Journal of Applied Phycology (2019) 31:375–387
https://doi.org/10.1007/s10811-018-1582-3

Microalgal/cyanobacterial biofilm formation on selected surfaces: the effects of surface physicochemical properties and culture media composition

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Received: 9 April 2018 / Revised and accepted: 11 July 2018 /Published online: 27 July 2018

Abstract

The increased interest in photosynthetic microorganisms for wastewater treatment processes has led to the demand for new biomass harvesting strategies. Biofilm systems have emerged as a good alternative to planktonic photosynthetic cultures. However, knowledge on the environmental aspects influencing microalgal/cyanobacterial biofilm formation is required. This study reports the influence of: (i) surface physicochemical properties of selected microorganisms (*Chlorella vulgaris*, *Pseudokirchneriella subcapitata*, *Synechocystis salina*, and *Microcystis aeruginosa*) and materials (copper—Cu; glass—G; poly(methyl methacrylate)—PMMA; polystyrene—PS; polyvinyl chloride—PVC; and AISI316 stainless steel—SS) and (ii) culture media composition (glucose-deficient and glucose-enriched media) on biofilm formation (up to 7 days), with constant temperature, light irradiation, and shaking conditions. Adhesion was assessed through thermodynamic prediction of adhesion and by in vitro adhesion assays on microtiter plates. In general, higher biofilm densities were observed after 7 days of experiment, and followed the order: SS > PS > G > PVC > PMMA> Cu. *M. aeruginosa* was the highest biofilm-former microorganism (2.1×10^6 CFU cm^{-2}), while *P. subcapitata* has shown lack of ability to adhere. Moreover, the higher biofilm formation ability was observed when glucose-deficient medium was used. Furthermore, the present results pointed out that the thermodynamic approach failed to predict the stochasticity of microalgal/cyanobacterial adhesion. In light of these findings, others factors must be considered when using predictive tools. Therefore, fine-tuning on photosynthetic biofilm formation can be obtained by optimizing the bulk fluid composition and the type of surface. In conclusion, the results show the potential of the selected microalgae/cyanobacteria for biofilm-based technology.

Keywords Adhesion · Biofilms · Cyanobacteria · Media composition · Microalgae · Surface physicochemical properties

Introduction

Over the past century, the potential of microalgae and cyanobacteria has been extensively explored in a wide range of applications. Current applications for these photosynthetic microorganisms range from human and animal nutrition to pharmaceuticals and cosmetics production (Singh et al. 2005; Spolaore et al. 2006; Del Campo et al. 2007; Priyadarshani and Rath 2012). For many years, microalgae and cyanobacteria have been applied in wastewater treatment processes since their use allows (i) low operational costs, (ii) no organic carbon requirements, (iii) the decrease of CO_2 emissions associated to wastewater treatment plants, (iv) the obtainment of an oxygenated effluent, (v) heavy metals removal in a safer way, (vi) the recycling of nitrogen and phosphorus into microalgal/cyanobacterial biomass, and (vii) the reduction in sludge formation (Aslan and Kapdan 2006; Ruiz et al. 2011; Cai et al. 2013; Wang et al. 2014). However, the major drawback associated to wastewater treatment using these microorganisms is the separation of the produced biomass from the treated effluent (Ruiz et al. 2011; Abdel-Raouf et al. 2012). Current methods used for microalgal harvesting include chemical flocculation, bioflocculation, gravitational sedimentation, filtration, electrocoagulation-flocculation, flotation, centrifugation, and their combination (Milledge and Heaven 2013; Gonçalves et al. 2015). However, these methods are time-consuming or expensive (Barros et al. 2015). In this sense, immobilization systems emerged as a good alternative, as they avoid further steps of biomass recovery from the effluent.

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Microalgal/cyanobacterial biofilms have gained particular attention in wastewater treatment systems since they can promote a rapid and efficient harvesting of biomass, leading to lower downstream processing costs (Abdel-Raouf et al. 2012). There are many reactors using microalgal biofilms for wastewater treatment. The Algal Turf Scrubber is one of the most used microalgal biofilm reactors for wastewater treatment. This system was invented and patented by Adey (1982) and has been widely used ever since by others (Jensen 1996; Schumacher et al. 2003; Stewart and Zivojnovich 2003) with some modifications. Several studies have shown satisfactory results regarding nutrient removal efficiencies (36–93%) and biomass productivities (5 to 20 g m^{-2} day^{-1}) using this system (Mulbry and Wilkie 2001; Wilkie and Mulbry 2002; Mulbry et al. 2005). Muñoz et al. (2009) compared a flat plate photobioreactor and a tubular photobioreactor, demonstrating that higher biochemical oxygen demand (BOD) removal rates can be obtained with microalgal biofilms (27 mg and 32 mg BOD L^{-1} h^{-1}, respectively). More recently, Johnson and Wen (2010) developed a polystyrene rocker system using different materials for microalgal and cyanobacterial attachment. Although the aim of these authors was to enhance biofuel production, this mechanism allowed nutrient removal efficiencies ranging between 70 and 100%. Boelee et al. (2011) designed and operated a rotating algal biofilm reactor at laboratory and pilot scales in order to maximize microalgal production in wastewaters resulting from the secondary treatment step of wastewater. With this system, high nutrient removal rates were reached with values of 14.1 and 2.1 g m^{-2} day^{-1} for total nitrogen and phosphorus, respectively.

Biofilms are an assemblage of cells attached to a substrate and embedded in a self-produced matrix of extracellular polymeric substances (EPS) (Donlan 2002; Christenson 2011; Christenson and Sims 2011). An ideal substrate for biofilm formation should present the following characteristics: non-toxicity, stability, and ability for biomass retention (Mallick 2002). Microbial adhesion to a substrate constitutes one of the main steps of biofilm development and strongly depends on several factors that can be divided into: (i) biological factors, (ii) physicochemical properties, and (iii) environmental conditions. Biological factors influencing microbial adhesion and biofilm formation include EPS production (Zhang and Fang 2001) and the presence of external structures, such as fimbriae, prosthecae, pili, or flagella, able to promote the contact between the microorganisms and the adhesion substrate. Physicochemical properties of the adhesion surfaces also influence the microbial attachment process. Finally, environmental conditions affecting photosynthetic biofilms formation include light (Hill et al. 1995), pH (Liehr et al. 1990), hydrodynamic conditions (Kokare et al. 2009), nutrient quality and quantity (Sekar et al. 2002; Kesaano and Sims 2014), temperature (Babu 2011), and carbon source (Yang and Gao 2003). Several studies have focused on the effects of surface physicochemical properties on microbial adhesion and biofilm formation, reporting that in general, microbial adhesion tends to occur preferably on hydrophobic surfaces (Holland et al. 2004; Sekar et al. 2004; Stanley and Callow 2007; Li et al. 2010; Ozkan 2012). However, other factors, such as culture medium and microbial species, can play an important role on microalgal/cyanobacterial attachment to a surface (Becker 1996; Johnson and Wen 2010; Irving and Allen 2011).

Although there are many studies focusing on surface physicochemical properties of several microorganisms and surface materials, studies comparing thermodynamic approaches and in vitro biofilm formation experiments are not very common or even absent for microalgae and cyanobacteria (Sekar et al. 2004; Irving and Allen 2011; Ozkan and Berberoglu 2011; Sirmerova et al. 2013; Gonçalves et al. 2015;). Accordingly, the aim of this study was to determine the surface physicochemical properties of *Chlorella vulgaris, Pseudokirchneriella subcapitata*, *Synechocystis salina*, and *Microcystis aeruginosa*, as well as those of selected surface materials (copper—Cu, glass—G, poly(methyl methacrylate)—PMMA, polystyrene—PS, polyvinyl chloride—PVC, and AISI316 stainless steel—SS) and evaluate their influence on biofilm formation. The influence of different medium compositions was also assessed through biofilm formation in vitro experiments. These microorganisms were selected because they have been extensively used in several applications, namely in wastewater treatment processes (Moreira-Santos et al. 2004; Memon et al. 2014; Gonçalves et al. 2015. The surface materials were selected because: (i) Cu is a control surface due to its cellular toxicity (Stauber and Florence 1987; Trevors and Cotter 1990; Levy et al. 2007; Grass et al. 2011); (ii) Cu, PVC, and SS are widely used in industrial applications (Carvalho et al. 2006, Kim et al. 2013); (iii) G, PMMA, and PS are transparent surfaces, allowing light penetration, which can be relevant for the growth of photosynthetic organisms (Booth 1971; Knauss and Schapery 1999); and (iv) PMMA and PVC are commonly used as biofilm adhesion support in wastewater treatment processes (Wuertz et al. 2003; Muñoz et al. 2009; Kesaano and Sims 2014).

