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1 **A modelling approach for the assessment of energy recovery and**
2 **impact on the water line of sludge pre-treatments**

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23 **Abstract**

24 A simple, easy-to-use, first-order model was elaborated to predict the methane production and the
25 release of ammoniacal nitrogen (N-NH₃) to the digestate in full-scale anaerobic digestion (AD)
26 processes. The study used long-term, semi-continuous AD tests, carried out with samples of primary
27 sludge (PS), raw waste activated sludge (WAS), WAS after a thermo-alkali pre-treatment (90°C, 90
28 min, 4 g NaOH/100 g TS) and mixed sludge (PS/treated WAS), to calibrate and validate the model.
29 The results of both the experimental activities and the phase of model tuning demonstrated that the
30 proposed model was capable to provide reliable information to completely characterize the AD
31 process, thus overcoming the limitations due to discontinuity of experimental tests. Furthermore, it
32 was demonstrated that low-temperature thermo-alkali pre-treatments could increase the values of the
33 model parameters, namely methane production after an infinite time (B₀, +70%) and hydrolysis
34 constant (k, +450%), and made them comparable to those obtained by the application of commercial,
35 high-energy demanding treatments (e.g. Cambi). Finally, the issue concerning the release of N-NH₃
36 to digestate was deemed to be very worthy to being investigated because, after pre-treatments, the
37 cost for nitrogen removal in the water line, through the traditional processes of nitrification –
38 denitrification, could increase even by 140%.

39

40

41 **Keywords:** anaerobic digestion; ammonia; primary sludge; waste activated sludge; energy analysis;
42 hydrolysis rate

43

44

45 **Highlights**

- 46 • Traditional WWTPs must increase their capacity in recovering resources
- 47 • Hybrid pre-treatments increase both methane production and nitrogen release
- 48 • Nitrogen release must be controlled in the application of sludge pre-treatments
- 49 • A simple, first-order model was validated to predict nitrogen release to the digestate
- 50 • Extra nitrogen in the water line due to pre-treatments raises treatments costs by 140%

51 **1. Introduction**

52 According to the circular economy roadmap, wastewater treatment plants (WWTPs) have to become
53 “ecologically sustainable” technological systems in the near future [1], that means more efficient, less
54 energy demanding and capable to support resource recovery [2]. At the same time, WWTPs must
55 remain effective in maintaining their fundamental task, i.e. to provide a constant and adequate water
56 pollution control, so as to protect human health and the environmental quality against conventional
57 and emerging contaminants. In the framework of the broad spectrum of strategies for resource and
58 energy recovery, anaerobic digestion (AD) processes, still frequently seen just as a profitable way to
59 stabilize sludge, will have to become a cornerstone. In fact, AD processes offer lots of advantages for
60 the transition of traditional WWTPs to water resource recovery facilities (WRRFs), such as: very high
61 energy efficiency [3], versatility in terms of feed [4], medium to high pathogens inactivation [5],
62 potentiality for nutrients (N, P and K) recovery [6] and for carbon-based building blocks production
63 through fermentation to VFAs [7], effectiveness in degrading compounds that are recalcitrant to
64 aerobic biodegradation [8].

65 The AD of primary and secondary sludge produced in a WWTP is a mature technology. However,
66 the energy recovery from secondary sludge (also known as waste activated sludge, WAS) still remains
67 at quite low values, at most up to 7% of the energy available in the wastewater [9]. Those low values
68 depend on the nature of WAS, in fact, the presence of protective extracellular polymeric substances
69 and the rigid structure of the microbial cell walls determine low hydrolysis rate and poor bio-methane
70 productivity [10]. In order to enhance the energy recovery, WAS pre-treatment technologies, such as
71 physical, thermal, and chemical treatments, or a combination of them, could be required before AD
72 [11].

73 In the direction of the fulfillment of the circular economy package’s objectives, the sludge line of
74 existing WWTPs must be revamped through the introduction of interventions aimed at improving the
75 efficiency of the AD process in each of its phases, from the thickening of sludge to the final treatment
76 of digestate. However, such interventions are expensive and can be justified only on the basis of
77 reliable results coming from extensive experimental campaigns [12]. The gap between the results
78 obtained at a lab or pilot scale and a WWTP running at the full scale can be filled with a modelling
79 approach, capable of describing the complexity of a system influenced by a number of operational
80 parameters [13]. Several mathematical models and, more recently, machine learning applications
81 have been developed, with the fundamental aim of understanding and optimizing the implementation
82 of AD processes, thus eventually achieving more efficient functioning in WWTPs [14]. Quantitative
83 models can support the designer not only in the reactor design and scale-up, but also in evaluating
84 energy balance and economic sustainability, through the assessment of the dynamic behavior of key-

85 process variables in a wide range of experimental conditions [15]. In 2002 the IWA Task Group for
86 the Mathematical Modelling Processes published the Anaerobic Digestion Model No.1 (ADM1) [16].
87 ADM1 was aimed at providing a complete modelling of the fundamental AD mechanisms, through
88 the description of the dynamics of 24 species which are involved in 19 conversion processes of both
89 physico-chemical (namely disintegration-hydrolysis) and biological (namely acidogenesis,
90 acetogenesis and methanogenesis) nature. However, the complexity of ADM1 and the large number
91 of input parameters required by the model, such as COD fractionation or VFAs, the latter arising from
92 the process intermediate stages, which are not routinely measured in a WWTP, significantly reduces
93 its application [17]. If these measurements are not available, it is crucial to make significant reductions
94 to the model, which can make the validity of AD simulations questionable [18]. For example, Tolessa
95 et al. [19] had to resort to an extensive literature survey, combined with Monte Carlo analysis and a
96 Gaussian Mixture Model approach, to account for parameter variability, leading to a probabilistic
97 estimate of steady-state biogas production from agricultural residue substrates. Surrogate models,
98 containing a limited number of parameters, have been developed, calibrated and validated [20].
99 However, calibration and validation processes of such models have often been carried out by using
100 the results of BMP essays [21], which present evident differences with continuous, full-scale
101 processes, for what concerns, among others, the representativity of the tested substrate and the
102 evolution of the AD process. Other empirical models have been developed using a set of statistical
103 and mathematical techniques, known as response surface methodology (RSM), artificial neural
104 network (ANN) [22] or a combination of the two above-mentioned approaches [23]. Recently,
105 Parthiban et al. [24] developed a second order model where the output neurons were biogas and
106 biomethane, while the input neurons were thermophilic temperature, organic loading rate (OLR), pH,
107 agitation time, and hydraulic retention time (HRT). However, calibration and validation of RSM or
108 ANN models is based on the output of a large number of bench-scale tests and the mathematical form
109 of the obtained response variable does not have a direct relationship with the dynamics of an AD
110 process.

111 In this framework, in order to shorten the calculation procedure and make biogas production estimates
112 easier, a simple model for the description of the production of methane in time, $B(t)$, was proposed
113 [25]. The model was based on a first-order kinetic rate reaction, such as that shown in Equation (1)

$$114 B(t) = B_0 (1 - e^{-kt}) \quad (1)$$

115 The model proved to be capable to adequately capture the overall performance of mesophilic and
116 thermophilic AD processes through the two parameters namely B_0 and k . B_0 was the specific methane
117 production after an infinite HRT, that is the theoretical amount of methane produced by the whole
118 amount of biodegradable VS in the substrate, and k was the hydrolysis constant. Differently from

119 other experiences reported in literature (see, for example, the recent study of Tamang et al. [26]), that
120 used biochemical methane potential (BMP) tests to assess B_0 and k parameters, in the above-
121 mentioned study the two model's parameters were quantified by making use of long-term semi-
122 continuous AD tests. That kind of tests was deemed more reliable than BMP tests for model
123 calibration. The data obtained from the tests allowed the determination of the optimal sets of values
124 of the two parameters (B_0 , k) by using the best fit algorithms as done by Wei et al. [27].

125 On the grounds of above, the present study contributes to the current literature by further validating
126 the already proposed model, in order to make it a simple, easy-to-use tool useful to provide
127 information concerning not only the bio-methane productivity of organic substrates, but also the
128 impact of the release of ammoniacal nitrogen ($N-NH_3$) following to the application of pre-treatments.
129 Specifically, the present study had a two-fold aim: firstly, to definitely verify the goodness of the
130 already proposed model through the digestion of pure and mixed sludge and, secondly, to assess if a
131 similar approach could be used to predict the release of ($N-NH_3$), from a substrate to the digestate,
132 during an AD process. For what concerns the first aim, long-term, semi-continuous AD tests were
133 carried out on samples of primary sludge (PS), raw WAS and WAS after a thermo-alkali pre-
134 treatment ($90^\circ C$, 90 min, 4 g NaOH/100 g TS) with the aim of obtaining B_0 and k for each substrate.
135 The model's parameters were validated with an AD test involving a mixture of PS and treated WAS.
136 With reference to the second aim, it is well known that ammonia (NH_3), which is produced during
137 the anaerobic degradation of nitrogenous organic matter (e.g. proteins, amino acids, urea and nucleic
138 acids), is a common inhibitor of AD processes [28]. Furthermore, sludge pre-treatments boost the
139 release of $N-NH_3$ to digestate, with possible technical and economic impacts onto the removal of
140 nitrogen from wastewater, when the liquid fraction of the digestate is recirculated back to the water
141 line. Except for the study of Alejo et al. [29], this topic has not been broadly addressed by the scientific
142 literature. The study wants to fill this gap, thus proposing a model for the quantification of the amount
143 of $N-NH_3$ released to the digestate and providing a rough, preliminary estimate of the costs that a
144 WWTP must bear to cope with the increase of nitrogen loads in the water line.

145

146 **2. Materials and Methods**

147 **2.1 Substrates**

148 Samples of primary sludge (PS) and waste activated sludge (WAS) were collected from the outlet of
149 the gravity pre-thickeners of the Castiglione Torinese WWTP (located 20 km from Turin, NW Italy)
150 once a week. The inoculum used for the start-up of the long-term AD tests, described in Section 2.2,
151 was obtained from one of the anaerobic digesters fed with WAS in the same WWTP.

