Effect of Assisted Ultrasonication and Ozone Pretreatments on Sludge Characteristics and Yield of Biogas Production

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Abstract: The effects of ultrasonic and ozonation pretreatments on organic solubilization, anaerobic biodegradability, and biogas production were elucidated in this study. Two pretreatment methods for batch anaerobic digestion for biogas recovery with the same material and experimental conditions were the focus for comparison. Anaerobic digestion experiments were conducted at ambient temperature with the solid retention time set to 25 days. The obtained results indicated that the soluble chemical oxygen demand increased from 0.344 without pretreatment to 1.023 and 1.228 g/L with ultrasound and ozone pretreatments, respectively, whereas the yields of biogas production in the 25 days increased by 32.3 and 52.9% via ultrasonic irradiation and ozonation relative to the control case, respectively. The biodegradability of the organic compounds of the samples for the cases of ultrasound, ozone pretreatments, and control achieved 55.9, 64.31, and 39.18%, respectively, in terms of chemical oxygen demand (COD) removal efficiencies. The physicochemical characteristics of sludge flocs were investigated via scanning with particle sizes, which were obviously affected by pretreatments of sonication and ozonation, resulting in finer particles than in the control case.

Keywords: sludge pretreatment; solubilization; biogas; ultrasonic; ozonation

1. Introduction

The waste sludge generated in the wastewater treatment process—which contains organic materials, toxic substances such as pathogens, heavy metals, and other contaminants—can cause environmental damage and affect public health. Furthermore, sludge treatment increases the total operating costs of the wastewater treatment plant (WWTP) by up to 50% [1]. Recently, two distinct fields of waste treatment and energy recovery are being closely connected. Finding new energy sources
that are cleaner, cheaper, and obtainable from alternative materials is a top priority. According to the estimation of the European Union (EU) policies, renewable energy consumption, accounting for at least 20% of the total energy demand, will have to be achieved by 2020 [2]. Kothari et al. [3] completed a review of the options available to overcome these situations from a worldwide viewpoint, which promotes energy production from different wastes, such as those derived from industrial, agricultural, and domestic sources. Depending on the technology used and kind of waste, there are many distinct methods that can be used to recover the energy. Munster and Lund [4] suggested that anaerobic digestion (AD) is one of the best alternative technologies for energy production from organic matter.

Anaerobic digestion is a well-known technology that is technologically simple, with low energy demand, and can be used to convert organic materials from many types of wastewater, solid wastes, and biomass into methane [5]. Biogas produced from AD process is mainly composed of 55–75 vol % methane (CH$_4$), which can be used as a fuel not only for heating and co-generation, but also for transportation. AD has several benefits: (1) converting the biomass and organic wastes to the environmental-friendly energy of CH$_4$, which can be stored and transported; (2) producing valuable fertilizer from the disposal sludges; and (3) reducing the environmental hazard of disposal sludge. This is why the AD-related processes and techniques have attracted such attention and are continuously being upgraded and optimized. In the AD process, four stages of digestion occur: hydrolysis, acidogenesis, acetogenesis, and methanogenesis, which are supported by the activities of endemic microorganisms [6]. However, AD is a slow process with a solids retention time (SRT) of around 20 to 30 days [1]. Hydrolysis of particulate organics in sludge is the rate-limiting step in sludge digestion in the anaerobic digestion process [7].

