Effects of high salinity wastewater on methanogenic sludge bed systems
S. B. Ismail, P. Gonzalez, D. Jeison and J. B. van Lier

ABSTRACT

The attainable loading potentials of anaerobic sludge bed systems are strongly dependent on the growth of granular biomass with a particular wastewater. Experiments were conducted to determine the effects of high salinity wastewater on the biological and physical properties of methanogenic sludge. Sodium concentration of 5 g/L and 15 g/L were added to the influent of upflow anaerobic sludge bed (UASB) systems. After 100 days of operation, the methanogenic activity, extracellular polymeric substances (EPS), and granular strength were analyzed. The results show a high removal of organic matter but with accumulating propionate concentrations in the effluents. Meanwhile, wash-out of active methanogenic biomass in the effluent of the reactors was observed, likely as a result of the high Na+ concentrations. The rinsed biomass was characterized by a considerable specific methanogenic activity (SMA) on acetate, propionate and hydrogen as the substrates. On the other hand, results show that the SMA evolution was not affected by high salt concentrations. Also the amount and composition of extracellular polymeric substances (EPS) were similar in all sludges. However, results clearly show a sharp drop in the granule strength as a result of high Na+ concentration.

Key words | bacterial immobilization, granule strength, high salinity, sodium, UASB reactor

INTRODUCTION

The biological treatment of high salinity wastewater is becoming a topic of increasing importance in the industrialized world. Lefebvre et al. (2007) reported that the treatment of saline and hypersaline wastewater could represent as much as 5% of worldwide effluent treatment requirements. This concerns both seawater-based agro food industries, such as fish processing, as well as concentrated wastewaters, such as those coming from chemical industries and distilleries. Moreover, owing to increased water efficiency in industrial processes and developments towards loop closure, the resulting wastewaters are more concentrated and increasingly characterised by harsh environmental conditions for biological treatment, such as high salinity and high temperatures. At present, granular sludge based anaerobic high-rate reactors, such as the upflow anaerobic sludge blanket (UASB) and the expanded granular sludge bed (EGSB) are most commonly applied to treat concentrated, organically polluted wastewaters (Van Lier 2008). High-rate anaerobic treatment offers the possibility of efficient wastewater treatment with low operational cost, high removal of organic carbon and small footprint size. However, the success of the anaerobic high-rate systems and particularly the expanded bed reactors is dependent upon a successful sludge granulation process. The excellent settling properties and high strength of the granular configuration minimize biomass wash-out, resulting in high solids retention times and concomitant high loading capacities reaching up to 40 kgCOD/m^3.d (Van Lier 2008). Also, the dense cell packing in granular aggregates optimizes the interspecies exchange of metabolites and
hence the overall activity (Lettinga 1995; Quarmby & Forster 1995; Ghangrekar et al. 1996; Britz et al. 2002).

The presence of high Na⁺ concentrations is negatively impacting the anaerobic treatment process, affecting the physiology of the microorganisms as well as the morphology of the required granular consortia. It has already been reported that a sodium concentration exceeding 10 g/L strongly inhibits methanogenesis (Gourdon et al. 1989; Kugelman & McCarty 1965). Rinzema et al. (1988) found that at neutral pH, sodium concentration exceeding 5 g/L inhibited aceticlastic methanogenic activity of granular sludge, reflecting the sensitivity of *Methanosaeta* spp. towards sodium.

Several research works have reported that the presence of calcium ions had a noticeable influence in the granulation process. Calcium concentration of 150–300 mg/L in wastewater had a positive effect on the granulation of anaerobic sludge and the presence of Ca²⁺ enhanced the mechanical strength and the settle ability of the granules (Yu et al. 2001). Ca²⁺ likely acts as a bivalent cationic bridge between negatively charged functional groups, being part of the bacteria themselves and/or extracellular polymeric substances (EPS) (Sobeck & Higgins 2002). In addition, granules have been found to have a net negative charge, which results of EPS-related functional groups. EPS, also called bio-glue, are sticky materials secreted by cells, contain variable proportions of protein, polysaccharides, nucleic acids, humic-like substances, lipids, and heteropolymers such as glycoprotein (Frolund et al. 1996). So far, no researches are conducted on the impact of high Na⁺ concentrations possibly affecting the stabilizing role of Ca²⁺ in granular sludge.

