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Acceleration of microalgal biofilm formation on PET by surface engineering

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Abstract−Biofilm-based microalgal cultivation has recently received great attention owing to its low harvesting cost, but the main problem in practicing it is the low rate of attachment on solid carriers. The aim of this research is to introduce novel physical and wet chemical surface engineering methods to provide more favorable polymeric surfaces for microalgal adhesion. PET threads were used as a substrate in the treatments. The surface of the threads was treated with chromic acid, sodium hydroxide and sandpaper. The chemical composition, surface morphology, topography and contact angle of the threads were characterized. The threads were placed in a biofilm-based cylindrical photobioreactor as a bed for attachment. Two freshwater single-cell microalgae, *Scenedesmus dimorphus* and *Chlorella vulgaris*, were cultivated in the photobioreactor to assess the attachment rate of the threads. The analysis of SEM and AFM images confirmed the creation of new grooves. The AFM image analysis showed 323%, 184% and 11.5% increase in the surface roughness, while there were 73%, 51%, and 30% rates of reduction in the contact angles for the treatments with acid, sandpaper and base, respectively. Creation of new grooves, increase of the surface roughness and decrease of the contact angle led to an increase in the microalgae attachment rate. The best results were achieved with acid treatment. It led to a remarkable increase in the attachment rate of *S. dimorphus*. However, the attachment of *C. vulgaris* cells was not efficient. This research is the first to apply a surface engineering method to increase the microalgal attachment rate in biofilm-based systems. The insight that is provided can be of benefit for further studies in this field.

Keywords: Surface Modification, Microalgal Biofilm, Attachment Rate, Surface Roughness, Biomass Harvesting

INTRODUCTION

Microalgae are industrially cultivated for different usages, as in food industries and wastewater treatment, but there is a major obstacle associates with their cultivation. Both typical methods of microalgal cultivation, i.e., open and closed systems, produce algal suspensions with total biomass concentrations in the range of 1-4 $g \cdot l^{-1}$ [1]. Thus, the production process requires an additional step of biomass harvesting. This step is crucial because it involves 20-30% of the total cost of biomass production [2]. An alternative was proposed to grow microalgae as a photosynthetic biofilm in which microalgal cells attach to a solid carrier [1,3]. This method resulted in the reduction of water and energy requirement for algae cultivation and an increase of cell density in the reactor, which altogether led to the cost reduction in the process [1,4-7]. Nevertheless, the low rate of attachments on solid carriers is a main problem; the accumulation of a desired content of a biofilm may last 30 days [7]. High cell densities have already been used by some researchers to increase attachments rates, but this procedure requires a concentrated inoculum [1,5,8].

Formation of a microalgal biofilm on a surface can be influenced by some physicochemical and biological factors such as the type of species and substratum, surface characteristics and the surrounding liquid [5]. Surface properties, which are usually characterized as the morphology, topography and chemistry of the surface, are the most efficacious factors [9]. Rough and porous surfaces serve as large surface areas to form biofilms [10]. Moreover, the cell-surface contact can be manipulated through the introduction of hydrophilic functional groups [11].

The type of substratum has recently been the subject of some studies in which different polymeric substrata have been widely used for microalgal attachment [1,4,5,8,12]. Little research has been conducted to modify surface characteristics by engineering methods such as laser micromachining texturing [1,5,13,14] or to provide better surfaces for microalgal adhesion. The objective of this study was to introduce physical and wet chemical surface modification procedures to accelerate the rates of microalgal settlement, adhesion and attachment on polymeric substrata. Surface functionalization through wet chemical modifications is a classical approach in which liquid reagents are used to chemically etch the polymeric surface layers in substrata. Sulfuric acid-dichromate solution and sodium hydroxide are two reagents widely used in the surface preparation of plastics [15-20]. The substitution of hydrophobic functional groups can cause a better cell-substratum interaction [11]. This technique can be implemented in laboratories because it does not require any specialized equipment [16].

In this study, three different treatment methods were utilized to alter the surfaces of PET threads that were then characterized by surface analytical techniques. The three differently treated threads were then used at a laminar flow in a cylindrical glass photobioreactor containing microalgae to provide attachment surfaces. The laminar flow regime resulted in a better attachment of single-cell microalgae than the turbulent regime did [1,4]. In the experiments, untreated PET was used as a control. In addition, different thermo-

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dynamic and theoretical models were tried for the comprehensive analysis of the attachment experimental data.

MATERIALS AND METHODS

1. Surface Modification

Commercially available PET threats were used as the substratum. Choosing them was based on the criteria of inexpensiveness, reusability, and ease of accessibility [12]. The threads were wiped with acetone to clean the surfaces before treatment. Then, they were divided into four equal sets. Each set consisted of approximately 500 threads of 9 cm in length and 400-600 μm in diameter. The first set was etched with a sulfuric acid-dichromate solution at 70-80 °C for 2 minutes, according to the Standard Practice for Preparation of Surfaces of Plastics-D 2093 [21]. The second one was etched with sodium hydroxide (10 N) at 95 °C for 5 minutes according to the procedure previously reported [20]. The third one was modified with three passes of sandpaper no. 80. The fourth set was used as a control one without any treatment. These sets will be further discussed in the attachment experiments section.

2. PET Threads Characterization

Chemical composition of the PET threads was determined (n=2) by Fourier transform infrared spectroscopy-attenuated total reflection (FTIR-ATR, Nicolet iS10, USA). Moreover, the surface morphology and the topography of the threads were characterized by field emission scanning electron microscopy (FESEM, Hitachi 4160, Japan) and atomic force microscopy (AFM, Nano vizard II, Germany) in a contact mode, respectively, to visually investigate the effects of the surface modification techniques on the three experimental sets as compared to the control set. The AFM images were further analyzed by the JPK Data Processing software to estimate the characteristics of the surfaces. Up to 90 images (three images for each thread) were prepared by AFM in high quality to assess the topographical properties of different treated threads. In addition, image processing was carried out by WSxM 5.0 to determine fractal dimensions of the surfaces. It is a criterion to estimate autosimilar characteristics of the surfaces. The skewness and kurtosis of the frequency distributions of the images were analyzed using MATLAB® R2010a (The MathWorks, Inc., USA). The influence of surface modification on the contact angle (CA) and the surface energy was also measured (n=3) by the sessile drop method using an optical contact angle meter (OCA, Dataphysics Instruments GmbH, Germany).

