# Coupled CFD-DEM modelling to predict how EPS affects bacterial biofilm deformation, recovery and detachment under flow conditions

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## Abstract

The deformation and detachment of bacterial biofilm are related to the structural and mechanical properties of the biofilm itself. Extracellular polymeric substances (EPS) play an important role on keeping the mechanical stability of biofilms. The understanding of biofilm mechanics and detachment can help to reveal biofilm survival mechanisms under fluid shear and provide insight about what flows might be needed to remove biofilm in a cleaning cycle or for a ship to remove biofilms. However, how the EPS may affect biofilm mechanics and its deformation in flow conditions remains elusive. To address this, a coupled computational fluid dynamic – discrete element method (CFD-DEM) model was developed. The mechanisms of biofilm detachment, such as erosion and sloughing have been revealed by imposing hydrodynamic fluid flow at different velocities and loading rates. The model, which also allows adjustment of the proportion of different functional group of microorganisms in the biofilm, enables the study of the contribution of EPS towards biofilm resistance to fluid shear stress. Furthermore, the stress-strain curves during biofilm deformation have been captured by loading and unloading fluid shear stress to study the viscoelastic properties of the biofilm.

**Keywords**: biofilm deformation, mechanical properties, discrete element method, computational fluid dynamics, shear modulus

# 1 Introduction

Bacterial biofilms are initiated by reversible attachment of planktonic bacteria to a surface. Bacteria are then irreversibly attached to the surface and develop cell-cell cohesion. Matured biofilms are embedded in extracellular polymeric substances (EPS) which are produced by bacteria themselves (Flemming & Wingender, 2010). The formation of biofilm helps bacteria to survive in harsh environments such as fluid flows (Banerjee et al., 2019). It was found that bacteria in biofilms are much more resistant to antibiotics than in planktonic state (Davies, 2003). Biofilms have dramatic impacts for a wide range of industries. For example, biofilms play an important role in bioremediation since they are able to convert toxic pollutants to harmless products (Singh, Paul, & Jain, 2006; Yadav & Sanyal, 2019). Biofilms are also essential in wastewater treatment (Capdeville & Rols, 1992; Sehar & Naz, 2016). However, the accumulation of biofilms in industrial pipelines and drinking water systems may lead to biocorrosion (Abe, Skali-Lami, Block, & Francius, 2012; Klapper, Rupp, Cargo, Purvedorj, & Stoodley, 2002). Additionally, biofilms adhered to marine surfaces is an important trigger of accelerated biofouling (Antunes, Leão, & Vasconcelos, 2019). The biofilms attached to the ship hull would increase the frictional drag which resulting in a high fuel consumption (de Carvalho, 2018). The emergence of biofilms allow pathogenic bacteria to survive in diverse environments (Tasneem et al., 2018). Besides, pathogen transmission is of concern to public health and can cause infection when the cells detach from the biofilm (Brindle, Miller, & Stewart, 2011). Therefore, a greater understanding of biofilm detachment in different hydrodynamic conditions may help to control the biofilm-related infection (Stoodley, Cargo, Rupp, Wilson, & Klapper, 2002).

In biofilms, the EPS is a self-produced matrix which majorly consists of polysaccharides, extracellular DNA (eDNA) and protein (Erskine, MacPhee, & Stanley-Wall, 2018; Gloag et al., 2013; Yadav & Sanyal, 2019). It provides many functions, such as adhesion to surfaces and

cohesion to maintain the mechanical stability of the biofilm system (Flemming, Neu, & Wozniak, 2007). The production of EPS is essential during biofilm development since bacteria cells could be immobilized by EPS (Flemming & Wingender, 2010). EPS production could be responsively regulated, for example, it was found that EPS production could be affected by EPS biosynthetic genes (Ali et al., 2000; Song, Xiong, Kong, Wang, & Ai, 2018). Besides, mutant strains could cause the overproduction of EPS to help the biofilm position in the beneficial environment (Hibbing, Fuqua, Parsek, & Peterson, 2010; Pahala G. Jayathilake et al., 2017). All these could affect the EPS amount in biofilms. Biofilms may also increase the strength of the matrix by increasing EPS production when subjected to mechanical stresses at intermediate time scales (e.g. 1 hour) (Shaw, Winston, Rupp, Klapper, & Stoodley, 2004). However, it is difficult to quantify EPS by microscopy or chemistry analysis due to the complexity of the chemistry, as well as extraction and purification techniques. Although EPS is complex, the computational modelling could be simplified to represent its physical function rather than the polymer components, hence, to gain better understanding of the contribution of EPS production to biofilm mechanical properties.

