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Identification of synergistic impacts during anaerobic co-digestion of organic wastes

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KEYWORDS

Anaerobic digestion; Codigestion; Slaughterhouse; Modelling; LCFA inhibition

ABSTRACT

Anaerobic co-digestion has been widely investigated, but there is limited analysis of interaction between substrates. The objective of this work was to assess the role of carbohydrates, protein and lipids in co-digestion behaviour separately, and together. Two sets of batch tests were done, each set consisting of the mono-digestion of three substrates, and the co-digestion of seven mixtures. The first was done with pure substrates -cellulose, casein and olive oil- while in the second slaughterhouse waste -paunch, blood and fat- were used as carbohydrate, protein and lipid sources, respectively. Synergistic effects were mainly improvement of process kinetics without a significant change in biodegradability. Kinetics improvement was linked to the mitigation of inhibitory compounds, particularly fats dilution. The exception was co-digestion of paunch with lipids, which resulted in an improved final yield with model based analysis indicating the presence of paunch improved degradability of the fatty feed.

1 INTRODUCTION

Cattle slaughterhouses process meat for human consumption, animal by-products (e.g. meat, bone and blood meal, tallow and skin) and generate a large variety of solid and liquid waste (Cuetos et al., 2008). The latter represents 5-10% of the total animal weight depending on the degree of further processing of the slaughtered animals, with the majority of waste being cattle paunch, or undigested feed (Jensen et al., 2013). Cattle slaughterhouse waste (SHW), which includes multiple waste streams such as stomach and intestinal content, fat, manure, blood and rendering residues, has emerged as an industrial waste with strong potential to recover energy and nutrient resources through waste management. SHW is considered a good substrate for anaerobic digestion, however, the composition of SHW is highly variable with methane yields ranging between 230 and 700 LCH_4 $kg^{-1}VS$ (Edstrom et al., 2003; Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012). Anaerobic treatment of SHW also includes risks associated with the high concentration of ammonia (NH_3) and/or long chain fatty acids (LCFA), potential inhibitors of the methanogenic activity (Cuetos et al., 2008). Ammonia inhibition is related to its capacity to diffuse into microbial cells and disruption of cellular homeostasis (Kayhanian, 1999), whereas LCFAs adsorb onto the cell membrane, interfering with membrane functionality (Palatsi et al., 2009; Chen et al., 2008). Since ammonia is a by-product of protein acidification and LCFAs are an intermediate product from the degradation of fat, oil and grease, slaughterhouse wastewater as well as other high-value wastes are high-risk, with inhibition being directly linked to the composition. Nevertheless, process instability and inhibition may be minimised through anaerobic co-digestion, which uses the degradation properties of a mixture of wastes to mitigate or dilute specific compounds (Mata-Alvarez et al., 2011).

Anaerobic co-digestion (AcoD) is a process where two or more substrates with complementary characteristics are mixed for combined treatment. AcoD has been reported as a feasible solution to overcome ammonia and LCFA inhibition and to improve the methane yield of SHW digestion. SHW have been successfully co-digested with biowaste (Zhang and Banks, 2012), manure (Hejnfelt and Angelidaki, 2009) and mixture of biowaste and manure (Edstrom et al., 2003; Murto et al., 2004; Alvarez and Liden, 2008; Cuetos et al., 2008). In AcoD, the improvement in methane production is mainly a result of the increase in organic loading rate (Astals et al., 2013); however, when possible, it is important to choose the best co-substrate and blend ration in order to: (i) favour positive interactions, i.e. synergisms, macro- and micro-nutrient equilibrium and moisture balance; (ii) dilute inhibitory or toxic

compounds; (iii) optimise methane production and (iv) enhance digestate stability (Astals et al., 2011; Mata-Alvarez et al., 2011). Even though all these factors should be considered, the decisions on the ratio between wastes had been typically simplified to the optimisation of the carbon-to-nitrogen (C/N) ratio, where optimum reported values vary from 20 to 60 (Alvarez et al., 2010; Mata-Alvarez et al., 2014; Wang et al., 2012). At the present time, there is limited knowledge about how waste composition (carbohydrates, protein and lipids) influences AcoD performance or whether interactions between substrates enhance or attenuate inhibition thresholds, degradation rates, or biogas yields on individual substrates. The degradation of carbohydrates, protein and lipids occur by different metabolic pathways, with varying rates and methane yields (Angelidaki and Sanders, 2004) and therefore knowledge about the influence of the substrate macro-composition would enhance the understanding and utility of potential and/or novel AcoD applications.

Reliable AcoD modelling is required to predict, in a clear and quantifiable manner, the effect of mixing two or more wastes in a digester and remove potentially negative impacts from mixing based on random or heuristic decisions (Astals et al., 2011; Mata-Alvarez et al. 2011). In addition, a better mechanistic understanding of how different feeds mix may reduce the time and costs associated with laboratory experiments as well as improve co-substrate selection and dose rates (Mata-Alvarez et al., 2014). Models are also useful to estimate important biochemical parameters such as biodegradability, hydrolysis rate and inhibition constant, which are critical in AD design, performance and troubleshooting (Batstone et al., 2009; Jensen et al., 2011). Recent nonlinear parameter estimation methods can provide quantitative and rigorous analysis of the impacts of AcoD (Batstone et al., 2003 and 2004).

The aim of the present study was to identify the interactions (synergisms and antagonisms) between carbohydrates, protein and lipids that take place during anaerobic co-digestion, focusing on process kinetics and anaerobic biodegradability of the substrates for a mechanistic model-based understanding of AcoD. This aims at identifying AcoD opportunities and, consequently, improving the anaerobic digestion of slaughterhouse and other similar wastes.

