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ASSESSMENT OF BIOCONVERSION PERFORMANCE AFTER ALKALINE FERMENTATION PROCESS TO RECOVER BIOGAS AND NUTRIENTS

Timóteo Silva Ferreira[1], Tales Abreu Tavares de Sousa[2*], Hidelbrando J. F. Diógenes[2], Israel Nunes Henrique[3], Valderi Duarte Leite[1], Wilton Silva Lopes[1], and José Tavares de Sousa[1]

[1] Sanitary and Environmental Engineering Department, State University of Paraíba (UEPB), Campina Grande - PB, Brazil

[2] Post-Graduate Program of Civil and Environmental Engineering, Federal University of Paraíba (UFPB), João Pessoa - PB, Brazil

[3] Institute of Water Sciences and Technology, Federal University of West Pará (UFOPA), Santarém - PA, Brazil

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Abstract: Alkaline fermentation of two distinct types of waste activated sludge was assessed in order to evaluate the recovery of by-products, such as nutrients, organic matter as a source of bioenergy, volatile fatty acids, and water. Also, to reduce the amount of solids to be disposed of, and the total management costs. Sludge 1 was from conventional activated sludge, and Sludge 2 was from a sequential batch reactor set for biological phosphorus removal. In the laboratory three different fermentation processes were provided in parallel, firstly treating Sludge 1, followed by Sludge 2. Treatment A was set as the control, wherein the fermentation process occurred without alkali addition, Treatment B was alkaline fermentation with a controlled pH 10 of pre-solubilized sludge; and Treatment C was alkaline fermentation at a controlled pH 10. The results indicated that alkaline fermentation significantly reduced the volatile suspended solids to 45-50 %, which was improved for both sludges when pre-solubilized, to 59-60 %. Also, comparing the biogas production test of Treatment A to the other conditions set, both increased almost 4, and 3 times, for B and C, respectively. Orthophosphate, chemical oxygen demand, carbohydrates, and proteins in soluble fractions significantly increased under alkaline fermentation in comparison with the control, and all of these parameters were boosted with pre-solubilization.

Keywords: Alkaline Solubilization, Resource Recovery, Circular Economy, Sludge Fermentation

* Correspondence to: Tales Abreu Tavares de Sousa. E-mail: mrtales@hotmail.com

INTRODUCTION

At wastewater treatment plants (WWTPs) operated by activated sludge processes, a significant amount of carbon in sludge form is generated as a final by-product. The management of waste activated sludge (WAS) to properly treat and be disposed of has been reported as 50-60 % of the total operational costs of the activated sludge-based WWTPs (Kroiss, 2004). However, in view of the circular economy, these high management costs must be minimized. WAS is a renewable source which can provide the recovery of nutrients, water, carbon, and bioenergy (Gherghel *et al*., 2019). Taking this into account, environmental engineering projects have been designed to provide the maximum recovery of resources, i.e. bioenergy, phosphorus, biopolymers, volatile fatty acids (VFA), and water (Chimuca *et al*., 2020; Leng *et al*., 2020; Liu *et al*., 2020; Schambeck *et al*., 2020; Sousa *et al*., 2020; Tobin *et al*., 2020; Zhang *et al*., 2020). Anaerobic digestion is increasingly applied in the treatment of WAS, mainly in WWTPs, and even in landfills, due to the efficiency of the bioconversion process, reducing the final amount of solids, and stabilizing the biomass. In a controlled anaerobic reactor it is a potential methane recovery method to generate bioenergy as a renewable energy source, or, to recovery the VFA, which can potentially reduce the traditional VFA production is based on non-renewable petrochemical sources, which gonna to mitigate greenhouse gas (GHG) emissions (Appels *et al*., 2008; Ilmas *et al*., 2018; Maciel and Jucá, 2012; Ramos-Suarez *et al*., 2021).

However, anaerobic digestion and its bioconversion of WAS is limited due to its recalcitrance, demanding a high solids retention time to hydrolyze the sludge (Neumann *et al*., 2016; Sheng and Yu, 2006; Stuckey and McCarty, 1984). Due to this, the fermentation process with alkaline pH control is commonly investigated to treat urban or agricultural organic waste, like sludge, aiming for carbon recovery in the form of VFA, carbohydrates, proteins, bioenergy, and nutrients (Bi *et al*., 2014; Cheah *et al*., 2019; Li *et al*., 2014; Lin *et al*., 2017; Liu *et al*., 2016). In addition, alkaline fermentation of WAS has recently been reported as being able to improve the hydrolysis rate, mainly at pH 10, increasing the VFA production (Liu *et al*., 2018; Wang *et al*., 2019) driven by the increased activity of hydrolytic enzymes and acidogenic bacteria, inhibiting methanogenic activity (Xiao *et al*., 2015). Furthermore, alkaline fermentation provides sludge solubilization, disintegrating the floc and releasing soluble organic matter which results in higher bioconversion and greater biogas production (Fang *et al*., 2014). It is a monophasic process, where the fermentation simultaneously improves the sludge hydrolysis, improving its biodegradability and maximizing the production of VFA (Chen *et al*., 2017). These indicators provide a basis for highlighting fermentation as a very effective strategy to maximize the recovery of resources like bioenergy, obtaining by-products from WAS, and mitigating GHG emissions (Veluswamy *et al*., 2021; Wang *et al*., 2016).

