

CHAPTER 2: LITERATURE REVIEW

2.1 SULPHUR CYCLE

The sulphur cycle (Figure 2.1) is, like the carbon and nitrogen cycles, an essential process in nature. However, due to human activities, the cycle can be easily disturbed, both on a local and on a global scale (Kuenen & Robertson, 1992). One of the major environmental pollutants in the sulphur cycle is the formation of SO_2 and other sulphur compounds by the burning of fossil fuels, due to global industrialization. The other major environmental contributor to the sulphur pollution is the formation of SO_4 as a consequence of mining operations. The sulphur cycle consists of several steps, including an oxidative and a reductive component, which in a natural ecosystem should be in balance. On the reductive side, sulphate and sulphur function as electron acceptors in the metabolic pathways, used by a wide range of anaerobic bacteria. On the oxidative side of the cycle, reduced sulphur compounds serve as electron donors for anaerobic phototrophic bacteria, which gain their energy from (sun)light or provide growth energy for the colourless sulphur bacteria. From an industrial management perspective, the best way to manipulate the sulphur cycle is to produce sulphur, which being insoluble, can be easily recovered.

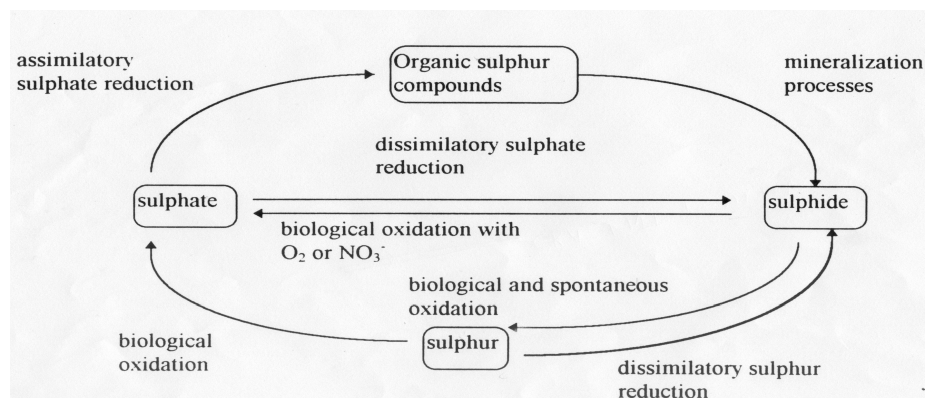


Figure 2.1: The Biological Sulphur Cycle (Pfenning & Widdel, 1982)

2.2 IMPACT OF MINING AND MINE EFFLUENTS ON THE ENVIRONMENT

Mining almost always impacts on the natural water environment. These impacts can be beneficial as some mine waters are of good enough quality that they can be used for public supply (Banks *et al.* 1996). The potential magnitude of environmental impacts associated with excess mine water discharging from old mine workings can

be established through the evaluation of excess mine water production, the geochemical properties of mine water, the safe environmental level to which the rising mine water can be allowed to increase before impacting on the groundwater and surface water resources, as well as probable surface decant points if the old workings were allowed to fill and decant. Dewatering in mining operations is essential for the safety of the mine workers. The consequences of dewatering of mines can include surface or groundwater pollution if the mine water is of poor quality and is discharged to the natural environment without prior treatment.

Underground mining tends to have less conspicuous impacts on surface water than an open pit, surface mining. But all types of mining have the potential to directly disrupt the ground water flow, which can affect surface waters that are in hydraulic continuity with affected groundwater systems (Booth 2000). However, the impact on the natural water environment arising from the act of mining itself tends to be relatively localized and limited when compared to other mining related impacts, such as those associated with dewatering and seepage of contaminated leachate from waste rock piles and tailings dams (Younger & Wolkersdorfer, 2004). Waste products from both mining and mineral processing operations are often contained in large heaps or in tailings dams. Seepage of contaminated leachate from waste rock piles and tailing dams is a significant cause of surface and ground water pollution in many mining areas. This kind of water pollution often occurs when the mine is in operation and without remediation can persist long after mine closure (Younger & Wolkersdorfer, 2004). This is the case in the operation of the South African mining industry, which inherited the legacy of the past regarding contaminated mine water.

The metals and salts containing mine effluents can deplete the oxygen in the receiving waters, which can have strong impacts on the survival of invertebrates and fish present in these receiving water bodies. Oxidation of Fe^{2+} to Fe^{3+} is a particular problem in the affected streams due to the precipitation of voluminous orange/red rusty coatings of ferric hydroxides/oxyhydroxides, called “yellow buoy” in the USA and “ochre” in the United Kingdom. The formation of these iron hydroxides/oxyhydroxides can have detrimental effects on the aquatic biota.

The pH of the mine water is usually acidic and can be as low as 2. When the pH is maintained below 6.5 for an extended period, it can result in decreased reproduction and growth of fish and aquatic invertebrates (Ikuta & Kitamura, 1995).

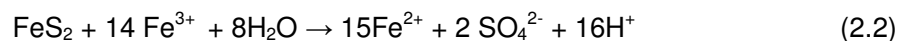
A significant cause of surface water pollution is contaminated leachate from waste rock piles and tailing dams in most mining districts. Younger (1997) states that re-vegetated waste rock piles can continue to release acidic leachates over several decades from shallow water table systems perched within the spoil. Drainage of leachate through the unlined bases of old tailings dams is also known to produce polluted surface and ground water (Manzano *et al.* 1999; Johnson, 2000).

2.3 ACID MINE DRAINAGE (AMD)

The formation of AMD is primarily a function of the geology, hydrology and mining activities. It is formed due to complex geo-chemical and microbial reactions, which occur when water and oxygen come into contact with pyrite in the coal seam. Bacterial oxidation of sulphide minerals is the major factor in the formation of acid mine drainage, a common environmental problem in coal mining regions. When pyrite is first exposed during mining operations, it is slowly oxidised according to reaction 2.1:



This reaction depicts the oxidation of pyrite by oxygen, when sulphur is oxidized to sulphate and ferrous iron is released. As can be seen by the reaction (2.1), 2 moles of acidity are formed for each mole of pyrite. The ferrous iron formed is converted to ferric iron due to the biological oxidation of ferrous (Fe^{2+}) to ferric ions (Fe^{3+}), which can react with more pyrite according to reaction 2.2:



When more Fe^{2+} ions are formed, the bacterial oxidation to Fe^{3+} continues, thus initiating a cycle referred to as the *propagation cycle*. The breakdown of pyrite leads ultimately to the formation of Fe^{2+} and SO_4^{2-} ions, resulting in acidic water, with a pH as low as 2. Furthermore, pyrite, occurring in coal discard heaps can be oxidized with similar results as for the mine water effluents. The run-off from coal mining discards often causes contamination of ground waters (Madigan *et al.* 1997; Younger *et al.* 2002). Under undisturbed conditions, the coal is not exposed to air, water or bacteria.

2.4 ACID MINE DRAINAGE TREATMENT TECHNOLOGIES

Before 1980, the only proven technologies for mine water treatment were the active treatment methods. By active treatment is meant conventional waste water engineering applied to mine waters (Younger *et al.* 2002). Therefore, in most cases the mine effluents can be treated following the design of infrastructure for similar unit processes in ordinary waste water treatment plants.

2.4.1 Physical and Chemical Technologies

Due to salination by AMD and the associated scaling and biocorrosion problems, as well as increased environmental awareness among the general population, methods are being investigated to remove the high sulphate concentration of AMD. Physical (reverse osmosis, electrodialysis and ion exchange) and chemical methods (precipitation with barium salts and limestone neutralisation followed by lime precipitation, for Mg removal) have been tested and applied.

2.4.1.1 The Barium removal technology

Kun (1972) studied sulphate removal using barium carbonate (BaCO_3) producing barium sulphate (BaSO_4). Volman (1984) and Maree *et al.* (1989) demonstrated that BaSO_4 could be reduced efficiently and economically with coal under thermic conditions to produce barium sulphide (BaS). The BaS can then be re-used for the sulphate removal process. For certain mine waters this technology can be applied and the benefit of the technology is that the chemicals required for the technology can be retrieved and re-used, which results in substantial savings on running/operation costs.

