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Microbial degradation of poultry feather biomass in a constructed bioreactor and evaluation of degraded product as a biofertilizer

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Abstract: Poultry waste management is a rising source of worry due to its detrimental effects on the environment and associated health issues. In response, recycling poultry manure using biological processes has become a potential strategy. Bioreactors are one of these techniques that have gained popularity. The ideal conditions for the microbial breakdown and production of organic fertilizers and biogas from poultry waste are provided by bioreactors. By using this approach, trash is managed effectively and greenhouse gas emissions are decreased, supporting sustainable agriculture practices. By dwelling on negative effects of chicken waste on the environment and human health, it has become an urgent need to manage such waste by developing a bioreactor for it. In this study, Stenotrophomonas maltophilia KARUNA5 strain isolated from poultry waste in our earlier studies was used and developed a lab-scale bioreactor which was optimized for poultry waste treatment. Shake flask studies at laboratory scale were conducted to optimize the conditions of bioreactor which helped us to identify key factors that contributed to the feather degradation to a large extent. These factors included a 2.0 % inoculum size of the culture (1O. D620nm), a feather concentration of 1.5 % in a whole feather medium with pH 8.0 kept on shaker (agitation rate of 140 rpm) at a 30°C for 6 days. The 78.5 % of feather degradation was observed. The lab-scale bioreactor was designed which could efficiently degrade the poultry waste, offering a sustainable method of waste management. Further, the foliar (liquid fertilizer) potential of the feather hydrolysate product was evaluated with chemical analysis (NPK analysis) showed the NPK Σ 2.68 % w/w (>1.15 minimum) fulfilling the specification of fertilizer.

Keywords — Bioreactor, Poultry waste, Shake flask experiment, NPK ratio

I. INTRODUCTION

Feathers are an important by-product in the poultry industry as they account for 5–7 % of the body weight of chickens. It is estimated that approximately several million tons of feathers could be generated annually from the poultry industry globally [1],[2]. Due to inappropriate disposal, this oversupply has created several environmental problems as well as serious pollution and health dangers [3],[4],[5]. Feathers are mainly composed of keratin, a durable and resilient proteinaceous biopolymer [6]. Keratin is a fibrous protein that is present in feathers, hairs, nails, hooves, and horns of birds and animals [7]. According to a study by Pereira et al. (2014), the intricate structure of keratin is characterized by a significant amount of cross-linking, hydrogen bonding, and disulfide bonding, making it very difficult to degrade and thereby presenting challenges for its efficient degradation [3],[6]. In recent years, research has focused on converting this waste into valuable products, such as amino acids, fertilizers, and biopolymers. As a result, there is an urgent need for the development of an environmentally friendly and affordable technique for the degradation of feathers. The microbial degradation of feathers is a multifaceted process that necessitates the fine-tuning of several factors such as aeration, pH, temperature, and substrate concentration to achieve optimal outcomes. To optimize microbial growth and metabolism in a controlled environment, bioreactors are crucial tools.

To ensure the uniform dispersion of microbial populations, they offer the essential variables of the environment, nutrients, gasses, and appropriate mixing. Bioreactor has

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been considered as a promising tool as it allows mass propagation of bacterial cultures under a controlled environment. It is defined as "a self-contained, sterile environment that capitalizes on liquid nutrient inflow and outflow systems, designed for intensive culture with maximal opportunity to monitor and thus to control microenvironmental conditions (agitation, aeration, temperature, and pH)" [8]. These bioreactors have been used in a variety of bioconversion processes, from wastewater treatment to food processing and biofuel production [9].In the case of feather degradation, bioreactors can offer solutions to overcome the challenges of microbial growth and enzyme production in submerged cultures [10].

This study intends to assess and improve the microbial breakdown of feathers in a shake flask experiment and this study also concentrates on the optimization of various parameters for microbial cultures such as pH, aeration, inoculum size and density, and feather concentration for efficient degradation of feather waste which apply to the bioreactor for efficient handling of feather waste. The study aims to design, fabricate, and develop a bioreactor prototype for efficient, cost-effective, and sustainable management of feather waste. Results from this study can offer insight into possibilities for the industrial application of microbial feather degradation and the production of liquid biofertilizer.

II. MATERIALS AND METHODS

2.1 Microbial strain for poultry waste treatment and recycling: *Stenotrophomonas maltophilia* KARUNA5 strain which was isolated from poultry waste in our earlier studies [11], was used throughout this study. The strain was screened for its feather degradation potential, keratinase enzyme activity, and growth on St. feather meal medium, and caseinolytic activity on the St. milk agar plate. Isolate's 16S rRNA gene sequence has already been deposited into the NCBI website with the GenBank accession number (LC-271188.1) and the isolate was maintained on St. Nutrient agar slant at a temperature 4◦C in the refrigerator till the further use.

