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Full-scale and laboratory-scale anaerobic treatment of citric acid production wastewater

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Key words: anaerobic digestion, citric acid production wastewater, methanogenesis, sulphate reduction, sulphidogenesis, sulphide toxicity, thermophilic digestion.

Abstract

This paper reviews the operation of a full-scale, fixed-bed digester treating a citric acid production wastewater with a COD: sulphate ratio of 3–4 : 1. Support matrix pieces were removed from the digester at intervals during the first 5 years of operation in order to quantify the vertical distribution of biomass within the digester. Detailed analysis of the digester biomass after 5 years of operation indicated that H_2 and propionate-utilising SRB had outcompeted hydrogenophilic methanogens and propionate syntrophs. Acetoclastic methanogens were shown to play the dominant role in acetate conversion. Butyrate and ethanol-degrading syntrophs also remained active in the digester after 5 years of operation.

Laboratory-scale hybrid reactor treatment at 55 °C of a diluted molasses influent, with and without sulphate supplementation, showed that the reactors could be operated with high stability at volumetric loading rates of 24 $kgCOD.m^{-3}.d^{-1}$ (12 h HRT). In the presence of sulphate (2 g/l^{-1}; COD/sulphate ratio of 6 : 1), acetate conversion was severely inhibited, resulting in effluent acetate concentrations of up to 4000 $mg.l^{-1}$.

Introduction

Many industrial wastewaters contain sulphate at varying concentrations and COD: SO_4^{2-} ratios (Colleran et al. 1994). The presence of sulphate in wastewaters undergoing anaerobic treatment presents operational problems due to competition between sulphate-reducing bacteria (SRB) and other anaerobes (syntrophs, methanogens) for common organic (low molecular weight acids, alcohols, etc.) and inorganic substrates (H_2). Sulphate is a normal constituent of wastewaters from the brewing, paper/pulp, edible oil and molasses-based fermentation industries. For example, the COD and sulphate concentrations of wastewater from a sugarcane molasses alcohol production plant was reported by Carrondo et al. (1983) to average 50.6 and 2.9 $g.l^{-1}$, respectively. Szendry (1983) reported a COD content of up to 95 $g.l^{-1}$ and a sulphate content of up to 6 $g.l^{-1}$ in the rum distillery slops serving as influent to a full-scale upflow anaerobic filter at the Bacardi Corporation rum distillery in San Juan, Puerto Rico. Wastewaters from industrial fermentative production of citric acid from sugarbeet molasses typically contain COD and sulphate concentrations of up to 30 and 4.5 $g.l^{-1}$, respectively (Svardal et al. 1993). Effluents from the paper and board industries vary greatly in COD content and organic chemical composition, with sulphate concentrations typically ranging from 1–2 $g.l^{-1}$ (Puhakka et al. 1990). The highest wastewater sulphate concentrations are associated with the industrial production of edible oil fatty acids. The effluents from edible oil refineries can have a sulphate content of up to 40–50 $g.l^{-1}$, with a COD/sulphate ratio of 1 or even less (Hoeks et al. 1984; Rinzema et al. 1986).

During anaerobic treatment of these wastewaters, two distinct mineralisation processes occur – i.e. sulphidogenesis and methanogenesis. SRB species carry out dissimilatory sulphate reduction (Widdel 1988), utilising sulphate as external electron acceptor and

generating sulphide as electron sink. The production of sulphide adversely affects full-scale methanogenic wastewater treatment systems via (i) reduction of the methane yield; (ii) potential sulphide inhibition of syntrophic and methanogenic bacteria; (iii) corrosion, malodour problems and additional gas purification costs associated with the presence of H_2S in the produced biogas; (iv) malodour and oxygen demand problems associated with sulphide dissolution in the treated effluent, leading to a possible requirement for effluent post-treatment.

With wastewaters of COD/sulphate ratios greater than 10 : 1, adverse effects of sulphate reduction are minimal and methanogenesis during wastewater treatment proceeds without significant negative effects (Rinzema and Lettinga 1988). At the other extreme, with COD/sulphate ratios of 1 or lower, sulphidogenesis will prevail and sulphidogenic wastewater treatment may be of commercial interest in the context of sulphur recovery for re-use (Visser 1995). However, most industrial wastewaters display COD/sulphate ratios of between 1 and 10. Knowledge of the factors determining the outcome of competition between SRB and syntrophic and methanogenic anaerobes at these intermediate COD/sulphate ratios is limited and requires investigation if anaerobic treatment of these wastewaters is to be optimised (O'Flaherty et al. 1998a).

This paper reviews the data obtained during full-scale anaerobic treatment of citric acid production wastewater under mesophilic conditions. Results from laboratory-scale thermophilic studies are also presented. Citric acid production wastewaters exhibit a COD/sulphate ratio of 3-4 : 1. Consequently, active competition for organic and H_2 substrates occurs under these conditions, resulting in potential favouring of sulphidogenesis over methanogenesis and potential sulphide toxicity against individual trophic groups. The factors underlying the competition outcome under laboratory-scale and full-scale conditions are discussed in this paper.

Materials and methods

Laboratory-scale reactors

Laboratory-scale quarter-packed upflow hybrid reactors (Henry et al. 1996), combining a granular sludge bed in the lower section and an upper filter section consisting of random-packed polyethylene cascade rings (diameter = 10 mm), were used for the thermophilic experiments described in this paper (Figure 1).

