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REVIEW ARTICLE

An overview of sulphate reducing bacteria in treating the sulphate-rich wastewater

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Abstract

Efficient removal of pollutants from sewage is essential for maintaining the sustainability of the ecosystem, which means that effective biological methods must be explored. Compared to traditional physical and chemical methods, bioremediation is an attractive alternative method because of its low-cost, maintains ecological balance and helps rebuild the polluted environment. In particular, the sustainable bioremediation technology based on sulphate-reducing bacteria (SRB) is considered to be one of the best treatment schemes to alleviate environmental pollution. The present paper provides a brief summary of the approach used to remove pollutants using sulphate-reducing bacteria, an obligate anaerobic bacterium. SRB are recognized for their capacity to convert sulphate into hydrogen sulfide, which facilitates the precipitation of heavy metals, degradation of organic pollutants and forms a large number of metal sulfides. The analysis delves into the biological processes utilized by SRB, the ideal conditions for their effectiveness and the potential advantages and obstacles associated with integrating SRB into wastewater treatment facilities. Additionally, it confronts challenges such as odor control, hydrogen sulfide mitigation and microbial survival. By examining of current studies and technological progress, this analysis underscores the potential of SRB as a sustainable and effective remedy for enhancing wastewater treatment and mitigating environmental contamination.

Key words: anaerobe; electron donors; microbial consortia; sulphate reduction; sulphate-reducing bacteria; wastewater

Introduction

Nearly eighty percent of the water used daily for domestic activities is discharged as wastewater. Similarly, industries generate a substantial amount of wastewater, the quality and quantity of which vary according to the product and manufacturing method. Consequently, wastewater has emerged as a significant concern for a large portion of the population. With one-quarter of the world's population experiencing water scarcity, the major goal of wastewater treatment plants is to supply clean water while reducing environmental pollution. Wastewater is generally characterised as sewage or non-sewage. Sewage is wastewater produced by home activities that contains primarily urine and faeces. This category also includes toilet water from hotels, schools, restaurants and hospitals. Non-sewage water includes wastewater from industrial activity, storm water and runoff from washing clothing and cleaning utensils. Other well-known terms that are gaining attention as wastewater categories include blackwater, greywater and yellow water (1). As the population expands rapidly, water consumption also increases, resulting in a significant rise in sewage production. In India, the total sewage generated amounts to 72368 MLD (Million Liters Per Day), surpassing the installed capacity of sewage generated. The operational and developmental capacity of these plants stands at 26869 MLD, treating only 28 % of the total sewage water generated, which amounts to 20236 MLD (2).

Sulfate (SO_4^{2-}) is a major ion commonly found in natural waters, as well as in municipal and industrial wastewater. While generally regarded as non-toxic, elevated sulphate concentrations can disrupt its natural environmental cycle and pose health risks when consumed over extended periods. High levels of sulphate in water, particularly concentrations above 600 mg/L, may result in issues such as dehydration, gastric upset and a laxative effect, in addition to contributing to scaling in pipes and public water systems (3). For these reasons, sulfate ions must be eliminated from wastewater before it is released into the atmosphere. While numerous physical, biological and chemical strategies exist for sulfate removal from wastewater, adsorption is recognized as a straightforward, economical and effective solution (4). But the drawback is that regenerating spent adsorbents for reuse can be technically challenging and costly, particularly for certain types of adsorbents or under specific operating conditions. Regeneration processes may require harsh chemicals or energy-intensive procedures, impacting the sustainability of the treatment system and it is highly pH dependent (5).

A promising substitute for conventional techniques is the newly developed anaerobic wastewater treatment technology, in which SRB completely break down organic debris (6). SRB-based biological treatment techniques have shown encouraging promise in the treatment of sulphate and heavy metal-containing wastewater. It is cost effective and economically attractive. The ideal removal conditions are attained with a pH of 7.19, initial sulphate concentration of 2153.15 mg/L, a COD/ SO_4^{2-} ratio of 2.72 and a COD ethanol/COD total ratio of 1. These parameters resulted in a remarkable sulphate removal rate of 98 % (7). The breakdown of organic matter in anaerobic environments is a multi-phase process involving many bacterial groups coordinated and syntrophic actions. As shown in Fig. 1 (8, 9), these include fermentative bacteria, obligatory hydrogen-producing acetogens, perhaps homoacetogens, methanogens and sulphate-reducing organisms. This review delves into the application of SRB in the removal of sewage pollutants, with a specific emphasis on their frequently overlooked role in the eradication of pathogens. It offers a detailed perspective by integrating microbiological, operational and engineering factors, supported by bibliometric network visualizations that illustrate current research trends. The review also highlights the synergistic interactions within microbial consortia and their practical applications in bioreactor systems, while exploring new opportunities for the application of SRB in carbon capture and sustainable bioenergy generation.

Literature studies

Extensive searches were carried out across different subjects using a wide range of keywords. A bibliometric analysis was executed based on data collected from Scopus and network visualization was performed using VOS viewer. From the 605 documents initially gathered from Scopus, only those with more than five citations were included, resulting in a selection of 421 documents. When sources were used as the unit of analysis (Fig. 2), a total of 243 were identified within the research area of sulphate-reducing bacteria. Among that 90 met the criteria of having a minimum of 3 documents of a source and a minimum of 2 citations.

In Fig. 3, the network visualization depicts the connections among keywords related to sulphate-reducing bacteria and their associated subjects generated through R 4.4.2 software. The clusters are distinguished by color: the red cluster centers on molecular and genetic research (such as 16S rRNA, phylogeny and genetic growth), the green cluster focuses on biochemical processes (including hydrogen sulfide, oxidation and metabolism) and the blue cluster showcases applied environmental and industrial applications (like wastewater treatment, heavy metal removal and bioremediation). The interconnected nodes illustrate collaborative research themes that link microbiology, biochemistry and environmental science.

Sources and characteristics of sulphate-rich wastewater

One of the most prevalent anions in the environment is sulphate. It is frequently found in high amounts and is a prevalent component of many natural streams and wastewaters. Sulphate is also generated through anthropogenic activities. Human-made sources typically stem from industrial activities such as edible oil production, molasses fermentation, tanneries, food processing, coal-fired power plants and paper mills (10). Besides, natural sulphate can arise from processes like the oxidation of sulfide ores in acid mine drainage (AMD), dissolution of sulphate minerals and photochemical reactions in seawater involving volcanic SO_2 and H_2S. Sulphate becomes a pollutant when excessive levels are dumped into the natural environment, posing various environmental risks (11). The maximum limit of sulphate in water meant for human consumption is recommended at 250 mg L^{-1} (12)(13). However, the general requirements for discharge of effluents are limited upto 1000 mg L^{-1} (14). The Bureau of Indian Standards 10500 says that maximum concentration of sulphate in drinking water should not exceed 200 mg L^{-1} (15). Fig. 4 shows the sulfur cycle occurring within sewer system, shows the reduction of

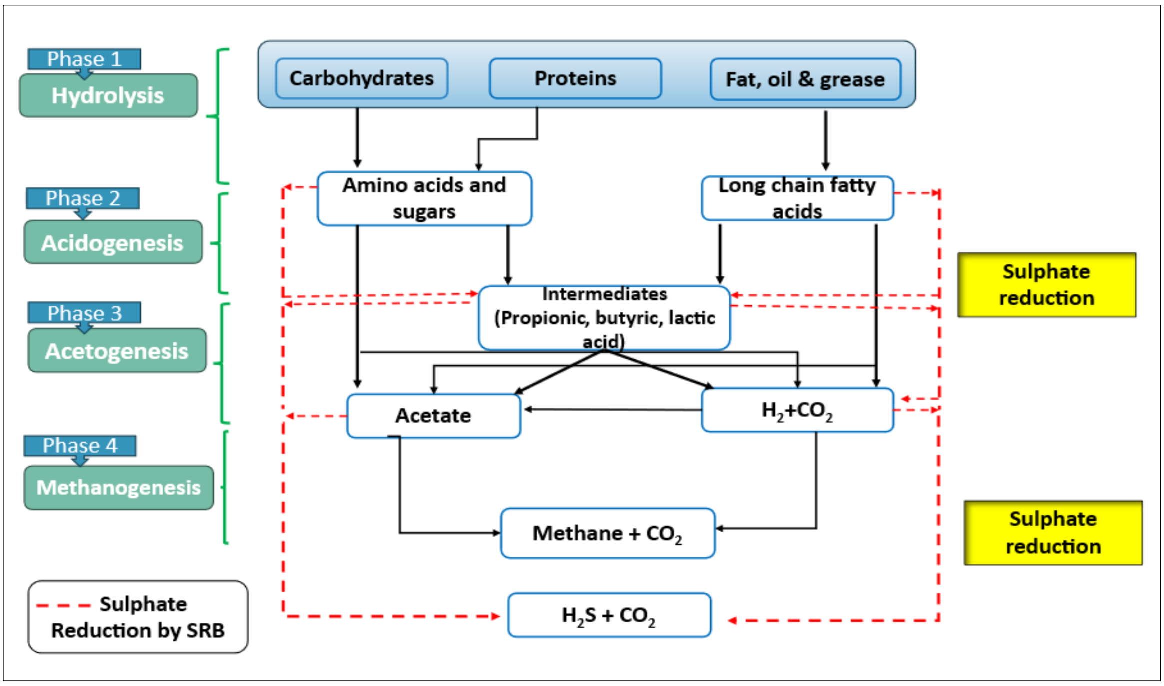

Fig. 1. Anaerobic degradation of organic compounds in the presence of sulphate.

