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## MODELING OF SOLUBILIZATION DYNAMICS OF MANURE ORGANIC MATTER AND PHOSPHORUS AS A FUNCTION OF pH CONTROL AND ENZYME SUPPLEMENTATION

The potential use of commercial enzyme and pH control has been investigated for enhancing dairy manure fermentation and modeling dissolved organic matter and orthophosphate (PO<sub>4</sub>-P) dynamics of fermenters. Anaerobic lab-scale batch fermenters (initial total solids concentration (TS<sub>0</sub>) = 3.8 wt. %) were fed with separated dairy manure solids and operated under pH controls (5 and 9.5). The enzyme-supplemented alkaline fermenters clearly outperformed the acidic fermenters in terms of chemical oxygen demand (COD) solubilization: ca. 50% vs. 20%, respectively. Soluble PO<sub>4</sub>-P in the acidic fermenters was comparably higher but constituted less than 20% of total phosphorus. Better soluble COD (>80%) and soluble PO<sub>4</sub>-P (>70%) yields were noted for the dilute fermenters (TS<sub>0</sub> = 0.6–0.8 wt. %). An existing model was retrofitted, calibrated and validated for simulating dynamics of soluble COD, volatile fatty acids, and soluble orthophosphate under various pH and enzyme conditions.

### SYMBOLS

- $C_e$  – concentration of hydrolytic enzymes, mg COD·dm<sup>-3</sup>
- $C_{is}$  – concentration of insoluble substrate, mg COD·dm<sup>-3</sup>
- $C_{mo}$  – concentration of monomer species, mg COD·dm<sup>-3</sup>
- $C_{NH_4-N}$  – concentration of ammonia, mg N·dm<sup>-3</sup>
- $C_{pp}$  – concentration of particulate orthophosphate, mg P·dm<sup>-3</sup>
- $C_{prot}$  – Nitrogen content of particulate proteins, mg N·dm<sup>-3</sup>
- $C_{sp}$  – concentration of soluble orthophosphate, mg P·dm<sup>-3</sup>
- $C_{ss}$  – concentration of high-molecular-weight soluble substrate, mg COD·dm<sup>-3</sup>

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$C_{VFA}$	– concentration of volatile fatty acids, $\text{mg COD}\cdot\text{dm}^{-3}$
$C_{Xa}$	– concentration of acidogenic bacteria, $\text{mg COD}\cdot\text{dm}^{-3}$
$C_{Xm}$	– concentration of methanogenic bacteria, $\text{mg COD}\cdot\text{dm}^{-3}$
$k_{amm}$	– ammonification rate constant for proteins, $\text{d}^{-1}$
$k_{dis}$	– dissolution rate constant for particulate orthophosphate, $\text{d}^{-1}$
$k_{h, is}$	– hydrolysis rate constant for insoluble substrate, $\text{d}^{-1}$
$k_{h, s}$	– hydrolysis rate constant for soluble substrate, $\text{dm}^3\cdot\text{mg}^{-1}\cdot\text{d}^{-1}$
$k_{pre}$	– precipitation rate constant for dissolved orthophosphate, $\text{d}^{-1}$
$\mu_{max, a}$	– maximum specific growth rate for acidogens, $\text{d}^{-1}$
$\mu_{max, m}$	– maximum specific growth rate for methanogens, $\text{d}^{-1}$
$K_a$	– half-saturation coefficient for monomer species, $\text{mg COD}\cdot\text{dm}^{-3}$
$K_m$	– half-saturation coefficient for volatile fatty acids, $\text{mg COD}\cdot\text{dm}^{-3}$
$K_n$	– half-saturation coefficient for ammonia, $\text{mg N}\cdot\text{dm}^{-3}$
$Y_a$	– yield for acidogens on monomer species, $\text{g COD}\cdot\text{g}^{-1}\text{COD}$
$Y_m$	– yield for methanogens on VFAs, $\text{g COD}\cdot\text{g}^{-1}\text{COD}$
$Y_{N/X}$	– weight fraction of nitrogen in bacterial cell, $\text{g N}\cdot\text{g}^{-1}\text{COD}$
$d_a$	– decay rate constant for acidogens, $\text{d}^{-1}$
$d_m$	– decay rate constant for methanogens, $\text{d}^{-1}$

## 1. INTRODUCTION

In the United States (US), number of the large concentrated animal feeding operations (CAFOs) increased by 234% between 1982 and 2002 [1]. It is estimated that more than 40% of the total livestock and 335 million dry tons of manure are produced in the CAFOs [2]. Currently, the CAFOs are regarded point sources that may pollute nation's surface and groundwater through manure discharges, and the federal government is regulating the CAFOs from the standpoint of water quality [3, 4]. Integrated manure treatment (IMT) is emerging as an alternative to protect water quality. The IMT systems include a combination of the physical, chemical, and biological processes typically used for domestic and industrial wastewater treatment. Full-scale IMT plants have been constructed to remove organic matter and inorganic pollutants (e.g., nutrients, metals) from the liquid or semi-solid manure streams of the CAFOs [5]. Some of the IMT plants include anaerobic digestion units delivering a valuable product, namely biogas [6]. The IMT systems can be further improved to increase their product yields or even diversify the products that can be delivered.

