Thermophilic Anaerobic Digestion Modeling of Lignocellulosic Hot Water Extract using ADM1

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Abstract

Lignocellulosic biomass is abundant and can become a major feed for anaerobic digestion methane production if its natural recalcitrance is overcome by pretreatment. Bio-degradable organic molecules were extracted by hot water (to produce "hydrolysate") from wood (Norway spruce). A high rate anaerobic sludge bed reactor fed the hydrolysate was modeled by the IWA Anaerobic Digestion Model No.1 (ADM1). Biodegradability kinetics for the hydrolysate material was obtained from batch tests at thermophilic condition, and the hydrolysis kinetic coefficient of carbohydrate (k_{hyd_ch}) found. Thus obtained $k_{hyd\_ch} = 0.44$ d^{-1} was used to simulate UASB reactor performance at 55°C and comparing results to measured parameters from an experimental reactor at five different organic loading rates. The simulation results correlated well with the experimental results for biogas production rate, biogas composition and chemical oxygen demand. This shows that ADM1 is a powerful tool to predict the behavior of thermophilic anaerobic digestion (AD) of pretreated lignocellulosic feed using standard ADM1 parameters except for hydrolysis kinetics. Hydrolysis was identified as the overall rate limiting step in AD of such feed in UASB.

Keywords: thermophilic anaerobic digestion, OLR, lignocellulosic hydrolysate, hydrolysis kinetic, ADM1

List of symbols

Symbol	Description [Unit]
COD	Chemical Oxygen Demand [g COD]
CSTR	Continuous flow Stirred Tank Reactor
HRT	Hydraulic Retention Time [day]
HWE	Hot Water Extraction
OLR	Organic Loading Rate [g COD/L · day]
IN	Inorganic Nitrogen [kmol/ m^3]
k_{hyd}	Hydrolysis kinetic coefficient
k_{dis}	Disintegration kinetic coefficient
S_ac	Soluble acetate [kg COD/m^3]
S_I	Soluble inert [kg COD/m^3]
S_su	Soluble Monosaccharides [kg COD/m^3]
s_COD	Soluble Chemical Oxygen Demand [g COD]
SRT	Solid Retention Time [day]
t_COD	Total Chemical Oxygen Demand [g COD]
UASB	Upflow Anaerobic Sludge Blanket
VFA	Volatile Fatty Acids
X_ch	Particulate carbohydrates [kmol/ m^3]
X_I	Particulate inert [kmol/ m^3]

1 Introduction

Serious environmental pollution due to exhaustive use of fossil fuel has demanded an environment-friendly technology to convert woody biomass or its waste residue to biofuel. Woody biomass, especially Norway spruce, is found abundantly in Norway and requires efficient methods to break its recalcitrance for faster conversion. Biodegradation of lignocellulosic material is difficult because of the complex structures of lignin and other cell wall polysaccharides. As a result, anaerobic microorganisms are not able to easily use this lignocellulosic material and biogas production is hampered (David *et al.*, 2018).Various pre-treatment methods have been tested to overcome this problem (Karuppiah and Azariah, 2019; Taherzadeh and Karimi, 2008). The main purpose of pre-treatment is to break the lignin which is the protective layer for cellulose and hemicellulose (Patinvoh *et al.*, 2017). Also decreasing the crystallinity of cellulose and solubilizing the hemicellulose enhances the digestion (Karuppiah and Azariah, 2019). Hot Water Extraction (HWE) as a proposed pre-treatment process for lignocellulosic material cooks woody biomass in the water at high temperature and pressure (Amidon and Liu, 2009; Therasme *et al.*, 2018) in order to produce liquid hydrolysate. The liquid product, after hot-water extraction, includes monosaccharides, polysaccharides, acetic acid, degraded lignin, and other low molecular weight extractable substances (Amidon and Liu, 2009).

