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Alkaline and acid solubilisation of waste activated sludge

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ABSTRACT

The influence of acidic and alkaline conditions on the solubilisation process of waste activated sludge (WAS) was investigated using HCl and NaOH at pH 2, 10, 11 and 12. The rise in concentration of solubilised compounds, the influence of reaction time, and the influence of the concentration of total solids (TS) during the solubilisation process were determined. Physical and chemical tests demonstrated that pre-treatment provided a release of compounds from the sludge floc matrix into the soluble fraction, characterising the solubilisation process. The highest degree of WAS solubilisation was observed when a pH of 12 was applied. Although largest effects were already attained after 0.25 h, WAS solubilisation continued reaching an increase in total dissolved solids by a factor 10.4 after 720 hrs. Under these conditions, the dissolved organic carbon (DOC), proteins, and carbohydrates resulted in releases up to 15, 40 and 41 times, respectively; phosphorus increased 5.7 times. Results indicate that by applying alkaline pre-treatment, higher TS concentrations can be treated per reactor volume compared to non-pre-treated WAS. Aerobic and anaerobic biodegradability tests showed increased bioconversion potentials in full-scale treatment plants. The respirometry tests ratify the improvement in solubilisation, with O_2 consumption rates increasing 1.4 times, concomitant with an additional 261 $mg{\cdot}L^{-1}$ of the COD used, which represents 90% bioconversion of waste activated sludge. Biomethanisation test indicated an increase of 3.6 times relative to the blank.

Key words | alkaline solubilisation, chemical solubilisation, pre-treatment of WAS, sludge hydrolysis

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HIGHLIGHTS

- Increased biodegradability of sludge identified by respirometric test.
- Increased applicable sludge loading rates per reactor volume.
- Increased degree of depolymerisation, characterised by the release of COD, TKN, phosphorus, protein, and carbohydrate in soluble fraction.
- The alkaline process significantly favours the solubilisation of microbial aggregates over the acid process.

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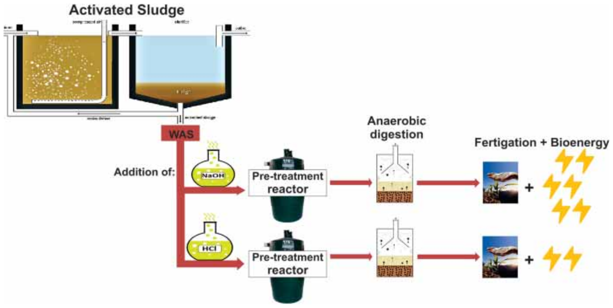
GRAPHICAL ABSTRACT



INTRODUCTION

The activated sludge process is a widely applied technology for the treatment of municipal wastewater. Being developed and vastly implemented in industrialised countries, it is also applied in the less prosperous countries worldwide. For instance, in Latin American countries, the activated sludge process is the second most applied technology in numbers of wastewater treatment plants (WWTPs); however, it treats the major part of the total accumulated flows ($m^3.s^{-1}$) of the sewage, i.e. 58% (Noyola *et al.* 2012). In this treatment process, the organic matter and contaminants are partly converted to excess sludge, which consists of new bacterial mass and entrapped organics. The waste activated sludge (WAS) represents less than 2% of the total sewage volume treated; however, it must be properly treated and disposed of (Foladori *et al.* 2010). In fact, WAS is the main residue of the activated sludge-based WWTPs and its production has been increased with the increase in installed treatment capacity. Meanwhile, the possibilities for treatment and disposal are limited or have even been decreased in certain countries (Bougrier *et al.* 2008). WAS management has been recognised as one of the most difficult and expensive problems in the sanitary engineering field (Tchobanoglous *et al.* 2003). The handling of this fraction may represent more than 50% of the total operational costs of WWTPs (Kroiss 2004; Neyens *et al.* 2004; Appels *et al.* 2008; Pilli *et al.* 2011; Tsadilas *et al.* 2014).

Although WAS is a costly residue due the expensive management process, it is rich in specific resources. Therefore, recovery of resources from WAS could allow for adding economic value to the produced waste. With this in mind, there is a lot of research going into developing technologies to treat and reduce the WAS produced at these WWTPs, meanwhile recovering specific resources for reuse (Campo *et al.* 2018; Sun *et al.* 2018; Wei *et al.* 2018; Zahedi *et al.* 2018), even in countries like Brazil (Rosa *et al.* 2015; Pinto-Júnior *et al.* 2020). The economic value aggregated by the recovery of resources provides possibilities for reduction in exploitation costs; resources that are increasingly considered are bioenergy, phosphorus, biopolymers, volatile fatty acids (VFAs), and water (Chimuca *et al.* 2020; Leng *et al.* 2020; Liu *et al.* 2020; Schambeck *et al.* 2020; Sousa *et al.* 2020; Zhang *et al.* 2020). The VFAs can be consumed as an external carbon source during the biological phosphorus removal process, denitrification process, and anaerobic digestion. Anaerobic digestion of WAS delivers biogas, which is recognised as a renewable bioenergy source. Nutrients can be recovered in various ways, such as via struvite precipitation from the sludge reject water, which produces by-products that can be used as agricultural fertilizers. Alternatively, the treated effluent can be used for fertigation in irrigated agriculture or city greening. Finally, the reclaimed water can also be used as process water in industry or other urban reuse applications.

In a review paper, Gonzalez *et al.* (2018) report that WAS is mostly composed of proteins and humic substances with bacterial biomass, carbohydrates, and DNA. These biodegradable compounds offer a source of renewable organic molecules. However, organic molecules recovery is limited, because all compounds are embedded into an organised matrix of microbial aggregates and extracellular mass, which in WAS are present in floc form. The extracellular mass consists of extracellular polymeric substances (EPS), which strengthen the microbial communities' physical structure and decreases their biodegradability (Stuckey & McCarty 1984). EPS is an important constituent of the floc matrix, acting as the bound material to maintain the mechanical stability of microbial aggregates, contributing to the surface properties of floc, floc strength, and floc formation, as well as representing the major organic fraction in activated sludge (Sponza 2003; Sheng & Yu 2006; Wang *et al.* 2014). In addition, EPS is a complex structure that contains glycan chains bound to peptides, which protect the microorganisms from cell lysis and dewatering; it is a nutrient source, and makes the biodegradation process difficult (Elefsiniotis & Oldham 1994; Sheng *et al.* 2010; Tian *et al.* 2015). Therefore, upfront disintegration of EPS in a pre-treatment step will facilitate the recovery of resources from WAS.

The idea of the pre-treatment is to rupture the sludge floc structure and to cause lysis of the microbial cells (Carrère *et al.* 2010) through forced solubilisation. Different strategies are being developed to not only enhance sludge reduction (Guo *et al.* 2020), but also increase its bioavailability as a renewable source of resources. Such strategies can be physical, chemical, thermal, biological or any combination of these (Carrère *et al.* 2010; Jain *et al.* 2015; Gonzalez *et al.* 2018). Therefore, solubilisation is applied as a pre-treatment step, as it has been designed to increase the biodegradable sludge fraction and reduce the fraction of refractory compounds; thus, transforming particulate organic matter into soluble organic matter.

