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Improvement of the activity of anaerobic sludge by low-intensity ultrasound

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Abstract

This paper aims to study the enhancement effect of low-intensity ultrasound on anaerobic sludge activity and the efficiency of anaerobic wastewater treatment. Dehydrogenate activity (DHA) and the content of coenzyme F_{420} were detected to indicate the change of activity of anaerobic sludge induced by ultrasound at 35 kHz. Single-factor and multiple-factor optimization experiments showed that the optimal ultrasonic intensity and irradiation period were 0.2 W/cm^2 and 10 min, respectively, and the biological activity was enhanced dramatically under the optimal condition. The chemical oxygen demand (COD) removal efficiency was increased by ultrasonic treatment and the COD in the effluent was 30% lower than that of the control (without exposure). The hypothetical mechanism of biological activity enhancement by ultrasound was also discussed according to the results.

Keywords: Low-intensity ultrasound; Anaerobic sludge; DHA; Coenzyme F_{420}; Ultrasonic intensity; Irradiation period

1. Introduction

Increasingly strict environmental legislation is forcing water and sewerage companies to undertake more advanced levels of wastewater treatment, which leads to greater energy use and sludge production at sewage treatment processes. Anaerobic wastewater treatment processes may offer a solution to the increasing energy use and sludge production at sewage treatment for their small amounts of stabilized sludge and additional methane produced (Leitão et al., 2006). But the development and implementation have not been as quick as was expected by numerous researchers and practitioners. This delay appears for several reasons (Switzenbaum, 1995; Zakkour et al., 2001). Anaerobic reactors are often hard to start up for the tough culturing of anaerobic microorganisms. Anaerobic processes may not treat sewage to levels suitable for direct discharge, and post-treatment following anaerobic treatment is required in certain instances. Therefore, methods for enhancing the bioactivity of anaerobic organics and improving the efficiency of anaerobic wastewater treatment are required urgently.

Many researchers have found that ultrasonic stimulation has the function of promoting the activity of enzyme, cell growth and cell membrane permeability (Barton et al., 1996; Liu et al., 2003; Pitt and Ross, 2003). Lin and Wu (2002) have found that the low power ultrasonic exposure can significantly stimulate the shikonin biosynthesis of the *Lithospermum erythrorhizon* cells at certain ultrasound doses (power density $\leq$113.9 mW/cm^3 and irradiation periods 1–8 min). Meanwhile, the shikonin excreted from the cells also increased due partially to the increase of the cell membrane permeability induced by sonication. Pitt and Ross (2003) found that ultrasound with low frequency of 70 kHz and low acoustic intensity of lower than 2 W/cm^2 increased the growth rate of the *Staphylococcus epidermidis* cells compared to those without ultrasonic irradiation. Liu et al. (2003) found that ultrasonic stimulation could promote the growth and proliferation of *Oryza sativa* Nipponbare cells in the suspension culture with

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the optimal stimulation of 5 s, while with longer agitation, its growth and proliferation were inhibited. Schläfer et al. (2002) have applied ultrasound to enhance a wastewater treatment process for the food industry, and the improvement of biological activity was successfully demonstrated at the frequency of 25 kHz and the power input of 0.3 W/l.

To sum up, the optimal ultrasonic intensity and irradiation period are varied in each biological process enhanced by ultrasound. Thus the aim of this paper is to explore the feasibility of using low-intensity ultrasound to intensify anaerobic biological treatment of sewage and indicate the change of activity of anaerobic sludge induced by ultrasound. The sonication conditions containing ultrasonic intensity and irradiation period were optimized and the efficiency of organic removal enhanced by low energy ultrasonic irradiation was also observed. This paper also presented the hypothetical explanation of the mechanism of biological activity enhancement stimulated by ultrasound.

