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ARTICLE *in* ENVIRONMENTAL SCIENCE & TECHNOLOGY · OCTOBER 2010

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Are Compatible Solutes Compatible with Biological Treatment of Saline Wastewater? Batch and Continuous Studies Using Submerged Anaerobic Membrane Bioreactors (SAMBRs)

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Received January 24, 2010. Revised manuscript received August 15, 2010. Accepted August 23, 2010.

This study investigated fundamental mechanisms that anaerobic biomass employ to cope with salinity, and applied these findings to a continuous SAMBR. When anaerobic biomass was exposed to 20 and 40 g NaCl/L for 96 h, the main solute generated de novo by biomass was trehalose. When we separately introduced trehalose, *N*-acetyl-β-lysine and potassium into a batch culture a slight decrease in sodium inhibition was observed. In contrast, the addition of 0.1 mM and 1 mM of glycine betaine dramatically improved the adaptation of anaerobic biomass to 35 g NaCl/L, and it continued to enhance the adaptation of biomass to the salt for the next three batch feedings without further addition. No shift in archaeal microbial diversity was found when anaerobic biomass was exposed in batch mode to 35 g NaCl/L for 360 h, and no changes were found when glycine betaine was added. The dominant species identified under these conditions were *Methanosarcina mazeii* and *Methanosaeta* sp. The addition of 5 mM glycine betaine to a continuous SAMBR at 12 h hydraulic retention time (HRT), and operation in batch mode for 2 days can significantly enhance saline (35 g NaCl/L) synthetic sewage degradation. In addition, the injection of 1 mM of glycine betaine into a SAMBR for five subsequent days also significantly enhanced dissolved organic carbon (DOC) removal from sewage under these conditions. The main compatible solutes generated by anaerobic biomass after 44 days exposure to 35 g NaCl/L in a SAMBR were *N*-acetyl-β-lysine and glycine betaine. Finally, the addition of 1 mM glycine betaine to the medium was beneficial for anaerobic biomass in batch mode at 20 °C under saline and non saline conditions.

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1. Introduction

Saline wastewater is discharged by many industries (*1, 2*), and represents around 5% of the total wastewater discharged (*1*). However, sodium is known to inhibit anaerobic treatment of organics, and given the advantages of anaerobic digestion such as methane evolution and low sludge production, it would be desirable if these wastewaters could be treated anaerobically. Nevertheless, several studies using anaerobic biomass reported adequate organic removal by employing strategies such as a long biomass adaptation period to salinity (*3, 4*), bioaugmentation with halophilic microorganisms (*5, 6*), marine inocula (*7*), membrane bioreactors (*8, 9*) and anaerobic digestion followed by aerobic treatment (*10*). However, most of these studies only investigated a gradual increase in salinity despite the fact that it is often highly variable in many industrial wastewaters (*2*). Moreover, in many industries salinity only increases during certain short periods of the year, while in some salinity can suddenly increase due to process instability and this could be a serious drawback for biological treatment. Based on the above considerations, this study examined a new approach for the effective treatment of highly fluctuating saline wastewater in a SAMBR by employing strategies that enabled the cells to cope with stress conditions through the use of compatible solutes.

There are two fundamental strategies for cells to survive under osmotic stress; (a) cells increase the intracellular ion concentration (mainly potassium) in order to balance the external osmotic pressure, and all intracellular enzymes have to adapt to the new conditions, the so-called "salt in strategy" (*11*). (b) Many microorganisms accumulate organic solutes intracellularly called "compatible solutes". The high external osmotic pressure is balanced within the cytoplasm by organic compatible solutes without the need for any special adaptation of the intracellular enzymes, and compatible solutes also serve as protein stabilizers in the presence of high ionic strength inside the cell (*11*). These solutes can be synthesized by the cell, or provided by the medium, but for most species uptake from the medium is energetically more favorable than synthesis (*12*). Methanogenic archaea isolated from moderately saline environments showed an accumulation of β-glutamine, α-glutamate, *N*-acetyl β-lysine and glycine betaine (*11, 13, 14*).