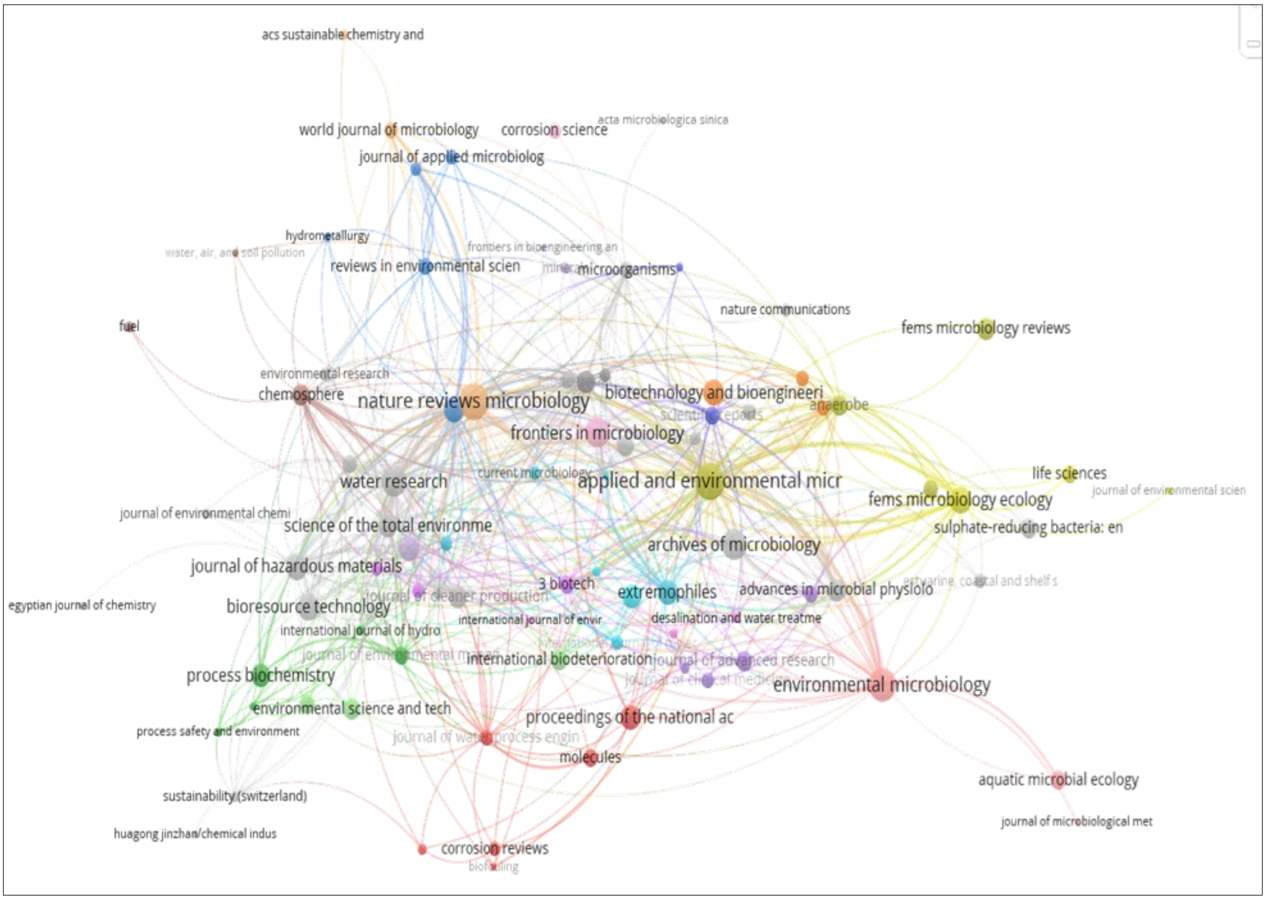

Fig. 2. Network visualisation of sources like journals/institutions as unit of analysis related to SRB.

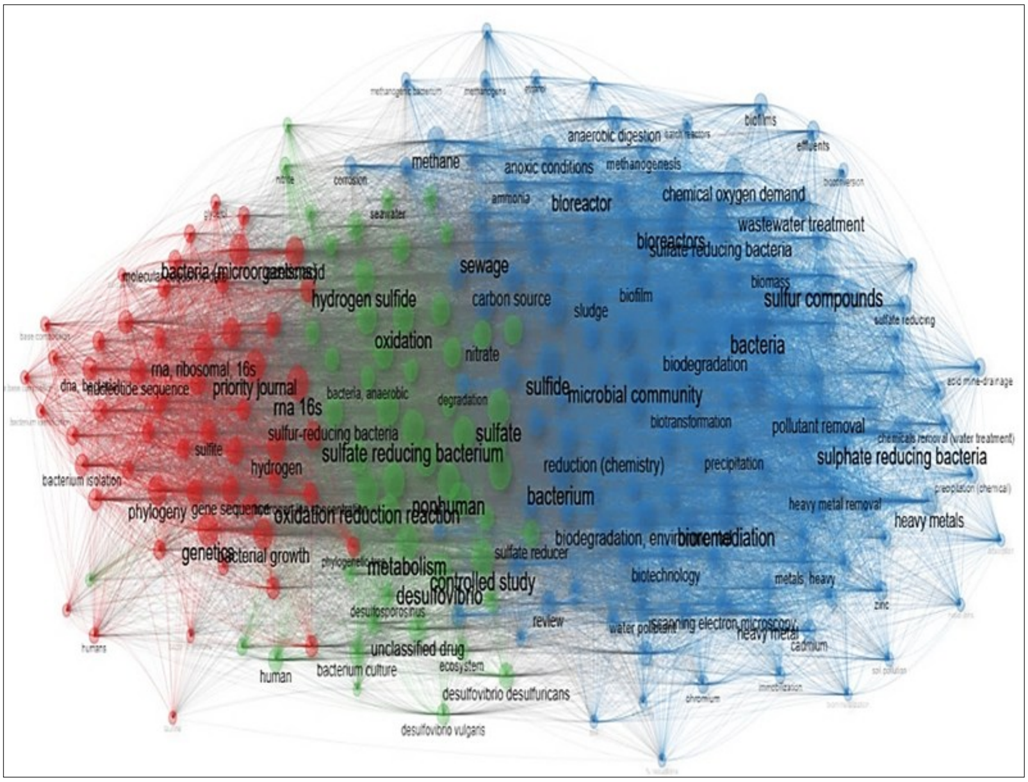

Fig. 3. Network visualization of keywords as unit of analysis related to SRB.

sulphate to H_2S gas by the SRB community and oxidation of H_2S to sulphuric acid by the SOB community or directly oxidized (16).

