# Assessment of bioconversion performance after alkaline fermentation process to recover biogas and nutrients

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# ABSTRACT

Alkaline fermentation of two distinct types of waste activated sludge was assessed 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, treating Sludge 1, followed by Sludge 2. Treatment A was 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 (VSS) to 45% treating Sludge 1, and to 50% treating Sludge 2, which was significantly improved for both sludges when pre-solubilized achieving 59% and 60%, respectively. Also, comparing the biogas production test of treatment A to the other conditions set, both increased almost four, and three times, for B and C, respectively. Orthophosphate, chemical oxygen demand, carbohydrates, and proteins in soluble fractions significantly increased with pre-solubilization. Comparing both sludges, the one from biological phosphorus removal systems achieved higher VSS reduction, and a significantly higher rate of orthophosphate release.

Keywords: Alkaline solubilization; Resource recovery; Circular economy; Sludge fermentation

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# 1. 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 [1]. However, in view of the circular economy, these high management costs must be minimized. WAS is a renewable source that can provide the recovery of nutrients, water, carbon, and bioenergy [2]. Thus, environmental engineering projects have been designed to provide the maximum recovery of resources, that is, bioenergy, phosphorus, biopolymers, volatile fatty acids (VFA), and water [3–9]. 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 recover the VFA, which can potentially reduce the traditional VFA production is based on nonrenewable petrochemical sources, which going to mitigate greenhouse gas (GHG) emissions [10-13].

However, anaerobic digestion and its bioconversion of WAS are limited due to its recalcitrance, demanding a high solids retention time to hydrolyze the sludge [14–16]. 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 [17-21]. 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 [22,23] driven by the increased activity of hydrolytic enzymes and acidogenic bacteria, inhibiting methanogenic activity [24]. Furthermore, alkaline fermentation provides sludge solubilization, disintegrating the floc and releasing soluble organic matter, which results in higher bioconversion and greater biogas production [25]. It is a monophasic process where the fermentation simultaneously improves the sludge hydrolysis, improving its biodegradability and maximizing the production of VFA [26]. 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 [27].

The pre-treatment of the WAS is constantly associated with 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, for example, thermal-alkaline, alkaline-enzymatic, enzymatic, and ultrasonic [19,23,28–32]. Alkaline solubilization as a pre-treatment can significantly influence the rheological properties, metal binding, organic adsorption capacities, and flocculation properties [33–35]. 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 [33,36,37].

Therefore, this study aims to treat WAS by alkaline fermentation in a laboratory-scale reactor set to recover resources such as nutrients and biogas, as well as reduce the final suspended solids concentration. The study will also assess and compare alkaline fermentation of two types of WAS as substrates to identify which releases more resources. In addition, the sludge pre-solubilization is assessed regarding optimizing the methanogenic bioconversion, to increase methane production as a maximized renewable source of bioenergy.

# 2. Materials and methods

### 2.1. Waste activated sludge 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 12 d; the other sludge was from a sequential batch reactor (SBR) configured for biological phosphorus removal with a sludge age of 5 d. 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 7 d at room temperature, then the supernatant of both was discarded to concentrate the solids, and the remaining sludges were stored at 4°C.

#### 2.2. Anaerobic sludge applied as inoculum

Anaerobic sludge from the UASB (up-flow anaerobic sludge blanket) reactor, also operated at EXTRABES and fed by municipal sewage, was used as fermentation inoculum with volatile suspended solids (VSS) concentration of  $30.65 \pm 3.2$  g L<sup>-1</sup>. Using sodium acetate (C<sub>2</sub>H<sub>3</sub>NaO<sub>2</sub>) and sucrose (C<sub>12</sub>H<sub>22</sub>O<sub>11</sub>), 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. [38].

# 2.3. Experimental procedures

The sludges applied as substrate were classified as Sludge 1, the WAS from the conventional activated sludge reactor, and Sludge 2, the WAS from the SBR. The experimental procedures started with the characterization of Sludges 1 and 2 at instant 0 h, presented in Table 1, both applied as influents in the alkaline fermentation process. Organic material predominance was identified in both sludges, despite the total suspended solids (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 [39,40]. Indeed, this sludge was from a sequential batch reactor set for biological phosphorus removal.