Pretreatment of sludge before the anaerobic digestion process is often applied to fracture the slowly biodegradable solids into easily biodegradable solids in sludge [8]. In previous studies, chemical (acid, alkali, catalyzed oxidation, etc.), physical (heating, irradiation, etc.), and biological pretreatment techniques have been investigated in sludge pretreatment. The goal of the pretreatment is to create appropriate conditions for digestion processes that increase the availability of consumption materials for microorganisms. The assistance provided by ultrasonic pretreatments was investigated by Riau et al. [9], Pili et al. [10], Braguglia et al. [11], Martin et al. [12], and Houtmeyers et al. [13]. The organic and produced biogas were increased by 20 (sludge disintegration degree (DD$_{COD}$) and 42%; 0.1 (soluble chemical oxygen demand ($S_{COD}$)/total chemical oxygen demand (COD)); 9 (DD$_{COD}$) and 35%; 81.5 (total organic carbon (TOC)) and 35%, and 1741 ($S_{COD}$) and 95%, respectively. Ozonation pretreatment methods also produced more biodegradable biogas, as shown in some previous work such as Weemaes et al. ($S_{COD}$ of 29%) [14], Beszedes et al. (biological oxygen demand (BOD$_5$)/COD increase 96%) [15], Cheng and Hong (DD$_{COD}$ of 18%) [16], and Kumar et al. ($S_{COD}$ of 18%) [17]. The produced biogas was used as the renewable energy source and supplemented for the input energy, which were presented by Pili et al. [10] and Braguglia et al. [11] at energy ratios of 1 and 0.8.

Herein, the main objective was to assess the biogas yield obtained from the AD of the waste sludge pretreated by ultrasound at the high frequency of 37 kHz and ozone generated from oxygen. The dosage and irradiation times of O$_3$ and ultrasound used in this study were 60 s and 20 min, while other studies needed a much longer reaction time [9–17] and higher dosage. Batch experiments were conducted to evaluate the influence of the pretreatment of digested sludge on the improvement in the biodegradability, if the digested sludge was digested again by anaerobic digestion. By examining the COD, $S_{COD}$, and volatile solid (VS) resulting after ultrasonic irradiation and/or ozonation, the mechanism of sludge disruption is investigated and discussed. The mainly biopolymers present in the sludge as proteins, cellulose, fats (lipid), and lignin are high-molecular compounds. The mechanism in this study was indicated to aid in decomposing high-molecular organic compounds into lower-molecular organic compounds, which could be easier consumed by microorganisms. The target digested sludge was obtained from a fishery wastewater treatment plant, which is typical in coastal countries like Vietnam.
2. Materials and Methods

2.1. Sludge Samples

Experimental sludge was collected from the thickening tank at a fishery wastewater treatment plant (WWTP) in Danang, Vietnam. The 20 L raw sludge, after primary settling and undergoing aerobic process, was collected for each experiment of this study. The characteristics of material, such as the COD, $S_{COD}$, total solid (TS), and VS of the sludge were examined.

2.2. Sludge Pretreatment Conditions

2.2.1. Ultrasonication Treatment

The ultrasonicator used was an Elmasonic S 300H from Elma, Singen, Germany, with an operating frequency of 37 kHz and supply power of 250 W. The sludge volume for pretreatment was 5 L, and it was treated with ultrasonic irradiation of 37 kHz for 20 min. During ultrasonication, the experimental temperature was maintained around 25–27 °C (by tap water) in order to avoid sludge hydrolysis by heating of ultrasonication (Figure 1).

![Figure 1. Scheme of sludge pretreatment by ultrasonication.](image)

2.2.2. Ozonation Treatment

Ozonation was performed with an ozone generator (Ozonia, LAB2B, Duebendorf, Switzerland) using 99% pure oxygen as a source to avoid the formation of NOx. To produce fine ozone bubbles, a stone diffuser was used. The ozonation system is illustrated in Figure 2. The experimental conditions applied were an ozone dose of 0.02 g O$_3$/g TS, gas flow rate of 0.5 L/min, controlled via flow meter (MFC5850E, Brooks, Hatfield, PA, USA), and ozone inlet concentration of 15 mg/L in 1 L sludge volume. Ozone concentrations in gas phase, before and after reaction with sludge, were measured during oxidation every 60 s with an ultraviolet (UV) analyzer (UV 245, Shimadzu, Kyoto, Japan), in order to calculate the amount of ozone that was transferred. The dosage and irradiation times of O$_3$ and ultrasound used in this study were 60 s and 20 min, while other studies needed a much longer reaction time [9–17] and higher dosage.
2.3. Anaerobic Digestion Assays

