

Research Article

Studying the Formation of Biofilms on Supports with Different Polarity and Their Efficiency to Treat Wastewater

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The main objective of this study was the evaluation of biofilm formation onto different supports and of biofilm efficiency to treat wastewater. Two different reactors were used, one with porous polyvinyl alcohol gel (PVA) biocarrier and another with a high-density polyethylene (PE) biocarrier. The reactor performance was evaluated and the biofilm formed was analyzed with potentiometric mass titrations. The biofilm formation was monitored with diffuse reflectance spectroscopy. The presence of the support did not alter the nature of the biofilm. However, the quantity of the biofilm formed was higher when polar surface groups were present on the support.

1. Introduction

One promising technology for wastewater treatment is the use of biofilms attached to inert solid surfaces. It has been applied to wastewater treatment for nitrogen removal [1], reduction of organic constituents [2], and abatement of specific toxic compounds such as phenol [3, 4], Cr(VI) [5], 2,4-dichlorophenol [6], and phthalic acid esters [7].

The biofilms are formed when bacteria are immobilized onto a solid surface. This immobilization of bacteria implies either attachment or entrapment of microorganisms and results in several advantages over free or suspended bacteria. The most important advantageous characteristics of the biofilms related to wastewater treatment include stability, tolerance against toxic compounds, effective treatment of high volumetric loadings, and coexistence of aerobic and anoxic metabolic activity within the same unit process.

Biofilm formation occurs through sequential steps. First, bacteria are attached to a solid surface. This process involves electrostatic forces, acid-base interactions, and Brownian motion forces. Then, the bacteria multiply and embed themselves in a matrix composed of extracellular polymeric substances (EPS) produced from themselves. Surface proteins are also regularly present in the biofilm matrix but their presence has been mainly related to the initial attachment of microbial cells to surfaces [8].

The ideal support medium must be nontoxic to the microorganisms and have good mass transfer characteristics, stable chemical properties, and adequate structural strength [9]. These supports can be hydrophobic like polyethylene (PE) or with polar surface species like polyvinyl alcohol (PVA). In the present study, two bench scale reactors with different support for biofilm formation were operated for the treatment of municipal wastewater. In the first reactor, the solid support is PVA and in the second it is PE. The main difference of these two supports is the -OH surface groups on the PVA that increases the hydrophilic character of the support in the first reactor. The main objective of this study is to see if the presence of the surface polar groups on PVA support results in higher amount of biofilm and better performance of the reactor. The performance of the different reactors was evaluated based on the removal efficiencies of organic compounds, nitrogen, and phosphorus.

2. Materials and Methods

2.1. Reactors Set-Up and Operation. Two 1.5 L glass beakers were used as bench scale activated sludge bioreactors operating at 25°C. Continuous aeration was provided by air pumps with one air diffuser in each reactor. Start-up of the reactors was conducted by the addition of 150 mL of activated sludge

taken from the aeration tank of the wastewater treatment plant at the University of Patras (Greece) campus having a mixed liquor suspended solids (MLSS) content of 3200 mg/L. This wastewater treatment plant operates as an oxidation ditch activated sludge system with partial nitrification. The activated sludge was taken fresh and was acclimatized to the new conditions. Acclimatization time may vary according to the newly introduced substances in the influent and their concentrations. It has been reported that in the case of low phenol concentrations activated sludge microfauna could be acclimatized in 3-4 days after the introduction [10]. In this study, an acclimatization period of 12 days (three cycles) was allowed for the activated sludge to adapt to the experimental conditions.

Besides the 150 mL of activated sludge, the reactors were also filled with municipal wastewater and biocarriers up to 1.0 L total volume. Each reactor was operated in subsequent cycles of 4 days. At the end of each cycle, sludge sedimentation was taking place by turning off the air pumps (adapted from [5, 11]). 300 mL of the supernatant water was withdrawn from each reactor for further analysis and was replaced with 300 mL of municipal wastewater. The total operation time of the reactors was 44 days.

