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Anaerobic Digestion from Residue of Industrial Cassava Industrialization with Acidogenic and Methanogenic Physical Separation Phases

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Abstract

A trial was carried out in a continuous regimen, using a completely stirred tank reactor, at acidogenic phase, and a hybrid reactor (upflow anaerobic sludge blanket + fixed bed) at methanogenic phase at room temperature. The residue to be treated came from a flour and cassava meal industry, and the reactors operated for 300 d with affluent chemical oxygen demand (COD) concentrations of 7500, 9000, 11,000, and 14,000 mg/L. The final results showed a biogas production with a content of 80% methane and an average reduction of COD and free cyanide of nearly 96 and 98%, respectively. The separation of phases selected bacterial groups. At acidogenic phase, a predominance of propionic, *n*-butyric, and *n*-valeric acids, as well as a biomass composed of 95% fermentative bacilli, which were responsible for a 90% reduction in free cyanide concentration, was observed. At methanogenic phase, a predominance of methanogenic bacteria that came only from the *Methanothrix* genus was observed. The bacteria were responsible for high levels of organic matter removal and methane production.

Index Entries: Anaerobic digestion; cassava; methanogenic phase; acidogenic phase.

Introduction

In Brazil, culture expansion of cassava has been linked to the importance of cassava as an energetic food for some communities, as a forage for animals, and as an industrial raw material for indoor and outdoor markets *(1)*.

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The southern region of Brazil is responsible for 18% of the national cassava production, and Paraná is the largest producer, nearly 600 t/d, creating a huge amount of residues. The *manipueira*, water that constitutes the cassava, is produced during the compression phase, and it pollutes the environment the most. One ton of cassava can produce from 300 to 450 L of *manipueira* (liquid residue).

The cassava is also called *aipim* or *macaxeira*, and it can be found in varieties called docile, which means that it is edible. The varieties that are industrialized are called *wild* or *bitter*. These consist of nearly 30% meal and their humidity content can reach 70%.

The wild varieties of cassava have CN^- contents >100 ppm, and this can lead to intoxication or death in humans and animals. The whole plant is toxic, especially the leaves and between the rinds; it has carcinogenic β-D-glucosideum called phaseololunatosideum, or linamarina, that by hydrolysis produces glucose, acetone, and cyanidric acid in equimolar quantities. This glucosideo is soluble and easily decomposed by endogenic *linamarase* enzymatic action, especially when the roots are pulled out and cut. The released cyanidric acid is one of the most powerful and well-known biocides, which affects any type of aerobic species, harming the oxygen flow *(2)*.

The majority of industries that process cassava also produce *manipueira*, which is deposited into rivers and streams. Its infiltration in soils or fermentation in opened tanks harms the productive sector, i.e., cattle raising, because the rivers and streams are used as drinking sources for animals.

During the 1960s, with the petroleum crisis, articles about the biological treatment of *manipueira* began to appear. New methods of producing energy were being developed and people were beginning to learn more about the ecology. This helped researchers rediscover the aerobic process, in part solving two problems: pollution control and energy production.

The global composition of produced biogas during anaerobic digestion varies according to the environmental conditions present in the reactor. This composition changes quickly during the initial period of the process. There is a reasonable production of gas in reactors that operate stably. Nevertheless, the carbonic gas production in relation to methane can vary substantially, depending on the characteristics of the organic compost to be degraded. For the processing of domestic sewage, typical proportions of methane and carbon dioxide in biogas are 70–80% and 20–30%, respectively.

Several researchers have been studying a one-phase system of anaerobic process. They have concluded that because of the existence of two groups of bacteria—those that form acids and those that consume acids—there are different nutritional needs, sensibility to the environment, and reaction velocity on the same environment. The process was unsettled and therefore it was difficult to maintain stability.

The causes of the system's instability are owing to an excess of volatile acids, promoted by many fermentative bacteria that act out on the present organic matter in the residue, causing a decrease in pH values, produced gas volume, and percentage of produced methane inside a biodigester *(3,4)*.

Pohland and Ghosh *(5)* suggested a separation of acidogenic and methanogenic bacteria in groups, so that each group could work in different physical environments.

