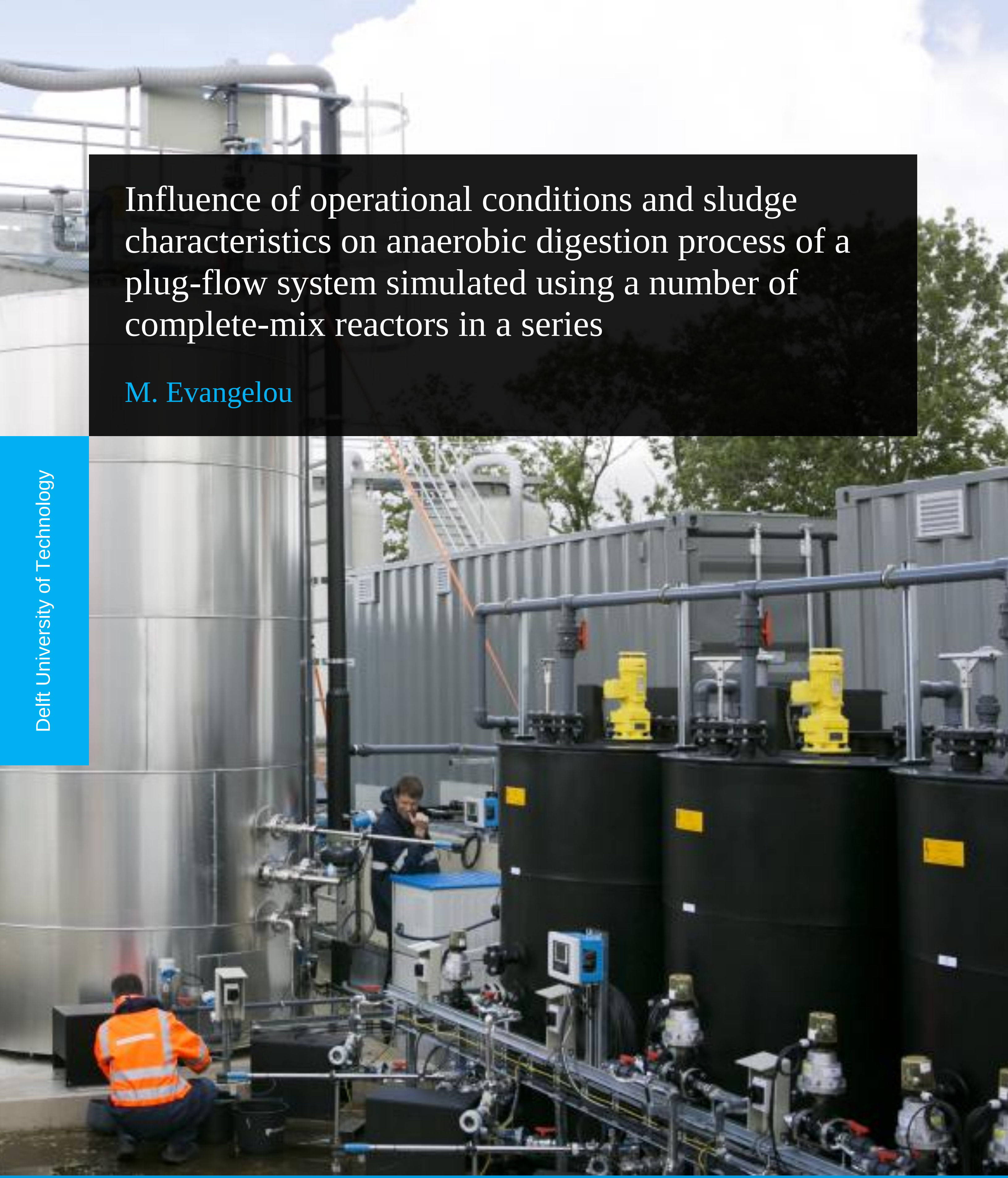

Influence of operational conditions and sludge characteristics on anaerobic digestion process of a plug-flow system simulated using a number of complete-mix reactors in a series

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Influence of operational conditions and sludge characteristics on anaerobic digestion process of a plug-flow system simulated using a number of complete-mix reactors in a series

By

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This thesis is confidential and cannot be made public.

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Contents

Abstract

Royal HaskoningDHV (RHDHV) has developed a new technology called Ephyra® to enhance sludge digestion process. It is based on plug flow which is simulated using four completely stirred tank reactors in series. The results obtained under lab conditions and on pilot scale, using sludge from Tollebeek WWTP and Lelystad WWTP, show that Ephyra® achieves higher breakdown of sludge and biogas production which can be 10 to 20 % higher than in conventional digesters. Degradation efficiency of sludge depends on organic loading rate and recirculation rate, which are both interrelated with hydraulic retention time. Therefore, this study investigated this pre-treatment technology with particular emphasis on the effects of recirculation rate and organic loading rate on anaerobic digestion process using a calibrated model developed in BioWin® 4.1. Also, it was researched which feedstock characteristics influence the anaerobic processes, and also if the number of the Ephyra reactors have an impact on the performance of the system. The results indicate that recirculation rate plays a significant role on the operation of the Ephyra system. It was found that the best ratio of recirculation which optimizes the performance of the system is 8%. To increase the removal of total COD in the effluent OLR has to be decreased from the default value. Moreover, the steady state simulations of the calibrated model indicate that decreasing the number of the Ephyra® reactors from 4 to 3 does not change the removal efficiency of the system, while a more stable performance can be achieved. To investigate the influence of different feedstock characteristics, measurements from a lab-scale plant inoculated with sludge obtained from Amsterdam WWTP were examined. However, they couldn't help to answer with more confidence which characteristics have an important role on the performance of the system.

1 Introduction

Anaerobic processes are defined as biological processes conducted by the action of several groups of microorganisms where organic matter is metabolized in an environment free of dissolved oxygen or its precursors and is converted into a gaseous mixture consisting of methane (60-70%) and carbon dioxide (30-40%) [1]. The diverse group of microorganisms involved and a number of interdependent metabolic stages taking place under anaerobic conditions constitute anaerobic digestion; a much more complex process than aerobic processes [1]. Anaerobic digestion is a plain process which reduces the mass of organic pollutants and pathogens which are transferred from the liquid to the solid phase during conventional biological wastewater treatment [1] [2] [3]. Disposal of sludge during municipal wastewater treatment constitutes a great part of the transporting and processing costs of a wastewater treatment plant, and that's why bioenergy production is not the only driver of anaerobic digestion. In Netherlands, about 50% of total treatment costs of sewage sludge are related with sludge disposal and processing [3] [4]. Anaerobic digestion combined with pre-treatment and post-treatment technologies consists a sustainable solution for handling and recycling organic wastes, and meets the objectives of the Kyoto agreement and contributes to prevent issues related with human and animal health and food safety [5].

The major groups of microorganisms involved in the process are acidogenic bacteria, hydrogen producing acetogenic bacteria, acetoclastic methanogens and hydrogenotrophic methanogens. The main steps of anaerobic digestion are hydrolysis, acidogenesis (fermentation), acetogenesis and methanogenesis. During hydrolysis complex organic compounds like proteins, carbohydrates and lipids are transformed into soluble products such as amino acids, sugars and long-chain fatty acids by extracellular enzymes which are produced by acidogenic bacteria. Often the hydrolysis step is the rate-limiting step, due to the lack of free accessible surface of the particles, or because waste contains lipids and/or significant amount of particulate matter. Hydrolysis products are fermented to a mixture of volatile fatty acids, alcohols and other simpler organic compounds by acidogenic bacteria. During acidogenesis, large amounts of both hydrogen and carbon dioxide are also produced. If hydrogen is removed by methanogens then acetate production is promoted, otherwise more reduced acids are generated like propionate and butyrate. Acidogenesis is the most rapid conversion process and results in a rapid acid formation. Therefore, there is a danger of VFA accumulation if they are not degraded in the subsequent steps. During acetogenesis higher volatile fatty acids, such as propionate and butyrate, produced during acidogenesis are converted to acetate, carbon dioxide and hydrogen. Acetogenesis is thermodynamically impossible under high hydrogen partial pressure because acetogenic bacteria are obligate hydrogen producers and are inhibited by hydrogen. Acetogenic bacteria grow slow with doubling times in the order of days. Then methanogenesis is accomplished by two distinct groups of microorganisms that produce methane and carbon dioxide. The two groups are acetoclastic methanogens which produce 70% of methane by converting acetic acid, and hydrogenotrophic methanoges which generate the remaining methane from carbon dioxide and hydrogen. Anaerobic digestion requires long start-up time due to the very slow growth of acetoclastic methanogens. There two main kind of acetoclastic methanogens; *Methanosarcina* which have a coccoid shape, have high maximum growth rates and low substrate affinity, and *Methanosaeta* which are filamentous, have a low maximum growth rate and very high affinity. On the other hand, hydrogenotrophic methanogens have much higher maximum growth rates and they are responsible for process stability under varying conditions [1] [2] [6].

Some factors, such as organic loading rate, hydraulic retention time, solid retention time, microbiology, environmental factors and reactor configuration, influence the anaerobic digestion process and they must be given the necessary attention during the design and operation of the system. Organic loading rate (OLR) is very important in designing and sizing of anaerobic digesters [2]. The organic loading rate is defined as the amount of organic matter expressed as volatile solids or COD of the feeding substrate that must be treated by a certain volume of anaerobic digester in a certain period of time [7]. Hydraulic retention time (HRT) indicates the time the sludge mixture remains in the reactor in contact with biomass. It is defined as the ratio of the liquid volume of the digester and the volumetric flow rate of the sludge leaving the digester [2] [7]. On the other hand solid retention time (SRT) controls microbial mass in order to achieve a given degree of waste stabilization. Higher SRT maintains more stable operation of digester, better shock loading tolerance and a quick recovery from toxicity [2] [8].

Environmental conditions which have a significant role on comforting microorganisms and succeed stable operation of the system are pH, alkalinity, temperature, feedstock composition and toxic compounds and inhibitors. The pH influences the function of all microorganisms, some on greater degree than others [1] [2]. An acceptable enzymatic activity of acid forming bacteria occurs above pH 5.0, but for methane forming bacteria is above pH 6.2. Most anaerobic bacteria perform well within a range of 6.8 and 7.2 [8]. Temperature has an impact on reactions rates and the number of microorganisms., with acetoclastic methanogens being the most sensitive population in anaerobic digestion. Feedstock should provide the system with the required nutrients for growth of existing microbial cell and synthesis of new cells. Substance like ammonia, heavy metals, halogenated compounds, sulphide and sulphate are substances which could inhibit anaerobic digestion processes [1] [2]. Fats and oils require longer retention times than those of conventional anaerobic digesters, so larger digester volumes are needed [9]. Alkalinity is the measure of buffering capacity to neutralize the acids and maintain proper conditions for methane fermentation. It helps to stabilize the pH in the optimum range of methane formers. Ammonium ions (NH_4^+) are produced in a digester as a result of bacterial degradation of amino acids and proteins. When organic compounds degraded carbon dioxide and ammonia is released. Bicarbonate alkalinity is the primary source of carbon for methane forming bacteria, and ammonia dissolves in water along with carbon dioxide forming ammonium bicarbonate. At the same time, degradation of organic compounds produces organic acids which destroy alkalinity. Values of pH below 6.0 or above 8.0 are not allowed as they are toxic to methane forming bacteria. So to maintain a stable pH a high level of alkalinity is required [8] [10] [11]. Alkalinity can be a better parameter than pH value for monitoring anaerobic digestion. The relation of VFA to alkalinity is more important than the absolute quantity of VFA as it gives more warning of impending digester problems, while pH may not indicate a problem until is too late. The ratio of VFA to alkalinity should be between 0.05 and 0.15 [10] [11].

Feedstocks ideal for renewable energy generation are wastes and residues rich in organic matter like municipal sludge, animal manure, agriculture residues and food wastes. However, they are characterised by slow digestibility due to the rate-limiting hydrolysis step. Their digestibility could be increased by using different pre-treatment technologies. Pre-treatment technologies can be distinguished to physical, mechanical and biological. Different substrates require different pre-treatment methods to achieve a higher digestibility [2] [12]. First steps of anaerobic digestion, hydrolysis and acidification, are separated from acetogenesis and methanogenesis when biological pre-treatment is used. It is also called pre-acidification or multi-stage fermentation and a common configuration is two-stage digestion system, but also plug-flow digesters are used [12].

Plug-flow reactor along with continuous stirred tank reactor (CSTR) is the main configurations used in anaerobic digestion. CSTR is the most basic bioreactor configuration. Complete mixing occurs instantaneously and uniformly throughout the reactor as fluid particles enter the reactor. In order to provide high retention times, which are required to sustain the slow grow of methanogens, large reactor volumes are needed, which increase the costs [1] [13]. In a plug-flow reactor fluid particles pass through the reactor with little or no longitudinal mixing and exit from the reactor with the same sequence in which they enter due to the high length to width ratio [13]. Using a plug-flow reactor VFA generation and biogas production can be separated in time and/or space. When influent doesn't contain the required microorganisms, recirculation is needed to prevent microorganisms to wash-out and sustain microbial growth process in the plug-flow reactor [14].

Studies utilizing a plug flow digester showed that acidogenesis and methanogenesis separation is achieved along the reactor which improved the stability and efficiency of the system. Royal HaskoningDHV with aim to enhance the sludge digestion process has developed a new technology called Ephyra®. Ephyra® is based on plug flow combined with process control rather than a completely mixed digester system. The plug flow is simulated using completely stirred tank reactors in series. In this compartment system phase separation of anaerobic digestion processes occurs [15] [16] [17]. The physical separation of anaerobic digestion processes helps maintaining proper concentrations of acidogenes and methanogens in each reactor and enhances acidogenesis and methanogenesis rates [17]. The lab-scale and pilot-scale plants were operated using sludge from Tollebeek WWTP and Lelystad WWTP, and Ephyra® was simulated using four CSTR in a series. The results obtained showed that Ephyra® achieved higher degradation of sludge and biogas production which can be 10 to 20 % higher than in conventional digesters.

During the operation of the pilot-scale plant a recirculation line from the fourth tank to the first one was necessary, in order to avoid the accumulation of volatile fatty acids (VFA) in the first compartment. An increased amount of VFAs causes process imbalance if their concentration exceeds the pH buffer capacity of the anaerobic digestion process. An intership report from a previous study states that recirculation is a key factor for this particular system [18]. Recycling of methanogenic effluent into an acidification reactor could enhance the buffer capacity and system stability, and hydrolysis of organic matter could be promoted greatly. Also, after a long period of recirculation problems may occur related to accumulation of VFAs and other inhibitory substances [19].

Degradation efficiency of sludge depends on organic loading rate and recirculation rate, which are both interrelated with hydraulic retention time. An increase of organic loading rate results in rise of biogas production, but only until a critical point. After this point, the organic material increases so much that inhibits bacteria’s work inside the digester [20].

Although much knowledge about the processes behind Ephyra® system has been obtained a lot of (scientific) questions still exist. The goal of this master thesis is to extent the knowledge of the anaerobic digestion in general and the Royal HaskoningDHV technology in particular. The main objective of this research is investigating the influence of different operational parameters on digestion of municipal wastewater sludge and biogas production, in order to maximize sludge degradation.

2 Research questions

The study focuses on answering the followed questions related with operational parameters and sludge characteristics with aim to obtain more knowledge about Ephyra®.

a) What is the best ratio of recirculation in order to optimize system’s performance? The term best ratio is defined as the fraction which prevents VFAs accumulation in the acidification reactor, maintains suitable buffer capacity and increases sludge degradation. To what extent is the digestibility increased at optimal recirculation rate compared to the degradation achieved at the recirculation rate used during the lab-scale and pilot-scale plants?

b) Can the increase of organic loading rate enhance the degradation of sludge and optimize biogas production, and to what extent? At which point does the OLR increase cause inhibition of bacteria's activity?

c) Could the number of compartments decrease, while total hydraulic retention time remains the same, in order to decrease capital and operational cost without significant effects on the process?

d) Does Ephyra® has the same performance when it is inoculated with different feedstock? Which sludge characteristics have an important influence on the performance of the system?

In order to answer the abovementioned questions a model was developed using BioWin® 4.1. After calibration and validation of the model it was used to predict the system's performance in different operational conditions. During the research, methods like literature study on sludge treatment, data analysis and experimental research on digestion performance were applied to answer the research questions.

3 Modelling approach

3.1 Model description and structure

BioWin® 4.1 was the main tool used to answer the research questions. BioWin® was developed by EnviroSim and uses a general Activated Sludge/Anaerobic Digestion (ASDM) model. It has more than fifty state variables and over seventy process expressions. These expressions were used to describe the biological processes typically occurring in wastewater treatment plants. An advantage of the model is that it does not require transferring model outputs to another model to be analysed, and therefore it decreases the complexity [21].

Another model that is widely used for modelling anaerobic digestion is the Anaerobic Digestion Model No. 1 (ADM1), published by the International Water Association [22]. It is a very detailed and comprehensive model for anaerobic digestion processes. For a correct implementation of ADM1 a detailed characterization of the sludge is required, and many parameters are difficult or impossible to measure. The data measured during the pilot-scale operation could not be used to describe in so much detail the influent characteristics of the sludge. Also, ammonia release in the digester and return of the digester supernatant to the liquid line is important for anaerobic digestion simulation. However, ADM1 does not include nitrogen release on organism decay and does not maintain a nitrogen mass balance [23] [24]. Also, ADM1 does not include a pH calculator, but pH value is considered a very important parameter for anaerobic digestion systems. These particularities consisted ADM1 not suitable for this study.

EnviroSim itself states in the manual that pH is very difficult to model as there are many reactions and complex components influencing pH value. BioWin uses a mixed kinetic/equilibrium based on approach for pH simulation. The pH model is implemented based on equilibrium of phosphate, carbonate, ammonium, VFA and other typical strong ions in wastewater such as Mg^{+2} and Ca^{+2}, and it incorporates activity coefficients based on the ionic strength of the solution. It also based on gas-liquid transfer of NH_3, CO_2, N_2, H_2, CH_4, NO_2 and O_2, and on biological activity which could affect the compounds included in the model [25].

The anaerobic digestion model of BioWin consists of three different functions operated by four kinds of organisms. As the influent biomass enters the anaerobic digester it undergoes anaerobic decay. Then, heterotrophic organisms hydrolyse particulate matter which may be present in the influent or as a product of anaerobic decay, and produce readily biodegradable COD, phosphate and soluble organic nitrogen. Ammonia is produced through ammonification of soluble organic nitrogen mediated by heterotrophs. Phosphate and ammonia are removed from the solution via struvite and calcium phosphates precipitation. Complex readily biodegradable COD is fermented by ordinary heterotrophic organisms (OHOs) to acetate, propionate, hydrogen and carbon dioxide. The process is governed by the dissolved hydrogen concentration. Propionate conversion to acetate, carbon dioxide and hydrogen is mediated by growth and decay of propionic acetogens. Methane is produced through growth and decay of two groups of obligate anaerobic microorganisms, acetoclastic and hydrogenotrophic methanogens. Acetoclastic methanogens convert acetate to methane and carbon dioxide, and hydrogenotrophic methanogens convert carbon dioxide and hydrogen to methane and water. All the processes are modified to account the environmental conditions, nutrient limitations and pH inhibition [21].

The hydrolysis rate is the product of a hydrolysis rate coefficient, the sum of the ordinary heterotrophs and phosphate accumulating organisms and a Monod expression. The default value of hydrolysis rate coefficient in BioWin® is 2.1 d^{-1} and is defined as the rate constant for hydrolysis of the slowly biodegradable organics into readily degradable substrate. Under anoxic and anaerobic conditions an efficiency factor is applied in order to adjust the process to the environmental conditions. The information obtained after personal communication with EnviroSim was that testing of BioWin® 4.0 has led to the adjustment of the rate reduction factor for hydrolysis under anaerobic conditions in anaerobic digestion, which is called anaerobic hydrolysis factor (AD), at 0.5 [-]. As such, this is the default AD factor for BioWin® 4.1. Therefore the actual anaerobic hydrolysis rate coefficient is 0.5 x 2.1 = 1.05 d^{-1}.

BioWin® simulates volatile fatty acids (VFAs) as the sum of propionate and acetate concentrations. Other kinds of acids are not included in the calculations. The COD influent element is used to define the feed characteristics in BioWin®. Two influential fractions are the fraction of total influent COD which is readily biodegradable (Fbs), and the fraction of the readily biodegradable COD which is VFAs (Fac). However, according to the equations of BioWin the VFAs that are used to define the fraction Fac consist only from acetate. Hence the concentration of propionate in the influent is zero.

$$VFA = Sbsa + Sbsp \qquad eq. 1$$

$$Fbs = \frac{Sbsc + Sbsa}{Total\ COD} \qquad eq. 2$$

$$Fac = \frac{Sbsa}{Sbsc + Sbsa} \qquad eq. 3$$

where: Sbsa = acetate concentration
Sbsp = propionate concentration
Sbsc = readily biodegradable COD (complex) concentration

3.2 Model implementation and initial conditions

The anaerobic digestion systems, Ephyra® and a reference system, are configured and modelled using BioWin® 4.1. software. Ephyra® system consists of four completely stirred tank reactors (CSTR) in a series where sludge is treated before it is fed in the final CSTR. The reference system consists of one CSTR and it is used to compare its performance with Ephyra®.

The initial conditions and most of the model variables were defined based on the measurements of the pilot-scale plant. The average input characteristics of Ephyra® and reference system are presented in Table and Table 2 respectively. All the measurements which were used in a later stage for model calibration and validation can be found in Appendix A.

Table 1: Influent characteristics of Ephyra® system based on the average values measured at pilot-scale plant

Parameter	Unit	Average values
Total COD	mg/l	67778
Soluble COD	mg/l	8502
TKN	mg/l	3333
TP	mg/l	1508
VFA	Mg/l	2501
NH_4-N	mg/l	461
PO_4-P	mg/l	652
Ca	mg/l	857
Mg	mg/l	318

Table 2: Influent characteristics of reference system based on the average values measured at pilot-scale plant

Parameter	Unit	Average values
Total COD	mg/l	58842
Soluble COD	mg/l	6500
TKN	mg/l	2770
TP	mg/l	1310
VFA	Mg/l	2232
NH_4-N	mg/l	324
PO_4-P	mg/l	435
Ca	mg/l	880
Mg	mg/l	263

There were no values available of infuent's pH and alkalinity value of the pilot-scale plant at Tollebeek. Recommended operational values of alkalinity of sludge for anaerobic digesters and values of hydrolysis rate coefficient found in literature are presented in Table 3 and Table 4.

Table 3: Hydrolysis rate coefficient values of anaerobic digestion systems from literature

Hydrolysis rate coefficient	Reference
Different mixed sludge: 0.23-0.43 d^{-1} at mesophilic conditions	Haghighatashar et al. (2015) [26]
Mixture of primary and secondary sludge: 0.15 d^{-1} at 35°C experimental results Primary sludge from domestic WWTP: 0.007-0.99 d^{-1} at 35-60°C Primary sludge from domestic WWTP: 0.4-1.2 d^{-1} at 35°C Secondary sludge: 0.168-0.6 d^{-1} at 35°C	B. K. Ahring (2003) [27]

Table 4: Alkalinity values of anaerobic digestion systems from literature

Alkalinity [mg $CaCO_3$/L]	Reference
4000-5000	Sperling et al. (2007) [28]
2000-4000	Pohland & Bloodgood (1963) [29]
2000-4000	Metcalf & Eddy (2002) [13]
2000-3000	Sanin et al. (2011) [30]

In order to run the model without problems related with VFA accumulation, at the beginning it was assumed that the hydrolysis rate coefficient of Ephyra® system was 0.11 d^{-1} and the sludge had a high alkalinity of 100 mmol/L (≃5 g $CaCO_3$/L). The value of pH was set at 7.2 which is the default value of BioWin®.

Model configurations of both system can be seen in Figure 1. The four CSTRs in a series of the Ephyra® system model have a sludge volume of 1.9 m^3 each and a headspace of 1.0 m^3. The final Ephyra® CSTR has a volume of 11.5 m^3 and a headspace of 1 m^3. The temperature was set at 35°C in all the compartments. A recirculation line from the fourth to the first reactor was simulated with a constant rate of 5%. The reference system consists of one completely stirred tank reactor with a volume of 19.1 m^3 and a headspace of 1 m^3. The temperature was kept constant at 35°C.

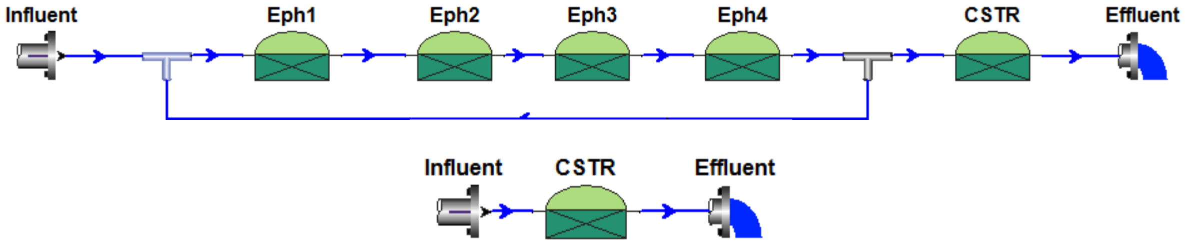


Figure 1: BioWin schematic flow diagram of the pilot-scale plant (up Ephyra® system and down the reference system)

3.3 Sensitivity analysis

Sensitivity analysis is a pure mathematical approach, and it measures the effects of perturbing model input factors on model outputs. In normal usage of sensitivity analysis and to obtain a global picture of sensitivity of the system the effect of change is investigated using a one-at-a-time (OAT) approach [31] [32].

The results of sensitivity analysis give the opportunity to select the most influential kinetic parameters. Kinetic parameter estimation determines model accuracy with respect to predicting the dynamic behaviour of the biogas system. Stoichiometric parameters are not included in model calibration as their value cannot be changed. But it should be given attention on the error of their true value and predict model deviation [33]. Sensitivity analysis was also conducted using the influent characteristics in order to investigate the influence of error in influent characterisation. Hence, sensitivity analysis comprised 19 kinetic and 26 stoichiometric parameters and 8 wastewater fractions, for both reference and Ephyra® system.

The sensitivity coefficients are the relative difference in the model outputs for each variable. It was selected to calculate the normalised sensitivity coefficients, which according to EPA guidelines represent the percentage change in the output variable resulting for a certain percentage change in each input variable [34]. It was selected to perturb the input variable by 10% change. The change of output was determined in respect to chemical oxygen demand, volatile fatty acids, pH, gas production and methane potential.

$$\text{Normalised sensitivity coefficient: } S_{i,j,} = \frac{\Delta y_j / y_j}{\Delta x_i / x_i} \qquad eq. 4$$

where: Δy_j = difference of output
y_j = output variable
Δx_i = change in input variable
x_i = input variable

Classification:

$S_{i,j} < 0.25$:	no significant influence
$0.25 < S_{i,j} < 1$:	influential
$1 < S_{i,j} < 2$:	very influential
$S_{i,j} > 2$:	extremely influential

3.4 Calibration and validation

The software contains two operational modules, so calibration of both models was conducted in two stages, a steady state module based on constant conditions and an interactive dynamic simulator. The parameters selected by the sensitivity analysis were estimated by minimizing the difference between the measurement and model outputs. Real time measurements of COD, VFAs, gas production and methane content were used for calibration. The average values of data collected from the pilot-scale plant were used for steady state calibration. Dynamic influent wastewater characteristics were used for dynamic simulation.

For calibration, the values of the parameters found to be more influential from the sensitivity analysis were changed. The outcome of the model was evaluated each time that a parameter was changed and depending on the result the attemp was eliminated or adjusted again. This was repeated until the best fit of data with that predicted by BioWin was achieved. Calibration targets were set for the above mentioned parameters at 10% relative percent difference between model and plant data.

Once the parameters were estimated, it was necessary to determine confidence behind the model and asses the parameter accuracy. Model validation was conducted in two stages: direct and cross validation. First, the model was checked if it can reproduce the data that has been used for parameter estimation (direct validation). Direct validation was conducted by running steady state simulations using the average influent concentrations. Then, to complete model's validation and to test that the model can reproduce the behaviour of the system a different set of data is used to identify the performance of the model (cross validation) [31]. The predictive capacity of both models was validated by comparing model outputs with dynamic measurements of chemical oxygen demand (COD) volatile fatty acids (VFAs), gas production and methane content.

4 Materials and methods

4.1 Experimental setup

New experimental data were obtained from the existing experimental setup in the technical research centre of Royal HaskoningDHV. The measurements were carried out in two anaerobic digestion systems, a 3-compartment Ephyra® system and a reference system (Figure 2). Both systems were operated by daily feeding strategy as continuously mixed reactors. Reactors were kept continuously mixed at 50 rpm. Both configurations were designed to have the same hydraulic retention time (HRT = 20 days) in order to be comparable.

The Ephyra® system was consisted of:

- A pump, which was feeding the system with a continuous flow of 0.9 L/d.
- Three completely stirred tank reactors with a volume of 3.6 L each (diameter is 12.4 cm and height is 29.5 cm) and an effective volume of 2 L. Each one of these compartments had a gas sampling point and a liquid sampling point.
- A completely stirred tank reactor with a volume of 18 L and an effective volume of 12 L, with a gas and liquid sampling point.
- Recirculation line from the 3rd to the 1st compartment, with a ratio of 5% of total sludge volume per day. Recirculation was kept constant during the whole experiment.

The reference system was consisted of:

- A pump which was feeding the system with a continuous flow of 0.78 L/d.
- A completely stirred tank reactor with a volume of 18 L and an effective volume of 15.7 L. The reactor had a gas and liquid sampling point.

The system was inoculated with a mixture of primary and secondary sludge obtained from Amsterdam WWTP. The sludge was composed of 50% secondary sludge and 50% primary sludge based on dry solids concentration. The temperature was maintained at 35°C throughout the experiment. In order to achieve a steady state the reactors were left to equilibrate over two or three hydraulic retention times.

Biomass samples were collected from the influent and effluent to determine initial inputs of the model and effluent characteristics. The concentrations of COD, VFAs, dry solids and organic dry solids, and the values of pH were measured periodically in each compartment of both systems. The produced gas of each compartment was collected in gasbags to determine methane content.

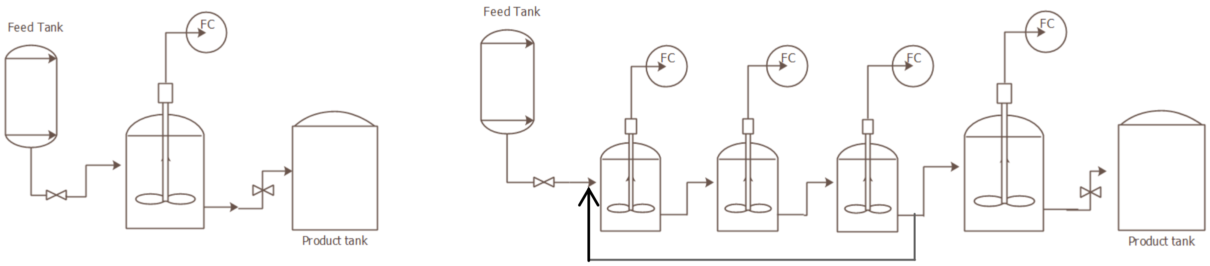


Figure 2: A schematic diagram of experimental setup. (left reference system and right Ephyra® system)

4.2 Analytical methods

Measurements were performed on total or soluble samples. Sludge samples were collected from each compartment, centrifuged for 10 min at 10000 rpm to remove solids, and then filtered through a glass fibre filter with 0.45 µm pore size. The supernatant was then analysed to determine soluble COD and VFAs concentrations in each compartment. Sludge samples were also collected to characterise the influent and effluent of each system. The samples were analysed for total COD, total phosphorus (TP), total nitrogen (TN), magnesium (Mg), calcium (Ca), ammonia (NH_4-N) and phosphate (PO_4-P) content. Also, samples were used to determine pH, alkalinity, total solids (TS) and volatile solids (VS) of the sludge.

Alkalinity and pH were measured using a digital meter. Total COD measurement was obtained on sludge sample after it was mixed using a vortex machine for 5 minutes. Concentrations of total and soluble COD, TP, TN, PO_4-P, NH_4-N, Mg and Ca were determined using Hach Lange cuvette tests. Concentrations of total dry solids and organic dry solids were determined according to APHA standard methods [35]. For the measurement of total dry solids, a well-mixed sample was transferred in a pre-weighed dish and it was dried in an oven at 103 to 105 ^{o}C for at least 24 hours. Then, it was cooled in a desiccator to balance temperature and weight. Then to measure volatile dry solids, the cooled dish with the sampe was ignitited at 550 ^{o}C for at least 1 hour. The dish was cooled in a desiccator to balance temperatue and weight. Total and volatile dry solids were calculated using the equations 5 and 6. The amount of VFAs was measured by a gas chromatography (GC) with a FID detector (Agilent 7890A USA) (Helium as carrier gas with a flow rate of 1.8 mL/min; Column: Agilent 19091F-112 (240^{o}C, 25 m x 320 µm x 0.5 µm, Oven temperature: 80^{o}C). Gas composition was determined using a portable gas detector from Binder GmbH.

$$Total\ dry\ solids\ (TS) = \frac{A-B}{C-B} \cdot 100\ \% \qquad eq.5$$

$$Volatile\ dry\ solids\ (VS) = \frac{A-D}{A-B} \cdot 100\ \% \qquad eq.6$$

where: A = weight of dried residue and dish
B = weight of dish
C = weight of wet sample and dish
D = weight of residue and dish after ignition at 550 ^{o}C

Biochemical methane potential (BMP) tests were conducted to define hydrolysis rate coefficient istead of the methane yield. Hydrolysis rate coefficient was determined separately for all the compartments for both systems. BMP tests are conducted to determine the methane yield of an organic material under anaerobic conditions. The test ends when the cumulative biogas curve closely approaches an asymptote, often after 30 days of incubation [36]. For BMP tests there are many guidelines available with aim the standarization of BMP test. However, high variability on the resutls have been observed for the same substrate, as the process of anaerobic degradation is a dynamic system where microbiological, biochemical and physio-chemical aspects are closely linked and it is a very complex process. Using the first part of the experimental curve of the cumulative biogas production, it is possible to determine the constant k_h (d^{-1}) using a first order hydrolysis model:

$$\frac{dS}{dt} = -k_h\, S \qquad eq.7$$

where S is the biodegradable substrate, t the time and k_h the first order hydrolysis rate coefficient. Integration of the above equation can give the followed relation:

$$\ln\frac{B_\infty - B}{B_\infty} = -k_h\, t \qquad eq.8$$

where $B_\infty$is the ultimate methane production and B is the cumulative methane produced at a given time t. The value of the first order hydrolysis constant can be defined as the slope of the linear curve obtained usually the first 5 days. This value is characteristic of a given substrate and gives information about the time required to generate a given ratio of the ultimate methane potential [37] [38] [39].

In literature is recommended to perform the test at least in triplicates for substrate, blank and control assays. The assay was performed in closed vessels with volume between 100 ml and 2 litres [38]. Due to small amount of sludge obtained from the digesters of the lab-scale plant at Royal HaskoningDHV, it was decided to conduct the assays with a total volume of 100 ml, and for determination of k_h of the first and second compartment only two assays were used for substrate, blank and control instead of three. The inoculum to substrate ratio was 2:1, based on volatile solids (VS).

The experiment was performed using the AMPTS II analytical device, which measures online bio-methane flows produced from the anaerobic digestion of the substrate. The experimental set-up consisted of a water bath with 15 slots of reaction vessels. The temperature of the water bath was adjusted in such a way that the experiment was performed under mesophilic conditions. The calculated amount of inoculum, substrate and stock solutions (phosphate buffer and macronutrients and micronuntrients) were added in the bottles, and pH was measured in all the bottles. A mechanical stirring device was mounted on the bottles to mix their content during the test. All the bottles were flushed with N_2 for about 2 minutes to achieve anaerobic conditions. The produced biogas was flowing to a wash bottle that was connected to the reaction vessels. Wash bottles were filled with caustic solution (3M NaOH with a colour indicator). Then, methane was flowing from the wash bottles to the flow cell array unit to measure the volume of the produced biogas. The data of produced methane in NmL per time unit were saved in a data file for further analysis.

In order to calculate hydrolysis rate coefficient equation 8 was solved in terms of cumulative methane production which is the Gompertz equation:

$$B = B_0\left(1 - e^{-kt}\right) \qquad eq.9$$

Using the data obtained from the BMP tests and the tool *Solver* of Excel the optimal solution of the equation was found for each assay. The particular tool adjusts the parameters B_0 and k_h in order to find the best fit of data to the equation. It finds the optimum when the sum of squared residuals is a minimum. The residual is the difference between the actual value of the dependent variable, which in this case was the cumulative methane production during the BMP tests, and the value B predicted by the *Solver*.

5 Results and discussion

5.1 Data analysis

The measurements and lab analysis of samples from the pilot-scale plant from both Ephyra® and reference system were taken during the period from October 2014 until August 2015. Analysis of these data gave some critical points regarding the data which were used for model calibration and validation. Data considered relevant for discussion and analysis are graphed in this section.

First, it has to be mentioned that Ephyra® and the reference system were fed with different substrate during the operation of the pilot-scale plant at Tollebeek. The Ephyra® system was inoculated with a mixture of primary and secondary sludge from Tollebeek WWTP and secondary sludge from Lelystad WWTP. The substrate was composed of 60% of secondary sludge and 40% of primary sludge based on dry solids concentration. On the other hand, the reference system was fed with a mixture of primary and secondary sludge only from Tollebeek WWTP with percentages of 40% and 60% respectively based on dry solids concentration. Both systems were operated as semi-continuously systems. The mixture of primary and secondary sludge was stored in a buffer tank and the total amount of sludge collected in a day was fed in 48 batches a day.

Temperature and pH were measured online for each compartment. It was observed that the pH values measured in the reactors increased step wise, from 6.2 (average value) in the first reactor up to 6.9 (average value) in the fourth compartment (Figure 3). Figure 4 shows that ammonium concentration increased from an average value in the influent of 460 mg/L to 1500 mg/L through the Ephyra® compartments and then it was slightly increased up to 1620 mg/L in the last CSTR.

