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Assessment of Bacterial Sterilization Efficiency Using a Custom-Designed Cold Atmospheric Pressure Plasma Jet System

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ABSTRACT

The present research work, an optimized sterilization method has been introduced using atmospheric pressure cold plasma. A developed pin-to-pin atmospheric pressure setup of cold yield plasma, the working operational parameters of the designed setup are equal to (11kV, 50µA, 17kΩ, 10L/min and 0.3mm, 11mm) for the electrode potential, the plasma current, ballast resistance and airflow rate, the electrode pins diameter and spacing respectively. The disinfection process was subjected on flat glasses substrates contaminated with several bacteria types including, *Proteus*, *Pseudomonas*, *Klebsiella pneumoniae*, *Escherichia coli*, *methicillin-resistant Staphylococcus aureus* (MRSA) and *Staphylococcus aureus*. The study demonstrates the dependent of the bacterial sterilization on the exposure time. The rates of exposure time of the contaminated slides were chosen in a relatively short time period equals 5, 10, 15 and 25 min the process was repeated statistically to confirm the effects. Sterilization efficiency was measured by counting the remaining colonies after treatment and standardizing against a 0.5 McFarland reference. The results expose that; all the bacteria type is eliminated at different exposure time. Since the *E. coli* bacteria elimination time is 10min, the *S. aureus* bacteria removal attended at 25min. this study shows that the cold plasma with suitable setup design and operational parameters can implemented for successful sterilization method as this type of sanitization has several practical advantages.

Keywords: Cold Atmospheric Pressure Plasma, Cold plasma sterilization, *Proteus* sterilization, *Pseudomonas* sterilization, *Klebsiella pneumoniae* sterilization,

INTRODUCTION

The physical or chemical sterilization process aims to eradicate all germs, including viruses, fungi, bacteria and resistance microbes. This process is essential in environments that require a high standard of safety and hygiene such as medical facilities, research laboratories, food and pharmaceutical industries (Acosta-Gnass *et al*., 2010; Favero *et al*., 2013; Bharti *et al*., 2022). Due to developments in functional response and genetic compositions in microbes, there is an urgent need for safer, non-thermal sterilization technique (Wani *et al*., 2022). Despite the multitude of traditional sterilization methods, which include chemical sterilization, radiation sterilization such as ultraviolet (UV) and ionizing radiation using gamma ray, based on the process, the materials to be sterilized and the purpose of sterilization (Bharti *et al*., 2022; Chauhan *et al*., 2020; Favero *et al*., 2013). These methods have disadvantages especially when used with heat sensitive materials or chemicals, they also have a negative impact on the environment and leave behind toxic waste (Elsheikh *et al*., 2024). Among these techniques cold atmospheric pressure plasma is one of the most prominent. This active medium comprises charged particles, free radicals and low energy ultraviolet light, which collectively compromise the bacterial cell wall and disrupt essential functions through both physical and chemical mechanisms (Marsit *et al*., 2017; Stoffels *et al*., 2008; Zhao *et al*., 2024). Cold plasma advances an excellent option in medical, biological and micro-technical, based on its proven efficient and effective in sterilizing surfaces and materials (Deepak *et al*., 2018; Katsigiannis *et al*., 2022).

To study bacterial sterilization, a cold atmospheric pressure plasma system utilizing tungsten pins was developed. The system's parameters were meticulously adjusted to generate an innovative cold plasma capable of effectively sterilizing microorganisms. This system was employed to assess its efficacy in sterilizing six bacterial strains: *Proteus*, *Pseudomonas*, *Klebsiella pneumoniae*, *Escherichia coli*, *Staphylococcus aureus* (MRSA), and *Staphylococcus aureus.*

MATERIALS AND METHODS

In Fig. (1), the tungsten pin-to-pin cold atmospheric plasma jet system is schematically depicted. The discharge circuit was powered by a negative DC voltage source rated at (11.66 kV, 50 µA), which was connected to a pair of tungsten pin electrodes through a ballast resistor of 17 kΩ. These electrodes, each with a diameter of 0.3 mm, were aligned linearly and positioned opposite each other at the outer diameter of a Pyrex glass airflow tube with an inner diameter of 11 mm. The tungsten pins extended 2 mm beyond the end of the tube to ensure direct interaction with the airstream. An air compressor provided air at a controlled flow rate of 10 L/min to facilitate the generation of plasma. Fig. (2) shows a photograph of experimental setup of plasma jet systems used.

