Simultaneous removal of ammonium-nitrogen and sulphate from wastewaters with an anaerobic attached-growth bioreactor

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Abstract Some industrial wastewaters may contain ammonium-nitrogen and/or sulphate, which need to be removed before their discharge into natural water bodies to eliminate their severe pollution. In this paper, simultaneous removal of ammonium-nitrogen and sulphate with an anaerobic attached-growth bioreactor of 3.8 L incubated with sulphate reducing bacteria (SRB) was investigated. Artificial wastewater containing sodium sulphate as electron acceptor, ammonium chlorine as electron donor and glucose as carbon source for bacteria growth was used as the feed for the bioreactor. The loading rates of ammonium-nitrogen, sulphate and COD were 2.08 gN/m^3·d, 2.38 gS/m^3·d, 104.17 gCOD/m^3·d, respectively, with a N/S ratio of 1:1.14. The results demonstrated that removal rates of ammonium-nitrogen, sulphate and COD could reach 43.35%, 58.74% and 91.34%, respectively. Meanwhile, sulphur production was observed in effluent as well as molecular nitrogen in biogas, whose amounts increased with time substantially, suggesting the occurrence of simultaneous removal of ammonium-nitrogen and sulphate. This novel reaction provided the possibility to eliminate ammonium-nitrogen and sulphate simultaneously with accomplishment of COD removal from wastewater, making wastewater treatment more economical and sustainable.

Keywords Ammonium-nitrogen; anaerobic attached-growth bioreactor; sulphate; sulphur

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Introduction

With the rapid economic development worldwide, a large quantity of domestic and industrial wastewaters discharge into natural water bodies daily, in which ammonium (NH_4^+) and sulphate (SO_4^{2-}) are frequently found. If these wastewaters are not treated effectively, ammonium-nitrogen and sulphate present in wastewaters will lead to serious contamination of the environment (Nguyen and Tanner, 1998; Jong and Parry, 2003). Such wastewater represents a tremendous challenge for biological treatment due to the inhibition of a high concentration of ammonium-nitrogen and sulphate to microorganism activities in bioprocesses (Ziomer and Shrout, 2000; Sawayama *et al.*, 2004). At present, the treatment processes for these kinds of wastewater mainly focus on the removal of ammonium-nitrogen and sulphate separately. The traditional method available for sulphate removal is to reduce sulphate into sulphide with the aid of sulphate reducing bacteria (SRB) under anaerobic conditions (Mizuno *et al.*, 1998; Pol *et al.*, 2001). However, this process has many disadvantages such as high operational cost, excessive sludge production, poor treating efficiencies etc., particularly the possibility of secondary contaminations to the atmosphere caused by hydrogen sulphide gas (H_2S). Concerns about treating wastewater containing high concentration of ammonium-nitrogen have been increasing, and large numbers of processes have been reported recently such as air stripping (Budzianowski and Koziol, 2005), chemical precipitation (Li *et al.*, 1999) and ANAMMOX (Fux *et al.*, 2004). Nevertheless, these methods also reveal some drawbacks, for example, high treating cost, small treating capacity or potential secondary pollution, which may possibly limit their practical applications, particularly in wastewater treatment

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plants. Therefore, efforts should be toward developing a renewable process that might be capable of removing ammonium-nitrogen and sulphate within wastewater simultaneously.

Fernando *et al.* (2001) once found that total Kjeldahl nitrogen (TKN) and sulphate of influent were removed in an anaerobic fluidised-bed reactor treating vinasse from an ethanol distillery of sugar beet molasses, and molecular nitrogen (N_2) was found in the biogas of the reactor. According to this accidental phenomenon, they predicted that a biochemical reaction shown in Eq. (1) perhaps happened, which could remove ammonium-nitrogen and sulphate simultaneously.

$$SO_4^{2-} + 2NH_4^+ \xrightarrow{\text{bacteria}} S + N_2 + 4H_2O \quad \Delta G^0 = -47.8\,\text{KJ/mol} \tag{1}$$

The objective of this paper is to investigate the potential of simultaneous removal of ammonium-nitrogen and sulphate under anaerobic conditions. To achieve this goal, an anaerobic attached-growth bioreactor has been designed to feed artificial wastewater containing a mixed substrate of ammonium chlorine and sodium sulphate. Simultaneous biodegradation of ammonium-nitrogen and sulphate has been monitored by changing operational conditions, including the level of COD, sulphate, ammonium as well as pH. Meanwhile, sulphur in effluent and nitrogen in biogas have been analysed respectively in order to verify the simultaneous removal of ammonium-nitrogen and sulphate from wastewater. The potential biochemical reaction pathway in such a system is also discussed.