152 The Castiglione Torinese WWTP is one of the facilities run by SMAT (Società Metropolitana Acque
153 Torino), the company that manages the integrated water service in the Metropolitan City of Turin.
154 The WWTP has a treatment load of approximately 2,000,000 population equivalent (p.e.). The AD
155 process is carried out in six digesters with average HRTs of 14.8 and 18.6 days for WAS and PS
156 respectively.

157 Details of the water and sludge line of the Castiglione Torinese WWTP were provided in a previous
158 paper [30]. Shortly, the WWTP has a standard configuration that includes the following treatment
159 phases: preliminary treatments (grating and sand/oil removal), primary settling, pre-denitrification,
160 biological oxidation with a solids retention time (SRT) of approx. 30-35 days, secondary settling and
161 final filtration on a dual media, sand – anthracite, bed.

162 A total of 2 plus 4 gravity pre-thickeners are used in the ordinary operation of the WWTP with the
163 aim of increasing the TS content of WAS and PS, respectively, before AD. WAS has a final TS
164 content, before AD, in the order of 3%, obtained with the addition of 0.5 g of a cationic polyelectrolyte
165 / 100 g TS. PS and WAS account for 64% and 36%, by weight (b.w.), on a TS basis, of the overall
166 amount of sewage sludge produced in the WWTP.

167 After being screened using a 40-mesh sieve to remove large particles, the sludge samples were stored
168 in 10 L polypropylene tanks at 4 °C prior to AD tests. Table 1 shows the average characteristics of
169 the two substrates, namely PS and WAS, averaged over the duration of the AD tests.

170

171 Table 1. Average characteristics of the two substrates, PS and WAS, used in the tests

	Primary sludge	Waste activated sludge
Total solids (TS, %)	2.56	3.05
Volatile solids (VS, %)	1.86	2.04
pH	6.11	7.20

172

173 Total and volatile solids were obtained as described in Section 2.3.

174 2.2 Reactors set up and experimental tests

175 WAS was used in the AD tests as a raw or thermo-alkali pretreated substrate. The thermo-alkali pre-
176 treatment (4 g NaOH/100 g TS, 90°C, 90 min) was carried out in a batch reactor. The operating
177 conditions for pre-treatments were fixed on the basis of the results obtained in a previous work [31],
178 that compared the performance of thermal, alkali and thermo-alkali pre-treatments for the
179 enhancement of methane production from WAS. The reactor used for the pre-treatment had a working
180 volume of 35 L and was completely stirred with an electric propelled shaker. The heat was transferred
181 to the sludge through three electrical band resistances, placed on the lateral surface of the reactor,
182 with an electric power of 2.6 kW each. The temperature inside the reactor was controlled by an open
183 source single-board microcontroller (Arduino).

184 The digestion tests were performed with two apparatus. The first digester was a continuous stirred
185 reactor (CSR) with a total volume of 12 L (operating volume, 10 L), equipped with a water jacket,
186 for the temperature control, and gasometers and systems for on-line monitoring of the volume and
187 composition of the biogas (see details in [25]). Mixing inside the digester was obtained through biogas
188 recirculation for 15 min every hour.

189 The second digester was a CSR with a total volume of 300 L (operating volume, 240 L), equipped
190 with an 80 L gasometer and an electronic system for on-line monitoring of the biogas volume and
191 composition (see details in [32]). Mixing inside the digester was obtained through an alternate biogas
192 recirculation (15 min on / 15 min off).

193 A total of four long-term, semi-continuous digestion tests were carried out. Details of the tests are
194 reported in Table 2. The substrate used in test n.4 was a mixture (50/50 by volume, b.v.) of PS and
195 thermo-alkali pre-treated WAS. In all tests the operations of substrate supply and digestate extraction
196 were carried out five days a week, from Monday to Friday.

197

198 Table 2. Details of the AD tests

Test number	Substrate	Reactor	Temperature regime	HRT (d)	Duration (d)	OLR kgVS/m ³ ·d
1	PS	CSR 10-L	Mesophilic, 38°C	20	158	0.93±0.13
2a	WAS	CSR 240-L	Mesophilic, 38°C	15	112	1.43±0.31
2b	WAS	CSR 240-L	Mesophilic, 38°C	20	46	1.22±0.36
3a	Treated WAS	CSR 240-L	Mesophilic, 38°C	20	29	1.28±0.32
3b	Treated WAS	CSR 240-L	Mesophilic, 38°C	20	90	0.56±0.15
4	Mixed sludge	CSR 240-L	Mesophilic, 38°C	20	108	1.03±0.08

199

200 **2.3 Analytical methods**

201 Total and volatile solids (TS, VS) were determined according to the Standard Methods [33]. The total
202 volatile fatty acid (tVFA) concentration, as acetic acid (CH₃COOH) equivalent, and the total
203 alkalinity (TA) were obtained by a potentiometric titration, according to the Nordmann method, by
204 using a SI Analytics automatic titrator. Specifically, a sample of 20 mL of digestate was titrated with
205 a 0.1 N sulfuric acid (H₂SO₄) solution up to pH 5.0, so as to calculate the TA value, expressed in
206 mg/L of calcium carbonate (CaCO₃). Then the tVFA value was obtained after a second titration step
207 from pH 5.0 to pH 4.4.

208 The soluble COD (sCOD) and ammonium ion (NH₄⁺) were determined according to the Standard
209 Methods [33] on the liquid phase of the substrates (raw and pre-treated sludge) or digestate. The
210 liquid phase was obtained after an initial centrifugation at 15,000 rpm for 10 min and a subsequent
211 filtration of the supernatant on a 0.45 μm nylon membrane filter, as recommended by Roeleveld and
212 van Loosdrecht [34].

213 The elemental composition analysis was carried out on samples of PS and WAS dried at 105 °C and
214 on the residual ashes after combustion at 600 °C. A Flash 2000 ThermoFisher Scientific CHNS
215 analyzer was used for the elemental analysis, assuming that the oxygen content of the substrate was
216 the complementary fraction towards C, H, N, S contents. The results of the elemental analysis were
217 used to calculate the theoretical COD and the theoretical methane production (B_{th}), according to the
218 Buswell model, of the two substrates.

219

220 **2.4 The mathematical model to predict the methane production in an AD process**

221 Interventions on the sludge line of existing WWTPs, such as the introduction of pre-treatments or
222 change of the digestion scheme, from one-stage to two-stage, can be justified only on the basis of
223 reliable results coming from extensive experimental campaigns. Mathematical models can help in
224 filling the gap between the results of tests carried out at a lab or pilot scale and the operation of the
225 WWTP at a full scale. In a previous work [25], a simple model was proposed and validated through
226 a series of AD tests carried out in mesophilic and thermophilic conditions. The above-mentioned
227 model was based on a first-order rate reaction, such as that shown in Equation (1), and contained two
228 parameters, namely the biochemical methane potential (B₀) and the hydrolysis rate (k). B₀ is the
229 maximum amount of methane that a substrate can produce after an AD process of infinite duration;
230 k is the first-order kinetic constant that describes the velocity at which the substrate is made available
231 for the AD process.

$$232 \quad B(t) = B_0 (1 - e^{-kt}) \quad (1)$$

233 As it is well-known, an AD process consists of the four steps namely hydrolysis, acidogenesis,
 234 acetogenesis and methanogenesis. Hydrolysis is the only step in which microorganisms are not
 235 directly involved. In fact, that process is merely a surface phenomenon, in which particulate and
 236 polymeric matters are degraded through the action of eso-enzymes. After hydrolysis, the produced
 237 smaller molecules can cross the cell barriers and be used by microorganisms for the production of
 238 intermediate and final AD products [35]. The hydrolysis phase is generally the rate-limiting step
 239 during an AD process of particulate substrates [36]. WAS is a typical particulate and complex
 240 substrate hard to biodegrade. If hydrolysis is assumed to be the limiting step of AD, and no other
 241 inhibition phenomena occur, the methane production can be modelled through a first-order rate
 242 reaction, such as that shown in Equation 1.

243 Equations 2-5 represent the complete set of equations necessary to describe an AD process in a CSR,
 244 when hydrolysis is assumed to be the limiting step and the substrate is made of particulate matter.

$$245 \quad B(t) = VS(t) \cdot k \cdot B_0 \cdot V \quad (2a)$$

$$246 \quad B(t) = VS_b(t) \cdot k \cdot Y \cdot B_{th} \cdot V \quad (2b)$$

$$247 \quad \frac{dVS_b(t)}{dt} = \frac{q(t) \cdot VS_{b,in}(t)}{V} - \frac{q(t) \cdot VS_b}{V} - k \cdot VS_b(t) \quad (3)$$

$$248 \quad \frac{dVS_{nb}(t)}{dt} = \frac{q(t) \cdot VS_{nb,in}(t)}{V} - \frac{q(t) \cdot VS_{nb}}{V} \quad (4)$$

$$249 \quad \frac{dNVS(t)}{dt} = \frac{q(t) \cdot NVS_{in}(t)}{V} - \frac{q(t) \cdot NVS}{V} \quad (5)$$

250 In Equation 2a the daily methane production, at the time t, B(t), is related with the amount of volatile
 251 solids, VS, at the same time frame, the hydrolysis rate constant (k), the biochemical methane potential
 252 (B₀) and the volume of the reactor (V). With reference to Equation 2a, it is important to keep in mind
 253 that the substrate fed to the digester is made of volatile (VS) and non-volatile solids (NVS or fixed
 254 solids) and that not all the VS are degradable in an AD process, even after an infinite time. Equations
 255 (3-5) describes the mass balance of biodegradable VS (VS_b), non-biodegradable VS (VS_{nb}) and NVS
 256 as a sum of (i) the input of fresh substrate, (ii) the output of the digested product and (iii) the
 257 degradation term where applicable. The time-change of the three kind of solids is a function of both
 258 volumetric flow rate (q) and volume (V).

