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Ozone microbubbles treatment with different gas–liquid mixing conditions and its application on printing and dyeing wastewater and *Escherichia coli*

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ABSTRACT

The low solubility of ozone in water limits its oxidation reaction rate, while microbubbles can enhance the gas–liquid mass transfer process and effectively improve the mass transfer rate of ozone. In this study, we set up an ozone microbubble generation system, with an internal recycle, a self-made releasing device using 3D printing and other designs to keep the system running steadily, with constant pressure, high ozone concentrations, and slow microbubble dissipation rate. This study investigated the ozone feed rate and optimization of mixing conditions through the test. With the increase of ozone feed rate, the average concentration of ozone in the solution tends to increase linearly, then decreases slightly after reaching the maximum 13.75 mg/L. On a stable running of the system, the ozone concentration of the solution could reach 13.29 mg/L. The ozone microbubble was then investigated by calculation and measurement of bubble disappearance time, particle size and mass transfer capacity. This study found the particle size of bubbles was about 42.66 μm and K_{La} (20) was 10.712 min^{-1}. The system's applications were then studied to demonstrate the effect of ozone microbubbles. Under strong alkaline conditions and without acid adjustment, COD of printing and dyeing wastewater could reach Chinese first-class A discharge standards after treatment with ozone microbubbles. Another experiment proved that ozone microbubble solution of 8 mg/L had more than 99% killing effect on *Escherichia coli*.

Keywords: Ozone microbubble; Mixing condition; Mass transfer capacity; Wastewater treatment; Disinfection

1. Introduction

Ozone is a strong oxidizing agent that affects our modern world in a variety of ways [1]. Due to its high oxidation potential, ozone has persistently been used in both industrial and private applications, such as organic compounds treatment [2], biodegradability improvement of wastewater [3], disinfection, and odour removal. Moreover, it has the advantage of no residue and, unlike conventional disinfectants, it does not cause bacteria to acquire resistance. Combined with these strengths, it is believed to have a wide range of applications in the manufacturing, medical [4] and food industries [5].

However, the maximum ozone concentration of the solution that can be achieved with typical systems could only reach less than 10 mg/L even when the maximum capacity of ozone generator to produce ozone was 50 g/h [6]. The relatively low solubility of ozone in water [7] limits its oxidation reaction rate. Microbubbles can enhance the process of gas–liquid mass transfer. At present, microbubbles have been applied in aquaculture [8], soilless cultivation [9],

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agriculture [10], water environment management and other fields. It can also effectively improve the ozone mass transfer rate [11].

To combine the strengths of ozone and microbubbles in the treatment of wastewater so as killing bacteria, an ozone microbubble generation system with high ozone concentrations and slow microbubble dissipation rate was set up in this study.

2. Materials and methods

2.1. Main materials

All reagents including Methylene blue (MB) solution, H_2SO_4 (c = 1 mol/L), NaOH (c = 1 mol/L) and Luria-Bertani (LB) medium were analytical chemicals and were from Sinopharm Chemical Reagent Co., (China). The *Escherichia coli* used in the experiment was isolated and purified from the wastewater of a hospital in Zhenjiang city, Jiangsu Province, China and its concentration was adjusted to 10^9 CFU before use.

2.2. Main instruments

We used a LZB-4W, 4WB gas flow meter in our main experiment, and a LZB-3W, 3WB gas flow meter in the part of simulated printing and dyeing wastewater (Xiangjin, China) to adjust the gas flow of the system. We used a LB K24 liquid flow meter (Lungbor, China) to adjust the liquid flow of the system. We used a YJ-200B ozone gas concentration online monitor purchased by Guangzhou Yuejia Environmental Protection Technology Co., (China) to record the ozone gas concentration. We detected the ozone concentration of the solution by a CL6587 ozone concentration detector (B&C Electronics, Italy). We observed, photographed and videotaped the microbubbles by a 1080FHD electron microscope (LEYES, China). We adjusted the pH of the simulated printing and dyeing wastewater with H_2SO_4 and NaOH and measured the pH with a FE28-Micro pH meter (Mettler Toledo, Swiss).

2.3. Ozone microbubble generation system

The system is shown in Fig. 1. The system is mainly composed of air compressor, molecular sieve tower, ozone generator, high-voltage power supply, gas–liquid mixing pump, gas–liquid mixing tank, etc.

