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ORIGINAL PAPER

Effect of fluctuations in salinity on anaerobic biomass and production of soluble microbial products (SMP_s)

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Abstract This study investigated the acclimation potential of batch fed anaerobic biomass with salinities of 0–50 gNaCl l^{-1}. Anaerobic biomass was acclimatized to salinities up to 20 gNaCl l^{-1}over a period of 35 days, with 3 consecutive feedings. After this period the biomass was subjected to non-saline conditions to simulate fluctuating feed compositions. High activity was obtained after the first exposure to non saline conditions for biomass previously exposed to 30 gNaCl l^{-1}. Short exposure (2–48 h) to high salinity (40 gNaCl l^{-1}) did not reduce biomass activity when it was re-subjected to normal conditions. The sensitivity of each anaerobic bacterial group showed that propionate utilisers were the most affected by sudden changes in salinity. Using size exclusion chromatography (SEC) it was found that biomass exposed to concentrations of salinity above 30 $gNaCl^{-1}$ produced higher molecular weight soluble microbial products (SMPs) which were present in the culture for longer periods than the control indicating that the effluent was more difficult to degrade. With the sudden removal of salinity anaerobic biomass can easily readapt to normal conditions without any high MW compounds being produced. These findings highlight the fact that anaerobic biomass is able to overcome sharp decreases in salinity in contrast with aerobic biomass as reported in the literature.

Keywords Anaerobic biomass · Recovery from salinity · Bacterial sensitivity to salinity · Soluble microbial products · Sodium inhibition

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Introduction

Organics in highly saline wastewaters are poorly biodegraded by conventional wastewater treatment plants with non adapted biomass due to the toxic effect of sodium in the wastewater. High concentrations of salts (10 gNaCl l^{-1}) can cause cell plasmolysis and the death of microorganisms due to the dramatic increase in osmotic pressure (Kargi and Dincer 1998; Kempf and Bremer 1998). Boardman et al. (1995) investigated the toxicity of sodium in batch assays using clam wastewater as a biomedia and substrate; a 10-fold decrease in activity was found for a 3-fold increase in Na^+ concentration from 4.2 gNa^+ l^{-1} to 12.6 g l^{-1}. Tuin et al. (2006) used a laboratory-scale (1.5 l) Upflow Anaerobic Sludge Blanket (UASB) reactor for the pre-treatment of a chemical wastewater which contained complex organics, high concentrations of sulphate (5-7 gSO_4^{2-} l^{-1}) and high salinity. At 16 gNaCl l^{-1} the COD removal was only 40%, while the sulphate removal was 80%. However, the high sulphate concentrations are likely to

encourage sulphate reducing organisms to compete with methanogenesis so no clear conclusions can be drawn from this work with respect to sodium inhibition of anaerobic biomass. Debaere et al. (1984) found no sodium inhibition up to 29.01 $gNa^+ l^{-1}$, and only at 37.4 $gNa^+ l^{-1}$ did the methanogenic activity decrease to 50%. Rovirosa et al. (2004) studied the performance of a downflow anaerobic fixed bed reactor treating saline (15 $gNaCl l^{-1}$) synthetic domestic wastewater. Start up lasted 335 days by gradually increasing the NaCl from 5 to 15 $g l^{-1}$ so the bacteria could acclimatise. After 590 days COD removal at 15 $gNaCl l^{-1}$was 68 and 92% for an organic loading rate (OLR) of 3.8 $gCOD l^{-1} day^{-1}$ and 0.47 $g l^{-1} day^{-1}$, respectively. Mendez et al. (1995) studied the treatment of a seafood processing wastewater (10.7–23.2 $gCOD l^{-1}$, 10–20 $gNaCl l^{-1}$, 1.9–2.7 $gSO_4^{2-} l^{-1}$) in thermophilic (TAF) and mesophilic anaerobic filters (MAF). Adaptation to salinity was achieved during a nine month prolonged start-up period; after this the OLR was increased gradually to 9 and 14 $gCOD l^{-1} day^{-1}$ in the TAF and MAF, respectively. Under these conditions the COD removal was 73% for the TAF and 64% for the MAF. In the above work, the long start up period of anaerobic biomass acclimating to salinity could be a serious drawback for the process. Due to the long acclimation period from the above studies, this study investigated anaerobic acclimation potential under different saline concentrations in order to determine if the long start up period could be reduced.

Salinity is often highly variable in many industrial wastewaters (Lefebvre and Moletta 2006), and several studies found that a sudden reduction in salinity often caused more severe effects in aerobic biomass than a stable but high concentration of salinity (Kinncannon and Gaudy 1966; Kinncannon and Gaudy 1968; Burnett 1974). However, to the best of our knowledge no studies have examined the performance of anaerobic biomass after a sudden removal in salinity for varying periods of exposure to salt conditions. Several researchers have found contradictory results for the acclimation of biomass to sodium, and this can be attributed to factors such as; the effect of other ions present in the wastewater, and, low concentrations of potassium acting as an antagonist to sodium. However, low concentrations of magnesium and calcium can also act synergistically towards sodium (Kugelman and McCarty 1965). Other factors which could have been important are; different inocula, different types of reactors, different feed strategies, different substrates and different test methods. Moreover, little work has been done to characterize the molecular weight of organic compounds in the effluent from saline wastewater treatment plants. Apart from sodium which causes toxicity to the biomass, it is possible that the soluble organics produced under osmotic stress may contribute to the poorer performance of the process, and these compounds are known generically as Soluble Microbial Products (SMPs). Due to their low biodegradability, SMPs are important in terms of achieving current discharge standards and effectively set the upper limit for treatment efficiency (Barker and Stuckey 1999). It is also possible that certain fractions of the SMPs could be inhibitory or even mutagenic.

