

Theoretical and Practical Aspects of Biological Sulfate Reduction: A Review

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Abstract

The major environmental impact of discharge of excessive quantities of sulfate is the pollution of the surface and ground water supplies which can be harmful to life forms and therefore the need for an efficient treatment system arises. Biological sulfate reduction offers the advantage of less sludge production, less operational cost and efficiency in comparison to the physicochemical processes. Depending upon the feeding and operating conditions as well as microbes used, a number of intermediates are formed that may greatly affect the overall performance of bioreactor. This article extensively explores the bacterial community, formation of intermediates and desirable end products, theoretical and practical aspects of various environmental and operating conditions, and performance of bioreactors used for treating sulfate rich wastewater along with process biokinetics involved in biological sulfate reduction.

Keywords: Sulfate reduction, sulfide, sulfate reducing bacteria, bioreactors, biokinetics.

Introduction

Sulfate is one of the most abundant anions present in the environment. It appears naturally with various water streams in dissolved forms or as insoluble salts like barite (BaSO_4), epsomite ($\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$) and gypsum ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$), as well as generated through oxidation of sulfide ores in acid mine drainage (AMD) [1]. It is also discharged in effluents from various industries such as mining, animal husbandry, food processing, pulp and paper wastewaters, dye and detergent manufacture industries [2]. Both physico chemical and biological treatment options are practiced for the remediation sulfate rich wastewater. Amongst the various treatment options, bioreduction of sulfate is considered as an efficient method [3], owing to less sludge handling, flexibility of operation along with low cost of treatment. It is a microbial driven process in which a specialized group of microorganisms called sulfate reducing bacteria (SRB) are responsible [4]. The SRB represent a diverse group of obligate anaerobes which thrive in the anoxic environments containing organic materials and sulfate [5]. The basic mechanism in sulfate reduction lies with the utilization of carbon source/s such as hydrogen, ethanol, methanol, glucose, lactate, sugarcane, and wood etc. as electron donor/s and sulfate as terminal electron acceptor. However depending upon the process conditions, many factors such as pH, temperature, influent $\text{COD}/\text{SO}_4^{2-}$ ratio, and electron donor may influence the outcome of bacterial substrate competition and sulfate reduction [6]. For example the SRB have been seen to thrive in various sediments characterized by very low temperatures and availability of organic matter [7, 8]. Even the presence of heavy metals in the system [9] and undissociated sulfide have been seen to affect the process efficiency as a whole [10, 11]. A number of reactor configurations such as batch reactor, sequential batch reactors, anaerobic filters, fluidized bedreactors, membrane bioreactors, hybrid anaerobic reactors and Upflow Anaerobic Sludge Blanket (UASB) reactors used for the biological reduction of sulfate have been described in the literature, each kind of reactor configuration providing its own flexibility in terms of operation and efficiency [12]. Various studies on biokinetics of sulfate reduction has been done for the estimation of biokinetic coefficients such as specific growth rate, half velocity constant, decay rate etc while utilizing various micro-organisms and electron donors. The biokinetic analysis assists in the prediction of population dynamics in terms of substrate consumption and growth of microbial population thus ensuring the design and process performance of various bio-reactors in practical scales. Various conventional and mathematical models have been used by researchers to investigate, control and predict treatment plant operation performance and optimizing the process at the same time [13, 14].

The present manuscript aims to provide an overview of various effects caused due to sulfate from various sources along with the process biochemistry and community structure of sulfate reducing bacteria. Various factors and conditions that determine the process outcome have also been highlighted along with bioreactors used so far and the associated biokinetics of sulfate reductions.

1. Sources and occurrence of sulfate rich wastewater

Sulfate is one of the most abundant anions found in the environment. It is a common constituent of many natural waters and wastewaters, and is sometimes present in high concentrations. Wastewaters generated from various industrial activities such as pulp and paper industries, mining and mineral processing, production of explosives, scrubbing of flue gases, petrochemical industries, galvanic processes, battery, paint and chemical manufacturing, food processing (molasses, seafood, edible oil), and pharmaceutical industries [15] are the main source of most anthropogenic emissions. Other industrial activities involved in the production of fertilizers, dyes, glass, soaps, textiles, fungicides and leather also release high sulfate bearing effluents [16]. Certain industrial effluents may contain large concentration of sulfate (**Error! Reference source not found.**) while domestic sewage contains typically less than 500 mgL^{-1} .

In the present scenario, large quantities of sulfate are being released into the environment but very less attention has been given to its mitigation owing to its relatively low direct environmental risk compared with other pollutants. Sulfate becomes a pollutant when it is discharged into the natural environment in excess amounts leading to various environmental hazards.

Wastewater source	Process from which sulfate rich effluent is generated	Sulfate (mgL ⁻¹)	Reference
Mining		20,800	Bai et al. [17]
Tannery industry	Deliming , pickling ,tanning , retanning , dyeing, greasing	2500-3000	Galiana-Aleixandre et al. [18]
		3190	Boshoff et al. [19]
Chemical industry	Washing of sulfonation reaction products in presence of sulfuric acid	180,000-284000	Sarti and Zaiat. [20]
Drug industry		500-600	Rao et al. [21]
TNT (trinitrotoluene) manufacturing process		5400	Lens et al. [15]
Electroplating industry		2000	Song et al. [22]
Galvanic industry		200 – 50,000	Tichy et al. [23]
Mining industry		100 – 20,000	Banks et al. [24]
Citric acid		2500-4500	Colleran et al.[25]
Flue gas scrubbing		1000 – 2000	Dijkman[26]
Alcohol production		2900	Lens et al. [27]
Sea food processing	Wastewaters originating from mussel, tuna, and octopus cooking manufacturing	2100-2700	Mendez et al. [28]
	Fish-meal production wastewaters	600	
Textile industry		2690	Kabdasli et al. [29]
Pulp & paper industry	Thermomechanical pulping	200-700	Habets & de Vegt[30]
	Chemo-thermomechanical pulping	1200-1500	
Molasses fermentation		4600-6300	Lo et al. [31]
		2500 – 3450	Carrondo et al. [32]

Table1: Industries producing sulfate rich wastewaters.

Excessive quantities of released sulfate can lead to pollution of the surface and ground water supplies posing health threat to life forms and therefore it needs to be treated before being discharged to maintain its level within the permissible limits [33]. The upper concentration limit of sulfate in water intended for human consumption is recommended at 250 mgL⁻¹ [34, 35] whereas the general standards for discharge effluents is limited upto 1000 mgL⁻¹ [36]. The BIS standard 10500 [37] states that maximum concentration of sulfate in drinking water should not exceed 200 mgL⁻¹.

2. Effects of sulfate in the environment:

Sulfate becomes a pollutant if it is released in excess leading to various environmental hazards and impacts upon its discharge into the natural environment. It is only mildly hazardous in comparison with toxic metals and for this reason many countries have not set any guidelines for sulfate in drinking water. The presence of high sulfate in water creates bad odour, colour and taste which lead to human health problems [38]. The taste threshold of sulfate in drinking water has been fixed between 300 to 400 mgL⁻¹. However, at concentrations above 600 mgL⁻¹,

sulfate can affect the taste of water and can have laxative effects [39]. High concentration has been reported to cause diarrhea and dehydration in human beings [40]. Infants are more prone to the higher sulfate concentration than adults. Hence it has been prescribed that water having sulfate more than 400 mgL⁻¹ should not be used for making infant food. Even animals are also sensitive to high levels of sulfate.

High levels of sulfate present in the tailings (piles or dumps) from coal and some metal-bearing ores (especially those rich in pyrite and chalcopyrite) are readily oxidized by water and oxygen, resulting in acid mine drainage (AMD) creating several problems in coal and ore producing countries [16]. It has deleterious impacts on environment and many aquatic systems [41, 42].

