

Article



Effects of Short Retention Times and Ultrasound Pretreatment on Ammonium Concentration and Organic Matter Transformation in Anaerobic Digesters Treating Sewage Sludge

Matías Olivera^{1,2}, Felipe Barriga¹, Patricio Neumann^{1,3}, Gloria Gómez^{1,2} and Gladys Vidal^{1,2,*}

- ¹ Water Research Center for Agriculture and Mining (CRHIAM), ANID Fondap Center, Victoria 1295, Concepcion 4030000, Chile; gibaudec@gmail.com (M.O.); felipe.barriga.azocar@gmail.com (F.B.); pneumann@ubiobio.cl (P.N.); gloriagomezosorio@gmail.com (G.G.)
- ² Engineering and Environmental Biotechnology Group (GIBA-UDEC), Environmental Sciences Faculty and Center EULA-Chile, Universidad de Concepcion, Concepcion 4030000, Chile
- ³ Basic Sciences Department, Faculty of Sciences, University of Bío-Bío, Chillan 3800708, Chile
- * Correspondence: glvidal@udec.cl

Abstract: Anaerobic digestion of sewage sludge is limited at the hydrolysis stage of the process. The goal of this study was to assess the effects of sludge retention times and ultrasound pretreatment on the ammonium concentration and organic matter transformation in anaerobic digesters treating sewage sludge. To achieve this, two laboratory-scale semicontinuous anaerobic digesters were operated for a period of over 70 d, including a control reactor and another fed by pretreated sludge. Both anaerobic systems were fed with mixed sludge (50%/50% primary/secondary treatment) in mesophilic conditions (37 °C), with solid retention times (SRT) of 7.5 d (Phase I) and 3 d (Phase II). The performance of the anaerobic digestion process was assessed in terms of the methane yield and the total and soluble chemical organic demand, total solids, and volatile solids removal. The results showed that the ultrasound pretreatment caused an increase of around 22.2% in CODt removal for an SRT of 7.5 d. Meanwhile, an SRT of 3 d resulted in a decrease of up to 92.4% in CODt removal. The performance in terms of biogas production and organic matter removal was significantly affected by the SRT reduction to 3 d, showing that the process is not viable in these conditions.

Keywords: ammonium; anaerobic digestion; pretreatment; retention time; sewage sludge; ultrasound

1. Introduction

1.1. Application and Limitations of Anaerobic Digestion for Sewage Sludge Treatment

Recent developments in sewage treatment plants (STPs) have tended to be focused on the recovery of the resources contained in wastewater, including nutrients, energy vectors, and organic matter. Energy recovery from the sludge generated by STPs not only allows for a significant reduction in the operating costs of water treatment but also contributes to reducing the environmental burdens of the process and to improving the environmental performance of the wastewater treatment sector [1,2]. The main wastewater treatment system worldwide is aerated activated sludge, which can remove between 55-95% of the biological oxygen demand (BOD_5) [3,4], but at the same time it generates large quantities of sludge, which must be treated before its final disposal [5]. Sewage sludge is composed of two main fractions: primary sludge, comprising solids removed during primary physical treatment, and secondary sludge, made up of the microorganisms that grow during the biological treatment and are then separated from the treated water during secondary settling. Anaerobic digestion (AD) has been widely implemented since the beginning of the XX century to stabilize sewage sludge, as the process results in a significant transformation of organic matter into biogas, mainly composed of methane (CH₄) and carbon dioxide (CO₂). However, the rate and extent of organic matter transformation during the AD of



Citation: Olivera, M.; Barriga, F.; Neumann, P.; Gómez, G.; Vidal, G. Effects of Short Retention Times and Ultrasound Pretreatment on Ammonium Concentration and Organic Matter Transformation in Anaerobic Digesters Treating Sewage Sludge. *Fermentation* **2024**, *10*, 63. https://doi.org/10.3390/ fermentation10010063

Academic Editor: Alessio Siciliano

Received: 20 November 2023 Revised: 16 December 2023 Accepted: 20 December 2023 Published: 17 January 2024



Copyright: © 2024 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (https:// creativecommons.org/licenses/by/ 4.0/). sewage sludge is limited at the hydrolysis stage of the process, mainly due to the complexity and low biodegradability of organic matter [6,7]. One of the most important barriers is the presence of bacterial flocs formed by microorganisms during wastewater treatment, which include a complex polymeric matrix formed by high-molecular-weight compounds that are excreted by the microorganisms [8].

1.2. Use of Pretreatments to Improve the AD Process

The application of pretreatments in AD is intended to facilitate the hydrolysis stage of the process, intensifying the conversion of organic matter and making lower SRTs possible. This can result in significant reductions in the capital costs of the process, as lower SRTs imply that smaller reactors can be used for treatment [9]. A wide array of pretreatments technologies has been proposed, including thermal, chemical, and mechanical processes. To improve the performance of pretreatment processes, hybrid methods based on the integration of different phenomena have also been studied (such as thermo-chemical or mechanical–biological processes), which in some cases can result in synergistic effects over organic matter solubilization and the observed improvements during the hydrolysis stage of AD [6,10,11].