Hence, the objective of this study was to examine the ability of anaerobic biomass to acclimate to saline conditions in a relatively short time under batch feeding, and study the performance of this biomass during a sudden reduction in salinity after a short/long time period exposure to saline conditions. In addition, the performance of this biomass was evaluated when re-exposed to salinity after a period under non-saline conditions. Under these conditions, evaluation of the sensitivity of each anaerobic bacteria group was measured by monitoring volatile fatty acid (VFA) concentrations over time. Finally, the SMPs in the batch reactors at different NaCl concentrations were characterized in terms of their Molecular Weight (MW) over time.

Methods

Determination of volatile suspended solids (VSS) was performed according to Standard Methods (APHA 1999) The composition of biogas was determined using a Shimadzu GC-TCD fitted with a Porapak N column (1,500 × 6.35 mm). The carrier gas was helium at a flow rate of 50 ml min^{-1}, and the column, detector and injector temperatures were 28, 38 and 128°C, respectively. The peak areas were calculated and printed out on a Shimazdu Chromatopac C-R6A integrator. Calibration gases were accurate to ±5%. Samples of 1 ml were collected using 1 ml plastic syringes (Terumo), and the coefficient of variance (COV) for 10 identical samples was ±2%.

Glucose and VFAs were measured on a Shimadzu (model 10A) high performance liquid chromatography (HPLC) system with an auto-sampler using an Aminex HPX-87H ion exclusion column (300 × 7.8 mm). The sample volume was 50 µl, the column was maintained at 55°C, and the eluent was 0.01 M H_2SO_4 at a flow rate of 0.7 ml/min. VFAs were detected with UV at 210 nm, while glucose detection was by refractive index (RI). The detection limit was 10 mg l^{-1} for glucose and 5 mg l^{-1} for VFAs, and the COV for 10 samples was within ±8% for both parameters.

For Size Exclusion Chromatography (SEC) an Aquagel OH-30 column (Polymer Labs) was used with DI water as the eluent at a flow rate of 1 ml min^{-1}.The sample volume was 50 µl, and the column was maintained at ambient temperature with both UV and RI detectors being used to detect the separated components. Standards of linear polyethylene glycol (PEG) were used; hence the results obtained are quoted relative to these linear compounds.

The serum bottle experiments were conducted using the media and serum bottle techniques developed by Owen et al. (1979). Anaerobic biomass was obtained from a conventional sewage sludge digester (Mogden, UK) that was operated under low salinity. The anaerobic biomass was maintained in a batch reactor (5 l) and fed with glucose as a substrate for 3 months at an OLR of 2 gCOD l^{-1} day^{-1}, nutrients according to Owen et al, and salinity less than 2 gNaCl l^{-1}. The reactor was fed under batch mode every 7 days (6 days of mixing and 1 day for the biomass to settle and then the supernatant was removed and new feed added). Biomass from this reactor was used as an inoculum in 165 ml serum bottles (2 gVSS l^{-1}). Anaerobic conditions were achieved by flushing the bottles with N_2 (70%) and CO_2 (30%) at a flow rate of 0.5 l min^{-1}. To each serum bottle, 95 ml of anaerobic media was added (Owen et al. 1979), then 5 ml of glucose to give a final concentration of 2 gCOD l^{-1} before capping it with a leak proof Teflon seal. The serum bottles were placed in a Gallenkamp Orbital Incubator Shaker at a constant temperature and shaker speed of 37°C and 200 rpm, respectively. In the re-feeding, biomass was removed by centrifugation (3,000 rpm for 10 min), and then placed back into the serum bottles and new media added with the same concentration of salinity and substrate. During the transfer of biomass N_2/CO_2 was purged into the bottles to maintain anaerobic conditions. In the experiments where the conditions changed from saline to non-saline (less than 1 gNaCl l^{-1}), the biomass was washed for 1 min with DI water to minimise any sodium that may have been absorbed by the biomass, and osmotic shock. At the beginning of the experiment 5 samples for each concentration of salinity were set up. In each feeding one of the sample was used for VFA and SMP analysis, The COV for CH_4 was ±3% between 5 different samples, while for VFAs for 3 identical samples was ±8%.