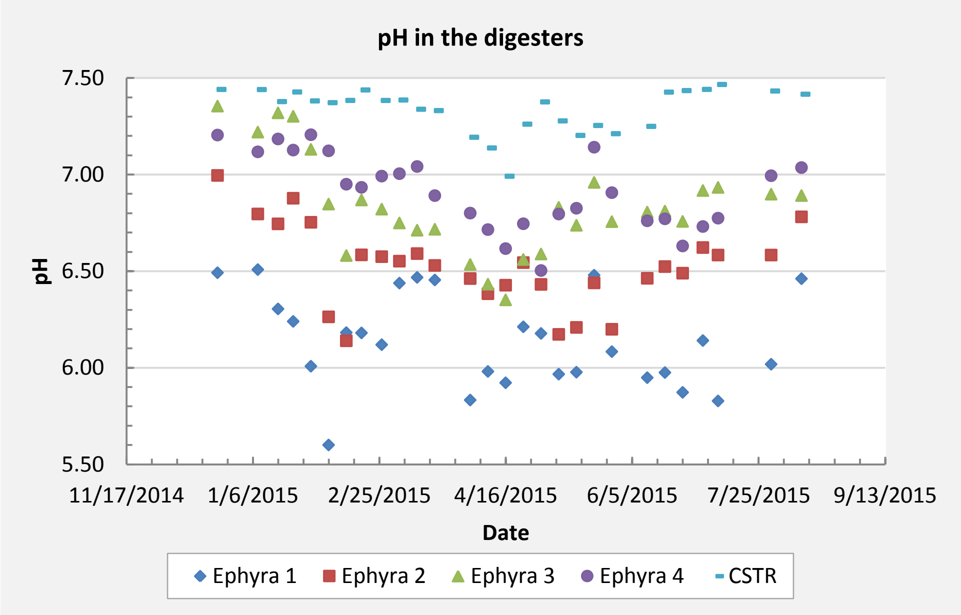


Figure 3: Online pH measurements in each reactor of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek

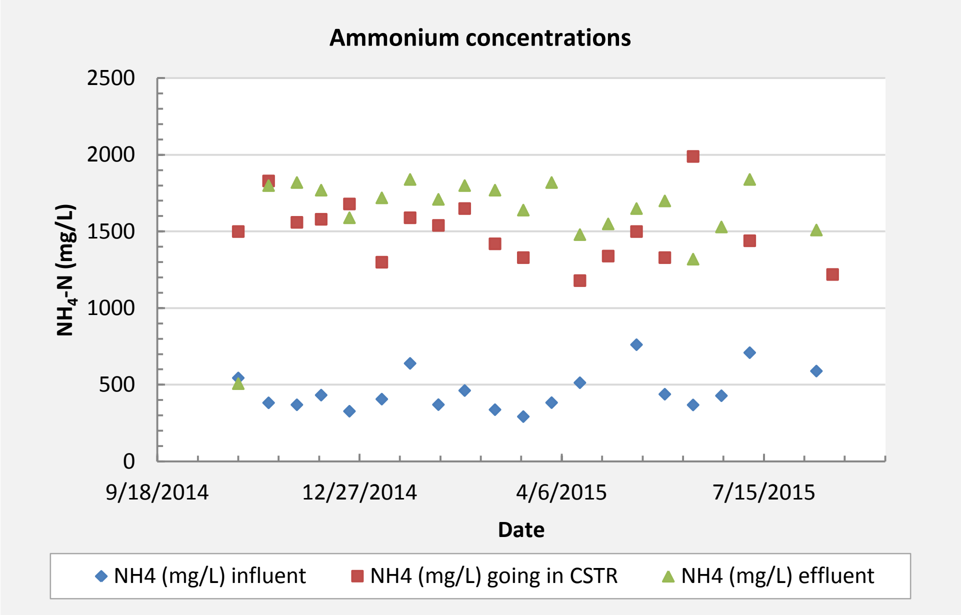


Figure 4: Ammonium concentration measurements of influent sludge, sludge going into the final CSTR and effluent flow of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek

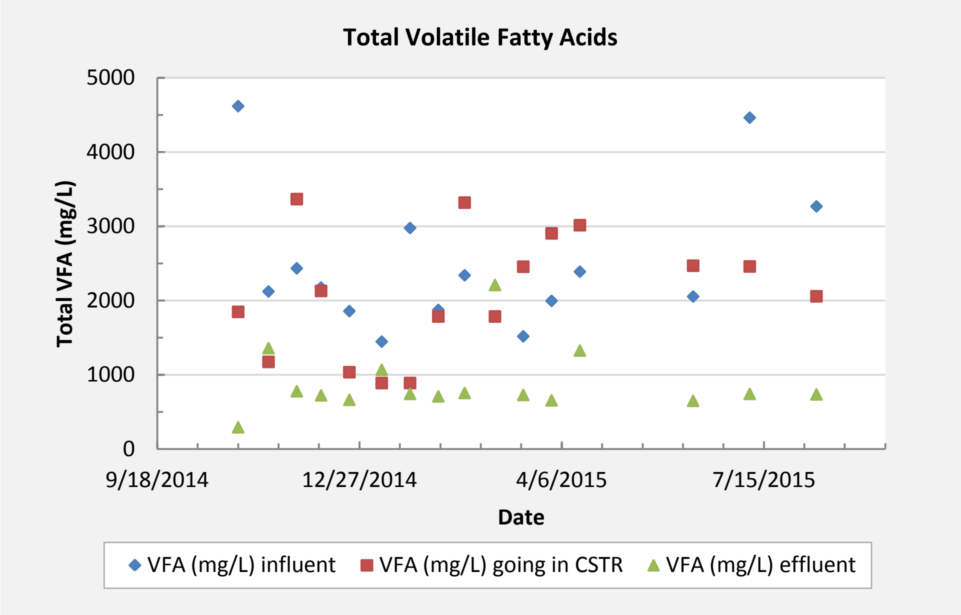


Figure 5: Total VFA concentrations of influent sludge, sludge going into the final CSTR and effluent flow of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek

It can be seen from Figure 5 that the total concentrations of VFAs going in and out the Ephyra® reactors had no certain pattern. From the analysis of samples from each reactor, the main types of VFAs measured in the digesters were propionic acid and acetic acid. Total concentration of VFAs increased in the first compartment about 59% compared to the input average value of 3965 mg/L. Then in the second compartment VFAs decreased about 30% compared to the concentrations measured in the first compartment. Then in the third compartment a slight increase of 5% is observed during most of the days, although according to values obtained on 17th and 25th of February VFAs decreased about 30%. In

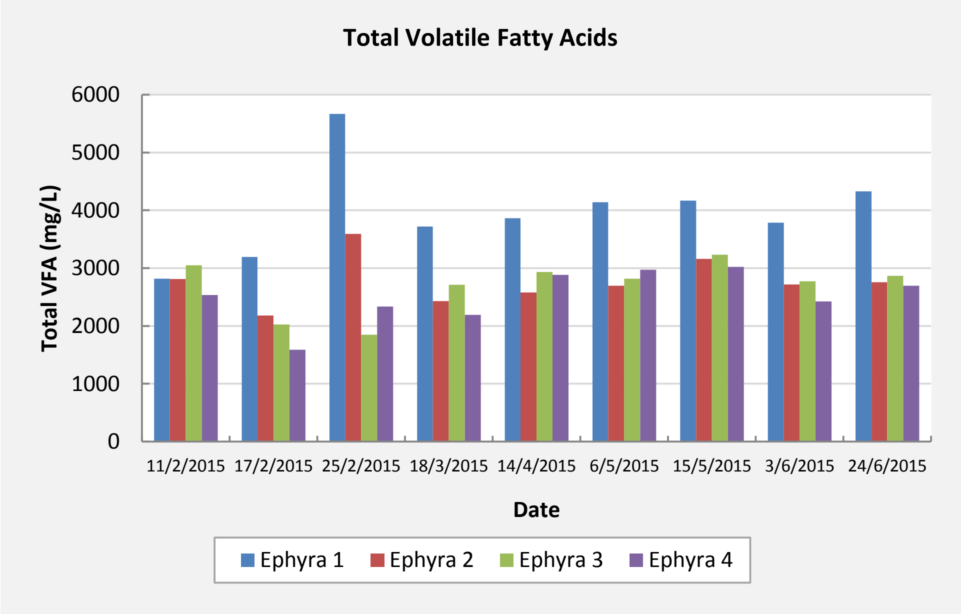


Figure 6: Total VFA concentrations in each compartment of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek

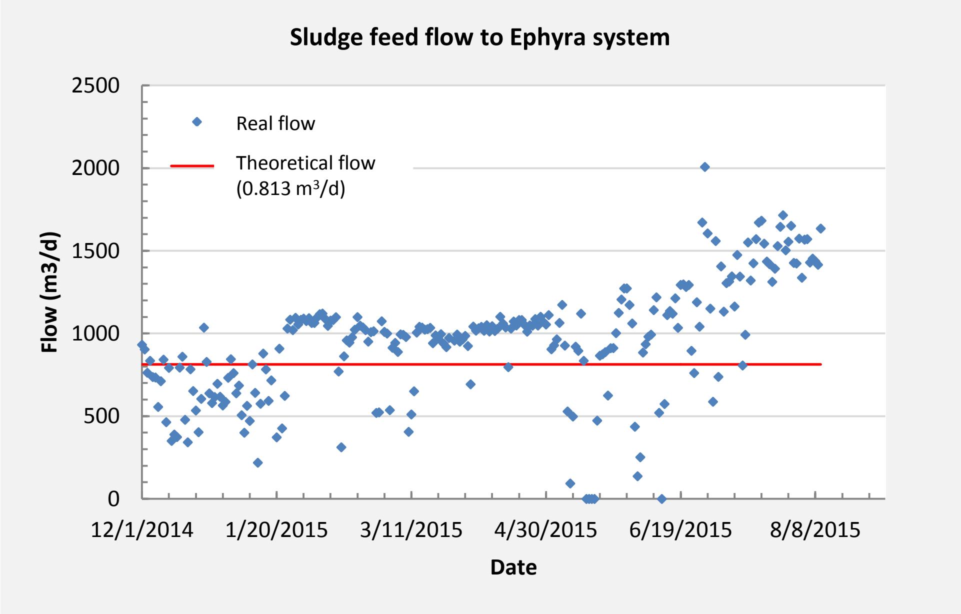


Figure 7: Sludge feed flow of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek with a theoretical flow of 0.813 m^3/d

the fourth reactor VFAs again decreased about 8% most of the days, while on 25th of February and 6th of June increased about 14%. Then in the final CSTR, VFAs are consumed and their concentration decreased about 97% compared to the average concentration of 2518 mg/L in the fourth Ephyra® compartment (Figure 6). It has to be noticed that propionic acid was present in high concentrations in all the compartments. On the other hand, acetic acid was present in high concentrations in the first compartment and was in concentrations comparable with those of propionic acid. Its concentration decreased significantly in the subsequent reactors. (see Appendix A)

In this case study, it was assumed that the hydraulic retention time (HRT) is equal to the solid retention time (SRT), as the system is considered to be an ideal plug flow system. Conversion of volatile solids to gaseous products in an anaerobic digester is controlled by HRT, which affects the rate and the extent of methane production [8]. The theoretical volume of the four Ephyra® compartments during the initial calculations was assumed to be 6.5 m^3 (1.625 m^3 each) with hydraulic retention time of 8 days (2 days each). Therefore, the feed flow was calculated to be 0.813 m^3/d. The final CSTR has a volume of 11.5 m^3 and a HRT of 14.15 days based on these calculations. The whole Ephyra® system had a theoretical HRT of 22.15 days. The reference system was designed to have the same HRT. However during the operation of the pilot scale plant the volume of sludge in each Ephyra® compartment was kept around 1.9 m^3. The feed flow of the system during the first weeks was slightly lower than the theoretical flow of 0.813 m^3/d, and later it was above this value (Figure 7). The average flow rate was 0.91 m^3/d. As a result the HRT of the system was fluctuating; it was in a range between 9 days and 71 days (**Figure 8**).

Concentrations of soluble COD did not seem to have a certain pattern through the Ephyra® reactors, and it decreased slightly in the final CSTR compared to the effluent of Ephyra® compartments (Figure 9). The total COD concentrations decrease about 33% in the effluent of Ephyra® compartments compared to the influent concentration of 67778 mg/L, and a total decrease of total COD for the whole system of about 52% based on the average values was observed. According to the dry solids measurements, the total solids concentration of the influent sludge mixture was 5.5 g/L. The calculated degradation through Ephyra® tanks based on dry solids was 28% in average, and the maximum degradation percentage observed was 64% (Figure 10). The whole system achieved a degradation of 36% in average, with a maximum degradation reaching a percentage of 69% (Figure 11). The gas flow measured in Ephyra® tanks was about 10.3 m^3/d with a methane content of 60% in average. The gas flow measured in the final CSTR was 7.3 m^3/d in average with a methane yield of 70% in average. The influent total COD concentration of the reference system was 58842 mg/L. The total COD removal efficiency of the reference system was about 48% and the gas production was about 11.4 m^3/d.

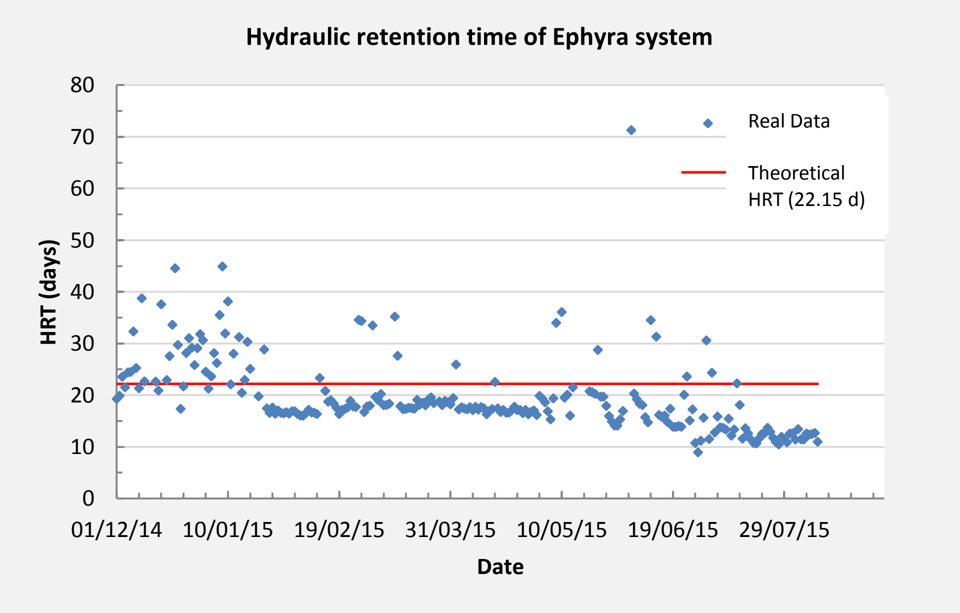


Figure 8: Hydraulic retention of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek with a theoretical HRT of 22.15 days

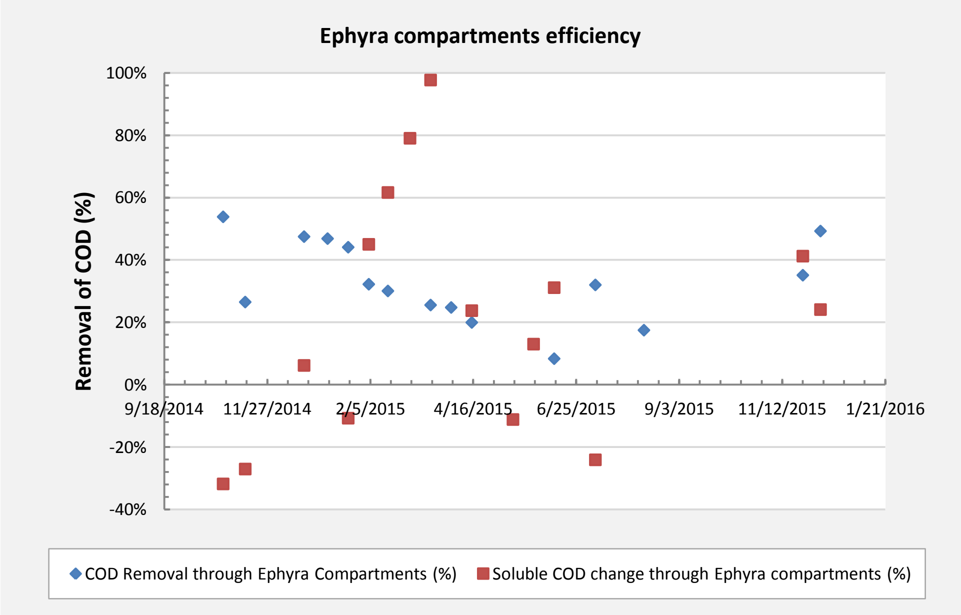


Figure 9: Removal efficiency based on total COD and soluble COD concentrations of pilot-scale Ephyra® system for anaerobic digestion of sludge from WWTP in Tollebeek

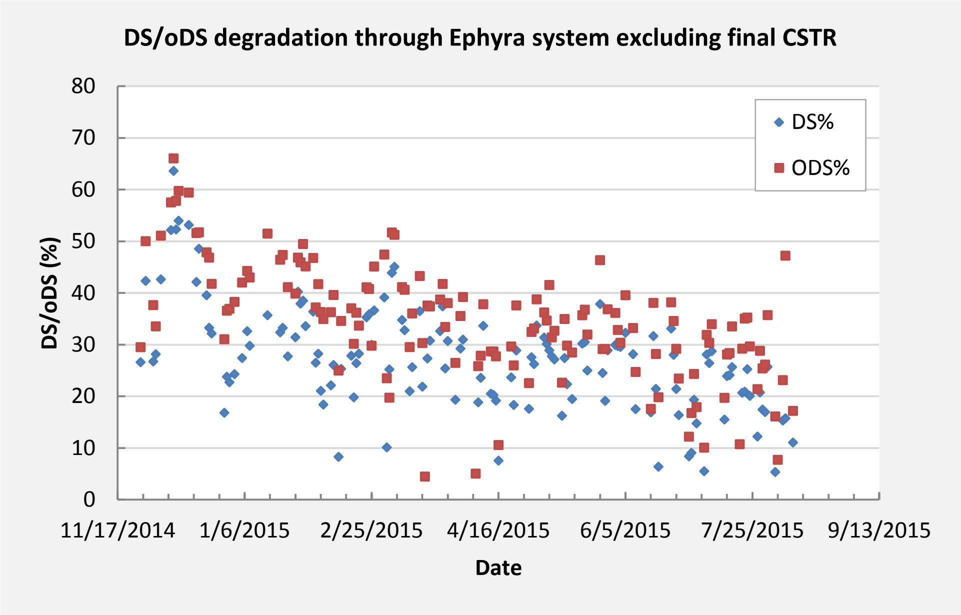


Figure 10: Degradation based on dry and organic dry solids concentrations of pilot-scale Ephyra® system excluding the final CSTR, for anaerobic digestion of sludge from WWTP in Tollebeek

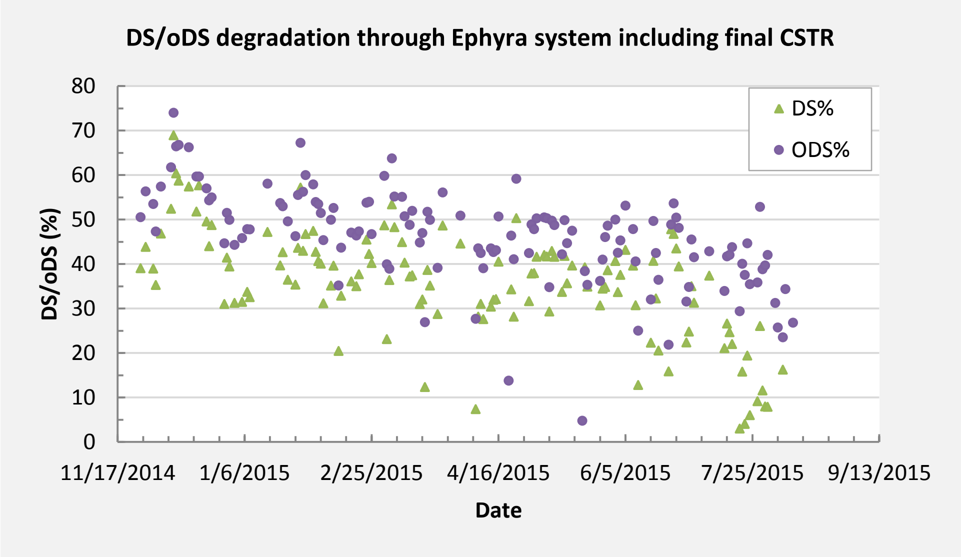


Figure 11: Degradation based on dry and organic dry solids concentrations of pilot-scale Ephyra® system including the final CSTR, for anaerobic digestion of sludge from WWTP in Tollebeek for Ephyra®system

5.2 Sensitivity analysis

The results of the sensitivity analysis conducted for Ephyra® system show that 6 model parameters may be considered for parameter estimation.

As it can be seen from Figure 12a a decrease of the k_h value by 10% does not really influence the system. Also, from the same graph it can be seen that an increase in hydrolysis rate coefficient by 10% does not influence so much the system, except from a slight increase in pH values and a small decrease in VFA concentration. However, major deviations of hydrolysis rate coefficient caused changes on the performance of the system. Therefore, hydrolysis rate is a key parameter for calibration of the model.

The results of sensitivity analysis for Ephyra® system show that other 5 parameters were influential (4 kinetic parameters and 1 wastewater fraction). The results are presented in Appendix B and in figures which follow. Based on the results these parameters are:

- *Acetoclastic maximum specific growth rate of methanogens:* an increase of this parameter causes increase in pH in 4th compartment, decrease of VFAs in 3rd and 4th compartment, and therefore increase in methane content in 3rd and 4th compartment. It also reduces VFAs concentration and gas production in the CSTR. A decrease of the default value results in a significant increase in VFAs in CSTR and decrease in methane content in 3rd and 4th compartment. (see Figure 12b)
- *H2-utilizing max specific growth rate of methanogens:* an increase of this parameter results in a decrease in VFAs concentrations in the 4th compartment and increase in methane content in the 1st compartment. Decreasing the value of this specific parameter leads to a significant decrease in gas flow and methane content in the 1st compartment. (Figure 12c)
- *Acetoclastic substrate half sat. of methanogens:* VFAs concentrations are analogous to this parameter, and has a major impact on the final CSTR. (Figure 12d)
- *Acetoclastic anaerobic decay rate of methanogens:* VFAs concentration in all the reactors is analogous to this parameter, with the most change presented in the final CSTR (Figure 12e)
- *Fbs – Readily Biodegradable (including acetate):* In general, pH and VFA concentrations are influenced by readily biodegradable fraction. As it can be seen a 10% increase of this fraction results in increase

of VFAs and decrease of pH in all compartments. It seems to influence more the operation of Ephyra$^{®}$ compartments than the final CSTR. Also, an increase in Fbs leads to increase of gas production in the final CSTR. Decrease of Fbs fraction by 10% results in a decrease in gas flow in the 1st compartment and final CSTR, and an increase in methane content in 3rd and 4th compartment. (Figure 12f)

Normalised sensitivity coefficients of stoichiometric parameters showed that even a small error of their true value didn't result in model deviation. Meanwhile, sensitivity analysis showed that the model outputs were sensitive to errors on total COD, pH, flow and alkalinity values in the influent. An error of 10% in measurements of total COD could lead to a major deviation of pH, VFA production and total COD degradation in all compartments. Also, it resulted to an alteration of gas flow and methane content in the 1st, 4th compartment and in CSTR (see Figure 12h). Flow variations influenced VFA concentration and gas production. A decrease of flow resulted in a decrease in VFA in 3rd and 4th compartment and in CSTR. But an increase of the flow mostly led to increase of VFAs concentration in CSTR. Methane content in Ephyra$^{®}$ compartments decreased while flow was increased (Figure 12g). Influent pH values influence the methane content in Ephyra$^{®}$ compartments (Figure 12i). Errors on alkalinity value mainly influence pH and methane content in Ephyra$^{®}$ compartments (Figure 12j).

Sensitivity analysis was also conducted for the reference system. The results showed that 4 kinetic parameters and 1 waste water fraction influence the performance of the system.

- *Hydrolysis rate coefficient:* an increase of 10% causes a decrease in total COD in the effluent, so the gas flow increases. A decrease of the default value causes the opposite result (Figure 13a).
- *Acetoclastic maximum specific growth rate of methanogens:* a reduction of the default value by 10% results in increase in VFA. (Figure 13b).
- *Acetoclastic substrate half sat.:* an increase of the default value results in increase in VFA concentration (Figure 13c).

  Acetoclastic anaerobic decay rate: an increase of the default value results in increase in VFA concentration, and a decrease causes the opposite result (Figure 13d).
- *Fbs – Readily Biodegradable (including acetate):* an increase of the default value results in decrease in total COD in the effluent and hence increase in gas flow (Figure 13e).

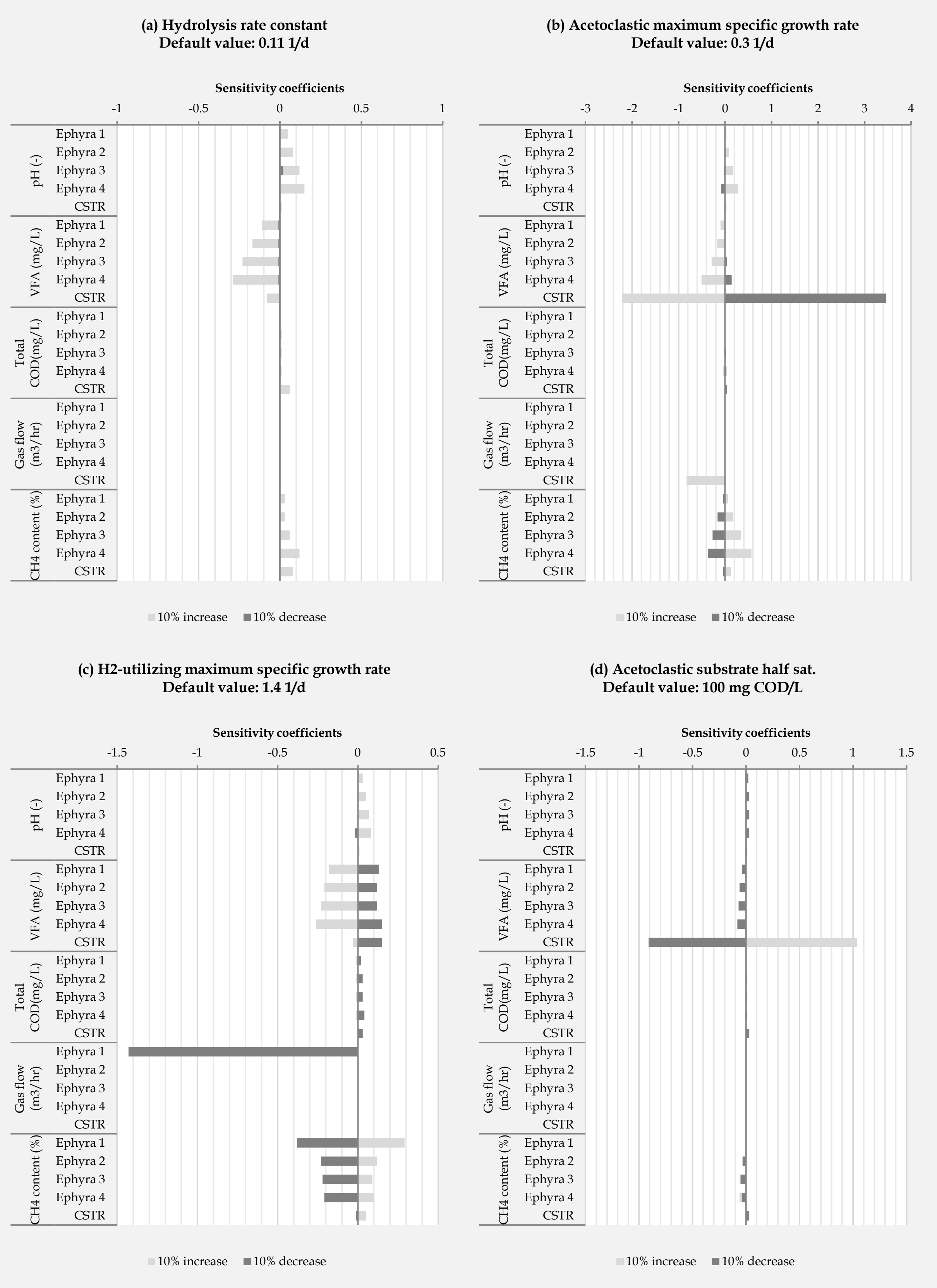
Sensitivity analysis showed that the model outputs of the reference system are sensitive to errors of total COD influent concentration and flow. An error in total COD could lead to deviations of effluent total COD and VFA production (Figure 13f). Flow variations influence VFA concentration, effluent COD and gas flow (Figure 13g).

Comparing the results of analysis, for both Ephyra$^{®}$ system and reference system, it can be seen that readily biodegradable fraction influences total COD concentration in the effluent. As it is a characteristic of the sludge, it has to be fixed in order to fit the input data. The default value of BioWin results in very high concentrations of VFA. Readily biodegradable COD fraction can be calculated according to Lei et al. (2006) as follows [40]:

$$\mathrm{F_{bs}}=\frac{[\mathrm{ffCOD_{inf}\text{-}ffCOD_{eff}}]}{[t\mathrm{COD}_{inf}]} \qquad eq.10$$

where: F_{bs} = readily biodegradable influent COD fraction (%)
$ffCOD_{inf}$ = flocculated and filtered influent COD concentration (mg/L)
$ffCOD_{eff}$ = flocculated and filtered effluent COD concentration (mg/L)
$tCOD_{inf}$ = total influent COD concentration (mg/L)

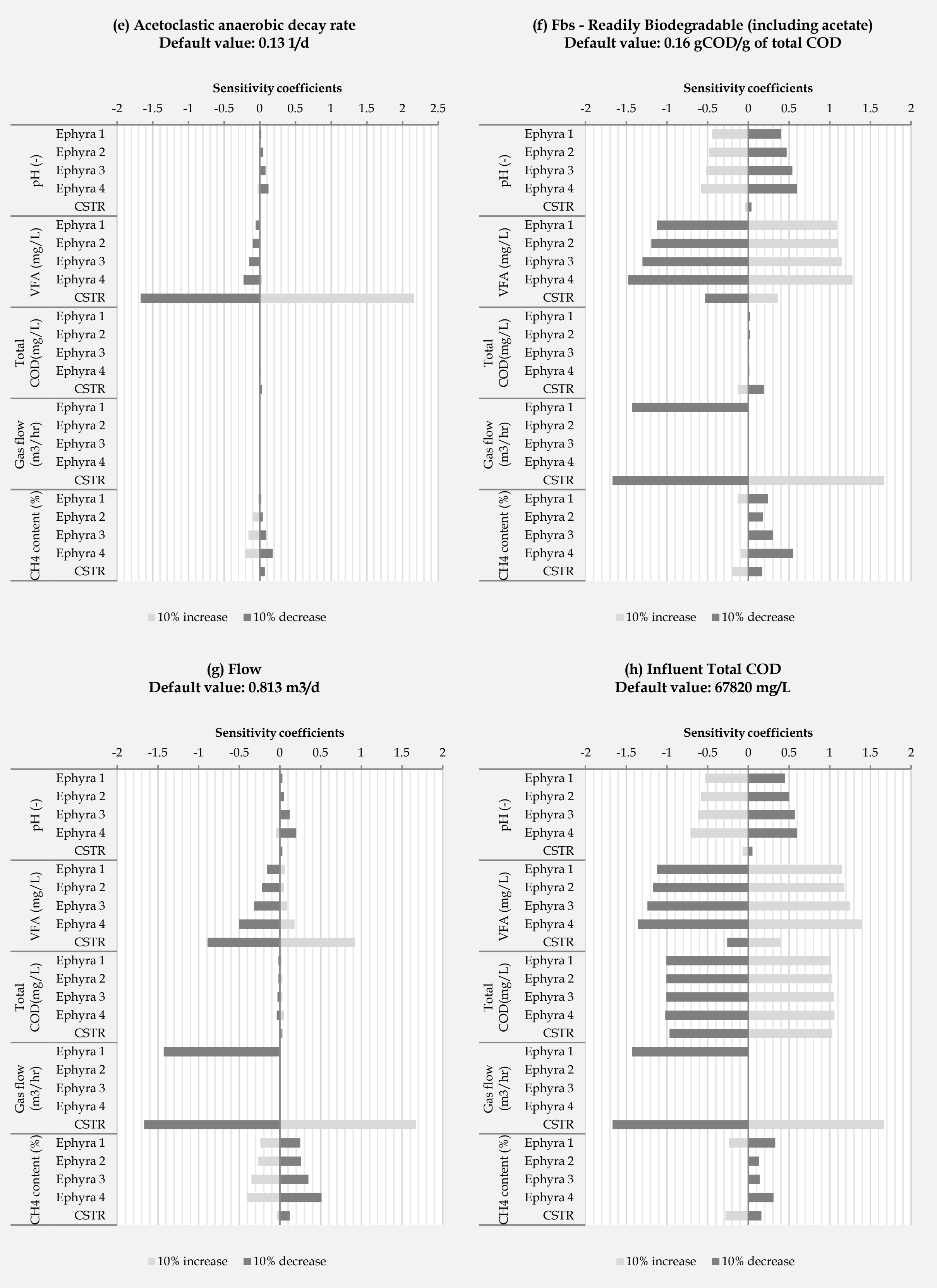
The model created at this point couldn't achieve the total COD removal efficiency which was calculated during the pilot-scale plant operation. Therefore, it was required to estimate the parameters which are


(a) Hydrolysis rate constant
Default value: 0.11 1/d

(b) Acetoclastic maximum specific growth rate
Default value: 0.3 1/d

(c) H2-utilizing maximum specific growth rate
Default value: 1.4 1/d

(d) Acetoclastic substrate half sat.
Default value: 100 mg COD/L


(e) Acetoclastic anaerobic decay rate
Default value: 0.13 1/d
(f) Fbs - Readily Biodegradable (including acetate)
Default value: 0.16 gCOD/g of total COD
(g) Flow
Default value: 0.813 m3/d
(h) Influent Total COD
Default value: 67820 mg/L
Sensitivity coefficients
-2 -1.5 -1 -0.5 0 0.5 1 1.5 2 2.5
pH (-)
VFA (mg/L)
Total COD(mg/L)
Gas flow (m3/hr)
CH4 content (%)
Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
CSTR
10% increase
10% decrease

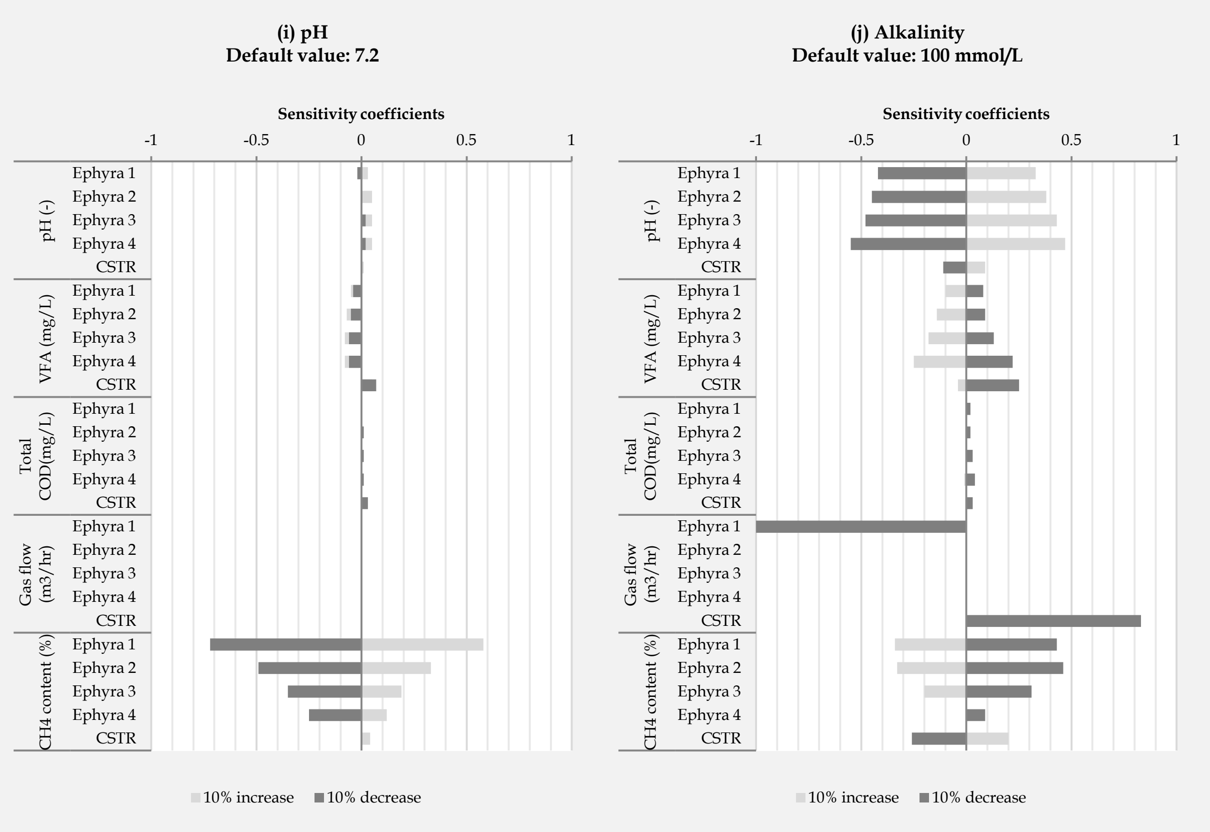
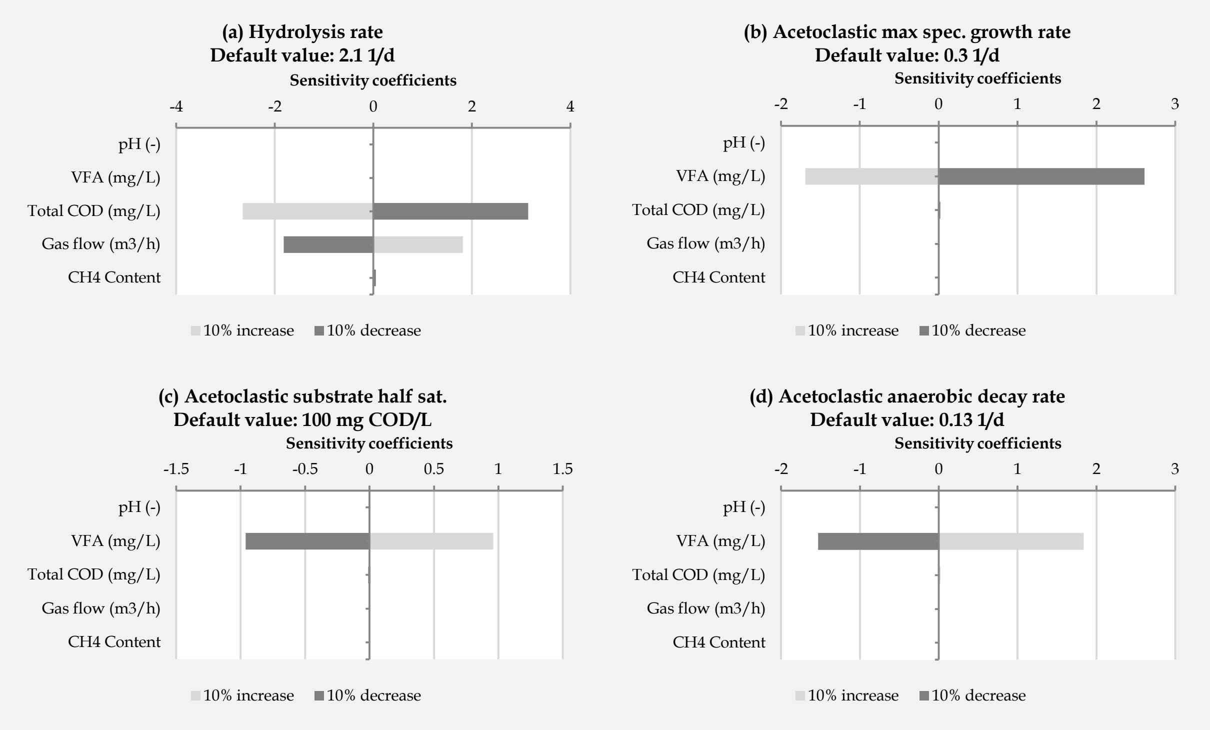


Figure 12: Sensitivity analysis results of pilot-scale Ephyra system model by changing (a) hydrolysis rate coefficient (b) acetoclasitc maximum specific growth rate (c) H_2-utilizing maximum specific growth rate (d) acetoclastic substrate half sat. (e) acetoclastic anaerobic decay rate (f) readily biodegradable fraction (g) flow (h) influent total COD (i) pH (j) alkalinity



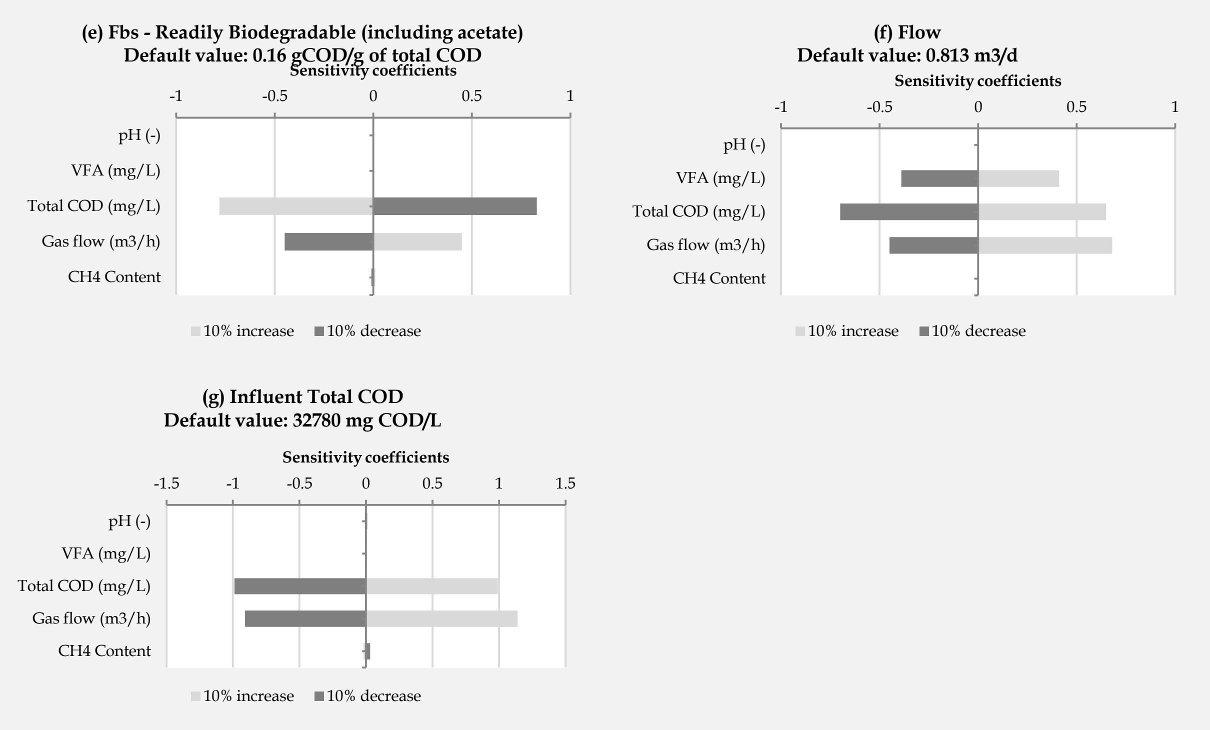


Figure 13: Sensitivity analysis results of pilot-scale reference system model by changing (a) hydrolysis rate coefficient (b) acetoclasitc maximum specific growth rate (c) acetoclastic substrate half sat. (d) acetoclastic anaerobic decay rate (e) readily biodegradable fraction (f) flow (g) influent total COD

influencing total COD concentration in the effluent. Based on the sensitivity analysis results the parameter that most influences the effluent total COD concentration was the hydrolysis rate coefficient. Since hydrolysis is the first step and it is also believed to be the rate-limiting step of anaerobic diigestion process, it was decided that both models were going to be calibrated by adjusting hydrolysis rate. Then, in order to have a better fit of the model outcome to the real data further parameters could be changed.

