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*Article*

# Co-digestion of extended aeration sewage sludge with whey, grease and septage: experimental and modelling determination.

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**Abstract:** Potential of co-digestion mixing thickened secondary sludge (TS) from extended aeration wastewater treatment plant and locally available substrates (whey, grease and septage) has been studied using three steps. The first step was a batch test to determine biological methane potential (BMP) of different mixtures of the three co-substrates with TS. The second step has been carried out with lab-scale reactors (20 L) simulating anaerobic continuous stirred tank reactors fed by three mixtures of co-substrates determined according to previous step results. Modelling using ADM1 as a mechanistic model was applied in the third step to help understanding the co-digestion process. According to BMP step, septage used as co-substrate has a negative effect on performance and addition of 10 to 30% grease or 10% whey would lead to a higher production of biogas and with an increase of the methane content. The results from the reactor showed less evidence of the positive effects observed with the BMP assay. Protein and lipid fractions of particulate biodegradable COD are important variables for digester stability and methane production as predicted by modelling. Results of simulations with ADM1 model adapted to co-digestion confirmed that this model is a powerful tool to optimize the process of biogas production.

**Keywords:** Anaerobic co-digestion; ADM1; BMP; biogas

## 1. Introduction

Activated sludge process is the most widely applied process to treat domestic wastewater due to its well-known facility design and operation parameters. Among diverse activated sludge processes, the extended aeration activated sludge system (EAASS) is the most widespread technology for the treatment of domestic wastewater in France with more than 60% of WWTP and a capacity greater than 2000 PE [1]. Extended aeration process is often better at handling organic loading and flow fluctuations and have a relatively low sludge production due to a long sludge age, as there is a greater detention time for the substrate's assimilation by microorganisms [2]. Despite the low yield of sludge, it is still necessary to reduce the mass to be removed valorizing it as much as possible. For this purpose, anaerobic digestion is nowadays the most efficient way [3]. In France, 97 domestic wastewater treatment plants (WWTP) were equipped with anaerobic digestion units in 2018, out of the nearly 20,000 WWTP in France at the time [4]. Almost half of the 97 installations equipped with digester are activated sludge units with extended aeration. These facilities treat the effluents of about 30 million person-equivalent (PE), close to 30% of the total PE. The biogas produced was mainly used on site (boiler) and for cogeneration of electricity. Sewage sludge from an activated sludge extended aeration treatment is less fermentable than primary sludge and has therefore naturally lower methanogenic power

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[5]. However, it seems important to characterize the performance achieved in digestion with this kind of sludge.

Co-digestion of extended aeration activated sludge (EAAS) with other organic waste with higher biological methane potential (BMP) is a possible way to enhance the biogas production and the fraction of $CH_4$ [6-7]. Digesters are also usually oversized and addition of co-substrates can help to enhance gas and electricity production at low extra costs [8]. The extra power produced may cover the energy needs of wastewater treatment at a reasonable cost [9]. In France, although the operators sometimes add fats to the mixture, co-digestion is still rarely used due to regulatory constraints.

Anaerobic digestion is a complex process, relatively slow, quite sensitive to variations in loads applied and with a microflora also quite fragile in the presence of toxicants. The co-digestion of substrates of different nature amplifies its complexity, its relative fragility of the digestion process and the qualitative and quantitative variability of the biogas produced. Co-digestion applied to EAAS presents several advantages. Sludge can be mixed with different types of organic waste, both in order to enhance biogas production and to valorize the co-substrates via biogas production. Indeed, some of the co-substrates cannot always be easily treated on their own such as septage from septic tanks, grease from grease traps or whey from cheese makers. Severe environmental pollution in many countries is caused by discharge of large quantities of these compounds [10].

Many studies on co-digestion have been conducted with agricultural or municipal organic waste [11] but few with EAAS.

The main issue for co-digestion process consists in balancing C/N ratio, but also other several parameters in the co-substrate mixture such as macro- and micronutrients, pH, inhibitors/toxic compounds, biodegradable organic matter and dry matter [**10,** 12-13].

The objective of this study was to assess the potential of co-digestion mixing thickened secondary sludge from extended aeration wastewater treatment plants with locally available substrates using wet mesophilic anaerobic digestion process. A first step consisted in batch-test to determine the methane potential of different mixtures of co-substrates in order to find the most favorable proportions and to characterize biodegradation kinetics and the biogas production. A second step has been carried out with lab-scale reactors simulating anaerobic continuous still tank reactors fed by three mixtures of co-substrates determined according to previous test results. Simulation using ADM1 as a mechanistic model was applied in a third step to help understanding of the co-digestion process.

To date, in order to simulate anaerobic digestion process and more particularly the kinetics of the various reactions, the most advanced mechanistic model is ADM1 (Anaerobic Digestion Model N°1) published by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes [14-15]. More simple models could be used [16] but the choice of the mechanistic model ADM1 is justified by infrequency data, erroneous data or hard to obtain [17]. In this work we did not make any particular extensions to the ADM1 initial model but we have adapted it by incorporating parameter data and inputs specific to co-digestion.

## 2. Materials and Methods

### *2.1 BMP test*

BMP test was carried out according to the main conditions defined [18], using glass bottles having a volume of 1075mL, closed by a septum, with a reaction volume being 470mL and an overhead gas of 605mL. The bottles were kept at 37 ° C in a thermostatically controlled chamber. Stirring was carried out by magnetic stirrers. Inoculum consisted in extended aeration sludge from a local WWTP of 19,500 PE (Haute-Savoie, France) and was pre-incubated for 3 to 5 days to deplete residual biodegradable compounds. The

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added volume of inoculum was 30mL/bottle. Dilution of substrates was realized with a buffered nutrient solution (pH 7) [19]. Biogas production has been determined by pressure variation over time using a manometric method with classical thermodynamic conversion in volume. At the beginning and end of the test (end of biogas production), DM, VS and COD were measured using standardized methods [20]. Biogas composition ($CO_2$, $N_2$ and $CH_4$) was determined by gas chromatography with katharometer as detector (Shimadzu GC-2014, with Supelco stainless steel column: 60/80 carboxen 1000, 15ft x 1/8in).

Kinetic parameters of biogas production have been calculated using modified Gompertz equation, useful when lag phase is present [21]. This equation (eq. 1), which represents sigmoidal curves, allows to access the lag time. To take into account two successive phases which may correspond to the consumption of two substrates sequentially, the equation 1 has been modified (eq. 2).

$$Xg = K \cdot e^{\left(-e^{\left(\frac{k}{K}e^{(\lambda - t)+1}\right)}\right)} \quad (1)$$

$$Xg = K_1 \cdot e^{\left(-e^{\left(\frac{k_1}{K_1}e^{(\lambda_1 - t)+1}\right)}\right)} + (K_2 - K_1) \cdot e^{\left(-e^{\left(\frac{k_2}{K_2}e^{(\lambda_2 - t)+1}\right)}\right)} \quad (2)$$

With:

Xg: biogas production (Lbiogas $gVS^{-1}$ or $COD^{-1}$); $K_i$: ultimate biogas production ($L_{biogas}$ $gVS^{-1}$ or $gCOD^{-1}$); $k_i$ maximum rate of biogas production ($t^{-1}$); $\lambda_i$: lag phase (t).

Three parameters are significant for the kinetics of biogas production (Xg): the ultimate potential for the production of biogas ($K_i$); the maximum rate of biogas production ($k_i$) and the lag phase ($\lambda_i$).

