



Co-digestion and model simulations of source separated municipal organic waste with cattle manure under batch and continuously stirred tank reactors

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1 **Co-digestion and model simulations of source**
2 **separated municipal organic waste with cattle**
3 **manure under batch and continuously stirred tank**
4 **reactors**

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15 **Abstract**

16 This study investigates the co-digestion of source separated municipal organic waste
17 (SSMOW), pretreated using a biopulper, and cattle manure both in batch and
18 continuous stirred tank reactors. The optimum co-digestion feeding mixture was
19 consisted of 90% SSMOW and 10% cattle manure on organic matter basis, yielding 443
20 mLCH₄/gVS. The high performance of the co-digestion was explained by the fact that
21 the efficient pulping pretreatment boosted the methane production from SSMOW and
22 that the added livestock slurry provided the buffer capacity to avoid inhibition occurred
23 by intermediates' accumulation. Moreover, batch assays focused on the effect of
24 inoculum to substrate ratio (ISR) were performed. Results showed that the reduction of
25 ISR had slight impact on extending the lag phase, without affecting the rest kinetic
26 parameters. The efficiency of the co-digestion process in continuously fed reactor was
27 comparable with the results obtained from the batch assay (i.e. <95% of the maximum
28 expected value). Finally, the outputs from an applied mathematical model were in good
29 agreement with the experimental data obtained from the continuous reactor operation,
30 demonstrating that the BioModel can serve as a reliable tool to predict the process
31 performance under real-scale conditions.

32

33 **Keywords**

34 Source separated municipal organic waste; anaerobic digestion; methane; kinetics;
35 modeling

36

37 **1. Introduction**

38 Anaerobic digestion (AD) of source separated municipal organic waste (SSMOW) is

39 considered as a competitive to the traditional (e.g. composting, landfilling, incineration)
40 waste management solution as the organic matter is efficiently degraded producing
41 bioenergy and also, biofertilizer [1,2]. In terms of bioenergy production, SSMOW can
42 ensure high biogas yielding operation [3–5]. Specifically, the presence of soluble
43 carbohydrates, proteins and lipids derived from the kitchen waste residues [6] settles
44 SSMOW as a very interesting substrate for AD.

45 Despite the fact that SSMOW consists mainly of degradable components, non-
46 degradable fractions (e.g. plastics) can be also found, as impurities. Thus, a well-
47 performing separation step can increase process efficiency by initially discarding the
48 non-degradable materials and subsequently, a suitable pretreatment method can boost
49 the deconstruction of previously intact organic matter [7–9]. In industrial perspective, it
50 was previously shown that the integration of two rather dissociated processes into a
51 single and straightforward step is able to remarkably enhance the AD sustainability
52 [10].

53 In this framework, pulping technology similar to the process used in paper industry
54 can combine these two steps namely separation and pretreatment steps that are needed
55 prior to AD of SSMOW, into a single process. A biopulper can separate the degradable
56 organic matter and sort-out the non-degradable that can be subsequently recycled,
57 reused or recovered [11]. In addition, the installed milling machinery assists the
58 pretreatment of organic matter improving the biodegradability of SSMOW. In fact, a
59 previous study demonstrated that the pretreatment of SSMOW with pulping technology,
60 led to more than 390 mLCH₄/gVS under different reactor configurations (i.e. batch
61 assays, fed-batch and continuous stirred tank reactors (CSTR)) [5].

62 Notwithstanding the high bioenergy output, SSMOW is a very acidic waste, and on

63 top of this, the AD process is prone to be inhibited at increased organic loads [1]. Thus,
64 it is crucial to ensure high bioenergy output avoiding risks of acidification incidents and
65 indeed, co-digestion can serve as a potential solution to such inhibition problems. More
66 specifically, cattle slurry is able to increase the pH towards higher levels and hinder
67 reactor's acidification due rapid volatile fatty acids (VFA) accumulation [12]. In
68 addition, various hydrolytic and fermentative microbes which accelerate the
69 disintegration process are already present in the livestock manure. So, the dissimilar
70 biochemical characteristics of SSMOW and manure substrates can be combined to
71 create a proper feedstock mixture. Furthermore, the usage of livestock slurries into the
72 biogas sector is promoted by the policy-makers by the granted subsidies as mean to
73 solve the manure treatment problem through AD [13]. Thus, co-digestion strategies
74 using livestock manures are highly exploited.

