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Supplementary Information

Paper Title: Anaerobic co-digestion of sludge and organic food waste – performance, inhibition and impact on the microbial community

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AMPTS II

AMPTS II is an analytical device developed for on-line measurements of ultra-low bio-methane flows produced from anaerobic digestion of biologically degradable substrates (Bioprocess Control AB, 2010). The instrument setup can be divided into three units:

Unit A (sample incubation unit): consists of 15 glass bottles each with a volume of 500 ml excluding extra volume for head space (ca 100 ml). The bottles are closed by an aluminum screw thread including a motor allowing for continuous stirring during the trials.

Unit B (CO_2-fixation unit): consists of a multi-point magnetic stirrer, magnet, rubber stopper with two metal tubes and 15 glass bottles of 100 ml for each reactor. Generated biogas in the sample incubation unit has to pass through a separate bottle containing an alkali solution and thus this setup is used to trap CO_2 and H_2S. Each bottle of the CO_2-fixation unit is prepared with 80 ml of alkali solution (3M NaOH) and a pH-indicator (0.4 % Thymolphthalein) to provide enough OH^- ions for fixation of all CO_2 and H_2S produced (Bioprocess Control AB, 2010).

Unit C (gas volume measuring device): the volume of CH_4 gas released from unit B is measured by using a wet gas flow measuring device with a multi-flow cell arrangement (15 cells). The measuring device works according to the principle of liquid displacement and buoyancy enabling monitoring of ultra-low gas flows. Digital pulses are generated when a defined volume of gas flows through the device. An integrated embedded data acquisition system is used to record, display and analyses the results (Bioprocess Control AB, 2010).

The calculation of the methane potential is performed as,

$$MP = \frac{V_{substrate\&inoculum} - V_{inoculum}\frac{m_{inoculum,sample}}{m_{inoculum,blank}}}{\mathrm{m}_{VS,substrate}}$$

where:

MP is the normalized volume of gas produced per gram VS of substrate added;

$V_{substrate\&inoculum}$ is the mean value of the accumulated volume of gas produced from the reactors with both inoculum and substrate;

$V_{inoculum}$ is the mean value of the accumulated volume of gas produced by the three blanks;

$m_{inoculum,sample}$ is the mass of inoculum in the reactors including samples/substrates;

$m_{inoculum,blank}$ is the mass of inoculum in the blank reactors; and

$mVS_{,substrate}$ is the mass of VS of the substrate.

Model Structure

As a first attempt the model structure from Arnell et al. (2016) – originally adapted from Zaher et al. (2009) – was used for this study [1,2]. Figure S.1 illustrates the virtual separation of the hydrolysis reaction from the other processes of the IWA Anaerobic Digestion Model No. 1 [3].

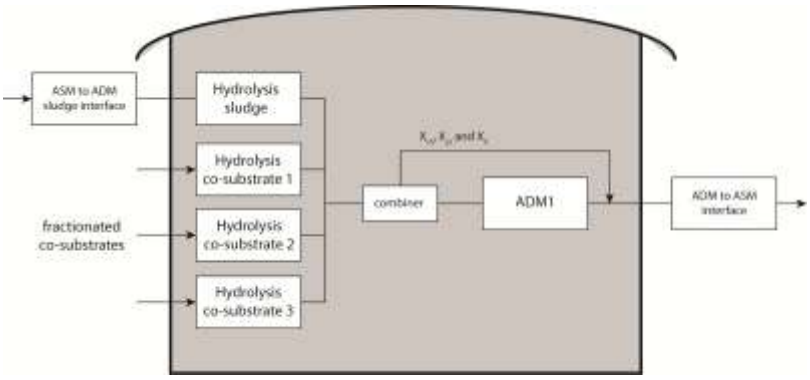

Figure S.1. Model structure initially applied in the study. Figure from (Arnell et al., 2016).

The initial simulations using this model (Figure S.2) indicated problems in the dynamics when increasing the load. The average gas production followed the data well at OLR of 1 g VS/m³ VAD/d and at the end of the experiment with OLR at 2 g VS/m³ VAD/d. However, immediately after the load increase the measured gas production in the experiments increased much more rapidly than the modelled production. It was hypothesized that this was due to the virtual separation of hydrolysis and remaining processes of ADM1, creating a hydraulic delay for the load increase to the subsequent processes of ADM1.