Materials and methods

Anaerobic attached-growth bioreactor

As shown in Figure 1, the anaerobic attached-growth bioreactor in column shape used in this study had a total volume of 3.8 L, consisting of a suspended-growth zone (0.5 L), attached-growth zone (2.5 L), effluent zone (0.5 L) and gas collection zone (0.3 L). Constant temperature of 35 ± 1 °C inside the reactor was realized via a temperature sensor connected with temperature controller (MWZK-02, China) and heating threads bonded

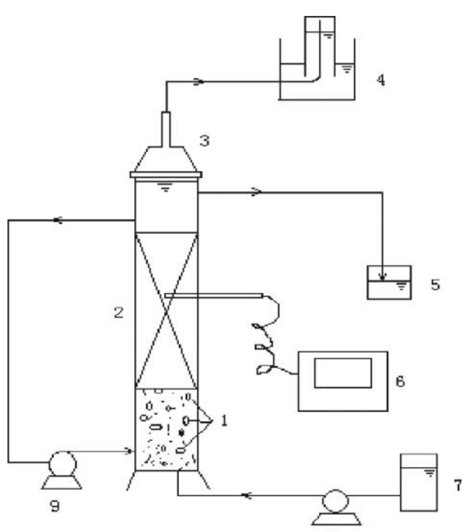

Figure 1 Schematic of anaerobic attached-growth bioreactor. 1 Suspended-growth zone; 2 Attached-growth zone; 3 Gas collection zone; 4 Gas collection apparatus; 5 Effluent zone; 6 Temperature controller; 7 Influent zone; 8 Influent pump; 9 Recirculating pump

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around the reactor. One peristaltic pump was used to batch-feed artificial wastewater from the bottom of the reactor into the system and the other peristaltic pump was used to recirculate sludge and water in the suspended-growth zone to achieve uniform mixing. The recycle rate was 250 ml/min. Biogas was collected by water proofing in solution with the addition of 0.05 mol/L sulphate acid to preserve H_2S from dissolving in water. In order to increase the biomass inside the reactor, sponge cubes (8 mm × 8 mm × 8 mm) made of polyurethane were applied as attached-growth media, which were washed with distilled water three times before use. The sponge cubes were light, fibrous, flexible, porous and corrosion-resistant, providing much space for bacteria to attach.

Substrate and inoculums

Artificial wastewater contained sodium sulphate (as electron acceptor) and ammonium chlorine (as electron donor), and glucose (as carbon source for bacteria growth) was used as the feed to the reactor. The ratio of nitrogen to sulphur was 1:1.14 and pH was in the range of 7.8 ∼ 8.5. The reactor was fed semi-continuously with 375 mL of substrate fed into the reactor every 3 days. The initial concentrations of ammonium chlorine, sodium sulphate and chemical oxygen demand (COD) were 50 mgN/L, 57 mgS/L and 2500 mg/L, respectively. Also potassium phosphate dibasic (K_2HPO_4, 0.1 mol/L) was fed into the reactor as nutrition for bacteria growth. Although a lot of kinds of wastewaters were treated at high volumetric loading rates, there were many successful examples of treating wastewater at low volumetric loading rates. Reginatto *et al.* (2005) cultivated bacteria to treat slaughterhouse wastewater at low ammonium nitrogen volumetric loading rates. Fernando *et al.* (2001) treated vinasse from an ethanol distillery of sugar beet molasses at low sulphate and COD volumetric loading rates. Also considering some other reasons such as high concentration of ammonium-nitrogen was toxic to anaerobic sludge inoculated, high concentration of glucose could be used as electron donor instead of ammonium by microorganisms, high concentration of sulphate could make SRB grow better rather than microorganisms responsible for simultaneous removal of ammonium-nitrogen and sulphate, low volumetric loading rates of sulphate, ammonium-nitrogen and COD were used here to cultivate bacteria in the reactor. On the basis of combined volume of suspended-growth and attached-growth zones, it could be calculated that the volumetric loading rate of ammonium-nitrogen, sulphate and COD were 2.08 gN/m^3·d, 2.38 gS/m^3·d and 104.17 gCOD/m^3·d, respectively. The bioreactor was inoculated with the anaerobic activated sludge collected from an anaerobic continuous stirred tank reactor (CSTR) used to treat wastewater enriched in sulphate. The concentration of sludge was 80.19 g/L and the volume of sludge inoculation was 0.75 L.

