Improve bio-activity of anaerobic sludge by low energy ultrasound

Yichun Zhu, Xin Li, Maoan Du, Zuwen Liu, Hui Luo and Tao Zhang

ABSTRACT

This research focused on ultrasound-enhanced bio-activity of anaerobic sludge. Low energy ultrasound irradiation can increase the bio-activity of anaerobic sludge. Ultrasonic parameter, characteristics of anaerobic sludge and experimental conditions are important parameters which affect the enhancement effect on anaerobic sludge. In order to assess the effects of characteristics of anaerobic sludge and experimental conditions on ultrasonic irradiation of anaerobic sludge, experiments with different characteristics of anaerobic sludge were carried out and analyzed with the content of coenzyme F420 and dehydrogenase activity (DHA). The results showed that anaerobic sludge bio-activity was impacted by the initial temperature, initial chemical oxygen demand (COD), sludge concentration, and stirring during the ultrasonic process. Optimal performance was achieved when sound frequency, power density, and ultrasonic irradiation period was 20 kHz, 0.1 W/mL, and 10 min, respectively, under which the wastewater COD removal efficiency was increased by 12.9 percentage points. The results indicated that low temperature could affect the anaerobic sludge irradiation effect, while intermittent stirring could enhance the bio-activity of anaerobic sludge irradiation effect and low substrate concentration improved anaerobic sludge activity by ultrasound.

Key words | anaerobic sludge, coenzyme F420, dehydrogenase activity, organic loading rate, sludge concentration, ultrasonic irradiation

INTRODUCTION

Low energy ultrasound can promote biological metabolism (Barton et al. 1996; Tiehm et al. 1997); the mechanism includes: promoting mass transfer (Prausnitz et al. 1993; Lin & Wu 2002), increasing enzyme activity (Sakakibara et al. 1996; Wu & Lin 2002) and accelerating cell growth (Dai et al. 2005; Liu et al. 2005). With appropriate energy, ultrasonic vibration with special energy can lead to the motion of cellular matter in activated sludge and circular exchange of material in and outside the cells. As a sequence, permeability of the cell membrane increases, and then the contact possibility between enzymes and substrate increases, mass transfer of pollutants from the liquid phase into the cell wall accelerates, and synthesis rate of protein and microbial activity will increase as well.

Ultrasound irradiation under appropriate parameters can increase sludge bio-activity and enhance biological treatment efficiency of sludge. Owing to the complexity and diversity of flora in activated sludge, ultrasonic operation parameters are different for activated sludge with different properties. However, organic degradation efficiency differs considerably based on these parameters, and ultrasonic pretreatment using inappropriate parameters can even inhibit sludge metabolism. There are many factors influencing the treatment performance of ultrasonic irradiation, and the irradiation effects are different for sludge in different growth periods. Therefore, it is necessary to investigate how the ultrasonic enhancement effect is influenced by the sludge properties.

Research on the use of low energy ultrasonic irradiation technology to treat wastewater is still in its initial stage. Previous studies included: ultrasound parameter optimization, promotion mechanisms analysis and factors influencing the ultrasonic enhancement process. Schläfer et al. (2000, 2002) used ultrasonic irradiation aerobic mixtures at a frequency of 25 kHz and an input energy density of 0.3 W/L throughout the wastewater treatment process. The results showed that low intensity ultrasound both promoted and inhibited the biological treatment of wastewater. Some
optimization experiments showed that the optimal ultrasound parameters were 0.2 W/cm² and 10 min where the total phosphorus concentration in the effluent was 35–50% lower than that of the control group (without ultrasonic irradiation) (Xie et al. 2008). Duan et al. (2011) studied the effect of low intensity ultrasound with a frequency of 25 kHz and ultrasonic intensity of 0.3 W/cm², demonstrating that continuous irradiation for 4 min can increase the total nitrogen removal efficiency by anaerobic ammonia oxidation by approximately 25.5%. Single-factor and multiple-factor optimization experiments showed that the optimal ultrasonic intensity and irradiation period were 0.2 W/cm² and 10 min, respectively, and the anaerobic sludge biological activity was enhanced dramatically under the optimal condition. The COD removal efficiency was increased by ultrasonic treatment and the effluent COD was 30% lower than that of the control group (without exposure) (Xie et al. 2009). Weak ultrasound (0.33 W/mL, 20 min, 20 kHz) significantly enhanced the anaerobic methane production of both original and flocculated biosolids, without fully destroying the floc structure. Confocal laser scanning microscope images indicated that the flocculated/sonicated biofloc exhibited a looser structure than non-flocculated/sonicated biofloc and thus achieved a higher methane production rate and accumulated yield (Chu et al. 2002).

