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Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge

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Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge / Chiappero, M., Demichelis, F., Lin, X., Liu, C., Frigon, D., Fiore, S.. - In: PROCESS SAFETY AND ENVIRONMENTAL PROTECTION. - ISSN 0957-5820. - STAMPA. - 131:(2019), pp. 28-37. [10.1016/j.psep.2019.08.034]

Availability:
This version is available at: 11583/2756532 since: 2019-10-15T11:45:30Z

Publisher:
Elsevier

Published
DOI:10.1016/j.psep.2019.08.034

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http://dx.doi.org/10.1016/j.psep.2019.08.034

(Article begins on next page)

30 October 2026

Manuscript Details

Manuscript number	PSEP_2019_1124_R1
Title	Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge
Article type	Full Length Article

Abstract

This work analyzed the feasibility of pre-treatments to improve the anaerobic digestion (AD) of waste activated sludge (WAS) at 20 °C. We investigated different physicochemical pre-treatments (thermal at 115 °C, thermo-alkaline at pH 10 and 70 °C and ozonation at 190 mg-O3 L-1) by comparing their performances about COD solubilization and sludge disintegration rate. Best performances were obtained by thermo-alkaline pre-treatment, followed by thermal and ozonation; results were consistent with literature. Pre-treated WAS was fed to 12 1-L anaerobic digesters operated in semi-continuous mode. Thermal and thermo-alkaline reactors produced biogas yields (0.30-0.36 m3 kg-1 VS in standard conditions, 65-70 % methane) analogous to mesophilic conditions. The economic assessment of the scale-up of the whole process demonstrated that thermo-alkaline pre-treatment made AD at 20 °C economically profitable for WAS generated by a 20,000 PE WWTP.

Keywords	anaerobic digestion; biogas; low-temperature; pre-treatment; semi-continuous; waste activated sludge
Taxonomy	Water Treatment, Treatment, Sustainable Economy, Energy Engineering
Manuscript region of origin	Europe

Corresponding Author	Silvia Fiore
Corresponding Author's Institution	Politecnico di Torino
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Turin, August 28, 2019

Dear Prof. Stefano Dionisi, Associate Editor of Process Safety and Environmental Protection,

We would like to submit the revised version of the manuscript PSEP_2019_1124 **"Investigation of pre-treatments aimed at improving low-temperature anaerobic digestion of waste activated sludge"** by Marco Chiappero, Francesca Demichelis, Xuan Lin, Chenxiao Liu, Dominic Frigon and Silvia Fiore.

The manuscript was revised and improved according to the Reviewers' comments.

Thank you very much for your time and consideration

Sincerely yours,

Silvia Fiore (corresponding author, on behalf of all authors)

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- Semi-continuous anaerobic digestion was performed at 20 °C on waste activated sludge

- Physicochemical pre-treatments were investigated to improve biogas yields

- The assessment was based on COD solubilization and on disintegration rate

- Thermo-alkaline pre-treatment (0.09 g NaOH/g TS, 70 °C, 60 min) gave best results

- Biogas yields (0.30-0.36 m^3/kg VS, 65-70 % CH_4) were analogous to 35 °C conditions

Investigation of pre-treatments improving low-temperature anaerobic digestion of waste activated sludge

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Abstract

This work analyzed the feasibility of pre-treatments to improve the anaerobic digestion (AD) of waste activated sludge (WAS) at 20 °C. We investigated different physicochemical pre-treatments (thermal at 115 °C, thermo-alkaline at pH 10 and 70 °C and ozonation at 190 mg-O_3 L^{-1}) by comparing their performances about COD solubilization and sludge disintegration rate. Best performances were obtained by thermo-alkaline pre-treatment, followed by thermal and ozonation; results were consistent with literature. Pre-treated WAS was fed to 12 1-L anaerobic digesters operated in semi-continuous mode. Thermal and thermo-alkaline reactors produced biogas yields (0.30-0.36 m^3 kg^{-1} VS in standard conditions, 65-70 % methane) analogous to mesophilic conditions. The economic assessment of the scale-up of the whole process

demonstrated that thermo-alkaline pre-treatment made AD at 20 °C economically profitable for WAS generated by a 20,000 PE WWTP.

Keywords: anaerobic digestion; biogas; low-temperature; pre-treatment; semi-continuous; waste activated sludge.

Abbreviations: AD, anaerobic digestion; COD, chemical oxygen demand; DR, disintegration rate; EU, European Union; HRT, hydraulic retention time, OLR, organic retention time; OZ, ozone pre-treatment; PE, person equivalent; R_X, removal of X; S_{COD}, solubilization ratio; SBP, specific biogas production; SMP, specific methane production; SRT, solids retention time; TA, thermo-alkaline pre-treatment; TH, thermal pre-treatment; TS, Total Solids; VS, Volatile Solids; WAS, waste activated sludge; WWTP, wastewater treatment plant

1. Introduction

Annual waste activated sludge (WAS) production in EU is expected to reach 13 Mt of dry solids by 2020 (Milieu Ltd et al., 2008), due to Urban Wastewater Treatment Directive 91/271/EC and to the restrictive limits on nutrients removal imposed by current legislations (Panepinto et al., 2016). In addition, WAS management could be responsible of up to 50 % of the operating costs in a wastewater treatment plant (WWTP) (Appels et al., 2008). Consequently the implementation of environmental and economic sustainable WAS management technologies is crucial for any WWTP. WAS disposal strategies in EU-27 between 2006 and 2010 were mostly based on agricultural

reuse (44 %), incineration (22 %), composting (15 %), landfilling (11 %) and others (8 %) (Eurostat, 2018).

In EU-27 aerobic and anaerobic digestion (AD) are the most common WAS stabilization approaches (Kelessidis and Stasinakis, 2012). Anaerobic stabilization processes are usually preferred over aerobic ones for medium-sized WWTPs and larger because biogas can partially cover the energy requirements of the plant. AD is a complex degradation process involving four main phases: hydrolysis, acidogenesis, acetogenesis and methanogenesis (Van Lier et al., 2008). Hydrolysis, consisting in the disintegration of cells and complex organic structures into polymers, followed by their hydrolysis to simpler monomers, is regarded as the rate-limiting step (Bakhshi et al., 2018). The flocs structure and the presence of extra polymeric substances around the cells make WAS disintegration particularly critical (Zhen et al., 2017). This limitation is generally overcome by increasing the operating temperature to enhance the microbial activity (Appels et al., 2008). Mesophilic (35 °C) anaerobic digesters usually serve medium and large scale WWTPs and they can be energy self-sufficient for WWTPs sized over 50,000 person equivalents (PE). However, 70 % of Italian WWTPs are below 20,000 PE (Istat, 2018), a situation rather common throughout the EU. Moreover, AD processes can be limited by high requirements of thermal energy in colder climate countries (Rajagopal et al., 2017). In this framework, developing psychrophilic (below 20 °C) AD of WAS could be strategic; it has lower energy demand and has as main challenges the lower rate of fermentation as a consequence of decreased temperature and the low biodegradability of WAS during the initial phase of hydrolysis (Dev et al., 2019). Psychrophilic AD has been previously investigated for wastewater (Gomec, 2010), animal manure (Saady and Massé, 2016) and food waste (Rajagopal et al., 2017).

To our knowledge very few studies are available about low-temperature AD processes implemented on WAS (Bakhshi et al., 2018; Dev et al., 2019), however the key role of an adapted inoculum was already demonstrated (Dolejs et al., 2018).

Biological, mechanical, thermal, chemical processes and their combinations have been extensively investigated as pre-treatments within mesophilic AD processes applied on WAS deriving from urban (Carrère et al., 2010) and industrial wastewater (Demichelis et al., 2018a). Thermal pre-treatment is well-established at full-scale (Zhen et al., 2017). The application of heat in a wide temperature range (60-180 °C) can disintegrate cell walls and membranes of the active biomass in WAS, leading to partial solubilisation of intracellular components (Tyagi and Lo, 2011). Alkaline pre-treatments were reported to induce the disruption of cells due to high pH values and reactions between the alkali agent and cell walls (Tyagi and Lo, 2011). However, an excess of alkali may inhibit AD (Carrère et al., 2010). For this reason, alkaline processes have been often combined with thermal treatment, with the aim of reducing both alkali dose (Ruffino et al., 2016) and process temperature (Uma Rani et al., 2012). A recent study (Bakhshi et al., 2018) comparing AD of WAS at 35 °C with AD at 20 °C after pre-treatment with ozone, revealed the latter to produce more energy. However, additional research about pre-treatments implemented on low-temperature AD of WAS is strongly needed, with a specific focus on enhancing the produced biogas compared to the energy spent in the process. The present work was aimed at assessing the technical feasibility of low-temperature (20 °C) AD of raw and pre-treated WAS to investigate whether its efficiency could be comparable with a mesophilic process. Compared to (Bakhshi et al., 2018), the adopted approach involved the optimization of process parameters and the comparison of three physicochemical pre-treatments (thermal, thermo-alkaline,

92 ozonation), assessing the increase in WAS solubilization and biodegradability in terms
93 of solids removal and biogas production. An assessment of the economic profitability of
94 the scale-up of the overall process chain concluded the research.

95 **2. Material and methods**

96 *2.1. Waste activated sludge*

97 12 WAS samples were collected once per week from Régie d'Assainissement des Eaux
98 du Bassin La Prairie (RAEBL) WWTP (240,000 PE) in Saint Catherine, Quebec,
99 Canada. The treatment outline was made of preliminary processes, biological process
100 and settling. WAS samples were diluted from an initial total solids (TS) content of 4 - 5
101 %-wt to approximately 3 %-wt TS prior pre-treatments to achieve a constant TS amount
102 during the tests.