The pre-treatment of the WAS is constantly associated to the alkaline fermentation process, aiming for higher resource recovery due to the previous solubilization process of the sludge, which damages the organized floc structure—releasing inner floc material such as organic matter and nutrients—by different treatment technologies, e.g. thermal-alkaline, alkaline-enzymatic, enzymatic, and ultrasonic (Carrère *et al*., 2010; Gonzalez *et al*., 2018; Liu *et al*., 2018, 2016, 2019; Pang *et al*., 2020; Yan *et al*., 2010). Alkaline solubilization as a pre-treatment can significantly influence the rheological properties, metal binding, organic adsorption capacities, and flocculation properties (Omoike and Chorover, 2004; Sheng *et al*., 2005; Zhao *et al*., 2018). Also, alkaline pre-treatment is simple and easy to operate, and has been reported as being able to provide a significant increase in VFA production (Gomec and Speece, 2003; Zhang *et al*., 2015; Zhao *et al*., 2018).

Therefore, the objective of this study is to treat was by alkaline fermentation in a laboratory scale reactor set to provide the recovery of resources such as nutrients and biogas, as well as reducing the final suspended solids concentration. The study will also assess and compare alkaline fermentation of two types of was as substrates in order to identify which one releases more resources. In addition, the sludge pre-solubilization is assessed in regards to optimizing the methanogenic bioconversion, with the objective of increasing methane production as a maximized renewable source of bioenergy.

MATERIALS AND METHODS

Waste activated sludge (WAS) as a substrate

Two different aerobic sludges were used as substrates to be compared: one was from a conventional activated sludge system with a sludge age of twelve days; the other sludge was from a sequential batch reactor (SBR) configured for biological phosphorus removal with a sludge age of five days. Both reactors were operated at EXTRABES (*Estação experimental de tratamentos biológicos de esgoto sanitário*), fed by municipal sewage from Campina Grande – PB (Brazil). Each sludge was collected separately over seven days at room temperature, then to concentrate the solids the supernatant of both was discarded and the remaining sludges were stored at 4°C.

Anaerobic sludge used as inoculum

Anaerobic sludge from the UASB (upflow anaerobic sludge blanket) reactor, also operated at EXTRABES and fed by municipal sewage, was used as fermentation inoculum with a volatile suspended solids (VSS) concentration of 30.65 ± 3.2 $g.L^{-1}$. Using sodium acetate ($C_2H_3NaO_2$) and sucrose ($C_{12}H_{22}O_{11}$), both pre-diluted, the inoculum was conditioned to pH 4.5 ± 0.2, to have a majority of acidogenic bacteria. This was a methodology adapted from Wang *et al.* (Wang *et al.*, 2013).

Experimental procedures

The sludges used as substrate were classified as Sludge 1, the WAS from the conventional activated sludge reactor; and Sludge 2, the WAS from the SBR. As shown in **Fig. 1**, three different treatment processes were set to treat each sludge separately in two experimental batches, and to compare the results: A, B, and C. Treatment A was set as the control, wherein the pH was not adjusted during the experimental procedures; with treatments B and C, the pHs were adjusted to 10, and every 24 hours they were readjusted to maintain the pH at 10. However, the difference in both was just an alkaline pre-treatment of the sludge that was performed only in Treatment B. The alkaline pre-treatment was carried out at pH 12 for 8 hours, stirred at 200 rpm on a stirring table (New Brunswick Scientific, mod. G 33) at room temperature (23-27 °C), and sodium hydroxide (NaOH) was added to raise the pH (de Sousa *et al.*, 2021). For the fermentation process NaOH was added to raise the pH, and when necessary hydrochloric acid (HCl) was added to acidify it (X. Ma et al., 2019).

The fermentation assay outlined in **Fig. 1** for both substrates was carried out simultaneously in 18 reactors, which were Erlenmeyer flasks (1000 ml) with used volume of 600 ml, operated in an open system. Five-hundred ml of substrate and 100 ml of inoculum were added to each reactor. The reactors were kept under constant stirring at 120 rpm on a shaker table (New Brunswick Scientific, mod. G 33) for ten days at room temperature. Each of the three reactors corresponded to the triplicate of the three treatment sets (A, B, and C) carried out for both substrates. Additionally, three reaction times were assessed, (72, 144, and 240 hours), and the instant 0 h was the start point assessed as the control. The samples collected at 72 and 144 h of reaction time for the physical-chemical characterization were 30 ml, designed to not exceed 10% of the used volume. The solubilization efficiency was calculated in Equation 1 for soluble COD (CODs), wherein the difference of influent and effluent COD_S was divided by total COD.

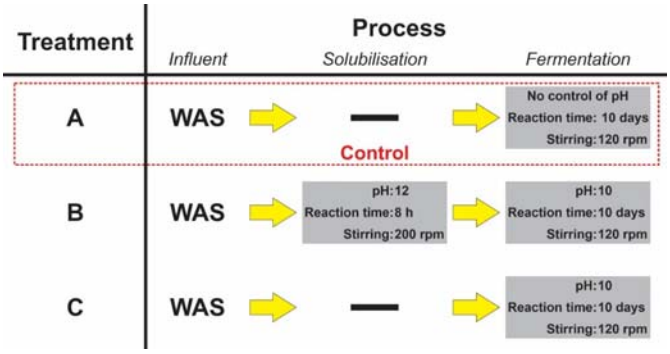

Fig. 1 Experimental Scheme of the alkaline fermentation treatments set.

$$Efficiency_{COD_S}: \frac{COD_{S_{effluent}} - COD_{S_{influent}}}{COD_{total}} x\ 100\% \qquad (1)$$

