Proceedings of International Conference on Heat Exchanger Fouling and Cleaning - 2013 (Peer-reviewed)
June 09 - 14, 2013, Budapest, Hungary
Editors: M.R. Malayeri, H. Müller-Steinhagen and A.P. Watkinson
Published online
www.heatexchanger-fouling.com

BIOFILM FORMATION UNDER TURBULENT CONDITIONS: EXTERNAL MASS TRANSFER *VERSUS* SHEAR STRESS

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ABSTRACT

This work investigates the effect of flow rate variation on the development of *Escherichia coli* biofilms formed in a flow cell system under turbulent conditions. Two flow rates were tested corresponding to Reynolds numbers of 4350 and 6720. The higher flow rate favored planktonic growth whereas the lower flow rate enhanced biofilm formation. Despite this, similar glucose consumption values were obtained in the whole system for both flow rates. Estimation of the external mass transfer coefficients by empirical correlations indicated that as the flow rate increased 1.5 fold, the external mass transfer coefficient increased 1.4 fold. Estimation of the shear stress in the flow cell was done by computational fluid dynamics and simulations indicated that the average shear stress increased 2.0 fold at the higher flow rate.

Our results indicate that although external mass transfer is favored by an increase in flow velocity, the increase in shear stress had a negative impact on biofilm development. These results may have implications on biofouling control procedures where the use of high pressure/shear stress regimes and the use of cleaning/disinfection agents, that have to be transported from the bulk solution to the liquid-biofilm interface, is common practice.

INTRODUCTION

The majority of microorganisms inhabiting hydrated environments prefer to live in cooperative communities called biofilms (Nikolaev and Plakunov, 2007).

Bacterial biofilms can have advantageous or detrimental effects. Although biofilms can be used in processes like bioremediation (Singh et al., 2006), wastewater treatment or production of various chemicals (Qureshi et al., 2005), they can also cause food spoilage (Shi and Zhu, 2009), potable water contamination in distribution systems (Batté et al., 2003) and bioufouling on heat exchangers (Melo and Flemming, 2010).

Escherichia coli has been used as a model microorganism for biofilm studies particularly in the health (Wood, 2009) and food industry sectors. It has been shown that *E. coli* is capable of attaching to food contact surfaces in industry (Dourou et al., 2011) and that this event can promote contamination by several microorganisms other than *E. coli* (Castonguay et al., 2006).

Hydrodynamic conditions are determinant in cell adhesion to a surface (Brooks and Trust, 1983; Chen et al. 2005; Mohamed et al., 2000). The fluid surrounding a biofilm provides the primary source of nutrients and is the vehicle for molecule delivery and removal. It has been observed that, in most cases, access to nutrients controls biofilm growth (Roberts and Stewart, 2004). Furthermore, for a given system, the flow rate dictates the shear stress imposed on the biofilm (Teodósio et al., 2013). This is of particular relevance in the food industry since the efficiency of cleaning-in-place procedures depends on the shear stresses generated by the cleaning fluid.

With this work we tried to understand the effect of changing the flow rate on the development of *E. coli* biofilms formed under turbulent conditions. The hydrodynamics of this system were simulated for two flow conditions by computational fluid dynamics (CFD). The wall shear stress and mass transfer coefficients were estimated in order to assess the relative effects of enhanced nutrient delivery *versus* increased shear stress on biofilm development.

MATERIALS AND METHODS

Mass transport

In this work the nutrient transport from the bulk solution to the liquid-biofilm interface was characterized by the external mass transfer coefficient (*Km*) calculated by a correlation between the Sherwood number (*Sh*) as a function of the Reynolds number (*Re*) and Schmidt number (*Sc*) (Perry and Green, 1997):

$$Sh = 0.023\,\mathrm{Re}^{0.83}\,Sc^{1/3} \quad (1)$$

$$Km = \left(Sh\ D/d\right) \quad (2)$$

Numerical simulation

The Fluent CFD code (version 6.3.26, Fluent Inc.) was used in the numerical simulation of the flow field in the semi-circular flow cell reactor as described in Teodósio et al. (2012a). The computational mesh (1250472 hexahedral cells) was created using Gambit 2.3.26 mesh generator (Fluent Inc.). The shear-stress transport (SST) version of the *k-ω* model (Menter, 1994) was used, which effectively merges the *k-ε* model (Launder and Spalding, 1972) and the standard *k-ω* model (Wilcox, 1998). The mass and momentum equations were solved along with the ones from the adopted turbulence model for the whole flow cell although the results shown on this work only concern the region were the coupons are located. The validity of the numerical simulations was confirmed by streak photography

as described in Teodósio et al. (2012a).