2.4.1.2 The limestone neutralisation and precipitation technology

It was demonstrated that limestone (CaCO_3) instead of lime (Ca(OH)_2) can be utilized for neutralization of acid water, resulting in a 50% saving in operating costs (Maree *et al.* 2003). The other advantages of the use of limestone are that limestone is safer to handle than lime and that the pH after neutralisation cannot exceed a pH of 8. The limestone neutralisation technology consists of the following stages: the CaCO_3 handling and dosing, CaCO_3 -neutralization and gypsum crystallization to achieve neutralised water and partial sulphate removal.

2.4.2 Biological treatment

The biological sulphate reduction technology is particularly beneficial to mining industries experiencing acid mine drainage problems, as it results in removal of

sulphate, in a pH increase of the treated water and often in metal removal. The SRB utilize organic products as the carbon and energy source, providing electrons, while sulphate is used as the terminal electron acceptor. The products of biological sulphate removal are sulphide and alkalinity. Sulphide production often results in metal-sulphides precipitation, e.g FeS, since most AMDs contain high concentrations of iron. Due to the production of alkalinity, the pH of the treated water often increases to neutral values.

Biological treatment of AMD can be applied after neutralisation and partial sulphate removal, which is advantageous for two reasons:

- a) It is cheaper to use limestone than a carbon and energy source
- b) For biological treatment a neutral pH is more favourable for the SRB

There are two options for the biological treatment, namely the passive and active treatment technologies, both of which will be discussed as both treatment systems have applications in South Africa.

2.4.2.1 Passive treatment

Passive treatment requires little maintenance and can find its application in rural mining areas, however, it can only treat relative small volumes of mining effluents (Pulles, 2000). "Passive treatment is the deliberate improvement of water quality using only naturally-available energy sources (e.g. gravity, microbial metabolic energy, photosynthesis), in systems which require infrequent (albeit regular) maintenance in order to operate effectively over the entire system design life" (Younger *et al.* 2002). Thus passive treatment technologies use natural materials to promote naturally occurring chemical and biological processes. Particular contaminant removal processes are optimized by manipulating the environmental conditions to obtain a cost effective technology. For this purpose, locally sourced materials, such as carbonate rocks and organic substrates, are utilised (Younger *et al.* 2002).

The advantage of a passive treatment system is that it can be used for more than 10 years with minimal requirement for operator intervention and costly maintenance. The ecological advantage is that they include constructed wetlands, which provide wildlife habitat and can have substantial values of social and ecological values (Hawke & José, 1996, Younger 1998). The plant-microbe associations in wetlands can serve both as the reactor and as source of carbon for the sulphate reduction and

water quality improvement (Batchelor *et al.* 1998). A wide range of electron donors, such as manure, spent mushroom compost, peat, sawdust and woodchips have been used. The natural occurring vegetation or specifically planted vegetation can be used as a continuous source of reduced carbon (Johnson, 2000). Passive treatment systems occur in North America as well as in Europe (UK and Spain) and it is the technology of choice for long-term use, wherever the hydrogeochemical prognosis is favourable and land space is available. These systems are usually applied in situations where mining was stopped many years ago and where no funds are available for costly high-tech solutions for the treatment of the remaining acid mine waters. In this kind of situation, where acid mine water needs to be treated, a relatively cheap passive treatment system can be operated with low maintenance and little supervision.

With the above mentioned conditions in mind, a novel South African passive treatment system has been developed, called the Integrated Managed Passive Treatment Process Technology (IMPI). This development is the result of many years of collaborative research between water professionals, research institutions and mining companies (Heath, 2002). The IMPI technology focussed both on the microbiology as well as on the chemical engineering of the processes by fundamentally investigating the breakdown and use of lignocellulose material, observing the sulphate reduction followed by the sulphide production as well as the reactor hydraulics (Heath, 2002). The IMPI technology can treat one Ml per day of mine water at a relatively low capital cost of R3 million to remove one ton of sulphate per day at an operating cost of R 0.60 per m³ (Heath, 2002).

2.4.2.2 Active treatment

The emphasis of the investigation described in this thesis will be on active biological sulphate reduction technology. A major advantage of the active technology is the increased rate of reaction, which in turn allows for larger volumes of effluents to be treated. Sulphate-rich effluents can be treated biologically when SRB and organic matter are present. In the presence of sulphate, but also of sulphite (SO₃²⁻) and thiosulphate (S₂O₃²⁻), SRB are able to use several intermediate products of the anaerobic mineralization process. Besides the direct methanogenic substrates, such as hydrogen, formate, acetate, methanol and pyruvate (Bock *et al.* 1997), they can also use propionate, butyrate, higher and branched fatty acids, lactate, ethanol and higher alcohols, fumarate, succinate, malate and aromatic compounds (Colleran *et al.* 1995). In sulphidogenic breakdown of VFA, two oxidation patterns can be

distinguished. Some SRB are able to completely oxidize VFA to CO₂ and sulphide as end-products, whereas other SRB can only carry out an incomplete oxidation of VFA with acetate and sulphide as end-products. The carbon sources most commonly used are listed in Table 2.1 (Maree, 1988). Some of these substrates will be discussed in greater detail in section 2.11.1.

Table 2.1. Organic substrates, most commonly used for biological sulphate removal

Acetate	Ethanol	Glycerol	Pyruvate
Alanine	Formate	Lactate	Succinate
Butyrate	Fructose	Malate	Sucrose
Citrate	Glucose	Propionate	Tartrate

Although the biological sulphate removal is an attractive option, worldwide not many active full scale operations are in operation, however several demo-scale plants have been constructed. Research is still being conducted to find the optimal reactor configuration (UASB, EESB, Completely Mixed), to maintain the biomass and to identify a cost effective carbon and energy source, as discussed in this thesis.

2.5 REACTOR DESIGN

Due to the development of improved reactor configuration, anaerobic, as opposed to the traditionally aerobic, treatment of wastewater was established as a feasible option. As the biological sulphate removal also occurs under anaerobic conditions, similar reactor configurations as typically used for the anaerobic COD removal, can be used for biological removal of high sulphate concentrations. A biological sulphate reduction process was developed at the CSIR, Pretoria, South Africa (Maree & Strydom, 1985; Maree *et al.* 1986). This three-stage process (anaerobic - aerobic – anaerobic) when treating mining effluents, employed up-flow, packed bed reactors for anaerobic treatment, followed by an activated sludge system for aerobic treatment. Once the biological sulphate reduction process had been proven, efforts concentrated on obtaining the most efficient reactor design. Among these are the Upflow Anaerobic Sludge Bed (UASB) Reactor (Lettinga, *et al.* 1980), the Fluidized Bed (FB) Reactor (Iza, 1991) and the Anaerobic Filter (AF) (Young & McCarty, 1969). These reactors are based on sludge immobilization and sludge retention, so that high biomass concentrations can be maintained in the reactors and high organic loading rates can be applied. The advantage of sludge immobilization and the

formation of biofilms is that wash-out of only small particles of the biomass occurs. To avoid sludge loss due to wash-out, the addition of a clarifier with a sludge return cycle to the reactor can be considered. However, due to the surface area of the clarifier, which is in contact with the atmosphere, it can be assumed that a fair amount of air will be introduced into the anaerobic reactor. A reactor system based on this principle was introduced by Maree *et al.* (1997) as the single-stage, completely-mixed reactor configuration, which removed sulphate and sulphide simultaneously, due to air diffusion into the reactor system.