The system works as follows: an air pump draws air into the system. The nitrogen in the air is removed by molecular sieve towers, which then release oxygen. The gas enters the ozone generator's gap. A high voltage supply is used to apply a high frequency voltage between the plates. The oxygen molecules were sufficiently broken down, ionized, and overexcited to form gaseous ozone. The gas–liquid mixing pump produces excess water. This water induces the device to use negative pressure to suck in ozone produced by the ozone generator. The excess water and ozone enter the gas–liquid mixing tank through the gas–liquid mixing pump. Ozone microbubble solution is formed after mixing at high pressure in the mixing tank. In order to obtain higher ozone concentration, part of the ozone microbubbles is returned to the gas–liquid mixing tank, where they are mixed with ordinary water and ozone. Finally, ozone microbubble solution is released through the releasing device.

We designed a releasing device using 3D printing to adapt to ozone microbubble generation system, with the bubble size reaching tens to hundreds of microns. The structure of the releasing device is shown in Fig. 2. A portion of the solution from the pump's outlet returns to the gas–liquid mixing tank. This design prevents the gas–liquid mixing tank from suddenly losing fluid after the power is switched off. The pump can also be protected if there is a particular volume of water in the tank. In addition, the system has a vent valve for the gas–liquid mixing tank, which prevents too much gas from entering the tank. If there is too much ozone in the gas–liquid mixing tank, part of it will not dissolve in the water quickly enough, causing the tank's pressure to drop rapidly and a corresponding malfunctioning of the pump.

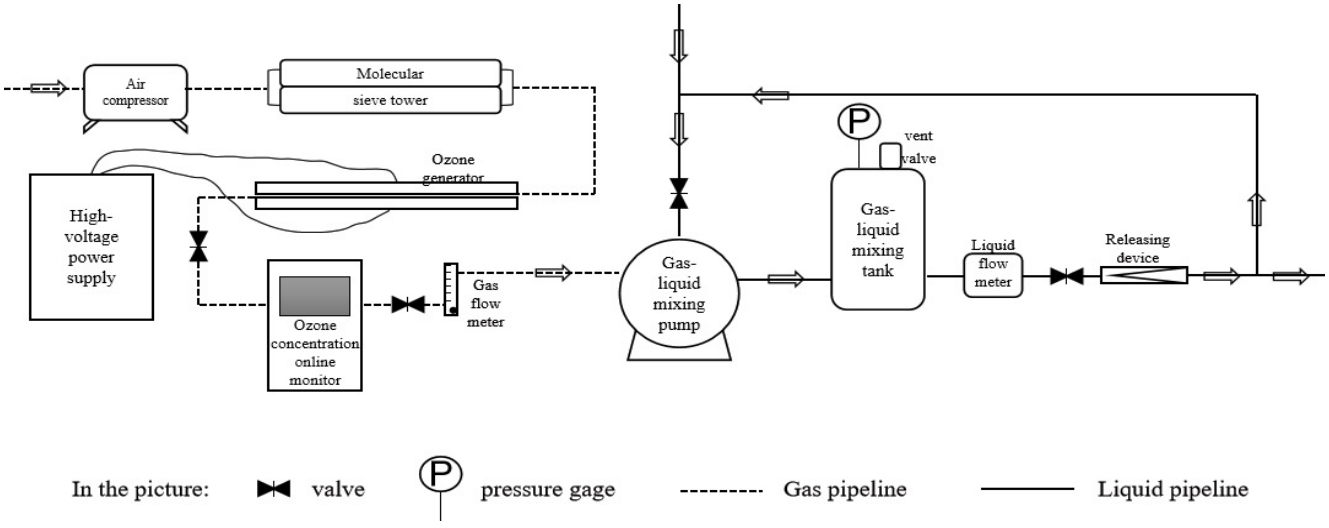

Fig. 1. Ozone microbubble generation system.

2.4. Experimental methods

In this study, the oxygen source was 90% O_2, and the oxygen flow was stable. The water valve was fully open at the beginning of operation, and the inlet flow was 10.1 L/min. Under the conditions of temperature 26°C and humidity 70%, ozone feed rate was adjusted by gas flow meter respectively. Then the ozone microbubbles in the solution were enriched through the system circulation, and the ozone concentration at the outlet of the release device was measured when ozone concentration was stabilized.