Culture conditions

Culture conditions were similar to those previously described by Teodósio et al. (2011). An intermediate tank containing 4 L of a culture medium containing 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) at pH = 7.0 was inoculated with an overnight grown culture of *Escherichia coli* JM109(DE3). When the optical density values (O.D.), reached 1 (610 nm), the culture was used to inoculate the recirculating tank (at a flow rate of 1.4 L h^{-1}) already containing 5 L of sterile water, under aerated conditions. System feeding started 18 h after inoculation at a flow rate of 0.025 L h^{-1} with a culture media consisting of 0.55 g L^{-1} glucose, 0.25 g L^{-1} peptone, 0.125 g L^{-1} yeast extract and phosphate buffer (0.188 g L^{-1} KH_2PO_4 and 0.26 g L^{-1} Na_2HPO_4), pH = 7.0.

Flow cell system

The flow cell system (Fig. 1) used to produce the biofilm is composed of a recirculating tank, peristaltic and centrifuge pumps and one vertical semi-circular flow cell reactor (1.83 cm of hydraulic diameter and 110 cm length) with 10 removable coupons on its flat wall (Teodósio et al., 2011). Two conditions were tested, *Re* = 6720, corresponding to a flow rate of 374 L h^{-1} and *Re* = 4350 corresponding to a flow rate of at 242 L h^{-1}. The temperature was kept constant at 30 ºC since this *E. coli* strain is capable of forming high biofilm amounts at this temperature (Teodósio et al., 2012b). Three independent experiments were performed for each condition. Biofilm formation was monitored for 9 days and sampling was performed as described by Teodósio et al. (2011).

Sampling and analysis

Biofilm wet weight, O.D. and glucose consumption determinations were performed as described by Teodósio et al. (2011). Although the amount of biofilm was directly expressed through wet weigh determination, the concentration of planktonic cells in the system was assayed indirectly through the O.D. Glucose consumption by planktonic and sessile cells was determined by mass balance assuming chemostat conditions as detailed in Teodósio et al. (2011). Average standard deviation on the triplicate sets was below 25% for the wet weight, below 22% for the O.D. and below 17% for the glucose consumption.

Statistical analysis

Paired *t*-test analyses were performed to estimate whether or not there was a significant difference between the results originated from three independent experiments for each hydrodynamic condition. Each time point was evaluated individually using the three independent results obtained in one condition and the three individual results obtained on the other condition.

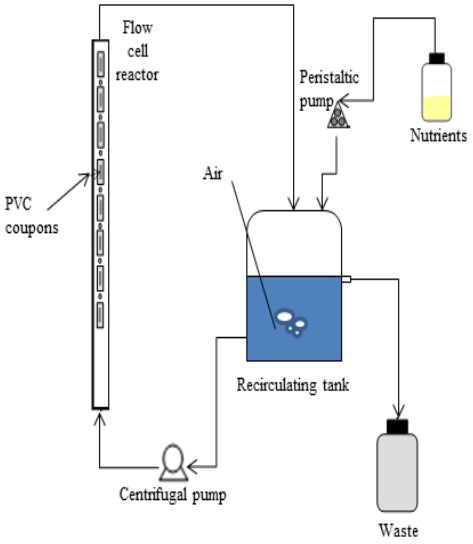

Fig. 1 Schematic representation of the biofilm producing system.

Whenever statistically significant differences were obtained from the different time points (confidence level of 95%, $p < 0.05$), these were marked in Fig. 2 with (*).

RESULTS

Two flow conditions were used (*Re* = 4350 and 6720) in order to study the effect of the flow rate in biofilm development. Table 1 presents the average wall shear stress (σ) inside the flow cell reactor simulated by CFD and the Schmidt (*Sh*) and external mass transfer coefficients (*Km*) which were calculated using eq. 1 and eq. 2. Figure 2 represents the average results obtained for planktonic cell concentration, biofilm wet weight and glucose consumption originating from three independent experiments for each hydrodynamic condition.

