Fecal coliform removal in a sulfate reduction, autotrophic denitrification and nitrification integrated (SANI) process for saline sewage treatment

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ABSTRACT

The Sulfate Reduction, Autotrophic Denitrification, Nitrification Integrated (SANI) process has been specially designed to treat saline wastewater. In the process no biological excess sludge is produced. SANI process also has the added advantages of cost and footprint reduction when compared to traditional activated sludge processes. In the SANI pilot plant, the fecal coliform removal efficiency in the sulfate reducing up-flow sludge bed (SRUSB) was found to be 1.4 log, whereas that in the subsequent anoxic and aerobic reactors it was 0.6 and 0.2 log, respectively, leading to a relatively high overall coliform removal of 2.2 log. Sulfide was confirmed to be toxic to fecal coliform and the contact time between the sulfide produced and coliform in the SRUSB played an important role in the removal.

Key words | fecal coliform removal efficiency, saline sewage, SANI process, sulfate reduction, sulfide

INTRODUCTION

As capacity of landfills for sewage sludge is to be suppressed in Hong Kong, a novel Sulfate Reduction, Autotrophic Denitrification, Nitrification Integrated (SANI) process was invented by the Hong Kong University of Science & Technology in partnership of the Delft University of Technology to minimize excess sludge significantly (Lau et al. 2006; Tsang et al. 2009; Wang et al. 2009). This new biological nitrogen removal (BNR) process makes use of sulfate originating from seawater toilet flushing to reduce organic matter through sulfate-reducing bacteria and subsequently conducts autotrophic denitrification with sulfide resulted from the sulfate reduction. The sulfide is completely in dissolved form due to an increase in alkalinity in the sulfate reduction. Because the three major microbial populations in the SANI process: SBR, autotrophic denitrifiers and nitrifiers are all slow growers, this process produces little excess sludge. The main SANI process bioreactor is essentially a Sulfate-reducing Up-flow Sludge Bed (SRUSB) reactor. This reactor provides dissolved sulfides for autotrophic denitrification in the subsequent anoxic bioreactor of the SANI process. Nitrification takes place in the third bioreactor of the process. This process conveniently oxidizes the toxic sulfide residues back to sulfate such that sewage can be safely discharged into the ocean. In a first successful demonstration, 95% COD and 74% nitrogen removal efficiency were achieved without excess sludge withdrawal (Tsang et al. 2009; Wang et al. 2009). The low sludge production is related to the use of autotrophic and sulfate reduction processes.

In addition to removing undesirable physical, chemical and biological constituents, sewage treatment should reduce the number of pathogenic microorganisms in the effluent for safe discharge. These microorganisms which are always present in sewage are disease-causing bacteria.
and viruses, hence sewage treatment effluent should be disinfected to ensure that its pathogenic content is within the stipulated discharge standards. Sewage treatment effluent can be disinfected by the addition of chlorine or ozone, or exposure to ultraviolet (UV) radiation. UV disinfection is ideal in terms of minimizing disinfection byproducts. However it is more expensive in comparison with chlorination and/or ozonation, making disinfection a cause for concern. Therefore, there is a need to find alternative methods of disinfection, or ways to reduce the required disinfectant dose with regard to treating Hong Kong’s saline sewage. SANI process produces sulfide which could be of help in removing more pathogens from sewage, thus earning a higher disinfection credit than conventional activated sludge processes. As no studies have previously been carried out regarding the disinfection capacity of the SANI process, this study aims to investigate the fecal coliform removal efficiency of the SANI process and examine the reasons behind this removal in addition to evaluate the pollutant removal performance of the process. If the SANI process can demonstrate high coliform removal efficiency prior to the addition of any disinfectant, it will be an added advantage to this novel process in terms of reducing disinfectant dose as well as the formation of disinfection byproducts (DBPs).

**MATERIALS AND METHODS**

This study comprised three investigations: (1) the fecal coliform removal in the SANI pilot plant that has been recently completed; (2) the fecal coliform removal under controlled conditions in the laboratory-scale SANI system which has been in operation for over 500 days (Wang et al. 2009); and (3) the effect of sulfides on fecal coliform removal in the SANI process.

