

Enzymatic Pre-Treatment of Chicken Feather Waste to Generate Biogas

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ABSTRACT

Feather is produced in large amounts as a waste in poultry slaughterhouses. Only 60-70% of the poultry slaughterhouse products is edible for human. 16-20% of the slaughterhouse by-products contains keratin; from this 7-9% consists of feather with 50-70% moisture content. Treatment of poultry feather anaerobically is a challenge, because of the complex, rigid, and fibrous structure of keratin, the main component of feathers. Under anaerobic conditions, poultry feather degrades poorly, which is the main obstacle for anaerobic digestion. The reported value of chicken feather methane potential is 0.19– 0.20Nm³/kg VS, which is only one third of the theoretical value. It is an excellent raw material for biogas production due to high protein content. Dried chicken feathers contain about 90% proteins with 0.2 methane potential (m³ kg⁻¹ VS added) and 0.05 methane potential (m³ kg⁻¹ wet weight). The keratin-content of feather is difficult to digest, so physical, chemical and/or biological pre-treatment are needed in practice, which have to be set according to the utilization method. Feather was degraded with the help of enzyme and then digested in anaerobic bioreactors in different ratios. We used cattle slurry as control without feather. Our main objectives for this study were to determine the effect of enzymatic pre-treatment, biogas methane potential of feather waste and the most effective treatment ratios. Chicken feather waste can also be digested or used with other feedstock as a co-digestion substrate.

KEYWORDS: Chicken feather, methane, enzymatic, co-digestion, proteins, anaerobic digestion

INTRODUCTION

Based on our research on the poultry meat we have found that according to the FAO, the estimation of global poultry meat production was approximately 137 million tons in 2020, with growth anticipated in China, the Europe, Britain, Brazil and other countries. New investments in processing capacity are expected to increase production in the Europe and the UK, although the effects of Covid-19, as well as HPAI, are still to be determined as this data was very crucial but there were few hindrances to compile the effects of covid-19. About 5–7 % of the total weight of a normal chicken is feathers. The poultry industries produce about few billion tons of chicken feathers annually worldwide, whereas in 2020, the consumption of poultry meat in India was found to be over 4.0 million metric tons. Chicken feather waste is disposed off at landfill site as a major

practice today. The feather protein (keratin) is insoluble, respectively with high structural stability and capability of resistance for proteolytic microbes because of high degree of cross-linking by cysteine disulphide, hydrogen bonding, and hydrophobic interactions. Physical, chemical and/or biological pre-treatment are needed in practice, which has to be set according to the utilization. The biodegradation of keratin occurs for proteases specify. Many species of soil bacteria are able to degrade the keratin. The *Bacillus* spp. was identified as the most beneficial feather-degrading microbe in soil. The study was to focus on *Bacillus*, especially on *Bacillus licheniformis*. Many researchers have stated that the isolation of *Bacillus licheniformis* KK1 from nature which produce extracellular protease and can degrade well the feather because of enzymatic ability.

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Previously, feather powder was produced which was used for animal feedstuff in few parts of our country. Chicken feather has high protein which makes it an excellent raw material for biogas production. Dried feather contains 90 - 91% proteins and has 0.2 methane potential ($\text{m}^3 \text{kg}^{-1} \text{VS}$) and 0.05 methane potential ($\text{m}^3 \text{kg}^{-1} \text{wet weight}$). This paper shows the effect of enzymatic pre-treatment of chicken feather and the anaerobic digestion of pre-treated feather. Limited information is available about direct application of feather waste as raw material for biogas production and methane potential of feather waste. In this paper, we describe the most effective treatment ratios. The effect of enzymatic pre-treatments was expressed in the percentage of the controls. Feather waste recycling with anaerobic digestion provides an environmentally friendly way of utilization.