3. Results and discussion

3.1. Optimization of gas–liquid mixing conditions

3.1.1. Effect of ozone feed rate change on concentration of ozone in solution

In this study, the maximum capacity of ozone generator to produce ozone was 50 g/h, and the water inlet flow was 10.1 L/min. The ozone concentration at the outlet of ozone generator was detected to be 87 mg/L. The ozone feed rate was adjusted by gas flow meter to 17.4, 34.8, 52.2, 69.6, 87 mg/min, respectively. The effect of different ozone feed rate on the concentration of ozone was then investigated. The ozone concentration data detected by CL6587 ozone concentration detector were accurate to two decimal places, each data was measured three times in the experiment, and the average value was taken. The result is shown in Fig. 3.

It was found in the experiment that under this condition, the ozone flow was unstable when the ozone feed rate was 87 mg/min, and the device did not absorb water and did not work because the ozone intake was too high in a short time. In practical operation, the ozone feed rate should be increased slowly, and the ozone intake progress should not be continued when the ozone flow was gradually unstable.

Under this condition, when the ozone feed rate of the device is set at 69.6 mg/min and below, the air volume is stable and can be operated. As can be seen from Fig. 3, in the working state, the larger the ozone feed rate is set, the more ozone mixes into the solution, the higher the concentration of O_3 in the solution at the outlet of the mixing pump will be, and the higher the concentration of O_3 in the solution released by the releasing device will be when the bubble disappears by half or completely. When the ozone feed rate is 69.6 mg/min, the concentration of the solution O_3 at the outlet

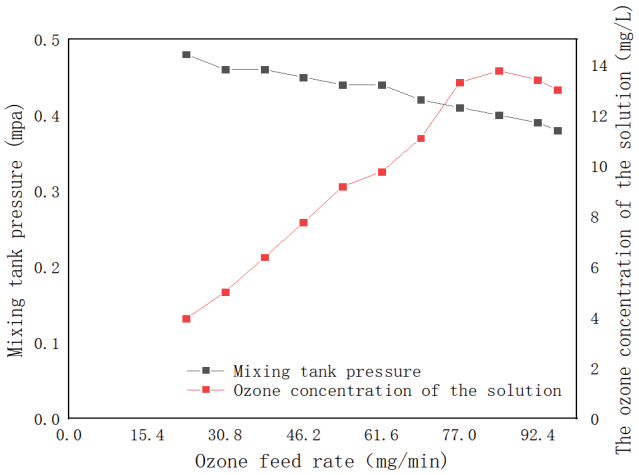

Fig. 3. Ozone concentration of solution at different ozone feed rates.

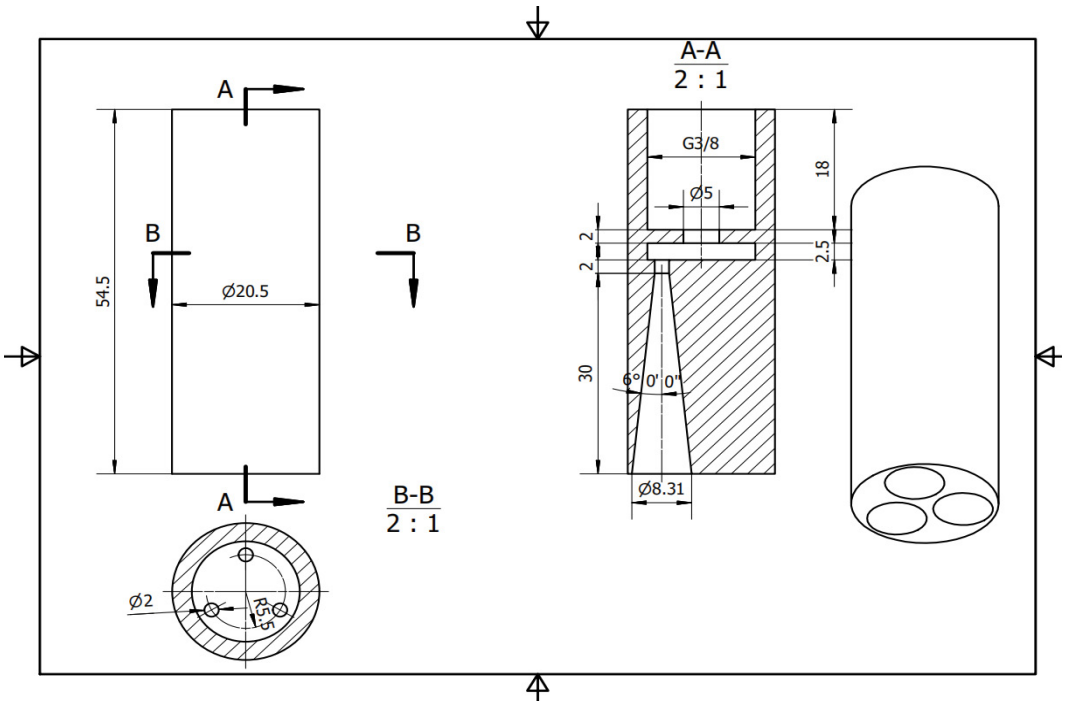

Fig. 2. Self-made releasing device and its related parameters.

