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RESEARCH NOTE

METHANE PRODUCTION IN ANAEROBIC SLUDGES SUPPLEMENTED WITH TWO SUPPORT MATERIALS AND DIFFERENT LEVELS OF ACETATE AND SULPHATE

M. ASCENSION MUÑOZ[1], JOSE M. SANCHEZ[1], JOSE M. RODRÍGUEZ-MAROTO[Ⓜ2], JUAN J. BORREGO[Ⓜ1*] and MIGUEL A. MORIÑIGO[1]

[1]Department of Microbiology and [2]Department of Chemical Engineering, Faculty of Sciences, University of Malaga, 29071-Malaga, Spain

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Abstract—The influence of two support materials, diabase and polyvinyl chloride (PVC), on the methanogenesis from anaerobic sludges supplemented with different concentrations of acetate and sulphate was evaluated by applying a mathematical model according to the response surface technique. The results obtained suggest that the addition of both support materials did not exert significant effects on the methanogenesis from domestic sludges. The best conditions to produce methane were obtained with the lowest concentration of sulphate (1 mM) and with the highest concentrations of acetate (75–100 mM). © 1997 Elsevier Science Ltd

Key words—methanogenesis, support materials, acetate, sulphate reduction

INTRODUCTION

In recent years, several types of reactors have been developed for the anaerobic biological treatment of wastewaters, such as anaerobic filters, upflow anaerobic sludge blankets (UASB) and expanded/fluidized bed reactors (Lettinga *et al.*, 1980; Song and Young, 1986; Sanz and Fernández-Polanco, 1989). All these systems are designed to avoid the loss of the biomass by washing using different mechanisms (Lettinga *et al.*, 1984; Albagnac, 1990; Vos *et al.*, 1990). These reactors are very efficient to separate solids from liquor phase (Hickey *et al.*, 1991), because the retention of high levels of active cell biomass is an important requisite for the development of high-rate treatments (Fukuzaki *et al.*, 1990). The microbial biomass retention is accomplished by the development of biofilms on support surfaces (Kida *et al.*, 1990; Perez Rodriguez *et al.*, 1992; Borja *et al.*, 1993; Muñoz *et al.*, 1994), in anaerobic filters and expanded/fluidized beds, but in the UASB system this effect is achieved by the granule formation (Lettinga *et al.*, 1984; Lettinga and Hulshoff Pol, 1991).

Different materials have been used as inert supports for the biomass retention. These support materials are differentiated on the basis of their surface roughness and porosity (Hickey *et al.*, 1991). Several authors have used efficiently clay materials as supports to immobilize microorganisms in the anaerobic digestion (Perez Rodriguez *et al.*, 1989, 1992; Muñoz *et al.*, 1994). On the other hand, polymeric materials, especially reticulated polyurethane foam, have been considered as an excellent substrate for the colonization of methanogenic microbiota (Poels *et al.*, 1984; Isa *et al.*, 1986a; Fukuzaki *et al.*, 1990), although there are results which suggested a poor biofilm development on polyvinyl chloride (Murray and van den Berg, 1981; Sanchez *et al.*, 1994).

High-rate systems, such as expanded/fluidized bed reactors, are being applied in the anaerobic treatment of wastewater which contains relatively high concentrations of solids, toxicants or sulphate (Suidan *et al.*, 1983; Nakhla *et al.*, 1989). The latter, at high levels, may interfere with the methanogenic process, resulting in a low methane yield. This competition between the sulphate reduction and methanogenesis depends on both the physico-chemical conditions and the composition of methanogens present in the anaerobic digestion (Robinson and Tiedje, 1984; Conrad *et al.*, 1986).

In the present work, we have studied the effects exerted on the methanogenesis process by the addition of different support materials to domestic sludges, supplemented with different levels of acetate and sulphate; in addition, we have determined the

*Author to whom all correspondence should be addressed.

optimal conditions for the methane production in these conditions.

