Biotechnology Letters Vol 12 155-160 (1990)

EVOLUTION OF MICROBIAL ACTIVITIES AND POPULATIONS IN GRANULAR SLUDGE FROM AN UASB REACTOR

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SUMMARY.

With granular sludges grown in an UASB reactor fed with a mixture of acetate and propionate, it is shown that (I) growth of propionate-utilizing bacteria is responsible for the increase of the VSS content of the granular sludge, acetoclastic microflora did not grow or little, (II) there is not a stoichiometric relationship between substrate removal and observed methane production, and (III) contrary to the common practice the best way to present data on bacterial concentrations in sludges is: bacteria/g VSS, which will provide a reliable basis for comparisons between different works from various authors.

INTRODUCTION.

The Upflow Anaerobic Sludge Blanket (UASB) reactor is a well established process for the anaerobic treatment of various kind of waste waters (Lettinga et al., 1980). Its good performances depends on the formation of a bed of well settling and highly active granular sludge, with a low sludge volumetric index (SVI) and a high methanogenic activity (Lettinga et al., 1980). This kind of anaerobic reactor might be an economic way for treating waste waters in developing countries (Noyola et al., 1988) if means are available to deal with the inoculum. Unfortunately, in spite of numerous studies, there is still a need for basic information on the characterization of the parameters that control the granulation of the sludge and its microbial composition. Such data are important for the optimization of anaerobic reactors operation and start-up.

In this paper some aspects of anaerobic microbial populations of granular sludge from an UASB reactor are discussed, particularly substrate consumption activities and microbial composition.

MATERIAL AND METHODS.

UASB reactor. A 4.5 liter reactor was inoculated with activated sludge collected from a quiescent anaerobic zone of an aeration tank of a municipal waste water treament plant, this sludge had a high mineral fraction (73% of TSS) due to small particles and grit. The reactor was operated for a year at 35°C, at an hydraulic retention time of 2 days, performing a 90% COD removal with a synthetic waste water containing acetic acid (3.5 g/l) and propionic acid (1 g/l) as energy and carbon sources, and a salt medium previously described (Noyola et al., 1988).

Experimental conditions. The anaerobic technique of Hungate (1969) and Balch and Wolfe (1976) was used throughout this study to prepare the cultivation media; a gassing manifold (Balch et al., 1979) and an anaerobic chamber were used for the operation of inoculation and addition of the substrates. Sodium sulfide was used as reductant.

Kinetics experiments. The sampled granular sludge (1 mm to 2 mm of diameter) was allowed to stay 24 hours under vacuum, in the air lock of the anaerobic hood, in order to allow the residual substrates to be biologically removed and to allow the gas produced to escape more easily; then, the sample was flushed with nitrogen. Controls containing no substrate only showed a residual methane production. Once, inside the anaerobic chamber, 10 ml of sample were distributed per serum-bottle (60ml) which already contained 10 ml of a mineral solution (Noyola et al., 1988). At time zero of the experiments, the substrates were added in known excess, in order to be in saturating conditions. Each experiment was run in duplicate and incubated at 35°C.

Bacteria counts. The anaerobic bacteria were quantified using the most probable number (MPN) technique with 5 tubes per dilution; sludge granules were dispersed in the anaerobic chamber using a Potter. For the OHPA bacteria (Obligate Hydrogen Producing Acetogenic Bacteria) the same culture media as for methanogenic bacteria were used, except for the sulfate salts which were changed for chloride salts. Sludges for kinetics experiments and bacterial counts were sampled at the reactor bottom.

Analytical techniques. Total suspended solids (TSS) and volatile suspended solids (VSS) were determined in duplicate using Standards Methods (1976). Acetate was analyzed by gas chromatography, using a FID detector and a stainless steel column packed with Porapack Q (80–100 mesh). Methane was analyzed using the same conditions as acetate but the samplings and injections were made by means of a Hamilton pressure-lock syringe.