Anaerobic digestion (AD) is a favorable technique due to its low environmental footprint (Kamali *et al.*, 2016) and high energy recovery by methane production. Thermophilic AD (50-57°C) is known as a faster method compared to mesophilic AD (30-40°C) since the choice of temperature affects the growth of microorganism via influencing the kinetic parameters of the main anaerobic reactions. The temperature can play a key role regarding system stability, with poorer yield and process stability for thermophilic AD, but better biogas and digestate quality have also been reported (Gebreeyessus and Jenicek, 2016). The higher temperature can also prevent AD culture contamination (Xia *et al.*, 2013) but may have higher thermal energy requirement (Eddy *et al.*, 2013). Therefore, it is

interesting to test thermophilic AD of lignocellulosic hydrolysate.

Empirical methods based on pilot plant results are usually used to scale up thermophilic AD for various feed stocks. Mathematical modeling and simulation of AD can speed up such design work and provide the opportunity to test a wider range of AD process conditions at lower cost than piloting.

The Anaerobic Digestion Model No.1 (ADM1) (Batstone *et al.*, 2002) has been applied for different AD systems and its performances studied for various substrates and reactor configurations (Gehring *et al.*, 2013).

ADM1 is structured in several main steps including disintegration and hydrolysis, acidogenesis, acetogenesis and methanogenesis. The first order kinetics describe the extracellular solubilization processes such as disintegration and hydrolysis (1), while the intracellular biochemical reactions are described by Monod-type kinetics (Batstone *et al.*, 2002b; Kaparaju *et al.*, 2009).

$$\rho = k_{hyd\_ch} \cdot X_{hyd\_ch} \qquad (1)$$

ρ = hydrolysis rate of solid substrate (kg COD solid substrate m^{-3} d^{-1} where COD = chemical oxygen demand), X_{hyd_ch} = solid carbohydrate concentration (kg COD solid substrate m^{-3}), k_{hyd_ch} = temperature dependent kinetic parameter for hydrolysis (d^{-1}).

Recommended relevant model parameters for most ADM1 reactions are published (Batstone *et al.*, 2002a) but not key kinetic parameters for thermophilic high rate hydrolysate digestion. Therefore, the aim of this study was to determine kinetic parameters for thermophilic AD of thermally hydrolyzed Norwegian spruce. This involved parameter estimation based on batch experiments and model evaluation based on continuous flow tests with increasing organic loading rates of this new substrate.

2 Materials and Methods

AD of hydrolysate from hot water extraction of lignocellulosic Norwegian spruce (*Picea abies*) is tested in batch and continuously fed UASB lab scale reactors with increasing load and modeled by ADM1.

2.1 Material Characterization

2.1.1 Substrate

Hydrolysate from 300 minutes hot water extraction at 140°C was used as substrates for both batch and continuously fed UASB reactors. Macronutrients (Table 1) and micronutrients (Table 2) were added in to provide required nutrients, COD:N:P ratio of 350:5:1 *(*Baeta *et al.*,2013). Initial pH was adjusted by NaOH to around 7. Substrate organics and ammonium are given in Table 3.

Table 1: Composition and concentration of macronutrients in substrate.

Macronutrients	
Type of chemical	***Concentration (mg L^{-1})***
NH_4Cl	1245.4
$(NH_4)H_2PO_4$	148.4
$(NH_4)_2HPO_4$	49.8
$MgCl_2{\cdot}6H_2O$	599.2
$CaCl_2{\cdot}2H_2O$	211.7
$NaHCO_3$	2800

Table 2: Composition and concentration of micronutrients in substrate.

Micronutrients	
Type of chemical	***Concentration (mg L^{-1})***
Yeast Extract	10
$FeCl_3{\cdot}6H_2O$	0.8
$ZnCl_2$	20.8
$MnCl_2{\cdot}4H_2O$	0.19
$(NH_4)_6Mo_7O_{24}{\cdot}4H_2O$	0.26
$AlKO_8S_2{\cdot}12H_2O$	0.04
$CoCl_2{\cdot}6H_2O$	0.8
$NiCl_2{\cdot}6H_2O$	2.08
H_3BO_3	0.48
$CuCl_2{\cdot}2H_2O$	1.28
HCl	80

Table 3: Substrate organics and ammonium.