**Pilot plant**

The pilot-scale SRUSB reactor had a diameter of 1,600 mm, a mixed liquid height of 2,200 mm and a liquid volume of 7,000 L. The nominal hydraulic retention time (HRT) was 18 hrs, while the temperature within the reactor was maintained at an average of 30°C during the trial period. The anoxic filter and aerobic filters were of similar diameter as the SRUSB, however the height of the biological filtration zone was 2,500 mm and the liquid volume was 7,400 L. The nominal HRTs of these two reactors were 4 hrs. All three reactors were inoculated with activated sludge obtained from a local sewage treatment works, and the anoxic and aerobic filters were packed with polypropylene plastic media. This works runs an MLE process treating 240,000 m³/day saline sewage, which anaerobic sludge digester is operated with an HRT of 20 days under a mesophilic condition. Influent sewage was from the Tung Chung Sewage Pumping Station in Hong Kong. The influent characteristics were such that the COD ranged from 300–600 mg COD/L, total nitrogen from 50–90 mg N/L, SO₄²⁻ from 400–600 mg SO₄²⁻/L or 134–200 mg S/L and chloride from 3,000–6,000 mg/L. The sulfate reduction bioreactor of the plant was operated for over 225 days, which reached a steady-state condition. Fecal coliform tests as described in section 2.6 below were carried out on the influent feed, SRUSB effluent, anoxic effluent and final effluent over a period of one month after the plant was in steady-state operation (Figure 1).

**Lab-scale setup**

The lab-scale SANI system treated 330 L per day synthetic saline sewage. To simulate the characteristics of Hong Kong’s saline sewage in terms of salinity, seawater and tap water were proportionally (1:4.4 in volume) mixed with the stock solution to achieve representative influent concentrations of COD, sulfate, nitrogen and chloride (265 mg COD/L, 500 mg SO₄²⁻/L or 167 mg S/L, 30 mg N/L ammonium nitrogen and 3,000 to 4,000 mg/L chloride on average). The system was developed and operated in a temperature controlled chamber (30°C), where all three reactors were inoculated with activated sludge obtained from a local secondary sewage treatment works. The system was operated for more than 500 days, reaching a steady state. Detailed information on the lab-scale system configuration, operation conditions and performance data can be found in our previous papers (Tsang et al. 2009; Wang et al. 2009).
Fecal coliform cultivation

An initial bacteriological study of the lab-scale system showed that its existing fecal coliform concentration was negligible. Fecal coliform bacteria were cultivated from the influent feed to the SANI pilot plant in Tung Chung so that a known concentration of \(10^8\) CFU/100 ml could be fed to the system. Influent feed to the pilot plant was collected and the procedures outlined in the fecal coliform test were carried out in order to obtain a colony of fecal coliform, which was then transferred to 20 mL of cultivation broth and incubated for 18 hrs at 36.5°C. This cultivation process yielded a fecal coliform concentration of about \(10^9\) CFU/mL. Cultivation broth was prepared by suspending 6 g of Tryptone Soya Broth in 200 mL of ultra pure water and autoclaving it in order to prevent contamination. To obtain a pure colony of fecal coliform, 100 μL of broth was diluted and the fecal coliform test was carried out again, from which a colony was picked up and cultivated. This purification process was repeated five times. Finally 1-ml samples of the purified fecal coliform were stored in the 4°C fridge until they were needed for use. Prior to feeding fecal coliform to the lab-scale system, samples were centrifuged and washed with influent feed three times so as to remove traces of the cultivation broth.