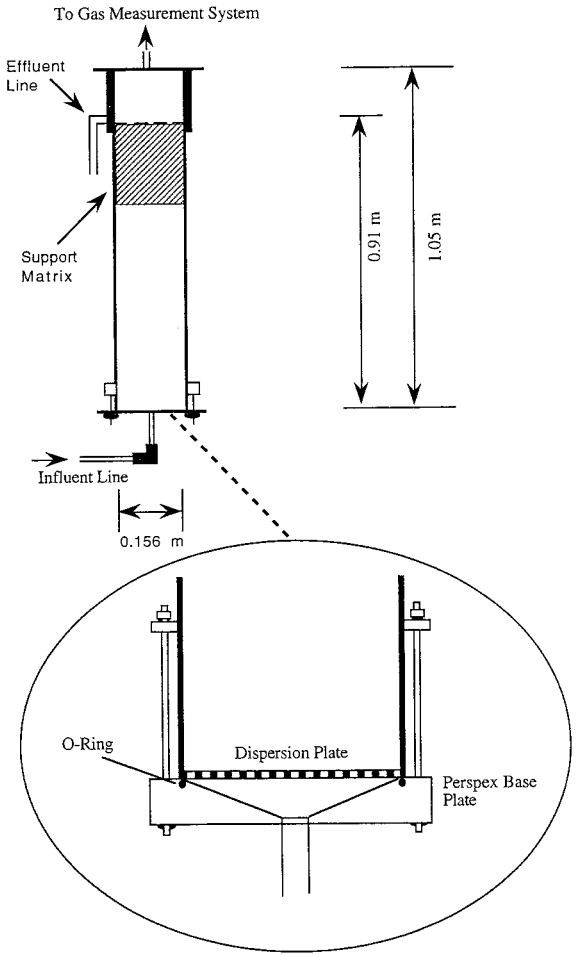

Figure 1. Schematic diagram of laboratory-scale hybrid reactors used for thermophilic anaerobic treatment of simulated citric acid production wastewaters.

Source of full-scale biomass

The full-scale, fixed-bed digester installed to treat wastewater from the Archer, Daniels and Midlands (ADM) citric acid production plant at Ringaskiddy, Co. Cork, Ireland has been described by O'Flaherty et al. (1998b). Biomass samples were removed periodically from the reactor during the first five years of operation, as described by Colleran et al. (1994) and O'Flaherty et al. (1998b).

Analytical techniques

Maximum specific methanogenic activity profiles of all sludge samples were determined against the direct methanogenic substrates, acetate and H_2/CO_2, and the syntrophic substrates, propionate, butyrate and ethanol, using the pressure transducer technique described previously (Colleran and Pistilli 1994; Coates et al. 1996). The same activity test procedure was also carried out in the presence of 60 mm bromoethane sulphonic acid (BES) and 2 mm sodium molybdate (Mb), specific inhibitors for methane-producing bacteria (MPB) and sulphate reducing bacteria (SRB), respectively (Oremland and Capone, 1988). Direct substrate degradation measurements were also performed in the presence and absence of sulphate and specific inhibitors, as described by O'Flaherty et al. (1998a).

Volatile suspended solids (VSS) were determined according to Standard Methods (APHA 1985). Volatile fatty acids (VFA), alcohol and gas analyses were carried out by gas chromatography, as described by Henry et al. (1996). Sulphate and sulphide analyses were carried out, respectively, by the turbidometric and colourimetric procedures described in Standard Methods (APHA 1985).

Results

Operation of the full-scale ADM Ringaskiddy reactor

Following intensive laboratory-scale and pilot-scale studies, a full-scale digester was commissioned to treat the ADM-Ringaskiddy wastewater in 1990. A fully-packed, upflow anaerobic filter design was chosen by the company concerned (Biotim). The support matrix consisted of polypropylene cascade rings (0.175 by 0.05 m) which were randomly packed within the 8000 m^3 digester. The digester was seeded with 600 m^3 of granular sludge developed on wastewater from a potato-chip production factory in the Netherlands. The sludge had an average VSS content of 4% and a COD conversion efficiency of 0.65 $kg.VSS^{-1}.d^{-1}$ (calculated with respect to acetate) (Derycke et al. 1993; Colleran et al. 1994). The maximum previous exposure of the seed sludge to sulphide was 20 $mg.l^{-1}$. Consequently, a start-up strategy utilising 50% dilution of the influent wastewater and supplementation of the organic carbon content by molasses addition was employed (Colleran et al. 1994). The time taken for the biomass to adapt to the design COD load and influent COD/sulphate ratio was approximately nine months, excluding factory shut-down periods (Derycke et al. 1993). The lengthy start-up time was largely attributable to the need to tailor the operation of the anaerobic plant to the capacity of the subsequent nitrification stage (Derycke et al. 1993; Colleran et al. 1994). The adaptation period was shown to coincide with an initial decrease in the granular nature of the suspended anaerobic sludge within the reactor, coinciding with the development of a matrix-associated biofilm and a flocculant interstitial biomass (Derycke et al. 1993).

Table 1. Design and operational performance of the ADM, Ringaskiddy full-scale anaerobic wastewater treatment plant

Process parameter	Design	Operational
Influent sulphate ($g.l^{-1}$)	4.0	3.43
Influent COD/SO_4^{2-} ratio	3.0	3.61
Hydraulic retention time (days)	1.4	1.4
COD reduction efficiency (%)	50	52
BOD reduction efficiency (%)	70	80
Volumetric loading rate ($kgCOD.m^3.d^{-1}$)	8.5	9.0
Biogas output (m^3/h)	950	1053
CH_4 in biogas (%)	60–65	65.5
H_2S in biogas (%)	5.0	4.8

The design characteristics and operational performance achieved on completion of start-up are presented in Table 1. Mass balance calculations at steady-state indicated that approximately 370 mg sulphide-$S.l^{-1}$ exited from the reactor in the biogas. Approximately 10% of the reduced sulphate-S was not detected as sulphide in the effluent and biogas. This discrepancy was probably due to deposition of sulphur compounds in the reactor, uptake of sulphur for bacterial growth and possible losses of sulphur compounds during effluent sampling (Derycke et al. 1993).