3. Media and Culture Maintenance

Two freshwater microalgae, *Scenedesmus dimorphus* and *Chlorella vulgaris*, which are known to exist in natural waterway biofilms [1], were kindly provided by [22]. Stocks of *S. dimorphus* and *C. vulgaris* were cultivated in Z8 [23] and Bold's Basal Media (BBM), respectively. Vitamin B12 was not included in the latter case. The stocks of both microalgae were grown photoautotrophically in 250-mL Erlenmeyer flasks shaken at 100 rpm on an orbital shaker. These culture media were maintained at room temperature

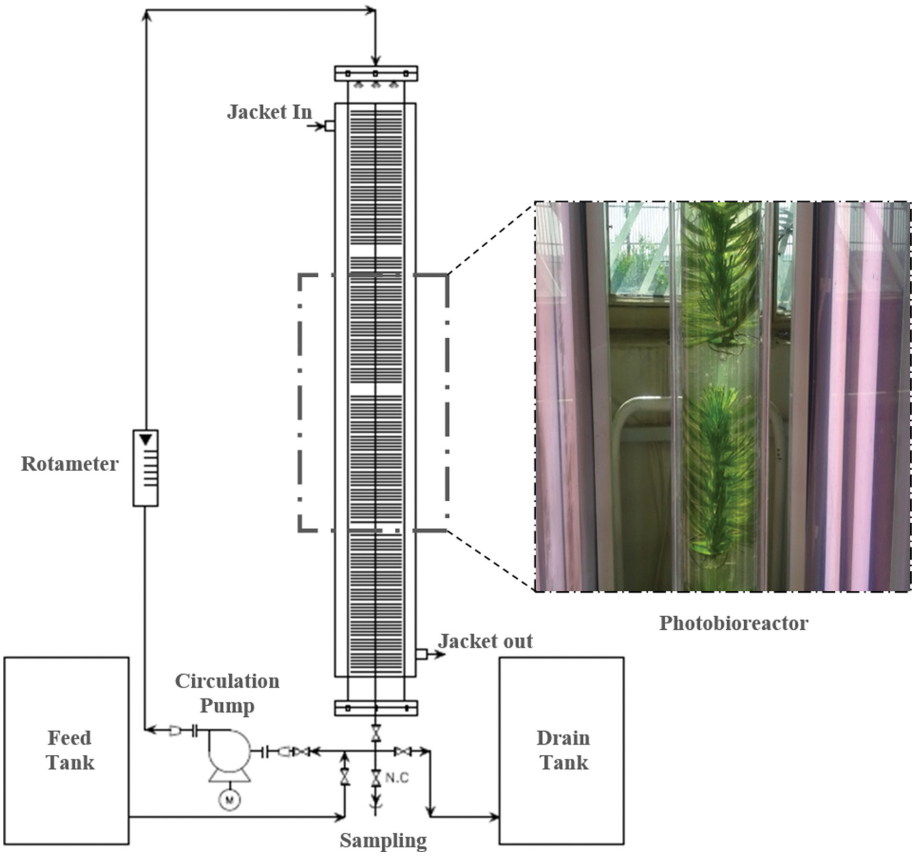

Fig. 1. Schematic diagram of cylindrical glass photobioreactor containing modified threads.

(25±2 °C) under 16 h : 8 h light : dark periods of illumination with six 36 W-cool white fluorescent lamps at an approximate intensity of 2,000 lux, which was measured by a digital light meter (TES, 1330A, Taiwan). Fresh media were prepared by adding 10% inoculum of the original culture every two weeks [1].

4. Experimental Setup

A biofilm-based cylindrical-shaped photobioreactor, including a water jacket, was used with an inner diameter of 0.09 m, a height of 1 m, and working volume of 6 L. This kind of photobioreactor has small footprint, large surface area for light absorbance, and simple construction [24]. As shown in Fig. 1, the four sets of threads were fixed in a four-part moveable shaft located in the center of the photobioreactor. This was done according to a reported procedure [7].

The photobioreactor was filled with Z8 Medium and BBM for the attachment experiments of *S. dimorphus* and *C. vulgaris*, respectively. In previous studies, a wide range of cell densities, from 10^5 to 10^7 of cells per mL, has been used for inoculation in attachment experiments [1,5,8]. In our experiments, the inocula were cultivated for 10 days to reach 10^6 mL of cells per mL. The temperature inside the photobioreactor was adjusted at 30 °C with a water jacket. The primary and final pH values were measured with a pH meter (Sartorius PT-10, Germany). The culture media were circulated by means of a pump (MD-20RZ, SPC magnet pump) with a flow rate of 20 mL/s to create a laminar condition for attachment inside the photobioreactor. Each experiment set was conducted for 30 days under continuous illumination with eight 36 W-cool white fluorescent lamps at an average intensity of 1,700 lux. Sampling was done twice during the experimentation; in each set of treatments, 15 threads were selected and cut crossly in liquid nitrogen to measure the biofilm thickness through SEM [25]. As SEM imaging requires dried samples [26,27], the thread samples were dehydrated through the freeze-drying (LYOVAC GT3, Germany) method after freezing in liquid nitrogen [27,28]. Moreover, 20 threads of different treated sets were sampled, and the attached biofilm was scrapped with tongs, dried at 103 °C and then measured gravimetrically to evaluate the total biomass productivity in terms of mg/m^2 [1]. Optical density was also measured at 750 and 540 nm for *S. dimorphus* and *C. vulgaris* respectively. The surface of the threads was imaged microscopically at various locations using a stereo microscope (Stemi 2000-C, Zeiss) to investigate the spatial structure of the biofilm formed on the threads [29].

5. Statistical Analysis

The experimental data were statistically analyzed by the Minitab software version 14.0 (Minitab Inc., USA). The measured values were expressed as mean±st.dev. Before each statistical analysis, the normality and the equal variance of the experimental data were analyzed by the Anderson-Darling test and Bartlett's test respectively. Then, the effects of different factors on the responses were analyzed using the single-factor ANOVA test. Moreover, the Tukey's method was applied to compare the experimental data. The statistical analyses were conducted at a 95% confidence interval.

RESULTS AND DISCUSSION

Modified PET threads characteristics first were analyzed, then the effect of different modification methods on *S. dimorphus* and *C. vulgaris* cell attachments on PET threads was assessed.

1. Surface FTIR Spectrum of the Threads

Fig. 2 shows the surface FTIR spectrum of the modified threads as compared to the control sample. Similar functional groups were found in the literature, which proves these threads were genuinely PET [30]. Preference of plastic substances such as PET over other materials in microalgal attachments lies in the physical and chemical considerations of some studies; in our review of the literature, some reports recommended the use of nonpolar materials such as plastic and Teflon rather than glass or metal for microalgal attachment. It is believed that the recommended materials provide better attachment [10,31]. The analysis of the surface characteristics of PET in the literature indicated that it has higher wettability and lower CA (~72.5°) among other polymers [32,33]. Reusability, availability and affordability can be mentioned as other characteristics of PET.