The release of inner floc components implies a decrease in high molecular weight organic matter in the aggregates. It's hypothesized that the increased bioavailability is due to the shift of extracellular proteins, polysaccharides, and enzymes from the inner layers of sludge flocs to the outer layers (Chu *et al.* 2002; Vaxelaire *et al.* 2008; Yu *et al.* 2008; Erden *et al.* 2010), likely also allowing increased cell lysis during pre-treatment. The released inner compounds could potentially be used in anaerobic digestion, leading to an increase in methane production (Elliott & Mahmood 2007; Zahedi *et al.* 2016). Therefore, the percentage of inorganic matter in digested sludge tends to increase, meanwhile improving the sludge dewaterability (Wawrzynczyk *et al.* 2007).

The chemical solubilisation process can be conducted by alkaline or acid pre-treatment at a specific pH and it has been reported as being able to provide floc and cell disruption, resulting in a decrease in particle size (Liao *et al.* 2002; Kim *et al.* 2003; Doğan & Sanin 2009; Wang *et al.* 2012; Xiao *et al.* 2015). A low pH, and thus high H^+ concentration, can destabilise the isoelectric point of macro molecules, such as proteins. Applying a high pH results in deprotonation of macro molecules, which changes the electric charge. The applied pH variation can significantly influence the rheological properties, metal binding, organic adsorption capacities, extraction efficiency of EPS, and flocculation properties (Omoike & Chorover 2004; Sheng *et al.* 2005). Knowledge of the effect of pH changes on EPS tertiary and quaternary structure, its surface properties, and electrostatic charge is still limited (Wang *et al.* 2012); however, it is closely linked to the EPS physicochemical properties (Dogsa *et al.* 2005; Wang *et al.* 2012). The flocculation and deflocculating processes of EPS are related to the applied pH levels (Salehizadeh & Shojaosadati 2001; Sheng *et al.* 2010). However, although particle size reduction has been reported for alkaline and acid solubilisation, subsequent cellular damage to the process may reduce the quality of solubilised material (Guo *et al.* 2014; Xiao *et al.* 2015). Therefore, it is necessary to compare both solubilisation processes in order to evaluate the quality of solubilised material for later biological processing or possible recovery of products.

In alkaline pre-treatment, it is common to use alkalis such as sodium hydroxide (NaOH), potassium hydroxide (KOH), magnesium hydroxide ($Mg(OH)_2$), and calcium hydroxide ($Ca(OH)_2$); however, NaOH is the most frequently used due to its high solubilisation rate using relatively low dosages (Jih-Gaw *et al.* 1997; Tian *et al.* 2015). Conversely, chemical species such as hydrochloric acid (HCl), sulfuric acid (H_2SO_4), phosphoric acid (H_3PO_4), and nitrous acid (HNO_2) are commonly used for acid pre-treatment. Acid solubilisation is also reported as a pre-treatment applied to increase methane production by improving the solubilisation rate (Jain *et al.* 2015). However, several authors disagree about the best pH value to perform solubilisation, as well as the best reaction time. This requires a comparison between acid and alkaline pre-treatment to define the most relevant parameters in the solubilisation process, as well as the necessary identification of the best reaction time. Additionally, few studies have evaluated the influence of total solids (TS) concentration, and thus the applied chemicals-TS ratio. Thus, the purpose of this work was to investigate the effect of acid and alkaline pre-treatment in sludge solubilisation, releasing the inner compounds. Different pre-treatment conditions were proposed to identify: (I) the effects of acid and alkaline solubilisation; (II) the effects of chemical concentrations and their respective reaction times; (III) the optimal TS concentration; and (IV) the increase in biodegradability.

MATERIALS AND METHODS

Operational procedures

The sludge for the experimental procedure was produced in an activated sludge system using a sequencing batch reactor (SBR), fed by municipal sewage from Campina Grande – PB (Brazil). The SBR operation was characterised by four cycles daily, and the age of the sludge was eight days. The oxygen concentration in the medium was maintained at about 2 $mgO_2{\cdot}L^{-1}$.

The work was developed in four steps, with the first three designed to identify the best solubilisation condition, and the last to investigate the biodegradability increase: Step I – Chemical (acid and alkaline) pre-treatment effects in sludge solubilisation; Step II – Influence of different alkali concentrations and their respective reaction times; Step III – Influence of the concentrations of TS on chemical solubilisation of sludge; and Step IV – Assessment of increase in biodegradability.

Step I – Chemical (acid and alkaline) pre-treatment effects in sludge solubilisation

In Step I, the influences of acid (pH 2) and alkaline (pH 12) pre-treatments in sludge solubilisation were analysed and compared with a control sample of an unaltered pH. For this step, the concentration of TS was adjusted to 20 $g{\cdot}L^{-1}$, according to the recommendations of Bi *et al.* (2014). For the acid pre-treatment, the pH was adjusted to 2, using hydrochloric acid (HCl). To finish the acidic solubilisation process, NaOH was used to return the pH to the initial level. During the assays of alkaline pre-treatment, NaOH was used to raise the pH to 12 and HCl to turn it back. It is important to point out that HCl was chosen because it does not contain oxygenated anions that may act as an alternative electron acceptor, affecting the performance of subsequent methanogenesis, and it is the most accessibly priced acid. NaOH was selected as a highly efficient alkali in the solubilisation of organic sludge particles when compared to others, such as $Ca(OH)_2$ (Cho *et al.* 2013).

In order to determine the necessary chemical dosage (HCl and NaOH) to adjust the pH values of each pre-treatment (acid and alkaline), a titration test was applied, adding the chemical compounds in 1 M of concentration. The same procedure was performed to reach the neutral pH of the sample. The amounts used to raise and decrease the pH levels were calculated in mmol of chemical species (HCl or NaOH) per grams of sludge TS. The required dosages of HCl and NaOH for each pre-treatment are listed in Table 1.

Samples of sludge (100 mL) were mixed in distinct erlenmeyers (250 mL) for three different setups reproduced in triplicate. The first setup entailed acid pre-treatment with a dosage of 0.011 mmol $HCl{\cdot}gTS_{sludge}^{-1}$ to adjust the pH to 2; the second was alkaline pre-treatment at pH 12 adjusted by the dosage of 6.25 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$, and the third had no pre-treatment, referred to as the control sample. The mixtures were kept under constant stirring at 200 rpm on a shaker table (New Brunswick Scientific, mod. G 33) for 48 h at room temperature, approximately 25 °C. At the end of the shaking, both samples were neutralised, each one with its respective reagent, NaOH or HCl.

Table 1 | Dosages of NaOH and HCl required to start and finish the acid and alkaline solubilisation process

pH	Starting dosage (t_0)	Neutralisation dosage (t)
2	0.011 $mmolHCl{\cdot}gTS_{sludge}^{-1}$	2.75 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$
10	1.25 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$	0.004 $mmolHCl{\cdot}gTS_{sludge}^{-1}$
11	2.50 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$	0.007 $mmolHCl{\cdot}gTS_{sludge}^{-1}$
12	6.25 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$	0.021 $mmolHCl{\cdot}gTS_{sludge}^{-1}$

Step II – Influence of different alkali concentrations and their respective reaction times

Based on the results from Step I, the influence of different alkali concentrations and the solubilisation reaction times were investigated. According to the literature, pH values below 10 do not show good sludge solubility (Xiao *et al.* 2015; Cai & Liu 2018). So, aiming at significant levels of sludge solubility by alkaline pre-treatment, the pH levels investigated in this step were 10, 11 and 12, based on the literature (Vlyssides & Karlis 2004; Doğan & Sanin 2009; Park *et al.* 2012; Uma Rani *et al.* 2012; Kavitha *et al.* 2014). The reaction times were 0.25, 0.5, 1, 3, 6, 9, 15, 24, 36, 48, 96, 144, 192, 240, 384, 528 and 720 h, at room temperature (25 °C). The reaction time started at the time (t_0), when the sludge was subjected to a specific pH, and finished (t) with the neutralisation. As in the previous step, the pH control was performed with NaOH and HCl and the TS concentrations were 20 $g{\cdot}L^{-1}$.

