IJNRD.ORG ISSN : 2456-4184
INTERNATIONAL JOURNAL OF NOVEL RESEARCH AND DEVELOPMENT (IJNRD) | IJNRD.ORG
An International Open Access, Peer-reviewed, Refereed Journal

# Waste Chicken Feathers Undergo Microbial Degradation in Submerged and Solid-State Fermentation

**[1] Piyusha Agrawal, [2] Satwik Sahay Bisarya, , [3] Chhaya Pandey and [4]Santosh Kumar**

[1]Research Scholar, Department of Applied Microbiology and Biotechnology, Dr. Harisingh Gaur Vishwavidyalay, Sagar, (M.P.) India
[2]Associate Professor, Faculty of Agriculture Science and Technology, Madhyanchal Professional University, Bhopal, (M.P.) India
[3]Academic Coordinator, Madhyanchal Professional University, Bhopal, (M.P.) India
Assistant Professor, Faculty of Agriculture Science and Technology, AKS University, Satna, (M.P.) India

***Abstract:*** The poultry business is quickly growing and generates millions of tonnes of feather waste each year. The justifiable use of keratinaceous byproducts is required due to the widespread production of industrial wastes that contain these materials. Many studies say that the chemical treatment of keratin waste is an eco-destructive method since it produces secondary contaminants. Keratin waste may be effectively treated by using keratinase, a digestive enzyme produced by a range of microorganisms (bacteria and fungi). The treatment of the resistant pollutant keratin as well as the production of the commercially significant enzyme keratinase are two advantages of the new and environmentally beneficial method of microbial degradation of keratin waste. In this work, fungal species are isolated, described, and potentially used to break down waste chicken feathers through submerged and solid-state fermentation. Aspergillus (A.) flavus was recognized and described as an isolated fungus. In a 30-day study, it seemed that A. flavus in submerged and solid-state fermentation processes lowered the weight of the feathers by 74 and 8 percent, respectively. In submerged fermentation, the growth media's pH was raised from 4.8 to 8.35. Therefore, it is advised to use keratinolytic microorganisms to treat keratinaceous wastes in order to get remediation advantages on both sides.

***IndexTerms* - \Poultry industry, Aspergillus flavus, Biodegradation, Economical bioremediation.**

## INTRODUCTION

Due to the high concentration of hydrogen and disulfide bonds in keratin, a structural protein that is insoluble, fibrous, and stubborn in nature. The integuments of many vertebrates, including fish, reptiles, birds, and mammals, include compact keratin, which ranks third in nature after chitin and cellulose due to its abundance (Kreplak et al., 2004; Bragulla and Homberger, 2009; Mckittrick et al., 2012; Meyers et al., 2008; Lange et al., 2014; Huang et al., 2015). Due to the existence of many disulfide (S-S) cross-linkages, keratin proteins are resistant to chemical and/or mechanical disintegration (Korniowicz-Kowalska, 1997b). Only keratinolytic microorganisms and some insects, such as moths, are capable of degrading keratin effectively because they secrete keratinases, which may work in tandem with other enzymes to break down complicated cross-linked bonds in keratin. (Lange et al., 2016; Jin et al., 2017).

protein demands in Pakistan's population are being efficiently met by more than 25,000 poultry farms. The consequence of chicken processing facilities is a significant volume of poultry waste (Abedullah and Bukhsh, 2007; Hussain et al., 2015; Khan et al., 2015). Each year, waste from slaughtering chickens totaling four million tonnes is collected (Onifade et al., 1998; Gousterova et al., 2005). However, it must be handled because this waste is linked to a number of infections. Typically, dirt is burned or disposed of with poultry manure (Suzuki et al., 2006; Ghaffar et al., 2018). Only a few research have examined the degradation of fungal keratin, whereas most have concentrated on the production of keratinase by bacteria (Gupta and Ramnani, 2006; Brandelli et al., 2010; Korniowicz-Kowalska and Bohacz, 2011; Gupta et al., 2013; Sahni et al., 2015). In the environmentally friendly and cost-effective treatment of keratin waste, fungal keratinases may be a key component (Gradisar et al., 2005; Huang et al., 2015).

Keratinases are produced by filamentous fungi employing two different fermentation processes. Solid-state fermentation (SSF) and submerged fermentation (SmF) are examples of these fermentation processes (Battaglino et al., 1991; Krishna, 2005). According to Subramaniyam and Vimala (2012), SmF provides more than 75% of the total enzymatic output, which plays a significant role in the manufacturing of enzymes on an industrial scale. For microorganisms that require high moisture concentrations for development, the SmF technique is more suited (Subramaniyam and Vimala, 2012). Microbes are cultivated using the SSF method on a wet and solid medium (Pandey, 2003; Hölker and Lenz, 2005; Mitchell et al., 2006). The goal of the current study was to identify, characterize, and use keratinolytic fungi for the treatment of chicken (keratin) waste using SmF and SSF methods. This was done in consideration of the remarkable therapeutic characteristics of fungi.

## MATERIALS AND METHODS

### Collection of samples and establishment of a pure culture of a keratinolytic fungus strain

Decaying feathers from feather-dumping sites in the region of Kasur were gathered in order to isolate keratinolytic fungi. Applied and Environmental Microbiology Laboratory, Department of Wildlife and Ecology, University of Veterinary and Animal

Sciences, Lahore (Ravi Campus, Pattoki), Pakistan received the collected samples and processed them here. Isolation of fungal strains was done through Keratin Bait Technique. The isolated fungal strain was then pure cultivated.

Species identification

Based on macroscopic and microscopic characteristics, fungi in pure culture were morphologically recognized. The fungal colony's macroscopic characteristics, including form, size, colour, and texture, were examined by colouring the fungus with lactophenol-cotton blue. Microscopic characteristics such as septation of hyphae, phialides, vesicles, and conidiophores were visible.

**The isolation of the fungus was identified molecularly.**

By using 18S rRNA gene sequencing, the pure cultivated keratinolytic fungal isolate was subsequently recognized at the molecular level (Jing et al., 2015; Saini et al., 2015). The required sequence was derived from the forward and reverse sequences, and the sequence editing was done using Bio-Edit sequence alignment editor version 7.0.9.0 (Tom Hall, Ibis Biosciences, Carlsbad, California). Using the Clustal W program of the Molecular Evolutionary Genetics Analysis (MEGA 6) software from GenBank were arranged beside the most similar recovered sequence (Tamura et al., 2013). According to Saitou and Nei (1987), the Neighbor-Joining technique was utilized to determine the evolutionary history. In the bootstrap test (100 repetitions), duplicate tree percentages were mixed with closely related taxa next to the branches (Felsenstein, 1985). The evolutionary distances used to determine the phylogenetic tree's branch lengths were in the same units as the scaled-down tree's branch lengths. According to Tamura and Nei (1993), the evolutionary distances were computed using the number of base substitutions per site. The gamma distribution was used to display the rate difference between locations (shape parameter = 1). The total number of places and data points in the final dataset was 734.