MATERIALS AND METHODS

Experimental conditions

Sludge samples were anaerobically collected from an anaerobic digestor of a wastewater treatment plant at Estepona (Malaga, Spain). The sludge possessed the following characteristics: Chemical Oxygen Demand (COD), about 2900 ppm; volatile fatty acids (VFA), 98 ppm; alkalinity, 2445 ppm; suspended solids 14,740 ppm; and a pH ranging between 7.0 and 7.2. Sludges contained sulphate concentrations lower than 100 ppm. Only acetate and propionate (0.75 mM and 0.4 mM, respectively) were detected. Methanogenic and sulphate-reducing bacteria were enumerated by the three-vials most probable number (MPN) technique using the media and methodology described by Maestrojuan (1987). Microbial counts of the sludge were 10^8 methanobacteria per 100 ml and between 10^6 and 10^7 sulphate-reducing bacteria per 100 ml. Batch experiments were performed using samples containing 40 ml from anaerobic digestor and 10 ml of a mixture of primary and activated sludges (1:4 v/v, respectively). All the operations were conducted while the samples were gassed with nitrogen. Sludge samples were incubated with gentle shaking at 36°C for 66 days in 118-ml total capacity serum bottles, stopped with butyl rubber plugs and sealed with aluminium caps. The values of pH were monitored periodically.

Chemicals

Concentrations of sodium acetate (12, 24, 50, 75, and 100 mM), sodium sulphate (1, 10, and 20 mM) and the support materials diabase and polyvinyl chloride (2, 4, and 6 cc per 50 ml of sludge) were used in the experimental conditions. Diabase was synthesized at 600°C from volcanic rocks and compacted at 260°C, porosity, 97%; water absorption, 80%. Polyvinyl chloride (PVC) had a density of 0.34 g ml^{-1}; porosity lower than 5%; the PVC tube was broken into pieces of 1.6–2-mm diametre size. Acetate and sulphate solutions were added to the vials under nitrogen atmosphere before being sealed.

Gas-chromatography determinations

The methane proportion in the biogas formed in the control and test conditions was monitored over time. Methane in the gas phase was determined by flame ionization gas-chromatography (Hewlett-Packard 5790A) with a DEGS column on CHRM W 80/100 that was held at 30°C with a nitrogen carrier flow rate of 20 psi and injector and detector temperatures of 240 and 260°C, respectively.

Mathematical modelling

A mathematical model was developed according to the response surface technique (Stoch de García Asensio, 1974; Murphy, 1977), applying a factorial design (3^3), where a follow-up of the selected function evolution (methane yielded per sludge volume unit), depending on the time until a maximal residence period of 66 d was considered. The ratio volume of methane produced/volume of sludge (Y), expressed as ml of CH_4/50 ml of sludge has been chosen as objective function. The level of acetate (A), sulphate (T) (expressed both as mM), and diabase and PVC (expressed as cc of support material/50 ml of sludge) were the variables of operation.

The first design explored three concentrations of each support, three concentrations of sulphate and three concentrations of acetate in the range 0–24 mM. The second design did the same for another range of acetate concentrations between 50 and 100 mM.

Table 1. Values of methane production (expressed as ml/50 ml of digestor sludge) obtained from the experiments carried out with different concentrations of acetate and sulphate and without addition of support materials

Concentrations of sulphate (mM)	Concentrations of Acetate (mM) 0[a]	12	24	50	75	100
0[a]	6.5	6.5	7.3	7.3	7.8	7.8
1	8.2	6.5	6.5	7.7	8.0	8.0
10	7.5	6.5	6.7	7.5	7.7	8.0
20	7.8	6.5	6.7	7.5	7.7	8.0

[a]Control conditions with concentrations of acetate and sulphate present in the digestor sludge used in this study.

RESULTS AND DISCUSSION

The results obtained in experiments carried out with digestor sludge supplemented with different levels of acetate and sulphate but without support materials are given in Table 1. It could be observed that the only addition of sulphate produced an increase in the production of methane, whilst in the case of acetate this increase was achieved only from a final concentration of acetate of 24 mM. The combined use of different concentrations of acetate and sulphate produced increases in methane volumes from 1 and 50 mM of sulphate and acetate, respectively. In Fig. 1, the percentages of methane production obtained from the digestor sludge supplemented only with different concentrations of diabase and PVC are represented. It can be observed that the addition of support materials enhanced the methane production compared to control. The highest values of methane yield were obtained with the lowest concentration of diabase. This moderate improvement in the methanogenesis exerted by the mineral support materials was previously observed by Muñoz (1991) and Muñoz *et al.* (1994) with different mineral supports. These findings may be explained by the fact that high concentrations of mineral supports release several ions to the liquor, which provokes modifications of the environmental conditions, such as pH changes as suggested by Sorlini *et al.* (1990). However, in vials supplemented with PVC, pro-