Up to now, low energy ultrasound enhanced wastewater treatment studies have mainly concentrated on ultrasonic irradiation for aerobic biological treatment processes (Schläfer et al. 2000, 2002; Xie et al. 2008; Zhang et al. 2008; Zhou et al. 2014). There are few studies about the enhancement effect and influential factors for ultrasound affecting anaerobic biological treatment (Chu et al. 2002; Xie et al. 2009; Zhu et al. 2014). With the development of third generation anaerobic technologies, especially hybrid anaerobic reaction technologies, these treatment methods have drawn more and more attention. Anaerobic treatment is popular in treating high concentration industrial wastewater (Pérez et al. 1999), and has also been applied to municipal wastewater treatment for organics removal or pretreatment (Lettinga et al. 1993; Lew et al. 2009; Ayaz et al. 2011; Reyes et al. 2011; Song & Gao 2013). The problems for anaerobic treatment of low concentration wastewater are mainly restricted to low mass transfer rate and microbial metabolism rates at ambient temperature. It is noted that low intensity ultrasonic irradiation can compensate for the above disadvantages, and significant advantages in enhancing the anaerobic treatment process for low concentration wastewater can be achieved. Our previous research suggested that, when frequency, input sound density and ultrasonic irradiation time were 20 kHz, 0.1 W/mL and 10 min respectively, the enhancement effect for anaerobic sludge activity was appreciable (Zhu et al. 2014).

In this experiment, the content of coenzyme F₄₂₀ and dehydrogenase activity (DHA) were used to evaluate the anaerobic sludge activity. The influence of initial COD, sludge concentration, initial temperature and agitation condition on enhanced bio-activity effect of ultrasound irradiation for anaerobic sludge was investigated. The purpose of this study is to reveal the principles for enhancing anaerobic sludge activity by ultrasound irradiation. It is expected that the research results can provide referenced data for anaerobic wastewater treatment enhanced by ultrasound.

MATERIALS AND METHODS

Ultrasound apparatus

The ultrasonic apparatus used in this study was a JY88-IIN probe ultrasonic generator (Xinzhi ultrasonic cell grinder, Ningbo, China), with a frequency of 20 kHz and variable powers from 0 to 250 W. Diameter of the probe was 6 mm, and the ultrasonic irradiation mode was intermittent. During sludge irradiation, the probe was immersed in the sludge at a depth of 10 mm. The reactor was a 150 mL glass beaker. During the test, the sludge was placed directly in the beaker, and the reactor surface was covered with plastic films (Figure 1).
Experimental materials

Anaerobic sludge samples were obtained from an anaerobic baffled reactor (ABR). The influent COD of the sewage in the ABR was controlled at 500–600 mg/L, nitrogen and phosphorus were provided by ammonium chloride and sodium hydrogen phosphate, making biochemical oxygen demand (BOD):N:P = 200:5:1, and by adding the right amount of NaHCO₃ to control water alkalinity at 500 mg/L or more (Zhu et al. 2014). Properties of the anaerobic sludge after treatment were as follows: the moisture content of sludge was 98%; the pH was 6.5–7.5; MLSS was 20 g/L. The ratio of volatile solids (VS) was approximately 60%.