The observed specific CH_4 and H_2S yield obtained at steady-state was 0.58 $Nm^3.kgCOD^{-1}$ removed (Colleran et al. 1994; Finnegan 1994). This value exceeded the theoretically possible yield and was attributed by Derycke et al. (1993) to the presence of betaine in the digester influent. Betaine accounts for up to 1.6% of the dry weight of sugar beet (Davies and Dowden 1936). However, betaine is incompletely oxidised in the standard COD assay procedure. Consequently, the actual influent organic carbon is significantly underestimated by the COD assay. Derycke et al. (1993) calculated that the 'true' COD value of the

influent was approximately 35% higher than the COD determined analytically. This implies that the volumetric COD loading rate applied to the reactor was higher than presented in Table 1 and that the influent COD/sulphate ratio was c. 5.6 : 1, rather that 3–4 : 1. When the underestimation of influent COD is taken into account, the true COD removal efficiency was considered to be in excess of 60–65% and the CH_4 + H_2S yield approximated the normal value of 0.35 $Nm^3.kgCOD^{-1}$ removed (Derycke et al. 1993).

Biomass distribution in the full-scale ADM digester during the first 5 years of operation

In order to monitor the development of biofilm on the matrix pieces during operation of the full-scale digester, a sampling device was installed during commissioning (Colleran et al. 1994). This enabled the periodic removal of a vertical core of matrix pieces through a specially designed opening in the digester roof. The matrix pieces were packed into three cages from which individual pieces could be removed or introduced prior to returning the cages to the digester. The cages were designated as Bottom, Mid, and Top and were located, within the reactor, about 2, 4 and 6 m, respectively, above the base. Individual support pieces removed from the digester were found to be associated with a loose layer of biomass which separated from the pieces during transit to the laboratory. A more tightly attached biomass layer remained associated with the matrix pieces during transit. These two biomass fractions were separately quantified and defined as 'loose sludge' and 'biofilm', respectively (Colleran et al. 1994; O'Flaherty et al. 1998b).

Table 2 illustrates the vertical profile of matrix associated biomass at three sampling times during the first five years of operation of the digester. At all sampling dates and at all levels, the amount of loose sludge associated with the matrix pieces was greater than the amount of biofilm. After 2 years of operation, the matrix-associated biomass was found to be uniformly distributed throughout the reactor. However, after 4 and 5 years of operation, the matrix pieces in the Mid and Top sections had significantly greater amounts of associated biomass than in the Bottom section, and than in any of the three sections after two years of operation (Table 2). It was also noted that the quantity of biofilm VSS per matrix piece in the Mid and Top sections in 1995 had significantly decreased relative to 1994 values, although the total matrix-associated biomass in these sections remained much the same as in 1994 (Table 2).

Table 2. Vertical distribution of biofilm and loose sludge associated with matrix pieces during 5 years of operation of the ADM reactor

	gVSS per Matrix Piece		
	Loose sludge	Biofilm	Total VSS
1992 (2 years of operation)			
Top	6.6	3.9	10.5
Middle	8.2	3.8	12.0
Bottom	8.6	3.4	12.0
1994 (4 years of operation)			
Top	20.21	17.67	37.88
Middle	17.46	14.86	32.32
Bottom	3.41	0.19	3.60
1995 (5 years of operation)			
Top	29.20	4.52	33.72
Middle	29.45	4.48	33.93
Bottom	9.22	4.38	13.60

The design of the matrix sampling device did not allow accurate sampling of the liquor VSS content at different heights within the digester. In 1992, a composite liquid sample was obtained from the different levels and shown to have a VSS content of 20 $g.l^{-1}$ (Colleran et al. 1994). This suggested that, at least in 1992, the suspended biomass represented the predominant form of biomass retained within the digester. Calculations by Biotim suggested an overall biomass retention of 8 $kgVSS.m^{-3}$reactor volume in 1992. Analysis of composite sludge samples in 1994 and 1995, and of samples removed from the liquid sampling ports throughout the 5 years of operation confirmed the dominance of suspended and loose matrix-associated sludge in the inventory of retained biomass within the digester. The data presented in Table 2 highlight the decreased contribution of matrix biofilm, after 5 years of operation, to the total biomass retained within the digester.

Specific methanogenic Aactivity of suspended and matrix-associated biomass in the ADM digester

The specific methanogenic activity (SMA) profiles of suspended, loose matrix-associated, and biofilm VSS samples taken from the full-scale digester in 1995 are compared with the SMA profile of the sludge used to seed the digester in 1990 (Table 3). The seed sludge displayed an SMA profile typical of its origin, with high specific activities against the direct substrates, ac-

Table 3. Specific methanogenic activity profiles of sludge and of suspended and matrix-associated biomass sampled from the ADM digester after 5 years of operation

Substrate	Seed sludge	Specific methanogenic activity (ml CH_4(STP).g^{-1}.VSS.d^{-1})		
		1995 suspended sludge	1995 matrix loose sludge	1995 matrix biofilm
Acetate	303.4	144.0	85.3	28.4
Propionate	73.7	0.0	1.2	0.0
Ethanol	482.0	135.0	63.6	77.5
Butyrate	89.2	73.3	120.0	40.9
H_2/CO_2	348.0	13.5	29.0	5.1

Table 4. Specific methanogenic activity profiles of matrix-associated biomass on polypropylene support pieces introduced to the ADM reactor in 1994 and sampled in 1995

Substate	Specific Methanogenic Activity (mlCH_4(STP).gVSS^{-1}.d^{-1})			
	Loose sludge		Biofilm	
	μ	δ	μ	δ
Acetate	203.9	11.5	42.8	5.0
Propionate	5.4	0.6	1.8	0.2
Butyrate	84.0	9.0	41.1	3.8
Ethanol	155.6	11.3	62.0	8.6
H_2/CO_2	45.2	3.3	20.7	2.9

μ = mean; δ = standard deviation; n = 3.

etate and H_2/CO_2, and the indirect substrate, ethanol. The syntrophic activities against propionate and butyrate were lower than against ethanol, but were also typical of a sludge developed on potato-processing wastewater.

After 5 years of operation, the composite suspended sludge sample converted acetate to methane, without any lag phase, at an average rate of 144 ml CH_4.gVSS^{-1}.d^{-1} (Table 3). The loose sludge associated with the matrix pieces and the matrix biofilm also converted acetate to methane without a lag phase, but with lower maximum conversion rates (28.4–85.6 ml CH_4.gVSS^{-1}.d^{-1}). The specific acetoclastic activities (Table 4) of loose sludge and biofilm VSS associated with matrix pieces that had been introduced to the digester during the 1994 sampling period and removed for analysis in 1995 were between 1.5- and 2.4-fold higher than the activities obtained with equivalent loose sludge and biofilm samples associated with matrix pieces that had been present in the digester since commissioning in 1990.