5.3 Lab research results

To generate more information related with Ephyra® a new lab research was undertaken, using sludge mixture from Amsterdam wastewater treatment plant. The sludge mixture was comprised 50% from primary sludge and 50% from secondary sludge based on dry solids concentration. The influent characteristics of the sludge mixture were determined and are shown in Table 5. All the measurements taken can be found in Appendix C.

The initial mixture of primary and secondary sludge had a pH of 6.0. So, it was decided to add a small amount of sodium hydroxide (NaOH) in order to increase the pH up to 6.5. Measurements of pH, which were taken twice a week in each reactor along with temperature measurement, showed that the pH was between a range of 7.2 and 7.5 in all the reactors. These values were considered high compared with the pH values measured during the pilot-scale plant. Only the last day when the system was shut down the pH was in a range of 6.7 and 6.8 in all the reactors. As it is mentioned in a previous section, alkalinity values weren't available for Tollebeek and Lelystad sludge. Alkalinity of Amsterdam sludge was measured about 60 mmol/L (≃3 g $CaCO_3$/L). This value is 2 g $CaCO_3$/L lower than the alkalinity value which was set in the model during the sensitivity analysis. Hence, this particular influent parameter could be further adjusted during the calibration pocess.

Total COD concentration of the influent was about 30% lower than the average influent concentration of total COD measured during the pilot scale plant operation. As it can be seen from Table 7, a high deviation was observed in measurements of total and soluble COD concentrations which indicated that there was an error in measurements. This error in measurements could be a result of the sampling and preparation method. During the period that samples were obtained for analysis different incidents, like overflow of the Ephyra® system due to blocking of tubes and temperature lower than 35°C due to low flow of heated water in the tubes around the reactors, disturbed the operation of the system. Another hypothesis justifying this deviation is that the mixture of sludge, which was prepared every two weeks with new sludge obtained from Amsterdam's WWTP, had different characteristics.

Soluble COD concentrations didn't have a certain pattern for both Ephyra® and reference systems as it can be seen from the measurements in Appendix C. This was also an observation which was also seen during the pilot scale plant operation. Soluble COD concentrations in the Ephyra® compartments were high during the first two weeks when the measurements took place. The highest soluble COD concentration measured was 3.8 g/L, and then the concentrations decreased at about 0.8 g/L after four weeks. The high deviation of soluble COD concentrations in combination with the values of pH measured in each reactor might be an indication that the system the first weeks when the measurements took place was not yet in steady state conditions . Based on the measurements for the Ephyra® system taken at the lab of Royal HaskoningDHV, which are shown in Table 7 and Table 8Table 8, the reduction of total COD concentrations and degradation of dry solids took place mainly in the first compartment. A reduction of total COD concentration of about 43% through the Ephyra® compartments was observed, where 39% of total COD removal took place in the first reactor. About 45% removal of total COD concentration was achieved through the whole system in average. Based on the dry solids values in Table 8 a degradation of about 40% was achieved through the Ephyra® compartments and about 44% through the whole system. The fact that reduction of total COD was achieved mainly in the first compartment may be caused either due to blocking of the tubes connecting the compartments, or due too high recirculation of sludge from the third to the first compartment. Another reason it might be that the influent sludge had a very high readily biodegradable fraction of COD which was immediately hydrolysed and utilized in the first tank. The measurements of total COD for the reference system showed a reduction of about 44% in average and a degradation of dry solids of about 42% in average. Therefore, it could be concluded that the Ephyra® system could achieve a slightly higher degradation than the reference system.

Table 5: Average values of influent characteristics of sludge mixure from Amsterdam WWTP of lab-scale systems

Parameter	Unit	Average values
Total COD	mg/l	52325
Soluble COD	mg/l	4114
TP	mg/l	1025
pH (after NaOH)	-	6.5
Alkalinity	mg $CaCO_3$/l	2958
NH_4-N	mg/l	499
PO_4-P	mg/l	525
Ca	mg/l	216
Mg	mg/l	389

Table 6: Gas production and methane yield in Ephyra compartments of lab-scale system using Amsterdam sludge

Element	Gas production (L/day)	Methane yield (%)
Ephyra 1	1.85 ± 0.98	56.0 ± 3.8
Ephyra 2	1.65 ± 0.67	61.2 ± 2.9
Ephyra 3	1.36 ± 0.22	59.7 ± 3.4

Table 7: Total and soluble COD measurements in each tank of lab-scale Ephyra system using Amsterdam sludge

Element	Total COD (g/L)	Soluble COD (g/L)
Feed	52.6 ± 3.3	4.1 ± 2.0
Ephyra 1	32.0 ± 2.9	1.9 ± 1.2
Ephyra 2	31.4 ± 2.7	1.7 ± 1.3
Ephyra 3	30.0 ± 1.9	1.8 ± 1.3
Ephyra CSTR	29.0 ± 3.8	1.5 ± 1.2

Table 8: Dry and volatile solids measurements in each tank of lab-scale Ephyra system using Amsterdam sludge

Element	Dry Solids (%)	Volatile Solids (%)
Feed	4.1 ± 0.2	81.3 ± 1.5
Ephyra 1	2.4 ± 0.1	69.1 ± 0.3
Ephyra 2	2.4 ± 0.2	68.7 ± 0.5
Ephyra 3	2.5 ± 0.1	69.3 ± 0.7
Ephyra CSTR	2.3 ± 0.0	65.7 ± 0.3
Reference	2.4 ± 0.0	67.3 ± 0.8

Gas from each tank was collected in gasbags in order to measure the volume and the methane content. It has to be mentioned that it wasn't possible to measure the gas production of the final Ephyra® CSTR and of the CSTR of the reference system due to leakage. There was also a problem with measuring gas flow in the 3rd compartment as most of the time there was leakage or gas flow wasn't high enough.Analysis of the measurements of biogas production showed that the gas production of the 2nd tank was higher than the 1st one the first two times that the gas production of each tank was measured. However, in later measurements, gas generation was higher in the first compartment. As it can be seen by the average values in Table 6, gas production was higher in the 1st tank which is expected as degradation of easily biodegradable COD takes place mainly in the first compartment, and then it decreased in the subsequent reactors. Hence, the results of total COD, dry solids and organic dry solids were in correlation with the measurements of biogas production in each tank. Even if gas production was higher in the 1st tank the methane yield in the 1st tank was lower than the 2nd tank. Then CH_4 yield seemed to decrease again in the 3rd tank compared to the methane potential in the 2nd tank. However due to the problems faced when collecting biogas from the 3rd reactor these values cannot be considered reliable.

The result of VFA analysis showed that there was no accumulation of acids inside the reactors. The analysis was conducted three times for both systems with a dilution factor of 2. Each time the outcome of the analysis was the same.

The day that both systems were shut down all the sludge was collected in order to conduct the BMP tests. When the compartments were emptied, struvite precipitation was observed inside the first reactor. The results of the BMP tests can be seen in Figure 14, which shows the cumulative normalized gas production over time for all the assays. From Figure 14, it can be seen that the sludge from Ephyra® 1 is more active than the other inoculums as it generated a higher gas production compared to the other reactors. This outcome was consistent with the measurements of total COD concentrations, dry solids, organic dry solids and gas production.

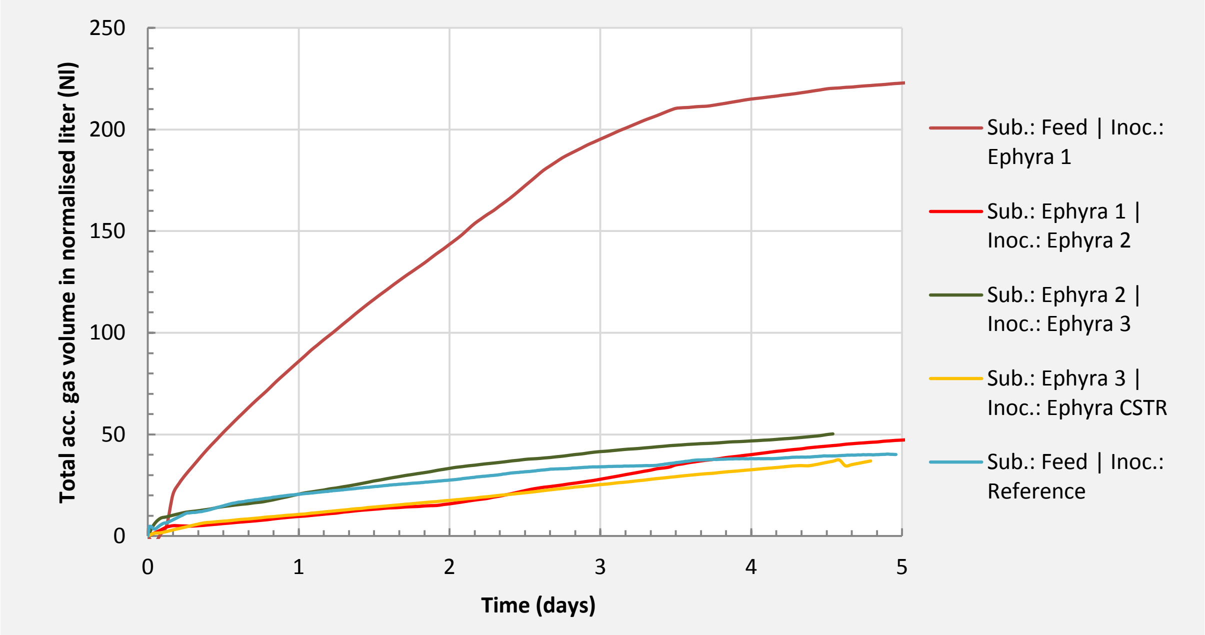


Figure 14: Cumulative normalized gas production for all the BMP assays

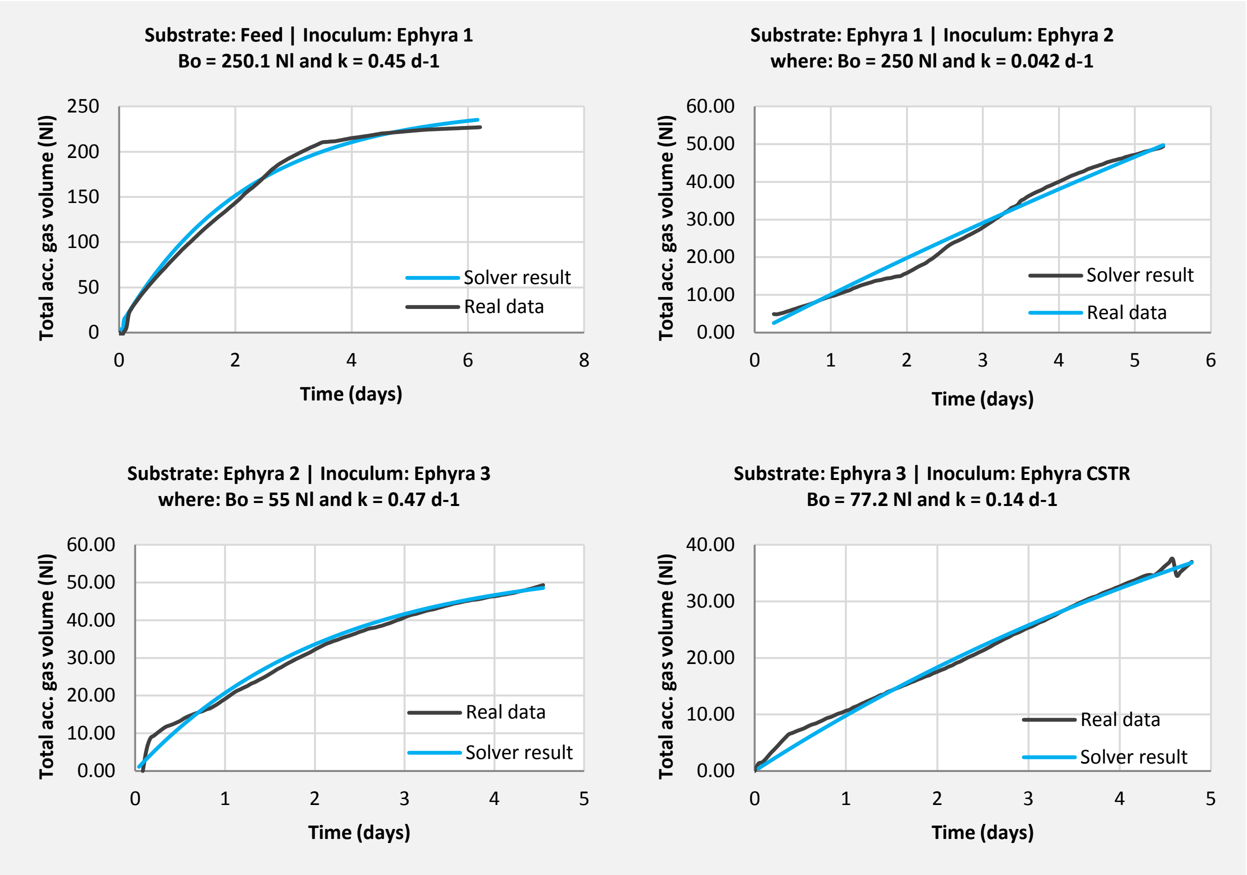
The main purpose of conducting the BMP tests was to determine the hydrolysis rate coefficient of each compartment and compare hydrolysis rate coefficients between Ephyra® and reference system. On the base of the initial approach, the data obtained from the BMP test and the tool *Solver* of Excel were used in order to find the optimal solution of equation 9. The outcome is presented in Figure 15 and Figure 16. The results presented in Table 10 show that this method was appropriate for all the cases, except for the case where sludge from Ephyra 2 was used as inoculum. For this particular case it can be seen from Table 10 that k_h was very low. A reason of this low hydrolysis rate coefficient could be the unexpected high concentration of dry solids which is listed in Table 9 (8.3%), which is not consistent with the measurements of dry solids shown in Table 8. The method could be applicable for this case if the BMP tests could run for more days. So it was decided for this case to define the hydrolysis rate coefficient as the slope of the linear part using a trend line. The slope calculation gave a hydrolysis rate coefficient is 0.43 d^{-1}. It could be concluded that hydrolysis rate coefficient is the same in the Ephyra® compartments and then in the final CSTR is lower.

Table 9: Total and volatile solids of each substrate and inoculum used

Sample	TS (%)	VS (%)
Feed 1	3.4	80.8
Ephyra 1	5.4	47.1
Ephyra 2	8.3	43.0
Ephyra 3	3.6	50.3
Ephyra CSTR	2.0	64.5
Feed 2	3.0	78.7
Reference	2.4	70.1

Table 10: Hydrolysis rate coefficient calculated from BMP test using fitting of Gompertz equation

Substrate	Inoculum	k_h (day)
Feed	Ephyra 1	0.45
Ephyra 1	Ephyra 2	0.04
Ephyra 2	Ephyra 3	0.47
Ephyra 3	Ephyra CSTR	0.14
Feed	Reference	0.67


Substrate: Feed | Inoculum: Ephyra 1
Bo = 250.1 Nl and k = 0.45 d-1

Substrate: Ephyra 1 | Inoculum: Ephyra 2
where: Bo = 250 Nl and k = 0.042 d-1

Substrate: Ephyra 2 | Inoculum: Ephyra 3
where: Bo = 55 Nl and k = 0.47 d-1

Substrate: Ephyra 3 | Inoculum: Ephyra CSTR
Bo = 77.2 Nl and k = 0.14 d-1

Figure 15: Optimal solution (B_0 and k_h) of Gompertz equation for each assay of the lab scale the Ephyra® system using the tool *Solver* of Excel

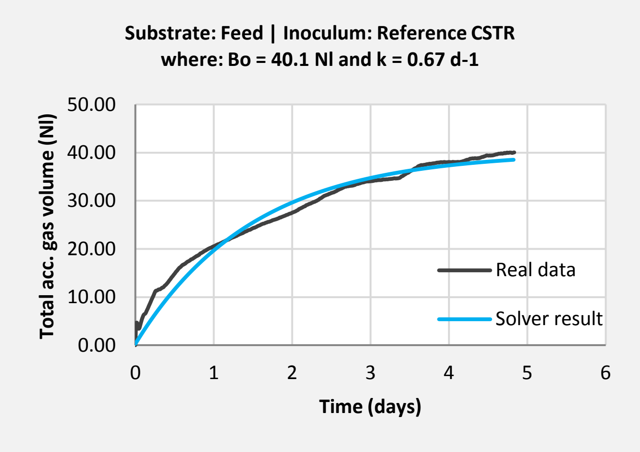


Figure 16: Optimal solution (B_0 and k_h) of Gompertz equation for assay of the lab scale reference system using the tool *Solver* of Excel

Even if a lower hydrolysis rate was expected in the reference system, the analysis of the data showed that the CSTR of the reference system had a higher hydrolysis rate coefficient than the Ephyra® system reactors. It can be seen from Figure 16 that at about 3.5 days a sudden increase was observed on gas production. It was attempted to determine the hydrolysis rate coefficient again by subtracting that increase and by ignoring that certain part, but in both cases the hydrolysis rate coefficient was higher than 0.67 d^{-1}.

At the time that the research was carried out many guidelines were available for standarization of a BMP test protocol, and as it is mentioned in a previous section hydrolysis rate coefficient could be defined as the slope of the linear curve of the cumulative gas production over time obtained the first 5 days. However, an article by Holliger et al. (2016) was published after the end of the lab research which provides further guidelines that have to be followed for a successful BMP test. According to the article the test finishes when daily methane production during three consecutive days is smaller than 1% of the accumulated volume of methane. Also, it is stated that inoculum should be taken from an active anaerobic digester at steady state which is digesting complex organic matter. Characteristics that define a good inoculum quality are pH between 7.0 and 8.5, NH_4^+ concentration smaller than 2.5 g $N\text{-}NH_4$/L, VFA concentration smaller than 1.0 g CH_3COOH/L, and an alkalinity higher than 3 g$CaCO_3$/L [37]. Concentration of acetate should be measured in order to verify whether hydrolysis or methanogenesis is the rate limiting step. When there is accumulation of soluble organic matter then methanogenesis is the rate limiting step [37] [41]. For this case where hydrolysis rate coefficient needed to be defined, it should be made sure that hydrolysis was controlling the overall rate of the reaction. The result of VFA analysis using samples from each reactor during the operation of the lab-scale system showed that there was no accumulation of acids inside the reactors. But, the concentration of VFA wasn't measured before starting the BMP tests. So, it is unclear whether hydrolysis was the rate-limiting step. Even if hydrolysis was the rate-limiting step the results may be unrealistic as the content of the lab reactors was chosen as the inoculum for the BMP tests, instead of active digested sludge from a full-scale plant. Also, because the AMPTS machine has only 15 slots and it was needed to conduct the BMP assays for five different substrates, some of the sludge was stored in the fridge at 4°C for more than five days. The sludge from the CSTR of the lab reference system was stored for at least 1.5 week in the fridge at 4°C. This may altered the outcome of the tests.

5.4 Model application

5.4.1 *Calibration process*

As mentioned before, it was decided to calibrate both systems by adjusting hydrolysis rate coefficient, which it is the most influential of all parameters for estimation. Then, in order to have a better fit of the data further parameters were changed. In the model calculations for struvite precipitation and stripping of ammonia were also included.

BioWin model simplifies the hydrolysis process by using one common hydrolysis rate coefficient and it is modelled based on Monod kinetics. Ephyra® technology is a pre-treatment step to enhance anaerobic digestion performance. Calibration of the model showed that hydrolysis rate is a crucial parameter for the efficiency of the process. It increases solubilisation of the organic substances and more volatile solids become biodegradable.

First the Ephyra® system model was generated using as influent characteristics the values presented in Table 1. The Ephyra® compartment characteristics were adjusted according to the theoretical values, which were calculated in the initial calculations by the company (volume: 1.623 m^3, headspace: 0.5 m^3, depth 2.0 m). The flow rate was also set at the calculated value (=0.813 m^3/d). At this point, no attention was given at the output of the final CSTR as it was considered more appropriate to focus on the Ephyra® compartments and try to find out how they are interrelated. After a series of steady state simulations, it was realised that the model output didn't change significantly when hydrolysis rate coefficient was increased above a certain value while it was kept the same in all four reactors. The maximum reduction of total COD concentration achieved was 22%, contradicting with the pilot and lab experiments where 33% was found.

So, it was decided to simulate Ephyra® as one compartment with a volume of 6.5 m^3 in order to find which hydrolysis rate coefficient gives a total COD concentration in the effluent of the reactor close to that of the real measurements of the pilot-scale plant. After a number of steady simulations it was found that a hydrolysis rate coefficient of 1.35 d^{-1} was needed to achieve the reduction of total COD required. Then, two models with two and three Ephyra® reactors respectively were developed, with a total volume of all the reactors of 6.5 m^3. Calibration process showed that hydrolysis rate coefficient should not be the same in all the reactors but it should increase gradually in order to achieve the reduction of total COD concentration required. As mentioned before, hydrolysis rate coefficient is a characteristic of the substrate and is affected by various parameters. In literature, studies on two-stage anaerobic digestion and plug-flow reactors can be found ([15] [16] [42] [43] [44] [45] [46]). However, none of them mentions or questions the possibility of different hydrolysis rate coefficients in the compartments.

Hydrolysis process is the least defined process in anaerobic digestion. It is usually considered to be the rate-limiting step of anaerobic digestion due to lack of availability of free accessible surface area of the particles, and not because of lack of enzyme activity [6]. Hydrolysis rate coefficient depends on various parameters such as pH, temperature, retention time, and the presence of other compounds. According to Chen et al. (2006) hydrolysis rate was increased in different environments at room temperatures in the following order: neutral, acidic, alkaline, and found that hydrolysis rate increases with pH [47]. Substrate is a complex organic material constituting of carbohydrates, proteins and lipids. Hydrolysis is carried out by exo-enzymes called hydrolases and different processes take place during hydrolysis, as each particulate has its own hydrolysis rate coefficient and each one is affected differently by other parameters [48]. Moreda (2016) mentions that the nature of substrate might affect hydrolysis rate coefficient. He cites as an example the need for specific enzymes which have to be generated in the inoculum to hydrolyse lipids, and as a result a lower hydrolysis rate coefficient is expected in a nonacclimated inoculum [49]. Primary sludge and secondary sludge (waste activated sludge, WAS) have very different hydrolytic characteristics. Secondary sludge cannot be hydrolysed as easy as primary sludge, because it is more difficult to hydrolyse living biomass biologically. That is the reason why hydrolysis rate coefficient of secondary sludge can be one order of magnitude lower than primary [50] [51].

Having in consideration all these information the possibility of different hydrolysis rate coefficients in each compartment can be justified if the effluent of each tank, which is the influent of the next compartment, is considered to be substrate with significant differences in its composition. As mentioned in model description, BioWin considers one hydrolysis rate coefficient for all different particulates of the substrate. In this case, a hypothesis is that hydrolysis rate coefficient in each compartment is related with a different particulate of the substrate. When two substrates are present in one reactor microorganisms consume first the preferred substrate and when it is completely consumed then they start consuming the less preferred substrate [52]. Another point that has to be noticed is that the sludge fed in the pilot-scale plant contained higher concentrations of lipids compared with a “normal” sludge because effluent from a fishery was disposed in the sewer which transported wastewater to the treatment plant. As mentioned above lipids require specific enzymes to be hydrolysed. Therefore, another speculation was that these enzymes might need time to be generated and thus the hydrolysis rate coefficient in the 1st and 2nd tank was lower.

However, having in regard the results of the BMP test the scenario of rise of hydrolysis rate coefficient through the compartments was discarded. According to Labatut et al (2011) hydrolysis rate coefficient calculated from BMP tests cannot be used for modelling continuous digester as they are conducted under diluted conditions and they predict lower methane production [53]. Jimenez et al. (2015) mention that a drawback of BMP tests is the lack of ability to predict continuous digester performances [36]. But these do not reject the fact that the results of the BMP tests conducted using the sludge from the lab-scale plant show that hydrolysis rate coefficient is the same in all the Ephyra® reactors.

In order to achieve the degradation required, hydrolysis rate coefficient should be increased higher than the default value of the model (1.05 d^{-1}). However, even with a high value of alkalinity in the feed (5 $gCaCO_3/L$) and a pH value of 7.2, the increase of hydrolysis rate coefficient without changing any other parameter resulted in collapse of the model as the reactors acidify. Observing the results of sensitivity analysis and the equations based on which the model is built (see Appendix D) it can be seen that methanogens also play a significant role on the model output. The growth rates, half saturation and anaerobic decay values of methanogens found in literature are presented in Table 11 and Table 12. The default values of maximum specific growth rate of acetoclastic and hydrogenetrophic methanogens in BioWin are 0.3 d^{-1} and 1.4 d^{-1} respectively. Comparing the default values with the values in Table 11 it could be concluded that there was potential for further increase of their value.

By the end of the calibration process, hydrolysis rate coefficient k_h was set at 1.35 d^{-1} in all the Ephyra® compartments. The hydrolysis rate coefficient of the final CSTR had to be lower than the Ephyra® tanks to fit better the data (0.825 d^{-1}). The acetoclastic methanogens’ growth rate was adjusted at 0.46 d^{-1}. The wastewater fractions were set as the default values for raw wastewater, except from the readily biodegradable fraction (F_{bs}) and the fraction of the readily biodegradable COD which is VFAs (F_{ac}). Using equation 2 and the data from the pilot scale the average value of Fbs fraction was estimated about 0.05 [-]. Also, the fraction of readily biodegradable COD which is VFAs was calculated using equation 3 and was adjusted at 0.50 [-]. The fraction of influent TKN which is ammonia was decreased from 0.66 to 0.15. Other kinetic parameters which were changed are acetoclastic substrate half saturation and acetoclastic anaerobic decay which were changed from 100 mgCOD/L and 0.13 d^{-1} to 90 mgCOD/L and 0.12 d^{-1} respectively for all the reactors.

Table 11: Maximum growth rates of methanogens under mesophilic conditions according to literature

Microorganism		μ_{max} (d^{-1})	Reference
Methanosarcina (acetoclastic)	barkeri	0.46-0.69	A.J.M. Stams, et. al. (2003) [54]
	mazei	0.49-0.53	
Methanosaeta (acetoclastic)	soehngenii	0.08-0.29	
	concilii	0.21-0.69	
Methanosacrina (acetoclastic)		0.12	Van Lier, et al. (2008) [6]
Methanosaeta (acetoclastic)		0.71	
Hydrogenotrophic methanogens		2.85	
Hydrogenotrophic methanogens		3.02	Koster & Koomen (1988) [55]
Acetoclasic methanogens		0.33	D.J. Batstone, et al. (2002) [56]
Hydrogenetrophic methanogens		2.00	

Table 12: Half saturation and anaerobic decay values of methanogens under mesophilic conditions based on literature

Microorganism	K_s (mgCOD/L)	k_d (d^{-1})	Reference
Methanosacrina	30		Van Lier, et al. (2008) [6]
Methanosaeta	300		
Methanosacrina	200-280		Shah, et al. (2014) [57]
Methanosaeta	10-50		
Methanosacrina	280	0.098	Conklin, et al. (2006) [58]
Methanosaeta	49	0.0064	
Acetoclastic	165-930	0.011-0.037	Gujer & Zehnder (1983) [59]
Hydrogenotrophic	0.6		
Acetoclastic	30-965		Vavilin & Lokshina (1996) [60]

Data analysis showed that the concentration of total VFAs remained high in compartments, mainly the concentration of propionic acid. Examining the equations used by BioWin it can be seen that the concentration of propionate depends on the activity of the propionic acetogens (Appendix D). Stams et al. (1992) explain that propionic acetogens growth depends on the type of methanogens. They measured growth rates under thermophilic conditions between 0.25 d^{-1} to 0.32 d^{-1} in cultures with both acetoclastic and hydrogenotrophic methanogens, while in cultures with hydrogenotrophic methanogens lower growth rates between 0.07 d^{-1} and 0.19 d^{-1} were determined [61]. A dissertation on propionic acid degradation under anaerobic conditions reports that propionic acetogens in co-culture with hydrogenotrophic methanogens had growth rates in a range between 0.06 d^{-1} and 0.19 d^{-1}, and can reach even lower growth rates under sulfate-reducing conditions from 0.024 d^{-1} to 0.069 d^{-1} [62]. Therefore, the growth rate of propionic acetogens was decided to be decreased from 0.25 d^{-1} to 0.22d^{-1}. Adjusting the activity of microorganisms involved in the anaerobic digestion made it possible to decrease the alkalinity and pH of the influent to lower values, at 2 $gCaCO_3/L$ and 6.0 [-] respectively. All the adjusted kinetic parameters of the Ephyra® system can be found in Table 15.

Table 13: Growth rates of propionic acetogens according to literature

Prevail methanogens	Conditions	Growth rate (d^{-1})	Reference
both acetoclastic and hydrogenotrophic	thermophilic	0.25 - 0.32	Stams et al. (1992) [61]
hydrogenotrophic	thermophilic	0.07 - 0.19	
hydrogenotrophic	mesophilic	0.06 - 0.19	M. Felchner-Zwirello (2013) [62]
hydrogenotrophic	sulfate-reducing conditions	0.024 - 0.069	

Table 14: Adjusted influent wastewater fractions for Ephyra® system model

Fraction name	Default	Adjusted
Fraction of total influent COD which is readily biodegradable	0.16	0.05
Fraction of readily biodegradable COD which is VFAs	0.15	0.50
Fraction of influent TKN which is ammonia	0.66	0.15

Table 15: Calibrated kinetic parameters for Ephyra® system model

Name	Default	Adjusted
Hydrolysis rate coefficient (Ephyra tanks) [d^{-1}]	1.05	1.35 d^{-1}
Hydrolysis rate coefficient (final CSTR) [d^{-1}]	1.05	0.825 d^{-1}
Acetoclastic methanogens max. spec. growth rate [d^{-1}]	0.30	0.46 d^{-1}
Acetoclastic substrate half saturation [mgCOD/L]	100.00	90.00
Acetoclastic anaerobic decay [d^{-1}]	0.13	0.12

Calibration process showed that increasing only hydrolysis rate coefficient or acetoclastic methanogens growth rate resulted in collapse of the system. High concentrations of acids could significantly change the substrates available for methanogens and their activity. In anaerobic digestion process is necessary that all the steps are balanced because if one stage is inhibited then the other stages are also inhibited. For example if the hydrolysis step is inhibited then methane production is decreased as the substrate for the next stages will be limited. And when methanogenesis is inhibited then acids produced in previous stages are accumulating and the system collapses [8]. So, increasing only the hydrolysis rate coefficient resulted in VFA accumulation and methanogenesis became the rate-limiting step. On the other hand when acetoclastic methanogens growth rate was increased while hydrolysis rate remained constant, soluble organic matter was consumed, and hydrolysis became the rate-limiting step.

Based on the concentration of methanogens in Table 16, acetoclastic methanogens concentration prevails over that of hydrogenotrophic methanogens. There was potential for increase of the hydrogenotrophic methanogens growth rate based on the values in Table 11. An attemp was made to change this growth rate, but it didn't affect the model output in respect to COD concentrations, VFA concentrations and gas production. So, the growth rate of hydrogenotrophic methanogens wasn’t changed and it was preferred to keep it as its default value. After the end of the model calibration and simulations, another research which was running at the time showed that hydrogenotrophic organisms were the dominant methanogens in all the reactors of the pilot-scale plant.

The graphs of output of the final calibrated Ephyra® model is presented in Appendix E. Although, model’s pH values were higher than those observed in the real case, the BioWin model predicted all the other variables. The steady state simulation gave a total COD concentration reduction in the effluent of

Ephyra® tanks of 33%, which is the same efficiency measured during the operation of the pilot-scale plant. The whole system achieved a total reduction of 52% in average. The dynamic simulation using the daily flows measured at the pilot-scale plant show that 11 out of 16 measurements have a relative difference with the model output smaller than 10%, 12 out of 16 measurements have a difference smaller than 15 %, and only 4 out of 16 have a difference between 15% and 20%. Total COD concentration is decreased gradually through Ephyra® compartments. Reduction efficiency of each tank is about 10% when comparing its effluent total COD concentration with the concentration of the previous tank, except from the 1st tank which removes about 8% of the influent total COD concentration.

Table 16: Steady state solution of Ephyra® model

Elements	Eph1	Eph2	Eph3	Eph4	CSTR
pH [-]	6.9	7.1	7.1	7.1	7.3
Total COD [mg/L]	62646	56083	5065/	45164	32586
Volatile fatty acids [mg/L]	4313	2737	2551	2284	27
Acetate [mgCOD/L]	2677	513	90	66	22
Meth. - acetoclastic [mgCOD/L]	220	558	694	785	491
Meth.-hydrogenotr. [mgCOD/L]	163	254	333	406	270
Heterotr. Organ. (OHO) [mgCOD/L]	1545	1569	1589	1608	830
Polyph. accum. org. (PAO) [mgCOD/L]	6	6	5	5	3
Propionate [mgCOD/L]	1636	2224	2461	2218	5
Propionic acetogens [mgCOD/L]	30	36	70	136	217
Off gas flow rate [m^3/hr]	0.09	0.16	0.14	0.14	0.29
Off gas Methane [%]	66	65	62	62	65

The graphs presenting VFAs concentrations in Appendix E show a good correlation with the real data from the Ephyra system of the pilot plant, except from the first reactor where the model gave VFA concentration about 1000 mg/L higher than the real data in almost half of the measurements. Observing the composition of VFA concentration, i.e. the concentrations of acetate and propionate, these higher concentrations of VFA in the 1st reactor of the Ephyra® system were a result of higher acetate concentration. In all the other reactors of the Ephyra® system, both acetate and propionate concentrations have a good correlation with real data. During the pilot scale plant operation, the high concentrations of propionate in the reactors wasn't explained. Hydrogen concentration has an important role on anaerobic digestion process and is an indicator of digester's health. Propionate degradation is possible when concentration of hydrogen is maintained at low levels by methanogens, as only in low hydrogen partial pressures the conversion of the acids is thermodynamically possible [63] [64]. Model's calibration showed that the concentration of hydrogen was kept low in all the compartments and it was maintained at concentrations between 0.16 and 0.41 mg/L. So, it wasn't the reason behind the high concentration of propionate. The small HRT of each Ephyra® reactor didn't help the growth of propionic acetogens which had a low growth rate of 0.22 d^{-1}. Propionic acetogens did not have sufficient time to grow in each tank and convert propionate to acetate, carbon dioxide and hydrogen. So, high concentrations of propionate were maintained inside the tanks.

It can be seen that there was a sharp increase in volatile fatty acids concentration of 150% in the 1st compartment as it is increased from 1695 mg/L in the influent to 4313 mg/L in the 1st compartment. This happens because heterotrophs, which release exo-enzymes, grow very fast, they hydrolyse influent particular matter and readily biodegradable COD is immediately completely fermented [8]. There is a decrease in VFA concentration in the 2nd compartment of 37% compared with the 1st tank, and it continues to decrease in in the 3rd and 4th tank, based on the result of the steady state simulation shown in Table 16. From the concentrations of acetate and propionate it can be seen that propionate is the dominant acid in all the Ephyra® tanks, except in the first one where acetat prevails propionate. Acetate decreases from 2677 mg/L in the 1st tank to 513 mg/L in the 2nd compartment, and then in 3rd tank decreases by 81%. In the 4th reactor it exists at a concentration of 66 mg/L.

According to the steady state solution of the calibrated model shown in Table 16, the gas production from all four Ephyra® compartments is in average 12.7 m^3/d which is about 2.4 m^3/d higher than the average gas production observed during the pilot-scale plant operation. The final CSTR of the Ephyra® system produces about 7.0 m^3/d which is only 0.3 m^3/d lower than the average value measured at the real situation. The methane potential of the Ephyra® reactors according to the model is 64% which 5% higher than the CH_4 yield measured at the pilot-scale. The methane content of the final CSTR of the Ephyra® system at the real case was 71% in average, but the model gives a methane potential of 65%. The higher gas production of the four Ephyra® reactors is probably a consequence of the higher pH which is simulated by BioWin. The pH is around 7.0 and it favors the bacterial growth in the digesters and they produce better biogas yield as less CO_2 is stripped.

There is a population of ordinary heterotrophic organisms (OHOs) already established in the influent about 1356 mg/L. Their concentration is increasing in each compartment about 1%. Phosphate accumulating organisms (PAOs) are present in much lower concentrations than OHOs, and they are almost negligible. Concentrations of propionic acetogens in the 1st compartment is 20 mg COD/L. Their concentration increases through the compartments. An increase of 83% can be seen in the 2nd compartment, then in the 3rd tank their concentration is almost doubled in respect with the previous tank as shown in Table 16. In the 4th reactor the concentration of propionic acetogens increases from 70 mg COD/L in the 3rd tank to 136 mg COD/L. Propionic acetogens exists in the lowest concentrations compared to all the microorganisms involved in anaerobic digestion process.