Cavitation has been extensively used as a pretreatment for increasing the biodegradability of sewage sludge. This process consists in the formation, growth, and collapse of vapor cavities within microseconds, generating intense shockwaves that cause localized hotspots with temperatures between 1000–15,000 K and pressures from 500 to 5000 bar [12]. Ultrasound is one of the most widespread pretreatment processes, which fulfils the objective of disintegrating the structure of the polymer matrix via phenomena such as cavitation, facilitating the interaction of microorganisms and extracellular enzymes with the substrate and favoring the transformation of high-molecular-weight molecules into simpler compounds during hydrolysis [10,13]. Even though the use of pretreatments shows significant effects on the AD process, including an increase in organic matter removal efficiency, higher biogas production, and a higher level of sludge stabilization [14,15], not all technologies are currently economically viable, mainly due to the energy requirements associated with such processes, especially at small scales [16,17]. Ultrasound is among the most widespread processes at an industrial scale, allowing for the treatment of sludge with solids concentrations of up to 10% TS, with energy consumption values below 10 kWh/ m^3 , which could explain its broad reach [17,18].

Although one of the main objectives of applying ultrasound before AD is the reduction in the SRT, most previous articles evaluate SRTs in the range of from 7.5 to 30 days [7,15,19]. Under these conditions, pretreatment results in increased methane yields, without affecting the process stability or generating the accumulation of intermediate compounds. Therefore, the assessment of shorter SRTs is important in order to evaluate the viability of this strategy, the impact of pre-treatment on the process, and potential operational issues such as ammonia accumulation or microorganism washout.

1.3. Ammonium as an Inhibitor of the AD Process

Despite the advantages of AD for sewage sludge treatment, the process is vulnerable to the inhibition of biological activity caused by the accumulation of some chemical compounds. AD is a complex process, during which organic compounds such as alcohols, ketones, long-chain fatty acids, and nitrogenous compounds such as ammonia and ammonium are generated [20]. The accumulation of some of these compounds in the anaerobic reactor can produce inhibitory effects on biological activity, mainly affecting acetotrophic methanogens [21]. Specifically, ammonium inhibition in the reactor is a recurring problem due to the high content of proteins and other nitrogenous compounds in sewage sludge, as well as the presence of dissolved ammonium in the same substrate.

AD inhibition due to the presence of ammonium can be presented at variable concentrations (NH_4^+ -N de 0.9–1.5 g/L) and is significantly affected by pH and the acclimation of the microorganisms [22]. Inhibition of the reactor occurs because free NH_3 can enter

through the cell membrane, mainly affecting the homeostasis of methanogenic archaea [18]. Under conditions of neutral pH (6.8–8.9) and mesophilic temperature (35–37 °C), the concentration of NH_3^+ -N and NH_4^+ -N are in equilibrium [23]. As the free ammonium fraction increases at higher pH, the inhibition phenomenon tends to be more pronounced under more basic conditions. Belmonte et al. [24] showed that the activity of methane-producing archaea is associated with the presence of free ammonium and that at concentrations greater than 40 mg NH_3^+ -N/L, between 56 and 84% of the methanogenic activity can be inhibited.

Many pathways have been proposed for ammonia inhibition, including changes in the intracellular pH of methanogens, an increase in the requirement of maintenance energy, and the inhibition of specific enzymatic reactions. Ammonia can affect methanogenic microorganisms mainly in two ways: (i) the ammonium ion can directly inhibit methaneproducing enzymes and/or (ii) the hydrophobic ammonia molecule can passively diffuse into bacterial cells, causing a proton imbalance or potassium deficiency [25].

Considering the above, the objective of this study was to assess the effects of an ultrasound pretreatment on the ammonium concentration and the operating performance of anaerobic digestion of sludge at low solid retention times. The pretreatment process has the potential to intensify the AD process, reducing the volume of the reactor and the capital costs of the process. However, this configuration can lead to the inhibition of biological activity due to a higher NH_3 release and its accumulation inside the system, as well as the potential washout of active biomass due to the low SRT. In this scenario, the quantification of the effects of both phenomena on the stability and performance of the process is necessary (COD_t removal, methane production), along with the identification of potential operational limits.

2. Materials and Methods

2.1. Sewage Sludge and Bacterial Inoculum

The mixed sewage sludge samples were obtained from the wastewater treatment plant of the city of Concepción (Chile), located in Hualpén Commune, Bío-Bío Region ($36^{\circ}48'$ S, $73^{\circ}08'$ W).

