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ORIGINAL PAPER

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Long-term adaptation of methanol-fed thermophilic (55 °C) sulfate-reducing reactors to NaCl

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Abstract A laboratory-scale upflow anaerobic sludge bed (UASB) reactor was operated during 273 days at increasing NaCl concentrations (0.5–12.5 g NaCl l^{-1}) to assess whether the stepwise addition of the salt NaCl results in the acclimation of that sludge. The 6.5-l thermophilic (55 °C), sulfidogenic [a chemical oxygen demand (COD) to SO_4^{2-} ratio of 0.5] UASB reactor operated at an organic loading rate of 5 g COD l^{-1} day^{-1}, a hydraulic retention time of 10 h and was fed with methanol as the sole electron donor. The results show that the adaptation of the thermophilic, sulfidogenic methanol-degrading biomass to a high osmolarity environment is unlikely to occur. Sulfide was the main mineralization product from methanol degradation, regardless of the NaCl concentration added to the influent. However, sulfide production in the reactor steadily decreased after the addition of 7.5 g NaCl l^{-1}, whereas acetate production was stimulated at that influent NaCl concentration. Batch tests performed with sludge harvested from the UASB reactor when operating at different influent salinities confirmed that acetate is the main metabolic product at NaCl concentrations higher than 12.5 g l^{-1}. The apparent order of NaCl toxicity towards the different trophic groups was found to be: sulfate-reducing bacteria > methane-producing archaea > acetogenic bacteria.

Keywords NaCl · Methanol · Methanogenesis · Salinity · Sulfate reduction · Thermophilic · UASB reactor

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Introduction

Constraints in water supply and restrictive environmental regulations are stimulating those responsible for industrial processes to re-examine their water management strategies. As a result, reuse/recycling of process water is becoming a valuable tool to reduce both freshwater intake and effluent disposal. One of the problems of recycling process water by deliberately closing the water loops is the toxicity that can be exerted due to a build-up of high salt concentrations in the circuit water. In addition, wastewater treatment processes, such as the biodesulfurization of flue gases, produce hot wastewaters that must be treated before their reuse as scrubbing water. As a consequence, there is a need for heat- and salt-tolerant biological wastewater treatment processes, which overcome the problems associated with these waters, i.e. enzyme denaturation by high temperatures [9] and cell decay due to osmotic shock in high osmolarity environments [8].

The effect of sodium on the methanization of seafood wastewater has been studied extensively [4, 6]. Mesophilic and thermophilic (up to 55 °C) high-rate methanogenic treatment of seafood wastewater proceeds successfully at NaCl concentrations of 15–25 g l^{-1}. In contrast to methanogenic reactors, little is known about the effect of sodium salts on the performance of sulfate-reducing reactors. The addition of 25 g NaCl l^{-1} was found to completely inhibit methanol degradation in a thermophilic (55 °C) upflow anaerobic sludge bed (UASB) reactor fed with methanol as the sole electron donor and having an excess of sulfate [chemical oxygen demand (COD) to SO_4^{2-} ratio of 0.5; 15].

For the methanization of seafood wastewaters, many authors suggest the stepwise increase of salt levels as a strategy for the adequate acclimation of the sludge to high salinity [4, 11]. Thus, a gradual selection for salt-tolerant microorganisms occurs in an initially non-adapted inoculum sludge. The aim of this work was to assess whether stepwise addition of NaCl is also suitable

for acclimation of sludge cultivated in a thermophilic (55 °C), sulfidogenic UASB reactor (COD/SO_4^{2-} ratio of 0.5) fed with methanol as the sole electron donor. This was investigated by monitoring the performance of a laboratory-scale UASB reactor subjected to increasing influent NaCl concentrations and assessing the maximum specific activity of the sludge harvested from the reactor when operating at different influent salinities.

Materials and methods

Experimental set-up

To investigate the aims of this work, a 6.5-l UASB reactor was operated during 273 days fed with methanol as the sole electron donor and carbon source and in excess of sulfate (COD/SO_4^{2-} ratio of 0.5). The reactor, described in detail by Vallero et al. [15], had an internal diameter of 0.10 m and height of 1 m. The reactor was equipped with a water-jacket, maintaining the reactor temperature at 55 °C. Effluent recycling was applied to obtain a superficial liquid upflow velocity of 1 m h^{-1}. The reactor was operated at a pH of 7.5 (± 0.2), using an automatic pH control device.