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, treatment A was set as the control, wherein the pH was not adjusted during the experimental procedures; with treatments B and C, the

#### Table 1

Characterization of Sludges 1 and 2 used as influent in the fermentation process

Parameters	Sludge 1	Sludge 2
pH	$7.5 \pm 0.1$	$7.5 \pm 0.2$
Total suspended solids (gTSS L <sup>-1</sup> )	$35 \pm 0.9$	$48 \pm 1.3$
Volatile suspended solids (gVSS L <sup>-1</sup> )	$24 \pm 0.2$	$30 \pm 0.2$
Ratio of VSS/TSS (-)	0.70	0.62
Chemical oxygen demand (gCOD L <sup>-1</sup> )	32 ± 3	$42 \pm 3$
Soluble chemical oxygen demand (gCOD <sub>s</sub> L <sup>-1</sup> )	$0.25 \pm 0.03$	$0.41\pm0.04$
Soluble carbohydrates (mg L <sup>-1</sup> )	$26 \pm 3.0$	$32 \pm 3.5$
Soluble proteins (mg L <sup>-1</sup> )	$80 \pm 10.2$	$102 \pm 14.2$
Ammonia nitrogen (mg N–NH <sup>+</sup> <sub>4</sub> L <sup>-1</sup> )	$192 \pm 3.4$	$285 \pm 5.3$
Orthophosphate (mg $P-PO_4^{3-}L^{-1}$ )	$23 \pm 1.4$	$44 \pm 3.0$
Total phosphorus (mg L <sup>-1</sup> )	$314 \pm 5.6$	$450\pm6.2$



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

pHs were adjusted to 10, and every 24 h 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 h, stirred at 200 rpm on a stirring table (New Brunswick Scientific, mod. G 33) at room temperature (23°C–27°C), and sodium hydroxide (NaOH) was added to raise the pH [41]. For the fermentation process, NaOH was added to raise the pH, and when necessary hydrochloric acid (HCl) was added to acidify it [42].

The fermentation assay outlined in Fig. 1 for both substrates was carried out simultaneously in 18 reactors, which were Erlenmeyer flasks (1,000 mL) with a used volume of 600 mL, operated in an open system. 500 mL of the 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 10 d 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 h), 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 Eq. (1) for soluble chemical oxygen demand  $(COD_s)$ , wherein the difference between influent and effluent  $COD_s$  was divided by total chemical oxygen demand (COD).

$$Efficiency_{COD_{s}} : \frac{COD_{setfluent} - COD_{sinfluent}}{COD_{total}} \times 100\%$$
(1)

# 2.4. Biogas production tests

The fermented substrates after the 10 d of operation were submitted separately to the biogas production tests. The tests were carried out in closed reactors designed to prevent gas leakage, with a 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 [43]. 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. [44]. The experimental biogas production tests were calculated, and to improve the discussion, the modified Gompertz model [Eq. (2)] was applied Jiunn-Jyi et al. [45].

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

where *M* is the accumulated biogas production at STP (mL gVSS<sup>-1</sup>), *P* is the potential biogas production (mL gVSS<sup>-1</sup>),  $R_m$  is the maximum biogas production rate (mL d<sup>-1</sup>),  $\lambda$  is the lag-phase time (d), *t* is the incubation time (d), and *e* equals 2.718.

#### 2.5. 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 [46] were phosphorus (P), ammonia nitrogen (N–NH<sup>+</sup><sub>4</sub>), 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. [47], and carbohydrate analysis was performed using the method described by Dubois et al. [48].

# 3. Results and discussion

# 3.1. Fermentation influence on sludge solubilization

# 3.1.1. Soluble COD

The soluble fraction increase of COD was assessed to indicate sludge solubilization after the fermentation process. The profile of COD<sub>s</sub> 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<sup>-1</sup> for Sludge 1, and 414 mg L<sup>-1</sup> 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 mgCODs L-1, which means increases of 1,880, 13,250, and 10,250 mgCOD<sub>s</sub> L<sup>-1</sup>, respectively. In contrast, the final concentrations after Sludge 2 treatments were 2,700, 18,500, and 14,000 mgCOD<sub>s</sub> L<sup>-1</sup>, which means increases of 2,286, 18,086, and 13,586 mgCOD<sub>s</sub> L<sup>-1</sup> after treatments A, B, and C, respectively. Knowing the total COD concentrations (Table 1), the increase of COD<sub>s</sub> 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.

Alkaline fermentation provides sludge floc disintegration, which indicates the 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<sub>s</sub>, reaching a factor of 5.5–7.5 times the COD<sub>s</sub> influent, which is significantly lower than the factor reached by treatment C (32-41 times). Interestingly, alkaline conditions inhibit methanogenesis, providing VFA generation [49]. This methanogenesis inhibition, and the increase in VFA production, are constantly reported in the 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 mg COD L<sup>-1</sup> [50]; a solubilization efficiency of 38% was achieved and VFA increased from 16 to 1,248 mg COD L-1 [51]; and 53% of efficiency with 2.9 kg m<sup>-3</sup> of accumulated VFA [52]. Thus, the significant  $\overline{COD}_{s}$  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) [41,53–55]. Therefore, the  $BOD_{20}/COD$  ratio can be significantly reduced by alkaline pre-treatment with pH 10 and upwards [56]. 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 [28,31,57].