The ultimate potentials were estimated from the average of the last values of the cumulative biogas production curves. The kinetics parameters were estimated using the solver function of the spreadsheet by maximizing the coefficient of determination ($R^2$) between experimental data and calculated ones

In order to quantify the synergy in anaerobic co-digestion, kinetics parameters obtained for the different mixture assays were normalized by the parameters from the mono-digestion test with TS according to equation 3:

$$N_p = \frac{P_{mix}}{P_{TS}} - 1 \quad (3)$$

Where Np represents the normalized parameter; $P_{mix}$: parameter value of BMP co-digestion assay; $P_{TS}$: parameter value of the reference TS BMP assay.

Four co-substrates have been tested, extended aeration thickened sludge (TS), grease (G), septage from septic tank (SP) and whey (W) (Table 1). Results were compared to a blank (inoculum + acetate or cellulose). Thickened sludge and grease (produced during the pre-treatment of the process) have been collected at the local wastewater treatment plant, septage collected from septic tanks of individual houses around the local wastewater treatment plant and whey came from the cheese factory of the agricultural school of La Motte-Servolex, (Savoie, France).

**Table 1**. Main characteristics of BMP substrates and inoculum.

| | **Inoculum** | **Thickened sludge** | **Grease** | **Septage** | **Whey** |
|---|---|---|---|---|---|
| **DM ($g \cdot l^{-1}$)** | 36.5 | 36.8 | 44.8 | 42.3 | 55.8 |
| **VS ($g \cdot l^{-1}$)** | 22.2 | 25.6 | 37.2 | 26.3 | 48 |

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| | | | | | |
|---|---|---|---|---|---|
| **COD (g·l$^{-1}$)** | 42.4 | 65.9 | 72.5 | 23.2 | 69.4 |
| **COD/VS** | 1.9 | 2.57 | 1.95 | 0.88 | 1.45 |

The experimental plan adopted was determined by the proportion of COD expressed as a percentage of thickened sludge / co-substrate: 90/10; 70/30; 50/50. Each proportion was performed in triplicate. Nominal COD content was equal to 6.4 g for each bottle.
The initial substrate over inoculum ratio (S/I) expressed in COD was equal to 4.

*2.2 Bioreactor's test*

Reactors were cylindrical tanks with volume of 15 L and a headspace volume of 5 L (Figure 1). Bioreactors were run as continuous stirred tank reactors (CSTRS) without biomass recycling so that the sludge retention time (SRT) was equal to the hydraulic retention time (HRT).

The HRT was fixed to 15 days providing a minimum acceptable SRT according to results of previous BMP tests and to literature recommendations [3, 22-23]. Reactors were mixed by continuous pumping (2L/min). Reactors were heated by external thermostatic water bath maintained to 35°C. Sludge was fed at regular intervals every day to help maintain steady-state conditions in the digester.

Substrates and co-substrates had the same origin as for the BMP test.

Biogas production was determined by a volumetric pulse meter comprising 2 chambers filled with water in communication regulated by a solenoid valve. Each pulse corresponded to a volume of 20mL of water displaced by the increase in pressure inside the reactor. Biogas production was also determined by measuring the pressure inside the reactor using an accurate 0.1mbar pressure gauge (Fig.1).

The contents of dry matter (DM), volatiles (VS), total COD (CODt), particulate COD (CODp) and soluble COD (CODs) were carried out according to standardized methods (APHA, 1998) on average 3 times per week.

pH and temperature were measured continuously using a specific probe inside the reactor.

The VFA contents and the composition of the biogas were measured by gas chromatography, approximately once every ten days. The analysis conditions are detailed in Table 2.

**Table 2**. Main characteristics for VFA and biogas analysis

| VFA analysis | |
|---|---|
| Type of detector | FID |
| Column type | Perkin Elmer capillary column Elite-FFAP, 15m x 0.53mm I.D. x 1µm |
| Injection mode | Split less |
| Carrier gas | $N_2$ |
| Temperature of injection | 250°C |
| Temperature of detection | 275°C |
| Flow of carrier gas | 7.5ml/min |
| Injection volume | 2µl |
| Temperature program | T1=80°C<br>t1=2min<br>r1=8°C/min<br>T2=120°C<br>t2=10min |

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| | |
|---|---|
| | r2=45°C/min<br>T3=175°C<br>t3=2min |
| Analysis for biogas composition | |
| Type of detector | Katharometer |
| Column type | Supelco custom column 60/80 carboxen 1000, 15ft x 1/8in O.D. x 2.1mm I.D. |
| Injection mode | Split less |
| Carrier gas | He |
| Temperature of injection | 250°C |
| Temperature of detection | 250°C |
| Flow of carrier gas | 10ml/min |
| Injection volume | 500µl |
| Temperature program | T1=100°C<br>t1=17min<br>r1=20°C/min<br>T2=120°C<br>t2=12min |

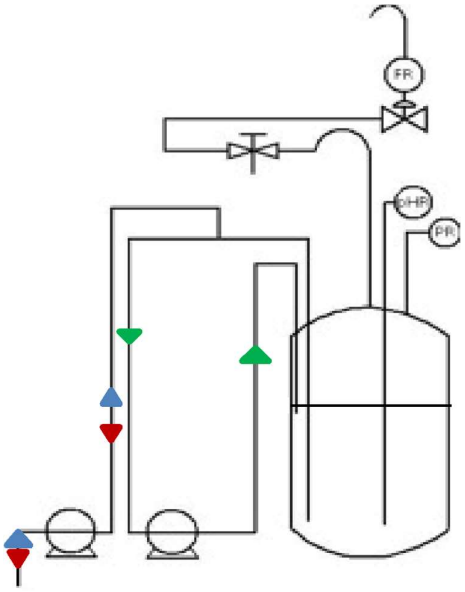


**Figure 1**. Diagram of a reactor.

FR: volumetric pulse meter; PR: pressure gauge; pHR: pH and temperature probe. In green agitation loop and red and blue arrows the feeding or withdrawal loop.

*2.3 Statistical analysis:*

Statistical analysis was performed using parametric (one-way and two-way ANOVA) or non-parametric (Friedman analysis) tests and the difference between reactors was expressed as significant at a significance level of $p < 0.05$. The p value is the estimated probability of rejecting the null hypothesis (hypothesis of "no difference" of the test when that hypothesis is true).

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The non-parametric statistical test was carried out when data did not meet criteria for a parametric test (normally distributed, equal variance). Post-hoc tests were conducted in order to decide which groups were significantly different from each other based on F-test for Ryan's test (ANOVA) and based upon the average rank differences of the groups for Friedman test.

Statistical analyses were performed using the StatEL software (adSciences, France).

*2.4 Modelling and simulation*

The ADM1 model takes into account two extracellular steps (disintegration and hydrolysis of substrates) and three steps of microbial anaerobic metabolism (acidogenesis, acetogenesis and methanogenesis). These reactions are carried out by seven groups of microorganisms and three types of enzymes. The model assumes constant stoichiometry coefficients with inhibitions of the degradation of volatile fatty acids by hydrogen. The model also considers the inhibition of the growth of microorganisms by pH and the inhibition of acetoclastic methanogenesis by hydrogen and ammonium [19]. The ionic balance, the gas-liquid exchanges are also integrated. This model considers the mortality of bacterial cells and their recycling into biomass.

ADM1 model has been implemented in AQUASIM 2.0 by the IWA Task Group for Mathematical Modelling of Anaerobic Digestion Processes. AQUASIM 2.0 is an open-source software developed in 1998 for the identification and simulation of aquatic systems [24]. Furthermore, AQUASIM allows to conduct sensitivity analysis and estimation parameters, being divided into variables, processes, compartments and links interfaces.

The modelling approach is based on the material balances and considering a continuous stirred tank reactor with output levels equal to those everywhere inside the reactor.

For this study the ADM1 model was implemented in AQUASIM as a differential and algebraic equation (DAE) and used an ideal CSTR compartment.