PVA reactor included 75 mL of PVA-gel beads (Kuraray Co., Ltd., Japan). The gel beads are 4 mm diameter spheres and are slightly heavier than water (specific gravity of 1.025 g/cm³), hydrophilic in nature, and have a very porous structure with only 10% solids and 90% water and a continuum of passages 10–20 μm in diameter tunnelling throughout each bead [12, 13]. PE reactor was filled with 150 mL of moving-bed biocarriers (Model K1, Veolia Water, France), which consist of high-density polyethylene rings and are slightly lighter than water (specific gravity of 0.95 g/cm³). They have a cylindrical shape with 7 mm length and 9 mm diameter with a cross inside and fins on the outside. Biomass grows mainly on the protected area inside the cylinder even though some biomass also grows outside between the fins.

2.2. Physicochemical Analyses. All operating parameters of the two reactors in their effluent solutions were measured using a HACH DR 2400 Spectrophotometer and the corresponding reactants and glassware. Chemical oxygen demand (COD) was measured by the Reactor Digestion Method (Method 8000, Hach), ammonium nitrogen (NH₄⁺-N) by the Nessler Method (Method 8038, Hach), nitrate-nitrogen (NO₃⁻-N) by the Chromotropic Acid Method (Method 10020, Hach), and orthophosphates (PO₄³⁻) by the Ascorbic Method (Method 8048, Hach).

2.3. Potentiometric Mass Titration. The study of the acid-base behavior of the biofilm was performed using the method of PMT [14]. The electrolyte solution (0.1 M NaNO₃) was prepared by using NaNO₃ (Merck, analytical grade) dissolved in triply distilled water (Jencons, Autostill).

The PMTs were performed at a constant temperature (25 ± 0.1°C), under N₂ atmosphere. The suspensions were equilibrated for 12 h and then a small amount of base (0.2 mL of 1 M NaOH) was added to deprotonate the charged sites,

rendering the surface negative, without exceeding pH 10 for the suspension of activated sludge. This was chosen because, above pH 10, significant cell lysis occurs and may interfere with the buffering measurements [15].

After 3–5 min, the new equilibration pH value was recorded and noted as the initial pH. The suspension was then titrated by adding automatically small volumes of a certified volumetric standard of 0.1 M HNO₃ and the pH was recorded as a function of the volume of titrant added to the suspension. The overall time of the titration was less than 1 h. This was done to ensure short time duration of the sludge especially in high (>9.5) and low (<4.5) pH values to avoid cell alteration due to pH change. All PMTs took place in a thermostated double-walled Pyrex vessel equipped with a magnetic stirrer and a Perspex lid with holes for the electrodes and the acid and nitrogen gas inlets. The pH during the titration experiments was monitored by a TIM 800 Radiometer Copenhagen Autoburette System and was recorded with the Timtalk 8, version 2.0 software.

By applying the mass balance equation for the H₃O⁺ ions for each titration curve, the H⁺ consumption on the biofilm was determined. This can serve as a measure of the biofilm quantity and also can show differences, if any, of the active species on the surface of each biofilm.

2.4. Absorption and Diffuse Reflectance (DR) Spectrometry. Using a UV-vis spectrophotometer (Varian Cary 3) equipped with an integration sphere for the DR experiments, the absorption spectra of the activated sludge were recorded at various pH values in the range 200–800 nm at 25°C. DR spectrometry uses Kubelka Munk equation and $F(R)$ is the outcome. For solid samples, the technique measures the reflectance since the visible light cannot penetrate the sample. Instead of the reflectance, the $F(R)$ which is an expression taking into account the absorption and the scattering of the sample can be used. These values are proportional to the absorbance of the sample.

The DR spectra of the biofilm formed on PVA were recorded after various contact times of the PVA with the activated sludge (i.e., 5 days, 1 month, and 2 months). In all cases, the DR spectra were collected on wet samples whereas wet virgin PVA was used as the reference sample. The samples were mounted in quartz cells, providing a sample thickness >3 mm to guarantee the “infinite” sample thickness.

3. Results

3.1. Reactors Evaluation. The two reactors demonstrated good removal efficiencies of organic matter with effluent COD levels well below the secondary effluent discharge limit (Table 1). The removal of organic load was efficient at the 4th day of operation. A similar pattern of removal was observed for the case of NH₄⁺-N concentrations. The nitrification process was very efficient with NH₄⁺-N levels decreasing from 24 mg/L (initial value) to 0.02 mg/L (4th day).

There were no significant differences in the phosphate removal efficiencies between the different reactors, suggesting that the use of biocarriers offers no benefit with respect

TABLE 1: Reactors effluent concentrations (mg/L) at 4, 28, and 44 days.