**Optimizing the fungal isolates growing conditions**

The fungal isolate was inoculated on Malt Extract agar (MEA) following molecular analysis, and it was cultured at 28oC for 14 days. Then, many factors were optimized, including temperature, pH, inoculum size, and incubation time. For the purpose of temperature optimization, fungal spores were cultured for 7 days at 20, 30, and 40oC on Petri plates with MEA. At each temperature, a different number of colonies appeared. For pH optimization, the number of colonies on each petri plate was counted after 7 days of incubation at 28oC with pH 5, 7, and 9. By inoculating petri plates with 0.1, 0.2, 0.3, 0.4, and 0.5 mL of fungal spores and incubating at 28oC for 7 days, colonies were counted to determine the ideal inoculum size. The number of colonies was counted every day for a period of five days after the inoculated petri plates were incubated at 28oC.

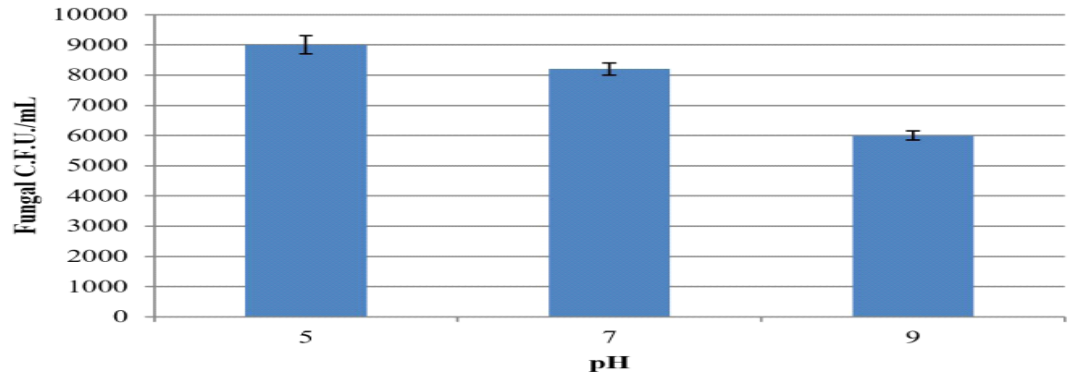


**Figure 1.** Optimization of pH for the fungal strain by using MEA.

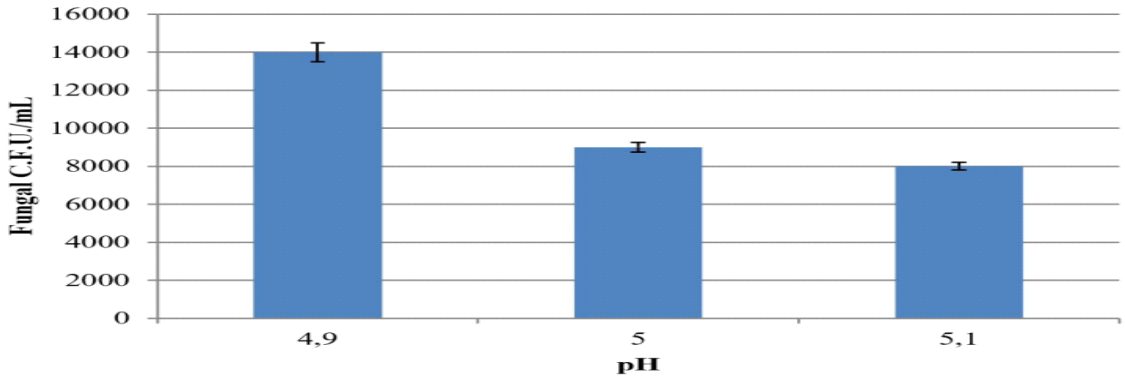


**Figure 2.** Optimization of pH for the fungal strain by using MEA.

**Feather degradation ability of fungal isolates under SmF and SSF**

The ability of the fungal isolate to degrade feathers in SmF and SSF was assessed using dried feather meal (2% w/v) as a growth substrate and basal salt medium had the following composition (gm per liter): K2HPO4, 1; KH2PO4, 0.46; MgSO4.7H2O, 0.05; H2O, 1000 ml). Basal medium and feather meal were used to make the inoculum as a source of carbon and energy. 100 ml of the autoclaved media was added to a sterile 250 ml Erlenmeyer flask along with 1 g of feather meal. Through the use of a sterile inoculating loop, fungal spores were inoculated into the medium. pH was kept at 4.8 and the flask was incubated at 28oC. After 14 days, the maximum growth was seen. For all subsequent tests, a fungal inoculum was created using this culture.

To completely remove moisture, feathers were properly cleaned with distilled water and then oven dried at for some time. The experiment was carried out in sterile 250 ml Erlenmeyer flasks with 100 ml of basal medium and 1 gm of sterile feathers. After being autoclaved and cooled, flasks containing medium and feathers were inoculated with fungal spores. Six sets of flasks with medium inside were inoculated with 0.5 ml of the fungal spores and incubated at pH 4.8 and 28oC. Triplicates of each experiment were carried out. The control flasks did not have any inoculation. For solid-state fermentation, flasks were incubated at 28oC under static conditions whereas for submerged fermentation flasks were incubated at 28oC, 110 rpm for 30 days. Feathers were

dried for some hours at 80oC to eliminate moisture and weighed to measure the degree of deterioration caused by the isolated fungal strain under solid state and SmF. Weight variations were seen in each pair of flasks.

**STATISTIC EVALUATION**

Using General Linear Model (GLM) techniques and a factorial layout, the data were analyzed using a Completely Randomised Design (CRD). Duncan's Multiple Range (DMR) test was used to separate the means with the aid of SAS 9.1 for Windows (SAS Institute Inc., 2002). At $P < 0.05$, differences between means were deemed significant.

**RESULTS**

Isolation of a pure culture of keratinolytic fungal strain

The goal of the current investigation was to separate a keratinolytic fungal strain from feather byproducts. The isolated strain was pure cultivated, and its phenotype and genotype were further determined.

Species identification

On the MEA medium, the isolated fungal strain showed maximal growth in 3 days. The colony was spherical in shape and had a powdery texture. Its colour was olive green with white margins. It was possible to see rough spiny conidiophores containing vesicles by colouring the fungal hyphae. The vesicles were globose, the hyphae were non-septate, the conidia were terminal, and the vesicles were covered in loosely radiating uniseriate phialides. Conidia were immediately linked with vesicles and there were no metulae.

The fungal isolate was identified molecularly

BLAST search of its 18S rDNA nucleotide sequence showed that it belonged to the Aspergillus genus and had a 98% similarity with Aspergillus flavus (ATCC 16883).

Optimizing the fungal isolate's growing conditions

Most colonies were seen at pH 5 (Figures 1 and 2). The fungal strain displayed varying growth in response to changes in temperature, pH, inoculum size, and incubation time. At 28oC, the fungal strain exhibited the fastest development (Figure 3). The fungal strain was then incubated at 28, 29, 31, and 32oC to get the best temperature. At 28oC, most colonies were visible (Figure 4). Utilizing previously optimized parameters, the inoculum size was optimized, and maximal growth was seen at this size (Figure 5). The highest number of fungal colonies were seen after 3 days of incubation at the ideal temperature, pH, and inoculum size.

