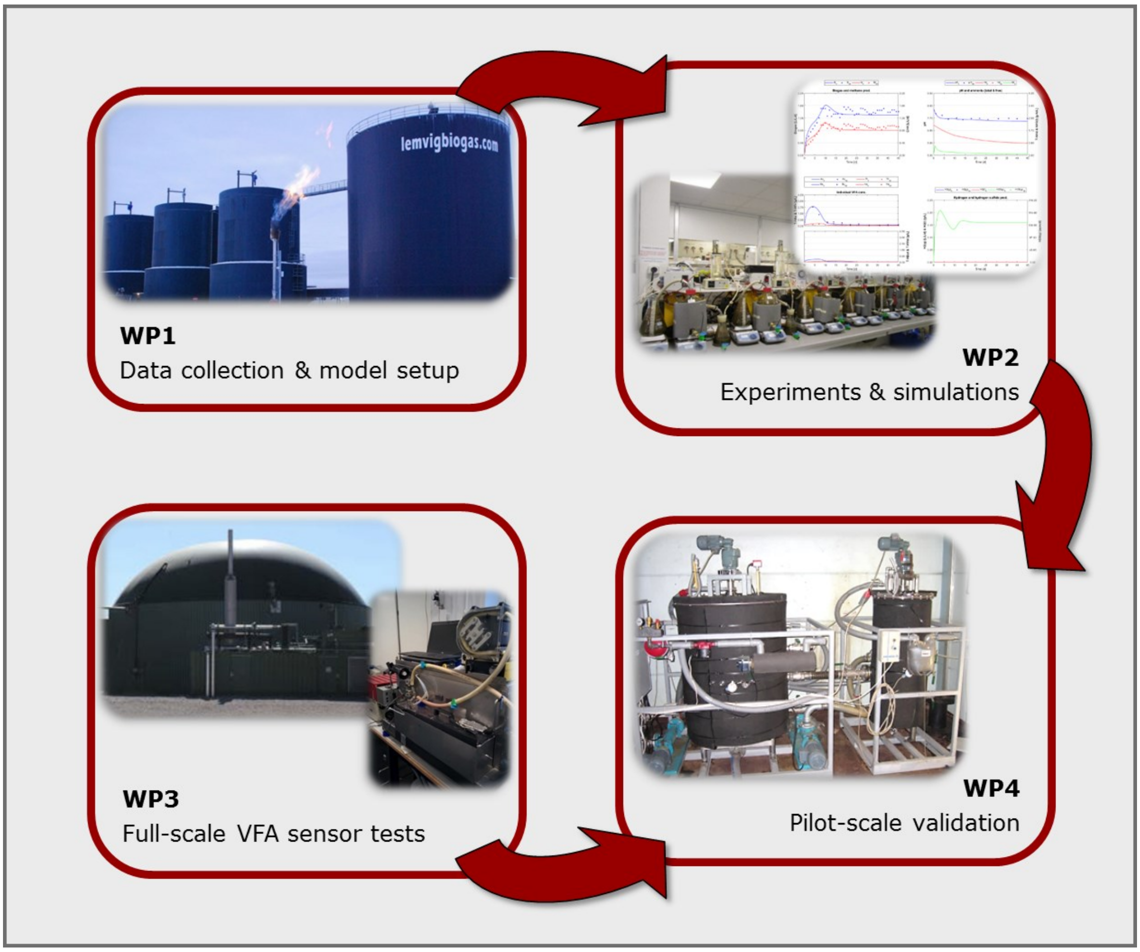
Improving synergy of co-digestion process



Final report prepared in fulfilment of the Energinet.dk project no. 12197

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Nomenclature

AD	- Anaerobic digestion
AcoD	- Anaerobic co-digestion
Biopulp	- Source-separated municipal solid waste
BMP	- Biochemical methane potential
CA	- Conference abstracts
CSTR	- Continuously stirred tank reactor(s)
GC	- Gas chromatography
GTO	- Glycerol trioleate
HRT	- Hydraulic retention time
LCFA	- Long-chain fatty acid(s)
LHS	- Latin Hypercube Sampling
M	- Milestone(s)
MN	- Cow manure
NH4-N	- Total ammonium nitrogen
OLR	- Organic loading rate
OP	- Orange peel
PRCC	- Partial Rank Correlation Coefficient
RP	- Reporting period(s)
SP	- Scientific publication(s)
SS	- Steady-state
SW	- Cast seaweed
TKN	- Total Kjeldahl nitrogen
TS	- Total solid(s)
VFA	- Volatile fatty acid(s)
VS	- Volatile solid(s)
WP	- Work package(s)

Final report

1.1 Project details

Project title	Improving synergy of co-digestion process Forbedring synergi af samudrådningproces
Project identification (program abbrev. and file)	Energinet.dk project no. 12197
Name of the programme which has funded the project	ForskEL
Project managing institution (name and address)	Technical University of Denmark, Department of Environmental Engineering, Miljøvej, Building 113, DK-2800 Kgs. Lyngby. Telephone: +45 45251429, Email: iria@env.dtu.dk
Project partners	Lemvig Biogasanlæg Amba, LINKOGAS A.M.B.A
CVR (central business register)	30 06 09 46
Date for submission	2018.12.10

1.2 Short description of project objective and results

English:
The main objective of this project was to establish the optimal operation criteria for achieving high efficiency and stability in manure co-digestion processes, through the optimization of substrate mixing, feeding strategies and operation strategies. Continuous reactor experiments, online VFA monitoring and pilot-scale validation, together with mathematical modeling were performed in a systematic way to fulfill the project goals. The main outcome of the project was a set of strategy guidelines for operation, like the use of biopulp in monodigestion, which can be applied practically in centralized biogas plants for optimizing the manure co-digestion process. Additional outcomes were a comprehensive mathematical model to perform co-digestion simulations and an online VFA sensor.

Dansk:
Hovedformålet med dette projekt var at etablere de optimale driftskriterier for opnåelse af høj effektivitet og stabilitet i samudrådningsprocesser af gylle gennem optimering af substratblanding, fodringsstrategier og driftsstrategier. Kontinuerlige reaktoreksperimenter, online VFA-overvågning og pilotskala validering, sammen med matematisk modellering blev udført på en systematisk måde, for at opfylde projektmålene. Projektets hovedresultat var et sæt strategibestemmelser for drift,

som brugen af biopulp ved monodigestion, som kan anvendes praktisk taget i centraliserede biogasanlæg til optimering af samudrådningsprocesser af gylle. Yderligere resultater var en omfattende matematisk model til at udføre samudrådningssimuleringer, og en online VFA sensor.

1.3 Executive summary

Present report provides a summary of the major results and conclusions obtained during the timeframe of the project for the anaerobic co-digestion (AcoD) of manures with various organic substrates. In general, it was found that the long-term process stability, methane yield and productivity of the different co-digestion scenarios largely depends on the substrate characteristics and the co-digestion ratio fed to the continuously operated reactors. Furthermore, an important aspect of the findings was that higher methane potentials are not always beneficial from a process operation point of view, for there exists a delicate balance between the amount of high-potential organic co-substrates added to the reactors and the buffer capacity of the manure substrate. More specifically, this buffering capacity proved to be essential for leveling unwanted process dynamics, such as volatile fatty acid (VFA) accumulation, pH fluctuation and the increase of free ammonia or hydrogen sulfide in the reactors' liquid phase, to list the most crucial ones. Furthermore, a comprehensive mathematical modeling framework was developed and used as an optimization tool for different co-digestion processes. Through simulations generated with the model, biogas plant operators would be able to find the optimal co-digestion scenarios easier, while reducing the number of necessary analyses and their costs significantly.

The project covered various aspects of the research process concerning anaerobic co-digestion with manures: from data collection, substrate characterization and mathematical process modeling, through running laboratory experiments, monitoring digestion performance and simulating experimental results, to scaling the optimal co-digestion scenario from laboratory to pilot-scale. Work package 1 (**WP1**) covered data collection and substrate characterization, which was continued throughout the project, as new experiments were carried out, new samples were received from full-scale reactor operations, or new information became available about processes reported in literature. Moreover, the design of a mathematical bioconversion model (BioModel) was also extended under WP1, in order to make it suitable for the simulation of diverse co-digestion scenarios.

Building on the information from WP1, the simulation and validation of various co-digestion scenarios under **WP2** was carried out as part of the project realization, by collecting detailed process information from a large-scale plant and laboratory co-digestion experiments, and developing an anaerobic bioconversion model. Experimental information was collected continuously and stored in an internal database, providing the basis for model optimization and the standardization of simulation steps. Through creating so-called digital twins of each co-digestion case studied and describing the model substrates in a uniform manner, running the various scenario simulations was improved, the results became comparable and valuable conclusions could be made about all case studies.

Regarding the online VFA sensor that was tested and installed at the Granja San Ramón biogas plant according to **WP3** of the project, it was concluded that the tool generates reliable results and can provide valuable information about different process states. By applying the novel sensor to full-scale reactor monitoring, and comparing the measured VFA concentrations with those determined using gas chromatography (GC), it was found that the agreement between the two datasets is of high quality, making the VFA sensor an ideal alternative to laboratory GC analyses. Given that changes in the concentration of VFA in anaerobic digestion (AD) are sensitive to environmental changes, and these changes reflect the state of the microbial community involved, the online VFA sensor can provide a reliable early warning system in case of process imbalances.

In the final step of the project (**WP4**), the knowledge generated during the experimental and modeling tasks was applied to setting up a pilot-scale AD unit, using biopulp as substrate and operated at mesophilic conditions. Although previous experiments involved the co-digestion of substrates with manures, one major finding of the project was that biopulp in itself is suitable for anaerobic treatment and has a significantly higher methane potential (490 mL/gVS) than other co-digestion scenarios tested. Furthermore, by using biopulp in monodigestion, sourcing of the necessary substrate became feasible even in an urban environment, where the availability of manures is limited. This opened potentially new ways for the treatment of organic wastes using AD technologies, allowing for the transfer of the concept from well-established rural environments to novel, less explored urban settings.