Inoculum and medium

The inoculum was collected from a thermophilic laboratory-scale (6.5 l) UASB reactor operating under similar conditions as used in the present work [15]. The sludge was stored during 3 months at 4 °C prior to inoculation. Sulfide was the main mineralization product of methanol degradation in this sludge, with acetate as a secondary product [15].

The synthetic influent contained methanol as the sole electron donor. Sulfate was added as sodium sulfate to provide a COD/SO_4^{2-} ratio of 0.5 (g COD g^{-1} SO_4^{2-}). Thus, theoretically all methanol could be degraded via sulfate reduction. The influent of both reactors was further supplied with a basal medium and a trace elements solution as described by Vallero et al. [15]. Basal medium was added to the main flow at a ratio of 2.22 ml basal medium g^{-1} COD in the influent. NaCl was selected as a model compound to increase the salinity of the influent.

Experimental design

The UASB reactor operated at a hydraulic retention time (HRT) of 10 h and an organic loading rate (OLR) of 5 g COD l^{-1} day^{-1} throughout the experiment. The NaCl concentration in the influent was increased stepwise from 0.5 g NaCl l^{-1} added at day 28 up to 12.5 g NaCl l^{-1} added at days 201–245. The conductivity of the reactor mixed liquor was measured to assess the salinity applied to the reactor (Fig. 1C). At day 246, NaCl was omitted from the influent in order to assess the reversibility of the NaCl-induced effects.

Maximum specific activity

Sludge samples were harvested from the UASB reactor at days 31, 103, 145, 200 and 241 (corresponding to an influent NaCl concentration of, respectively, 0.5, 3.5, 7.5, 10 and 12.5 g NaCl l^{-1}) and activity tests were performed to assess the effect of NaCl on the maximum specific activity (MSA). The batch vials were supplemented with a gradient series of NaCl (up to 25 g NaCl l^{-1}), as indicated in Figs. 2, 3 and 4. The MSA was determined as described by Vallero et al. [15].

Batch toxicity assays were performed with sludge sampled at day 241 to assess the effect of NaCl on methanol degradation and to determine the 50% inhibition concentration (IC_{50}) of NaCl on the methanol depletion rate. Experimental procedures were similar to those applied for the maximum specific activity tests. Figure 4 shows the NaCl concentrations added to the batch vials for the sludges harvested at day 241. Control vials were assayed without NaCl addition. The percentage of toxicity was determined by comparing the activity in NaCl supplemented vials with that of control vials. The final pH and the amount of volatile suspended solids (VSS) for each vial were determined upon completion of the test. All experiments were performed in duplicate.

Analysis

VSS were analyzed according to standard methods [1]. Sulfide was determined photometrically, as described by Trüper and Schlegel [14]. Methanol, acetate and methane were measured by gas chromatography, as described by Weijma et al. [16]. The volume of biogas produced in the UASB reactor was measured as described by Vallero et al. [15].

Results

UASB reactor operation

Reactor start-up (background salinity)

Complete methanol degradation was only obtained 4 days after the start-up (Fig. 1A). After that day, methanol started to accumulate in the effluent, indicating that the reactor was operating under overloading conditions. Methane production rates as high as 1.32 g COD l^{-1} day^{-1} were observed in the first days of reactor operation (Fig. 1C), although the reactor was inoculated with a sludge grown in a sulfidogenic methanol-fed thermophilic UASB that exhibited no methanogenic activity in the presence of sulfate [15]. Methane production started to decrease 7 days after reactor start-up and no methane was detected after day 26 (Fig. 1B). Acetate production accounted for about 57% of the electron flow in the first days of operation. Sulfide production increased steadily at the expense of methane production 7 days after reactor start-up, reaching a sulfide production rate as high as 2.1 g COD l^{-1} day^{-1} at day 18. Sulfide was the main mineralization product prior to the addition of salt in the reactor, accounting for 50 ± 21% of the electron flow, whereas acetate and methane accounted for 40 ± 10% and 10 ± 10% of the consumed methanol COD, respectively.

