Effect of sulphate on pelletisation in the UASB system with glucose as substrate

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Abstract

In a UASB system treating a mixture of a carbohydrate (glucose) and sulphate (SO_4^{2-}), at a low influent SO_4^{2-}/COD ratio virtually all the SO_4^{2-} was reduced. Above some upper influent SO_4^{2-}/COD ratio, the mass of SO_4^{2-} reduced became virtually constant, at 0,12 mg SO_4^{2-}/mg influent COD. As the influent SO_4^{2-}/COD ratio increased from a low value so pellet formation concomitantly decreased, from ± 0,08 mg VSS/mg influent COD at trace influent SO_4^{2-} concentration, to ± 0,04 mgVSS/mg influent COD at excess influent SO_4^{2-} concentration. The lower pellet production is proposed to be due to competition for hydrogen (H_2) substrate between the hydrogenotrophic methanogens and sulphidogens. However, the kinetics of H_2 utilisation reported in the literature for these two species in chemostats, do not provide a consistent explanation for the behaviour observed in the UASB systems; possibly the structure of the pellet itself, or the distribution of organisms in the pellet, influence their respective rates of H_2 utilisation.

Introduction

Sam-Soon *et al.* (1987) proposed a hypothesis for pelletisation in upflow anaerobic sludge bed (UASB) reactors. In this hypothesis, one of the prerequisites for pellet formation is an environment of high hydrogen partial pressure (high $\bar{p}H_2$). They concluded that in a UASB system even though a substrate (e.g. carbohydrate) does produce hydrogen at a sufficiently high rate to generate a high $\bar{p}H_2$ zone, secondary reactions (e.g. sulphate, SO_4^{2-}, reduction) may abstract hydrogen, thereby reducing $\bar{p}H_2$ and hence limiting pelletisation. This conclusion is supported partly by the observations of Russo (1987). He observed that in a UASB system treating a paper re-pulping waste (50 per cent carbohydrate, 50 per cent short-chain fatty acids (SCFA), with COD ≈ 5 000 mg/ℓ) containing sulphate (SO_4^{2-} ≈ 300 mg/ℓ), pellet formation was limited and of poor quality. He observed complete removal of the influent sulphate with production of hydrogen sulphide, and pellet size varying inversely to the influent sulphate concentration. However, the evidence of Russo (1987) is qualitative only. Furthermore, the re-pulping waste is complex so that it is not possible to isolate quantitatively the effect of sulphate on pellet production using this waste.

This paper investigates the response of a UASB system to a glucose substrate when dosed with a number of different concentrations of sulphate.

Biochemical background

Sulphate reduction

Sulphate reducing microorganisms (sulphidogens) utilise similar intermediate anaerobic fermentation products as the methanogens; both groups have species that utilise hydrogen or acetic acid as energy sources.

Within the sulphidogen group, the principal species mediating sulphate reduction is believed to be *Desulfovibrio desulphuricans* (Thauer, 1982). This species utilises hydrogen (H_2) as energy source (electron donor) and sulphate (SO_4^{2-}) as terminal electron acceptor. With H_2 as electron donor, reduction of SO_4^{2-} can be expressed as:

$$SO_4^{2-} + 4H_2 \rightarrow H_2S + 2H_2O + 2OH^- \quad (1)$$

That is, for 1 mol SO_4^{2-} reduced, 4 mol H_2 are oxidised. (In hydrogenotrophic methanogenesis, where 1 mol CO_2 is reduced, 4 mol H_2 also are oxidised, Sam-Soon *et al.*, 1990). Eq. (1) also shows that reduction of 1 mol SO_4^{2-} by 4 mol H_2 produces 2 mol OH^-, that is, 2 mol $H_2CO_3^*$ alkalinity, i.e. 100 mg $H_2CO_3^*$ alkalinity as $CaCO_3$ (Loewenthal *et al.*, 1986). As alkalinity is generated, the pH of the medium would tend to rise.

Sulphidogens such as *Desulfotomaculum acetoxidans* and *Desulfobacter postgatei* can use acetic acid (HAc) as energy source and SO_4^{2-} as terminal electron acceptor. With HAc as electron donor, reduction of SO_4^{2-} can be expressed as:

$$CH_3COOH + SO_4^{2-} \rightarrow H_2S + 2CO_2 + 2OH^- \quad (2)$$

That is, for 1 mol SO_4^{2-} reduced, 1 mol HAc is oxidised. (In acetoclastic methanogenesis acetic acid is decarboxylated to give CH_4 and CO_2, Sam-Soon *et al.*, 1990). Eq. (2) shows that reduction of 1 mol SO_4^{2-} by oxidation of 1 mol HAc generates 2 mol $H_2CO_3^*$ alkalinity (100 mg alkalinity as $CaCO_3$).

Sulphate reduction with acetate as energy source has been observed in anaerobic digestion operating on medium salinity waters (ionic strength = ± 0,148) by Middleton and Lawrence (1977). However, from studies on microbial sulphate reduction, Laanbroek and Pfennig (1981) concluded that acetate-oxidising, sulphate reducing organisms generally are not present in low salinity environments such as anaerobic digesters. Isa *et al.* (1986) observed in anaerobic digestion studies that sulphate reduction was not promoted with acetate as substrate. These studies would indicate that in "normal" anaerobic digestion operating on low salinity influents, sulphate reduction most likely takes place with H_2 as the sole energy source.

Experimental scope

Two experimental studies were undertaken, designated the high/low $\bar{p}H_2$ and the high $\bar{p}H_2$ UASB reactor systems respectively. In the high/low $\bar{p}H_2$ reactor system, sufficient pelletised sludge was available, and the loading was selected so that both the high and low $\bar{p}H_2$ zones could develop fully and the fermentation reactions go to completion. This allowed monitoring of the changes in

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Received 12 March 1990; accepted in revised form 5 June 1990.

product formation and utilisation in the profiles under different influent sulphate concentrations. In the high $\bar{p}H_2$ reactor system, from the behavioural patterns observed in the high/low $\bar{p}H_2$ reactor system, the sludge mass was reduced (to the degree experimentally practical) so that the high $\bar{p}H_2$ zone was dominant. This ensured that pellet destruction, which occurs in the low $\bar{p}H_2$ zone, would be minimised, thereby allowing the specific VSS yield to be estimated.

High/low $\bar{p}H_2$ reactor system

Experimental

A UASB reactor, effective volume 9 ℓ, was seeded with 3 ℓ of pelletised sludge obtained from a high/low $\bar{p}H_2$ UASB reactor, at 30°C, fed with glucose. In the study reported here glucose also served as the sole organic carbon source, at a COD concentration of approximately 5 500 mg/ℓ. The feed was supplemented with trace elements and nutrients for organism growth (details of composition are given by Sam-Soon *et al.,* 1987), but with excess NH_3- N, and buffered by addition of 1,6 mg alkalinity as $CaCO_3$ per mg influent COD by addition of $NaHCO_3$. The feed rate was maintained constant at 15 ℓ/d to give a loading rate of 27,5 kgCOD/m³ sludge bed volume per day for the 3 ℓ sludge bed (a loading approximately equal to the loading that would be implemented at full scale). With this loading rate experience had shown (Sam-Soon *et al.,* 1990) that the sludge mass of 3 ℓ would be more than adequate to ensure that the fermentation reactions go to completion well before the flow reaches the top of the bed.

