BIOLOGICAL PRETREATMENT TO ENHANCE BIOGAS POTENTIAL OF WASTE CHICKEN FEATHERS

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ABSTRACT

The poultry industry produces large amounts of waste and has a high energy demand. Currently, most of this waste is disposed of in landfills, causing soil and water pollution. However, by properly managing and recycling this waste, valuable energy and nutrients can be obtained. Chicken feathers, rich in keratin, are a potential substrate for anaerobic digestion, which allows for waste treatment, energy production, and the generation of useful by-products such as fertilizers. Due to the insolubility and difficulty of degrading keratin, this protein must be hydrolyzed for use in anaerobic digestion. In this work, we studied the pretreatment of chicken feathers using a bacterial consortium with keratinolytic activity to obtain a substrate for methane production. Bacterial consortium was isolated from poultry farm waste disposal site and their proteolytic and keratinolytic activities were determined. Subsequently, feather hydrolysis by the bacterial consortium was evaluated by measuring free amino groups, soluble proteins, keratinase activity and volatile solids/total solids (VS/TS) ratio indicating high biodegradability, therefore demonstrating their potential for anaerobic digestion. Finally, the biochemical methane potential (BMP) of the obtained hydrolysate was determined. The biological pretreatment increased PBM to 494.74 ± 0.26 $NmLCH_4$/gVS, compared to 41.45 ± 0.22 mL $NmLCH_4$/gVS for untreated feathers.

Keywords: Agro-industrial waste; microbial pretreatment; chicken feather hydrolysate and biogas production.

1 INTRODUCTION

Poultry industry in Uruguay generates huge amounts of waste and at the same time it has a high energy demand. According to a study carried out by UdelaR and Biovalor project, 25.181 t/year of organic solid waste are produced in this sector, which affects 29% of the organic waste generated in the country (dry basis).[1,2] Currently, these wastes are disposed of in landfills or directly on the ground, posing a significant problem as they are a major source of soil and water contamination.[2] However, this waste can provide valuable energy and nutrients if properly managed and recycled.[3,4,5] One option is to use this waste to produce biogas through anaerobic digestion, which would allow poultry companies to use this renewable energy source and avoid transportation costs by treating the waste in the same plant. Additionally, the byproduct of this process can improve soils in agriculture.[6]

Feathers are an important waste product of the poultry industry because they represent between 5-7% of the body weight of domestic fowl.7 In 2015, this translated into the generation of 7,800 tons of feathers locally.[1,2] Feathers are composed of 90–92% keratin and 1–8% lipids, leading to a relatively high theoretical methane yield.[8] However, the biodegradability of feathers is relatively low due to the complex, rigid and fibrous structure of keratin that imparts high stability and resistance to degradation. Application of appropriate pretreatment methods hydrolyses the feathers and breaks down their strong structure into the corresponding amino acids and small peptides that enhance the biogas potential of the feathers.[9] Feathers can be hydrolyzed under mild conditions and with high efficiency by microorganisms. A useful strategy for obtaining microorganisms with keratinolytic activity is the isolation of microorganisms from natural environments such as samples of waste from poultry farms and raw feathers since the waste generated in agricultural farms constitutes a rich source of microbial diversity.[10] Particularly interesting is the use of a consortium of microorganisms for the degradation of keratin since an interspecific interaction between microbes can result in more robust to environmental fluctuations and microbial cooperation via complementary metabolic pathways.[11]

In the present work, we have isolated from decomposing feathers that were discarded in landfills to a bacterial consortium 8CS. The microbial diversity of 8CS was determined by 16S rRNA gene massive sequencing, showing that Bacillus and Staphylococcus genera were present at an abundance 98.21 and 1.52%, respectively. Keratinolytic activity of bacterial consortium 8CS was determined and feather microbial degradation was carried out in basal medium. Biodegradation was measured as soluble protein and free amino groups content, and keratinase activity. Physicochemical analysis of the hydrolysate was performed, and volatile solids/total solids (VS/TS) ratio was over 60% indicating high biodegradability, therefore demonstrating their potential for anaerobic digestion. Finally, feather hydrolysate produced by microbial degradation was evaluated as a substrate for biochemical methane (PBM) production.

2 MATERIAL & METHODS

***Source and preparation of chicken feathers*.** Freshly plucked white chicken feathers were collected from a poultry farm (San Ramón, Canelones, Uruguay) and transferred to the laboratory in a clean plastic bag. The chicken feathers were washed with tap water were chopped into smaller parts and sterilized at 180ºC for 60 minutes.

***Microorganism isolation*.** Waste samples from a poultry farm were taken and resuspended in minimal medium with 1% feather meal. The mixture was incubated at 37ºC with orbital shaking at 150 rpm until microbial growth was observed. This process was repeated twice under the same conditions. After microbial development, an inoculum was prepared from the final growth and used to inoculate feather meal agar plates and Tryptic Soy Agar (TSA) plates with 5% feather meal. The plates were incubated at 37°C until colonies developed. All colonies were isolated and preserved for future experiments. Bacterial strains were maintained as frozen cultures in 17% glycerol at -20°C and -70°C.

Microbial consortium identification. Microbial consortium 8CS was grow overnight in tryptone yeast extract broth (TYE) at 37°C, 200 rpm. Cells were centrifugated and DNA extracted. Massive sequencing of 16S rRNA and archaeal genes was performed. Sequencing was performed on the Ion Torrent Personal Genome Machine platform of the Instituto de Investigaciones Biológicas Clemente Estable (IIBCE). The analysis of the data obtained is carried out with the QIIME software.