2.2. Shake flask experiment for optimization of the bioreactor culture system: The growth kinetics of Stenotrophomonas maltophilia KARUNA5 study was done in St. Whole feather medium ([(g/L):0.5 g of NH4Cl, 0.5 g of NaCl, 0.3 g of K2HPO4, 0.4 g of KH2PO4, 0.1 g of MgCl2·6H2O, 0.1 g of yeast extract, and 1 g of defatted whole chicken feathers.) (Kim et al., 2001). Inoculum 1 % (1O.D 650nm) of Stenotrophomonas maltophilia KARUNA5 was added to this medium and incubated on a rotary shaking incubator at 120 rpm and 30°C for 7 days. And feather degradation percentage was calculated by the weight loss method [12], [13]. Optimized process parameters for feather degradation in bioreactor evaluated with shake flask experiment with OVAT (One variable at a time) method with four independent variables like incubation time, inoculum size, feather concentration, pH, aeration, and agitation speed.

2.2.1. Optimization Incubation time for feather degradation: The sterile whole feather media were inoculated with the optimized size of inoculum and were incubated on an orbital shaker at 120 rpm for varying periods 24 hrs. to 168 hrs with an interval of 24 hrs and the feather degradation was assessed. All the other conditions (including pH, media, inoculum size, and substrate concentration) in the above flasks were identical. After a particular time, interval, after incubation % of feather degradation was determined with the weight loss method [14], [15].

2.2.2. Optimization Inoculum size for feather degradation: The inoculum size for feather degradation was optimized to obtain maximum feather degradation. The Basal feather medium was inoculated with different percentages (1.0 to 5.0 % with an interval of 1 % of inoculum with 1 O.D. at 620 nm) and then flasks were incubated at 30° C for 6 days under shaker conditions maintained at 120 rpm. After six days of incubation % of feather degradation was determined with the weight loss method [13].

2.2.3. Optimization of feather concentration for feather degradation: The concentration of feathers was optimized to obtain maximum feather degradation. The St. whole feather medium was supplemented with different concentrations (0.5 to 5.0%) of whole feathers with an interval of 0.5 % and subsequently, each flask was inoculated with 2 % inoculum of 1 O.D at 620 nm adjusted with U.V. visible spectrophotometer (Bio Era, India) and incubated at 30°C for 6 days under shaker conditions maintained at 120 rpm. After six days of incubation % of feather degradation was determined with the weight loss method [12], [13],[16]

2.2.4. Optimization of aeration and agitation speed for feather degradation: The effect of static and aeration (agitation) conditions and rate of aeration on keratinase production and feather degradation were determined by incubating the whole feather media at static and shaker conditions along with previously optimized conditions. To determine the optimized agitation speed orbital shaker speed was adjusted to 40, 80, 120, 160, and 200 rpm speed at 30°C (Mahdi et al., 2020). All the other conditions (including pH, media, inoculum size, and substrate concentration) in the above flasks were identical and all flasks were inoculated with 2 % inoculum with 1 O.D. at 620 nm and incubated at 30°C for 6 days. After seven days of incubation % of feather degradation was determined with the weight loss method [17].

2.2.5. Optimization of pH for feather degradation: For optimization of pH for the maximum percentage of feather degradation, six different values of pH (i.e., 5.0, 6.0, 7.0,

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8.0, 9.0, and 10.0) were adjusted in whole feather medium with the above-optimized feather concentration using Tris buffer, phosphate buffer, and sodium carbonate for acidic, neutral and alkaline conditions respectively. Each flask was inoculated with 2 % inoculum 1 O.D. at 620 nm adjusted with a U.V. visible spectrophotometer (Bio Era, India), and incubated at 30°C for 6 days under shaker conditions maintained at 140 rpm. After six days of incubation, % of feather degradation was determined with the weight loss method [13], [18], [3].

2.2.6. Evaluation of Feather degradation (%) with weight loss method: Substrate weight loss (%) was used to measure the biodegradation of substrate by Stenotrophomonas maltophilia KARUNA5 during fermentation. Following fermentation, the feather remnants were filtered out of the culture supernatant and collected. Following a thorough washing, the gathered leftover feathers were dried for 48 hours at 100°C in a hot air oven. Feathers' biodegradation was quantified as a percentage of weight loss relative to the substrate's initial dry weight (both before and after incubation). The uninoculated flask with feathers was used as the control for the assessment.

Feather degradation (%) =

(Initial weight-Final weight/Initial weight X100)

[12].

2.2.7. Bioreactor Design and experimental setup for feather degradation

2.2.7.1. Design Stage: In order to complete the Bioreactor, facts and principles gathered from several sources formed the foundation for the bioreactor design. Precise dimensions and material specifications were considered in the development of the engineering layout and analysis. For the fabrication stage, the design and creation of the schematic layout were crucial. Materials availability and operating costs were also taken into account.