In this study, a three-dimensional individual-based model (IbM) of biofilm was developed by coupling the computational fluid dynamics approach (CFD) with the discrete element method (DEM). This model was implemented on NUFEB (https://github.com/nufeb) which is an open-source tool for individual-based modelling of microbial communities (Li et al., 2019). NUFEB integrates CFD-DEM solver SediFoam (https://github.com/xiaoh/sediFoam) which provides a flexible interface between LAMMPS (Large-scale Atomic/Molecular Massively Parallel Simulator) (Plimpton, 1993) and OpenFOAM (Open-source Field Operation and Manipulation) (Greenshields, 2017). The framework enables us to describe the fluid induced biofilm deformation and detachment subjected to different flow velocities. In this work, we model a bacterial mutant that can produce the same type of EPS at different levels. Different EPS

amount can be obtained by varying the relevant kinetic parameters in the model. We predicted the effect of EPS amount on the mechanical properties of biofilms and biofilm detachment.

## 2 Methodology

The processes of biofilm growth and biofilm deformation were decoupled in this study, that is, fluid flow was applied to a pre-grown biofilm. The pre-grown biofilm was "grown" under static condition for 5.3 days (no fluid) using the NUFEB individual based model which was described in (Pahala Gedara Jayathilake et al., 2017). The kinetic parameters for biofilm growth are provided in supporting information (Table S1). Only bacteria growth, division and EPS production were considered in this study. Then the two-way coupling between the solid biofilm and computational fluid dynamic was adopted to investigate the deformation and detachment of biofilm under different hydrodynamic conditions. The simulation domain is displayed in Figure 1, with the pre-grown biofilm positioned on the inlet side of the channel. The diameter of the involved particles is in the micrometry range (0.7 to 1.4 μm) based on the stochasticity of the biological system (Pahala Gedara Jayathilake et al., 2017). The fluid flow was applied along the top wall while the left and right wall have the cyclic boundary conditions (channel dimensions [L×W×H]: 200×30×50 $\mu m^3$), these boundary conditions were also applied to the front and back walls to reduce computational effort. A no-slip boundary condition was adopted on the bottom wall where the fluid velocity is zero. The details of cohesion between all the particles are discussed in Section 2.6.

### 2.1 Fluid-induced biofilm deformation and detachment

The EPS volume ratio, which is the ratio of the total volume of EPS to the volume of the whole biofilm, was varied based on bacteria species and growth conditions. The EPS growth yield coefficient was varied from 0.12 to 0.22 (g $COD_{EPS}$/g $COD_S$) which corresponds to EPS/biofilm ratio of 20 % to 51 % in this study. In order to investigate the biofilm deformation

and detachment events, the biofilm with 46 % EPS was subjected to inlet flow velocity between 0.1 m/s and 0.4m/s (Reynold number from 3.75 to 15) for a duration of 40 ms. In the next simulation, the inlet fluid velocity was kept at 0.3m/s to study the effect of EPS production on biofilm deformation and detachment. The detachment coefficient, which is defined as the ratio of the volume of detached biofilm clusters to the total volume of preformed biofilm, was calculated during the initial 14 ms (before biofilm washed away from the particle wall). Cluster detachment from the biofilm was defined as erosion if the particle number of the cluster was less than 1000 and sloughing if the particle number of the detached cluster exceeded 1000. The EPS amount, the mean and maximum heights, the roughness and porosity of different biofilms are summarized in Supporting Information (Table S2).