2. Materials and methods

2.1. Instrument of ultrasonic irradiation

The schematic diagram of ultrasonic irradiation system is shown in Fig. 1. An ultrasonic cleaning bath (Model DL-60D, Shanghai ZhiSun Instruments Co., Ltd., Shanghai, China) was used to treat the anaerobic sludge in the serum bottle. The bath has a fixed frequency of 35 kHz and variable powers from 0 to 80 W.

2.2. Materials

Anaerobic condensed sludge without digestion from a municipal sewage treatment plant (Beijing area) was used for this study, and the concentration of this sludge was as follows: mixed liquor suspended solids (MLSS) was 36.11 g/l, mixed liquor volatile suspended solids (MLVSS) was 15.32 g/l. The samples were preserved at 4 °C before use.

The real sewage used for this study was taken from the university eatery with the COD of over 10,000 mg/l.

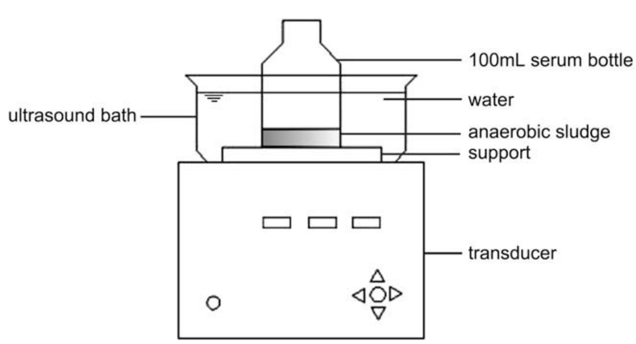

Fig. 1. Schematic diagram of ultrasonic irradiation system.

2.3. Experimental procedures

Anaerobic sludge samples were put into six serum bottles (100 ml) which were cleaned by 99.5% N_2 for several minutes, each bottle took 40 ml sludge. All the bottles were then capped with rubber stoppers and fixed at the center of the ultrasonic bath. All treatments were triplicate and each data point reported in the results was the mean of three reduplicate measurements.

2.3.1. Single-factor optimization experiment

The sludge samples were irradiated with different ultrasonic intensity from 0 to 1.0 W/cm^2 with the same irradiation period of 10 min, and then these sludge samples were incubated at 35 °C with shaking. DHA and the content of coenzyme F_{420} were detected after the incubation to determine the optimal ultrasonic intensity. Then, other sludge samples were stimulated at the optimal ultrasonic intensity with different irradiation periods, and the same operation as above was repeated to determine the optimal irradiation period.

2.3.2. Uniform design method

Uniform design was proposed by Fang (1980), based on quasi-Monte Carlo method or number-theoretic method. The uniform design has its own features, such as its functional agility of arranging experiment runs and its robustness against model uncertainty (Liang et al., 2001). So it would be a good candidate for this study. The ultrasonic intensity and irradiation period were considered to be the main factors influencing the sludge activity. Based on the single-factor optimization experiment as mentioned above, $U_5(5^2)$ (Table 1) was chosen for the experimental design.

Five sludge samples were exposed to different ultrasonic intensity and irradiation periods according to Table 1, and then they were incubated at 35 °C with shaking. DHA and the content of coenzyme F_{420} were detected to determine the optimal ultrasonic intensity and irradiation period.

2.3.3. Real sewage disposal

Three sludge samples were exposed to ultrasound with the optimal intensity and irradiation period, while other three without ultrasonic exposure were treated as control. Real sewage was put into these six serum bottles to be treated and the removal efficiency of COD was determined.

Table 1
$U_5(5^2)$

No.	Irradiation period	Ultrasonic intensity
1	1	2
2	2	5
3	3	3
4	4	1
5	5	4

2.4. Analytical methods

The mechanism of measuring DHA is that 2,3,5-triphenyl tetrazoliumchloride (TTC) is used as hydrogen receiver in cell respiration, and the reduced TTC forms a reddish color substance named triphenyl formazon (TF) which is proportional to its concentration and can be measured colorimetrically (Liu et al., 2005).