**EVALUATION OF FEATHER DEGRADATION IN SMF AND SSF BY FUNGAL ISOLATES**

The use of keratin as a substrate is crucial for determining how well bacteria can break down keratin. This study evaluated the keratinolytic capacity of fungal isolates to break down chicken feathers. As time went on, keratin degradation activity rose but feather weight decreased. In both fermentation procedures, different amounts of feathers deteriorated throughout the course of successive days. Feather loss varied dramatically between SmF and SSF. Feathers initially weighed 1 g during SmF. After the incubation period of five days, the feather-reduction process was begun. No decrease was noticed in the initial five days. The largest level of feather loss, or 74%, was seen on day 30 during the incubation period. The feathers' weight was reduced by a maximum of 0.74 g. The findings revealed that keratinolytic activity rose with time: 30 days are more than 25 days, 20 days, 15 days, 10 days, and 5 days. A 0.2% drop was seen throughout the first 5 days of SSF. The largest level of feather loss, or 8%, was visible on the 20th day of incubation. After 20 days, there was no change in the feather degradation until the 30th day of incubation. On day 20, there was an 8% drop in featherweight at its greatest. The findings demonstrated that keratinolytic activity increased with time: 20 days are longer than 15 days, 10 days, and 5 days. As keratin was broken down by A. flavus in SmF and SSF, the pH shifted from acidic to alkaline. The pH rises as keratin breakdown releases S, N, and O ions as well as a number of other chemicals. On the day (30th day) when the greatest feather degradation was seen, the pH in SmF increased noticeably to 8.35 (control pH = 4.8). The maximal pH in SSF was 7.81 (control pH = 4.8) on the day that the largest feather degradation was visible (20th day). Results indicated that pH rose as keratinolytic activity rose (Figures 6 to 9).

**DISCUSSION**

The goal of the current investigation was to assess the ability of A. flavus to break down keratin in SmF and SSF. To test if a keratinolytic fungus species could degrade chicken feathers (keratin) under both conditions (SmF and SSF), it was isolated from chicken feather waste. The isolated strain was recognized as A. flavus visually, and molecular characterization revealed a stronger similarity to A. flavus. The isolated fungus possessed the morphological characteristics listed below, including a green colour, uniseriate phialides, and globose conidia. These characteristics nearly matched the findings of Hedayati et al. (2007), who said that A. flavus is either uniseriate or biseriate and ranges in colour from yellow to green. Globose to subglobose in shape is conidia.

At pH 4.8 and 28oC, this isolated fungal strain grew at the fastest rate; at 40oC, activity was reduced. According to Samapundo et al. (2007), the isolated fungus thrived at a temperature within the range of A. flavus. The highest level of keratinolytic activity, according to Cai and Zheng (2009), was recorded at 28oC. According to Kote et al. (2009), pH 5 was the pH at which A. flavus was most active. In comparison to other fungal species, A. flavus is regarded as an exceptional generator of keratinase (Friedrich et al., 1999). With shaking conditions for bacteria and static conditions for fungus, SmF is mostly employed for keratinase synthesis (Riessen and Antranikian, 2001; Nam et al., 2002). Keratinase synthesis in the solid form is very briefly discussed in a few papers (De Azeredo et al., 2006; Esawy, 2007). In a 30-day study, the keratinolytic capability of submerged and solid-state fermentation was evaluated. Feather degradation of keratin by A. flavus in SmF has received a small number of reports, while fungal keratin degradation in SSF has received even fewer. According to the findings, A. flavus had 9 times more keratin degradation in SmF than in SSF. According to the majority of investigations, microorganisms degrade SSF materials more quickly than SmF. According to Mazotto et al. (2013), A. niger's keratinolytic activity was 7 times higher in SSF than in SmF. The outcomes of the current study, however, were wholly unexpected. After 5 days of incubation, the keratinolytic activity in SmF began, and it grew with time, reaching 4 weeks. At day 30 of the incubation phase, chicken feather deterioration peaked (74%). According to Korniowicz-Kowalska (1997a), the loss of substrate is an obvious indicator of keratinolytic activity. In the current investigation, keratinolytic activity on chicken feathers by A. flavus in SmF was almost twice as high as its activity in Muhsin and Hadi's (2002) study. The strain utilized in the current investigation may be characterized as strongly keratinolytic based on this percentage. According to Kunert (2000), highly keratinolytic microorganisms are those that can break down keratin

by more than 40% in just 60 days under submerged circumstances. Chrysosporium articulatum strain demonstrated 63.7% feather loss after 42 days in liquid culture, according to Bohacz (2017). This deterioration is also less than what A. flavus is already producing. While A. flavus had poorer keratinolytic activity in SSF, i.e., 8%, a strain of Chrysosporium keratinophilum displayed weaker keratinolytic activity, i.e., 35%. The 20th day had the highest level of degrading activity; after that, it remained constant. The least amount of keratin breakdown in SSF has ever been documented. The degradation of keratin is characterized by a change in pH. Evidently, the pH of the post-liquid culture media also showed a notable rise (4.8 to 8.3). In solid-state keratin fermentation, pH also tended to be more alkaline. pH variations and keratinolytic activity were directly correlated. The pH rose as keratinolytic activity did, indicating an alkaline state. In the early stages of SmF, pH was low (6.7), and at this pH, less keratinolytic activity was seen. Additionally, Cai and Zheng (2009) noted that less keratinolytic activity occurred at low pH. On the day of peak keratinolytic activity in liquid culture, a maximum pH of 8.3 was noted. According to Cai et al. (2008), the pH level of the medium rose to 8.5 during the synthesis of keratinase. Degradation of keratin, the release of keratinase, and the substantial amounts of ammonia, sulphur, and other substances are what cause the pH to move towards alkalinity (Saparrat et al. 2007). According to Kaul and Sumbali (1999), fungi with higher keratinolytic activity have a tendency to make the post-culture medium more alkaline than fungi with lower keratinolytic activity. Both Hasija et al. (1990) and Elades et al. (2010) showed similar pH changes.

The generation of keratinase, an important industrial enzyme, and the effective breakdown of feather waste may both be accomplished using A. flavus, it has been determined. Future research is needed to investigate restorative process kinetics at pilot and commercial scales for the practical application of keratinolytic fungus. The current investigation was carried out on a lab scale.

