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Desalination and Water Treatment

A brief review on possible approaches towards controlling sulfate-reducing bacteria (SRB) in wastewater treatment systems

Ze-hua Liu\textsuperscript{ab}, Abdul Majid Maszenan\textsuperscript{b}, Yu Liu\textsuperscript{bc} & Wun Jern Ng\textsuperscript{bc}

\textsuperscript{a} College of Environment and Energy, South China University of Technology, Guangzhou 510006, China, Tel. +86 20 39380507

\textsuperscript{b} Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, CleanTech One, Singapore 637141, Singapore, Tel. +65 6513 7363 (A.M. Maszenan), +65 6790 4104 (Y. Liu), +65 6790 6813 (W.J. Ng)

\textsuperscript{c} School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

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A brief review on possible approaches towards controlling sulfate-reducing bacteria (SRB) in wastewater treatment systems

Ze-hua Liu\(^{a,b,*}\), Abdul Majid Maszenan\(^b\), Yu Liu\(^{b,c}\), Wun Jern Ng\(^{b,c}\)

\(^a\)College of Environment and Energy, South China University of Technology, Guangzhou 510006, China, Tel. +86 20 39380507; email: zehualiu@scut.edu.cn (Z.-H. Liu)

\(^b\)Advanced Environmental Biotechnology Centre, Nanyang Environment and Water Research Institute, Nanyang Technological University, CleanTech One, Singapore 637141, Singapore, Tel. +65 6513 7363; email: CMaszenan@ntu.edu.sg (A.M. Maszenan), Tel. +65 6790 4104; email: CYLiu@ntu.edu.sg (Y. Liu), Tel. +65 6790 6813; email: WJNg@ntu.edu.sg (W.J. Ng)

\(^c\)School of Civil and Environmental Engineering, Nanyang Technological University, 50 Nanyang Avenue, Singapore 639798, Singapore

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**ABSTRACT**

Anaerobic processes in wastewater treatment and excess sludge digestion are desirable as these offer the prospect of energy recovery via the methane gas generated. However, hydrogen sulfide (H\(_2\)S) generated from reduction of sulfate by sulfate reducing bacteria (SRB) during the process, is inhibitory to the methane producing bacteria (MPB). The SRBs and MPBs also compete for utilization of a key substrate in methanogenesis, volatile fatty acids. For development of effective methods to mitigate the adverse impact of SRBs on methanogenesis, it is important there is better understanding of the SRBs and this can begin with knowing which species are likely to be present in wastewater treatment. With this objective in mind, species of SRBs isolated from wastewater treatment systems reported in the literature have been summarized in this paper and discussed.

**Keywords:** Anaerobic digestion; SRB species; Hydrogen sulfide; Wastewater treatment systems

1. Introduction

Anaerobic digestion (AD) is a method engineered to decompose organic matter by a variety of anaerobic micro-organisms under oxygen-free conditions. The process has a number of advantages related to cost of operation which is important to the industry. These advantages include much lower production of excess sludge, and energy recovery via the biogas generated [1–3]. Notwithstanding these advantages there is a perception that AD processes are not easy to operate stably and this may be due to “unexpected” issues such as competition and inhibition [1,4]. An example is the inhibition caused by H\(_2\)S which may be produced by a group of micro-organism commonly referred to as the sulfate reducing bacteria (SRBs). Inhibition by H\(_2\)S is related to two aspects. Firstly

*Corresponding author.


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there can be competition for utilization of substrates, the volatile fatty acids (VFAs), between the SRBs and methane producing bacteria (MPBs), in which the SRBs are better able to compete [5]. With successful competition for the substrates, the SRBs generate \( \text{H}_2\text{S} \) and the undissociated form is more inhibitory to MPBs than SRBs [1]. Apart from inhibiting the MPBs, the gaseous \( \text{H}_2\text{S} \) in the biogas generated is strongly corrosive to the gas handling equipment which follows [6]. To reduce incidence of \( \text{H}_2\text{S} \) inhibition, chemical, and biological technologies are available. The chemical technologies include precipitation by addition of metal salts and addition of oxidizing reagents such as chlorine, hydrogen peroxide as well as potassium permanganate, to oxidize the sulfide. The biological methods include biological oxidation of \( \text{H}_2\text{S} \) by sulfide-oxidizing bacteria and activity inhibition of the SRBs [6]. Among these efforts, inhibition of SRB activity is theoretically most desirable as it would prevent formation of the sulfide ion in the first instance. To achieve this, better understanding of the SRBs such as its growth properties is necessary.