of the mixing pump is the highest, and the concentration of the solution O_3 at the outlet of the mixing pump exceeds the 20 mg/L range. The concentration of the solution O_3 released by the releasing equipment when the bubble disappears half or completely is 8.92 mg/L and 5.74 mg/L, respectively.

3.1.2. Changes of ozone concentration in solution with ozone feed rate change at lower ozone intake concentration

The ozone generation efficiency in summer may be greatly affected in practice because the device is not easy to cool down when using the ozone generator with high ozone output. Therefore, this part considers replacing the ozone generator with smaller specifications, so that the intake ozone concentration can be slightly reduced accordingly. In this study, the specification of ozone generator was 20 g/h, and the water inlet flow was 8.6 L/min. The ozone concentration at the outlet of ozone generator was detected to be 77 mg/L. The ozone feed rate was adjusted by gas flow meter to 23.1, 30.8, 38.5, 46.2, 53.9, 61.6, 69.3, 77, 84.7, 92.4, 96.3 mg/min, and the effect of different ozone feed rates on ozone concentration in the solution was investigated. It is observed from the experiment before that the pressure of the mixing tank will decrease with the increase of ozone feed rate and will immediately drop to 0mpa when the ozone feed rate is too large. At the same time, the device does not absorb water, so it is considered to observe the pressure change at the same time. The result is shown in Fig. 4.

As can be seen from the red curve in Fig. 4, under this condition, with the increase of ozone feed rate of the device, more and more ozone mixes into the solution, and the average concentration of O_3 in the solution released by the release device first tends to increase linearly. After that, the average concentration of the released solution O_3 reached the maximum 13.75 mg/L when the ozone feed rate was 84.7 mg/min. Subsequently, the ozone feed rate continues to increase, the gas inhalation process is gradually unstable, the release of the release solution O_3 concentration slightly reduced.

It can be seen from the black curve in Fig. 4, under the condition of the inlet flow valve fully open, only to adjust the ozone feed rate, the pressure of mixed solution on mixing tank decrease slowly with the increase of ozone feed rate and tend to be linear. When ozone feed rate is 0 mg/min, the mixing tank pressure is about 0.5 mpa. When ozone feed rate is 23.1 mg/min, the mixing tank pressure is 0.48 mpa. When ozone feed rate increase to 77 mg/min, the pressure rises to 0.41 mpa. Continue to increase ozone feed rate, this progress becomes unstable, and the highest ozone feed rate of 96.25 mg/min leads to minimum pressure of 0.38 mpa.

Based on the conclusions in Fig. 3 and above, when the ozone feed rate was 84.7 mg/min, the concentration of the release solution O_3 reached the maximum of 13.75 mg/L. When the ozone feed rate is 77 mg/min, the intake is stable and the concentration of the released solution O_3 is close to the highest, and the concentration can reach 13.29 mg/L. The latter has the best effect after comprehensive consideration.

3.2. Basic properties of ozone microbubbles

3.2.1. Determination of dissipation time of microbubbles in solution

The dissipation time of ozone microbubbles was measured in a measuring cylinder with a measuring range of 250 mL. After the experimental device is opened in advance until the operation is stable, the solution is injected into the measuring cylinder to the maximum measuring range. A laser pointer is used to observe the microbubble disappearance interface while a stopwatch is used to record the time from the beginning to disappear to complete disappearance of the microbubbles.