## 2.2 Biofilm deformation-recovery test

The responses of biofilm to a rapid fluctuating shear stress were analysed immediately prior to biofilm failure. To save the computational efforts, the fluid shear stress was applied to the biofilm for 3 ms (loading cycle) and then stopped immediately. Afterwards, the biofilm was allowed to relax for 17 ms (unloading cycle). During the loading period, the fluid shear stress was increased by increasing the fluid velocity from 0 m/s at a constant acceleration. For the biofilm with 46 % EPS, deformation-recovery tests were carried out by exposing the biofilm to the ramping flow with different accelerations: 20 $m/s^2$, 30 $m/s^2$ and 40 $m/s^2$, respectively. Then the biofilms with 40 % and 51 % EPS were subject to the increasing fluid velocity at the acceleration of 40 $m/s^2$ to investigate the effect of EPS amount on mechanical response of biofilm. The shear strain in this simple shear test was defined as the angle change between the front edge of biofilm with the left channel wall (Figure S1). The shear modulus was calculated as follows:

$$G = \frac{\sigma_{xz}}{\alpha} \tag{1}$$

where α is the shear strain, $\sigma_{xz}$ is the fluid induced shear stress on the biofilm which was computed globally by LAMMPS (Thompson, Plimpton, & Mattson, 2009). In this section, three planes (y = 5 μm, y = 15 μm and y = 25 μm) were selected to measure the deformation angle thus to obtain the averaged shear strain (Figure S2).

## 2.3 Motion of bacterial and EPS agents

During biofilm deformation and detachment, the bacterial and EPS particles motion are tracked by DEM on a Lagrangian framework:

$$m_i \frac{d\vec{v}_i}{dt} = \vec{f}_{c,i} + \vec{f}_{coh,i} + \vec{f}_{fp,i} \tag{2}$$

where $\vec{v}_i$ is the velocity of the particle *i*; $m_i$ is the particle mass; $\vec{f}_{c,i}$ is the contact force among collided particles (Xia, Jayathilake, Li, Zuliani, & Chen, 2021), $\vec{f}_{coh,i}$ is inter-particle cohesive force, $\vec{f}_{fp,i}$ is the fluid-particles interaction force.

## 2.4 Locally-Averaged Navier-Stokes equations for fluids

The fluid flow is solved by locally-averaged incompressible Navier-Stokes equation in which the fluid density $\rho_f$ is constant:

$$\nabla \cdot \left(\epsilon_s \vec{U}_s + \epsilon_f \vec{U}_f\right) = 0, \tag{3}$$

$$\frac{\partial(\epsilon_f \vec{U}_f)}{\partial t} + \nabla \cdot \left(\epsilon_f \vec{U}_f \vec{U}_f\right) = \frac{1}{\rho_f}(-\nabla p + \epsilon_f \nabla \cdot \vec{R} + \vec{F}_{fp}) \tag{4}$$

$\epsilon_s$ is solid volume fraction while $\epsilon_f$ is fluid volume fraction which equals to (1-$\epsilon_s$). $\vec{U}_s$ and $\vec{U}_f$ are particle velocity and fluid velocity, respectively. $\vec{F}_{fp}$ is the fluid-particle interaction force. $\nabla p$ is the pressure gradient, $\vec{R}$ is the stress tensor consisting of viscous stress and Reynolds stress, only viscous stress was computed since the Reynolds number is small here (3.75 to 15). The Eulerian field $\epsilon_s$, $\vec{U}_s$ and $\vec{F}_{fp}$ are calculated by averaging the Lagrangian information of particles (Sun, Xiao, & Sun, 2018).

## 2.5 Fluid-particle interaction

In this model, the fluid-particle interaction force $\vec{f}_{fp,i}$ consists of a drag force and lift force. For the particle $i$, the drag force model is expressed as (Sun et al., 2018):

$$\vec{f}_{fp,i}^{drag} = \frac{V_{p,i}}{\epsilon_{f,i}\epsilon_{s,i}} \beta_i\left(\vec{U}_{f,i} - \vec{u}_{p,i}\right) \tag{5}$$

where $V_{p,i}$ is the volume of the particle $i$, $\vec{U}_{f,i}$ and $\vec{u}_{p,i}$ are the fluid velocity and particle velocity, respectively. $\epsilon_{f,i}$ is the fluid volume fraction while $\epsilon_{s,i}$ is the solid volume fraction, $\beta_i$ is the drag correlation coefficient which is used to convert terminal velocity correlation to drag correlation (Syamlal, Rogers, O'Brien, & Documentation, 1993).