Enhancing hydrolysis and fermentation of manure fibers can promote the production of readily biodegradable organic (e.g., volatile fatty acids, VFA) materials. The steps undertaken for enhanced fermentation can also dissolve particulate phosphorus (P), i.e., the predominant P fraction in the animal manure. Then, the readily biodegradable organics can be used for various purposes, such as: (i) biogas production by anaerobic digestion, (ii) supporting biological nutrient removal processes, and

(iii) development of mixed bacterial culture for the synthesis of polyhydroxyalkanoates (PHAs). PHAs are biodegradable plastics exhibiting similar characteristics as their conventional and nonbiodegradable counterparts of polypropylene and polyethylene. In addition, solubilized P can be harvested as high-grade, slightly soluble magnesium (Mg) and/or calcium (Ca) phosphate minerals from the liquid stream.

Fibers constitute ca. 50% of dry matter in dairy manure [7]. Cellulose constitutes almost half of the fibers with the remaining portion being equally distributed between hemicellulose and lignin. Although hemicellulose and cellulose are insoluble in water, an array of natural organisms is capable of hydrolyzing these slowly biodegradable polymers by secreting the hemicellulase and cellulase enzymes. Supplementation with the commercially available cellulase enzymes (0.2–0.8 wt. %) has been reported to increase the soluble organic yield from cattle manure by ca. 30% under anaerobic fermentation conditions [8]. Chemical treatment alternatives have also been investigated to solubilize P and organic matter in the solid wastes and by-products including but not limited to, the animal manure. Some of the treatments are quite harsh since they require a simultaneous use of heat (100–130 °C) and reactive chemicals (e.g., H<sub>2</sub>SO<sub>4</sub>, H<sub>2</sub>O<sub>2</sub>) [7, 9, 10].

On the other hand, relatively weaker chemical interventions appear useful to enhance the solubilization process. For instance, more than 50% of the total organics can be converted into a soluble form enriched with the short-chain VFA when the activated sludge is fermented under anaerobic and alkaline conditions (i.e., pH > 9) [11]. The alkaline pH is suggested to improve the soluble organic yield by inhibiting the methanogens. The pH adjustment also influences P partitioning between the dissolved and solid phases. A strong ( $R^2 = 0.87$ ) relationship has been established between extracted animal manure P (i.e., poultry litter) and pH suggesting the use of acidic extractants for P recovery purposes [12]. Enzyme supplementation has also been used as an alternative method to solubilize organic matter from animal manure. Under mesophilic conditions, the cellulase supplementation (0.2–0.8%) increased the dissolved organic yield (i.e., dissolved chemical oxygen demand, COD) of anaerobically fermented cattle manure by 20% [8]. The fermenter pH was not adjusted in this study.

Models capable of simulating manure organic matter and P solubilization can be instrumental in the development, design, and operation of IMT systems. Mechanistic models have been proposed to simulate the hydrolysis and anaerobic fermentation of the organic matter taking place in the sewage sludge or animal manure: [13, 14]. Surface-limiting reactions were used to describe the hydrolysis of organic matter in the models. However, solubilization of organic matter and particulate P under pH-controlled, anaerobic environments has not been addressed in the previous modeling studies.

The main objective of this study was, therefore, to investigate the potential use of commercial enzyme and pH control for enhancing dairy manure fermentation, and to model dissolved organic matter and orthophosphate dynamics in the fermenters.

## 2. EXPERIMENTAL DESIGN

*Separated dairy manure solids (SDMS)* were collected from a farm housing 1200 cows (Middleton, WI, USA). Manure was firstly dry-scraped and then flushed into a manure pit. The flushed manure was subjected to the mechanical separation using an on-farm solid-liquid separator. The solids obtained from the separator were used as substrate in the lab-scale batch anaerobic fermenters.

*Anaerobically digested dairy manure.* Two different dairy manure digestates were obtained for seeding the lab-scale fermenters. The first sample (Seed 1) was taken from the effluent of an on-farm, full-scale thermophilic anaerobic digester (Vernon County, WI, USA). The second sample (Seed 2) was collected from the effluent of an on-farm, full-scale mesophilic anaerobic digester (Nekoosa, WI, USA). Both of the seed samples were maintained at their source digester temperatures for 1 d prior to their use in the lab-scale fermenters.

*Lab-scale batch anaerobic fermentation.* An experimental design including two pH and temperature levels and three types of commercial cellulase enzymes was used (Table 1). The enzymes were Accellerase 1500 (A) (Danisco US Inc.; Rochester, NY, USA); Cellulase (C) from *A. niger* (Sigma Aldrich Co., St. Louis, MO, USA), and CeluStar CL (CS) (Dyadic International, Inc., Jupiter, FL, USA).

Table 1

Experimental design for the lab-scale batch anaerobic fermentation

Reactor	pH	Temperature [°C]	Seed weight [g]	SDMS [g]	Water [g]	TS wt. %]	VS [wt. %]	Enzyme
S1	5	50	Seed 1 (160)	100	525	3.9	3.4	A
C1-1				–	625	0.65	0.48	–
C1-2				100	525	3.9	3.4	–
S2	5	38	Seed 2 (160)	100	525	3.9	3.4	C
C2-1				–	625	0.65	0.48	–
C2-2				100	525	3.9	3.4	–
S3	9.5	50	Seed 1 (160)	100	525	3.9	3.4	CS
C3-1				–	625	0.65	0.48	–
C3-2				100	525	3.9	3.4	–
S4	9.5	38	Seed 2 (160)	100	525	3.9	3.4	CS
C4-1				–	625	0.65	0.48	–
C4-2				100	525	3.9	3.4	–

SDMS – separated dairy manure solids, TS – total solids, A – accellerase 1500 (Danisco US, Inc., Rochester, NY, USA), C – cellulase from *A. niger* (Sigma Aldrich, St. Louis, MO, USA), CS – CeluStar CL (Dyadic International, Inc., Jupiter, FL, USA), VS – volatile solids.