As can be seen from Fig. 5, the microbubble dissipation at different scales is approximately proportional to time, and the dissipation rate is stable. It takes 257 s for the microbubbles to completely dissipate in a 250 mL scale at a dissipation rate of about 1 mL/250 mL per second. Ozone concentration decreases with microbubble dissipation. The concentration of ozone in the solution began to decrease from 9.47 mg/L. With the decrease of the concentration of ozone,

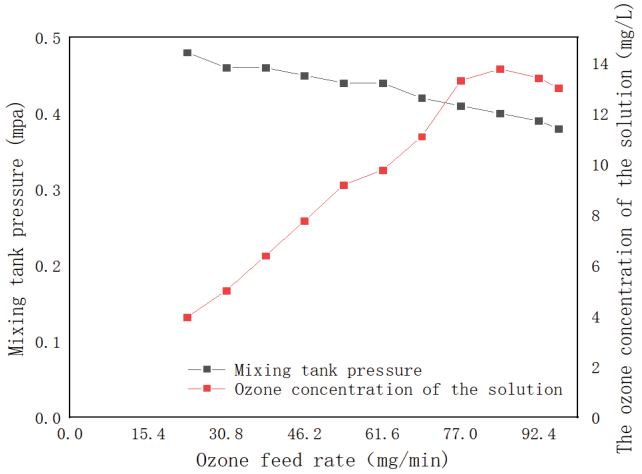

Fig. 4. Ozone concentration of solution and mixing tank pressure at different ozone feed rates.

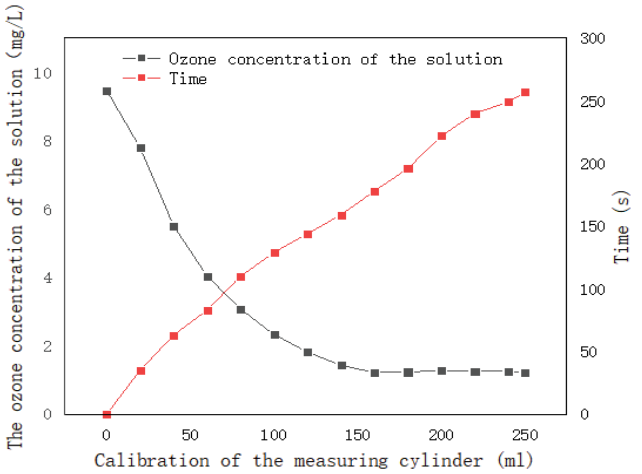

Fig. 5. Microbubble dissipation time and its concentration at different scales.

the solution separated from the saturation state of ozone, and the ability of solution to dissolve ozone increased. At the same time, the decay rate gradually decreased, and tended to be stable after 140 s. The decay rate was slow when the concentration of ozone in solution decreased to 1.82 mg/L. After 257 s, the microbubbles disappeared, and the ozone concentration decreased to 1.21 mg/L and remained almost unchanged for a period.

3.2.2. Calculation and measurement of microbubble size

The internal viscosity of water was 0. 001025 Pa·s, the internal viscosity of ozone was 1. 56 mPa·s, g was 9.8 N/kg, the floating distance of bubbles was 0.58 m, and the observed bubble dissipation time was about 400 s. The bubble diameter is about 42.66 μm.

The formula and calculation steps are as follows [12,13]:

$$u_t(H-R)=\frac{2\Delta\rho g r^2}{9\mu}\cdot\frac{\mu+\mu'}{2\mu+3\mu'} \tag{1}$$

$$r^2=\frac{u_t(H-R)\cdot 9\mu}{2\Delta\rho g}\cdot\frac{2\mu+3\mu'}{\mu+\mu'} \tag{2}$$

where μ is the internal viscosity of water (Pa·s); μ′ is the ozone internal viscosity (mPa·s); Δρ is the density difference (kg/m^3); u_t (H–R) is the bubble terminal velocity (m/s).

$$u_t(H-R)\le\frac{0.58}{400}=0.00145\text{m/s} \tag{3}$$

$$d=\sqrt{\frac{12\mu u_t(H-R)}{\Delta\rho g}}\le 4.266\times10^{-5}\text{m}=42.66\mu\text{m} \tag{4}$$

As can be seen from Fig. 6, the solution is rich in micron-sized bubbles within the visual range of the microscope. The bubble particle size calculated by bubble dissipation time is consistent with the particle size obtained by microscope observation.

3.2.3. Investigation of mass transfer capacity of ozone microbubbles

It is known that the ozone mass transfer coefficient at any water temperature is:

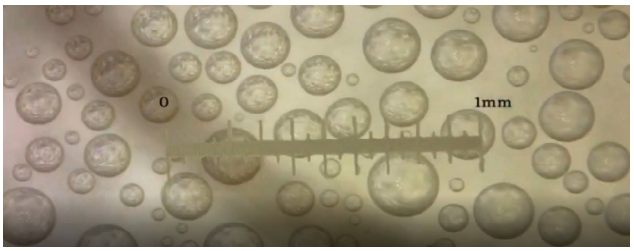

Fig. 6. Measurement of bubble size under electron microscope.

$$K_{L_a}(T)=\ln\frac{C_s-C_1}{C_s-C_2}\times\frac{60}{t_2-t_1}\left(\text{min}^{-1}\right) \tag{5}$$

where T is the test water temperature (°C); C_s is the saturated dissolved ozone concentration (mg/L); C_1 and C_2 is the ozone concentration at t_1 and t_2 (mg/L); t_1, t_2 is the reading time of C_1 and C_2 (min).

