https://doi.org/10.48047/AFJBS.6.15.2024.6292-6303

African Journal of Biological Sciences

Journal homepage: http://www.afjbs.com

Research Paper Open Access

Bio-Hydrolysation of Chicken Feather Waste and Fish Waste Using *B. licheniformis* and *B. subtilis* for the Production of Organic Liquid Fertilizer

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Volume 6, Issue 15, Sep 2024

Received: 15 July 2024

Accepted: 25 Aug 2024

Published: 05 Sep 2024

doi: 10.48047/AFJBS.6.15.2024.6292-6303

Abstract

In this present investigation, the amino acids and keratin proteins rich in chicken feather waste and fish wastes were hydrolysed into organic liquid fertilizer by bio- hydrolysation using *B. licheniformis* and *B. subtilis*. With the optimized bioprocess parameters such as 10 ml inoculum volume, incubation temperature at 37 ℃, agitation rate at 175 rpm for the incubation of degradation medium at 8.5 pH value with 12.5 g of chicken feathers separately, 12.5 g of fresh solid fish separately and 12.5 g of combined waste of chicken feathers and fresh solid fish waste separately, bio-hydrolysation was established. Incubated flasks were observed at the different time intervals of the 1st day, 4th day, 8th day, and 12th day and samples were collected after the 12th day. Corresponding collected samples were filtered and centrifuged. The supernatants obtained were analysed to determine nitrogen content as 7500 mg/L, 4240 mg/L, and 6104 mg/L, phosphorous content as 1295 mg/L, 2100 mg/L, and 1850 mg/L, potassium content as 4710 mg/L, 5608 mg/L, and 5300 mg/L, total amino acid content as 4.38%, 2.15%, and 3.96% and total antioxidant capacity as 190 µg/ml, 240 µg/ml and 228 µg/ml, respectively. Thus, the liquid fertilizer was produced by an eco- friendly approach through microbial degradation.

Keywords: Organic liquid fertilizer, Chicken feather waste, Fish waste, Bio-hydrolysation, Digestion, Nitrogen content

Introduction

In industrial processes through a biotechnological approach, the cultivation of microorganisms in nutritional media is essential and of economic importance to aid their growth. Preparing the media using organic nitrogen sources is more expensive (Subosa et al., 2016). Therefore, the detection of novel sources of low-cost primary source of nitrogen for microbial culture media is an important approach in industrial and biotechnological research (Salamony et al., 2024). One of the cost-effective approaches to producing low-cost primary sources of nitrogen media is the byproducts of protein hydrolysis (Ali et al., 2021). Moreover,bioactive peptides resulting from animal proteins are known for their biotic action and helpfuleffects on plants (Moreno-Hernández et al., 2020)

The global production of chicken feathers is regarded as a major source of bioactive peptides and amino acids (Romero-Garay, 2022). On the other hand, the presence of amino acids, soluble proteins, and peptides in hydrolysate promotes the growth of microorganisms in the rhizosphere, which enhances nutrient uptake and utilization from the soil. Hydrolysate enhances soil's water-holding capacity, C/N ratio, and mineral content (Bhari et al., 2021).

However, the old protein extraction methods such as acid-base and high-temperature treatments have many restrictions, including high energy involvement, unmanageable process, denaturing of amino acids, reduced product quality, and decline in the development of microorganisms (Sharma et al., 2022; Chen et al., 2022; Li, 2021). In the present world, microbial degradation of keratin waste into value-added products has appeared as an alternate green environmental technology that helps in financial development and eco-friendly sustainability (Shen et al., 2022; El-ghonemy and Ali, 2021; Li et al., 2020; Huang et al., 2020).

Bacterial and fungal keratinolytic microorganisms were utilized by many researchers (Mini et al., 2017; Zhang et al., 2016) to degrade chicken feathers because it is an environmentally friendly process (Fang et al., 2017; Daroit and Brandelli, 2014). Hydrolyzation of chicken feathers was previously reported by many researchers using *B. megaterium* KC405251 (Iqtedar et al., 2017), B. subtilis DB 100 (Zaghloul et al., 2011) and *B. amyloliquefaciens* K11 (Yang et al., 2016). The hydrolyzed products of chicken feathers exhibit antioxidant properties (Wan et al., 2015).