The average wall shear stress for the higher *Re* was approximately 2 fold higher than for the lower *Re*. It was also estimated that for the higher *Re*, the *Sh* and *Km* values were 1.4 fold higher than the results obtained with the lower *Re*.

Regarding planktonic cell concentration (Fig. 2a), a similar behavior ($P > 0.1$) was observed until day 4 for both conditions. From this day onwards, the O.D. increased for the higher *Re* and this only started to occur one day later and at a slower rate for the lower *Re* until day 8, when a constant concentration was reached for both conditions. Higher planktonic cell concentrations were generally obtained from day 5 for the higher *Re* with statistically significant differences obtained between days 5 and 7. For both studied cases, the growth curves, followed the classical growth pattern (Bühler et al., 1998).

Biofilm wet weight (Fig. 2b), showed a slight increasing tendency for the higher *Re* during the experimental time. For the lower *Re* a distinct behavior was observed, since a marked increase in biofilm wet weight was obtained between days 3 and 7. The maximum biofilm wet weight was reached on day 7 for *Re* = 4350, and it was 57% higher than that obtained for *Re* = 6720.

Table 1 Average wall shear stresses predicted by CFD. Calculated values for the *Sh* number using correlation (eq. 1) and the *Km* (eq. 2).

Re	***σ* / Pa**	***Sh***	***Km* / (m s^{-1})**
4350	0.183	252	9.64x10^{-6}
6720	0.365	362	1.38x10^{-5}

Glucose consumption increased thorough the experiment (Fig. 2c) and with the exception of day 2, consumption profiles for both hydrodynamic conditions were statistically similar ($P > 0.05$).

DISCUSSION

This work assesses the effects of increasing the flow rate on biofilm formation in turbulent conditions. A 54% increase in the flow rate (starting from a Reynolds number of 4350) caused a 2 fold increase in the estimated wall shear stress and a 1.4 fold increase in the calculated external mass transfer coefficient. These two effects are contradictory for biofilm formation because although the transport of nutrients and cells is favored at a higher *Re*, the increased shear stress may promote biofilm erosion (or sloughing) and can also prevent initial adhesion (Stoodley et al., 1998; Telgmann et al., 2004)

Thus, a higher biofilm formation was expected at higher *Re* if nutrient transport from the bulk solution was limiting biofilm development. Instead, we have obtained a higher biofilm formation at a lower *Re*.

Until day 3, similar amounts of biofilm were formed in both conditions and it seems that a balance occurs between shear forces and external nutrient transport effects. From day 3 onwards a higher amount of biofilm was formed at the lower *Re*. This may happen because under a higher *Re* biofilm cohesion is affected by the shear stress or internal mass transport limitations begin to occur (Stewart and Franklin, 2008). The first hypothesis seems to be more plausible since a higher concentration of planktonic cells with the higher *Re* suggests a higher biomass detachment due to the stronger shear forces. A higher amount of planktonic cells was obtained at a higher *Re* probably due to the fact that they are more sensitive to nutrient transport from the liquid than to shear stress. One should bear in mind that at the cell surface the relative velocity between liquid and planktonic cells is very small.

The hydrodynamic conditions also affect the proportion of microbial cells and exopolysaccharides (EPS) produced by the biofilms (Melo and Vieira, 1999; Vieira and Melo, 1999). It has been shown that stronger shear forces may increase EPS production by biofilms (Liu and Tay, 2002). For the higher *Re*, the EPS production was probably higher than the formation of new microbial cells resulting in a smaller fraction of the active layer. Under a lower *Re* although a higher amount of biomass was formed, a higher percentage of the biofilm layer can be inactive due to the depletion of substrate inside them (Vieira and Melo, 1999).