103 *2.2. Pre-treatments*

104 Thermal (TH), Thermo-alkaline (TA) and Ozone (OZ) pre-treatments were selected
105 according to previously discussed literature, optimized (see Supplementary Material)
106 and compared about the increased solubilization of the sludge, expressed as chemical
107 oxygen demand (COD). Pre-treatments were performed just after WAS sampling, then
108 the sludge was stored at 4 °C until use. Two assessment parameters were adopted:
109 solubilization ratio (S_{COD}), defined as the ratio between soluble and total COD
110 (respectively sCOD and tCOD), and disintegration rate (DR), which is the ratio between
111 the increase in sCOD due to the pre-treatment and the maximum possible variation in
112 sCOD (Kim et al., 2013; Li et al., 2012) (Eq.1).

113 $$DR(\%) = \frac{sCOD_T - sCOD_o}{tCOD_0 - sCOD_0} \cdot 100 \quad (1)$$

where $sCOD_T$ is the soluble COD after treatment; $sCOD_0$ and $tCOD_0$ are respectively the soluble and the total COD before treatment.

2.2.1. Thermal and Ozone pre-treatment

Thermal pre-treatment (TH) was carried out in a pressure cooker (Instant Pot, IP-DUO80) on 0.75 L WAS samples at 115 - 118 °C and 0.8 bar for 30 min. Temperature value was chosen according to previous studies (Tyagi and Lo, 2011; Carrere et al., 2010) while the 30 min extent was defined after three COD solubilization tests (T1, T2 and T3) (Table 1 and Supplementary Material) performed on 0.3 L WAS samples. The effect of the cooling phase at the end of TH was investigated by testing two cooling modes: at room temperature and in ice-bath, the latter aiming to abruptly interrupt the effect of the temperature at the end of pre-treatment.

Ozone pre-treatment (OZ) was performed as in Bakhshi et al. (2018), adopting an average dose of 190 mg O_3 L^{-1}.

Table 1. Operating conditions of COD solubilisation tests for the optimization of thermal and thermo-alkaline pre-treatments.

Thermal tests	operating conditions		
	pre-treatment time (min)	cooling time (min)	cooling mode
T1	30, 60, 90, 120	30 – 40	room temperature
T2	10, 20, 30, 45, 60, 90, 120	15 - 20	ice bath
T3	30, 60, 90, 120	30 - 40	ice bath
	30, 60, 90, 120	15 - 20	room temperature
Thermo–alkaline tests	operating conditions		
	target pH	pre-treatment time (min)	
TA1, TA2, TA3	9	0, 30, 60, 90, 120	

	10	0, 30, 60, 90, 120
	11	0, 30, 60, 90, 120

130

131 *2.2.2. Thermo-alkaline pre-treatment*

132 Thermo-alkaline pre-treatment (TA) was operated at 70 °C for 60 min with 0.09 g
133 NaOH g^{-1} TS (defined after three COD solubilisation tests TA1, TA2 and TA3) (Table 1
134 and Supplementary Material). Different doses of 5 N NaOH were added to 0.6 L WAS
135 samples until pH 9, 10 and 11, then each sample was split into five 0.1 L subsamples
136 further treated at 70 °C in a water bath for increasing time intervals (measured after
137 target temperature value was reached). Sludge samples were then cooled and pH was
138 adjusted to 7.0 - 7.5 with 10 N HCl.

139 *2.3. Anaerobic Digestion tests*

140 Twelve AD reactors (3 for each pre-treatment and 3 fed with raw WAS) were operated
141 at 20 °C in semi-continuous mode for 80 days with solids retention time (SRT) and
142 hydraulic retention time (HRT) equal to 15 days (Uma Rani et al., 2012). Each reactor
143 consisted of a 1-L Pyrex glass bottle, equipped with a polypropylene screw thread cap,
144 wrapped in aluminium foil and mixed through a magnetic stirrer (model 801, Apera
145 Instruments). Two holes in the cap allowed feeding and biogas collection in a 1-L gas
146 bag (30226-U, Supelco, Sigma-Aldrich). The experimental procedure started with a
147 start-up phase (30 days), in which the reactors were filled up to 0.8 L with digestate
148 from the mesophilic digester of the RAEBL WWTP as inoculum (Table 1). During the
149 start-up phase, lasted two SRTs, three times per week the reactors were fed by OZ-
150 WAS. Afterwards, the test phase lasted 50 days, corresponding to 3.3 SRTs, as it may
151 be assumed that steady state was reached after 3 SRTs (Bakhshi et al., 2018; Liao et al.,

2006). During the test phase, the four types of feeds (raw WAS, TH, OZ, TA) and the digestate from the reactors were characterized once per week evaluating soluble and total COD, total solids (TS) and volatile solids (VS). Biogas production was measured every 2 - 4 days and biogas was characterized at the end of the test phase. Digestate pH was checked at each feed (3 times per week).

2.4. Analytical procedures

Chemical oxygen demand (COD) was measured through colorimetric method 5220D (APHA-AWWA-WEF, 2005). Prior sCOD analysis the samples were centrifuged at 20 x 10^3 g (Legend Micro 21, Sorvall™, Thermo Fisher Scientific centrifuge) and the supernatant was filtered through a 0.45 μm membrane. TS and VS were analyzed by gravimetric methods 2540B and 2540E (APHA-AWWA-WEF, 2005). pH was measured with a Thermo Fisher Scientific 710A Orion pH/ISE meter. Daily specific biogas production (SBP) was measured through water displacement (Bakhshi et al., 2018) and referred to standard conditions. Methane content in biogas was analyzed by means of an Agilent 7820A gas chromatograph equipped with a PoraPLOT Q capillary column (25 m × 0.32 mm × 10 μm, Agilent) and a TCD detector.

2.5. Sensitivity analysis

All analyses were carried out in triplicates and average values are reported in the study together with standard deviation. Statistical tests on experimental data were carried out using data analysis extension of Microsoft Excel 2016. A correlation test investigated the presence of linear correlation between pairs of variables, considering significant those having $p < 0.05$.

174 *2.6. Scale up evaluation*

175 Energy and economic assessments were performed and simulated using SuperPro
176 Designer® 8.0 software considering three scenarios: S0 - AD at 35 °C of WAS coming
177 from secondary settling; S1 - AD at 35 °C of WAS coming from the same WWTP
178 considered in this study; S2A - TH pre-treatment + AD at 20 °C; S2B - THA pre-
179 treatment + AD at 20 °C; S2C - OZ pre-treatment + AD at 20 °C. In S0 and S1
180 scenarios, sludge characteristics and biogas yields were respectively based on (Ruffino
181 et al., 2016) and (Bakhshi et al., 2018). S2 scenarios were simulated considering the
182 experimental data gathered in this work.

183 *2.6.1 Energy assessment*

184 The energy assessment was carried out under thermodynamic equilibrium and steady
185 state conditions, considering atmospheric air (79 % $_{v/v}$ N_2 and 21 % $_{v/v}$ O_2), assuming
186 valid the ideal gas law and negligible gas leaks from connecting pipes (Mehr et al.,
187 2017). The net energy load (Q_n), expressed in MJ/d, was calculated considering the
188 seasonal temperature average variations in Europe (IPCC, 2017) and it was expressed as
189 the sum of energy consumed (Qc) and energy produced (Q_p) (Eq. 2).

190 $$Q_n = Q_c + Q_p \quad (2)$$

191 Qc was the sum of: energy required (Q_{req}) to heat the pre-treatment units (TH at 118 °C,
192 TA at 70 °C, O at 20 °C) and AD reactor (at 20 °C) (Eq. 3); energy to mix (Q_{mix}) the
193 pre-treatment units and AD reactor (Eq.4); energy losses (Q_{loss}) from external and
194 ground walls of AD reactor (Eq. 5); energy to transfer ozone (Q_{O3}) to the inoculum and
195 to perform OZ pre-treatments (Eq. 6).

196 $$Q_{req} = m_{sludge} \cdot c_{sludge} \cdot (T_{reac} - T_{in}) \quad (3)$$

where m_{sludge} is the sludge mass flow rate [kg/d], while T_{reac} and T_{in} are respectively the reactor and inlet temperatures, and c_p is the specific heat capacity (4200 $\frac{J}{kg\,°C}$)

$$Q_{mix} = P_{mix} \cdot t_{mix} \quad (4)$$

whereP_{mix} is the mixing power [J/h] and t_{mix} the required time to mix the sludge [h/d]

$$Q_{loss} = U_{ug} \cdot A_{ug} \cdot (T_{reac} - T_{gr}) + U_{ext} \cdot A_{ext} \cdot (T_{reac} - T_{ext}) \quad (5)$$

where according to (Mehr et al., 2017) U_{ug} and U_{ext} are respectively the coefficients of heat transfer for underground walls (2.33 $\frac{W}{m2\,°C}$.) and external walls (0.93 $\frac{W}{m2\,°C}$.); A_{ug} and A_{ext} are respectively the areas of underground walls and external walls; T_{gr} and T_{ext} are respectively the temperatures of underground walls and partial walls.

$$Q_{O3} = O_{3\,dose} \cdot m_{sludge} \cdot Elec_{\,O3} \quad (6)$$

where $Elec_{\,O3}$ is the energy required to perform OZ and according to (Bakhshi et al., 2018), equal to 12.5 $\frac{kWh}{kgO3}$.

Q_p was the sum of two items: energy from methane production (Q_{CH4}) (Eq. 7) and energy from heat recovery (Q_r) (Eq. 8)

$$Q_{CH4} = V_{CH4} \cdot \eta_{el} \cdot 39.4\,\frac{MJ}{m3} \quad (7)$$

where η_{el} is assumed to be 0.35.

$$Q_r = \eta \cdot (T_{ex-hot} - T_{ex-cold}) \cdot m_{sludge} \cdot c_{sludge} \quad (8)$$

where η is the heat exchanger efficiency equal to 70% according to (Ruggeri et al., 2015), T_{ex-hot} is the temperature of the AD reactor (20 °C) and $T_{ex-cold}$ depends on the season.

The energy sustainability is achieved if the energy sustainability index (ESI) (Eq. 9) is higher than 1.