One hundred million tons of fish are harvested worldwide per year and about fifty million tons of fish are rejected as processing waste (Hassan and Heath, 1986; Kim, 2011a). Ecofriendly organic fertilizer can be produced from the rejected fish waste using various methodologies (Kim, 2011b). Nitrogen, phosphorus, and Potassium are abundantly available in fish waste. The presence of Nitrogen supports leaf, protein, and chlorophyll development. Phosphorous promotes root, flower, and fruit development. Potassium helps in stem and root growth and protein synthesis (Kim and Lee, 2009). The microbial hydrolysation of solid fish waste could be achieved and used for the production of organic liquid fertilizer for plant growth (Eguchi et al., 1998; Gao et al., 2006; Gildberg, 1992). Based on the literature, *Bacillus flexus* and *Pseudomonas stutzeri* were previously reported for the production of liquid organic fertilizer solid fish waste (Rishitha and Muralidhara Rao, 2019). Only limited studies have been established for the production of liquid organic fertilizer from the combined waste material of fresh solid fish waste and chicken feathers waste by bio-hydrolysation.

The hydrolysis of chicken feathers and fish waste into organic fertilizer via bacterial fermentation with *B. licheniformis* and *B. subtilis* has not received much attention. The current work investigates the use of chicken feathers and fish waste to produce liquid fertilizer by fermentation using *B. licheniformis* and *B. subtilis*.

Materials & Methods

Materials

All chemicals and solvents used in the present work to hydrolyse chicken feathers and fish waste were analytical grade and bought from Sisco Research Laboratory (SRL) in Mumbai, India. Fresh solid fish waste (gills, fins, head, tail, bones, viscera) and chicken feathers were gathered from Chidambaram's local fish markets and chicken stores in Tamil Nadu, India. *Bacillus licheniformis* (MCC No-2297) was obtained from the National Centre for Microbial Resources (NCMR), Pune, Maharashtra. *Bacillus subtilis* was received from Annamalai University's Microbiology Department in Chidambaram.

Methods

Bacterial culture

The nutrient agar (NA) medium (beef extract 3.0 g, peptone 5.0 g, agar 20.0 g, NaCl 0.5 g, and 1000 ml of distilled water) has been prepared and autoclaved for 20 minutes at 121 ℃ at 15 psi pressure. After autoclaving, the medium was allowed to cool before being transferred to petri plates and solidified. After burning, the sterilized inoculating loop was allowed to cool. A little amount of each bacterial strain (*B. licheniformis* and *B. subtilis*) was selected and streaked over the appropriate petri plates with solidified nutritional agar medium. The plates were incubated at 37 ℃ for 24 hours and checked for growth (Arbia et al., 2013).

Preparation of Raw Material

Chicken feathers collected from the local chicken shops were cleaned with tap water to remove blood, other impurities, and micro-organisms. The cleaned chicken feathers were pretreated by washing with warm water and soap solution. The pretreated feathers were dried in an oven at 121℃ for 5 min and cut into 1.5-2.5 cm long fibres (Figure 1). Fresh solid fish waste collected from local fish markets was cleaned in running water to remove impurities and cut into a size of less than 5 mm x 5 mm and autoclaved at 121 ℃ with 15 psi pressure for 20 minutes. After autoclaving, the fish oil was extracted from the fish waste using hot water.