1.4 Project objectives

1.4.1 Project organization and implementation

The project covered a period of three and a half years, from 1st of July in 2014 to 30th of June in 2018. In order to realize the overarching aim of the project, namely to establish the optimal operation criteria for achieving high efficiency and stability in manure co-digestion processes, the following individual project objectives were identified:

- Setup an existing advanced bioconversion model for the simulation of co-digestion situations;
- Use the extended model to investigate the optimal operation scenarios for various manure co-digestion processes and create a database for storing the characteristics of the main substrates digested by Danish biogas plants;
- Validate the co-digestion model simulation results under different operation scenarios, using the data from lab-scale continuous reactor experiments and full-scale biogas plants;
- Test the suggested operation strategy in a pilot-scale biogas reactor and evaluate the synergistic effect in larger scale operation compared with modeling results and lab-scale experiments;
- Demonstrate the applicability of a VFA sensor at a full-scale biogas plant and utilize the sensor for process optimization in pilot-scale operation.

These individual objectives were then divided into four thematic WP and covered different operative aspects of the project, such as:

- WP1. collecting reactor operation data, and characterizing substrates and reactor samples from biogas plants to setup the co-digestion model;
- WP2. running laboratory-scale reactor co-digestion experiments, modeling and simulating the various scenarios;
- WP3. preparing and applying a VFA sensor for the online monitoring of a full-scale biogas plant;
- WP4. testing the optimal operation strategies for improving co-digestion efficiency in a pilot-scale biogas reactor.

Finally, in order to ensure the timely realization of the work outlined in the WP, five independent project milestones (M) were defined. These were tightly connected to the content of the WP and were spread out throughout the whole project period. The M respectively were:

- M1. Model setup and validation from full-scale data (due December 2014);
- M2. Simulation results of different co-digestion scenarios (due May 2015);
- M3. Validation results of VFA sensor for monitoring full-scale biogas reactor (due February 2016);
- M4. Establish co-digestion strategy to be tested in pilot biogas reactor (due November 2017);
- M5. Validation and suggestion of co-digestion strategy for full-scale biogas plant applications (due June 2018).

As a result of executing the project work packages, several valuable outputs have been generated. Firstly and most importantly, operation strategy guidelines were created, for optimizing AD processes and to be practically applied by full-scale biogas plants. These involved

- the use of tested organic by-products in co-digestion with manure, increasing methane yields, improving the carbon to nitrogen ratio and ensuring process stability;
- the use of biopulp as a single substrate in AD or its co-digestion with manure (if available), for increasing methane yields in urban biogas plants;
- the continuous monitoring of VFA concentration as the most critical process variable indicating imbalances;
- the consideration and simulation of microbial growth lag during the initial (dynamic) phase of starting up AD processes, helping the more accurate estimation of the expected biogas output.

Furthermore, the comprehensive bioconversion model – now extended for the simulation of co-digestion scenarios –, along with the substrate database characterizing typical co-substrates applied in Danish biogas plants, were both made available for further research and development. Last but not least, the full-scale applicability of the online VFA sensor and the efficiency of the pilot-scale reactor experiment were demonstrated for the first time, marking a significant step in the development and commercialization of the project results.

1.4.2 Risks

Two major risks were associated with the project execution. The first one considered the external partners of the project, more specifically their availability for collaboration and potential abstention from participating in the project. Meanwhile, the second risk was related to the pilot-scale reactor experiment, and concerned the possibility of incurring delays or complications prior to or during the operation of the setup.

1.4.3 Project development

In general, the project followed the targeted milestones well and the flow of project delivery agreed with the work outlined in the WP. However, there were three minor changes to the initial project outline, which had to be considered.

Firstly, during the third reporting period (July to December 2015), it was acknowledged that opposite initial plans, Linkogas would not be able to participate in the project. Therefore, the plan was slightly changed, and instead of the collection of process data from two full-scale plants, it was agreed that only Lemvig biogas plant would provide such data. Meanwhile, the missing process information was collected from additional pilot- and laboratory-scale experiments carried out at DTU, along with experimental data reported in the scientific literature.

The last modification to the original plans was the extension of the overall project period. This was deemed necessary due to a change in project personnel, the inability of a project partner to deliver full-scale process data and consequently additional time needed to test the model with supplementary process data. The extension was limited to 12 months, and the project ended successfully on the 31st of July 2018.

1.4.4 Challenges

The challenges faced during project delivery were related to the timely management of the project tasks, given the subsequent abstention of Linkogas and the complications with the pilot-scale reactor setup. These challenges, however, were considered during the risk assessment of the project (see section 1.4.2), therefore no unexpected challenges can be listed.

1.5 Project results and dissemination of results

1.5.1 WP1

WP1 involved the collection of reactor operation data, the characterization of substrate and reactor samples from biogas plants and the setup of the bioconversion model, used later in WP2 to simulate different co-digestion scenarios. In the beginning of the project, the biogas plant partners involved in the project were contacted, in order to discuss the collection of process information and the reactor and substrate samples outlined in the project plan. These information and samples would later be collected periodically during the whole project period, providing the input data subsequent model validation and long-term, full-scale application.

Moreover, technical results such as the initial model formulation and preliminary simulation results were also achieved during reporting period 1 (**RP1**), the latter based on substrate characteristic and operation data of manure co-digestion processes from earlier lab-scale experiments (see the first scientific publication or SP1). Using the initial model (referred to as BioModel), simulations could predict biogas production, pH and VFA concentrations well in most cases. However, at times when biogas reactors were overloaded, the predicted biogas production was higher than the measured values. This indicated that the model was less sensitive to disturbances or overload, compared to a physical setup. Therefore, optimization of the model inhibition parameters and continuous model validation were planned for later periods, parallel with lab-scale reactor experiments. Nevertheless, during this period the requirements for the first milestone (**M1**) of the project were fulfilled.

In **RP2**, an intensive sample collection from Lemvig biogas plant was carried out, during the period from May-July 2015. The samples originated from both manure and industrial waste storage thanks, and all reactor samples were collected two times per week, for characterization and subsequent analyses at DTU Environment. Such characterized substrate and reactor data would then be used as model input, for the simulation of the full-scale biogas reactor operation. Furthermore, these would also serve as reference data during the validation of model predictions. Meanwhile, as the other biogas plant partner (Linkogas) was in the process of installing new instruments during this period, sample collection from this plant was moved to August 2015.

Later in 2015 (**RP3**), another set of samples were received from Lemvig biogas plant. Sampling was performed once a week for a period of sixteen consecutive weeks. Substrate samples consisted of 1) raw slurry (a mixture of cattle, pig and mink manure) and 2) mixed industrial waste streams from companies working in partnership with Lemvig plant. In addition to the above, samples from Lemvig's thermophilic reactors were also received, along with various process data covering the sampling period. Substrate and reactor samples were then fully characterized. Results of the characterization were combined with information about full-scale operational state variables and were used as input for the BioModel. Finally, the output of the BioModel was validated by comparison to the full-scale process data retrieved from the Lemvig plant. As Linkogas was unable to provide samples and process information until this time, their involvement in the project was postponed indefinitely.

In late 2016 (**RP5**), it was confirmed that the Linkogas biogas plant was unable to deliver the agreed reactor operation data and samples, due to construction works taking place at the plant. From a project perspective, this meant that additional laboratory experiments and literature data collection had to be performed, so that the supplementary input data for model validation would be supplied.

Concerning technical results, the work realized in this period was disseminated in two scientific articles (SP4 and SP5) published in this period. Data collected from Lemvig biogas plant and analyzed previously were used for running full-scale simulations in the BioModel, the results of which can be seen in Figure 1, in terms of biogas, methane and carbon dioxide productivities and as compared to the actual values measured by the plant.

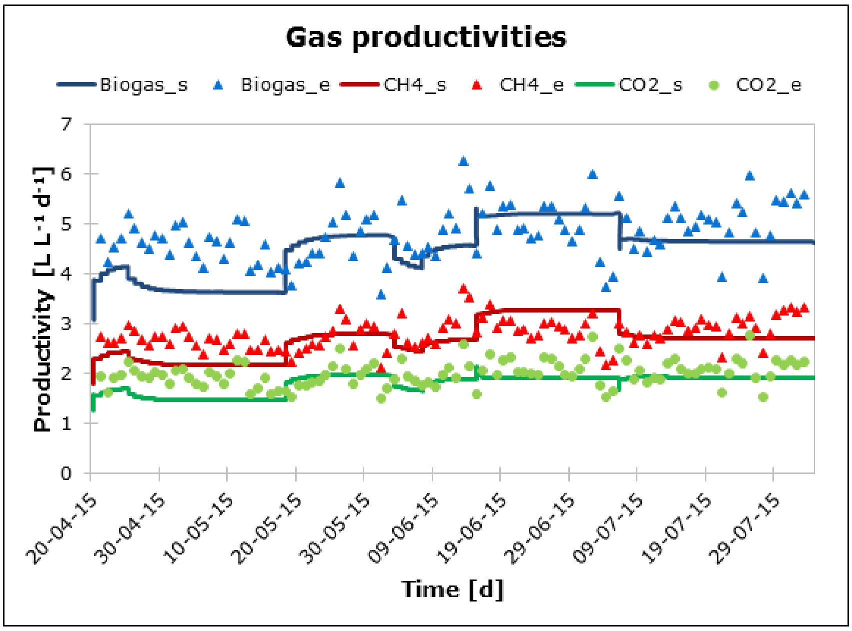

Figure 1: Simulated and measured biogas, methane and carbon dioxide productivities for Lemvig Biogas Plant's primary anaerobic digesters (2400 m^3). *_s* indicates simulated data, while *_e* stands for experimental results.

