Similar evolution in $\delta^{13}$CH$_4$ and model-predicted relative rate of aceticlastic methanogenesis during mesophilic methanization of municipal solid wastes

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ABSTRACT

Similar evolution was obtained for the stable carbon isotope signatures $\delta^{13}$CH$_4$ and the model-predicted relative rate of aceticlastic methanogenesis during mesophilic methanization of municipal solid wastes. In batch incubations, the importance of aceticlastic and hydrogenotrophic methanogenesis changes in time. Initially, hydrogenotrophic methanogenesis dominated, but increasing population of Methanosarcina sp. enhances aceticlastic methanogenesis. Later, hydrogenotrophic methanogenesis intensified again. A mathematical model was developed to evaluate the relative contribution of hydrogenotrophic and aceticlastic pathways of methane generation during mesophilic batch anaerobic biodegradation of the French and the Chinese Municipal Solid Wastes (FMSW and CMSW). Taking into account molecular biology analysis reported earlier three groups of methanogens including strictly hydrogenotrophic methanogens, strictly aceticlastic methanogens (Methanosaeta sp.) and Methanosarcina sp., consuming both acetate and H$_2$/H$_2$CO$_3$ were considered in the model. The total organic and inorganic carbon concentrations, methane production volume, methane and carbon dioxide partial pressures values were used for the model calibration and validation. Methane isotopic composition ($\delta^{13}$CH$_4$) evolution during the incubations was used to independently validate the model results. The model demonstrated that only the putrescible solid waste was totally converted to methane.

Key words | isotope $^{13}$C, mathematical model, mesophilic anaerobic digestion, Methanosarcina sp, municipal solid waste

INTRODUCTION

The principal substrates of methanogenic archaean are acetate and hydrogen/carbonic acid (Zinder 1993):

$$\text{CH}_3\text{COOH} + H_2O \rightarrow \text{CH}_4 + H_2\text{CO}_3$$  \hspace{1cm} (1)

$$4H_2 + H_2\text{CO}_3 \rightarrow \text{CH}_4 + 3H_2O$$  \hspace{1cm} (2)

It is traditionally considered (Batstone et al. 2002) that aceticlastic methanogenesis contributes to about 70% of the total CH$_4$ production, the reminder being produced from CO$_2$ and H$_2$. However, the differences in environmental factors as well operational conditions can affect the behaviour and the fate of aceticlastic and hydrogenotrophic methanogens (Demirel & Scherer 2008) changing an impact of aceticlastic and hydrogenotrophic methanogenesis. Determination of stable carbon isotope signatures of the CH$_4$ ($\delta^{13}$CH$_4$) produced allows a coarse estimate of
the relative contributions of the hydrogenotrophic and aceticlastic pathways as methanogenesis from H₂/CO₂ results in lower δ¹³CH₄ values than the aceticlastic metabolism (Conrad 2005).

*Methanosarcina* sp. and *Methanosaeta* sp. were identified as the two known types of methanogens capable of metabolizing acetate (Ferry 1993). *Methanosarcina* having a lower value of half-saturation coefficient $K_S$ for acetate consumption dominates at acetate concentrations below 1 mM while *Methanosaeta* having a higher $K_S$ and ability to use as an energy source not only acetate but also other substrates including H₂/H₂CO₃ dominates above this acetate level.

In the developed industrial countries (France) cellulose and hemicellulose are the major biodegradable components of MSW. Contrarily, in the developing countries (China) the major biodegradable fraction is the ready-degradable putrescible waste. Earlier, the mathematical models were developed to describe anaerobic digestion of putrescible (Vavilin et al. 2008) and cellulosic (Qu et al. 2009b) wastes. In this study, taking into account the (fluorescent in situ hybridization) FISH and (automated ribosomal intergenic spacer analysis) ARISA a mathematical model was developed to evaluate the significance of hydrogenotrophic and aceticlastic pathways of methane generation during anaerobic biodegradation of complex FMSW and CMSW. Model results were then compared to methane stable carbon isotopic signature evolution during the incubations.