The sludge samples were extracted from the outlet chamber where the primary and secondary sludge converge. This unit is situated before the sludge treatment in the anaerobic reactors and after its thickening. Subsequently, the samples were taken to the laboratory and stored at 4 °C until their use in the assays, a process repeated twice a month throughout the period. The anaerobic inoculum was extracted from the anaerobic digestion recirculation line in the same treatment plant.

2.2. Ultrasound Pretreatment of Sludge

The pretreatment was carried out under batch conditions. Beakers of 1 L with 600 mL of mixed sludge were used, and mechanical agitation was applied to optimize the homogeneity of the pre-treatment. The specific energy applied was 2000 kJ/kgTS at a frequency of 26 kHz [7], using a UP200ST ultrasonic laboratory homogenizer (Hielscher Ultrasonics GmbH, Berlin, Germany).

2.3. Operation of the Anaerobic Digesters

Two laboratory-scale anaerobic digestors were operated: one fed with raw sludge without pretreatment (control reactor; CR) and another fed with pretreated sludge (pretreatment reactor; PTR). The reactors had a total capacity of 9 L and were operated with a reaction volume of 6 L. The temperature of the system was kept constant at 37–38 °C during the experimental period [7,15] through the recirculation of hot water from a thermostatic bath. The production of biogas generated by the reactors was measured by volume displacement in an automated system that used an automatic digital counter [26]. A schematic representation of the laboratory-scale anaerobic digestors is presented in Figure 1.



Figure 1. Schematic representation of the laboratory-scale anaerobic digestors.

The CR and PTR reactors were operated at two solid retention times (SRTs): Phase I = 7.5 days and Phase II = 3 days, which lasted 35 and 23 days, respectively. The average applied organic loading rate (OLR) for Phases I and II was 3.6 and 6.4 kgVS/L-d for CR and 3.5 and 5.6 kgVS/L-d for PTR, respectively. Biogas production was registered daily. Daily methane production was estimated using the following expression:

$$V(CH_4)\frac{mL}{d} = \frac{V_b \frac{mL}{d} \times \% CH_4}{100}$$
(1)

where V (CH₄): volume of CH₄ produced daily; V_b : volume of biogas produced daily; and %CH₄: percentage of CH₄ in biogas.

Biogas composition (CO₂ and CH₄) was measured through a gas chromatograph equipped with a thermal conductivity detector (TCD). The performance of the digestion process was assessed in terms of the removal of total and soluble chemical organic demand (COD_t, COD_s, respectively), TS, and VS for both reactors, with a periodicity of at least two times a week. The pH of the reactors was measured daily, while other important operation and stability parameters such as electrical conductivity (EC), oxidation–reduction potential (ORP), intermediate (IA) and total alkalinity (TA), and NH₄⁺-N were determined once a week. In addition, the efficiency of organic matter conversion during digestion was evaluated in the hydrolysis (H), acidogenesis (A), and methanogenesis (M) stages, according to the methodology proposed by El-Mashad et al. [27]., as described in Equations (2)–(4):

$$H(\%) = (CH_4 - COD + COD_{S-E}) / (COD_{T-I}) \times 100$$
⁽²⁾

$$A(\%) = (CH_4 - COD + COD_{VFA-E}) / (COD_{T-I}) \times 100$$
(3)

$$M(\%) = (CH_4 - COD) / (COD_{T-I}) \times 100$$
(4)

where

 CH_4 -COD: methane output, expressed as COD (g/day); COD_{S-E} : soluble COD in the reactor effluent (g/day); COD_{T-I} : total COD in the influent sludge (g/day); COD_{VFA-E} : VFA in the reactor effluent, expressed as COD (g/day).

2.4. Analytical Methods

The COD_t, COD_s, TS, TSS, and VS concentrations were determined according to Standard Methods for the Examination of Water and Wastewater [28]. CODt and CODs were analyzed using the colorimetric method. Both were determined with an absorbance at 600 nm using a Shimadzu UV-VIS spectrophotometer (UV-1800). To obtain COD_s, centrifugation of the sludge samples was performed at 3900 rpm for 5 min, followed by a serial filtration of the supernatant with filters with 1.5-µm and 0.7-µm pore sizes, respectively. TS were determined by drying the sample using a Memmert oven at 104 °C. Then, the VS were determined using the same TS sample, exposing it in a JSR muffle (JSMF-30T) at a temperature of 550 $^{\circ}$ C for 1 h, after which the sample was placed in a desiccator at a temperature of 20 °C for subsequent weighing using an analytical balance. EC, pH, and ORP were determined using a multi-parametric instrument OAKTON PC650 (Vernon Hills, IL, USA). Alkalinity was determined by titration in order to reach a pH of 5.75, equivalent to IA, followed by titration until reaching a pH of 4.3, corresponding to TA. The ammonia concentration was determined using a Merck-Millipore Spectroquant[®] (Merck KGaA, Darmstadt, Germany) photometric test (2.0–150 mg NH₄⁺-N/L). The VFA and biogas compositions (CO_2 and CH_4) were measured using a Shimadzu GC 2014 gas chromatograph with dual-lame ionization and thermal conductivity detectors (FID/TCD) equipped with an AOC 20i autosampler.