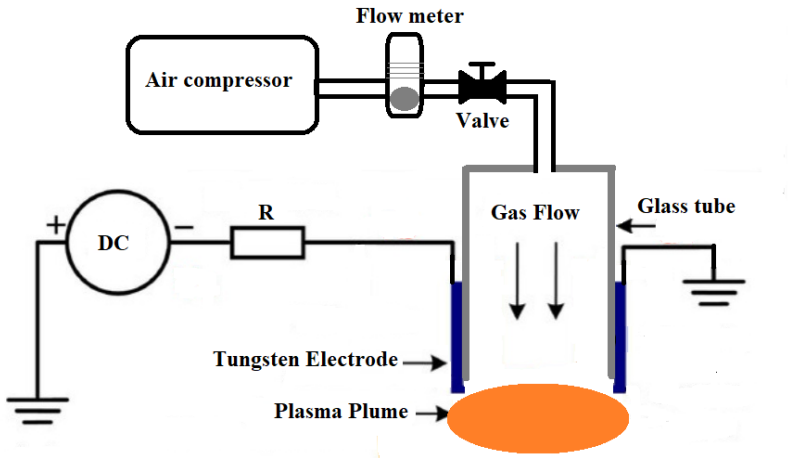

Fig.1: Schematic of the designed plasma jet system.

Fig. 2: A photograph the experimental setup of plasma jet systems

To assess the bacterial sterilization efficacy of the designed cold plasma system, a nutrient agar culture plate was prepared for culturing bacterial samples, which were incubated at 37° C for 24 h. Subsequently, a bacterial suspension was prepared for each bacterial type. These suspensions were exposed to plasma for varying durations (5, 10, 15, and 25 min). The specimen was placed on a glass slide and positioned in the path of the plasma jet emerging from the nozzle of the tube. The system was activated while maintaining a constant distance of 2 mm between the nozzle and specimen surface, as shown in Fig. (3), this figure illustrates the glowing tube used in this research work to sterilization process. After each exposure period, a swab was obtained from each plasma-treated bacterial suspension. These samples were subsequently re-cultured on fresh nutrient agar plates and incubated under identical conditions (37° C for 24 h). Upon completion of the incubation period, the number of bacterial colonies formed was enumerated and compared to the initial bacterial count prior to treatment, using a McFarland tube at a concentration of 0.5 as a reference to ascertain the bacterial killing rate attributable to plasma exposure. (Table 1) represents the effects of plasma exposure on the viability of bacterial colonies from various bacterial species across different time intervals.

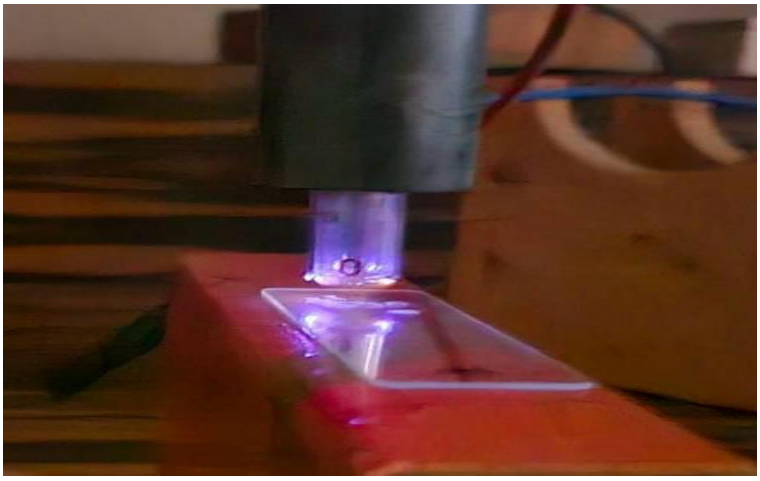

Fig. (3): Sterilization of a bacterial sample on a glass slide utilizing a cold atmospheric pressure plasma jet.

Table 1: Effects of plasma exposure on the viability of bacterial colonies from various bacterial species across different time intervals.

