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Revisiting simultaneous sulfate reduction and ammonium oxidation in wastewater treatment – from inexplicable experimental observations to extended mechanistic hypotheses†

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Over the last two decades, reference has been made to the 'sulfammox' conversion, comprising the anaerobic oxidation of ammonium with sulfate, with nitrogen gas (N_2) and elemental sulfur (S^0) as the main end products. However, this phenomenon has been associated with inexplicable experiment results in terms of variable end products and unclear reaction stoichiometry, besides the fact that it has been reported to occur under both heterotrophic and autotrophic conditions. This contribution sheds light on the 'sulfammox' phenomenon through a comprehensive revisit of experimental observations. The hypothesis for sulfammox-related reaction mechanisms was systematically extended, considering other end products than N_2 and S^0, and as well as potential syntrophic bioprocesses. This resulted in additional reactions which were more general than the specific sulfammox one and which were denoted by the term – simultaneous sulfate reduction and ammonium oxidation (SRAO). Multiple thermodynamically feasible reaction pathways of SRAO under heterotrophic and autotrophic conditions were identified in a systematic and intelligible way, and compared against previously reported experimental results regarding reactor performance and microbial community analysis.

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Water impact

This work involves an emerging microbial process, namely simultaneous sulfate reduction ammonium oxidation, which has brought challenge to the current knowledge on the natural nitrogen and sulfur cycles. Unravelling this new phenomenon can help us better understand nitrogen and sulfur evolution in natural environment, and enable practical application in biological wastewater treatment as the real-world impacts.

1. Introduction

Anaerobic ammonium oxidation (anammox), which utilises ammonium as electron donor and nitrite as electron acceptor, has been well investigated and become an established autotrophic nitrogen removal process in wastewater treatment.[1] Over the last two decades, it has become clear that nitrite was not the only possible electron acceptor for microorganisms to oxidise ammonium under anaerobic condition.[2] Anaerobic simultaneous sulfate reduction and ammonium oxidation (SRAO) was for the first time observed by Fdz-Polanco[3] when running an anaerobic digestion reactor with high sulfate and ammonium concentration, where nearly 50% of ammonium "disappeared" in the experiment, leading to the postulation of a new anaerobic ammonium oxidation phenomenon (eqn (1)). The Gibbs free energy for this reaction was calculated as ΔG = −47.8 kJ mol^{-1},[4] indicating its thermodynamic feasibility.

$$SO_4^{2-} + 2NH_4^+ \rightarrow S^0 + N_2 + 4H_2O \quad (1)$$

This specific reaction (eqn (1)) was later termed sulfammox[5] – in contrast to SRAO, which is a more general term. The SRAO phenomenon was recently examined by ^{15}N-label isotope analysis.[6,7] In their studies, the experiment conditions were indicated to be strictly anaerobic, and the initial substrate was

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only ^{15}N-labeled ammonium with sulfate. After the incubation of biomass, which was acclimated in the continuous-flow reactors, $^{15}N_2$ was detected to be formed, indicating that $^{15}NH_4^+$ was oxidised without oxygen, nor with other known electron acceptors, such as nitrite. The discovery of the SRAO phenomenon has brought a new challenge to currently known biological N and S cycles, and offers new options for the design of wastewater treatment processes.

At present, the mechanism of SRAO conversion in wastewater treatment remains uncertain. Fdz-Polanco described the overall reaction (eqn (1)) as a combination of sulfate reduction to sulfide and ammonium oxidation to nitrite (eqn (2)), sulfur-based denitrification (eqn (3)), and anammox (eqn (4)).

$$3SO_4^{2-} + 4NH_4^+ \rightarrow 3S^{2-} + 4NO_2^- + 4H_2O + 8H^+ \quad (2)$$

$$3S^{2-} + 2NO_2^- + 8H^+ \rightarrow 3S^0 + N_2 + 4H_2O \quad (3)$$

$$2NO_2^- + 2NH_4^+ \rightarrow 2N_2 + 4H_2O \quad (4)$$

The postulated reaction mechanism described above seems to ignore that both the anammox (eqn (4)) and sulfur-based denitrification (eqn (3)) reaction are typically inhibited in the presence of organic carbon, as prevailing in the anaerobic digestion reactor studied by Fdz-Polanco. Schrum *et al.* investigated SRAO in a sub-seafloor sediment environment and proposed incorporating organic carbon into the overall reaction (eqn (5)) by combining sulfate reduction to sulfide, and ammonium oxidation to nitrate (eqn (6)) and heterotrophic denitrification (eqn (7)), but neither explanation nor further discussion was given.

$$10H^+ + 2NH_4^+ + 2SO_4^{2-} + 10\text{Org. } e^- \rightarrow 2HS^- + N_2 + 8H_2O \quad (5)$$

$$NH_4^+ + SO_4^{2-} \rightarrow HS^- + NO_3^- + H_2O + H^+ \quad (6)$$

$$20\text{Org. } e^- + 4NO_3^- + 24H^+ \rightarrow 2N_2 + 12H_2O \quad (7)$$

Meanwhile, Liu *et al.* demonstrated the SRAO phenomenon in the absence of organic carbon but in the presence of bicarbonate (denoted as autotrophic conditions),[8] who directly adopted the mechanism from Fdz-Polanco. Following their study, other researchers also successfully achieved the SRAO conversion in their experiments,[9,10] both under heterotrophic (organic carbon) and autotrophic (bicarbonate) conditions. Nevertheless, they encountered inexplicable results, such as variable end products, unclear stoichiometry, and the involvement of a complex microbial community. This seems to indicate that the SRAO conversion is likely the result of multiple reactions rather than a single process, with potentially different underlying mechanisms under heterotrophic and autotrophic conditions.

Previously published review papers regarding SRAO phenomenon mainly summarised publication and citation records, potential environmental affecting factors[11] and operational parameters affecting practical reactor operation.[12] Other review studies, Liu *et al.*[13] and Wu *et al.*[14] recognised that the SRAO reactions could take place under both heterotrophic and autotrophic conditions, however they did not address potential differences in the corresponding reactions and underlying mechanisms. It is hereby clear that more fundamental insight needs to be gained to fully understand the SRAO phenomenon.

This contribution comprises a comprehensive revisit of experimental observations of the SRAO phenomenon under heterotrophic and autotrophic conditions, in order to identify and explain the (seemingly) inexplicable observations. Subsequently, a systematic extension of the hypothesis for the underlying reaction mechanism was made in order to reveal the SRAO phenomenon, considering all possible SRAO reactions, including also NO_3^-, NO_2^-, and S^{2-} as possible end products, besides N_2 and S^0 which are considered in the sulfammox reaction. Multiple thermodynamically feasible pathways were identified. Furthermore, SRAO-related microbial communities reported in the literature have been reviewed and compared against the possibility of SRAO being an elementary or complex (combined) reaction. Lastly, the reaction kinetics were assessed based on batch tests in the literature.

2. Revisiting the experimental observations of the SRAO phenomenon

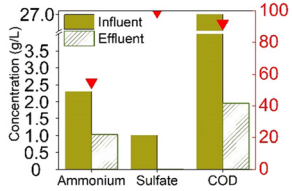
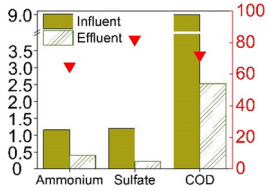
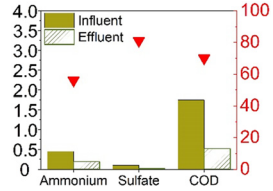
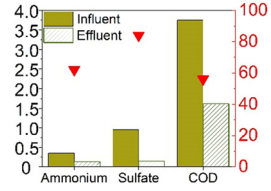
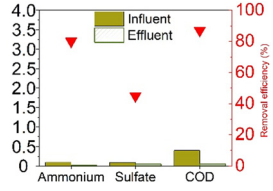
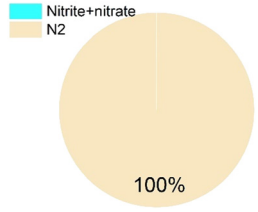
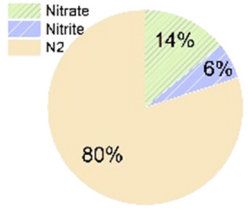
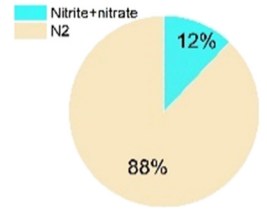
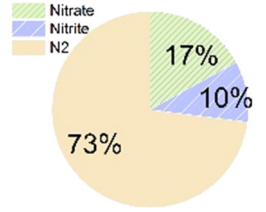
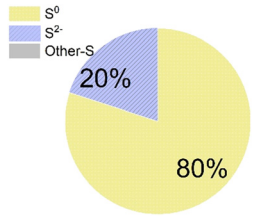
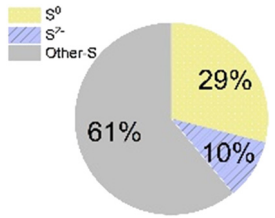
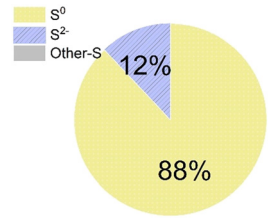
The experimental observations of studies on SRAO phenomenon were revisited. In order to investigate the end products and reaction stoichiometry, the steady state results obtained for continuous-flow reactors with or without organic carbon are summarised separately and subsequently compared.