2.5. Statistical Analysis

An inferential statistical analysis of the results was carried out, for which the paired t test was used for the variables that met the normality criterion and the Kruskal–Wallis test was used in the case of non-normally distributed data. Normality was evaluated using Shapiro-Wilk's tests. All tests were performed using a significance level (α) of 0.05 using the statistical program INFOSTAT version 2017.

3. Results and Discussion

Table 1 summarizes the characterization parameters for the sludge samples before (RS) and after ultrasound pre-treatment (US). The average value of VS and COD_t in RS was 24.6 and 52.5 g/L during the experimental period, respectively. Soluble CODs accounted for approximately 18.4% of the COD_t. Average values of 0.6 g NH₄⁺-N/L and 3.29 mS/cm were observed for ammonia and EC, respectively, similar to previous reports from other authors [29].

Table 1. Characterization of mixed sludge without and with pre-treatment before anaerobic digestion.

Parameter (n)	Unit	Range		Average	
		RS	US	RS	US
pH (11)	-	5.59-5.74	5.46-5.90	5.66 ± 0.1	5.76 ± 0.1
Conductivity (11)	mS/cm	1.71-4.90	3.03-5.87	3.29 ± 1.1	3.65 ± 0.9
ORP (11)	mV	-203.6 - 85.7	-283.8 - 87.3	-171.86 ± 61	-165.05 ± 70.8
COD _t (11)	g/L	31-97.6	26.4-113.8	52.5 ± 18.6	58.9 ± 24.3
COD _s (11)	g/L	5.8-18.1	5.6-23.4	9.7 ± 6.9	11.5 ± 9.3
Total solids (11)	g/L	17.4-40.9	16.8-37.7	29.6 ± 9.3	30.8 ± 8.2
Volatile solids (11)	g/L	13.5-31.5	13.1-28.6	24.6 ± 6.8	23.7 ± 6.5
NH4 ⁺ -N (10)	g/L	0.2-1.5	0.2 - 1.4	0.6 ± 0.5	1.0 ± 0.5
Acetic acid (7)	g/L	0.17-0.28	0.22-0.39	0.21 ± 0.05	0.29 ± 0.06
Propionic acid (7)	g/L	0.19-0.40	0.36-0.59	0.36 ± 0.08	0.51 ± 0.09
Butyric acid (7)	g/L	0.06-0.25	0.13-0.31	0.13 ± 0.08	0.23 ± 0.08
N-valeric acid (7)	g/L	0.05-0.12	0.07-0.16	0.08 ± 0.03	0.12 ± 0.03
Total VFA *	gCOD/L	0.53-0.93	0.93-1.36	0.83 ± 0.15	1.16 ± 0.16

n: number determinations; RS: raw sludge; US: ultrasonicated sludge; Total VFA (volatile fatty acids). *: Obtained by stoichiometric calculation theoretical chemical organic demand of acetic, propionic, butyric, and N-valeric acid.

Conversely, the average value of VS and COD_t in the ultrasonicated sludge showed values of about 23.7 g/L and 30.8 g/L, respectively, without significant differences with RS (p > 0.05). Soluble COD showed an increase of about 1.8 g/L or 18.6%, corresponding to a solubilization factor of around 4.2%, which was similar to previous studies [7,15]. The average value for the total VFA concentration was also increased by the pretreatment, with average values of 0.83 ± 0.15 and 1.16 ± 0.16 gCOD_t/L for RS and US, respectively, being obtained. Previous studies showed divergent results regarding the effects of ultrasound pretreatment over the VFA concentration. In terms of the total concentration, Liu et al. [10] reported that concentrations of VFAs in waste activated sludge ranged between 0.75 and 0.9 gCOD_t/L, while Xue et al. [11] found that acetate concentrations increased 2 to 4 times after the application of a US pretreatment.

In terms of the performance of the DA process, Figure 2 summarizes some of the most important operating parameters of the digesters during the experimental period.



Figure 2. Conditions of operational parameters of: (**a**) ORP, (**b**) pH, (**c**) alkalinity ratio IA/TA, and (**d**) EC. For the control reactor (**III**) and reactor with pretreatment (**D**) at SRTs (solids retention times) of 7.5 and 3 days.

During all stages of the process, both CR and PTR presented negative ORP values. Even though the reactors operated with a variable ORP, ranging from -267 to -169 and -248 to -184 mV for CR and PTR, respectively, these values are characteristic of anaerobic conditions and were similar to previous studies such as those by Amani et al. [30] and Neumann et al. [7] (-225 and -300 mV). During the 7.5 d SRT operational period (Phase I), the average pH values were about 7.27 ± 0.1 and 7.09 ± 0.1 for CR and PTR, respectively, with corresponding ranges between 7.1-7.6 and 6.9-7.2. The reduction in the SRT from 7.5 d to 3.5 d between Phases I and II caused a noticeable decrease in pH, reaching values of 6.6 and 6.8 for CR and PTR, respectively. This result is attributable to the higher organic load associated with the reduction in the SRT, which could have led to the potential accumulation of intermediate compounds such as VFA and indicates a potential overloading of the systems in these conditions.

