Anaerobic model for high-solids or high-temperature digestion – additional pathway of acetate oxidation

B. Wett, I. Takács, D. Batstone, C. Wilson and S. Murthy

ABSTRACT

Current anaerobic digestion models cannot properly simulate processes that are operated under high solids concentrations or high temperatures. A modification to existing models has been implemented by adding important missing degradation pathways, to accommodate these systems without artificially recalibrating the model parameters. Specifically, we implemented the alternate acetate oxidizing mechanism that is more tolerant to ammonia than the standard aceticlastic pathway. Inhibition values were estimated and an empirical function has been used to apply ammonia inhibition. The model also relates metabolic activity to un-ionised species such as undissociated acetic acid as substrate (although not obligatory for all organisms) and unionised ammonia as inhibitor. The model relies on an equilibrium chemistry module (e.g. including the phosphate buffer), resulting in more accurate pH predictions, which is crucial for proper modeling of CO_2 and NH_3 stripping. Calibration results from three case-studies modeling thermal hydrolysis and subsequent digestion of sludge are presented.

Key words | acetate oxidation, ACOX, ADM1, ammonia inhibition, anaerobic digestion, ASDM, Cambi, modeling, thermal hydrolysis

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INTRODUCTION

Anaerobic digestion is a widely used and sustainable technology for treatment of various sludges and highly concentrated wastes. The metabolic pathways in anaerobic digestion are well known (Pavlosthatis & Girald-Gomez 1991), and a standardized and research-based model was published as the Anaerobic Digestion Model No. 1 (ADM1) in 2002 (Batstone *et al.* 2002). Another widely used model is the anaerobic digester model part of the general activated sludge – anaerobic digestion (ASDM) model implemented in BioWin (Comeau & Takács 2008). The ASDM is targeted specifically at mesophilic anaerobic digestion of primary and waste sludges. The ADM1 is targeted at a wider range of applications, including higher temperature operation, and high-rate anaerobic treatment of industrial wastewaters. Its broader focus has slightly compromised application to domestic applications, specifically around the input definition, which is not readily applicable to activated sludge.

As with all mechanistic models, both ADM1 and ASDM contain simplifications resulting in certain deficiencies from an engineering perspective:

- The ADM1 (Figure 1) (a) requires an extension to readily represent primary and waste activated sludge as an input (this was addressed by Nopens *et al.* (2009)), (b) has a non-intuitive input set that needs complex characterization or an input model, (c) does not include phosphate (and acid conjugates) as components, and (d) does not consider precipitation.
- The ASDM does not contain an ammonia inhibition term for acetoclastic biomass. ASDM simplifies particulate substrate degradation and hydrolysis processes so the model can be used more easily in practice in conjunction with existing activated sludge models and waste stream characterization techniques. Neither model contains the acetate oxidation mechanism, which is the primary biochemical pathway under high ammonia conditions (Karakashev *et al.* 2006) and high temperatures (Ho *et al.* 2013).

The importance of domestic sludge digestion is particularly becoming more important and under more diverse operating conditions for whole system modeling, and in

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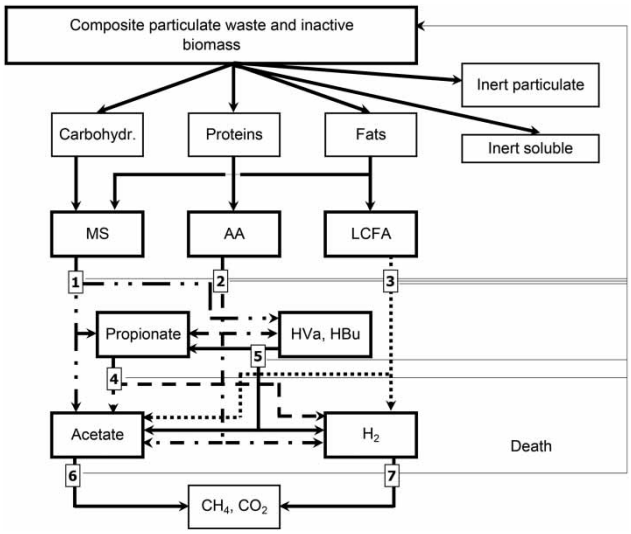

