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Effect of inoculum to substrate ratio (I/S) on municipal solid waste anaerobic degradation kinetics and potential

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ABSTRACT

The goal of this study is to evaluate the impact of the inoculum to substrate ratio (I/S) on the anaerobic degradation potential of municipal solid waste (MSW). Reconstituted MSW samples were thus incubated under batch anaerobic conditions and inoculated with an increasing amount of inoculum originating from a mesophilic sludge digester. I/S tested values were 0 (no inoculum added), 0.015, 0.03, 0.06, 0.12, 0.25, 1, 2 and 4 (gVM_{inoculum}/gVM_{waste}). The results indicate that the apparent maximal rate of dissolved organic carbon accumulation is reached at I/S = 0.12. Under this level, the hydrolysis process is limited by the concentration of biomass and can thus be described as first order kinetics phenomena with respect to biomass for I/S ratios below 0.12. The maximum methane production rate and the minimal latency are reached at a ratio of 2. In addition to that, both methane signature and ARISA show a shift in the methanogenic populations from hydrogenotrophic to acetoclastic.

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1. Introduction

Methane production from waste is a topic of great interest since it is closely related to both greenhouse gas reduction and energy production. This phenomenon occurs under anaerobic conditions such as anaerobic digesters or landfills. For digestion to proceed properly, sufficient amount of active inoculum, consisting of a complex community of microbes catalyzing a series of interdependent biochemical reactions (hydrolysis, acidogenesis, acetogenesis and methanogenesis), is required. The case study of inoculum to substrate ratio is therefore important and interesting in order to determine the efficiency of waste anaerobic degradation.

Optimal values of I/S ratios are poorly documented for municipal solid waste. However, previous studies on maize and straw have shown a great decrease of methane production for an I/S ratio below 0.25 expressed in g of Volatile Matter (gVM) of biomass per gVM of waste; the maximum specific methane production nevertheless increases until an I/S ratio of 2 (Hashimoto, 1989; Raposo et al., 2006).

Comparable conclusions have also been documented on solid potato waste (Parawira et al., 2004). Moreno-Andrade and Buitron (2003) have also reported that increasing the concentration of initial biomass can help the system overtake inhibition phenomena.

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Other studies have also reported that the increase of I/S ratio can positively impact the methane production rate and the total volume produced for certain substrates such as sunflower oil cakes between I/S ratio from 0.5 to 3 (Raposo et al., 2009). On swine slurry, an increase of I/S also increases the rate of methane production (Gonzalez-Fernandez and Garcia-Encina, 2009).

Working on inoculation with bovine rumen fluid, Lopes et al. (2004) have shown that the I/S ratio of 0.18 (kg/kg) is the most performant compared to ratios 0, 0.05 and 0.11 in terms of methane production rate and volume, and amount of methane in the biogas.

On kitchen waste, the use of a granular inoculum can prevent acidification of the system for I/S ratios between 0.4 and 2 when the alkalinity to COD ratio was 37 mg_{NaHCO3} g_{COD}⁻. It was further determined that a ratio of 0.7 is an advisable way of preventing acidification, since the biodegradability and the maximum methane production rate presents no significant difference when the alkalinity decreased from 44 to 22 mg_{NaHCO3} g_{COD}⁻ (Neves et al., 2004).

A large study on food and green waste (equally mixed or in monosubstrate incubation) has been realized by Liu et al. (1999), in thermophilic conditions for four I/S ratios (0.2, 0.25, 0.32 and 0.62) and in mesophilic conditions for only one ratio (0.32). Results showed that the biogas yield increased with the ratio in each condition and about 80% of the biogas production was obtained during the first 10 days of digestion. At the I/S ratio of 0.32, the biogas and methane yields from mesophilic digestion of food waste, green waste and their mixture were lower than the yields obtained at thermophilic temperature (Liu et al., 1999).



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The objective of this study is therefore to better characterize the impact of various I/S ratios on the anaerobic degradation of MSW.

2. Materials and methods

2.1. Experimental system

26.85 g of dry reconstituted solid waste (40 g wet mass) were used in batch reactors (1.1 L glass bottles). Waste composition was defined according to the French average waste composition determined during the MODECOM national characterization campaign of 1993 (Ademe, 1993) (Table 1).

Fractions were shredded to approximately 20-mm. The waste was placed in batch reactors (1.1 L glass bottles) and submerged in 680 mL of a carbonate buffer solution ($K_2CO_3 + NaHCO_3$). The buffer solution was prepared in a way to provide enough carbonates for buffering a Volatile Fatty Acids (VFAs) accumulation of 0.1 M and to keep final Na⁺ and K⁺ concentrations lower than the inhibition threshold values of 8 g L⁻¹ and 12 g L⁻¹ respectively (Kugelman, 1965). The pH value was around 7. The addition of buffer was performed to avoid an acid inhibition and to compare the effect of the I/S ratio on the kinetics and potential of methane production and not on the process stability.

These bioreactors were hermetically closed with a screw cap and a septum that enables liquid sampling. At the beginning of each experiment, the headspace was purged with helium to reach less than 0.5% of O₂. Reactors were incubated at 35 °C ± 2 °C during all the duration of the test without mixing.