2.2.7.2. Fabrication Stage: This stage covers the fabrication of the prototype based on the design specifications. Proper selection of construction materials was ensured to successfully obtain the functionality of the **Bioreactor. Assumptions**

Per day feather waste generation from 500 chickens is approximately 50 g.

From the batch study, The optimal incubation time for one cycle (one cycle = one complete process of degradation) is two days. Therefore, Waste generation for two days = 2 x 50 = 100 g The optimal feed concentration is 2%. Thus, for 100 g waste 6 L capacity bioreactor is required.

2.2.7.3. Experimental setup : A bioreactor system was developed for the efficient degradation of poultry waste. Figure 1 depicts the design of the bioreactor experiment which utilized an internal circulation airlift mechanism. The bioreactor's total capacity was 7 L, with a corresponding working volume of 6.2 L. Copper pipe with perforations was used for aeration, and the airflow was regulated through the Filter Regulator Lubricator (FRL) system. The external vessel was made of mild steel, and the internal vessel was made of stainless steel. A pressure cooker was used for the sterilization process.

Table 1. shows the design of the bioreactor system developed for optimal degradation of poultry feather waste. The table presents the specifications of the external and internal vessels. These design characteristics were carefully chosen to promote efficient feather and poultry waste degradation in the bioreactor system. Photographic and cross-sectional views of the developed bioreactor system are shown in Figure 8A and B.

Table 1. Specifications of bioreactor used.

Mild Steel Vessel	Size	Diameter - 31cm Height - 33cm
	Volume	25 L
	Surface Area	195 cm^2
Steel vessel	Size	Diameter - 19 cm Height - 25 cm
	Volume	7 L
	Surface area	60 cm^2
	Effective volume	6.2 L
Effective Volume of Reactor		6.2 L

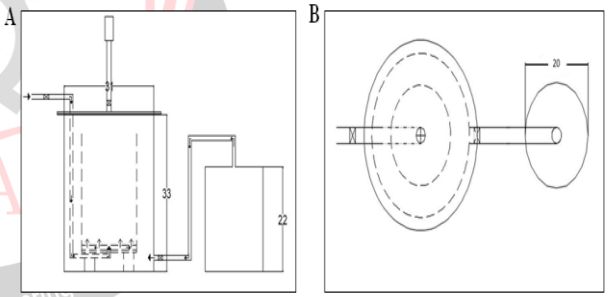

Figure 1. Schematic layout of the bioreactor for poultry waste disintegration. (A) a) Sectional view of bioreactor, (B) Top view of the bioreactor (All dimensions in cm).

2.3. Evaluation of feather hydrolysate: Phosphorus content from feather hydrolysate is evaluated by the molybdate-blue method feathers were subjected to analysis of elemental composition. Nitrogen was determined by the Microkjeldahl method, Phosphorus by the Molybdenum method, and potassium determination by the Gravimetric method [19].

III. RESULTS

3.1. Shake flask experiment for optimization of bioreactor culture system: The growth kinetics of Stenotrophomonas maltophilia KARUNA5 was studied in St. Whole feather medium. Inoculum of Stenotrophomonas maltophilia KARUNA5 was added to this medium and incubated on a rotary shaking incubator at 120 rpm and 30°C for 7 days. and feather degradation percentage was

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calculated by the weight loss method.

3.1.1. Incubation time: The effects of the Incubation time on feather degradation are shown in Figure 1. The highest feather degradation in the shaking flask was obtained with an incubation time of 144 hrs. (37.16 %). As incubation time increases there is an increase in feather degradation. This observation is consistent up-to 144 hrs. for feather degradation. After incubation of 168 hrs., it shows slight decrease in degradation percentage (37.1 %). Degradation rapidly increases after 24 hrs. of incubation.

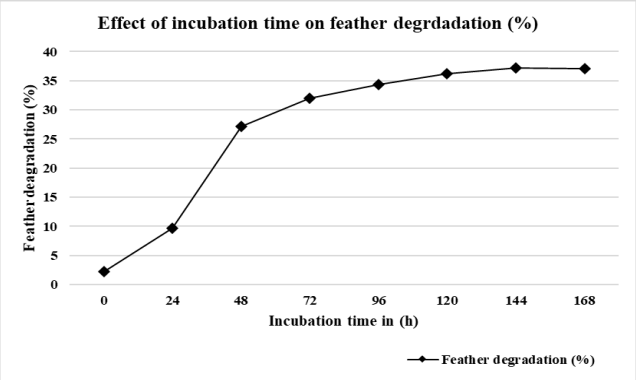

Figure 2. Effect of Incubation time on feather degradation %

3.1.2. Inoculum size: Inoculum size and density influences distribution of nutrients as well as feather degradation. The effects of the inoculum size on feather degradation are shown in Figure 2. The highest feather degradation was obtained with the inoculum size of 2 % with 1O.D. at 620 nm (47.73 %) and it significantly shows the highest feather degradation. Degradation of feathers is directly proportional to the increase in inoculum size up to 2.0 % thereafter it shows decline in feather degradation and the lowest feather degradation observed with 5 % inoculum (29.41 %).