**METHODS**

**Batch experimental study**

Typical FMSW and CMSW were reconstituted according to average compositions in order to simulate representative MSW from developed and developing countries, respectively (Vigneron et al. 2007). A weight fraction was 28.8 and 61.6% (putrescible waste) and 25.3 and 7.1% (cellulosic waste contained in paper and cardboard) for FMSW and CMSW, respectively. About 31 g of CMSW and FMSW were incubated at 35 ± 2°C in 1.1 l glass bottles and with liquid volume of 0.68 l. 1.5 g of centrifuged anaerobic sludge from a municipal wastewater treatment plant was added into both batch reactors as an inoculum. Buffered solution containing 0.15 M of NaHCO₃ and 0.15 M of K₂CO₃ was added into every reactor. One control reactor containing only the inoculum was used to measure the background methane production.

The gas composition in the headspace of the reactors was analyzed immediately after the equilibration by connecting the bottle to a gas chromatograph (μGC CP4900, Varian) equipped with parallel chromatographic columns coupled with thermal conductivity detectors. The gas samples for analysis of δ¹³CH₄ were periodically collected by a gas syringe, transferred into 7 ml vacuum serum tubes and stored for later isotopic analysis. The analysis was performed using a Trace GC Ultra (Thermo Electron Corporation) attached to a Delta V plus isotope ratio mass spectrometer via a GC combustion III. Typical δ¹³C uncertainty, quantified by replicate measurements of different samples, was ± 0.2‰.

During the 160 days of operation, about 20 leachate samples were recovered from each reactor. For every sample, 6 ml of liquid was withdrawn through the septum. Raw samples (3 ml) were stored at −20°C for analysis of TIC and TOC measured with a BIORITECH 700 analyzer. The other 3 mL were centrifuged at 13,000 rpm for 10 min. Supernatants were recovered and stored at −20°C for physico-chemical analysis. Acetic acid, propionic acid and butyric acid in the leachate were measured using a Thermo Quest-Trace GC 2000 (Thermo Quest, Italy) equipped with a flame ionization detector (FID) and DB-WAXetr Capillary Column. The pellets were used for DNA extraction and FISH observations. For each reactor, 9–10 samples corresponding to different sampling dates were used. The detailed description of experiments including isotopic data, the ARISA and FISH analysis were reported earlier (Qu et al. 2009a).

**Model**

Polymer hydrolysis/acidogenesis and acetogenesis/methanogenesis were included in the simplified model as the two possible rate-limiting steps of the overall anaerobic digestion process (Figure 1). Three groups of methanogens including strictly hydrogenotrophic methanogens, strictly aceticlastic methanogens (*Methanosaeta* sp.) and...
Methanosarcina sp. consuming both acetate and \( \text{H}_2/\text{H}_2\text{CO}_3 \) were considered in the model for MSW. The dimensionless functions \( I_h \) and \( I_m \) describing the inhibition of hydrolysis and aceticlastic methanogenesis by the high VFA concentration were calculated using the functions described previously (Vavilin et al. 2008):

\[
I(\text{VFA}) = \frac{1}{1 + (\text{VFA}/K_i)^n},
\]

where \( K_i \) is the inhibition coefficient and \( n \) is the degree index written as \( K_{ih} \), \( K_{im} \) for hydrolysis and aceticlastic methanogenesis, respectively. The wide range of possible proton sources, including inorganic buffers, and a lack of corresponding data, made it difficult to accurately compute pH. Thus, in the model the pH data was approximated by the two exponential functions (Qu et al. 2009b). Some kinetic coefficients are shown in the Table 1.

### Hydrolysis and acidogenesis

CMSW and FMSW were assumed to contain different proportion of putrescible and recalcitrant waste as well as cellulosic material contained in office paper (OP) and cardboard (CD). The hydrolysis and acidogenesis of different fractions of MSW were described as the simple first-order reactions inhibited by high VFA concentration:

\[
\begin{align*}
\frac{dW_i}{dt} &= -k_i I_{hi} W_i, \\
\frac{d\text{H}_2}{dt} &= \sum \chi_i \frac{H_2}{K_i I_{hi} W_i}, \\
\frac{d\text{VFA}}{dt} &= \sum \chi_{\text{VFA}} k_i I_{hi} W_i, \\
\frac{d\text{H}_2\text{CO}_3}{dt} &= \sum \chi_{\text{H}_2\text{CO}_3} k_i I_{hi} W_i,
\end{align*}
\]

