Improvement of activated sludge bacteria growth by low intensity ultrasound

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Abstract. Influence of low intensity ultrasound (US) on growth rate of bacteria separated from aerobic activated sludge was studied. In order to reveal the optimal ultrasonic conditions, specific oxygen uptake rate (SOUR) of activated sludge was first detected and results showed that the maximum SOUR was obtained (increased by 40\%) at US intensity of 0.3 Wcm\textsuperscript{-2} and irradiation time of 10min. Under the optimal conditions, 2 species of bacteria isolated from activated sludge were sonicated and then cultivated for 36h, and increment of 6\% and 10\% of growth rate were detected for the 2 species of bacteria, respectively, indicating US irradiation of suitable parameters effectively improved activated sludge bacteria growth.

1. Introduction
As a new technology in the wastewater biological treatment field, low intensity ultrasound (US) can enhance the wastewater purification effect with simple equipment, low cost and convenient combination with various biological treatment processes. Yan et al. [1] applied low intensity US to the Sequencing Batch Reactor (SBR) process, the specific oxygen uptake rate (SOUR) increased by 14\%, and the effluent COD decreased by more than 40\%. In sewage anaerobic treatment and removal of nitrogen and phosphorus processes, the low intensity US showed significant improving effects [2-4]. Duan et al. [5] and Yu et al. [6] enhanced the Anammox process using low intensity US, and a maximum of 2.6 times increment of TN (total nitrogen) removal effect was obtained.

Many studies in biological fields have indicated that the stable cavitation effect induced by low intensity US increases the permeability of cell membranes, and the consequent acceleration of the mass transfer thus increases the bacteria growth [7-11]. In order to study the influence of low intensity US on activated sludge bacteria, the appropriate ultrasonic irradiation conditions were first explored by SOUR detection, and then the growth rate of activated sludge bacteria irradiated under these conditions was detected and compared with the control.

2. Materials and Methods

2.1. Materials
Aerobic activated sludge was from a pump room of returning sludge of a wastewater treatment plant (WWTP) in Zhengzhou, China. The wastewater used was from a primary settling tank in the same WWTP with the water quality, as shown in table 1.

2.2. Ultrasonic irradiation apparatus
An ultrasonic cleaning bath (DL-60D, Shanghai ZhiSun Instruments Co., Ltd., Shanghai, China) with a fixed frequency of 35 kHz and variable powers from 0–80 W was used to insonate the activated sludge in a beaker. During exposure, the beaker containing activated sludge was immersed in the sonic bath and fixed at the center of the support, and the activated sludge sample was stirred gently.

Table 1. Water quality (mgL⁻¹).

| Contaminant | COD⁰ₓ | BOD₅ | NH₃-N | TP | SS |
|-------------|-------|------|-------|----|----|
| Concentration | 409.5 | 282.3 | 43.1 | 7.6 | 70.1 |

2.3. Experimental processes

2.3.1. Optimal ultrasonic irradiation conditions. An optimization test of ultrasonic irradiation conditions was conducted with the sludge activity index of SOUR. The activated sludge samples were first sonicated with different ultrasonic intensities for 20 min and then detected for SOUR. Then, the US intensity inducing the highest SOUR was adopted to irradiate the sludge samples for different times to discern the optimal ultrasonic irradiation time length.

2.3.2. Influence of low intensity US on growth rate of activated sludge bacteria. Single colonies of bacteria samples separated from activated sludge were sonicated under the optimal ultrasonic irradiation conditions. Their growth curves were then ascertained according to the photoelectric turbidimetry results, which showed the influence of US on bacterial growth rate by comparison with the unsonicated control.

2.4. Analytical methods
The mixed liquor suspended solids (MLSS), single colony separating and purification, gram stain and photoelectric turbidimetry were determined using Standard Methods [12,13]. SOUR was measured by a self-made respirometer, as shown in figure 1.

Figure 1. Experimental setup for measuring SOUR.

The activated sludge sample was washed with distilled water three times to ensure all organic compounds were removed, and then saturated with oxygen by feeding of fully-aerated wastewater. During the measurement, the conical flask was carefully closed with no air bubbles left, and the activated sludge sample was mixed by a magnetic stirrer (Shanghai Sile Appliance Factory, Shanghai, China) at 150 rpm. The change of dissolved oxygen (DO) was detected by an oxygen electrode (HI9146, HANNA) to calculate SOUR (mgO₂(gMLSS.min⁻¹)) according to the slope of the linear parts of the recorded DO profiles (Equation 1).

\[
\text{SOUR} = \frac{\text{DO}_1 - \text{DO}_2}{\text{MLSS} \times (t_2 - t_1)}
\]  (1)
All the tests were conducted in triplicate at a temperature of 25-26°C.

3. Results and Discussion

3.1. Optimal Ultrasonic irradiation conditions

Figure 2. Effects of US irradiation conditions on sludge activity (a: US intensity; b: irradiation time).

The influence of US intensity on sludge activity is shown in figure 2(a). Initially, sludge activity progressively increased with the increment of US intensity before the maximum of SOUR was achieved at 0.3 Wcm⁻². Then, continuous increments of US intensity caused a decline of SOUR, and the SOUR was even inferior to the control when the intensity exceeded 0.5 Wcm⁻².

