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ORIGINAL RESEARCH

The study of plant growth promoter production from leather industrial solid waste

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Abstract

Purpose The leather industry produces huge quantities of proteinous solid wastes and is being dumped or improperly utilized resulting in economical losses or causing an environmental problem. An efficient, low-cost procedure was developed to convert animal fleshings into plant growth promoters (PGP).

Method The fleshings were hydrolyzed and analyzed for moisture, fat, ash, and nitrogen contents. The protein hydrolysate is incorporated with phosphorous and potassium in a suitable method to convert into plant growth promoters. The obtained product is characterized and field application studies have been conducted with ladyfinger plants (*Abelmoschus esculentus*).

Results The detailed characterization of PGPs through physicochemical analyses gave a deeper understanding of microstructures. Spectroscopic features and FT-IR studies confirmed the potential of PGP. The bio-metric results have shown that the application of plant growth promoter yielded better results and chemical characteristics.

Conclusion Hence this study provided a viable solution for the conversion of proteinous solid wastes into plant growth promoters.

Keywords Protenious waste, Leather, Nitrogen, Phosphorous, Ladies finger, Biometric analysis

Introduction

Various kinds of liquid, solid and gaseous emissions generated during leather processing pose a major challenge to the environment. Solid wastes create a major problem for the leather industry in terms of both their composition and quantity. A high amount of reusable waste is generated in the leather industry. It is possible to recycle these products and even use them as raw materials for different industries. The leather industry generates a large quantity of solid wastes based on proteins, fatty matters, and salts which are difficult to process due to the high content of water (50-80%). Out of 1000 kg of rawhide, nearly 800 kg is generated as solid wastes in leather processing and only 150 kg of the raw material is converted into the leather (Francisco et al. 2011). At an average consumption of 45-50 m^3 of the waste liquor and 800 kg of solid wastes per ton of raw-

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hide are discharged by the leather industry (Pati et al. 2014; Sundar et al. 2011a).

During the leather processing, the skins and hides are plumped up by alkalis and the adipose layer which is the lowest layer of the skin together with the underlying fatty tissue is scraped off and disposed of as waste. There is a lot of research work has been carried out to utilize the fleshing wastes (Kolomaznik et al. 2008; Kumar et al. 2010; Ozgunay et al. 2007). Fleshings find use in the manufacture of glue, gelatin, chicken feed supplement, plastics, amino acids, artificial sausage casings, surface-active agents, artificial leather, raw material for fungicides and bactericides, and dog chew (Sundar et al. 2011b). Of those mentioned, the use of animal feed supplements has received the most attention since it offers a potential for large-scale utilization. The possible use of fleshing fat in the production of biodiesel (fatty acid methyl esters) and fleshing hydrolysate by an alkali digestion method was investigated. Both deemed fleshings and residual hair might well constitute an important source of protein with interesting uses as biological fertilizers in agriculture or horticulture (Nustorova et al. 2006; Gousterova et al. 2008; Ranjit and Jyoti 2013). But still only lesser amounts of fleshings are consumed as fertilizer because of a few shortcomings which are addressed in the present research work (Ashmead and Hsu 1985; Ashmead et al. 1986; Davidson et al. 2013).

Recent climate models predict that incidences and duration of drought and heat stress periods are increasing in many regions, negatively affecting our major crops, and thus food security. Therefore, major challenges for agriculture are to enhance crop yields in more resource-efficient systems and to stabilize plant development and yield formation under biotic and abiotic stress conditions (El-Samad et al. 2011). In this context, among the many plant nutrients, nitrogen, phosphorus, and potassium (NPK) play a crucial role in many physiological processes vital to the growth, yield, quality, and stress resistance of all crops.

