Antonie van Leeuwenhoek **67:** 29–46, 1995.

Anaerobic treatment of sulphate-containing waste streams

Emer Colleran, Siobhain Finnegan & Piet Lens
Department of Microbiology, University College, Galway, Republic of Ireland, Ireland

Accepted 4 October 1994

Key words: anaerobic competition, anaerobic digestion, methanogenesis, sulphidogenesis, sulphate reduction, sulphide toxicity

Abstract

Sulphate-containing wastewaters from the paper and board industry, molasses-based fermentation industries and edible oil refineries present difficulties during anaerobic treatment, leading to problems of toxicity, reduction in methane yield, odour and corrosion. The microbiology and biochemistry of dissimilatory sulphate reduction are reviewed in order to illustrate the potential competition between sulphate reducers and other anaerobes involved in the sequential anaerobic mineralisation process. The theoretical considerations which influence the outcome of competition between sulphate reducers and fermentative, syntrophic, homoacetogenic and methanogenic bacteria are discussed. The actual outcome, under the varying influent organic composition and strength and sulfate concentrations which prevail during digestion of industrial wastewaters, may be quite different to that predicted by thermodynamic or kinetic considerations. The factors governing competitive interactions between SRB and other anaerobes involved in methanogenesis is discussed in the context of literature data on sulphate wastewater treatment and with particular reference to laboratory and full-scale digestion of citric acid production wastewater.

Introduction

Major advances have been made, over the past two decades, in our understanding of the microbiology of anaerobic digestion and in the development of high-rate reactor designs based either on biomass retention or biomass recycle. These advances have largely counteracted early feelings of unreliability associated with the process and have resulted in the widespread adoption, throughout the world, of anaerobic digestion for industrial wastewater treatment. Anaerobic digestion is now generally regarded as an established, but still evolving, technology with a sound theoretical basis for reactor design and operation and with extensive operational experience at full-scale (Iza et al. 1991).

Retained biomass reactors, such as the Upflow Anaerobic Sludge Bed (UASB), the Fixed-Bed/Fixed Film process or the Fluidised/Expanded Bed system, have been mainly used at full-scale for the treatment of wastewaters from the food-processing sector. Current research is focussed on extending the range of anaerobic treatment to more recalcitrant industrial wastewaters which may contain compounds that are either poorly degraded anaerobically or may be toxic to the various bacterial groups involved in the anaerobic digestion process.

Sulphate is a common constituent of many industrial wastewaters. It is the form of sulphur taken up by most bacterial species and its reduction prior to incorporation into biological compounds (cysteine, methionine, co-enzyme A, etc.) is referred to as assimilatory sulphate reduction. Under anaerobic conditions, sulphate can act as an external electron acceptor for a group of bacteria that can couple the oxidation of reduced organic or inorganic compounds to the reduction of sulphate for bioenergetic purposes. This process is known as dissimilatory sulphate reduction and the bacteria involved are known as the sulphate reducers or sulphate-reducing bacteria (Widdel 1988). The difference between the two processes in terms of sulphate transformation is exemplified by the fact that one milligram of sulphate-sulphur supplies the growth requirements for approximately 200 mg of *Klebsiella aerogenes* cells (assimilatory) but only 0.5 to 1.0 mg of

cell mass for dissimilatory *Desulfovibrio* sp. (Rivers-Singleton 1993).

During anaerobic treatment of sulphate-containing industrial wastewaters, two alternative mineralization processes can, consequently, occur – i.e. sulphate reduction or methanogenesis. The outcome of the competition between sulphate-reducing bacteria and the bacterial groups involved in methanogenesis will not only dictate the composition of the biogas produced, but will also determine the feasibility, or otherwise, of methanogenic treatment of the wastewater.

Molasses-based fermentation industries typically generate effluents with both a high COD and high sulphate concentration (Pipyn & de Smedt 1991). Such industries include alcohol distilleries, yeast production plants and factories producing citric acid or monosodium glutamate. The wastewater from a cane molasses alcohol production plant was reported by Carrondo et al. (1983) to have an average COD and sulphate concentration of 50.6 and 2.9 $g.l^{-1}$, respectively. The influent distillery slops treated in the full-scale, down-flow anaerobic filter at the Bacardi Corporation rum distillery in San Juan, Puerto Rico has a COD content of up to 95 $g.l^{-1}$ and a sulphate content of 6 $g.l^{-1}$ (Szendry 1983). Effluent from citric acid production from sugar beet molasses can have a COD content of up to 30 $g.l^{-1}$ and a sulphate content of 2.5 to 4.5 $g.l^{-1}$ (Svardal et al. 1993). Wastewaters from the paper and board industry vary greatly in chemical composition but typically contain sulphate in the range of 1–2 $g.l^{-1}$ (Puhakka et al. 1990). The highest wastewater sulphate concentrations are associated with the industrial production of fatty acids. These so-called 'acid waters' from edible oil refineries can have a sulphate content as high as 40–50 $g.l^{-1}$, with a COD/sulphate ratio of 1 or less (Hoeks et al. 1984; Rinzema et al. 1986). Clearly, the presence of sulphate at such high concentrations may be expected to promote dissimilatory sulphate reduction and to present problems in the application of anaerobic digestion technology to this diverse range of wastewaters.

Problems associated with anaerobic treatment of high sulphate wastewaters

In aerobic environments, sulphate is the most stable and most abundant form of sulphur and is generally regarded as being non-toxic. At very high concentrations in wastewaters (ca. 10 $g.l^{-1}$), salt toxicity effects leading to significant inhibition of methanogenesis may, however, be encountered (Rinzema et al. 1986). By contrast, sulphide, which is the most energetically stable form of sulphur under anaerobic conditions, is highly reactive, corrosive and toxic to microorganisms, plants, animals and man (Widdel 1988). Consequently, the problems associated with anaerobic treatment of high-sulphate wastewaters are primarily linked to the production of sulphide by dissimilatory sulphate-reducing bacteria.

The most obvious effect on methanogenesis is a reduction of the methane yield per unit COD converted. Oxidation of 2 g COD is required for the reduction of 1 g SO_4^{2-} – S to 1 g S^2 – S (Kühl & Jorgenson 1992). In terms of sulphate, the reduction of 1.5 g SO_4^{2-} will require oxidation of 1 g COD, resulting in a decrease of 0.233 m^3 in the methane (STP) yield for every kg SO_4^{2-} reduced during anaerobic treatment (Anderson et al. 1982).

Hydrogen sulphide dissociates in water according to the following equations (Garrels & Christ 1965):

$$H_2S \rightleftharpoons H^+ + HS^- (K_1 = 1.0 \times 10^{-7})$$
$$HS^- \rightleftharpoons H^+ + S^{2-} (K_2 = 1.0 \times 10^{-14})$$

Toxicity of sulphide is pH dependent since only the unionised hydrogen sulphide form is able to pass through the cell membrane (Speece 1983). Above pH 8–9, virtually all dissolved sulphide is present in the ionised form. At the neutral pH values typical of methanogenic systems, approximately 20–50% of the dissolved sulphide is present in the undissociated H_2S form.

There is considerable discrepancy in the literature with respect to the levels of sulphide required for inhibition of methanogenesis. Which step of the methanogenic conversion process is most adversely affected by sulphide is still not clear, although Lawrence et al. (1966) showed that methane production ceased first, followed by a build-up of volatile fatty acids. This is in agreement with recent studies by Maillacheruvu et al. (1993) which showed that fermentative bacteria are far less prone to sulphide toxicity than either sulphate-reducing or methanogenic bacteria.

The sulphide levels reported in the literature as being inhibitory to methane formation may be summarised as being in the range 100–800 $mg.l^{-1}$ dissolved sulphide or approximately 50–400 $mg.l^{-1}$ undissociated H_2S (Parkin et al. 1990). For example, Speece & Parkin (1983) found that methane production from an unacclimated batch digester was inhibited by a sulphide level as low as 50 mg S^{2-} – $S.l^{-1}$ (1.6 mM).

By contrast, Kroiss & Plahl-Wabnegg (1983) reported that an unionized H_2S level of 50 mg.l^{-1} inhibited acetoclastic methanogenesis by about 50%, with complete inhibition only occurring at a free H_2S level of ca. 200 mg.l^{-1}.

The literature on sulphide toxicity is highly complex. Information on pH is rarely included, making comparison on the basis of unionized H_2S levels impossible. In addition, the data presented has been obtained with chemostat completely stirred Jowe Reactors (CSTR) and retained biomass systems. Recent studies by Maillacheruvu et al. (1993) clearly show that sulphide toxicity is mediated at lower concentrations in suspended growth systems than in anaerobic filters. Levels of 60–75 mg H_2S-S.l^{-1} (150–200 mg dissolved sulphide.l^{-1}) were found to cause stress in all suspended growth systems tested. By contrast, propionate-fed filters could withstand 200 mg H_2S-S.l^{-1} and dissolved sulphide (DS) levels of 1000 mg S.l^{-1}. With acetate-fed filters, hydrogen sulphide levels in excess of 125 mg S.l^{-1} caused no inhibition and DS levels of 400 mg S.l^{-1} could be tolerated with no adverse effect (Maillacheruvu et al. 1993). These data confirm the earlier findings of Speece & Parkin (1983) that sulphide levels up to 400 mg of S^{2-}-S.l^{-1} had no effect on methane production from a submerged anaerobic filter and higher levels of 800 mg S^{2-}-S.l^{-1} only reduced methane formation by about 30%. It is apparent from these results that biofilm or granular/flocculent sludge reactors present a much more complex system than completely mixed reactors in the context of sulphide toxicity. Clearly, factors, such as substrate transport inside the biofilm/granule/floc; the site of sulphate reduction and its proximity to the site of methanogenesis; the diffusion of unionized H_2S and dissolved sulphide; pH gradients etc., can have quite significant effects on the toxicity of sulphide generated within a reactor to the populations involved in methanogenesis.

Literature data on the sensitivity of sulphate-reducing bacteria to sulphide toxicity is quite contradictory. Isa et al. (1986a) concluded that sulphate-reducing bacteria were not affected by high levels of hydrogen sulphide. By contrast, Widdel (1988) reported inhibition of *Desulfotomaculum acetooxidans* at hydrogen sulphide concentrations of 85 mg.l^{-1}. Using a *Desulfovibrio* enrichment, Reis et al. (1992) reported complete inhibition of lactate conversion at a hydrogen sulphide concentration of 547 mg.l^{-1} (16.1 mM). Hilton & Oleszkiewicz (1988) concluded that sulphate-reducing bacteria are more sensitive to elevated levels of dissolved total sulphide than are methane-producing bacteria. Since sulphate-reducing bacteria exhibit an absolute requirement for iron, the toxicity of sulphide was initially assumed to be indirect and to result from iron unavailability due to the formation of insoluble FeS (Postgate 1984; Isa et al. 1986b). However, at least for *Desulfovibrio* sp. growing on lactate, Reis et al. (1992) showed that H_2S toxicity was not linked to iron sequestration and that it was both direct and reversible.