Results

Exposure to three batch feedings at different salt concentrations

Anaerobic biomass not adapted to sodium was subjected to different concentrations of NaCl in order to examine their acclimation potential. During the first exposure (Fig. 1a) concentrations above 20 gNaCl l^{-1} caused significant inhibition, while the biomass exposed to 10 and 20 gNaCl l^{-1} produced slightly less methane compared to the control after

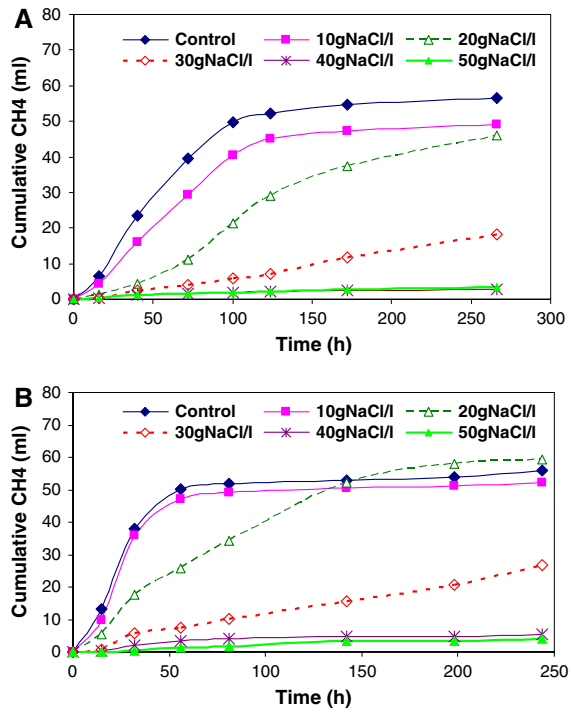

Fig. 1 (**a**) First batch exposure of non acclimatized biomass to concentrations of 0–50 gNaCl l^{-1}. (**b**) Third batch exposure of non acclimatized biomass to concentrations of 0–50 gNaCl l^{-1}

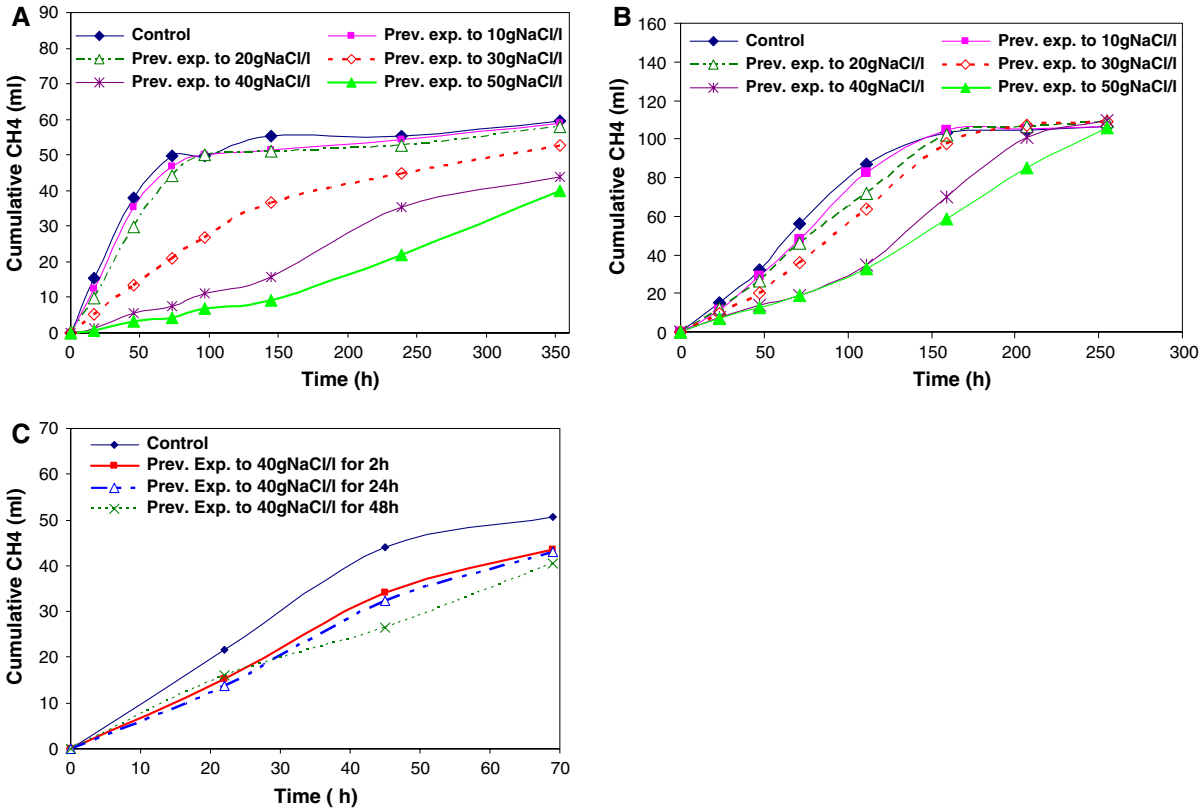

Fig. 2 (**a**) First exposure of biomass to non saline conditions after a long exposure to salinity. (**b**) Second exposure of biomass to non saline conditions after a long exposure to salinity. (**c**) Exposure of biomass to non saline conditions after 2, 24 and 48 h exposure to salinity

265 h. At concentrations of 40–50 gNaCl l^{-1}, even after 265 h the methane production was negligible. In the next feedings, the media was replaced by new media with the same salinity and substrate concentration (2 gCOD l^{-1}). After the third batch feeding, the biomass that exposed to 10 gNaCl l^{-1} immediately produced the same amount of methane as the control while the biomass exposed to 20 gNaCl l^{-1} reached the same methane production level of the control after 142 h. With biomedia containing higher concentrations of salt (30 gNaCl l^{-1}), the decrease in inhibition was substantially less, while above and including 40 gNaCl l^{-1} inhibition was not reduced even after the third feeding.

