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Invited review

Bacteria of the sulphur cycle: An overview of microbiology, biokinetics and their role in petroleum and mining industries

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ABSTRACT

Bacteria of the sulphur cycle, in particular sulphate reducing and sulphide oxidizing bacteria, are of immense importance from the industrial and environmental point of views. While biogenic production of H_2S by sulphate reducing bacteria creates severe processing and environmental problems for the petroleum industry and agriculture sector, when used in a properly designed and controlled bioreactor sulphate reducing bacteria could play an instrumental role in the treatment of acid mine drainage, a major environmental challenge faced by the mining industry. Biooxidation of sulphide and intermediary sulphur compounds carried out by sulphide oxidizing bacteria are crucial in biotreatment of acid mine drainage and in the bioleaching of refractory minerals. Moreover, sulphide oxidizing bacteria are known as major players in the in situ removal of H_2S from the onshore and offshore oil reservoirs and are used in the ex situ processes for the treatment of sour gas and sulphide laden waters. Owing to the numerous environmental and industrial applications, the bacteria of the sulphur cycle have been subject of numerous studies. The present article aims to provide an overview of the microbiology, biokinetics, current and potential applications of the bacteria of sulphur cycle and the reactions which are carried out by these versatile microorganisms. Special consideration is given to the role of these bacteria in the biotreatment of acid mine drainage, oil reservoir souring and the treatment of H_2S-containing gaseous and liquid streams.

Contents

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1. Introduction

Microorganisms play an important role in the global cycle of various elements such as sulphur, nitrogen, carbon and iron. Sulphur occurs in variety of oxidation states with three oxidation states of −2 (sulphide and reduced organic sulphur), 0 (elemental sulphur) and +6 (sulphate) being the most significant in nature. Chemical or biological agents contribute to transformation of sulphur from one state to another. A biogeochemical cycle which describes these transformations is comprised of many oxidation-reduction reactions. For instance, H_2S, a reduced form of sulphur, can be oxidized to sulphur or sulphate by a variety of microorganisms. Sulphate, in turn, can be reduced back to sulphide by sulphate reducing bacteria. A simplified schematic of the microbial sulphur cycle demonstrating the fundamental reactions is presented in Fig. 1. The sulphur cycle consists of oxidative and reductive sides. Sulphate on the reductive side functions as an electron acceptor in metabolic pathways used by a wide range of microorganisms and is converted to sulphide. On the oxidative side, reduced sulphur compounds such as sulphide serve as electron donors for phototrophic or chemolithothrophic bacteria which convert these compounds to elemental sulphur or sulphate [1]. A situation in which the reductive and oxidative sides of this cycle are not in balance could result in accumulation of intermediates such as sulphur, iron sulphide and hydrogen sulphide. Sulphur disproportionation, carried out by some species of sulphate reducing bacteria and other highly specialized bacteria, is an energy generating process in which elemental sulphur or thiosulphate functions both as electron donor and electron acceptor. Sulphur disproportionation results in simultaneous formation of sulphate and sulphide [2]. In addition to the inorganic sulphur compounds, a vast array of organic sulphur compounds (i.e. sulphur containing proteins) are synthesized by microorganisms and considered part of the microbial sulphur cycle. Other organic sulphur compounds such as dimethyl sulfide, dimethyl disulphide, dimethyl sulfoxide, methanethiol, and carbon disulphide are also involved and affect the microbial sulphur cycle.

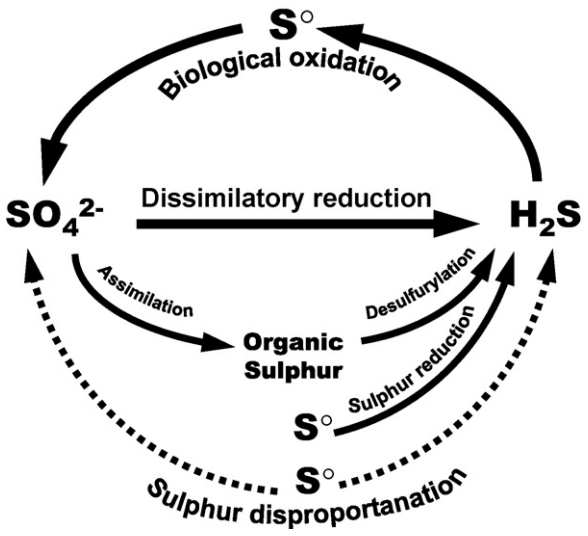

Fig. 1. Schematic representation of microbial sulphur cycle.

The bacteria of the sulphur cycle, specifically sulphate reducing and sulphide oxidizing bacteria play an instrumental role in many environmental and industrial settings. The activity of these bacteria in some cases creates severe environmental or processing problems, while their utilization under carefully controlled conditions could resolve and alleviate other processing and environmental problems, especially those encountered in the petroleum and mining industries. For instance, sulphate reducing bacteria are known as the causative microorganism for biogenic production of H_2S in oil reservoirs (souring) and the associated corrosion which occurs during the production, transportation and processing of the crude oil and various petroleum products. Generation and emission of H_2S from livestock operations, especially manure pits, has been partly attributed to the activity of sulphate reducing bacteria. On a positive note, sulphate reducing bacteria can be utilized in conjunction with sulphide oxidizing bacteria to tackle the problem of acid mine drainage, a severe environmental challenge facing the mining industry. Apart from the contribution in biotreatment of acid mine drainage, sulphide oxidizers play a key role in bioleaching of refractory minerals, in situ removal of H_2S from oil reservoirs and biological treatment of sour gases and waters contaminated with sulphide, with the latter being produced in large quantities in the enhanced oil recovery processes by water flooding. While sulphide oxidizers contribute in resolving a number of environmental and processing issues faced by the mining and petroleum industries, their negative impacts through unwanted oxidation of sulphide minerals and waste rocks, a major factor in generation of acid mine drainage in the first place should not be overlooked.

The present manuscript aims to provide an overview of the microbiology, biokinetics, current and potential applications of the bacteria of the sulphur cycle, specially in biotreatment of acid mine drainage, oil reservoir souring and the treatment of H_2S-containing gaseous and liquid streams.

2. Processing and environmental applications of sulphur cycle bacteria

2.1. In situ control of H_2S production in oil reservoirs

Biogenic production of H_2S in oil reservoirs subjected to water flooding (souring) is a serious concern for the oil industry. Toxicity of H_2S, accelerated corrosion of pipeline, production and processing equipment, and decrease in efficiency of secondary oil recovery due to plugging of the oil bearing strata by biomass and precipitated metal sulfides are some of the problems associated with souring. Furthermore, the necessity for the removal of H_2S prior to the use

of oil, gas, and before recycling of the produced water increases the cost of production. Sulphate reducing bacteria (SRB) are believed to be major players in souring of oil reservoirs. Thermochemical sulphate reduction and dissolution of sulphidic components of the reservoir rock are considered as other contributing factors [3,4]. Souring is observed both in shallow reservoirs where sulphate reduction by mesophilic sulphate reducers is prevalent and in deep offshore reservoirs where injection of seawater provides a source of sulphate for the activity of thermophilic SRB [5].

Strategies for control of souring in oil reservoirs include the removal of sulphate from water prior to injection [6], amendment of injection water with molybdate and nitrite [7–9], application of biocides such as glutaraldehyde, diamines and tetrakishydroxymethylphosphonium sulphate [10–12] and exposure of water to microwave and ultrasonic irradiations [13]. Although biocides are frequently used to tackle the souring and biocorrosion, their efficiency could be hindered by the presence of SRB in protective biofilms and the emergence of biocide resistant strains of SRB [10,12]. Toxic and corrosive nature of biocides is also a cause for concern [9,14]. In recent years a microbial approach relying on the amendment of injected water with nitrate or a combination of nitrate and nitrate-reducing, sulphide-oxidizing bacteria (NR-SOB) has emerged as an attractive option to control souring. Studies in model laboratory systems [5,10,15–26], and a number of field tests both in onshore and off shore reservoirs [27,28] have shown the effectiveness of this approach. Biooxidation of sulphide by NR-SOB resident in the oil reservoirs or those which are introduced together with nitrate, specially in the laboratory systems has been described as one of the underlying mechanism for the decrease in the sulphide level in oil reservoirs or model laboratory systems subjected to this treatment.

2.2. Treatment of acid-mine drainage and bioleaching of sulphide minerals

Mining and mineral processing generate large quantities of waste rocks and tailings, usually rich in sulphidic compounds. Exposure of sulphide minerals to air and water, and activities of indigenous microbial populations results in formation and release of acid mine drainage (AMD). AMD is an acidic stream which contains high levels of sulphate and metallic ions [29]. Generation of waste streams rich in sulphate and metallic ions is not limited to mining and mineral processing; other industrial activities such as flue-gas scrubbing, galvanic processes, battery, paint and chemical manufacturing discharge effluents with similar characteristics [30–32]. Formation of AMD and its release into natural waters has serious environmental impacts. Sulphate content of AMD contributes to the total dissolved solids of the receiving water. Under proper conditions sulphate may be biologically reduced to sulfide with associated problems of odor and severe corrosion risk. The acidic nature and presence of heavy metals can lead to permanent ecological damage of the receiving water body. Conventionally, AMD and other acidic sulphate-containing wastewaters are treated by passive methods or lime neutralization. The passive treatment usually takes place behind manmade dams or reed beds and is based on naturally occurring processes such as oxidation, reduction, adsorption and precipitation. Aerobic wetlands, compost wetlands and anoxic limestone drains are used for passive treatment of AMD. Large land requirements, build up of heavy metals in the wetland, formation of H_2S and sludge are some of the drawbacks of the passive treatment. Active treatment is based on the same fundamental processes governed in the passive treatment. However, in this case the efficiency of the process is increased by careful control of the process conditions. Limestone neutralization, ion exchange, liquid membrane extraction, reverse osmosis, solvent extraction and biological treatment are typical examples of active methods. Costs associated with liquid membrane extraction, reverse osmosis, solvent extraction has hindered the application of such approaches for the treatment of AMD. Active biological treatment of AMD and other wastewaters containing sulphate and metals, as represented in Fig. 2, consists of three main sub-processes. First, SRB convert the sulphate content of AMD to sulfide, using suitable carbon and energy sources. The produced sulfide is then mixed with the incoming AMD. This increases the pH and results in precipitation of metals as sulphide. In the absence of sufficient metal ions either an oxidizing agent or sulphide-oxidizing bacteria (SOB) are used to convert the remaining sulphide to elemental sulphur. Active biological treatment of AMD offers several advantages, including the permanent removal of sulphur and metals, production of clean water and possibility for the recovery of value metals.

Bioleaching of sulphide minerals is another process in which sulphide oxidizers play an important role. Although the original view which classified the bioleaching mechanisms as direct (direct oxidation of the sulphur moiety of the mineral by bacterial enzymatic system) or indirect (oxidation of metal sulphide by ferric iron and bacterial oxidation of the resulting ferrous iron) has gone thorough extended scrutiny and most importantly the indirect mechanism has been singled-out as the most relevant mechanism, the role of sulphide oxidizers in transformation of intermediary sulphur compounds, specifically sulphur to sulphuric acid is still recognized as one of the important sub-processes involved in the bioleaching of sulphide minerals [33].

2.3. Biological removal of H_2S from gaseous and liquid streams

Hydrogen sulphide (H_2S) is a highly toxic, corrosive and flammable gas with an unpleasant odour. Natural gas, whether produced from a condensate field or associated with an oil reservoir, frequently contains hydrogen sulphide [34]. Biogas, a value added product of anaerobic digestion of sludge and agricultural wastes also contains H_2S [35]. In the pulp and paper industry, exhaust gases from processing equipment such as rotary kilns, evaporators and washers used in the Kraft process contain H_2S [36]. In landfills, emission of gaseous pollutants such as H_2S generally occurs from ventilated pipes and landfill surfaces. Emission of H_2S from landfills has become more significant as landfills receive large quantities of construction and demolition wastes. Conversion of sulphate of the disposed gypsum is one the main reason for emission of H_2S [37]. Removal of H_2S from gaseous streams is essential prior to use to control corrosion during transportation and distribution, and to prevent sulphur dioxide emission upon combustion and subsequent acidic deposition [38–40]. Sulphide in the dissolved form is considered an undesirable component of many wastewaters, solid and liquid wastes such as those generated in the livestock operations, and in produced waters recovered from the oil fields subjected to water flooding [5,41–43]. Options for the treatment of sulphide-laden streams include well-established physicochemical processes such as Claus, Alkanolamine, Lo-Cat and Holmes-Stretford [36,44], and biological processes. Operation at high pressures and temperatures, as well as the need for expensive chemicals make the physicochemical processes energy and cost intensive. In addition, the physicochemical processes are generally developed for the treatment of gaseous streams and are feasible when large volumes of polluted stream with high sulphide content are treated. Biological methods, by contrast, operate around the ambient temperature and pressure, can handle smaller volumes of the contaminated stream and could remove sulphide even at low concentrations [45,46]. Biological alternatives for the treatment of sulphide-laden streams which rely on oxidation of sulphide to elemental sulphur or sulphate are categorized as direct and indirect. The indirect method relies on the oxidizing power

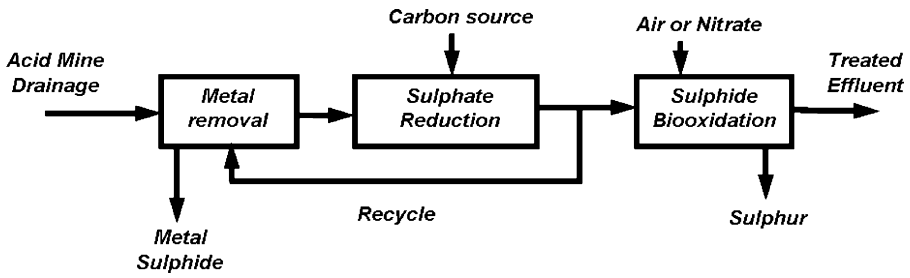

Fig. 2. Simplified flow diagram of AMD biotreatment process.

of ferric iron for conversion of sulphide to elemental sulphur, and the catalytic activity of iron-oxidizing bacteria for the regeneration of ferric iron [47]. In the direct approach, photoautotrophic or chemolithotrophic sulphide oxidizing bacteria convert the sulphide to elemental sulphur or sulphate [45,48–55]. Given the prevalence of sulphur compounds in various wastewaters, utilization of microbial fuel cell type reactors for the treatment of such streams could turn these wastewaters into a valuable source of energy. Recent studies have explored the idea of biological removal of sulphate and sulphide from waste streams in microbial fuel cell type reactors for the purpose of energy generation [56,57].

