Effects of shear stress on the secretion of extracellular polymeric substances in biofilms

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Abstract Movement of biofilm into the suspended state as a result of erosion and sloughing is inevitable. The causes for this have been widely attributed to the presence of high shear stress, excess nutrients or a combination of both parameters. It is believed that the extracellular polymeric substances (EPS) of biofilm contribute to the mechanical stability of the biofilm; however, the effect of changing shear stress on the secretion of biofilm EPS is not well understood. This research examined the EPS of a biofilm quantitatively during the change in shear stress. Biofilm was grown in a rotating drum biofilm reactor and subjected to three different shear stresses: 0.1022 N/m^2 at 100 rpm, 0.1533 N/m^2 at 150 rpm and 0.2044 N/m^2 at 200 rpm. Results indicate that a sudden increase of shear stress caused a drastic increase of the EPS-polysaccharides in the biofilm. However, 20 days after each shear increase, the EPS-polysaccharides secretion dropped back to the previous values before the shear change. Physical properties of the biofilm such as porosity were also monitored and the result showed that higher shear stress produced less porous but denser biofilm. Another notable effect when the shear stress was increased was that more erosion of biofilm occurred.

Keywords Biofilm; extracellular polymeric substances (EPS); EPS-polysaccharides; EPS-protein; rotating drum biofilm reactor; shear stress; sloughing

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Introduction

Biofilm accumulation is the net result of physical, chemical, and biological processes; it involves adsorption, attachment, desorption, and detachment (Characklis, 1990). During biofilm accumulation, detachment occurs when the external forces (shear stress) are greater than the internal strength of the biofilm matrix (Horn *et al.*, 2003; Peyton, 1996). Detachment is caused by a combination of processes, including abrasion, sloughing, erosion, and predator grazing; in that, sloughing refers to a rapid, massive loss of biofilm (Howell and Atkinson, 1976; Horn *et al.*, 2003). Sloughing may have major impacts on biofilm reactor performance and cause widely fluctuating output (Howell and Atkinson, 1976). When sloughing occurs, it may take several weeks to regenerate the microbial population and regain the treatment efficiency. Therefore, successful maintenance of a biofilm is the key to achieving the stability and performance of a fixed film system, and obtaining satisfactory treatment results.

It is understood that shear stress is an important parameter in a biofilm system. Shear stress can affect the morphological characteristics of biofilm, indicated by biofilm thickness, density, and surface shape. These characteristics are important indicators for the stability and performance of a biofilm reactor (Kwok *et al.*, 1998). Several researchers have reported that higher shear stress caused the biofilm to lose its surface roughness and become smoother and more compact; it also caused an increase in biofilm density but a decrease in biofilm thickness and porosity (Liu and Tay, 2001, 2002; Kwok *et al.*, 1998; van Loosdrecht *et al.*, 1995, 2002). Furthermore, biofilms were found to be more stable when grown in a turbulent flow condition (Pereira *et al.*, 1995, 2002). Ohashi and Harada

(1996) and Chen *et al.* (1998) explained that an increase in dry density caused the biofilm strength to increase, thus making it more resistant to sloughing. On the other hand, a decrease in shear stress by switching to laminar flow conditions produced thick and fluffy biofilm, which is more prone to sloughing (van Loosdrecht *et al.*, 2002). Besides the shear stress, other factors such as growth rate and nutrient concentrations could also cause sloughing. Picioreanu *et al.* (2001) found that detachment rates were higher amongst biofilms which had a higher growth rate. Nutrient-rich environments produce thicker biofilms and they are associated with more frequent sloughing events (Trulear and Characklis, 1982).

However, other conclusions about the effects of shear stress have also been reported, which are contradictory to the results mentioned above. Nicolella *et al.* (1997) found that the detachment rate increased when the shear stress was increased, and dropped by a small amount when the shear stress was decreased. Peyton and Characklis (1993) and Peyton (1996) found that biofilm thickness was only affected by the substrate loading – it increased to a maximum of 30 mm; shear stress, however, was found to have no significant effect on the thickness. Soini *et al.* (2002) reported that an increase in the shear stress caused a decrease in biofilm density and that biofilms preferred to grow in regions having laminar flow or less shear stress. These findings clearly contradict the results summarized earlier wherein an increase in the shear stress produced a smoother and stable biofilm which minimized sloughing. The contradictory results suggest that more study is needed to understand the dynamics of a biofilm system.

