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-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
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Order of Authors	Marco Chiappero, Francesca Demichelis, Xuan Lin, Chenxiao Liu, Dominic Frigon, Silvia Fiore
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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)

Prof. Dr. Silvia Fiore
Department of Engineering for Environment, Land and Infrastructures (DIATI),
Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129 Torino, Italy
E-mail: silvia.fiore@polito.it

Silvia Fiore

- 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

Marco Chiappero[a], Francesca Demichelis[a], Xuan Lin[b], Chenxiao Liu[b], Dominic Frigon[b], Silvia Fiore[a,*]

[a]DIATI (Department of Environment, Land and Infrastructure Engineering), Politecnico di Torino, Corso Duca degli Abruzzi 24, 10129, Torino, Italy

[b]Department of Civil Engineering and Applied Mechanics, McGill University, Sherbrooke St. West 817, H3A 0C3, Montreal, Quebec, Canada

***Corresponding author**: Prof Silvia Fiore, silvia.fiore@polito.it

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

2. Material and methods

2.1. Waste activated sludge

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

2.2. Pre-treatments

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

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

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

2.2.2. Thermo-alkaline pre-treatment

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

2.3. Anaerobic Digestion tests

Twelve AD reactors (3 for each pre-treatment and 3 fed with raw WAS) were operated at 20 °C in semi-continuous mode for 80 days with solids retention time (SRT) and hydraulic retention time (HRT) equal to 15 days (Uma Rani et al., 2012). Each reactor consisted of a 1-L Pyrex glass bottle, equipped with a polypropylene screw thread cap, wrapped in aluminium foil and mixed through a magnetic stirrer (model 801, Apera Instruments). Two holes in the cap allowed feeding and biogas collection in a 1-L gas bag (30226-U, Supelco, Sigma-Aldrich). The experimental procedure started with a start-up phase (30 days), in which the reactors were filled up to 0.8 L with digestate from the mesophilic digester of the RAEBL WWTP as inoculum (Table 1). During the start-up phase, lasted two SRTs, three times per week the reactors were fed by OZ-WAS. Afterwards, the test phase lasted 50 days, corresponding to 3.3 SRTs, as it may 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^{TM}, 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 $p < 0.05$.

2.6. Scale up evaluation

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

2.6.1 Energy assessment

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

$$Q_n = Q_c + Q_p \tag{2}$$

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

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

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 ($4200 \frac{J}{kg\ °C}$)

$$Q_{mix} = P_{mix} \cdot t_{mix} \tag{4}$$

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

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

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.

$$Q_{O3} = O_{3\ dose} \cdot m_{sludge} \cdot Elec_{\ O3} \tag{6}$$

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)

$$Q_{CH4} = V_{CH4} \cdot \eta_{el} \cdot 39.4 \frac{MJ}{m3} \tag{7}$$

where η_{el} is assumed to be 0.35.

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

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.

$$\mathrm{ESI} = \frac{\mathrm{Q\,p}}{\mathrm{Q\,c}} \tag{9}$$

2.6.2 Economic assessment

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

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

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

WWTP; 2. Decreasing the costs of the final disposal of the digestate (whose volume is inferior compared to WAS).

Labour cost is considered an addition to the current staff of the WWTP; consequently 2, 3, 4 and 5 workers were hypothesized respectively for WWTPs serving 5,000 to 20,000 PE; 50,000 to 200,000 PE; 500,000 PE and 1,000,000 PE.

The revenues came from the surplus of energy produced in the plant from AD and heat recovery. The annual income was calculated as the difference between the revenue and the amortization for the first 5 years and operational costs. The profitability was evaluated through: return of interest (ROI) (Eq. 11), net present value (NPV) (Eq. 12) assuming 20 years plant lifetime with 5 % discount on the future cash flows to the present value, according to (Demichelis et al. 2018b).

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

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

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

where *t* is the plant lifetime, C_t is the net cash flow during period *t*, C_0 is the initial capital investment and *d* is the discount rate. To conclude the economic profitability assessment, Payback time is the time required to regain the investment cost.