Cellulase content of A, C, and CS was 5–10%, 8%, and 4%, respectively. A, C, and CS dosages were 0.08, 0.08, and 0.12 cm<sup>3</sup> per g of SDMS, respectively. The reactors S1, S2, S3, and S4 were run in triplicates. Two types of control reactors were used to investigate the effect of (i) the enzyme supplementation and (ii) seed material on the fermentation products (Table 1). The fermenter pH was checked every other day and adjusted with 10 M NaOH or concentrated HCl when necessary.

The fermenters were regularly sampled to determine the short-chain (C2–C6) volatile fatty acids (VFA), soluble COD, and the dissolved reactive P (DRP) for 12–22 d. The samples were centrifuged at 3000 rpm for 5 min followed by filtration with 0.45 µm membrane syringe filters. DRP was determined using an automated molybdate-ascorbic acid method (QuikChem 10-115-01-1-A) on a Lachat QuikChem flow injection analyzer (FIA) 8000 (Hach Company, Loveland, CO, USA). Soluble COD was analyzed using the closed reflux colorimetric method [15]. Short-chain VFAs were determined individually using a gas chromatograph. The COD equivalent concentration of each VFA species was determined and the sum of the species' COD equivalent concentrations was reported as the VFA concentration [16]. Analysis of VFAs was performed on a Shimadzu Scientific GC2010 gas chromatograph equipped with a Restek Stabilwax-DA (15 m, 0.53 mm ID) column. The carrier gas was helium at the rate of 30 cm<sup>3</sup>·min<sup>-1</sup>.

*Fermentation model.* The P-related processes were incorporated into an existing fermentation model developed by von Munch et al. [14]. The model includes anaerobic hydrolysis and fermentation reactions, but excludes methanogenesis. Eleven state variables and related mass balance equations were used in the batch fermentation model. Equations ((8)–(24)) given by von Munch et al. [14] were adapted for modeling the batch reactors in this study.  $Y_{NX}$  as a fixed model parameter was assumed to be 0.0875 g N·g<sup>-1</sup> COD in this study [14]. Inorganic P dissolution and precipitation reactions were considered first order with respect to  $C_{pp}$  and  $C_{sp}$ , respectively (Eq. (1)). Influence of microbial activity on P dynamics was captured by incorporating the heterotrophic growth, fermentation, and lysis processes from the activated sludge model No. 2d (ASM2d) into Eq. (1) with their corresponding coefficients [17]:

$$\begin{aligned}
 -\frac{dG_{pp}}{dt} = \frac{dC_{sp}}{dt} = & k_{dis}C_{pp} - k_{pre}C_{sp} - 0.004 \frac{\mu_{max,a}C_{mo}C_{NH_4-N}C_{Xa}}{(K_a + C_{mo})(K_n + C_{NH_4-N})} \\
 & - 0.02 \frac{\mu_{max,m}C_{VFA}C_{NH_4-N}C_{Xm}}{(K_m + C_{VFA})(K_n + C_{NH_4-N})} + 0.01 \frac{(1 - Y_a)\mu_{max,a}C_{mo}C_{NH_4-N}C_{Xa}}{(K_a + C_{mo})(K_n + C_{NH_4-N})} \\
 & + 0.01(d_aC_{Xa} + d_mC_{Xm})
 \end{aligned} \tag{1}$$

All the variables in the above equation have been defined in the section Symbols. Equation (1) represents an extension of the model developed by von Munch et al. [14] for fermentation of animal manures. Initial concentrations of the analytically quantifiable state variables (e.g.,  $C_{sp}$ ,  $C_{pp}$ ,  $C_{NH_4-N}$ ) were estimated using initial total solids concentrations of the reactors and the coefficients derived from the literature on cattle manure (Table 2). The remaining state variables were estimated using Eqs. (30)–(36) given by von Munch et al. [14].

Table 2

Coefficients used for estimating concentrations  
of the initial state variables in dairy manure batch fermenters

Sample	TCOD:TS [g·g <sup>-1</sup> ]	NH <sub>4</sub> -N:TS [g·g <sup>-1</sup> ]	TKN:TS [g N·g <sup>-1</sup> ]	VFA:TS [g COD·g <sup>-1</sup> ]	SP:TS [mg P·g <sup>-1</sup> ]	PP:TS [mg P·g <sup>-1</sup> ]
Raw dairy manure (substrate)	1.09 [19–24]	0.01 [6, 19–24]	0.03 [19–22, 24]	0.10 [19, 23–25]	0.2 [6]	6 [6]
Anaerobically digested dairy manure (seed)	0.93 [22, 26]	0.04 [6, 22, 26]	0.07 [22, 26]	0.04 [23, 24]	0.2 [6]	7 [6]

Initial values of the state variables used for the calibration are presented in Table 3.