Parameter	***Measured value (g L^{-1})***
t-COD	22± 2
s-COD	20 ± 2
NH_4^+	0.30 ± 0.02
Acetate	0.59 ± 0.09
VFA	0.59 ± 0.09
Arabinose	1.63 ± 0.02
Galactose	1.67 ± 0.05
Glucose	1.55 ± 0.05
Xylose	1.95 ± 0.04
Mannose	5.1 ± 0.1
Total sugars	11.9 ± 0.3

2.1.2 Inoculum

Granular sludge inoculum (Table 4) used was from a mesophilic industrial internal recirculation reactor treating paper mill effluent. The sludge was adapted for thermophilic condition for 53 d before being used in the batch test and 20 d adaptation till stable operation in the UASB test.

Table 4: Properties of granular sludge.

Parameters	*Values*
Density (kg m^{-3})	1.00 – 1.09
Diameter (mm)	0.6 – 2.7
Settling velocity (m h^{-1})	68 – 71
Total Solids (g L^{-1})	181.0
Volatile Solids (g L^{-1})	119.4
pH	7.46

2.2 Batch Reactors Set up

100 mL syringes were used as batch reactors in accordance with (Østgaard *et al.*, 2017) with 15 mL inoculum. They were fed 4, 6.7, 13.4 and 20 mL of hydrolysates (Table 3) with three parallels for each COD loadings including control blank reactors to correct for biogas generated from the inoculum, all operated for 38 d at 55°C.

2.3 UASB Reactors Set up and Operation

Two parallel glass vessel reactors (Bergland *et al.*, 2015) with 345 mL liquid volume were used as UASB reactors. Half the reactor volumes were filled with granular sludge (Table 4). The substrate was kept cool (4 °C). Culture adaptation started at 35°C and organic loading rate, OLR = 0.65 [gCOD $L^{-1}d^{-1}$] followed by 2°C daily increases to 55°C. Then, followed the 52 d test period with step load increases (Table 5).

Table 5: The UASB operation conditions as hydraulic (HRT) and organic (OLR) loading rates.

Time interval	*HRT [d]*	*OLR [gCOD $L^{-1}d^{-1}$]*
From d 1 to d 16	34.5	0.65
From d 17 to d 27	17.25	1.29
From d 28 to d 37	11.5	1.94
From d 38 to d 48	8.62	2.59
From d 49 to d 53	5.75	3.88

2.4 Analytical Methods

UASB biogas production was monitored continuously and gas composition measured twice a week by gas chromatography (SRI 8610-C) as described in (Bergland *et al.*, 2015). Liquid phase COD (total and soluble), volatile fatty acids (VFAs), including acetate, propionate, butyrate, iso-butyrate, valerate, iso-valerate, iso-caprionate, caprionate and heptanoic acid, pH and ammonium content (NH_4^+) were sampled and measured as described in (Bergland *et al.*, 2015). Batch reactor biogas production was measured manually in accordance with Østgaard *et al.* (2017).

2.5 Modelling and Simulation Methods

The Anaerobic Digestion Model No. 1 (ADM1) was applied to model the processes with stoichiometric coefficients, equilibrium coefficients and dynamic states and algebraic variables as proposed by (Batstone *et al.*, 2002b), for all biochemical and physio-chemical processes, with the following exception: lignocellulosic hydrolysate as feed is introduced here. The only model modification assumed necessary is the hydrolysis of this new substrate and, considering the characteristics of the substrate and inoculum (Table 3 and 4), input values for simulation (Table 6) are based on some assumptions:

- 10 percent of the total feed COD is inert (based on batch tests).
- One-third of total inert is particulate inert ($input_{X_{I_{in}}}$) and two-thirds is soluble inert ($input_{S_{I_{in}}}$).
- The feed amount of biodegradable particulate carbohydrates ($input_{X_{Ch_{in}}}$) used is assumed to be total particulates minus the inert fraction (2).
- The input of biodegradable soluble sugars ($input_{S_{su_{in}}}$) used as all soluble organics (s_{COD}) minus soluble inert and acids (dominated by acetic acid so used measured ($input_{S_{ac_{in}}}$) (3).

