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ANAEROBIC MODELING FOR IMPROVING SYNERGY AND ROBUSTNESS OF A MANURE CO-DIGESTION PROCESS

D. M. F. Lima[1], J. A. D. Rodrigues[2*], K. Boe[3], M. Alvarado-Morales[3], L. Ellegaard[4] and I. Angelidaki[3]

[1]São Carlos Engineering School, (EESC), University of São Paulo, (USP),
Av. Trabalhador São-Carlense 400, CEP: 13.566-590, São Carlos - SP, Brazil.
[2]Mauá School of Engineering, (EEM), Mauá Institute of Technology, (IMT),
Square Mauá 1, CEP: 09580-900, São Caetano do Sul - SP, Brazil.
E-mail: rodrigues@maua.br
[3]DTU, Environment, Department of Environmental Engineering,
Technical University of Denmark, Building 113, Kongens Lyngby, Denmark.
[4]Burmeister & Wain Scandinavian Contractor A/S, Denmark.

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Abstract - Biogas production is becoming increasingly important in the environmental area because, besides treating wastewaters, it also generates energy. Co-digestion has become more and more powerful since it is possible, with the use of abundant and cheap substrates, to dilute the inhibitory effects of various other substrates, making the process of anaerobic digestion more efficient and stable. Biogas process modelling describes the kinetics and stoichiometry of different steps in the anaerobic digestion process. This mathematical modelling provides an understanding of the processes and interactions occurring inside the biogas system. The present work investigated the interactions between different simple co-substrates (carbohydrate, lipid and protein) and real co-substrates (corn silage, fodder beet, grass and wheat straw) under co-digestion with manure, in order to verify synergetic effects. Subsequently, some experiments were reproduced, in order to evaluate the synergy obtained in the previous simulation and validate the model.
Keywords: Methane; Anaerobic digestion; Modelling; Co-digestion; Manure.

INTRODUCTION

The biogas process is a sustainable process for energy production, waste treatment and recycling of organic waste nutrients. Co-digestion of manure with several organic wastes is a common practice applied in the Danish centralized biogas plants for more than two decades (Angelidaki and Ellegaard, 2003). The methane yield from manure is relatively low; thus, co-digestion of manure with organic substrates with a high content of lipids, proteins or carbohydrates, is necessary for the biogas plant's economy. The positive effects of the co-digestion concept have been widely reported (Atandi and Rahman, 2012). Co-digestion of manure with organic wastes has economic advantages from improved biogas yield, cost saving by economy of scale and the sharing of equipment, and easier handling of mixed wastes (Li *et al*., 2009). Moreover, co-digestion of concentrated organic substrate with manure has an advantage in the form of a synergy effect as manure is a good substrate for dilution of inhibitory wastes, rich in nutrients important for microbial growth and has strong buffer capacity for maintaining a stable pH. Recently, attention has been paid to co-digestion of manure with energy crops in order to boost the bio-

*To whom correspondence should be addressed gas production for reaching the goal of renewable energy contribution to the energy supply in Denmark. This is a new situation for biogas operation and there is urgent need for clarification of the interaction of the substrates and for establishing the best operational conditions and mixing ratios for optimal utilization of these potential biomasses. The outcomes of such co-digestion processes have not yet been foreseen and optimized.

Previous studies have shown that several industrial organic wastes could not be efficiently treated alone due to their inhibitory effect, for example, due to high salt content or ammonia. These wastes showed better process stability when co-digested with manure than when treated alone (Fang *et al.*, 2011a; 2011b). However, the application of co-digestion is sometimes associated with process imbalance from potential inhibiting compounds in the co-substrate such as ammonia from protein or long chain fatty acids from lipid wastes. Previous investigation also pointed out that the process imbalances in the Danish centralized biogas plants are often directly related to the addition of industrial organic wastes (Nielsen and Angelidaki, 2008). Addition of protein-rich co-substrate increased the ammonia concentration in the biogas reactor and led to an increased residual biogas loss (suboptimal process conditions). There is a need to find the optimal method for operation of co-digestion processes in order to avoid process imbalance and inhibition and to obtain the full benefits of co-digestion.

Although the biogas process is a naturally-occurring process which has been known for centuries and industrially exploited for decades. It is complex and there is a constant demand for a better understanding and improvement of the process. The biogas process is the interaction of many bacterial groups that interact directly and indirectly and is also a versatile process that can degrade a variety of organic wastes. This means there are many parameters that have to be considered in order to understand the process in detail. Mathematical modelling provides an understanding of processes and interactions occurring inside the biogas system. Model results can be used to demonstrate important inhibition patterns and suggest guidelines for optimal substrate mixing and operation of biogas reactors. Biogas process modelling has been widely studied during the last two decades. Several models has been proposed to describe the kinetics and stoichiometry of different steps in the anaerobic digestion process. A comprehensive Anaerobic Digestion Model 1 (ADM1) (Batstone *et al.*, 2002) is a generic model widely used in different anaerobic digestion applications. Moreover, a comprehensive biogas process model for simulation of a manure co-digestion system was developed at DTU, which has been the basis for the ADM1 (Angelidaki *et al.*, 1999). This model has provided insight into the important interactions in the manure-based biogas process.

The DTU model has been used to illustrate some specific operational aspects of the co-digestion process (Ellegaard and Angelidaki, 2009). The biogas model results illustrated the equilibrium interaction between different ionic components and their effects on pH and reactor stability. This is importante when dealing with protein-rich substrates, which could result in high ammonia concentration in the reactor, especially for thermophilic operation. Moreover, the model result also showed that the addition of carbohydrate or lipids to manure could enhance the biogas production rate, and also increase process stability, as seen from lower VFA concentration under steady state conditions. A possible synergy effect from co-digestion of protein wastes together with carbohydrate or lipid wastes was also shown. From the model simulation, easily degradable carbohydrates provide extra energy to microorganisms, which increase the cell mass production and enhance the assimilations of ammonia into cell mass. Moreover, the higher biogas production from easily degradable substrate also increases dissolved CO_2, which decreases pH and reduces free ammonia concentration in the reactor. Thus, the addition of easily degradable carbohydrates to reactors treating protein waste would have a positive effect in counteracting the ammonia inhibition. Furthermore, it was suggested from the model simulation results that the key factor to neutralize the ammonia load from protein wastes was to mix protein with carbohydrate or lipid wastes, which increases the assimilation of ammonia into biomass and reduces pH by modifying the cation-anion balance. Nevertheless, the interesting process information as described above has neither been verified by lab-scale experiments, nor by comparing with full-scale reactor operation data. These findings are important and could help improve the efficiency and stability of the biogas plant. However, there is a need for verification of these scenario predictions before practical guidelines for operation can be made.