It appears that present and potential environmental and industrial applications for the bacteria of sulphur cycle are numerous. Anaerobic reduction of sulphate and biooxidation of sulphide are two key reactions in biological sulphur cycle and have a central role in many of these applications and therefore will be discussed in the remainder of this article.

3. Anaerobic reduction of sulphate, elemental sulphur and thiosulphate

3.1. Sulphate reducing bacteria (SRB)

Sulphide can be produced by anaerobic microorganisms as a result of the breakdown of proteins to amino acids and further degradation of amino acids to sulphide, or direct reduction of sulphate to sulphide by SRB. Sulphate reduction may occur through either assimilatory or dissimilatory pathways. The assimilatory pathway generates reduced sulphur compounds for biosynthesis of amino acids and proteins and does not lead to direct excretion of sulphide. In dissimilatory reduction, sulphate (or sulphur) is reduced to inorganic sulfide by obligatory anaerobic sulphate or sulphur reducing bacteria [58].

Assimilatory and dissimilatory reduction of sulphate both begin with the activation of sulphate by adenosine triphosphate (ATP). The attachment of sulphate to ATP, resulting in the formation of adenosine phosphosulphate (APS) is then catalyzed by enzyme ATP sulphurylase. In dissimilative reduction, the sulphate moiety of APS is reduced directly to sulphite (SO_3^{2-}) by the enzyme APS reductase. In assimilative reduction, another phosphorus atom is added to APS to form phosphoadenosine phosphosulphate (PAPS). PAPS is then reduced to sulphite. Once sulphite is formed, it is converted to sulphide by the enzyme sulphite reductase. In dissimilative reduction, the sulphide is excreted, while in assimilative reduction, the sulphide is incorporated into organic sulphur compounds [59].

SRB encompass a diverse group of obligate anaerobes which thrive in the anoxic environments containing organic materials and sulphate. SRB utilize organic compounds or hydrogen as electron donor in reduction of sulphate to sulphide according to Eq. (1) [58]. In most instances the electron donor and the carbon source are the same compound. However, when H_2 is used as an electron donor, supply of CO_2 or organic compounds such as acetate as the carbon source is required:

$$SO_4^{2-} + 8e^- + 4H_2O \rightarrow S^{2-} + 8OH^- \quad (1)$$

Sulphate reducing bacteria fall into three major branches: (i) the δ-subclass of proteobacteria (more than 25 genera), (ii) the gram positive bacteria (*Desulfotomaculum*, *Desulfosporosinus*), (iii) branches formed by *Thermodesulfobacterium* and *Thermodesulfovibrio*, with the sulphate reducers in the third branch (iii) being thermophilic, while the other two branches (i and ii) encompass psychrophilic, mesophilic and thermophilic species [60]. As far as the metabolic functionality is concerned, SRB are classified into two groups of complete oxidizers (acetate oxidizers) which have the ability to oxidize the organic compound to carbon dioxide, and incomplete oxidizers (non-acetate oxidizers) which carry out the incomplete oxidation of the organic compound to acetate and CO_2. Some species of the genera *Desulfobacter*, *Desulfobacterium*, *Desulfococcus*, *Desulfonema*, *Desulfosarcina*, *Desulfoarculus*, *Desulfoacinum*, *Desulforhabdus*, *Desulfomonile*, as well as *Desulfotomaculum acetoxidans*, *Desulfotomaculum sapomandens* and *Desulfovibrio baarsii* belong to the group of complete oxidizers [58,60–62]. The incomplete oxidizers include *Desulfovibrio*, *Desulfomicrobium*, *Desulfobotulus*, *Desulfofustis*, *Desulfotomaculum*, *Desulfomonile*, *Desulfobacula*, *Archaeoglobus*, *Desulfobulbus*, *Desulforhopalus* and *Thermodesulfobacterium* [59,62]. The growth kinetics for incomplete oxidizers is generally faster than the complete oxidizers. However, the former are less versatile as far as the nutritional requirements are concerned [61,62]. Sulphur-reducing bacteria, the other group of obligate anaerobes responsible for production of sulphide consist of genera such as *Desulfuromonas*, *Desulfurella*, *Sulfurospirrilium* and *Campylobacter*. These bacteria can reduce sulphur to sulphide but are unable to reduce sulphate to sulphide [60].

3.1.1. Electron donors (energy and carbon sources)

As reported by Lens et al. [63] and Rabus et al. [60], a variety of compounds could serve as electron donor and often simultaneously as carbon source for SRB. These include but are not limited to hydrogen, monocarboxylic acids such as formate, acetate, propionate, butyrate, lactate and pyruvate, dicarboxylic acids like malate, fumarate, succinate, alcohols including methanol, ethanol, 1-propanol, 2-propanol, 1-butanol, and glycerol, as well as acetaldehyde [60]. Amino acids, furfural, methylated nitrogen and sulphur compounds, polar aromatic hydrocarbons, aromatic hydrocarbons, and saturated hydrocarbons are among the other compounds which are utilized by SRB. Table 1 summarizes the chemical reactions and the standard free energies for oxidation of common organic compounds utilized by SRB. For further details the readers are referred to the article by Rabus et al. [60] which provides an excellent review on the metabolisms of various electron donors.

To increase the feasibility of the AMD biotreatment, attempts have been made to sustain the anaerobic reduction of sulphate using inexpensive carbon sources such as saw dust, hay, alfalfa, wood chips, manure, sewage sludge, peat, pulp mill, molasses and

Table 1
Oxidation of various electron donors coupled to reduction of sulphate and the corresponding Gibbs free energy [58].

Reaction		ΔG° (kJ/reaction)
Hydrogen : $4H_2 + SO_4^{2-} \rightarrow 4H_2O + S^{2-}$	(2)	−123.98
Acetate : $CH_3COO^- + SO_4^{2-} \rightarrow H_2O + CO_2 + HCO_3^- + S^{2-}$	(3)	−12.41
Formate : $4HCOO^- + SO_4^{2-} \rightarrow 4HCO_3^- + S^{2-}$	(4)	−182.67
Pyruvate : $4CH_3COCOO^- + SO_4^{2-} \rightarrow 4CH_3COO^- + 4CO_2 + S^{2-}$	(5)	−331.06
Lactate : $2CH_3CHOHCOO^- + SO_4^{2-} \rightarrow 2CH_3COO^- + 2CO_2 + 2H_2O + S^{2-}$	(6)	−140.45 or −178.06
Malate : $2(OOCCH_2CHOHCOO)^{2-} + SO_4^{2-} \rightarrow 2CH_3COO^- + 2CO_2 + 2HCO_3^- + S^{2-}$	(7)	−180.99
Fumarate : $2(OOCCHCHCOO)^{2-} + SO_4^{2-} + 2H_2O \rightarrow 2CH_3COO^- + 2CO_2 + 2HCO_3^- + S^{2-}$	(8)	−190.19
Succinate : $4(OOCCH_2CH_2COO)^{2-} + 3SO_4^{2-} \rightarrow 4CH_3COO^- + 4CO_2 + 4HCO_3^- + 3S^{2-}$	(9)	−150.48

compost [64]. Application of recalcitrant substrates like saw dust and wood chips together with a readily biodegradable compound such as manure or sludge usually results in improved performances [65–71]. Considering the inability of SRB to utilize complex organic substrates directly, the presence of other anaerobic bacteria capable of degradation of these compounds to simpler molecules is essential in sustaining the reduction of sulphate. Furthermore, the synergism and/or competition among acidogens, methanogens and SRB have been reported as the determining factors in the overall performance of a system utilizing these complex substrates [64,72].

3.1.2. Electron acceptors

In addition to sulphate, most species of SRB can utilize thiosulphate and sulphite as electron acceptors. Some species of SRB belonging to *Desulfohalobium*, *Desulfofustis*, *Desulforomusa* and *Desulfospirs* are reported to grow with elemental sulphur [60]. Reduction of sulphonates and dimethylsulphoxides by SRB has been demonstrated [73,74]. Other non sulphur-containing electron acceptors utilized by SRB include nitrate and nitrite [75,76], ferric iron [77,78], arsenate, chromate and uranium [79–81], and surprisingly O_2, considering the strict anaerobic nature of SRB [82,83].

3.1.3. Environmental pH

SRB are known to thrive in the environments with pH in the range 5–9 [84]. pH values outside this range usually results in reduced activity [64]. Visser et al. [85] reported that the sulphate reducers from an anaerobic reactor grew optimally at pH values in the range 6.9–8.5 and tolerated pH values as high as 10. The presence of SRB in various acidic environments such as sediments of acidic ponds and acid mine drainage, as well as isolation of acidophilic or acid tolerant strains of SRB have been reported by various researchers [31,86–90]. Fortin et al. [89] isolated an SRB strain from the acidic and slightly oxidizing environment in an abandoned mining site, although attempts to grow this strain at pH values below 5.5 was unsuccessful. Johnson et al. [88] reported the growth of an acid tolerant SRB strain belonging to *Desulfotomaculum* genus in an environment with a pH of 2.9. Kolmert and Johnson [31] observed that a mixed acidophilic SRB culture was able to grow at a pH of 3.0, supporting the view expressed by Postgate [58] that mixed SRB cultures are more tolerant of extreme conditions when compared with pure cultures. Recently Kimura et al. [91] reported the establishment of a defined mixed culture on glycerol, with the ability of dissimilatory reduction of sulphate at pH values in the range 3.8–4.2. The culture was comprised of a sulphate reducing bacterium with 94% gene identity to *Desulfosporosinus* and a non-sulphate reducer, which shared 99% gene identity with *Acidocella aromatica*. Despite the efficient treatment of acid mine drainage at pH values as low as 2.5 [92] and demonstration of sulphate reduction under very acidic conditions [87,88], the existence of the truly acidophilic SRB is yet to be proved.

3.1.4. Temperature

SRB encompass both mesophilic and thermophilic strains with the growth and sulphate reduction kinetics being affected significantly by temperature [93–95]. Stetter et al. [93] isolated a number of thermophilic strains of SRB from the Thistle reservoir. Using a mixed SRB population, Moosa et al. [96] showed a significant increase in sulphate reduction rate as temperature increased from 20 to 35 °C. Increase of temperature to 40 °C led to decreased bacterial activity. Tsukamato et al. [92] observed that the efficiency of acid mine drainage treatment was not affected by temperatures as low as 6 °C. Prolonged and successful operation of on-site reactors employing SRB at low temperatures in the range 2–16 °C [97] and 1–8 °C [98,99] has been reported. It should be pointed out that acclimation of SRB to low temperatures needs an extended period but once the population is acclimatized the effect of temperature becomes insignificant [92,99,100]. Table 2 summarizes the growth conditions for a number of sulphate reducers.

3.1.5. Inhibitory effects of metallic ions and sulphide

The activity of SRB is influenced by the presence of metallic ions. This is particularly important since acid mine drainage usually contains metallic ions such as iron, zinc, copper, manganese and lead which may be toxic or inhibitory to SRB employed for the treatment of such streams. The inhibitory and toxic level of metallic ions has been subject of several studies [86,108–118]. According to the results, heavy metals at low concentrations could promote the activity of SRB, while inhibitory or even lethal effects are observed at high concentrations [64]. Summarizing the available literature, Utgikar et al. [116] report the range of toxic levels, defined as concentration causing the cessation of sulphate reduction as: 2–50 mg Cu/L, 13–40 mg Zn/L, 75–125 mg Pb/L, 4–54 mg Cd/L, 10–20 mg Ni/L, 60 mg Cr/L, 74 mg Hg/L. One should note that the tolerance of metallic ions is species dependent [111,112,119] and that the simultaneous presence of metals such as Ni and Zn or Cu and Zn could induce synergistic or cumulative toxic effects [64]. Utgikar et al. [117] reported that the toxic effects of binary mixtures of Cu and Zn were significantly higher than what was expected based on the additive individual metal toxicity. Contrary to common belief that only soluble metallic ions can be toxic

Table 2
Temperature range for growth of a number of SRB.

SRB[a]	Temperature (°C)	
	Range	Optimum
Desulfobacter [61]	28–32	
Desulfobulbus [61]	28–39	
Desulfomonas [61]	–	30
Desulfosarcina [61]	33–38	
Desulfovibrio [61]	25–35	
Thermodesulforhabdus norvegicus [101]	44–74	60
Desulfotomaculum luciae [102]	50–70	
Desulfotomaculum solfataricum [103]	48–65	60
Desulfotomaculum thermobenzoicum [104]	45–62	55
Desulfotomaculum thermocisternum [105]	41–75	62
Desulfotomaculum thermosapovorans [106]	35–60	50
Desulfacinum infernum [107]	64	–

[a] All species listed in this table are neutrophile.

or inhibitory, Utgikar et al. [114] demonstrated that insoluble metallic compounds, especially metal sulphides, could affect the activity of SRB by deposition on the surface of the cells and blocking the access to the substrate and other nutrients.