The main objective of our present study is to elucidate the effects of high sodium concentrations on the biological and physical properties of anaerobic granular sludge in continuously fed UASB reactors. The biological properties of the obtained sludge were determined by assessing the specific methanogenic activities (SMA) on defined substrate and the quantity and composition of the extracellular polymeric substances (EPS). Characterization of the physical properties were done by determining the granule strength. Furthermore, the effects of high Na⁺ concentration on Ca²⁺ leaching from methanogenic granules is assessed in batch tests.

### MATERIALS AND METHODS

#### Inoculum characteristics

The reactors were inoculated with sludge from a full-scale UASB reactor treating wastewater from a styrene and propene-oxide production plant of Shell, Moerdijk, the Netherlands. The industrial wastewater contains a sodium concentration in the range 10–15 g/L, with acetic acids and benzoic acids as main sources of COD. The sludge was adapted to such high salinity wastewater for more than 10 years (Biothane International, personal communication). This inoculum was used for both the UASB experiments and the batch tests.

#### Continuous UASB reactor experiments

The experiments were performed in a temperature controlled room at 30 ± 2°C using three glass UASB reactor with a volume of 3 litres. The UASB reactors were equipped with a reversed funnel phase separator. After passing the gas through a concentrated sodium hydroxide solution for removing carbon dioxide and through a column filled with soda lime pellets with indicator (Merck Art. no. 6839, Darmstadt, FRG), methane production was monitored by a wet-test gas meter (Schlumberger, Dordrecht, The Netherlands). All reactors (R1, R2 and R3) were fed with 70% of acetate, 20% of gelatine and 10% of ethanol as substrate. The media were prepared in distilled water. The influent parameters for the reactors are given in Table 1.

The upflow liquid velocity was controlled by liquid recirculation and was fixed at 0.8 m/h. The initial organic loading rate (OLR) was 2.5 kgCOD/m³d, gradually increasing to 18 kgCOD/m³d. Reactor R1 was operated at a hydraulic retention time (HRT) of 12 h, which was 10 times shorter than the HRT of reactors R2 and R3. Higher loading rate were imposed on the system by increasing the flow rate of a separated peristaltic pump (Watson Marlow 202) for the concentrated feed stock solution.

#### Table 1 | Influent parameters for the three reactors; R1, R2 and R3

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<th>R1</th>
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<td>COD (g/L)</td>
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<td>Na⁺ (g/L)</td>
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Batch incubations: effects of high salt concentration on methanogenic granules

Batch tests were performed in 0.25 litre bottles to study Ca$^{2+}$ leaching from anaerobic granular sludge by adding high sodium concentration, i.e. 20 gNa$^+$/L in absence of feeding for 30 days. Two sets of batch bottles were prepared with 20 gNa$^+$/L and a blank as control. The bottles were placed on a reciprocal shaker at 30°C. The leaching test was performed in duplicate.

Specific methanogenic activity (SMA) assessments

The SMA was determined in duplicates by pressure increase in experiments performed in 117 mL serum bottles, with 50 mL of media as described by (Vallero et al. 2002). Biomass concentration was 1 gVSS/L. Acetate, propionate, butyrate were used as sole substrates, at an initial concentration of 1.5 gCOD/L. The SMA was evaluated as the maximum specific methane production rate.

Analytical techniques

Volatile fatty acids (VFA) analysis were performed as described elsewhere (Jeison & van Lier 2007). Particle size distribution was measured by laser diffraction (Mastersizer 2000, Malvern UK) and is presented based on volume distribution. Calcium content in the methanogenic sludge was determined by Vista-MPX CCD with simultaneous inductively coupled plasma-optical emission spectroscopy (ICP-OES)(Varian, Australia). For analysis of the extracellular polymeric substances (EPS) content of the granular sludge, a cation exchange resin (CER) was used (Frolund et al. 1996). Bubble column test as described by Pereboom (1997) was used to determine the sludge granule strength. Sludges were examined by scanning electron microscopy (SEM) coupled with an energy dispersive x-ray (EDX) detector to determine the spatial distribution of calcium (Alphenaar et al. 1994). Cross-sectioned granules were prepared as described in details by Alphenaar et al. (1994), except for the cryo-protectant which was replaced by sucrose. Carbohydrates were analyzed by using the anthrone methods (Raunkjaer et al. 1994), meanwhile protein concentrations were determined based on Lowry et al. (1951). Biomass yield analysis was done by measuring the amount of biomass produced related to the amount of substrate consumed in a specific period. All other analysis were carried out according to Standard Methods for the Examination of Water and Wastewater (APHA 1998).