By contrast, the specific hydrogenophilic methanogenic activities of composite sludge and matrix-associated loose sludge and biofilm biomass sampled in 1995 were very low by comparison with the SMA value of the seed sludge against H_2/CO_2 (Table 3). A low SMA value against H_2/CO_2 was also obtained with loose sludge and biofilm samples from support pieces introduced into the digester in 1994 and sampled in 1995 (Table 4).

With respect to syntrophic substrates, relatively high SMA values were obtained for 1995 loose sludge and biofilm samples against ethanol (62.0–155.6 ml CH_4.gVSS^{-1}.d^{-1}) and butyrate (40.9–120.0 ml CH_4.gVSS^{-1}.d^{-1}) (Tables 3 and 4). By contrast, the SMA values obtained against propionate for all sludge samples tested in 1995 (Tables 3 and 4) were negligible and not significantly different from blank values obtained without substrate.

Effect of sulphate and inhibitors on the conversion of direct and indirect methanogenic substrates

Addition of sulphate or molybdate (an inhibitor of sulphate-reducing bacteria) to activity test vials containing 1995 digester biomass samples with acetate as substrate did not result in any enhancement or decrease in the rate of acetate conversion (O'Flaherty et al. 1998b). This suggested that acetoclastic methano-

genesis was responsible for acetate utilisation by the composite, loose sludge and biofilm components of the reactor biomass after 5 years of operation. Addition of sulphate to H_2/CO_2 test vials resulted in immediate utilisation of the substrate (O'Flaherty et al. 1998b). This utilisation was inhibited by molybdate but was completely unaffected by inclusion of BES (an inhibitor of acetoclastic and hydrogenophilic methanogens). Consequently, it can be concluded that SRB were responsible for the majority of H_2 utilisation by the digester biomass.

Addition of sulphate to test vials containing butyrate or ethanol as test substrates did not significantly affect the rate of substrate conversion by ADM digester biomass sampled in 1995 (O'Flaherty et al. 1998b). Rapid degradation rates were obtained in the presence and absence of molybdate, indicating that competition was taking place between sulphidogenic and methanogenic members of the biomass consortia involved in ethanol and butyrate utilisation. Inhibition of the conversion of both substrates to methane was observed when BES was included in test vials (with or without sulphate addition), suggesting that methanogenesis from acetate was a dominant stage in the mineralisation of these compounds by the digester biomass.

By contrast, addition of sulphate to test vials containing propionate as test substrate resulted in greatly enhanced rates of substrate conversion and methane production (Figure 2). This enhanced rate of propionate conversion was inhibited by the simultaneous inclusion of either molybdate or BES in test vials, suggesting that SRB species were catalysing incomplete conversion of propionate to acetate which was subsequently converted by acetoclastic methanogens to methane. The inhibition data obtained confirmed the conclusion from uninhibited activity test vials that an active propionate syntrophic population was not present within the digester after 5 years of operation (Figure 2).

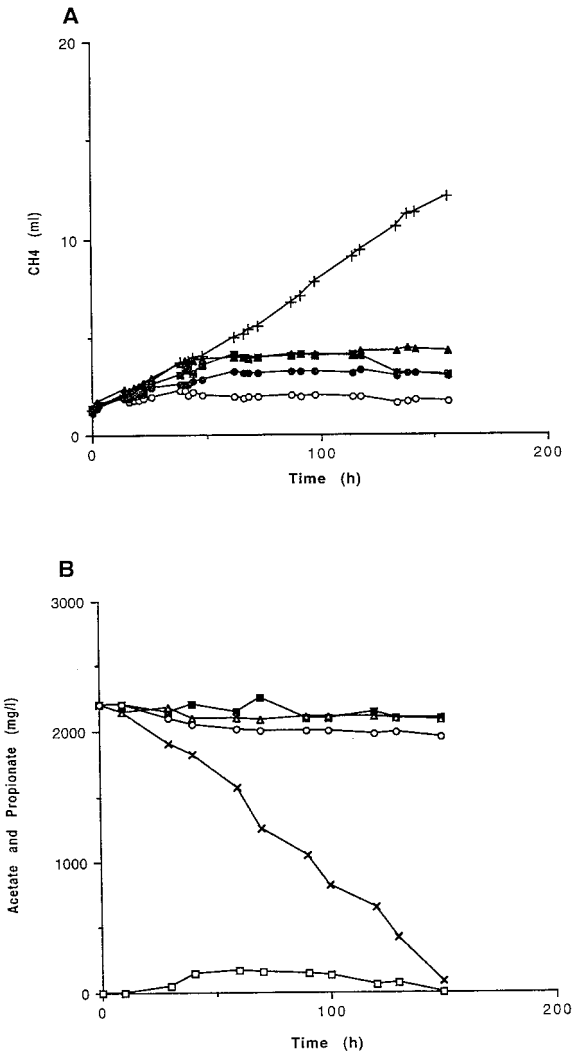

Figure 2. Evaluation of the ability of 1995 composite sludge samples from the full-scale ADM digester to utilise propionate. (A) ml methane produced in activity test vials in the absence of sulphate and inhibitors (–■–) and in the presence of sulphate (-+-), sulphate plus molybdate (–▲–), and sulphate plus BES (–○–). Blank (no propionate) vials (–●–). (B) Propionate degradation in activity test vials in the absence and presence of sulphate and specific inhibitors. Symbols as for A, except for intermediate test vial concentrations of acetate (–□–) in the presence of sulphate.

