

## THE EFFECT OF pH PROFILES IN METHANOGENIC AGGREGATES ON THE KINETICS OF ACETATE CONVERSION

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**Abstract**—Due to the conversion of acetic acid into the weaker carbonic acid and methane, the pH inside methanogenic aggregates is higher than in the bulk liquid. The pH profiles in aggregates were measured with pH microelectrodes. These profiles strongly determine the macro-kinetics of the aggregate, by their influence on the values of the growth parameters  $K_s$  and  $\mu_{\max}$ . Acetate transport resistances were not limiting for the conversion rate in methanogenic aggregates. Nevertheless, the effectiveness factor  $\eta$  did not approach unity, but amounted to 0.57–0.62 in the acetate concentration range relevant for most methanogenic reactors. The value of  $\eta$  is determined almost entirely by the pH profiles inside the aggregates. It was concluded that for the physical/mathematical description of the conversion in methanogenic aggregates, information on the pH gradients and the pH dependency of the growth parameters is indispensable.

Batch experiments showed that acetate uptake by aggregates was not coupled directly to methanogenesis. Consumed acetate was not converted instantaneously to methane, suggesting the conversion to proceed via a pool of acetate or reserve material.

**Key words**—methanogenesis, acetate digestion, aggregates, microenvironment, mass transfer, microelectrodes, macro-kinetics, growth, pH

### NOMENCLATURE

- $D$  = substrate diffusion coefficient ( $\text{m}^2/\text{s}$ )
- $D_{\text{eff}}$  = effective diffusion coefficient ( $\text{m}^2/\text{s}$ )
- $k$  = mass transfer coefficient ( $\text{m}/\text{s}$ )
- $K_s$  = dissociation constant ( $\text{mol}/\text{m}^3$ )
- $K_s$  = Monod saturation constant ( $\text{mol}/\text{m}^3$ )
- $K'_s$  = Monod saturation constant for unionized acetic acid ( $\text{mol}/\text{m}^3$ )
- $K'_s$  = pH dependent Monod saturation constant, related to total substrate ( $\text{mol}/\text{m}^3$ )
- $r$  = distance from centre ( $\text{m}$ )
- $r_s$  = reaction rate ( $\text{mol}/\text{m}^3\text{s}$ )
- $R$  = aggregate radius ( $\text{m}$ )
- $S$  = total substrate concentration ( $\text{mol}/\text{m}^3$ )
- $S_b$  = total substrate concentration in the bulk ( $\text{mol}/\text{m}^3$ )
- $S_i$  = total substrate concentration at aggregate interface ( $\text{mol}/\text{m}^3$ )
- HAc = unionized acetic acid concentration ( $\text{mol}/\text{m}^3$ )
- Ac<sup>−</sup> = ionized substrate concentration ( $\text{mol}/\text{m}^3$ )
- $V_{\text{agg}}$  = conversion rate per aggregate ( $\text{mol}/\text{s}$ )
- $V_{\text{max}}$  = maximal conversion rate of an aggregate without pH micro-profile ( $\text{mol}/\text{s}$ )
- $Y_{\text{bio}}$  = biomass yield ( $\text{g dry wt}/\text{mol}$ )
- $Y_p$  = product yield ( $\text{mol}/\text{mol}$ )
- $X$  = biomass concentration ( $\text{g}/\text{m}^3$ )
- $\delta$  = diffusional boundary layer thickness ( $\text{m}$ )
- $\eta$  = effectiveness factor (—)
- $\mu'_{\max}$  = maximal specific growth rate at pH 7.0 ( $1/\text{s}$ )
- $\mu_{\max}$  = maximal specific growth rate at prevailing pH ( $1/\text{s}$ )

### INTRODUCTION

For the treatment of wastewater with a high organic matter content anaerobic processes are increasingly

applied. Anaerobic wastewater treatment is a multi-step process in which soluble organics are degraded to fatty acids and, subsequently, converted to methane and carbonate. Methanogenesis involves a complex interaction of conversions by various microbial species (Zeikus, 1980; Dubourgier *et al.*, 1987). The digestion of acetate is responsible for 65–96% of the methane formation (Weber *et al.*, 1984). Moreover, conversion of acetate to methane is considered as the rate limiting step in the methane production from wastewater (McCarthy, 1964). Therefore, knowledge of the kinetics of this process is necessary for the design and operation of methane reactors.

The Monod equation is widely used for the modelling of the anaerobic digestion process. The kinetic parameters that describe the performance of a bio-reactor to a large extent are the maximum specific growth rate ( $\mu_{\max}$ ), the substrate saturation constant ( $K_s$ ) and the product yield ( $Y_p$ ). Usually, these constants are determined from a series of continuous flow or batch experiments with enrichment or pure cultures (Zehnder *et al.*, 1980; Huser *et al.*, 1982; Yang and Okos, 1987). This method is very elaborate, especially with methanogens. A more serious disadvantage is the fact that in high performance bio-reactors methanogens do not grow in suspension, but form aggregates with a typical diameter of 1–3 mm. Therefore, suspended growth experiments can be considered as studies on artifacts. Conversely, the aim of this study is to determine the kinetic

parameters of the methanogenic acetate conversion on intact aggregates.