259 On the basis of the elemental composition of the VS, it is possible to calculate the theoretical methane
 260 production of the substrate, B_{th}, (see Equation 2b) by referring to Equation 6:

$$261 \quad B_{th} = VS_{in} \cdot \frac{COD_{in}}{VS_{in}} \cdot 0.350 \frac{Nm^3 CH_4}{kg COD} \quad (6)$$

262 B_0 is always smaller than B_{th} , because not all the VSs are biodegradable (i.e presence of VS_{nb} into the
 263 substrate fed to digester) and, in minor measure, because of the anabolic activity of anaerobic
 264 microorganisms. Furthermore, in a real case, the specific methane production (SMP) of a substrate is
 265 smaller than B_0 , because, as in Equation 7, both the hydrolysis process (the rate of which is quantified
 266 by the kinetic constant, k) and the duration (HRT) of the AD process limit the methane production.
 267 Equation 7 provides the solution at steady condition of Equation 2a.

$$268 \quad SMP = B_d(t) = \left(1 - \frac{1}{1+k \cdot HRT}\right) B_0 \quad (7)$$

269 The Y parameter, reported in Equation 2b, is the absolute biodegradation (or degradation extent), that
 270 is the ratio between B_0 and B_{th} , an intrinsic characteristic of the substrate. Because of the relationship
 271 between B_0 and VS_b , and between B_{th} and VS , Y can also be defined as the ratio between VS_b and
 272 total VS , as in Equation 8.

$$273 \quad Y = \frac{B_0}{B_{th}} = \frac{VS_b}{VS} \quad (8)$$

274 The optimal set of B_0 and k values, capable of describing the trend of the SMP observed in the
 275 experimental tests carried out in a continuous mode, was obtained by minimizing the objective
 276 function (J). Function J is the residual sum of squares (RSS) between the measured data and the data
 277 predicted by the model, as stated in Batstone et al. [37]. If the RSS are normally distributed, a critical
 278 value (J_{crit}), that defines the surface of the parameter uncertainty region, can be defined by using the
 279 F distribution, as in Equation 9 [27].

$$280 \quad J_{crit} = J_{min} \left(1 + \frac{p}{N_{data}-p} \cdot F_{\alpha,p,N_{data}-p}\right) \quad (9)$$

281 where N_{data} is the number of measured data, p is the number of parameters, and $F_{\alpha,p,N_{data}-p}$ is the value
 282 of the F distribution for α , p , and $N_{data}-p$. An α value of 0.05 was used to estimate the 95% confidence
 283 regions.

284

285 **2.5 The mathematical model to predict the ammonia release from the substrate to the digestate**

286 A new first-order kinetic model was proposed with the aim to predict the amount of $N-NH_3$ released
 287 to the digestate. During an AD process, nitrogen is released to the digestate, as a consequence of
 288 hydrolytic processes, under the two forms of ammonia (NH_3) and ammonium ion (NH_4^+), collectively
 289 called ammonia nitrogen (AN). The relative abundance of each of the two forms is regulated by a pH
 290 and temperature depending equilibrium ($pK_a = 9.25$ at $25^\circ C$, see Supplementary Materials, Section
 291 1, SM1). The assessment of the $N-NH_3$ amount into the digestate is of capital importance for two
 292 main reasons. Firstly, concentrations of $N-NH_3$ higher than 1800 – 2000 mg/L into the digesting

293 material have an adverse effect on the activity of the acetoclastic methanogenic microorganisms, that
 294 reduce the production of methane [38]. In the ADM1 the inhibition of methanogens due to free NH₃
 295 is modeled as a non-competitive inhibition process [16]. Details concerning the equation and the
 296 default value of the inhibition parameter K_{INH3} are reported in SM2. Secondly, the liquid phase of the
 297 digestate, after solid – liquid separation, is often recirculated back to the water line, with an evident
 298 impact of the residual AN forms on the mass and energy balances of the biological processes, namely
 299 nitrification and denitrification.

300 The release of AN (and, depending on the pH, of the N-NH₃ fraction) to the digestate is limited by
 301 the hydrolysis process, that transforms the feedstock's proteins, firstly, into amino-acids and, finally,
 302 into AN and VFAs. The “perN-NH₃” parameter was introduced to indicate the ratio between the
 303 maximum amount of N-NH₃ that the AD substrate can potentially release to the digestate, and the
 304 amount of VS fed to the digester. A correspondence can be identified between B₀, that is the
 305 maximum amount of producible methane, and “perN-NH₃”, that is the maximum amount of
 306 releasable N-NH₃. The model is described by Equation 10:

$$307 \quad \frac{dN-NH_3(t)}{dt} = \frac{q \cdot N-NH_3(in)(t)}{V} - \frac{q \cdot N-NH_3(t)}{V} + perN - NH_3 \cdot k \cdot VS \quad (10)$$

308 Where

309 N-NH_{3(in)}, is the concentration (g/m³) of ammoniacal nitrogen into the substrate fed to the digester

310 N-NH₃ is the concentration (g/m³) of ammoniacal nitrogen into the digesting material

311 The solution of Equation 10 at steady state (SS) is that described by Equation 11:

$$312 \quad N - NH_3(SS) = N - NH_3(in) + perN - NH_3 \cdot \frac{k \cdot HRT}{1+k \cdot HRT} \cdot VS_{in} \quad (11)$$

313 All the mathematical models used to predict methane production (as in Section 2.4) and NH₃ release
 314 from the substrate, were implemented into the graphical programming environment Simulink-
 315 Matlab® (Simulink 9.2, solver method ode23t).

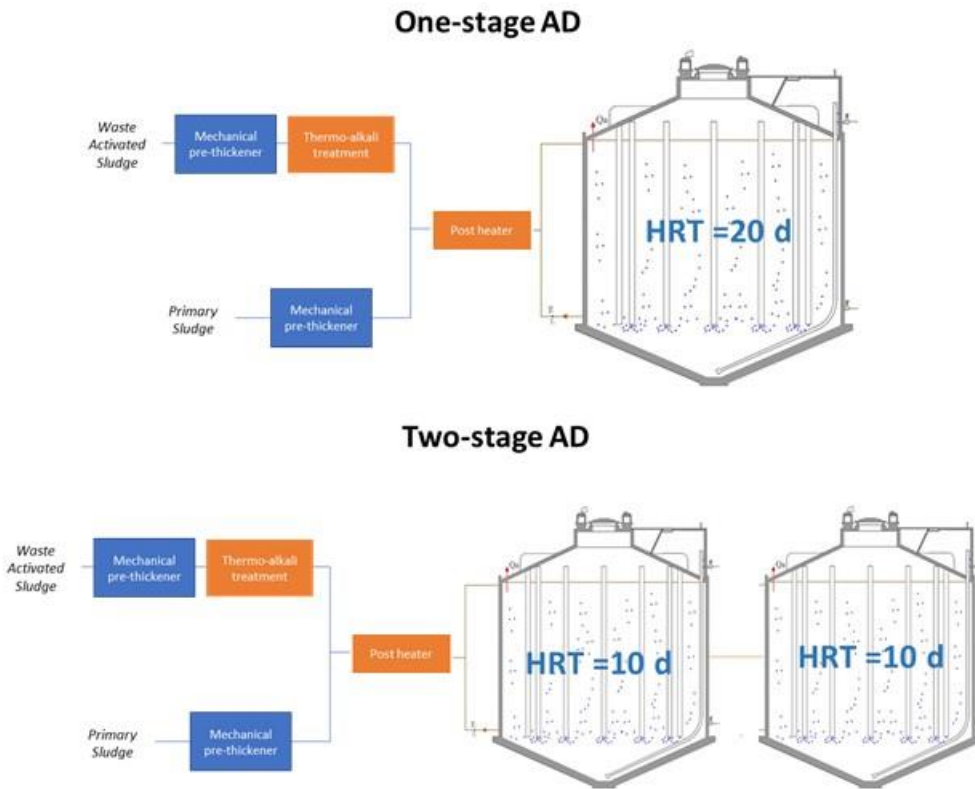
316

317 **2.6 Energy analysis of future scenarios**

318 The values of B₀ and k parameters, obtained from the model application (see Section 2.4), were used
 319 to compare two possible future configurations of the WWTP sludge line (see Figure 1). The energy
 320 balances reported in this section were written with reference to a WWTP's configuration where the
 321 produced biogas is combusted in combined heat and power (CHP) units with a thermal and electrical
 322 efficiency of 42.4% and 41.9% respectively. However, it should be emphasized that, at the time the
 323 experimentations were carried out, the produced biogas was burned in the CHP engines. Today, 2023,

324 the biogas produced from the AD is sent to an upgrading and purification unit, which was designed
 325 for biomethane generation.

326



327

328

329 Figure 1. One-stage and two-stage AD sludge treatment line configuration

330

331 In the first scenario, thermo-alkali pre-treatments (90°C, 90 min, 4 g NaOH/100 g TS) were
 332 introduced for WAS and the AD process of PS and WAS was carried out in traditional one-stage
 333 digesters. In the second scenario, other than the introduction of the thermo-alkali pre-treatment for
 334 WAS, the AD process was carried out according to a two-stage scheme. The HRT of the modelled
 335 digesters was assumed equal to 20 and 10+10 days for the first and second scenario, respectively. For
 336 both scenarios, the thickening of PS and WAS was considered to be obtained with dynamic
 337 thickeners, that would substitute the gravity thickeners presently used in the WWTP. The heat
 338 recovered from the thermo-alkali pre-treated WAS was used to pre-heat the PS.