Exposure to no salt for two batch feedings after three feedings with salt

After the third batch with a total exposure to salinity of 35 days, the biomass was then fed with a new medium with NaCl less than 1 g l^{-1} at 2 gCOD l^{-1} glucose. Results in Fig. 2a show that the slowest rate of methane production was from the biomass that was pre-exposed to the highest sodium concentration. The biomass pre-exposed to salt concentrations up to 20 gNaCl l^{-1} showed a high activity despite previous acclimation of the biomass to salinity. In the second feeding under organic shock conditions (4 gCOD l^{-1} glucose), the recovery was significantly higher especially for biomass pre-exposed to higher concentrations of salinity (Fig. 2b). The biomass previously exposed to 50 gNaCl l^{-1} in the second feeding (with salt less than 1 gNaCl l^{-1}), produced the same amount of methane as the control after 250 h (Fig. 2b).

Exposure to no salt after a short period at 40 gNaCl l^{-1}

40 gNaCl l^{-1} was added to non acclimatised to salinity anaerobic biomass for 2, 24 and 48 h. During

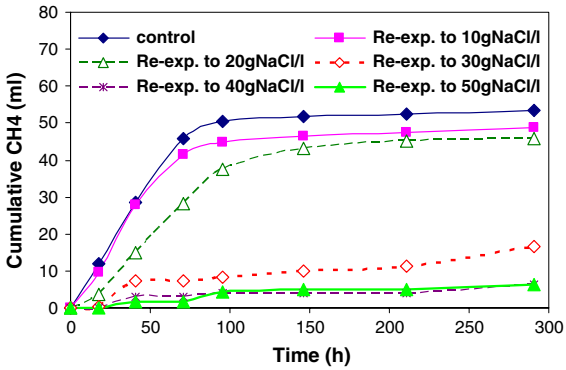

Fig. 3 Re-exposure of biomass to salinity after a period under non saline conditions

this period all the biomass samples showed negligible methane production compared to the control shown in Fig.1a. When the biomass was returned to non saline conditions it produced 40 ml CH_4 in 69 h, while the control produced around 50 ml for 2 gVSS l^{-1} (Fig. 2c). Exposure to 40 gNaCl l^{-1} for 2, 24, and 48 h did not result in any significant difference in methane production after the sudden removal of salinity.

Re-exposure to salt

After the two batch feedings with non saline media, the biomass was then re-exposed to the initial salinity concentrations. It can be seen from Fig. 3 that the biomass exposed to 10 and 20 gNaCl l^{-1} maintained almost the same activity of the third feeding before the exposure to no sodium. The methane production of biomass exposed to concentrations of salt higher than 30 gNaCl l^{-1} (Fig. 3) remained at the same low level as the third exposure to salinity, so no conclusion for the re-exposure to salinity can be drawn for this level of salinity.

Sodium effects on VFA production

Up to 20 gNaCl l^{-1} VFAs were catabolised by the anaerobes in 150 h and this is an indication of good performance (Fig. 4a). However, when the biomass was exposed to concentrations higher than 20 gNaCl l^{-1} this resulted in an accumulation of VFAs. As can be seen from Fig. 4b and c, the main VFAs forming under saline conditions were acetate and propionate, and increasing the concentration of sodium chloride resulted in an increase in VFAs (Fig. 4a). With biomass exposed to 20 gNaCl l^{-1} acetate was consumed after 90 h, whereas propionate was only consumed completely after 160 h. At concentrations higher than 20 gNaCl l^{-1} the acetate concentration was higher than the propionate. This occurs due to the higher overall production of acetate over propionate (seen from the control biomass), however, even after 250 h the concentration of these acids had not started to decrease significantly as a result of a severe inhibition of the propionic utilizers and methanogens. Glucose was found only in the first 11 h at concentrations of 362 mg l^{-1} and 453 mg l^{-1} for biomass exposed to 40 and 50 gNaCl l^{-1}, respectively.

The role of formic acid in methanogenesis under high salinity

Formic acid was found only during the initial period at a concentration of 40 mg l^{-1} for the control at 17 h, while for biomass exposed to the highest salinity (50 gNaCl l^{-1}) 43 mg l^{-1} was produced after 44 h. This was an indication that salinity was not inhibitory to the formic acid formers. Moreover, it is likely the highest percentage of the CH_4 was produced by the reductive methanogens due to the fact that the acetoclastic methanogens were significantly inhibited. However, to confirm that reductive methanogens were not inhibited formic acid was fed to the biomass (Dolfing and Bloemen 1985). Anaerobic biomass was initially exposed to 2 gCOD l^{-1} of formic acid for 25 days in serum bottles to increase the population and activity of reductive methanogens. In the next batch test the same concentration of formic acid was used with salinities of 20 and 40 gNaCl l^{-1}. From the first feeding at 40 gNaCl l^{-1} methane production almost reached the methane control level after 140 h (Fig. 4d) compared with the biomass fed glucose (Fig. 3) which in the same time only produced 4.5 ml of CH_4, and hence it was clear that salinity did not inhibit reductive methanogenesis.