In active bacterial aggregates, profiles of substrate and product will develop. In methanogenic aggregates pH profiles are also formed by the conversion of acetic acid into the weaker carbonic acid. These profiles are dependent on the size of the aggregate and the complex interaction of diffusion, kinetics of the conversion processes and the distribution of the bacterial activity. Since the kinetics and the pH profile are interdependent, calculations on this system are very complex. In this study the pH profiles present in methanogenic aggregates were actually measured and this information was used to calculate substrate profiles and conversion rates.

Comparison of measured conversion rates with calculations using a structured mathematical model enabled the estimation of the relevant growth parameters, and the significance of pH profiles in aggregates for reactor operation was evaluated.

## MATERIALS AND METHODS

### Measurements

Methanogenic aggregates were sampled from a pilot-scale Internal Circulation reactor (Paques BV, Balk, The Netherlands) treating brewery wastewater (Heineken BV, 's-Hertogenbosch, The Netherlands). Since this reactor is 17 m high, precautions had to be taken to prevent the aggregates from damage by fast pressure drops during sampling. Therefore, sampling was done in a vessel pressurized with CO<sub>2</sub> at 2.5 atm. After the vessel was filled with reactor content, the pressure was released slowly.

Activity tests on intact aggregates and micro-profile measurements were performed in a mineral medium containing NH<sub>4</sub>Cl (5 mol/m<sup>3</sup>), KCl (0.6 mol/m<sup>3</sup>), NaH<sub>2</sub>PO<sub>4</sub> (0.5 mol/m<sup>3</sup>), Na<sub>2</sub>SO<sub>4</sub> (0.08 mol/m<sup>3</sup>), MgCl<sub>2</sub> (0.062 mol/m<sup>3</sup>), CaCl<sub>2</sub> (0.001 mol/m<sup>3</sup>), citric acid (0.1 mol/m<sup>3</sup>), Na<sub>2</sub>MoO<sub>4</sub> (6 × 10<sup>-6</sup> mol/m<sup>3</sup>), NaSeO<sub>4</sub> (3 × 10<sup>-5</sup> mol/m<sup>3</sup>), NiSO<sub>4</sub> (1.5 × 10<sup>-4</sup> mol/m<sup>3</sup>), FeCl<sub>3</sub> (0.005 mol/m<sup>3</sup>), MgCl<sub>2</sub> (0.003 mol/m<sup>3</sup>), CoCl<sub>2</sub> (0.0005 mol/m<sup>3</sup>), ZnO (0.00125 mol/m<sup>3</sup>), CuCl<sub>2</sub> (0.00025 mol/m<sup>3</sup>) and H<sub>3</sub>BO<sub>3</sub> (0.00025 mol/m<sup>3</sup>). These nutrients were supplied with the desired concentration of acetate, flushed with N<sub>2</sub> and adjusted to pH 7.0. The conversion rate of aggregates was measured in 500 ml serum bottles containing 750–1500 mg dry wt. The incubation temperature was 30°C and the acetate concentration was maintained at 90–100% of the desired value by addition of 1900 mol/m<sup>3</sup> acetate of pH 5.0. During these fed-batch cultures, the pH of the medium varied from 6.9 to 7.1. Acetate consumption was determined by gas chromatography. Methane production was measured with mariotte bottles containing 1500 mol/m<sup>3</sup> NaOH.

The effectiveness factor ( $\eta$ ) was determined from the increase of the conversion rate upon disintegration of aggregates and calculated as the quotient of the activity before and after disintegration. Disintegration of the aggregates in separate cells or fragments smaller than 50  $\mu$ m was achieved in 2 h with a magnetic stirrer at 250 rpm.

Measurements of pH profiles were performed in a flow-cell using pH-microelectrodes with a tip diameter of 1  $\mu$ m, as described previously (de Beer and Van den Heuvel, 1988). The dimensions of the aggregate were determined with a vernier calliper. Since aggregates are not perfectly spherical, a characteristic diameter was calculated from the mean diameters in the  $x$ ,  $y$  and  $z$  direction. The Sauter mean diameter of the sample was determined with an image analysing system (Shieh *et al.*, 1981).

### Calculations

Substrate profiles in spherical microbial aggregates can be obtained from a substrate mass balance comprising diffusion and reaction:

$$(D_{\text{eff}}/r^2)d(r^2dS/dr)/dr = -r_s \quad (1)$$

The reaction rate depends on the specific growth rate  $\mu$ , the local biomass concentration  $X$  and the biomass yield. Neglecting maintenance, substrate consumption can be described with Monod kinetics:

$$-r_s = \mu X/Y_{\text{bio}} = (\mu_{\text{max}} X/Y_{\text{bio}})S/(K_s + S) \quad (2)$$

As the acetate concentration range applied is well below the substrate inhibiting concentration (Speece, 1988) no inhibition kinetics were considered. Graef and Andrews (1973) postulated that unionized acetic acid is the actual substrate for methanogens. Accordingly, the reaction rate is described by

$$\mu X/Y_{\text{bio}} = (\mu_{\text{max}} X/Y_{\text{bio}}) \text{HAc}/(K'_s + \text{HAc}) \quad (3)$$