# 3.1.2. Carbohydrates, proteins, and ammonia nitrogen $(N-NH_{\star}^{*})$

The concentrations of carbohydrates, proteins, and ammonia nitrogen in soluble fraction for both sludges are presented separately by influent and effluent of each



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

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 favors to solubilizing EPS and damage the cell, and the inner floc constituents are released in the soluble fraction of the sludge [50,53]. The increases of proteins in the soluble fractions were 538, 2,166, and 1,256 mg L<sup>-1</sup> for Sludge 1; and 380, 1,652, and 955 mg L<sup>-1</sup> for Sludge 2 for treatments A, B, and C, respectively. The increases of carbohydrates were 161, 934, and 390 mg L<sup>-1</sup> for Sludge 1, and 118, 730, and 296 mg L<sup>-1</sup> for Sludge 2, for treatments A, B, and C, respectively. The concentration increase in the soluble fraction of proteins and carbohydrates is explained as a dissociation consequence not only of EPS matrix, but also of VFA [58,59].

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<sup>-1</sup> 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<sup>-1</sup>, and proteins, 955–1,256 mg L<sup>-1</sup>), 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 carbohydrates 730-934 mg L-1, and proteins 1,652–2,166 mg L<sup>-1</sup>, 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 increase COD<sub>s</sub>. Therefore, proteins and carbohydrates do not necessarily have the same increase for the COD<sub>s</sub> curve [60], since a sludge solubilization rate can exceed that of biological degradation of proteins and carbohydrates by acidogenic bacteria [52,61,62]. 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 bio-production process of bioenergy, or of short chain fatty acids, as well as inhibiting methanogenesis, providing VFA accumulation [22,63]. 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 [13,64]. 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 [13,65]; 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 the ammonia nitrogen concentration was lower than the influent in treatments under alkaline conditions. The drop in N might be attributed to the ammonification process due to the pH under alkaline conditions: the fraction of released organic nitrogen-containing compounds was transformed into ammonia nitrogen, then  $\rm NH_4^+$  was deionized to  $\rm NH_3$  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 [41,66–68]. 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 [69], 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 [20,41,66,67]. 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 [70].

#### 3.1.3. 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  $23 \pm 1.4$  mg L<sup>-1</sup> for Sludge 1, and  $44 \pm 3.0$  mg L<sup>-1</sup> for Sludge 2. The orthophosphate concentrations in effluent for treatments A, B, and C, were 85, 200, and 170 mg L<sup>-1</sup> for Sludge 1, and 155, 397, and 353 mg L<sup>-1</sup> for Sludge 2, respectively. The alkaline fermentation results were also better than the fermentation with no pH control, which was previously reported in the literature that assessing the sludge fermentation varying the pH from 4 to 12, and reported pH 10 as the most favorable [71].

The pre-treatment applied in line with alkaline fermentation significantly improved the orthophosphate release. Liu et al. [31] assessed the phosphorus release by four different WAS pre-treatments, reporting significant 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 efficiently releasing orthophosphate, which can be boosted by the alkaline pre-treatment. This is especially the case for treating WAS from biological phosphorus removal systems containing biomass poly-P. The orthophosphate increase is caused by the release of phosphorus from the EPS rupture, dead microbial cells, or phosphorus-accumulating organisms [72].

#### 3.2. 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 d, 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



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.

were only 90.7 NmL gSSV<sup>-1</sup>. The average biogas production for treatment C was 269.4 NmL gSSV<sup>-1</sup>, 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<sup>-1</sup>, 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 to 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 modeled using the modified Gompertz model, and the results are presented in Fig. 4 and Table 3. The  $R^2$  coefficient

values were more significant 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 the 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 by 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 Table 2

Carbohydrates, proteins, and ammonia nitrogen concentrations in soluble fraction at influent and effluent of the three treatments set

Treatment	Parameters	s Carbohydrates (mg L <sup>-1</sup> )	Proteins (mg L <sup>-1</sup> )	Ammonia nitrogen (mgN–NH <sub>4</sub> <sup>+</sup> L <sup>-1</sup> )
Sludge 1	Influent	$26 \pm 3.0$	$80 \pm 10.2$	192 ± 22
	Effluent A	$187 \pm 19$	$618 \pm 25$	$556 \pm 28$
	Effluent B	$960 \pm 30$	$2246 \pm 32$	$115 \pm 12$
	Effluent C	$416 \pm 21$	$1336 \pm 17$	$131 \pm 16$
Sludge 2	Influent	$32 \pm 3.5$	$102 \pm 14.2$	$285 \pm 31$
	Effluent A	$150 \pm 11$	$482 \pm 38$	$614 \pm 29$
	Effluent B	$762 \pm 13$	$1754 \pm 20$	$94 \pm 14$
	Effluent C	$328 \pm 12$	$1057 \pm 16$	$210 \pm 21$

expected [73], which indicates the increase in salinity inside the anaerobic reactor. However, according to the literature, it does not necessarily inhibits anaerobic bioconversion [74,75]. A previous study from this group reported an increase in biogas production even pre-treating sludge by alkaline methodology with the addition of NaOH and HCl [41].

