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Micro Oxidation Sterilization by Non-Thermal Plasma Technology

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ABSTRACT

This study was carried out to compare the efficiency of sterilization by using non-thermal plasma technology with other traditional sterilizations and to study more on the concept of non-thermal plasma technique on kitchenware. Different kitchenware was used during this study. They were stainless steel plates, plastic plates and frying pan. *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* were used as contaminants and were grown on Brain Heart Infusion agar and broth (BHI) medium. Standard Gram staining method and light microscopy were used to observe the characteristics of the bacteria. Plasma chamber was used to expose the kitchenware directly to plasma. They were exposed at different durations. There was completely no growth of bacteria after 30 minutes of exposure to plasma for all three different conditions applied on those specific kitchenware suggesting this to be the optimum time point reach by this plasma chamber for sterilization purpose.

Keywords: *Micro oxidation, sterilization, Plasma technology, non-thermal.*

INTRODUCTION

Sterilization is the process of killing all forms of microbial existence in or on particular objects. In microbiological term, sterile material represents no living organisms at all [1]. One type of sterilization method is chemical sterilization where this technique occupies a treatment of preparations to be sterilized with selected chemicals in either gaseous or liquid form. Gaseous sterilization is done by exposure to a gas that destroys microorganisms. The most commonly used gas for sterilization is ethylene oxide and formaldehyde [2]. However, ethylene oxide has some drawbacks as it residues being absorbed on devices after sterilization process where it is highly toxic, carcinogenic and mutagenic. Same goes with formaldehyde too [3]. So, gaseous sterilization by using plasma is the best. It is a safer technique to be applied and free from harmful properties compared to the other types of gaseous used before.

Plasma is an incompletely or entirely ionized gas comprising of various elements, such as electrons, ions, atoms, and molecules [4]. It is an efficient biological disinfectant [5] for microorganisms. There are two categories of plasma which are thermal plasma and non-thermal plasma. Thermal plasma is where almost all its elements are at equilibrium condition. The non-thermal plasma is not in the equilibrium condition. It differs significantly between the electrons and the other particles such as ions, atoms, and molecules. Non-thermal plasma

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is also known as cold plasma [6], produce a variety reactive constituents, including charged particles and UV radiation, without increasing temperature. Oxidation is a reaction of a substance with oxygen as the electrons were lost during the oxidation process. These tiny substances converted into volatile compounds that can be pumped away. It also referred as micro oxidation [6].

Since, non-thermal plasma (NTP) was reported to have shown advantages such as using low temperature and under appropriate situation [7], injury of the objects or materials can be reduced [3], used for inactivation of surface contaminants [8], eliminate the yield of toxic by-product [8] and also affordable cost effective methods [9], therefore, our aim of this study were to compare the efficiency of sterilization by using non-thermal plasma technology with other traditional sterilizations and to study more on the concept of non-thermal plasma technique on kitchenware.

MATERIALS AND METHOD

Preparation of culture media and bacterial strains

The Brain Heart Infusion agar and broth (BHI) medium were prepared based on needs. *Escherichia coli (E. coli)* and *Staphylococcus aureus (S. aureus)* were grown on Brain Heart Infusion (BHI) broth medium with suspension of 10 ml at 37°C for 24 h.

Contamination of Surfaces

E. coli and *S. aureus* were employed as the target to be sterilized. In this experiment, the kitchenware chosen to be used were stainless steel plate, plastic plate and frying pan. Three items of each kitchenware would be sterilized in four different time points in three variable types of condition each. The three conditions applied are (1) normal washing without any bacteria inoculated on its surface, (2) inoculation with *E. coli* on each surfaces of kitchenware and (3) inoculation with *S. aureus* on each surfaces of kitchenware by using swabbing technique.

Chamber Cleaning

Sterile the chamber surface with alcohol swab to avoid any contamination during the experiment.

Plasma Treatment

The plasma generator was set at 110V and 50-Hz frequency for all experiments. All the kitchenware (control and contaminated surfaces) were introduced into the plasma chamber for 10, 20 and 30 minutes duration. These plates were exposed directly to the plasma. At a certain pressure, sterilization gases (air, O_2, H_2O_2, N_2, H_2O) were fed into the chamber, separately and were allowed to flow at a specific rate.

After plasma treatment, surface of all kitchenware used were swabbed by using sterile cotton swab on BHI agar medium before and after located in the plasma chamber. Then all the petri dishes were incubated for 24 hours at 37°C. Sterilization effect of plasma O_2 was inspected by comparing the number of colonies with and without plasma treatment.

haracterization of experiment (Confirmation test)

Gram staining and microscopic morphology observation were used to observe the bacteria.

Gram Staining

It is a differential staining technique used to characterize bacteria as Gram positive and Gram negative. Standard Gram staining method was used. The fixed bacterial smear is subjected to Crystal Violet, Iodine Solution, Alcohol (decolorizing agent) and Safranin respectively. Gram-positive bacteria retain crystal violet and hence appear deep violet in color, while Gram negative bacteria lose the crystal violet and are counterstained by the Safranin. Hence they appear red in color. After Gram staining bacteria were observed under a Light Microscope to observe their shape and arrangements.