2.2. Inoculum

Anaerobic sludge used as inoculum was sampled in a full scale mesophilic anaerobic digester operating as a continuous stirred

Table 1	
Composition of reconstituted municipal	solid waste.

Fraction	Wet mass in reactor (g)	Dry Matter (%)	Volatile Matter (%)	%C	%N
Meat	0.63	2.4	4.8	47.6	15.2
Potato	2.45	9.1	32.8	27.3	na
Ground coffee	0.29	1.1	2.6	52.4	2.1
Bread	0.43	1.6	1.1	42.2	1.8
White paper	1.85	6.9	4.7	34.0	0.0
Newspaper	1.47	5.5	4.0	42.4	0.1
Magazine	1.38	5.1	3.6	31.3	0.1
Cardboard	0.92	3.4	2.8	38.6	0.1
Corrugated cardboard	1.53	5.7	4.2	38.3	0.1
Wool	0.24	0.9	0.7	45.9	15.1
Cotton wool	0.31	1.1	0.8	42.1	0.0
Synthetic	0.24	0.9	0.6	63.8	16.8
Hay	0.43	1.6	1.1	43.0	1.0
Hard wood	1.02	3.8	2.8	46.6	0.1
Polyethylene	2.04	7.6	5.0	28.6	na
PET	0.15	0.6	0.4	na	na
Polyolefin	0.27	1.0	0.7	na	na
PVC	0.46	1.7	1.1	na	na
Polystyrene	0.46	1.7	1.1	92.1	0.0
Milk carton	0.36	1.3	1.0	51.4	0.1
Cigarette paper	0.06	0.2	0.2	37.9	0.0
Diaper	0.13	0.5	0.3	na	na
Tissue	0.37	1.4	1.0	41.6	0.1
Glass	5.21	19.4	12.7	na	na
Aluminum	0.26	1.0	0.7	0.0	0.0
Iron	1.24	4.6	3.1	0.0	0.0
Rocks	2.45	9.1	6.0	na	na
Battery	0.20	0.7	0.5	na	na

tank reactor (HRT = 30d) treating sludge from a wastewater treatment plant. Samples were stored in hermetically closed containers at $4 \circ C$ until use.

Dry Matter (DM) and Volatile Matter (VM) were determined using standard procedure (AFNOR, 2000). Their values were respectively $12.2 \pm 1.9\%$ and $4.9 \pm 0.7\%$. Biomass was recovered by centrifugation (9420g, 15 min) and target amounts of inocula were introduced in the reactors.

The studied I/S ratios were 0 (no inoculation, only indigenous inoculum of reconstituted waste is present) ; 0.015; 0.03; 0.06; 0.12; 0.25; 1; 2; 4 (gVM_{inoculum}/gVM_{waste}). All reactors were run in triplicate except for ratio 4 (one reactor only, because of material constraint). Due to the important volume of sludge necessary for reaching an I/S ratio of 4 and to experiment in a working volume of 680 mL, this single last reactor has been conducted with less waste matter. The reactors were followed until reaching a plateau of methane production; this represented a monitoring period of at least 160 days.

Triplicate blank experiments (inoculum without waste) were launched in order to determine the amount of endogenous methane production per gram of sludge. This amount $(6.1 \pm 1.8 \text{ mL}_{CH4} \text{ STP})$ was substracted from all experiments accordingly. It represented only 4.1% of the mean of the methane production of all inoculated reactors.

2.3. Gas phase

2.3.1. Biogas production measurement

To measure gas production, the biogas volume is expressed as biogas STP. Biogas accumulation in the headspace was measured using a differential manometer (Digitron 2082P). As the volume of the headspace was constant, the ΔP could be converted into a volume of biogas produced at standard pressure.

In the reactor:

$$Ph(t) \times Vh = nRTi$$

The excess pressure was removed under atmospheric conditions, at a pressure of 1013.25 mbar.

$$Ph \times Vh = nRTi = Vb \times Patm$$

$$Vb = \frac{ph \times Vh}{Patm}$$
$$Pcb(t) = \sum_{i=0}^{t} Vb(i)$$

where Ph(t) corresponds to the pressure in the headspace at time t (mbar); Vh, volume of headspace (mL); n, number of moles of gas (moles); R, gas constant (J K⁻¹ mol⁻¹); Ti, temperature of incubation (K), constant at 35 °C (308.15 K); Vb, volume of biogas produced (mL gDM⁻¹ STP); *Patm*, atmospheric pressure equal to 1013.25 mbar; *Pcb*, cumulated biogas production (mL gDM⁻¹ STP).

All the results below were thus given at STP (Standard Temperature and Pressure) conditions.

$$Vm(t) = Vb(t) \times \%m(t)$$

$$Pcm(t) = \sum_{i=1}^{t} Vm(i)$$

where *Vm* correspond to the volume of methane produced; *Vb*, volume of biogas produced (mL gDM⁻¹ STP); *%m*, the percentage of methane in the biogas; *Pcm*, cumulated methane production (mL gDM⁻¹ STP).

The methane production will be expressed in this paper as *Pcm*, corresponding to the cumulated methane production at the end of

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