Bio-Hydrolysation of Chicken Feathers by Co-cultivation of *B. licheniformis* and *B. subtilis*

The degradation medium (Glucose-3 g, KH_2PO_4-0.7 g, K_2HPO_4-1.4 g, $MgSO_4$-0.1 g, NaCl-0.1 g, distilled water-250 mL) was prepared. 12.5 g dried chicken feathers with reduced length were taken in 500 ml Erlenmeyer flasks along with degradation medium at the pH value of 8.5. Three Sets of Erlenmeyer flasks of 500 mL capacity were autoclaved with 250 mL of degradation medium and chicken feathers. After autoclaving, the flasks were cooled. The bacterial cultures (*B. licheniformis* and *B. subtilis*) were introduced (10 mL) into the conical flasks containing degradation medium with chicken feathers under aseptic conditions and one flask was kept as control without inoculation. The conical flasks containing slurry with inoculation and without inoculation were incubated in a temperature-controlled shaker at 37 ℃ and at 175 rpm for 12 days. After 12 days, the fermented feather hydrolysate was

filtered through Whatman No.1 filter paper. The filtered isolates were centrifuged at 10,000 rpm for 10 mins. 1% lactate was added to the supernatant for the purpose of storage. The supernatant was analysed for the presence of nutrients required for plant growth.

Bio-Hydrolysation of Solid Fish Waste by Co-cultivation of *B. licheniformis* and *B. subtilis*

The medium (Glucose-3 g, KH_2PO_4-0.7 g, K_2HPO_4-1.4 g, $MgSO_4$-0.1 g, NaCl-0.1 g and distilled water-250 mL) applicable for the degradation of solid fish waste was prepared. Solid fish waste of 12.5 g with the reduced size was taken in 500 mL Erlenmeyer flasks containing 250 mL of degradation medium with pH value of 8.5. Six Erlenmeyer flasks with 500 mL capacity were autoclaved with 250 mL of degradation medium and solid fish waste with reduced size. After cooling the autoclaved flasks, the bacterial strains (*B. licheniformis* and *B. subtilis*) were inoculated (10 mL) into the conical flasks containing degradation medium with solid fish waste at reduced size under aseptic conditions and one flask was used as control without inoculation. The control flask and inoculated flasks were incubated in a shaker by maintaining constant temperature of 37 ℃ at 175 rpm for 12 days. After 12 days, the fermented solid fish waste hydrolysate was filtered through filter paper with Whatman No.1. At 10,000 rpm for 10 mins, the filtered isolates were centrifuged. For storage, 1% lactate was added to the supernatant. The nutrients available for the plant growth in the supernatant was analysed.

Bio-Hydrolysation of Chicken Feathers and Solid Fish Waste by Co-cultivation of *B. licheniformis* and *B. subtilis*

The appropriate medium was prepared and used for the degradation of chicken feathers and solid fish waste (Glucose-3 g, KH_2PO_4-0.7 g, K_2HPO_4-1.4 g, $MgSO_4$-0.1 g, NaCl-0.1 g, distilled water-250 ml). 12.5 g of dried chicken feathers with reduced length and solid fish waste with the reduced size were engaged in 500 mL Erlenmeyer flasks containing degradation medium at the pH value of 8.5. Three Sets of Erlenmeyer flasks of 250 mL capacity were taken and autoclaved with 250 mL of degradation medium in the presence of dried chicken feathers with reduced length and solid fish waste with the reduced size. The autoclaved flasks were cooled. 10 mL bacterial cultures (*B. licheniformis* and *B. subtilis*) were inoculated into the conical flasks containing degradation medium with chicken feathers at the reduced length and solid fish waste at the reduced size under aseptic conditions and one

flask was considered as control without inoculation. The slurry containing in conical flasks with inoculation and without inoculation were incubated in a rotary shaker with the temperature controlled at 37^{o}C and at 175 rpm for 12 days. After 12 days, the digested feather and solid fish waste hydrolysate was filtered using Whatman No.1 filter paper. For 10 mins, the filtered isolates were centrifuged at 10,000 rpm. Lactate with 1% was introduced in to the supernatant for the storage purpose. The presence of nutrients in the supernatant was analysed for plant growth.

Statistical Analysis

Experiments conducted in the present research work were in triplicates. The nitrogen content, phosphorous content, potassium content and total amino acid content results observed from the present study were subjected to one way analysis of variance (ANOVA). With the usage of the statistical analysis, the standard deviation was analyzed and computed for the three final values obtained from the present study and the values were found to be less than 5% of mean values.

