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The combined effects of shear stress and mass transfer on the balance between biofilm and suspended cell dynamics

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Abstract

This work investigates the effect of shear stress and mass transfer on the development of biofilms in a flow cell that mimics industrial piping. The shear stress and maximum flow velocity were estimated by computational fluid dynamics and the external mass transfer coefficient was calculated using empirical correlations for Reynolds numbers ranging from 100 to 10000. The effect of two flow rates on the development of *Escherichia coli* biofilms under turbulent flow conditions was assessed and it was observed that biofilm formation was favored at the lowest flow rate. Additionally, estimations of the shear stress and external mass transfer coefficient indicate that both parameters increase with increasing flow rates. Thus it seems that biofilm formation was being controlled by the shear stress that promoted biofilm erosion/sloughing and not by mass transfer which would potentiate biofilm growth.

Our results indicate that not only efficient pre-treatment units are required on water recirculation loops in order to reduce the effective concentration of bacteria and nutrients, but also that high flow rates are preferred at all times to reduce the buildup of bacterial biofilms. For instance, high flow rates should be used during cleaning and disinfection cycles because the increase in shear stress will promote biofilm detachment and also potentiate the effect of biocides and other cleaning agents due to the increased mass transfer from the bulk solution to the surface of the biofilm.

Keywords

Biofilms, *Escherichia coli*, mass transfer, shear stress

Introduction

Water scarcity and the increasing costs of water supply and wastewater disposal underlie the growing concern on reducing water usage and wastewater discharge in industry. With technological improvements and the consequent legislation opening to the use of alternative water qualities (e.g. grey water utilization) and social acceptance of water reuse, water systems integration is being implemented in many industries in order to ensure an efficient use of the water resources [1, 2]. This technology entails the integration of the wastewater generated by an industrial equipment/process after its treatment or directly in the industrial lines by continuous loops of water recirculation [3, 4]. The potential for water saving in industry is enormous since it has been estimated that the industrial sector is responsible for up to 25% of the total water consumption in the world [5]. Some examples of water saving through reuse can be found on the textile industry where water savings can reach 52% per ton of product [6], or in the food industry where savings between 20 and 50% can be reached [2]. For common industrial settings, it has been estimated that the maximum reuse fraction can reach up to 93% [5].

However, this water saving technology can promote biofilm formation since a concentration effect of bacteria and nutrients can occur after some reutilization cycles [7]. The buildup of biofilms in the wastewater recycling/reuse units starts with the transport of microorganisms and nutrients to the walls of the equipment/pipes and the consequent attachment and biofilm growth [8]. Additionally, the increase in the system residence time will also facilitate biofilm growth. This accumulation process promotes a reduction of the flow area, the increase of the pressure drop until complete clogging of the equipment/pipe, pitting corrosion phenomena and heat transfer resistance [9, 10]. Moreover, biofilms can serve as hosts for pathogenic microorganisms which become more resistant to disinfecting agents and promote the contamination of the fluids flowing through the pipes by erosion/sloughing from the biofilm [8, 11]. It has been estimated that biofilm development in industrial process

lines may represent up to 30% of the plant operating costs [8]. These include cleaning and disinfection costs, (since a decrease in chemical treatment efficiency implies a higher biocide consumption), costs associated with frequent production downtimes (to perform the cleaning), maintenance and repair costs (due to the earlier and faster equipment degradation by the biofilms) and the increased costs associated with wastewater treatment (which contains a higher concentration of the chemicals used in the cleaning/disinfection process).

In industry, chemical and mechanical actions are combined to remove the biofilms, however some limitations to an efficient disinfection process are observed [12, 13]. The mechanical action of scrubbing and scraping may be abrasive and leave scratches that can eventually lodge some microorganisms and promote biofilm development. Some equipment contains crevices and dead spaces that are hard to reach during cleaning and will function as a niche for future biofilm development. Thus, biofilm-related problems do not solely arise from microorganisms which have suddenly invaded the system, but are sometimes a result of a set of several conditions, such as nutrient concentration [14] and absence of inhibiting factors on biofilm development. Understanding the factors that control the onset and maturation of biofilms in closed loop water systems is key to reduce process downtimes that are necessary for system cleaning and reduce the probability of further contamination. It is known that one of the major determinants for biofilm development is the hydrodynamic conditions of the system [15, 16]. These conditions will dictate the shear stress on the surfaces where the biofilms form and also the mass transfer of nutrients/biocides and bacteria from the bulk medium to the biofilm.