Figure 1 | The anaerobic model as implemented including biochemical processes: (1) acidogenesis from monosaccharides (MS), (2) acidogenesis from amino acids (AA), (3) acetogenesis from long-chain fatty acids (LCFA), (4) acetogenesis from propionate, (5) acetogenesis from butyrate (HBu) and valerate (HVa), (6) acetiklastic methanogenesis, and (7) hydrogenotrophic methanogenesis (Batstone *et al.* 2002).

the current context of enhanced sludge digestion (Carrère *et al.* 2010). The current paper discusses modifications which remove the limitations of existing digester models or extend their range of applicability, respectively, particularly where ammonia inhibition is a controlling mechanism.

METHODS

Ammonia inhibition impacts on digestion modeling

Mesophilic sludge digestion systems typically do not experience ammonia inhibition for normal total suspended solids (TSS) concentrations of 4–6% in the feed. In high-solids digestion systems (organic solid waste digestion or sludge digestion systems using pre-treatment processes), the ammonia concentration can reach inhibitory levels. At approximately 2,500 mg NH_4-N/L and corresponding alkaline pH beyond 7.5 the free ammonia shows an inhibitory impact on methanogenic growth (Angelidaki *et al.* 1993).

Free ammonia toxicity causes a bottleneck in particularly acetate cleavage (Figure 2). In the case of ammonia inhibition of aceticlastic methanogenesis a metabolic side-route over acetate oxidation (ACOX) to H_2 can become dominant. This adaptation process on microbial community level has been observed earlier (Karakashev *et al.* 2006; Wilson 2009), and also the significance for a complete modeling approach

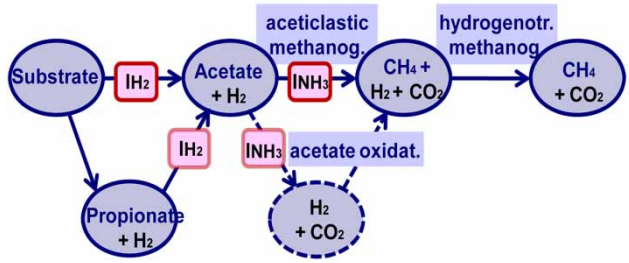

Figure 2 | Process scheme via acetate oxidation (ACOX): inhibition from released ammonia (INH_3) slows down the main route of aceticlastic methanogenesis and can cause a shift towards the metabolic alternative of acetate oxidation and consecutive hydrogenotrophic methanogenesis.

has been outlined (Batstone *et al.* 2002) but so far it has not been implemented. Compared to ADM1 the ACOX model approach suggests the following improvements:

- ACOX adds acetate oxidation to model this diversion under high loaded and high temperature anaerobic systems without recalibration of the model parameters.
- ACOX relates metabolic activity to un-ionised species such as undissociated acetic acid as substrate (although not obligatory for all organisms) and unionised ammonia as inhibitor.
- ACOX incorporates all important chemical species and activity coefficients in the equilibrium chemistry module (such as the phosphate buffer), resulting in more accurate pH predictions, which is crucial for proper modeling of both CO_2 and NH_3 ionization and gas transfer.

RESULTS AND DISCUSSION

Calibration of ammonia inhibition on aceticlastic methanogens and acetate oxidizers

Samples of biomass adjusted to high ammonia concentrations were taken from a continuous bench-scale digester for thermally hydrolysed sludge (THD) (process conditions in Table 1) and from a parallel conventional mesophilic digester (MAD). Ammonia concentration was stepwise diluted and cumulative gas production was measured from batch-bottles (Wilson 2009). Initial slopes of cumulative biogas production curves were used to determine the degree of inhibition imposed by unionised ammonia. These data are plotted as the fraction of biogas production activity remaining versus the concentration of unionised ammonia. For example, a reduction in the biogas generation rate by 20% would be plotted as an inhibition factor (INH_3)