Analytical methods

To measure sulphate and nitrite (NO_2^-), liquid samples were filtrated with a 0.45 μm filter and injected into an ion chromatograph (DIONEX4500i, USA) equipped with an inhibitory type conductivity detector and an Ionpac column (AG4A AS4A-SC, 4 mm). The flow rate of carrier liquid was 1.0 mL/min. Another ion chromatograph (DIONEX2010i, USA) was used to measure ammonium, which worked at carrier gas flow rate of 1.5 mL/min equipped with an Ionpac column (HPIC CS-1 CG-1). N_2 and H_2S were analysed by gas chromatography (Agilent 4890D, USA) equipped with a thermal conductivity detector and a molecular screen column (5 Å). The temperatures of column, injector and detector were 60 °C, 100 °C and 100 °C respectively. Measurements for COD and MLVSS were taken according to the *Standard Methods* (APHA, 1995). The sulphur element was analysed by a method described by Wang (1998) using a spectrophotometer (UV-2550, Japan) and pH was measured by pH meter model (pHs-3C, China).

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Results and discussion

Biodegradation of ammonium-nitrogen and sulphate

After the anaerobic attached-growth bioreactor was operated for 156 days, sulphur and N_2 appeared in the effluent and biogas. The variation of ammonium-nitrogen and sulphate is shown in Figure 2. The removal of ammonium decreased while the removal of sulphate increased, when the pH of the influent was maintained at 7.9 during the first stage. From the 30th day, the removal of both ammonium and sulphate decreased with increase of pH in the influent. Changing pH to 8.3 at the 45th day resulted in a stable removal of ammonium and sulphate after slight fluctuation for a short time. Within the whole reaction course, pH in the reactor was maintained to be a stable value of 7.8. If only the simultaneous removal of ammonium and sulphate reaction occurred in the reactor, the developing trend of sulphate should be close to ammonium instead of the opposite trend, which is shown in Figure 2. During the initial period, traditional sulphate reduction dominated in the reactor, while simultaneous removal of ammonium and sulphate reaction reacted slightly. After adjusting pH up to 8.5, the sulphate reduction was inhibited and simultaneous removal of ammonium and sulphate was prosperous. Moderating pH to 8.3 led to the balance of two reactions, and removal of ammonium-nitrogen and sulphate trended to be stable. The sulphate reducing bacteria (SRB) and the microorganisms that were responsible for simultaneous removal of ammonium-nitrogen and sulphate were speculated to be competitively based on the phenomena observed in the reactor mentioned above.

From the 225th day, the loading rate of influent COD was changed from 104.17 gCOD/m^3·d to 33.33 gCOD/m^3·d while the other influent parameters were kept constant, the removal rates of ammonium-nitrogen and sulphate are shown in Figure 3. The removal rate of sulphate decreased and the removal rate of ammonium increased while influent COD decreased. Initially, sulphate reduction occurred in the reactor at the highest rate. When influent COD decreased, the activities of SRB were inhibited, resulting in an increase of ammonium removal, likely due to the enhancement of reaction between ammonium and sulphate. Thus, low concentration of COD was able to promote simultaneous removal of ammonium and sulphate reaction.

Production of sulphur and molecular nitrogen

It was observed that the colour of effluent changed from colour-less to yellow gradually in the anaerobic attached-growth bioreactor after operation for 156 days. The cumulative amounts of sulphur increased with time during 180 ~ 222 days as shown in Figure 4.