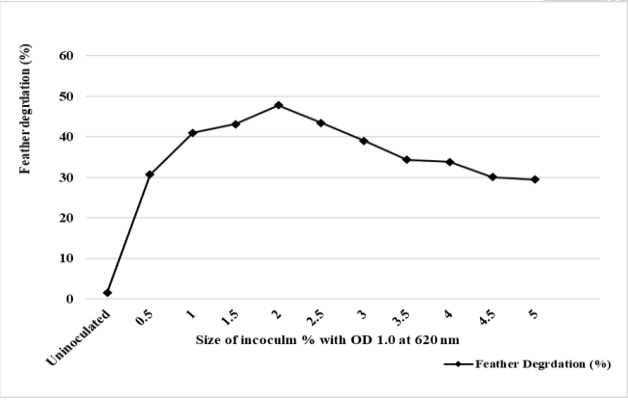

Figure 3. Effect of Size on inoculum on feather degradation.

3.1.3. Concentration of feather: The effect of feather concentration on feather degradation is shown in Figure 3. The highest feather degradation was obtained with a feather concentration of 1.5 % (49.41 %). Feather degradation rate is declined above this concentration e.g., at feather concentration 2.0 % (45.37 %) and 2.5 % (44.68 %)

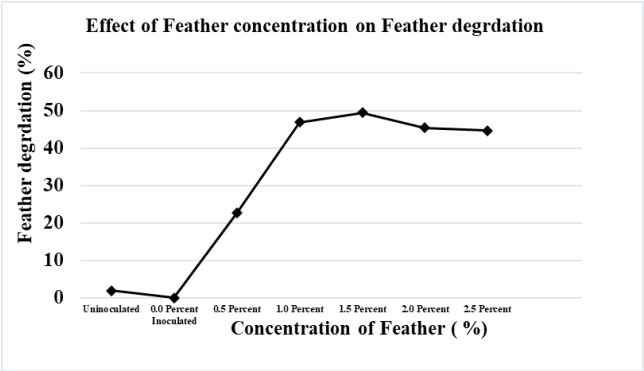

Figure 4. Effect of feather concentration on feather degradation %

3.1.4. Aeration speed: The effects of the static and agitation conditions on feather degradation are shown in Figure 5. And Figure 6 The highest feather degradation is obtained with the agitation flask as it shows significant degradation of feathers. The study was conducted to determine optimum agitation rate. The effects of the agitation speed on feather degradation are shown in Figure 7. The maximum feather degradation is obtained with the agitation speed of 140 rpm.

Figure 5. Effect of static and agitation on feather degradation.

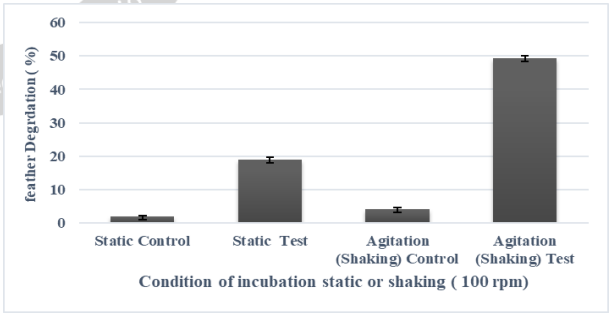

Figure 6. Effect of static and agitation on feather degradation.

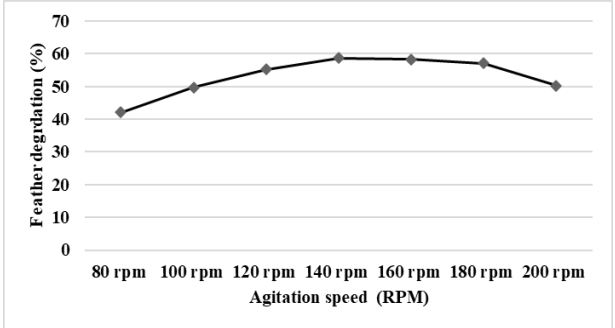

Figure 7. Effect of Agitation speed on Feather Degradation.