Different sulphur compounds could also inhibit the activity of SRB with the inhibitory effect increases in the following order: sulphate < thiosulphate < sulphite < total sulphide < H_2S [64]. Sulphide can exist in different forms such as H_2S, HS^- and S^{2-} with the environmental pH being a determining factor in the proportion of the present ionic species. As stated by Lens et al. [63] at pH values up to 6.0 the produced hydrogen sulphide exists mainly in the undissociated form and as the pH increases it dissociates into HS^-. Thus, for environmental pH values in the range 6.0–9.0 a mixture of H_2S and HS^- exists in the solution and the level of H_2S decreases as pH is increased in this range. At pH values above 8.5 HS^- dissociates further to S^{2-} and eventually S^{2-} becomes the sole species at pH values above 10.

The exact mechanism of sulphide inhibition is not fully understood and different views exist. Generally, the inhibitory effect of sulphide has been attributed to either permeation of undissociated H_2S into the cells and destruction of the proteins thereby making the cell inactive [58], or reaction of H_2S with metals and precipitation as metal sulphide which deprives the SRB from the trace metals essential for activation of their enzymes [120,121]. However, the reversibility of sulphide inhibition shown in different works has challenged the validity of the first mechanisms [120,122]. Recently, Utkigar et al. [115] proposed the deposition of the metal sulphide on the bacterial cells as another reason for inactivity of SRB. Uncertainty also exists on whether total sulphide or only the undissociated H_2S should be considered when the subject of inhibition is investigated. Hilton and Oleskiewicz [123] observed the inhibition of SRB under alkaline condition and concluded that a direct relationship existed between the total sulphide concentration and the extent of inhibition. By contrast Reis et al. [120] demonstrated that the inhibition of SRB correlated better with the level of undissociated H_2S than total sulphide. This is in agreement with the theory stating that only undissociated H_2S could permeate through the bacterial cell membrane [124] and the observations by O'Flaherty and Colleran [125] who demonstrated that the increase of pH in the range 6.8–8.5 could lead to toleration of higher sulphide levels. The inhibitory levels reported in terms of total sulphide fall in the range 64–2059 mg/L [122,123,125,126], and those for undissociated H_2S vary from 57 to 550 mg/L [85,120,126].

3.2. Biokinetics of sulphate reduction and bioreactor configurations

A variety of reactor configurations such as stirred tank [96,127,128], up-flow anaerobic sludge bed (UASB), fluidized-bed [129–132] packed bed [20,30] and membrane [32,133] reactors have been used to study anaerobic reduction of sulphate and treatment of acid mine drainage.

3.2.1. UASB and fluidized-bed reactors

Utilization of ethanol by a mixed culture of SRB was investigated by Nagpal et al. in a batch stirred tank reactor [121] and a fluidized bed reactor [130]. In the stirred tank reactor ethanol was oxidized mainly to acetate and production of CO_2 was insignificant. Comparing the bacterial yield and growth observed with ethanol with those reported for the lactate in the literature indicated a lower yield and slower growth with ethanol. Utilization of SRB in a fluidized-bed reactor fed with ethanol led to a maximum sulphate reduction rate of 6.3 g/(L day) at a retention time of 5.1 h. The incomplete oxidation of ethanol led to an effluent with a high level of COD. Addition of an inoculum containing complete oxidizer *Desulfobacter posgatei* did not alleviate the problem.

Competition among thermophilic SRB, methanogens and acetogens was investigated by Weijma et al. [95] in an expanded granular sludge bed reactor operated at 65 °C and a pH of 7.5 with methanol as carbon source. Methanol was used mainly for reduction of sulphate and only at a minor level for methane and acetate productions. A follow-up study revealed that the system under investigation was capable of removing both sulphite and sulphate with the removal rates up to 21.1 g/(L day) and 14.4 g/(L day), respectively [134]. Using a similar system, Weijma et al. [135] showed that lowering the pH from 7.5 to 6.0 or decreasing the COD/SO_4^{2-} ratio from 6 to 0.34 favored the reduction of sulphate. The inhibitory effect of sulphide on methanogens was only observed when total sulphide concentration was above 1.2 g S/L.

Kaksonen et al. [131] investigated the treatment of an acidic waste stream containing zinc and iron in up-flow anaerobic sludge blanket (UASB) and fluidized-bed reactors, using lactate as carbon and energy source. In either case the maximum reduction rate of sulphate was around 2.3 g/L-day at a residence time of 16 h. The corresponding removal rate of zinc in UASB and fluidized-bed reactors was 0.35 and 0.25 g/(L day), respectively, while a similar removal rate for iron (0.08 mg/(L day)) was observed in both systems. In a relevant study, Kakasonen et al. [129] used ethanol and studied the removal of zinc and iron from an influent with a pH of 3.0 in a fluidized-bed reactor. The decrease in residence time in the range 20.7–6.1 h increased the rates for the reduction of sulphate, removal of the zinc and iron, and oxidation of ethanol, with the maximum rates being 2.6, 0.6, 0.3 and 4.3 g/(L day), respectively. The produced alkalinity led to a pH of 8.0 in the reactor. The accumulation of acetate was reported for retention times below 12 h. Using 16S rRNA gene cloning libraries and denaturing gradient gel electrophoresis (DGGE) fingerprinting, Kakasonen et al. [136] identified a large number of proteobacterium sequences in the ethanol-fed reactor. Sequences clustering with *Nitrospira* phylum were abundant in the lactate-fed reactor. Some sequences from each reactor were closely related to known sulphate reducers including *Desulfobacca acetoxidans*, *Desulforhabdus amnigenus* and *Desulfovibrio*.

3.2.2. Packed-bed reactors with inert packing

Treatment of an acidic lignite mine water was reported by Glombitza [137] who used immobilized SRB in a fixed-bed reactor fed with methanol. Based on the results, a three stage pilot scale process similar to what presented in Fig. 1 was designed. Glombitza et al., however, used hydrogen peroxide for oxidation of excess sulphide to sulphur. The system was operated successfully for several months with a metal removal close to 100% and an effluent with a pH of 6.9. Foucher et al. [138] used a two step process to treat a real effluent from Chessy–Les–Mines. In this process, a sulphate reducing fixed-bed reactor fed with a mixture of CO_2 and H_2 was used in conjunction with a gas stripper for separation of H_2S from the effluent. The stripped H_2S was then injected into a well-mixed reactor containing the mine effluent. Treatment of an actual mine effluent, initially cleared from its metal content through precipitation, resulted in 90–95% sulphate removal. The maximum sulphate reduction rate observed during the treatment of mine effluent was 0.2 g/(L h) at a residence time of 21.6 h.

Using various combinations of glycerol, lactate and ethanol as potential electron donors, Kolmert and Johnson [31] investigated the tolerance of acidic conditions of three populations of acidophilic SRB (a-SRB), neutrophilic SRB (n-SRB) and a mixture of acidophilic and neutrophilic SRBs in packed-bed bioreactors. Sulphate reducing capacity of the reactors containing a-SRB and mixture of a-SRB and n-SRB were similar and lower than that with n-SRB. Elimination of glycerol virtually had no effect. Subsequent elimination of lactate, however, decreased the reduction rate of sulphate to zero in reactors with a-SRB and mixture of a-SRB, n-SRB. The reactor with n-SRB remained unaffected when lactate was eliminated. The acid

tolerance of each population was evaluated by stepwise decrease of the influent pH from 4.0 to 2.25. Sulphate reduction rate was relatively constant especially in the reactor with a mixture of a-SRB and n-SRB for pH values around or above 3.0. With lower pH values sulphate reduction rate was insignificant in all three reactors.

Jong and Parry [30] studied the removal of Cu, Zn, Ni, Fe, Al, Mg and As in an up-flow packed-bed reactor with methanol as carbon and energy source. Activity of SRB increased the pH from 4.5 (in the influent) to 7.0 (in the effluent) and led to removal of at least 97.5% of Cu, Zn, Ni, 77.5% As and 82% Fe.

Baskaran and Nemati [29] carried out the anaerobic sulphate reduction in packed-bed reactors inoculated with a consortium of SRB enriched from the produced water of a Canadian oil reservoir. The reactor performance, as assessed by volumetric sulphate reduction rate, was dependent on the total surface area of the carrier matrix provided for passive immobilization of SRB. Among the three tested matrices (sand, biomass support particles and glass beads) sand displayed a superior performance and a maximum reduction rate of 1.7 g/(L h) was achieved at the shortest residence time of 0.5 h. At a constant feed sulphate concentration, increases in sulphate volumetric loading rate caused the reduction rate to pass through a maximum. Contrary to the pattern reported for the freely suspended cells [128], the increases in feed sulphate concentrations led to lower reaction rates with immobilized SRB. Wang and Banks [139] reported the effective treatment of an alkaline sulphate rich leachate originated from a landfill in an anaerobic filter with immobilized SRB. The inhibitory effects of accumulated sulphide on both SRB and methanogenic populations were overcome by dosing of the filter with $FeCl_3$. The reduction of sulphate was identified as the dominant mechanism responsible for the removal of COD from the leachate. The low production rate of methane (2 m^3 of for every 1 m^3 of treated leachate) together with the costs associated with $FeCl_3$ dosing and possible blockage of the filter with precipitated sulphide were identified as the main impediments in large scale application of the system.

3.2.3. Packed-bed reactors with organic containing packing

The suitability of oak chips, spent oak, spent mushroom compost, sludge from a waste paper recycling plant and organic rich soil for the treatment of an acidic waste was investigated by Chang et al. [140]. Although reactors packed with spent oak, spent mushroom compost and sludge outperformed the other waste materials in short term, the ultimate performance in all cases were similar. Cellulose polysaccharides were the main component of the waste materials consumed in the process. Considering the inability of SRB in direct utilization of cellulose, it was concluded that other anaerobes had converted the cellulose polysaccharides to fatty acids and alcohol which were in turn used by SRB. Harris and Ragusa [141] used a 50:50 mixture of finely cut rye grass as a rapidly decomposable organic and a high cation exchange clay soil as a pH buffering agent for the treatment of an acidic mine water. Application of this mixture increased the pH of AMD from 2.3 at the inlet to 5.0 near the top of the reactor and supported the establishment of an active SRB population over a short period.

Using column reactors, Waybrant et al. [67] investigated the effectiveness of permeable reactive barriers consisting of layers of silica sand, pyrite and organic material for the purpose of sulphate and metal removals from a simulated mine drainage with a pH of 5.5–6.0. Two organic mixtures, one consisting of leaf mulch, saw dust, sewage sludge and wood chips, and the other containing leaf mulch and saw dust were tested. Both mixtures supported the growth of SRB and removal of the Fe, Zn and Ni. However, sulphate reduction rate in the system packed with a mixture of leaf mulch saw dust, sewage sludge and wood chips decreased as the experiment progressed, while with a mixture of leaf mulch and saw dust a relatively constant sulphate reduction rate maintained.

Zagury et al. [71] evaluated the suitability of six organic materials including maple wood chips, sphagnum peat moss, leaf compost, conifer compost, poultry manure and conifer saw dust for reduction of sulphate and removal of metallic ions from a waste stream in batch systems. Each organic material, ethanol, a mixture of leaf compost, poultry manure and maple wood chip, as well as the same mixture spiked with formaldehyde were tested. The mixture of organics with and without formaldehyde was the most effective substrate followed by ethanol and maple wood chip, while the lowest sulphate reduction and metal removal rates was observed with poultry manure despite its high carbon content.

One of the problems associated with the use of inexpensive organic materials is the deterioration of the treatment process due to exhaustion of the organic components accessible to SRB. The possibility of recovering the activity in a reactor packed with spent manure through amendment with methanol and lactate was investigated by Tsukamoto and Miller [142]. While addition of either compound led to reactivation of the system, methanol was found to be more effective. In a pilot scale system with low sulphate and iron removal efficiencies (7% and 32%, respectively) amendment with ethanol increased the removal efficiencies of sulphate and metal to 69% and 93% respectively. In a subsequent study Tsukamoto et al. [92] compared the effects of ethanol and methanol amendments on reactivation of sulphate reducers residing in the spent manure matrix. The acclimation of SRB for utilizing ethanol was faster than that for methanol. Application of low temperatures and pH led to a longer acclimation period. Decreasing the temperature to values as low as 6 °C had little effect on the performance of the system when the bacteria acclimated to ethanol at room temperature.

