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Efficacy of Poultry Feather Decomposition by Native Isolates of *Bacillus* Sp. And it's Impact on Mineralization.

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ABSTRACT

The study was conducted in preparation of feather compost, characterization and effect of feather compost in the fields. During the study period *Bacillus subtilis* was used to degrade the feather material. Initially, decomposed soil samples were collected in the poultry feather dumping places. *Bacillus subtilis* was isolated as best native species and used to degrade the feather material. The degradation of chicken feathers was observed through morphological level changes. During the feather degradation for temperature were recorded on 20th day (42.1°C) and decreased trend was observed up to 40th day (25°C) respectively. The high moisture content on 15th day (41%) and decreased moisture content in feather compost in 40 day (29%). The maximum available nutrient content was estimated at after 40 days of inoculation of FDS 15 such as 88mg, 9.10mg and 72.0 mg per kg of N, P and K respectively under sand 2 kg + 30 % of cleaned feather (T1). The decomposition of feather was faster in the treatment which inoculated FDS 15 except control which might be due to drastic multiplication FDS15 bacteria. The minimum available nutrient of 19mg, 5.85mg and 56.5mg in N, P and K respectively recorded under control (sand alone). The results of mineralization estimate showed that FDS15 *Bacillus subtilis* was increase the decomposition process of feather even increase the quantity of substrate (feather) which leads to convert the higher content of nutrients in the soil. Generally, the pH and EC of the substrate were on par with treatments. It indicates that the inclusion of the organisms in poultry waste compost was successful in producing an odourless, pathogen free product with complete biological degradation waste in 40 days of the feather. The application of this system may improve the rapid disposal of poultry waste as well as the public and environmental health in the region.

Keywords: Poultry feathers, Degradation, *Bacillus subtilis*, Mineralization, pot culture experiments

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INTRODUCTION

Biodegradation of poultry processing waste is an alternative avenue for creating a viable end product with visible benefits to the primary producers in environmental and economic management strategies (Kim and Patterson, 2000). At present, the poultry industry manages feather waste by either steam or chemical treatment to produce feather meal (Valtcho and Zheljzakov, 2005) and either untreated or treated forms of feathers used as fertilizer or disposed as landfill (Pettett and Kurtböke 2004). Feathers are rich with pure keratin protein (Moran *et al.*, 1966). Investigations into feather degradation began in 1950s on alpha keratin using keratinolytic *Bacillus* sp. and *Microsporium canis*)

The growing concern for environmental production coupled with the urgent search for potentially useful materials has led to the development of a number of technologies for the bioconversion of keratinous waste into recoverable products (Shih, 1993; Matsumoto, 1996). The disposal of large quantities of keratinous wastes from leather industries, agricultural industry or from a slaughterhouse by dumping piles of unusable animal hides in a natural biological zone may lead to a severe environmental impact.

Biodegradation of intensively farmed animal waste is now viewed as an alternative avenue for creating a viable end product with visible benefits to the primary producers in environmental and economic management strategies (Kim and Patterson, 2000). A combination of specific waste products and process producers via biological methods would lead not only to an improved consumable product, but also to consumer confidence in waste management practices considering their high content of useful compounds, animal wastes could have a great potential for many applications. For this purpose, the destruction of the rigid keratin structure is necessary. Degradation of keratin wastes a material is usually achieved by thermal hydrolysis in dilute acid or base, or enzymatic digestion by specific keratinases (Jou *et al.*, 1999). Disposal pits and trench burial incineration are also common methods used for disposal of disease mortality. In each of these processes, however, the outcome has limitations with respect to quality, cost efficiency as well as environmental management (Kim and Patterson, 2000). The hair, feather and sheep wool contain approximately 90% keratin and use in composting. It is one of the more economical and environmentally safest methods of recycling the feather (Ichida *et al.*, 2001).

Actinomycetes are widely distributed in nature and have a major role in the degradation of organic matters. Investigation of feather degradation began in the 1950s on alpha keratin using keratinolytic *Bacillus* sp. and *Microsporium canis* (Daniels, 1953) and also actinomycetes reported to break down feathers such as *Streptomyces pactum* and *Streptomyces albidoflavus* (Ignatova *et al.*, 1999; Bressollier *et al.*, 1999). Therefore, the present study is aimed to develop an ecofriendly process of feather composting using indigenous actinomycetes to replace the currently used environmentally hazardous ones.