Effect of stepwise increase in the influent salt concentration on reactor performance

Low salinity (0.25–3.5 g NaCl l^{-1}) When the reactor operated at influent NaCl concentrations up to 2 g NaCl l^{-1} (day 76), methanol removal efficiencies of around 75% were obtained (Fig. 1A). It dropped to around 50% when the reactor was operated at 2–3 g NaCl l^{-1}

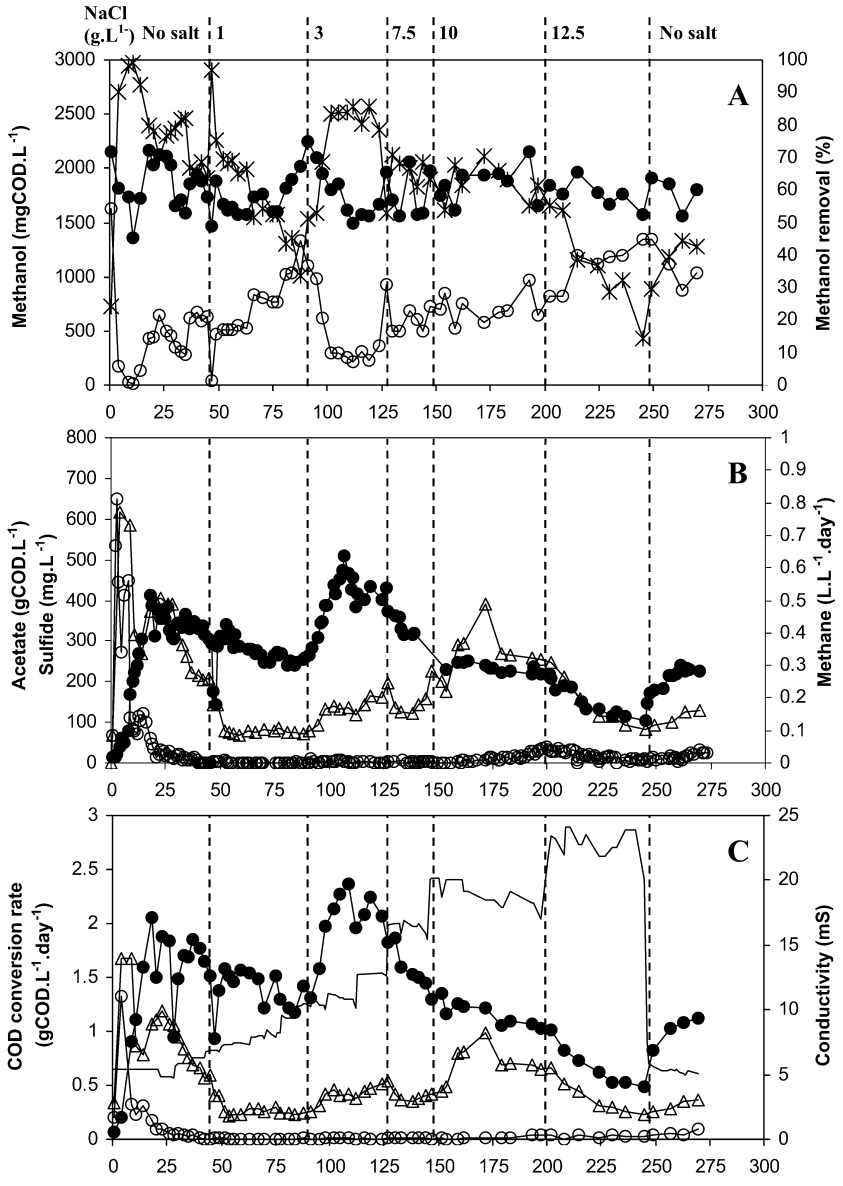

Fig. 1A–C Process performance of the upflow anaerobic sludge bed (UASB) reactor. **A** Evolution of the methanol concentration in the influent (*black circles*) and effluent (*white circles*) and the methanol removal efficiency (*crosses*). **B** Evolution of sulfide (*black circles*), methane (*white circles*) and acetate (*triangles*) concentrations. **C** Evolution of the chemical oxygen demand (COD) conversion rate to sulfide (*black circles*), methane (*white circles*) and acetate (*triangles*) and the conductivity (*solid line*)

(days 76–98). The sulfide production rate decreased from 2.05 g COD l^{-1} day^{-1} at day 18 to 1.18 g COD l^{-1} day^{-1} at day 84 (3 g NaCl l^{-1} in the influent; Fig. 1C). The acetate production rate also decreased steadily, from 1.19 g COD l^{-1} day^{-1} at day 23 to 0.21 g COD l^{-1} day^{-1} at day 54 (Fig. 1C) and to 0.25 g COD l^{-1} day^{-1} at day 98, when the influent NaCl concentration was further increased to 3.5 g l^{-1}. Sulfide was the main mineralization product during the 69 days (days 28–97) when the reactor was subjected to low NaCl concentrations, accounting for about $79.0 \pm 7.8\%$ of the electron flow, whereas acetate and methane accounted for about $20.0 \pm 7.3\%$ and $1.0 \pm 0.5\%$ of the consumed methanol COD, respectively.