Experimental procedures

Based on the research group's previous results, ultrasonic irradiation enhancement tests were carried out under a power input of 0.1 W/mL and an irradiation period of 10 min. For each test, the sludge of volume 100 mL was placed in a 150 mL glass beaker, and the pH was controlled at approximately 7. The probe was 1 cm beneath the liquid's surface. Different kinds of sludge were exposed to the ultrasonic irradiation with the same irradiation period of 10 min.

Effects of sludge on biological activity by ultrasound

When we studied the effect of initial temperature of sludge on biological activity by ultrasound, anaerobic sludge samples were controlled at 20 g/L, and the temperature was controlled at 20 °C. Synthetic wastewaters with different COD concentrations were added into Erlenmeyer flasks; samples were then cultivated in a shaking table at a constant temperature to keep the temperature in the flasks at 30 ± 2 °C for 4 hours. After the ultrasonic irradiation conditions described above, the content of coenzyme F₄₂₀ and sludge DHA were measured before and after the irradiation treatment. Table 1 shows the COD concentration added into the Erlenmeyer flasks. The anaerobic sludge was controlled at 20 g/L, and the temperature was controlled at 20 °C. Ultrasonic irradiation treatments were carried out with no stirring, continuous stirring and intermittent stirring. The intermittent stirring cycle was 2 min, and slow stirring was carried out five times in each cycle (Sakakibara et al. 1996). After the ultrasonic irradiation under the conditions described above, the content of coenzyme F₄₂₀ and sludge DHA were measured before and after the irradiation treatment.

Finally the anaerobic sludge was controlled at 20 g/L, and the temperature was controlled at 20 °C. The initial COD before sludge sampling was 600 mg/L. Intermittent stirring was applied during the ultrasonic process. After the ultrasonic irradiation under the conditions described above, the sludge was put into a sealed bottle prefilled with nutrient liquid (BOD₅:N:P = 200:5:1), and then cultured in a shaking table at a constant temperature to keep the temperature in the bottle at 30 ± 2 °C. The sealed bottle volume was 300 mL, and the sludge concentration was 6.7 g/L. After 8 hours, the COD value was measured.

<table>
<thead>
<tr>
<th>No.</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD (mg/L)</td>
<td>200</td>
<td>600</td>
<td>1,000</td>
<td>2,000</td>
<td>5,000</td>
</tr>
</tbody>
</table>

When we studied the effect of total solids (TS) concentration on biological activity by ultrasound, the temperature of the anaerobic sludge samples was controlled at 20 °C. The sludge was condensed by gravity method and approximately 40 g/L of concentrated sludge was obtained. Then, the samples were processed with distilled water to prepare 5, 10, 15, 20, 25, 30 and 40 g/L individual samples. After the ultrasonic irradiation under the conditions described above, the content of coenzyme F₄₂₀ and sludge DHA were measured before and after the irradiation treatment.
Parameter analysis

The sludge DHA test was carried out by the spectrophotometric method using 2,3,5-triphenyltetrazolium chloride (TTC) (Yang et al. 2002; Xie et al. 2009). TTC is used as a hydrogen receiver in cell respiration, and the reduced TTC forms a substance named triphenyl formazan (TF), whose reddish color is proportional to its concentration and can be measured colorimetrically. The following materials and reagents were added to two sets of centrifuge tubes (20 mL): 0.5 mL 0.36% Na₂SO₃, 1.5 mL Tris-HCl buffer, 2.0 mL anaerobic sludge suspension, 0.5 mL 0.4% TTC and 1.0 mL synthetic sewage (COD = 4,000 mg/L). After shaking, the sample tubes were placed into a black hopper and set in a water-bath boiler at a constant temperature (37 °C) immediately. All samples were shaken slightly for reaction time of 60 min. A drop of oil of vitriol was added into sample tubes to end the reaction, and 5 mL ethyl acetate was supplemented to sample tubes separately. All samples were mixed thoroughly and extracted for 6 min (90 °C), then centrifuged at 4,000 rpm for 10 min. The supernatants of the samples were colorimetrically measured at 480 nm, and the absorbance was obtained. Then the absorbency was used to compare with the standard curve and the DHA of samples were calculated. One unit of DHA is defined as the activity catalyzing the reduction of 1 mg of TF per hour. The specific DHA is the DHA measured per gram mixed liquor volatile suspended solids (VSS). Coenzyme F₄₂₀ was tested using the ultraviolet spectrophotometry method (Wu et al. 1986; Xie et al. 2009). COD determinations were done according to *Standard Methods for the Examination of Water and Wastewater* (APHA et al. 1998).