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

3. Results and discussion

3.1. Pre-treatments

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

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

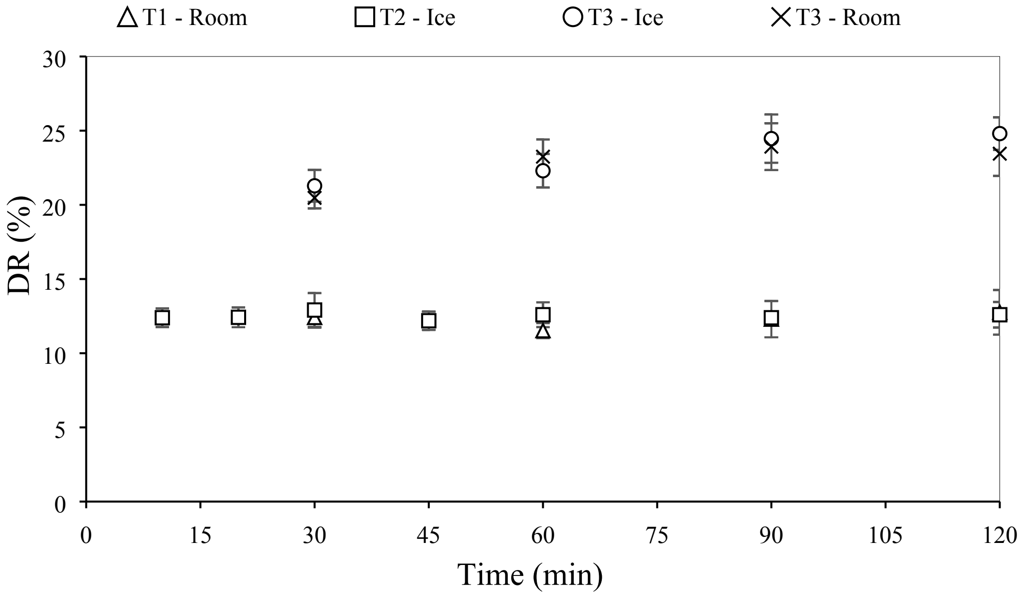
sCOD (g O_2 L^{-1})	1.3 ± 0.1	2.7 ± 1.0	9.9 ± 1.3	4.0 ± 1.1	14.3 ± 0.8
sCOD/tCOD (%)	-	7.3 ± 3.2	26.2 ± 3.6	10.2 ± 2.9	37.8 ± 4.0
DR (%)	-	-	20.5 ± 4.2	3.7 ± 0.9	33.3 ± 3.3

*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)