Biogas production tests

The fermented substrates after the ten days of operation were submitted separately to the biogas production tests. The test were carried out in closed reactors designed to prevent gas leakage, with digital manometer (MPX5700AP) coupled to the reactor, which constantly recorded the internal reactor pressure; this was carried out under standard temperature and pressure (STP) conditions (Ramos *et al.*, 2020). The tests were performed in an incubator set to 35 °C under constant homogenization, the substrate/inoculum rate was 1:2 to guarantee the ratio of food and microorganisms at 0.5. The biogas production tests were performed following an adapted methodology of Holliger *et al.*, (Holliger *et al.*, 2016). The experimental biogas production tests were calculated, and to improve the discussion the modified Gompertz model (Equation 2) was applied (Jiunn-Jyi *et al.*, 1997).

$$M = P \times exp\left\{-exp\left[\frac{R_m{}^e}{P} \times (\lambda - t) + 1\right]\right\} \qquad (2)$$

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

Analytical parameters

Physical-chemical characterization was performed for all samples, before and after the fermentation and pre-treatment. The measured parameters following the procedures of Standard Methods for the Examination of Water and Wastewater (Bridgewater *et al.*, 2012) were phosphorus (P); ammonia nitrogen ($N\text{-}NH_4^+$); chemical oxygen demand (COD); and total solids (TS) and their fractions, such as fixed dissolved solids (FDS) and volatile dissolved solids (VDS). To determine the soluble fractions, the sludge samples were centrifuged at 12,000 rpm for 15 min, and then the supernatant was filtered through a membrane with a mesh size of 0.45 µm. Protein analysis was performed applying the Lowry method modified by Frølund *et al*. (Frølund *et al*., 1995), and carbohydrate analysis was performed using the method described by Dubois *et al*. (Dubois *et al*., 1956).

RESULTS AND DISCUSSION

The characterization of sludges 1 and 2 at instant 0 h are presented in **Table 1**, both used as influents in the alkaline fermentation process. Organic material predominance was identified in both sludges, despite the VSS/TSS ratio being lower in Sludge 2. This lower proportion has been reported in the literature as an indication of the inorganic material in polyphosphate form due to the presence of phosphate accumulating organisms (Henrique *et al*., 2010; van Haandel and van der Lubbe, 2012). Indeed, this sludge was from a sequential batch reactor set for biological phosphorus removal.

Fermentation influence on sludge solubilization

Soluble COD (COD_S)

The soluble fraction increase of COD was assessed to indicate sludge solubilization after the fermentation process. The profile of COD_S over time by each treatment are represented by graphs for Sludge 1 (**Fig. 2A**) and Sludge 2 (**Fig. 2B**). The influent COD_S values were 250 $mg.L^{-1}$ for Sludge 1, and 414 $mg.L^{-1}$ for Sludge 2 (**Table 1**). Then, after the fermentation process treating Sludge 1, the concentration of the final effluent of treatments A, B, and C were 2,130, 13,500, and 10,500 $mgCOD_S.L^{-1}$, which means increases of 1,880, 13,250, and 10,250 $mgCOD_S.L^{-1}$, respectively. In contrast, the final concentrations after Sludge 2 treatments were 2,700, 18,500, and 14,000 $mgCOD_S.L^{-1}$, which means increases of 2,286, 18,086, and 13,586 $mgCOD_S.L^{-1}$ after treatments A, B, and C, respectively. Knowing the total COD concentrations (**Table 1**), the increase of COD_S could be calculated as efficiencies (Eq.: 1), which were 5.9%, 41.4%, and 32.0% in relation to treating Sludge 1, and 5.4%, 43.1%, and 32.3% for Sludge 2, respectively for treatments A, B and C.

Table 1. Characterization of sludge 1 and 2 used as influent in the fermentation process.

Parameters	Sludge 1	Sludge 2
pH	7.5 ± 0.1	7.5 ± 0.2
Total suspended solids ($gTSS.L^{-1}$)	35 ± 0.9	48 ± 1.3
Volatile suspended solids ($gVSS.L^{-1}$)	24 ± 0.2	30 ± 0.2
Ratio of VSS/TSS (-)	0.70	0.62
Chemical oxygen demand ($gCOD.L^{-1}$)	32 ± 3	42 ± 3
Soluble chemical oxygen demand ($gCOD_S.L^{-1}$)	0.25 ± 0.03	0.41 ± 0.04
Soluble carbohydrates ($mg.L^{-1}$)	26 ± 3.0	32 ± 3.5
Soluble proteins ($mg.L^{-1}$)	80 ± 10.2	102 ± 14.2
Ammonia nitrogen ($mgN\text{-}NH_4^+.L^{-1}$)	192 ± 3.4	285 ± 5.3
Orthophosphate ($mg\ P\text{-}PO_4^{3-}.L^{-1}$)	23 ± 1.4	44 ± 3.0
Total phosphorus ($mg.L^{-1}$)	314 ± 5.6	450 ± 6.2