During the period between January and June 2017 (**RP6**), two aspects of the BioModel had been improved, using data from laboratory experiments carried out at DTU and from the scientific literature. On one hand, experimental data from an in situ biogas upgrading process and other experiments reported in the literature were used to implement hydrogen flow dependency in the BioModel. On the other hand, a simplified version of the BioModel had been created and calibrated with data from a laboratory experiment, in order to account for the microbial growth lag that was conceptualized earlier (see section 1.5.2).

With regards to technical results, the achievements of this period were disseminated in a scientific article that was published in this period (SP7) and two scientific abstracts that were submitted for a conference (CA1 and CA2). As reported in SP7, data collected from various laboratory experiments were used to extend the BioModel to simulate anaerobic co-digestion scenarios with in-situ biogas upgrading. During the same period, a simplified version of the BioModel was developed and extended with microbial growth lag calculation, in order to be applied for the simulation of another laboratory experiment carried out at DTU (see Figure 2).

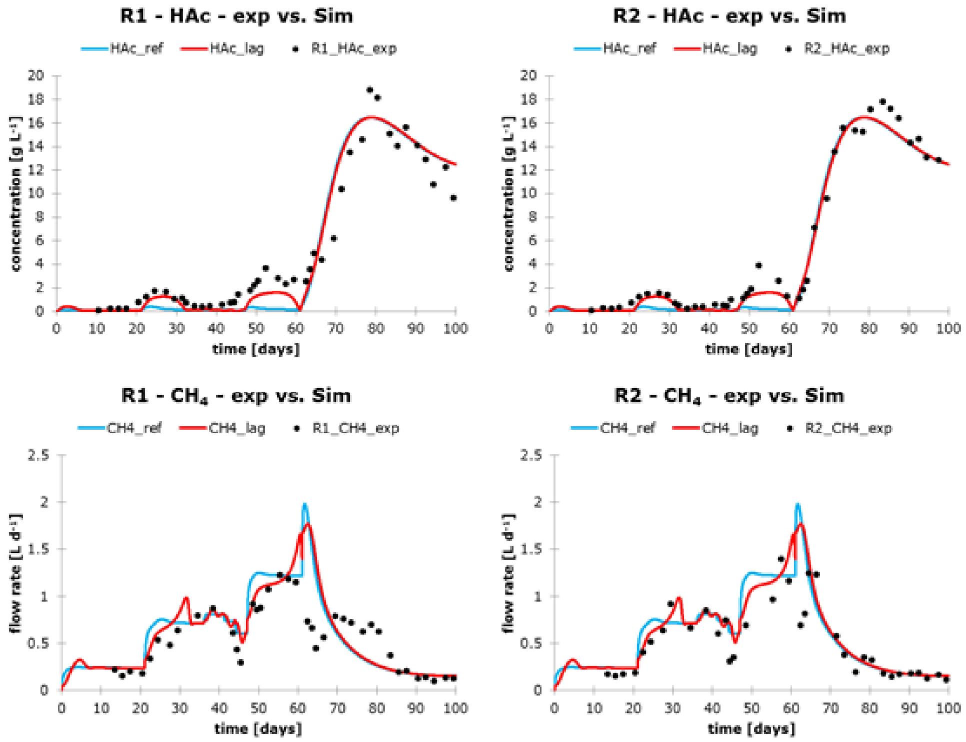

Figure 2: Simulated acetic acid concentrations (*HAc*) and methane flow rates (*CH4*) without and with microbial growth lag (*ref* and *lag*) from the simplified and extended BioModel, compared to the corresponding experimental data (*HAc_exp* and *CH4_exp*) from the two reactors (*R1* and *R2*) of the glucose experiment

During the two reporting periods of 2017 (**RP6** and **RP7**), the collection of reactor operation data focused on two experiments. On one hand, process and substrate characterization data were gathered from a laboratory experiment, where the monodigestion of manure and its co-digestion with meadow grass were investigated. Meanwhile, the other experiment involved the co-digestion of manure and biopulp, which was later published in a scientific journal (see additional references 2 or AR2). Although the article covered both batch and continuous tests, data was only collected from the continuous experiment.

Finally, 2018 marked the beginning of the pilot-scale experiment, during which period (**RP8**) data was collected from the pilot-scale reactor. For more details on the experiment, the reader is referred to section 1.5.4. With regards to dissemination, three scientific abstracts were submitted for a conference in this period (CA3, CA4 and CA5), the content of which was developed in RP6 and RP7.

1.5.2 WP2

Work on WP2 started in 2015 (**RP2**), building on data collected and generated during tasks covered in WP1 and with the aim to run and simulate lab-scale reactor experiments of different co-digestion scenarios. During this initial period, the BioModel was used to simulate the co-digestion of manure with different co-substrate combinations, at various organic loading rates, in order to investigate the effect of co-substrate composition and the organic loading rate on the performance of biogas reactors. The co-digestion process was simulated in 3 scenarios, and scenario 3 (manure co-digested with gelatin alone, compared to the co-digestion of gelatin with another co-substrate) was also tested in a lab-scale reactor experiment (Figure 3).

Figure 3: Experimental setup of lab-scale CSTRs

Technical results of this period were collected in the following points:

- Results from the simulation of co-digestion scenario 1 showed that increasing concentrations of glucose, glycerol trioleate (GTO) and wheat straw in the feed could improve methane yields significantly, while the addition of gelatin was not favorable.
- From the simulation results of co-digestion scenario 2, the methane production and yield from different substrate mixtures was strongly dependent on the type of co-substrates.
- According to the co-digestion simulations of scenario 3, methane production in an ammonia inhibited reactor was best improved by adding GTO, followed by glucose and wheat straw, respectively, while the effect of long-chain fatty acid (LCFA) inhibition was not critical.
- Methane production from different co-digestions followed the same trend as in the simulation results.

It was also in this period that the second milestone (**M2**) was fulfilled, although subsequent simulations of different co-digestion scenarios were also generated. Already during the period of July to December 2015 (**RP3**), six agricultural organic by-products were tested, to identify the best co-digestion substrate that can be combined with manure for optimal biogas production in a CSTR (SP2). Regarding the above, the following could be summarized:

- Meadow grass had the highest BMP value (388 ± 30 NmL g-VS-1) among all substrates tested.
- On the basis of BMP, the substrates ranked as follows: meadow grass > spring barley, winter wheat, winter barley, ryegrass > rapeseed > manure.

- Co-digestion of manure with agricultural by-products resulted in only additive and not synergistic methane production.
- Continuous co-digestion of raw meadow grass with manure increased the methane production rate of CSTR reactor by 114% compared to the manure alone.
- The response of the model was compared to experimental data from the reactor fed with increasing meadow grass addition (see Figure 4), and the simulations were qualitatively in good agreement with the measured values.

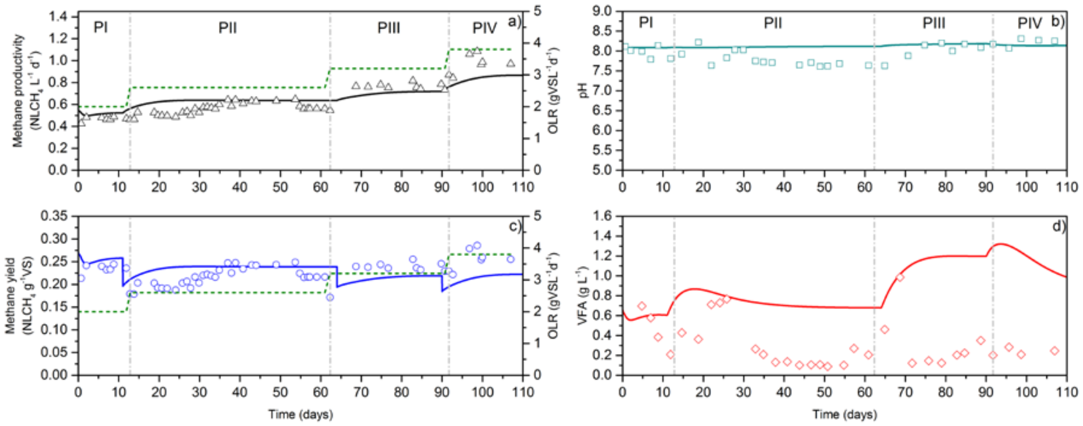

Figure 4: Experimental data and model simulation of the CSTR experiment with manure and meadow grass. Points are experimental values and straight lines are model simulations. Dashed lines mark the OLR. PI (2 g-VS L^{-1} d^{-1}), PII (2.6 g-VS L^{-1} d^{-1}), PIII (3.2 g-VS L^{-1} d^{-1}) and PIV (3.8 g-VS L^{-1} d^{-1}).

Considering another part of the work carried out, Lemvig's full-scale reactor performance was also documented in this period, followed by preliminary simulations to predict the dynamics of the full-scale reactor using the BioModel. The model was able to forecast process performance in terms of gas composition and pH reliably.

In early 2016 (**RP4**) and in order to further extend the range of application of the BioModel framework, two complex organic wastes were tested to identify the best co-digestion scenarios that can result from their combination with manure, for optimal biogas production in a CSTR (see submission 1 or SU1).

Parallel to the experimental tests, the BioModel itself was also extended during RP4. Model development aimed to improve the simulation results obtained from running the BioModel – when compared to experimental datasets – and prove that it is capable of forecasting gas and intermediate production trends from both manure- and wastewater sludge-based co-digestion scenarios in a satisfactory manner. For this end, the model output was analyzed concerning its sensitivity to select model parameters and subsequently parameters deemed sensitive were estimated on different levels of complexity.

