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THE KINETIC BASIS OF A NEW START-UP METHOD TO ENSURE THE RAPID GRANULATION OF ANAEROBIC SLUDGE

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ABSTRACT

The control of the granulation process seems to be a kind of microbial selection: the enrichment of Methanothrix sp. against the Methanosarcina sp. Because of a lower K_S value of Methanothrix, keeping a low (below 200 $mg.dm^{-3}$) acetate concentration has been advised to be beneficial for granulation. This method results a 70-100 days start-up time. This approach assumes Monod-type kinetics, although a substrate inhibition model may describe better the biomethanation of acetate. We found that the best fit was yielded by a Haldane-type equation modified by an inhibition response coefficient (n). The major difference between the kinetics of raw and granular sludges has been manifested in this dimensionless parameter. The n was 4 times higher for raw sludge (3.6-4.1) than for granular sludge (0.95-1.13) which means that the granular sludge (formed mainly by Methanothrix sp.) is less sensitive to substrate inhibition. Continuous UASB experiments gave a similar result: the n-value continuously decreased (from 2.3 to 0.2) following granule formation. On the basis of the above findings we developed a new strategy for granulation control which ensured fast (35-40 day) granulation on carbohydrate-containing wastewaters.

KEYWORDS

UASB-reactor; granular sludge; kinetics; substrate inhibition; start-up

INTRODUCTION

Satisfactory performance of Upflow Anaerobic Sludge Blanket (UASB) reactors (Lettinga et al., 1980) depends on the formation of compact sludge granules. The granulation of anaerobic sludge is a special case of microbial aggregation. The wide variety of factors affecting this process makes different hypotheses possible. According to the different approaches we divided the results of studies on microbial aggregation presented previously into three groups.

1. Physico-chemical approach. The basis of this theory is that the behaviour of bacterial cells can be described by the classical theory of colloid stability (Calleja, 1984). Accordingly, microbial

aggregation is mainly influenced by the characteristics of microbial cell wall and some physico-chemical factor of the medium.

2. Physiological approach. Evidence was obtained indicating that bacterial cells are often covered by extracellular polymers (Dolfing et al., 1985). They play an important role in the formation of a supporting matrix for cell aggregates. Production of biopolymers and thus the cell aggregation is affected by the nutritional balance, e.g. by carbohydrate-containing substrate (Harada et al., 1988).

3. The ecological approach is the most complex approach, which integrates the previous ones, with special emphasis on the inter-microbial connections like the symbiosis, or the competition for substrates (Zeikus et al., 1985).

Our investigations were based on the last description. The shape and structure of the granule are determined by dense balls of filaments of Methanothrix soehngenii. Another acetoclastic bacterium often isolated from anaerobic ecosystems, Methanosarcina barkeri, and in the case of complex substrates, non-methanogenic bacteria may also be embedded in this matrix.

The main criterion of granulation is the selection of filament-forming bacteria. Most authors agree that this can be carried out by making use of the much lower half rate constant of Methanothrix sp. compared to that of Methanosarcina sp. This means that the acetate concentration has to be lower than 200 $mg.dm^{-3}$ during the start-up, otherwise growth of Methanosarcina sp. is promoted (Hulshoff Pol, 1989). Nevertheless, half rate constants considered in this theory were yielded by assumption of Monod-type kinetics, but several authors reported the validity of substrate-inhibition models for description of acetate biomethanation (Bolle et al., 1986).

MATERIALS AND METHODS

Sludges. Eight different samples were studied. Three of them (samples 6, 7 and 8) represented a granulation process in a 200 dm^3 UASB reactor, taken at the start, and at the 23rd and 93rd days of operation respectively. Their characteristics (the origin and structure of sludges, the types of substrates which were fed to reactors before taking samples) are summarized in Table 1. All digester plants and laboratory scale bioreactors were operated in the mesophilic temperature range.

Determination of gas production rate. Two batch procedures (A and B) were used. In Method A the gas production was followed by monitoring the pressure in 50 cm^3 Erlenmeyer flasks, while in Method B the volume of biogas produced was directly measured in 500 cm^3 serum bottles. Specific gas production rate as $cm^3.g^{-1}VSS.h^{-1}$ was determined with a 0-4.5 $g.dm^{-3}$ acetate concentration range. Detailed descriptions of these methods and sludge characterisation have been presented elsewhere (Morvai et al., in press/a).

Acetate concentration was determined by a Chrom 4 gas chromatograph (Laboratorní Pristoje, Prague, Czechoslovakia), equipped with a 2.5 m glass column, filled with GP 10% SP 1200/1% H_3PO_4 on 80/100 Chromosorb WAW (Supelco, Bellefonte, USA; Catalogue no:1-1965), and a flame ionization detector. The temperature of the thermostatically controlled oven was 140°C.