75 However, the chemical composition of both substrates is not consistent but is
76 strongly dependent on different parameters, which will in turn affect the final methane
77 productivity. For instance, the major origin of SSMOW can influence positively (e.g.
78 food residues) or negatively (e.g. green waste) the final bioenergy output [14]. On the
79 other hand, nutritional feedstock composition, moisture content, animal species and
80 growth stage are among the parameters that markedly affect manure's biogas
81 productivity [15]. Hence, a universal feeding recipe for the biogas plants is not possible
82 and thus, the optimum feedstock composition should always be independently examined
83 within the framework of co-digestion applications.

84 Apart from the optimum co-digestion mixture, other kinetic parameters of the AD
85 process are equally important and should be evaluated. For example, the achievement of
86 a rapid and efficient disintegration of organic matter is assigned to the ratio between

87 added feedstock and active biomass [16]. Indeed, organic overload inhibits the
88 methanogenic community due to VFA accumulation and over-acidification [17]. Thus,
89 kinetic parameters such as lag phase, hydrolysis and methane rate are influenced by the
90 inoculum to substrate ratio (ISR) [18]. The imbalance between rapid hydrolysis-
91 acidification and slow methanogenesis causes organic overload to the archaeal species
92 which could not fully utilise the fed substrate [19]. Hence, it is crucial to secure an
93 efficient feeding strategy to avoid toxicity that can eliminate the methanogenic activity.

94 Furthermore, operational parameters (e.g. reactor's configuration) play an important
95 role towards co-digestion process optimisation. For instance, batch reactors can
96 efficiently provide information about the duration of lag phase, maximum biogas yield,
97 methane and hydrolysis rate. In contrast, CSTR are more appropriate to examine issues
98 as microbiome's acclimatization at long term operation. Experiments are laborious and
99 time consuming and therefore can only cover few experimental conditions. On the
100 contrary, the outcome of both lab-scale reactor set-ups after data interpretation can be
101 extremely useful as input for modeling simulations in order to expand testing at various
102 conditions, and thereby improve the understanding of the AD system. Specifically,
103 reliable mathematical models can reveal in advance the bottlenecks that limit the
104 methane production (e.g. lag phase, substrates inhibition etc.) and highlight the
105 operational conditions (e.g. hydraulic retention time, organic loading rate) that optimise
106 process efficiency [20]. Hence, through reliable simulation outputs, the application of
107 SSMOW for AD can be generalised in the direction of stable and high-yielding biogas
108 production.

109 The aim of the present work was to provide a comprehensive research on
110 exploitation of SSMOW as a major influent substrate for biogas digesters and to

111 generate a dataset based on continuous reactor operation monitoring that would be used
112 as input for mathematical modeling. Thus, mono- and co-digestion batch assays using
113 SSMOW, pretreated using a biopulper, and cattle manure as the co-substrate were
114 initially conducted. A subsequent batch set was performed to evaluate the kinetics of the
115 most promising feeding mixture and to identify potential problems related to process
116 inhibition at different ISR. Moreover, a continuously fed digester was set up to monitor
117 and evaluate further the effect of the co-digestion process. Finally, a mathematic model
118 (BioModel) was used to simulate the co-digestion process and validate the accuracy of
119 the experimental work.