$$input_{X_{Ch_{in}}} = (total\ particulate) - \left(input_{X_{I_{in}}}\right) \quad (2)$$

$$input_{S_{su_{in}}} = (s\_COD) - \left(input_{S_{I_{in}}}\right) - \left(input_{S_{ac_{in}}}\right) \quad (3)$$

Table 6: Parameters used for simulation in the ADM1 model

Type of parameter	*Formula for calculation*	*Amount*	*Unit*
t_COD		22.31	kgCOD m^{-3}
s_COD		20.04	kgCOD m^{-3}
Total particulate	= (t_COD - s_COD)	2.27	kgCOD m^{-3}
Inert (10% t-COD)	= (0.1 * t_COD)	2.23	kgCOD m^{-3}
Input_X_I_in	= (1/3 * inert)	0.74	kgCOD m^{-3}
Input_S_I_in	= (2/3 * inert)	1.49	kgCOD m^{-3}
Input_X_C_in		0.00	kgCOD m^{-3}
Input_X_Ch_in	= [(total particulate) - (input_X_I_in)]	1.53	kgCOD m^{-3}
Input_S_ac_in		0.63	kgCOD m^{-3}
Input_S_su_in	=[(s_COD) - (input_S_I_in) - (input_S_ac_in)]	17.92	kgCOD m^{-3}
Input_S_IN_in		0.016	mol L^{-1}
Volume		0.00035	m^3
Temperature		328	K

- The feed ammonium concentration, "Input_S_IN_in" is set to 0.016 $mol\ L^{-1}$ based on the added nutrients (Tables 1 and 2).
- Disintegration and hydrolyze kinetic parameters for thermophilic high rate was not specified by (Batstone *et al.*, 2002b). The values for mesophilic high rate (Table 7) are therefore used for the hydrolysis of protein and lipids while the hydrolysis kinetic factor of carbohydrate k_{hyd_ch} was assumed representative for the substrate and obtained from the batch test using equation 4. Disintegration high rate is assumed the same as disintegration solid.

$$B = B_0 (1\text{-}e^{-kt}) \qquad (4)$$

B_0 is the total biogas production and B is the biogas production at the given time t and k= k_{hyd_ch}.

Table 7: Kinetic parameters used for disintegration and hydrolysis as recommended by (Batstone *et al.*, 2002b) and as used for the unique conditions tested here.

Parameter	**Proposed by (Batstone et al. 2002b)**			***Used in this project***
	Meso-phillic high-rate (35°C)	*Mesophilic solids (35°C)*	*Thermo-phillic solids (55°C)*	*Thermo-phillic high-rate (55°C)*
$k_{dis}\ (d^{-1})$	0.4	0.5	1.0	1.0
$k_{hyd\_ch}(d^{-1})$	0.25	10	10	0.44
$k_{hyd\_pr}(d^{-1})$	0.2	10	10	0.2
$k_{hyd\_li}(d^{-1})$	0.1	10	10	0.1

High rate reactors (e.g. UASB) are characterized by long solids retention time compared to hydraulic retention time (SRT >> HRT), modeled by a t_res_x (difference between sludge and hydraulic retention time) factor. Its value is however unknown, so it was assessed by simulating different t_res_x values.

3 Results and Discussion

The k_{hyd_ch} parameter is first estimated by the batch experiment and then the model evaluated by comparison to the UASB test.

3.1 Hydrolysis Kinetic Coefficient

Hydrolysis kinetic coefficient (k_{hyd_ch}) for the carbohydrate was calculated based on Eq. 4 and batch data (Figure 1) to be 0.44 d^{-1} with low standard deviations between the parallels.