Despite these insights into cell mechanisms, only Yerkes et al. (*15*) successfully found that the addition of small concentrations of betaine (1−10 mM) to pure cultures of *Methanosarcina* and *Methanosaeta*; in sucrose fed batch assays, continuous stirred-tank reactor (CSTRs), fluidized bed reactors (FBR) and upflow anaerobic sludge blanket bioreactor (UASB) reactors reduced sodium toxicity. Vyrides and Stuckey (*16*) found that anaerobic biomass exposed to 35 g NaCl/L for 28 days showed little acclimatization to salinity. In contrast, anaerobic biomass that had not been exposed to salinity before but had glycine betaine added to the saline medium increased its acclimation potential significantly (*16*). However, Vallero et al. (*17*) using a mixture of compatible solutes found no significant reduction in sodium toxicity in thermophilic sulfate reducing bacteria (SRBs) in a UASB reactor and batch assays. Aside from these findings, no studies have examined the effect of betaine addition on a mixed anaerobic culture in a bioreactor for the treatment of saline wastewater.

Apart from the generation of compatible solutes, and the accumulation of extracellular polysaccharides (EPS) by anaerobic biomass under salinity (*16*), microbial evolution

10.1021/es903981k
Published on Web 09/10/2010

TABLE 1. Operational Strategy for SAMBR-A and SAMBR-B

days	HRT (h)	NaCL (g/L)	sewage DOC (mg/L)	glycine betaine addition strategy for SAMBR-A
0−13	12	0.2	145	
14−33	12	35	145	day 14/addition of 1 mM glycine betaine
34−52	12	0.2	145	
53−73	12	35	145	days 54−64/addition of 0.1 mM glycine betaine
74−81	12	0.2	145	
82−84	batch	35	145	day 82/addition of 5 mM Betaine and operation for 2 days in batch mode
85−92	12	35	145	
93−96	6	35	145	
97−126	12	35	145	days 115−119/addition of 1 mM betaine

could also be an important factor in the adaptation of biomass to salinity. Lefebvre et al. (*18*), using nonadapted biomass in batch reactors, found that increases in salinity had little effect on the microbial diversity of anaerobic biomass, and that the salinity mostly influenced the biodegradation rate. The same group found in other studies (*19, 20*) that the diversity of a salt-tolerant ecosystem treating hypersaline (21−57 gTDS/L) industrial wastewater could be similar to that of a nonsalt-tolerant one. Considering this data, we decided to examine the shift in the archaeal population under saline conditions in the presence and absence of compatible solutes in the media.

Apart from the rapid uptake of glycine betaine by cells under salinity, Chattopadhyay (*21*) reported that glycine betaine can be used as a cryoprotectant for bacteria. Under low temperatures, the glycine betaine maintains the cell membrane fluidity and protects the cellular proteins from cold aggregation. Cleland et al. (*22*) also found that glycine betaine can be effective as a cryoprotectant for prokaryotic organisms.

The aim of this study was 4-fold: first to find the main compatible solutes that were generated under salinity, and investigate which compatible solutes could reduce sodium inhibition when added to anaerobic biomass. Second, to study the effect of varying concentrations of glycine betaine on anaerobic biomass and their long-term effect under batch saline conditions; archaeal diversity was also investigated with and without glycine betaine under saline conditions. Third, to examine various strategies of glycine betaine addition to a SAMBR, and compare these with a SAMBR operating under the same saline conditions but without addition of glycine betaine. Finally, to investigate the effect of glycine betaine addition in batch mode on the performance of anaerobic biomass at 20 °C under saline and nonsaline conditions.

2. Materials and Methods

2.1. Laboratory Scale SAMBRs. Anaerobic biomass was obtained from a conventional sewage sludge digester (Mogden, UK), and maintained in a batch reactor (5 L) fed with glucose as a substrate for 3 months at an organic loading rate (OLR) of 2 gCOD/L.day and a salinity of less than 2 gNaCl/L. The biomass from this reactor was used as an inoculum for the serum bottle experiments (at concentration of 2 gVSS/L), and as an inoculum in the SAMBRs (3 L) with the same configuration as that used by Akram and Stuckey (*23*). A Kubota flat sheet membrane module (polyethylene) was used in this study with a surface area of 0.1 m^2 and a pore size of 0.4 μm. The reactors were maintained at 35 ± 1 °C, and the biomass was continuously mixed using headspace biogas (at 5 L/min) that was pumped through a stainless steel tube diffuser to generate coarse bubbles. A computer data logging system was used to monitor transmembrane pressure (TMP). During the 130 days of operation of the SAMBRs, TSS were approximately constant in the range of 5−6 g/L, while the VSS were in the range of 3.5−4.5 g/L. The operational strategy that was followed for SAMBR-A and B can be seen in Table 1. The reactors were fed with a synthetic sewage recipe (465 ± 20 mg COD/L or 145 mg DOC/L ± 20) comprised of peptone (0.22 g/L), meat extract (0.15 g/L) urea (0.01 g/L), K_2HPO_4 (0.008 g/L) and $NaHCO_3$ (200 mg/L).