To investigate the effect of sulphate (SO_4^{2-}) the feed was supplemented with SO_4^{2-} in the form of anhydrous sodium sulphate. Initially, the pelletised sludge was acclimatised to a sulphate concentration of 100 mgSO_4^{2-}/ℓ. In less than three weeks 90 per cent of the SO_4^{2-} was reduced to sulphide and the overall COD, NH_3-N and SO_4^{2-} removals became constant; it was accepted that a steady state had been established. Subsequently on average it was found that when SO_4^{2-} concentration was increased, three weeks were needed before steady state was attained.

Six influent SO_4^{2-} concentrations were monitored, i.e. 100, 200, 400, 1 000, 3 000 and 5 000 mgSO_4^{2-}/ℓ. With each run at a selected SO_4^{2-} concentration, samples were taken along the line of flow once a steady state had been attained, and measurements were made of: total soluble COD, short-chain fatty acids [SCFA - propionic (HPr), acetic (HAc)], free and saline ammonia (NH_3-N), total Kjeldahl nitrogen (TKN-N), hence organic nitrogen (orgN) by difference, SO_4^{2-} and pH. Measurements were made according to the methods described by Sam-Soon *et al.* (1990). Sulphate concentration was determined by the turbidimetric method as outlined in Standard Methods (1985). For measurement of total soluble COD, the sample was first stripped of the sulphide by bubbling nitrogen gas through the sample (the S^{2-} is oxidised to SO_4^{2-} during the COD test and hence will give an inflated COD value).

The VSS production was not monitored in this study, but the level of the sludge bed was maintained at 3 ℓ (port No. 5) by daily wasting, or when the sludge bed volume declined below 3 ℓ (with increasing SO_4^{2-} in the influent) the bed volume was left undisturbed.

Result/discussion

The profiles obtained under the different SO_4^{2-} concentrations are shown in Figs. 1 to 6. The responses of various parameters to the different influent SO_4^{2-} concentrations were calculated, and are summarised in Table 1. Also given in Table 1 are masses of glucose processed, by port No. 1 and after passing through the system, for the various influent SO_4^{2-} concentrations; these values were calculated using the procedures set out by Sam-Soon *et al.* (1990) in their **Appendix 1** and **2.**

Sulphate reduction

The SO_4^{2-} removals by port No. 1 and after passing through the system, are shown plotted against influent SO_4^{2-} concentration in Fig. 7 for the various experiments. From Fig. 7 and Figs. 1 to 6 at port No. 1, except for a small persistent residual SO_4^{2-} concentration of about 10 mgSO_4^{2-}/ℓ, removal of SO_4^{2-} was virtually complete for influent SO_4^{2-} concentrations up to about 600 mgSO_4^{2-}/ℓ, i.e. the system was SO_4^{2-} limited. At high influent SO_4^{2-} concentrations, an approximately constant SO_4^{2-} removal was obtained by port No. 1 (± 620 mgSO_4^{2-}/ℓ) and the excess SO_4^{2-} passing through the rest of the system was only marginally affected, to give maximum system SO_4^{2-} removal of ± 650 mgSO_4^{2-}/ℓ (Table 1). This implies that the system exhibited an upper limit to SO_4^{2-} removal capacity of approximately 0,12 mgSO_4^{2-}/mg influent COD. This upper limit arises from one of two possibilities; the system was limited either by mass supply of H_2 substrate or by competitive kinetics of H_2 utilisation by the sulphidogens and methanogens.

TABLE 1
RESPONSE OF THE LOW/HIGH $\bar{p}H_2$ SINGLE UASB REACTOR WITH GLUCOSE AS SUBSTRATE TO VARIOUS INFLUENT SO_4^{2-} CONCENTRATIONS

Influent SO_4^{2-}	ΔCOD mgCOD/ℓ		ΔNH_3 mgN/ℓ		ΔSO_4^{2-} mg/ℓ		HAc mg/ℓ		HPr mg/ℓ		Glucose processed mgCOD/ℓ	
mg/ℓ	Port No. 1	System	Port No. 1	System	Port No. 1	System	Port No. 1	Effluent	Port No. 1	Effluent	Port No. 1	System
0	2 272	-	89,2	-	0	0	1 244	0	944	0	-	-
100	2 979	5 504	86,8	86,8	90	91	867	0	833	0	5 778	5 653
200	3 014	4 997	79,8	93,0	180	180	955	0	777	0	5 202	5 146
400	3 353	5 042	73,7	84,8	379	390	578	0	444	0	4 950	5 199
1 000	3 455	5 051	66,5	73,3	590	590	231	0	371	0	4 574	5 193
3 000	3 572	4 964	65,6	69,6	620	650	244	0	356	0	4 662	4 990
5 000	3 596	5 000	64,9	70,7	615	645	256	0	312	0	4 486	5 025

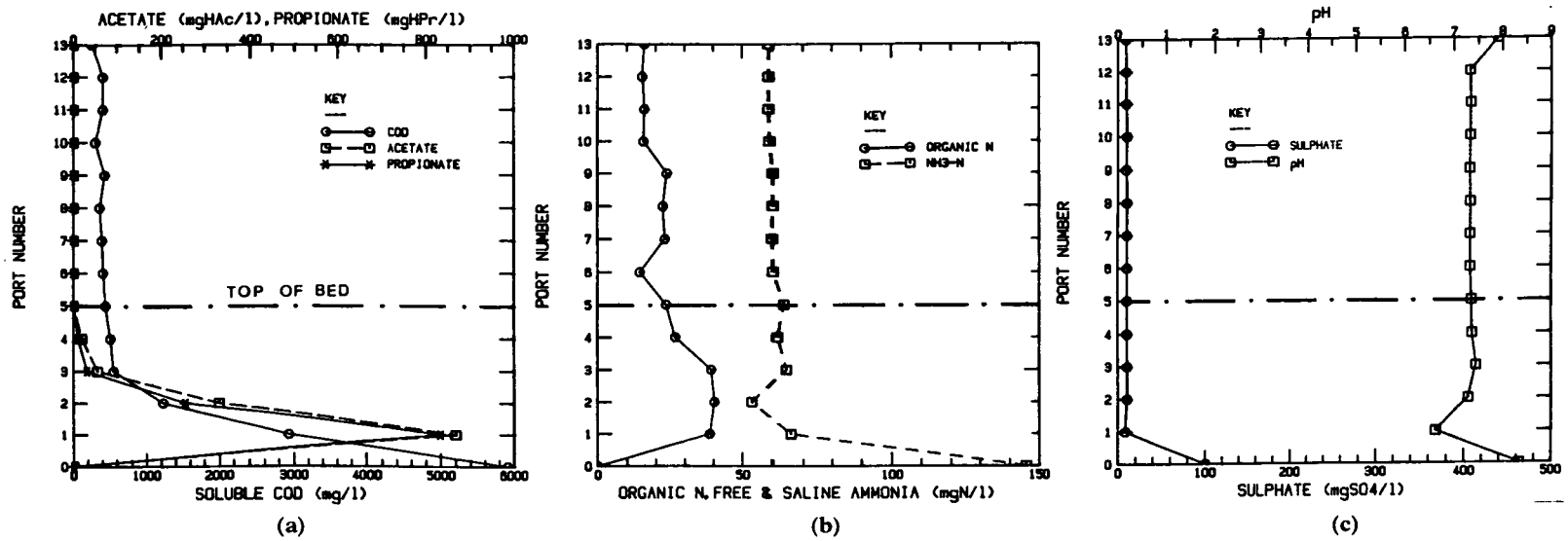

Figure 1

Concentration and pH profiles observed in single high/low $\bar{p}H_2$ UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 100 mgSO_4^{2-}/ℓ)