Fig. 2 reveals some differences between the functional groups of the control and those of the chemically etched threads, which means that chemical etchings affect the surface functional groups. The FTIR spectra of the acid-treated samples show an increase in the intensity and a shift in some peaks, compared to the control. To account for these changes shown in the spectra, the literature was searched but no report was found about the treatment effects of chromic acid on PET threads. Therefore, the changes may be attributed to the presence of dichromate [34]. The higher absorbance intensity (up to two orders of magnitude) in some peaks is also explained by the presence of more concentrated functional groups on the surface of the acid-treated samples than in the control samples. This can be related to the cross-linking bonds cleavage on the PET surface. New bands are also observed between 890 and 1,250 cm^{-1}, which may be attributed to the presence of sulfonic groups [35]. There exist a new broad absorption band near 3,035 cm^{-1} and a peak at 3,500 cm^{-1}, which refer to the hydroxyl groups [35,36]. The FTIR spectrum of the base-treated samples show the ester groups bond cleavage of the surface polymer in the presence of sodium hydroxide, leading to the substitution of carboxyl and hydroxyl groups by the surface functional groups [37,38]. The FTIR spectrum of the sandpaper-treated samples indicates less absorbance intensity in those samples than in the control. It can be related to the reduction of the negatively charged groups on the PET surface.

Substitution of surface functional groups can affect cell-substrate interaction with an increase in the wettability of PET [11,37]. The presence of oxygen-containing functional groups, like hydroxyl (-OH) and carboxyl (-COOH), on the surface of PET threads leads to more hydrophilic properties [39].

2. Modified Threads Morphology and Topography

Using a stereo microscope, certain changes were observed in the appearance of the modified threads. Modification with an acid made the threads thin and created several cracks across them. In modification with a base, however, the threads were covered with shallow pores, lost their polished surface and compared to the control samples reflected less light under the stereo microscope. Finally, modification with sandpaper made long raised grooves with different thicknesses.

Fig. 3 (Left) shows the FESEM results of the modified threads

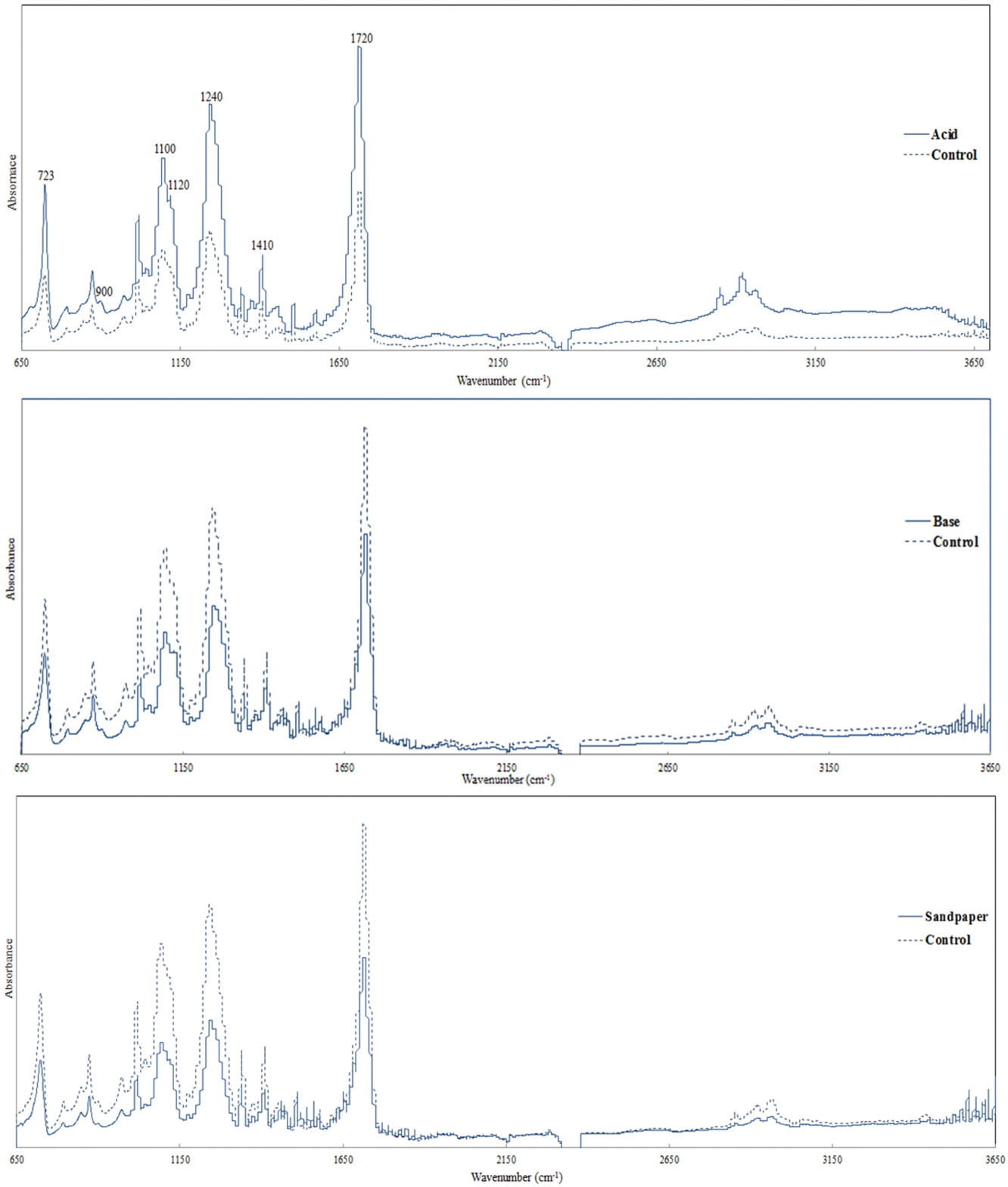

Fig. 2. Surface FTIR spectrum of modified threads compared to control (n=2).

and the control samples in 700x magnification. A comparison of the images reveals that the surface roughness of the threads modified whether with acid, base or sandpaper had gravely increased, while the control sample had a low roughness. These modified surfaces pave the way for the better settlement of microalga. Indeed, more pores and grooves in the surface of threads serve as a perpendicular barrier in the water stream of microalgae inside a photobioreactor [1].