Anaerobic digestion (AD) tests were performed in 5 L batch reactors (AF-10-2, Miyamoto Co., Osaka, Japan) in a pilot-scale lab. Two reactors were placed in a water bath at ambient temperature under a stirring speed of 20 rpm (Figure 3). The pretreated sludges were filled in reactors with 5 L volume. To simulate anaerobic conditions with minimized oxygen gas, pure nitrogen gas was flushed twice into reactors for 2-3 min each time before starting the AD test. The yield of biogas was quantified by liquid displacement in a column filled with a dissolved saline solution to minimize biogas dissolution. The biogas volume was measured every day, whereas COD and VS were monitored every 5 days. After each AD test, the sludge was exposed for 25 days at room temperature of 28–35 °C.

Figure 2. Flow chart of sludge pretreatment by ozonation.

Figure 3. Flow chart of anaerobic digestion pilot—AF-10-2. Note: ① reactor, ② biogas container; ③ control panel, ④ water bath; ⑤ motors, ⑥ temperature sensor, ⑦ pressure meter, and ⑧ gas valves.
2.4. Analytical Methods

Volatile solid and soluble chemical oxygen demand were measured according to Standard Methods 20th edition [18]. The term solubilization represents the transfer from the particulate fraction of the sludge to the soluble fraction of the sludge. These two fractions were obtained by centrifugation of the samples at 6000 rpm for 15 min, using a Universal 320 (Hettich Zentrifugen, Tuttlingen, Germany). The retained particulate fraction after centrifugation was then dried to produce the total solids. Chemical oxygen demand was analyzed following SMEWW 5220C:2012. The flocs structure was scanned by a field-emission scanning electron microscope (FE-SEM) S4800, Hitachi, Japan, following the method of Goldstein et al. [19].

2.5. Statistical Analysis

All the analyses were performed three times in order to assess the precision and data errors. The results are presented as mean values with the standard deviation. All data in this study were created in the graphs via the Microsoft Excel software (Microsoft Office version 2010); the values performed the statistical analysis and fitted the experimental data, showing accurately the tendency lines of the experiment in this work.

3. Results and Discussion

3.1. Effect of Pretreatments on Characteristics of Sludge

The sludge characteristics of the control and pretreated samples were analyzed with regard to the COD, SCOD, TS, and VS. The data are listed in Table 1. During the sonication and ozone pretreatments, the COD, TS, and VS remained almost constant, although slightly changed versus the control. Similar trend variations in COD and TS were also observed by Bougrier and colleagues [20,21] and Shao et al. [22]. However, the SCOD significantly increased when sludge was pretreated with ultrasound and ozone, increasing from 0.344 g/L in raw sludge to 1.023 g/L and 1.228 g/L, respectively. These increasing effects on SCOD were also reported by Bougrier et al. [20], Shao et al. [22] and Chu et al. [23]. Although these previous works reported the effects of pretreatment processes, the best process was not identified. Furthermore, Zhen et al. [24] noticed that sludge from various sources with different operation conditions may exhibit different characteristics. They thus indicated the necessity to propose standardization to enable assessment and comparison. The characteristics of sludge shown in Table 1 indicate high COD concentration and ratios of VS/TS of about 64, 70, and 66% for initial, ultrasound, and ozone-treated sludges, respectively. The results demonstrate that the substrate of the three sludges was mainly composed of organic matter that is appropriate for microorganisms to use in AD.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Unit</th>
<th>Study Control $^a$</th>
<th>Ultrasonication</th>
<th>Ozonation</th>
<th>Reference $^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>SCOD</td>
<td>g/L</td>
<td>0.344 ± 0.05</td>
<td>1.023 ± 0.08</td>
<td>1.228 ± 0.06</td>
<td>[12] 0.08</td>
</tr>
<tr>
<td>TS</td>
<td>g/L</td>
<td>174.1 ± 1.11</td>
<td>158.2 ± 1.76</td>
<td>164.0 ± 1.45</td>
<td>[13] 23</td>
</tr>
<tr>
<td>VS</td>
<td>g/L</td>
<td>111.33 ± 1.23</td>
<td>112.0 ± 1.34</td>
<td>109.23 ± 1.88</td>
<td>13.5</td>
</tr>
</tbody>
</table>

$^a$: Before pretreatment (i.e., without pretreatment); $^b$: The reference of raw sludge from municipal wastewater. COD: chemical oxygen demand. SCOD: soluble chemical oxygen demand. TS: total solid. VS: volatile solid.