219 $$\mathrm{ESI} = \frac{\mathrm{Q\,p}}{\mathrm{Q\,c}} \quad (9)$$

220

221 *2.6.2 Economic assessment*

222 The economic analysis was aimed to define the minimum plant size able to be
223 economically profitable considering 365 working days per year. The assessment was
224 based on the experimental data presented in this work and related to existing AD plants
225 (Table 2), while costs evaluation was consistent with Chemical Engineering Plant Cost
226 Index (Peters and Timmerhaus, 2003). Economic analysis considered capital and
227 operational costs and revenues. Capital costs were made of fixed capital investment
228 (FCI, consisting in equipment purchase for plant construction and working capital cost,
229 which is 6.5 % of FCI) (Pommerat et al, 2017). The cost of land wasn't taken into
230 account since the AD plant was hypothesized in the WWTP area. A 5-years
231 amortization with a 2 % interest was assumed for the capital costs (Eq. 10):

232 $$A[Euro] = C_0 \cdot \frac{i \cdot (1+i)^n}{(1+i)^n - 1} \quad (10)$$

233 where A is the amortization cost, C_o is the initial capital cost i is the interest and n the
234 number of years considered for amortization. Operational costs included utilities,
235 digestate disposal and labor costs (Table 2). Sludge collection and transport were not
236 accounted since the AD plant was hypothesized in the WWTP area. This assumption
237 was the core of the further assessment of the scale-up of the overall process. Our idea
238 was to optimize WAS management in WWTPs through an on-site process, with two
239 positive consequences: 1. Biogas/methane production, which is needed to heat the
240 digester and could eventually, if in excess, be valorized to fulfil the energy needs of the

241 WWTP; 2. Decreasing the costs of the final disposal of the digestate (whose volume is
242 inferior compared to WAS).
243 Labour cost is considered an addition to the current staff of the WWTP; consequently 2,
244 3, 4 and 5 workers were hypothesized respectively for WWTPs serving 5,000 to 20,000
245 PE; 50,000 to 200,000 PE; 500,000 PE and 1,000,000 PE.
246 The revenues came from the surplus of energy produced in the plant from AD and heat
247 recovery. The annual income was calculated as the difference between the revenue and
248 the amortization for the first 5 years and operational costs. The profitability was
249 evaluated through: return of interest (ROI) (Eq. 11), net present value (NPV) (Eq. 12)
250 assuming 20 years plant lifetime with 5 % discount on the future cash flows to the
251 present value, according to (Demichelis et al. 2018b).

252 $$ROI\ [\%] = \frac{Annual\ net\ profit}{Initial\ total\ investment} \cdot 100 \quad (11)$$

253 NPV represents the scenario profitability for the plant lifetime (20 years) considering a
254 5 % discount on the future cash flows to the present value. NPV > 0 means that the
255 process is profitable.

256 $$NPV\ [Euro] = \sum_{t-1}^{T} \frac{C_t}{(1+d)^t} - C_0 \quad (12)$$

257 where t is the plant lifetime, C_t is the net cash flow during period t, C_0 is the initial
258 capital investment and d is the discount rate. To conclude the economic profitability
259 assessment, Payback time is the time required to regain the investment cost.
260
261 **Table 2.** Details of economic analysis: capital and operational costs and energy values

Investment costs		
Equipment	Unit	reference

Reactor	€/m^3	2514.7	Dahiya et al., 2018
Stirrer	€/kW	46465.3	Akeberg and Zacchi, 2000
Operational costs			
Inoculum	€/m^3	4.1	Wingren et al. 2003
NaOH	€ /kg	0.27	Sigma-Aldrich, 2018
Digestate disposal	Euro/t	0.55	Arpa, 2017
Labour	€/year	44978	Eurostat, 2018
Revenue			
Energy value	€/kWh	0.22	SNAM, 2018

262

263 **3. Results and discussion**

264 *3.1. Pre-treatments*

265 The characteristics of inoculum, raw WAS and pre-treated WAS are shown in Table 3.

266

267 **Table 3.** Physico-chemical characteristics of inoculum, raw WAS and pre-treated WAS
268 (TH: thermal pre-treatment; OZ: ozone pre-treatment; TA: thermo-alkaline pre-
269 treatment)

Parameter	inoculum	raw WAS	TH	OZ	TA
Dose				189 ± 53mg O_3 g^{-1} TS	0.08 ± 0.01g NaOH g^{-1} TS
pH	-	6.2 ± 0.3	6.0 ± 0.2	6.1 ± 0.3	7.3* ± 0.3
TS (g L^{-1})	25.5 ± 0.5	33.1 ± 1.9	33.2 ± 2.1	33.2 ± 2.0	36.0 ± 2.4
VS (g L^{-1})	17.2 ± 0.4	25.3 ± 1.4	25.3 ± 1.6	25.4 ± 1.5	25.3 ± 1.5
VS / TS (%)	-	76.9 ± 1.7	76.2 ± 1.8	76.5 ± 1.8	70.5 ± 1.9
tCOD (g O_2 L^{-1})	17.2 ± 0.6	37.8 ± 2.9	37.8 ± 2.0	39.2 ± 3.3	38.0 ± 2.7

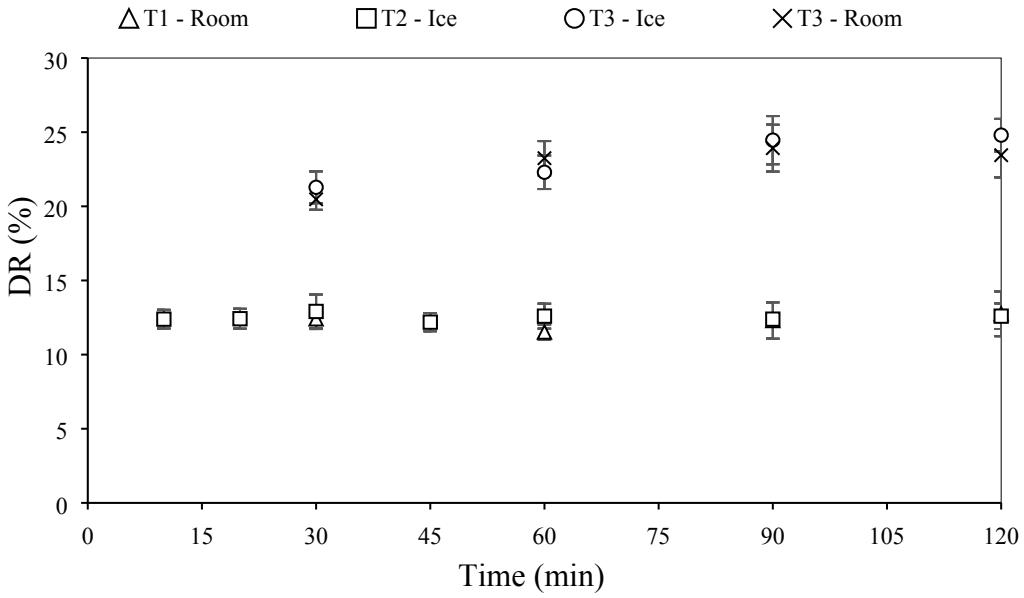
sCOD (g O_2 L^{-1})	1.3 ± 0.1	2.7 ± 1.0	9.9 ± 1.3	4.0 ± 1.1	14.3 ± 0.8
sCOD/tCOD (%)	-	7.3 ± 3.2	26.2 ± 3.6	10.2 ± 2.9	37.8 ± 4.0
DR (%)	-	-	20.5 ± 4.2	3.7 ± 0.9	33.3 ± 3.3

*after pH conditioning

3.1.1. Optimization of operating conditions

TH was operated at 115 – 118 °C. Higher temperatures (160 - 180 °C) were shown to be more efficient (Bougrier et al., 2008; Carrère et al., 2010) but high energy demanding (Appels et al., 2010) Low-temperature (< 100 °C) pre-treatments need longer durations (from hours to days) (Ferrer et al., 2008; Xu et al., 2014). Therefore an intermediate temperature value was preferred (Ennouri et al., 2016; Jeong et al., 2007). The influence of the pre-treatment time on COD solubilization was investigated within three tests (T1, T2 and T3) (Table 1). The obtained DR values were: 11 – 13 % in T1 and T2; 21 – 25 % in T3 (Figure 1), while the starting ratio between sCOD and tCOD before treatment was comparable (around 8 %). Full details about T1, T2 and T3 tests are in Supplementary Material. The gathered results are consistent with literature: Kim et al. (2003) obtained 10.4 % DR by autoclaving WAS (38.0 g L^{-1} TS) at 121°C and 1.5 atm for 30 min; a thermal pre-treatment on WAS at 120 °C for 30 min led to 22 – 23 % DR (Jeong et al., 2007); heating WAS (14.26 ± 2.18 g L^{-1}) at 121 °C and 1 bar for 15 min produced 15.7 % DR (Salsabil et al., 2010). A slight influence of time on solubilization of sludge during a thermal pre-treatment of WAS at 130 °C was already observed (Valo et al., 2004). Our research did not find a significant influence of pre-treatment time in improving COD solubilization, as DR was already stable after 30 min. The cooling mode at room temperature or in ice-bath did not determine significant influences on COD solubilization (see Figure 1).