Furthermore, the IA/TA ratio values during the 7.5 d SRT stage presented averages of 0.7 ± 0.1 and 0.5 ± 0.1 for CR and PTR, respectively. These values were greater than those reported by Neumann et al. [7], who observed values of 0.28 and 0.25 for the same SRT. During the 3 d SRT stage, average values of 0.6 ± 0.1 were reached in both reactors, and in the period between days 35 and 50, values of over 0.7 were observed. These conditions represent a reduced buffer capability of the medium inside the reactor, which may be

indicative of a potential inhibition associated with the accumulation of VFAs and could lead to the acidification of the reactor [9].

Figure 3 shows the efficiency of the reactors regarding COD_t removal, VS removal, and biogas yield under the experimental conditions. During Phase I, CR and PTR achieved average CODt removal efficiencies of 27% and 33%, respectively, with a statistically significant increase in COD_t removal associated with the pretreatment equivalent to a 22.2% compared to RC (Figure 3a; p < 0.05). In terms of VS removal (Figure 3b), no significant differences (p > 0.05) were observed between CR and PTR, with average removal efficiencies of 30% and 31%, respectively (7.5 d SRT), being obtained. This was consistent with the findings of previous studies [7], who state that the main effect of ultrasound pretreatment over sludge is the solubilization of a part of the organic matter, without affecting the biodegradability of the particulate fraction of the substrate. This was also consistent with the observed increase in biogas yield during the digestion. During operation Phase I, an increase in biogas yield of approximately 30% in PTR with respect to CR was observed (Figure 3c). Braguglia et al. [31] reported that in similar operational conditions (semicontinuous operation, 10 d SRT, and temperature of 37 °C) an ultrasound pretreatment performed at a specific energy of 2500 kJ/kgTS resulted in increases in methane yield equivalent to 27%. Overall, our results suggest that the fraction of organic matter that is solubilized during the application of ultrasound is in turn transformed into biogas during the anaerobic digestion process. Therefore, the increase in biogas yield observed in PRT with respect to CR (Figure 3c) was not associated with VS removal but rather with the observed increase in COD_t removal.



Experimental phase and SRT (d)

Figure 3. Boxplot summarizing the operational performance of the reactors, expressed in terms of their removal efficiencies and methane yields. (a) COD_t , (b) VS, and (c) CH_4 production. Control reactor (\blacksquare) and reactor with pretreatment (\Box).

However, in the case of Phase II, the shortening of the SRT resulted in significant reductions in terms of biogas yield and COD_t and VS removal for both reactors compared

to the previous phase (Figure 3a,b,c, respectively). This effect was most likely generated by a combination of system organic overload and microorganism washout, which can occur when operating with SRTs that are above the specific growth rate of microorganisms. Due to their lower growth rate, this issue is especially relevant for methanogenic organisms, and according to Appels et al. [9], it could happen when digesters are operated with SRTs below 10 d. However, the specific value when washout occurs depends on the characteristics of the active biomass inside the digester.

The organic matter conversion efficiency for the different steps of the AD process is summarized in Figures 4a and 4b for SRTs of 7.5 and 3 d, respectively. The main difference between CR and PTR in both stages of operation was related to a greater hydrolysis extent in PTR compared to CR, which was consistent with the expected effect generated by US pretreatment on hydrolysis rates [32]. On the other hand, decreasing the SRT from 7.5 days to 3 days led to an observable reduction in the extent of organic matter transformation during acetogenesis and methanogenesis and the accumulation of soluble COD_s inside the digester. This reinforces the hypothesis of microorganism washout as the main factor behind the decreased performance of the systems at this SRT, since no observable accumulation of intermediate compounds such as VFAs were noticed.



Figure 4. Organic matter conversion efficiency in the different anaerobic digestion stages in the control reactor (CR) (**a**) and the reactor with pretreatment (PTR) (**b**) at SRTs (solids retention times) of 7.5 (■) and 3 days (□).

The ultrasound pretreatment also led to a significant increase in the input load of ammonia and VFA to the digestion process. Over the full experimental period, the input load of NH_4^+ -N and VFAs in PTR was 40% and 28% greater compared to CR, respectively, mainly associated with the solubilization effect caused by the pretreatment and the release of these compounds during the ultrasound application (Table 1).