where \( W_i \) (\( W_r, W_p, W_c \)) are the ready-degradable (putrescible), recalcitrant, cardboard and paper waste concentrations; VFA and \( \text{H}_2\text{CO}_3 \) are the concentrations of volatile fatty and carbonic acids; \( d\text{H}_2/dt \) is the rate of hydrogen production; \( k_i \) is the corresponding first-order rate coefficient, \( I_{hi} \) are the hydrolysis inhibiting functions; \( \chi_{\text{VFA}}, \chi_{\text{H}_2\text{CO}_3}, \chi_{\text{H}_2\text{CO}_3} \) are the corresponding stoichiometric coefficients. The stoichiometric coefficients for monosaccharides being the main products of enzymatic degradation of cellulose presented in OP and CD were appointed according to the equation:

\[
\text{C}_6\text{H}_{12}\text{O}_6 + 4\text{H}_2\text{O} \rightarrow 2\text{CH}_3\text{COOH} + 2\text{H}_2\text{CO}_3 + 4\text{H}_2
\]

Cellulosic waste degradation was described similar to that was presented by Qu et al. (2009b).

### Aceticlastic methanogenesis

The aceticlastic methanogenic process was performed by both strictly aceticlastic methanogens (Methanosaeta sp.) and Methanosarcina sp. consuming acetate (VFA) and \( \text{H}_2/\text{H}_2\text{CO}_3 \). For simplicity, acetogenesis was not considered separately in the model. The VFA’s consumption, methane and \( \text{H}_2\text{CO}_3 \) production rates during this step were

<table>
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<th>Table 1</th>
<th>Kinetic coefficients of hydrolysis and methanogenesis, and initial biomass concentration</th>
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<td>First-order rate constant of hydrolysis ( k ) (d(^{-1}))</td>
<td>( \text{Cellulose in OP and CD: 0.012; PW: 0.025} )</td>
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<td>Maximum specific rate of substrate consumption ( \rho_m ) (d(^{-1}))</td>
<td>( \text{Acetate: 3.3 (Methanosarcina sp.), 0.3 (Methanosaeta sp.)} )</td>
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<tr>
<td>Half-saturation coefficient of acetate consumption ( K_S ) (g/l(^{-1}))</td>
<td>( \text{H}_2/\text{H}_2\text{CO}_3: 10 \text{ (Hydrogenotrophs), 2 \text{ (Methanosarcina sp.)}} )</td>
</tr>
<tr>
<td>Initial biomass concentration ( B ) (g/l(^{-1}))</td>
<td>( \text{1.6 (Methanosarcina sp.), 0.1 (Methanosaeta sp.)} )</td>
</tr>
<tr>
<td></td>
<td>( \text{0.0006 (Methanosarcina sp.), 0.3 (Methanosaeta sp.), 0.0001 (Hydrogenotrophs)} )</td>
</tr>
</tbody>
</table>
described by the following equations:

\[
\frac{dVFA}{dt} = -\rho_{m\text{saet}} I_mB_{\text{saet}} \frac{VFA}{K_{\text{VFA}s\text{aet}} + VFA} - \rho_{m\text{sarAc}} I_mB_{\text{sar}} \frac{VFA}{K_{\text{VFA}s\text{ar}} + VFA},
\]

(6)

\[
\frac{dCH_4}{dt} = \text{Vol} \times I_m \left[ (1 - Y_{\text{saet}}) \rho_{m\text{saet}} B_{\text{saet}} \frac{VFA}{K_{\text{VFA}s\text{aet}} + VFA} + (1 - Y_{\text{sarAc}}) \rho_{m\text{sarAc}} B_{\text{sar}} \frac{VFA}{K_{\text{VFA}s\text{ar}} + VFA} \right]
\]

(7)

\[
\frac{dH_2CO_3}{dt} = -(1 - Y_{\text{sarAc}}) \rho_{m\text{sarAc}} I_mB_{\text{sar}} \frac{VFA}{K_{\text{VFA}s\text{ar}} + VFA} + (1 - Y_{\text{sat}}) \rho_{m\text{saet}} I_mB_{\text{saet}} \frac{VFA}{K_{\text{VFA}s\text{aet}} + VFA},
\]