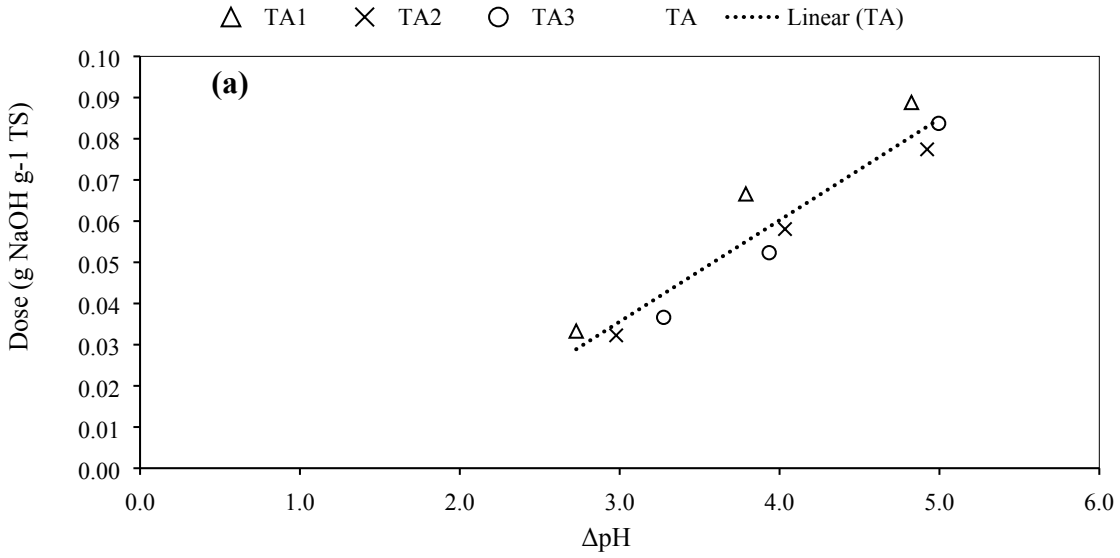
Figure 1. Optimization of thermal pre-treatments through COD solubilisation tests (T1, T2, T3) with two cooling modes (ice bath and at room temperature)

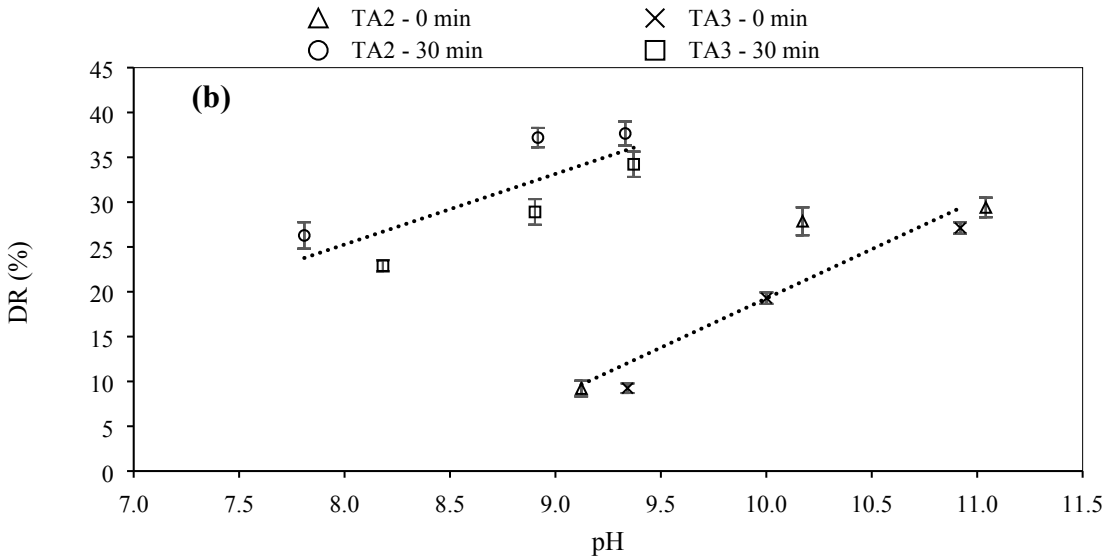


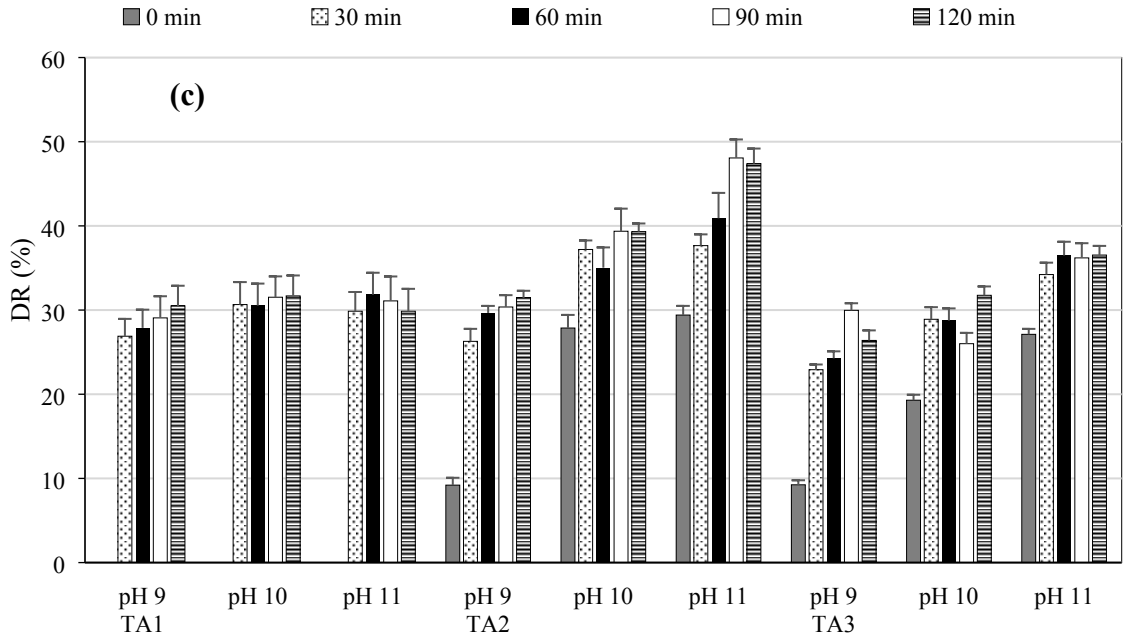
TA was based on sodium hydroxide, which was found to determine better solubilisation than other alkali agents (Kim et al., 2003). The alkali doses corresponding to pH 9, 10 and 11 were selected from literature (Uma Rani et al., 2012; Xu et al., 2014). The temperature value of 70 °C was chosen as a compromise between pre-treatment performance and energy costs (Kim et al., 2013; Ruffino et al., 2016). The effect on COD solubilization of the alkali dose and the thermal pre-treatment time was assessed by three tests: TA1, TA2 and TA3 (see section 2.2.1 and Figure 2). Full details are reported in Supplementary Material. The doses of NaOH needed for reaching pH 9, 10 and 11 were recorded during each test (Figure 2a). It was found a significant linear positive correlation between the alkali dose and the pH increase ($r(7) = 0.954$, $p < 0.05$). Figure 2b shows the DR trends for different pH values (and doses of NaOH) with and without any thermal pre-treatment at 70 °C for increasing times. An enhancement of

306 COD solubilisation linked to pH variations was observed: an increase of alkali dose
307 from pH 9 to 11 at room temperature determined a DR increase from 10 % to almost 30
308 % revealing a significant positive linear correlation between the initial pH and DR ($r\ (4) = 0.928$, $p < 0.05$). These DR values are in good agreement with previous studies
310 (Ruffino et al., 2016; Li et al. 2012). As for tests T1, T2 and T3, COD solubilisation
311 was linked to pH variations showing a significant linear correlation between pH and DR
312 ($r\ (4) = 0.815$, $p < 0.05$). However, comparing DR values obtained by adding NaOH
313 with or without thermal pre-treatment, it seemed that the lower duration of the pre-
314 treatment emphasised the effect of pH increase (Figure 2b). Further confirmations of
315 these patterns are in Supplementary Material. Figure 2c shows DR values obtained for
316 each combination of pH and thermal pre-treatment time in TA1, TA2 and TA3. The
317 results of each test were grouped into pH 9, 10 and 11 and each dose presented a group
318 of bars corresponding to increasing heating times from left to right. Overall, DR values
319 ranged from almost 10 % after the lower dose of NaOH to close to 50 % after the
320 thermal pre-treatment. As already observed, the effect of an increased dose of NaOH (0
321 min) is evident from TA2 and TA3, as well as the increase of DR due the thermal pre-
322 treatment (0 min versus 30 min). However, a variation of the pre-treatment time did not
323 seem to enhance COD solubilisation at 70 °C. For instance, in TA1 at pH 9 DR values
324 varied from 27 % to 30 % for pH 10 and pH 11 were respectively 31 – 32 % and 30 –
325 32 %. In TA3 DR values after the thermal pre-treatment at pH 9, 10 and 11 varied
326 respectively from 23 % to 30 %, from 29 % to 32 % and from 34 % to 37 %, Only TA2
327 seemed to suggest a slight effect of the duration on COD solubilisation. These results
328 are in agreement with Appels et al. (2010): a moderate increase of sCOD was observed,
329 if compared to higher temperatures, when heating WAS at 70 °C for 15 - 60 min;

330 furthermore, their results were in line with the outcomes of our thermal tests T1, T2 and
331 T3. A significant enhancement of COD solubilization, reaching DR values of 32 % in
332 TA1, 41 % in TA2 and 36 % in TA3 was observed, thanks to synergic effects of TA
333 pre-treatment, while avoiding the use of a high NaOH dose and its possible inhibition
334 problems (Li et al., 2012; Penaud et al., 1999) as well as limiting the energy
335 expenditures due to higher temperatures and pre-treatment durations. On the grounds of
336 the gathered results, we adopted the combination of pH 11 (0.089 g NaOH g^{-1} TS) and
337 60 min pre-treatment time as optimum for the subsequent AD tests.
338
339
340 **Figure 2**. Optimization of thermo-alkaline pre-treatments through COD solubilisation
341 tests (TA1, TA2, TA3): (a) dose of NaOH as a function of the pH increase; (b)
342 Disintegration Rate as a function of pH after 0 min and 30 min of TH; (c) Disintegration
343 Rate for different combinations of pH and treatment times







344

345

346 *3.1.2. Effect of pre-treatments on solubilization and characteristics of waste activated*
347 *sludge*

348 The optimal operating conditions for TH (115 – 118 °C for 30 min), OZ (190 mg O_3 L^{-1})
349 and TA (0.09 g NaOH g^{-1} TS at 70 °C for 60 min) pre-treatments were selected as
350 described in section 3.1.1. Table 1 reports the mean physico-chemical characteristics of

raw and pre-treated WAS according to the optimal operating conditions adopted during the AD tests. The pH of raw WAS, equal to 6.2, was close to the values measured on TH and OZ samples. However, pH 9.0 - 9.5 of WAS after TA was adjusted to 7.3 ± 0.3 before AD tests, to reach the optimum pH range for methanogenic bacteria, equal to 6.5 - 7.2 (Appels et al., 2008). TS were around 32 - 33 g L^{-1} for raw WAS, TH and OZ, while TA showed an increase up to 36 g L^{-1} as a result of the use of NaOH. Higher TS compared to raw WAS after alkali addition were previously observed (Valo et al., 2004), investigating a thermo-alkaline pre-treatment on WAS at 130 °C and pH 10. VS were stable for all samples around 25 g L^{-1}. Accordingly, the organic content of TA sludge was affected by TS variation down to 70 % compared to the 76 – 77 % of other samples.

