

Chicken Feather Waste – A Threat to the Environment

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ABSTRACT: Chicken feathers are considered to be the waste products of the poultry industry and can create a solid waste problem in the environment. It is estimated typically every bird has up to 125 grams of feather and the weekly worldwide production of feather waste is about 3000 tons. Disposal of this bulk waste is a global environmental problem accounting to pollution of soil and ground water sources. The present work is aimed to evaluate the impacts of degradation of chicken feathers on the environment. In the present research work the feathers were analyzed for parameters like p^H, proteins, weight loss of feather. The analytical results revealed that the p^H values indicate the alkaline nature of feathers and the changes in p^H is an indicator and pre-conditioner for keratinolysis. The increase in total protein content indicates the biodegradation of feather. In addition the weight loss of feather also indicates the rapid degradation of feather. The present research story indicates a preliminary methodology to degrade the feather to prevent the pollution caused by feather in soil and ground water. However future studies can result in the generation of a proper and suitable methodology for industrial level disposal.

KEYWORDS: Feather, degradation, waste, pollution, soil and ground water.

I. INTRODUCTION

Chicken feathers are waste products of the poultry industry create a serious solid waste problem [1]. It is estimated that 400 million chickens are processed every week. Typically as each bird has up to 125 grams of feather, the weekly worldwide production of feather waste is about 3000 tons. Disposal of this bulk waste is a global environmental problem accounting to pollution of land and underground water resources.

Feather constitutes over 90% protein, and composed of two types of Keratins α and β . Keratin is a fibrous and insoluble structural protein extensively cross linked by disulfide bonds. Keratin consists of a number of amino acids but largely made up of cysteine, lysine, proline, and serine [2] [3]. These amino acids tend to cross-link with one another by forming disulfide or hydrogen bonds resulting in fibers that are tough, strong, lightweight, and with good thermal and acoustic insulating properties [4] [5]. Keratinases are emerging to play a vital role in the degradation of keratin and its conversion into digestible animal feed of higher nutritive value.

A cost effective fermentation media with stretch as carbon source and soyabean meal as nitrogen source was designed along with optimization of physical parameters of fermentation resulting a yield of > 500KU/ml [6]. However for most of the applications of Keratinase including bioconversion of feather into animal feed, leather processing etc., crude or partially purified enzyme is sufficient.

The poultry feathers pollute the soil, or the burnt which again pollutes the air. In both the cases the presence of sulphur dioxide in feathers bears the danger behind heavy pollution. Use of Keratinolytic microorganisms for feather degradation is an economical, environmental friendly alternative. Keratinases which are produced by these Keratinolytic organisms could be used to degrade waste and further the digested products could be an excellent material for producing animal feed, fertilizers or natural gas.

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II. SCOPE OF THE STUDY

Around 24 billion chickens, per year, are killed across the world which is discarding four billion pounds (18, 14,369 tones) of poultry feather. This mammoth size of discarded feather, apart from polluting the soil or air, also causes various human ailments including chloresis, mycoplasmas and fowl chlorea view on the magnitude of pollution arising from feathery waste. Tradition disposal strategies of feather are expensive and difficulty. They are often burned in incineration plants, buried in landfills, or recycled into low quality animal feeds. However, these disposal methods are restricted or generate greenhouse gases that pose danger to the environment. By keeping in view this study is taken to degrade the feather in eco-friendly way using the microorganisms *Tricophyton mentagrophytes* and *Tricophyton terrestre*.

III. OBJECTIVES

To assess the keratin degrading potential of *T.mentagrophytes* and *T. terrestre* by conducting assays in vitro.
To suggest a feasible method to degrade feather waste in real time environment.

IV. MATERIALS AND METHODS

Soil sample collection: Soil samples were collected from the feather dumping sites to screen for Keratinophilic fungi by adopting standard method [7]. Glassware and spatula were sterilized by keeping them in the Hot air oven at 180°C for one hour. The sterilized glassware was opened at the field and spatulae were used to dig out the soil. Soil sample was obtained at a depth of 2-5 cm from the surface and the soil samples were transferred to screw cap test tubes and sterile plastic bags and were processed at room temperature.