Technical results originating from the co-digestion tests can be listed as follows:

- Organic matter present in orange peels (OP) and seaweed (SW) is easily biodegradable, therefore eliminating the need of harsh and expensive pretreatments.
- Co-digestion of OP and SW with manure resulted in higher methane yields and stable AD process.
- Orange peels (OP) and/or seaweed (SW) were successfully co-digested with manure reaching a methane yield of 264.52 ± 5.17 and 220.53 ± 5.19 NmLCH4 g-VS-1 added, respectively.
- During the entire experimental period inhibition either by limonene in OP or phenolic compounds in SW was not observed.

Considering the BioModel extension through sensitivity analyses and parameter estimation, and the subsequent model validation, the below technical results can be mentioned:

- Results of the combined uncertainty and sensitivity analysis from different co-digestion scenarios (for simulated acetic acid and methane gas production) can be seen in Figure 5.
- The analysis showed that out of the 38 parameters tested, there were 14 whose values greatly influenced the output (i.e. sensitive), while the rest did not play a significant role in shaping the results (i.e. robust).

The effect of the sensitive parameters was divided into three segments: whether it was exerted on 1) biogas and methane production, 2) the pH and the amount of soluble ammonia or 3) the concentration of VFA, considering the output of the simulation.

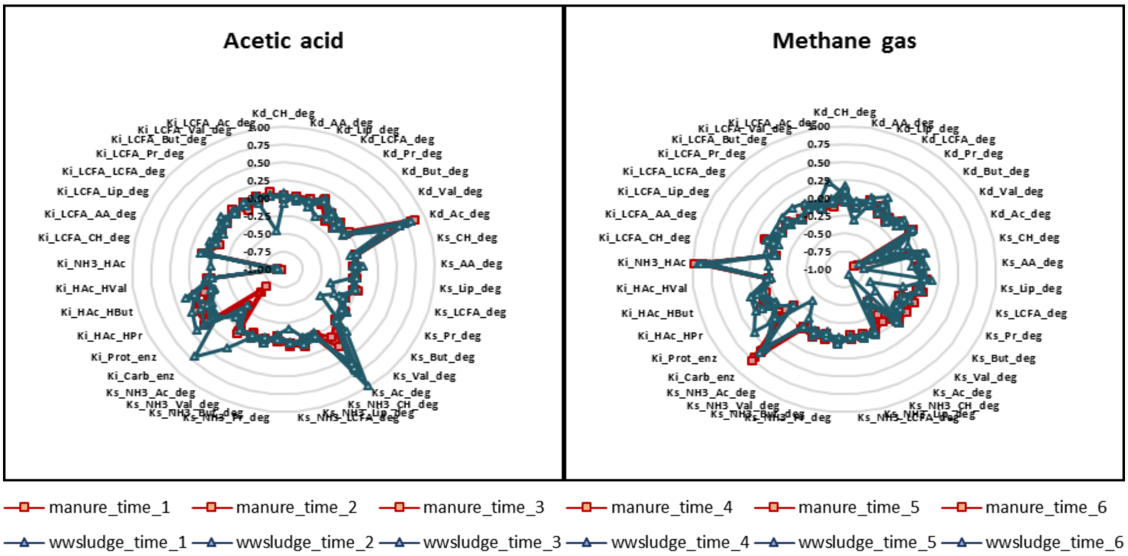

Figure 5: The effect of the 38 select parameters on forecasted acetic acid and methane gas production, at different time steps of the manure-based and wastewater slurry-based co-digestion simulations, where positive values indicate synergistic effects, negative values show inverse relationships and the magnitude of the values represents the strength of the effects

- The comparison of the three segments – for both manure- and sludge-based co-digestion scenarios – is shown in Figure 6, with parameters being organized according to the average magnitude of their effect.
- Although some parameters behaved differently in the two case scenarios, the trends were similar overall. This implies that out of all parameter-output relations, the ones describing ammonia inhibition, the inhibition originating from enzymatic hydrolysis and the trends in the growth and death of acetic acid degrading microorganisms are the most influential.

As a result of model development, several improvements were made to how the BioModel works, compared to its earlier software structure. In Figure 7, a part of the so-called "input sheet" is presented, which is used to define substrate compositions in a user-friendly way.

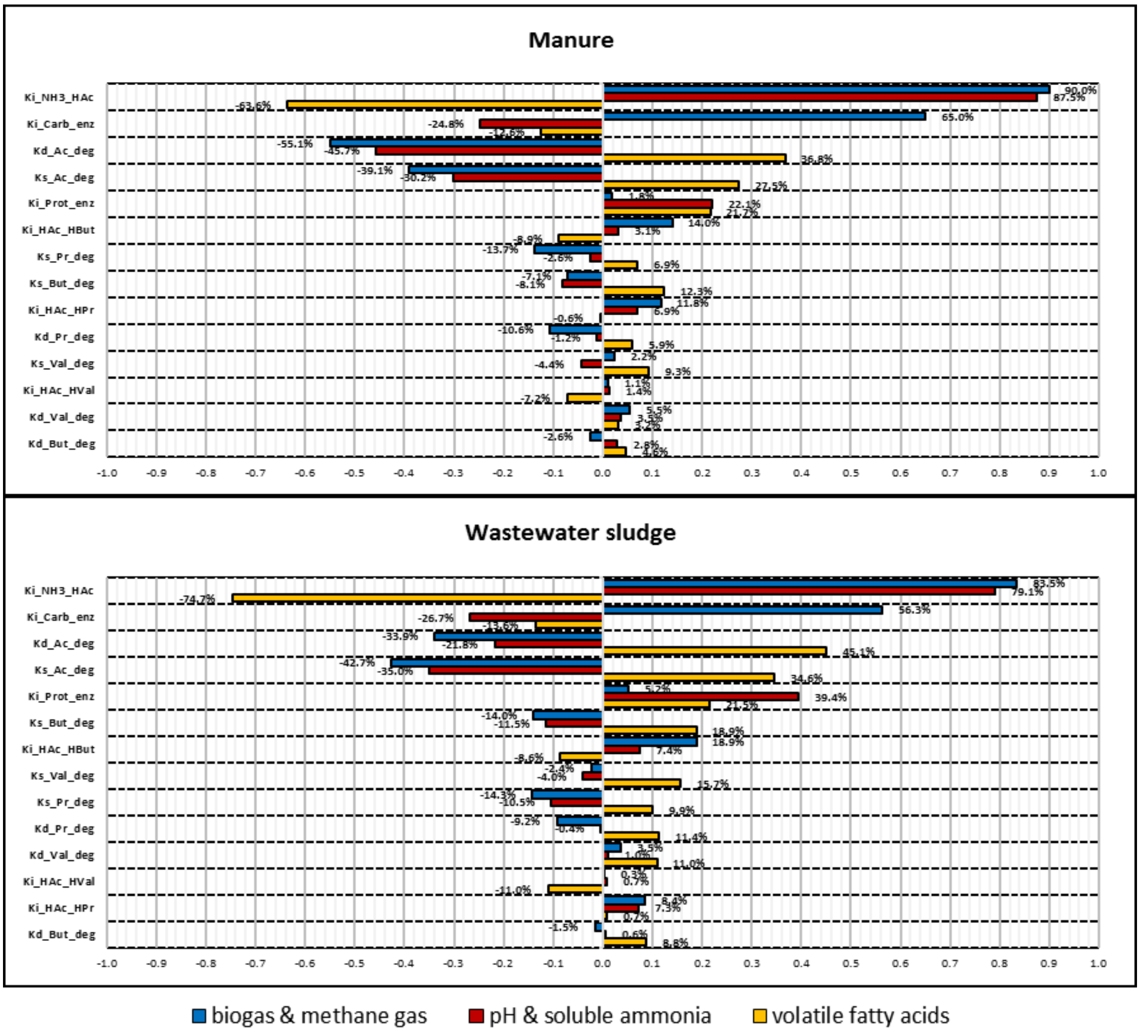

Figure 6: The effect of the 14 sensitive parameters on forecasted gas production, pH, soluble ammonia and VFA concentrations, where positive values indicate synergistic effects, negative values show inverse relationships and the magnitude of the values represents the strength of the effects

Meanwhile, the source code of the MatLab model implementation was also changed and extended, in order to make data visualization and parameter estimation possible in an automated way. These parts of the running model (the built-in graphical display and the parameter estimation module) can be viewed in Figure 8.

Model input data 2 - **THE 1ST SUBSTRATE**

	Period and basic information		**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**
	Source's file name		Han_M(OLR3.2	an_M+GTO(OLF	an_M+GTO(OLF	Han_M+GTO(OL	M+GTO(OLR4)+	M+GTO(OLR4)+NH3(5)				
	TS	[g/L]	0,62									
	VS	[g/L]	0,48									
	Total Carbohydrate	[g/L]										
	Total Protein	[g/L]										
	Total Lipid	[g/L]										
	Total N	[g-N/L]	3,48									
	NH4-N	[g-N/L]	2,10									
	Total VFA	[g/L]	5,61									
	Volumetric ratio in the feed											
		[%]	100,00	100	100	100	100	100				
	Component concentrations											
Carb	Insoluble	[g/L]	4,8600	4,8600	4,8600	4,8600	4,8600	4,8600				
Carb	Inert	[g/L]	27,5400	27,5400	27,5400	27,5400	27,5400	27,5400				
Carb	Soluble (Glucose)	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
Protein	Insoluble	[g/L]	1,5456	1,5456	1,5456	1,5456	1,5456	1,5456				
Protein	Inert	[g/L]	6,1824	6,1824	6,1824	6,1824	6,1824	6,1824				
Protein	Soluble (Amino acids)	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
Lipid	Insoluble	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
Lipid	Inert	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
Lipid	Soluble (GTO)	[g/L]	2,5000	5,0820	8,0820	14,0820	14,0820	14,0820				
Lipid	LCFA	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
VFA	Propionic acid	[g/L]	0,5800	0,5800	0,5800	0,5800	0,5800	0,5800				
VFA	Butyric acid	[g/L]	0,0700	0,0700	0,0700	0,0700	0,0700	0,0700				
VFA	Valeric acid	[g/L]	0,0200	0,0200	0,0200	0,0200	0,0200	0,0200				
VFA	Acetic acid	[g/L]	4,7200	4,7200	4,7200	4,7200	4,7200	4,7200				
ssolved	T-NH4	[g-N/L]	2,1000	2,1000	2,1000	2,1000	4,0000	5,0000				
ssolved	CH4	[g/L]	0,0000	0,0000	0,0000	0,0000	0,0000	0,0000				
ssolved	CO2	[g/L]	2,8800	2,8800	2,8800	2,8800	2,8800	2,8800				

info | calc_aid | INO | SUB | COSUB_1 | COSUB_2 | COSUB_3 | EXP | labset | fullset | kin | ion | yco

