

GAS DISCHARGE PLASMA AS A NOVEL TOOL FOR BIOFILM DESTRUCTION

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Summary:

Biofilms are bacterial communities embedded in an exopolysaccharidic matrix with a complex architectural structure. Bacteria in biofilms show different properties from those in free life thus, conventional methods of killing bacteria are often ineffective against biofilms. The use of plasmas potentially offers an alternative to conventional sterilization methods since plasmas contain a mixture of charged particles, chemically reactive species, and UV radiation. 4 and 7 day-old single-species and mixed biofilms were produced using two bacterial species. Gas discharge plasma was produced by using an Atomflo™ reactor (Surfx Technologies) and bacterial biofilms were exposed to it for different periods of time. Our results show that a 10-minute plasma treatment was able to kill 100% of the cells in most cases. Optical emission spectroscopy was used to study plasma composition which is then correlated with the effectiveness of killing. These results indicate the potentiality of plasma as an alternative sterilization method.

Key words: bacterial biofilms, atmospheric pressure plasmas, sterilization methods, biofilm destruction

1 Introduction

A biofilm is a highly structured bacterial community embedded in an exopolysaccharidic matrix with a complex architectural structure that adheres to solid surfaces or interfaces exposed to nutrients. Microorganisms in biofilms show different properties, including a different antibiotic resistance, from those in planktonic life [1]. Biofilms play an important role in bacterial pathogenesis in humans, animals, and plants, and in bacterial attachments to surfaces such as in medical devices, dental waterlines, etc. Biofilms commonly colonize many household surfaces, including toilets, sinks, countertops and cutting boards; they cause pipe plugging, corrosion and water contamination in industries. Effective bacterial biofilm destruction poses a very serious problem, since the microbial communities are much more resistant to conventional methods of killing bacteria (such as antibiotics, sterilization by heat, sterilization by chemical processes and UV radiation) than are individual microbes. Moreover, there are no proven methods for destroying biofilms *in situ*. The limitations of the current sterilization methods (some of which include the use of environmentally undesirable techniques such as sterilization by chemical processes) demand the development of alternative techniques. The use of plasma offers a very promising and more widely applicable alternative to conventional sterilization methods as plasmas contain a mixture of charged particles, chemically reactive species produced by plasma chemical reactions, and UV radiation, all of which have been shown to destroy bacteria.

2 Materials and Methods

Four and seven day-old biofilms were produced in polystyrene microplates by adding a suspension of a gram-negative bacteria, *Rhizobium gallicum*. 96-wells microplates were chosen since the internal size of each well is exactly the size of the plasma reactor applicator ensuring a uniform exposure of cells. After the desired period of time, planktonic cells were removed from the wells and plates were subjected to discharge plasmas under controlled conditions and varying exposure times (0 to 60 minutes). Biofilms were then destroyed by sonication, and bacteria suspended, diluted and plated onto Petri dishes. Colony-forming units (CFUs) were determined after 24 hours of incubation. In order to evaluate the onset of the killing process, additional experiments were performed using shorter exposure times (0 to 5 minutes).

Gas discharge plasma was produced by using an AtomfloTM 250 reactor (Surfx Technologies, CA). It consists of two perforated rectangular plates separated by a gap 1.6 mm across. The upper aluminum electrode is connected to a 100-W RF power supply (13.56 MHz), the lower electrode is grounded. The size of the plasma showerhead is 0.25 inches wide by 1.0 inch across. For the microbiological experiments, an atmospheric pressure plasma jet was generated by using a He flow of 20.4 L/min, a secondary gas flow (N₂) of 0.305 L/min and an input power of approximately 4.8 watts. The plasma applicator was mounted such that the showerhead was only a few millimeters away from the biofilm.

Emission spectroscopy studies were carried out for the same plasma conditions stated above. The experimental set-up for these studies consisted of a 0.5 nm resolution spectrometer in conjunction with a CCD detector (Ocean Optics HR-2000). The output of the CCD detector was fed into the computer for data storage and analysis. To optimize the collection of light, a fiber optics was used to focus the light from the discharge plasma onto the entrance slit of the monochromator.

3 Results and Discussion.

Almost complete killing was achieved after a 5-minutes treatment with plasma (Figures 1 and 2). Both types of biofilms were similarly destroyed by plasma treatment suggesting that the methodology might be effective regardless the age of the biofilm. Further research is carried out with different bacterial species and mixed biofilms. D values were around 2 minutes for both types of biofilms.