In this work, computational fluid dynamics (CFD) tools were used to simulate the hydrodynamics in a flow cell system that mimics industrial piping [17, 18]. The maximum flow velocity and average wall shear stress were estimated for Reynolds numbers (*Re*) between 100 and 100000. Mass transfer coefficients were also calculated in order to assess the effects of nutrient transport on biofilm development. A flow cell system was then used to observe experimentally the effect of two distinct flow rates (*Re* = 4350 and 6720) on planktonic cell concentration and biofilm formation using *Escherichia coli* JM109(DE3).

Materials and methods

Mass transport estimation and flow simulation

In this work the rate of nutrient transport from the bulk solution to the liquid-biofilm interface for *Re* ranging from 100 to 10000 was quantified by the external mass transfer coefficient (*Km*) obtained from the appropriate correlations (for laminar and turbulent flow regimes).

The Sherwood number (*Sh*) for a fully developed concentration profile in laminar flow conditions (*Re* between 100 and 1000) has a constant value of 3.66 [19]. For turbulent flow, the Sherwood numbers were calculated by correlation (1) as a function of *Re* valid in the range between *Re* = 2100 and 35000 and Schmidt number (*Sc*) in the range between *Sc* = 0.6 and 3000 [19].

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

From the Sherwood number, the external mass transfer coefficient can be calculated by:

$$Km = \left(Sh\ D/d\right) \tag{2}$$

The Fluent CFD commercial code (version 6.3.26, Fluent Inc.) was used for the numerical simulation of the flow field (for *Re* ranging from 100 to 10000) in the flow cell reactor as described in Teodósio et al. [18]. These simulations enabled the determination of the wall shear stress and the maximum flow velocity in the flow cell at different *Re.*

Flow cell system and culture conditions

The flow cell system used to produce the biofilms was previously described by Teodósio et al. [17], consisting of a recirculating tank, one vertical semi-circular flow cell reactor (hydraulic diameter of 18.3 mm) with 10 removable coupons, peristaltic and centrifuge pumps (Fig. 1).

Escherichia coli JM109(DE3) was used throughout this work to produce the biofilms using the culture conditions described by Teodósio et al. [17]. This strain was selected because it was shown to be a good biofilm

producer at this working temperature [20]. 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, was used to feed the system during the experiment, at a flow rate of 0.025 L h^{-1}. Temperature was kept at 30 ºC and the air flow rate in the tank was 108 L h^{-1}.

Sampling and analysis

Three independent experiments were performed to characterize the planktonic cell growth and the biofilm formed under each flow condition. Biofilms were formed at *Re* = 6720, corresponding to a flow rate of 374 L h^{-1} and at *Re* = 4350 corresponding to a flow rate of at 242 L h^{-1}.

Biofilm formation was monitored for 8 days and during this period the recirculating tank was fed with the culture medium previously described. Biofilm wet weight, optical density (at 610 nm) and glucose consumption determinations were performed as described by Teodósio et al. [17]. Average standard deviation on the triplicate sets was below 25% for the wet weight, below 22% for the Optical Density (O.D.) and below 17% for the glucose consumption.

Experimental results are an average of those obtained from the three independent experiments for each flow 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. Paired *t*-test analyses were performed to estimate whether or not there was a significant difference between these results. When a confidence level greater than 95% was obtained ($P < 0.05$), these time points were marked with an x.

Results

The hydrodynamic simulation of the flow cell reactor was made for both laminar and turbulent flow regimes ($100 \leq Re \leq 10000$) using CFD which enabled the determination of the wall shear stress and maximum flow velocity. Nutrient transport by the fluid flow was characterized through *Sh* and K_m which were calculated by correlations.

In fig. 2a it is possible to see the values obtained for K_m and *Sh*. For laminar flow, *Sh* and K_m values remain constant for *Re* between 100 and 1000. For the turbulent flow regime both parameters increase and raising *Re* from 5000 to 10000 (2.0-fold) increases the external mass transfer coefficient by a factor of 1.8 fold.

