The inactivation of Chlorella spp. with dielectric barrier discharge in gas-liquid mixture

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Abstract. The inactivation of Chlorella spp. with high voltage and frequency pulsed dielectric barrier discharge in hybrid gas-liquid reactor with a suspension electrode was studied experimentally. In the hybrid gas-liquid reactor, a steel plate was used as high voltage electrode while a quartz plate as a dielectric layer, another steel plate placing in the aqueous solution worked as a whole ground electrode. A suspension electrode is installed near the surface of solution between high voltage and ground electrode to make the dielectric barrier discharge uniform and stable, the discharge gap was between the quartz plate and the surface of the water. The effect of peak voltage, treatment time, the initial concentration of Chlorella spp. and conductivity of solution on the inactivation rate of Chlorella spp. was investigated, and the inactivation mechanism of Chlorella spp. preliminarily was studied. Utilizing this system inactivation of Chlorella spp., the inactivation rate increased with increasing of peak voltage, treatment time and electric conductivity. It was found that the inactivation rate of Chlorella spp. arrived at 100% when the initial concentration was $4 \times 10^6$ cells mL$^{-1}$, and the optimum operation condition required a peak voltage of 20 kV, a treatment time of 10 min and a frequency of 7 kHz. Though the increasing of initial concentration of the Chlorella spp. contributed to the addition of interaction probability between the Chlorella spp. and O$_3$, H$_2$O$_2$, high-energy electrons, UV radiation and other active substances, the total inactivation number raise, but the inactivation rate of the Chlorella spp. decreased.

1. Introduction
The gas-liquid mixture dielectric barrier discharge (DBD) is a leading advanced oxidation water treatment technology that can produce large amounts of active substances which contain high energy electron, ion and plasma, and generate O$_3$, H$_2$O$_2$. This technique have the advantages of high treatment efficiency, time-energy saving and without secondary pollution [1-4]. Currently, there is none research of using gas-liquid mixture DBD to kill Chlorella spp. This paper utilized non-thermal plasma generated from gas-liquid mixture DBD to kill the Chlorella spp., investigated the impact of parameters on the inactivation of Chlorella spp., concluding discharge peak voltage, the treatment time, solution conductivity and Chlorella spp. initial concentration, and preliminarily investigated the killing mechanism.

2. Experiment

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2.1 Instrument
The experimental setup is shown in figure 1. The reactor made of Plexiglas was 160×160×150 mm, the quartz plate was as a dielectric layer and the electrodes plate was stainless. Suspended electrode was immersed in the surface of solution with the mesh size of 5×5 mm. Discharge gap covered between the quartz plate and the solution surface. The voltage of high voltage pulse power was 0-20 kV, the frequency was 0-10 kHz, voltage probe model was Tektronix P6015A, the current probe model was Pearson 2878, and the oscilloscope model was Tektronix TDS2024B which detected the output voltage and current waveforms.

The concentration of *Chlorella spp.* in laboratory was 4×10^7 cells mL^-1, and the conductivity was 12 mS·cm^-1. In this paper, d stands for the discharge gap, f stands for the frequency, U is the peak voltage, σ is conductivity of the solution, c represents the initial concentration of *Chlorella spp.*, t represents the discharge time.

![Figure 1. Scheme of experimental device.](image)

2.2 Methods of test
Each experiment recycling treated 800 mL *Chlorella spp.* solution in the reactor, then took 100 mL solution after discharge treatment and 100 mL without discharging to be cultured for 5 days under the same conditions. The microscopy was used to count the number of *Chlorella spp.* directly, and calculated the killing rate of the *Chlorella spp.* as shown in equation 1.

\[
\text{The inactivation rate} \left(\%\right) = \frac{C_0 - C_1}{C_0} \times 100\%
\]

(1)

C_0 is the concentration of *Chlorella spp.* without discharging treatment after 5 days, C_1 is the concentration of *Chlorella spp.* under discharge treatment after 5 days.

3. Results and discussion

![Figure 2. The gas-liquid mixture DBD topography (d=5 mm, f=7 kHz, U=20 kV, pH=6.85, σ=1.0 μS cm^-1).](image)
Figure 2 is the topography of gas-liquid DBD while the ultra-pure water was injected into the reactor, it was clear that there were numbers of discharge plasma channels, and the discharge was intense, uniform and stable.