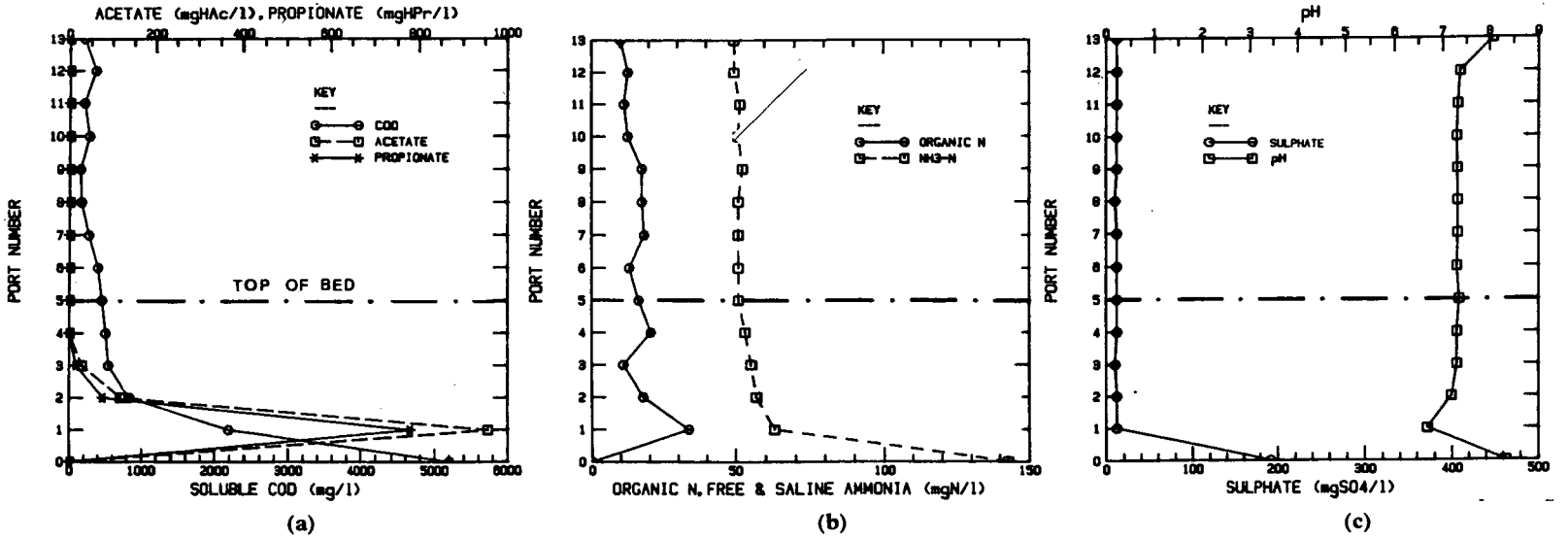

Figure 2

Concentration and pH profiles observed in single high/low $\bar{p}H_2$ UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 192 mgSO_4^{2-}/ℓ)

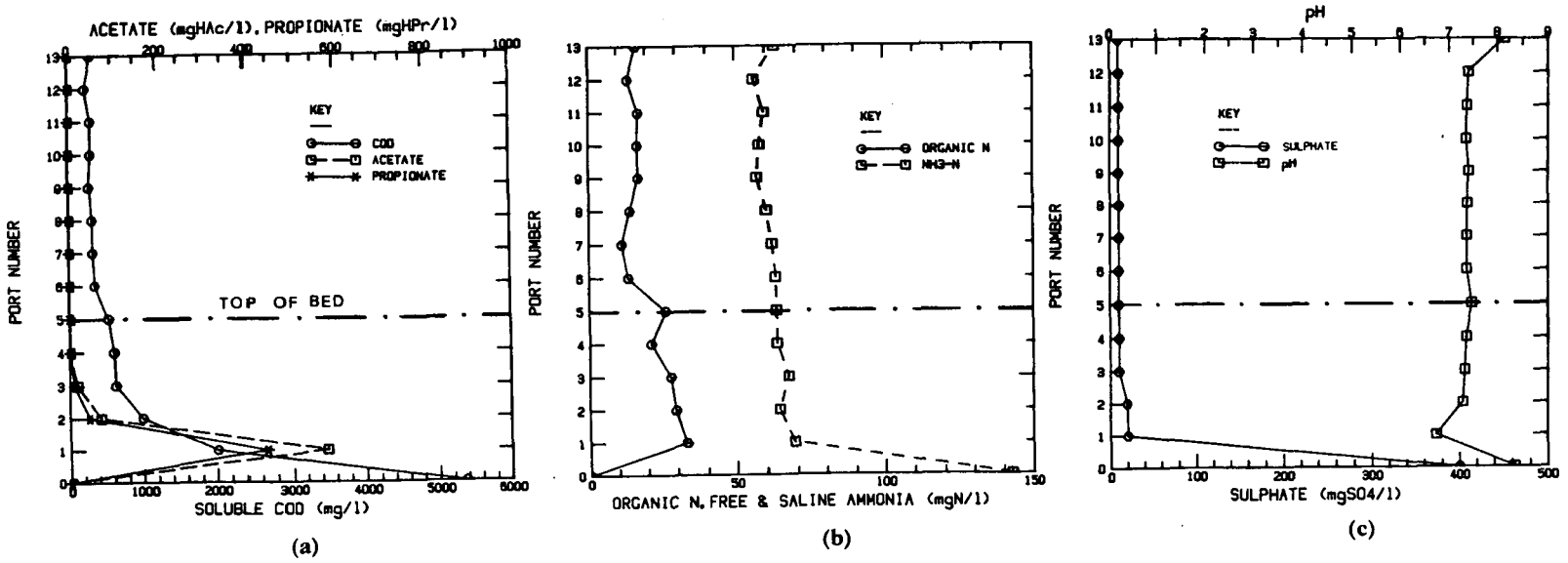

Figure 3

Concentration and pH profiles observed in single high/low $\bar{p}H_2$ UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 400 mgSO_4^{2-}/ℓ)