120

121 **2. Materials and methods**

122 **2.1 Inoculum**

123 Thermophilic inoculum was provided by a well performing lab-scale reactor fed with
124 cattle manure. The digestate was sieved to remove the remaining organic matter and
125 stored in thermophilic incubator for 10 days to reduce the background biogas
126 production. The major physicochemical characteristics of the inoculum, after the
127 degassing process, were pH: 8.36, Total Solids (TS): 26.70 ± 0.20 g/L, Volatile Solids
128 (VS): 17.54 ± 0.22 g/L, Chemical Oxygen Demand (COD): 24.78 ± 1.19 g/L, Total
129 Kjeldahl Nitrogen (TKN): 2.32 ± 0.09 g-TKN/L, Ammonium Nitrogen: 2.06 ± 0.10 g-
130 NH_4^+ /L and total Volatile Fatty Acids (TVFA): 0.25 ± 0.05 g/L.

131

132 **2.2 Substrates**

133 SSMOW of approximately 25% (v/v) industrial and 75% (v/v) household waste were
134 collected from Gemidan Ecogi A/S after pulping process, as previously described [11].

135 In brief, municipal waste is inserted into a pulper equipped with a helical rotor. The
136 rotor agitates to disperse the bio-degradable organic matter without damaging the non-
137 degradable fraction. Subsequently, the two fractions are separated using a perforated
138 plate. Cattle manure was collected from Hashøj biogas plant. The substrates were
139 diluted with tap water to reach the same content of organic matter to prevent pumping,
140 mixing and clogging problems in the lab scale reactors. After dilution and mixing, the
141 substrates were stored in plastic bottles at -20°C until usage. The main chemical
142 characteristics of the prepared substrates are presented in Table 1.

143

144 **2.3 AD experiments**

145 Biochemical Methane Potential (BMP) assays were initially performed based on
146 Angelidaki et al. [21] in order to define the bioenergy production of the used substrates
147 under mono- and co-digestion trials (i.e. 80:20, 60:40, 40:60 and 20:80 on VS basis).
148 Triplicate glass reactors were used, with total and working volume of 547 and 200 mL,
149 respectively. The inoculum represented 80% of the working volume and the organic
150 load was 2 gVS/L. Prior to incubation, the batch reactors were flushed with pure N₂ to
151 replace the remaining oxygen and achieve anaerobic conditions. Subsequently, they
152 were placed in a thermophilic incubator (54 ± 1 °C). Based on the results from the first
153 BMP test, the optimum mixing ratio of substrates in the feedstock was determined.
154 Then, a second BMP test was set up to examine the effect of ISR on the AD of the used
155 substrates. Specifically, batch co-digestion experiments were established at three
156 different ISR on VS basis (i.e. 0.5, 1.5 and 3.0) keeping the amount of inoculum
157 constant in all batches [22]. Samples for VFA determination and methane content were
158 taken during the incubation period. For both BMP tests, daily manual stirring was

159 conducted to avoid the creation of dead zones and monitoring of methane production
160 was performed twice a week until cease of methane production was observed ($p < 0.05$).

161 Moreover, a continuously stirred tank reactor (CSTR) with 9.0 L total and 7.5 L
162 working volume was used to examine the AD of the mixed feedstock under continuous
163 mode operation. The reactor was initially filled with the same inoculum as the batch
164 assays and flushed with pure N₂ to ensure anaerobic conditions. Based on the results
165 from the BMP tests, the influent feedstock consisted of 90% SSMOW and 10% cattle
166 manure, in terms of VS. The hydraulic retention time was set at 15 days by supplying
167 125 mL of feedstock four times per day using a peristaltic feeding pump. The organic
168 loading rate of the reactor was set to 2.3 gVS/L/d. Biogas and liquid samples were taken
169 directly from CSTR at a sequence of twice a week to measure methane concentration,
170 pH and VFA composition. The CSTR was operated at thermophilic conditions (54 ± 1
171 °C) using silicone thermal jacket. The biogas volume was quantified daily with a gas
172 meter based on water displacement principle and the bioenergy production was
173 calculated.