Parameter	Influent concentration (mg/L)	Discharge limit* (mg/L)	PVA reactor			PE reactor		
			4th day	28th day	44th day	4th day	28th day	44th day
COD	250	125	13	26	25	36	39	45
NH ₄ ⁺ -N	24	15*	0.02	0.15	0.18	0.02	0.22	0.25
NO ₃ ⁻ -N	5.0	15*	21	9.4	4.1	23	35	35
PO ₄ ³⁻ -P	13	2	5.6	3.3	4.4	4.4	3.4	4.0

*Based on Council Directive 91/271/EEC concerning urban wastewater treatment adopted on 21 May 1991, there is a limit (15 mg/L) only for total nitrogen.

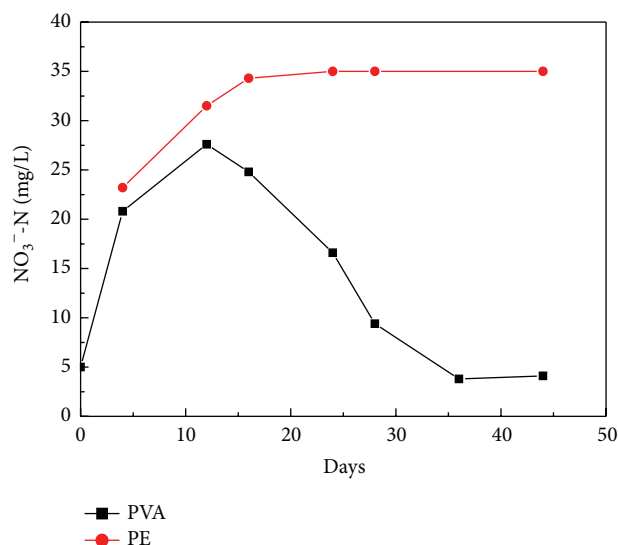


FIGURE 1: Nitrate-nitrogen (NO_3^- -N) concentrations in PVA (squares) and PE (circle) as a function of operation time.

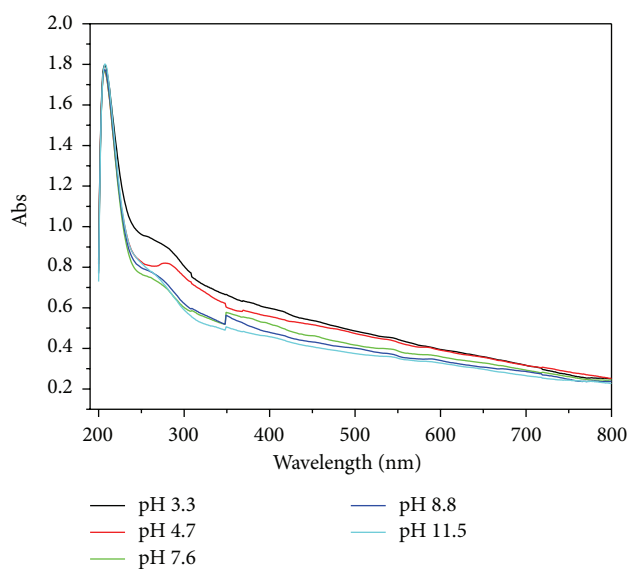


FIGURE 2: Absorption (Abs) spectra of activated sludge suspensions at different pH values.

to this parameter. Unfortunately, the effluent phosphates did not comply with the discharge limits.

Finally, the levels of nitrate (Figure 1) trended upward until the 12th day of operation in the two reactors. However, from that point denitrification appeared to occur in PVA reactor, demonstrating a pronounced reduction in NO_3^- -N levels, which decreased significantly until leveling off at approximately 5 mg/L by the 36th day of operation. This occurrence of denitrification only in the PVA reactor indicates that the porous PVA-gel support provides a superior niche for formation of a stable anoxic zone in the protective core of the gel beads as has been previously suggested by others [16, 17]. In addition, PE reactor could not achieve reduction of nitrogen to levels well below the discharge limit (Table 1). This difference in the reactor activities could be attributed to the presence of PVA and the easier formation of biofilm in this support medium. The biomass into the PVA reactor is a mixture of the initial biomass and the biofilm formed on the surface of the PVA. Probably, when the ratio of these two forms of biomass changes in favour of the biofilm, then the reactor acts in a more efficient way for nitrate-nitrogen. This is an evidence that the presence of PVA acts beneficially for the formation of biofilm and also for the reactor's efficiency.