The concentration of the unionized acetic acid depends on the pH and, for the relevant pH range of 7.0–8.0,  $\text{Ac}^- \approx S$  and since the acid–base reaction is in equilibrium momentarily

$$\text{HAc} = (\text{H}^+ \cdot S)/K_a \quad (4)$$

Equation (3) can be rewritten as

$$\mu X/Y_{\text{bio}} = (\mu_{\text{max}} X/Y_{\text{bio}})S/(K''_s + S) \quad (5)$$

with

$$K''_s = K'_s \cdot K_a/\text{H}^+ \quad (6)$$

The saturation constant  $K''_s$  refers to the total concentration of both forms of acetate and depends on the pH. Equation (1) can now be rewritten as

$$(D_{\text{eff}}/r^2)d(r^2dS/dr)/dr = (\mu_{\text{max}} X/Y_{\text{bio}})S/(K''_s + S) \quad (7)$$

This equation cannot be solved analytically, therefore, a Runge–Kutta iteration was used with boundary conditions (i)  $dS/dr = 0$  at  $r = 0$ , and (ii)  $D_{\text{eff}}(dS/dr)_{(r=R)} = k(S_b - S_s) = D(S_b - S_s)/\delta$ . The experimental relation between  $\mu_{\text{max}}$  and the pH from Van den Berg *et al.* (1975) was approximately by a Gaussian curve:

$$\mu_{\text{max}} = \mu'_{\text{max}} \cdot e^{-0.5(\text{pH} - 7)/0.62)^2} \quad (8)$$

where  $\mu'_{\text{max}}$  represents the value of  $\mu_{\text{max}}$  at pH = 7.0. The effective diffusion coefficient of acetate inside the aggregates was conservatively assumed to be  $4.2 \times 10^{-10}$  m<sup>2</sup>/s, which is 34% (Speece, 1988) of the value in pure water (Perry and Green, 1984).  $Y_{\text{bio}}$  was set at 1.5 g biomass dry wt/mol acetate (de Zeeuw, 1984).

Equation (7) was used to calculate the conversion rate of a single aggregate  $V_{\text{agg}}$  at different bulk substrate concentrations. Comparison of calculated conversion rates at various substrate concentrations with measured data enabled determination of  $\mu'_{\text{max}}$  and  $K_s$  at pH 7.

Effectiveness factors ( $\eta$ ) at various acetate concentrations in the bulk liquid ( $S_b$ ) were calculated using

$$\eta = V_{\text{agg}}/(V_{\text{max}}S_b/(K_s + S_b)) \quad (9)$$

where  $V_{\text{max}}$  is the theoretical maximal conversion rate per aggregate without diffusion resistance, i.e. with a constant acetate concentration and pH throughout the aggregate, and is given by

$$V_{\text{max}} = (\mu'_{\text{max}} X/Y_{\text{bio}})4/3\pi R^3 \quad (10)$$

### Electron microscopy

Aggregates were washed gently in 150 mol/m<sup>3</sup> NaCl, followed by fixation in 2.5% glutaraldehyde for 1 h.

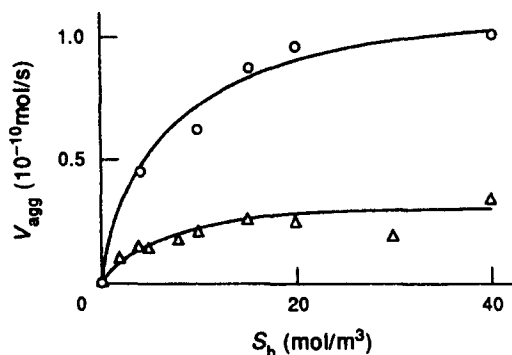


Fig. 1. Acetate consumption (○) and methane production (△) rates of methanogenic aggregates, incubated at different acetate concentrations in the bulk liquid.

Aggregates were dehydrated in an ethanol series from 15, 30, 45, 60, 70, 85 and 100%, made up with 150  $\text{mol/m}^3$  NaCl. Subsequently, the aggregates were submitted to critical-point drying in liquid carbon dioxide. Dry aggregates were mounted on SEM stubs with silver paint, sputter