RESULTS AND DISCUSSION.

Two series of experiments were performed during an eight month period. The first one was run 3 months after the start-up of the UASB reactor. Time-course of methane production from acetate and propionate were recorded (Fig. 1) and the kinetic parameters were calculated (Table 1, 1st series).

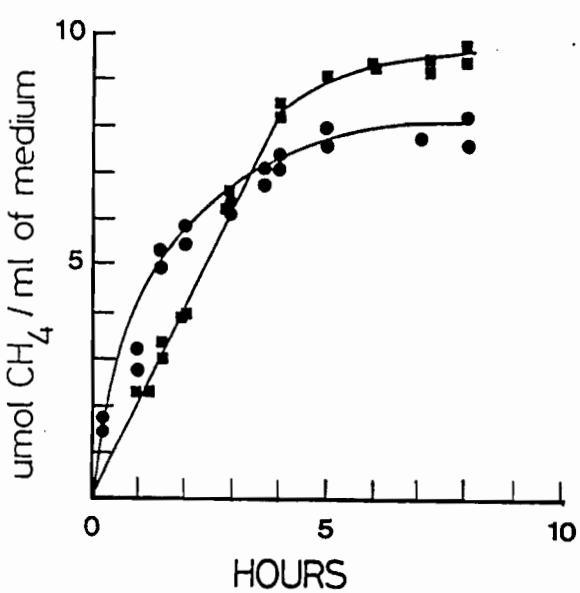

Fig. 1. time course of methane production from acetate (●) and propionate (■) by granular sludges, 3 months after the UASB reactor start-up.

The maximum velocity (Vmax) and specific activity (Asp = Vmax/VSS) of methanogenesis from acetate is higher than the parameters calculated for propionate (Table 1, 1st series). Lower activities were calculated by Dolfing (1985): 0.3 to 1.14 mmol/g VSS/h for the acetate and 0.36 to 0.42 mmol/g VSS/h for the propionate. This author also observed that the activity of the acetoclastic microflora is higher than that of the propionate users. The granular sludge used by Dolfing (1985) were sampled from an industrial UASB digestor (Holland), fed with waste waters from a sugar beet processing plant. We can see that the results obtained from two different geographic areas (temperate and tropical) and two different types of effluent are in the same order of magnitude. Some differences would be expected mainly in relation with the origin of the inoculum.

Table 1. Kinetic parameters for methane production by granular sludge.

	So mM	TSS g/l	VSS %	Vmax mmol/l/h	Asp mmol/g VSS/h	Vmax/g TSS mmol/g TSS/h
Acetate:						
1st series	7.8	28.4	27	10.8	1.4	0.38
2nd series	11.7	13	53	6.4	0.9	0.49
Propionate:						
1st series	5.4	28.4	27	4.3	0.55	0.15
2nd series	10	13	53	4.5	0.64	0.35

Eleven months after start-up, in order to study the sludge evolution, the same parameters were measured in a second series of experiments. The time-course of experiments is presented in Fig. 2 and the calculated kinetic parameters in Table 1, 2nd series. The methanogenic activity from acetate remained greater than that from propionate. The VSS fraction increased twice from 27 % to 53% (% TSS) and the Vmax calculated per gram of TSS, increased 1.3 times with acetate and 2.3 times with propionate as substrates. Then, on the basis of proportionality between microbial biomass and Vmax, the increase in VSS (%TSS) can be related with the increase of the Vmax (per g TSS) of propionate degradation. The increase of biomass can mainly be explained by the growth of the population of the propionate-using bacteria: the increase of methanogenic specific activity with propionate, together with the decrease of the activity with acetate, are also arguments that support this observation, since those values are calculated per gram of VSS as a whole (acetoclastic plus OHPA biomass). This implies that the acetoclastic microflora did not grow or little. To date, scarcely anything is available about physiology of complex anaerobic microbial populations within anaerobic reactors, even if efforts are made to understand them on the basis of individual properties of defined microbial groups. In the case of the acetoclastic bacteria[4], the hypothesis is made that they could be present under a physiological state corresponding at an uncoupling between growth and energy metabolism, since acetate consumption is accomplished in our reactor apparently without significant growth. In anaerobic digestion this phenomenon might be very important, because one of the great advantages of this process is the generation of small amounts of sludge which is generally attributed to the slow generation time of anaerobic bacteria. In view of the former results, we emitt the hypothesis that uncoupling energy production and growth would also act in favor of a low biomass