Batch test

The main purpose of this study was to investigate the effects of sulfide ions on sulfate-reducing bacteria (SRB). It was reported that 50% inhibition of SRB occurred at a total sulfide concentration of approximately 500 mg/L (Okabe et al. 1992). They implied that sulfide could be toxic to microorganisms, especially in the unionized form of hydrogen sulfide (H₂S), as it may be cell membrane permeable. Similarly Reis et al. (1992) reported that SRB inhibition was observed at a H₂S concentration of 547 mg/L. O’Flaherty et al. (1998) concluded that sulfide inhibition on SRB is related to H₂S concentrations at pH 6.8 to 7.2, while the inhibition at pH 7.2 was related to the total sulfide concentration. In the SANI process the influent sulfate concentration was 400–600 mg/L, hydrogen sulfide inhibition should not be a cause for concern because 100% sulfide was present in dissolved form due to an increase in pH beyond 8 (Wang et al. 2009). However, the sulfide concentrations present in the SRUSB reactor could still be toxic to certain pathogens, leading to higher coliform removal efficiencies. The coliform removal in this paper is an overall term which may include both kill and inactivation.

Based on the above, batch tests were carried out in order to verify the hypothesis if sulfide toxicity can be a reason for fecal coliform die-off in the SRUSB reactor. Fecal coliform was cultivated as described above. Standard sulfide solution was prepared by following the Standard Methods (APHA 1998). Phosphate buffer was prepared by mixing the required volumes of 1 M K₂HPO₄ and 1 M KH₂PO₄ solutions to obtain a desired pH. 10.29 g of NaCl was added to 1 L buffer solution so that 80 ml buffer will contain 0.5 g Cl⁻ ions such that the characteristics of Hong Kong’s
sewage could be more closely simulated. All tests were carried out at room temperature (25°C).

Stoppard glass bottles of volume 110 ml were used in the experiment, so that head space and hence mixing with air could be minimized. All equipment used was autoclaved, and all solutions used were either autoclaved or passed through 0.22 μm pore sized filters in order to prevent bacterial contamination. Under each of the tested conditions, 2 blank samples containing only 80 ml buffer solution, 20 ml ultra pure water and fecal coliform, as well as 2 samples containing 80 ml buffer solution, 20 ml sulfide and fecal coliform were tested. The fecal coliform concentration was maintained to the order of 10^9 CFU/100 ml, and the sulfide concentration at 200 mg/L. Four batch tests were carried out: (1) pH 6 buffer at a contact time of 6 hrs; (2) pH 7.5 buffer at a contact time of 6 hrs; (3) pH 7.5 buffer at a contact time of 12 hrs; (4) pH 7.5 buffer at a contact time of 18 hrs. All samples were continuously stirred during the contact time. Tests were carried out under acidic and basic conditions at a contact time of 6 hours in order to determine if H2S (predominant species under acidic conditions) is more toxic to fecal coliform bacteria than HS^- (predominant species under basic conditions).

The tests were carried out immediately after mixing of chemicals and coliforms as well as at the end of the contact period in order to determine the pH, sulfide and coliform concentrations. Sulfide concentration was determined according to the Standard Methods (APHA 1998).

Fecal coliform test

Fecal coliform tests were carried out to comply with the methodology prescribed in Method 9222 D., Fecal Coliform Membrane Filter Procedure, of the Standard Methods (APHA 1998). The M-FC medium was prepared by suspending 3.7 g of laboratory grade commercially prepared M-FC powder in 100 mL of ultrapure water. 1 mL of 1 g rosolic acid powder in dissolved in 100 mL of 0.2 N NaOH was added to 100 mL of the medium, heated near boiling, and cooled to below 50°C prior to use. All samples were diluted as appropriate to produce membrane filter counts within the range of 20–60 fecal coliform colonies. 3 identical samples were tested in each case to ensure accuracy. The filtration process was carried out under sterilized conditions, in the presence of an alcohol lamp. All equipment used in conjunction with the filtration process was sterilized appropriately. Samples were filtered through sterile 0.45 μm membrane filters under partial vacuum. Strict measures were taken during the filtration process to prevent contamination and/or cross-contamination of the sample being tested. Upon completion of the filtration process, the membrane filter was placed on 1.8 mL medium added to a sterile absorbent pad placed in a sterile culture dish. The dish was then placed in a waterproof plastic bag, inverted, anchored in a water bath and incubated for 20 ± 2 hours at 44.5°C. Colonies which appeared in blue were counted and converted to CFU/100 mL.