Table 1 | Modeled biomass compositions over a range of systems

	Units	Fermenter	Mesophilic digester	Digestion of thermally hydrolysed sludge
Solids retention time	days	3	20	15
Temperature	°C	25	35	42
Input total dry substance	%	5.3	5.3	10.5
pH	–	4.92	7.32	7.85
Volatile solids removal	%	17.3	51.9	56.9
Volatile fatty acids	mgCOD/L	6,828	180	2,772
Total ammonia $NH_3 + NH_4$	mgN/L	326	1,139	2,918
Fermenting organ.	mgCOD/L	9,367	2,108	777
Acetogens	mgCOD/L	9	32	71
Aceticlastic methanogenesis	mgCOD/L	11	383	5
Acetate oxidizers	mgCOD/L	10	10	1,042
Hydrogenotrophs	mgCOD/L	228	258	1,448

of 0.80, thus a high INH_3 value (i.e. close to 1.0) would be indicative of little methanogenic inhibition. Previous research shows that ammonia inhibition of aceticlastic methane generation is relatively mild at low unionised ammonia concentrations and follows a logistic reduction in methanogenesis as ammonia is increased, theoretically leading to full inhibition of methanogenesis at sufficiently high concentrations (Angelidaki & Ahring 1994). As such, the following log transformation has been applied to calculate $LINH_3$ values via least-squares regression (Wett *et al.* 2009):

$$LINH_3 = \frac{1}{1 + e^{(-\text{slope}\{I,NH_3\}(k\{I,NH_3\} - [NH_3]))}} \quad (1)$$

where slope$\{I,NH_3\}$ is a curve fitting parameter, and $k\{I, NH_3\}$ is the molar unionised ammonia concentration at which $INH_3 = 0.50$.

Methanogenic inhibition factors (INH_3) related to the initial slope of cumulative biogas production curves from batch ammonia toxicity assays are presented in Figure 3. The logarithmic inhibition function Equation (1) was calibrated to the parameters given in the Appendix (available online at http://www.iwaponline.com/wst/069/047.pdf). It is clear that the degree of methanogenic inhibition attributable to NH_3 is less for THD than MAD at equivalent NH_3 concentrations. The model that was applied to these data exhibited good statistical fit. Coefficients of determination (R^2) of $LINH_3$ for THD and MAD were 0.947 and 0.951, respectively. The relative insensitivity of the THD culture to free ammonia inhibition relative to the MAD culture

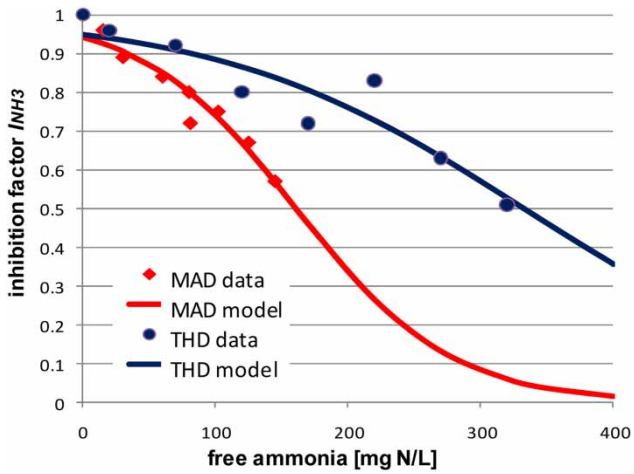

Figure 3 | Inhibition factors (INH_3) related to the measured initial slope of cumulative methane production from batch ammonia toxicity assays using sludge from a mesophilic digester (MAD) and a thermal hydrolysis digestion system (THD) and fitted model profiles.

becomes particularly pronounced as the free ammonia concentration exceeds 150 mg N/L. It is thus hypothesized that methanogenesis during THD is less dependent on ammonia-sensitive aceticlastic methanogenesis, and, rather, non-aceticlastic methanogenesis from HAc is important for THD.

Population shift at increasing ammonia concentrations at Cambi pilot at Blue Plains