3.2.4. Membrane reactors

Application of SRB for the treatment of acid mine drainage and other metal containing streams is limited by inhibitory effects of heavy metals and sulphide, and extreme acidity of the waste stream. To circumvent these issues Chuichulcherm et al. [32] proposed the use of an extractive membrane reactor which prevented the direct contact between the SRB and the waste stream. The system consisted of a fluidized-bed reactor with sulphidogenic population and a membrane reactor. The sulphide produced in the fluidized-bed was pumped to the shell side of the membrane reactor where sulphide diffused through the silicon rubber membrane and precipitated with the metallic ions in the wastewater flowing through the tube side. Operating this system with a synthetic waste stream containing 0.25 g zinc/L, resulted in 90% removal of zinc. Precipitation of zinc sulphide on the membrane surface was identified as the main draw back. The use of membrane reactors is equally important when hydrogen and carbon dioxide gases are used as electron donor and carbon source, respectively. In a conventional approach the mixture of these gases is injected directly into the sulphate reducing reactor. The necessity of compression and recycling of a large volume of gas to overcome the mass transfer limitations, as well as safety issues arising from the use of pressurized hydrogen are some of the drawbacks. The use of a membrane reactor in which the mixture of CO_2 and H_2 is injected into the tube side, while a waste stream flows through the shell side has been proposed as an attractive option by Tabak and Govind [133], who summarized the main advantages of this system as: facilitation of H_2 mass transfer due to larger surface area of microporous membrane when compared with the surface area of gas bubbles; preventing the contamination of the exhaust gases with H_2S; establishment of SRB biofilm on the surface of the membrane resulting in increased biomass hold-up, although this may act as a barrier against the transfer of gases through the membrane; lower capital and operating costs due to a smaller reactor volume and absence of a recycle stream.

Table 3 summarizes the performance of various reactor configurations as reported in different works. Included in this table are the

Table 3
Operating conditions and sulphate reduction biokinetics in various bioreactors used to treat sulphate-containing streams.

Reference	Source of bacteria	Bioreactor	Matrix for establishment of biofilm	Carbon and energy source(s)	Temperature (°C)	pH	Influent sulphate concentration (g/L)	HRT (h)	Sulphate volumetric reduction rate (g/(L h))
Moosa et al. [128]	Waste-water treatment plant	Continuous flow stirred tank	–	Acetate, peptone	35	8	1–10	90–48	0.007–0.017
Tabak and Govind [133]	Anaerobic digester sludge and New York/New Jersey harbor sediments	Gas sparged membrane reactor	–	CO_2 and H_2	25	8.3	5.4	Batch	0.025
Weijma et al. [95]	Sludge from a sulphate reducing reactor	Expanded granular sludge blanket	–	Methanol	65	7.5	3.8	3.5	0.625
Kaksonen et al. [131]	Methanogenic sludge and mine sediments	Up-flow anaerobic sludge blanket	–	Lactate	35	2.3–5.6	1–2.2	16	0.096
Nagpal et al. [130]	Mixed SRB	Fluidized-bed	Porous glass beads	Ethanol	–	6.9–7.3	2–2.5	5.1	0.264
Kaksonen et al. [129]	Methanogenic sludge and mine sediments	Fluidized-bed	Silica	Lactate	35	3–3.2	2	6.1[a]	0.179[a]
Baskaran and Nemati [29]	Produced water of an oil reservoir	Packed-bed	Sand	Lactate	22	7	1–5	0.5–2.7[a]	1.7–0.68[a]
			BSP	Lactate	22	7	1	5.3[a]	0.2[a]
			Glass bead				1	28.6[a]	0.04[a]
Jong and Parry [30]	Water from the wetland filter of a mine site	Packed-bed	Coarse sand	Lactate	25	4.5	2.5	16.2[a]	0.02[a]
Waybrant et al. [67]	Water from anaerobic zone of a creek	Packed-bed	Sand, pyrite, reactive mixture[b]	Reactive mixture[b]	–	6.5	3.7	–	0.005[a]
Glombitza [137]	Water from a lignite mine	Packed-bed	Porous ceramic carriers	Methanol	–	2.9–3.2	2	12[a]	0.13[a]
			Crushed lava rocks					4.2[a]	0.13[a]
Tsukamoto and Miller [142]	Spent manure	Packed-bed	Spent manure	Methanol	23–26	4.2	0.9	6.6[a]	0.067[a]
Foucher et al. [138]	–	Packed-bed	Special packing	H_2, CO_2, acetate	30	2.5	5.8[c] (0.6–0.8)	21.6[d]	0.2[d]
Lin and Lee [143]	Digested sludge	Packed-bed	Plastic ballast rings	Acetate	35	7	0.9	60[d]	0.013[d]
Kolmert and Johnson [31]	Derelict mine sites	Packed-bed	Porous glass beads	Ethanol, lactate, glycerol	–	4	1.4	49.3[d]	0.021[d]
Chang et al. [140]	Anaerobic digester fluid	Packed-bed	Waste material[e]	Waste material[e]	25	6.8	2.6	480[d]	0.005[d]

[a] Calculated based on the void volume of reactor.
[b] Leaf mulch and saw dust.
[c] Due to existence of a recycle stream the concentration of sulphate entering the reactor was around 0.6–0.8 g/L.
[d] Calculated based on the total volume of reactor.
[e] Oak chips, spent mushroom compost, organic rich soil, sludge from waste paper recycling plant.

source of microbial cultures, operating conditions such as pH, temperature and sulphate concentration and finally, the performance of the reactor in terms of volumetric reduction rate of sulphate. The variations in the microbial cultures and experimental conditions applied in each work complicate the accurate assessment and as such careful consideration is required when comparing the kinetic data reported in different works.

Large scale application of anaerobic sulphate reduction as a part of Paques Thioteq process for the treatment of metal containing effluents has been reported [144]. The Paques Thioteq process which has been tested in a zinc mine in North America consists of a biological stage in which elemental sulphur is reduced to sulphide under anaerobic conditions. The produced sulphide is then transported by a carrier gas into a second stage where it contacts with the metal containing effluent resulting in precipitation of metallic ions as sulphide.

3.3. Sulphate reducing bacteria in oil reservoirs

The ability of hydrocarbon metabolism in the absence of molecular oxygen has been reported for several species of denitrifying, ferric iron reducing and sulphate reducing bacteria [145,146]. The utilization of hydrocarbons by SRB is regarded as one of the main sources of sulphide and sulphur during the maturation of oil reservoirs, with sulphur being formed by incomplete oxidation of sulphide [147]. Using the sediments from Guaymas basin in the Gulf of California, Rueter et al. [148] developed an anoxic enrichment with sulphate reducing activity in the presence of crude oil at 60 °C. The culture also displayed sulphate reduction with n-decane as carbon source. A pure culture referred to as strain TD3 was isolated from this enrichment which had the ability to oxidize n-alkanes (C_6–C_{16}), with the best growth occurred in the C_8 to C_{12} range. Fatty acids from C_4 to C_{18} were also utilized by this strain but no growth was observed on H_2, ethanol or lactate. The optimal growth for TD3 which exhibited a new, deep branch within the sulphate reducing eubacteria of the delta subdivision occurred at 55–65 °C and a pH around 6.8. Rueter et al. [148] also used the water phase of a North Sea oil tank at Wilhelmshaven as an inoculum and developed a mesophilic sulphate-reducing enrichment with the ability to oxidize alkylbenzenes. Further work on this enrichment led to isolation of two new strains of sulphate reducing bacteria designated as strains oXyS1 and mXyS1, with o-xylene and m-xylene being the substrate for these strains, respectively [149]. In addition to o-xylene, strain oXyS1 was able to utilize toluene, o-ethyltoluene, benzoate and o-methylbenzoate, while strain mXyS1 oxidized toluene, m-ethyltoluene, m-isopropyltoluene, benzoate and m-methylbenzoate, as well as m-xylene. It was shown that both isolates were capable of anaerobic reduction of sulphate to sulphide in the presence of crude oil. Based on the sequence analyses of 16S rRNA genes, starin oXyS1 showed the highest similarities to *Desulfobacterium cetonicum* and *Desulfosarcina variabilis*, while the closest relative to strain mXyS1 was identified as *Desulfococcus multivorans* [149]. Enrichment of ethylbenzene-degrading sulphate reducing bacteria from the anoxic marine sediments of different locations in Western Europe (Canale Grande in Venice, Italy; the Bay of Arcachon, France; and the Wadden Sea in the North Sea at Horumersiel, Germany) and North America (Eel Pond in Woods Hole, Mass, USA; and Guaymas basin in the Gulf of California, Mexico) was reported by Kinemeyer et al. [150]. A pure culture, strain EbS7, which was isolated from the Guaymas basin enrichment showed complete mineralization of ethylbenzene coupled to reduction of sulphate. Strain EbS7 was closely related to marine sulphate reducing bacteria strains NaphS2 and mXyS1 which grew anaerobically with naphthalene and m-xylene, respectively. Strain EbS7, however, did not oxidize naphthalene, m-xylene or toluene. Phenylacetate, 3-phenyl propionate, formate, n-hexonate, lactate and pyruvate were reported as other compounds utilized by EbS7 [150]. Benzene-dependent anaerobic reduction of sulphate by a marine sulphate reducing culture originated from the sediments of a Mediterranean lagoon, Etang de Berr, France was reported recently by Musat and Widdel [151]. Phylogenic analysis indicated a high diversity of phylotypes related to sulphate reducing deltaproteobacteria, including *Desulfobacterium anilinii*, other *Desulfobacterium* spp., *Desulfosarcina* spp. and *Desulfotignum* spp.

Recent work by Kniemeyer et al. [152] suggests that SRB are also able to thrive in seep area and the gas reservoirs where short chain hydrocarbons such as propane and butane are plentiful. SRB can use these short chain hydrocarbons, thus altering the composition of the gas and contributing to production of sulphide. Using the sediments collected at hydrocarbon seep area in the Gulf of Mexico and the Guaymas basin in the Gulf of California, Kniemeyer et al. [152] enriched SRB cultures which thrived on propane or n-butane as the sole substrate at 12, 28 or 60 °C. Further work led to isolation of a mesophilic pure culture, designated as strain BuS5, that used only propane or n-butane and was affiliated with *Desulfosarcina*/*Desulfococcus*. The thermophilic enrichment growing at 60 °C on propane was dominated by *Desulfotomaculum* like SRB.

The ability of SRB in utilizing various hydrocarbons from crude oil has severe consequences for the petroleum industry both in the underground oil reservoirs and in the surface facilities. For instance the frequently observed increases in concentration of H_2S (souring) in the onshore and offshore oil reservoirs subjected to water flooding and the associated problems such as contamination of oil, gas and produced water with sulphide, plugging of the oil bearing rock formation and accelerated corrosion in the production, processing and storage facilities could be attributed to the activity of SRB [147]. Control of biogenic sulphide production which improves the quality of the produced oil and gas and decreases the cost of production could be achieved through elimination of sulphate from the water prior to injection, suppression of SRB with biocides or metabolic inhibitors such as nitrite and molybdate, and addition of nitrate to the injection water.

Reinsel et al. [153] reported that continuous addition of 0.71–0.86 mM nitrite to the Berea sandstone columns containing SRB from an oil field completely inhibited the production of H_2S. Using microbial cultures originated from the produced water of the Coleville oil field, Saskatchewan, Canada, Nemati et al. [7] observed that the inhibitory level of nitrite or molybdate was dependent on the composition of the SRB culture. With a pure culture of *Desulfovibrio* strain Lac6, H_2S production stopped by addition of 0.25 mM nitrite or 0.095 mM molybdate, while 4 mM nitrite or 0.47 mM molybdate was required in the case of a consortium of SRB. A combination of 2 mM nitrite and 0.095 mM had a similar effect on the SRB consortium. This confirmed the synergism of nitrite and molybdate in containment of souring as reported previously by Hitzman et al. [154].

Gardner and Stewart [12] studied the effects of glutaraldehyde and nitrite on biogenic production of H_2S in a continuous reactor with a mixed SRB biofilm originated from the produced water of the Chevron Lost Hills oil field in California. Following the establishment of biofilm and production of H_2S, the liquid medium was flushed from the bioreactor and the biofilm was exposed to a solution of 500 mg glutaraldehyde/L for 7 h. The production of sulphide resumed 73 h after reinstatement of the nutrient flow. Treatment with 1 mM nitrite suppressed the activity of SRB. However, with nitrite the recovery of the SRB biofilm was observed 28 h after reinstatement of the nutrient flow.

Inhibition of sulphide production by an SRB consortium originated from the produced water of Coleville oil field through application of nitrite, molybdate, and six biocides including bronopol (thiol inactivator), formaldehyde and glutaraldehyde (cross linking agents), benzalkonium chloride and cocodiamine

(cationic surfactants), and tetrakishydroxymethylphosphonium sulphate (THPS) was investigated by Greene et al. [155]. The level of the individual agents required to stop the production of sulphide were determined as 5, 3, 4, 6, 5 and 0.1 mM for nitrite, molybdate, bronopol, formaldehyde and glutaraldehyde, and THPS, respectively, 50 mg/L of benzalkonium chloride and 0.003% (v/v) cocodiamine. Synergism was observed when a mixture of two biocides or a combination of nitrite or molybdate with a biocide was used. The synergistic mixtures included glutaraldehyde and formaldehyde, cocodiamine and benzalkonium chloride. Bronopol, glutaraldehyde, and to a lesser extent benzalkonium chloride interacted synergistically with most other compounds. Considering the strong synergy observed between nitrite and glutaraldehyde, nitrite and benzalkonium chloride, nitrite and bronopol, Greene et al. recommended the use of nitrite with either of these biocides to decrease the required level of biocides and risk associated with biocide toxicity.

Addition of nitrate to the injection water is another option which has been proved successful in control of biogenic sulphide production both in the model laboratory systems and in the field tests conducted in onshore and offshore oil reservoirs. One of the earliest field tests was performed in the Coleville oil field, located in Saskatchewan, Canada in 1996 [156]. Continuous addition of 500 ppm ammonium nitrate to injection water over a period of 50 days resulted in complete removal of sulphide from one of the two injectors employed, and a 50–60% reduction in the sulphide content of coproduced water from two adjacent producing wells. Monitoring the dynamics of the microbial community by reverse sample genome probing (RSGP), Telang et al. [156] observed that application of nitrate increased the population of a nitrate reducing sulfide-oxidizing bacterium (NR-SOB) designated as *Thiomicrospira* strain CVO.