Overall, the optimized pre-treatments seemed to enhance the sludge solubilisation. The sCOD of pre-treated samples increased compared to raw WAS: the sCOD of TA sludge raised by 4.3 times, sCOD of TH by 2.6 times while sCOD of OZ by 0.5 times. Moreover, the disintegration rate values after different pre-treatments were: DR_{TA} > DR_{TH} > DR_{OZ}. DR_{TA} value of 33 % was consistent with the results of TA1, TA2 and TA3 tests (see Supplementary Material) for pH 11 and 60 min. This value can be compared with other studies: Ruffino et al. (2016) and Campo et al. (2018) obtained DR values of 25 - 30% on WAS after thermo-alkaline pre-treatment at 70 °C for 90 min dosing 0.04 - 0.08 g NaOH g^{-1} TS. DR values of 64.8 % and 68.7 % were found by Kim et al. (2013) treating WAS with 0.1 M (about 0.24 g NaOH g^{-1} TS) and 0.2 M of NaOH at 75 °C for 6 hours. Demichelis et al. (2018a) achieved 39 % DR after a thermo-alkaline treatment (0.08 g NaOH g^{-1} TS) for 15 min at 50 °C on industrial WAS. In addition, 21 % DR_{TH} (achieved in October-December 2017) was close to the results of

test T3 (November 2017) but significantly different from those of T1 and T2 (September 2017), in accordance with the previous hypothesis. These results were in agreement with literature: a thermal treatment on WAS at 121 °C for 30 min gave a DR of 10.5 % (Kim et al., 2003); at 121 °C under 1 bar for 15 min a led to a DR of 15.7 % (Salsabil et al., 2010). DR_{OZ} around 4 % was significantly lower than the values achieved from other pre-treatments. The dose of 190 mg O_3 L^{-1} (corresponding to 0.01 g O_3 g^{-1} TS) adopted in the present study seemed to be too low to determine a significant COD solubilisation. The reported optimum dose of O_3 ranged between 0.05 and 0.5 g O_3 g^{-1} TS (Zhen et al., 2017). Bougrier et al. (2006) adopted 0.16 g O_3 g^{-1} TS obtaining a DR value around 22 % for WAS. In conclusion, TH and TA pre-treatments were able to enhance WAS solubilisation, while the adopted ozone dose was too low.

Table 4. Physico-chemical characteristics of digested sludge of each group of reactors after three SRTs (SBP: specific biogas production; SMP: specific methane production)

Parameter	Raw WAS	TH	OZ	TA
pH	7.00 ± 0.10	7.09 ± 0.08	7.06 ± 0.05	7.28 ± 0.05
TS (g L^{-1})	32.3 ± 0.04	24.0 ± 2.3	29.6 ± 0.3	28.1 ± 0.3
TS removal (%)	1.0 ± 0.1	26.5 ± 7.0	9.4 ± 0.8	14.1 ± 1.1
VS (g L^{-1})	23.5 ± 0.3	16.6 ± 1.2	20.9 ± 0.4	16.7 ± 0.2
VS removal	6.5 ± 1.2	33.8 ± 4.8	16.7 ± 1.7	33.2 ± 0.7
VS/TS (%)	72.5 ± 0.9	69.2 ± 1.4	70.6 ± 0.8	59.6 ± 1.4
VS/TS removal (%)	5.5 ± 1.2	9.8 ± 1.8	8.0 ± 1.0	22.3 ± 1.8
tCOD (g O_2 L^{-1})	37.4 ± 0.8	27.9 ± 3.8	31.7 ± 0.9	28.1 ± 2.8
sCOD (g O_2 L^{-1})	1.8 ± 0.02	3.6 ± 0.2	2.4 ± 0.1	3.7 ± 0.2
SBP (m^3 kg^{-1} VS_{IN})	0.21 ± 0.03	0.30 ± 0.03	0.24 ± 0.02	0.36 ± 0.001
CH_4 content (%)	69.3 ± 1.2	66.3 ± 1.6	69.2 ± 0.9	70.5 ± 0.2
SMP (m^3 kg^{-1} VS_{IN})	0.15 ± 0.02	0.20 ± 0.02	0.17 ± 0.02	0.25 ± 0.001

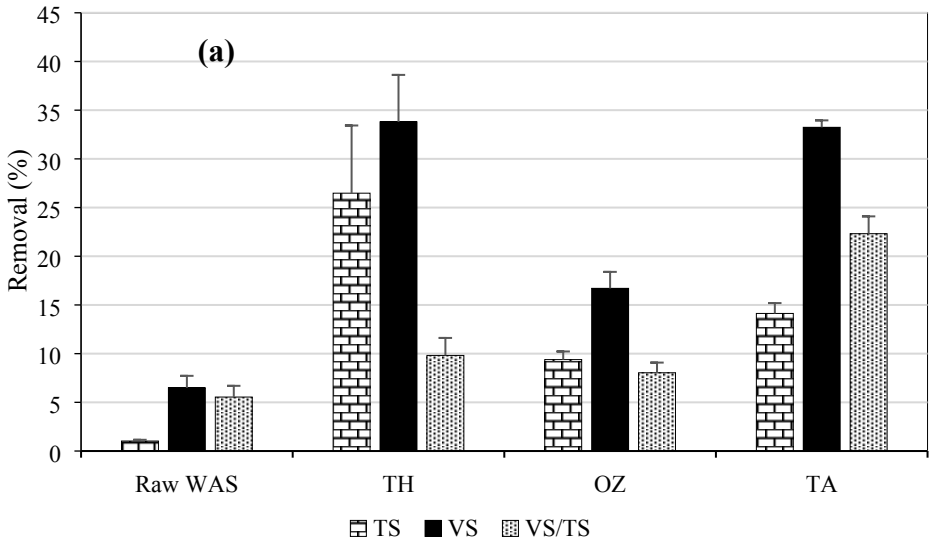
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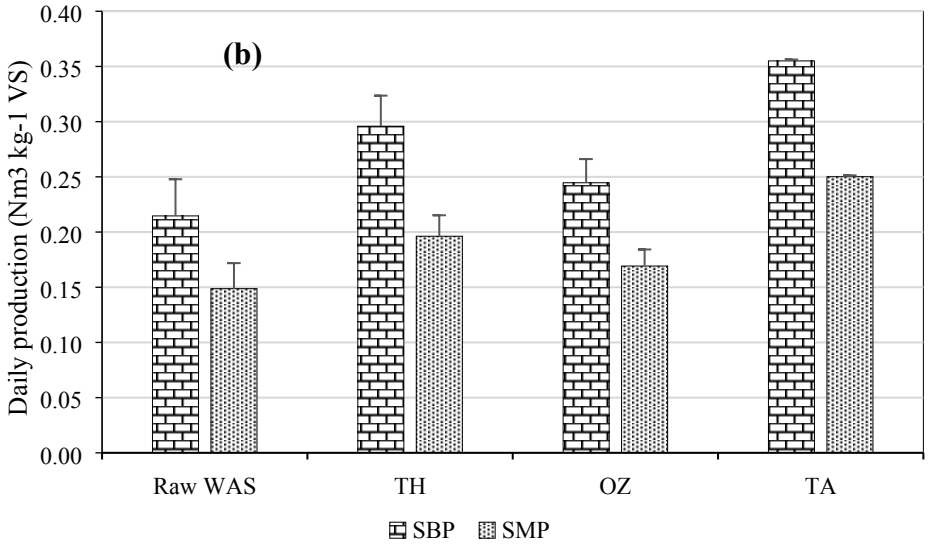
390 *3.2. Anaerobic digestion tests*

391 The performances of the different pre-treatments in improving AD were compared in
392 terms of solids removal and biogas production. Table 4 summarizes the mean physic-
393 chemical characteristics of digested sludge of each group of triplicate reactors at steady
394 state. Overall, after three SRTs the pH values of digestate were close to neutrality. The
395 daily organic loading rate (OLR) was evaluated for all AD tests at each feeding
396 operation to prevent any overload problems (data not shown). The average daily OLR
397 was 1.7 g VS_{IN} $L^{-1}d^{-1}$ for all reactors.