2.1. Steady-state results under heterotrophic conditions

The earliest study to observe sulfammox was by Fdz-Polanco,[3] involving an anaerobic digestion reactor fed with diluted vinasse from an ethanol distillery plant. Since then, a number of studies aimed to reproduce the observations under heterotrophic conditions, all of them were using synthetic wastewater organic carbon (chemical oxygen demand – COD) addition. Regarding the reactor type, most of them used a continuous flow reactor with or without biofilm carrier, except for Zhu *et al.*,[15] who used a sequencing batch reactor (Table S5†). Table 1a shows the steady state experimental results under heterotrophic conditions reported in the literature. The influent ammonium concentration ranged from 38 mg N/L to 2300 mg N/L, the influent sulfate concentration ranged from 48 mg S/L to 1200 mg S/L, and the influent COD ranged from 400 mg L^{-1} to 27 000 mg L^{-1}. The removal efficiency of ammonium ranged from 55% to 80%, without a clear relation to influent concentration (Fig. S1†), nor to the ammonium/sulfate ratio (Table S1†). Regarding sulfate, the removal efficiency was mostly higher, between 85% to 100%, except for Zhu *et al.*, where the sulfate

Table 1 Review of steady state experimental results of SRAO reported in the literature, for both heterotrophic and autotrophic conditions, ordered from high to low ammonium concentration. The red triangles indicate the removal efficiency. The pie charts indicate the proportion of N and S end products relative to the converted N and S compounds

1a. Heterotrophic conditions (with organic carbon in the influent)

	(Fdz-Polanco, 2001)[3]	(Wang *et al.*, 2017)[16]	(Zhao *et al.*, 2006)[41]	(Sabumon, 2008)[37]	(Zhu *et al.*, 2022)[15]
Inf/eff					
N product				Not given	
S-product				Not given	Not given

1b. Autotrophic conditions (without organic carbon but carbonate)

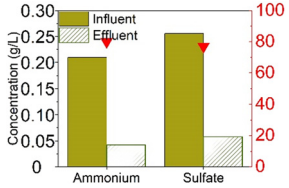
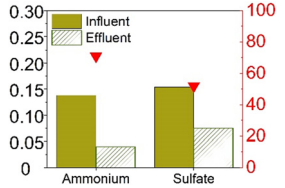
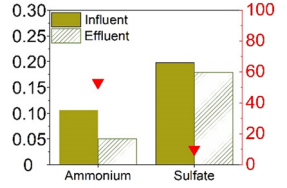
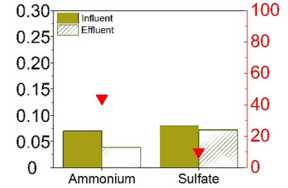
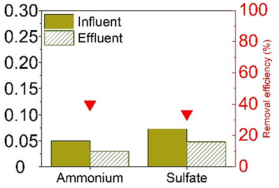
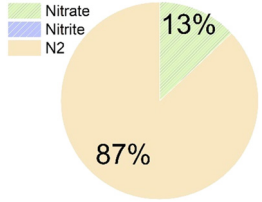
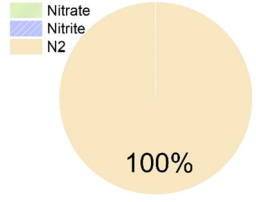
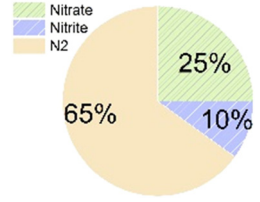
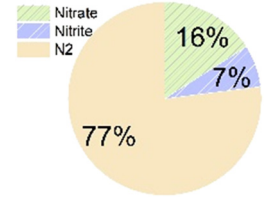
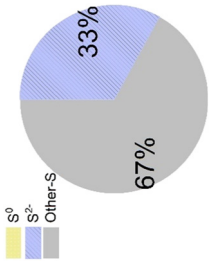
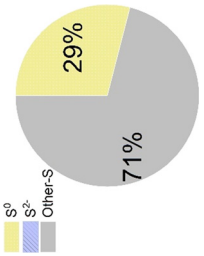
	(Liu *et al.*, 2008)[8]	(Zhang *et al.*, 2019)[39]	(Zhang *et al.*, 2009)[40]	(Qin *et al.*, 2021)[17]	(Yang *et al.*, 2009)[9]
Inf/eff					
N product					Not given

Table 1 (continued)

1b. Autotrophic conditions (without organic carbon but carbonate)					
	(Liu *et al.*, 2008)[8]	(Zhang *et al.*, 2019)[39]	(Zhang *et al.*, 2009)[40]	(Qin *et al.*, 2021)[17]	(Yang *et al.*, 2009)[9]
S product	Not given	Not given	Not given		

removal efficiency was only 50%. In the case of Zhu *et al.*, COD was completely consumed, while in the former studies, sulfate was largely used up. In sum, there was always 20% to 45% of the influent ammonium remained unconverted, whereas sulfate had a high removal efficiency (>85%) as long as COD was available.

As for the end products, N_2 was always the main end product (73% to 100% of the converted ammonium), while NO_2^- and NO_3^- were not always observed (Table 1), accounting for about 0–10%, and 0–15% of the converted ammonium, respectively. As for the sulfur compounds, elemental S was the main end product, corresponding to 29–88% of the converted sulfur, while some sulfide was produced as well (10–20% of the converted sulfur). In the case of Wang *et al.*,[16] the reported total effluent sulfur species concentrations (Table S1†) corresponded to only 39% of the influent sulfur, while 61% of the converted sulfur seemed to be unidentified.

2.2. Steady-state results under autotrophic conditions

Table 1 provides a summary of the steady-state experimental results under autotrophic conditions (see Table S1† for corresponding numerical values). The influent ammonium concentration ranged from 50 mg N/L to 225 mg N/L, the influent sulfate concentration ranged from 73 mg S/L to 256 mg S/L. All studies were using synthetic wastewater and intended to examine the sulfammox reaction. Three studies used an up-flow reactor with or without biofilm carrier, while other studies applied a sequencing batch reactor and rotating contactor (Table S5†). The ammonium conversion efficiency decreased with decreasing influent ammonium concentration, from 80% down to 40% (Table S1 and Fig. S1†). The sulfate removal efficiency ranged between 10 and 78%, the highest sulfate removal efficiencies were obtained for the highest influent sulfate and the highest influent ammonium concentrations (Table S1 and Fig. S1†).

In terms of the end products, N_2 was the main end product (65% to 100% of the converted ammonium), while NO_2^- and NO_3^- were observed as well, accounting for 7–10%, and 13–25% of the converted nitrogen. As for the end products from sulfate conversion, the reported concentrations by Qin *et al.*[17] and Yang *et al.*[9] corresponded to one third of the converted sulfate ending up as sulfide and elemental sulfur, respectively, while two thirds remained unidentified in both studies (Table S1†).

2.3. Seemingly inexplicable experimental observations

Comparing the results under heterotrophic and autotrophic conditions, the ammonium removal efficiency was positively correlated to the influent ammonium concentration (Fig. S1c†) under autotrophic conditions, while no clear relation was found under heterotrophic conditions (Fig. S1a†). The removal efficiency of sulfate was found very high (>85%) under heterotrophic conditions (Fig. S1b†), except in the case where organic carbon was depleted (Table 1). Under autotrophic conditions, the sulfate removal efficiency was

lower than under heterotrophic condition, probably due to absence of heterotrophic sulfate reduction. In both cases, a significant part of the ammonium remained unconverted. This may be due to the depletion of another component which is limiting the reaction, or it could be attributed to a slow reaction rate, possibly preventing the system from reaching steady state.

According to the sulfammox reaction mechanism and stoichiometry postulated by Fdz-Polanco (eqn (1)), the converted ammonium: sulfate molar ratio is expected to be about 2 : 1. However, in the experimental results (Table 1 and S1†), the stoichiometric ammonium: sulfate molar ratio ranges between 0.6–7.2 in the presence of organic carbon and between 0.6–8.9 in the presence of bicarbonate, which significantly deviates from the expected value 2.0. This suggests that eqn (1) is not the (only) reaction occurring; the relatively wide range of consumption ratios observed also points towards the interference of one or more other reactions with the conversion of ammonium and/or sulfate. Some researchers hypothesised that the observed wide ammonium: sulfate ratio could be due to other bioprocesses, such as sulfate reduction and sulfur-based denitrification, interfering with the sulfammox phenomenon.[13] Indeed, sulfate reduction, when taking place, increases sulfate consumption, while sulfur-based denitrification re-generates sulfate[3] and thus reduces the net sulfate consumption. Overall, sulfate reduction could explain a higher sulfate consumption under heterotrophic conditions, and sulfur-based denitrification could explain a lower sulfate consumption under autotrophic conditions. However, the converted ammonium: sulfate molar ratio was found higher (>2 : 1) under heterotrophic condition, whereas it was found lower (<2 : 1) under autotrophic condition (Table S1†), which is contradictory to the explanation above.

Nitrogen gas and elemental sulfur are typically considered as the end products of the sulfammox reaction (eqn (1)). However, nitrite and nitrate were also formed, under both heterotrophic and autotrophic conditions, albeit not in fixed proportions (Table 1), which suggests they could be either intermediate products or byproducts. Besides, the heterotrophic condition tended to have elemental sulfur and sulfide as main products, while the sulfur products were mostly unidentified under autotrophic conditions (Table 1).