Sludge characteristiscs. Determination of suspended solids (X_{SS}), volatile suspended solids (X_{VSS}) and sludge volume index (SVI_{30}) were made according to APHA (1985). Packed cell volume (PCV) was derived from the sediment volume fraction of a sample after 10 min at 1500 X g centrifugation related to dried solid mass content.

Study on influence of acetate concentration on granulation. Experimental set-up and operational parameters have been described earlier (Morvai et al. 1990). Reactors and operational parameters for start-up experiments with carbohydrate wastewater also have been described (Morvai et al., in press/b).

TABLE 1. Characterisation of Sludges Examined

Sample number	Sludge structure	Origin of sample	Substrate
1	Non-granular	Digester plant	Communal sludge
2	Non-granular	Digester plant	Communal sludge
3	Non-granular	Laboratory scale UASB reactor	Synthetic acetate wastewater
4	Granular	Laboratory scale UBF reactor*	Synthetic carbohydrate wastewater
5	Granular	Laboratory scale UBF reactor*	Synthetic carbohydrate wastewater
6	Non-granular	Digester plant	Communal sludge
7	Non-granular	Pilot scale UASB reactor	Molasses wastewater
8	Granular	Pilot scale UASB reactor	Molasses wastewater

* UBF: Upflow Blanket Reactor - The combination of an UASB reactor and an anaerobic filter.

DESCRIPTION OF KINETICS OF ACETATE BIOMETHANATION

Acetate may become inhibitory to the acetoclastic methanogens at higher concentrations. Inhibition kinetics were introduced by Andrews (1969) into the description of the anaerobic digestion process. He used an equation published originally by Haldane (1930) for enzyme reactions.

Comparing several substrate inhibition models (Morvai et al., in press /a), we found that the most successful description was obtained by using the following, modified Haldane-type equation:

$$q = \frac{q_{max}}{1 + S/K_S + (K_I/S)^n} \qquad (1)$$

where q is a specific gas production rate, q_{max} is its maximum value in the absence of inhibition , K_S is a half rate constant and K_I is the inhibition constant, while n is the inhibition response coefficient. S represents substrate concentration. According to the deduction presented by Yang and Okos (1987) a close relationship can be assumed between the q value and the specific growth rate (μ).

The above equation successfully described the anaerobic decomposition of toxic materials, for example the biodegradation of phenolic compounds (Dwyer et al.,1986) and the anaerobic treatment of solid waste landfill leachate (Wu et al.,1988). In the case of n=1, Equation 1 is the

original Haldane relationship between specific gas production rate and substrate concentration.

The parameters of the Haldane- (and Andrews-) type equations represent the values which would be observed in the absence of inhibition. As Figure 1 shows, this fictive maximum specific growth rate (μ_{max}) is far from the detectable maximum of the Haldane-type function (μ_{max}'). Accordingly, half rate velocity constants also belong to these "imaginary" values. Consequently, it is difficult to compare kinetic parameters of the different models, although there is a common range of the two curves yielded by substitution of the same values into both models.

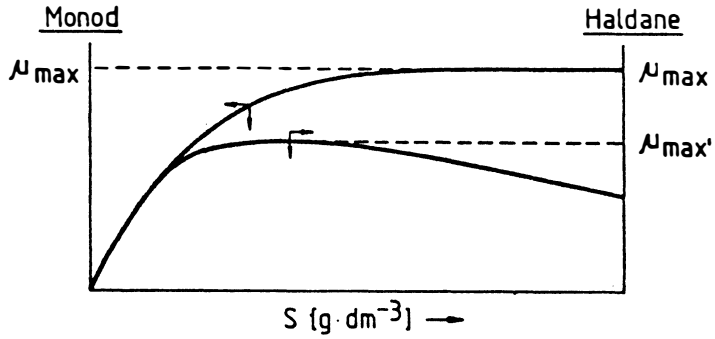

Fig. 1. The specific growth rate as a function of the substrate concentration for the Monod and Haldane equations

In order to improve the model described by Equation 1, we substituted A, B and C, considered as empirical constants for q_{max}, K_S and K_I respectively:

$$q = \frac{A}{1 + (B/S) + (S/C)^n} \qquad (2)$$

The real, detectable maximum specific gas production (q_{max}') can be calculated by the following expression:

$$q_{max}' = \frac{A}{1 + \left(\frac{B^n.n}{C^n}\right)^{1/(n+1)} + \left(\frac{B}{C.n}\right)^{n/(n+1)}} \qquad (3)$$

K_S' and K_I', the values of concentration at which q is equal to $q_{max}'/2$, can be calculated by a simple iteration program.