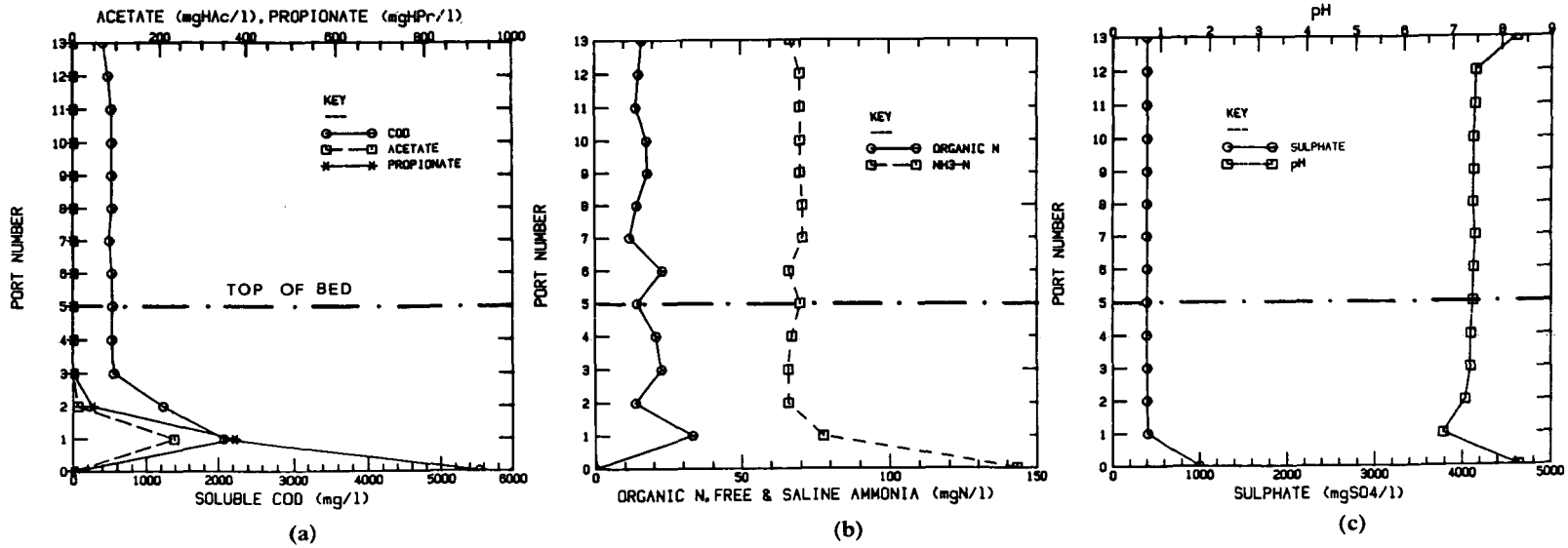

Figure 4

Concentration and pH profiles observed in single high/low $\bar{p}H_2$ UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 1 000 mgSO_4^{2-}/ℓ)

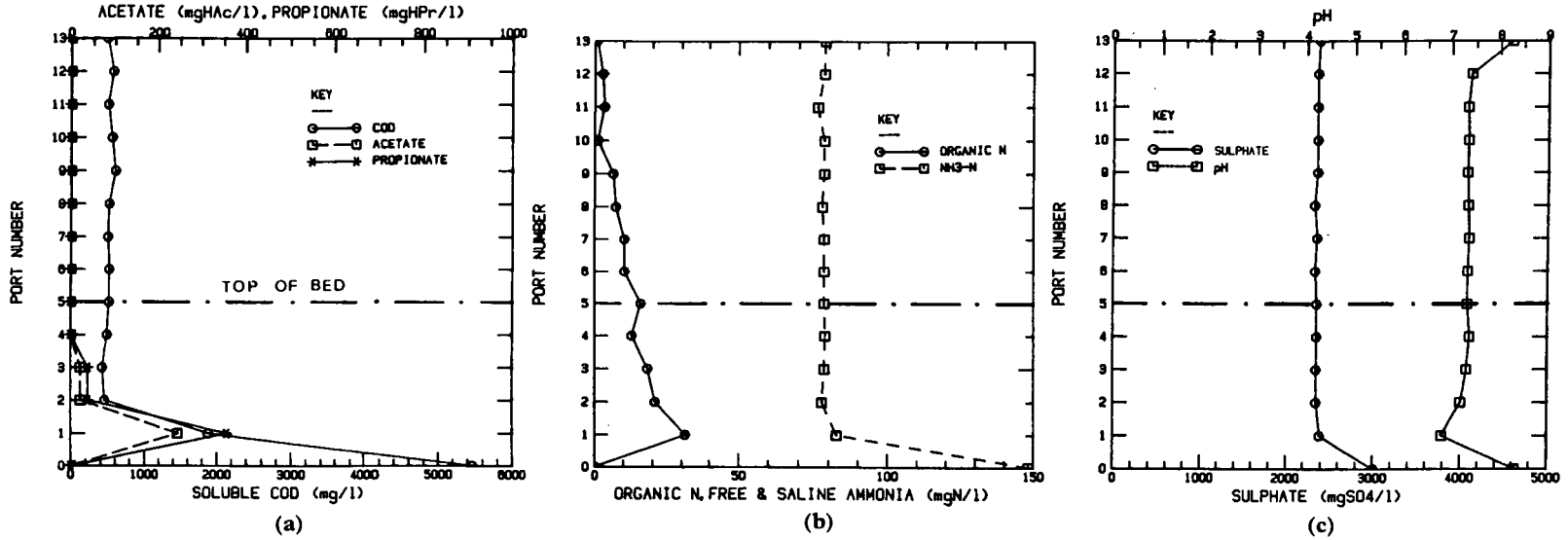

Figure 5

Concentration and pH profiles observed in single high/low pH_2 UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 3 000 mgSO_4^{2-}/ℓ)

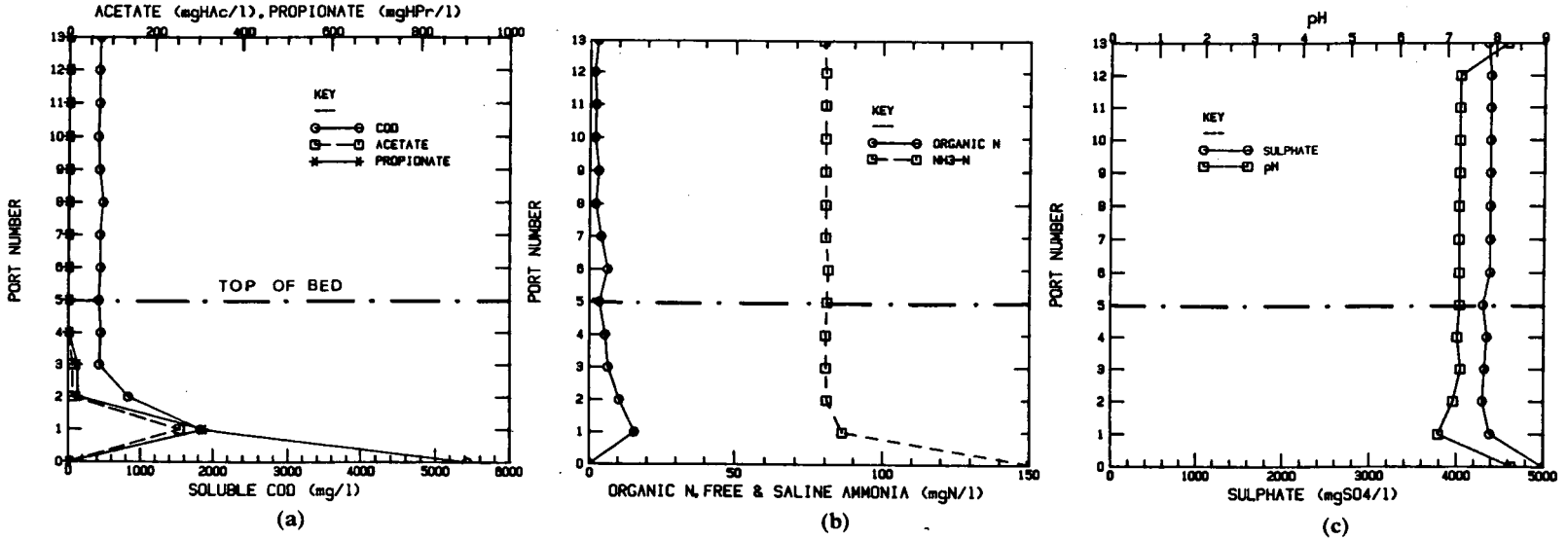

Figure 6

Concentration and pH profiles observed in single high/low pH_2 UASB system with glucose substrate and sulphate (influent COD concentration ≈ 5 500 mg/ℓ; flow rate = 15 ℓ/d; influent SO_4^{2-} concentration = 5 000 mgSO_4^{2-}/ℓ)

