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Biofilm Removal using Phosphate Buffer Saline

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ABSTRACT

Biofilm represents a serious threat to water, medical and health facilities. Dealing with such an engineering problem may be pre or post formation. Generally, there is no pre-treatment method could stop biofilm formation completely while post treatment cost a lot of expenses and resources such as biological and steam techniques. This gives the potential to explore a method helping to remove and disinfect biofilms completely. In its quantification, Phosphate Buffer Saline (PBS) was used to release biofilm from coupons that mounted in various laboratory scale reactors. This gave the potential for using this substance to remove biofilm after its foundation. To explore that, the two controlling variables of exposure time and PBS concentration were examined against biofilm percentage removal Using 5X dilution factor will do the desired job at any exposure time. Results were promising and gave a strong potential for practical and commercial use.

1. Introduction

As a result of their links to medical instruments, pneumonia, urinary catheter diseases, as well as chronic wounds, biofilms have always been at the centre of healthcare investigation[1-3]. Dispersion of pathogens via a biofilm, whether it occurs within a host from medical devices or directly on a near-patient surface, indicates a higher probability of infection[4]. By estimates, biofilms are the root cause of 65–80% of all microbial and chronic diseases[5].

While biofilms have been linked to recurring infections, unsuccessful therapies, and extended stays in hospitals, they pose an important clinical dilemma in modern health care. When microbes, especially bacteria, attach to the surfaces of medical instruments such as mechanical ventilators, prosthetic joints, and intravenous catheters, complex bacterial structures are formed. Following attachment, they create an extracellular polymeric material (also called EPS that keeps them from both conventional antibiotic therapy and host immune system responses[6]. Biofilm creation is thought to be the cause of more than 80 percent of hospital-acquired infections that persist, particularly among patients with devices that are implanted and those with weakened immune systems. Because biofilms enable microorganisms to survive in the human body for a prolonged amount of time, they grow resistant to elevated concentrations of antibiotics, making detection and therapy harder.

Innovative methods of therapy that specifically target biofilms have been the focus of a growing amount of investigation in recent decades. These typically include bacteriophage-based therapy, photodynamic treatment, the use of nanomaterials, and the

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enzyme-mediated decomposition of EPS. Although these novel techniques resulted in encouraging outcomes, most of them are still in the research or early clinical stages and still encounter multiple challenges in terms of practical application, protection and efficacy.

As a theory for this work, to remove biofilm from any surface, the Extracellular Polymeric Substances [7] should be dissolved or detached. When addressing a previous biofilm quantification method mentioned in some literatures [8], Phosphate Buffer Saline was used to do such a job. The mentioned technique used PBS to dissolve, or detach, biofilm in a solution as an introduction to the viable count test that used to quantify biofilm. This phenomenon gave the potential for using PBS as an industrial biofilm remover. The removal will transfer biofilm bacteria to planktonic, which leaves them exposed to various well-known biocides. This should give surfaces free of bacteria as well as free of biofilm. This should reduce healthcare-associated diseases and enhance disinfection effectiveness within healthcare settings; a practical aspect has been carried out to determine how well this process destroyed biofilms when compared to traditional strategies.

2. Methodology

This set of experiments aims to build a biofilm on a coupon "microscope slide", then apply PBS on them. This should weaken the attachment force between biofilm and the surface as an introduction to remove biofilm using external mechanical force such as Teflon scrubber or cloth wiping.

The Biofilm reactor

The biofilm reactor consists of a bacterial broth reservoir equipped with a low-speed "1 rpm" motor attached to its cap. The motor shaft extended to hold biofilm coupons. Figure 1 illustrates the setup. Every biofilm building process was implemented along 3 days period. All the biofilm building experiments were conducted under room standard conditions.

Nutrient broth

This was obtained from Millipore brand. This broth was cultivated with Escherichia Coli K-12 bacterial strain obtained from Sigma-Aldrich. After mixing the broth, a bacterial inoculate was poured into it and incubated for 24 hours at 37 °c in the incubator of Isotherm mark. On the second day, biofilm coupons were hung on the reactor shaft and the motor was turned on.