2.6 MICROORGANISMS IN THE ANAEROBIC BIOREACTOR

2.6.1 Sulphate Reducing Bacteria (SRB)

Ten genera of dissimilatory SRB are currently recognised and are placed in two broad physiological subgroups (Postgate, 1984, Rinzema & Lettinga, 1988, Madigan *et al.* 1997). The genera in group I, *Desulfovibrio*, *Desulfomonas*, *Desulfotomaculum*, and *Desulfobulbus* utilize lactate, pyruvate, ethanol, or certain fatty acids as carbon and energy sources, reducing sulphate to hydrogen sulphide. The genera in group II, *Desulfobacter*, *Desulfococcus*, *Desulfosarcina*, and *Desulfonema*, specialise in the oxidation of fatty acids, particularly acetic acid, reducing sulphate to sulphide. The SRB are all obligate anaerobes and strict anaerobic techniques must be used for their cultivation. SRB are widespread in aquatic and terrestrial environments that become anaerobic due to active decomposition processes. The best known genus is *Desulfovibrio*, which is common in aquatic habitats or water-logged soils containing abundant organic material and sufficient levels of sulphate. *Desulfotomaculum* consist of endore-spore forming rods primarily found in soil. *Desulfomonas* can be isolated from the mammalian intestine. Certain SRB, among which are the *Desulfosarcina*, *Desulfococcus* and certain species of *Desulfovibrio*, are unique in their ability to grow chemolithotrophically with H₂ as electron donor, sulphate as electron acceptor and CO₂ as sole carbon source (Autotrophic growth).

2.6.2 Acetogenic Bacteria (AB)

Acetate is an important intermediate degradation product in an anaerobic reactor and can be produced by both AB and homoacetogenic bacteria. Homoacetogenic bacteria are obligate anaerobes that utilize CO₂ as a terminal electron acceptor, producing acetate as the sole product of anaerobic respiration. Electrons for the reduction of CO₂ to acetate can be derived from H₂, a variety of C₁ compounds,

sugars, organic acids, alcohols, amino acids and certain nitrogen bases. Many homoacetogens can also reduce NO_3^- and $\text{S}_2\text{O}_3^{2-}$. However, CO_2 reduction is probably the major reduction of ecological significance (Madigan *et al.* 1997).

2.6.3 Methanogenic bacteria (MB)

The MB are CO_2 reducing bacteria, belonging to a major group of Archaea. They utilize H_2 as the electron donor, to produce CH_4 from CO_2 according to equation (2.3):



When growing on H_2 and CO_2 , the MB are autotrophic, with CO_2 serving as both carbon source and electron acceptor. In addition to CO_2 , some alcohols, formate, methanol and several methylamines can be converted to methane by certain MB species. The three classes of methanogenic substrates known are listed below:

- 1 The CO_2 substrates, CO_2 , CO and formate (HCOO^-)
- 2 Methyl groups (CH_3OH) (through reduction)
- 3 Acetate: CH_3COO^- (equation 2.4)



2.6.4 Cellulose degrading microorganisms

Cellulose degrading microorganisms are ubiquitous and are found in various environments including soils, sediments, compost heaps and the gut of vertebrate herbivores such as the ruminants (Coughlan and Mayer, 1992). They include protozoa, fungi and bacteria, aerobes and anaerobes, mesophiles and thermophiles. In the natural environment, cellulose is mainly oxidized by aerobic fungi and bacteria, producing CO_2 and water, while only 10% is converted by anaerobic microorganisms producing methane and carbon dioxide. The anaerobic digestion of cellulose utilising rumen fluid as the inoculum will be discussed in this thesis. The level of microorganisms in the rumen is as high as typically found in any other natural habitat. These bacteria are adapted to live in a slightly acidic environment between pH 5.5 and 7.0 at a preferred temperature of 39–40 °C. The steady supply of food and continuous removal of fermentation products and food residues maintain relatively constant conditions, in which an extremely dense population develops (Hungate, 1966). The diversity amongst rumen bacteria is striking, which may be due to the

complex feed intake by the ruminants. The feed typically contains carbohydrates, proteins, fats and numerous other organic compounds and minerals (Hungate, 1966).

Already in 1832, Karel Sprenger published that decomposition of plant materials in the rumen was known to give rise to volatile substances which, at that time, were assumed to consist of acetic and butyric acids. Hungate in 1966 writes “the ruminant and the rumen microbial population exist in an equally beneficial relationship in which many of the plant materials consumed by the mammalian host are digested and fermented by the rumen microbes to form chiefly carbon dioxide, methane and volatile acids.” The rumen is a complex ecosystem where microorganisms thrive in symbiotic relationship that facilitates fibre digestion. Therefore, anaerobic degradation of plant material can be executed efficiently using the bacteria, fungi and protozoa occurring in the rumen as they produce cellulose fibre degrading enzymes (Lee *et al.* 2000). Cellulose degradation in anaerobic environments can be carried out by different *Clostridium* species, producing glucose and cellobiose, which are then fermented to lactate, acetate, ethanol, CO₂ and H₂. Ljungdahl and Eriksson, (1985) described the fermentation of sugars to produce carbon dioxide and hydrogen according to Equation (2.5)



The hydrogen-utilizing bacteria assimilate hydrogen and use it for the reduction of CO₂ to acetate or methane, sulphate to H₂S or nitrate to ammonia or N₂. The end product of the degradation process depends on the nature of the hydrogen-utilizing bacterium in the second stage, which in our studies will be mainly the SRB, producing hydrogen-sulphide.

The anaerobic species of cellulose degraders comprise *Acetivibrio cellulolyticus*, *Bacteroides cellulosolvens* and *Fibrobacter succinogenes*, *Caldocellum saccharolyticum*, the *Clostridium* species, the *Erwinia* species and the *Ruminococcus* species. Ruminococci have been isolated from cattle and sheep rumen fluid in Africa, Europe and the USA (Hungate, 1966). Several species of the most primitive group of fungi (anaerobe Chytridomycete) are well known for their ability to degrade cellulose in the gastrointestinal tracts of ruminant animals (Carlile & Watkinson, 1997; Lynd *et al.* 2002).

Cellulose fibers are imbedded in a matrix of structural biopolymers, primarily hemicellulose and lignin, which comprise 20 - 35 and 5 - 30% of plant dry weight, respectively (Lynd *et al.* 1999). Many bacteria can grow on cellulose producing enzymes that catalyse the degradation of soluble derivatives of cellulose. However few bacteria synthesise the complete enzyme system, which can totally hydrolyse the crystalline material found in nature (Coughlan and Mayer, 1992). Hemicellulose is a plant carbohydrate, which forms a large percentage of the forage consumed by ruminants. Its digestion is similar to that of cellulose and is almost completely digested in the rumen (McAnally, 1942). In contrast to cellulose that is crystalline, strong, and resistant to hydrolysis, hemicellulose has a random, amorphous structure with little strength. It is easily hydrolysed by dilute acid or base, but nature provides an arsenal of hemicellulase enzymes for its hydrolysis (Marchessault & Sundararajan, 1993).

2.6.4.1 *Acetivibrio cellulolyticus*

This anaerobic bacterium attaches itself to cellulose and produces acetic acid, hydrogen, carbon dioxide and traces of propanol, butanol and ethanol. It was first isolated from municipal sewage sludge.

2.6.4.2 *Bacteroides cellulosolvens (Fibrobacter succinogenes)*

The genus of *Bacteroides* includes species of obligately anaerobic, mesophilic, non-sporeforming gram-negative rods (Holdeman *et al.* 1984). They form an important part of the cellulolytic rumen flora (*Bacteroides succinogenes*).

2.6.4.3 *Caldocellum saccharolyticum*

These species are thermophilic, anaerobic, cellulolytic bacteria (Reynolds *et al.* 1986). The best three isolated strains can hydrolyse cellulose and lignin cellulose, comparable to *Clostridium thermocellum*.

2.6.4.4 *Clostridium* species

Most clostridia are mesophilic, which includes *Clostridium cellobioparum*, which is isolated from rumen fluid.

2.6.4.5 *Erwinia* species

These species are responsible for the soft rot of crops, both in the field and in storage. The bacteria secrete hydrolytic enzymes, including pectinases, cellulases, proteases and nucleases into extracellular fluids.

2.6.4.6 Ruminococcus species

These species are after the Bacteroides (Fibrobacter) group, the most important cellulose-digesting of the rumen flora. These species of rumen origin ferment cellulose and various sugars, to produce acetate, formate, succinate, ethanol, hydrogen and carbon dioxide as major end products (Bryant, 1986).