The following materials and reagents were added to two sets of centrifuge tubes (50 ml): 0.5 ml 0.36% Na_2SO_3, 0.5 ml 0.0577% $CoCl_2$, 1.0 ml Tris–HCl buffer, 1.0 ml anaerobic sludge suspension, 0.5 ml 0.4% TTC and 1.0 ml synthetic sewage (COD = 4000 mg/l). After shaking, the sample tubes were placed into a black hop-pocket and set in a water-bath boiler at a constant temperature (37 °C) immediately. All samples were shaken slightly for reaction for 30 min. A drop of oil of vitriol was added to sample tubes to finish 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 4000 rpm for 10 min. The supernatants of the samples were colorimetrically measured at 485 nm, and the absorbency 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 μg of TF per hour. The specific DHA activity is the DHA activity measured per gram MLVSS.

COD was measured using Standard Methods (HACH COD system, HACH Company, Loveland, CO.; American Public Health Association, American Water Works Association, Water Pollution Control Federation, 1995). The measurement of coenzyme F_{420} with spectrophotometry was determined using the method proposed by Wu et al. (1986). The measurement of the ultrasonic intensity was carried out with a standard calibration ultrasound needle hydrophone (CS-3). The pinhead of the hydrophone was placed 5 mm over the bottom of the serum bottle. The voltage results were expressed with a digital oscillograph (DSO-2902), and translated to ultrasonic intensity number.

2.5. Calculation methods

The definition of the improving percentage (Re) of organic removal efficiency is as follows:

$$Re = (C_c - C_u)/C_c$$

where C_c is the COD concentration in the effluent of the control and C_u is the COD concentration in the effluent of the samples with ultrasonic exposure.

3. Results and discussion

3.1. Influence of ultrasonic intensity on biological activity

The essence of the biological sewage treatment is a reactive process promoted by enzymes. The disposal of the organic pollutants in the sewage is catalyzed by microbial enzymes, and the dehydrogenation is the key approach of the biochemical reaction, so DHA can be a good indicator of the active fraction of anaerobic sludge. The result of exposure with different ultrasonic intensities on DHA is detailed in Fig. 2, and it showed that at the ultrasonic intensity of 0.2 W/cm^2, DHA increased to the maximum, and above that value DHA decreased drastically. When ultrasonic intensity exceeded 0.6 W/cm^2, the activity of anaerobic sludge was inhibited by sonication, resulting in lower DHA than that of the control (without exposure).

Methanogenesis is the final process of the anaerobic degradation of biomass, and a central electron carrier in methanogenic metabolism is the deazaflavin derivative coenzyme F_{420} (De Poorter and Keltjens, 2001), so the content of F_{420} in the sludge can be used as a biological tool to estimate the methanogenic potential activity (Wu et al., 1986). The results are also detailed in Fig. 2. The changes of F_{420} showed the same trend with the DHA. The content of F_{420} increased to the maximum at the same ultrasonic intensity of 0.2 W/cm^2 and it was lower than the control when the intensity exceeded 0.4 W/cm^2.

Therefore, low-intensity ultrasound can promote the biological activity of anaerobic sludge remarkably. The possible reason is that through function of low-intensity ultrasound, the cell will be hurt in some ways and make cell membrane flaw, which promote the permeability and selectivity of cell membrane and wall and accelerate all sorts of nutrition or molecule transport between cell membranes (Dai et al., 2003). In addition, ultrasound can also increase the activity of enzymes and modifies the metabolism of cells (Liu et al., 2003). But if the intensity of ultrasound goes beyond some range, cavitation will occur and lead to cell death, so in this experiment the DHA and the content of F_{420} was lower than the control when the intensity exceeded 0.4 W/cm^2. While, when the ultrasonic intensity is higher than 0.4 W/cm^2, the content of F_{420} increased which is quite different from the changes of DHA. The definite mechanism is unknown yet, but there are