Results and Discussion

Bio-Hydrolysation of Chicken Feathers by Co-cultivation of *B. licheniformis* and *B. subtilis*

The hydrolysation of washed and dried raw chicken feathers with reduced length using the bacterial cultures (*B. licheniformis* and *B. subtilis*) was established in batch mode. The impact of bioprocess parameters such as of 10 mL inoculum volume, incubation temperature at 37 ℃, agitation rate of rotating shaker at 175 rpm during the incubation of 500 mL Erlenmeyer flasks containing 250 mL of degradation medium at the pH value of 8.5 with dried chicken feathers at the reduced length of 1.5 cm to 2.5 cm on hydrolysation was studied. Incubated flasks were observed for the hydrolysation at different time intervals such as 1st day, 4th day, 8th day and 12th day after inoculation (Figure 2). With these optimised bioprocess parameters, the hydrolysed solution of dried raw chicken feathers was filtered and centrifuged. After centrifugation, the supernatant was analysed to determine nitrogen content as 7500 mg/L, phosphorous content as 1295 mg/L, potassium content as 4710 mg/L, total amino acid content as 4.38% and total antioxidant capacity as 190 µg/mL.

From the result, it was observed that the digestion of chicken dried raw feathers was found to be increased with increase in the incubation time. The increase in incubation time may increase the activity of microorganisms (Swetlana and Jain, 2010). The presence of dried

chicken feathers may also act as a nutrient apart from digestion medium for the microbial growth and thereby increases the digestion rate. The same types of result were previously reported for the treatment of dried chicken feathers using *B. licheniformis* and *B. subtilis* (Mousavi et al., 2013).

Bio-Hydrolysation of Solid Fish Waste by Co-cultivation of *B. licheniformis* and *B. subtilis*

The solid fish waste of 12.5 g with the reduced size was hydrolysed using the bacterial cultures (*B. licheniformis* and *B. subtilis*) in batch process. The effect of various bioprocess parameters such as 10 mL size of inoculum volume, incubation temperature at 37 ℃, rate of agitation in rotating shaker at 175 rpm during the incubation of Erlenmeyer flasks (500 ml) containing degradation medium of 250 ml at the pH value of 8.5 with the solid fish waste of the reduced size on bio-hydrolysation was conducted. Incubated flasks were detected for the hydrolysation after inoculation at the different time intervals of 1st day, 4th day, 8th day and 12th day (Figure 3). By maintaining these bioprocess parameters, the hydrolysed solution of solid fish waste was filtered and centrifuged. After filtration and centrifugation, the supernatant was collected to determine nitrogen content as 4240 mg/L, phosphorous content as 2100 mg/L, potassium content as 5608 mg/L, total amino acid content as 2.15% and total antioxidant capacity as 240 µg/mL.

According to the results of bio-hydrolyzation, the digestion of solid fish waste increased as the incubation contact time increased (Hassan and Heath, 1986). The increased incubation period may increase bacteria' contact time with fish waste. The presence of solid fish waste may also serve as a nutrient for microbial strain growth, increasing digestion rate. A similar pattern of results was previously reported for the treatment of solid fish waste using *B. licheniformis* and *B. subtilis* (Kim, 2011).

Bio-Hydrolysation of Chicken Feathers and Solid Fish Waste by Co-cultivation of *B. licheniformis* and *B. subtilis*

Using the bacterial cultures (*B. licheniformis* and *B. subtilis*) in batch mode, the washed & dried raw chicken feathers with reduced length and solid fish waste with the reduced size was digested and hydrolysed. With the influence of various biochemical process parameters such as inoculum volume size of 10 mL, incubation temperature of 37 ℃, shaking speed of agitation in rotating shaker at 175 rpm for the incubation of Erlenmeyer flasks (500 mL) having the degradation medium of 250 mL at 8.5 pH value with the chicken feathers and

solid fish waste, bio-hydrolysation was established. For the bio-hydrolysation study, incubated flasks were analysed after inoculation at the different period of intervals such as 1st day, 4th day, 8th day and 12th day (Figure 4). By keeping these bioprocess parameters, the hydrolysed solution of digested medium with washed & dried raw chicken feathers with reduced length and solid fish waste with the reduced size was filtered and centrifuged. The collected supernatant after filtration and centrifugation was allowed to determine nitrogen content as 6104 mg/L, phosphorous content as 1850 mg/L, potassium content as 5300 mg/L, total amino acid content as 3.96% and total antioxidant capacity as 228 µg/ml.