Research has shown that the process of anaerobic digestion is available with physical separation of phases, that its stability is superior in relation to the conventional process in the unique phase, and that it can be implemented at a low cost. In addition, it destroys organic matter more quickly and has higher methane production.

Fernandes *(6)* and Sampaio *(7)* observed that the treatment of industrialized cassava residue using the anaerobic digestion process with physical separation of phases was a good option, and they also observed important parameters that mediate on it.

The aim of the present study was to investigate anaerobic digestion from the residue of industrialized cassava using physical separation phases, and to determine parameters that lead to maximum efficiency in methane production, and a reduction in chemical oxygen demand (COD) and free cyanide.

Materials and Methods

Substratum

The effluent used as a source of carbon was the *manipueira* from Indemil, a cassava flour and meal industry in Paranava, PR, Brazil. The *manipueira* was collected at the industry in 20-L bottles. At the laboratory, these bottles were left resting, so that sand and other undesirable materials would be decanted. This liquid was sifted and then stored in 2-L bottles in refrigerators at 4°C.

The effluent preparation of daily nourishment, *manipueira*, was taken out of the freezer 24 h before use and allowed to thaw gradually at 6°C. Its concentration was determined, and then it was diluted in distilled water, so there would be no change in effluent composition. The trial was carried out in four stages (stages 1, 2, 3, and 4), and the effluent concentrations were 7500, 9000, 11,000, and 14,000 mg/L of COD, respectively.

The tank that contained the final effluent of nourishment from the system was kept under low temperature, to be free of microorganisms that could lead to an undesirable loss, or a significant change in carbon source.

Experimental Setup

The acidogenic reactor (the completely stirred tank reactor) was made of acrylic with a 20 cm diameter and an 18 cm height and worked under room temperature with a hydraulic retention time (HRT) of 1 d. The methanogenic reactor was made of a polyvinyl chloride column with a 20 cm diameter and 64 cm height. It was a hybrid reactor (upward flow and bed reactor of fixed sludge + bed), and as stuffing material, pieces of dry bamboo were used, measuring about 2 × 2 cm and filling up 40% of the reactor total volume.

The effluent nourishment of the methanogenic reactor was kept at a pH ranging from 7.0 to 8.0, plus the addition of 1.0 *N* NaOH, in a neutralization tank, and the reactor operated at room temperature and HRT of 4 d.

Analytical Methods

Volatile Acidity and Alkalinity

The volatile acidity (VA) and alkalinity (AL) were obtained by titrimetric methods according to the methodology described by Silva *(8)*.

Chemical Oxygen Demand

The COD was obtained by the micro-method, according to the methodology described by Tavares *(9)*.

Determination of Volume and Gas Composition

The gas volume was measured by moving a saline-acidulate solution, corrected for normal conditions of temperature and pressure (dry gas). The gas content was determined by gas chromatography (electric conductivity detector) *(10)*.

Free Cyanide

Free cyanide concentration was determined by the direct potentiometry method, using a selected electrode to cyanide, according to the methodology described by Marins *(11)*.

Analysis of Microorganisms

Bacteria that worked on the process, photos of phase contrast, and fluorescences were analyzed with a BH-2 Olympus microscope adapted with a C-35AD-4 Olympus camera at the Universidade de São Paulo, São Paulo, Brazil.

Results and Discussion

During the anaerobic digestion process with physical separation phases, to establish a continuous regime of work, fermented *manipueira* that came from the acidogenic reactor was fed to the methanogenic reactor. In this trial, results that refer to the second stage were presented and obtained over 73 d of the system's operation and were estimated according to the system's observed parameters.

Volatile Acidity

The results presented in Figs. 1 and 2 show that the methanogenic phase of the system worked very stably and that the conversion of volatile acids in biogas was nearly 93%. This degree of conversion may be considered satisfactory, when compared to Sampaio's results *(7)*, which had a reduction of 92.66% to an organic concentration 33% less than the one used in our trial.

