

## An interesting approach for Biofilm disruption by Thermocouple device

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### Abstract

Microbial biofilm are an aggregate of microbial population encompassed in a matrix of self secreted extracellular polymeric substances (EPS). In various sectors from human health to industries biofilm bring with it an array of harmful impacts. For instance, biofilm linked diseases like chronic otitis, chronic osteomyelitis, urinary tract infections, cystic fibrosis-associated lung infection etc. cannot be treated with regular antibiotics since profound use of the same have led to the existence of several drug resistant strains. Effective techniques for combating this bacterial biofilm are still a challenging issue to the scientific community. Towards this direction, in the present study efforts have been put to use electric shock generated by a thermocouple device to disrupt bacterial biofilm formation. The uniqueness of the procedure lies with the method of generation of the electric shock, wherein a difference in temperature has been exploited to create the shock. Using this method, electric shocks of 105 $\mu$ V was applied for different durations on bacterial biofilm formed by *Pseudomonas* sp. and *Staphylococcus* sp. In each case a difference in response was observed which led to the conclusion that *Staphylococcal* biofilm as well as *Pseudomonas* biofilm are susceptible to the electric shocks. Hence, this interesting method of usage of low voltage electric shock could be a potential method to fight against biofilm threats.

**Keywords:** Bacterial biofilm, thermocouple device, low voltage electric shock, *Pseudomonas* sp., *Staphylococcus* sp.

### Introduction

In nature microorganisms can live in either free-living form or as biofilm (Chakraborty et al. 2020). Biofilm is defined as an organized array of homogenous or heterogeneous cluster of microorganisms embedded on a self-secreted matrix made of extracellular polymeric substances (EPS) (Cortes et al. 2011). In many cases, microbial biofilm have executed antibiotic resistance property and an ease to escape host immune responses (Gupta et al. 2016). In a report published by National Institutes of Health (NIH) it was mentioned that 80% of the microbial infections were biofilm linked in human hosts which included urinary tract infections, catheter infections, formation of dental plaques and cystic fibrosis (Yakandawala et al 2006; Cortes et al. 2011). Moreover, in various industrial sectors, biofilm formation in fluid carrying pipelines have resulted in disrupting the efficiency of the machines by hampering the heat transfer as well as reducing the rate of liquid flow in the storage tanks such as fermenters or in heat exchangers (Patel et al. 2014; Chan et al. 2019). In this context, Gupta et al. (2016) stated multiple microorganisms like *Pseudomonas fluorescens*, *Pseudomonas aeruginosa*, *Escherichia*