RESULTS AND DISCUSSION

Batch experiments on kinetics of acetate biomethanation.

To the gas production rates measured at different acetate concentrations we fitted a curve according to Equation 2. Parameters A, B, C and n for the best fit were calculated by non-linear least-squares method with a microcomputer program (Valkó and Vajda, 1989). The "real" maximum gas production rates (q_{max}') calculated by Equation 3, the half rate constants corresponding to these values (K_S' and K_I') and the inhibition

response coefficients (n) are summarized in Table 2. According to several authors, the unionized acetic acid is assumed to be the substrate (Andrews, 1969). For easy comparison with kinetic parameters reported previously, we also present our K_S' and K_I' values expressed as unionized acetic acid concentration ($K_S'_{HAc}$ and $K_I'_{HAc}$). Our results in both expressions are similar to those reported elsewhere and reviewed by Wiesmann (1988).

TABLE 2 Modified Kinetic Parameters Obtained from the Batch Experiments

Sample	q_{max}' [$cm^3.g^{-1}.h^{-1}$]	K_S' [g.acetate.dm^{-3}]	K_I'	$K_S'_{HAc}$ [mg HAc.dm^{-3}]	$K_I'_{HAc}$	n [-]
I. Non-granular sludges:						
1	0.85	0.09	2.14	0.510	12.2	4.08
2	1.22	0.11	2.57	0.625	14.16	3.92
3	4.54	0.31	2.26	1.76	12.8	4.11
II. Granular sludges:						
4	6.40	0.025	5.94	0.148	33.7	1.00
5	2.94	0.07	6.50	0.398	36.9	1.00
III. Development of granular sludge in the UASB-experiment:						
6	3.53	0.24	2.85	1.36	21.8	2.28
7	6.48	0.17	1.18	0.966	233	0.39
8	7.17	0.29	6.47	1.65	5600	0.23

Comparing the parameters for both types of sludges, we only found significant differences in n. Its value was 4 times as high for non-granular sludges than for granular ones irrespective of the measurement method and the origin of the sample. This means that the granular sludge, formed mainly by Methanothrix-like bacteria, is less sensitive to inhibition caused by high substrate concentration. This statement can be supported by results of kinetic studies on pure cultures of methanogens. While substrate inhibition was reported for several strains of Methanosarcina (Yang and Okos, 1987), no inhibition was found even above 2.5 g acetate.dm^{-3} concentration when the isolated culture of Methanothrix soehngenii was investigated (Huser et al., 1982).

A continuous UASB experiment with molasses wastewater (Sample 6, 7 and 8) gave a similar result: the n-value continuously decreased following the enrichment of Methanothrix-like bacteria and granule formation.

Influence of acetate concentration on sludge characteristics

Five reactors were operated at the same hydraulic retention time (0.45d) but at different feed substrate concentrations (0.3-3.0 g acetate.dm^{-3}). Two of them were stopped because of their very low gas production, thus, only the performance of three reactors fed with 3.0 , 2.0 and 1.0 g.dm^{-3} acetate solution were recorded. After 140 days of operation the sludge was removed from each reactor. Their characteristics and main operational parameters are summarized in Table 3.

Only the sludge of Reactor 1 contained granules with 1-2 mm diameter. These granules were less firm and dense than the ones grown on starch-sucrose substrate and were less stable during storage. Our results show that the higher the organic load, the better the settling ability (smaller SVI_{30}) and the more compressible (smaller PCV) the sludge. These results are supported by visual observation of the sludge bed. As usual in UASB reactors, the sludge bed first expanded, then separated into a sludge bed and a blanket. Bed separation was observed earlier in

Reactor 1 than in Reactor 2, while in Reactor 3 this phenomenon did not take place during the test period.

The organic content of the sludges (X_{VSS}/X_{SS}) in Reactor 1 was less than in the others, indicating that growth was limited by substrate inhibition. (Initial X_{VSS}/X_{SS} was 0.45.) Nevertheless satisfactory COD-removal efficiency of this reactor (E_{COD}>0.82) and the granular structure of the sludge prove that the favourable sludge characteristics are not caused by, but only concident with the relatively high ash content. It was the high acetate concentration which exerted a selection pressure during the operation.

