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The impact of material properties, nutrient load and shear stress on biofouling in food industries

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ABSTRACT

In the food industry, biofilm formation in pipes, equipment and cooling systems increases maintenance costs, decreases operational efficiencies and is a source of contamination. Shear stress, nutrient load and surface material are important variables affecting the biofilm onset in industry. In this work, the combined impacts of these variables were assessed using three different materials (glass, copper and stainless steel), two nutrient loads (high and low nutrient medium) and two hydrodynamic conditions (static and dynamic). Initial adhesion and biofilm formation were studied in microplates using *Escherichia coli* as a model organism.

Surface material was the factor with the strongest impact and adhesion/biofilm formation were correlated with surface hydrophobicity. However, the impact of this variable was dependent on the nutrient load and imposed shear stress. It was also found that, for the majority of the situations tested, initial attachment performance is a good predictor of biofilm formation behaviour and that the effects observed during attachment are amplified during biofilm maturation. Since shear stress is a major determinant in cell adhesion, the results of this study may find application in industrial systems operating at flow rates between 0.001 and 600 $m^3 h^{-1}$ depending on tube material and diameter.

1. Introduction

In industry, microorganisms are more difficult to eradicate when they deposit and adhere to equipment surfaces and piping systems. After this initial step, microorganisms start producing extracellular polymeric substances which protect them from cleaning and disinfection protocols. This community of microorganisms, known as biofilm, can cause serious problems when it starts to spread in process lines. In the food industry, the formation of this biological fouling in piping, process equipment, cooling towers or heat exchangers can lead to an increase in maintenance costs, decrease of equipment operational efficiencies and can be a source of contamination (Brooks and Flint, 2008).

Escherichia coli has been reported as one of the most persistent foodborne microorganisms (Dourou et al., 2011; Sagong et al., 2011; Shi and Zhu, 2009). Moreover, it is also a ubiquitous microorganism in natural water systems which are commonly used in industrial cooling systems (Casani et al., 2005). *E. coli* has been found in vegetable process industries, meat industries and ready-to-eat products (Srey et al., 2013) which have different compositions and thus different nutrient loads. Additionally, the water used in the cooling systems of these industries may also have different nutrient compositions. Several authors have been reporting the impact that different nutrient loads may have on biofilm development. Jackson et al. (2002) tested the effect of LB medium (with 0.2% glucose) and colony-forming antigen medium (lacking glucose) on *E. coli* biofilm formation and verified that the presence of glucose in the media inhibited biofilm formation. Gomes et al. (2014) tested the effect of glucose, peptone and yeast extract load on *E. coli* biofilm formation under static and shaking conditions (0–0.07 Pa) and verified that higher glucose concentrations enhanced *E. coli* adhesion in the first 24 h, but variation in

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peptone and yeast extract concentration had no significant impact on biofilm formation. Teodósio et al. (2011) observed that at higher shear stresses (0.6 Pa) the amount of *E. coli* biofilm formed under two different nutrient loads was similar, concluding that hydrodynamics were probably controlling biofilm formation.

In industrial lines, bacterial adhesion and biofilm growth may be controlled by the shear forces applied on the surface by the fluid flow (Lelièvre et al., 2002). Shear forces can be controlled by either design of the equipment/pipe diameter or the flow rate used during operation, with hygienic design being preferred due to the cost and limitations of increasing the flow rate (Jensen et al., 2005). The specific hydrodynamic conditions of each system will determine the bacterial concentration on the surface, the structure of biofilm matrix and the nutrient mass transport (Melo and Vieira, 1999). Higher flow velocities (or higher shear forces) are usually preferred in industry since they prevent bacterial adhesion and increase the rate of biofilm detachment (Vieira et al., 1993). However, there are some process lines where lower velocities are necessary and lower velocity zones may also be found in equipment with complex geometries, the so-called dead zones (Walid et al., 2013). Additionally, zones of stagnant flow may be found in wet surfaces and this is also a suitable place for bacterial adhesion and biofilm development (Ganesh and Anand, 1998).

The selection of a material to be used as food contact and processing surface is complex. The material should be non-toxic when in contact with food, suitable for the place where it will be used (pipes, equipment or free surfaces), resistant to corrosion, easily cleanable, etc. (Van Houdt and Michiels, 2010). Stainless steel, glass and copper have been intensively used in food industries (Bonsaglia et al., 2014; Brooks and Flint, 2008; Melo and Flemming, 2010; Shi and Zhu, 2009; Van Houdt and Michiels, 2010). Stainless steel tubing is often used because of its hygienic status (low soiling level and/or high cleanability) (Jullien et al., 2003) and the ability to resist corrosive damage (Flint et al., 2000). Copper and its alloys have desirable properties for industrial applications due to their high thermal conductivity, corrosion resistance and antimicrobial effects (Grass et al., 2011; Wilks et al., 2005) and have been traditionally used, for instance, in heat exchanger tubing in cane and beet sugar refineries (AISI, 1976). Glass is a surface that has no effect on the final product smell or taste and its transparency is also advantageous (Muller-Steinhagen and Zettler, 2011).