Elevated concentrations of sulphates in potable water may result in an unpalatable flavor and excessive levels (>1000 mg/L) can lead to ailments such as diarrhoea. Recently, there has been a close connection between increasing sulphate levels and adverse environmental consequences. Sulphates have the potential to eliminate aquatic vegetation while promoting algal blooms, causing significant disruptions to ecosystems. Ruminants like moose and cattle are vulnerable to sulphates as their digestive systems can transform them into toxic hydrogen sulfide. Furthermore, sulphates can generate deposits on stream beds, covering areas that aquatic organisms require for shelter

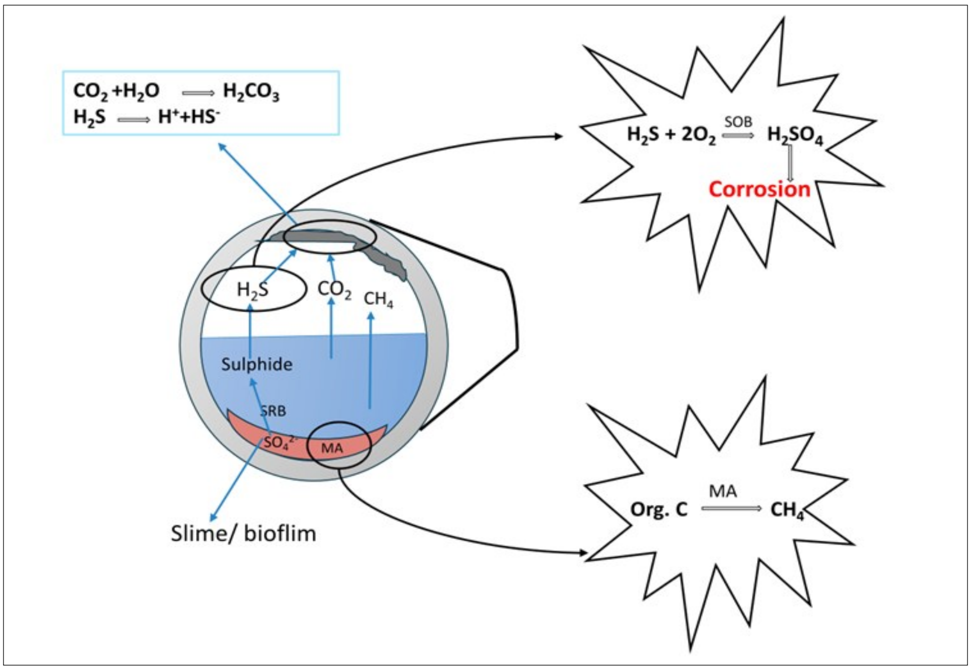

Fig. 4. Sulfur cycle occurring within the sewer pipe.

and reproduction (17). In sewer systems characterized by anaerobic conditions, sulphate-reducing bacteria convert sulphate into hydrogen sulfide (H_2S), which results in both corrosion of the sewer infrastructure and the release of unpleasant odors (18). It also influences chemical and biological properties of the wastewater. Industrial and municipal effluents are commonly acknowledged as the main sources of heavy metal pollution. The increase in industrial activities leads to the generation of large amounts of waste and the pollution of wastewater with elevated levels of heavy metals. The discharge of heavy metal ions into the environment, due to industrial progress and the growth of urban areas, is now a major global issue (19). The removal of sulphate from wastewater is crucial for various reasons. Firstly, it's vital for environmental preservation as elevated sulphate levels can contribute to water pollution, harming aquatic ecosystems and it aids in controlling odours. Additionally, sulphate removal is necessary to prevent corrosion in infrastructure caused by sulphate-reducing bacteria. Moreover, it safeguards downstream water bodies from the adverse effects of untreated wastewater with high sulphate levels, as shown in Table 1. The ailments including gastrointestinal problems, which manifest as symptoms like nausea, vomiting and metabolic acidosis, can be brought on by elevated levels of SO_4^{2-} (20).

Distribution and classification of SRB

SRB are strictly anaerobic prokaryotes found in diverse habitats (Fig. 5) lacking oxygen, including paddy soils, rhizosphere of plants, underground pipelines, freshwater sediments, mud volcanoes, lakes, marshes, petroleum reserves and industrial wastewater (29, 30).

Martinus Beijerinck isolated the first SRB in the year 1895, which was later classified as *Desulfovibrio desulfuricans* (31). By the 1970s, SRB had been categorized into three genera namely: *Desulfovibrio, Desulfotomaculum* and *Desulfomonas.* In 1965, the classification of SRB belonging to the genera *Desulfotomaculum* and *Desulfovibrio* was expanded due to their ability to produce spores (32). Advances in biotechnology led to the isolation and naming of several new genera of SRB. In the 1984 edition of the Bergey's Manual of Systematic Bacteriology, the classification of SRBs was expanded to include eight genera: *Desulfovibrio, Desulfomonas, Desulfobulbus, Desulfotomaculum, Desulfococcus, Desulfobacter, Desulfosarcina and Desulfonema* (33).

A reclassification of SRB proposed into six clusters, owing to rapid advances in molecular biology: *Desulfotomaculum, Desulfobubus, Desulfobacterium, Desulfobacter, Desulfococcus-Desulfonema-Desulfosarcina* and *Desulfovibrio-Desulfomicrobium.* SRB have recently been identified in five domains with totally 41 genera and 168 species (34). Notably, *Desulfovibrio* and *Desulfotomaculum* emerged as the most common SRB taxa used in wastewater treatment. The sulfur cycle depends heavily on microorganisms and SOB and SRB are two important bacterial families that are involved in this cycle (35). These bacterial groups are widely distributed ecologically and they are found in habitats with minimum quantities of oxygen, nitrates and oxidized metals and different degrees of sulphate reduction (36). Numerous prokaryotic species, such as bacteria and archaea, are part of SRB. Ubiquitous habitats for these common species include the edges of oil fields, marine sediments, hydrocarbon seeps, PG deposits and wastewater from industrial operations. These areas are also known to be rich in SO_4^{2-}. They are composed of a gram-positive sporulating species called *Desulfotomaculum* and several Gram-negative taxa, including *Desulfovibrio*, *Desulfobulbus, Desulfobacterium, Desulfosarcina* and *Desulfococcus*. Some genera are thermophilic, such as *Thermosulfobacterium* and *Thermodesulfovibrio* (37). Some SRB

Table 1. Industries producing sulphate rich wastewaters

Wastewater source	Sulphate (mg L^{-1})	Reference
Tannery industry	1500-2000	(21)
Drug industry	100-3000	(22)
Mining industry	1500	(23)
Citric acid	3000	(24)
Alcohol production	1000-3000	(22)
Sea food processing	2800	(25)
Textile industry	1568. 6	(26)
Pulp & paper industry	100- 500	(27)
Molasses fermentation	1000-4000	(28)

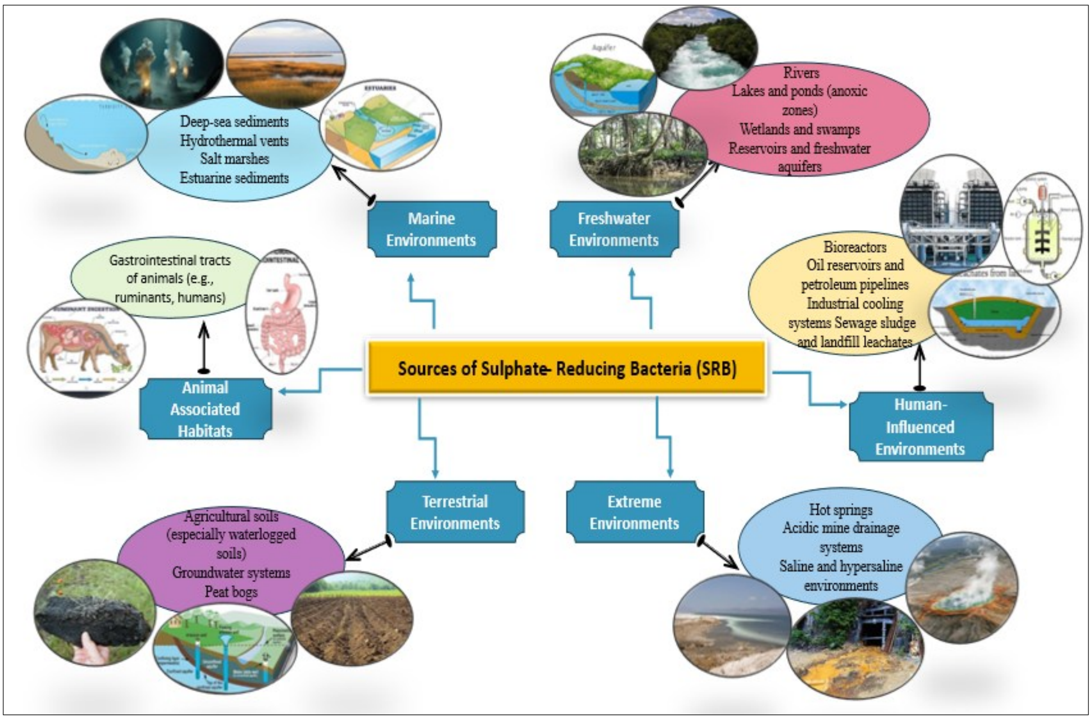

Fig. 5. Sources of SRB from different habitats.