The extracellular polymeric substances (EPS) represent the construction material in the biofilm that allows the cells to maintain stable microconsortia and establish synergistic relationships (Wingender *et al.*, 1999). EPS also contribute to the mechanical stability of the biofilms (Mayer *et al.*, 1999), enabling them to withstand considerable shear forces. Characklis (1990) pointed out that a change in EPS volume, especially in the presence of hydrodynamic shear forces, may result in sloughing. Tay *et al.* (2001) studied aerobic granules in three column sequential aerobic sludge blanket reactors and found that high hydrodynamic shear forces seem to stimulate the production of cellular polysaccharides. They concluded that the formation and stability of aerobic granules are dependent on the hydrodynamic shear force-associated cellular polysaccharides production. However, is the stimulated production of EPS short-lived or long-lived? This question remains to be answered. An answer to this question will provide a better understanding of the effect of shear stress on the secretion of biofilm EPS and the role of EPS in a biofilm system. Therefore, the objective of this study was to determine the effect of shear stress on the secretion of biofilm EPS over long-term monitoring of a biofilm reactor. In this study, a rotating drum biofilm reactor, with polycarbonate material as substratum, was used to grow biofilm and the EPS was monitored over a period of 120 days.

Materials and methods

Rotating biological reactor. A jacketed rotating drum biofilm reactor (purchased from Biosurface Technologies, Bozeman, Montana, USA) was used to grow biofilm. It consists of a rotating inner drum enclosed within a stationary outer cylinder forming an annular space in between. The inner rotating drum consists of 20 removable polycarbonate sampling slides on which the biofilm grows. The reactor has a port at the bottom to facilitate recycling. Provision for ports on the upper portion of the reactor helps to remove the excess liquid from the reactor in the form of effluent.

The reactor was seeded with 50% activated sludge from an aeration basin obtained from the Lowell Wastewater Treatment Facility in Lowell (MA) and 50% nutrient media. The reactor was placed in a batch mode while constantly replacing 50% of the reactor

Table 1 Nutrient composition (Zhang *et al.*, 1999)

	Conc. in reactor (mg/l)
Organics	
Beef extract	41.76
Yeast extract	45.93
Peptones	41.76
Glucose	29.48
Urea	29.48
Inorganic	
NH_4Cl	1.67
$NaHCO_3$	156.44
K_2HPO_4	18.37
KH_2PO_4	7.11
$MgSO_4 \cdot 7H_2O$	18.37
$FeCl_2 \cdot 2H_2O$	0.25
$CaCl_2 \cdot 2H_20$	24.56
$Na_2HPO_4 \cdot 7H_2O$	27.56

volume daily with a mixture of activated sludge and nutrient media in a 1:1 ratio for a period of 7 days. After the seeding process, the reactor was run continuously and receiving a feed concentration of ~150 mg/L COD by mixing the concentrated nutrient media with deionized water in a ratio of 1:3.6. The resulting concentrations are shown in Table 1. The organics and deionized water used to make the feed were all autoclaved at 121 °C and 29 psia to obtain completely sterile conditions. Air was filtered through a gas purifier consisting of anhydrous $CaSO_4$ (Drierite) and molecular sieves and introduced into the reactor via the recycle line just before entering the reactor. A motor mounted on top of the reactor was used to control the rotational speed of the inner cylinder. The reactor temperature was maintained by circulating water stored in an incubator through a water jacket outside of the reactor (Figure 1).

Operating conditions. The concentrated feed and water were pumped through separate pump heads and their mixture was fed into the reactor at a flow rate of 9.3 ml/min resulting in a hydraulic retention time of 98 minutes. The contents of the reactor were recycled at a rate of 350 ml/min to ensure complete mixing (Figure 1). The influent to the reactor was maintained at an average pH of 7.8 ± 0.3 and the dissolved oxygen was maintained between 2.5–3.5 mg/L. The influent COD to the reactor was maintained at an average value of 153 ± 10 mg/L and the effluent at an average of 18.3 ± 7.0 mg/L.

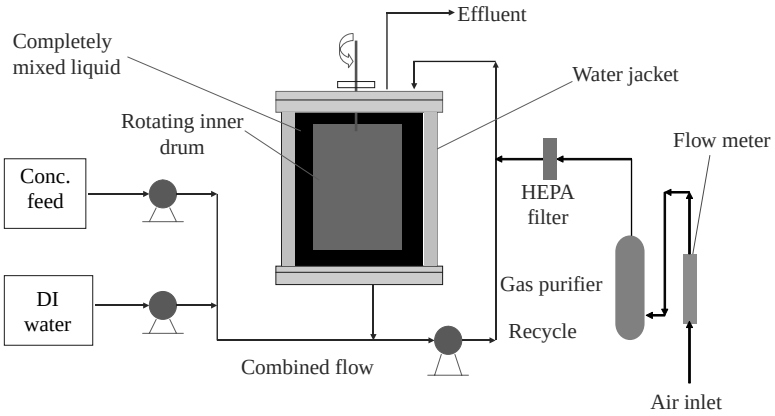

Figure 1 Biofilm reactor setup

The temperature of the reactor was maintained at 23.4 ± 0.9 °C throughout the experiments. The reactor was run for 20 days before the first sample was taken.