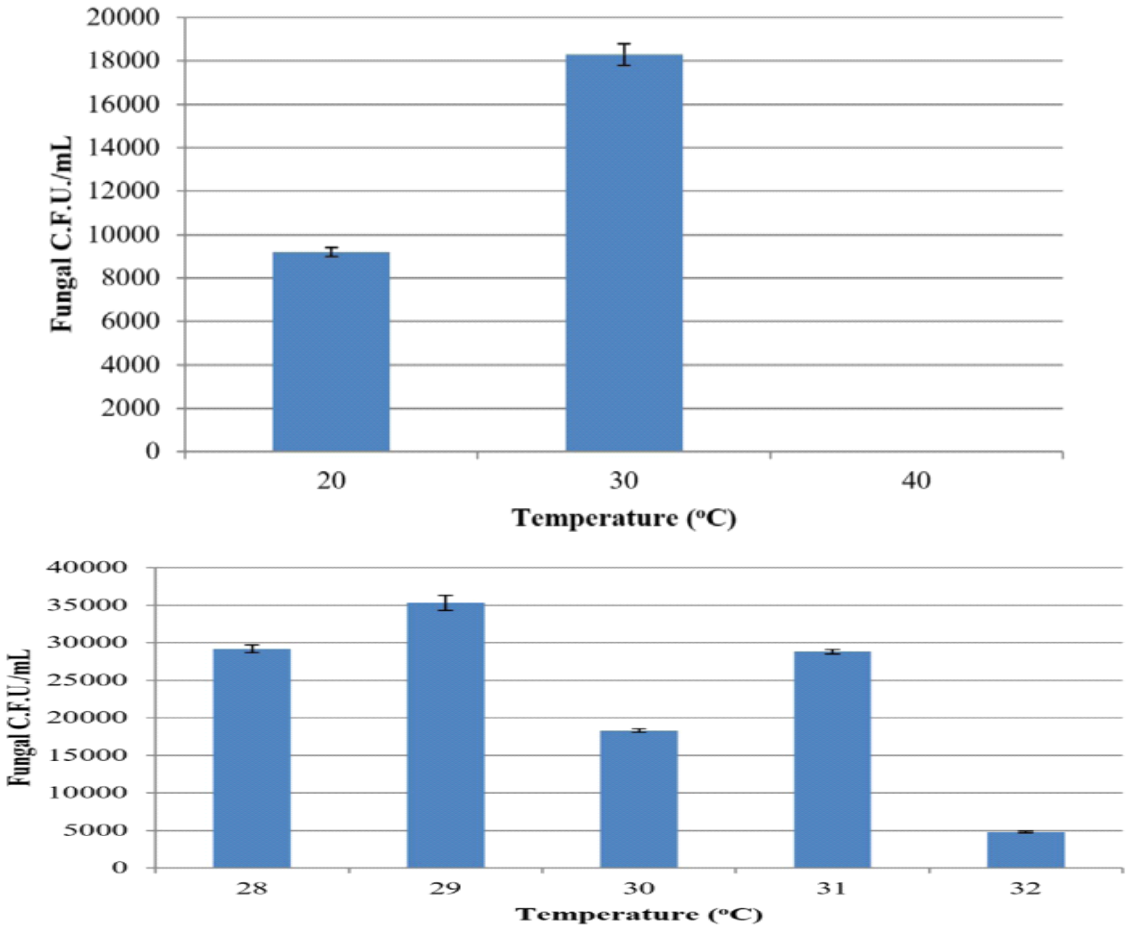


**Figure 4.** Optimization of temperature for the fungal strain by using MEA.

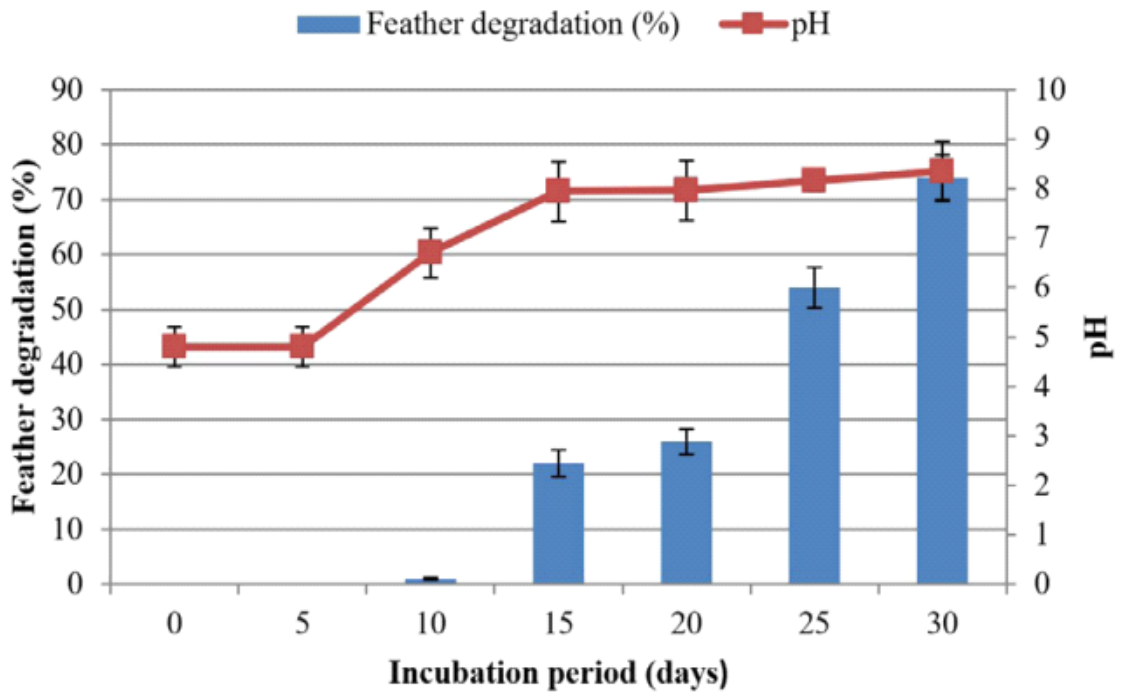


**Figure 6.** Feather degradation and pH changes in post-culture SmF medium.

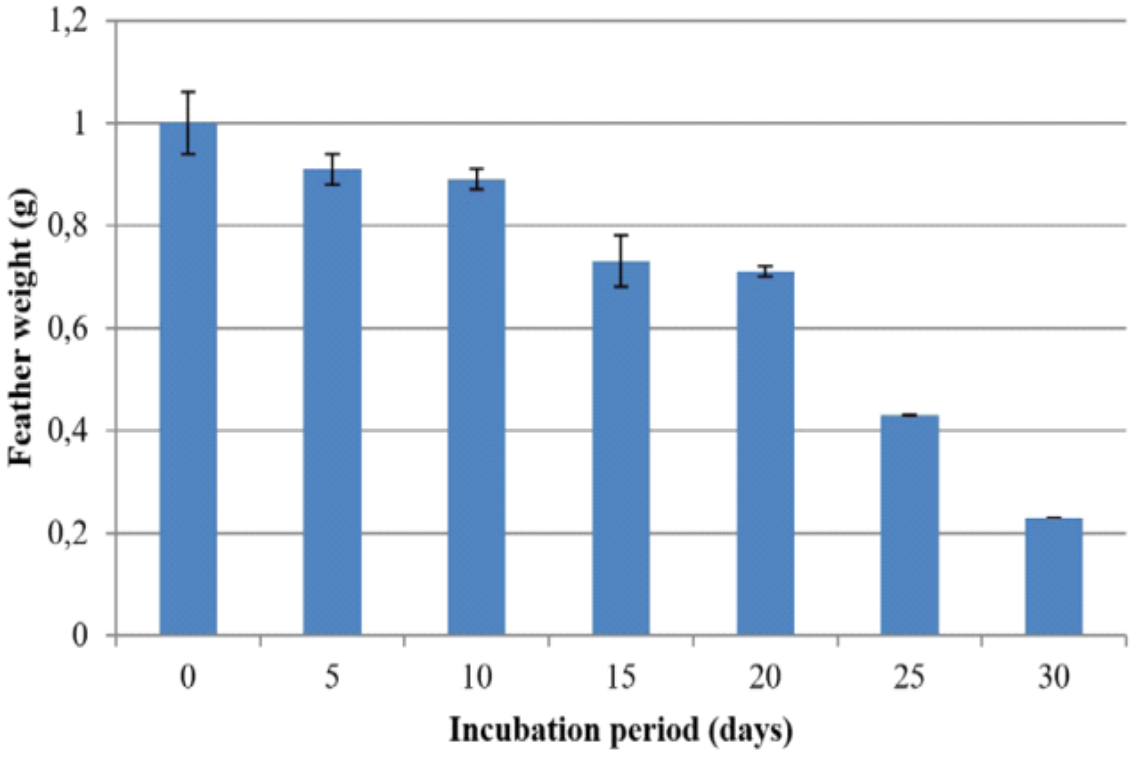


**Figure 7.** Feather weight after incubation during SmF.

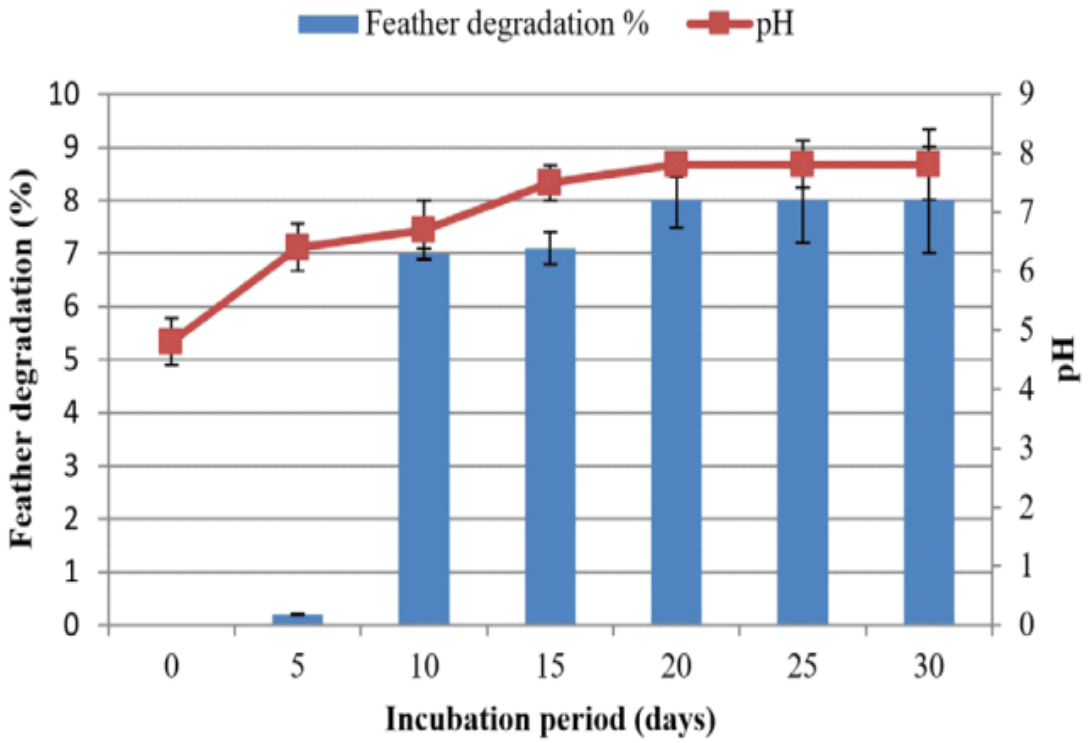


**Figure 8.** Feather reduction and pH change in post-culture medium during SSF.

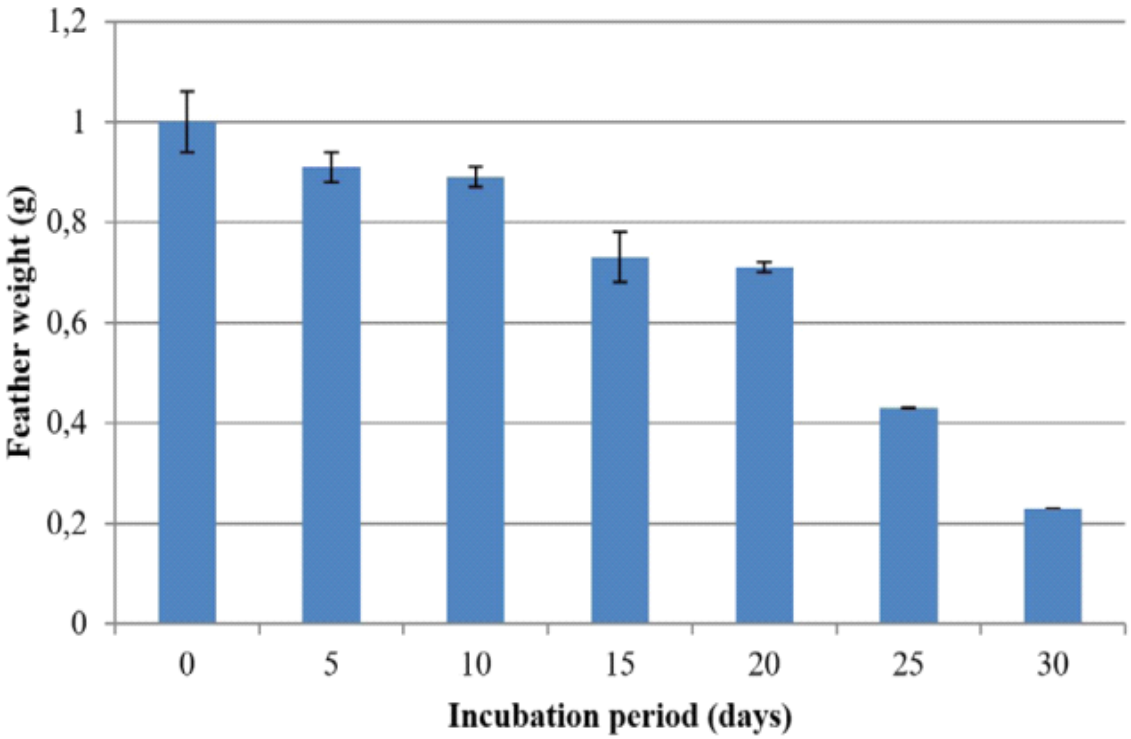


**Figure 7.** Feather weight after incubation during SmF.

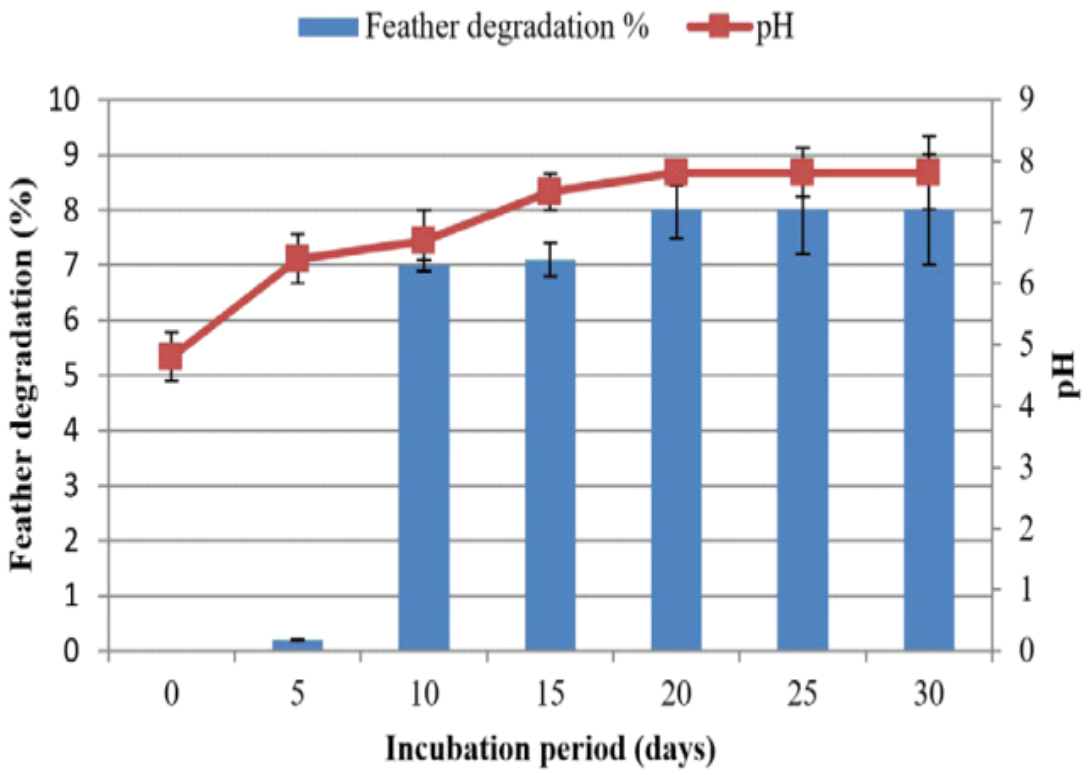


**Figure 8.** Feather reduction and pH change in post-culture medium during SSF.

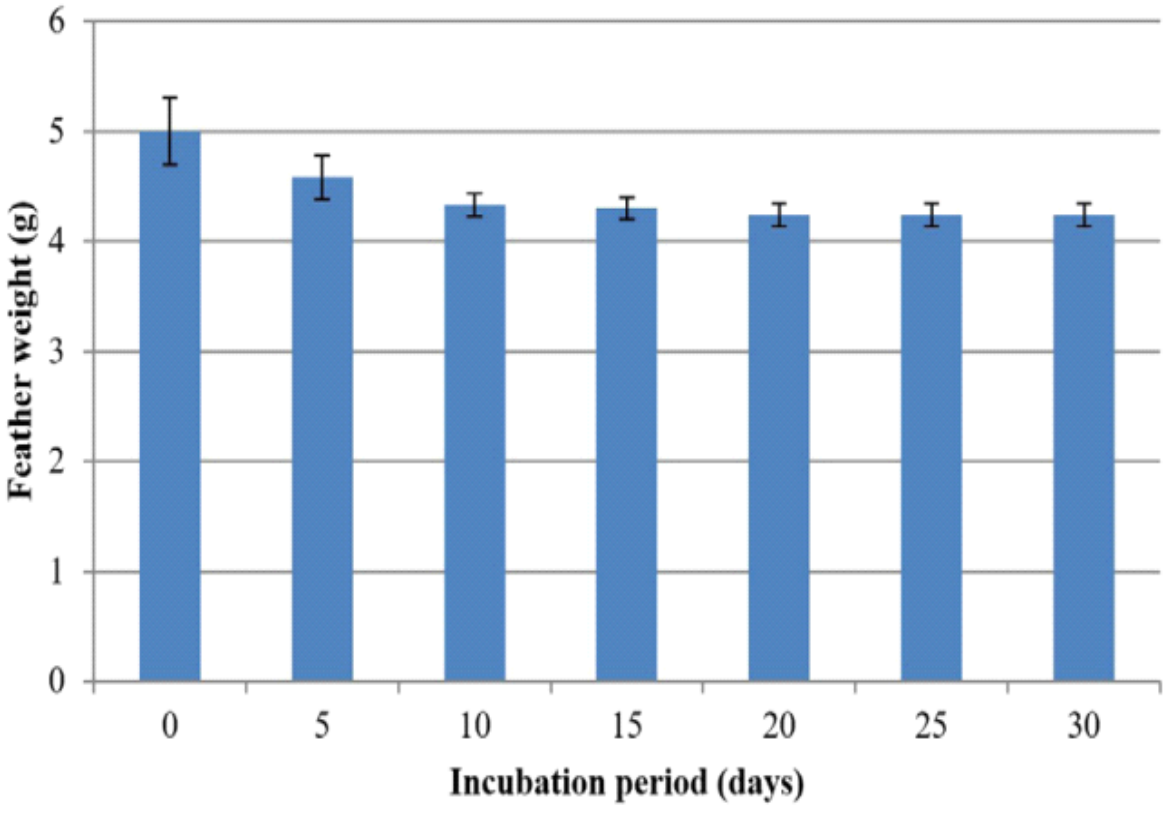


**Figure 9.** Feather weight after incubation in SSF.

**REFERENCES**

**1)** ABEDULLAH and K. BUKHSH, 2007. An examination of the problems and economics of chicken production in Pakistan's Faisalabad. 27, p. 25–28, Pakistan Veterinary Journal.

**2)** HUERGO, M., PILOSOF, A.M.R., and BARTHOLOMAI, G.B., 1991. BATTAGLINO, R.A. The conditions necessary for Aspergillus oryzae to produce protease during solid-state fermentation. 