(8)

where \( \rho_{m\text{saet}}, \rho_{m\text{sarAc}} \) are the maximum specific rate of VFA utilization by \( \text{Methanosaeta} \) sp. and \( \text{Methanosarcina} \) sp.; \( B_{\text{saet}}, B_{\text{sar}} \) are the concentration of aceticlastic methanogens \( \text{Methanosaeta} \) sp. and \( \text{Methanosarcina} \) sp., respectively; \( K_{\text{VFA}s\text{aet}}, K_{\text{VFA}s\text{ar}} \) are the half-saturation constant for VFA utilization by \( \text{Methanosaeta} \) sp. and \( \text{Methanosarcina} \) sp., respectively; \( \text{CH}_4 \) is the methane volume produced by aceticlastic methanogenesis; \( \text{Vol} \) is the liquid volume; \( Y_{\text{saet}}, Y_{\text{sarAc}} \) are the corresponding biomass yield coefficients.

**Hydrogenotrophic methanogenesis**

\( H_2CO_3 \) and \( H_2 \) consumption, and methane production during this step was expressed by:

\[
\frac{dH_2CO_3}{dt} = -0.25 \left[ \rho_{m\text{hyd}} I_mB_{\text{hyd}} \frac{PH_2}{K_{PH_2\text{hyd}} + PH_2K_{H_2CO_3\text{hyd}} + H_2CO_3} + \rho_{m\text{sarH}_2} I_mB_{\text{sarH}_2} \frac{PH_2}{K_{PH_2\text{sarH}_2} + PH_2K_{H_2CO_3\text{sarH}_2} + H_2CO_3} \right]
\]

(9)

\[
\frac{dH_2}{dt} = -\rho_{m\text{hyd}} I_mB_{\text{hyd}} \frac{PH_2}{K_{PH_2\text{hyd}} + PH_2K_{H_2CO_3\text{hyd}} + H_2CO_3} - \rho_{m\text{sarH}_2} I_mB_{\text{sarH}_2} \frac{PH_2}{K_{PH_2\text{sarH}_2} + PH_2K_{H_2CO_3\text{sarH}_2} + H_2CO_3}
\]

(10)

\[
\frac{dCH_4^{H_2/H_2CO_3}}{dt} = \text{Vol} \times 0.25 \left[ I_m(1 - Y_{\text{sarH}_2}) \rho_{m\text{sarH}_2} B_{\text{sarH}_2} \frac{PH_2}{K_{PH_2\text{sarH}_2} + H_2K_{H_2CO_3\text{sarH}_2} + H_2CO_3} + (1 - Y_{\text{hyd}}) \rho_{m\text{hyd}} B_{\text{hyd}} \frac{PH_2}{K_{PH_2\text{hyd}} + H_2K_{H_2CO_3\text{hyd}} + H_2CO_3} \right]
\]

(11)

where \( \rho_{m\text{hyd}}, \rho_{m\text{sarH}_2} \) are the maximum specific rate of \( H_2/H_2CO_3 \) utilization by hydrogenotrophic methanogens and \( \text{Methanosarcina} \) sp., respectively; \( B_{\text{hyd}} \) is the concentration of hydrogenotrophic methanogens; \( PH_2 \) is the \( H_2 \) partial pressure; \( K_{PH_2\text{hyd}}, K_{PH_2\text{sarH}_2}, K_{H_2CO_3\text{hyd}}, K_{H_2CO_3\text{sarH}_2} \) are the half-saturation constant for \( H_2 \) and \( H_2CO_3 \) utilization by hydrogenotrophic methanogens and \( \text{Methanosarcina} \) sp.; \( \text{CH}_4^{H_2/H_2CO_3} \) is the methane volume produced from \( H_2/H_2CO_3 \).