The expected increased number of biogas plants in the near future will lead to an increasing need for finding more suitable co-substrates for the biogas process. The biomasses that have recently gained increasing interest in Denmark are cellulosic residues such as grass, straws, cast seaweed, etc. Some ongoing projects are investigating the potential of this biomass for biogas production. Another potential organic waste in Denmark that has not been fully utilized for biogas production is the organic fraction of municipal household wastes. The co-digestion model could also be applied to investigate the synergy effect on process stability and methane yield of these new types of co-substrates. Furthermore, co-digestion of manure with energy crops would give insight in this co-digestion situation. This will support the utilization of a wider range of organic wastes in the future biogas plants.

In this context, the main objective of the present work was *to investigate synergy effects between* simple substrates (carbohydrates, lipids and proteins) and also complex substrates (corn silage, fodder beet, grass and wheat straw) with manure, using the Biomodel developed by Angelidaki *et al*. (1999), an anaerobic digestion model developed by the Bioenergy Group as an alternative to the DTU model used previously. The present work also tried to check the reliability of the model by reproducing real results obtained in experiments.

MATERIALS AND METHODS

Model Description and Dynamics

The Biomodel describes complex substrates by its main components (carbohydrates, lipids, and proteins). An initial material flow for each type of organic matter is included separately and these materials merge into common intermediates and products while the degradation proceeds. Additional intermediates derived from the more-detailed substrate definition (LCFA, amino acids, valerate, and hydrogen sulfide) and their effect on kinetics and chemical equilibria are also included. Lastly, the decay of dead-cell mass has been included in the model to complete the mass balance and overall yield.

Figure 1 shows the main pathways of the process. The model involves two enzymatic processes: (A) hydrolysis of undissolved carbohydrates, and (B) of undissolved proteins, and eight bacterial groups: (1) glucose-fermenting acidogens, (2) lipolytic bacteria, (3) LCFA-degrading acetogens, (4) amino acid-degrading acidogens, (5) propionate-, (6) butyrate-, (7) valerate-degrading acetogens, and finally (8) aceticlastic methanogens.

The model includes free ammonia inhibition of the acetoclatic step and inhibition of the hydrolytic steps by total VFA concentration. Product inhibition of VFA degradation to acetate was further included along with inhibition of all the bacterial steps by LCFA.

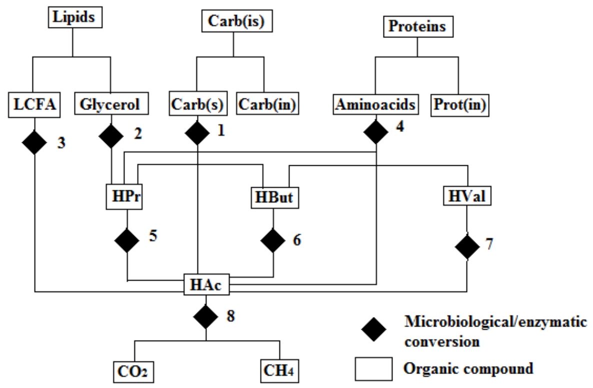

Figure 1: Main pathways for anaerobic degradation of organic matter used in the model.

Free ammonia inhibition results in VFA accumulation, which decreases the pH and pushes the ammonia ionization equilibrium towards lower concentrations of free ammonia. This positive feedback interaction allows the model to simulate situations with elevated VFA levels due to ammonia inhibition, as is often seen in manure-based processes (Angelidaki and Ahring, 1993).

VFA inhibition of the initial hydrolytic steps accounts for the apparent loss of biogas potential often seen in inhibited reactors. Acetate inhibition of the degradation of higher VFAs accounts for the maintenance of these, while LCFA inhibition accounts for the interruption effect seen in the cases of LCFA accumulation due to overdosing of lipids to biogas reactors (Angelidaki and Ahring, 1992).

Inhibitions, interactions, yield coefficients, fixed stoichiometry of the components of the substrates and kinetic constants and equations applied in the model are described in Angelidaki *et al*. (1999).

Separate hydrogen kinetics have been omitted (merged into other steps), as hydrogen turnover is faster compared to the other steps of the process. Including hydrogen kinetics and inhibitions as suggested by Mosey (1983) can present unrealistic, fast and dynamic behaviors, suggesting the regulatory role of hydrogen can be exaggerated. Also, separate glycerol kinetics have been omitted and merged with the GTO step as glycerol only accounts for a very limited fraction of the material flow and there is no proof that it is an important inhibitor.

The substrate in the model was defined by its organic and inorganic composition. The organic components that describe the substrate were carbohydrates, proteins, lipids and their degradation intermediates. Dead-cell mass was accounted for in the model and assumed gradually to decay into carbohydrates and proteins, thus becoming new substrate. The inorganic components included in the model were ammonia-N, phosphate-P, carbonate-C, hydrogen sulfide, anions (A^-), and cations (Z^+). The net concentration of Z^+ represents cations such as Ca^{2+}, Mg^{2+}, and K^+ and plays an important role in determining the pH and buffer balance of the process.

The bacterial death rate has generally been assumed to be 5% of the maximum growth rate. A first-order decay of dead cell mass with a rate of 0.01 h^{-1} has been used. The effect of pH on the growth rate was described by a Michaelis pH function, normalized to give a value of 1.0 as the center value, as previously described. Temperature dependency of the growth rate of all the bacterial steps was included as described previously. Gas and liquid were assumed to be in quasi-stationary equilibrium and the distribution of the volatile components between the gas and liquid phases was determined as previously described (Angelidaki *et al*., 1999)

RESULTS AND DISCUSSION

Simulation Using Simple Substrates (Pure Glucose, Protein and Lipid)

The first topic studied using the model was the potential benefits of co-digestion.

As shown in the model description, it is expected that increasing the substrate load would lead to higher VFA level and a loss of efficiency. However, if the organic load increases without increasing the hydraulic load (in practice by raising VS% in the substrate supply), the requirements for growth rate and thus the substrate concentration do not change after the equilibrium has occurred, showing that significant chemical factors were not adversely affected. This could correspond to a situation where there is an addition of a product with very high lipid (oil/grease) concentration (fish oil, bleaching earth, among others), or an addition of a slightly more modest scale energy crop with high VS% content and good biodegradability.