398

399 **Figure 3**. Results of anaerobic digestion test at 20 °C: (a) solids removal (TS, VS and
400 VS/TS); (b) specific biogas production (SBP) and specific methane production (SMP)





401

402 *3.2.1. Solids removals*

403 The first two main objectives of AD of any substrate are the reduction of the solids
404 content, assessed by the removal of TS, and its stabilisation (evaluated through the
405 removal of VS and VS/TS ratio). Figure 3a shows TS removal (R_{TS}) for the different
406 samples after three SRTs. It could be pointed out that TS removal was almost negligible
407 in the case of raw WAS. However, R_{TS} exceeded 25 % for TH, was around 15 % for TA
408 and about 10 % for OZ. The difference between removals of TH and TA could have
409 been affected by NaOH addition, as previously mentioned. Hence there was not
410 evidence of a better performance of TH compared to TA in terms of TS removal. VS
411 removal showed a clear difference between TH and TA. The higher mineral content
412 after TA helped to reduce VS/TS ratio of the digested sludge. Further confirmation
413 comes from the fact that VS removal for TH and TA were around 33 – 34 %. In the case
414 of OZ, R_{VS} was about 17 % and lower than 7 % for raw WAS. These results seemed to
415 be partially consistent with the increased COD solubilization of WAS by pre-treatments.
416 On one hand, the low COD solubilization induced by OZ corresponded to low removals
417 of TS and VS. On the other hand, the solubilization occurred for TA appeared to be

418 significantly larger than that of TH, however this was not followed by higher solids
419 removals during subsequent digestion process.
420 The gathered results in terms of solids removals can be compared with other studies
421 carried out in mesophilic conditions. The results of TA were lower than those of Xu et
422 al. (2014): in their study a thermo-alkaline treatment at pH 11 with NaOH for 10 h at 90
423 °C (DR = 43.7 %) and a thermal treatment at 70 °C for 9 h (DR = 27.9 %) led to VS
424 removal respectively equal to 46.2 % and 43.7 %, while 38.9 % was recorded for raw
425 WAS from batch AD tests. It can be noticed that both the duration and temperature of
426 thermo-alkaline pre-treatment were higher in Xu et al. (2014) than in the present study
427 and the removal of VS was significant also for the untreated WAS. However, our results
428 are consistent with the ones achieved by (Uma Rani et al., 2012) from AD tests in semi-
429 continuous mode after a thermo-alkaline pre-treatment on WAS with NaOH at pH 12
430 and 60 °C for 60 min, which resulted in a removal of TS and VS concentrations of 25.1
431 % and 33 %, in comparison with 9.6 % and 17 % for raw WAS. In addition, VS
432 removal gathered from TH was comparable to the results of Kim et al. (2003) related to
433 a thermo-alkaline pre-treatment with NaOH (7 g L^{-1}) at 121 °C for 30 min and a thermal
434 pre-treatment at 121 °C for 30 min, which determined VS removals respectively about
435 45 % and 30 %, in comparison with less than 15 % for raw WAS. On the contrary,
436 higher solids removals were found by Ennouri et al. (2016) who performed a thermal
437 pre-treatment at 120 °C and 1.5 atm for 30 min on urban and industrial WAS
438 determining respectively 74 % and 71 % VS removals in comparison with 48 % and 56
439 % for raw WAS. It was not possible to find literature data concerning TS and VS
440 removal during AD of WAS at 20 °C. In conclusion, it could be stated that WAS

441 biodegradability at 20 °C could be enhanced by TH and TA pre-treatments, while the
442 biodegradability of raw WAS seemed very low.

443

444 *3.2.2. Biogas and methane production*

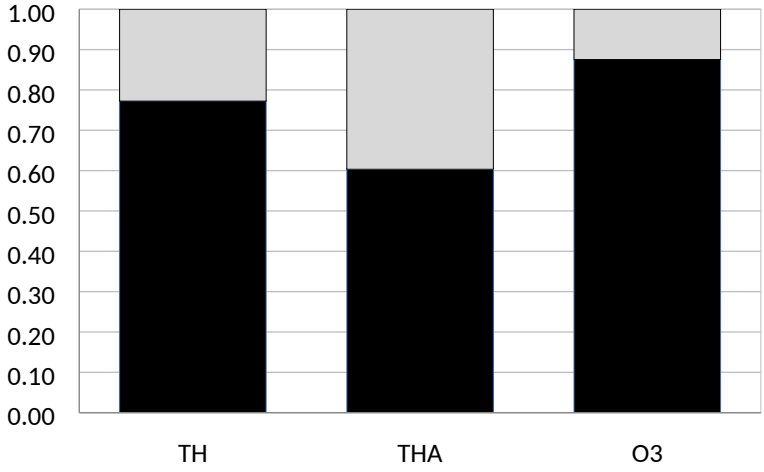
445 Figure 3b shows SBP and SMP values achieved from AD at 20 °C at steady state. SBP
446 for raw WAS was around 0.2 m^3 kg^{-1} VS_{IN} with 70 % methane. SBP values were
447 enhanced by pre-treatments: biogas production increased, compared to raw WAS, of 65
448 % for TA, 38 % for TH and 14 % for OZ. In addition, SBP values are consistent with
449 enhanced COD solubilization due to pre-treatments. TH and TA pre-treatments resulted
450 in similar VS removal values but TA led to higher biogas production. Methane
451 concentration appeared promising for all samples, ranging between 65 and 70 %-vv
452 (Table 4). Different pre-treatments did not seem to affect methane percentage in
453 comparison with raw WAS. As a result, SMP revealed an increase, compared to raw
454 WAS, of 68 % for TA, 32 % for TH and 14 % for OZ. SMP values are consistent with
455 the increased solubilization of organic matter produced by different pre-treatments, even
456 though TH and TA seemed to be equivalent in terms of solids removals. Overall, SBP
457 values varied from 0.21 (raw WAS) to 0.36 Nm^3 kg^{-1} VS_{IN} (TA), while SMP values
458 from 0.15 (raw WAS) to 0.25 m^3 kg^{-1} VS_{IN} (TA), in agreement with (Dolejs et al.,
459 2018), who observed SMP values equal to 0.22 m^3 kg^{-1} of COD added.
460 The results of this work are consistent with those of other studies carried out in
461 mesophilic conditions. Considering TH, a thermal pre-treatment on WAS at 121 °C for
462 60 min enhanced SBP from 0.35 to 0.42 L g^{-1} VSS_{IN} (Barjenbruch and Kopplow, 2003),
463 while at 90 °C for 60 min increased SBP from 0.035 to 0.377 L g^{-1} VS (Appels et al.,
464 2010). The increase of methane production due to TA was comparable with the results

of (Kim et al., 2013): a thermo-alkaline pre-treatment of WAS (12 g TS L^{-1}) with NaOH at 75 °C for 6 h achieved 70 % increase of methane production. As already mentioned, a thermo-alkaline pre-treatment of WAS (3 % TS) with NaOH (pH 11) at 90 °C for 10 h to an increase of biogas production from 0.396 to 0.605 L g^{-1} VS (Xu et al., 2014). Our TA results are also comparable with SBP values obtained by Ruffino et al. (2016), who performed a thermo-alkaline pre-treatment with 0.04 g NaOH/g TS at 70 °C for 90 min on WAS (5-6 % TS), obtaining an increase of the biogas production from 0.236 to 0.299 m^3 kg^{-1} VS (+26.8 %) through mesophilic AD in batch mode.

3.3. Scale-up: energy assessment

The energy assessment was performed for three scenarios (see section 2.6). The calculated ESI values were higher than 1 for all scenarios: from 200,000 PE for S0; from 100,000 PE for S1 and from 2,000 PE for S2 A, B and C. In details, ESI for S0 was 1.03 ± 0.10; for S1 1.04±0.08 and S2 A (TH + AD), B (TA +AD) and C (OZ +AD) were 2.09 ± 0.11, 2.63 ± 0.09 and 1.03±0.11 respectively. The standard deviation considered the temperature variation of WAS from January to December. The achieved ESI values are in agreement with (Ruggeri et al., 2015; Bakhshi et al., 2018). Among the three configurations of S2, S2-B (TA +AD) reached the highest ESI value, since the energy consumption due to the pre-treatment was the lowest (Figure 4). The percentage contribution in energy consumption of TH, TA and OZ were 77.22 %, 60.33 % and 87.53 % respectively and the detailed energy for S2 A, B, C are reported in Table 5.

Figure 4. Relative amounts of energy consumed for pre-treatments (black) and AD (grey) processes



488

489

490 **Table 5**. Details of energy assessment

	TH	THA	O
Q c[MJ/d]	104.57	83.76	209.30
Qreq for treatment [%]	58.11	40.87	2.73
Qmix for treatment [%]	0.57	1.42	0.57
Q O_3 for treatment [%]	0.00	0.00	73.61
Q req for AD [%]	5.46	6.81	2.59
Q mix for AD[%]	27.27	40.16	13.62
Q loss for AD[%]	4.50	10.73	4.53
Q O_3 for inoculum of AD [%]	4.09	0.01	2.35
Q p [MJ/d]	218.25	220.31	216.32
Q CH4 [%]	12.50	15.61	10.64
Q r [%]	87.50	84.39	89.36
ESI	2.09	2.63	1.03

491

492 *3.4. Scale-up: economic assessment*

493 The economic assessment was aimed to detect the minimum plant size that could be

494 profitable comparing the three different scenarios S0, S1 and S2 A, B, C (Figure 5).

495 According to (Eurostat, 2018), 24.99 kg/y PE of sludge were produced in EU28. The
496 detailed economic assessment (Table 6) proved for S0 a partial economic profitability at
497 500,000 PE and complete profitability after 1,000,000 PE with NPV equal to 19.05 M€,
498 ROI 67.69 % and payback time of 5 years. According to (Arnò et al, 2017), the
499 economic profitability should be reached at 50,000 PE combining AD of WAS and
500 organic fraction of municipal solid waste. For S1 a partial economic profitability was
501 achieved after 5-years amortisation after 1,000,000 PE, but ROI < 0, NPV < 0 and
502 payback time >20 y were obtained. Hence, for S1 the economic sustainability was not
503 reached.
504 For S2, the minimum plant size to reach the economic sustainability was equal to
505 50,000 PE for S2-A and 20,000 PE for S2-B, whereas S2-C didn't reach the economic
506 sustainability. Considering S2 proposed configurations, S2-B reached the best
507 performances with ROI equal to 45.16 %, NPV 0.21 M€ and payback time after 3 years.
508 No data are available for economic assessment of low temperature AD in literature,
509 nevertheless the economic profitability for 50,000 PE for S2-B exhibited the same order
510 of magnitude of mesophilic AD of WAS (Rosa et al., 2018; Zhang et al, 2019).