*coli*, *Staphylococcus aureus*, *Staphylococcus equi* etc. to be potent biofilm forming organisms (Gupta et al. 2016). Hence, in this study, *Staphylococcus* sp. as well as *Pseudomonas* sp. have been selected as test organisms. *Staphylococcus* sp. is a round shaped, gram-positive bacteria which colonize on any site of the human body leading to development of chronic diseases including osteomyelitis, medical device implants, endocarditis, and persistence in the cystic fibrosis lung (Yarwood and Schlievert 2003; Paharik and Horswill 2016). On the other hand, *Pseudomonas* sp. is a gram-negative, rod shaped bacteria which is accountable for multiple acute diseases like urinary tract, kidney infections, bacteraemia etc. (Sharma et al. 2014; Gupta et al. 2016). In each case, a drug resistance property is noticed where the antibiotics get hindered in the EPS layer, thereby, failing to penetrate through the biofilm structure and reach the target sight (Stewart and Costerton 2001). Hence, scientists all over the world are coming up with new techniques like phage therapy (Khalifa et al. 2015) in some cases, than conventional antibiotics, especially regarding multidrug-resistant biofilm infections. The objective here was to isolate an anti- *Enterococcus faecalis* bacteriophage and to evaluate its efficacy against planktonic and biofilm cultures. *E. faecalis* is an important pathogen found in many infections, including endocarditis and persistent infections associated with root canal treatment failure. The difficulty in *E. faecalis* treatment has been attributed to the lack of anti-infective strategies to eradicate its biofilm and to the frequent emergence of multidrug-resistant strains. To this end, an anti- *E. faecalis* and *E. faecium* phage, termed EFDG1, was isolated from sewage effluents. The phage was visualized by electron microscopy. EFDG1 coding sequences and phylogeny were determined by whole genome sequencing (GenBank accession number KP339049), use of silver nanoparticles (Siddique et al. 2020), antimicrobial peptides (de la Fuente-Núñez and Hancock 2015), polysaccharides (Valle et al. 2006), chelating-agents (Abraham et al. 2012), enzymes (Saggu et al. 2019) and combinatorial approaches (Das et al. 2016; Tagliaferri et al. 2019) to attenuate microbial biofilm. In this context, a novel strategy was introduced by Kim et al. (2015) which claimed an increase in antibiotic potential when these are used in combination with alternating (AC), direct (DC) or superimpose (SP) potential (Kim et al. 2015). Moreover, another report stated that application of 2000 $\mu$ A of current can reduce the biofilm formation by *Staphylococcus epidermis* (Ruiz-Ruigomez et al. 2016). Thus, in this study we have focused on using low voltage electric shock produced by a thermocouple device for potential disruption of biofilm. A thermocouple device consists of bi- metal junctions maintained at different temperatures which develop a thermo-emf and provides enough practical applications. Efforts have been put to examine the inhibitory effect of low voltage electric shock (105 $\mu$ V) on *Pseudomonas*

and Staphylococcal biofilm formed under laboratory conditions. The use of an open circuit to generate an electric shock for disruption of bacterial biofilm may provide a novel, in-expensive and flexible approach towards the control of threats caused by microbial biofilm.

## MATERIALS AND METHODS

### Estimation of formed bacterial biofilm by crystal violet assay

To study the bacterial biofilm formation the strains of *Pseudomonas sp* and *Staphylococcus sp* were isolated from the soil. Characterisations of the strains were performed according to Holt et al. 1994 and Dong and Cai, 2001. For examining the biofilm forming potential of the tested organisms, 100 µl of overnight culture was inoculated in 5ml of fresh media and incubated in Petri plates (4.5 cm diameter) and incubated for 24h, 48h and 72. Post incubation the formed biofilm was estimated by conducting the crystal violet assay (CV assay) as mentioned in Tribedi and Sil 2014.

### Thermocouple device

A thermocouple device consists of two dissimilar electrical conductors forming a bi-metallic junction which can produce electric voltage. The thermo emf generated by it can be estimated by using Spot Galvanometer that shows deflections proportional to the potential difference generated. In our experimental setup the sensitivity of the instrument was 8µv/division. A thermocouple made of iron-copper for generation of electric shock was used for our experiment. Temperature difference was maintained by using distilled water at room temperature and ice. This experimental setup is depicted in Figure 1.

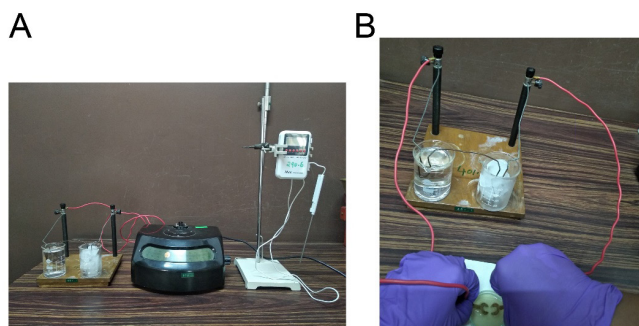


Fig 1. Thermocouple device. A. Thermocouple device connected with spot galvanometer. B. Experimental setup for electric shock application