On this basis, sludge samples were irradiated for 0-30 min with the US intensity of 0.3 Wcm⁻² to obtain the optimal irradiation time length. As shown in figure 2(b), the activated sludge achieved the maximum SOUR (increased by 40% compared with the control) when irradiated for 10 min, and longer irradiation time caused a downward trend. Therefore, the optimal ultrasonic irradiation conditions were 0.3 Wcm⁻² of US intensity and 10 min of irradiation time length, and a higher intensity or longer irradiation time would bring adverse influences on the sludge activity.

US is characterized by the cavitation effect. Stable cavitation that is gentle and regular occurs when US intensity is low enough, thus inducing oscillation of cavitation bubbles and consequent acoustic microstreaming in the medium, which in turn causes shear stress on cells around cavitation bubbles [14]. Besides, the vibration energy of medium particles increases due to the mechanical vibration induced by US. In combination with the stable cavitation and mechanical vibration effects, the substrate entering active sites of enzymes or cells and the product entering the liquid medium are both improved by low intensity US through thinning the mass transfer boundary layer and accelerating solute particle movement [15,16]. Therefore, enzymatic activity and cell metabolism are enhanced when irradiated by low intensity US. However, when US intensity exceeds a limit, transient cavitation occurs and induces cavitation bubbles to shrink until they collapse, and the produced high temperature and high pressure in the bubbles causes the formation of radical and powerful shockwave and jet flow. Thus, the resultant cell structure damage and enzyme inactivation decrease the cell activity. Therefore, there is always an optimal US intensity, below which no significant enhancing effect would be achieved, and above which the cell activity would be inhibited or even destroyed.

Activated sludge is an ecological population of microbes, and the individual differences to US induce an inconformity in adaptive intensity for different microorganisms. However, most of the microorganisms adapted to ultrasonic irradiation of low intensity and showed notable increments of sludge activity as shown in figure 2(a), and with increasing intensity the sludge activity was also enhanced. When the intensity increased beyond the optimal value (0.3 Wcm⁻² in this test), the amount of unadaptive microbes increased and the sludge activity declined to even lower than the control,
indicating that excessive US intensity (beyond 0.5 Wcm\(^{-2}\) in this test) induced severe destruction of the microbial population.

Furthermore, US-induced shear stress may inflict minor wounds on the cell surface. When irradiation time is short enough, the smaller wounds may be bearable for most cells, and the cell activity is improved with the produced increments of cell permeability and extra and intracellular material exchange. However, an excessive irradiation time may deteriorate the wound and thus cause a decline of sludge activity. Therefore, US intensity of 0.3 Wcm\(^{-2}\) and irradiation time of 10 min were used to conduct ultrasonic irradiation on activated sludge bacteria.

3.2. Influences of ultrasonic irradiation on cell growth rate

3.2.1. Separation and purification of activated sludge bacteria. Activated sludge samples were separated and purified to obtain 2 species of bacteria prior to gram staining, and their morphological characteristics were as shown in table 2.

| Bacteria | Community form                  | Gram stain | Cell shape   |
|----------|---------------------------------|------------|--------------|
| W        | white, hyaline, round, regular  | G-         | rod          |
|          | edge                            |            |              |
| Y        | yellow, hyaline, round, regular | G+         | spherical    |
|          | edge                            |            |              |

3.2.2. Influences of ultrasonic irradiation on cell growth rate. Bacteria W and bacteria Y were sonicated with a US intensity of 0.3 Wcm\(^{-2}\) for 10 min. A comparison of the growth rates with the control over the course of 36 h is shown in figure 3.

Figure 3. Influences of ultrasonic treatment on the growth of bacteria (a: bacteria W; b: bacteria Y).

As shown in figure 3, the growth rate of bacteria W and bacteria Y increased by 6% and 10%, respectively, after being sonicated, and the t-test analysis indicated that the results had a significant difference.

Considerable research on cell growth accelerated by low intensity US has been conducted in the medical, food and pharmaceutical fields. Gao et al. [15] investigated the effects of low intensity US on the growth of Saccharomyces cerevisiae cells, and notable promotion of growth rate was achieved. Pitt and Ross [16] sonicated S.epidermidis, E.coli and P.aeruginosa with low intensity US, and obvious
acceleration was observed in the growth of all the three species of bacteria. Similarly, activated sludge bacteria showed a significant improvement in growth rate after being sonicated by low intensity US. In combination with the above SOUR test results, it can be speculated that the enhancement of sludge activity induced its growth acceleration.

In addition, more significant improvement of growth rate was observed for bacteria Y than bacteria W, which were G+ and G- respectively. Likewise, Pitt and Ross [16] observed different degree improvement effects of US on different species of bacteria, among which the improvement effects followed the descending order: S. epidermidis (G+) > E.Coli (G-) > P. aeruginosa (G-). Therefore, the difference of the cell wall structures of gram-positive bacterium and gram-negative bacterium may lead to a difference in the improvement effects of low intensity US on bacteria growth.

4. Conclusions
- The optimal irradiation conditions of low intensity US were 0.3 Wcm\(^{-2}\) of US intensity and 10 min of irradiation time length, with which a 40% improvement of SOUR could be achieved over the control.
- Bacteria W and bacteria Y were separated from the activated sludge, and their growth rates accelerated by 6% and by 10%, respectively, after being sonicated under optimal irradiation conditions.

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