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 [41,76]. 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. However, it also provides an increase in refractory organic matter, such as humic substances and lignins [77]. This negatively influences the methanization, decreasing the potential production of methane as the humic content increases.

# 3.3. 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<sub>s</sub>, 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 reduction 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 boosted with the pre-solubilization process. However, to evaluate its suitability for the 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

# Table 3

Parameters Gompertz' model [Eq. (1)], methane yield, and methane production rate

Substrate	Modified Gompertz model			Yield
	Р	$R_{_m}$	$R^2$	Biogas
	NmL gVSS <sup>-1</sup>	$mL  d^{\scriptscriptstyle -1}$	-	NmL gVSS <sup>-1</sup>
Effluent A	86	6.4	0.9583	91
Effluent B	341	29.2	0.9358	346
Effluent C	274	16.9	0.9506	269

presented in the literature [41], 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. [78]. Lastly, treatment B achieved the highest orthophosphate release for both sludges (Table 4), due to the applied solubilization process. Comparing the effluent concentration to the influent, the increases were 8.6 and 9.2 times for Sludges 1 and 2, respectively.

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. So, this is an achievement to support the engineering project, which should know the primary treatment objective.

Fig. 5. Mass balance schematic diagram of treatment processes B and C treating Sludges 1 and 2 separately.

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		
	А	В	С	А	В	С
Increase of CODS (%)	5.9	41.4	32.0	5.4	43.1	32.3
Release of $P-PO_4^{3-}$ (%)	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

# 4. Conclusion

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 confirmed as a technology that could become an alternative 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 the greater bioavailability of soluble organic matter, such as proteins and carbohydrates.

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# **Conflict of interest**

The authors declare that they have no conflict of interest. The funding sources were not involved in the study design; the collection, analysis, or interpretation of the data; the writing of the report; or the decision to submit the article for publication.

# References

- H. Kroiss, What is the potential for utilizing the resources in sludge?, Water Sci. Technol., 49 (2004) 1–10.
- [2] A. Gherghel, C. Teodosiu, S. De Gisi, A review on wastewater sludge valorisation and its challenges in the context of circular economy, J. Cleaner Prod., 228 (2019) 244–263.
- [3] L. Leng, W. Zhang, S. Leng, J. Chen, L. Yang, H. Li, S. Jiang, H. Huang, Bioenergy recovery from wastewater produced by hydrothermal processing biomass: progress, challenges, and opportunities, Sci. Total Environ., 748 (2020) 142383, doi: 10.1016/j.scitotenv.2020.142383.
- [4] B. Zhang, Z. Zhao, N. Chen, C. Feng, Z. Lei, Z. Zhang, Insight into efficient phosphorus removal/recovery from enhanced methane production of waste activated sludge with chitosan-Fe supplementation, Water Res., 187 (2020) 116427, doi: 10.1016/j. watres.2020.116427.
- [5] W. Liu, H. Yang, J. Ye, J. Luo, Y.Y. Li, J. Liu, Short-chain fatty acids recovery from sewage sludge via acidogenic fermentation as a carbon source for denitrification: a review, Bioresour. Technol., 311 (2020) 1–10.
- [6] C.M. Schambeck, B.S. Magnus, L.C.R. de Souza, W.R.M. Leite, N. Derlon, L.B. Guimarães, R.H.R. da Costa, Biopolymers recovery: dynamics and characterization of alginate-like exopolymers in an aerobic granular sludge system treating municipal wastewater without sludge inoculum, J. Environ. Manage., 263 (2020) 1–11.
- [7] J.F.J. Chimuca, J.T. de Sousa, W.S. Lopes, V.D. Leite, C.S.A. do Canto, Decentralized treatment of domestic sewage in dynamic membrane bioreactor, Desal. Water Treat., 197 (2020) 76–89.
- [8] J.T. de Sousa, M.L.D. de Luna, I.N. Henrique, V.D. Leite, W.S. Lopesa, H.W. Pearsona, Compact sewage treatment systems for rural sanitation, J. Urban Environ. Eng., 14 (2020) 78–86.
- [9] T. Tobin, R. Gustafson, R. Bura, H.L. Gough, Integration of wastewater treatment into process design of lignocellulosic biorefineries for improved economic viability, Biotechnol. Biofuels, 13 (2020) 1–17.