Materials and methods

Microorganisms and culture conditions

The microalgae *Chlorella vulgaris* CCAP 211/11B and *Pseudokirchneriella subcapitata* CCAP 278/4 were obtained from Culture Collection of Algae and Protozoa (UK), while the cyanobacteria *Synechocystis salina* LEGE 06079 and *Microcystis aeruginosa* LEGE 91344 were obtained from the Laboratory of Ecotoxicology, Genomic and Evolution – CIIMAR (Centre of Marine and Environmental Research of the University of Porto, Portugal). Stock solutions of these microorganisms were prepared in OECD (Organization for

Economic Co-operation and Development) test medium (OECD 2011) with the following composition (per liter), 250 mg $NaNO_3$, 12 mg $MgCl_2{\cdot}6H_2O$, 18 mg $CaCl_2{\cdot}2H_2O$, 15 mg $MgSO_4{\cdot}7H_2O$, 45 mg KH_2PO_4, 0.08 mg $FeCl_3{\cdot}6H_2O$, 0.1 mg $Na_2EDTA{\cdot}2H2O$, 0.185 mg H_3BO_3, 0.415 mg $MnCl_2{\cdot}4H_2O$, 3 μg $ZnCl_2$, 1.5 μg $CoCl_2{\cdot}6H_2O$, 0.01 μg $CuCl_2{\cdot}2H_2O$, 7 μg $Na_2MoO_4{\cdot}2H_2O$, and 50 mg $NaHCO_3$. Growth medium was sterilized by autoclaving at 121 °C for 15 min. The cells were incubated in 500-mL flasks at room temperature (24.0 ± 1.0 °C), under continuous fluorescent light with an irradiance of approximately 120 μmol photons m^{-2} s^{-1} at the surface of the flasks (Gonçalves et al. 2015). Agitation was obtained by bubbling atmospheric air (filtered through 0.22-μm cellulose acetate membranes, Orange Scientific, Belgium) at the bottom of the flasks.

Surface contact angles

Microalgal and cyanobacterial suspensions in the exponential growth phase were harvested, washed twice, and resuspended in saline solution (0.85%, *w*/*v*, NaCl) to obtain a final concentration of about 5.0×10^6 cells mL^{-1}. Microbial lawns were prepared by filtering the previously washed suspensions using 0.45-μm nitrocellulose membrane filters (Advantec MFS, Inc., Japan) until complete clogging of the membranes. The surface materials used in this experiment were Cu, G, PMMA, PS (bacterial grade), PVC, and SS. The surfaces were washed with commercial detergent and sterile water, and sterilized under UV light for 30 min. Contact angle measurements of the above-described lawns and the surface materials were performed using the sessile drop method, as described by Busscher et al. (1984). The measurements were carried out at room temperature using water, formamide, and α-bromonaphthalene (Sigma-Aldrich, Portugal) as reference liquids. Determination of contact angles was performed automatically using an OCA 15 Plus (Dataphysics, Germany) video-based optical contact angle measuring instrument that allows image acquisition and data analysis. Contact angle measurements (at least 20 determinations for each liquid and for each suspension/material) were performed in three independent experiments.

Surface physicochemical parameters and hydrophobicity

Surface hydrophobicity of the selected microorganisms and surface materials were determined using the approach of Van Oss (1995), which allows the assessment of the absolute degree of hydrophobicity of any surface in comparison with their interaction with water. These determinations were performed as described by Gonçalves et al. (2015).

Free energy of adhesion determinations

The free energy of adhesion between the selected microorganisms and surface materials was determined according to Simões et al. (2008). When studying the interaction between surfaces *i* (microalgal/cyanobacterial cells) and *I* (surface materials) that are immersed or dissolved in water, the total interaction energy ($\Delta G_{adhesion}$, in mJ m^{-2}) can be assessed through the following expression:

$$\begin{aligned}\Delta G_{adhesion} = {} & \gamma_{il}^{LW} - \gamma_{iw}^{LW} - \gamma_{Iw}^{LW} \\ & + 2\left[\sqrt{\gamma_w^+}\left(\sqrt{\gamma_i^-} + \sqrt{\gamma_I^-} - \sqrt{\gamma_w^-}\right) + \sqrt{\gamma_w^-}\right] \\ & \times \left(\sqrt{\gamma_i^+} + \sqrt{\gamma_I^+} - \sqrt{\gamma_w^+}\right) - \sqrt{\gamma_i^+\gamma_I^-} - \sqrt{\gamma_i^-\gamma_I^+}\end{aligned} \quad (1)$$

Thermodynamically, adhesion is expected to occur if $\Delta G_{adhesion} < 0$, while if $\Delta G_{adhesion} > 0$, microbial adhesion is not favored.

Biofilm formation in vitro assays

In vitro biofilm formation assays were performed based on the method proposed by Meireles et al. (2015). In these experiments pieces of Cu, G, PMMA, PS, PVC, and SS were used as biofilm substrates. The dimensions of the pieces were 1.0 × 0.9 cm, with a thickness ranging from 0.1 to 0.2 cm. The pieces were washed with commercial detergent and sterile water, and sterilized under UV light for 30 min. Additionally, biofilm formation experiments were performed using two different media: OECD test medium (OECD 2011) and a synthetic medium simulating a domestic effluent (synthetic effluent, SE), both protein-free. The OECD test medium is an inorganic medium presenting some macronutrients, such as inorganic nitrate and phosphate, and a wide variety of inorganic salts, the trace elements required for microalgal/cyanobacterial growth. Composition of this culture medium is the same as the one described by Gonçalves et al. (2015). On the other hand, the SE used in this study is a modified version of the one proposed by Gebara (1999), having the following composition (per liter), 300 mg $C_6H_{12}O_6$, 62 mg $NaNO_3$, 10 mg $MgSO_4{\cdot}7H_2O$, 11 mg KH_2PO_4, 1 mg $MnSO_4{\cdot}H_2O$, 0.46 mg $CaCl_2$, and 0.05 mg $FeCl_3{\cdot}6H_2O$. This culture medium also contains inorganic sources of nitrogen and phosphorus. However, it contains an organic carbon source (glucose). The initial pH of the culture media was 7.1 and 6.7 for the OECD and SE test media, respectively.

After cleaning and sterilization, the coupons were inserted in 12-well microtiter plates (Orange Scientific, Braine-l'Alleud, Belgium). Microalgal/cyanobacterial suspensions of the selected microorganisms were centrifuged at 16800×*g*

for 15 min (5810 R Eppendorf, Germany). After centrifugation, the supernatant was discarded and the suspensions were diluted in each of the culture media evaluated in this study to obtain a final concentration of 1.0×10^6 cells mL^{-1}. Then, 3 mL of each cell suspension was added to the respective well with the coupon already inserted. The plates were placed in a KS 130 orbital shaker (IKA Werke, Germany) with constant agitation of 160 rpm. Biofilm formation was allowed to occur for 24, 72, and 168 h at room temperature and constant light intensity (approximately 120 μmol photonsm^{-2} s^{-1}, as the one used for stock solutions preparation). After these periods, the coupons were placed in 15-mL tubes containing saline solution and microalgal/cyanobacterial cells were removed from the coupons by vigorously vortexing for 1 min. These suspensions were further used for quantification of biofilm cells. Additionally, the pieces containing the adhered cells were analyzed by scanning electron microscopy (SEM) to evaluate the organization of cells at the surface of the selected materials. Three independent experiments were performed for each coupon in each condition.

Quantification of biofilm cells

Biofilm cells were quantified through colony forming units (CFUs) determinations. To determine the number of CFUs, the necessary dilutions in saline solution were prepared and plated on Bold Basal medium (Hoff and Bold 1963) supplemented with agar (1.5%, *w/v*), using the motion drop method (Reed and Reed 1948). These assays were carried out in duplicate. The cells on the studied substrate pieces were expressed in terms of CFU per square centimeter, according to Eq. 2:

$$\mathrm{CFU\ cm^{-2}} = \frac{\frac{N}{V_P} \cdot V_S \cdot D}{A} \quad (2)$$

where N is the number of CFUs, V_P is the volume plated (0.01 mL), V_S is the volume of saline solution in which biofilms were resuspended, D is the dilution factor, and A is the total area of each substrate (cm^2).

SEM observations

For SEM observations, the samples were fixed, successively dehydrated, and dried, according to the method described by Gomes et al. (2013).

Statistical analysis

The statistical analysis was performed using the statistical software SPSS 22.0 (SPSS Inc., USA) at a significance level of 0.05. The surface physicochemical parameters were analyzed using paired-samples *t* test. Furthermore, the influence of strain, culture media, and material, as well as a combination of these factors, in the different parameters studied were evaluated through three-way-ANOVA.

Results and discussion

Surface physicochemical properties of the selected microorganisms

Contact angles measurements allowed the determination of the surface tension parameters of *C. vulgaris*, *P. subcapitata*, *S. salina*, and *M. aeruginosa*. These results are presented in Table 1 in a decreasing order of free energy of hydrophobic interaction (from the most hydrophilic to the most hydrophobic). It is possible to observe that all microbial surfaces were hydrophilic ($\Delta G_{sws}^{TOT} > 0$ mJ m^{-2}). In a previous study of Gonçalves et al. (2015), aiming to evaluate the influence of surface physicochemical properties of microalgae and cyanobacteria on their sedimentation kinetics, similar ΔG_{sws}^{TOT} values were obtained for *C. vulgaris*, *P. subcapitata*, and *M. aeruginosa* (52.5, 13.8, and 43.4 mJ m^{-2}, respectively) using the OECD test medium for microbial growth. Ozkan and Berberoglu (2013c) studied the surface physicochemical properties of *Synechocystis* sp. and verified a hydrophilic character (ΔG_{sws}^{TOT} of 10.9 mJ m^{-2}) for this cyanobacterium, which corroborates the data obtained in this study for *S. salina*.