Medium salinity (3.5–5.0 g NaCl l^{-1}) After the reactor was supplied with 3 g NaCl l^{-1} at day 84 (Fig. 1B), the sulfide production rate increased from 1.18 g COD l^{-1} day^{-1} at day 84 (low salinity period) to 2.59 g COD l^{-1} day^{-1} at day 107, when 4 g NaCl l^{-1} was applied to the reactor (Fig. 1C). As for the production of sulfide, the acetate production rate also increased after the influent salinity was elevated to 3 g NaCl l^{-1}, rising from 0.23 g COD l^{-1} day^{-1} at day 84 to around 0.45 g COD l^{-1} day^{-1} between days 98–124 (Fig. 1C). The methanol removal efficiency increased as a consequence of the higher sulfide and acetate production, with an average removal efficiency of about 82% between days 102–124 (Fig. 1A). Methane was hardly detected during this period. As for the low salinity concentrations, sulfide was also the main mineralization product when the reactor was subjected to medium NaCl concentrations, accounting for $83.0 \pm 1.5\%$ of the electron flow. Acetate and methane production, respectively, accounted for about $16.0 \pm 1.5\%$ and $0.5 \pm 0.5\%$ of the consumed methanol COD of this period.

High salinity (7.5–12.5 g NaCl l^{-1}) Increasing the influent NaCl concentration of the reactor to 7.5 g l^{-1} caused a steady decrease in sulfide production (Fig. 1B), accompanied by a drop in the methanol removal efficiency to around 65% between days 126–145 (Fig. 1A). The sulfide production rate decreased from 2.06 g COD l^{-1} day^{-1} at day 124 to 1.45 g COD l^{-1} day^{-1} at day 144; and it dropped further to 1.03 g COD l^{-1} day^{-1} after the influent NaCl concentration was increased to 10 g NaCl l^{-1} at day 145 (Fig. 1C). Acetate production was not affected by the presence of 7.5 g NaCl l^{-1} in the influent, as evidenced by the relatively constant acetate production rate of 0.41 g COD l^{-1} day^{-1} (Fig. 1C). In contrast to the decrease in sulfide production, acetate production steadily increased upon the addition of 10 g NaCl l^{-1} to the influent, increasing to 0.99 g COD l^{-1} day^{-1} at day 172. Low methane production rates were detected when operating the reactor with an influent NaCl concentration of 10 g l^{-1} (Fig. 1B), never exceeding more than 2% of the electron flow.

Increasing the influent NaCl concentration to 12.5 g l^{-1} at day 201 resulted in a steady decrease in both sulfide and acetate production (Fig. 1B). Sulfide production and acetate production rates as low as, respectively, 0.48 g COD l^{-1} day^{-1} and 0.23 g COD l^{-1} day^{-1} were measured at day 245 (Fig. 1C). A methanol removal efficiency of only 14% was measured at day 245 (Fig. 1A). Even though the production of acetate was stimulated by the presence of high NaCl concentrations, sulfide was still the main mineralization product of methanol degradation, accounting for about $62 \pm 9\%$ of the electron flow. Acetate and methane production accounted for the remaining $36 \pm 8\%$ and $2.0 \pm 1.5\%$ of the consumed methanol COD, respectively.

Effect of salt omission on the recovery of reactor performance (background salinity)

Immediately after omission of NaCl from the influent of the UASB reactor (Fig. 1B), sulfide production increased and sulfide production rates as high as 1.12 g COD l^{-1} day^{-1} were achieved at the end of the experiment (Fig. 1C). Acetate production remained relatively low after switching to no-salt conditions, achieving acetate production rates similar to those observed when the reactor was operating at low NaCl concentrations (0.25–3.0 g l^{-1}; Fig. 1B). Rather low methane production rates were detected in this period, accounting for less than $3 \pm 1\%$ of the electron flow. Sulfide was the main mineralization product at the end of the experiment, accounting for $74 \pm 2\%$ of the electron flow, whereas acetate accounted for the remaining $23 \pm 1\%$ consumed methanol COD.