Excessive quantities of released sulfate can lead to pollution of the surface and ground water supplies posing health threat to life forms and therefore it needs to be treated before being discharged to maintain its level within the permissible limits [33]. In the aquatic environment, the natural sulfur cycle would be altered due to excessive release of sulfate and sulfide formed due to sulfate reduction. Sulfate ions also lead to increase in the conductivity and corrosion potential of receptor water bodies as they are one of the main contributors of mineralization of water [43]. These anions promote the corrosion and scaling in pipes, structures and equipment; fouling and deposition in boilers; and acidification of soils and blockage of soil pores, retarding irrigation or water drainage systems [44]. Torres-Sanchez et al.[45] observed high density and low depth pitting on the surface of stainless steel AISI 304 exposed to the action of SRB. Various other researchers have proved that the presence of SRB accelerates the process of corrosion in metals [46, 47].

3. Sulfate reducing bacteria (SRB) and bacterial community structure:

Dissimilatory sulfate reduction process which utilizes sulfate ions as electron acceptors for anaerobic respiration is mediated by sulfate reducing bacteria and archaea [48]. So far, the known SRB can be grouped into seven phylogenetic lineages, five within the bacteria and two within the archaea based on comparative analysis of 16S rRNA sequences [49]. Maximum sulfate-reducers are found within the Deltaproteobacteria (~ 23 genera) which includes the typical sulfate reducer *Desulfovibrio*, followed by the Clostridia (*Desulfotomaculum*, *Desulfosporosinus* and *Desulfosporomusa* genera) which are low G+C gram-positive SRB [50]. Only thermophilic SRB occur within Nitrospirae (*Thermodesulfo vibrio* genus), Thermodesulfobacteria (*Thermodesulfobacterium* genus) and Thermodesulfobiaceae (*Thermodesulfobium* genus). Within the Archaea, SRB is divided into the Euryarchaeota (*Archaeoglobus* genus) and the Crenarchaeota (*Thermocodium* and *Calditerrivirga* genera).

SRB are capable of utilizing sulfate as an electron acceptor for growth and convert it to sulfide. Sulfate reducers can be divided into two metabolic groups on the basis of their substrate utilization. The first group comprises of those species which are capable of complete oxidation of the substrates to carbon dioxide while the second group includes those which can oxidize the substrate to acetate and not completely to carbon dioxide. Representatives of the genera *Desulfomonas*, *Desulfococcus*, *Desulfobacter*, *Desulfosarcina*, *Desulfotomaculum*, *Desulfonema*, *Desulfoarculus*, *Desulfoacinum*, *Desulforhabdus*, *Desulfomonile*, as well as *Desulfotomaculum acetoxidans*, *Desulfotomaculum sapomandens* and *Desulfovibrio baarsii* are capable of degrading organic compounds (e. g. acetate) completely [4, 5, 48, 51] while *Desulfobulbus*, *Desulfovibrio*, *Desulfomicrobium*, *Desulfobotulus*, *Desulfofustis*, *Desulfotomaculum*, *Desulfomonile*, *Desulfobacula*, *Archaeoglobus*, *Desulfobulbus*, *Desulforhopalus* and *Thermodesulfo bacterium* are some of SRB which are not complete oxidizers [5, 51]. SRB have the ability to utilize a broad range of electron donors, including lactate, propionate, acetate, and hydrogen [52]. Lactate can be consumed both by complete oxidizers as well as incomplete oxidizers, while hydrogen can be utilized more by

incomplete oxidizers and very less by most complete oxidizers. The morphology, carbon source, pH and temperature range for growth of some SRB species which have been isolated are presented in Table 2.

Species	Morphology and size	Carbon and energy source	pH	Temperature (°C)	References
<i>Desulfovibrio aminophilus</i> sp.	Curved, Gram-negative, non-spore forming cells (0.2 × 3.0-4.0 µm)	Formate, alanine, aspartate, leucine, isoleucine, valine, and methionine, H ₂ /CO ₂ and ethanol	7.5	35	Baena et al. [53]
<i>Desulfotomaculum acetoxidans</i>		Acetate	6.6 - 7.6	30°C- 40	Crine et al. [54]
<i>Desulfobacter postgatei</i>		Acetate	6.2 - 8.4	25°C - 35	
<i>Desulfovibrio aerotolerans</i>	Curved, 1×2–5µm , non-spore-forming cells	Lactate, pyruvate, H ₂ ,acetate, ethanol and glycerol	6.9	29	Mogensen et al. [55]
<i>Desulfotomaculum arcticum</i> sp.	spore-forming	pyruvate	7.1–7.5	44	Vandieken et al. [56]
<i>Thermodesulfobacterium hveragerdense</i> sp.nov.	Gram negative ,rod, with an average cell size of 2.8 × 0.5 µm, non-spore forming	lactate, pyruvate and H ₂	7.0	70 -74	Sonne-Hansen and Ahring[57]
<i>Thermodesulfovibrio islandicus</i> sp.nov.	Gram negative ,vibrio-shaped rod with an average cell size of 1.7 × 0.4 µm, non-spore forming		7.0	65	
<i>Desulfosporosinus acidiphilus</i>	Gram negative, non-motile, curved rods, 4-7 ×0.8-1.0 µm	H ₂ , lactate, pyruvate, glycerol, glucose, and fructose	5.2	35	Alazard et al. [58]
<i>Desulfobagelida</i> sp.	Gram-negative, 3.1×5.4-6.2µm	acetate, propionate, butyrate, lactate and hydrogen,	7.1-7.6	7	Knoblauch et al. [59]

Table 2 Morphology, carbon source, pH and temperature range for growth of some SRB species

4. Biochemistry involved in sulfate reduction: formation of Intermediates and end products

The biological sulfate reduction process is mediated in dissolved oxygen deficient environment by a group of microorganisms known as sulfate reducing bacteria (SRB). Though many microbes generate H₂S metabolically, sulfate often being the primary source of H₂S, the process is normally a small-scale one involving the incorporation of sulfur into cell protein and its subsequent degradation by catabolic and autolytic processes [4].



Under anaerobic conditions, heterotrophic sulfate reducing bacteria (SRB) use sulfate as the terminal electron acceptor for the degradation of electron donors like various organic compounds and hydrogen [4]. In the absence of dissolved oxygen and nitrate, sulfate is converted to sulfides by acting as a source of electron acceptor. During

this process, sulfate after being activated to adenosine-phosphosulfate (APS), is reduced to sulfite, which is further reduced to sulfides as the final end products. Sulfate transport in SRB has been proposed to be driven by a proton symport, which follows chemi-osmotic principles of transport. However, sulfide moves across membranes by diffusion and not by an active transport process [60]. Once within the cytoplasm, the sulfate is reduced to sulfide in a series of reactions driven by various enzymes. The reduction of sulfate to sulfide in dissimilatory sulfate reduction is mediated by three enzymes which occur within the cell cytoplasm [61] and the pathway as shown in Fig.1 is comprised of the following four steps catalyzed by membrane bound enzymes [62].

Step 1: Transfer of sulfate inside the bacterial cell

Step 2: Activation of internal sulfate to adenosine 5' phosphosulfate (APS) with adenosine triphosphate (ATP) mediated by enzyme ATP sulfurylase.

Step 3: Reduction of APS to sulfite by APS reductase

Step 4: Finally reduction of sulfite to sulfide by sulfite reductase

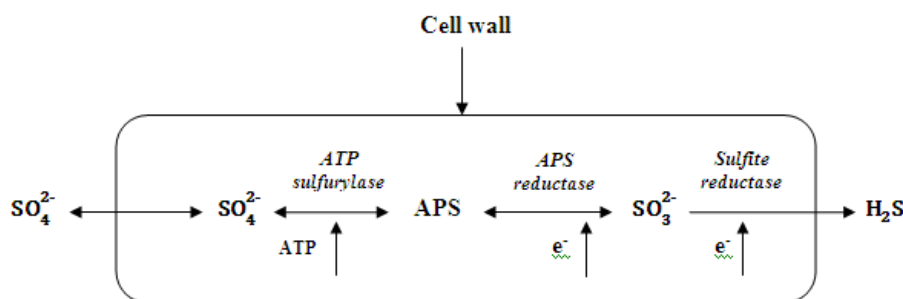


Fig.1 Pathway of dissimilatory sulfate reduction [50]

The similarity between assimilatory and dissimilatory sulfate reduction lies in the presence of enzyme ATP sulfurylase, which leads to formation of APS, mediated by enzyme APS reductase for formation of sulfite. The main difference lies in the reversible transition from external sulfate to sulfite in dissimilatory sulfate reduction as compared to assimilatory sulfate reduction. Sulfite has been conceived to be an intermediate of sulfate reduction in sulfate reducing bacteria like *Desulfovibrio*, sp, as well as in other microorganisms and higher plants [63]. So far two mechanisms have been proposed to describe the reduction of sulfite to sulfide: (i) by direct reduction of sulfite to sulfide without the formation of any intermediate compound and (ii) through the formation of trithionate and thiosulfate as proposed by Kobayashi et al.[64]. With the reduction of six electrons, the direct reduction of sulfite to sulfide takes place leading to the formation of sulfide through a single step only and is catalyzed by sulfite reductase enzyme [65]. In the reduction of sulfite to sulfide through the trithionate pathway[50], the first step involves the reduction of sulfite to trithionate catalyzed by trithionate reductase with the reduction of two electrons. In the second step trithionate is converted to thiosulfate with the reduction of two electrons in the presence of enzyme thiosulfate reductase. In the final step sulfide is formed from thiosulfate with release of again two electrons.