Table 2. Isolation and characteristics of SRB

Genera	Morphology	pH	Growth T (°C)
Desulfovibrio	No spores, curved rods	4.5-7.3	25-40
Desulfomicrobium	Rod-shaped, no spores	8.5	25-40
Desulfobollus	Curved	4.5-6.9	25-40
Desulfovirga	Rod shaped	6.6-7.4	20-40
Desulfobacterium	Round, rod-shaped	6.5-7.4	20-35
Desulfotomaculum	Straight or curved rod	6.4-7.3	25-40, 40-65
Thermodesulfobacterium	Arc, rod	7.0	65-70
Desulfobacter	Round, rod-shaped, without spores	6.2-8.4	20-23
Desulfococcus	Spheroidal, no spores	6.7-7.6	28-35
Desulfosarcina	Stacking, without spores	6.5-7.0	33
Desulfobacca	Oval to rod	6.5-8.3	27-47
Desulfomonile	Rod-shaped, spheroidal	6.8-7.2	37
Desulfoglaeba	Rod to oval end	6.5-7.2	31-37
Desulfobulbus	Oval, shape of Lemon, No spores	6.0-7.8	25-40
Desulfonema	Screw shape, no spores	7.0-7.2	28-32

taxa, such as *Desulfobulbus, Desulfotomaculum* and *Desulfovibrio*, can survive in microaerobic environments even though they are strictly anaerobes (35). The isolation and characteristics of some genera of SRB are shown in Table 2 (38).

The assays conducted comprised the Voges-Proskauer reaction, Methyl red test, Indole production, Malonate utilization, Esculin hydrolysis, Oxidase production and the assessment of sugar utilization, which included Arabinose, Xylose, Adonitol, Rhamnose, Cellobiose, Melibiose, Saccharose, Raffinose, Trehalose and Glucose. Additional tests included Gram staining, ONPG, Lysine utilization, Ornithine utilization, Urease activity, Phenylalanine deamination, Nitrate reduction, H_2S production, Citrate utilization and Catalase activity. Evaluations were also conducted on the use of lactose, sorbitol and sucrose. A detailed analysis of the colony's attributes was conducted, considering its size, form, elevation, perimeter, texture, color and composition. Furthermore, gram staining was used in microscopic analysis to identify structural characteristics and determine if the strains were Gram-positive or Gram-negative (39).

Physiology of sulphide production in sewers

Electron-donor metabolism

SRB plays a significant role in the biogeochemical cycles of both sulphur and carbon. In the carbon cycle, sulphate reducers were initially believed to have a negligible role until the early 1980s. At that time, the *Desulfovibrio* and *Desulfotomaculum* species were identified, which utilized hydrogen and various organic compounds like ethanol, formate, lactate, pyruvate, malate and succinate for their growth. These SRB typically incompletely oxidize carbon compounds, resulting in the production of acetate. Sulphate-reducing bacteria that break down organic compounds into carbon dioxide typically utilize acetate as a growth substrate. These bacteria employ two distinct pathways for acetate oxidation. One pathway, known as the modified citric acid cycle, is utilized by *Desulfobacter postgatei* (Fig. 6), (40). The other is known as the acetyl-CoA pathway, is employed by species such as *Desulfobacterium, Desulfotomaculum* (Fig. 7), (40), *Desulfococcus and Desulfobacca acetoxidans.* Over the last 25 years, numerous new sulphate reducers have been identified. These microorganisms possess the capability to thrive on a diverse range of substrates, including sugars (41), amino acids and one-carbon compounds like methanol, carbon monoxide and methanethiol. Sulphate reducers can also grow by dismutating compounds such as thiosulphate, sulphite and sulphur, which results in the production of both sulphate and sulphide (42). In 1976 early workers suggested that anaerobic

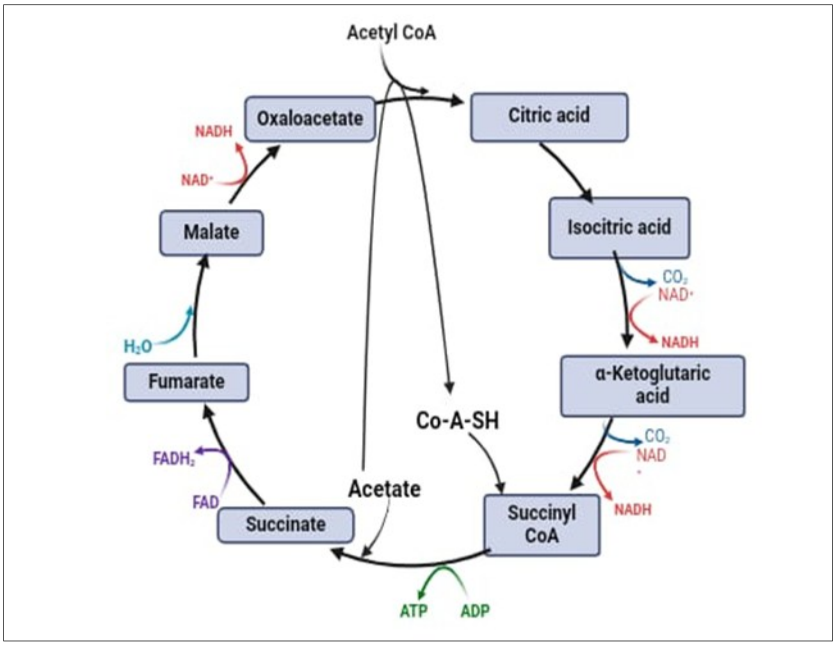

Fig. 6. Pathway of acetate oxidation via the citric acid cycle in *Desulfobacter postgatei* (40).

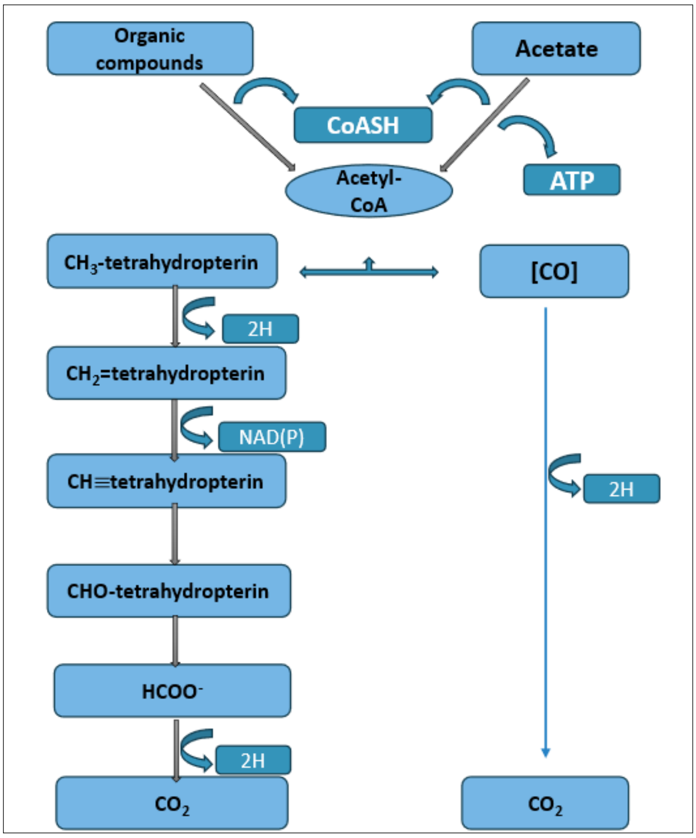

Fig. 7. Non-cyclic carbon monoxide dehydrogenase pathway by *Desulfotomaculum acetooxidans* (40).

methane oxidation might be connected to sulfate reduction. The archaea involved are typically related to the *Methanosarcina* genus, while the sulfate-reducing bacteria are associated with the *Desulfosarcina-Desulfococcus, Desulfobulbus,* or *Desulfobacter* genera (43).