RESULTS AND DISCUSSION

Fecal coliform removal in pilot plant

Tests were carried out with grab samples collected from the influent feed, SRUSB effluent, anoxic effluent and final effluent over a period of one month. Figure 2 shows the coliform concentration in each of the reactors of the pilot plant. It can be seen that the removal efficiency is the greatest in the SRUSB averaging 1.4 ± 0.1 log, while the overall removal efficiency of the SANI system averages 2.2 ± 0.1 log.

To evaluate the potential role of sulfide on coliform removal we plotted the relationship between the sulfide concentration and the fecal coliform removal efficiency in the pilot scale SRUSB reactor where H2S was not present (see Figure 3). The non-linear correlation coefficient was calculated to be 0.83, indicating that there is a positive relationship.
The correlation between the sulfide concentration in the reactor and the coliform removal.

Fecal coliform removal in lab-scale SRUSB reactor

Fecal coliform in a concentration of $10^8$ CFU/100 ml was continuously fed to the laboratory scale SANI system and was monitored over a period of 2 months. Attention was paid to the SRUSB reactor as the coliform removal efficiency was found to be the highest of the system. The coliform removal efficiency of this SRUSB reactor is shown in Figure 4, which average was found to be 0.9 log units.

It was observed that the coliform removal in the lab-scale SRUSB was lower than that of the pilot plant. This could be attributed to different sulfide concentrations and/or HRTs in these two reactors. The sulfide concentration in the lab-scale SRUSB was only 80 mg/L, much lower than 120 mg/L in the pilot-scale USSB reactor, also the HRT in the lab-scale SRUSB was only 6 hours, which was lower than the 18 hour HRT in the pilot-scale SRUSB. The pH, and temperature averaged 6.5 and 30°C respectively in the both the lab and pilot plant.

### Table 1 Batch test results on the effect of sulfide on coliform reduction

<table>
<thead>
<tr>
<th>Contact time (hr)</th>
<th>Final (pH)</th>
<th>Initial fecal coliform (CFU/100 ml)</th>
<th>Final fecal coliform (CFU/100 ml)</th>
<th>Log kill $S^{2-}$ (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>6</td>
<td>6.52</td>
<td>$8 \times 10^8$</td>
<td>$4.5 \times 10^7$</td>
<td>1.25</td>
</tr>
<tr>
<td></td>
<td>6.52</td>
<td>$8 \times 10^8$</td>
<td>$4.1 \times 10^7$</td>
<td>1.3</td>
</tr>
<tr>
<td></td>
<td>6.02</td>
<td>$8 \times 10^8$</td>
<td>$7.7 \times 10^8$</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td>6.02</td>
<td>$8 \times 10^8$</td>
<td>$7.3 \times 10^8$</td>
<td>0.04</td>
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<tr>
<td></td>
<td>7.73</td>
<td>$7.7 \times 10^8$</td>
<td>$6.1 \times 10^7$</td>
<td>1.10</td>
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<tr>
<td></td>
<td>7.73</td>
<td>$5.4 \times 10^8$</td>
<td>$4.6 \times 10^7$</td>
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<tr>
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<td>$4.2 \times 10^8$</td>
<td>$4.1 \times 10^8$</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>7.45</td>
<td>$3.6 \times 10^8$</td>
<td>$3.4 \times 10^8$</td>
<td>0.02</td>
</tr>
<tr>
<td>12</td>
<td>7.69</td>
<td>$4.1 \times 10^8$</td>
<td>$6.1 \times 10^6$</td>
<td>1.83</td>
</tr>
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<td></td>
<td>7.7</td>
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<td>$4.7 \times 10^6$</td>
<td>1.89</td>
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<td>7.47</td>
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<td>$5.0 \times 10^8$</td>
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<tr>
<td>18</td>
<td>7.85</td>
<td>$3.8 \times 10^8$</td>
<td>$4.3 \times 10^6$</td>
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<tr>
<td></td>
<td>7.86</td>
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<td>$3.3 \times 10^6$</td>
<td>1.93</td>
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<tr>
<td></td>
<td>7.48</td>
<td>$2.6 \times 10^8$</td>
<td>$2.4 \times 10^8$</td>
<td>0.03</td>
</tr>
<tr>
<td></td>
<td>7.49</td>
<td>$5.5 \times 10^8$</td>
<td>$5.4 \times 10^8$</td>
<td>0.07</td>
</tr>
</tbody>
</table>