- [10] B. Ilmas, K.A. Mir, S. Khalid, Greenhouse gas emissions from the waste sector: a case study of Rawalpindi in Pakistan, Carbon Manage., 9 (2018) 645–654.
- [11] L. Appels, J. Baeyens, J. Degrève, R. Dewil, Principles and potential of the anaerobic digestion of waste-activated sludge, Prog. Energy Combust. Sci., 34 (2008) 755–781.
- [12] F.J. Maciel, J.F.T. Jucá, Biogas recovery in an experimental MSW cell in Brazil: lessons learned and recommendations for CDM projects, Greenhouse Gas Meas. Manage., 2 (2012) 186–197.
- [13] M. Ramos-Suarez, Y. Zhang, V. Outram, Current perspectives on acidogenic fermentation to produce volatile fatty acids from waste, Rev. Environ. Sci. Biotechnol., 20 (2021) 439–478.
- [14] P. Neumann, S. Pesante, M. Venegas, G. Vidal, Developments in pre-treatment methods to improve anaerobic digestion of sewage sludge, Rev. Environ. Sci. Biotechnol., 15 (2016) 173–211.
- [15] D.C. Stuckey, P.L. McCarty, The effect of thermal pretreatment on the anaerobic biodegradability and toxicity of waste activated sludge, Water Res., 18 (1984) 1343–1353.
- [16] G.P. Sheng, H.Q. Yu, Characterization of extracellular polymeric substances of aerobic and anaerobic sludge using three-dimensional excitation and emission matrix fluorescence spectroscopy, Water Res., 40 (2006) 1233–1239.
- [17] Y.-K. Cheah, C. Vidal-Antich, J. Dosta, J. Mata-Álvarez, Volatile fatty acid production from mesophilic acidogenic fermentation of organic fraction of municipal solid waste and food waste under acidic and alkaline pH, Environ. Sci. Pollut. Res., 26 (2019) 35509–35522.
- [18] L. Lin, R.-h. Li, Y. Li, J. Xu, X.-y. Li, Recovery of organic carbon and phosphorus from wastewater by Fe-enhanced primary sedimentation and sludge fermentation, Process Biochem., 54 (2017) 135–139.
- [19] H. Liu, H. Xiao, B. Yin, Y. Zu, H. Liu, B. Fu, H. Ma, Enhanced volatile fatty acid production by a modified biological pretreatment in anaerobic fermentation of waste activated sludge, Chem. Eng. J., 284 (2016) 194–201.
- [20] W. Bi, Y. Li, Y. Hu, Recovery of phosphorus and nitrogen from alkaline hydrolysis supernatant of excess sludge by magnesium ammonium phosphate, Bioresour. Technol., 166 (2014) 1–8.
- [21] Y. Li, Y. Hu, G. Wang, W. Lan, J. Lin, Q. Bi, H. Shen, S. Liang, Screening pretreatment methods for sludge disintegration to selectively reclaim carbon source from surplus activated sludge, Chem. Eng. J., 255 (2014) 365–371.
  [22] X. Wang, Y. Li, Y. Zhang, Y. Rong Pan, L. Li, J. Liu, D. Butler,
- [22] X. Wang, Y. Li, Y. Zhang, Y. Rong Pan, L. Li, J. Liu, D. Butler, Stepwise pH control to promote synergy of chemical and biological processes for augmenting short-chain fatty acid production from anaerobic sludge fermentation, Water Res., 155 (2019) 193–203.
- [23] H. Liu, P. Han, H. Liu, G. Zhou, B. Fu, Z. Zheng, Full-scale production of VFAs from sewage sludge by anaerobic alkaline fermentation to improve biological nutrients removal in domestic wastewater, Bioresour. Technol., 260 (2018) 105–114.
- [24] B. Xiao, C. Liu, J. Liu, X. Guo, Evaluation of the microbial cell structure damages in alkaline pretreatment of waste activated sludge, Bioresour. Technol., 196 (2015) 109–115.
- [25] W. Fang, P. Zhang, G. Zhang, S. Jin, D. Li, M. Zang, X. Xu, Effect of alkaline addition on anaerobic sludge digestion with combined pretreatment of alkaline and high pressure homogenization, Bioresour. Technol., 168 (2014) 167–172.
- [26] Y. Chen, X. Jiang, K. Xiao, N. Shen, R.J. Zeng, Y. Zhou, Enhanced volatile fatty acids (VFAs) production in a thermophilic fermenter with stepwise pH increase – investigation on dissolved organic matter transformation and microbial community shift, Water Res., 112 (2017) 261–268.
- [27] R. Wang, Y. Li, W. Chen, J. Zou, Y. Chen, Phosphate release involving PAOs activity during anaerobic fermentation of EBPR sludge and the extension of ADM1, Chem. Eng. J., 287 (2016) 436–447.
- [28] H. Pang, X. Pan, L. Li, J. He, Y. Zheng, F. Qu, Y. Ma, B. Cui, J. Nan,Y. Liu, An innovative alkaline protease-based pretreatment approach for enhanced short-chain fatty acids production via a short-term anaerobic fermentation of waste activated sludge, Bioresour. Technol., 312 (2020) 1–9.