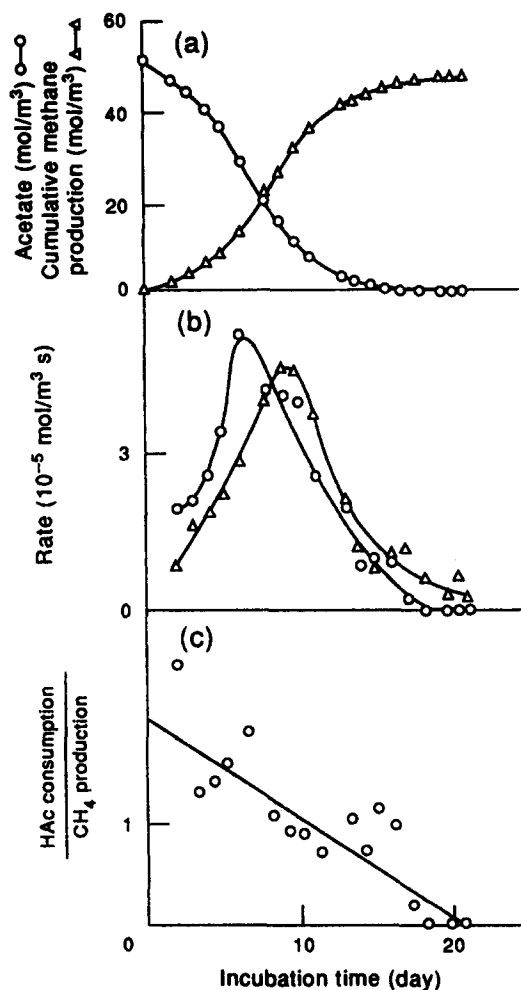


Fig. 2. Relation between the acetate consumption and the methane production by methanogenic aggregates during a long term incubation. (a) Acetate concentration (○) and cumulative methane production (△), (b) acetate consumption (○) and methane production rate (△) and (c) ratio of acetate consumption rate and methane production rate during the experiment.

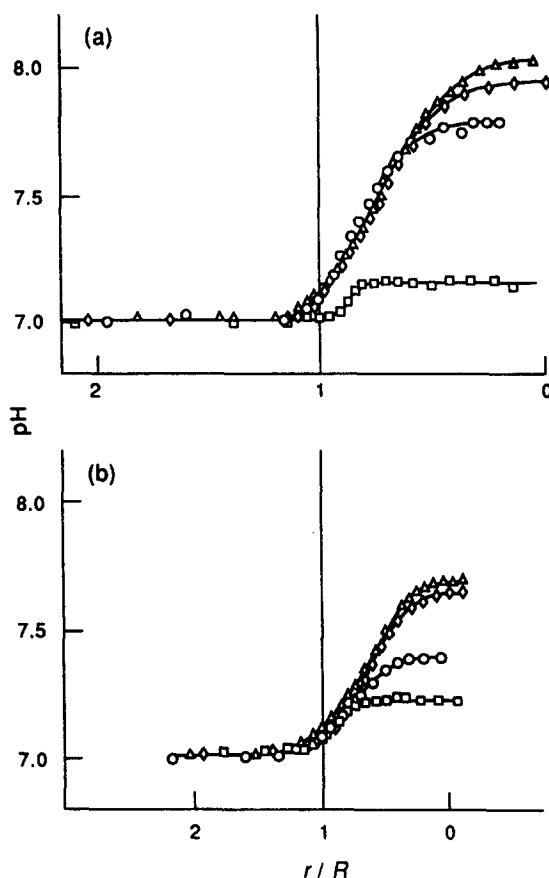


Fig. 3. pH microprofiles measured in methanogenic aggregates with a characteristic diameter of  $2.2 \times 10^{-3} \text{ m}$  (a) and  $1.3 \times 10^{-3} \text{ m}$  (b), incubated in acetate concentrations of 0  $\text{mol/m}^3$  (□), 5  $\text{mol/m}^3$  (○), 10  $\text{mol/m}^3$  (◇) and 15  $\text{mol/m}^3$  (△).

coated with gold/palladium, and examined in a scanning electron microscope (ISI-DS 130).

## RESULTS

Electron microscopy revealed that the aggregates consisted almost exclusively of rod-shaped bacteria, most likely belonging to the genus *Methanothrix*, which are commonly found in sewage digesters (Zehnder *et al.*, 1980).

Acetate consumption and methane production rates of aggregates at different acetate bulk concentrations in fed-batch are presented in Fig. 1, showing maximal conversion rates were reached at acetate concentrations of 15–20  $\text{mol/m}^3$ . From these results, it is clear that the consumption rate of acetate in the activity test was about three times higher than the production rate of methane.

To investigate the stoichiometry of the microbial conversion, acetate consumption and methane production by aggregates were monitored during a long term batch experiment, which was terminated after acetate was depleted and methanogenesis had stopped. The results of this experiment are pictured in Fig. 2. During the first 8 days of the experiment

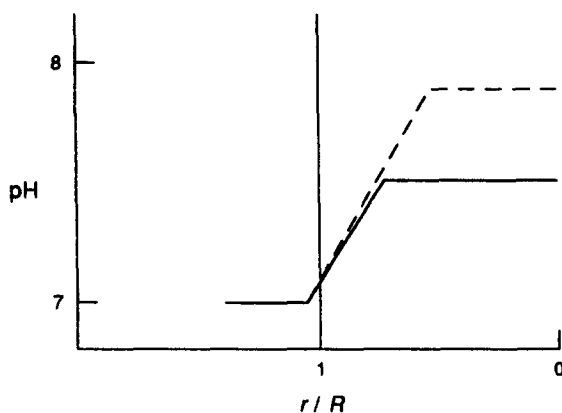


Fig. 4. Simplified pH profiles used in the structured model for calculations on transport and conversion in methanogenic aggregates, at acetate concentrations of 5 (solid line) and 10 mol/m<sup>3</sup> or more (dotted line).