511 **Table 6.** Economic assessment of the three investigated scenarios (wV = working volume, V = volume)

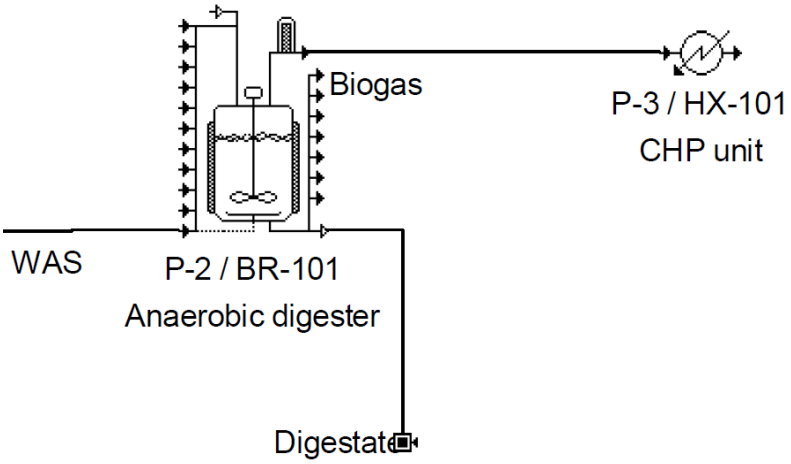
		PE	5.000	10.000	20.000	50.000	100.000	200.000	500.000	1.000.000
		wV unit pre-treatment [m^3]	0.34	0.68	1.36	3.40	6.79	13.59	33.96	679.29
		V unit pre-treatment [m^3]	0.42	0.85	1.70	4.25	8.49	16.98	42.46	849.11
		AD reactor wV [m^3]	5.09	10.19	20.38	50.95	101.89	203.79	509.47	10189.32
		AD reactor V [m^3]	6.37	12.74	25.47	63.68	127.37	254.73	636.83	12736.64
S0		Investment cost [M€]	0.03	0.033	0.05	0.09	0.17	0.09	0.59	3.8
		Operational cost [M€]	0.05	0.07	0.07	0.15	0.17	0.08	0.08	0.81
		Revenues [M€]	<0	<0	<0	<0	0.003	0.03	0.1	2.62
		Profitability first 5 years	<0	<0	<0	<0	-0.21	-0.74	-0.11	0.99
		Profitability after 5 years	<0	<0	<0	<0	-0.17	-0.05	0.012	1.81
		ROI [%]	<0	<0	<0	<0	1.74	29.32	16.44	67.68
		NPV [M€]	<0	<0	<0	<0	<0	<0	<0	19.05
		Payback time [y]	>20	>20	>20	>20	>20	>20	>20	5
S1		Investment cost [M€]	0.03	0.03	0.05	0.095	0.17	0.095	0.59	3.88
		Operational cost [M€]	0.05	0.07	0.07	0.15	0.17	0.082	0.09	0.81
		Revenues [M€]	-0.003	-0.004	-0.004	-0.003	-0.008	0.010	0.065	2.10
		Profitability first 5 years	-0.08	-0.11	-0.13	-0.25	-0.34	-0.16	-0.62	-2.59
		Profitability after 5 years	-0.05	-0.075	-0.079	-0.16	-0.17	-0.07	-0.021	1.29
		ROI [%]	<0	<0	<0	<0	<0	<0	<0	<0
		NPV [M€]	<0	<0	<0	<0	<0	<0	<0	<0
		Payback time [y]	>20	>20	>20	>20	>20	>20	>20	>20
S2	TH+AD	Investment cost [M€]	0.03	0.03	0.03	0.07	0.29			
		Operational cost [M€]	0.07	0.08	0.09	0.18	0.22			
		Revenues [M€]	0.02	0.04	0.09	0.22	0.42			

	Profitability first 5 years	-0.06	-0.04	-0.01	0.01	0.13
	Profitability after 5 years	-0.05	-0.03	0.00	0.03	0.19
	ROI [%]	<0	<0	<0	<0	43.53
	NPV [M€]	<0	<0	<0	0.32	2.06
	Payback time [y]	>20	>20	>20	3	8
TA+AD	Investment cost [M€]	0.03	0.03	0.03	0.07	0.29
	Operational cost [M€]	0.07	0.08	0.09	0.18	0.22
	Revenues [M€]	0.02	0.05	0.11	0.27	0.53
	Profitability first 5 years	-0.05	-0.03	0.01	0.07	0.25
	Profitability after 5 years	-0.05	-0.03	0.02	0.08	0.30
	ROI [%]	<0	<0	45.16	99.67	86.32
	NPV [M€]	<0	<0	0.21	0.99	3.55
	Payback time [y]	>20	>20	3	2	2
OZ+AD	Investment cost [M€]	0.03	0.03	0.08	0.16	0.29
	Operational cost [M€]	0.07	0.08	0.09	0.20	0.26
	Revenues [M€]	0.01	0.01	0.03	0.07	0.14
	Profitability first 5 years	-0.08	-0.08	-0.08	-0.16	-0.18
	Profitability after 5 years	-0.07	-0.07	-0.07	-0.13	-0.12
	ROI [%]	<0	<0	<0	<0	<0
	NPV [M€]	<0	<0	<0	<0	<0
	Payback time [y]	>20	>20	>20	>20	>20

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514 **Figure 5.** Process outlines of the considered scenarios: S0, S1, S2: (A) TH + AD; (B)
515 TA + AD; (C) OZ + AD

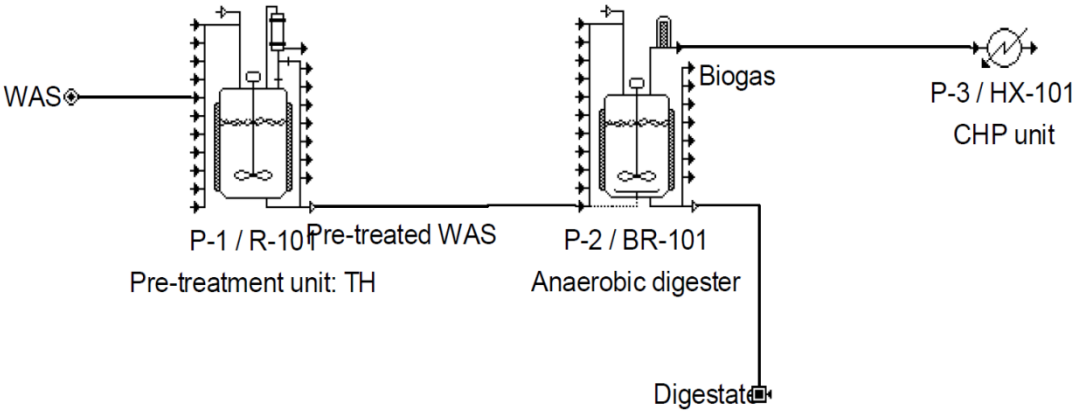


516 S0, S1

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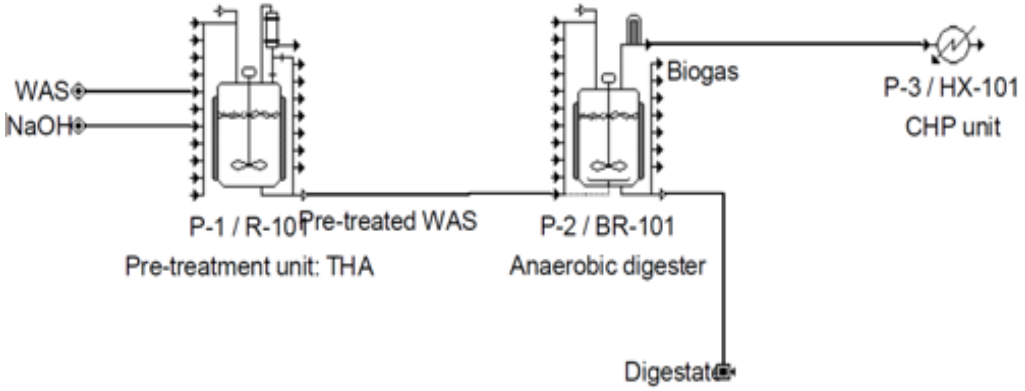


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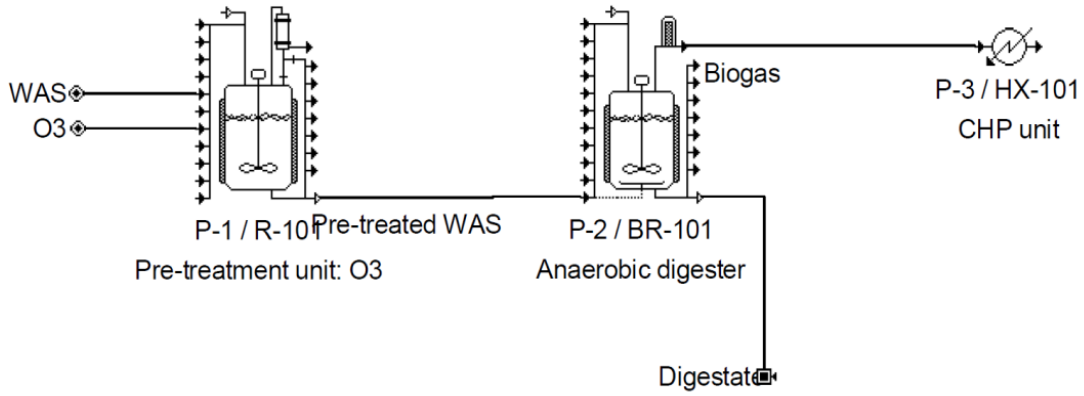
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532 **4. Conclusions**

533 This work investigated physic-chemical pre-treatments to improve the anaerobic
534 digestion of waste activated sludge at 20 °C. Thermo-alkaline pre-treatment, followed
535 by thermal and ozonation, achieved the best performances, in agreement with literature.
536 Biogas and methane yields obtained from semi-continuous reactors after thermal and
537 thermo-alkaline pre-treatments (0.30 - 0.36 m^3 kg^{-1} VS, 65 -70 % methane) were
538 equivalent to literature data referred to mesophilic conditions. The economic assessment

539 of the scale-up of the whole process demonstrated that thermo-alkaline pre-treatment
540 made AD at 20 °C was economically profitable for WAS generated by a 20,000 PE
541 WWTP. Anaerobic digestion at 20 °C was demonstrated to have a promising potential
542 and considering the almost complete lack of literature studies about psychrophilic
543 processes, further research is urgently needed.