generation with active metabolic properties in anaerobic reactors.

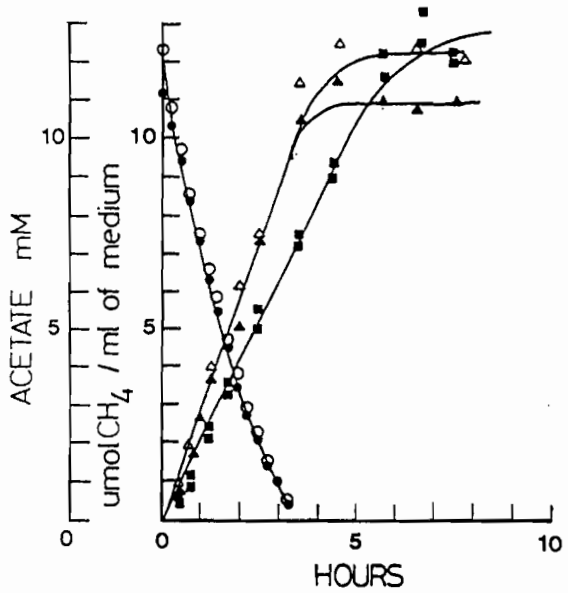

Fig. 2. Kinetics of acetate consumption (○, ●) and methane production from acetate (▲, △) and propionate (■) (duplicate experiments) by granular sludges, 11 months after the UASB reactor start-up.

Dolfing (1985) has calculated degradation activities of acetate and propionate from the curves of methane production, in order to characterize the mass transfer limitations of the substrate in the granular sludge. However, in his study the mass transfer limitations of the methane evolved from the biomass within the granules to the atmosphere, was ignored. In case that gas transfer limitation exists, it might not be accurate to calculate substrate degradation in the liquid phase from evolved methane data, since the observed kinetics of both substrates removal and methane production could not be correlated. Taking into account these considerations, the acetate degradation parameters were determined from the curve of acetate consumption (Fig. 2), in order to know if the methane production was stochiometrically related with the substrate removal. The Vmax, Asp and apparent constant of affinity (K'm) were calculated using the following linearized Michaelis-Menten equation: $(\ln So/St)/t = (-1/K'm) \times (So-St)/t + Vmax/K'm$, where S is the initial (o) or time (t) acetate concentration (Robinson and Characklis, 1984). The calculated Vmax were 9.5 mmol/l/h (Table 2) for the acetate degradation and 6.4 mmol/l/h for the methane production (Table 1, 2nd series), so there is a difference of 48.4% between the two parameters. Furthermore, whereas acetate quite desappeared after 3 hours, a slight methane production is still registered during one hour more (Fig. 2). This could be explained by the presence of tiny gas bubbles that remain stuck to the granules, after substrate desappearance, and by the consumption of remaining acetate adsorbed onto the granules. Without gas transfer limitation the Vmax calculated in both cases must be equal (according to 1 mol Acetate ——> 1 mol CH4), but as we can note, in the case of granular sludge this phenomenon should be taken into account since the Vmax are quite different between acetate degradation and methane production. Then, it must be concluded that with granular sludge, it is not correct to perform kinetics studies of substrate consumption only on the basis of methane production.