However, the higher NH_4^+ -N and VFA load in PTR did not lead to a significant accumulation of these compounds inside the digester. Figure 5 shows the operating parameters of the AD with and without pretreatment and the stability of the process with both substrates. As previously mentioned, although the NH_4^+ -N concentration in

the sonicated sludge was 40% higher (1 mg/L) than in the raw sludge (0.6 mg/L), the parameters indicate that both reactors were able to buffer the input load. In terms of pH (Figure 5b), both reactors showed stability during the operational period at a 7.5 d SRT, with a slightly lower value for PTR compared to CR. Furthermore, the IA/TA ratios were lower for PTR compared to CR for the most part of the experiment, which could be associated with a higher presence of bicarbonate alkalinity in this system, as the concentration of VFAs was also slightly higher for this reactor compared to CR (0.86 ± 0.1 gCOD/L versus 0.76 ± 0.1 gCOD/L).



Figure 5. Behavior of the monitored operational parameters: (**a**) NH_4^+ -N, (**b**) pH, (**c**) alkalinity ratio (IA/TA), and (**d**) ORP. Data for the control reactor (**I**) and reactor with pretreatment (**I**) at solids retention times of 7.5 and 3 days.

However, and as previously discussed, from day 65 on, a decrease in the pH values in both reactors was observed, with a sharper trend in the case of PTR. In both reactors, the total VFA concentration was increased compared to the previous stage (Phase I), with values of 0.72 ± 0.1 gCOD/L and 1.04 ± 0.1 gCOD/L for CR and PTR during this stage, respectively, likely associated with the decreased capacity of the system to transform organic matter due to biomass washout. The ORP observed in both reactors during the entire operation was within the range commonly reported for stable anaerobic processes, which different authors situate between -200 mV and values below -300 mV [9,16,33].

Although the higher NH_4^+ -N and VFA loads in PTR due to the ultrasound pretreatment could have led to a detrimental effect over the functioning of the microbial consortia, this effect was not observed in the anaerobic digestion stability parameters, including the NH_4^+ -N, pH concentration, IA/TA ratio, and ORP. On the other hand, the ultrasound led to significant effects over the biogas yield and the overall performance of the process, even though the specific energy applied was lower than in many prior studies. For example, Cesaro and Belgiorno [34] obtained an increase in biogas production equivalent to 16% in a reactor fed with secondary ultrasonicated sludge with respect to a control reactor but using a specific energy of 15,000 kJ/kgTS.

The application of ultrasound as a pretreatment technology in a real-scale STP was also studied in the literature. Çelebi et al. [35] evaluated the effect of anaerobic digestion with or without ultrasound pretreatment on the fuel properties of sludge. The results of this study showed that the ultrasound pretreatment provided 32% more methane in a real STP. However, the energy consumption of this pretreatment overcomes the benefits of a higher methane yield. For this reason, more studies are necessary to optimize these processes and thus achieve favorable energy cost balances.

It is important to note that our study did not include an assessment of the microbial community structure of the system and the effects that different operational conditions have on them and that the operating period for each solids retention time was limited. Future studies should address these issues as well as evaluate the hypotheses arising from our results. Finally, the importance of assessing the economic aspects of the process is highlighted, considering the capital and operational costs of its implementation.

4. Conclusions

In this study, we evaluated the impact of ultrasound pretreatment and reduced retention times on the ammonia concentration and the overall performance of anaerobic digestion of sewage sludge. Our findings reveal that the ultrasound application led to significant improvements in methane yield and organic matter removal during the AD of mixed sludge. Specifically, during the initial phase of the experiment (SRT 7.5 days), the organic matter removal and methane production were approximately 22% and 30% higher in the pretreatment reactor (PTR) compared to the control reactor (CR).

However, a notable decline in organic matter removal efficiencies and methane production occurred when the solids retention time (SRT) was reduced to 3 days, primarily attributed to biomass washout effects. Despite a 40% increase in the ammonium load in PTR due to the pretreatment, the process exhibited no significant signs of instability during the 7.5-day SRT period.

Notably, during the final phase of the experiment, the washout effect resulting from further reducing the SRT led to an 89% decrease in organic matter removal efficiencies. This highlights the unfeasibility of operating under such conditions, even with the application of ultrasound pretreatment to the sludge.

Author Contributions: Conceptualization, M.O. and G.V.; methodology, M.O., P.N. and G.V.; software, G.G.; validation, M.O., P.N., G.G. and G.V.; formal analysis, M.O.; investigation, M.O.; resources, G.V.; data curation, M.O.; writing—original draft preparation, M.O., P.N., F.B., G.G. and G.V.; writing—review and editing, M.O., P.N., G.G. and G.V.; visualization, G.G.; supervision, G.V. and P.N.; project administration, G.V.; funding acquisition, G.V. All authors have read and agreed to the published version of the manuscript.

Funding: This research was funded by Agencia Nacional de Investigación y Desarrollo grant number ANID/FONDAP/15130015 and ANID/FONDAP/1523A0001.

Institutional Review Board Statement: Not applicable.

Informed Consent Statement: Not applicable.