### Application of low voltage electric shock on bacterial biofilm formation

The culture plates were inoculated and incubated for 24h, 48h and 72h to check the biofilm formation by the tested organisms, *Pseudomonas sp.* and *Staphylococcus sp.* Thereafter, the established bacterial biofilm was treated with a low voltage electric shock (105µV) for 10 min using a thermocouple device. A temperature difference of 25°C was maintained by using ice and distilled water kept at room temperature to generate the necessary low voltage electric shock. The electrodes were held at a distance of 0.5cm inside the Petri plates with its ends touching the bottom surface where biofilm formation occurs. The plates were subjected to electric shock at three different time frames, at an interval of 24h for 3 days. Throughout the

experiment untreated plates were used as control. Finally, CV assay was performed to calculate the amount of biofilm present after 24h time interval of each treatment with electric shock.

### Application of an increased duration of electric shock on bacterial biofilm formation

The bacterial culture was inoculated and the biofilm formation was allowed till 72h. After every 24h for the 3 days of incubation the established bacterial biofilm was treated with low voltage electric shock (105µV) using a thermocouple device. This shock was generated by maintaining a temperature difference of 25°C using ice and distilled water kept at room temperature and applied for 15 min. The electrodes were held at a distance of 0.5cm inside the Petri plate. Untreated plates were used as control and CV assay was performed after 24h of electric shock application. Absorbance was recorded at 580nm after performing the CV assay. The same method was followed for both *Pseudomonas sp* and *Staphylococcus sp* simultaneously.

## Result and Discussion

### Estimation of biofilm formation by crystal violet assay

To determine the biofilm formation efficiency of the tested *Pseudomonas sp.* and *Staphylococcus sp.* the crystal violet assay was performed. The assay has been quite frequently employed in multiple literature to examine the extent of microbial biofilm development (Mukherjee et al. 2013; Sharma et al. 2015). The result of the CV assay performed at an interval of 24h for 3 days showed a successful biofilm formation by the tested organisms (Table 1). The data indicates that with time the amount of formed biofilm increased. In this context, *Staphylococcus sp.* was found to execute a better biofilm forming potential at 72 h in comparison to *Pseudomonas sp* at identical laboratory conditions (Table 1, Fig 2).

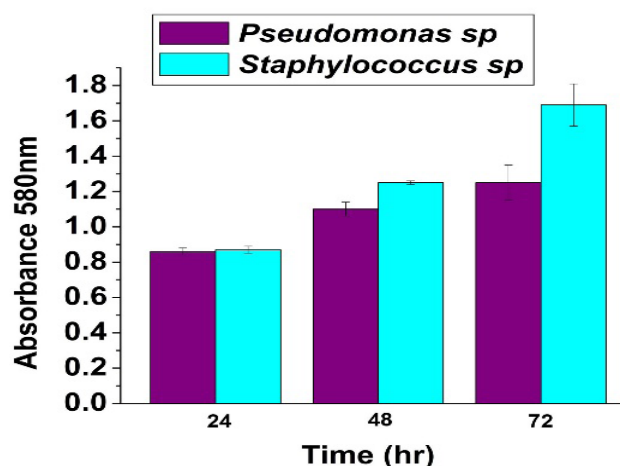


Fig 2. Standardisation of Biofilm formation by *Pseudomonas sp* and *Staphylococcus sp.* CV assay was performed after 24hr, 48hr and 72hr of inoculation, for biofilm estimation of both *Pseudomonas sp.* and *Staphylococcus sp.* Absorbance measured at 580nm.

Table 1. Crystal violet assay for biofilm estimation

Time (h)	<i>Pseudomonas sp</i>		<i>Staphylococcus sp</i>	
	OD 580nm	SD	OD 580nm	SD
24				
48				
72				

24	0.86	0.02	0.875	0.02
48	1.10*	0.04	1.12*	0.02
72	1.25*	0.10	1.69*	0.12

\*Absorbance was measured with 10 & 20 fold dilution.

