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## ► To cite this version:

A. Coutu, D. Dochain, S. Mottelet, L. André, M. Mercier-Huat, et al.. Dynamical model development and parameter identification for solid-state anaerobic digestion of shellfish products: Application to *Mytilus edulis*. *Bioresource Technology Reports*, 2023, 22, pp.101458. 10.1016/j.biteb.2023.101458 . hal-04099654

**HAL Id: hal-04099654**

**<https://normandie-univ.hal.science/hal-04099654v1>**

Submitted on 30 Oct 2024

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# 1 **Dynamical model development and parameter identification for solid-state** 2 **anaerobic digestion of shellfish products: Application to *Mytilus edulis***

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## 10 **Abstract**

11 A simplified AM2 model was developed to characterize mussel solid-state anaerobic digestion. This  
12 model considers two different substrates for mussels' degradation: the mussel meat and the  
13 mussel juice obtained after sanitization. This model was implemented to characterize the  
14 anaerobic degradation of *Mytilus edulis* species. This model was verified, implemented, and  
15 validated in 60L batch reactors in mesophilic conditions. Two different experiments were used to  
16 calibrate kinetics using reaction invariants and an interior point optimization method. A  
17 conditioning study and a sensitivity analysis were done and had shown a better sensitivity with  
18 delayed substrate injections throughout the experiment with a factor of 10. An 88.6%  
19 accumulation of methane yield of the BMP measurement was observed, corresponding to 57.7%  
20 volatile removal with a minimum mass balance of 96.1%. Additionally, the model proposed in this  
21 study was able to successfully predict the two characteristic methane yield peaks observed during  
22 solid-state anaerobic digestion.

## 23 **Keywords**

24 Anaerobic digestion; Mathematical modeling; Sensitivity analysis; Inhibition; Kinetic parameters

## 25 **1 Introduction**

26 Shellfish aquaculture consists of domestic shellfish farming by humans. Mussels are among the  
27 most popular farmed shellfish in the world, with an increasing worldwide production of over 2  
28 million tonnes per year, with China, Chili, and Spain as the main producers (FAO, 2020). *Mytilus*  
29 *edulis*, otherwise known as blue mussels, is the third more farmed species after *Mytilus*  
30 *galloprovincialis* and *Mytilus chilensis* with over 10 % of global mussel production. France is one of  
31 the main producers of this last species with an annual production of 47,000 t (FAO, 2020).  
32 However, only 660 kg.t<sup>-1</sup> is suitable for human consumption (Vareltzis and Hundeland, 2012),  
33 resulting in a large amount of waste that could be valued. Many parts of the mussel could indeed  
34 be used: byssal thread, shell, and mussel meat are sources of fat, protein, carbohydrates, and other  
35 bioactive compounds. These by-products from mussel wastes could be valorized as functional  
36 ingredients for animals (Sardenne *et al.*, 2019; Afrose *et al.*, 2016) or humans (Vijaykrishnaraj *et al.*,  
37 2016; Zhang *et al.*, 2013), as building material (Martínez-García *et al.*, 2020; Martínez-García *et al.*,  
38 2019) or as soil improvement to improve soil fertility and microbial activity (Fernandez-Calviño *et al.*,  
39 2018; Messiga *et al.*, 2016) or soil decontamination (DiLoreto *et al.*, 2016; Fernandez-Calviño *et al.*,  
40 2015; Seco *et al.*, 2014). Compounds of chemical interest could be extracted from mussel wastes as  
41 bioactive proteins, polyunsaturated fatty acids, enzymes, mineral compounds, or pigments (Naik  
42 and Hayes, 2019; Pintado *et al.*, 1999). Another way to valorize mussel wastes is anaerobic  
43 digestion (AD). AD is a biological process that consists of the degradation of an organic substrate by  
44 a microbial consortium to produce biogas and digestate. This process kinetics may be divided into 4  
45 main steps which are hydrolysis, acidogenesis, acetogenesis, and methanogenesis (Kothari *et al.*,  
46 2014; Li *et al.*, 2011; Amani *et al.*, 2010). Solid-state anaerobic digestion (SS-AD) is defined by a

47 total solid content higher than 15 % and is less common in industrial applications but is more  
48 efficient in the digestion of high solid content feedstock like cattle manure or corn silage  
49 (Karthikeyan and Visvanathan, 2013; André *et al.*, 2018). This approach reduces the reactor size,  
50 the amount of water used, and thus the amount of energy required. However, many scientific  
51 challenges still exist in this process due to the lack of knowledge on SS-AD including local  
52 accumulation of inhibitors as volatile fatty acids (VFA) due to the medium heterogeneity (André *et*  
53 *al.*, 2018).

54 AD of *Mytilus edulis* have already been implemented (Wollak *et al.*, 2018; Akizuki *et al.*, 2018) with  
55 great methane yield between  $310 \text{ m}^3 \cdot \text{t}_{\text{VS}}^{-1}$  and  $490 \text{ m}^3 \cdot \text{t}_{\text{VS}}^{-1}$  using one-step and two-step processes.  
56 Other studies showed that optimal conditions are reached when alkalinity is controlled (Murto *et*  
57 *al.*, 2004). Concerning *Mytilus edulis*, the salt concentration is an important parameter to  
58 guarantee optimal methane production and avoid process inhibitions (Zhang *et al.*, 2017; Anwar *et*  
59 *al.*, 2016; Kimata-Kino *et al.*, 2011). *Mytilus edulis* SS-AD was already carried out in an Upflow  
60 Anaerobic Sludge Blanket (UASB) with a methane yield of  $330 \text{ m}^3 \cdot \text{t}_{\text{VS}}^{-1}$  (Nkemka and Murto, 2013)  
61 attesting the SS-AD feasibility while maintaining process efficiency, but the digestion of mussels  
62 including shells gave low methane production. A better understanding of the phenomenon is  
63 necessary to provide a better degree of predictability regarding methane production.  
64 Mathematical modeling can be a useful understanding tool for representing biological kinetics  
65 through equations (Du *et al.*, 2021; Fdez.-Güelfo *et al.*, 2011). This understanding could help to  
66 implement some prediction and control tools to optimize methane production for SS-AD (Zhou *et*  
67 *al.*, 2020; Donoso-Bravo *et al.*, 2011). Different SS-AD models were developed in the literature  
68 (Coutu *et al.*, 2022; Xu *et al.*, 2015) including modeling of perfectly mixed systems using ordinary  
69 differential equation systems (ODE) to reach a compromise between the model complexity and the  
70 kinetic parameters identifiability as the AM2 model (Bernard *et al.*, 2001). The current models of

71 anaerobic digestion are simple models such as the Gompertz model, perfectly mixed models such  
72 as the ADM1 model and its derivatives, heterogeneous models such as the distributed model and  
73 its derivatives for solid-state anaerobic digestion, and statistical models such as the logistical model  
74 (Liu *et al.*, 2023). However, no model available in the literature is adapted to a substrate such as  
75 *Mytilus edulis* due to the complexity of its degradation. Indeed, the degradation of the mussel and  
76 the released juice of the mussel with the risks of inhibitions that this implies cannot be simply  
77 modeled using the models available in the literature. This study aimed to mathematically develop  
78 an innovative modified AM2 model to characterize complex substrates SS-AD such as *Mytilus edulis*  
79 SS-AD. This model was verified, implemented, and validated in 60L batch reactors in mesophilic  
80 conditions using the asymptotic observers' method, which is not used much in the literature and is  
81 yet a very practical method to obtain as much experimental information as possible from the  
82 measurements made.

## 83 **2 Materials and methods**

### 84 *2.1 Physicochemical characterization of substrate and inoculum*

85 Undersize *Mytilus edulis* mussels (MeM) used for SS-AD were sampled from the CultiMer France  
86 workshop (Vivier-sur-Mer, France). These mussels were separated and crushed (with a thickness of  
87 12 mm) from marketable bouchot mussels with a mussel sizer and a grinder on the sorting line,  
88 randomly sampled and transported to the UniLaSalle Polytechnic Institute (Beauvais, France). A  
89 sanitizing step during 1h at 70°C (Klarstein 60L, Germany) was realized. During these operations,  
90 mussels released a liquid called released juice (RJ) which was considered a different substrate than  
91 mussels during SS-AD. Liquid bovine manure (LM) was sampled from the farm of the UniLaSalle  
92 Polytechnic Institute (Beauvais, France) and was used as *inoculum* to bring the microbial  
93 consortium. LM was filtered by a mesh with 5 mm diameter holes to avoid solid clogging in the  
94 recirculation pipe.

95 The total solid content (TS) and the volatile solid (VS) content of MeM, RJ, and *inoculum* were  
96 determined by a 105°C drying for 24h and combustion at 550°C for 2h (APHA, 1988). The pH was  
97 measured with a pH meter (Mettler Toledo, Switzerland), and the total volatile fatty acid content  
98 (VFA) and the buffer capacity (TAC) were determined with an automatic titrator (Mettler Toledo,  
99 Switzerland) by two titrations using sulfuric acid. For VFA/TAC measurement, samples were  
100 centrifuged at 9000 rpm for 20 min to remove the micro-organisms which could induce an  
101 intracellular content release. Chemical Oxygen Demand (COD), Ammonium concentration, Calcium,  
102 Sodium, and total Nitrogen concentration were determined using WTW kits (WTW, Germany). All  
103 measures have been triplicated. The biochemical methane potential (BMP) of each substrate was  
104 measured using an AMPTS I device (Automatic Potential Test System, Bioprocess Control, Sweden)  
105 according to Holliger et al. (2016). All the results are reported in **Table 1**.