2.2. Analytical Methods. Determination of volatile suspended solids (VSS) was performed according to standard methods (*24*). The composition of biogas was determined using a Shimadzu GC-TCD fitted with a Porapak N column (1500 × 6.35 mm). The carrier gas was helium at a flow rate of 50 mL/min, and the column, detector and injector temperatures were 28, 38, and 128 °C, respectively. The coefficient of variance (COV) for 10 identical samples was ±2%. Total organic carbon (TOC) was measured with a Shimadzu 5050 (Shimadzu, UK) TOC analyzer. Before TOC analysis, the biomass was removed by centrifugation and filtration (0.45 μm). The COV for five samples was within ±2%. TOC analysis was used instead of COD due to the interference of chloride ions in the saline samples.

For size exclusion chromatography (SEC) an Aquagel OH-30 column (Polymer Laboratories) was used with DI water as the eluent at a flow rate of 0.4 mL/min. The sample volume was 50 μL, and the column was maintained at ambient temperature with the UV detector set at 254 nm, a wavelength where most aromatic compounds and humic substances can be identified (*25*). Standards of polyethylene oxide (PEO) and polyethylene glycol (PEG) were used to calibrate the system and detected by refractive index (RI) detector. The results obtained are quoted relative to these linear standards.

The serum bottle experiments were conducted using the media and serum bottle techniques developed by Owen et al. (*26*). Duplicate samples were run for each condition and the COV was less than ±2%. The biomass concentration in the serum bottle was 2 g VSS/L.

DNA Extraction and PCR Amplification. The DNA was extracted using the UltraClean Microbial Genomic Isolation Kit (MoBio, Carlsbad, CA) according to the manufacturer's instructions. The extracted genomic DNA was used as a template in the PCR to amplify 16S rRNA genes. Archaeal fragments suitable for subsequent denaturing gradient gel electrophoresis (DGGE) were amplified with the primer combination 344fGC-915r as described by other workers (*27, 28*). The 50 μL PCR reaction mixtures contained 50 ng of template DNA, each deoxynucleoside triphosphate at a concentration of 200 μM, 3 mM $MgCl_2$, each primer at a concentration of 0.3 μM, 1 U Taq DNA polymerase (Invitrogen) and 0.5 mM PCR buffer supplied by the manufacturer. The same PCR reaction protocol followed by (*29*) was employed in this study.

DGGE, Cloning and Sequencing. The products of the PCR reaction were mixed with 5 μL of DGGE loading buffer (1% bromophenol blue, 1% xylene cyanol and 70% glycerol) and were loaded onto a 10% polyacrylamide gel (37.5:1, acrylamide/bisacrylamide) prepared with a denaturing gradient of 40−60% (100% denaturing gradient corresponds to 7 M urea and 40% formamide). The gel was run at 60 V for 20 h

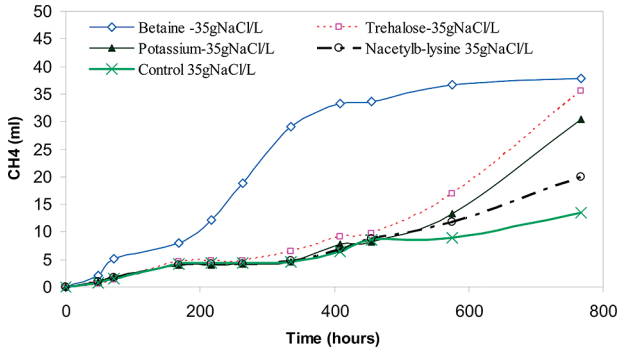

FIGURE 1. Methane production from anaerobic biomass in the presence of 1 mM of various compatible solutes in the medium under 35 gNaCl/L.