The wide range in conversion efficiencies, converted substrate ratios, as well as the variety in the type and share of the observed end products, point out the value of a systematic and thorough investigation of all possible reactions which may either constitute an integral part of the SRAO reactions or take place in parallel.

3. Systematic extension of the hypothesis for the SRAO reaction mechanism

So far, two mechanisms have been put forward in the literature to explain the simultaneous sulfate reduction and ammonium oxidation, as denoted by eqn (2)–(4)[3] and eqn (6)–(7),[4] respectively. However, these may not be the only possible pathways. The objective of this section is to

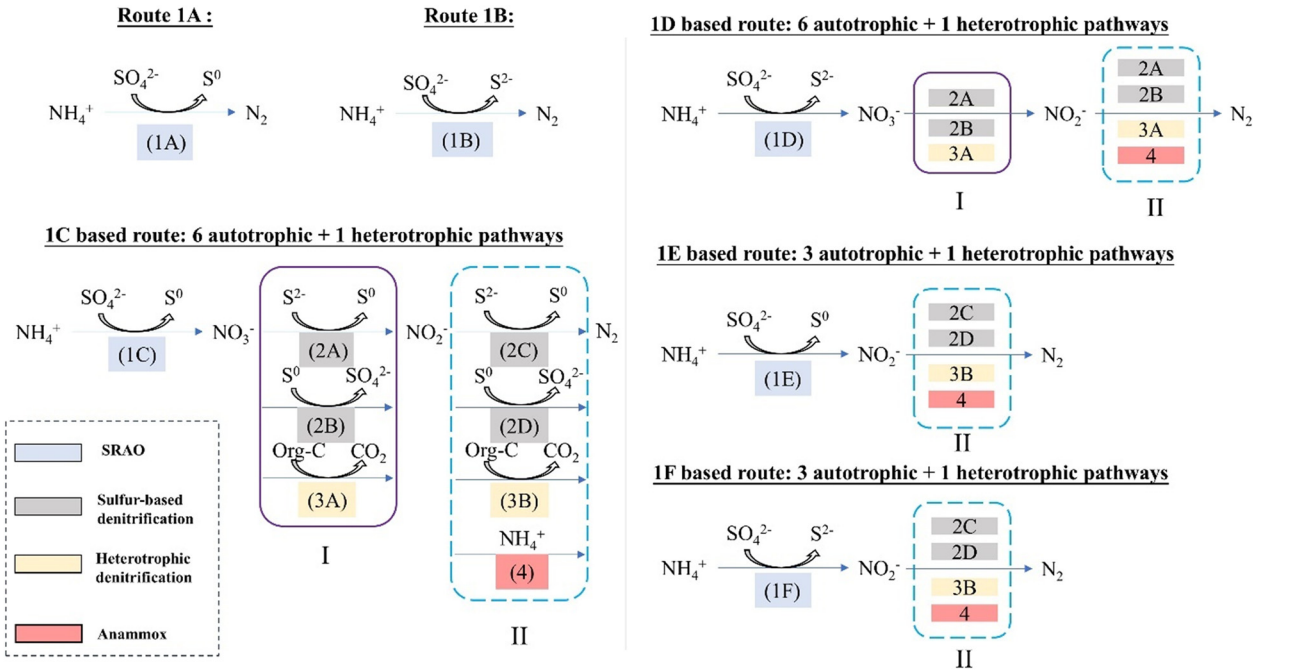


Fig. 1 Extended hypothesis: overview of elementary SRAO reactions (1A, 1B, 1C, 1D, 1E, 1F) and of complex SRAO reactions with nitrogen gas as end product (18 combinations for autotrophic conditions and 4 combinations for heterotrophic conditions). Box I and box II group bioprocesses which are repeated. The reaction numbers and corresponding colours are consistent with Table 2; a full description is given in section 3.2.

systematically identify all thermodynamically feasible pathways which result in simultaneous conversion of ammonium and sulfate. In this study, the term SRAO was adopted to denote all biochemical reactions in which sulfate reduction and ammonium oxidation take place simultaneously, considering more possible end products. Additional reaction pathways were identified in a systematic way, thus extending previous hypotheses.

Elementary SRAO reactions, *i.e.* consisting of a single reaction step, are considered first and their thermodynamic feasibility will be evaluated. Next, an inventory is made of complex SRAO reactions, which are thermodynamically feasible combinations of elementary SRAO reactions with other, syntrophic reactions. A graphical overview of elementary and complex SRAO reactions is given in Fig. 1; their stoichiometry and thermodynamic feasibility is summarized in Table 2 (elementary SRAO) and Tables 3 and 4 (complex SRAO). This will be elaborated on in the following sections.

3.1. Elementary SRAO reactions

In this study, the term SRAO is defined to denote all biochemical reactions in which sulfate reduction and ammonium oxidation take place simultaneously. By differentiating between nitrogen gas, nitrate and nitrite as three possible end products for the ammonium conversion, and between elemental sulfur and sulfide as products for the sulfate reduction, six elementary SRAO reactions are obtained (Table 2).

For each of these six reactions, the standard Gibbs free energy change ΔG_0 has been calculated (adapted to physiological conditions, *i.e.*, pH = 7.0, atmospheric pressure, 25 celsius degree; the ionic strength was assumed to be ideal (I = 0); the detailed methodology is described in ESI† S4), showing that only two reactions (1A:N_2–S^0 and 1B:N_2–S^{2-}, Table 2) are thermodynamically feasible, both of which have nitrogen gas as the end product of ammonium conversion. The remaining four SRAO reactions, with nitrite or nitrate as the end product, are thermodynamically infeasible (1C:NO_3^-–S^0, 1D:NO_3^-–S^{2-}, 1E:NO_2^-–S^0, and 1F:NO_2^-–S^{2-}, Table 2).

The observation of nitrite and nitrate present in the experiments (Table 1) seems contradictory to the fact that the SRAO reactions of which they are the end product, are thermodynamically infeasible (1C, 1D, 1E, and 1F, Table 2).

It is important to recognise that the SRAO reactions listed in this paper are only the catabolic reactions. In practice, metabolic reactions combine both catabolism (energy supply) and anabolism (microorganism growth).[18] It could be that the nitrate production during SRAO results from the anabolic reaction coupled to the catabolic reactions 1A and 1B, analogous as for the anammox stoichiometry.[19,20]

Assuming a typical microorganism composition of $C_5H_7O_2N$, as suggested by Haug and McCarty,[21] the biomass growth reaction can be written as:

$$5HCO_3^- + NH_4^+ + 20e^- + 24H^+ \rightarrow C_5H_7O_2N + 13H_2O \quad (8)$$

Table 2 Elementary SRAO reactions (eq. 1A–1F) and potential syntrophic bioprocesses (eq. 2A–5). Substrates are indicated in green, while produced compounds are denoted in red. The colours of the reactions correspond to the ones in Fig. 1. All ΔG_0^* have been corrected to match physiological conditions (pH = 7, T = 25 °C)

Reaction number	Substrate/product ↘ Microbial process ↓	NH_4^+	NO_3^-	NO_2^-	N_2	S^{2-}	S^0	SO_4^{2-}	H^+	H_2O	Organics (methanol/acetate/glucose)	CO_2	ΔG_0 (kJ mol^{-1} per equation)	ΔG_0 (kJ mol^{-1} e^{-1}) (electron)
Elementary SRAO reactions														
1A	Elementary SRAO with products N_2–S^0	−2			1		1	−1		4			−47.8	−8.0
1B	Elementary SRAO with products N_2–S^{2-}	−8			4	3		−3	8	12			−551.9	−23.0
1C	Elementary SRAO with products NO_3^-–S^0	−3	3				4	−4	−2	7			1302.1	54.3
1D	Elementary SRAO with products NO_3^-–S^{2-}	−1	1			1		−1	2	1			309.9	38.7
1E	Elementary SRAO with products NO_2^-–S^0	−1		1			1	−1		2			312.4	52.1
1F	Elementary SRAO with products NO_2^-–S^{2-}	−4		4		3		−3	8	4			878.2	36.6
Syntrophic bioprocesses														
2A	S-denitrification (NO_3^-; S^{2-})		−1	1		−1	1		−2	1			2.2	1.1
2B	S-denitrification (NO_3^-; S^0)		−3	3			−1	1	2	−1			−364.9	−60.8
2C	S-denitrification (NO_2^-; S^{2-})			−2	1	−3	3		−8	4			−297.9	−49.7
2D	S-denitrification (NO_2^-; S^0)			−2	1		−1	1					−670.2	−111.7
3A	Hetero-denitrification (NO_3^-)		−2	2						4/3(methanol); 1(acetate); 1(glucose)	−2/3(methanol); −1/2(acetate); −1/6(glucose)	2/3(methanol); 1(acetate); 1/6(glucose)	−314.1(methanol); −298.7(acetate); −330.8(glucose)	−78.5(methanol); −74.7(acetate); −82.7(glucose)
3B	Hetero-denitrification (NO_2^-)			−2	1				−2	3(methanol); 5/2(acetate); 5/2(glucose)	−1(methanol); −6/8(acetate); −3/12(glucose)	1(methanol); 3/2(acetate); 3/2(glucose)	−776.4(methanol); −753.4(acetate); −801.6(glucose)	−129.4(methanol); −125.6(acetate); −133.6(glucose)
4	Anammox	−1		−1	1					2			−357.5	−119.2

