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

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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_2S) 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_2S which may be produced by a group of micro-organism commonly referred to as the sulfate reducing bacteria (SRBs). Inhibition by H_2S is related to two aspects. Firstly

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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 H_2S and the undissociated form is more inhibitory to MPBs than SRBs [1]. Apart from inhibiting the MPBs, the gaseous H_2S in the biogas generated is strongly corrosive to the gas handling equipment which follows [6]. To reduce incidence of H_2S 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 H_2S 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 respirations 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 δ-*Proteobacteria* with genera *Desulfovibrio*, *Desulfobacterium*, *Desulfobacter*, *Desulfobulbus*, *Desulfomicrobium*, *Desulfomonas*, *Desulfococcus*, *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 *Desulfotomaculum*, *Desulfosporosinus*, and *Desulfosporomusa*; (d) the *Euryarchaeota* with the genus *Archaeoglobus*; and (e) the C*renarchaeota* with the genus *Thermocladium* and *Caldirvirga* [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 H_2S 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℃, 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℃ 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. H_2S 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 H_2S was

Table 1
SRB isolated from wastewater

Category	Genus	Specie	Source	Shape	G+C (%)	Growth conditions: Temperature[a]	pH	Substrate and electron transfer and other main conclusions	Ref.
Mesophilic *δ-Proteobacteria*	*Desulfovibrio*	*Legallis*	Wastewater digester	Rod	55	22–43 (35)[b]	5–9.2 (7.3–7.5)	(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	[19]
		Marrakechensis	Olive wastewater	Rod	65.1	20–50 (37)	6.5–8.5 (7)	(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	[20]
		Aminophilus	Dairy wastewater	Vibrios	66	25–40 (35)	6.7–8 (7.5)	(1) In the presence of acetate as a carbon source, it is possible to grow on ethanol or H_2 plus CO_2 in the presence of sulfate; (2) Sulfate, sulfite, and thiosulfate serve as electron acceptor instead of elemental sulfur, fumarate, and nitrate	[21]

(*Continued*)

Table 1
(*Continued*)

Category	Genus	Specie	Source	Shape	G+C (%)	Growth conditions			Ref.
						Temperature[a]	pH	Substrate and electron transfer and other main conclusions	
		Carbinolicus	Food wastewater	Rod	65	5–44 (37–38)	5.3–8.7 (7–7.3)	(1) Instead of nitrate, nitrite, and fumarate, sulfate, sulfite, thiosulfate, and elemental sulfur can serve as electron acceptor, and finally reduced to H_2S (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	[22]
		Mexicanus	Cheese wastewater	Vibrio	66	20–40 (37)	6.3–8.2 (7.2)	(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	[23]
Mesophilic δ-*Proteobacteria*	*Desulfovibrio*	*Paquesii*	Wastewater	Vibrio to spiral	62.2	10–45	6.5–8.5	(1) Sulfate, sulfite, and thiosulfate reduced to H_2S 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	[24]
		Aerotolerans	Activate sludge	Vibrio	57.2	3–37 (29)	6.4–7.8 (6.9)	(1) Sulfate, sulfite, thiosulfate, and elemental sulfur reduced to H_2S; (2) Nitrate, nitrite, and ferric ion are not utilized as electron acceptors; (3) Lactate, H_2, pyruvate, methanol, ethanol, glycerol can	[25]

							be utilized as electron donors in the presence of sulfate; (4) Strict anaerobic growth, while its non-growth culture can tolerate oxygen for a short period of exposure	
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_2S; (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	[26]
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 C2-C9 straight chain primary alcohols can be completely oxidized in the presence of excess sulfate	[27]
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	[28]

(*Continued*)

Table 1
(*Continued*)

Category	Genus	Specie	Source	Shape	G+C (%)	Growth conditions: Temperature[a]	pH	Substrate and electron transfer and other main conclusions	Ref.
Mesophilic δ-proteobacteria	*Desulfoglaeba*	*Alkanexedens*	Oily wastewater storage	Rod to oval end	53.6	17–50 (31–37)	4.5–8.2 (6.5–7.2)	(1) Tolerance to NaCl up to 55 g/L; (2) Sulfate and thiosulfate serve as electron acceptors, while alkanes (C_6-C_{12}), pyruvate, butyrate, and hexanoic acid are electron donors	[29]
	Desulfobacca	*Acetoxidans*	Anaerobic granular sludge	Oval to rod	51.1	27–47 (36–40)	6.5–8.3 (7.1–7.5)	(1) Acetate serves as the only electron donor and it is completely oxidized to CO_2; (2) Sulfate, sulfite, and thiosulfate serve as electron acceptors, and reduced to H_2S	[30]
Thermophilic gram-negative bacteria	*Thermodesulfovibrio*	*Aggregans*	Sludge granules	Vibrios	35.2	45–70 (60)	6–8.5 (6.5–7)	(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	[31]
		Thiophilus	Sewage sludge	Vibrios	34	45–60 (55)	6–8.5 (7–7.5)	(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	[31]
Gram-positive bacteria	*Desulfotomaculum*	*Alcoholivorax*	Wastewater	Rod	48	33–53 (44–46)	6–7.5 (6.4–7.3)	(1) Sulfate, sulfite, thiosulfate, and elemental sulfur serve as electron acceptors;	[32]

						(2) Alcohols and carboxylic acid are electron donors in the presence of sulfate; (3) Fermentative growth on pyruvate	
Carboxydivorans	Sludge in anaerobic reactor	Rod	45.6	30–68 (55)	6–8 (6.8–7)	(1) CO (100% of the gas) can serve as the sole electron donor in the presence or absence of sulfate; (2) H_2/CO_2, 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	[33]

[a] °C.

[b] The values in the parenthesis in columns of temperature and pH mean optimal points.

produced but there was possibly sufficient O_2 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 H_2S production, addition of nitrate to the wastewater treatment systems have been reported [6]. Heukelelekian 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 there is also the growing concern associated with incomplete nitrate reduction and consequent production of nitrogen oxides. Of the 3 approaches identifies, 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/waste 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 SRBs identified in this paper are obligate anaerobes, and 14 of the 16 SRB species cannot utilize nitrate/nitrite as electron acceptors. On the basis of 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.

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