The assistance of ultrasound and ozonation for pretreatment could enhance the COD, TS, and VS, as indicated in previous works of [9–17]. If a high-energy dosage is used for pretreatment, the TS and VS will become destructed sludge flocs and the insoluble organics will transform into soluble form. The SE dosed in this work induced TS, increased SCOD, and VS showed negligible change. However, the authors intended to reduce the SE used in this study for energy balances. Due to strong oxidation
of O₃, it was easy to break the cell wall of the flocs and release the substrate, so that \(S_{\text{COD}}\) content was higher than ultrasonic pretreatment in this study.

### 3.2. COD and Biopolymers Solubilisation

The concentration of soluble biopolymers also increased significantly after ultrasonic and ozonation pretreatments (Table 1), by 197.38\% \((1.023–0.344)/0.344\) and 256.98\% \((1.228–0.344)/0.344\) of \(S_{\text{COD}}\), respectively, relative to the control case. These results indicate the interactions induced by the ultrasonic and ozone pretreatments enhanced the formation of more soluble biodegradable substrates from the waste sludge. This was possible because ultrasound can break down longer biopolymers to shorter ones that can be more easily biodegraded. Ozone was not only able to solubilize organics, but also converted nonbiodegradable solids into biodegradable ones.

Table 2 indicates that VS and \(S_{\text{COD}}\) removal efficiencies of the control case changed slightly after 10 days, demonstrating that the hydrolytic bacteria already used the soluble COD and VS for biogas production. However, the hydrolytic bacteria for the pretreated cases used the substrate until the 25th day for anaerobic digestion.

### Table 2. Chemical oxygen demand (COD) and volatile solids (VS) removal efficiencies of digested sludge by anaerobic digestion (AD) every five days over the experimental period (25 days).

<table>
<thead>
<tr>
<th>Retention Time (Days)</th>
<th>VS Removal Efficiency (%)</th>
<th>COD Removal Efficiency (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without Pretreatment</td>
<td>With Ultrasound</td>
</tr>
<tr>
<td></td>
<td>Without Pretreatment</td>
<td>With Ultrasound</td>
</tr>
<tr>
<td>5</td>
<td>2.52</td>
<td>8.34</td>
</tr>
<tr>
<td>10</td>
<td>18.85</td>
<td>29.34</td>
</tr>
<tr>
<td>15</td>
<td>41.43</td>
<td>45.89</td>
</tr>
<tr>
<td>20</td>
<td>42.60</td>
<td>55.81</td>
</tr>
<tr>
<td>25</td>
<td>43.15</td>
<td>58.21</td>
</tr>
</tbody>
</table>

The above enhancement effects were similar to those reported by Kim et al. [25], when alkali addition with ultrasound on sewage sludge disintegration induced synergistic COD solubilization. Tian et al. [26] reported that using an individual ultrasonic pretreatment method resulted in an increase of 20.7\% biodegradability in sewage sludge. Salihu and Alam [27] illustrated the optimal doses of ozone as 0.05 to 0.5 g O₃/g TS for sludge (organic wastes) solubilization. Ozone doses from 0.06 to 0.16 g O₃/g TS were examined by Chu et al. [23], applying a microbubble ozonation process that improved sludge solubilization efficiency by 15–30\%. Moreover, complete mineralization occurred when the ozone doses applied were too high [28], resulting in loss of methane production. Thus, this study used the ozone dose based on the concentration range of Salihu and Alam [27].

### 3.3. Biogas Production

The digested sludge without and with ultrasonication and ozonation pretreatments was anaerobically digested in AD reactors to determine the effect of pretreatment methods on biogas yield. Throughout the anaerobic digestion assay, the biogas production was recorded every day during 25 days of digestion.