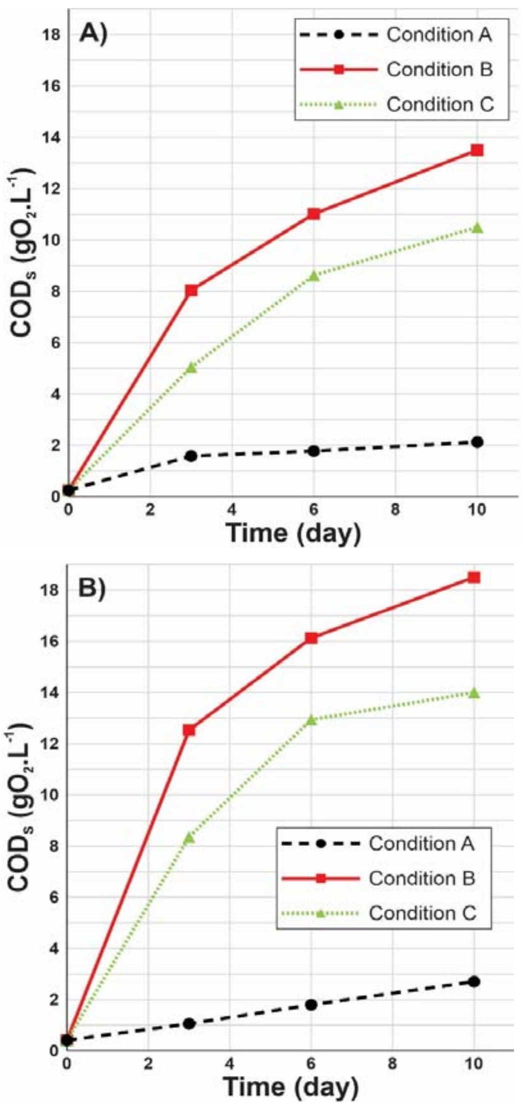

Fig. 2 Soluble COD profile over the reaction time at treatment process set for sludge 1 (A), and sludge 2 (B).

Table 2. Carbohydrates, proteins, and ammonia nitrogen concentrations in soluble fraction at influent

Treatment	Parameters	Carbohydrates (mg.L^{-1})	Proteins (mg.L^{-1})	Ammonia nitrogen (mgN-NH_4^+.L^{-1})
Sludge 1	Influent	26 ± 3.0	80 ± 10.2	192 ± 22
	Effluent A	187 ± 19	618 ± 25	556 ± 28
	Effluent B	960 ± 30	2246 ± 32	115 ± 12
	Effluent C	416 ± 21	1336 ± 17	131 ± 16
Sludge 2	Influent	32 ± 3.5	102 ± 14.2	285 ± 31
	Effluent A	150 ± 11	482 ± 38	614 ± 29
	Effluent B	762 ± 13	1754 ± 20	94 ± 14
	Effluent C	328 ± 12	1057 ± 16	210 ± 21

Alkaline fermentation provides sludge floc disintegration, which indicates occurrence of the hydrolysis process and particulate matter reduction. This leads to a high COD_S concentration becoming available, which was noticed after Treatment C for both sludges. The fermentation process with no pH control at alkaline condition (A) increased the COD_S, reaching a factor of 5.5-7.5 times the COD_S influent, which is significantly lower than the factor reached by Treatment C (32-41 times). Interestingly, alkaline conditions inhibit methanogenesis providing VFA generation (Ma *et al.*, 2016). This methanogenesis inhibition, and the increase in VFA production, are constantly reported in literature for WAS fermentation with an alkaline condition. For example, the literature reports that a solubilization efficiency of 57% was achieved, and VFA increased from 162 to 4,527 mgCOD.L^{-1} (Li *et al.*, 2017); a solubilization efficiency of 38% was achieved and VFA increased from 16 to 1,248 mg COD.L^{-1} (Gao *et al.*, 2011); and 53% of efficiency with 2.9 kg.m^{-3} of accumulated VFA (Wang *et al.*, 2017). Thus, the significant COD_S increase must be due to VFA production.

Furthermore, alkaline fermentation previously treated by alkaline solubilization, in Treatment B, reached the highest factor—43-53 times the COD_S in influent. A strong alkaline condition promotes sludge floc rupture, damages walls and membranes of the cell, and solubilizes extracellular polymeric substances (EPS) (de Sousa *et al.*, 2021; Guo *et al.*, 2014; Li *et al.*, 2012; Xu *et al.*, 2018). Therefore, the BOD_{20}/COD ratio can be significantly reduced by alkaline pre-treatment with pH 10 and upwards (Wonglertarak and Wichitsathian, 2014). Thus, the higher COD_S concentrations for both sludges were achieved due to the pre-treatment process associated with the alkaline fermentation. Therefore, with the objective of sludge solubilization and VFA production, the alkaline fermentation previously solubilized at pH 10 and upwards is recommended in this work, however the economic viability of each WWTP must be studied (do Ó *et al.*, 2021; Liu *et al.*, 2019; Pang *et al.*, 2020).

Carbohydrates, proteins, and ammonia nitrogen (N-NH_4^+)

The concentrations of carbohydrates, proteins, and ammonia nitrogen in soluble fraction for both sludges are presented separately by influent and effluent of each treatment process set in **Table 2**. In relation to the analysis of proteins and carbohydrates, they are the main constituents of the EPS matrix. Alkaline conditions are favorable to solubilize EPS and damage the cell, and the inner floc constituents are released in the soluble fraction of the sludge (Guo *et al.*, 2014; Li *et al.*, 2017). The increases of proteins in the soluble fractions were 538 mg.L^{-1}, 2,166 mg.L^{-1}, and 1,256 mg.L^{-1} for Sludge 1; and 380 mg.L^{-1}, 1,652 mg.L^{-1}, and 955 mg.L^{-1} for Sludge 2 for treatments A, B, and C, respectively. The increases of carbohydrates were 161 mg.L^{-1}, 934 mg.L^{-1}, and 390 mg.L^{-1} for Sludge 1, and 118 mg.L^{-1}, 730 mg.L^{-1}, and 296 mg.L^{-1} for Sludge 2, for treatments A, B, and C, respectively. The concentration increase in soluble fraction of proteins and carbohydrates is explained as a dissociation consequence not only of EPS matrix, but also of VFA (Flemming and Wingender, 2010; Sheng *et al.*, 2010).