Maximum specific activities

Evolution of the MSA of the sludges in the absence of salt

The highest methanol degradation rates (1,019 mg COD g^{-1} VSS day^{-1}) were obtained with sludge harvested at day 31, when only 0.5 g NaCl l^{-1} was added to the influent of the UASB reactor (Fig. 2A). Surprisingly, the methanol depletion rate dropped to 624 mg COD g^{-1} VSS day^{-1} for sludge sampled at day 103, despite the fact that methanol removal in the UASB reactor at day 103 was as high as that at day 31 (Fig. 1A). The methanol depletion rate increased with time; and values as high as 908 mg COD g^{-1} VSS day^{-1} were obtained when operating the reactor with an influent NaCl concentration of 10 g l^{-1} (Fig. 2A). The increase to 12.5 g

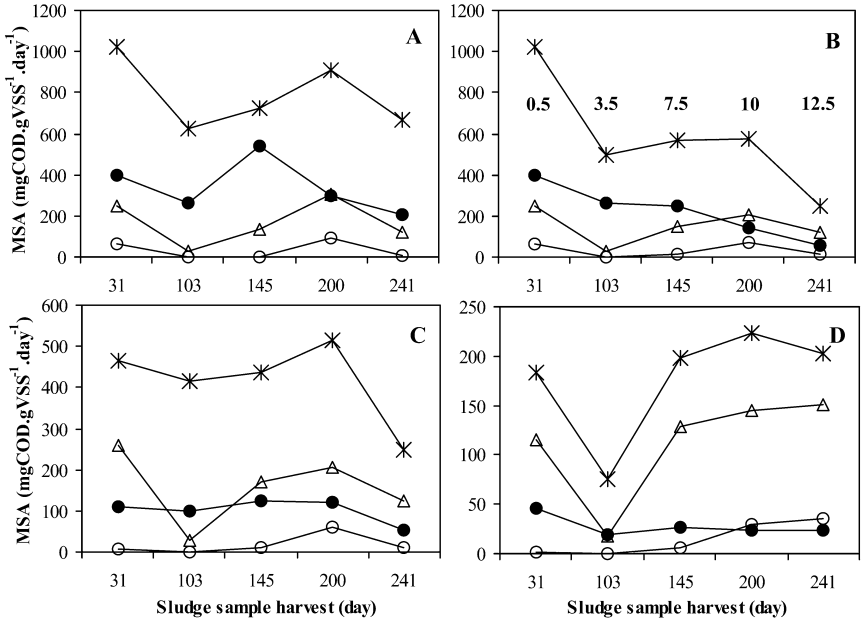

Fig. 2A–D Evolution of the methanol depletion rate and the acetate, methane and sulfide formation rates during activity assays performed with the sludge harvested from the UASB reactor at different days and inoculated with different salinities. **A** No salt added, **B** actual reactor salinity, **C** 12.5 g NaCl l^{-1}, **D** 25.0 g NaCl l^{-1}. Note the difference in the maximum specific activity scale of the figures. Methanol (*crosses*), sulfide (*black circles*), methane (*white circles*), acetate (*triangles*), volatile suspended solids (*VSS*)

NaCl l^{-1} in the influent of the reactor caused a drop in the methanol depletion rate to 671 mg COD g^{-1} VSS day^{-1}, despite the fact that the vials were not amended with any supplementary salt (Fig. 2A). Both the acetate and sulfide production rates followed the same pattern as the methanol depletion rate, except that the sulfide production rate had already dropped for sludge sampled when the reactor was operating at a NaCl concentration of 10 g l^{-1} (Fig. 2A).

Sulfide was the main mineralization product in the vials without NaCl amendment, accounting for more than 60% of the electron flow (Fig. 3A), independent of the salt concentration added to the reactor. Acetate production was as high as the sulfide production only with the sludge sampled at day 200, when the reactor operated at an influent NaCl concentration of 10 g l^{-1} (Fig. 3A). Methane production was detected only at considerable concentrations at day 200, accounting for 13% of the electron flow (Fig. 3A). Methane was also detected for sludges harvested at days 31 and 241, accounting for 8% and 4% of the electron flow, respectively (Fig. 3A).

Effect of salt in the SMA of sludges cultivated at different salinities

All rates decreased with the increase of salinity, independent of the salt concentration on which the sludge was cultivated (Fig. 2B–D). As for the vials without NaCl amendment, the methanol depletion rate decreased for sludge harvested at day 103, despite the good performance of the reactor at that time (Fig. 3B–D). In the vials amended with similar NaCl concentrations, a significant decrease in activity was observed only for vials inoculated with sludge harvested at day 241, when the reactor operated at an influent NaCl concentration of 12.5 g l^{-1} (Fig. 2B–D). A similar pattern was found for the sulfide production rate (Fig. 2B, C), except for the sludge sampled at day 241 (influent NaCl concentration of 12.5 g l^{-1}), when the acetate production rate exceeded the rate measured for sludge sampled between days 145–200 (influent NaCl concentration of 10 g l^{-1}; Fig. 2D).