Trudinger and Loughlin [66] reported that trithionate and thiosulfate formation can take place by chemical reactions in the culture medium with high sulfite concentrations or abiotically by reaction between sulfite and sulfide [52] and that neither trithionate nor thiosulfate are obligatory intermediates in the sulfite reduction pathway. However, formation of thiosulfate [67] and trithionate [64] as intermediates in the reduction of sulfite by *Desulfovibrio vulgaris* has been reported. Findley and Akagi [68] have even provided evidence about the

generation of both sulfur atoms of thiosulfate from sulfite and, also reduction of the outer sulfur atom to sulfide and regeneration of the inner sulfur atom back to sulfite during thiosulfate reduction. Trithionate and thiosulfate formation as intermediates with whole cells of sulfate reducing bacterium *Desulfovibrio desulfuricans* supports the trithionate pathway of sulfite reduction [67]. Thus the trithionate pathway of sulfite reduction may be a fully functional biochemical process [50].

5. Treatment of sulfate rich wastewater

Normally sulfate containing wastewaters can be treated using physicochemical and biological methods. However, biological treatment is preferred due to the overlying limitations of separation and appropriate disposal of the solid phase, relatively high cost and energy consumption involved in physicochemical methods [69]. Biologically, sulfate bearing wastewater is generally treated by anaerobic processes [70-72]. There are several factors that affect biological sulfate degradation efficiency and formation of end products.

5.1. Factors influencing sulfate reduction:

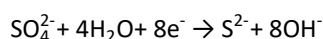
A) pH

Sheoran et al. [73] reported that SRB has two threshold inhibition levels, one for the undissociated sulfide (H_2S) and the other for the total sulfide. The state of sulfide solely depends on the pH of the environment as shown in Fig. 3. Most of the SRB's are reported to be neutrophilic [48] and prefer an environment having pH between 7.5-8. However various acid tolerant species have also been seen to thrive for sulfate reduction at pH as low as 3.8 [74], while some species have been found to be alkaliphilic and the highest pH seen to support the growth of SRB's has been reported to be 10 [75]. Below pH 5, activity of SRB's is reduced considerably whereas at neutral pH their activity is enhanced. The inactivity of SRB's at low pH is mainly attributed to the acidification of the cytoplasm which inhibits the formation of a proton motive force. At a pH less than 7.2, un-dissociated H_2S is dominant and reaches the threshold limit while at a pH above 7.2, the total sulfide is responsible for the inhibitory effect [76]. At pH of 8.5, the HS^- further dissociates into the sulfide dianion (S^{2-}) form and becomes the predominant sole species at pH value above 10 [5, 77]. The SRB are less sensitive to total sulfide when the pH is increased from 6.8 to 8.0 and more sensitive to the undissociated sulfide concentration. At low pH the produced hydrogen sulfide exists in undissociated form and as the pH increases it dissociates into HS^- and S^{2-} .

Most sulfidogenic bioreactors have been operated around neutral pH. In order for SRB to survive, a pH in the range of 5–8 is required [78] while outside this range, the rate of microbial sulfate reduction generally declines. Low pH (<5) normally inhibits sulfate reduction and increases the solubility of metal sulfides [79]. Below a pH of 4, bioreactors have been less successful. However, Elliott et al. [80] reported the presence of SRB activity at a pH of 3.0 in an anaerobic upflow bioreactor. Kolmert and Johnson [81] reported the growth of a mixed acidophilic SRB culture in a medium with a pH of 3.0 thus supporting the view by Postgate [4] that mixed SRB cultures are more tolerant to extreme conditions than pure cultures. Sulfate reduction has also been reported to occur at a pH of 10 [75]. However, significant reduction rates have been shown until a pH of 8.0 where a volumetric activity of $25 \text{ SO}_4^{2-} \text{ g Ld}^{-1}$ was reported [82].

B) Electron donors/carbon source:

Lens et al. [15] reported the diversity of SRB in their carbon source utilization and the metabolic activities. The carbon and energy source provides the energy for the growth and maintenance of SRB based on the reaction given below:



The electrons required for the sulfate reduction are generated by the oxidation of a carbon source (e.g, lactate, acetate, and propionate).

The ATP produced, using the energy released from oxidation of the organic carbon is utilized for the reduction of sulfate to sulfide. In most instances, the electron donor and the carbon source are the same compound. Only when hydrogen is used as the electron donor, CO₂ is used as the carbon source. A minimum COD/SO₄²⁻ mole ratio of 0.67 is required for achieving theoretically possible removal of sulfate [83]. Various organic compounds such as sewage sludge, leaf mulch, molasses animal and manure have been used as carbon sources in addition to the low molecular weight organic compounds. Some of the commonly used electron donors are hydrogen, formate, methanol, ethanol, molasses, lactate, acetate, propionate and butyrate, sugar, hydrocarbons and organic waste [3]. Table 3 shows the advantages and disadvantages of different carbon sources.

Carbon Source	Advantage(s)	Disadvantage(s)
Hydrogen,	More efficient utilization by SRB than methanogens	Only a few anaerobes can grow with hydrogen as sole energy source
Acetate	-	SRB cannot completely oxidize acetate even with excess sulfate levels
Methanol	Readily available and cost effective	Low growth rate of SRB
Ethanol	SO ₄ ²⁻ conversion efficiency	Slow growth rate of SRB , produces acetate
Molasses	Low cost , readily available	Accumulation of non-biodegradable content reduces biomass activity and COD removal. High VFA generation
Lactate	Most SRB can utilize it	Complete lactate oxidation not achieved by some SRB species
Sugar	Easily degraded under anaerobic conditions.	Costly
Hydrocarbons	-	Free energy change low as estimated for the methane oxidation
Organic waste (sewage sludge, animal manure, leaf mulch, wood chips, sawdust, cellulose.)	Cost effective.	Very less utilization rate

Table 3: Electron donors and carbon sources for SRB [3]

c) COD /SO₄²⁻ ratio:-

COD /SO₄²⁻ ratio appears to be a key factor in the regulation of sulfate reduction as it determines the competition between SRBs and methanogenic bacteria (MB) for monomeric (e.g sugar, amino acids) and H₂ or acetate compounds [84]. In addition, COD/SO₄²⁻ also determines the electron flow during sulfate reduction and methanogenesis. It has been reported theoretically that conversion of 1 mol of sulfate requires 0.67 mol of COD or electron donor [83, 85]. When this ratio is decreased i.e there is more sulfate available, then the amount of organic matter required by the biomass for sulfate reduction is not present and its then that an addition of an external source of organic matter, preferably carbon source/electron donor is required. Conversely the sulfate reduction is also hampered when this ratio is increased as the electron transport to the sulfate reducing bacteria decreases. In fact, when the ratio increases beyond a certain value, there is competition between methane formers and sulfate reducers for acetate. Choi and Rim [83] indicated that sulfate reducers and methane formers are very competitive

at a ratio of 1.7 to 2.7 and observed that methane producers dominate at higher ratio while sulfate reducers dominate at lower ratios. Chou et al. [86] studied the competition reaction kinetics of SRB and MB at different COD/SO₄²⁻ ratios by finding out the values of mass fraction of SRB and MB i.e f(SRB) and f(MB) respectively. They found out that f(SRB) continued to be higher up-to a COD /SO₄²⁻ of 1.3 indicating that SRB could outcompete MB for bacterial growth. However, as the ratio was increased upto 2 and 3, the mass fraction of MB became more than mass fraction of SRB's. The following Table 4 shows various studies carried out at different COD /SO₄²⁻ ratio along with the sulfate and organic matter removal efficiencies.

