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Effects of hydrodynamic cavitation, low-level thermal and low-level alkaline pre-treatments on sludge solubilisation

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ABSTRACT

WAS is a polluting and hazardous waste generated in WWTPs that must be treated to prevent pollution and human health risks. Anaerobic digestion is the most used process for sludge stabilization. However, it must be improved in terms of both speed and extend of degradation. With the purpose of reducing the energy and chemical consumption linked to sludge treatment, in this study, different anaerobic digestion pre-treatments such as low-level mechanical (hydrodynamic cavitation, 2 bar), low-level thermal (50 °C) and low-level alkaline (NaOH, KOH and $Ca(OH)_2$, pH 10) methods, and a combination thereof, were tested as strategies to improve sludge solubilisation. When the pre-treatments were used alone, the alkaline pre-treatment showed the highest sludge solubilisation. Among the alkaline reagents tested, NaOH and KOH led to higher DD_{PCOD} (41.6 and 39.4%), while only 8.4% was achieved by using $Ca(OH)_2$. However, the low-level hydrodynamic cavitation assisted thermo-alkaline pre-treatment was the most efficient in terms of both sludge solubilisation (DD_{PCOD} = 53.0%) and energy efficiency (EE = 64.5 mgΔSCOD kJ^{-1}). The synergetic effects of the combined pre-treatment were also confirmed by the highest release of EPS. Furthermore, cytometric analyses showed that the main mechanism involved in sludge solubilisation for the investigated pre-treatments was flocs disintegration rather than cell lysis.

1. Introduction

Waste activated sludge (WAS) is an undesirable by-product of many wastewater treatment plants (WWTPs) with activated sludge processes. It contains organic contaminants and pathogens that would cause potential risk to the ecological environment if it was not properly treated. Anaerobic digestion (AD) is the most traditional and widely employed sludge treatment to stabilize sewage sludge with the benefits of methane production, mass reduction and improved dewatering properties of the digested sludge. AD process involves four stages: hydrolysis, acidogenesis, acetogenesis and methanogenesis. However, hydrolysis of the microorganism cells in the WAS occurs very slowly and limits the efficiency of AD process. In order to improve the rate limiting hydrolysis and the digestion performance, different pre-treatment methods such as thermal, chemical and mechanical were investigated in the literature [1–3]. All these methods can be successfully applied before AD to disrupt the complex WAS floc structure and to release extra- and intra- cellular polymeric substances into soluble phase along with solubilisation of particulate organic matters.

Thermal pre-treatments, originally used to enhance WAS dewaterability [4], were found to be effective in increasing biogas production and WAS biodegradability. Performance of the thermal process heavily relies upon treatment temperature and time used. Previous studies suggested a wide range of temperatures (50–250 °C) to enhance the effectiveness of AD of WAS. Ennouri et al. [5] found an optimum temperature of 120 °C, investigating the effects of different temperatures (ranged from 60 to 120 °C) for thermal pre-treatment on the efficiency of AD of urban and industrial WAS in terms of methane production, volatile solid (VS) biodegradability and the microbial community structure. Higgins et al. [6] observed a reduction in viscosity and the improvement in dewaterability pre-treating WAS thermally at temperatures ranged from 130 to 170 °C. Similar results on WAS dewaterability were obtained by Li et al. [7] treating the WAS from 140 to 220 °C. Although thermal pre-treatment could effectively disintegrate WAS and promote the biogas production, their application requires large consumption of energy [8].

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Chemical pre-treatments (acidic or alkali) can employ different chemical reagents for disintegrating and hydrolysing cell walls and membranes, thus increasing the solubility of organic matter contained within cells. HCl, H_2SO_4, H_3PO_4 and HNO_3 are usually used as acids [9], while NaOH, KOH, $Mg(OH)_2$ and $Ca(OH)_2$ and CaO are the most used alkali agents [10,11]. However, the use of alkali agents seems to be more promising in enhancing WAS acidification [12] and methanogenesis [13]. In addition to deforming cell walls and membranes, favouring availability of sludge organic matter for enzymatic attacks, the presence of alkalis in chemical processing were found to have additional effects on increasing biogas production [10,11] and on facilitating sludge dewatering [14]. Even if chemical pre-treatments could be used to accelerate hydrolysis during AD, and thereby to reduce digestion time and enhance biogas production, these pre-treatment methods need large consumption of reagents and, then, high operating costs [15].

Mechanical pre-treatments act by destroying bacterial cell walls and membranes resulting in either an increase of specific surface area and a release of extra- and intra-cellular [16]. An increased surface area provides better contact between substrate and anaerobic bacteria. Moreover, many of the intra-cellular constituents, including cytoplasm and nuclei acids, are readily biodegradable. These conditions lead to an acceleration of the AD process. Many mechanical pre-treatments such as sonication [17–19], hydrodynamic cavitation (HC) (Venturi [20], orifice plates [21], stator and rotor assembly [22], high-pressure homogenizer [23,24]), lysis-centrifuge [25] and grinding [26,27] were investigated in the literature. Among these methods, HC is nowadays taking a more prominent role in the field of wastewater treatment [21,22,28], mainly due to ease of operation, moderate energy consumption, no odour generation, flexibility and capability to vary the required intensities of cavitational conditions [29]. With all of these advantages, a hybrid advanced oxidation reactor based on the combined use of hydrodynamic cavitation, acoustic cavitation, ozone and electrochemical oxidation/precipitation was proposed at commercial scale by Gogate et al. [30] for the treatment of various types of polluted water.

Recently, the positive properties of individual methods have been effectively combined in order to get synergetic effects between thermal, chemical and mechanical pre-treatments. When alkaline agents, high temperature and HC pre-treatments were simultaneously applied, an increase in treatment efficiencies has been detected. This is in accordance with the results reported by Lee and Han [21], in which the synergetic effects in terms of methane production were observed treating activated sludge by using a pre-treatment based on HC combined with alkaline treatment, and by Seo et al. [31], in which a combined HC system under alkaline conditions improved the hydrogen production from cheese whey. Similarly, Hilares et al. [32] observed the highest efficiencies for the sugarcane bagasse treatment by considering HC combined with the thermo-alkaline pre-treatment.

The main objective of the present work is to evaluate the influence of HC assisted thermo-chemical pre-treatment on sludge solubilisation, aiming to reduce the energy and chemical consumption linked to sludge pre-treatment. With this main purpose, the prefix “low-level” used throughout the manuscript for the investigated thermal, chemical and mechanical pre-treatments refers to low-level temperature, chemicals and energy inputs, respectively.

Therefore, a low-level thermal treatment at 50 °C, a low-level alkaline treatment at 10 pH, and a low-level HC treatment at low inlet pressure (2 bar) were tested.

Firstly, individual effects of low-level thermal (T), low-level alkaline (ALK) and low-level mechanical (HC) pre-treatments, and the combined effect of thermo-alkaline (T-ALK), thermo-mechanical (T-HC), and mechanical-alkaline (HC-ALK) pre-treatments on sludge solubilisation were carried out. Then, the influence of the combined HC assisted thermo-alkaline pre-treatment (T-HC-ALK) were investigated, by combining the previous pre-treatment methods. Process efficiencies were evaluated in terms of increase in WAS solubilisation. Mechanisms involved in the WAS solubilisation were further investigated by extracellular polymeric substances (EPS) and flow cytometry analyses.