Table 3

Estimated initial concentrations of state variables  
preceding model calibration in reactors  
S1–S4, C1-2, C2-2, C3-2, C4-2

State variable	Value
$C_{is}$ , mg COD·dm <sup>-3</sup>	33253
$C_{ss}$ , mg COD·dm <sup>-3</sup>	1069
$C_{mo}$ , mg COD·dm <sup>-3</sup>	0
$C_{VFA}$ , mg COD·dm <sup>-3</sup>	1813
$C_e$ , mg COD·dm <sup>-3</sup>	119
$C_{Xa}$ , mg COD·dm <sup>-3</sup>	4875
$C_{Xm}$ , mg COD·dm <sup>-3</sup>	542
$C_{NH_4-N}$ , mg N·dm <sup>-3</sup>	759
$C_{prot}$ , mg N·dm <sup>-3</sup>	191
$C_{pp}$ , mg P·dm <sup>-3</sup>	238
$C_{sp}$ , mg P·dm <sup>-3</sup>	9

*Calibration of the model.* The model was calibrated to simulate three different reactor types: (A) acidic fermenters (it should be noted that enzyme supplementation did not make a difference to the solubilization of organic matter in acidic reactors), (B) alkaline fermenters without enzyme supplementation, and (C) alkaline fermenters with

enzyme supplementation. This calibration approach was justified by the organic matter and P solubilization performances of the batch fermenters. Measured soluble COD and PO<sub>4</sub>-P data from the mesophilic fermenters of S2, C4-2, and S4 (representing reactor types A, B, and C, respectively) were imported to PottersWheel toolbox [18] functioning under Matlab platform (Mathworks, Natick, MA, USA). Initial values of both the model parameters and the state variables were fitted using the PottersWheel. The following decision rules were strictly followed: a) initial values of the state variables (Table 3) should not be changed by more than 20%, b) the yield values should not exceed one, and c)  $k_{h,is}$  should be smaller than  $k_{h,s}$ . The rule (a) was set to compensate for uncertainty associated with the initial value estimation procedure. The other rules (b, c) were dictated by the model structure. The best set of the fitted model parameters delivering the lowest  $\chi^2$  sum was used to construct the calibrated model for each reactor type of interest:

$$\chi_m^2 = \sum_{i=1}^n \frac{(y_{\text{measured}} - y_{\text{simulated}})^2}{\sigma_{\text{measured}}^2} \quad (2)$$

$$\chi^2 = \sum_{m=1}^3 \chi_m^2 \quad (3)$$

where  $\chi_m^2$  is the goodness-of-fit indicator for the  $m$ th observed variable,  $y$  – observed variable's concentration at time  $t$  (mg COD·dm<sup>-3</sup> or mg P·dm<sup>-3</sup>),  $\sigma$  – standard deviation of the observed variable's concentration at time  $t$  (mg COD·dm<sup>-3</sup> or mg P·dm<sup>-3</sup>), and  $n$  – the number of the measured data points. The  $\sigma^2$  values were available for S2 and S4 and directly used in Eq. (2), whereas the  $\sigma^2$  values for C4-2 were estimated using the error model of the PottersWheel.

*Sensitivity analysis.* One dimensional sensitivity analysis was performed to assess the sensitivity of the observed variables to the model parameters and the initial values of the state variables. Using the sensitivity analysis feature of the PottersWheel, the model was simulated, as the input parameter of interest was changed using multipliers between 0.1 and 10. Time period used for the simulations was 15 d.

An indicator  $\hat{y}/\hat{y}_0$  was used to assess the observed variable's sensitivity to the input parameter:

$$\frac{\hat{y}}{\hat{y}_0} = \frac{\sum_{i=1}^n \frac{y_i}{n}}{\sum_{i=1}^n \frac{y_{0,i}}{n}} \quad (4)$$

where  $y_i$  and  $y_{o,i}$  are the variable's simulated concentrations at time  $t$  using the changed input parameter and the calibrated model (mg COD·dm<sup>-3</sup> or mg P·dm<sup>-3</sup>), respectively, and  $n$  is the total number of simulated data points. An observed variable was decided to be sensitive to a particular input parameter, if  $\hat{y}/\hat{y}_o < 0.5$  or  $\hat{y}/\hat{y}_o > 1.5 < 0.5$  as the input parameter was changed using the multiplicands.

*Model validation.* The model calibrated for the different reactor types was further tested for its validity. The model calibrated for the acidic fermentation (with or without enzyme supplementation) was validated using the VFA, soluble COD, and soluble PO<sub>4</sub>-P time series of S1, C1-2, and C2-2. The measured soluble COD time series data (up to 15 d) of the anaerobic mesophilic batch fermenters were also used for the validation. These fermenters were fed with cattle manure and operated under acidic conditions. The acidic pH was a consequence of souring and was not controlled externally as opposed to the pH control scheme used in this study. The model calibrated for the alkaline fermentation was validated using the observed variable time series of C3-2 (no enzyme supplementation) and S3 (enzyme supplementation). All the fitted model parameters were fixed during the simulations, whereas the initial values of the state variables were allowed to change less than 20%. A linear regression equation ( $y = mx$ ) was fitted for each observed variable using the measured and simulated data as the explanatory ( $x$ ) and response ( $y$ ) variables, respectively.

Coefficients of determination ( $R^2$ ), slope ( $m$ ), and average percent error ( $\bar{e}$ ) (Eq. (5)) were used to assess the prediction performance of the calibrated model

$$\bar{e} = \frac{\sum_{i=1}^n \frac{y_{\text{measured},i} - y_{\text{simulated},i}}{y_{\text{measured},i}}}{n} \times 100 \quad (5)$$

where  $y_{\text{measured}}$  and  $y_{\text{simulated}}$  are the measured and simulated variable concentrations at a given time  $t$  (mg COD·dm<sup>-3</sup> or mg P·dm<sup>-3</sup>), respectively, and  $n$  is the number of the measured data points.