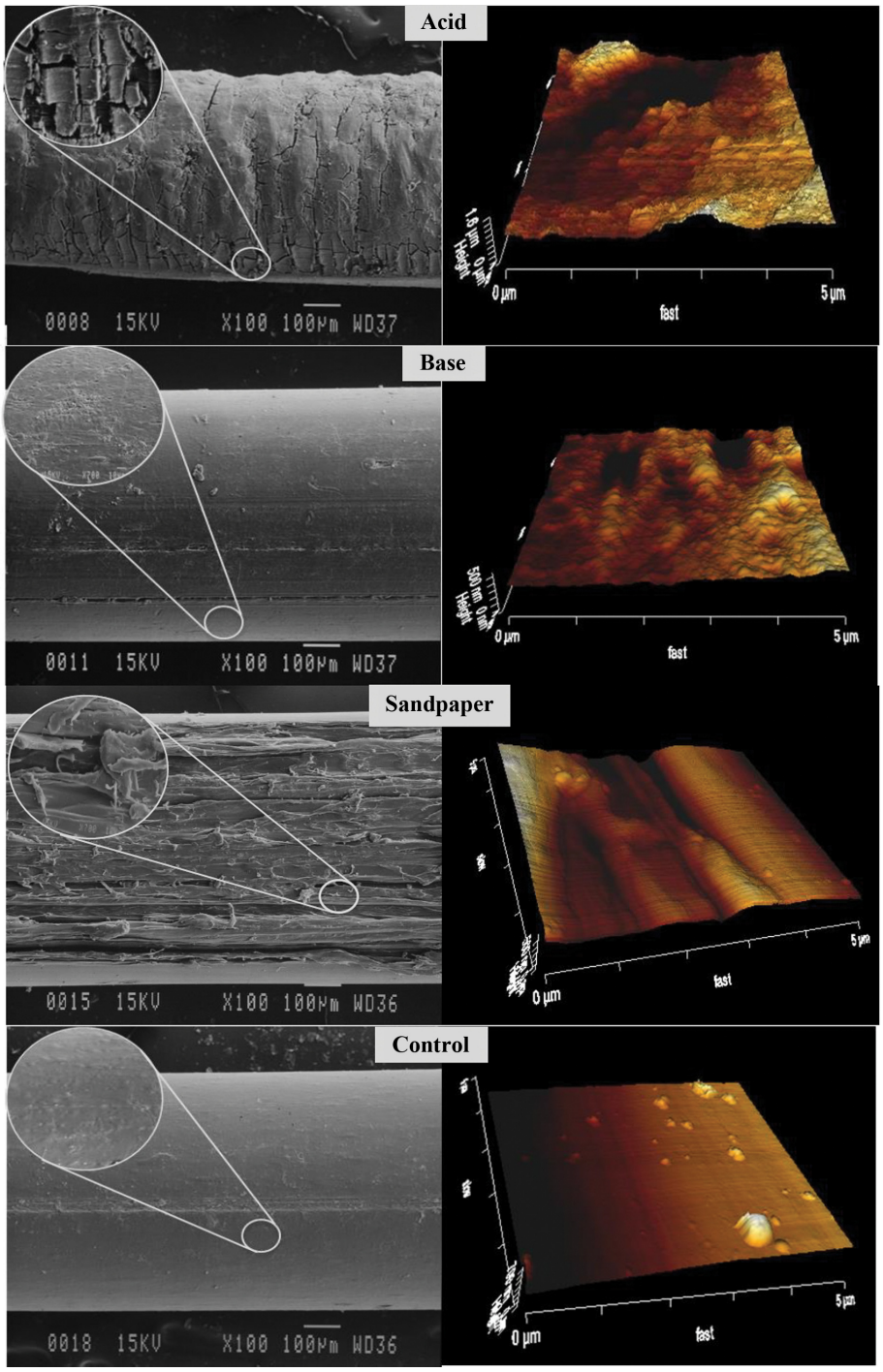

Fig. 3. FESEM (Left) and AFM (Right) Images of threads surfaces; modified with acid, base, sandpaper and control.

Fig. 3 (Right) shows the topography of the surface of the threads using AFM. As indicated in the image, the surface of the control samples is smooth, while that of the modified threads has various surface pores or grooves. It suggests that the threads modified with acid or sandpaper have prominent surface grooves while base-modified threads are covered with shallow pores.

As many as ninety different images of the modified threads were taken by AFM. The images were analyzed by the JPK software. The results are summarized in Table 1.

The statistical analysis indicated that the effect of surface modification with acid, base and sandpaper on the threads was significant ($P<0.000$). Moreover, Tukey's test proved the presence of different intervals of surface roughness; the surface modified with a concentrated acid demonstrated a relatively higher rate of roughness, but the one modified with a concentrated base was relatively less rough. Compared with the control, there were noticeable changes in the average roughness of surfaces modified with acid (323%), sandpaper (184%) and base (11.5%). The increased roughness of

Table 1. Surface properties of modified threads (Number of sample images=90; P<0.05)

Type of threads	Acid	Base	Sandpaper	Control
Average height (mean±st.dev, nm)	4.2 ± 1.6^{b}	5.21 ± 0.29^{a}	5.2 ± 1.2^{a}	5.52 ± 0.58^{ab}
Average roughness (mean±st.dev, nm)	440 ± 34^{a}	116 ± 36^{c}	295 ± 55^{b}	104 ± 25^{c}
RMS roughness (nm)	566.67^{a}	136.97^{a}	345.64^{b}	129.19^{c}
RMS roughness (%)	15.16	2.60	6.20	2.28
Peak to valley roughness (mean±st.dev)	2940±897	720±364	1399±789	694±243
Fractal dimension	1.09-1.52	0.66-1.49	1.26-1.69	1.37-1.81
Skewness (mean±std. error)	-0.34 ± 0.13^{b}	-0.02 ± 0.10^{ab}	-0.14 ± 0.10^{ab}	0.13 ± 0.06^{a}
Kurtosis (mean±std. error)	3.33 ± 0.20^{a}	2.42 ± 0.22^{a}	2.94 ± 0.09^{a}	2.53 ± 0.24^{a}

Table 2. Contact angle analysis of different treatments with Tukey comparison (n=3; P<0.000; mean±st.dev)

Parameter / Type of treatment	Avg. of contact angle (mean±st.dev)	Percentage of CA variation$^{\varphi}$ (in mean values)	Avg. of surface energy (mN/m) mean±st.dev	Percentage of SE variation$^{\varphi}$ (in mean values)	Polar surface energy (mN/m)	Dispersive surface energy (mN/m)
Control	67 ± 5^{a}	-	47 ± 15^{a}	-	5.8	42.09
Base	47 ± 22^{ab}	30%	58 ± 26^{ab}	23%	33.99	24.39
Sandpaper	33 ± 9^{bc}	51%	73 ± 29^{ab}	55%	67.65	5.67
Acid	18 ± 4^{c}	73%	78 ± 3^{ab}	66%	72.7	5.47

φ based on the control

PET surfaces through base treatment is already reported in the literature [38], but, to our best knowledge, no report exists about acid- or sandpaper- modified PET.