Exposure Time (min)	Number of colonies					
	E. coli	*Klebsiella*	*Proteus*	*Pseudomonas*	*S. aureus*	*S. aureus* (MRSA)
5	1	349	11	5	79	3
10	0	3	8	1	20	1
15	0	0	1	0	2	0
25	0	0	0	0	0	0

RESULTS AND DISCUSSION

Following exposure to cold plasma, a significant reduction in the number of bacterial colonies was observed in all samples. Fig. (4) demonstrates a clear correlation between the duration of exposure and the efficacy of eliminating bacterial cells. This figure shows that the percentage of eradicated cells increased progressively with extended exposure times until complete eradication was achieved at time period equals (25 min). The effectiveness of these agents varied depending on the duration of exposure and the specific type of bacteria involved.

At an initial exposure duration of 5 min, the plasma exhibited a partial bactericidal effect. *Escherichia coli* was notably affected, with only one colony surviving, whereas *Pseudomonas aeruginosa* and methicillin-resistant *Staphylococcus aureus* (MRSA) displayed low survival rates. In contrast, *Klebsiella pneumoniae* and *Staphylococcus aureus* demonstrated greater resistance, with 340 and 79 colonies, respectively.

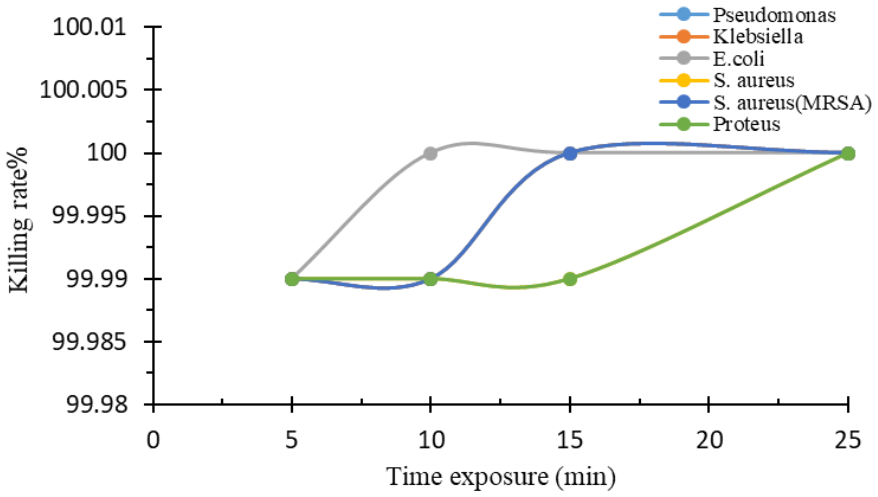

Fig. 4: Correlation between exposure duration and the percentage of bacterial eradication.

Increasing the exposure time to 10 min resulted in the plasma effectively eliminating most bacterial species. *E. coli* was completely eradicated, while the number of Klebsiella colonies dropped from 349 to just 3, and *S. aureus* colonies reduced from 79 to 20. Extending the exposure to 15 min led to near-total destruction of most strains, with *Pseudomonas*, *Staphylococcus aureus* (MRSA), *Klebsiella pneumoniae*, and *E. coli* being entirely eliminated, leaving only one colony of *Proteus* and two colonies of *Staphylococcus aureus*. After a 25-minute exposure period, no bacterial colonies were detected, indicating that all bacterial strains under investigation were completely sterilized, as shown in the photographic Fig. (5).