- [29] H. Carrère, C. Dumas, A. Battimelli, D.J. Batstone, J.P. Delgenès, J.P. Steyer, I. Ferrer, Pretreatment methods to improve sludge anaerobic degradability: a review, J. Hazard. Mater., 183 (2010) 1–15.
- [30] A. Gonzalez, A.T.W.M. Hendriks, J.B. van Lier, M. de Kreuk, Pre-treatments to enhance the biodegradability of waste activated sludge: elucidating the rate limiting step, Biotechnol. Adv., 36 (2018) 1434–1469.
- [31] J. Liu, S. Deng, B. Qiu, Y. Shang, J. Tian, A. Bashir, X. Cheng, Comparison of pretreatment methods for phosphorus release from waste activated sludge, Chem. Eng. J., 368 (2019) 754–763.
- [32] Y. Yan, L. Feng, C. Zhang, C. Wisniewski, Q. Zhou, Ultrasonic enhancement of waste activated sludge hydrolysis and volatile fatty acids accumulation at pH 10.0, Water Res., 44 (2010) 3329–3336.
- [33] J. Zhao, D. Wang, Y. Liu, H.H. Ngo, W. Guo, Q. Yang, X. Li, Novel stepwise pH control strategy to improve short chain fatty acid production from sludge anaerobic fermentation, Bioresour. Technol., 249 (2018) 431–438.
- [34] A. Omoike, J. Chorover, Spectroscopic study of extracellular polymeric substances from *Bacillus subtilis*: aqueous chemistry and adsorption effects, Biomacromolecules, 5 (2004) 1219–1230.
- [35] G.P. Sheng, H.Q. Yu, Z. Yu, Extraction of extracellular polymeric substances from the photosynthetic bacterium *Rhodopseudomonas acidophila*, Appl. Microbiol. Biotechnol., 67 (2005) 125–130.
- [36] C.Y. Gomec, R.E. Speece, The role of pH in the organic material solubilization of domestic sludge in anaerobic digestion, Water Sci. Technol., 48 (2003) 143–150.
- [37] D. Zhang, X. Li, S. Jia, L. Dai, J. Zhao, Y. Chen, X. Dai, A review: factors affecting excess sludge anaerobic digestion for volatile fatty acids production, Water Sci. Technol., 72 (2015) 678–688.
- [38] J. Wang, H. Liu, B. Fu, K. Xu, J. Chen, Trophic link between syntrophic acetogens and homoacetogens during the anaerobic acidogenic fermentation of sewage sludge, Biochem. Eng. J., 70 (2013) 1–8.
- [39] A.C. van Haandel, J. van der Lubbe, Handbook Biological Wastewater Treatment. Design and Optimisation of Activated Sludge Systems, 2nd ed., IWA Publishing, London, 2012.
- [40] I.N. Henrique, J.T. de Sousa, B.S.O. de Ceballos, D.P. Brasil, Biological phosphorus removal in sequencing batch reactors with different solid retention times, Eng. Sanit. Ambient., 15 (2010) 197–204.
- [41] T.A.T. de Sousa, F.P. do Monte, J.V.N. Silva, W.S. Lopes, V.D. Leite, J.B. van Lier, J.T. de Sousa, Alkaline and acid solubilisation of waste activated sludge, Water Sci. Technol., 83 (2021) 2980–2996.
- [42] X. Ma, J. Ye, L. Jiang, L. Sheng, J. Liu, Y.Y. Li, Z.P. Xu, Alkaline fermentation of waste activated sludge with calcium hydroxide to improve short-chain fatty acids production and extraction efficiency via layered double hydroxides, Bioresour. Technol., 279 (2019) 117–123.
- [43] R. de O. Ramos, T.L.Q. de Albuquerque, W.S. Lopes, Sistema de monitoramento on-line de biorreatores (SISMOBIO), 2020.
- [44] C. Holliger, M. Alves, D. Andrade, I. Angelidaki, S. Astals, U. Baier, C. Bougrier, P. Buffière, M. Carballa, V. de Wilde, F. Ebertseder, B. Fernández, E. Ficara, I. Fotidis, J.C. Frigon, H.F. de Laclos, D.S.M. Ghasimi, G. Hack, M. Hartel, J. Heerenklage, I.S. Horvath, P. Jenicek, K. Koch, J. Krautwald, J. Lizasoain, J. Liu, L. Mosberger, M. Nistor, H. Oechsner, J.V. Oliveira, M. Paterson, A. Pauss, S. Pommier, I. Porqueddu, F. Raposo, T. Ribeiro, F.R. Pfund, S. Strömberg, M. Torrijoset, M. van Eekert, J. van Lier, H. Wedwitschka, I. Wierinck, Towards a standardization of biomethane potential tests, Water Sci. Technol., 74 (2016) 2515–2522.
- [45] L. Jiunn-Jyi, L. Yu-You, T. Noike, Influences of pH and moisture content on the methane production in high-solids sludge digestion, Water Res., 31 (1997) 1518–1524.
- [46] L. Bridgewater, American Public Health Association, American Water Works Association, and Water Environment Federation, Standard Methods for the Examination of Water and Wastewater, 22nd ed., 2012.