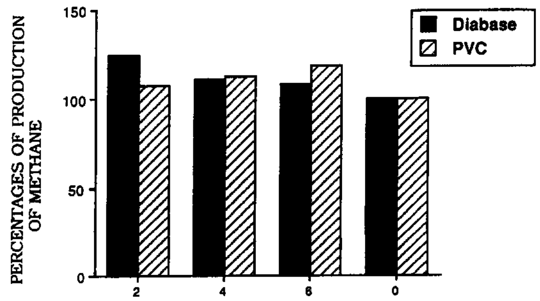

Fig. 1. Percentages of methane produced with different levels of diabase and PVC (concentrations are expressed as cc of support material/50 ml of sludge). The concentration 0 corresponds to the control conditions.

Table 2. Experimental designs and operational conditions in methane production depending on the acetate concentrations using vials with diabase as support material and supplemented with sulphate

Concentration of diabase[a]	Concentration of sulphate[b]	Concentration of acetate[b] 0	12	24	50	75	100
2	1	7.6[c]	9.1	6.0	9.7	10.1	10.1
	10	4.8	8.7	8.0	9.6	8.5	5.5
	20	6.1	8.3	9.1	9.5	9.2	8.5
4	1	6.6	8.6	5.0	9.9	10.4	10.4
	10	4.8	8.3	8.9	9.2	10.1	7.3
	20	5.4	8.4	9.4	9.5	9.2	8.2
6	1	5.9	8.7	5.8	9.7	9.8	10.1
	10	4.5	8.7	8.6	9.2	8.6	9.1
	20	5.9	8.1	8.8	9.3	8.6	7.3

[a]Concentration expressed as cc of support material/50 ml of sludge.
[b]Concentration expressed in Mmol.
[c]Concentration expressed as ml of methane/50 ml of sludge.
Equations of methane production in the experimental design corresponding to different acetate concentrations:
For 0, 12, and 24 mM of acetate: $Y = K1 + K2A + K3A^2 + K4A^2T + K5AT^2$ (where $K1 = 5.73$; $K2 = 0.47$; $K3 = 2.06 \times 10^{-2}$; $K4 = 8.03 \times 10^{-4}$; $K5 = -5.65 \times 10^{-4}$ (relative error = 0.068) — A: acetate concentration; T: sulphate concentration).
For 50, 75, and 100 mM of acetate: $Y = K1 + K2A + K3T + K4AT + K5T^2$ (where $K1 = 10.30$; $K2 = 3.63 \times 10^{-3}$; $K3 = -0.12$; $K4 = -2.05 \times 10^{-3}$; $K5 = 1.04 \times 10^{-2}$ (relative error = 0.0623)).

duction increases were recorded at the highest concentrations of the support material due to the optimal development of the methanogenic biofilm on the high surface of this material (Verrier *et al.*, 1988).

The operational conditions and the results obtained using two experimental designs depending on the sulphate and acetate concentrations in vials supplemented with diabase and PVC are given in Tables 2 and 3. The equations were obtained by a computer program, which reproduces the experimental values adequately (Figs 2 and 3). This allows us to know the influence of the variables to achieve the optimal production of methane. In both designs, the influence of the increase of the support material concentrations used in these experimental conditions did not show statistically significant differences, although a light negative effect on the methane production could be detected when there was an increase in the concentration of diabase in vials supplemented with 1 mM of sulphate.