The model is solved as a system with the biochemical reactions integrated explicitly as a system of 26 differential equations (7 groups of bacteria and archaea, 19 catalyzing processes), three physico-chemical liquid phase processes, 15 equations for the mechanisms of inhibition and eight implicit algebraic variables (per reactor). This approach is a tool to overcome the inherent 'stiffness' problem due to the fast hydrogen and pH dynamics (now explicitly algebraically calculated), which results in some numerical solvers encountering difficulty in solving the system of ODEs [17].

The values and the sensitivity of the parameters were determined by statistical fitting of the experimental data observed in steady state using AQUASIM 2.0 which is able to perform linear sensitivity analysis with respect to a set of selected parameters and to perform parameters' estimation by using the weighed least squares method [24-25].

Our approach to adapt ADM1 model to co-digestion was to characterize the actual feed mix with the stoichiometric composition of composite particulate chemical oxygen demand (CODp) i.e. carbohydrates (Xch), proteins (Xpr), lipids (Xli) and inerts (Xi). This approach was successful in terms of model predictions [26] and similar to input model for fractionation of COD described by Arnell et al [27]. As described by Feucken et al [28] ADM1 was modified by introducing three state variables for fractionated particulate COD (Xch, Xpr, and Xli) and three corresponding hydrolysis processes (first-order hydrolysis). In AQUASIM stoichiometric composition of substrate mix has been determined by repeating the corresponding characterization using dynamic inputs.

The biodegradable part of particulate COD has been considered based on COD analysis during the experiment. The ultimate methane potential and hydrolysis parameter ($k_{hyd}$) are estimated by fitting to BMP data using the defined model (eq 1 or eq 2).

Specifically, to take into account mixture composition feeding, fractionation of COD based on substrates proportion have been applied on inputs. For example, with TS/W mixture 70/30, soluble amino-acids input has been calculated from following equation:

$$(0.7f_{aa\text{-}TS} + 0.3f_{aa\text{-}W}) \,.\, CODs \quad (4)$$

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Where $f_{aa\text{-}TS}$: fraction of amino acids in thickened sludge; $f_{aa\text{-}W}$: fraction of amino-acids in whey; CODs: soluble COD in g/L.

Missing input state variables required for ADM1 have been found from a dataset containing the characterization and fractionation of organic substrates coming from literature [29].

Some kinetic rate equations have been modified to take into account inhibition linked to substrate concentration. Non-competitive substrate inhibition has been modeled by the Andrews-Haldane equation (eq 5).

$$mu_X = \frac{mu_{maxX}.S_X}{K_s + S_X + \frac{S_X^2}{K_i}} \quad (5)$$

Where: $mu_{maxX}$: degradation rate constant in kg COD kg $COD^{-1}$ $d^{-1}$; $S_X$: substrate concentration in kg COD $m^{-3}$; $K_s$: half saturation constant in kg COD $m^{-3}$; $K_i$: inhibition constant in kg COD $m^{-3}$.

## 3. Results

### *3.1 BMP test*

#### 3.1.1 Thickened sludge and grease mixtures

Biogas production per gram of VS is stimulated by the presence of grease (Figure 2A).

A first phase of biogas production with a very visible plateau has been observed with 100% grease and less and less visible depending on the decrease in the proportion of grease in the mixture. This behavior has been observed with BMP tests carried out with solid fat waste and with a comparable S / I ratio [30] or with grease trap sludge mixed with sewage sludge [31]. The presence of 10% grease accelerated the production of biogas and greatly increased the methane potential by a factor of approximately 1.5 compared to thickened sludge alone (Table 3).

The mixture of thickened sludge and grease in equal parts led to the same biogas production as the fat alone but with a shorter lag phase. Note that the increase in the ultimate production of biogas was not directly proportional to the increase of the share of grease, which translates a phenomenon of inhibition.

#### 3.1.2 Thickened sludge and septage mixtures

Beyond 10% of sewage matter in the mixture of sludge and septage, a very strong negative effect on the production of biogas was observed (Figure 2B). The mixture with 30% of septage led to an inhibition of about 36% of the potential of the sludge alone and about 60% for the mixture with 50% of septage.

With 10% septage, there was a positive effect on the first phase of production but not for the second phase (Table 3). As modelling two-phase kinetics is not adequate to describe exactly the shape of the curve, a kinetic with three successive phases was tested, the last with a lag phase of about 25 days.

#### 3.1.3 Thickened sludge and whey mixtures

The mixtures of 10% and 30% whey accelerated and increased the potential for biogas production per gram of VS (Figure 2C) compared to sludge alone.

The equal part mixing led to the same potential as the sludge alone but later because the second phase started later with a latency time of more than 14 days (Table 3). The production of biogas was lower for pure whey as substrate.

Modelling by the modified Gompertz model was satisfactory with coefficients of determination between observed and estimated values of around or above 0.99 (Table 3). This allowed us to quantify the parameters of the specific production kinetics of biogas per gram of volatile matter in the mixtures. The mixtures of 10% grease and 10 to 30% whey with the sludge thus appear to be very interesting for co-digestion.

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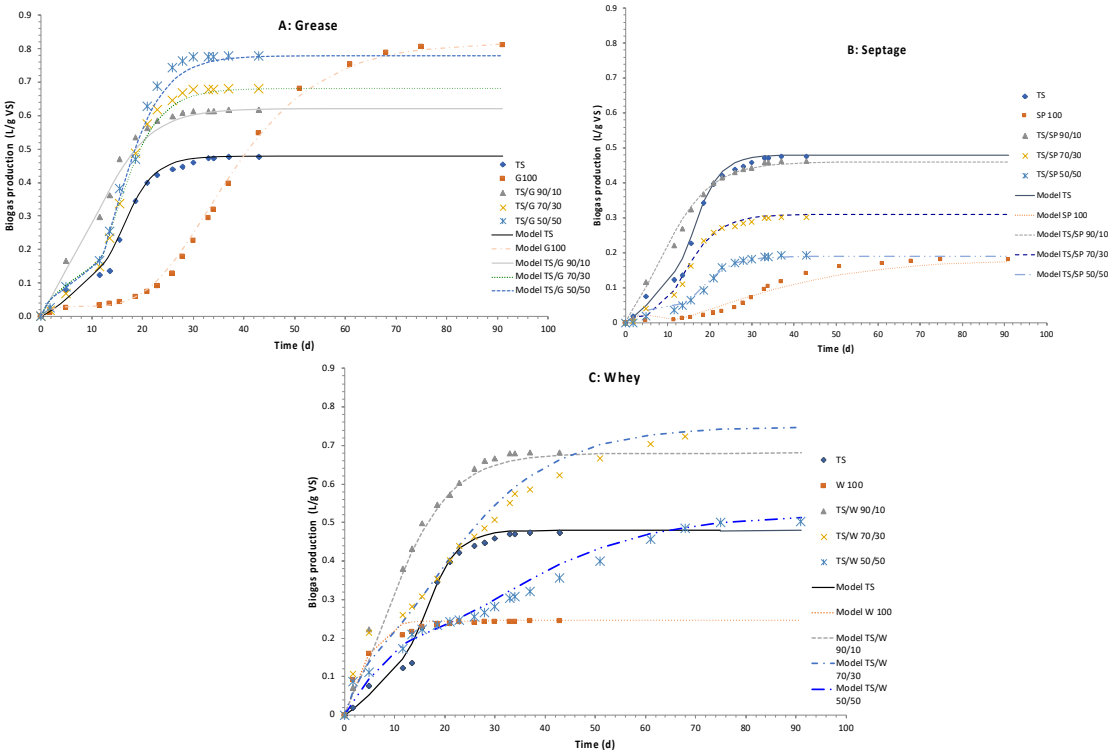