Acetoclastic methanogens are present in the 1st compartment in a concentration of 221 mg COD/L. They are increased by 153% in the 2nd tank and then they rise in the 3rd compartment only by 24%. Based on the results in Table 16, a smaller rise of their concentration about 13% is observed in the 4th tank compared with the 3rd tank. Hydrogenotrophic methanogens exist in a lower concentration in the 1st compartment (162 mg/L). In the 2nd tank, their concentration increases about 57%, then in the 3rd tank by 31%, and finally in the 4th tank by 22%. Their increase through the compartments is much lower compared to acetoclastic methanogens. Concentration of acetoclastic methanogens increases from 221 mg/L in the 1st tank up to 785 mg/L in the 4th tank. On the other hand, hydrogenotrophic methanogens increase from 162 mg COD/L up to 406 mg COD/L. The higher concentration of methanogens in the last compartment is expected as methanogens require high retention times in anaerobic digesters in order to ensure the growth of a large population [8]. The increase in methanogens concentration justifies the low gas flow in the first tank compared with the flow in the subsequent compartments. Methane potential is in a range between 61% and 66% and a small decrease is observed between the 1st and the 4th reactor.

The reference system model was calibrated using dynamic flows and influent concentrations measured at the pilot-scale plant. The dynamic simulation result of the calibrated reference model can be found in Appendix E. To achieve a good correlation with the real data of the pilot plant, hydrolysis rate coefficient was estimated at 1.08 d^{-1}. This value is lower than that of the Ephyra® system reactors.

As mentioned in data analysis section, the reference system at the pilot plant was fed with different substrate than the Ephyra® system. In order to be able to compare the outcome of the two models, the reference system model should be simulated using as influent the characteristics of the Ephyra system model, after it was calibrated using the data from the reference pilot system. Other kinetic parameters which were changed were maximum growth rate of acetoclastic methanogens, acetoclastic substrate half saturation and acetoclastic anaerobic decay, and they were adjusted at the same values found during the calibration of the Ephyra® system model (Table 17).

The steady state solution presented in Table 18 shows that the reference system model using as influent the data of the pilot reference system achieves a degradation of 49% and generates a biogas flow of 10.6 m^3/d. It can be seen that VFAs in the CSTR are present in very low concentrations and the dominant acid is acetate. Then, in order to be able to compare the outcome of the two models, a steady state solution was simulated using as influent the characteristics of Ephyra® system. The output is shown in Table 19. The reference system which is fed with the same influent characteristics of the Ephyra® system model achieves 40% removal of total COD concentration and produces 15.1 m^3/d biogas with 64% methane yield.

Table 17: Calibrated kinetic parameters for reference system model of the pilot plant

Name	Default	Adjusted
Hydrolysis rate coefficient [d^{-1}]	1.05	1.08 d^{-1}
Acetoclastic methanogens max. spec. growth rate [d^{-1}]	0.30	0.46 d^{-1}
Acetoclastic substrate half saturation [mgCOD/L]	100.00	90.00
Acetoclastic anaerobic decay [d^{-1}]	0.13	0.12
Propionic acetogens max. spec. growth rate [d^{-1}]	0.25	0.22

Table 18: Steady state solution of reference system model using as input the characteristics of pilot reference system

Characteristics of reference CSTR	Value
pH [-]	7.0
Total effluent COD [mg/L]	30359
Volatile fatty acids [mg/L]	44
Acetate [mgCOD/L]	40
Meth. - acetoclastic [mgCOD/L]	296
Meth.-hydrogenotr. [mgCOD/L]	159
Heterotr. Organ. (OHO) [mgCOD/L]	622
Polyph. accum. org. (PAO) [mgCOD/L]	3
Propionate [mgCOD/L]	5
Propionic acetogens [mgCOD/L]	76
Off gas flow rate [m^3/hr]	0.44
Off gas Methane [%]	63

Table 19: Steady state solution of reference system model using as input the characteristics of pilot Ephyra® system

Characteristics of reference CSTR	Value
pH [-]	7.0
Total COD [mg/L]	40734
Volatile fatty acids [mg/L]	50
Acetate [mgCOD/L]	45
Meth. - acetoclastic [mgCOD/L]	396
Meth.-hydrogenotr. [mgCOD/L]	211
Heterotr. Organ. (OHO) [mgCOD/L]	872
Polyph. accum. org. (PAO) [mgCOD/L]	4
Propionate [mgCOD/L]	6
Propionic acetogens [mgCOD/L]	101
Off gas flow rate [m^3/hr]	0.63
Off gas Methane [%]	64

Comparing the results of steady state simulations of the Ephyra® and reference system it can be concluded that Ephyra® system achieves a higher reduction of total COD degradation of about 12% and can produce 23% higher biogas in average. From Table 16 and Table 18 it can be seen that microorganisms are present in higher concentrations in the Ephyra® system than in the reference system. Therefore, Ephyra® system promotes the growth of microorganisms and achieves process separation. The estimated hydrolysis rate coefficients demonstrate that Ephyra® enhances the overall hydrolysis rate of the system. That's why Ephyra® achieves higher sludge degradation and biogas production.

5.4.2 Recirculation rate

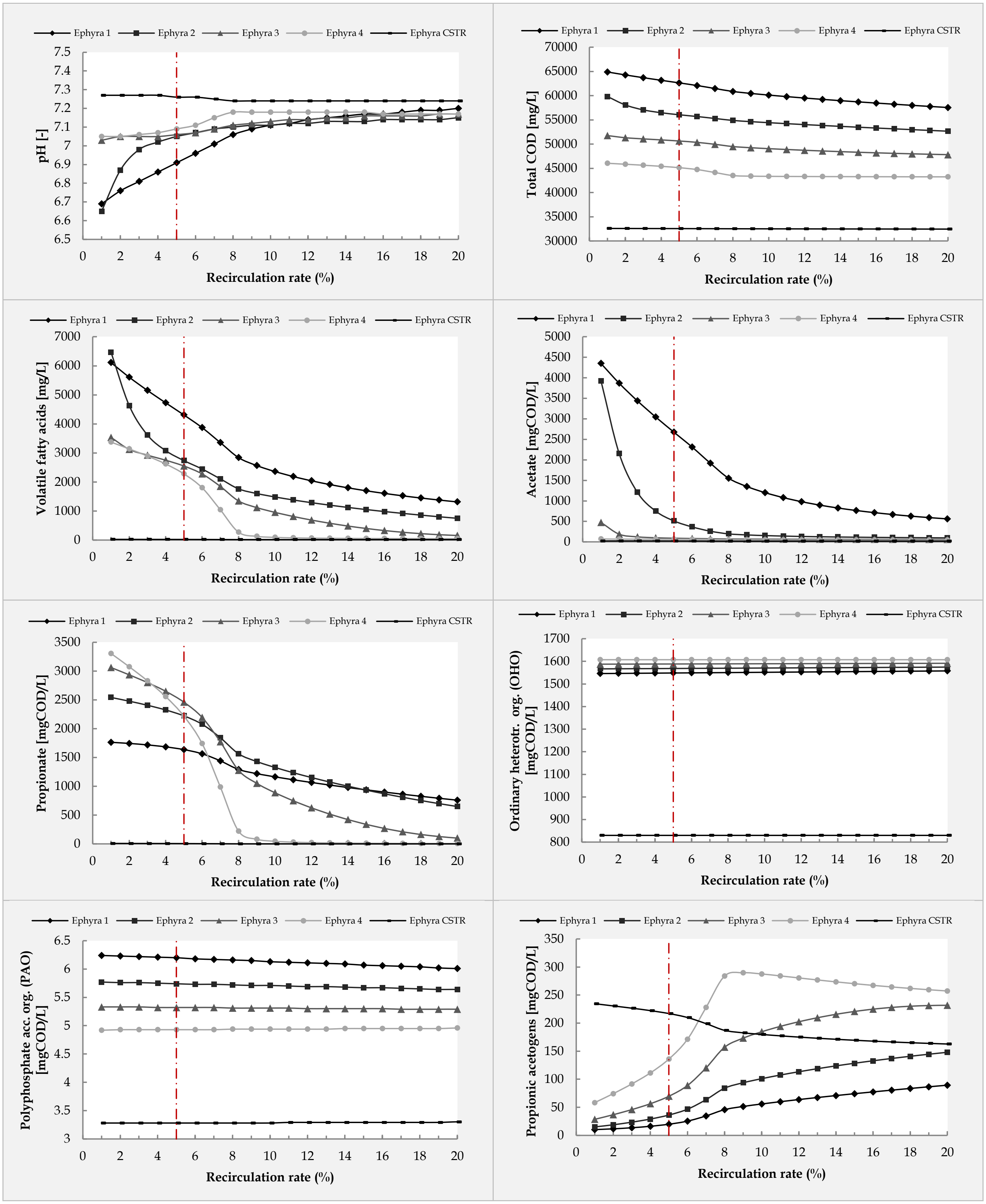
A recirculation line from the 4th to the 1st reactor was required for proper operation of the system. The model shows that the system collapses as pH drops below 6.0 [-] and the system acidifies without a recirculation line. Many studies have shown that recirculation improves the efficiency and stability of the systems. According to Namsree et al. (2012) anaerobic digestion of pineapple pulp using a plug-flow reactor was improved with recirculation and biogas production was increased [16]. A study on two-stage anaerobic system treating leaf vegetable waste by Zhuang et al. (2015) showed that the system without recirculation was sensitive to high OLR and VFA consumption was inhibited in the methanogenic reactor [45]. Effluent recirculation can help buffer the rapid increase of VFA produced in the first tank and maintain a proper pH for microorganisms to grow [46] [65]. Alkalinity is returned in the first reactor which improves pH stability and it could support the hydrolysis process and improve the performance of anaerobic digestion [42]. A portion of established population of methanogens is reintroduced in the first reactor and in this way biogas production is enhanced [44] [66] [67].

The results of steady state simulations under different recirculation rates can be found in Appendix F. Figure 17 shows that increase of recirculation rate of sludge from the 4th to the 1st reactor up to 8% increases the degradation of the organic substances in the effluent of the four Ephyra® reactors. Further increase of the recirculation rate does not change the concentration of total COD. The changes in OHOs and PAOs concentration may be considered insignificant in all the compartments. The rise of the recirculation rate results in a higher pH in all the compartments establishing suitable conditions for further growth of organisms. The value of pH in the all the reactors increases constantly as the recirculation rate increases until the fraction of sludge volume which is recirculated is set up to 9%. Changes of pH are very small at rates higher than 9%. The rise of pH enhances microorganisms' activity and therefore a higher amount of VFAs is consumed in all the reactors. It can be seen that recirculation rate favors the growth of acetoclastic methanogens in the 1st and 2nd reactor and the establishment of propionic acetogens in the 3rd and 4th reactor. As a consequence the increase of recirculation rate enhances acetate consumption in the first two tanks and consumption of propionate in the last two.

As mentioned before, with recirculation an active population of methanogens is transferred in the first reactor and higher methane yield is achieved in the first reactors. It can be seen Figure 17 that the concentration of acetoclastic methanogens increased in the first two reactors as the recirculation rate increases. At the same time concentration of hydrogenotrophic methanogens increases slightly in all the compartments, except in the 4th reactor where their concentration decreases slightly for recirculation rates higher than 8%. Increase of recirculation rate further than 5% promotes gas production and causes a rapid increase in methane potential in the first tank. However, the influence of recirculation on gas flow and methane yield is not so significant for rates higher than 8%. It has to be noticed that recirculation causes a small reduction of gas production in the 4th reactor and it stabilizes at rates higher than 10%. The total gas production of the Ephyra® compartments doesn't seem to be influenced by the raise of the recirculation rate. It increases from 0.53 m^3/hr at a recirculation rate of 5% to 0.55 m^3/hr for recirculation rates higher than 8%.

The organisms which are affected in the final CSTR from recirculation applied on the Ephyra® system are propionic acetogens and both species of methanogens. Increase of recirculation rate has as a result a decrease of these populations in the final CSTR and therefore a smaller volume of gas is produced with a lower methane yield. Gas production and methane potential stabilizes at 0.26 m3/hr and 62% for recirculation rates higher than 8%.

Observing all the graphs in Figure 17 it can be seen that the best ratio of recirculation which optimizes the performance of the system is 8%. Using this fraction, a removal efficiency of total COD of 36% is


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
pH [-]
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
0
2
4
6
8
10
12
14
16
18
20
Recirculation rate (%)
Total COD [mg/L]
70000
65000
60000
55000
50000
45000
40000
35000
30000
Volatile fatty acids [mg/L]
7000
6000
5000
4000
3000
2000
1000
0
Acetate [mgCOD/L]
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Propionate [mgCOD/L]
3500
3000
2500
2000
1500
1000
500
0
Ordinary heterotr. org. (OHO) [mgCOD/L]
1700
1600
1500
1400
1300
1200
1100
1000
900
800
Polyphosphate acc. org. (PAO) [mgCOD/L]
6.5
6
5.5
5
4.5
4
3.5
3
Propionic acetogens [mgCOD/L]
350
300
250
200
150
100
50
0

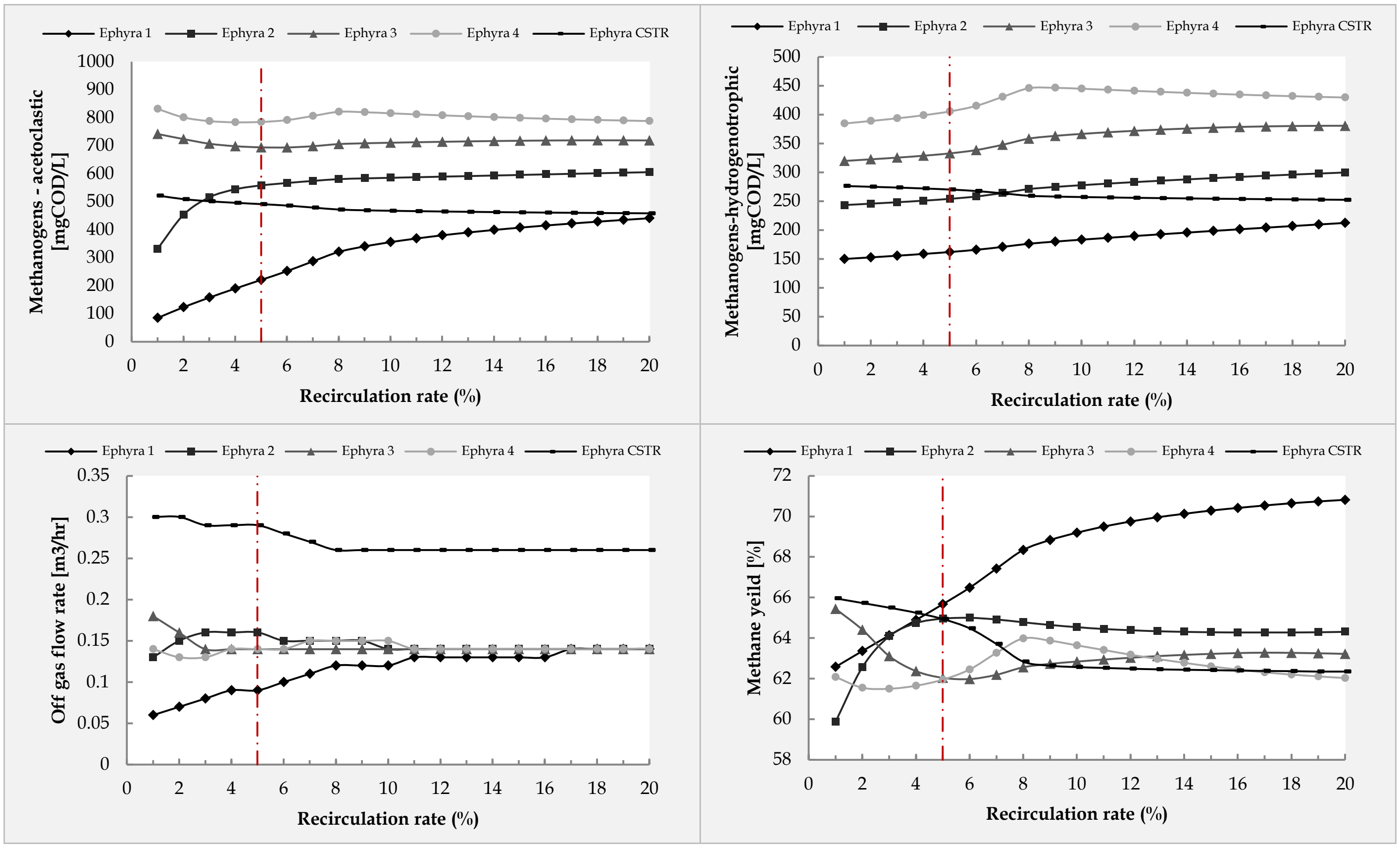


Figure 17: Effect of recirculation rate of sludge from the 4th to the 1st reactor of Ephyra system model on different variables and operational conditions: pH, total COD, VFA, acetate, propionate, OHOs, PAOs, propionic acetogens, acetoclastic methanogens, hydrogenotrophic methanogens, off gas flow rate and methane yield

achieved through the four Ephyra® compartments and 52% through the whole system. Therefore, the recirculation rate improves the COD removal efficiency of the Ephyra® compartments, while the removal efficiency of the whole system remains the same. Better conditions are maintained in Ephyra® compartments and the possibility of the system to collapse is smaller. The gas production of the Ephyra® system is 13.4 m^3/d with a methane yield 65%, and the final CSTR generates 6.3 m^3/d and a methane yield of 63%. So, a recirculation rate of 8% increases gas production from the Ephyra® reactors and decreases gas generation in the final CSTR by 0.7 m^3/d in both cases. It can be seen that application of higher recirculation rates can achieve the same degradation efficiency; however higher recirculation fractions should be avoided to prevent operational problems.

5.4.3 Organic loading rate

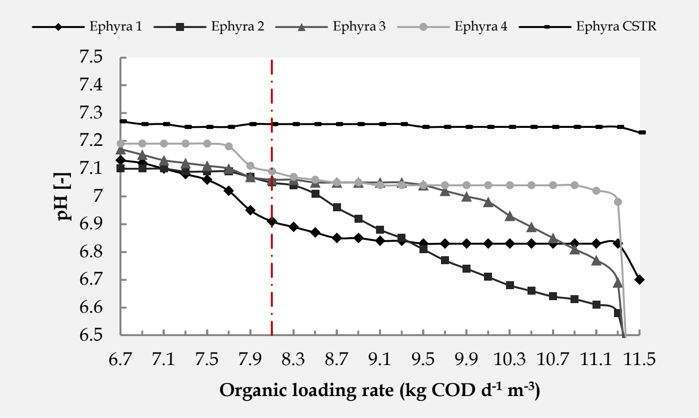
As mentioned in the introduction, OLR is expressed in respect of amount of organic matter treated by a certain volume of the digester. In this case it was decided to express OLR as the amount of total COD fed and have to be treated by the volume of the four anaerobic digesters comprising the Ephyra® system. In practise to investigate the influence of OLR on the performance of the system the flow is changed. So, it is clear that OLR is interrelated with HRT. As the flow increases a higher OLR and a shorter HRT is applied.

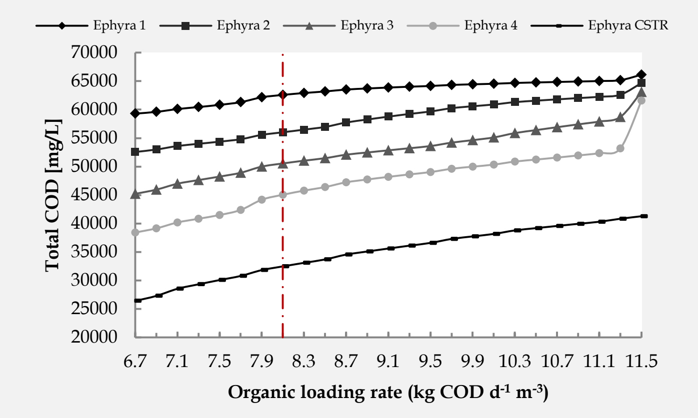
According to Yadvika et al (2004) organic loading significant affects gas production, as a decrease in OLR increases CH_4 yield, and above an optimum feed rate no further production of gas is observed [67]. A study on treating sugar-beef processing wastes in an anaerobic acidification reactor reports that acidification products were increased with increase of OLR which had as a consequence the inhibition of methanogenesis [68]. Other studies have shown that hydraulic retention time is a very important operational parameter for two-stage anaerobic digesters, as they report that it affects the operation of acidogenic reactors, while other researchers have reported that it has no significant influence [69]. A research on two-stage anaerobic digestion of poultry slaughterhouse waste showed that high organic loadings and short HRT causes accumulation of VFA and as consequence the process is inhibited and overloaded [70]. Higher organic loading and smaller HRTs support VFA production and enhance acidogenic organisms' growth, and therefore suppress methanogens activity [43].

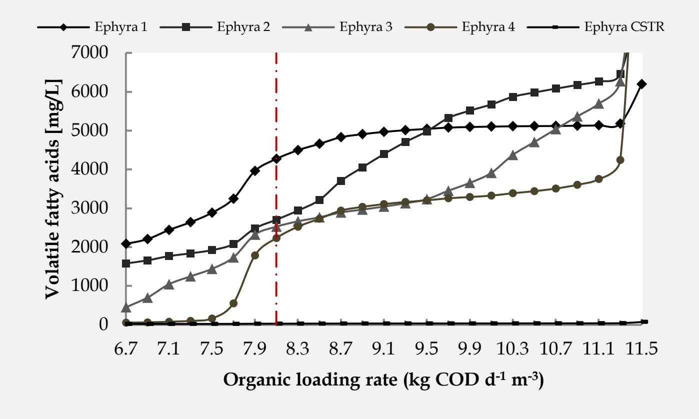
The pilot-scale plant was operated with an average feed rate of 0.91 m^3/d and a HRT of 21.0 days. The OLR of the pilot plant study was 8.1 kg COD m^{-3} d^{-1} for the 4-compartment Ephyra® system with a recirculation rate of sludge from the 4th to the 1st reactor of 5%. A number of steady state simulations were run to investigate the influence of OLR on the performance of the system. The detailed results can be found in Appendix G. It can be seen from Figure 18 that as the OLR increases further than 8.1 kg COD m^{-3} d^{-1} a slight decrease on pH value is observed in 1st and 4th reactor which stabilizes for OLR higher than 8.7 kg COD m^{-3} d^{-1}. Rapid reduction of pH can be seen in the 2nd reactor, and pH in the 3rd reactor is dropped for OLR higher than 9.5 kg COD m^{-3} d^{-1}. The same pattern appears also on the increase in VFA concentrations. Increase of OLR and decrease of HRT results in accumulation of VFAs and the anaerobic digestion process is inhibited. Hydrolysis process is suppressed in the first reactors and the sludge which is fed in the subsequent reactors without being hydrolysed causes the drop of pH and reduction of total COD removal efficiency of the system. Acetate and propionate concentrations are highly depend on HRT and ORL. Feeding the system with higher amount of organic matter favors the production of acetate in the 1st and 2nd reactors and propionate in the 4th reactors. In the 3rd reactor acetate production starts to be promoted for OLR higher than 9.5 kg COD m^{-3} d^{-1}. It has to be noticed that applying OLRs lower than 7.5 kg COD m^{-3} d^{-1} results in acetate concentrations lower than 200 mg/L in the 2nd, 3rd and 4th reactor and almost zero concentration of propionate in the 4th tank. At this OLR propionate concentration in 1st, 2nd and 3rd reactor is between 1350 mg/L and 1750 mg/L, and as the OLR is reduced and thus the HRT increases the concentration decreases. Therefore, decreasing OLR encourages transformation of VFA into biogas.

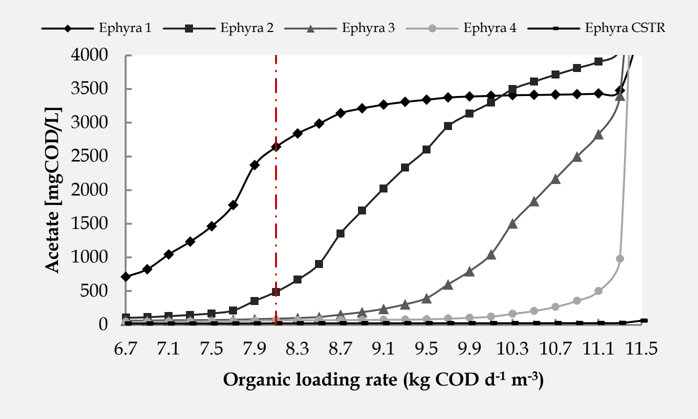
Changes on heterotrophic organisms' concentrations are insignificant in the Ephyra® compartments, but they increase constantly in the final CSTR as the OLR rises. The observations related with propionate concentration are justified by propionic acetogens concentration. It can be seen that for OLR smaller 8.1 kg COD m^{-3} d^{-1} their concentration increases significant. A study investigating the changes on microbial communities in hybrid anaerobic reactors reports that higher OLR and shorter HRT decrease the archaea diversity and reduce methane yield [71]. The same observation is made here as the Ephyra® system model shows that methanogens concentration is decreased in the Ephyra® compartments when OLR increases. When applying higher OLRs and thus shorter HRTs, the organisms are washed out of the system faster. The total gas production from all four Ephyra® reactors decreases from 12.7 m^3/d at 8.1 kg COD m^{-3} d^{-1} to 11.5 m^3/d at 11.1 kg COD m^{-3} d^{-1}.

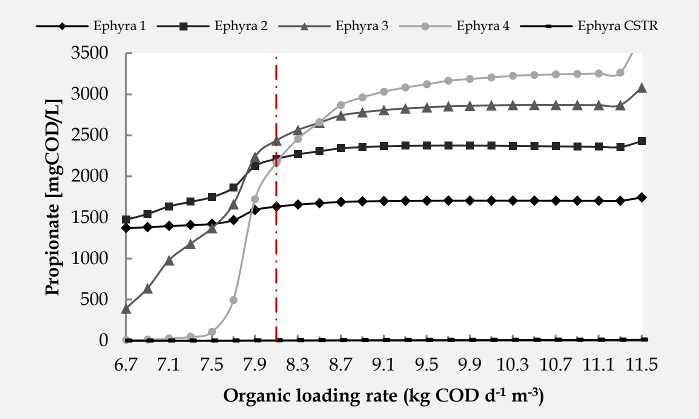
It is mentioned above that an increase in OLR can support to increase biogas production. However, the model shows that increase of OLR from the default value doesn't real help in generation of more biogas in the Ephyra® compartments. Also, it isn't observed a certain pattern of biogas production in the Ephyra® reactors. On the other hand, all the variables and organisms concentrations show that the gas production of the final CSTR is improved as the OLR increases. All the microorganism population present in the final CSTR exist in higher concentrations and VFAs are completely consumed as the OLR increases. Gas flow and methane yield increase constantly with the OLR. However, the rise of OLR results in higher

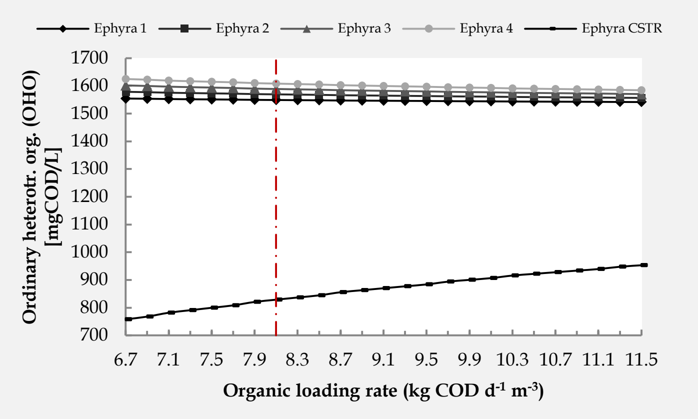

Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
pH [-]
7.5
7.4
7.3
7.2
7.1
7
6.9
6.8
6.7
6.6
6.5
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})

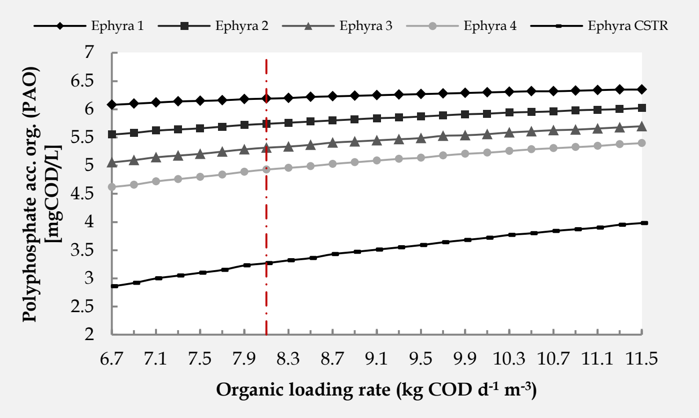

Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Total COD [mg/L]
70000
65000
60000
55000
50000
45000
40000
35000
30000
25000
20000
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})

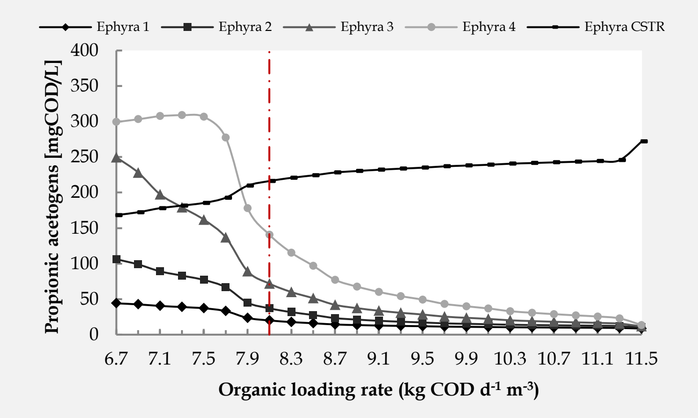

Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Volatile fatty acids [mg/L]
7000
6000
5000
4000
3000
2000
1000
0
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Acetate [mgCOD/L]
4000
3500
3000
2500
2000
1500
1000
500
0
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Propionate [mgCOD/L]
3500
3000
2500
2000
1500
1000
500
0
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Ordinary heterotr. org. (OHO) [mgCOD/L]
1700
1600
1500
1400
1300
1200
1100
1000
900
800
700
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Polyphosphate acc. org. (PAO) [mgCOD/L]
7
6.5
6
5.5
5
4.5
4
3.5
3
2.5
2
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})


Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
Propionic acetogens [mgCOD/L]
400
350
300
250
200
150
100
50
0
6.7
7.1
7.5
7.9
8.3
8.7
9.1
9.5
9.9
10.3
10.7
11.1
11.5
Organic loading rate (kg COD d^{-1} m^{-3})

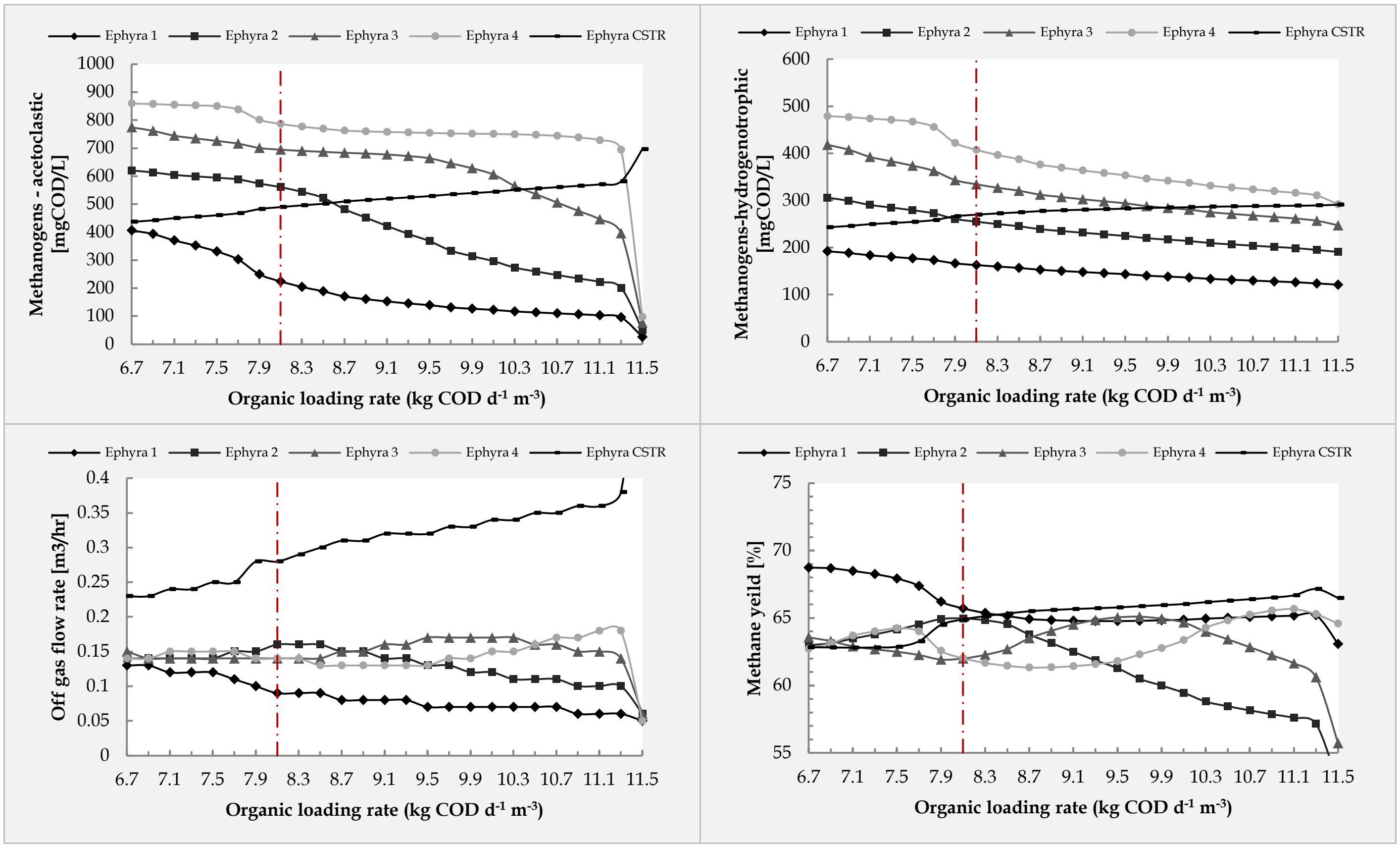


Figure 18: Effect of organic loading rate of Ephyra system model on different variables and operational conditions: pH, total COD, VFA, acetate, propionate, OHOs, PAOs, propionic acetogens, acetoclastic methanogens, hydrogenotrophic methanogens, off gas flow rate and methane yield

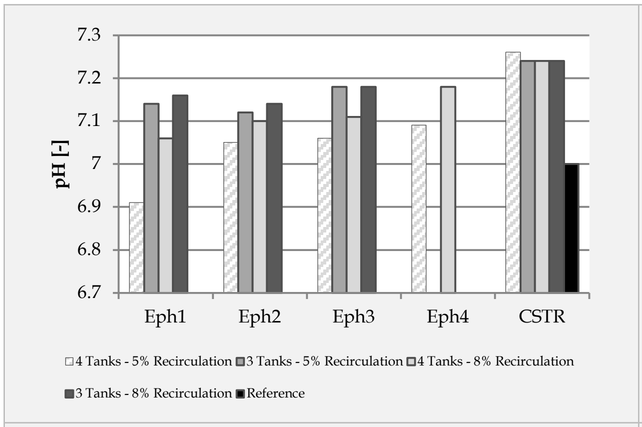
COD concentrations in the effluent. Each increase in OLR by 0.2 kg COD m^{-3} d^{-1} results in an increase of effluent total COD concentration about 350-850 mg/L, and it finally collapses at an OLR 11.5 kg COD m^{-3} d^{-1}.

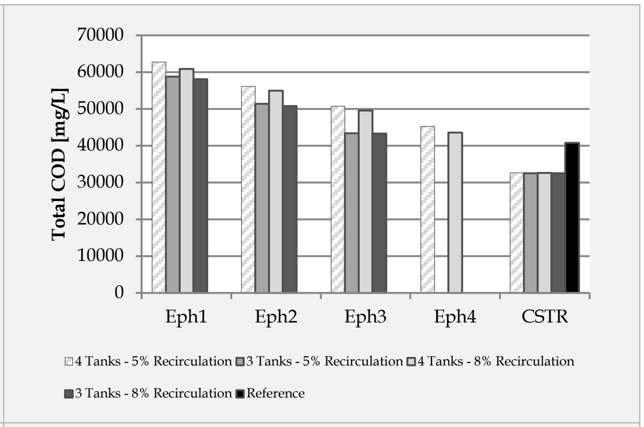
It is concluded that in order to increase the reduction of effluent total COD concentration then a lower OLR than 8.1 kg COD m^{-3} d^{-1} has to be applied. For each decrease in OLR by 0.2 kg COD m^{-3} d^{-1} results in a decrease in total HRT of about 0.5 days and a decrease in total COD concentration between 700 mg/L and 1200 mg/L. An OLR of 6.7 kg COD m^{-3} d^{-1} corresponds to a HRT of 25.5 days, and it can achieve almost 6 g/L more COD reduction. So, an increase in HRT from 21 days to 25.5 days can increase the removal efficiency of the system by 9%.

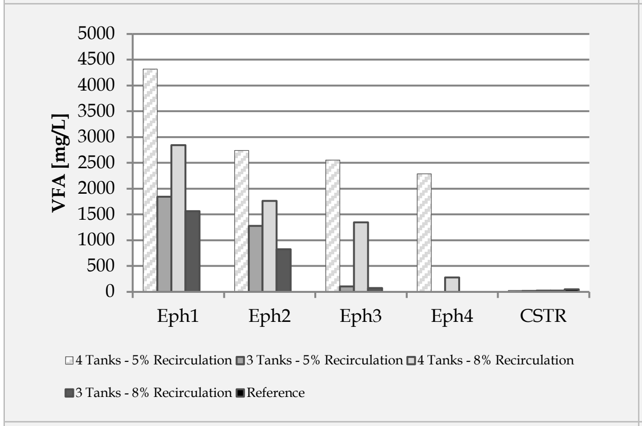
5.4.4 Number of tanks

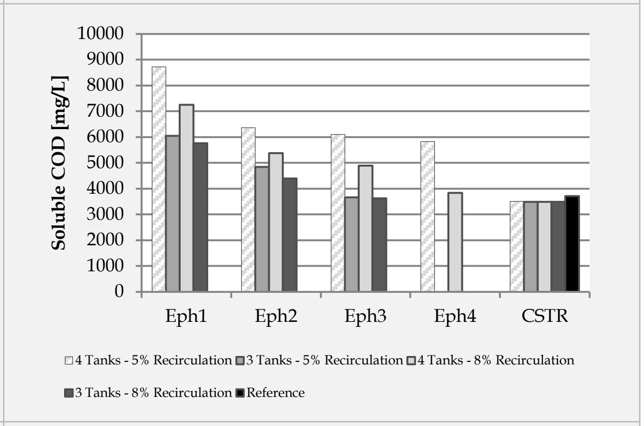
In order to answer to the third research question a model is created with three Ephyra® compartments, which have a total volume of 7.6 m^3 (2.53 m^3 each) in order to keep the same hydraulic retention time. All other parameters are kept the same. The detailed results can be found in Appendix H.

