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Methanogenic activity optimization using the response surface methodology, during the anaerobic co-digestion of agriculture and industrial wastes. Microbial community diversity

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ABSTRACT

The anaerobic co-digestion of manure, agriculture and industrial wastes for methane production depends on the nutritional condition to develop the microbial community. The effect of each substrate concentrations, as well as their interactive effects on specific methanogenic activity and microbial community diversity were investigated in this work. A central composite design and the response surface methodology were applied for designing the anaerobic co-digestion batch test at 35 and 55 °C. It was analyzed the anaerobic sludge by specific methanogenic activity (SMA) and using molecular techniques (terminal restriction fragment length polymorphism, TRFLP). The results showed a significant interaction among the substrates and an enhancement of the methane production and SMA response caused by the three components. Rice straw had lower influence on SMA than clay residues, due to the mineral content and the beneficial ammonia nitrogen adsorbent properties of the latter. The optimum condition for mesophilic and thermophilic anaerobic co-digestion of pig manure, rice straw and clay mixture allowed SMA values of 1.31 and 1.38 gCH_4-COD/gVSSd^{-1}, respectively. The TRFLP analysis showed the effect of rice straw and clay addition on microbial community diversity at both temperatures. The acetotrophic methanogens belonging to the order *Methanosarcinales* (*genera Methanosarcina* and *Methanosaeta*) dominated in mesophilic condition, whereas at thermophilic conditions dominated *Methanomicrobiales* and *Methanobacteriales* order. The optimization allowed identifying the substrate interaction effects in a concentration range with a reduced number of experiments. Besides, the model validation proved to be useful for defining optimal combination of wastes in anaerobic system.

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1. Introduction

The co-digestion of different wastes may improve nutrient balance and cause synergy effects, overcoming substrate deficits [1]. Moreover, this type of waste management may improve methane yield and increase the efficient use of equipment by processing different waste streams in a single facility. The co-digestion of manure and industrial organic wastes has been widespread in Europe [2] and reports on industrial applications of this concept have been published [3–9].

Manures are an abundant source of organic material that can be used as feedstock in anaerobic digesters [10,11]. However, manures often contain concentrations of ammonia greater than necessary for microbial growth, what may inhibit the anaerobic digestion [12,13]. On such cases, the anaerobic digestion of pig manure could be enhanced using agriculture wastes as co-substrates, due to their high content of carbon and subsequent improvement of carbon nitrogen (C/N) ratio [1]. This way, rice straw could be a promising feedstock biomass as co-substrate for pig manure anaerobic digestion, mainly due to the low costs of this waste biomass [14].

In addition, some clays and zeolite have been described as a means to reduce ammonia inhibition in the anaerobic digestion of manure [12,15–19]. Angelidaki and Ahring [16] used bentonite clay for the anaerobic thermophilic digestion of cattle manure. Milan et al. [17], used a natural zeolite, a modified zeolite [15] and a mixture of clinoptilolite, mordenite, montmorillonite and others [18], for the anaerobic digestion of pig waste. Tada et al. [19], also found that natural mordenite had a synergistic effect on the Ca^{2+} supply as well as on NH_4^+ removal during the anaerobic digestion of a sludge with high ammonia content (NH_4Cl 4500 mg L^{-1}). On the other hand, the metal contained on clays could be used by the anaerobic microorganisms as part of their enzyme structure and has a significant effect on the anaerobic degradation of VFA, being this an ongoing research subject [20–24].

Despite the advantages of the co-digestion process, the addition of co-substrate from a different typology can provoke cell toxicity, that is why the optimization of substrate concentration, temperature and others factors that affect the co-digestion process is necessary [1,25–27]. Response surface methodology (RSM) is feasible to solve this kind of problem, since it is a statistical technique for designing experiments, building models, evaluating the effects of several factors and searching optimum conditions for desirable responses, maintaining a reduced number of experiments. With RSM, the interaction of possible influencing parameters on methane production can be effectively evaluated [28]. Furthermore, central composite design is a fractional factorial design effective for sequential experimentation to obtain a reasonable amount of information for testing lack of fit while a large number of design points are not involved [28].

In this sense, different molecular methods have allowed [29,30] a better approach to the physiology of the microorganisms involved in this process. The terminal restriction fragments length polymorphism analysis (TRFLP) is an effective tool to determine the molecular composition of the microbial community and the relative abundance of certain species [31]. This tool has been successfully applied in different ecosystems such as methanogenic reactors [32,33]. The interactive effects of substrate concentration on methane production from co-digestion of pig manure, rice straw and clay residues as inorganic additives have not been reported yet. Consequently, the main objective of this work was to investigate the effect of pig manure, rice straw and clay residue concentration, as well as their interactive effect, on the specific methanogenic activity and microbial community diversity at mesophilic and thermophilic conditions.

2. Methods

2.1. *Inoculum and wastes sources*

Two anaerobic inoculums were used depending on the temperature tested: adapted to mesophilic (35 ± 2 °C) and to thermophilic (55 ± 2 °C) conditions, both fed with pig manure collected at the Veterinary School at Autonomy National University of Mexico (UNAM). Rice straw was collected from Rice Cuban Enterprise "Sur del Jibaro" and the clay residue was taken from the oil clarification process at Refinery and Petrochemical Industry "Sergio Soto", both located in the province of Sancti Spíritus. The pig manure was kept at 4 °C and the rice straw and clay residual were kept at environmental temperature until use. The characteristics of the substrates are shown in Table 1.

Experiments were carried out in batch tests, containing mineral medium (1%), vitamins solutions (1%), micronutrients (1%), resazurin (0.1%) and cystein (1 g L^{-1}). Rice straw was pretreated by size reduction. The oily residue was removed from clay using absorbent paper. All bottles were inoculated in an anaerobic chamber and incubated at mesophilic condition (35 ± 2 °C) or thermophilic condition (55 ± 2 °C), during 30 days. Bottles with inoculum without substrate, and inoculum with pig manure were used as blanks.

2.2. *Analytical techniques*

The anaerobic process was monitored by means of total suspended solids (TSS), volatile suspended solids (VSS), pH (pH-conductivity meter, OAKTON, EUTECH Instrument, Singapore), and alkalinity, determined according to the Standard Methods for the Examination of Water and Wastewater [34]. The alkalinity ratio (α) was calculated as the quotient of partial alkalinity (at pH 5.75) and total alkalinity (at pH 4.3).

2.3. *Methane production and specific methanogenic activity*

The methane production was determined every day by gas chromatography (Fisher Gas Partitioner model 1200 with a thermal conductivity detector and a Porapak Q column). Subsequently, the total methane in the bottle gas space was determined. SMA was calculated with the slope of the accumulated methane production curve (mL d^{-1}) in the first 5 days, divided by the amount of VSS introduced in the bottle