acetate consumption was higher than methane production, conversely, after day 8 methane production exceeded the acetate consumption [Fig. 2(b)]. After the acetate was depleted completely at day 17, the methane production continued for a period of 4 days. As a consequence, the ratio of the acetate consumption rate and the methane production rate, during the experiment, initially exceeded 1, subsequently decreased and was eventually reduced to 0 [Fig. 2(c)]. Upon termination, the total amount of consumed acetate was 51 mmol, from which 48 mmol of methane was produced, resulting in an overall stoichiometry of 1.06. Clearly, during the experiment acetate consumption and methane production were not synchronous. Part of the acetate consumed was not converted instantaneously to methane, although eventually its conversion to methane was stoichiometric.

In an additional experiment in medium that, in contrast to the usual medium, was buffered with 10 mol/m<sup>3</sup> phosphate at a pH value of 7, no signifi-

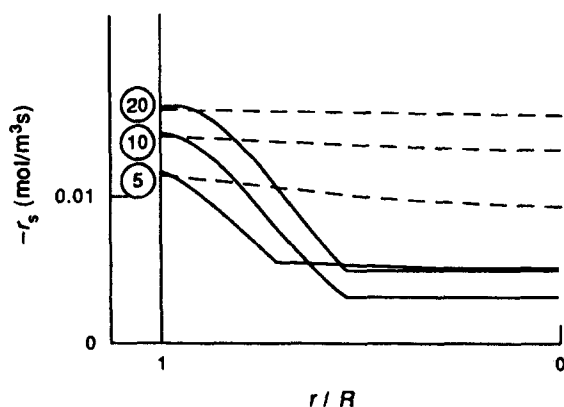


Fig. 5. Activity profiles, calculated from growth parameters based on methane formation, in methanogenic aggregates, with a diameter of  $1.64 \times 10^{-3}$  m, at different acetate concentrations with (solid line) and without (dotted line) pH gradients. Encircled numbers indicate the acetate concentration in the bulk liquid.

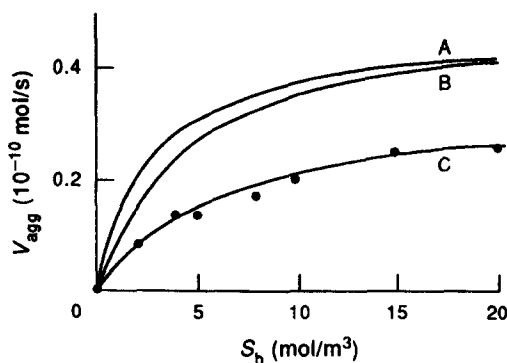


Fig. 6. Conversion rate of a single aggregate at various acetate concentrations in the bulk liquid. Calculated (A) for an aggregate with no diffusion resistance for acetate and without pH gradients, (B) for an aggregate with diffusion resistance for acetate and without pH gradients and (C) for an aggregate with both diffusion resistance for acetate and pH profiles from Fig. 4. Solid circles indicate measured values.

cant influence of the substrate concentration on the conversion rate was observed. In the acetate concentration range of 5–30 mol/m<sup>3</sup>, the maximal conversion rate was already reached at a concentration of 5 mol/m<sup>3</sup> (data not shown).

Incorporation of the developing pH micro-profiles in the structured model is essential, since the growth parameters  $\mu_{max}$  and  $K_s$  are strongly dependent on the local pH value. Therefore, pH profiles in aggregates were measured at various acetate concentrations in the bulk liquid under steady state conditions. These measurements were performed on 50 aggregates. The average characteristics diameter of all the aggregates in which profiles were measured was 1.64 mm, while the Sauter mean of the total sample was 1.58 mm. In Fig. 3 a few characteristic pH profiles are presented. The pH inside aggregates was higher than the bulk pH, as could be expected since the stronger acetic acid was replaced by the weaker carbonic acid. Also in the absence of acetate in the bulk liquid, pH gradients were observed although very small. In a concentration range from 0 to 10 mol/m<sup>3</sup>, relevant for most anaerobic reactors, the maximal pH value inside the aggregates increased with higher acetate concentrations in the bulk. Further increase of the acetate concentration only caused a slight increase of the internal pH. At an acetate concentration of 5 mol/m<sup>3</sup> the pH increased to a depth of  $280(\pm 40)$   $\mu$ m, while a plateau was apparent in the centre. At acetate concentrations of 10 mol/m<sup>3</sup> or higher, a pH increase was observed down to a larger depth of  $410(\pm 60)$   $\mu$ m. In aggregates incubated in medium, buffered with 10 mol/m<sup>3</sup> phosphate, no pH gradients developed (data not shown).