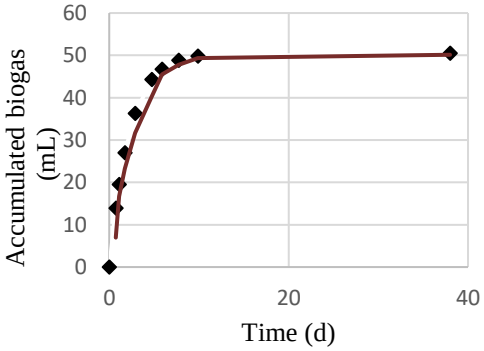

Figure 1. Batch test data and fitted line to calculate k_{hyd_ch}

3.2 Sludge Retention Time

The t_res_x value (difference between sludge and hydraulic retention time in the UASB) cannot be measured so it was assessed by simulations using different t_res_x values equal to 5, 15, 25 and 40 d. The experimental and simulated results correlated quite well for all measured parameters for t_res_x = 40 d, as seen for COD in Figure 2. Total COD was lower than simulated while simulated soluble COD was close to measured value after the first 17 d with the lowest load. t_res_x > 40 d was also tested (not shown) but without significant effects on concentrations so t_res_x = 40 d was used for the following simulations.

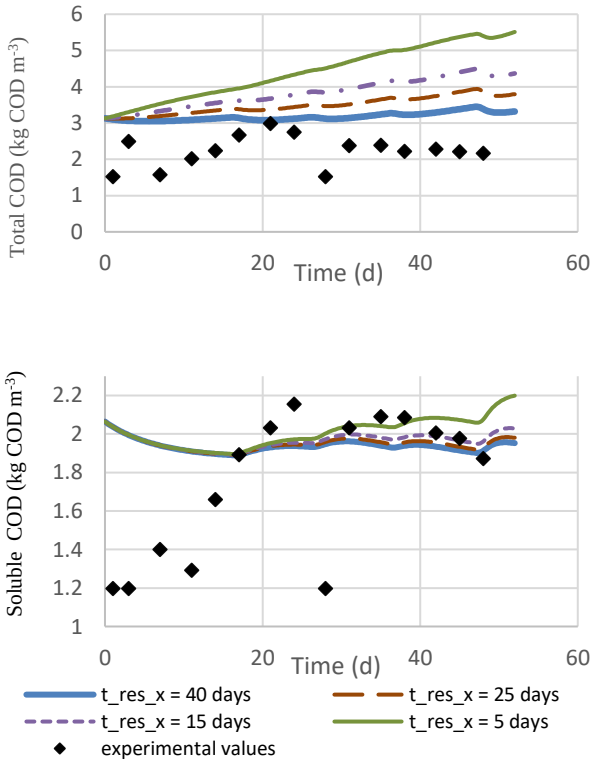

Figure 2. Total and soluble UASB effluent COD values.

3.3 Biogas Production

The measured and simulated biogas production rate (Figure 3) deviated initially until day 12, perhaps due to incomplete adaptation to thermophilic conditions. Thereafter the biogas production was close to the simulated values for the lowest OLRs indicating adapted culture. The model predicted somewhat higher yields than measured at the higher ORLs. The increases in biogas production following each step increase in OLR were predicted well but not the subsequent drops in production. These deviations show that some adaptation to higher loads are needed and that such is not accounted for in ADM1.

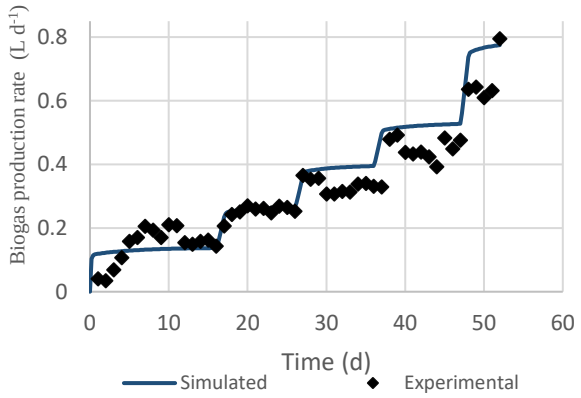

Figure 3. Simulated and experimental values for UASB biogas production rate (L d^{-1}).