## 106 *2.2 Experimental set-up*

107 Two batch reactors made of polyethylene with a total volume of 60 L (considering a 50 cm height  
108 and a 39 cm diameter) were used for one run of experiments under mesophilic conditions.  
109 Experiments are (R1) and (R2). Each reactor was equipped with a plastic holder to separate the  
110 liquid and the solid phases. The reaction process took place in the liquid phase. A grid with holes of  
111 5 mm diameter was placed on the plastic holder to avoid solid blockages due to pieces of shells in  
112 the recirculation pipe. The liquid phase composed of inoculum and RJ was recirculated in each  
113 reactor with an external peristaltic pump (Masterflex, USA) respecting a liquid flow rate of 15 L.h<sup>-1</sup>,  
114 for 15 min each hour. Thus, the liquid phase was spread out across the top of the solid phase inside  
115 the reactor. Each reactor was connected to a biogas flow counter (Drum gas meter TG05, Ritter,  
116 Germany), and biogas production was continuously measured and daily averaged. The biogas  
117 composition was daily measured with a biogas analyzer (MGA300 multi-gas analyzer ADC gas

118 analysis Ltd., Hoddesdon United Kingdom) and manually verified with a portative biogas analyzer  
119 (Multitec 540 Sewerin, Germany) to avoid measurement drift due to daily recalibration.

120 RJ brings a non-negligible quantity of volatile content which contributed to VFA accumulation and  
121 could cause biological inhibitions (Karthikeyan and Visvanathan, 2013; Siegert and Banks, 2005). To  
122 study the RJ impact on MeM SS-AD, different addition strategies were adopted on each reactor.  
123 The inoculum/substrate ratio was similar in the two experimented conditions ( $I/S=0.41$ ) to  
124 compare the experimental results. Each reactor was filled with 12 kg of MeM and 23 kg of LM. RJ  
125 was added in each reactor following a different strategy for each reactor: 3.5 kg of RJ was placed  
126 inside the first reactor (R1) at the beginning of the experiment and 5 constant additions of 0.7 kg of  
127 RJ were made in the second reactor (R2) during day 4, day 7, day 10, day 14, and day 17 with a  
128 ratio  $I/S=0.41$ .

129 Once these reactors were filled, each of them was sealed and the temperature was held at a  
130 constant value of 37°C with a thermostatically controlled water bath for each experiment for 41  
131 days. Each experiment is described in **Fig 1**. At the end of each experiment, mass balances were  
132 determined.

## 133 *2.3 Mathematical model implementation*

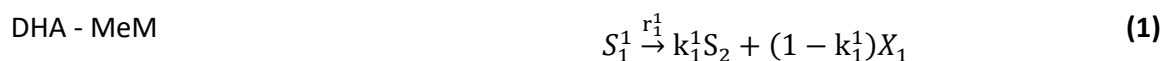
### 134 *2.3.1 Modeling assumptions*

135 Let us first consider the following assumptions for the derivation of the dynamical model of the  
136 process. First of all, all the state variables are expressed in COD units, and in consequence carbon  
137 dioxide does not appear in the model because it could not be oxidized. Moreover, the  
138 disintegration, hydrolysis, and acidogenesis steps have been gathered in a single step named DHA  
139 and modeled by first-order kinetics as proposed in Bollon *et al.* (2011). This assumption could be  
140 made because MeM and RJ are mostly composed of proteins, lipids, and carbohydrates whose

141 hydrolysis is the limiting step. In addition, the acidogenic step is very fast in comparison with the  
 142 hydrolysis step. Instead of different volatile fatty acids, only a generic equivalent acetic acid was  
 143 considered (Bernard *et al.*, 2001). As a consequence, the acetogenesis step was removed from the  
 144 model. These assumptions allow us to reduce the number of parameters to be determined.  
 145 Regarding biomass growth, the methanogenic biomass is assumed to be constant, which means  
 146 that microbial growth and death are neglected. Indeed, less than 10% of the organic part of  
 147 substrates is turned into biomass (Batstone *et al.*, 2002) and this assumption allows us to identify  
 148 the kinetic parameters. Ammonia was not considered in this model because there was always a  
 149 very constant concentration of ammonia in all measurements. Hydrogen is an intermediate gas in  
 150 SS-AD and its concentration is negligible, therefore hydrogen was not considered either. Methane  
 151 was assumed to have negligible solubility in the liquid phase and therefore the methane liquid-gas  
 152 transfer was neglected to simplify the model. Finally, RJ was supposed more easily degradable than  
 153 MeM due to the solutes' accessibility, and all the organic substrate entering the batch reactor was  
 154 assumed fully biodegradable.

### 155 2.3.2 Anaerobic digestion model and reaction kinetics

156 A three-reaction-based biological kinetic model scheme was used for this study to provide a simple  
 157 and understandable representation of the phenomenon. In this model, the DHA biomass ( $X_1$ )  
 158 hydrolyses and converts MeM ( $S_1^1$ ) and RJ ( $S_1^2$ ) into VFA ( $S_2$ ) during the DHA step. Then VFA is  
 159 converted into methane ( $CH_4$ ) during the methanogenesis step. The following equations present  
 160 the model reactions:





Methanogenesis



161 A first-order kinetic was used for DHA steps, and a Haldane kinetic model with acid concentration  
162 inhibition was used to consider for methanogenesis step for the accumulated methane yield.  $k_1^1$   
163 and  $k_1^2$  represent respectively the acidogenesis and methanogenesis conversion rates,  $(1 - k_1^1)$   
164 and  $(1 - k_1^2)$  represent respectively the acidogenesis biomass and methanogenesis biomass  
165 growth rates. A Peterson matrix summarizes these kinetics in **Table 2**. In this table,  $\mu_1^1$  represents  
166 the DHA rate of  $X_1$  other  $S_1^1$ ,  $\mu_1^2$  represents the DHA rate of  $X_1$  other  $S_1^2$ ,  $K_{S_2}$  represents the half-  
167 saturation constant associated with  $S_1^1$  and  $S_1^2$ ,  $\mu_2^{\max}$  represents the maximum growth rate of  $X_2$   
168 other  $S_2$  and  $K_I$  is the inhibition constant associated with the consumption of  $S_2$ . The dynamical  
169 system obtained for mesophilic SS-AD is composed of 5 ordinary differential equations (ODE):

$$\frac{dS_1^1}{dt} = -r_1^1 \quad (4)$$

$$\frac{dS_1^2}{dt} = -r_1^2 \quad (5)$$

$$\frac{dS_2}{dt} = k_1^1 r_1^1 + k_1^2 r_1^2 - r_2 \quad (6)$$

$$\frac{dX_1}{dt} = (1 - k_1^1) r_1^1 + (1 - k_1^2) r_1^2 \quad (7)$$

$$\frac{dCH_4}{dt} = k_2 r_2 \quad (8)$$

170

### 171 2.3.3 State variables initialization

172 There are 5 state variables in the model, a lower number than other models of the literature as the  
173 ADM1 (Batstone *et al.*, 2002) and modified solid-state models (Bollon *et al.*, 2011; Abbassi-  
174 Guendouz *et al.*, 2012; Coutu *et al.*, 2022), due to the previous assumptions. The total COD of LM,

175 MeM ( $S_1^1$ ), and RJ were measured. RJ contained initial VFA extracted from mussels during  
176 sanitizing. This is why COD of RJ was divided into initial VFA ( $S_2^0$ ) and initial RJ ( $S_1^2$ ). Initial VFA  
177 content was measured and initial RJ was deduced from this value. The COD of biomass was divided  
178 into DHA biomass ( $X_1$ ) and methanogenic biomass ( $X_2$ ) respecting a 25%-75 % ratio according to  
179 Gavala et al. (2003).  $X_2$  was supposed to be constant all along SS-AD per the modeling  
180 assumptions.

#### 181 2.3.4 Mass balance model

182 A total mass balance and a first simulation were led to perform a model validation of the  
183 initialization conditions and during calibration and validation steps. This step allows us to verify  
184 mass conservation. The total mass balance expressed in equation (9) meets equation (10).

$$\text{Total mass balance} = S_1^1 + S_1^2 + S_2 + CH_4 \quad (9)$$

$$\frac{dS_1^1}{dt} + \frac{dS_1^2}{dt} + \frac{dS_2}{dt} + \frac{dCH_4}{dt} = 0 \quad (10)$$

185

## 186 2.4 Computational aspects

### 187 2.4.1 Calibration and Validation steps

188 Calibration was performed on the experiment (R2) for which RJ was added at constant intervals  
189 with constant amounts. This procedure allowed us to generate data that better scan the kinetics  
190 curves. 7 stages were identified in this experiment: stage 1 represents the period during which only  
191 MeM was consumed and RJ was absent from the reactor. Stages 2 through 6 represent the periods  
192 between each RJ addition. Finally, stage 7 represents the period during which all the RJ and the  
193 MeM were consumed and only the remaining VFA was consumed. The identification of these  
194 different stages allowed us to determine the kinetic parameters of the DHA step and the

195 monitoring of unmeasured state variables as presented in section 2.4.3. The validation step was  
196 conducted on the experiment (R1) for which all RJ was injected into the reactor at the beginning of  
197 the experiment. The cumulative methane production, the methane flow rate, and the VFA  
198 concentration were then compared with the simulated values to validate the calibration step. 2  
199 periods were identified in the first experiment (R1): a first stage of rapid consumption of RJ with a  
200 little degradation of MeM, and a second stage during which only the remaining MeM was  
201 consumed. The kinetic parameters of the DHA were also determined in this experiment to validate  
202 the values obtained during the calibration step.

#### 203 2.4.2 *Identifiability of model parameters and unmonitored variables*

204 The notion of reaction invariants (Dochain and Vanrolleghem, 2001) allows writing a part of  
205 process dynamics independently of the reaction kinetics. This property is very helpful when one or  
206 more variables are not accessible for measurement. Reaction invariants rely on the mass balance  
207 or part of the mass balance to determine the concentration of one or more of the solutes in the  
208 process. This method is applied in this part to determine hydrolysis parameters  $k_1^1$  and  $k_1^2$  and  
209 substrate concentrations  $S_1^1$  and  $S_1^2$ .