Biofilm measurement

Biofilm was measured using both the standard crystal violet assay (CV) [9] [10] as well as optical density methods [11] where the penetrated light through the biofilm built on a coupon was measured as those two variables are inversely proportional. Crystal violet was used to observe the existence of biofilm, while optical density was used for quantitative measurements.

Experiments frequency

Every point in Figure 2 represents the average of 3 replicants.

3. Results and discussion

Results are represented by the percentage biofilm removal estimated by optical density versus multiple exposure time periods in seconds and as well as PBS dilutions. It has been shown that the more both of time and PBS concentrations the more removal of biofilm.

The equation ruling the phenomena is:

$$Biofilm\ removal\ \% = \frac{a}{b} \times 100$$

Where:

a= penetrated light in Luxes for biofilm containing coupon after treatment.

b= penetrated light in Luxes for biofilm containing coupon before treatment.

Biofilm removal represents a serious challenge to water and medical facilities. Many of previous studies dealt with this problem but targeting equipment with water passage such as dentists' chair or ventilating machines [12, 13]. For those kinds of devices, many solutions were suggested. Most of them dealing with passing various disinfectants through the targeted pipes [1, 14]. The main problem with those techniques is the need to relatively long time that starts from 30 minutes in best cases.

Nearly all application heading to replace those pipes rather than treat them even with the slightly higher cost [15, 16].

Regarding various apparent surfaces on the same facilities, the matter is totally different. Disinfection does not have this long exposure time especially for vertical or inclined ones. Also, biofilm formation on such surfaces was not taken into account because it was belief that the process cannot be completed since it is lacking one of the biofilm essentials, that is, water. This presumption may be correct but not in this case. Water provided through daily practice of cleaning and disinfection. Even this amount of substrate is very low, it represents enough environment to build biofilm. Furthermore, this cycle of moisture-drought gives a big potential for biofilm formation as it provides the process with stress which drives it to form [17, 18].

One of the driving forces for introducing the current work is the lack of practical method for biofilm removal. The danger of biofilm is huge and expandable. It may represent a stronghold for any pathogenic microorganisms that come to the surface and not have the time to build its own biofilm [19]. Or in worst cases, they may develop more aggressive strains starting from both pathogenic and non-pathogenic ones. Therefore, biofilm is a big challenge that should be treated under zero tolerance strategy [20].

When taking a look to Figure 2, results justified the assumed hypothesis that using PBS is a reliable and practical solution for biofilm

Figure 1: The biofilm reactor assembly

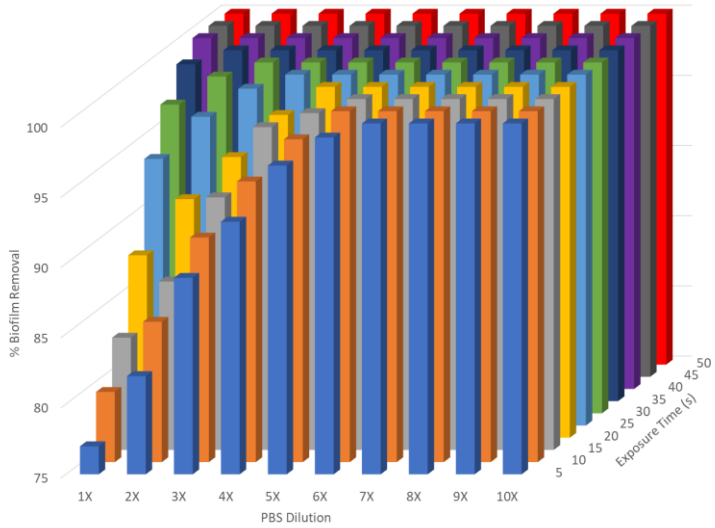

Figure 2: Effect of Exposure time (sec.) and PBS concentration on percentage biofilm removal