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3.1.5. pH

The effects of the pH on feather degradation are shown in Figure 8. The highest feather degradation was obtained with the pH value 8.0 as it shows maximum 81.61 % feather degradation. Other values of feather degradation percentage are as follows: at acidic pH 4.0 (6.54%), pH5.0 (22.1%) and at alkaline pH 10 (49.68%) 11(22.66%) and pH 12 (3.21%).

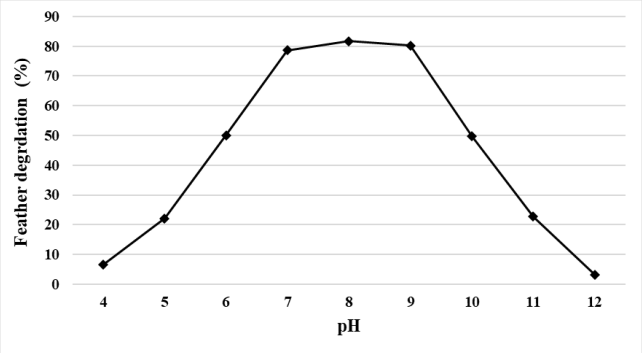

Figure 8. Effect of pH on feather degradation.

3.2. Feather degradation with designed bioreactor

Figure 9A and B demonstrates an overhead view of the bioreactor system. These figures offer insight into the complex design of the bioreactor system developed to promote efficient poultry waste degradation. An internal circulation airlift bioreactor was used, with a total volume of 7 L and a working volume of 6.2 L. The external vessel was made up of mild steel and the internal vessel was made up of stainless steel. The aeration systems comprised of copper pipe with perforations for the air exit. A pressure cooker was used for the sterilization. Filter Regulator Lubricator (FRL) was then used to filter air and regulate the airflow coming from the aerator. Along with optimized conditioned deduced from shake flask experiment feather degradation studied conducted with bioreactor with fermentation medium volume 6.2 L after the 6 days of incubation with optimized parameter equivalent with results of shake flask experiment (pH-8.0, agitation rate 140 rpm, feather concentration 1.5 % and inoculum size 2.0 % it showed 51 % degradation of feather substrate. Feather hydrolysate separated from reactor assessed for fertiliser potential by evaluation of TKN, Phosphorus and Potassium concentration.

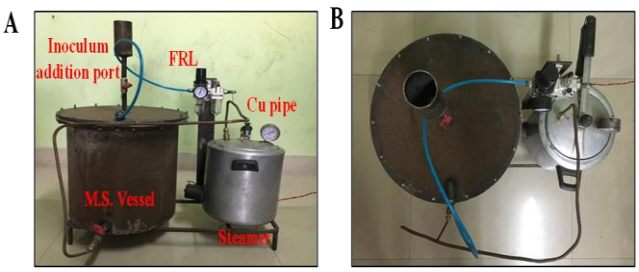

Figure 9. Experimental setup of (A) keratin waste degradation bioreactor, (B) Photographic top view of bioreactor.

3.6. Analysis of the hydrolysate

The pH of hydrolysate ranged between 8 to 10. For the application of hydrolysate as fertilizer, pH should be 6.5 to

7.5 as per FCO standard. Thus, H3PO4 was used to adjust the pH and then analysis was carried out. Methods of analysis are referred to IS:6092: 2004 and FAD7(2189). The hydrolysate obtained in the present study had TKN, P, and K values as shown in Table 2. These values are according to Schedule – IV, Part-A of Specifications of Organic Fertilizers as per Biofertilizer and Organic Fertilizers in Fertilizer (Control) Order 1985 of Government of India (Annexure-II) [10].

Table 2. Characteristics of chicken feather hydrolysate.

Parameter	Chicken feather hydrolysate (% w/w)
Total Kjeldahl Nitrogen (TKN)	2.41 ± 0.54
Phosphorous (P)	0.15 ± 0.05
Potassium (K)	0.12 ± 0.07
∑NPK	2.68 (≥1.5 minimum)

IV. DISCUSSION

In currant study bacterium Stenotrophomonas maltophilia KARUNA5 showing feather degradation ability was isolated from poultry waste. Potential of isolate screened on the basis of keratinase enzyme activity, Proteolytic activity on skimmed milk agar and ability to degrade feather in mineral medium. This strain behaves in the similar fashion to Stenotrophomonas maltophilia K279a [20], Vibrio strain kr2 [21], Flavobacterium sp Kr6, [22]. Fermentation medium play crucial role in feather degradation similar to our study Whole feather medium was also used by several researchers for feather degradation as well as production of keratinase enzyme include Stenotrophomonas maltophilia strain L1 shows maximum degradation at temperature 40^0C and pH between 7.0 to 8.0 [23]. These results are resembled with present study. In a recent study, Derhab et al. (2023) used Plackett–Burman design and reported mineral medium containing 0.3% K2HPO4, 0.05% KH2PO4, 0.01% MgSO4.7H2O, 0.05% yeast extract and 1% chicken feathers as optimum for keratinase production by Bacillus cereus L10, where 94 % feather degradation was achieved. Bacillus sp. CN2 showed 86.70% degradation of native feathers in 24 hrs, and the enzyme yield (195.05 ± 6.65 U/mL) peaked at 48 hrs [24]. Shaha and Vaidhya, (2017) reported use of 1% feather concentration in optimization studies. Shah and Vaidya, (2017) achieved 73.34 % - 75.6 % feather degradation with Stenotrophomonas maltophilia strain K279a. The optimum feather degradation by Stenotrophomonas maltophilia DHHJ was observed at pH of 7.8 [25]. Stenotrophomonas