##### 210 2.4.2.1 *Hydrolysis parameters determination*

211 The yield constants  $k_1^1$  and  $k_1^2$  were first identified during the anaerobic digestion. For this, the  
212 periods during which only MeM was consumed allowed to determine the constant  $k_1^2$  while the  
213 periods for which both substrates were consumed allowed to determine  $k_1^1$ . Two assumptions  
214 were made to use the reaction invariant method:

- 215 • The methanogenesis step is the limiting kinetic step
- 216 • The methane produced in aqueous form is instantaneously transferred to the gas phase

217 These assumptions resulted in a  $k_2$  constant and  $\frac{dCH_{4,L}}{dt} = 0$ . The reaction invariant used for the  
 218 determination of  $k_1^1$  is  $Z_2$  defined in equation (11). Based on the assumptions presented above,  
 219 equations (12), (13), and (14) were obtained and the coefficient  $k_1^2$  was calculated by integration  
 220 from the experimental data.

$$Z_2 = k_1^2 S_1^2 + FOS + \frac{CH_{4,L}}{k_2} \quad (11)$$

$$\frac{dZ_2}{dt} = k_1^2 \frac{dS_1^2}{dt} + \frac{dFOS}{dt} = -r_2 = -\frac{Q_{CH_4}}{k_2} \quad (12)$$

$$k_1^2 \int_{S_1^{2,i}}^{S_1^{2,f}} dS_1^2 + \int_{FOS^i}^{FOS^f} dFOS = -\frac{1}{k_2} \int_{t^i}^{t^f} Q_{CH_4} dt \quad (13)$$

$$k_1^2 = -\frac{(CH_{4,g}^f - CH_{4,g}^i) + k_2(FOS^f - FOS^i)}{k_2(S_1^{2,f} - S_1^{2,i})} \quad (14)$$

221 Knowing the value of  $k_1^2$ , the same method was applied over the periods during which both  
 222 substrates were consumed to determine  $k_1^1$ . The considered reaction invariant  $Z_1$  is presented in  
 223 equation (15). Under the assumptions made earlier, equations (16), (17), and (18) were obtained.  
 224  $k_1^1$  was then determined by integration from the experimental data. Results are presented in **Table**  
 225 **3**.

$$Z_1 = k_1^1 S_1^1 + k_1^2 S_1^2 + FOS + \frac{CH_4}{k_2} \quad (15)$$

$$\frac{dZ_1}{dt} = k_1^1 \frac{dS_1^1}{dt} + k_1^2 \frac{dS_1^2}{dt} + \frac{dFOS}{dt} = -\frac{Q_{CH_4}}{k_2} \quad (16)$$

$$k_1^1 \int_{S_1^{1,i}}^{S_1^{1,f}} dS_1^1 + k_1^2 \int_{S_1^{2,i}}^{S_1^{2,f}} dS_1^2 + \int_{FOS^i}^{FOS^f} dFOS = -\frac{1}{k_2} \int_{t^i}^{t^f} Q_{CH_4} dt \quad (17)$$

$$k_1^1 = -\frac{(CH_{4,g}^f - CH_{4,g}^i) + k_1^2(S_1^{2,f} - S_1^{2,i}) + k_2(FOS^f - FOS^i)}{k_2(S_1^{1,f} - S_1^{1,i})} \quad (18)$$

#### 226 2.4.2.2 Unmonitored state variables tracking with asymptotic observers

227 The method of reaction invariants was also used to determine the evolution of unmeasured state  
 228 variables. For this, the property of reaction invariants was used to estimate their value and to  
 229 deduce the state variable values as a function of time. Thus, the reaction invariant  $Z_2$  was  
 230 estimated to determine the evolution of  $S_1^2$  during periods when only MeM was consumed and the  
 231 reaction invariant  $Z_1$  was estimated to determine  $S_1^1$  during periods when both substrates were  
 232 consumed. The expressions presented in equations (19) and (20) allowed the estimation of  $Z_1$  and  
 233  $Z_2$  considering the concentration of methane gas. The expression of these two reaction invariants  
 234 allowed us to deduce the curve shapes of  $S_1^2$  and  $S_1^1$  using equations (21) and (22).

$$\widehat{Z}_2 = k_1^2 S_1^{2,t} + FOS^t + \frac{CH_{4,g}^t}{k_2} \quad (19)$$

$$\widehat{Z}_1 = k_1^1 S_1^{1,0} + k_1^2 S_1^{2,0} + FOS^0 + \frac{CH_{4,g}^0}{k_2} \quad (20)$$

$$\widehat{S}_1^2 = \frac{1}{k_1^2} \left( \widehat{Z}_2 - FOS - \frac{CH_{4,g}}{k_2} \right) \quad (21)$$

$$\widehat{S}_1^1 = \frac{1}{k_1^1} \left( \widehat{Z}_1 - FOS - \frac{CH_{4,g}}{k_2} - k_1^2 S_1^2 \right) \quad (22)$$

### 235 2.4.2.3 DHA kinetic parameters determination

236 The kinetic parameters of the DHA step for RJ and MeM were determined differently. Indeed, the  
237 experimental  $S_0$  concentration was known at the beginning and the end of the experimental data  
238 set and allowed us to integrate the DHA first-order kinetics to directly determine  $\mu_1^1$  and  $\mu_1^2$   
239 according to equations (23) and (24). Results are presented in **Table 3**.

$$\begin{cases} \frac{dS_1^1}{dt} = -r_1^1 = -\mu_1^1 S_1^1 X_1 \\ \frac{dS_1^2}{dt} = -r_1^2 = -\mu_1^2 S_1^2 X_1 \end{cases} \quad (23)$$

240

$$\begin{cases} \mu_1^1 = \frac{\ln\left(\frac{S_1^1(t=t_i)}{S_1^1(t=t_f)}\right)}{\int_{t_i}^{t_f} X_1 dt} \\ \mu_1^2 = \frac{\ln\left(\frac{S_1^2(t=t_i)}{S_1^2(t=t_f)}\right)}{\int_{t_i}^{t_f} X_1 dt} \end{cases} \quad (24)$$

### 241 2.4.2.4 Haldane kinetic parameters determination

242 In practice, it is difficult to obtain the kinetic parameters of a Haldane kinetic model. Indeed, even  
243 if the parameters are structurally identifiable like a Monod model (Aborhey and Williamson, 1978),  
244 the presence of uncertainty and noise as well as the number of experimental data, particularly over  
245 inhibition makes these parameters often practically unidentifiable (Dochain and Vanrolleghem,  
246 2001). Thus, the set of parameters determined by an optimization method may not be unique. The  
247 method developed here from the literature aims at maximizing the accuracy of the obtained data.  
248 The estimation of the Haldane kinetic parameters was performed by minimizing an objective  
249 function considering the three measured state variables: the cumulative methane production, the  
250 methane flow rate, and the VFA concentration. This function has also considered arbitrarily chosen

251 weights to possibly balance the weight of one state variable over the others in the identification  
 252 process. The objective function is defined in equation (25) as a function of the parameter set to be  
 253 determined  $p = [\mu_2^{max}; K_{S2}; K_I]$ .

$$\begin{aligned}
 J(p) = \sum_{n=1}^N & \left[ \sigma_1 (CH_{4,i}^{sim}(\hat{p}) - CH_{4,i}^{exp})^T (CH_{4,i}^{sim}(\hat{p}) - CH_{4,i}^{exp}) \right. \\
 & + \sigma_2 (Q_{CH_4,i}^{sim}(\hat{p}) - Q_{CH_4,i}^{exp})^T (Q_{CH_4,i}^{sim}(\hat{p}) - Q_{CH_4,i}^{exp}) \\
 & \left. + \sigma_3 (FOS_i^{sim}(\hat{p}) - FOS_i^{exp})^T (FOS_i^{sim}(\hat{p}) - FOS_i^{exp}) \right] \quad (25)
 \end{aligned}$$

254 where J is the objective function. N represents the number of experimental points, and  $\sigma_1, \sigma_2$  and  
 255  $\sigma_3$  the weights assigned to each state variable, equal to 1, 0.5, and 2, respectively. An interior point  
 256 optimization method was used to perform the nonlinear optimization of the objective function  
 257 under constraints. The problem was solved with the SciIPOpt toolkit on Scilab 6.0 (ESI Group). The  
 258 relative convergence tolerance was chosen equal to  $1 \times 10^{-3}$ . The constraints and initialization for  
 259 each kinetic parameter were found in the literature and arbitrarily chosen (Zaher et al., 2009;  
 260 Müller et al., 2002). These data are illustrated in **Table 3**.

#### 261 2.4.3 Sensitivity analysis and Conditioning of the objective function

262 The vector of the state variables studied for the calibration of Haldane kinetics depended on time,  
 263 other state variables, and model parameters as shown in equation (26). The sensitivity of each  
 264 parameter is defined by equation (27) and the model sensitivity matrix is determined by equation  
 265 (28).

$$X = (CH_4, Q_{CH_4}, FOS), \quad p = (\mu_2^{max}, K_{S2}, K_I) \quad (26)$$

$$S(t) = \frac{\partial x}{\partial p} \quad (27)$$

$$\frac{\partial S(t)}{\partial t} = \frac{\partial f(t, x, p)}{\partial x} S + \frac{\partial f(t, x, p)}{\partial p}, \quad \frac{\partial x}{\partial t} = f(t, x, p) \quad (28)$$

266 The determination of the sensitivity matrix allowed us to determine the sensitivity of the output  
267 variables for each input parameter of the calibration step. The calculations were performed using  
268 the complex-step derivation approximation method (Martin et al., 2003). Moreover, the  
269 approximation of the objective function allowed us to draw the curves of the function according to  
270 the values of the parameters to be determined and according to the domains of existence defined  
271 for these parameters. The objective function was plotted as a function of each pair of parameters  
272 to be determined in order to determine the quality of the conditioning of the objective function  
273 (Munack, 1989).