2. SRBs in wastewater treatment systems

SRBs comprise a metabolically versatile group of micro-organisms of many different families and genera. SRBs were formerly considered obligate anaerobic micro-organisms which use sulfate or other oxidized sulfur compounds as the terminal electron acceptor. However, this understanding is now considered not completely correct [7]. Since the 1990s, examples have been found of SRBs which could reduce oxygen and nitrate for energy in oxic conditions wherein their respiration only occurred in the microaerophilic environment and this would decline or halt at increased concentrations of oxygen [8,9]. Later it was proven that some SRBs favored use of oxygen as the electron acceptor, with nitrate/nitrite next, and sulfur compounds as the least favored [10].

SRBs include Bacteria and Archaea, with complex physiology, and various properties have been used in their classification [11]. Most of the SRBs described to date belong to one of five phylogenetic lineages: (a) the mesophilic \( \delta \)-Proteobacteria with genera Desulfovibrio, Desulfbacterium, Desulfohalobacter, Desulfobulbus, Desulfomonos, Desulfofoccus, Desulfomonile, Desulfonema, and Desulfosarcina; (b) the thermophilic gram-negative bacteria with the genus Thermodesulfovibrio, Thermodesulfobacterium, and Thermodesulfobium; (c) the gram-positive bacteria with the genus Desulfothiobacillus, Desulfosporosinus, and Desulfosporomusa; (d) the Euryarchaeota with the genus Archaeoglobus; and (e) the Crenarchaeota with the genus Thermocladium and Caldilinea [12–15]. Important parameters such as morphology, mobility, guanidine and cytosine (G+C) content of DNA, and growth properties are included in Table 1. Only species isolated from wastewater related sources have been included. SRBs belonging to the Euryarchaeota and Crenarchaeota have not been reported found in wastewater related sources.

3. Possible approaches towards controlling SRBs

Given the competition SRBs may pose to MPBs and that the \( \text{H}_2\text{S} \) generated by the SRBs has inhibitory, corrosive, and odorous properties, there is interest in methods which can be applied to control numbers of SRBs in a population or to control their activities. The following discussion is on three possible approaches and possibility of success:

(1) The temperature and pH range suitable for SRB growth have been reported to be from 3 to 70°C, and 4.5 to 9.2, respectively. This would suggest that neither temperature nor pH can be effectively used to eliminate SRBs. It has been reported in the literature, to reduce competitive utilization of VFAs between SRBs and MPBs, a short-term low temperature shock at 12–15°C over 3 d was performed on an upflow anaerobic sludge bed reactor. No significant change in utilization of COD by the SRBs was noted. Changing the pH in the same reactor also had not helped [16].