As can be seen from Fig. 7, the concentration of ozone in the solution dropped sharply in a short time after reaching 800 s, and then fluctuated greatly. Therefore, it was considered that the concentration of ozone in the solution reached saturation at 800 s, so C_s = 11.55. K_{La} (20) = ln[(11.55–1.01)/(11.55–8.87)] × 60/(9–1.33) = 10.712 (min^{-1}).

3.3. Treatment effect of ozone microbubble on simulated printing and dyeing wastewater

3.3.1. Effect of ozone gas flow

In this part of the study, the water source was changed to simulate printing and dyeing wastewater. Due to the small amount of simulated printing and dyeing wastewater in the experimental configuration, the pump with smaller flow should be replaced for this experiment. Refer to the gas–liquid mixing conditions obtained from previous experiments, the specification of ozone generator was 20 g/h, and the water inlet flow was 5.3 L/min at the beginning of operation. The simulated printing and dyeing wastewater is a newly prepared MB solution, the initial pH is adjusted to 7, and the initial COD is about 125 mg/L.

In the experiment, it was found that MB solution could be decolorized completely within 10 min under various ozone feed rates or pH conditions, as can be seen from Fig. 8. Therefore, ozone microbubble solution treatment time was set as 10 min. The ozone feed rate was adjusted by gas flow meter to be 3.85, 7.7, 15.4, 23.1, 30.8 and 38.5 mg/min respectively, to investigate the influence of different intake on the COD removal of MB solution.

As can be seen from Fig. 9, under this condition, COD of the solution decreases rapidly within 3 min and then slowly with the increase of reaction time. With the increase of gas intake, the concentration of ozone in the solution increases,

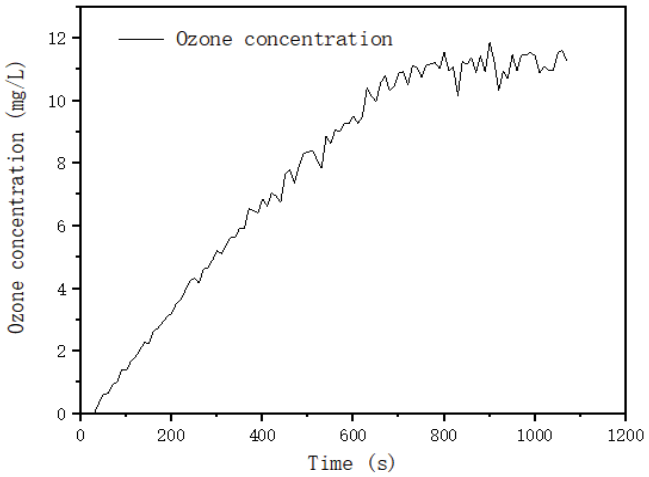

Fig. 7. Ozone dissolution curve.

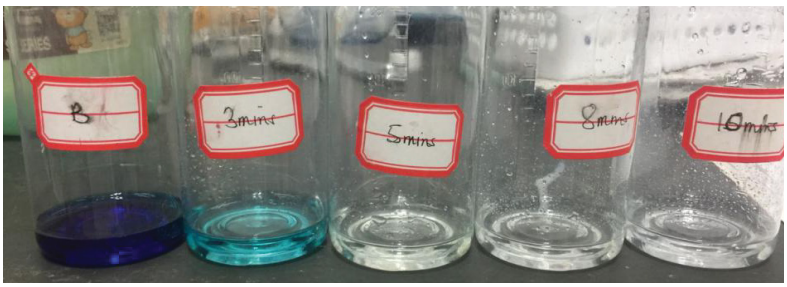

Fig. 8. The decolorization effect of MB solution treated with ozone microbubble solution for 0~10 min.

