Effect of high salinity on the fate of methanol during the start-up of thermophilic (55°C) sulfate reducing reactors

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Abstract
Two 6.5 L lab-scale upflow anaerobic sludge bed (UASB) reactors were operated at 55°C fed with methanol as the sole electron and carbon source and in excess of sulfate (COD/SO$_4^{2-}$ of 0.5) in order to investigate the effect of high wastewater salinity on the start-up period. The first reactor (UASB I) was operated without NaCl addition, while the second reactor (UASB II) was fed with 25 g.L$^{-1}$ of NaCl in the first 13 days of operation. Successful start-up of UASB I was achieved, with full methanol conversion (100% elimination) to methane gas (methane production rate up to 3.66 gCOD.L$^{-1}$.day$^{-1}$). Despite the detection of sulfide from day 15 onwards in UASB I, methane was the main mineralization product when operating at an organic loading rate (OLR) of 5 gCOD.L$^{-1}$.day$^{-1}$ and a hydraulic retention time (HRT) of 10 hours. Sulfide and acetate started to be produced after salt omission from the influent in UASB II at day 13, with no detection of methane. Acetate was the main product when operating at an OLR of 10 gCOD.L$^{-1}$.day$^{-1}$ and HRT of 6.5 hours in both reactors. Apparently, the methane producing bacteria (MPB) are the trophic group most sensible to the NaCl shock.

Keywords
Methanol degradation; salt effects; sulfate reduction; thermophilic anaerobic processes; UASB reactor

Introduction
The characteristics of different wastewaters produced by some (industrial) processes, such as temperature and salinity, are far from the physiological optima for the microorganisms. Furthermore, with the current trend to close water cycles in industry, there is an increasing need for hot and salt tolerant treatment processes. Therefore, it is of utmost importance to evaluate the possibility of anaerobic bio-transformations at elevated temperature and high salinity conditions. As a result, the range of utilisation of biological-based processes can be extended to very interesting conditions for industrial wastewater treatment.

The effect of sodium on anaerobic digestion was already studied extensively for the treatment of seafood process wastewater (Guerrero et al., 1997; Feijoo et al., 1995; Omil et al., 1995a; Méndez et al., 1995). Mesophilic and thermophilic methanization of these wastewaters are not hindered at NaCl concentrations ranging from 15 to 25 g.L$^{-1}$.

In recent years, bioreactors for sulfate removal have been designed aiming at the magnification of anaerobic sulfate reduction (Hulshoff Pol et al., 2001). In the presence of sulfate, the sulfate reducing bacteria (SRB) will proliferate in anaerobic bioreactors, where they may compete with the MPB and the homoacetogenic bacteria (AB) for common substrates such as hydrogen, acetate and methanol. The latter compound deserves special attention, as it is often relied on in biotechnology as an inexpensive and efficient electron donor for inorganic wastewaters, such as in thermophilic (65°C) sulfate reduction (Weijma et al., 2000), denitrification (Chen et al., 1993) and dehalogenation (DiStefano et al., 1992) processes.

Methanol can be completely converted to methane and carbon dioxide in high rate anaerobic reactors either in mesophilic (Florencio et al., 1994) and thermophilic (Paulo et al., 2001) conditions. The presence of sulfate does not exert considerable effects on the methanol conversion on mesophilic conditions (Weijma et al., 2000). In contrast, the presence of sulfate...
greatly affects the methanol conversion under thermophilic (65°C) conditions, as sulfide accounted for more than 80% of the consumed methanol-COD in an EGSB treating sulfur-rich wastewater fed with methanol as sole electron donor (Weijma et al., 2000).

Whether sulfate reduction is the main end product of methanol degradation in bioreactors operated at 55°C with methanol as the sole electron and carbon source, and how this is affected by high salinity is still unclear. The aim of this work is to assess the influence of high salinity (25 gNaCl.L⁻¹) on the performance of a thermophilic (55°C) UASB reactor fed with methanol as the sole electron donor and high sulfate concentration (COD/SO₄²⁻ ratio of 0.5).

Material and methods
Two bench-scale (6.5 L) UASB reactors were operated under identical operational conditions during 63 days, except the influent salt concentration. One reactor (UASB I) was operated as control reactor, whereas 25 gNaCl.L⁻¹ was supplemented to the influent of the second reactor (UASB II) in the first 13 days of the experiment. The UASB reactors (Figure 1), described in detail by Omil et al. (1996a), had an internal diameter of 0.10 m and height of 1 m.