As per the result obtained from the bio-hydrolyzation, the digestion of chicken feathers and solid fish waste was found to increase with increase in the incubated interaction time (Hassan and Heath, 1986). The increase in interaction time may increase the contact time of microorganisms with the chicken feathers and solid fish waste and increase in the number of collisions that occur between the molecules (Swetlana and Jain, 2010). The existence of chicken feathers and solid fish waste may also act as nutrients for the microbial strains growth and thereby increases the bio- hydrolysation reaction rate. As per the literature, the results obtained from the present study was found to have similar trend of the results previously reported for the treatment of chicken feathers and solid fish waste using bio-hydrolysation (Mousavi et al., 2013; Kim, 2011).

Conclusion

From the present investigation, fresh solid fish waste and chicken were feathers collected from local fish markets and chicken shops were effectively utilised and converted into organic liquid fertilizer by bio-hydrolyzation using *B. licheniformis* and *B. subtilis.* The production of organic liquid fertilizer is depending on the hydrolysation and digestion of fresh solid fish waste and chicken feathers. With the optimised bioprocess parameters, the samples collected from the hydrolysed solution of dried raw chicken feathers separately, fresh solid fish waste separately and combined waste material of fresh solid fish waste and chicken feathers waste separately were filtered and centrifuged. After filtering and centrifuging the corresponding hydrolysed solution, the supernatant was analysed to determine the nitrogen content phosphorous content, potassium content, total amino acid content and total antioxidant capacity. The results obtained from the present investigation revealed that the bio-hydrolysation of fresh solid fish waste and chicken feathers was found to be increased with increase in incubation time. The present investigation concluded that the fresh solid fish

wastes and chicken feather wastes were efficiently hydrolysed using *B. licheniformis* and *B. subtilis* to attain the organic liquid fertilizer through environmentally friendly approach.

Conflict of interest

Authors state that there is no conflict of interest

References

1. Ali, M.F., Hossain, M.S., Moin, T.S., Ahmed, S. and Chowdhury, A.M.S. (2021). Utilization of waste chicken feather for the preparation of eco-friendly and sustainable composite. Clean Eng Technol. Volume 4, 100190, ISSN 2666-7908, https://doi.org/10.1016/j.clet.2021.100190

2. Arbia, W., Arbia, L., Adour, L. and Amrane, A. (2013). Chitin extraction from crustacean shells using biological methods–a review. Food Technol Biotech. Mar 29, 51(1), 12-25.

3. Bhari, R., Kaur, M. and Singh, R.S. (2021). Chicken feather waste hydrolysate as a superior biofertilizer in agroindustry. Curr Microbiol. 78, 2012–2230.

4. Chen, H., Gao, S., Li, Y., Xu, H, Li, W. and Wang, J. (2022). Valorization of livestock keratin waste: application in agricultural fields. Int J Environ Res Publ Health. 19, 6681, https://doi.org/10.3390/ijerph19116681Academic.

5. Chen, Q. (2021). Structure, application, and biochemistry of microbial keratinases. Front Microbiol. 12, 674345, https://doi.org/10.3389/fmicb.2021.674345.

6. Daroit, D.J. and Brandelli, A. (2014). A current assessment on the production of bacterial keratinases. Crit. Rev. Biotechnol. 34:372–84. https://doi.org/10.3109/07388551.2013794768

7. Eguchi, Y., Bela, J.S. and Shetty, K. (1998). Simulation of Somatic Embryogenesis in Anise (Pimpinella anisum) Using Fish Protein Hydrolysates and Proline. J herb spices & med plants. 5(3), 61-8.