Precipitation of non-alkaline trace metals, such as cobalt, nickel, etc., that are essential nutrients for methanogens, may result in an indirect inhibition of methanogenesis of some industrial wastewaters (Isa et al. 1986b; Maillacheruvu et al. 1993). This, however, is unlikely to be a major problem since it can be easily overcome by nutrient supplementation.

Other problems associated with anaerobic treatment of high sulphate wastewaters result from the presence of sulphide in the biogas and in the effluent. Hydrogen sulphide, even at concentrations of ≤ 2 ppm, is recognisable by its distinctive smell and may cause significant malodour problems. Although burning of H^2S-containing biogas is feasible and removes the malodour problem, the resultant release of SO_2- to the atmosphere results in acid rain and is subject to strict licencing requirements. The presence of H_2S in biogas may also cause severe problems of corrosion, necessitating costly sulphide stripping techniques. The presence of dissolved sulphide in the effluent after anaerobic treatment also gives rise to malodour problems and to enhanced oxygen demand. Post-treatment of the effluent may be necessary, depending on the sulphide concentration, and is generally accomplished either by chemical precipitation with iron salts or biological or chemical oxidation (Rinzema & Lettinga 1986; Rinzema 1988).

The presence of sulphate can also have beneficial effects during anaerobic treatment of wastewaters. The majority of methanogens lack assimilatory sulphate reductases (Daniels et al. 1986) and their sulphur requirements are satisfied by a combination of inorganic sulphide and organic sulphur compounds, such as cysteine, glutathione, etc. Consequently, the production of sulphide by dissimilatory species during anaerobic treatment may enhance methanogenesis by satisfying the sulphur growth requirements of the methanogens. The optimal level of sulphide supplementation to methanogen culture media recommended by the literature is in the range 1–25 mg S.l^{-1} (Scherer & Sahm 1981). The requirement for low levels

of sulphide-S has also been proven for methanogenic reactors. For example, Khan & Trottier (1978) showed that the addition of sulphate at concentrations up to 25 mg sulphate $S.l^{-1}$ (0.8 mM) stimulated methanogenesis of cellulose in tissue paper pulp. Replacement of sulphate by sulphide at the same sulphur concentration was shown by these authors to give equal stimulation of methanogenesis.

Sulphide produced during digestion by dissimilatory sulphate reduction can also have beneficial effects due to the precipitation of toxic heavy metals, such as chromium, copper, zinc, etc. (Lawrence & McCarty 1965; Tursman & Cork 1989).

Microbiology and biochemistry of dissimilatory sulphate reduction

Knowledge of the complex microbiology and biochemistry of dissimilatory sulphate reduction is essential in order to understand the factors which control competition between methanogenesis and sulphidogenesis in digesters treating sulphate-containing wastewaters.

Sulphate-reducing bacteria may be defined as obligate anaerobes (eubacteria and archaebacteria) that utilise sulphate (or other oxidised sulphur compounds) as an electron acceptor during the dissimilation of organic compounds for energy gain (Pfennig et al. 1981; Gibson 1990). Although unified by the common property of carrying out sulphate reduction as a principal component of their bioenergetic processes, recent research has highlighted the enormous morphological, ecological, nutritional and metabolic diversity found among this bacterial group (Hansen 1993). Campbell & Postgate (1965) first proposed that sulphate-reducing bacteria be organised into two major genera, rod-shaped, sporeforming species with a low G + C content (*Desulfotomaculum*) and non-sporeforming vibrios and spirillae with a high G + C content (*Desulfovibrio*). More recent classification of sulphate-reducing genera recognises 13 distinct eubacterial genera and one archaebacterial genus (Widdel & Hansen 1991).

Based on their metabolic capacities, sulphate-reducing bacteria appear to fall naturally into two categories – those species or genera that are capable of complete oxidation of organic compounds to CO_2 and those that carry out incomplete oxidation, usually to acetate as end-product (Widdel 1988). The inability of the latter group to oxidise substrates completely to CO_2 usually reflects the absence of a biochemical pathway for the oxidation of acetyl CoA to CO_2 (Hansen 1993). Incomplete degradation of a particular substrate does not, in all cases, imply degradation to the level of acetate. For example, some *Desulfovibrio* strains do not oxidise propanol beyond the propionate level because they lack the metabolic pathway for propionate oxidation to acetate (Hansen 1993).

Genera capable of complete oxidation include *Desulfobacter, Desulfobacterium, Desulfococcus, Desulfosarcina, Desulfomonile, Desulfonema, Desulfoarculus* and *Archaeoglobus*. Incomplete oxidisers include *Desulfomicrobium, Desulfobulbus, Desulfobotulus, Thermodesulfobacterium* and the majority of the species of the genera *Desulfovibrio* and *Desulfotomaculum* (Widdel & Hansen 1991).

Studies over the past 15–20 years have demonstrated the enormous diversity of energy substrates used by sulphate-reducing bacteria. Prior to 1977, only a limited number of low molecular weight metabolic intermediates were thought to act as substrates for sulphate reducers. These included H_2, formate, lactate, pyruvate, dicarboxylic acids, such as malate and fumarate, some primary alcohols, alanine and glycerol. Although sulphate reducers do not appear to be able to hydrolyse polymeric substrates, recent research has indicated that their substrate range is extremely diverse and includes representatives of virtually all classes of monomeric compounds (Hansen 1993). Table 1 summarises the range of energy substrates now known to be metabolised by sulphate reducers.

In general, strains with novel substrate oxidation abilities have been isolated more readily from marine and estuarine environments than from freshwater habitats. This may be a logical consequence of the fact that freshwater environments are often sulphate-limited whereas the higher levels of SO_4^{2-} in marine environments may have facilitated the evolution of a wider range of species in response to the greater role of sulphate reducers in marine mineralization. Table 1 includes straight chain alkanes as energy substrates for sulphate reducers. The isolation of a sulphate reducer capable of complete oxidation of C_{12} to C_{18} alkanes by Aeckersberg et al. (1991) marks the first recorded evidence for complete mineralisation of saturated aliphatic hydrocarbons under strict anaerobic conditions.

Growth of sulphate reducers on acetate

Given the importance of acetate as an intermediate in methanogenesis, the ability of sulphate reducers to

Table 1. Energy substrates for sulphate-reducing bacteria*.

Compound class	Individual compounds used
Aliphatic monocarboxylic acids	Formate, acetate, propionate, butyrate, isobutyrate; 2 methyl butyrate, 3 methylbutyrate, 3 methylvalerate, fatty acids up to C_{20}, pyruvate, lactate.
Dicarboxylic acids	Succinate, fumarate, malate, oxalate, maleinate, glutarate, pimelate
Alcohols	Methanol, ethanol, propanol-1 and 2, butanol-1 and 2, isobutanol, pentanol-1, ethylene glycol, 1-2 propanediol, 1-3 propanediol, glycerol
Amino acids	Glycine, serine, alanine, cysteine, cystine, threonine, valine, leucine, isoleucine, aspartate, glutamate, phenylalanine
Sugars	Fructose, glucose, mannose, xylose, rhamnose
Aromatic compounds	> 35 known aromatics, including benzoate, phenol, indole, resorcinol, catechol, p-cresol, quinoline, nicotinic acid, phenylacetate, vanillin, syringaldehyde, trimethoxybenzoate, etc.
Miscellaneous	Very varied group including betaine, choline, furfural, acetone, cyclohexanone, etc.
Inorganic compounds	H_2CO_2

* Hansen (1993).

couple acetate oxidation to sulphate reduction is of particular interest. Organisms capable of significant growth rates on acetate belong to the genera *Desulfobacter* and *Desulfotomaculum*. Completely oxidising sulphate reducers, such as *Desulfovibrio baarsi* and members of the *Desulfococcus, Desulfosarcina, Desulfobacterium* and *Desulfonema* genera, oxidise acetate much more slowly and sometimes without any substantial formation of cell mass (Widdel 1988).

The best known acetate-utilisers belong to the genus *Desulfobacter*, which is one of the least nutritionally versatile of the sulphate-reducing genera. The majority of *Desulfobacter* species can only grow on acetate, although some may also use H_2 and/or ethanol (Widdel 1988; Gibson 1990). *Desulfobacter* species were initially enriched from brackish waters or marine sediments and their growth rates are accelerated in saline media. This group oxidises acetate via a variation of the citric acid or TCA cycle. As shown in Fig. 1 for *Desulfobacter postgatei*, acetate activation proceeds via transfer of the co-enzyme A group from succinyl CoA (Hansen 1993). The cycle differs from that found in aerobic bacteria by having a ferredoxin-dependent α-ketoglutarate dehydrogenase, a membrane-bound NAD-independent malate synthase and an ATP-citrate lyase instead of a citrate synthase (Möller et al. 1987). The cycle allows formation of ATP by substrate level phosphorylation (Fig. 1).

Desulfotomaculum acetooxidans is a freshwater acetate-utiliser that grows more slowly on acetate (doubling time of 30 hr) than the *Desulfobacter* species. This organism does not possess a citric acid cycle, as evidenced by the absence of the key enzyme, α-ketoglutarate dehydrogenase (Schauder et al. 1986). Instead, it shares with a number of other complete oxidisers (*Desulfococcus* species; *Desulfovibrio baarsi, Desulfobacterium* sp. etc.) a non-cyclic pathway involving cleavage of the two-carbon unit into a methyl and carbon monoxide moiety, each of which is oxidised independently to CO_2 (Fig. 2). Acetate activation in *Dtm. acetooxidans* requires expenditure of ATP (unlike in *Desulfobacter* spp.) and the non-cyclic pathway does not allow substrate-level-phosphorylation. Although most reducing equivalents in *Dtm. acetooxidans* are generated at a more favourable redox potential than in *Desulfobacter*, thereby possibly allowing greater synthesis of ATP by electron transport-associated phosphorylation (Thauer 1988), the above disadvantages may explain the slower growth rate of *Dtm. acetooxidans* than *Desulfobacter* on acetate (Hansen 1993).

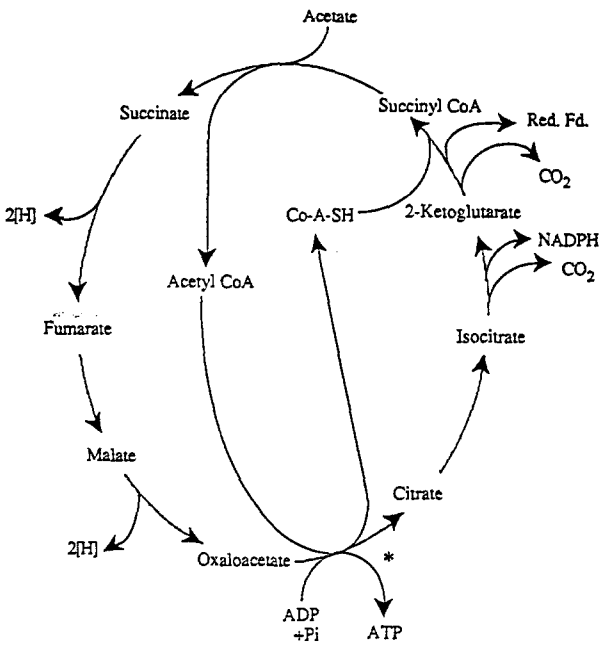

Fig. 1. Pathway of acetate oxidation via the citric acid cycle in *Desulfobacter postgatei* (Hansen 1993).