339 Equations 12 and 13 were used to calculate the SMP for the one-stage and two-stage process
 340 respectively.

341
$$SMP = \left(1 - \frac{1}{1+k \cdot HRT}\right) B_0 \quad (12)$$

342
$$SMP = \left[1 - \frac{1}{1+kHRT_1} \frac{1}{1+kHRT_2} \right] B_0 \quad (13)$$

343 The heat amounts involved in the energy analysis of the two scenarios were calculated as in the
344 follow.

345 The overall amount of heat, recovered from the biogas combustion in the CHP units, was calculated
346 as in Equation 14

347
$$Q_1 = (q_{PS} \cdot \%VS_{PS} \cdot SMP_{PS} + q_{WAS} \cdot \%VS_{WAS} \cdot SMP_{WAS}) \cdot LHV_{CH_4} \cdot \eta_1 \quad (14)$$

348 where:

349 q_{PS} = volumetric flow rate of PS, m³/d

350 $\%VS_{PS}$ = concentration of VS into the PS, kg VS/m³ PS

351 SMP_{PS} = specific methane production of PS, Nm³ CH₄/kg VS

352 q_{WAS} = volumetric flow rate of the WAS, m³/d;

353 $\%VS_{WAS}$ = concentration of VS into the WAS, kg VS/m³ WAS

354 SMP_{WAS} = specific methane production of WAS, Nm³ CH₄/kg VS

355 LHV_{CH_4} = lower heating value of methane, 35.259 MJ/Nm³

356 η_1 = efficiency of heat generation of the CHP unit

357 The generated heat can be used for the thermo-alkali pre-treatment of the WAS, as in Equation 15

358
$$Q_2 = \frac{q_{WAS} \cdot c_p \cdot (T_p - T_1)}{\eta_2} \quad (15)$$

359 where:

360 c_p = specific heat capacity of sludge, kJ/m³·°C

361 T_p = temperature of the pre-treatment, °C

362 T_1 = temperature of the environment, °C

363 η_2 = efficiency of heat transfer from the CHP unit to the cold, raw WAS

364

365 The heat transferred to the WAS in the pre-treatment process could be efficiently used to support the
366 AD process, that is to heat the cold PS and compensate the heat losses across the walls and roof of
367 the digesters. The heat necessary to support the temperature-controlled AD process was calculated as
368 in Equation 16:

369
$$Q_3 = \frac{(q_{PS} + q_{WAS}) \cdot c_p \cdot (T_2 - T_1) + n \cdot Q_a}{\eta_3} \quad (16)$$

370 where:

371 T_2 = temperature of the digestion process, 38°C

372 n = number of reactors

373 Q_a = heat losses across the walls and roof of digester(s)

374 η_3 = efficiency of the heat transfer from the pre-treated WAS to the cold PS;

375

376 The system is energy self-sustainable provided that (i) the heat generated from the biogas combustion
377 (Q_1) is sufficient to support the pre-treatment of WAS and (ii) the heat recovered from the pre-treated
378 WAS (Q_2) is sufficient to support the AD of PS and WAS (Q_3). The combination of Equations (15)
379 and (16) allows to calculate the volumetric flow rate of PS that makes the AD process energy self-
380 sustainable Equation (17):

381
$$q_{PS} = \frac{1}{(T_2 - T_1)} \cdot \left\{ q_{WAS} \cdot [\eta T_p + T_1(1 - \eta) - T_2] - \frac{n \cdot Q_a}{c_p} \right\} \quad (17)$$

382

383

384 3. Results and Discussion

385 3.1 Validation of the mathematical model to predict the methane production in an AD process

386 Four long-term, semi-continuous tests were carried out with the aim of validating the mathematical
387 model proposed in Ruffino et al. [25] and recalled in Section 2.4 of this paper. From the data of
388 methane production recorded in the first three tests, that involved PS, raw WAS and pre-treated WAS,
389 the B_0 and k parameters were obtained for each substrate. After the calibration phase, the couple of
390 parameters obtained for each substrate was validated by referring to the trend of VS into the digestate.
391 Finally, the model was completely validated by using the results of the fourth AD test, that was carried
392 out with the mixed sludge (PS – pre-treated WAS, 50/50 b.v.).

393

394 3.1.1 Tests and model calibration for the single substrates (PS, WAS, pre-treated WAS)

395 The raw formula of the VS of the two substrates (PS and WAS), the COD/VS ratio and the theoretical
396 biogas and methane production according to the Buswell equation were calculated from the results
397 of the elemental composition analysis (C, H, N and O content). The values of the above-mentioned
398 parameters are reported in Table 3. It can be seen that the theoretical methane production of the two
399 substrates, B_{th} , was equal to 0.62 and 0.52 Nm^3/kg VS for PS and WAS, respectively.

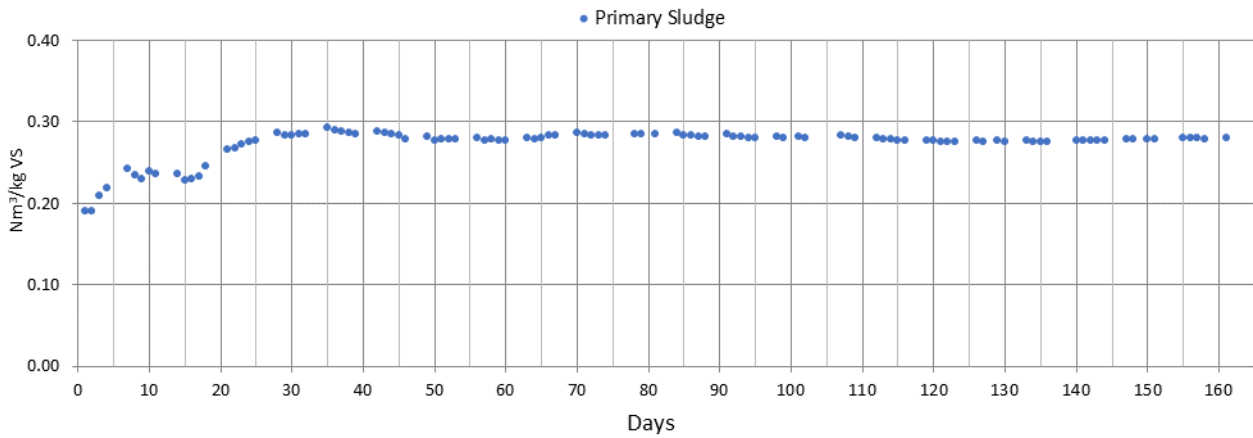
400

401 Table 3. PS and WAS parameters obtained from the elemental composition analysis

	PS	WAS
VS raw formula	$C_{10.6}H_{18.2}O_{4.1}N$	$C_{6.8}H_{11.8}O_{3.2}N$
COD/VS (g O_2/g VS)	1.76	1.49
Theoretical biogas production (Nm^3/kg VS)	1.06	0.96
Theoretical methane production (Nm^3/kg VS)	0.62	0.52

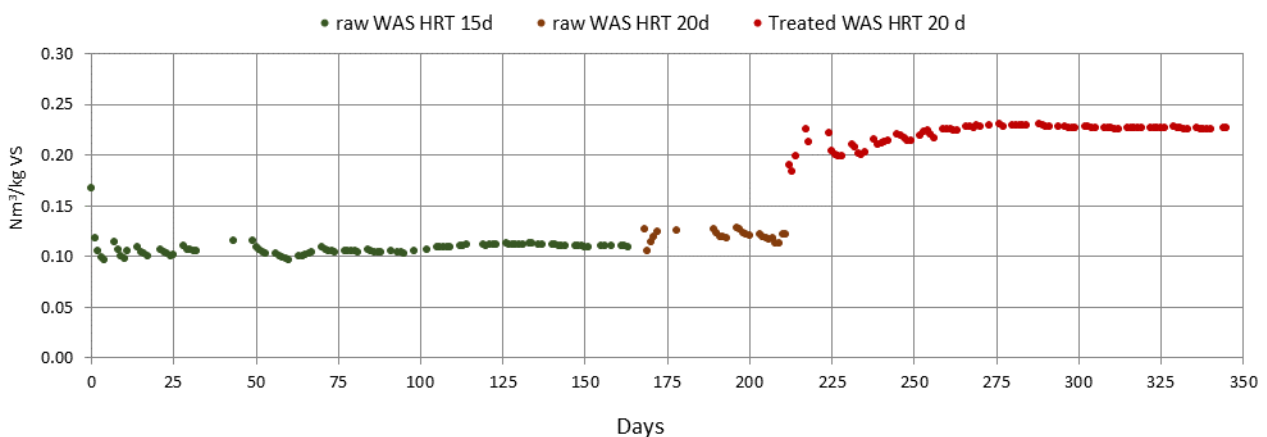
402

403 Figure 2 shows the evolution of the SMP during the approx. 160-day lasting digestion test that
 404 involved the PS. It can be seen that after approximately 35-40 days a steady value of SMP equal to
 405 $0.280 \text{ Nm}^3/\text{kg VS}$ was reached. These findings confirmed the results obtained in a previous work
 406 [39].



407
 408 Figure 2. Trend of the SMP for PS (test n.1)

409
 410 Figure 3 shows the combination of the results obtained in the two tests involving raw and thermo-
 411 alkali pre-treated WAS (test n. 2 and n.3, respectively). The whole study had been lasted for approx.
 412 one year. Figure 3 shows that, after a start-up phase lasting approximately two months, the SMP of
 413 the raw WAS reached the steady value of $0.110 \text{ Nm}^3/\text{kg VS}$ (HRT = 15 days). That SMP value had
 414 been maintained for approx. 200 days, with an only moderate change in SMP (+ 9%) due to the
 415 increase in the HRT, from 15 to 20 days, that intervened after 169 days from the beginning of the
 416 test.