Effect of sudden removal of salt on anaerobic bacteria by monitoring VFAs

VFA production (acetic, propionic and n-butyric acids) under no salt conditions after long exposure of the biomass to salinity is shown in Fig. 5a–c. The

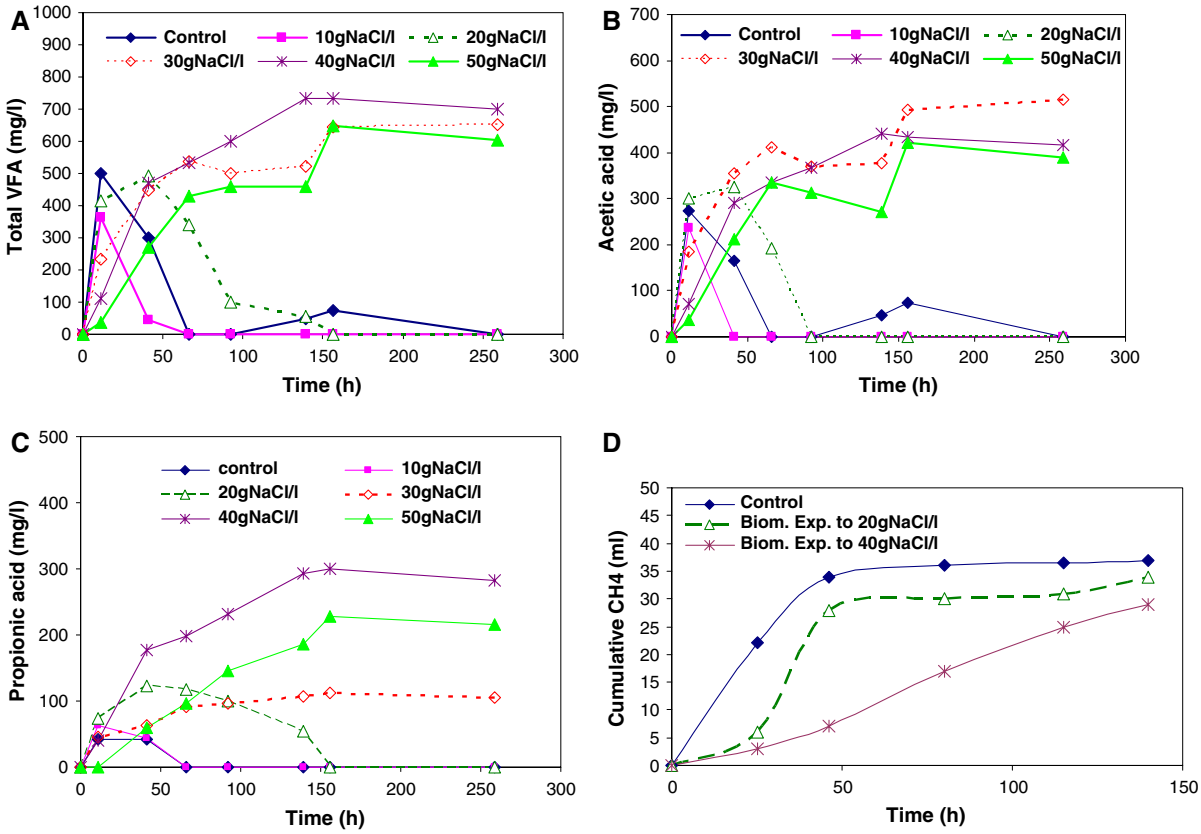

Fig. 4 (**a**) Total VFA production in the first batch feeding for biomass exposed to 0–50 gNaCl l^{-1}. (**b**) Acetic acid production in the first batch feeding for salinity concentrations of 0–50 gNaCl l^{-1}. (**c**) Propionic acid production in the first batch feeding for salinity concentrations of 0–50 gNaCl l^{-1}. (**d**) Cumulative CH_4 production from biomass exposed to no salinity, and 20 and 40 gNaCl l^{-1}. Formic acid used as a sole carbon source

biomass that was previously exposed to the highest salt conditions showed the slowest degradation of VFAs. For biomass previously exposed to up to 30 gNaCl l^{-1} (Fig. 5a–c), propionic acid was the only remaining acid. For biomass previously exposed to 40 and 50 gNaCl l^{-1} the remaining acids were acetic, propionic and n-butyric.

Chromatographic analysis of supernatant from biomass exposed to 0-50 gNaCl l^{-1}

In SEC the organics with a low molecular weight (MW) go deeper into the gel pores and so take a longer time to elute, whereas the high MW compounds are quickly excluded from the column. As can be seen from Fig. 6a, the presence of salt (40 gNaCl l^{-1}) after the first 24 h resulted in much higher intensities (concentrations) of soluble organics compared to biomass exposed to no salt, while biomass exposed to 20 gNaCl l^{-1} had signal intensities between the two. It appears that the presence of higher NaCl concentrations caused a greater accumulation of SMPs with higher MWs, especially those eluting at 9–10 min, corresponding to compounds in the range 1.147–7.12 kDa; these compounds did not appear in cultures with no salt. This result suggests that biomass exposed to high salinity produced higher MW compounds. The results after 48 h (Fig. 6b) show that for biomass exposed to 20 gNaCl l^{-1} there were no peaks at 9–10 min, while the other peaks appeared at the same retention time, but with a lower signal. This may be due to the degradation of the organics in the initial bulk liquid and the simultaneous accumulation of methane. On the other hand, biomass exposed to 40 gNaCl l^{-1} resulted in the generation of two new peaks at 7–8 min and 10–11 min. The same behaviour was found for biomass exposed to 50 gNaCl l^{-1} from 24 to 48 h (Fig. 6c).