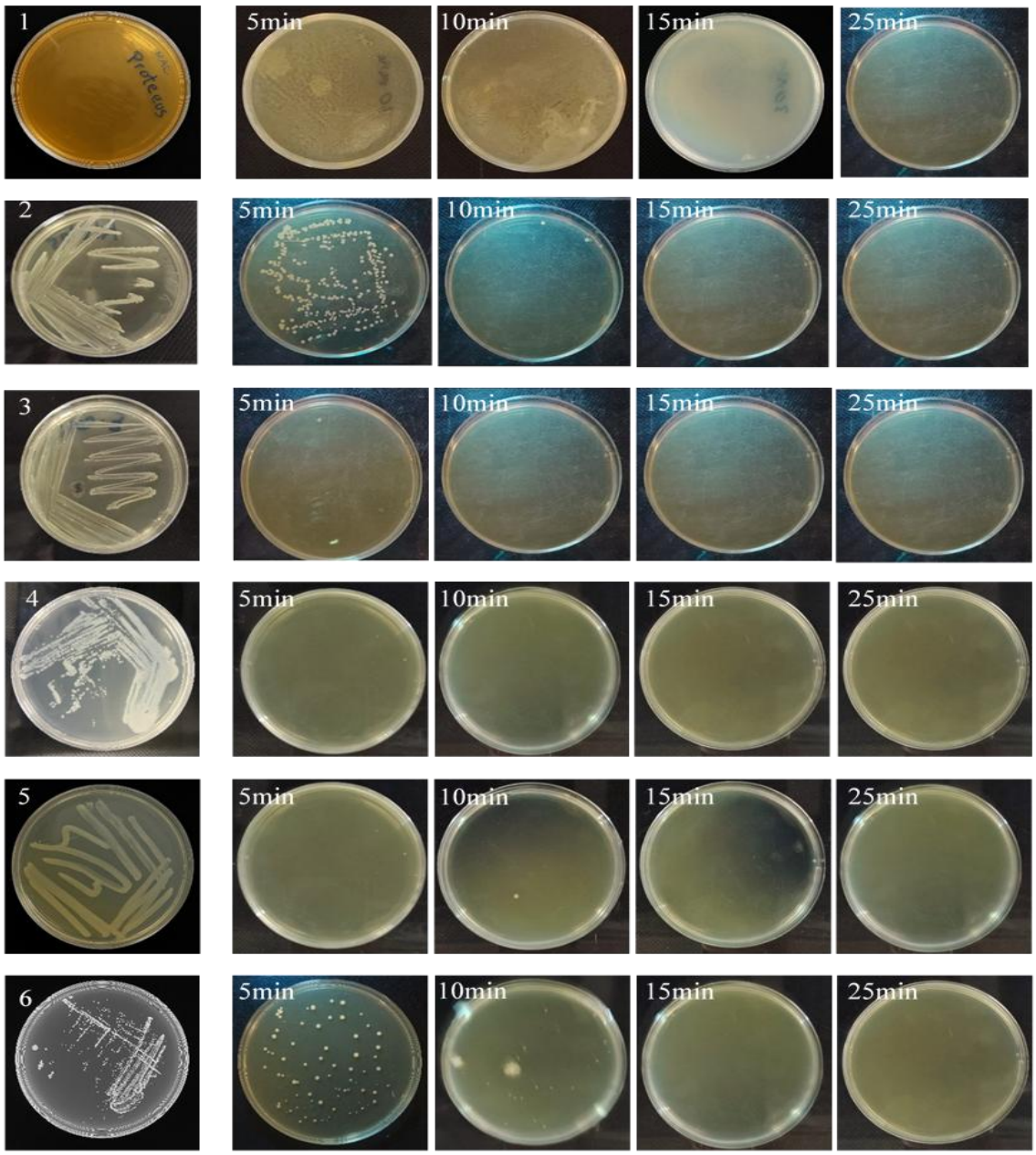

Fig. 5: represents the photograph elementation of the bacterial colonies type (1- *Proteus*, 2- *Klebsiella pneumoniae*, 3- *E.coli*, 4- *Pseudomonas*, 5- *Staphylococcus aureus* (MRSA), and 6- *Staphylococcus aureus*)

(Table 2) represents the percentage of bacteria eradicated by comparing the number of remaining colonies with those from a sample taken from a McFarland tube at 0.5 (1.5×10^8 CFU/mL). These samples were obtained from the Biology Department, College of Science at Mosul University.

Table (2): Percentage reduction of CFU (%) following exposure to cold atmospheric pressure plasma over varying durations.

Time exposure (min)	*E. coli*	*Klebsiella*	*Proteus*	*Pseudomonas*	*S. aureus*	*S. aureus* (MRSA)
5	99.99	99.99	99.99	99.99	99.99	99.99
10	100	99.99	99.99	99.99	99.99	99.99
15	100	100	99.99	100	99.99	100
25	100	100	100	100	100	100