An archaeal clone library was constructed from digested biosolids from pilot-scale anaerobic digestion systems treating raw and thermally hydrolyzed combined (primary and

secondary) wastewater sludge. In total, 161 genomic DNA inserts (88 from thermally hydrolyzed biosolids, 73 from raw biosolids) were recovered from successful clones. Data from highly similar sequences (greater than 99% sequence similarity) were defined as operational taxonomic units (OTUs). Each sequence was searched for phylogenetic relatives using the nucleotide BLAST (BLASTn) program (Altschul *et al.* 1990). For anaerobic digestion of conventional sludge (MAD), methanogens belonging to OTUs having greater than 99% similarity to *Methanosarcina barkeri* dominated (66% of total clones). The minority population closely matched an uncultured *Methanomicrobiales* archaeon (97% similar to *Methanospirillum hungateii*, approximately 22% of clones). It is thought that this methanogenic population structure reveals a preference for aceticlastic methanogenesis (direct acetate cleavage) rather than acetate oxidation (via hydrogen as an intermediate). In contrast, the digester treating THD was dominated by hydrogenotrophic *Methanoculleus bourgensis* (100% similarity) at 77% of total clones. The remainder of this methanogenic community consisted of near equal ratios of hydrogenotrophic *Methanospirillum hungatei* (97% similarity, 10% of library) and aceticlastic *Methanosarcina* sp. (100% similarity, 13% of library). It is apparent from these data that high solids anaerobic digestion of THD results in selective pressure for the growth of hydrogenotrophic methanogens relative to aceticlastic methanogens.

Both functional groups of acetate consumers (cleaver and oxidizer) are more sensitive to ammonia relative to hydrogenotrophic methanogens, but acetate cleavers are most sensitive. It is presumed that this selective pressure is due to the channeling of acetic acid through a non-aceticlastic pathway, thus producing additional hydrogen and carbon dioxide as methanogenic substrates. These experimental data are shown in Figure 4 (left). Applying the described model ACOX which incorporates acetate oxidation under high unionised ammonia (i.e. high solids) concentrations, a similar shift towards hydrogenotrophic methanogenesis is observed (Figure 4, right).

Steady state validation run – varying solids concentration at Cambi pilot at Welsh Water

Cambi pilot tests were conducted by Welsh Water to mimic variations of total dried solids (DS) concentration in the digester feed at constant load. The corresponding variation in solids retention time (SRT) was between 14.7 and 15.8 days. Validation runs of the ACOX model could match the more pronounced increase in volatile fatty acids (VFA) at DS concentrations in the feed exceeding 9% solids (Figure 5).

Dynamic validation run – start-up of Cambi digestion at Oxley Creek

An analysis of the Oxley Creek Cambi plant was done based on Batstone *et al.* (2010). During start-up (Figure 6(a)), the ammonia concentration increased (Figure 6(b)). As it passed through 0.008 M ammonia as NH_3, an acute response in acetate concentration up to 2,500 mg/L was observed. This was initially managed by reducing load to the digester, but eventually took 30 days to decrease fully to background levels (approx. 300 mg L^{-1}). The digesters have remained at this level for over 3 years. The retention time at the point of overload was 22–25 days, and input solids concentration approximately 9%.

Only data from digester 1 are presented here, but a similar response was observed in digester 2 (Batstone *et al.* 2010) where acetate peaked up to about 4,500 mg/L

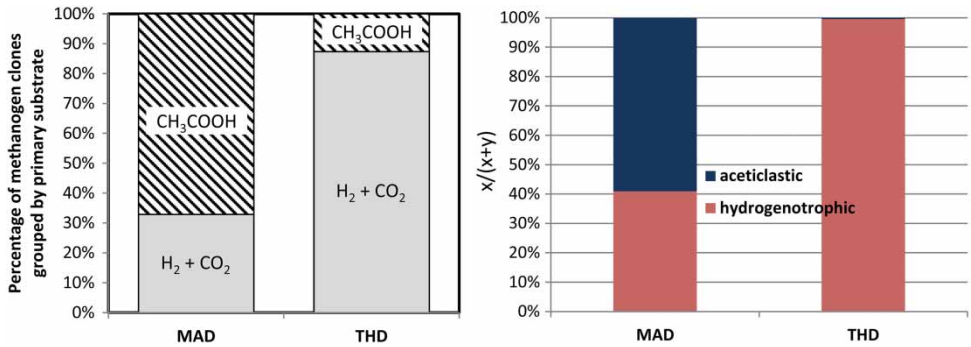

Figure 4 | Fractions of methanogenic organisms (percentage of total methanogenic population) grouped by primary substrate methane and hydrogen – based on phylogenetic measurements (left; from Wilson 2009) and model simulations (right).