**Figure 2.** Cumulated biogas production for different mixtures in BMP assays.

**Table 3.** Kinetic parameters and biogas production rates per removed VS of BMP assays.

| Reactor | λ1 (d) | λ2 (d) | k1 ($LgVS^{-1}d^{-1}$) | k2 ($LgVS^{-1}d^{-1}$) | K1 ($LgVS^{-1}$) | K2 ($LgVS^{-1}$) | $R^2$ | Biogas ($L/gVSr^{-1}$) |
|---|---|---|---|---|---|---|---|---|
| **TS 100** | 1.5 | 13 | 0.015 | 0.051 | 0.2 | 0.48 | 0.993 | 0.67 |
| **G 100** | 0 | 21 | 0.012 | 0.025 | 0.03 | 0.82 | 0.998 | 1.28 |
| **TS/G 90/10** | 0 | 6.5 | 0.027 | 0.04 | 0.18 | 0.62 | 0.993 | 0.98 |
| **TS/G 70/30** | 0 | 10.4 | 0.027 | 0.054 | 0.1 | 0.68 | 0.997 | 1.01 |
| **TS/G 50/50** | 0 | 10.4 | 0.028 | 0.058 | 0.09 | 0.78 | 0.996 | 1.15 |
| **SP 100** | 1 | 8 | 0.004 | 0.017 | 0.22 | 0.05 | 0.977 | 0.07 |
| **TS/SP 90/10** | 0 | 6.5 | 0.02 | 0.026 | 0.24 | 0.46 | 0.993 | 0.64 |
| **TS/SP 70/30** | 0 | 8.2 | 0.01 | 0.023 | 0.02 | 0.31 | 0.993 | 0.37 |
| **TS/SP 50/50** | 0 | 14.1 | 0.008 | 0.017 | 0.05 | 0.19 | 0.995 | 0.23 |
| **W 100** | 0 | NaN | 0.034 | NaN | 0.245 | NaN | 0.975 | 0.32 |
| **TS/W 90/10** | 1 | NaN | 0.035 | NaN | 0.68 | NaN | 0.993 | 1.03 |
| **TS/W 70/30** | 0 | 4 | 0.021 | 0.021 | 0.1 | 0.75 | 0.991 | 1.16 |
| **TS/W 50/50** | 0 | 16.3 | 0.019 | 0.012 | 0.2 | 0.52 | 0.985 | 0.74 |

TS: Thickened sludge; G: Grease; SP: Septage; W: Whey.

Values of kinetic parameters and biogas production per removed VS have been standardized taking into account TS test as reference and applying equation 3 (Table 4).

**Table 4.** Normalized parameters taking into account TS as reference.

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| Reactor | λ1 | λ2 | k1 | k2 | K1 | K2 | Biogas | Score1 | Score2 | Score3 |
|---|---|---|---|---|---|---|---|---|---|---|
| **TS 100** | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 | 0.00 |
| **G 100** | -1.00 | 0.62 | -0.20 | -0.51 | -0.85 | 0.71 | 0.91 | 0.06 | 0.00 | -0.12 |
| **TS/G 90/10** | -1.00 | -0.50 | 0.80 | -0.22 | -0.10 | 0.29 | 0.46 | 0.39 | 0.39 | 0.39 |
| **TS/G 70/30** | -1.00 | -0.20 | 0.80 | 0.06 | -0.50 | 0.42 | 0.51 | 0.35 | 0.37 | 0.30 |
| **TS/G 50/50** | -1.00 | -0.20 | 0.87 | 0.14 | -0.55 | 0.63 | 0.72 | 0.43 | 0.45 | 0.35 |
| **SP 100** | -0.33 | -0.38 | -0.73 | -0.67 | 0.10 | -0.90 | -0.90 | -0.34 | -0.39 | -0.26 |
| **TS/SP 90/10** | -1.00 | -0.50 | 0.33 | -0.49 | 0.20 | -0.04 | -0.04 | 0.21 | 0.16 | 0.25 |
| **TS/SP 70/30** | -1.00 | -0.37 | -0.33 | -0.55 | -0.90 | -0.35 | -0.45 | -0.17 | -0.20 | -0.21 |
| **TS/SP 50/50** | -1.00 | 0.08 | -0.47 | -0.67 | -0.75 | -0.60 | -0.66 | -0.32 | -0.37 | -0.35 |
| **W 100** | -1.00 | NaN | 1.27 | NaN | 0.23 | NaN | -0.52 | 0.49 | 0.42 | 0.58 |
| **TS/W 90/10** | -0.33 | NaN | 1.33 | NaN | 2.40 | NaN | 0.54 | 1.15 | 1.27 | 1.39 |
| **TS/W 70/30** | -1.00 | -0.69 | 0.40 | -0.59 | -0.50 | 0.56 | 0.73 | 0.33 | 0.32 | 0.29 |
| **TS/W 50/50** | -1.00 | 0.25 | 0.27 | -0.76 | 0.00 | 0.08 | 0.10 | 0.06 | -0.02 | 0.04 |

A score was determined corresponding to the calculated average value of NP taking a positive value for lag phases. This score is used to compare and rank the results. In the calculation of score 1, the same weight is assigned for each of the parameters, which is open to criticism. For score 2, lag phase 2, k1, k2 (if present) and Biogas have been multiplied by a factor 2 to take into account the importance of these parameters. Considering the residence time in the digester, in this case the parameters lag phase 2, K1 and k1 must be considered as essential and assigned a greater weight to them i.e. lag phase 2, K1 and k2 multiplied by 2 (Score3 in Table 4). Whatever the calculated score was, septage used as co-substrate had a negative effect. Addition of 10 to 30% grease or 10% whey would be the most preferable looking at the different scores.

3.1.4 VS removal ratio.

Looking at the removal of volatile solids, they were for all the tests on average higher than 60% and in line with the observed kinetics (Fig. 3).

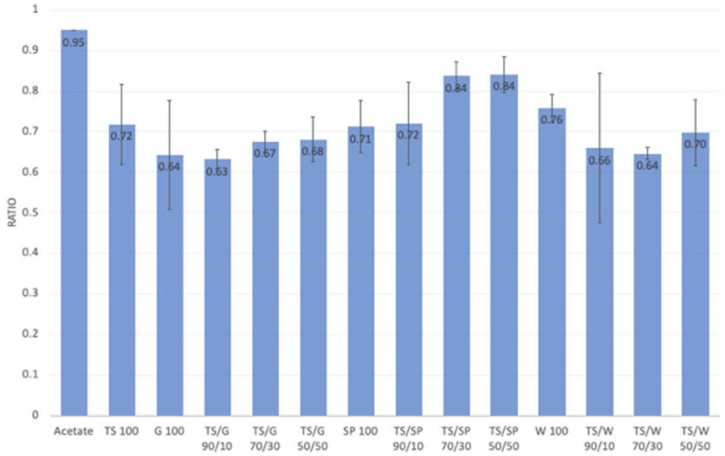


**Figure 3.** VS removal ratios with BMP assays.

3.1.5 Methane production

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A significant variability in the proportion of methane in the biogas produced was observed for most of the mixtures tested (Table 5). This variability was very low for mixtures with 10% co-substrates for grease and septage.

Grease and whey alone produced few quantities of methane. This was due to an inhibition of the methanogenic flora and only the hydrolysis-acetogenesis phase was carried out.

The 10% mixture of grease produced more methane than the thickened sludge alone and confirms its interest for co-digestion.

