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Effects of sulfide on the integration of denitrification with anaerobic digestion

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The effects of sulfide on the integration of denitrification with anaerobic digestion using anaerobic effluents of cassava stillage as carbon source were investigated. Batch tests indicated that nitrate reduction efficiencies decreased from 96.5% to 15.8% as sulfide/nitrate (S/NO_3^-–N) ratios increased from 0.27 to 1.60. At low S/NO_3^-–N ratios (0.27–1.08) anaerobic acidogenesis was accelerated. Nitrate was reduced to nitrite via sulfur-based autotrophic denitrification, after which the formed nitrite and residual nitrate were converted to N_2 via heterotrophic denitrification. Increases in the S/NO_3^-–N ratio (1.60) caused a shift (76.3%) in the nitrate reduction pathway from denitrification to dissimilatory nitrate reduction to ammonia (DNRA). Sulfide concentrations (S/NO_3^-–N ratio of 1.60) suppressed not only heterotrophic denitrification but also acidogenesis. The potentially toxic effect of sulfide on acid production was mitigated by its rapid oxidation to sulfur, allowing the recovery of acidogenesis.

[**Key words:** Anaerobic acidogenesis; S/NO_3^-–N ratio; Denitrification; Dissimilatory nitrate reduction to ammonia; Electron-flow]

Industrial wastewaters typically contain high levels of nitrogen and carbon compounds that must be treated before they can be discharged safely. Studies of anaerobic denitrification have shown that it is a cost effective, environmentally friendly process to achieve simultaneous denitrification and methanogenesis in a single bioreactor. Moreover, a wide variety of substrates can be treated, ranging from synthetic high-strength organic wastewater, such as glucose, methanol, and peptone, to industrial wastewaters, including those from fish canneries and breweries (1–4).

The nature of the carbon source is a key factor determining both the nitrate reduction pathway and the carbon utilization pattern (5–6). For example, propionate and butyrate rather than acetate are preferably utilized by denitrifiers (6–7). Akunna et al. (5) found that when volatile fatty acids (VFAs) were the electron donors, the preferred nitrate reduction pathway was denitrification; but in the presence of glycerol or glucose, the dissimilatory nitrate reduction to ammonia (DNRA) pathway predominated. In addition, the utilization of various electron donors, i.e., dextrin/peptone, propionate, acetate, or H_2/CO_2, by nitrate reducers at an initial COD/NO_3^-–N ratio of 10 differentially impacts methanogenesis, ranging from its complete inhibition to its full recovery (8). Thus, nitrate utilization patterns and methanogenesis activity are strongly dependent on the nature of the electron species, which in turn results in differences in chemical oxygen demand (COD) and total nitrogen (TN) removal efficiencies.

Besides organic carbon, other inorganic substrates in industrial wastewater, such as sulfide and ferrous iron, can serve as potential electron donors in reactions by nitrate reducers. In the study of Tugtas and Pavlostathis (9), denitrification was the predominant pathway in sulfide-amended methanogenic cultures in which dextrin/peptone was the carbon source. Autotrophic denitrification was also demonstrated in a sulfide-rich anaerobic digester by the oxidation of sulfide (10), but in other studies sulfide inhibited heterotrophic denitrifiers, which resulted in a shift in the nitrate reduction pathway to DNRA (11–13). Autotrophic denitrifiers, heterotrophic denitrifiers, and anaerobic digestion bacteria, when present within the same reactor, may compete significantly with each other for nitrate as the electron acceptor. In addition, the interaction of nitrate reduction with sulfide oxidation and the subsequent effects on nitrate utilization patterns, carbon acidogenesis, and methanogenesis might alter the performance of anaerobic processes. Hence, it remains to be experimentally determined whether, in the presence of sulfide, denitrification can be successfully integrated with anaerobic digestion in the treatment of industrial wastewaters. Indeed, the effect of sulfide on anaerobic acidogenesis is poorly understood and similar examinations of nitrate utilization patterns have produced inconsistent results.