Fig. 2b shows that maximum flow velocities between 0.013 and 0.722 $m.s^{-1}$ and average wall shear stress ranging from 0.002 to 1.19 Pa can be achieved for *Re* ranging from 100 to 10000 in this flow cell system. As expected, increasing *Re* increases the maximum flow velocity and the wall shear stress. For laminar flow, raising *Re* from 100 to 200 (2.0-fold) increases the maximum flow velocity by 1.8 fold and the wall shear stress by 2.1 fold. For the turbulent regime, raising *Re* from 5000 to 10000 (2.0-fold) increases the maximum flow velocity by 1.9 fold and the wall shear stress by 3.1 fold.

A flow cell reactor was used to assess the influence of two different flow rates on *Escherichia coli* JM109(DE3) biofilm development under turbulent flow conditions (*Re* = 4350 and 6720). Figure 3 represents the average results obtained for biofilm wet weight, planktonic cell concentration and glucose consumption originating from three independent experiments for each hydrodynamic condition.

Regarding biofilm wet weight (Fig. 3a), a slight increase was observed for the higher *Re* during the experimental time. On the other hand, for the lower *Re*, 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 on day 8 for *Re* = 6720 (57% lower than the maximum value obtained for the less turbulent regime).

Planktonic cell concentration (Fig. 3b), had a similar behavior ($P > 0.05$) for both flow conditions until day 4. Between days 4 and 5, a 84% increase in O.D. was obtained for the higher *Re*. For the lower *Re*, the planktonic cell concentration only started to increase one day later and at a slower rate. Between days 5 and 7 the planktonic cell concentration values became closer for the two tested hydrodynamic conditions, although higher cell concentrations were obtained with the higher *Re* ($P < 0.05$). At the end of experiment (day 8), the maximum value of O.D. reached for *Re* = 4350 was 44% lower than the maximum value obtained for *Re* = 6720.

In fig. 3c it is possible to observe that glucose consumption in the whole system increased throughout the experiment and, with the exception of day 2, consumption profiles for both hydrodynamic conditions were statistically similar ($P > 0.05$).

Discussion

In most industrial settings the flow regime is turbulent [8, 21] but even in these cases, certain zones within equipment may have laminar flow characteristics namely when crevices, depressions or dead-zones are found [22].

In laminar flow conditions, the influence of the shear forces is less significant and therefore initial cell adhesion is facilitated in this case [23]. Moreover, since the external mass transfer in laminar flows does not improve with higher flow velocities, some nutrient transport limitations can be anticipated. Thus, thicker biofilms are likely to be formed, with a more porous matrix in order to favour nutrient and oxygen delivery to the deeper layers [23]. During cleaning-in-place (CIP) procedures, the transport of cleaning agents to the biofilm surface can be a limiting step in the disinfection process. Jensen and Friis [24] tried to predict the cleanability of closed food-process equipment based only on the critical wall shear stress obtained by CFD and observed that shear stress alone was insufficient to completely remove the contamination. They concluded that there are some effects such as mass transfer of the detergent solutions to the surface that are very likely to have a strong influence in the cleaning process. Thus, an improvement in the external mass transfer rate can result in a reduction of disinfectant consumption and increase the cleaning efficiency. Our results show that under laminar flow conditions, a variation of 2.0-fold on *Re* (from 100 to 200) promotes an increase of 2.1 fold in shear stress but with no effect on the external mass transfer coefficient. Under these flow conditions, the ratio between the convective and the diffusive mass transport is constant (since the *Sh* is unchanged). In turbulent flow conditions, to promote the same increase in the shear stress (2.1 fold), it would be necessary to increase *Re* only by 1.5 fold (instead of 2.0-fold) which would promote an increase of 1.4 fold in the external mass transfer coefficient. Thus, contrary to laminar flow conditions, a slight increase of *Re* in cleaning operations during turbulent flow besides improving the external mass transfer (which can be beneficial for the transport of cleaning products) promotes a strong increase in the shear forces and turbulent burst phenomena that have a determinant role on biofilm removal [23]. Moreover, shear forces will promote biomass loss from the external biofilm layer where the cells that exhibit the highest growth rate and are responsible for biofilm growth are located [25]. This increase in shear stress can be achieved by a modest increase in the fluid velocity. Although this scenario entails a slightly higher water flow rate during cleaning, the same (or better) cleaning performance may be achieved within a shorter operating time, thus decreasing the overall consumption of water and chemicals.