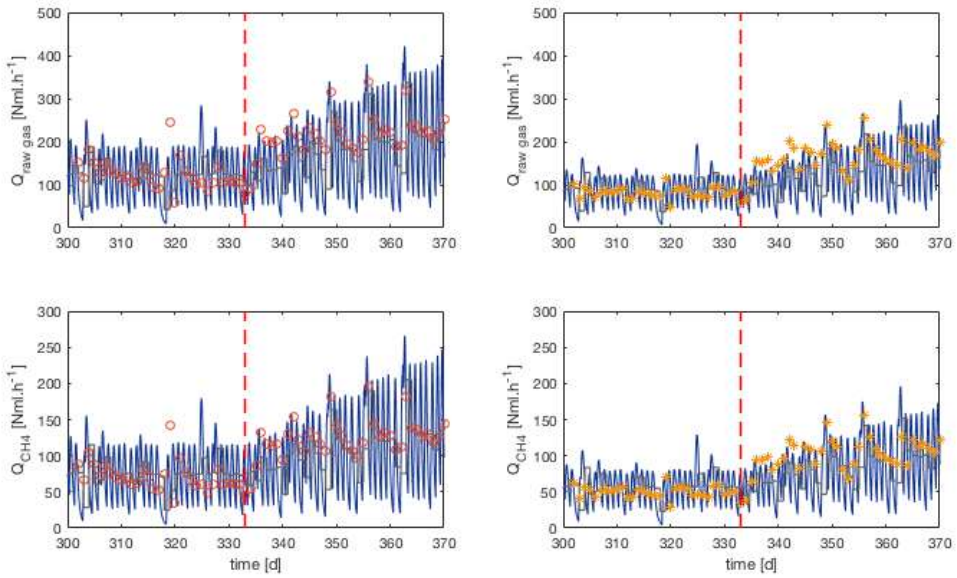

Figure S.2. Gas production for continuous lab-scale reactor R1 (a) and the reference reactor R2 (b). Total gas flow (top) and methane flow (bottom). Markers represent data for daily production, blue lines represent modelled instantaneous production and grey lines modelled daily production using the structure of Zaher *et al.* (2009). The red dashed lines mark the time for load increase.

To test this hypothesis the ADM1 was modified. Three new state variables (X_{ch2}, X_{pr2} and X_{li2}) were introduced for hydrolysis of the secondary substrate (i.e. organic fraction of municipal solid waste, OFMSW). The other state variables of the secondary substrate were assumed to follow the same processes and rates as for the primary substrate (i.e. mixed sewage sludge). For these, a common concentration of the mix was calculated. Three new hydrolysis reactions were introduced for X_{ch2}, Xp_{r2} and X_{li2} with a separate hydrolysis rate. This way the individual characteristics, in terms of composition and degradation kinetics, could be maintained and dynamic simulations featured. The model structure is illustrated in Figure S.3. In this example, the model is limited to two substrates, however, the concept could be multiplied to model an arbitrary number of substrates. One drawback of the concept is that the model code must be up-dated to extend the number of substrates. The simulation results using this model are shown in the main paper.

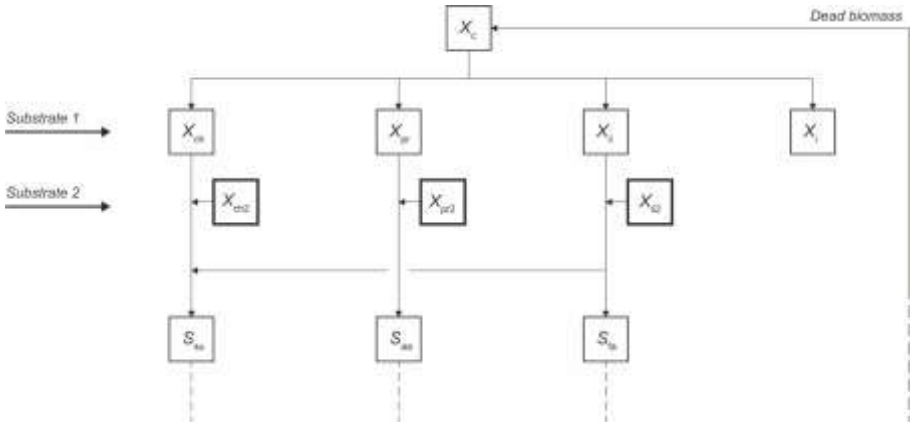

Figure S.3. Model structure for the digester feed and hydrolysis of two substrates.

Measured Data on Substrates

The following physico-chemical analyses were performed for each substrate: dry solids (DS, SS-EN 12880:2000), chemical oxygen demand (COD_{Cr}, ISO 15705:2002(E)), COD filtered (LCK 114), VS (SS-EN 12879:2000), Kjeldahl nitrogen (SS-EN 13342), total nitrogen (TN, LCK 338), lipids (NMKL 131), ammonium (NH4-N, LCK 302) and volatile fatty acids (VFA, LCK 365). Analysis results are tabulated in Table S.1.

Table S.1. Raw data from the physico-chemical analysis on mixed sewage sludge and organic fraction of municipal solid waste (OFMSW).

