studies

The results on application of compost at laboratory scale significantly enhanced the growth and yield of chilly crops. Further, field studies were conducted to evaluate the influence of compost on growth and yield of paddy, amaranthus, chilly and bhendicrops and the results are shown in Table 3. The RBD used for the application of compost indicated that use of 500 kg/acre of compost along with required dose of fertilizer significantly increased the growth and yield of the crops compared to the control fields, which were without the compost. The increase in yield of various crops were 20.0, 34.5, 31.0 and 19.5 % with paddy, amaranthus, bhendi and chilly crops, respectively (Table 3). This increase could be due to the addition of fermentation liquor containing the bacterium and the protein rich keratin hydrolysate in the composting materials, which may accelerate the degradation of organic matter along with the soil microflora in attaining the ideal C/N ratio of the product. Moreover, the addition of compost to the soil could increase the water holding capacity and other mineral content of the soil as reported by Tamreihao et al. (2019) using feather hydrolysate. The enhanced yield of crops would lead to the increase in profit for the farmers thereby leading to the improvement in their livelihood.

4. Conclusion

In the present study, the degradation of animal hair waste using keratinase producing *Brevibacteriumluteolum* MTCC 5982 at pilot scale in 500 L fermentor was standardized. The fermentation liquor containing the bacterium and the keratin hydrolysate is utilized for compost preparation without discharging any waste to the environment. The addition of compost enhances the yield of crops and reduces the consumption of the chemical fertilizer to about one-third. The process provides a sustainable solution for the disposal of hair waste of the tanning industries and benefit the agricultural industry by reducing the import and ensure financial returns, thereby stands alone as an exemplary model for circular economy.

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Table 1

Profile of different parameters during composting process at pilot level (500 kg)

Parameters	Days							
	Initial	2	4	6	8	10	12	14
pH	7.6±0.2	7.9±0.2	8.0±0.2	8.2±0.2	7.8±0.2	7.6±0.2	7.6±0.2	7.4±0.2
Temperature (^{o}C)	33±2	45±2	47±2	47±2	48±2	41±2	35±2	34±2
Moisture (%, w/w)	55±2	56±2	55±2	55±2	54±2	54±2	55±2	55±2
CFU/g(× 10^{5})	18±4	47±7	115±10	162±8	190±10	198±12	209 ±10	205±10
Organic carbon (%)	46.52	41.91	35.55	31.60	31.09	28.08	25.49	24.80
Total nitrogen (%)	1.18	1.10	1.07	1.19	1.22	1.25	1.29	1.29
C/N ratio	39.42	38.10	33.22	26.55	25.49	22.46	19.75	19.22

Table 2:

Analysis of compost prepared at 5, 50 and 500 kg level

Parameters	Fertilizer control (Acceptable limit for city compost)	5 kg	50 kg	500 kg
pH	6.5-7.5	7.17	7.46	7.3
Conductivity (mS/cm)	Less than 4.0	1.46	1.51	1.67
Moisture (%)	15.0-25.0	15.80	17.36	16.21
Total nitrogen (%)	Minimum 0.8	1.22	1.25	1.29
Total phosphate P_2O_5 (%)	Minimum 0.4	0.21	0.20	0.23
Potassium as K_2O (%)	Minimum 0.4	1.94	2.45	2.38
Organic carbon (%)	Minimum 12.0	23.18	25.72	24.80
C/N ratio	<20	19.0	20.6	19.22

Table 3:

Effect of compost on crop yield

Crops	Yield in kg. per acre		Increase in yield (%)
	Control	With compost	
Paddy	2625 ± 55	3150 ± 50	20.0 ± 3.0
Amaranthus	7045 ± 150	9473 ± 120	34.5 ± 3.5
Bhendi	5406 ± 95	7082 ± 75	31.0 ± 3.0
Chilly	517 ± 20	618 ± 20	19.5 ± 4.0

Figure legends

Fig. 1. Preparation of organic compost at pilot level (500 kg)

Fig. 2. Structural and molecular breakdown of keratin by keratinase

Fig.3. Scale up of hair degradation at 50 L and 500 L level in fermentor. (a) Degree of hydrolysis and keratinase production and (b) Profile of pH and dissolved oxygen during degradation