Different NaCl concentrations strongly affected the fate of methanol (Fig. 3B–D). Sulfide was the main mineralization product only for vials amended with less than 7.5 g NaCl l^{-1} (Fig. 3B–D). Acetate became the main fate of methanol degradation for vials amended with more than 10 g NaCl l^{-1}, independent of the influent NaCl concentrations imposed to the reactor (Fig. 3B–D). For instance, acetate production always accounted for more than 70% of the electron flow in the vials amended with more than 25 g NaCl l^{-1}, independent of the influent sodium concentration (Fig. 3D). Considerable methane production (around 16%) was only detected in vials inoculated with sludge sampled at day 200 (10 g NaCl l^{-1} influent), independent of the salt concentration amended to the vials (Fig. 3B–D). In addition, considerable methane production (about 17%) was detected in vials amended with 25 g NaCl l^{-1} and inoculated with sludge sampled at day 200 (10 g NaCl l^{-1} reactor) and at day 241 (12.5 g NaCl l^{-1} influent).

IC_{50} of NaCl of sludge cultivated at high salinity (12.5 g NaCl l^{-1})

The IC_{50} concentration of NaCl in sludge sampled at day 241 (12.5 g NaCl l^{-1} influent) was 6.90 g l^{-1} (Fig. 4A). This corresponds to an IC_{50} concentration for Na^+ of 5.1 g l^{-1}, when the sodium introduced into the medium via both sodium sulfate and sodium

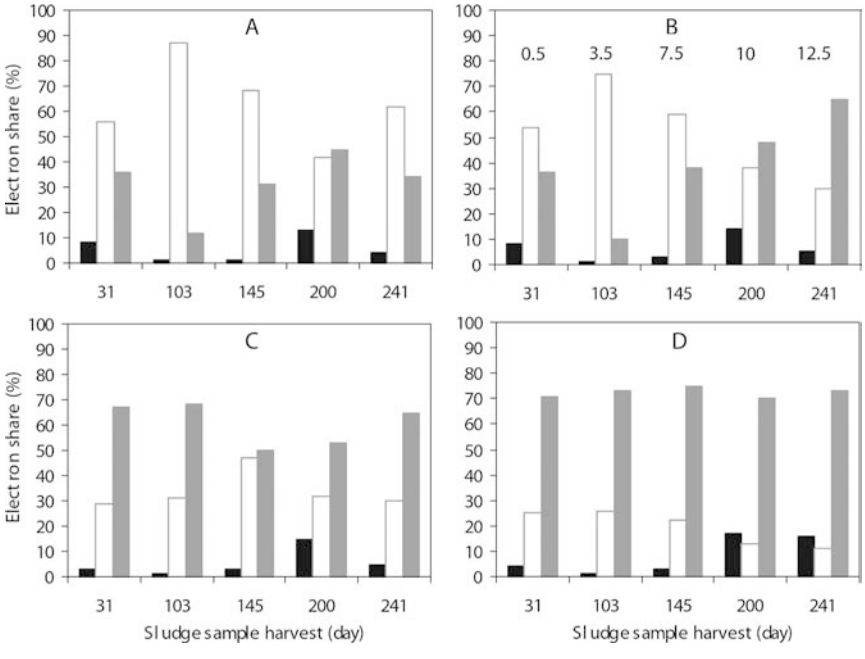

Fig. 3A–D Evolution of product formation during methanol degradation (%) during activity tests performed with sludge harvested from the UASB reactor at different days and inoculated with different salinities. **A** No salt added, **B** actual reactor salinity, **C** 12.5 g NaCl l^{-1}, **D** 25.0 g NaCl l^{-1}. Sulfide (*black columns*), methane (*white columns*), acetate (*gray columns*)