**Biomass growth**

The Monod functions with a single substrate or two substrates were used to describe biomass growth of aceticlastic methanogens \( \text{Methanosaeta} \) sp. and \( \text{Methanosarcina} \) sp. and hydrogenotrophic methanogens by:

\[
\frac{dB_{\text{saet}}}{dt} = Y_{\text{saet}} \rho_{m\text{saet}} I_mB_{\text{saet}} \frac{VFA}{K_{\text{VFA}s\text{aet}} + VFA} - k_{\text{daet}} B_{\text{saet}}.
\]

(12)

\[
\frac{dB_{\text{sar}}}{dt} = I_mB_{\text{sar}} \left[ Y_{\text{sarAc}} \rho_{m\text{sarAc}} \frac{VFA}{K_{\text{VFA}s\text{ar}} + VFA} + Y_{\text{satH}_2} \rho_{m\text{sarH}_2} \frac{PH_2}{K_{PH_2\text{sarH}_2} + PH_2K_{H_2CO_3\text{sarH}_2} + H_2CO_3} \right] - k_{\text{daar}} B_{\text{sar}}.
\]

(13)

\[
\frac{dB_{\text{hyd}}}{dt} = Y_{\text{hyd}} \rho_{m\text{hyd}} B_{\text{hyd}} \frac{PH_2}{K_{PH_2\text{hyd}} + PH_2K_{H_2CO_3\text{hyd}} + H_2CO_3} - k_{\text{dahyd}} B_{\text{hyd}}.
\]

(14)

where \( k_{\text{daet}}, k_{\text{daar}}, k_{\text{dahyd}} \) are the specific biomass decay coefficients of the respective microorganisms.

**Gas pressure**

The partial gas pressures of \( \text{CO}_2, \text{H}_2 \) and \( \text{CH}_4 \) were computed according to \( \text{Vavilin et al. (1995)} \).

\[
\frac{dP_i}{dt} = \frac{RT}{V_{\text{gas}}} \left( -\text{TR}_i + \sum_{i=1}^{N} \frac{P_i}{P_j} \right)
\]

(15)

where \( R \) is the universal gas constant, \( T \) is the temperature, \( V_{\text{gas}} \) is the volume of gas phase, \( P_i \) is the total gas pressure, and \( \text{TR}_i \) is the rate of mass transfer exchange between
gaseous and liquid phases, \( N \) is the total number of gases. The total gas pressure was appointed to 1 bar. It was assumed that initially 100% of gas phase was occupied by the inert gas (helium) and the exponential function was used to describe a quick decrease of helium partial pressure in that short period when helium was substituted by the gases produced. Taking into account the dissociation of \( H_2CO_3 \) and using the Henry’s law for \( CO_2 \), we determine \( CO_2 \) mass exchange as:

\[
TR_C = \text{Vol} \times K_{LV}[K_{HC} P_C - H_2CO_3/(1 + K_{DC}/H)],
\]

where \( K_{LV} \) is the coefficient of mass exchange between the liquid and gas phases; \( K_{HC} \) is the Henry constant for \( CO_2 \); \( P_C \) is the partial pressure of \( CO_2 \); \( K_{DC} \) is the constant of \( H_2CO_3 \) dissociation; \( H \) is the proton concentration. \( H_2 \) as well as \( CH_4 \) were assumed insoluble in water.

**Model-predicted relative rate of aceticlastic methanogenesis**

The relative rate of aceticlastic methanogenesis giving a ratio between the production rates of methane from acetate and both acetate and \( H_2/H_2CO_3 \) was computed by:

\[
f = [dCH_4_{Ac}/dt]/[dCH_4_{tot}/dt].
\]

All numerical simulations were performed using MATLAB software. For complex CMSW and FMSW the same parameter values were used as for OP, CD and PW previously obtained (Qu et al. 2009b; Vavilin et al. 2008) except for the coefficients to describe the pH values. It should be noted that an overpressure occurred in the closed bottles during the quick stage of hydrolysis/acidogenesis and the gas release was undertaken during samplings. The model could not adequately describe partial pressure changes at the initial time.