### Effect of low voltage electric shock on bacterial biofilm formation

Biofilm formation in *Pseudomonas sp* was found to decrease when treated with an electric shock of 105µV generated by the thermocouple device. However, a more prominent decrease in the amount of biofilm present was noticed when the shock was given to the culture after 48h and 72h of incubation (Table 2A & Fig 3A).

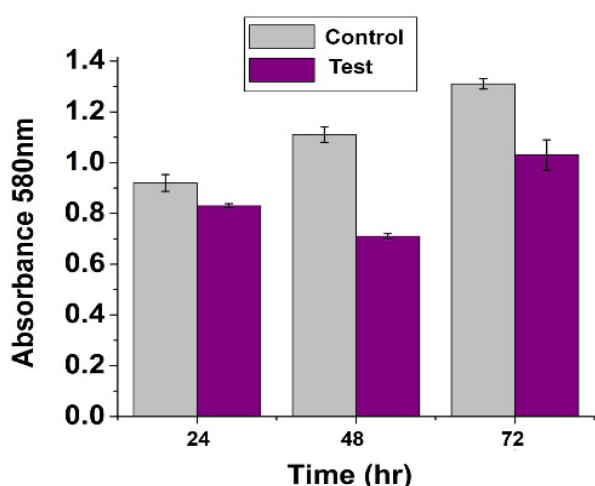


Fig 3A. Effect of 105 µV of electric shock for 10 mins on *Pseudomonas sp* Biofilm. Bacterial culture was allowed to form biofilm for 24, 48 and 72hrs and subjected to 105µV of electric shock for 10mins at 24hr, 48hr and 72hr. CV assay was performed after 24hrs of electric shock treatment for each of the culture.

Table 2A. Effect of low voltage electric shock, 105µV on *Pseudomonas sp* biofilm

Time (in h) for Electric Shock (with respect to inoculation time)	Time (in h) for CV assay(with respect to inoculation time)	<i>Pseudomonas sp</i>	
		Control	Test
24h	48h	0.92	0.83
48h	72h	1.11*	0.71
72h	96h	1.31*	1.03*

\*Absorbance was measured with 10-fold dilution.

The *Staphylococcus sp* too showed sensitivity towards the electric shock (table 2B & Fig 3B). However, the data indicates that the *Staphylococcus sp* is more sensitive towards the electric shock compared to the *Pseudomonas sp*. In both the bacteria the effect of the electric shock is better if it's given after 48 h of inoculation or biofilm formation. As the culture grows older, the biofilm formed tend to become more resistant towards the electric shock as depicted by the CV assay performed on the 72h culture for both *Pseudomonas* and *Staphylococcus*.

Table 2B. Effect of low voltage electric shock on *Staphylococcus sp* biofilm

Time (in hr) for Electric Shock (with respect to inoculation time)	Time (in hr) for CV assay (with respect to inoculation time)	<i>Staphylococcus sp</i>	
		Control	Test
24h	48h	0.91	0.89
48h	72h	1.19*	0.64
72h	96h	1.58*	1.04*

\*Absorbance was measured with 10-fold dilution.

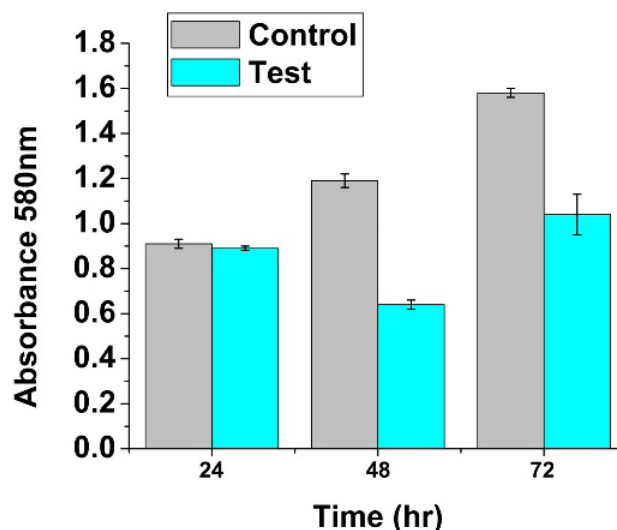


Fig 3B. Effect of 105µV electric shock for 10mins on *Staphylococcus* biofilm. Bacterial culture was allowed to form biofilm for 24, 48 and 72hrs and subjected to 105µV of electric shock for 10mins at 24hr, 48hr and 72hr. CV assay was performed after 24hrs of electric shock treatment for each of the culture.