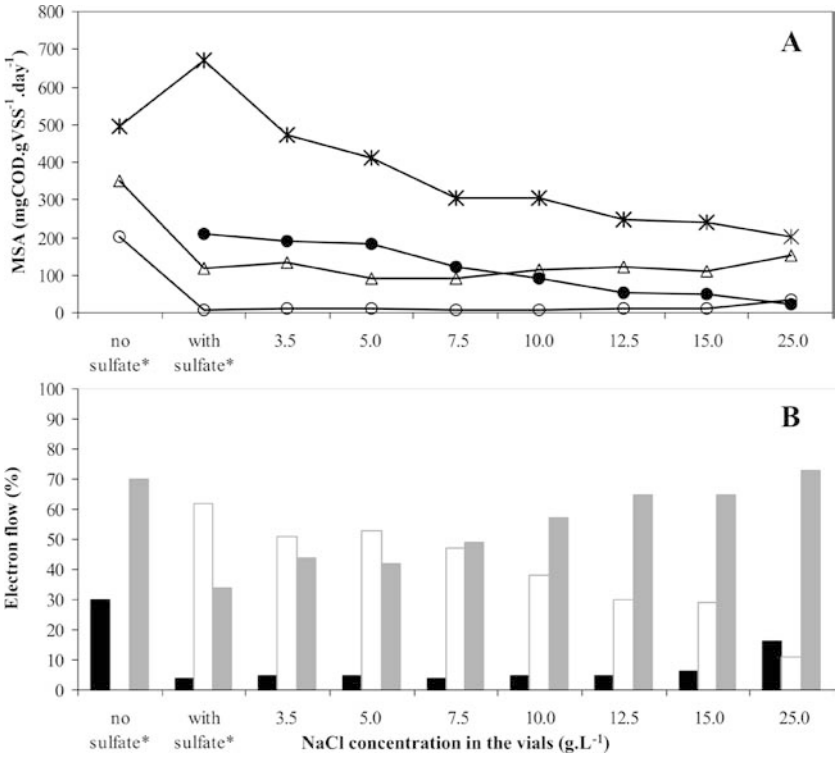

Fig. 4A, B Effect of salt concentration on the maximum specific activity (**A**) and electron flow (**B**) on day 241 (influent NaCl concentration of 12.5 g l^{-1}). **A** Rates are given for methanol depletion (*crosses*) and the formation of acetate (*triangles*), methane (*white circles*) and sulfide (*black circles*). **B** Product formation from methanol degradation. Sulfide (*black columns*), methane (*white columns*), acetate (*gray columns*)

bicarbonate is also considered. In the absence of sulfate, higher acetate and methane production rates occurred (Fig. 4A), with acetate and methane accounting for, respectively, 70% and 30% of the electron flow (Fig. 4B). As observed for sludges harvested at previous days, the presence of NaCl in different concentrations strongly affected the fate of methanol under thermophilic conditions (Fig. 4B). Acetate was the main mineralization product in vials amended with more than 7.5 g NaCl l^{-1} (Fig. 4B), whereas sulfide predominated as the end-product in vials amended with 5 g NaCl l^{-1} or less (Fig. 4B). The methane concentration upon termination of the experiment increased with increasing NaCl concentration (Fig. 4B). However, methane accounted for maximally 16% of the electron flow for the vials amended with 25 g NaCl l^{-1} (Fig. 4B).

Discussion

Long term adaptation to NaCl stress

Stepwise acclimation of the biomass to NaCl was an ineffective strategy to treat saline sulfate-rich wastewaters using methanol as electron donor (Fig. 1). Thus, the adaptation of thermophilic (55°C), sulfidogenic methanol-degrading biomass to a high osmolarity environment is unlikely to occur. The results obtained in this work contrast with previous papers which affirm that stepwise exposure of sludge to increasing salt concentration is necessary for the successful treatment of salt-rich wastewaters [5, 12, 13]. These authors worked, however, with the anaerobic treatment of seafood wastewaters, which have a rather complex composition and contain many different substrates, contrasting with the single substrate (methanol) applied in this study. In addition, seawater was used as process water and it may have contained synergistic cations (e.g. K^+, Mg^{2+}) or provoked the growth of halotolerant seawater microorganisms in the sludge.

In previous work, a higher IC_{50} value of 9.30 g NaCl l^{-1} was obtained in a UASB reactor operating at low influent salt concentrations [15]. The lower IC_{50} value of 6.90 g NaCl l^{-1} obtained in this work suggests a limited extent of adaptation by the sludge to high salinity (Fig. 4A). However, one must consider that only partial recovery of the activity might have occurred when re-suspending sludge cultivated in the UASB reactor at 12.5 g NaCl l^{-1} in fresh medium (no NaCl). Thus, the control vials probably had a lower activity compared with sludge cultivated in fresh medium (Figs. 1, 4A). The only-partial reversibility of the deleterious effect of salt towards the granular sludge suggests that a period of time is needed to re-establish stable performance when switching from high-salt to low-salt influent concentrations. This was confirmed by the only-partial recovery of the UASB reactor even 26 days after switching the influent NaCl concentration from 12.5 g l^{-1} to no salt at day 246 (Fig. 1).