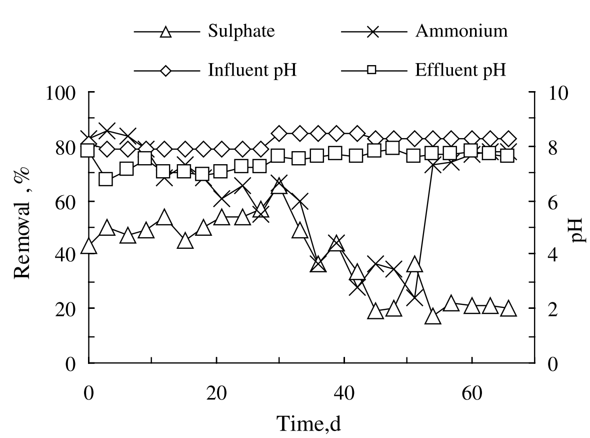

Figure 2 Removal rate of ammonium-nitrogen and sulphate

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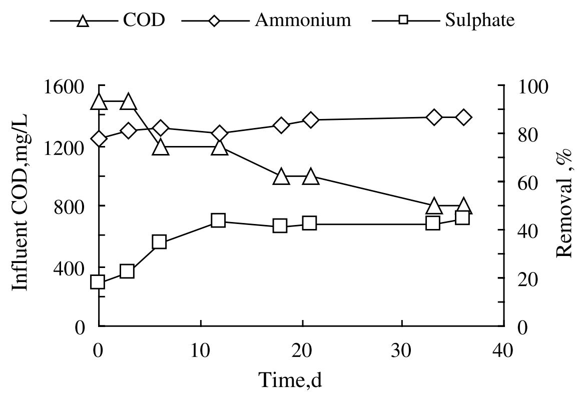

Figure 3 COD in influent and removal rate of ammonium-nitrogen and sulphate

The reactor was operated under sulphate loading rate of 2.38 gS/m^3·L, resulting in an average sulphate removal rate of 34.0%. As well, cumulative amounts of 0.9 mgS in the form of H_2S and 89.97 mgS in the form of elemental sulphur were found in the effluent respectively, indicating that 88.24% of the sulphate removed was converted into sulphur.

The quantity of N_2 was also analysed along with the detection of sulphur. Figure 5 revealed that the amounts of N_2 from gas collection apparatus also increased with time. When the ammonium loading rate was 2.08 gN/m^3·d, average ammonium removal rate of 55.4% and accumulative 73 mgN as N_2 were obtained. In terms of the analysis above, 50.28% of ammonium-nitrogen removed from the reactor was converted to N_2. This demonstrated that relative amount of sulphur in effluent and nitrogen in biogas was 1:1.23.

Effect of ammonium loading rate on simultaneous removal of ammonium and sulphate

In order to further investigate what concentration of ammonium was suitable for the reaction between ammonium-nitrogen and sulphate, the sludge of the anaerobic attached-growth reactor was inoculated to three different bottles in an anaerobic shaking bed after the reactor was operated for 192 days. The effective volume of each bottle was 400 mL and 100 mL of wastewater were fed into the bottles every 3 days. The concentration of sulphate, COD and pH of influent were the same in the three bottles. The loading rates of COD and sulphate were 583.33 g/m^3·d and 33.33 g/m^3·d respectively. Different concentrations of ammonium-nitrogen were used, which were 50 mgN/L, 250 mgN/L, 450 mgN/L respectively. And the corresponding volume loading rates were 4.17 gN/m^3·d,