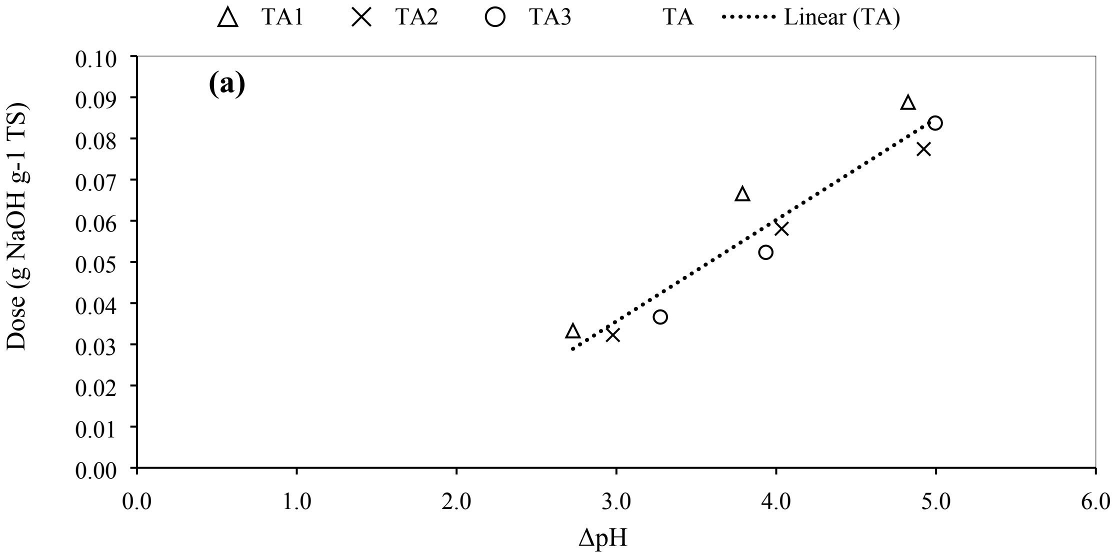


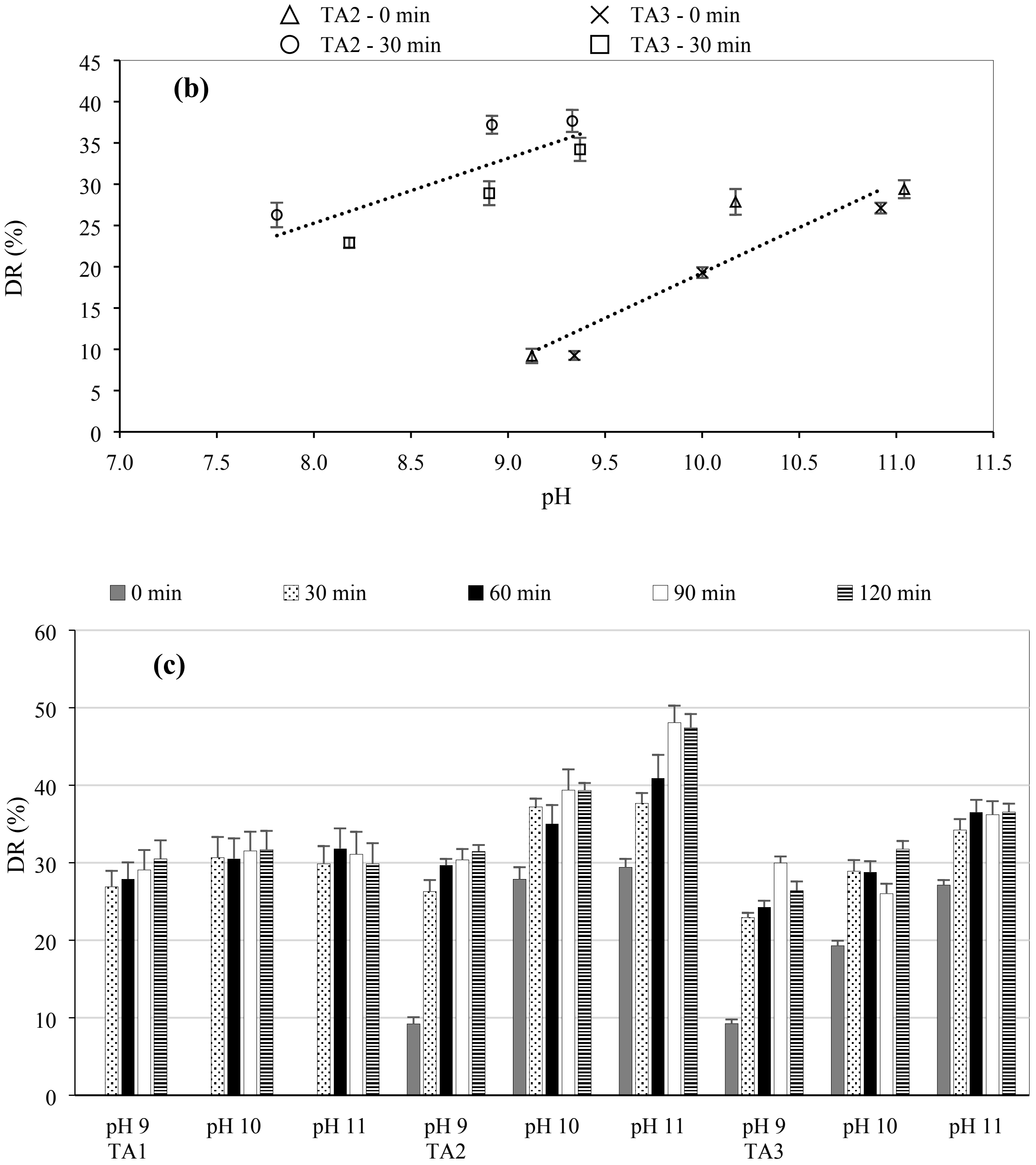
TA was based on sodium hydroxide, which was found to determine better solubilisation than other alkali agents (Kim et al., 2003). The alkali doses corresponding to pH 9, 10 and 11 were selected from literature (Uma Rani et al., 2012; Xu et al., 2014). The temperature value of 70 °C was chosen as a compromise 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