The Lifshitz van der Waals component, γ_s^{LW}, was similar ($p > 0.05$) for all studied microorganisms, with values ranging from 35.8 ± 1.0 to 38.1 ± 1.8 mJ m^{-2}. Previous studies (Ozkan and Berberoglu 2013c; Gonçalves et al. 2015) provided γ_s^{LW} values of 35.0 and 37.8 mJ m^{-2} for different microalgae and cyanobacteria. LW forces, usually attractive, result from instantaneous asymmetrical distribution of electrons in molecules (the higher the value of the LW component, the more apolar is the surface and, therefore, the lower would be its affinity for polar liquids) (Ozkan and Berberoglu 2013b). Accordingly, similar results obtained for this parameter indicate that cell wall composition of the studied microorganisms may be similar (Latala et al. 2009; Wurdack 1923). In the study performed by Ozkan and Berberoglu (2013c), differences in the LW component observed for green algae and diatoms were attributed to differences in their cell wall chemical composition. Electron donor and acceptor parameters give information about the properties of molecules present in the surfaces studied: higher γ_s^+ indicate the presence of positively charged molecules and higher γ_s^- indicate the presence of negatively charged ones (Janczuk et al. 1993). In this study, all microorganisms presented electron donor properties, with the predominance of the electron donor component, γ_s^-. This polar

Table 1 Contact angles and surface physicochemical properties of the studied microorganisms and surface materials

Surface	Contact angles (°)			Surface tension parameters and free energy of hydrophobic interaction (mJ m^{-2})				
	θ_W	θ_B	θ_F	γ_s^{LW}	γ_s^{AB}	γ_s^-	γ_s^+	ΔG_{sws}^{TOT}
M. aeruginosa	32.3 ± 6.5	32.8 ± 11.3	38.6 ± 9.4	37.1 ± 4.1	8.2 ± 6.0	52.8 ± 5.9	0.5 ± 0.4	35.4 ± 8.5
C. vulgaris	37.1 ± 4.8	36.3 ± 2.9	38.9 ± 5.3	36.2 ± 1.2	8.1 ± 4.0	46.7 ± 8.4	0.5 ± 0.3	28.1 ± 13.0
S. salina	34.6 ± 3.9	37.2 ± 2.4	34.2 ± 5.7	35.8 ± 1.0	11.3 ± 3.9	45.8 ± 4.3	0.8 ± 0.6	25.5 ± 6.8
P. subcapitata	38.9 ± 9.3	31.4 ± 5.1	39.1 ± 7.9	38.1 ± 1.9	5.7 ± 4.1	44.1 ± 9.6	0.3 ± 0.3	24.5 ± 14.4
SS	51.3 ± 3.4	30.7 ± 7.0	48.9 ± 4.3	38.2 ± 2.6	2.5 ± 2.7	35.1 ± 3.2	0.1 ± 0.1	12.3 ± 5.3
PS	93.9 ± 3.3	30.6 ± 1.3	99.9 ± 5.6	38.4 ± 0.5	0.0 ± 0.0	19.2 ± 3.8	0.0 ± 0.0	− 18.5 ± 9.0
PMMA	56.6 ± 8.5	13.0 ± 1.4	53.6 ± 4.4	43.3 ± 0.2	0.1 ± 0.3	32.5 ± 11.9	0.0 ± 0.0	− 19.1 ± 6.8
PVC	66.9 ± 6.7	23.9 ± 2.9	53.9 ± 10.2	40.6 ± 0.9	2.2 ± 2.3	17.7 ± 4.1	0.1 ± 0.2	− 22.6 ± 5.2
G	59.8 ± 3.5	41.0 ± 2.1	28.4 ± 1.4	34.1 ± 0.9	13.8 ± 1.4	11.6 ± 3.2	4.2 ± 0.6	− 22.6 ± 10.5
Cu	93.5 ± 5.3	23.5 ± 2.9	79.1 ± 4.2	40.8 ± 0.9	0.0 ± 0.0	6.2 ± 4.9	0.0 ± 0.0	− 62.1 ± 21.3

Values are presented as the mean ± standard deviation of three independent experiments

θ_W contact angle using water (°), θ_B contact angle using α-bromonaphthalene (°), θ_F contact angle using formamide (°), γ_s^{LW} Lifshitz van der Waals component of the surface tension (mJ m^{-2}), γ_s^{AB} acid-base component of the surface tension (mJ m^{-2}), γ_s^- electron donor component of the surface tension (mJ m^{-2}), γ_s^+ electron acceptor component of the surface tension (mJ m^{-2}), ΔG_{sws}^{TOT} free energy of hydrophobic interaction (mJ m^{-2})

character may be due to the presence of excessive molecules of oxygen or polar groups on microbial surfaces (Van Oss 1995, 2006; Gonçalves et al. 2015). Additionally, the values determined for the studied microorganisms were not statistically different ($p > 0.05$), with the exception of *M. aeruginosa*. Regarding the acid-base or polar component, the values determined for the studied species were not statistically different ($p > 0.05$). In the study of Ozkan and Berberoglu (2013c), similar γ_s^{AB} values were obtained for *Nannochloris* sp., *Nannochloris oculata*, *Chlorella vulgaris*, *Scenedesmus dimorphus*, *Afrocarpus falcatus*, *Botryococcus sudeticus*, and *Botryococcus braunii*, ranging from 0.0 to 5.1 mJ m^{-2}. Acid-base forces result from electron transfer interactions between polar components of the involved surfaces, which can be attractive (hydrophobic attraction) or repulsive (hydrophilic repulsion) (Gonçalves et al. 2015). In this study, the γ_s^{AB} component represents a measure of hydrophilic repulsion, since all microorganisms presented a hydrophilic character $\left(\Delta G_{sws}^{TOT} > 0\right)$.

Surface physicochemical properties of the selected materials

The surface tension parameters were also determined for the selected surface materials. As for microbial surfaces, these results are presented in Table 1 in the decreasing order of free energy of hydrophobic interaction. The ΔG_{sws}^{TOT} values obtained were statistically different ($p < 0.05$) between the studied materials, with the exception of the SS-PMMA, PS-G, PS-PVC, and PVC-G combinations. As it can be observed from Table 1, contact angles determined using the polar liquids on the studied surfaces (with the exception of Cu and PS) present values below 90°, meaning that surface-liquid interactions dominate the system. Only SS presented a hydrophilic character ($\Delta G_{sws}^{TOT} > 0$ mJ m^{-2}). In fact, surfaces presenting higher free energy of hydrophobic interaction are more hydrophilic, which is the case of SS (Visser 1995; Kokare et al. 2009). In contrast, Cu, G, PMMA, PS, and PVC are hydrophobic surfaces, due to the negative value determined for ΔG_{sws}^{TOT}. Similar ΔG_{sws}^{TOT} values have already been reported for Cu (− 79.6 mJ m^{-2}) and G (− 13.8 and − 14.8 mJ m^{-2}) (Teixeira et al. 2005; Simões et al. 2007). However, it is important to notice that it is likely that Cu and G were probably contaminated with a hydrophobic material (e.g., hydrocarbon from the air) before the experiments. Some authors reported similar ΔG_{sws}^{TOT} values for PMMA, with values of about − 18.9 (Teixeira et al. 2005) and − 16.8 mJ m^{-2} (Teixeira and Oliveira 1999; Oliveira et al. 2001). In the study performed by Lopes et al. (2005) PMMA was also determined as hydrophobic, but the absolute value of ΔG_{sws}^{TOT} obtained was higher (36.3 mJ m^{-2}) than the one determined in this study (19.1 ± 6.8 mJ m^{-2}). The free energy of hydrophobic interaction determined for PVC (− 22.6 mJ m^{-2}) is in accordance with the results of Teixeira and Oliveira (1999) and Oliveira et al. (2001), who reported ΔG_{sws}^{TOT} values of about 22.0 mJ m^{-2}. Nevertheless, it was found that ΔG_{sws}^{TOT} values determined for PS were lower than those reported in the literature. Typical ΔG_{sws}^{TOT} values reported for this surface material are − 29.3 (Van Oss 2005) and − 32 mJ m^{-2} (Simões et al. 2010). These contradictions may result from different nature, finishing, or cleaning treatment of the materials used (Simões et al. 2007).

The γ_s^{LW} parameter was statistically different ($p < 0.05$) between the surface materials, with the exception of PS-SS and Cu-PVC combinations. This parameter values ranged from 38.2 (for SS) to 40.2 mJ m^{-2} (for Cu). Comparing electron acceptor (γ_s^+) and donor (γ_s^-) parameters, it is possible to conclude that all surfaces are electron donors. Data obtained for γ_s^+ and γ_s^- between the studied materials was statistically different ($p < 0.05$). The theory developed by Van Oss et al. (1987, 1988, 1989) predicts the non-existence of electron acceptor parameters, which corroborates with the γ_s^+ values (close to zero). Looking at γ_s^{AB}, the values determined ranged from 0.0 ± 0.0 to 13.8 ± 1.4 mJ m^{-2}. In general, the γ_s^{AB} values were statistically different ($p < 0.05$) between the surface materials evaluated in this study, except for the PS-SS, PVC-SS, Cu-PMMA, and Cu-PS combinations.