Nitrogen (N) is the most vital nutrient for plant growth and productivity. Although there is about 78% N_2 in the atmosphere, it is unavailable to the growing plants. The atmospheric N_2 is converted into plant-utilizable forms by biological N_2 fixation (BNF) which changes nitrogen to ammonia by nitrogen-fixing microorganisms using a complex enzyme system known as nitrogenase (Kim and Rees 1994; Sudadi 2012). Even though Potassium **(**K) constitutes about 2.1–2.3% of the earth's crust and eighth most abundant element, large agricultural areas of the world are reported to be deficient in K availability, including ¾ of the paddy soils of China, and 2/3 of the wheat belt of Southern Australia (Wedepohl 1995; Mengel and Kirkby 2001). Additionally, with intensive agricultural production systems, K becomes a limiting element, in particular in coarse-textured or organic soils (Goulding and Loveland 1986). Lower Potassium fertilizer application in the context of unbalanced fertilization may result in a significant depletion of available soil K reserves, and thus in decreased soil fertility. Phosphorus (P), the second important plant growth-limiting nutrient after nitrogen, despite large reservoir in the soil, the number of available forms to plants is generally low (Khan et al. 2009). To overcome the P deficiency in soils, there are frequent applications of phosphatic fertilizers in agricultural fields. Plants absorb fewer amounts of applied phosphatic fertilizers and the rest is rapidly converted into insoluble complexes in the soil. But the regular application of phosphate fertilizers is not only costly but is also environmentally undesirable. This has led to a search for an ecologically safe and economically reasonable option for improving crop production in low P soils.

Hence a need arose to find alternative advantageous scientific solutions (Sirbu et al. 2008; Lima et al. 2010;

Giacometti et al. 2011). So, in the present research work it has been studied in detail to utilize the leather industry solid wastes to produce a value-added product viz., plant growth promoter (Ravindran et al. 2014; 2015). Nitrogen (N), phosphorous (P), and potassium (K) are the most important macronutrients required for the growth and development of plants (Salminen et al. 2001; Nogueira et al. 2009; Robertson and Vitousek 2009; Römheld and Kirkby 2010).

Materials and methods

Materials

Sheepskin fleshings from a commercial leather processing industry, commercial-grade KOH, and orthophosphoric acid were employed for the study.

Hydrolyzation and enrichment with P and K

The alkaline animal fleshings are treated with NH_4Cl (3-5% *w/v*) to remove sulfide and calcium salts used during depilation of hair and fiber opening of skins. Then fleshings are washed thoroughly with water to get rid of all alkalis and impurities until the pH reaches 8.0. The fleshing is characterized for pH, moisture content (SLC 5 1996), total solids, fat content (SLC 4 1996), total Kjeldahl nitrogen (TKN), protein (Enechi and Emilia 2013), and ash content (Vogel 1989). Then the fleshings are minced to 2 mm size and hydrolyzed using 1% KOH and the hydrolyzed mixture was kept in the oven for 12 hours at 90^{o}C. The resultant hydrolyzed solution's alkalinity (pH 13) was neutralized with a mixture of phosphoric acid and water (1:3). Thus, the required plant nutrients, phosphorus, and potassium were incorporated with protein hydrolysate which is rich in nitrogen content.

Stabilization of PGP

Then the hydrolyzed and neutralized proteinous solution is blended with shredded dried agricultural residues of almond leaves, sand, and inert china clay to bring stability to the formulation in various compositions (Table 1). Almond leaves maintain pH levels and sand and inert china clay is employed for concentration and stability of PGP during its storage which does not interfere with other PGP characteristics. The semi-dried mixture is fed into a mincer to obtain pellets and dried at ambient temperature. The obtained plant growth promoter composition is characterized and field studies are undertaken.

Table 1 PGP formulations

Composition	Hydrolysed solution (Parts)	Almond leaves powder (Parts)	China clay (Parts)	Sand (Parts)
PGP – 1	5	2	1	1
PGP – 2	5	1	2	1
PGP – 3	5	1	1	1

Physico – chemical analysis of PGP

The plant growth promoter was analyzed for pH, TKN, Total Organic Carbon (Vogel 1989), Total Calcium and magnesium, and total phosphorus (SLC 3 1996; AOAC 2000) in triplicate according to official methods and the results were averaged. The pH and electrical conductivity were determined using distilled water

suspension of the PGP mixture in the ratio of 1:10 (*w/v*) that had been agitated mechanically for 30 min and filtered. Ash content and total solids were carried out according to standard methods (SLC 6 1996). Micro-Kjeldahl method is used for TKN estimation and Total Calcium and magnesium were done by Triacid extract method. Total phosphorus was measured colorimetrically with molybdenum in sulfuric acid using Varian-Cary 100 UV-visible spectrophotometer. Total potassium was determined, after digesting the sample in a diacid mixture (conc HNO_3: conc $HClO_4$, 4:1, *v/v*), by flame photometer.