Furthermore, human pathogenic organisms are known residents of poultry farms. For example, (Craven *et al.*, 2001) concluded that the populations of *Clostridium perfringens* followed throughout the production and processing of the waste were at levels of 94% in the bird faecal and caecal samples and 53% on wall swabs and 29% on the workers' boots. (Mallinson and Snoeyenbos, 1989) also showed that the untreated poultry waste provided an ideal environment for pathogenic species such as *Vibrio* and *Salmonella* to thrive. In addition, as a result of the continuous use of the glycolpeptide antibiotics as a feed supplement, resistant enterococci and salmonella organisms compete with normal microflora constituting a significant human health risk (Collignon, 1999).

Actinomycetes were also reported to break down feathers such as *Streptomyces pactum* and *Streptomyces albidoflavus* isolated from soil showed a broad range of enzymatic activity (Ignatova *et al.*, 1999; Bressollier *et al.*, 1999). The protein rich concentrates feather meal generated for poultry feed can also be applied for organic farming as a semi slow-released fertilizer (Safranek and Goos, 1982). Even though number of bacteria is identified for decomposing of poultry feather still lack of suitable and fast multiplying bacteria for the same. So, the current research is taken to isolate native bacteria for decomposing of feather and its influence on mineralization and plant growth under in vitro condition.

MATERIALS AND METHODS

Preparation of Sand Feather composts

Chicken feather was washed with tap water and detergent. After making them fat free, the solvent was evaporated, washed three to four times with distilled water and air-dried. In the preparation of feather 5%, 10%, 20%, 30% and commonly each sand 2 kg taken and autoclaved at 121° C for 15 min. Then, it was placed mixed uniformly in a plastic bin. The preparation was then inoculated with 10% *Bacillus subtilis* culture (10^8 cfu/ml) and separated control measured. The feathers were kept for degradation for 40 days, respectively.

The plastic bin was covered with polythene bag use to avoid contamination and was moisture maintained with sterilized water at 30 to 40%. The combination of poultry feather, sand and inoculation with native isolates bacteria such as 5% feathers + sand 2kg + 10 % culture (T4) , 10% feathers + sand 2kg + 10% culture (T3), 20% feathers + sand 2kg + 10% culture (T2), 30% feathers + sand 2kg +10% culture (T1) and each combination the control also studied without culture.

Determination of temperature and moisture

In the plastic bin of feather sand compost sample was taken and recorded the temperature and moisture content at 10 days intervals. The temperature was continuously monitored by a Salmoiraghi Co. thermometer. Process temperature was determined every 10 days intervals by inserting the thermometer 20 cm deep in three different locations into the decomposition of feather materials in the plastic bin. The moisture content was also expressed based on wet weight, which gives the percentage of original wet weight sample containing water (Bressollier *et al.*, 1999). Initial weight of the compost was measured and final weight of the compost after drying is also determined, there by water loss can be calculated, which indicates the original moisture content of the compost.

Determination of pH and determination of total nutritional composition of sand feather compost

The samples were also determined the pH, electrical conductivity, Nitrogen, Phosphorus and Potassium present in the decomposition of feather sand were analyzed with the help of Tamil Nadu Agricultural University, KVK (Krishi Vigyan Kendra), Vriddhachalam, Cuddalore Dt, Tamil Nadu.

In vitro experiment on plant growth

The soil samples were prepared as that mineralization of feather. The same could be filled in five pots. The groundnut crop variety VRI 2 was chosen for pot culture experiment. Seven seeds were sown per pot including control pot, where no compost was supplemented. The pots were watered every day to keep water-holding capacity of soil up to 60%. To assess the effectiveness of sand feather compost in pot experiments were carried out in triplicate.

RESULTS AND DISCUSSION

Preparation of Sand Feather compost

During composting process of keratin materials were observed for morphological changes occurred due to the colonization of bacillus subtilis feather and depicted in respectively (Fig- 1). The final sand feather compost obtained after degradation was represented in a plastic bin (Fig-2). The Damage to feathers, including delaminating of the rachis was evident on 10th day and *Bacillus subtilis* was tightly associated with the feather in the inoculated plastic bin.