Free energy of adhesion

In order to predict the ability of the selected microorganisms to adhere on the surface materials, the free energy of interaction, also known as free energy of adhesion, was calculated according to the thermodynamic approach represented in Eq. 1. As presented in Table 2, similar values of $\Delta G_{adhesion}$ were obtained for the selected microorganisms. Comparing the values obtained for each surface material, it is possible to observe that the total interfacial energy of the system was lower for Cu and G, with $\Delta G_{adhesion} < 0$ mJ m^{-2}. These results suggest that all studied microorganisms are expected to adhere on Cu and G surfaces. On the other hand, $\Delta G_{adhesion}$ was positive for SS, PMMA, PS, and PVC, in the decreasing order of free energy of adhesion. These results indicate that the interaction between microalgal/cyanobacterial cells and these surface materials is not thermodynamically favored and, consequently, it is not expected to occur. Comparing these surface materials, it is noticeable that adhesion may be thermodynamically less favorable on SS surfaces, due to the highest $\Delta G_{adhesion}$ values: values determined for each microorganism ranged between 19.7 ± 4.3 and 25.2 ± 4.3 mJ m^{-2}.

Table 2 Free energy of adhesion ($\Delta G_{adhesion}$, mJ m^{-2}) between the studied microorganisms and surface materials

Surface	*C. vulgaris*	*P. subcapitata*	*S. salina*	*M. aeruginosa*
SS	22.1 ± 9.0	21.1 ± 8.4	19.7 ± 4.3	25.2 ± 4.3
PMMA	10.1 ± 4.3	10.3 ± 3.3	7.9 ± 4.1	13.2 ± 5.3
PS	9.4 ± 5.4	11.4 ± 3.3	9.2 ± 3.9	12.9 ± 5.2
PVC	7.9 ± 4.4	7.9 ± 4.3	5.9 ± 3.8	10.9 ± 5.0
G	− 8.8 ± 3.7	− 10.5 ± 3.9	− 8.0 ± 3.0	− 3.4 ± 1.9
Cu	− 11.3 ± 4.4	− 14.7 ± 7.8	− 13.9 ± 5.6	− 12.1 ± 5.7

Values are presented as the mean ± standard deviation of three independent experiments

To better understand the relationship between the free energy of hydrophobic interaction and the free energy of adhesion, data from the ΔG_{sws}^{TOT} for all surface materials were plotted against data from the $\Delta G_{adhesion}$ (for each microorganism). So, a good linear relationship was obtained, with coefficient of determination values (R^2) of 0.994, 0.967, 0.985, and 0.982 for *C. vulgaris*, *P. subcapitata*, *S. salina*, and *M. aeruginosa*, respectively. This strong correlation is not surprising, as the thermodynamic approach is based on the surface physicochemical properties of the selected microorganisms and surface materials. Additionally, the positive relationship between free energy of hydrophobic interaction of the studied materials and free energy of adhesion shows that the larger the ΔG_{sws}^{TOT} (more hydrophilic), the larger became the $\Delta G_{adhesion}$.

Biofilm formation on the selected materials using different culture media

The density of attached cells varied with time, culture media, and surface among all microorganisms. Figure 1 presents the log CFUs per square centimeter determined on each of the surface materials using both culture media after 24, 72, and 168 h of incubation.

Comparing the biofilm formation ability for the different microorganisms, a significant difference ($p < 0.05$) was observed between the strains used in this study. *C. vulgaris* presented the highest ability to form biofilms, followed by *M. aeruginosa*, *S. salina*, and *P. subcapitata* (shown a lack of biofilm formation ability). In fact, *P. subcapitata* was unable to form biofilms on the selected materials; it was not possible to present the number of CFUs for this microalga. The inability of *P. subcapitata* for biofilm formation might be related to the high net zeta potential already reported for this microorganism (Gonçalves et al. 2015). According to Gonçalves et al. (2015), zeta potential determined for *P. subcapitata* was − 48.1 ± 0.9 mV, indicating that this microalga tends to be stable in the dispersed form.

For those microorganisms with biofilm formation ability, initial adhesion occurred after 24 h of incubation. Irving (2011), while studying the initial adhesion of *C. vulgaris* on various surfaces, obtained cell densities between 1.3×10^3 and 2.5×10^4 cells cm^{-2} after 7 days of growth on Petri dishes. Additionally, Sekar et al. (2004) investigated the attachment of *C. vulgaris* on pieces of several surface materials and found maximum attachment after 48 h of experiment. Additionally, Ozkan and Berberoglu (2013a), while measuring the strength of adhesion of *C. vulgaris* attached to different substrata, found maximum attachment after 10 h, which resulted in a density of 2.5×10^4 cells cm^{-2}. Comparing the values obtained by the authors stated above with this study, it is possible to see that the present study reached higher biofilm densities for *C. vulgaris*, with

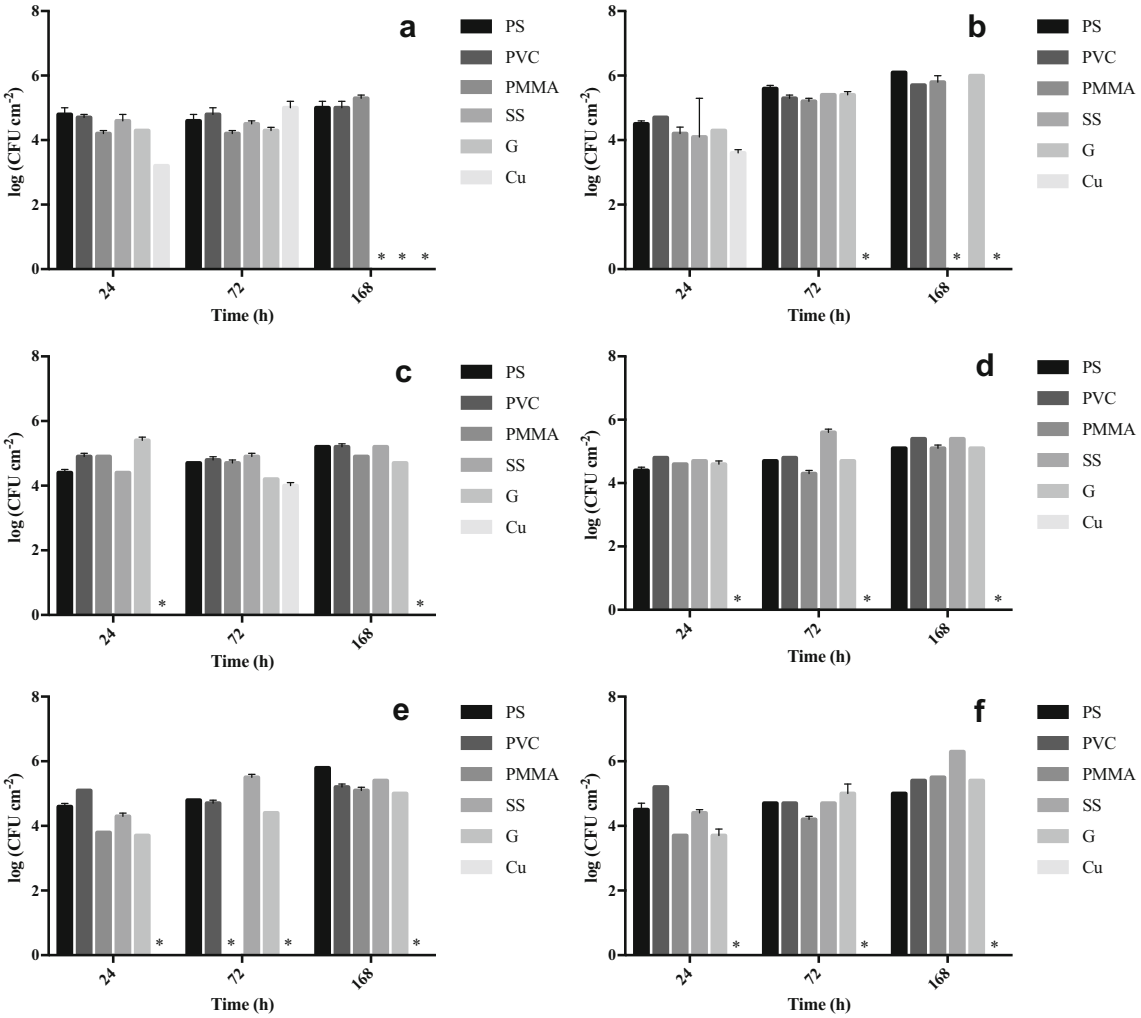

Fig. 1 Number of adhered cells (log CFU cm^{-2}) of *C. vulgaris* (**a** and **b**), *S. salina* (**c** and **d**), and *M. aeruginosa* (**e** and **f**) on the studied surface materials after 24, 72, and 168 h incubation in SE (**a**, **c,** and **e**) and OECD media (**b**, **d**, and **f**). The symbol * represents that no CFU was detected. Error bars correspond to the standard deviation of the mean determined for three independent experiments

maximum values of about 1.3×10^6 CFU cm^{-2}. However, it is important to notice that in those studies they used flow chambers to induce biofilm formation, whereby the environmental and hydrodynamic conditions were different from those applied in this study. Comparing the different time points evaluated, it was possible to verify that, in general, the number of biofilm cells increased along time for all microorganisms and the highest biofilm density was reached after 7 days (168 h) of incubation. However, in a few cases, the number of CFUs decreased after 72 h and increased at 168 h of experiment, meaning that biofilm formation occurred within the first 24 h. The decreasing in the cell density after 72 h was not statistically significant ($p < 0.05$). These results may be related to the low EPS concentrations evidenced by SEM micrographs (Fig. 2c) and to the homogeneous distribution of microbial cells in the coupons (Fig. 2a), which results in a lower biofilm cohesion and helps to explain the low number of biofilm cells. Figure 2 shows representative SEM micrographs of SS coupons after 24 h of incubation with *C. vulgaris*. Such behaviour has already been reported in the literature (Menicucci Jr. 2010; Irving and Allen 2011; Kesaano and Sims 2014). This fact can also be related to nutrient depletion, which leads to cell migration from a surface with non-adsorbed nutrients to another location more favorable for their growth (Donlan 2002). In the study performed by Korber et al. (2003), the authors found that microorganisms can detach from a surface and remain planktonic or reattach after a few hours, depending on the microorganism. Furthermore, according to Horn et al. (2003), biofilm detachment can also be influenced by internal strength decrease, through hydrolysis of EPS. Although several studies on microbial attachment and detachment can be found in the literature, the mechanisms involved in detachment are not well established and further studies need to be done (Wilson et al. 2004). Other issues worth noting are (i) to know if the biofilm resists mechanical detachment, once initial cell adhesion occurs and the biofilm