FT-IR spectroscopy of PGP

One gram of high yielding sample of PGP III was ground well along with KBr and a pellet was prepared for FT-IR analysis. Fourier transform infrared spectroscopy (FT-IR) analyses were performed on FT/IR-4700 type A in the absorbance mode ranging the wavenumber from 4000 to 600 cm^{-1}.

Differential scanning calorimetry (DSC) of PGP

Differential Scanning Calorimetry (DSC) analyses are conducted in Netzsch DSC 200 PC Differential Scanning Calorimeter. Samples were placed in an aluminum crucible and heated at a heating rate of 10 ^oC min^{-1} from 25 to 400 ^oC under an inert nitrogen atmosphere flowing at 40 mL min^{-1}.

Experimental design and procedures

The three compositions of PGP pellets were applied once to the soil before planting fresh ladies' finger seeds according to the quantities mentioned in Table 4. The seeds were sowed 8-9 inches apart and 2 inches deep and the germination was observed on the sixth day and a detailed study is undertaken for its growth parameters.

Biometric observations of plants

As ladies' finger matures between 45-60 days the biometric observations were done between these days by randomly selecting plants in the net plot area of individual treatment. The growth attributes of plant height and girth, number of leaves, and number and size of the pods were recorded as per the standard procedures and the mean values obtained were expressed as per the SI system of units.The height of the plant from the ground level to the tip of the main stem was measured on 45^{th} and 60^{th} days and expressed in centimeters. Yield attributes like number of leaves, pod length, individual pod weight, number of pods per plant, and pod yield were recorded as per the standard procedures and the mean values obtained were expressed as per the SI system of units (Starling et al. 1998). The length and breadth of the pod were measured at random which were recorded from the calyx end to the tip from all treatments and the mean was expressed in cm. The mean weight of all pods per plant was recorded and expressed in grams.

Analysis of plant samples

The plant samples were collected at the post-harvest stage and analyzed for the nutrient content as per the methods prescribed. The characterization of plant samples such as nitrogen content, organic carbon content, total calcium, and total magnesium content was done (Fleck and Munro 1965).

Results and discussion

The proteinous wastes of the leather processing industry are characterized and converted as plant growth promoter (PGP) formulation and evaluated for their efficacy. The action of PGP from fleshings is similar to that of organic manure and it is a slow nitrogen release

agent since the final protein breakdown is accomplished with the aid of soil microorganisms. In this context, plant growth promoters (PGP) are considered promising biofertilizers, may provide the available forms of NPK to the plants, and hence a viable substitute to chemical phosphatic fertilizers where the availability is insufficient (Khan and Zaidi 2006).

Characterization of proteinous fleshing and hydrolysed solution

Table 2 describes the main chemical characteristics of proteinous fleshings and hydrolyzed solution which was carried out on a dry weight basis.

The yield of proteinous fleshings to the hydrolyzed solution was found to be 80-88%. The collagen was shown to be a good alternative for synthetic nitrogen sources for the growth of plants with its sufficient quantity of nitrogen content of the hydrolyzed solution.

Table 2 Characteristics of proteinous fleshings and hydrolysed solution

Parameters	Proteinous fleshings	Hydrolyzed solution
pH	8±0.3	13±0.3
Moisture content	85±3%	75±2%
Total solids	25±1%	13±1%
Fat Content	5±0.5%	2±0.2%
Ash Content	17±3%	2±0.1%
Total Nitrogen	15±0.5%	12±1%
Total Protein	78±1%	67±1%

Characterization of plant growth promoter

Plant growth promoters (I, II and III) were characterized for their chemical contents and shown in Table 3.