The necessary volumes of HCl and NaOH to be added to reach pH 10 and 11 were determined using the titration test, the same method applied for pH 12 in the first step. The volumes used to raise and decrease the pH were calculated and are presented in Table 1. At that moment, 51 erlenmeyers were separated into three groups of 17 flasks each. Each group corresponded to each pH investigated (10, 11 and 12) with their respective dosages (1.25, 2.50 and 6.25 mmol $NaOH{\cdot}gTS_{sludge}^{-1}$). Thereafter, similar to the first step, 100 mL of sludge was added to each erlenmeyer and stirred at 200 rpm on a stirring table (New Brunswick Scientific, mod. G 33) at room temperature (25 °C) during the 17 different proposed reaction times. At the end of each time period, the pre-treated sludge sample in each pH range was neutralised with HCl. All these experimental procedures were carried out in triplicate.

Step III – Influence of the TS concentrations on chemical solubilisation of sludge

In the third step, the influence of the alkali/TS concentration on the alkaline pre-treatment was investigated. Following the results from previous steps, the alkaline pre-treatment with the best solubilisation was selected as the optimal pH. This pH was subsequently used to perform Step III with a focus on the concentration of solids. For better comparison, reaction times of 24 and 48 hours were selected for this step. The sludge had the TS concentration adjusted to 30 g·L^{-1}. From this concentration, distilled water was used to prepare sludge samples of 25, 20, 15, 10 and 5 g·L^{-1}. In Table 2, the amounts of water and sludge used to adjust each condition of this step are presented. The necessary dosages of NaOH and HCl for each condition are also presented in the table.

The alkalis were added to six erlenmeyers for each concentration of solids, as shown in Table 2; experiments were carried out in triplicate. To maintain the mixture, the same methodology from the previous steps was performed, i.e., stirring at 200 rpm on a stirring table (New Brunswick Scientific, mod. G 33) at room temperature (25 °C). The same procedure was applied for the reaction time of 24 hours and was repeated for 48 hours' reaction time. The solubilisation process was halted, applying HCl titration for pH neutralisation.

Step IV – Assessment of increase in biodegradability

In addition to the physical and chemical tests applied to evaluate the solubilisation potential of the sludge, the biodegradability of the released organic matter was assessed in Step IV. The respirometry test was applied for aerobic biodegradability investigation. Meanwhile, the biomethane accumulation test was used to measure the improvement in anaerobic biodegradability. Therefore, respirometry and biomethane accumulation tests were carried out for both the raw (control sample) and the pre-treated sludge. Selection of the pre-treatment conditions for Step IV were based on the results of previous experiments, giving the best solubilisation results.

Table 2 | Variation of TS and their respective dosages of NaOH and HCl

TS (g·L^{-1})	Sludge (mL)	H_2O (mL)	mmolNaOH·gTS$^{-1}_{sludge}$	mmolHCl·gTS$^{-1}_{sludge}$
30	100	0	9.50	0.030
25	83	17	8.00	0.026
20	67	33	6.25	0.021
15	50	50	4.75	0.016
10	33	67	3.25	0.010
5	17	83	1.75	0.005

Respirometry tests. The aerobic respirometry test procedures followed the detailed procedures as outlined in literature (van Haandel & Catunda 1982; Spanjers & Vanrolleghem 1995; Vanrolleghem *et al.* 1999). The applied methodology allowed evaluation of the oxygen uptake rate (OUR), also known as the respiration rate, differentiating the removal rate of readily and slowly biodegradable organic matter by heterotrophic organisms. The applied methodology allowed for calculating both the catabolised and anabolised fractions during the aerobic bacterium metabolism (van Haandel & Catunda 1982; Costa *et al.* 2007; Sousa *et al.* 2013). The test was carried out with both raw and pre-treated sludge and results were evaluated making use of the following COD fractions:

- COD filtered (COD_f), which reflects the soluble COD available for bio-oxidation; prior to analysis, liquid samples are filtered using a 0.45 μm paper filter;
- COD used (COD_u), which is the biodegradable COD fraction that was metabolised and measured in the respirometry test (Sousa *et al.* 2013);
- COD_u is divided into COD readily biodegradable (COD_{rb}), and COD slowly biodegradable (COD_{sb}) following the approach of Sousa *et al.* (2013). During the respirometry test, the transition in oxidation of COD_{rb} to COD_{sb} is markedly indicated by a sudden sharp drop in the oxygen uptake rate.

Biomethane accumulation test. The biomethane accumulation test quantifies the methanogenic bioconversion of a given substrate. The test was carried out in a closed reactor, designed to prevent gas leakage, guaranteeing internal pressure assessment. A pressure sensor was coupled to the reactor and connected to an Arduino board with a digital manometer (MPX5700AP) that constantly recorded the internal reactor pressure. Continuous data logging was provided, generating graphs with pressure increase over time. The tests were performed with both raw and pre-treated sludge and carried out under standard temperature and pressure (STP) conditions. The experimentally assessed cumulative biogas production in the batch assays was calculated to biomethane based on the chromatography analysis of the gas phase. The graphs were mathematically interpreted using the modified Gompertz model (Equation (1)) (Jiunn-Jyi *et al.* 1997):

$$M = P \times \exp\left\{-\exp\left[\frac{R_m^e}{P} \times (\lambda - t) + 1\right]\right\} \qquad (1)$$

where M is the accumulated biogas production at STP ($mL.gVSS^{-1}$), P is the potential biogas production ($mL{\cdot}gVSS^{-1}$), R_m is the maximum biogas production rate ($mL{\cdot}day^{-1}$), λ is the lag-phase time (days), t is the incubation time (days), and e equals 2.718.

Analytical parameters

Physicochemical characterisation was performed for all samples, before and after the pre-treatment, and always in triplicate. The sludge samples were centrifuged at 3,500 rpm for 30 min, and then the supernatant was filtered over a membrane with a mesh size of 0.45 μm. The measured parameters of the filtrate following the procedures of Standard Methods (Bridgewater *et al.* 2012) were orthophosphate (P as PO_4^{-3}); total kjeldahl nitrogen (TKN); chemical oxygen demand (COD); total dissolved solids (TDS), and their fractions: fixed dissolved solids (FDS) and volatile dissolved solids (VDS). Protein analysis was performed applying the Lowry method modified by Frølund *et al.* (1995), and carbohydrate analysis was performed using the method described by Dubois *et al.* (1956). The determination of dissolved organic carbon (DOC) was performed using a high temperature combustion analyser (COT Multi N/C 3,100 Analytik Jena, Germany).

The biogas composition of the gas samples was determined via gas chromatography (7890A GC; Agilent Technologies, USA) only at the end of the test using a thermal conductivity detector operated at 200 °C and oven temperature ramping from 40 to 100 °C. The system operated with two columns: an HP-PLOT Molesieve GC Column 30 m × 0.53 mm × 25.00 μm and an HP-PLOT U GC Column, 30 m, 0.53 mm, 20.00 μm (Agilent Technologies, USA). The carrier gas was helium at a constant flow rate of 10 mL min^{-1}.