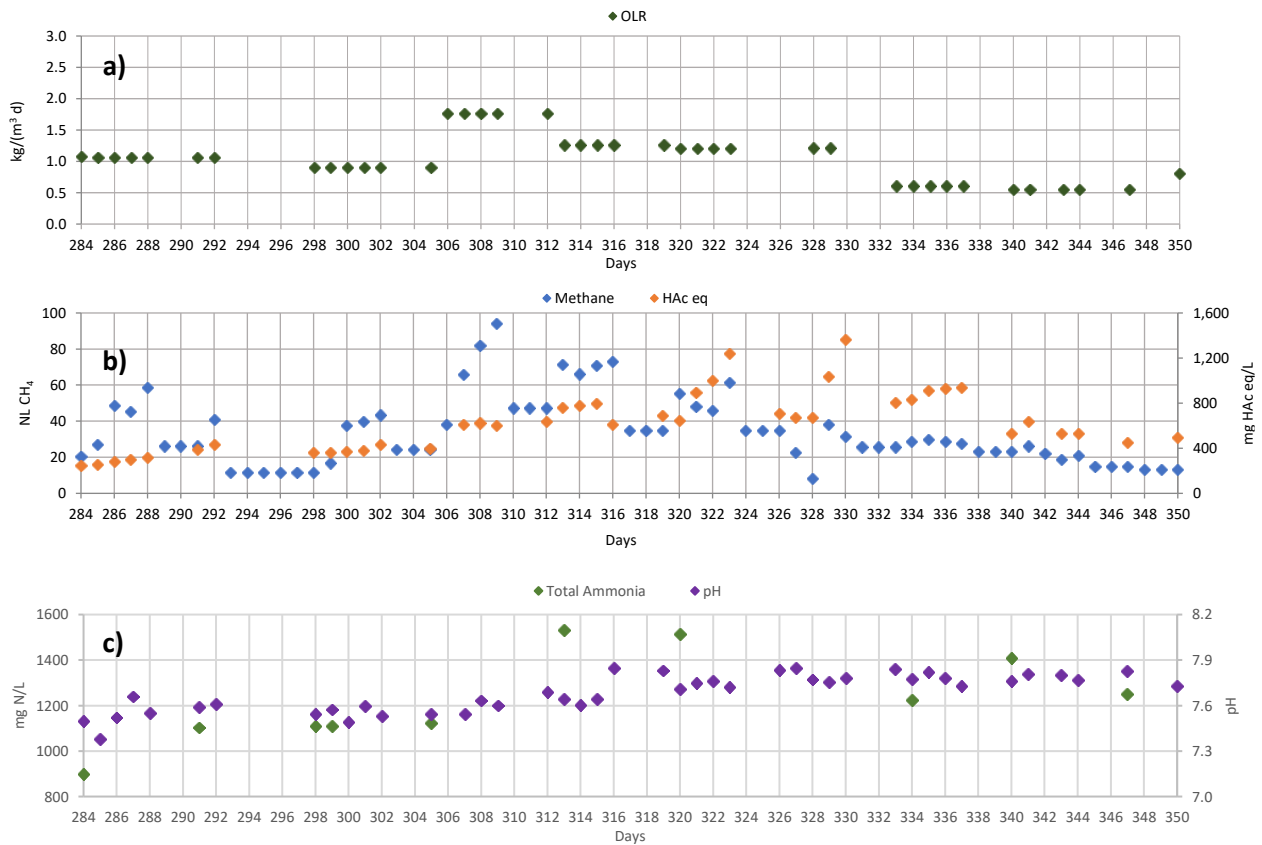
417
 418 Figure 3. Trend of the SMP for raw and thermo-alkali pre-treated WAS (tests n.2 and 3)

419
 420 As expected, the thermo-alkali pre-treatment (90°C , 90 min, 4 g NaOH/100 g TS) of the WAS
 421 promoted the solubilization of particulate organic matters [40], thus determining an increase in the

422 sCOD [10]. The observed disintegration rate was in the order of 40% (data not shown), in line with
 423 the values found in the tests carried out at a smaller scale [31]. The pH of the WAS after the pre-
 424 treatment was in the order of 8.5.

425

426 Figure 4 shows the detail of the results of the digestion test involving the thermo-alkali pre-treated
 427 WAS (test n.3).



428

429 Figure 4. Details of the digestion test (n.3) involving the thermo-alkali pre-treated WAS: trend of (a)
 430 daily organic loading rate (OLR) (b) daily methane production and total acidity concentration; (c) N-
 431 NH₃ and pH.

432

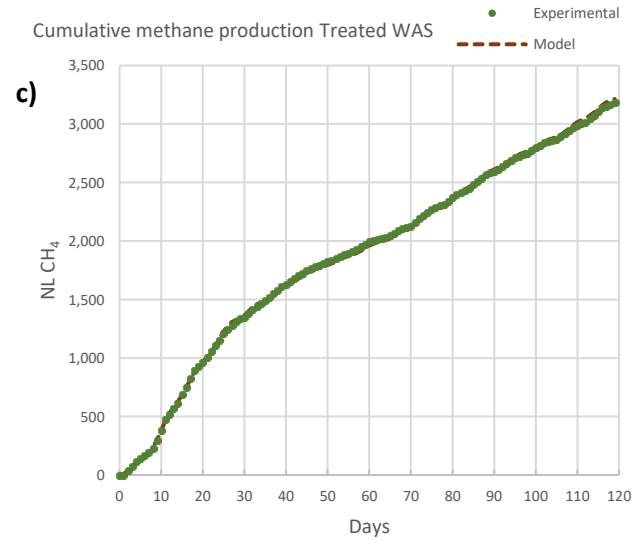
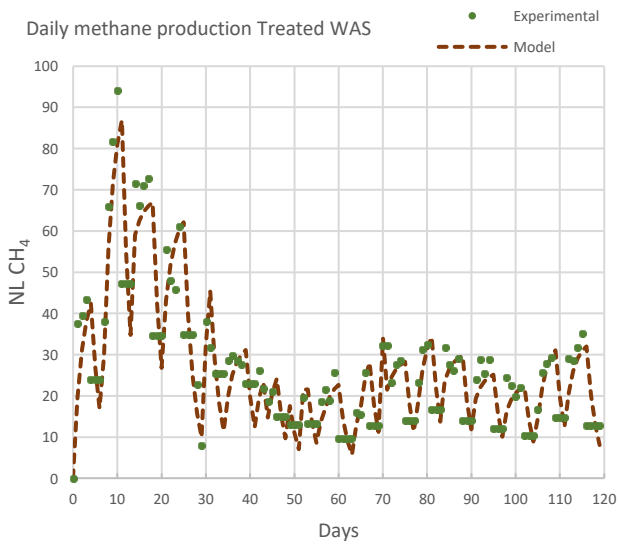
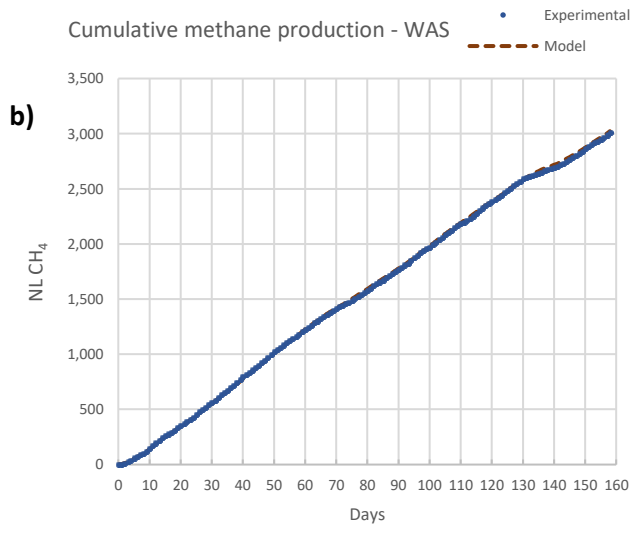
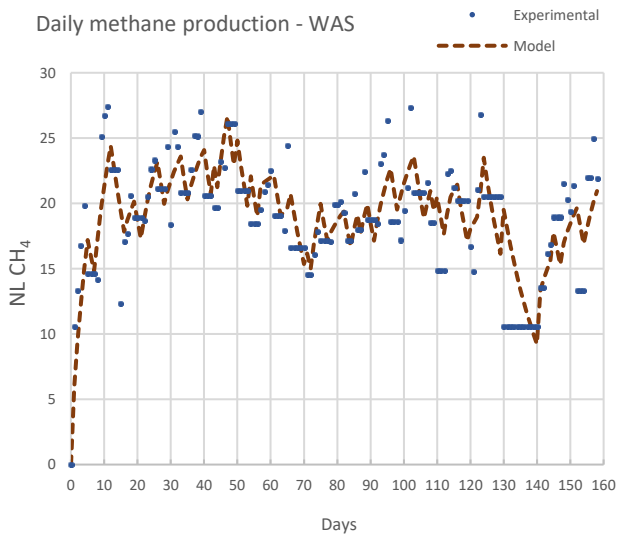
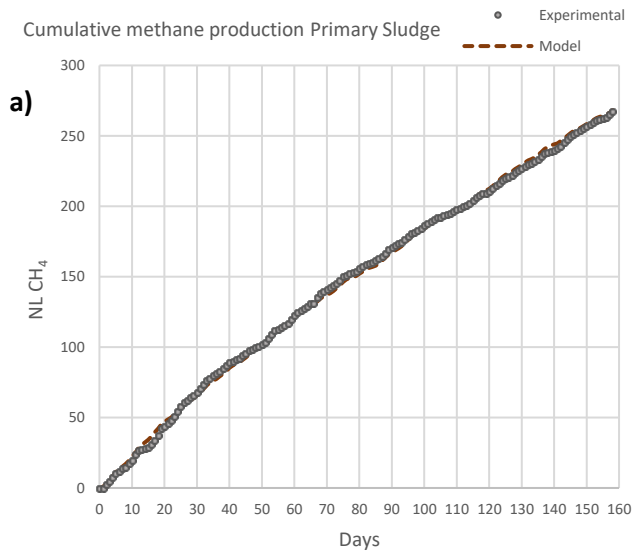
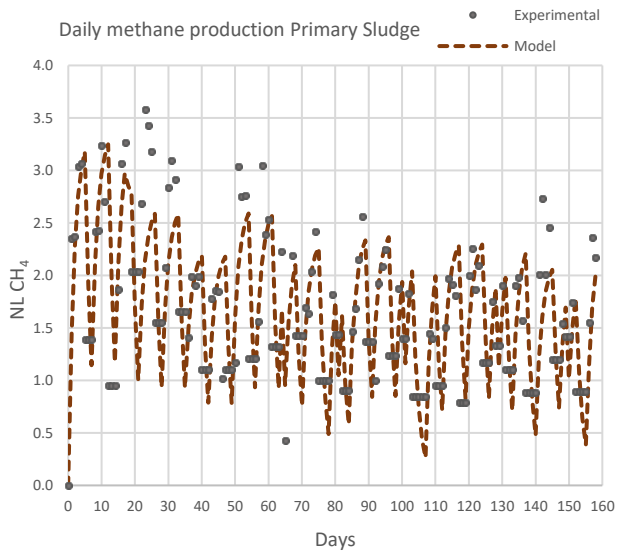
433 It can be seen from Figures 4a and 4b that the substrate fed at an OLR value of approx. 1.3 kg VS/m³·d
 434 (phase “a” of test n.3) determined an evident instability of the system already after 20 days from the
 435 beginning of the test. The daily methane production dropped from 50-60 NL to less than 20 NL. The
 436 instability was due to an increase of the concentration of N-NH₃ into the digestate, from 1000 mg/L
 437 (the first day of the test) to 1500 mg/L (the 21st day, Figure 4c), that inhibited methanogens thus
 438 determining a reduction in the methane production and an evident accumulation of acidic species
 439 (total VFAs) as observed, for example, in Capson-Tojo et al. [41]. Figure 4a shows that the
 440 concentration of total VFAs rose from approx. 400 mg acetic acid equivalent/L to values of more

