Anaerobic batch degradation of solid poultry slaughterhouse waste

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Abstract We studied anaerobic batch degradation of solid poultry slaughterhouse wastes with different initial waste and inoculum concentrations and waste-to-inoculum ratios and simulated the dynamics of the process with a new generation <METHANE> model. Our modelling results suggest that inhibited propionate degradation by long-chain fatty acids (LCFA) and inhibited hydrolysis by a high propionate concentration constituted the rate-limiting step in the waste degradation. Palmitate was the most abundant LCFA in the assays. Within 27 days of incubation, up to 0.55 to 0.67 m3 of methane (STP)/kg VS added was produced under the studied conditions. Lower waste-to-inoculum ratios exhibited a faster onset and rate of specific methane production. In all the assays, ammonification occurred within 3 to 6 days and accounted for 50 to 60% of total nitrogen.

Keywords Anaerobic digestion; inhibition; long-chain fatty acids; poultry slaughterhouse waste; simulation model

Introduction

Poultry slaughterhouses produce quantities of lipid and protein rich wastes, which are now mostly banned as, e.g., animal feed. In this situation, anaerobic digestion could become a worthwhile treatment option, because the wastes are valuable sources of energy and fertiliser. However, anaerobic digestion has so far rarely been applied to slaughterhouse wastes alone on an industrial-scale (Banks, 1994; Tritt and Schuhardt, 1992), and previous studies have reported difficulties maintaining a long-term stable semi-continuous digestion of these wastes, apparently due to inhibition by ammonia or long-chain fatty acids (LCFA; e.g. Salminen and Rintala, 1999).

The anaerobic biodegradation of complex organic materials is a multi-step conversion process, in which solid materials, such as polycarbohydrates, proteins, and lipids, are first hydrolysed, polycarbohydrates to sugars and alcohols, proteins to polypeptides, and amino acids and lipids to LCFA and glycerol. Fermentative acidogenic bacteria subsequently ferment the intermediates to volatile fatty acids (VFA), hydrogen (H2), and carbon dioxide (CO2). Ammonia is a by-product of amino acid fermentation. Hydrogen-producing acetogenic bacteria metabolise VFAs longer than acetate and neutral compounds larger than methanol to acetate, H2 and CO2, which methanogenic bacteria further convert to methane and CO2. LCFA and VFAs are syntrophic substrates, i.e., their breakdown depends on the removal of H2 from the media by anaerobic micro-organisms. The breakdown of LCFA is usually regarded as the rate-limiting step in the degradation of complex substrates (Angelidaki and Ahring, 1995; Novak and Carlson, 1970; Rinzema et al., 1994). Already at very low concentrations, LCFA, especially unsaturated LCFA, are suggested inhibitory to syntrophic acetogenic and methanogenic bacteria (Angelidaki and Ahring, 1992; Hanaki et al., 1981; Koster and Cramer, 1987; Rinzema et al., 1994; Roy et al., 1985). Non-ionised ammonia reportedly inhibited methanogenesis at initial concentrations ranging from 33.
approximately 0.1 to 1.1 g-N/l (Angelidaki and Ahring, 1993; De Baere et al., 1984; Hansen et al., 1998; Hashimoto, 1986).

Because experiments with the anaerobic process are time consuming, labour intensive, and expensive, modelling can become a powerful tool to develop and operate such a process. <METHANE> is a generalised model of anaerobic digestion used successfully to describe degradation of complex organic material (Vavilin et al., 1994).

The objective of our study was to investigate the anaerobic batch degradation of solid poultry slaughterhouse wastes using different initial waste and inoculum concentrations and waste-to-inoculum ratios. We modelled the dynamics of the process with a new generation <METHANE> model.

Materials and methods

Waste
Solid poultry slaughterhouse waste (minced and mixed fractions of bonemeal, 42.1 weight%; blood, 15.8 weight%; offal and guts, 31.6 weight%, and autoclaved [5 min at 120ºC] feather, 10.5 weight%; from Atria Ltd., Nurmo; total solids (TS) 31.2%, volatile solids (VS) 26.0%, ammonia 380 mg-N/l, Kjeldahl-N 24.3 g-N/l, proteins 15.2%, lipids 10.0%) was used as substrate in the assays.