RESULTS AND DISCUSSION

Step I – Chemical (acid and alkaline) pre-treatment effects in sludge solubilisation

To compare the chemical pre-treatment by alkaline and acid solubilisation methods, the average results are shown in Table 3. Both methods were identified as being able to solubilise the sludge; however, the alkali was more effective than the acid. Comparing to raw sludge, the DOC for acid treatment increased 1.3 times, whereas for the alkali the increase was 15 times. The better alkaline solubilisation compared to acidic is in accordance with Cai & Liu (2018), they worked with the same reagents, also at pH 2 and 12, and reported a higher increase in DOC, dissolved nitrogen, and biopolymers in the soluble fraction after alkaline treatment. The limited effects of acid solubilisation were reported to be due to absence of cell lysis (Sahinkaya 2015), with only the occurrence of sludge floc breakage being identified (Bi *et al.* 2014).

Conversely, the VDS concentration had a significant and similar increase in both kinds of pre-treatments, reaching a factor 10–11 for both acid and alkaline treatment. Increase in FDS was a factor of about 4 for the acid treatment compared with the raw sludge, and 10 for the alkaline solubilisation. Significant increases in FDS were noticed, which can be explained by dissolved salt formation, resulting from the addition of NaOH and HCl (Tchobanoglous *et al.* 2003). However, the suspended solids decrease, in agreement with the high FDS increase in alkaline solubilisation, highlights the release of inorganic material trapped in the floc structure. These results are in accordance with the literature, which reported a decrease in particle size after both alkaline (Kim *et al.* 2003; Doğan & Sanin 2009; Xiao *et al.* 2015) and acid (Liao *et al.* 2002; Wang *et al.* 2012; Guo *et al.* 2014)

Table 3 | Characterisation before and after the alkaline and acid pre-treatments, and for all parameters the number of samples was 9

Parameters	Raw sludge $\bar{x} \pm \delta$	Acid treatment (pH 2) $\bar{x} \pm \delta$	Alkaline treatment (pH 12) $\bar{x} \pm \delta$
Dissolved organic carbon ($mg{\cdot}gTS^{-1}$)	0.9 ± 0.1	1.2 ± 0.2	13.7 ± 0.7
Total dissolved solids ($mg{\cdot}gTS^{-1}$)	48 ± 4	286 ± 5	501 ± 6
Volatile dissolved solids ($mg{\cdot}gTS^{-1}$)	13 ± 1	132 ± 3	136 ± 3
Fixed dissolved solids ($mg{\cdot}gTS^{-1}$)	36 ± 2	154 ± 4	365 ± 5
TKN ($mg{\cdot}gTS^{-1}$)	5.9 ± 0.4	8.3 ± 0.5	14.2 ± 0.7
Phosphorus ($mg{\cdot}gTS^{-1}$)	0.2 ± 0.04	0.22 ± 0.05	1.40 ± 0.15
Protein ($mg{\cdot}gTS^{-1}$)	2.8 ± 0.17	8.9 ± 0.48	131.1 ± 1.94
Carbohydrate ($mg{\cdot}gTS^{-1}$)	1.2 ± 0.1	4.4 ± 0.2	23.7 ± 0.7

treatments. At low pH levels, protonation of the medium occurs, destroying microbial aggregation (Liao *et al.* 2002; Wang *et al.* 2012). Therefore, compared to alkaline treatment, the acid solubilisation with lowest cell damage (Guo *et al.* 2014) is limited to floc disruption while the alkaline goes beyond breaking the floc, damaging the cell membrane.

Compared to the best alkaline result, the soluble fraction of TKN and phosphorus had a limited increase during acid solubilisation, reaching 1.4 times for each. In contrast, the alkaline solubilisation reached 2.4 times the soluble TKN concentration, and 8.8 times the phosphorus compared with the raw sludge. Obtained results indicate that an inner fraction of the flocs was released, and the alkaline conditions resulted in cell damage. This agrees with Guo *et al.* (2014), who concluded that EPS rupture was highest for the NaOH solubilisation process, with increased damage to cell walls and cell membranes.

The breakdown of cells in sludge increases the bioavailability of proteins, carbohydrates, and nucleic acids (Kim *et al.* 2010). Therefore, the increase in soluble protein and carbohydrate concentrations was also investigated. In acid treatment, the protein and carbohydrate concentrations were 3.2 and 3.7 times higher than in the raw sludge. In contrast, the alkaline treatment increased protein concentrations 47 times, while the carbohydrate concentrations were 20 times higher than raw sludge. These results are comparable to those reported by Xiao *et al.* (2015). Comparing these results with the literature, HCl dosage as an acid pre-treatment is effective for COD solubilisation at pH values of 1–2 (Devlin *et al.* 2011; Sahinkaya 2015). However, carbohydrate and protein solubilisation are still limited to an increase of only 4 and 6 times, respectively (Devlin *et al.* 2011). In alkaline solubilisation, the carbohydrate increase corroborates with the literature better than acid solubilisation (Guo *et al.* 2014). The release of soluble proteins in the alkaline treatment was almost 15 times higher than in the acid pre-treatment; this higher release was previously reported in the literature (Liu *et al.* 2019). Similar results were observed with different treatment methods, for instance ultrasonication (Sahinkaya 2015).

All these increases are consistent with each other; however, comparing both treatments shows that alkaline solubilisation offered better release of inner organic molecules. Other researchers also suggest that although acidic pre-treatment is able to solubilise organic matter, alkaline pre-treatment is more effective (Chen *et al.* 2007; Guo *et al.* 2014; Maspolim *et al.* 2015). In summary, the efficiency of the alkaline method made it a better option for WAS solubilisation.

Step II – Influence of different alkali concentrations and their respective reaction times

The pH range investigation was performed by varying pH values incrementally from 10 to 11, and then to 12, with the objective of selecting the best pH for the solubilisation process of WAS regarding the best reaction time, as explained in the methodology. The proposed pH values were based on literature, as was explained in methodology.

In Figure 1, the dissolved organic carbon levels for pH 10, 11 and 12 are shown versus time (logarithmic time scale). Interestingly, the results of pH 11 and 12 were already noteworthy after the initial 15 minutes (0.25 h). A more pronounced effect at pH 12 was particularly noticeable over time, while conditions at pH 10 and 11 do not show the same significant organic carbon release. Especially at pH 10, the worst solubilisation was noted from the first 15 minutes through to the total 720 hours, with an accumulated release of just 3.8 $\text{mgDOC}\cdot\text{gTS}^{-1}$, which means a total of 75.5 $\text{mgDOC}\cdot\text{L}^{-1}$. At pH 11, despite initial results being near to pH 12, the difference was remarkable throughout the remaining time period. Conversely, the improved solubilisation in the first 9 hours at pH 12 was 11 times, which represents an addition of 10 $\text{mgDOC}\cdot\text{gST}^{-1}$ in the soluble fraction. After these 9 hours, the fast concentration-increasing process slowed down, and in 720 hours the total amount achieved was 16,14 $\text{mgDOC}\cdot\text{gTS}^{-1}$, of which about half the value was released before 9 h of the experiment.

The dissolved solids (DS) concentration assays over time are summarised in Figure 2. The observed DS increase for all three pH values indicates that alkaline solubilisation increases the soluble fraction. However, the increase in concentration of fixed solids was clearly higher than the volatile solids increase, which was previously explained as a consequence of salt formation due to the addition of NaOH and HCl (Tchobanoglous *et al.* 2003). The salts residue indicates the increase in salinity of the medium, which, according to the literature, does not necessarily inhibit anaerobic bioconversion (Miranda *et al.* 2014; Sierra *et al.* 2018). Compared to the DOC results in Figure 1, the VDS solubilisation results clearly showed a pH dependency; the better release at pH 12 was also remarkable.

