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Research Article

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Competition of autotrophic and heterotrophic denitrification in anaerobic biofilm wastewater treatment process

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ABSTRACT

The desulfurization-denitrification biofilm reactor with an anaerobic sulfate reduction bioreactor as pretreatment was operated for 370 days. And autotrophic and heterotrophic denitrification was found to be coexistence in this process. In anaerobic sulfate reduction reactor (AR), the influent sulfate was increased from 900 mgSO_4^{2-}/l to 2100 mgSO_4^{2-}/l and the sulfide generating rate was high at 97.5%. In autotrophic and heterotrophic (mixotrophic) desulfurization-denitrification reactor (MR), HRT was decreased from 6.63h to 3.31h. The sulfide was removed of 100% and predominantly oxidized to sulfur. TOC was further removed of 86.6% and the level of heterotrophic denitrification happened in MR ranged from 35.7% to 59.9%. The final average removals of sulfate and organic carbon were 93.8% and 97.8% at volumetric loadings of 7200 mgSO_4^{2-}/(l·d) and 4800 mgC/(l·d), respectively. The sulfate was finally converted to sulfur which could be reused and would not cause secondary pollution. Furthermore, nitrate and nitrite added as electron acceptors were finally converted to nitrogen gas and the removals were both up to 100%.

Key words: Denitrification, Nitrite, Nitrate, Sulfide, Biofilm

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INTRODUCTION

The excess sulfate is bad for natural waters. It may bring death to aquatic animals and plants, and break the ecological balance. But with rapid development of world economy, much wastewater containing sulfate was discharged by pharmacy, papermaking, tannery and mining industries. In the past thirty years, the anaerobic reduction of sulfate to sulfide was a traditional microbial process for sulfate removal [1]. However, the generated hydrogen sulfide gas in this process would bring secondary pollution to the environment [2], inhibit the sulfate reduction bacteria (SRB) and increase the difficulty for the methane recovery [3]. Therefore two-phase anaerobic digestion process and some physical-chemical processes [4], such as air stripping, chemical precipitation and electrodialysis were explored for the sulfate removal. But these processes had some advantages: high investing and operating costs, repairing difficulty, generating much chemical sludge and non-thorough prevention of sulfurous compounds pollution [5].

In order to avoid these disadvantages, some bacterial species have been introduced. The sulfide can be oxidized under denitrifying conditions using chemoautotrophic bacteria [6]. It has also been reported that the sulfide can be removed by oxygen and nitrate under autotrophic conditions [7]. But the organic carbon remained in wastewater from sulfate reduction limits the application of autotrophic denitrification. In view of this, the mixotrophic desulfurization-denitrification process can be introduced to sulfate-laden organic wastewater treatment. And in wastewaters containing nitrogenous contaminants, nitrates and nitrites generated after nitrification can be circulated to the effluents of sulfate reduction process to serve as electron acceptors.

In this work, the anaerobic attached-growth biofilm reactors were set up to remove sulfate, sulfide, nitrate, nitrite and organic carbon (TOC) simultaneously. The autotrophic and heterotrophic denitrification coexisted in this bioprocess. In AR, the sulfate was predominantly reduced to sulfide. Little hydrogen sulfide gas was leaked to atmosphere. In MR, the sulfide was predominantly oxidized to sulfur which could be collected for reuse, and the organic carbon compounds were further removed. Meanwhile, nitrate and nitrite could be reduced to nitrogen gas instead of nitrous oxide which brought pollution to the atmosphere [8]. Few processes have been reported to remove sulfate, organic carbon, nitrate and nitrite simultaneously up to the present.

EXPERIMENTAL SECTION

Design of experiments: The AR and MR were both anaerobic attached-growth bioreactors in column shape illustrated in Fig. 1. The volumes of AR and MR were 5.1 l and 3.52 l, respectively. The temperatures in AR and MR were maintained at (35±0.2) ^{o}C and (30±0.2) ^{o}C, respectively. The sludge inoculated in AR was 1.4 l from a continuous stirred tank reactor treating sulfate-rich wastewater, giving the biomass concentration of 15.3 MLVSSg/l. The MR was inoculated with 1.5 l of the sludge collected from a secondary sediment tank used to treat municipal wastewater, giving the biomass concentration of 15.19 MLVSSg/l. In order to increase the biomass inside two bioreactors, sponge cubes (8 mm × 8 mm × 8 mm) were applied as attached-growth media. Before it was linked to AR, MR was operated solely and fed with artificial wastewater containing sulfide, TOC, nitrate and nitrite to acclimate the microorganisms for mixotrophic desulfurization- denitrification.