Preparation of medium: Sabouraud's Dextrose Agar (SDA) was used to culture the Keratinophilic fungi of soil sample. Three grams of Sabouraud's dextrose agar was weighed and mixed with 100ml distilled water and P^H of the medium was adjusted to 7.2 ±0.5. The medium was weighed sterilized in an autoclave at 121°C, 15 lb/inch². After cooling the medium, Chloramphenicol was added as a antibacterial compound. Then the medium was poured into different Petri plates and were labeled properly.

Isolation of fungi: Serial Dilutions were carried out for soil sample. From the sample, one gram of soil was taken and dilutions were employed till 10⁻⁵. Later, 0.1 ml serial diluted soil solution from each test tube (10⁻² & 10⁻⁵) were inoculated on to the respective culture medium and left at room temperature(25°C) for the growth of Keratinophilic fungi. When growing colonies were observed they were identified microscopically.

Identification of fungi: After the preparation of pure cultures, the cultures, and morphological characteristics of fungal colonies and their identification was done by referring pictorial atlas of soil and seed fungi and key to species [Tsuneo Watanabe- second edition] and their morphology was studied by staining them with Lactophenol cotton Blue Dye. Then the stained organisms were observed and 40x as well as oil immersion 100x objectives with the help of Microphotography technique using Labomed Digi-3 Digital Research Microscope, equipped with image analysis software, prog Res Capture Pro.

Experimental setup: The steps for 21 day analysis with regular intervals of 7 days include.

CONTROL-1:50ml sterilized mineral medium + 5ml of spore suspension of *T.mentagrophytes* and *T. terrestre*.

CONTROL-2:50ml sterilized mineral medium + 500mg of chopped and sterilized feathers.

Test Sample: 50ml sterilized mineral medium+500mg of chopped and sterilized feathers. +5ml of spore suspension of *T.mentagrophytes* and *T. terrestre*.

Procedure: 50ml of mineral medium (K₂HPO₄-1.5gm, MgSO₄.7H₂O-0.025gm, CaCl₂-0.025gm, FeSO₄.7H₂O-0.015gm, ZnSO₄.7H₂O-0.005gm, Water-50ml) was taken in 250ml Erlenmeyer flask. Then 5ml of spore suspension of *T.mentagrophytes* and *T. terrestre* suspension containing 10⁵spores/ml of test fungi was inoculated to the mineral medium at P^H-7. 500mg of chopped and sterilized feather were also added. These steps were maintained in triplicate with regular shaking. The flasks are incubated at 28± 2°C. The samples were filtered at regular intervals of 7 days using preweighed Whatman's no:42.

The Biochemical tests carried out include pH, Total protein-(Bradford method, 1976), Weight loss, Keratinolytic activity, pH determined by p^H meter (model meter Systronic), Total protein by Bradford Method with reagents Coomassie Brilliant Blue (CBB) and Bovine Serum Albumin (BSA).

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Coomassie Brilliant Blue Solution: In a volumetric flask, 100mg Coomassie Brilliant Blue G-250 was dissolved in 50ml of 95% ethanol. Later 100 ml of 85% Phosphoric acid was added and diluted up to 500ml with distilled water. This was filtered through Whatmann No.1 filter paper and stored in cold room at 4°C.

Procedure: To 1ml of sample, 2ml of Coomassie Brilliant Blue solution was added and incubated for 30min. for color development and absorbance was recorded at 595nm. Amount of protein released in the culture filtrate was calculated with the help of standard prepared by using Bovine Serum Albumin (BSA) as the substrate.

Weight Loss: The Whatman's no: 42 filter paper is used to filter the sample was pre weighed. After the filtration filter paper was dried at room temperature and final weight was noted down. Net weight loss of the feather was calculated.

Keratinolytic activity: Reagents: Sodium Hydroxide, Formaldehyde, Sodium Sulfite

Preparation of soluble Keratin protein: Feather collected from the dumping site was washed with distilled water and then chopped in small pieces. Treated with 1% Sodium Sulfite and hydrolyzed in 0.4% NaOH at 90°C for two hours. The resulted solution was centrifuged and 10% of the feather protein was treated with 0.4% Formaldehyde and 1% Sodium Sulfite. pH was adjusted to 11 by adding NaOH or HCl.

Procedure: 100ml Agar medium was prepared and to this 0.06% of feather protein was added. The organisms *Tricophyton mentagrophytes* and *Tricophyton terrestre* were inoculated by digging the wells in the medium. Then plates are incubated at 37°C. The zone of inhibition was observed on the medium and the diameter was calculated.

