

Integration of sulphate reduction, autotrophic denitrification and nitrification to achieve low-cost excess sludge minimisation for Hong Kong sewage

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Abstract An integrated anaerobic–aerobic treatment system of sulphate-laden wastewater was proposed here to achieve low sludge production, low energy consumption and effective sulphide control. Before integrating the whole system, the feasibility of autotrophic denitrification utilising dissolved sulphide produced during anaerobic treatment of sulphate rich wastewater was studied here. An upflow anaerobic sludge blanket reactor was operated to treat sulphate-rich synthetic wastewater (TOC = 100 mg/L and sulphate = 500 mg/L) and its effluent with dissolved sulphide and external nitrate solution were fed into an anoxic biofilter. The anaerobic reactor was able to remove 77–85% of TOC at HRT of 3 h and produce 70–90 mg S/L sulphide in dissolved form for the subsequent denitrification. The performance of anoxic reactor was stable, and the anoxic reactor could remove 30 mg N/L nitrate at HRT of 2 h through autotrophic denitrification. Furthermore, sulphur balance for the anoxic filter showed that more than 90% of the removed sulphide was actually oxidised into sulphate, thereby there was no accumulation of sulphur particles in the filter bed. The net sludge productions were approximately 0.15 to 0.18 g VSS/g COD in the anaerobic reactor and 0.22 to 0.31 g VSS/g NO_3^- -N in the anoxic reactor. The findings in this study will be helpful in developing the integrated treatment system to achieve low-cost excess sludge minimisation.

Keywords Anaerobic treatment; autotrophic denitrification; excess sludge minimisation; sulphate reduction

Introduction

Treatment and disposal of excess sludge generated from secondary sewage treatment plants become an urgent issue in many densely populated urban areas such as Hong Kong due to the restricted land applications and limited landfill capacity. Sludge minimisation can provide a feasible solution to the sludge problem. Japan and the EU have formed special task forces to work on possible in-line treatment options to minimise excess sludge within a secondary sewage treatment plant, among which thermal, ultrasonic and ozone in-line treatment options have been studied extensively. In this regard, we have been conducting research studies on various sludge minimisation options for more than six years. Our two major research tasks that have been completed studied the options of “chemically stimulated energy uncoupling” and an oxic-settling-anaerobic (OSA) process (Chen *et al.*, 2000, 2003). However, these options, mainly realised through cell disintegration and lysis, are either expensive or require extra footprint.

Therefore, more cost-effective and space-saving approaches for sludge minimisation have become necessary. In this respect, application of anaerobic treatment shows great promise, which has been widely reported in the literature. It has long been known that the anaerobic treatment produces significantly less sludge and requires less energy than

aerobic processes (Foresti, 2002). However, nitrification cannot be achieved in the anaerobic reactor, thus another treatment phase for nitrification is essential. Therefore, application of such a combined scheme is required for organic and nitrogen removal, which has been extensively studied (Akunna *et al.*, 1994; Edriksen and Ahring, 1996; Del Pozo and Diez, 2003). In such a system, heterotrophic denitrification and methanogenesis are both attained in the anaerobic reactor. Overall sludge production in such a combined system is effected by the competition between denitrifiers and other anaerobes for substrates. As the sludge production for heterotrophic denitrification (0.4 g VSS/g COD) is much higher than that for anaerobic reactions (0.1 g VSS/g COD), diversion of the substrate from methanogenesis towards denitrification will increase the overall sludge production, which has been confirmed by Inamori *et al.* (1996). In addition, the methanogenesis is much slower than the denitrification, which requires a very long HRT in removing COD completely.