35, no. 3, Applied Microbiology and Biotechnology, p. 292-296. PMid: 22622927; DOI: 10.1007/BF00172714.

**3)** J. BOHACZ, 2017. The statistical optimisation of feather mass loss and keratinolytic soil fungus of the genus Chrysosporium's biodegradation of feather waste keratin. 33, no. 1 of the World Journal of Microbiology & Biotechnology, p. PMid:27885567, dx.doi.org/10.1007/s11274-016-2177-2.

**4)** H.H. BRAGULLA and D.G. HOMBERGER, 2009. In simple, layered, keratinized, and cornified epithelia, keratin proteins' structures and functions are described. 516-559 in Journal of Anatomy, vol. 214, no. 4. PMid:19422428. Available at: http://dx.doi.org/10.1111/j.1469-7580.2009.01066.x.

**5)** BRANDELLI, D. J. DAROIT, and A. RIFFEL. Microbiological keratinases' biochemical properties, as well as how they are produced and used. Vol. 85, No. 6, Applied Microbiology and Biotechnology, pp. 1735–1750. PMid:20039036. D.x.doi.org/10.1007/s00253-009-2398-5.

**6)** CAI, C.; ZHENG, X.D.(2009). Using the response surface approach, the medium is optimized for keratinase synthesis in the hair substrate by a novel Bacillus subtilis KD-N2. 