For both sludges, the fermentation without pH control achieved the lowest concentration of proteins and carbohydrates in the effluent. Treatment A increased 118-161 mg.L^{-1} of carbohydrates, and 380-538 mg.L^{-1} of proteins which respectively means 3.7-6.2 and 3.7-6.7 times the influent value; whereas Treatment C released more than twice as many as Treatment A (carbohydrates, 296-390 mg.L^{-1}, and proteins, 955-1,256 mg.L^{-1}), meaning 9-15 and 9-16 times the influent of soluble carbohydrates and proteins, respectively. Treatment B was the most significant increase in the concentrations of carbohydrate 730-934 mg.L^{-1}, and proteins 1,652-2,166 mg.L^{-1}, which means almost 22-36 and 16-27 times the influent soluble concentration of carbohydrates and proteins, respectively. Alkaline fermentation is a synergistic process, wherein chemical solubilization occurs simultaneously with the substrate's biological degradation. This directly influences the organic material concentrations in the soluble fraction over the fermentation reaction time, like proteins and carbohydrates, which are not the only by-products that make COD_S increase. Therefore, proteins and carbohydrates do not necessarily have the same increase for the COD_S curve (Su *et al*., 2013), since a sludge solubilization rate can exceed that of biological degradation of proteins and carbohydrates by acidogenic bacteria (Chen *et al*., 2004; Wang *et al*., 2017; Wu *et al*., 2009). Thus alkaline fermentation can be considered a sludge treatment technology that provides the recovery of resources due to a significant release of proteins and carbohydrates that can be used in the bioproduction process of bioenergy, or of short chain fatty acids, as well as inhibiting methanogenesis, providing VFA accumulation (Feng *et al*., 2009; Wang *et al*., 2019). Traditional VFA production is based on non-renewable petrochemical sources, which is reported to cause serious negative environmental effects, such as GHG emissions without energy recovery (Atasoy *et al*., 2018; Ramos-Suarez *et al*., 2021). Thus, recovery of VFAs in WWTPs is a sustainable and economically viable production process that naturally reduces the demands of VFA production by the petrochemical industry, reducing GHG emission (Ramos-Suarez *et al*., 2021; Veluswamy *et al*., 2021); a reduction which may be boosted with a closed reactor.

Different from all of the parameters assessed, the ammonia nitrogen concentration was only higher after fermentation with no pH control under alkaline conditions. It is noticeable that with treatments under alkaline conditions, the ammonia nitrogen concentration was lower than the influent. The drop in N might be attributed to ammonification process due the pH under alkaline conditions: the fraction of released organic nitrogen-containing compounds was transformed into ammonia nitrogen, then NH_4^+ was deionized to NH_3 and stripped from the liquid. The fermentation reactors operated in an open system under alkaline conditions may have allowed ammonia gas to escape, which coincided with what has been reported in the literature (de Sousa *et al*., 2021; Leite *et al*., 2018; Li *et al*., 2018; Ma *et al*., 2012). Additionally, the organic matter, especially microbial cells and EPS, contains proteins in its constitution that also can be degraded during the VFA fermentation, releasing a high concentration of ammonia nitrogen in the liquid medium by hydrolytic and acidogenic bacteria (Ye *et al*., 2020), which also must have escaped due to the stripping process.

When comparing ammonia nitrogen concentration in effluent after treatments B and C, the pre-treatment provided a larger reduction in nitrogen, which must also be a consequence of the alkaline conditions (Bi *et al*., 2014; de Sousa *et al*., 2021; Leite *et al*., 2018; Ma *et al*., 2012). Although alkaline fermentation followed by pre-treatment is an efficient treatment technology of WAS that can be applied in WWTPs with nutrient recovery, this nitrogen removal reduces the potential for agricultural use of stabilized sludge (Toutian *et al*., 2020).