Under such conditions, for the supplementary substrate, it is expected to achieve values close to the theoretical maximum methane yield presented in Table 1 (DTU model characteristics and ratios), given that the addition does not lead to an increase in nutrient losses. This is the opposite to the intuitive notion that increased load may increase the damage to the process. Also, some authors reported unexpectedly high yields upon adding some supplementary substrates.

The above explanation is solely based on Monod and can be applied properly for a simple pure culture process with only one reaction step. In a manure biogas process where methane production is almost exclusively determined by the acetate reaction, something similar is expected. However, it is difficult to assess, since an increase in the substrate feeding and the biogas production can affect the CO_2

Table 1: DTU model characteristics and ratios.

		General		DTU Model					
Ratios (excluding leaching losses)		Assumed degradation degree	Buswel methane potential	Maximum methane potential	Percentage compared to Buswel	Cell mass ($C_5H_7NO_2$)	NH_3/NH_4^+ balance	Percentage of methane via H_2/CO_2	Organic intermediate products (model), besides acetate and H_2/CO_2
Component	Chemical formula	%	nL-CH_4.g-VS^{-1}	nL-CH_4.g-VS^{-1}	%	g.g-VS^{-1}	mg-N.g-VS^{-1}	%	-
Celulose	$(C_6H_{10}O_5)_n$	50	0.207	0.170	82.0	0.075	-9.35	21.4	Glucose, Propionate, Butyrate
Protein (Gelatin)	$CH_{2.03}O_{0.6}N_{0.3}S_{0.001}$	80	0.316	0.268	84.9	0.100	108.24	10.8	Amino acids, Propionate, Butyrate, Valerate
Lipid (Glycerin tri-oleate)	$C_{57}H_{104}O_6$	100	1.014	0.924	91.2	0.186	-23.04	29.9	LCFA, Propionate
Dissolved carbohydrate	$C_6H_{12}O_6$	100	0.373	0.306	82.0	0.136	-16.84	21.4	Propionate, Butyrate
Acetate	CH_3COOH	100	0.373	0.353	94.5	0.041	-5.14	0.0	None
Propionate	CH_3CH_2COOH	100	0.530	0.467	88.2	0.126	-15.63	42.8	None
Butyrate	$CH_3CH_2CH_2COOH$	100	0.636	0.568	89.3	0.137	-17.03	19.9	None
LCFA	$C_{18}H_{34}O_2$	100	1.013	0.924	91.2	0.178	-22.12	29.4	None

balance between gas and liquid, affecting pH and ammonium/ammonia balance and, through these changes, affecting important mechanisms of inhibition. To illustrate this theme, a series of simulations was carried out in which the concentration of different substrates was increased relative to a base level of digestion of manure.

The starting point was a thermophilic digestion (55 °C) with residence time of 15 days and only manure as substrate, which may be briefly described as follows: VS 4.6% (46 $g.L^{-1}$), of which: 33.8 $g.L^{-1}$ carbohydrate, 85% insoluble, 74 mM VFA (4.5 $g.L^{-1}$ as acetate, 4.8 $g.L^{-1}$ with current VFA absorption), 4.9 $g.L^{-1}$ of protein and 2.5 $g.L^{-1}$ of lipid, and N-tot.NH_4-N^{-1} 3.2/2.5 $g.L^{-1}$. Dissolved CO_2, phosphate and cations were also added to the "synthetic manure" (which roughly corresponds to a thin mix of cattle and pig slurry), trying to provide a digested pH value of 8.04. The model dictates that the digestion gives a methane yield of 0.73 nL-CH_4.L-$reactor^{-1}$.day^{-1} and runs with a VFA level of 11.7 mM (0.702 $g.l^{-1}$ calculated as acetate).

Table 2 shows the process data and gas outcome after long-term stabilization by increasing the concentration of different substrate types (additional supply of pure concentrated substrate).

As can be seen from the results, the model shows that the VFA level actually decreases upon adding pure dissolved carbohydrate or lipid, while the opposite happens for the protein. This means that, at least for carbohydrate and lipid, synergies can be achieved by co-digestion rather than independent digestion, in which a yield slightly below 100% of the maximum yield model would be expected due to losses in the effluent (unreacted VFA).

The resulting extra yield responses for dissolved carbohydrate and lipid were, respectively, 118 and 112%, while, with the addition of protein, it reached only 96% of the methane yield obtained with manure only (0.733 nL-CH_4.L-$reactor^{-1}$.day^{-1}).

For dissolved carbohydrate, due to the advantage of N load, there was somewhat of a reduction by "absorption" of NH_4^+-N, with additional cell growth. At the same time, the CO_2 partial pressure increased, which resulted in more dissolved CO_2 in the liquid phase and a small acidification (lower pH), with a further reduction in the concentration of NH_3.

For the lipid, due to the positive effect of receiving NH_4^+-N in a reaction medium with low amounts of this compound, the pH was not affected. On the other hand, there was a reduction in the CO_2 partial pressure, occasioning an increase in biogas production due to the stripping of CO_2. This resulted in the maintenance of the VFA level as the pH rose and led to a higher ammonia concentration despite falling ammonium levels.

Table 2: Results of simulation by adding pure (100% concentrated) supplementary substrates.

Scenario:	Unit	Basis (Pure manure)	+10 $g.L^{-1}$ dissolved carbohydrate	+10 $g.L^{-1}$lipid (GTO)	+10 $g.L^{-1}$ protein (Gelatin)
pH	-	8.05	7.98	8.03	8.05
NH_4^+.NH_3-N^{-1}	$mg.L^{-1}$	2577	2462	2354	3222
VFA total	mM	11.62	8.84	9.8	40.2
* Acetate	$g.L^{-1}$	0.444	0.297	0.346	1.550
* Propionate	$g.L^{-1}$	0.172	0.151	0.154	0.605
* Butyrate	$g.L^{-1}$	0.076	0.065	0.067	0.241
* Valerate	$g.L^{-1}$	0.106	0.114	0.121	0.357
CO_2 (aq.)	$g.L^{-1}$	11.43	11.61	10.69	11.6
Methane	nL/L-$reactor^{-1}$.day^{-1}	0.733	1.001	1.425	0.707
Biogas	nL/L-$reactor^{-1}$.day^{-1}	1.074	1.604	2.057	1.041
Methane content	%	68.25%	62.41%	69.3%	67.9%
Extra methane	nL/L-$reactor^{-1}$.day^{-1}	-	+0.268	+0.692	-0.026
Extra methane	NL.g-VS^{-1}	-	+0.402	+1.038	-0.039
Extra methane compared model max	%	-	118.23[a]	112.30[b]	-14.60[cc]

OBS: dissolved carbohydrate here is calculated as cellulose units with molecular mass 162 g/mol. Expressed as a true sugar (molar mass 180 $g.mol^{-1}$) with a dissolved carbohydrate scenario dosage of +11.1 $g.L^{-1}$ the specific VS extra allocation would be correspondingly lower. Additional yield in% remains unchanged.