The fractal analysis demonstrated the existence of fractal components in the modified PETs. As the surface of the control was covered by some roughness, the fractal dimension was placed in a quite higher range. However, AFM has some limitations in measuring steep walls that are seen abundantly on the surface of acid-modified PET. As a result, the relatively lower amount of fractal dimension represents smoother surface by mistake. The amount of fractal dimensions of base-modified PET confirmed that the roughness was affected by treatment.

The skewness and kurtosis of the frequency distribution of an image describe the asymmetry and flatness of the surfaces, respectively. The positive mean value of skewness obtained for the control means that the surface of the threads was covered by more peaks than valleys. The modification methods created a negative value of skewness [40], which means that the modified surfaces were covered by more valleys and tended to increase fluid retention and wettability [41]. The kurtosis mean value of the modification with acid was higher than three, so the surface had peaked pores [42].

3. Modified Thread Contact Angle

The changes in the CA of the modified threads are reported in Table 2. Those results were achieved with the use of samples with six different CAs (right and left of the drop), as practiced in some other studies such as [43,44].

Through an ANOVA test, it was realized that surface modification can significantly affect the surface CA (P<0.000). According to the criterion of CA for surfaces [16], the threads modified with base, sandpaper and acid were located in a complete wettability zone (the percentage of decrease were 73%, 51% and 30% for acid, sandpaper and base respectively).

A low CA, which is suggestive of high wettability, can cause better adhesion of microalgal cells [10,45,46]. In addition, the surface energy of treated threads is increased as a result of introducing polar functional groups during the treatment process [47].

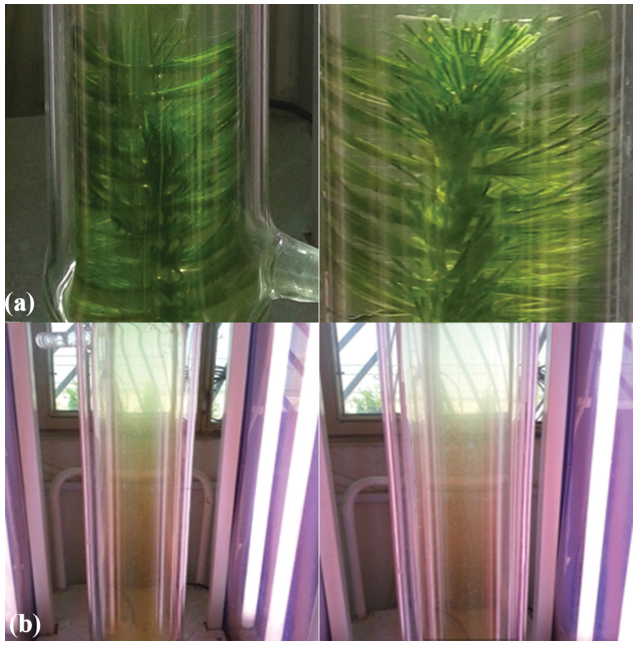

Fig. 4. Cultivation of *S. dimorphus* in photobioreactor containing modified threads: after ten days (a); after removing threads from the photobioreactor (b).

Treatment led to morphological (higher RMS roughness), chemical (increased oxygen-containing functional groups) and physical (higher availability of hydrophilic groups) changes on the surface of PET which, in turn, caused an increase in the surface area and a decrease in the surface hydrophobicity [36,37].

4. Results of Microalgal Attachment on the Modified Threads

4-1. *S. dimorphus* Attachment on the PET Threads

As mentioned, *S. dimorphus* was cultivated in a photobioreactor to assess the rate of attachment on different treated threads. The pH values of the culture media were mostly found to be in the range of 7.5 to 10.9. Fig. 4(a) shows the *S. dimorphus* culture column after 10 days. The cultivation of *S. dimorphus* in a photobioreactor containing the modified threads resulted in efficient cell attachment on the PET threads as the polymeric surface finally turned dark green after ten days. Bubbling was also observed during the microalgae cultivation inside the photobioreactor, which indicated the aliveness of biolayers [7]. Fig. 4(b) shows the retention of the culture medium after the threads were removed [43]. The culture medium stayed clear as long as the cell count of the samples was negligible. Compared to other studies in the field, the present research showed that modification can lead to a remarkable decrease in the time of attachment from thirty days [7] to ten days.