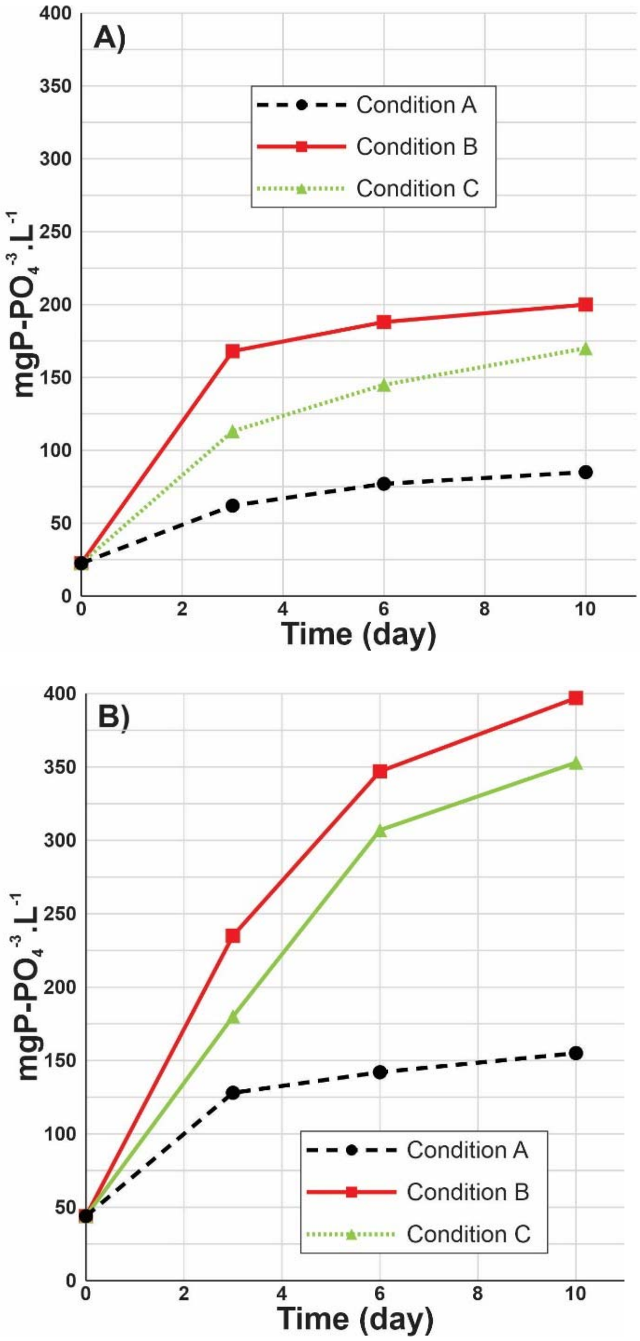

Fig. 3 Orthophosphate profile over the reaction time at fermentation treatments set for sludge 1 (A), and sludge 2 (B).

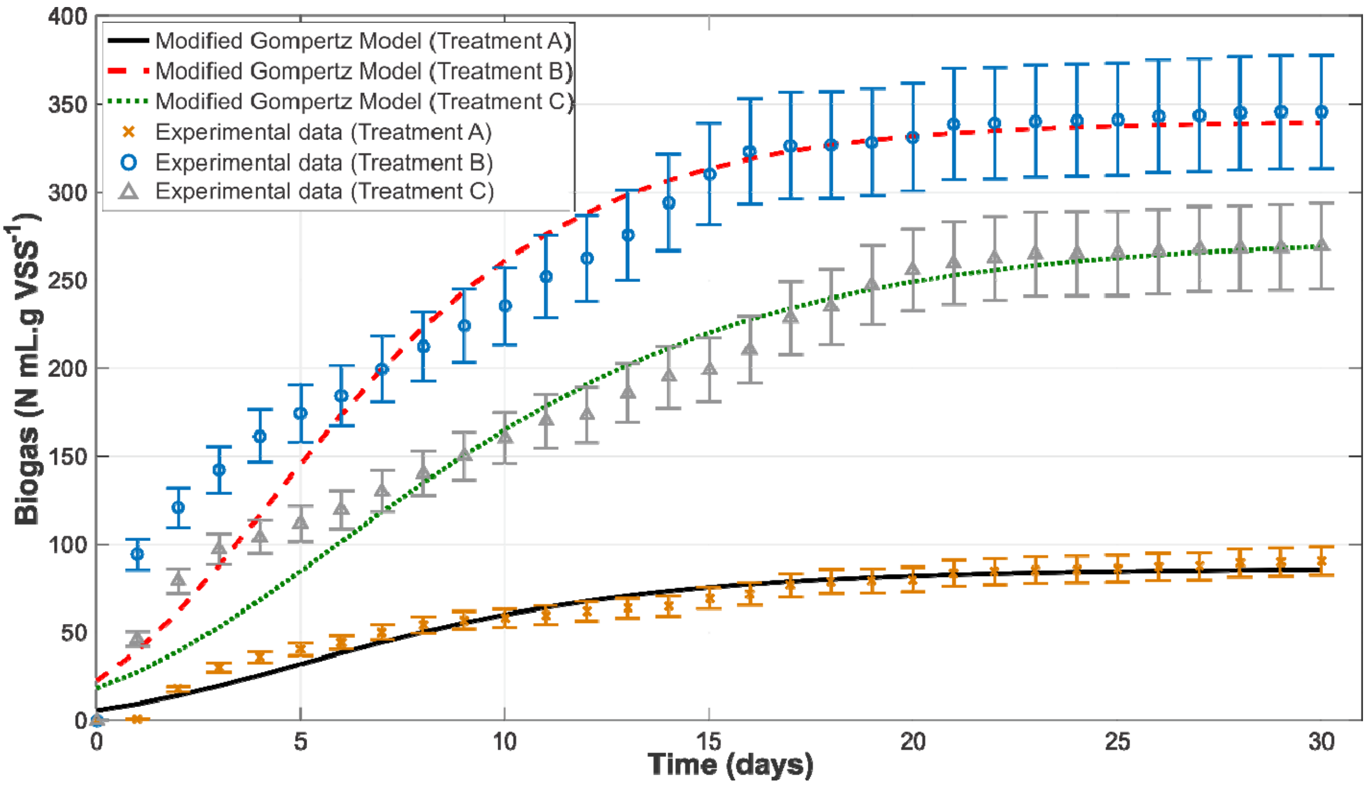

Fig. 4 Biogas production curves over time of fermented sludge of treatments A, B and C.