Reactor type	COD/SO ₄ ²⁻	COD removal efficiency	SO ₄ ²⁻ removal efficiency	Reference
SBBR	1-1.5	48-95	84-98	Archilha et al. [87]
ABR	6	>85	96.8	Vossoughi et al. [88]
UASB	0.67-1.5	100	94	Velasco et al. [89]
Batch	2.6	92.6	>92	Cao et al. [90]
EGSB	6	>65	>85	de Smul et al. [6]
FBR	1.17	87	91	Thabet et al. [91]
SBBR	3.67±0.19	32	99	Sarti and Zaiat[20]
UASB	1	67.4	85.6	Rodriguez et al. [92]
UASB	6.67	-	>95	Sipma et al. [93]
CSTR	1.2	99±0.6	86±0.5	Oyekola et al. [94]
CSTR	2	N.A	99	Zhao et al. [95]
Upflow hybrid reactor	1.3	>90	>90	Sabumon[96]
Batch	0.7	>85	>90	
Batch	1.6	N.A	97.4	Wang et al. [97]
Continuous	2.7	N.A	>94.6	

SBBR: Sequential batch biofilm reactor; UASB: Upflow anaerobic sludge blanket reactor; FBR: Fixed bed reactor; EGSB: Expanded granular sludge bed reactor; ABR: Anaerobic baffled reactor; CSTR: Continuous stirred tank reactor

Table 4 Sulfate and COD removal at different COD /SO₄²⁻ ratio

D) Temperature:

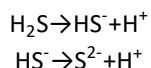
Sulfate reducers can grow over a wide range of temperature. Some can thrive at temperature as low as 5°C [98] while others have been reported to grow at temperature above 50°C [99, 100]. Sulfate reducing bacteria can be classified into mesophiles (growth temperature < 40°C), moderate thermophiles (growth temperature 40-60°C) and extreme thermophiles (>60°C). But most of the studies conducted so far in laboratory scale for sulfate reduction show that majority of the sulfate reducers such as *Desulfobacter hydrogenophilus*, *Desulfobacter curvatus*, *Desulfovibrio latus*, *Desulfovibrio vibrioformis* and *Desulfovibrio halotolerans* are mesophilic in nature. Arrhenius plot has been employed in order to gain an insight of the adaptation of bacteria for sulfate reduction in low temperature regions like marshy areas, deep sea, sediments etc

Ingvorsen et al. [101] investigated the effect of temperature on sulfate reduction on concentrated sludge and native sludge. They found out that the exponential phase was attained after 6 hr when temperature was 20°C as against 20 hr when temperature was 5°C. Various studies by Pallud & Cappellen[102] and Sawicka et al. [103] on samples obtained from marshes and sediments show that the sulfate reduction rates increase with increase of temperature values of 20-30°C. The E_a and Q₁₀ values found in the studies showed that the temperature range of 20-30°C is optimum for sulfate reduction. de Smul et al. [6] found that the optimum sulfate reduction rate were maintained at a temperature of 33°C with the expanded sludge granular bed (ESGB) reactors fed with ethanol and

ethylene glycol. In addition to that, they also observed the suppression of overall sulfidogenic activity in contrast to methanogenesis which became active once the temperature was increased to 55°C. Similar results were also found by Sulaiman Al-Zuhair[14] and Moosa et al. [104] where fastest drop in sulfate concentration was observed at temperature of 35°C. Studies by Moosa et al. [105] on anaerobic sulfate reduction across a temperature of 20-35°C observed that the values of bio-kinetic parameters namely specific growth rate (μ_m) and yield (Y) did not change with temperature while K_s declined to a value of 0.949 from 0.016 kg dry weight m^{-3} and k_d value increased from 0.008 to 0.038 per hour.

E) Sulfide

The toxicity of sulfide is regarded as being pH dependent because in the pH range of 6-8, sulfide exists as a mixture of HS^- and H_2S . Undissociated hydrogen sulfide (H_2S) becomes the dominant sulfide species at pH lower than 6 [106]. The chemical reactions taking place for the sulfide species is governed by the equations given below:-



The total Hydrogen sulfide is found out as below

$$H_2S_{total} = H_2S_{aq.} + HS^-$$

Two theories have been postulated so far for sulfide inhibition. The first one states that the undissociated sulfide molecule can pass through the cell membrane, making the cell inactive by destroying the bacterial proteins [4, 107] and interfering with the metabolic coenzymes by formation of sulfide bond [108]. The other one is applicable when there are heavy metals in the system, which states that due to the precipitation of heavy metals the sulfate reducing bacteria are deprived of the essential trace nutrients used as cofactors and hence their growth gets inhibited [109]. However it was seen that the sulfide toxicity is reversible and the normal cell growth and sulfate reduction rates are attained as soon as sulfide is removed from the system [11, 110]. Table 5 depicts the effects of sulfide on the sulfate reducing bacteria at different concentration levels studied so far.

Organism	Reactor	Sulfide inhibition level (mgL ⁻¹)		Reference
		Un-dissociated sulfide (mgL ⁻¹)	Dissolved sulfide (mgL ⁻¹)	
Mixed culture	CSTR	290	---	Moosa & Harrison [106]
		---	1000	
Mixed culture	Continuous	---	1000	Icgen and Harrison [111]
Wet granular sludge	UASB	---	100	Lopes et al. [112]
<i>Desulfovibrio Desulfuricans</i>	Batch culture	---	251	Okabe et al. [11]
	Continuous chemostat	---	250	
Mixed culture	Ethanol- lactate fed FBR	---	613.44	Nevatalo et al. [113]
<i>Desulfovibrio Desulfuricans</i>	Bacterial culture		34.08	Truong et al. [114]
<i>Desulfovibrio Desulfuricans</i>	Chemostat	---	212±23	Okabe et al. [115]
Granular Sludge	UASB	---	115	Lopes et al. [116]
SRB growing on lactate and sulfate	---	---	547	Reis et al. [117]
Mixed culture	Chemostat	---	150-200	Krishnanand and Parkin

				[110]
AMD treatment sludge	Serum vials	302.6	1781	O'Flaherty et al. [118]
Anaerobic hybrid reactor sludge	Packed up-flow hybrid reactor	258.4	2736.5	O'Flaherty & Colleran[119]