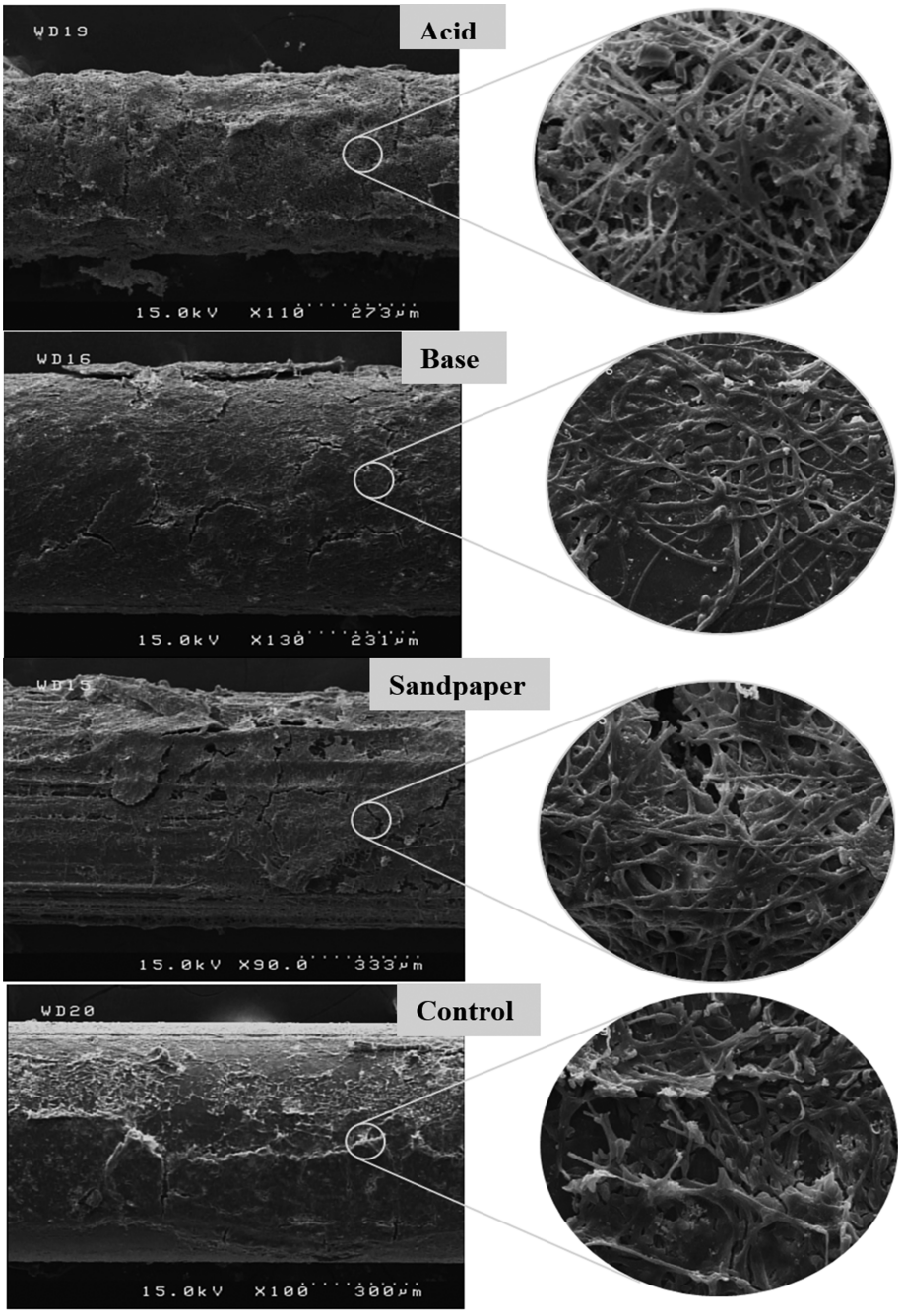

Fig. 5. SEM's images of biofilm formed on the threads modified with acid, base, sandpaper and control in 100 and 1,000x magnification.

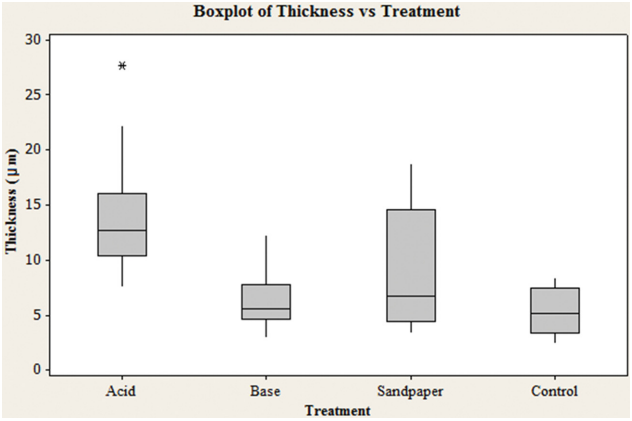

Fig. 6. Comparison of biofilm thickness formed on different modified threads (Number of samples=60).

This result, i.e., an increase in the attachment rate, causes a decrease in the overall time of industrial processes, which can be of great importance in the development of biofilm culturing processes.

4-1-1. Formation of a *S. dimorphus* Biofilm on the PET Threads

Surface etching provides deeper spaces for the settlement of microorganisms. It leads to increased cell immobility and attachment. The next stage after the adhesion of initial cells is colonization on the surface of the threads. In this stage, microorganisms start to secrete exopolysaccharide in order to attach firmly to the surface and protect themselves from shear stress [10,48]. Fig. 5 presents the inside views of the biofilm structures formed on different modified threads. As it can be seen, in comparison with the base-treated samples and the control samples, the biofilms formed on the threads modified with acid and sandpaper are better bonded to the substratum.

4-1-2. *S. dimorphus* Biofilm Thickness in Comparison

Fig. 6 presents a boxplot for the distribution of the thickness of the *S. dimorphus* biofilms formed on different threads. The results were extracted from sixty samples evaluated via SEM. The mean and the median of the biofilm thicknesses (μm) in the modified threads were 13.92 and 12.65 for the acid-modified ones, 6.35 and 5.58 for the base-modified ones, 8.76 and 6.75 for modification with sandpaper and 5.34 and 5.2 for the control samples. The measured values for acid and sandpaper treatments lie within wider ranges than the base-treated and the control samples. The figure also shows higher values of thickness for the acid and sandpaper modifications, as compared to the base modification and the control. The median value of the control samples is almost equal to the mean, which implies a normal distribution in the experimental data. The median values of the other treatments are lower than their mean values, suggesting that the majority of the values are lower than the mean. In addition, compared to the control samples, the increase in the mean thickness of the biofilms was found to be 19% and 64% for modification with base and sandpaper, respectively. A remarkable increase of 160% was also achieved through the treatment with acid. In line with the findings of this study, researchers observed higher values of thickness for *S. obliqqus* using different substrata on non-sterile wastewater, which can be related to the higher densities of the inocula [1].

4-1-3. Comparison of the Biofilms in Terms of Dry Weight

The results of Tukey's test ($p<0.000$) on the dried biomass productivity (mg/m^2) showed that the dry weights varied in the range of 7.45 ± 0.04^a for acid, 6.68 ± 0.07^b for base, 7.06 ± 0.04^c for sandpaper modification and 6.1 ± 0.1^d for the control threads. In comparison to the controls, the threads modified with base, sandpaper and acid had an increase rate of 9.5%, 15.7% and 22% in their dry weight, respectively. It is worth mentioning that, when the threads were modified (especially with acid or sandpaper), it became hard to harvest the formed biofilms. It was because the cells are placed in the surface grooves and resist detachment from the surface by secreting exopolysaccharide threads. The EPS of the biofilms prevented removal of the whole thread biomass of the biofilms; however, it has a benefit for the continuation of cultivation in that further inoculation can occur by the remaining colonies [1]. If it is desired to harvest entire biofilms of modified threads, the best way is to use a sharp blade.

The findings on the dry weight were in good agreement with those on the biofilm thickness, confirming the increased values of the treated threads. Surface modification caused an increase of roughness, a decrease of CA and an increase of surface energy, compared to the control threads. The increased roughness provided a larger surface area available for biofilm formation [10].

The CA decrease also caused more attraction and a closer contact of water droplets and, in consequence, more colonization of the microalgal cells [11]. In contrast, the effect of surface energy on the adhesion of microalgae varied from case to case. In the current research, the adhesion of *S. dimorphus* was directly related to the increased surface energies even though different results have been reported in other studies such as [5,47,48].

Based on the results, modification with acid provided a thicker biofilm than the other treatments do. Three possible reasons can be mentioned in this regard. First, it may be related to the functional groups which cause the better adhesion of *S. dimorphus* cells. The significantly higher biofilm formation in the acid-treated threads may be attributed to the presence of full or partial positive charges of the treated PET functional groups. It may also be attributed to the presence of divalent cations in the culture medium and the high level of the negatively charged groups on the surface [11]. Secondly, according to the results in Table 1, the grooves on the acid-modified surfaces were deeper than on the others samples, which provided more space for biofilm formation [5]. Thirdly, according to the results in Fig. 3, the direction of the acid-modified grooves was perpendicular to the flow stream [1].