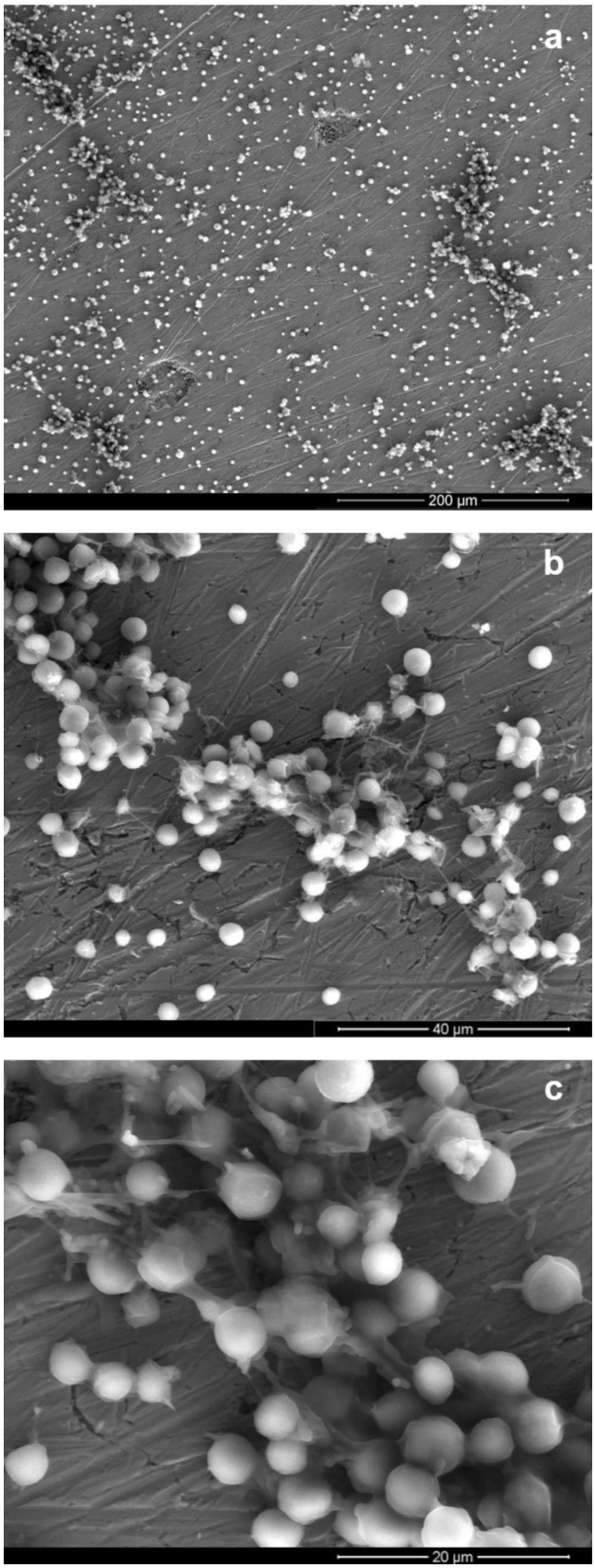

Fig. 2 SEM micrographs of *C. vulgaris* attached on SS. **a** Magnification of 500×. **b** Magnification of × 2500. **c** Magnification of × 5000

is established, and (ii) to compare the effect of physicochemical properties on both short-term and long-term experiments. Gross et al. (2016) found that both initial cell attachment and long-term attached growth are influenced by surface physicochemical properties of the microorganisms and the materials. Conversely, Irving (2011) found that although hydrophobicity could not be correlated with surface colonization, either in batch and continuous experiments, significant differences were observed in batch unlike continuous experiments, in which similar attachment patterns were found on both hydrophobic and hydrophilic surfaces. Furthermore, concerning biofilm growth, some authors stated that once initial colonization occurs on the surface material, the attachment of other cells can be much easier (Ozkan and Berberoglu 2013b; Katarzyna et al. 2015). The same researchers claimed that biofilm integrity is mainly governed by keeping the biofilm thickness at appropriate limits. Clearly, with increasing biofilm thickness, the bottom cell layers can become nutrient starved and light limited, leading to biofilm sloughing (Gross 2015). So, it is crucial to study biofilm growth kinetics over long-term experiments to find the most beneficial time for biofilm harvesting, in order to achieve higher biofilm density and thickness, while avoiding biofilm sloughing.

Regarding the surface materials, significant differences ($p < 0.05$) were observed in biofilm formation on the selected materials for all time points (for most cases). For the 24 h, the combinations PS-PVC, PS-Cu, SS-PVC, SS-Cu, PMMA-PVC, PMMA-Cu, G-Cu, and PVC-Cu presented statistically significant differences ($p < 0.05$). For the 72 h, significant differences ($p < 0.05$) were found between PS-Cu, SS-Cu, G-Cu, PVC-Cu, SS-PVC, SS-G, and SS-PMMA combinations. At 168 h, significant differences ($p < 0.05$) were observed between all the surface materials-Cu combinations.

In general, the degree of biofilm formation was found to follow the sequence SS > PS > G > PVC > PMMA> Cu (which corresponds to an average of adhered cells of $1.9 \times 10^5 > 1.8 \times 10^5 > 1.6 \times 10^5 > 1.4 \times 10^5 > 1.2 \times 10^5 > 5.2 \times 10^3$ CFU cm^{-2}). The degree of biofilm formation was based on which surface had the highest number of cells adhered (average of CFU cm^{-2}) for the overall experiments, with both culture media and for all microorganisms. In the study performed by Sekar et al. (2004), the authors reported that surface colonization was higher on stainless steel, followed by titanium, PMMA, and glass with values of biofilm cells of about 8.0×10^3, 7.0×10^3, 6.8×10^3, and 3.8×10^3 cells cm^{-2}, respectively.

Comparing the effect of different media compositions on biofilm formation, Fig. 1 shows that the number of CFUs determined when using the OECD test medium was higher than the one determined with SE. However, the differences were only statistically significant ($p < 0.05$) for 168 h. This increased biofilm formation ability under low nutrient concentrations explains the differences observed when using the

selected culture media. Microalgae and cyanobacteria are mixotrophic, meaning that they can use both light and organic carbon as energetic source. Since the OECD test medium does not contain an organic carbon source, microalgae and cyanobacteria tend to be under stress conditions when grown on that medium. Previous studies (Fields et al. 2014; Kesaano et al. 2015) also proposed that environmental stress and nutrient deprivation enhances microalgal/cyanobacterial biofilm development, nutrient uptake, and lipid accumulation.

The influence of the microorganism, culture medium, surface material, and the combined effect of these variables on biofilm formation was assessed using a three-way-ANOVA (Table 3). Analysis of Table 3 shows that the combined effect of these factors presents a significant effect on biofilm formation ($p < 0.05$), especially for longer periods of incubation (72 and 168 h).

Relationship between surface physicochemical properties and biofilm formation

Although the influence of surface physicochemical properties on bacterial adhesion has been extensively studied, only a few studies focused on a systematic analysis of microalgal/cyanobacterial biofilm formation on different materials (Sekar et al. 2004; Irving and Allen 2011; Ozkan and Berberoglu 2011; Sirmerova et al. 2013). Many researchers have reported that thermodynamic approaches alone cannot be used to surely predict bacterial attachment and biofilm formation (Morra and Cassinelli 1998; Li and Logan 2004; Chae et al. 2006; Simões et al. 2010). However, this fact is not well established for photosynthetic microorganisms and for the broad range of materials/surfaces used in this study. Comparison between the thermodynamic prediction of adhesion (Table 2) and the biofilm formation results present in Fig. 1 confirmed that microalgal and cyanobacterial biofilm formation cannot be predicted exclusively by thermodynamic approaches. For example, although $\Delta G_{adhesion}$ values determined for PMMA, PS, PVC, and SS assumed a positive value for both microalgae and cyanobacteria, proposing that the adhesion to these materials was thermodynamically unfavorable, the in vitro assays demonstrated the opposite.