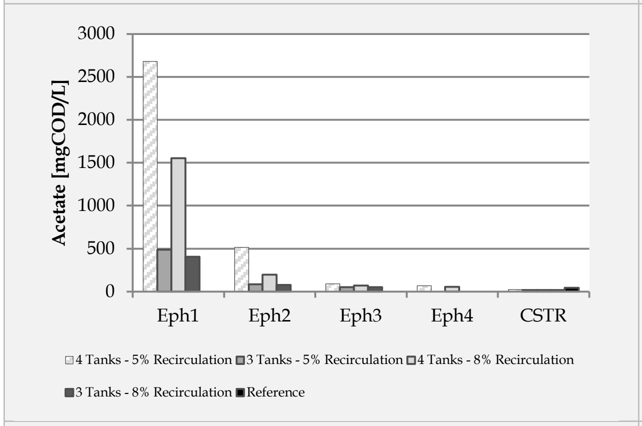
It can be seen from Figure 19 that when the HRT of each tank increases from 2.0 days to 2.7 days better conditions are maintained inside the reactors. A higher pH is maintained in all the compartments and lower VFA concentrations are observed since a better environment is established for the organisms to

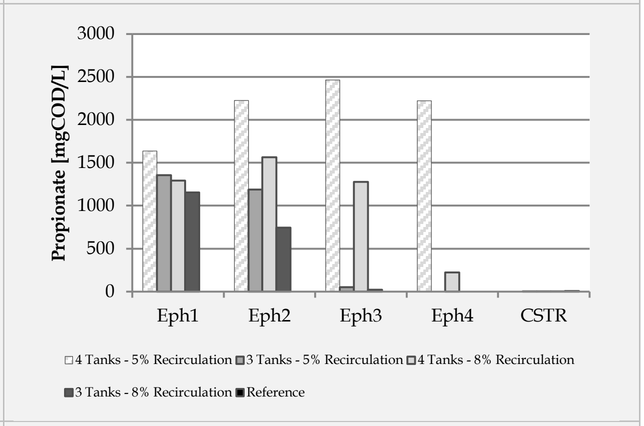

pH [-]
7.3
7.2
7.1
7
6.9
6.8
6.7
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference

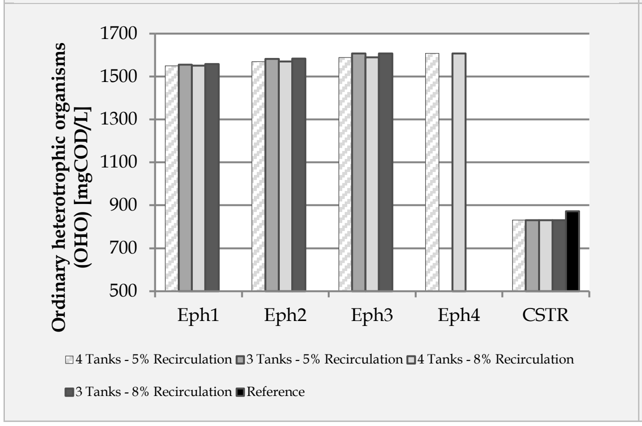

Total COD [mg/L]
70000
60000
50000
40000
30000
20000
10000
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference

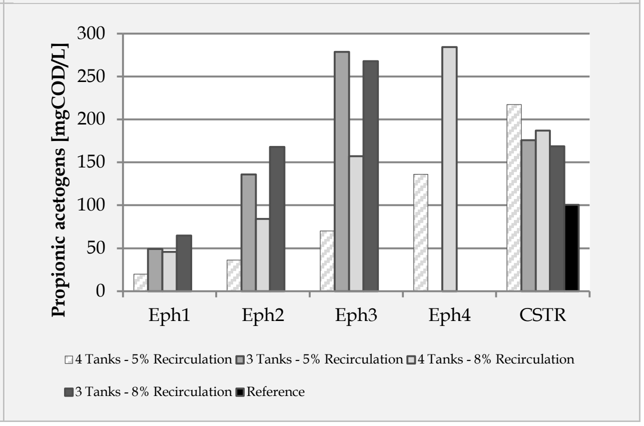

VFA [mg/L]
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference


Soluble COD [mg/L]
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference


Acetate [mgCOD/L]
3000
2500
2000
1500
1000
500
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference


Propionate [mgCOD/L]
3000
2500
2000
1500
1000
500
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference


Ordinary heterotrophic organisms (OHO) [mgCOD/L]
1700
1500
1300
1100
900
700
500
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference


Propionic acetogens [mgCOD/L]
300
250
200
150
100
50
0
Eph1
Eph2
Eph3
Eph4
CSTR
4 Tanks - 5% Recirculation
3 Tanks - 5% Recirculation
4 Tanks - 8% Recirculation
3 Tanks - 8% Recirculation
Reference

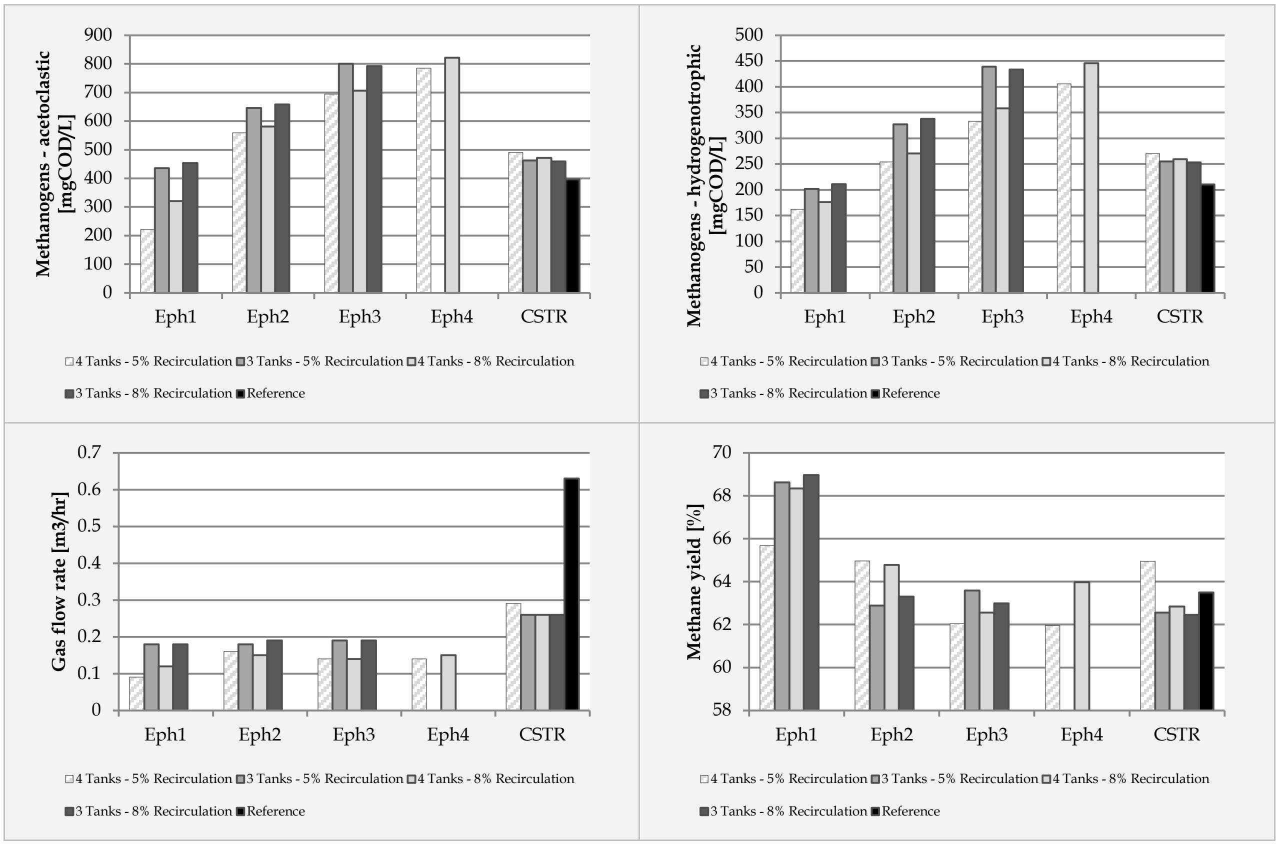


Figure 19: Influence of reducing number of Ephyra® tanks from 4 to 3 on system's performance with 5% and 8% recirculation rate of sludge on different variables and operational conditions: pH, total COD, VFA, acetate, propionate, OHOs, PAOs, propionic acetogens, acetoclastic methanogens, hydrogenotrophic methanogens, off gas flow rate and methane yield

consume them. The effluent VFA concentration is about 100 mg/L which in comparison with the 4-compartment system (2284 mg/L) is significant lower. It can be observed that the concentration og acetate is about 500 mg/L in the 1st compartment and it almost consumed in the subsequent tanks. Also, it can be seen that concentration of propionate is decreased rapidly through the compartments. In the second tank propionate concentration is almost half the concentration of the 2nd tank of the 4-compartment system, and in the third compartment almost all of it has been consumed. The reason behind this phenomenon is the higher retention time. Figure 19 shows that both species of methanogens and propionic acetogens are present in higher concentrations in all the Ephyra® reactors for the 3-compartment system. Propionic acetogens grow slower than the other organisms and the higher retention time of each tank supports them to grow in higher concentrations. The increase of heterotrophs' concentration is insignificant compared to the other organisms. The total COD concentration in the effluent of the 3-compartment Ephyra® system is lower about 1810 mg COD/L, but the effluent concentration from the final CSTR remains the same compared with 4-compartment Ephyra® system. The gas production of the four Ephyra® reactors increases by 0.5 m^3/d in the case of the 3-tanks system, but the total gas production of the whole system seems to be independent of the number of tanks.

An extra steady simulation was run to investigate the number of the tanks with higher recirculation rate. To run the simulation the optimum rate of 8% which was found in the previous section was used. It can be seen from the graphs in Figure 19 that the 3-compartment system with a recirculation rate of 8% can achieve slightly higher consumption of VFAs, so changes on pH value are very small. Acetate concentrations increase while propionate concentration decreases. Small changes on propionic acetogens and both species of methanogens concentrations are observed. Total biogas production remains the same and methane potential decreases slightly. The total COD reduction of the system remains the same, however better conditions are maintained inside the Ephyra® reactors.

To investigate whether a further decrease of the number of tanks, from 3 to 2, results in the same or higher degradation, an extra simulation was run. Based on the results presented in Figure 20 decreasing the number of the Ephyra® compartments from 3 to 2 results in decrease of the total degradation of the system. A 3-compartment Ephyra® system achieves a decrease of total COD concentration in the effluent about 52%. On the other hand, 2-compartment Ephyra® system achieves a decrease of total COD concentration in the effluent of about 47%.

Therefore, it can be concluded that the number of Ephyra reactors can be decreased from 4 to 3 in order to decrease capital and operational cost without significant effects on the performance and the efficiency of the process. At the same time it gives the advantage on the system for better monitoring and more stable performance as a lower concentration of VFAs is maintained throughout its operation and the possibility of failure due to VFA accumulation is decreased.

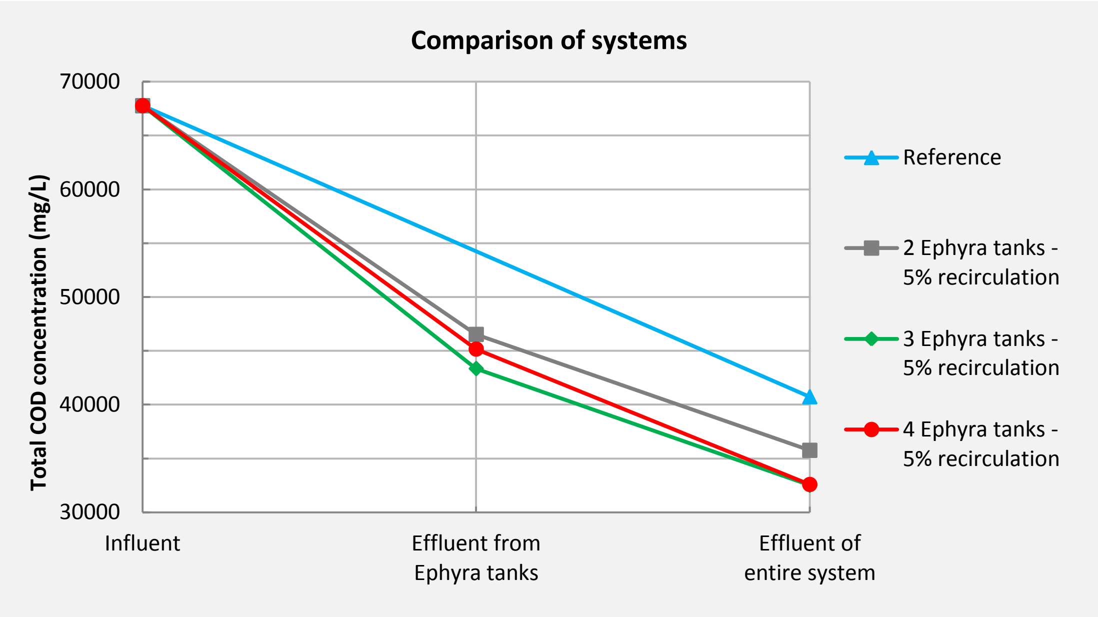


Figure 20: Comparison of reference system, and 2-compartment, 3-compartment and 4-compartment Ephyra® system at a recirculation rate of 5% regarding total COD concentration

5.4.5 Feedstock characteristics

During the process of the calibration it was made clear that the influent characteristics of the sludge play a significant role on the performance of the Ephyra® system. Also, it was shown before in the results of the sensitivity analysis that some of the wastewater fractions have a great influence on the model output. By increasing slightly the fraction of the readily biodegradable COD causes a collapse of the system as a higher amount VFA is generated. The fraction of influent TKN which is ammonia also plays an important role on anaerobic digestion performance. The influent ammonia concentration simulated by the model is around 500 mg/L in average. Steady state simulations showed that the process is inhibited for influent concentrations of ammonia higher than 1780 mg/L. Taking into considerations these results, the Ephyra® system would probably face some operational problems if it would be feed with aquaculture sludge, swine manure or poultry manure. Previous studies have shown that anaerobic digestion of aquaculture sludge was inhibited by the free ammonia levels, and swine and poultry manure contain high nitrogen concentration resulting in ammonia accumulation during digestion [72] [73]. In order to answer the fourth research question more precisely real data were examined.

The lab-scale system was operated using a different mixture of sludge obtained from Amsterdam WWTP, comprised of 50% primary sludge and 50% secondary sludge on dry solids base. The results could be used to investigate if the system has the same efficiency when is fed with different feedstock. The performance of the pilot-scale plant at Tollebeek and the lab-scale plant fed with Amsterdam sludge was evaluated for their ability to reduce the total COD and dry solids concentration in their effluent. Degradation efficiency for both systems is presented in Table 20 and Table 21. It can be seen that the total removal of COD is higher at the pilot-scale plant, but the total reduction of dry solids is higher for the lab-scale plant. If only the Ephyra® compartments efficiency is observed then for both cases dry solids and total COD concentration reduction is higher when the system is fed with Amsterdam sludge. In general, tests using sludge obtained from Amsterdam WWTP have shown that it gave higher degradations.

Table 20: Reduction of total COD concentration for pilot-scale plant using Tollebeek and Lelystad sludge, and lab scale plant fed with Amsterdam sludge

	Ephyra tanks	Entire Ephyra system
Pilot-scale plant *(Tollebbek and Lelystad sludge)*	33%	52%
Lab-scale plant *(Amsterdam sludge)*	43%	45%

Table 21: Reduction of dry solids concentration for pilot-scale plant using Tollebeek and Lelystad sludge, and lab scale plant fed with Amsterdam sludge

	Ephyra tanks	Entire Ephyra system
Pilot-scale plant *(Tollebbek and Lelystad sludge)*	28%	36%
Lab-scale plant *(Amsterdam sludge)*	40%	44%

Using the total and soluble COD measurements the Fbs fraction was calculated around 0.05 in average, which is the same value estimated for the pilot-scale plant. However, because the VFA measurements showed zero concentrations inside the reactors the Fac fraction couldn't be determined. In an attempt to simulate the lab-scale system using BioWin, the solution couldn't be found. The pilot-scale model showed that the model was sensitive to flow variations, so probably BioWin can't find a solution for the lab system due to the small volumes of the flow and the tanks. Comparing the hydrolysis rate coefficient determined using the BMP tests (0.45 d^{-1}) and determined through model calibration (1.35 d^{-1}) it can be seen that there is a significant difference between these values. The value determined through calibration is higher than typical values found in literature. Also in a previous section is stated that BMP tests cannot be used for modelling as they predict lower methane production. Bearing in mind that primary and secondary sludge have different hydrolytic characteristics and that the two cases which are compared had different portions of primary and waste activated sludge no conclusion can be made based on these hydrolysis rate coefficient values.

BioWin model doesn't have the ability to distinguish primary and WAS as a feed or define a more detailed substrate composition. The hydrolytic characteristics of the sludge play an important role on the performance of the system as it was observed from the steady state and dynamic simulations. A way to simulate the characteristics of primary and secondary sludge is to simulate the full-scale wastewater plants from which sludge is obtained, and feed the primary and secondary flows to the Ephyra® system. However, this particular case study investigates a pilot-scale plant and BioWin doesn't provide the ability to store primary and secondary sludge in a tank in order to feed it in a later stage to Ephyra® system.

In addition to hydrolytic characteristics, specific components of the feed influence system's performance. Dinopoulou et al. (1988) found that propionic acid concentration is highly depended on substrate composition. It is reported that when the substrate is constituted by easily biodegradable carbohydrates then the anaerobic digestion products are mainly acetic and butyric acid. But when the system is fed with more complex substrate then production of propionic acid is promoted [17]. Moreover, particulate components of sludge such as proteins, carbohydrates and fats play an significant role on anaerobic digestion, as their degradation leads to formation of VFAs which are the substrate for microorganisms during acetogenesis and methanogenesis. Sludge with high protein content results to high amounts of ammonia that could inhibit the anaerobic processes, but along with carbohydrates have the fastest conversion rates providing higher degradation rates. On the other hand, high fats contents increase significantly VFA concentrations, but they could provide higher biogas yields. Due to their low bioavailabity they require higher retention times [74]. The absence of VFA concentration in the compartments from the lab-scale plant makes it impossible to argue on the reason why Amsterdam sludge has a better performance.

Therefore, the lab-scale plant can't help to answer with more confidence which sludge characteristics have an important role on the performance of the system. However, it helps to argue on some of the above mentioned speculations. It is recommended in a future research to investigate the influence of substrate's composition on system's performance and efficiency.

6 Conclusions and recommendations

The results of this research show that:

- Calibration process results indicate that Ephyra® reactors have the same hydrolysis rate coefficient (1.35 d^{-1}), and then the final CSTR of the Ephyra® system has a lower hydrolysis rate coefficient (0.825 d^{-1}). The reference system has a lower hydrolysis rate coefficient than Ephyra® reactors (1.08 d^{-1}).
- The output of the final calibrated Ephyra® model shows that Ephyra® can achieve a total COD concentration reduction in the effluent of Ephyra® tanks of 33% and the whole system achieves a total reduction of 52% in average. Comparing these results with those obtained from the calibrated reference system model which achieved a removal of total COD concentration of 40%; it can be concluded that Ephyra® system achieves a higher reduction of total COD degradation of about 12% and can produce 23% higher biogas in average than the reference system.
- Ephyra® system promotes the growth of microorganisms and achieves process separation. It enhances the overall hydrolysis rate of the system and that's why Ephyra® achieves higher sludge degradation and biogas production.
- The high concentrations of propionate at the 4-compartment Ephyra® system are justified by the low growth rate of propionic acetogens. Due to the small HRT of each Ephyra® reactor (2.0 days) propionic acetogens do not have enough time to grow and convert propionate to acetate, carbon dioxide and hydrogen, and hence high concentrations of propionate are maintain inside the tanks.
- Recirculation line is necessary for the operation of the system because without it the whole system collapses as the reactors acidify. The rate of recirculation of sludge from the 4th reactor to the 1st tank that optimizes the performance of the system is 8%. It achieves a removal efficiency of total COD of 36% through Ephyra® reactors, while the total removal efficiency of the system remains the same.

- To increase the removal of total COD in the effluent then the default value of OLR (8.1 kg COD m^{-3} d^{-1}) has to be decreased. For each decrease in OLR by 0.2 kg COD m^{-3} d^{-1} results in a decrease on total HRT of about 0.5 days and a decrease in total COD concentration between 700 mg/L and 1200 mg/L. The model shows that an increase of HRT from 21 days to 25.5 days can increase the removal efficiency of total COD of the system by 9%.
- Capital and operational cost can be reduced by decreasing the number of Ephyra® reactors from 4 to 3 while the HRT remains the same, as it does not influence the removal efficiency of the system and better conditions and more stable performance of the anaerobic digestion process can be achieved.
- The measurements from the lab-scale plant show that sludge from Amsterdam WWTP seems to achieve higher efficiency than Tollebeek and Lelystad sludge. However, it can’t help to answer with more confidence which sludge characteristics have an important role on the performance of the system due to absence of detailed sludge characterization.

Alkalinity and pH have a great influence on the operation of the system. So, it is recommended in future operation of pilot-scale and/or full-scale plants to measure alkalinity and pH of sludge in the feed but also inside the reactors. If it is possible to measure VFA concentrations then it is preferable to measure and calculate the ratio of VFA to alkalinity, instead of measuring the pH value, as this ratio gives more warning of impending digester problems.

During the BMP tests, problems related with the sludge availabilty, but also the fact that it wasn't verified whether hydrolysis was the rate-limiting step, decrease the confidence behind the final result. It is suggested to run again the BMP tests to determine the kinetics and the methane potential of both substrates, i.e. Tollebeek and Amsterdam sludge. The outcome would provide more information to justify why these two substrates give different degradation efficiencies. The BMP tests should be conducted according to the guidelines and recommendations reported in the article by Holliger et al. (2016).

In future research and measurements, it is recommended to determine a more detailed characterisation of the feed, such as carbohydrates, proteins and fats, to investigate how these components influence the products of anaerobic digestion, the biogas production, and the overall performance of the system. Moreover, measurement of microorganisms population inside the digesters could give an insight on how the growth rates of microorganisms are interelated. Also, it could confirm whether the higher hydrolysis rate achieved by Ephyra system is the result of higher concentration of enzymes secreted by acidogenic bacteria.

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APPENDIX A: Pilot Scale Plant Data

Table 22: Influent characteristics of Ephyra® system at Tollebeek pilot plant

Date	Sol. COD (mg/L)	NH_4-N (mg/L)	Ortho-P (mg/L)	Residue (%)	Tot. COD (mg/L)	VFA (mg/L)	N kjeld. (mg/L)	Total P (mg/L)	Ca (mg/L)	Mg (mg/L)
28-10-2014	14800	545	630	6.5	87100	4617	3575	1365	1170	254
12-11-2014	5520	383	390	5.4	73980	2121	3510	1134	918	211
26-11-2014	8990	370	370	5.2	71760	2433	3068	988	988	187
8-12-2014	7830	433	480	6.1	77470	2174	3355	1342	915	262
22-12-2014	7710	328	490	5.7	68400	1857	3534	1767	1083	365
7-1-2015	6440	407	670	4.6	58420	1446	2944	1656	736	322
21-1-2015	10200	640	770	4.6	58880	2976	3404	1472	736	285
4-2-2015	7170	371	500	5.3	66780	1873	3498	1537	848	302
17-2-2015	8350	463	690	4.8	59520	2339	3360	1584	1008	374
4-3-2015	6590	338	650							
18-3-2015	6170	293	560	5.2	66040	1517	3380	1716	832	354
1-4-2015	5720	384	740	6	73200	1995	3720	1680	900	384
15-4-2015	7860	514	730	5	64500	2388	2650	1600	850	355
13-5-2015	13500	762	970							
27-5-2015	8580	439	830							
10-6-2015	8920	369	530	4.3	56330	2053	3053	1290	688	340
24-6-2015	9000	429	780							
8-7-2015	9780	710	970	6.1	73810	4462	3782	1952	1098	470
10-8-2015	8410	590	630	4.8	60480	3267	3168	1536	91	302
Minimum	5520	293	370	4.3	56330	1446	2650	988	91	187
Maximum	14800	762	970	6.5	87100	4617	3782	1952	1170	470
Average	8502	461	652	5.3	67778	2501	3333	1508	857	318
St. Deviation	2393	132	172	0.7	8543	955	306	251	254	72

Table 23: Influent characteristics to final CSTR of the Ephyra® system at Tollebeek pilot plant

Date	Sol. COD (mg/L)	NH_4-N (mg/L)	Ortho-P (mg/L)	Residue (%)	Tot. COD (mg/L)	VFA (mg/L)	N kjeld. (mg/L)	Total P (mg/L)	Ca (mg/L)	Mg (mg/L)
28-10-2014	10100	1500	370	4.1	40180	1847	3526	1476	943	299
12-11-2014	4030	1830	260	3.8	54340	1173	3572	1254	950	201
26-11-2014	12700	1560	460	3.3	46530	3365	3300	1188	825	129
8-12-2014	9720	1580	410	3.3	39270	2129	3267	1254	825	224
22-12-2014	8190	1680	540	3.7	35890	1034	3700	1702	851	481
7-1-2015	20900	1300	520	2.9	31030	888	3190	1595	812	247
21-1-2015	9110	1590	590	3.5	32900	888	3500	1925	840	560
4-2-2015	10400	1540	550	3.9	45240	1784	3783	1794	858	468
17-2-2015	13500	1650	680	3.2	41600	3318	3520	1088	768	246
4-3-2015	11800	1420	700	3.8	47500	1784	3572	1596	874	274
18-3-2015	12200	1330	700	3.9	49140	2455	3588	1560	858	289
1-4-2015				4.3	55040	2904	3741	1935	946	559
15-4-2015	9730	1180	610	4.0	51600	3014	2680	1640	1320	344
29-4-2015	13500	1340	660							
13-5-2015	12000	1500	760							
27-5-2015	9700	1330	730							
10-6-2015	11700	1990	550	4.3	51600	2468	3999	1720	903	383
8-7-2015	7430	1440	780	4.4	50160	2458	3740	2024	924	528
10-8-2015				4.3	49880	2056	3311	1419	99	284
18-8-2015	8810	1220	440							
Minimum	1180	260	31030	2.9	888	2680	1088	99	129	1180
Maximum	1990	780	55040	4.4	3365	3999	2024	1320	560	1990
Average	1499	573	45119	3.8	2098	3499	1573	850	345	1499
St. Deviation	10100	1500	370	4.1	40180	1847	3526	1476	943	299

Table 24: Effluent characteristics of the final CSTR of the Ephyra® system at Tollebeek pilot plant

Date	Sol. COD (mg/L)	NH_4-N (mg/L)	Ortho-P (mg/L)	Residue (%)	Tot. COD (mg/L)	VFA (mg/L)	N kjeld. (mg/L)	Total P (mg/L)	Ca (mg/L)	Mg (mg/L)
28-10-2014	1520	508	110	2.5	15750	292	1675	2025	925	925
12-11-2014	4040	1800	270	3.8	51680	1358	3572	1178	988	198
26-11-2014	5430	1820	270	3.2	32000	778	2080	1280	1024	224
8-12-2014	4520	1770	320	3.8	31160	722	3800	2736	1026	912
22-12-2014	3560	1590	240	3.4	31620	662	3298	1428	1054	218
7-1-2015	4330	1720	440	3.2	31360	1067	3520	1632	992	269
21-1-2015	5630	1840	540	3.1	29450	742	3410	1736	899	301
4-2-2015	5600	1710	490	3.2	31040	709	3520	1664	864	352
17-2-2015	5600	1800	480	3.0	32100	755	3600	1470	810	231
4-3-2015	5390	1770	560	3.3	31350	2209	3630	1815	858	462
18-3-2015	5390	1640	550	3.5	33250	728	3850	1820	875	490
1-4-2015	5400	1820	610	3.4	33660	655	3740	1632	884	279
15-4-2015	4680	1480	510	3.5	34300	1326	3325	1680	1050	385
29-4-2015	5540	1550	500							
13-5-2015	4630	1650	600							
27-5-2015	4800	1700	670							
10-6-2015	6050	1320	510	3.6	32760	649	3960	1836	936	360
24-6-2015	4830	1530	540							
8-7-2015	5020	1840	590	3.7	35150	742	3700	1813	1036	366
10-8-2015	5100	1510	430	4.9	38710	735	4753	4900	1127	235
Minimum	1520	508	110	2.5	15750	292	1675	1178	810	198
Maximum	6050	1840	670	4.9	51680	2209	4753	4900	1127	925
Average	4853	1618	462	3.4	32834	883	3465	1915	959	388
St. Deviation	1520	508	110	2.5	15750	292	1675	2025	925	925

Table 25: Influent characteristics of reference system at Tollebeek pilot plant

Date	Sol. COD (mg/L)	NH_4-N (mg/L)	Ortho-P (mg/L)	Residue (%)	Tot. COD (mg/L)	VFA (mg/L)	N kjeld. (mg/L)	Total P (mg/L)	Ca (mg/L)	Mg (mg/L)
28-10-2014	12300	381	520	1.4	21980	4556	1078	616	55	127
12-11-2014	6380	348	230	5.4	74520	1920	3240	1080	972	173
26-11-2015	9510	346	310	5.5	78100	2598	3190	770	990	149
8-12-2015	6860	369	460	6.4	74880	2001	3392	1728	1088	288
22-12-2014	4220	176	290	5.8	68440	1147	3016	1914	1160	360
7-1-2015	4370	298	490	4.3	52030	1333	2537	1548	817	284
21-1-2015	6490	530	510	4.2	52920	2244	2772	1344	756	252
4-2-2015	4830	267	300	4.8	61920	2454	2976	1248	864	235
4-3-2015	5390	341	430							
18-3-2015	6510	238	420	5.1	64770	1165	3060	1632	867	342
29-7-2015						3140				
26-8-2015				3.4	37740	1087	2074	1156	714	292
26-8-2015	2800	152	450							
9-9-2015	4910	283	480	4.0	48000	1796	2480	1120	960	264
23-9-2015	9930	480	770	6.0	70800	3577	3420	1560	1320	384
Minimum	2800	152	230	1.4	21980	1087	1078	616	55	127
Maximum	12300	530	770	6.4	78100	4556	3420	1914	1320	384
Average	6500	324	435	4.7	58842	2232	2770	1310	880	262
St. Deviation	2655	107	138	1.4	16940	1041	666	388	312	81

Table 26: Effluent characteristics of reference system at Tollebeek pilot plant

Date	Sol. COD (mg/L)	NH_4-N (mg/L)	Ortho-P (mg/L)	Residue (%)	Tot. COD (mg/L)	VFA (mg/L)	N kjeld. (mg/L)	Total P (mg/L)	Ca (mg/L)	Mg (mg/L)
28-10-2014	2750	1710	110	3.1	27280	637	3007	1178	1085	195
12-11-2014	3350	1650	170	3.6	33840	782	3312	1332	1152	223
26-11-2014	3460	1670	170	3.4	28560	655	3196	1292	1122	252
8-12-2014	4340	1550	110	3.7	30932	682	3293	1443	111	292
22-12-2014	5450	1850	380	3.1	29760	689	3410	1426	837	260
7-1-2015	2420	1290	260	3.1	28520	596	2914	1581	1023	236
21-1-2015	4980	1440	280	3.2	29440	675	2976	1504	960	230
4-2-2015	4440	1360	260	3.2	30080	529	3168	1504	992	269
4-3-2015	3930	1840	380							
18-3-2015	5260	1330	390	3.3	30030	612	3234	1683	924	363
29-7-2015						549				
18-8-2015	2690	824	330							
26-8-2015				3.2	31680	253	2560	1312	992	234
26-8-2015	2690	824	330							
9-9-2015	2750	844	310	3.3	31680	350	2739	1287	1089	224
23-9-2015	3510	928	280	3.7	37370	370	2812	1295	1110	244
Minimum	2420	824	110	3.1	27280	253	2560	1178	111	195
Maximum	5450	1850	390	3.7	37370	782	3410	1683	1152	363
Average	3716	1365	269	3.3	30764	568	3052	1403	950	252
St. Deviation	2750	1710	110	3.1	27280	637	3007	1178	1085	195

Table 27: Volatile fatty acids measurements in each tank of Ephyra system at Tollebeek pilot plant

Ephyra 1					
Date	Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Boterz. (mg/L)	Total VFA (mg/L)
11-2-2015	1433	811	134	441	2819
17-2-2015	950	1353	329	564	3196
25-2-2015	2385	1818	417	1049	5670
18-3-2015	1140	1504	313	762	3719
14-4-2015	1371	1646	208	638	3863
6-5-2015	1064	1876	282	916	4138
13-5-2015	46	2461	366	1295	4168
3-6-2015	871	1743	292	878	3784
24-6-2015	1467	1898	260	701	4329
Average	1192	1679	289	805	3965
St. Deviation	620	449	84	261	800

Ephyra 2					
Date	Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Boterz. (mg/L)	Total VFA (mg/L)
11-2-2015	1061	1023	202	526	2814
17-2-2015	350	1446	301	83	2181
25-2-2015	1234	2028	104	225	3591
18-3-2015	274	1791	290	78	2433
14-4-2015	58	1943	266	311	2578
6-5-2015	49	2256	368	23	2696
13-5-2015	41	2691	412	18	3162
3-6-2015	193	2008	401	115	2717
24-6-2015	427	1955	305	66	2756
Average	410	1904	294	161	2770
St. Deviation	442	471	98	167	409

Ephyra 3					
Date	Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Boterz. (mg/L)	Total VFA (mg/L)
11-2-2015	703	1740	317	290	3052
17-2-2015	172	1766	80	7	2025
25-2-2015	467	1289	68	24	1848
18-3-2015	220	2310	171	11	2712
14-4-2015	160	2448	293	31	2932
6-5-2015	39	2519	253	4	2815
13-5-2015	36	3030	165	2	3233
3-6-2015	113	2452	204	4	2773
24-6-2015	392	2292	165	20	2869
Average	256	2205	191	44	2696
St. Deviation	223	520	86	93	460

Ephyra 4					
Date	Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Boterz. (mg/L)	Total VFA (mg/L)
11-2-2015	322	2031	152	32	2537
17-2-2015	98	1483	3	3	1587
25-2-2015	190	2128	19	0	2337
18-3-2015	240	1903	41	11	2195
14-4-2015	191	2559	114	20	2884
6-5-2015	61	2816	90	4	2971
13-5-2015	47	2965	12	0	3024
3-6-2015	167	2200	50	8	2425
24-6-2015	373	2243	56	8	2697
Average	188	2259	60	10	2517
St. Deviation	112	461	50	11	453

Ephyra CSTR					
Date	Acetic acid (mg/L)	Propionic acid (mg/L)	Isobutyric acid (mg/L)	Boterz. (mg/L)	Total VFA (mg/L)
11-2-2015					0
17-2-2015					0
25-2-2015	233	142	20	9	404
18-3-2015					0
14-4-2015	11	0	0	0	11
6-5-2015	2	0	0	0	2
13-5-2015	4	0	0	0	4
3-6-2015	8	0	2	0	10
24-6-2015	134	0	4	3	141
Average	65	24	4	2	64
St. Deviation	97	58	8	4	135

Table 28: pH value and methane yield for Ephyra® and reference system at Tollebeek pilot plant

Date	Ephyra system							Reference system	
	CH_4%		pH					pH	CH_4%
	Eph. 1-4	CSTR	Eph. 1	Eph. 2	Eph. 2	Eph. 4	CSTR		
22-12-2014	63.32	67.78	6.49	7.00	7.35	7.21	7.44	7.21	62.96
7-1-2015	63.50	63.14	6.51	6.80	7.22	7.12	7.44	7.18	55.67
15-1-2015	61.01	64.25	6.30	6.75	7.32	7.18	7.38	7.15	58.90
21-1-2015	62.45	64.63	6.24	6.88	7.30	7.13	7.43	7.19	57.20
28-1-2015	56.14	61.60	6.01	6.75	7.13	7.21	7.38	7.18	55.21
4-2-2015	58.01	70.99	5.60	6.26	6.85	7.12	7.37	7.21	63.51
11-2-2015	56.04	73.54	6.18	6.14	6.58	6.95	7.38	7.17	62.71
17-2-2015	60.52	74.73	6.18	6.58	6.87	6.93	7.44	7.22	63.37

25-2-2015	57.02	71.31	6.12	6.58	6.82	6.99	7.38	7.19	65.50
4-3-2015	57.43	72.52	6.44	6.55	6.75	7.01	7.39	7.18	60.65
11-3-2015	59.97	71.96	6.47	6.59	6.71	7.04	7.34	7.24	59.19
18-3-2015	57.52	72.71	6.45	6.53	6.72	6.89	7.33	7.18	63.01
1-4-2015	55.17	71.54	5.83	6.46	6.53	6.80	7.19	7.08	62.40
8-4-2015	54.12	72.63	5.98	6.38	6.43	6.72	7.14	7.52	0.00
15-4-2015	53.11	72.27	5.92	6.43	6.35	6.62	6.99	6.68	62.20
22-4-2015	54.90	72.78	6.21	6.54	6.56	6.75	7.26	7.32	62.31
29-4-2015	55.97	73.31	6.18	6.43	6.59	6.50	7.38	7.95	62.20
6-5-2015	54.03	72.44	5.97	6.17	6.83	6.80	7.28	6.58	62.18
13-5-2015	55.65	73.01	5.98	6.21	6.74	6.83	7.20	6.57	61.53
20-5-2015	60.94	71.71	6.48	6.44	6.96	7.14	7.25	6.61	82.33
27-5-2015	59.40	72.16	6.08	6.20	6.76	6.91	7.21	6.64	61.37
10-6-2015	59.12	72.74	5.95	6.46	6.81	6.76	7.25	6.82	63.31
17-6-2015	59.99	72.69	5.98	6.52	6.81	6.77	7.43	6.75	63.62
24-6-2015	57.42	72.45	5.87	6.49	6.76	6.63	7.44	6.67	24.05
2-7-2015	61.80	72.89	6.14	6.62	6.92	6.73	7.44	6.76	63.20
8-7-2015	60.65	69.37	5.83	6.58	6.93	6.77	7.47	6.82	51.88
29-7-2015	59.93	72.96	6.02	6.58	6.90	6.99	7.43	7.01	69.35
10-8-2015	63.29	70.09	6.46	6.78	6.89	7.04	7.42	6.96	48.99
Average	58.52	70.86	6.14	6.53	6.84	6.91	7.34	7.04	58.17
Standard Deviation	3.05	3.40	0.24	0.21	0.25	0.19	0.11	0.32	14.61

Table 29: Biogas production for Ephyra® and reference system at Tollebeek pilot plant

Date	Ephyra 1-4 (m^3/d)	Ephyra CSTR (m^3/d)	Reference (m^3/d)
22-12-2015		2.1	13.3
7-1-2015	8.3	3.6	6.9
15-1-2014	8.2	2.3	1.2
21-1-2015		2.8	11.3
28-1-2015		3.9	14.7
4-2-2015		6.4	14.0
11-2-2015		8.1	14.5
17-2-2015		7.3	0.0
25-2-2015	9.6	6.2	0.0
4-3-2015	10.3	5.8	0.0
11-3-2015	10.3	7.1	16.5
18-3-2015	10.4	7.5	15.8
1-4-2015	9.3	8.8	2.5
8-4-2015	9.3	11.7	0.0
15-4-2015	9.1	9.5	0.0
22-4-2015	10.1	9.7	0.0
29-4-2015	11.6	10.5	0.0
6-5-2015	11.7	5.7	6.7
13-5-2015	12.2	10.0	0.0
20-5-2015	11.4	2.8	0.2
27-5-2015	12.6	6.3	0.0
10-6-2015	10.9	7.3	0.0
17-6-2015	8.8	7.5	0.0
24-6-2015	11.3	9.4	0.0
2-7-2015	8.3	6.7	0.1
8-7-2015	10.6		0.0
29-7-2015	11.2	7.9	0.0
10-8-2015		4.9	0.0
Average	10.3	7.3	2.4
Standard Deviation	1.3	2.6	6.2

APPENDIX B: Sensitivity Analysis Results: Normalised Sensitivity Coefficients for both Ephyra® and Reference system