On the first days of the system's operation, a variation in the values of VA reduction was observed because an adjustment phase was provoked by

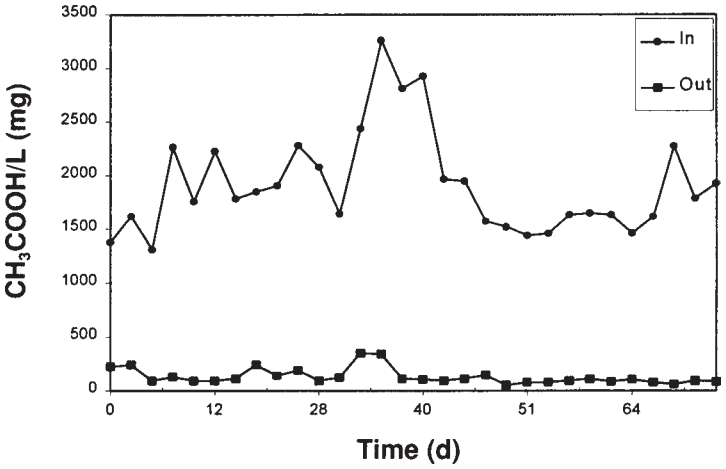

Fig. 1. Evolution of VA in the affluent and effluent. Days of operation.

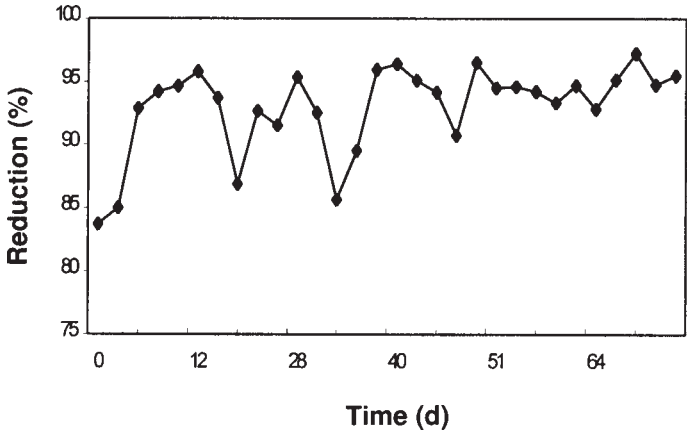

Fig. 2. Evolution of percentage of VA.

the increase in organic concentration. The stability of the methanogenic reactor was reached just after the introduction of a neutralization tank and a gas–liquid system separation, which allowed a maintenance of its pH between 7.0 and 8.0 and better performance of the methane bacteria.

Alkalinity

The performance of methanogenic bacteria in producing CO_2 and CH_4 depends on the capacity of AL buffering on the high concentration of volatile acids. The most important aim of AL in anaerobic digestion is to produce a means for bacterial activities in the process.

The ideal value for the VA/AL relation in anaerobic digestion has not yet reached a consensus among researchers. This rate depends on internal and external conditions of reactor operation, as well as the type of effluent.

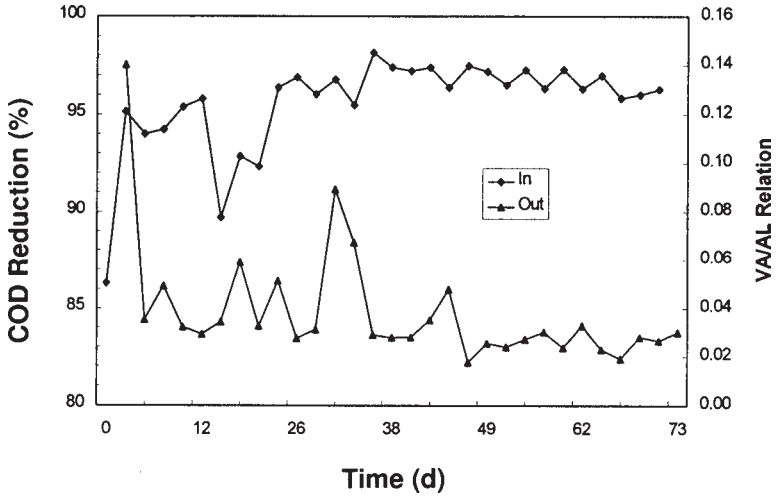

Fig. 3. Evaluation of evolution of COD reduction with the evaluation of VA/AL rate.