Therefore, in this work, first-stage anaerobic effluents of cassava stillage (CS) were used as substrates in batch assays aimed at evaluating the synergistic effect of organic carbon sources and sulfide, as electron donors, on nitrate reduction and anaerobic acidogenesis at different S/NO_3^-–N ratios. The predominant nitrate reduction pathway as a function of carbon and sulfide was determined. Finally, we propose a general reductive pathway for electron flow in an anaerobic system containing carbon, sulfide, and nitrate.

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TABLE 1. Characteristics of the anaerobic cassava stillage effluent collected from a cassava ethanol plant in Jiangsu Province, China.

Parameter	Average value
Total chemical oxygen demand (mg/L)	2500
Soluble chemical oxygen demand (mg/L)	1500
Volatile fatty acids (mg/L)	360
Soluble total nitrogen (mg/L)	585
NH_4^+–N (mg/L)	235
pH	7.5

MATERIALS AND METHODS

Inoculum and substrate Excess sludge (total suspended solids: 4–5 g/L) obtained from the secondary sedimentation tank of the Quyang sewage treatment plant (Shanghai, China) was used as the inoculum. The substrate, anaerobic CS effluent, was obtained directly from the full-scale continuous stirred tank reactor used in the first-stage anaerobic digestion process at the Taicang cassava ethanol plant (Jiangsu, China). Table 1 summarizes the characteristics of the substrate. After their collection, the anaerobic CS effluents were stored in a refrigerator at 4 °C until needed.

Batch assays To understand the effect of sulfide on nitrate reduction, three batch assays were conducted that differed in their nitrate and sulfide concentrations, as shown in Table 2. The reactors consisted of 250-mL serum bottles containing 150 mL of anaerobic CS effluent supplemented with nitrate (250 ± 20 mg NO_3^-–N/L) and different initial concentration of sulfide (0–428.3 mg S/L) from which precipitation loss with residual metals in the suspension was deducted. The fed serum bottles were sealed with a butyl-rubber stopper and the headspace air was displaced by helium gas to maintain anaerobic conditions. A 50-mL inoculum was then added to each serum bottle, followed by shake-incubation at 120 rpm and 37 °C. The batch test was performed in duplicate.

Analytical methods Liquid samples for sulfide analysis were collected periodically using syringes and filtered immediately through 0.45-μm filters to minimize oxidation loss. Liquid samples for the analysis of other parameters were treated with 0.2 mL of 2 M zinc chloride to precipitate the remaining sulfide and then centrifuged at 11,000×*g* for 10 min (Multifuge X1R, Thermo Scientific, USA). The resulting supernatants were filtered through 0.22-μm filters and the filtrates were stored in a refrigerator until their analysis.

Sulfide analysis was conducted using the methylene blue method (14). Briefly, the samples were diluted with 6.9 mL of water and reacted with 2 mL of zinc acetate, 1 mL of *N,N*-dimethyl-*p*-phenylenediamine dihydrochloride, and 0.1 mL of ammonium ferric alum [$Fe(NH_4)(SO_4)_2 \cdot 12H_2O$]. Absorbance was then measured at 665 nm. Soluble chemical oxygen demand (SCOD) was determined by placing 2 mL of COD reagent into a series of vials (Hach, Loveland, CO, USA). The vials were heated in a COD reactor (Hach DRB200) for 120 min after which the absorbance was measured using a spectrophotometer (Hach DR3900). Ammonium, nitrate, nitrite, and sulfate ion concentrations were determined as described by Xie et al, (6) and using Dionex ICS-3000 ion chromatography (Dionex, Sunnyvale, CA, USA). The concentration of soluble TN was determined by a total organic carbon (TOC)/TN analyzer (Shimadzu TOC-L CPN CN200). The suspended solids concentrations were determined by standard methods (15). Each experiment was conducted in duplicate under identical conditions. The maximum relative error of the measurements was <5%.