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

furthermore, their results were in line with the outcomes of our thermal tests T1, T2 and T3. A significant enhancement of COD solubilization, reaching DR values of 32 % in TA1, 41 % in TA2 and 36 % in TA3 was observed, thanks to synergic effects of TA pre-treatment, while avoiding the use of a high NaOH dose and its possible inhibition problems (Li et al., 2012; Penaud et al., 1999) as well as limiting the energy expenditures due to higher temperatures and pre-treatment durations. On the grounds of the gathered results, we adopted the combination of pH 11 (0.089 g NaOH g^{-1} TS) and 60 min pre-treatment time as optimum for the subsequent AD tests.

Figure 2. Optimization of thermo-alkaline pre-treatments through COD solubilisation tests (TA1, TA2, TA3): (a) dose of NaOH as a function of the pH increase; (b) Disintegration Rate as a function of pH after 0 min and 30 min of TH; (c) Disintegration Rate for different combinations of pH and treatment times





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

The optimal operating conditions for TH (115 – 118 °C for 30 min), OZ (190 mg O_3 L^{-1}) and TA (0.09 g NaOH g^{-1} TS at 70 °C for 60 min) pre-treatments were selected as 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.

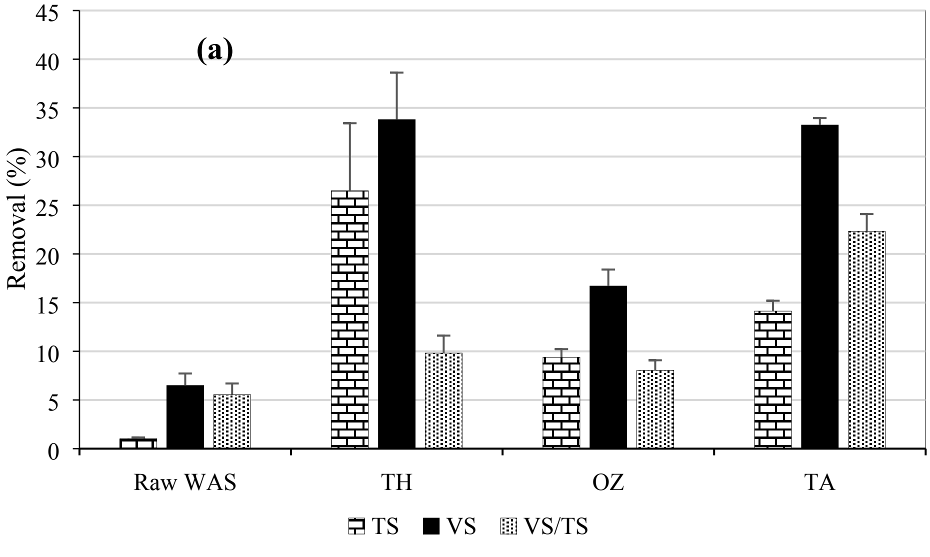
Table 4. Physico-chemical characteristics of digested sludge of each group of reactors after three SRTs (SBP: specific biogas production; SMP: specific methane production)