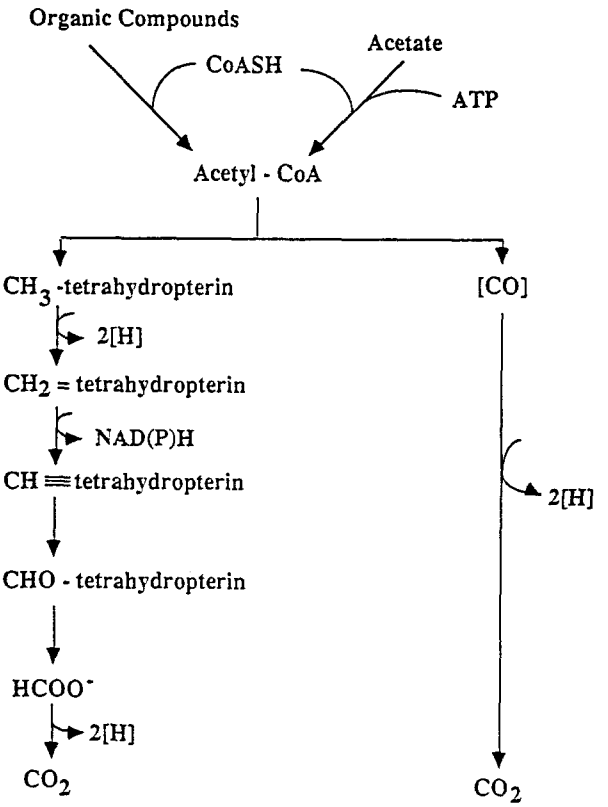

Fig. 2. Non-cyclic carbon monoxide dehydrogenase pathway for oxidation of acetyl groups by *Desulfotomaculum acetooxidans* and by complete oxidizers growing on higher carbon compounds (Thauer 1988; Hansen 1993).

It is interesting to note that the mechanism used for acetate degradation in these species represents an inversion of the pathway for acetate synthesis in homoacetogenic bacteria. An interesting parallel is also seen between the non-cyclic acetate oxidising pathway used by eubacteria, such as *Desulfotomaculum*, and the pathway used by the archaebacterial complete oxidiser, *Archaeoglobus fulgidus*. During growth on lactate or pyruvate, the latter species uses an analogous branched, non-cyclic pathway involving the oxidation of carbon monoxide and methyl moieties. However, in the archaebacterial species, the folic acid carriers used by eubacteria for methyl and other C-1 compound carriage are replaced by typical archaebacterial carriers, such as methanopterin and methanofuran, and coenzyme F_{420} is used instead of NAD(P) as redox carrier (Möller-Zinkhan & Thauer 1990).

Growth of sulphate reducers on propionate

Propionate is, after acetate, probably the most important fermentation end-product in many natural ecosystems (Cummings 1981; Gibson 1990) and is also a key intermediate in anaerobic digesters. All *Desulfobulbus* species can grow on propionate as sole carbon and energy source and oxidise it incompletely to acetate and CO_2 (Widdel 1988; Gibson 1990). Several of the completely oxidising bacteria (e.g. *Desulfococcus, Desulfonema, Desulfobacterium* etc.) also utilise propionate, converting it completely to CO_2, but the majority of these species grow rather slowly with propionate as sole energy source (Hansen 1993). *Desulfobulbus* species oxidise propionate via a randomising pathway (Fig. 3) involving transcarboxylation of propionyl-CoA (using oxaloacetate as donor) to methylmalonyl CoA followed by isomerization to succinyl CoA (Stams et al. 1984; Hansen 1993). The pathway is energetically quite favourable as shown in the following equation:

$$4CH_3CH_2COO^- + 3SO_4^{2-} \rightarrow$$
$$4CH_3COO^- + 4HCO_3^- + 3HS^- + H^+$$
$$\Delta G^{01} = -150.6kJ$$

Nearly all *Desulfobulbus* isolates can also utilise ethanol, propanol or H_2 and a few strains slowly oxidise butyrate or 2-methylbutyrate to acetate (Widdel 1988).

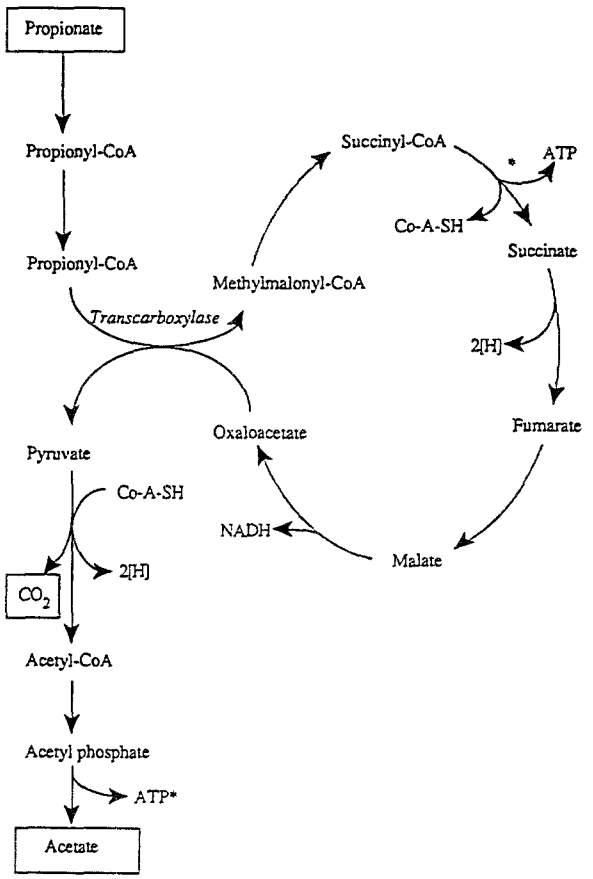

Fig. 3. Pathway for the incomplete oxidation of propionate by *Desulfobulbus propionicus* (Stams et al. 1984; Hansen 1993). * Substrate level phosphorylation.

Growth of sulphate reducers on butyrate, higher fatty acids and alcohols

Both butyrate and ethanol can act as significant fermentation intermediates during anaerobic digestion. Butyrate and higher fatty acids are utilised by species of both the incompletely and completely oxidising sulphate reducers. Even-numbered fatty acids are converted by incomplete degraders to acetate whereas uneven-numbered fatty acids yield acetate and propionate (Widdel 1988). Complete oxidisers, such as *Desulfosarcina, Desulfonema, Desulfobacterium* sp. etc., can utilise fatty acids up to chain lengths of C 10–16, yielding CO_2 as sole product. Both complete and incomplete oxidisers utilise the β-oxidation pathway. Isobutyrate utilisation has been reported only for some complete oxidisers – e.g. marine species such as *Desulfonema limicola* (Widdel 1988). Isobutyrate is converted to acetyl CoA and CO_2 via a pathway similar to that found in *Desulfobulbus* (Stieb & Schink 1989; Hansen 1993).

The ability to utilise ethanol is very common among incompletely and completely oxidising sulphate reducers. Utilisation of longer chain primary alcohols for energy gain by incomplete oxidisers generally yields the corresponding acid and requires provision of a carbon source, such as acetate or acetate plus CO_2, in the medium (Hansen 1993). Only a few strains of sulphate-reducing bacteria, such as *Desulfococcus multivorans*, appear to utilise secondary alcohols such as 2-propanol and 2-butanol (Widdel 1988).

Growth of sulphate-reducers on H_2 and formate

A large number of incompletely and completely oxidising sulphate reducers can grow on H_2 as sole energy source (Widdel 1988). This facility is particularly prevalent among *Desulfovibrio* sp. which are often enriched from natural environments and digesters on H_2. Thermodynamically, growth on H_2 is more favourable than growth on acetate or other reduced 2, 3 or 4 C-compounds (Thauer et al. 1977).

$$4H_2 + SO_4^{2-} + H^+ \rightarrow 4H_2O + HS^-$$
$$\Delta G^{01} = -152.2kJ$$

Growth of incomplete oxidisers, such as *Desulfovibrio* sp., is usually much more rapid on H_2 than is growth of complete oxidisers, such as *Desulfosarcina variabilis, Desulfonema limicola, Desulfococcus niacini* and *Desulfobacterium autotrophicum* (Widdel 1988). Although all of the H_2 utilising species were initially assumed to be capable of autotrophic growth, it was subsequently ascertained that only the complete oxidisers are capable of true autotrophic growth on H_2/CO_2 (Mechelas & Rittenberg 1960; Widdel 1988). Incompletely oxidising *Desulfovibrio* sp. require, in addition to CO_2, at least a 2-carbon compound, such as acetate, for assimilation and cell synthesis. When acetate is provided, labelling studies show that about two thirds of the cell material is derived from acetate with the remaining third coming from CO_2 (Badziong et al. 1979). This stoichiometry is suggestive of a reductive carboxylation of acetyl-CoA to pyruvate as the first step in assimilatory cellular synthesis in *Desulfovibrio* species (Badziong et al. 1979). Complete oxidisers, on the other hand, already possess the enzymes of one or other of the two dissimilatory acetyl-CoA oxidation pathways (Widdel 1988). Presumably, during autotrophic growth on H_2/CO_2, these pathways are reversed to allow assimilatory formation of acetyl-CoA

which is then used, as described above, as the starting point for cellular synthesis (Jansen et al. 1984).

Many sulphate reducers capable of growing on hydrogen can also grow on formate as sole electron and energy source. This is not a universal rule, however, since some species which utilise H_2 cannot grow on formate (e.g. *Desulfobulbus propionicus*) and some formate-utilisers, such as *Desulfovibrio baarsii*, cannot grow on H_2 (Widdel 1988).

Electron acceptors utilised by sulphate reducers

Although sulphate reducers utilise sulphate as their usual electron acceptor, reducing it in the process to sulphide, the majority of species can also utilise sulphite or thiosulphate if added as electron acceptors to culture media (Widdel & Pfennig 1984; Cypionka et al. 1985). Cell yields during growth on sulphite or thiosulphate are higher than on sulphate since activation at the expense of ATP is not necessary as is the case with respect to sulphate (Widdel 1988). The inability of some species to utilise sulphite or thiosulphate, e.g. *Desulfonema magnum, Desulfovibrio sapovorans* etc., appears to be correlated with the lack of an appropriate transport system for these sulphur compounds (Cypionka et al. 1985).

Desulfovibrio desulfuricans, a limited number of other *Desulfovibrio* strains, *Desulfobulbus propionicus* and *Desulfobacterium catecholicum* can also utilise nitrate as terminal electron acceptor, reducing it to ammonia rather than to dinitrogen as in true denitrifying bacteria (McCready et al. 1983; Szewzyk & Pfennig 1987; Widdel 1988). *Desulfobacterium catecholicum* is the only complete oxidiser known so far to be capable of dissimilatory nitrate as well as sulphate reduction (Szewzyk & Pfennig 1987).