TABLE 3 Characteristics of Sludges Obtained from Parallel Experiments with Acetate as Sole Carbon Source

Parameters	Reactor 1	Reactor 2	Reactor 3
I. Operational parameters:			
B_V [kg COD.m^{-3}.d^{-1}]	2.35	4.71	7.06
B_X [kg COD.kg^{-1}VSS.d^{-1}]	0.4	0.8	1.2
II. Sludge characteristics:			
X_{VSS}/X_{SS} [-]	0.54	0.60	0.62
SVI_{30} [$cm^3.g^{-1}$]	80.8	93.1	130.2
PCV [$cm^3.g^{-1}$]	10.7	14.1	17.4
SVI_{30}/PCV [-]	7.54	6.60	7.46

Start-up on carbohydrate wastewaters

The above findings are not in contradiction with most of the papers reporting that organic loads lower than 0.3 kg COD.kg^{-1}VSS.d^{-1} (Hulshoff Pol et al., 1984), or keeping the acetate concentration lower than 0.2 g.dm^{-3} (Wiegant, 1988; Hulshoff Pol, 1989) are advisable for control of granulation. A comparison of the shape of the curves describing the kinetics of acetate biomethanation by raw and granular sludges indicates that two concentration ranges seem to be beneficial for microorganisms generating granular sludge. Both the low acetate level (0-0.2 g.dm^{-3}), owing to the lower K_S-values of Methanothrix sp., and the higher than 1.5 g.dm^{-3} level, owing to the decreased growth of non-filamentous bacteria limited by substrate inhibition, may ensure the granulation (Figure 2).

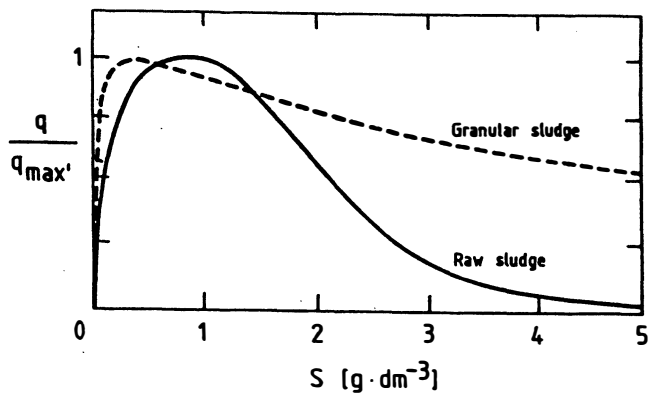

Fig. 2. Comparison of the kinetics of acetate biomethanation by raw (n=4) and granular (n=1) sludge.

The basis of the usual, widely accepted operation method for first start-up of UASB reactors is the stepwise increase of the organic load, after 80% reduction of biodegradable COD has been achieved. This method results in a continuous selection of sludge particles by the enrichment of filamentous bacteria (dependent on relatively low acetate level) and the washing out of poorly settling sludge particles formed mainly by Methanosarcina sp. caused by both the liquid upflow and the gas production (Lettinga et al., 1985). In contrast to the former our strategy for rapid granule formation is based on the complete separation of the two different (physiological and physical) selection factors at the time of start-up. Our method resulted in a non-continuous selection divided into two periods.

In the first period the aim was the enrichment of filamentous bacteria against others. For this reason we ensured relatively good conditions for the growth of Methanothrix-like microorganisms, while increasing appearance of bacterial filaments was observed (physiological influence). The granular structure was still not characterising the sludge.

In the second period the most important selection pressure was an increased upflow liquid velocity. The poorly settling sludge particles were washed out of the reactor (hydraulic factor), which resulted in a dense sludge bed, formed by macroscopic, well settling aggregates. Full size granules developed within a week after the beginning of this period, which makes possible the increasing of the organic load to the planned value of continuous operation.

The above described method was tested during the start-up of 21.3 and 200 dm^3 laboratory reactors. Each experiment resulted in a decrease in the time required for granulation; with starch-sucrose and molasses waste-water complete granulation was attained within 40-45 days of operation. The results of several previously reported granulation experiments are given in Table 4.

TABLE 4 Results of Experiments Aimed at the Granulation of Anaerobic Sludge

Substrate	B_X at start-up [kg COD.kg^{-1}VSS.d^{-1}]	Time required for granulation [days]	Reference
Molasses waste-water	0.5 -0.6	33- 45	Wu et al. 1987
Acetate	0.3	not observed	Hulshoff Pol et al. 1983
Propionate	0.9	56-100	Hulshoff Pol et al. 1983
Brewery wastewater	0.28-0.63	41- 60	Wu et al. 1985
Sucrose wastewater	0.07-0.4	130-160	Wu et al. 1985
Sucrose wastewater	0.1 -0.38	36- 70	Sierra-Alvarez et al. 1988
Carbohydrate	0.4 -1.2	28- 45	this study
Molasses wastewater	0.4 -1.2	23- 37	this study