### Effect of increased duration of electric shock on bacterial biofilm formation

Considering an effect of low voltage electric shock (105µV for 10min) on disruption of bacterial biofilm, attempts were made to investigate the effect of increased duration of the same strength of electric shock on the bacterial biofilm formation. On increasing the duration of 105µV electric shock to 15 min, to a 72h old biofilm, a significant decrease in the absorbance in the CV assay was observed for both *Pseudomonas* and *Staphylococcus* (Table 3 and Fig 4). This decrease in absorbance confirmed a successful effect of increased duration of the electric shock on the 72h old bacterial biofilm. This data also indicates that to disrupt the biofilm developed over higher time frames an increased duration of the shock is also required.

Table 3. Effect of low voltage electric shock for 15mins on *Pseudomonas sp* and *Staphylococcus sp* biofilm

Organism	Control	Test
<i>Pseudomonas sp</i>	1.32*	0.78
<i>Staphylococcus sp</i>	1.55*	0.82

\*Absorbance was measured with 10-fold dilution.

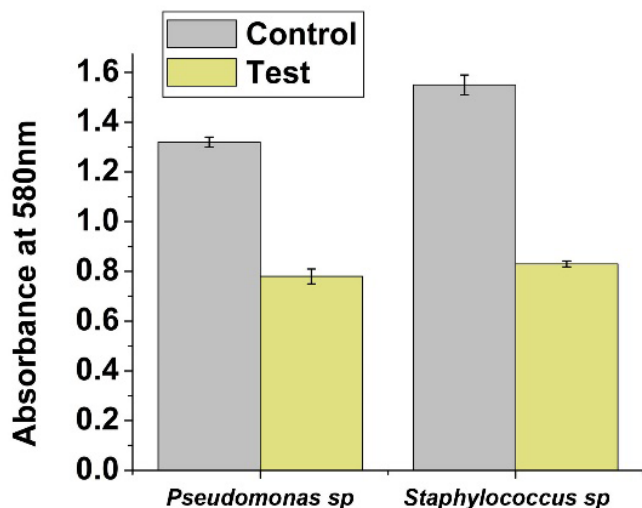


Fig 4. Effect of 105  $\mu$ V electric shock for 15 mins on *Pseudomonas* and *Staphylococcus* biofilm. Biofilm formation was carried for 72hrs and then the cultures were treated with 105 $\mu$ V of electric shock for 15mins. CV assay was performed after 24hrs of the shock application.

### Conclusion

The study indicates the possibility of using mild electric shock to disrupt bacterial biofilm formation. The advantage of this approach lies in the fact that the thermo-electric voltage can be varied by increasing or decreasing the temperature difference. Based on the observation it can be claimed that the duration for which the biofilms were subjected to electric shock plays an important role in the process of disruption. This signifies that the reduction in the amount of biofilm is proportional to time duration of exposure and the applied voltage. The data also indicates that biofilm from different organisms will show different extent of inhibition on treatment of the biofilms with electric shocks. However, creating a steady temperature difference for the generation of the wide ranges of electric shocks in the laboratory condition can be challenging; though it opens a new avenue for designing efficient instruments for the process. The exact mechanism of how biofilm is being disrupted by this method has not been the look out of this study but the concept can lead to the development of a device highly effective and widely applicable in biofilm disruption technologies.

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### Conflict of interest

The authors declare that they have no conflict of interest.

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