Fermentative capabilities of sulphate reducers

In the absence of sulphate or any other feasible inorganic electron acceptor, some sulphate-reducing bacteria can oxidise a limited range of organic substrates using typical fermentative reactions that are independent of exogenous electron acceptors (Hansen 1993). The ability of these species to grow fermentatively is obviously of ecological advantage in ecosystems, such as freshwater sediments or anaerobic wastewater treatment plants, where sulphate limitation may be encountered.

Sulphate reducers capable of fermentative growth may be subdivided into two types: (i) those that ferment organic compounds irrespective of the prevailing partial pressure of hydrogen and (ii) those that can only carry out fermentation when the H_2 partial pressure is maintained at a very low level (Hansen 1993). Malate and fumarate are fermented by a number of *Desulfovibrio, Desulfobacterium* and *Desulfosarcina* sp., irrespective of the H_2 partial pressure, yielding acetate, CO_2 and succinate as fermentation end-products (Widdel & Pfennig 1984; Widdel 1988). Other substrates fermented independent of the H_2 partial pressure include pyruvate, lactate or ethanol plus CO_2, choline, glycerol, serine and fructose (Widdel 1988; Hansen 1993). Some species, such as *Desulfobulbus propionicus*, ferment lactate to propionate, acetate and CO_2 (Laanbroek et al. 1982). Propionate is formed via the same randomising pathway as used by *Propionibacterium* sp. (Stams et al. 1984). Consequently, depending on whether sulphate is present or not, *Desulfobulbus propionicus* can either oxidise propionate via dissimilatory sulphate reduction or form propionate during fermentation of lactate or alcohols such as ethanol and propanol (Laanbroek et al. 1982).

Some *Desulfovibrio* species and a limited number of other sulphate reducers (e.g. *Desulfotomaculum nigrificans*) ferment substrates, such as ethanol, lactate, glycerol, propionate, 1,2-propanediol and 1,3-propandiol, only if the H_2 partial pressure is maintained at a low level by hydrogenophilic methanogens (Widdel 1988; Hansen 1993). As shown in the following equations, sulphate reducers growing under these conditions use protons as electron acceptors, using H_2 as their electron sink.

$$Lactate + 2H_2O \rightarrow acetate + HCO_3^- + H^+ + 2H_2$$

$$Ethanol + H_2O \rightarrow acetate + H^+ + 2H_2$$

Consequently, these organisms function in a similar way to the obligate hydrogen-producing acetogenic (OHPA) bacteria and can only be cultivated in co-culture with hydrogen-consuming methanogens (McInerney & Bryant 1981; Traore et al. 1983). This syntrophic association provides yet another example of interspecies hydrogen transfer under anaerobic conditions.

The importance of syntrophic associations between fermenting sulphate reducers and hydrogenophilic methanogens in anaerobic digesters treating wastewaters containing low sulphate levels has only recently begun to be addressed. Heppner et al. (1992) fed propionate as sole substrate to a laboratory-scale fluidised-bed reactor which had been seeded with an

enrichment capable of complete propionate oxidation (Wollersheim et al. 1989). Analysis of sand particle biofilm after 200 days of operation revealed the presence of large numbers of bulb-shaped *Desulfovibrio* sp. Subsequent investigations confirmed the presence of *Desulfovibrio propionicus* which was shown to be growing syntrophically on propionate with a range of H_2-utilising methanogens and the acetate utiliser, *Methanothrix soehngenii* strain HI (Heppner et al. 1992). These findings were consistent with the following reactions for propionate conversion by the biofilm bacteria:

$$CH_3CH_2COO^- + 2H_2O \rightarrow CH_3COO^- + CO_2 + 3H_2 \quad (i)$$
$$\Delta G^{\circ'} = +76.1kJ; \Delta G' = -8.4kJ$$

$$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^- \quad (ii)$$
$$\Delta G^{\circ'} = -35.8kJ; \Delta G' = -22.7kJ$$

$$3H_2 + 0.75CO_2 \rightarrow 0.75CH_4 + 1.5H_2O \quad (iii)$$
$$\Delta G^{\circ'} = -101.7kJ; \Delta G' = -12.6kJ$$

where $\Delta G^{\circ'}$ is the Gibbs' standard free energy change at pH 7.0 and $\Delta G'$ is Gibbs' free energy change at *in situ* concentrations and pH 7.0.

Detailed analyses were carried out by Wu et al. (1991) on the metabolic activity and microbial composition of granular sludge from a UASB reactor treating brewery wastewater. These studies revealed that propionate-degrading sulphate reducers present in the granule were capable of syntrophic fermentation of propionate with H_2 and/or formate-consuming methanogens in the absence of sulphate whereas, in the presence of sulphate, propionate oxidation was exclusively coupled to sulphate reduction. Molybdate inhibition studies also showed that sulphate reducers capable of fermentative growth on ethanol were involved, although not exclusively, in the syntrophic oxidation of ethanol in the absence of sulphate.

To date, there is no evidence from pure culture or enrichment studies that sulphate reducers can ferment fatty acids or aromatic compounds in the absence of sulphate. Consequently, it is unlikely that fermentative sulphate reducers can substitute for the OHPA bacteria known to be capable of syntrophic growth on fatty acids or aromatics (Hansen 1993).

Competition between sulphate reducers and other bacteria involved in anaerobic mineralisation

In aerobic environments, individual heterotrophic microorganisms are capable of carrying out complete mineralisation of organic compounds to CO_2 and H_2O. By contrast, anaerobic ecosystem mineralisation requires the sequential, cooperative and syntrophic involvement of different groups of bacteria with widely different substrate ranges and thermodynamic and kinetic characteristics (Vosjan 1982; Gibson 1990). Under anaerobic conditions, organic compounds are partially oxidised in a successive manner, with the end-products of each oxidation stage acting as substrates for the next member of the food chain until complete mineralisation is achieved. The route taken depends primarily on the nature of the electron acceptors available and on the partial pressure of hydrogen prevailing in the ecosystem (Widdel 1988; Gibson 1990; Zinder 1993).

Figure 4 illustrates the possible anaerobic pathways of organic compound degradation under methanogenic and sulphidogenic conditions. In the absence of sulphate (or other oxidised inorganic electron acceptors, such as nitrate, Fe^{3+}, etc.), organic compounds are mineralised to CH_4 and CO_2 by a bacterial consortium involving fermentative species, obligate hydrogen-producing acetogens (OHPA), hydrogenophilic and acetoclastic methanogens and, to an unknown extent, homoacetogenic bacteria (Zehnder et al. 1982; Zinder 1993). In the presence of sulphate, competition between sulphate reducers and the anaerobic bacteria involved in methanogenesis can occur at a number of different levels in the stepwise degradation process:

- competition between sulphate reducers and fermentative bacteria for monomeric starting compounds, such as sugars, amino acids, etc.,
- competition between sulphate reducers and OHPA species for intermediate fermentation products, such as propionate, butyrate, ethanol, etc.,
- competition between sulphate reducers and homoacetogenic bacteria for H_2, and
- competition between sulphate reducers and methanogens for direct methanogenic substrates, such as H_2 and acetate.

Competition between sulphate reducers and fermentative bacteria

It is generally agreed that sulphate reducers do not effectively compete against the fast-growing fer-

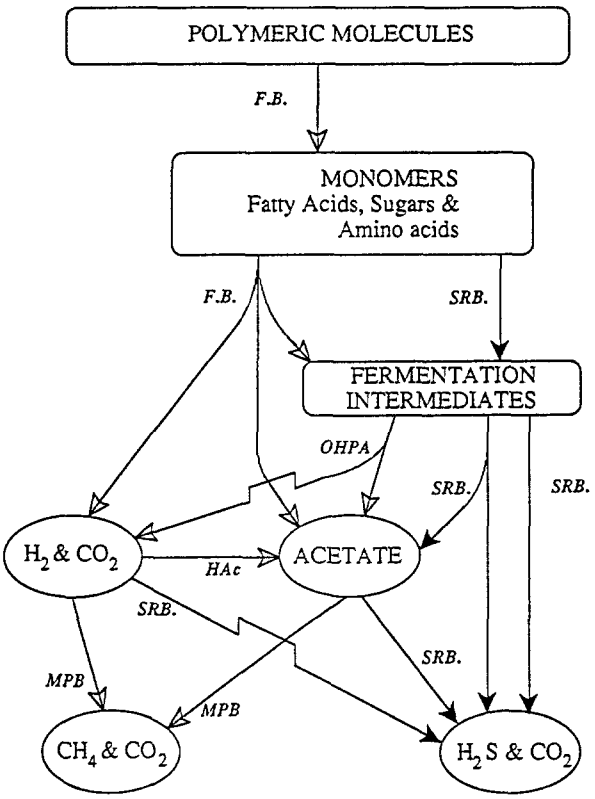

Fig. 4. Pathways of organic compound degradation under methanogenic and sulphidogenic conditions. (FB – fermentative bacteria; OHPA – obligate hydrogen-producing acetogens; HAc – homoacetogenic bacteria; MPB – methane-producing bacteria; SRB – sulphate-reducing bacteria).

mentative bacteria involved in polymer hydrolysis and monomer degradation in anaerobic environments (Postgate 1984; Widdel 1988). Since sulphate reducers cannot hydrolyse polymers, competition cannot take place at the initial polymer hydrolysis level (Hansen 1993). Although a limited number of sulphate reducers capable of sulphidogenic and fermentative growth on sugars, amino acids, etc. has been isolated from a variety of environments (Table 1), it is likely that, in natural ecosystems and in anaerobic digesters, sulphate reducers are more likely to be involved in the ultimate and penultimate stages of mineralisation than in the initial fermentative stage (Postgate 1984; Rivers-Singleton 1993).

Competition between sulphate reducers and OHPA bacteria

As indicated in Fig. 4, the involvement of sulphate-reducers in the further mineralisation of intermediate compounds, such as propionate, butyrate, ethanol, lactate, etc., is potentially quite complex and may involve different cooperative associations depending on the sulphate and sulphide levels, the substrate concentration and the partial pressure of hydrogen (Widdel 1988; Gibson 1990). In theory, four different scenarios for SRB involvement are feasible:

- complete oxidation of fermentation intermediates to CO_2 and sulphide by sulphate reducers;
- incomplete oxidation of fermentation intermediates to acetate by sulphate reducers coupled to acetate conversion by acetoclastic methanogens;
- syntrophic degradation of fermentation intermediates by OHPA species with sulphidogenic utilisation of the H_2 and, possibly, the acetate products, and
- fermentative growth of sulphate reducers on substrates, such as propionate, ethanol, etc., in the absence of sulphate and in syntrophic association with H_2 and acetate-consuming methanogens.