Electron acceptor metabolism

Sulfate serves as the terminal electron acceptor for the growth of sulphate reducers. Sulfate, however, is not excellent electron acceptor for microbes biologically. With an E' value of -516 mV, the sulphate-sulphite redox pair is too negative to be reduced by intracellular electron carriers present in sulphate reducers, such as ferredoxin or NADH, which have E' values of -398 mV and -314 mV, respectively. As a result, before reduction, sulphate must be activated by ATP sulphurylase, producing adenosine-phosphosulphate (APS) and pyrophosphate. Pyrophosphate is then hydrolyzed by pyrophosphatase to form two phosphate molecules.The redox couple APS-sulphite plus AMP has an E' value of -60 mV, enabling the reduction of APS using reduced ferredoxin or NADH. After APS is reduced, AMP is produced. ATP-dependent adenylate kinase then uses AMP to turn it into two molecules of ADP. Thus, for sulfate to activate, two ATP molecules are required. Sulphite is reduced to create sulfide and the redox pair sulfite-sulfide's E value is -116 mV.

SRB can use hydrogen and sulphate as their sole energy sources and sulphate reduction is linked to electron-transport

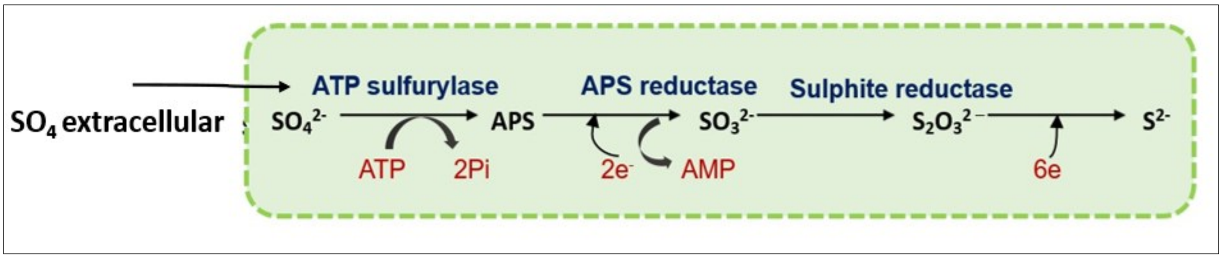

Fig. 8. Pathway of dissimilatory sulphate reduction.

phosphorylation. To compensate for the ATP consumed during sulphate activation, more than two ATP molecules must be produced through electron-transport phosphorylation (32). Fig. 8 illustrates the dissimilatory sulphate reduction pathway. In 1979 previous workers estimated a net gain of one ATP molecule per sulphate reduced, based on a comparison of the ATP yields from a *Desulfovibrio* strain grown with hydrogen and sulphate versus hydrogen and thiosulphate (44). Additionally, sulphate reducers can convert other sulphur compounds (such as thiosulphate, sulphite and sulphur) to sulphide and can also reduce nitrate and nitrite to ammonium (45). Numerous SRB utilize fumarate as an electron acceptor, while certain marine SRB employs dimethylsulphoxide for this purpose.

Factors influencing the sulphate reduction

pH

Sulphate (SO_4^{2-}) from the earth's crust is a major cause of pollution in domestic and industrial wastewaters. This lowers pH levels and speeds up the dissolving of metals, which causes acidic drainages to occur. The efficiency of biological sulphate reduction as a remediation technique can be impacted by several variables, including high sulfide concentrations, pH values and temperature. Studies have shown that sulphur-reduced species are more harmful to the microbial consortia at low pH (3.5) and low temperature (10 °C). On the other hand, mesophilic temperatures (25 °C) and almost neutral pH (6.2) are ideal for achieving upto 95 % reduction of SO_4^{2-} (46). Sulphate reduction reaches its highest rate at the pH range of 7-7.5 (47). When the pH is less than 5 or greater than 9, inhibition can be observed and when the pH is less than 2, there is no activity (48). The pH fluctuations have a significant impact on SRB activity, which in turn has a large impact on sulphate consumption as well as the synthesis of acetate and H_2S. Therefore, pH management has a significant impact on heavy metal removal and sulphate removal in the SRB process. In the treatment of acid mine wastewater using an anaerobic packed-bed reactor, the influent water is maintained at a pH of 7 by adding alkaline substances. This leads to metal precipitation before the enrichment of SRB due to the presence of these alkaline chemicals (49). Most of the metal precipitation occurs when the metals combine with sulfides produced by SRB, as metals like Fe, Cu, Ni and Zn cannot be effectively precipitated at pH 7 but can be completely and efficiently precipitated at pH levels above 9.5 (47). It can have a major impact on numerous microbial metabolic pathways. Among those (48) identified one such mechanism: electron donor dissociation and homeostasis. It has a significant impact on treatment progress and the energy performance of electron donors. As a result, good pH regulation is critical for maximizing electron donor use (48). Notably non-ionic substrates such as glycerol, hydrogen, sugars and alcohols are better suitable for fermentation at low pH values than at higher pH ranges (50).

Temperature

Temperature is a key factor influencing the effectiveness of biological sulphate reduction (51). The global-scale seasonal wastewater temperature can be determined by considering various factors. For instance, considering a sewer buried at a depth of 6.1 m (about 20 feet), with soil water moisture estimated as the average between field capacity and permanent wilting point, a wastewater flow rate of around 11 L per second (0.25 million gallons per day) with a density of 1000 kg/m^3 and an initial wastewater temperature of 17.8 °C, it is projected that roughly 75 % of global wastewater temperatures fall within the range of 6.9-34.4 °C over a year (52). Mesophilic sulphate-reducing bacteria (SRBs) are the most common strains, whereas thermophilic SRBs are also present. However, temperatures above 35 °C can decrease SRB function due to bacterial inactivation and protein denaturation (53). Moderately thermophilic SRB prefer a temperature of 50°C, whereas thermophilic SRB flourish in temperature between 65 and 70 °C (54). Temperature and the growth rate of SRB have been found to be strongly correlated, with a notable decrease in growth rate occurring outside of the ideal temperature range. Low temperatures reduced the effectiveness of passive biochemical reactors, leading to reduced SRB activity, alkalinity and removal efficiencies for sulphate and heavy metals. Furthermore, the removal of heavy metals and sulphate decreased from 70-90 % in summer (14-18 °C) to 0-39 % in winter (approximately 5 °C) (55). Temperature variations will alter the mean structural composition of membrane lipids (56).

Sulfide concentration

The presence of anaerobic SRB promotes sulphate reduction and the metabolism of sulfur-containing amino acids, resulting in the sulfide generation. These sulfides play several critical roles in the SRB process. The ability of SRB to create sulfides is an important factor in determining metal removal efficiency. Even after several dilutions, an SRB system capable of abundantly producing sulfides demonstrates long-term and very effective metal precipitation (57). Furthermore, sulfide has a reducing capacity due to the low valence state of sulfur, allowing it to decrease oxidized metals and lessen their toxicity (49). The composition of the salt affects SRB activity; sodium (Na^+) and potassium (K^+) have a greater impact than magnesium (Mg^{2+}) (58). The use of biochar can help to reduce the negative effects of high sulfide levels on SRB. It is critical to note that both too much and too little sulfide can be damaging to the heavy metal removal process. Sulfide comes in several forms, including H_2S, HS and S^2, each with varying amounts of toxicity to SRB. The most dangerous type is undissociated H_2S, which can significantly reduce SRB metabolic activities without completely stopping them (59). H_2S can impede cytochrome oxidase, which transports electron donors from respiratory substrata to molecular oxygen, hence preventing microbial metabolism (59).