Change of shear stress. After the seeding, the biofilm was grown at 100 rpm for 48 days; after that, the rotational speed was increased to 150 rpm within seconds and the reactor was run for another 44 days; after that, the rotational speed was increased to 200 rpm within seconds and the reactor was run for another 28 days. The increase in rotational speed created a higher shear stress on the biofilm growing on the slides. During each different shear stress-induced running condition, a number of samples were obtained and the following analyses were conducted on the biofilm samples.

Measurement of biofilm physical properties. Total solids (TS) of the biofilm and total suspended solids (TSS) of the effluent were measured according to APHA (1998). Biofilm porosity was calculated according to Zhang and Bishop (2001).

EPS extraction and measurement. Biofilm samples were periodically obtained from the sampling slides and the EPS were extracted by a steaming method (Zhang *et al.*, 1999). The EPS yields in the extracts were represented by protein and polysaccharides since they are the predominant components (Lazarova and Manem, 1995). A modification of the Bradford (1976) method, called the Coomassie procedure (Micro range, Pierce Chemicals) was used to quantify protein, with bovine serum albumin (BSA) as the standard. Polysaccharides quantification was carried out by the phenol–sulfuric acid method (Gerhardt *et al.*, 1994) with dextrose as the standard.

Determination of shear stress. The shear stress exerted on the biofilm can be changed by varying the rotating speed of the inner cylinder. The shear stress was calculated according to the method described by Rasmussen and Ostgaard (2003). A linear relationship was obtained between the shear stress and the rotational speed. For the reactor used, the shear stress was calculated to be 0.1022 N/m^2 at 100 rpm, 0.1533 N/m^2 at 150 rpm, and 0.2044 N/m^2 at 200 rpm.

Results and discussion

Variation in the EPS with shear stress. All EPS data were normalized with respect to the TS of biofilm obtained. The data obtained at 100, 150, and 200 rpm in Figure 2

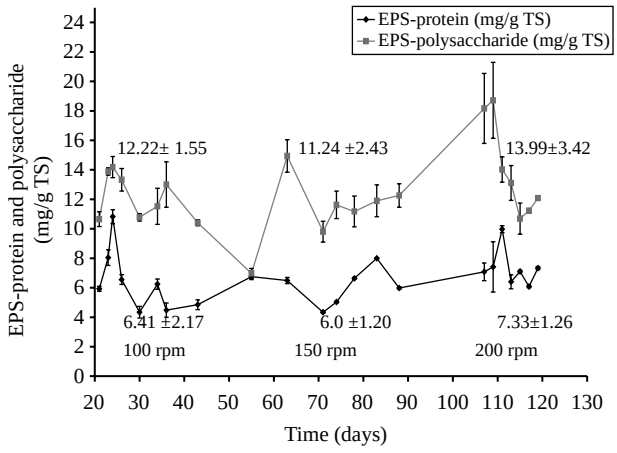

Figure 2 EPS secretion as a function of shear

indicate that there was no change of EPS-protein when the shear stress was increased from 100 rpm to 150 rpm, and then to 200 rpm (6.4 ± 2.2, 6.0 ± 1.2, and 7.5 ± 1.4 mg protein/g TS, respectively). The EPS-polysaccharides values at 100, 150, and 200 rpm were 12.22 ± 1.55, 11.24 ± 2.43, and 14.9 ± 3.4 mg/g TS, respectively, indicating no significant change occurred when the reactor was running at different shear stress. However, a drastic increase in the EPS-polysaccharides was observed within 20 days after each increase in the shear stress. And it seems that the EPS-polysaccharides were stimulated to a much higher value (18.17 ± 2.58 mg/g TS) when the shear stress was increased from 150 rpm to 200 rpm comparing to the value that was stimulated to (14.94 ± 1.10 mg/g TS) when the shear stress was increased from 100 rpm to 150 rpm. Sloughing occurred immediately after each increase in the rotational speed (results not shown). The drastic increase in the EPS-polysaccharides seems to be the direct response of the microbial communities to help biofilm re-establish after most of the biofilm was sloughed off. Once the biofilm was re-established and reached a quasi-steady state, the increase in shear stress had no effect on the secretion of biofilm EPS; the secretion of EPS dropped back to the original value seen at 100 rpm.