[a] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (0.402 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.340 nL CH4.g-VS^{-1}) – Table 4.

[b] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (1.038 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.924 nL CH4.g-VS^{-1}) – Table 4.

[c] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (-0.039 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.268 nL CH4.g-VS^{-1}) – Table 4.

For protein, disappointing results were obtained (actually damage by the addition in the protein-based process, obtaining a negative "extra" yield). A great enhancement in N load occurred due to NH_4^+-N released by the decomposition of protein, with probable inhibition of the process.

On the whole, the results illustrate the importance of ammonia, CO_2, and the pH equilibrium, which, at least for the thermophilic processes, are important regulatory factors. For a mesophilic process, disparities between the relative yields of the different substrate types probably are smaller.

It should be emphasized that the above is based on the addition of pure 100% concentrated substrates containing no interfering inorganic compounds. Such substrate types are probably not exactly typical in practice, but certain types, such as petroleum products, glycerin/LCFA and energy crops, which basically only contains carbohydrates (biodegradable cellulose), eventually can present similar behavior.

A lipid/protein ratio of approx. 5:1 on VS basis neutralizes the N load of protein, while for cellulose (insoluble carbohydrate) or sugar (dissolved glucose) a ratio of approximately 11:1 and 6:1, respectively, is required. The calculation should be possible taking into account the dilution (if the substrate is not 100% concentrated manure) or the inorganic content of NH_4^+-N. For protein, which constitute a major portion of slaughterhouse waste, animal products, among others, a suitable supplementary substrate is not necessary; it is not convenient to add too high concentrations of this substrate or it can be neutralized by co-digestion with N absorbing raw material types.

Normally, steady and continuous or regular loads in the biogas process are necessary for the safest mode of operation, with gradual changes in feed composition considered, if necessary. This avoids any risk of accumulation of inhibitory intermediates and the composition of microorganisms will be in balance with the substratum or in connection with gradual changes in position to make adjustments. Also, chemical resistance and gas dynamics will be stable.

Simulation Using Complex Substrates (Corn Silage, Fodder Beet, Grass and Wheat Straw)

After the assays using pure concentrated substrate (carbohydrate, protein and lipid), simulations using real substrates were performed. Table 3 shows the process data and gas outcome after long-term stabilization by adding different co-substrate types: corn silage, fodder beet, grass and wheat straw. The composition of each substrate is described in Table 4.

Table 3: Results of simulation by adding real co-substrates.

Scenario:	Unit	Basis (Pure manure)	Manure + Corn silage	Manure + Fodder beet	Manure + Grass	Manure + Wheat straw
pH	-	8.05	7.99	7.96	8.00	8.00
$NH_4^+.NH_3$-N^{-1}	$mg.L^{-1}$	2577	2491	2357	2553	2524
VFA total	mM	11.62	9.54	7.76	10.92	10.45
* Acetate	$g.L^{-1}$	0.444	0.328	0.245	0.378	0.375
* Propionate	$g.L^{-1}$	0.172	0.156	0.139	0.166	0.166
* Butyrate	$g.L^{-1}$	0.076	0.068	0.059	0.072	0.073
* Valerate	$g.L^{-1}$	0.106	0.123	0.116	0.160	0.116
CO_2 (aq.)	$g.L^{-1}$	11.43	11.47	11.33	11.42	11.50
Methane	nL/L-reactor^{-1}.day^{-1}	0.733	0.921	0.991	0.901	0.864
Biogas	nL/L-reactor^{-1}.day^{-1}	1.074	1.438	1.598	1.387	1.324
Methane content	%	68.25	64.05	62.02	64.96	65.26
Extra methane	nL/L-reactor^{-1}.day^{-1}	-	+0.188	+0.258	+0.168	0.131
Extra methane	nL.g-VS^{-1}	-	+0.282	+0.387	+0.252	0.196
Extra methane compared model max	%	-	112.79[a]	137.22[b]	102.43[c]	104.51[d]

[a] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (0.282 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.250 nL CH4.g-VS^{-1}) – Table 4.
[b] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (0.387 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.282 nL CH4.g-VS^{-1}) – Table 4.
[c] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (0.196 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential (0.188 nL CH4.g-VS^{-1}) – Table 4.
[d] The "extra methane compared model max" is obtained by comparing the extra methane achieved in the simulation (0.252 nL CH4.g-VS^{-1}) with the DTU model maximum methane potential l (0.246 nL CH4.g-VS^{-1}) – Table 4.

Table 4: Substrate and co-substrate characterization.

Parameters	Unit	Experimental data				
		Manure	Grass	Wheat straw	Corn silage	Foder beet
TS	%	-	17	85	33	18
VS	%	4.6	15,4	82.4	31.8	16.6
Total Glucose	$g.L^{-1}$	33.8	107.6	779.5	284.1	139.3
Glucose insoluble (%)	%	84.9	82.9	100.0	63.9	20.9
Glucose insoluble	$g.L^{-1}$	28.7	89.25	779.45	181.5	29.16
Glucose (%)	%	15.1	17.1	0.0	36.1	79.1
Glucose	$g.L^{-1}$	5.1	18.36	0.0	102.63	110.16
Glucose inert	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
GTO	$g.L^{-1}$	2.5		16.15	7.26	0.72
LCFA	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
Protein insoluble	$g.L^{-1}$	4.9		28.05	0.0	13.32
Protein inert	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
VFA total	$g.L^{-1}$	4.8	0.0	0.0	0.0	0.0
*Acetate	$g.L^{-1}$	3.1	0.0	0.0	0.0	0.0
*Propionate	$g.L^{-1}$	1.6	0.0	0.0	0.0	0.0
*Butyrate	$g.L^{-1}$	0.1	0.0	0.0	0.0	0.0
*Valerate	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
CH_4	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
CO_2	$g.L^{-1}$	2.88	0.0	0.0	0.0	0.0
H_2S	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
Z^+	$g\text{-}K.L^{-1}$	6.75	2.98	2.12	1.96	3.66
$H_2PO_4^-$	$g.L^{-1}$	0.55	0.0	0.0	0.0	0.0
A^-	$g.L^{-1}$	0.0	0.0	0.0	0.0	0.0
NH_3-N	$g.L^{-1}$	2.5	0.0	0.0	0.0	0.0
CAB*	$meq.kg\text{-}TS^{-1}$	-	450	64	152	521