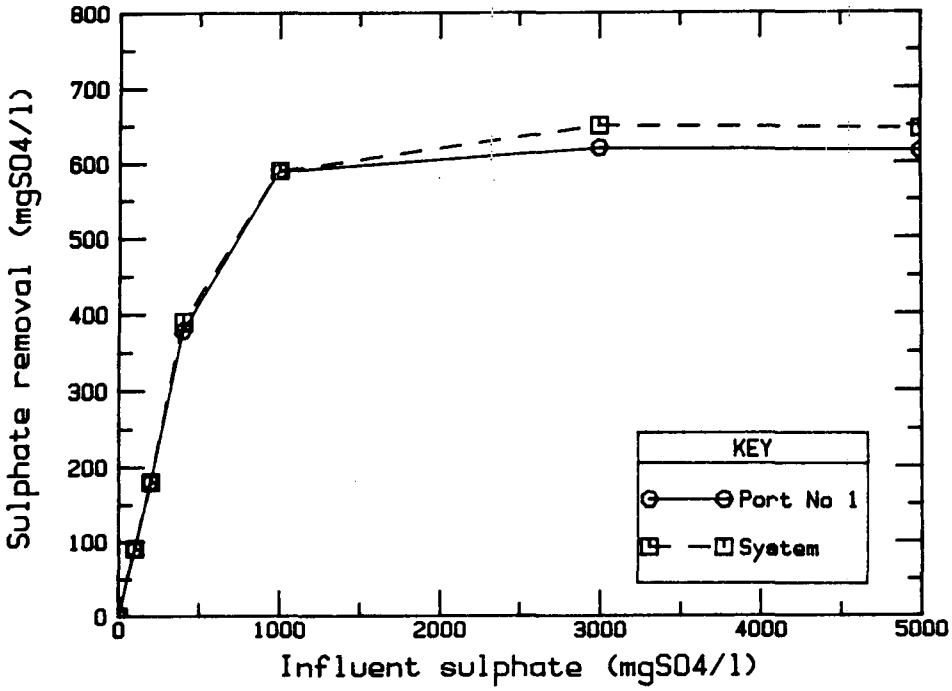

Figure 7
SO_4^{2-} removal, by port No. 1 and through the system, versus influent SO_4^{2-} concentration for single high/low $\bar{p}H_2$ UASB system with glucose as substrate

It is unlikely that the system was limited by mass supply of H_2. From Sam-Soon *et al.* (1990), in the complete fermentation of glucose approximately 10 per cent of the COD appears in acidogen mass; 30 per cent of the COD passes via H_2 to CH_4 and hydrogenotrophic methanogen, *M. Strain AZ*, mass (cell + polypeptides/amino acids); and 60 per cent of the COD passes via acetic acid to CH_4 (acetogen mass and acetoclastic methanogen mass are considered to be negligible, Sam-Soon *et al.*, 1990). Thus, for influent glucose of 5 500 mgCOD/ℓ, a mass of 0,3 x 5 500 = 1 650 mgCOD/ℓ of H_2 is generated (and subsequently utilised). Assuming that all the H_2 generated is available to the sulphidogens, then 1 650/0,67 = 2 463 $mgSO_4^{2-}$/ℓ can be reduced [reduction of 1 $mgSO_4^{2-}$ utilises 0,67 mgCOD of H_2, Eq. (1)], a mass very much more than the maximum measured SO_4^{2-} removal of ± 650 $mgSO_4^{2-}$/ℓ. Clearly, the system with excess SO_4^{2-} must have been limited by competition for H_2 substrate between sulphidogens and methanogens or the relative availability of H_2 to these two organism species.

One particularly puzzling aspect of the responses is that SO_4^{2-} removal was virtually complete by port No. 1 for excess influent SO_4^{2-} systems. Taking as an example the system with influent SO_4^{2-} concentration of 5 000 $mgSO_4^{2-}$/ℓ, a substantial propionic acid (HPr) concentration still was present at port No. 1 (312 mgHPr/ℓ, Table 1). In the low $\bar{p}H_2$ zone above port No. 1, conversion of HPr to HAc by acetogenesis will take place as follows:

$$CH_3CH_2COOH + 2H_2O \rightarrow CH_3COOH + CO_2 + 3H_2 \quad (3)$$

From Eq. (3), for 1 mol HPr converted, 3 mol H_2 are generated, that is, for 1 x 74 x 1,512 = 112 gCOD HPr converted, 3 x 1 x 16 = 48 gCOD (H_2) are generated. Thus for the system with influent SO_4^{2-} of 5 000 $mgSO_4^{2-}$/ℓ, a mass of (312 x 1,512 x 48)/112 = 202 mgCOD/ℓ of H_2 was generated by HPr oxidation above port No. 1. If the H_2 generated should have been available to the sulphidogens, then a removal of 202/0,67 = 300 $mgSO_4^{2-}$/ℓ should have been attained above port No. 1. However, a removal of only 30 $mgSO_4^{2-}$/ℓ was observed. Possible causes for this behaviour are discussed in the section **Pellet formation.**

Profiles

The profiles obtained under different influent SO_4^{2-} concentrations are shown in Figs. 1 to 6. The concentration profiles show patterns of behaviour similar to those of a UASB system with glucose as substrate and no SO_4^{2-} present in the feed (Sam-Soon *et al.*, 1990) except that as the influent SO_4^{2-} concentration increased so the magnitudes of some parameters were increasingly affected. In all instances the fermentation reactions (in the high and low $\bar{p}H_2$ zones) essentially were complete by port No. 3 (1,8 ℓ).

The data at sampling port No. 1 were analysed, as this reflects closest the behaviour in the lower active (high $\bar{p}H_2$) zone - the acetate and propionate profiles exhibit peak values at this sampling port (Sam-Soon *et al.*, 1987; 1990). However, this does not imply that the high $\bar{p}H_2$ zone extended up to sampling port No. 1; this could not be determined experimentally as it was not possible to obtain samples below port No. 1.

At port No. 1, from Figs. 1 to 6, the most striking feature of the profiles is that for influent SO_4^{2-} concentrations up to about 600 $mgSO_4^{2-}$/ℓ, as the influent SO_4^{2-} concentration increased, the concentrations of both the SCFAs, acetic and propionic, decreased significantly. To obtain more information of this aspect, the masses of glucose processed by port No. 1 and through the system (Table 1) were plotted against SO_4^{2-} removal by this port and through the system respectively, see Fig. 8. Also plotted was the total COD of the SCFAs, acetic and propionic, at port No. 1. From Fig. 8, at port No. 1 the COD of the glucose processed decreased linearly as SO_4^{2-} removal increased. This would imply that the removal of SO_4^{2-} inhibited the processing of glucose by the

acidogens. That the removal of SO_4^{2-} and not the influent SO_4^{2-} concentration inhibited processing of glucose, suggests that the inhibition of the acidogens was due to H_2S generated in SO_4^{2-} reduction, Eq. (1). Elimination of H_2S inhibition in a UASB reactor system may not be feasible; stripping of H_2S by, for example, gassing will disturb the plug flow nature of the reactor.