(2) The literature review (Table 1) has indicated all species reported other than Desulfovibrio aerotolerans, are obligate anaerobes. They had shown no growth but could tolerate an aerobic environment for at least 13 h. Their ability of reducing sulfate could be immediately recovered once the anoxic environment was re-established. The requirement for a strictly anaerobic growth condition offers possibility of an approach for inhibiting SRB growth and activity. In Tang et al. [17] study, inhibition of SRBs was successfully obtained through micro-aeration at a municipal solid-waste digester. \( \text{H}_2\text{S} \) in the biogas had then decreased from 680 to below 5 mg/L, while production of methane gas was little affected. Dissimilatory sulfite reductase genes associated with SRBs were, nevertheless, detected in the presence of the micro-aeration condition. This would suggest that \( \text{H}_2\text{S} \) was
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<tr>
<th>Category</th>
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<tr>
<td>Mesophilic δ-</td>
<td>Desulfovirio</td>
<td>Legallis</td>
<td>Wastewater digester</td>
<td>Rod</td>
<td>55</td>
<td>22–43 (35)</td>
<td>5–9.2 (7.3–7.5)</td>
<td>(1) Sulfate, sulfite, thiosulfate, elemental sulfur, and fumarate serve as electron acceptor instead of nitrate and nitrite; (2) Lactate, pyruvate, fumarate, ethanol, succinate, and hydrogen serve as electron donors in the presence of sulfate as terminal electron acceptor. Lactate is incompletely oxidized to acetate; (3) Substrates that cannot be utilized include acetate, propionate, malate, valerate, formate, methanol, glycerol, mannitol, mannose, xylose, casamino acids, fructose, glucose, and ribose.</td>
<td>[19]</td>
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<tr>
<td>Marrakechensis</td>
<td></td>
<td></td>
<td>Olive wastewater</td>
<td>Rod</td>
<td>65.1</td>
<td>20–50 (37)</td>
<td>6.5–8.5 (7)</td>
<td>(1) Sulfate, sulfite, thiosulfate, elemental sulfur, and fumarate serve as electron acceptor instead of nitrate and nitrite; (2) Strictly anaerobic, but with limited growth in the absence of sulfate under air in basal medium containing lactate and yeast extract; (3) Hydrogen and formate can only be utilized in the presence of acetate.</td>
<td>[20]</td>
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<tr>
<td>Aminophilus</td>
<td></td>
<td></td>
<td>Dairy wastewater</td>
<td>Vibrios</td>
<td>66</td>
<td>25–40 (35)</td>
<td>6.7–8 (7.5)</td>
<td>(1) In the presence of acetate as a carbon source, it is possible to grow on ethanol or H₂ plus CO₂ in the presence of sulfate; (2) Sulfate, sulfite, and thiosulfate serve as electron acceptor instead of elemental sulfur, fumarate, and nitrate.</td>
<td>[21]</td>
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<th>Substrate and electron transfer and other main conclusions</th>
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<td></td>
<td><em>Carbinolicus</em></td>
<td>Food wastewater</td>
<td>Rod</td>
<td>65</td>
<td>5–44 (37–38)</td>
<td>5.3–8.7 (7–7.3)</td>
<td></td>
<td>(1) Instead of nitrate, nitrite, and fumarate, sulfate, sulfite, thiosulfate, and elemental sulfur can serve as electron acceptor, and finally reduced to H$_2$S; (2) It can grow on hydrogen, formate, and ethanol with acetate as carbon source in the presence of CO$_2$, while growth on methanol requires the presence of yeast extract</td>
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<td></td>
<td><em>Mexicanus</em></td>
<td>Cheese wastewater</td>
<td>Vibrio</td>
<td>66</td>
<td>20–40 (37)</td>
<td>6.3–8.2 (7.2)</td>
<td></td>
<td>(1) Sulfate, sulfite, thiosulfate, and elemental sulfur serve as electron acceptor instead of nitrate, nitrite, and fumarate; (2) Formate and H$_2$ (with acetate), pyruvate, casamino acids, and ethanol can serve as electron donors in the presence of thiosulfate</td>
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<tr>
<td>Mesophilic δ-</td>
<td>Desulfocibrio</td>
<td><em>Paquesii</em></td>
<td>Wastewater</td>
<td>Vibrio to spiral</td>
<td>62.2</td>
<td>10–45</td>
<td>6.5–8.5</td>
<td>(1) Sulfate, sulfite, and thiosulfate reduced to H$_2$S under strict anaerobic condition, with incomplete oxidation of organics to acetate; (2) Hydrogen, formate, pyruvate, fumarate, lactate, succinate, malate, ethanol, and glycerol serve as electron donors in the presence of sulfate</td>
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<td>Proteobacteria</td>
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<td>(1) Sulfate, sulfite, thiosulfate, and elemental sulfur reduced to H$_2$S; (2) Nitrate, nitrite, and ferric ion are not utilized as electron acceptors; (3) Lactate, H$_2$, pyruvate, methanol, ethanol, glycerol can</td>
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<td></td>
<td><em>Aerotolens</em></td>
<td>Activate sludge</td>
<td>Vibrio</td>
<td>57.2</td>
<td>3–37 (29)</td>
<td>6.4–7.8 (6.9)</td>
<td></td>
<td>(1) Sulfate, sulfite, thiosulfate, and elemental sulfur reduced to H$_2$S; (2) Nitrate, nitrite, and ferric ion are not utilized as electron acceptors; (3) Lactate, H$_2$, pyruvate, methanol, ethanol, glycerol can</td>
</tr>
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</table>
Desulfobulbus *Elongatus*  
Mesophilic Industrial digester  
Rod 59  20–40 (35)  6–7.8 (7)  
(1) Instead of nitrate, sulfate, sulfite, and thiosulfate serve as electron acceptor and are reduced to H$_2$S;  
(2) Propionate, lactate, pyruvate, ethanol, propanol are incompletely oxidized to acetate in the presence of sulfate;  
(3) Hydrogen is used as electron donor in the presence of acetate and CO$_2$ as carbon sources  