The long-term adaptation might have failed because of the lack of good attachment properties of the salt-tolerant microorganisms, leading to their wash-out from the reactor. In order to verify the presence of

salt-tolerant microorganisms in the sludge, a parallel UASB reactor was run at a HRT of around 80 h, using the same inoculum (data not shown). Very low methanol removal efficiencies (around 25% of methanol was converted to acetate) were obtained when operating the reactor at a conductivity of 30 mS cm^{-1} (corresponding to about 20 g NaCl l^{-1} in the reactor bulk), despite the fact that a sufficient hydraulic retention time (80 h) allowed the growth of halotolerant species present in the granules or as cell suspension (data not shown). The absence of halotolerant microorganisms in the sludge cultivated in this work is further confirmed by the batch tests. Indeed, higher activities were obtained for the batch vials amended with lower salt concentrations (Fig. 4A); and this was also found in the activity tests performed with sludge cultivated in the UASB reactor at lower salinities (Fig. 2A, B).

In view of the problems due to high salinity, viz. the limited extent of adaptation and reduction in methanol degradation kinetics coupled to a suppression of sulfidogenesis, further research must be oriented towards alternative ways to overcome salinity stress in sulfidogenic bioreactors. For this, the potential application of compatible solutes, i.e. organic compounds accumulated by halotolerant microorganisms in response to increases in salinity [8], or special microorganisms adapted to high salinity, the so-called halophiles, need to be applied for the anaerobic treatment of saline sulfate-rich waste streams. Recently, a whole range of autotrophic and heterotrophic sulfate-reducing species was described that are able to grow under high salinity, such as the halophile *Desulfobacter halotolerans* [2]. To the best of our knowledge, there are so far no reports on the successful immobilization of these halophilic sulfate-reducing bacteria (SRB) in bioreactor sludges. Such an approach will become essential, as it could push the current operation limits of sulfate-reducing bioreactors to more extreme working conditions.

Effect of NaCl on the fate of methanol degradation

This study shows that, in agreement with Vallero et al. [15], acetogenic bacteria (AB) displayed a lower sensitivity to NaCl than SRB, as sulfide production in the reactor steadily decreased after the addition of 7.5 g NaCl l^{-1}, whereas acetate production was stimulated at this influent NaCl concentration (Fig. 1B, C). This study further confirms that sulfide is the main mineralization product from methanol degradation in a thermophilic (55 °C) UASB reactor operating at an OLR of 5 g COD l^{-1} day^{-1} and a HRT of 10 h, regardless of the NaCl concentration added to the influent. Despite the fact that sulfide was the predominant metabolic product of methanol degradation when operating the reactor at an influent NaCl concentration up to 12.5 g l^{-1} (about 62% of the electron flow), batch experiments showed that acetate is the main metabolic product at higher NaCl concentrations (Figs. 3C, D, 4B).

Although methane production by methane-producing archaea (MPA) was very low in the UASB reactor (Fig. 1B, C), a methanogenic population was cultivated in the bioreactor when operating at higher salinities, as considerable methane production was detected in batch vials amended with 25 g NaCl l^{-1} at days 200 and 241, even exceeding the production of sulfide (Fig. 3D). The apparent order of toxicity of NaCl towards the different trophic groups was found to be: SRB > MPA > AB. Thus, acetate can be expected to be the main mineralization product of methanol degradation for influent NaCl concentrations higher than 12.5 g l^{-1}.

In principle, it is expected that the produced acetate would be further converted to H_2S or CH_4, as a number of both SRB [17] and MPA [3, 10] are able to oxidize acetate to CO_2 under thermophilic conditions. However, neither methane nor sulfide production was detected in batch tests inoculated with acetate as the single substrate (data not shown). The lack of acetate degradation in sulfate-reducing thermophilic bioreactors is well reported, even under low salinity conditions [15, 16]. The production of acetate is undesirable in sulfate-reducing bioreactors, as it induces the need for further treatment steps, either when the water is meant for re-use or when sulfide is to be biologically converted to elemental sulfur [7].

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