In order to further minimise the sludge production, we, therefore, propose a low-cost treatment scheme, as shown in Figure 1, for Hong Kong sewage containing around 500 mg/L with a COD-to-sulphate ratio of about 0.6 due to the seawater toilet flushing practice. With such a relatively high sulphate level, sulphate reducing bacteria (SRB) will out-compete methane-producing bacteria (MPB) for organic matters under anaerobic condition because of their higher specific growth rate, and low residual COD level can be achieved in the anaerobic reactor under a short HRT condition (Widdel, 1988). Thus, sulphate reduction will become predominant in the anaerobic reactor for COD removal, thereby producing a certain extent of sulphide. The sulphide produced, as an electron donor, is then utilised for autotrophic denitrification in a subsequent anoxic reactor, before wastewater entering nitrification in the aerobic reactor. In this case, sludge production would be further reduced, since yield coefficient of autotrophic denitrification is significantly lower than that of heterotrophic denitrification.

Autotrophic denitrification using various sulphur sources as an electron donor has been widely studied (Driscoll and Bisogni, 1978; Furumai *et al.*, 1996; Koenig and Liu, 1996; Klerbezem and Mendez, 2002), but only limited information is available on autotrophic denitrification directly utilising sulphide in an anaerobic effluent. Hence, the prime objectives of this study are (1) to examine the feasibility of autotrophic denitrification utilising dissolved sulphides produced during the anaerobic treatment of sulphate containing wastewater with an aim to develop an integrated treatment system with low sludge production; (2) to determine the net sludge production of the proposed system; and (3) to examine the fate of sulphur in this autotrophic denitrification as well as the sulphate reduction.

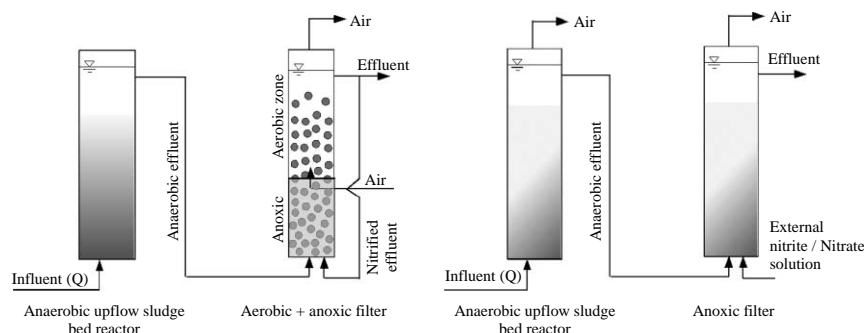


Figure 1 Schematic diagram of the proposed treatment system employing autotrophic denitrification (left-hand side) and the experimental setup (right-hand side)

Materials and methods

System configuration and operating conditions

In order to investigate the feasibility of autotrophic denitrification utilising dissolved sulphides, an anaerobic upflow sludge bed (UASB) reactor followed by an anoxic filter, as shown in Figure 1, was used in this stage before integrating the nitrification compartment. The UASB reactor was fabricated using a transparent acrylic cylinder of 100 mm internal diameter and 400 mm height. The anoxic filter (AF) having an internal diameter of 100 mm and a height of 285 mm was packed with polypropylene plastic media (size = 28–31 mm). Both reactors were inoculated initially with activated sludge obtained from a local sewage treatment plant and were continuously operated in a temperature-controlled chamber at $30 \pm 5^\circ\text{C}$. Mixing in both reactors was achieved through internal water recycling. After sufficient microbial growth was attained, the HRT was lowered in a stepwise manner. Finally, the UASB was optimised with the HRT of 3 h and the organic loading rate of 0.86 kg TOC/m^3 per day. The anoxic biofilter fed with anaerobic effluent and external nitrate solution, was then operated at the HRT of 2 h. Nitrate concentration of 30 mg N/L was continuously maintained in the anoxic influent, so nitrate loading rate of 0.36 kg N/m^3 per day was achieved. Throughout this experimental period, besides monitoring performance of the system, the fate of sulphur and sludge production were also determined.