2.7 PRODUCTS OF THE MICROBIAL ACTIVITY IN THE RUMEN

Most rumen species produce acetic acid as the final fermentation product. One fourth of the species forms propionate e.g. *Selenomonas*, which is usually second in abundance (Hungate, 1966, Bergman 1990). Ruminal VFA production is closely related to ruminal pH (Russell & Dombrowski, 1980). Hydrogen is a major intermediate in organic matter degradation in the ruminal ecosystem (Hungate, 1966; Wolin & Miller, 1988). It is produced by fermentative microorganisms and can potentially be used by MB, SRB and the AB, to produce acetate. Interspecies hydrogen transfer between H₂-producing and H₂-utilizing microorganisms allows growth of fermentative and hydrolytic microorganisms (Morvan *et al.* 1996). In the rumen, H₂ is used by MB to reduce CO₂ and produce CH₄, while in the sulphidogenic bioreactor it is used by SRB for biological sulphate reduction.

2.8 COMPETITION FOR SUBSTRATE IN THE ANAEROBIC REACTOR

When considering the affinity of the SRB, the AB and the MB for substrates such as acetate, CO₂ and H₂, it is evident that these groups of bacteria may out-compete each other for their preferred substrate. In the sulphate reducing stage, a complete reduction of sulphate to sulphide is desired. Channelling of reducing equivalents towards the SRB is enhanced by the ability of the SRB to effectively compete with other anaerobic bacteria for the available organic substrate and the sensitivity of other bacteria for sulphide (Lens *et al.* 1998b). The anaerobic process can become very complex in the presence of sulphate, because SRB will compete with MB for compounds such as formate and hydrogen, and with AB for compounds such as propionate and butyrate (Colleran *et al.* 1995). Until recently, only limited investigations have been conducted on the likely outcome of the competition between SRB and MB. Once the factors, influencing the outcome of this competition are known and applied, they can avoid the risk of process failure. Moreover, practical engineering manipulations could force the bacteria to either go the sulphidogenic or the methanogenic route.

O'Flaherty *et al.* (1998) studied the population structure of biomass from a full-scale anaerobic reactor after 5 years of operation, with the purpose to obtain an improved understanding of long-term competition between SRB and other anaerobic microorganisms, such as the MB, the AB and other (syntrophic) bacteria. The results showed that SRB carried out an incomplete oxidation of propionate to acetate. It was observed that the SRB and syntrophic bacteria competed for butyrate and ethanol. However, in the case of hydrogen, the SRB out-competed the MB, which confirmed the results of other studies, which demonstrated that H₂ and CO₂ are primarily used by the SRB, provided that sufficient sulphate is available (Visser, 1995). It is thought that the SRB keep the hydrogen concentration below the threshold level required by the MB (Lovley, 1985). Oude Elferink *et al.* (1994) showed that the hydrogen utilizing SRB (HSRB) gain more energy from the consumption of molecular hydrogen, have a higher substrate affinity, growth rate and cell yield than the hydrogen utilizing methanogenic bacteria (HMB). These authors also suggested that in the presence of sulphate, compounds, such as alcohols, lactate, propionate and butyrate, may be oxidized directly by the SRB without the intermediate formation of hydrogen. They presented the following conclusions from their investigation:

- SRB will compete with MB for hydrogen, formate and acetate.
- In general, SRB have better growth kinetic properties than MB, since SRB have a higher growth rate than the MB and the conditions in a sulphidogenic reactor generally favour the SRB
- Reactor conditions, such as pH, temperature, sulphate and sulphide concentrations, can influence the microbiological processes in the bioreactor and can determine whether these processes will proceed via the sulphidogenic or the methanogenic pathway.

O'Flaherty *et al.* (1998) further showed that acetogenic bacteria also played a role in the utilisation of H₂ and CO₂ in their study of the microbial activity in an anaerobic reactor. It was shown that even after 5 years of reactor operation, the SRB failed to out-compete the acetate utilizing MB. In general, the findings of O'Flaherty *et al.* (1998) were a confirmation by those of Harada *et al.* in (1994). They showed that when the sulphate concentration in the bio-reactor increased from 30 to 100 to 600 mg SO₄/ℓ, the SRB utilized almost 5, 30 and 40-75% of the COD present. It was observed that propionate accumulated significantly when no or low levels of sulphate were present. Therefore, it can be deduced that SRB strongly contribute to the degradation of propionate to acetate. The study of Harada *et al.* (1994) indicated

furthermore that the activity of the HMB decreased with increasing sulphate concentrations. It can be assumed that the SRB contribute to the degradation of propionate to acetate using hydrogen. It was also shown that the SRB were poor competitors of MB for acetate. Only during long-term operation, the SRB started to out-compete the MB for acetate.

Omil *et al.* (1997) also studied the competition between acetate utilizing MB and SRB, operating two UASB reactors, at a reactor pH of 8. The UASB reactors received VFA mixture of acetate, propionate and butyrate (5:3:2), on the basis of COD and only acetate, respectively, at different COD: Sulphate ratios. It was found that in the presence of excess sulphate concentration (COD: Sulphate concentration ratio < 0.67), the SRB became predominant in relation to the MB, when the reactors were operated from 250 to 400 days.

2.9 CARBON AND ENERGY SOURCES FOR BIOLOGICAL SULPHATE REMOVAL

Since the 1970's the application of anaerobic wastewater treatment has increased dramatically. Some of the advantages of anaerobic treatment over aerobic treatment are the low energy input required and the low sludge yield. The main advantage is that the end product of the anaerobic degradation of organic matter is the production of methane gas (CH_4), a potential energy source. However, as already indicated, when sulphate forms part of the organic waste, the SRB will use the available organic matter as their carbon and energy source to reduce sulphate with hydrogen sulphide, partly as gas and partly dissolved in the treated water, as the end product. Due to this reason, many operators of anaerobic treatment plants consider sulphate rich effluents troublesome, as during anaerobic treatment of these wastewaters, the reactor will turn sulphidogenic rather than methanogenic. When treating AMD or other sulphate containing industrial effluents, which contain no or insufficient electron donor and carbon source for a complete sulphate reduction, addition of an appropriate electron donor is required. The selection of the electron donor depends on the costs of the added electron donor per unit reduced sulphate and on the pollution potential of the additive in the waste stream.

2.9.1 Traditional carbon and energy sources

2.9.1.1 Sucrose

The studies of Greben *et al.* (2000a and 2000b) showed that sucrose can be used as the carbon and energy source for the biological sulphate removal. When operating a single stage completely mixed biological sulphate removing reactor, using sucrose as the carbon and energy source, the experimental volumetric and specific sulphate reduction rates (maximum) were determined to be 10.4 g SO₄/(l.d) and 0.79 g SO₄/(g VSS.d) respectively (Greben *et al.* 2000b). When sucrose is used as the carbon and energy source, the SRB can utilize sucrose and produce hydrogen (reaction 2.6), which can be utilized by the SRB, according to reaction (2.7). Some SRB species are unique in their ability to grow with H₂ as electron donor, sulphate as electron acceptor and CO₂ as sole carbon source.

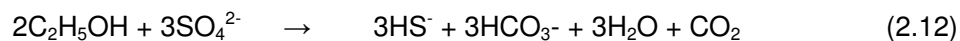
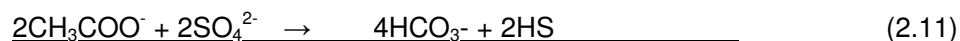
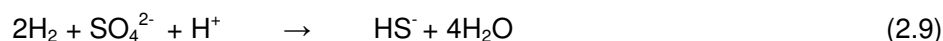


2.9.1.2 Ethanol

Ethanol in the presence of AB and SRB represents a substrate that can be oxidized to acetate, which then can be oxidized by the acetate utilizing SRB, such as *Desulfuromonas acetoxidans* and *Desulfobacter postgatei*. These microorganisms are often unable to metabolise lactate and pyruvate, but can oxidize ethanol completely to CO₂. The reactions involved are (2.8-2.12):



The produced hydrogen can be used as the energy source by the SRB in the presence of sulphate:



Ethanol has been identified as an intermediate during the degradation of organic matter in most anoxic ecosystems (Kaspar and Wuhrmann, 1978; Lovley *et al.* 1982; Schink *et al.* 1985). Szewzyk & Pfennig (1990) concentrated on the competition for ethanol by the SRB and other fermenting bacteria during their investigations. The

results in continuous culture showed that SRB are able to successfully compete with fermenting bacteria under low substrate concentrations. This confirms the important role of the SRB in the anaerobic degradation process. The study of Szewzyk & Pfennig (1990) showed that SRB compete successfully with the fermenting bacteria in the process of organic degradation.