The pre-treatment at pH 10 and 11 maintained lower solubilisation levels; for pH 12, the better release of organic molecules from inner floc was confirmed, analysing solubilised proteins, carbohydrates (Figure 3), phosphorus (Figure 4), and TKN. For pre-treatment at pH 12, the noticeable results for protein and carbohydrate in 9 hours were 69.2 and 14.7 $\text{mg}\cdot\text{gST}^{-1}$, respectively, which represent a 15

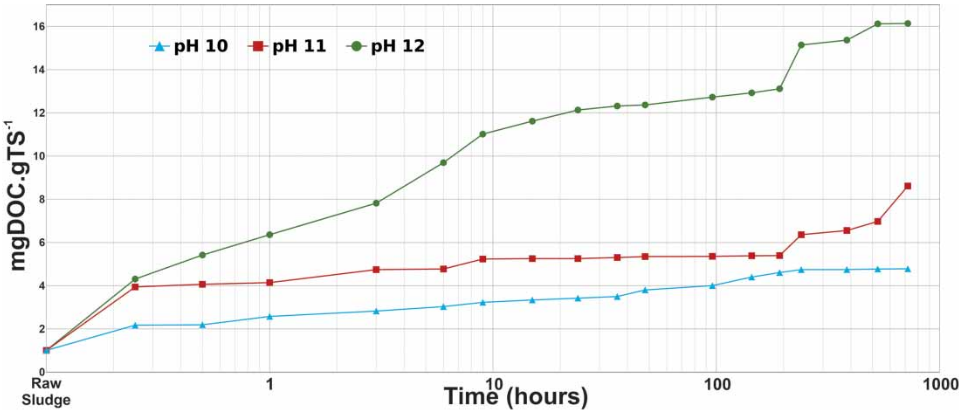


Figure 1 | pH influence in dissolved organic carbon (DOC) concentration profile through time.

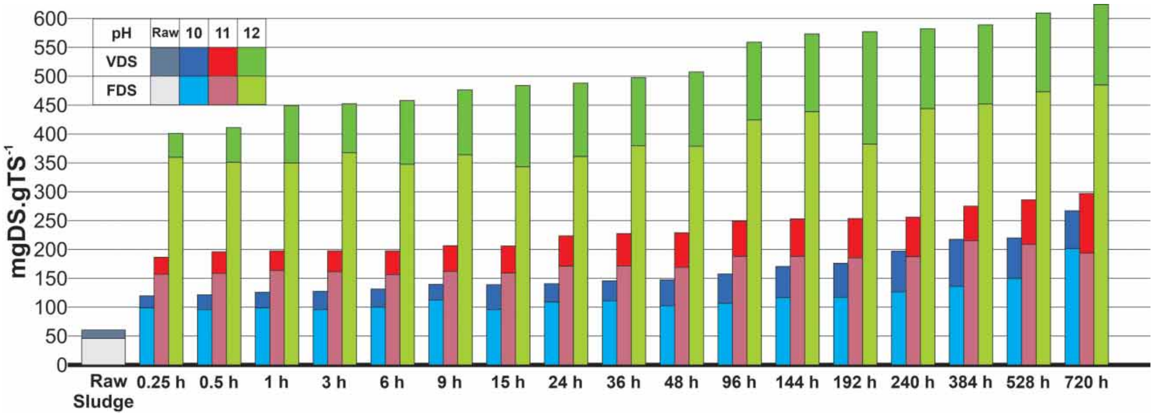


Figure 2 | Concentration of dissolved solids before and after alkali addition, and their behaviour through time.

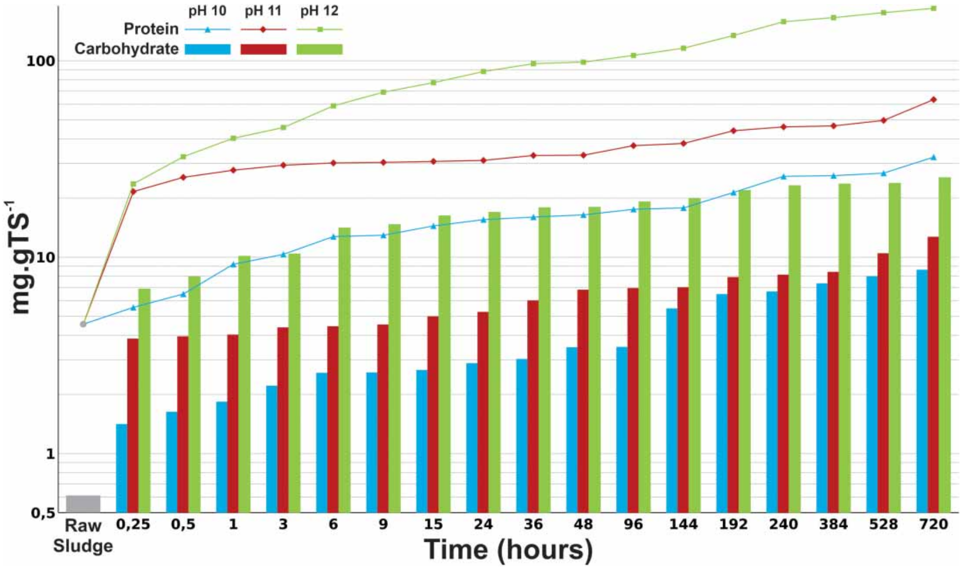


Figure 3 | Protein and carbohydrate concentration before and after alkali addition, and their behaviour through time.

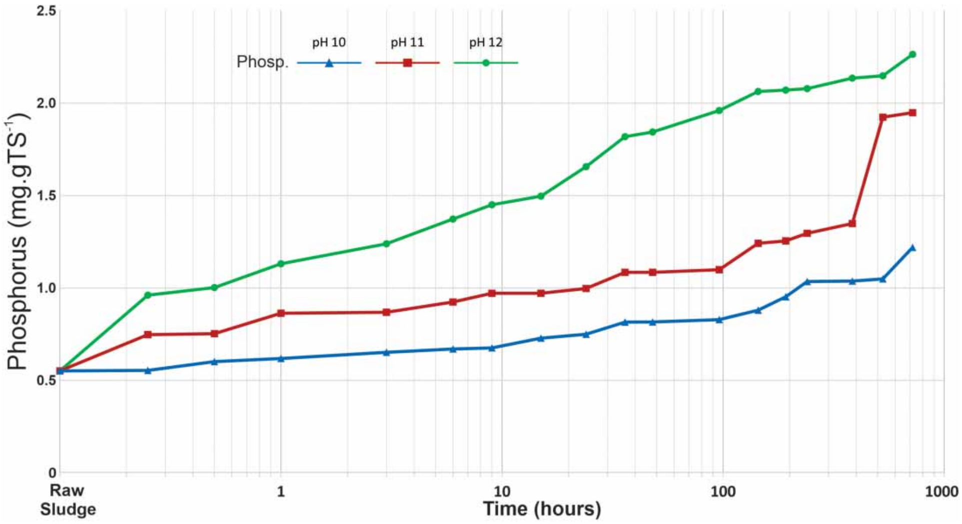


Figure 4 | Phosphorus concentration before and after alkali addition, and its profile through time.

and 24 times increase compared with raw sludge. Assuming the amount released after 720 hours as the total potential, then the protein concentration in 9 h was only 37%, reaching 52% in 36 hours despite the reaction time being four times higher. When investigating the alkaline solubilisation, Chen *et al.* (2007) reported better results for higher pH values and longer reaction times. Despite the fact that they worked with a maximum pH of 11 and 336 h, the improvement through time with higher pH values was in accordance with this present work.