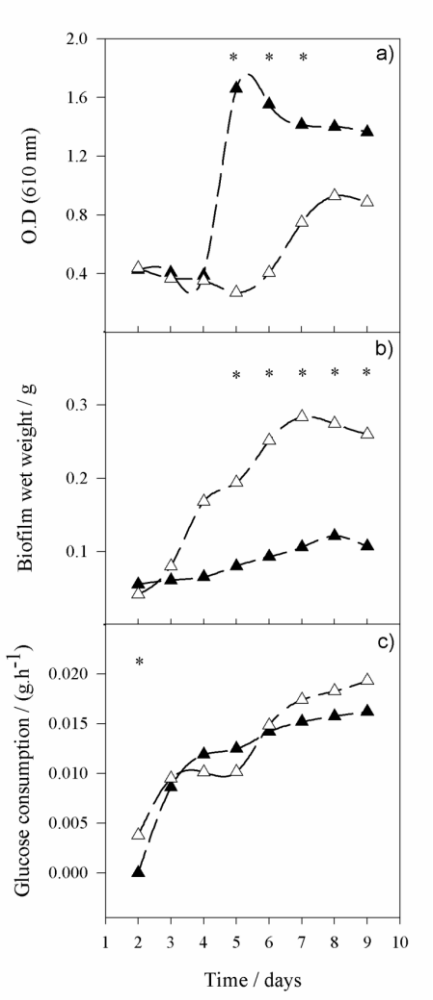

Fig. 2 Time-course evolution of: a) optical density in the recirculating tank, b) biofilm wet weight, c) glucose consumption in the system. Closed symbols – higher flow rate (*Re* = 6720), open symbols – lower flow rate (*Re* = 4350). Time points marked with * are those for which a statistical difference was found between both conditions (confidence level greater than 95%, $P < 0.05$)

Since shear stress is a major determinant in cell adhesion to a surface (Chen et al., 2005; Finger et al., 1996), the results presented in this work are of particular relevance to predict the onset of biofilms in different systems.

The shear stress values that were obtained in this system are in the range of those found in industrial and biomedical settings, namely in the human cardiovascular system, where shear stresses between 0.076 and 3.4 Pa can be obtained (Michelson, 2002; Ross et al., 1998); in the rennet-induced coagulation of milk during cheese making, where values below 0.5 Pa are common (Konuklar and Gunasekaran, 2002); or in membrane processes used for wastewater remediation, where average shear stresses of 0.25 can be obtained (Ahmed et al., 2011).

CONCLUSIONS

The results demonstrate that the flow cell reactor here described can be used for the simulation of biofilm formation in different systems in industrial and biomedical settings taking into consideration the shear stress values that were achieved.

The data presented on this work indicates that biofilm formation was favored at the lowest flow rate because shear stress effects were more important than mass transfer limitations. Furthermore, operation at higher flow rates not only reduces biofilm formation but also favors the transport of biocides and other cleaning agents during CIP (cleaning in place) procedures and increases cell detachment from the biofilm.

Due to the similarities of the shear stress in this system and those occurring in industrial and biomedical settings it is likely that the results obtained on this work can be used in biofouling control in industrial settings as well as in the design of biomedical devices where biofilm development must be minimized.

ACKNOWLEDGMENTS

The authors acknowledge the financial support provided by the Operational Programme for Competitiveness Factors – COMPETE, European Fund for Regional Development – FEDER and by the Portuguese Foundation for Science and Technology – FCT through Projects PTDC/EBB-BIO/102863/2008 and PTDC/EBB-BIO/104940/2008. Manuel Moreira Alves and Filipe Silva (CEFT, Faculty of Engineering, University of Porto) are acknowledged for the CFD simulations.

NOMENCLATURE

Symbol	Unit	Description
d	m	hydraulic diameter
D	$m^2\ s^{-1}$	molecular diffusivity of growth-limiting nutrient in water ($7.0x10^{-10}$ $m^2\ s^{-1}$ at 30 ºC for glucose)
Km	$m\ s^{-1}$	external (liquid) mass transfer coefficient
Re	dimensionless	Reynolds number ($\rho\ v\ d\ \mu^{-1}$)
Sc	dimensionless	Schmidt number ($\mu\ \rho^{-1}\ D^{-1}$)
Sh	dimensionless	Sherwood number ($Km\ d\ D^{-1}$)
t	s	time
v	$m\ s^{-1}$	flow velocity
μ	$kg\ m^{-1}\ s^{-1}$	Viscosity ($8.007\ x10^{-4}$ $Pa.s^{-1}$ at 30ºC for water)
ρ	$kg\ m^{-3}$	Density (995.647 $Kg.m^{-3}$ at 30ºC for water)

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