Thermophilic anaerobic digestion of methanol in UASB reactor

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Abstract A 5.1 L laboratory scale upflow anaerobic sludge bed (UASB) reactor was operated at 55°C over 130 days in order to investigate the feasibility of treating methanol-containing wastewater under thermophilic conditions, focussing on start-up and process stability. Batch assays were conducted to elucidate the most probable pathway for methanol conversion. The results demonstrated a good performance, with a chemical oxygen demand (COD) removal averaging 82% throughout the experiment. No significant VFA accumulation was detected in the effluent, even with bicarbonate concentration exceeding 20 mM. Acetate was the main component of the VFA at relatively low organic loading rates (OLR). At high OLR, the main components were propionate and butyrate. Reactor performance was hardly affected when the system was exposed to non-optimal conditions, i.e., temperature drop, overloading and no feeding. Good thermophilic granular sludge was retained in the reactor. Washout of biomass was not severe during the experiment. From the pathway analysis it could be concluded that indirect pathways play an important role in the methanol degradation by the cultivated consortia.

Keywords Anaerobic; methanol; pathway; thermophilic; UASB reactor

Introduction
Methanol can be the main pollutant in some specific wastewaters and may be formed under natural conditions as an intermediate in the decomposition of organic matter. Coal-gasification plants, evaporator condensate of pulp and paper industries, potato-starch producing factories and landfill leachates are examples of wastewater where methanol can be present.

Methanol can be converted to methane via several pathways. It can be directly converted to methane by methylotrophic methanogens. The second possibility is its transformation to acetate by acetoogens followed by acetate cleavage to methane by acetoclastic methanogens, or conversion to hydrogen and carbon dioxide by homoacetogens followed by methanogenesis from hydrogen and carbon dioxide by hydrogenotrophic methanogens. The anaerobic treatment of methanolic wastewaters under mesophilic conditions has been investigated by many researchers (Lettinga et al., 1979; Minami et al., 1991; Nishio et al., 1993; Florencio, 1994; Bhatti et al., 1996; Fukuzaki and Nishio, 1997). The feasibility seems to remain questionable and doubts about the operation of a stable treatment process still persist, due to unpredictable accumulation of volatile fatty acids (VFA) in the effluent which may cause failure of the treatment process due to inhibition of the methanogens, especially at low pH-values. Technically, methylotrophic methanogens is not inhibited at low pH, the degree of inhibition is strongly dependent on the concentration of the undissociated form of the fatty acids.

Thermophilic treatment is an attractive alternative, particularly when the wastewater is discharged at high temperatures (van Lier, 1995). A typical example of wastewater is the evaporator condensate of pulp and paper industries, where methanol is the main pollutant (Minami et al., 1991) and it is discharged at high temperatures. Thermophilic treatment is also an alternative for mesophilic digestion due to the higher metabolic rates of the bacteria
involved and, consequently, the higher maximum specific methanogenic activities. The results obtained with thermophilic treatment of various types of wastewaters are very promising (van Lier, 1995), although many authors have reported several drawbacks of thermophilic reactors, such as: high susceptibility to temperature increases, feed interruptions and shock loads.

So far, very little is known about the anaerobic thermophilic digestion of methanol-containing wastewater. The main objective of this research was to investigate the feasibility of treating methanol-containing wastewater under thermophilic conditions (55°C), focusing on start up, process stability, and the assessment of the probable pathway for methanol conversion to methane.

Methods

Continuous experiments

The thermophilic anaerobic degradation was studied in continuous and batch reactor systems. A glass UASB reactor equipped with a water jacket, with a working volume of 5.1 L was used (Figure 1).

The reactor was equipped with a double wall connected to a 55°C waterbath recirculator (Julabo, MB-Basis, Germany). Biogas was collected in a gas-solid-liquid separator and led through a waterlock filled with a 16% NaOH solution to remove CO₂ from the gas. Thereafter the gas passed through a column filled with soda lime pellets with indicator. Subsequently, the gas flow was measured with a wet-type precision gas meter (Meterfabriek Dorfrecht, The Netherlands).