Table 5: Toxicity levels of sulfide in Sulfate reducing bacteria

F) Heavy metals

The capacity of various heavy metals to react with the functional groups of enzymes and deactivating them in the process results in toxic effects on microorganisms including SRB which are generally utilized in bioremediation process. The heavy metals are even capable of substituting essential ions on cellular sites causing denaturation of proteins [120]. The main criteria on which the removal ability of the SRB depends, is the metal concentration in solution which may lead to decrease in metabolic activity of the bacteria or even death when the metal concentration is very high. The toxicity concentrations of heavy metals for SRB have been reported to range from a few mgL^{-1} to as high as 100 mgL^{-1} [9]. Martins et al [121] reported that the variation of the metal species also plays a very important role imparting toxicity to the SRB. For instance, the less mobile arsenate As(V) is more toxic than arsenite As(III), while inorganic species are more toxic as compared to their methylated counterparts [122]. Various resistance mechanisms such as sequestration or transformation to other chemical species have been observed in different organisms as a means of tolerance to the toxic effects exhibited by various metal ions [123]. Jong and Parry [124] reported the decrease in sulfate reduction with increasing initial concentrations of metals which might be attributed to firstly to reduction in metabolic activity of SRB due to metal toxicity. The second reason could be the partial blockage of the sand-bed of the reactor leading to severe mass transfer limitations which are common at higher metal concentrations. The toxic concentrations of some heavy metals as reported by Hao et al. [125] employing a mixed culture of SRB for studying the effects of heavy metals on sulfate reduction are Zn ($25\text{--}40 \text{ mgL}^{-1}$), Pb ($75\text{--}80 \text{ mgL}^{-1}$), Cu ($4\text{--}20 \text{ mgL}^{-1}$), Cd ($>4\text{--}20 \text{ mgL}^{-1}$), Ni ($10\text{--}20 \text{ mgL}^{-1}$) and Cr (60 mgL^{-1}). The sulfate removal IC₅₀ (concentration causing 50% inhibition of SRB sulfate removal efficiency) for Cu was reported to be 156 mgL^{-1} [22] in contrast to 1.02 mgL^{-1} as reported by Sani et al. [9] who used *D. desulfuricans* strain along with a specific metal toxicity medium containing constituents that did not result in any abiotic precipitation of metal ions. Important studies on toxicity levels of various metals have been listed in Table 6. This comparative difference shows that the chemical and physicochemical properties of the environment surrounding the SRB play an important role in determining the level of metal toxicity and inhibition in SRB [124]. In the studies conducted by Martins et al. [121], the inhibition of sulfate reducing ability of the SRB was significant in the presence of zinc concentration of 150 mgL^{-1} and copper concentration of 80 mgL^{-1} .

Metal	SRB	Toxic concentration (mgL^{-1})	Reference
Zinc	Mixed culture	150	Martins et al. [121]
	Mixed culture	25-40	Hao et al. [125]
	<i>Desulfovibrio vulgaris</i>	20	Cabrera et al. [120]
	<i>Desulfomicrobium</i> sp.	>125	Azabou et al. [126]
Copper	Mixed culture	80	Martins et al. [121]
	Mixed culture	4-20	Hao et al. [125]
	<i>Desulfovibrio vulgaris</i> and <i>Desulfovibrio</i> sp.	9	Cabrera et al. [120].
	<i>Desulfovibrio desulfuricans</i> G20	2	Sani et al. [9]

	<i>Desulfomicrobium</i> sp.	>10	Azabou et al. [126]
Lead	Mixed culture	75-80	Hao et al. [125]
Iron	<i>Desulfomicrobium</i> sp.	>60	Azabou et al. [126]
Chromium	Mixed culture	60	Hao et al. [125]
	<i>Desulfovibrio vulgaris</i> and <i>Desulfovibrio</i> sp.	>15	Cabrera et al. [120]
Nickel	Mixed culture	10-20	Hao et al. [125]
	<i>Desulfovibrio vulgaris</i> and <i>Desulfovibrio</i> sp.	>8.5	Cabrera et al. [120]
Cadmium	Mixed culture	4-20	Hao et al. [125]
Manganese	<i>Desulfovibrio vulgaris</i> and <i>Desulfovibrio</i> sp.	>10	Cabrera et al. [120]

Table 6 Toxicity levels of various metals

5.2. Anaerobic bioreactors for sulfate reduction

Batch/ semi-batch/sequential bioreactors

Batch reactor using anaerobic reactors and serum vials as a process is based on the scientific assumption that biodegradation of various pollutants can be achieved by periodic exposure of the microorganisms to defined processes and conditions. Important parameters such as time and frequency of exposure of microorganisms to different pollutant concentration can be set irrespective of any inflow conditions. It offers many advantages over other continuous reactors in terms of flexibility of operation such as operation even at low retention time, control over microbial population and operation with variety of reactor designs. Sequential batch reactor and sequencing batch biofilm reactor are the most common configuration of batch reactors used for sulfate removal. These reactors offers specific conditions for operating the system at high cellular retention times once it promotes microbial immobilization or cellular adhesion in some inert support such as porcelain rings [127], mineral coal [128], and polyurethane foam[87]. The operation of such reactor comprises of feeding liquid influent, anaerobic biological reactions, settling of sludge, decantation and then drawing the final effluent [129]. Some batch reactors used in sulfate removal process are listed in Table 7.

Batch reactor	Substrate	Microbial culture	Sulfate loading rate ($\text{g L}^{-1} \text{h}^{-1}$)/Inflow concentration (g L^{-1})*	Reduction rate/% reduction *	Temperature	pH	HRT (h)	Reference
500 ml Erlenmeyer flask	lactate	<i>Desulfovibrio desulfuricans</i> ATCC 5575	0.8*	---	35	7	10	Okabe et al.[11]
An SBR	Butanol	Mixed culture	0.02	0.017	25±1	5.9-6.5	48	Sarti & Zaiat[20]
ASBBR	Ethanol	Mixed culture	0.041	0.035	31±2	---	48	Sarti et al.[128]
An SBBR	Sodium lactate	Mixed culture	1.6*	82*	30±2	7.2-8.4	24	Mohan et al.[127]
Anaerobic reactors	Sucrose	Mixed culture	1.2*	>90*	35±1	6-6.5	8	Mizuno et al.[130]
ASBR	Sucrose, starch	Mixed culture	1.5	1.33	30	8±0.1	19.16	Costabile et al.[131]

	,meat extract, soybean oil							
ASBR (fed batch)	-do-	-do-	4.5	3.88	-do-	7.9±0. 2	-	
Glass reaction flasks	Maple wood chips, Maple sawdust, Composte d poultry manure, Leaf compost	Anaerobically digested sludge	5.5 *	99*	22±1	5.45- 5.51	192- 384	Neculita&Zagury[1 32]
SBR	Sodium lactate and lactic acid	Granular sludge	1.2*	100*	30	7	5	Torner-Morales &Buitrón[133]
1L cylinder Semi batch reactor	silage	Mixed culture	>1	0.034	30	5.5	20- 40	Wakeman et al.[134]

Table 7: Performance of various batch reactors in sulfate removal

Flow through bioreactors

Different flow through bioreactor configurations have been reported in literature for anaerobic reduction of sulfate. Some of the common bioreactor configurations include continuous stirred tank reactors [104, 105, 135]; membrane reactors [136]; packed bed reactors [124, 137, 138]; and up-flow anaerobic sludge blanket reactors [139, 140]. These bioreactors can be classified into two main groups based on the mixing regime of the influent [141]. The bioreactors with completely mixed regime can be subdivided into CSTR and MBR based on the biomass retention characteristic of the reactor. Biomass retention increases biomass concentrations, which is especially important in sulfidogenic bioreactors because of the low growth rate of anaerobic microorganisms. In case of incompletely mixed or gradient type bioreactor, the bioreactor can be categorized into PBR and UASB based on the use or non-use of the carrier material, respectively. These reactors with gradient mixing regime are mainly used for soluble, low suspended solid wastes [142]. In case of these bioreactors, the activity of the bioreactor is determined by the activity of the biomass and the biomass concentration.

Continuous stirred tank reactors (CSTR)

In this reactor, mixing is done by a mechanical stirrer resulting in a completely mixed system. However, CSTRs are subjected to washout of biomass [107]. Biomass retention has been enhanced by employing sedimentation systems and cationic flocculants [143]. The performance of CSTR in treating sulfate rich wastewaters with different substrate and under varying volumetric loading rates has been investigated by various researchers (Table 8). Moosa et al.[104] investigated the effects of initial sulfate concentration and its volumetric loading on the kinetics

of reaction and activity of sulfate-reducing bacteria. The increase in initial concentration of sulfate in the range 1.0–10.0 kg m⁻³ enhanced the reaction rate from 0.007–0.17 kg m⁻³ h⁻¹.