2. Materials and methods

2.1. *Source and characteristic of WAS*

Sludge samples used in this study were obtained from urban WWTP in Trento, Italy. Since sludge solubilisation was found to be higher for sludge with higher solids content [28], thickened sludge, with total solid content (TS) of $43.5 \pm 0.5\,g\,L^{-1}$, was collected at ambient temperature ($T = 20 \pm 2\,°C$), after the dynamic thickening process treating excess WAS of a nitrification/denitrification process. The physical and chemical characteristics of the thickened sludge used were: pH 6.5 ± 0.2, $TS = 43.5 \pm 0.5\,g\,L^{-1}$, volatile solids $(VS) = 35.7 \pm 0.5\,g\,L^{-1}$, total chemical oxygen demand $(TCOD) = 23{,}970 \pm 300\,mg\,L^{-1}$, soluble COD $(SCOD) = 234.0 \pm 5.0\,mg\,L^{-1}$, total Kjeldahl nitrogen $(TKN) = 1808 \pm 5.0\,mg\,L^{-1}$, ammonia nitrogen $(NH_4{}^+\text{-}N) = 55.0 \pm 1.0\,mg\,L^{-1}$, total phosphate $(P_{TOT}) = 737.0 \pm 15.0\,mg\,L^{-1}$.

2.2. *Sludge pre-treatment efficiency*

The pre-treatment efficiency was evaluated by measuring the improvement of solubilisation of sludge in terms of SCOD increase (Eq. (1)) and the ratio of change in soluble chemical oxygen demand (SCOD) after cavitation to particulate chemical oxygen demand ($PCOD_0 = TCOD - SCOD_0$) (Eq. (2)).

$$\Delta SCOD\ (mg\ L^{-1}) = SCOD_t - SCOD_0 \tag{1}$$

$$DD_{PCOD}\ (\%) = \frac{(SCOD_t - SCOD_0)\times 100}{PCOD_0} = \frac{(SCOD_t - SCOD_0)\times 100}{(TCOD - SCOD_0)} \tag{2}$$

where $SCOD_t$ is the soluble COD of the treated sludge by using a pre-treatment method [$mg\,L^{-1}$] at the time t, $SCOD_0$ is the soluble COD of the untreated sludge [$mg\,L^{-1}$], TCOD is the total COD of the untreated sludge [$mg\,L^{-1}$], and $PCOD_0$ is the particulate COD of the untreated sludge [$mg\,L^{-1}$].

2.2.1. *Low-level thermal pre-treatment*

Low-level thermal (T) pre-treatment was performed using the experimental setup showed in Fig. 1, where V_4 and V_5 control valves were kept closed for the entire test to exclude HC process, and only the bypass line was used (V_6 and V_7 control valves were kept fully opened for the entire test).

For each thermal experiment, thickened WAS at ambient temperature was used to fill the 50 L insulated feed tank (1), which was equipped with an external water bath (6). The sludge sample was heated at 50 °C by using two immersion resistances (5) (1.5 kW, Rommelsbacher TS 1502).

Once the desired temperature of 50° C was reached, it was kept constant for the entire duration of the experiment of 1 h. Temperature was decreased or increased, by allowing to cold water to enter through valve V_1 or by using the two immersion resistances, respectively. In order to homogenise its content and to ensure a uniform distribution of temperature, WAS was recirculated in a closed loop circuit by using a Mohno pump (2) with a flow rate of $2\,m^3\,h^{-1}$ (3.0 kW nominal power, Netzsch Pumps & Systems GmbH Germany). Temperature and pH of sludge were continuously monitored in the main tank.

2.2.2. *Low-level alkaline and thermo-alkaline pre-treatments*

Low-level alkaline (ALK) and thermo-alkaline (T-ALK) pre-treatments were conducted with three different alkaline agents (NaOH, KOH, and $Ca(OH)_2$). Fig. 2 shows a schematic representation of the

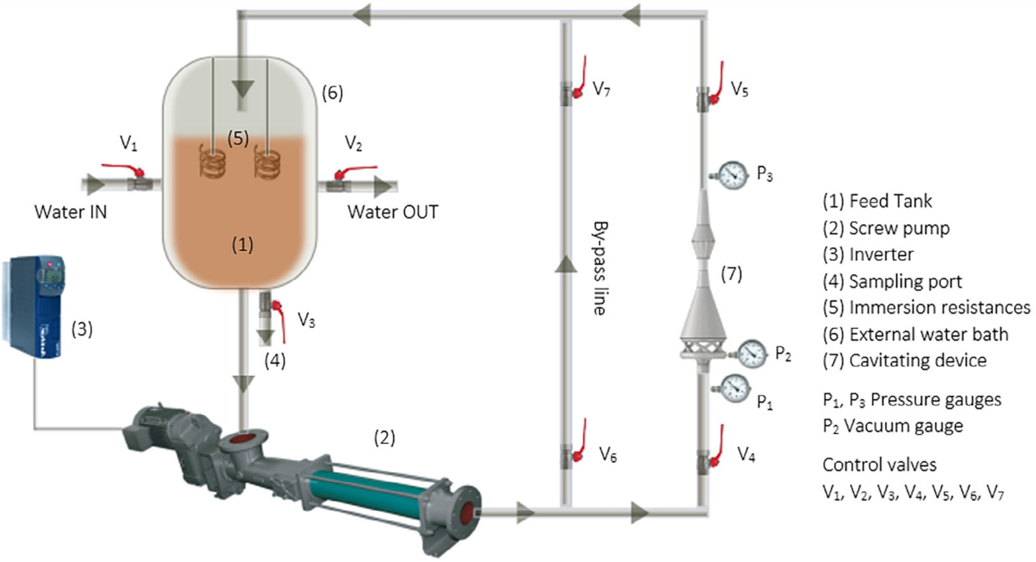


Fig. 1. Schematic representation of low-level thermal/HC experimental setup.

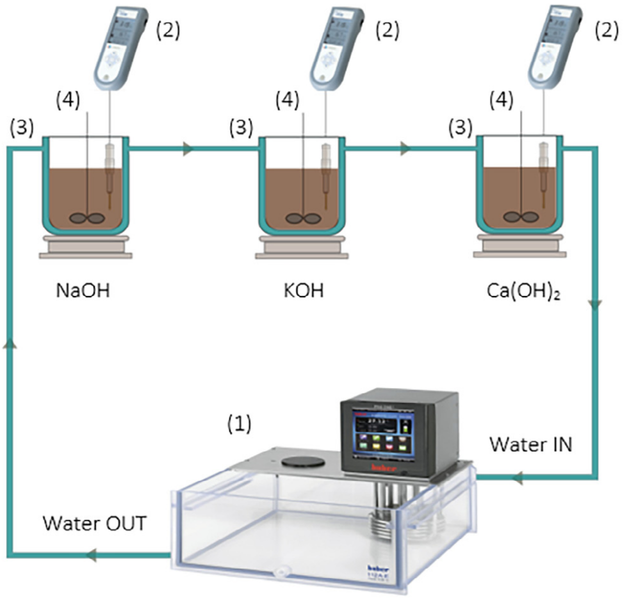
experimental setup used for the alkaline pre-treatments. Details are as follows: (1) a thermostatic bath; (2) temperature and pH probes; (3) n. 3 glass thermal reactors with a capacity of 1.8 L each; (4) mechanical stirrers.

The three different reagents were simultaneously tested using a reactor for each of them as shown in Fig. 2. The temperature was adjusted at the desiderated value by means of the thermostatic bath, and it was kept constant at 20 °C and 50 °C during the entire duration of alkaline and thermo-alkaline tests, respectively.