RESULTS

Sulfur and nitrogen transformations in the integrated system at different S/NO_3^-–N ratios Batch tests with additions of sulfide and nitrate into the anaerobic reactors were conducted for 70 h. A blank reactor without sulfide and nitrate additions was also included for comparison. The sulfur and nitrogen profiles as a function of the reaction time are shown in Fig. 1. Under the tested conditions, sulfide could be completely removed within 10 h at S/NO_3^-–N ratios ranging from 0.27 to 0.72 (Fig. 1A). With further increases in the S/NO_3^-–N ratio, the sulfide removal efficiency decreased; for example, only 39.6% of the sulfide was removed when the S/NO_3^-–N ratio was 1.60. Sulfate concentrations remained low (10–20 mg/L) when sulfide was added during the experiment (Fig. 1B) and were slightly higher than the value of the blank reactor (5 mg/L). The numerous straw-yellow, granular, filamentous insoluble solids in the liquid phase of the reactors were confirmed to be elemental sulfur (S^0) that had formed in the system.

Fig. 1C and D shows the changes in nitrate and nitrite concentrations as a function of reaction time. Nitrate removal was strongly related to the sulfide level in the system. In the blank reactor without sulfide addition, the nitrate removal efficiency was almost 98.1% at 26 h, without a lag time. When a small amount of sulfide (68.5 mg S/L) was added, corresponding to an S/NO_3^-–N ratio of 0.27, nitrate removal occurred mostly after a lag phase of 10 h, during which time almost all the sulfide was removed (Fig. 1A). Under these conditions, nitrate removal occurred within 33 h, with a removal efficiency of 96.5%. Further increases in the sulfide content (to 288.3 mg S/L), corresponding to an S/NO_3^-–N ratio of 1.08, severely suppressed the nitrate removal efficiency. As shown in Fig. 1D, the nitrate reduction intermediate nitrite failed to reach concentrations above 25 mg/L during a reaction time of 10–20 h, and it was rapidly consumed within 26 h. The ammonia concentration increased over the course of the reaction in all tests and with similar trends, probably reflecting anaerobic degradation (Fig. 1E). The consistent decrease insoluble TN during nitrate removal at S/NO_3^-–N ratios below 1.08 suggested that denitrification was the primary nitrate reduction pathway in the reactors (Fig. 1F).

Effect of S/NO_3^-–N ratios on anaerobic acidogenesis As shown in Fig. 2A, the pH in the anaerobic reactor increased from 7.5 to 8.0 after the addition of nitrate but without sulfide addition. When sulfide was added, there was a further increase in the pH of up to 8.5. The addition of nitrate alone resulted in the immediate utilization of SCOD, whereas sulfide addition enhanced the SCOD concentration dramatically over the reaction time, although part of it was consumed during nitrate reduction (Fig. 2b). The increased SCOD was attributable to the increase in pH, which resulted from the generation of hydroxyl ion during sulfide hydrolysis. Thus, under alkaline conditions, sulfide stimulated the hydrolysis and degradation of organics.

The observed effect of the influent S/NO_3^-–N ratios on SCOD was indicative of the production and consumption of VFAs, as shown in Fig. 3. Acetic and propionic acids along with low levels of butyric and valeric acids were detected in all tests. In the blank reactor, without sulfide and nitrate, total VFAs generally increased from 200 to 300 mg COD/L. VFAs were rapidly consumed within 10 h after the addition of nitrate whereas their consumption was retarded by the addition of a low concentration of sulfide (initial S/NO_3^-–N = 0.27). At higher initial S/NO_3^-–N ratios (0.72 and 1.08), there was very little consumption such that VFAs accumulated. It was also observed that VFA production generally increased with increasing S/NO_3^-–N ratios.

At the highest S/NO_3^-–N of 1.60, anaerobic acidogenesis activity was initially suppressed during the first 20 h. Only after

TABLE 2. Treatments used in the batch assays.

	Blank	0	1	2	3	4
S^{2-} (mgS/L)[a]	0	0	68.5 ± 2.3	186.8 ± 10.1	288.3 ± 21.5	428.3 ± 23.0
NO_3^-–N (mgN/L)[a]	0	263.7 ± 15.0	250.1 ± 11.9	261.2 ± 13.8	266.9 ± 18.9	267.0 ± 14.3
S/NO_3^-–N	–	0	0.27	0.72	1.08	1.60
COD/NO_3^-–N	–	9.48	10.00	9.57	9.37	9.36
COD/S	–	–	37.04	13.29	8.68	5.85

[a] Mean ± SD.