Table 3 Complex SRAO reactions under autotrophic conditions. The Gibbs free energy change for thermodynamically feasible reactions is written in bold

Pathway	Substrate/product↘ Microbial process↓	NH_4^+	NO_3^-	NO_2^-	N_2	S^{2-}	S^0	SO_4^{2-}	H^+	H_2O	ΔG_0 (kJ mol^{-1} Eq^{-1})	ΔG_0 (kJ mol^{-1} e^{-1}) (electron)
Complex autotrophic SRAO reaction 1 (same stoichiometry as route 1A in Fig. 1)												
Pathway 1	Elementary reaction eqn (1A)	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 2	2*1C+2*2B+3*2D	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 3	(1C+2B)/3+4	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 4	2*1D+2*2A+2D	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 5	1D+2A+4	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 6	2*1E+2D	−2			1		1	−1		4	**−47.8**	**−8.0**
Pathway 7	1E+4	−2			1		1	−1		4	**−47.8**	**−8.0**
Complex autotrophic SRAO reaction 2 (same stoichiometry as route 1B in Fig. 1)												
Pathway 8	Elementary reaction eqn (1B)	−8			4	3		−3	8	12	**−551.9**	**−23.0**
Pathway 9	1F+4*4	−8			4	3		−3	8	12	**−551.9**	**−23.0**
Other complex autotrophic reactions												
Pathway 10	2*1C+6*2A+3*2C	−6			3	−15	23	−8	−40	32	1466	81.4
Pathway 11	2*1C+6*2A+3*2D	−6			3	−6	11	−5	−16	20	608.4	33.8
Pathway 12	(2*1C+2*2B)/3+2C	−2			1	−3	5	−2	−8	8	311.3	51.9
Pathway 13	1C+3*2A+3*4	−6			3	−3	7	−4	−8	12	1185	65.8
Pathway 14	2*1D+2*2A+2C	−2			1	−3	5	−2	−8	8	311.3	51.9
Pathway 15	6*1D+2*2B+3*2D	−6			3	6	−5	−1	16	4	**−880.8**	**−48.9**
Pathway 16	6*1D+2*2B+3*2C	−6			3	−3	7	−4	−8	16	236.1	13.1
Pathway 17	3*1D+2B+3*4	−6			3	3	−1	−2	8	8	**−508.5**	**−28.2**
Pathway 18	2*1E+2C	−2			1	−3	5	−2	−8	8	311.3	51.9
Pathway 19	1F+2*2C	−4			2	−3	6	−3	−8	12	281.5	−23.5
Pathway 20	1F+2*2D	−4			2	3	−2	−1	8	4	**−463.1**	**−38.6**

Table 4 Complex SRAO reactions under heterotrophic conditions

Pathway	Substrate/product↘ Microbial process↓	NH_4^+	NO_3^-	NO_2^-	N_2	S^{2-}	S^0	SO_4^{2-}	H^+	H_2O	CO_2	Organics (methanol/acetate/glucose)	ΔG_0 (kJ mol^{-1} Eq^{-1})	ΔG_0 (kJ mol^{-1} e^{-1}) (electron)
Pathway 1	2*1D+3A+3B	**−2**			**1**	**2**		**−2**	**2**	**19/3(methanol); 11/2(acetate); 11/2(glucose)**	**5/3(methanol); 20/8(acetate); 10/4(glucose)**	**−5/3(methanol); 10/8(acetate); 5/12(glucose)**	−470.7(methanol); −432.3(acetate); −512.6(glucose)	−47.1(methanol); −43.2(acetate); −51.3(glucose)
Pathway 2	1F+2*3B	**−4**			**2**	**3**		**−3**	**4**	**10(methanol); 9(acetate); 9(glucose)**	**2(methanol); 3(acetate); 3(glucose)**	**−2(methanol); 6/4(acetate); 1/2(glucose)**	−674.6(methanol); −628.6(acetate); −725.0(glucose)	−52.2 (methanol); −52.4(acetate); −60.4(glucose)
Pathway 3	2*1C+3*(3A+3B)	**−6**			**3**		**8**	**−8**	**−10**	**27(methanol); 49/2(acetate); 49/2(glucose)**	**5(methanol); 60/8(acetate); 30/4(glucose)**	**−5(methanol); 30/8(acetate); 5/4(glucose)**	−667.3(methanol); −552.1(acetate); −793.0(glucose)	−22.0(methanol); −18.0(acetate); −26.4(glucose)
Pathway 4	2*1E+3B	**−2**			**1**		**2**	**−2**	**−2**	**7(methanol); 13/2(acetate); 13/2(glucose)**	**1(methanol); 12/8(acetate); 6/4(glucose)**	**−1(methanol); 6/8(acetate); 1/4(glucose)**	−151.6(methanol); −128.6(acetate); −176.8(glucose)	−25.2(methanol); −21.4(acetate); −29.5(glucose)

in which the electrons are provided by the electron donor (ammonium, in this case), which is then converted to nitrate:

$$NH_4^+ + 3H_2O \rightarrow NO_3^- + 10H^+ + 8e^- \quad (9)$$

By balancing the redox half-reactions eqn (8) and eqn (9), the anabolic reaction is obtained:

$$An: 5HCO_3^- + \frac{7}{2}NH_4^+ \rightarrow C_5H_7O_2N + \frac{5}{2}NO_3^- + H^+ + \frac{11}{2}H_2O \quad (10)$$

The overall metabolism reaction (eqn (11)) results from the combined anabolic and catabolic reactions:[18]

$$Met = \lambda_{Cat} \cdot Cat + A \quad (11)$$

in which λ_{Cat} is determined based on the experimentally measured yield coefficient (g VSS biomass per g substrate), which is not yet available for sulfammox, or more general, for SRAO.

A second, alternative or even complementary explanation for the occasional presence of nitrite/nitrate is that the thermodynamically infeasible SRAO reactions are combined

with syntrophic bioprocesses, resulting in complex SRAO reactions that are thermodynamically feasible.

Possible syntrophic bioprocesses that may occur under such conditions are sulfur-based denitrification (2A to 2D in Table 2), heterotrophic denitrification (3A to 3B), and anammox (4 in Table 2). In what follows, it will be investigated which combinations of thermodynamically infeasible SRAO reactions (1C to 1F) with syntrophic reactions result in thermodynamically feasible overall reactions. The focus hereby lies on the identification of thermodynamically feasible reactions which result in the conversion of ammonium to nitrogen gas, as aimed for during wastewater treatment.

3.2. Complex SRAO reactions

3.2.1. Identification methodology. The identification of all possible reaction mechanisms, leading to the overall conversion of ammonium to nitrogen gas along with sulfate reduction to either elemental sulfur or sulfide, was performed in a systematic way, starting from the six elementary SRAO reactions. A schematic overview of the involved SRAO and syntrophic conversions is given in Fig. 1; the reaction numbers refer to Table 2.

The SRAO reactions in which ammonium is converted to nitrogen gas with sulfate reduced to either elemental sulfur (1A in Table 2) or sulfide (1B in Table 2) constitute two obvious reaction mechanisms, in the form of elementary reactions.

The third SRAO reaction (1C) combines the oxidation of ammonium to nitrate with the reduction of sulfate to elemental sulfur. The produced nitrate can then be reduced to nitrite *via* sulfur-based denitrification (2A or 2B), or heterotrophic denitrification (3A). Subsequently, nitrite will be further reduced to nitrogen gas *via* sulfur-based denitrification (2C or 2D), or heterotrophic denitrification (3B), or anammox (4). The mechanisms involving heterotrophic denitrification require the presence of organic carbon, while the others take place under autotrophic conditions.

The fourth SRAO reaction (1D) connects ammonium oxidation to nitrate, and sulfate reduction to sulfide. The subsequent processes, *i.e.*, nitrate reduction to nitrite, and nitrite reduction to nitrogen gas, are the same as described in the 1C based route, briefly denoted by (dotted) boxes I (nitrate to nitrite) and II (nitrite to nitrogen gas) in Fig. 1.

In the fifth (1E) and sixth (1F) SRAO reactions, ammonium is oxidised to nitrite, and sulfate is reduced to elemental sulfur or sulfide, respectively. Then nitrite is reduced to nitrogen gas in the same way denoted by box II (nitrite to nitrogen gas).

Overall, there are totally 24 possible pathways described in Fig. 1, in which 20 of them are not involving organic carbon, while 4 of them are. Therefore, 20 pathways under autotrophic conditions and 4 pathways under heterotrophic conditions are obtained.

3.2.2. Complex SRAO reactions under autotrophic conditions. All 20 possible SRAO pathways under autotrophic conditions, with nitrogen gas as end product (illustrated in Fig. 1) are listed in Table 3 in terms of overall reaction stoichiometry and standard Gibbs free energy change. The overall reactions are obtained as combinations of SRAO with nitrite/nitrate as products and syntrophic bioprocesses in which nitrite and nitrate are consumed, namely sulfur-based denitrification and anammox. The calculation process is detailed in the ESI† (Tables S2 and S3).

Interestingly, some of the pathways were found to result in the same overall reactions. More specifically, seven complex pathways resulted in the same thermodynamically feasible overall reaction ('complex autotrophic SRAO reaction 1' in Table 3) as elementary reaction 1A, whereas two pathways led to another thermodynamically feasible overall reaction ('complex autotrophic SRAO reaction 2' in Table 3) as elementary reaction 1B. This indicates that reactions 1A and 1B may be either elementary reactions or complex (combined) reactions.