Using representative microbial cultures enriched from the Coleville produced water, Nemati et al. [5] reported that the addition of nitrate and an NR-SOB culture dominated by *Thiomicrospira* sp. CVO to a growing SRB consortium inhibited the production of sulphide by this consortium immediately. This was followed by oxidation and removal of the present sulphide. The addition of nitrate alone did not impose an inhibitory effect but stimulated the activity of the NR-SOB which were present at low concentration in the SRB culture, leading to the removal of sulphide. Based on the results of a follow-up study, Green et al. [8] suggested that the production of nitrite by NR-SOB during the oxidation of sulphide was the main reason for the observed inhibition. Furthermore, it was shown that the SRB which contained periplasmic nitrite reductase (Nrf) could overcome this inhibition by further reducing nitrite to ammonia [8,157]. Utilizing electrochemical techniques including potentiodynamic scan and linear polarization, and representative cultures from the Coleville oil field, Rempel et al. [23] studied the dynamics of the corrosion during the nitrate- and nitrite-mediated control of biogenic sulphide production. The addition of nitrate or a combination of nitrate and NR-SOB to a mid exponential phase SRB culture led to oxidation and removal of the present sulphide. Addition of nitrite inhibited the production of sulphide immediately and led to the removal of sulphide through nitrite mediated oxidation of sulphide. In all three cases accelerated corrosion rates occurred during the oxidation and removal of sulphide. With nitrate and NR-SOB or nitrate, corrosion occurred locally with the maximum corrosion rates being 0.72 and 1.4 mm $year^{-1}$, respectively. With nitrite extent of pitting was less pronounced and maximum corrosion rate (0.3 mm $year^{-1}$) was lower than those observed with other control methods.

In order to simulate the reservoir biological environment, Hubert et al. [20] used continuous up-flow packed-bed bioreactors inoculated with Coleville produced water and studied the impacts of nitrate and nitrite addition on production of H_2S by SRB biofilms. The amount of nitrite or nitrate required to prevent the activity of SRB was dependent on the level of the available electron donor, Na-lactate. Hubert et al. recommended the use of 0.7 mol nitrate or 0.8 mol nitrite per each mole of present Na-lactate to suppress the activity of SRB. Addition of nitrate did not change the composition of the microbial community, whereas application of nitrite led to emergence of two nitrate reducing strains, designated as NO_3A and NO_2B as the major members of the microbial community. Devising carbon steel coupons in continuous up-flow packed bioreactors with established SRB biofilm, Hubert et al. [21] observed that continuous addition of 20 mM nitrite or 17.5 mM nitrate stopped the production of H_2S. Nitrite addition eliminated the corrosion of carbon steel coupons, while in the presence of nitrate localized corrosion occurred, with the observed corrosion rates varied in the range 0.04–0.11 mm $year^{-1}$. These results were in agreement with those reported by Rempel et al. [23], implying that control of souring through addition of nitrite would be a preferred option in order to reduce the extent of corrosion. In a follow-up study, Hubert and Voordouw [25] isolated several NRB including *Sulfurospirillum* and *Thauera* spp. from the effluent of these bioreactors. It was shown that *Sulfurospirillum* sp. coupled the reduction of nitrate to nitrite and ammonia with oxidation of lactate or sulphide. Cocultures of *Sulfurospirillum* sp. strain KW with *Desulfovibrio* sp. starins Lac3, lac6, Lac15 indicated that heterotrophic nitrate reducing activity of *Sulfurospirillum* sp. strain KW and its ability to produce inhibitory levels of nitrite were the key factors in outcompetition of SRB in these cocultures.

Using most probable number (MPN) method, Eckford and Fedorak [16] examined the make-up of the nitrate reducing bacteria (heterotrophic NRB vs NR-SOB) in the produced water of five oil fields in the western Canada. The number of heterotrophic NRB was equal or greater than the number of NR-SOB in 80% of the tested samples. Nitrate amendment of the produced waters in some cases stimulated a large increase in population of heterotrophic NRB and NR-SOB and a rapid decrease in concentration of present sulphide, while with others only NR-SOB were stimulated and removal of sulphide was much slower [17]. Eckford and Fedorak suggested that stimulation of heterotrophic NRB was required for the rapid removal of sulphide from the oil field produced waters.

Okabe et al. [19] studied the effects of nitrate and nitrite on in situ production of sulphide in an activated sludge immobilized agar gel film. Measurements of O_2, H_2S, NO_3^- and NO_2^- concentration profiles by microelectrodes indicated that addition of nitrate or nitrite at concentrations in the range 0.3–1 mM forced the sulphide reduction zone into the deeper parts of the gel and reduced the extent of sulphide production. The in situ production of sulphide quickly recovered to the original levels as soon as the addition of nitrate or nitrite stopped. Okabe et al. concluded that the addition of nitrite or nitrate did not kill the SRB but induced competition between heterotrophic NRB and SRB for common electron donor and enhanced the oxidation of the produced sulphide.

Using aerobic bacteria, SRB, NRB and NR-SOB cultures originated from an oil field in Dahran, Saudi Arabia, Kjellerup et al. [26] studied the effects of nitrate (100 mg/L), nitrite (100 mg/L), and combination of nitrate (100 mg/L) and molybdate (35 mg/L) on biogenic production of sulphide in continuous flow reactors. Nitrite alone and a combination of nitrate and molybdate reduced the production of sulphide, while nitrate alone had no effect. Molecular techniques showed a diverse bacterial population in these systems but no shift in the composition of microbial community was observed following these treatments.

Myhr et al. [18] investigated the impacts of nitrite and nitrate addition on production of sulphide by an SRB consortium enriched from the produced water of Statfjord oil filed in North sea, using model columns containing crude oil as the carbon source. Injection of 0.5 mM nitrate or 0.12 mM nitrite for 2.5–3.5 months led

to complete elimination of sulphide from these systems. Kaster et al. [24] enriched two thermophilic SRB cultures, designated as NS-tSRB1 and NS-tSRB2, from the produced water of the Ekofisk in the Norwegian sector of North Sea. Sequencing of rDNA indicated the presence of *Thermodesulforhabdus norvegicus* in the NS-tSRB1 culture and *Archaeoglobus fulgidus* in the NS-tSRB2 culture. Nitrate at a concentration of 10 mM had no effect on production of H_2S by these cultures, whereas 0.25 mM nitrite inhibited the reduction of sulphide. Addition of 1 mM nitrite to up-flow packed-bed bioreactors with established biofilms of NS-tSRB1 or NS-tSRB2 at 60 °C reduced the concentration of the sulphide to a negligible level, whereas addition of 1 mM nitrate had no effect on H_2S production. Tests conducted at the Halfdan and Skjold oil fields in North Sea have proved the efficiency of nitrate addition in controlling the production of sulphide in these offshore reservoirs [27,28].

In summary, the results of research in the model systems and field tests reveal the efficacy of nitrate or nitrite addition in control of biogenic production of sulphide. Various mechanisms have been proposed for the decrease in sulphide level following the amendment of these systems with nitrate or nitrite. These include: (1) the preferential use of nitrate as an electron acceptor instead of sulphate by some species of SRB, (2) suppression of SRB activity as a result of competition between heterotrophic NRB and SRB for common electron donor, and outcompetition of SRB, (3) oxidation of present sulphide by NR-SOB, which either added or already present in the system, and (4) inhibition of SRB activity by added nitrite, followed by nitrite mediated oxidation of sulphide. As indicated earlier some species of SRB possess high nitrite reductase activity which allows them to overcome this inhibition by reducing nitrite to ammonia. The intermediate compounds such as nitrite, NO and N_2O which are produced during the reduction of nitrate by heterotrophic NRB or NR-SOB could also hamper the activity of SRB. It should be pointed out that in some cases more than one mechanism may be involved in the control of biogenic sulphide production.

4. Biooxidation of hydrogen sulphide and sulphur

The biological removal of sulphide from liquid or gaseous streams can be classified as direct and indirect methods. In the direct approach photoautotrophic or chemolithotrophic sulphide oxidizing bacteria use sulphide as an electron donor and convert it to sulphur or sulphate. Photoautotrophs use CO_2 as the terminal electron acceptor, while with chemolithotrophs oxygen (aerobic species) or nitrate and nitrite (anaerobic species) serve as terminal electron acceptors. In the indirect method oxidation of reduced sulphur compound is carried out chemically by ferric iron as the oxidizing agent, and iron oxidizing bacteria is used to regenerate the ferric iron for further use [47].

4.1. Photoautotrophic oxidation of sulphide

Phototrophic oxidation of sulphide is an anaerobic process which is carried out by green sulphur bacteria such as *Chlorobium,* and purple sulphur bacteria such as *Allochromatium* [59]. These bacteria utilize H_2S as an electron donor for CO_2 reduction in a photosynthetic reaction referred to as the van Niel reaction as described below [46,59]:

$$2H_2S + CO_2 \xrightarrow{\text{Light}} 2S^\circ + CH_2O\ (\text{carbohydrate}) + H_2O,$$
$$\Delta G^\circ = 75.36\ \text{kJ mol}^{-1} \quad (10)$$

Madigan and Martinko [59] characterize the photoautotrophic growth by two distinct set of reactions: the light reaction in which light energy is conserved as chemical energy, and the dark reaction in which CO_2 is reduced to organic compounds using the stored energy. This energy is supplied in form of adenosine triphosphate (ATP), while the electrons for reduction of CO_2 is supplied through NADH, which is produced by reduction of NAD^+ by electrons originating from sulphide, elemental sulphur or thiosulphate.

The majority of the purple sulphur bacteria store the produced elemental sulphur as globules within the cell. Further oxidation of sulphur results in formation and release of sulphate from the cells [59]. The purple sulphur bacteria encompass many genera such as *Chromatium*, *Thioalkalicoccus*, *Thiorhodococcus*, *Thiocapsa*, *Thiocystis*, *Thiococcus*, *Thiospirillum*, *Thiodictyon*, and *Thiopedia*. Of special interest are the genera *Ectothiorhodospira*, *Thiorhodospira* and *Halorhodospira* because unlike other purple sulphur bacteria, the sulphur produced by these bacteria resides outside the cell [59]. Although light seems to be the main source of energy for photoautotrophic sulphide oxidizers, lithoautotrophic growth in the absence of light has been documented for certain purple sulphur bacteria such as *Allochromatium vinosum* and *Thiocapsa roseopersicina* [158].

Green sulphur bacteria, encompassing key genera such as *Chlorobium*, *Prosthecochloris*, *Pelodictyon*, *Ancalochloris* and *Chloroherpeton*, use H_2S as an electron donor, oxidizing it first to elemental sulphur and then to sulphate. However, unlike the majority of purple sulphur bacteria, the produced sulphur resides outside the cell. In addition, due to the existence of the chlorosomes, an efficient light harvesting structure, green sulphur bacteria are able to grow and function at light intensities much lower than that required by any other phototrophic organisms [59].

4.2. Chemolithotrophic sulphide oxidation

The chemolithotrophic sulphide oxidizers (also referred to as colorless sulphur bacteria) have diverse morphological, physiological and ecological properties, and are able to grow chemolithotrophically on reduced inorganic sulphur compounds such as sulphide, sulphur and thiosulphate and in some cases organic sulphur compounds like methanethiol, dimethylsulphide and dimethyldisulphide [1,59].

The first step in oxidation of sulphide involves the production of sulphite through transfer of six electrons from sulphide to the cell electron transport system and subsequently to the terminal electron acceptor. The terminal electron acceptor is primarily oxygen, as many sulphur chemolithotrophs are aerobic. However, some species can grow anaerobically using nitrate or nitrite as the terminal electron acceptor. Oxidation of sulphite to sulphate could occur through two different pathways. In the most widespread pathway the enzyme sulphite oxidase transfers electrons from sulphite directly to cytochrome c with concomitant formation of ATP as a result of electron transport and proton motive force. In the second pathway sulphite oxidation occurs through a reversal of the activity of adenosine phosphosulphate reductase. This reaction produces one high energy phosphate bond by converting adenosine monophosphate (AMP) to adenosine diphosphate (ADP). When thiosulphate is used as electron donor, it is split into elemental sulphur and sulphite, both of which are then oxidized to sulphate [59].

The colorless sulphur bacteria encompass many genera such as *Thiobacillus*, *Acidithiobacillus*, *Achromatium*, *Beggiatoa*, *Thiothrix*, *Thioplaca*, *Thiomicrospira*, *Thiosphaera*, and *Thermothrix* to name a few. The genus *Thiobacillus*, one of the most studied groups, consists of several gram-negative and rod-shaped species which utilize oxidation of sulphide, sulphur and thiosulphate for generation of energy and growth [159]. Oxidation of reduced sulphur compounds generates significant acidity and thus several species of *Thiobacillus* are acidophilic. One such species, *Acidithiobacillus ferrooxidans* can also grow chemolithotrophically by the oxidation of ferrous iron. *Achromatium*, a spherical sulphur-oxidizer, is common in fresh water sediments containing sulphide. Similar to

Chromatium, *Achromatium* store elemental sulphur internally as granules which eventually disappear as sulphur is further oxidized to sulphate [59]. Organisms of *Beggiatoa* genus, residing in habitats rich in sulphide such as sulphur springs, decaying seaweed beds, and waters polluted with sewage exist in the form of long and gliding filaments of large diameters. The filaments are usually filled with sulphur granules. The growth of *Beggiatoa* and other filamentous bacteria can cause a severe settling problem, referred to as bulking, in wastewater treatment plants and industrial waste lagoons. *Thioploca* and *Thiothrix* are the other filamentous sulphur-oxidizing bacteria. *Thioploca* species, found in the ocean floor off the coast of Chili and Peru carry out the anoxic oxidation of sulphide with simultaneous reduction of nitrate. *Thioploca* has the ability to store significant amounts of nitrate intracellularly which supports the extended period of anaerobic respiration with H_2S as electron donor. *Thiothrix* is also a filamentous sulphur-oxidizer in which the filaments group together at their ends forming rosettes. In most other aspects *Thiothrix* resembles *Beggiatoa* [59].