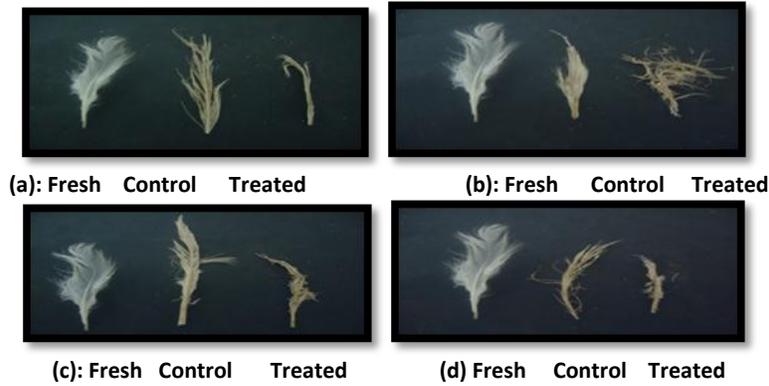


Figure-1: Feather observation: (image)

a) Sand+feather 5% + culture 10%, b) Sand+feather10%+ culture 10%
 c) Sand+feather20%+ culture 10% c) Sand+feather30%+ culture 10%

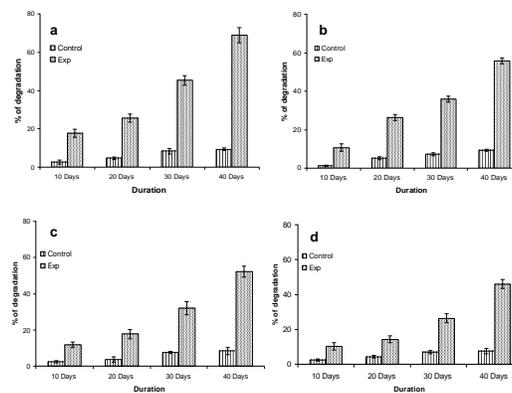


Fig-3, Degradation of feather compost

a) Sand+feather 5% + culture 10%, b) Sand+feather10%+ culture 10%
 c) Sand+feather20%+ culture 10%, d) Sand+feather30%+ culture 10%

Fig-1: Feather Decomposition Moisture 30 to 40%, 10 ml culture inoculated, sterilized sand and sterilized feather used.

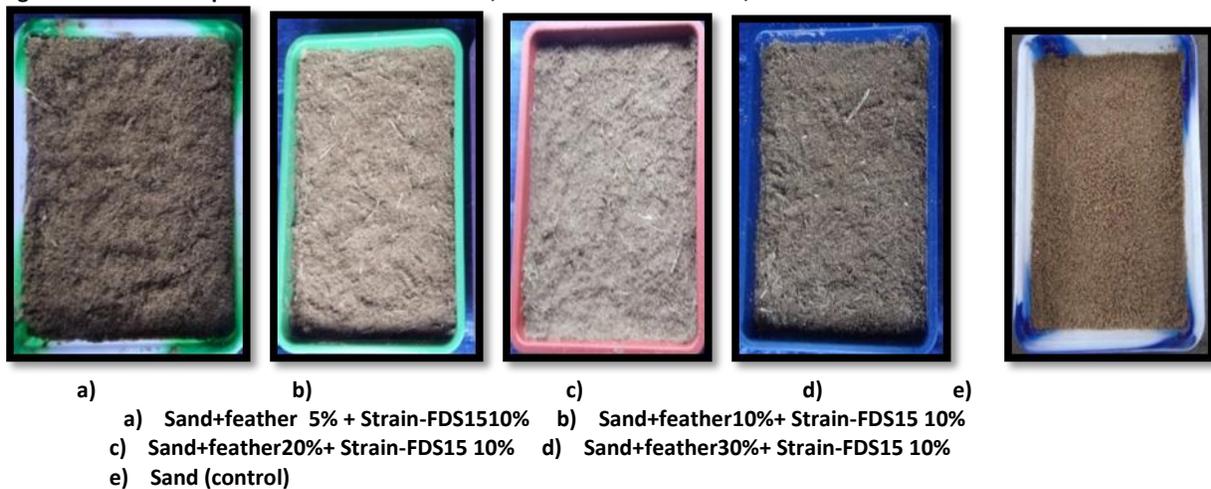
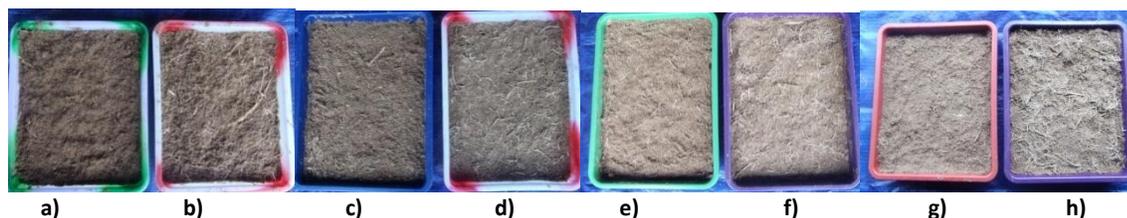