Table 3 Influence of the different variables evaluated in this study on cell adhesion obtained at different time periods

Variables in study	*p* values		
	CFU cm^{-2} at 24 h	CFU cm^{-2} at 72 h	CFU cm^{-2} at 168 h
Strain	< 0.05	< 0.05	< 0.05
Material	< 0.05	< 0.05	< 0.05
Medium	0.43	0.55	< 0.05
Strain × material	< 0.05	< 0.05	< 0.05
Strain × medium	0.12	< 0.05	< 0.05
Material × medium	0.63	< 0.05	< 0.05
Strain × material × medium	0.86	< 0.05	< 0.05

Results are shown as the *p* value obtained through the statistical test three-way ANOVA (significance level was set at 0.05)

On the other hand, data obtained from the analysis of physicochemical properties indicated that all microorganisms should have higher ability to adhere to Cu and G surfaces. In fact, G was one of the materials with more biofilm on its surface, as previously predicted by the thermodynamic approach. Nevertheless, Cu was the surface material presenting the lowest number of biofilm cells. Also, in most cases, there was no growth on Cu surfaces. Indeed, Cu has been reported as a toxic material to microalgae and cyanobacteria (Stauber and Florence 1987; Leale 1998; Lombardi et al. 2007; Jamers et al. 2013). Additionally, several authors (Van Leeuwen 1999; Costas and Lopez-Rodas 2006; Gregor et al. 2008; Hadjoudja et al. 2010) reported that *M. aeruginosa* is more sensitive to Cu than *C. vulgaris*, which corroborates data obtained; the adhesion experiments showed that there were no cyanobacterial cells adhered on Cu surfaces and, on the other hand, *C. vulgaris* was able to slightly attach to this material. The theoretical and in vitro adhesion of *C. vulgaris* to Cu, G, PMMA, PS, and SS has already been reported (Sekar et al. 2004; Irving and Allen 2011; Ozkan and Berberoglu 2013a, 2013b; Sirmerova et al. 2013). In these studies, the thermodynamic approach was not always in accordance with the adhesion assays, proving that more factors should be accounted in the prediction models.

Considering data obtained for surface physicochemical properties and comparing with those obtained for biofilm formation, a linear relationship was found. It is important to notice that for the linear association analysis, only data from 24-h biofilms using the OECD test medium were used, since the first day is critical for microalgal attachment and biofilm development (Di Pippo et al. 2009). The results obtained for Cu were not included, since, in general, there was no biofilm formation on this surface material. As it can be seen in Fig. 3, the correlation obtained was not good ($R^2 = 0.111$). However, there is a negative relationship, since lower $\Delta G_{adhesion}$ values correspond, in general, to a higher number of biofilm cells. As it was described above, lower values of $\Delta G_{adhesion}$ are strongly associated with lower values of ΔG_{sws}^{TOT} (hydrophobic surfaces). So, linking this correlation to the information presented in Fig. 3, it is possible to see that this relationship corroborates the hypothesis that cell adhesion tends to occur on hydrophobic surfaces rather than on hydrophilic ones, as proposed by several authors (Holland et al. 2004; Sekar et al. 2004; Stanley and Callow 2007; Li et al. 2010). In these studies, several microorganisms were used namely *Phaeodactylum tricornutum*, *C. vulgaris*, *Nitzschia amphibia*, *Chroococcus minutus*, *Nitzschia closterium*, *Amphora coffeaeformis* var. *perpusilla*, *Craspedostauros*

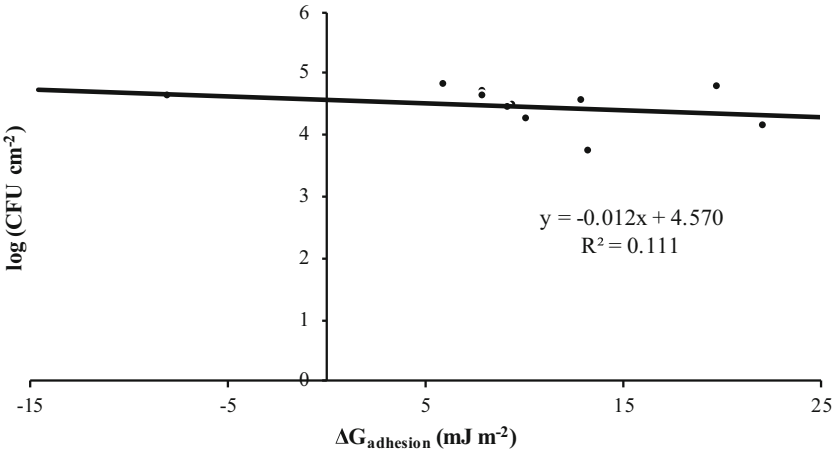

Fig. 3 Linear regression between the number of colony forming units (CFU cm^{-2}) and the free energy of adhesion ($\Delta G_{adhesion}$, mJ m^{-2})

australis, and *Navicula perminuta*. The discrepancies observed between the adhesion based on the thermodynamic theory and on biofilm formation in vitro assays can be explained by the non-consideration of environmental and microbiological parameters in the predictive approaches.

Despite the attempts of many researchers to describe microbial adhesion behaviour from individual physicochemical measurements of microorganisms and surfaces, the models proposed so far have significant limitations. Although the Van Oss (1995) approach is one of the most currently employed methods to predict microbial adhesion, it also has some limitations. Firstly, this model tends to ignore the hydrogen-bonding interaction of bacteria and surfaces with the liquid medium (Ista 2011). Secondly, it assumes smooth surfaces with a homogenous distribution of cells that are similar themselves (Ista 2011; Ista and López 2013). According to the DLVO theory, higher effective radius of particles leads to more repulsive electrostatic interactions. Accordingly, microalgae and cyanobacteria can reduce their effective radius of interaction with the surface material through some biological mechanisms, such as EPS production and the presence of extracellular motility appendages that can favor adhesion and biofilm formation (Doyle 2000; Sinde and Carballo 2000; Chaves 2004; Simões et al. 2007). In fact, Characklis (1990) reported that the transition from reversible to irreversible attachment is usually mediated by EPS, through the change from a weak interaction between microorganisms and surfaces to a permanent linkage. Furthermore, several studies suggested that the attachment of microalgae/cyanobacteria may be enhanced by the presence of bacteria on substrates by means of interspecific interactions (Bridier et al. 2014). Indeed, natural biofilms are mainly complex consortia of autotrophs, heterotrophs, and EPS (Hodoki 2005; Irving and Allen 2011; Shen et al. 2015). Besides, surface properties, such as roughness and texture, which have also been reported in the literature as key factors for microbial adhesion, are not included in the thermodynamic approach and could explain the differences observed between the prediction approach and the in vitro experiments (Sekar et al. 2004; Hodoki 2005; Shen et al. 2015). This lack of relationship between theoretical/thermodynamic and in vitro adhesion is well described for bacteria (Oliveira et al. 2001; Simões et al. 2007). However, there is still a lack of knowledge on the mechanisms involved in adhesion and biofilm formation of microalgae and cyanobacteria.

In conclusion, a comprehensive study has been performed to determine the surface physicochemical properties of selected microalgae, cyanobacteria, and surface materials and to understand the influence of these properties on biofilm formation. The influence of media composition was also assessed in this study. Free energy of hydrophobic interaction values showed that SS was hydrophilic, whereas Cu, G, PMMA, PS, and PVC were hydrophobic surfaces. Also, the selected microorganisms presented a hydrophilic character. Concerning the ability of the selected microorganisms to form biofilms on the referred materials, *M. aeruginosa* presented the highest biofilm formation ability, followed by *S. salina* and *C. vulgaris*, with maximum biofilm densities of 2.1×10^6, 1.3×10^6, and 2.9×10^5 CFU cm^{-2}, respectively. *P. subcapitata* was the exception, as the results showed a lack of biofilm formation ability. Furthermore, the degree of biofilm formation was found to follow the order SS > PS > G > PVC > PMMA> Cu. Also, a significant difference ($p < 0.05$) between the number of biofilm cells when using the OECD test medium and SE was observed for 168 h, confirming that microbial adhesion may be enhanced by exposing microorganisms to nutrient stress conditions. Comparison between the thermodynamic theory and the experimental assays showed that adhesion may be underestimated when predicted exclusively by the thermodynamic approach and that its prediction depends on many other factors apart from surface physicochemical properties. Therefore, other factors should be taken into account, particularly the type and characteristics (presence/absence of extracellular appendages, production of

EPS) of the microorganism, the bulk media composition, and the type of adhesion surface.

Acknowledgements This work was the result of the projects: POCI-01-0145-FEDER-030219; POCI-01-0145-FEDER-006939 (Laboratory for Process Engineering, Environment, Biotechnology and Energy - UID/EQU/00511/2013) funded by the European Regional Development Fund (ERDF), through COMPETE2020 – Programa Operacional Competitividade e Internacionalização (POCI) and by national funds, through FCT - Fundação para a Ciência e a Tecnologia; NORTE-01-0145-FEDER-000005 - LEPABE-2-ECO-INNOVATION, supported by North Portugal Regional Operational Programme (NORTE 2020), under the Portugal 2020 Partnership Agreement, through the European Regional Development Fund (ERDF); SFRH/BD/88799/2012; 0302_CVMAR_I_1_P..