Synthetic wastewater

Synthetic stock solution was prepared with the following composition: glucose, 19.57 g/L; sodium acetate, 26.1 g/L; yeast extract, 9.78 g/L; NH_4Cl , 4.16 mg/L; K_2HPO_4 , 1.92 g/L; KH_2PO_4 , 0.72 g/L; $\text{MgCl}_2 \cdot 6\text{H}_2\text{O}$, 8.32 g/L; CaCl_2 , 5.2 g/L, trace solution, 2.50 mL/L. The composition of trace mineral solution was: $\text{FeCl}_3 \cdot 6\text{H}_2\text{O}$, 15.0 g/L; H_3BO_3 , 1.5 g/L; CuSO_4 , 0.2 g/L; KI , 0.3 g/L; $\text{MnSO}_4 \cdot \text{H}_2\text{O}$, 1.0 g/L; $(\text{NH}_4)_6\text{Mo}_7\text{O}_{24} \cdot 4\text{H}_2\text{O}$, 0.4 g/L; $\text{ZnSO}_4 \cdot 7\text{H}_2\text{O}$, 1.2 g/L; $\text{CoCl}_2 \cdot 6\text{H}_2\text{O}$, 1.5 g/L; $\text{C}_6\text{H}_5\text{Na}_3\text{O}_7 \cdot 2\text{H}_2\text{O}$, 10.0 g/L. For simulating the characteristics of Hong Kong sewage in term of salinity and sulphate concentration, seawater with average sulphate concentration of 2700 mg/L was dosed into the synthetic stock solution. The actual feed solution was prepared every 2 days by diluting synthetic stock solution with tap water to adjust the TOC and sulphate concentration of about 100 and 500 mg/L (166 mg S/L), respectively, before injecting it into the reactor. Nitrate solution was prepared with sodium nitrate and stored at 4°C .

Sample analysis

During the experiments, influent and effluent for both reactors were collected on a regular basis. The total organic carbon (TOC) concentration in a sample was analysed with a TOC analyser (Shimadzu model TOC-700A). TOC instead of COD was adopted in this study because COD measurement can be interfered with by dissolved sulphide and chloride ions. A correlation between TOC and COD was established and the conversion factor of 2.65 was obtained, which was used to convert TOC to COD. The concentrations of nitrite, nitrate and sulphate were analysed by ion chromatograph (Dionex-100) equipped with a conductivity detector and IonPac AS9-HC analytical column. Dissolved sulphide concentration in a sample was measured using an iodometric method (APHA, 1998). The biomass concentration, pH and alkalinity were also measured according to the *Standard Methods* (APHA, 1998).

Results and discussion

Organic removal

In this study, synthetic wastewater with TOC of 100 mg/L (\sim COD of 265 mg/L) and sulphate concentration of 500 mg/L was operated at an organic loading of 0.86 kg/TOC/m³ per day for 35 days. The average organic removal efficiency in the anaerobic reactor was 77% at a HRT of 3 h. The effluent-dissolved sulphide concentration ranged from 70 to 90 mg S/L, which indicated the presence of dynamic sulphidogenic activity. With the intention of estimating the sulphidogenic activity in the anaerobic reactor, the ratio of the amount of COD removed (calculated from the TOC measurements) to the amount of sulphate reduced was calculated. The observed ratio was about 0.75 during this experimental period (Figure 2 and Figure 3), which was close to the theoretical COD/SO₄²⁻ ratio of 0.67. This showed the majority of the COD in the anaerobic reactor were removed by sulphate reducing bacteria. At the average influent TOC concentration of 50 mg/L, from days 25 to 35, no significant shift of COD/SO₄²⁻ ratio was observed, but there was lower dissolved sulphide production (\sim 35 mg S/L) due to the carbon limitation. However, the competitiveness between SRB and MPB in the long term should be further studied by closely monitoring the COD/SO₄²⁻ ratio in the anaerobic reactor, as this may affect the performance of the whole treatment system.