8. El Salamony, D.H., Hassouna, M.S,. Zaghloul, T.I., Zhen He. and Abdallah, H.M.(2024). Bioenergy production from chicken feather waste by anaerobic digestion and bioelectrochemical systems, Microb Cell Factories. 23 (2024) 102, https://doi.org/10.1186/s12934-024-02374-5.

9. El-ghonemy, D.H. and Ali, T.H. (2021). Effective bioconversion of feather-waste Keratin by Thermo-Surfactant Stable Alkaline Keratinase produced from Aspergillus sp. DHE7 with promising biotechnological application in detergent formulations. Biocatal Agric Biotechnol. 35, 102052, https://doi.org/10.1016/j. bcab.2021.102052.

10. Fang, Z., Zhang, J., Du, G. and Chen, J. (2017). Rational protein engineering approaches to further improve the keratinolytic activity and thermostability of engineered keratinase KerSMD. Biochem Eng J. 127, 147–53. https://doi.org//10.1016/J.BEJ.2017.08.010

11. Gao, M.T., Hirata, M., Toorisaka, E. and Hano, T. (2006). Acid-hydrolysis of fish wastes for lactic acid fermentation. Bioresour Technol. Dec 1, 97(18), 2414-20.

12. Gildberg, A. (1992). Recovery of proteinases and protein hydrolysates from fish viscera. Bioresour technol. Jan 1, 39(3), 271-6.

13. Hassan, T.E. and Heath, J.L. (1986). Biological fermentation of fish waste for potential use in animal and poultry feeds. Agric wastes. 15(1), 1-5.

14. Huang, Y., Łęz, M., Her, F. and Busk, P.K. (2020). Novel keratinolytic enzymes, discovered from a talented and efficient bacterial keratin degrader. Sci Rep. 10, 10033, https://doi.org/10.1038/s41598-020-66792-2

15. Iqtedar, M., Qazi, J.I., Baqri, N., Mirza, S.S., Abdullah, R., Kaleem, A. and Naz, S. (2017). Bioconversion of agro-industrial feather waste utilizing thermophilic Bacillus megatherium D1. Romanian Biotechnol Lett. 22(1),12234-12239.

16. Kim, J.K. (2011a). Scaled-up bioconversion of fish waste to liquid fertilizer using a 5 L ribbon-type reactor. J Environ Manag. 92(10), 2441-6.

17. Kim, J.K. (2011b). Cost-effectiveness of converting fish waste into liquid fertilizer. Fish Aquat Sci. 14(3), 230-3.

18. Kim, J.K. and Lee, G. (2009). Aerobically biodegraded fish-meal wastewater as a fertilizer. Environ Res J. 3, 219-36.

19. Li, Q. (2021). Structure, application, and biochemistry of microbial keratinases. Front Microbiol. 12, 674345, https://doi.org/10.3389/fmicb.2021.674345.

20. Li, Z., Liang, S., Ke, Y., Deng, J., Zhang, M., Lu, D., Li, J. and Luo, X. (2020). The feather degradation mechanisms of a new Streptomyces sp. isolate SCUT-3. Commu. Biol. 3, 191, https://doi.org/10.1038/s42003-020-0918-0.

21. Mini, K.D., Sampath, K.S. and Mathew, J. (2017). Dehairing of cattle hide by keratinase enzyme of Aspergillus flavuss125. Int J Pharm Biol. Sci. 8: b204–209.

22. Moreno-Hernández, J.M., Benítez-García, I., Mazorra-Manzano, M.A., Ramírez-Suárez, J.C. and Sánchez, E. (2020). Strategies for production, characterization and application of protein-based biostimulants in agriculture: A review. Chil J Agric Res. 80, 274–289

23. Mousavi, S., Salouti, M., Shapoury, R. and Heidari, Z. (2013). Optimization of keratinase production for feather degradation by Bacillus subtilis. Jundishapur J Microbiol. Oct 31, 6(8).