Additional thermodynamically feasible pathways were identified (Table 3). Since their substrates are not only ammonium and sulfate, they do not strictly match the definition of SRAO. Still, they may take place under the same conditions.

From the above analysis, it is clear that multiple reactions, with different stoichiometries, may take place simultaneously. This may explain the wide range of ammonium to sulfate conversion ratios and of end products reported in the sulfammox literature. Besides, the substrate consumption ratios and intermediate product accumulation will also be influenced by reaction kinetics, which is likely to differ as well between the different SRAO reactions.

3.2.3. Complex SRAO reactions under heterotrophic conditions. From the combination of elementary SRAO reactions and heterotrophic denitrification (Fig. 1), four possible heterotrophic complex SRAO reactions with nitrogen gas as end product were identified (eqn (12)–(15)):

$$8H^+ + 2SO_4^{2-} + 2NH_4^+ + 10\text{Org. } e^- \rightarrow 2S^{2-} + N_2 + 8H_2O \quad (12)$$

$$8H^+ + 3SO_4^{2-} + 4NH_4^+ + 12\text{Org. } e^- \rightarrow 3S^{2-} + 2N_2 + 12H_2O \quad (13)$$

$$40H^+ + 8SO_4^{2-} + 6NH_4^+ + 30\text{Org. } e^- \rightarrow 8S^0 + 3N_2 + 32H_2O \quad (14)$$

$$8H^+ + 2SO_4^{2-} + 2NH_4^+ + 6\text{Org. } e^- \rightarrow 2S^0 + N_2 + 8H_2O \quad (15)$$

The overall reaction stoichiometries and standard Gibbs free energy changes for various organic carbon sources (methanol, acetate, glucose) are listed in Table 4 (see ESI† – Table S4 for the detailed calculations). The resulting reactions are all thermodynamically feasible, for the three organic carbon sources considered.

It is clear that the combined occurrence of multiple feasible heterotrophic SRAO reactions may lead to a wide range of ammonium : sulfate conversion ratios and different end products (S^{2-} and/or S^0), as observed in the experimental results.

4. Functional microorganisms for SRAO

Since the first reports on the sulfammox, the functional microorganism(s) behind have remained unidentified. In this section, the SRAO-related microbial community analysis results from the literature are reviewed and analysed in light of the extended reaction mechanism hypothesis.

4.1. Dedicated strain *versus* consortium

The most challenging puzzle comes from the functional microorganisms of the elementary SRAO reactions, for which various results have been reported in the literature.

A commonly encountered hypothesis is that SRAO is carried out by anammox, through a dedicated metabolic pathway.[22] Some latest studies were also in favour of this hypothesis,[7,23] for instance, Liu *et al.*[8] and Zhang *et al.*[24] found *Candidatus Brocadia sapporoensis* prevailed in their SRAO reactors, which was potentially dedicated SRAO strain. However, this postulation seems only applicable for SRAO under autotrophic conditions, since it is contradictory to the fact that SRAO was discovered in anaerobic digestion reactor with high COD concentration, where anammox bacteria could not survive. Alternatively, as a similar hypothesis, SRAO could be carried out by another dedicated strain, other than anammox. Two strains were isolated from the biomass of SRAO reactors and claimed to be dedicated SRAO strains, namely *Bacillus benzoevorans*[25] and Bacillus cereus.[26] However, so far none of these strains nor consortium have been publicly verified and acknowledged for their function of conducting SRAO.

The other commonly encountered hypothesis is that SRAO is performed by consortium, for instance, Derwis *et al.* suggested a connection related to *Thauera* and *Chloroflexi*.[27] Wimalaweera *et al.* indicated *Desulfovibrio* and *Sulfurospirillum* were the key species of SRAO.[28]

4.2. Comparison against experimental observations

A summary of microbial communities in SRAO reactors and their putative function is given in Table 5. These studies used high-throughput sequencing as analysis method. The hypothesised functional microorganisms were categorised into the dedicated strain or consortium, as proposed in the corresponding study. In general, strains with diverse functions were found in these studies. Under autotrophic conditions, *Planctomycetes* were the most suspected organisms for carrying out SRAO as a dedicated strain, while in the other studies, different combinations of consortium were suggested. As for heterotrophic conditions, there was limited literatures available – a consortium between denitrifiers and sulfate reduction bacteria was the most likely combination.

For the syntrophic bioprocesses, autotrophic sulfur-based denitrification and (or) anammox were found in each study under autotrophic conditions, whereas heterotrophic denitrification was present as a major component in studies under heterotrophic conditions. This matches well what was

Table 5 Microbial communities in SRAO reactors and their putative functions (coloured cells correspond with microbial abundance >5%)

Conditions	Nitrification	Autotrophic denitrification	Heterotrophic-denitrification	Anammox	Sulfate reduction	Anaerobic fermentation	Microorganism hypothesized to be responsible for SRAO	Reference
Autotrophic, anaerobic (purged by argon)	*Nitrosomonas sp. Nm47*	*Paracoccus denitrificans strain IAM12479*	Not mentioned	*Planctomycetales bacterium clone T13J-B80; planctomycete clone MPR114*	*Desulfacinum subterraneum*	*Acidobacteriaceae bacterium clone EfT107_A12*	**Dedicated strain** *Planctomycete*	36
Autotrophic, anaerobic (only indicated), 35 °C	*Not mentioned*	*Thiobacillus (3%);*	*Denitratisoma (5%); Pseudomonas (<1%) Limnobacter (4%)*	*Fimbriimonadales (3%); Candidatus_Brocadia (1%)*	*Desulfatiglans (3%) Desulfurivibrio (2%)*	**Spirochaeta_2 (2%)*	**Consortium** anammox, sulfate reduction and sulfide oxidation bacteria	17
Autotrophic, anaerobic (only indicated), 35 °C	*Nitrosomonas (15%)*	*Thauera (16%)*	*Bacillus (2%); Comamonadaceae (3%) Limnobacter (1%)*	*Candidatus Brocadia (<1%)*	*Thauera (16%)*	—	**Consortium** *Nitrosomonas* and *Thauera*	27
Autotrophic, anaerobic (only indicated), 27 °C	*Nitrosomonas (2%)*	*Thiobacillus (1%); Sulfurimonas (1%)*	*Denitratisoma (2%); Comamonadaceae (2%)*	*Candidatus Kuenenia (2%); Candidatus Brocadia (3%);*	*Desulfuromusa (2%) Deltaproteobacteria (2%)*	*Saprospiraceae (4%)*	**Dedicated strain** *Planctomycete Brocadia*	31
Autotrophic, anaerobic (purged by nitrogen gas), 35 °C	*SBR1031 (27%)*	*Crenothrix (2%) ; Thiobacillus (2%)*	*PHOS-HE 36 (4%); OLB-13(3%); Denitratisoma (2%); Limnobacter (2%)*	*Candidatus Brocadia (8%); Candidatus Kuenenia (2%); Phycisphaeraceae (1%)*	*Desulfobacterota*	*SJA-28 (2%); Bryobacter (2%)*	**Consortium** *Crenothrix* and *Desulfobacterota*	6
Autotrophic, anaerobic (purged by nitrogen gas), 30 °C	*Nitrosomonas; Nitrosospira; Nitrospira; Nitrolancea (all <0.5%)*		*Comamonas (17%)*	*Candidatus_Anammoxoglobus (<0.5%); Candidatus_Brocadia (<0.5%)*	*Desulfovibrio* (<0.5%); , *unclassified_p_Desulfobacterota* (<0.5%)	—	**Dedicated strain** *Planctomycete Brocadia*	24
Heterotrophic (organic load ~150 mg COD per L d^{-1}), anaerobic (purged by nitrogen gas), 35 °C	*SBR1031 (10%)*	—	*Pseudomonas (15%); PHOS-HE 36 (2%); Bacillus (2%) Limnobacter (10%)*	—	*Desulfuromonadaceae (5%)*	*Anaerolineaceae (2%); Rhodococcus (2%)*	**Consortium** denitrifiers and sulfate reduction bacteria	15
Heterotrophic, (organic load ~9 kg COD per m^3 d^{-1}), anaerobic (dissolved oxygen <0.15 mg L^{-1}), 36 °C	—	—	*Pseudomonas (2%); Alcaligenes (1%); Acinetobacter (2%)*	—	*Desulfovibrio (8%); Desulfomicrobium (2%); Desulfocurvus (1%)*	—	**Consortium** *Desulfovibrio* and denitrifiers	16

postulated in the extended hypothesis, in light of SRAO under autotrophic and heterotrophic conditions.

Furthermore, the presence of nitrifiers, *i.e.*, *Nitrosomonas* was found abnormally accumulated under such an anaerobic condition. The abundance of *Nitrosomonas* could somehow reach 15% of the microbial community.[27] There are two possibilities for this: either all studies failed in keeping the environment anaerobic strictly[29] and keeping the system from photosynthesis effect, or the nitrifiers may be related to the SRAO process, even though there is no known pathways of nitrifiers capable of oxidising ammonium in the absence of oxygen.[30] A simple mass balance can be made to check whether SRAO is caused by oxygen intrusion: assuming the dissolved oxygen concentration in these experiments was 8–10 mg L^{-1}, and all of this could be used for aerobic nitrification of ammonium to nitrate, this would correspond to 1.8–2.2 mg N/L nitrate produced (stoichiometry coefficient: 4.57 mg O_2/mg NO_3^-). However, according to Table 1, the total nitrogen removal efficiency was typically over 40%, corresponded with a net nitrogen loss more than 100 mg N/L. Thus it can be seen that the unoptimized experimental conditions (*e.g.* oxygen intrusion) are most likely not (the only) cause of SRAO, albeit equipment failures and other major experimental design errors could not be completely excluded.