4.2.1. Electron donors (energy and carbon sources)

In terms of energy and carbon sources, the colorless sulphide-oxidizers are classified into four groups. (i) Obligate chemolithotrophs need an inorganic source for energy, and use CO_2 as their carbon source. Despite the classification as "obligate" autotrophs, many species have been shown to benefit from small amount of supplemented carbon compounds [160,161]. Many species of *Thiobacillus*, at least one species of *Sulfolobus*, and all of the known species of *Thiomicrospira* belong to this category. (ii) Facultative chemolithotrophic sulphide oxidizers can grow either chemolithoautotrophically with carbon dioxide and an inorganic energy source, or heterotrophically with complex organic compounds as carbon and energy source, or mixotrophically using both pathways simultaneously. Some species of *Thiobacilli*, *Thiosphaera pantotropha*, *Paracoccus denitrificans* [162] and certain *Beggiatoa* [163] are typical examples of facultative chemolithotrophic sulphide oxidizers. (iii) Chemolithoheterotrophs are characterized by the ability to generate energy from oxidation of reduced sulphur compounds, while being unable to fix CO_2. A few species of *Thiobacillus* and some *Beggiatoa* strains fall into this category. (iv) Chemoorganoheterotrophs such as *Thiobacterium* and *Thiothrix* and some species of *Beggiatoa* can oxidize reduced sulphur compounds without deriving energy from them. These organisms use this reaction as a means for detoxifying the metabolically produced hydrogen peroxide [164,165]. As indicated earlier, sulphide, elemental sulphur, and thiosulphate are the most common sulphur compounds utilized by chemolithotrophic sulphide-oxidizers.

4.2.2. Electron acceptors

Oxygen is a universal electron acceptor used by the colorless sulphide oxidizers. However, the degree of aerobiosis that can be tolerated by different species varies. The electrons produced during the oxidation of sulphur compounds are transferred to the dissolved oxygen and O_2 is reduced to H_2O. The important reactions involved in chemolithotrophic oxidation of sulphide, sulphur and thiosulphate under aerobic conditions can be summarized as [59]:

$$H_2S + \tfrac{1}{2}O_2 \rightarrow S^\circ + H_2O, \quad \Delta G^\circ = -209.4\,\text{kJ/reaction} \tag{11}$$

$$S^\circ + \tfrac{3}{2}O_2 + H_2O \rightarrow {SO_4}^{2-} + 2H^+, \quad \Delta G^\circ = -587.1\,\text{kJ/reaction} \tag{12}$$

$$H_2S + 2O_2 \rightarrow {SO_4}^{2-} + 2H^+, \quad \Delta G^\circ = -798.2\,\text{kJ/reaction} \tag{13}$$

$${S_2O_3}^{2-} + H_2O + 2O_2 \rightarrow 2{SO_4}^{2-} + 2H^+, \quad \Delta G^\circ = -818.3\,\text{kJ/reaction} \tag{14}$$

Various colorless sulphur bacteria grow differently under anaerobic conditions, one of the best known pathways is the use of nitrate or nitrite as terminal electron acceptors. Oxidation of sulphide under denitrifying conditions could lead to formation of sulphur, sulphate and nitrite or nitrogen based on the following reactions [166]:

$${S^{2-}} + 1.6{NO_3}^- + 1.6H^+ \rightarrow {SO_4}^{2-} + 0.8N_2 + 0.8H_2O, \quad \Delta G^\circ = -743.9\,\text{kJ/reaction} \tag{15}$$

$${S^{2-}} + 0.4{NO_3}^- + 2.4H^+ \rightarrow S^\circ + 0.2N_2 + 1.2H_2O, \quad \Delta G^\circ = -191.0\,\text{kJ/reaction} \tag{16}$$

$${S^{2-}} + 4{NO_3}^- \rightarrow {SO_4}^{2-} + 4{NO_2}^-, \quad \Delta G^\circ = -501.4\,\text{kJ/reaction} \tag{17}$$

$${S^{2-}} + {NO_3}^- + 2H^+ \rightarrow S^\circ + {NO_2}^- + H_2O, \quad \Delta G^\circ = -130.4\,\text{kJ/reaction} \tag{18}$$

As stated by Cardoso et al. [166], conversion of sulphide to sulphate coupled to complete denitrification (Eq. (15)) consumes four times more nitrate when compared with conversion to sulphur (Eq. (16)). In the case of complete oxidation of sulphide to sulphate, complete denitrification to nitrogen (Eq. (15)) decreases the amount of required nitrate by a factor of 2.5 when compared with incomplete denitrification to nitrite (Eq. (17)). Oxidation of sulphur and thiosulphate under denitrification can be represented by the following reactions:

$$S^\circ + 1.2{NO_3}^- + 0.4H_2O \rightarrow {SO_4}^{2-} + 0.6N_2 + 0.8H^+, \quad \Delta G^\circ = -547.6\,\text{kJ/reaction} \tag{19}$$

$${S_2O_3}^{2-} + 1.6{NO_3}^- + 0.2H_2O \rightarrow 2{SO_4}^{2-} + 0.8N_2 + 0.4H^+, \quad \Delta G^\circ = -765.7\,\text{kJ/reaction} \tag{20}$$

A few species such as *Thiobacillus thioparus* can only reduce nitrate to nitrite, while others could carry-out the complete reduction of nitrate to nitrogen. *Thiobacillus denitrificans* and *Thiomicrospira denitrificans* are two known obligately chemolithotrophic sulphur bacteria with the ability of reducing nitrate to nitrogen. Of these two, *Thiobacillus denitrificans* is able to grow under either aerobic or fully anaerobic conditions. *Thiomicrospira denitrificans* grows well anaerobically but can grow aerobically only under extremely low oxygen concentrations [59,167]. To compare with these obligate chemolithotrophic species, the facultative species such as *Thiosphaera pantotropha* are less efficient in anaerobic growth. Some of the facultative species such as *Thiobacillus versutus* and *Paracoccus denitrificans* even lose their sulphide oxidizing capability under anaerobic conditions [59]. Sulphide-dependent reduction of nitrate to nitrogen has been shown for *Beggiatoa* [168]. Of the sulphur oxidizers belonging to archaebacteria, *Sulfolobus* species are more dependent on oxygen, although the use of ferric iron and molybdate as electron acceptor has been reported under microaerobic conditions [59]. The anaerobic growth with hydrogen as electron donor and sulphur as electron acceptor has been observed for members of the genus *Acidianus,* making these sulphur-oxidizers or sulphur-reducers depending on the prevailed conditions [169].

4.2.3. Environmental pH and temperature

Colorless sulphur bacteria are diverse as far as the growth pH and temperature are concerned. Growth at pH values in the range 1–9 and temperatures ranging from 4 to 90 °C have been reported. The acidophilic sulphur bacteria such as *Acidothiobacillus ferrooxidans* and *Thiobacillus acidophilus* are abundant in acid mine drainage

streams and are capable of mixotrophic growth on iron and sulphur components of pyrite. *Sulfolobus* and *Acidianus* are the other acidophilic sulphur bacteria which play an instrumental role in the process of bioleaching of sulphide minerals. One should note that the optimal pH varies among the species of sulphur oxidizing bacteria and the outcome of competition for common substrate in the mixed cultures is dictated mainly by pH.

The majority of well-studied chemolithotrophic sulphide oxidizers are mesophilic, the *Thiobacillus* being the only genera encompassing both mesophiles and thermophiles. Other important thermophilic genera include *Sulfolobus*, *Acidianus* and *Thermothrix.* Table 4 presents the growth conditions for a number of phototrophic and chemolithotrophic sulphide-oxidizing bacteria.

4.3. Kinetics of sulphide biooxidation

4.3.1. Phototrophic Biooxidation Kinetics

Studies on phototrophic biooxidation of sulphide in general identify the simultaneous control of gas flow rate and reactor photon flux as important factors in optimizing the van Niel reaction [38]. Kobayashi et al. [172] studied the removal of sulphide from an anaerobic waste treatment effluent by phototrophic bacteria in a packed column, as well as in a submerged system. At a retention time of 24 h and with a loading rate of 107 mg S^{2-}/day, 95% removal was achieved in the packed column. In the submerged system, at a retention time of 0.66 h and a sulphide loading rate of 36.2 mg/(L h), 98% of the sulphide was removed. The end product composed of both sulphate and elemental sulphur.

Kim and Chang [173] compared removal rate of H_2S in an immobilized-cell and sulphur-settling free-cell reactors, using *Chlorobium thiosulphatophilum*. Both fed-batch and continuous operations were studied. The immobilized-cell reactor achieved a removal rate of 0.26 μmol/(min mg protein L), which was higher than the removal rate of 0.11 μmol/(min mg protein L) in the free-cell reactor of the same volume. The removal rate for a larger free-cell reactor with cell recycle was 0.21 μmol/(min mg protein L). The light-energy requirements of the immobilized cell and free cell reactors for an H_2S removal rate of 2 mM/(L h) were 600 and 850 W/m^2, respectively.

Henshaw et al. [48] studied the biooxidation of sulphide by *Chlorobium limicola* in a suspended-growth CSTR. The system was able to achieve a sulphide removal rate of 3.2 mg/(L h), with 100% conversion to elemental sulphur. Using *Chlorobium limicola* in a fixed-film continuous flow photoreactor Henshaw and Zhu [45] obtained 100% conversion at a sulphide loading rate of 286 mg/(L h) with the end product being elemental sulphur. In a relevant study with *Chlorobium limicola* complete conversion of sulphide to elemental sulphur at a maximum loading rate of 1451 mg/(L h) was reported by Syed and Henshaw [174]. Syed and Henshaw [175] also compared the performance of a tubular fixed-film photoreactor with light emitting diodes (LEDs) and infrared light bulbs as the energy sources. Based on the modified van Niel curve generated for the LEDs and infrared bulb, Syed and Henshaw concluded that for the same light intensity, the system with LEDs was able to handle loading rates 1.3–1.7 fold higher than those for the system with infrared bulbs. The highest sulphide loading rate resulting in complete sulphide removal in the system with LEDs was 338 mg/(L h).

An enrichment of green sulphur bacteria was employed by Hurse and Keller [176] in a substratum-irradiated photosynthetic biofilm reactor. With a maximum sulphide concentration of 11.5 mg/L and flow rates in the range 1.11 and 1.18 mL/min, a maximum sulphide removal rate of 2.08 g/m^2 d was achieved. The end products of the sulphide oxidation were elemental sulphur and sulphate.

Borkenstein and Fischer [177] investigated the removal of sulphide by a mutated strain of *Allochromatium vinosum* (strain 21D) which was unable to oxidize intracellular sulphur to sulphate, making it ideal for a desulphurization process with sulphur as a by-product. The sulphide removal process consisted of three successive fed-batch sections. Each section was initiated with photoorganoheterotrophic growth using malate and acetate to achieve high cell concentrations. After each sulphide addition, the culture grew photolithoheterotrophically with malate/acetate and sulphide. The highest sulphide removal rate achieved in this system was 49.3 μM/h.

A summary of the recent works on biological removal of sulphide by phototrophic sulphide-oxidizers are presented in Table 5. The data include the microbial culture, reactor configuration and light source, operating conditions and reported removal rates, as well as the main end products. For the ease of comparison where possible, the reported rates have been recalculated in terms of a consistent unit of g/(L h).

4.3.2. Chemolithotrophic biooxidation kinetics

4.3.2.1. Aerobic biooxidation of sulphide. The chemolithotrophic biooxidation of sulphide has been investigated using a number of organisms including *Thiobacillus denitrificans, Thiomicrospira* sp. CVO, and *Acidithiobacillus thiooxidans* AZ11, as well as mixed cultures. Sublette and Sylvester [49] studied oxidation of sulphide by *Thiobacillus denitrificans* in a small scale reactor. At loading rates of 4–5 mmol H_2S/(h g) biomass, with an agitation rate of 300 rpm and an environmental pH of 7.0, H_2S was not detected in the outlet gas. No elemental sulphur was detected in the reactor and sulphate accumulated in the medium as H_2S was removed from the feed gas.

Ongcharit et al. [180] immobilized *Thiobacillus denitrificans* by co-culturing it with floc-forming heterotrophs and used it in a continuously stirred tank reactor (CSTR). The maximum sulphide removal rate in the CSTR with biomass recycle was 3.2 mmol/(L h). The sulphide was oxidized to sulphate. Lee and Sublette [51] employed the immobilized *Thiobacillus denitrificans* cells in an upflow bubble column and achieved complete sulphide removal at loading rates in the range 12.7–15.4 mmol/h. The product of sulphide oxidation in this case was also sulphate.