As has been mentioned, granulation was followed by the determination of kinetic parameters in the 200 dm^3 reactor. During this experiment the n-value decreased from 2.3 to 0.2 following the enrichment of

Methanothrix-like bacteria and granule formation. Based on light microscopic examination, we found that the seed sludge became more rich in filamentous microorganisms (corresponding to the lower n value) than that observed in the case of other samples of raw sludge. The most remarkable differences were observed between samples 6 and 7; the changes took place during the first 23 days of operation. The q_{max}' increased intensively, showing the multiplication of acetoclastic methanogens. At the same time, both n and K_S' decreased, suggesting the assumption concerning the selection of Methanothrix. This has also been proved by light microscopy. Further operation did not lead to remarkable changes, either in q_{max}' or in n, although granulation was completed by the 37th day and large granules with 3-4 mm diameter developed in the reactor, up to the 93rd day, when Sample 8 was taken (Figure 3).

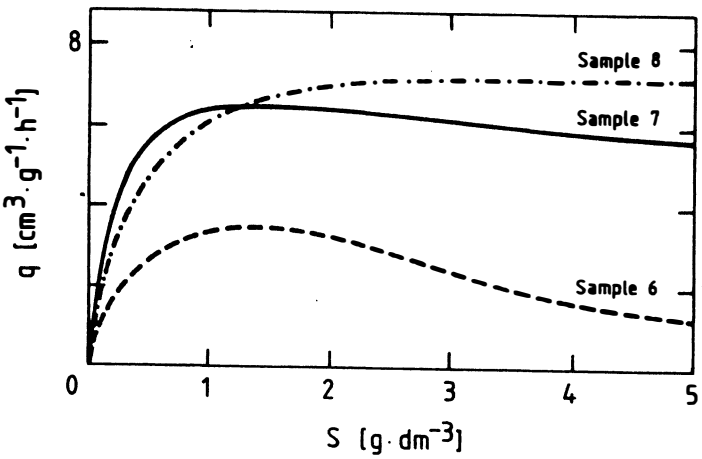

Fig. 3. Change in the kinetic parameters during granulation. (Parameters for each curve are given in Table 2.)

The relatively high acetate concentration applied during the start-up raised the subject of neutralization. The possibilities of decreasing the base demand were investigated earlier (Czakó et al., 1987).

CONCLUSIONS

Comparing the validity of different models we found that a modified Haldane-equation yielded the best fit to sets of kinetic data obtained from batch studies on the methane fermentation of acetate.

Comparing the parameters of the above equation for granular and non-granular sludges obtained from different sources, we found significant differences in the inhibition response coefficient (n). Its value was about 4 for raw and about 1 for granular sludge, proving the reduced sensitivity of granular sludge formed mainly by filamentous (Methanothrix-like) bacteria to substrate inhibition. A continuous UASB experiment with molasses wastewater gave similar results: the value of this coefficient continuously decreased as granulation proceeded.

Based on the above, two concentration ranges seem to be beneficial for microorganisms generating the granular sludge:

- low acetate level (0-0.2 $g.dm^{-3}$), owing to the lower K_S values of Methanothrix sp.;

- much higher (S > 1.5) concentration, owing to the decreased growth of non-filamentous bacteria limited by substrate inhibition.

A new start-up method was developed based on the above findings. During test runs granulation was completed within 40 days both in 21.3 and 200 dm^{-3} laboratory UASB reactors.

NOMENCLATURE

μ	$[h^{-1}]$	Specific growth rate
q	$[cm^3.g^{-1}.h^{-1}]$	Specific gas production rate
K_S	$[g.dm^{-3}]$	Half rate constant
K_I	$[g.dm^{-3}]$	Inhibition half rate constant
(Subscript "max" represents their maximum values and superscript "'" represents the measurable values)		
n	$[-]$	Inhibition response coefficient
S	$[g.dm^{-3}]$	Substrate (acetate) concentration
X_{SS}	$[g.dm^{-3}]$	Suspended solids concentration
X_{VSS}	$[g.dm^{-3}]$	Volatile suspended solids concentration
B_V	$[kgCOD.m^{-3}.d^{-1}]$	Volumetric load
B_X	$[kgCOD.kg^{-1}VSS.d^{-1}]$	Organic load
E_{COD}	$[-]$	Efficiency of COD removal
SVI_{30}	$[cm^3.g^{-1}]$	Sludge volume index
PCV	$[cm^3.g^{-1}]$	Packed cell volume

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