Widdel (1988) proposed that complete or incomplete sulphidogenic oxidation of fermentation intermediates should be favoured over the OHPA syntrophic route due to the insensitivity of the former to hydrogen partial pressures. In the presence of sulphate, incompletely-oxidising SRB are thought to outcompete completely-oxidising species due to the faster growth rate of the former on fermentation intermediates such as propionate, ethanol, lactate, etc. (Widdel 1988; McCartney & Oleszkiewicz 1993). It should, however, be pointed out that for some intermediates, such as isobutyrate, isovalerate, acetone or aromatic compounds, incompletely-oxidising SRB species capable of utilising these compounds have not so far been isolated and their metabolism by sulphate reducers must always, therefore, be complete.

In marine sediments, between 75 and 99% of organic substrate electrons appears to be scavenged by SRB species (Isa et al. 1986a). In saltmarsh sediments, propionate and butyrate are known to be utilised directly by sulphate-reducing bacteria (Banat & Nedwell 1983). In digesters treating high sulphate wastewaters, neither the fermentation intermediates nor sulphate are likely to be limiting. Consequently, the outcome of competition for intermediates, such as butyrate, propionate, etc. may be quite different to the outcome which prevails in sediments where organic substrates and/or sulphate may be limiting. Both in natural environments and in digesters, hydrogen levels are generally low and sulphate reducers are known to successfully outcompete hydrogenophilic methanogens because of

their more favourable thermodynamic and kinetic characteristics (Lovley & Phillips 1987; Widdel 1988; Zinder 1993). Consequently, syntrophy between OHPA bacteria and hydrogenophilic SRB species is a feasible scenario in the presence of sulphate.

In the absence of sulphate, the role of sulphate-reducing bacteria in natural habitats and in digesters is both unclear and of considerable current research interest. Consortia consisting of fermentatively-growing sulphate reducers in syntrophy with H_2-consuming methanogens have been isolated from a variety of natural environments and shown to be capable of degradation of lactate, 3-trimethoxybenzoate, tetrachloromethane, 1,2-dichloroethane, furfural, glycerol and propanediol (Gibson 1990). The involvement of sulphate reducers in propionate and ethanol fermentation to acetate and H_2 in anaerobic digesters treating low sulphate wastewaters has also recently been confirmed by Wu et al. (1991) and Heppner et al. (1992).

The potential competitive interactions between sulphate-reducing bacteria and bacteria involved in the penultimate stages of organic mineralisation, both in the absence and presence of sulphate, are complex and conclusions as to the likely outcome of competition await detailed information on the substrate affinity, thermodynamics and growth kinetics of the various trophic groups involved.

Competition between sulphate reducers and homoacetogenic bacteria

Few studies have addressed the possibility of competition between sulphate reducers and homoacetogenic bacteria in anaerobic environments. To some extent, this reflects the lack of knowledge of the role played by homoacetogenic bacteria, particularly in anaerobic digesters. From thermodynamic and substrate affinity considerations, H_2-oxidising sulphate reducers should effectively outcompete homoacetogens under the conditions prevailing in digesters (Zinder 1993). Since homoacetogens are also capable of heterotrophic growth on a wide range of organic compounds, it has been suggested that their mixotrophic ability, coupled with their versatility, may be the primary factor in determining their prevalence in anaerobic digesters (Zehnder & Stumm 1988). The degree to which competition occurs between sulphate reducers and homoacetogens for organic substrates, either in the presence or absence of sulphate, has not so far been researched.

Competition between sulphate reducers and methanogens

Studies on potential competition between sulphate reducers and methanogens have been confined chiefly to the substrates H_2 and acetate (Widdel 1988; Zinder 1993). Early studies of marine sediments suggested that methanol and methylamines were converted to methane even when high levels of sulphate were present (Oremland & Polcin 1982), suggesting that sulphate reducers could either not utilise these compounds for growth or that they were outcompeted by methylotrophic methanogens. More recent reports have provided evidence both for the existence of methanol-utilising sulphate reducers (Florencio 1994) and for the sulphate-dependent oxidation of methanol in marine sediments (King et al. 1983). However, oxidation of methanol in sulphate-rich sediments or anaerobic digesters may not necessarily reflect successful competition of methylotrophic sulphate reducers over methylotrophic methanogens since the oxidation of methanol can also be mediated by syntrophic associations of methylotrophic acetogens and H_2 and/or acetate-utilising SRB species (Zeikus et al. 1980; Heijthuisjsen & Hansen 1986; Florencio 1994; Florencio et al. 1994).

In natural environments and in digesters, hydrogen and acetate are the key intermediates through which organic matter is channeled during both methanogenic and sulphidogenic mineralisation (Widdel 1988). Consequently, any consideration of competition between SRB and MPB species must focus primarily on the consumption kinetics of hydrogen and acetate. Thermodynamic considerations are often used to predict the outcome of competition between SRB and MPB species for both of these substrates (Widdel 1988; Zinder 1993; McCartney & Oleszkiewicz 1993). As indicated in Table 2, ΔG° and $\Delta G^{\circ'}$ values predict that sulphate reducers should outcompete methanogens for both H_2 and acetate. In practice, however, the actual free energy changes are dependent on the activities of the reactants and products of each reaction and, consequently, the data presented in Table 2 may not accurately predict the competition outcome in natural environments or digesters with varying substrate and product levels (McCartney & Oleszkiewicz 1993).

Michaelis-Menten kinetics are regarded as being more useful in evaluating competition between SRB and MPB species (Widdel 1988). As indicated in Table 2, SRB species have a higher affinity for hydrogen than methanogens. This higher affinity, coupled with yield

Table 2. Free energy, apparent Km and minimum substrate threshold values for hydrogenophilic and acetoclastic methanogens and sulphate reducers.

	$\Delta G°$ (kJ/mole H_2 or C_2)	$\Delta G°'$ (kJ/rxn)	Apparent Km (μM)	Minimum threshold (nM)
$4H_2 + CO_2 \rightarrow CH_4 - 2H_2O$	- 32.7	- 135	5–13	23–75
$4H_2 + HSO_4^- \rightarrow HS^- + 4H_2O$	- 38.0	- 152	2	7
$CH_3COO^- + H_2O \rightarrow CH_4 + HCO_3^-$	- 28.2	- 31	*3–5 × 10^3	0.5–1.2 × 10^6
			**0.5–1 × 10^3	5-70 × 10^3
$CH_3COO^- + SO_4^{2-} \rightarrow HS^- + 2HCO_3^-$	- 39.5	- 47	0.2 × 10^3	± 1 × 10^3

* *Methanosarcina* sp.; ** *Methanothrix* sp. (Widdel 1988; Cord-Ruwisch et al. 1988; McCartney 1991; Zinder 1993).

coefficient data, suggest that SRB should effectively outcompete MPB under growth conditions and at limiting substrate levels (Widdel 1988; McCartney & Oleszkiewicz 1993). Tursman & Cork (1989) proposed that SRB have a higher affinity for hydrogen than MPB due to the location of the hydrogenase enzyme in the periplasmic space in the former rather than in the cytoplasm as in MPB.

Kinetic data also suggest that SRB should successfully outcompete *Methanosarcina* sp. at the low acetate concentrations prevailing in natural environments and digesters (Table 2). The kinetic advantage of SRB species over *Methanothrix* sp. is not so clearcut because of the significantly greater affinity of *Methanothrix* sp. than *Methanosarcina* sp. for acetate (Isa et al. 1986a; Zinder 1993). Apparent Km values for acetate utilisation by *Methanosarcina* sp. are 3–5 mM whereas those for *Methanothrix* sp. are typically less than 1 mM (Table 2).

Given the very low levels of H_2 and acetate that may prevail in natural environments and in steady-state digesters, a comparison of minimum substrate threshold values may be a more useful guide for prediction of the outcome of competition between SRB and MPB species (Zinder 1993). Cord-Ruwisch et al. (1988) determined the threshold concentrations for H_2 for a variety of anaerobes and concluded that there was an inverse correlation between the free energy available for the reaction and the threshold value. Threshold values for H_2 for sulphate reducers were found to be lower than for methanogens (Table 2), indicating that SRB species can lower the H_2 partial pressure below the level capable of being utilised by hydrogenophilic methanogens. The limited threshold values reported for acetate (Table 2) confirm the competitive advantage of *Methanothrix* over *Methanosarcina* sp. Typical minimum threshold values for *Methanothrix* are 0.5–1 mM whereas those for *Methanothrix* are in the micromolar range (Zinder 1993). Although only limited data are available for SRB species, studies with *Desulfobacter postgatei* suggest a minimum threshold for acetate of ± 1 μM (Ingvorsen et al. 1984). The indicated ability of SRB to outcompete MPB for acetate at very low concentrations was confirmed in freshwater sediments by Lovley & Phillips (1987) who showed that the prevailing acetate concentration under methanogenic conditions was approximately 5 μM (i.e. close to the minimum threshold for *Methanothrix soehngenii*) whereas it was reduced to 2 μM by the addition of sulphate.

In anaerobic digesters, numerous studies have confirmed that SRB successfully outcompete methanogens for H_2 during digestion of sulphate-containing wastewaters (Mulder 1984; Rinzema 1988; Rinzema & Lettinga 1986; McCartney 1991; Zinder 1993). Literature data on the outcome of competition for acetate in digesters are contradictory, with some authors showing preferential acetate utilisation by SRB and the majority indicating successful competition by acetoclastic MPB species in the presence of influent sulphate. The discrepancy between the results obtained can, to some extent, be related to the type of anaerobic reactor design used in the investigations. Experiments with CSTR reactors (no sludge retention) showed that acetate was consumed by SRB species in the presence of high influent sulphate levels (Middleton & Lawrence 1977), whereas in reactors with biomass retention (UASB, Anaerobic Filter), the opposite situation prevailed, with acetate being preferentially converted to methane (Mulder 1984; Hoeks et al. 1984; Isa

et al. 1986a, b; Yoda et al. 1987; Rinzema & Lettinga 1988).

Various theories have been put forward to explain the apparent competitive advantage of acetoclastic MPB over sulphate-reducers in retained biomass reactors. Isa et al. (1986a, b) concluded that the successful competition of acetoclastic methanogens can be attributed to their superior capability to colonise support materials and to the relatively high concentrations of acetate prevailing in the reactors under test. Yoda et al. (1987) concluded that acetotrophic methanogens predominated because of their higher growth rate than SRB at acetate concentrations greater than 8 mg COD.l^{-1}. Other theories put forward include the nature of the seed sludge used and the duration of the experimental trial (i.e. not enough time allowed for sulphate reducers to become dominant with a non-sulphate adapted seed sludge); differential inhibition by produced sulphide; nutrient limitation (e.g. the high iron demand of sulphate reducers) and the fact that retained biomass reactors are often under-loaded with respect to the retained biomass (i.e. non-optimal growth conditions).