at 60 °C in TAE buffer (40 mM TRIS-HCl, 20 mM Acetic acid and 1 mM EDTA -pH 8.0). The gel was stained with SYBR Green I in TAE buffer for 30 min, and visualized under blue light. Prominent bands were excised from the gels, DNA eluted from the bands in sterile deionized water overnight, reamplified with the PCR primers 344F−915R, and purified using a high pure PCR product purification kit (Roche) prior to sequencing. The PCR product was cloned with the TOPO TA cloning kit (Invitrogen) into *E. coli* according to the manufacturer's instructions. Recombinant plasmids in *E. coli* were picked and grown up overnight in LB media (1% bacto-tryptone, 0.5% yeast extract and 1% NaCl) and plasmids extracted using the QIAprep spin miniprep kit (QIAgen), following the manufacturer's instructions. Sequencing reactions were performed direct from the plasmids by Cogenics (Takeley, Essex, UK).

NMR Analysis. The intracellular solutes were extracted with boiling 80% ethanol as described by ref *30*. Freeze-dried extracts were dissolved in distilled water and analyzed by NMR. Proton NMR spectra were acquired in a Bruker DRX500 spectrometer with 5 mm inverse detection probe head at 25 °C, with presaturation of the water signal, a 90° flip angle, and a repetition delay of 6.9 microseconds. Formate was added to the samples as a concentration standard.

3. Results and Discussion

3.1. NMR Analysis of Anaerobic Biomass after 96 h under Saline Conditions. Anaerobic biomass was exposed to 0, 20, and 40 g NaCl/L for 48 and 96 h, and then withdrawn and subjected to NMR analysis to identify the compatible solutes that accumulated during exposure to a salt stress environment. The results showed that trehalose was the dominant solute accumulated under anaerobic conditions, and its concentration for biomass exposed to 20 and 40 g NaCl/L (96 h) was in the range of 0.027−0.175 μmol/mg dry weight. The control biomass not exposed to osmotic stress did not contain trehalose (data not shown).

3.2. Addition of Different Compatible Solutes and Potassium to Anaerobic Biomass. Based on the above NMR data and previous studies of anaerobic mixed cultures under salinity (*16*), serum bottle experiments were carried out by adding the following osmoprotectants separately to the medium: 1 mM trehalose, 1 mM glycine betaine, 1 mM *N*-acetyl-β-lysine and 1 mM potassium. As can be seen from Figure 1, glycine betaine was the most effective in alleviating sodium inhibition in anaerobic biomass, and after 576 h had produced 36.6 mL CH_4. Interestingly, despite trehalose being the dominant compatible solute produced de novo in the first 96 h, addition of it to the medium reduced sodium inhibition, but not to the same degree as the glycine betaine (Figure 1). This agrees with the study in ref *31* who found that trehalose was the main solute generated in the atrazine-degrading bacterium *Pseudomonas* sp. strain ADP. When

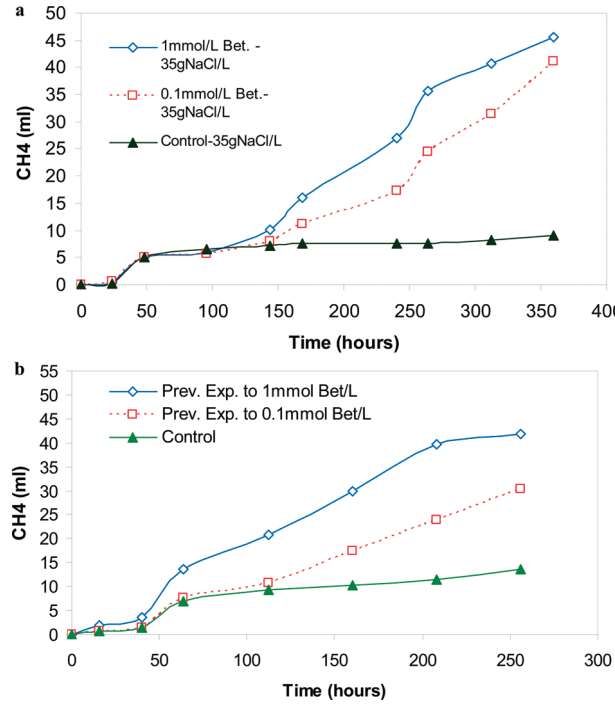

FIGURE 2. (a) Methane production from anaerobic biomass in 35 gNaCl/L with addition of glycine betaine. (b) Methane production from anaerobic biomass after three batch feeding in 35 gNaCl/L where the media was separated from the cells after each batch.