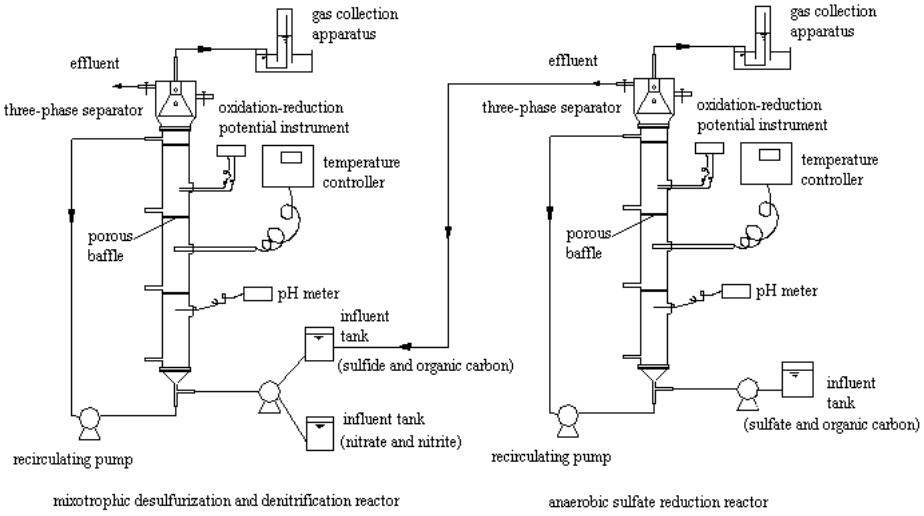

Fig. 1 Schematic diagrams of the anaerobic attached-growth bioreactors

Substrate: Artificial wastewater (solution (1)) containing sodium sulfate as sulfate source, glucose as organic carbon source, sodium bicarbonate as inorganic carbon source and potassium dihydrogen phosphate as phosphorus source for bacteria growth was used as the feed to AR. The solution (1) was diluted by tap water to supply other microelements nutrition for microorganisms. The pH was adjusted by using 1 mol/l sodium carbonate. The effluent of AR was fed to MR as influent, where the artificial wastewater (solution (2)) containing potassium nitrate and sodium nitrite was added as electron acceptors. The pH of solution (2) was adjusted to 6.5 by 1 mol/l hydrochloric acid.

Analytical methods: To measure sulfate, nitrate and nitrite, liquid samples were filtrated with a 0.45 μm filter and injected into an ion chromatography (DIONEX ICS 3000, USA) equipped with an inhibitory type conductivity detector and an Ionpac column (AG4A AS4A-SC, 4 mm). The sulfide was measured by the spectrophotometer (UV-2550, Japan). Nitrogen gas was analyzed by gas chromatography (Agilent 4890D, USA). Measurements for the concentrations of TOC and inorganic carbon (IC) were taken by the TOC analyzing instrument (TOC-VCPH，Japan). The images of microorganisms were taken by scanning electron microscope (HITACHI S-4700, Japan). All the items mentioned above were analyzed according to APHA. Two liquid samples for analyzing were taken from each bioreactor every two days. And each sample was analyzed for three times.

RESULTS AND DISCUSSION

Degradation of substrates and generation of sulfide in AR: The influent TOC and IC were 1200 mgC/l and 150 mgC/l, respectively, while the influent sulfate was increased from 900 mgSO_4^{2-}/l to 2100 mgSO_4^{2-}/l. The hydraulic

retention time (HRT) was maintained at 6 h. The average removal of sulfate was 93% during 231st d to 370th d (steady state).

The sulfate reduction in this research was different from the traditional sulfate reduction process, because it was the pretreatment of mixotrophic desulfurization-denitrification and sulfide was required to be remained in the bioreactor as much as possible under high sulfate removal conditions. The pH in the bioreactor was kept at around 7.9, because high pH was beneficial to remaining sulfide in wastewater. Therefore, the sulfide generating rate in this research was higher than the normal level[9]. When the sulfate was decreased from 2100 mgSO_4^{2-}/l to 1800 mgSO_4^{2-}/l, the sulfide generating rate was above 100%. This phenomenon was accounted for the sulfide accumulation in the bioreactor. The sulfide in effluent reached about 540 mgS^{2-}-S/l and the sulfide generating rate reached 97.5% during the steady state. The sulfide concentration of 540 mgS^{2-}-S/l was higher than the value reported by some other traditional sulfate reduction researches[10].