Methane (CH₄) production was continuously measured. The hydraulic retention time (HRT) was calculated based on the flow rate of effluent. Influent and effluent samples were taken twice per week to analyse the methanol and VFA concentration. The sludge bed height was measured every day. The biogas composition and suspended solid COD were measured when the organic loading rate (OLR) was increased. The reactor was inoculated with 1170 g granular wet sludge from a pilot plant UASB reactor treating paper mill wastewater at 55°C (Paques Biosystems BV, Balk, The Netherlands). Table 1 presents the operating conditions of the UASB reactor. The OLR was always increased when about 90% of methanol conversion was achieved. Effluent recirculation was imposed to the system. Methanol was used as the sole organic carbon source. The concentration in the stock solution was 2.7 gCOD.L⁻¹ (day 0–60) and 5.4 gCOD.L⁻¹ (day 61–130). 0.33 g bicarbonate was added per 1 g methanol.L⁻¹, to ensure pH stability.

The reactor was supplemented with macro and micro-nutrients. 2.22 ml of a nutrient

![Figure 1 Schematic view of the experimental set-up](image-url)
stock solution was supplied for each gram influent COD.L⁻¹, stock solution contained (g.L⁻¹): From day 0–27: NH₄Cl (0.28), K₂HPO₄•3H₂O (0.27), Na₂S (0.0317), CaCl₂•2H₂O (0.01), yeast (0.1) and 1 millilitre of trace element solution. From day 28–130: NH₄Cl (7.5), K₂HPO₄•3H₂O (2.12), MgSO₄•H₂O (1.5), CaCl₂•2H₂O (0.3), yeast (0.5) and 4.5 millilitre of trace element solution.

The trace elements solution contained (mg.L⁻¹): FeCl₂•4H₂O (2000), H₃BO₃ (50), ZnCl₂ (50), CuCl₂•2H₂O (38), MnCl₂•4H₂O (500), (NH₄)₆MoO₂₄•4H₂O (50), AlCl₃•6H₂O (90), CoCl₂•6H₂O (2000), NiCl₂•6H₂O (92), Na₂SeO₃•5H₂O (194), EDTA (1000), Resazurine (200), HCl 36% (1%). All chemicals were of analytical grade.

Batch experiments

Activity assays. 120 ml glass serum vials were filled with 50 ml basal medium containing (g.L⁻¹): NH₄Cl (0.28), K₂HPO₄•3H₂O (0.33), MgSO₄•7H₂O (0.1), CaCl₂•2H₂O (0.01), yeast (0.1) and one millilitre of trace element solution. Before adding the sludge and substrate, all bottles containing basal medium were incubated in a waterbath with shaker (TUV, GLF 1083, Germany) at 55°C and 50 rpm. Sludge was added to the vials at a VSS concentration of about 1 g.L⁻¹. Methanol or acetate was added as the substrate at a concentration of 2 gCOD.L⁻¹. When methanol was used as a substrate, 2.52 g NaHCO₃ per litre of basal medium was added, to ensure pH stability. The vials were sealed with butyl rubber stoppers and the gas headspace was flushed for 5 minutes with N₂/CO₂ (70:30). After various periods of time, gas samples were taken and analysed for CH₄. The pH, as well as the exact amount of VSS in each bottle was measured after the test was completed. The specific methanogenic activity (SMA) was calculated from the linear increase of the CH₄ concentration in the beginning of the experiment, when no lag phase was observed, divided by the exact amount of VSS. The assay was performed in triplicate, using the bottles without substrate as blank.

Pathway analysis. The presence of specific bacterial subpopulations in the sludge was studied by using batch activity tests to which specific inhibitors, 30 mM bromoethane sulfonic acid (BESA, Sigma, USA) and 0.25 g.L⁻¹ Vancomycin (Acros, Belgium) were added for blocking a metabolic pathway. Figure 2 represents the general strategy used for blocking the competitive reactions.

The 120 ml serum bottles were filled with 25 ml basal medium when H₂/CO₂ was the substrate. Pure H₂ was injected to give a pressure of 1.74 atm, equivalent to 2.0 g COD.L⁻¹. When methanol and acetate were used as the substrate, the experimental set-up and conditions of the assays were the same of the activity test, except the shaking speed (100 rpm).

The bottles were placed horizontally in a water-bath to optimise mass transfer of hydrogen from gas to liquid phase. Liquid and gas samples were taken periodically to analyse
substrate consumption and product formation. The apparent substrate affinity $K_m$ and maximum substrate degradation activity $V_{max}$ on methanol and acetate were estimated using the same conditions of the specific methanogenic activity assay. Apparent $K_m$ and $V_{max}$ were estimated from the substrate depletion curve, by using a Michaelis–Menten derived equation and a non-linear regression routine for parameter estimation.