COD/SO_4^{2-} ratio

Changes in the organic substrate (COD) to sulphate ratio (COD/SO_4^{2-}) have been found to have a considerable impact on the efficiency of pollutants removal by mixed SRB cultures. In theory, under ordinary conditions, reducing 1g of SO_4^{2-} requires 0. 67 g of COD. The COD/SO_4^{2-} ratio influences the competition between SRB and other microbes, which determines heavy metal and sulphate removal efficiency. This is because SRB and other bacteria use the same carbon sources for growth. When the COD/SO_4^{2-} ratio falls below the predicted value of 0.67, all electrons are driven to SO_4^{2-} (49). SRB has a competitive advantage over methane producing archaea (MPA) when the ratio is less than 1.7 due to the higher usage of electrons for sulphate reduction (60). Studies reveal that effluent with a COD/SO_4^{2-} ratio of 8 has lower acetate levels than other ratios, presumably due to the use of electrons from acetate for methane generation (60). Generally, the biodegradable fraction of COD in domestic wastewater ranges from 200 to 500 mg/L, necessitating a minimum sulphate concentration of 300-750 mg/L to ensure the dominance of SRB. As long as the COD/SO_4^{2-} ratio remains below 0.67, fluctuations in COD and sulphate levels in the influent do not impact the dominance of SRB. Even a significant increase in sulphate concentration from 500 to 2500 mg/L, resulting in a lower COD/SO_4^{2-} ratio has a minimal effect on SRB in wastewater treatment plants (6).

Oxygen

SRBs are stringent anaerobes, therefore even little levels of oxygen found in urban wastewater can be problematic for them. Numerous investigations have documented cases of SRB failures in low-oxygen conditions (6). SRB can remain active in the presence of oxygen by forming aggregates. The formation of granules allows SRB to thrive in anaerobic zones within these granules. Although SRB typically thrive in anaerobic environments, they possess molecular and physiological mechanisms to sustain activity in low oxygen levels often found in municipal wastewater (6).

Carbon sources

The variety of SRB in terms of their utilization of carbon sources and metabolic functions (61). The carbon and energy sources play a crucial role in providing the necessary energy for the growth and upkeep of SRB, as illustrated by the following reaction:

$$SO_2 + 4H_2O + 8e^- \rightarrow S^{2-} + 8OH^- \ (61)$$

To facilitate sulphate reduction, the necessary electrons are produced through the oxidation of carbon sources such as lactate, acetate, or propionate. Recent studies have been focusing on demonstrating the efficacy of lactate- sulphate combinations (62). Different types of organic substances like sewage sludge, leaf mulch, molasses, animal manure and low molecular weight organic compounds have been utilized as carbon sources (6). It is also proved that the prevalence of SRB in conditions specific to home wastewater, with both acetate and propionate. The typical VFA concentration of residential wastewater, acetate and propionate, is encouraging for establishing a dominant active SRB population in the tank (6).

ORP (Oxidation Reduction Potential)

Redox potential, which is essential to SRB activity, is used to show the general redox properties of compounds in an aqueous solution (6). The presence of redox substances like nitrate, nitrite and zero valent iron influences ORP (Fig. 9). Zero valent iron (ZVI) has been reported in various studies to enhance SRB activity by reducing ORP in the solution, thus creating a more conducive environment for SRB sulphate reduction (49). For biological sulphate reduction to occur, a minimum of at least -150 mV is required with -300 mV being a good indicator of a strong reducing environment (63). Micro-aeration can either stop sulphur from reducing into H_2S or oxidise H_2S to elementary sulphur. In the reference digester (without micro-oxygen infusion), the H_2S content was higher than 4000 ppm. However, there are risks to digester function and safety when adding oxygen to an anaerobic environment. Thus, Nghiem's research proposes the use of ORP to control the injection of oxygen and provide a micro-aeration state to control the generation of H_2S. Although research in the lab has demonstrated that micro-

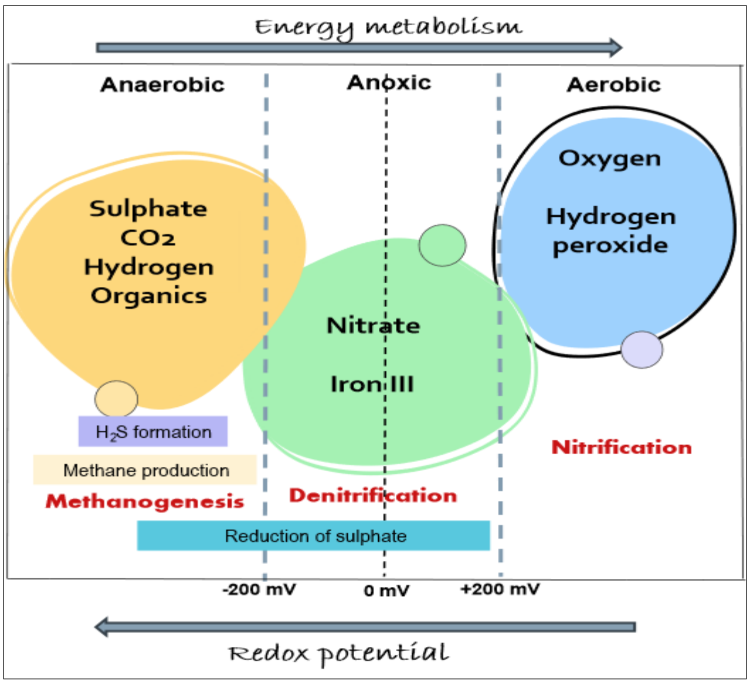

Fig. 9. Wastewater septicity & redox potential- how they relate.

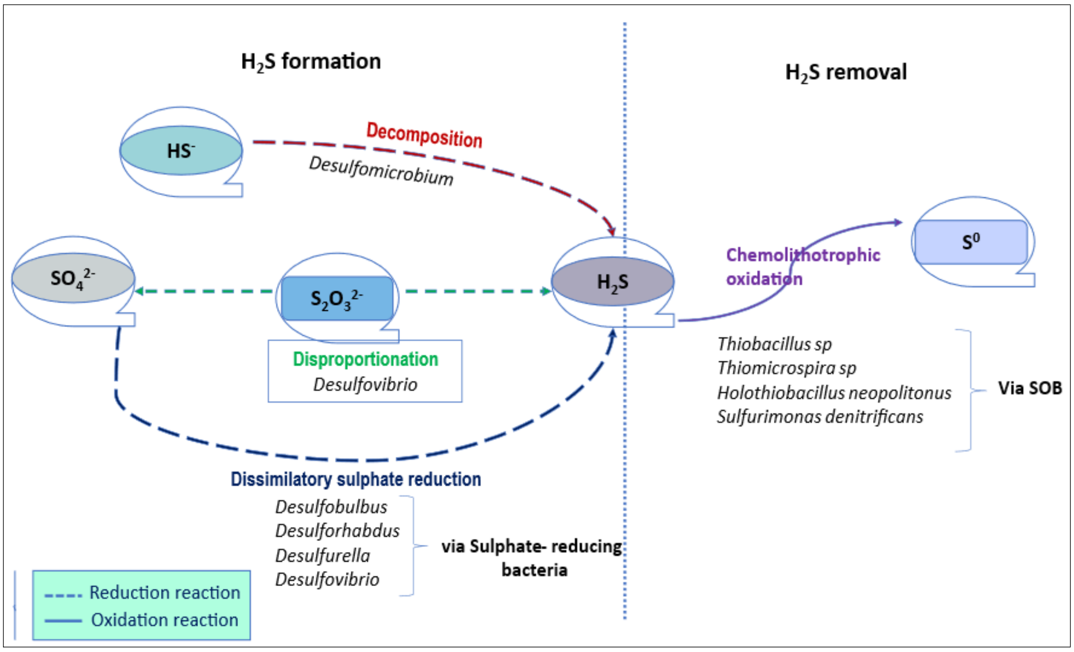

Fig. 10. Redox reaction takes place during anaerobic digestion.

oxygen infusion can effectively lower the H_2S concentration in biogas, no real-world pilot or full-scale demonstration of this technique has been made (64).

The presence of sulphur-oxidizing microorganisms such as *Thiobacillus* is crucial for the conversion of H_2S to elemental sulphur (Fig. 10). These microorganisms occur naturally in anaerobic digestion, so no artificial inoculation is necessary. Their autotrophic nature allows the use of biogas as a carbon source for carbon dioxide, potentially improving the quantity and quality of biogas produced by anaerobic digestion (65).