In addition, the lift force on the particle $i$ is calculated by the following formula (Sun et al., 2018; Van Rijn, 1984; Zhu, Zhou, Yang, & Yu, 2007):

$$\vec{f}_{fp,i}^{lift} = C_l(\rho_f\mu)^{0.5} d_{p,i}^2\left(\vec{U}_{f,i} - \vec{u}_{p,i}\right) \times \frac{\omega_i}{|\omega_i|^{0.5}} \tag{6}$$

where $C_l$ is the lift coefficient equals to 1.6, $\omega_i = \nabla \times \vec{U}_{f,i}$ is the curl of the flow velocity interpolated to the center of particle $i$.

## 2.6 Cohesive force among different particles

The cohesive force among the particles was computed by using the equation below:

$$\vec{F}_{coh,i} = -\frac{A}{6} \frac{64 r_i^3 r_j^3 (s + r_i + r_j)}{(s^2 + 2r_i s + 2r_j s)^2 (s^2 + 2r_i s + 2r_j s + 4r_i r_j)^2} \vec{n}_{ij} \tag{7}$$

where $A$ is the cohesive strength, and $s$ is the separation distance between the particle surface. A minimum separation distance $s_{min}$ is implemented when the separation distance between the two particles equals zero ($s = 0$). In this work, five different values of cohesive strength were used for the interactions of bacterial cells with bacterial cells, bacteria cells with EPS particles, bacteria cells with particle-wall, EPS particles with the particle-wall, EPS particles with EPS particles. Since EPS plays a significant role on binding the bacterial cells, the cohesive strength driven by EPS was assumed to be three orders of magnitude larger than that for bacteria (Fang, Chan, & Xu, 2000). The mechanical parameters of the simulations are listed in Table 1. The

fluid density is $10^3$ kg $m^{-3}$ and the fluid dynamic viscosity is $10^{-3}$ kg $m^{-1}$ $s^{-1}$. The contact model for the colloid particles has been introduced in (Xia et al., 2021).

# 3 Results and discussion

## 3.1 Flow effect on biofilm deformation and detachment

In this study, the time was relatively short during the flow tests, therefore, bacterial growth and EPS production were negligible. Deformation and detachment of the biofilm with 46 % EPS at four inlet flow velocities are shown in Figure 2 and 3, which are representations of the biofilm at 14 ms and 40 ms time points, respectively. At the lowest inlet flow velocity of 0.1 m/s, the biofilm elongated along with the flow direction and detachment occurred at the rear part of the biofilm (Figure 2A). Because of the gradient fluid shear force along the z direction and the patchy structure of the biofilm, the top of the biofilm deformed much more than its bottom. Biofilm deformation was dominant during exposure to this lowest level fluid shear force and only erosion occurred. No further erosion or detachment were observed between 14 ms and 40 ms (Figure 3A).

When the inlet flow velocity was increased to 0.2 m/s, detachment at the rear part of the biofilm occurred as early as 3 ms (Figure S3). Compared to the lowest inlet fluid velocity (i.e. 0.1 m/s), detachment frequency increased sharply, both biofilm erosion and sloughing took place (Figure 2B). The continuous detachment events led to a decrease in the volume of the remaining biofilm. Comparing Figure 2A and 2B, it is clear that higher flow velocity led to more biofilm detachment during the same time period as expected. However, there was still some biofilms adhere on the surface at 40 ms when the inlet flow velocity is 0.2 m/s (Figure 3B).