Figure 7: Examples of how the "input sheet" is built up

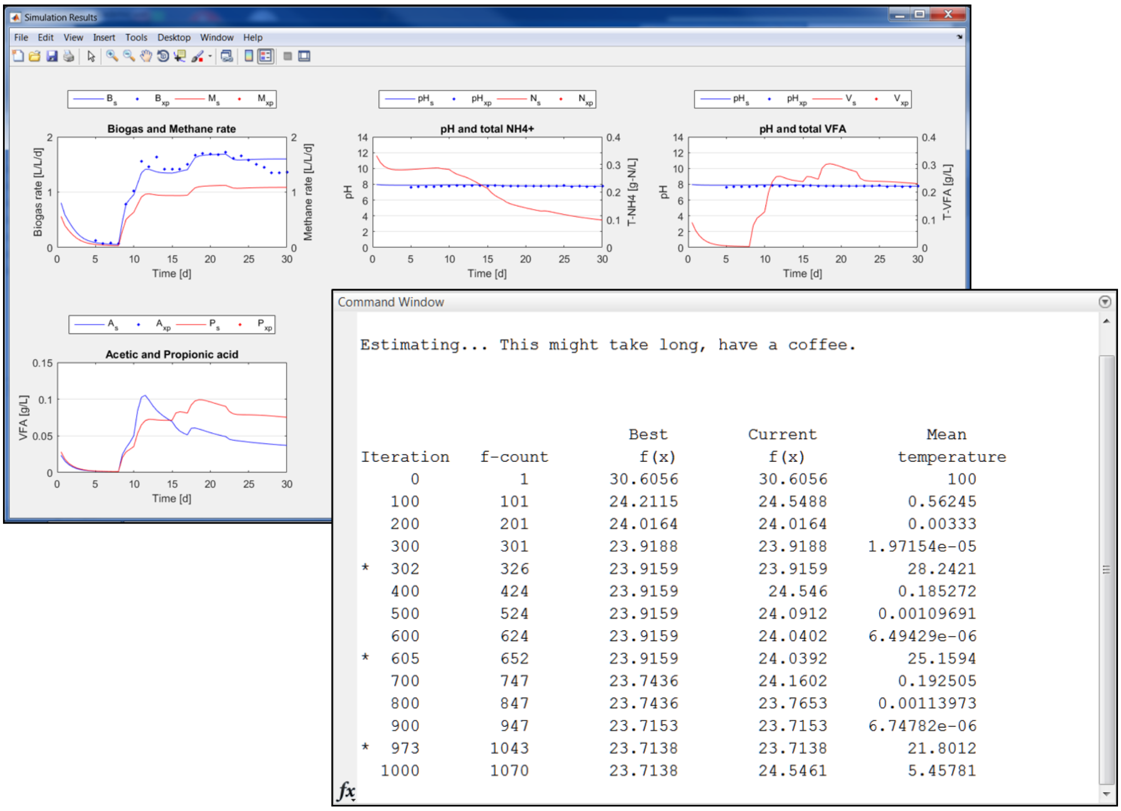

Figure 8: Extracts of the running BioModel (graphical display above and parameter estimation module below)

Still in RP4, simulations were carried out with manure- and wastewater slurry-based co-digestion scenarios as well, in order to validate the accuracy of the BioModel. Already until now, simulation results were found to be in fairly good agreement with experimental datasets used for comparison (see Figures 9 and 10), while independent proof (AR1) was found that the BioModel can potentially provide output data of high quality, often superior to other AD models currently publicly available. This was especially evident in the case of manure-based co-digestion scenarios.

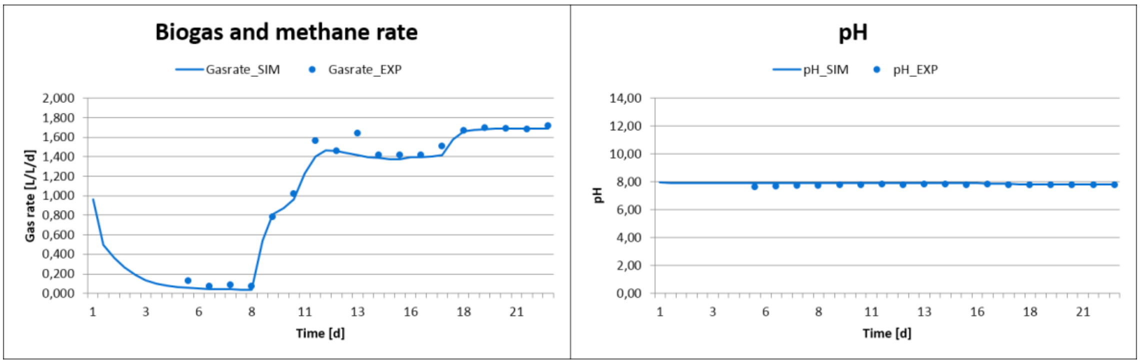

Figure 9: Biogas, methane and pH simulation results of a manure-based co-digestion scenario, compared to experimental results

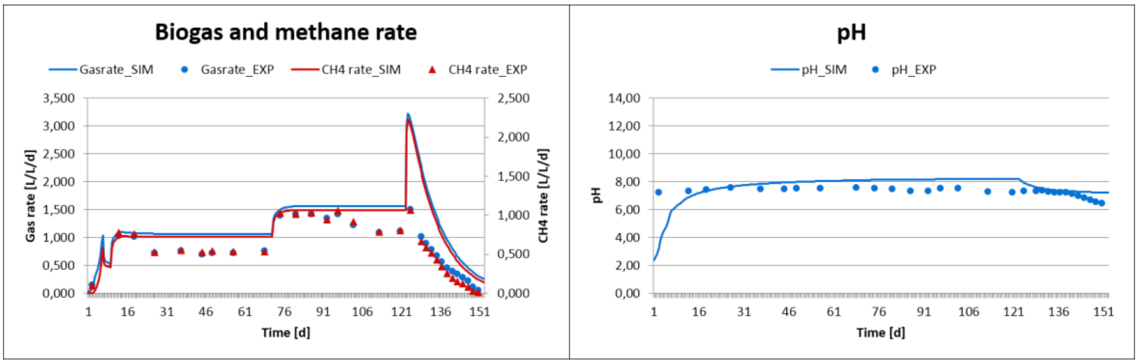

Figure 10: Biogas, methane and pH simulation results of a wastewater slurry-based co-digestion scenario, compared to experimental results

The dissemination of results in RP4 was carried out by the publication of a scientific article (SP3).

In the second half of 2016 (**RP5**) and due to the completion of the experimental element of this WP during RP4, no further experimental work was carried out under this WP. Meanwhile the BioModel, whose applicability had been extended to both manure- and wastewater sludge-based co-digestion scenarios previously, was further developed to better account for dynamic changes in substrate feeding. In addition, using the results of the model's sensitivity analysis and parameter estimation also carried out during RP4, the most influential model parameters were optimized and the model was validated with data from three different experimental lab-scale anaerobic co-digestion scenarios.

Furthermore, it was observed that the model was often reacting faster than the real situation, upon changes. Therefore, in order to achieve better simulation results for experimental cases where dynamic changes in substrate feeding take place, the model's microorganic growth simulation module had been revised. As starting point, a microbial growth lag phase was implemented in a simplified AD model. The simplified AD model consisted of a two-step bioconversion process: glucose degradation to acetic acid by acidogenic microorganisms and further to methane by acetoclastic methanogens. The technical results achieved were:

- Growth latency was defined in terms of the simulated process and growth lag was distinguished from time delays (Figure 11).
- Growth lag was implemented in the simplified model, as a transfer function dependent on time.
- Simulations were performed with and without microbial death, product inhibition and growth lag considered (Figure 12).
- Results of the growth lag implementation and the simulations are planned for submission to a conference during the following reporting period and will also be used for the extension of the BioModel with growth lag dynamics (second step of implementation).

Another part of the modeling work involved the use of optimized parameters to test the BioModel for the simulation of three select experimental cases (see SP6). The cases were manure- or wastewater-sludge based co-digestion experiments carried out in lab-scale and either at thermophilic or mesophilic conditions.