174

175 **2.4 Analytical methods**

176 The standard methods for the examination of water and wastewater were followed
177 for TS, VS, pH, COD, NH₄⁺ and TKN measurements [23]. The elementary chemical
178 composition was used to define the carbon to nitrogen ratio (C/N) of both substrates.
179 Gas chromatography (GC-TRACE 1310) equipped with a thermal flame ionisation
180 detector (FID) was used to determine the methane content of all biogas reactors and to
181 quantify the VFA accumulation (GC-TRACE 1300) [5]. The content of micro- and
182 macro- nutrients in both substrates was determined using inductively coupled plasma

183 with optical emission spectrometry (ICP-OES). All measurements were performed in
184 triplicate samples.

185

186 **2.5 Computational methods**

187 The modified Gompertz equation was used to describe the kinetics of the BMP tests:

$$188 \quad M(t) = M_0 \times \exp \left\{ -\exp \left[\frac{R_{max} \times e}{M_0} (\lambda - t) + 1 \right] \right\}$$

189 where, $M(t)$ is the produced CH₄ yield over time t (mL/gVS), M_0 stands for the final
190 CH₄ yield (mL/gVS), R_{max} is the maximum CH₄ production rate (mL/gVS/d), λ
191 represents the lag phase (day) and e is Euler's constant (2.7183).

192 The co-digestion of cattle manure with SSMOW under continuous mode operation
193 was evaluated using the extended dynamic bioconversion model (BioModel) [24]. First
194 order kinetics was used to simulate hydrolysis and Monod kinetic was used for the rest
195 AD steps. Moreover, inhibition of VFA to hydrolysis, acetate to acetogenesis, ammonia
196 to methanogenesis and pH to all AD steps was examined.

197

198 **2.6 Statistical analysis**

199 Tukey post hoc test ($p < 0.05$) and one-way analysis of variance (ANOVA) was
200 followed to determine the statistically significant variations among mono- and co-
201 digestion samples using the software Graphpad Prism (Graphpad Software, Inc., San
202 Diego, CA). The prediction accuracy of the regression analyses were evaluated using
203 the coefficient of determination (R^2) and root mean square error (RMSE).

204

205 **3. Results and discussion**

206 **3.1 Mono- and co-digestion of SSMOW and cattle manure**

207 The first set of batch assays was conducted to define the maximum methane yield of
208 SSMOW and cattle manure and to reveal the most efficient co-digestion mixture using
209 these substrates (Fig. 1). Among different feedstocks, the usage of cattle manure as a
210 sole substrate was associated with the lowest biomethanation potential (181 ± 6
211 mL/gVS). The limited biodegradability is attributed to the presence of biofibers, as a
212 result of the animal nutrition, which are mainly composed of lignin molecules [13]. In
213 contrast to cattle manure, the obtained methane yield using SSMOW was significantly
214 higher (464 ± 69 mL/gVS, $p < 0.05$). The increased bioenergy production is attributed
215 to both biomass composition (i.e. high lipid and protein content, negligible
216 lignocellulosic biofibers) and applied pulping pretreatment before AD. Indeed,
217 Khoshnevisan et al. [5] found that the mono-digestion of SSMOW pretreated with a
218 biopulper led to similar results (490 mL/gVS) under mesophilic conditions and
219 Naroznova et al. [11] found almost the same methane yield (469 mL/gVS) with the
220 present study under thermophilic conditions.

221 With respect to co-digestion experiments, the higher the contribution of SSMOW in
222 the feedstock the higher the methane production. Especially, the highest methane output
223 was produced using 20% of cattle manure and 80% of SSMOW on VS basis in the
224 feedstock (382 ± 16 mL/gVS). As expected, the addition of SSMOW in the feedstock
225 boosted the biogas production. The results can be ascribed to two parameters: 1)
226 compositional differences related to the biodegradable organic polymers with dissimilar
227 theoretical BMP value, and 2) significant variation of co-substrates' C/N ratio (Table 1).
228 Specifically, SSMOW contained increased amounts of lipids and soluble carbohydrates