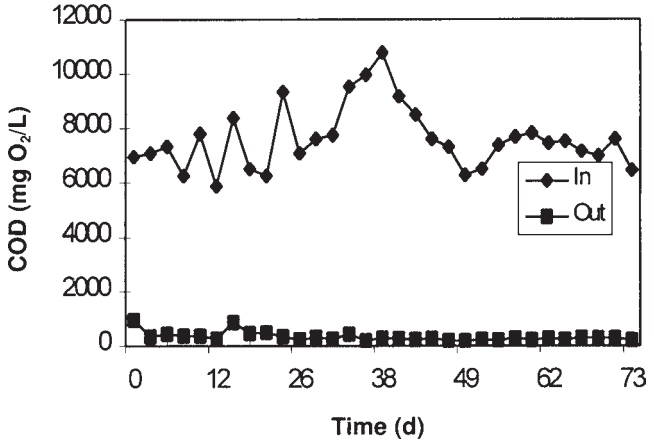

Fig. 4. Evolution of soluble COD in the affluent and effluent.

Figure 3 shows that in the present trial, the methanogenic reactor operated at a rate of an average VA/AL of 0.043. This value corresponds to propitious methanogenic bacterial activity: no accumulation of acids and high alkalinity. Figure 3 also shows that the stability on COD reduction percentage was reached for low values of the VA/AL rate. The VA/AL rate was between 0.018 and 0.08, and the removal efficiency of COD was nearly 95.6%. These values are similar to the ones obtained by Sampaio *(7)*.

Chemical Oxygen Demand

The evolution of soluble COD in the methanogenic reactor (*see* Fig. 4) showed that the system operated in normal conditions, because soluble and regular values of COD of the effluent (about 132 mg of O_2/L) were

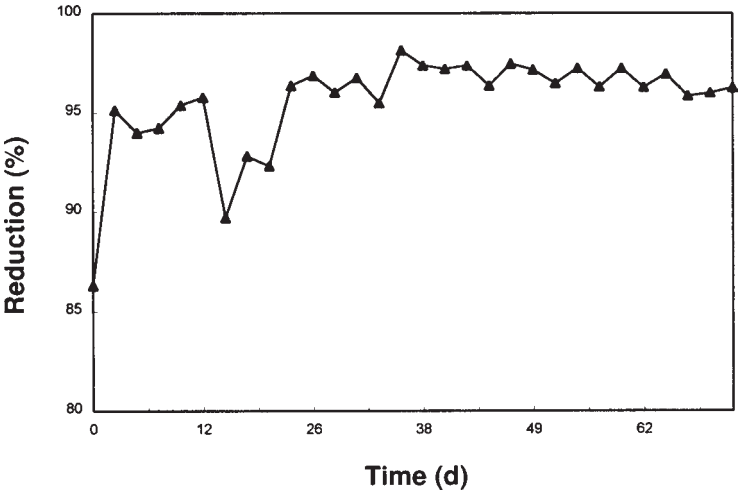

Fig. 5. Evolution of removal efficiency from the soluble COD.

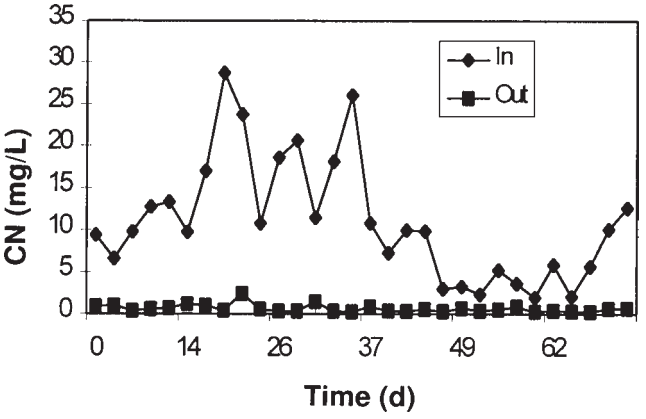

Fig. 6. Evolution of free cyanide concentration.

obtained. Variation in COD values of entrance was owing to the feed flow, which changed between 5.0 and 5.5 L/d, causing a float of HRT and a fermentation into the acidogenic reactor by acidogenic bacteria. The efficiency of COD removal was nearly 93%, as shown in Fig. 5.