Also, from Fig. 8, the COD of the SCFAs decreased as the SO_4^{2-} removal increased. This decrease in SCFAs (ΔCOD_{SCFA}) appears to correlate closely with the reduction in COD of glucose processed ($\Delta COD_{Glucose}$), $\Delta COD_{SCFA}/\Delta COD_{Glucose}$ = 1,08. Thus, the reduction in the mass of glucose processed by port No. 1 accounts for a substantial fraction of the observed reduction in SCFA concentration at this port. The remaining reduction in SCFA probably was due to changes in SCFA production and utilisation, caused by changes in $\bar{p}H_2$ in the profiles as a result of H_2 utilisation by the sulphidogens. More detailed experimental information on the high $\bar{p}H_2$ zone will be required to clarify this latter aspect.

Pellet formation

As the influent SO_4^{2-} concentration increased, it was observed qualitatively that pellet formation decreased. At trace SO_4^{2-} concentrations, excess sludge growth (above the 3 ℓ level) required regular wasting to maintain this selected sludge volume. However, as the influent SO_4^{2-} concentration increased so the excess sludge wasted declined and eventually the sludge volume declined to below 3 ℓ; for influent SO_4^{2-} concentrations of 1 000, 3 000 and 5 000 $mgSO_4^{2-}/\ell$ the sludge volume stabilised at 2,3 ℓ. The pellet bed volume stabilised because at these SO_4^{2-} concentrations pellet break-up in the low $\bar{p}H_2$ zone equalled the pellet production in the high $\bar{p}H_2$ zone (The decrease in pellet formation with increased influent SO_4^{2-} concentration was measured quantitatively, see later).

The decrease in pellet formation was substantiated by the NH_3-N uptake. A plot of NH_3-N uptake against SO_4^{2-} removal, through the system and by port No. 1, is shown in Fig. 9. It is apparent that NH_3-N uptake (by port No. 1 and through the system) was linearly related to SO_4^{2-} removal; the higher the SO_4^{2-} removal the lower the NH_3-N uptake. This behaviour was due to decreased pellet formation with increased SO_4^{2-} removal, causing a correspondingly lower NH_3-N requirement for polypeptide synthesis.

The decrease in pellet formation can be ascribed to the decreased availability of H_2 substrate to the hydrogenotrophic methanogen *M.* Strain *AZ* in the lower active (high $\bar{p}H_2$) zone, due to removal of H_2 by sulphidogen mediated SO_4^{2-} reduction. [In the upper active (low $\bar{p}H_2$) zone, H_2 is utilised for methane production, not for pellet formation, Sam-Soon *et al.*, 1990]. The mass of H_2 utilised by the sulphidogens below port No. 1 (which most closely reflects the behaviour in the high $\bar{p}H_2$ zone) can be estimated as follows: From Eq. (1), for 1 mol SO_4^{2-} reduced, 4 mol H_2 are oxidised. That is, for 1 $mgSO_4^{2-}$ reduced 0,67 mgCOD of H_2 are oxidised. From Table 1, the maximum SO_4^{2-} removed by port No. 1 was ± 620 $mgSO_4^{2-}/\ell$ = 9,30 gSO_4^{2-}/d for influent SO_4^{2-} concentrations of 3 000 to 5 000 $mgSO_4^{2-}/\ell$. Thus, for this SO_4^{2-} removal, 9,3 x 0,67 = 6,2 gCOD/d of H_2 were oxidised.

The approximate mass of H_2 generated below port No. 1 can also be estimated. For the influent SO_4^{2-} concentration of 5 000 $mgSO_4^{2-}$, in complete fermentation approximately 30 per cent (see earlier) of the COD in the glucose processed by port No. 1 (4 486 mgCOD/ℓ, Table 1) would have appeared as H_2 to be utilised subsequently, i.e. 0,3 x 4 486 = 1 346 mgCOD(H_2)/ℓ. However, the fermentation was incomplete by port No. 1; 256 mgHAc/ℓ and 312 mgHPr/ℓ remain (Table 1). The residual HAc would not have resulted in further H_2 generation; it would have been converted to CH_4 by acetoclastic methanogens. The HPr had a potential for H_2

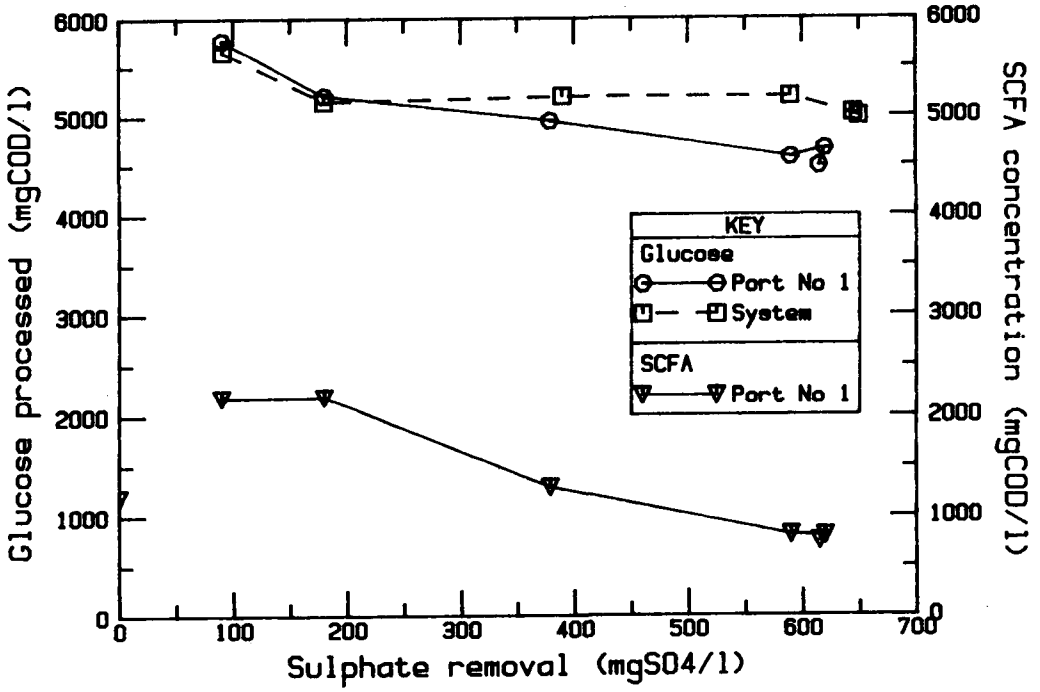

Figure 8
Glucose processed, by port No. 1 and through the system, and total short chain fatty acid (SCFA) concentration (COD units) at port No. 1 versus SO_4^{2-} removal for single high/low pH_2 UASB system with glucose as substrate