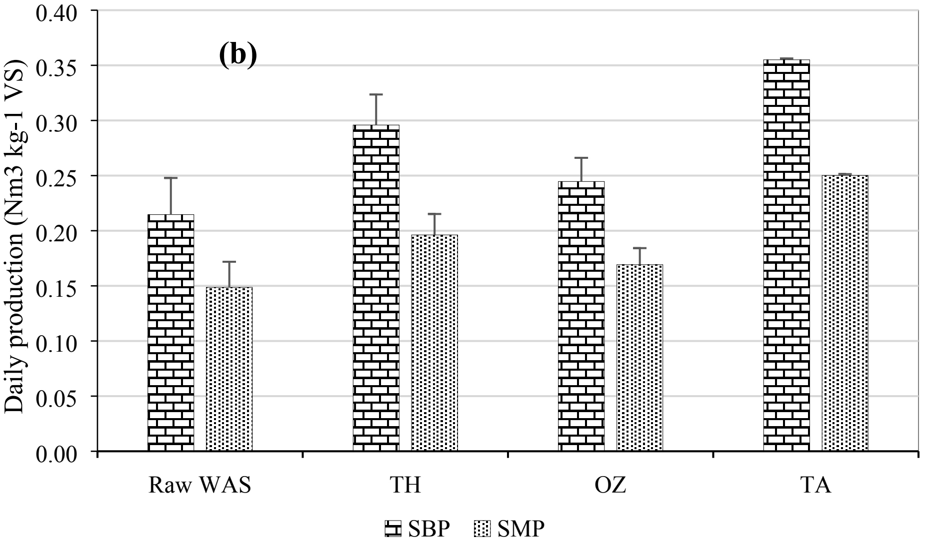
Parameter	**Raw WAS**	**TH**	**OZ**	**TA**
pH	7.00 ± 0.10	7.09 ± 0.08	7.06 ± 0.05	7.28 ± 0.05
TS (g L^{-1})	32.3 ± 0.04	24.0 ± 2.3	29.6 ± 0.3	28.1 ± 0.3
TS removal (%)	1.0 ± 0.1	26.5 ± 7.0	9.4 ± 0.8	14.1 ± 1.1
VS (g L^{-1})	23.5 ± 0.3	16.6 ± 1.2	20.9 ± 0.4	16.7 ± 0.2
VS removal	6.5 ± 1.2	33.8 ± 4.8	16.7 ± 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

3.2. Anaerobic digestion tests

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

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





3.2.1. Solids removals

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

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

3.2.2. Biogas and methane production

Figure 3b shows SBP and SMP values achieved from AD at 20 °C at steady state. SBP for raw WAS was around 0.2 m^3 kg^{-1} VS_{IN} with 70 % methane. SBP values were enhanced by pre-treatments: biogas production increased, compared to raw WAS, of 65 % for TA, 38 % for TH and 14 % for OZ. In addition, SBP values are consistent with enhanced COD solubilization due to pre-treatments. TH and TA pre-treatments resulted in similar VS removal values but TA led to higher biogas production. Methane concentration appeared promising for all samples, ranging between 65 and 70 %-vv (Table 4). Different pre-treatments did not seem to affect methane percentage in comparison with raw WAS. As a result, SMP revealed an increase, compared to raw WAS, of 68 % for TA, 32 % for TH and 14 % for OZ. SMP values are consistent with the increased solubilization of organic matter produced by different pre-treatments, even though TH and TA seemed to be equivalent in terms of solids removals. Overall, SBP values varied from 0.21 (raw WAS) to 0.36 Nm^3 kg^{-1} VS_{IN} (TA), while SMP values from 0.15 (raw WAS) to 0.25 m^3 kg^{-1} VS_{IN} (TA), in agreement with (Dolejs et al., 2018), who observed SMP values equal to 0.22 m^3 kg^{-1} of COD added.

The results of this work are consistent with those of other studies carried out in mesophilic conditions. Considering TH, a thermal pre-treatment on WAS at 121 °C for 60 min enhanced SBP from 0.35 to 0.42 L g^{-1} VSS_{IN} (Barjenbruch and Kopplow, 2003), while at 90 °C for 60 min increased SBP from 0.035 to 0.377 L g^{-1} VS (Appels et al., 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

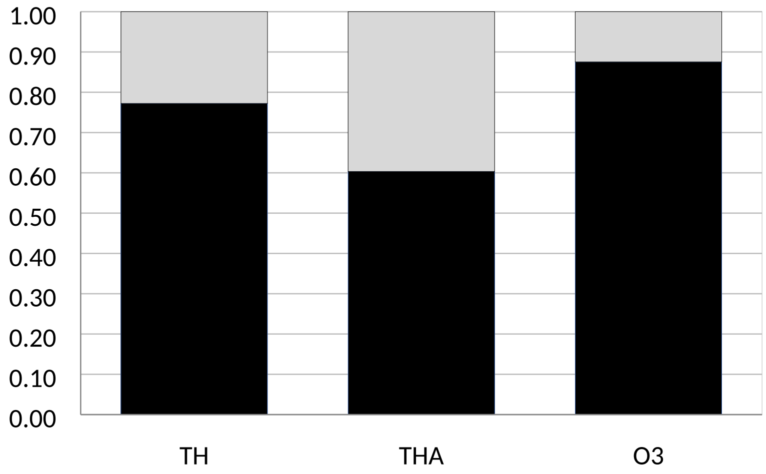


Table 5. Details of energy assessment

	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

3.4. Scale-up: economic assessment

The economic assessment was aimed to detect the minimum plant size that could be profitable comparing the three different scenarios S0, S1 and S2 A, B, C (Figure 5).