		Common		Kinetic parameters - Acetogens											
		Hydrolysis half sat. (-)		Max spec. growth rate (1/d)		Substrate half sat. (mgCOD/L)		Acetate inhibition (mgCOD/L)		Anaerobic decay rate (1/d)		Aerobic/anoxic decay rate (1/d)			
	Default	**0.0600**		**0.2500**		**10.0000**		**10000.00**		**0.0500**		**0.5200**			
		0.0660	**0.0540**	**0.2750**	**0.2250**	**11.0000**	**9.00**	**11000.000**	**9000.0000**	**0.0550**	**0.0450**	**0.5720**	**0.4680**		
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02		
	Ephyra 2	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.02	0.03	0.03	0.03		
	Ephyra 3	0.07	0.07	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03		
	Ephyra 4	0.07	0.07	0.05	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03		
	CSTR	0.01	0.01	0.01	0	0	0	0	0	0	0	0	0		
VFA	**Ephyra 1**	-0.06	-0.06	-0.06	-0.03	-0.04	-0.04	-0.05	-0.04	-0.04	-0.05	-0.04	-0.04		
	Ephyra 2	-0.09	-0.09	-0.09	-0.04	-0.06	-0.06	-0.07	-0.05	-0.06	-0.07	-0.06	-0.06		
	Ephyra 3	-0.12	-0.12	-0.13	-0.03	-0.07	-0.07	-0.09	-0.05	-0.06	-0.08	-0.07	-0.07		
	Ephyra 4	-0.15	-0.15	-0.19	0	-0.07	-0.07	-0.11	-0.04	-0.05	-0.1	-0.07	-0.07		
	CSTR	-0.04	-0.04	-0.04	0.18	0.1	0.02	0.04	0.08	0.11	0.02	0.06	0.06		
COD	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0		
	Ephyra 2	0	0	0	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01	0.01		
	Ephyra 3	0	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
	Ephyra 4	0	0	0	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01		
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03		
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0		
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0		
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0		
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0		
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0		
Methane	**Ephyra 1**	0.02	0.02	0.02	-0.01	0	0	0.01	-0.01	0	0	0	0		
	Ephyra 2	0.01	0.01	0.02	-0.08	-0.04	-0.04	-0.02	-0.06	-0.05	-0.03	-0.04	-0.04		
	Ephyra 3	0.02	0.02	0.06	-0.13	-0.05	-0.05	-0.02	-0.09	-0.07	-0.03	-0.05	-0.05		
	Ephyra 4	0.05	0.05	0.12	-0.17	-0.05	-0.05	0	-0.1	-0.08	-0.01	-0.05	-0.05		
	CSTR	0.04	0.04	0.05	0.01	0.02	0.02	0.03	0.02	0.02	0.03	0.02	0.02		

		Kinetic parameters - Methanogens											
		Acetoclastic max spec. growth rate (1/d)		H2-utilizing max. speci. growth rate (1/d)		Acetoclastic substrate half sat. (mgCOD/L)		Acetoclastic methanol half sat. (mgCOD/L)		H2-utilizing CO2 half sat. (mmol/L)		H2-utilizing substrate half sat. (mgCOD/L)	
	Default	**0.3000**		**1.4000**		**100.0000**		**0.5000**		**0.1000**		**1.0000**	
		0.3300	**0.2700**	**1.5400**	**1.2600**	**110.0000**	**90.0000**	**0.5500**	**0.4500**	**0.1100**	**0.0900**	**1.1000**	**0.4500**
pH	**Ephyra 1**	0.03	0	0.03	0	0.02	0.02	0.02	0.02	0.02	0.02	0	0.03
	Ephyra 2	0.08	-0.02	0.05	0	0.02	0.03	0.03	0.03	0.03	0.03	0.02	0.03
	Ephyra 3	0.17	-0.03	0.07	0	0.03	0.03	0.03	0.03	0.03	0.03	0.02	0.07
	Ephyra 4	**0.28**	-0.08	0.08	-0.02	0.03	0.03	0.03	0.03	0.03	0.03	0	0.07
	CSTR	0.03	-0.01	0.01	0	0	0.01	0	0	0	0	0	0.01
VFA	**Ephyra 1**	-0.1	-0.01	-0.18	0.13	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	0.05	-0.15
	Ephyra 2	-0.17	0	-0.21	0.12	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	0.04	-0.17
	Ephyra 3	**-0.29**	0.04	-0.23	0.12	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	0.04	-0.19
	Ephyra 4	**-0.51**	0.14	**-0.26**	0.15	-0.07	-0.08	-0.07	-0.07	-0.07	-0.07	0.06	-0.21
	CSTR	**-2.22**	**3.46**	-0.03	0.15	**1.04**	**-0.91**	0.06	0.06	0.06	0.06	0.12	-0.01
COD	**Ephyra 1**	0	0.01	-0.01	0.02	0	0	0	0	0	0	0.01	-0.01
	Ephyra 2	-0.01	0.01	-0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	-0.01
	Ephyra 3	-0.02	0.02	-0.01	0.03	0.01	0.01	0.01	0.01	0.01	0.01	0.02	-0.01
	Ephyra 4	-0.04	0.03	-0.01	0.04	0.01	0.01	0.01	0.01	0.01	0.01	0.03	0
	CSTR	0.02	0.04	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	**-1.43**	0	0	0	0	0	0	**-1.43**	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	**-0.83**	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	0.06	-0.04	**0.29**	**-0.38**	0	0	0	0	0	0	-0.21	0.22
	Ephyra 2	0.18	-0.16	0.12	-0.23	-0.04	-0.03	-0.04	-0.04	-0.04	-0.04	-0.15	0.08
	Ephyra 3	**0.34**	**-0.27**	0.09	-0.22	-0.06	-0.05	-0.05	-0.05	-0.05	-0.05	-0.15	0.05
	Ephyra 4	**0.57**	**-0.37**	0.1	-0.21	-0.06	-0.04	-0.05	-0.05	-0.05	-0.05	-0.15	0.06
	CSTR	0.13	-0.04	0.05	-0.01	0.02	0.03	0.02	0.02	0.02	0.02	0	0.05

		Kinetic parameters - Methanogens											
		H2-utilizing methanol half sat. (mgCOD/L)		**Acetoclastic propionic inhibition (mgCOD/L)**		**Acetoclastic anaerobic decay rate (1/d)**		**Acetoclastic aer./anox. decay rate (1/d)**		**H2-utilizing anaerobic decay rate (1/d)**		**H2-utilizing aer./anox. decay rate (1/d)**	
	Default	**0.5000**		**10000.0000**		**0.1300**		**0.6000**		**0.1300**		**2.8000**	
		0.5500	**0.4500**	**11000.0000**	**9000.0000**	**0.1430**	**0.1170**	**0.6600**	**0.5400**	**0.1430**	**0.1170**	**3.0800**	**2.5200**
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.03	0.03	0.03	0.02	0.02	0.05	0.03	0.03	0.02	0.03	0.03	0.03
	Ephyra 3	0.03	0.03	0.05	0.02	0	0.08	0.03	0.03	0.03	0.05	0.03	0.03
	Ephyra 4	0.03	0.03	0.07	0	-0.03	0.12	0.03	0.03	0.02	0.05	0.03	0.03
	CSTR	0	0	0.01	0	0	0.01	0	0	0	0.01	0	0
VFA	**Ephyra 1**	-0.04	-0.04	-0.05	-0.04	-0.03	-0.06	-0.04	-0.04	0	-0.09	-0.04	-0.04
	Ephyra 2	-0.06	-0.06	-0.08	-0.05	-0.03	-0.1	-0.06	-0.06	-0.01	-0.11	-0.06	-0.06
	Ephyra 3	-0.07	-0.07	-0.1	-0.04	-0.02	-0.15	-0.07	-0.07	-0.02	-0.12	-0.07	-0.07
	Ephyra 4	-0.07	-0.07	-0.12	-0.02	0.03	-0.23	-0.07	-0.07	-0.01	-0.13	-0.07	-0.07
	CSTR	0.06	0.06	-0.01	0.14	**2.16**	**-1.67**	0.06	0.06	0.09	0.03	0.06	0.06
COD	**Ephyra 1**	0	0	0	0	0	0	0	0	0.01	0	0	0
	Ephyra 2	0.01	0.01	0	0.01	0.01	0	0.01	0.01	0.01	0	0.01	0.01
	Ephyra 3	0.01	0.01	0.01	0.01	0.01	0	0.01	0.01	0.01	0	0.01	0.01
	Ephyra 4	0.01	0.01	0.01	0.02	0.02	-0.01	0.01	0.01	0.02	0	0.01	0.01
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	0	0	0.01	-0.01	-0.02	0.02	0	0	-0.1	0.1	0	0
	Ephyra 2	-0.04	-0.04	-0.01	-0.07	-0.1	0.04	-0.04	-0.04	-0.09	0.02	-0.04	-0.04
	Ephyra 3	-0.05	-0.05	0	-0.11	-0.16	0.09	-0.05	-0.05	-0.1	-0.01	-0.05	-0.05
	Ephyra 4	-0.05	-0.05	0.02	-0.13	-0.21	0.18	-0.05	-0.05	-0.1	0	-0.05	-0.05
	CSTR	0.02	0.02	0.04	0.01	-0.01	0.07	0.02	0.02	0.01	0.04	0.02	0.02

		Wastewater Fractions							
		Fbs - Readily Biodegradable (including acetate) (gCOD/g of total COD)		Fac - Acetate (gCOD/g of readily bodegradable COD)		Fxsp - Non-colloidal slowly biod. (gCOD/g of slowly biodegradable COD)		Fus - Unbiodegradable soluble (gCOD/g of total COD)	
	Default	**0.1600**		**0.1500**		**0.7500**		**0.0500**	
		0.1760	**0.1440**	**0.1650**	**0.1350**	**0.8250**	**0.6750**	**0.0550**	**0.0450**
pH	**Ephyra 1**	**-0.45**	**0.4**	0	0.03	0.02	0.02	0.02	0.02
	Ephyra 2	**-0.48**	**0.47**	0.02	0.05	0.03	0.03	0.03	0.03
	Ephyra 3	**-0.52**	**0.54**	0.05	0.08	0.07	0.07	0.07	0.07
	Ephyra 4	**-0.58**	**0.6**	0.05	0.1	0.07	0.07	0.07	0.07
	CSTR	-0.04	0.04	0.01	0	0.01	0.01	0.01	0.01
VFA	**Ephyra 1**	**1.09**	**-1.12**	0.01	-0.12	-0.06	-0.06	-0.06	-0.06
	Ephyra 2	**1.1**	**-1.19**	-0.03	-0.15	-0.09	-0.09	-0.09	-0.09
	Ephyra 3	**1.15**	**-1.3**	-0.05	-0.18	-0.12	-0.12	-0.12	-0.12
	Ephyra 4	**1.28**	**-1.48**	-0.08	-0.21	-0.15	-0.15	-0.15	-0.15
	CSTR	**0.36**	**-0.53**	-0.03	-0.05	-0.04	-0.04	-0.04	-0.04
COD	**Ephyra 1**	-0.01	0.02	0.01	0	0	0	0	0
	Ephyra 2	0	0.02	0.01	0	0	0	0	0
	Ephyra 3	0	0.01	0.01	0	0	0	0	0
	Ephyra 4	0.02	0	0.01	0	0	0	0	0
	CSTR	-0.13	0.19	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	**-1.43**	**-1.43**	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0
	CSTR	**1.67**	**-1.67**	0	0	0	0	0	0
Methane	**Ephyra 1**	-0.13	0.24	0.16	-0.12	0.02	0.02	0.02	0.02
	Ephyra 2	0.1	0.18	0.11	-0.09	0.01	0.01	0.01	0.01
	Ephyra 3	0.08	**0.3**	0.09	-0.04	0.02	0.02	0.02	0.02
	Ephyra 4	-0.09	**0.55**	0.09	0.02	0.05	0.05	0.05	0.05
	CSTR	-0.2	0.17	0.05	0.04	0.04	0.04	0.04	0.04

		Wastewater Fractions							
		Fup - Unbiodegradable particulate (gCOD/g of total COD)		**FZbpa - Propionic acetogens COD fraction (gCOD/g of total COD)**		**FZbam - Acetoclastic methanogens COD fraction (gCOD/g of total COD)**		**FZbhm - H2-utilizing methanogens COD fraction (gCOD/g of total COD)**	
	Default	**0.1300**		**0.0001**		**0.0001**		**0.0001**	
		0.1430	**0.1170**	**0.00011**	**0.00009**	**0.00011**	**0.00009**	**0.00011**	**0.00009**
pH	**Ephyra 1**	-0.03	0.07	0.02	0.02	0.03	0.02	0.02	0.02
	Ephyra 2	-0.02	0.08	0.03	0.03	0.05	0.03	0.03	0.03
	Ephyra 3	0	0.12	0.07	0.07	0.08	0.03	0.07	0.07
	Ephyra 4	0.02	0.13	0.08	0.07	0.12	0.03	0.08	0.07
	CSTR	0	0.03	0.01	0.01	0.01	0	0.01	0.01
VFA	**Ephyra 1**	-0.05	-0.07	-0.06	-0.05	-0.07	-0.04	-0.06	-0.05
	Ephyra 2	-0.07	-0.1	-0.1	-0.08	-0.11	-0.06	-0.09	-0.08
	Ephyra 3	-0.1	-0.13	-0.13	-0.1	-0.16	-0.07	-0.12	-0.11
	Ephyra 4	-0.12	-0.18	-0.17	-0.12	-0.22	-0.07	-0.15	-0.14
	CSTR	-0.02	-0.06	-0.06	-0.02	-0.14	0.06	-0.04	-0.04
COD	**Ephyra 1**	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0.01	0	0
	Ephyra 3	0.01	0	0	0.01	0	0.01	0	0
	Ephyra 4	0.01	0	0	0.01	-0.01	0.01	0	0
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	-0.08	0.11	0.02	0.01	0.03	0	0.03	0.01
	Ephyra 2	-0.03	0.06	0.03	-0.01	0.06	-0.04	0.02	0.01
	Ephyra 3	-0.01	0.06	0.05	-0.01	0.1	-0.05	0.03	0.02
	Ephyra 4	0	0.1	0.09	0.01	0.15	-0.05	0.06	0.05
	CSTR	0.01	0.07	0.05	0.04	0.06	0.02	0.04	0.04

		Stoichiometric parameters - Common													
		Biomass volatile fractions (VSS/TSS)		**Endogenous residue volatile fractions (VSS/TSS)**		**N in endogenous residue (mgN/mgCOD)**		**P in endogenous residue (mgP/mgCOD)**		**Endogenous residue COD:VSS ratio (mgCOD/mgVSS)**		**Particulate substrate COD/VSS ratio (mgCOD/mgVSS)**		**Particulate inert COD/VSS ratio (mgCOD/mgVSS)**	
	Default	**0.9200**		**0.9200**		**0.0700**		**0.0220**		**1.4200**		**1.6000**		**1.6000**	
		1.0000	**0.9200**	**1.0000**	**0.9200**	**0.0770**	**0.0630**	**0.0242**	**0.0198**	**1.5620**	**1.2780**	**1.7600**	**1.4400**	**1.7600**	**1.4400**
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 3	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 4	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
VFA	**Ephyra 1**	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 2	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06
	Ephyra 3	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	Ephyra 4	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	CSTR	0.07	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
COD	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 4	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 3	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	Ephyra 4	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	CSTR	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

		Stoichiometric parameters - Acetogens													
		Yield (-)		H2 yield (-)		CO2 yield (-)		N in biomass (mgN/mgCOD)		P in biomass (mgP/mgCOD)		Fraction to endog. residue (-)		COD:VSS ratio (mgCOD/mgVSS)	
	Default	**0.1000**		**0.4000**		**1.0000**		**0.0700**		**0.0220**		**0.0800**		**1.4200**	
		0.1100	**0.0900**	**0.4400**	**0.3600**	**1.1000**	**0.9000**	**0.0770**	**0.0630**	**0.0242**	**0.0198**	**0.0880**	**0.0720**	**1.5620**	**1.2780**
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.02	0.03	0.03	0.02	0.02	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 3	0.03	0.03	0.05	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 4	0.03	0.03	0.05	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	CSTR	0.01	0	0.05	-0.04	-0.01	0.03	0	0.01	0	0	0	0	0	0
VFA	**Ephyra 1**	-0.04	-0.05	-0.05	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 2	-0.05	-0.07	-0.07	-0.05	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06
	Ephyra 3	-0.06	-0.09	-0.08	-0.06	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	Ephyra 4	-0.05	-0.1	-0.09	-0.05	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	CSTR	0.08	0.04	0.09	0.04	0.05	0.08	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
COD	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0.01	0	0	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 4	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	0	0	0.02	-0.02	-0.01	0.01	0	0	0	0	0	0	0	0
	Ephyra 2	-0.05	-0.03	0.03	-0.11	-0.06	-0.01	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 3	-0.07	-0.04	0.08	-0.19	-0.1	0	-0.06	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	Ephyra 4	-0.07	-0.03	0.15	-0.25	-0.12	0.02	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	CSTR	0.05	0	0.24	-0.19	-0.07	0.11	0.02	0.03	0.02	0.02	0.02	0.02	0.02	0.02

		Stoichiometric parameters - Methanogens											
		Acetoclastic yield (-)		Methanol acetoclastic yield (-)		H2-utilizing yield (-)		Methanol H2-utilizing yield (-)		N in acetoclastic biomass (mgN/mgCOD)		N in H2-utilizing biomass (mgN/mgCOD)	
	Default	**0.1000**		**0.1000**		**0.1000**		**0.1000**		**0.0700**		**0.0700**	
		0.1100	**0.0900**	**0.1100**	**0.0900**	**0.1100**	**0.0900**	**0.1100**	**0.0900**	**0.0770**	**0.0630**	**0.0770**	**0.0630**
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.02	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.02	0.03	0.02	0.03
	Ephyra 3	0.02	0.07	0.03	0.03	0.03	0.05	0.03	0.03	0.03	0.03	0.03	0.05
	Ephyra 4	0	0.08	0.03	0.03	0.02	0.05	0.03	0.03	0.03	0.03	0.03	0.03
	CSTR	0.01	0	0	0	0	0.01	0	0	0	0.01	0	0.01
VFA	**Ephyra 1**	-0.03	-0.06	-0.04	-0.04	-0.04	-0.05	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 2	-0.04	-0.09	-0.06	-0.06	-0.06	-0.07	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06
	Ephyra 3	-0.03	-0.12	-0.07	-0.07	-0.06	-0.08	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	Ephyra 4	-0.01	-0.15	-0.07	-0.07	-0.06	-0.08	-0.07	-0.07	-0.07	-0.07	-0.07	-0.08
	CSTR	0.15	-0.04	0.06	0.06	0.07	0.06	0.06	0.06	0.06	0.07	0.06	0.06
COD	**Ephyra 1**	0	0	0	0	0.01	0	0	0	0	0	0	0
	Ephyra 2	0.01	0	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 3	0.01	0	0.01	0.01	0.01	0	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 4	0.02	0	0.01	0.01	0.02	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	CSTR	0.04	0.02	0.03	0.03	0.04	0.02	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	-0.02	0.02	0	0	-0.07	0.08	0	0	0	0	-0.01	0.01
	Ephyra 2	-0.08	0.02	-0.04	-0.04	-0.09	0.02	-0.04	-0.04	-0.04	-0.04	-0.04	-0.03
	Ephyra 3	-0.12	0.03	-0.05	-0.05	-0.1	-0.01	-0.05	-0.05	-0.06	-0.05	-0.06	-0.05
	Ephyra 4	-0.13	0.05	-0.05	-0.05	-0.09	-0.01	-0.05	-0.05	-0.05	-0.05	-0.06	-0.04
	CSTR	0.04	0.01	0.02	0.02	0.01	0.03	0.02	0.02	0	0.04	0.02	0.03

		Stoichiometric parameters - Methanogens											
		P in acetoclastic biomass (mgP/mgCOD)		**P in H2-utilizing biomass (mgP/mgCOD)**		**Acetoclastic fraction to endog. Residue (-)**		**H2-utilizing fraction to endog. Residue (-)**		**Acetoclastic COD:VSS ratio (mgCOD/mgVSS)**		**H2-utilizing COD:VSS ratio (mgCOD/mgVSS)**	
	Default	**0.0220**		**0.0220**		**0.0800**		**0.0800**		**1.4200**		**1.4200**	
		0.0242	**0.0198**	**0.0242**	**0.0198**	**0.0880**	**0.0720**	**0.0880**	**0.0720**	**1.5620**	**1.2780**	**1.5620**	**1.2780**
pH	**Ephyra 1**	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.03	0.03	0.03	0.02	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 3	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 4	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0
VFA	**Ephyra 1**	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 2	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06
	Ephyra 3	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	Ephyra 4	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	CSTR	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06	0.06
COD	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 3	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 4	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01	0.01
	CSTR	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	0	0	0	0	0	0	0	0	0	0	0	0
Methane	**Ephyra 1**	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 2	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 3	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	Ephyra 4	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	CSTR	0.03	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02	0.02

		Influent characteristics																	
		Flow (m3/d)		COD (mg COD/L)		TKN (mg/L)		TP (mg/L)		pH [-]		Alkalinity (mmol/L)		ISS Influent (mgISS/L)		Calcium (mg/L)		Magnesium (mg/L)	
	Default	0.81		67820.00		3357.35		1524.78		7.20		100.00		2600.00		825.25		321.94	
		0.89	0.73	74602	61038	3693.1	3021.6	1677.2	1372.3	7.40	7.00	110.00	90.00	2860.0	2340.0	907.78	742.73	354.13	289.75
pH	Ephyra 1	0	0.03	-0.53	0.45	0.17	-0.15	0	0.03	0.03	-0.02	0.33	-0.42	0.02	0.02	0.02	0.02	0.02	0.02
	Ephyra 2	0.02	0.05	-0.58	0.5	0.2	-0.17	0.02	0.03	0.05	0	0.38	-0.45	0.03	0.03	0.02	0.03	0.02	0.03
	Ephyra 3	0	0.12	-0.62	0.57	0.22	-0.17	0.02	0.05	0.05	0.02	0.43	-0.48	0.03	0.03	0.03	0.03	0.03	0.03
	Ephyra 4	-0.05	0.2	-0.71	0.6	0.23	-0.2	0.02	0.05	0.05	0.02	0.47	-0.55	0.03	0.03	0.03	0.03	0.03	0.03
	CSTR	-0.01	0.03	-0.07	0.05	0.04	-0.04	-0.03	0.04	0.01	0	0.09	-0.11	0	0	0	0	0	0
VFA	Ephyra 1	0.06	-0.16	1.15	-1.12	-0.07	0	-0.04	-0.05	-0.05	-0.04	-0.1	0.08	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 2	0.05	-0.22	1.18	-1.17	-0.1	-0.01	-0.06	-0.06	-0.07	-0.05	-0.14	0.09	-0.06	-0.06	-0.06	-0.06	-0.06	-0.06
	Ephyra 3	0.09	-0.32	1.25	-1.24	-0.13	0	-0.07	-0.08	-0.08	-0.06	-0.18	0.13	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	Ephyra 4	0.18	-0.5	1.4	-1.36	-0.16	0.03	-0.06	-0.08	-0.08	-0.06	-0.25	0.22	-0.07	-0.07	-0.07	-0.07	-0.07	-0.07
	CSTR	0.92	-0.89	0.4	-0.26	0	0.14	0.04	0.08	0.05	0.07	-0.04	0.25	0.06	0.06	0.06	0.06	0.06	0.06
COD	Ephyra 1	0.02	-0.02	1.02	-1.01	0	0.01	0	0	0	0	0	0.02	0	0	0	0	0	0
	Ephyra 2	0.03	-0.02	1.03	-1.01	0	0.01	0.01	0.01	0.01	0.01	0	0.02	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 3	0.03	-0.03	1.05	-1.01	0	0.02	0.01	0.01	0.01	0.01	0	0.03	0.01	0.01	0.01	0.01	0.01	0.01
	Ephyra 4	0.05	-0.04	1.06	-1.02	0	0.02	0.01	0.01	0.01	0.01	-0.01	0.04	0.01	0.01	0.01	0.01	0.01	0.01
	CSTR	0.04	0.02	1.03	-0.97	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03	0.03
Gas flow	Ephyra 1	0	-1.43	0	-1.43	-1.43	0	-1.43	0	-1.43	0	0	-1.43	0	0	0	0	0	0
	Ephyra 2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	Ephyra 4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
	CSTR	1.67	-1.67	1.67	-1.67	0	0	0	0	0	0	0	0.83	0	0	0	0	0	0
Methane	Ephyra 1	-0.24	0.25	-0.24	0.33	0.34	-0.32	0.18	-0.18	0.58	-0.72	-0.34	0.43	0	0	0	0	0	0
	Ephyra 2	-0.27	0.26	0.11	0.13	0.15	-0.19	0.08	-0.15	0.33	-0.49	-0.33	0.46	-0.04	-0.04	-0.04	-0.04	-0.04	-0.04
	Ephyra 3	-0.35	0.35	0.14	0.14	0.09	-0.17	0.02	-0.12	0.19	-0.35	-0.2	0.31	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	Ephyra 4	-0.41	0.51	-0.01	0.31	0.12	-0.19	-0.01	-0.08	0.12	-0.25	0.01	0.09	-0.05	-0.05	-0.05	-0.05	-0.05	-0.05
	CSTR	-0.04	0.12	-0.28	0.16	0.1	-0.08	-0.07	0.11	0.04	0	0.2	-0.26	0.02	0.02	0.02	0.02	0.02	0.02

Kinetic parameter	**Default**	**Increase**	**Decrease**		**Reference system**				
Common		**10.00%**	**10.00%**		**pH**	**VFA**	**COD**	**Gas flow**	**Methane**
Hydrolysis rate (1/d)	2.1000	2.3100	1.8900	+	0	0.01	**-2.65**	**1.82**	-0.02
				-	0.01	-0.01	**3.14**	**-1.82**	0.05
Hydrolysis half sat. (-)	0.0600	0.0660	0.0540	+	0	0	0.03	0	0
				-	0	0	-0.03	0.23	0
Acetogens									
Max spec. growth rate (1/d)	0.2500	0.2750	0.2250	+	0	-0.05	0	0	0
				-	0	0.07	0	0	0
Substrate half sat. (mgCOD/L)	10.0000	11.0000	9.0000	+	0	0.04	0	0	0
				-	0	-0.04	0	0	0
Acetate inhibition (mgCOD/L)	10000.0	11000.0	9000.0	+	0	0	0	0	0
				-	0	0	0	0	0
Anaerobic decay rate (1/d)	0.0500	0.0550	0.0450	+	0	0.04	0	0	0
				-	0	-0.04	0	0	0
Aerobic/anoxic decay rate (1/d)	0.5200	0.5720	0.4680	+	0	0	0	0	0
				-	0	0	0	0	0
Methanogens									
Acetoclastic max spec. growth rate (1/d)	0.3000	0.3300	0.2700	+	0	**-1.69**	-0.01	0	0
				-	0	**2.61**	0.02	0	0
H2-utilizing max. speci. growth rate (1/d)	1.4000	1.5400	1.2600	+	0	0	-0.01	0	0.01
				-	0	0	0.02	0	-0.02
Acetoclastic substrate half sat. (mgCOD/L)	100.0000	110.0000	90.0000	+	0	**0.96**	0.01	0	0
				-	0	**-0.96**	-0.01	0	0
Acetoclastic methanol half sat. (mgCOD/L)	0.5000	0.5500	0.4500	+	0	0	0	0	0
				-	0	0	0	0	0
H2-utilizing CO2 half sat. (mmol/L)	0.1000	0.1100	0.0900	+	0	0	0	0	0
				-	0	0	0	0	0
H2-utilizing substrate half sat. (mgCOD/L)	1.0000	1.1000	0.9000	+	0	0	0.01	0	-0.01
				-	0	0	-0.01	0	0.01
H2-utilizing methanol half sat. (mgCOD/L)	0.5000	0.5500	0.4500	+	0	0	0	0	0

				-	0	0	0	0	0
Acetoclastic propionic inhibition (mgCOD/L)	10000.0	11000.0	9000.0	+	0	0	0	0	0
				-	0	0	0	0	0
Acetoclastic anaerobic decay rate (1/d)	0.1300	0.1430	0.1170	+	0	**1.84**	0.02	0	0
				-	0	**-1.53**	-0.01	0	0
Acetoclastic aerobic/anoxic decay rate (1/d)	0.6000	0.6600	0.5400	+	0	0	0	0	0
				-	0	0	0	0	0
H2-utilizing anaerobic decay rate (1/d)	0.1300	0.1430	0.1170	+	0	0	0.01	0	-0.01
				-	0	0	-0.01	0	0.01
H2-utilizing aerobic/anoxic decay rate (1/d)	2.8000	3.0800	2.5200	+	0	0	0	0	0
				-	0	0	0	0	0
Wastewater fractions									
Fbs - Readily Biodegradable (including acetate) (gCOD/g of total COD)	0.1600	0.1760	0.1440	+	0	0	**-0.78**	**0.45**	0.01
				-	0	0	**0.83**	**-0.45**	-0.01
Fac - Acetate (gCOD/g of readily bodegradable COD)	0.1500	0.1650	0.1350	+	0	0	0.08	0	0.02
				-	0	0	-0.08	0.23	-0.02
Fxsp - Non-colloidal slowly biodegradable (gCOD/g of slowly biodegradable COD)	0.7500	0.8250	0.6750	+	0	0	0	0	0
				-	0	0	0	0	0
Fus - Unbiodegradable soluble (gCOD/g of total COD)	0.0500	0.0550	0.0450	+	0	0	0.01	0	0
				-	0	0	-0.01	0	0
Fup - Unbiodegradable particulate (gCOD/g of total COD)	0.1300	0.1430	0.1170	+	0	0	0.03	0	-0.01
				-	0	0	-0.03	0	0.01
FZbpa - Propionic acetogens COD fraction (gCOD/g of total COD)	0.0001	0.00011	0.00009	+	0	0	0	0	0
				-	0	0	0	0	0
FZbam - Acetoclastic methanogens COD fraction (gCOD/g of total COD)	0.0001	0.00011	0.00009	+	0	-0.01	0	0	0
				-	0	0.01	0	0	0
FZbhm - H2-utilizing methanogens COD fraction (gCOD/g of total COD)	0.0001	0.00011	0.00009	+	0	0	0	0	0
				-	0	0	0	0	0
Stoichiometric parameter									
Common									
Biomass volatile fractions (VSS/TSS)	0.9200	1.0000	0.8400	-8.70%	0	0	0	0	0

				-8.70%	0	0.01	0	0	0
Endogenous residue volatile fractions (VSS/TSS)	0.9200	1.0000	0.8400	+	0	-0.01	0	0	0
				-	0	0.01	0	0	0
N in endogenous residue (mgN/mgCOD)	0.0700	0.0770	0.0630	+	0	0	0	0	0
				-	0	0	0	0	0
P in endogenous residue (mgP/mgCOD)	0.0220	0.0242	0.0198	+	0	0	0	0	0
				-	0	0	0	0	0
Endogenous residue COD:VSS ratio (mgCOD/mgVSS)	1.4200	1.5620	1.2780	+	0	0	0	0	0
				-	0	0	0	0	0
Particulate substrate COD/VSS ratio (mgCOD/mgVSS)	1.6000	1.7600	1.4400	+	0	0	0	0	0
				-	0	0	0	0	0
Particulate inert COD/VSS ratio (mgCOD/mgVSS)	1.6000	1.7600	1.4400	+	0	0	0	0	0
				-	0	0	0	0	0
Acetogens									
Yield (-)	0.1000	0.1100	0.0900	+	0	0	0.01	0	0.01
				-	0	0	-0.01	0.23	-0.01
H2 yield (-)	0.4000	0.4400	0.3600	+	0.01	0	0	0	0.07
				-	0	0	0	0.23	-0.06
CO2 yield (-)	1.0000	1.1000	0.9000	+	0	0	0	0.23	-0.03
				-	0	0	0	0	0.03
N in biomass (mgN/mgCOD)	0.0700	0.0770	0.0630	+	0	0	0	0	0
				-	0	0	0	0	0
P in biomass (mgP/mgCOD)	0.0220	0.0242	0.0198	+	0	0	0	0	0
				-	0	0	0	0	0
Fraction to endogenous residue (-)	0.0800	0.0880	0.0720	+	0	0	0	0	0
				-	0	0	0	0	0
COD:VSS ratio (mgCOD/mgVSS)	1.4200	1.5620	1.2780	+	0	0.01	0.11	0	0.01
				-	0	-0.01	-0.11	0.23	-0.01
Methanogens									
Acetoclastic yield (-)	0.1000	0.1100	0.0900	+	0	0	0	0	0
				-	0	0	0	0	0

Methanol acetoclastic yield (-)	0.1000	0.1100	0.0900	+	0	0	0.07	0	-0.02
				-	0	0	-0.07	0.23	0.02
H2-utilizing yield (-)	0.1000	0.1100	0.0900	+	0	0	0	0	0
				-	0	0	0	0	0
Methanol H2-utilizing yield (-)	0.1000	0.1100	0.0900	+	0	0	0	0	0
				-	0	0	0	0	0
N in acetoclastic biomass (mgN/mgCOD)	0.0700	0.0770	0.0630	+	0	0	0	0	0
				-	0	0	0	0	0
N in H2-utilizing biomass (mgN/mgCOD)	0.0700	0.0770	0.0630	+	0	0	0	0	0
				-	0	0	0	0	0
P in acetoclastic biomass (mgP/mgCOD)	0.0220	0.0242	0.0198	+	0	0	0	0	0
				-	0	0	0	0	0
P in H2-utilizing biomass (mgP/mgCOD)	0.0220	0.0242	0.0198	+	0	0	0	0	0
				-	0	0	0	0	0
Acetoclastic fraction to endog. Residue (-)	0.0800	0.0880	0.0720	+	0	0	0	0	0
				-	0	0	0	0	0
H2-utilizing fraction to endog. Residue (-)	0.0800	0.0880	0.0720	+	0	0	0	0	0
				-	0	0	0	0	0
Acetoclastic COD:VSS ratio (mgCOD/mgVSS)	1.4200	1.5620	1.2780	+	0	0	0	0	0
				-	0	0	0	0	0
H2-utilizing COD:VSS ratio (mgCOD/mgVSS)	1.4200	1.5620	1.2780	+	0.02	0.03	0.03	0.03	0
				-	0.02	0.03	0.03	0.03	0
Influent characteristics / operational parameters									
Flow (m3/d)	0.8130	0.8943	0.7317	+	0	**0.41**	**0.65**	**0.68**	0
				-	0	**-0.39**	**-0.7**	**-0.45**	0
COD (mg COD/L)	32780.00	36058.00	29502.00	+	-0.01	0	**0.99**	**1.14**	-0.02
				-	0.01	0	**-0.99**	**-0.91**	0.03
TKN (mg/L)	2086.3400	2294.974	1877.706	+	0.03	0	0	0	0.07
				-	-0.03	0	0	0.23	-0.07
TP (mg/L)	1010.1700	1111.187	909.153	+	-0.03	0	0	0.23	-0.03
				-	0.03	0	0	0	0.03

pH [-]	7.2000	7.4000	7.0000	+	0.01	0	0	0	0.09
				-	-0.01	0	0	0.23	-0.13
Alkalinity (mmol/L)	100.0000	110.0000	90.0000	+	0.06	0.01	0	0.23	-0.03
				-	-0.06	-0.01	0	0	0.03
ISS Influent (mgISS/L)	1300.0000	1430.000	1170.000	+	0	0	0	0	0
				-	0	0	0	0	0
Calcium (mg/L)	619.6000	681.5600	557.6400	+	0	0	0	0	0
				-	0	0	0	0	0
Magnesium (mg/L)	205.2000	225.7200	184.6800	+	0	0	0	0	0
				-	0	0	0	0	0

APPENDIX C: Lab research results

Table 30: Soluble COD measurements in mg/L of lab-scale plant using Amsterdam sludge

Date	Influent	Ephyra 1	Ephyra2	Ephyra 3	Ephyra CSTR	Reference
12-2-2016	5900				477.5	
12-2-2016	5775				490	
24-2-2016					1450	1355
24-2-2016					1435	1335
26-2-2016		2220	1204	1316	778.5	1670
26-2-2016		2260	1212	1292	757.5	1694
29-2-2016		3455	3855	3820	3465	3885
29-2-2016		3405	3860	3740	3430	3885
4-3-2016	2480	1020	954	1130	710	720
4-3-2016	2300	1025	921	1180	700	700
7-3-2016		730	860	830	885	955
7-3-2016		685	905	810	880	955
Average	4114	1850	1721	1765	1288	1715
Standard Deviation	1992	1151	1325	1258	1054	1196

Table 31: Total COD measurements in mg/L of lab-scale plant using Amsterdam sludge

Date	Influent	Ephyra 1	Ephyra 2	Ephyra 3	Ephyra CSTR	Reference
9-12-2015	57863				24568	
25-1-2016	48631					
12-2-2016	51075				23300	
12-2-2016	50775				22175	
24-2-2016					28300	30225
24-2-2016					28550	30625
14-3-2016		27820	30400	30540	24140	26160
14-3-2016		27800	29040	29820	24380	25900
18-3-2016		33820	34760	32680	34380	34300
18-3-2016		33060	34980	32700	33980	35180
21-3-2016		31920	33100	28280	29200	30560
21-3-2016		31200	31700	28240	29400	27780
23-3-2016	52500	35080	29120	28820	28300	28420
23-3-2016	54950	35100	28140	28300	28560	29180
Average	52632	31975	31405	29923	27889	29833
Standard Deviation	3306	2910	2659	1893	3868	3081

Table 32: Dry and organic dry solids measurements of lab-scale plant using Amsterdam sludge

	Mass of cup (grams)	Mass of cup and sludge sample (grams)	Mass of cup and sludge sample after 105^0C (grams)	Mass of cup and sludge sample after 550^0C (grams)	Dry solids (%)	Organic Dry solids (%)
Monday, 14-3-2016						
Ephyra 1	0.9639	22.0926	1.4613	1.1164	2.35	69.34
Ephyra 2	0.9647	22.9298	1.4599	1.1195	2.25	68.74
Ephyra 3	0.9659	20.8740	1.4813	1.1202	2.59	70.06
Ephyra CSTR	0.9602	20.3531	1.4069	1.1130	2.30	65.79
Reference	0.9743	21.9730	1.4752	1.1347	2.39	67.98
Friday, 18-3-2016						
Ephyra 1	0.9888	19.2408	1.4575	1.1333	2.57	69.17
Ephyra 2	0.9776	19.9547	1.4633	1.1273	2.56	69.18
Ephyra 3	0.9925	20.1099	1.4696	1.1395	2.50	69.19
Ephyra CSTR	0.9866	28.3045	1.6086	1.1981	2.28	66.00
Reference	0.9814	24.9711	1.5628	1.1762	2.42	66.49
Monday, 21-3-2016						
Ephyra 1	0.9672	22.6926	1.4792	1.1271	2.36	68.77
Ephyra 2	0.9920	25.1213	1.5956	1.1839	2.50	68.21
Ephyra 3	0.9891	24.2722	1.5282	1.1575	2.32	68.76

Ephyra CSTR	0.9826	22.6795	1.4863	1.1569	2.32	65.40
Reference	0.9782	15.6871	1.3262	1.0917	2.37	67.39

Table 33: Data analysis of dry and organic dry solids measurements of lab-scale plant using Amsterdam sludge

	Influent	Ephyra 1	Ephyra 2	Ephyra 3	Ephyra CSTR	Reference
Dry solids (%)						
Number of samples	52	3	3	3	3	3
Average	4.1	2.4	2.4	2.5	2.3	2.4
Standard Deviation	0.2	0.1	0.2	0.1	0.0	0.0
Minimum	3.7	2.4	2.3	2.3	2.3	2.4
Maximum	4.8	2.6	2.6	2.6	2.3	2.4
Organic Dry solids (%)						
Number of samples	52	3	3	3	3	3
Average	81.3	69.1	68.7	69.3	65.7	67.3
Standard Deviation	1.5	0.3	0.5	0.7	0.3	0.8
Minimum	78.1	68.8	68.2	68.8	65.4	66.5
Maximum	83.9	69.3	69.1	70.1	66.0	68.0

Table 34: Gas production and methane potential of Ephyra® compartments of lab-scale plant using Amsterdam sludge

	Gas production (L/d)			Methane yield (%)		
Date	Ephyra 1	Ephyra 2	Ephyra 3	Ephyra 1	Ephyra 2	Ephyra 3
19-2-2016	0.35	0.47		62.2	65.2	63.9
4-3-2016	1.40	1.93		51.1	56.2	
7-3-2016				54.7	60.9	
11-3-2016	2.56	2.00	1.16	57.3	61.3	56.1
14-3-2016	2.73	2.07	1.60	55.1	62.2	56.6
16-3-2016	2.20	1.80	1.33	52.5	59.4	59.7
18-3-2016	0.35	0.47		59.0	63.2	62.4
Average	1.85	1.65	1.36	56.0	61.2	59.7
Standard Deviation	0.98	0.67	0.22	3.8	2.9	3.4