441 1000 mg acetic acid equivalent/L. Consequently, in order to avoid that the digestion process was
442 completely compromised, the OLR was decreased by 50%, by mixing the feedstock with an equal
443 volume of tap water (50:50 by volume). As it can be seen from Figure 3, the digestion process had
444 been carried out with the dilute feedstock for approx. 100 days, and it evidenced a SMP of 0.230
445 $\text{Nm}^3/\text{kg VS}$, approx. 110% more than the value observed for the raw WAS.

446 As shown by the Figures provided in SM3, the daily production of methane of the three substrates
447 was heavily affected by the frequency of the digester feeding. In fact, it was not possible to keep the
448 HRT at a constant value, because the digester was fed only five days per week. Consequently, it was
449 verified whether, with the aid of the first order kinetic model described in Section 2.4, the raw data
450 collected from the experimentation could be used to provide a complete description of the AD
451 process, in terms of B_0 and k . In fact, the raw data alone cannot be considered sufficient to quantify
452 the substrate production at a fixed HRT.

453 The data of methane production obtained from the long-term, semi continuous tests were fit with the
454 first order kinetic model described in Section 2.4. In the phase of model calibration, the optimal set
455 of B_0 and k values were found by minimizing the objective function J . Details concerning the calculus
456 of the two parameters, namely the number of experimental data used for the model calibration and
457 the range of the values into which the optimal values of B_0 and k were searched for are reported in
458 SM4. A very good agreement between the experimental and the calculated data was obtained, as
459 shown in Figure 5a, 5b and 5c for the PS, raw and pre-treated WAS.

460



461

462 Figure 5. Daily and cumulative volumes of methane produced during the AD test involving the PS
 463 (a, test n. 1), the raw WAS (b, test n. 2) and the pre-treated WAS (c, test n. 3)

464 The model parameters that characterized the three substrates, PS, WAS and thermo-alkali treated
465 WAS, namely B_0 , k and biodegradability (Y) are reported in Table 4.

466

467 Table 4. Values of the B_0 and k parameters and biodegradability (Y) for the three substrates, PS, WAS
468 and thermo-alkali treated WAS

Substrate	B_0 ($\text{Nm}^3 \text{CH}_4/\text{kgVS}$)	k ($1/\text{d}$)	Y (%)
Primary sludge	0.300 ± 0.000	0.520 ± 0.040	49 ± 0
Raw WAS	0.147 ± 0.000	0.085 ± 0.000	28 ± 0
Pre-treated WAS	0.250 ± 0.000	0.465 ± 0.020	48 ± 0

469

470 It can be seen from the figures of Table 4 that, the SMP obtained in the experimental test for the PS,
471 equal to $0.280 \text{ Nm}^3/\text{kg VS}$, approached the value obtainable from an AD process of infinite duration,
472 being the difference between the experimental SMP and B_0 of only 7%. The biodegradability was in
473 the order of 50%. The effect of the thermo-alkali pre-treatment on WAS was not only an increase in
474 the amount of biodegradable organic matter, from 28% to 48%, and, consequently, in the produced
475 methane (B_0 , + 70%), but, above all, an increase in the rate at which the substrate was made available
476 for the digestion process (k , + 447%). An increase in the k allows the AD process to be performed
477 with shorter HRTs and, consequently, with smaller reactors [42]. It was evident that the thermo-alkali
478 pretreatment modified the behavior of the WAS in an AD process, thus making it quite similar to that
479 of PS, in terms of biodegradability and biogas potential production.

480 The results predicted by the model for the pre-treated WAS make the introduction of the thermo-
481 alkali pre-treatment in the sludge line of the Castiglione Torinese WWTP a promising and beneficial
482 option. Oosterhuis et al. [43] obtained similar results after the introduction of a pilot-scale Cambi
483 thermo-hydrolysis process, running at 165°C and 6 bars for 20 minutes, at the Hengelo WWTP (The
484 Netherlands). They observed an increase in the Y parameter from 26% to 42% after WAS pre-
485 treatment. In the present study, the pre-treatment carried out in less severe conditions (90°C , 30 min,
486 in the presence of NaOH) determined an increase in the substrate biodegradability (Y) from 28% to
487 48%. Gianico et al. [44] observed values of the maximum methane production parameter (B_0) of
488 $0.154 \text{ Nm}^3 \text{CH}_4/\text{kgVS}$, for raw WAS, and of $0.223 \text{ Nm}^3 \text{CH}_4/\text{kgVS}$ for the WAS after a thermal lysis
489 process (134°C , 3 bars, 30 min). Gianico et al. [44] carried out the pre-treatment under conditions
490 that were milder than those of a typical thermo-hydrolysis process. It can be seen that the values by
491 Gianico et al. [44] were very similar to those found in this study. Recently, Guerrero Calderon et al.
492 [42] demonstrated that a free nitrous ammonia pre-treatment could increase the rate of hydrolysis (k)
493 of WAS from 22-33% to 54-66%, depending on the presence of primary treatments in the water line
494 of a WWTP. They observed k values of 0.20 d^{-1} for raw WAS and of $0.28 - 0.34 \text{ d}^{-1}$ for treated WAS.

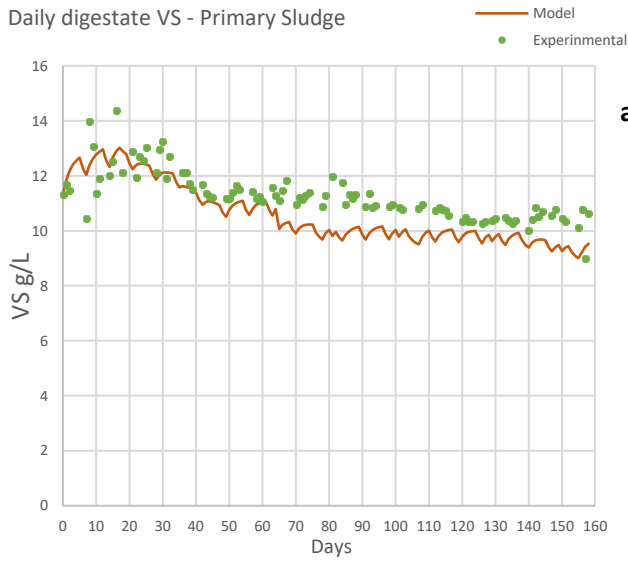
495 The thermo-alkali pre-treatment carried out in this study seemed to have a larger /more intense impact
496 on the rate at which the organic substrate was made available for the AD process. He et al. [45] tested
497 a pre-treatment method based on the reflux of the digestion liquid back to the WAS digestion unit
498 (pH 9.5 for 24 h). The extent of the maximum methane production parameter (B_0) found by He et al.
499 [45] (282.5 mL/gVS) was quite close to that of this study (0.250 NmL/g VS). Finally, Kim et al. [46]
500 evaluated the potential of a series of lower ($< 100^\circ\text{C}$) and higher ($> 100^\circ\text{C}$) thermal pre-treatments
501 applied to samples of dewatered sludge collected from a municipal WWTP and a brewery WWTP in
502 Hongcheon, South Korea. The study demonstrated that thermal pre-treatments (mainly carried out at
503 high temperatures) had a very good capacity in improving the methane production of the substrate
504 (+81% with respect the control), but their potentiality was in general lower than that of the
505 combination of milder temperatures and alkali substances.

506

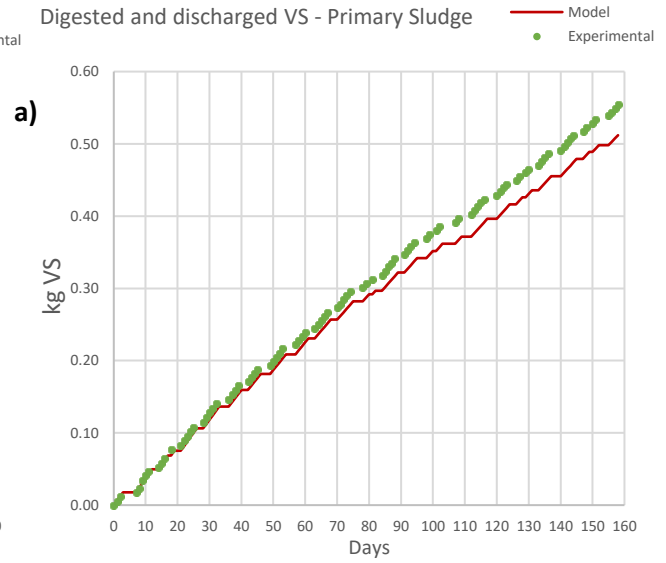
507 3.1.2 Model validation for the single substrates (PS, WAS, pre-treated WAS)

508 After the calibration, the model was validated for each of the three substrates by using the VS
509 remaining into the digestate after the digestion process. The phase of model validation made use of a
510 strong hypothesis, that is that the nature and composition of the biodegradable VS was the same of
511 non-biodegradable VS. Therefore, also the COD/VS ratio for both biodegradable and non-
512 biodegradable organic matter was the same. The model validation consisted in the comparison of the
513 daily amount of VS found in the digestate with the daily amount of VS predicted by the model. Figure
514 6 shows a very good agreement between the experimental and calculated data. The error values
515 between the sets of experimental and predicted data were equal to 7.3 %, 1.3% and 1.8% for PS, raw
516 WAS and thermo-alkali WAS respectively.