V. RESULTS

Changes in pH: With both *Tricophyton mentagrophytes* and *Tricophyton terrestre*, a gradual increase in pH of the medium up to 21 days was resulted. The medium, set up for feather hydrolysis has shown the pH of 7.72±0.2, 8.5±0.2 and 10.8±0.1 with *T.mentagrophytes* and shows pH of 7.68±0.3, 8.72±0.2 and 10.27±0.1 with *T. terrestre* for 7th, 14th and 21st days respectively against control pH 7.0 and the data is shown in the Table 1.

Table 1: Changes in levels during feather degradation.

S.NO.	ORGANISM	0 TH (DAY) (CONTROL)	7 TH DAY	14 TH DAY	21 ST DAY
1.	<i>Tricophyton mentagrophytes</i>	7.00	7.72±0.2	8.5±0.2	10.8±0.1
2.	<i>Tricophyton terrestre</i>	7.00	7.68±0.3	8.72±0.2	10.27±0.1

Estimation of protein: Maximum amount of protein was released by *T.mentagrophytes* and *T.terrestre*. (Fig. 2) in 21 days sample (9.01±0.1 mg/ml, 8.4±0.3 mg/ml), there is gradual increase amount of protein up to 21 days. A remarkable amount of protein was released by Keratinolytic activity by both the organisms and the values are 1.8±0.4mg/ml, 5.97±0.2mg/ml, and 9.01±0.1mg/ml with *T.mentagrophytes* and 3.2±0.1, 6.13±0.3 and 8.4±0.3 with *T.terrestre* for 7th, 14th, and 21st days respectively against control 0µg/ml (Table 2).

Table 2: Release of protein during feather degradation (mg/ml).

S.NO.	OGRANISM	0 th day (CONTROL)	7 th day	14 th day	21 st day
1.	<i>Tricophyton mentagrophytes</i>	-	1.8±0.4mg/ml	5.97±0.2mg/ml	9.01±0.1mg/ml
2.	<i>Tricophyton terrestre</i>	-	3.2±0.1mg/ml	6.13±0.3mg/ml	8.4±0.3mg/ml

Amount of Weight Loss: In both the cases of setup with *Tricophyton mentagrophytes* and *Tricophyton terrestre* there was a gradual increase of weight of up to 21 days (Fig. 3). The net weight loss in the samples in 125mg, 189 mg and

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348 mg with *Tricophyton mentagrophytes* and 103mg, 187 mg and 298mg with *Tricophyton terrestre* for 7th, 14th, and 21st days respectively against control 0mg (Table 3).

Table 3: Weight loss of feather substrate during degradation (mg).

S.NO	ORGANISM	0 TH DAY (CONTROL)	7 TH DAY	14 TH DAY	21 ST DAY
1.	<i>Tricophyton mentagrophytes</i>	-	125mg	189mg	348mg
2.	<i>Tricophyton terrestre</i>	-	103mg	187mg	298mg

Determination of Keratinolytic activity: In both the cases of setup with *Tricophyton mentagrophytes* and *Tricophyton terrestre* the maximum zone of inhibition was observed on 21st day (11mm and 9mm). It is evident from the results (Fig. 4) that Keratinolytic activity of samples is 3mm, 5mm and 11mm with *Tricophyton mentagrophytes* and 3mm, 5mm and 9mm with *Tricophyton terrestre* for 7th, 14th and 21st days respectively (Table 4).

Table 4: Keratinolytic activity of feather degradation extracts (mm).

S.NO.	ORGANISM	0 th DAY (CONTROL)	7 TH DAY	14 TH DAY	21 ST DAY
1.	<i>Tricophyton mentagrophytes</i>	-	3mm	5mm	11mm
2.	<i>Tricophyton terrestre</i>	-	3mm	5mm	9mm