Cold plasma has demonstrated significant efficacy in biological sterilization processes owing to its capacity to generate bioactive components that adversely affect bacterial cells, leading to their destruction or inactivation (Zhu *et al*., 2022). These components include reactive oxygen and nitrogen free radicals, such as ozone, hydroxide radicals, nitrogen oxides, and hydrogen peroxide, which target the bacterial cell wall and membrane, resulting in the oxidation of essential components and causing structural and functional damage. Additionally, ultraviolet rays produced by plasma directly damage DNA, impeding cell division and inducing apoptosis (Vatansever *et al*., 2013; Al-Shehri, 2021; Tvrdá *et al*., 2020). When active particles in plasma jet collide localized heat shocks are formed, which damages the cell's proteins and biological components (Morabit *et al*., 2021). Additionally, the generated charged particles such as ions and electrons cause cracks or holes in the cell membrane, which allow cell contents to flow out and degrade (Zhu *et al*., 2024). The results of this study demonstrate that bacteria's response to plasma is influenced by their cellular structure, where gram-negative bacteria such as *Klebsiella pneumoniae* and *Proteus* are more resiste due to their outer membrance, which is rich in lipopolysaccharides (LPS) and serves as a barrier against oxidizing agents (Al-Oqaidy *et al*., 2010). While gram-positive bacteria because of their lack an outer membrane and have a thick peptidoglycan layer, they are more susceptible to free radical penetration (Al-Allaf *et al*., 2014).

One important discovery of this study is that plasma can totally eradicate antibiotic resistant *Staphylococcus aureus* (MRSA) germs, as well as methicillin-resistant bacteria. As these bacteria are a major concern in medical environments and are difficult to eliminate using traditional sterilization procedures. These finding implicitly to the effectiveness and efficiency of cold plasma as a biological sterilization tool, as it is considering one of the effective sterilization methods against resistant bacteria. The device's efficiency is affected by factors including exposure time, type of bacteria and operating parameters such as discharge voltage, gas type and flow rate.

CONCLUSIONS

The results indicate that cold plasma technology, which offers a safer alternative to traditional methods, could be a valuable tool in various sterilization applications. Based on research on bacterial sterilization using cold plasma jet system operating at atmospheric pressure between specially designed tungsten pin-to-pin, cold plasma is a promising and relatively safe technology for biological sterilization, and is particularly effective against resistant bacterial strains. Several important factors influence the effectiveness of this technique, including gas type, flow rate, discharge voltage, exposure time and bacterial species.

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تصميم منظومة بلازما باردة تعمل تحت الضغط الجوي الاعتيادي ودراسة أدائها في التعقيم البكتيري

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الملخص

في هذا البحث، تم تقديم طريقة تعقيم محسنة باستخدام البلازما الباردة ذات الضغط الجوي، وهي عبارة عن جهاز متطور يعمل بالضغط الجوي من نوع دبوس الى دبوس لإنتاج البلازما الباردة. المعلمات التشغيلية للجهاز المصمم هي كما يلي: (11 كيلو فولت، 50 ميكرو أمبير، 17 كيلو أوم، 10 لتر/دقيقة، و0.3 ملم، 11 ملم)، بالنسبة الى جهد القطب الكهربائي، تيار البلازما، مقاومة الصبورة، معدل التدفق، قطر الاقطاب الكهربائية والكثافة بن الاقطاب على التوالي. تم إجراء عمليات التطهير على ركائز زجاجية مسطحة ملوثة بعدة أنواع من البكتيريا، بما في ذلك بروتيوس، الزائفة الزنجارية (Pseudomonas)، كليبسييلا الرئوية، الاشريشية القولونية، المكورات العنقودية الذهبية المقاومة للمثيسيلين (MRSA)، والمكورات العنقودية الذهبية. توضح الدراسة اعتماد تعقيم البكتريا على مدة التعريض تم اختيار معدلات مدة تعريض للشرائح الملوثة في فترات زمنية قصيرة نسبيا تساوي (5, 15, 15 و25) دقيقة، وتكررت العملية احصائياً لتأكيد التأثيرات. تم قياس كفاءة التعقيم عن طريق حساب المستعمرات المتبقية بعد المعالجة وتوحيدها مقابل مرجع 0.5 ماكفرلاند. أظهرت النتائج ان جميع انواع البكتريا تم القضاء عليها في أوقات تعرض مختلفة. نظراً لأن وقت القضاء على البكتريا الاشريشية القولونية هو10 دقائق، فأن ازالة بكتريا المكورات العنقودية الذهبية تمت في 25 دقيقة. تظهر هذه الدراسة ان البلازما الباردة ذات التصميم المناسب والمعلمات التشغيلية يمكن تنفيذها كطريقة تعقيم ناجحة، حيث ان هذا النوع من التعقيم له العديد من المزايا العملية.

الكلمات الدالة: البلازما الباردة عند الضغط الجوي، تعقيم بالبلازما الباردة، تعقيم البروتيوس، تعقيم السيدوموناس، تعقيم الكليبسيلا