Figure 3 shows the voltage and current waveforms under the same conditions as in figure 2, there were two discharges in a pulse period; the power was applied to start the first discharge, while the surface charge accrued. When the electric field direction changed, and the surface charge accumulated to a certain extent to lead the second discharge.

![Figure 3](image-url)

**Figure 3.** The gas-liquid mixture DBD voltage-current waveforms (d=5 mm, f=7 kHz, U=20 kV, pH=6.85, σ=1.0 μS cm⁻¹).

3.1. Effect of discharge peak voltage

![Graph](image-url)

**Figure 4.** Effect of peak voltage on the inactivation rates of *Chlorella spp.* (t=10 min, c=4×10⁶ cells mL⁻¹, σ=3 mS cm⁻¹).

The higher the discharge peak voltage was, the lower survival number of *Chlorella spp.* was, and the higher the kill rate was. When the discharge intensity increased, the number of oxidizing groups
concluding the hydrogen peroxide, hydroxyl and ozone increased, the effect of membrane permeability increased, resulting to cell broken [5, 6]. UV light and the shock wave enhanced killing effects of *Chlorella spp*.

In figure 4, the kill rate of *Chlorella spp* was 97.58% with the peak voltage of 21 kV. The cells of *Chlorella spp* cultured for 5 days were observed by microscopic; the numbers were essentially the same in each day. The cells may had died, but not broken and suspended in solution, so the microscope cannot judge that they were dead. Therefore, we identified the inactivation rate was 100%.

### 3.2. Effect of treatment time

![Figure 5](image)

**Figure 5.** Effect of treatment time on the inactivation rates of *Chlorella spp*. (U=20 kV, c=4×10^6 cells mL\(^{-1}\), \(\sigma\)=3 mS cm\(^{-1}\)).

Figure 5 shows that the longer the discharge treatment time was, the higher the inactivation rate was. The various factors of UV, reactive species, electric field strength and the shock wave had accumulated superimposed effect with the time continuing [7], which affected the activity of *Chlorella spp* and resulted in an increase of the kill rate.

### 3.3. Effect of solution conductivity

![Figure 6](image)

**Figure 6.** Effect of conductivity on the inactivation rates of *Chlorella spp*. (U=20 kV, c=4×10^6 cells mL\(^{-1}\), t=6 min).
In figure 6, after discharging 6 min, the inactivation rate of *Chlorella* spp. with the conductivity of 12 mS cm$^{-1}$ was 13% more than the conductivity of 3 mS cm$^{-1}$. The conductivity increased and the number of survival cells decreased, the kill rate raised up. The addition of conductivity led to the electrical resistance decreasing, the total current in the circuit rising, the voltage applied to discharge gap increasing, so the discharge was more intense and resulted a higher inactivation rate.

3.4. Effect of initial concentration

In Figure 7, the inactivation rates of *Chlorella* spp. with the initial concentration of $4\times10^5$ cells mL$^{-1}$, $4\times10^6$ cells mL$^{-1}$, $4\times10^7$ cells mL$^{-1}$ were 100%, 97.58% and 94.38%. The inactivation rate of *Chlorella* spp. decreased with the addition of the initial concentration. Though the cells had more probability to contact with hydrogen peroxide, ozone and other active substances in the solution, the number of death cells increased, the initial concentration was too large to decrease the killing rate.

![Figure 7. Effect of initial concentration on the inactivation rates of *Chlorella* spp. (U=20 kV, t=10 min, $\sigma$=12 mS cm$^{-1}$).](image)

4. Conclusion

(1) The concentrations of hydrogen peroxide and ozone in solution by gas-liquid mixture DBD increased with the increasing of the discharge peak voltage and treatment time.

(2) Utilizing this system inactivation of *Chlorella* spp., the inactivation rate increased with increasing of peak voltage, treatment time and electric conductivity, and decreased with the addition of initial content.

(3) The inactivation rate of *Chlorella* spp. arrived at 100% when the initial content was $4\times10^6$ cells mL$^{-1}$, and the optimum operation condition required a peak voltage of 20 kV, a treatment time of 10 min.

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