De Smul *et al.* (1997) indicated that a sulphate reduction efficiency of 80-85% was obtained when the reactor pH was controlled above pH of 7.8, using ethanol as the energy source. They also found that in their reactors, the oxidation of ethanol proceeded mainly via acetate, but due to the higher reactor pH at 7.8, the ASRB out-competed the MB, confirming the findings of Visser (1995).

Greben *et al.* 2000b showed that ethanol could be used for the treatment of AMD in laboratory scale reactors obtaining a sulphate reduction rate of 6.8 g SO₄/ℓ.d, while Maree *et al.* (2004) described a sulphate removal rate of 12 g SO₄/ℓ.d operating a demonstration plant at Navigation Mine (Witbank, South Africa), using ethanol as the carbon and energy source to which sugar was added. Greben *et al.* (2002) indicated that adding 0.25 g/ℓ of sucrose to technical ethanol 96 % (1 ml ethanol/ℓ feed water) as the carbon and energy source resulted in a good sulphate reduction rate as well as in biomass growth.

2.9.1.3 Methanol

Braun & Stolp (1985) and Nanninga & Gottschall (1986) reported the use of methanol as electron donor for sulphate removal. Davidova and Stams (1996) researched the degradation of methanol in anaerobic sludge at temperatures over 60 °C. They found that a consortium of bacteria, obtained from anaerobic granular sludge could degrade methanol at 65 °C via sulphate reduction and acetogenesis. Tsukamoto and Miller (1999) proved sulphate reduction by using a combination of lactate and methanol as the substrate, followed by only methanol. Weijma (2000) described high sulphate removal rates of 4-7 g SO₄ (ℓ/d) at a HRT of 10 h when using methanol as the carbon and energy source operating under thermophilic conditions.

2.9.1.4 Hydrogen and Carbon dioxide mixture

Hydrogen gas is a clean and sustainable fuel, which can be considered an important alternative energy resource. Jules Verne, the well-known science fiction author, wrote as early as 1874 in his book “The mysterious Island” that hydrogen gas (H₂) is the “fuel” of the future. Hydrogen can be produced both chemically and biologically.

Verne (1874) indicated that it would be produced from a “plentiful” source, such as water. Water can be split by electrolysis into gaseous hydrogen and oxygen. To generate electricity for the electrolysis, other energy sources, such as coal combustion, have to be employed. Thus, in order to produce hydrogen chemically, another energy source needs to be provided, whereas for biologically produced hydrogen, fermented waste material can be used.

SRB can use hydrogen and CO₂ as energy source and carbon source, respectively, for the reduction of sulphate, which serves as the electron acceptor. The utilisation of hydrogen as energy source for biological sulphate removal has been reported by Du Preez *et al.* (1992) and van Houten, (1996). SRB have the advantage over MB, when H₂ is used as the energy source (Visser, 1995; Oude Elferink, 1998).

The study of Schutte & Maree (1989) reported on the autotrophic sulphate reduction using hydrogen. They operated both under batch and under continuous conditions, using the same upflow packed bed reactor for both experiments. The results showed that the sulphate removal efficiency was 91% at a hydraulic retention time (HRT) of 2.4 days. The need of the SRB for CO₂ was illustrated by omitting the CO₂, namely when the CO₂ flow to the reactor was stopped, the sulphate reduction ceased. When, however, the CO₂ flow was restored, the sulphate removal efficiency was brought back to previous levels. Schutte & Maree (1989) ascribed the dependence of SRB on CO₂ because in an anaerobic environment, syntrophic bacteria utilize carbon dioxide, forming intermediates such as lactate, ethanol and other carbon sources, which can then be used by SRB as source of carbon. The investigations of Van Houten (1996) confirmed this finding, showing that the hydrogen utilizing SRB (HSRB) are not autotrophic, so they do not assimilate CO₂ but are dependent on other anaerobes to produce acetate, which the SRB require as an additional carbon source. Acetate is formed by the homoacetogens, a group of obligate anaerobes, which utilize CO₂ as a terminal electron acceptor, producing acetate as the sole product of anaerobic respiration. Under H₂ limiting conditions, insufficient amounts of acetate become available for the HSRB, which may result in the predominance of HMB. It can also be assumed that under CO₂ limiting conditions, no acetate is produced, thus limiting or inhibiting the SRB respiration (Hulshoff-Pol *et al.* 1998).

When in the study, described by Schutte & Maree (1989), the hydrogen supply was stopped, the sulphate reduction ceased as well. This result led to the assumption that both growth and sulphate reduction seems to be strictly dependent on the

presence of an energy source (H_2). It can thus be concluded that when H_2 is available as the energy source and CO_2 as the electron acceptor for the homoacetogens, acetate will be produced, which the HSRB can use as carbon source for the reduction of sulphate present in e.g. the mine waste water.

Van Houten (1996) reported that the use of a mixture of H_2 and CO_2 (vol.:vol.as 80%:20%), resulted in a sulphate reduction rate of 30 g SO_4 /(l .d) This rate was achieved within 10 days of operation at 30 °C using a gas-lift reactor, which provided good gas-mass transfer rates, with pumice as carrier material for the SRB. When examining the structure of the biomass, the results showed that the *Desulfovibrio* spp. and the *Acetobacterium* spp. were the most abundant microorganisms present. This confirmed the assumption that H_2 , provided in the reactor, was consumed both by the SRB and the homoacetogens, which formed biofilms on the pumice particles.

2.9.1.5 Synthesis gas,

The studies of Du Preez *et al.* (1992), operating both under continuous as well as under batch conditions, showed that sulphate reduction was achieved using synthesis gas. This gas mixture can be generated from any material containing carbon and hydrogen and is readily available, as several industries dispose of it as a waste product. It originates from industrial sources such as steam and methane, through the oxidation of fuel oil and through coal gasification. The resultant mixture contains 29.7% hydrogen, 7.9% carbon dioxide, 59.1% carbon monoxide and 2.9% nitrogen gas and can be used as the energy and carbon source for SRB. When feeding this mixture, at 35 ml/min, to a 20 l packed bed reactor, with pelletized ash as support medium, an average sulphate reduction of 2 g SO_4 /day was achieved, feeding artificial SO_4 rich (1 350 mg SO_4 /l) feed water. When the SO_4 concentration in the feed increased to 2 000 mg SO_4 /l, the sulphate reduction was 3.3 g SO_4 /day.

2.9.2 Complex organic products as alternative carbon and energy sources

2.9.2.1 Complex organic products

Probably the cheapest carbon and energy source to be used in the biological sulphate reduction technology is sewage and other types of industrial waste liquors. McKinney and Conway (1957) discussed sulphate as a possible terminal electron acceptor for anaerobic biological waste treatment and Pipes (1960) developed a process with potential practical application using activated sludge. Domka *et al.* (1977) surveyed a variety of municipal wastes, such as sewage, dairy waste and

sugar plants as carbon and energy sources for biological sulphate reduction (Postgate, 1984). Although sewage is a relative cheap product, the question in South Africa is whether enough sewage is available in the areas where AMD is produced. Recently, Rose (2000) and Joubert (2005) applied the use of primary sewage sludge as the carbon and energy source for the biological treatment of sulphate in AMD, operating the so-called Rhodes Biosure process. It is based on the hydrolysis of complex carbon sources in a novel Falling Sludge Bed Reactor, providing an easily accessible feed for SRB activity.

Algae can be considered a bio-waste product and have been also been used as a carbon and energy source. Boshoff *et al.* (1996) have investigated the anaerobic fermentation of waste-grown micro algae produced in waste stabilisation ponds and the linked production of sulphide by SRB. Waste Stabilisation Pond (WSP) technology involves the large-scale application of algal photosynthesis and the role of SRB in the anaerobic compartments of these systems. The study of Rose *et al.* (1996) also described the biological sulphate removal from a tannery effluent using dried *Spirulina spp.* as the organic substrate.