Desulfovirga *Adipica*  
Anaerobic digester  
Rod 60  20–36 (35)  6.6–7.4 (7)  
(1) Under strict anaerobic condition, sulfate, sulfite, thiosulfate, and elemental sulfur serves as electron acceptor instead of nitrate;  
(2) Pyruvate, lactate, C$_1$-C$_9$ straight chain fatty acids, C$_4$-C$_6$ iso-fatty acids, and C$_2$-C$_9$ straight chain primary alcohols can be completely oxidized in the presence of excess sulfate  

Desulfatirhabdium *Butyrativor*  
Anaerobic reactor  
Oval to rod 55.1  15–37 (28–30)  6.5–8.0 (7.0)  
(1) Growth on H$_2$/acetate, formate, ethanol, pyruvate, propionate, propanol, 1-butanol, 2,3-butandiol, fumarate, and succinate in the presence of electron acceptor;  
(2) Sulfate and thiosulfate serve as electron acceptors instead of sulfite and nitrate;  
(3) No growth on H$_2$/CO$_2$, methanol, acetone, lactate, malate, glucose, or fructose  

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<tr>
<td>Mesophilic δ-proteobacteria</td>
<td><em>Desulfoglaeba</em></td>
<td><em>Alkanexedens</em></td>
<td>Oily wastewater storage</td>
<td>Rod to oval end</td>
<td>53.6</td>
<td>17-50 (31-37)</td>
<td>4.5-8.2</td>
<td>(1) Tolerance to NaCl up to 55 g/L; (2) Sulfate and thiosulfate serve as electron acceptors, while alkanes (C₆-C₁₂), pyruvate, butyrate, and hexanoic acid are electron donors</td>
<td>[29]</td>
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<td></td>
<td><em>Desulfobacca</em></td>
<td><em>Acetoxidans</em></td>
<td>Anaerobic granular sludge</td>
<td>Oval to rod</td>
<td>51.1</td>
<td>27-47 (36-40)</td>
<td>6.5-8.3</td>
<td>(1) Acetate serves as the only electron donor and it is completely oxidized to CO₂; (2) Sulfate, sulfite, and thiosulfate serve as electron acceptors, and reduced to H₂S</td>
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<td>Thermophilic gram-negative bacteria</td>
<td><em>Thermodesulfovibrio</em></td>
<td><em>Aggregans</em></td>
<td>Sludge granules</td>
<td>Vibrios</td>
<td>35.2</td>
<td>45-70 (60)</td>
<td>6-8.5</td>
<td>(1) Lactate, hydrogen, formate and pyruvate serve as electron donors in the presence of sulfate; (2) Organics are incompletely oxidized to acetate; (3) Sulfate and thiosulfate serve as electron acceptor in the presence of lactate, instead of sulfite, elemental sulphur, nitrate, and fumarate</td>
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<td></td>
<td><em>Thiophilus</em></td>
<td></td>
<td>Sewage sludge</td>
<td>Vibrios</td>
<td>34</td>
<td>45-60 (55)</td>
<td>6-8.5</td>
<td>(1) Lactate, hydrogen, formate and pyruvate serve as electron donors in the presence of sulfate; (2) Organics are incompletely oxidized to acetate; (3) Sulfate, sulfite, and thiosulfate serve as electron acceptor in the presence of lactate, instead of elemental sulfur, nitrate, and fumarate</td>
<td>[31]</td>
</tr>
<tr>
<td>Gram-positive bacteria</td>
<td><em>Desulfotomaculum</em></td>
<td><em>Alcolithoxan</em></td>
<td>Wastewater</td>
<td>Rod</td>
<td>48</td>
<td>33-53 (44-46)</td>
<td>6-7.5</td>
<td>(1) Sulfate, sulfite, thiosulfate, and elemental sulfur serve as electron acceptors;</td>
<td>[32]</td>
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</table>
produced but there was possibly sufficient O2 caused by the micro-aeration to oxidize the sulfide. Micro-aeration would therefore be a possible approach for controlling SRB activity and mitigating their activity.

