Biotechnology Letters **23:** 1889–1892, 2001.

Analysis of the methane production in thermophilic anaerobic reactors: use of autofluorescence microscopy

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Received 10 July 2001; Revisions requested 23 July 2001/10 September 2001; Revisions received 5 September 2001/21 September 2001; Accepted 24 September 2001

Key words: activity, anaerobic process, biogas, methanogens, waste water treatment

Abstract

Methanogenic activity in thermophilic, anaerobic reactors was determined by comparing the amount of methane generated in single- and two-stage systems with the size of the methanogenic population, as determined by microscopy. The methanogenic activities were 2.71×10^{-9} ml methane cell^{-1} d^{-1} and 1.10×10^{-9} ml methane cell^{-1} d^{-1} for 10 and 4 days of the hydraulic retention time (HRT), in the single-stage system. In the two-stage system, 7.49×10^{-9} ml methane cell^{-1} d^{-1} in the acidogenic reactor and 1.56×10^{-9} ml methane cell^{-1} d^{-1} in the methanogenic reactor for 4 days of the HRT. A high correlation was evident between the methane production and methanogenic population $[0.1354 \ln(x) - 2.1375](R^2\ 0.8619)$.

Introduction

The anaerobic treatment of industrial wastewater has a number of potential benefits, including low energy consumption, low excess sludge production, enclosure of odours and aerosol (Fynn & Withmore 1982, van der Berg & Kennedy 1981). The parameters normally employed in the control of anaerobic digestion, such as the percentage of COD removal, the concentration of volatile fatty acids and the amount and composition of biogas generated in the process, are not always representative of the composition and physiological state of the biomass contained within the system (Jawed & Tare 1999). Consequently, and in order to acquire more detailed information in respect of this biomass, other parameters have also been used in the characterization of the microorganisms responsible for the anaerobic processes. Traditionally, volatile solids have been the parameter of choice in the measurement of anaerobic biomass; in the case of activity analyses, the tests used have permitted the evaluation of the maximum activity attainable by these microorganisms under standard test conditions, and these do not necessarily coincide with those in the treatment unit itself (Lazarova & Manem 1995).

Methanogenic activities are normally calculated by comparing the rate at which the substrate is consumed, or the amount of methane that is generated by the process, with the total biomass contained in the system. However, the results obtained with the parameter most commonly used in the determination of biomass, volatile suspended solids (VSS), are not always representative of the minority groups involved in the anaerobic treatment process (Solera *et al*. 2001).

In this study, the methanogenic activity in thermophilic anaerobic reactors has been determined by comparing the amount of methane generated in each system with the size of the methanogenic population, as determined by microscopy. These activity measurements have then been compared with those obtained by more classic means (relative to VSS). Single- and two-phase thermophilic anaerobic agitator tanks were employed, operating at different hydraulic retention times.

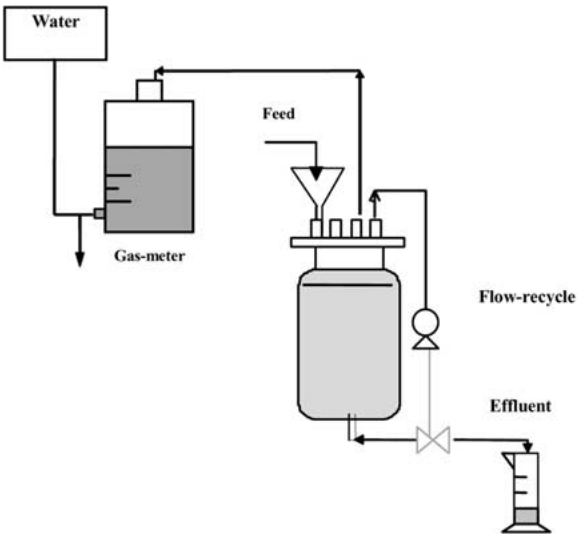

Fig. 1. Diagram of the CSTR used in the experimental protocol. Schematic representation of the laboratory-scale continuously stirred tank reactor with no recycling of biomass used in the study. The active volume was 2 l.