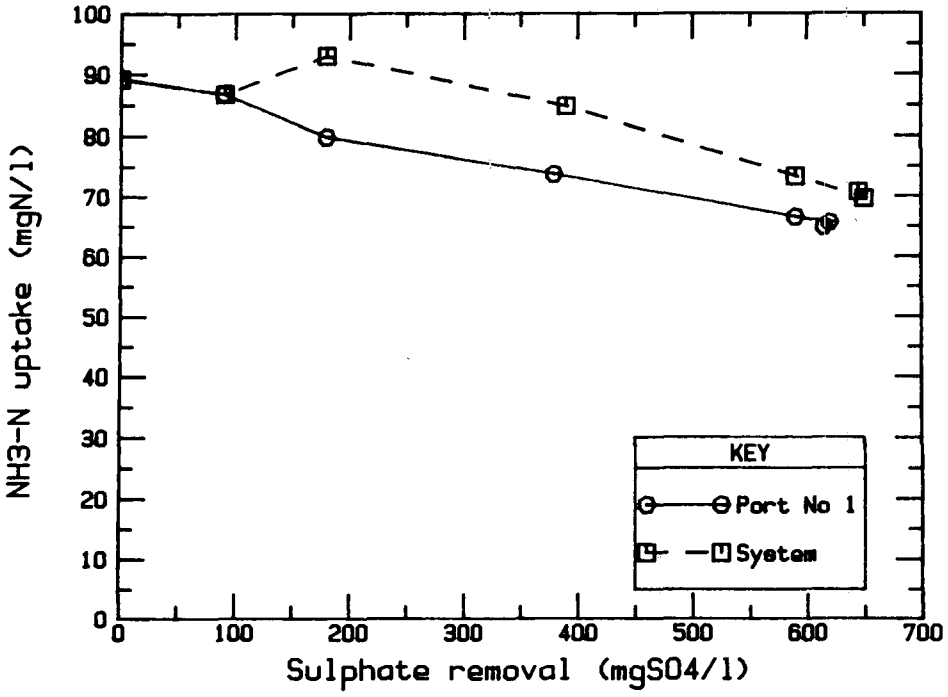

Figure 9
NH_3-N uptake, by port No. 1 and through the system, versus SO_4^{2-} removal for single high/low pH_2 UASB system with glucose as substrate

generation; conversion to HAc by acetogens would have generated 202 mgCOD/ℓ of H_2 (see section **Sulphate reduction** above). Thus, below port No. 1 approximately 1 346 - 202 = 1 144 mgCOD/ℓ = 17,2 gCOD/d of H_2 had been generated.

Comparing the H_2 generated below port No. 1 (17,2 gCOD/d) with the H_2 utilised in SO_4^{2-} reduction (6,2 gCOD/d), it is evident that an appreciable removal of H_2 by sulphidogen mediated SO_4^{2-} reduction took place below port No. 1, the zone where pelletisation takes place (high $\bar{p}H_2$ zone, Sam-Soon *et al.*, 1987; 1990). This gave rise to a substantially decreased availability of H_2 substrate to the hydrogenotrophic methanogen *M.* Strain *AZ* [(6,2/17,2) x 100 = 36 per cent reduction]. Consequently, pelletisation was substantially reduced.

One may now raise the question why (even at an excessively high influent SO_4^{2-}concentration of 5 000 mgSO_4^{2-}/ℓ) the H_2 substrate was not completely utilised by the sulphidogens for SO_4^{2-} reduction, or, in fact, by methanogens for methane production. The literature provides some information on this aspect. Kristjansson *et al.* (1982) have shown that the maximum specific substrate utilisation rate for the hydrogenotrophic methanogen *M.* Strain *AZ (M. arboriphilus)* [≈ 54 μmol H_2/(mg dry weight.h)] is approximately 5 times that for the hydrogenotrophic sulphidogen *D. vulgaris* [≈ 9,8 μmol H_2/(mg dry weight.h)]. However, the half saturation coefficient (K_s) value for the methanogens (6 μmol H_2) they found to be about 5 times that of the sulphidogens (1 to 2 μmol H_2). The rates of H_2 utilisation of the respective hydrogenotrophic species up the UASB profile will depend on the $\bar{p}H_2$ and the organism masses. Quantitatively, insufficient information is available to formulate their rates of H_2 utilisation. Qualitatively, in the state of high $\bar{p}H_2$ (below port No. 1) the reported relative substrate utilisation rates of the two hydrogenotrophs should have favoured the pellet forming *M.* Strain *AZ* to complete dominance, but this did not occur; pellet formation occurred but at a reduced rate, and substantial SO_4^{2-} reduction was obtained in the high $\bar{p}H_2$ zone.

From the maximum specific substrate utilisation rates and half saturation coefficients, under low $\bar{p}H_2$ (i.e. above port No. 1), the sulphidogens should be able to compete more successfully with the methanogens for the H_2 substrate than under high $\bar{p}H_2$. That is, above port No. 1 significant SO_4^{2-} removal should have taken place - for the 5 000 mgSO_4^{2-}/ℓ system, 202 mgCOD (H_2)/ℓ was generated above port No. 1 giving maximum potential SO_4^{2-} removal of 300 mgSO_4^{2-}/ℓ (see section **Sulphate reduction**). However, negligible removal of SO_4^{2-} (only ± 30 mgSO_4^{2-}/ℓ) was observed.

From the discussion above, it is apparent that the reported kinetics for the hydrogenotrophic methanogens and sulphidogens cannot explain their behaviour observed in the UASB system. Possibly, in the UASB system the pellet structure itself, and the proximity of organisms to each other within the pellet exerts an influence on the relative kinetics of the methanogens and sulphidogens - Kristjansson *et al.* (1982) conducted their experiments in chemostats and supplied H_2 substrate by bubbling gas through the liquid supernatant. In the UASB system the H_2 is generated within the pellet by the action of the acidogens and acetogens and transfer of H_2 occurs across microscopic interspecies distances (McCarty and Smith, 1986). Since the methanogens are the agents responsible for the development of the pellet, they may have an advantage over the sulphidogens for obtaining the H_2 substrate. Clearly, this aspect requires further investigation.

TABLE 2
RESPONSES OF THE HIGH pH_2 REACTOR AT EXCESS AND AT LIMITING SO_4^{2-} INFLUENT CONCENTRATIONS

Parameter	Excess SO_4^{2-} system	Limited SO_4^{2-} system
Influent SO_4^{2-} (mg/ℓ)	5 000	1,06
Flow rate (ℓ/d)	15	15
Loading (gCOD/d)	76	77
COD in (mg/ℓ)	5 080	5 100
COD out (mg/ℓ)	2 276	3 155
COD removed per day (gCOD/d)	42,06	29,18
COD removal (%)	55	43
NH_3-N (mgN/ℓ) in	173,6	173,6
NH_3-N (mgN/ℓ) out	115,6	86,8
Dissolved orgN - effl. (mgN/ℓ)	4,1	33,6
Sludge VSS (gVSS/ℓ)	30,40	35,34
Sludge TSS (gTSS/ℓ)	33,17	37,95
Volume of sludge wasted (mℓ/d)	100	180
VSS generated (mgVSS/d)	3 040	6 361
Net yield (mgVSS/mg infl. COD)	$4,0 \times 10^{-2}$	$8,3 \times 10^{-2}$
Nature of VSS generated	Fines and pellets with $\phi \approx$ 0,5-1,0mm	Mainly pellets with $\phi \approx$ 1,0-3,0mm
SCFA (effluent)		
Acetate (mgHAc/ℓ)	690	987
Propionate (mgHPr/ℓ)	495	815
Butyrate (mgHBr/ℓ)	0	0

High $\bar{p}H_2$ UASB system

Experimental

In order to obtain an estimate of the VSS yield with excess SO_4^{2-} in the influent, two 3 ℓ UASB reactors were set up to operate in parallel as high $\bar{p}H_2$ reactors. The first reactor was fed a trace of SO_4^{2-} (1,06 mgSO_4^{2-}/ℓ) sufficient for normal anaerobic growth. The second reactor was fed an excess of SO_4^{2-} (5 000 mgSO_4^{2-}/ℓ). The first reactor was seeded with pelletised sludge obtained from the lower active zone of a single UASB system fed with glucose substrate (influent COD concentration ≈ 5 000 mg/ℓ, flow rate = 15 ℓ/d, NH_3-N = 173,6 mgN/ℓ); the reactor was seeded up to the 2nd port (equivalent to port No. 2 in a single UASB system), a volume of 0,7 ℓ. The second reactor likewise was seeded with 0,7 ℓ of sludge obtained from the lower active zone of the single high/low $\bar{p}H_2$ UASB reactor described in the previous section, also with glucose as substrate (influent COD concentration ≈ 5 000 mg/ℓ, flow rate = 15 ℓ/d, NH_3-N = 173,6 mgN/ℓ, SO_4^{2-} ≈ 5 000 mg/ℓ). Both systems that supplied the seed sludges had been operating in a stable state for six months.