- [47] B. Frølund, T. Griebe, P.H. Nielsen, Enzymatic activity in the activated-sludge floc matrix, Appl. Microbiol. Biotechnol., 43 (1995) 755–761.
- [48] M. Dubois, K.A. Gilles, J.K. Hamilton, P.A. Rebers, F. Smith, Colorimetric method for determination of sugars and related substances, Anal. Chem., 28 (1956) 350–356.
- [49] H. Ma, X. Chen, H. Liu, H. Liu, B. Fu, Improved volatile fatty acids anaerobic production from waste activated sludge by pH regulation: alkaline or neutral pH?, Waste Manage., 48 (2016) 397–403.
- [50] X. Li, Y. Peng, B. Li, C. Wu, L. Zhang, Y. Zhao, Effects of alkali types on waste activated sludge (WAS) fermentation and microbial communities, Chemosphere, 186 (2017) 864–872.
- [51] Y. Gao, Y. Peng, J. Zhang, S. Wang, J. Guo, L. Ye, Biological sludge reduction and enhanced nutrient removal in a pilotscale system with 2-step sludge alkaline fermentation and A<sub>2</sub>O process, Bioresour. Technol., 102 (2011) 4091–4097.
- [52] D. Wang, Y. Liu, H.H. Ngo, C. Zhang, Q. Yang, L. Peng, D. He, G. Zeng, Xi. Lie, Approach of describing dynamic production of volatile fatty acids from sludge alkaline fermentation, Bioresour. Technol., 238 (2017) 343–351.
- [53] X. Guo, J. Liu, B. Xiao, Evaluation of the damage of cell wall and cell membrane for various extracellular polymeric substance extractions of activated sludge, J. Biotechnol., 188 (2014) 130–135.
- [54] D.-c. Xu, C.-q. Zhong, K.-h. Yin, S.-h. Peng, T.-t. Zhu, G. Cheng, Alkaline solubilization of excess mixed sludge and the recovery of released phosphorus as magnesium ammonium phosphate, Bioresour. Technol., 249 (2017) 783–790.
- [55] H. Li, C. Li, W. Liu, S. Zou, Optimized alkaline pretreatment of sludge before anaerobic digestion, Bioresour. Technol., 123 (2012) 189–194.
- [56] W. Wonglertarak, B. Wichitsathian, Alkaline pretreatment of waste activated sludge in anaerobic digestion, J. Clean Energy Technol., 2 (2014) 118–121.
- [57] K.D.S. do Ó, T.A.T. de Sousa, I.N. Henrique, V.D. Leite, R. de O. Ramos, J.T. de Sousa, Assessing of alkaline and enzymatic pre-treatment: comparison as WAS solubilisation process to maximize its potential as carbonaceous source, Biomass Convers. Biorefin., (2021) 1–12, doi: 10.1007/s13399-021-01834-x.
- [58] H.C. Flemming, J. Wingender, The biofilm matrix, Nat. Rev. Microbiol., 8 (2010) 623–633.
- [59] G.P. Sheng, H.Q. Yu, X.Y. Li, Extracellular polymeric substances (EPS) of microbial aggregates in biological wastewater treatment systems: a review, Biotechnol. Adv., 28 (2010) 882–894.
- [60] G. Su, M. Huo, Z. Yuan, S. Wang, Y. Peng, Hydrolysis, acidification and dewaterability of waste activated sludge under alkaline conditions: combined effects of NaOH and Ca(OH), Bioresour. Technol., 136 (2013) 237–243.
- [61] Y. Chen, A.A. Randall, T. McCue, The efficiency of enhanced biological phosphorus removal from real wastewater affected by different ratios of acetic to propionic acid, Water Res., 38 (2004) 27–36.
- [62] H. Wu, D. Yang, Q. Zhou, Z. Song, The effect of pH on anaerobic fermentation of primary sludge at room temperature, J. Hazard. Mater., 172 (2009) 196–201.
- [63] L. Feng, Y. Chen, X. Zheng, Enhancement of waste activated sludge protein conversion and volatile fatty acids accumulation during waste activated sludge anaerobic fermentation by carbohydrate substrate addition: the effect of pH, Environ. Sci. Technol., 43 (2009) 4373–4380.