The measured pH profiles were used to calculate acetate concentration profiles and conversion rates in aggregates. For this purpose simplified mean pH profiles were composed as given in Fig. 4. The measurement of pH profiles revealed that they also extended outside the actual aggregate, indicating a

Table 1. Effectiveness factors  $\eta$  of aggregates at various acetate bulk concentrations (A) calculated using the structured model when no pH profiles occur, (B) calculated using the structured model including pH profiles and (C) values obtained from the increase of activity upon disintegration into cells

$S_b$ (mol/m <sup>3</sup> )	Effectiveness factor $\eta$		
	A	B	C
5	0.92	0.57	0.62
10	0.96	0.62	0.42
15	0.98	0.63	0.61
20	0.99	0.63	0.53

diffusive boundary layer outside the biofilm (Fig. 3). Therefore, in the calculations on substrate transport and conversion the presence of such a boundary layer of 50  $\mu$ m thickness was assumed. Calculations were performed using the average aggregate diameter of 1.64 mm.

By comparing calculated conversions at various acetate concentrations in the bulk liquid with the fed-batch measurements on aggregates presented in Fig. 1, a fit based on acetate consumption was found using  $\mu'_{\max} = 6.9 \times 10^{-7} \text{ s}^{-1}$  and  $K_s = 2.1 \text{ mol/m}^3$ , while based on methane production a fit was found with  $\mu'_{\max} = 2.1 \times 10^{-7} \text{ s}^{-1}$  and  $K_s = 2.6 \text{ mol/m}^3$ .

Calculations of the local activities in the aggregates are pictured in Fig. 5, showing that even for homogeneously distributed active biomass, the conversion rate strongly depended on the location, although acetate penetrated the whole aggregate and its concentration was close to that of the bulk liquid under the applied conditions. It was calculated that the acetate concentrations in the centre of aggregates incubated in 5, 10 or 20 mol/m<sup>3</sup> acetate amounted to 3.73, 8.59 and 18.33 mol/m<sup>3</sup>. The difference between the local conversion rates calculated with and without pH profiles, shows that the local conversion rate is much more influenced by the pH value than by the acetate concentration. The low activity in the centre of the aggregates is caused mainly by the high local pH value. Figure 5 shows that the activity in the centre of the aggregates may even decrease at higher substrate concentrations due to the more pronounced pH profile, although the activity of the total aggregate is positively related to the substrate concentration (Fig. 6).

In Fig. 6 measured and calculated conversion rates of a single aggregate at various acetate concentrations in the bulk liquid are presented. These calculations were done to establish the influence of the mass

transfer resistance for acetate and the role of pH profiles on the conversion rate and the effectiveness factor. The differences of the curves underline the importance of the pH profiles. From comparison of curves (A) and (B) it is clear that the influence of acetate transport limitation by external and internal diffusion resistances is insignificant for the conversion rate in aggregates. Comparison of curves (B) and (C) shows that the local pH values determine the conversion rate to a much higher extent.

In Table 1 values for effectiveness factor  $\eta$  are presented that were calculated with the structured model with and without pH profiles, together with values determined from the increase of the conversion rate upon disintegration of aggregates. In all cases,  $\eta$  is almost independent of the substrate concentration in the bulk. Experimental values are in agreement only with those calculated using the structured model if pH micro-profiles were taken into account.

## DISCUSSION

The value for  $\mu'_{\max}$  of *Methanothrix soehngenii* found in previous studies with suspended pure and enrichment cultures are slightly higher but of the same order of magnitude as our estimations for methanogenic aggregates based on acetate consumption rates (see Table 2). However, the value for  $\mu'_{\max}$  obtained from methane production is significantly lower. Estimations of  $K_s$  based on acetate consumption and methane production resulted in values 2–5 times higher as found by other authors.

The estimations presented in this study are based on the assumption that methanogenesis and growth are strictly coupled by a constant yield factor under all experimental conditions. Although the conclusive verification of this assumption is difficult, it is supported by the observation that methanogenesis by *Methanobacterium*, growing on H<sub>2</sub> and CO<sub>2</sub>, is strictly coupled to biomass growth in the pH range from 7.5 to 8.9 (Zehnder and Wuhrmann, 1977). Most yield factors on acetate found previously under different conditions are in the range from 1.3 to 1.95 g/mol (Graef and Andrews, 1973; Weber *et al.*, 1984; Yang and Okos, 1987; de Zeeuw, 1984), however, a value of 2.82 has been reported as well (Zehnder *et al.*, 1980). If the latter value is used, the estimation of  $\mu'_{\max}$  based on acetate consumption becomes  $1.3 \times 10^{-7} \text{ s}^{-1}$ , which is in between the two values reported previously for *Methanothrix soehngenii*

Table 2. Comparison of results from this study with those obtained in studies on suspended bacteria

$\mu'_{\max}$ (1/s)	$K_s$ (mol/m <sup>3</sup> )	Source
$8.9 \times 10^{-7}$	0.47	<i>Methanothrix soehngenii</i> (Zehnder <i>et al.</i> , 1980)
$1.6 \times 10^{-6}$	0.70	<i>Methanothrix soehngenii</i> (Huser <i>et al.</i> , 1982)
$1.1 \times 10^{-6}$	1.17	Disintegrated aggregates, pH 7.3, based on acetate consumption (Hamelers and Koster, 1986)
$6.9 \times 10^{-7}$	2.1	Intact aggregates, based on fitting of acetate consumption with model calculations (this study)
$2.1 \times 10^{-7}$	2.6	Intact aggregates, based on fitting of methane production with model calculations (this study)

(Zehnder *et al.*, 1980; Huser *et al.*, 1982), and close to the value obtained for disintegrated aggregates (Hamelers and Koster, 1986). No conclusive answer can be found for the question why the estimation of  $K_s$  is relatively high as compared to other authors. In this study  $K_s$  was determined by fitting the macrokinetics of intact aggregates with a mathematical model, while the other authors made use of suspended bacteria. The latter method might be more precise. Alternatively, growth parameters of bacteria in aggregates could be different from those of freely suspended bacteria.