Figure- 4, In vitro experiment on groundnut plant growth

T1-30% feather compost, T2-20% feather compost T3-10% feather compost, T4-5% feather compost T5-control

Feather Decomposition Moisture 30 to 40%, 10 ml culture inoculated, sterilized sand and sterilized feather used



- a) Sand 2kg , feather 5% and Strain-FDS15
- b) control
- c) Sand 2kg + feather 10% and Strain -FDS15
- d) control
- e) Sand 2kg + feather 20% and Strain-FDS15
- f) control
- g) Sand 2kg + feather 30% and Strain-FDS15
- h) control

Bioreactors of compost materials of straw, chicken feathers and poultry litter will, when inoculated with active feather degrading bacteria, enhances keratin utilization (Ichidia., *et al* ,2001). Composting of residual feather seems to require the presence of a co-substrate for composting and nitrogen conservation. Recent works have been published on the biodegradation of animal wastes using specific microbial populations (Gushterova., *et al* , 2005); Tiquia., *et al* , 2005) obtained 50% carbon conversion when composting the wastes from the poultry industry with high nitrogen content. This indicated high biodegradability of protein of animal origin under composting conditions. *Bacillus subtilis* gradually eroded feather surface indicating that the keratin molecule was being digested and shows the growth of the feather unit. The effective degradation for sand, feather compost of interval duration 10th, 20th, 30th and 40th days (Fig- 3) However, in the present work specific Bacteria used in enhancing the keratin degradation and it was very effective.

Change in Temperature:

A wide range of temperature was found to be essential during the process of feather compost were carefully monitored and change in temperature was noticed as shown as respectively. In feather compost, there was a peak attainable in composting temperature of about 39° C on 10th day, remains stabilized until 20th day and there is a quick drop in temperature on 30th day of about 35.8°C as available carbon was utilized. This peak temperature remained/stabilized up to 10th day and for 33 day there was a quick drop in temperature indicating the depleting level of organic matters and enzyme production by *Bacillus subtilis*. This gradual decrease in temperature continued and on 40th day the final temperature recorded in the compost was 25.5° C. The nature and population size of microorganism in feather composting depend on a number of factors, one of which is temperature. Similar variations in temperature levels were found during keratin degrading experiments, carried out in composting piles for the development of environmental friendly bio-fertilizer (Lyndall *et al.*, 2004). The

composting process is usually carried out within the thermophilic range of temperature permitting the disinfection of the final product (Salter and Cuyler, 2003)

pH:

The final pH values of the feather sand composts were between 6.90 and 7.85. The pH value in feather compost was decreased slightly in the neutral range during the processing period. Initial pH value in feather compost was neutral (pH 6.90). There was a peak attainment of pH on 10th day indicates the optimum pH for composting activity by *Bacillus subtilis*. It was also observed that there was a gradual increase in pH after peak attainment. Interestingly, during the process of composting, a wide range of pH changes. This is due to *Bacillus subtilis* getting rid of excess nitrogen by intensive delimitation and ammonia production. Any further increase in pH is limited by the volatility of ammonia, which escapes in the form of gas (Kunert, 2000).

Change in Moisture Content

The moisture content of the sand feather compost was recorded during the process and different in the moisture content was observed the sampling. The moisture content feather compost was less indicated rapid loss in moisture during feather degradation. The initial phases of sand, feather composting 41%, 10th day moisture raised 43% and 40th day moisture reduced to 31%. Throughout the composting process of feather, the moisture loss was observed, mainly due to metabolic activity of organisms that generates heat energy or due to environmental conditions such as, over ventilation, (Hogan., *et al*, 1989). The end of composting in feather observed decrease in moisture content of about 46%, (Hayashida., *et al*, 1988) reported similar findings on the removal of 0.78 g water/g dry weight of starting material. Compost samples with normal, non-moisture content may be biologically impaired, low moisture reduces respiration whereas, high moisture content increases anaerobic condition. It was also reported that the drop in moisture content below 31% slowed down the biological reactions. So, 50-60% of moisture content are the optimum range for the process of composting.