Table 3 Characteristics of plant growth promoter

Composition	**pH**	**N (%)**	**C (%)**	**H (%)**	**S (%)**	**C/N ratio**	**C/H ratio**	**TOC (%)**	**Ca (%)**
PGP - I	7.1±0.2	3±0.1	3±0.1	9.3±1	0.65±0.01	1	0.3	10.5±1	0.8±0.01
PGP – II	7.3±0.2	4±0.1	3.6±0.1	10±1	0.62±0.01	0.9	0.4	10±1	0.8±0.01
PGP – III	6.5±0.2	3±0.1	7.9±0.1	9±1	0.3±0.01	2.4	0.9	9.7±1	0.74±0.01

Spectroscopic features of PGP

The FT-IR spectra of PGP III are shown in Fig. 1. The characteristic bands found in the infrared spectra of proteins mainly include the Amide I and Amide II bands. The bending vibration of the NH_2 group appears as a shoulder at 1630 cm^{-1} in the FT-IR spectrum. The NH stretching vibration gives rise to the amide bands at 3390 and 3465 cm^{-1}. The amide I vibration, absorbing near 1652 cm^{-1}, arises mainly from the C═O stretching vibration with minor contributions from the out-of-phase CN stretching vibration and the NH in-plane bend. A band at 1510 cm^{-1} observed in the IR spectrum may be assigned to the stretching vibration of the aromatic ring and NH bending motion in Try and Trp. The sharp bands at 2882 cm^{-1}, 2989 cm^{-1}, 2890 cm^{-1} indicate the possible presence of C-H stretching. A medium band in the IR spectrum at 1029 cm^{-1} can also be attributed to the ρ (NH_2) and C-O stretching vibration. The bands at 1635 and 1480 cm^{-1} correspond to the

stretching of aromatic C=C (Nakanishi and Solomon 1977). Consequently, the peak at 1635 cm^{-1} might indicate the presence of aromatic rings in anthocyanins (Merlin et al. 1994).

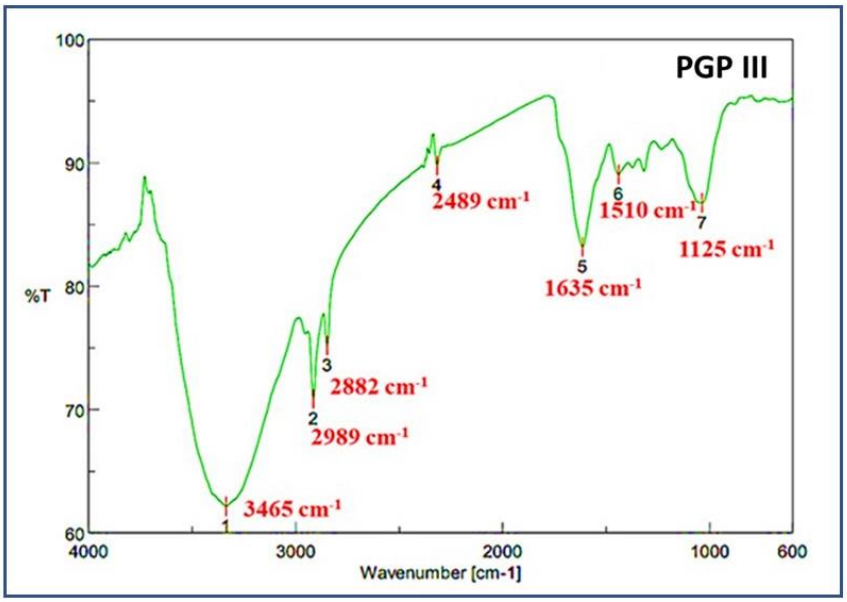

Fig. 1 FT-IR Spectra of PGP III

Differential scanning calorimetry analysis

Differential Scanning Calorimetry (DSC) revealed thermal behavior of the samples of PGP III which were resulted in better yields. A typical DSC trace is shown in Fig. 2.