Analyses

Liquid samples for methanol and VFA analysis were centrifuged at 17,000g for 5 min., diluted with a 3% formic acid solution, and stored at 4°C. VFA was determined by chromatography. The GC (HP 5890A, Palo Alto, USA) was equipped with 2 m × 4 mm glass column, packed with Supelcoport (100–200 mesh) coated with 10% Fluorad FC 431. Operating temperatures were: column, 130°C; injection port, 200°C; flame ionisation detector, 280°C. N$_2$ saturated with formic acid at 20°C is used as carrier gas (30 ml). Methanol was analysed in the same way as VFA except for the oven temperature, which was 70°C.

Biogas composition ($\text{CH}_4$, $\text{CO}_2$, $\text{N}_2$) was determined with a Packard Becker GC model 433 (Delft, The Netherlands) equipped with two columns connected in parallel (split 1:1): 1.5 m × 1/8" Teflon packed with Chromosorb 108 (60–80 mesh) and 1.2 m × 1.8" stainless steel packed with mol. sieve 5A (60–80 mesh). Helium was used as a carrier gases (45 ml/min). Temperatures were column, 40°C; injection port, 100°C; and hot wire detector, 100°C. Injection volume was 100 µl.

Methane was determined in a Packard-Becker 438/S gas chromatography (Delft, The Netherlands). Injection volume was 100 µl. A 2 m × 2 mm stainless steel column was used packed with Poropak Q (80–100 mesh) The temperature of the column, injection port and flame ionisation detector were 60, 200 and 220°C, respectively. N$_2$ was used as carrier gases (20 ml/min).

Hydrogen was determined by GC with a Hewlett-Packard 5890 gas chromatography equipped with a thermal conductivity detector (TCD) and molecular sieve 25H (60–80 mesh). Column size: 1.5 m × 6.4 mm. Argon was used as carrier gas at a flow rate of 25 ml/min. Temperatures were: column, 40°C; injection port 110°C; and detector 125°C. Injection volume was 100 µl or 1000 µl depending on the concentration.

The gas samples were taken by a pressure-lock syringe (Alltech, USA). The gas standards were incubated at 55°C in order to prevent unexpected errors (Kim and Daniel, 1991).
Results and discussion
The performance of the reactor

Table 1 describes the operating conditions and performance data of the reactor. During the first 28 days of operation, the reactor presented a poor performance, with total VFA accumulation up to 563 mgCOD.L⁻¹. From day 28 onwards, the nutrient stock solution was replaced and the performance of the reactor improved immediately. No significant accumulation of VFA was detected in the overall continuous experiment, even when relatively high OLR were applied (Table 1, Figure 3). Less than 2% of the influent COD was detected as VFAs after day 40, when total VFA concentration were kept below 100 mgCOD.L⁻¹.

In phase II, the reactor accommodated immediately to the OLR increase. Decrease in the methane production rate was detected in some periods, however it could be attributed to the gas outlet clogging by floc biomass. The sludge bed increased constantly and gradually, even though floc and spongy biomass was washed out due to the high gas loading rates. Significant disintegration of granular sludge occurred at this phase. In phase III, the reactor took more time to recover from the increasing OLR due to some undesirable temperature and load shocks. The performance recovered however, without any retardation for the temperature shock (day 77, 35°C, during about 17 h) and overloading shock (day 87, 67 gCOD.L⁻¹.d⁻¹, about 21 hours). The recovery from a period without feed supply (day 96, about 7 h) took 3 days, suggesting high maintenance energy requirement. These results show that, the reactor performance is quite stable when exposed to non-optimal conditions.

Theoretical calculation was applied to compare the biomass washout and bacterial growth, based on the biomass COD conversion factor of 1.45 and biomass yield of...
0.05 gVSS. gCOD\(^{-1}\) (Pavlostathis and Giraldo-Gomez, 1991). The bacterial growth was higher than the biomass washout (Figure 4), explaining the continuous increase in the sludge bed in Ppases II and III (suspended solid COD was not measured during phase I).