2 MATERIALS AND METHODS

2.1 Chemical analytical methods

Analyses of the total fraction were performed directly on the raw samples. For analyses of the soluble fraction, the samples were centrifuged at 4,000 g for 5 minutes and then the supernatant was filtered through a 0.45 μm PES Millipore® filter. Total solids (TS) and volatile solids (VS) were measured according to standard methods procedure 2540G with minor modifications (APHA, 2005). Specifically, samples were dried overnight, at least 16 hours, in a Clayson OM1000ME oven set at 103 °C and afterwards samples were volatilised in a BTC 9090 muffle furnace (heating ramp from room temperature to 550 °C and held for 3 hours). Total chemical oxygen demand (CODt) and soluble chemical oxygen demand (CODs) were measured using Merck COD Sprectroquant® test, range 500-10000 mg L^{-1}, and by a SQ 118 spectrophotometer (Merck, Germany). Volatile fatty acids (acetic, propionic, butyric and valeric) and ethanol were analysed by an Agilent 7890A gas chromatograph equipped with a Phenomenex ZB-FFAP column (15 m length, 0.53 mm internal diameter and 1.0 μm film) and a flame ionization detector. The chromatograph oven program was as follows: hold 2 min at 60 °C, ramp to 240 °C at 20 °C min^{-1}, and hold 2 min. Injector and detector temperature was set at 220 °C and 300 °C respectively; 12.5 mL min^{-1} of high purity Helium at 8.6 psi was used as carrier gas. Nitrogen and phosphorous ions (NH_4^+, NO_2^-, NO_3^-, PO_4^{3-}), total Kjeldahl nitrogen (TKN) and phosphorous (TKP) were determined by a Lachat Quik-Chem 8000 flow injection analyser using the methods (QuickChem®) developed by the instrument provider (Lachat Instruments, US). Metals ions were determined by an inductively coupled plasma optical emission spectrometer (ICP-OES) Perkin Elmer Optima 7300 DV, which uses 15 L min^{-1} of high purity Argon as plasma gas. Prior to plasma analysis, samples were digested (15 min at 200 °C) with 10% nitric acid in a MARS Xpress microwave. Total and soluble carbohydrates were analysed by the anthrone method using glucose as standard (Smith et al. 1985). Total and soluble protein was determined by the bicinchoninic acid method using bovine serum albumin as standard (Raunkjær et al. 1994). Oil and grease were determined by a Wilks Enterprise, Inc. InfraCal TOG/TPH analyser, where S-316 was used as extraction solvent.

2.2 Biochemical methane potential test

Biochemical methane potential (BMP) tests were carried out according to Angelidaki et al. (2009) in 240 mL glass serum bottles at mesophilic temperature. All tests contained 120 mL

inoculum, the amount of substrate that met an inoculum to substrate ratio (ISR) of 2 (VS-basis) and deionised water, added to make up the total test volume to 160 mL. Bottles were flushed with 99.99% N_2 gas for 1 min (4 L min^{-1}), sealed with a rubber stopper retained with an aluminium crimp seal and stored in temperature-controlled incubators (37 ± 1°C). Tests were mixed by inverting once per day. Blanks containing inoculum and no substrate were used to correct for background methane potential in the inoculum. All tests and blanks were carried out in triplicate, and all error bars indicate 95% confidence in the average of the triplicate. Biogas volume was measured by manometer at the start of each sampling event. Accumulated volumetric gas production was calculated from the pressure increase in the headspace volume (80 mL) and expressed under standard conditions (0 °C, 1 bar). At each sample event, the biogas composition (CH_4, CO_2 and H_2) was determined using a PerkinElmer Autosystem 1022 Plus gas chromatograph equipped with a thermal conductivity detector.

Two sets of BMP tests were done in order to assess the role of carbohydrates (Ch), protein (Pr) and lipids (Li) in AcoD. Each set of tests consisted of the mono-digestion of three substrates, representative of carbohydrates, protein and lipids, and the co-digestion of 7 mixtures, performed in VS-basis (Fig. 1). The first set of BMPs was done with pure substrates, i.e. cellulose, casein and olive oil, whereas in the second set of BMPs complex substrates from an slaughterhouse, i.e. paunch, blood and dissolved air flotation fat sludge (DAF), were used as sources of carbohydrate, protein and lipid, respectively. More details about the performance of the tested mixtures are shown in Table I (pure substrates) and Table II (slaughterhouse waste) at supplementary data.

2.3 Model implementation and data analysis

Mathematical analysis of the BMPs was based on the IWA Anaerobic Digestion Model No. 1 (ADM1). As hydrolysis step is considered the rate-limiting step during the AD of complex substrates, the BMPs were modelled using a first order kinetic (eq. 1) (Jensen et al., 2011).

$$r = \left(0_{t< t_{delay}} , \sum_i \left(f_i \cdot k_{hyd,i} \cdot S_i \cdot C_i \cdot I \right)_{t>t_{delay}} \right) \quad \text{(eq. 1)}$$

where r is the process rate (mL COD L^{-1} day^{-1}), f_i is the substrate biodegradability for substrate i (-), $k_{hyd,i}$ is the first order hydrolysis rate constant of the substrate (day^{-1}), S_i is the substrate concentration (g VS L^{-1}), C_i is the COD-to-VS ratio of the substrate, I is the inhibition factor and t_{delay} is the lag-phase. Biodegradability (f_i) is used for model-based

analysis in order to normalise analysis between substrates. f_i can be converted to methane yield (B_0) using the conversion factors provided at the bottom of Tables V and VI (supplementary data), with material with a COD:VS of 1 having a conversion factor of 350 mL CH_4 g^{-1} VS (B_0/f=350). The inhibition factor was included to model LCFA inhibition when lipids were either mono- or co-digested, where the non-competitive inhibition function was used (eq. 2).

$$I = \frac{K_{I,li}}{S_{li} + K_{I,li}} \quad \text{(eq. 2)}$$

where I is the LCFA inhibition factor, which range from 0 (total inhibition) to 1 (no inhibition), S_{li} is the lipid concentration and $K_{I,li}$ is the inhibition constant (g VS L^{-1}).