#### 3.3.1. Daily Biogas Production from Anaerobic Digestion of Digested Sludges Pretreated by Ultrasound and Ozonation

Figure 4 shows the daily biogas production from anaerobic digestion of digested sludge without and with ultrasound and ozonation pretreatments. It illustrates that the evolution of the daily biogas production during the period of anaerobic digestion could be divided into several stages. At the beginning, due to the adaptation of bacteria to the substrate in the digested sludge, little biogas was produced in the first four days. The biogas production rate (biogas yield/day, RBG) was higher during
around the 5th to the 11th day. Afterward, the RBG started to decrease slightly until the 18th day. This dramatic drop was caused by the depletion of the substrate. The biogas production rates of the control case were generally lower than those of the pretreated cases. Moreover, it dropped below 2 L/d after the 13th day for the control case. This can be attributed to the gradual depletion of the soluble organics in sludge that were directly detrimental to the anaerobic microorganisms in the system.

![Figure 4](image_url)  
**Figure 4.** The daily biogas productions from anaerobic digestion of digested sludges pretreated by ultrasound and ozonation.

### 3.3.2. Cumulative Biogas Production from Anaerobic Digestion of Digested Sludge Pretreated by Ultrasound and Ozonation

The cumulative biogas production from anaerobic digestion of digested sludge both with and without ultrasound and ozonation pretreatments is shown in Figure 5. Biogas production significantly improved with the pretreatments. After 25 days, the assisted ultrasonication and ozonation pretreatment increased biogas yields 32.3% and 52.9% relative to control case, respectively. The enhancement effect on biogas yield by pre-ozonation was much higher than that by pre-ultrasonication, with a relative increase of 20.6%. The improvement in biogas yields occurred because more soluble organics were released under ultrasonication and ozonation pretreatments in comparison with the case without pretreatment. Wang et al. [29] reported methane production from waste-activated sludge with ultrasonic pretreatment increasing 64% compared with untreated sludge. Lafitte-Trouque and Forster [30] demonstrated that biogas production rates from AD of ultrasound pretreated waste-activated sludge were higher than those of untreated sludge. The study of Tian et al. [26] indicated that methane production from pretreated sewage sludge increased around 10% with the same energy input of 9 kJ/g TS and applying ultrasound at 20 kHz. Tian et al. [5] also reported an enhancement of methane production of 28.3% from sewage sludge pretreated by ultrasound. The batch tests results showed pre-ozonation was able to improve biodegradability of sludge better than ultrasonic pretreatment.

The results from this work were further compared with previous studies on sewage treatment using thermal pretreatments at medium (60–80 °C) and high (130–170 °C) temperatures and high pressure (up to 21 bar), which resulted in a biogas production increase in the range of 30 to 80% [31]. The produced biogas of 54% at 90 °C and 50% at 200 °C was reported by Campo et al. [32] and Ortega et al. [33]. Takashima and Tanaka [34] reported the improvement in methane production of 130% to 200% when thermal pretreatment at 120 °C was applied to sewage sludge. Although the conditions in the pretreatment processes and target sludges were different, the results of this study on the enhancement effects of pretreatments on biogas production were similar to or even better than those of previous studies. Boni et al. [35] used 50,000 kJ/kg TS, which resulted in a biogas gain of only 30%. Resulting from Figures 4 and 5, ozonation pretreatment greatly increased the ultimate yield of biogas versus ultrasonication and control experiments. On the seventh experimental day, the highest biogas production increased by 0.12 L/g COD for ozone, and 0.11 and 0.8 L/g COD for ultrasound and
control, respectively. Meanwhile, the ultimate yield increased by 1.32 L/g COD (ozone), 1.14 L/g COD (ultrasound), and 0.86 L/g COD (control). Both O₃ and ultrasound pretreatment are employed in real scale and have obtained the achievement of increasing biogas yield by around 35%, sludge discharge, and dewater reduction [36].