- [64] M. Atasoy, I. Owusu-Agyeman, E. Plaza, Z. Cetecioglu, Biobased volatile fatty acid production and recovery from waste streams: current status and future challenges, Bioresour. Technol., 268 (2018) 773–786.
- [65] G.K. Veluswamy, K. Shah, A.S. Ball, A.J. Guwy, R.M. Dinsdale, A techno-economic case for volatile fatty acid production for increased sustainability in the wastewater treatment industry, Environ. Sci. Water Res. Technol., 7 (2021) 927–941.
- [66] H. Ma, S. Zhang, X. Lu, B. Xi, X. Guo, H. Wang, J. Duan, Excess sludge reduction using pilot-scale lysis-cryptic growth system integrated ultrasonic/alkaline disintegration and hydrolysis/ acidogenesis pretreatment, Bioresour. Technol., 116 (2012) 441–447.
- [67] V.D. Leite, J.M.R. Paredes, T.A.T. de Sousa, W.S. Lopes, J.T. de Sousa, Ammoniacal nitrogen stripping from landfill leachate at open horizontal flow reactors, Water Environ. Res., 90 (2018) 387–394.
- [68] X.L. Li, L. Zhang, B.K. Li, C.Y. Wu, Y.Z. Peng, Alkaline fermentation and elutriation of waste activated sludge for short chain fatty acids abstraction, J. Chem. Technol. Biotechnol., 93 (2018) 138–145.
- [69] M. Ye, J. Luo, S. Zhang, H. Yang, Y.-Y. Li, J. Liu, In-situ ammonia stripping with alkaline fermentation of waste activated sludge to improve short-chain fatty acids production and carbon source availability, Bioresour. Technol., 301 (2020) 1–6.
- [70] V. Toutian, M. Barjenbruch, C. Loderer, C. Remy, Pilot study of thermal alkaline pretreatment of waste activated sludge: seasonal effects on anaerobic digestion and impact on dewaterability and refractory COD, Water Res., 182 (2020) 1–48.
- [71] S. Shi, G. Xu, Identification of phosphorus fractions of biofilm sludge and phosphorus release, transformation and modeling in biofilm sludge treatment related to pH, Chem. Eng. J., 369 (2019) 694–704.
- [72] L.-K. Ju, H.K. Shah, J. Porteous, Phosphorus release in aerobic sludge digestion, Water Environ. Res., 77 (2005) 553–559.
- [73] G. Tchobanoglous, F.L. Burton, H.D. Stensel, Metcalf & Eddy, Inc., Wastewater Engineering Teatment and Reuse, J. Wastewater Eng., 4th ed., 2003.
- [74] A.C. Miranda, W.S. Lopes, A.V. Haandel, Influência da concentração de sal na atividade do lodo, Hydro, 10 (2014) 36–43.
- [75] J.D. Muñoz Sierra, M.J. Oosterkamp, W. Wang, H. Spanjers, J.B. van Lier, Impact of long-term salinity exposure in anaerobic membrane bioreactors treating phenolic wastewater: performance robustness and endured microbial community, Water Res., 141 (2018) 172–184.
- [76] H. Chen, H. Yi, H. Li, X. Guo, B. Xiao, Effects of thermal and thermal-alkaline pretreatments on continuous anaerobic sludge digestion: performance, energy balance and, enhancement mechanism, Renewable Energy, 147 (2020) 2409–2416.
- [77] S. Ma, H. Hu, J. Wang, K. Liao, H. Ma, H. Ren, The characterization of dissolved organic matter in alkaline fermentation of sewage sludge with different pH for volatile fatty acids production, Water Res., 164 (2019) 1–9.
- [78] Y. Chen, R. Ruhyadi, J. Huang, W. Yan, G. Wang, N. Shen, W. Hanggoro, A novel strategy for improving volatile fatty acid purity, phosphorus removal efficiency, and fermented sludge dewaterability during waste activated sludge fermentation, Waste Manage., 119 (2021) 195–201.

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