### 274 **3 Results and discussion**

#### 275 *3.1 Batch reactors performance*

276 Mass balances were determined at the end of each experiment, with a minimal value of 96.1%  
277 attesting to the absence of local batch failures. The VS removal during these experiments was  
278 determined with an average value of  $57.7 \pm 0.1\%$ . During preliminary experiments, when  
279 substrates were not immersed in the liquid phase, a VS removal of 64% was observed with the  
280 same experiment duration. This observation could be explained by a strong VFA production at the  
281 beginning of experiments which could cause a temporary inhibition impacting the methane yield  
282 (Wollak *et al.*, 2018). Accumulated methane yield and VFA concentration are represented in **Fig. 2**  
283 for each reactor. Two methane flow production peaks could be observed at the beginning and the  
284 end of the experiment. This behavior is typical of solid-state anaerobic digestion (André *et al.*,  
285 2015; Degueurce *et al.*, 2016; Riggio *et al.*, 2017) and the main challenge for solid-state anaerobic  
286 digestion is to consider this behavior in a mathematical model (Coutu *et al.*, 2022). The experiment  
287 was stopped after 42 days to remain realistic about the real operating time of batch reactors and



288 to respect the usual industrial constraints. The accumulated methane yield reached was  
289 respectively 99.5% and 88.6% of the BMP measurement at the end of experiments for the reactors  
290 (R1) and (R2) attesting to great experimental conditions.

### 291 *3.2 Identifiability of model parameters and unmonitored variables*

292 Equations (14) and (18) were used on the results of the experiment (R2) to determine the values  
293 and standard deviations of the yield coefficients  $k_1^1$  and  $k_1^2$ . Equation (14) was used to determine  
294 the  $k_1^2$  coefficient in the stage where only MeM was consumed. This stage is identified in the  
295 experiment (R2) as stage 1 before the first injection of RJ. The coefficient  $k_1^1$  was then identified  
296 over stage 2, representing the first RJ injection, using equation (18). All the results obtained are  
297 presented in **Table 3**. No outliers were observed during this step, with a value of  $k_1^1$  obtained of  
298 0.977 and a value of  $k_1^2$  of 0.987.

299 In order to validate these values, the parameters  $k_1^1$  and  $k_1^2$  were determined with the same  
300 method using the results of the experiment (R1). The hypothesis was made that the experiment  
301 (R1) was divided into 2 stages: a first stage with degradation of both substrates and rapid  
302 degradation of the RJ, and a second stage where only the MeM was consumed. Equation (14) was  
303 used in the second stage of the experiment (R1) and equation (18) in the first stage. The values  
304 obtained were  $k_1^1=0.976$  and  $k_1^2=0.987$ , which validated the calibration performed previously.

305 The values of parameters  $\mu_1^1$  and  $\mu_1^2$  were also obtained using equations (24) from the data  
306 obtained from the experiment (R2).  $\mu_1^2$  was first determined in stage 1 and then  $\mu_1^1$  was  
307 determined between each addition of RJ in stages 2 to 6. The values obtained for the calibration of  
308 these two parameters were  $\mu_1^1=3.15 \cdot 10^{-2} \text{ h}^{-1}$  and  $\mu_1^2=1.42 \cdot 10^{-4} \text{ h}^{-1}$ . To validate these results,  $\mu_1^1$  and  
309  $\mu_1^2$  were also determined in the experiment (R1). The second identified stage of (R1) was used to  
310 determine  $\mu_1^1$  and then the first identified stage of (R1) was used to determine  $\mu_1^2$ . The values

311 obtained were  $\mu_1^1=1.68.10^{-2} \text{ h}^{-1}$  and  $\mu_1^2=1.44.10^{-4} \text{ h}^{-1}$ . These values were of the same order of  
312 magnitude as the values obtained during the calibration, which validated the calibration of the  
313 parameters  $\mu_1^1$  and  $\mu_1^2$  from the experiment (R2).

314 In order to obtain the unmonitored variables in the experiment (R1), the reaction invariant notion  
315 was also used (Dochain et al., 1992). The monitoring of these state variables allowed validation of  
316 state variables simulated from the calibration data on the experiment (R1). Equations (21) and (22)  
317 were used and the results obtained are presented in **Fig 3**.

### 318 *3.3 Calibration of Haldane kinetic parameters*

319 The calibration step of Haldane kinetic parameters aimed to obtain the best fitting with the  
320 calibration data set of the experiment (R2). Two different data sets were used to calibrate and  
321 validate this set of parameters through 3 state variables: the cumulated methane production, the  
322 VFA concentration, and the methane flow rate observed respectively in experiments (R2) and (R1).  
323 The calibration step was carried out by trial and error to obtain the best dataset possible. First of  
324 all, a conditioning study of the objective function was done to determine if the objective function  
325 was well-conditioned. Then a minimization procedure of the objective function was done using an  
326 interior point optimization method.

#### 327 *3.3.1 Conditioning of the objective function*

328 The study of the conditioning of the objective function allowed us to determine if the Haldane  
329 kinetic parameters were identifiable. To perform this study, the value of the objective function  
330 presented in equation (25) was determined by discretization by varying each parameter over the  
331 calibration interval considered. The calibration interval is presented in **Table 3**. A discretization  
332 step was arbitrarily chosen to divide the calibration interval into 20 equal parts for each parameter,  
333 which represented 8000 estimations of the objective function. Once the values of the objective

334 function were obtained as a function of each set of parameters, the objective function was plotted  
335 as a function of the parameters associated in pairs. The result of this conditioning is presented in  
336 **Fig 4**. In these figures, it is evident that the objective function was ill-conditioned because the  
337 objective function as a function of each pair of parameters was represented by a very flattened  
338 ellipse (Dochain and Vanrolleghem, 2001). This first observation resulted in probably poor practical  
339 identifiability, which corroborated the assumptions made earlier. A sensitivity analysis was done  
340 following this conditioning study to determine which parameters were practically identifiable  
341 (Robinson et al., 1985).

### 342 3.3.2 *Sensitivity analysis*

343 In many biological models, kinetic parameters are highly correlated, which can result in "valley"  
344 behaviors in which several combinations of parameters can describe the same data similarly. It is  
345 therefore interesting to plot sensitivity functions to determine the practical identifiability of the  
346 studied model. The sensitivity analysis was here conducted by considering the impact of the three  
347 parameters to be determined on the three measured state variables present in the Haldane  
348 kinetics. The same curve shapes could be observed for the different parameters considered. This  
349 behavior could be observed for each state variable, which meant that the kinetic parameters were  
350 not identifiable and therefore there was not a unique solution for the set of parameters to be  
351 determined. Moreover, the sensitivity of each state variable to the  $K_i$  parameter was much lower  
352 than other parameters, with an order of magnitude of  $10^{-8}$  against  $10^{-4}$ . However, the presence of  
353 RJ injections allowed increasing the sensitivity of the different state variables to the parameters to  
354 be determined with a 10 factor. The consequence was an improvement in the identifiability of  
355 parameters during the calibration step. This phenomenon is consistent with the observations made  
356 in the literature (Vanrolleghem et al., 1995) and allowed to confirm the use of the data set from  
357 the experiment (R2) for the calibration step. Results of the sensitivity analysis are illustrated in **Fig**

358 5 for the cumulated methane production sensitivity to the  $K_{S2}$  parameter. Following this  
359 observation, the objective of the calibration step was to obtain the optimal set of parameters in  
360 order to fit the model with the experimental observations of (R2).

### 361 3.3.3 Calibration results

362 The calibration of Haldane kinetic parameters was done to obtain the best fitting with  
363 experimental results from the experiment (R2). This step was carried out by trial and error to find  
364 the best data set with optimal parameters. Calibration results are illustrated in **Fig 6**. The  
365 simulation results represented by continuous lines were close to the experimental data which were  
366 represented by dots. The Haldane kinetics obtained are presented in **Table 3**. These parameters  
367 values were very different from other studies in the literature. While  $\mu_2^{max}$  was in line with the  
368 literature (Zaher et al., 2009; Müller et al., 2002), the  $K_{S2}$  calibration value was a little high and  $K_I$   
369 was very low in comparison with values obtained from the literature. This difference in behavior  
370 could be explained by slower anaerobic digestion and the presence of inhibition phenomena  
371 specific to MeM and RJ.

### 372 3.4 Model validation

373 Validation steps were previously conducted on each parameter determination and a global  
374 validation was done considering experiment (R1). Results are illustrated in **Fig 6**. The simulation  
375 done correctly reproduced the global behavior of each solute for a complete period of 45 days. The  
376 main quality of this simplified model is the consideration of a low number of parameters, which  
377 allowed a faster and easier calibration step. However, the calibration step was very sensitive to the  
378 initialization step and parameter bounds, which could be validated by sensitivity analysis. Although  
379 the simulated curve representing VFA concentration was representative of the experimental data,  
380 the simulated methane yield showed a deviation from the experimental values. This deviation is

381 due to the presence of 2 peaks of methane production characteristic of the SS-AD. The model  
382 developed in this paper allows the analysis of the behavior and evolution of the biomass and the  
383 different chemical species present. The two peaks of methane production were modeled, which  
384 is impossible with the usual models of the literature. This model is a first step to characterize  
385 complex co-substrates as MeM and RJ with a simple model using few parameters but this model  
386 could potentially evolve into a spatially distributed model introducing new parameters and using  
387 new experiments to fit perfectly with the methane production curve.

### 388 *3.5 Discussion about possible inhibitions*

389 The specific behavior of MeM and RJ digestion could be due to high VFA concentrations (until 19  
390  $\text{g.L}^{-1}$  during our preliminary experiments) (Khartikheyan and Visvanathan, 2013) or ammonia  
391 concentrations (between 4  $\text{g.L}^{-1}$  and 5  $\text{g.L}^{-1}$  during our experiments). These values could be a source  
392 of inhibition (Amani et al., 2010) but the acclimatization of the inoculum was carried out upstream,  
393 which makes it possible not to impact the anaerobic digestion (Chen et al., 2008; Yenigun and  
394 Demirel, 2013). Another possibility is an inhibition of sodium chloride (Feijoo et al., 1995) but just  
395 like ammonia, the acclimatization of the inoculum was carried out upstream and there is no  
396 possible impact on the methane yield (Kimata-Kino et al., 2011). The results obtained by this study  
397 also showed that the released fluid has its importance in the SS-AD phenomenon and should not  
398 be lost during MeM grinding. The potential presence of such inhibitions could modify the methane  
399 yield and VFA accumulation curve shapes. Not all of these modifications were considered in the  
400 mathematical model of this study and could potentially falsify the calibration results. Indeed, the  
401 Haldane kinetics for Methanogenesis step used in this study considered inhibitions on  
402 methanogenic biomass but inhibitions on other biomass were neglected and could potentially vary  
403 the calibration results. In this study, the methane yields observed were consistent with the  
404 literature (Wollak et al., 2018; Akizuki et al., 2016; Nkemka and Murto, 2013), attesting robust

405 experimental results to modeling the phenomenon of MeM and RJ SS-AD. However, further study  
406 will be needed to improve the fit between the model and the experimental curves by better  
407 characterizing the inhibitions of this process.

