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Biogas from slaughterhouse wastewater anaerobic digestion is driven by the archaeal family *Methanobacteriaceae* and bacterial families *Porphyromonadaceae* and *Tissierellaceae*

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ABSTRACT

Currently, global demand for energy has grown and the search for new ecological energy sources is one of the mostly significant issues we face. The digestion of alternative sources of carbon in anoxic environment produces gas of high calorific value, which is a promising source of alternative energy. Thus, this work aimed to evaluate the biogas production of waste originated from a slaughterhouse industry of pigs and poultry, and from the dairy industry, and to characterize the physicochemical properties and microbiological composition of the biogas-producing biomass. Residues were collected and physicochemical and microbiological parameters were evaluated in four different stages of biogas production. At the end of 42 days, approximately 26 L of methane and 12 L of other gases were produced. The high amount of biogas/methane observed was related to the families *Porphyromonadaceae*, *Tissierellaceae*, and *Methanobacteriaceae*. Although less than 6% of the total reads lack classification at any taxonomic level, our analysis showed that about 50% of the sequences did not present a homologue sequence at the genus level in public databases. Knowledge about changes in the microbial composition and their dominance can provide tools for manipulation, isolation, and inoculation of the microorganisms inside the bioreactors to maximize methane production.

1. Introduction

Throughout the last century, global demand for energy has grown. Presently, about 80% of all primary energy in the world is derived from fossil fuels [1]. Burning fossil fuels causes associated problems such as the emission of greenhouse gases, mainly CO_2 [2]. The limited supply of fossil fuels and the increased environmental worries concerning gas emission have driven research for alternative sources of energy, called biofuels [3]. The search for new environment-friendly energy sources is one of the most significant issues in the 21st century [4].

Wastewater from the slaughterhouse industry may contain blood, manure, hair, fat, feathers, and bones, which need to be treated and stabilized before being discarded in fields to avoid contamination of the environment [5,6]. These residues are generally subject to primary treatment that includes activated sludge processing, which produces a large amount of waste-activated sludge [7]. In this process, an efficient removal of organic matter is achieved only by anaerobic digestion [5], which produces gas of high calorific value, called biogas, a promising source of alternative energy [8].

Biogas is generated by the activity of specific anaerobic microorganisms capable of decomposing organic matter [9] derived from waste plants, animal droppings, sewage, sludge wastewater from treatment plants, and others [10] in a process called Anaerobic Digestion (AD). The source of raw materials and the

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operational conditions during AD (temperature, pH, N and C content) play an important role in defining the chemical composition of the biogas [8]. Raw biogas is comprised by around 50–70% of methane (CH_4), 30–45% of carbon dioxide (CO_2), and trace amounts of other components, such as water, hydrogen sulfide (H_2S), siloxanes, halogenated hydrocarbons, and ammonia (NH_3) [11]. The methane portion of biogas is of great interest to energy production due to its potential for heat and power generation [12], lower production costs, and easy access to raw material that allows for its production [13].

The source of raw materials and operational conditions also determine the microbiological composition of biogas-producing biomass [9]. The most abundant microbial classes usually found in biogas reactors with environmental samples are Methanomicrobia, Actinobacteria, Bacteroidetes, Bacilli and Clostridia [14]. Chen et al. [15] analyzed the microbial composition of the anaerobic digestate of dairy manure and food waste incubated at 35 °C and 50 °C. It was observed that the most abundant phyla were Bacteroidetes, ranging from 46 to 69% at 35 °C, and 16–28% at 50° C; Firmicutes, from 20 to 45% at 35 °C, and 45–62% at 50 °C; and Proteobacteria, from 2 to 5% at 35 °C, and 4–7% at 50 °C. However, changing the source of raw material to silage and the digestion temperature to 50 °C, the phylum Thermotogae was the most abundant (>50% of all sequences) [16].