In the first design, at 0–24 mM of acetate and 1–20 mM of sulphate (Tables 2 and 3), both lineal and square influences with both supports were observed, which makes difficult to analyse each influence separately. The predicted levels of methane to be produced with different concentrations of

Table 3. Experimental designs and operational conditions in methane production depending on the acetate concentrations using vials with PVC as support material and supplemented with sulphate

Concentration of PVC[a]	Concentration of sulphate[b]	Concentration of acetate[b] 0	12	24	50	75	100
2	1	10.0[c]	8.1	8.4	9.7	10.2	10.0
	10	4.5	8.5	8.9	9.7	9.5	8.8
	20	4.1	8.4	8.7	4.1	9.4	7.8
4	1	6.0	7.8	9.3	9.9	10.1	10.2
	10	5.4	6.3	8.9	9.2	9.3	8.9
	20	4.0	8.2	8.6	5.1	9.0	7.9
6	1	6.2	9.0	10.0	9.9	10.0	10.3
	10	5.1	8.6	8.5	9.7	8.9	8.6
	20	4.1	7.3	8.9	4.6	9.3	7.5

[a]Concentration expressed as cc of support material/50 ml of sludge.
[b]Concentration expressed in Mmol.
[c]Concentration expressed as ml of methane/50 ml of sludge.
Equations of methane production in the experimental design corresponding to different acetate concentrations:
For 0, 12, and 24 mM of acetate: $Y = K1 + K2A + K3T + K4AT + K5A^2$ (where $K1 = 7.06$; $K2 = 0.21$; $K3 = -0.15$; $K4 = 6.64 \times 10^{-3}$; $K5 = -5.66 \times 10^{-3}$ (relative error = 0.0784) — A: acetate concentration; T: sulphate concentration).
For 50, 75, and 100 mM of acetate: $Y = K1 + K2T + K3AT + K4A^2 + K5A^2T$ (where $K1 = 10.82$; $K2 = -1.42$; $K3 = 3.30 \times 10^{-2}$; $K4 = -7.71 \times 10^{-5}$; $K5 = -2.00 \times 10^{-4}$ (relative error = 0.0627)).

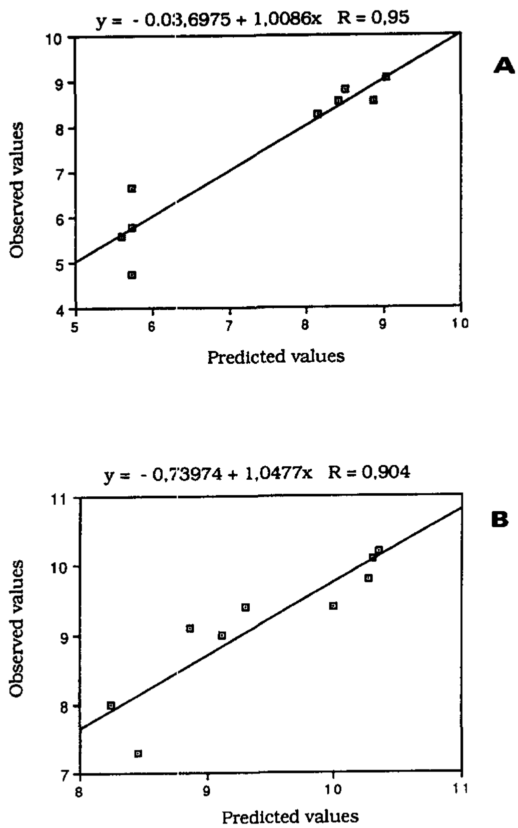

Fig. 2. Relationship between observed and predicted values in the experiments conducted to evaluate the effect of sulphate (1, 10, and 20 mM), acetate (A: 0, 12, and 24 mM; B: 50, 75, and 100 mM) and diabase (2, 4, and 6 cc/50 ml sludge).

acetate and sulphate and 2 cc of diabase and PVC per 50 ml of sludge are shown in Figs 4 and 5. The presence of low levels of sulphate (1 mM) exerted a positive effect on the methane production when PVC was used (Table 3). At 13 mM of sulphate and higher levels of acetate (approximately 18–19 mM), the maximal methane production (about 9.18 ml per 50 ml of sludge) was obtained from the vials supplemented with diabase (Fig. 4), whilst in the vials to which PVC was added, this maximal production (about 9 ml per 50 ml of sludge), was achieved at the highest concentration of acetate used in this design (24 mM). The increase in the concentration of sulphate with a constant level of acetate provokes a reduction in the methane production, except with 24 mM of acetate (Fig. 5). The positive effect of acetate and low amounts of sulphate used separately have been also reported by several authors (Tursman and Cork, 1989; Muñoz *et al.*, 1992, 1994).