A possible explanation of the difference between the  $\mu'_{\max}$  based on methane production and acetate consumption may be found in the fact that both processes are not tightly coupled. Acetate consumed in the initial phase of the cultures was only partly converted directly to methane, the other part being stored in an unknown pool, which was eventually converted to methane. This phenomenon was also observed in the initial phase of batch experiments by Huser *et al.* (1982). De Zeeuw (1984) found that the biomass yield of methanogenic bacteria when growing on acetate was three times higher than when growing on methanol. Since protein formation was the same for both substrates, it was hypothesized that most of the consumed acetate was converted to polymeric reserve material. This could explain our observation, that in the batch culture gas production continued for some time after acetate was depleted. In addition, the persistence of small pH profiles inside aggregates upon depletion of substrate may be the result of this process. Since the biochemistry and physiology of this acetate consumption and storage process is unknown, activity measurements based on acetate consumption may lead to erroneous conclusions on the kinetics of methanogenesis. Therefore, estimation of kinetic parameters based on methane production is to be preferred for aggregates, and the following discussion is based on these values. Comparison of our observations with previous measurements on suspended growing cells and disintegrated aggregates leads to the conclusion that  $\mu'_{\max}$  of aggregated bacteria, used in this study, is lower. Possibly, this can be attributed to the use of different strains, however, an interesting electron microscopic study on aggregates of *Methanosarcina* spp showed that only the bacteria in the outermost cell-layers of these aggregates divide and grow (Bochem *et al.*, 1982). The absence of growth in deeper regions cannot be due to starvation, since the substrate concentration and the pH hardly change over a cell layer of approx. 20  $\mu\text{m}$ . This suggests that cell division and growth in aggregates may be subjected to regulation.

The effectiveness factors  $\eta$  based on measured methane production as well as those calculated with and without pH profiles (Table 1) are almost independent of the substrate concentration in the bulk, while the measured values (column C) were far from unity.

The explanation is that the influence of acetate transport resistance on the conversion rate is negligible for the relevant  $S_0$  range. Even at high substrate concentrations  $\eta$  did not approach a value of 1, since the elevated internal pH value reduced  $\mu_{\max}$  and thus the maximal conversion rate. The extent of the pH profiles depends on the size of the aggregate. The value of  $\eta$  of disintegrated aggregates does amount to 1 because even in the largest fragments (50  $\mu\text{m}$ ) no significant pH profiles can develop. The effectiveness factors obtained after disintegration are much lower than those calculated with fixed kinetic parameters (no pH profiles taken into account), but are in good agreement with the calculations using pH dependent kinetics and comprising the pH profiles. Therefore, it can be concluded that the model is reliable and pH profiles are of great importance for the behaviour of methanogenic aggregates.

Reactor behaviour is expected to be strongly influenced by the pH profiles occurring in aggregates. A decrease of the bulk pH may result in a lower internal pH and a concomitant decrease of the local  $K_s$  value, leading to a higher biomass activity, especially at low substrate concentrations. Increase of the influent pH buffer capacity may have the same effect. Moreover, it enables establishment of the optimal pH value for  $\mu_{\max}$  in the whole aggregate.

The measurements of the pH profiles revealed the existence of a boundary layer of approx. 50  $\mu\text{m}$ , surrounding the aggregates. This layer increased the surface pH value by approx. 0.1 units. Thereby it influenced the pH profiles inside the aggregate to some extent and thus the kinetics of the conversion. An acetate boundary layer also might influence the transport rate of substrate towards the biofilm. However, since the influence of the acetate transport rate on the conversion rate was negligible, the boundary layer for acetate was not significant. Since the pH boundary layer was small it is unlikely that enhancement of external transport by mixing will increase the conversion rate significantly in methanogenic reactors.

At the moment reliable acetate selective micro-electrodes are not available. However, such an electrode would not enable measurement of unionized acetic acid, the actual substrate for methanogenesis, which is determined by the total acetate concentration and the pH. Therefore, information on the acetate profiles without knowledge of the local pH is not sufficient. Moreover, according to calculations with the structured model, the acetate concentration in aggregates remains almost constant and is close to the bulk concentration.