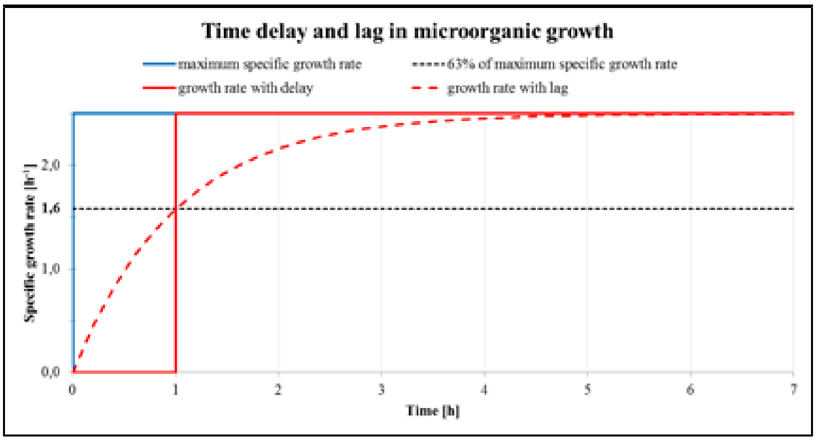

Figure 11: Visual explanation for the difference between delay and lag in a microbial cell growth context

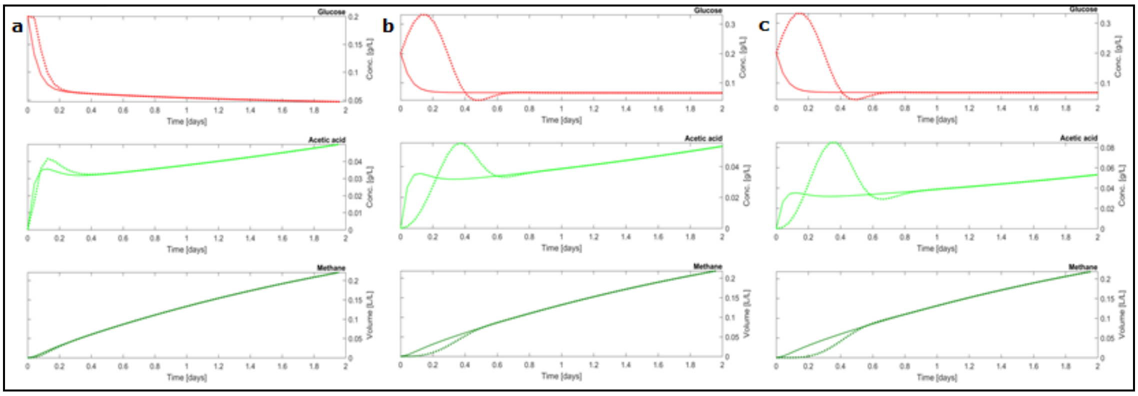

Figure 12: Simplified model simulation with growth lag (a), growth lag and microbial death (b), and growth lag, death and product inhibition (c) implemented in the model.

The first simulation involved an experiment with manure, co-digested with lignocellulosic material retrieved from agricultural lands. Results of the simulation showed improved curve fit, compared to the simulation using parameter values from earlier experiments. The second simulation was carried out using data from an experiment with manure, but this co-digested with a mixture of food waste and various lignocellulosic garden wastes. The improvement in curve fit is well represented by the ammonia simulation. For the third simulation, data from a wastewater-sludge experiment was used, which was co-digested with the effluent of an olive oil processing facility. Here the level of improvement is lower, however it was due to conditions outside the scope of the simulation.

During the second half of 2017 (**RP7**), the microbial growth lag functionality developed in the simplified model during RP6 was implemented in the BioModel. Furthermore, data collected earlier from a manure and meadow grass co-digestion experiment (see section 1.5.1) was used to run simulations with the extended BioModel, to see if the improved model could generate more accurate simulations of the study than the original one. In order to make the no-lag and lag simulation cases directly comparable, the pH simulation was restricted to vary between values measured experimentally, while the microbial growth lag time – effective for acetoclastic methanogens – was fixed in 3 hours. Results of the comparison can be seen in Figure 13.

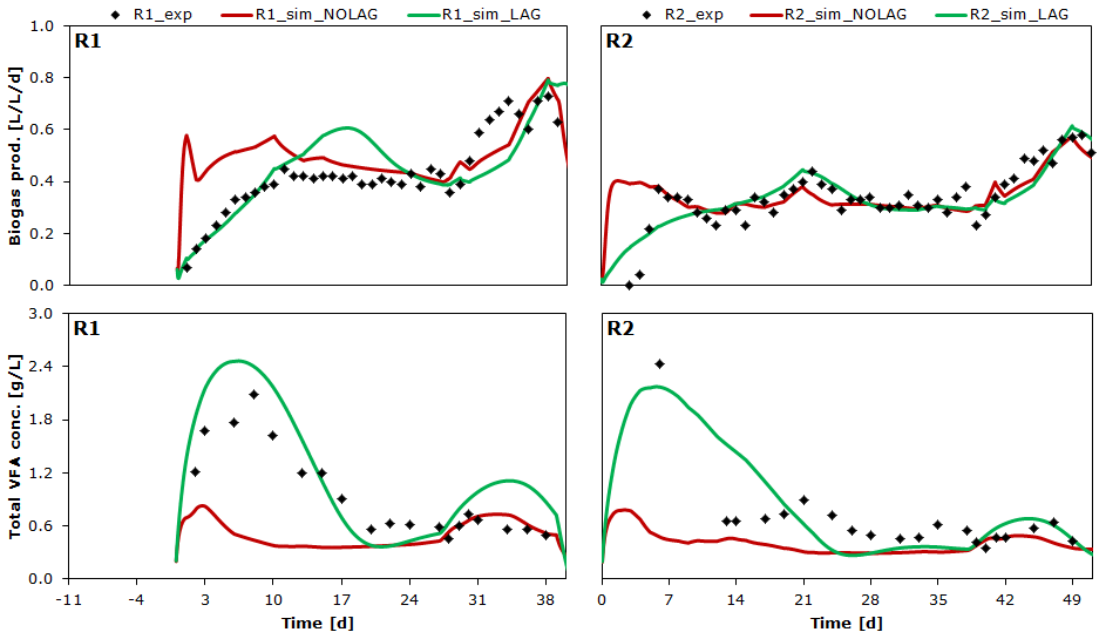

Figure 13: Comparison of BioModel outputs when simulating the manure monodigestion period of a co-digestion experiment. The experimental and simulated biogas production and VFA concentration for reactor 1 (R1) is on the left, while those for reactor 2 (R2) are on the right. Black diamonds mark experimental values, red lines the simulations with the original BioModel and green lines the simulations with the lag implementation of the model.

The shift in the timeframe of the two reactors (R1 and R2) was due to an initial breakdown and restart of R1, delaying the final start date by 11 days. However, for visualization purposes the ends of the manure monodigestion periods and the beginnings of the manure and meadow grass co-digestion periods in the two reactors (day 27 and 38, respectively) are shown in the same part of the subgraphs.

Above figures indicated that the BioModel with the lag phase implementation generated better simulation results, especially in terms of the fits between experimental and simulated total VFA concentrations. Regarding biogas production rates, the improvement was also significant, although a slight production overshoot was simulated in R1, right after the system reached steady-state production. Nevertheless, as microbial metabolism remains a highly complex and dynamic process to model, such a lag implementation provides a reasonable alternative, while maintaining low computational requirements and increasing simulation accuracy.

Following the implementation and validation of the lag functionality in the BioModel, and still during RP7, the process and substrate information gathered from the biopulp co-digestion experiment during the same period (see section 1.5.1) was used to prove model flexibility in yet another scenario. Biopulp as a substrate was unlike previous organic wastes or residues tested, and had a promisingly high methane potential, for which reason this was considered an interesting simulation case. The outcome of the simulations is shown in Figure 14.

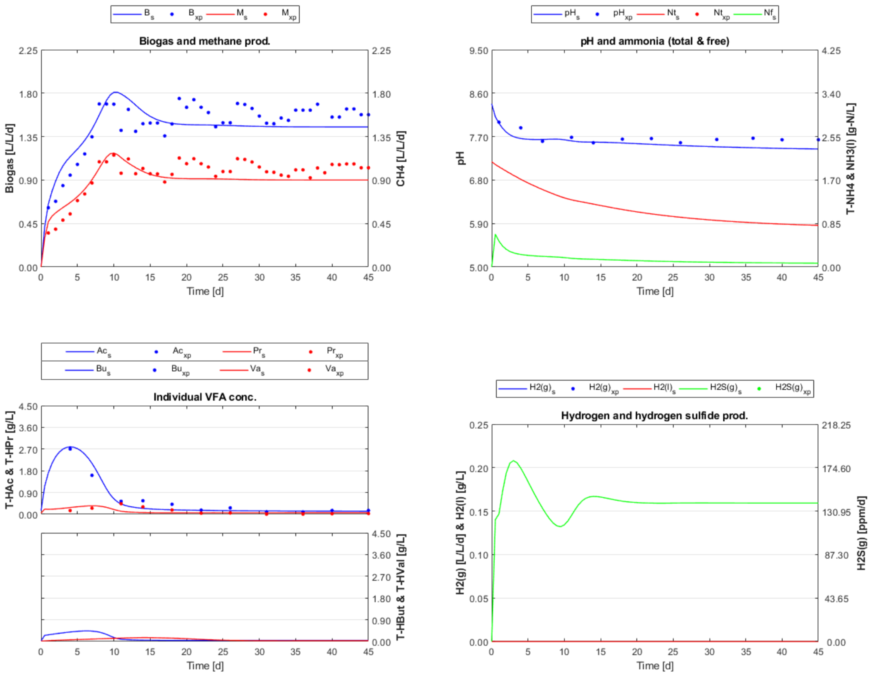

Figure 14: Simulation output from the BioModel, showing biogas and methane production rates, pH, ionic and free ammonia levels, VFA and hydrogen sulfide concentrations.

As it appeared from the results, the BioModel captured the experimental trends with high accuracy, as far as the variables with available measurement data are concerned. Unlike the simulations run by the authors of AR2, the initial lag in the variable trends was due to the growth lag of the methanogenic archaea, and not an initially lower concentration of microbial functional groups. While conceptually different, this approach agreed better with the general consensus, regarding the reduced activity of microbial groups when exposed to new substrates or fed after a nutrient deprivation period.

Moreover, the high quality of simulations showed that the BioModel was readily applicable for the simulation of biopulp-based systems as well. Given the conclusion of AR2 and the results of the simulation, it was therefore agreed that the pilot-scale reactor experiment outlined in WP4 would be carried out with biopulp only, using the model to simulate the experiment. The experiment and the simulations were carried out in 2018 (**RP8**) and are covered in detail in section 1.5.4.