Feather Structure:

Feathers are composed of 90–95 % of proteins and 5–10 % of lipids. Keratin is the main protein component which is highly specialized fibrous protein with mechanical strength and protective abilities. Apart from this keratin is also the main component of wool, nails, hair, horn, and hoofs. Keratin is distinguished from the other structural proteins by its relatively high cysteine content, which enables it to form disulphide bonds, that serve as structural elements, thereby stabilizing the molecule. The amounts of different amino acids in feather depend on the age of the bird age, and data vary in the literature. While feathers generally have high cysteine content along with high concentrations of serine, proline, and acidic amino acids, they are deficient in some essential amino acids, like methionine and histidine.

The secondary structure of feather keratin comprises 41% α -helix and 38% β -sheet configurations, and 21% disordered regions. The polypeptide chain comprises of approximately 4 – 40 residues and the α -helix are a right-handed coil of amino acid residues, with 3.6 amino acid residues making up a complete turn of the helix. The β -pleated sheet is formed when 2 (or more) segments of the amino acid chain overlap each other. The strands are stretched out and lie parallel or antiparallel to each other. Hydrogen bonds are formed between the different polypeptide chains. The secondary structure and the cysteine content are the two most important properties; they determine the physical and chemical qualities of feathers. Feathers are insoluble in alkalis, weak acids, and water. They are very resistant against attacks by most proteolytic enzymes, as a result of the numerous inter- and intramolecular disulphide cysteine bonds, hydrogen bonds, and hydrophobic interactions.

Materials and Methods:

Slaughterhouse poultry feathers were collected from a local slaughterhouse industry in and around Pune. Chicken feathers from slaughterhouse were autoclaved at 100°C, 30 min (whole feather) and pre-treated with keratinolytic bacteria or followed autoclaving was dried at 80°C for 10 hour. It was chopped into 2-4 mm sieve (feather powder) with stainless steel homogenizer and was used by C and N content analyzing. The sterilization of wet poultry feather from slaughterhouse was completed by using an autoclave. The dry matter (DM), organic dry matter (oDM) and ash content were determined according to the standard methods. Carbon and nitrogen content of chicken feather were analyzed.

Enzymatic pre-treatment of feather:

Proteolytic enzyme, also called protease, proteinase, or peptidase is one of the most important groups of commercial enzymes. Several industries are using this kind of enzymes in a purified form, including textile, leather, dairy, and detergent industries. Keratinases are a particular type of proteolytic enzymes, possessing the ability to degrade insoluble keratin-rich substrates. Most of the keratinases reported to date are serine proteases, but a few are metalloproteinase. Keratinolytic metalloproteinase contain mainly Ca^{2+} and Zn^{2+} . Keratin degradation by enzymes is a promising technology but it has some limitations and disadvantages too. The main disadvantage of using enzymes is the high cost of the enzyme production, mostly related to the purification steps.

Anaerobic Digestion Experiment:

Anaerobic batch digestion was carried out using either feather hydrolysate or total broth of biologically pre-treated chicken feathers as substrates. The assays were carried out under mesophilic conditions ($38 \pm 1^\circ \text{C}$). The inoculum was obtained from a CSTR reactor treating municipal solid waste and cattle slurry and operating at mesophilic conditions at a municipal corporation. The inoculants were filtered through a 2-mm porosity sieve to remove large and undigested particles, and then acclimated for 5 days in an incubator at 37 °C prior to use. Substrates with loading of 0, 5, 10 and 20% amount of solubilized volatile solids for feather hydrolysate and volatile solids of both solubilized and un-degraded feathers was used, and then inoculum corresponding to 0.5 g VS was added, keeping a VS ratio (VS substrate to VS inoculum) at 1:2 in all setups. Quantity parameters of raw materials were measured before setting up experiments, and after that was calculated and average weight of dry matter

percentage and VS percentage by each of the experiments were noted down and mentioned in the Table-1 below. Table-2 shows the average parameters of untreated chicken feathers from 05 samples. The highest total solid content percentage and VS percentage was calculated by the control experiment without pre-hydrolysed feathers. The pH in each reactor was adjusted to 7.0 using hydrochloric acid solution. Water and inoculum instead of substrate were used as blank to disclose any methane production by the inoculum itself. The reactors were then placed in an incubator at 38 ± 1 °C, and they were shaken manually once a day during the incubation period of 60 days. All experimental setups were performed in triplicates. Gas samples were taken twice a day at the beginning and once a day towards the end of the digestion period from the headspace of each reactor using a pressure-tight syringe, Gas measurement and analysis were carried out with the help of a local company.

