

ANAEROBIC BIOMASS EVALUATION DURING BIODIGESTION OF SOLID WASTES AMENDED WITH ORGANIC SUBSTRATES AND USING LEACHATE AS A SEED

Baldochi V*, Varesche M, Povinelli J, Vazoller R

Departamento de Hidráulica e Saneamento da Escola de Engenharia de São Carlos -
Universidade de São Paulo
Av. Dr. Carlos Botelho, 1465, CEP: 13560-250, São Carlos, SP, Brasil

In order to evaluate the enriched biomass activities from leachate produced during solid waste anaerobic degradation, five different organic substrates were individually tested in batch conditions during six months of operation time, at $35^{\circ}\text{C} \pm 1^{\circ}\text{C}$. Reactors were serum bottle flasks filled with organic fraction from urban solid wastes, inoculated with leachate and amended with mineral medium plus each organic source studied: acetate, butyrate, lactate, butanol and methanol. Control reactors were done without addition of leachate. Direct light and fluorescence microscopic observations showed different kinds of bacterial morphologies, with predominance of coccus and sarcinas fluorescent cells. Free or forming chains rod cells seem to be the main morphology without fluorescence during the experiment. Sporulated morphologies were observed as well. Reactors without leachate addition presented less bacterial morphologies and fluorescence species diversity. The methanogenic potential values suggested high cell activities in all substrates tested with leachate, specially organic acids. In that case, leachate methane production, and it was possible to concluded that leachate can be a potential inoculum for fresh refuse. The experiments also allowed to enrich anaerobic bacterial species during the metabolism of leachate amended reactors, and probably *Methanosarcina* sp and *Methanococcus* sp species could be selected from this environment.

Palabras clave: solid wastes; leachate; anaerobic digestion; anaerobic bacteria; methanogenic potential

INTRODUCTION

The state of knowledge on the microbiological aspects during anaerobic biodigestion of solid wastes is still rather sketchy, even though these process has been used worldwide on a large scale, as its natural occurrence inside of landfills or its induced occurrence in different kinds of anaerobic biodigesters.

Historically, little attention has been given to fundamental research on refuse decomposition. Such research is necessary to improve sanitary landfills design for enhanced methane production and minimization of adverse environmental impacts resulting from poorly designed or operated landfills (BARLAZ, 1989). The anaerobic biological activities in landfills are comparable with those that occurring in anaerobic bioreactors, where a complex process coordinated by a microbial metabolism take place. COLLERAN et al. (1991) pointed out the low growth velocities of methanogens and others species inside of biodigesters. This situation is further complicated by the fact that solid wastes have great amounts of polymeric organic matter as polyssacharides, lipids and proteins, then hydrolysis and acidogenesis are the an important steps to the start-up of solid wastes anaerobic processes degradation, followed by acetogenic and methanogenic metabolisms.

Many species involved on the solid wastes anaerobic processes were isolated and identified. FIELDING at al. (1988) described *Methanosarcina* sp as an important methanogenic genus founded in landfill samples. This data is in agreement with VAZOLLER et al (1992) observations, who also pointed out isolations of *Methanobrevibacter* sp, *Methanobacterium* sp and the acidogens *Megasphaera elsdenii* e *Selenomonas* sp. BARLAZ et al (1990) enumerated some others important species as *Clostridium perfringens*, *Lactobacillus* sp, *Bacillus* sp, *Propionibacterium* sp, *Pseudomonas* sp and *Cellulomonas fermentans* from diverse landfill

samples, and *Methanosarcina barkeri*, *Methanogenium* sp, and various strains of *Methanobacterium* sp as a methanogenic species.

Methodology for the characterization of anaerobic microorganisms is an increasingly requirement for monitoring and evaluating solid wastes anaerobic samples and leachate. Although methods of determining gas evolution have been developed and attempts made to optimize the refuse methanogenic fermentation no reference has been made to the operative pathways or the relative contributions of each, and to the most common microorganisms with in systems inoculated with leachate. This study was initiated to identify some aspects of organic substrates anaerobic degradation inoculated with leachate, using small reactors. To give an estimation of the bacterial metabolic groups activities from leachate, methanogenic potential of organic samples enriched was determined, and microscopic observations provided the bacterial identification morphologies. The results obtained allowed to help the microbiological aspects of two 179 ℓ of anaerobic reactors, one of them with leachate recycle.

MATERIAL AND METHODS

Substrate: Fresh organic fraction of urban solid waste was obtained from Novo Horizonte Recycling and Composting Plant, Novo Horizonte City, São Paulo State, Brazil. Inoculum: leachate from a 8 years old landfill, neutralized with sodium bicarbonate (50 g/ℓ).