The second experimental design 3^3 was performed to verify if higher levels of acetate could produce an increase in the methane production. In this new experimental design the concentrations of sulphate and support materials were maintained, and the acetate concentrations added were 50, 75 and 100 mM (Tables 2 and 3). From the statistical analysis of the results, it may be concluded that with both support materials, a higher methane production was achieved compared to that obtained in the first design. The highest methane productions were obtained at 75 and 100 mM of acetate and 1 mM of sulphate. Similar results were obtained using raw sludges of anaerobic digestors supplemented with several sulphate concentrations (1–20 mM) (Phelps *et al.*, 1985; Ueki and Ueki, 1990; Muñoz *et al.*, 1992). This improvement in the methanogenesis may be due to the more effective colonization of certain support surfaces by the methanogenic bacteria than that carried out by sulphate-reducing bacteria (Isa *et al.*, 1986b; Yoda *et al.*, 1987; Sanchez *et al.*, 1994). Furthermore, Yoda *et al.* (1987) reported that this colonization by the methanogens was efficient at high acetate levels.

In the experiments conducted with diabase, the lowest methane productions were achieved with 100 mM acetate concentration and 10–20 mM of sulphate, whereas with PVC the worst results were obtained with 50 mM of acetate and 20 mM of sulphate. These results could be explained by enrichment of the sulphate-reducing and

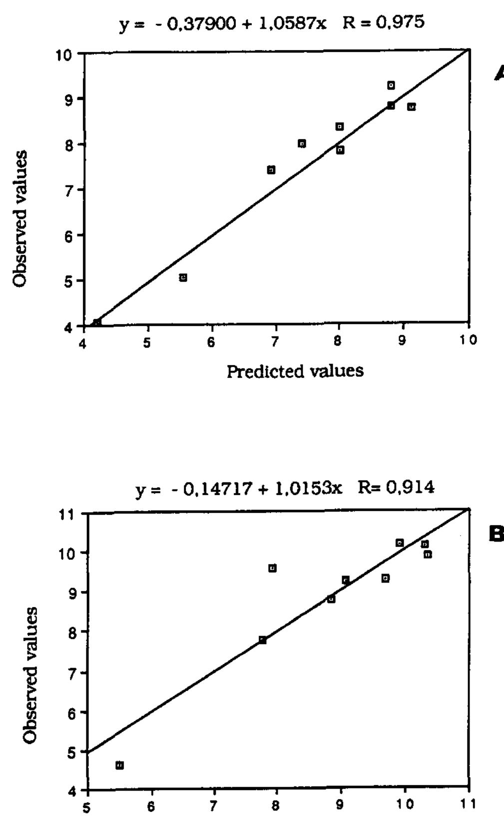

Fig. 3. Relationship between observed and predicted values in the experiments conducted to evaluate the effect of sulphate (1, 10, and 20 mM), acetate (A: 0, 12, and 24 mM; B: 50, 75, and 100 mM) and PVC (2, 4, and 6 cc/50 ml sludge).