It was already demonstrated that the biogas volume and its chemical composition change over the anaerobic incubation period [17,18]. Knowledge about changes in the microbial composition and their dominance during the incubation period can provide tools for manipulation of the microorganisms inside the bioreactors to maximize biomass conversion and methane production, boosting the use of industrial wastes to generate sustainable energy. Thus, this work aims to: 1) assess the production of biogas, especially methane, of waste derived from one industry that slaughter pigs and poultry, and also manufactures dairy products, and 2) determine the physicochemical properties and the microbiological composition of the biomass in different stages of the biogas production.

2. Material and methods

2.1. Biogas production experiment

Two liters of different waste generated by one industry that slaughter pigs and poultry and manufactures dairy products were collected in sterile pots. The different types of waste were mixed proportionally to the amount generated by this industry (Table 1) and the biomass generated was used for biogas production.

The batch-fed lab-scale digester had a height of 0.18 m and a diameter of 0.12 m representing a total volume of approximately 2 L and a working volume of 1.8 L. In the reactor, 1.26 L of the biomass described in Table 1 were added to 0.54 L of digestate (from previous anaerobic digestion), and were incubated in anaerobic conditions at 35 °C for 42 days. The reactor design and the biogas measurements were performed according to procedures described by Konrad et al. [19]. This experiment was carried out in triplicates The methane content (%) of the biogas was measured daily with a specific methane measurement sensor (Advanced Gasmitter, manufactured by PRONOVA Analysentechnik GmbH & Co).

During the experiment, four sampling points were chosen to evaluate the physicochemical parameters and the microbiological composition of the biogas-producing biomass. Samples were collected in four stages: P1 (Point 1)–Before anaerobic incubation (day 1); P2 (Point 2) High biogas production and methane percentage (day 7); P3 (Point 3)–Low biogas production with high methane percentage (day 25); P4 (Point 4)–End of the experiment (day 42).

2.2. Physicochemical analysis of the biomass

The physicochemical parameters evaluated in each reactor were: Biochemical Oxygen Demand (BOD), Chemical Oxygen Demand (COD), Total Organic Carbon, Total Nitrogen, Conductivity, pH, and Solid Composition. BOD was determined through the *Oxytop* respirometric system (*Wissenschaftlich-Technische Werkstätten GmbH* (WTW) - *Oxitop* IS 6), according to procedures described by Reuschenbach et al. [20]. Parameters as COD, total organic carbon, and total nitrogen were determined through standard methods for water and wastewater examination [21]. The pH was measured using a pH meter (Digimed brand, model DM-20), and Conductivity was measured using a conductivity meter (Bel brand, model W12D). Total Solids (TS), Volatile Solids (VS), and Fixed Solids (FS) were evaluated using the gravimetric method according to procedures described in the Official Methods of Analysis of the Standard Methods for Evaluation of Water and Wastewater [22].

A comparison between each of the physicochemical parameters in the four sampling points analyzed was performed through One-Way ANOVA, with means compared through Tukey's test ($p < 0.01$) using the Assistat 7.6 beta software (available at www.assistat.com).

2.3. Microbiological analysis

The microbiological study was performed with molecular biology techniques using high-throughput DNA sequencing analysis. The DNA was extracted using the NucleoSpin® Soil kit (Macherey-Nagel). Polymerase Chain Reaction (PCR) was used to amplify a partial segment of *16S rRNA* gene using the F515 and R806 primers; and PCR conditions were those described by Bates et al. [23]. The ~250 bp *16S rRNA* gene fragments obtained were subjected to high-throughput sequencing in an Ion PGM sequencer (Thermo Fisher) following the manufacturer's protocol.

The *16S rRNA* reads were submitted to quality control that retained sequences with a minimum length of 100 bp and trimmed to remove low quality bases for the minimum Phred score of 30 using PRINSEQ [24]. The remaining sequences were dereplicated

Table 1
Composition of the biogas producing biomass.

Biomass composition	Percentage (%)
Float sludge from the wastewater treatment plant of the pig slaughterhouse	13
Pig blood	4
Float sludge from the wastewater treatment plant of the manufacture of dairy products	5.75
Activated sludge from the wastewater treatment plant of the manufacture of dairy products	5.75
Activated sludge and float sludge from the wastewater treatment plant of a poultry slaughterhouse	16
Poultry blood	5.5
Pig manure	50