RESULTS AND DISCUSSION

The effect of initial temperature of sludge on biological activity by ultrasound

*Figure 2* shows the effect of initial temperature on anaerobic sludge activity when enhanced by ultrasonic irradiation. As the figure shows, when the initial temperature of sludge was 0–10 °C, as the temperature increased, DHA was enhanced significantly. When the initial temperature of sludge was 10–20 °C, DHA slowed down and no longer changed with the temperature. DHA was highest in this temperature range. After the temperature rose to 40 °C, DHA then decreased. These results indicate that, when the temperature was low, the sludge viscosity was high, which prevented parts of the sludge from receiving effective ultrasonic irradiation. Moreover, the cavitation threshold of sludge at the low temperature was high, inhibiting ultrasonic cavitation to some extent (Niemczewski 1999). As the temperature gradually increased, the effect was weakened. When the temperature increased to 20 °C, the cavitation threshold of the sludge decreased and the energy released from cavitation increased (Wang et al. 2012), so DHA was significantly enhanced. However, after reaching 20 °C, the increase of the temperature no longer obviously enhanced DHA. This was primarily because the effective irradiation could be achieved in the glass beaker after the temperature reached 20 °C. The ultrasonic irradiation process itself would produce a small temperature rise, and, after reaching 20 °C, increase of the temperature no longer had an obvious impact. When the temperature increased to 40 °C, DHA then declined. It might be related to cavitation at the steady-state leading to the boundary layer for mass transfer to be thin, which accelerated the motion of the solute particles, and improved the mass transfer and diffusion rate for the reactant entering the active site of the enzyme or cell and the products entering the liquid phase. As the temperature rose, the sludge cavitation threshold was lowered, converting the stable cavitation into a transient cavitation. The resulting high temperature and high pressure caused the formation of free radicals and the generation of a powerful shock wave and jet. As a result, cell structures and enzymes activity were partially destroyed, and the DHA reduced (Pitt & Ross 2005).

*Figure 2* | DHA and F₄₂₀ changes with temperature by ultrasound.

Variations of coenzyme F₄₂₀ were the same as DHA. However, when the temperature was 20–50 °C, the coenzyme F₄₂₀ increased with the increase of temperature. After reaching 40 °C, coenzyme F₄₂₀ concentration also remained at a relatively high level, because the temperature
sensitivity of methane bacteria was higher than that of the anaerobic acidification bacterium. As the temperature increased, its growth rate increased gradually, reaching the maximum value at the optimum temperature range. As such, coenzyme F₄₂₀ increased with the increase of temperature. Meanwhile, a different study showed that different ultrasonic conditions can activate or inactivate an enzyme’s catalytic effect, and different enzymes may perform differently in the same ultrasonic conditions (Wu & Lin 2002).

The effect of total solids concentration on biological activity by ultrasound

Figure 3 shows the effect of TS concentration on the ultrasonic enhancement. This figure indicates that as the sludge concentration increased, coenzyme F₄₂₀ decreased, but the decrease rate slowed down gradually. When the sludge concentration was 5 g/L, the concentration of coenzyme F₄₂₀ increased by 100%, and the input ultrasonic specific energy consumption was 12 kJ/g TS₀. When the sludge concentration was 20 g/L, the concentration of coenzyme F₄₂₀ increased by 54.55%, with the input ultrasonic specific energy consumption of 3 kJ/g TS₀. When the sludge concentration was 40 g/L, the concentration of coenzyme F₄₂₀ increased by 40.91%, and the input ultrasonic specific energy consumption was only 1.5 kJ/g TS₀. When the sludge concentration was 5–20 g/L, coenzyme F₄₂₀ decreased rapidly as the sludge concentration increased. When the sludge concentration was higher than 20 g/L, the coenzyme F₄₂₀ still decreased as sludge concentration increased, but the decreasing slowed down greatly.