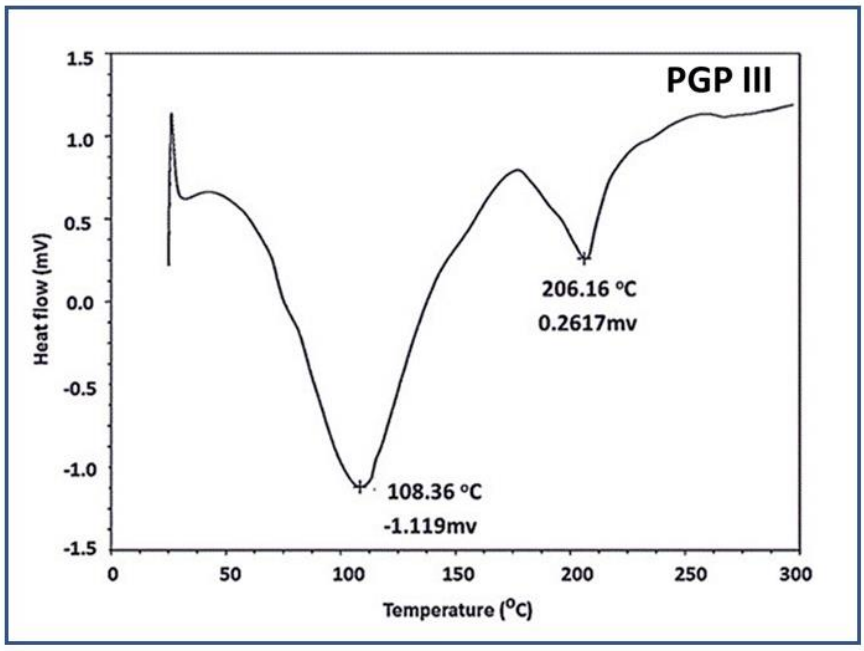

Fig. 2 DSC curve of PGP III

It is possible to note that in the range between 108 ºC and 206 ºC there are only endothermic events. It was seen that decomposition starts at 108° C and at that step absorbed water was evaporated. PGP containing protein with phosphorous and potassium exhibited peak temperature at 206 ºC. The studies on synthetic fertilizers also confirmed similar thermal behavior (Gorbovskiy et al. 2016).

Influence of plant growth promoter on growth attributes

Nitrogen in plant growth promoter is in the organic form and it is naturally released in soil and made available for plant uptake after mineralization processes of the organic matter (Sirbu et al. 2009). Consequently, N is slowly released in soil following environmental conditions. Soil temperature and humidity and redox potential are the main factors that affect the mineralization process (Ciavatta et al. 2012). In this research plant growth and yield attributes, nutrient contents, and shoot length parameters were investigated to confirm the effects of PGP.

The results of plant height, plant girth at 45th and 60th days are presented in Tables 4 and 5 of different compositions and quantities of PGP. From the results, it was observed that 45th day the highest plant heights were 45 cm, and the plant girth where 3.2 cm was recorded for the application of 0.50% PGP III which was significantly higher when compared to all other treatments.

Table 4 The biometric values (on 45th day) of the plant in different concentrations (0.25%, 0.5% and 0.75%) of plant growth promoter

Application Dose	Plant height (cm)	No. of leaves	Girth (cm)	No. of pods	Average length of Pods (cm)	Average breadth of Pods (cm)	Fresh Pod weight (gm)
Control	30	8	2.8	2	6	3	7
PGP I 0.25%	32.5	7	2.5	2	6.5	2.5	9.6
PGP I 0.50%	30.0	8	2.7	3	6.5	3.5	9.7
PGP I 0.75%	34.0	7	2.9	2	7.5	3.5	10.2
PGP II 0.25%	38.0	10	3.0	3	7.0	4.0	9.5
PGP II 0.50%	40.5	8	2.9	2	7.5	4.0	13
PGP II 0.75%	41.0	8	3.0	3	8.5	4.5	11.5
PGP III 0.25%	42.0	10	3.2	3	8.5	5.5	10.5
PGP III 0.50%	45.0	11	3.2	3	11.5	6.0	11.0
PGP III 0.75%	44.0	10	3.1	3	11.5	5.5	11.5

On the 60th day, the highest plant height (51 cm) and plant girth (3.5 cm) was recorded due to the application of 0.50% PGP II which was significantly higher when compared to all other treatments. The yield with all the compositions was ranged between 5.6 cm and 11.5 cm. Among the treatments, the maximum pod length (14.5 cm) was obtained with 0.50% PGP III which was recorded highest yield and the lowest pod length (6.2 cm) was observed with control where PGP is not employed. On the 60th day, the maximum pod breadth (6.6 cm)

was obtained with 0.50% PGP III and was recorded as the highest yield. The lowest pod breadth (3.3 cm) was recorded with control.

The results show that the composition and quantity of PGP III are found to be easily available to the soil to assimilate and nutrients were absorbed by the plants.