* CAB [meq/kgTS] = {(Na/23.0+K/39.1)-(Cl/35.5+S/32.0)}*1000

As can be seen from the results, the model shows that the VFA level actually decreases with the addition of the four co-substrates. This means that synergies can be achieved by co-digestion rather than independent digestion, as was observed when pure concentrated carbohydrate and lipid were added. The resulting extra yield responses for corn silage, fodder beet, grass and wheat straw were, respectively, 113, 137, 102 and 104%.

Analyzing the composition of the co-substrates used, they have similar protein and lipid compositions. The main difference between them is in glucose, especially in the soluble and the insoluble fraction. The fodder beet, which was the co-substrate that presented the best synergy level, has the highest soluble glucose concentration and the smallest insoluble glucose concentration. In sequence, corn silage presents a high concentration of soluble and insoluble glucose. Probably this was the reason why it does not reach the same level of synergy of the fodder beet. Grass and wheat straw presented the worst synergy levels among the four co-substrates. These two substrates have high concentrations of insoluble and low concentrations of soluble glucose (especially the wheat straw). Yet they showed synergic effects. The ammonia inhibition probably was not a problem in the synergy process for any of the co-substrates tested.

A good synergy raw material for manure is characterized primarily by very easily degradable carbohydrate, low protein and low CAB numbers. Some lipid content can help to highlight the specific methane yield. Among the four real substrates tested in the simulations, corn and beets seem to be promising candidates, while grass and wheat straw are less suitable, although they can be used together with manure since they presented synergic effects. Especially for the straw, some practical problems (it is an immediate "food competitor" substrate) must be solved prior its utilization.

Mathematical Model Validation Using Simple Co-Substrates (Glucose, Protein and Lipid)

In order to evaluate the synergy obtained in the previous simulation and validate it, several experiments were reproduced in which the co-digestion of manure with glucose, sodium oleate (representing lipids) and gelatine (representing proteins) was tested (Kougias *et al*., 2013).

In their work, Kougias *et al*. (2013) investigated the effect of using simple substrates (glucose, proteins and lipids) and the effect of organic loading rate (OLR) on foaming in continuous stirred tank reactors (CSTR), in order to identify the correlation between OLR, substrate composition, foam formation and process performance (methane production). The experiment was carried out in CSTRs with a working volume of 1.5 L, operating temperature of 54 ± 1 °C, and hydraulic retention time (HRT) of 15 days. The OLR of the reactors was increased by adding the different simple co-substrates to the manure. In the first experiment the influent manure was supplemented with gelatine as a representative of proteins, while in the second experiment it was supplemented with Na-Oleate, as a representative of lipids. The third experiment was used to investigate the effect of OLR with addition of glucose. Each experiment was divided into four periods. During each period, the glucose, gelatine or Na-Oleate concentration and the OLR were increased, in order to analyze the effect of each component on the process.

Figures 2 and 3 show the daily methane yield and the VFA and pH, respectively, for the co-digestion of manure and pure glucose from the experiments and the simulation. There was a gradual increase in the glucose concentration and the simulation data fitted very well the experimental reactor data, especially for the addition of low concentration of glucose (+10 and +20 gGlucose.L^{-1}). Even for the high glucose concentration values (+35 and +50 gGlucose.L^{-1}), the behavior of the system was well predicted. The difference in the values obtained in the steady-state for the last two concentrations tested can be related to the foaming. According to Kougias *et al*. (2013), with the addition of high glucose concentrations there was an increase in the biogas production (predicted by the model). But this increase in the biogas production also increased the bubbling in the system and stimulated the microbial production of bio-surfactants, resulting in the formation of stable and thick foam which could not be destroyed by stirring, only by vigorous manual shaking of the reactor. This way, there was probably a lack of equilibrium between the gas and liquid phase due to the oversaturation of the liquid phase with the biogas and loss of methane by leaching in the effluent. The model could not predict the effect of the foaming formation.

Figures 4 and 5 present the daily methane yield and the VFA and pH, respectively, for the co-digestion of manure and pure lipid and glucose for the experiments and the simulation. There was a gradual increase first in the lipid concentration, then in the glucose concentration and at last in both the lipid and glucose concentrations.

The model fitted well the experimental results, especially in the last step. In the second and third period of operation the experimental results were below the simulation results. This fact can be related to the time adaptation of the microorganisms because at first just lipid was added and then glucose was added, and not considering inhibition mechanisms. This way the different microorganisms responsible for the utilization of the two co-substrates added would need time to adapt to the changes in the feed composition, which was reached only at the end of the experiment. Microbial community shifts as a consequence of the exposure to LCFA pulses have previously been reported (Baserba *et al*. 2012). So it is possible to assume that the model is not able to predict the adaptation time required when effluents with high concentrations of lipids are used.

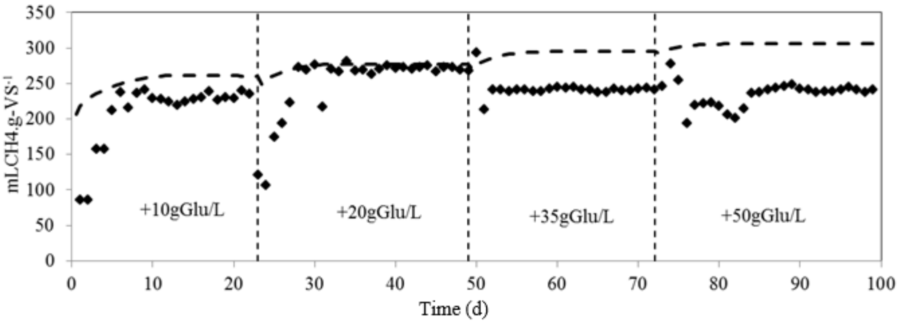

Figure 2: Experimental data (♦) and simulation (- - -) for co-digestion of manure and pure glucose (gradual addition) regarding methane yield.