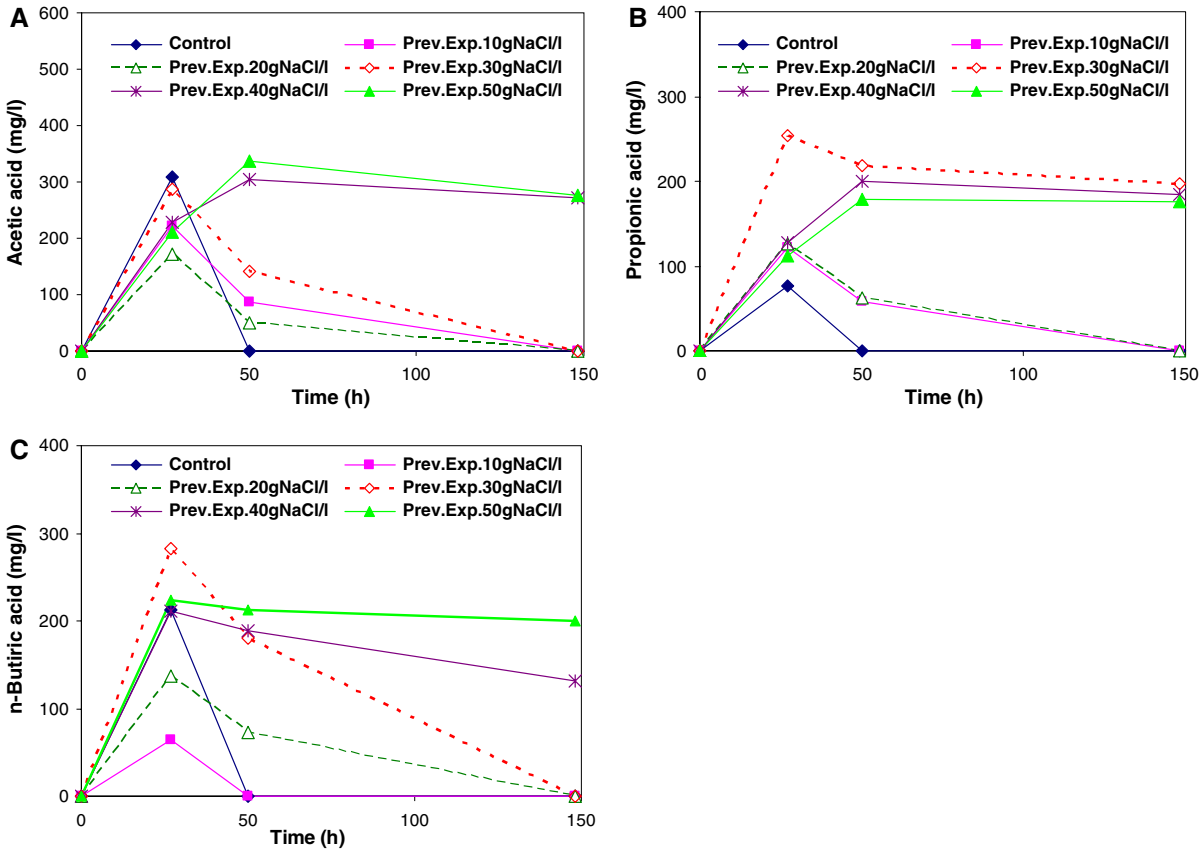

Fig. 5 (**a**) Acetic acid production in the first batch feeding at non saline conditions after the exposure to saline conditions for concentrations of 0-50 gNaCl l^{-1}. (**b**) Propionic acid production in the first batch feeding under non saline conditions after exposure to saline conditions for concentrations of 0-50 gNaCl l^{-1}. (**c**) N-Butyric acid production in the first batch feeding under non saline conditions after exposure to saline conditions for concentrations of 0-50 gNaCl l^{-1}

For the biomass after 48 h, the compounds at 7–8 min and 10-11 min may have been produced from the partial degradation of the compounds at 4–5 min and 9–10 min respectively (Fig. 6b, c).

Chromatographic analysis of effluent from biomass exposed to no salinity after a long exposure to 20 and 40 gNaCl l^{-1}

The chromatograph of the biomass that was subjected to no salinity after a long exposure to saline conditions (20 and 40 gNaCl l^{-1}) is shown in Fig. 7. The higher peak at around 13 min is probably VFAs and intermediates of glucose. The lower peak eluted at about 5 min is high MW, probably as a results of cell lysis. This peak has a higher intensity than for the biomass previously exposed to 40 and 20 gNaCl l^{-1} compared with the biomass that was not exposed to saline conditions before. The intensity of the peak at 13 min for the biomass is considerably lower compared with the biomass exposed to saline conditions.

Discussion

The results of re-feeding under batch conditions show that the acclimation potential is rapid for biomass exposed to 10 gNaCl l^{-1}, and relatively fast for biomass exposed to 20 gNaCl l^{-1}. Even with biomass exposed to 30 gNaCl l^{-1} acclimation is possible at a slower rate. However, biomass that was exposed to 40–50 gNaCl l^{-1} over a period of 270 h showed no acclimation, even after the third refeeding.