The porous sponge cubes as media in AR could provide a high specific surface area for microbial growth and also provided a shelter for bacteria that encountered sulfide toxicity. Furthermore, the media were beneficial to prevent sulfide release of the bioreactor as H_2S gas and increased the sulfide concentration. The microorganisms attached on the media were shown in Fig. 2. There were microorganisms composed of Bacilli-like bacteria, vibrio-like bacteria and cocci-like bacteria. And the Bacilli-like bacteria were predominant. It was probably interpreted that the concomitancy of different bacteria could endure different environmental conditions.

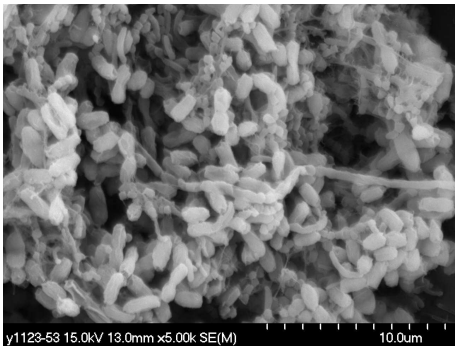

Fig.2 Scanning electron microscopic image of microorganisms attached on media in AR

Degradation of sulfide and generation of sulfur in MR: The effluent of AR (solution (3)) was fed to MR from 231st d and the abiotic oxidation of sulfide to sulfur by oxygen was about 10% when solution (3) was pumped to MR. Then 486 mgS^{2-}-S/l of sulfide was remained in solution (3). The nitrate and nitrite in solution (2) were 800 mgNO_3^--N/l and 800 mgNO_2^--N/l, respectively. The volumetric ratio of solution (3) to solution (2) was 4:1. The average concentrations of sulfide, sulfate, TOC, nitrate and nitrite fed to MR were 388.8 mgS^{2-}-S/l, 100.8 mgSO_4^{2-}/l, 144 mgC/l, 160 mgNO_3^--N/l and 160 mgNO_2^--N/l, respectively. All the concentrations were obtained by mixing solution (2) and solution (3).

As shown in Fig. 3, the sulfide removal was up to 100% for different HRT and 11.5% of sulfate was reduced to sulfide in MR during 271st d to 370th d at HRT of 3.31 h. Therefore the sulfide from sulfate reduction in MR was about 3.86 mgS^{2-}-S/l and 392.66 mgS^{2-}-S/l of sulfide was finally used as electron donors for sulfide-utilizing denitrification. The major biochemical conversions involved were given in Eq (1) to Eq (4).

$$5S^{2-} + 2NO_3^- + 12H^+ \rightarrow 5S + N_2 + 6H_2O \quad (1)$$

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

$$12NO_3^- + C_6H_{12}O_6 \rightarrow 12NO_2^- + 6H_2O + 6CO_2 \quad (3)$$

$$8NO_2^- + C_6H_{12}O_6 \rightarrow 4N_2 + 4H_2O + 4HCO_3^- + 2CO_3^{2-} \quad (4)$$

The stoichiometry of Eq. (1) and Eq. (2) indicated that sulfide of 392.66 mgS^{2-}-S/l could be converted to sulfur by nitrate and nitrite of (68.7-114.5) mgN/l. The average removals of nitrate and nitrite at HRT of 3.31 h were 99.4% and 99.5%, thus nitrate of 159mgN/l and nitrite of 159.2mgN/l were used for mixotrophic denitrification. As the nitrogen compounds were also needed for heterotrophic denitrifiction, although 318.2mgN/l was higher than the theoretical amount for oxidization of sulfide to sulfur, the oxidization of sulfide to elemental sulfur still predominated in the bioreactor, which was demonstrated by the low concentration of sulfate in MR.

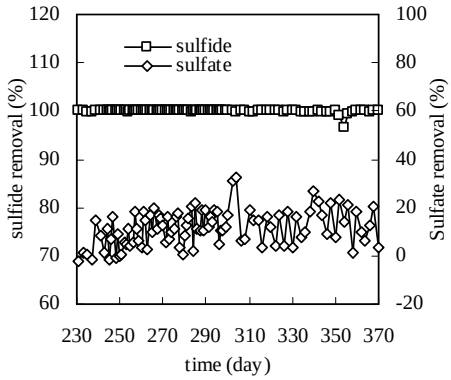

Fig.3 Removals of sulfide and sulfate as a function of time in MR

The main ingredient of biogas analyzed was nitrogen gas. There were not any H_2S and nitrous oxide (N_2O) in the biogas to pollute environment. The ORP was -430mV at sulfide loading of 2819.1 mgS^{2-}-S/(l·d).