It is notable that pH 12 was more efficiently disrupting the sludge floc and releasing organic material from the inner floc to the dissolved fraction. The damages to EPS, cell walls, and cell membranes are reported as the reason for all these soluble fraction increases (Valo *et al.* 2004; Bi *et al.* 2014; Xu *et al.* 2018). The phosphorus concentration (Figure 4) increased immediately (0.25 h) at pH 12; however, at pH 10 it was insignificant and at pH 11 the good results appeared only after 528 h. The release of phosphorus at high pH levels might be of interest when phosphorus recovery is pursued (Bi *et al.* 2014; Xu *et al.* 2018), and at pH 12, the total released amount after 720 h was 1.7 mg·gST^{-1}, which represents a total of 34.2 mg per litre of sludge treated.

The average particle size of sludge during alkaline pretreatment was reported to significantly decrease (Kim *et al.* 2003; Doğan & Sanin 2009; Xiao *et al.* 2015), which indicates disruption of the flocs. Regarding cell disruption, Xiao *et al.* (2015) observed in alkaline solubilisation that most cell wall damage occurs at pH 10–12.5, and cell membrane damage occurs at pH 9–12.5. In addition, the microbial cell breakup is usually accompanied by the substantial increase in soluble P, carbon, protein, and carbohydrate concentrations (Bougrier *et al.* 2005; Zhao *et al.* 2007). Therefore, the results at the end of this step corroborated with Doğan & Sanin (2009), who reported that at pH 11 cell damage is much less compared to pH 12–12.5.

In contrast with other parameters, a decrease in TKN concentrations was identified after the alkaline pre-treatment, and decreased more throughout the reaction time. The drop in TKN might be attributed to stripping of NH_3 at high pH values. Apparently, a fraction of released organic nitrogen-containing compounds were transformed into ammonia nitrogen by ammonification. Subsequently, NH_4^+ is deionised to NH_3 at the applied pH and stripped from the liquid (Ma *et al.* 2012; Bi *et al.* 2014; Leite *et al.* 2018).

Regarding alkaline reaction time, the alkaline solubilisation occurs mainly at the first hours of the treatment process, as shown in Figures (1, 2, 3 and 4). The soluble fraction increased as long as the experiment lasts for all different pH values with different rates, as shown in the figures. Thus far, there is no uniformity of criteria among existing research regarding the best alkaline reaction time. Li *et al.* (2008) reported that the alkaline solubilisation process develops in two phases, an initial and fast phase (0.5 h) followed by a slower phase. Sheng & Yu (2006) hypothesized that the sludge floc has a multiple-layer structure with two evident and distinct regions, one stable and another dispersed. Most likely, the EPS binding structure governs the stability of microbial aggregates, and in the case of the alkaline treatment process, the early stage quickly solubilises the weakly bound (dispersible) fraction, while during the second stage, the tightly bound (stable) part is damaged over time. It seems that the microbial resistance fades over time as a consequence of depolymerisation, and loses the capacity to survive in adverse conditions. In the profile presented in Figure 3, the maximum protein release rate occurred in the first 9 hours. However, in this time only 37% of the maximum release was reached. Therefore, a profile of 48 hours was chosen, reaching 53% of total release. Based on this, the amounts released by alkaline pre-treatment at pH 12 after 48 h of reaction time were 12.4 mgDOC·gTS^{-1}, 508 mgDS·gTS^{-1}, 129 mgVDS·gST^{-1}, 379 mgFDS·gST^{-1}, 98.4 mg·gTS^{-1} of protein, 18.0 mg·gTS^{-1} of carbohydrates and 1.8 mgP·gTS^{-1}, which represents a fraction in percentage of the total released after 720 hours of 76.6, 81.3, 92.1, 78.1, 53.2, 70.7 and 81.4%, respectively. The conclusion of the two first experimental steps is that alkaline pre-treatment is better than acid pre-treatment, and high pH values (pH 12) are more effective in solubilising the sludge. However, the most optimal reaction time is difficult to determine since solubilisation increases with increasing reaction time. Therefore, other factors such as application scenario and economic factors likely must be considered for determining the most optimal reaction time.

Step III – Influence of TS concentrations on chemical solubilisation of sludge

Based on previous steps and the observed protein release, the third step was performed in alkaline conditions (pH 12), applying 2 reaction times: 24 and 48 hours. In Step III, the impact of alkali/TS concentration was investigated, which may influence the disintegration performance of the sludge (Sahinkaya 2015). In Figure 5, six distinct TS concentrations of pre-treated sludge are shown (5, 10, 15, 20, 25, 30 g·L^{-1}) at 24 and 48 hours of reaction time, including the raw sludge with no alkaline treatment. The parameters measured were DOC in Figure 5(a), TKN in Figure 5(b), phosphorus (P) in Figure 5(c) and dissolved solids with their respective fractions in Figure 5(d).

It is important to highlight that regarding each of the analysed parameters, the concentration of the control sample (raw sludge) increased proportionally to the increase in the concentration of alkaline exposed solids. Distinct concentrations of solids had no influence on the increase in monitored parameters, not even when doubling the reaction time. Their increase ratios remained the

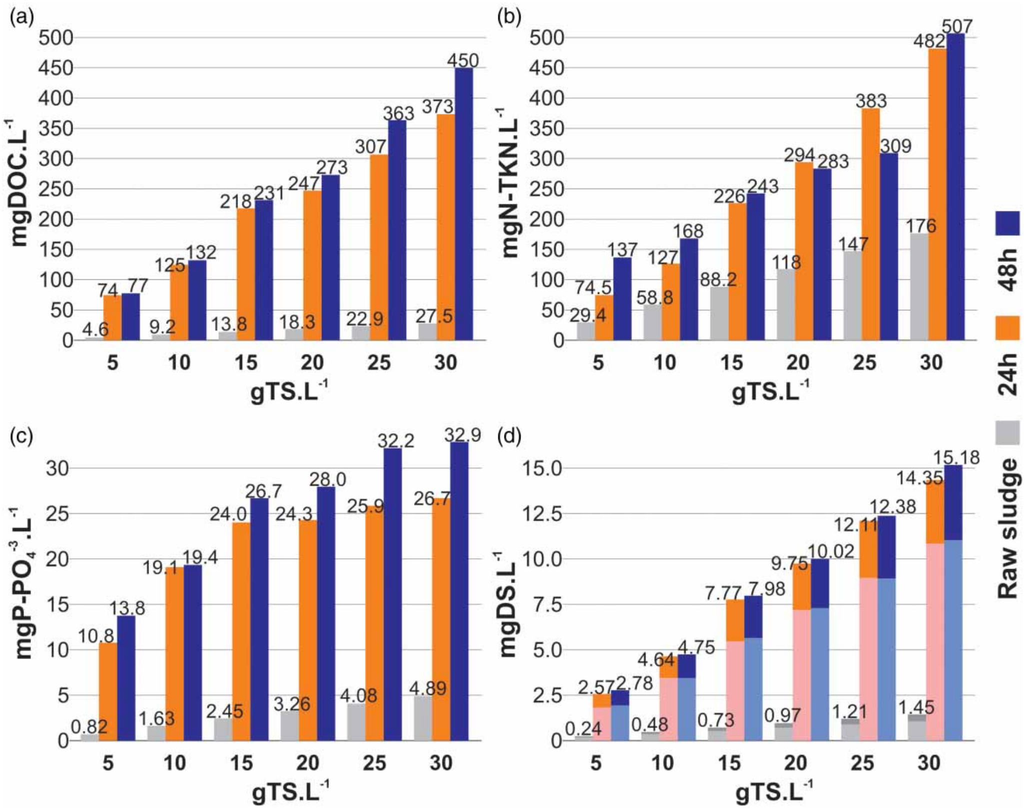


Figure 5 | For two reaction times the influence of concentration of solids to (a) DOC; (b) TKN, (c) phosphorus (P); (d) dissolved solids and their volatile and fixed fractions.

same: 0.9 $\mathrm{mgCOD \cdot gTS^{-1}}$, 5.9 $\mathrm{mgTKN \cdot gTS^{-1}}$, 0.2 $\mathrm{mgP \cdot gTS^{-1}}$, 48.3 $\mathrm{mgDS \cdot gTS^{-1}}$, 12.7 $\mathrm{mgVDS \cdot gTS^{-1}}$ and 35.7 $\mathrm{mgFDS \cdot gTS^{-1}}$.