Data Availability Statement: Data has been included in the present paper.

Conflicts of Interest: The authors declare no conflict of interest.

References

- 1. Mainardis, M.; Buttazzoni, M.; Gievers, F.; Vance, C.; Magnolo, F.; Murphy, F.; Goi, D. Life cycle assessment of sewage sludge pretreatment for biogas production: From laboratory tests to full-scale applicability. *J. Clean. Prod.* **2021**, 322, 129056. [CrossRef]
- Neumann, P.; Riquelme, C.; Cartes, C.; Kuschel-Otárola, M.; Hospido, A.; Vidal, G. Relevance of sludge management practices and substance modeling in LCA for decision-making: A case study in Chile. *J. Environ. Manag.* 2022, 324, 116357. [CrossRef] [PubMed]
- 3. Tchobanoglous, G.; Burton, F.; Stensel, D. Wastewater Engineering: Treatment and Reuse; McGraw-Hill: New York, NY, USA, 2004.
- 4. Chen, G.; van Loosdrecht, M.C.M.; Ekama, G.A.; Brdjanovic, D. *Biological Wastewater Treatment: Principles, Modelling and Design*, 2nd ed.; IWA Publishing: London, UK, 2023.
- Mikkelsen, L.H.; Keiding, K. Physico-chemical characteristics of full-scale sewage sludges with implications to dewatering. *Water Res.* 2002, *36*, 2451–2462. [CrossRef] [PubMed]
- 6. Nguyen, V.K.; Chaudhary, D.K.; Dahal, R.H.; Trinh, N.H.; Kim, J.; Chang, S.W.; Hong, Y.; La, D.D.; Nguyen, X.C.; Ngo, H.H.; et al. Review on pretreatment techniques to improve anaerobic digestion of sewage sludge. *Fuel* **2021**, *285*, 119105. [CrossRef]
- Neumann, P.; Barriga, F.; Álvarez, C.; González, Z.; Vidal, G. Process performance assessment of advanced anaerobic digestion of sewage sludge including sequential ultrasound-thermal (55 °C) pre-treatment. *Bioresour. Technol.* 2018, 262, 42–51. [CrossRef]
- Nielsen, P.H.; Thomsen, T.R.; Nielsen, J.L. Bacterial composition of activated sludge—Importance for floc and sludge properties. Water Sci. Technol. 2004, 49, 51–58. [CrossRef]
- 9. Appels, L.; Baeyens, J.; Degrève, J.; Dewil, R. Principles and potential of the anaerobic digestion of waste-activated sludge. *Prog. Energy Combust. Sci.* 2008, *34*, 755–781. [CrossRef]
- Liu, Y.; Li, X.; Kang, X.; Yuan, Y.; Du, M. Short chain fatty acids accumulation and microbial community succession during ultrasonic-pretreated sludge anaerobic fermentation process: Effect of alkaline adjustment. *Int. Biodeterior. Biodegrad.* 2014, 94, 128–133. [CrossRef]
- 11. Xue, Y.; Liu, H.; Chen, S.; Dichtl, N.; Dai, X.; Li, N. Effects of thermal hydrolysis on organic matter solubilization and anaerobic digestion of high solid sludge. *Chem. Eng. J.* 2015, 264, 174–180. [CrossRef]
- 12. Bhat, A.P.; Gogate, P.R. Cavitation-based pre-treatment of wastewater and waste sludge for improvement in the performance of biological processes: A review. J. Environ. Chem. Eng. 2021, 9, 104743. [CrossRef]
- 13. Khanal, S.K.; Grewell, D.; Sung, S.; Van Leeuwen, J. Ultrasound applications in wastewater sludge pretreatment: A review. *Crit. Rev. Environ. Sci. Technol.* 2007, *37*, 277–313. [CrossRef]
- 14. Carrère, H.; Dumas, C.; Battimelli, A.; Batstone, D.J.; Delgenes, J.P.; Steyer, J.P.; Ferrer, I. Pretreatment methods to improve sludge anaerobic degradability: A review. *J. Hazard. Mater.* **2010**, *183*, 1–15. [CrossRef] [PubMed]
- Neumann, P.; González, Z.; Vidal, G. Sequential ultrasound and low-temperature thermal pretreatment: Process optimization and influence on sewage sludge solubilization, enzyme activity and anaerobic digestion. *Bioresour. Technol.* 2017, 234, 178–187. [CrossRef] [PubMed]
- 16. Ariunbaatar, J.; Panico, A.; Esposito, G.; Pirozzi, F.; Lens, P.N.L. Pretreatment methods to enhance anaerobic digestion of organic solid waste. *Appl. Energy.* 2014, 123, 143–156. [CrossRef]
- 17. Cano, R.; Pérez-Elvira, S.I.; Fdz-Polanco, F. Energy feasibility study of sludge pretreatments: A review. *Appl. Energy.* 2015, 149, 176–185. [CrossRef]
- Pilli, S.; Bhunia, P.; Yan, S.; LeBlanc, R.J.; Tyagi, R.D.; Surampalli, R.Y. Ultrasonic pretreatment of sludge: A review. Ultrason. Sonochem. 2011, 18, 1–18. [CrossRef] [PubMed]
- 19. Pérez-Elvira, S.; Fdz-Polanco, M.; Plaza, D.; Garralón, G.; Fdz-Polanco, F. Ultrasound pre-treatment for anaerobic digestion improvement. *Water Sci. Technol.* 2009, *60*, 1525–1532. [CrossRef] [PubMed]
- 20. Chen, Y.; Cheng, J.J.; Creamer, K.S. Inhibition of anaerobic digestion process: A review. *Bioresour. Technol.* **2008**, *99*, 4044–4064. [CrossRef]
- 21. Procházka, J.; Dolejš, P.; MácA, J.; Dohányos, M. Stability and inhibition of anaerobic processes caused by insufficiency or excess of ammonia nitrogen. *Appl. Microbiol. Biotechnol.* **2012**, *93*, 439–447. [CrossRef]
- 22. Yenigün, O.; Demirel, B. Ammonia inhibition in anaerobic digestion: A review. Process Biochem. 2013, 48, 901–911. [CrossRef]
- 23. Sung, S.; Liu, T. Ammonia inhibition on thermophilic anaerobic digestion. *Chemosphere* 2003, 53, 43–52. [CrossRef] [PubMed]
- 24. Belmonte, M.; Hsieh, C.F.; Figueroa, C.; Campos, J.L.; Vidal, G. Effect of free ammonia nitrogen on the methanogenic activity of swine wastewater. *Electron. J. Biotechnol.* **2011**, *14*, 11. [CrossRef]
- 25. Rajagopal, R.; Massé, D.I.; Singh, G. A critical review on inhibition of anaerobic digestion process by excess ammonia. *Bioresour. Technol.* **2013**, *143*, 632–641. [CrossRef] [PubMed]
- 26. Veiga, M.C.; Soto, M.; Méndez, R.; Lema, J.M. A new device for measurement and control of gas production by bench scale anaerobic digesters. *Water Res.* **1990**, *24*, 1551–1554. [CrossRef]
- 27. El-Mashad, H.M.; Zeeman, G.; Van Loon, W.K.P.; Bot, G.P.A.; Lettinga, G. Effect of temperature and temperature fluctuation on thermophilic anaerobic digestion of cattle manure. *Bioresour. Technol.* **2004**, *95*, 191–201. [CrossRef]
- 28. APHA/AWWA/WEF. Standard Methods for the Examination of Water and Wastewater, 23rd ed.; American Public Health Association: Washingto, DC, USA, 2017.