The model was also used to simulate much higher loads than tested (not shown) by continuing the step increases used in the test and it predicts that much higher loads than tested can be applied while maintaining stable operation and biogas yield. This is important for the economy of such fuel production plants since capital cost depends on reactor size. This exercise also demonstrates how the model can be used to evaluate conditions that would be very time-consuming and costly to test experimentally.

3.4 Limiting Step

The increase in OLRs showed no VFAs accumulation during the experiments while the model predicts temporary VFA accumulations after each step increases (Figure 4). Both measured and simulated values are, however, low and far enough the tolerable level for smooth reactor performance: < 2 % of acetic, butyric and propionic acid concentrations of 2.4, 1.8 and 0.9 ($kg\ m^{-3}$), respectively, reported to be of threshold value (Kim *et al.*, 2002). This implies that the reactors had stable conditions with no signs of limitations of the methanogenesis at the tested loads.

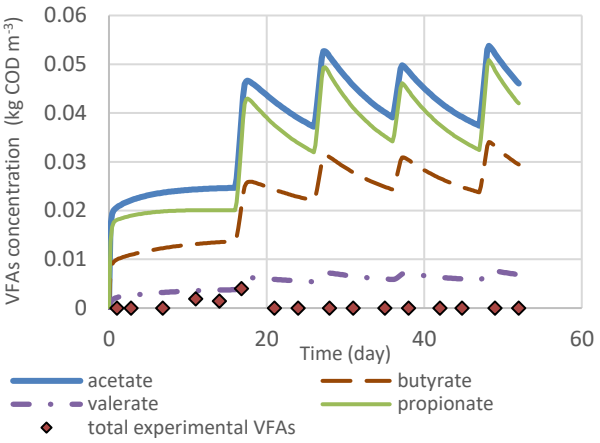

Figure 4. VFAs concentration experimental and simulated values for 52 days by increasing organic loading rate (OLR).

This is also seen by the stable methane content in the biogas, simulated and measured (Figure 5). These results also confirm that it was a correct modeling assumption to only adapt hydrolysis of ADM1 to the given conditions while the latter stages (methanogenesis etc.) were kept according to (Batstone *et al.*, 2002), since these were not limiting steps for the overall process performance. This implies that hydrolysis is the rate limiting step of lignocellulosic hydrolysate AD in UASB. This seems reasonable given that 90 % of feed organics is soluble (Table 3).

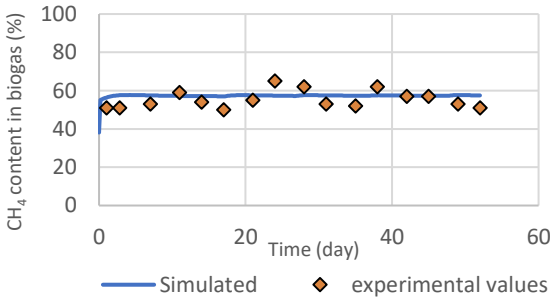

Figure 5. Simulated and experimental UASB biogas methane content.

3.5 Biomass

The active biomass cannot be accurately measured by existing methods so mass development of the main groups of microorganism in AD is studied by simulations (Figure 6). The figure shows that sugar consuming bacteria will be most abundant on such feed. The time allowed for each OLR tested, except the first, was too short to reach true steady state (even if biogas production stabilized) since biomass was still increasing for all seven microbial groups at the end of each OLR test. This supports the above suggestion that some deviations between simulated and measured value can be due to too short time for the slow growing AD

microorganism to adapt to the higher loads. AD cultures have been reported to require months to adapt to new conditions (Nordgård *et al.*, 2017).