36, no. 7, pp. 875-883, Journal of Industrial Microbiology & Biotechnology. PMid:19350297; dx.doi.org/10.1007/s10295-009-0565-4.

**7)** LOU, B.G., X.D. ZHENG, and C.G. CAI, 2008. Mutant Bacillus subtilis strain produces keratinase and breaks down keratin. Zhejiang University's journal. Science B, volume 9, issue 1, pages 60–67. PMid:18196614. http://dx.doi.org/10.1631/jzus.B061620.

**8)** L. A. I. DE AZEREDO, M. B. DE LIMA, R. R. R. COELHO, and D. M. G. FREIRE, 2006. Streptomyces sp. 594 produces thermophilic proteases in submerged and solid-state fermentations with feather meal. 641-647 in Journal of Applied Microbiology, volume 100, issue 4. the following URL: 10.1111/j.1365-2672.2005.02791.x. PMid:16553718.

**9)** A 2010 study by ELADES, L., CABELLO, M., VOGET, C., GALARZA, B., and SAPARRAT, M. fungus isolated from soils of Argentina's "Parque Costero del Sur" biosphere reserve were tested for alkaline keratinolytic activity. 2105-2111 in World Journal of Microbiology & Biotechnology, vol. 26, no. 11. The citation is 10.1007/s11274-010-0389-4.