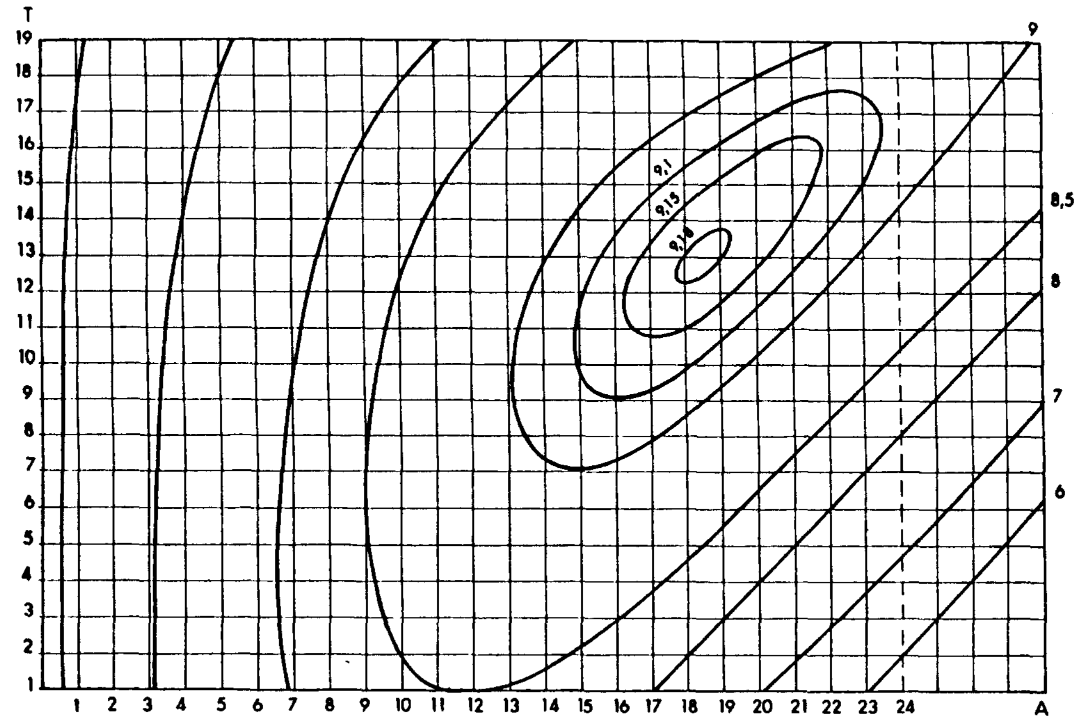

Fig. 4. Levels of methane produced with 2 cc of diabase and different concentrations of acetate (A) and sulphate (T).

methanogenic populations which has been observed by Sanchez *et al.* (1994) in the sludges supplemented with support materials, compared to those without support materials. The low methane productions obtained with 10–20 mM of sulphate was also reported by Yoda *et al.* (1987), who observed that

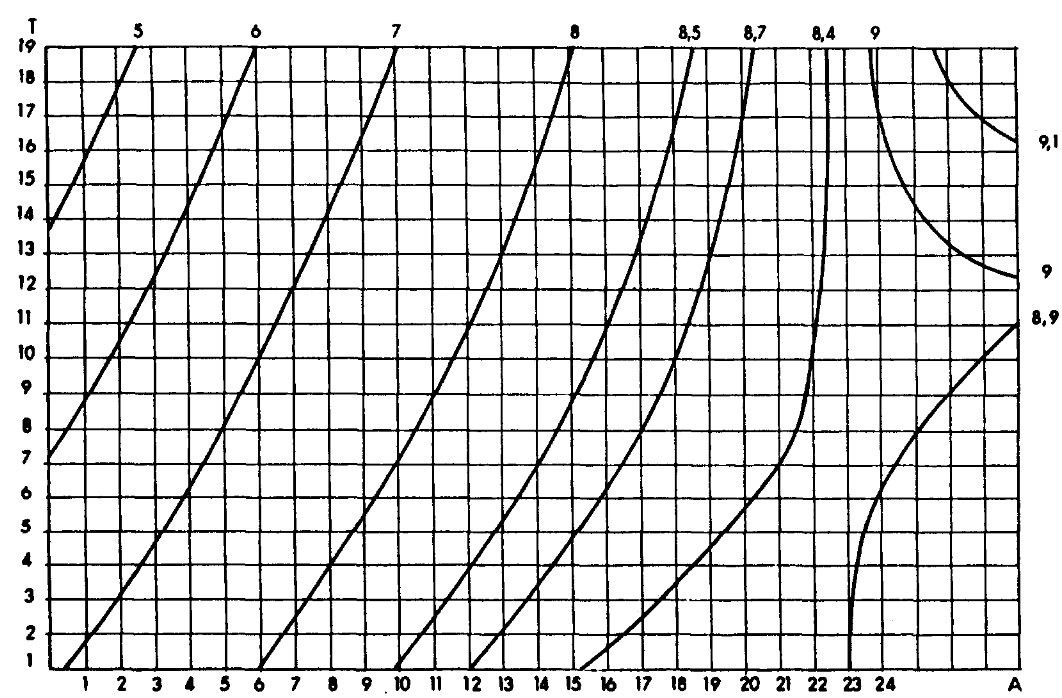

Fig. 5. Levels of methane produced with 2 cc of PVC and different concentrations of acetate (A) and sulphate (T).