Thermophilic laboratory-scale treatment of simulated citric acid production wastewaters

Laboratory-scale hybrid reactors were utilised to study the adaptation of mesophilic anaerobic sludge to thermophilic operation in the presence and absence of influent sulphate; to determine the maximum volumetric loading rates applicable, and to evaluate the role of SRB species in the thermophilic conversion of indirect and direct substrates (propionate, butyrate, ethanol, acetate and H_2/CO_2) to methane. Since short-term trials may not adequately assess population changes in the biomass of retained biomass digesters, a very long-term trial of approximately 900 days was carried out.

Two hybrid reactors were seeded with mesophilic sludge that had not been adapted to a high influent sulphate concentration and was similar in terms of specific methanogenic activity profile to the sludge used

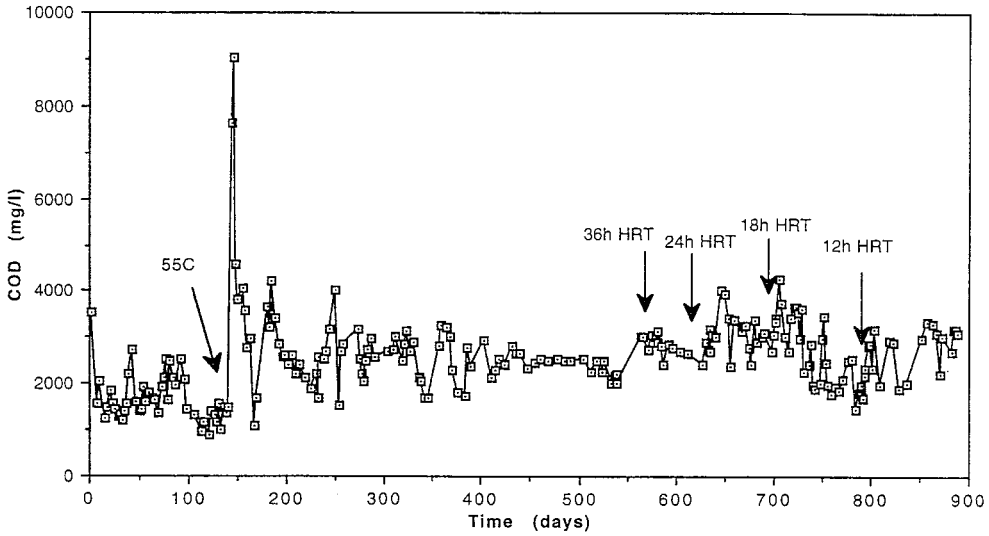

Figure 3. R1 (control) reactor effluent COD concentration throughout the laboratory-scale thermophilic treatment trial of simulated citric acid production wastewater.

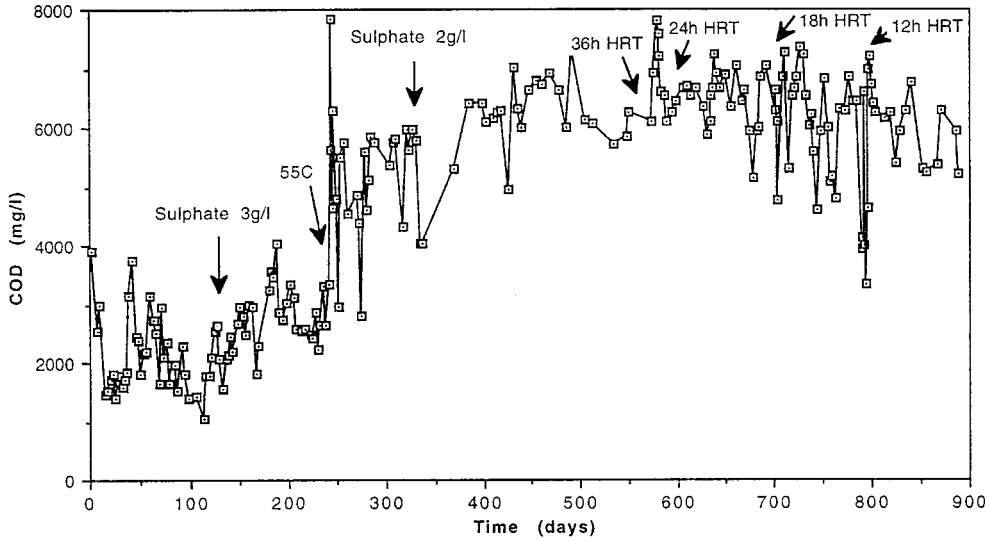

Figure 4. R2 (test) reactor effluent COD concentration throughout the thermophilic treatment trial of simulated citric acid production wastewater.

to seed the full-scale ADM digester. Both reactors were started up mesophically (35 °C) on a two-day hydraulic retention time (HRT) at a volumetric loading rate of 6 kgCOD.m^3.d^{-1}. The feed to both reactors consisted of diluted sugar-beet molasses with a COD concentration of 12 g.l^{-1} and containing a very low sulphate concentration (<200 mg.l^{-1}). Both reactors were operated at this loading rate, influent composition and temperature for 113 days. Figures 3 and 4 illustrate the effluent COD concentration from both reactors during this period. The COD removal efficiency for both reactors averaged approximately 90% during this period, increasing to approximately 95% by day 113. Effluent VFA concentrations decreased to less than 500 mg.l^{-1} during this period (Figures 5 and 6). The percentage methane in the biogas from both reactors ranged between 50 and 60% during this start-up period.