- 29. Ruffino, B.; Campo, G.; Genon, G.; Lorenzi, E.; Novarino, D.; Scibilia, G.; Zanetti, M. Improvement of anaerobic digestion of sewage sludge in a wastewater treatment plant by means of mechanical and thermal pre-treatments: Performance, energy, and economical assessment. *Bioresour. Technol.* **2015**, *175*, 298–308. [CrossRef]
- 30. Amani, T.; Nosrati, M.; Sreekrishnan, T.R. Anaerobic digestion from the viewpoint of microbiological, chemical, and operational aspects—A review. *Environ. Res.* 2010, *18*, 255–278. [CrossRef]
- Braguglia, C.M.; Gianico, A.; Mininni, G. Comparison between ozone and ultrasound disintegration on sludge anaerobic digestion. *J. Environ. Manag.* 2012, 95, 139–143. [CrossRef]
- Dhar, B.P.; Nakhla, G.; Ray, M.B. Techno-economic evaluation of ultrasound and thermal pretreatments for enhanced anaerobic digestion of municipal waste activated sludge. *Waste Manag.* 2012, 32, 542–549. [CrossRef]
- 33. Gerardi, M.H. The Microbiology of Anaerobic Digesters; John Wiley and Sons: Hoboken, NJ, USA, 2003.
- Cesaro, A.; Belgiorno, V. Sonolysis and ozonation as pretreatment for anaerobic digestion of solid organic waste. Ultrason. Sonochem. 2013, 20, 931–936. [CrossRef]
- Çelebi, E.B.; Aksoy, A.; Sanin, F.D. Maximizing the energy potential of urban sludge treatment: An experimental study and a scenario-based energy analysis focusing on anaerobic digestion with ultrasound pretreatment and sludge combustion. *Energy* 2021, 221, 119876. [CrossRef]

Disclaimer/Publisher's Note: The statements, opinions and data contained in all publications are solely those of the individual author(s) and contributor(s) and not of MDPI and/or the editor(s). MDPI and/or the editor(s) disclaim responsibility for any injury to people or property resulting from any ideas, methods, instructions or products referred to in the content.