After a mixing time of 15 min to homogenise sludge content, 5 M NaOH and 5 M KOH solutions and $Ca(OH)_2$, respectively, were added to about 1.5 L of thickened WAS contained in each reactor in order to adjust the pH of the treated sludge. The samples were then continuously mixed for 1 h and the pH was monitored throughout the tests. Table 1 summarizes the amount of each reagent that was added at the beginning of the test in each thermal reactor to achieve a pH of 10.

Table 1
ALK and T-ALK pre-treatments characteristics.

Reagent	NaOH	KOH	$Ca(OH)_2$
ALK pre-treatment at environmental temperature of 20 °C			
Reagent Volume/Weight	19.8 mL	21.8 mL	5.6 g
Weight of treated WAS (g)	1493	1565	1486
pH	9.79	9.84	9.87
TS ($g\,L^{-1}$)	41.5	41.5	41.5
T-ALK pre-treatment at temperature of 50 °C			
Reagent	NaOH	KOH	$Ca(OH)_2$
Reagent Volume/Weight	21.6 mL	21.6 mL	6.1 g
Weight of treated WAS (g)	1622	1556	1605
pH	9.81	9.93	9.71
TS ($g\,L^{-1}$)	40.5	40.5	40.5



(1) Thermostatic bath; (2) Temperature / pH probes; (3) Glass thermal reactors; (4) Mechanical stirrers

Fig. 2. Schematic representation of low-level alkaline experimental setup.

2.2.3. Low-level mechanical, thermo-mechanical and mechanical-alkaline pre-treatments

Low-level mechanical (HC), thermo-mechanical (T-HC) and mechanical-alkaline (HC-ALK) pre-treatments tests were performed by means of the same experimental setup used for thermal pre-treatment (Fig. 1), where a modified swirling jet reactor (HC reactor), previously described in Mancuso el al. [28,33,34], was connected to the screw pump.

The feed tank was filled with 50 L of thickened WAS. Firstly, the temperature was adjusted to the desiderate values by using the two immersion resistances located inside the main tank, while the sludge was recirculated through the by-pass line for about 15 min in order to homogenise its content. At this stage, valves V_6 and V_7 were kept fully opened, whereas valves V_4 and V_5 were kept closed in order to avoid activated sludge to flow through the HC reactor. Then, in order to generate cavitation, V_4 and V_5 control valves were kept opened for the entire HC test, while the valves of by-pass line (V_6, V_7) were kept fully closed. The HC system consisted of a closed loop circuit, where 50 L of WAS were continuously HC-treated for 1 h. Temperature was kept constant throughout the experiments (with a variation of ± 3.0 °C), by using the heating and cooling system as described in Section 2.2.1. The inlet pressure upstream to the cavitation system was kept at 2 bar by adjusting the frequency of the pump inverter.

The (HC) test was conducted at 20 °C, while the thermo-HC (T-HC) pre-treatment was conducted at 50 °C. For the mechanical-alkaline (T-ALK) pre-treatment test, a 5 M NaOH solution was added into the feed tank to the sludge at 20 °C to attain a final pH of about 10, as soon as the by-pass line was closed, and the HC reactor was used. Table 2 gives a summary of the hydrodynamic conditions in the performed experiments.

2.3. Low-level HC assisted thermo-alkaline pre-treatment

The simultaneous combination of thermal, alkaline and cavitation effects was investigated. In this combination, the effect of HC at 2 bar, 50 °C and pH 10 (NaOH dosage, 5 M) on sludge disintegration was studied, using the experimental set-up shown in Fig. 1. In the HC assisted thermo-chemical experiments (T-HC-ALK), the sludge was mixed, heated at 50 °C and immediately HC-treated after the addition of the desired dosage of NaOH into 50 L of sludge. Then, the sludge was kept at 50 °C during the remaining part of the pre-treatment period (1 h).

2.4. Extracellular polymeric substances (EPS)

Extracellular polymeric substances (EPS) represent a complex high molecular-weight extracellular biopolymer, consisted of polysaccharides, proteins, nucleic acids, DNA residuals and cell debris [35]. They are produced by several pathways including excretion by microorganisms, lysis, hydrolysis and adsorption of organic matter from wastewater. In the sewage sludge, proteins and carbohydrates are considered as the most representative constituents of EPS. EPS can be classified into soluble EPS and bound EPS. In this study, the soluble fraction was analysed. For the extraction of soluble EPS, centrifugation (200 mL; 4000 × g; room temperature; 20 min) and following filtration (1.5 µm fibre-glass paper) were used [36,37].

In order to determine the polysaccharides and proteins content, the soluble fractions of EPS were analysed in a spectrophotometer. Soluble polysaccharide concentration was measured according to Dubois et al. [38] using glucose as standard. Proteins were measured according to Frolund et al. [39], using bovine serum albumin as reference protein.

2.5. Bacterial cell fluorescent staining and cytometric tests

Bacterial cell fluorescent staining was performed according to Foladori et al. [40]. To distinguish intact and permeabilised bacteria, cells were stained with SYBR-Green I (SYBR-I, 1:30 dilution of commercial stock; from Invitrogen, USA; lex = 495 nm, lem = 525 nm) diluted in dimethyl sulfoxide (DMSO, Merck, Germany) and Propidium Iodide (PI, stock solution concentration 1 mg mL^{-1}; Invitrogen, USA; lex = 536 nm, lem = 617 nm). An amount of 10 µL of both fluorochromes was added to 1 mL of bacterial suspension containing about 10^6 e 10^7 cells mL^{-1}. Samples were incubated in the dark for 15 min at room temperature. SYBR-I is capable of staining all cells, whereas the polarity of PI allows it to penetrate only cells with permeabilised membranes, which can be considered as dead [41]. In the permeabilised cells, the simultaneous staining with SYBR-I and PI activates energy transfer between the fluorochromes. Therefore, intact bacteria emit green fluorescence, while permeabilised bacteria emit red fluorescence.

FCM analyses were performed with an Apogee-A40 flow cytometer (Apogee Flow Systems, UK) equipped with an Ar laser (488 nm) [40,42]. Collected signals were green and red fluorescence, acquired with logarithmic gain and Forward Angle Light Scatter (FALS), which is related to cell size [43]. Data acquisition gates were set on green and red fluorescence distribution to eliminate non-bacterial particles and debris. At least 10,000 cells were analysed for each sample in a few minutes, providing good statistical data. Results of green and red fluorescence measured for each stained cell were analyzed in order to evaluate populations of dead and viable cells. The accuracy of the staining method and the FCM analysis was previously evaluated with epifluorescence microscopy [41].

2.6. Analytical methods

Untreated and treated sludge samples were collected during each pre-treatment test. Before being analysed, they were cooled to ambient temperature, and stored at 4.0 °C. TS, VS, TSS, TCOD, SCOD, TKN, $NH_4{}^+$-N of WAS were measured according to the standard methods [44]. Prior to SCOD and $NH_4{}^+$-N determinations, sludge samples were centrifuged at 5000*g* and the liquid supernatant was filtered using cellulose nitrate membrane of pore size 0.45 µm by compression. pH was monitored by using a Crison 25 portable pH-meter. All the analyses were performed in duplicate and the results were expressed as average of the values.

2.7. Calculations

The efficiencies of each pre-treatment were evaluated as function of the specific energy (SE) applied to the sludge (Eq. (3)).

Table 2
Hydrodynamic operating conditions for the HC, T-HC and HC-ALK pre-treatments.

HC, T-HC and HC-ALK pre-treatments				
Ecowirl configuration	Inlet pressure (bar)	Frequency (Hz)	Flow rate[1] ($m^3 h^{-1}$)	Flow velocity[1,2]($m s^{-1}$)
Standard [28]	2	59.0	4.6	4.2

[1] Values calculated using water as working fluid.