RESULT AND DISCUSSION

Based on the results obtained from the experiment, the condition of normal washing for stainless steel plate shows a few colonies grow on the media at 0 minute before put into the plasma chamber and the colonies become fewer at 10 minutes after put into the chamber. No colony grows at 20 minutes and 30 minutes after plates were put into the plasma chamber. Another condition is contamination of plate surface with *E. coli* strains show the growth of colonies all over the media at 0 minute before the plate was put into the chamber and the colonies become lesser at 10 minutes after put into the plasma chamber. There are totally no colony grows at 20 and 30 minutes' time points. For surface contamination with *S. aureus*, the results obtained same

with the *E. coli* contamination before. So, overall of these three conditions show no colony grows at 30 minutes after put the plate into plasma chamber. The result has been summarized and can be referred in **Table 1.**

Table 1: Experiments carried out on Stainless Steel Plates

Type of condition	Before placed in the chamber	After placed in the chamber		
	0 min	10 min	20 min	30 min
Normal washing	Few colonies grow on media	Fewer colonies grow than before (0 min)	No colony grows	No colony grows
Escherichia coli (*E. coli*) swab (on surface)	Colonies grow overall the media	Less colonies grow than before (0 min)	No colony grows	No colony grows
Staphylococcus aureus (*S. aureus*) swab (on surface)	Colonies grow overall the media	Less colonies grow than before (0 min)	Lesser colonies grow than after 10 min	No colony grows

These 3 types of condition were also applied on another kitchenware which is plastic plate and frying pan. The results were shown in **Table 2** and **Table 3** below.

Table 2: Experiments carried out on Plastic Plates

Type of condition	Before placed in the chamber	After Placed in the chamber		
	0 min	10 min	20 min	30 min
Normal washing	No colony grows	No colony grows	No colony grows	No colony grows
Escherichia coli (*E. coli*) swab (on surface)	Colonies grow overall the media	No colony grows	No colony grows	No colony grows
Staphylococcus aureus (*S. aureus*) swab (on surface)	Colonies grow overall the media	Less colonies grow than before (0 min)	14 colonies grow on the media (lesser)	No colony grows

Table 3: Experiments carried out on Frying Pan

Type of condition	Before placed in the chamber	After Placed in the chamber		
	0 min	10 min	20 min	30 min
Normal washing	No colony grows	No colony grows	No colony grows	No colony grows
Escherichia coli (*E. coli*) swab (on surface)	Colonies grow overall the media	No colony grows	No colony grows	No colony grows
Staphylococcus aureus (*S. aureus*) swab (on surface)	Colonies grow overall the media	Less colonies grow than before (0 min)	Lesser colonies grow than 10 min	No colony grows

The colonies grown were subjected to Gram staining which showed Gram positive cocci in clusters. This microorganism is facultative anaerobes and is expected to be *Staphylococcus aureus.*

As there are completely no colony grows at each 30 minutes for all three different conditions applied on those specific kitchenware, so this is the optimum time point reach by this plasma chamber for sterilization purpose.

Plasma O^{-2} technology produces a corona-effect without sparking. Each tube has 100 discharge points producing an abundant stream of oxygen plasma for effective and continuous sterilization and purification of air and surfaces. The unit produces a controlled and continuous high energy electron discharge across the glass wall of the plasma tube. This splits the oxygen molecules in the air to form negatively-ionized oxygen plasma. One of the oxygen radicals found in the plasma include hydrogen peroxide (H_2O_2), a very powerful disinfectant and cleanser. When it encounters bacteria, it quickly oxidizes some of the components of the cell membrane causing the bacteria to die quickly. Therefore, it could be a very efficient biocidal against bacteria. The plasma treatment can effectively inactivate a wide range of microorganisms including spores and viruses. This low- pressure oxygen plasma has been shown to degrade lipids, proteins and DNA of cells.

The plasma chamber is also not an ozone-generator. Ozone generators produce high levels of ozone which are toxic to human. The plasma chamber complies with the World Health Organization (WHO) standards on ozone emission (less than the permitted level of 0.05ppm).

Some of the general characteristic of this plasma chamber are - (1) using the non-thermal plasma technology, (2) small in size, compact and silent chamber, (3) consumed low energy, (4) designed for 24 hours operation and (5) maintenance-free.

The plasma is highly reactive and purifies both air and surfaces by killing bacteria and viruses, 98% odor neutralization and toxic gases, cleansing the air of dust and particulates, reduce aerobic bacteria, mold and fungus up to 90% germ sterilization and freshening the air with negative ions.

CONCLUSION

Cold plasma treatment is a promising technology which acts rapidly and does not leave toxic residual on processed parts of kitchenware (on its surface). The temperature rise also can be kept to an acceptable level. The cold plasma is an emerging disinfection method that approach for reducing the microbial populations on the surface of kitchenware at 30 minutes as the optimum time taken.

Ethical Clearance- Not required

Source of Funding- Self

Conflict of Interest - Nil

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