Experimental equipment: Twenty 350 ml and four 1,000ml of serum bottle flasks were closed with rubber stoppers and aluminum screw caps. The gas analyses were done by gas chromatography (Chromatograph model Gow Mac series 150) with a thermal conductivity detector and with Porapak Q column (80-100 mesh, aluminum tubing with 2,0 m x 1/4"). The column was operated at 75 °C with hydrogen as the carrier gas. The optical microscopy observations were made an Olympus microscope model BH2 and micrographs were taken by an Exposure Control Unit Olympus. The volumetric production of biogas was measure with volumetric siring.

Experimental procedure: 100g (77 g/g of dry matter) and 600 g (180 g/g of dry matter) of refuse were respectively incubated in ten 350 ml and six 1,000 ml of serum bottle flasks, under nitrogen atmosphere, at 35±1°C. The bottles inoculated with leachate neutralized were kept with 23 % of moisture. The not inoculated bottles received water, at 25 % of moisture. After sixty days, the enrichment tests were carried out as follow: the refuse incubated in 350 ml serum bottles received 100 ml of mineral medium (ZINDER & KOCH, 1984) plus each carbon sources (duplicate): sodium acetate, 40 mM; sodium butyrate, 20 mM; methanol, 20 mM; and buthanol, 20 mM; under nitrogen atmosphere. The 1,000 ℓ serum flasks bottles were utilized as controls, and were incubated in duplicate with inoculum (250 ml) and no inoculum (250 ml of mineral medium). The enrichments evolution were analyzed during the start-up, and after 73 and 107 days of incubation time, when the new feedings of organic sources, at the same final concentrations, were done. Gas samples were analyzed weekly, and optical microscopy observations were done before the second and third feedings.

RESULTS AND DISCUSSION

Table1 showed the methanogenic potential values from each organic source studied, and Figures 1 to 6 presented the gas evolution during the experiments.

Table 1 . Methanogenic potential values from each organic source a long the experiments ($\text{m}^l \text{CH}_4$ /g. dry solid waste .day)

Organic source	no inoculated	no inoculated	inoculated	inoculated
Sodium acetate	0,03	0,04	0,2	0,21
Sodium butyrate	0,04	0,03	0,21	0,18
Sodium lactate	0,05	0,05	0,17	0,18
Buthanol	0,13	0,04	0,22	0,14
Methanol	0,05	0,04	0,17	0,11
Controls	0,04	0,04	0,16	0,15

Figures 7 and 8 showed the different morphologies of cells from the enrichments.

The differences between methanogenic potential values from inoculates and not inoculated systems are remarkable (Table 1) . During the leachate enrichments acetate utilization was faster than other organic sources, and microscopic observations showed the enrichment of sarcina fluorescent cells, one acetotrophic methanogen. Fig. 7 (a) it is observed the sarcina morphology and (b) the fluorescence of individual cells , that is possible to be the individual coccoid cells of sarcina according Bergey's Manual (1989) or even coccus belonged to genus *Methanococcus* sp. The methane present in the biogas from acetate were 55 %. The values of methanogenic potential on methanol can be related to methanogenic sarcina genus, Fig. 7 (f) , specially with *Methanosarcina* barkeri. These kind of methanogenic can used both substrates. It was noticed sarcina fluorescent cells (Fig c, d) in sodium lactate enrichment as well. The lactate can be transformed to acetate by some kind of acetogens, like sulphate-reducing bacteria, and then used by acetotrophic methanogens. However, it was not possible confirm the presence of these sulphate reducing species. Fig 7 (e) showed coccus fluorescent mophologies from reactor feeding buthanol. The morphologies observations were very similar with those described by literature , and sarcina and coccus morphologie are predominant. The comparison between the cell morphologies from those observed from leachate (Fig.8-g,h,i,and j) of biodigester operated with recycle it is possible confirmed the predominance of fluorescent coccus . In these observations , a rod shape cell not fluorescent is also present (Fig 8 g and i) . A close interaction between culture can explained the presence of two kinds of morphologies during hydrolisis, fermentation and methanogenesis.

The neutralized leachate could help the interactions between methanogenic and non-methanogenic cells , but it was the real source of bacteria during the experiments, considering the very low methanogenic potential of not inoculated reactors.