1.5.3 WP3

The preparation and application of the DTU VFA sensor for online monitoring of a full-scale biogas plant was carried out early in the project, more specifically in the first two years. The sensor, which had been built earlier, was modified and tested in the laboratory at DTU Environment during **RP1**. Afterwards, the sensor was installed and tested at the farm-scale biogas plant called Granja San Ramón situated in Valencia, Spain, with the purpose of monitoring their process. The VFA sensor was applied at the biogas plant in collaboration with our research partner, AINIA technology center, Spain. During the test period, the reactor liquid samples were collected for GC analysis to verify the reliability of the sensor output (Figure 15). Due to some technical challenges concerning the auto-sampling system of the reactor, our partner AINIA had to work on solving this problem before the online monitoring application could be continued.

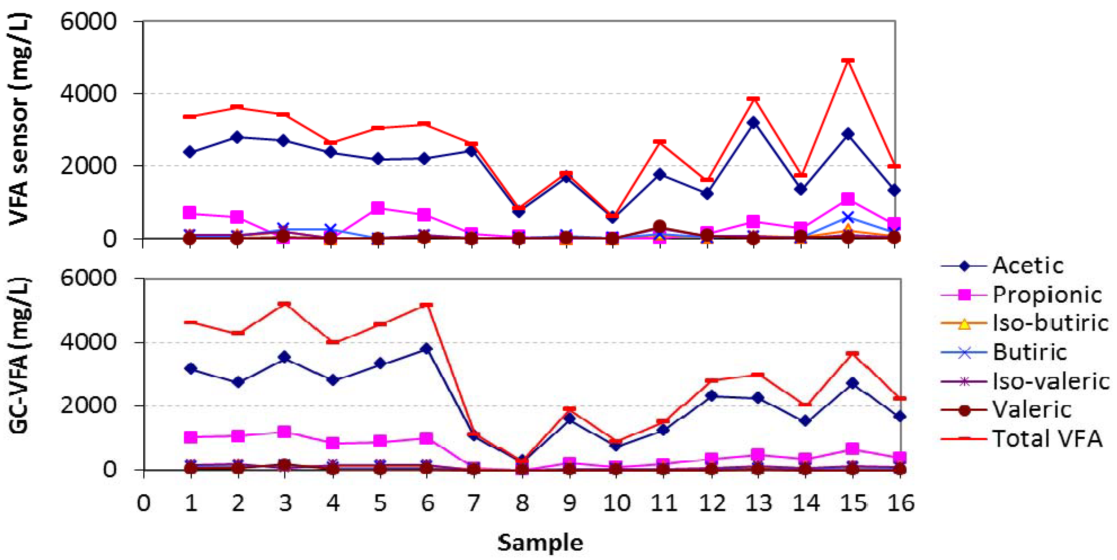

Figure 15: Results from VFA sensor compared to GC analysis

The aim of this work was to demonstrate the applicability and reliability of the VFA sensor in full-scale application. The sensor could later be applied for pilot-scale reactor optimization, through intensive process monitoring and high frequency data recording, potentially preventing the breakdown of the biological process. Overall, relevant technical results in RP1 were the preparation, installation and initial testing of the VFA sensor at the Granja San Ramón biogas plant. Early on in the following year (**RP2**), testing and validation of the VFA sensor at the biogas plant concluded, therefore fulfilling the third milestone (**M3**) on time.

1.5.4 WP4

Work on WP4 began later in the project, given that it was directly or indirectly dependent on the outcome of all the other WP. It involved the running of a pilot-scale reactor (Figure 16) and testing of the optimal operation strategies, for improving co-digestion efficiency. The first tasks addressed from WP4 considered milestone four (M4), that is the establishment of a co-digestion strategy to be tested in a pilot-scale reactor. This became possible during the first half of 2016 (**RP4**), by which time a large number of experiments and simulation results had been generated.

Figure 16: The reactor setup used in the pilot-scale experiment

However, due to the continued generation of experimental and simulated data, along with the steady development of the BioModel, several co-digestion strategies had been considered during the subsequent periods of the project, therefore making it more challenging to select the optimal one for the pilot-scale experiment. Moreover, the setup of the pilot-scale facility was delayed by several months due to the necessity of reparations, the installation of additional safety equipment and the subsequent accreditation of the facility (ATEX) that was deemed necessary.

It was therefore not until 2018 (**RP8**) that the pilot-scale experiment could be carried out. In the meantime, experiments and later simulations with biopulp as a co-substrate showed that high biogas yields (490 mL $g\text{-}VS^{-1}$) and process stability can be achieved, even with the addition of only a small amount of manure (see section 1.5.2). Eventually, biopulp was selected as a single substrate for testing in the pilot-scale reactor experiment, without the addition of manure. This decision about the test strategy, although slightly different from the original milestone 4, also marked the fulfilment of **M4**.

The experimental setup was transported from DTU to the Avedøre wastewater treatment plant of BIOFOS during 2017, and was scheduled to begin operation in 2018. The reactor would be inoculated with the effluent of a manure-based full-scale reactor, and would be operated at mesophilic conditions (37 °C), with an HRT of 15 days and an initial OLR of approximately 2-2.5 g-VS L^{-1} d^{-1}.

However, as the experiment started in the middle of the winter of 2017/2018, and due to the outside placement of the pilot-scale facility, complications arose regarding the activity of the inoculum and the feeding of the liquid substrate. This led to an initial gas production lag in the process (presumably due to the low activity of methanogenic microbial groups) and a corresponding accumulation of VFA, therefore the feeding had to be interrupted and the recovery of the process closely monitored. Nevertheless, the same initial period provided suitable process data for simulations, where testing of the previously developed microbial growth lag module of the BioModel could be tested under pilot-scale conditions. The results of the simulation can be seen in Figure 17, compared with experimental data and focusing on methane productivity, pH and acetic and propionic acid concentration.

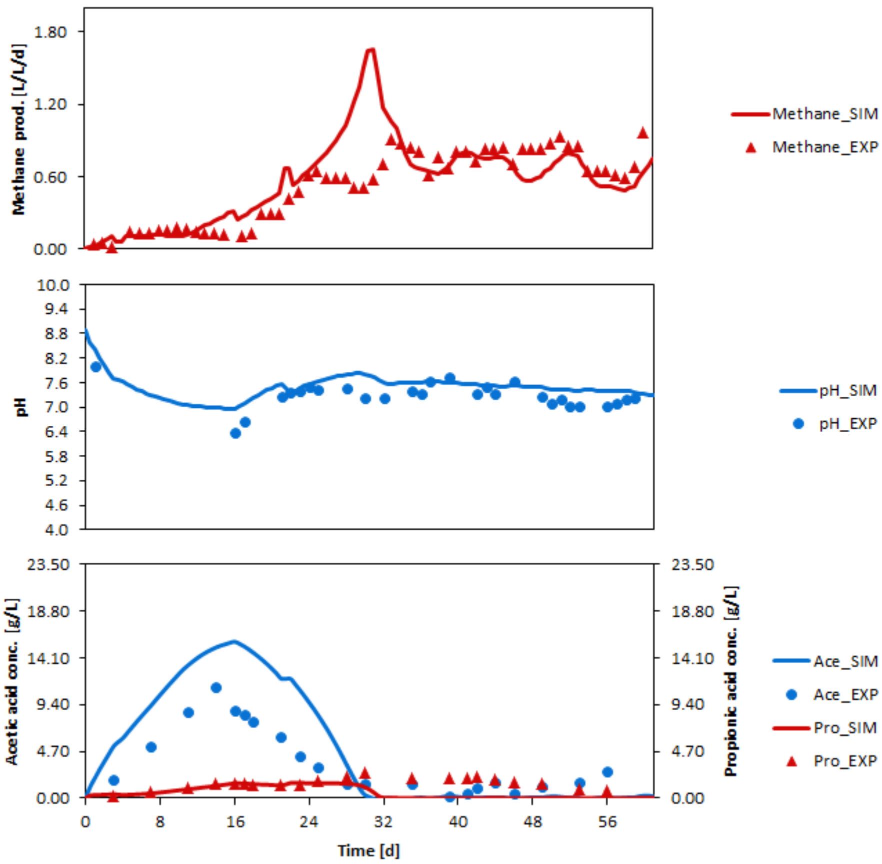

Figure 17: Experimental and simulated methane productivity, pH and acetic and propionic acid profiles in the initial operational period of the pilot-scale reactor

While the experiment eventually reached steady-state operation and showed biogas productivities similar to expectations, the simulation of this initial period showed the excellent potential and versatility of the BioModel, when modeling full-scale processes. While the simulation results also showed that certain, dynamic aspects of the process are still challenging from a simulation perspective (e.g. suppressing the simulated overshoot at the end of the lag period around day 30), they gave a good representation of the future that modeling full-scale AD processes offer.

In conclusion, by validating the pilot-scale biopulp digestion experiment using simulations with the BioModel, milestone 5 (**M5**) was finally fulfilled. The only remaining task was therefore to define the implications of the results and provide suggestions to biogas plants on how to utilize them. However, as this involved continuous consulting with the plants, the timeframe of this activity would span beyond the scope of present project.

1.5.5 Results in light of expectations

Overall, the project milestones were fulfilled successfully and the work outlined in the WP was realized according to the original plans. Despite slight changes to the sources of process data (i.e. the abstention of Linkogas biogas plant) and minor delays with the setup of the pilot-scale reactor, the project generated a significant amount of useful information on various co-digestion scenarios, which can be used effectively by biogas plants, consultants or other interested parties. Furthermore, the main project objective to establish the optimal operation criteria for highly efficient and stable manure co-digestion processes was also realized, through the analysis and comparison of the co-digestion scenarios tested. Using a combination of experimental and modeling tools, as well as batch, continuous and pilot-scale reactors during the project work, the different aspects of process efficiency and stability (e.g. biological, operational, etc.) could be examined successfully. Regarding the unexpected results of the project, the most important finding was related to the use of biopulp in monodigestion scenarios. Although the project work focused on manure-based systems and co-digestion setups involving different organic wastes as substrates, this finding showed that high efficiency and stability AD processes can be established even in urban environments, using only municipal organic wastes.