These results are mainly influenced by ultrasonic cavitation. The cavitation effects under the same ultrasound conditions were similar. When the sludge concentration was relatively low, the cavitation threshold was also low, facilitating the cavitation phenomenon. When the sludge concentration was low, the force acting on sludge per unit volume was large, so F₄₂₀ value was high. Moreover, when the specific energy consumption of ultrasound irradiation was high, parts of the cells were killed, and the substance inside the cell dissolved and was released outside. Macromolecules were disintegrated and then used by archaea, promoting the metabolism of methanogenic archaea. With the increase of soluble organics and cell activity, the speed of anaerobic fermentation accelerated greatly (Neis et al. 2000; Chu et al. 2002). As the sludge concentration increased, the ultrasound utilization per unit of energy consumption increased, leading to decrease of ultrasonic energy consumption and the force acting on sludge per unit volume. Meanwhile, the increased sludge concentration also increased sludge viscosity and reduced liquidity. Given that only parts of the organism can be effectively irradiated, the growth rate of the coenzyme F₄₂₀ decreased as the increase of sludge concentration. This is consistent with the law of energy conservation.

The change in DHA is just the opposite of coenzyme F₄₂₀. As the sludge concentration increased, the DHA increased gradually. When the sludge concentration was between 5 and 20 g/L, DHA increased slowly with the increase of sludge concentration; when the sludge concentration increased from 20 to 30 g/L, DHA increased significantly. The maximum DHA was achieved when the sludge concentration was 30 g/L. After that, DHA decreased with the increase of sludge concentration. This may be ascribed to the kind of biological reaction that took place, and there is an optimal ultrasonic intensity and irradiation time. Inappropriate intensity and irradiation time cannot increase sludge activity (Liu et al. 2003). For this experiment, ultrasonic parameters were optimized at a sludge concentration of 25 g/L. Therefore, it is assumed that the sludge concentration most suitable for the optimized parameters were 25–30 g/L.

Lin & Wu (2002) found that different enzymes performed differently in the same ultrasonic conditions, which could explain the different trends of coenzyme F₄₂₀ and DHA. Schläfer et al. (2000) also noted that activated sludge was slurry composed of a variety of organisms, and organic and inorganic substances. As such, the concentration, type, physiological status, and the biological structure of the sludge can also influence the effect of ultrasonic irradiation. This makes the optimized parameters for the ultrasound narrow in application.
Effect of initial COD on biological activity by ultrasound

Figure 4 shows how ultrasonic irradiation promotes anaerobic sludge activity at different initial COD concentrations. The figure shows that as initial COD increases, the increased percentage of coenzyme F420 declines. When the initial COD value is higher than 2,000 mg/L, the percentage increase of coenzyme F420 decreases slowly with the increase of initial COD. The DHA trend is similar to coenzyme F420, but the percentage increase of DHA follows a rising trend when initial COD is greater than 1,000 mg/L. In the case of low organic loading, microorganisms experience lack of substrate and the initial activity is low. As such, ultrasound irradiation can cause sludge floc breakage (Salsabil et al. 2009), leading to release of extrapolymeric substances from the sludge, and an increase of protein and nucleic acid in the wastewater. This increases the substrate consumption for the solution. At the same time, as the enzyme activity increases with ultrasonic stimulation, sludge activity further increases. When the organic loading increases gradually, this influence gradually weakens. Hence, low substrate concentrations support improvements in anaerobic sludge activity. This phenomenon facilitates anaerobic biological treatment with ultrasonic enhancement for wastewater with low pollutant concentrations.