The model was implemented in Aquasim 2.1d. Parameters and their uncertainty were simultaneously estimated, with a 95% confidence limit, as for Batstone et al. (2003 and 2009). Parameters uncertainty was estimated based on a two-tailed t-test on parameter standard error around the optimum, and non-linear confidence regions were also tested to confirm the linear estimate was representative of true confidence. The objective function used was the sum of squared errors (χ^2), where average data from triplicate experiments were used as the model target.

2.4 Substrates and inoculum origin

Pure substrate included analytical grade cellulose and casein purchased from Sigma-Aldrich® and white-label refined olive oil, which contains mainly palmitic, oleic and linoleic acid (AOCS, 2013) (see characterisation at Table III of supplementary data). Slaughterhouse wastes, i.e. paunch, blood and DAF sludge, were obtained from a slaughterhouse of Queensland (Australia). Table 1 shows a basic characterisation of the SHW. A complete physical-chemical characterisation of SHW is provided in the supplementary data (Table IV). The CODt of cellulose and olive oil were calculated by multiplying the VS concentration by the theoretical oxygen demand of cellulose (1.07 g COD g^{-1} VS) and oleic acid (2.89 g COD g^{-1} VS), respectively, while the CODt of DAF sludge, which could not be analysed due to analytical interferences, was estimated by multiplying its VS concentration by 3.0 g COD g^{-1} VS. The inoculum, which had a specific methanogenic activity of 0.2 g COD CH_4 g^{-1} VS day^{-1} (37 ºC), was collected from a stable full-scale anaerobic digester that treats mixed sewage sludge at a conventional configuration municipal WWTP in Queensland (Australia). The

inoculum was degasified at 37 °C during 1 week prior starting the assays (Angelidaki et al., 2009); however, no acclimation period to the pure substrates or SHW was performed. Although, parameters such as: origin, concentration, activity, pre-incubation, acclimation and storage, might affect the substrate degradation kinetics and/or inhibition thresholds, the synergism mechanism should remain unchanged, but in a different extent, of the inoculum characteristics (Alvarez et al., 2010).

3 RESULTS AND DISCUSSION

3.1 Biomethane potential tests results

3.1.1 Pure substrates

Methane production of cellulose and casein followed first order process kinetics with B_0 values of 318 ± 5 and 431 ± 6 mL CH_4 g^{-1} VS, respectively; whereas olive oil, with a B_0 of 831 ± 32 mL CH_4 g^{-1} VS, showed a sigmoidal profile (Fig. 2). B_0 values and their uncertainty were outputs of the BMP modelling. Olive oil shape was probably due to LCFA inhibition of the methanogens, although the initial olive oil concentration (4.8 g L^{-1}) was far above the reported half maximal inhibitory concentration (IC_{50}) values for LCFA, which range from 50 to 1500 mg L^{-1} (Palatsi et al., 2009). In addition, the short lag phase (1.5 days) indicated that LCFA adsorption was followed rapidly by conversion through methanogenesis, which is in contrast to the normal longer lag period (> 10 days) corresponding to a strong inhibition of the methanogens (Hwu et al., 1998; Salminen et al., 2000; Palatsi et al., 2009). The shorter lag period can likely be related to the relatively high inoculum-to-lipid ratio used in the present tests (Hwu et al., 1998; Salminen et al., 2000).

To compare the response from pure substrates with those from co-digestion, a simple prediction curve based on the combination of substrates over time and proportioned to the amount of substrate present was generated. Fig. 2 shows the three pure substrates (top left), and predicted and actual curves for each mixture. These demonstrate a clear kinetic advantage caused by mixing substrates, but without any impact on methane yield (net B_0). Kinetic improvement where mixtures present high concentration of olive oil (i.e. 50%Ch - 50%Li; 50%Pr - 50%Li; 33%Ch - 33%Pr - 33%Li and 17%Ch - 17%Pr - 66%Li) was clearly due to attenuation of inhibition. This could be a consequence of both the lower LCFA concentrations in the mixture and the synergy between substrates. It can be established that substrate diversification improved the AD rate and reduced the inhibitory effect of LCFA. The present results are in agreement with Kuang et al. (2002) who concluded that the addition of glucose (carbohydrate) and cysteine (protein), either singly or in combination, decreased LCFA inhibition and improved the formation of granular biomass in high rate anaerobic reactors. Feeding glucose and/or cysteine to an LCFA inhibited digester also stimulates the degradation of LCFA and the growth of methanogenic archaea to enable a rapid recovery of digester performance (Kuang et al. 2006).

3.1.2 Cattle slaughterhouse wastes

As Table 1 shows, paunch, blood and DAF are high in carbohydrates, protein and lipids, respectively. When the SHW mono-digestion BMP results were compared with the results obtained from the pure substrates there was very strong overlap in methane profiles when comparing both the casein and blood tests, and the olive oil and DAF sludge tests (Fig. I at supplementary data). In contrast, paunch, due to its lignocellulosic composition, presented a flattened profile and reduced B_0 compared to cellulose. Paunch, blood and DAF sludge presented B_0 of 237 ± 12, 417 ± 7 and 832 ± 35 mL CH_4 g^{-1} VS, respectively. Again, B_0 values and their uncertainty were obtained through the BMP modelling. When the B_0 values where compared with the values reported by Hejnfelt and Angelidaki (2009) there was a good agreement in the B_0 of blood (450 mL CH_4 g^{-1} VS), whereas the B_0 reported for fat (560 mL CH_4 g^{-1} VS) was much lower than in the present study. Differences in the B_0 of fat be can be related to fat origin and structure. The B_0 of paunch is in the range of those values reported for paunch and lignocellulosic agricultural wastes (Tong et al., 1990; Tritt et al., 1991). DAF sludge showed LCFA inhibition similar to the olive oil test.