According to (Eurostat, 2018), 24.99 kg/y PE of sludge were produced in EU28. The detailed economic assessment (Table 6) proved for S0 a partial economic profitability at 500,000 PE and complete profitability after 1,000,000 PE with NPV equal to 19.05 M€, ROI 67.69 % and payback time of 5 years. According to (Arnò et al, 2017), the economic profitability should be reached at 50,000 PE combining AD of WAS and organic fraction of municipal solid waste. For S1 a partial economic profitability was achieved after 5-years amortisation after 1,000,000 PE, but ROI < 0, NPV < 0 and payback time >20 y were obtained. Hence, for S1 the economic sustainability was not reached.

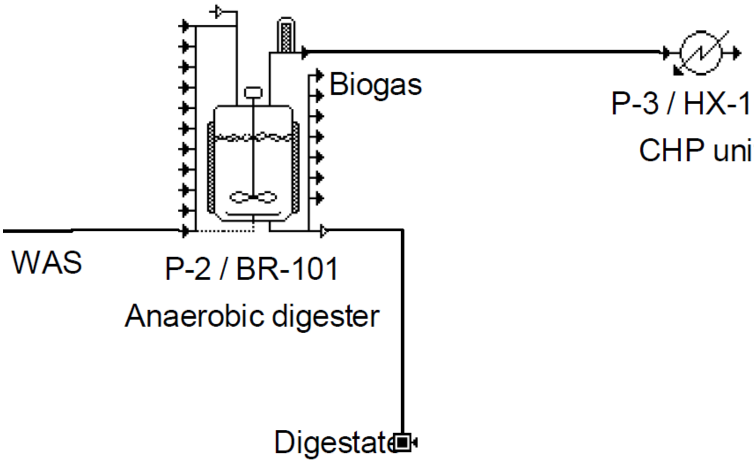
For S2, the minimum plant size to reach the economic sustainability was equal to 50,000 PE for S2-A and 20,000 PE for S2-B, whereas S2-C didn't reach the economic sustainability. Considering S2 proposed configurations, S2-B reached the best performances with ROI equal to 45.16 %, NPV 0.21 M€ and payback time after 3 years. No data are available for economic assessment of low temperature AD in literature, nevertheless the economic profitability for 50,000 PE for S2-B exhibited the same order of magnitude of mesophilic AD of WAS (Rosa et al., 2018; Zhang et al, 2019).

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

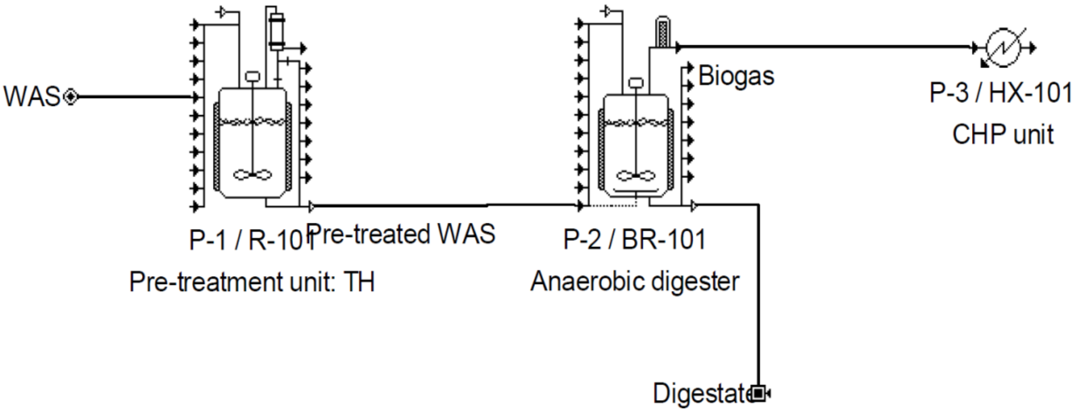
		PE	5.000	10.000	20.000	50.000	100.000	200.000	500.000	1.000.000
		wV unit pre-treatment [m^3]	0.34	0.68	1.36	3.40	6.79	13.59	33.96	679.29
		V unit pre-treatment [m^3]	0.42	0.85	1.70	4.25	8.49	16.98	42.46	849.11
		AD reactor wV [m^3]	5.09	10.19	20.38	50.95	101.89	203.79	509.47	10189.32
		AD reactor V [^{m3}]	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