229 that can boost bimethanation compared to the recalcitrant cattle manure [5]. On the
230 contrary, the high content of nitrogen into cattle manure leads to decreased C/N ratio.
231 Thus, during co-digestion trials the markedly higher C/N of SSMOW increased the
232 overall value. Accordingly, Zhang et al. [12] examined the co-digestion of food waste
233 with cattle manure and concluded that the optimal C/N ratio was 15.8. The findings are
234 in accordance with the present co-digestion experiments where a C/N ratio of 16.9 was
235 associated with the highest methane yield. Moreover, the preference for conducting co-
236 digestion strategies instead of using pure substrates is also induced by the micro-
237 nutrients composition. Specifically, livestock slurries can supplement the required trace
238 elements for high enzymatic activity that are occasionally presented in negligible
239 concentrations in SSMOW [5]. For instance, cattle manure can serve as Mg^{2+} source to
240 stimulate the fermentation process and additionally, decrease Na^+ toxicity which can be
241 detected in high levels in SSMOW depending on their origin (e.g. food residues)
242 [12,25]. In accordance, the content of Mg^{2+} into the cattle manure (9.5 mg/gTS) was
243 significantly higher compared to SSMOW (1.9 mg/gTS). On the hand, SSMOW had
244 slightly higher content of Na^+ than manure, 9.5 and 7.3 mg/gTS respectively. However,
245 the content of Na^+ was not high to provoke any salinity stress to the microbial cells [26].
246 Furthermore, the addition of livestock slurry can overcome the occasional lack of Ca^{2+}
247 into the SSMOW (i.e. when green waste corresponds to the major fraction), which is
248 mandatory for the growth of methanogenic archaea [27]. Nevertheless, green waste
249 represented only a minor fraction into the used SSMOW and thus, a Ca^{2+} deficiency
250 was not observed into the biowaste (19.7 mg/gTS) compared to manure (23.6 mg/gTS).

251 In order to limit the co-digestion mixtures to only four but at the same time to be
252 able to define the maximum methane output using both substrates, a mathematical

253 mixture design approach was followed [28]. Linear, quadratic and full cubic equations
254 were used to fit the experimental data from the BMP tests and subsequently, R^2 and
255 RMSE were used to evaluate the prediction accuracy (Table 2). In fact, the cubic model
256 had the best prediction quality (i.e. highest R^2 , lowest RMSE). The response
257 optimisation using the full cubic model showed that 90% SSMOW in the feedstock
258 mixture can lead to even higher methane production than the 20:80. While the
259 calculated value (i.e. 10:90) was slightly lower compared to the highest BMP that was
260 obtained at the mono-digestion of SSMOW (i.e. 0:100), these two methane yields did
261 not differ significantly ($p > 0.05$). Hence, a mixture containing 10% of cattle manure
262 and 90% of SSMOW was further examined, due to the relatively high methanation and
263 the high interest with respect to the political and economic frame conditions. The
264 selected feedstock composition was used for the second batch assay and subsequently,
265 to the CSTR operation. Additionally, the results from the second BMP test were used to
266 evaluate the full cubic model output.

267

268 **3.2 Effect of ISR to the AD of SSMOW with cattle manure**

269 In the second batch assay, the effect of inoculum to substrate ratio (ISR) was
270 elucidated. The results indicated that the methane yield of the selected co-digestion
271 mixture was not affected by the ISR as insignificant statistical differences were
272 detected. Additionally, the average value of the recorded methane yield (443 ± 8
273 mL/gVS) was slightly higher but significantly meaningless ($p > 0.05$) with the predicted
274 value (419 mL/gVS), validating the accuracy of the cubic model obtained from the first
275 BMP test.