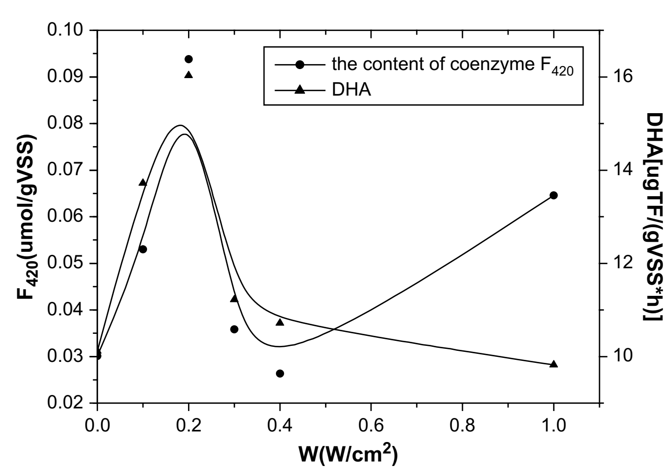

Fig. 2. Influence of ultrasonic intensity on the content of coenzyme F_{420} and DHA (with the same ultrasonic irradiation period of 10 min).

several possible explanations. Onyeche et al. (2002) used 20 kHz, 200 W ultrasound to disrupt the concentrated stabilized sewage sludge and showed that subsequent anaerobic digestion of the ultrasonically disrupted sludge could improve biogas production with reduced sludge quantity because the ultrasound could break down the higher organic molecules of sludge into simpler molecules for easier bacterial metabolism. So it was possible that when the ultrasonic intensity increased to 1.0 W/cm^2 the sludge cells may lyse partially which can lead to the decrease of DHA drastically, while the inner light organic substances released into the sludge water may promote the metabolism of rest bacteria and increase the content of F_{420}. In addition, the cavitational events induced by high-intensity ultrasound can increase the permeability of cell membranes greatly, leading to the easier release of cellular contents (Guzman et al., 2001). So during the measurement (Wu et al., 1986) the coenzyme F_{420} may be extracted easier by the alcohol when the ultrasonic intensity increased to 1.0 W/cm^2.

3.2. Determination of optimal ultrasonic irradiation period

Fig. 3 shows the changes of DHA and the content of coenzyme F_{420} of anaerobic sludge exposed at 0.2 W/cm^2 for various irradiation periods. The activity of anaerobic sludge is significantly influenced by irradiation period. At the irradiation period of 10 min, both the DHA and the content of coenzyme F_{420} increased to their peak value. As the irradiation time was prolonged, the DHA decreased gradually, and at all exposure time tested in the experiment, ultrasound with intensities of 0.2 W/cm^2 produced positive effects on the activity.

With relatively short irradiation time, cell damage and membrane permeability induced by ultrasound which could accelerate substance exchange and biological activity appears to be temporary and reversible, but with the prolonged exposure time, the flaw may expand and the structure of cell wall will be destroyed, which leads to the decrease of activity. Schläfer et al. (2000) found that only a few steps in intracellular metabolisms would be supported by ultrasound and others are not or are even inhibited. Therefore, in this experiment long-time ultrasonic irradiation can lead to the decrease of DHA. But when the irradiation time exceeded 20 min, the content of F_{420} increased just as the ultrasonic intensity increased to 1.0 W/cm^2. We assumed the reason may be the same too.