Anaerobic digestion of sulphate-rich industrial wastewaters

Anaerobic digestion has been successfully applied to a variety of sulphate-rich wastewaters both at laboratory and full-scale (Szendry 1983; Hoeks et al. 1984; Mulder 1984; Rinzema et al. 1986; Svardal et al. 1993; Visser et al. 1992, 1993a, b; Alphenaar et al. 1993). In general, the data obtained suggest that, for most COD:SO_4^{2-} ratios examined, acetate was largely consumed by methanogens whereas sulphate reducers appeared to successfully outcompete MPB for hydrogen. This has given rise to the general assumption that, during anaerobic digestion of sulphate-containing wastewaters, 70% of available COD is converted to methane via acetate, with 30% going to H_2S via hydrogen (Rinzema & Lettinga 1988; Maillacheruvu et al. 1993). Should the level of sulphide become high, it is further suggested that this would stress methanogenesis and lead to lowered methane production (Maillacheruvu et al. 1993). However, this model is rather simplistic and clearly does not take into account competitive interactions between sulphate reducers and the anaerobic species catalysing stages higher up the methanogenic pathway. Neither does it allow for differential levels of inhibition by sulphide on the various trophic groups involved in both methanogenesis and sulphidogenesis.

The extent to which sulphate reduction may dominate at low COD/SO_4^{2-} ratios is illustrated by the studies of Visser et al. (1993b) and Alphenaar et al. (1993). For example, Visser and coworkers treated a laboratory-scale UASB reactor with 5 mg.l^{-1} chloroform for 5 days to terminate methanogenesis and then fed it an influent containing 2,500 mg.l^{-1} COD and 5,000 mg.l^{-1} SO_4^{2-} for a 180 day trial period (Visser et al. 1993b). No methane production was detected from this 'sulphidogenic' reactor throughout the experiment and, towards the end of the trial, a COD conversion rate of 0.9–1.0 g COD.g^{-1}VSS.d^{-1} was achieved. In a parallel 'sulphidogenic/methanogenic' (i.e. mixed) reactor which had not been treated with chloroform, the percentage of organic COD used by SRB on the same influent was about 50% at the start of the experiment and gradually increased to 80% over the first 150 days of feeding (Visser et al. 1993b). This was correlated with an increase in the proportion of acetate being used for sulphate reduction. The length of time required for the shift from acetotrophic methanogenesis to acetotrophic sulphidogenesis was attributed by Visser et al. (1993b) to the very long biomass retention times typical of UASB reactors.

This study, and related studies by Alphenaar and coworkers (Alphenaar et al. 1993; Alphenaar 1994), open up the feasibility of treating industrial wastewaters which contain a very low COD:SO_4^{2-} ratio in sulphidogenic reactors in which methanogenesis is completely suppressed.

Studies in the authors' laboratory have been concerned with the anaerobic treatment of the high sulphate wastewater generated by the fermentative production of citric acid from sugar beet molasses (Colleran et al. 1994). One of the largest citric acid production plants in the world is located in Ringaskiddy, Co. Cork, Ireland. In 1990, the company (ADM) decided to adopt an anaerobic/aerobic treatment route and commissioned the construction of a 8000 m^3 anaerobic digester. The reactor design chosen was the upflow, fully-packed, fixed-bed type and the support material used was polypropylene cascade rings.

Table 3 summarises the design characteristics of the plant and compares these with the operational performance one year after the plant was started up. It is apparent that, with an influent COD:SO_4^{2-} ratio of 3.6, an average COD loading rate of 8.6 kgCOD.m^{-3}.d^{-1} and a HRT of 1.4 days, the full-scale reactor achieved stable methanogenesis and a BOD_5 removal efficiency

Table 3. Design and operational characteristics of the full-scale anaerobic digester treating citric acid production wastewater at ADM Ringaskiddy, Co. Cork, Ireland.*

Process parameter	Design	Operation
COD reduction (%)	50	52
BOD reduction (%)	70	80.8
Influent sulphate (g/l)	2.5–4.3	3.43
Effluent sulphate (mg/l)	–	250
Influent COD/sulphate ratio	3.1	3.6
Total effluent sulphide (mg/l)	400	580
Biogas production (m^3/d)	18,450	26,272
CH_4 content of biogas (%)	55.0	65.5
H_2S content of biogas (%)	5.0	4.8

* Colleran et al. (1994).

of 81%. In practice, the digester exceeded the design load of 62 tonnes COD.d^{-1} and performed with a high degree of operational stability. Analysis of the influent and effluent sulphate and the produced CH_4 and H_2S during steady state operation indicated that the partitioning of the electron flow between the SRB and MPB averaged 18% and 82%, respectively.

Parallel studies (Fig. 5) with a laboratory-scale hybrid reactor in which the influent COD and sulphate concentrations and the COD:SO_4^{2-} ratio were varied over a 380 day trial period were also carried out (Finnegan 1994). It is apparent from Fig. 5 that, as the influent sulphate concentration was increased and the COD:SO_4^{2-} ratio decreased from day 60 onwards, the percentage electron flow via methanogenesis gradually decreased. Between day 100 and day 325, as the influent sulphate was increased from 2.5 to 4.0 g.l^{-1} and the COD:SO_4^{2-} ratio was decreased from 1.9 to 1.2, the percentage electron flow via sulphidogenesis increased from 38 to 52% (Fig. 5). At the lowest COD:SO_4^{2-} ratio tested (day 215–320), the reactor operated with great stability, achieving CODt and CODs removal efficiences of 71 and 81%, respectively. Increasing the influent COD concentration to 12 g.l^{-1}, while maintaining the SO_4^{2-} concentration constant at 4 g.l^{-1} over the following 15 days, increased the COD:SO_4^{2-} ratio to 3 and was accompanied by a very rapid decrease in the percentage electron flow via sulphidogenesis to its final value of 18% (Fig. 5). These results clearly indicate that the partitioning of electron flow via sulphidogenesis and methanogenesis is governed mainly by the influent COD:SO_4^{2-} ratio, rather than by the actual influent SO_4^{2-} concentration. The data also suggest that an increase in the COD:SO_4^{2-} ratio can induce a rapid change in the pattern of electron flow in a retained biomass hybrid reactor. This is in contrast to the findings of Visser et al. (1993a).

After almost a year of operation on a high influent SO_4^{2-} concentration, the most significant changes in the specific methanogenic activity profiles of the retained biomass, in both the laboratory- and full-scale reactors, from that of the seed sludge was a decrease to almost negligible levels of the specific methanogenic activity against propionate and butyrate (Colleran et al. 1994). Although little is known about the metabolism of butyrate in anaerobic reactors treating high sulphate wastewaters, considerable information has been accumulated with respect to propionate conversion (Mulder 1984; Hilton & Archer 1988; Rinzema & Lettinga 1988; Parkin et al. 1990; McCartney 1991; McCartney & Oleszkiewicz 1991; Maillacheruvu et al. 1993).

The absence of significant levels of propionate in effluent samples, coupled with the very low specific methanogenic activity of the biomass against propionate, suggest that growth of propionate-degrading OHPA bacteria was inhibited under the high influent SO_4^{2-} conditions applied. This might suggest that propionate was being exclusively catabolised by SRB or that the influent sugars were being metabolised by a route which did not generate propionate as an intermediate. Support for the latter hypothesis is provided by the findings of McCartney & Oleszkiewicz (1991) that an inoculum which had acclimated to high lactate and high sulphate conditions (COD:SO_4^{2-} ratio of 1.6) utilised a completely different pathway from an inoculum acclimated to a lactate/SO_4^{2-} feed with a COD:SO_4^{2-} ratio of 3.7. In the former, lactate was metabolised incompletely by an SRB species to acetate and propionate was not an intermediate of the overall process. In the latter, lactate was fermented to acetate and propionate, with propionate being exclusively utilised by incompletely-oxidising SRB.

The involvement of incomplete propionate oxidisers, such as *Desulfobulbus propionicus*, in propionate degradation during digestion of sulphate-containing wastewaters has been shown by many authors (Rinzema & Lettinga 1988; Parkin et al. 1990; Wu et al. 1991; Heppner et al. 1992; Maillacheruvu et al. 1993). *Desulfobulbus propionicus*-like cells were shown to be numerous in both suspended biomass and biofilm samples from the hybrid reactor receiving high influent sulphate levels whereas they were absent from a parallel reactor receiving the same feed but without sulphate supplementation (Finnegan 1994). The very

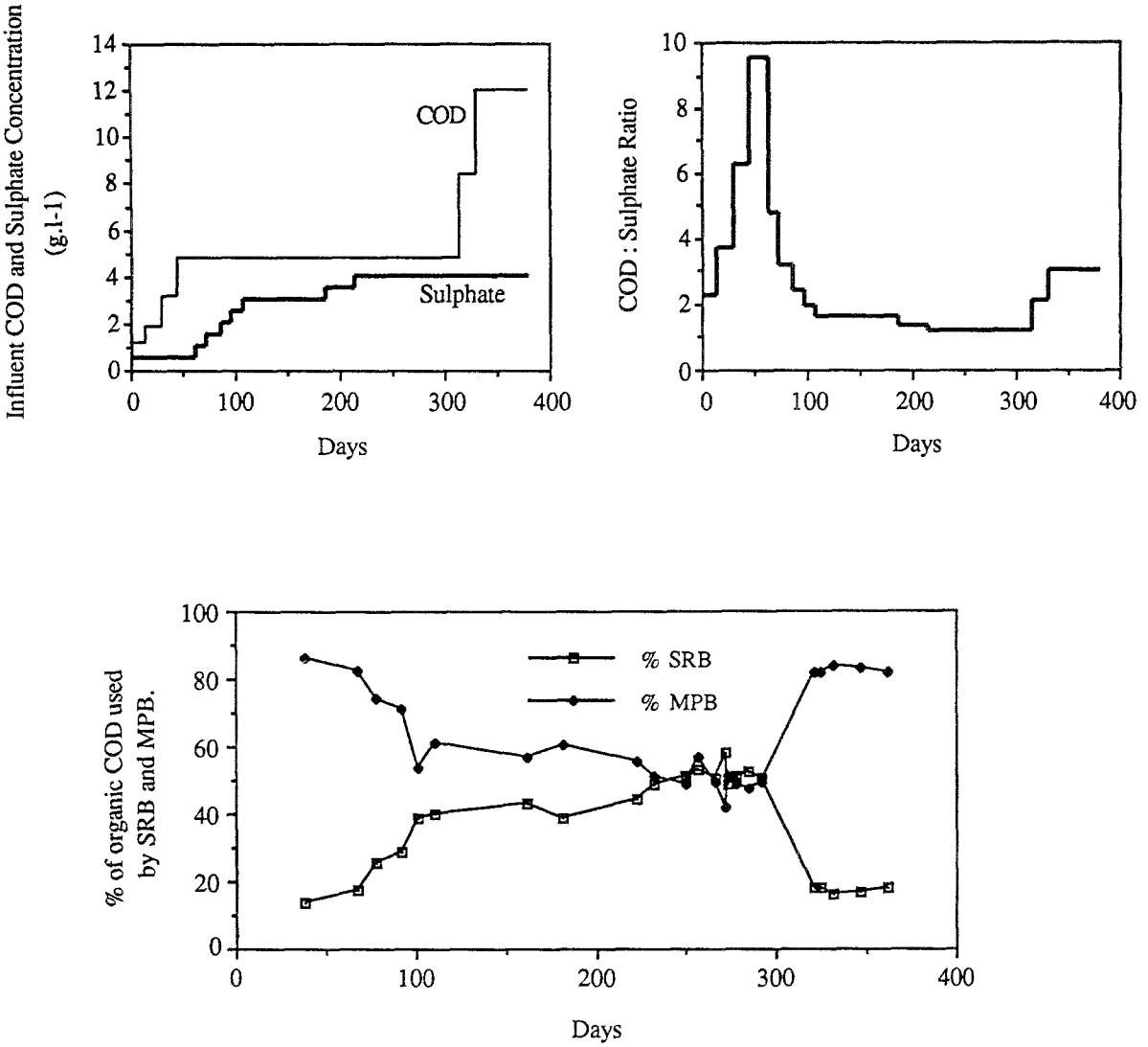

Fig. 5. Influent COD and sulphate concentration, influent COD: sulphate ratio and percentage utilisation of influent COD by sulphate-reducing bacteria (SRB) and methane-producing bacteria (MPB) during a 380 day trial of a laboratory-scale hybrid reactor treating simulated citric acid production wastewater.