Inoculum
Sludge (TS 3.3%, VS 2.1%, Kjeldahl-N 1.7 g/l) from a mesophilic (35ºC) digester in a municipal sewage treatment plant (Viinikka, Tampere, Finland) was used as inoculum in the assays.

Assays
Assays were run in 118 ml vials with a liquid volume of 50 ml. A waste mixture, 0.75 g/vial (3.9 g VS/l) or 1.50 g/vial (7.8 g VS/l), was added into the vials. Assays without added substrate were assayed to evaluate inoculum performance alone. Inoculum (20 or 40 ml) was then transferred into the vials and distilled water was added to volume (50 ml). The vials were flushed with N2/CO2 (80%/20%) and sealed with butyl rubber stoppers and aluminium crimps. Finally, Na2S (9H2O (0.25 g/l) was added to remove any residual O2. The vials were then incubated in shaken cultures at 35ºC. Assays were run using 6 replicates with 4 vials sacrificed for analyses during incubation.

Model
The new generation <METHANE> model used here is based on an earlier model (Vavilin et al., 1994). Slaughterhouse wastes were assumed a mixture of proteins, lipids, and carbohydrates. Hydrolysis, acidogenesis, acetogenesis, and methanogenesis as induced by different groups of microorganisms were described. Four groups of variables and equations were included in the model:

1. Suspended solid concentrations (Xk, k=1, 2, 3)

\[ V \frac{dX_k}{dt} = q_f \cdot X_{Fk} - q_{BX} \cdot X_k - V \cdot \theta_{Xk} \]

where \( X_{Fk} \) = influent concentrations of suspended solids; \( q_f \) = feed flow rate; \( q_{BX} \) = discharge rate of excess suspended solids including biomass; \( \theta_{Xk} \) = rates of solids transformation; \( V \) = volume of liquid phase.

2. Active biomass concentrations (Bi, i=1,2, . . . ,10)

\[ V \frac{dB_i}{dt} = q_f \cdot B_i - q_{BX} \cdot B_i + V \cdot \theta_{Bi} \]
where $B_F$=influent concentrations of active bacteria; $\Theta_B$=growth rates of various sub-populations.

3. Soluble substrate concentrations ($S_j$, $j=1,2,\ldots,13$)

$$V \cdot \frac{dS_j}{dt} = \eta_j \cdot (S_F - S_j) + V \cdot \Theta_{S_j} \cdot \text{TRS}_j$$

where $S_F$=influent concentrations of soluble substrates; $\Theta_{S_j}$=rates of soluble substrate transformation; $\text{TRS}_j$=rate of mass exchange between gaseous and liquid phase. Note that $\Theta_B = \Theta_{S_j} / Y_j$, where $Y_j$=yield coefficient.

4. Partial gas pressures ($P_l$, $l=1,\ldots,5$)

$$\frac{dP_l}{dt} = \frac{RT}{V^g} \left[ -\text{TRS}_l + \sum_n \text{TRS}_n \cdot \frac{P_l}{P_T} \right]$$

where $R$=universal gas constant; $T$=temperature (K); $V^g$=volume of gas phase; $P_T$=total gas pressure.

The rate of the main limiting substrate transformation by the $i$-th group of bacteria ($\Theta_{S_i}$) was expressed as a product of several functions:

$$\Theta_{S_i} = \Theta_m \cdot FT_i \cdot FL_i \cdot FI_i \cdot B_i$$

where $\Theta_m$=maximum specific rate of limiting substrate consumption by $i$-th group of microorganisms under optimum conditions with biomass concentration $B_i$; $FT_i$, $FL_i$, $FI_i$=functions describing temperature dependence, mechanism of substrate limitation, and inhibition, respectively. Inhibition processes by VFA (propionate and butyrate) and total LCFA were considered here using the generalised function of non-competitive inhibition:

$$FI_i(I) = \left[ 1 + \left( \frac{I}{K_{I2}} \right)^{\frac{\Theta_m}{\Theta_m-K_{I1}}} \right]^{-1}$$

where $I$=inhibition agent; $K_{I1}$, $K_{I2}$=inhibition constants where the process rate decreases twice and 100 times, respectively. Experiment pH data was approximated in a stepwise manner to describe pH in the model. The inhibiting impact of hydrogen was given by the function

$$FI_i(H_2) = [1 + (P_{H2}/K_{H2})^4]^{-1}$$

where $K_{H2}$=constant of hydrogen inhibition. Inhibition processes by ammonia were not taken into account here.