Dissolved sulphide in the anaerobic effluent

In the proposed treatment system, a sufficient amount of dissolved sulphides produced in the anaerobic reactor is critical for the subsequent autotrophic denitrification. With TOC concentration of 100 mg/L, 70–90 mg/L of dissolved sulphides could be produced and total dissolved sulphides accounted for 86% on average, but no less than 72%. Hence,

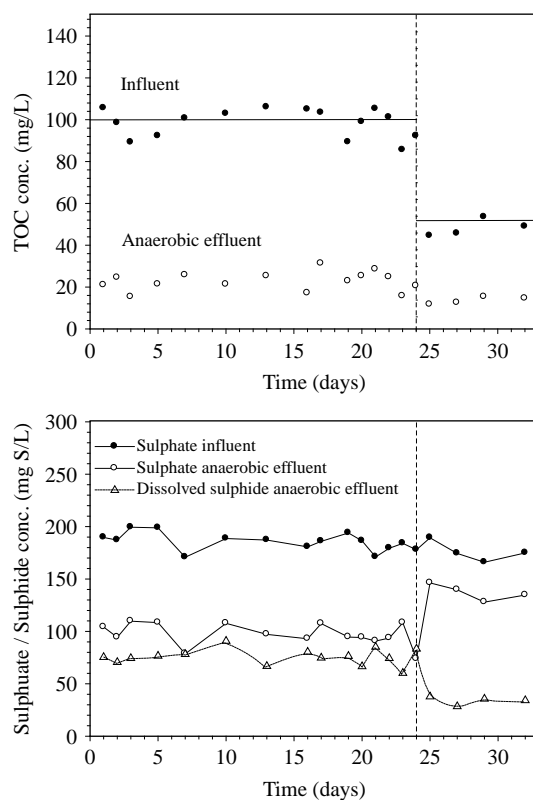


Figure 2 Performance of the anaerobic reactor at 3 h HRT

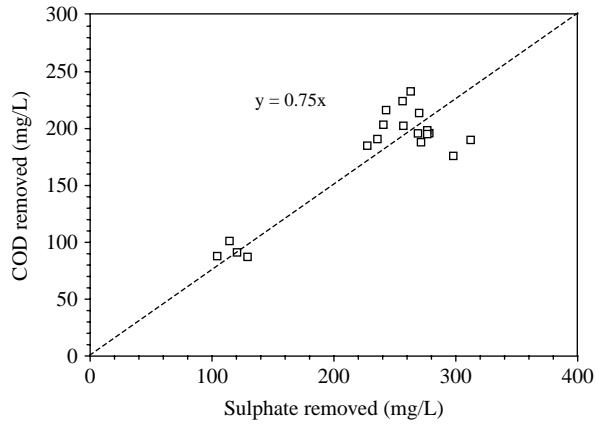


Figure 3 Relationship between COD removed and sulphate removed in the anaerobic reactor

the majority of sulphides produced through the sulphidogenic pathway were in dissolved form and the extent of dissolved sulphides was sufficient for subsequent denitrification in the anoxic reactor.

Nitrate removal

Performance of the anoxic reactor at a HRT of 2 h is shown in Figure 4. At an average feed nitrate concentration of 30 mg N/L, the performance of the anoxic reactor with a

