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Optimization of the specific methanogenic activity during the anaerobic co-digestion of pig manure and rice straw, using industrial clay residues as inorganic additive

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HIGHLIGHTS

- Rice straw and clay additive enhanced methane production on pig manure anaerobic digestion.
- Rice straw enhanced the cumulative methane production in long term.
- Clay reduced the inhibition effect of pig manure due to ammonia nitrogen adsorption.
- Response surface methodology was successfully applied to define model and optimize SMA.

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ABSTRACT

The effect of pig manure, rice straw and clay residue concentrations, as well as their interactive effects on specific methanogenic activity (SMA) at mesophilic and thermophilic conditions were investigated in this work. A central composite design and the response surface methodology (RSM) were applied for designing the anaerobic co-digestion experiments, in order to optimize conditions to enhance methane production. The results showed a significant interaction among the substrates and an enhancement of the methane production and SMA response caused by the three components. The clay residues had a positive effect to reduce the inhibition of SMA caused by high concentration of pig manure due to the ammonia nitrogen adsorbent properties of clay demonstrated in this study by the Freundlich isotherm analysis. Thus, it was corroborated the positive effect of clay as inorganic additive for stimulating pig manure anaerobic digestion. The optimum condition for mesophilic anaerobic co-digestion of pig manure, rice straw and clay mixture was obtained for SMA values of 1.31 and 1.38 gCH_4-COD $gVSS^{-1}$ d^{-1} at mesophilic and thermophilic conditions, respectively. The optimization of the SMA using RSM made possible to identify 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 considering their anaerobic co-digestion. SMA was also a good response variable for that purpose.

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

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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 [13,15–20]. Angelidaki and Ahring [17] used bentonite clay for the anaerobic thermophilic digestion of cattle manure. Milan et al. [18] used a natural zeolite, a modified zeolite [16] and a mixture of clinoptilolite, mordenite, montmorillonite and others [19], for the anaerobic digestion of pig waste. Tada et al. [20] 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 [21–25].

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 [1,26–28]. 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 [29]. With RSM, the interaction of possible influencing parameters on methane production can be effectively evaluated [30,31]. 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 [29].

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 at mesophilic and thermophilic conditions, using a central composite design and the response surface methodology.

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 residual 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 used. 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 [32]. 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 (inoculum) using the proper conversion factor to report it as: gCH_4-COD $gVSS^{-1}$ d^{-1}.

2.4. Central composite design experiments

In order to optimize the concentration of each waste and to analyze the effect of their interaction, a 2^3 factorial central composite design was used based on the *STATISTICA* software (version 8.0, StatSoft Inc., USA). The factorial design was amplified by six axial points, and two replications of center points. The center runs provided a means for estimating the experimental errors and a measure of lack of fit. The axial points were added to the factorial design for estimating the model curvature.

The experiments were tested in three replicates at both temperature conditions (35 and 55 °C) for the selected variables: manure (A), straw (B) and clay (C), in concentration ranges of 9.1–36.6, 7.0–17.6 and 0.8–8.3 gVSS L^{-1}, respectively. The effect of each factor was evaluated under both low and high range of the remaining factors. The concentration of each waste was chosen as three independent variables in this experiment design; SMA was the dependent variable and was calculated as the average of the replicates.

The dependent variable was fitted using a predictive polynomial quadratic equation in order to correlate the response variable to the independent variables. The general shape of the predictive polynomial quadratic equation is:

$$Y = N_0 + \sum_{i=1}^{k} N_i x_i + \sum_{i=1}^{k} N_{ii} x_i^2 + \sum_{i=1}^{k} \sum_{j=1}^{k} N_{ij} x_i x_j \qquad (1)$$

where Y is the response (dependent variable SMA); X_i is the input variable (concentration of manure, straw and clay), which influence the response variable Y; N_0 the i linear coefficient; N_{ii} the quadratic coefficient and N_{ij} is ij interaction coefficient.

Each independent variable and their interaction were compared to verify the feasible hypothesis regarding the treatment effect and their estimation with 0.05 of confidence level. The data normality was confirmed through the construction of the residue normal probability graphic. The standardized effects of the independent variables and their interactions on the dependent variable were also calculated by preparing a Pareto chart.

The quality of fit of the model equation was expressed by the determination coefficient (R^2) and the adjusted determination coefficient (R^2_{adj}). The model statistical significance was determined by a Fisher test (F-test) based on the p-value with 95% of confidence level. Also, the correlation coefficient (R), the Durbin–Watson (DW) statistic, the sum of squares (SS), the middle sum of squares (MSS), and chi-square (χ^2) test were used to analyze the statistical significance of the quadratic model.