The concentrations of the various compounds in the treated samples did not increase proportionally, but the difference for organic carbon (Figure 5(a)) and dissolved solids (Figure 5(b)) between the different concentrations of solids were low. As seen in Figure 5(a) ($\mathrm{mg \cdot L^{-1}}$), the DOC concentrations converted to $\mathrm{mg \cdot g^{-1}}$ after pre-treatment for TS of 5, 10, 15, 20, 25 and 30 $\mathrm{g \cdot L^{-1}}$ were 14.9, 12.5, 14.5, 12.4, 12.3 and 12.4 $\mathrm{mg \cdot gTS^{-1}}$ at 24 hours of reaction time, and 15.5, 13.2, 15.4, 13.7, 14.5 and 15.0 $\mathrm{mg \cdot gTS^{-1}}$ at 48 hours, respectively. Results showed that the highest solubilisations were observed at 5 and 15 $\mathrm{g \cdot L^{-1}}$ for both reaction times. However, statistical analysis showed no significant differences among the released DOC measured per g TS (Table 4). In addition, among all concentrations of solids investigated, the average for 24 hours was 13.2 of $\mathrm{mgDOC \cdot gTS^{-1}}$ and 14.6 $\mathrm{mgDOC \cdot gTS^{-1}}$ for 48 h, while the standard deviations were 1.2 and 0.9 respectively. Such standard deviations imply an acceptable coefficient of variation – less than 10%. Similar results occurred with DS and its fraction (Figure 5(d)). These results indicate that sludge disintegration had the same efficiency for alkaline pre-treatment at pH 12 for the concentration range studied (5–30 $\mathrm{gTS \cdot L^{-1}}$).

As the total concentration of solids increased, so did the concentrations of TKN (Figure 5(b)) and phosphorus (Figure 5(c)) for both parameters and both reaction times. However, the ratio between P and concentration of solids decreased significantly with increasing solids, for both reaction times. In addition, statistical analysis indicated a significant difference for phosphorus and TKN in Table 4. Bi *et al.* (2014) identified that the release efficiency of $\mathrm{PO_4^{-3}}$ and $\mathrm{NH_4^+}$ decreases with the increase in the concentration of solids. In addition, the authors reported that the concentration of solids in the range of 20–30 $\mathrm{g \cdot L^{-1}}$ provides the biggest release of $\mathrm{PO_4^{-3}}$ and $\mathrm{NH_4^+}$. Different to this study, the lowest concentration of solids was responsible for the greater release of orthophosphate and TKN. As cell damage occurred (Li *et al.* 2008), nitrogenous organic matter, such as protein, was available for TKN release. The stripping process, as discussed in Step II, is an explanation for the observed results. However, for phosphorus, Bi *et al.* (2014) provided a reasonable explanation: the water content of the sludge is low with a high concentration of solids, which resulted in a decrease in intracellular material leaching to the aqueous phase.

In Figure 6, the release of carbohydrates and proteins at the different applied solids concentrations is shown. The profile for both were similar to the behaviour of phosphorus, which might be attributed to the same phenomenon. The results of step III indicate that a higher concentration of solids (30 $\mathrm{g \cdot L^{-1}}$) allows for a higher volume of sludge that can be treated per reactor volume, being six times

Table 4 | Amount of compounds released according solids concentration after alkaline solubilisation by pH 12

		Total solids (TS) concentration					
Parameters	**Reaction time**	**5 g**	**10 g**	**15 g**	**20 g**	**25 g**	**30 g**
Dissolved organic carbon ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	14	12	14	11	11	12
	48 h	15	12	15	13	14	14
Total dissolved solids ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	466	416	470	439	436	430
	48 h	508	427	484	453	447	458
Volatile dissolved solids ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	135	106	141	115	113	104
	48 h	155	117	142	123	125	125
Fixed dissolved solids ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	330	309	329	324	323	326
	48 h	352	309	342	329	322	332
TKN ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	9	7	9	9	9	10
	48 h	21	11	10	8	6	11
Phosphorus ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	3	2	1	1	1	1
	48 h	2	2	2	1	1	1
Protein ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	94	91	116	109	97	93
	48 h	91	103	154	128	121	131
Carbohydrate ($\mathrm{mg \cdot gTS^{-1}}$)	24 h	27	20	24	21	22	20
	48 h	27	21	27	22	25	26

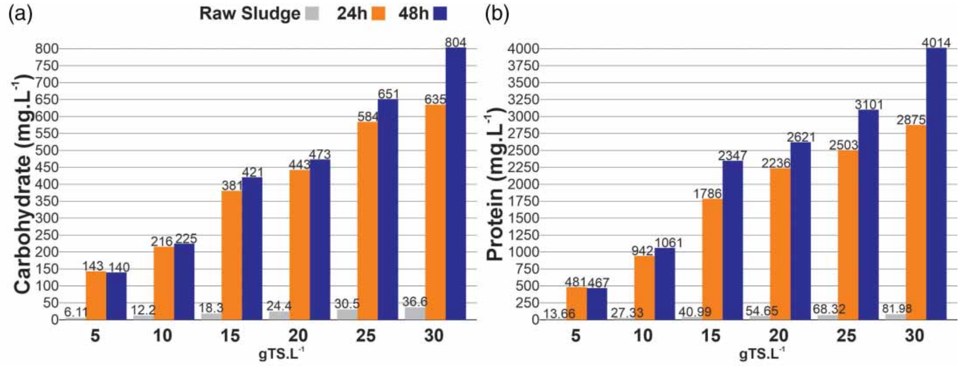


Figure 6 | The influence of concentration of solids on two different reaction times for (a) carbohydrates and (b) protein.

higher than the lowest concentration of 5 g·L^{-1}. However, the amounts of proteins and carbohydrates released from the TS, shown in Table 4, was significantly different according to the statistical analysis. In general, it is important to note that the alkali treatment performs well for TS solubilisation under the investigated range (5–30 g·L^{-1}), making pre-adjustments unnecessary for the concentration of TS, avoiding further economic investment. However, the final application of WAS will likely determine the most favourable concentration of solids.

Step IV – Assessment of increase in biodegradability

Theoretically, the bacterial metabolism in aerobic processes is divided into two fractions: one part of the organic material undergoes oxidative catabolism, while the other part forms new bacterial mass during anabolism (Marais & Ekama 1976). The fraction of organic matter used in bacterial metabolism can be quantified by the consumption of dissolved oxygen. Per definition, 1 gram of dissolved oxygen consumed is equivalent to 1 gram of oxidized COD (Tchobanoglous *et al.* 2003). In Table 5, the respirometry test results are shown.