In addition, the heterotrophic denitrifiers were observed to be present together with the anaerobic fermentation bacteria even under autotrophic conditions, which indicated biomass decay within the sludge. It could be that the heterotrophic microorganisms were being fed by organic carbon from the decay of autotrophic biomass. This suggests a mixotrophic conditions, where the autotrophic SRAO and heterotrophic SRAO could take place simultaneously.

Furthermore, in the latest studies, the functional genes were detected (Table 6). For sulfur metabolism, *sat* (sulfate adenylyltransferase), *apr* (adenylylsulfate reductase), *asr* (anaerobic sulfite reductase), and *sqr* (sulfide quinone oxidoreductase) were often found, indicating sulfate reduction took place. As for nitrogen metabolism, *hao* (hydroxylamine oxidoreductase), *nar* (nitrate reductase), *nap* (periplasmic nitrate reductase), *nirS* (nitrite reductase (NO-forming)), *nirK* (copper-contating nitrite reductase), and amt (ammonium transporter) were detected, indicating co-occurrence of ammonium oxidation, nitrite oxidation and denitrification.

Overall, the experimental results in the literature match the postulation in the extended hypothesis well. The mass balance convinced that oxygen intrusion might not be (the only) reason for the cause of SRAO. Multiple syntrophic bioprocesses are observed to be involved and have played a significant role in the complex reaction of SRAO. Biomass decay was found to occur, which suggests a mixotrophic conditions, where autotrophic and heterotrophic SRAO could take place simultaneously.

5. SRAO kinetics

Fig. 2 shows the batch test results of SRAO under autotrophic conditions. Notably, ammonium and sulfate depletion occurred only in Liu *et al.* (2008),[8] with the experiment lasting for 220 hours, whereas the other studies lasted less than 24 hours, with the removal efficiency all less than 50%. It suggested that the reaction of SRAO was slow and might prevent the reactors in the literature from reaching steady state.

When the substrate concentration is fixed, higher biomass concentration is expected to result in higher substrate removal efficiency. In Fig. 2, a comparison between Zhu *et al.* (2022)[15] and Prachakittikul *et al.* (2016),[36] which had similar initial ammonium and sulfate concentrations, shows that the removal efficiencies of ammonium and sulfate were 10% and 8% (0.607 g VSS L^{-1}), and 12% and 8%(4.9 g VSS L^{-1}), respectively. Hence it seemed that higher volatile suspended solid concentration did not correspond to higher removal efficiency. The possible reason for this could be the functional microorganism only constitutes a small portion of the total biomass concentration.

Table 6 Main functional genes detected in the reactors where SRAO occurred

Reference	Main functional genes related to sulfur metabolism	Main functional genes related to nitrogen metabolism
Liu *et al.*, 2024[23]	*asrBC*, *sat*, *aprAB*, *sqr*	*hao*, *nar*, *amt*, *nirS*, *nosZ*
Wimalaweera *et al.*, 2025 (ref. 28)	*cysK*, *sqr*, *aprAB*	*nifDKH*, *narGHI*, *napAB*, *nosZ*
Yin *et al.*,2025 (ref. 7)	*dsrA*,*dsrB*,*sat*, *aprB*, *soxA*,*soxX*,*soxZ*,*soxB*,*sqr*	*hzs*,*hdh*,*nirK*,*narI*,*napA*,*napB*,*norC*
Zhang *et al.*, 2025 (ref. 24)	*aprA*,*sat*	*amtB*,*hao*,*napA*,*narI*,*narH*,*narG*,*nirK*,*nirS*, *norC*,*norB*,*nosZ*,*nxrB*,*nxrA*

*The functional genes for sulfur metabolism are *aprA* (adenosine-5′-phosphosulfate reductase alpha subunit), *aprB* (adenosine-5′-phosphosulfate reductase beta subunit), *aprAB* (adenosine-5′-phosphosulfate reductase alpha and beta subunit), *asrBC* (assimilatory sulfite reductase), *cysK* (cysteine synthase), *dsrA* (dissimilatory sulfite reductase alpha subunit), *dsrB*(dissimilatory sulfite reductase beta subunit), *sat* (sulfate adenylyltransferase), *soxA* (sulfur oxidation protein A), *soxX* (sulfur oxidation protein X), *soxZ* (sulfur oxidation protein Z), *soxB* (sulfur oxidation protein B), *sqr* (sulfide:quinone oxidoreductase). **The functional genes for nitrogen metabolism are *amt* (ammonium transporter), *amtB* (ammonium transporter B), *hao* (hydroxylamine oxidoreductase), *hdh* (hydrazine dehydrogenase), *hzs* (hydrazine synthase), *napA* (periplasmic nitrate reductase A),*napB* (periplasmic nitrate reductase B), *napAB* (periplasmic nitrate reductase A and B), *nar* (nitrate reductase), *narG* (membrane-bound nitrate reductase G),*narGHI* (membrane-bound nitrate reductase complex), *narH* (membrane-bound nitrate reductase H), *narI* (membrane-bound nitrate reductase I), *nirK* (copper-containing nitrite reductase), *nirS* (cytochrome cd_1 nitrite reductase), *nifDKH* (nitrogenase complex),*norB* (nitric oxide reductase B), *norC* (nitric oxide reductase C), *nosZ* (nitrous oxide reductase), *nxrA* (nitrite oxidoreductase A), *nxrB* (nitrite oxidoreductase B).

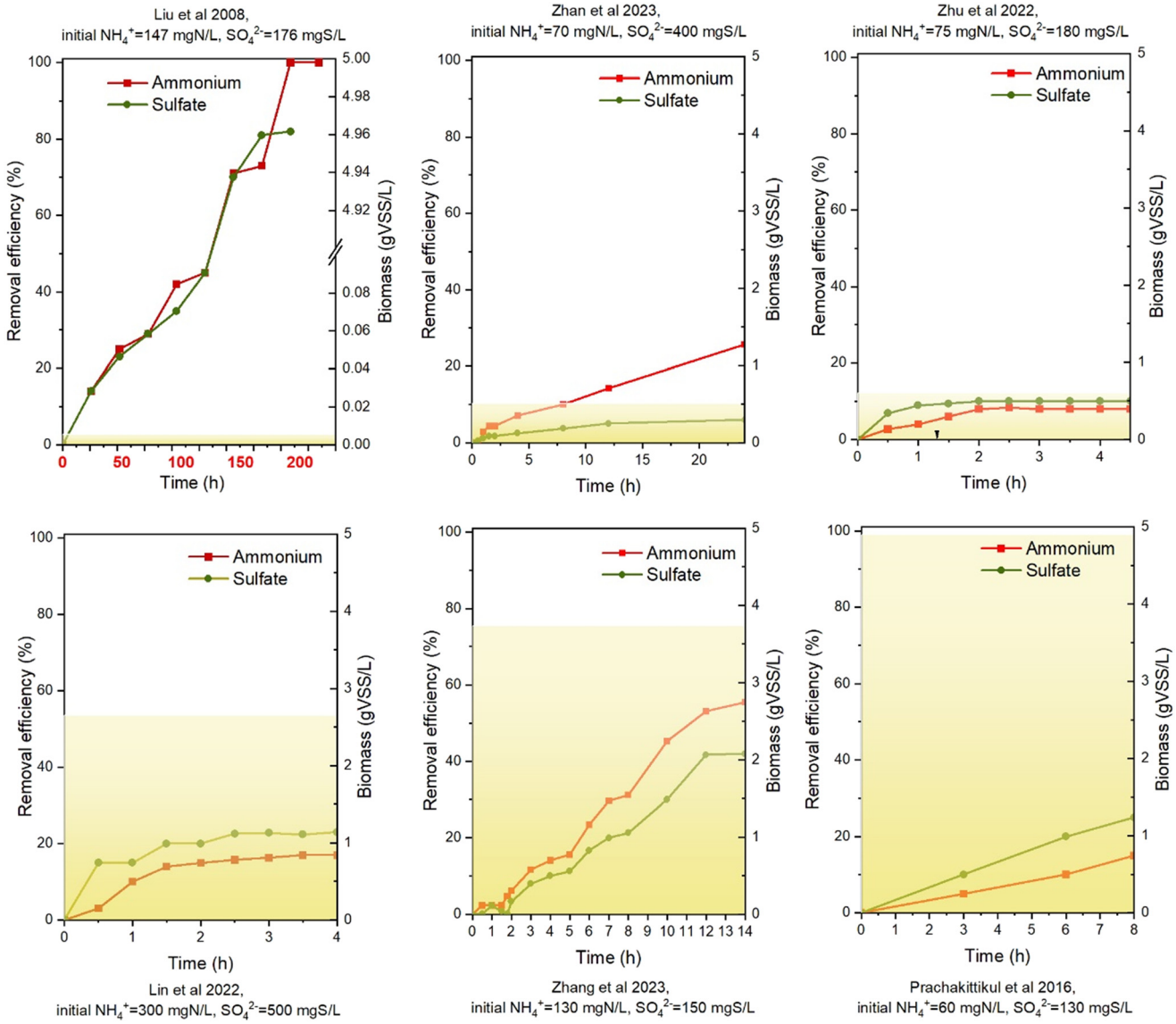


Fig. 2 Batch tests of SRAO under autotrophic condition (colour area presents for biomass concentration). The figures have been ordered by biomass concentration from low to high, as indicated in yellow colour block.