On day 141, the operating temperature of the control reactor (R1) was increased to 55 °C in a single step. This was accompanied by an immediate increase in effluent COD (Figure 3), largely contributed by

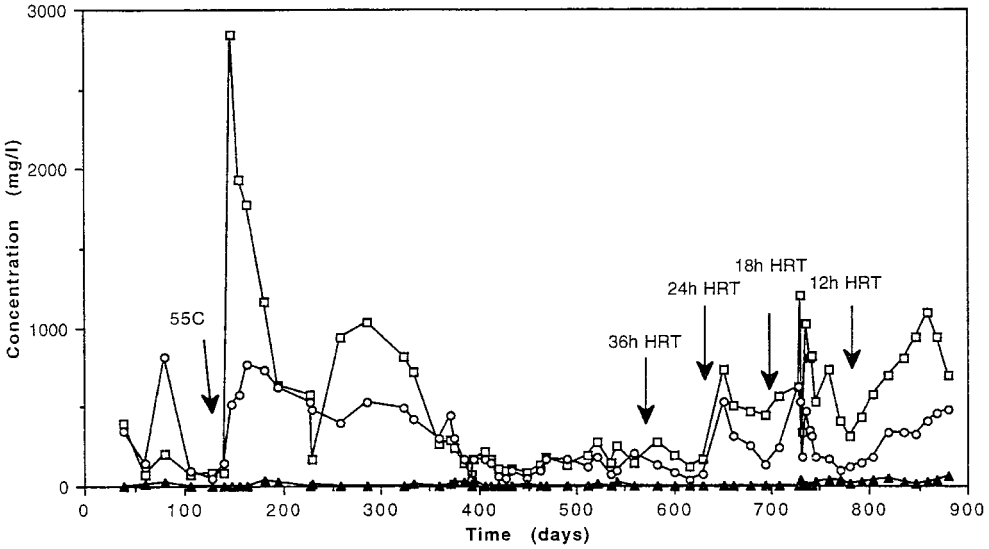

Figure 5. Figure 5: Individual effluent VFA concentrations from the R1 (control) reactor throughout the thermophilic laboratory-scale trial. Acetate (–□–); Propionate (–○–); butyrate (–△–).

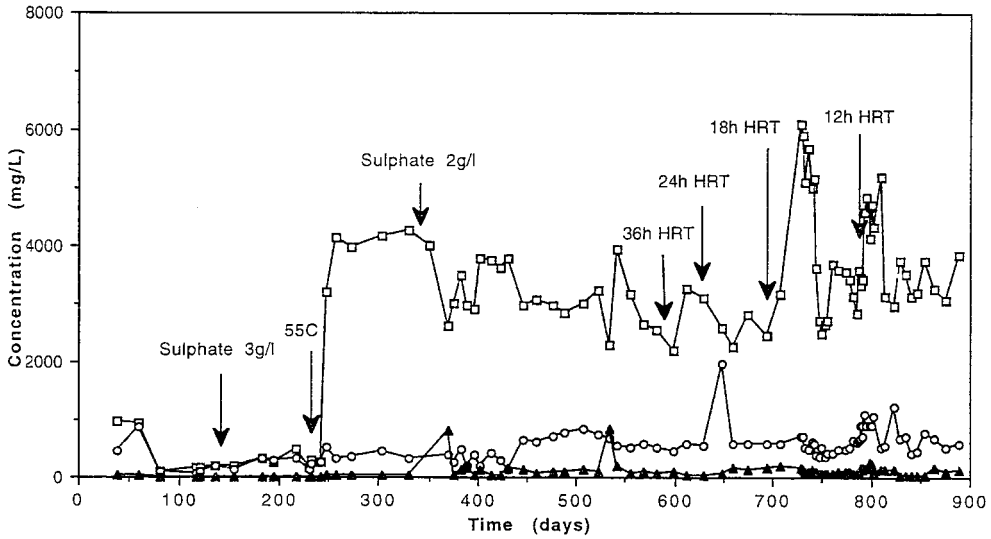

Figure 6. Figure 6: Individual effluent VFA concentrations from the R2 (test) reactor throughout the thermophilic laboratory-scale trial. Acetate (–□–); Propionate (–○–); butyrate (–△–).

an increase in effluent acetate, propionate and butyrate concentrations (Figure 5). Over the subsequent 438 days of operation at unchanging volumetric load and influent COD concentrations, the effluent COD stabilised at approximately 2500 mg.l^{-1} (Figure 3), consisting mainly of acetate and propionate in approximately equal concentrations (Figure 5). Between day 400 and 579, the performance of the R1 reactor was very stable, with marginal changes in the effluent COD and VFA concentrations and with a percentage methane in the biogas of approximately 50%.

Magnesium sulphate (3 g sulphate.l^{-1}) was added to the R2 influent on day 114, without changing the temperature of operation. The amount of sulphate added resulted in an influent COD/sulphate ratio of 4 : 1. The inclusion of sulphate in the reactor feed was accompanied by a decrease in COD removal efficiency to approximately 80%, which was correlated with a relatively small increase in effluent VFA (Fig-

ure 6) and a decrease in the percentage methane of the biogas to an average of 45%. On day 243, the operating temperature of R2 was changed, in a single step, to 55 °C. This was accompanied by a dramatic increase in effluent COD to an average of 5700 mg.l^{-1} (Figure 4) and a reduction in COD removal efficiency to approximately 50%. The methane content of the produced biogas also dramatically decreased to an average of 12–15%. The corresponding increase in effluent VFA was almost completely attributable to acetate, which increased to an average concentration of approximately 4000 mg.l^{-1} (Figure 6). The effluent acetate concentration remained unchanged until day 343 when the influent sulphate concentration to R2 was reduced to 2g.l^{-1}, resulting in a decrease in the influent COD to sulphate ratio to 6 : 1. The effluent acetate concentration subsequently decreased and stabilised at approximately 3000 mg.l^{-1} (Figure 6). The effluent propionate concentration, however, increased from about day 430 onwards, stabilising at 600 to 800 mg.l^{-1} (Figure 6). Reduction of the influent COD/sulphate ratio was not accompanied by any increase in COD removal efficiency which continued to decrease to an average of 45%, corresponding to an effluent COD concentration of approximately 6500 mg.l^{-1} (Figure 4). The decrease in influent sulphate concentration was accompanied, however, by an improvement in the percentage methane of the produced biogas to approximately 25%.

Following a slight improvement in R2 COD removal efficiency between days 550 and 578, the HRT was reduced to 36 h on day 579 for both R1 and R2. The HRT was again reduced to 24, 18 and 12 h on days 634, 707 and 790, respectively. These reductions in HRT corresponded to increases in the volumetric COD loading rate applied to both reactors to 8, 12, 18 and 24 kgCOD.m^{-3}.d^{-1}. The increased loading rate applied to R1, in the absence of influent sulphate, was accommodated with minimal decreases in COD removal efficiency and transient increases in effluent acetate and propionate concentrations (Figure 5). On conclusion of the trial, R1 was still achieving a COD removal efficiency of greater than 75% at the high volumetric loading rate of 24 kgCOD.m^{-3}.d^{-1} (12 h HRT).