Table 4. Variation of pH values in the experimental designs and operational conditions depending on acetate and sulphate concentrations added to vials with different support materials

Support material	Concentration of acetate (mM)	Concentration of sulphate (mM) 0	1	10	20
Digestor sludge (without supports)	0	7.20	6.91	6.86	6.89
	24	5.50	5.90	5.79	5.88
	50	N.T.	5.99	5.8	5.77
	75	N.T.	5.59	5.61	5.60
	100	N.T.	5.62	5.58	5.57
Diabase (2 cc/50 ml)	24	5.89	5.89	5.83	5.90
	50	6.02	5.98	5.80	5.91
	75	6.04	6.01	6.03	6.15
	100	6.05	5.90	6.00	6.03
PVC (6 cc/50 ml)	24	5.60	5.68	5.69	5.73
	50	5.79	5.71	5.75	5.81
	75	6.05	5.77	5.89	5.90
	100	5.91	5.87	5.8	5.75

N.T. = Not tested.

methanogens competed with sulphate-reducing bacteria at a volumetric load of about 300 mM of acetate.

Sulphate-reducing bacteria compete with methanogens for acetate and hydrogen, and the former possess more specialized mechanisms for their uptake (Lovley and Klug, 1983; Robinson and Tiedje, 1984; Conrad *et al.*, 1986). For this reason, at high concentrations of acetate and low levels of sulphate (about 100 and 1 mM, respectively), there will not be competition between methanogenic and sulphate-reducing bacteria, and even the sulphate reduction directs the flow of the electron transfer towards the methanogenesis by the microbial degradation of volatile acids (Ueki and Ueki, 1990; Ueki *et al.*, 1992). However, at higher concentrations of sulphate (between 10 and 20 mM) the sulphate-reduction could replace the methanogenesis. In addition, at higher concentrations of both anions, a synergic effect could be produced, and the metabolism of sulphate-reducing bacteria would provoke a high concentration of acid products and a decrease in the pH values (about 6 with both supports) (Table 4). Previous studies have reported a relationship among the toxicity of the hydrogen sulphide, total sulphide, pH and temperature (Koster *et al.*, 1986). Visser *et al.* (1993), working at thermophilic temperature (55°C), and Koster *et al.* (1986), at mesophilic conditions have demonstrated that for granular sludges precultured in the presence of sulphate, the toxic effect of free hydrogen sulphide on the acetoclastic methanogenic activity depends on the pH value, the sensitivity to hydrogen sulphide being higher at alkaline pH than at neutral or acid pH. On the contrary, the sensitivity to total sulphide was lower at alkaline pH.

In a similar study previously carried out with sepiolite (Muñoz *et al.*, 1994) we found a clearly negative effect of sulphate at high concentrations of sepiolite used on the methanogenesis. On the contrary, in the present study the influence of different concentrations of support materials (PVC and diabase) does not seem to be so clear as in the case of sepiolite. The production of methane achieved with sepiolite as support material and with a concentration of acetate ranging between 0 and 24 mM was higher than with diabase or PVC (15 and 25%, respectively).

The highest production of methane obtained using 2 cc of sepiolite/50 ml of sludge was achieved with 1 mM of sulphate and 24–45 mM of acetate (Muñoz *et al.*, 1994). On the other hand, when diabase or PVC are used as supports, the best results were achieved also with 1 mM of sulphate, but with higher levels of acetate (75–100 mM). These results can be due to a better colonization of the methanogenic population, especially acetoclastic, on sepiolite than on PVC and diabase (Sanchez *et al.*, 1994); in this way, with about 1 mM of sulphate and lower levels of acetate (24–45 mM) high methane productions can be achieved (Muñoz *et al.*, 1994).

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