#### 408 **4 Conclusions**

409 A simplified AM2 model was developed to characterize *Mytilus edulis* SS-AD. This model was  
410 verified, implemented, and validated in 60L batch reactors in mesophilic conditions. A better  
411 sensitivity with delayed substrate injections throughout the experiment with a factor of 10. These  
412 results gave a correct approximation of solutes behavior with an accumulated methane yield  
413 representing 88.6% of the BMP measurement, and a volatile removal of 57.7% attesting to great  
414 experimental conditions. and could identify the two methane production peaks characteristics of  
415 SS-AD but the results did not allow for prediction with enough accuracy to implement control tools  
416 to optimize methane production. Further work is needed with new considerations to better  
417 understand the phenomenon of *Mytilus edulis* solid-state anaerobic digestion. A further study  
418 could be done to evolve this model into a spatially distributed model with more parameters in  
419 order to fit perfectly with the methane production curve.

#### 420 **Acknowledgments**

421 The authors gratefully thank Cultimer France, the ANRT, the region of Bretagne, the GALPA, and  
422 the FEAMP for the support provided for this work and the Ph.D. grant of Maël Mercier-Huat. The  
423 authors are very grateful to Rejanne Le Bivic for her carefully reading and English revision of the  
424 manuscript.

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593 **Figure captions**

594 **Fig 1.** Schematic representation of the experimentation set up

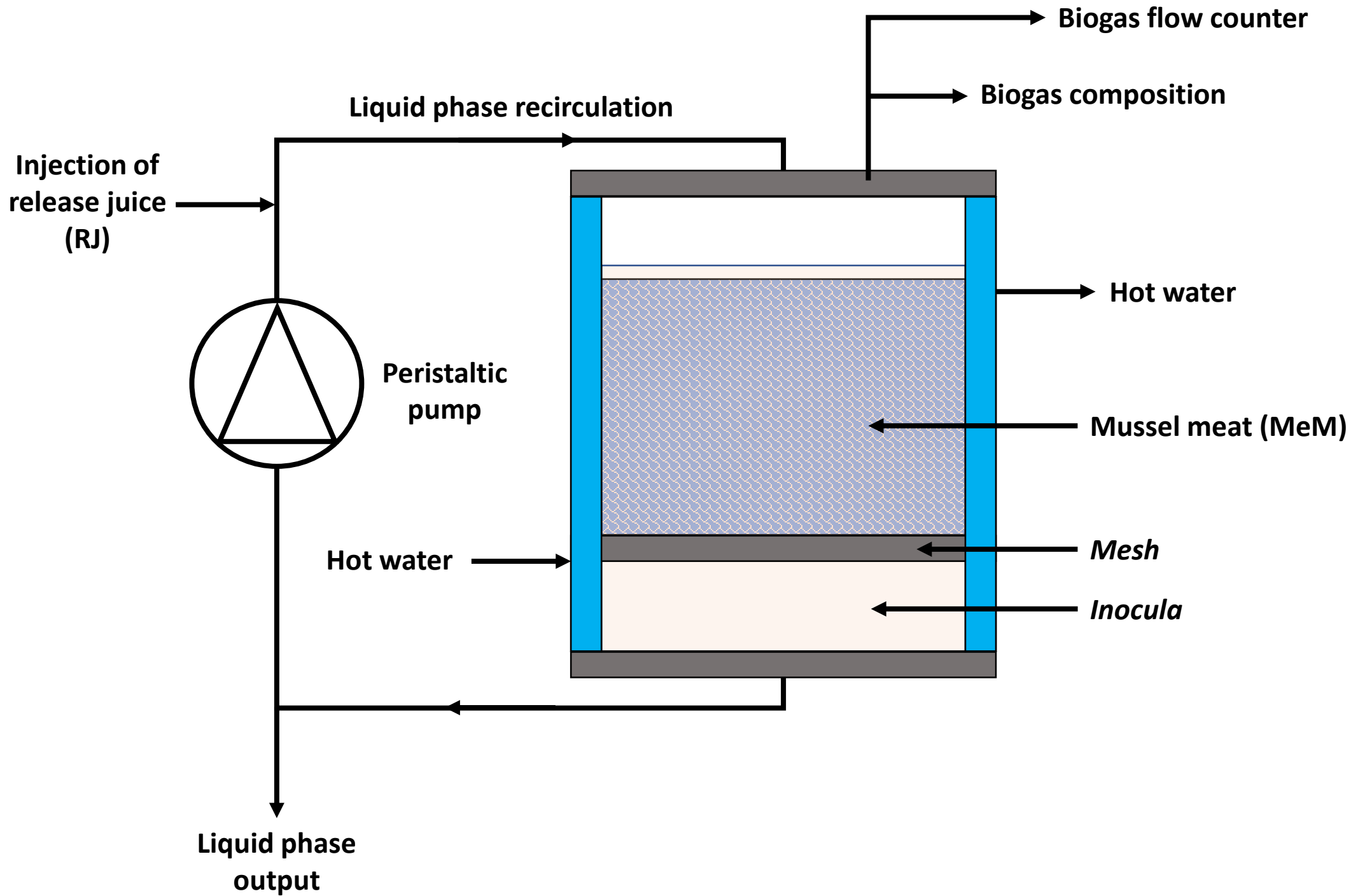
595 **Fig 2.** Solid-state anaerobic digestion performance on experiment (R1) with A : cumulated methane  
596 production, B: VFA concentration and C: methane flow rate and experiment (R2) with D :  
597 cumulated methane production, E: VFA concentration and F: methane flow rate and experiment.  
598 Different considered stages are represented on each experiment in blue and red zones