Applications of SRB

Pathogen removal

Wastewater treatment can potentially contribute to the transmission of infectious diseases caused by waterborne pathogenic microorganism and became an important human health concern. A wide variety of pathogens can be found in domestic wastewater, including helminths, viruses, enteric viruses, noroviruses, adenoviruses and protozoa, as well as bacteria, enterococci, Salmonella, coliforms and E. *coli* among others (66). The study emphasized the effectiveness of various onsite wastewater treatment systems in pathogen removal, noting that systems incorporating SRB showed promise due to their unique biological processes (66). A study demonstrated that SRB could proliferate in domestic wastewater treatment systems and reduce pathogen levels under pilot-scale conditions (67). Numerous studies have demonstrated that adding SRBs to wastewater treatment systems can significantly reduce the number of various pathogens, including coliforms and faecal coliforms. Most people agree that there is a positive effect on lowering pathogens in the treated effluent, even if the precise level of efficiency may differ based on the treatment settings and particular strains of SRBs used. SRBs can produce antimicrobial chemicals as byproducts of their metabolic activities and outcompete harmful bacteria for resources. Furthermore, the anaerobic environments produced by SRB activity prevent aerobic pathogens from surviving and proliferating (67).

Heavy metal removal

The anaerobic conversion of sulphate to sulfide by SRB is necessary for the removal of both sulphate and heavy metal from wastewater. There are two phases to SRB's heavy metal wastewater treatment method. First, SRB oxidizes simple organic compounds like lactate and acetate in anaerobic environments by using sulphate as an electron acceptor. This process produces hydrogen sulfide and bicarbonate ions. Second, insoluble metal sulfide precipitates are created when biologically produced hydrogen sulfide combines with dissolved heavy metal (29). Table 3 shows some recent research in SRB efficiency for heavy metal removal in the sulphate rich wastewater.

Nitrogen removal

Municipal wastewater has ammonium concentrations ranging from 27 to 100 mg/L (75). In contrast, levels of domestic wastewater typically range from 39 to 60 mgL^{-1} (76). Plants obtain nitrogen to support their growth and protein synthesis through the uptake of NH_4^+ and NO^{3-}. This essential nutrient can be obtained from two sources: natural bacterial fixation or synthetic fertilizers. Additionally, NH_4^+ undergoes a series of reactions involving oxidation and reduction of nitrogen atoms, leading to its biological conversion into atmospheric nitrogen (77). Sulphate -reducing bacteria, or SRBs, are key players in the denitrification processes that remove nitrogen from wastewater. By enhancing the conversion of nitrate to nitrogen gas and employing sulphate as an electron acceptor, SRB extract nitrogen from wastewater. Recent studies have shown that integrating SRB with anammox processes can further increase the efficiency of nitrogen removal while reducing operating costs, generating less sludge and improving treatment performance (67).

Decreased sludge production

In recent years, there has been a growing focus on reducing the amount of sludge disposal because of strict environmental regulations (6). The amount of sludge generated in a wastewater treatment plant (WWTP) is a mere 1 % (dewatered sludge is 0. 5 %) of the total volume of influent wastewater that needs to be treated. To ensure the smooth and efficient operation of WWTPs, it is imperative to remove waste sludge, which includes inert solids and excessive biomass, to prevent their build-up within the system. SS production has become an issue of intense

Table 3. Efficiency of SRB in heavy metal removal from sulphate-rich wastewater

Pollutants	Initial metal conc (ppm)	Removal efficiency	Treatment	References
Cu^{2+}	100	64 %	SRB–Cu/Fe system	(68)
Mn^{2+}	25	95 %	SRB-bioreactor	(69)
Cr (VI)	50	95 %	SRB-bioreactor	(57)
Ni^{+}	150	>98 %	Fe^{0} -SRB	(70)
Zn^{2+}	100	73 %	SRB–Cu/Fe system	(68)
Hg^{2+}	50	99.9 %	SRB	(71)
Sb(V)	20	80a.35 %	Iron-oxidizing bacteria and SRB	(72)
Cd^{2+}	< 600	77.6-96.4 %	SRB	(73)
As(V)	5	78 %	Fe (II) and SRB	(74)
Cu^{2+}	100	30-100 %	SRB	(57, 68)
Zn^{2+}	100	91-100 %	SRB	(57, 68)
Pb	200	96 %	SRB- bioreactor	(57)

debate in recent years due to the management and disposal challenges associated with the increasing amounts of sludge produced annually, especially in the most developed countries (78). One of the most environmentally and economically sustainable solutions is the agricultural reuse of biologically treated SS (79), such as after thermophilic anaerobic digestion of SS in centralized plants (80).

Chemical removal of sulphide

The main sources of hydrogen sulfide include chemical process byproducts, bacterial sulfate reduction (BSR) and microorganism breakdown of organic matter (81). According to OSHA (Occupational Safety and Health Administration), Fig. 11 shows the physiological effects on the human body of varying H_2S concentrations and exposure times.

Precipitation by metal salts is a key method in wastewater treatment, especially for removing heavy metals and phosphates. AMD, a problematic form of wastewater produced by mining activities, can contain high levels of metals, metalloids and sulphate. To address this issue, metal sulfide precipitation has been investigated as a method to recover or eliminate these harmful substances from AMD. One approach involves using H_2S in a sulfidogenic process to reduce the sulphate content in AMD through biogenic sulfide precipitation. By using biological competition exclusion approaches, it is possible to create an environment where bacteria that can outcompete SRB can survive and develop, which will inhibit the growth of SRB. By encouraging the growth of bacteria that compete with SRB for electron donors and bacteria that can directly remove H_2S, injecting nitrate or nitrite can improve SRB control (49). Rhizobiaceae and Xanthophytaceae proliferated upon the introduction of nitrate, whereas chlorate suppressed the microbial community and significantly decreased the number of sulphate-reducing species, showing toxicity. The development of Thiomonaceae and Thiobaceae, which oversee elemental sulfur reduction and sulfide oxidation, respectively, is aided by the introduction of perchlorate. Together with biological competition, this sulphur oxidation-reduction cycle aids in controlling the amount of sulfur in wastewater (82). According to recent research, nitrate has a more inhibitory impact than perchlorate since the study's enriched perchlorate-reducing bacteria are unable to use certain substrates like alkylbenzene, which nitrate-reducing bacteria can readily consume (83). The content of sulfide is significantly reduced in a chlorine dioxide system when nitrate-reducing bacteria are present. It is more economical to investigate the combined effects of low concentrations of chlorine dioxide and metabolic inhibitors, including nitrite (84). The utilization of metal salts and SRB

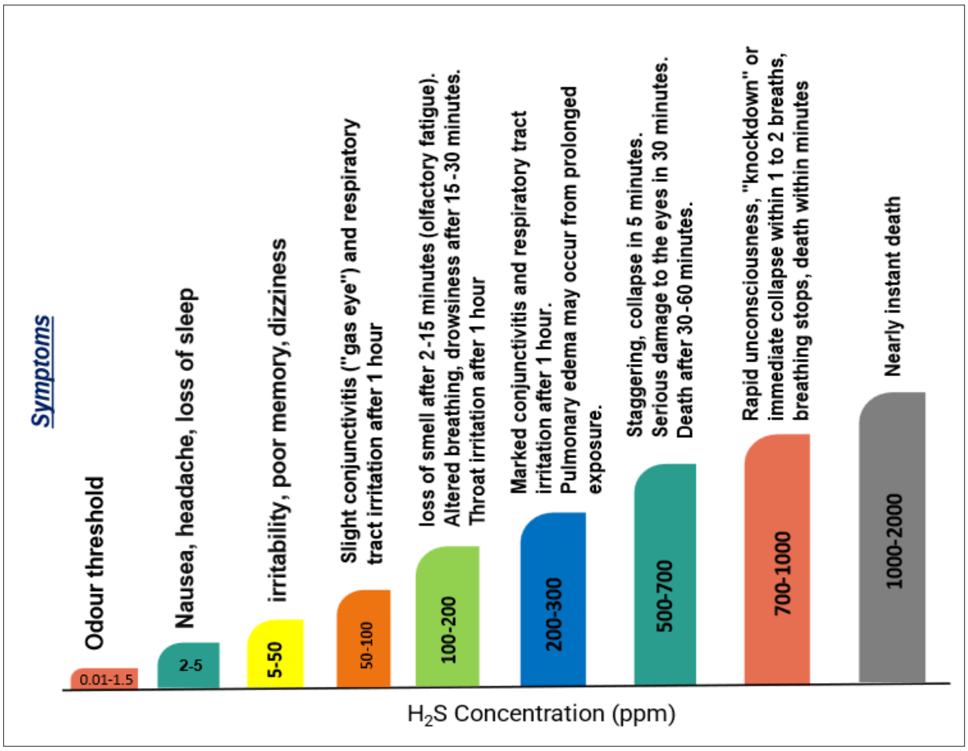

Fig. 11. Physiological influence on human H_2S.

activity is an effective method for treatment. SRB efficiently eliminates heavy metals and sulphate from wastewater by converting sulphate into sulfide. This sulfide then reacts with metal salts to produce insoluble metal sulfides. The biogenic sulfide precipitation technique is advantageous for industrial wastewater treatment due to its remarkable selectivity, cost-effectiveness and ability to operate within a wide pH range (49).