Free Cyanide

Because of a great reduction in the concentration of free cyanide in acidogenesis, the methanogenic reactor received a lower charge of cyanide, thereby benefiting acidogenic bacterial activities. Figure 6 shows the evolution of free cyanide concentration at the reactor entrance and exit. The percentage of free cyanide reduction (about 92%) is also presented in Fig. 7, and this result clearly shows that the two-phase anaerobic process had good performance.

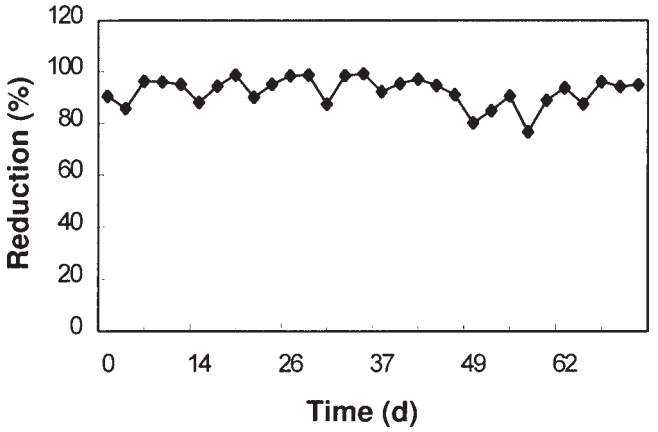

Fig. 7. Percentage of evolution of free cyanide reduction.

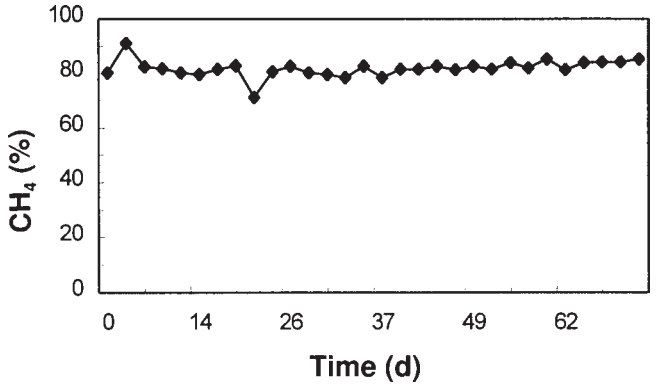

Fig. 8. Percentage of evolution of CH_4.

Produced Gas

During the methanogenic phase of the anaerobic digestion process with physical separation of phases, the efficiency of biogas production (CO_2 and CH_4) can be directly related to a reduction in the percentage of volatile acids. The greater the reduction, the greater the amount of obtained gas. Biogas production was almost 14.4 L/d and had an average CH_4 content of 80% (*see* Fig. 8). The mean mass of COD that was removed daily in the methanogenic reactor was 41.2 g and, it acquired 0.35 L of produced gas per gram of removed COD.

Microbiology and Route of Methane Production on a Two-Phase System of Anaerobic Digestion

In this trial, we studied bacterial populations of acidogenic and methanogenic reactors by optic microscopy to investigate microorganisms that were participating in the process. We also determined their final prod-

ucts by parallel analysis of the mass spectrum of *manipueira*, which was the system affluent for the acidogenic and methanogenic reactor liquids, and the system effluent that was considered as already treated.

Samples were prepared for analysis of the mass spectrum using the *manipueira* that was collected at the industry immediately after the cassava was compressed, and in a collected sample after the trip from Paranavaí to Maringá. This was done to evaluate the potential of fermentation of *manipueira* from the collection to its storage in freezers.

It was seen that the fermentation potential was considerable, because the analyses showed that the number of composts just after the trip increased in great proportion, with a predominance of alcohols of great molecular weight and traces of formic acid among other associations of carboxylic groups, amines, and ethers. Therefore, this characterizes superficially the substratum that is affluent to the acidogenic reactor.