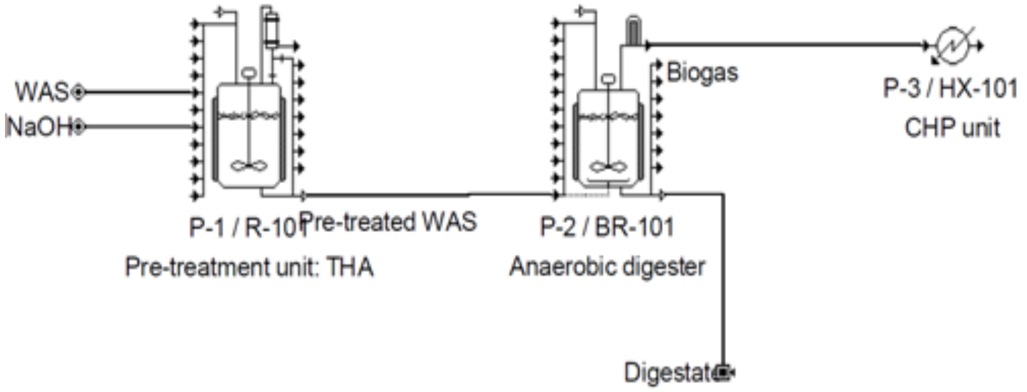
Figure 5. Process outlines of the considered scenarios: S0, S1, S2: (A) TH + AD; (B) TA + AD; (C) OZ + AD



S0, S1



S2-A



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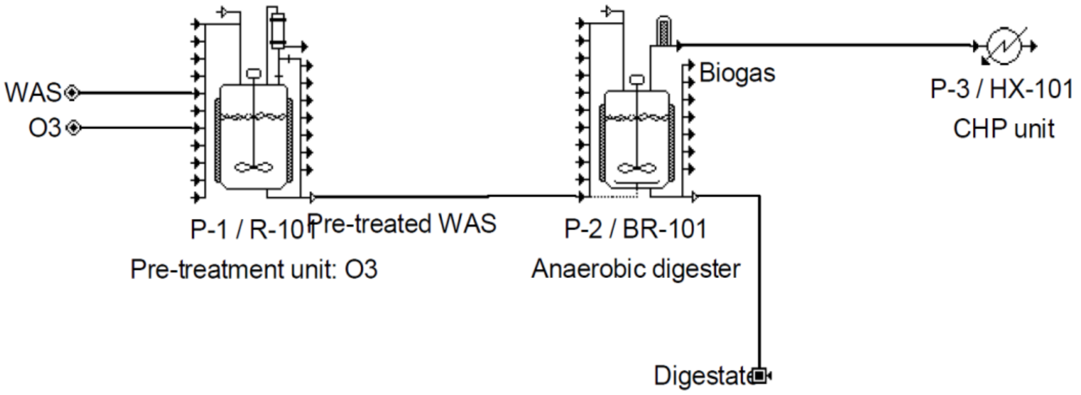

S2-C

4. Conclusions

This work investigated physic-chemical pre-treatments to improve the anaerobic digestion of waste activated sludge at 20 °C. Thermo-alkaline pre-treatment, followed by thermal and ozonation, achieved the best performances, in agreement with literature. Biogas and methane yields obtained from semi-continuous reactors after thermal and thermo-alkaline pre-treatments (0.30 - 0.36 m^3 kg^{-1} VS, 65 -70 % methane) were equivalent to literature data referred 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 was economically profitable for WAS generated by a 20,000 PE WWTP. Anaerobic digestion at 20 °C was demonstrated to have a promising potential and considering the almost complete lack of literature studies about psychrophilic processes, further research is urgently needed.

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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