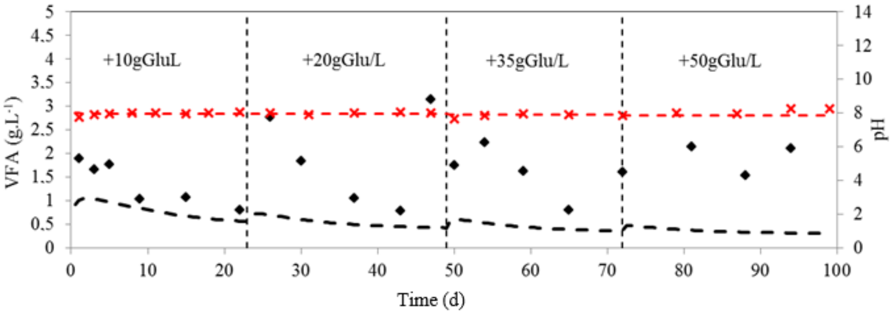

Figure 3: Experimental data (♦ - VFA and X - pH) and simulation (-- - VFA and -- - pH) for co-digestion of manure and pure glucose (gradual addition) regarding VFA and pH.

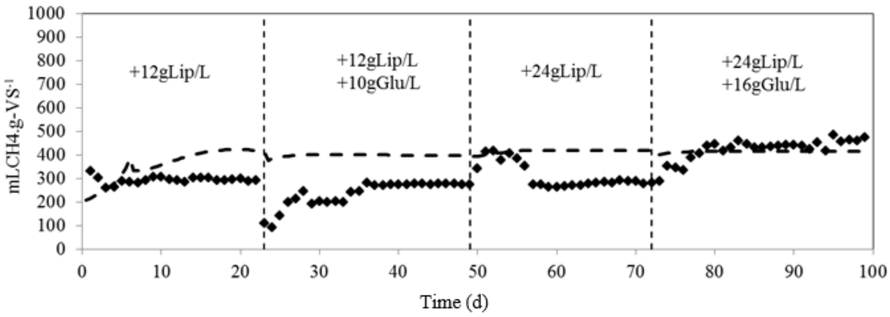

Figure 4: Experimental data (♦) and simulation (- - -) for co-digestion of manure and pure lipid and glucose (gradual addition) regarding methane yield.

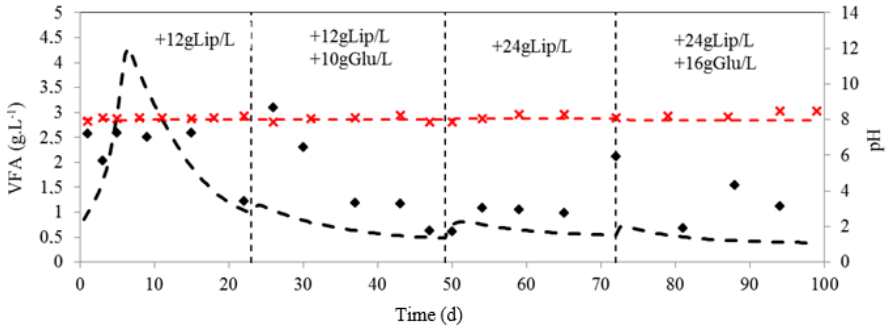

Figure 5: Experimental data (♦ - VFA and X - pH) and simulation (-- - VFA and -- - pH) for co-digestion of manure and pure lipid and glucose (gradual addition) regarding VFA and pH.

Foam formation had no adverse effect on methane production as it was only seen in operation with the addition of glucose. This was especially evident in period 4 (period with higher foam production) of the experiment with addition of lipid, where the model fitted well the experimental results.

Figures 6 and 7 show the daily methane yield and the VFA and pH, respectively, for the co-digestion of manure and pure protein and glucose for the experiments and the simulation. As in the other experiments using simple co-substrates, it is possible to see that there was a gradual increase in the protein and glucose concentrations.

The simulation data fitted well the experimental reactor data, especially for the addition of low concentration of glucose (+10 and +20 gGlucose.L^{-1}). Even for the high glucose concentration values (+35 and +50 gGlucose.L^{-1}), the behavior of the system was well predicted. The difference in the values obtained in the steady-state for the last two concentrations tested can be related to the foaming.

The results presented by the simulation fitted well the experimental data, which is very important for the model taking into account the practical importance of co-digestion of effluent with high protein concentration and the problems of inhibition that can

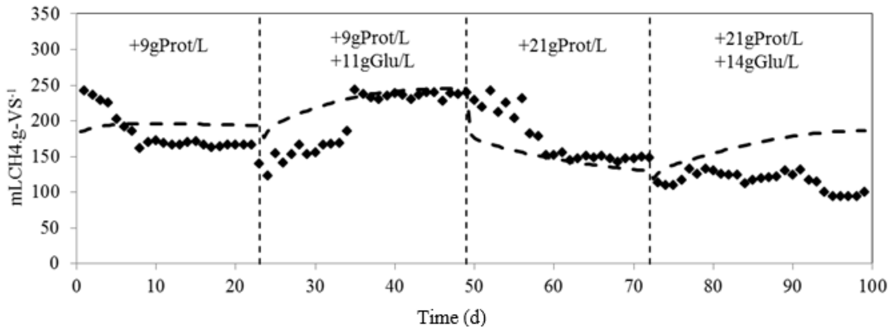

Figure 6: Experimental data (♦) and simulation (- - -) for co-digestion of manure and pure protein and glucose (gradual addition) regarding methane yield.