### 3. RESULTS AND DISCUSSION

#### 3.1. ORGANIC MATTER AND PHOSPHORUS RELEASE PERFORMANCE OF FERMENTERS

Organic matter and P release in the acidic and alkaline fermenters levelled off within 6 d (Fig. 1). Increasing the operational temperature from 38 to 50 °C did not improve the organic matter and P release significantly (Table 4). The values given in Table 4, are the peak values observed during the course of the anaerobic fermentation. The ob-



servation times of the peak values in various columns for a given reactor do not necessarily overlap. Under the acidic conditions, less than 20% of the total COD was released into the dissolved phase in S1, C1-2, S2, and C2-2.

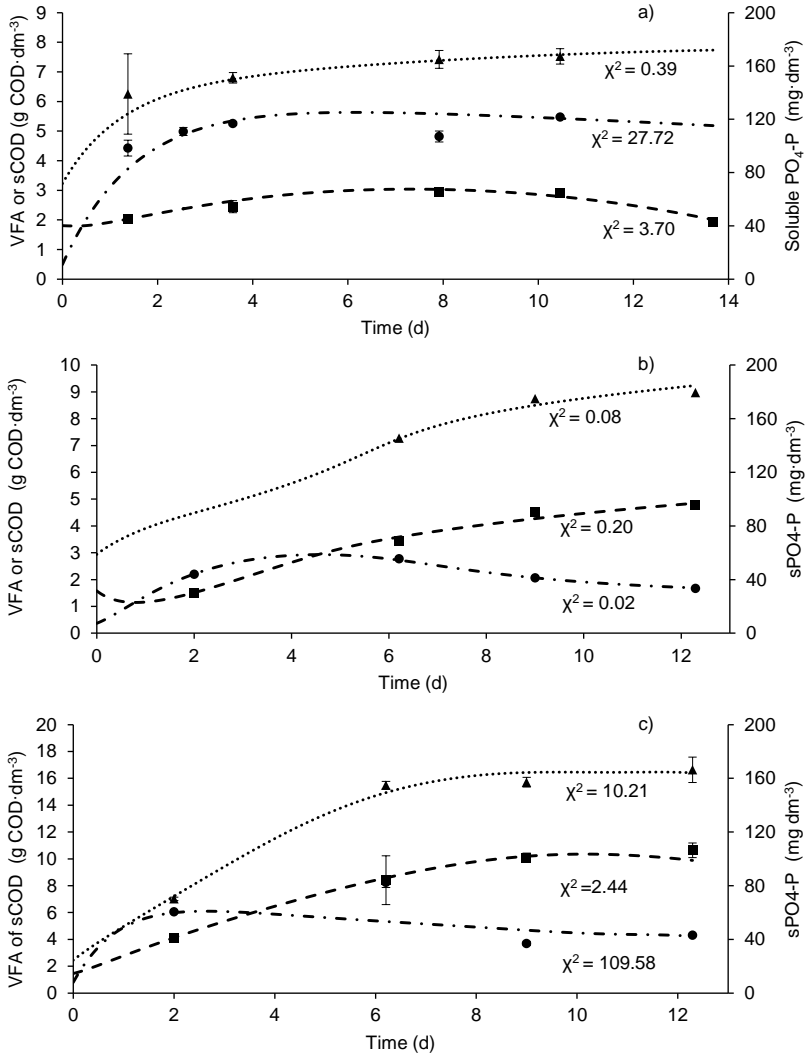


Fig. 1. Measured vs. simulated VFA, soluble COD, and soluble PO<sub>4</sub>-P data of fermenters: a) acidic + mesophilic + enzyme (S2), b) alkaline + mesophilic (C4-2), c) alkaline + mesophilic + enzyme (S4). VFA, g·dm<sup>-3</sup> – ■, - - -, soluble COD, g·dm<sup>-3</sup> – ▲, ·····, soluble PO<sub>4</sub>-P, mg·dm<sup>-3</sup> – ●, · - ·. Measured and simulated data represent solid symbols and dashed lines, respectively. Error bars ( $\pm$  standard deviation) are shown where applicable

The increase of sCOD attributable to the enzyme supplementation appeared to be quite limited in the acidic fermenters (Fig. 1 and Table 4). In the previous study, less than 20% of the total COD in the cattle manure was released into the dissolved phase in the mesophilic batch fermenters spiked with cellulase [8]. The fermenters were not seeded, and the cellulase dose varied between 3.9 and 7.8 mg·g<sup>-1</sup> of dry manure. Under acidic conditions of this study, enzyme addition had a more pronounced effect in the mesophilic fermenters than in the thermophilic reactors.

Table 8

Maximum values observed for organic matter and phosphorus release from dairy manure

Reactor	sCOD:VS [g·g <sup>-1</sup> ]	VFA:VS [mg COD·g <sup>-1</sup> ]	sPO <sub>4</sub> -P:TP [g·g <sup>-1</sup> ]
S1 <sup>a</sup>	0.19	58	0.16
C1-1 <sup>b</sup>	0.26	51	0.75
C1-2 <sup>a</sup>	0.18	59	0.16
S2 <sup>a</sup>	0.23	90	0.17
C2-1 <sup>c</sup>	0.43	65	0.92
C2-2 <sup>a</sup>	0.18	50	0.16
S3 <sup>a</sup>	0.52	288	0.10
C3-1 <sup>b</sup>	0.92	59	0.13
C3-2 <sup>a</sup>	0.28	81	0.05
S4 <sup>a</sup>	0.51	308	0.12
C4-1 <sup>c</sup>	0.89	58	0.76
C4-2 <sup>a</sup>	0.27	146	0.08

<sup>a</sup>Initial TS and VS are ca. 3.8 and 3.3 wt. %, respectively.