	Mixed Sludge	**OFMSW**
DS [%]	7.63	18.60
VS [%]	81.0	92.9
Ash [%]	19.0	7.1
NH_4 [mg N/l]	99.8	511.2
COD filt [mg COD/l]	10 305	47 677.5
COD tot [mg COD/l]	57 230	124 160
TN [mg N/l]	3 320	5 184
VFA [mg COD/l]	1 597	10 040

Raw protein (N x 6.25) [% of DS]	21	20
Raw lipids [g/100 g]	1.24	2.98
Raw lipids [% of DS]	17.8	16.6

Microscopy for Population Analysis

Results of microscopic investigations by FISH technique are depicted in Figures S.4 and S.5.

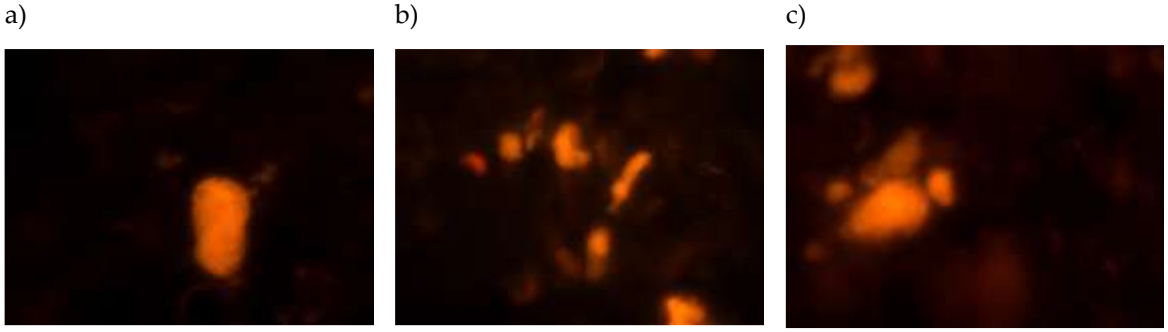

Figure S.4. Increase of Methanosaetaceae (cell clusters containing densely packed rod-shaped cells) from start to OLR 2.0 g VS/m^3 V_{AD}/d, in R1 and R2: a) at start (inoculum), b) in R1 at OLR 1.0 g VS/m^3 V_{AD}/d, c) in R2 at OLR 2.0 g VS/m^3 V_{AD}/d.

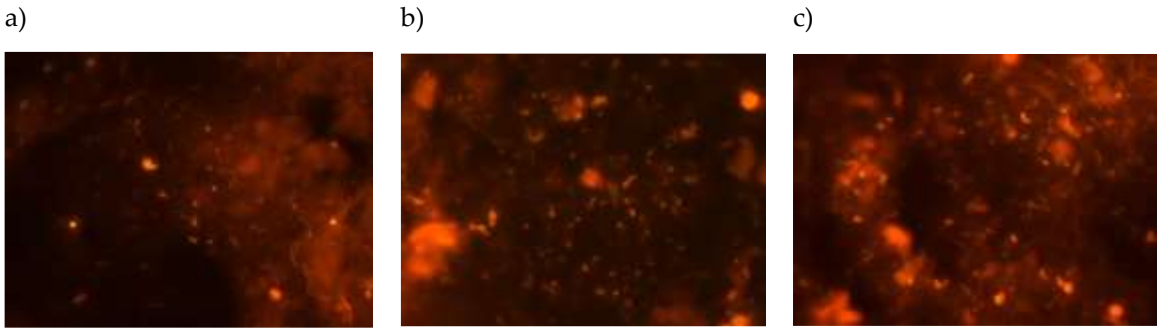

Figure S.5. Increase of Methanomicrobiales (rod-shaped and coccoid cells) from start to OLR 2.0 g VS/m^3 V_{AD}/d, in R1: a) at start (inoculum), b) in R1 at OLR 1.0 g VS/m^3 V_{AD}/d, c) in R1 at OLR 2.0 g VS/m^3 V_{AD}/d.

References

1. Arnell, M.; Astals, S.; Amand, L.; Batstone, D.J.; Jensen, P.D.; Jeppsson, U. Modelling anaerobic co-digestion in Benchmark Simulation Model No. 2: Parameter estimation, substrate characterisation and plant-wide integration. *Water Research* **2016**, *98*, 138-146, DOI: 10.1016/j.watres.2016.03.070.
2. Zaher, U.; Li, R.; Jeppsson, U.; Steyer, J.P.; Chen, S. GISCOD: General Integrated Solid Waste Co-Digestion model. Water Research **2009**, *43(10*), 2717-2727, DOI: 10.1016/j.watres.2009.03.018.
3. Batstone, D.J.; Keller, J.; Angelidaki, R.I.; Kalyuzhnyi, S.V.; Pavlostathis, S.G.; Rozzi, A.; Sanders, W.T.M.; Siegrist, H.; Vavilin, V.A. Anaerobic Digestion Model No. 1. *Report IWA Scientific and Technical Report* **2002**, *No. 13*, IWA Publishing, London, UK.