[2] Velocity of the flow through the narrowest reduced area of the orifice plate [28].

$$\mathrm{SE}(\mathrm{kJ\ kgTS^{-1}}) = \frac{P_{abs} \times t_1}{(\mathrm{V}\times\ \mathrm{TS})} \qquad (3)$$

where P_{abs} is the total absorbed powers by the pre-treatment, t_1 is the treatment time, V is the volume of the treated sludge [L] and TS is the sludge solid content [$g\,L^{-1}$]. For all the pre-treatments in which was used the experimental setup as previously described in Fig. 1, SE was calculated by using Eq. (3) and considering as absorbed power the energy associated with the pump and the immersion resistances [$P_{abs} = (P_{res} \times t_1) + (P_{pump} \times t_1)$], where P_{res} is the absorbed power by the immersion resistances used to heat the sludge at 50 °C, P_{pump} is the pump absorbed power [W] by the system. On the contrary, for the alkaline and thermos-alkaline pre-treatments (Fig. 2), SE was again calculated by using Eq. (3), but this time in its determination it was taking into account the absorbed power due to the use of the thermostatic bath and the mechanical stirrers [$P_{abs} = (P_{bath} \times t_1) + (P_{mix} \times t_1)$], where P_{bath} is the absorbed power by the thermostatic bath used to heat the sludge at 50 °C, and P_{mix} is the mixer absorbed power [W].

Further, according to Zhang et al. [45], the energy efficiency (EE), expressed as mgΔSCOD kJ^{-1}, was calculated as the mg of SCOD-increase per unit of energy supplied (Eq. (4)). Higher EE values correspond to higher removal efficiencies.

$$\mathrm{E}(\mathrm{mg\Delta SCOD\ kJ^{-1}}) = \frac{(\mathrm{V}\times\Delta\mathrm{SCOD})}{(P_{abs} \times t_1)} \qquad (4)$$

3. Results and discussions

Type of used pre-treatments, operating conditions and efficiencies measured at the end of each test are summarized in Table 3. In following sub-sections, results of the effect of pre-treatment methods individually and simultaneously used on WAS solubilisation are discussed. The synergistic effect of combined pre-treatments was evaluated by comparing them with individual pre-treatments.

3.1. Preliminary sludge pre-treatment study

3.1.1. The effect of temperature on sludge disintegration

The effect of low-level thermal (T) pre-treatment at 50 °C on COD solubilisation is showed in Fig. 3. Results were compared with values of non-pre-treated WAS at environmental temperature of 20 °C.

As shown in Fig. 3, the low-level thermal (T) pre-treatment led to an increase in COD solubilisation of WAS. SCOD immediately increased from 224 to 4941 mg L^{-1} in about 5–10 min as the temperature was increased from 20 to 50 °C. The SCOD further increased slightly with increasing the time of thermal treatment, reaching 5941 mg L^{-1} and a DD_{PCOD} of 22.3% with an operating time of 1 h. Previous studies confirmed that at temperatures below 100 °C (low-level thermal pre-treatments [46]) an increase in WAS solubilisation with both temperature and time can be detected [47–50], while for temperatures higher than 100 °C (high-temperature thermal pre-treatments) the temperature is a more important factor than the treatment duration [51]. In this experiment, by using the experimental setup showed in Fig. 1, an intensive increase of SCOD and DD_{PCOD} has been achieved increasing the temperature from 20 to 50 °C, while only a minor increase of SCOD and DD_{PCOD} has been measured with treatment time. This intensive increase during the heating process could be related to the heating system used in the experimental setup, that could create during the heating process from 20 to 50 °C local high temperatures, causing a higher SCOD increase. Once the temperature of 50 °C was achieved, the heating loss were minimal during the 1 h treatment, as the tank was insulated, and supposedly local high temperatures were no further achieved. However, this effect could be avoided in future applications by using heating elements not in direct contact with the sludge sample to treat.

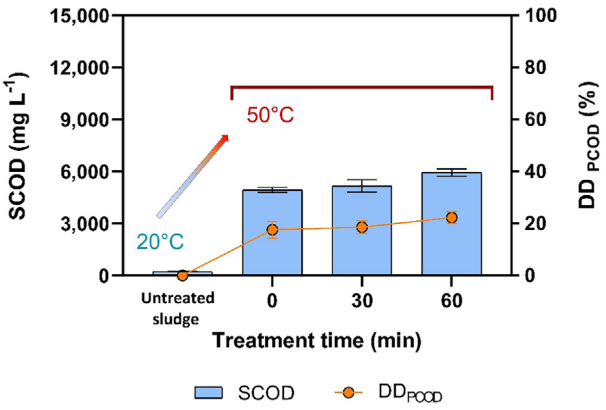


Fig. 3. Effect of low-level temperature thermal (T) pre-treatment on COD solubilisation.

Low-level thermal (T) treatment has been applied in this study in order to reduce the pre-treatment costs. Even if some authors reported that the energetic expense in high-temperature thermal pre-treatments could be balanced by the increment in sludge biodegradability and the use of sludge residual heat in the maintenance of digester temperature [52], low-level thermal pre-treatments imply a much lower energy consumption and a higher ease of operations. The temperature of 50 °C

Table 3
Pre-treatments characteristics, tests efficiencies, and economic comparison.

Type of pre-treatment	Temperature (°C)	Reagent	Treatment time (h)	DD_{PCOD} (%)	ΔSCOD (mg L^{-1})	SE (kJ $kgTS^{-1}$)	EE (mgΔSCOD kJ^{-1})	Energy Cost[1] (€ $kgTS^{-1}$)	Reagent Cost[2] (€ $kgTS^{-1}$)	Total Cost (€ $kgTS^{-1}$)
Thermal (T)	50	–	1	22.3	5,941	2,855	39.3	0.140	–	0.140
Alkaline (ALK)	20	NaOH	1	41.6	11,554		2.3	0.049	0.234	0.283
		KOH		39.4	10,554	993	2.1	0.049	0.187	0.236
		$Ca(OH)_2$		8.4	2,244		0.4	0.049	0.641	0.690
Thermo-alkaline (T-ALK)	50	NaOH	1	46.5	12,347		2.4	0.140	0.251	0.391
		KOH		44.0	11,678	2,855	2.3	0.140	0.187	0.327
		$Ca(OH)_2$		16.8	4,466		0.9	0.140	0.698	0.838
Mechanical (HC)	20	–	1	1.4	617	1,655	3.4	0.081	–	0.081
Thermo-mechanical (T-HC)	50	–	1	23,5	6,600	3,351	38.2	0.164	–	0.164
Mechanical-alkaline (HC-ALK)	20	NaOH	1	44.2	8,899	1,489	51.5	0.073	0.242	0.315
Thermo-mechanical and alkaline (T-HC-ALK)	50	NaOH	1	53.0	10,673	3,186	64.5	0.156	0.239	0.395

[1] Values calculated considering the cost of 1 kWh = 0.1762 € – Enel Energia Elettrica 2019.

[2] Values calculated considering the following costs for the reagents: a) NaOH = 58.48 € kg^{-1}; b) KOH = 30.74 € kg^{-1}; c) $Ca(OH)_2$ = 114.42 € kg^{-1}; Costs of reagents were taken from the Sigma-Aldrich S.r.L. website.