To further quantify the reaction rate, the reaction rate of SRAO has been calculated based on batch test results from the literatures (Table 7) The first four hours were chosen for the calculation to ensure a fair comparison of the reaction rates (see ESI† S6).

Under autotrophic conditions, the ammonium removal rate amounted to 0.6–124.1 mg N per g VSS L^{-1} h^{-1} (Table 7), showing a two order-of-magnitude variation. The highest reaction rate, 124.1 mg N per g VSS L^{-1} h^{-1}, was observed in Liu *et al.* (2008),[8] where SRAO was indicated to be induced by inoculating anammox sludge into the reactor. Also Lin *et al.* (2022)[32] reported a relatively high ammonium removal rate, namely 15.08 mg N per g VSS L^{-1} h^{-1}. All other studies had a much lower reaction rate, from 0.60 to 2.81 mg N per g VSS L^{-1} h^{-1}. N isotope analysis also indicated the reaction rate to be very slow, with a rate of 2.4 mg N per g VSS L^{-1} h^{-1}.[6]

For heterotrophic condition, the available literature was limited thus only Wang *et al.* (2017)[16] could be used for the calculation. According to this study, the reaction rate was 9.24–21.22 mg N per g VSS L^{-1} h^{-1} for ammonium removal, and around 90 mg S per g VSS h^{-1} for sulfate removal, which were averagely 2 times faster than the reaction rate under autotrophic condition.

Overall, the SRAO reaction rates were very low, which may explain the low removal efficiency in the literature. The rate-limiting step of SRAO, *i.e.* whether it is sulfate reduction or ammonium oxidation, is hard to unravel, as they are not independent from each other. In the future study, a longer hydraulic retention time could be adopted in the experiments to deplete the substrates. Also, more favourable environmental conditions, *e.g.*, thermophilic conditions and high substrate concentrations could be examined to facilitate SRAO conversions.

Table 7 Average SRAO reaction rates calculated from batch tests after 4 hours (see ESI S6 for the methodology)

Batch volume (L)	Initial NH_4^+ (mg N)	NH_4^+ consumption (mg N)	Initial SO_4^{2-} (mg S)	SO_4^{2-} consumption (mg S)	Biomass (g L^{-1})	Ammonium reaction rate (mg N per g VSS L^{-1} h^{-1})	Sulfate reaction rate (mg S per g VSS L^{-1} h^{-1})	Ref.
Autotrophic condition								
0.1	14.7	0.27	17.6	1.28	0.0544	124.1	588.2	8
0.25	75	12.5	125	28	2.654	15.08	33.76	32
0.3	15	1.05	16	1.92	4.900	0.60	1.1	36
0.7	49	3.5	280	7	0.509	2.81	5.61	6
1.5	112.5	9	270	25.5	0.607	1.32	3.73	15
1.5	195	30	225	48	3.730	0.89	1.15	31
Heterotrophic condition								
0.5	64.75	45.33	600	432	4.215	10.76	88.84	16
0.5	129.5	54.39	600	414	4.215	12.9	91.12	16
0.5	194.5	38.95	600	414	4.215	9.24	88.84	16
0.5	388.75	89.41	600	438	4.215	21.22	91.12	16

6. Application potential of SRAO

Despite the mechanism behind SRAO is still under investigation (as it is inherently being the result of multiple bioprocesses), its practical application in wastewater treatment process is definitely viable, which could be a supplement to lower substrate removal cost and energy consumption.[27] Due to its slow kinetics, several researchers indicated that it is better to combine SRAO with other techniques when making the process design,[38] instead of implementing SRAO alone. One example is the one from Zhang *et al.*,[31] who combined anammox and SRAO in mature landfill leachate treatment containing high concentrations of sulfate. In their study, SRAO acted as a good supplementary process to remove nitrogen (27.5% contribution rate). Another scenario is the combination of SRAO with sulfur-based autotrophic denitrification[32] and potentially also with anammox.[7] Sulfur-based denitrification produces sulfate as the end product from sulfide oxidation, which can be in turn used by SRAO to oxidise ammonium, where SRAO could also become a good supplement in the system.

In addition, concerns may also arise on the emission of nitrous oxide (N_2O), a potent greenhouse gas. SRAO is most likely the result of multiple bioprocesses, some of which are known and some not. Even for the known ones, the N_2O emissions are uncertain, for instance, heterotrophic denitrification may be both a sink or source of N_2O; the studies on N_2O emissions from sulfur-based denitrification showed contradictory results – some indicated it produces more N_2O,[33] while the rest indicated it reduces N_2O.[34,35] Besides, the mechanistic of ammonium oxidation of SRAO remains unknown, which warrants further investigation. The overall situation of N_2O emissions will be thus very complicated, taking into account all the elements and the interactions between them.

7. Conclusions

Simultaneous sulfate reduction and ammonium oxidation (SRAO) has emerged as an intriguing biological conversion in wastewater treatment, on which this study sheds the following light:

- Experimental results reported in the literature showed inexplicable results, such as a wide range of reaction stoichiometry, and variation in the types and proportions of observed end products.
- A systematic extended hypothesis for the underlying reaction mechanism was developed, considering multiple possible end products and the interaction with syntrophic bioprocesses, leading to complex SRAO reaction pathways under autotrophic and heterotrophic conditions.
- The thermodynamic feasibility of the complex SRAO reactions was calculated and discussed.
- Analysis of the functional microorganisms reported in the literature strengthened the hypothesis that SRAO is likely the result of multiple bioprocesses, with different functional organisms, and different possible combinations between heterotrophic conditions and autotrophic conditions.
- The reaction rate of SRAO was calculated as being very low.

Data availability

The data used in this study was selected from the literature, and could be found in Table S1 in ESI.†

Conflicts of interest

There are no conflicts to declare.

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References

1 M. Zheng, H. Li, H. Duan, T. Liu, Z. Wang, J. Zhao, Z. Hu, S. Watts, J. Meng, P. Liu, M. Rattier, E. Larsen, J. Guo, J. Dwyer, B. Van Den Akker, J. Lloyd, S. Hu and Z. Yuan, One-year stable pilot-scale operation demonstrates high fexibility of