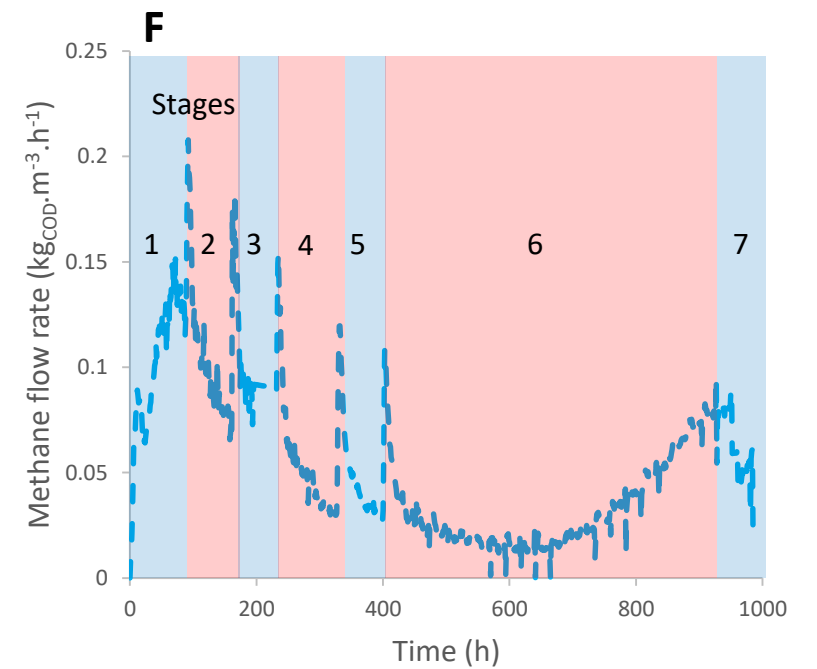
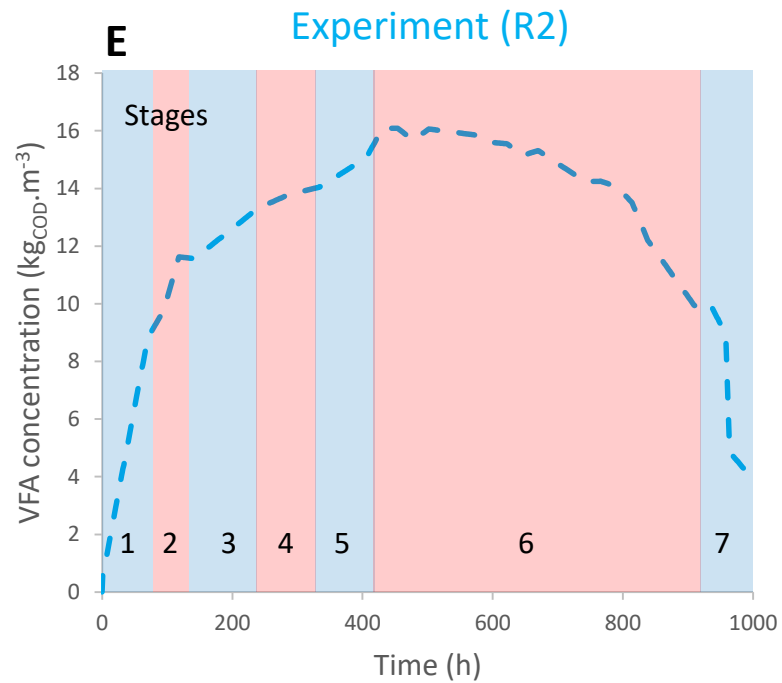
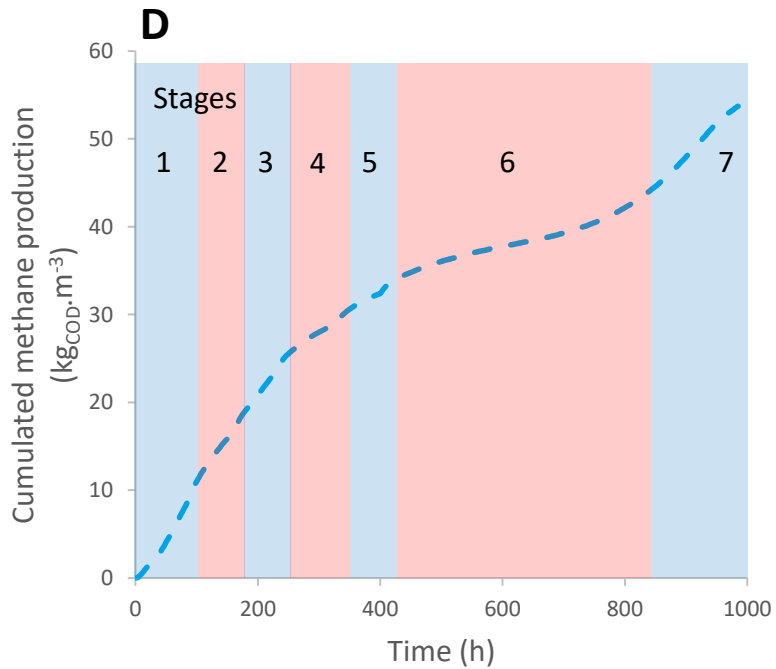
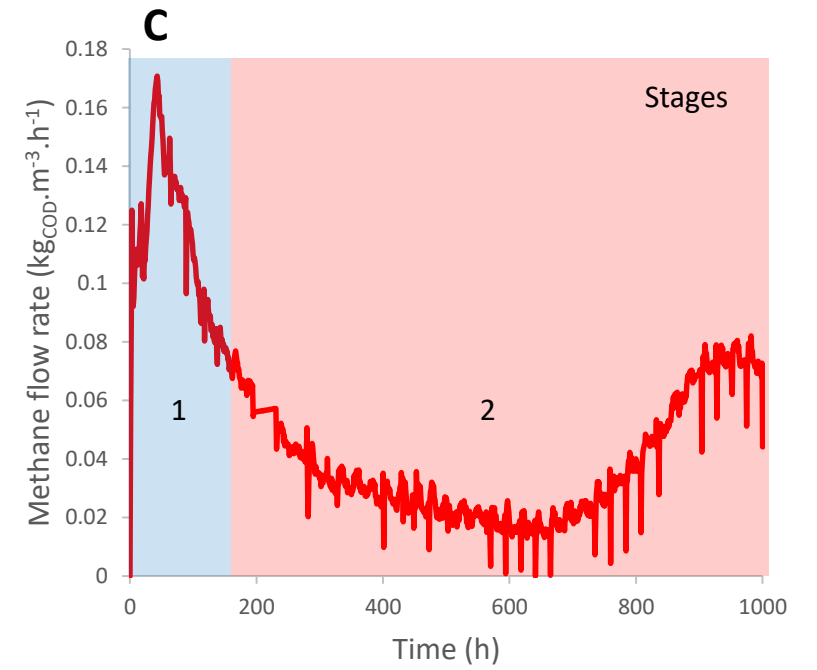
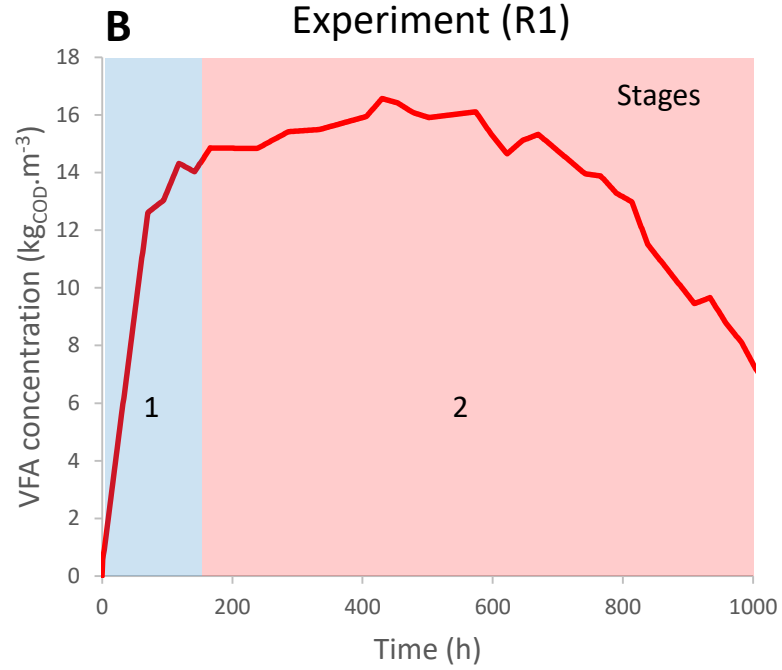
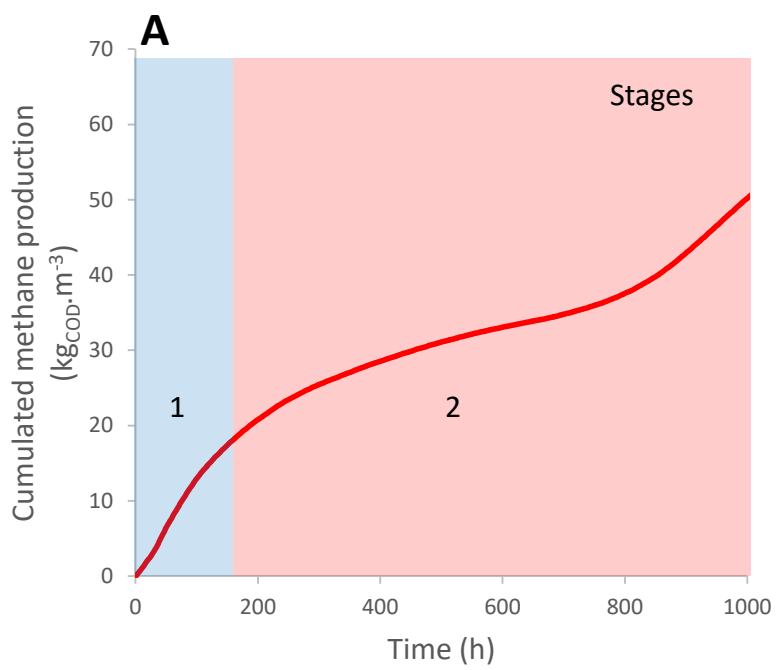
599 **Fig 3.** Unmonitored substrate concentrations of MeM and RJ all along SS-AD

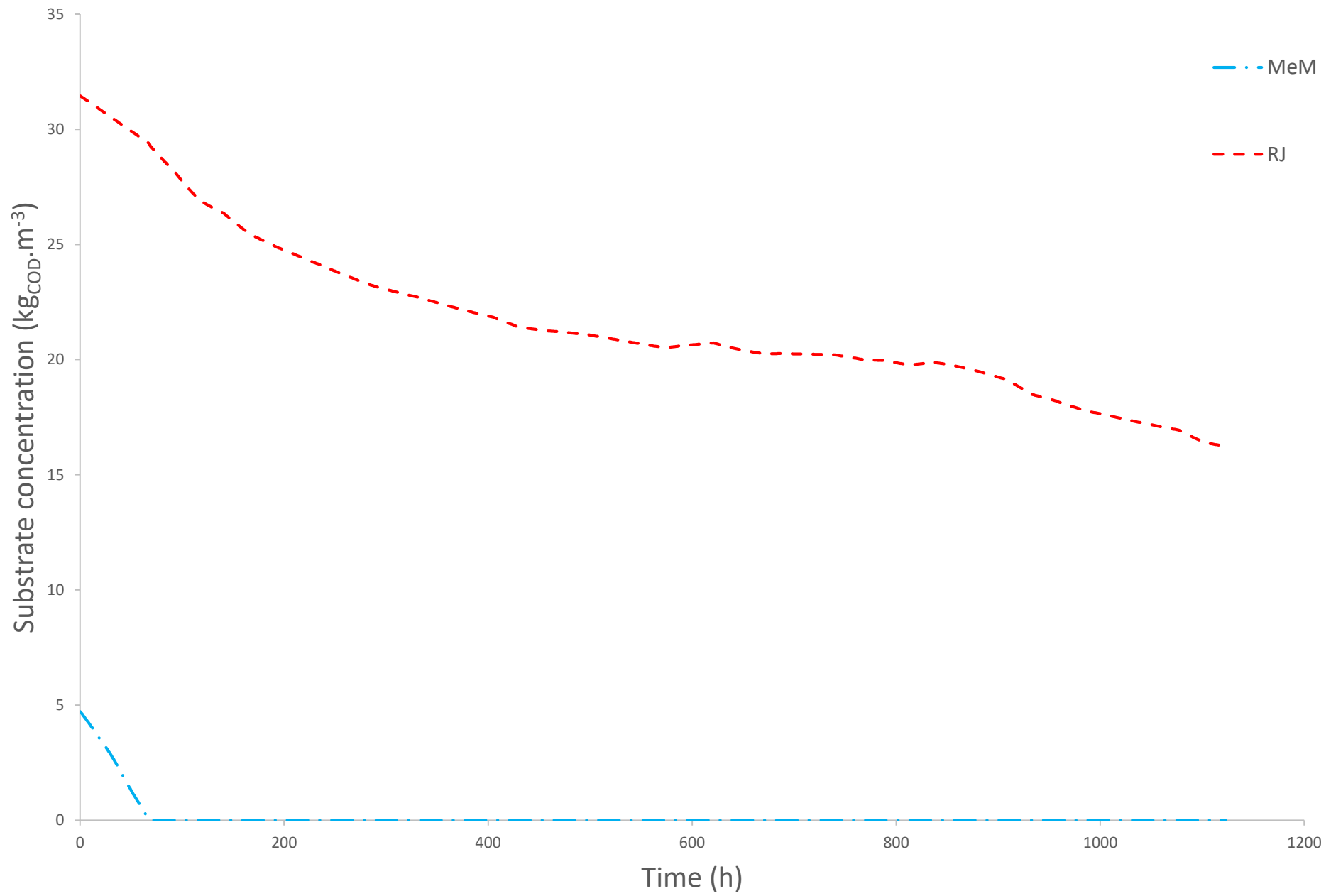
600 **Fig 4.** Conditioning study of the objective function for each pair of parameters with A :  $\mu_2^{max}$  and  
601  $K_{S2}$ , B :  $K_I$  and  $K_{S2}$  and C :  $\mu_2^{max}$  and  $K_I$