<sup>b</sup>Initial TS and VS are 0.8 and 0.5 wt. %, respectively.

<sup>c</sup>Initial TS and VS are 0.6 and 0.5 wt. %, respectively.

The enzyme addition was very effective under the alkaline conditions and almost doubled the COD released from the manure (Fig. 1 and Table 4). Approximately half of the total COD placed in reactors S3 and S4 was converted into the soluble COD. VFA constituted only ca. 15–40% of the soluble COD in the acidic fermenters. On the other hand, 50–60% of the soluble COD in the alkaline fermenters, namely S3, S4, and C3-2, was comprised by VFA. The VFA to soluble COD ratio was substantially lower (ca. 7%) in C3-1 and C4-1. This could be attributed to the fact that the reactors were not seeded and their microbial concentrations were low. Acetic acid was the predominant VFA species regardless of the fermenter: acetic acid constituted 60–90% of the VFA in S1, C1-2, S2 and 50–75% of the VFA in S3, S4, C4-2. No VFA species other than acetic acid was determined in C2-1, C3-1, and C4-1. The soluble PO<sub>4</sub>-P released was less than 20% of the TP in S1, C1-2, S2, and C2-2 under the acidic conditions. P release performance of the alkaline fermenters (S3, C3-2, S4, C4-2) was worse (<15%) than the acidic fermenters discussed above.

Percent COD and P released into the dissolved phase was substantially higher in the control fermenters that solely received only seed (C1-1, C2-1, C3-1, C4-1) (Table 4). Total solids' content of these fermenters was ca.  $6000\text{--}8000\text{ mg}\cdot\text{dm}^{-3}$  as opposed to ca.  $38,000\text{ mg}\cdot\text{dm}^{-3}$ , i.e., TS content of the remaining fermenters. Hence, the dilute fermenters only had ca. 20% of the total COD and total P present in the concentrated fermenters which explains the higher soluble COD and P yields given below. More than 80% of the total COD was released in C3-1 and C4-1 under the alkaline conditions (Table 4). On the other hand, more than 70% of the TP was released in C1-1 and C2-1 under the acidic conditions. A soluble  $\text{PO}_4\text{-P}$  spike ( $59\text{ mg}\cdot\text{dm}^{-3}$ ) was observed in C4-1 within the first six days of the fermentation. The soluble  $\text{PO}_4\text{-P}$  subsequently decreased to  $10\text{ mg}\cdot\text{dm}^{-3}$  and remained stable.

Our results showed that mesophilic, alkaline ( $\text{pH} = 9.5$ ), enzyme-supplemented anaerobic reactors could be used for enhanced fermentation under IMT schemes. These fermenters can potentially dissolve 50% of the total COD and deliver an effluent enriched with VFA. Concurrent dissolution of organic matter and P within the same fermenter appears to be unfeasible. Other reactor alternatives for high efficiency P dissolution should be investigated in the future studies. The dilute batch fermenters fed with anaerobically digested manure exhibited exceptionally high soluble COD and soluble  $\text{PO}_4\text{-P}$  yields, which contradicts with the above statements. Excessive need for water ( $100\text{ dm}^3\cdot\text{kg}^{-1}$  dry manure) can be an important obstacle that may work against scaling up the dilute fermenters for IMT.

### 3.2. MODEL CALIBRATION AND SENSITIVITY ANALYSIS

The model parameters were calibrated for enabling the simulations of VFA, soluble COD, and soluble  $\text{PO}_4\text{-P}$  in the acidic fermenters, and in the alkaline fermenters without and with enzyme supplementation. The calibration was performed separately fitting the model to the experimental data collected from S2, C4-2, and S4 (Fig. 1). The fitted model was not able to simulate the soluble  $\text{PO}_4\text{-P}$  peak observed in S4, and the presence of this outlier contributed to the relatively high  $\chi^2$  value (Fig 1c). Since the standard deviation of the S2 and S4 measured data was usually low,  $\chi^2$  values of the corresponding fits were rather high (Fig 1a, c) as compared to the  $\chi^2$  values of the C4-1 fits where the standard deviation of the measured data was estimated (Fig. 1b). Overall, the best fits captured the analyte dynamics of the fermenters well.

A comprehensive sensitivity analysis was performed to investigate effects of the model parameters, and the initial values of the state variables on the behaviors of the response variables of soluble COD, VFA, and soluble  $\text{PO}_4\text{-P}$ . It must be noted that the inferences made hereafter were based on the values assumed by the indicator,  $\hat{y}/\hat{y}_0$ . The observed variables were found insensitive to  $K_n$  and  $k_{\text{amm}}$  regardless of the reactor type (Table 5). On the other hand,  $\mu_{\text{max,a}}$ ,  $d_a$ , and  $d_e$  emerged as the critical parameters that significantly influenced both soluble COD and VFA independent of the fermenter type.

$k_{h,s}$  was also observed not to be an important parameter determining the soluble organic concentration in the alkaline fermenters, whereas  $k_{h,is}$  continued to limit the soluble organic, particularly soluble COD yield.