Reduction of the HRT from 48 to 12 h, while maintaining the influent COD to sulphate ratio constant at 6 : 1, also resulted in transient increases in effluent COD from R2 (Figure 4). These were correlated primarily with increases in the effluent acetate concentration and with minor increases in effluent propionate levels (Figure 6). On conclusion of the trial, the COD removal efficiency attained by R2 had stabilised at 45%, with effluent acetate and propionate concentrations remaining constant at approximately 3500 and 600 mg.l^{-1}, respectively (Figure 6). Despite the four-fold decrease in HRT and increase in volumetric COD loading rate applied to R2 over the final 312 days of the trial, the performance of the reactor remained essentially unchanged. The methane content of the produced biogas also continued to average 20–25% during the period of loading rate increases.

Discussion and conclusions

The data presented on the operation of the full-scale reactor indicate that it is possible to seed a full-scale, fixed-bed digester with granular sludge developed on a potato-processing wastewater with a low sulphate content. The start-up regime utilised allowed the sludge to gradually adapt to an increasing COD/sulphate concentration in the influent (Derycke et al. 1993). Once the adaptation phase had been completed, the digester continued to treat the citric acid production wastewater with a high degree of process stability and operational efficiency. It was observed by Derycke et al. (1993) that the seed sludge lost its granular character within the first 50 days of operation of the full-scale digester. This was not considered to be important since the polypropylene support material was shown to effectively retain the flocculant sludge that subsequently developed within the digester. Studies of the amount and distribution of the retained biomass within the digester during its first 5 years of operation indicated that the true biofilm component played a minor role in the COD conversion capacity of the reactor. The quantity of interstitial flocculant sludge and loose sludge associated with the matrix pieces greatly exceeded the amount of matrix biofilm, suggesting that the role played by the costly support material used in the fully-packed reactor was to ensure flocculant biomass retention, rather than provide a support surface for development of a biofilm that could play an important role in the COD conversion capacity of the digester. The data obtained suggest that the support material could have been restricted to the upper third or quarter of the digester (i.e. a hybrid reactor design) without any adverse effect on reactor operation. Apart from initial capital cost considerations, the use of a hybrid reactor design would minimise potential subsequent problems of scaling of support matrix pieces,

with consequent reduction of the active volume of the digester.

O'Flaherty et al. (1998b) showed that the highest amount of biofilm VSS linked to support matrix pieces was attached to matrix pieces introduced to the reactor in 1994, and sampled in 1995. A four-fold greater difference in biofilm VSS was observed between support pieces introduced in 1994 and those present in the reactor for the five years prior to sampling in 1995. It was also noted that the SMA values obtained for matrix-associated loose sludge sampled in 1994 and 1995 were significantly higher than for corresponding biofilm samples, irrespective of the year of introduction of the matrix pieces to the digester (O'Flaherty et al. 1998b). It was concluded by these authors that the loose sludge represented the outer, most active layer of the in-reactor biofilm which, being less tightly attached to the support material, became detached during transit to the laboratory. The higher specific activity values observed for biomass in outer biofilm layers by Hoehn and Ray (1973) was attributed by these authors to higher substrate and oxidant concentrations, and lesser problems of mass transfer limitation in the outer than in the inner layers of the biofilm.

The presence of betaine in the citric acid production wastewater was shown by Derycke et al. (1993) to result in an underestimation of the actual COD concentration of the influent wastewater. As a result, the true COD to sulphate ratio of the influent was 5.6 : 1 rather than 4 : 1. Betaine has been reported to be anaerobically biodegradable (Finnegan 1994) and this was confirmed by betaine biodegradability tests (results not shown). The non-detectability of betaine in the influent COD analyses also means that the volumetric COD loading rate applied to the digester was higher than that presented in Table 1.

Specific methanogenic activity (SMA) analysis, in the presence and absence of specific SRB and methanogen inhibitors, highlighted the role played by SRB in the operation of the full-scale digester. The data obtained clearly showed that SRB were responsible for propionate conversion by the digester biomass. The SRB degradation of propionate involved an incomplete conversion to acetate, such as that catalysed by *Desulphobulbus* species. Incomplete SRB oxidation of propionate to acetate has also been reported by many other authors to be characteristic of sulphate-adapted sludges treating a variety of different wastewaters (Mulder 1984: Ueki et al. 1988; Qatibi et al. 1990; Hepner et al. 1992; Zellner & Neudvrfer 1995; Omil et al. 1996). Detailed studies of the syntrophic, methanogenic and SRB populations of the ADM digester sludge showed that propionate-utilising SRB exhibited higher growth rates than the propionate-utilising syntrophic species of non-sulphate adapted sludges (O'Flaherty 1997; O'Flaherty et al. 1998a). The propionate-utilising SRB in the ADM biomass also displayed a significantly higher affinity for sulphate than that exhibited by either butyrate or ethanol-utilising SRB or butyrate or ethanol syntrophs in sulphate and non-sulphate adapted sludges (O'Flaherty 1997; O'Flaherty et al. 1998a). The propionate-utilising SRB in the ADM digester in 1995 also displayed a marginally higher affinity for sulphate than that displayed by the H_2-utilising SRB also shown to be present in the digester (O'Flaherty 1997; O'Flaherty et al. 1998a).

Although competition was shown to be taking place between SRB and syntrophs for butyrate and ethanol in the full-scale sludge, the data obtained suggested that, by contrast with propionate, an active population of butyrate and ethanol syntrophs was present in the full-scale digester biomass after 5 years of operation. Successful competition at mesophilic temperatures between butyrate syntrophs and butyrate-utilising SRB has also been reported by Visser et al. (1993). Overmeire et al. (1994) suggested that the maintenance of butyrate and ethanol syntrophs in retained biomass reactors may be attributed to mass tansfer limitation of substrate or sulphate in the inner layers of biofilms or within sludge granules. The affinity of SRB for reduced substrates follows the order H_2 > propionate > other organic electron donors (Laanbroek et al. 1984), suggesting that butyrate and ethanol syntrophs are more likely to outcompete SRB than are propionate syntrophs under conditions where substrate concentrations are reduced by mass transfer limitations.