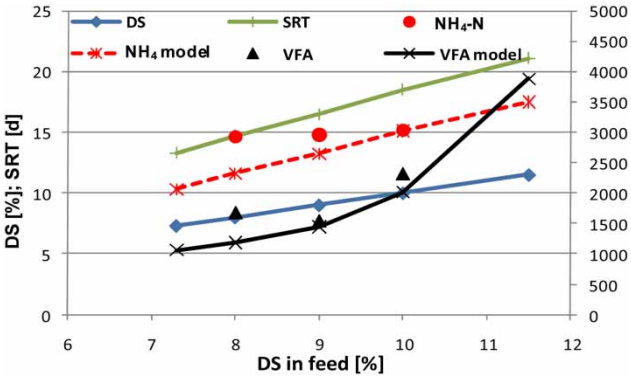

Figure 5 | Measured (Welsh Water Cambi pilot) and simulated acetate response to varying feed solids concentrations (sludge characteristics of Blue Plains).

and then was degraded even at continued ramp-up of load. A similar initial ramp-up of digester load was applied by the model simulating digester solids build-up (Figure 7(a)) and acetate peak (Figure 7(b)) although again the influent characterization of Blue Plains (and not Oxley Creek) was used. The main difference is the final acetate level at constant load. The measured acetate effluent concentration was unusually low compared to all other Cambi-data sets under investigation and also compared to the model result. This fact can be explained by a lower observed pH-level of about 7.55 compared to 7.85, meaning a free ammonia concentration of about 125 compared to 225 mg N/L in the modeled scenario.

Spot samples of Oxley Creek THD sludge showed high abundance of *Methanosarcina* (facultative oxidizer/cleaver) indicating acetate oxidation as the potential metabolic route. However this does not mean a complete wash-out of *Methanosaeta* as predicted for a model environment at ideally homogeneous conditions (compare Figures 4 and 7(c)). *Methanosaeta* as the main acetate cleaver is usually seen in environments with low VFA levels (Karakashev *et al.* 2006).