The smaller amount of the surface functional groups in the sandpaper and base treatments caused the better attachment of *S. dimorphus* than in the control group. The roughened and less negatively charged surface of the sandpaper-treated threads led to better attachment, compared to the base treated [5].

Contrary to the results, it is recommended to utilize sandpaper surface modification method due to the dangers involved in the use of acids, non-repeatability of chemical surface modification methods and toxic products resulting from the decomposition of polymers. Moreover, although modification with acid sounds possible on a laboratory scale, it falls short of use on an industrial scale [16].

4-2. *C. vulgaris* Attachment on the PET Threads

Through the formation of *C. vulgaris* biofilms on the modified threads, it emerged that the thread surfaces were not fully covered by the microalgal biofilm. This means that the strain was unable to form a proper biolayer during the culture period, which is similar to the findings of other studies in the literature [1,49].

4-3. Comparison of the Attachment Efficiencies of *S. dimorphus* and *C. vulgaris*

As previously mentioned, *S. dimorphus* and *C. vulgaris* were separately cultivated to form biofilms in a cylindrical photobioreactor. The results pointed to the lower attachment efficiency of *C. vulgaris* than *S. dimorphus*. Due to chemical complexity, no comprehensive thermodynamic model has been found to account for both microalgal attachments [13,50].

Several factors contributed to the formation of a stable biofilm of *S. dimorphus* in different stages. The intrinsic ability of *Scenedesmus* to settle better in a later growth phase [51] and the existence of flagella [52,53] caused the better attachment of the first cells of this strain to the surface, as compared to *Chlorella* [54,55].

The two microalgal strains have somewhat similar hydrophilic cellulose-based cell walls and negatively charged groups, which seem to have similar attraction and repulsion close to the PET surface. However, the cell wall of *C. vulgaris* is more hydrophilic than that of *S. dimorphus*. This is due to the higher zeta potential [39, 56] and more negatively charged groups on the cell wall of *C. vulgaris* [10,11,57,58]. These groups cause more repulsive electrostatic forces, which results in the reversible binding of *Chlorella* to the surface [39]. Moreover, pH values outside the optimum range cause massive repulsive interactions between the cells, which stops the further development of biofilms. This leads to the dispersion of *C. vulgaris* cells in the culture medium, while *S. dimorphus* tends to form a self-attached structure in experimental conditions [39,56].

The differences in shape and size of the cells play an important role in the efficiency of attachment [5]. *C. vulgaris* is spherical with diameter in the range of 2 to 3.5 μm [59,60]. In contrast, *S. dimorphus* is almond-shaped with an average cell length of 10 to 16 μm and cell diameter of 3 to 5 μm [5,61-63].

According to the diagram reported previously, if the dimension of microalgal cells is close enough to the surface dimensions of grooves and pores, those cells settle into the grooves [64]. As a result, the formation of cell-surface initial bonds is greatly accelerated. In contrast, more or less similar dimensions in this regard cause a great decrease in the surface bonds [5,64]. The settlement of *S. dimorphus* was increased due to the same length of most acid and sandpaper-treated grooves. This aided the microalgal cells to get adjusted well into the grooves [5,65]. The width of the pores on the base-treated surface was estimated to be in the range of 1 to 2 μm, which kept the cells of *C. vulgaris* from settling into the pores.

Contact point theory can help to explain the results of *C. vulgaris* adhesion onto the acid and sandpaper-treated surfaces. According to a proposed theory [5], the energy required for adhesion to a surface is proportional to the changes of the surface area. Thus, the fewer contact points, the more energy for adhesion. In the present study, there were few contact points for the settlement of the spherical cells of *C. vulgaris* on the acid and sandpaper-treated surfaces with a pore size mostly in the range of 5 to 10 μm.

Eventually, the secretion of extracellular polymeric substances (EPSs) strongly contributed to the stability of biofilms [56]. As indicated in Fig. 5, the EPSs stabilized the mature biofilm of *S. dimorphus*, while *C. vulgaris* cells were not able to secrete any EPS [66].

CONCLUSION

This study aimed at the effect of surface engineering on the attachment of two microalgae. The treatments resulted in a larger hydrophilic surface and an increase in the surface roughness. Due to intrinsic properties, the cultivation of *C. vulgaris* did not lead to biofilm formation, while *S. dimorphus* could efficiently form biofilms on the treated PET threads. The rate of *S. dimorphus* attachment increased about three times, and the total weight of the biomass rose up to 22% with acid treatment. Based on the results, prior to the selection of an engineering method, attention should be paid to the intrinsic properties of microalgal strains if an efficient attachment is to be achieved. With regard to the gaps in the literature, further studies are required on biofilm-based microalgal cultivation systems.

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DECLARATION OF AUTHORS' CONTRIBUTION

S. Danaee was in the charge of conceptualizing, designing, performing the experiments, analyzing the data and drafted the article. S. M. Heydarian and H. Ofoghi supervised the research and were responsible for the concepts, design, critical revision of the article for important intellectual contents, and the final approval of the article.

SUPPORTING INFORMATION

Additional information as noted in the text. This information is available via the Internet at http://www.springer.com/chemistry/journal/11814.

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Supporting Information

Acceleration of microalgal biofilm formation on PET by surface engineering

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In each replicate, spatial structure of the sampled biofilm of threads was analysed at the end of the test period. Different surface modification resulted to different biofilm formation (Fig. S1):

Although the biofilm formed on the acid-modified threads expected to be thicker, the sandpaper-modified threads presented disordered biofilm with different thicknesses along the threads. The biofilm formed on the base-modified threads looks more orderly than the two previous ones and bears more green spots compared to the control sample. This phenomenon can be related to the surface topography of pores and grooves of the threads where the sandpaper- and acid-etched surfaces were irregular compared to base-modified threads.