The observations were done during almost six months of operation time , at $35 \text{ }^\circ\text{C} \pm 1 \text{ }^\circ\text{C}$, wich could be seen an increasing of bacteria growth in the samples. According to BARLAZ et al (1990), leachate recycle can stimulate the microbial activities inside of anaerobic solid wastes biodigestion, but some results from different experiments in the past not confirm this potential. The conclusions of the present study are that leachate permitted to enrich some kind of bacteria, specially methanogenic sarcina morphology , and could be a very good inoculum of solid wastes anaerobic biodigesters.

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Fig.1 . Amended with sodium acetate reactors

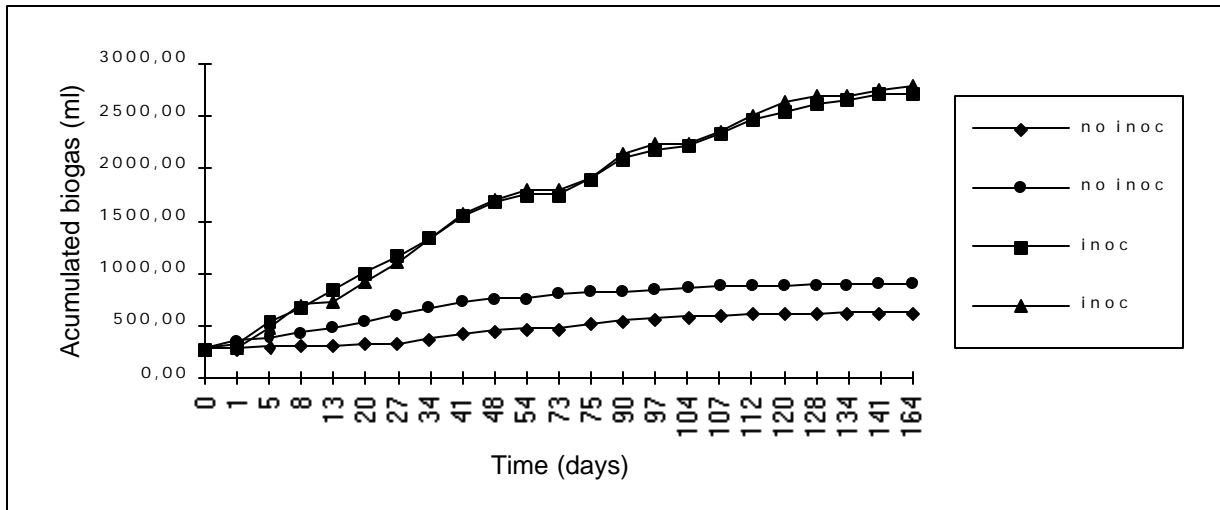


Fig.2 . Amended with sodium butyrate reactors