**Table 5.** Methane production with BMP assays

| | % $CH_4$ | | normoL $CH_4$ $gVSr^{-1}$ | |
|---|---|---|---|---|
| **BMP reactor** | **Average** | **SD** | **Average** | **SD** |
| **TS 100** | 62.13 | 9.3 | 0.416 | 0.085 |
| **G100** | 34.40 | 14.9 | 0.327 | 0.171 |
| **TS/G 90/10** | 69.85 | 1.6 | 0.669 | 0.082 |
| **TS/G 70/30** | 67.86 | 4.6 | 0.657 | 0.041 |
| **TS/G 50/50** | 62.34 | 17.3 | 0.699 | 0.109 |
| **SP 100** | 41.25 | 14.4 | 0.140 | 0.053 |
| **TS/SP 90/10** | 69.16 | 2.2 | 0.453 | 0.114 |
| **TS/SP 70/30** | 70.02 | 0.6 | 0.247 | 0.023 |
| **TS/SP 50/50** | 65.38 | 12.2 | 0.160 | 0.038 |
| **W 100** | 4.26 | 1.6 | 0.016 | 0.003 |
| **TS/W 90/10** | 62.06 | 5.9 | 0.719 | 0.258 |
| **TS/W 70/30** | 55.05 | 13 | 0.658 | 0.030 |
| **TS/W 50/50** | 43.95 | 2.1 | 0.296 | 0.028 |

These results show the possibility of using grease and whey as substrates for the anaerobic co-digestion of thickened WWTP sludge. 10 to 30% COD of the total load should not be exceeded with the S / I (in VS) ratios tested. These proportions would lead to a higher production of biogas and with an increased methane content.

However, it is not advisable to mix more than 10% of septage as a proportion of the total COD load. For this proportion there would be no increase in biogas production, but the methane concentration would be higher.

The second phase of the study with the CSTR reactors was carried out according to these conclusions concerning used ratios. The co-substrate septage from septic tank has not been tested in this experiment.

*3.2 Bioreactor test*

Volumetric loading rates before mixture with co-substrates were 1.1 ± 0.22 g VS $L^{-1}$ $d^{-1}$ and 1.8 g ± 0.7 COD $L^{-1}$ $d^{-1}$. During period of co-substrates addition loading rates were in the same range of values (Table 6).

**Table 6.** Volumetric loading rates during period of co-substrates mixture.

| **Volumetric loading rates** | **TS** | **TS/W** | **TS/G** |
|---|---|---|---|
| **COD** | 2.5±0.6 | 2.5±0.6 | 2.6±0.6 |

| | | | |
|---|---|---|---|
| **VS** | 1.1±0.3 | 1.3±0.5 | 1.3±0.2 |
| **DM** | 1.8±0.4 | 1.8±0.4 | 1.7±0.2 |

Mean values ± SD in g $L^{-1}$ $d^{-1}$

The ratios expressed in COD between the substrate and the co-substrate were nominally fixed in VS at 80/20 for the TS / W mixture and 60/40 for the TS / G mixture.

The COD, VS and DM measurements made it possible to calculate the real average ratios between substrate and co-substrate. The nominal ratios have been respected but only for DM. For VS and COD, the ratios are 70/30 for TS / W and close to 50/50 for TS / G.

3.2.1 Biogas production

Observed production of biogas was in the same order than expected values for well-conducted digester with waste activated sludge, 0.8 to 1.1 L g $VS_{removed}^{-1}$ $d^{-1}$ under the same conditions of loadings [32].

By expressing the daily production of biogas per gram of biomass (VS) in the reactor, the results show that it was the highest for the mixture with whey and the lowest for the mixture with grease. Mean values were respectively equal to 0.43 ± 0.11 L $d^{-1}$ $g_{VS}^{-1}$ (TS/W); 0.29 ± 0.05 (TS) and 0.11 ± 0.05 (TS/G). The differences are significant at $p < 0.01$ for the values of the mixtures in comparison with values of TS (Test of Dunnett, n=33).

3.2.2 Qualitative analysis of biogas

The Table 7 shows that the proportions of $CH_4$ and $CO_2$ were similar between the three feeding conditions.

The Kruskal-Wallis test does not allow the H0 hypothesis to be rejected, with the risk of error $p < 0.05$. No reactor stood out significantly from the others.

**Table 7.** Proportions of $CH_4$ and $CO_2$ in biogas.

| **Biogas composition (%)** | **TS** | **TS/W** | **TS/G** |
|---|---|---|---|
| **$CH_4$** | 71.3 | 69.7 | 68.7 |
| | ±1.3 | ±5.4 | ±1.5 |
| **$CO_2$** | 28.7 | 30.3 | 31.3 |
| | ±1.3 | ±5.4 | ±1.5 |

3.2.3 VS and COD removal

Analysis of the data over the entire period of the test showed a smaller reduction of volatiles and COD between input and output for mixing with grease, while removal was greater than 80% for other reactors (Table 8). This difference is statistically highly significant at $p < 0.000001$. The dispersion of the data around the mean value was also greater for the mixture with grease with a coefficient of variation of nearly 30%.

It can be observed that soluble COD had a lower removal yield than particulate COD as total COD yield was higher than soluble COD.

**Table 8.** VS and COD removal.

| **Removal (%)** | **TS** | | | **TS/W** | | | **TS/G** | | |
|---|---|---|---|---|---|---|---|---|---|
| | **VS** | **$COD_T$** | **$COD_S$** | **VS** | **$COD_T$** | **$COD_S$** | **VS** | **$COD_T$** | **$COD_S$** |
| **Average** | 82.2 | 82.4 | 60.2 | 87.2 | 87.3 | 77.4 | 64.8 | 65.1 | 47.1 |
| **Standard deviation** | 8.6 | 7.7 | 20.9 | 4.3 | 7.1 | 20.2 | 18.7 | 21.3 | 28.1 |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Median** | 83.1 | 84.2 | 67.7 | 88.3 | 89.1 | 82.4 | 69.7 | 72.1 | 56.1 |

The average VS removal efficiency has evolved linearly over the applied load for TS and TS / W mixtures but less clearly for the TS / G mixture (Fig. 4).

The mixture with grease was visually inhomogeneous and deposits of grease were observed in various parts of the reactor, this could explain the poorer efficiency of this mixture for co-digestion.

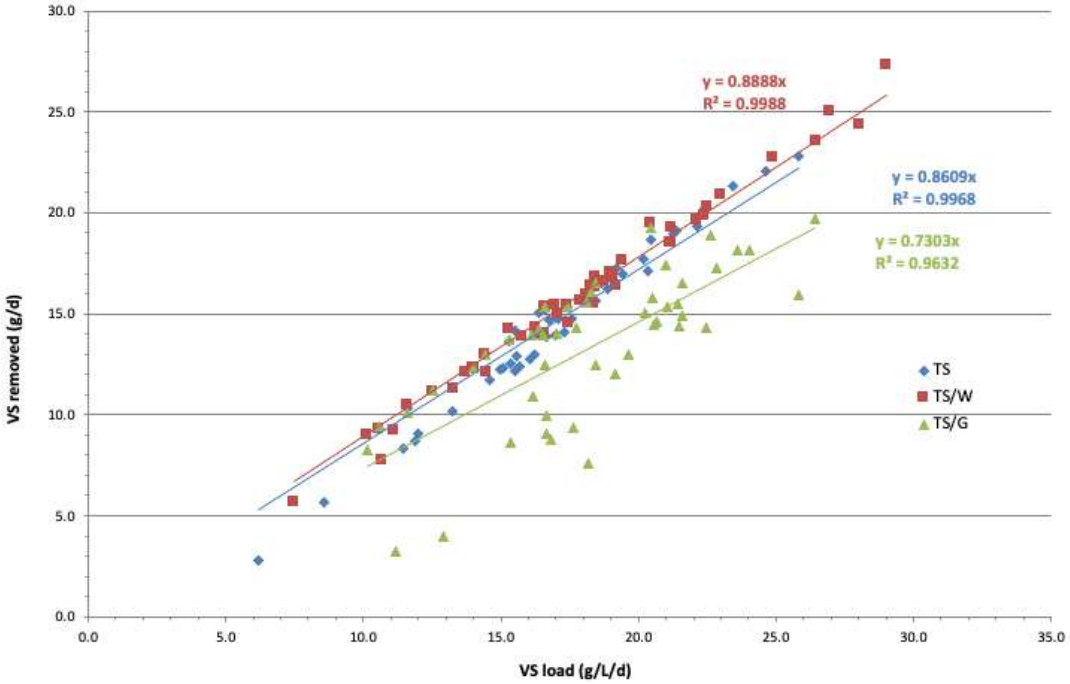


**Figure 4.** VS removed vs VS loads for TS, TS/W and TS/G.

3.2.4 VFA measurements

We observed a tendency for the accumulation of volatile fatty acids over time and more particularly for acetate (Fig. 5). Total VFA concentration between day 35 and day 70 did not exceed 0.6 g $L^{-1}$ except for TS reactor at day 70 due to an accumulation of acetate. The Kruskal-Wallis test does not allow the hypothesis H0 to be rejected, at the risk of error $p < 0.05$ and there were therefore no significant differences.