Table 5

Calibrated model parameters, and sensitivities of observed variables to the parameters

Parameter	Acidic solution pH $\approx$ 5	Alkaline solution, pH $\approx$ 9	
		Enzyme	No enzyme
$\mu_{max,a}$ , d <sup>-1</sup>	2.18 <sup>c</sup>	3.38 <sup>c</sup>	1.36 <sup>c</sup>
$\mu_{max,m}$ , d <sup>-1</sup>	0.22 <sup>a,c</sup>	1.95 <sup>c</sup>	8.60 <sup>a,c</sup>
$K_a$ , mg·dm <sup>-3</sup>	883 <sup>b,c</sup>	5427 <sup>c</sup>	548 <sup>a,c</sup>
$K_m$ , mg·dm <sup>-3</sup>	78 <sup>a,b,c</sup>	188 <sup>a,b,c</sup>	1459 <sup>a,c</sup>
$K_n$ , mg·dm <sup>-3</sup>	108 <sup>a,b,c</sup>	5.7 <sup>a,b,c</sup>	20.7 <sup>a,b,c</sup>
$d_a$ , d <sup>-1</sup>	0.66 <sup>c</sup>	0.60 <sup>c</sup>	1.00 <sup>c</sup>
$d_m$ , d <sup>-1</sup>	0.067 <sup>a,b,c</sup>	0.022 <sup>a,c</sup>	1.05 <sup>a,c</sup>
$d_e$ , d <sup>-1</sup>	4.43 <sup>c</sup>	0.25 <sup>c</sup>	1.13 <sup>c</sup>
$Y_a$ , g·g <sup>-1</sup>	0.38 <sup>c</sup>	0.31 <sup>c</sup>	0.81 <sup>b,c</sup>
$Y_m$ , g·g <sup>-1</sup>	0.96 <sup>a,b,c</sup>	0.041 <sup>a,c</sup>	0.058 <sup>a,c</sup>
$Y_e$ , g·g <sup>-1</sup>	0.94 <sup>a,c</sup>	0.011 <sup>c</sup>	0.001 <sup>c</sup>
$k_{h,is}$ , d <sup>-1</sup>	0.001 <sup>c</sup>	1.91 <sup>c</sup>	2.68 <sup>b,c</sup>
$k_{h,s}$ , dm <sup>3</sup> ·mg <sup>-1</sup> ·d <sup>-1</sup>	0.006 <sup>c</sup>	30 <sup>a,b,c</sup>	493 <sup>a,b,c</sup>
$k_{amm}$ , d <sup>-1</sup>	1.41 <sup>a,b,c</sup>	0.28 <sup>a,b,c</sup>	65.4 <sup>a,b,c</sup>
$k_{dis}$ , d <sup>-1</sup>	0.28 <sup>a,b</sup>	0.19 <sup>a,b</sup>	0.072 <sup>a,b</sup>
$k_{pre}$ , d <sup>-1</sup>	0.19 <sup>a,b</sup>	0.73 <sup>a,b</sup>	0.58 <sup>a,b</sup>

<sup>a</sup>The average soluble COD insensitive to this parameter.

<sup>b</sup>The average VFA insensitive to this parameter.

<sup>c</sup>The average soluble PO<sub>4</sub>-P insensitive to this parameter.

All the observed variables were insensitive to the initial values of  $C_{mo}$  and  $C_{prot}$  in both acidic and alkaline fermentation. It must be noted that the initial value of  $C_{mo}$  was set to zero prior to the calibration routine and the initial value of  $C_{mo}$  was kept under one for the validation simulations. The observed insensitivity of variables to the initial value of  $C_{mo}$  was a consequence of this approach. Additionally, the observed variables did not respond to the varying initial values of  $C_e$  in the acidic fermentation. The initial value of  $C_{sp}$  did not influence the observed variables including the soluble PO<sub>4</sub>-P significantly in the alkaline fermentation. Our results showed that it was not a prerequisite to choose the initial values of  $C_{prot}$  and  $C_{NH_4-N}$  properly for the accurate simulation of dissolved COD, VFA, and soluble PO<sub>4</sub>-P. Sensitivity of the dissolved COD to the initial value of  $C_{NH_4-N}$  in the enzyme-supplemented alkaline fermentation was an exception. Furthermore, the soluble PO<sub>4</sub>-P could be predicted effectively depending on the initial value of  $C_{pp}$ ,  $k_{pre}$ , and  $k_{dis}$ .