3.2. Biofilm Formation. One easy-to-use method employed for the first time in this study to observe the biofilm formation is the DR spectrometry. This method is based on the light absorption of the sample in UV and visible region of the spectrum and can be applied in most of the solid surfaces.

Figure 2 presents the absorption spectra of activated sludge suspensions at different pH values. Generally, there are not any significant differences in the spectra. There is a main peak at almost 210 nm and a shoulder at 275 nm. With decreasing pH of the suspension the shoulder at 275 nm is more pronounced. The light absorption of the activated sludge in the visible region (800–400 nm) is due to the dark brown colour of the sludge.

The DR spectra of the biofilm formed on the surface of the PVA are presented in Figure 3. For comparison purposes, the DR spectrum of the mixture of PVA and activated sludge is also presented. In this case, it is expected that no biofilm is formed since this sample is only a mixture without any contact time. The spectrum of the mixture shows the peak at 210 nm which is attributed to the activated sludge in accordance with Figure 2. The shoulder at 275 nm of the activated sludge is a well-defined peak shifted at 265 nm.

Generally, the peaks at 200–220 nm are $n-\sigma^*$ and/or $\pi-\pi^*$ transitions, which are found in many organic functional

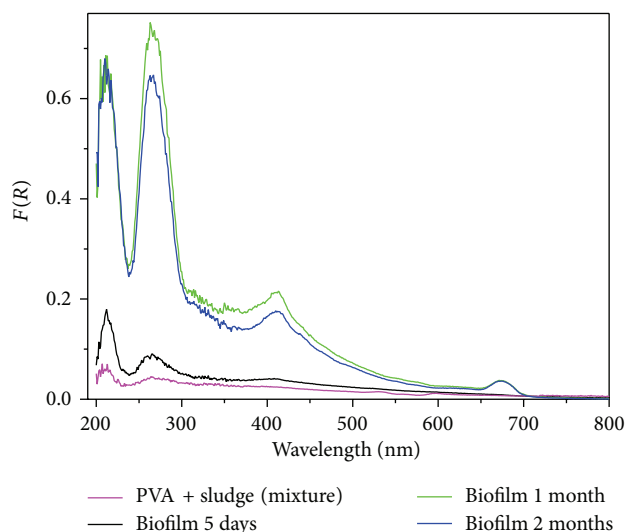


FIGURE 3: DR spectra of the biofilm formed on the surface of PVA support.

groups. On the other hand, the peak at 260~280 nm range is commonly attributed to π - π^* electron transitions in aromatic and polyaromatic compounds found in most conjugated molecules, including proteins [18–20].

Taking into account that the pH of the mixture is about 7.8, this peak may be evidence for the biofilm and it can be assumed that the biofilm formation is starting almost immediately when the activated sludge is in contact with the solid support. The DR spectra for the biofilm after 5 days, 1 month, and 2 months suggest that the biofilm formation is a dynamic process and is developing with time altering the analogy of the two main peaks, in favor of the peak at 265 nm until it reaches equilibrium after 1 month. The difference between the DR spectra at 1 month and that at 2 months is not significant suggesting a dynamic attachment and detachment of the biofilm on its support.

At about 1 month, the biofilm is fully grown as the intensity of $F(R)$ reaches the maximum value and two new peaks appear in the spectrum at 412 and 675 nm. One possibility is that these peaks associate with adsorbing processes from the solution to biofilm surface. It is well known that the biofilm, especially the EPS, can play an important role in the sorption of organic pollutants, inorganic ions, and nutrients [21, 22].

This spectroscopic study could not be done for the biofilm formation on PE, due to the shape of the solid support. In order to use the DR Spectroscopy, an “infinite” sample thickness (thickness > 3 mm) is necessary. The cylindrical shape of PE with 7 mm length and 9 mm diameter with a cross inside and fins on the outside could not fit in the cuvettes without destroying them or the biofilm formed on its surface.