The traditional Monod dependence, describing substrate limitation, was used for acido-genic, syntrophic, and acetotrophic methanogenic bacteria. For hydrogen consuming methanogens, the hydrogen consumption rate was determined according to the principle of a minimum between the Monod functions for $H_2$ and $CO_2$ as the limiting substrates. First-order kinetics with inhibition by VFA was applied to describe the polymer hydrolysis of solids. The following stoichiometric equations were selected after model calibration:

- Peptides $\rightarrow 0.1$ $H_2 + 1.7$ $CO_2 + 2.8$ Acetate + 0.1 Propionate + 2.1 Butyrate + 4 $NH_3$
- Lipids $\rightarrow 8.5$ $H_2 + 1.75$ Acetate + 2.5 Propionate + 2.0 Stearate
- Carbohydrates $\rightarrow 0.4$ $H_2 + 0.8$ $CO_2 + 1.3$ Acetate + 0.2 Propionate + 0.5 Butyrate
- Stearate $\rightarrow 1.0$ Palmitate + 2.0 $H_2 + 1.0$ Acetate
- Palmitate $\rightarrow 12.0$ $H_2 + 6.0$ Acetate + 1.0 Butyrate

In the model, the stearate concentration was calculated, for simplicity, as the sum of stearate and oleate concentrations and the palmitate concentration as the sum of palmitate and myristate concentrations.
Analyses

Methane content was analysed with a Perkin Elmer Autosystem XL gas chromatograph with a flame-ionisation detector and a PE Alumina column, as described elsewhere (Lepistö and Rintala, 1995). VFA (volatile straight and branched-chain fatty acids from C₂ to C₅) was analysed with the above chromatograph equipped with a flame-ionisation detector and a PE FFAP column, as described elsewhere (Lepistö and Rintala, 1995). Lipids were determined according to the *Official Methods of Chemical Analysis* (1990). LCFA (non-esterified long-chain fatty acids: C14:0, myristate; 16:0, palmitate; C18:0, stearate and C18:1(n-9), oleate) samples were extracted as described by Bligh and Dyer (1959). Chloroform was evaporated with N₂ and its residue dissolved in methyl-tert-butylether, methylated, and then analysed with a Hewlett Packard HP 6890 series gas chromatograph with a 5973 mass selective detector and an HP-5 column. The analysis was run in scan mode (mass range m/e 35-600, scan rate 1 scan/s). pH was measured with a 774 pH-meter (Metrohm) immediately after sampling to avoid pH changes due to loss of CO₂ in the liquid. Nitrogen was analysed with a Kjeltec system 1002 distilling unit (Tecator AB). Ammonia, TS, and VS were determined according to *Standard Methods for the Examination of Water and Wastewater*. (1985). The ammonia samples were filtered with glass fibre filter papers (Schleicher and Schuell). Total Kjeldahl nitrogen was determined according to the Tecator application note (Perstorp Analytical/ Tecator AB, 1995). Before distillation, the samples were digested with digester 2006 (Tecator AB). Protein content was calculated from the Kjeldahl-N content using a conversion factor of Kjeldahl-N × 6.25 (for meat).

Results and discussion

The comparative assays (Table 1) indicated that during the degradation of solid poultry slaughterhouse wastes, the different factors to a degree affected the concentrations of the individual intermediate compounds and the onset and rate of methane production (Figure 1). In general, however, the degradation patterns resembled each other and indicated rapid hydrolysis/acidogenesis, accumulation of LCFA and VFA, removal of LCFA and subsequently that of VFAs, and methane production (Figure 1). Similar patterns were reported for anaerobic batch degradation of sheep tallow (Broughton *et al.*, 1998), whole milk (Hanaki *et al.*, 1981), and capric acid (Rinzema *et al.*, 1994).