Materials and methods

The equipment consisted of a laboratory-scale continuously stirred tank reactor (CSTR), with no recycling of biomass (see Figure 1). In this type of reactor, the solid and hydraulic retention times coincide. Two types of system were used: single and two-phase reactors. The single-phase reactors were operated at two hydraulic retention times (HRT): 4 and 10 days (digesters R4 and R10, respectively). In the two-phase systems, the HRT of both the acidogenic (RA) and the methanogenic (RM) reactors was 4 days. The reactor temperature was maintained at 55 °C.

The reactors were fed with a wine distillery wastewater (vinasses) (15 g COD l^{-1}) and was supplemented with NaOH to maintain a neutral pH in the reactors, and pH 5.5 in the acidogenic reactor. Vinasses have a readily biodegradable fraction (80% of the total) (Pérez *et al*. 1997). The methanogenic reactor was fed with filtered acidogenic influent (pore size of 0.22 μm) to retain the acidogenic microorganisms.

In a previous study (Solera *et al*. 2001), the methanogenic population was determined by autofluorescence microscopy in the single- and two-stage reactors. We obtained the following percentages of methanogens in each of the systems studied: 16% in single-phase reactors, 0.17% in the acidogenic reactor and 26% in the methanogenic reactor.

All analytical measurements of the parameters used in the monitoring and control of the anaerobic digestion process were carried out in accordance with Clescerli (1990). The volume and composition of biogas were determined according to Nebot *et al.* (1995). The methanogenic population was determined by autofluorescence microscopy (Doddema & Vogels 1978, Jain *et al*. 1991). Biomass was determined by measuring volatile suspended solids, according to Clescerli (1990).

Results

Performance and operating parameters for the control of the anaerobic process are shown in Table 1. The single-phase reactors give total COD removal of 80%. This value coincides with the readily biodegradable fraction of the feed, mentioned earlier.

Table 2 shows the average results in respect of methanogen concentration (cells ml^{-1}), biomass (VSS) and activity in each of the systems studied. In the single-phase digesters, a decreased hydraulic retention times (HRT) is accompanied by an increase in the methanogenic population and a decrease of methanogenic activity with respect to both biomass and the concentration of methanogens. In systems with no biomass retention, decreased HRT is reflected by faster rates of dilution and, as a result, in a greater number of microorganisms leaving the system daily in the effluent. Consequently, a larger amount of substrate is consumed in the anabolic route, in which no methane is generated, in order to keep the size of the population in the steady state.

In the acidogenic system, the average values do not coincide. Hence, the activity values in respect of biomass are lowest, and those relating to methanogenic concentration highest in this digester. In this case, the methanogenic population represents less than 1% of the microbial population in the reactor (Solera *et al.* 2001). It therefore follows that total biomass is not a representative parameter for this minority group. In any event, it is worth pointing out that the activity values obtained in this research are lower than those recorded in studies performed under similar operating conditions and with similar reactors, but in which activity values referred to maximum specific methanogenic activity levels (Shang & Sung 1998).

Attempts were made to correlate the production of methane to the size of the methanogenic population and to the level of biomass present in the

Table 1. Performance and operation parameters for control of the anaerobic process.

Reactor	HRT (days)	OLR_o (g l^{-1} d^{-1})	COD_r (%)	pH	Biogas $l l^{-1}$ d^{-1})	Composition of gas (% v/v) (% v/v)		
						CH_4	CO_2	H_2
R10	10	1.44	82.1	7.35	0.47	82	18	0
R4	4	3.75	80.1	7.6	0.8	85	15	0
RA	4	3.79	30.1	5.53	0.18	66	29	7
RM	4	2.65	71.7	7.8	0.45	91	9	0

HRT = hydraulic retention times (days).
OLR = organic load rate; the food of the reactor, expressed in g COD_o per litre of reactor per day (COD_o = initial COD, 15 g l^{-1}).
COD_r = organic removal efficiency as a percentage of initial COD (COD_o).
Biogas: volume of gas generated as litre of biogas per litre of reactor per day.
RA = acidogenic reactor; RM = methanogenic reactor; R10 = single-stage reactor with a hydraulic retention times of 10 days; R4 = single-stage reactor with a hydraulic retention times of 4 days.