The sludge volume of 0,7 ℓ was well in excess of that required for the high $\bar{p}H_2$ zone in a reactor receiving a high influent SO_4^{2-} concentration; in the high/low $\bar{p}H_2$ reactor system described above the high $\bar{p}H_2$ zone lay below port No. 1 (0,3 ℓ). However, it was not practical to operate the reactor with a smaller volume (with a sludge bed up to say port No. 1) because the gas bubbles disrupted the plug flow state. Hence, the system had more than half the sludge volume in the low $\bar{p}H_2$ zone. However, compared with the high/low $\bar{p}H_2$ reactor system the low $\bar{p}H_2$ zone was reduced significantly, from about 2,7 to about 0,4 ℓ, so that pellet destruction would be minimised.

Every day the following parameters were measured on the influent and on the filtered effluent: COD, TKN, NH_3-N (and hence by difference, orgN) the SCFA propionic and acetic, and the VSS produced. The procedure for measuring the VSS was as follows: Each day the sludge volume above port No. 2 was drained and well mixed with the effluent, a sample was taken, centrifuged and the VSS determined.

Results

In Table 2 are listed the responses of the two high $\bar{p}H_2$ systems at excess and trace influent SO_4^{2-} concentrations. Taking the system with trace SO_4^{2-} as the reference, the system with excess SO_4^{2-} exhibited the following significant deviant responses:

- Mass COD removed per day was greater by 12,88 gCOD/d (42,06 compared to 29,18 gCOD/d).
- NH_3-N removal was lower by 28,8 mgN/ℓ (58,0 compared to 86,8 mgN/ℓ) and associated dissolved orgN generation similarly was lower, by 29,5 mgN/ℓ (4,1 compared to 33,6 mgN/ℓ).
- SCFA concentrations were lower (acetate 690 compared to 987 mgHAc/ℓ and propionate 495 compared to 815 mgHPr/ℓ).
- Net VSS specific yield was lower ($4,0 \times 10^{-2}$ compared to $8,3 \times 10^{-2}$ mgVSS/mgCOD influent).

For comparative purposes, the specific yield values are reported as mgVSS/mgCOD influent instead of the more usual mgVSS/mgCOD removed. This method of reporting was adopted

because it was not possible to sample the COD at the upper limit of the high $\bar{p}H_2$ zone - for the excess SO_4^{2-} system, at port No. 2 the $\bar{p}H_2$ was already so low that conversion of propionic to acetic acid was well advanced.

Discussion

In Table 2 the specific VSS yields for the two high $\bar{p}H_2$ systems are expressed in terms of the influent COD, viz. 8,3 x 10^{-2} and 4,0 10^{-2} mgVSS/mg influent COD for the trace SO_4^{2-} and excess SO_4^{2-} systems respectively. That is, the VSS yield for the trace SO_4^{2-} system was more than 2 times greater than the VSS yield for the excess SO_4^{2-} system. [Note that the yield for the trace SO_4^{2-} system (8,3 x 10^{-2} mgVSS/mg influent COD) was slightly less than that obtained in the high $\bar{p}H_2$ system reported by Sam-Soon *et al.* (1990) when expressed in a similar way (10,8 x 10^{-2} mgVSS/mg influent COD). The reason for this is that in the trace SO_4^{2-} system, the sludge bed included both high and low $\bar{p}H_2$ zones and hence some destruction of the VSS was to be expected in the low $\bar{p}H_2$ zone of the bed; in the study of Sam-Soon *et al.* (1990) the VSS yield was determined on a sludge bed comprising only a high $\bar{p}H_2$ zone so that pellet destruction would have been at a minimum].

The lower measured VSS yield for the excess influent SO_4^{2-} system compared to the trace SO_4^{2-} system is in agreement with observations on the high/low $\bar{p}H_2$ systems (see earlier). We have seen that the lower VSS yield is due to reduced pellet formation by the methanogen, *M. Strain AZ* which is caused by decreased availability of H_2 substrate due to removal of H_2 by the sulphidogens. The ratio of the specific VSS yields for the two systems [(4,0 x 10^{-2}):(8,3 x 10^{-2}) = 0,48:1] is in reasonable accord with the ratio of calculated availability of H_2 to the methanogens with or without excess sulphate (11:17,2 = 0,64:1, see the high/low $\bar{p}H_2$ section above).

The lower pellet formation in the excess SO_4^{2-} system is reflected also in the lower NH_3-N removal and the lower effluent orgN generated compared to their values in the trace SO_4^{2-} system. The lower effluent orgN concentration in the SO_4^{2-} excess system (4,1 mgN/ℓ compared to 33,6 mgN/ℓ for the SO_4^{2-} trace system) is in conformity with the hypothesis on pelletisation in that a decrease can be expected in the generation and release of amino acids by *M. Strain AZ* in the SO_4^{2-} excess system due to a reduction in H_2 availability to these organisms.

Conclusions

In a UASB system treating a mixture of glucose and sulphate (SO_4^{2-}):

- At a low influent SO_4^{2-}/COD ratio, virtually all the SO_4^{2-} is reduced, i.e. system is SO_4^{2-} limited. Above some upper influent SO_4^{2-}/COD ratio the mass of SO_4^{2-} reduced becomes virtually constant at ≈ 0,12 mgSO_4^{2-}/mg influent COD, i.e. system appears to be substrate limited.
- With influent SO_4^{2-}/COD increasing from an initially low value, pellet formation concomitantly decreases. When the influent SO_4^{2-}/COD reaches the upper limit of SO_4^{2-} reduction, pellet formation reaches its lower limit of production and thereafter stays at this limit for any further increase in influent SO_4^{2-}/COD ratio. The lower pellet production is ascribed to decreased availability of H_2 substrate to the pellet forming methanogens, due to removal of H_2 by sulphidogen mediated SO_4^{2-} reduction. The measured production of pellets at trace SO_4^{2-} (2 x 10^{-4} mgSO_4^{2-}/mg influent COD) and excess SO_4^{2-} (0,98 mgSO_4^{2-}/mg influent COD) were 8,3 x 10^{-2} and 4,0 x 10^{-2} mgVSS/mg influent COD respectively, i.e. ratio of 1:0,48. This ratio approximates the calculated ratio of H_2 availability to the methanogens without and with excess SO_4^{2-}, i.e 1:0,64.
- The reported kinetics of H_2 utilisation for the hydrogenotrophic methanogens and sulphidogens obtained from chemostat studies do not provide a consistent explanation for the observed relative utilisation of H_2 by these two species in the high and low $\bar{p}H_2$ zones of the UASB system. In the UASB system, possibly the structure of the pellet itself and the distribution of organisms within the pellet exert an influence on the utilisation of H_2 by the methanogens and sulphidogens.

Acknowledgements

This research was supported jointly by the Foundation for Research Development and the Water Research Commission of South Africa and this paper is published with their permission.

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