All AcoD mixtures between SHW presented an improvement in the digestion kinetics when compared with the theoretical predictions (Fig. 3). The lipid-rich SHW mixtures (50%Ch - 50%Li; 50%Pr - 50%Li; 33%Ch - 33%Pr - 33%Li and 17%Ch - 17%Pr - 66%Li) showed a greater improvement in the process kinetics than that observed for pure substrates, whereas the other mixtures presented a similar trend. In the lipid-rich mixtures, the increase of the slope in the cumulative methane production, related to the greater LCFA methanisation period, was observed at day 4-5 instead of day 7. Therefore, AcoD mitigated LCFA inhibition in the SHW tests similar to the pure substrate tests; where the reduction of LCFA inhibition could be related to the lower LCFA concentration in the mixture and the synergy between substrates. However, the increased mitigation of LCFA inhibition in the SHW tests compared to the pure substrate tests could be due to the adsorption of the LCFA on the surface of the paunch and/or blood, thus lowering the absorption of LCFA on the methanogen cell membrane. Consequently, the LCFA inhibition was further reduced and the methane production stimulated (Palatsi et al., 2009; Cuetos et al., 2010).

Two mixtures (50%Ch - 50%Li; 17%Ch - 17%Pr - 66%Li) resulted in a B_0 significantly higher than the theoretical prediction. The 15% difference between the theoretical B_0 and actual B_0 may be related to the capacity of the hydrolytic biomass present in the paunch to

further hydrolyse the DAF sludge (slurry with small fat conglomerates). This conclusion is supported by a COD balance, as the paunch and blood COD were not enough to justify the difference of 80 and 95 mL CH_4 g^{-1} VS, respectively, between the theoretical and actual B_0. Paunch refers to the stomach contents of cattle and contains rumen micro-organisms consisting of bacteria, protozoa, and fungi, which are highly efficient at hydrolysis of lignocellulosic material. Nevertheless, paunch also contains, in a minor degree, lipolityc biomass which is able to breakdown lipids to fatty acids (Kim et al., 2009). For paunch lipolityc biomass, the degradability of unprotected lipids has been estimated to be about 90%, while the hydrolysis of structural plant lipids is thought to be lower due to the need to remove surrounding cellular matrices (Kim et al., 2009). In any case, the presence of lipid-degrader biomass in the paunch may have improved the degradation rate and extent of DAF sludge in the aforementioned mixtures.

Small improvements in B_0 values were recorded in other AcoD mixtures, however, the difference between the theoretical and actual values were lower than 7%, and were considered not significant. The minor improvement in the process kinetics and B_0 recorded in the mixture between paunch and blood (50%Ch – 50%Pr) is in agreement with the result obtained by Elbeshbishy and Nakhla (2012) when co-digesting a 50% starch (carbohydrates) and 50% bovine serum albumin (protein) mixture (weight-basis). However, the same authors reported that the 80% starch and 20% bovine serum albumin mixture had a significant impact on the process kinetics and B_0 as both were much higher than the expected values (Elbeshbishy and Nakhla, 2012). Finally, it must be noted that the reported methane yields for mixed slaughterhouse are in the range of 400 - 600 mL CH_4 g^{-1} VS (Edstrom et al., 2003; Cuetos et al., 2008; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012). However, as shown by the results obtained in the present study, the methane yield and kinetic are greatly influenced by the SHW composition, with similar impacts and variability expected during full scale implementations.

3.2 Model-based parameter estimation

The kinetic parameters estimated in the present work, either mono- or co-digestion, are substrate biodegradability (f_i), degradation kinetic ($k_{hyd,i}$) and LCFA inhibition ($K_{I,li}$), which quantifies the fraction of material that may be degraded under anaerobic conditions and the speed of degradation. Table V (pure substrates) and Table VI (slaughterhouse wastes) at supplementary data show the model outputs and its 95% confidence interval when the 10 BMPs were simulated with a single set of parameters and when some variables were different for each BMP.

The comparison between the actual and modelled methane curves, when the 10 BMPs were simulated with a single set of parameters, for pure substrates and SHW are shown in Figure II and IV (supplementary data), respectively. The single set of parameters obtained for pure substrates lead to a better fit than the one obtained for SHW. Nonetheless, as a result of the interaction between substrates, a single set of parameters could not be used to reproduce all scenarios. Those results suggest that the interactions between substrates do not only depend on the macro-composition but also on other properties such as substrate structure. Consequently, the comparison between actual and modelled methane curves was done with the parameters obtained when some variables were different for each BMP. After analysing model outputs under several scenarios (data not shown), flexible variables were selected as follows: pure substrates scenario had different $K_{I,li}$ and t_{delay}, while SHW scenarios had different f_{ch}, f_{li}, $K_{I,li}$ and t_{delay}. This approach allowed to better quantification of the key interactions observed.

The high biodegradability for cellulose (90%), casein (81%) and olive oil (85%) are in agreement with the B_0 values obtained (Table V - supplementary data). Moreover, the agreement between the actual and the modelled B_0 for all scenarios confirmed the absence of any antagonism phenomena related to the organic matter intrinsic composition which could reduce substrate biodegradability. Blood (77%) and DAF sludge (82 – 99%) also presented high biodegradabilities in all scenarios while paunch, as lignocellulosic material, showed lower values (59 - 71 %) (Table VI - supplementary data). The high biodegradabilities of the SHW are in agreement with already reported values, which range from 70 to 90 % (Tritt et al., 1991; Hejnfelt and Angelidaki, 2009; Zhang and Banks, 2012; Jensen et al., 2013). Regarding the hydrolysis rate of each substrates ($k_{hyd,ch}$, $k_{hyd,pr}$, $k_{hyd,li}$) in AcoD conditions, model results indicate that they remain constant and similar to the values obtained under