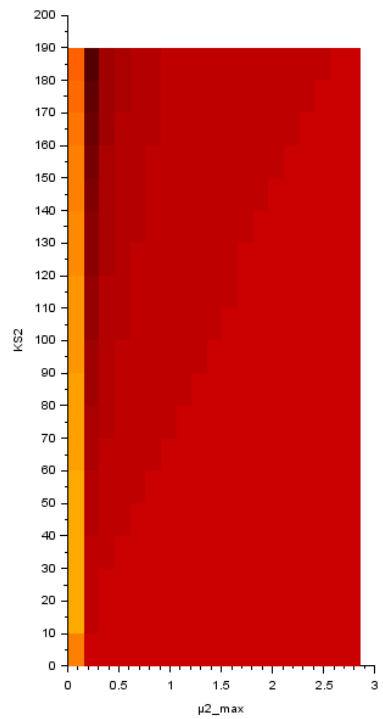
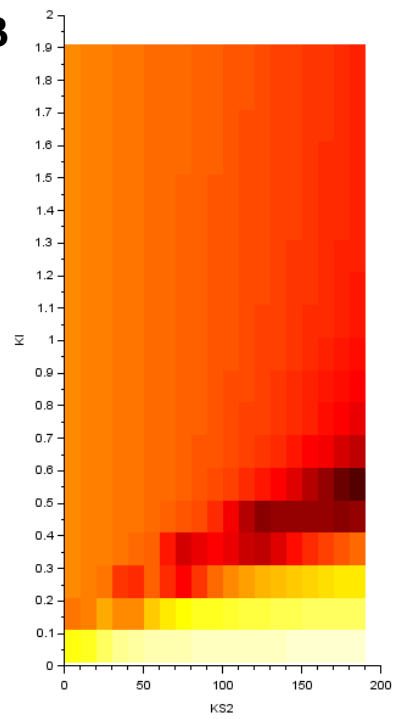
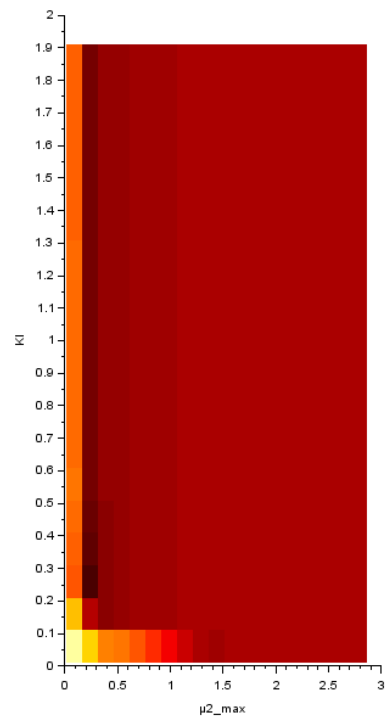
602 **Fig 5.** Results of sensitivity analysis concerning the cumulated methane production sensitivity to  $K_{S2}$   
603 parameter

604 **Fig 6.** A: Calibration step on A: cumulated methane production and VFA concentration, B: methane  
605 flow rate and Validation step on C: cumulated methane production and VFA concentration and D:  
606 methane flow rate; dots for average experimental values, lines for simulated values