**10)** M.A. ESAWY, 2007. Extracellular keratinase was isolated and only partially characterized from a brand-new mesophilic strain of Streptomyces albus AZA. pp. 808–817 in Research Journal of Agriculture and Biological Sciences, volume 3. 1985.

**11)** FELSENSTEIN, J. Phylogeny confidence limits: a bootstrap-based method. International Journal of Organic Evolution, Vol. 39, No. 4, Pp. 783-791, Evolution. PMid:28561359. http://dx.doi.org/10.1111/j.1558-5646.1985.tb00420.x.

**12)** FRIEDRICH, J., GRADISAR, H., MANDIN, D., and CHAUMONT, J.P. (1999). checking fungus for keratinolytic enzyme production. Pages 127–130 of Letters in Applied Microbiology, vol. 28, no. 2. http://dx.doi.org/10.1046/j.1365-2672.1999.00485.x.

**13)** GHAFFAR, A. IMTIAZ, A. HUSSAIN, A. JAVID, F. JABEEN, M. AKMAL, and J. I. QAZI, 2018. Keratinase synthesis by microbes and industrial uses: an overview. pp. 163–174 in International Microbiology, vol. 21, no. 4. PMid:30810899; dx.doi.org/10.1007/s10123-018-0022-1.

**14)** It was published in 2005 by GOUSTEROVA, A., BRAIKOVA, D., GOSHEV, I., CHRISTOV, P., TISHINOV, K., VASILEVA-TONKOVA, E., HAERTLÉ, T., and NEDKOV, P. Wastes containing keratin and collagen are broken down by recently discovered thermoactinomycetes or by alkaline hydrolysis. p. 335–340 in Letters in Applied Microbiology, vol. 40, no. 5. PMid:15836735. Available at: 10.1111/j.1472-7655.2005.01692.x

**15)** The authors are GRADISAR, H., FRIEDRICH, J., KRIZAJ, and JERALA (2005). Comparing the keratinases of Paecilomyces marquandii and Doratomyces microsporus to a few well-known proteases reveals similarities and differences between fungal keratinolytic proteases. Vol. 71, No. 7, Applied and Environmental Microbiology, p. 3420–3426. PMid:16000744, available at: http://dx.doi.org/10.1128/AEM.71.7.3420-3426.2005.

**16)** R. GUPTA and P. RAMNANI, 2006. An overview of potential uses for microbial keratinases. Vol. 70, No. 1, Applied Microbiology and Biotechnology, pp. 21–33. PMid:16391926; dx.doi.org/10.1007/s00253-005-0239-8.

**17)** GUPTA, R., SHARMA, R., and BEG, Q.(2013). The next generation of proteases for sustainable biotechnology: revisiting microbial keratinases. 33, no. 2, Critical Reviews in Biotechnology, pp. 216-228. PMid:22642703. Available at: http://dx.doi.org/10.3109/07388551.2012.68 5051.

**18)** HASIIA, S. K.; MALVIYA, H. K.; RAJAK, R. C.(1990). Some of the fungi found on the campus of the gelatin plant in Jabalpur, MP, have keratinolytic properties. 305–309 in Proceedings of the National Academy of Science India, volume 60.

**19)** A.C. PASQUALOTTO, M.T. HEDAYATI, P. WARN, P. A. BOWYER, and D. W. DENNING, (2007). Human pathogen, allergen, and mycotoxin producer Aspergillus flavus. 1677–1692 in Microbiology, volume 153, no. 6. PMid:17526826. http://dx.doi.org/10.1099/mic.0.2007/007641-0.

**20)** U. HLKER and J. LENZ, (2005). Are there any biotechnological benefits to solid-state fermentation? pp. 301-306 in Current Opinion in Microbiology, vol. 8, no. 3. PMid:15939353, http://dx.doi.org/10.1016/j.mib.2005.04.006.

**21)** Y. HUANG, P. K. BUSK, F. A. HERBST, and L. LANGE, 2015. Genome and secretome studies shed light on the new proteases from the nonpathogenic fungus Onygena corvine that break down keratin. Vol. 99, No. 22, Applied Microbiology and Biotechnology, pp. 9635-9649. PMid:26177915, dx.doi.org/10.1007/s00253-015-6805-9.

**22)** AHMAD, H., HUSSAIN, J., RABBANI, I., and ASLAM, S., 2015. An overview of Pakistan's poultry business. pp. 689–700 in World's Poultry Science Journal, volume 71, no. 4. PMid:26696690. DOI: 10.1017/S0043933915002366.

**23)** It was published in 2017 by JIN, H.S., PARK, S.Y., KIM, K., LEE, Y.J., NAM, G.W., KANG, N.J., and LEE, D.W. keratin substrates derived from recombinant chicken feathers were used to develop a keratinase activity test. e0172712 in PLoS One, volume 12, no. 2. PMid:28231319. Available at: http://dx.doi.org/10.1371/journal.pone.0172712.

**24)** A 2015 study by JING, L., ZHAO, S., XUE, J.L., ZHANG, Z., YANG, Q., XIAN, L., and FENG, J.X. Cellulase-hydrolyzed sugarcane bagasse was used as a carbon source in the isolation and characterisation of a new Penicillium oxalicum strain Z1-3 with improved cellobiohydrolase production. 77, p. 666–675. Industrial Crops and Products. http://dx.doi.org/10.1016/j.indcrop.2015.09.052.

**25)** 1999. KAUL, S., and SUMBALI, G. A comparison of the production of extracellular keratinases by keratinophilic fungi growing on the feathers of live chickens (Gallus domesticus). 19–24 in Mycopathologia, vol. 146, no. 1. the following URL: 10.1023/A:1007086720237.

**26)** In 2015, KHAN, M.B., ASAR, A.U., and NAWAZ, A. generating electricity from chicken farms at a lower cost and to the grid. Vol. 123, No. 2, pp. 5-8 of the International Journal of Computers and Applications. Ijca2015905233, available at http://dx.doi.org/10.5120.

**27)** 2011; KORNIOWICZ-KOWALSKA, T.; BOHACZ, J. Keratin waste biodegradation: theoretical and practical considerations. Waste Management (New York, New York), volume 31, issue 8, pages 1689–1701. PMid:21550224. Available at: 10.1016/j.wasman.2011.03.024.

**28)** Studies on the breakdown of keratin wastes by saprotrophic microfungi, KORNIOWICZ-KOWALSKA, T. 1997a. 1. standards for assessing keratinolytic activity. Pages. 51–79 in Acta Mycologica, vol. 32, no. 1. http://dx.doi.org/10.5586/am.1997.006.

**29)** TRINA KORNIOWICZ-KOWALSKA, 1997b. studies on how saprotrophic microfungi break down waste keratin. P. II. Balance of nitrogen and sulphur. 81–93 in Acta Mycologica, vol. 32, no. 1. http://dx.doi.org/10.5586/am.1997.007.

**30)** KOTE, N.V., A.G.G. PATIL, and V.H. MULIMANI, 2009. Thermostable endo-1,4 mannanase production from newly identified Aspergillus niger gr and Aspergillus flavus gr strains is optimised. Vol. 152, No. 2, Applied Biochemistry and Biotechnology, pp. 213-223. PMid:18597050. Available at: http://dx.doi.org/10.1007/s12010-008-8250-z.