![Figure 2](https://iwaponline.com/wst/article-pdf/60/12/3173/447450/3173.pdf)

**Figure 2** | Time profiles of the system variables during mesophilic anaerobic digestion of CMSW. The symbols represent values measured in the actual reactor and the lines are results obtained using the model.
RESULTS AND DISCUSSION

Mesophilic aceticlastic and hydrogenotrophic methanogenesis

Under the given sets of parameters the model describes the experimental data of anaerobic digestion of CMSW and FMSW reasonably well (Figures 2 and 3). The concentration of fast-growing hydrogenotrophic methanogens did not exceed 0.01 g l\(^{-1}\). The relative proportions of aceticlastic and hydrogenotrophic methanogenesis change during biodegradation. A peak value of hydrogen partial pressure arose quickly from the start. Because the growth rate of hydrogenotrophs was significantly higher than that of \textit{Methanosaeta} sp. the rate of CH\(_4\) production from H\(_2/\)H\(_2\)CO\(_3\) increases from the start and the relative proportion of aceticlastic methanogenesis reach its minimal value (panels ‘g’) after 15–20 days. With increasing population of hydrogenotrophs the hydrogen partial pressure decreased to a low level. Based on the FISH analysis of the inoculum originating from an anaerobic sludge digester we assumed that \textit{Methanosarcina} sp. were present initially at a much lower concentration than \textit{Methanosaeta} sp. However, the model predicts that after a delay \textit{Methanosarcina} sp. started to dominate among the methanogens (panels ‘i’), in agreement with the FISH observations. During the intensive aceticlastic methanogenesis causing an increase in the value of \(f\) (panels ‘g’) the partial pressure of carbon dioxide and methane decreased and increased, respectively (panels ‘e’). When VFA concentration reached low values then VFA (acetate) were consumed by \textit{Methanosaeta} sp. and H\(_2/\)H\(_2\)CO\(_3\) was consumed by hydrogenotrophs as well as \textit{Methanosarcina} sp. causing again a decrease in the value of \(f\) (panels ‘g’).

![Figure 3](https://iwaponline.com/wst/article-pdf/60/12/3173/447450/3173.pdf)

**Figure 3** | Time profiles of the system variables during mesophilic anaerobic digestion of FMSW.
As it was reported earlier (Qu et al. 2009a), in mesophilic conditions the trends of the isotope signature of the measured CH₄ (δ¹³CH₄) were similar for both CMSW and FMSW (panels ‘h’).

δ¹³CH₄ initially decreased during the slow initial methane production period to reach typical hydrogenotrophic values (around −70‰) which corresponds to the slight initial decrease in f values determined by the model (panels ‘g’). When active methane production began, the isotopic composition also started to increase (panels ‘h’) to reach their highest values at days 65 and 50 for CMSW and FMSW, respectively. This evolution of the methane isotopic signature illustrates the metabolic shift from hydrogenotrophic to aceticlastic pathway. During that period the relative proportion of aceticlastic methanogenesis determined by the model also increased (panels ‘g’) and became maximal at days 60 (CMSW) and 45 (FMSW). Then, δ¹³CH₄ decreased again (panels ‘h’) which corresponds to the decrease in f values (panels ‘g’). Methane stable carbon isotopic signature (δ¹³CH₄) monitoring allows then to independently validate the evolution of the relative proportion of aceticlastic methanogenesis along waste degradation determined by our model.

During the initial stage not only aceticlastic methanogenesis but also the hydrolysis of OP and PW were inhibited (not shown) by temporary accumulation of VFA. The model showed that the total volume of released methane agrees with the experimental data under assumption that in case of CMSW only the putrescible solid waste was biodegradable and for FMSW only 50% of other waste fractions (cellulosic, recalcitrant) were degraded in addition to the total degradation of putrescible waste. All in all, it can be concluded that only PW present in CMSW and FMSW was fully transformed into methane.

CONCLUSIONS

During batch mesophilic anaerobic digestion of the samples with CMSW and FMSW similar evolution was obtained for the model-predicted relative rate of aceticlastic methanogenesis and the stable carbon isotope signatures δ¹³CH₄ measured in experiment. Initially, hydrogenotrophic methanogenesis dominated, but increasing population of Methanosarcina sp. enhances aceticlastic methanogenesis. Later, hydrogenotrophic methanogenesis intensified again. The model results agreed well with hypothesis that under mesophilic conditions only the putrescible solid waste was totally converted to methane.

REFERENCES