mono-digestion conditions. Therefore, the improvement of the process kinetic is mainly linked to dilution of fats (with $K_{I,li}$ largely remaining static). This assessment can be confirmed by comparing the actual and expected profile of the unique mixture without lipids (50%Ch – 50%Pr) (Fig. 2 and 3) as well as its actual and the modelled profile (Fig. III and V - supplementary data), since the shape between profiles do not present significant differences. $K_{I,li}$ trends across all tests (Fig. 4) indicates a central tendency (~1.3 g VS L^{-1}) and remains reasonably constant independently of the lipid proportion in the digester medium. There is a minor trend for $K_{I,li}$ to increase with increased fats in SHW (i.e. inhibition to relax), and decrease in pure substrates (i.e. inhibition to strengthen), but both of these trends are weak and conflicted by outliers. For the two SHW mixtures that produced more methane than expected (50%Ch - 50%Li; 17%Ch - 17%Pr - 66%Li), it is important to highlight that the model estimated a DAF sludge biodegradability close to 100 %, much higher than when mono-digested, but not a significantly higher paunch biodegradability. This indicates that the presence of carbohydrates/paunch is possibly enhancing the degradability of fats, rather than fats enhancing the degradability of carbohydrates. Additionally, the presence of paunch seems to be important, rather than the amount (e.g. 17% fraction of paunch seems as effective as 66% fraction, with 33% being the outlier). From a technical point of view, process kinetics in the AcoD mixtures are linked to lipid derived inhibition and mitigation of this phenomenon rather than to other substrate properties, this indicates that the maximum sustainable loading rate of lipids to a process is largely determined by the LCFA inhibition constant of the anaerobic community at the operating temperature and not the AcoD mixture composition.

CONCLUSIONS

AcoD lead to an improvement of the AD kinetics. However, the ultimate methane potential is generally not affected. Mixing a carbohydrate and/or protein source to lipids is a feasible option to reduce LCFA inhibition, mainly due to dilution. The main exception to no-increase of degradability is that on the presence of paunch (carbohydrate) appeared to improve degradation of mixed fatty feeds to 100%, resulting in a higher ultimate methane potential.

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REFERENCES

(01) Alvarez, J.A., Otero, L., Lema, J.M. (2010). A methodology for optimising feed composition for anaerobic co-digestion of agro-industrial wastes. Bioresour. Technol. 101, pp. 1153-1158.

(02) Alvarez, R., Lidén, G. (2008). Semi-continuous co-digestion of solid slaughterhouse waste, manure, and fruit and vegetable waste. Renewable Energy 33(4), pp. 726-734.

(03) Angelidaki, I., Sanders, W. (2004). Assessment of the anaerobic biodegradability of macropollutants. Rev. Environ. Sci. Biotechnol. 3(2), pp. 117-129.

(04) Angelidaki, I., Alves, M., Bolzonella, D., Borzacconi, L., Campos, J.L., Guwy, A.J., Kalyuzhnyi, S., Jenicek, P., Van Lier, J.B. (2009). Defining the biomethane potential (BMP) of solid organic wastes and energy crops: a proposed protocol for batch assays. Water Sci. Technol. 59(5), pp. 927-934.

(05) AOCS - American Oil Chemists' Society. (2013). http://lipidlibrary.aocs.org/Lipids/tag1/index.htm. January 2013.

(06) APHA, 2005. 21st edition Standard Methods for the Examination of Water and Wastewater. American Public Health Association, Washington. ISBN 978-0-87553-047-5.

(07) Astals, S., Ariso, M., Galí, A., Mata-Alvarez, J. (2011). Co-digestion of pig manure and glycerine: Experimental and modelling study. J. Environ. Manage. 92(4), pp. 1091-1096.

(08) Astals, S., Nolla-Ardèvol, V., Mata-Alvarez, J. (2013). Thermophilic co-digestion of pig manure and crude glycerol: process performance and digestate stability. J. Biotechnol. 166, pp. 97-104.

(09) Batstone, D.J., Pind, P.F., Angelidaki, I. (2003). Kinetics of thermophilic, anaerobic oxidation of straight and branched chain butyrate and valerate. Biotechnol. Bioeng. 84(2), pp. 195-204.

(10) Batstone, D.J., Torrijos, M., Ruiz, C., Schmidt, J.E. (2004). Use of an anaerobic sequencing batch reactor for parameter estimation in modelling of anaerobic digestion. Water Sci. Technol. 50(10), pp. 295-303.

(11) Batstone, D.J., Tait, S., Starrenburg, D. (2009). Estimation of hydrolysis parameters in full-scale anerobic digesters. Biotechnol. Bioeng. 102(5), pp. 1513-1520.

(12) Chen, Y., Cheng, J.J., Creamer, K.S. (2008). Inhibition of anaerobic digestion process: A review. Bioresour. Technol. 99, pp. 4044-4064.

(13) Cuetos, M.J., Gómez, X., Otero, M., Morán, A. (2008). Anaerobic digestion of solid slaughterhouse waste (SHW) at laboratory scale: Influence of co-digestion with the organic fraction of municipal solid waste (OFMSW). Biochem. Eng. J. 40(1), pp. 99-106.

(14) Cuetos, M.J., Gómez, X., Otero, M., Morán, A. (2010). Anaerobic digestion and co-digestion of slaughterhouse waste (SHW): Influence of heat and pressure pre-treatment in biogas yield. Waste Manag. 30(10), pp. 1780-1789.

(15) Edström, M., Nordberg, Å., Thyselius, L. (2003). Anaerobic treatment of animal byproducts from slaughterhouses at laboratory and pilot scale. Appl. Biochem. Biotechnol.-Part A. 109(1-3), pp. 127-138.

(16) Elbeshbishy, E., Nakhla, G. (2012). Batch anaerobic co-digestion of proteins and carbohydrates. Bioresour. Technol. 116, pp. 170-178.

(17) Hejnfelt, A., Angelidaki, I. (2009). Anaerobic digestion of slaughterhouse by-products. Biomass Bioenergy 33(8), pp. 1046-1054.

(18) Hwu, C.S., Tseng, S.K., Yuan, C.Y., Kulik, Z., Lettinga, G. (1998). Biosorption of long-chain fatty acids in UASB treatment process. Water Res. 32(5), pp. 1571-1579.