A K'm value for acetate of 1.3 mM was found, which is slightly higher than those calculated by Dolfing (1985) with granular sludges

(Table 2). Kaspar and Wuhrmann (1978) with a completely stirred conventional digestor found a K'm of 0.32 mM (Table 2). The Km of pure cultures of acetoclastic bacteria are 0.7 mM for Methanothrix soehngenii (Huser et al., 1982), 1.2 mM for Methanothrix concilii (Patel, 1984) and 0.5 mM for Methanosarcina barkeri (Smith and Mah, 1978). This means that the different values found by the various authors with anaerobic sludges are of the same order of magnitude as those calculated for the pure cultures of methanogenic bacteria.

Table 2. Kinetics parameters of acetate degradation by granular sludge. (*) Data calculated in mmol/g VSS/h, from values in umol/g VSS/mn in the cited reference.

	this work UASB 4.5L	conventional reactor Kaspar et al., 1978	Industrial UASB Dolfing (1985)
Vmax,Ac mmol/l/h	9.5	0.63	_
Asp,Ac mmol/g VSS/h	1.4	_	0.3 a 1.14(*)
K'm mM	1.3	0.32	0.5 a 1

In order to characterize the different microbial populations of the granular sludge, the anaerobic bacteria were counted (Table 3). It is found that acetoclastic bacteria represents 30 % of the hydrogenophilic bacteria.

Table 3. Quantification of the different microbial groups in the granular sludges. E is for exponent of 10. I : this work, II : Dolfing et al., 1985, III : Dubourguier et al., 1987.

	Number of microorganisms per g VSS	per ml		
	I	I	II	III
Fermentative bacteria	3.E6	2.E7	1.E10	1.E9
Acetoclastic methanogens	3.E5	3.E6	1.E8	2.5.E9
Hydrogenophilic methanogens	1.E6	1.E7	1.E9	2.5.E9
OHPA: propionate users	2.E5	6.E6	1.E7	2.5.E8

In our reactor, the high fraction of hydrogen-using methanogenic bacteria is explained by their role in the syntrophic association with propionate users. Futhermore, it has been demonstrated that methanogenic acetoclastic bacteria evolved molecular hydrogen (15 Pa) when using acetate (Lovley and Ferry, 1985); then, we can expect that this hydrogen may acts as another substrate source for hydrogenophilic bacteria in anaerobic reactors, since a very tight relationship exists between these bacteria in a granule.

Dubourguier et al. (1988) and Dolfing et al. (1985) made counts by the MPN method using 3 tubes per dilution which makes difficult any comparison with our counts (5 tubes per dilution); furthermore, their data are reported as bacteria/ml, which is difficult to interpret in the case of solids as granular sludge: the amount of sludge (TSS) that can be taken out from a same reactor between two different samplings can vary markedly, since it is difficult to control the volume of liquid which run out in the same time with the sludge. Moreover, VSS

concentration in the sludge bed may vary widely for different reactors, preventing comparisons between them. In our laboratory, we often observed that two samples taken out from the same reactor at very short time intervals gave different results in term of bacteria/ml but same counts as bacteria/g VSS. Table 3 gives us an idea of such a difference when the counts are calculated as bacteria/ml or bacteria/g VSS. Wu et al. (1987) reported data as bacteria/ml and bacteria/g Suspended Solids, which goes in the sense of our assumption. Nevertheless, they did not report counts as bacteria/g VSS. Since the VSS content of a sludge is used as an indicator of biomass and to express most of the operation parameters of anaerobic reactors, it might appear more useful to report bacterial counts as bacteria/g VSS.

ACKNOWLEGMENTS: This work is part of a project supported by O.E.A. (Organizacion de los Estados Americanos) under the program "fuels by fermentation" and by the E.E.C. (European Economic Comunity) (grant No. C11.0197.Mexico (H)). J.P. Guyot is a visiting researcher from ORSTOM (Institut Francais de Recherche Scientifique pour le Developpement en Cooperation).

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