Removal of Microbial Contamination from Surface by Plasma

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Abstract. Microbial contamination is closely associated with human and environmental health, they can be tested on food surfaces, medical devices, packing material and so on. In this paper the removal of the microbial contamination from surface using plasma treatment is investigated. The Escherichia coli (E. coli) has been chosen as a bio-indicator enabling to evaluate the effect of plasma assisted microbial inactivation. Oxygen gas was as the working gas. The plasma RF power, plasma exposition time, gas flow and the concentration of organic pollutant were varied in order to see the effect of the plasma treatment on the Gram-negative germ removal. After the treatment, the microbial abatement was evaluated by the standard plate count method. This proved a positive effect of the plasma treatment on Gram-negative germ removal. The kinetics and mathematical model of removal were studied after plasma treatment, and then the removing course of E. coli was analyzed. This work is meaningful for deepening our understanding of the fundamental scientific principles regarding microbial contamination from surface by plasma.

1. Introduction
Contamination of surfaces by micro-organisms is a major source of problems in health care [1]. For example, surgical instruments were microbially contaminated, which would lead to increased surgical infection incidence [2, 3]. Furthermore, microbiological contamination of food surfaces causes some foodborne illnesses. In 2015, FoodNet identified 20107 confirmed cases of infections, 4531 hospitalizations and 77 deaths caused by nine pathogens transmitted through food at 10 sites, which encompassed 15% of the US population [4]. To avoid risks to the safety of patients and personnel, therefore, controlling microbial contamination has become an important issue and a hot research topic in the worldwide.

In general, many different methods have been used to inactivate microorganism, which widely used two methods including autoclaving and exposure to gases (ethylene) oxide. Both methods, although effective, suffer from drawbacks such as exposure to extremely high temperatures (> 100 °C) in the case of autoclaves and toxic exposure in the case of ethylene oxide. Another concern with the latter is long aeration process (up to 24 h) and, importantly, creates a serious threat for both personnel and the environment [5]. Especially, due to some thermo sensitive materials or food, the decontamination technique of microbial from surfaces of those materials and food, without using toxic materials or high temperatures, is arrestive more and more in the medical field and food industry [4,6,7]. For these reasons, development of new cold techniques of microbial elimination is extremely important. Plasma-based inactivation technique is one of the most serious current alternatives to gaseous sterilization. As a chemically active medium, plasma is formed by excitation, dissociation, and ionization of any gaseous or vaporous substance, including nontoxic substances and even inert gases. Such active particles exist only while the discharge glows, and they disappear almost immediately after turning off the discharge. Furthermore, adequate processes do affect only slightly the bulk material, do not produce toxic by-products, are fast and cost effective. These circumstances completely solve the problems of safety,
ecology and economy. Therefore, elimination of microbial contamination by low temperature plasma has already been regarded as one of the most promising inactivation techniques [1,4,8-12]. For a fair estimation of the efficiency and application range of the plasma removal technique, it is necessary to first investigate the main factors engaged in the gas-discharge plasma. In accordance with these reasons, the paper firstly discusses the influence of the treatment conditions and organic pollutant on the removing efficiency of bio-indicator, Escherichia coli (E. coli), in an oxygen discharge generator. Then, the removing kinetics is discussed on the basis of above obtained data.

2. Experimental

2.1. Materials
E. coli 8099 slant lawn incubated at 37°C for 24 h was oscillated and eluted by phosphate buffer solution (PBS), and then the eluent was diluted to form certain concentration suspensions of bacilli. This suspension was taken 0.01 mL to spread uniformly on 25×25 mm glass sheet and dried naturally at room temperature [13].

2.2. Decontamination testing
The experimental arrangement used is shown schematically in Fig.2-1. RF generator is the type of SY-500W, whose frequency is 13.56 MHz and output power can be adjusted continuously in order to match SP-II matcher. Reflection power can be close to zero by using and adjusting inductive coupled discharge. The reaction chamber is Pyrex glass tube (length 120 mm, diameter 45 mm), where inductance-coupling discharge is applied.

During the experiment, sheets contaminated by bacilli were placed on the carrier and inserted into the reactor in order to carry out the treatment. The purity of oxygen was more than 99.99%. The conditions of treatment were at a discharge power of 20-100 W, exposition time of 20-120 s, and oxygen flow of 20-100 cm³/min. After treatments, several process steps must be applied to the test sheets: first the bacilli have been removed from the sheets into phosphate buffer solution (PBS), then they were transferred to Petri dish which contained nutritional agar and incubated at 37°C for 48 h prior to determining the resulting number of colony forming units. Germicidal effect (GE) was determined by the following equation,

\[ GE = \log N_u - \log N_i \]  

Where \( N_0 \) and \( N_t \) are the number of colony forming units of control and exposed, respectively [13,14].

2.3. Influence of organic pollutant measurement
Serum of moggy was as the organic pollutant and mixed pro-rata with suspensions of bacilli. The concentration of serum was set by 0, 20%, 40%, 60%, respectively. The mixed solution was used to
make samples and then treated them in above mentioned reactor. The method and calculation were in the way described in section 2.1 and 2.2.

3. Results and Discussion

3.1. Germicidal Effect at Various Treatment Conditions

For studying the germicidal efficiency of oxygen plasma, the samples were positioned at the center of the induction coil in the Pyrex glass tube and the GE was as a function of the plasma RF power, the exposition time and the oxygen flow. The GE showed a strong dependence on above mentioned treatment conditions, which are plotted in Figs. 3-1.