Screening of proteolytic activity. From an overnight preculture of 8CS in Tryptic Soy Broth (TSB) was taken and inoculated in the center of a Petri dish with milk agar medium. The plates were Incubated at 37ºC and then the clear zone formation was examined.

Screening of keratinolytic activity. Screening for keratinolytic activity was performed in two ways: 1) an overnight preculture of 8CS was inoculated in the center of a Petri dish with minimal medium added with 1% feather meal. The plate was incubated at 37°C until growth was visualized. It was then revealed by adding a solution Coomassie Brilliant Blue G250 dye from (Sigma), destained and then the clear zone formation was examined; 2) preculture was inoculated in a test tube with minimal medium and 1 feather as a source of carbon and nitrogen. The tube was incubated at 37°C until growth was visualized and feather degraded.

Biological pretreatment of feathers by microbial consortium 8CS. Microbial consortium 8CS was grow overnight in TYE medium at 37°C, 200 rpm. Cells were centrifuged, washed, and resuspended in minimal medium and repeated twice to remove residual TYE. Cells were inoculated (OD=0.12-0.16) into minimal feather medium, incubated at 37°C, 200 rpm for 192 h.

Protein determination. The protein content was determined by Bradford's method using bovine-serum albumin (Sigma) as a standard (SIGMA) with bovine-serum albumin (Sigma) as a standard.[12]

Keratinase activity determination. Measure of keratinase activity was as described by Vermelho et al.[15]. Ninhydrin method was used for detecting free amino groups as described Sun et al.[16] and soluble protein content was done as described Church et al.[17]

Biological methane production. Methane production was conducted through anaerobic digestion of the hydrolysate obtained from feather degradation by the 8CS microbial consortium. The methanogenic biochemical potential (PBM) was evaluated in batch cultures under anaerobic conditions using an inoculum adapted for methane production, following Angelidaki et al. [18]

3 RESULTS & DISCUSSION

Microbial consortium isolation and screening of proteolytic activities. The microbial consortium 8CS was isolated from feathers discarded in landfills at a poultry farm using an enrichment technique. 8CS displayed extracellular protease activity, indicated by a clear zone when grown on milk agar plates (Fig. 1a). In a liquid minimal medium with an intact feather, complete feather degradation occurred within 72 hours (Fig. 1c), showing that 8CS produces extracellular keratinases. Additionally, this activity was evident when 8CS was streaked on feather meal plates, as a clear zone formed around the microbial growth (Fig. 1b).

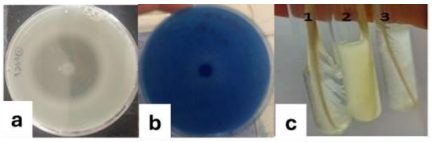


Figure 1 Screening of proteolytic activity of 8CS. a) the microbial growth on milk agar; c) microbial growth on meal feather agar; d) the growth of 8CS on chicken feather (tube 2) compared to an uninoculated tube and a tube inoculated with a bacteria without keratinolytic activity.

Microbial consortium identification. Through massive sequencing it was identified that from the different genera that composes the microbial consortium the 8CS, the most predominant was *Bacillus* (Fig. 2).

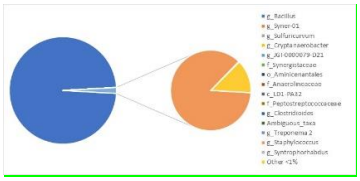
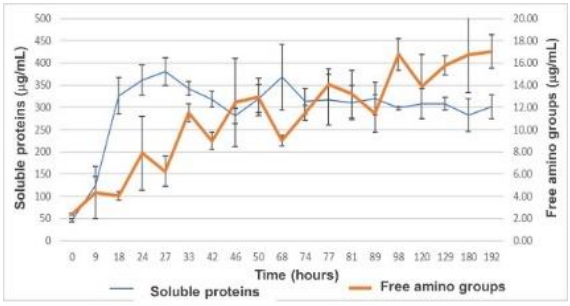


Figure 2. Relative abundance of the 8CS microbial consortium at the genus level according to 16S rRNA analysis.

Biological pretreatment of feathers by microbial consortium 8CS. To evaluate keratin degradation in feathers, soluble protein concentration and free amino groups were measured (Graphical 1). The hydrolysate from the 8CS consortium showed a high concentration of free amino groups at the end of the study, with a maximum keratinase activity of 41 U/mL after 53 hours of incubation. These findings align with previous reports on Bacillus species strains, such as *B. licheniformis* and *B. pumilus*, which showed maximum enzyme activities of 50.58 and 35.74 U/mL, respectively, after 48 hours in a feather-based culture medium. Physicochemical analysis of feather hydrolysates by the 8CS consortium and control feathers used in methane production assays showed a VS/TS ratio over 60%, indicating high biodegradability and potential for anaerobic digestion.



Graphical 1. Study of the hydrolysis of chicken feathers using the 8CS microbial community.

Biological methane production. The biochemical potential for methane production (PMB) was measured for feather hydrolysate produced by the 8CS consortium. A control group using whole feathers from the same batch was included for comparison. Results showed that methane production was significantly higher in the biologically hydrolyzed chicken feathers (494.74 ± 0.26 mL CH_4/gSV) compared to the control (41.45 ± 0.22 mL CH_4/gSV), indicating that the 8CS consortium enhanced methane production.

4 CONCLUSION

Biogas production from feathers faces significant challenges and generally produces low results due to the difficult-to-degrade structure of beta-keratin. In this study, a microbial consortium capable of degrading chicken feathers was isolated and identified. With this consortium, chicken feathers generated as waste at a local poultry farm were hydrolyzed, resulting in a substrate suitable for methane production. Evaluation of the PBM using this substrate showed that biological hydrolysate of the feathers led to increased methane production compared to untreated feathers.

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