Membrane bioreactors (MBR)

It is the combination of a membrane process with a suspended growth reactor and is relatively new in the field of sulfate reduction. The advantage of this configuration is that almost complete biomass retention can be obtained which is especially useful in slow growing processes [144]. Membrane bioreactors commonly adopt a biomass retention system relying on the difference in density between the sludge and the reactor liquor, resulting in settling or floatation of the sludge. Vallero et al.[145]investigated the sulfate reducing potential of anaerobic membrane reactor in salt rich wastewaters using a 6L submerged anaerobic membrane bioreactor (SAMBaR) inoculated solely with *Desulfobacter halotolerans* (Table 5). A sulfate reduction rate up to 6.6 g SO₄²⁻L⁻¹ d⁻¹ was achieved in the SAMBaR at an HRT of 9 h including the backflow of permeate used for back flushing. Mizuno et al.[146]investigated the biological sulfate removal in the acidogenic bioreactor with an ultra-filtration membrane system at 35°C using sucrose as the sole organic substrate. The efficiency of sulfate removal by sulfate reduction reached about 100% in the membrane bioreactor, and 55 to 87% of sulfide was removed from the permeate by membrane filtration.

Upflow anaerobic sludge blanket reactor (UASB)

The UASB reactor was developed for methane production from highly concentrated organic wastewater [147]. It is a robust system in which the produced methane gas provides the mixing of the reactor liquor. However, in sulfate reducing reactors mixing depends solely on the upflow of the waste stream, since the gases produced during sulfate reduction stay mainly in solution [144].Lens et al. [148] investigated the effect of the superficial liquid up-flow velocity on the acidifying and sulfate reducing capacity of thermophilic (55°C; pH 6.0) granular sludge bed reactors treating partly acidified wastewater. Synthetic wastewater containing starch, sucrose, lactate, propionate and acetate and a low sulfate concentration (COD/SO₄²⁻) ratio of 10 was fed at an HRT of about 5 h and volumetric organic loading rates (OLR) ranging from 4.9 to 40.0 g CODL⁻¹d⁻¹. At the end of the experiment, the sulfate level of the influent was slightly increased to a COD/SO₄²⁻ ratio of 8. When imposing an OLR of 40.0 g CODL⁻¹d⁻¹, the acidification efficiency dropped to 80% and the sulfate reduction efficiency decreased to 50% in the UASB reactor producing acetate and propionate. At the higher organic loading rates, propionate was converted to n-butyrate and n-valerate. The effluent sulphide concentration was always below 200 mgL⁻¹, of which about 90% was present as undissociated H₂S (under the given conditions of pH 5.8-6.1 and 55°C).

Packed bed reactors (PBR):

In this reactor, a carrier material is used to obtain well settable biomass by biofilm formation on the carrier material in contrast to granulation in a UASB. The carrier material provides a large surface area for bio-film formation [107]. The use of different packed-bed reactors with various combinations of carrier material, carbon source and bacterial group is reported in literature for treating sulfate rich wastewaters (Table 8). Brahmacharimayum and Ghosh [138] operated a PBR packed with polyurethane foam particle as the packing material to study its feasibility on SO₄²⁻ reduction under different feeding and operating conditions of HRT, COD/SO₄²⁻ ratio and SO₄²⁻ concentration. Chen et al. [149] used a packed-bed bioreactor using sea sand as carrier matrix to study the kinetics and stoichiometry of sulfide formation. Waybrant et al. [150] investigated the effect of packing reactive mixtures which were basically waste products. Elliott et al. [80] conducted experiments in a PBR to investigate the effect of pH on the anaerobic sulfate reduction. Chang et al.[137] demonstrated that solid waste materials including oak chips, spent oak from shiitake mushroom farms (SOS), spent mushroom compost (SMC), sludge from a wastepaper recycling plant (SWP) and organic-rich soil (ORS) can be used as electron donors and immobilization matrices to treat ARD. Kolmert and Johnson [81] investigated the tolerance of mixed SRB culture to

acidic environment in an up-flow packed-bed bioreactor, using porous glass beads as a carrier matrix. The average volumetric reduction rates of 0.010–0.013 gL⁻¹d⁻¹ were achieved in bioreactors containing mixed culture of acidophilic and neutrophilic SRB with a feed pH of 4.0. Kolmert and Johnson [81] reported that sulfate reduction occurred at a pH of 3.0 but with a lower rate. Jong and Parry [124] used an up-flow packed-bed bioreactor with sand as carrier matrix for anaerobic reduction of sulfate with mixed culture of SRB. The highest volumetric reduction rate of 0.019 gL⁻¹h⁻¹ was observed at a volumetric loading rate of 0.155 gL⁻¹h⁻¹ at 25°C. Foucher et al. [151] successfully used CO₂ and H₂ as carbon and energy source to treat Chessy mine drainage in an upflow packed-bed bioreactor with a special packing to provide good mass transfer between hydrogen and liquid. Lin and Lee [152] studied anaerobic sulfate reduction in a fixed bed bio-film column bioreactor with Plastic Ballast rings as the supporting media for bio-film formation.

Bacterial group	Substrate	Reactor Type	Temp. (°C)	Feed pH	Volumetric reduction rate (gL ⁻¹ h ⁻¹)	Reference
Mixed SRB	acetate	CSTR	35	8±0.2	0.076	Moosa et al. [105]
Mixed SRB	acetate	CSTR	35	8±0.2	0.184	Moosa et al. [104]
Activated sludge	molasses	CSTR	30	4.5-5.5	-	Ren et al. [153]
<i>Desulfobacter halotolerans</i>	acetate, ethanol	MBR	33±1	7.2±0.2	0.276	Vallero et al. [145]
Sulfate reducing bacteria	sucrose	MBR	35	6-6.5	-	Mizuno et al. [146]
Granular methanogenic sludge	acetate	UASB	32±1	8.3	0.584	Muthumbi et al. [154]
Mixed culture	methanol	UASB	30		0.016	Weijima et al. [155]
Mixed sludge	Starch, sucrose, lactate, acetate, propionate	UASB	55	6	-	Lens et al. [148]
Mixed SRB	Lactate	PBR	25	4.5	0.019	Jong and Parry [124]
Anaerobic sludge From anaerobic digesters	Landfill leachate	PBR	37	6.5	0.015	Thabet et al. [91]
Mixed SRB	Lactate	PBR	22	7	0.228	Baskaran and Nemati[156]
Mixed SRB	H ₂ &CO ₂ + sodium acetate	PBR	25	2.55	0.20	Foucher et al. [151]
Mixed SRB	Lactate	PBR	30	7.4±0.2	0.067	Brahmacharimayum and Ghosh[138]

Table 8: Performance of UASB, CSTR, MBR and PBR used for treating sulfate rich wastewater

5.3. Bioreactors employed for microaerobic process:

The major problem associated with the anaerobic treatment of sulfate-rich wastewater is the production of sulfide. The sulfide so produced is an undesirable product as it is reported to severely impair methanogenesis [157], emanates unpleasant odor, causes corrosion of materials, affects human health and lowers the quality of biogas especially when sulfide content of biogas is above 0.7% by volume [158]. Sulfide is one of the most toxic pollutants having a characteristic “rotten eggs” odor perceptible in fresh air in a dilution of 0.002 mgL⁻¹ of air [159]. Different sulfide removal techniques exist [160], including chemical precipitation as well as gas scrubbing in combination with chemical or biological oxidation processes. The biological reduction process can be made more effective if the sulfide produced can be converted to some other harmless and useful product such as elemental sulfur. As an alternative, introduction of limited quantities of oxygen/air to anaerobic bioreactors can be considered.

Under oxygen-limiting conditions, sulfur is the major end product of the sulfide oxidation, whereas under fully oxygenated condition, sulfide will be completely oxidized to sulfate [161]. Elemental sulfur production is favorable because it is neither inhibitory nor highly soluble, forming a solid precipitate that may produce dense sludge which settles well. The Gibb's free energy (ΔG_0 , kJmol⁻¹) calculated for the reactions involved in sulfide oxidation (Table 9) suggests that the reactions are feasible.