Rishitha, M. and Muralidhara Rao, D. (2019). Bioconversion of fish waste into a liquid fertilizer and its impact on semi- arid tropical crops. Res. J. Life Sci. Bioinform. Pharm. Chem. Sci. 5(1), 903-912. 24. Romero-Garay, M.G., Montalvo-González, E., Hernández-González, C., Soto- Domínguez, A., Becerra-Verdín, E.M. and GarcíaMagaña, M.D.L. (2022). Bioactivity of peptides obtained from poultry by-products: A review. Food Chem. 13, 100181.

25. Sharma, C., Timorshina, S., Osmolovskiy, A. and Misri, J. (2022). Chicken feather waste valorization into nutritive protein hydrolysate: role of novel thermostable keratinase from Bacillus pacificus. Front. Microbiol. 13, 1–15, https://doi. org/10.3389/fmicb.2022.882902.

26. Shen, N., Yang, M., Xie, C., Pan, J., Pang, K., Zhang, H. and Wang, Y. (2022). Isolation and identification of a feather degrading Bacillus tropicus strain Gxun - 17 from marine environment and its enzyme characteristics. BMC Biotechnol. 22, 1–13, https://doi.org/10.1186/s12896-022-00742-w.

27. Subosa, B.N., Mirando, J.S., Velasquez, A.P., Alamar, V.L., Capinpin, G.G., Sablan, K.A. and De Leon, R.L. (2016). Production of Peptone From Chicken Feathers. Philipp Eng J. 37(1), 63-7

28. Swetlana, N. and Jain, P. C. (2010). Feather degradation by strains of Bacillus isolated from decomposing feathers. Braz J Microbiol. 41, 196-200.

29. Wan, M.Y,, Dong, G., Yang, B.Q. and Feng, H. (2015). Identification and characterization of a novel antioxidant peptide from feather keratin hydrolysate. Biotechnol. Lett. 38, 643–649. https://doi.org/10.1007/s10529-015-2016-9

30. Yang, L., Wang, H.L., Bai, Y., Luo, H., Shi, P., Huang, H. and Yao, B. (2016). Construction of a rapid feather-degrading bacterium by overexpression of a highly efficient alkaline keratinase in its parent strain Bacillus amyloliquefaciens K11. J. Agric. Food Chem. 64, 78–84. https://doi.org/10.1021/acs.jafc.5b04747

31. Zaghloul, T.I., Embaby, A.M. and Elmahdy, A.R. (2011). Biodegradation of chicken feathers waste directed by Bacillus subtilis recombinant cells: scaling up in a laboratory scale fermentor. Bioresour. Technol. 102, 2387–2393. https://doi.org/10.1016/j.biortech.2010.10.106

32. Zhang, R.X., Gong, J.S., Su, C., Zhang, D.D., Tian, H., Dou, W.F., Li, H., Shi, J.S. and Xu, Z.H. (2016). Biochemical characterization of a novel surfactant-stable serine keratinase with no collagenase activity from Brevibacillus parables CGMCC 10798. Int. J. Biol. Macromol. 93:843–851. https://doi.org/10.1016/j.ijbiomac.2016.09.063

Figure caption:

Figure 1. (a) Dried chicken feathers at the reduced length (b) Raw solid fish waste.