2.9.2.2 Production of Volatile Fatty Acids from complex organic material

Volatile Fatty Acids are products of the anaerobic digestion of complex organic material, forming methane as the final product of the process. The effective conversion of complex organic material into methane depends on the combined activity of a diverse microbial population consisting of various genera of obligate and facultative anaerobic bacteria. Koster (1988) showed that the activities of the mixed population present in an anaerobic digester can be summarised as seven distinct processes:

- Hydrolysis of suspended solids
- Fermentation of amino acids and sugars
- Anaerobic oxidation of long-chain fatty acids
- Anaerobic oxidation of intermediary products, mainly volatile fatty acids, such as butyric acid (C4), Propionic acid (C3) and Acetic acid (C2).
- Non-methanogenic conversions of acetate and hydrogen
- Acetoclastic and acetotropic methanogenesis
- Hydrogenotrophic methanogenesis

These processes can be arranged as four distinct metabolic stages (McInerney *et al.* 1980) as depicted in Figure 2.2.

➤ *Hydrolysis*

During the hydrolysis process, complex, non-soluble organic compounds are solubilized by exoenzymes excreted by hydrolytic microorganisms. Basically, hydrolysis is the conversion of polymers into monomers.

➤ *Acidogenesis*

During the acidogenesis, soluble organic compounds, including the products of hydrolysis, are converted into organic acids, such as butyric, propionic and acetic acids.

➤ *Acetogenesis*

In the acetogenesis process, the products of the acidogenesis are converted into acetic acid, hydrogen and carbon dioxide.

➤ *Methanogenesis*

In the methanogenesis process, methane is produced from acetic acid or from hydrogen and carbon dioxide. Methane can also be formed from other substrates, of which methanol and formic acid are the most important.

For purposes of using degradation products of organic waste as carbon and energy sources for biological sulphate reduction, the hydrolysis/fermentation processes are the most relevant. The anaerobic degradation of organic material in a methanogenic reactor will differ from that in a sulphidogenic reactor, due to the presence of sulphate and SRB. When sulphate is present in the wastewater, the SRB are able to couple the oxidation of organic compounds and hydrogen to sulphate reduction (Oude Elferink, 1998). Therefore, for the purpose of this study, the oxidation of organic compounds will be presented as occurring in a sulphidogenic reactor.

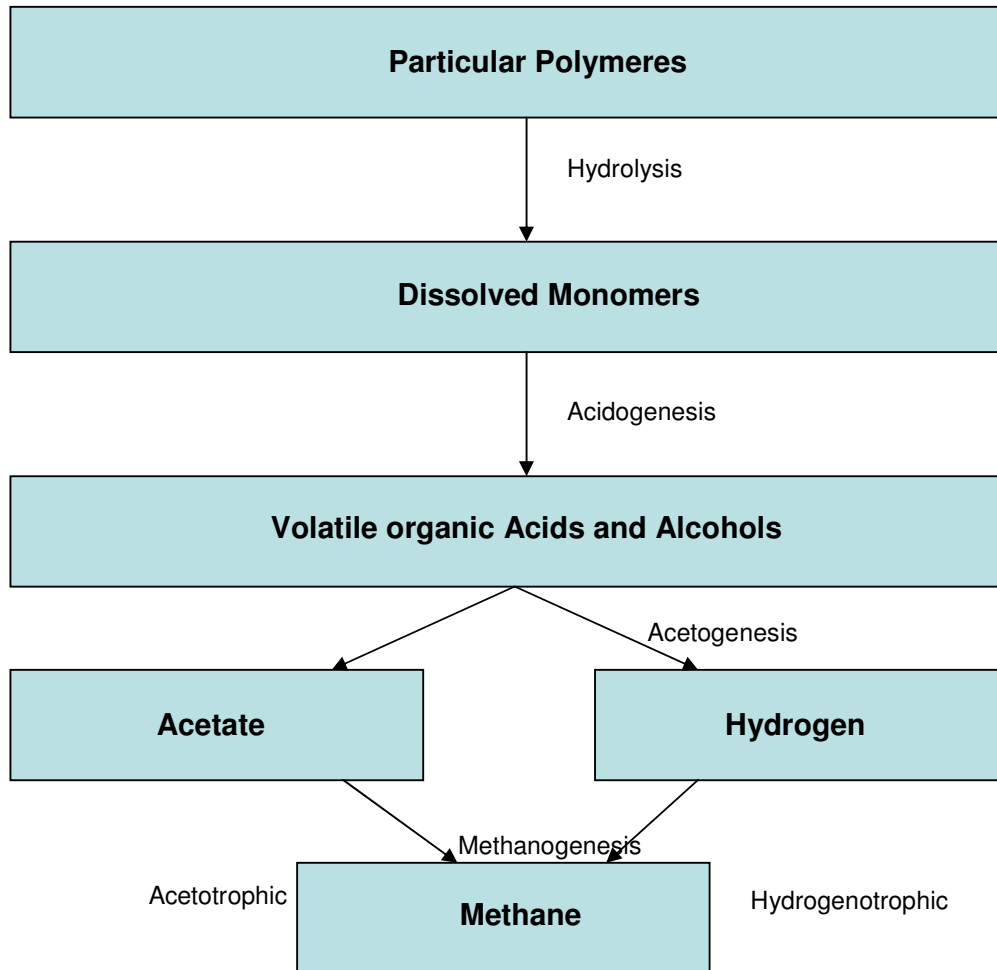


Figure 2.2 Metabolic stages and products in the anaerobic digestion of complex organic matter

Hydrolysis

In experiments on the fermentation of organic waste products to methane, the hydrolysis varied with the reaction time. Degradation percentages of 59% of the hemicellulose and 34% of the cellulose, respectively, were achieved when the reaction time was 15 days, whereas at 100 days reaction time, the degradation percentage increased to 96% and 76%, respectively (Lequerica *et al.* 1984).

The optimum pH for hydrolysis is different for various substrates. When degrading carbohydrates, the hydrolysis and acidogenesis processes proceed at maximum rates at pH 5.5-6.5 (Zoetemeyer *et al.* 1982; Zoetemeyer, 1982).

The rate and extent to which a substrate may be hydrolyzed is also influenced by the accessibility of the substrate by the exoenzymes (Hobson, 1982). This is especially true for the anaerobic digestion of fibrous materials, in which the cellulosic and hemicellulosic microfibrils are aggregated and embedded within the liquefied cell wall matrix. The crystallinity and surface area of the fibers are the most important features which determine the accessibility for exoenzymes (Fan *et al.* 1980). In some cases, physical pretreatment methods, such as heating or milling are applied. Alternatively, chemical treatment, comprising of scaling in NaOH is an option, whereas microbial pretreatment based on the capacity of “White Rot” fungi to degrade lignocellulose may be considered a more environmentally friendly option (Koster, 1988; Wicklow *et al.* 1980). In the hydrolysis step of the total degradation process, the particle size of the organic waste product influences the speed of the hydrolysis, due to the accessibility for the exoenzymes. In a fermentation reactor the methane production increased just over three times when the particle size was decreased from 20 to 1.3 mm (Hills and Nakano, 1984). In general, the slow rate of hydrolysis of organic waste products can be the limiting factor in the application of one-stage anaerobic digestion.

➤ *Fermentation process*

When defining the fermentation processes, one considers those processes that do not involve oxygen or nitrate as electron acceptors. Compared to aerobic processes, the anaerobic fermentation reactions result in smaller amounts of biomass attained per mole of substrate and the production of large amounts of fermentation products (Gottschalk, 1979). The fermentation products present after the degradation process depend on environmental conditions. When fermenting glucose in a separate acid producing reactor, the main products are butyric acid, acetic acid, hydrogen and carbon dioxide. However, when interrupting the feed supply for a period of 1–24 h, the fermentation pattern changed to an increased production of propionic and acetic acid. The study of Zoetemeyer *et al.* (1982) showed the influence of the pH on the fermentative bacteria. They showed that at pH values < 6 (pH=5.7), the main fermentation product of glucose is butyric acid, while the propionic acid concentration decreased. When the pH was increased, a successive change from butyric to lactic acid and subsequent change from lactic acid to acetic acid, ethanol and formic acid was observed. The product pattern of the fermentation also depends on the type of organic waste (Cohen, 1983). An increase in acetic, propionic and valeric acids was observed, when hydrolyzing and fermenting gelatine in a separate acid-producing reactor at pH values > 6.