Table 1: Percentage error margines for results in figure 2

PBS Dil. / Time (s)	1X	2X	3X	4X	5X	6X	7X	8X	9X	10X
5	±4	±4	±4	±5	±3	±4	±7	±5	±6	±4
10	±3	±5	±4	±5	±5	±6	±6	±6	±4	±5
15	±6	±4	±2	±2	±3	±4	±3	±3	±3	±4
20	±5	±6	±5	±4	±4	±4	±4	±3	±3	±3
25	±6	±5	±4	±3	±2	±3	±3	±5	±5	±5
30	±4	±6	±4	±5	±5	±5	±5	±5	±5	±5
35	±5	±3	±2	±3	±3	±3	±3	±4	±3	±5
40	±4	±4	±4	±4	±4	±4	±4	±4	±4	±4
45	±5	±6	±3	±6	±5	±5	±5	±5	±5	±5
50	±6	±5	±4	±3	±2	±3	±4	±6	±4	±3

removal. Moreover, it has a fabulous advantage over other techniques. That is, biofilm here is released from surface not dissolving its EPS. This gives the advantage of removing biofilm without spreading the embedded microorganisms. Such a mechanism is well-known when quantifying biofilm by viable count using PBS [21]. The use of such a technique includes using a homogenization by a prob homogenizer in order to release microorganisms from this “float” biofilm agglomerates. Also, it could be concluded that surface-biofilm represents a weaker attachment force than that of biofilm-biofilm one.

Results show that the biofilm percentage removal is clearly increased by increasing PBS concentration and exposure time. Those two variables are exactly hypothesized in this work. To obtain 100% biofilm removal we either increasing PBS concentration or increasing exposure time. Both directions gave the same results. The aim of this technique is removing biofilm without spreading the included microorganisms. Therefore, choosing any concentration is a trade-off matter. That is, when PBS price is high, we choose going towards low concentration and longtime treatment and vice versa if the labour price is high.

The main technique for biofilm removal from horizontal surfaces is drowning by excess PBS solution and scrabbing with Teflon scrubber. For the vertical surfaces this technique does not work. The best usable method should be wiping using moisten cloth with PBS. Therefore, dealing with horizontal surfaces is easier.

Many other factors may affect biofilm detachment and enhance surface disinfection such as temperature degree, surface material, or microorganism type [4, 5]. For the first factor, it is hard to manipulate temperature degree for the targeted surface as there is no practical method without side effects to do so in addition to cost issues. Surface materials may be suggested for manufacturers before manufacturing not after.Microorganisms type cannot be controlled and should be dealt with as it is. Therefore, the suggested technique is the most reliable and practical one.

Relying on optical density is the best path to obtain percentage biofilm removal. That is, since the desired measurement function depends on both initial and final biofilm quantification, this suggests using non-distractive measurement method. Those kinds of measurements are divided into two subcategories; microscopy and optical techniques [22, 23]. The first is limited to very small area of the coupon which leads to lack of representivity. The second is perfect as it works on the whole sample area.

Surface roughness is an important factor in dealing with biofilm [24, 25]. More rough surfaces have more surface area and terrain which helps microorganisms for more attachment especially in earlier stages which make it more resilient to the applied shear forces. This may be the reason behind detaching

biofilm as a coherent layer rather than melt it completely in a useful phenomenon from the practical point of view. Therefore, manufacturing any operating theatres and laboratories equipment and furniture should be with surfaces as smoother as they could be to facilitate biofilm removal [26]. Even tough, the current technique is effective with both rough and smooth surfaces because it de-attach biofilm in bulk.

Flow regime during biofilm formation in the biofilm reactor is a fairly sensible factor helping us to drive the process faster [27]. Most studies claim that mixing Reynolds number values should be kept laminar for more biofilm formed per time [28, 29]. In our reactor design, this dimensionless group was of a value of 82. This value is transition and near laminar, as limits are: less than 10 is laminar, between 10 and 10^4 is transition, and more than 10^4 is turbulent. As a support to calculations, an ink drop test was implemented by comparing between stagnant and running reactors, the results support the assumption of laminar flow, hence faster biofilm formation process.

4. Conclusions

Biofilm represents a stronghold of microorganisms against any disinfection process. Removing this aggregate from various surfaces is an important step of the whole disinfection process. Disinfection of free-living bacteria does not be considered a complete method unless removing biofilm first.

The removing process by PBS is a promising, cost effective, and less labour consuming process. It is strongly recommended to use this technique to prevent infections and any concerns in various medical and health facilities.

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