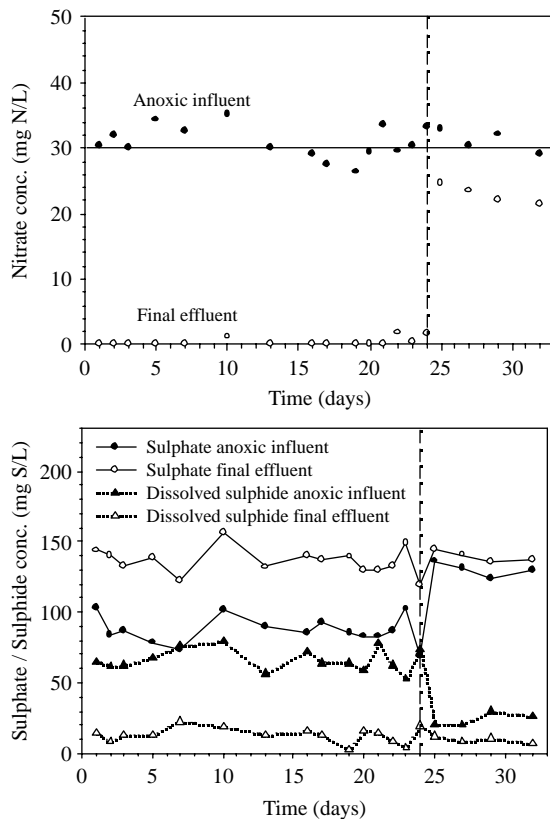


Figure 4 Performance of the anoxic reactor at 2 h HRT

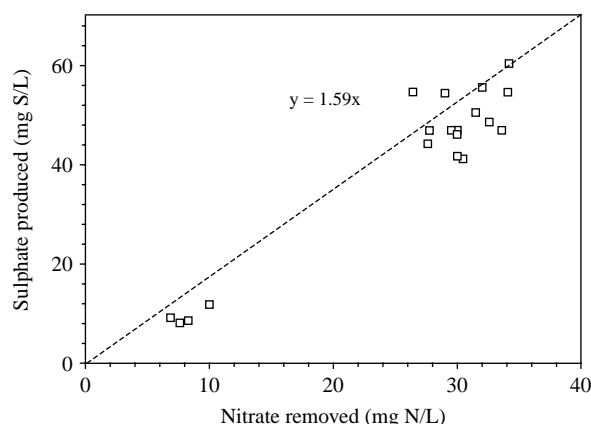
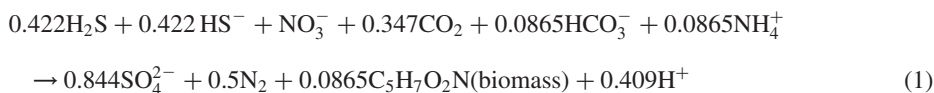


Figure 5 Relationship between sulphate produced and nitrite removed in the anoxic reactor

nitrate loading rate of 0.36 kg N/m^3 per day was stable, and 99% nitrate removal was achieved in the anoxic reactor with dissolved sulphides of the anaerobic effluent as an electron donor during the entire operation. The effluent nitrate concentration was generally below 0.5 mg N/L and no accumulation of nitrite was observed in the effluent, which suggested that nitrate was completely converted to nitrogen gas. From days 25 to 35, nitrate removal efficiency dropped to 25–30% due to the limited dissolved sulphide concentration of 40 mg S/L in the anaerobic effluent, but no nitrite accumulation was observed. This demonstrated the effect of feed dissolved sulphide concentration on nitrate removal and also the sensitivity of anoxic reactor to the level of sulphide concentration.

In order to verify further the feasibility of autotrophic denitrification, the stoichiometric ratio was compared with the results. The stoichiometry of autotrophic denitrification using sulphide as electron donors are as follows (Driscoll and Bisogni, 1978):



The consumptive ratio of sulphide-S to NO_3^- -N removed (S/N ratio) is 1.93. This ratio would vary with the type of sulphur compound used (elemental sulphur, thiosulphate or sulphide) as the electron donor. In some previous studies, different S/N ratios were reported even with the same type of sulphur source. It was suggested that column configuration, operational conditions significantly influenced the bacteria species present in a system (Gu et al., 2004). In this study, the S/N ratio observed ranged from 1.35 to 2.06 with an average of 1.59, which was close to the stoichiometric value, indicating that autotrophic denitrification was predominant in the anoxic tank (Figure 5). Under the condition of sulphide limitation, as discussed previously, the S/N ratio also did not show any significant changes. Furthermore, the results showed that residual TOC ($15 \sim 25 \text{ mg/L}$) in the feed did not promote the growth of heterotrophic denitrifiers under this operating condition.