Degradation of TOC in MR: The organic carbon compounds disappeared with sulfide, nitrite and nitrate from the bioreactor. Fig. 4 illustrated the TOC removal and variation of pH in MR. This simultaneous respiratory process could be explained in terms of the microbial diversity present in the bioreactor shown in Fig. 5, where it could be possible to find groups of microorganisms simultaneously carrying out the biological reduction of nitrate and nitrite using glucose and sulfide as electron donors. The autotrophic denitrification happened together with heterotrophic denitrification in MR. And the level of heterotrophic denitrification in the bioreactor could be indicated by TOC removal. TOC fed to MR was about 144 mgC/l and its average removal was 85.1% at HRT of 3.31 h. Then the corresponding amount of nitrate and nitrite was (113.5-190.6) mgN/l according to the stoichiometric reactions for the heterotrophic denitrification shown in Eq. (3) and Eq. (4). Because the total removed nitrogen (NO_3^-, NO_2^-) was 318.2 mgN/l, the level of heterotrophic denitrification was (35.7-59.9)%. This result indicated that the heterotrophic denitrification would predominate in the bioreactor if glucose was mostly consumed by nitrite, while the autotrophic denitrification would predominate in the bioreactor if glucose was mostly consumed by nitrate. The level of heterotrophic denitrification happened in MR depended on the categories of electron acceptors.

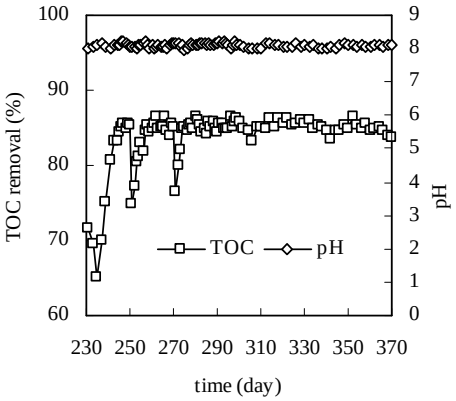

Fig.4 pH variation and TOC removal as a function of time in MR

The TOC removal decreased sharply at each start of HRT changing for the influent shock load and increased gradually to a steady value when the microorganisms in MR adapted to the environment. Furthermore, the effect of influent shock load on TOC removal decreased with HRT decreasing as shown in Fig. 4. TOC removal could reach 86.6% at TOC loading 1044.1mgC/(l·d). Fig. 5(a) and Fig. 5(b) showed the microorganisms attached on the surface and middle layer of media, respectively. The surface was scraggly with a great deal of micro-holes, which were the channels for microorganisms acquiring nourishment from outside and removing excretion from inside. There were filamentous bacteria, Bacilli-like bacteria, vibrio-like bacteria and cocci-like bacteria on the surface. It was generally thought that the filamentous bacteria could form a matrix suitable for the other non-filamentous bacteria to attach on. As shown in Fig. 5(b), the Bacilli-like bacteria were predominant. Different groups of bacteria were distributed on different layers of the biofilm. It was probably interpreted that the concomitancy of different bacteria could endure different environmental conditions and increased the rate of substrate transfer. Fig. 5(c) and Fig. 5(d) showed that the dominant

microorganisms in the inner layer of biofilm were long rod. The effluent pH was around 8.1 for hydrogen ion consumption during sulfur formation according to Eq. (1) and Eq. (2) as illustrated in Fig. 4.

During the whole anaerobic reduction and mixotrophic desulfurization denitrification process, the removals of sulfate and TOC were 93.8% and 97.8%, respectively.