they added trehalose to the medium no positive effect was noted, and only the addition of glycine betaine induced salt tolerance (*31*). The addition of 1 mM *N*-acetyl-β- lysine slightly alleviated sodium inhibition (20 mL CH_4 in 768 h). The uptake of glycine betaine by anaerobic biomass is a strategy to cope with salinity, however, the uptake of trehalose does not seem to be a dominant mechanism. The addition of potassium also had a positive effect on reducing sodium inhibition compared with the control, and after 576 h the bottle had produced 13.5 mL CH_4, however, its effect was less positive compared with glycine betaine. An early study by Kugelman and McCarty (*32*) found that low concentrations of potassium can reduce the sodium inhibition of methanogens, and contributed to reactivating the damaged enzymes. It is likely that the increase in performance of biomass with a low concentration of potassium under high salinity is due to a "salt in strategy", and not to the reactivation of the damaged enzymes. Nevertheless, these results highlight the fact that anaerobic biomass prefers the "compatible solutes strategy" for osmoregulation rather than the "salt in strategy".

3.3. Effect of Different Glycine Betaine Concentrations on Anaerobic Biomass. As can be seen from Figure 2a, addition of 0.1 mM betaine to the medium can be beneficial for anaerobic biomass, and at 35 g NaCl/L the CH_4 production after 360 h was 41.2 mL. When 1 mM of betaine was added the CH_4 production was 45.6 mL in 360 h, while under the same conditions without any compatible solutes the biomass produced 9.1 mL after 360 h. These results show that a concentration as low as 0.1 mM of glycine betaine can be beneficial for anaerobic biomass, and this strategy has considerable potential not only for effective treatment, but also from an economic point of view.

3.4. Prolonged Effect of Glycine Betaine on Anaerobic Biomass. In order to study the long-term effect of glycine betaine, the medium was removed by centrifugation after each batch and replaced with a new medium (35 g NaCl/L) without any addition of compatible solutes in any of the samples for the following four batch feedings. As can be seen

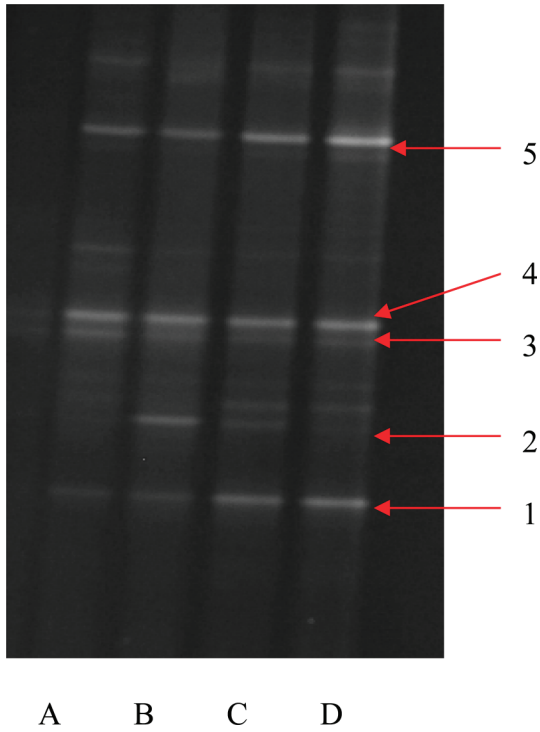

FIGURE 3. DGGE fingerprint of anaerobic biomass under saline and nonsaline conditions. (A) the initial biomass withdrawn from the CSTR; (B) the biomass after 360 h in a serum bottle under normal conditions (2 g glucose COD/L); (C) the biomass that was subjected to 35 g NaCl/L; and (D) the anaerobic biomass that was subjected to 35 g NaCl/L with 1 mM glycine betaine added.

from Figure 2b, after the third feeding the anaerobic biomass that was previously exposed to 1 mM of glycine betaine (first feeding) increased its activity and produced 41.8 mL CH_4 in 256 h. The biomass that was previously exposed to 0.1 mM of glycine betaine produced 30.4 mL CH_4 in 256 h, while the control anaerobic biomass produced 13.7 mL in 256 h. The above results demonstrate the beneficial effect of glycine betaine over time, even when it is not added continuously. It is likely that the anaerobic biomass maintains the betaine inside the cell so it can act as osmoprotectant over time.