544

545 **Acknowledgements**

546 The authors would like to thank RAEBL for the support to experimental activities. This
547 research was supported in part by a Natural Sciences and Engineering Research Council
548 of Canada grant (CRDPJ 500865-16) obtained in collaboration with Air Liquide
549 Canada. The authors declare no conflict of interest. Author's contributes: Marco
550 Chiappero performed the experimental activity, analyzed the results and wrote the draft
551 of the manuscript; Francesca Demichelis supported data analysis and performed the
552 energy and economic assessments; Xuan Lin and Chenxiao Liu contributed to the
553 experimental activity; Dominic Frigon and Silvia Fiore planned and supervised the
554 research and revised the manuscript.

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Table A1. Results of COD solubilisation tests T1, T2 and T3: determination of the optimum treatment time for thermal pre-treatment. Standard deviation values are given.

Test	Operating conditions		tCOD ($g\ O_2\ L^{-1}$)	sCOD ($g\ O_2\ L^{-1}$)	$sCOD_0$ / $tCOD_0$ (%)	($sCOD_t$ - $sCOD_0$) /$sCOD_0$ (%)	DR (%)
	Cooling mode	Treatment time (min)					
T1	Room temperature	0	49.26 ± 0.41	3.98 ± 0.10	8.1 ± 0.2	-	-
		30	-	9.60 ± 0.21	-	141 ± 8	12.4 ± 0.7
		60	-	9.20 ± 0.13	-	131 ± 6	11.5 ± 0.5
		90	-	9.55 ± 0.45	-	140 ± 14	12.3 ± 1.2
		120	-	9.76 ± 0.58	-	145 ± 17	12.8 ± 1.5
T2	Ice bath	0	94.27 ± 0.95	7.47 ± 0.13	7.9 ± 0.2	-	-
		10	-	18.23 ± 0.40	-	144 ± 8	12.4 ± 0.6
		20	-	18.25 ± 0.43	-	144 ± 8	12.4 ± 0.7
		30	-	18.68 ± 0.85	-	150 ± 13	12.9 ± 1.1
		45	-	18.05 ± 0.39	-	142 ± 7	12.2 ± 0.6
		60	-	18.40 ± 0.58	-	146 ± 10	12.6 ± 0.8
		90	-	18.23 ± 0.08	-	144 ± 4	12.4 ± 0.3
		120	-	18.40 ± 0.61	-	146 ± 10	12.6 ± 0.9
T3	Ice bath	0	42.01 ± 0.49	3.31 ± 0.06	7.9 ± 0.2	-	-
		30	-	11.54 ± 0.33	-	249 ± 13	21.3 ± 1.1
		60	-	11.94 ± 0.36	-	261 ± 14	22.3 ± 1.1
		90	-	12.77 ± 0.55	-	286 ± 19	24.5 ± 1.6
		120	-	12.91 ± 0.34	-	290 ± 13	24.8 ± 1.1
	Room temperature	0	42.01 ± 0.49	3.31 ± 0.06	7.9 ± 0.2	-	-
		30	-	11.23 ± 0.19	-	240 ± 9	20.5 ± 0.7
		60	-	12.30 ± 0.36	-	272 ± 14	23.2 ± 1.2
		90	-	12.56 ± 0.53	-	280 ± 19	23.9 ± 1.6
		120	-	12.38 ± 0.50	-	274 ± 18	23.4 ± 1.5

Table A2. Results of COD solubilisation tests TA1, TA2 and TA3: determination of the optimum dose and treatment time for thermo-alkaline pre-treatment. Standard deviation values are given.

Test	Operating conditions		pH	Dose (gNaOH g^{-1}TS)	tCOD (g O_2 L^{-1})	sCOD (g O_2 L^{-1})	($sCOD_t$ - $sCOD_0$) /$sCOD_0$ (%)	DR (%)
	pH target	Treatment time (min)						
TA1	9	Before NaOH	6.42	0	30.62 ± 2.02	1.65 ± 0.09	-	-
		0	9.14	0.033	-	-	-	-
		30	7.77	0.033	-	9.44 ± 0.09	471 ± 29	26.9 ± 2.1
		60	7.69	0.033	-	9.73 ± 0.12	488 ± 31	27.9 ± 2.2
		90	7.66	0.033	-	10.07 ± 0.33	509 ± 39	29.1 ± 2.6
		120	7.65	0.033	-	10.49 ± 0.16	534 ± 34	30.5 ± 2.4
	10	Before	6.41	0	30.62 ± 2.02	1.65 ± 0.09	-	-
		0	10.20	0.067	-	-	-	-
		30	8.98	0.067	-	10.53 ± 0.33	537 ± 40	30.7 ± 2.7
		60	8.93	0.067	-	10.50 ± 0.30	535 ± 39	30.5 ± 2.6
		90	8.88	0.067	-	10.78 ± 0.17	552 ± 35	31.5 ± 2.5
		120	8.85	0.067	-	10.83 ± 0.12	555 ± 34	31.7 ± 2.4
	11	Before	6.32	0	30.62 ± 2.02	1.65 ± 0.09	-	-
		0	11.15	0.089	-	-	-	-
		30	9.64	0.089	-	10.30 ± 0.11	523 ± 32	29.9 ± 2.3
		60	9.52	0.089	-	10.88 ± 0.24	558 ± 38	31.8 ± 2.6
		90	9.44	0.089	-	10.66 ± 0.43	545 ± 44	31.1 ± 2.9
		120	9.43	0.089	-	10.30 ± 0.35	523 ± 40	29.9 ± 2.7
TA2	9	Before	6.15	0	34.07 ± 0.56	2.49 ± 0.07	-	-
		0	9.12	0.032	-	5.39 ± 0.20	117 ± 11	9.2 ± 0.9
		30	7.81	0.032	-	10.79 ± 0.37	334 ± 20	26.3 ± 1.5
		60	7.80	0.032	-	11.85 ± 0.11	377 ± 13	29.7 ± 0.8
		90	7.72	0.032	-	12.08 ± 0.33	386 ± 20	30.4 ± 1.4
		120	7.66	0.032	-	12.42 ± 0.10	399 ± 14	31.4 ± 0.8
	10	Before	6.14	0	34.07 ± 0.56	2.49 ± 0.07	-	-
		0	10.17	0.058	-	11.29 ± 0.39	354 ± 21	27.9 ± 1.6
		30	8.92	0.058	-	14.23 ± 0.17	472 ± 17	37.2 ± 1.1
		60	8.94	0.058	-	13.55 ± 0.66	445 ± 32	35.0 ± 2.4
		90	8.87	0.058	-	14.92 ± 0.74	500 ± 36	39.4 ± 2.7
		120	8.88	0.058	-	14.90 ± 0.10	499 ± 16	39.3 ± 1.0
	11	Before	6.12	0	34.07 ± 0.56	2.49 ± 0.07	-	-
		0	11.04	0.077	-	11.77 ± 0.22	374 ± 16	29.4 ± 1.1
		30	9.33	0.077	-	14.38 ± 0.28	478 ± 20	37.7 ± 1.3
		60	9.24	0.077	-	15.40 ± 0.84	520 ± 40	40.9 ± 3.0
		90	9.26	0.077	-	17.67 ± 0.55	611 ± 31	48.1 ± 2.2
		120	9.17	0.077	-	17.46 ± 0.41	602 ± 26	47.4 ± 1.8
TA3	9	Before	6.07	0	39.58 ± 0.80	2.87 ± 0.04	-	-
		0	9.34	0.037	-	6.27 ± 0.14	118 ± 7	9.2 ± 0.5
		30	8.18	0.037	-	11.29 ± 0.08	293 ± 6	22.9 ± 0.6
		60	8.03	0.037	-	11.78 ± 0.19	310 ± 9	24.3 ± 0.8
		90	8.04	0.037	-	13.87 ± 0.14	383 ± 8	30.0 ± 0.8
		120	7.94	0.037	-	12.56 ± 0.34	337 ± 14	26.4 ± 1.2
	10	Before	6.07	0	39.58 ± 0.80	2.87 ± 0.04	-	--
		0	10.00	0.052	-	9.96 ± 0.13	246 ± 7	19.3 ± 0.6
		30	8.90	0.052	-	13.48 ± 0.43	369 ± 17	28.9 ± 1.4
		60	8.76	0.052	-	13.43 ± 0.43	367 ± 17	28.8 ± 1.4
		90	8.78	0.052	-	12.42 ± 0.38	332 ± 15	26.0 ± 1.3
		120	8.68	0.052	-	14.53 ± 0.24	406 ± 11	31.8 ± 1.1
	11	Before	5.93	0	39.58 ± 0.80	2.87 ± 0.04	-	-
		0	10.92	0.084	-	12.83 ± 0.03	346 ± 5	27.1 ± 0.6
		30	9.37	0.084	-	15.44 ± 0.39	437 ± 16	34.2 ± 1.4
		60	9.29	0.084	-	16.27 ± 0.47	466 ± 19	36.5 ± 1.6
		90	9.32	0.084	-	16.16 ± 0.52	462 ± 21	36.2 ± 1.7
		120	9.24	0.084	-	16.29 ± 0.21	467 ± 11	36.5 ± 1.1