There are several reports in the literature describing the individual effects of nutrient media, hydrodynamics and surface properties on biofilm development, but little is known about the combined impact of these three factors. The aim of the present study was to examine the combined effect of two nutrient loads (high and low nutrient medium), two hydrodynamic conditions (static and dynamic) and three materials typically used in food industry (glass, copper and stainless steel) on *E. coli* adhesion and biofilm formation. The applicability of the obtained results to industrial settings is also discussed.

2. Material and methods

2.1. Preparation of bacterial strain

E. coli JM109(DE3) from Promega (USA) was used in this study because it has shown a good biofilm forming ability in a variety of biofilm reactors operated at different shear stresses (Moreira et al., 2013, 2014b; Teodósio et al., 2012). Additionally, it was shown that the biofilm formation ability of this strain is similar to other *E. coli* strains which are often used for antimicrobial susceptibility and disinfection tests (Gomes et al., 2014). The strain was grown overnight at 30 °C and 120 rpm in 0.2 L of inoculation medium previously described by Teodósio et al. (2011). This medium consisted of 5.5 g L^{-1} glucose, 2.5 g L^{-1} peptone, 1.25 g L^{-1} yeast extract in phosphate buffer (1.88 g L^{-1} KH_2PO_4 and 2.60 g L^{-1} Na_2HPO_4), pH 7.0. Then, the cells were centrifuged (3202 g, 10 min, 25 °C) and washed twice with saline solution (8.5 g L^{-1} NaCl in distilled water). The pellet was resuspended and the cellular suspension was adjusted to a final concentration of approximately 7.6×10^8 cells mL^{-1}, determined by optical density at 610 nm (OD = 1).

2.2. Surface preparation

Coupons with dimensions of 1 × 1 cm made from glass (GLA; Vidraria Lousada, Lda, Portugal), stainless steel 316 (SS; F. Ramada, Portugal) and copper (Cu; Neves & Neves, Lda, Portugal) were prepared. SS, Cu and GLA were selected because of their common use in heat exchange equipment and pipes in food processing lines (Bonsaglia et al., 2014; Brooks and Flint, 2008; Melo and Flemming, 2010; Shi and Zhu, 2009; Van Houdt and Michiels, 2010).

All materials were immersed in a solution of 5% (v/v) commercial detergent (Sonasol Pril, Henkel Ibérica S.A.) for 30 min with gentle shaking (Azevedo et al., 2006). To remove any remaining detergent, coupons were rinsed in ultrapure water and immersed in 96% (v/v) ethanol for 30 min (Gomes et al., 2015). After being rinsed again with ultrapure water and air-dried, all coupons were autoclaved for 15 min at 121 °C (Gomes et al., 2015) before being used in contact angle measurements and for adhesion and biofilm assays.

2.3. Surface free energy measurements

The surface energy components of the tested materials (GLA, SS and Cu) were determined after measuring the contact angles of the surfaces by the sessile drop method using a contact angle meter (OCA 15 Plus, Dataphysics, Germany). These measurements were carried out at room temperature (25 ± 2 °C) with three pure liquids: water, formamide and α-bromonaphthalene (Sigma-Aldrich Co., Portugal). Reference values for surface tension components were obtained from the literature (Janczuk et al., 1993). Contact angle data were obtained from at least 25 determinations for each liquid and surface. Afterwards, the hydrophobicity of the surfaces was evaluated by the method of van Oss et al. (1988). In this approach, the degree of hydrophobicity of a given material (i) is expressed as the free energy of interaction between two entities of that material immersed in water (w)–ΔG_{iwi}. If the interaction between the two entities is stronger than the interaction of each entity with water ($\Delta G_{iwi} < 0$ mJ m^{-2}), the material is considered hydrophobic. Conversely, if $\Delta G_{iwi} > 0$ mJ m^{-2}, the material is hydrophilic. ΔG_{iwi} was calculated from the surface tension components of the interacting entities, according to Eq. (1):

$$\Delta G_{iwi} = -2\left(\sqrt{\gamma_i^{LW}} - \sqrt{\gamma_w^{LW}}\right)^2 + 4\left(\sqrt{\gamma_i^+\gamma_w^-} + \sqrt{\gamma_i^-\gamma_w^+} - \sqrt{\gamma_i^+\gamma_i^-} - \sqrt{\gamma_w^+\gamma_w^-}\right) \quad (1)$$