Wolin (1976, 1979) showed the importance of hydrogen production and utilisation in the fermentation reactor. Removal of hydrogen, e.g. by the hydrogenotrophic bacteria, such as hydrogen consuming SRB (HSRB), can influence the kinds of products formed by the fermentative bacteria. When hydrogen is consumed by HSRB or HMB, the fermentative bacteria can produce further oxidized products than they would be able to at increased hydrogen levels, which supplies more energy per unit of substrate to the bacteria. This indicates that when HSRB are present, keeping the hydrogen partial pressure low, other organisms use the electrons generated in the fermentation process for hydrogen production rather than for the production of ethanol (Reddy *et al.* 1972). This observation may be significant when using the fermentation products of organic waste for the biological reduction of sulphate in the fermentation tank, when sulphate and SRB are present.

2.9.2.3 Anaerobic oxidation of Long-Chain Fatty Acids

The anaerobic degradation of long-chain fatty acids occurs by β -oxidation (Jeris & McCarty, 1965). When long chain fatty acids with an even number of carbons are oxidized, the fermentation products are acetate and hydrogen, but when acids with an uneven number of carbons are oxidized, the products are propionate and hydrogen. Anaerobic β -oxidation of long-chain fatty acids is thermodynamically unfavourable, unless the hydrogen partial pressure is maintained at a very low level (Hanaki *et al.* 1981). The affinity for hydrogen exhibited by HSRB is higher than that of HMB, and therefore the HMB are out-competed by HSRB in environments where a sufficient amount of sulphate is present (Robinson & Tiedje, 1984).

2.10 THE OXIDATION OF ORGANIC COMPOUNDS IN A SULPHIDOGENIC REACTOR

Compared to the MB, SRB are very diverse in terms of their metabolic capabilities. Acetate is the product, when oxidizing the C3 and C4 fatty acids, as is the case in the hydrolysis of the C3 and C4 fatty acids. The hydrolysis products as well as the oxidation products in the presence of sulphate of propionate and butyrate are acetate and hydrogen (Table 3.2). Both autotrophic and heterotrophic growth on hydrogen is possible. The hydrogen utilisation of the SRB will be discussed in a following section.

2.10.1 Fatty Acids in the sulphidogenic reactor

In 1928, Rubentschik as well as Baars isolated SRB capable of growing on fatty acids. Their work was in agreement with the observations of Hoppe-Seyler (1886) who had already documented the complete conversion of cellulose carbon, to carbon dioxide accompanied by sulphide production when sufficient sulphate was added. However, Postgate (1984) when reviewing the work done by Rubentschik and Baars referred to their findings as “historical errors”. Widdel and Pfenning (1977) confirmed that SRB appear to have a large share in the mineralization of organic material. They isolated several new species of SRB capable of growing on fatty acids (Rinzema & Lettinga, 1988). Since then, there has been no doubt that the SRB are able to oxidize VFA and that the SRB can use all important intermediates in the anaerobic degradation of organic matter (Table 2.2).

Another important factor in the competition of SRB, MB and AB is the COD/SO₄ ratio in the fermentation reactor. This ratio determines which part of the organic material can be degraded via the sulphate reduction. The COD/SO₄ ratio in the sulphate removing reactor indicates the COD concentration versus the sulphate concentration in the reactor (mg/l). The theoretical ratio value is 0.67, which indicates that, at that reactor ratio, all COD present will theoretically be used for the sulphate degradation. If the ratio is > 0.67, the MB and AB can participate in the degradation process as well. The propionate-oxidizing species of SRB (*Desulfobulbus propionicus*) can ferment lactate and ethanol in the absence of sulphate (Stams *et al.* 1984). Direct oxidation of hydrogen by the SRB and indirect hydrogen consumption by incomplete oxidation of propionate and higher fatty acids can be expected if sufficient sulphate is present. Under high sulphate concentrations, a sharp decrease in methanogenesis can be observed.

Both *Desulfobulbus propionicus* and acetogenic bacterial species grow on propionate. Visser (1995) showed that the propionate degrading AB are out-competed by the SRB due to the better growth kinetic property of the latter. He furthermore showed the crucial role of the SRB in the anaerobic degradation of butyrate and propionate in sulphate rich environments. When no sulphate is present the propionate concentration will increase in the reactor. The results of his study showed that the competition between the SRB and AB for propionate depends on the COD/SO₄ ratio. At COD/SO₄ ratios of about 10, the predominant route is a syntrophic oxidation of propionate by acetogens coupled to sulphate reduction by the

generated hydrogen. Under conditions of oversupply of sulphate (COD/SO₄ ratio of 0.5) the propionate is degraded mainly by direct oxidation by SRB.

Table 2.2. Acetogenic and methanogenic reactions, and sulphate-reducing reactions involved in the degradation of organic matter in methanogenic bioreactors and sulphate-reducing bioreactors, respectively.

Syntrophic Acetogenic reactions		
Propionate + 3 H ₂ O	→	Acetate ⁻ + HCO ₃ ⁻ + H ⁺ + 3 H ₂
Butyrate ⁻ + 2 H ₂ O	→	2 Acetate ⁻ + H ⁺ + 2 H ₂
Lactate ⁻ + 2 H ₂ O	→	Acetate ⁻ + HCO ₃ ⁻ + H ⁺ + 2 H ₂
Ethanol + H ₂ O	→	Acetate ⁻ + H ⁺ + 2 H ₂
Methanogenic reactions		
4 H ₂ + HCO ₃ ⁻ + H ⁺	→	CH ₄ + 3 H ₂ O
Acetate ⁻ + H ₂ O	→	CH ₄ + HCO ₃ ⁻
Sulphate-reducing reactions		
4 H ₂ + SO ₄ ²⁻ + H ⁺	→	HS ⁻ + 4 H ₂ O
Acetate ⁻ + SO ₄ ²⁻	→	2 HCO ₃ ⁻ + HS ⁻
Propionate ⁻ + 3/4 SO ₄ ²⁻	→	Acetate ⁻ + HCO ₃ ⁻ + 3/4 HS ⁻ + 1/4 H ⁺
Butyrate ⁻ + 1/2 SO ₄ ²⁻	→	2 Acetate ⁻ + 1/2 HS ⁻ + 1/2 H ⁺
Lactate ⁻ + 1/2 SO ₄ ²⁻	→	Acetate ⁻ + HCO ₃ ⁻ + 1/2 HS ⁻ + 1/2 H ⁺
Ethanol + 1/2 SO ₄ ²⁻	→	Acetate ⁻ + 1/2 HS ⁻ + 1/2 H ⁺ + H ₂ O

2.10.2 Competition for propionate and butyrate

As already indicated, in the anaerobic fermentation reactor in which a high sulphate concentration is present, the SRB will compete with the AB for butyrate and propionate. It is expected that for wastewater with an excess of sulphate, the SRB become predominant, because of their better growth kinetic properties. SRB grow much faster when sulphate is present than the syntrophic consortia (Oude Elferink, 1998). The C3 and C4 fatty acids are oxidized to acetate and hydrogen by the AB, followed by the hydrogen conversion via sulphate reduction. Harmsen *et al.* (1996) showed direct propionate oxidation by the SRB.

Other studies have shown that the SRB can be present (up to 15%) in the methanogenic sludge of the total biomass in an anaerobic fermentation reactor, even when no sulphate is present (Raskin *et al.* 1996). Under those conditions, the SRB grow similarly to the AB by oxidizing ethanol and lactate to acetate. Certain SRB can in the absence of sulphate, oxidize propionate in syntrophic association with hydrogen consuming anaerobes, while in the presence of sulphate they couple propionate to sulphate reduction. Growth of SRB on butyrate without the presence of sulphate has so far not been demonstrated (Oude Elferink, 1998).