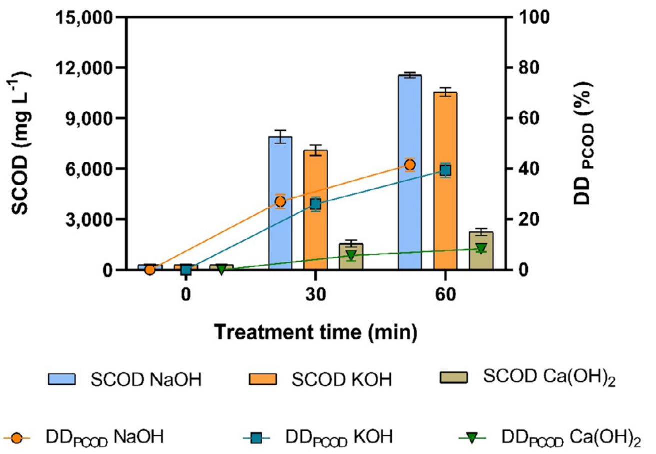


Fig. 4. Effect of low-level alkaline (ALK) pre-treatment on COD solubilisation at environment temperature of 20 °C.

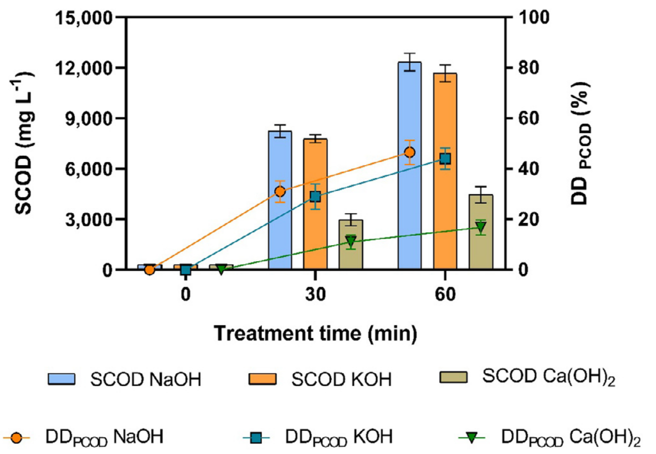


Fig. 5. Effect of low-level thermo-alkaline (T-ALK) pre-treatment on COD solubilisation when heating at 50 °C.

was further chosen as this temperature is very close to that required by following treatment units such as, for example, the AD process, in which the heating of activated sludge at about 37 °C would no longer be necessary, resulting in a decrease of operating costs for the AD. Moreover, it should be highlighted that low-level thermal (T) treatments do not result in toxic compounds, while temperatures higher than 180 °C could lead to the production of recalcitrant soluble organics or toxic and inhibitory intermediates, hence reducing the sludge biodegradability [53–55].

3.1.2. The effect of alkalization and temperature on sludge disintegration

Low-level alkaline (ALK) pre-treatments were performed at pH 10 with various alkaline agents NaOH, KOH, and Ca(OH)$_2$ at ambient temperature of 20 °C. NaOH and KOH were easily added to the sludge to be treated. By gently mixing the vessel, pH reached the value of 10 after about 5 min. On the contrary, the use of Ca(OH)$_2$ was more time demanding, as it required about 10–15 min to dissolve and reach the pH of 10. Results on disintegration degree and COD solubilisation are shown in Fig. 4.

At the beginning of each test, the reagents were added to WAS as described in Section 2.2.2. Because the pH remained constant for all the duration of each alkaline test regardless of the reagent that was used, it was not necessary to add further reagents to WAS. Among all the alkaline agents tested, monobasic agents, NaOH and KOH, were the most efficient in terms of sludge solubilisation with the highest DD$_{PCOD}$ of 41.6 and 39.4%, achieved after 1 h of chemical treatment, respectively. A lower disintegration degree was detected by using the dibasic agents, Ca(OH)$_2$, (Table 3). These findings are most probably due to a not complete dissolution of dibasic alkali agents. Therefore, NaOH and KOH have most favoured the main disintegration reactions (i.e. hydrolysis of organic matter) and the sludge biodegradability was improved. By using the same alkaline agents, Kim et al. [11] at 20 °C obtained comparable degree of solubilisation: 40.0, 37.0, and 15.0% with NaOH, KOH, and Ca(OH)$_2$, respectively. In the study conducted by Lee and Han [21], the values of DD$_{PCOD}$ varied as a function of pH: for pH below or equal to 11, Ca(OH)$_2$ was found to be more efficient in terms of sludge solubilisation, while for pH higher than 11, the effectiveness followed the order of NaOH > KOH > Ca(OH)$_2$ with a maximum DD$_{PCOD}$ of 40.1% at pH 13 with NaOH.

Furthermore, in this study, the same alkaline agents were used to investigate the effect of the combination of low-level alkaline and thermal (T-ALK) pre-treatments on sludge solubilisation. Results are showed in Fig. 5. As for the low level alkaline tests conducted at 20 °C, the pH was constant for all the duration of each test, then any reagent was further added to WAS. Contrarily to what reported in section 3.1.1., in those experiments where has been used the experimental setup showed in Fig. 2, any significant increase in SCOD has been detected as the temperature was increased from 20 to 50 °C. Then, raising the treatment time, an increase in SCOD was measured for each alkaline agents utilized, but depending on the alkaline agent tested, different disintegration degrees were observed. Again, as obtained at 20 °C, at 50 °C monobasic agents resulted in higher solubilisation percentages than dibasic ones. NaOH was the most efficient reagent in terms of sludge solubilisation, with the highest DD$_{PCOD}$ of 46.5% achieved after 1 h of chemical treatment. These results agree with previous studies of Kim et al. [11] and Penaud et al. [10] that observed higher solubilisation percentages for monobasic agents rather than dibasic ones, working at 121 °C and 140 °C, respectively. Influence of NaOH and Ca(OH)$_2$ on sludge solubilisation were also investigated by Rajan et al. [56] that observed higher solubilisation ratios of TCOD to SCOD by using NaOH rather than Ca(OH)$_2$, both at 20 and 38 °C.

Comparing Fig. 5 with Fig. 4, it can be observed that the combined low-level thermal and low-level alkaline (T-ALK) pre-treatments enhanced organic matter solubilisation, even if the synergistic effect was not so detectable.

As the low-level thermal (T) and low-level alkaline (ALK) pre-treatment alone were observed to achieve 22.3 and 41.6% COD solubilisation, an additive sum of 63.9% should have been expected for COD solubilization by applying the combined (T-ALK) pre-treatment. However, the experimental results did not confirm the synergetic effect, as DD$_{PCOD}$ resulting from the simultaneous (T-ALK) pre-treatment reached only 46.5%, which is more than that of (T) and (ALK) pre-treatment individually, but lower than their sum. The most probable reason of this result may be that temperature and pH used in this study were not sufficiently high to observe synergetic effects. Even if higher synergetic effects could be expected at higher pH, in this study pH of 10 was selected for the alkaline and the thermo-alkaline pre-treatments in order to reduce chemical usage.

3.1.3. The effect of HC on sludge disintegration

HC alone was tested as low-level mechanical (HC) pre-treatment at 2 bar and 20 °C in order to have a bench-scale HC experiment (Table 3). This value of inlet pressure was selected in order to minimize the costs related to the use of HC. Under these conditions a minimal energy was supplied to the system, but the obtained results showed that (HC) treatment alone at 2 bar was slightly effective in sludge solubilisation. Results on disintegration degree and COD solubilisation are shown in Fig. 6.