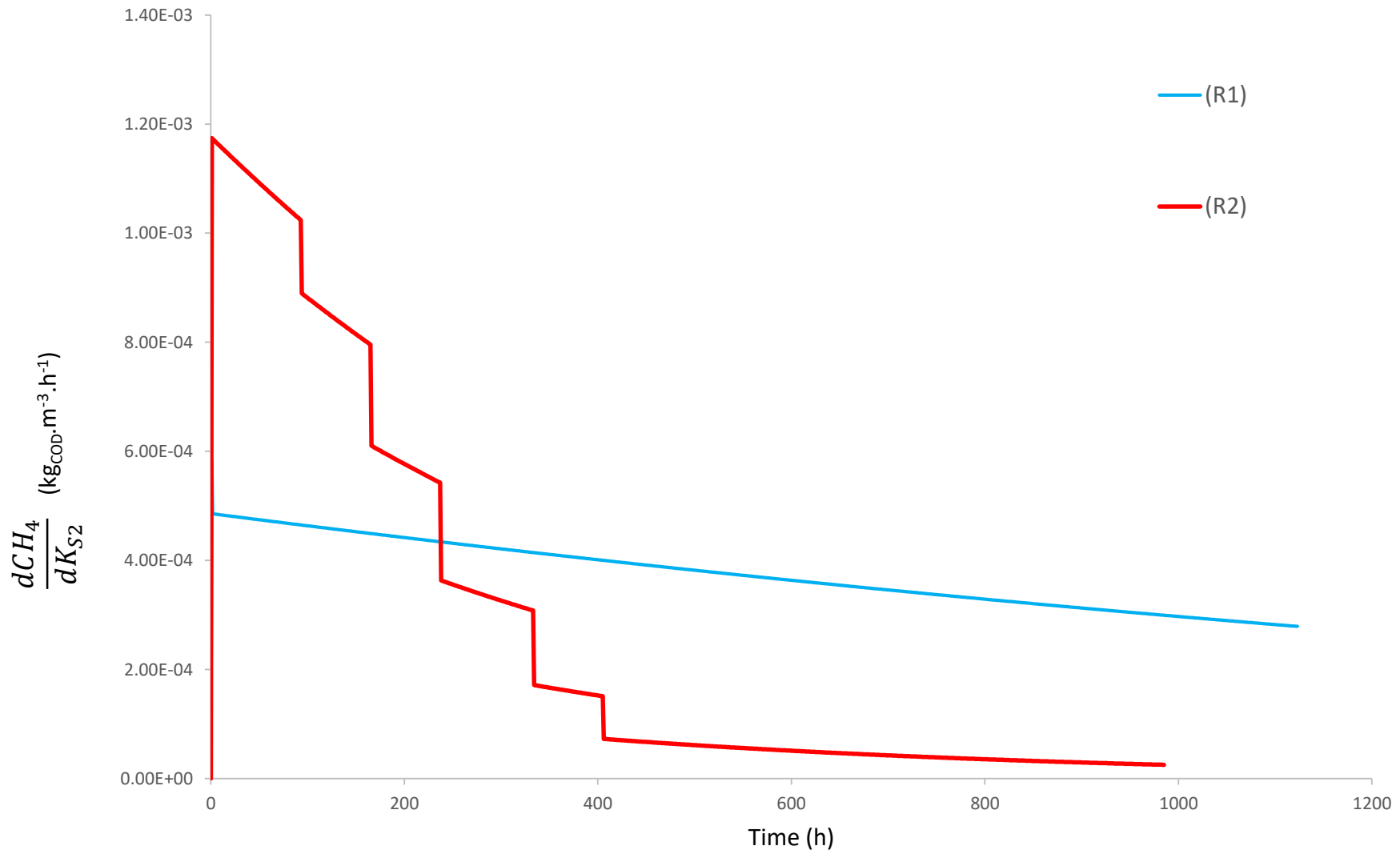


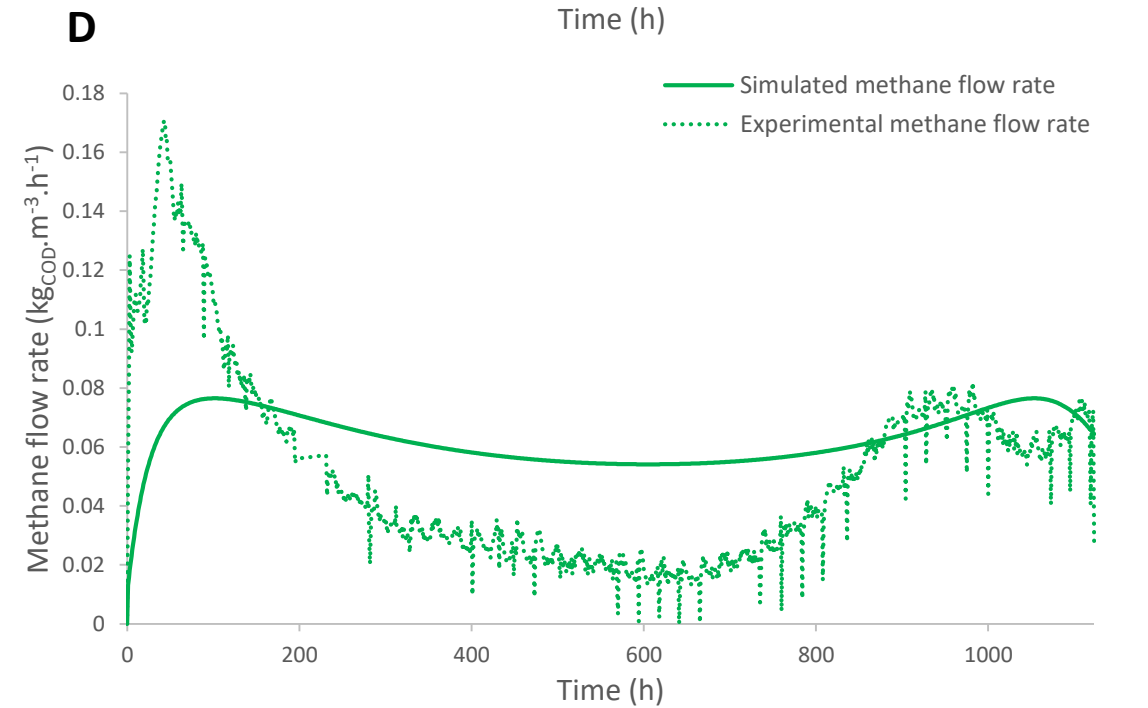
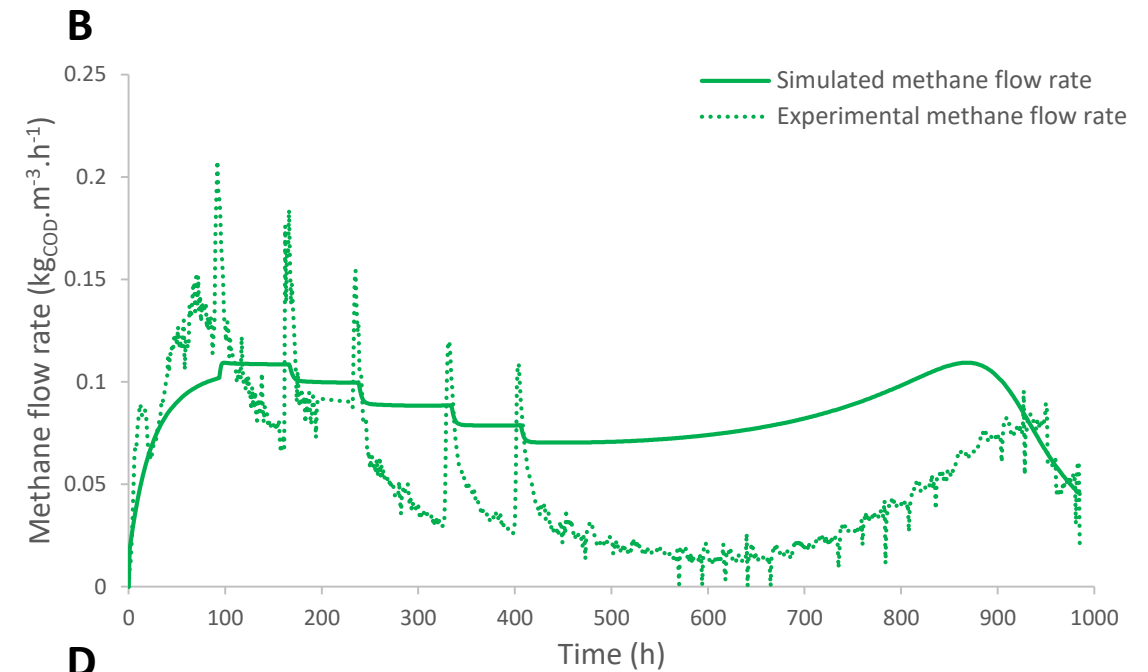
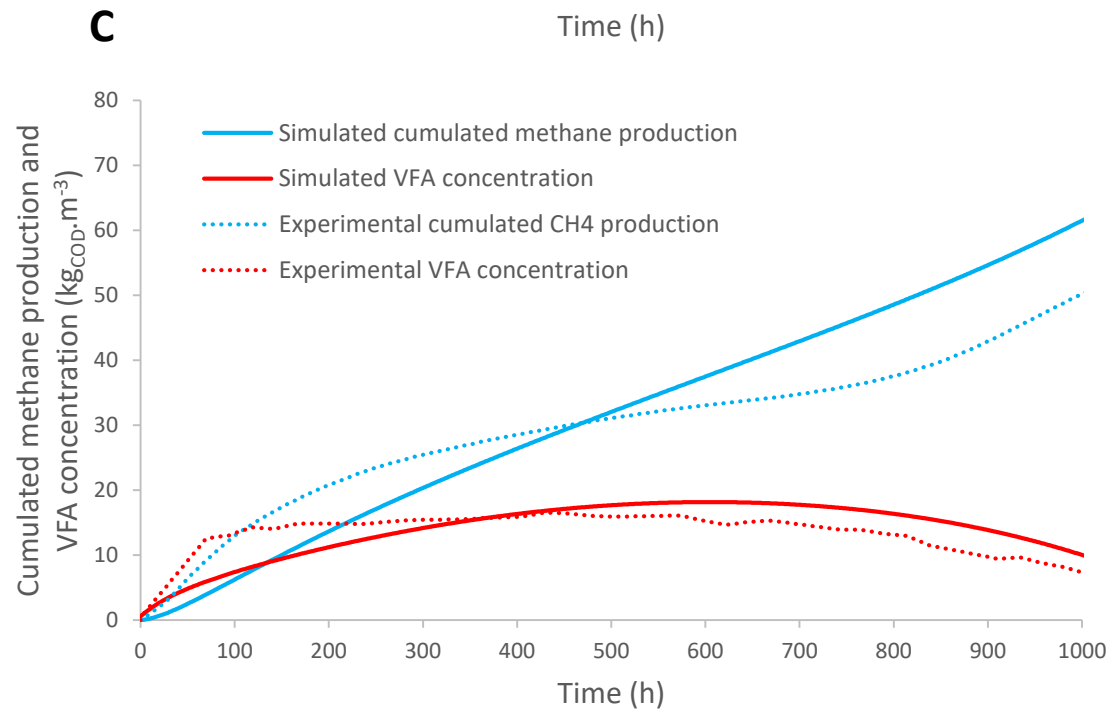
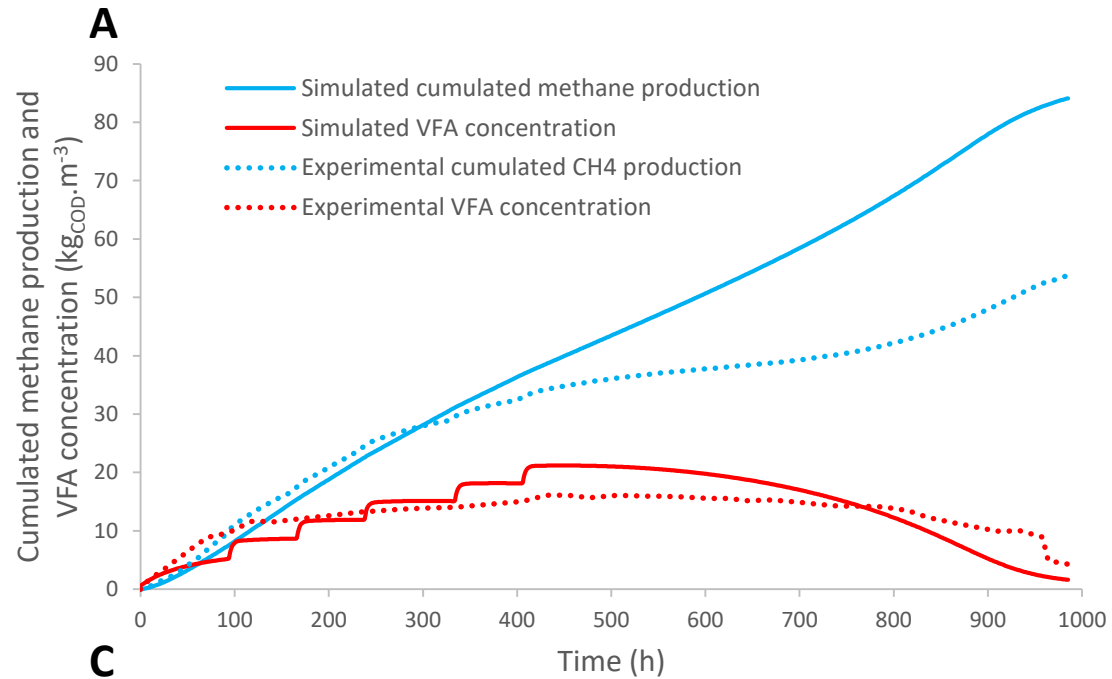




**A****B****C**







**Table 1** Chemical characteristics of inoculum and substrates used

	Unit	Run 1			Run 2		
		Initial MeM	Initial RJ	Initial LM	Initial MeM	Initial RJ	Initial LM
TS	%	67.2 ± 0.1	8.2 ± 0.1	3.8 ± 0.1	68.8 ± 1.6	12.4 ± 0.3	2.6 ± 0.1
VS	% <sub>TS</sub>	14.5 ± 0.5	62.4 ± 0.1	62.9 ± 0.2	12.2 ± 1.5	75.5 ± 0.7	51.5 ± 0.3
pH	-	-	5.3 ± 0.1	8.2 ± 0.1	-	5.7 ± 0.1	8.1 ± 0.1
VFA	g <sub>HAc</sub> .L <sup>-1</sup>	-	8.5 ± 0.4	0.05 ± 0.05	-	5.0 ± 0.1	0.0 ± 0.1
TAC	g <sub>CaCO3</sub> .L <sup>-1</sup>	-	0.2 ± 0.2	3.9 ± 0.1	-	0.05 ± 0.05	6.7 ± 0.1
BMP	N <sub>CH4</sub> .kg <sub>VS</sub> <sup>-1</sup>	277 ± 11	306 ± 8	-	277 ± 11	306 ± 8	-

**Table 2** Peterson matrix of the model kinetics

Step	$S_1^1$	$S_1^2$	$S_2$	$X_1$	$CH_4$	Reaction rate
DHA - MeM	-1		$k_1^1$	$(1 - k_1^1)$		$r_1^1 = \mu_1^1 S_1^1 X_1$
DHA - RJ		-1	$k_1^2$	$(1 - k_1^2)$		$r_1^2 = \mu_1^2 S_1^2 X_1$
Methanogenesis			-1		$k_2$	$r_2 = \mu_2^{\max} \frac{S_2 X_2}{S_2 + K_{S_2} + \frac{S_2}{K_I}}$

**Table 3** Kinetic parameters initialization and values obtained from calibration step

Parameter	Initialization value	Calibration value	Interval	Unit
$k_1^1$	-	$0.976 \pm 0.001$	-	-
$k_1^2$	-	$0.987 \pm 0.001$	-	-
$\mu_1^1$	-	$3.150 \pm 1.068$	-	$10^{-2} \cdot h^{-1}$
$\mu_1^2$	-	$1.422 \pm 1.313$	-	$10^{-4} \cdot h^{-1}$
$\mu_2^{max}$	2.670	$12.46 \pm 1.204$	[0.1 – 30]	$10^{-2} \cdot h^{-1}$
$K_{S2}$	3.450	$145.3 \pm 12.67$	[0.1 – 200]	$kg_{COD} \cdot L^{-1}$
$K_I$	1.440	$0.401 \pm 0.061$	[0.1 – 50]	$kg_{COD} \cdot L^{-1}$