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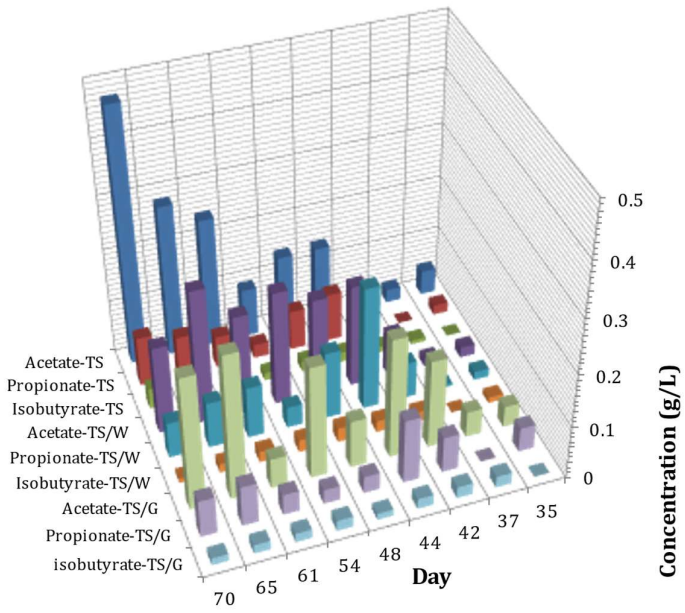


**Figure 5**. VFA concentrations at different days.

*3.3 Modelling with ADM1*

3.3.1 Biogas production per reactor

One of the greatest interests in modelling is to simulate the biogas production according to variation of feeding with the estimation of the biogas composition. The comparison of biogas production per reactor between the simulated and experimental values shows that the simulation with thickened sludge presents a satisfactory adjustment ($R^2$=0.704) but with a slight underestimation. With whey mixture the amplitude of the variations observed experimentally is not correctly simulated ($R^2$=0.321) and for the mixture with grease there is a slight underestimation ($R^2$=0.643) (Fig. 6).

The fractionation of the organic matter into elementary substrate (amino acids, long chain fatty acid, sugars), adjusted to the different mixtures and consideration of biodegradability of particulate matter are insufficient to achieve a complete simulation of real phenomenon. With grease mixture, Monod's equations were modified by inhibition equation (eq. 5) using parameter estimation to determine the value of the inhibition coefficient.

Before the addition of co-substrate, real biogas production per reactor was slightly higher (statistically different at P<0.00005) for reactor TS_W (Table 9). This higher production was shown with simulation but without statistically significant difference.

The addition of whey as co-substrate did not influence significatively the average biogas production per reactor both for the experiment and for simulation. The addition of grease showed a similar behavior between experience and simulation. This influence was statistically different at P<0.00001 for the experiment and P<0.005 for simulation. In case of the experiment, the average production was lower (9.82 L $d^{-1}$ vs 11.6 L $d^{-1}$ for TS) and also lower in case of simulation (9.11 L $d^{-1}$ vs 10.01 L $d^{-1}$ for TS).

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**Table 9.** Comparison between experimental and modeling results for daily biogas production per reactor.

| | | Experimental results (L $d^{-1}$) | | | Modeling results (L $d^{-1}$) | | |
|---|---|---|---|---|---|---|---|
| | | TS | TS_W | TS_G | TS | TS_W | TS_G |
| **Before co-substrate addition** | **Average** | 7.32 | 8.81* | 7.02 | 5.74 | 6.75 | 4.71 |
| | **+/- SD** | 1.32 | 0.98 | 0.50 | 2.01 | 2.64 | 1.61 |
| | **Median** | 6.50 | 9.00 | 6.90 | 5.10 | 5.58 | 3.91 |
| **After co-substrate addition** | **Average** | 11.61 | 11.94 | 9.82* | 10.01 | 10.18 | 9.11* |
| | **+/- SD** | 1.52 | 2.70 | 2.05 | 1.74 | 1.96 | 1.47 |
| | **Median** | 11.80 | 12.30 | 9.90 | 10.79 | 10.43 | 9.17 |

*Statistically different

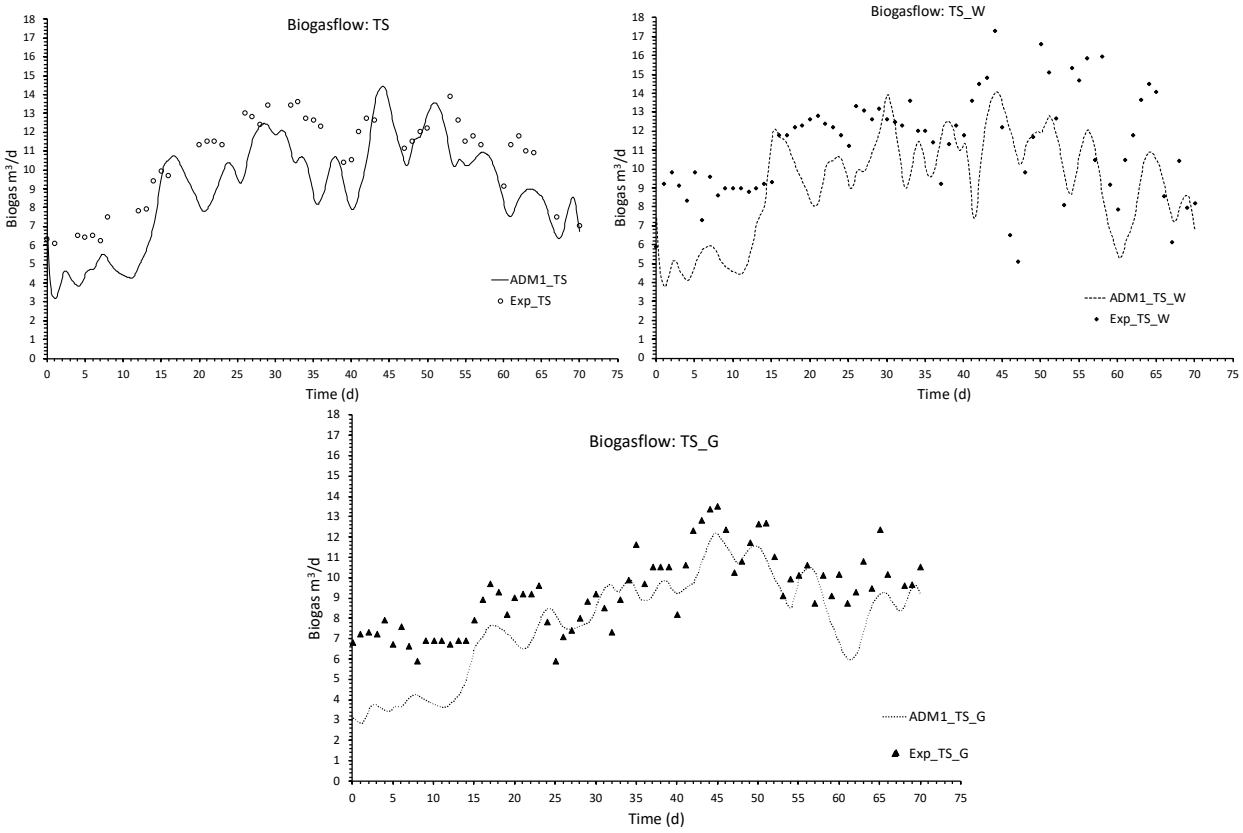


**Figure 6.** Comparison of biogas flow between experiments and modelling.

3.3.2 Biogas composition

The simulated composition of the biogas did not show an effect of the addition of the co-substrate (Fig. 7). This confirms the observations of the experimental values.