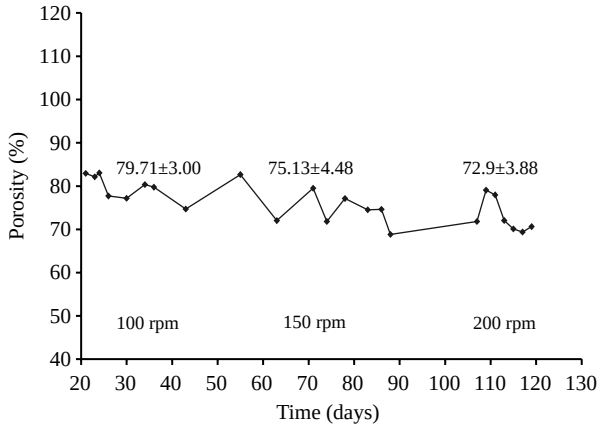

Figure 3 Porosity

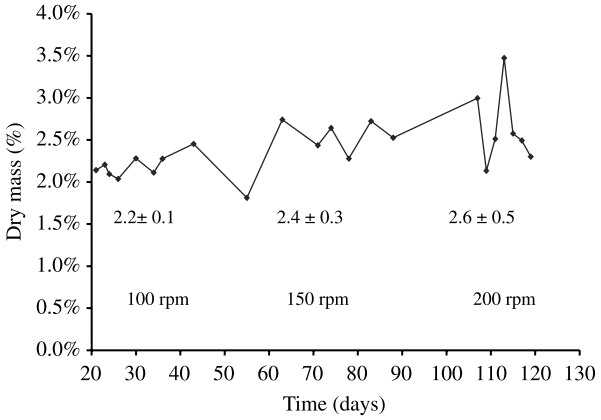

Figure 4 Dry mass as a function of shear

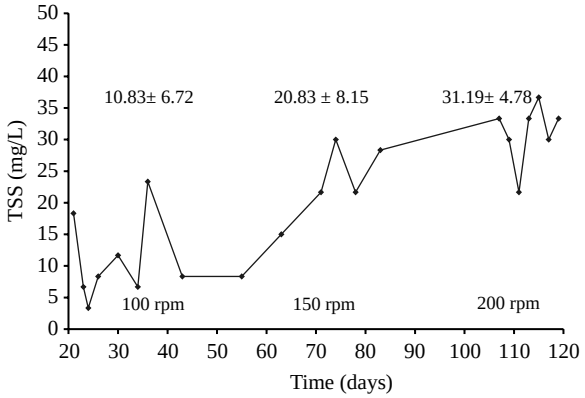

Figure 5 TSS in the effluent as a function of shear

Porosity. A distinctive change in the biofilm porosity was observed. The porosity decreased with the increase in shear stress (Figure 3). It decreased from an average of 79.71 ± 3.00% at 100 rpm to an average of 72.90 ± 3.88% at 200 rpm.

TS of the biofilm and TSS in the reactor effluent. TS of biofilm and TSS in the effluent were also plotted out to illustrate the effects of shear stress. As the shear stress increased, the dry solids content increased from an initial value of 2.2 ± 0.1% to a final value of 2.6 ± 0.5% (see Figure 4). This suggests a denser biofilm was formed as a result of higher shear stress. Higher shear stress also caused more erosion as indicated by the TSS in the reactor effluent (erosion process is where small pieces of biofilm are constantly broken off and carried into the suspended state). Figure 5 clearly shows that the TSS (in mg/L) in the effluent increased from 10.83 ± 3.23 mg/L to 20.83 ± 8.15 mg/L, then to 31.19 ± 4.80 mg/L as the shear increased from 100 rpm to 150 rpm, then to 200 rpm.

Conclusions

This research monitored the EPS secretion at three different shear stresses using a biofilm reactor. Sudden increase of the shear stress on a biofilm not only caused sloughing (results not shown) but also stimulated the secretion of EPS-polysaccharides, which is a probable mechanism for the microbial communities to re-establish after a sloughing event. However, once the biofilm was re-established and reached a quasi-steady state, the increase in the shear stress had no effect on the secretion of biofilm EPS any more. The shear force was found to have a marked effect on the physical characteristics of the biofilm. An increase in the shear stress produced less porous but denser biofilm, and caused more erosion.

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