**Methanogenic activity**

After adaptation on methanol, the SMA on this substrate increased by 110%; conversely, the SMA on acetate decreased by 55.6% (Table 2), indicating the growth of a new population. The apparent substrate affinity \(K_m\) and maximum substrate degradation rate \(V_{\text{max}}\) of the cultivated sludge were estimated (Table 3). The high affinity for acetate, indicates that acetate could be converted rapidly, if produced from methanol. It also explains why acetate concentration was always low in the effluent. The non-adapted population to methanol coupled with the lack of nutrients, might explain the poor performance of the reactor during the first 28 days of the experiment.

**Pathway analysis**

Methanol was converted to methane within about one day when no inhibitor was supplied. Addition of vancomycin to the medium resulted in a decrease in the SMA by 55% (Table 4).

**Table 2** The specific methanogenic activity for the different sludge. Standard deviations are given in brackets as mean value of triplicate culture

<table>
<thead>
<tr>
<th>Sludge type</th>
<th>pH</th>
<th>T(°C)</th>
<th>Substrate</th>
<th>Methanogenic activity (g COD CH(_4).g VSS. d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Seed sludge</td>
<td>7.0</td>
<td>55</td>
<td>Methanol</td>
<td>0.423 (0.010)</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>55</td>
<td>Acetate</td>
<td>1.17 (0.046)</td>
</tr>
<tr>
<td>Cultivated sludge</td>
<td>7.0</td>
<td>55</td>
<td>Methanol</td>
<td>0.89 (0.050)</td>
</tr>
<tr>
<td></td>
<td>7.0</td>
<td>55</td>
<td>Acetate</td>
<td>0.52 (0.030)</td>
</tr>
</tbody>
</table>

**Table 3** The apparent substrate affinity \(K_m\) and maximal substrate degradation rate \(V_{\text{max}}\) of cultivated sludge

<table>
<thead>
<tr>
<th>Substrate</th>
<th>(K_m) (g COD L(^{-1}))</th>
<th>(V_{\text{max}}) (g COD g VSS-1 d(^{-1}))</th>
</tr>
</thead>
<tbody>
<tr>
<td>Methanol</td>
<td>0.043 (0.004)</td>
<td>2.28 (0.152)</td>
</tr>
<tr>
<td>Acetate</td>
<td>0.123 (0.022)</td>
<td>1.41 (0.029)</td>
</tr>
</tbody>
</table>

*Standard deviation given in brackets as mean value of duplicate culture*
The addition of BESA to the medium, caused methanogenesis from methanol to completely cease. Notably, only after a lag phase of 2 days, methanol was stochiometrically converted to acetate. Nonetheless, a significant contribution of the methanol-acetate or methanol-H₂/CO₂-acetate pathway in the methanol degradation cannot yet be excluded.

Results show that the acetoclastic activity was 1.4 times higher than the acetogenic activity, indicating that a methanol conversion via acetate might occur without the build-up of acetate in the medium.

The above results give a general indication about the methanol conversion via the different pathways. Syntrophic conversion seems to play an important role in the methanol degradation by the cultivated consortia. The importance of each of the pathways is presently being investigated (results not yet known).

The hydrogenotrophic methanogenic activity found in our cultivated sludge was relatively high. Hydrogenotrophic methanogens play a key role in the overall process by maintaining the very low partial pressure of H₂ (< 10 Pa), necessary for the metabolism of the syntrophic bacteria. This high activity coupled to the high acetoclastic activity, and high apparent affinity for acetate is essential to keep the concentrations of acetate and higher VFA in the effluent low, when methanol is converted by non-methylotrophic bacteria.

Conclusions
The satisfactory reactor performance at an OLR up to 47.3 gCOD.L⁻¹ and a 3.2 h HRT demonstrates the feasibility of the thermophilic treatment of methanol-containing wastewater by using a one-stage UASB reactor.

No significant VFA accumulation was detected in the effluent, even with bicarbonate concentration exceeding 20 mM. Acetate was the main component of the VFA at relatively low OLR (below 20 gCOD.L⁻¹.d⁻¹), and at high OLR (above 30 gCOD.L⁻¹.d⁻¹), propionate and butyrate were the main VFAs accumulating.

The reactor was characterised by a stable performance even when exposed to non-optimal conditions, such as, a temperature drop (to 35°C), overloading (67 g.L⁻¹.d⁻¹) and no feeding (during 7 hours). The recovery from interruption in feed supply required more time than from the other two shocks.

The thermophilic granular sludge was appropriately retained in the reactor. Biomass wash-out was low throughout the experimental period.

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References