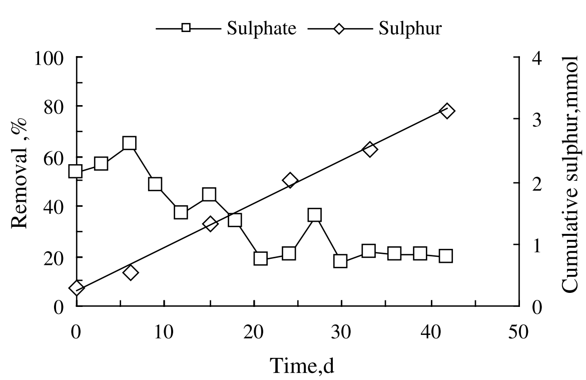

Figure 4 Variation of the amounts of sulphur in effluent

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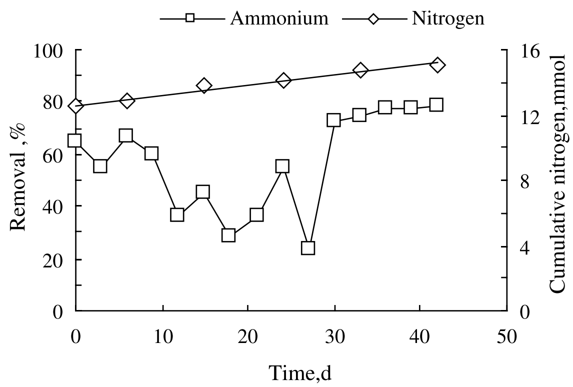

Figure 5 Variation of the amounts of nitrogen in biogas

20.8 gN/m^3·d and 37.5 gN/m^3·d. After the three bottles were operated for about one month, the removal rates of sulphate and ammonium as well as other parameters were analysed with results shown in Figure 6.

Figure 6(a) illustrated that removal rates of COD and sulphate were high, in contrast with ammonium removal of 1.024%, when influent ammonium loading rate was maintained at 4.17 gN/m^3·d. At this time, sulphate reduction dominated in the bottle while SRB was in high use of COD. The reaction, which could remove ammonium-nitrogen and sulphate simultaneously, did not proceed well. After the influent ammonium loading rate was increased to 20.8 gN/m^3·d, the rates of COD and sulphate declined. The removal rate of ammonium was still low as illustrated in Figure 6(b). Although SRB in the bottle was inhibited and the removal rate of sulphate decreased, the sulphate reduction still dominated and simultaneous removal of ammonium-nitrogen and sulphate reaction did not perform well till now. The removal rate of COD increased; also, the removal rate

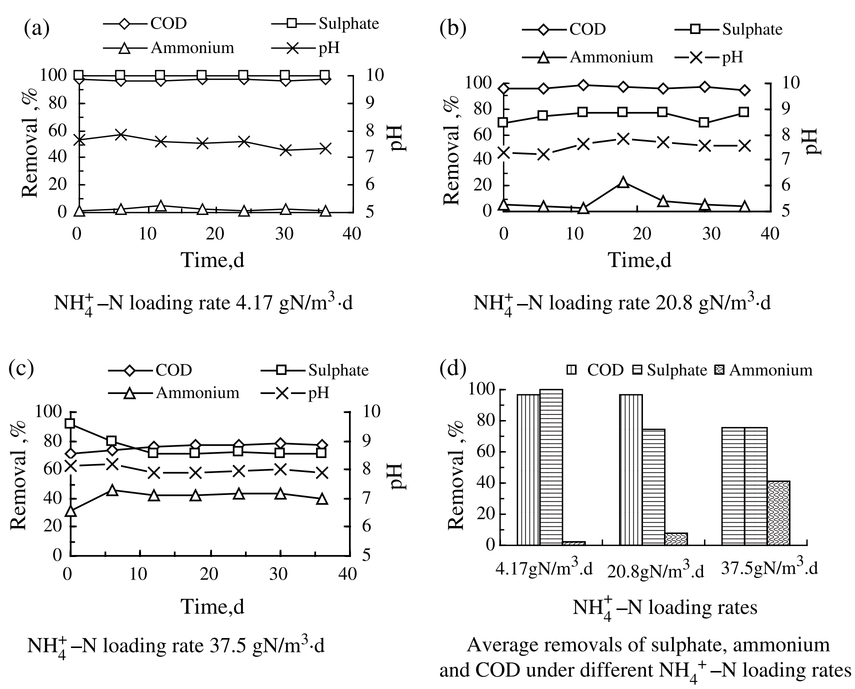

Figure 6 Removal of ammonium-nitrogen and sulphate with different ammonium volume loading rates