1 h of (HC) pre-treatment at 2 bar led to a DD$_{PCOD}$ of only 1.4%, and an increase in SCOD from 228 to 617 mg L^{-1} was observed. However, as reported in previous studies, higher values of the disintegration can be easily obtained by varying HC operating conditions (i.e. inlet

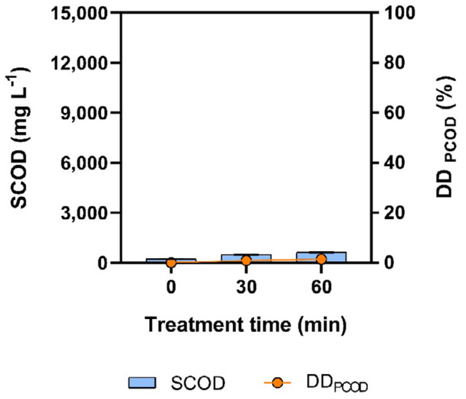


Fig. 6. Effect of low-level mechanical (HC) pre-treatment on COD solubilisation at environment temperature of 20 °C.

pressure) and the (HC) treatment time [21,22]. Indeed, higher disintegration degrees can be achieved in relatively shorter treatment times if higher inlet pressures to the HC system are applied. Alternately, with the same energy supplied to the HC system, similar efficiencies in terms of sludge solubilisation can be obtained by using lower inlet pressures and treatment times higher than 1 h.

3.1.4. *The effect of HC and temperature on sludge disintegration*

The effect of the combined low-level thermal and low-level mechanical (T-HC) pre-treatment on sludge solubilisation was investigated (Fig. 7). By using the experimental setup showed in Fig. 1, SCOD immediately increased from 306 to 4723 mg L^{-1} in about 5–10 min as the temperature was increased from 20 to 50 °C, according to the results reported in section 3.1.1 and 3.1.2. Then, the SCOD further increased to 6600 mg L^{-1} with an operating time of 1 h of (T-HC) pre-treatment.

Compared with the low-level thermal (T) pre-treatment alone (section 3.1.1) and the (HC) pre-treatment alone (section 3.1.3), the combination of HC with low-level thermal (T-HC) pre-treatment led to a slight improvement in the sludge solubilisation, due to synergistic effects of two different sludge disintegration mechanisms.

In this combined treatment synergistic effect was detected as the DD_{PCOD} resulting from the simultaneous (T-HC) treatment reached 23.5%, which was equal to the sum of low-level thermal (T) and (HC) pre-treatment individually, being 22.3 and 1.4%, respectively. The most probable reason of this synergistic effect may be the different disintegration mechanisms of thermal (T) and (HC) treatment. There is a possibility that, after being weakened by thermal attack, the structure of the cell walls and organic material was more vulnerable to the mechanical and chemical attacks produced by the (HC) treatment. However, the HC-thermo (T-HC) pre-treatment had only a slight synergetic effect on WAS solubilisation, this effect was attributable to the low inlet pressure of the (HC) pre-treatment used in this study.

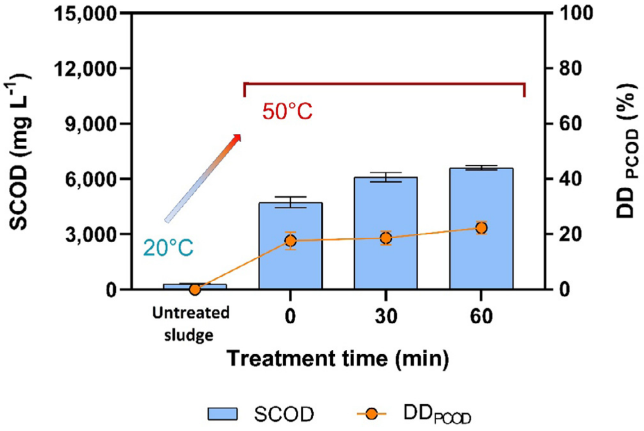


Fig. 7. Effect of low-level thermo-mechanical (T-HC) pre-treatment on COD solubilisation when heating sludge at 50 °C.

Synergetic effects of thermal treatments with acoustic and hydrodynamic cavitation treatments have been reported in literature. In their study Şahinkaya and Sevimli [57] observed that both sonication and low-temperature thermalization disintegrated the WAS, and further the disintegration of sludge was improved significantly by the combined pre-treatment. Similarly, Wett et al. [58], studying the disintegration of sludge pre-treated at 19–21 bar pressure and 160–180 °C for 1 h, showed that the combined pre-treatment induced advanced COD-solubilisation (SCOD/TCOD = 43.0%) and specifically complete destruction of cell mass which was hardly degradable in conventional digestion. Yeneneh et al. [59] observed a complementary synergy between ultrasonic and microwaves, which led to an enhancement of sludge disintegration, floc destruction, cell wall disruption and release of soluble organics.

3.1.5. *The effect of HC and alkalization on sludge disintegration*

The effect of the combined low-level alkaline and low-level mechanical (HC-ALK) pre-treatment on sludge solubilisation was investigated (Fig. 8). After 1 h of combined (HC-ALK) treatment, SCOD increased from 118 to 8899 mg L^{-1}.

DD_{PCOD} was increased up to 44.2% by the (HC) treatment at 2 bar in combination with the alkaline treatment, showing a synergistic effect of the two different sludge disintegration mechanisms in sludge solubilisation.

The experimental results confirmed that the combined (HC-ALK) treatment showed a more pronounced effect and achieved 44.2% COD solubilization, as compared with 1.4% and 41.6% obtained by using the (HC) pre-treatment and the alkaline pre-treatments with NaOH, respectively. The synergistic improvement was +1.2%, calculated as 44.2 − (1.4 + 41.6) = 1.2%. Also in this case, the most probable reason of this synergistic effect may be the different disintegration mechanisms of alkaline and HC treatment: after being damaged by alkaline attack organic compounds were more exposed to the mechanical and chemical attack produced by the HC treatment. As observed in the HC-thermo pre-treatment, the (HC-ALK) pre-treatment has only a slight synergetic effect on WAS solubilisation, again attributable to the low inlet pressure of the (HC) pre-treatment applied in this study.

HC has been employed in literature in order to improve the efficiency of alkaline pre-treatments. Hilares et al. [32] founded that the alkaline assisted HC treatment was a potent and promising approach to pre-treat lignocellulosic biomass. Authors combined HC (0.3 and 0.03 MPa for 45 min) with an alkaline pre-treatment with NaOH (0.1–0.5 M) to treat sugarcane bagasse, increasing the enzymatic digestibility. Sludge solubilization by alkaline assisted HC treatment was studied by Şağban et al. [60] in terms of SCOD and STKN increments.

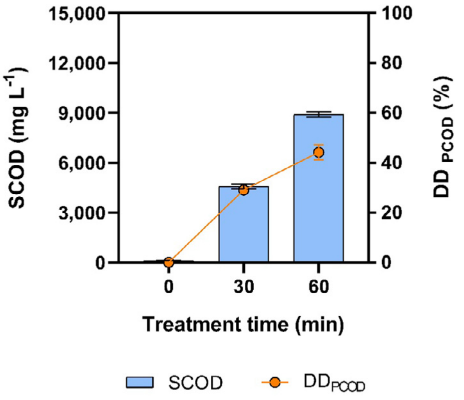


Fig. 8. Effect of low-level mechanical-alkaline (HC-ALK) pre-treatment on COD solubilisation at pH 10 (T = 20 °C).

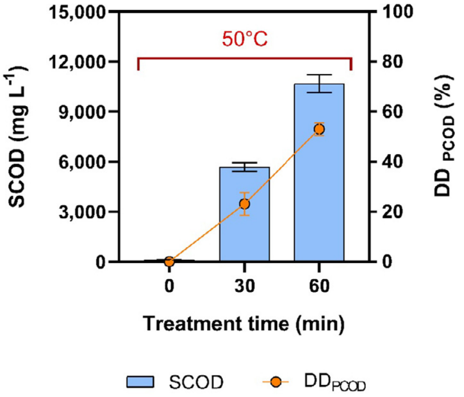


Fig. 9. Effect of combined low-level HC assisted low-level thermo-alkaline (T-HC-ALK) pre-treatment on COD solubilisation at pH 10 when heating sludge at 50 °C.