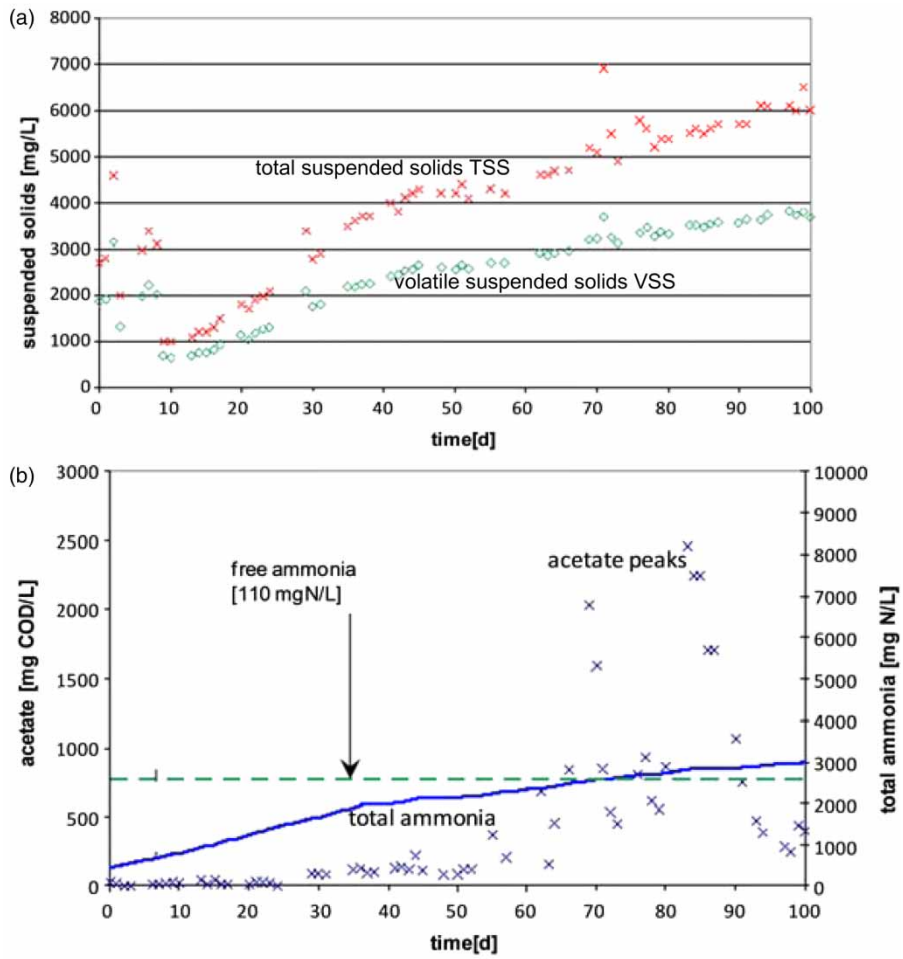

Figure 6 | Measured profiles of total and volatile solids (a) and ammonia and acetate (b) during start-up (100 days) of the Cambi digesters in Brisbane (Batstone *et al.* 2010).

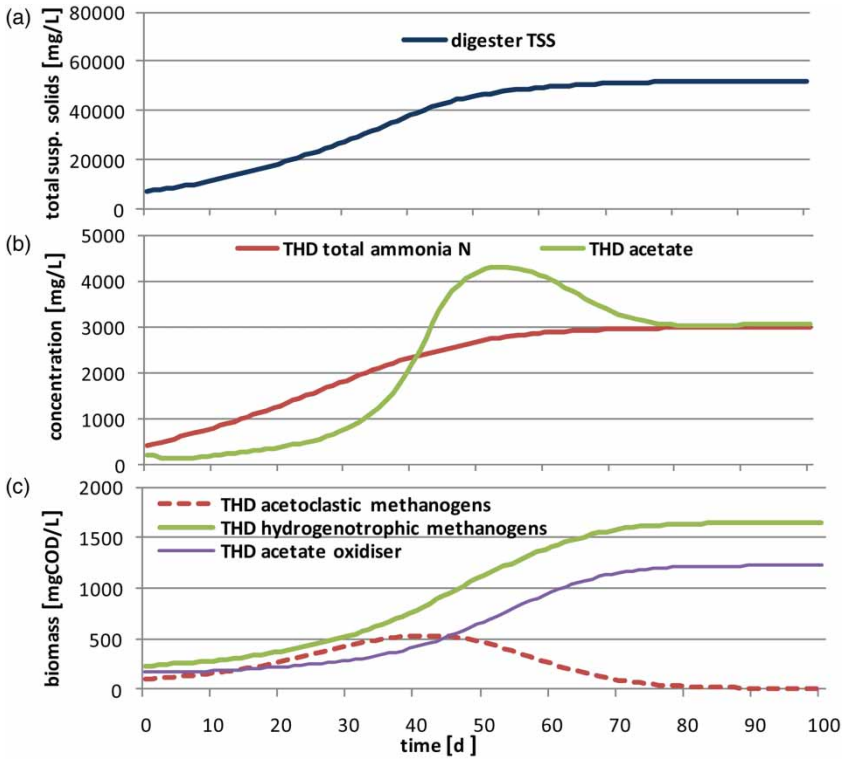

Figure 7 | Simulated increase in digester solids concentration (a) and ammonia concentration and corresponding acetate accumulation (b), and simulated population dynamics of aceticlastic methanogens, acetate oxidizers and hydrogenotrophic methanogens (c) during digester start-up period of 100 days.

Generic model applicability to a broad range of digester types

The described model approach has been applied to simulate four types of anaerobic processes in order to demonstrate the range of applicability. A short summary of the main variables of these digesters operated at different SRT and temperature is given in Table 1.

CONCLUSION

The current versions of anaerobic digestion models (ADM and ASDM) do not consider acetate oxidation or its increase in tolerance to high free ammonia conditions. These limitations could be extended under high temperature conditions (thermophilic temperature range between 45 and 60 °C). The proposed model extensions help to mimic dynamic adaptation to a toxic process environment by a shift between different sensitive populations, to accommodate these high solids or high temperature anaerobic systems without artificially recalibrating the model parameters. Presented case-studies (change in feed-solids and ramping start-up load) demonstrate the model's ability to describe the transition in process conditions and adherent process instability. This is a significant improvement that allows for anaerobic digestion to be used by practitioners wanting to employ and evaluate these types of processes for design, upgrade or operations.

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