3.5. Archaeal Evolution under Salinity. DGGE analysis was used to investigate the shift in Archaea populations under saline conditions with the addition of glycine betaine, and without. Figure 3 shows the anaerobic biomass DGGE fingerprint of the following biomass: (A) the initial biomass withdrawn from CSTR; (B) biomass after 360 h in a serum bottle under normal conditions (2 g glucose COD/L), (C) biomass that was subjected to 35 gNaCl/L, and (D) anaerobic biomass that was subjected to 35 g NaCl/L with 1 mM betaine added. As can be seen from Figure 3, there was very little change in the archaeal population after 360 h when the anaerobic biomass was subjected to 35 g NaCl/L. There was also very little change observed in archaeal species with the addition of glycine betaine. However, the intensity of band number 1 (Figure 3) is stronger for biomass under saline conditions (C and D) than under low salinity (A and B), indicating that there is a larger population of the species that the band represents in the more saline conditions. This is in contrast to the intensity of band number 2 (Figure 3), which shows the opposite and is less intense under saline conditions. These results show that there are no major changes in archaeal diversity when the culture conditions change from low salinity to high salinity, and there are no changes even when glycine betaine was added under saline conditions. Sequence analysis of the dominant bands (*4*) and (*5*) showed the presence of *Methanosaeta* sp. and *Methanosarcina mazeii*, and there is a strong indication that these species are halotolerant. Lefebvre et al. (*18*) also reported that *Methanosaeta* sp. were present in anaerobic batch reactors under 60 g NaCl/L. However, if there is no significant shift in the archaeal microbial community with salinity then the factors that govern the survival of anaerobic biomass under high salinity must be the generation of compatible solutes, and the production of EPS (*16*).

3.6. Strategies of Glycine Betaine Addition to SAMBR. Two SAMBRs were used in order to investigate different strategies of addition of glycine betaine under high fluctuations in salinity (0 and 35 g NaCl/L) during continuous operation, and these are summarized in Table 1. During the first strategy the SAMBRs were operated under low salinity (200 mg NaCl/L), at 12 h HRT, for 13 days and both SAMBRs had similar performance (Figure 4). On day 14, salinity was increased to 35 g NaCl/L and 1 mM Betaine was added to SAMBR-A: on day 33 SAMBR-A contained 87.6 mg and 100.4 mg DOC/L in the effluent and bulk, respectively. SAMBR-B, which had no betaine added, contained 97.5 mg and 109.3 mg DOC/L in the effluent and bulk, respectively. On day 34, salinity was reduced to a low level (200 mg NaCl/L) for 18 days and under these conditions the performance of both SAMBRs improved (Figure 4). On day 53 the salinity was increased suddenly to 35 g NaCl/L, and 0.1 mM of betaine was added to SAMBR-A from day 54 until day 64: as a result, on day 73 SAMBR-A had 86.5 mg DOC/L in the effluent and

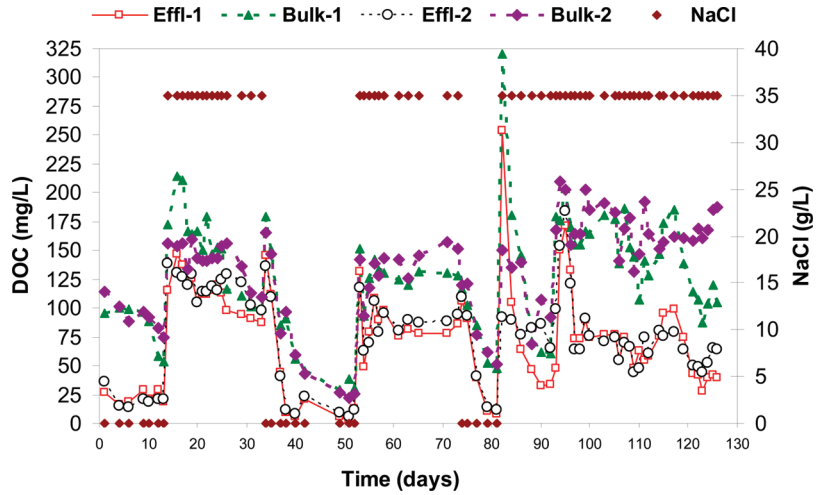

FIGURE 4. DOC removal (%) in the reactor and effluent for SAMBRs. Influent 145 ± 20 mg/L.