The synergistic effect was observed for the combination of low-level alkaline treatment with an initial pH level of 9 and HC with an inlet pressure of 5 bar. A higher inlet pressure of 12 bar was used for the HC treatment combined with the alkaline treatment at 9 pH by Suschka et al. [61] in their study. Furthermore, Grubel et al. [62] showed that the adopted alkaline (pH ≈ 9) assisted HC treatment (12 bar) resulted in 22.0–27.0% higher biogas production and 13.0–28.0% higher biogas yield.

3.2. *The synergistic effects of low-level HC assisted thermo-alkaline treatment*

Finally, in the current study, the combination of low-level thermal (50 °C) and low-level alkaline (pH = 10) treatments with low-level (HC) treatment (at 2 bar) for sludge solubilisation was investigated (Fig. 9). Results after 1 h of treatment showed the highest sludge solubilisation efficiency obtained in this study, and the SCOD increased from 118 to 10,673 mg L^{-1}. The sludge solubilising effect of low-level thermo and low-level alkaline (T-ALK) treatment was significantly enhanced after treating the sludge with HC treatment. The DD$_{PCOD}$ efficiency resulting from the simultaneous low-level HC assisted thermo-alkaline (T-HC-ALK) treatment reached 53.0%, which was more than the sum of low-level thermal and low-level alkaline (T-ALK) and low-level (HC) pre-treatment individually, being 46.5% and 1.4%, respectively. The synergistic improvement was +5.1%, calculated as 53.0 − (1.4 + 46.5) = 5.1%. This has therefore the potential to achieve better results using less chemicals, heat and energy. The thermo-alkaline treatment could hydrolyse the organic matter, and HC treating simultaneously emphasizes the solubilising effect, which could possibly accelerate the release of more extra and intracellular material (proteins, carbohydrates, lipids, RNA and DNA) into the bulk liquid. Higher synergistic DD$_{PCOD}$ could be reached at higher inlet pressures, and merits therefore more research.

3.3. *Energetic and economic comparisons*

Table 3 reports the summary of the results of each pre-treatment investigated in this study. Further, both the energy efficiency (EE) and the specific supplied energy (SE) have been calculated and reported for each investigated pre-treatment. According to our aim to develop a low cost-effective pre-treatment to increase sludge biodegradability, the SE applied in this study are relatively low as compared with SE applied in other studies for thermal, alkaline and mechanical treatments [28,63].

However, in order to compare the effectiveness of each investigated pre-treatment, in terms of both SCOD solubilisation and energy consumption, the EE index was used (Table 3).

The low-level mechanical (HC) pre-treatment at 2 bar showed low solubilisation degrees (DD$_{PCOD}$ = 1.4%), requiring high amounts of specific energy, having a low value EE of 3.4 mgΔSCOD kJ^{-1}. This result is most likely due to the very low pressure (2 bar) at the inlet of the HC system applied in this study. Higher EE values have been previously reported for higher HC inlet temperature by Mancuso et al. [28]. Low-level alkaline (ALK) and low-level thermo-alkaline (T-ALK) pre-treatments provided higher values of solubilisation (DD$_{PCOD}$ = 41.6 and 46.5% using NaOH as reagent), even if high values of specific energy (SE) were observed, resulting in the lowest EE values (EE = 2.3 mgΔSCOD kJ^{-1} and EE = 2.4 mgΔSCOD kJ^{-1} respectively). On the contrary, higher COD solubilisation degree were detected by using low-level thermal, thermo-mechanical and mechanical-alkaline pre-treatments, for which higher values of EE were observed, 39.3, 38.2. and 51.5 mgΔSCOD kJ^{-1}, respectively.

However, the highest value of the EE (64.5 mgΔSCOD kJ^{-1}) was achieved for the combined low-level HC assisted thermo-alkaline (T-HC-ALK) pre-treatment, which showed the highest DD$_{PCOD}$ (53.0%), highlighting not only the synergetic effect between HC and thermo-alkaline pre-treatments in terms of sludge solubilisation, but also the sustainability of the system in terms of energy consumption.

The efficiency of the pre-treatments is not only based on the effectiveness of the different pre-treatments (individually or simultaneously applied), but also on their economic performances. Table 3 shows the economic comparison of the different pre-treatments investigated in this work. The total cost associated with each pre-treatment was calculated by considering the total amount of energy required by the different methods and eventually the costs related to the use of chemicals (NaOH, KOH and $Ca(OH)_2$). The total amount of energy required by the different methods was estimated by considering the SE (Table 3), providing a specific cost for each kg of TS that has been treated. The use of reagents showed the highest costs among the pre-treatments investigated, highlighting how the cost linked to the use of reagents for sludge conditioning cannot be neglected. On the contrary, the low-level mechanical (HC) treatment confirmed to be the most energy-efficient treatment. Relatively low costs have also been detected for the low-level thermal (T) pre-treatment, and its combination with the low-level mechanical (T-HC) pre-treatment.

3.4. *The effect of various pre-treatments on EPS*

The released of soluble proteins and carbohydrates was investigated for low-level thermal pre-treatments (T, T-ALK, T-HC-ALK). Low-level thermal (T) pre-treatments, alone or in combination with low-level alkaline (ALK) and low-level (HC) treatments, caused a high release of soluble proteins and carbohydrates. The measured increases in proteins and carbohydrates content in the bulk liquid suggested that cell breakdown and EPS solubilisation occurred.

The amount of soluble proteins and carbohydrates in the untreated sludge were 1010 μg proteins mL^{-1} and 249 mg glucose L^{-1}, respectively. Low-level thermal (T) pre-treatments achieved soluble proteins and carbohydrates concentrations in the range of 1700–2050 μg proteins mL^{-1} and 600–700 mg glucose L^{-1}, respectively (Fig. 10).

This evidence is consistent with the increase in SCOD concentrations, since soluble proteins and carbohydrates are the two predominant organic matters in the SCOD of sewage sludge.

Moreover, for combined pre-treatments (T-ALK and T-HC-ALK), the release of proteins was higher than in the thermal treatment alone, indicating a very clear synergistic effect. Therefore, it can be said that the low-level thermal (T) pre-treatment had weakened the cell walls and led to the release of more proteins when combined with the alkaline and hydrodynamic cavitation treatments. Although the concentration of carbohydrates was higher in each pre-treatment compared to the untreated sludge, only slight differences were detected between pre-treatments, and any clear correlation have been found in this study.

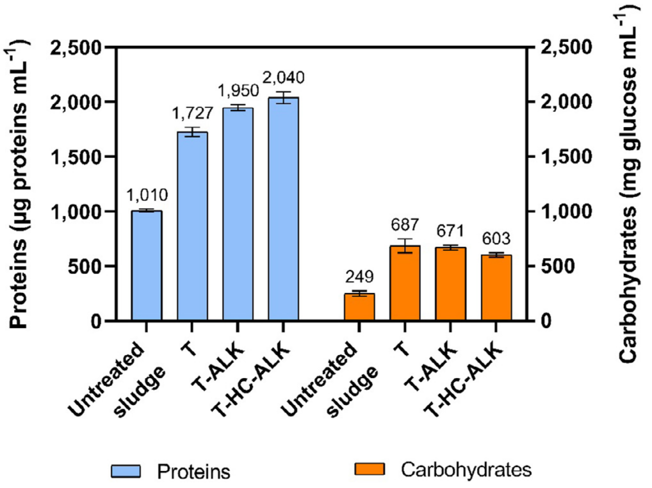


Fig. 10. Effect of low-level thermal pre-treatments on the concentrations of soluble proteins and carbohydrates.