According to Speece (1996), toxicity should be interpreted as an adverse effect, not necessarily

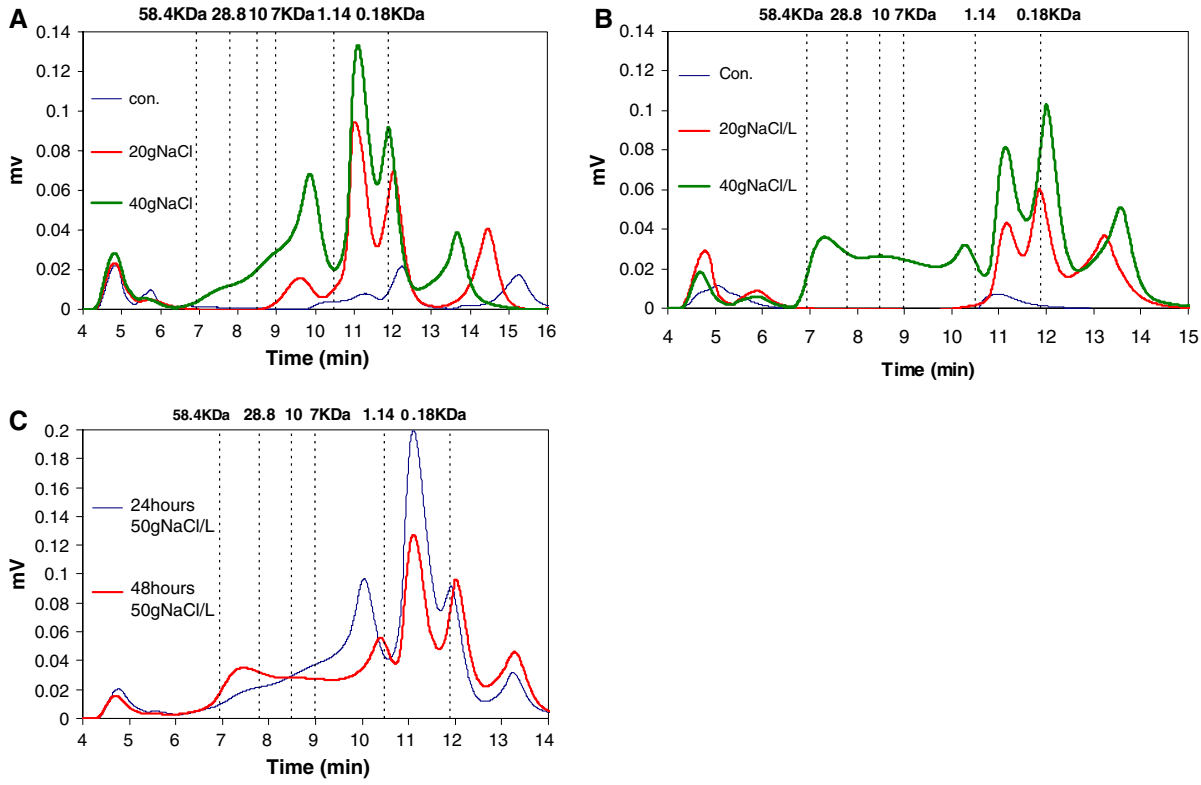

Fig. 6 (**a**) MW after 24 h for control, biomass exposed to 20 and 40 gNaCl l^{-1}. (**b**) MW after 48 h for control, biomass exposed to 20 and 40 gNaCl l^{-1}. (**c**) MW for biomass exposed to 50 gNaCl l^{-1} at 24 and 48 h

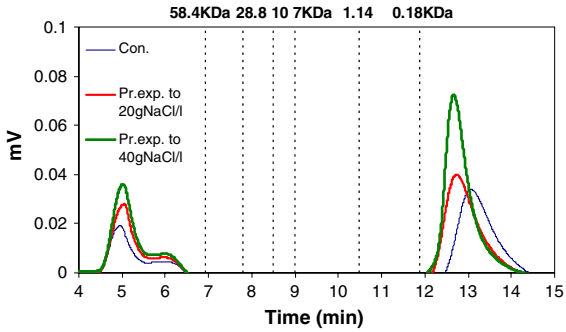

Fig. 7 MW after 24 h under non-saline conditions for biomass that was previously exposed to 0, 20 and 40 gNaCl l^{-1}

lethal, on bacterial metabolism, whereas inhibition should be considered as an impairment of bacterial function. The effect of a sudden removal of salinity from aerobic systems is probably lethal on aerobic bacterial populations (Kinncannon and Gaudy 1966, 1968); Burnett (1974) using activated sludge showed BOD removal efficiencies dropped from 95 to 25%, with an increase of 3.5% in the effluent solids, when a freshwater feed was changed to a salty feed, but when returning to a freshwater feed the removal efficiency reduced to 8%. Kinncannon and Gaudy (1966), using an aerobic sludge, found more severe effects with the sudden removal of salinity than an increase. Comparing the results of his study with that of Kinncannon and Gaudy (1966); Burnett (1974) showed that anaerobic biomass performs better than aerobic biomass under sudden changes from saline to fresh water conditions. In the present study, some continuing methane production following a sudden removal of salinity after long exposures to high salt concentrations (40–50 gNaCl l^{-1}) indicates that the biomass was not fully destroyed. Moreover, a doubling of the activity after the second feeding, and recovery of its initial activity in term of total methane (100%) over a short time (250 h) without salt indicated that anaerobic bacteria were only temporarily deactivated by high salinity, so sodium caused more of an adverse effect than impairment with anaerobic bacteria. With biomass exposed to lower concentrations of 30 gNaCl l^{-1} the initial activity was

re-established faster. When the anaerobic biomass was returned to non saline conditions after a short period (2–48 h) under highly saline conditions (40 gNaCl l^{-1}), it produced approximately 80% of the amount of methane as the control after three days. This has practical implications in anaerobic treatment as a temporary salinity shock (up to 48 h) would appear to only partially inhibit methanogenesis when the biomass is returned to normal conditions.