Phosphorus release

The orthophosphate profiles are presented in **Fig. 3**, and indicate an increase over the retention time under all treatment conditions set for both sludges. The influent orthophosphate concentrations were 23 ± 1.4 mg.L^{-1} for Sludge 1, and 44 ± 3.0 mg.L^{-1} for Sludge 2. The orthophosphate concentrations in effluent for treatments A, B, and C, were 85, 200, and 170 mg.L^{-1} for Sludge 1, and 155, 397, and 353 mg.L^{-1} for Sludge 2, respectively. The alkaline fermentation results were also better than the fermentation with no pH control, which was previously reported in literature that assessing the sludge fermentation varying the pH from 4 to 12, and reported pH 10 as the most favorable (Shi and Xu, 2019).

The pre-treatment applied in line with alkaline fermentation significantly improved the orthophosphate release. Liu *et al.* (Liu *et al.*, 2019) assessed the phosphorus release by four different WAS pre-treatments, reporting considerable solubilization efficiency for all technologies, however chemical solubilization under alkaline conditions was the highest efficiency achieved. Therefore, it is possible to identify alkaline fermentation as being able to efficiently release orthophosphate, which can be boosted by the alkaline pre-treatment. This is especially the case for treating WAS from biological phosphorus removal systems, which contains biomass poly-P. The orthophosphate increase is caused by the release of phosphorus from the EPS rupture, from dead microbial cells, or from phosphorus-accumulating organisms (Ju *et al*., 2005).

Biogas production test

The biogas production test was performed in triplicate with effluents from the three fermentation treatments set which treated Sludge 1. The test lasted for 30 days, and it was observed that the biogas production curves diverged on the first day of the assay (**Fig. 4**). Furthermore, the biogas production stabilizations were reached at different retention times for each one of the treated effluents assessed. The substrate bioconversion from Treatment A was too limited in comparison to the other two substrates, which were only 90.7 NmL.gSSV^{-1}. The average biogas production for Treatment C was 269.4 NmL.gSSV^{-1}, almost three times higher than the control, which indicated that alkaline fermentation controlled at pH 10 provided higher bioavailability in sludge to be bioconverted to biogas. Treatment B stood out with the highest biogas production, at 345.8 NmL.gSSV^{-1}, confirming the solubilization process efficiency previously applied to the alkaline fermentation controlled at pH 10. Comparing treatments B and C, the production increase was 84 % higher in Treatment B due the alkaline solubilization, which was significantly different. The biogas production efficiencies were directly proportional to the solubilization efficiencies identified in the parameters previously discussed: COD_S, carbohydrates, proteins, and orthophosphate.

Experimental data of cumulative biogas production were modelled using the modified Gompertz model, and the results are presented in **Fig. 4** and **Table 3**. The R2 coefficient values were greater than 0.93, indicating that the use of this model to describe the data is appropriate. Also, all three investigated treatments resulted in profiles with significant differences. Furthermore, the adaptation period of microbial consortia to each substrate, and to the prevailing specific environmental conditions, was higher with substrate from Treatment A which indicates better bioavailability in sludge treated under alkaline fermentation. Note that calculated P in **Table 3** corroborated with experimental data, so the reliability of the experiment was confirmed by the similarity of experimental data and the modified Gompertz model.

According to **Fig. 4**, the potential biogas production has the same profile as the maximum biogas production rate, which can be confirmed looking at **Table 3**, where the theoretical values (*P*) were approximated to the experimental ones (*biogas*). It is important to point out that a salt formation due to the addition of NaOH and HCl was expected (Tchobanoglous *et al.*, 2003), which indicates the increase in salinity inside the anaerobic reactor. However, according to literature, not necessarily inhibits anaerobic bioconversion (Miranda *et al.*, 2014; Muñoz Sierra *et al.*, 2018), previous paper from this group reported an increase in biogas production even pre-treating sludge by alkaline methodology with addition of NaOH and HCl (de Sousa *et al.*, 2021).

The lowest rate was also with Treatment A, 2.6 times higher than it was with Treatment C, and almost 4.6 times higher with Treatment B (**Table 3**). Potential biogas production and R_m were evidently enhanced after alkaline solubilization, which corroborates with the literature (Chen *et al.*, 2020; de Sousa *et al.*, 2021). Therefore, it is conclusive that fermentation of WAS under a pH controlled at 10 was effective at improving the VFA production performance, which is necessary to produce biogas in anaerobic digestion.

Table 3. Parameters Gompertz' model (Eq. 1), methane yield, and methane production rate.

Substrate	Modified Gompertz model			Yield
	P	R_m	R^2	biogas
	$NmL.gVSS^{-1}$	$mL.d^{-1}$	-	$Nml.gVSS^{-1}$
Effluent A	86	6.4	0.9583	91
Effluent B	341	29.2	0.9358	346
Effluent C	274	16.9	0.9506	269

However, it also provides an increase of refractory organic matter, such as humic substances and lignins (Ma *et al.*, 2019). This negatively influences the methanization, decreasing the potential production of methane as the humic content increases.