3.3. Potentiometric Mass Titration (PMT) of the Biofilm.

There are many studies in the literature presenting titration data for each individual microorganism (e.g., [23]) but for more complicated systems, like activated sludge or biofilm, the available data are rather limited. In this study, the biofilm

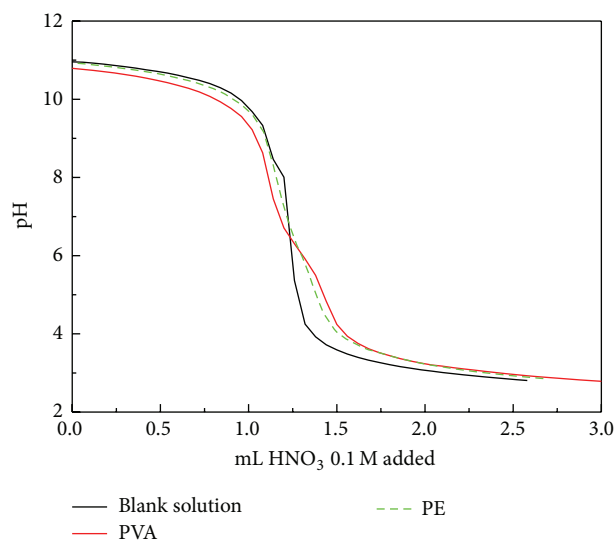


FIGURE 4: Potentiometric titrations curves for the blank solution and the biofilms formed on PVA and PE supports.

formed onto the surface of the solid support was titrated according to the PMT procedure. The amount of the solid support used was the same in order to be able to compare the biofilm formation.

In Figure 4, it can be seen that there is a common intersection point at pH 6.4 for the three curves. On the left of the intersection point, the titration curves of the biofilm have lower pH values than the solution. The order of the curves with the higher pH is blank > PE > PVA. The lower values of pH for the biofilm curves show that a quantity of H^+ was released from the biofilm in the solution lowering the pH and charging negatively the biofilm. On the right of the common intersection point, the order of the curves is inverted (PVA > PE > blank). The biofilm in this pH region is positively charged because a quantity of H^+ was consumed on the surface. Only at pH equal to 6.4, the suspensions and the solution have the same acid-base behavior. This means that the point of zero charge, pzc, of the biofilm is 6.4. This value is in accordance with the usually observed negative charges of EPS (at natural water or wastewater pH) due to the large amount of surface functional groups, which also explains the high dependence of the charge with solution pH.

The common intersection point implies that the biofilm formed on the surface of the two different supports has the same characteristics. Otherwise the point of zero charge, pzc, of each biofilm would be different and a common intersection point would not appear in the diagram. Taking this observation into account, it can be concluded that the different surface species present on the surface of each support and their nature in terms of polarity (polar or not polar) do not alter the mechanism of the biofilm formation.

One other observation is that the amount of biofilm in the case of PVA support is more than the amount of biofilm for PE support. This is more obvious when the amount of H^+ consumed by the biofilm is plotted against suspension pH (Figure 5). These curves can be produced from the titration

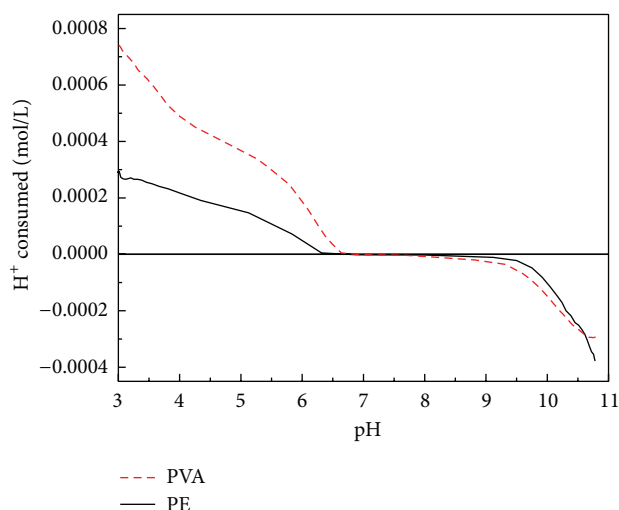


FIGURE 5: The amount of H^+ consumed by the surface of biofilms formed on PVA and PE supports.

curves using the mass balance equation for H^+ ions in the solution (more specifically, the blank titration curve is subtracted from the suspension titration curve). Detailed procedure for the calculation can be found in literature [24]. Figure 5 shows that the H^+ ions consumed on the surface of biofilm or released in the solution are important at pH region above 9 and below 5. At the pH region between 6 and 8, the H^+ consumption is almost zero. This means that in the above region the biofilm cannot act as a buffer and possible changes in pH solution can occur. Taking into account that the nature of the biofilm formed onto the two different supports is the same and the amount of solid support used for the titration is also the same, the curves clearly show that the amount of the biofilm is higher in the PVA case. The H^+ ions added in suspension titrate the surface acid-base sites and these sites are more in the PVA support especially at $pH < 7.0$.