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maltophilia K279a showed 73.34% feather degradation over a pH range between 5.0 and 9.0 [20]. Derhab et al. (2023) reported optimum feather degradation from Bacillus cereus L10 in 5 days for incubation. Bacillus safensis LRF3X and Penicillium sp., required 8 and 7 days respectively for visual degradation of feather [27], [28]. Stenotrophomonas maltophilia KARUNA5 showed optimum inoculum size 2.0 % for feather degradation. Similarly a high cell density of 1.0 O.D.600nm (using pregrown cell mass) was used by Yusuf et al. (2019) for production of keratinase from Alcaligenes sp. AQ05-001 since the culture was immobilised in gellan gum and Jani et al. (2014) used 0.75 O.D.670nm for production of keratinase as well as feather degradation from Streptomyces sp. and Saccharothrix xinjiangensi.

Same result as ours i.e. 2% inoculum size for maximum feather degradation was also reported by Shah and Vaidya (2017) and Moridshahi et al. (2021) for Stenotrophomonas maltophilia K279a. and Bacillus tequilensis BK206 respectively. The employment of bioreactors for waste management has been explored in various studies. For instance, Gavrilescu and Chisti (2005) highlighted the significance of bioreactors as a cost- and environmentally-friendly method of managing different forms of waste. They noted the potential of bioreactors to improve waste degradation rates while minimizing environmental pollution. Additionally, research has demonstrated that the employment of microbes in bioreactors can efficiently break down keratin. Guo et al. (2020) used a bioreactor containing mixed populations of bacteria for the degradation of chicken feathers. With a decrease in feather size and an increase in nutrient release, they claimed to have achieved a 64.5% degradation rate. Similarly, Oh and Lee, (2018) employed Bacillus licheniformis for the degradation of keratin waste in a bioreactor system and achieved a 91.2 % degradation of keratin waste. Which is slightly greater than our studies. Feather hydrolysate produced from currant studies suite all required criterion of liquid fertiliser which was demonstrated by evaluating TKN, P, and K from sample with specified methods these results are matched with past research include Biswas et al. (2020) reported poultry waste compost has very high potential as fertiliser because of presence of more than 1.53 % available Nitrogen which act as alternative to nitrogen based chemical fertiliser like urea. Nurdiawati et al. (2019) analysed poultry waste for ultimate concentration of N, P and K and it showed that high N content (0.12 %), P content (0.31 %), K content (0.15 %) reflects good qualities as fertiliser.

V. Conclusion

In this study, a bioreactor system was employed to degrade poultry waste, yielding significant results. The findings indicate that the use of such a system can effectively decompose keratin waste with promising results for use in waste management and related fields. These results are in line with prior research on the potential of bioreactors as an efficient method of waste decomposition, thereby reducing environmental pollution. The optimization of feather degradation and keratinase production using Stenotrophomonas maltophilia KARUNA5 has significant potential in utilizing waste feathers effectively. Additionally, the newly designed lab-scale bioreactor efficiently degraded the keratin waste, which could have profound implications for waste management. The analysis of the produced feather hydrolysate exhibited the existence of NPK ratio with fertiliser potential indicating its possible application in the agricultural industry.

Conflicts of Interest: The authors declare no conflict of interest.

References

[1] Jingwen Q, Wilkens c, Barrett K, Meyer A s (2020). Microbial enzymes catalyzing keratin degradation: classification, structure, function, Biotechnology Advances, (44) 107607.

[2] Li, Q. (2019) Progress in Microbial Degradation of Feather Waste. Front. Microbiol 10, 2717.

[3] Cai, C. G., Lou, B. G., & Zheng, X. D. (2008). Keratinase production and keratin degradation by a mutant strain of Bacillus subtilis. Journal of Zhejiang University: Science B, 9(1), 60–67.

[4] Zhao W., Yang R., Zhang Y., Wu L. (2012) Sustainable and practical utilization of feather keratin by an innovative physicochemical pretreatment, high-density steam flash explosion. Green. Chem. OI, 10.1039.