(3) Fourteen out of the sixteen SRB species shown in Table 1 did not have nitrate/nitrite utilization capability. To prevent H2S production, addition of nitrate to the wastewater treatment systems have been reported [6]. Heukelekian attributed the method's success to the preferred reduction of nitrate over sulfate under oxygen deficient conditions [18]. However, since the bulk of the species identified did not have nitrate utilization, it is possible nitrate is inhibitory to SRBs but this would need to be confirmed.

Of the 3 approaches identified, manipulating temperature and pH had not been successful. This would then suggest that both mesophilic and thermophilic anaerobic systems can be affected by SRBs. Similarly 2-phase anaerobic systems with an acidogenic reactor preceding the methanogenic reactor can also be adversely impacted by SRBs. The use of nitrates to control SRB activity has been more successful and this can be applied to situations where septic conditions can develop—as in sewer lines on an "as needed" situation. This, however, unlikely can be a suitable method for application on an anaerobic reactor treating wastewater or a digester treating sludges. Nitrates applied continuously can adversely impact methanogenesis and this can develop as in sewer lines on an "as needed" situation. Of the 3 approaches identified, it would therefore seem micro-aeration to be the most viable for control of SRBs in wastewater treatment systems.

4. Summary

Various species of SRBs isolated from sources associated with wastewater/wastewater treatment and reported in the literature have been identified. These belong to both mesophilic δ-Proteobacteria, and thermophilic gram-negative bacteria or gram-positive bacteria. The temperature range over which SRBs can be found would suggest both mesophilic and thermophilic systems can be affected. Their occurrence over a broad band of pH values would suggest manipulating pH is unlikely to be a successful control method. However, all 16 listed SRB species cannot utilize nitrate/nitrite as electron acceptors. On the basis of

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Carboxydovorans Sludge in anaerobic reactor Rod 45.6 30–68 (55) 6–8 (6.8–7)

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(2) Alcohols and carboxylic acid are electron donors in the presence of sulfate;
(3) Fermentative growth on pyruvate
(1) CO (100% of the gas) can serve as the sole electron donor in the presence or absence of sulfate;
(2) H2/CO2, pyruvate, lactate, glucose, fructose, maltose, ethanol, glycerol, alanine, and serine can be utilized in the presence of sulfate;
(3) Sulfate, sulfite and thiosulfate are electron acceptors, instead of elemental sulfur

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application costs (nitrates are likely more expensive than air) and lower risk of producing metabolites which may result in environmental issues (e.g. greenhouse gas), the obligate anaerobe characteristic may be better exploited. This may be done so via development of micro-aeration techniques for application on anaerobic systems for wastewater and wastes treatment.

References