In the different assays, the highest total LCFA ranged from 4.1 mM to 12.0 mM, the concentrations being below the detection limit after 27 days of incubation (Table 1). Palmitate was the most abundant LCFA (up to 9.0 mM) in the assays, whereas oleate, stearate, and myristate were present up to 2.1, 0.7 and 0.5 mM, respectively (Figure 1).

The degradation patterns were simulated with kinetic constants (Table 2), obtained by model calibration with data from all the assays. The model, assuming an inhibition of hydrolysis by VFA, an inhibition of acetogenesis by LCFA, and a stearate transformation by H₂, fitted the experiments reasonably well, as shown for the assay with the lowest waste-to-inoculum ratio (assay 2, Figure 2). According to the model, polymer hydrolysis/acidogenesis brought about a rapid accumulation of VFA and LCFA, which inhibited hydrolysis and acetogenesis, respectively (day 3). The stearate concentration began to plummet because stearate was consumed faster than it was produced. However, increasing hydrogen became inhibitory to stearate consumption (day 9): the stearate concentration initially increased but began to drop (day 12), as the growth of hydrogenotrophic methanogens lowered the concentration of hydrogen. Because of the low number of syntrophs consuming palmitate during the first 12 days, palmitate was consumed at a lower rate than produced, driving up its concentration, which began to drop on day 16 after a rapid growth of the syntrophs. When the palmitate and stearate concentrations became non-inhibitory to butyrate and propionate consumption, a rapid acetogenesis accompanied by a rapid hydrolysis followed, setting off...
a rapid methanogenesis. All in all, the model suggested that syntrophic LCFA degradation was the rate-limiting step at the beginning of all the assays, whereas in the final stage of the degradation, polymer hydrolysis turned out to be the rate limiter (Figure 2). Hence LCFA degradation is most likely the critical part in continuous digestion processes, an assumption which can be verified only upon processing enough data on digesters treating poultry wastes on a continuous basis.

Acetate was the most abundant VFA in all the assays and was consumed before propionate (Figure 1). In all the assays, methane production started after a 6 to 9 day lag. Lower waste-to-inoculum ratios exhibited a slightly faster onset and a higher methane production rate (Figure 1). The model suggested that the rate-limiting step, indicated by delayed methane production, rather than being the result of inhibited aceticlastic methanogenesis, was caused by LCFA inhibiting propionate degradation, followed by high propionate inhibiting hydrolysis. During model calibration we discovered that aceticlastic methanogenesis was not inhibited. On the other hand, in the anaerobic degradation of tallow, hydrolysis resulted in a rapid inhibition of LCFA degrading syntrophic acetogens and the subsequent accumulation of LCFA inhibiting methanogenesis (Broughton et al., 1998).

Rinzema et al. (1994) observed that LCFA inhibited both acetogenesis and methanogenesis and that obligate hydrogen producing acetogens and hydrogenotrophic methanogens recovered first from the inhibition, bringing about the recovery of acetoclastic methanogenesis.

In the model, we assumed that acetogenesis was inhibited by the total LCFA concentration, whereas in practice inhibition is caused by various LCFA. Unsaturated LCFA are suggested more inhibitory to anaerobic micro-organisms than saturated LCFA (Roy et al., 1985; Angelidaki and Ahring, 1992). An enhanced inhibitory effect on methanogenesis of a mixture of LCFA by synergism of an individual LCFA has been reported (Koster and Cramer, 1987). Inhibition by LCFA is reportedly determined by the ratio of LCFA to biomass (Rinzema et al., 1994), whereas contradictorily inhibition is related primarily to the concentration of LCFA (Galbraith et al., 1971).

The average methane yields within 27 days ranged from 0.55 to 0.67 m³ of methane (STP)/kg VS added (Table 1). The highest waste-to-inoculum ratio exhibited a slightly lower methane yield by day 27, while the yields were similar after 35 days of incubation.
Figure 1  Specific cumulative methane productions (above) and LCFA (left) and VFA (right) concentrations during batch assays.
Table 2  Model parameters for simulation presented in Figure 2