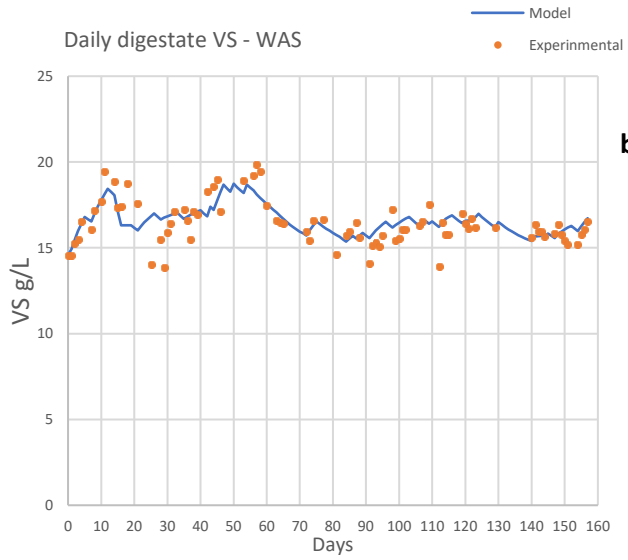
Daily digestate VS - Primary Sludge



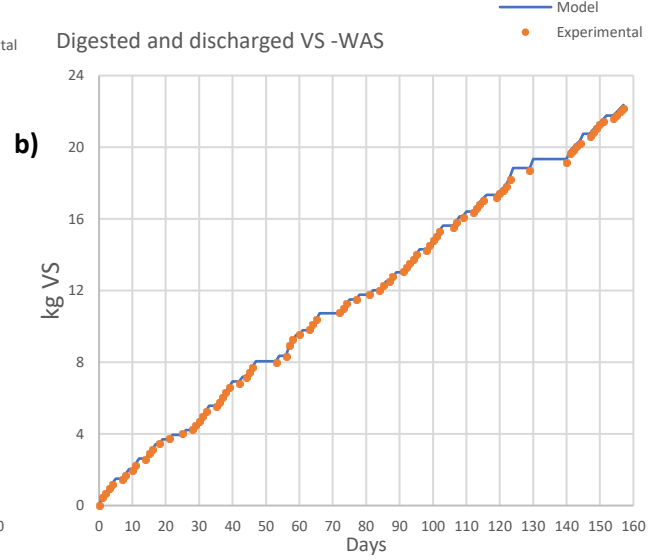
Digested and discharged VS - Primary Sludge



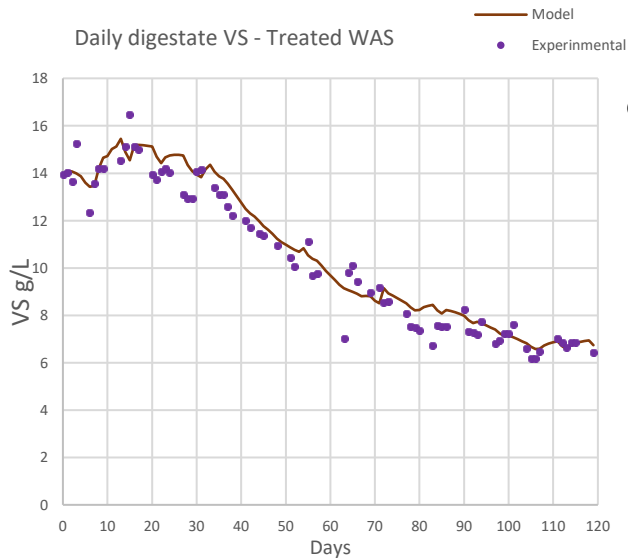
Daily digestate VS - WAS



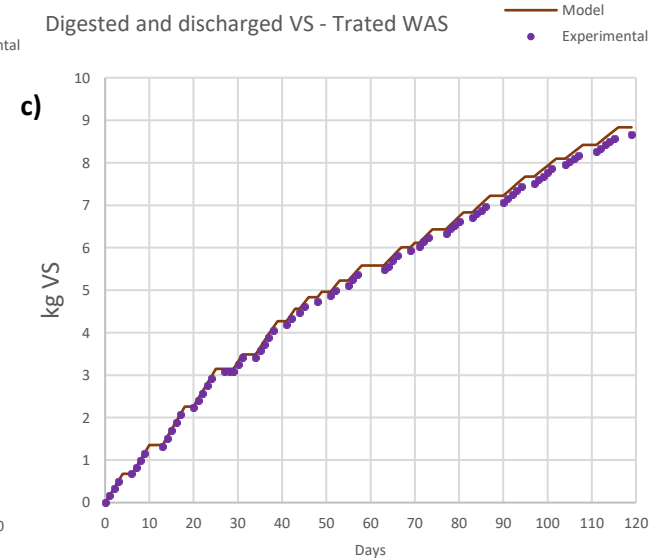
Digested and discharged VS - WAS



Daily digestate VS - Treated WAS



Digested and discharged VS - Treated WAS

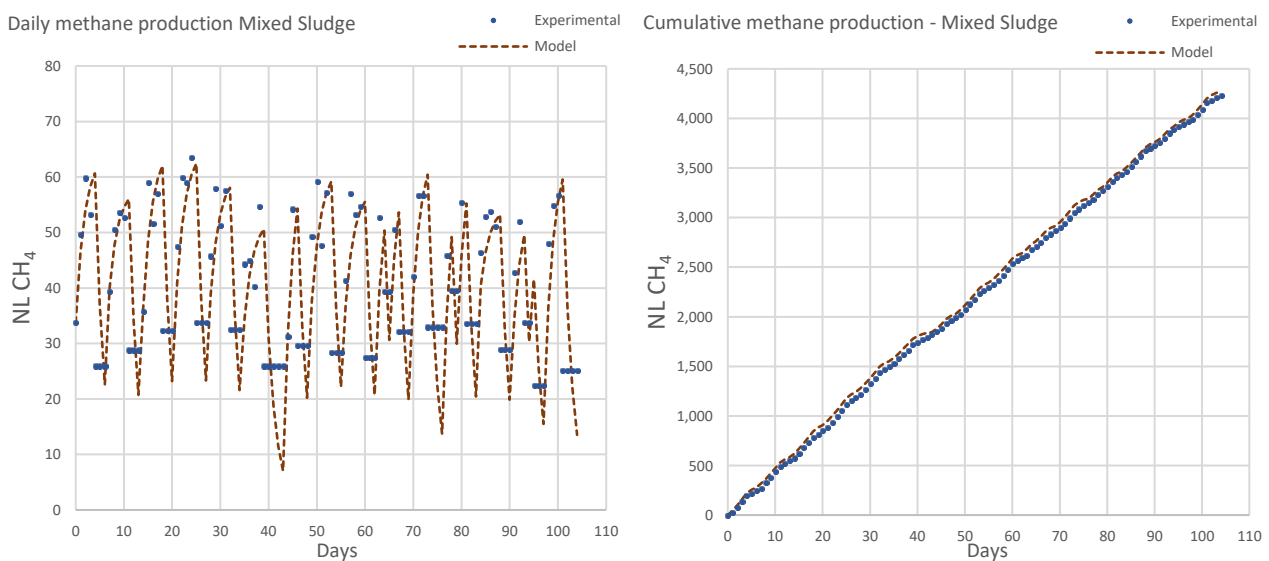


518 Figure 6. Daily digestate VS concentrations and cumulative discharged VS during the semi-
519 continuous AD tests involving the PS (a, test n.1), raw WAS (b, tests n.2) and treated WAS (c, tests
520 n.3).

521

522 3.1.3 Model validation for the mixed sludge and assessment of WWTP's new configurations

523 The model, calibrated and preliminary validated as described in Sections 3.1.1 and 3.1.2, was further
524 validated by using the results of the fourth AD test, carried out with the mixed sludge, 50/50 b.v. PS
525 and pre-treated WAS. Figure 7 shows the daily and cumulative volumes of methane produced during
526 the AD test.



527

528 Figure 7. Daily and cumulative volumes of methane produced during the AD test involving the mixed
529 sludge (test n. 4)

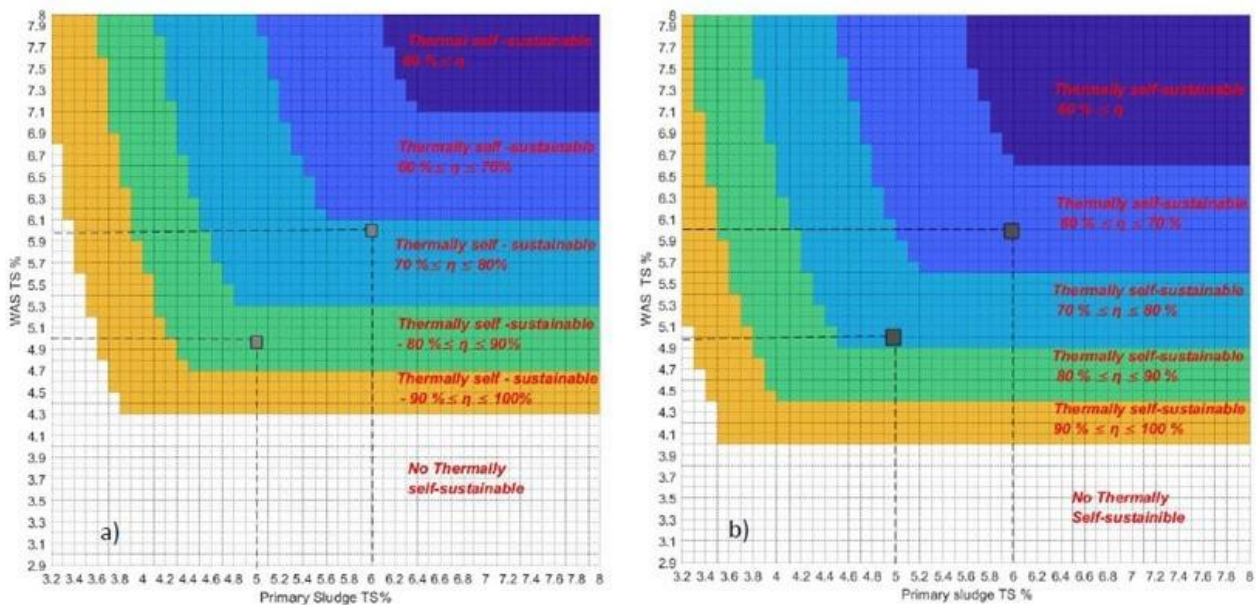
530

531 Values of B_0 and k found individually for the PS and the pre-treated WAS were used to predict the
532 methane production of a digester with an HRT of 20 days fed with the mixed sludge. The error values
533 of only 1.1% between the sets of experimental and predicted data, as in Figure 7, demonstrated that
534 the proposed model was robust and could be successfully used even to predict the production of
535 methane from an AD process where the feedstock was a mixture of substrates.