128.7 mg DOC/L in the bulk. SAMBR-B with no betaine added had a slightly higher DOC in the effluent (94.4 mg DOC/L) and a significantly higher DOC in the bulk (151.5 mg DOC/L). On day 74 a sudden decrease in salinity was imposed in order to investigate how the reactors responded, and both SAMBRs showed fast recovery (Figure 4). On day 82, salinity was increased to 35 g NaCl/L and 5 mM Betaine was added to SAMBR-A; for the following 2 days the SAMBRs were operated in batch mode to eliminate any risk of washout of glycine betaine from the reactor. On day 92, at 12 h HRT and 35 g NaCl/L, SAMBR-A showed significantly higher removal of DOC; 34.4 mg DOC/L in the effluent and 60.8 mg DOC/L in the bulk, while SAMBR-B had 65.2 mg and 92.5 mg DOC/L in the effluent and bulk, respectively. On day 93, the HRT was reduced from 12 to 6 h and this resulted in a decrease in performance of SAMBR-A; on day 96 the effluent and bulk for this reactor was around 133 mg and 170 mg DOC/L, respectively. SAMBR-B showed similar low performance (Figure 4). On day 97, the HRT was increased to 12 h and in the following days both SAMBRs showed almost the same performance (Figure 4). From day 115 until 119, 1 mM betaine was added to SAMBR-A, and this caused a decrease of the DOC on day 126−39.4 mg/L and 104.9 mg/L for the effluent and the bulk, respectively. Under these conditions SAMBR B showed a lower recovery; on day 126 the DOC in the effluent was 64.1 mg/L and bulk was 187 mg/L.

The above results show that in a continuous reactor (12 h HRT) the addition of 1 mM Betaine slightly alleviates sodium inhibition, and the strategy of adding 0.1 mM glycine betaine for 10 days slightly improved the performance of a continuous SAMBR. On the other hand, the addition of 5 mM glycine betaine and operation in batch mode for 2 days significantly enhanced organic degradation in the SAMBRs. Nevertheless, this high performance was dissipated when the HRT was reduced from 12 to 6 h. This was probably due to the rapid disruption of the cycle between solutes and biomass (*16*). Another strategy that resulted in high performance was the injection of 1 mM glycine betaine for 5 days. Despite the positive effects of adding glycine betaine to anaerobic biomass under batch saline conditions, several betaine addition strategies were not so effective under continuous operation. This could be due to the time required for the glycine betaine to be taken up by the anaerobic biomass, and some of the glycine betaine may have been biodegraded by anaerobic bacteria (*33*). Also, the continuous exposure of organic substrates to anaerobic biomass may slow down their adaptation to saline conditions. The high performance of anaerobic biomass when subjected to changes between saline and nonsaline conditions is in agreement with previous work (*34*).

3.7. NMR Analyses of Anaerobic Biomass from SAMBRs. The main solutes accumulated by anaerobic biomass from SAMBRs using medium with (SAMBR-A) and without added betaine (SAMBR-B) are trehalose, glycine-betaine, and *N*-acetyl-β-lysine. NMR analysis of the intracellular solutes of biomass collected from SAMBR-A on day 85, after exposure to salt stress for two days, showed the accumulation of glycine-betaine and *N*-acetyl-β-lysine (Figure 5). In contrast, no compatible solute was accumulated in biomass from SAMBR-B on day 85. The lower performance of SAMBR-B may be related to the absence of glycine-betaine in the medium; biomass in SAMBR-A accumulated glycine-betaine that was most probably taken up from the medium. At day 126 the intracellular content of glycine-betaine and *N*-acetyl-β-lysine decreased notably in the biomass collected from SAMBR-A (12-fold and 5-fold, respectively), while the biomass from SAMBR-B accumulated *N*-acetyl-β-lysine as a strategy to adapt to salinity (Figure 5). After 44 days of exposure to salinity (day 126) the biomass (SAMBR-A) was already acclimatized to salinity and reduction in the solute pool was probably due to catabolism of osmoprotectants.