In order to keep the reactor temperature at 55°C, the reactors were equipped with a double wall, through which water, heated in a thermostatic waterbath (Julabo, Seelbach, Germany), was recirculated. Effluent recycling was applied to obtain a superficial liquid upflow velocity (V_up) to 1 m.h⁻¹. Both reactors were fed using peristaltic pumps (Watson Marlow 501 U and 505 S, Falmouth, Cornwall, UK). Concentrated stock solutions and basal medium were added to the main influent flow using vertical axis peristaltic pumps (Gilson Minipuls 3 and 2, respectively, Villiers, France). The pH in the reactors was controlled by automatic pH control, by adding concentrated NaOH or HCl solutions in the recirculation system. The pH was measured with a sulfide-resistant Flushtrode pH electrode (Hamilton Flushtrode, Hilkomij bv, Rijswijk, The Netherlands) connected to the automatic pH controller with two changeable setpoints to adjust the pH. The pH of both reactors was maintained at 7.0 (±0.2) throughout the experiment.

Both reactors were inoculated with a sludge growing in two thermophilic (55°C) lab-
scale (6.5 L) UASB reactors treating a synthetic paper-mill wastewater at pH 6 (Lens et al., 2001). Both reactors were started-up with about 2 L of inoculum sludge, corresponding to approximately 40 g of volatile solids (VS) per reactor.

Both reactors were fed with a synthetic influent, containing methanol as the sole electron donor. Sulfate was added as sodium sulfate to provide a COD/sulfate ratio of 0.5 (gCOD per gSO_4^{2-}), so theoretically all methanol could be degraded via sulfate reduction. Sodium bicarbonate (0.25 g.L^{-1}) was added as buffer agent. The influent of both reactors was further supplied with a basal medium consisting of (g.L^{-1}): NH_4Cl (7.5), K_2HPO_4 (2.10), MgSO_4.7H_2O (1.5), CaCl_2.2H_2O (0.3), yeast extract (0.5) and a trace element solution prepared according to Zehnder et al. (1980), adding 4.5 mL per litre of basal medium consisting of (mg.L^{-1}): FeCl_2.4H_2O (2000), H_3BO_3 (50), ZnCl_2 (50), CuCl.3H_2O (38), MnCl_2.4H_2O (500), (NH_4)MoO_2.4H_2O (50), AlCl_3.6H_2O (90), CoCl.6H_2O (1000), NiCl_2.6H_2O (92), Na_2SeO_3.5H_2O (194), EDTA (1000), resazurine (200) and HCl 36% (1 mL). Basal medium was added to the main flow at a ratio of 2.22 mL per gram influent COD. Table 1 summarises the operational parameters applied to UASB I and UASB II in the different experimental periods. Note that the Na^+ concentrations given in Table 1 refer to the contribution of sodium from both NaCl and Na_2SO_4 addition.

Sulfide was determined photometrically as described by Trüper and Schlegel (1964). Methanol, VFA and methane were measured by gas chromatography (GC), as described by Weijma et al. (2000). The volume of biogas produced in the UASB reactors was measured with a wet-type precision gas meter (Schlumberger Industries, Dordrecht, The Netherlands), after passing through a waterlock filled with 3N NaOH solution and a column filled with soda lime pellets to remove H_2S and CO_2 from the gas.

**Results and discussion**

During the first week of operation, methanol was completely converted to methane in UASB I at high methane production rates (3.66 gCOD.L^{-1}.day^{-1} at day 10) and neither acetate nor sulfide were detected (Figure 2A,B). Sulfide started to be produced from day 15 onwards, but methane was clearly the main mineralization product in the UASB I during the first 42 days of operation (Figure 2B), accounting for 87% of the consumed methanol-COD. In contrast to the successful start-up of UASB I, high influent sodium chloride concentrations (25 gNaCl.L^{-1}) appeared to completely inhibit the methanol degradation in the UASB II (Figures 3A,B). Upon omission of NaCl from the influent from day 13 onwards, partial methanol consumption was observed within 7 days (Figure 2A versus Figure 3A).

![Image](https://iwaponline.com/wst/article-pdf/45/10/121/424894/121.pdf)

**Table 1** Operational parameters applied to the UASB I and UASB II reactors (HRT = Hydraulic Retention Time; OLR = Organic Loading Rate; COD = Chemical Organic Demand; SLR = Sulfate Loading Rate)