276 Based on the outcome of linear regression (i.e. high R^2 , low RMSE), the modified

277 Gompertz equation had high prediction accuracy. Its applicability to predict similar co-
278 digestion processes has been previously shown [29,30]; and thus, the kinetic analysis
279 was based on the modified Gompertz model. Apart from the values of methane
280 production, the rest kinetic parameters varied markedly upon the different inoculum to
281 substrate content. It was demonstrated that the higher the amount of inoculum the
282 shorter was the lag phase (Table 3 and Fig. 2). The observations are in agreement with
283 studies examining the effect of substrate to inoculum ratio on wastes from
284 municipalities and livestock industry [16,31]. Indeed, high load of substrate in parallel
285 with limited content of active biomass could lead to reactor's acidification and therefore
286 inhibition [18,22]. In the present work, the lowest pH value (i.e. 6.66) was observed
287 during the 3rd incubation day (Fig. 3a) and was directly connected with the
288 accumulation of TVFA (Fig. 3b) which resulted in limited methane production (Fig. 2).
289 Acetate represented the highest portion of produced intermediates, indicating that the
290 initial three steps of AD were efficiently conducted and only the methanogenesis was
291 partially inhibited during the start-up period. However, on day 8 the TVFA levels of
292 batch assays set at ISR of 0.5 were low and on the 12th day the methane production was
293 similar with the rest ISRs. Hence, the intermediates were efficiently consumed by the
294 methanogenic community and the initially observed accumulation did not lead to
295 irreversible inhibition. In a recent study, the methanogenic community was clearly
296 inhibited at low ISR in continuously fed reactors with SSMOW [5]. The inhibition was
297 depicted by accumulation of VFA and especially acetate concentration, drop of pH, and
298 subsequently, extension of lag phase compared to control operation. However, in the
299 present study irreversible inhibition was not detected.

300 Results obtained from the second batch set showed that the decrease of ISR had only

301 a slight impact on extending the lag phase during the co-digestion of SSMOW with
302 cattle manure. The strong buffer capacity of livestock slurry alleviated the overload of
303 the inoculum that otherwise can occur at low ISR [18].

304

305 **3.3 Continuous mode co-digestion of SSMOW and cattle manure**

306 CSTR operation is better to mimic the co-digestion of SSMOW with cattle manure
307 to real conditions compared to BMP assays. At steady state conditions, the methane
308 yield of the CSTR was relatively high (437 ± 20 mL/gVS, Fig. 4a) corresponding to
309 96% of the maximum expected output based on the results from the second BMP assay.
310 Typically, the methane production of a continuous reactor reaches 70-90% of the BMP
311 value [32], which highlights the high efficiency of the investigated system. In this
312 context, the reactor did not face any technical challenges and after seven days of
313 operation reached almost the maximum bioenergy production. Moreover, during the
314 second HRT the overall process performance was already stable. During the whole
315 experimental period, the methane content in biogas was rather constant ($65.3 \pm 2.3\%$),
316 pH was stable (7.65 ± 0.06) and the VFA were efficiently processed by the AD
317 microbiome and were not accumulated (Fig. 4b). Regarding the individual VFAs, acetic
318 and propionic acids were the dominant intermediates during the whole experimental
319 period. Nevertheless, acetic and propionic acid were always significantly lower than the
320 suggested inhibition indicator of 2.4 and 1.8 g/L respectively [33]. In addition, the ratio
321 between acetic to propionic acid was always higher than 1.0 g/L validating the well-
322 performing AD process [34].

323 The increased performance of CSTR was in accordance with the simulation outputs,
324 as the BioModel described efficiently both bioenergy production and biochemical

325 parameters (Fig. 4). Indeed, the BioModel has a wide range of applicability using
326 various organic substrates as crop residues, food waste, cheese waste, livestock slurries,
327 wastewater sludge and SSMOW [5,35,36] and thus, it is reliably designed to simulate
328 efficiently various co-digestion scenarios. In addition, BioModel considers also
329 ammonia inhibition which is a major problem during the AD of either livestock slurries
330 or SSMOW [37]. However, the used substrates were diluted with water in the present
331 study and thus, the concentration of ammonium nitrogen was low. More specifically,
332 the free ammonia was calculated to be less than 0.05 g/L at these conditions and on top
333 of this, no inhibition was indicated in the simulation. In parallel, both CSTR monitoring
334 and BioModel simulations showed that the physicochemical parameters (e.g. TVFA
335 accumulation or pH increase), which are directly connected with ammonia problems,
336 were within optimal range for AD process. To sum up, the overall reactor performance
337 was good as concluded by both experimental and modeling aspects. SSMOW pretreated
338 with biopulper can easily lead to high bioenergy output without instabilities and
339 therefore, it should be highly considered as a primary feedstock for full-scale biogas
340 plants.