mainstream anammox application, *Water Res.:X*, 2023, **19**, 100166.
2 X. Kong, D. Castarède, E. S. Thomson, A. Boucly, L. Artiglia, M. Ammann, I. Gladich and J. B. C. Pettersson, A surface-promoted redox reaction occurs spontaneously on solvating inorganic aerosol surfaces, *Science*, 2021, **374**, 747–752.
3 F. Fdz-Polanco, New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions, *Water Res.*, 2001, **35**, 1111–1114.
4 H. N. Schrum, A. J. Spivack, M. Kastner and S. D'Hondt, Sulfate-reducing ammonium oxidation: A thermodynamically feasible metabolic pathway in subseafloor sediment, *Geology*, 2009, **37**, 939–942.
5 E. E. Rios-Del Toro and F. J. Cervantes, Anaerobic ammonium oxidation in marine environments: contribution to biogeochemical cycles and biotechnological developments for wastewater treatment, *Rev. Environ. Sci. Biotechnol.*, 2019, **18**, 11–27.
6 M. Zhan, W. Zeng, H. Liu, J. Li, Q. Meng and Y. Peng, Simultaneous nitrogen and sulfur removal through synergy of sulfammox, anammox and sulfur-driven autotrophic denitrification in a modified bioreactor enhanced by activated carbon, *Environ. Res.*, 2023, **232**, 116341.
7 S. Yin, Y. Wang, C. Hou, J. Wang, J. Xu, X. Jiang, D. Chen, Y. Mu and J. Shen, Deciphering the key role of biofilm and mechanisms in high-strength nitrogen removal within the anammox coupled partial S0-driven autotrophic denitrification system, *Bioresour. Technol.*, 2025, **419**, 132020.
8 S. Liu, F. Yang, Z. Gong, F. Meng, H. Chen, Y. Xue and K. Furukawa, Application of anaerobic ammonium-oxidizing consortium to achieve completely autotrophic ammonium and sulfate removal, *Bioresour. Technol.*, 2008, **99**, 6817–6825.
9 Z. Yang, S. Zhou and Y. Sun, Start-up of simultaneous removal of ammonium and sulfate from an anaerobic ammonium oxidation (anammox) process in an anaerobic up-flow bioreactor, *J. Hazard. Mater.*, 2009, **169**, 113–118.
10 E. Rikmann, I. Zekker, M. Tomingas, T. Tenno, L. Loorits, P. Vabamäe, A. Mandel, M. Raudkivi, L. Daija, K. Kroon and T. Tenno, Sulfate-reducing anammox for sulfate and nitrogen containing wastewaters, *Desalin. Water Treat.*, 2016, **57**, 3132–3141.
11 D. Grubba, J. Majtacz and J. Mąkinia, Sulfate reducing ammonium oxidation (SULFAMMOX) process under anaerobic conditions, *Environ. Technol. Innovation*, 2021, **22**, 101416.
12 R. Madani, J. Liang, L. Cui, D. Zhang, T. Otitoju, R. Elsalahi and X. Song, Novel simultaneous anaerobic ammonium and sulfate removal process: A review, *Environ. Technol. Innovation*, 2021, **23**, 101661.
13 L.-Y. Liu, G.-J. Xie, D.-F. Xing, B.-F. Liu, J. Ding, G.-L. Cao and N.-Q. Ren, Sulfate dependent ammonium oxidation: A microbial process linked nitrogen with sulfur cycle and potential application, *Environ. Res.*, 2021, **192**, 110282.
14 T. Wu, J. Ding, L. Zhong, H.-J. Sun, J.-W. Pang, L. Zhao, S.-W. Bai, N.-Q. Ren and S.-S. Yang, Sulfate-reducing ammonium oxidation: A promising novel process for nitrogen and sulfur removal, *Sci. Total Environ.*, 2023, **893**, 164997.
15 Y. Zhu, S. Yang, W. Wang, L. Meng and J. Guo, Applications of Sponge Iron and Effects of Organic Carbon Source on Sulfate-Reducing Ammonium Oxidation Process, *Int. J. Environ. Res. Public Health*, 2022, **19**, 2283.
16 D. Wang, B. Liu, X. Ding, X. Sun, Z. Liang, S. Sheng and L. Du, Performance evaluation and microbial community analysis of the function and fate of ammonia in a sulfate-reducing EGSB reactor, *Appl. Microbiol. Biotechnol.*, 2017, **101**, 7729–7739.
17 Y. Qin, Q. Wei, Y. Zhang, H. Li, Y. Jiang and J. Zheng, Nitrogen removal from ammonium- and sulfate-rich wastewater in an upflow anaerobic sludge bed reactor: performance and microbial community structure, *Ecotoxicology*, 2021, **30**, 1719–1730.
18 R. Kleerebezem and M. C. M. van Loosdrecht, A generalized method for thermodynamic state analysis of environmental systems, *Crit. Rev. Environ. Sci. Technol.*, 2010, **40**, 1–54.
19 M. Strous, E. Pelletier, S. Mangenot, T. Rattei, A. Lehner, M. W. Taylor, M. Horn, H. Daims, D. Bartol-Mavel, P. Wincker, V. Barbe, N. Fonknechten, D. Vallenet, B. Segurens, C. Schenowitz-Truong, C. Médigue, A. Collingro, B. Snel, B. E. Dutilh, H. J. M. Op Den Camp, C. Van Der Drift, I. Cirpus, K. T. Van De Pas-Schoonen, H. R. Harhangi, L. Van Niftrik, M. Schmid, J. Keltjens, J. Van De Vossenberg, B. Kartal, H. Meier, D. Frishman, M. A. Huynen, H.-W. Mewes, J. Weissenbach, M. S. M. Jetten, M. Wagner and D. Le Paslier, Deciphering the evolution and metabolism of an anammox bacterium from a community genome, *Nature*, 2006, **440**, 790–794.
20 M. Jia, C. Castro-Barros, M. Winkler and E. I. P. Volcke, Effect of organic matter on the performance and N_2O emission of a granular sludge anammox reactor, *Environ. Sci.: Water Res. Technol.*, 2018, **4**, 1035–1046.
21 R. Haug and P. McCarty, Nitrification with Submerged Filters, *J. - Water Pollut. Control Fed.*, 1972, **44**, 2086–2102.
22 D. R. Shaw, M. Ali, K. P. Katuri, J. A. Gralnick, J. Reimann, R. Mesman, L. Van Niftrik, M. S. M. Jetten and P. E. Saikaly, Extracellular electron transfer-dependent anaerobic oxidation of ammonium by anammox bacteria, *Nat. Commun.*, 2020, **11**, 2058.
23 L.-Y. Liu, X. Wang, C. Dang, Z. Zhao, D. Xing, B. Liu, N.-Q. Ren and G. Xie, Anaerobic ammonium oxidation coupled with sulfate reduction links nitrogen with sulfur cycle, *Bioresour. Technol.*, 2024, **403**, 130903.
24 Z. Zhang, C. Zhang, Y. Yang, Z. Zhang, K. Guo, X. Zhang, Z. Qin, J. Huang and Y. Li, Roles of nitrite in facilitating nitrogen and sulfur conversion in the hybrid bioreactor of sulfate-reduced ammonium oxidation and anaerobic ammonium oxidation, *Bioresour. Technol.*, 2025, **419**, 132085.
25 J. Cai, J. Jiang and P. Zheng, Isolation and identification of bacteria responsible for simultaneous anaerobic ammonium and sulfate removal, *Sci. China:Chem.*, 2010, **53**, 645–650.
26 R. Madani, J. Liang, L. Cui, R. H. Elsalahi, T. Otitoju, D. Zhang, X. Song, Y. Ma and S. Liu, Novel Simultaneous Removal of Ammonium and Sulfate by Isolated Bacillus cereus Strain from Sewage Treatment Plant, *Water, Air, Soil Pollut.*, 2022, **233**, 185.

27 D. Derwis, J. Majtacz, P. Kowal, H. E. Al-Hazmi, J. Zhai, S. Ciesielski, G. Piechota and J. Mąkinia, Integration of the sulfate reduction and anammox processes for enhancing sustainable nitrogen removal in granular sludge reactors, *Bioresour. Technol.*, 2023, **383**, 129264.

28 I. Wimalaweera, F. Zuo, Q. Tang, Q. Sui, S. Jinadasa, S. Weragoda, T. Ritigala, R. Weerasooriya, Y. Wang, H. Zhong, M. Makehelwala and Y. Wei, Synchronised removal of nitrogen and sulphate from rubber industrial wastewater by coupling of Sulfammox and sulphide-driven autotrophic denitrification in anaerobic membrane bioreactor, *Bioresour. Technol.*, 2025, **416**, 131785.

29 Z. Bi, D. Wanyan, X. Li and Y. Huang, Biological conversion pathways of sulfate reduction ammonium oxidation in anammox consortia, *Front. Environ. Sci. Eng.*, 2020, **14**, 38.

30 B. Ni and Z. Yuan, Recent advances in mathematical modeling of nitrous oxides emissions from wastewater treatment processes, *Water Res.*, 2015, **87**, 336–346.

31 F. Zhang, S. Ren, H. Liang, Z. Wang, Y. Yan, J. Wang and Y. Peng, Efficient nitrogen removal and partial elemental sulfur recovery by combined nitrite-Anammox and sulfate-Anammox: A novel strategy for treating mature landfill leachate, *J. Cleaner Prod.*, 2023, **399**, 136553.

32 W. Lin, J. Feng, K. Hu, B. Qu, S. Song, K. He, C. Liu, Y. Chen and Y. Hu, Sulfidation forwarding high-strength Anammox process using nitrate as electron acceptor via thiosulfate-driven nitrate denitratation, *Bioresour. Technol.*, 2022, **344**, 126335.

33 Y. Liu, L. Peng, H. H. Ngo, W. Guo, D. Wang, Y. Pan, J. Sun and B. Ni, Evaluation of nitrous oxide emission from sulfide- and sulfur-based autotrophic denitrification processes, *Environ. Sci. Technol.*, 2016, **50**, 9407–9415.

34 W. Yang, Q. Zhao, H. Lu, Z. Ding, L. Meng and G.-H. Chen, Sulfide-driven autotrophic denitrification significantly reduces N_2O emissions, *Water Res.*, 2016, **90**, 176–184.

35 Y.-F. Deng, D. Wu, H. Huang, Y.-X. Cui, M. C. M. van Loosdrecht and G.-H. Chen, Exploration and verification of the feasibility of sulfide-driven partial denitrification coupled with anammox for wastewater treatment, *Water Res.*, 2021, **193**, 116905.

36 P. Prachakittikul, C. Wantawin, P. Lek Noophan and N. Boonapatcharoen, ANAMMOX-like performances for nitrogen removal from ammonium-sulfate-rich wastewater in an anaerobic sequencing batch reactor, *J. Environ. Sci. Health, Part A: Toxic/Hazard. Subst. Environ. Eng.*, 2016, **51**, 220–228.

37 P. C. Sabumon, Development of a novel process for anoxic ammonia removal with sulphidogenesis, *Process Biochem.*, 2008, **43**, 984–991.

38 L. Wu, Z. Yan, J. Li, S. Huang, Z. Li, M. Shen and Y. Peng, Low temperature advanced nitrogen and sulfate removal from landfill leachate by nitrite-anammox and sulfate-anammox, *Environ. Pollut.*, 2020, **259**, 113763.

39 D. Zhang, L. Cui, H. Wang and J. Liang, Study of sulfate-reducing ammonium oxidation process and its microbial community composition, *Water Sci. Technol.*, 2019, **79**, 137–144.

40 L. Zhang, P. Zheng, Y. He and R. Jin, Performance of sulfate-dependent anaerobic ammonium oxidation, *Sci. China, Ser. B:Chem.*, 2009, **52**, 86–92.

41 Q.-L. Zhao, W. Li and S.-J. You, Simultaneous removal of ammonium-nitrogen and sulphate from wastewaters with an anaerobic attached-growth bioreactor, *Water Sci. Technol.*, 2006, **54**, 27–35.