1.5.6 Material benefits and employment

Although the validation of the VFA sensor was successful, the further development of the product requires continued negotiations between the project stakeholders, which would potentially concern financial implications at a later time. However, the mathematical model can be used for the efficient monitoring and control of biogas processes at various scales, providing economic benefits to biogas plants and contributing to the development of the biogas industry as a competitive energy providing sector.

1.5.7 Dissemination of project results

Dissemination of the results generated during the project was realized in multiple ways. Firstly, the seven scientific publications that were generated as a direct or indirect result of this project meant an increase from the originally planned 3-4 and showed that the project developed in a healthy manner. In addition, the project work contributed to the submission of more than five conference abstracts and the subsequent oral or poster presentations, which increased the outward exposure of the project results and its stakeholders significantly. Last but not least, a constant communication between project partners about the planning and delivery of the project ensured the strengthening of existing relationships and created new connections between the academic and commercial representatives, promoting future collaborations substantially.

1.6 Utilization of project results

Project outcomes were categorized as material products or methodologies, where the former group relates to the

- online VFA sensor;
- mathematical model;
- substrate database.

Meanwhile, methodologies concern the operation guidelines defined for running successful co-digestion processes, such as the

- use of tested organic by-products in co-digestion with manure, so as to increase methane yields and improve the carbon to nitrogen ratio, while maintaining process stability;
- use of biopulp in monodigestion or co-digestion with manure (if available), for achieving high methane yields even in urban environments;
- use of microbial growth lag simulation to estimate process dynamics during reactor startup;
- continuous monitoring of VFA to point out process imbalances.

Results of the project are expected to be utilized primarily by the participating biogas plants and DTU, for process optimization and further research purposes, respectively. The disseminated findings of the project can further be used by other biogas plants and industrial stakeholders operating relevant AD systems, for instance in the wastewater treatment sector.

Regarding the commercial output of the project, the validated VFA sensor prototype could serve as a potentially marketable product; however, this aspect requires additional planning steps to take place. So far, this process neither did involve the creation of a business plan or the analysis of a market potential, nor the assessment of market competition. With regards to intellectual property, no patent resulted from the project and none is expected in the future.

Project results contributed to the development of the biogas industry directly, by providing systematic guidelines for efficient plant operation and innovative tools (an online VFA sensor, mathematical model and a substrate database) that can support the monitoring and management of biogas plants. As many biogas plants operate under suboptimal conditions, above outputs can be used during various phases of their process optimization activities and can increase the productivity of their co-digestion processes significantly (AR3, AR4), often by more than 10-20%.

Through this knowledge transfer process, it is also considered that the transition to 100% renewable energy by 2050 in Denmark could be accelerated. More specifically, by sharing such information with the plants partnering in the project, as well as additional industrial partners getting involved later during the project (i.e. the Granja San Ramón biogas plant in Spain or BIOFOS' wastewater treatment plant in Avedøre, Denmark), the project results could be utilized directly, contributing to the above national goal.

The project was carried out by a postdoc, instead of a PhD student. Besides that, some PhD and several MSc students have partly been involved in the work. Some of the results were used as examples in teaching, where modelling of the anaerobic digestion was taught. In the teaching notes general kinetics were described.

1.7 Project conclusion and perspective

The results of the project contributed to the development of next generation biogas processes, which take advantage of mathematical models in plant management. Integrated models can access and collect information from substrate databases and online process monitoring equipment, such as VFA sensors. In turn, the simulations generated with these models can assist plant operators in monitoring and controlling the plant infrastructure, while supporting plant managers in forecasting the long-term dynamics of the process. Whether applied as a standalone tool or combined with advanced control algorithms, the presented modeling framework can therefore offer significant benefits during the planning and steering of full-scale biogas processes, and improves the competitiveness of the biogas industry as a whole.

Moreover, the extended model can be used for the simulation of various co-digestion scenarios, due to the new functionalities developed and the systematic model validation process followed throughout the project. These simulations can provide a reference for biogas plant managers, who are constantly looking for high yield and stably digestible substrates. Using the model to forecast the dynamics of new and untested co-digestion scenarios, plant managers can significantly reduce their costs by avoiding laboratory experiments, and the time spent between initial tests and the approval of new substrates for full-scale application.

Finally, through the collection and analysis of several experimental co-digestion datasets, a number of strategic operation guidelines have been defined as a result of the project. These guidelines covered various aspects of the co-digestion process, like the optimal choice of substrates, the relevance of microbial growth lag during process startup and the constant monitoring of VFA to ensure process stability. These guidelines, although not being substitutes for detailed process knowledge, offer biogas plant constructors and managers some important advice and points to consider, for designing and operating their plants efficiently.

Annex

Publications:

SP1. Lima, D. M. F., Rodrigues, J. A. D., Boe, K., Alvarado-Morales, M., Ellegaard, L., Angelidaki, I. 2016. ANAEROBIC MODELING FOR IMPROVING SYNERGY AND ROBUSTNESS OF A MANURE CO-DIGESTION PROCESS. Brazilian Journal of Chemical Engineering, 33(4), 871-883. DOI:10.1590/0104-6632.20160334s20150314

SP2. Søndergaard, M. M., Fotidis, I. A., Kovalovszki, A., Angelidaki, I. (2015). Anaerobic co-digestion of agricultural byproducts with manure for enhanced biogas production. Energy & Fuels, 29(12), 8088-8094. DOI:10.1021/acs.energyfuels.5b02373

SP3. Pan, X., Angelidaki, I., Alvarado-Morales, M., Liu, H., Liu, Y., Huang, X., Zhu, G., 2016. Methane production form formate, acetate and H2/CO2; focusing on kinetics and microbial characterization. Bioresour. Technol. 218, 796-806. DOI:10.1016/j.biortech.2016.07.032

SP4. Awais, M., Alvarado-Morales, M., Tsapekos, P., Gulfraz, M. and Angelidaki, I., 2016. Methane production and kinetic modeling for co-digestion of manure with lignocellulosic residues. Energy & Fuels. DOI: 10.1021/acs.energyfuels.6b02105

SP5. Alvarado-Morales, M., Tsapekos, P., Awais, M., Gulfraz, M. and Angelidaki, I., 2017. TiO2/UV based photocatalytic pretreatment of wheat straw for biogas production. Anaerobe. DOI: 10.1016/j.anaerobe.2016.11.002

SP6. Kovalovszki, A., Alvarado-Morales, M., Fotidis, I. A., Angelidaki, I., 2017. A systematic methodology to extend the applicability of a bioconversion model for the simulation of various co-digestion scenarios. Bioresource Technology. DOI: 10.1016/j.biortech.2017.03.101

SP7. Lovato, G., Alvarado-Morales, M., Kovalovszki, A., Peprah, M., Kougias, P. G., Rodrigues, J. A. D., & Angelidaki, I., 2017. In-situ biogas upgrading process: modeling and simulations aspects. Bioresource Technology. DOI: 10.1016/j.biortech.2017.08.181

Submissions:

SU1. Negro, V., Alvarado-Morales, M., Fino, D., Ruggeri, B., Angelidaki, I., 2016. Anaerobic co-digestion of orange peels and seaweed with cow manure.

Conference abstracts:

CA1. Alvarado-Morales, M., Kovalovszki, A., Fotidis, I. A., Angelidaki, I., 2017. A robust methodology to extend the applicability of a bioconversion model for the dynamic simulation of various anaerobic co-digestion scenarios http://ad15.medmeeting.org/3169?lang=en

CA2. Kovalovszki, A., Alvarado-Morales, M., Ellegaard, L., Angelidaki, I., 2017. Introduction of lag phase of microbial growth upon changes, during modeling of the anaerobic digestion process
http://ad15.medmeeting.org/3169?lang=en

CA3. Kovalovszki, A., Alvarado-Morales, M., Angelidaki, I., 2018. Bioaugmentation modeling in anaerobic digestion: the dynamics of methanogenesis
http://www.biogas-science2018.it/

CA4. Kovalovszki, A., Alvarado-Morales, M., Ellegaard, L., Ghofrani Isfahani, P., Angelidaki, I., 2018. Microbial growth lag implementation in a complex bioconversion model
http://www.biogas-science2018.it/

CA5. Mazarji, M., Alvarado-Morales, M., Tsapekos, P., Nabi-Bidhendi, G., Mahmoodi, NM., Angelidaki, I., 2018. Impact of graphene on ZnO assissted photocatalysis for degradation of lignin-rich substrates by UV/Iodide process
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AR1. Weinrich, S., Nelles, M., 2015. Critical comparison of different model structures for the applied simulation of the anaerobic digestion of agricultural energy crops. Bioresource technology, 178, 306-312.
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AR2. Tsapekos, P., Kougias, P. G., Kuthiala, S., Angelidaki, I., 2018. Co-digestion and model simulations of source separated municipal organic waste with cattle manure under batch and continuously stirred tank reactors. Energy Conversion and Management, 159, 1-6.
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AR3. Søndergaard, M. M., Fotidis, I. A., Kovalovszki, A., Angelidaki, I., 2015. Anaerobic co-digestion of agricultural byproducts with manure for enhanced biogas production. Energy & Fuels, 29(12), 8088-8094.
DOI: 10.1021/acs.energyfuels.5b02373

AR4. Kougias, P. G., Angelidaki, I., 2018. Biogas and its opportunities – A review. Frontiers of Environmental Science & Engineering, 12, 1-12.
DOI: 10.1007/s11783-018-1037-8