RESULTS AND DISCUSSION

UASB experiments

Figure 1 presents the effluent VFA concentration and applied loading rate during the operation of the UASB reactors, fed with partly acidified substrate. OLR was increased gradually from 7 until 18 kgCOD/m$^3$.d. The VFA concentration in the effluents dropped to a lower level as the operation proceeded except immediately after increasing the OLR. The decreasing of VFA concentration shows that the reactors performed well respective to the OLR. The single-day peaks sometimes occurring prior to the OLR increase can be ascribed to technical disturbances. Reactor performance, however, was restored before loading rate increments were imposed to the system. The remnant fraction of VFA in the effluents were mainly composed of propionate. At the end of the operation, propionate concentration in the effluent was about 80% in reactor R3, whereas reactors R1 and R2 had only 25%.

Considerable sludge washed-out was observed in the effluent of the reactor operated at 5 gNa$^+$/L and with a low influent COD concentration (reactor R1). At the end of the reactor operations about 45 g of sludge were washed out from reactor R1. In contrast, only about 15 g of sludge were rinsed from reactors R2 and R3. This difference can be attributed to the different HRT at which the reactors were operated. The applied low HRT introduces a high selection pressure into the reactor R1, resulting in rinsing of the poor settling sludge and biomass grown in suspension.

Biological properties

The specific methanogenic activity (SMA) was measured for the washed-out sludge from each reactors at 25, 50 and 75 days of operation. The washed-out sludge exerted an extremely high SMA especially for reactor R1, as depicted in
Figure 2. Very likely, the high loading rate and concomitant high biomass yield led to effluent suspended solids consisting predominantly of methanogenic biomass. Notably, the influent COD concentration in reactor R1 was 5 gCOD/L, whereas in reactors R2 and R3 this was 50 gCOD/L, resulting in relatively long HRTs in reactors R2 and R3.

Figure 3 presents the SMA of the sludge measured at the end of the experimental period, when the reactors were characterized by a loading rate of 18 kgCOD/m³·d and 95% of COD removal. The used inoculum of each reactor showed a relatively low SMA on all substrates tested. Interestingly, with regard to the inoculum, the measured SMA does not give any clear differences comparing the various Na⁺ concentrations, while applying the same substrate. Apparently, the inoculum can tolerate high Na⁺ concentration, a finding which agrees with the previously published Na⁺ response curve (Jeison et al. 2008).

The EPS contents of the sludges were analyzed at the end of reactor operation (Table 2). In the sludge samples from the reactors R1, R2, and R3, the amounts of EPS were somewhat higher compared to the inoculum. Likely, this can be attributed to the presence of gelatine in the feed.

Figure 2 | Effluent VFA concentration and applied OLR during the operation of the UASB reactors. ■ Organic Loading Rate (kgCOD/m³·d); □ R1-5 gNa⁺/L; ▇ R2-5 gNa⁺/L; ▪ R3-15 gNa⁺/L.

Figure 2 | SMA of washed-out sludge measured with acetate (C2), propionate (C3), butyrate (C4) and hydrogen (H2) as the substrates. (□) 25 day of operations; (□) 50 day of operations; (□) 75 day of operations. Error bars represent the variance between duplicates.
Results also show that the amounts of protein are higher compared to polysaccharides, resulting in a protein/polysaccharides ratio between 16 to 33 (see Table 2). Strikingly, the total EPS amount increased for all reactor sludges irrespective the Na\(^+\) concentration applied in the feed, i.e. 5 or 15 gNa\(^+\)/L.