Sulphur balance

Sulphur balance was conducted for both anaerobic and anoxic reactors throughout the experimental periods in order to study the fate of sulphur in the whole system. In the sulphur balance, with the consideration of total dissolved sulphide and sulphate only, sulphur recovery in both reactors was already close to 100%. For the anaerobic reactor, it

was evident that most of the influent sulphate ended up in dissolved form and the unknown sulphur mostly accounted for less than 10%, suggesting that loss of biogas hydrogen sulphide and accumulation of elemental sulphur were trivial. In a previous study, incomplete sulphide oxidation to elemental sulphur in the anoxic bioreactor with high-rate operation was reported (Kleerbezem and Mendez, 2002). However, in this experiment, sulphur balance showed more than 90% of dissolved sulphide was completely oxidised to sulphate through the autotrophic denitrification, which implied that the anoxic reactor could be operated at a higher nitrate loading rate if sufficient sulphide was provided.

Sludge production

The estimation of sludge production in both reactors is based on the correlation of COD and nitrate–nitrogen removal with total biomass increase in the reactors. Owing to the low sludge production in both reactors, biomass yield was estimated every 30 days using the following expressions:

UASB: Biomass yield

$$= \frac{\text{Total biomass in UASB} + \text{Biomass washout in UASB effluent}}{\text{COD}_{\text{in}} - \text{COD}_{\text{out}}} \quad (2)$$

$$\text{AF: Biomass yield} = \frac{\text{Total biomass in AF} + \text{Biomass washout in AF effluent}}{\text{NO}_3^- - \text{NO}_3^-} \quad (3)$$

Under the anaerobic condition, the cell synthesis generally is not only dependent on the amount of organic degradation, but also related to the types of organic matter involved. In this study, with the organic loading rate of 0.86 kg TOC/m³ per day, yield coefficient was found to vary between 0.15 and 0.18 g VSS/g COD in the anaerobic reactor, which was slightly higher than other applications. It is probably due to the use of glucose that provided more energy for synthesis and can also support a more diversified microbial community in the reactor.

For the autotrophic denitrification, various theoretical biomass yield coefficients were reported in the literature. The theoretical yield values calculated from the bioenergetic techniques of McCarty's method for several sulphur sources (sulphide, thiosulphate and elemental sulphur) vary between 0.683 and 0.704 g VSS/g NO₃⁻-N (Bisogni and Driscoll, 1977). By using the "energy dissipation" method developed by Heijnen, Kleerbezem and Mendez (2002) suggested another yield value for denitrification using sulphide was approximately 0.45 g VSS/g NO₃⁻-N (0.34 C-mol/S-mol or 0.25 C-mol/N-mol), which is equivalent to 0.16 g VSS/g COD, if the COD/nitrate conversion ratio of 2.86 for heterotrophic denitrification is applied. Different predicted yield values were reported even for using same type of sulphur source that were mainly due to the effects of assumptions inherent in the methodologies. In this study, total biomass in the anoxic reactor was composed of biomass attached on the media and biomass entrapped within the media. The attached biomass accounted for the 80–90% of total biomass. Observed biomass yield varied from 0.22 to 0.31 g VSS/g NO₃⁻-N in the anoxic reactor. Comparing with the theoretical values, lower biomass yields were found probably due to the endogenous decay and experimental error. However, no purposeful biomass wastage was required during the entire experimental period, thereby showing the low sludge production of autotrophic denitrification.

Conclusions

The results of this study demonstrated the feasibility of autotrophic denitrification utilising dissolved sulphides produced through the sulphidogenic pathway in saline sewage effluent. Excellent and stable organic and nitrate removal could be attained in the UASB reactor and anoxic filter, respectively. Feeding the synthetic wastewater with COD of 300 mg/L and sulphate concentration of 500 mg/L, 60–90 mg S/L dissolved sulphides produced anaerobically were sufficient for the removal of 30 mg N/L nitrate, which is commonly found in the nitrified effluent. Close to 100% sulphur recovery was found in both reactors, which implied that accumulation of elemental sulphur and loss of biogas hydrogen sulphide were not significant. During the experimental periods, the net sludge productions were approximately 0.22–0.31 g VSS/g NO_3^- -N in the anoxic reactor and 0.15–0.18 g VSS/g COD in the anaerobic reactor. Based on the findings of this study, we could develop further an integrated anaerobic–aerobic treatment system incorporating autotrophic denitrification for complete treatment of sulphate-laden wastewater. The major advantages of such a treatment system would be low energy consumption, a low sludge production due to removal of organic matter entirely by sulphate reducers, and effective sulphide control. Further studies will be focused on the effects of different parameters on autotrophic denitrification, and integration of the whole proposed treatment system. Furthermore, we will characterise the microbial community in the integrated treatment system using molecular techniques.