APPENDIX D: Equations on which the anaerobic digestion model of BioWin is built

1. $Anaerobic\ decay\ of\ OHO = -\ OHOs + 0.18\ endogenous\ products + 0.82\ Slowly\ bio.COD\ (part.) + 0.06\ Part\ bio.org.N + 0.02\ Part.bio.org.P$

2. $Sequestration\ of\ acetate\ by\ PAO = 0.89\ Stored\ PHA\ -\ 0.51\ Released\ stored\ polyP - Acetate + 0.11\ Dissolved\ H_2 + 0.51\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.12\ Magnesium + 0.03\ Calcium$

3. $Sebquestration\ of\ propionate\ by\ PAO = 0.51\ Stored\ PHA - 0.29\ Released\ stored\ polyP - Propionate + 0.49\ Dissolved\ H_2 + 0.29\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.07\ Magnesium + 0.02\ Calcium$

4. $Anaerobic\ PAO\ lysis\ decay = -PAO + 0.25\ Endogenous\ products + 0.60\ Readily\ biod.COD\ (complex) + 0.04\ Ammonia + 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.15\ Sol.inert\ COD + 0.01\ Sol.inert\ TKN$

5. $PHA\ release\ on\ ana.decay\ of\ PAO = -\ Stored\ PHA + Acetate$

6. $PP - LO\ release\ on\ ana.decay\ of\ PAO = -R\ eleasable\ stored\ polyP +\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.24\ Magnesium + 0.06\ Calcium$

7. $PP - HI\ release\ on\ ana.decay\ of\ PAO = -Fixed\ stored\ polyP +\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.24\ Magnesium + 0.06\ Calcium$

8. $Cleavage\ of\ PP\ for\ ana.maintenance = -\ Releasable\ stored\ polyP + 0.24\ Magnesium + 0.06\ Calcium$

9. $Hydrolysis\ of\ X_S\ COD = -Slowly\ bio.COD\ (part.) + Readily\ bio.COD\ (complex)$

10. $Hydrolysis\ of\ X_{ON} = -Part.bio.org.N$

11. $Hydrolysis\ of\ X_{OP} = -Part.bio.org.P +\ PO_4 - P\ (Solub.\&\ Me\ Complexed)$

12. $Adsorption\ of\ colloidal\ COD = Slowly\ bio.COD\ (part.) - Slowly\ bio.COD\ (colloid)$

13. $Ammonification = +Ammonia\ N - Sol.bio.org.N$

14. $Assimilative\ nitrate\ reduction - OHO = -4.57\ OHOs + 1.32\ Ammonia\ N - Nitrate + 0.01\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.13\ Total\ CO_2$

15. $Assimilative\ nitrite\ reduction - OHO = -3.43\ OHOs + 1.24\ Ammonia\ N - Nitrite + 0.08\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.1\ Total\ CO_2$

16. $Decay\ of\ endogenous\ products\ to\ X_{SP} = -Endogenous\ products + Slowly\ bio.COD\ (part.) +\ 0.07\ Part.bio.org.N + 0.02\ Part.bio.org.P$

17. $Fermentation\ of\ SBSC\ by\ OHO\ (low\ H_2) = OHO - 10\ Readily\ biod.COD\ (complex) + 5.5\ Acetate + 3.5\ Dissolved\ H_2 - 0.07\ Ammonia\ N - 0.02\ PO_4 -$

$P\ (Solub.\&\ Me\ Complexed) + 0.06\ Total\ CO_2$

18. $Fermentation\ of\ SBSC\ by\ OHO\ (high\ H_2) = OHO - 10\ Readily\ biod.COD\ (complex) + 2\ Acetate + 7\ Dissolved\ H_2 - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed)$

19. $Growth\ of\ propionic\ acetogens = Propionic\ acetogens + 5\ Acetate - 10\ Propionate + 4\ Dissolved\ H_2 - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.08\ Total\ CO_2$

20. $Decay\ of\ propionic\ acetogens = -Propionic\ acetogens + 0.08\ Endogenous\ products + 0.92\ Slowly\ bio.COD\ (part.) + 0.06\ Part.bio.org.N + 0.02\ Part.bio.org.P$

21. $Growth\ of\ methanogens - acetoclastic = Acetoclastic\ methanogens - 10\ Acetate + 2.26\ Dissolved\ Methane - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.14\ Total\ CO_2$

22. $Growth\ on\ methanol\ of\ methanogens - acetoclastic = Acetoclastic\ methanogens - 10\ Propionate + 2.26\ Dissolved\ Methane - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 0.05\ Total\ CO_2$

23. $Decay\ of\ methanogens - acetoclastic = -Acetoclastic\ methanogens + 0.08\ Endogenous\ products + 0.92\ Slowly\ bio.COD\ (part.) + 0.06\ Part.bio.org.N + 0.02\ Part.bio.org.P$

24. $Growth\ of\ methanogens - hydrogenotrophic = Hydrogenotrophic\ methanogens - 10\ Dissolved\ H_2 + 2.26\ Dissolved\ Methane - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed) - 0.16\ Total\ CO_2$

25. $Growth\ on\ methanol\ of\ methanogens - hydrogenotrophic = Hydrogenotrophic\ methanogens - 10\ Propionate - 3.33\ Dissolved\ H_2 + 3.09\ Dissolved\ Methane - 0.07\ Ammonia\ N - 0.02\ PO_4 - P\ (Solub.\&\ Me\ Complexed)$

26. $Decay\ of\ methanogens - hydrogenotrophic = -Hydrogenotrophic\ methanogens + 0.08\ Endogenous\ products + 0.92\ Slowly\ bio.COD\ (part.) + 0.06\ Part.bio.org.N + 0.02\ Part.bio.org.P$

27. $Struvite\ precipitation/redissolution = -0.58\ Ammonia\ N - 1.27\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 10.09\ ISS\ Influent - Magnesium$

28. $HDPprecipitation/redissolution = -0.39\ PO_4 - P\ (Solub.\&\ Me\ Complexed) + 2.62\ Hydroxy - dicalcium - phosphate - Calcium$

29. $HDAprecipitation/redissolution = -15.73\ Hydroxy - dicalcium - phosphate + 12.53\ Hydroxy - apatite - Calcium$

30. $H_2\ stripping = -Dissolved\ H_2$

31. $NH_3\ stripping = -Ammonia\ N$

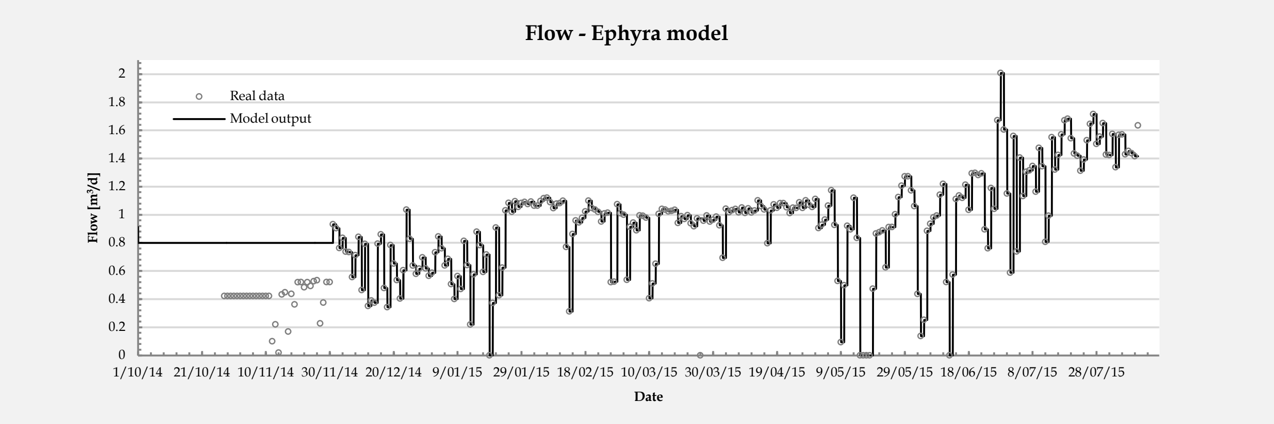
32. $N_2\ stripping = -Dissolved\ nitrogen\ gas$

33. $N_2O\ stripping = -Nitrous\ Oxide\ N$

34. $CH_4\ stripping = -Dissolved\ methane$

35. $CO_2\ stripping = -Total\ CO_2$

APPENDIX E: Output of Calibrated Models (both Ephyra® and Reference system)


Flow - Ephyra model
Real data
Model output
Flow [m³/d]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/10/14
21/10/14
10/11/14
30/11/14
20/12/14
9/01/15
29/01/15
18/02/15
10/03/15
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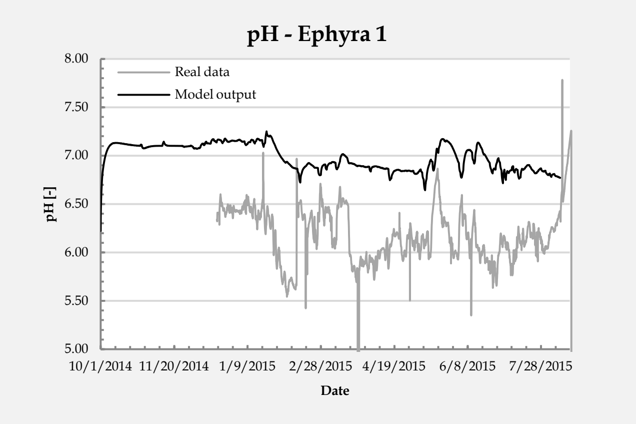
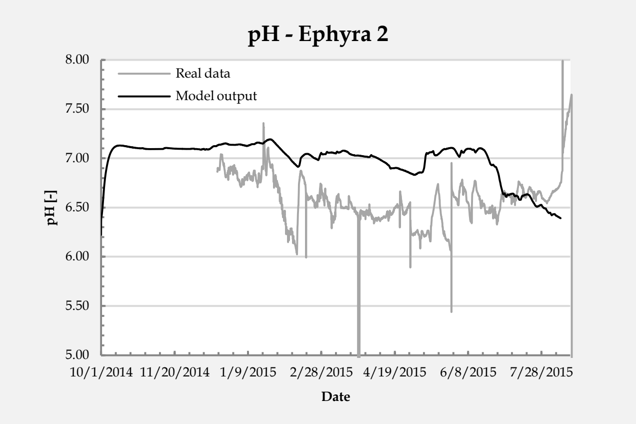
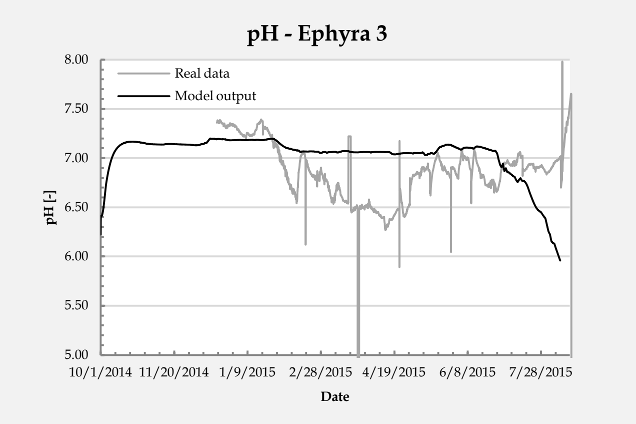
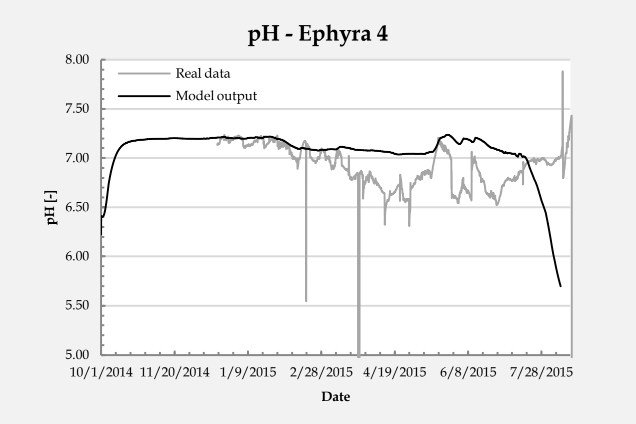
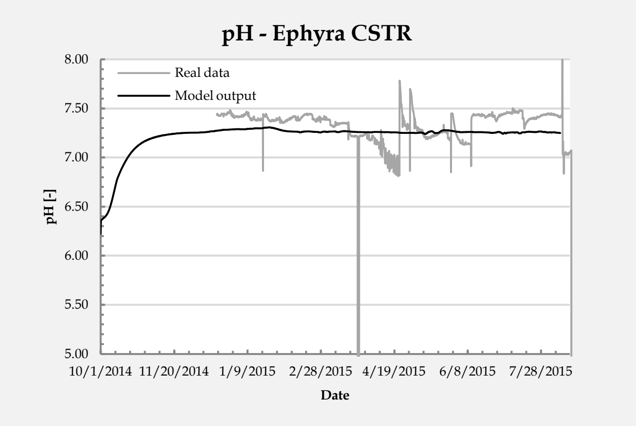
18/06/15
8/07/15
28/07/15
Date


pH - Ephyra 1
Real data
Model output
pH [-]
8.00
7.50
7.00
6.50
6.00
5.50
5.00
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


pH - Ephyra 2
Real data
Model output
pH [-]
8.00
7.50
7.00
6.50
6.00
5.50
5.00
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


pH - Ephyra 3
Real data
Model output
pH [-]
8.00
7.50
7.00
6.50
6.00
5.50
5.00
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


pH - Ephyra 4
Real data
Model output
pH [-]
8.00
7.50
7.00
6.50
6.00
5.50
5.00
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


pH - Ephyra CSTR
Real data
Model output
pH [-]
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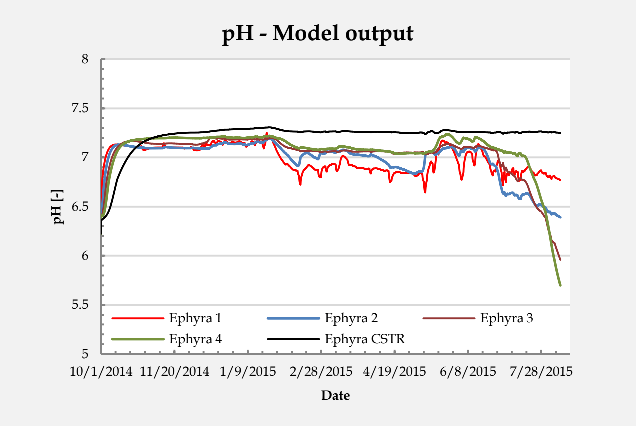
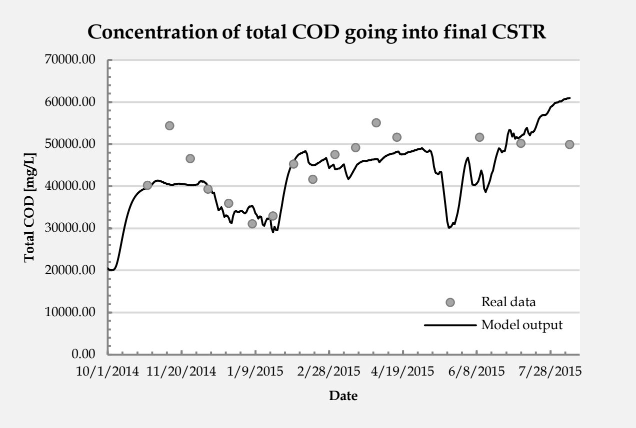
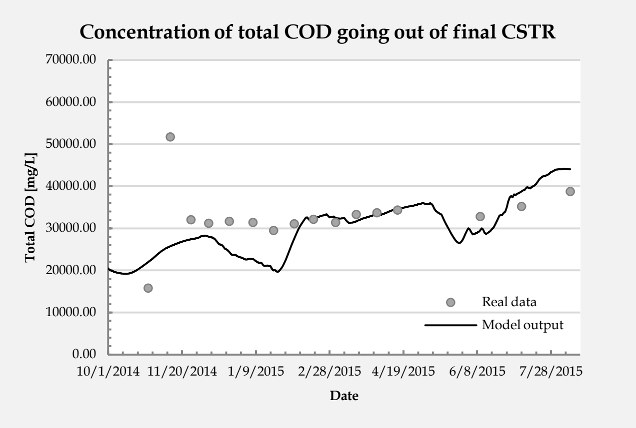
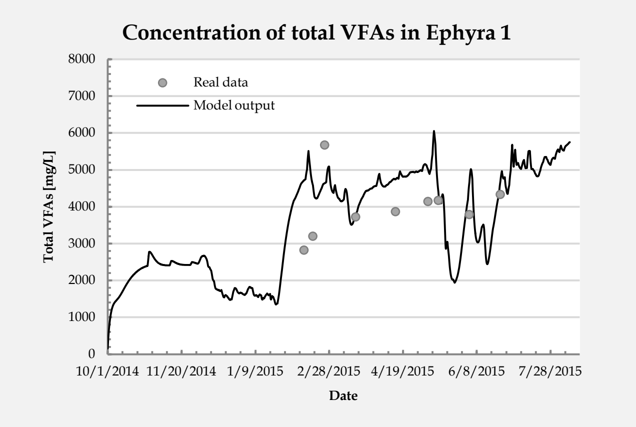
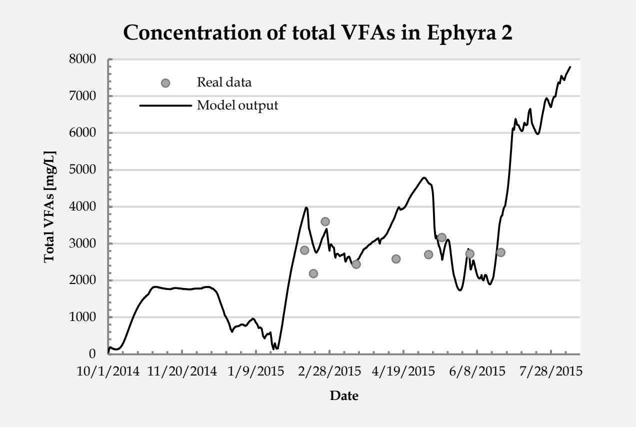
7.50
7.00
6.50
6.00
5.50
5.00
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

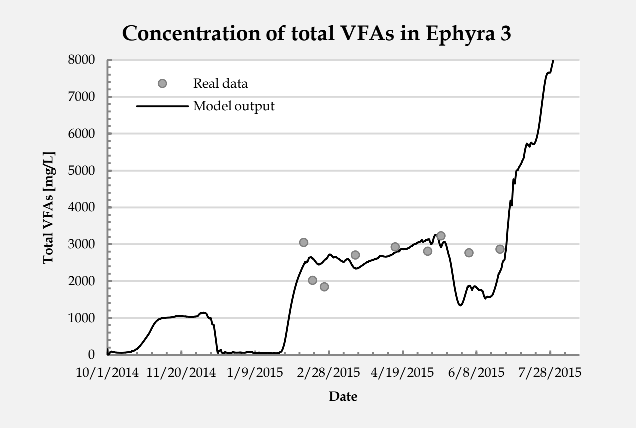

pH - Model output
pH [-]
8
7.5
7
6.5
6
5.5
5
Ephyra 1
Ephyra 2
Ephyra 3
Ephyra 4
Ephyra CSTR
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

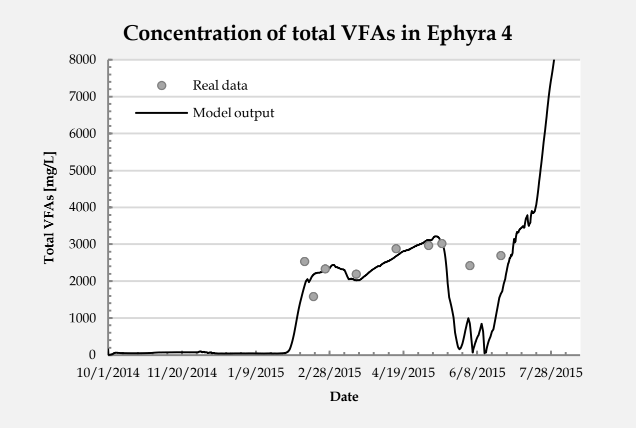

Concentration of total COD going into final CSTR
Total COD [mg/L]
70000.00
60000.00
50000.00
40000.00
30000.00
20000.00
10000.00
0.00
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

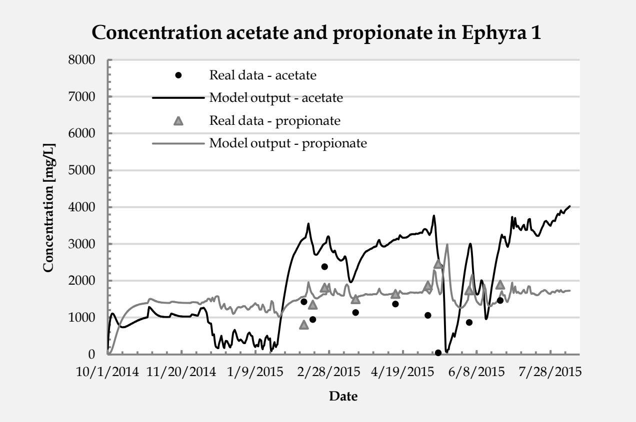

Concentration of total COD going out of final CSTR
Total COD [mg/L]
70000.00
60000.00
50000.00
40000.00
30000.00
20000.00
10000.00
0.00
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

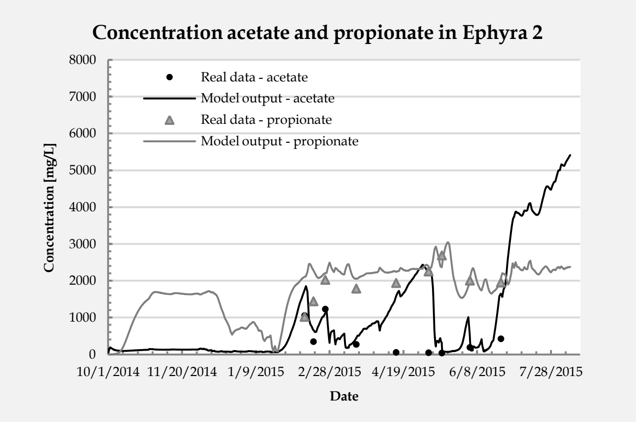

Concentration of total VFAs in Ephyra 1
Total VFAs [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

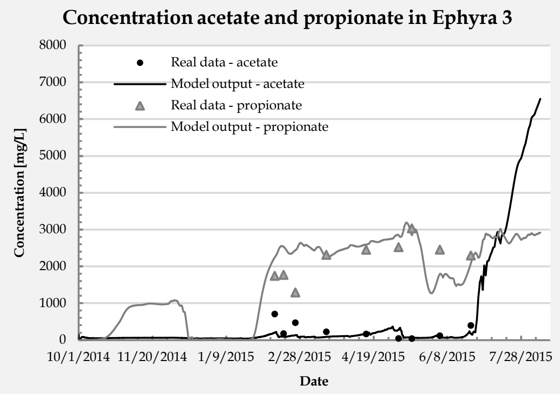

Concentration of total VFAs in Ephyra 2
Total VFAs [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


Concentration of total VFAs in Ephyra 3
Total VFAs [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

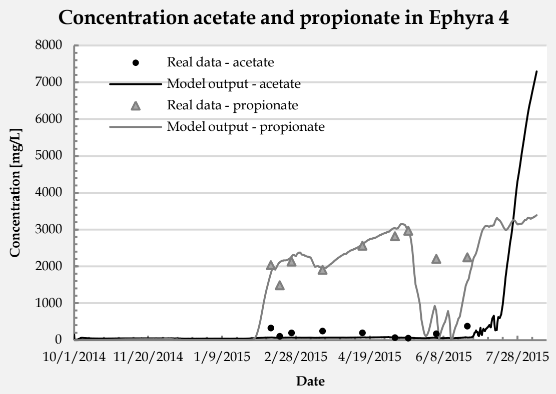

Concentration of total VFAs in Ephyra 4
Total VFAs [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data
Model output
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date


Concentration acetate and propionate in Ephyra 1
Concentration [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data - acetate
Model output - acetate
Real data - propionate
Model output - propionate
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date

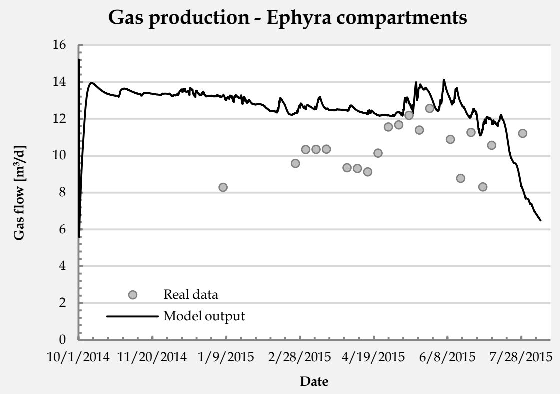

Concentration acetate and propionate in Ephyra 2
Concentration [mg/L]
8000
7000
6000
5000
4000
3000
2000
1000
0
Real data - acetate
Model output - acetate
Real data - propionate
Model output - propionate
10/1/2014
11/20/2014
1/9/2015
2/28/2015
4/19/2015
6/8/2015
7/28/2015
Date



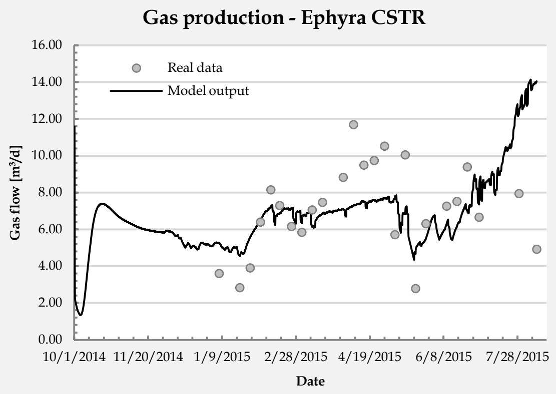
Concentration acetate and propionate in Ephyra 3



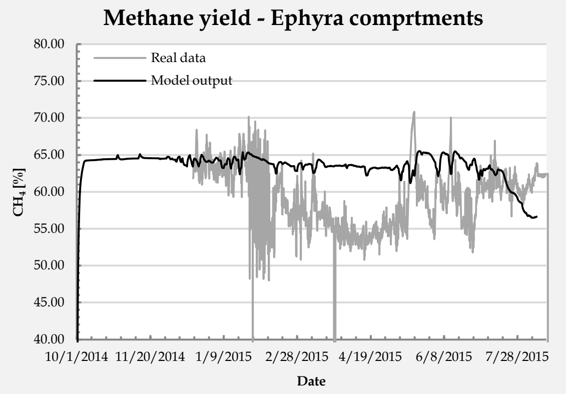
Concentration acetate and propionate in Ephyra 4



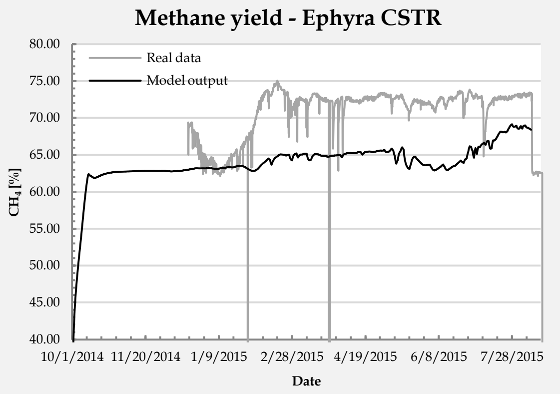
Gas production - Ephyra compartments



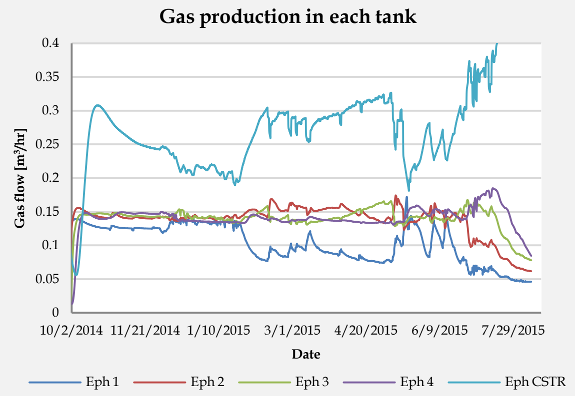
Gas production - Ephyra CSTR



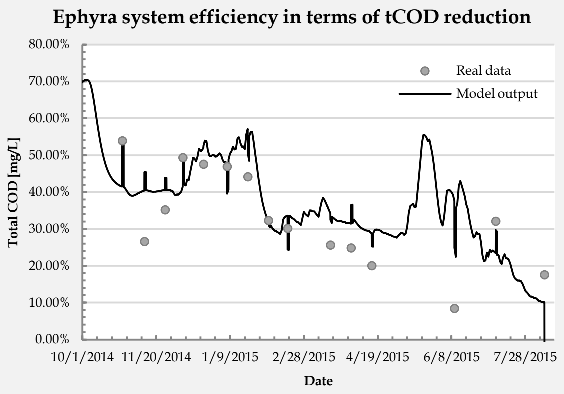
Methane yield - Ephyra comprtments



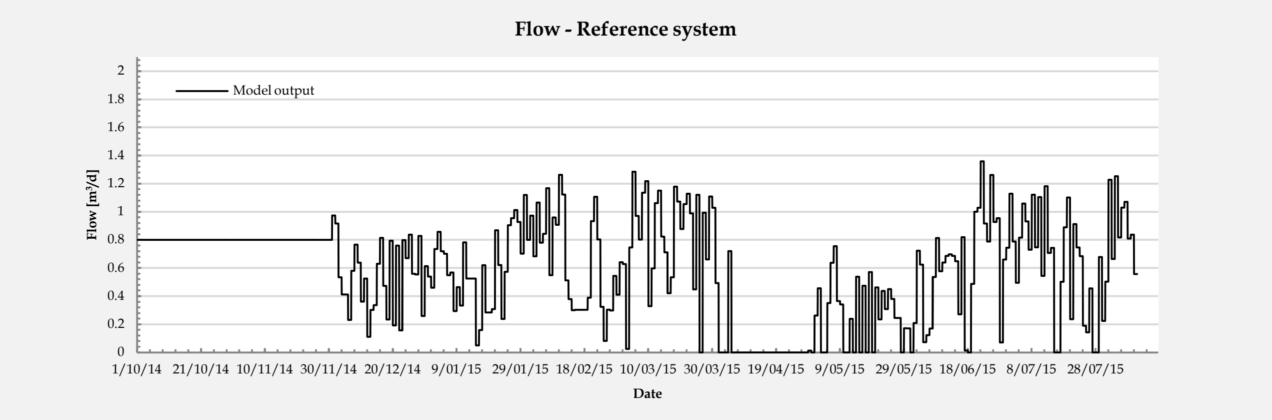
Methane yield - Ephyra CSTR

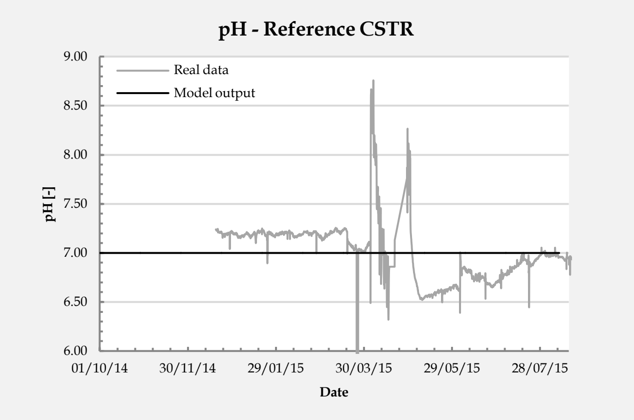


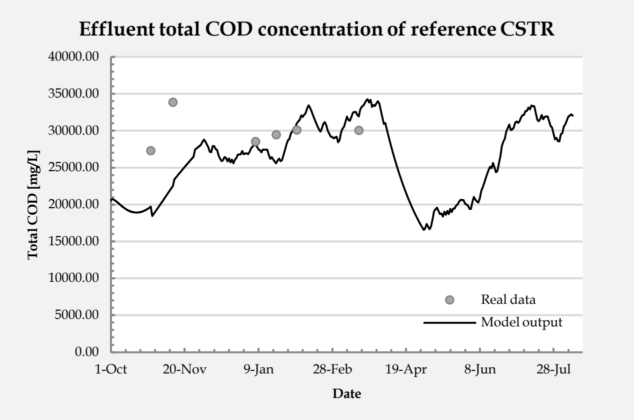
Gas production in each tank

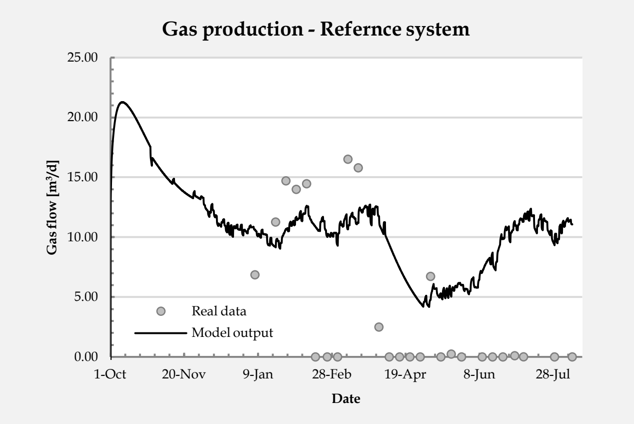


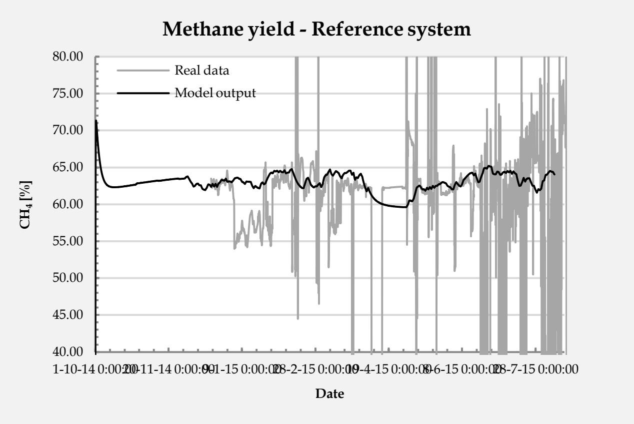
Ephyra system efficiency in terms of tCOD reduction


Flow - Reference system
Model output
Flow [m³/d]
2
1.8
1.6
1.4
1.2
1
0.8
0.6
0.4
0.2
0
1/10/14
21/10/14
10/11/14
30/11/14
20/12/14
9/01/15
29/01/15
18/02/15
10/03/15
30/03/15
19/04/15
9/05/15
29/05/15
18/06/15
8/07/15
28/07/15
Date


pH - Reference CSTR
Real data
Model output
pH [-]
9.00
8.50
8.00
7.50
7.00
6.50
6.00
01/10/14
30/11/14
29/01/15
30/03/15
29/05/15
28/07/15
Date


Effluent total COD concentration of reference CSTR
Real data
Model output
Total COD [mg/L]
40000.00
35000.00
30000.00
25000.00
20000.00
15000.00
10000.00
5000.00
0.00
1-Oct
20-Nov
9-Jan
28-Feb
19-Apr
8-Jun
28-Jul
Date


Gas production - Refernce system
Real data
Model output
Gas flow [m³/d]
25.00
20.00
15.00
10.00
5.00
0.00
1-Oct
20-Nov
9-Jan
28-Feb
19-Apr
8-Jun
28-Jul
Date


Methane yield - Reference system
Real data
Model output
CH₄ [%]
80.00
75.00
70.00
65.00
60.00
55.00
50.00
45.00
40.00
1-10-14 0:00:00
20-11-14 0:00:00
9-1-15 0:00:00
28-2-15 0:00:00
19-4-15 0:00:00
8-6-15 0:00:00
28-7-15 0:00:00
Date

APPENDIX F: Steady state simulations results for different recirculation rates

Table 35: Results of steady state simulations under different recirculation rates of Ephyra system model using BioWin

Ephyra® 1

Rate [%]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
0	6.62	65590.93	6706.82	11091.17	4928.52	39.64	147.25	1545.91	6.26	1778.31	9.26	0.05	61.76
1	6.69	64900.06	6114.48	10504.33	4351.66	84.89	149.91	1546.53	6.24	1762.82	10.18	0.06	62.58
2	6.76	64288.88	5611.59	10005.51	3868.29	123.13	152.66	1547.14	6.23	1743.31	11.55	0.07	63.36
3	6.81	63720.67	5156.52	9553.75	3438.77	157.62	155.54	1547.75	6.22	1717.74	13.48	0.08	64.13
4	6.86	63178.01	4729.38	9129.48	3045.66	189.8	158.6	1548.36	6.21	1683.73	16.11	0.09	64.89
5	6.91	62645.84	4312.74	8715.52	2676.54	220.71	161.94	1548.98	6.2	1636.2	19.78	0.09	65.67
6	6.96	62098.06	3876.06	8281.78	2312.21	252.05	165.82	1549.59	6.18	1563.85	25.3	0.1	66.48
7	7.01	61483.83	3359.44	7769.26	1918.23	287.04	170.78	1550.2	6.17	1441.21	34.52	0.11	67.42
8	7.06	60872.5	2843.47	7257.23	1551.94	320.81	176.28	1550.81	6.16	1291.53	45.76	0.12	68.34
9	7.09	60468.51	2569.29	6983.16	1349.96	340.37	180.01	1551.43	6.15	1219.33	51.4	0.12	68.83
10	7.11	60124.21	2364.24	6777.07	1200.41	355.61	183.33	1552.04	6.13	1163.83	55.82	0.12	69.19
11	7.12	59809.36	2193.03	6604.23	1079.62	368.6	186.52	1552.65	6.12	1113.41	59.87	0.13	69.49
12	7.14	59515.6	2046.02	6455.17	979.84	379.96	189.62	1553.26	6.11	1066.18	63.69	0.13	69.74
13	7.15	59238.17	1917.77	6324.53	896.28	390.05	192.64	1553.87	6.1	1021.48	67.32	0.13	69.95
14	7.16	58973.77	1804.49	6208.61	825.52	399.13	195.6	1554.48	6.09	978.97	70.79	0.13	70.12
15	7.17	58719.92	1703.38	6104.64	764.98	407.41	198.5	1555.09	6.07	938.4	74.13	0.13	70.28
16	7.17	58474.75	1612.28	6010.49	712.7	415.02	201.35	1555.7	6.06	899.58	77.34	0.13	70.41
17	7.18	58236.76	1529.5	5924.53	667.14	422.1	204.16	1556.31	6.05	862.36	80.46	0.14	70.53
18	7.19	58004.83	1453.72	5845.45	627.12	428.73	206.93	1556.92	6.04	826.6	83.48	0.14	70.64
19	7.19	57778.03	1383.92	5772.24	591.73	434.99	209.67	1557.53	6.02	792.2	86.41	0.14	70.73
20	7.2	57555.68	1319.3	5704.12	560.22	440.92	212.37	1558.14	6.01	759.08	89.28	0.14	70.81
21	7.2	57337.21	1259.22	5640.46	532.03	446.58	215.05	1558.75	6	727.19	92.06	0.14	70.89
22	7.21	57122.18	1203.17	5580.76	506.67	452	217.7	1559.36	5.99	696.5	94.78	0.14	70.96
23	7.21	56910.21	1150.72	5524.6	483.76	457.21	220.32	1559.97	5.98	666.95	97.42	0.14	71.02
24	7.21	56700.98	1101.5	5471.61	462.97	462.23	222.91	1560.58	5.96	638.53	99.98	0.14	71.07
25	7.22	56494.22	1055.2	5421.5	444.03	467.07	225.47	1561.19	5.95	611.17	102.48	0.14	71.12
26	7.22	56289.68	1011.56	5373.99	426.7	471.77	228	1561.79	5.94	584.86	104.9	0.14	71.16