(19) Jensen, P.D., Ge, H., Batstone, D.J. (2011). Assessing the role of biochemical methane potential tests in determining anaerobic degradability rate and extent. Water Sci. Technol. 64 (4), pp. 880-886.

(20) Jensen, P.D., Carney, C., Sullivan, T., Batstone, D.J. (2013). Anaerobic digestion as a treatment strategy to enable resource recovery in intensive food production. In: Proceeding of the 13th World Congress on Anaerobic Digestion, Santiago de Compostela, Spain.

(21) Kayhanian, M. (1999). Ammonia inhibition in high-solids biogasification: an overview and practical solutions. Environ. Technol. 20, pp. 355–365.

(22) Kim, E.J., Huws, S.A., Lee, M.R.F., Scollan, N.D. (2009). Dietary transformation of lipid in the rumen microbial ecosystem. Asian-australas. J. Anim. Sci. 22(9), pp. 1341-1350.

(23) Kuang, Y., Lepesteur, M., Pullammanappallil, P., Ho, G.E. (2002). Influence of co-substrates on structure of microbial aggregates in long-chain fatty acid-fed anaerobic digesters. Lett. Appl. Microbiol. 35(3), pp. 190-194.

(24) Kuang, Y., Pullammanappallil, P., Lepesteur, M., Ho, G.E. (2006). Recovery of oleate-inhibited anaerobic digestion by addition of simple substrates. J. Chem. Technol. Biotechnol. 81(6), pp. 1057-1063.

(25) Mata-Alvarez, J., Dosta, J., Macé, S., Astals, S. (2011). Codigestion of solid wastes: A review of its uses and perspectives including modelling. Crit. Rev. Biotechnol. 31(2), pp. 99-111.

(26) Mata-Alvarez, J., Dosta, J., Romero-Güiza, M.S., Fonoll, X., Peces, M., Astals, S. (2014). A critical review on anaerobic co-digestion achievements between 2010 and 2013. Renew. Sust. Ener. Rev. 36, pp. 412-427.

(27) Murto, M., Björnsson, L., Mattiasson, B. (2004). Impact of food industrial waste on anaerobic co-digestion of sewage sludge and pig manure. J. Environ. Manage. 70(2), pp. 101-107.

(28) Palatsi, J., Laureni, M., Andrés, M.V., Flotats, X., Nielsen, H.B., Angelidaki, I. (2009). Strategies for recovering inhibition caused by long chain fatty acids on anaerobic thermophilic biogas reactors. Bioresour. Technol. 100(20), pp. 4588-4596.

(29) Raunkjær, K., Hvitved-Jacobsen, T., Nielsen, P.H. (1994). Measurement of pools of protein, carbohydrate and lipid in domestic wastewater. Water Res. 28(2), pp. 251-262.

(30) Salminen, E., Rintala, J., Lokshina, L.Ya., Vavilin, V.A. (2000). Anaerobic batch degradation of solid poultry slaughterhouse waste. Water Sci. Technol. 41(3), pp. 33-41.

(31) Smith, P.K., Krohn, R.I., Hermanson, G.T., Mallia, A.K., Gartner, F.H., Provenzano, M.D., Fujimoto, E.K., Goeke, N.M., Olson, B.J. and Klenk, D.C. (1985). Measurement of protein using bicinchoninic acid. Anal. Biochem. 150(1), pp. 76-85.

(32) Tong, X., Smith, L.H., McCarty, P.L. (1990). Methane fermentation of selected lignocellulosic materials. Biomass 21(4), pp. 239-255.

(33) Tritt, W.P., Kang, H. (1991). Ultimate biodegradibility and decay rates of cow paunch manure under anaerobic conditions. Bioresour. Technol. 36(2), pp. 161-165.

(34) Wang, X., Yang, G., Feng, Y., Ren, G., Han, X. (2012). Optimizing feeding composition and carbon-nitrogen ratios for improved methane yield during anaerobic co-digestion of dairy, chicken manure and wheat straw. Bioresour. Technol. 120, pp. 78-83.

(35) Zhang, Y., Banks, C.J. (2012). Co-digestion of the mechanically recovered organic fraction of municipal solid waste with slaughterhouse wastes. Biochem. Eng. J. 68, pp. 129-137.

Table 1. Basic characterisation of the slaughterhouse wastes

Parameter	Units	Paunch	Blood	DAF sludge
TS	g kg^{-1}	117	187	360
VS	g kg^{-1}	106	178	353
CODt	g O_2 kg^{-1}	106	266	1053
CODs	g O_2 kg^{-1}	2.5	253	3.7
VFA	g kg^{-1}	0.64	1.86	0.52
Oil and grease	g kg^{-1}	4.5	1.5	265
Total proteins	g kg^{-1}	10.2	129.5	11.8
Soluble proteins	g kg^{-1}	1.7	128.2	0.4
Total carbohydrates	g kg^{-1}	55.5	3.7	0.6
Soluble carbohydrates	g kg^{-1}	1.6	0.1	0.4
TKN	g kg^{-1}	0.60	26.7	1.2
TKP	g kg^{-1}	0.21	0.20	0.29
Ammonium	mg N kg^{-1}	143	391	49
Phosphate	mg P kg^{-1}	161	164	162