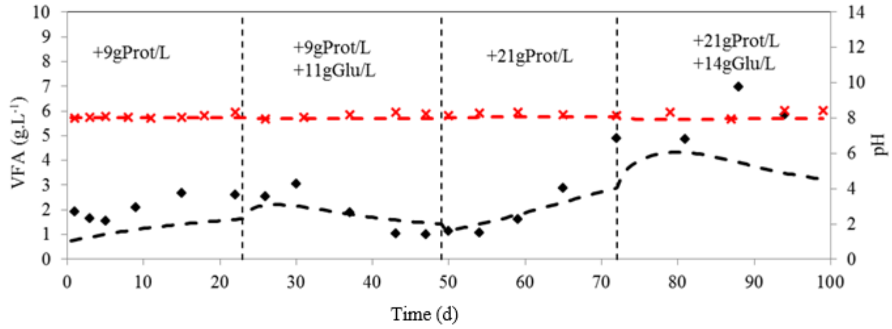

Figure 7: Experimental data (♦ - VFA and X - pH) and simulation (-- - VFA and -- - pH) for co-digestion of manure and pure lipid and glucose (gradual addition) regarding VFA and pH.

occur when using substrates with this characteristic due to the increase in ammonia concentration. This point can be seen for the addition of +21 gProtein.L^{-1} in period 3 of the operation, which ended up having a negative effect on methane production, as can be observed both in the simulation and the experimental results.

According to the model, the addition of glucose in period 4 should decrease this inhibition effect caused by the high concentration of protein; however, this occurred with a lower intensity than in the experimental data. As in the operation with addition of lipids, foaming had no adverse effect on methane production, only seen in the operation with addition of glucose. In periods 2 and 3 the model fitted well the experimental data, even with a significant production of foam in these periods.

Mathematical Model Validation Using Complex Co-Substrates (Sugar Beet)

In order to evaluate the synergy obtained in the previous simulation and validate it, some experimental data were simulated, in which the co-digestion of manure with sugar beet pulp was tested (Fang *et al*., 2011a).

In their study, Fang *et al*. (2011a) investigated the co-digestion of sugar beet pulp and cow manure for biogas production, in order to evaluate the process efficiency and stability. A 4.5 L continuously stirred tank reactor (CSTR) with 3 L working volume was used, and the reactor was operated at a hydraulic retention time of approximately 20 days and temperature of 55 °C. The reactor was fed automatically four times per day. Co-digestion of sugar beet pulp and cow manure without dilution with water was tested at different substrate ratios and organic loading rates to compare the effect of substrate composition on reactor performance.

Figures 8, 9 and 10 show the organic load rate applied to the system, the daily methane yield and the daily methane production, respectively, from the co-digestion of manure and sugar pulp beet for the experiments and the simulation. It is important to focus the comparison between the experimental data and the simulation not between the exact values obtained, but on the trend of the behavior presented by the process. This is because experiments in reactors with real and complex effluents generally present many operational problems, causing the various peaks and declines shown by the experimental data, which are not represented by the model. This fluctuation can be

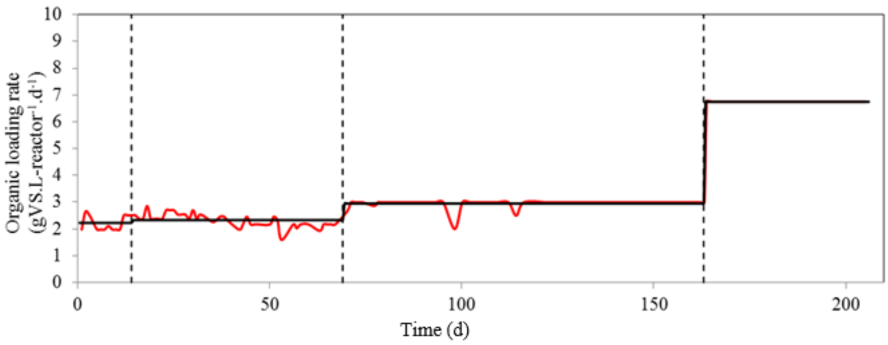

Figure 8: Experimental data (▬) and simulation (▬) for co-digestion of manure and sugar pulp beet – Organic load rate.

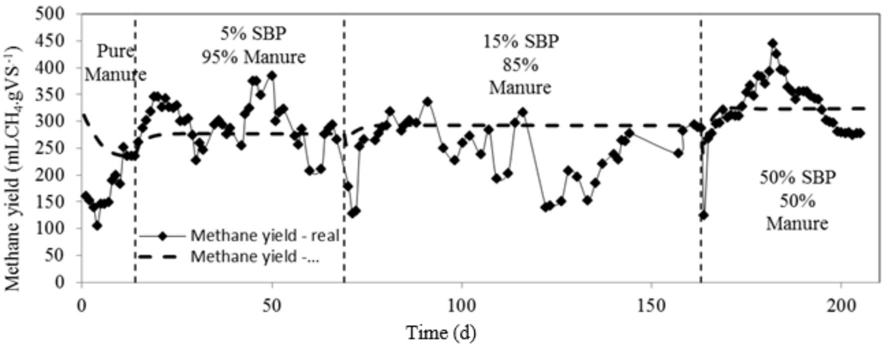

Figure 9: Experimental data (-♦-) and simulation (- - -) for co-digestion of manure and sugar pulp beet – Methane yield.

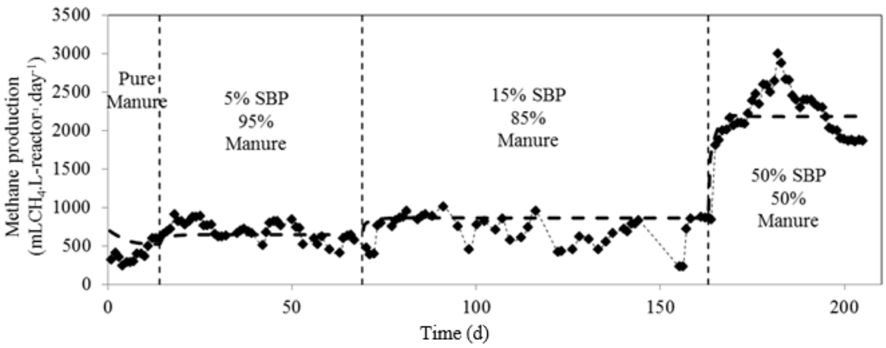

Figure 10: Experimental data (-♦-) and simulation (- - -) for co-digestion of manure and sugar pulp beet – Methane production.

observed, for example, in Figure 8, that shows the organic load rate applied to the system. There were various fluctuations in the experimental values which had a direct effect on methane production.

The addition of sugar beet pulp, especially in the last step of the experiment (50% of sugar beet and 50% of manure), increased substantially the methane production ($mLCH_4.L\text{-}reactor^{-1}.day^{-1}$), which was already expected in view of the high concentration of easily degradable glucose present in the composition of the co-substrate used (139.15 $g.L^{-1}$; Fang *et al.*, 2011a). The methane yield ($mLCH_4.gVS^{-1}$) did not show great changes, as was expected, taking in account the way this parameter is calculated.