**Batch test**

Table 1 shows the results of the batch tests. At a contact time of 6 hours tests were carried out at pH 6 and 7.5 in order to observe the effect of the change in pH, and hence change in $HS^-/H_2S$ ratio on the coliform removal efficiency. Table 1 shows that the coliform removal efficiency was not significantly affected by pH and a longer contact time between fecal coliform and sulfide increased the decay of fecal coliforms. However, Figure 5 reveals that when the contact time was above 12 hours the contact time effect on the coliform decay is less relevant. This confirms that the pilot-scale SRUSB
reactor killed more coliform than the lab-scale SRUSB since the former has a longer HRT.

Comparison with freshwater wastewater treating processes

Table 2 compares the performance of the SANI process SRUSB reactor to traditional up-flow anaerobic sludge bed (UASB) reactors and conventional activated sludge treatment units.

The coliform removal efficiency in the SRUSB reactor is greater than or comparable to both aerobic and anaerobic bioreactors that treat freshwater wastewater. The pH of the cited UASB (unit no. 6) reactor could not be found, however in such traditional UASB reactors the pH varies from 7–8. Likewise, the operation conditions of the traditional activated sludge units (units no. 1–3) were not included in the cited source. However it was given that the total HRT of the activated sludge plants averaged 18 hours, where conventionally 10 hours are spent on primary and secondary sedimentation, and 8 hours are spent on aeration. In the activated sludge plant (unit no. 4) 18 hours was spent on extended aeration while 4 hours was spent on sedimentation. The pathogen removal in activated sludge processes is mainly attributed to filtration and aggregation to the sludge and predation by protozoa in the bioreactors (van der Drift et al. 1977; Gerba 2008), while in the SANI process the pathogen removal is likely due to removal of E. Coli cells in the sulfate reduction bioreactor (SRUSB) through sulfide toxic effect. Hence, SRUSB can likely remove more coliforms than an aeration tank in activated sludge, though the overall removal efficiency of SANI process and activated sludge could be similar.

CONCLUSIONS

The overall fecal coliform removal of the SANI process pilot plant was 2.18log units. The fecal coliform removal in its SRUSB reactor is higher than or comparable to conventional bioreactors.

ACKNOWLEDGEMENTS

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REFERENCES

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Table 2 | Comparison of the SRUSB Reactor with conventional treatment

<table>
<thead>
<tr>
<th>Unit No.</th>
<th>Process</th>
<th>HRT (hrs)</th>
<th>pH</th>
<th>Temperature (°C)</th>
<th>Coliform removal (log)</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Primary sedimentation</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.4</td>
<td>Lucena et al. (2004)</td>
</tr>
<tr>
<td>2</td>
<td>Aeration tank (activated sludge)</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>1</td>
<td>Lucena et al. (2004)</td>
</tr>
<tr>
<td>3</td>
<td>Flocculation aided secondary clarification</td>
<td>–</td>
<td>–</td>
<td>–</td>
<td>0.7</td>
<td>Lucena et al. (2004)</td>
</tr>
<tr>
<td>4</td>
<td>Activated sludge plant</td>
<td>22</td>
<td>6.8–7.5</td>
<td>25</td>
<td>2.06 ± 0.26</td>
<td>Wen et al. (2009)</td>
</tr>
<tr>
<td>5</td>
<td>Facultative ponds</td>
<td>49 days</td>
<td>8.3–8.9</td>
<td>20–26</td>
<td>2.8</td>
<td>Lucena et al. (2004)</td>
</tr>
<tr>
<td>6</td>
<td>UASB</td>
<td>12</td>
<td>–</td>
<td>10–30</td>
<td>0.9</td>
<td>Tawfik et al. (2008)</td>
</tr>
<tr>
<td>7</td>
<td>Pilot-scale SRUSB</td>
<td>18</td>
<td>6–7</td>
<td>30</td>
<td>1.42</td>
<td></td>
</tr>
</tbody>
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