27	7.22	56087.17	970.33	5328.85	410.79	476.33	230.5	1562.4	5.93	559.53	107.26	0.14	71.2
28	7.22	55886.5	931.31	5285.88	396.14	480.76	232.98	1563.01	5.92	535.17	109.56	0.14	71.24
29	7.23	55687.52	894.32	5244.9	382.6	485.09	235.43	1563.62	5.9	511.72	111.79	0.14	71.27
30	7.23	55490.11	859.22	5205.77	370.05	489.31	237.86	1564.22	5.89	489.16	113.96	0.15	71.3

Ephyra® 2

Rate [%]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
0	5.88	62842.64	9732.94	13268.85	7055.24	111.37	238.25	1566.79	5.78	2677.71	11.97	0.07	53.92
1	6.65	59842.05	6470.37	10054.33	3926.81	331.13	243.13	1567.21	5.77	2543.56	14.9	0.13	59.88
2	6.87	58100.34	4631.34	8239.73	2152.71	452.69	245.52	1567.61	5.76	2478.63	18.71	0.15	62.57
3	6.98	57087.99	3616.43	7235.73	1208.65	515.61	248.01	1568.01	5.76	2407.77	23.39	0.16	64.09
4	7.02	56498.18	3078.69	6700.81	752.45	544.27	250.72	1568.41	5.75	2326.24	28.95	0.16	64.74
5	7.05	56083.29	2736.95	6358.42	513.42	558.28	253.88	1568.81	5.74	2223.53	36.12	0.16	64.96
6	7.07	55716.05	2445.42	6064.83	367.27	566.86	257.94	1569.21	5.73	2078.15	46.5	0.15	65
7	7.09	55313.76	2106.94	5723.56	262.85	574.33	263.92	1569.61	5.73	1844.09	63.54	0.15	64.92
8	7.1	54910.51	1762.84	5376.65	197.52	580.75	270.83	1570.02	5.72	1565.32	83.97	0.15	64.78
9	7.11	54661.23	1602.71	5214.71	172.1	583.43	274.57	1570.42	5.71	1430.61	93.66	0.15	64.65
10	7.11	54447.16	1484.29	5094.97	155.99	585.53	277.58	1570.82	5.71	1328.3	100.93	0.14	64.54
11	7.12	54246.22	1381.17	4990.84	144.12	587.57	280.33	1571.21	5.7	1237.05	107.37	0.14	64.45
12	7.12	54053.96	1288.11	4897.04	134.92	589.61	282.91	1571.61	5.69	1153.2	113.25	0.14	64.39
13	7.13	53868.48	1202.93	4811.32	127.56	591.66	285.34	1572.01	5.69	1075.37	118.68	0.14	64.34
14	7.13	53688.61	1124.31	4732.31	121.54	593.71	287.65	1572.41	5.68	1002.77	123.71	0.14	64.31
15	7.13	53513.54	1051.33	4659.08	116.52	595.75	289.85	1572.81	5.67	934.81	128.41	0.14	64.29
16	7.14	53342.64	983.27	4590.86	112.26	597.78	291.96	1573.21	5.67	871.02	132.8	0.14	64.28
17	7.14	53175.41	919.55	4527.07	108.59	599.8	293.98	1573.61	5.66	810.96	136.92	0.14	64.28
18	7.14	53011.44	859.71	4467.22	105.39	601.8	295.92	1574.01	5.65	754.32	140.8	0.14	64.28
19	7.14	52850.42	803.39	4410.95	102.57	603.78	297.8	1574.4	5.64	700.82	144.47	0.14	64.29
20	7.15	52692.13	750.34	4358	100.07	605.74	299.62	1574.8	5.64	650.27	147.93	0.14	64.31
21	7.15	52536.41	700.4	4308.19	97.83	607.67	301.38	1575.2	5.63	602.57	151.19	0.14	64.33
22	7.15	52383.15	653.41	4261.36	95.82	609.56	303.07	1575.6	5.62	557.59	154.25	0.14	64.35
23	7.15	52232.22	609.25	4217.39	93.99	611.41	304.71	1575.99	5.62	515.25	157.13	0.14	64.37

24	7.16	52083.52	567.8	4176.14	92.33	613.23	306.29	1576.39	5.61	475.46	159.83	0.14	64.4
25	7.16	51936.95	528.93	4137.5	90.82	614.99	307.82	1576.79	5.6	438.12	162.34	0.14	64.42
26	7.16	51792.41	492.55	4101.35	89.43	616.72	309.3	1577.18	5.6	403.12	164.69	0.14	64.45
27	7.16	51649.8	458.53	4067.57	88.15	618.4	310.72	1577.58	5.59	370.38	166.87	0.14	64.47
28	7.16	51509.04	426.77	4036.07	86.97	620.04	312.09	1577.98	5.58	339.81	168.89	0.14	64.5
29	7.17	51370.05	397.18	4006.74	85.87	621.63	313.42	1578.37	5.58	311.31	170.76	0.14	64.52
30	7.17	51232.75	369.67	3979.5	84.85	623.18	314.7	1578.77	5.57	284.83	172.47	0.14	64.55

Ephyra® 3

Rate [%]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
0	5.41	59771.91	12467.98	15961.56	8906.21	186.99	305.14	1587.33	5.33	3561.77	14.6	0.07	60.73
1	7.03	51784.09	3535.9	7126.25	474.14	741.9	319.85	1587.6	5.33	3061.76	28.64	0.18	65.44
2	7.05	51337.59	3122.67	6689.34	187.75	723.73	322.65	1587.8	5.33	2934.92	36.88	0.16	64.41
3	7.05	51095.36	2924.54	6476.92	123.74	707.06	325.57	1587.99	5.33	2800.8	46	0.14	63.11
4	7.05	50884.27	2750.52	6296.28	101.19	698.12	328.83	1588.19	5.32	2649.33	56.5	0.14	62.37
5	7.06	50657.84	2551.29	6094.31	90.46	694.03	332.88	1588.38	5.32	2460.83	69.85	0.14	62.04
6	7.07	50375.33	2279.55	5822.01	83.48	693.71	338.56	1588.57	5.32	2196.07	89.05	0.14	61.98
7	7.09	49966.99	1849.74	5393.64	77.14	698.18	347.65	1588.77	5.32	1772.6	120.38	0.14	62.19
8	7.11	49502.52	1348.76	4895.36	71.75	705.73	358.25	1588.96	5.31	1277.01	157.2	0.14	62.56
9	7.12	49258.37	1121.21	4669.05	69.58	708.59	362.99	1589.16	5.31	1051.63	173.38	0.14	62.73
10	7.13	49065.29	957	4505.76	68.08	710.56	366.36	1589.35	5.31	888.92	184.76	0.14	62.84
11	7.14	48891.35	816.51	4366.06	66.83	712.32	369.2	1589.54	5.31	749.68	194.32	0.14	62.94
12	7.14	48731.01	692.88	4243.13	65.75	713.94	371.65	1589.73	5.3	627.14	202.58	0.14	63.03
13	7.15	48582.25	583.64	4134.49	64.78	715.39	373.78	1589.92	5.3	518.86	209.71	0.14	63.11
14	7.15	48444.07	487.56	4038.9	63.91	716.65	375.6	1590.11	5.3	423.65	215.81	0.14	63.17
15	7.16	48315.95	404.02	3955.74	63.13	717.68	377.12	1590.3	5.3	340.89	220.91	0.14	63.22
16	7.16	48197.65	332.76	3884.74	62.42	718.46	378.35	1590.49	5.3	270.34	225.01	0.14	63.26
17	7.16	48089.08	273.69	3825.78	61.77	718.95	379.27	1590.68	5.29	211.91	228.1	0.14	63.28
18	7.16	47989.95	226.46	3778.51	61.18	719.15	379.9	1590.87	5.29	165.28	230.22	0.14	63.27
19	7.17	47899.46	190.09	3741.96	60.64	719.06	380.26	1591.06	5.29	129.45	231.43	0.14	63.25
20	7.17	47816.26	162.87	3714.44	60.13	718.74	380.39	1591.25	5.29	102.74	231.89	0.14	63.22

21	7.17	47738.73	142.74	3693.92	59.66	718.25	380.35	1591.44	5.28	83.08	231.78	0.14	63.17
22	7.17	47665.35	127.8	3678.53	59.21	717.65	380.19	1591.63	5.28	68.59	231.27	0.14	63.12
23	7.17	47594.95	116.53	3666.8	58.79	716.99	379.96	1591.82	5.28	57.75	230.5	0.14	63.06
24	7.17	47526.68	107.88	3657.66	58.39	716.3	379.68	1592.01	5.28	49.49	229.56	0.14	63
25	7.17	47459.95	101.08	3650.39	58.01	715.61	379.37	1592.2	5.27	43.07	228.53	0.14	62.94
26	7.17	47394.35	95.65	3644.48	57.66	714.93	379.05	1592.39	5.27	37.99	227.44	0.14	62.89
27	7.17	47329.62	91.22	3639.6	57.32	714.26	378.73	1592.58	5.27	33.89	226.33	0.14	62.83
28	7.17	47265.56	87.55	3635.51	57.01	713.62	378.41	1592.77	5.27	30.54	225.2	0.14	62.78
29	7.17	47202.04	84.48	3632.03	56.71	713	378.09	1592.95	5.26	27.77	224.09	0.14	62.73
30	7.17	47138.95	81.88	3629.05	56.44	712.41	377.78	1593.14	5.26	25.45	222.98	0.14	62.68

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Rate [%]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
0	5.22	56561.66	15126.08	18622.15	10661.93	251.05	353.69	1607.41	4.92	4464.15	17.08	0.07	63.84
1	7.05	46073.63	3378.11	6919.27	72.81	831.57	384.64	1607.58	4.92	3305.3	58.21	0.14	62.08
2	7.05	45872.24	3142.75	6680	67.98	801.21	389.02	1607.58	4.93	3074.77	74.2	0.13	61.55
3	7.06	45668.74	2898.29	6435.83	67.49	787.89	393.67	1607.58	4.93	2830.81	91.55	0.13	61.5
4	7.07	45442.7	2624.23	6163.24	66.85	783.63	398.94	1607.57	4.93	2557.38	111.22	0.14	61.65
5	7.09	45164.09	2284	5825.36	65.6	784.86	405.6	1607.57	4.93	2218.4	135.99	0.14	61.95
6	7.11	44775.33	1805.99	5350.95	63.31	791.45	415.2	1607.56	4.93	1742.68	171.33	0.14	62.45
7	7.15	44161.83	1047.39	4598.21	59.05	806.22	430.75	1607.56	4.93	988.33	228.08	0.15	63.27
8	7.18	43536.83	276.11	3831.94	53.99	821.25	445.79	1607.56	4.94	222.12	284.06	0.15	63.98
9	7.18	43414.48	134.2	3688.8	52.21	819.69	446.47	1607.55	4.94	82	289.86	0.15	63.87
10	7.18	43377.19	98.3	3650.85	51.08	815.87	444.92	1607.54	4.94	47.23	287.64	0.15	63.64
11	7.18	43356.07	82.23	3632.75	50.13	812.02	443.08	1607.53	4.94	32.1	284.21	0.14	63.41
12	7.18	43340.75	73.04	3621.66	49.3	808.42	441.24	1607.52	4.94	23.74	280.57	0.14	63.18
13	7.18	43328.29	67.05	3613.92	48.56	805.09	439.46	1607.51	4.94	18.49	276.99	0.14	62.97
14	7.18	43317.54	62.84	3608.13	47.91	802.03	437.77	1607.5	4.95	14.93	273.56	0.14	62.78
15	7.18	43307.96	59.75	3603.63	47.34	799.21	436.18	1607.49	4.95	12.41	270.33	0.14	62.6
16	7.17	43299.28	57.45	3600.1	46.87	796.61	434.69	1607.48	4.95	10.58	267.31	0.14	62.45
17	7.17	43291.36	55.74	3597.36	46.48	794.23	433.3	1607.47	4.95	9.26	264.51	0.14	62.31

18	7.17	43284.1	54.5	3595.27	46.2	792.03	432.02	1607.45	4.95	8.3	261.92	0.14	62.2
19	7.17	43277.39	53.64	3593.74	46	790	430.84	1607.44	4.95	7.64	259.53	0.14	62.11
20	7.17	43271.15	53.07	3592.66	45.88	788.12	429.73	1607.43	4.96	7.19	257.33	0.14	62.03
21	7.17	43265.26	52.7	3591.91	45.82	786.36	428.7	1607.42	4.96	6.88	255.27	0.13	61.98
22	7.17	43259.64	52.49	3591.41	45.81	784.7	427.72	1607.41	4.96	6.68	253.35	0.13	61.93
23	7.17	43254.21	52.38	3591.07	45.82	783.12	426.78	1607.39	4.96	6.55	251.53	0.13	61.89
24	7.17	43248.93	52.33	3590.85	45.86	781.61	425.87	1607.38	4.96	6.47	249.8	0.13	61.86
25	7.17	43243.77	52.34	3590.71	45.91	780.16	424.99	1607.37	4.96	6.43	248.15	0.13	61.83
26	7.17	43238.69	52.38	3590.63	45.97	778.76	424.14	1607.35	4.97	6.41	246.57	0.13	61.81
27	7.17	43233.69	52.44	3590.59	46.04	777.4	423.3	1607.34	4.97	6.4	245.05	0.13	61.79
28	7.17	43228.75	52.52	3590.59	46.12	776.08	422.49	1607.33	4.97	6.41	243.59	0.13	61.77
29	7.17	43223.86	52.62	3590.61	46.2	774.8	421.69	1607.31	4.97	6.42	242.19	0.13	61.75
30	7.17	43219.02	52.72	3590.65	46.28	773.54	420.91	1607.3	4.97	6.45	240.84	0.13	61.74

Ephyra® CSTR

Rate [%]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
0	7.26	32740.7	53.72	3565.73	45.22	682.19	280.34	830.38	3.27	8.5	274.8	0.53	65.86
1	7.27	32617.72	28.9	3508.45	21.86	521.37	276.39	830.43	3.28	7.04	234.6	0.3	65.96
2	7.27	32609.7	28.71	3507.48	22.17	508.6	275.05	830.43	3.28	6.54	230.63	0.3	65.73
3	7.27	32602.06	28.22	3506.2	22.2	500.91	273.64	830.43	3.28	6.02	226.64	0.29	65.5
4	7.27	32594.32	27.5	3504.62	22.06	495.5	272.08	830.43	3.28	5.44	222.28	0.29	65.25
5	7.26	32586.13	26.53	3502.57	21.78	490.75	270.17	830.43	3.28	4.75	217.05	0.29	64.94
6	7.26	32576.95	25.11	3499.63	21.29	485.53	267.58	830.43	3.28	3.82	209.99	0.28	64.48
7	7.25	32565.61	22.89	3494.96	20.44	478.54	263.55	830.43	3.28	2.45	199.1	0.27	63.71
8	7.24	32553.86	20.76	3490.27	19.55	471.42	259.25	830.43	3.28	1.21	187.16	0.26	62.84
9	7.24	32546.78	20.46	3489.47	19.46	468.79	257.86	830.43	3.28	1	182.79	0.26	62.64
10	7.24	32540.6	20.45	3489.31	19.48	467.08	257.03	830.43	3.28	0.97	179.9	0.26	62.57
11	7.24	32534.69	20.48	3489.27	19.53	465.66	256.33	830.42	3.29	0.96	177.43	0.26	62.53
12	7.24	32528.93	20.53	3489.26	19.57	464.43	255.7	830.42	3.29	0.96	175.2	0.26	62.49
13	7.24	32523.28	20.58	3489.27	19.62	463.32	255.13	830.41	3.29	0.96	173.17	0.26	62.46
14	7.24	32517.73	20.63	3489.28	19.66	462.32	254.61	830.41	3.29	0.97	171.3	0.26	62.44

15	7.24	32512.27	20.68	3489.3	19.7	461.41	254.12	830.4	3.29	0.98	169.58	0.26	62.42
16	7.24	32506.89	20.73	3489.32	19.74	460.59	253.67	830.4	3.29	0.99	168	0.26	62.4
17	7.24	32501.59	20.77	3489.35	19.78	459.83	253.26	830.39	3.29	1	166.55	0.26	62.38
18	7.24	32496.36	20.82	3489.37	19.81	459.15	252.88	830.39	3.29	1.01	165.23	0.26	62.37
19	7.24	32491.2	20.86	3489.4	19.84	458.52	252.53	830.38	3.29	1.01	164.02	0.26	62.35
20	7.24	32486.09	20.89	3489.43	19.87	457.94	252.21	830.38	3.3	1.02	162.92	0.26	62.35
21	7.24	32481.04	20.93	3489.46	19.9	457.4	251.9	830.37	3.3	1.03	161.89	0.26	62.34
22	7.24	32476.04	20.96	3489.48	19.92	456.9	251.62	830.36	3.3	1.04	160.94	0.26	62.33
23	7.24	32471.09	20.99	3489.51	19.95	456.42	251.35	830.36	3.3	1.05	160.04	0.26	62.33
24	7.24	32466.17	21.03	3489.54	19.97	455.96	251.08	830.35	3.3	1.05	159.19	0.26	62.32
25	7.24	32461.29	21.06	3489.56	20	455.52	250.83	830.35	3.3	1.06	158.38	0.26	62.32
26	7.24	32456.45	21.09	3489.59	20.02	455.1	250.58	830.34	3.3	1.07	157.6	0.26	62.32
27	7.24	32451.64	21.11	3489.62	20.04	454.69	250.34	830.33	3.3	1.07	156.86	0.26	62.31
28	7.24	32446.87	21.14	3489.64	20.06	454.29	250.11	830.33	3.3	1.08	156.15	0.26	62.31
29	7.24	32442.12	21.17	3489.67	20.08	453.9	249.88	830.32	3.31	1.09	155.46	0.26	62.31
30	7.24	32437.4	21.2	3489.69	20.1	453.53	249.65	830.31	3.31	1.09	154.8	0.26	62.3

APPENDIX G: Steady state simulations results for different Organic Loading Rates

Table 36: Results of steady state simulations under different organic loading rates of Ephyra system model using BioWin

Ephyra® 1

Rate [kg COD m^{-3} d^{-1}]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
6.7	7.13	59312.6	2082.15	6347.72	710.08	406.27	191.74	1554.03	6.08	1372.06	44.43	0.13	68.73
6.9	7.12	59621.11	2204.05	6489.73	822.13	393.44	188.25	1553.29	6.1	1381.92	42.93	0.13	68.68
7.1	7.1	60110.92	2439.4	6754.02	1042.61	370.58	183.23	1552.24	6.12	1396.79	40.71	0.12	68.48
7.3	7.08	60464.33	2640.21	6973.09	1232.51	352.27	179.98	1551.58	6.14	1407.7	39.18	0.12	68.25
7.5	7.06	60846.56	2883.8	7233.97	1461.7	331.03	176.74	1550.95	6.15	1422.1	37.42	0.12	67.92
7.7	7.02	61321.31	3244.12	7609.49	1776.33	302.69	172.89	1550.35	6.16	1467.79	33.43	0.11	67.37
7.9	6.95	62169.78	3959.65	8344.45	2369.54	249.59	166.04	1549.5	6.18	1590.12	23.69	0.1	66.21
8.1	6.91	62584.54	4271.94	8671.95	2640.66	224.16	162.45	1548.96	6.19	1631.28	20.21	0.09	65.71
8.3	6.89	62916.1	4495.74	8912.21	2839.16	204.43	159.3	1548.45	6.2	1656.59	17.9	0.09	65.37
8.5	6.87	63189.65	4659.74	9093.52	2986.52	188.73	156.4	1547.96	6.22	1673.22	16.25	0.09	65.14
8.7	6.85	63523.33	4829.64	9290.45	3140.99	170.49	152.4	1547.26	6.23	1688.65	14.5	0.08	64.92
8.9	6.85	63708.79	4907.76	9387.06	3212.95	160.86	149.91	1546.81	6.24	1694.81	13.66	0.08	64.84
9.1	6.84	63872.57	4966.43	9464.48	3267.66	152.66	147.53	1546.38	6.25	1698.78	13	0.08	64.79
9.3	6.84	64019.34	5010.7	9527.64	3309.45	145.55	145.25	1545.97	6.26	1701.25	12.47	0.08	64.77
9.5	6.83	64152.37	5043.99	9579.94	3341.28	139.31	143.04	1545.58	6.27	1702.71	12.02	0.07	64.77
9.7	6.83	64331.01	5078.36	9642.96	3374.59	131.27	139.87	1545.01	6.28	1703.77	11.48	0.07	64.79
9.9	6.83	64438.54	5093.27	9677.01	3389.23	126.63	137.83	1544.65	6.29	1704.03	11.17	0.07	64.83
10.1	6.83	64538.43	5103.32	9706.24	3399.25	122.48	135.85	1544.3	6.3	1704.07	10.9	0.07	64.87
10.3	6.83	64676.93	5112.27	9743.94	3408.48	116.91	132.97	1543.8	6.31	1703.8	10.54	0.07	64.95
10.5	6.83	64763.71	5116.16	9766.95	3412.72	113.48	131.12	1543.48	6.32	1703.44	10.33	0.07	65.01
10.7	6.83	64847.67	5120.02	9789.87	3417.02	110.13	129.31	1543.17	6.32	1703	10.15	0.07	65.07
10.9	6.83	64930.55	5125.68	9814.48	3423.12	106.75	127.55	1542.87	6.33	1702.56	9.98	0.06	65.12
11.1	6.83	65015.23	5136.25	9843.86	3434.03	103.1	125.82	1542.58	6.34	1702.22	9.82	0.06	65.17
11.3	6.83	65162.47	5180.04	9915.33	3477.68	95.87	123.3	1542.16	6.35	1702.36	9.57	0.06	65.21
11.5	6.7	66130.05	6195.59	10933.8	4452.1	25.78	120.66	1541.89	6.35	1743.49	8.7	0.05	63.07

Ephyra® 2

Rate [kg COD m^{-3} d^{-1}]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]

6.7	7.1	52539.46	1575.36	5153.9	103.13	620.25	305.78	1578.05	5.55	1472.23	106.18	0.14	62.98
6.9	7.1	52975.4	1654.08	5235.96	111.33	613.32	299.45	1576.7	5.58	1542.75	98.85	0.14	63.13
7.1	7.1	53587.92	1762.98	5351.05	128.41	604.34	290.51	1574.8	5.62	1634.57	89.09	0.14	63.47
7.3	7.09	53975.77	1835.35	5428.43	144.94	599.18	284.83	1573.61	5.64	1690.41	83.1	0.14	63.78
7.5	7.09	54356.39	1916.71	5515.53	168.35	594.35	279.22	1572.47	5.66	1748.36	77.03	0.14	64.13
7.7	7.09	54786.08	2073.54	5678.96	210.38	588.39	272.48	1571.38	5.69	1863.17	66.78	0.15	64.52
7.9	7.07	55549.82	2477.55	6093.26	353.1	573.57	260.62	1569.84	5.72	2124.44	45.04	0.15	64.92
8.1	7.05	56007.87	2701.12	6321.74	488.21	560.62	254.71	1568.87	5.74	2212.91	37.11	0.16	64.97
8.3	7.04	56465.65	2935.92	6559.71	667.47	543.87	249.62	1567.93	5.76	2268.44	31.71	0.16	64.83
8.5	7.01	56946.66	3208.8	6833.94	902.82	522.43	245.01	1567.04	5.78	2305.98	27.71	0.16	64.53
8.7	6.96	57720.44	3698.58	7322.63	1356.51	481.92	238.72	1565.77	5.8	2342.08	23.35	0.15	63.77
8.9	6.92	58246.69	4050.18	7672.15	1693.37	451.88	234.85	1564.96	5.82	2356.81	21.23	0.15	63.14
9.1	6.88	58754.2	4390.41	8010.01	2024.17	422.01	231.19	1564.18	5.84	2366.24	19.57	0.14	62.49
9.3	6.85	59228.13	4701.86	8319.23	2330.02	393.8	227.7	1563.44	5.85	2371.83	18.27	0.14	61.86
9.5	6.81	59662.65	4977.38	8592.93	2602.62	367.92	224.36	1562.72	5.87	2374.76	17.22	0.13	61.28
9.7	6.77	60240.71	5322.45	8936.16	2946.53	333.84	219.56	1561.68	5.89	2375.91	15.98	0.13	60.49
9.9	6.74	60581	5510.96	9124.07	3135.65	314.12	216.48	1561.03	5.91	2375.32	15.32	0.12	59.99
10.1	6.71	60890.2	5671.46	9284.36	3297.42	296.47	213.49	1560.39	5.92	2374.04	14.74	0.12	59.46
10.3	6.68	61304.74	5868.88	9482.28	3497.79	273.31	209.15	1559.48	5.94	2371.09	14.02	0.11	58.8
10.5	6.66	61555.25	5978.97	9593.15	3610.4	259.55	206.36	1558.9	5.95	2368.57	13.61	0.11	58.46
10.7	6.64	61790.73	6077.92	9693.14	3712.09	246.73	203.63	1558.34	5.96	2365.82	13.25	0.11	58.15
10.9	6.63	62016.24	6171.13	9787.56	3808.14	234.46	200.97	1557.8	5.98	2362.99	12.92	0.1	57.87
11.1	6.61	62238.88	6266.39	9884.09	3906.09	222.21	198.36	1557.27	5.99	2360.3	12.61	0.1	57.6
11.3	6.58	62604.41	6454.28	10073.42	4096.82	201.13	194.54	1556.51	6	2357.46	12.14	0.1	57.15
11.5	6.18	64674.65	8648.64	12239.18	6218.39	50.41	190.16	1556.01	6.02	2430.25	10.42	0.06	52.73

Ephyra® 3

Rate [kg COD m^{-3} d^{-1}]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
6.7	7.17	45235.23	449.97	4003.39	58.71	774.61	418.11	1601.59	5.06	391.26	249.59	0.15	63.57
6.9	7.15	45977.67	697.42	4248.88	61.17	762.01	407.6	1599.68	5.1	636.25	228.06	0.14	63.29
7.1	7.13	47017.85	1042.44	4591.06	64.64	744.75	392.39	1596.97	5.15	977.8	197.49	0.14	62.9
7.3	7.12	47657.85	1245.33	4792.37	66.82	734.9	382.94	1595.27	5.18	1178.5	179.28	0.14	62.68
7.5	7.11	48267.17	1438.72	4984.43	69.09	726.18	373.81	1593.65	5.21	1369.63	161.92	0.14	62.5

7.7	7.1	48936.34	1733.14	5277.09	72.55	716.33	362.64	1592.1	5.25	1660.59	137.05	0.14	62.26
7.9	7.07	50021.03	2319.54	5861.25	81.76	700.87	343.03	1589.89	5.29	2237.79	89.34	0.14	61.92
8.1	7.06	50574.33	2525.84	6068.37	89.09	694.78	334.1	1588.5	5.32	2436.75	72	0.14	62
8.3	7.06	51057	2663.68	6208.49	99.06	690.37	326.81	1587.16	5.34	2564.62	60.24	0.14	62.27
8.5	7.05	51497.56	2766.78	6315.3	113.56	687.13	320.47	1585.88	5.37	2653.22	51.58	0.14	62.69
8.7	7.05	52109.53	2890.67	6447.11	148.9	683.54	312.1	1584.06	5.41	2741.77	42.18	0.15	63.5
8.9	7.05	52496.16	2965.54	6528.07	185.06	680.99	307.08	1582.9	5.43	2780.49	37.59	0.15	64.05
9.1	7.05	52872.73	3042.31	6610.81	234.55	677.37	302.4	1581.78	5.45	2807.76	33.97	0.16	64.51
9.3	7.05	53245.93	3128.48	6702.33	301.63	671.99	297.99	1580.71	5.47	2826.86	31.06	0.16	64.85
9.5	7.04	53624.09	3233.22	6811.41	392.92	664.14	293.79	1579.68	5.49	2840.3	28.66	0.17	65.05
9.7	7.02	54223.87	3450.58	7032.74	596.82	646	287.79	1578.19	5.53	2853.75	25.7	0.17	65.1
9.9	7	54661.89	3653.63	7236.33	793.69	628.44	283.95	1577.25	5.54	2859.94	24.01	0.17	64.95
10.1	6.98	55137.56	3910.64	7492.07	1046.02	606.03	280.21	1576.34	5.56	2864.62	22.49	0.17	64.67
10.3	6.93	55901.63	4373.32	7949.94	1504.47	565.33	274.81	1575.03	5.59	2868.84	20.53	0.17	63.99
10.5	6.89	56420.55	4704.83	8277.05	1834.97	535.8	271.35	1574.19	5.61	2869.87	19.44	0.16	63.42
10.7	6.85	56932.35	5037.32	8604.7	2167.57	505.86	267.98	1573.38	5.63	2869.75	18.49	0.16	62.84
10.9	6.81	57433.17	5365.8	8928.15	2496.95	476.03	264.69	1572.59	5.64	2868.85	17.66	0.15	62.24
11.1	6.77	57929.15	5696.75	9253.9	2829.09	446.03	261.48	1571.84	5.66	2867.66	16.91	0.15	61.64
11.3	6.69	58728.86	6270.03	9818.21	3402.73	396.09	256.74	1570.74	5.68	2867.3	15.81	0.14	60.61
11.5	5.57	63127.11	11023.85	14506.96	7943.06	76.69	246.97	1569.99	5.7	3080.79	12.04	0.06	55.72

Ephyra® 4

Rate [kg COD m^{-3} d^{-1}]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
6.7	7.19	38409.22	50.62	3594.48	42.53	859.3	478.77	1624.31	4.62	8.09	299.46	0.14	62.72
6.9	7.19	39140.87	57.14	3604.39	44.39	857.38	476.91	1621.92	4.66	12.74	303.3	0.14	63.13
7.1	7.19	40173.9	73.51	3625.67	47.27	854.62	473.7	1618.52	4.72	26.24	307.67	0.15	63.7
7.3	7.19	40828.98	95.79	3650.83	49.17	852.66	471.02	1616.37	4.76	46.62	308.97	0.15	64.01
7.5	7.19	41483.75	156.84	3714.16	51.18	849.82	467.25	1614.32	4.8	105.66	306.64	0.15	64.24
7.7	7.18	42372.58	547.6	4103.62	54.79	837.95	455.7	1612.34	4.84	492.81	277.32	0.15	64.01
7.9	7.11	44171.33	1782.24	5327.87	62.5	801.21	421.98	1609.52	4.89	1719.73	178.02	0.14	62.57
8.1	7.09	45038.97	2229.49	5771.12	65.25	786.66	407.51	1607.74	4.93	2164.23	140.62	0.14	62.02
8.3	7.07	45760.22	2521.77	6060.79	67.14	776.57	396.46	1606.03	4.96	2454.64	115.34	0.14	61.67
8.5	7.06	46391.13	2727.25	6264.52	68.61	769.41	387.37	1604.39	4.99	2658.64	96.92	0.13	61.46

8.7	7.05	47222.69	2937.16	6473.05	70.62	762.64	376.02	1602.05	5.03	2866.53	77.17	0.13	61.33
8.9	7.05	47719.3	3032.72	6568.38	72.19	759.87	369.54	1600.56	5.06	2960.53	67.63	0.13	61.35
9.1	7.04	48179.93	3103.88	6639.83	74.24	757.81	363.69	1599.12	5.09	3029.65	60.16	0.13	61.43
9.3	7.04	48611.57	3158.03	6694.81	77.1	756.08	358.31	1597.74	5.12	3080.93	54.19	0.13	61.58
9.5	7.04	49020	3200.98	6739.24	81.25	754.53	353.28	1596.4	5.14	3119.73	49.29	0.13	61.8
9.7	7.04	49599.42	3254.22	6796.32	91.56	752.54	346.21	1594.49	5.18	3162.67	43.28	0.14	62.3
9.9	7.04	49969.49	3287.59	6833.64	102.99	751.53	341.74	1593.27	5.21	3184.6	39.89	0.14	62.77
10.1	7.04	50330.69	3322.9	6874.18	120.19	750.75	337.41	1592.09	5.23	3202.71	36.88	0.15	63.36
10.3	7.04	50861.5	3384.97	6945.84	161.67	749.28	331.2	1590.4	5.26	3223.3	33	0.15	64.28
10.5	7.04	51212.51	3437.26	7004.94	204.23	747.34	327.23	1589.31	5.29	3233.03	30.81	0.16	64.82
10.7	7.04	51567.62	3505.36	7079.64	265.09	743.89	323.39	1588.26	5.31	3240.27	28.89	0.17	65.25
10.9	7.04	51937.74	3601.38	7181.62	355.52	737.99	319.64	1587.25	5.33	3245.86	27.16	0.17	65.55
11.1	7.02	52345.65	3750.75	7335.81	499.84	727.93	315.97	1586.26	5.35	3250.91	25.54	0.18	65.67
11.3	6.98	53176.04	4241.07	7828.68	979.04	693.94	310.48	1584.84	5.38	3262.03	22.93	0.18	65.29
11.5	5.28	61573.81	13424.23	16904.47	9671.77	97.52	291.61	1583.82	5.4	3752.46	13.5	0.05	64.59

Ephyra® CSTR

Rate [kg COD m^{-3} d^{-1}]	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	PAO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
6.7	7.27	26436.5	19.65	3485.4	18.8	437.1	242.82	758.28	2.86	0.84	168.12	0.23	62.83
6.9	7.26	27316.85	19.68	3485.84	18.84	442.39	245.56	767.98	2.92	0.84	172.17	0.23	62.81
7.1	7.26	28561.33	19.74	3486.52	18.89	450.13	249.43	782.04	3	0.85	177.92	0.24	62.8
7.3	7.25	29344.22	19.81	3487.02	18.93	455.21	251.86	791.1	3.05	0.88	181.54	0.24	62.81
7.5	7.25	30092.8	19.98	3487.74	19.02	460.44	254.31	799.92	3.1	0.96	185.35	0.25	62.86
7.7	7.25	30811.28	21.04	3490.38	19.51	467.7	258.07	808.52	3.15	1.53	192.66	0.25	63.27
7.9	7.26	31837.18	24.73	3498.49	21.08	482.47	266.17	821.03	3.23	3.65	209.71	0.28	64.49
8.1	7.26	32483.97	26.32	3501.9	21.7	489.69	269.68	829.11	3.27	4.62	216.21	0.28	64.89
8.3	7.26	33104.04	27.5	3504.45	22.14	495.86	272.4	837.01	3.32	5.36	220.79	0.29	65.14
8.5	7.26	33699.38	28.45	3506.51	22.47	501.52	274.64	844.73	3.36	5.98	224.28	0.3	65.32
8.7	7.26	34549.54	29.59	3509.02	22.83	509.56	277.4	855.97	3.43	6.76	228.28	0.31	65.5
8.9	7.26	35089.77	30.22	3510.43	23.02	514.68	278.94	863.26	3.47	7.2	230.38	0.31	65.59
9.1	7.26	35610.23	30.76	3511.68	23.17	519.56	280.3	870.39	3.51	7.6	232.17	0.32	65.66
9.3	7.26	36112.03	31.26	3512.83	23.3	524.2	281.5	877.36	3.55	7.96	233.72	0.32	65.72
9.5	7.25	36596.23	31.73	3513.92	23.43	528.65	282.59	884.19	3.59	8.3	235.11	0.32	65.78

9.7	7.25	37291.61	32.39	3515.49	23.61	535.17	284.03	894.16	3.64	8.78	236.97	0.33	65.87
9.9	7.25	37735.86	32.83	3516.53	23.73	539.57	284.89	900.63	3.68	9.1	238.11	0.33	65.95
10.1	7.25	38165.6	33.27	3517.6	23.85	544.1	285.68	906.97	3.72	9.41	239.19	0.34	66.03
10.3	7.25	38784.59	33.95	3519.29	24.07	551.12	286.73	916.23	3.77	9.88	240.71	0.34	66.17
10.5	7.25	39181.17	34.44	3520.5	24.26	555.84	287.35	922.26	3.8	10.18	241.66	0.35	66.28
10.7	7.25	39565.69	35	3521.84	24.52	560.58	287.92	928.16	3.84	10.49	242.57	0.35	66.39
10.9	7.25	39938.81	35.67	3523.41	24.88	565.42	288.43	933.95	3.87	10.79	243.45	0.36	66.51
11.1	7.25	40301.27	36.55	3525.42	25.44	570.65	288.89	939.63	3.9	11.1	244.32	0.36	66.68
11.3	7.25	40827.44	38.84	3530.61	27.23	581.23	289.53	947.94	3.95	11.61	245.68	0.38	67.15
11.5	7.23	41266.55	72.75	3600.18	60.07	695.98	291.03	953.33	3.98	12.69	271.92	0.64	66.48

APPENDIX H: Steady state simulations results - Decreasing number of Ephyra® tanks from 4 to 3, with 5% and 8% recirculation rate

Table 37: Results of steady state simulations to investigate influence of decreasing number of Ephyra® tanks from 4 to 3, with 5% and 8% recirculation rate

4 Tanks - 5% Recirculation

Element	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
Eph1	6.91	62645.84	4312.74	8715.52	2676.54	220.71	161.94	1548.98	1636.2	19.78	0.09	65.67
Eph2	7.05	56083.29	2736.95	6358.42	513.42	558.28	253.88	1568.81	2223.53	36.12	0.16	64.96
Eph3	7.06	50657.84	2551.29	6094.31	90.46	694.03	332.88	1588.38	2460.83	69.85	0.14	62.04
Eph4	7.09	45164.09	2284	5825.36	65.6	784.86	405.6	1607.57	2218.4	135.99	0.14	61.95
CSTR	7.26	32586.13	26.53	3502.57	21.78	490.75	270.17	830.43	4.75	217.05	0.29	64.94

3 Tanks - 5% Recirculation

Element	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
Eph1	7.14	58751.11	1843.28	6046.78	488.74	435.98	201.87	1555.57	1354.55	49.23	0.18	68.62
Eph2	7.12	51328.86	1275.35	4847.34	86.24	646.26	327.36	1581.81	1189.11	135.89	0.18	62.89
Eph3	7.18	43353.02	103.37	3656.28	52.45	800.53	439.05	1607.41	50.92	278.52	0.19	63.59
CSTR	7.24	32513.99	20.73	3489.6	19.72	462.54	255.37	830.36	1	175.65	0.26	62.56

4 Tanks - 8% Recirculation

Element	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
Eph1	7.06	60872.5	2843.47	7257.23	1551.94	320.81	176.28	1550.81	1291.53	45.76	0.12	68.34
Eph2	7.1	54910.51	1762.84	5376.65	197.52	580.75	270.83	1570.02	1565.32	83.97	0.15	64.78
Eph3	7.11	49502.52	1348.76	4895.36	71.75	705.73	358.25	1588.96	1277.01	157.2	0.14	62.56
Eph4	7.18	43536.83	276.11	3831.94	53.99	821.25	445.79	1607.56	222.12	284.06	0.15	63.98
CSTR	7.24	32553.86	20.76	3490.27	19.55	471.42	259.25	830.43	1.21	187.16	0.26	62.84

3 Tanks - 8% Recirculation

Element	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
Eph1	7.16	58067.79	1561.86	5759.56	405.66	453.62	211.08	1557.19	1156.2	64.92	0.18	68.96
Eph2	7.14	50729.02	823.07	4397.93	78.99	658.17	337.56	1582.59	744.08	168.16	0.19	63.3
Eph3	7.18	43306.57	71.77	3619.74	50.03	792.14	433.63	1607.39	21.74	267.69	0.19	62.99
CSTR	7.24	32498.63	20.83	3489.53	19.83	459.49	253.49	830.35	1	168.81	0.26	62.46

Reference

Element	pH []	Total COD [mg/L]	VFAs [mg/L]	Sol. COD [mg/L]	Acetate [mgCOD/L]	Meth.-acetoclastic [mgCOD/L]	Meth.-hydrogenotr. [mgCOD/L]	OHO [mgCOD/L]	Propionate [mgCOD/L]	Prop. acetogens [mgCOD/L]	Gas flow [m3/hr]	Methane Yield [%]
CSTR	7	40734.07	49.87	3713.26	43.98	396.35	210.57	871.63	5.89	100.69	0.63	63.5