The observed high acetate-SMA values of the sludges from reactors R2 and R3 at the end of the UASB experiments indicate that growth and retention of methanogenic biomass was considerable, despite the high Na\(^+\) concentration applied. The calculated yield values were 0.04 ± 0.01 and 0.05 ± 0.01 gVSS-COD/COD\(_{converted}\) for 5 and 15 gNa\(^+\)/L, respectively. The obtained biomass yields are within the range reported in the anaerobic digestion model no.1, i.e. about 0.04–0.06 gVSS-COD/COD\(_{converted}\) (Batstone et al. 2002). In agreement with Figure 2, sludge retention was much poorer in Reactor R1, subjected to a short HRT, resulting in a relatively low SMA on acetate. Surprisingly, the SMA on propionate and, to a lesser extent, butyrate did increase in the reactor R1 sludge.

Physical properties

Abrasion experiments were performed in a bubble column to measure the granule strength of the inoculum and the sludge from each reactor at the end of the operational period. The strength of the granules was plotted in function of the production of the fines measured as volatile suspended solid after 1 minute of settling. All reactors presented lower strength granules in comparison with the inoculum. However, with the sludge from reactor R3, a higher concentration of fines was found. Apparently, the granule strength of this sludge was lowest (Figure 4). This finding supports our hypothesis that a high amount of sodium reduces the granule strength, producing a weak dispersed sludge.

Calcium leaching test

Batch tests were carried out to determine the calcium leaching from the granules as a results of high sodium
concentrations. Figure 5a shows a micrograph of an SEM image of an inoculum granule, which was embedded in a sucrose matrix. Qualitative chemical analysis by EDX present the relative weighted presence of calcium, sulphur, iron and phosphorus, being 63%, 20%, 14% and 3%, respectively (Figure 6). Calcium was detected as the element with the highest relative abundance. Figure 5b for spatial distribution mapping of the calcium by SEM-EDX shows that the calcium is evenly spread over the granule mass, having a high intensity in some specific areas.

Results of the leaching experiment clearly show an increase in the bulk liquid Ca\(^{2+}\) concentration at Na\(^{+}\) concentration of 20 g/L (Figure 7). Combining these results with the above-described results on granule strength, we postulate that the abundantly available Na\(^{+}\) replaces Ca\(^{2+}\) in the matrix, resulting in week granule structures. Previously, Grotenhuis (1988) also showed a deterioration of the granule strength upon Ca\(^{2+}\) removal. The bivalent Ca\(^{2+}\) has often been considered to bridge negatively charged sites on extracellular biopolymers, thus enhancing the matrix stability of an attached microbial community (Wloka et al. 2004). Therefore, calcium has often been introduced into the medium to increase the strength of anaerobic granules e.g. Teo et al. (2000). Our present results indicate that such Ca\(^{2+}\) displacement can be brought foreword by high Na\(^{+}\) concentration. A similar finding was made by Bruss et al. (1992) who observed Ca\(^{2+}\) displacement in aerobic activated sludge floc when exposing the flocs to high Na\(^{+}\) concentrations. This displacement resulted in deterioration in floc properties measured by supernatant turbidity and a high specific resistance to filtration (SRF).

Particle size distribution measurements did not show a clear impact of Na\(^{+}\) exposure on the granule size. The sludge granules were very small, with 50% of the particles smaller than 500 \(\mu\)m and 475 \(\mu\)m for the inoculum and 30 days incubated sludge, respectively. The surface weighted
mean of the inoculum and the granular sludge after incubation for 30 days, respectively, was 410 and 318 μm, with no particles bigger than 2,000 μm, which is the upper detection limit of the equipment (data not shown).

CONCLUSIONS

In summary, the SMA and EPS were not affected by high salinity concentrations, but a significant drop in granule strength was observed under conditions of long-term exposure to high Na⁺ concentrations. The performance of the granular sludge system shows the appropriateness of methanogenic inocula, pre-grown under saline conditions, for the anaerobic treatment of high salinity wastewater. The later conditions, however, cause a decrease in granule strength and washout of bacterial mass. Batch leaching test showed that high concentrations of monovalent cations could cause problems with granule properties.

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REFERENCES