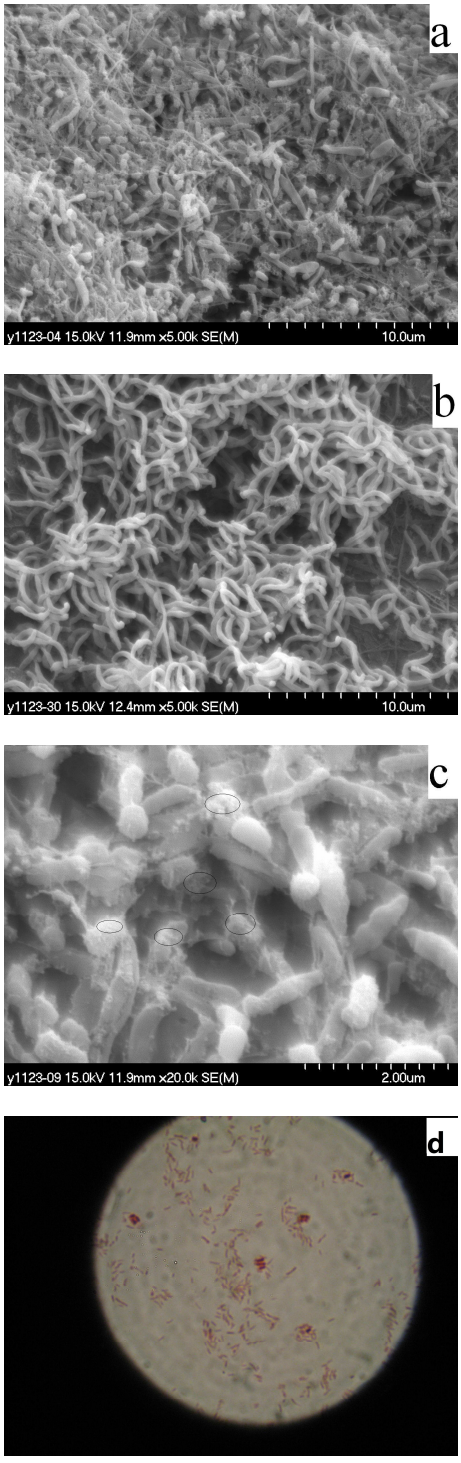

Fig.5 Scanning electron microscopic images and micrograph of microorganisms attached on media in MR

Degradation of nitrate and nitrite in MR: Fig. 6 illustrated the removals of nitrate and nitrite in MR. When HRT was maintained at 6.63 h, the nitrate removal was close to 100%, while nitrite removal increased gradually from 88.6% to 95.3%. The initial nitrite removal was not high for nitrite inhibition on the microorganisms. Nevertheless, the nitrite began to compete with nitrate for limited sulfide and TOC, when the microorganisms adapted the environment.

As shown in Eq. (1) and Eq. (2), 1mol of nitrate used (5/2) mol of sulfide while 1mol of nitrite used (3/2) mol of sulfide. The nitrate needed more sulfide than nitrite did. Thus the competitive power of nitrite was higher than that of nitrate for limited electron donors. And the nitrite removal increased to 99.9% while nitrate removal rate decreased to 99.1% at HRT of 4.14 h to 3.31 h. The nitrate and nitrite were almost removed and would not introduce new nitrogen pollution to the wastewater. [11] reported that nitrite can be generated during the sulfur-utilizing denitrification. But the opposite phenomenon that nitrite was removed instead of being generated appeared in this research.

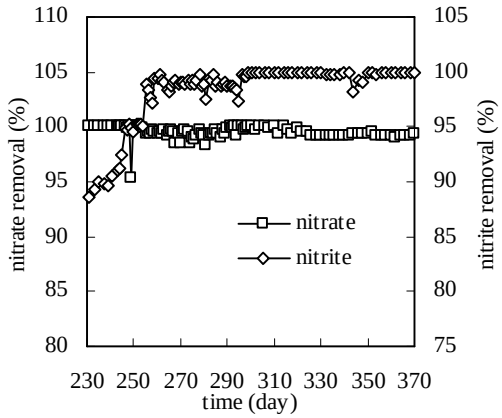

Fig.6 Removals of nitrate and nitrite as a function of time in MR

CONCLUSION

Based on the study of biofilm mixotrophic desulfurization - denitrification process, main conclusions could be drawn as follows.

(1) The sulfide generating rate in AR was 97.5%, which was higher than the normal level. The sulfide concentration of 540mgS^{2-}-S/l was close to the value that made the activity of methanogens decreased by 50% in AR. The sponge cubes as media in AR were beneficial to the high sulfide generating rate.
(2) Sulfide was removed up to 100% in MR when influent sulfide loading ranged from 1407.4mgS^{2-}-S/(l·d) to 2819.1mgS^{2-}-S/(l·d). Sulfide was predominantly oxidized to sulfur which could be reused, and sulfate of 11.5% was further reduced in MR at HRT of 3.31 h.
(3) The TOC removal could reach 86.6% at TOC loading of 1044.1mgC/(l·d). The autotrophic denitrification happened together with the heterotrophic denitrification whose level ranged from 35.7% to 59.9% in MR. The level of heterotrophic denitrification happened in the bioreactor depended on the categories of electron acceptors.

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