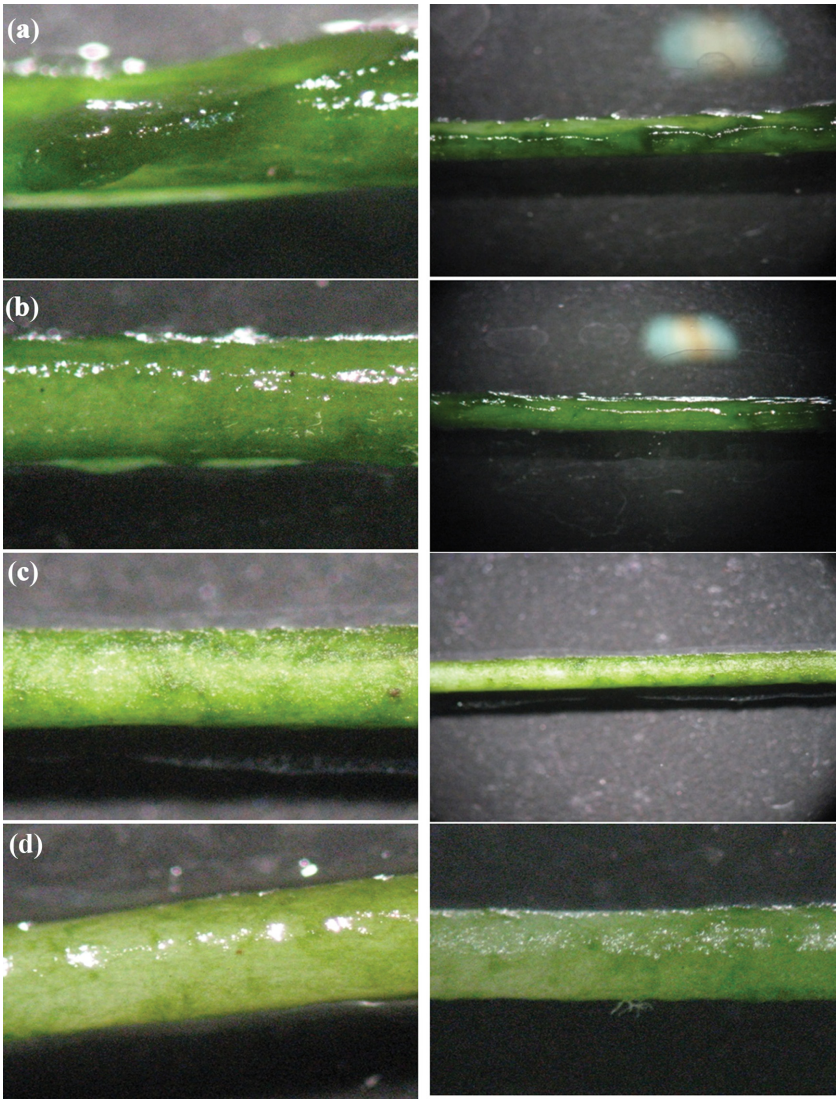

Fig. S1. Spatial structure of the biofilm formed on treated threads; modified by acid (a); base (b); sandpaper (c) and control (d).

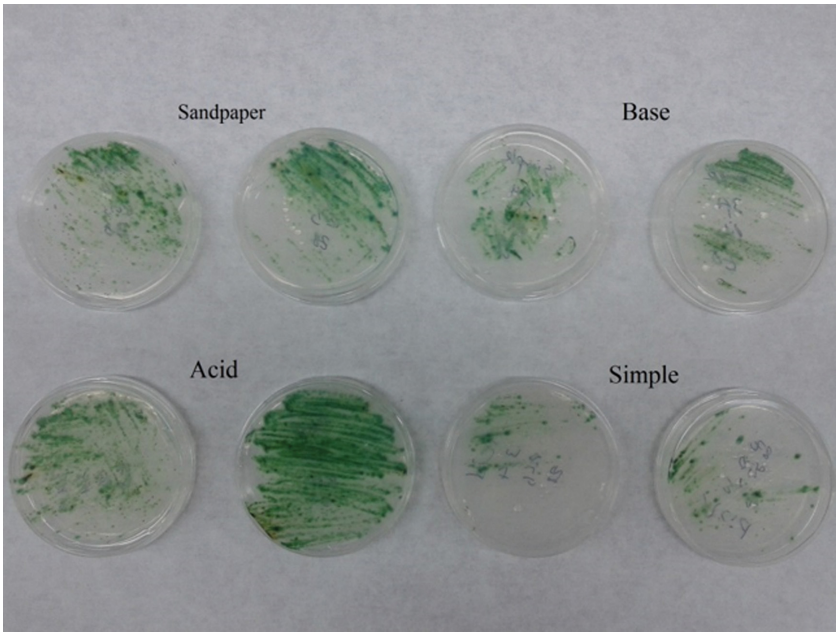

Fig. S2. Final cultivation of biofilms to investigate viabilities.

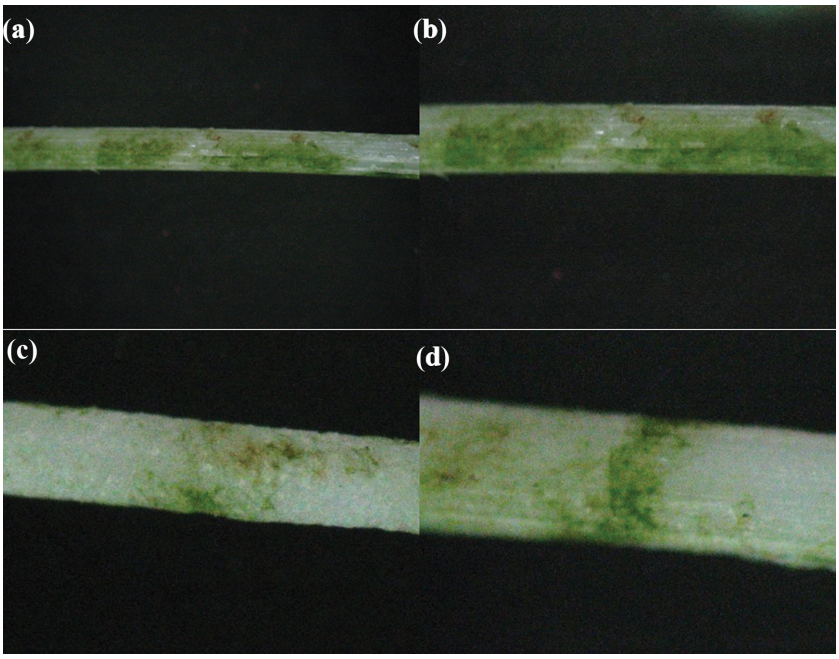

Fig. S3. Biofilm of microalga *C. vulgaris* formed on the threads; (a) & (b) control; (c) & (d) modified threads.

Analysis of the Viability of the Biofilm Formed on the Modified Threads

Viability of the *Scenedesmus* biofilms were analysed by sampling from the surface of the threads and culturing them on a solid environment. The results of this sampling and culturing were reported in Fig. S2. According to this figure, the microalgae have grown well in the petri dishes which deduced the biofilm was alive after the period of cultivation.

C. vulgaris Attachment on PET Threads

Fig. S3 shows the results of *C. vulgaris* biofilm formation on the modified threads. Results of *C. vulgaris* biofilm formation on the modified threads showed that thread surfaces have not fully covered by microalgal biofilm.

The results showed low attachment efficiency of *C. vulgaris* compared to *S. dimorphus*.