low specific methanogenic activity against propionate observed for biomass samples from the sulphate fed reactor at the end of the trial argues, to some extent, against the involvement of *Desulfovibrio propionicus* since Wu et al. (1991) and Heppner et al. (1992) have shown that, in the absence of sulphate, *Desulfovibrio propionicus* can play a fermentative role in syntrophy with H_2-utilising methanogens. Ongoing studies are designed to ascertain, more clearly, the pathways of sugar, propionate and butyrate catabolism and the possible differential inhibitory effects of produced sulphide on different trophic groups treating citric acid production wastewater at low influent COD:SO_4^{2-} ratios.

Conclusions

Anaerobic treatment of industrial wastewaters containing significant levels of sulphate presents an interesting challenge due to competition between sulphidogenesis and methanogenesis and the toxicity of hydrogen sul-

phide to the different trophic groups involved in both processes. Competition for electron flow can take place at a number of different levels in the sequential anaerobic degradation process. Because of their diverse substrate range, SRB species can, theoretically, compete for substrates against fermentative, syntrophic OHPA, homoacetogenic and methanogenic bacteria. Although thermodynamic and kinetic considerations generally favour SRB, in practice factors, such as the prevailing substrate concentration, differential sulphide toxicity, pH, temperature, biomass retention times in retained biomass reactors, substrate and product gradients in biofilms and granules, etc. may significantly affect the competition outcome.

It is clear from detailed studies carried out to date that high sulphate wastewaters can be anaerobically treated, even at full-scale, with a high degree of operational stability. The influent COD:SO_4^{2-} ratio appears to be the most significant factor in determining the partitioning of the electron flow between sulphidogenesis and methanogenesis. With increasing knowledge of sulphide toxicity and the factors controlling competition between SRB and other anaerobic trophic groups, it may well be possible in the future to digest wastewaters with very low COD:SO_4^{2-} ratios under entirely sulphidogenic conditions and to manipulate the operational conditions with intermediate COD:SO_4^{2-} ratios to enhance methanogenesis.

Acknowledgements

The authors acknowledge receipt of a joint Higher Education-Industry Cooperation grant from the Irish Science and Technology Agency and ADM Ringaskiddy. The financial support of Merck Sharpe & Dohme and Pfizers Chemical Corporation is also gratefully acknowledged.

References

Aeckersberg F, Bak F & Widdel F (1991) Anaerobic oxidation of saturated hydrocarbons by a new type of sulfate-reducing bacterium. Archives of Microbiology 156: 5–14

Alphenaar PA (1994) Anaerobic granular sludge: characterisation and factors affecting its functioning. Ph.D. Thesis, University of Wageningen, The Netherlands

Alphenaar PA, Visser A & Lettinga G (1993) The effect of liquid upward velocity and hydraulic retention time on granulation in UASB reactors treating wastewater with a high sulphate content. Bioresearch Technology 43: 249–258

Anderson GK, Donnelly T & McKeown KJ (1982) Identification and control of inhibition in the anaerobic treatment of industrial wastewaters. Process Biochemistry July/August: 28–41

Badziong W, Ditter B & Thauer RK (1979) Acetate and carbon dioxide assimilation by *Desulfovibrio vulgaris* (Marburg), growing on hydrogen plus sulfate and hydrogen plus thiosulfate as the sole energy sources. Archives of Microbiology 117: 209–214

Banat IM & Nedwell DB (1983) Mechanisms of turnover of C_2–C_4 fatty acids in high sulphate and low sulphate anaerobic sediments. FEMS Microbiology Letters 17: 107–110

Campbell LL & Postgate JR (1965) Classification of the spore-forming sulfate-reducing bacteria. Bacteriological Reviews 29: 359–363

Carrondo MJT, Silva JMC, Figueira MII, Ganho RMB & Oliveira JFS (1983) Anaerobic filter treatment of molasses fermentation wastewater. Water Science and Technology 15: 117–126

Colleran E, Finnegan S & O'Keeffe RB (1994) Anaerobic digestion of high sulphate-containing wastewater from the industrial production of citric acid. In: Proceedings of the 7th International Symposium on Anaerobic Digestion: Oral Papers (pp 160–169). RSA (Litho) Ltd., Goodwood, South Africa

Cord-Ruwisch R, Steitz H-J & Conrad R (1988) The capacity of hydrogenotrophic anaerobic bacteria to compete for traces of hydrogen depends on the redox potential of the terminal electron acceptor. Archives of Microbiology 149: 350–357

Cummings JH (1981) Short chain fatty acids in the human colon. Gut 22: 763–779

Cypionka H, Widdel F & Pfennig N (1985) Survival of sulfate-reducing bacteria after oxygen stress and growth in sulfate-free oxygen-sulfide gradients. FEMS Microbiology Ecology 31: 39–45

Daniels L, Belay N & Rajagopal BS (1986) Assimilatory reduction of sulfate and sulfite by methanogenic bacteria. Applied & Environmental Microbiology 51: 703–709

Finnegan S (1994) Anaerobic digestion of sulphate-containing wastewater from citric acid production. Ph.D. Thesis, University College Galway, Ireland

Florencio L (1994) The fate of methanol in anaerobic bioreactors. Ph.D. Thesis, University of Wageningen, The Netherlands

Florencio L, Field JA & Lettinga G (1994) Importance of cobalt for individual trophic groups in an anaerobic methanol-degrading consortium. Applied & Environmental Microbiology 60: 227–234

Garrels RM & Christ CL (1965) Solutions, Minerals and Equilibria. Harper & Row, New York

Gibson GR (1990) Physiology and ecology of the sulphate-reducing bacteria. Journal of Applied Bacteriology 69: 769–797

Hansen TA (1993) Carbon metabolism of sulfate-reducing bacteria. In: Odom JM & Rivers-Singleton JR (eds) The Sulfate-Reducing Bacteria: Contemporary Perspectives (pp 21–40). Springer-Verlag, New York

Heijthuijsen JHFG & Hansen TA (1986) Interspecies hydrogen transfer in co-cultures of methanol-utilising acidogens and sulfate-reducing or methanogenic bacteria. FEMS Microbiology Letters 38: 57–64

Heppner B, Zellner G & Diekmann H (1992) Start-up and operation of a propionate-degrading fluidized-bed reactor. Applied Microbiology and Biotechnology 36: 810–816

Hilton BL & Oleszkiewicz JA (1988) Sulfide-induced inhibition of anaerobic digestion. Journal of Environmental Engineering 114(6): 1377–1391

Hilton MG & Archer DB (1988) Anaerobic digestion of a sulfate-rich molasses wastewater: inhibition of hydrogen sulfide formation. Biotechnology Bioengineering 31: 885–888

Hoeks FWJMM, Ten Hoopen, Roels JA & Kuenen JG (1984) Anaerobic treatment of acid water (methane production in a sulfate-rich environment). In: Houwink EH & Van der Meer RR (Eds) Innovations in Biotechnology (pp 113–119). Elsevier Science, Amsterdam

Ingvorsen K, Zehnder AJB & Jorgensen BB (1984) Kinetics of sulfate and acetate uptake by *Desulfobacter postgatei*. Applied & Environmental Microbiology 47: 403–408

Isa Z, Grusenmeyer S & Verstraete W (1986a) Sulfate reduction relative to methane production in high-rate anaerobic digestion: Technical Aspects. Applied and Environmental Microbiology 51(3): 572–579

— (1986b) Sulfate reduction relative to methane production in high-rate anaerobic digestion: Microbiological Aspects. Applied and Environmental Microbiology 51(3): 580–587

Iza J (1991) Fluidised bed reactors for anaerobic wastewater treatment. Water Science and Technology 24: 109–132

Iza J, Colleran E, Paris JM & Wu W-M (1991) International workshop on anaerobic treatment technology for municipal and industrial wastewaters: summary paper. Water Science & Technology 24: 1–16

Jansen K, Thauer RK, Widdel F & Fuchs G (1984) Carbon assimilation pathways in sulfate reducing bacteria. Formate, carbon dioxide, carbon monoxide and acetate assimilation in *Desulfovibrio baarsii*. Archives of Microbiology 138: 257–262

Khan AW & Trottier TM (1978) Effect of sulfur containing compounds on degradation of cellulose to methane by a mixed culture obtained from sewage sludge. Applied and Environmental Microbiology 35: 1027–1034

King GM, Klug MJ & Lovley DR (1983) Metabolism of acetate, methanol and methylated amines in intertidal sediments of Lowes Cove, Maine. Applied & Environmental Microbiology 45: 1848–1853

Kroiss H & Plahl-Wabnegg F (1983) Sulphide toxicity with anaerobic wastewater treatment. In: Van der Brink WJ (Ed) Proceedings European Symposium on Anaerobic Wastewater Treatment (AWWT) (pp 72–78). TNO Corporate Communication Dept., The Hague, The Netherlands

Kühl M & Jorgenson BB (1992) Microsensor measurements of sulfate reduction and sulfide oxidation in compact microbial communities of aerobic biofilms. Applied & Environmental Microbiology 58: 1164–1174

Laanbroek HJ, Abee T & Voogd IL (1982) Alcohol conversions by *Desulfobulbus propionicus* Lindhorst in the presence and absence of sulfate and hydrogen. Archives of Microbiology 133: 178–184

Lawrence AW & McCarty PL (1965) The role of sulfide in preventing heavy metals toxicity in anaerobic treatment. Journal Water Pollution Control Federation 37: 392–409

Lawrence AW, McCarty PL & Guerin FJA (1966) The effects of sulfides on anaerobic treatment. International Journal of Air & Water Pollution 10: 207–221

Lovley DR & Phillips EJP (1987) Competitive mechanisms of sulfate reduction and methane production in the zone of ferric iron reduction in sediments. Applied & Environmental Microbiology 53: 2636–2641

Maillacheruvu KY, Parkins GF, Peng CY, Kuo W-C, Oonge ZI & Lebduschka V (1993) Sulfide toxicity in anaerobic systems fed sulfate and various organics. Water Environment Research 65(2): 100–109