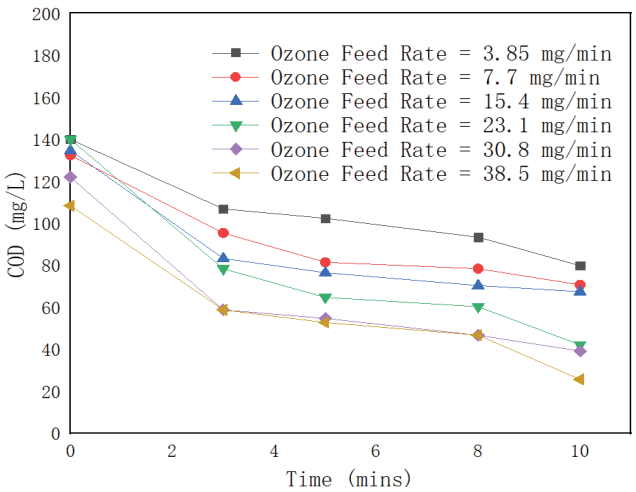

Fig. 9. The change of COD with time at different ozone flow.

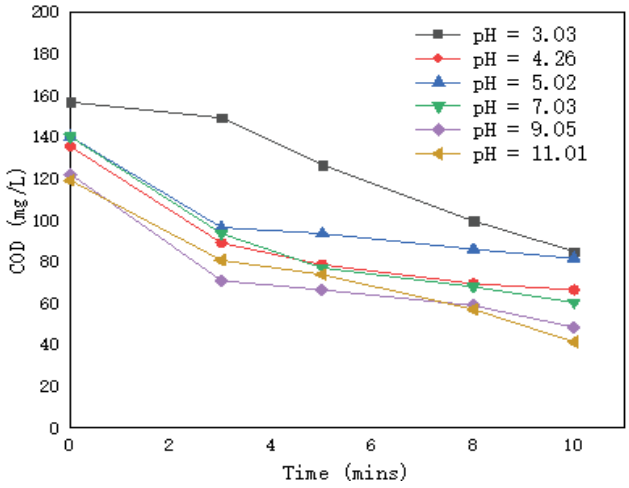

Fig. 10. The change of COD with time at different pH.

and the COD removal effect becomes better. When the ozone feed rate increases to 23.1 mg/min, the effluent COD can reach 42.1 mg/L, reaching Chinese first-class A discharge standard. When the ozone feed rate increases to 30.8 mg/min, the COD decreases to the lowest 39.13 mg/L, and the removal rate reaches 68%. The ozone feed rate continues to increase to 38.5 mg/min. Although COD can be reduced to 25.6 mg/L, the unstable gas volume is easy to cause the device to stop absorbing water. In general consideration, the optimal ozone feed rate is 30.8 mg/min, and the original device should also choose the highest ozone feed rate volume under the premise of stable ozone flow.

3.3.2. *Influence of pH*

Printing and dyeing wastewater is characterized by high alkalinity, usually up to more than 10. Usually, the treatment of printing and dyeing wastewater needs to adjust the pH of wastewater to 6~9 before treatment. In this study, ozone feed rate was 30.8 mg/min, and other conditions were consistent with the previous experiment. The pH of simulated printing and dyeing wastewater was adjusted by sulfuric acid and sodium hydroxide. COD increased slightly before treatment. The COD removal effect of simulated printing and dyeing wastewater under different pH was investigated.

As can be seen from Fig. 10, when pH is 3.03, COD removal is not obvious within 3 min due to the strong acidity of the solution. The COD of the solution decreases rapidly from 3 min to 10 min. As the initial pH increases, the COD removal effect becomes better, and the pH of the solution will quickly drop to about 4 within 3 min. Meanwhile, it can be seen from the figure that COD will decrease rapidly and then slowly. When the initial pH is increased to 9, the effluent COD can reach 48.2 mg/L, reaching Chinese first-class A discharge standard. When the initial pH continues to increase to 11, the effluent COD decreases to the lowest 41 mg/L, and the removal rate reaches 65.35%. Therefore, the printing and dyeing wastewater with strong alkalinity can be directly treated in practical application. This eliminates pretreatment and saves a lot of acid to regulate pH. At the same time, the strong alkalinity of printing and dyeing wastewater can promote the oxidation of ozone microbubbles and get better COD removal effect.

3.4. *The killing effect of ozone microbubble on Escherichia coli*

The *Escherichia coli* liquid with a concentration of 10^9 CFU was stored at 4°C–8°C as the standard liquid.It was diluted 10,000 times to obtain a solution of 10^5 CFU *Escherichia coli* for the experiment. A fully sterilized pipette head was used in the experiment. Use it to absorb 5 ml of bacterial liquid and add it to the centrifuge tube, then add 5 ml of sterile water and mix thoroughly. In the ultra-clean table, the bacteria liquid in 1ml centrifuge tube was absorbed by sterile pipet and inoculated on the LB medium to be used.