Table 1 – Average Total Solids and Volatile Solids percentage by different experiments

| Sample Number | 1 | 2 | 3 | 4 |
|--------------------------|-----|-----|-----|-----|
| Pre hydrolysed feather % | 0 | 5 | 10 | 20 |
| Total Solid % | 4.5 | 4.2 | 4.0 | 3.8 |
| Volatile Solid % | 3.6 | 3.2 | 2.8 | 2.4 |

Table 2 –Untreated Chicken Feathers showed completely different results

| Parameters | Chicken Feathers (average of 5 samples) |
|------------------------------|---|
| Total Solids % | 92 – 93 |
| Volatile Solids % of TS | 98 – 98.5 |
| Moisture % | 7 – 8 |
| Total organic Carbon % of TS | 55 |
| Total Nitrogen % of TS | 15 |
| Crude Proteins % of TS | 92 |
| C/N ratio | 3.7 |
| Fat Content % of TS | 1 |

*Note: We have found huge variations in the chicken feather parameters above ones were the average. The above table shows the untreated chicken feathers as it was observed that the moisture content varied based on the timing of collection so there was a huge variation in the samples.

Biogas production of enzyme treated feather

Feather keratin was hydrolysed by a commercial enzyme, EKABlend®, a subtilisin-like protease, with enzyme concentrations of 0.55–2.8 mL/g VS feather for 0, 2, or 24 hours at 58 °C, in order to improve the biogas yield in the resulting anaerobic digestion. According to the sCOD determination in the feather

hydrolysate, 18–40 % of the feather was solubilized after the enzymatic treatment. Enzymatic treatment under similar conditions was even more effective after an initial thermal treatment at 125 °C for 12 min, resulting in sCOD values increasing to 43–95 % of the theoretical maximum. During the following anaerobic batch digestion assays, up to 0.39 Nm³/kg VS methane yield was obtained. The combined treatment, i.e. thermal and enzymatic treatment, was less effective, with methane yields of 0.20–0.26 Nm³/kg VS. This was probably a result of undesirable compounds (e.g. ammonia) being formed. The statistical analysis revealed no interaction between the sCOD and the methane yield. Since EKABlend® is a thermophilic enzyme, able to function at 55 °C, a direct enzyme feeding strategy was also investigated. In this strategy, the enzyme is added directly to the digester, which facilitates the process, making it more economically feasible by saving time, since no additional treatment step is required. The results showed that the methane yield obtained (0.39 Nm³/kg VS) using this strategy did not differ from the yield obtained when the enzymatic treatment step was extended in time.

The biological method requires a thermal pre-treatment, followed by treatment with a recombinant enzyme possessing high keratinase activity. It is an environmentally friendly method, since it requires no application of chemicals. It is a very slow process and demands several days. Furthermore, the application of genetically modified organisms is strongly regulated, and not widely accepted. Enzymatic pre-treatment with EKABlend® is a fast and environmentally friendly method, but the process requires a relatively high enzyme load (0.45 mL/g VS feather). Consequently, the financial viability of this process greatly depends on the price of the enzyme.

Conclusion:

The compact structure of feather was successfully degraded, by using enzymatic pre-treatment method and

- The methane yield of feather was doubled compared to the yield of untreated feather, and 0.39 Nm³ CH₄/kg VS was acquired after the enzymatic pre-treatment.
- Increase in feather concentration from 5 to 20 % did not result in significant increase in the number of feathers degraded. Pre-treatment of feather wastes with Bacillus sp. was successful, as an average of 75.5 % of the chicken feather keratin was converted to soluble crude protein by the enzyme produced after 9 days of degradation.
- The economic viability of an industrial process, employing a chemical pre-treatment prior to the

anaerobic digestion, was also explored. Process capacities of at least 3650 tons /year would be viable under the suggested conditions.

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