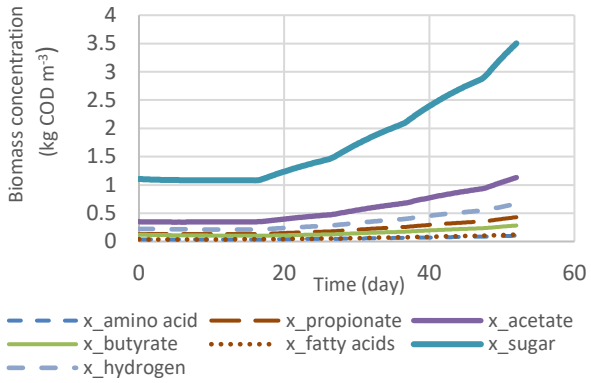

Figure 6. Simulated UASB biomass concentration development for the seven microbial groups in ADM1 during the 52 d test with increasing OLR.

3.6 Inhibition

The simulated inhibition (Figure 7) was low with slight hydrogen concentration inhibition of the propionate degradation (reducing the rate to around 80 % of maximum) and butyrate degradation (reduced to 90 %). The NH_3 concentration reduced the acetate degradation rate to 90% of not inhibited rate. There may however occur inhibition not accounted for due to unknown inhibitors in the feed. Lignocellulosic hydrolysate may contain furfural and HMF (5-hydroxymethylfurfural) that can inhibit microorganisms. This should be further studied and included in the model if relevant. If so, it could narrow the gaps between simulated and measured methane production (Figure 3).

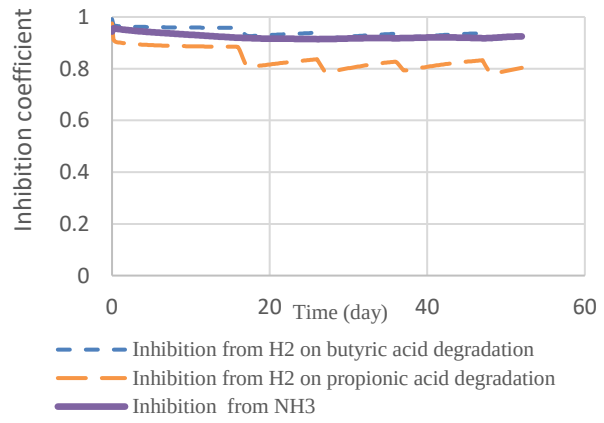

Figure 7. Simulated inhibitors coefficients (value 1 implies no inhibition and 0 implies complete inhibition).

Inorganic nitrogen may also cause AD inhibition both if in excess or in shortage, the latter typical for such feeds, compensated by ammonium supplement (Table 1). Measured and simulated ammonia levels and the inhibition simulations (Figure 7) show appropriate ammonia levels implying the amount of inorganic nitrogen added to this lignocellulosic substrate was appropriate.

Despite previous reports about the sensitivity of thermophilic AD and its poor stability, the efficient UASB reactor treatment of lignocellulosic hydrolysate suggests it is a good option for such feeds. The quite good predictability by the standard ADM1 with previously recommended process parameters support this claim and implies that ADM1 can be used in process design and to test process limitations.

4 Conclusion

- The study shows that the thermophilic AD of lignocellulosic hydrolysate in a UASB can be simulated well by the ADM1 model.
- The hydrolysis kinetic rate constant of carbohydrate, k_{hyd_ch}, was found to be 0.44 d^{-1} for thermophilic degradation of hydrolysate of Norwegian spruce.
- The low concentrations of effluent VFAs and CODs, simulated and measured, implies good digestion for the organic loading rates between 0.65 and 3.88 $g\ COD\ L^{-1}d^{-1}$ tested and implies that the last AD steps were not limiting the overall process performance.
- Hydrolysis appeared to be the overall rate limiting step in AD of such feed in UASB.
- The microbial granular sludge from mesophilic paper-mill effluent treatment adapted both to change from the mesophilic (35°C) to thermophilic (55°C) and to increased load.
- The largest simulated inhibition was from H_2 reducing the propionate degradation but there may have been some un-accounted for inhibitor(s) causing slightly less methane production than simulated at the higher loads.
- Thermophilic AD in UASB appears to be a good treatment option for lignocellulosic hydrolysate.
- The standard ADM1 can be used in process design for thermophilic UASB AD of lignocellulosic hydrolysate.

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