<table>
<thead>
<tr>
<th>Process</th>
<th>( m, \text{mM}/\text{mM}/\text{d} )</th>
<th>( Y, \text{mM}/\text{mM} )</th>
<th>( K_S, \text{mM, bar}^* )</th>
<th>( K_{I1}, K_{I2}, \text{mM, bar}^* )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hydrolysis</td>
<td>0.7***</td>
<td></td>
<td>12.03; 12.13 (VFA)</td>
<td></td>
</tr>
<tr>
<td>Acidogenesis</td>
<td>100.0</td>
<td>0.05</td>
<td>0.94</td>
<td></td>
</tr>
<tr>
<td>Acetogenesis (butyrate)</td>
<td>7.0</td>
<td>0.04</td>
<td>0.23</td>
<td>4.0; 9.0 (105 (LCFA)</td>
</tr>
<tr>
<td>Acetogenesis (propionate)</td>
<td>8.5</td>
<td>0.05</td>
<td>1.0</td>
<td>2.0; 9.0 (105 (LCFA)</td>
</tr>
<tr>
<td>Acetogenesis (palmitate)</td>
<td>44.0</td>
<td>0.02</td>
<td>0.39</td>
<td></td>
</tr>
<tr>
<td>Acetogenesis (stearate)</td>
<td>70.0</td>
<td>0.11</td>
<td>0.35</td>
<td>0.086 (hydrogen)</td>
</tr>
<tr>
<td>Methanogenesis (H2/CO2)</td>
<td>70.0</td>
<td>0.02</td>
<td>0.0012, 0.0001**</td>
<td></td>
</tr>
<tr>
<td>Methanogenesis (acetate)</td>
<td>36.4</td>
<td>0.01</td>
<td>1.33</td>
<td></td>
</tr>
</tbody>
</table>

\( \omega_m \) = specific maximum rate of substrate utilisation; \( Y \) = yield coefficient; \( K_S \) = half-saturation coefficient; \( K_{I1} \) and \( K_{I2} \) = inhibition constants; *gas components are expressed in bars; **two values corresponded to \( \text{H}_2 \) and \( \text{CO}_2 \) concentrations, respectively; ***first-order rate constant is expressed in days⁻¹

Figure 2  Time profiles of main model variables. Symbols: experimental data in assay 2; lines: model predictions (DSS – degradable suspended solids, B – butyrate, P – propionate, A – acetoclastic, Syntr – syntrophs, Palm – palmitate, Stear – stearate)
(Figure 1). The results (Table 1) showed complete degradation of waste-derived LCFA and VFA to methane, as also found in anaerobic batch treatment of tallow (Broughton et al., 1998). The methane yields were high compared to those of, e.g. cattle manure (approximately 0.20 m³/kg VS added) (Angelidaki and Ahring, 1994) and swine manure (approximately 0.30 m³/kg VS added) (Hansen et al., 1998). The high yield agrees with the high theoretical methane potential of proteins and lipids.

In all the assays, 50 to 60% of nitrogen was ammonificated within 3 to 6 days of incubation, indicating that ammonification did not significantly depend on the different factors. The resulting ammonia concentration ranged from 0.6 to 1.4 g-N/l in the assays, depending on the waste and inoculum concentrations, and did not significantly change during the rest of the assays. Thus 40 to 50% of nitrogen in the assays remained in the particulate fraction. The non-ionised ammonia concentrations were below reported initial inhibitory concentrations (ranging from approximately 0.1 g-N/l to 1.1 g-N/l, Angelidaki and Ahring, 1993; De Baere et al., 1984; Hansen et al., 1998; Hashimoto, 1986). Apparently buffered by ammonia, pH in all the assays remained between 6.8–7.5 during the runs, despite the periodically high VFA concentrations (Georgacakis et al., 1982).

Conclusions
Anaerobic digestion of solid poultry slaughterhouse wastes rich in proteins and lipids appears technically possible, but control of LCFA is likely to be the critical part of the process. The simulation model showed that the degradation patterns involved complicated feedback connections. The model suggests that the delay in methane production resulted, not directly from inhibited aceticlastic methanogenesis, but rather from LCFA inhibiting propionate degradation and the consequent high propionate inhibiting hydrolysis. Up to 0.55 to 0.67 m³ of methane/kg VS added was produced from the waste. Lower waste-to-inoculum ratios exhibited a faster onset and rate of specific methane production. Up to 50 to 60% of the waste nitrogen was ammonificated within 3 to 6 days of incubation.

References