Mass balance

The higher efficiencies of the fermentation process with pH controlled at 10 were confirmed using both sludges. This significantly reduced the final sludge amount, decreased the VSS, increased the COD_S, and released orthophosphate. To better represent and discuss these results, the mass balance of treatments B and C are presented in a schematic diagram in **Fig. 5**. After Treatment B, the decreasement range of suspended solids was 56-60 %, and 45-50 % after Treatment C (**Table 4**). Comparing these reduction efficiencies of treatments B and C to Treatment A, fermentation with no pH adjustment was too limited (12-13 %). Therefore, analyzing suspended solids reduction clearly revealed that alkaline fermentation at pH 10 is an efficient treatment technology of WAS and it can be boostered with the pre-solubilization process. However, to evaluate its suitability of application, it is necessary to comprehend that alkaline fermentation applied with or without pre-treatment is more than solely solids reduction, since it also provides recovery of by-products, like nutrients, biomethane, VFA, and water, and it reduces the costs of final disposal of WAS. Similar to the discussion presented in the literature (de Sousa *et al.*, 2021), the specific conditions and demands of the region where the proposed technology will be implemented must determine the economic feasibility, so a specific study must be performed to evaluate its local applicability.

In relation to the orthophosphate release, the fermentation process with no pH control was too limited, with only 37 and 67 mg in sludges 1 and 2, respectively. This represents a solubilization range of total phosphorus of 20-25 % for Treatment A (**Table 4**). However, the fermentation process with pH controlled at 10 achieved a solubilization efficiency of total phosphorus of 47 % treating Sludge 1, and 75 % treating Sludge 2 (**Table 4**). This represents orthophosphate increases of 7.3 and 8.8 times the influent concentration for Treatment C (**Fig. 5**). The higher efficiency of fermentation controlled at pH 10 to solubilize total phosphorus increasing orthophosphate concentration was previously reported by Chen *et al.* (Chen *et al.*, 2021). Lastly, Treatment B achieved the highest orthophosphate release for both sludges (**Table 4**), which was due to the solubilization process applied. Comparing the effluent concentration to the influent, the

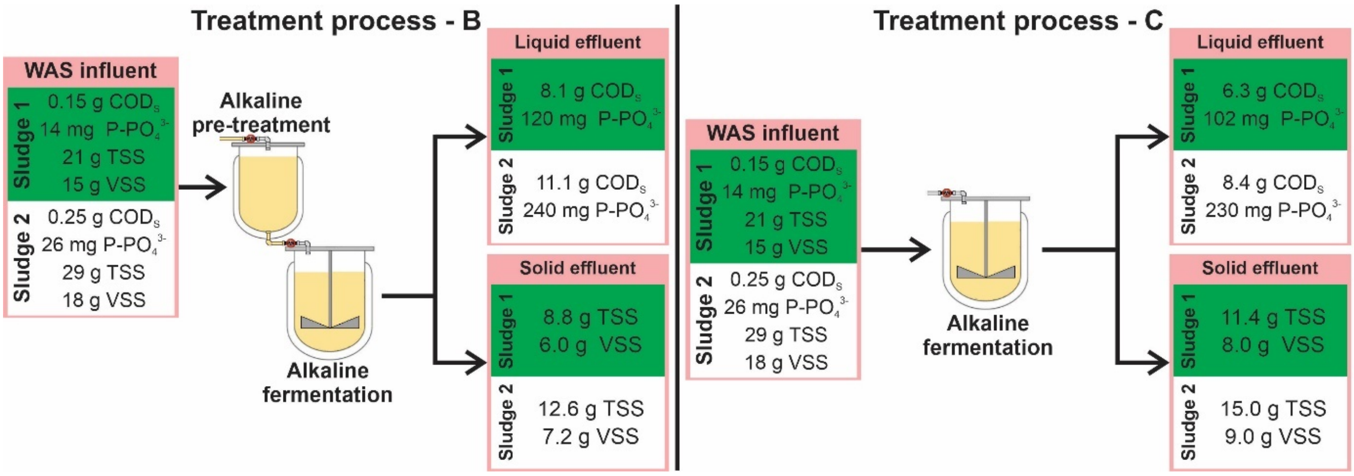

Fig. 5 Mass balance schematic diagram of treatment process B and C treating sludge 1 and 2

Table 4. Efficiency calculated of mass balance of each treatment set (A, B, and C) for both sludges according each parameter.

Substrate	Sludge 1			Sludge 2		
	A	B	C	A	B	C
Increase of COD_S (%)	5.9	41.4	32.0	5.4	43.1	32.3
Release of $P\text{-}PO_4^{3-}$ (%)	19.9	56.5	47.0	24.7	78.4	75.3
Reduction TSS (%)	13	58	45	12	56	48
Reduction VSS (%)	13	59	45	13	60	50

increases were 8.6 and 9.2 times for sludges 1 and 2, respectively.

This study concluded that alkaline fermentation efficiently treats WAS. Additionally, the significant release of nutrients and soluble organic matter indicates this technology can promote the recovery of resources such as nutrients, VFA, and biogas. At the same time, it reduces the final suspended solids concentration, which decreases the amount of final solids to be disposed of, and therefore the total management costs. The recovery of VFA and biogas is also a potential option for reducing the emission of GHG.

Alkaline pre-treatment was also confirmed as technology able to efficiently improve the release of nutrients, increase soluble organic matter, reduce volatile solids, and pre-solubilize the sludge, optimizing the generation of VFA and subsequent methanogenic bioconversion. Specifically, the biogas production after alkaline fermentation controlled at pH 10 produced almost 2.9 times more than the control condition, whereas the alkaline fermentation associated with pre-solubilization produced almost 3.8 times the control. This must be a consequence of greater bioavailability of soluble organic matter, such as proteins and carbohydrates.

When comparing both sludges, the alkaline fermentation was efficient for both, and also the pre-treatment process applied. The WAS from biological phosphorus removal systems, which contains biomass poly-P, achieved higher VSS reduction, and a significantly higher rate of orthophosphate release.

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