<table>
<thead>
<tr>
<th>Parameter</th>
<th>UASB I – Control reactor</th>
<th>UASB II – Salt-fed reactor</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Period I</td>
<td>Period II</td>
</tr>
<tr>
<td>Influent flow (litre.day^{-1})</td>
<td>15.84 ± 0.20</td>
<td>16.17 ± 0.71</td>
</tr>
<tr>
<td>HRT (hour)</td>
<td>9.85 ± 0.40</td>
<td>9.66 ± 0.41</td>
</tr>
<tr>
<td>OLR (gCOD.litre^{-1}.day^{-1})</td>
<td>4.12 ± 0.25</td>
<td>4.35 ± 0.72</td>
</tr>
<tr>
<td>COD (g.litre^{-1})</td>
<td>1.45 ± 0.19</td>
<td>1.74 ± 0.27</td>
</tr>
<tr>
<td>SLR (gSO_4^{2-}.litre^{-1}.day^{-1})</td>
<td>7.98 ± 1.24</td>
<td>8.89 ± 1.13</td>
</tr>
<tr>
<td>SO_4^{2-} (g.litre^{-1})</td>
<td>2.79 ± 0.45</td>
<td>3.60 ± 0.45</td>
</tr>
<tr>
<td>COD/SO_4^{2-} ratio</td>
<td>0.52 ± 0.03</td>
<td>0.49 (0.04</td>
</tr>
<tr>
<td>pH</td>
<td>7.15 ± 0.15</td>
<td>7.08 ± 0.11</td>
</tr>
<tr>
<td>NaCl (g.litre^{-1})</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Na^+ (g.litre^{-1})</td>
<td>1.26 ± 0.15</td>
<td>2.11 ± 0.28</td>
</tr>
</tbody>
</table>

*calculated values
and the sulfide and acetate production rates increased up to 0.91 and 1.09 gCOD.L⁻¹.day⁻¹, respectively, at day 32 (Figure 3B).

At first sight, the complete inhibition at high salinity (25 gNaCl.L⁻¹) contrasts the many reports of successful methanization of high saline (up to 12 g.L⁻¹ of sodium) seafood effluents, either in mesophilic (Soto et al., 1993; Omil et al., 1995b; Feijoo et al., 1995) or thermophilic (Méndez et al., 1995) anaerobic reactors. However, seafood wastewaters have a rather complex composition containing many different substrates, contrasting to the single substrate methanol wastewater applied in this study. Nonetheless, some aspects of methanization of seafood wastewaters in high-rate reactors cannot be disregarded, such as the need of a start-up procedure, which aims at acclimation of the biomass (Soto et al., 1993; Omil et al., 1996b). In order to achieve the development of active biomass in reactors treating saline influent, a feeding policy, in terms of salinity and organic loading rate must be applied. This can be achieved by long term exposure of the biomass to progressively higher salinity or application of an OLR corresponding to the theoretical maximum applicable organic load (Omil et al., 1995a).

Surprisingly, the MPB were strongly inhibited by the 13 day salt shock in UASB II, since no methane production was detected till day 32 (Figure 3B). As only sulfide and acetate were detected after omitting the salt from UASB influent (Figure 3B), it can be that inferred high NaCl concentrations have different effects to the SRB, MPB and AB. In fact, Kugelman and McCarty (1965) have shown that the mesophilic MPB exhibited a greater sensitivity to the toxic effects of cations than the acid formers. Also, the results indicate that
the microorganisms present in the inoculum sludge were predominantly freshwater species, that may be inhibited by marine NaCl concentrations (Widdel, 1988), as used in the start-up of this work. This suggests the need for immobilization of halotolerant SRB species in bioreactor sludges, such as the recently isolated *Desulfobacter halotolerans* (Brandt and Ingvorsen, 1997) and those present in marine and oil sediments (Widdel, 1988). To the best of our knowledge, there are so far no reports on this. Such an approach surely deserves careful attention as it could extend the application of methylotrophic systems to more salt rich wastewaters, i.e. less bleed is required in closed systems.

An alkaline pH shock (9.5) during approximately 8 hours at day 32 in UASB II caused a temporary inhibition in the sulfide and acetate production, but the system recovered within a few days (Figure 3B). Surprisingly, methane production was detected immediately after the pH shock, although methane production rates were very low when compared with UASB I during the same period (Figures 2B and 3B).

It should be noted that the sharp decrease of the methane production rate in UASB I between days 38–48 started even before the OLR was increased to 10 gCOD.L⁻¹.day⁻¹ at day 43 (Period III). The acetate production rate increased considerably in both reactors in the period with an OLR of 10 gCOD.L⁻¹.day⁻¹ (Period III), reaching values up to 2.94 gCOD.L⁻¹.day⁻¹ and 3.07 for UASB I and UASB II, respectively (Figures 2B and 3B). During this period, the sulfide production rate remained stable in both reactors, with average values of 0.74 gCOD.L⁻¹.day⁻¹ and 0.76 gCOD.L⁻¹.day⁻¹ for, respectively, UASB I and UASB II.

**Figure 3** A – Evolution of the methanol concentrations in the influent (●), effluent (○) and methanol removal efficiencies (×) for UASB II. B – Evolution of the COD conversion rate to sulfide (●), methane (○) and acetate (∆) for UASB II.
Conclusions
The results obtained in this research allow to conclude that:
- The application of 25 gNaCl.L⁻¹ at the start-up of UASB II leads to a complete process failure.
- Apparently, the methane producing bacteria (MPB) is the trophic group most sensible to the NaCl shock.
- Acetate was the main product when operating at an OLR of 10 gCOD.L⁻¹.day⁻¹ and HRT of 6.5 hours in both reactors.

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References

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