Table 5 The biometric values (on 60th day) of the plant in different concentrations (0.25%, 0.5% and 0.75%) of plant growth promoter

Application Dose	Plant height (cm)	No. of leaves	Girth (cm)	No. of pods	Average length of pods (cm)	Average breadth of pods (cm)	Fresh Pod weight (g)
Control	35	8	2.8	2	6.2	3.3	9
PGP I 0.25%	41	9	3	3	8.0	5.6	11.4
PGP I 0.50%	38	10	3.2	3	10.0	6.2	11.9
PGP I 0.75%	42	11	3.0	3	11.0	5.8	12.0
PGP II 0.25%	43	12	3.1	3	11.0	6.13	11.7
PGP II 0.50%	45	12	3.2	3	12.0	6.4	12.0
PGP II 0.75%	43	12	3.3	4	13.0	5.8	13.0
PGP III 0.25%	48	12	3.4	4	13.9	6.25	13.0
PGP III 0.50%	51	13	3.5	4	14.5	6.6	14.0
PGP III 0.75%	48	13	3.5	4	14.0	6.5	15.2

The yield was ranged between 9 to 14 gm per pod. Among the treatments highest weight (15.2 gm/gm) was observed with 0.75% PGP III followed by 0.50% PGP III (14 gm/gm) and 0.75% PGP III (13 gm/gm). The lowest pod weight (9 gm/gm) was observed with the control.

Effect of plant growth promoter on nutrient contents

Different doses of application of plant growth promoter influenced the essential nutrient uptake (N, OC, Ca, and Mg) in ladies' finger pot culture experiments (Khan et al. 2009; Zaidi et al. 2009) (Table 6). Nitrogen content in ladies' finger pot culture ranged between 0.9 and 1.5%. The maximum nitrogen uptake of ladies' fingers in 0.50% of PGP III (1.5%) followed by 0.75% of PGP III (1.4%). The calcium content of ladies' finger pot culture ranged from 100 to 120 mg/kg. The maximum calcium uptake in 0.50% of PGP III (120 mg/kg) followed by 0.75 % PGP I (115 mg/kg) and 0.75% of PGP III (114 mg/kg). The magnesium content in plants is below the detectable limit. The maximum organic carbon content of 5.5% was observed with 0.75% PGP III and the lowest content in the range is 4% was observed with control.

This research study showed that PGP-assisted planting gave good results in terms of plant height, number of leaves and pods, girth, length and breadth of pods, pod weight, and nitrogen in the plant (%). Furthermore, PGP III represents an economically beneficial and environmentally sound alternative to chemical fertilizers.

Table 6 Chemical characteristics of plant in different concentrations (0.25%, 0.5% and 0.75%) of plant growth promoter

Application Dose	Nitrogen %	Organic carbon %	Phosphorus %	Ca (mg/kg)
Control	0.9±0.02	4.0±0.1	0.2±0.001	90±3
PGP I 0.25 %	1.1±0.1	5.3±0.2	0.2±0.001	100±3
PGP I 0.50 %	1.2±0.1	5.3±0.2	0.3±0.001	111±3
PGP I 0.75 %	1.3±0.1	5.4±0.2	0.3±0.001	115±3
PGP II 0.25 %	1.1±0.1	4.3±0.2	0.2±0.001	100±3
PGP II 0.50 %	1.1±0.1	4.7±0.2	0.19±0.001	105±3
PGP II 0.75 %	1.0±0.1	5.0±0.2	0.2±0.001	110±3
PGP III 0.25 %	1.2±0.1	5.0±0.2	0.2±0.001	110±3
PGP III 0.50 %	1.5±0.1	5.2±0.2	0.3±0.001	120±3
PGP III 0.75 %	1.4±0.1	5.5±0.2	0.4±0.001	114±3

Conclusion

There is an urgent need to find value-added products from proteinous fleshing wastes because they are dumped or used to manufacture undervalued products. Hence in this study, the animal fleshings are converted into plant growth promoters and its effectivity was evaluated on plants and found to replace the existing synthetic fertilizers. The application of PK enriched-protein formulation resulted in N, P, and K contents in the vegetative parts of ladies' finger plants equivalent or superior to those obtained with commercial NPK formulations. The application of protein-based formulations, as a nutrient source for ladies' finger plants, showed promising agronomic results. Utilizing the tannery biowaste is not only managing the hazard but also making wealth out of waste.

Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest associated with this study.

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