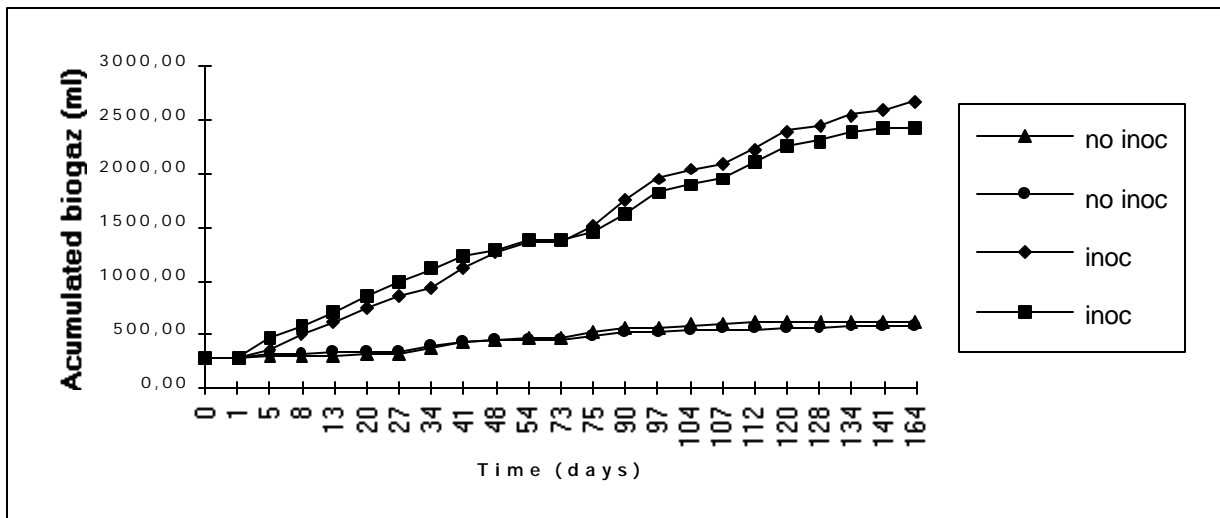


Fig.3 . Amended with sodium lactate reactors

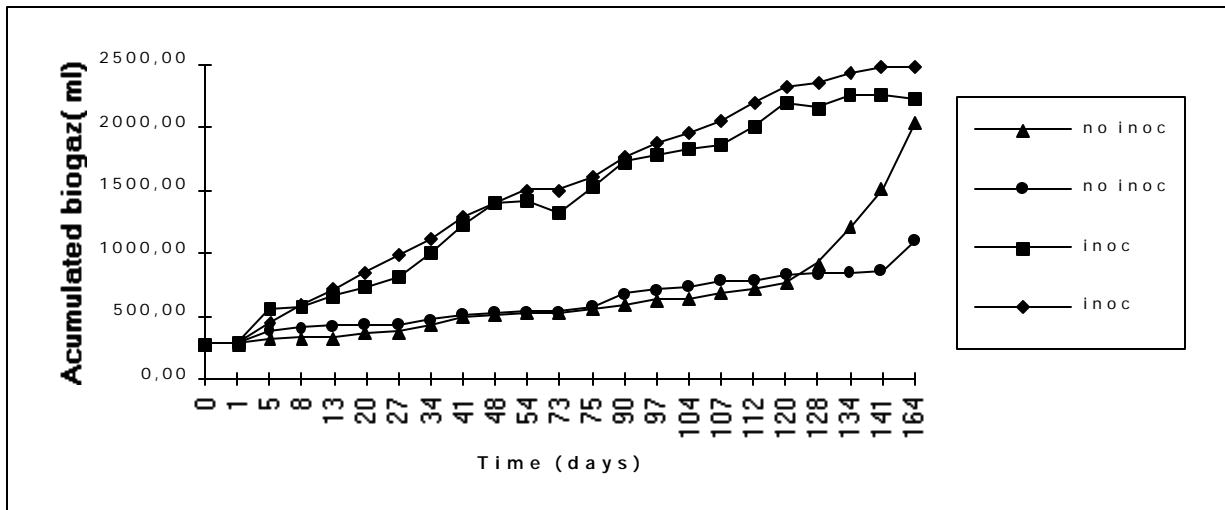


Fig.4 . Amended with buthanol reactors

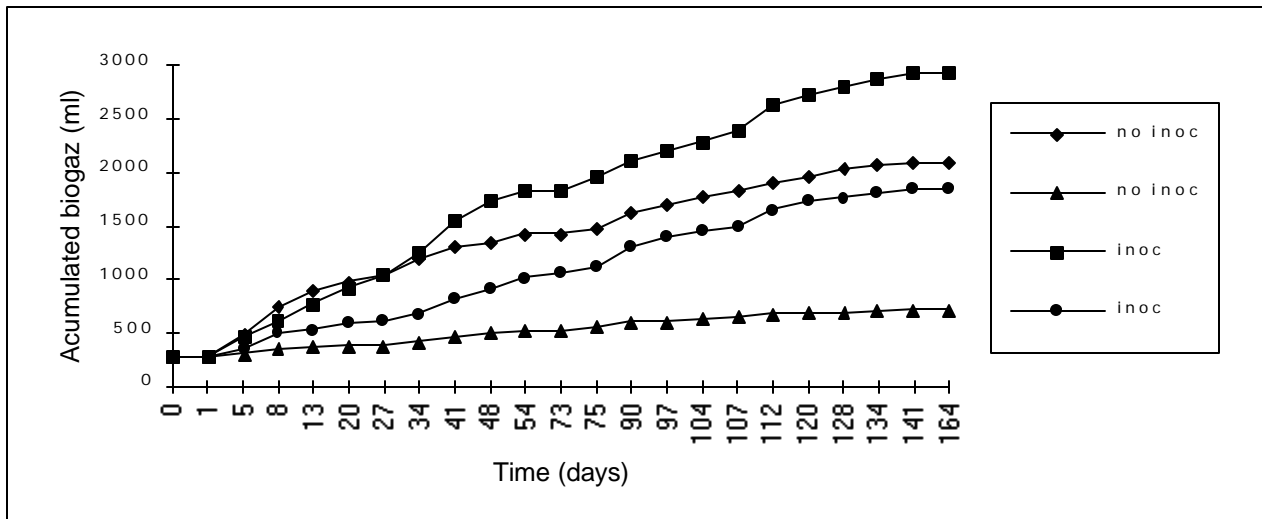


Fig.5 . Amended with methanol reactors

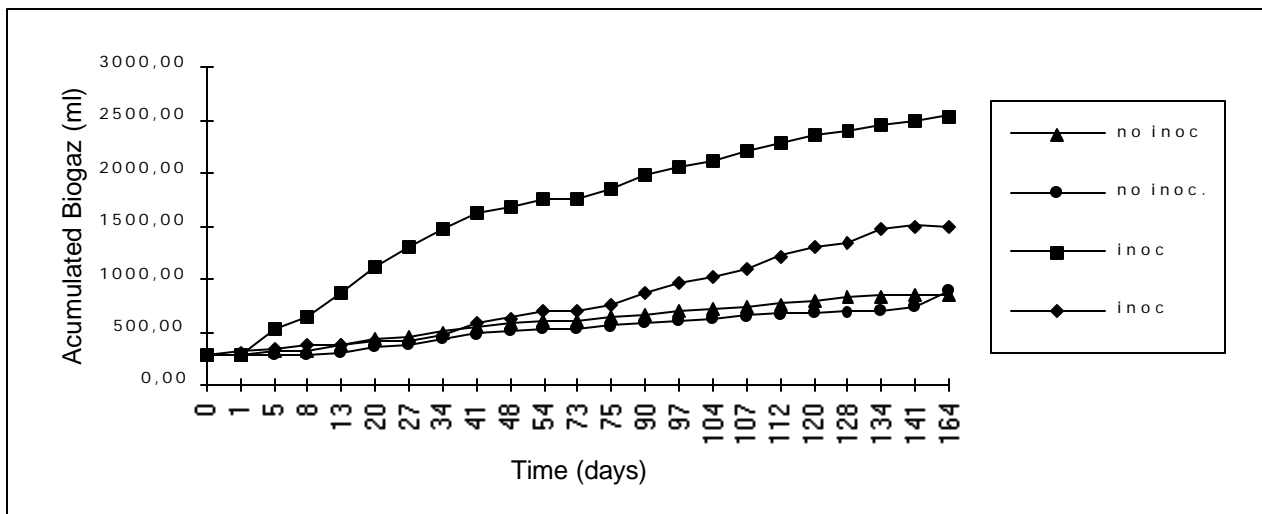
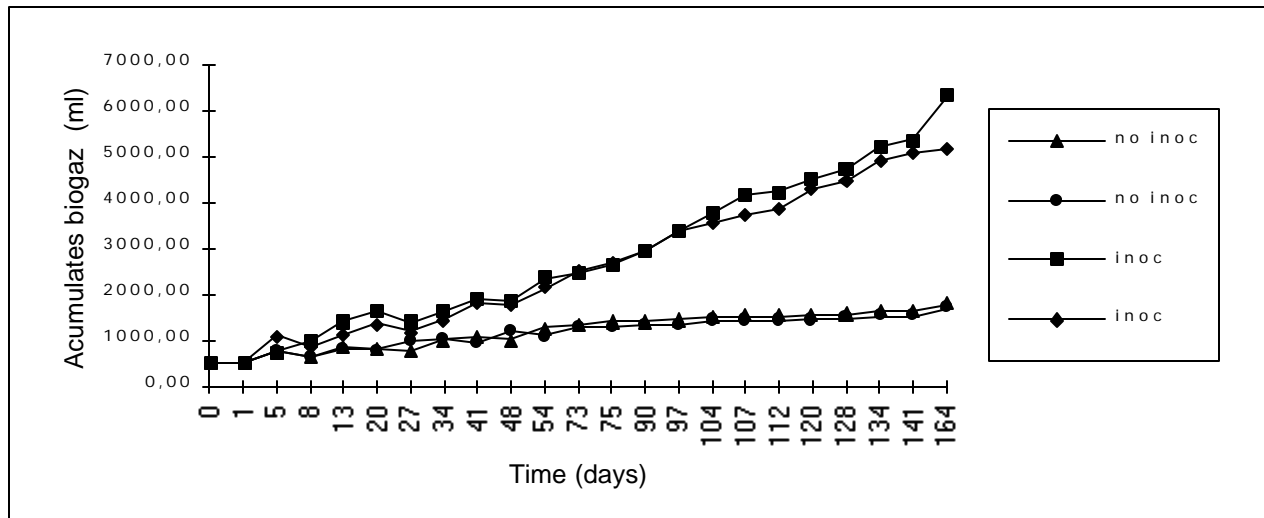


Fig.6 . Controls reactors



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