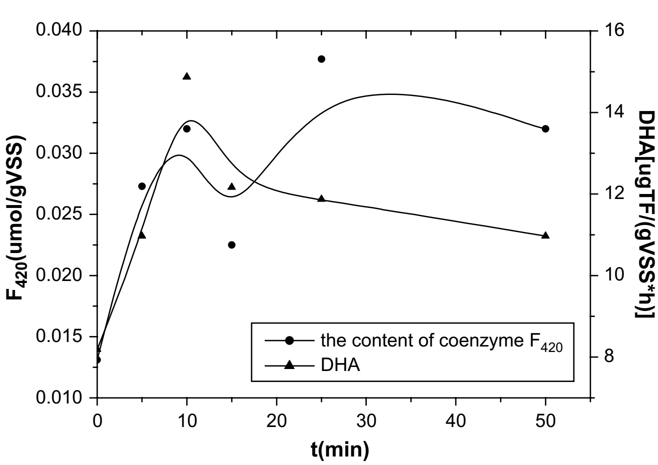

Fig. 3. Influence of ultrasonic irradiation period on the content of coenzyme F_{420} and DHA (with the same ultrasonic intensity of 0.2 W/cm^2).

3.3. Determination and analysis of uniform design

According to the single-factor tests above, we considered ultrasonic intensity and irradiation period as main factors influencing the activity of the sludge, the values of the five levels of these two factors settled are as follows:

Irradiation period: 5 min, 8 min, 10 min, 12 min, 15 min;
Ultrasonic intensity: 0.1 W/cm^2, 0.15 W/cm^2, 0.2 W/cm^2, 0.25 W/cm^2, 0.3 W/cm^2.

The design of the experiments and responding results are shown in Table 2. Note that the optimal results in Table 2: DHA = 15.83 μg TF/(g VSS h); F_{420} = 0.0453 μmol/g VSS. The corresponding ultrasonic condition was ultrasonic intensity of 0.2 W/cm^2 and irradiation period of 10 min. It showed a good agreement with the primary experiments.

3.4. The effect of sewage disposal treatment with and without ultrasound

The performances of the anaerobic sludge with/without ultrasonic irradiation are shown in Table 3. The result showed that the COD removal efficiency of the exposed samples was 3.60% higher than that of the control. The enhancement was not significant very much, because the COD removal efficiency of the control was over 87% that had very little room for improvement. We defined the reduction of the COD concentration in the effluent by ultrasonic irradiation as Re and the result showed that the Re was about 30% which can be obviously validated that the biological activity of sludge and the rate of COD degradation were promoted by ultrasonic irradiation.

So the next step of this research will be the construction of a lab-scale ASBR. With the volume of 12 l, simultaneously the optimal effect of ultrasound on biological activity and organic removal efficiency will be specified with further test results.

Table 2
The results of uniform design

No.	Irradiation period (min)	Ultrasonic intensity (W/cm^2)	DHA [μg TF/(g VSS h)]	F_{420} (μmol/g VSS)
1	5	0.15	12.32	0.0377
2	8	0.3	13.12	0.0084
3	10	0.2	15.83	0.0453
4	12	0.1	12.12	0.0244
5	15	0.25	13.42	0.0339

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

	With optimal ultrasound	Without ultrasound
COD of influent water (mg/l)	$10,837 \pm 336$	$10,837 \pm 336$
COD of effluent water (mg/l)	920 ± 46	1311 ± 65
Removal efficiency of COD (%)	91.51 ± 0.69	87.91 ± 0.98
Re (%)	29.78 ± 7.00	

4. Conclusions

(1) Low-intensity ultrasound can promote the biological activity of anaerobic sludge remarkably. DHA and the content of coenzyme F_{420} were detected to indicate the changes of activity of anaerobic sludge induced by ultrasound, the biological activity increased to the maximum with the ultrasonic intensity of 0.2 W/cm^2.
(2) The activity of anaerobic sludge is significantly influenced by irradiation time. At an irradiation period of 10 min, the biological activity of sludge increased to the maximum, but as the irradiation time was prolonged, it decreased gradually.
(3) The uniform design method also showed the great influence of the ultrasonic intensity and irradiation period, and the optimal ultrasonic condition was confirmed ultimately.
(4) The eatery's sewage was disposed by the anaerobic sludge with/without ultrasonic treatment. The result showed that the COD removal efficiency increased 3.60% after ultrasonic treatment and the effluent COD was about 30% lower than that of the control.

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