Following the differential potentiometric titration method proposed by Bourikas et al. [25], the data in Figure 5 were used to calculate a differential curve for each hydrogen consuming curve. These two curves are presented in Figure 6 clearly showing that the biofilm is heterogeneous with respect to the surface species that can participate in acid-base reactions. These surface species can be described by a variety of surface organic groups such as $-COOH$, $-OH$, and $-NH_2$. These groups can exchange H^+ altering the total surface charge and creating surface charged sites on the biofilm. These sites can explain the ability of the biofilm to adsorb and accumulate ions and nutrients and, also, the protective action of biofilm in hostile environments. These surface species and the EPS can act as a barrier against all possible hostile compounds.

4. Discussion

It is well known that the presence of the solid support is necessary for the biofilm formation. Although there are some articles about biofilm formation, there is no clear answer

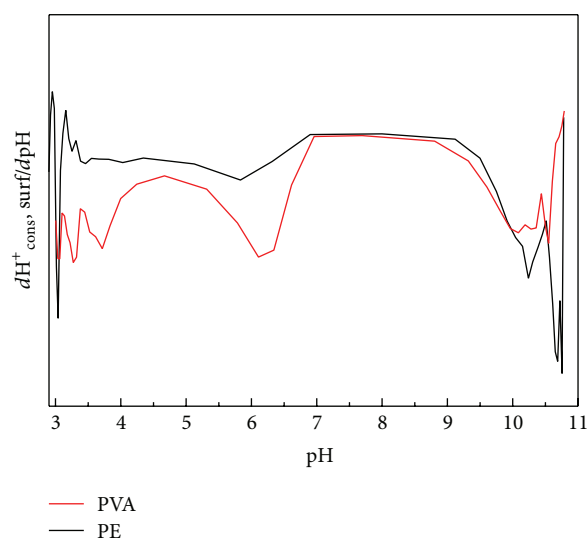


FIGURE 6: Differential potentiometric titrations curves for the biofilms formed on PVA and PE supports.

whether the polar groups on the surface of the support affect the formation of biofilm. Normally, hydrophobic materials attract hydrophobic bacteria that are sorbed with nonspecific interactions. On the other hand, the microbe cells are able to make stronger contact with polar groups on a polar surface compared to a hydrophobic surface and this increases the chances of adhesion and subsequent biofilm growth.

This study shows that the formation of biofilm can improve the removal efficiency of the reactor, as already known [26], but also presents for the first time the use of DR spectroscopy and PMT as quick and easy methods to determine the formation and the amount of biofilm on solid surfaces, respectively. DR spectroscopy can help significantly to observe the formation of biofilm especially in surfaces like polymers that are not highly absorbing in UV and visible regions of the spectrum. The DR spectroscopy can be used for the observation of the biofilm formation, as it can absorb light in UV and visible region of the spectrum. Also, PMT technique can act supplementary to other techniques, in order to provide more information for the biofilm characteristics such as the nature of the biofilm and the amount of H^+ consumed by the biofilm. The latter is closely related to the quantity of the biofilm formed.

5. Conclusion

The following conclusions can be drawn from the present study:

- The presence of suitable solid surface in the reactor can be beneficial for the waste treatment procedure due to the biofilm formation.
- The polarity of the solid support can affect the amount of biofilm formed and thus the reactor efficiency.

- (c) The solid support with polar surface groups helps better the biofilm formation compared to another support that is hydrophobic.
- (d) The polar groups of the support alter only the amount and not the acid-base behavior of biofilm.
- (e) DR spectroscopy can provide useful information about the formation of biofilm on a solid.
- (f) PMT can provide useful information about the nature and the amount of the formed biofilm.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

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