SRB were found to have outcompeted hydrogenophilic methanogens after 5 years of digester operation. This finding is in agreement with literature data indicating that, in reactors treating sulphate-containing wastewaters, H_2 oxidation is almost exclusively catalysed by SRB (Rinzema & Lettinga 1988; Visser et al. 1993; Harada et al. 1994; Uberoi & Bhattacharya 1995; Omil et al. 1996). This has been related to the more favourable kinetic parameters for SRB (Widdel, 1988; Oude-Elferink et al. 1994). It may also reflect the fact that hydrogenotrophic SRB have a lower hydrogen threshold concentration than hydrogenotrophic methanogens (Lovley et al. 1982). Consequently, during long-term stable operation of

the full-scale digester, the SRB may have maintained the H_2 concentration at a value which did not sustain hydrogenophilic methanogen maintenance or growth.

By contrast, it was shown that acetate-utilising SRB failed to outcompete acetoclastic methanogens even after 5 years of digester operation. Literature data on the outcome of competition between SRB and MPB for acetate are contradictory, with some authors reporting successful competition by SRB (Rinzema & Lettinga, 1988; Alphenaar et al. 1993; Stucki et al. 1993; Gupta et al. 1994), whereas others report long-term dominance by acetoclastic MPB (Mulder 1984; Rinzema et al. 1986; Iza et al. 1986; Visser et al. 1993; Omil et al. 1996). These discrepancies reflect differences in the prevailing acetate, sulphate and sulphide concentrations in the digestion systems under study and may also be influenced by the digester design utilised. Visser et al. (1993) reported that retained biomass reactors promoted dominance of acetoclastic methanogens over SRB. Omil et al. (1996) reported a selective washout of SRB from UASB reactors operating at upflow velocities of 4 and 6 $m.h^{-1}$. Iza et al. (1986) attributed the dominance of MPB over SRB in fixed-film reactors to the inferior attachment capabilities of acetoclastic SRB. Visser (1995) suggested that literature reports on the predominance of acetoclastic MPB may reflect the short-term nature of many laboratory-scale experiments and the initial low levels of acetate-utilising SRB present in non-sulphate adapted seed sludges. This was supported by Oude-Elferink et al. (1994) who calculated that, starting with a ratio of acetoclastic MPB/SRB of $10^4:1$ and with a biomass retention time in the reactor of 0.02 day^{-1}, it would take one year before the number of SRB equalled that of the MPB in the reactor. However, the results of the present study clearly showed that, even after 5 years of operation of the full-scale ADM digester, acetoclastic MPB continued to successfully outcompete acetoclastic SRB.

The thermophilic laboratory-scale hybrid reactor studies reported in this paper illustrate that mesophilic reactors quickly adopted to thermophilic operation after increasing the temperature of operation in a single step to 55 °C. Thermophilic treatment of a dilute molasses influent was shown to proceed with COD removal efficiencies of 75–80% (Figure 3) at volumetric COD loading rates of 6–24 $kgCOD.m^{-3}.d^{-1}$ (HRT of 2 d to 12 h). By contrast, a one-step increase in operating temperature from 37–55 °C of a reactor treating a sulphate-supplemented dilute molasses feed (COD/sulphate ratio of 4 : 1) was accompanied by a reduction in COD removal efficiency from 80–50% and a decrease in the methane percentage of the biogas to 12–15%. It was also accompanied by a dramatic increase in effluent acetate concentration to an average of 4700 $mg.l^{-1}$ (Figure 6). Reduction of the influent sulphate concentration to 2 $g.l^{-1}$ (COD/sulphate ratio of 6 : 1) resulted in an initial decrease in effluent acetate concentration to c. 3000 $mg.l^{-1}$ (Figure 6) and an initial decrease in effluent COD (Figure 4). The methane content of the produced biogas also initially improved from 12–15% to c. 25%. The test reactor was maintained at an influent sulphate concentration of 2 $g.l^{-1}$ and at the same volumetric loading rate and HRT until day 578. During this period, effluent COD effluent acetate concentrations fluctuated between 5000–7000 $mg.l^{-1}$ and 3000–4000 $mg.l^{-1}$, respectively (Figures 4 and 6). The propionate concentration in the effluent increased from the previous steady-state value of < 200 $mg.l^{-1}$ to 600-800 $mg.l^{-1}$ (Figure 6). Overall, the reduction in influent sulphate concentration did not result in any sustained improvement in the COD removal efficiency of the test reactor.

Step-wise increases in the volumetric loading rate applied to the test reactor, by reduction in the HRT from 48 to 12 h resulted in transient decreases in reactor performance but had no long-term negative impact on the operational efficiency of the reactor with the influent COD and COD: sulphate ratio under test (Figure 4).

Initial SMA analysis of the test reactor biomass on take down highlighted a very low acetoclastic MPB activity and an absence of acetate-utilising SRB. Since the specific methanogenic activity of acetoclastic methanogens was shown to be high in biomass sampled from the control R1 reactor (no influent sulphate supplementation), it may be inferred that the produced sulphide in R2 was inhibitory to the thermophilic acetoclastic methanogens. Studies on H_2 utilisation by R2 biomass at the end of the trial indicated that both hydrogenophilic methanogens and H_2-utilising SRB were present in the reactor, suggesting competition between these groups for produced hydrogen.

Since considerably less is known about the affinities of thermophilic syntrophs, methanogens and SRB for their respective substrates and about their sensitivities to sulphide inhibition than their mesophilic counterparts, ongoing detailed analysis of the thermophilic sludges developed in this study should yield valuable information on the factors determining the

outcome of competition between these various trophic groups at 55 °C.

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