Re-exposure to salinity after the two non-saline feedings showed that the biomass maintained most of the activity that had developed after the third batch feeding with salinity. Anaerobic biomass that had adapted to salinity levels up to 20 gNaCl l^{-1} can withstand variations in salinity quite effectively. The propionic acid utilisers seem to be the most sensitive group under high salinities as propionate was the last VFA biodegraded at salinities up to 20 gNaCl l^{-1}, and was present at high concentrations at higher salinities. This shows that the rate of biodegradation by the propionic utilizers was significantly decreased in the presence of NaCl. This finding is in agreement with Soto et al. (1993) who found that an increase in the sodium concentration from 5 to 12 gNa^{+} l^{-1} resulted in an increase in the propionate to acetate ratio from 0.7 to 1. However, Feijoo et al. (1995) using granular non-adapted biomass found that n-butyrate utilisers were the most sensitive group of bacteria. The contradictory results between the present study and the study of Feijoo et al. (1995) can possibly be attributed to different morphological characteristics of the biomass which had an effect on the sensitivity of each bacterial group. The acetoclastic methanogens were found to be the second most sensitive group to sodium in a mixed anaerobic biomass that had not been adapted to sodium. In contrast, the reductive methanogens that utilize H_2 and CO_2 were not affected severely by sodium toxicity. Liu and Boone (1991) used a sludge that was acclimated to salinity and found that the reductive methanogens were the least affected by sodium. However, Zheng et al. (2005) found less production of H_2 and a reduction of the percentage of H_2 in the gas at higher sodium concentrations indicating that there was inhibition of H_2 utilising microorganisms by sodium. In the present study, the production of formic acid by anaerobic biomass during the initial period, and the low inhibition at high salinity of reductive methanogens when formic acid was used as a substrate indicates that more substrate could be consumed through reductive methanogenesis under high salinity compared to non saline conditions. The glucose oxidisers showed a high tolerance to sodium as there was no remaining glucose after 40 h. As was mentioned above, the anaerobic biomass can function relatively well with sudden changes from saline to normal conditions. During this sudden change the propionate utilisers had the slowest degradation rate, and in general are the most sensitive to salinity fluctuations. The n-butyric utilisers were also affected significantly by the sudden reduction of salinity as the degradation rate of butyric acid was seriously decreased for biomass previously exposed to 40 and 50 gNaCl l^{-1}. However, under high salinity n-butyric utilizers did not show any inhibition, probably as a result of fast biodegradation of butyric acid under these conditions.

The low methane production rate at high salinity could also be due to the consumption of substrate by anaerobic biomass to generate compatible solutes and extracellular polysaccharides to survive under high osmotic conditions (Vyrides and Stuckey 2007). Another factor could be the high MW organics that are produced in the reactor, possibly due to the release of extracellular compounds during metabolism, enhancement of cell lysis, or stimulation of efflux mechanisms (Aquino and Stuckey 2006). These high MW SMPs with high salinity wastewaters are significantly higher compared to no salt wastewater, and hence the higher MW fractions of the COD are more slowly degraded resulting in poor performance of the anaerobic biomass. The results of this study show that the high MW compounds (SMPs) from biomass exposed to 40–50 gNaCl l^{-1} were hardly degraded as they remained in the medium over time, and these high MW compounds (SMPs) did not appear in concentrations lower than 20 gNaCl l^{-1}. These findings highlight the fact that to increase the performance of an anaerobic reactor treating saline wastewater, attention should be paid to the removal of the SMPs. The use of a membrane reactor could be a solution as it can separate the high MW compounds from the effluent (Aquino et al 2006). In the case of a sudden removal of salinity from biomass which had been exposed to saline conditions for a long time, the SMPs do not seem to cause any severe toxic effects.

Conclusions

- Anaerobic biomass can acclimatize to salinities up to 20 gNaCl l^{-1} over a period of around 35 days during 3 batch feedings.
- Anaerobic biomass previously exposed to salinities up to 50 gNaCl l^{-1} for 35 days can recover quickly to full activity under non saline conditions. The exposure to sodium can be considered more as an adverse effect on biomass metabolism than fatal, and the initial activity can be recovered over time.
- Anaerobic biomass re-exposed to 10 and 20 gNaCl l^{-1} retains almost the same activity that had been present before the exposure to no sodium for 40 days.
- Amongst biomass not adapted to salinity, the propionate utilisers seem to be the most sensitive group to sodium toxicity, while the acetoclastic methanogens were the second most sensitive. In contrast, the reductive methanogens and acid formers were not severely affected by sodium toxicity. With a sudden reduction in salinity, the propionate and n-butyric utilisers and acetoclastic methanogens were affected more.
- Results from SEC reveal that anaerobic biomass under high salinity release high MW SMPs that are difficult to biodegrade.

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