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of ammonium-nitrogen increased up to 46%. In Figure 6(c), we found the removal rate of sulphate was a little higher than that in Figure 6(b). The higher removal of ammonium indicated that simultaneous removal of ammonium-nitrogen and sulphate reaction was enhanced as well as sulphate reduction being inhibited. The sulphate being used as electron acceptor in simultaneous removal of ammonium-nitrogen and sulphate as well as the sulphate reduction resulted in a higher removal rate of sulphate. The relative amounts of influent nitrogen and sulphate-sulphur were 1:0.89, higher than the theoretical value of 1:1.14 according to the simultaneous removal of ammonium-nitrogen and sulphate reaction, when the influent ammonium loading rate was kept at 20.8 $gN/m^3 \cdot d$. This was likely due to the fact that although sulphate reduction was inhibited, the number of SRB was still great and microorganism accelerating simultaneous removal of ammonium-nitrogen and sulphate still had less chance to contact with ammonium, resulting in the inhibition of microorganism degradation activities, when the ratio of nitrogen and sulphur was maintained as 1:1.14. Increasing influent ammonium volume rate (Figure 6c) could strengthen the simultaneous removal of ammonium-nitrogen and sulphate reaction, which increased the odds of microorganisms contacting with ammonium.

Therefore, ammonium removal rate increased when influent ammonium loading rate was increased. Moreover, when the simultaneous removal of ammonium-nitrogen and sulphate reaction worked well, the methane production and sulphate reduction processes were inhibited. This phenomenon indicated that competition existed between methane-producing bacteria, SRB and microorganisms for removing sulphate and ammonium-nitrogen. The ammonium loading rate of 37.5 $gN/m^3 \cdot d$ was found to achieve better removal of ammonium-nitrogen and sulphate simultaneously, compared with the cases of 4.17 $gN/m^3 \cdot d$ and 20.8 $gN/m^3 \cdot d$. The comparison of different removal rates of sulphate, ammonium-nitrogen and COD under different ammonium loading rates is shown in Figure 6(d).

Discussion on the potential biochemical reaction pathways

Data and phenomena obtained from this experiment suggested that the conventional sulphate reduction reaction and ammonium oxidation reaction were not the only two reactions occurring in the reactor. It was known that under anaerobic conditions, N^{3-} within NH_4^+ was unable to accept excessive electrons, while S^{6+} in SO_4^{2-} could just accept electrons without any possibility to donate electrons. The NO_2^- found in the effluent indicated that NH_4^+ and SO_4^{2-} could react via equation (2), because in the influent there was only one kind of nitrogenous compound, which was ammonium. But equation (2) was not available for explaining the sulphur obtained in the effluent. Furthermore, in terms of this reaction, the amount ratio of S^{2-}-S and NO_2^--N obtained in effluent should be 1:0.58, but the ratio we got was 1:0.39 practically. The less nitrite produced meant a further reaction (equation 3) would took place following equation (2). The practical amount ratio of sulphur and N_2-N was 1:1.23, much lower in comparison with the theoretical value (1:0.29) according to equation (3), indicating that some NH_4^+ from the influent converted into N_2 (Equation 4). So the general biochemical reaction (equation 1) could happen thermodynamically which could be obtained by combining reactions involving nitrite formation and the ANAMMOX reaction.

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

$$S^{2-} + 2/3NO_2^- + 8/3H^+ \rightarrow S + 1/3N_2 + 4/3H_2O \quad (3)$$

$$2/3NO_2^- + 2/3NH_4^+ \rightarrow 2/3N_2 + 4/3H_2O \quad (4)$$

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In addition, it might be speculated that porous sponge padding in the reactor provided high concentration of substrate, sufficient porosity and surface for the growth of microorganisms, which could be propitious for simultaneous removal of ammonium and sulphate.