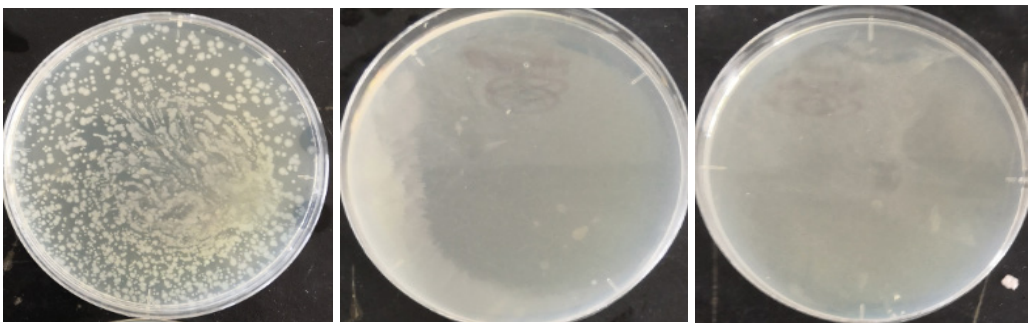

Fig. 11. The applications of the system in disinfection. Effect of 10^5 CFU resistant *Escherichia coli* solution treated with different concentration of hydroxyl radical solution.

Three replicates were set, and the bacteria liquid was evenly smeared on the medium with sterile spatula and served as the control group.

In the experiment of treating *Escherichia coli*, 5 mL 10^5 CFU bacterial liquid was added into the centrifuge tube after the pipette head was replaced each time. Add 5 ml of newly prepared ozone microbubbles of different concentrations to treat the solution, shake it slightly until it is evenly mixed, and treat it for 1 min. The treated bacterial liquid in 1 ml centrifuge tube was absorbed by sterile pipet and inoculated on LB medium. Three replicates were set, and then the bacterial liquid was evenly smeared on the plate with sterile spatula. The treatment group was made.

Put the evenly coated medium on the table for 30 min to allow the bacterial liquid to penetrate into the medium. Then invert the medium and culture it in a incubator.

The most suitable growth temperature of *Escherichia coli* is 37°C, so set the incubator culture temperature is 37°C. Considering that *Escherichia coli* will be dormant due to excessive drying, a certain humidity should be set. The humidity of the incubator was set at 99%.

As can be seen from Fig. 11, there were dense yellow dots on the media of the control group, indicating that *Escherichia coli* grew well in natural state. There were 1,973 spots in the control group, as indicated by the marker count. The sample treated with 8 mg/L ozone microbubble had 17 spots and killed 99.14% of *Escherichia coli*. At this time, the sterilization rate was over 99%. Further increase of ozone concentration can obtain higher sterilization rate. When the concentration of ozone microbubbles was increased to 16 mg/L, the samples were treated with 7 spots and 99.65% *Escherichia coli* were killed. At this point, the experiment is nearly complete sterilization.

4. Conclusion

The results show that, with the system increases its ozone feed rate, the average concentration of O_3 in the solution released by the release device tends to increase linearly. Then O_3 concentration will reach the maximum 13.75 mg/L. Subsequently, the ozone feed rate continues to increase, the gas inhalation process will be gradually unstable, and O_3 concentration will be slightly reduced. In this study, the optimal process parameters are as follows: oxygen source is 90% O_2, oxygen flow is 1 L/min, temperature is 26°C, humidity is 70%, specification of ozone generator is 20 g/h, ozone feed rate is 77 mg/min, water intake is 8.6 L /min, mixing tank pressure is 0.4 mPa. Under these conditions, the system can operate stably, and the ozone concentration of the solution can reach 13.29 mg/L.

The microbubbles dissipate for 257 s in a 250 mL measuring cylinder. The particle size of bubbles which this system produced is about 42.66 μm and K_{La} (20) is 10.712 min^{-1}.

The applications of the system in different fields all have good results in experiments. It is proved that COD of printing and dyeing wastewater can reach Chinese first-class A discharge standard after treatment of ozone microbubbles, under strong alkaline conditions without acid adjustment of pH. Ozone microbubble solution of 8 mg/L has more than 99% killing effect on *Escherichia coli* and higher concentration of the solution works better.

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