References

Abdel-Raouf N, Al-Homaidan A, Ibraheem I (2012) Microalgae and wastewater treatment. Saudi J Biol Sci 19:257–275

Adey WH (1982) Algal turf scrubber. US patent 4333263

Aslan S, Kapdan IK (2006) Batch kinetics of nitrogen and phosphorus removal from synthetic wastewater by algae. Ecol Eng 28:64–70

Babu M (2011) Effect of algal biofilm and operational conditions on nitrogen removal in waste stabilization ponds. Dissertation, UNESCO-IHE Institute for Water Education

Barros AI, Gonçalves AL, Simões M, Pires JC (2015) Harvesting techniques applied to microalgae: a review. Renew Sust Energ Rev 41: 1489–1500

Becker K (1996) Exopolysaccharide production and attachment strength of bacteria and diatoms on substrates with different surface tensions. Microbiol Ecol 32:23–33

Bischoff HW, Bold HC (1963) Phycological studies IV. Some soil algae from Enchanted Rock and related algal species. University of Texas Publication 6318:1–95

Boelee N, Temmink H, Janssen M, Buisman C, Wijffels R (2011) Nitrogen and phosphorus removal from municipal wastewater effluent using microalgal biofilms. Water Res 45:5925–5933

Booth C (1971) Methods in microbiology. Academic Press Inc, London

Bridier A, Briandet R, Bouchez T, Jabot F (2014) A model-based approach to detect interspecific interactions during biofilm development. Biofouling 30:761–771

Busscher HJ, Weerkamp AH, van der Mei HC, Van Pelt A, de Jong HP, Arends J (1984) Measurement of the surface free energy of bacterial cell surfaces and its relevance for adhesion. Appl and Environ Microbiol 48:980–983

Cai T, Park SY, Li Y (2013) Nutrient recovery from wastewater streams by microalgae: status and prospects. Renew Sust Energ Rev 19:360–369

Carvalho AP, Meireles LA, Malcata FX (2006) Microalgal reactors: a review of enclosed system designs and performances. Biotechnol Prog 22:1490–1506

Chae MS, Schraft H, Hansen LT, Mackereth R (2006) Effects of physicochemical surface characteristics of *Listeria monocytogenes* strains on attachment to glass. Food Microbiol 23:250–259

Characklis WG (1990) Biofilm processes. In: Characklis WG (ed) Biofilms. Wiley and Sons, New York, pp 195–231

Chaves LC 2004. Estudo da cinética de formação de biofilmes em superfícies em contato com água potável. Dissertation, University of Minho

Christenson L (2011) Algal biofilm production and harvesting system for wastewater treatment with biofuels by-products. Dissertation, Utah State University

Christenson L, Sims R (2011) Production and harvesting of microalgae for wastewater treatment, biofuels, and bioproducts. Biotechnol Adv 29:686–702

Costas E, Lopez-Rodas V (2006) Copper sulphate and DCMU-herbicide treatments increase asymmetry between sister cells in the toxic cyanobacteria *Microcystis aeruginosa*: implications for detecting environmental stress. Water Res 40:2447–2451

Del Campo JA, García-González M, Guerrero MG (2007) Outdoor cultivation of microalgae for carotenoid production: current state and perspectives. Appl Microbiol Biotechnol 74:1163–1174

Di Pippo F, Bohn A, Congestri R, De Philippis R, Albertano P (2009) Capsular polysaccharides of cultured phototrophic biofilms. Biofouling 25:495–504

Donlan RM (2002) Biofilms: microbial life on surfaces. Emerg Infect Dis J 8:881–890

Doyle RJ (2000) Contribution of the hydrophobic effect to microbial infection. Microbes Infect 2:391–400

Fields MW, Hise A, Lohman EJ, Bell T, Gardner RD, Corredor L, Moll K, Peyton BM, Characklis GW, Gerlach R (2014) Sources and resources: importance of nutrients, resource allocation, and ecology in microalgal cultivation for lipid accumulation. Appl Microbiol Biotechnol 98:4805–4816

Gebara F (1999) Activated sludge biofilm wastewater treatment system. Water Res 33:230–238

Gomes L, Moreira J, Miranda J, Simões M, Melo L, Mergulhão F (2013) Macroscale versus microscale methods for physiological analysis of biofilms formed in 96-well microtiter plates. J Microbiol Meth 95: 342–349

Gonçalves AL, Ferreira C, Loureiro JA, Pires JCM, Simões M (2015) Surface physicochemical properties of selected single and mixed cultures of microalgae and cyanobacteria and their relationship with sedimentation kinetics. Bioresour Bioprocess 2:1–10

Grass G, Rensing C, Solioz M (2011) Metallic copper as an antimicrobial surface. Appl Environ Microbiol 77:1541–1547

Gregor J, Jančula D, Maršálek B (2008) Growth assays with mixed cultures of cyanobacteria and algae assessed by in vivo fluorescence: one step closer to real ecosystems? Chemosphere 70:1873–1878

Gross MA (2015) Development and optimization of biofilm based algal cultivation. Dissertation, Iowa State University

Gross M, Zhao X, Mascarenhas V, Wen Z (2016) Effects of the surface physico-chemical properties and the surface textures on the initial colonization and the attached growth in algal biofilm. Biotechnol Biofuels 9:1

Hadjoudja S, Deluchat V, Baudu M (2010) Cell surface characterisation of *Microcystis aeruginosa* and *Chlorella vulgaris*. J Colloid Interf Sci 342:293–299

Hill WR, Ryon MG, Schilling EM (1995) Light limitation in a stream ecosystem: responses by primary producers and consumers. Ecology 76:1297–1309

Hodoki Y (2005) Bacteria biofilm encourages algal immigration onto substrata in lotic systems. Hydrobiologia 539:27–34

Holland R, Dugdale T, Wetherbee R, Brennan A, Finlay J, Callow J, Callow ME (2004) Adhesion and motility of fouling diatoms on a silicone elastomer. Biofouling 20:323–329

Horn H, Reiff H, Morgenroth E (2003) Simulation of growth and detachment in biofilm systems under defined hydrodynamic conditions. Biotechnol Bioeng 81:607–617

Irving TE (2011) Factors influencing the formation and development of microalgal Biofilms Dissertation, University of Toronto

Irving TE, Allen DG (2011) Species and material considerations in the formation and development of microalgal biofilms. Appl Microbiol Biotechnol 92:283–294

Ista L (2011) Substratum interfacial energetic effects on the attachment of marine bacteria. Dissertation, The University of New Mexico

Ista LK, López GP (2013) Thermodynamic analysis of marine bacterial attachment to oligo (ethylene glycol)-terminated self-assembled monolayers. Biointerphases 8:24

Jamers A, Blust R, De Coen W, Griffin JL, Jones OA (2013) Copper toxicity in the microalga *Chlamydomonas reinhardtii*: an integrated approach. Biometals 26:731–740

Janczuk B, Chibowski E, Bruque J, Kerkeb M, Caballero FG (1993) On the consistency of surface free energy components as calculated from contact angles of different liquids: an application to the cholesterol surface. J Colloid Interf Sci 159:421–428

Jensen KR (1996) Apparatus for water purification by culturing and harvesting attached algal communities US patent 5527456

Johnson MB, Wen Z (2010) Development of an attached microalgal growth system for biofuel production. Appl Microbiol Biotechnol 85:525–534

Katarzyna L, Sai G, Singh OA (2015) Non-enclosure methods for non-suspended microalgae cultivation: literature review and research needs. Renew Sust Energ Rev 42:1418–1427

Kesaano M, Sims RC (2014) Algal biofilm based technology for wastewater treatment. Algal Res 5:231–240

Kesaano M, Gardner RD, Moll K, Lauchnor E, Gerlach R, Peyton BM, Sims RC (2015) Dissolved inorganic carbon enhanced growth, nutrient uptake, and lipid accumulation in wastewater grown microalgal biofilms. Bioresour Technol 180:7–15

Kim YB, Jeong SH, Park JW (2013) Adsorption characteristics of microalgae on surface-modified photobioreactor system. J Biobased Mater Biol 7:80–84

Knauss W, Schapery RA (1999) Recent advances in fracture mechanics. Springer, Berlin

Kokare C, Chakraborty S, Khopade A, Mahadik K (2009) Biofilm: importance and applications. Indian J Biotechnol 8:159–168

Korber DR, Lawrence JR, Lappin-Scott HM, Costerton JW (2003) Growth of microorganisms on surfaces. In: Lappin-Scott HM, Costerton JW (eds.) Microbial biofilms. Cambridge University Press, Cambridge, UK p. 15–45

Latała A, Nędzi M, Stepnowski P (2009) Toxicity of imidazolium and pyridinium based ionic liquids towards algae. *Chlorella vulgaris, Oocystis submarina* (green algae) and *Cyclotella meneghiniana, Skeletonema marinoi* (diatoms). Green Chem 11:580–588

Leale J (1998) Effects of copper sulfate on benthic algae: a laboratory experiment. McNair Schol J 2:46–51

Levy JL, Stauber JL, Jolley DF (2007) Sensitivity of marine microalgae to copper: the effect of biotic factors on copper adsorption and toxicity. Sci Tot Environ 387:141–154

Li B, Logan BE (2004) Bacterial adhesion to glass and metal-oxide surfaces. Colloids Surf B 36:81–90

Li Y, Gao YH, Li XS, Yang JY, Que GH (2010) Influence of surface free energy on the adhesion of marine benthic diatom *Nitzschia closterium* MMDL533. Colloids and Surfaces B 75:550–556