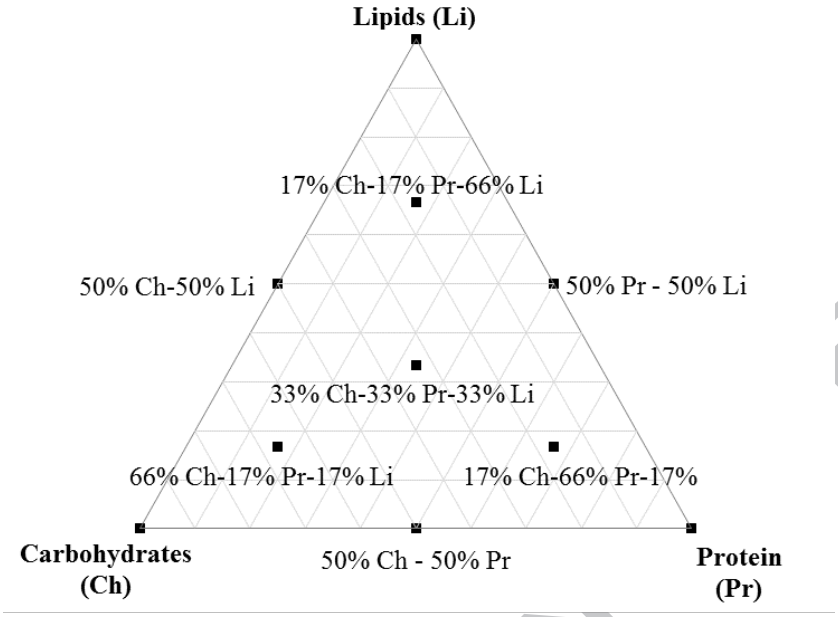

Fig. 1. Design of the co-digestion mixtures, organic mass basis (VS), between carbohydrates, protein and lipids

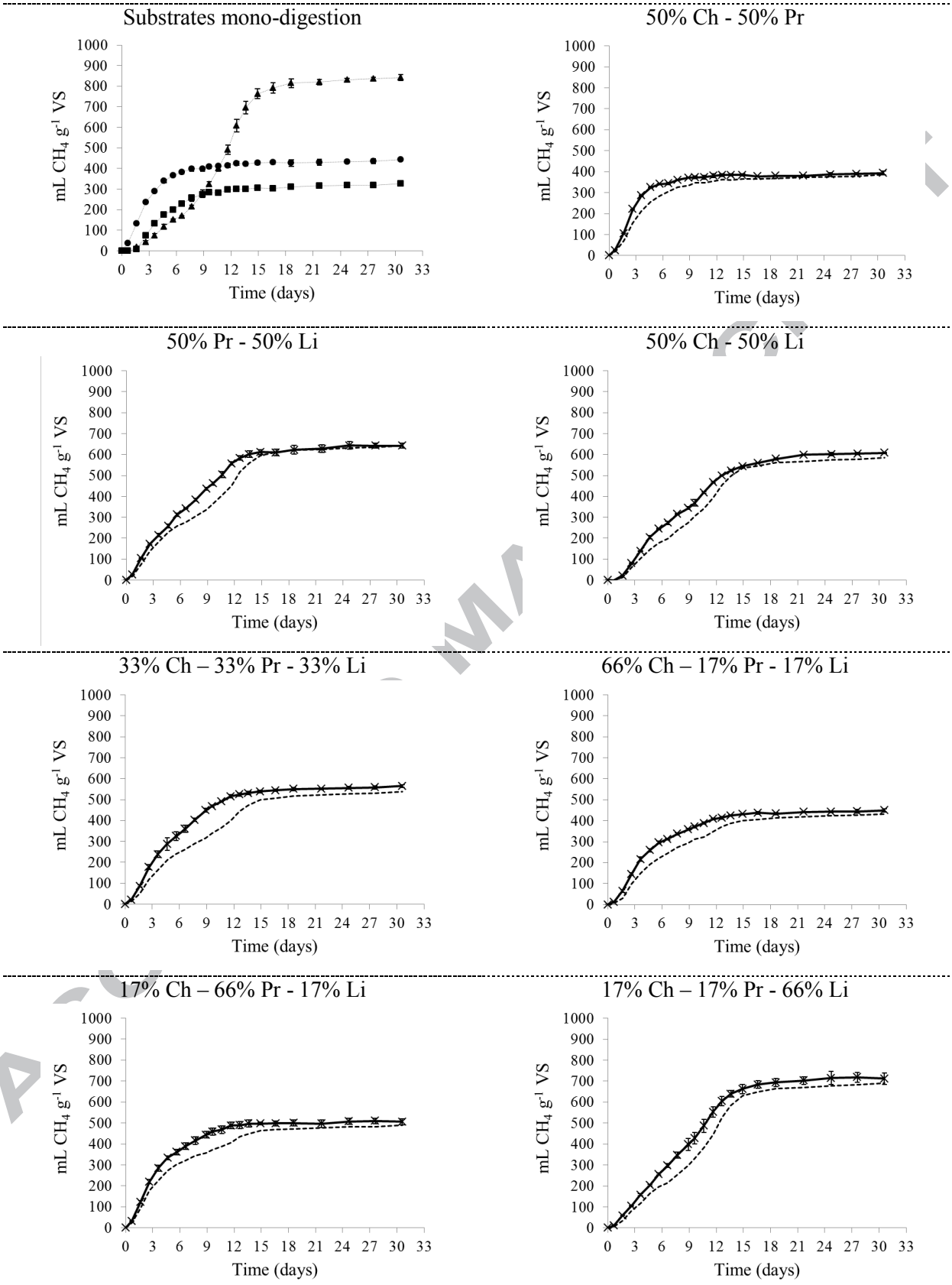

Fig. 2. Cumulative methane production in the course of time of pure substrates mixture (×), theoretical profile of the mixture (dashed line), cellulose (■), casein (●) and olive oil (▲).