2.10.3 Propionate utilisation treating sulphate rich effluent.

The study of Ghigliazza *et al.* (2000) concentrated on the biological treatment of gypsum-rich wastewater, using propionate as the organic carbon source. This carbon source was chosen as it is an important intermediate product, commonly found in anaerobic fermenting processes. The results of this study indicated that at a Feed: $Prop/SO_4^{2-}$ ratio of 1.31, a 99.5% SO_4 removal at a HRT of 2 days could be achieved. This ratio could approach 1, after a longer acclimatization period. This finding agreed with others indicating that sulphate removal efficiency improves with time (Visser, 1995). While good propionate utilisation as well as efficient sulphate reduction was observed, the acetate concentration increased to constant levels as high as 1.2 g/l. Utilisation of the produced acetate for further sulphate removal would be beneficial.

2.10.4 Acetate degradation

Acetate is the degradation product of the acetogenesis of the higher fatty acids ($>C_2$) and of the sulphidogenic activities of the propionate and butyrate utilizing SRB, mainly in the presence of sulphate. A specific ASRB (*Desulfotomaculum acetoxidans*) has been isolated from manure, rumen content and fresh water sediments contaminated with manure. This bacterium has a temperature optimum at 36 °C and does not grow at <10 °C. This observation suggests that *D. acetoxidans* is primarily an intestinal microorganism, which most likely is present in digested sewage sludge, the most frequently used inoculum for anaerobic water treatment systems (Rinzema & Lettinga, 1988).

Acetate is the primary substrate for the MB, however, SRB interfere with methane production in the presence of sulphate. Anaerobic degradation of organic material is accomplished through a series of successive and parallel microbial processes. Besides methane, hydrogen sulphide (H_2S) can be an important end-product of this

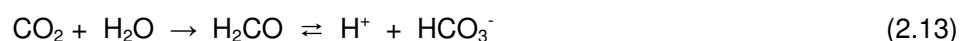
mineralization process (Rinzema & Lettinga, 1988). It has been discussed that when oxidizing propionate and butyrate, acetate is the end product. Visser (1995) and Omil *et al.* (1997) have shown that acetate is the most recalcitrant VFA under sulphidogenic conditions.

The studies of Greben *et al.* (2000a, 2000b) have shown that the remaining COD in the anaerobic sulphate removing reactor using sucrose and ethanol consist of acetate. Lens *et al.* (1998a) indicated that the acetate removal capacity is the limiting factor of sulphidogenic VFA removal, using different reactor systems (UASB and staged USSB). They had envisaged that in the staged reactor, the presence of acetate would allow the development an ASRB population. They concluded that the period of 138 days may not have been sufficiently long to allow the ASRB to multiply, since ASRB have a low growth rate. Visser (1995) also showed that ASRB require a long period of time to become a dominant population under sulphidogenic conditions. The results of his study confirmed that after prolonged operation of the reactor, the ASRB were able to out-compete the AMB for acetate. It took 250 days in the one reactor and 400 days in the other to observe the acetate concentration used by the ASRB increased from 50 till 90%. The results of Visser's study furthermore indicated that at a reactor pH > 7.7, the ASRB became the predominant organisms. Moreover, the ASRB can out-compete the AMB at sufficiently high sulphate concentrations in the reactor. It seems that the competition is mainly determined by the kinetic growth properties of the bacteria. Another important conclusion was that, when seed sludge cultivated on a substrate with low sulphate levels, the ASRB will be absent or only present in low quantities, whereas the SRB become the predominant organisms in the sulphidogenic reactor. The non-preference for acetate is a nutritional characteristic of SRB (Oude Elfink, 1998) and even ASRB e.g. *Desulforhabdus amnigenus*, still prefer propionate and butyrate to acetate.

Visser (1995) showed in his study that regarding acetate utilisation in a sulphidogenic reactor, contradictory results have been reported. Several factors are known to influence acetate utilisation in the reactor, such as the acetate and sulphate concentrations, the type of seed sludge, as well as the effect of temperature and pH. Similar observations were made regarding the growth of the propionate degrading sulphate reducers, which will decrease under sulphate limiting conditions.

- *Alkalinity production*

Methanogenesis as well as sulphidogenesis is dependent on the digester/reactor pH which preferably should be in the range 6.7-7.4 for the methanogenic activity. SRB can tolerate a similar pH range, however, they prefer a pH as high as 8.0-8.5. Under balanced reactor conditions, the biochemical reactions tend to automatically maintain the pH within the required pH range. The acidogenic reactions in the reactor would result in a pH reduction due to the production of organic acids. However, this effect is counteracted by the concomitant formation of bicarbonate buffering ions. The most important buffering system in anaerobic digestion is the equilibrium between dissolved carbon dioxide and bicarbonate (reaction 2.13).



- *Reactor temperature*

Anaerobic digestion can occur at two different temperature ranges, according to the two different groups of bacteria. The mesophilic MB grow and are active at temperatures up to 35-40 °C, while the thermophilic MB operate at temperatures over 50 °C, with an optimum between 55-75 °C. When the operating temperature is as low as 20-25 °C, the mesophilic MB population usually predominates.

- *Reactor type*

O'Flaherty *et al.* (1998) attributed the competition between the ASRB and AMB to the reactor type, since biomass retention promotes dominance of AMB over ASRB. Omil *et al.* (1997) reported a selective washout of SRB from UASB reactors, operating at up-flow velocities of 4 and 6 m/h. Iza *et al.* (1986) attributed the dominance of MPB over SRB in anaerobic filters to the inferior attachment capacity of ASRB. Another consideration may be that only a small numbers of ASRB are present in the seed sludge and it may take a long time for SRB to eventually displace the AMB. However, O'Flaherty *et al.* (1998) showed that the AMB out-competed the ASRB even after 5 years under full-scale conditions, when treating a sulphate containing citric acid production wastewater at a COD/SO₄ ratio of 12 g COD/1.4 g SO₄²⁻/ℓ, which ratio will favour the AMB.

2.11 BIOLOGICAL TREATMENT OF AMD: THE CHALLENGES

Mining contributes positively to the economy, but negatively to the environment, due to the production of contaminated effluents in the form of AMD, which should be treated so that it can either be discharged to a river system or re-used in the coal processing plant. Several treatment methods have been described, both in active

treatment plants as well as under passive conditions. The main pollutants in AMD are the acidity and salinity and in some cases high metal concentrations. The most cost effective treatment option to remove the acidity is to apply the limestone neutralisation technology, which will result in treated water with a neutral pH and a partial sulphate reduction to ≈ 2000 mg/l. In order to remove both the sulphate and the metals, the biological sulphate removal technology can be applied. The one product of biological sulphate removal is sulphide, which results in any heavy metals in the mine water being precipitated as metal-sulphides (MeS). The other product is alkalinity which assists in the pH increase of the treated water.

The most important factor for the biological sulphate removal technology is the need for a cost effective carbon and energy source (electron donor), while sulphate is the electron acceptor. Globally, many different carbon and energy sources have been described, varying from methanol, ethanol, sugar and gas mixtures, such as producer gas as well as a mixture of hydrogen and carbon dioxide. Recently, the emphasis has shifted to organic waste products, such as wheat straw, cow manure, mushroom compost and sewage sludge. These products all have cellulose in common. The advantage of the use of a bio-waste product is it can be used as energy source through the fermentation of cellulose to oligomers, monomers and ultimately volatile fatty acids, which then can be used as energy sources for biological sulphate removal.

2.12 CONCLUSIONS

The literature study has shown that the fermentation products from cellulose and hemicellulose, such as sugars, VFA, alcohols and hydrogen are favoured by SRB as carbon and energy sources. It has furthermore become evident that hydrogen as the final product of the degradation of organic product can be used by SRB in the reduction of sulphate and that the HSRB will out-compete the HMB for the utilisation of hydrogen in the presence of sulphate. SRB will select hydrogen, propionate, butyrate and acetate, in that order.

This information is important for the successful outcome of the study described in this thesis. The emphasis of the study therefore needs to be directed towards investigating which parameters are important for the production of VFA as well as the utilisation thereof for the biological sulphate reduction. The choice of the fermentative microbes to obtain the highest VFA production as well as the conditions under which

these microbes can be sustained will be investigated, as well as the use of the most applicable reactor system for maintaining a constant sulphate removal rate.

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