Wall shear stress and nutrient transport are the most important parameters that influence biofilm formation [14]. In industrial settings, turbulent flow is the predominant regime, thus it is interesting to study the effect of increasing the flow rate on biofilm formation under turbulent flow conditions. For the flow rates used in this work (242 and 374 L h^{-1}), an increase of 1.5 fold in the flow rate caused an improvement of 1.4 fold on the external mass transfer. Thus, if mass transfer effects were controlling biofilm growth, higher biofilm amounts would be expected at higher *Re*, since the transport of nutrients and cells is favored in these conditions. Instead, until day 3, similar amounts of biofilm were formed in both conditions, whereas from this day onwards a higher amount of biofilm was formed at the lower *Re*. It seems that in the first days a balance occurred between shear forces and external nutrient transport effects: although, nutrient transport to the biofilm surface is favoured at a higher *Re*, a lower shear stress (lower *Re*) tends to facilitate cell adhesion [26]. After the third day, the biofilm cohesion under a higher *Re* may have been affected by the stronger shear stress and turbulence intensity that promotes biomass detachment [26]. This hypothesis is supported by the higher planktonic cell concentration that was observed. Moreover, although a higher flow rate does not favour biofilm development, it favours planktonic cell growth since these cells are probably more sensitive to nutrient transport than to the shear stress. Another phenomenon associated with the increase of shear forces is the production of exopolysaccharides (EPS) [27]. It has been shown that biofilm growth originates from initially attached cells (which will result in an active layer) and not from cell deposition from the bulk liquid [21]. Biofilms formed under lower *Re* probably have a higher number of active cells, unlike the biofilms formed under higher velocities that are likely to have a higher EPS content [27]. Since the new microbial cells originate from the active layer, this can explain the higher biofilm amount obtained from day 3 onwards under a lower *Re*. Under lower flow velocities, this new layer will resist to the weaker shear forces.

On the other hand, the biofilm formed under higher fluid velocities, would be thinner and robust with a higher EPS content in order to withstand the strong shear forces [16].

Glucose consumption values in the whole system were similar for both flow conditions along the experimental time. Gikas and Livingston [28] observed, in a three phase air lift bioreactor, that even if suspended biomass does not represent a significant fraction of the total biomass it can contribute significantly to the total substrate uptake. Thus, substrate consumption in the system results from a combined action of both, planktonic and biofilm cells. This in an indication that the total microbial load on the system might be similar in both cases. It is interesting to observe that despite this fact, the amounts of biofilm formed and the concentration of planktonic cells are different in both situations (higher *Re* induced less biofilm and more planktonic cells). For industrial scenarios, like the operation of heat exchangers in cooling water systems, a certain amount of microbial load can be tolerated as long as it is not in the form of a biofilm. This is because biofilms cells are more difficult to eliminate and planktonic cells are immediately purged from the system in CIP. Additionally, it is the biofilm buildup that causes the problems associated with increased pressure drop, corrosion and pitting and increased heat transfer resistance [9, 10]. In these cases, if the operational conditions of a certain process are prone to stimulate microbial growth (for instance due to the high concentration of nutrients in recycle loops), it is wise to operate the system using conditions that reduce biofilm formation even if this means that planktonic concentrations may be increased.

The data presented on this work indicates that shear stress effects can be more important than mass transfer limitations on biofilm formation since biofilm growth was favored at lower *Re*. When higher fluid velocities are used, biofilm buildup is reduced and the transport of biocides and other cleaning agents during the CIP procedures is favored. Additionally, since cell detachment from the biofilm also increases, the effectiveness of the chemical treatment may be enhanced at higher flow velocities, as suspended cells are likely to be much more susceptible to the disinfecting agents.