Table 2. Methanogenic concentration, biomass and activity of methanogens in the experimental protocol.

Reactor	Methanogen (cell ml^{-1}) ($\times 10^{-8}$)	VSS (g l^{-1})	Activity[a] ml methane $cell^{-1}$ d^{-1} ($\times 10^{-9}$)	Activity[b] ml methane g VSS^{-1} d^{-1}
R10	1.29 ± 0.5	0.47 ± 0.09	2.71 ± 0.38	680 ± 150
R4	4.6 ± 0.8	1.57 ± 0.22	1.1 ± 0.08	350 ± 20
RA	0.17 ± 0.05	1.05 ± 0.09	7.4 ± 3.16	110 ± 20
RM	2.68 ± 0.27	0.89 ± 0.15	1.56 ± 0.14	480 ± 80

[a]Methanogenic activity determined with reference to methanogenic concentration and daily methane production.
[b]Methanogenic activity determined with reference to biomass (volatile suspended solids) and daily methane production.
RA = acidogenic reactor; RM = methanogenic reactor; R10 = single-stage reactor with a hydraulic retention times of 10 days; R4 = single-stage reactor with a hydraulic retention times of 4 days.

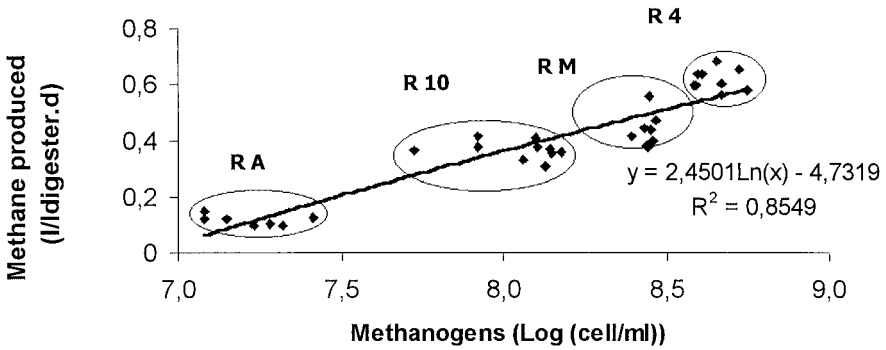

Fig. 2. Correlation between the production of methane and methanogenic concentration in the single- and two-stage anaerobic reactors. The slope of the curve represents methanogenic bacteria activity in the studied reactors: RA = results from acidogenic reactor; RM = results from methanogenic reactor; R10 = results from single-stage reactor with a hydraulic retention times of 10 days; R4 = results from single-stage reactor with a hydraulic retention times of 4 days.

systems studied. In the case of the former, this produced a logarithmic relationship (see Figure 2). No correlation was found between the production of methane and biomass. The slope of the curve represents methanogenic bacteria activity. The results are distributed in three distinct areas: the upper group corresponds to the R4 single-phase reactors and the RM methanogenic reactor, both of which produced similar methanogenic activity values and had larger methanogenic populations than the other reactor types under study; the intermediate group corresponds to the R10 digester, exhibiting an average methane yield and greater activity levels than the R4 and RM reactors; and the lower group corresponds to the acidogenic digester, with lower methane production, and a smaller methanogenic population than the other systems under analysis. This reactor is representative, primarily, of H_2-using methanogens. The activity of H_2-using methanogens was far higher in the acidogenic reactor than in the other digesters, which reflects that these methanogens develop far more quickly than their acetoclastic homologues.

Conclusion

Methanogenic activity may be determined with reference either to methanogenic concentration or to VSS values in single-phase systems operating under steady-state conditions. In two-phase systems, the methanogenic activity measurements determined with reference to methanogenic concentration provide more specific information regarding the physiological state of this population than with respect to total biomass.

Up to a certain, maximum level, there is a positive correlation between the production of methane in anaerobic reactors with no recycling of biomass and the size of the methanogenic population.

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