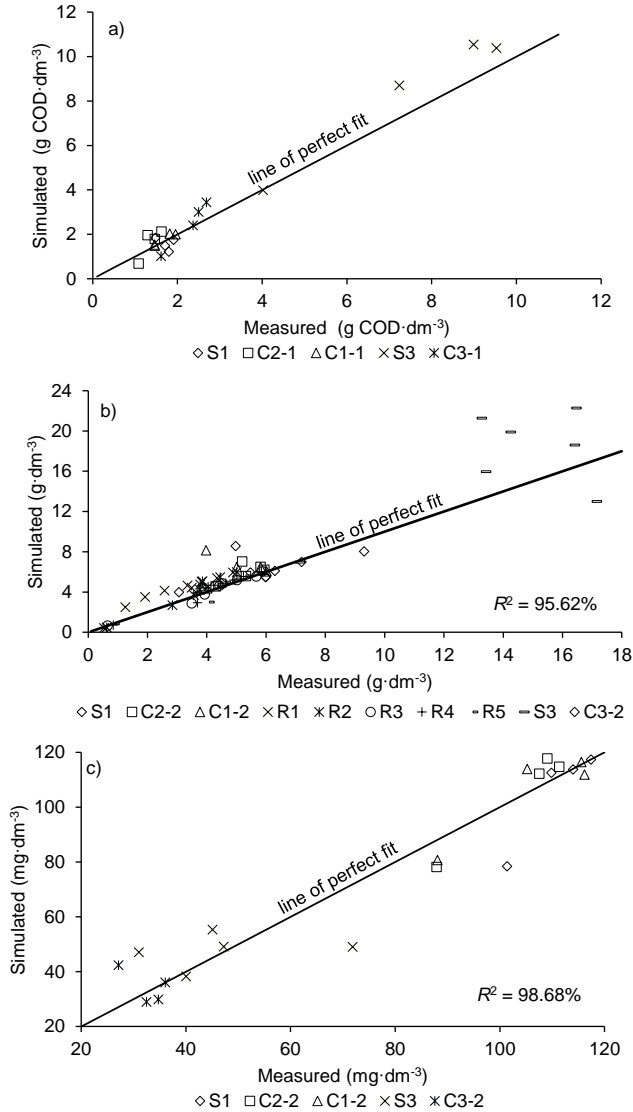


Fig. 2. Model validation results for VFA (a), soluble COD (b), and soluble PO<sub>4</sub>-P (c). Batch mesophilic data (R1-R5) after [8]

The fitted model parameters of  $k_{h, is}$  and  $k_{h, s}$  for the acidic fermentation were substantially lower than those used for the sludge prefermenters ( $k_{h, is} = 0.22 \text{ d}^{-1}$ ,  $k_{h, s} = 0.75 \text{ dm}^3 \cdot \text{mg}^{-1} \cdot \text{d}^{-1}$ ) [14]. The difference can be attributed to the relatively high fibrous (cellulose + hemicellulose) content of dairy manure: the fibers constitute approximately half of the manure solids [7] and their hydrolysis is quite slow due to their complex structure.

The fitted hydrolysis rate constants increased remarkably when the fermentation condition was switched from acidic to alkaline. The rate constants evidently responded to the improved hydrolysis of the slowly biodegradable organics under alkaline conditions. Other fitted parameters that were significantly higher than the literature values were  $d_e$ ,  $Y_a$ ,  $Y_e$  (acidic),  $\mu_{\max,m}$ ,  $d_m$ ,  $d_e$ ,  $Y_a$ ,  $Y_m$  (alkaline + no enzyme); and  $\mu_{\max,m}$ ,  $K_a$ ,  $d_e$ ,  $Y_e$  (alkaline + enzyme) [14]. Among the fitted parameters, only  $Y_e$  (alkaline + no enzyme) was lower than the value ( $0.01 \text{ d}^{-1}$ ) used for the sludge prefermenters [14].

### 3.3. MODEL VALIDATION

The model calibrated for the three fermenter types was validated using an independent data set (Fig. 2). Only the data generated in this study were used for validating the simulated VFA and soluble  $\text{PO}_4\text{-P}$ , whereas the data from the previous study were also used for validating the simulated soluble COD [8].  $R^2$  value observed for the VFA data ( $R^2 > 0.99$ ) was the highest, whereas  $R^2$  value determined for the soluble COD was the lowest ( $R^2 < 0.96$ ). For the soluble COD and VFA data, the outliers were mostly clustered above the line of perfect fit and caused the regression equations to have slopes larger than one. It must also be noted that the slope values were below 1.15, an indication of the calibrated model's general tendency to overestimate the soluble COD and VFA. On the other hand,  $\bar{e}$  of the data sets was in the range of 5–35% and 6–45% for VFA and soluble COD, respectively. Contrary to the soluble COD and VFA results, the calibrated model did not have an inclination to over- or underestimate soluble  $\text{PO}_4\text{-P}$ , which was corroborated by a regression equation slope approximating 0.99. The  $\bar{e}$  values determined for the data sets remained in a relatively narrow range of 5–23%.

Overall, the calibrated model appeared to simulate soluble COD, VFA, and soluble  $\text{PO}_4\text{-P}$  in the acidic and alkaline fermenters effectively. In future studies, the model can be used as a tool in the design/operation of the anaerobic fermenters (e.g., continuously stirred tank reactors) delivering high dissolved organic or dissolved P yield for the downstream units of the IMT systems.

## 4. CONCLUSIONS

Our experimental results showed that P solubilization was favored by the acidic pH, whereas alkaline pH promoted organic matter solubilization. Also, the commercial enzyme operating under alkaline pH delivered an improved soluble organic yield of  $0.5 \text{ g COD} \cdot \text{g}^{-1}$  volatile solids (VS). Sensitivity analysis of the model showed that  $\mu_{\max,a}$ ,  $d_a$ , and  $d_e$  were the critical parameters for the soluble COD and VFA. Soluble  $\text{PO}_4\text{-P}$  was mostly sensitive to  $C_{pp}$ ,  $k_{pre}$ , and  $k_{dis}$ . The calibrated model was validated ( $R^2 > 0.95$ ) and found to have a tendency to slightly overestimate soluble COD and VFA. The accuracy of the model is dependent on the initial values of the state variables, to which the observed

variables are sensitive. Readily available manure characterization data are quite limited for the modeling, which is not an uncommon issue in this field of study. This challenge was addressed using a simple estimation method requiring only the measured concentration of one analyte (total solids). To account for uncertainties associated with the estimated values, the initial state variable values were allowed to be fitted ( $\pm 20\%$ ) during the model calibration and validation. Measured data sets of the observed variables used for the calibration and validation were less than 15 d in duration. Effects of these factors on the prediction performance of the calibrated model need to be explored in the future studies.

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