536 The model, with the key parameters B_0 and k , obtained as described in Sections 3.1.1 and 3.1.2 and
537 listed in Table 4, was used to compare the two possible future configurations of the WWTP sludge
538 line described in Section 2.6. The two novel configurations include the introduction of the thermo-
539 alkali pre-treatment on WAS and a one-stage or two-stage digestion scheme.

540 The results of the calculations demonstrated that the introduction of the thermo-alkali pre-treatment
541 determined an increase in the methane production from the digestion of WAS of 144% and 167%,

542 for the one-stage and two-stage plant's configuration, respectively. Considering that WAS represents
 543 only 36% of the TS fed to the WWTP digesters, the increase in the total methane production from the
 544 AD process was of 25% and 34% for the one-stage and two-stage configuration respectively.
 545 The data reported in Figure 8 were calculated by referring to the energy analysis carried out as
 546 described in Section 2.6. Figure 8 shows the ranges of TS concentration in PS and WAS that make
 547 the digestion processes carried out at the WWTP under one-stage (left) or two-stage (right) scheme
 548 self-sustainable on a thermal point of view. The position and the amplitude of each zone depends on
 549 the TS content of the two sludge and on the efficiency in heat transfer from the CHPs to the WAS.
 550 As expected, low TS contents for both sludges, in the order of 4%, require a high heat transfer
 551 efficiency. Conversely, high thickening performances, capable to produce substrates with a TS
 552 content of 7% or more, can tolerate/admit lower heat transfer efficiencies.
 553



554
 555 Figure 8. Ranges of TS concentration in PS and WAS that make the digestion processes carried out
 556 at the WWTP under one-stage (left) or two-stage (right) scheme thermally self-sustainable
 557

558 3.2 Effect of the thermo-alkali pre-treatments on the ammoniacal nitrogen release

559 3.2.1 Validation of the mathematical model to predict the N-NH₃ release to the digestate

560 The concentration values of N-NH₃ into the digestate, coming from the two long-term digestion tests
 561 that involved the raw and pre-treated WAS, were used to calibrate the model presented in Section
 562 2.5. The searched value for that model was the “perN-NH₃” parameter, that depends on both the
 563 composition of the substrate and the amount of VS fed to the digester. Details on the number of
 564 experimental data used for the model calibration and the range of the values into which the optimal

565 value of “perNH₃” was searched for are reported in SM4. Table 5 lists the values found for the “perN-
 566 NH₃” parameter for the digestion tests involving either raw or pre-treated WAS.

567

568 Table 5. Parameters used to calibrate the model predicting the ammonia release to the digestate and
 569 values of the “perNH₃” parameter

	Number of experimental data	Range of “perN-NH ₃ ” (g N-NH ₃ / g VS _{fed})	“perN-NH ₃ ” (g N-NH ₃ / g VS _{fed})
WAS	5	0.0-0.1 (step 0.0005)	0.0475 ± 0.0025
Pre-treated WAS	16	0.0-0.1 (step 0.0005)	0.0655 ± 0.0025

570

571 The thermo-alkali pre-treatment, carried out on WAS, determined not only an increase in the B₀ and
 572 k parameters, as reported in Section 3.1.1, but also in the capacity of the substrate to release N-NH₃
 573 to the digestate (see Table 5). In fact, the “perN-NH₃” parameter increased from 4.75% to 6.55%
 574 when the pre-treatment was applied. These findings are in line with the results of previous studies.
 575 Chen et al. [7] evidenced an increase in TN, in the supernatant of a pre-treated WAS, from 40.50
 576 mg/L to 112.27 mg/L, 143.84 mg/L, and 248.94 mg/L after alkali, microwave irradiation and
 577 ultrasonication pre-treatment, respectively. Specifically, the addition of NaOH used in an alkali pre-
 578 treatment could determine a reaction of saponification between the alkali agent and phospholipids,
 579 that is the main component of cell membranes, thus disrupting the cell and determining the release of
 580 intracellular constituents such as proteins [7].

581 The developed model, calibrated with the parameters B₀, k and “perN-NH₃” found in the experimental
 582 tests, was used to predict (i) the production of methane (SMP, Nm³/kg VS), (ii) the consumption of
 583 biodegradable solids (VS_{effluent}/VS_{fed}) and (iii) the release of ammonia (“perN-NH₃”
 584 gNH₄⁺effluent/kg VS_{fed}) in full-scale AD processes, involving raw or pre-treated WAS. The HRT
 585 values considered in the AD processes were of 14.8 days, that is the value at which the digestion of
 586 WAS in the Castiglione Torinese WWTP was carried out during the experimental period, and 20.0
 587 days, that is the value used for the tests of this study. Table 6 details the results obtained from the
 588 application of the model.

589 Table 6. Results obtained from the application of the model predicting the methane production and
 590 ammonia release to the digestate

	One-stage AD			Two-stage AD	
	Raw WAS	Raw WAS	tWAS	Raw WAS	tWAS
HRT (d)	14.8	20.0	20.0	10+10	10+10
SMP (Nm ³ CH ₄ /kg VS)	0.083	0.093	0.225	0.104	0.242
VS _{effluent} /VS _{fed}	0.84	0.82	0.55	0.80	0.54
NVS _{effluent} /NVS _{fed}	1.0	1.0	1.0	1.0	1.0
perN-NH ₃ (gN-NH ₄ ⁺ /kg VS _{fed})	26.5	29.9	59.1	33.6	63.4

591

592 As it can be seen from the values of Table 6, the introduction of a thermo-alkali pre-treatment on the
 593 WAS in the sludge line of the Castiglione Torinese WWTP, at the present HRT value (14.8 days),
 594 could be of benefit for the methane production, that would increase by 175%. The results listed in
 595 Table 6 show that a larger amount of VS was consumed, with a decrease in the residual VS content
 596 of the digestate from 84% to 54% (two-stage AD scenario). It can also be seen that, especially for the
 597 scenario that considers the implementation of the pre-treatments, the increase of HRT from 14.8 to
 598 20.0 days had a very limited impact on the SMP and VS consumption.

599 Furthermore, according to the data of Table 6, it can be seen that the pre-treatment determined an
 600 increase of the amount of N-NH₃ released to the digestate of more than 100% (123% at an HRT of
 601 20 days and 139% at the same value of HRT but with a two-stage AD). As a consequence of that, the
 602 managers of the WWTP should carefully assess if the existing biological processes intended to
 603 nitrogen removal in the water line can cope with the increase in the AN load due to the implementation
 604 of pre-treatments in the sludge line. Otherwise, on-purpose made treatments for the reduction of the
 605 nitrogen load, such as a side-stream Anammox, should be considered.

606 It can be estimated that the electric energy necessary to remove the extra amount of AN, due to the
 607 introduction of the pre-treatment, in the water line through a nitrification – denitrification process
 608 increased from 129 kW to 254 kW, for the one-stage AD scenario (HRT = 20 days), and from 145
 609 kW to 273 kW, for the two-stage AD scenario (HRT = 10+10 days). These values were obtained by
 610 referring to the unit electric power demand for nitrogen removal in the water line, equal 4.0 kWh/kg
 611 N, as reported in [47]. Whether it was possible to treat the AN in the liquid fraction of the digestate
 612 with a dedicated, side stream process, such as an Anammox process, the electric energy demand for
 613 the nitrogen removal would be in the order of 48 kW for the present situation, and of approx. 105-
 614 114 kW considering a future introduction of thermo-alkali pre-treatments. At Castiglione Torinese
 615 WWTP, SMAT recently introduced a DEMON process treating the reject water of sludge dewatering
 616 after AD. The process, based on partial nitritation and anaerobic ammonium oxidation carried out by

617 anammox bacteria, is particularly efficient in treating high nitrogen load streams and less energy
618 intensive than the traditional nitrification-denitrification scheme.

619

620 **Conclusions**

621 This study demonstrated that:

- 622 • first-order models could overcome the limitations due to discontinuity in experimentation and
623 provide reliable parameters (B_0 , k , “perN-NH₃”) to describe the production of gas and the
624 release of ammonia to the digestate when changes are introduced in existing WWTPs;
- 625 • secondly, low-temperature thermo-alkali pre-treatments could achieve comparable results, in
626 terms of methane production (B_0) and velocity at which the substrate was made available for
627 AD process (k), of some commercial, high-energy demanding treatments (e.g. Cambi).
628 Specifically, the introduction of the thermo-alkali pre-treatment determined an increase in the
629 methane production from WAS of 144% and 167%, for a one-stage and a two-stage digestion
630 configuration respectively;
- 631 • finally, the issue concerning the ammonia release is very worthy to being investigated
632 because, after pre-treatment, the cost for nitrogen removal in the water line, through traditional
633 processes of nitrification – denitrification, could increase even by 139%.

634

635

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641

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