A typical emission spectrum is shown in figure 3. The characteristic features of the spectrum in the far ultraviolet are the NO γ -bands near 250 nm and an OH band around 309 nm. These reactive species have direct impact on the cells of microorganisms, and especially on their outmost membranes [2] and on the cell walls. The most prominent emission is due to the N₂ 2nd positive band.

We used the N₂ emissions to determine rotational temperatures of the plasma species. The second positive band system of N₂ and the first negative band system of N₂⁺ are the most frequently analyzed emission systems because the molecular constants describing these two transitions are well-known [3]. Thus, synthetic model spectra of these two band systems can be calculated easily and with high accuracy, and the rotational temperature can be determined from a fit of the measured emission spectrum (either rotationally resolved or unresolved) to the

synthetic spectrum. The rotational temperatures reflect the rotational population of the emitting species. If the emitting species are in equilibrium with the bulk gas in the plasma, then this temperature can be interpreted as the gas kinetic temperature in the plasma. Using the (0,1) band of the N₂ second positive system near 357 nm a plasma temperature of 325 K was calculated. This is significantly lower than the typical autoclaving temperature (394 K) therefore temperature is not responsible for biofilm destruction.

4 Conclusions

Our results clearly indicate the potentiality of plasma as an alternative sterilization method. Further research is being carried out to find conditions that completely destroy bacterial biofilms and to elucidate the mechanisms underlying the process.

References.

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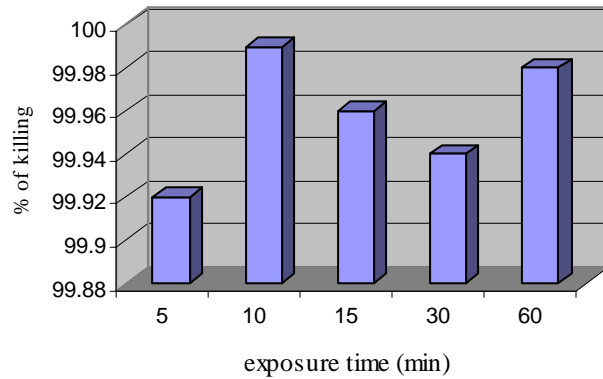


Figure 1. 4-day-old *Rhizobium gallicum* biofilm after plasma treatment

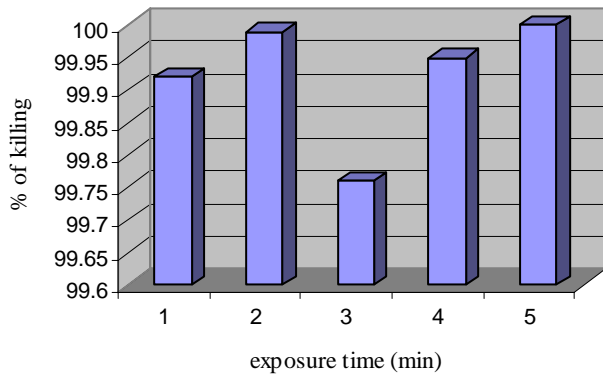


Figure 2. 7-day-old *Rhizobium gallicum* biofilm after plasma treatment

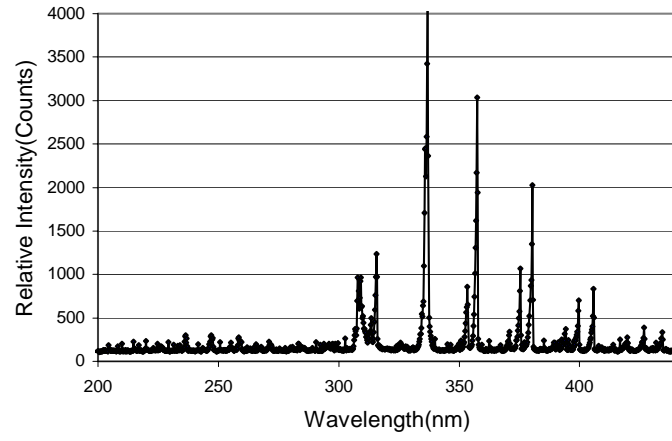


Figure 3: Relative plasma emission intensity as a function of wavelength.