Conclusions

Anaerobic attached-growth bioreactor applying SRB as inoculums was used to examine simultaneous removal of ammonium-nitrogen and sulphate using artificial wastewater as substrate. The main conclusions could be drawn as follows. Firstly, production of sulphur, molecular nitrogen and nitrite was observed in this system; moreover, the amounts of sulphur and nitrogen increased with time, demonstrating that the reaction dominating the simultaneous removal of ammonium and sulphate substantially existed. Secondly, removal efficiency of COD, ammonium-nitrogen and sulphate reached 91.34%, 43.35%, 58.74% respectively, while more efficient removal of ammonium was obtained when COD decreased. Thirdly, removal of ammonium could reach the highest level when concentration of ammonium was 450 mgN/L (37.5 gN/m^3·d), compared with 50 mgN/L (4.17 gN/m^3·d) and 250 mgN/L(20.8 gN/m^3·d), however, leading to a decrease of removal of COD and sulphate. Fourthly, in such a system, conventional sulphate reduction coupled to simultaneous removal of ammonium-nitrogen and sulphate was likely responsible for eliminating ammonium-nitrogen and sulphate. Furthermore, low removal of ammonium was obtained with high removal of sulphate, suggesting the existence of competition between sulphate reduction bacteria (SRB) and microorganisms that were responsible for using sulphate and ammonium as substrate for metabolisms. Lastly, conditions of low COD, high sulphate and high ammonium at pH of 7.8 could be selected to promote simultaneous removal of ammonium-nitrogen and sulphate.

Further study on the microbial examination and continuous run of the reactor are in progress. Also the operational conditions of the reactor at higher volumetric loading rates of substrates are currently being researched.

Acknowledgements

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References

APHA (1995). *Standard Methods for the Examination of Water and Wastewater*. 19th edn. American Public Health Association, Washington DC, USA.

Budzianowski, W. and Koziol, A. (2005). Stripping of ammonia from aqueous solutions in the presence of carbon dioxide – effect of negative enhancement of mass transfer. *Chemical Engineering Research & Design*, **83**(A2), 196–204.

Dean, J.A., Shang, J.F., Cao, S.J. and Xin, W.M. (1991). *Lange's Handbook of Chemistry*. Science book company, Beijing, pp. 9(1)–9(67) (in Chinese).

Fernando, F.P., Maria, F.P., Neivy, F., Miguel, A.U., Pedro, A.G. and Santiago, V. (2001). New process for simultaneous removal of nitrogen and sulphur under anaerobic conditions. *Water Research*, **35**(4), 1111–1114.

Fux, C., Marchesi, V. and Brunner, I. (2004). Anaerobic ammonium oxidation of ammonium-rich waste streams in fixed-bed reactors. *Water Science and Technology*, **49**(11–12), 77–82.

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Jong, T. and Parry, D.L. (2003). Removal of sulphate and heavy metals by sulphate reducing bacteria in short-term bench scale upflow anaerobic packed bed reactor runs. *Water Research*, **37**, 3379–3389.

Li, X.Z., Zhao, Q.L. and Hao, X.D. (1999). Ammonium removal from landfill leachate by chemical precipitation. *Waste Management*, **19**, 409–415.

Mizuno, O., Takagi, H. and Noike, T. (1998). Biological sulphate removal in an acidogenic bioreactor with an ultrafiltration membrane system. *Water Science and Technology*, **38**(4–5), 513–520.

Nguyen, M.L. and Tanner, C.C. (1998). Ammonium removal from wastewaters using natural New Zealand zeolites. *New Zealand Journal of Agriculture Research*, **41**(3), 427–446.

Pol, L.W.H., Lens, P.N.L., Weijma, J. and Stams, A.J.M. (2001). New developments in reactor and process technology for sulphate reduction. *Water Science and Technology*, **44**(8), 67–76.

Reginatto, V., Teixeira, R.M., Pereira, F., Schmidell, W., Furigo, A., Menes, R., Etchebehere, C. and Soares, H.M. (2005). Anaerobic ammonium oxidation in a bioreactor treating slaughterhouse wastewater. *Brazilian Journal of Chemical Engineering*, **22**(4), 593–600.

Sawayama, S., Tada, C., Tsukahara, K. and Yagishita, T. (2004). Effect of ammonium addition on methanogenic community in a fluidized bed anaerobic digestion. *Journal of Bioscience and Bioengineering*, **97**(1), 65–70.

Wang, G.Z. (1998). Analysis on the amounts of sulphur in ores and soil by spectrophotometer. *Analysis on Metallurgy*, **18**(3), 52–53 (in Chinese).

Ziomer, D.H. and Shrout, J.D. (2000). High-sulphate, high-chemical oxygen demand wastewater treatment using aerated methanogenic fluidized beds. *Water Environment Research*, **72**(1), 90–97.

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