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References

- Akunna, J., Bizeau, C., Moletta, R., Bernet, N. and Heduit, A. (1994). Combined organic-carbon and complete nitrogen removal using anaerobic and aerobic upflow filters. *Wat. Sci. Technol.*, **30**(12), 297–306.
- APHA, AWWA, WEF (1998). *Standard Methods for the Examination of Water and Wastewater*, 20th edition. American Public Health Association (APHA)/American Water Works Association (AWWA)/Water Environment Federation (WEF), Washington DC, USA.
- Del Pozo, R. and Diez, V. (2003). Organic matter removal in combined anaerobic–aerobic fixed-film bioreactors. *Wat. Res.*, **37**, 3561–3568.
- Driscoll, C.T. and Bisogni, J.J. (1978). Use of sulphur and sulphide in packed-bed reactors for autotrophic denitrification. *J. Wat. Pollut. Con. Fed.*, **50**, 569–577.
- Edriksen, H.V. and Ahring, B.K. (1996). Integrated removal of nitrate and carbon in an upflow anaerobic sludge blanket (UASB) reactor: operating performance. *Wat. Res.*, **30**, 1451–1458.
- Foresti, E. (2002). Anaerobic treatment of domestic sewage: established technologies and perspectives. *Wat. Sci. Tech.*, **45**(10), 181–186.
- Furumai, H., Hideki, T. and Fujita, K. (1996). Effects of pH and alkalinity on sulphur-denitrification in a biological granular filter. *Wat. Sci. Tech.*, **34**(1–2), 355–362.
- Chen, G.H., An, K.J., Saby, S., Brois, E. and Djafer, M. (2003). Possible cause of excess sludge reduction in an oxic-settling-anaerobic activated sludge process (OSA process). *Water Res.*, **37**, 3855–3866.
- Chen, G.H., Mo, H.K., Saby, S., Yip, W.K. and Liu, Y. (2000). Minimization of activated sludge production by chemically stimulated energy spilling. *Water Sci. Technol.*, **42**(12), 189–200.
- Gu, J.D., Qiu, W., Koenig, A. and Fan, Y. (2004). Removal of high NO_3^- concentrations in saline water through autotrophic denitrification by the bacterium *Thiobacillus denitrificans* strain MP. *Wat. Sci. Tech.*, **49**(5–6), 105–112.
- Inamori, Y., Takai, T., Yamamoto, Y., Katagai, N., Sankai, T. and Hirata, A. (1996). Sludge production characteristics of small-scale wastewater treatment facilities using anaerobic/aerobic biofilm reactors. *Water Sci. Technol.*, **34**(3–4), 379–387.

- James, J., Bisognit, A.M., Jr and Driscoll, C.T., Jr (1977). Denitrification using thiosulphate and sulphide. *J. Environ. Eng. Div., American Society of Civil Engineers*, **103**, 593–604.
- Kleerbezem, R. and Mendez, R. (2002). Autotrophic denitrification for combined hydrogen sulphide removal from biogas and post-denitrification. *Wat. Sci. Tech.*, **45**(10), 349–356.
- Koenig, A. and Liu, L.H. (1996). Autotrophic denitrification of landfill leachate using elemental sulphur. *Wat. Sci. Tech.*, **34**(5–6), 469–476.
- Widdel, F. (1988). Microbiology and ecology of sulphate- and sulphur-reducing bacteria. In Zehnder, A.J.B. (ed.), *Biology of Anaerobic Microorganisms*. John Wiley and Sons, New York, USA.