![Figure 5](image_url)

**Figure 5.** The cumulative biogas productions from anaerobic digestion of digested sludges pretreated by ultrasound and ozonation.

### 3.4. COD and VS Removal Efficiencies

Protein and carbohydrates were the two main components of COD in sludge. During microbial activities, organic matter in the sludge was used to produce biogas in the anaerobic digestion process. As shown in Table 2, COD and VS removal efficiencies using pretreated sludge in AD were generally higher than those using untreated sludge. On the 25th day, the COD removal efficiencies reached 39.18, 55.9, and 64.31% in the control, pre-ultrasonication, and pre-ozonation cases, respectively. In a study of the AD of pre-ultrasound treated sludge, Shao et al. [22] reported a COD removal efficiency of 25% at 10 days, which, however, is lower than that of 27.47% achieved in this study. Further concerning the organic removal rate of the anaerobic digestion process, Tian et al. [5] and Chu et al. [37] noticed that the rates in the first 10 experimental days were higher than those in the remaining retention times. Table 2 indicates that the COD removal efficiencies of un-pretreated, ultrasound, and ozonation pretreated cases were 27.87, 27.47, and 27.80% in the first 10 days, which were higher than 2.18 (39.18 – 37), 14.31 (= 55.9 – 41.59), and 21.15% (64.31 – 43.16) during days 16 to 25, respectively. The trends thus were consistent with those of Tian et al. [5] (20 days) and Chu et al. [37] (30 days). Regarding the VS removal efficiency, the results of this work showed prominent performance, with 58.21 and 61.06% for pretreatments with ultrasound and ozonation, respectively, versus 43.15% without pretreatment. Pre-ozonation was among the three cases compared.

### 3.5. Microstructure Based on SEM

In this study, floc structures and particle diameters of pretreated and control sludge samples were examined. All the images are shown in Figure 6. The particle size of untreated sludge (Figure 6a) was larger than that of ultrasound and ozone pretreated sludges. The sludge particles after ultrasonic irradiation at 37 kHz (Figure 6c) were rougher than after ozonation (Figure 6b), as determined using a microscope scale of 2 μm. The pretreatments resulted in the relative decrease in the particle sizes of sludge of 64.9% (from 45.3 μm to 15.9 μm). However, Li et al. [38] reported that the median diameter of sludge pretreated with an ozone dose of 0.012 g O₃/g TS slightly decreased from 45.3 μm to 43.1 μm, or even increased after the reflocculating process occurred. The study of Tian et al. [5] indicated that the particle size after pretreatment of ozone versus ultrasound demonstrated a negligible change but was still finer than without pretreatment. Nevertheless, the results of the effects of pretreatments on inducing the changes of physicochemical characteristics of sludge and the content of SCOD were
deduced from experiments at 37 kHz ultrasonic irradiation and ozone dose of 0.02 g O₃/g TS, which were different from the conditions used in other studies. The effects of pretreatments depend upon a series of sludge factors, such as COD, TS, VS, experimental time, and so on. The results and conclusions would be dependent on testing conditions.

**Figure 6.** The images from a scanning microscope for the floc structures of (a) untreated, (b) ozone, and (c) ultrasound pretreated sludge samples.
4. Conclusions

This study demonstrated that ultrasound- and ozone-assisted sludge pretreatments can enhance anaerobic biodegradability and biogas production. The soluble COD of sludge increased by 197.38% via ultrasonication and by 256.97% via ozonation, relative to those without pretreatment. After 25 days of anaerobic digestion, ultrasonic and ozone pretreatments prominently increased the yield of biogas production by 32.3% and 52.9%, respectively, in comparison with control case. The COD and VS removal efficiencies after 25 days anaerobic digestion with ozone pretreatment significantly improved by 64.31% and 61.06%, whereas the un-pretreated case only eliminated 39.18% and 43.15%, respectively. The results of this work showed that the soluble COD and ultimate yield of biogas using ozone pretreatment were better than those of ultrasonic pretreatment and the control.


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