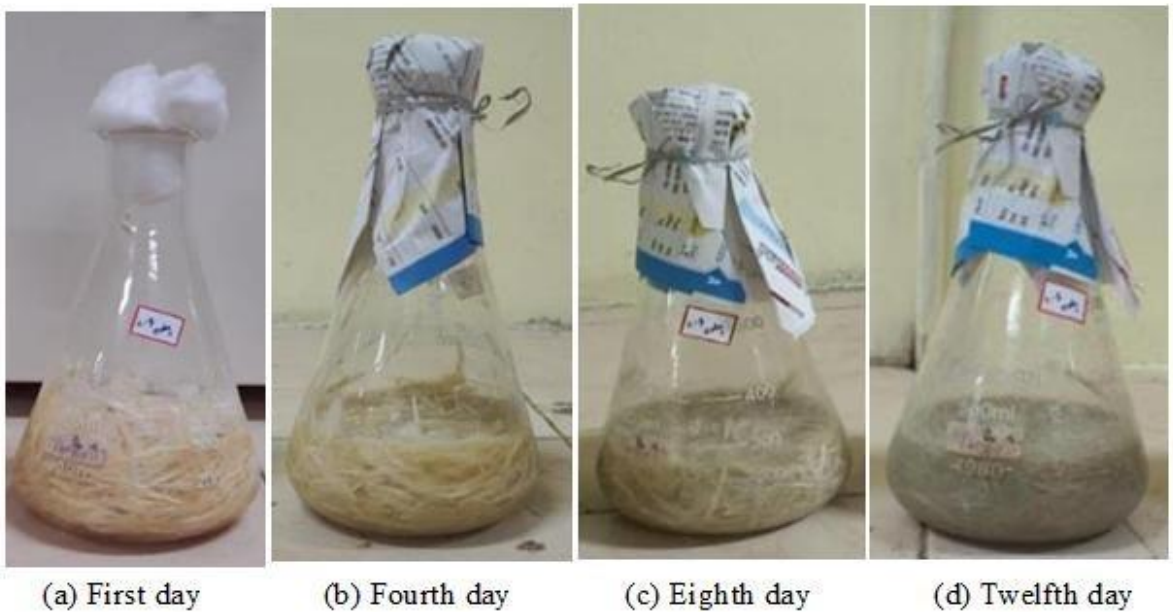

Figure 2. Bio- hydrolysation of dried chicken feathers on (a) First day (b) Fourth day (c) Eighth day and (d) Twelfth day

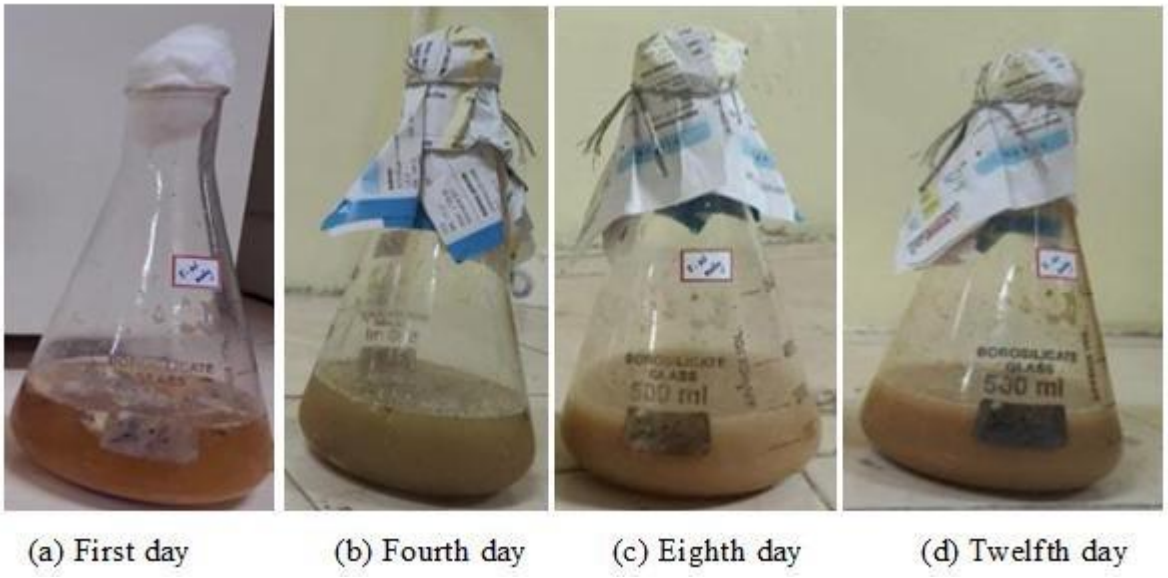

Figure 3. Bio- hydrolysation of Raw solid fish waste on (a) First day (b) Fourth day (c) Eighth day and (d) Twelfth day

Figure 4. Bio- hydrolysation of dried chicken feathers and Raw solid fish waste on (a) First day (b) Fourth day (c) Eighth day and (d) Twelfth day