CONCLUSIONS

The Biomodel developed by Angelidaki *et al.* (1999), an anaerobic digestion model developed by the Bioenergy Group as an alternative to the DTU model used previously, showed itself to be a great tool to understand and predict the behavior of the co-digestion process with manure for biogas production and wastewater treatment. The simulations with the co-digestion of simple substrates (carbohydrates, lipids and proteins) with manure and also of complex substrates (corn silage, fodder beet, grass and wheat straw) with manure showed synergistic effects for carbohydrate and lipid in the process, with a theoretical increase in the biogas production. For protein, disappointing results were obtained (actually damage in the protein-based process, obtaining a negative "extra" yield). There was a great enhancement in N load due to NH_4^+-N released by the decomposition of protein, with probable inhibition of the process. The next step was to check the reliability of the model by reproducing real results obtained in experiments of co-digestion of simple substrates and manure (Kougias *et al*., 2013) and complex substrates with manure (Fang *et al*., 2011a). The results of the simulations were quite good, proving the applicability of the Biomodel software.

Some points can be highlighted after the studies conducted with simulation and co-digestion: biomass with a high VS content (slurry) is preferable for co-digestion due to the prospect of synergies through reduced leaching losses; carbohydrate-containing biomass seems to be the most promising for achieving synergy; lipid (oil/grease) containing biomass is subject to be suitable with moderate synergy potential (but also due to the generally high VS concentrations); protein-containing biomass can be particularly problematic, especially if the process is already NH_3 stressed; mesophilic systems and systems with low VFA level are best suited to co-digestion of protein-rich biomass; with mixed types of biomass, or a mixture of several different types, it may be important to accompany the net N load effect; the CAB number looks like a useful evaluation parameter for the lack of pH effect; and a good synergy raw material for manure co-digestion is characterized primarily by highly liquid carbohydrate, low protein and low CAB numbers.

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REFERENCES

Angelidaki, I. and Ahring, B. K., Effects of free long-chain fatty acids on thermophilic anaerobic digestion. Applied Microbiology and Biotechnology, 37, 808-812 (1992).

Angelidaki, I. and Ahring, B. K., Thermophilic anaerobic digestion of livestock waste: The effect of ammonia. Applied Microbiology and Biotechnology, 38, 560-564 (1993).

Angelidaki, I. and Ellegaard, L., Codigestion of manure and organic wastes in centralized biogas plants. Status and future trends. Applied Biochemistry and Biotechnology, 109, 95-105 (2003).

Angelidaki, I., Ellegaard, L. and Ahring, B. K., A mathematical model for dynamic simulation of anaerobic digestion of complex substrates: Focusing on ammonia inhibition. Biotechnology and Bioengineering, 42, 159-166 (1993).

Angelidaki, I., Ellegaard, L. and Ahring, B. K., Modelling anaerobic codigestion of manure with olive mill effluent. Water Science Technology, 36, 263-270 (1997).

Angelidaki, I., Ellegaard, L. and Ahring, B. K., A comprehensive model of anaerobic bioconversion of complex substrates to biogas. Biotechnology and Bioengineering, 63, 363-372 (1999).

Atandi, E. and Rahman, S., Prospect of anaerobic co-digestion of dairy manure: A review. Environmental Technology Reviews, 1, 127-135 (2012).

Baserba, M. G., Angelidaki, I. and Karakashev, D., Effect of continuous oleate addition on microbial communities involved in anaerobic digestion process. Bioresource Technology, 106, 74-81 (2012).

Batstone, D. J., Keller, J., Angelidaki, I., Kalyuzhny, S. V., Pavlostathis, S. G., Rozzi, A., Sanders, W. T. M., Siegrist, H. and Vavilin, V. A., Anaerobic Digestion Model No. 1 (ADM1). IWA Publishing, London, UK (2002).

Breure, A. M., Mooijman, K. A. and van Andel, J. G., Protein degradation in anaerobic digestion: Influence of volatile fatty acids and carbohydrates on hydrolysis and acidogenic fermentation of gelatin. Applied Microbiology and Biotechnology, 24, 426-431 (1986).

Ellegaard, L. and Angelidaki, I., Optimering af biogasproduktionen. Forskning i Bioenergi, 30, 12-14 (2009). (In Danish).

Fang, C., Boe, K. and Angelidaki, I., Anaerobic co-digestion of by-products from sugar production with cow manure. Water Research, 45, 3473-3480 (2011a).

Fang, C., Boe, K. and Angelidaki, I., Anaerobic co-digestion of desugared molasses with cow manure; Focusing on sodium and potassium inhibition. Bioresource Technology, 102, 1005-1011 (2011b).

Hanaki, K., Matsuo, T. and Nagase, M., Mechanisms of inhibition caused by long chain fatty acids in anaerobic digestion process. Biotechnology and Bioengineering, 23, 1591-1610 (1981).

Koster, I. W. and Cramer, A., Inhibition of methanogenesis from acetate in granular sludge by long-chain fatty acids. Applied Microbiology and

Biotechnology, 53, 403-409 (1986).
Kougias, P. G., Boe, K. and Angelidaki, I., Effect of organic loading rate and feedstock composition on foaming in manure-based biogas reactors. Bioresource Technology, 144, 1-7 (2013).
Li, X., Li, L., Zheng, M., Fu, G., Lar, J. S., Anaerobic co-digestion of cattle manure with corn stover pretreated by sodium hydroxide for efficient biogas production. Energy Fuels, 24, 4635-4639 (2009).
McCarty, P. L., The methane fermentation. In: Heukelekian, H., Dondero, N. C., Editors. Principles and Applications in Aquatic Microbiology, New York, Wiley (1964).
Mosey, F. E., Mathematical modelling of the anaerobic digestion process: regulatory mechanisms for the formation of short-chain volatile fatty acids from glucose. Water Science and Technology, 15, 209-232 (1983).
Nielsen, H., B. and Angelidaki, I., Codigestion of manure and industrial organic waste at centralized biogas plants: Process imbalances and limitations. Water Science and Technology, 58, 1521-1528 (2008).
Pavlostathis, S. G., Miller, T. L. and Wolin, M. J., Kinetics of insoluble cellulose by continuous cultures of *Ruminococcus albus*. Applied Environmental Microbiology, 54, 2600-2663 (1988).