341

342 **4. Conclusions**

343 The present study demonstrated that the anaerobic co-digestion of SSMOW with
344 cattle manure is feasible and leads to high methane production. The kinetics of co-
345 digestion showed that high process performance can be achieved independently from
346 the inoculum to substrate ratio. Moreover, the mixed influent feedstock demonstrated
347 increased biodegradation efficiency which was similar at batch assays and continuous
348 reactor operation. Subsequently, the continuously fed reactor process was modelled

349 using the BioModel and the results allowed close fit to the experimental measurements.

350

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355

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470

471 **Figure captions**

472 **Fig. 1.** Methane yields of mono- and co-digestion tests of cattle manure and SSMOW

473

474 **Fig. 2.** Cumulative CH₄ production as a function of time during the co-digestion of
475 SSMOW with cattle manure at different ISR.

476

477 **Fig. 3.** pH change (a) and TVFA accumulation (b) as a function of time during the co-
478 digestion of SSMOW with cattle manure at different ISR.

479

480 **Fig. 4.** Experimental data and modelling simulations for bioenergy yield (a) and pH
481 alteration and TVFA accumulation (b) during the co-digestion o of SSMOW with cattle
482 manure in continuous mode operation

483

484 **Tables**485 **Table 1.** Characteristics of SSMOW and cattle manure

Characteristics	SSMOW	Cattle manure
pH	4.05	7.24
TS, g/L	40.65 ± 0.64	48.25 ± 0.23
VS, g/L	35.00 ± 0.67	35.00 ± 0.04
COD, g/L	62.34 ± 1.78	56.99 ± 1.63
TKN, g/L	1.23 ± 0.04	2.46 ± 0.08
NH₄⁺, g/L	0.29 ± 0.04	1.61 ± 0.08
C/N	19.01 ± 0.95	8.69 ± 0.43
TVFA, g/L	1.73 ± 0.05	6.73 ± 0.30
Acetate, g/L	1.54 ± 0.05	4.49 ± 0.29
Propionate, g/L	0.06 ± 0.00	1.19 ± 0.08
Iso-butyrate	0.01 ± 0.00	0.16 ± 0.00
Butyrate	0.11 ± 0.01	0.59 ± 0.02
Iso-valerate	0.01 ± 0.00	0.27 ± 0.08
Valerate	0.01 ± 0.00	0.05 ± 0.00

486

487 **Table 2.** Models summary statistics with BMP as response variable and VS share of
 488 SSMOW in the feedstock as regressor.

Model	Regression equations	<i>R</i>²	<i>RMSE</i>
Linear	$BMP = 2.503 \times VS + 204.063$	0.956	18.45
Quadratic	$BMP = -0.004 \times VS^2 + 2.946 \times VS + 198.153$	0.958	17.91
Cubic	$BMP = 0.001 \times VS^3 - 0.102 \times VS^2 + 6.505 \times VS + 182.566$	0.996	5.42

489

490 **Table 3.** Parameters of modified Gompertz equation fitting experimental results

491 obtained from the co-digestion of SSMOW with cattle manure at different ISR

<i>Modified Gompertz equation</i>	ISR		
	0.5	1.5	3.0
λ , days	3.11	2.63	1.95
R_{max} , mL/gVS/d	90	118	96
Measured BMP, mL/gVS	444	455	446
Predicted BMP, mL/gVS	442	452	438
Difference, %	0.5	0.7	1.9
R^2	0.999	0.999	0.999
RMSE	4.19	2.38	5.54

492