Various oxidizing agents, including chlorine, hydrogen peroxide and potassium permanganate, have been investigated in research to convert sulfide into less harmful substances such as sulphate. An example of this is a study that emphasized the utilization of a sulphate-reducing bioreactor combined with a sulfide-oxidizing fuel cell (SOFC), which successfully achieved both sulfide removal and electricity generation. This integrated system exhibited sulfide removal efficiencies of up to 93 %, demonstrating its promising prospects for practical implementations (85).

Efficiency of microbial consortia in sewage

Microbial consortia exhibit remarkable efficiency in the removal of diverse pollutants from sewage. These consortia, comprising different species of microorganisms working in synergy, offer enhanced stability and functionality in complex wastewater environments compared to treatments involving single species. Utilizing microbial consortia for the degradation of complex compounds proves more advantageous than employing isolated bacteria, as the former demonstrates greater adaptability and stability within the growth environment. Moreover, they create a suitable catalytic environment for each enzyme required in the biodegradation pathway (86). To maximize their pollutant removal capacity, pollutant-degrading microbial floras (PDBFs) have been developed and optimized. These consortia are cultivated and fine-tuned to effectively eliminate key pollutants such as ammonium nitrogen (NH_4+-N), total nitrogen (TN), total phosphorus (TP), nitrate nitrogen (NO_3+ -N) and chemical oxygen demand (COD) from simulated wastewater. Research suggests that by adjusting carbon and nitrogen sources, along with other culture conditions, the efficiency of these consortia can be significantly enhanced (85). A postgate medium is commonly used for SRB cultivation (87).

Fig. 12 shows that a diverse microbial community functions synergistically in a sulphate-rich environment or an anaerobic digester. In the process of cellulose hydrolysis, Clostridium species break down cellulose into cellobiose and glucose using their cell-bound cellulosomes (88). During fermentation, bacteroides species utilize the glucose and cellobiose to ferment them into organic acids like acetate, as well as gases such as hydrogen and carbon dioxide. Sulphate reduction is the process by which *Desulfovibrio* species convert sulphate into hydrogen sulfide (H_2S) by using the acetate and hydrogen created during fermentation (89). There may also be methanogenic archaea, which are active in methanogenesis. They generate methane (CH_3) by using H_2 and CO_2 or acetate, which further reduces the fermentation products and supports the SRB by lowering hydrogen pressure (90). As a result of this interaction, cellulose is effectively broken down and sulphate levels are reduced, with each group of microorganisms benefiting from the other's metabolic processes. Two essential substrates for the SRB, hydrogen and acetate, are produced by the fermentative bacteria. Fermentative bacteria benefit from low hydrogen partial pressure, which is maintained in part by the SRB. Consequently, an efficient and balanced ecosystem is created that can decompose cellulose in anaerobic environments with elevated sulphate levels.

Types of reactors used for sulphur recovery

Studies to convert sulphate or zero-valent sulfur (ZVS) into hydrogen sulfide have recently used laboratory-scale acidophilic bioreactors (91). There exists a range of bioreactor types, such as packed-bed reactors, gas-lift bioreactors (GLB), expanded granular sludge bed reactors (EGSB), fluidized bed reactors (FBR), submerged membrane bioreactors (MBR) and upflow anaerobic sludge blanket reactors (UASB) (92). For instance, the continuous stirred-tank reactor (CSTR) operates at a stable temperature of 35 °C and maintains a pH of 8. It uses acetate and peptone as energy sources and employs wastewater treatment

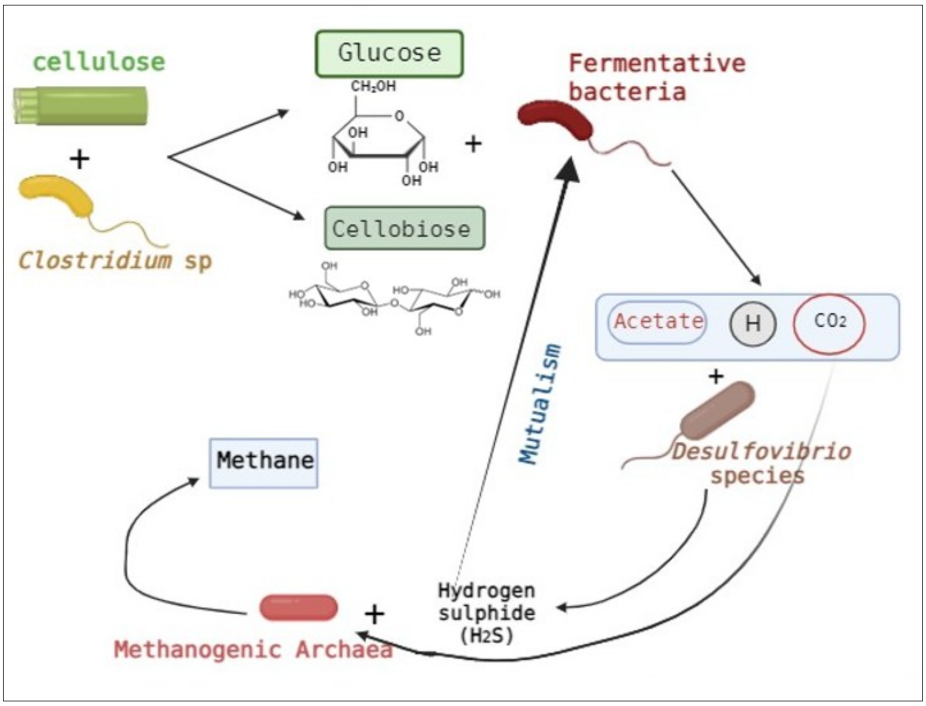

Fig. 12. Microbial consortium interactions.

plant sludge as the inoculum. The sulfate reduction rate ranges from 0.17 to 0.48 g/L/day, with a Hydraulic Retention Time (HRT) of 48-90 hr. Acidophilic bacterial consortia are typically utilized in effective low-pH sulfidogenic bioreactors, including MBRs, packed-bed reactors and UASB reactors (93). Moreover, PS (primary sludge) may be a possible source of sulfur. PS for biotransformation requires careful consideration of various bioreactor types and their unique characteristics, such as HRT (94). The decrease of SO_4^{2-} from PS in a continuous fluidized-bed reactor. As the organic electron donor, they employed a 90 % lactate and 90 % glycerol mixture. They evaluated two different HRTs, namely 9 and 15 hrs. Between the two HRT circumstances, the study observed no statistically significant difference in SO_4^{2-} reduction (73 % for 9 hrs and 75 % for 15 hrs) (95).

Conclusion

The integration of SRB into wastewater treatment offers a viable strategy for the simultaneous removal of organic matter and heavy metals. SRB's ability to reduce sulphate to hydrogen sulfide promotes metal precipitation and organic degradation, which results in reduced sludge production and the potential for metal recovery. Nonetheless, actual applications face challenges such as maintaining anaerobic conditions, managing hydrogen sulfide emissions and ensuring process stability across various types of wastewater. Future research should focus on optimizing bioreactor design, improving microbial interaction and refining operational control to support the practical deployment of SRB in actual sewage treatment systems.

Future prospects

Future research on SRB should prioritize investigating the SRB enzymes for development of novel biocatalyst.

Investigation of SRB role in carbon capture and storage for sustainable carbon management.

It is recommended Implement efficient reactor designs and sustainable electron donors with high applicability and minimal environmental impact to enhance SRB processes in industrial wastewater treatment.

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Authors contributions

Literature collection, conceptualization and writing original draft was done by MV. Supervision, visualization and critical revision was performed by MM. Supervision and reviewing was done by BA, KS. RD performed the supervision and visualization. Editing and critical reviewing was done by KG.

Compliance with ethical standards

Declaration of competing interest : The authors declare that they have no known competing interests exist.

Ethical issues : None

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