Effect of stirring condition on the ultrasound enhancement efficiency

Table 2 shows the influence of ultrasound irradiation on anaerobic sludge activity in different operating conditions. The table shows that intermittent stirring can slightly improve the enhancement effect on the sludge, while continuous stirring slightly reduces the enhancement effect. This may be because more organisms were exposed to ultrasonic irradiation more fully and evenly by the intermittent stirring, making the ultrasound energy utilization more efficient under the same specific energy consumption. Continuous stirring, however, puts the sludge in a flow state, shortening the continuous contact time between the organisms and the ultrasonic transducer’s radiating surface, reducing ultrasonic energy utilization efficiency eventually.

The ultrasound enhancement biological wastewater treatment experiment

Table 3 shows the COD value and removal efficiency of the anaerobic sludge after ultrasound-enhanced treatment, and after being cultured in the constant temperature shaker. The table shows the initial COD was 1,000 mg/L, while the final COD of the control group was 256 mg/L, indicating COD removal rate of 74.4%. For the ultrasound group, the final COD was 127 mg/L, indicating COD removal rate of 87.3%. This 12.9 percentage point difference between the control and the ultrasound test sample is higher than the removal rate reported in another study (Xie et al. 2009). This may be because the ultrasonic irradiation effect differs during different sludge growth phases (Yang & Huang 2002). The ultrasound irradiation enhancement effect for low sludge loading is greater; research also shows that ultrasonic enhancement effects differ greatly between different microbial species (Koch et al. 2000). The ultrasound clearly promotes organic compound degradation by anaerobic sludge.

Table 2 | F420 and DHA changes with mixing conditions by ultrasound

<table>
<thead>
<tr>
<th>Mixing condition</th>
<th>DHA (mg/gVSS)</th>
<th>F420 (umol/gVSS)</th>
</tr>
</thead>
<tbody>
<tr>
<td>No stirring</td>
<td>0.129</td>
<td>11.2</td>
</tr>
<tr>
<td>Intermittent stirring</td>
<td>0.141</td>
<td>12.6</td>
</tr>
<tr>
<td>Continuous stirring</td>
<td>0.132</td>
<td>11.4</td>
</tr>
</tbody>
</table>

Table 3 | Comparison of removal efficiency of COD with and without ultrasound

<table>
<thead>
<tr>
<th></th>
<th>With ultrasound</th>
<th>Without ultrasound</th>
</tr>
</thead>
<tbody>
<tr>
<td>COD of influent water (mg/L)</td>
<td>1,000 ± 32</td>
<td>1,000 ± 32</td>
</tr>
<tr>
<td>COD of effluent water (mg/L)</td>
<td>127 ± 6.4</td>
<td>256 ± 12.8</td>
</tr>
<tr>
<td>Removal efficiency of COD (%)</td>
<td>87.3 ± 1.0</td>
<td>74.4 ± 2.1</td>
</tr>
</tbody>
</table>
CONCLUSIONS

This study explored how sludge characteristics influence the enhancement of anaerobic sludge activity by using low energy ultrasound irradiation experiments. The aim was to describe the optimal conditions for improving the wastewater treatment efficiency of anaerobic sludge. When the ultrasonic frequency, ultrasonic energy density, and ultrasonic irradiation period were 20 kHz, 0.1 W/mL, and 10 min, respectively, the following experimental results can be achieved.

1. With the increase of temperature the ultrasound enhancement effect strengthened until the temperature reaches 20 °C. With the increase of sludge concentration DHA increased, while the content of coenzyme F420 decreased.

2. Low substrate concentration improves anaerobic sludge activity by ultrasonic irradiation; an intermittent stirring can improve the anaerobic sludge irradiation effect.

3. Low energy ultrasound can promote the biological activity of anaerobic sludge remarkably. When anaerobic sludge samples were controlled at 20 g/L, initial temperature at 20 °C, and using intermittent stirring operate, the content of coenzyme F420 and DHA increased by 59.8% and 192.3% respectively, and the COD removal reached 87.3%.

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