McCartney DM (1991) Effects of sulfate and sulfide on methanogenic and sulfate-reducing activity during degradation of simple organics: role of propionate and acclimation. Ph.D. Thesis, University of Manitoba, Canada

McCartney DM & Oleszkiewicz JA (1993) Competition between methanogens and sulfate reducers: effect of COD:sulfate ratio and acclimation. Water Environment Research 65: 655–664

McCready RGL, Gould WD & Cook FD (1983) Respiratory nitrate reduction by *Desulfovibrio* sp. Archives of Microbiology 135: 182–185

McInerney MJ & Bryant MP (1981) Anaerobic degradation of lactate by syntrophic associations of *Methanosarcina barkeri* and *Desulfovibrio* species and effect of H_2 on acetate degradation. Applied & Environmental Microbiology 41: 346–354

Mechelas BJ & Rittenberg SC (1960) Energy coupling in *Desulfovibrio desulfuricans*. Journal of Bacteriology 80: 501–507

Middleton AG & Lawrence AW (1977) Kinetics of microbial sulfate reduction. Journal Water Pollution Control Federation 49: 1659–1670

Möller D, Schauder R, Fuchs G & Thauer RK (1987) Acetate oxidation to CO_2 via a citric acid cycle involving an ATP-citrate lyase: a mechanism for the synthesis of ATP via substrate level phosphorylation in *Desulfobacter postgatei* growing on acetate and sulfate. Archives of Microbiology 148: 202–207

Möller-Zinkhan D & Thauer RK (1990) Anaerobic lactate oxidation to 3 CO_2 by *Archaeoglobus fulgidus* via the carbon monoxide dehydrogenase pathway: demonstration of the acetyl-CoA carbon-carbon cleavage reaction in cell extracts. Archives of Microbiology 153: 215–218

Mulder A (1984) The effects of high sulphate concentrations on the methane fermentation of wastewater. In: Houwink EH & Van der Meer RR (Eds) Innovations in Biotechnology (pp 133–143). Elsevier Science, Amsterdam

Nanninga HJ & Gottschal JC (1986) Anaerobic purification of wastewater from a potato-starch producing factory. I. Intermediary compounds and bacterial species involved. Water Research 20: 97–103

— (1987) Properties of *Desulfovibrio carbinolicus*, sp.nov., and other sulfate-reducing bacteria isolated from an anaerobic purification plant. Applied & Environmental Microbiology 53: 802–809

Oremland RS & Polcin S (1982) Methanogenesis and sulfate reduction: competitive and noncompetitive substrates in estuarine sediments. Applied & Environmental Microbiology 44: 1270–1276

Parkin GF, Speece RE, Yang CHJ & Kocher WM (1983) Response of methane fermentation systems to industrial toxicants. Journal of Water Pollution Control Federation 55: 44–53

Parkin GF, Lynch NA, Kuo W-C, Van Keuren EL & Bhattacharya SK (1990) Interaction between sulfate reducers and methanogens fed acetate and propionate. Research Journal WPCF 62(6): 780–788

Pfennig N, Widdel F & Truper HG (1981) The dissimilatory sulfate-reducing bacteria. In: Starr MP, Stolp H, Truper HG, Balows A & Schlegel HG (Eds) The Prokaryotes Vol I (pp 926–940). Springer-Verlag, Heidelberg

Pipyn P & de Smedt M (1991) BIOTIM pilot and full scale anaerobic and aerobic wastewater treatment plants on effluent from the molasses based fermentation industry. In: Verachtert H & Verstraete W (Eds) Proceedings of the International Symposium of Environmental Biotechnology. Part 1 (pp 169–171). Royal Flemish Society of Engineers, Belgium

Postgate JR (1984) The Sulfate-Reducing Bacteria. Cambridge University Press, Cambridge, England

Puhakka JA, Salkinoja-Salonen M, Ferguson JF & Benjamin MM (1990) Carbon flow in acetotrophic enrichment cultures from pulp mill effluent treatment. Water Research 24(4): 515–519

Reis MAM, Almeida JS, Lemos PC & Carrondo MJT (1992) Effect of hydrogen sulfide on growth of sulfate reducing bacteria. Biotechnology Bioengineering 40: 593–600

Rinzema A & Lettinga G (1986) Anaerobic treatment of sulfate-containing wastewater. In: Wise DL (Ed) Biotreatment Systems Vol III (pp 65–109). CRC Press, Boca Raton, USA

Rinzema A (1988) Anaerobic treatment of wastewater with high concentrations of lipids or sulphate. Ph.D. Thesis, University of Wageningen, The Netherlands

Rinzema A, Paardekooper A, de Vegt A & Lettinga G (1986) Anaerobic treatment of edible oil refinery wastewater in granular sludge UASB reactors. In: Anaerobic Treatment: A Grown-Up Technology (pp 205–217). Industrial Presentations (Europe) BV, Schiedam, The Netherlands

Rivers-Singleton JR (1993) The sulfate-reducing bacteria: an overview. In: Odom JM & Rivers-Singleton JR (Eds) The Sulfate-Reducing Bacteria: Contemporary Perspectives (pp 1–20). Springer-Verlag, New York

Schauder R, Eikmanns B, Thauer RK, Widdel F & Fuchs G (1986) Acetate oxidation to CO_2 in anaerobic bacteria via a novel pathway not involving reactions of the citric acid cycle. Archives of Microbiology 145: 162–172

Scherer P & Sahm H (1981) Influence of sulphur-containing compounds on the growth of *Methanosarcina barkeri* in a defined medium. European Journal of Applied Microbiology & Biotechnology 12: 28–35

Speece RE (1983) Anaerobic biotechnology for industrial wastewater. Environmental Science and Technology 17: 416A–427A

Speece RE & Parkin GF (1983) The response of methane bacteria to toxicity. In: Proceedings of the Third International Symposium on Anaerobic Digestion (pp 23–35). Evans & Faulkner Inc. Watertown, Massachusetts

Stams AJM, Kremer DR, Nicolay K, Weenk GH & Hansen TA (1984) Pathway of propionate fermentation in *Desulfobulbus propionicus*. Archives in Microbiology 139: 167–173

Stieb M & Schink B (1989) Anaerobic degradation of isobutyrate by methanogenic enrichment cultures and by a *Desulfococcus multivorans* strain. Archives of Microbiology 151: 126–132

Svardal K, Gotzendorfer K & Nowak O (1993) Treatment of citric acid wastewater for high quality effluent on the anaerobic-aerobic route. Water Science and Technology 28(2): 177–186

Szendry LM (1983) Start-up and operation of the Bacardi Corporation anaerobic filter. In: Proceedings of the Third International Symposium on Anaerobic Digestion (pp 365–377). Evans & Faulkner Inc. Watertown, Massachusetts

Szewzyk R & Pfennig N (1987) Complete oxidation of catechol by the strictly anaerobic sulfate-reducing *Desulfobacterium catecholicum* sp. nov. Archives of Microbiology 147: 163–168

Thauer RK (1988) Citric acid cycle, 50 years on. Modifications and alternative pathway in anaerobic bacteria. European Journal of Biochemistry 176: 497–508

Thauer RK, Jungermann K & Decker K (1977) Energy conservation in chemotrophic anaerobic bacteria. Bacteriology Reviews 41: 100–180

Traore AS, Fardeau M-L, Hatchikan EC, LeGall J & Belaich J-P (1983) Energetics of growth of a defined mixed culture of *Desulfovibrio vulgaris* and *Methanosarcina barkeri*: interspecies hydrogen transfer in batch continuous culture. Applied & Environmental Microbiology 46: 1152–1156

Tursman JF & Cork DJ (1989) Influence of sulphate and sulfate-reducing bacteria on anaerobic digestion technology. In: Mizrahi A & Van Wezel A (Eds) Biological Waste Treatment (pp 273–281). Alan R Liss Inc., New York

Visser A, Gao Y & Lettinga G (1992) Anaerobic treatment of synthetic sulfate-containing wastewater under thermophilic conditions. Water Science & Technology 25: 193–202

— (1993a) Effects of short-term temperature increases on the mesophilic anaerobic breakdown of sulfate-containing wastewater. Water Research 27: 541–550

Visser A, Alphenaar PA, Gao Y, van Rossum G & Lettinga G (1993b) Granulation and immobilisation of methanogenic and sulfate-reducing bacteria in high-rate anaerobic reactors. Applied Microbiology & Biotechnology 40: 575–581

Vosjan JH (1982) Respiratory electron transport system activities in marine environments. Hydrobiological Bulletin 16: 61–68

Widdel F & Pfennig N (1984) Dissimilatory sulfate or sulfur-reducing bacteria. In: Kreig NR & Holt JG (Eds) Bergey's Manual of Systematic Bacteriology, Vol. 1 (pp 663–679). Williams & Wilkins, Baltimore

Widdel F (1988) Microbiology and ecology of sulphate- and sulphur-reducing bacteria. In: Zehnder AJB (Ed) Biology of Anaerobic Microorganisms (pp 469–586). Wiley & Sons, New York

Widdel F & Hansen TA (1991) The dissimilatory sulfate- and sulfur-reducing bacteria. In: Balows A, Truper HG, Dworkin M, Hardner W & Schleifer K (Eds) The Prokaryotes (pp 3352–3378). Springer-Verlag, New York

Wollersheim R, Selz A, Heppner B & Diekmann H (1989) Enrichment of acetate-, propionate-, and butyrate-degrading co-cultures from the biofilm of an anaerobic fluidized bed reactor. Applied Microbiology and Biotechnology 31: 425–429

Wu W-M, Hickey RF & Zeikus JG (1991) Characterization of metabolic performance of methanogenic granules treating brewery wastewater: role of sulfate-reducing bacteria. Applied and Environmental Microbiology 57(12): 3438–3449

Yoda M, Kitagawa M & Miyaji Y (1987) Long term competition between sulfate-reducing and methane-producing bacteria for acetate in anaerobic biofilms. Water Research 21: 1547–1556

Zehnder AJB & Stumm W (1988) Geochemistry and biogeochemistry of anaerobic habitats. In: Zehnder AJB (Ed) Biology of Anaerobic Microorganisms (pp 1–38). John Wiley & Sons, New York

Zehnder AJB, Ingvorsen K & Marti T (1982) Microbiology of methane bacteria. In: Hughes DE, Stafford DA, Wheatley BI, Baader W, Lettinga G, Nyns E-J & Verstraete W (Eds) Proceedings of Second International Symposium on Anaerobic Digestion (pp 45–68). Elsevier Biomedical Press, Amsterdam

Zeikus JG, Lynd LH, Thompson TE, Krzyeki JA, Weimer PJ & Hegge PW (1980) Isolation and characterisation of a new, methylotrophic, acidogenic anaerobe, the Marburg strain. Current Microbiology 3: 381–386

Zinder SH (1993) Physiological ecology of methanogens. In: Ferry JG (Ed) Methanogens: Ecology, Physiology, Biochemistry and Genetics (pp 128–206). Chapman & Hall, London/New York.