It is interesting to note that there was a significant increase in the COD$_f$ fraction after pre-treatment, 5.6 times higher than raw sludge, which means an increase of 4,021 mgCOD$_f$·L^{-1}, or 201 mgCOD$_f$·gTS^{-1}. The biodegradable fraction of the pre-treated sludge (59%) was higher than the biodegradable fraction of raw sludge (34%). These results demonstrate that biodegradable organic matter is specifically solubilised during alkaline treatment. In addition, the concentrations of COD$_{rb}$ and COD$_{sb}$ were 11.5 mg·gTS^{-1} and 3.8 mg·gTS^{-1} for raw sludge, and 108.4 mg·gTS^{-1} and 37.4 mg·gTS^{-1} for pre-treated sludge, respectively. Despite the similar percentages of COD$_{rb}$ (F$_{rb}$) and COD$_{sb}$ (F$_{sb}$) for raw and pre-treated sludge, the consumption rate increased by 1.4 times. Results confirmed that 1,938 mg·L^{-1} of readily biodegradable organic matter was solubilised by the alkaline pre-treatment, which was rapidly assimilated by bacterial metabolism. Therefore, after pre-treatment, the sludge becomes more suitable for biological treatment.

In our study, the increased solubilisation of organic matter by alkaline pre-treatment coincided with increased respiration rates and extent of bioconversion, which agrees with observations in literature (Bougrier *et al.* 2008). However, contradicting studies report that methane production following COD solubilisation by pre-treatment had little or no improvement (Dhar *et al.* 2012; Nazari *et al.* 2017; Sapkaite *et al.* 2017). Therefore, biomethane accumulation test were performed to evaluate the effect of

Table 5 | Metabolised organic matter of the raw and pre-treated sludge during the respirometry test

	COD metabolised during the test			**Respirometric test**				
Substrate	**COD$_f$ (mg·L^{-1})**	**COD$_u$ (mg·L^{-1})**	**Bf (%)**	**COD$_{rb}$ (mg·L^{-1})**	**COD$_{sb}$ (mg·L^{-1})**	**F$_{rb}$ (%)**	**F$_{sb}$ (%)**	**Consumption rate mgO$_2$·L^{-1}·h^{-1}**
Raw sludge	882	304	34	229	75	75	25	19
Pre-treated sludge	4,903	2,914	59	2,167	747	74	26	27

Bf, Biodegradable fraction of COD during the test; COD$_f$, COD of filtered (mg); COD$_u$, COD used (mg); COD$_{rb}$, COD readily biodegradable (mg); COD$_{sb}$, COD slowly biodegradable (mg); F$_{rb}$, Fraction of COD readily biodegradable; F$_{sb}$, Fraction of COD slowly biodegradable.

pre-treatments on COD solubilisation and its respective biomethane production. Figure 7 shows the results of the tests performed with both raw and pre-treated sludge. Experiments were performed measuring the cumulative biogas production. The methane fraction was measured by gas chromatography at the end of the experiment and was 42.5% for the raw sludge sample and 48.2% for pre-treated sludge. Experimental data were modelled using the modified Gompertz model, and results are presented in Figure 7 and Table 6.

The duration of a lag-phase depends on the adaptation period of microbial consortia to a given substrate, and the prevailing specific environmental conditions such as pH, and solids content (Lankford *et al.* 1966; Jiunn-Jyi *et al.* 1997). The lag-phase for raw sludge was lower than for pre-treated sludge. The somewhat longer lag-phase might be attributed to the possible formation of inhibitory substances during pre-treatment. However, the accumulated biogas production profiles showed a remarkable increase in production rate after the pre-treatment process (Figure 7). After 13 days, the production rate of the solubilised sludge became insignificant. The total amount of accumulated methane was 122 $\mathrm{NmLCH_4{\cdot}gVSS^{-1}}$. However, for the raw sludge, the observed production rates were significantly lower, and after 37 days the accumulated methane productions were only 36 $\mathrm{NmLCH_4{\cdot}gVSS^{-1}}$, which means 29% of the achieved production for pre-treated sludge.

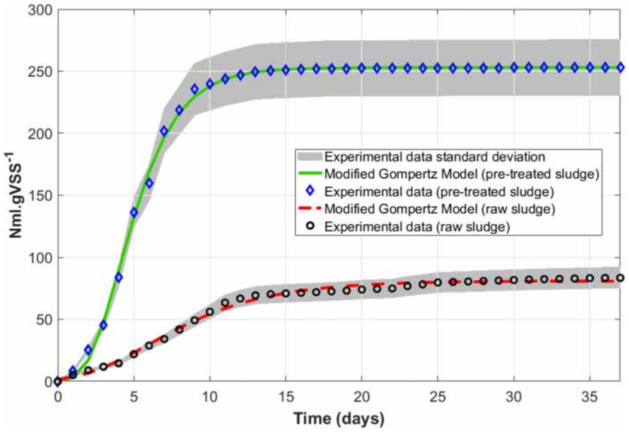


Figure 7 | Biogas production curves for raw and pre-treated sludge over time.

Table 6 | Parameters Gompertz' model (Equation (1)), methane yield, and methane production rate

	Modified Gompertz model				**Yield**	
Substrate	**P** **NmL·gVSS^{-1}**	**R$_m$** **mL·d^{-1}**	**λ** **d**	**R^2** **–**	**biogas** **NmL·gVSS^{-1}**	**CH$_4$**
Pre-treated sludge	253.0	44.3	1.99	0.9987	253.1	122.0
Raw sludge	80.9	6.8	1.62	0.9922	83.4	35.5

Considering that 1 gVSS ≅ 1.42 g COD, based on the composition of heterotrophic biomass, i.e. $C_5H_7O_2N$, a total amount of 497 mL (STP) CH_4 was expected at 100% of VSS conversion. Therefore, the observed 122 NmL CH_4 agrees with a VSS conversion of only 25%. Apparently, a large fraction of the VSS was non-biodegradable, which probably can be attributed to the fact that the sludge was derived from extended aeration.

Results of our current research clearly revealed that alkaline pre-treatment is an efficient solubilisation technology of WAS and is much more efficient than acidic solubilisation. The advantage of applying alkaline pre-treatment is more than solely biomethane production increase, since it also reduces the WAS disposal costs. Additionally, the recovery of nutrients and water might be considered as a final by-product. The specific conditions and demands of the region will determine the economic feasibility of the proposed technology, such as in the semi-arid climate zone prevailing in this study. To indicate if alkaline solubilisation is viable or not, a specific study must be performed to evaluated its applicability.

CONCLUSIONS

This paper quantified the effects of acid and alkali addition as pre-treatment for WAS. Results showed that alkali is much more effective than acid in solubilising WAS. In alkaline conditions, with pH 10, 11 and 12, the higher pH implied a higher sludge solubilisation rate. Also, at pH 12 the extent of sludge solubilisation was highest, while sludge floc inner layers were destroyed.

Alkaline solubilisation was equally efficient for both the lowest and the highest concentrations of solids. The latter is of high importance for practical applications, since efficient pre-treatment is possible at high TS concentrations.

Regarding the treatment time, which is expressed as reaction time, solubilisation increased over time; however, by far the highest impact is observed in the first few hours. Nonetheless, cost-benefit analysis will determine the most optimal reaction time.

Respirations tests revealed a distinct increase in oxygen uptake rate for applying the solubilised sludge compared to the raw sludge. Under anaerobic conditions, results of biomethane accumulation tests showed that both the rate and extent of methane production were distinctly improved.

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DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information.

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