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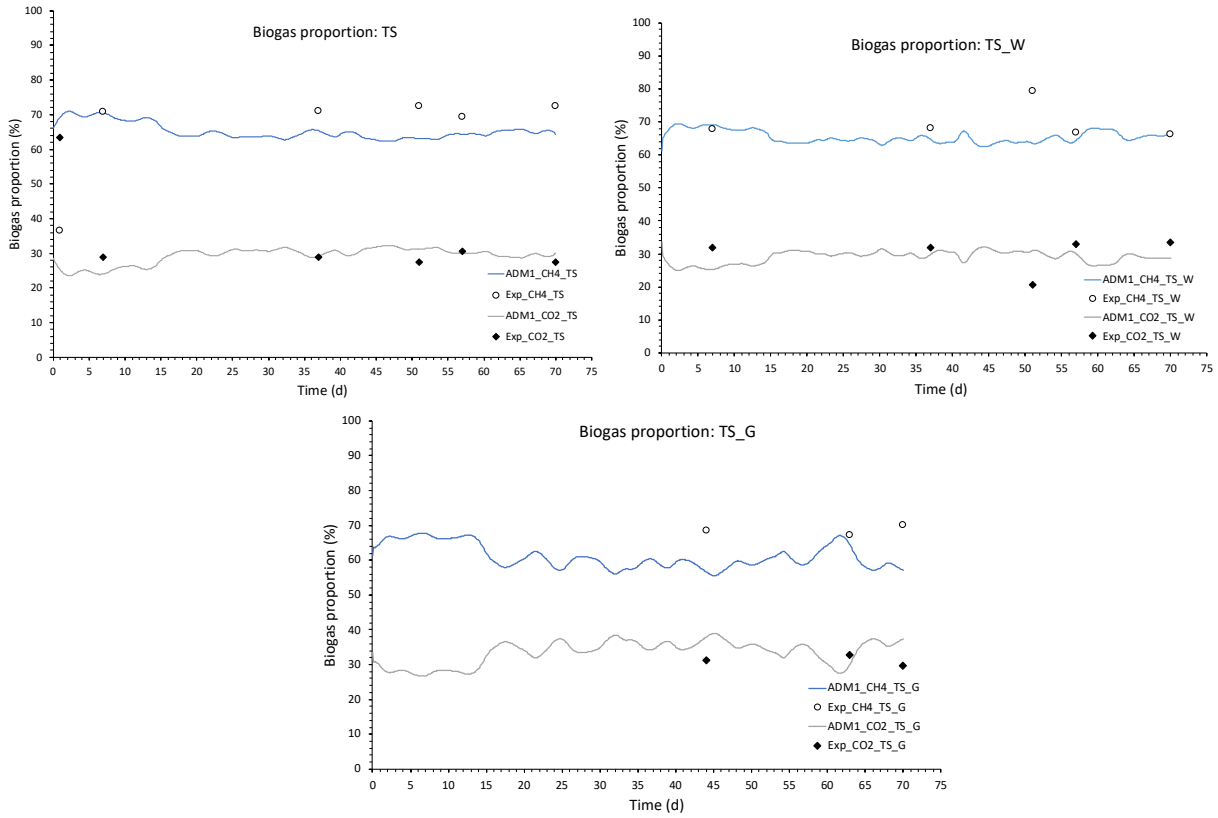


**Figure 7.** Comparison between experiments and modelling for biogas composition.

3.3.3 pH

Simulations shown a relative stability of the pH (Fig. 8) with correct adjustment to experimental values for thickened sludge, an underestimation for the mixture with grease and an overestimation for the mixture with whey.

It was not possible to adjust the modelling using realistic parameters responsible for the change in pH.

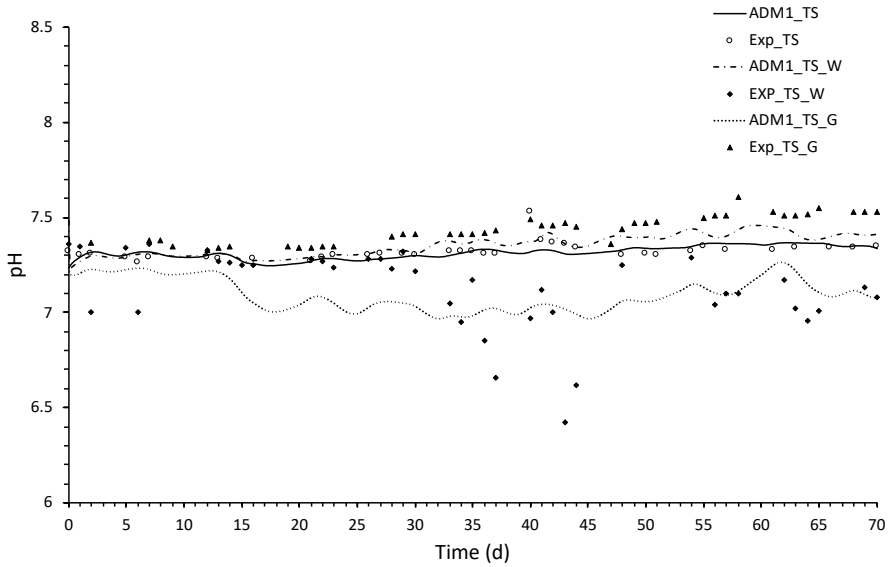


**Figure 8**. Comparison for pH evolution between experiments and modeling.

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3.3.4 Microbial degraders population distribution profiles obtained from simulation with ADM1

Experimental microbial population analysis has not been realized but it is possible to simulate with ADM1 growth rate and decay of the different microorganisms involved in the anaerobic digestion.

The microbial degraders population distribution obtained from simulation show that on day corresponding to the addition of co-substrates, profiles were similar between the three reactors (Figure 9). On day 70, at the end of the simulation period for experiment and simulation, profiles were different. Main differences were observed for long chain fatty acids degraders, sugar degraders and acetic acid degraders.