[5] Silva, L.C.F., Lima, H.S., Sartoratto, A., Sousa, M.P. de, Torres, A.P.R., Souza, R.S. de, de Paula, S.O., Oliveira, V.M. de, Silva, C.C. da, 2018. Effect of salinity in heterotrophic nitrification/aerobic denitrification performed by acclimated microbiota from oil- produced water biological treatment system. Int. Biodeterior. Biodegrad. (130): 1–7.

[6] Pereira, J.Q., Lopes, F.C., Petry, M.V., da Medina, L.F.C.,and Brandelli, A. (2014) Isolation of three novel Antarctic psychrotolerant feather-degrading bacteria and partial purification of keratinolytic enzyme fromLysobactersp.A03.Int Biodeterior Biodegrad 88:1–7.

[7] Ramnani R, Singh R, and Gupta R, (2005), "Keratinolytic potential of Bacillus licheniformis RG1: Structural and biochemical mechanism of feather degradation." Canadian Journal of Microbiology (51): 191-196.

 DOI : 10.35291/2454-9150.2023.0201

[8] Paek KY & Chakrabarty D (2003) Micropropagation of woody plants using bioreactor. In: Jain SM & Ishii K (eds) Micropropagation of Woody Trees and Fruits Kluwer Academic Publishers, Dordrecht (pp. 756–756).

[9] Kumar, D.S, Ray,S., (2014). Fungal lipase production by solid state fermentation-an overview. Anal. Bioanal. Tech. 5:230

[10] Nurdiawati, A., Suherman, C., Maxiselly, Y., Akbar, M. A., Purwoko, B. A., Prawisudha, P., & Yoshikawa, K. (2019). Liquid feather protein hydrolysate as a potential fertilizer to increase growth and yield of patchouli (Pogostemon cablin Benth) and mung bean (Vigna radiata). International Journal of Recycling of Organic Waste in Agriculture, 8(3), 221–232.

[11] Patil S and K Aruna, (2022) Screening and partial 16 S rRNA gene sequencing of keratinase producing Stenotrophomonas maltophilia KARUNA5 isolated from poultry waste. Res, I. J. A. 10 (11), 147-153.

[12] Jain R., Jain P. C., and Agrawal S. C. (2012), "Feather degradation by Streptomyces exfoliatus CFS 1068." Annals of Microbiology, 62(3), 973–978.

[13] Govinden Guvin and Puchooa Daneshwar (2012), "Isolation and characterization of feather degrading bacteria from Mauritian soil." African Journal of Biotechnology, (11) 7: 13591-13600.

[14] Mahdi, Z.S., Talebnia Roshan, F., Nikzad, M., Ezoji, H., 2020. Biosynthesis of zinc oxide nanoparticles using bacteria: a study on the characterization and application for electrochemical determination of bisphenol A. Inorg. Nano-Metal Chem. 1–9.

[15] Werlang, P. O., & Brandelli, A. (2005). Characterization of a novel feather-degrading Bacillus sp. strain. Applied Biochemistry and Biotechnology - Part A Enzyme Engineering and Biotechnology, 120(1), 71–79.

[16] Coello, N., & Vidal, L. (2005). Kocuria Rosea as a New Feather Degrading Bacteria. Applied Microbiology, 165–175.

[17] Deniz, I., Demir, T., Oncel, S. S., Hames, E. E., & Vardar-Sukan, F. (2021). Effect of Agitation and Aeration on Keratinase Production in Bioreactors Using Bioprocess Engineering Aspects. Protein Journal, 40(3), 388–395.

[18] Sousa M , Souza O, Maciel M., Cruz R, Rêgo M, Magalhães O, Pessoa-Júnior A, Porto A, Souza-Motta C. (2015), "Keratinolytic potential of fungi isolated fromsoil preserved at the Micoteca URM." European Journal of Biotechnology and Bioscience 3 (4): 10-15.

[19] Cortezi M, Cilli E, Contiero J (2008) Bacillus amyloliquefaciens: a new keratinolytic feather degrading bacteria. Curr Trends Biotechnol Pharm 2:170–177.

[20] Shah, M., & Vaidya, R. (2017). Partial characterization of keratinase from Stenotrophomonas maltophilia K279a and study of its dehairing potential. International Journal of Biotechnology and Biochemistry. 13(1):95–110.

[21] Sangali S and A Brandelli. 2000. Feather keratin hydrolysis by a Vibrio sp. strain kr2. J Appl Microbiol 89: 735–743.

[22] Riffel, A., and Brandelli, A. (2002). Isolation and characterization of a feather-degrading bacterium from the poultry processing industry. Journal of Industrial Microbiology and Biotechnology, 29(5) : 255–258).