The effects of dissolved oxygen concentration (DO) on the composition of end products was studied by Annachhatre et al. [181] in a fluidized bed reactor. At DO concentrations greater than 0.1 mg/L, sulphate was the main product. Increasing the sulphide loading rate increased the production of elemental sulphur. At DO concentrations less than 0.1 mg/L, sulphur was the main end product. Sulphide removal greater than 90% was achieved at sulphide loading rates of 0.13–1.6 kgS/(m^3 day). In a similar study van der Zee et al. [182] observed that when oxygen was introduced into the batch cultures (initial molar ratios of O_2 to sulphide: 0.53, 1.1 and 3.5) sulphide disappeared rapidly, and elemental sulphur and thiosulphate were formed. Substantial sulphate formation was only observed after the second injection of oxygen and only at the highest tested ratio of 3.5. Alcantara et al. [54] utilized a microbial consortium primarily consists of *Thiobacillus* to oxidize sulphide in a recirculation reactor system in which sulphide oxidation and liquid aeration were spatially separated, allowing for control of the oxygen concentration. Alcantara et al. reported that oxygen to sulphide ratios of 0.5–1.5 would result in partial oxidation of sulphide to elemental sulphur, and ratios of 1.5–2 would lead to complete oxidation to sulphate. Extent of sulphide oxidation at ratios below 0.5 was low.

Huang et al. [183] studied biofiltration of H_2S by autotrophic bacterium *Thiobacillus* sp. CH11, and heterotrophic bacterium *Pseudomonas putida* CH11, isolated from a swine wastewater. Concentration of H_2S applied to these biofilters was 60 ppm. At flow rates ranging from 18 to 93 L/h (retention times of 145 and 28 s, respectively) more than 95% of H_2S was removed in both systems. However, the removal efficiency with the heterotrophic cells was lower than that with the autotrophic cells for all tested flow rates. The effect of H_2S concentration (0–200 ppm) on the removal

Table 4
Growth conditions for a number of phototrophic and chemolithotrophic sulphide oxidizing bacteria.

Microorganisms	pH		Temperature (°C)		Carbon source(s)
	Range	Optimum	Range	Optimum	
Photolithotrophic species					
Chlorobium limicola [170]	6.5–7.0	6.8	–	25–35	CO_2
Chlorobium tepidum [170]	–	6.8–7.0	32–52	47–48	CO_2
Allochromatium vinosum [171]	6.5–7.6	7.0–7.3		25–35	CO_2
Chemolithotrophic species					
Acidithiobacillus thiooxidans [171]	0.5–5.5	2.0–3.0	10–37	28–30	CO_2
Acidithiobacillus ferrooxidans [171]	1.3–4.5	2.5	10–37	30–35	CO_2
Thiobacillus thioparus [171]	4.5–7.8	6.6–7.2	–	28	CO_2
Thiobacillus denitrificans [171]	–	6.8–7.4	–	28–32	CO_2
Thiomicrospira denitrificans [171]	–	7.0	–	22	CO_2
Thiomicrospira denitrificans sp. CVO [167]	5.5–8.5	–	–	5–35	CO_2, acetate
Acidianus ambivalens [169]	1.0–3.5	2.5	–	80	CO_2
Acidianus brierleyi [169]	1.0–6.0	1.5–2.0	45–75	70	CO_2, yeast extract, peptone, tryptone, casamino acids
Solfolobus metallicus [169]	1.0–4.5	–	50–75	65	CO_2
Solfolobus acidocaldarius [169]	1.0–6.0	2.0–3.0	55–85	70–75	yeast extract, tryptone, casamino acids, sugars
Thermothrix thiopara [170]	6.0–8.5	–	73	60–80	CO_2, organic compounds
Thermothrix azorensis [170]	6.0–8.5	7.0–7.5	76–78	60–87	CO_2

capacity of the biofilter was tested at 28–30 °C, using a flow rate of 150 L/h. The highest removal capacity of 25 g S/(m^3 h) was achieved in the heterotrophic biofilter with 100 ppm H_2S. Increase of H_2S concentration to 150 ppm caused an abrupt decrease in the removal efficiency. The biofilter with autotrophic cells achieved greater removal rates as the inlet concentration of H_2S increased to 200 ppm.

Duan et al. [184] studied the treatment of H_2S using a horizontal biotrickling filter packed with *Acidithiobacillus thiooxidans* immobilized on activated carbon and operated at 25–30 °C. The maximum sulphide removal rate achieved in the filter was 113 g H_2S/(m^3 h) with a removal efficiency of 96%. The liquid flowing through the reactor had an initial pH of 4.5, while the pH of the effluent was in the range 1.0–2.0. Examining the mechanism of H_2S removal, Duan et al. [185,186] reported the adsorption and biooxidation of H_2S as the main processes involved in the removal of H_2S. Analysis of the sulphur species in the medium and those deposited on the activated carbon revealed that sulphate was the main end product.

Lee et al. [187] identified *Acidithiobacillus thiooxidans* strain AZ11 as a species capable of oxidizing sulphur and sulphide in the presence of high sulphate concentrations and in extremely acidic conditions. The optimal pH for sulphur oxidation was determined as 1.5 and a maximum sulphur oxidation rate of 21.2 g S/g cell dry weight day was observed in the presence of 4.2 g sulphate/L. Using *A. thiooxidans* strain AZ11 in a biofilter, complete sulphide removal at concentrations up to 2200 ppm and loading rates of 670 g/(m^3 h) was achieved.

Using microbial consortia obtained from three hot pools around Lake Rotorua in New Zealand, Datta et al. [188] studied biotrickling filtration of H_2S at 40, 50, 60, and 70 °C. The microbial consortia consisted of several species including *Oceanobacillus*, *Virgibacillus*, *Bacillus*, *Orchobactrum*, *Rhizobium*, and *Desulfitobacterium*. The biofilters were operated aerobically and pH was maintained in the range 4.0–5.0. Addition of glucose and/or monosodium glutamate improved the performance of the biofilters. The maximum removal capacity approached 40 g H_2S/m^3 h, at temperatures up to 70 °C.

Ng et al. [189] studied the removal of H_2S in batch reactors packed by *Thiomonas* sp. immobilized on activated carbon or teflon disks and achieved maximum removal rates of 0.01 mg H_2S/min g carbon and 0.002 mg H_2S/min g teflon, respectively. The removal rate observed with fresh activated carbon particles in the absence of the cells was 66% of that obtained with bacteria immobilized on activated carbon. Ma et al. [190] used *Thiobacillus denitrificans* immobilized on granular activated carbon in a packed column to remove H_2S from waste gases. The removal efficiency was greater than 98% when retention times maintained in the range 25–50 s. Additionally, for H_2S concentrations in the range 110–120 mg/L and the overall loading rates ranging from 1.3 to 20.6 mg S/(L h), removal efficiencies greater than 96.8% were achieved. The maximum removal rate obtained in the reactor was 666.7 mg H_2S/(L day).

Krishnakumar et al. [44] proposed the use of a reverse fluidized loop reactor for sulphide oxidation. The reactor consists of an outer tube enclosing a draft tube. The aeration regime inside the reactor created a loop flow between the tubes, fluidizing the carrier particles loaded with *Thiobacillus denitrificans*. It was reported that molar sulphide to oxygen ratios of 0.6–1.0 led to sulphur production. Given the difficulties in maintaining the sulphide to oxygen ratio at this narrow range, maintaining an optimum redox potential was proposed as a mean to control the oxidation state of the end product. Redox potentials in the range −300 to −200 mV were reported to maximize sulphur production. The operation of the reactor without controlling the environmental pH resulted in a sulphide conversion of 90% at the maximum loading rate of 20 kg/(m^3 day), while maintaining the pH at 8.0 resulted in 100% conversion of sulphide at a loading rate of 19 kg/(m^3 day).

The aerobic chemolithotrophic oxidation of sulphide has been used in the Shell–Paques process for the removal of H_2S from low, medium and high pressure natural gas streams. In this process, the H_2S-containing gas stream contacts with an aqueous solution of sodium hydroxide in an absorber. The H_2S is absorbed into this solution and the treated gas which usually contains less than 4 ppm H_2S leaves the absorber. The resulting aqueous solution is then transferred to an aerated reactor where the sulphide oxidizing bacteria (*Thiobacillus* species) converts the H_2S to elemental sulfur. The sulfur slurry which is produced may be used for agricultural purposes or purified to a high quality sulfur cake [191].

4.3.2.2. Anaerobic biooxidation of sulphide. McComas et al. [192] proposed an anaerobic enrichment culture originated from the produced water of Coleville oil field in Saskatchewan, Canada as a novel biocatalyst for removal of sulphide. The culture was dominated by *Thiomicrospira* sp. CVO and contained another novel species, *Arcobacter* sp. FWKO B. Freely suspended cells were cultured in a bench-scale fermentor at a pH of 7.4 and 32 °C. The maximum loading of sulphide handled by the system was 5.8 mmol H_2S/(g biomass h) which was comparable to that achieved in a system with *T. denitrificans* under similar conditions. The enrichment culture, however, was more tolerant of extremes in pH and elevated temperatures, as well as salinity when compared with *T. denitrificans*. In batch studies, elemental sulphur appeared to be the main

Table 5
Operating conditions and biokinetics of sulphide removal in various bioreactors with phototrophic sulphide oxidizing bacteria.

Reference	Bacteria or culture source	Bioreactor	Matrix for biofilm establishment	Electron acceptor	Light source	Temperature (°C)	pH	Treated influent	Volumetric removal rate (g/(L h))[a]	End product(s)
Kim and Chang [173]	*Chlorobium limicola*	Fed batch immobilized cell	Strontium alginate	CO_2	Incandescent light bulb	30	6.8–6.9	H_2S gas: 4.2%	0.055	Sulphur
Kim et al. [178]		Sulphur settling free cell recycle reactor	–						0.083	
Henshaw et al. [48]	*Chlorobium limicola*	Continuous flow stirred tank	–	Bicarbonate	Infrared light bulb	30	6.8–7.2	Sulphide solution: 0.55 g/L	0.003	Sulphur
An and Kim [179]	*Chlorobium limicola*	Solar optical stirred tank	–	CO_2	Metal halide lamp (day and night)	30	6.9	H_2S gas: 3.6%	0.73 (μmol/min)/(mg protein/L)	Sulphur
					Sunlight (day)-metal halide lamp (night)				0.41 (μmol/min)/(mg protein/L)	
					Sunlight (day)				0.28 (μmol/min)/(mg protein/L)	
Henshaw and Zhu [45]	*Chlorobium limicola*	Fixed film continuous flow	Tygon tubing	Bicarbonate	Infrared light bulb	27	6.8–7.2	0.142 g/L	0.284	Sulphur
Syed and Henshaw [174]	*Chlorobium limicola*	Fixed film continuous flow	Tygon tubing	Bicarbonate	Infrared light bulb	27–29	6.8–7.0	0.164 g/L	1.451	Sulphur
Syed and Henshaw [175]	*Chlorobium limicola*	Fixed film continuous flow	Tygon tubing	Bicarbonate	Infrared light bulb	27–29	6.8–7.0	0.068 g/L	0.255	Sulphur
					Light emitting diode			0.063 g/L	0.338	
Kobayashi et al. [172]	Domestic wastewater treated in an anaerobic filter in subdued sunlight	Packed-bed	Raschig ring	Carbonate	Tungsten light bulb	–	7.0	Sulphide solution: 0.02 g/L	0.75×10^{-3}	Sulphate
		Submerged tubular	–						0.063	
Hurse and keller [176]	Lake sediments, wastewater from anaerobic digester	Substratum irradiated biofilm	Hollow illumination panels	CO_2	Filtered light from an incandescent light bulb	21 ± 1.5	–	0.011	0.092 g/(m^2 h)	Sulphur and sulphate
Brokenstein and Fischer [177]	*Allochromatium vinosum* 21D	Fed batch stirred tank	–	Malate/acetate	Neon tube	30	6.9	Sulphide solution: 0.02–0.04 g/L	0.002	Sulphur

[a]Unless stated otherwise all removal rates are in g sulphide/(L h).

Table 6
Operating conditions and biokinetics of sulphide removal in various bioreactors with chemolithotrophic sulphide oxidizing bacteria.