3.5. The effect of various pre-treatments on physiological status of bacteria

Floc disintegration and disruption of bacterial cells for low-level thermal pre-treatments (T, T-ALK, T-HC, T-HC-ALK) were studied, identifying free intact (alive) and free permeabilised (dead) cells simultaneously based on their membrane integrity. Populations of intact, dead, aggregates and destroyed cells were distinguished and quantified as shown in profiles of Fig. 11.

During the initial part of each pre-treatment mainly flocs disintegration occurred, instead of cell disruption, as showed by the decrease of aggregates and the increase of the total free cells (intact + permeabilised). Depending on the pre-treatment applied, cell lysis occurred with the increase of treatment time, as showed by the increase of measured destroyed cell.

Low-level thermal (T) pre-treatment acted more on flocs disaggregation than cell disruption. Due to the rising temperature of WAS from 20 to 50 °C, after 30 min of pre-treatment, aggregates were reduced from 32 to 6%, permeabilised cells increased from 25% to 49% and the percentage of intact cells was almost unaltered (44%). After 1 h of low-level thermal (T) treatment, intact cells decreased and a percentage of 31% was observed, while permeabilised cells increased.

The low-level thermo and low-level alkaline pre-treatment (T-ALK) resulted in a progressive conversion of aggregated cells into mainly dead cells. The synergetic effect of temperature and alkali agents led to a faster increase of permeabilised cells, as compared with the thermal pre-treatment. After 30 min of treatment, aggregates decreased from 56 to 18%, permeabilised cells increased from 2 to 67%, while intact cells were reduced significantly from 42 to 15%. After 1 h of treatment, aggregates were further reduced, furthermore a percentage of 2% of destroyed cells was observed, indicating cell lysis.

In low-level thermal and low-level mechanical pre-treatment (T-HC), the effect of HC at 50 °C on the status of bacteria was investigated. Due to the low inlet pressure of only 2 bar, thermal assisted HC (T-HC) mainly acted as flocs disintegration mechanism, instead of cell disruption mechanism, as showed by the decrease of aggregates and the increase of both permeabilised and intact cells. After 1 h of treatment, aggregates were further reduced, but destroyed cells were not observed.

Compared with the low-level thermal (T) pre-treatment alone, the combination of HC with low-level thermal (T-HC) pre-treatment did not led to a significant improvement of the effectiveness of the treatment in terms of cells lysis, mainly due to the low inlet pressure and the limited treatment time of 60 min.

Finally, in the combined low-level HC assisted thermo-alkaline (T-HC-ALK) pre-treatment, the combination of (HC) treatment with the thermo-alkaline (T-ALK) pre-treatment resulted in a slight increase of destroyed cells after 1 h of treatment, as result of synergic effects of the combined treatments: while mechanical shear forces created by HC disintegrated the sludge flocs, the temperature of 50 °C and the alkaline treatment helped HC to break down the undisrupted microorganism cells into soluble organic matters by destroying the chemical bonds of the cell wall and membrane.

4. Conclusions

The present work reports the use of HC at 2 bar as a low-level pre-

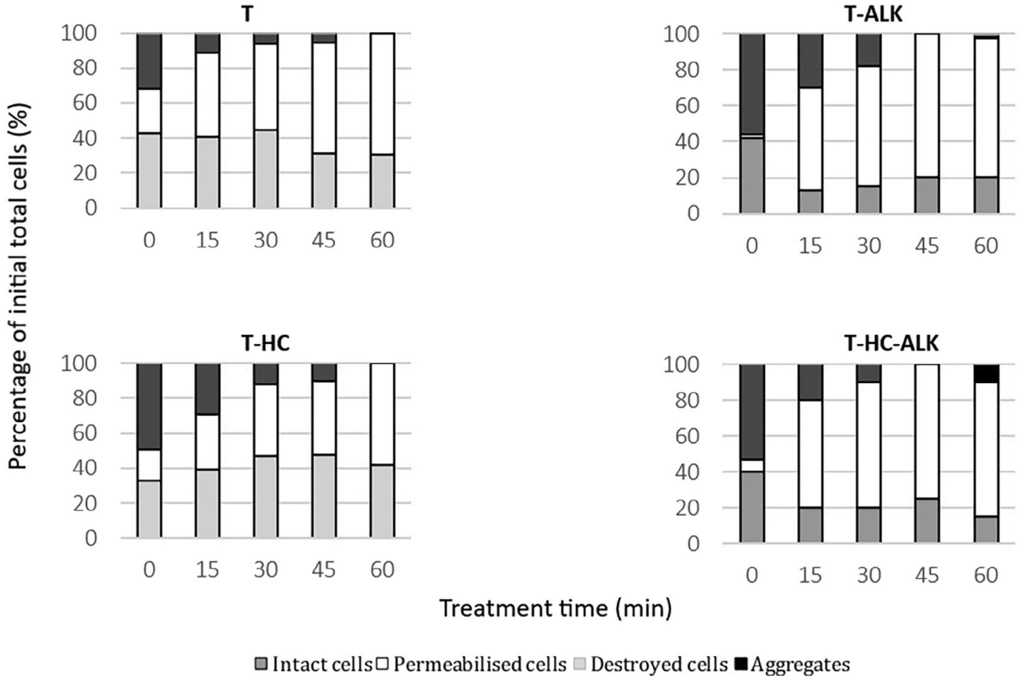


Fig. 11. Profiles of percentages of intact cells, permeabilised cells, destroyed cells and aggregates.

treatment technique before AD in combination with low-level thermo-alkaline treatments to increase WAS solubilisation. The effects of mechanical (2 bar), low-level thermal (50 °C) and alkaline (pH = 10) pre-treatments, alone and a combination of one or more treatments, were tested on sludge solubilisation. Results showed that some of these pre-treatments, if individually applied, led to very low sludge solubilisation degrees, mainly due to the smooth operative conditions of the applied treatments. For instance, in the case of (HC) alone, the lower efficiencies in terms of sludge solubilisation could be attributable to the very low inlet pressure (2 bar) considered during this study. Nevertheless, for the other pre-treatments, an increase in sludge solubilisation was observed. The combined treatments showed the highest efficiency. In particular, the low-level HC assisted low-level thermo-alkaline (T-HC-ALK) pre-treatment was the most efficient in terms of both sludge solubilisation and energy efficiency, showing the highest synergistic effect.

In this combined pre-treatment, cytometric analyses showed that while mechanical shear forces created by HC disintegrated the sludge flocs, the increase in temperature and the chemical hydrolysis as result of pH increase weaken the bacterial cells and dissolve external polymers, thus distinctively improving the effects of sludge solubilization. Cytometric analyses also revealed that after 30 min of treatment, regardless of the applied technology, a decrease in biomass viability occurred with an increase in sludge solubilisation, as consequence of dead cells and/or disrupted cells (cell lysis).

To conclude, the low-level HC assisted thermo-chemical technology offers a potential advantage for sludge solubilisation in terms of its less energy consumption and ease of operation, flexibility and capability to vary the required intensities of cavitational conditions. However, in order to obtain higher synergic effects, higher inlet pressures can be applied in the HC treatment when combined with low-level thermal-alkaline treatments.

Acknowledgements

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