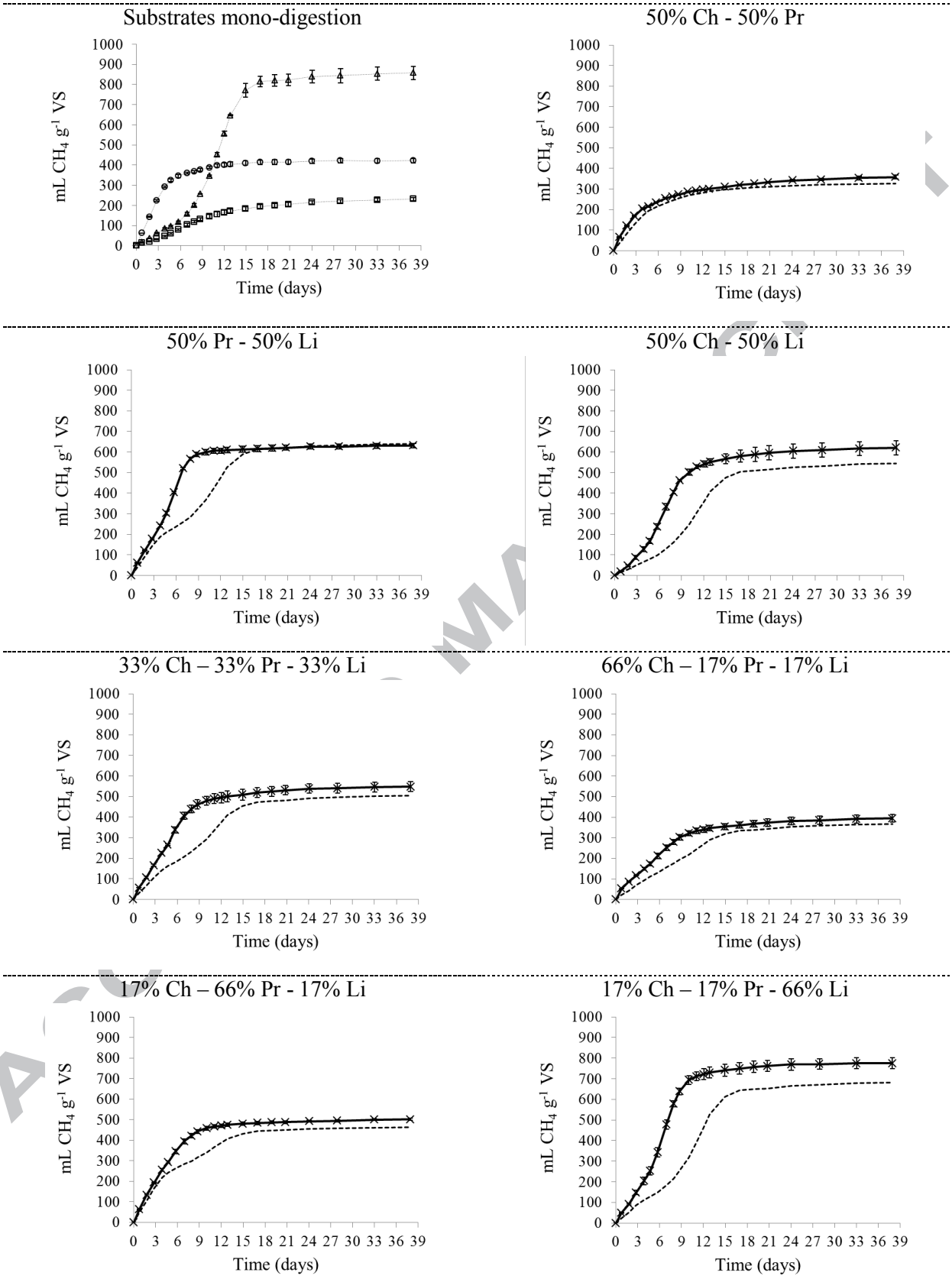

Fig. 3. Cumulative methane production in the course of time of each SHW mixture (×), theoretical profile (dashed line), paunch (□), blood (○) and DAF sludge (Δ).

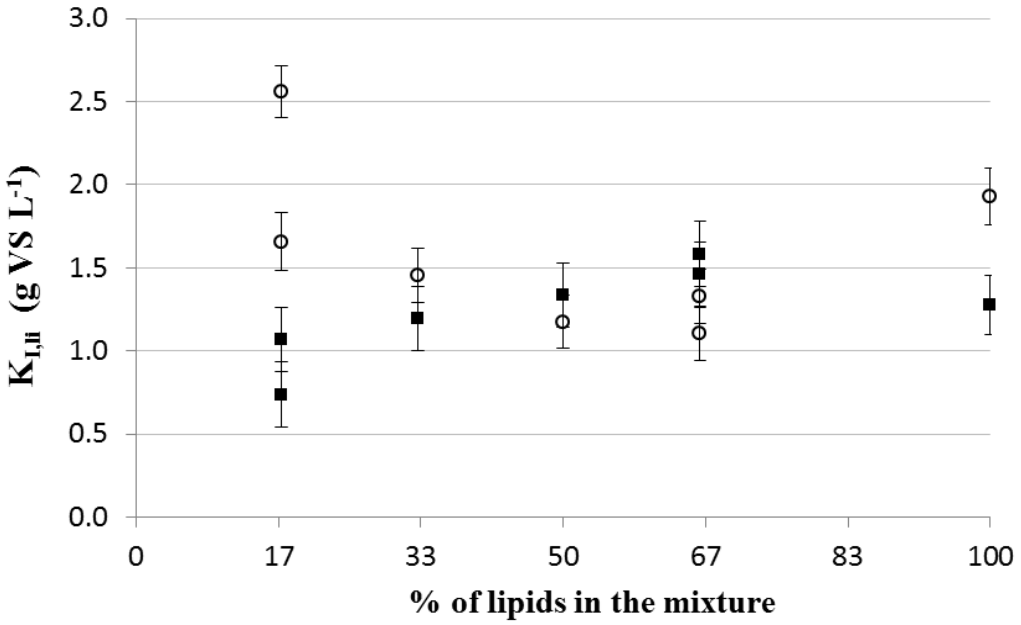

Fig. 4. Modelled lipid inhibition constant as function of the lipid percentage at (○) pure substrates and (■) SHW mono- and co-digestion.

HIGHLIGHTS

- Pure and slaughterhouse carbohydrate, protein, and lipid substrates were tested
- Modelling was used to quantify the impact of mixing substrates
- LCFA inhibition was substantial and detrimental with a K_I of 1.3 g VS L^{-1}
- Co-digestion did not increase ultimate biodegradability
- Co-digestion mitigated LCFA inhibition, mainly through dilution