Reference	Bacteria or culture source	Bioreactor	Matrix for biofilm establishment	Carbon source	Electron acceptor	Temperature (°C)	pH	Treated influent	Volumetric removal rate (g/(L h))[a]	End product(s)
Ongcharit et al. [180]	Co-culture of *Thiobacillus denitrificans* and floc forming heterotrophs	Stirred tank reactor with biomass recycle	–	CO_2	O_2 (from air)	–	–	H_2S gas: 1%	0.11	Sulphate
Lee and Sublette [51]	Co-culture of *Thiobacillus denitrificans* and floc forming heterotrophs	Uplflow bubble column with biomass recycle	–	CO_2	O_2 (from air)	30	–	Sulphide solution: 0.017 g/L	0.43–0.52	Sulphate
Annachatre and Suktrakoolvait [181]	Mixed culture of *Thiobacilli* from activated sludge	Fluidized-bed	–	Bicarbonate	O_2 (from air)	25–30	7.8	Sulphide solution: 0.48 g S/L	0.06	Sulphur and small amount of sulphate
Elias et al. [53]	Pig manure	Packed-bed with three modules	Pig manure and saw dust	Pig manure and saw dust	O_2 (from air)	25	8.4–6.8 (1st–3rd modules)	H_2S gas	0.045	Sulphur
Ng et al. [189]	*Thiomonas* sp.	Packed-bed filter	Activated carbon	–	O_2 (from air)	–	–	H_2S gas	0.01 mg H_2S/min g activated carbon loaded with cells	–
Alcantra et al. [54]	*Thiobacilli* consortium	Recirculation reactor system	–	Bicarbonate	O_2 (from air)	30	7.0–7.5	Sulphide solution: 2 g/L	0.15	Sulphur and sulphate
Cytryn et al. [55]	*Thiomicrospira denitrificans*, *Thiothrix*, sulphide oxidizing symbionts	Fluidized-bed	Sand	Organic content of waste stream	NO_3 and O_2	–	–	Sulphide solution: 0.02 g/L	0.24	–
Krishnakumar et al. [44]	*Thiobacillus denitrificans*	Reverse fluidized-bed	Polyethylene with added clay	Bicarbonate	O_2 (from air)	–	8.0	Sulphide solution: 0.25 g/L	1.11	Sulphur and sulphate
Duan et al. [184,185]	Activated sludge	Horizontal biotrickling filter	Activated carbon	–	O_2 (from air)	25–30	4.5	H_2S gas: 92 ppm	0.11	Sulphate
Lee et al. [187]	*Acidithiobacillus thiooxidans*	Packed-bed filter	Porous ceramic	CO_2	O_2 (from air)	–	–	H_2S gas: 2200 ppm	0.67	–
Ma et al. [190]	*Thiobacillus denitrificans*	Packed-bed	Activated carbon	Bicarbonate	O_2 (from air)	30–35	6.8–7.4	H_2S gas: 110–120 ppm	0.02	Sulphur
Datta et al. [188]	Sediments and water from hot pools of a lake	Biotrickling filter	NOVAINERT packing	Glucose and glutamate	O_2 (from air)	70	4.0–5.0	H_2S gas: 3.5%	0.04	–
Sublette and Sylvester [49]	*Thiobacillus denitrificans*	Batch stirred tank	–	CO_2	NO_3^-	30	7.0	H_2S gas: 0.5–1%	0.18–0.26 g/h g biomass	Sulphate
McComas et al. [192]	*Thiomicrospira* sp. CVO	Fed batch	–	CO_2	NO_3^-	32	7.4	H_2S gas: 1%	0.05	Sulphate and small amount of sulphur
Gadekar et al. [193]	*Thiomicrospira* sp. CVO	Continuous flow stirred tank	–	Bicarbonate	NO_3^-	22	7.0	Sulphide solution: 0.57 g/L	0.1	Sulphur

[a] Unless stated otherwise all removal rates are in g sulphide/(L h).

product of sulphide oxidation in a culture of *Thiomicrospira* sp. CVO and *Arcobacter* sp. FWKO B. According to Gevertz et al. [167] CVO does oxidize sulphide to sulphate when sulphide concentrations are low and nitrate is not limiting, but FWKO B oxidizes sulphide to elemental sulphur only.

Gadekar et al. [193] reported the reaction kinetics and stoichiometry of anaerobic sulphide oxidation by *Thiomicrospira* sp. CVO in batch and continuous systems. Utilizing NO_3 as electron acceptor, CVO was able to oxidize sulphide at concentrations as high as 19 mM. Sulphide oxidation proceeded in two distinct phases of formation of sulphur followed by conversion of sulphur to sulphate. In the continuous reactor, complete removal of sulphide was observed at loading rates up to 1.6 mM/h. At a sulphide to nitrate ratio of 0.28, 93% of the reaction products was sulphate, while at a ratio of 1.6 only 9.3% of sulphide was converted to sulphate.

Wang et al. [194] studied simultaneous desulphurization and denitrification by *Thiobacillus denitrificans*. The objective of this study was to maximize the production of elemental sulphur from sulphide and to study the effect of sulphide concentration (100, 200, 300, 400, and 500 mg/L) on the efficiency of the desulphurization and denitrification processes. Using a S^{2-}/NO_3 ratio of 5:3 and an initial sulphide concentration of 100 mg/L, 99% of the sulphide was removed, while with 300, 400 and 500 mg/L sulphide the percentage of sulphide removal were 67.9, 22.9 and 17.2%, respectively.

Sulphide oxidation under denitrifying conditions was also studied in a batch system by Cardoso et al. [166]. The culture used in this study was from an up-flow anaerobic sludge bed (UASB) reactor operated at a pH of 7.0 and 30 °C. Nitrate reduction coupled to thiosulphate oxidation was 4.6 and 9.5 times higher when compared to the rates observed during the oxidation of sulphide and elemental sulphur, respectively. High concentrations of sulphide inhibited the denitrification process, particularly affecting the conversion of nitrate to nitrite. At a sulphide-to-nitrate ratio of 2.5, nitrate was limiting, and no sulphate was detected, suggesting that the end product was elemental sulphur. At a sulphide-to-nitrate ratio of 0.62, sulphide conversion to sulphate approached a maximum, indicating that any ratio lower than 0.62 would produce a similar result.

Table 6 summarizes the recent literature data on biological removal of sulphide by chemolithotrophic sulphide oxidizing bacteria. Where possible, removal rates are recalculated and presented in terms of a consistent unit of g/(L h). In addition to variations in the microbial cultures, reactor configurations and experimental conditions, the possibility of chemical oxidation of sulphide in the systems operated under aerobic conditions or adsorption of sulphide on the matrices used for immobilization of the cells (i.e. activated carbon) which could have contributed to the reported sulphide removal rate make the comparison of the results rather difficult. Nonetheless, an evaluation of the data presented in Tables 5 and 6 indicates that sulphide removal rates in the systems with the biomass recycle or those utilizing attached bacteria are higher than those with freely suspended cells. The removal rates reported for phototrophic sulphide oxidizers are comparable to those achieved with for chemolithotrophs. However, the complicated nutritional and energy requirements of the photoautotrophs makes their chemolithotrophic counterparts a more favorable biocatalyst for oxidation and removal of sulphide. The use of phototrophic sulphide oxidizers could prove advantageous in the removal of sulphide during the anaerobic digestion of waste streams. Utilization of chemolitotrophs for this purpose requires a separate stage to prevent the exposure of the obligately anaerobic acetogens and methanogens to inhibitory levels of oxygen or nitrate, whereas phototrophs could be used directly in the anaerobic digester without any impact on the other microbial populations [164].

4.4. Indirect biological removal of sulphide

The indirect biological removal of sulphide is a two step process which can be described by the following reactions [43,47,195]:

$$H_2S + Fe_2(SO_4)_3 \rightarrow S^\circ + 2FeSO_4 + H_2SO_4 \quad (21)$$

$$4FeSO_4 + 2H_2SO_4 + O_2 \xrightarrow{\text{Iron oxidizing bacteria}} 2Fe_2(SO_4)_3 + 2H_2O \quad (22)$$

In the first step ferric iron serves as an oxidizing agent converting the sulphide to elemental sulphur (Eq. (21)). The produced ferrous iron is then oxidized to ferric iron using iron oxidizing bacteria such as *Acidithiobacillus ferrooxidans* (Eq. (22)). A similar approach can also be used for the removal of sulphur dioxide from flue gas according to the following reaction [195]:

$$SO_2 + Fe_2(SO_4)_3 + 2H_2O \rightarrow 2FeSO_4 + 2H_2SO_4 \quad (23)$$

Acidithiobacillus ferrooxidans is a chemoautotrophic aerobic bacterium which has the ability to oxidize iron and uses the derived energy to support carbon dioxide fixation and growth [195]. The kinetics of oxidation of ferrous iron by *Acidithiobacillus ferrooxidans* have been studied extensively, for both freely suspended cells as well as immobilized cells [195]. Other bacterial species capable of biooxidation of iron include *Leptospirillum ferrooxidans* [59] and *Sulpholobus acidocaldarius* [196].

Pagella and De Faveri [47] studied H_2S removal in a two stage bioprocess consisting of an absorber column for H_2S oxidation by ferric iron and a packed bed reactor with immobilized *A. ferrooxidans* for regeneration of ferric iron. The maximum reaction rate for sulphide oxidation was achieved at the maximum concentration of ferric iron of 1.2×10^{-4} mol/L and a pH of 1.5. At a gas flow rate of 100 L/h, H_2S at concentrations of 25, 50, and 100 ppm were removed completely.

Son and Lee [197] studied indirect oxidation of 20–510 ppm H_2S in the presence of *Acidithiobacillus ferrooxidans* in a single stage reactor. The inhibitory effect of H_2S on the iron-oxidizing bacteria led to development of a hybrid reactor in which the oxidation of sulphide by ferric iron took place in a well-mixed reactor, while the biological regeneration of ferric iron conducted in a packed bed reactor. The ferric iron medium regenerated by *Acidithiobacillus ferrooxidans* was able to achieve a 99.99% H_2S removal at a concentration of 2000 ppm and a gas flow rate of 1.22 L/min. Giro et al. [43] used a process consisting of a packed-bed reactor with PVC strands as a carrier matrix for *A. ferrooxidans* with an absorber column for oxidation of H_2S by ferric iron. With an inlet H_2S concentration of 20,000 ppm and a gas flow rate of 120 L/h, a removal efficiency close to 100% was achieved. The packed-bed reactor was operated at a temperature of 30 °C and the pH of the medium was adjusted to 1.7.

5. Concluding remarks

The bacteria of the sulphur cycle and the reactions which are carried out by them, specifically anaerobic reduction of sulphate and biooxidation of sulphide are of significant importance from the industrial and environmental point of views. Souring, a phenomenon occurring frequently in the offshore and onshore oil reservoirs decreases the quality of oil and gas and imposes severe corrosion risks in the production, transportation and processing facilities. Souring is caused by the sulphate reducing bacteria. Generation of H_2S in livestock operations which is a major impediment for the expansion of such operations is also attributed partly to the activity of sulphate reducing bacteria. While causing serious processing and environmental problems for the oil industry and agriculture sector, if used in a properly designed and carefully operated system, sulphate reducing bacteria can contribute in the treatment of acid mine drainage, a serious environmental problem

faced by the mining industry. Biooxidation of sulphide catalyzed by sulphide oxidizing bacteria is one of the key steps in biotreatment of acid mine drainage and is equally important in the bioleaching of sulphide minerals. Furthermore, sulphide oxidizing bacteria are instrumental in the in situ removal of H_2S from onshore and offshore oil reservoirs and in the ex situ treatment of sour gases and sulphide laden waters.

Owing to the widespread industrial and environmental applications, anaerobic reduction of sulphate and biooxidation of sulphide have been studied extensively. These studies cover a broad range of topics including the microbiological and genetic aspects, bioenergetics, kinetics and process engineering. Studies on anaerobic reduction of sulphate have evaluated the impacts of sulphate concentration, pH, temperature, carbon and energy sources, as well as the inhibitory effects of sulphide and metallic ions on the microbial growth and sulphate reduction kinetics. On the process engineering side, modeling of the reaction kinetics and improving the feasibility of the process through variations in reactor designs and utilization of inexpensive carbon sources have been the centre of attention. The challenges, however, remain in the isolation and characterization of acid tolerant species of SRB with the ability of complete oxidation of the carbon source, and in identifying inexpensive carbon sources which could be effectively utilized by the microbial population in treatment of acid mine drainage. Control of biogenic production of sulphide in model laboratory systems and in oil reservoirs thorough the removal of sulphate from injection water, addition of biocides and metabolic inhibitors to the injected water, as well as amendment of the reservoir by nitrate has been the other focal point of the research on anaerobic reduction of sulphate.

Biooxidation of H_2S in the gaseous streams and sulphide laden waters has been investigated using phototrophic or chemolithotrophic sulphide oxidizing bacteria. Among the phototrophic bacteria, *Cholorobium limicola* has attracted attention, possibly due to its ability in efficient oxidation of sulphide and extracellular deposition of the produced sulphur. Considering that light energy is one of the most influential factors on the performance of a phototrophic system, a variety of artificial light sources and solar energy have been evaluated. Nonetheless, supply of the light energy remains as one of the main constraints for the widespread application of phototrophs for the removal of sulphide. Further research for the development of an efficient and feasible system for the delivery of the light energy, specifically those relying on the solar energy is needed.

Although sulphide removal rates achieved with phototrophic bacteria are comparable to those reported for their chemolithotrophic counterparts, the simpler nutritional and energy requirements has made the latter a more attractive option. Chemolithotrophic oxidation of sulphide under aerobic conditions has been investigated extensively, using various species of sulphide oxidizing bacteria, especially those belonging to *Thiobacilli* genus. However, risks associated with the operation of the reactor under oxygen rich environment is a major concern, specially when a gaseous stream such as natural gas or biogas is treated. Biooxidation of sulphide under denitrifying conditions alleviates this risk and eliminates the aeration costs. As a result a number of research works have focused on this topic. Interest in anaerobic biooxidation of sulphide with chemolithotrophs also stems from the recent findings which identify this process as one of the underlying mechanisms in the control of souring in oil reservoirs subjected to nitrate amendment.

Composition of end products is another topic of interest as far as the research on sulphide biooxidation is concerned. There is a universal agreement among the researchers that regardless of the nature of the process (either aerobic or anaerobic) the ratio of sulphide to electron acceptor is a determining factor in the composition of end products, with higher ratios favoring the production of sulphur, the desirable end product.

The present article aimed to provide a brief overview of the bacteria of sulphur cycle and the instrumental role which they play in solving some of the environmental and processing problems encountered in the mining and petroleum industries. However, applications of the sulphur cycle bacteria is not limited to those discussed in this article and future research on topics such as an integrated process for biological removal of sulphide and denitrification of wastewaters, possibility of capturing CO_2 and finally development of microbial fuel cell type reactors for the treatment of sulphate, sulphide and nitrate containing streams with concomitant generation of energy could open up further opportunities for utilization of these versatile microorganisms.

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