Reaction	ΔG_0 (kJmol ⁻¹)	ΔG_0 (kJmol ⁻¹)	ΔG_0 (kJmol ⁻¹)
	pH = 7.0	pH = 7.8	pH = 8.4
$H_2S + 0.5O_2 \rightarrow S^0 + H_2O$	-203.8	-203.8	-203.8
$HS^- + 0.5O_2 \rightarrow S^0 + OH^-$	-209.1	-204.7	-201.2
$S^{2-} + 0.5O_2 + 2H^+ \rightarrow 2S^0 + H_2O$	-237.1	-227.6	-220.8
$H_2S + 2O_2 \rightarrow SO_4^{2-} + 2H^+$	-791.2	-800.0	-807.2
$HS^- + 2O_2 \rightarrow SO_4^{2-} + H^+$	-796.7	-801.2	-804.6
$S^{2-} + 2O_2 \rightarrow SO_4^{2-}$	-824.1	-824.1	-824.1

ΔG_0 : Standard Gibb's free energy for the reaction at pH = 7. ΔG_0 (kJmol⁻¹) values for the individual compounds for calculation are referred from Lide[162], Thauer et al.,[163], Stumm and Morgan,[164] and Rossini et al.,[165].

Table 9 Gibbs free energy values for the reactions involved in sulfide oxidation

As given in Table 9, by regulating the oxygen dosing, microaerobic environment can be created in anaerobic reactors where sulfides can be oxidized into elemental sulfur. In addition, such conditions are sufficient to maintain an appropriate reducing environment essential for microorganisms responsible for sulfate reduction. The hydrogen sulfide so formed is oxidized to various other products indicating an effective competition of sulfide-oxidizing microorganisms with other microorganisms for the available oxygen.

Mode of micro-aerobic regulation:

Direct introduction of oxygen/air into ‘anaerobic’ bioreactor systems for sulfide removal has been investigated previously during treatment of sulfate-rich wastewaters. By regulating the oxygen dosing, micro-aerobic conditions can be maintained in anaerobic reactors to maintain an acceptable reducing environment for anaerobic microorganisms to degrade the organic matter [157, 166, 167]. Biological hydrogen sulfide treatment processes is more favored nowadays compared to other traditional physico-chemical processes as it is less expensive and requires less or no utilization of chemicals [2, 168]. Biogas containing hydrogen sulfide from anaerobic treatment of high sulfate wastewaters can be reduced effectively both in fed-batch reactors [169], and in continuous reactors

[166, 167, 170] by providing limited oxygen supply. Microorganisms such as *Thiomicrospira* sp. and *Thiobacillus* sp. are capable of performing sulfide oxidation even in anaerobic conditions like those in the anaerobic sludge digester depending on the oxygen availability [5]. Pure cultures acclimatized to hydrogen sulfide, oxygen and nutrients are utilized in bio- scrubbers [171] and biotrickling filters [172, 173] to remove hydrogen sulfide biologically. In order to make biological hydrogen oxidation more cost effective, micro-oxygenation of the digester can be done as an alternative as the sludge already contains some sulfide oxidizing bacteria[174]. By supplying air or pure oxygen under micro-aerobic conditions to the headspace [175] removal of hydrogen sulfide in the biogas was achieved. Removal of only hydrogen sulphide from the biogas or the total dissolved sulphide was observed depending on the sludge or biogas recirculation and the oxygen supply point (headspace or liquid phase). Sludge recirculation resulted in the removal of hydrogen sulphide from the biogas while dissolved sulphide removal also occurred with bio-gas recirculation [176]. Micro-aerobic supply of oxygen or air is thus a very practical and feasible method for hydrogen sulfide removal from anaerobic digesters without causing much harm to the anaerobic digestion process [175, 176]. Oxygen or air was introduced either directly into the reactor [167, 169, 177] or into the combined flow of effluent and biogas, right before this mixture entered a reservoir acting as a gas/liquid separator [157]. The mode of oxygen dosing was done differently in each of the reactor studies. Khanal and Huang [157] applied an ORP system to monitor the oxygen dosing taking into account that the ORP varies linearly with the logarithm of oxygen concentration, the intrusion of oxygen, even at a level well beyond the detection limit of commercially available oxygen probe (0.1 mgL^{-1}), can be easily sensed by the ORP measurement. Chuang et al.[178] used DO and ORP sensors in a floated bed micro-aerobic reactor for a moderate degree of oxidation of hydrogen sulfide. van der Zee et al. [169] introduced a low airflow of $0.7\text{--}0.9 \text{ m}^3 \text{ m}^{-3} \text{ d}^{-1}$, corresponding to a super-stoichiometric ratio of 8– 10 mol O_2 per molS. Diaz et al.[176] maintained micro-aerobic conditions using the regulated flow of pure oxygen with a Cole-Parmer EW-32660-26 mass flow controller from an oxygen cylinder; when air was employed as an oxygen source and was injected into the headspace. A flow rate of $1.8 \pm 0.1 \text{ NmLmin}^{-1}$ representing $\sim 0.25 \text{ NL}$ of oxygen per L of feed sludge was provided to the sludge digesters to provide micro-oxygenation [175]. A controlled and continuous air injection (0.19 Lmin^{-1}) given at 40% volume of an up-flow hybrid sulphidogenic reactor affected sulfide oxidation inside the reactor and enhanced the sulfate reduction efficiency [96]. Xu et al.[179] achieved sulfate removal efficiency of 81.5% and S^0 recovery of 71.8% in an integrated sulfate reducing and sulfate oxidizing EGSB bioreactor under micro-aerobic conditions by providing Dissolved Oxygen dose of $0.10 - 0.12 \text{ mgL}^{-1}$ by adjusting aeration flow rate in a separate 5L vessel used as the aeration unit. Chemostats with working volume of 4L operated at $35 \pm 0.5^\circ\text{C}$ and a HRT of 15 days was maintained in a complete mixing condition by biogas recirculation at a flow rate of $3\text{--}4 \text{ Lmin}^{-1}$ through a cadet pump (Cole Palmer, Model 7530-65) [157]. Krishnakumar et al.[180] used a novel aerobic bioreactor, the reverse fluidized loop reactor (RFLR) (US Pat. No.6,544,421) with biofilm carrier particle for recovering sulfur from aqueous sulfide at an HRT around 90 minutes. The air supply into the reactor was regulated with an on-off controller to maintain the redox potential required levels. Chuang et al [178] operated a system composed of an upward-flow anaerobic sludge blanket (UASB) reactor and a floated bed micro-aerobic reactor packed with elastic porous carriers maintained at dissolved oxygen below 0.5 mgL^{-1} . An average of $70 \pm 6\%$ of sulfate was transformed to hydrogen sulfide in UASB reactor followed by the oxidation of most of the sulfide to elemental sulfur and sulfate in micro-aerobic reactor. At a HRT of 2.8 h, sulfide was almost completely removed in the microaerobic reactor. Diaz et al.[176] studied the performance of oxygen, air and nitrate for microaerobic removal of hydrogen sulfide in biogas from sludge in a 200-L digester with HRT of ~ 20 days. Hydrogen sulfide content was reduced from $15,811 \text{ mgN}^{-1} \text{ m}^3$ to less than $400 \text{ mgN}^{-1} \text{ m}^3$ when oxygen was supplied ($0.25 \text{ N m}^3 \text{ m}^{-3}$ feed) while introduction of air ($1.27 \text{ N m}^3 \text{ m}^{-3}$ feed) successfully removed more than 99% of the hydrogen sulfide content, with a final concentration of $\sim 55 \text{ mgN}^{-1} \text{ m}^3$. Two pilot-plant digesters with an HRT of $\sim 20 \text{ d}$ were micro-oxygenated at a rate of 0.25 NL per L of feed sludge with a removal efficiency higher than 98% [175]. Sulfide oxidation occurred in the headspace were different sulfide-oxidizing bacteria developed then, The supply of oxygen to the headspace was found to be the optimal dosing point resulting in elemental sulfur

formation due to different sulfide-oxidizing bacteria found present. Xu et al. [179] reported the successful operation of an integrated SRB + SOB expanded granular sludge bed (EGSB) reactor under microaerobic condition. At DO = 0.10–0.12 mgL⁻¹, the sulfate removal efficiency reached 81.5% and the recovery of S⁰ peaked at 71.8%, higher which is the highest reported so far. At DO > 0.30 mgL⁻¹ activities of SRB were inhibited, leading to failure of the SRB + SOB reactor. Performance of microaerobic reactors used for treating sulfate rich wastewaters is given in Table 10.