[23] Cai, C., & Zheng, X. (2009). Medium optimization for keratinase production in hair substrate by a new Bacillus subtilis KD-N2 using response surface methodology. Journal of Industrial Microbiology and Biotechnology, 36(7), 875–883.

[24] Lai, Y., Wu, X., Zheng, X., Li, W., & Wang, L. (2023). Insights into the keratin efficient degradation mechanism mediated by Bacillus sp. CN2 based on integrating functional degradomics. Biotechnology for Biofuels and Bioproducts. 16(1): 1–14.

[25] Wu, WL., Chen, MY., Tu, IF., (2017) The discovery of novel heat-stable keratinases from Meiothermus taiwanensis WR-220 and other extremophiles. Sci Rep (7): 4658.

[26] Derhab, N., Mabrouk, M. E. M., El, M. M., & Youssef, M. (2023). Thermostable keratinase from Bacillus cereus L10: optimization and some potential biotechnological applications. Biomass Conversion and Biorefinery, 0123456789.

[27] Zanphorlin LM, Facchini F, Vasconcelos F, Bonugli-Santos R, Rodrigues A, Sette L, Gomes E, Bonilla-Rodriguez G (2010) Production, partial characterization, and immobilization in alginate beads of an alkaline protease from a new thermophilic fungus Myceliophthora sp. J Microbiol 48 (3):331-336.

[28] Ezeme-nwafor, A., & George-okafor, U. O. (2022). Production and optimization of keratinase enzyme by Bacillus safensis LRF3X isolated from feather dump sites . May 2020.

[29] Gavrilescu, M., & Chisti, Y. (2005). Biotechnology — a sustainable alternative for chemical industry. 23, 471–499.

 DOI : 10.35291/2454-9150.2023.0201

[30] Biswas, S., Nandy, A., Islam, N., & Rafa, N. (2020). Environmental citizenship and solid waste management in Chattogram , Bangladesh. 135–150.

[31] Kim K. (2007). Purification and Characterization of a Keratinase from a Feather-Degrading. Mycobiology 35(4):219–225.

[32] Kurane, A. B., & Attar, Y. C. (2020). Studies on Keratinase enzyme using bacteria isolated from feather dumping soil and its application in Dehairing and Tannery waste management. International Journal of Scientific and Technology Research, 9(3), 5812–5817.

[33] Moridshahi R, Bahreini M, Sharifmoghaddam M, Asoodeh A. (2020). Biochemical characterization of an alkaline surfactant-stable keratinase from a new keratinase producer, Bacillus zhangzhouensis. Extremophiles. 24(5):693-704.

[34] Oh, H.S., Lee, C.H., (2018) Origin and evolution of quorum quenching technology for biofouling control in MBRs for wastewater treatment. J. Membr. Sci. 554, 331–345.

[35] Orosco, P., & Brandelli, A. (2005). Novel Feather-Degrading Bacillus sp. Strain Characterization of a Novel Feather-Degrading Bacillus sp. Strain. In Applied Biochemistry and Biotechnology (120).71-79

[36] Sayed, S.A., Saleh, S.M., Hasan, E.E. (2005). Removal of some polluting metals from industrial water using chicken feathers. Desalination, 181(1-3), 243-255.

[37] Sonwani, R. K., Giri, B. S., Jaiswal, R. P., Singh, R. S., & Rai, B. N. (2020). Performance evaluation of a continuously packed bed bioreactor: Bio-kinetics and external mass transfer study. Ecotoxicology and Environmental Safety, 201(06), 110860.

[38] Syed, D. G., Lee, J. C., Li, W. J., Kim, C. J., & Agasar, D. (2009). Production, characterization and application of keratinase from Streptomyces gulbargensis. Bioresource Technology, 100(5), 1868–1871.

[39] Van Blitterswijk, C., Thomsen, P., Hubbell, J., Cancedda, R., de Bruijn, J. D., Lindahl, A., Sohier, J., & Williams, D. (2008). Tissue Engineering. Tissue Engineering, 5(1), 43–63.

[40] Yusuf, I., Ahmad, S.A., Phang, L.Y., Syed, M.A., Shamaan, N.A., Khalil, K.A., Dahalan, F.A., Shukor, M.Y. (2016) Keratinase production and biodegradation of polluted secondary chicken feather wastes by a newly isolated multi heavy metal tolerant bacterium-Alcaligenes sp. AQ05-001. Journal of Environmental Management, 183(1):182-195.

[41] Zhou X, Idler W. W, Stevent A. C, Roopii D. R, and Steinerts P. M. (1998), “The Complete Sequence of the Human Intermediate Filament Chain Keratin.” J Biol Chem. 263 (30):15584–15589. M. Young, *The Techincal Writers Handbook.* Mill Valley, CA: University Science, 1989.

 DOI : 10.35291/2454-9150.2023.0201