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List of symbols

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 ($K_m\ d\ D^{-1}$)
V	$m\ s^{-1}$	flow velocity
M	$kg\ m^{-1}\ s^{-1}$	Viscosity
P	$kg\ m^{-3}$	Density

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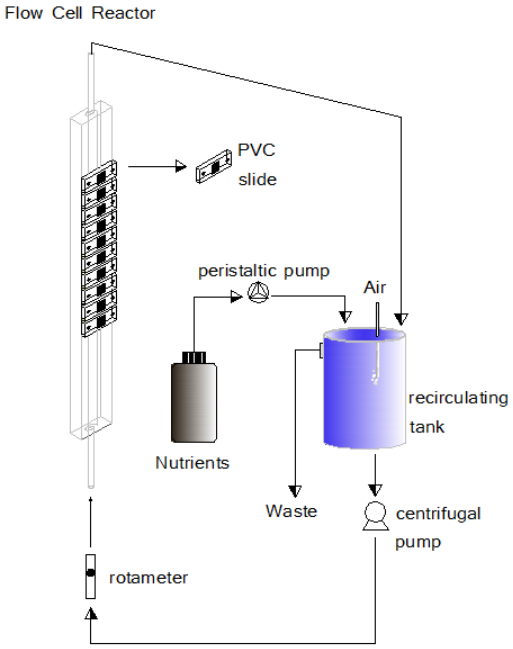

Fig. 1. Schematic representation of the biofilm producing system.

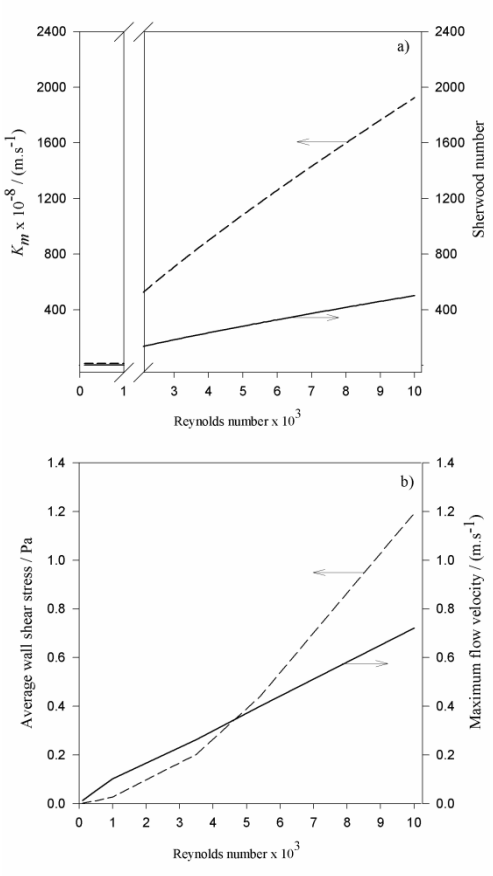

Fig. 2. a) Calculated values using correlations for the Sherwood number (solid line) and for the external mass transfer coefficient K_m (dashed line). Values for the transition zone ($1000 \leq Re \leq 2100$) were not represented due to the poor reliability of the results generated by empiric correlations in this zone. b) Average wall shear stress (dashed line) and maximum flow velocity (solid line) for *Re* ranging from 100 to 10000 predicted by CFD.

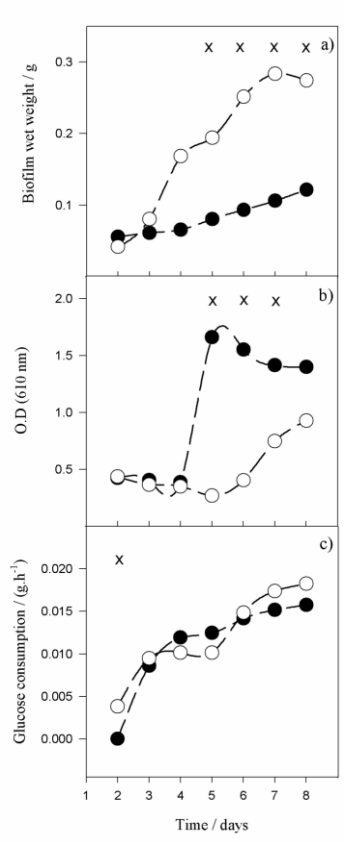

Fig. 3. Time-course evolution of: a) biofilm wet weight, b) optical density in the recirculating tank, c) glucose consumption in the system. Closed symbols – higher flow rate (*Re* = 6720), open symbols – lower flow rate (*Re* = 4350). Time points marked with x are those for which a statistical difference was found between both conditions (confidence level greater than 95%, $P < 0.05$)