The mass spectrum analysis from the liquid of the acidogenic reactor (complete mixture reactor) was verified among other composts, by the presence of propionic, *n*-butyric, *n*-valeric, and caproic acids, without detecting acetic acid. This indicated that hydrolysis and acidogenesis occurred in the acidogenic reactor. The acids we found are products of bacterial metabolism that produce the same acids.

Microbiological analysis of the same liquid indicated that the bacterial flora of the acidogenic reactor was formed in great majority by filaments and bacilli that were acidogenic, fermentative (fermentative and hydrolytic bacteria), and nonfluorescent. These are characteristics of the hydrolytic phase of fermentation, and this indicated that they belonged to the facultative anaerobic bacterial group.

Microbial populations were analyzed in the methanogenic reactor in 14 points, from 15 to 15 cm, beginning at the reactor bottom (upflow reactor). The highest concentration of bacterial flora was found at the reactor bottom, because of the presence of specific grains, which worked as a support to the microbial population and provide good development of microorganisms in reactors like the upflow anaerobic sludge blanket. The concentration of bacterial population decreased as the upward bed occurred, but all species were found.

Analyses of methanogenic reactor samples showed the presence of a huge methanogenic microbial population, among the fluorescent ones, with a predominance of curved and straight bacilli (nonfluorescent), thin bacilli (fluorescent), coconuts (fluorescent), bacteria from the *Methanothrix* genus (nonfluorescent), as well as the presence of *Methanosarcinas* sp. (fluorescent) bacteria.

Acids that came from the acidogenic reactor, but that were propionic, were found only on the bottom part of the methanogenic reactor. This finding indicates that the acetogenic phase was probably transitory between the two physical spaces of the process, and at this point, acetic acid was not detected either.

Acetogenic bacteria oxidized the final products in the acidogenic phase in an appropriate substratum for methanogenic bacteria, which were hydrogen, CO_2, and acetate. From the products that were produced by acidogenic bacteria, only hydrogen and acetate can be directly used by the methanogenics.

A certain amount of hydrogen is produced during the synthesis of acetic and propionic acids since the pH value in an aqueous environment decreases. Probably the produced hydrogen in the reactor's interphase can be consumed by methanogenic thin and straight bacilli and producers of methane via H_2 and CO_2. These bacilli helped in the process, decreasing the partial pressure of hydrogen in the environment and permitting the production of acetate to other bacteria.

The presence and predominance of *Methanothrix* bacteria (acetoclastic) showed that part of the methane production occurred via acetate consumption, its favorite substratum. These kinds of methanogenic bacteria are found in great amounts in anaerobic digestion.

The *Methanothrix* bacteria grow as filaments and organisms that belong to the *Methanosarcina* genus. They are also present in the observed system and grow as "coconuts," which together form a kind of "package."

Conclusion

The results show that physical separation of phases allowed good stability of the effluent that comes from the industrialization of cassava, as well as the stable operation of acidogenic and methanogenic reactors, on the conditions of operation used.

The average percentage of reduction in VA was 93%, expressing a good performance of methanogenic bacteria, even when kept at room temperature. The best efficiency of COD removal occurred at a VA/AL rate between 0.018 and 0.08. The removal efficiencies of COD and free cyanide were 93 and 92%, respectively. The average production of biogas was 14.5 L/d, with a content of 80% CH_4.

Microbiological analysis of bacterial flora that came from the acidogenic reactor indicated that there was a selection of microorganisms in this reactor, since it was predominantly formed by filaments and fermentative acidogenic bacilli, which were nonfluorescent and typical of the hydrolytic phase of fermentation, probably constant to the group of anaerobic facultative bacteria.

Microbiological analysis of the bacterial flora from the methanogenic reactor indicated that there was a huge population of microorganisms formed mainly of straight and thin bacilli, which are responsible for hydrogen use from the environment, lowering the partial pressure of hydrogen, and benefiting methane formation via CO_2 reduction. The *Methanothrix* uses acetate to produce methane, as well as coconuts and *Methanosarcinas*, which is typical of the methanogenic phase of the anaerobic process.

Acknowledgments

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