Determination of total nutritional composition of sand, feather compost

The end products of feather sand composting were analysed and percentage of macro nutrients such as N, P, K contents were determined. The feather composts showed macro nutrients in The maximum available nutrient content was estimated at after 40 days of inoculation of *Bacillus subtilis*, such as 88mg, 9.10mg and 72.0 mg per kg of N, P and K respectively under sand 2 kg + 30 % of cleaned feather (T1). The minimum available nutrient of 19mg, 5.85mg and 56.5mg in N, P and K respectively recorded under control (sand alone). Nitrogen is one of the important primary nutrient, serves as an essential component of amino acid, the basic structural units of proteins. As a component of nucleic acids, it is required for plant cell division and reproduction. Here the significant decrease in the nitrogen content of the compost was resultant of ammonification (NH^{+4}) process, which converted a fraction of organic nitrogen in to NH_3 and NH^{+4} ions (Tiquia SM, 2002). Phosphorus, an important primary nutrient supplies energy for growth and maintenance. It serves as an integral component of cell membranes, their by involved in selective permeability of cell membrane and also a part of the plant's energy transfer mechanism high energy phosphate ATP (McWilliams D, 2003). In the current study, feather compost contains of phosphorus. The processing of compost results in slight increase in pH. In such alkaline conditions, the availability of phosphorus to plants gets increased. Potassium as a primary nutrient activates certain enzymes in plants and regulates the opening and closing of stomata, which regulates airflow and in water transpiration out of the leaves. Wool and hair wastes have a nutrient source (N, P, K) for crops and evaluate their potential to improve soil biology and chemical properties (Valtcho and Zheljzkov, 2005). Based on this view, feather compost was considered as important compost for agriculture fields.

The final level of moisture content of the composts was within guidelines (Naylor and Girenes, 2002). All composts types used in this study resulted in faecal coliform reduction under the required Environmental Protection Agency level of 1000 cfu/g (Brinton, 2000) for end use. Elimination or reduction of pathogenic species and odour nuisances reduces the risks associated with disease dispersal, air quality problems, flies, vermin and scavenging animals, protection of surface and ground water. The feather degradation for mesophilic temperature should be a desirable characteristic because these microorganisms may achieve hydrolysis with reduced. The

Bacillus subtilis used in this study showed predominant growth activity between pH levels of 7.0 and 8.0. Findings agreed with (Guerra-Rodriguez., *et al.* 2001) who composted chestnut burr/leaf litter and poultry manure and also with (Ignatova., *et al.* 1999) who conducted studies on the digestion of keratinaceous substances

In vitro experiment on plant growth

The significant variation was observed in all the parameters with irrespective of days and samples. The results of the pot culture experiment showed that the combination of sand 2 kg + 30 % of cleaned feather (T1) with 10ml inoculation of FDS 15 was recorded significant increases in all biometric observations as compared to other combinations (T1, T2, T3 & T4) and sand alone (T5). At an early stage, 100 per cent germination was observed in all treatments, but later stages the establishment of the crop was poor in T2 (86 %), T3 & T4 (71 % each) and T5 (57 %). However, 100 % establishment was recorded in sand 2 kg + 30 % of cleaned feather (T1) with 10ml inoculation of FDS 15. It might be due to continues supply of sufficient nutrients for better establishment of the crop (Fig- 4).

CONCLUSION

The method used in this study might present an alternative to on farm composting to produce a community orientated and environmentally eco – friendly. This study is useful in rapid removal of the recalcitrant feather content with the release of valuable by products acceptable in land use application.

The pathogenic bacteria and fungi reduction is also noticed in feather composting. These characteristics increase the value of feather waste in agriculture field.

Alternative poultry manure composting strategies such as the one illustrated in this study would result in the reduction of retention time in the compost cycle performed and would decrease operational costs, which would in turn significantly increase the success of on farm composting. It would also relieve nursery facilities of environmental liability, farmers of liability and promote recycling while ensuring human and environmental health. In addition, further enzymatic studies on the keratinolytic performance of the *Bacillus* sp in this study might lead to the biological production of feather meal.

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