The GE changes undulately in a narrow range with increasing the RF power up to 80 W, as shown in Fig. 3-2, and subsequently, a large increase in GE shows between 80 and 100 W, the peak GE value reaches 3.42, i.e. the germicidal efficiency reaches 99.96%. The reason is that above 80 W of RF power, the ionization degree of oxygen gas and average energy of reactive species are augmented rapidly, consequently the probability of action on bacilli is enhanced.

![Figure 3-1. Effect of rf power on Germicidal Effect of E. coli](image1.png)

![Figure 3-2. Effect of exposition time on Germicidal Effect of E. coli](image2.png)

From Fig.3-2, the GE increases with increasing the plasma exposition time up to 50 s, then it tends to stable beyond 50 s. Clearly, the longer exposition time cannot help improving GE. The interaction between bacilli and reaction species has almost completed at 60 s. We consider that a longer treatment time than 60 s with a higher oxygen plasma RF power than 100 W causes heavy degradation and damages on the substrate, especially for the medical polymer [15-17]. Therefore we find that the optimum plasma treatment conditions are 100 W power and 60 s exposition time to obtain the maximum GE. For the subsequent investigation of the effect of oxygen flow on GE, we fix the two conditions for sample decontamination as the optimum plasma treatment conditions.

Fig.3-3, shows the effect of oxygen flow on GE. The GE increases firstly but decreases later with increasing oxygen flow. When discharge power and treatment time are fixed, the energy of system received is a constant. At low oxygen flow, though the frequency of reaction species colliding with the bacilli is less than that at high flow, the average energy of them is higher than that with high flow. Thus, under 40 cm$^3$/min, with increasing the gas flow, the efficiency of bacterial decontamination is enhanced and GE increases. Beyond 40 cm$^3$/min, the number of reaction species is large but the average energy and residence time is lower, so the action on bacilli is comparatively small. Consequently, when the oxygen flow increases, while other two conditions are fixed, the GE plays down. In a word, action on bacilli is the combining effect of the energy and the amount of reaction species and residence time. All these values in Figs.3-4 indicate that the optimum oxygen plasma treatment conditions are of 100 W, 60 s and 40 cm$^3$/min for the maximum GE from surface.
3.2. Influence of Organic Pollutant on Germicidal Effect

Fig. 5 shows the typical influence of different serum concentration of moggy on germicidal effect. From Fig. 3-4, germicidal effect plays down sharply with increasing the serum concentration of moggy. This conclusion indicates that elimination efficiency of bacilli by oxygen plasma is very unsatisfactory when organic pollutant, for example, the serum of moggy, is present. The reason could be as follows [18].

(1) Organic pollutant can form a protection course on microbial surface which can hold back the interaction between activated particles such as electron, ions and radicals and microorganisms so as to microorganisms produce adaptability to these activated particles. (2) The interaction between organic pollutant and activated particles can produce compound with lower solubility which has mechanical protecting action to microorganisms. (3) Some activated particles react to organic pollutant such as neutralization so as to their concentration acted on microorganisms reduces.

Germicidal kinetics from surface by oxygen. Using the Origin software to fit the experimental data, the kinetic curve of removing bacteria by oxygen plasma was obtained, as shown in Fig.3-5. After comparing with typical curves of inactivation kinetics of microorganism in classical report (Fig.3-6) [18], we found that the curve of germicidal kinetics of E. coli from surface by oxygen plasma belongs to typical concave curve. It indicates that E. coli was killed by oxygen plasma undergoing the steps as follows: susceptible stirps were changed to resistive ones before they were killed [13,19]. Based on the reported choices within the fitting functions of inactivation curves, the mathematical model of oxygen plasma decontamination was obtained, which is shown by equation (2).
3.3. n plasma

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\[
\log \frac{N(t)}{N_0} = A_1 + (A_2 - A_1) \left( 1 + e^{(R-1.999)} \right) (R = 0.999)
\]

(2)

Where \(A_1 = -1.51535\), \(A_2 = -3.34452\), \(B = 3.41965\).

Transforming above equation after filling the values of every parameters in it, the final function of inactivation kinetics of E. coli from surface by oxygen plasma is obtained as follows.

\[
\frac{N(t)}{N_s} = \frac{K_1}{1 + K_2 e^{\lambda_2 t} - \lambda_2 t}
\]

(3)

Where \(K_1 = 1.82917\), \(K_2 = 0.10794\), \(\lambda_1 = 0.29243\), \(\lambda_2 = 3.34452\).

4. Conclusions

In this paper, the removal of the microbial contamination from surface using plasma treatment is investigated. The main results are summarized as follows:

A) We found the optimum oxygen plasma treatment conditions were 100 W power, 60 s treatment time and 40 cm³/min oxygen flow to obtain the maximum germicidal effect of E. coli.

B) Because the influence of organic pollutant on germicidal effect was great, we also known the polluted appliances with blood and salt couldn’t be treated by this kind of oxygen plasma. If do, the cleaning procedure was absolutely necessary.

C) Fitting the experimental data using Origin software, the inactivation kinetics curve and mathematical model of oxygen plasma treatment were obtained, and then the obituary course of E. coli was analyzed. Equation (3), Constructed with the simple format, can provide the theoretic gist and guidance for forecasting the bacterial removal efficiency from surface, improving the treatment method and designing the plasma equipment.

This work is meaningful for deepening our understanding of the mechanism principles regarding microbial contamination from surface by plasma, providing valuable and feasible references for researchers and designers in microbial contamination control. Controlling the contamination of microorganisms of surfaces by promising technology plasma decreases the safety risks of environment and human.

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