Liehr SK, Suidan MT, Eheart JW (1990) A modeling study of carbon and light limitation in algal biofilms. Biotechnol Bioeng 35:233–243

Lombardi AT, Hidalgo TMR, Vieira AAH, Sartori AL (2007) Toxicity of ionic copper to the freshwater microalga *Scenedesmus acuminatus* (Chlorophyceae, Chlorococcales). Phycologia 46:74–78

Lopes F, Morin P, Oliveira R, Melo L (2005) The influence of nickel on the adhesion ability of *Desulfovibrio desulfuricans*. Colloids Surf B 46:127–133

Mallick N (2002) Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. Biometals 15:377–390

Meireles A, Machado I, Fulgêncio R, Mergulhão F, Melo L, Simões M (2015) Efficacy of antimicrobial combinations to reduce the use of sodium hypochlorite in the control of planktonic and sessile *Escherichia coli*. Biochem Eng J 105:115–122

Memon AR, Andresen J, Habib M, Jaffar M (2014) Simulated sugar factory wastewater remediation kinetics using algal-bacterial raceway reactor promoted by polyacrylate polyalcohol. Bioresour Technol 157:37–43

Menicucci Jr JA (2010) Algal biofilms, microbial fuel cells, and implementation of state-of-the-art research into chemical and Biol Eng laboratories. Dissertation, Montana State University

Milledge JJ, Heaven S (2013) A review of the harvesting of micro-algae for biofuel production. Rev Environ Sci Biotechnol 12:165–178

Moreira-Santos M, Soares AM, Ribeiro R (2004) An *in situ* bioassay for freshwater environments with the microalga *Pseudokirchneriella subcapitata*. Ecotox Environ Saf 59:164–173

Morra M, Cassinelli C (1998) Bacterial adhesion to polymer surfaces: a critical review of surface thermodynamic approaches. J Biomat Sci-Polym E 9:55–74

Mulbry WW, Wilkie AC (2001) Growth of benthic freshwater algae on dairy manures. J Appl Phycol 13:301–306

Mulbry W, Westhead EK, Pizarro C, Sikora L (2005) Recycling of manure nutrients: use of algal biomass from dairy manure treatment as a slow release fertilizer. Bioresour Technol 96:451–458

Muñoz R, Köllner C, Guieysse B (2009) Biofilm photobioreactors for the treatment of industrial wastewaters. J Hazard Mater 161:29–34

OECD (2011) Test No. 201: freshwater alga and cyanobacteria, growth inhibition test: organisation for economic co-operation and development

Oliveira R, Azeredo J, Teixeira P, Fonseca A (2001) The role of hydrophobicity in bacterial adhesion. BioLine 1:11–22

Ozkan A (2012) Development of a novel algae biofilm photobioreactor for biofuel production. Dissertation, University of Texas

Ozkan A, Berberoglu H (2011) Adhesion of *Chlorella vulgaris* on hydrophilic and hydrophobic surfaces. In: ASME 2011 International Mechanical Engineering Congress and Exposition. Denver, Colorado, pp 169–178

Ozkan A, Berberoglu H (2013a) Adhesion of algal cells to surfaces. Biofouling 29:469–482

Ozkan A, Berberoglu H (2013b) Cell to substratum and cell to cell interactions of microalgae. Colloids Surf B 112:302–309

Ozkan A, Berberoglu H (2013c) Physico-chemical surface properties of microalgae. Colloids Surf B 112:287–293

Priyadarshani I, Rath B (2012) Commercial and industrial applications of microalgae—a review. J Algal Biomass Utln 3:89–100

Reed R, Reed G (1948) "Drop plate" method of counting viable bacteria. Can J Res E 26:317–326

Ruiz J, Álvarez P, Arbib Z, Garrido C, Barragán J, Perales J (2011) Effect of nitrogen and phosphorus concentration on their removal kinetic in treated urban wastewater by *Chlorella vulgaris*. Int J Phytoremediat 13:884–896

Schumacher G, Blume T, Sekoulov I (2003) Bacteria reduction and nutrient removal in small wastewater treatment plants by an algal biofilm. Water Sci Technol 47:195–202

Sekar R, Nair K, Rao V, Venugopalan V (2002) Nutrient dynamics and successional changes in a lentic freshwater biofilm. Freshw Biol 47: 1893–1907

Sekar R, Venugopalan V, Satpathy K, Nair K, Rao V (2004) Laboratory studies on adhesion of microalgae to hard substrates. Hydrobiologia 512:109–116

Shen Y, Zhang H, Xu X, Lin X (2015) Biofilm formation and lipid accumulation of attached culture of *Botryococcus braunii*. Bioprocess Biosyst Eng 38:481–488

Simões LC, Simões M, Oliveira R, Vieira MJ (2007) Potential of the adhesion of bacteria isolated from drinking water to materials. J Basic Microb 47:174–183

Simões M, Simões LC, Cleto S, Pereira MO, Vieira MJ (2008) The effects of a biocide and a surfactant on the detachment of *Pseudomonas fluorescens* from glass surfaces. Int J Food Microbiol 121:335–341

Simões LC, Simoes M, Vieira MJ (2010) Adhesion and biofilm formation on polystyrene by drinking water-isolated bacteria. Antonie Van Leeuwenhoek 98:317–329

Sinde E, Carballo J (2000) Attachment of *Salmonella* spp. and *Listeria monocytogenes* to stainless steel, rubber and polytetrafluorethylene: the influence of free energy and the effect of commercial sanitizers. Food Microbiol 17:439–447

Singh S, Kate BN, Banerjee U (2005) Bioactive compounds from cyanobacteria and microalgae: an overview. Cr Rev Biotechn 25: 73–95

Sirmerova M, Prochazkova G, Siristova L, Kolska Z, Branyik T (2013) Adhesion of *Chlorella vulgaris* to solid surfaces, as mediated by physicochemical interactions. J Appl Phycol 25:1687–1695

Spolaore P, Joannis-Cassan C, Duran E, Isambert A (2006) Commercial applications of microalgae. J Biosci Bioeng 101:87–96

Stanley MS, Callow JA (2007) Whole cell adhesion strength of morphotypes and isolates of *Phaeodactylum tricornutum* (Bacillariophyceae). Eur J Phycol 42:191–197

Stauber J, Florence T (1987) Mechanism of toxicity of ionic copper and copper complexes to algae. Mar Biol 94:511–519

Stewart EA, Zivojnovich MJ (2003) Apparatus and method for harvesting and collecting attached algal communities. US patent 6572770B1

Teixeira P, Oliveira R (1999) Influence of surface characteristics on the adhesion of *Alcaligenes denitrificans* to polymeric substrates. J Adhes Sci Technol 13:1287–1294

Teixeira P, Lopes Z, Azeredo J, Oliveira R, Vieira MJ (2005) Physicochemical surface characterization of a bacterial population isolated from a milking machine. Food Microbiol 22:247–251

Trevors J, Cotter C (1990) Copper toxicity and uptake in microorganisms. J Ind Microbiol 6:77–84

Van Leeuwen HP (1999) Metal speciation dynamics and bioavailability: inert and labile complexes. Environ Sci Technol 33:3743–3748

Van Oss C (1995) Hydrophobicity of biosurfaces - origin, quantitative determination and interaction energies. Colloids Surf B 5:91–110

Van Oss C (2005) Interfacial forces in aqueous media. Marcel Decker Inc, New York

Van Oss CJ (2006) Interfacial forces in aqueous media. CRC Press, Boca Raton

Van Oss C, Chaudhury M, Good R (1987) Monopolar surfaces. Adv Colloid Interfac 28:35–64

Van Oss C, Good R, Chaudhury M (1988) Additive and nonadditive surface tension components and the interpretation of contact angles. Langmuir 4:884–891

Van Oss C, Ju L, Chaudhury M, Good R (1989) Estimation of the polar parameters of the surface tension of liquids by contact angle measurements on gels. J Colloid Int Sci 128:313–319

Visser J (1995) Particle adhesion and removal: a review. Particul Sci Technol 13:169–196

Wang M, Kuo-Dahab WC, Dolan S, Park C (2014) Kinetics of nutrient removal and expression of extracellular polymeric substances of the microalgae, *Chlorella* sp. and *Micractinium* sp., in wastewater treatment. BioresourTechnol 154:131–137

Wilkie AC, Mulbry WW (2002) Recovery of dairy manure nutrients by benthic freshwater algae. Bioresour Technol 84:81–91

Wilson S, Hamilton MA, Hamilton GC, Schumann MR, Stoodley P (2004) Statistical quantification of detachment rates and size distributions of cell clumps from wild-type (PAO1) and cell signaling mutant (JP1) *Pseudomonas aeruginosa* biofilms. Appl Environ Microbiol 70:5847–5852

Wuertz S, Bishop PL, Wilderer PA (2003) Biofilms in wastewater treatment: an interdisciplinary approach. IWA Publishing London, UK

Wurdack ME (1923) Chemical composition of the walls of certain algae. Ohio J Sci 28:181–191

Yang Y, Gao K (2003) Effects of CO_2 concentrations on the freshwater microalgae, *Chlamydomonas reinhardtii*, *Chlorella pyrenoidosa* and *Scenedesmus obliquus* (Chlorophyta). J Appl Phycol 15:379–389

Zhang T, Fang HH (2001) Quantification of extracellular polymeric substances in biofilms by confocal laser scanning microscopy. Biotechnol Lett 23:405–409