Type of reactor	Reactor volume (Litre)	Influent Sulfate (gL ⁻¹)	CO D/Sulfate ratio	Sulfate Removal efficiency (%)	S ⁰ recovered	Carbon source	Oxygen introduction	DO (mgL ⁻¹)	Aeration level (L/d)	Temp (°C)	Support material	HRT (hrs)	Reference
Down-flow fluidized bed reactor	2.3	3.9	0.66	75	52	Lactate	Filtered air at bottom of reactor		2.28	30	Finely ground LPDE without additives	24	[181]
		6.2	0.67	77	54				3.42				
EGSB	4	1±0.1	3	81.5 94.6	71.8 62.5	Lactate	Separate vessel for aeration to maintain DO	0.08-0.1 0.1-0.12	14.4 28.8	30±1	-	18	[179]
Chemo-stat	4	1 3 5	10 3.33 2	43.4 22.4 59	Dissolved sulfides UD	Glucose	Recycled biogas stream (pure O ₂)		-230 to -180 ORP	35±0.5	-	360	[157]
Pilot plant reactor	250	2.2	42.7 - 21.8	-	-	Sludge	Pure O ₂ Headspace	~0.25 NL of oxygen per L of feed sludge	1.8 ± 0.1 NmLmin ⁻¹	35±1	-	480	[175]

Table 10 Performance of microaerobic reactors used for treating sulfate rich wastewaters

Biokinetic coefficients:

The design of various waste water treatment processes based on hydraulic parameters are not sufficient considering the wide variation in the nature and composition of waste waters and various complex bio-chemical reactions taking place [182]. It is because of this that estimation of bio-kinetic coefficients is required. The estimation of the coefficients assists in prediction of population dynamics, design and process performance of various bio-reactors used in sulfate reduction. Following this, biokinetic models have been employed by various studies to control and predict treatment plant operation performance, optimize the plant design and the results of

scale-up pilot studies. Various Bio-kinetic models like Monod model, Contois Model, Chen and Hashimoto model and Stover-Kincannon model, have been used to study the nature of sulfate reduction. Table 11 and Table 12 respectively show the various Bio-kinetic models used and biokinetic coefficients obtained by different researchers. Along with these models various mathematical models which are modifications of the previously mentioned models that incorporate important factors such as pH, temperature, type of reactor and inhibitory substances have been also used by researchers [86, 102, 183-185]. All of these models used so far are growth models and are applicable up-to the growth phase of the bacterial growth curve.

Model	Equation	Plot	Slope	Intercept	Reference
Michalis-Menten	$v = \frac{V_m C_s}{K_m + C_s}$	$\frac{1}{v}$ vs $\frac{1}{C_s}$	$\frac{K_m}{V_m}$	$\frac{1}{V_m}$	Brandis-Heep et al. [186]
Monod	$\mu = \frac{\mu_{max} S}{K_s + S}$	$\frac{1}{\mu}$ vs $\frac{1}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1}{\mu_{max}}$	Monod [187]
Contois	$\mu = \frac{\mu_{max} S}{K_s'' X + S}$	$\frac{1}{\mu}$ vs $\frac{S_0}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1 - K_s}{\mu_{max}}$	Contois[188]
Chen and Hashimoto	$\mu = \frac{\mu_{max} S}{K_s S_0 + (1 - K_s) S}$	$\frac{1}{\mu}$ vs $\frac{X}{S}$	$\frac{K_s}{\mu_{max}}$	$\frac{1}{\mu_{max}}$	Chen & Hashimoto [189]
Kinetic model	$r_s = \left\{ \frac{\mu_{max} S}{K_s' S_0 X + S} - k_d \right\} \frac{X}{Y}$				Moosa et al. [105]
Stover –Kincannon model	$\frac{V}{Q(S_i - S_e)} = \frac{K_B S_i}{U_{max} V Q} + \frac{1}{U_{max}}$	$\frac{V}{Q(S_i - S_e)}$ vs $\frac{V}{Q S_i}$	$\frac{K_B}{U_{max}}$	$\frac{1}{U_{max}}$	Kosińska & Miśkiewicz[13]
First Order Growth model	$\ln(X) = \mu t - B$	$\ln X$	t	$-B$	Silvia N. Medircio[190]

Where X , bacterial population (concentration); B , constant; μ and μ_{max} are specific growth rate and maximum specific growth rate (time^{-1}); S_i (or S_0) and S (or S_e), threshold initial and effluent substrate (concentration); K_s, K_s'', K_B are the Half velocity constant (concentration); Y , biomass yield (growth of bacterial mass in g/ amount of substrate utilized in g); k_d , decay rate (time^{-1}); U_{max} , substrate utilization rate (concentration /time); Q , flow rate (volume /time); r_s , reaction rate (concentration/time); t , time

Table 11 Various models used so far to determine bio-kinetic coefficients

Reactor type	Carbon source	Major organisms	Model fit	Bio-kinetic parameters					References
				μ_m (h^{-1})	Y_{COD} (g biomass g^{-1} COD utilized)	Y (g biomass g^{-1} sulfate reduced)	K_s (g L^{-1})	V_{max}	
Chemostat	Lactate	Mixed Culture	Contois	0.2	0.08		0.6		Oyekola et al. [191]
500 ml fermenter flasks	Acetate + CO_2	<i>Desulfovibrio vulgaris</i>	---	0.15	---	0.08	---		Badziong & Thauer[192]
115 ml serum vials	Acetate	<i>Desulfobacter postgatei</i>	---	0.03	0.07				Brandis-Heep et al. [186]
Continuous chemostat	Acetate	<i>Desulfobacter postgatei</i>	Michaelis Menten equation	---	---	---	77 μM	3.2 $\text{mmol h}^{-1}\text{g}$	
Screw cap bottles	Acetate	<i>Desulfobacter vibrioformis</i>	----	---	4.6	---	---	---	Lien & Beeder[193]

50 ml screw cap bottles	Acetate	<i>Desulfobacter psychrotolerans</i>	---	---	0.07-0.075	---	---	---	Tarpgaard et al. [194]
2L Continuous mechanical stirred reactor	Lactate	<i>Desulfovibrio desulfuricans</i> Essex 6	---	0.054	0.04	0.07			Cooney et al. [195]
Batch culture, 60mL test tubes	Lactic acid	<i>Desulfovibrio desulfuricans</i> , strain NCIMB 9467	Monod	0.04	0.01	2.09	1092	---	Herrera et al. [196]
3L glass reactor	Ethanol	Mixed culture	Monod	0.012 - 0.013	---	---	0.2	---	Nagpal et al. [197]
300 & 1000mL Fermentation units ,	Glycerol	<i>Desulfovibrio baarsii</i> , <i>Desulfomicrobium</i> sp., and <i>Desulfatomaaculum</i> sp.	Mathematical model using Monod equation	0.02	0.28	0.23	2ml /L	---	Dinkel et al. [184]

Table 12 List of various bio-kinetic coefficients obtained for different SRB using bio-kinetic models

Conclusion:

The environmental and industrial impacts caused due to sulfate makes it imperative to search for various treatment methods wherein biological sulfate reduction stands as an efficient process. The sulfate reducing bacteria involved in the processes are ubiquitous in anoxic and anaerobic environments, not only remain versatile in their metabolism, but are also able to thrive even in harsh climate and pH conditions. They can also utilize a wide range of natural and synthetic carbon sources which defines their variability in terms of existence and functioning. Lower sludge production along with the generation of bio-film in attached growth processes like PBR, and membrane bio reactors makes it a more appropriate method to isolate the involved microorganisms from toxic environments. The choice of a suitable electron donor and process performance of a reactor for sulfate treatment depends upon the availability and effectiveness as well as operational costs involved. The recovery of sulfide to elemental sulfur is a great trend which makes it as one of the emerging technologies for sulfate removal. However, very few studies have been carried out on the effects of air flow rates on the sizes and settling behavior of the elemental sulfur particles under microaerobic conditions. Optimization studies would result in maximum sulfate removal along with maximum elemental sulfur generation. Biological sulfate reduction would become a cost effective option to treat the industrial sulfate rich wastewaters if the sulfur recovery from the simultaneous sulfate reduction and sulfide oxidation is improved.

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