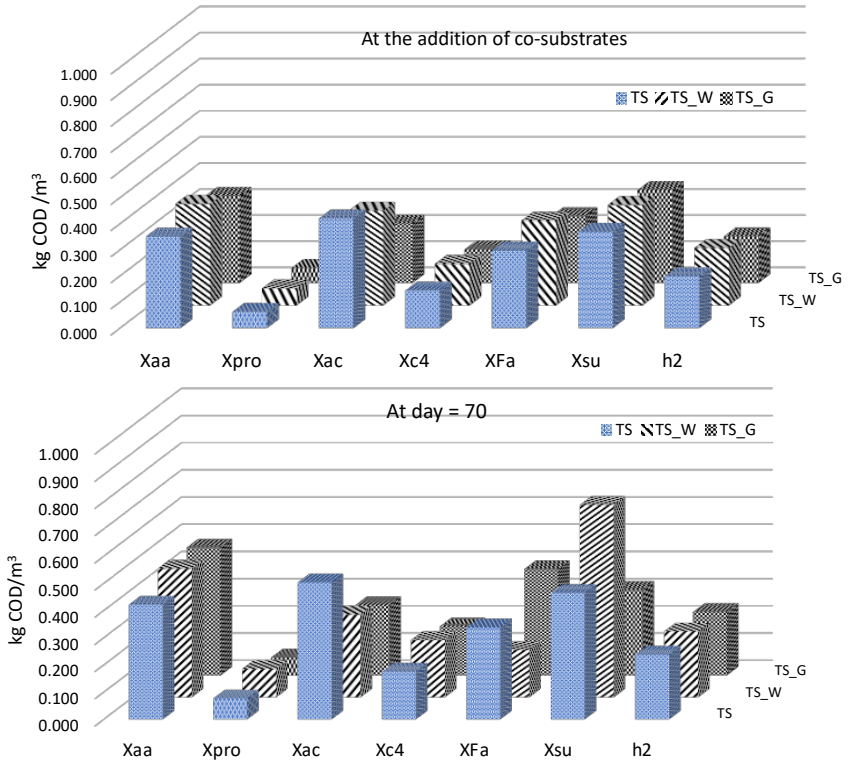


**Figure 9.** Biomass degraders distribution profiles obtained from simulation with ADM1. Xaa: aminoacid biomass; Xpro: propionic biomass; Xac: acetic acid biomass; Xc4: valeric/butyric biomass; Xfa: long chain fatty acid biomass; Xsu: sugar biomass; h2: hydrogen biomass.

## 4. Discussion

Among process parameters impacting heavily on quantity and quality of biogas production, co-digestion directly linked to feedstock type is one of the main variables [33].

The results obtained with the BMP tests confirmed the positive effects of co-digestion reported in the literature with compounds which do not inhibit anaerobic digestion [34-35].

These positive effects, especially on biogas production and methane are dependent on their proportion mixed with thickened extended aeration sludge. The results of the

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reactor test showed less evidence of the positive effects observed with the BMP tests and indicates mainly that co-digestion in this case had no adverse effects.

Feed degradable COD fraction is very important, as previously shown [27, 36-37] and can explain differences observed in BMP test and reactor test especially for less biodegradable substrate like septage and grease.

Protein and lipid fractions of particulate biodegradable COD are important state variables for digester stability and methane production as predicted by simulation [27] and confirmed in this study.

Concerning lipid fraction, laboratory and pilot scale anaerobic digesters have shown larger increases in gas production with addition of readily available high strength organic wastes such as fats, oils, and grease (FOG) [34, 38]. Co-digestion of mixed municipal sludge (primary and thickened sludge) with concentrated external organic wastes such as FOG can result in significantly high methane production at higher organic loading rates and lower digester HRT/SRT values as long as COD and VS destruction does not drop below an acceptable lower value [39]. However, anaerobic digestion of high lipid wastes has been reported to cause inhibition of acetoclastic and methanogenic bacteria, substrate, and product transport limitation, sludge flotation, digester foaming, blockages of pipes and pumps, and clogging of gas collection and handling systems.

The limit for FOG (grease interceptor waste) addition leading to an inhibited digestion process was identified to be between 20 and 40% (v/v) or 65.5 and 83.5% (w/w) of VS added [40]. This inhibition has been observed with BMP test and reactor test in this experiment.

Increased FOG levels extended the lag phase and eventually displayed strong or complete inhibition. Operating anaerobic digesters with FOG levels in the concentration range of 0.1–1.5% (v/v) enhanced biomethane production, resulting in a 2 to 19-fold increase. The highest biomethane production was observed at 1% FOG (19-fold increase), with a lag phase of 25 days. LCFAs were degraded by 80–90% during AD, following a 0.5–1.5% FOG loading. An addition of either 2 or 3% FOG permanently inhibited biomethane production due to high VFA accumulation (17–19 g/L) and low LCFA reduction (29 and 18%), respectively. Under optimum biomethane production (1–1.5% FOG), *Syntrophomonas* and *Fermentimonas* were abundant, indicating their role in LCFA degradation and acetogenesis [41].

To avoid inhibition during high FOG loadings, this would require microbial acclimatization or substrate pretreatment [8,42-43].

Concerning whey addition and dairy wastewater by extension, it is important to respect an organic matter load compatible with a CSTR process to avoid any failure of this non-adapted to high strength load process [44-45]. Indeed, whey has a high C/N ratio, a low pH and is rich in easily degradable sugars (mainly lactose), which could lead to rapid acidification in the digester and process inhibition [46-47]. A correlation study was made on whey/TS mixture reactor, between pH, COD load and production of biogas in an attempt to explain the significant variations observed. We did not obtain any significant correlations between these parameters. The highest level is between the pH and the production of biogas with a coefficient of determination $R^2$ of only 0.44 ($r = 0.66$ at $p = 0.00001$). The pH reduction observed with this reactor seems not due to accumulation of VFAs from acidogenesis step as shown by chemical analysis but probably to a weak buffering capacity of the mixture due to acidification as whey have a low pH. Indeed, if the change in pH may reflect the excess of VFA, it may also be a sign of a high production of NH4 (protein hydrolysis). It is therefore necessary to adapt the organic load to the buffering capacity of the reaction medium, the recommended VFA / ALK ratio is 0.2-0.5 [48].

To enhance nutrient balance with improved pH buffering and to optimize the production of methane, a C/N ratio of about 15-30/1 is required [11, 33] and can be reached by deploying co-digestion. Co-digestion of 10% to 30% whey with thickened sludge has shown a synergistic effect on biogas production in this study as also observed when mixing with poorly degradable substrates [49-50].

Results of simulations with ADM1 model adapted to co-digestion confirms that this model is a powerful tool to optimize the process of biogas production. Numerous publications have been noted indicating that the disintegration and hydrolysis steps are the limiting factors of co-digestion process [51].

To improve ADM1 simulation with whey co-substrate it may be possible to consider uptake of lactate and incorporate in the biochemical processes [52]. This does not seem necessary in our case because of the simulation results obtained and certainly of a too low concentration of lactic acid in the digester when the proportion in the mixture of whey is less than 20-30%. However, in the presence of significant lactate concentration, it would be advisable to add the metabolic pathway of transformation into methane which can lead to a complete conversion [53]:

$$2 \text{ lactate} + 3\ CO_2 \rightarrow 3 \text{ acetate} \rightarrow 3\ CH_4$$

Overall, it therefore seems important to respect the C/N ratios recommended for the whey/thickened sludge mixture or other co-substrate rich in nitrogen, to avoid excess nitrogen causing ammonia formation together with an increase in pH and consequently drop in methane yield or at the opposite a nitrogen availability depletion resulting in incomplete methanogenesis and a reduced biogas productivity.

If the results obtained with the BMP test could lead to a significant increase in production as observed in literature for dairy whey and grease sludge [54], biomethane and therefore energy, the reactor tests did not validate this hypothesis satisfactorily. As extended aeration plants request high operational costs due to high energy needs, 0.903 ± 0.509 kWh $m^{-3}$ [55] additional power via co-digestion should not be expected too much to cover energy needs. But reactor tests confirmed the good biodegradation efficiency and the main interest of co-digestion in this case is to recover energy from waste and effluents which would require even more energy to treat with this type of extended aeration process.

## 5. Conclusions

The results obtained show and confirm that it is possible to add local organic waste in anaerobic digesters of extended aeration wastewater treatment plant to treat the thickened sludge. This addition is even desirable in order to valorize these effluents into biogas. To define a good practice for the conduct of co-digestion, it will be necessary to respect the designed daily volume or mass loads as well as the residence time required for good degradation of the co-substrates. Adapted modelling using ADM1 makes it possible to estimate the proportions of the mixture to be respected based on data from BMP tests from the literature or from specific tests if necessary.

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