**31)** DUMAS, P., KREPLAK, L., DOUCET, J., and BRIKI, F. 2004. New features of stretched hard keratin fibres' transition from helix to sheet. 640–647 in Biophysical Journal, vol. 87, no. 1. PMid:15240497. Available at: http://dx.doi.org/10.1529/biophysj.103.036749.

**32)** S. KRISHNA, 2005. Infrared photodetectors using quantum dots in a well. 2142-2150 are found in Journal of Physics D, Applied Physics, vol. 38, no. 13. The URL is 10.1088/0022-3727/38/13/010.

**33)** KUNERT, 2000. keratinophilic fungus physiology. KUSHWAHA, R.K.S., and GUARRO, J., editors. Biology of other keratinophilic fungi and dermatophytes. Revista Iberoamericana de Micologia, 77–85, Bilbao.

**34)** 2014; LANGE, L.; BUSK, P.K.; and HUANG, Y. keratinaceous materials are degraded using a microbial composition. Denmark. WO 2014/169920 A2, 23 October 2014.

**35)** 2016; LANGE, L.; HUANG, Y.; BUSK, P.K. Keratin degradation by microorganisms in nature: a novel theory with application to industry. 2083–2096 in Applied Microbiology and Biotechnology, vol. 100, no. 5. PMid:26754820. Dx.doi.org/10.1007/ s00253-015-7262-1.

**36)** VERMELHO, A.B., DAMASO, M.C.T., COURI, S., and MAZOTTO, A.M., 2013. Comparison of submerged and solid-state fermentation in the degradation of feather waste by Aspergillus niger keratinases. 85, 189–195, International Biodeterioration & Biodegradation. https://dx.doi.org/10.1016/j. ibiod.2013.07.003.
**37)** MCKITTRICK, P. Y. CHEN, S. G. BODDE, W. YANG, E. E. Novitskaya, and M. A. Meyers, 2012. the keratin's composition, purposes, and mechanical characteristics. 64th volume of Journal of Management, pages 449-468. http://dx.doi.org/10.1007/s11837-012-0302-8.
**38)** M.A. MEYERS, P. CHEN, A. Y. LIN, and Y. SEKI, 2008. Structure and mechanical characteristics of biological materials. Pages 1–206 of Progress in Materials Science, volume 53, no. 1. 10.1216/j.pmatsci.2007.05.002, http://dx.doi.org/10.1016/j.
**39)** LUZ, L.F.L., KRIEGER, N., and BEROVIC, M., MITCHELL, D.A., et al., 2006. solid-state fermentation bioreactors. Oxford: Springer. http://dx.doi.org/10.1007/3-540-31286-2.
**40)** T.M. MUHSIN and R.B. HADI, 2002. keratin substrates are degraded by fungi that were isolated from sewage sludge. 185–189 in Mycopathologia, vol. 154, no. 4. PMid:12206319. DOI: 10.1023/A:1016335623534.
**41)** CHOE, E.A., HWANG, J.K., SUHARTONO, M.T., and PYUN, Y.R., 2002. NAM, G.W., LEE, D.W., LEE, H.S., LEE, N.J., KIM, B.C. Fervidobacterium islandicum AW-1, a recently discovered thermophilic anaerobe that produces keratinase, degrades native feathers. 538-547 may be found in Archives of Microbiology, vol. 178, no. 6. PMid:12420177; dx.doi.org/10.1007/s00203-002-0489-0.
**42)** AL-SANE, N.A., AL-MUSALLAM, A.A., and ALZARBAN, S., 1998. ONIFADE, A.A. Review of the potential biotechnological uses of keratin-degrading bacteria and their enzymes for enhancing the nutritional value of feathers and other keratins as sources for animal feed. pp. 1–11 in Bioresource Technology, vol. 66, no. 1. https://dx.doi.org/10.1016/S0960- 8524(98)00033-9.
**43)** PANDEY, 2003. fermentation in a solid state. pp. 81–84 in Biochemical Engineering Journal, vol. 13, no. 2-3. the following URL: 10.1016/S1369-703X(02)00121-3.
**44)** 2001. RIESSEN, S., and ANTRANIKIAN, G. discovery of the new thermophilic, anaerobic bacteria Thermoanaerobacter keratinophilus sp. nov. having keratinolytic activity. Pages 399–408 of Extremophiles, volume 5, issue 6. URL: 10.1007/s007920100209, PMID: 11778841.
**45)** PHUTELA, U.G., SAHOTA, P.P., and SAHNI, N., 2015. Review of bacterial keratinases and potential uses for them. pp. 768–783 in International Journal of Current Microbiology and Applied Sciences, volume 4.
**46)** SAINI, R., SAINI, J.K., A. MATHUR, A. PATEL, D. TULI, and R. R. SINGHANIA, 2015. Penicillium oxalicum increased cellulase synthesis for use in bioethanol. 240–246 in Bioresource Technology, vol. 188. PMid:25661515; DOI: 10.1016/j.biortech.2015.01.048.
**47)** 1987. SAITOU, N., and NEI, M. An innovative technique for rebuilding phylogenetic trees is the neighbor-joining approach. 4, no. 4, Molecular Biology and Evolution, p. 406-425. PMid:3447015.
**48)** SAMAPUNDO, S., DEVLIEGHERE, F., GEERAERD, A.H., DE MEULENAER, J.F. VAN IMPE, and J. DEBEVERE. modelling of the individual and combined effects of temperature and water activity on Aspergillus parasiticus and Aspergillus flavus radial growth on maize. pp. 517-529 in Food Microbiology, vol. 24, no. 5. PMid:17367685, available at: 10.1016/j.fm.2006.07.021.
**49)** ARAMBARRI, A.M., SAPARRAT, M.C.N., and BALATTI, P.A., 2007. Ulocladium botrytis LPSC 813 grown on carboxymethylcellulose within a pH range exhibits a growth response and extracellular enzyme activity. 44, no. 2, Biology and Fertility of Soils, 383-386. https://doi.org/10.1007/ s00374-007-0217-7.
**50)** Version 901 of Statistics from SAS Institute Inc. SAS Institute Inc., 2002, Cary, N.C.
**51)** R. SUBRAMANIYAM and R. VIMALA, 2012. A comparison of solid state and submerged fermentation for the synthesis of bioactive compounds. 480–486 in International Journal of Science and Nature, volume 3.
**52)** A study from 2006 by SUZUKI, Y., TSUJIMOTO, Y., MATSUI, H., and WATANABE, K. Animal proteins that are incredibly difficult to breakdown are broken down by thermophilic bacteria. 73–81 are included in Journal of Bioscience and Bioengineering, volume 102, no. 2. PMid: 17027867; DOI: 10.1263/jbb.102.73.
**53)** 1993. TAMURA, K., and NEI, M. estimation of the number of nucleotide alterations in the mitochondrial DNA's regulatory region between chimps and humans. 10, no. 3, Molecular Biology and Evolution, 512-526. PMid:8336541.

**54)** A 2013 study by TAMURA, K., STECHER, G., PETERSON, D., FILIPSKI, A., and KUMAR, S. Version 6.0 of the Molecular Evolutionary Genetics Analysis, or MEGA6. 30, no. 12, Molecular Biology and Evolution, p. 2725–2729. PMid:24132122, dx.doi.org/10.1093/molbev/mst197.