VI. DISCUSSION

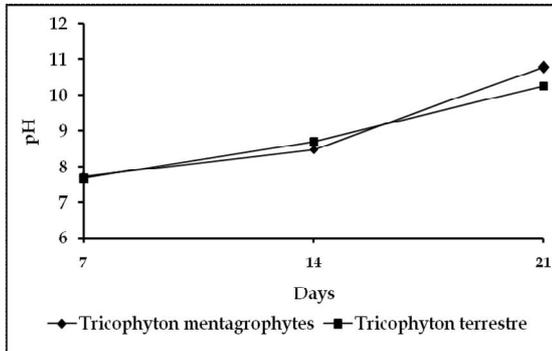
The pH in both cases increases (Fig. 1). The tendency towards alkalization of the medium may be do to the hydrolysis of feather. This change in pH was an indicator for as well as preconditioner for Keratinolysis [8] [9]. Total protein increased in both *T. mentagrophytes* and *T.terrestre* (Fig. 2). Increase in protein content value elucidates the biodegradation of feather. The waste feathers are the valuable organic sources because of their high protein content [9]. There is gradual increase in weight loss of the feather 25%, 37.7% and 69.7% with *Tricophyton mentagrophytes* and 20%, 37,4% and 59.6% with *Tricophyton terrestre* for 7th, 14th and 21st days respectively (Fig. 3). It shows rapid degradation of feather in the medium. The zone of inhibition indicates the degradation of Keratin in the medium.

A gradual increase in the Keratinolytic activity was observed up to 21 days (Fig. 4) and such a conclusion was in accordance with the findings of references [9] and [10]. Hence, it is evident from the present investigation that the fungal strains *Tricophyton mentagrophytes* and *Tricophyton terrestre* were potent Keratinolytic activity fungi against feather [11] and [12].

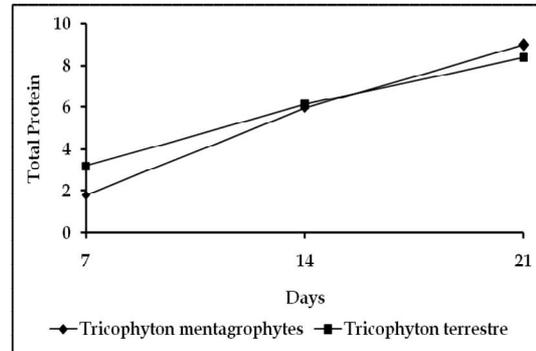
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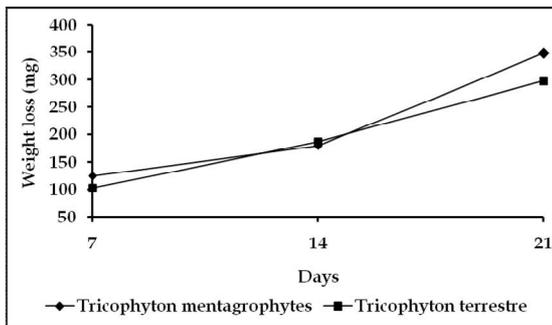
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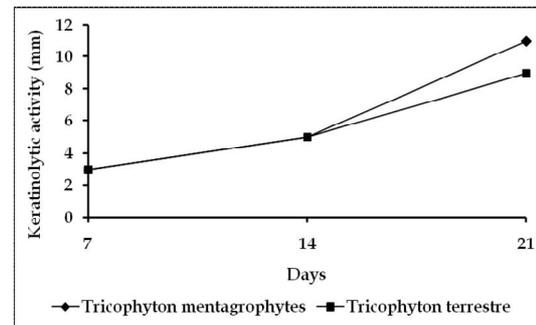
(Fig. 1)



(Fig. 2)



(Fig. 3)



(Fig. 4)

Fig. (1) Changes in the pH mineral medium after degradation Fig. (2) Estimation of total protein released after degradation Fig. (3) Weight loss of feather substrate after degradation Fig. (4) Determination of Keratinolytic activity

VII. CONCLUSION

Feather waste is generated in large amounts as a by – product of commercial poultry processing. Disposal of this waste is accounting in soil pollution and burning of this waste releases sulfur dioxide. The present study has found the key to degrade the feather, thus preventing the pollution caused by feather. Nevertheless this sole work may not be sufficient for direct large scale degradation of wastes but it can be suggested from this work that if further studies can construct a proper methodology for industrial level disposal, it will be worth anything to mitigate the environmental problem caused by feather waste.

From this work, *Tricophyton terrestre* can be recommended as it has very less potential to cause infections in humans or animal though it can be included under dermatophytes, while the other one, *T.mentagrophytes*, though it has shown a good degradation potential, it is not suggestible over *T.terrestre* do its dangerous potential as pathogen, which can immediately cause mycoses.

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