The medium pH buffer capacity strongly determined the macrokinetics of methanogenic aggregates by its influence on the pH profiles in aggregates. A phosphate concentration of 10 mol/m<sup>3</sup> completely prevented the development of pH profiles in aggregates and no influence of the substrate concentration on the conversion was found in the substrate

concentration range from 5 to 30 mol/m<sup>3</sup>, due to the low local  $K_s$  values. As a consequence, the theoretical prediction of Henze and Harremoës (1983) that mass transfer resistance in methanogenic biofilms is not significant, is only valid in well buffered media. The verification of this prediction by Dolfig (1985) was based on experiments in media buffered with 5 mol/m<sup>3</sup> phosphate, in which the pH profiles will be strongly reduced.

The conversion rate in aggregates is influenced by the transport resistance for protons. This can be understood by the notion that under physiological conditions proton concentrations are very low, in aggregates varying from 10<sup>-5</sup> to 10<sup>-4</sup> mol/m<sup>3</sup>, and 6 orders of magnitude smaller than the acetate concentration. The mass transfer resistances are sufficiently large to maintain small proton concentration gradients (maximal value of 2 × 10<sup>-1</sup> mol/m<sup>4</sup>), nevertheless leading to significant pH gradients. However, limitation of the conversion rate by acetate depletion in the aggregate, under typical reactor conditions, requires much larger concentration gradients. Supposing an acetate bulk concentration of 5 mol/m<sup>3</sup>, gradients should amount to at least 2 × 10<sup>3</sup> mol/m<sup>4</sup>, for which the mass transfer resistances are obviously insufficient. From this study it can be concluded that mass transfer resistance is important in methanogenic aggregates via the influence of the internal pH on the kinetics. This conclusion is relevant for most industrial reactors, since the phosphate concentration is usually well below 1 mol/m<sup>3</sup>. The bulk liquid of the Internal Circulation reactor, from which the investigated aggregates were obtained, is weakly buffered at a pH value of 6.8–7.1 by approx. 0.1 mol/m<sup>3</sup> phosphate and 20–25 mol/m<sup>3</sup> carbonate. It was calculated that at the typical acetate concentration in the reactor of 5 mol/m<sup>3</sup> the acetate concentration in the centre of aggregates amounts to 3.73 mol/m<sup>3</sup>. Using an alkalinity balance, it can be calculated that in such a medium the pH in the centre of aggregates amounts to 7.4, which is in good agreement with the presented microelectrode measurements.

The high internal pH reduced the activity in the centre of the aggregates to 15–30% of the activity in the peripheral zones. Moreover, the volume of the central part of aggregates ( $r/R < 0.5$ ) is only 10% of the total volume. Therefore, an additional assumption of a non-homogeneous biomass distribution, assuming an inactive centre, is insignificant for modelling.

Inhibition by sulphide is reported to be strongly influenced by the pH and it was found that inhibition of methane formation is related to the unionized sulphide (H<sub>2</sub>S) concentration (Koster *et al.*, 1986). These authors observed a lower inhibition rate due to sulphide on intact aggregates, as compared to disintegrated aggregates. Since the unionized fraction of sulphide is pH dependent, they concluded that the pH inside the aggregates must be higher than in the bulk liquid. Indeed, this conclusion is confirmed directly

by our profile measurements. It should be stressed that the mentioned sulphide inhibition experiments were performed in weakly buffered media containing 6 × 10<sup>-2</sup> mol/m<sup>3</sup> phosphate.

Previous measurements on aggregates from an upflow anaerobic sludge blanket reactor, fed with incompletely acidified wastewater, showed the presence of an acidifying process (de Beer and Van den Heuvel, 1988), resulting in pH profiles strongly deviating from those reported in this study. Moreover, pH profiles depend on the size of the aggregates, larger aggregates having more pronounced pH profiles. Since both the processes inside aggregates and their size distribution may depend on the reactor type and composition of the influent, the reported pH profiles may not be valid for aggregates from other reactors.

Knowledge of  $K_s$  and  $\mu_{\max}$  only, is not sufficient for the understanding of the behaviour of a methane reactor containing aggregates. This study shows that the mathematical description of the conversion in methanogenic aggregates requires a structured model, comprising pH micro-profiles and the pH dependency of the kinetics as postulated by Graef and Andrews (1973) and van den Berg *et al.* (1975). This result indicates clearly the central role that pH profiles play in methanogenic aggregates. Both  $\mu_{\max}$  and  $K_s$ , and thus the conversion rate, are strongly influenced by the local pH. Therefore, a mathematical description of the methanogenic conversion in aggregates is impossible without knowledge of the concomitant pH profiles. Since in most microbial conversions net proton consumption or production takes place, this conclusion seems relevant for many processes in which biofilms are involved.

## CONCLUSIONS

The low effectiveness factor of methanogenic aggregates converting acetate to methane and carbonate cannot be explained by mass transfer resistances for acetate. The macrokinetics of these aggregates can be explained by the pH dependent growth kinetics as postulated by Graef and Andrews (1973) assuming unionized acetic acid to be the actual substrate. Due to the high internal pH values the conversion rate in the aggregates is reduced kinetically. Therefore, decrease of the reactor pH may lead to an increase of the methanogenic activity. The mass transfer resistances were large enough for the development of pH profiles, but insufficient for the development of significant acetate profiles.

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