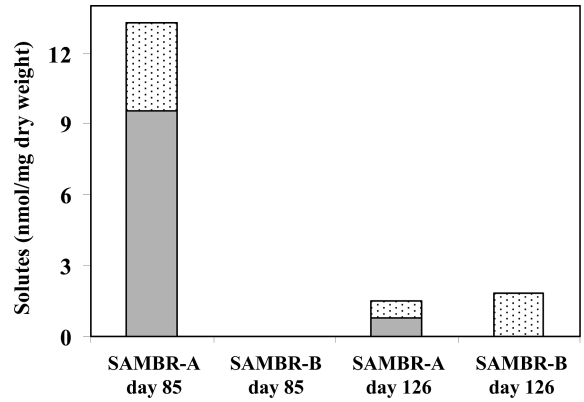

FIGURE 5. Accumulation of compatible solutes in suspended biomass from medium SAMBR-A and SAMBR-B, collected at days 85 and 126. *N*-acetyl-β-lysine (dotted); glycine-betaine (gray).

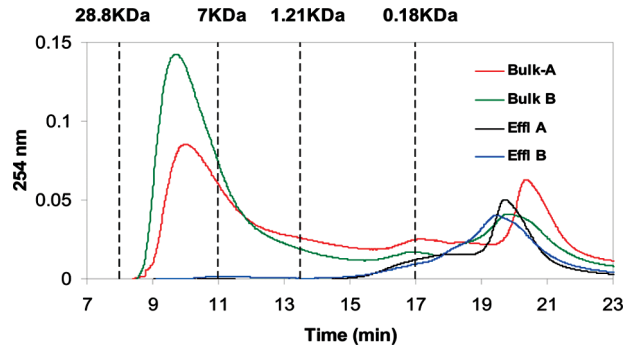

FIGURE 6. Size exclusion chromatograms inside the bioreactors (A) and (B) (bulk), and in the effluent.

3.8. Soluble Microbial Products (SMP) and TMP Pressure. Figure 6 shows the MW distribution of the reactor bulk and effluent for both SAMBRs at 254 nm on day 125. As can be seen, most of the organics present in both SAMBRs were in the 7−28.8 kDa MW range. The organics with a MW less than 0.18 KDa are likely to be volatile fatty acids (VFAs). The concentration of organics in SAMBR-A is 1.8 times less than that from SAMBR-B due to the presence of glycine betaine in SAMBR-A, and the rapid adaptation of anaerobic biomass to saline conditions. The exposure of anaerobic biomass in SAMBR-B to high salinity (35 g NaCL/L) resulted in the generation of EPS and release of SMPs (*16, 34*), and as a result more organics were present in SAMBR-B at higher concentrations; compared with SAMBR-A the addition of glycine betaine enhanced the acclimatization to salinity. However, the results of size exclusion chromatography showed that in the effluent only the low MW compounds (less than 0.18KDa) (Figure 6) were present due to filtration and rejection of the remaining higher MW compounds by the membrane and the biofilm. This highlights one of the positive attributes of a membrane bioreactor when treating toxic wastewater since it can maintain an effluent free from high MW organics, and achieve high COD removals. Clearly it can also maintain biomass within the reactor even when they are adapting, and enable them to grow and survive rather than being washed out of a suspended growth reactor.

During the first 14 days under low salinity and 9 LMH, the TMP for both SAMBRs was around 0.14 bar. The sudden increase in salinity on day 14 resulted in an increase in TMP by 0.06−0.1 bar and 0.06−0.18 bar for SAMBR-A and SAMBR-B, respectively. This difference may be due to the injection

of glycine betaine into SAMBR-A, and as a result the biomass adapted faster to saline conditions and released less SMPs.

3.9. Effect of Glycine Betaine Addition to Anaerobic Biomass at 20 °C under Saline and Nonsaline Conditions. In this study we examined biomass performance at 20 °C under saline and nonsaline conditions with and without the addition of 1 mM glycine betaine. The anaerobic biomass with glycine betaine produced 38.7 mL CH_4 in 408 h while the control biomass only produced 25.7 mL CH_4. At the same temperature with 35 gNaCl/L, biomass with glycine betaine generated 17.7 mL CH_4 while biomass without compatible solutes produced only 3.7 mL of CH_4. These preliminary results raise an interesting new approach for the biological treatment of wastewater under low temperatures. More research needs to be done in this area such as evaluating a variety of compatible solutes and different feeding strategies in order to explore the usefulness of this approach at low temperatures.

Acknowledgments

I.V thanks the A.G. Leventis Foundation for the award of a scholarship.

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ES903981K