High-frequent biofilm detachment events could also be captured by further increasing the inlet flow velocity to 0.3 m/s. At this higher flow, biofilm sloughing was the predominant behaviour during the detachment process. A small fraction of biofilm remained adhered to the surface at 14 ms (Figure 2C). The remainder biofilm, which in continuous exposure to the fluid shear

force, experienced more biofilm cluster detachment and only a layer of biofilm was left at 25 ms (Figure S4A). Interestingly, this remaining biofilm layer started rolling along the rough wall under the steady fluid shear force after then (Figure S4B, C and D). Finally, biofilm moved out of the original location (the initial surface occupied by the pre-grown biofilm) by this rolling motion at around 40 ms (Figure 3C). This phenomenon was firstly observed by Rupp et al. in their experiments, in which *S.aureus* microcolonies moved downstream by rolling in a flow cell (Rupp, Fux, & Stoodley, 2005).

At the highest inlet fluid velocity, 0.4 m/s, biofilm clusters with different sizes detached rapidly due to the high fluid shear force. Figure 2D displays the morphology of the biofilm after being subjected to the shear force for 14 ms, only a thin layer of biofilm remained adhered to the surface. Furthermore, the biofilm rolling motion was also captured at this flow speed. The biofilm rolled along the surface for several microseconds then lifted from the surface by the fluid (Figure S5A-D). Eventually, the biofilm was washed away along the direction of the fluid flow (Figure 3D).

## 3.2 EPS effect on biofilm deformation and detachment

To study how the EPS amount affected the deformation and detachment of biofilms, we examined a single fluid flow condition, 0.3 m/s sustained for a duration of 40 ms. For biofilms with small amount of EPS (20 % EPS), the biofilm clusters could easily detach (erosion dominated) from the parent biofilm at a high frequency. In addition, it can be seen that lots of bacteria departed from the biofilm (Figure S6). This may be due to limited EPS availability to immobilize the cells in biofilm (Flemming & Wingender, 2010).

When the EPS amount increased, the detachment frequency of biofilm decreased. Figure 4 shows the biofilms after being subjected to the fluid flow for 14 ms. It can be seen that the volume of detached biofilm decreased as the EPS amount increased. For the biofilm with low EPS amount (20 %, 32 % and 40 % EPS), most particles were detached but a thin layer biofilm

remained adhered to the particles wall at the end of 40 ms (Figure 5). However, when the EPS amount increased to 46 %, the biofilm was removed by a rolling motion. The results suggest that the rolling motion may depend on the amount of EPS. When the EPS amount further increased to 46 %, about half of the initial biofilm remained on the surface at 40 ms. It is evident that the same flow caused less detachments for biofilm with higher EPS amount, which further suggest that the biofilm with greater emergence of EPS appeared to be stiffer resist well against fluid flows.

The local detachment, such as erosion and sloughing, could be significantly captured within the simulation time of 14 ms. However, biofilm removal occurred after this period when the inlet fluid velocity was greater than 0.2m/s. Therefore, the detachment rate coefficient, which is defined as the ratio of the volume of detached biofilm cluster to the total volume of biofilm per millisecond, was calculated during the initial detachment period (14 ms) and adopted to describe the biofilms detachment behaviour under the range of hydrodynamic conditions. Each simulation was run for 3 replicates and the average results were calculated. As displayed in Figure 6A, the detachment rate coefficient increased with the inlet fluid velocity which agrees with previously reported experimental observations (Stoodley et al., 2002). There was no significant detachment until inlet flow velocity increased to 0.2 m/s, the coefficient increased sharply before the inlet flow velocity reached 0.3 m/s and then slowed down when the inlet fluid velocity was further increased. In keeping with the visual results (Figure 5), a negative correlation was found between the EPS amount and detachment rate coefficient when the inlet fluid velocity was kept constant (Figure 6.B). The detachment rate coefficient for the biofilm with 20 % EPS was approximately twice that for the biofilm with 51 % EPS. The results suggest that the resistance of the biofilm to the external fluid is largely attributable to the EPS amount. EPS is responsible for the mechanical stability of the biofilm due to its cohesive

properties, therefore, the biofilms with a greater density of EPS components are predicted to be more stable when exposed to the fluid flow.

### 3.3 Biofilm viscoelastic response during deformation-recovery test

Deformation of the biofilm with 46 % EPS was monitored for 3 ms as the fluid velocity was incrementally increased from 0 m/s at a constant acceleration. Then the fluid flow stopped, the biofilm was allowed to relax for 17 ms. The stress-strain curve was obtained from the loading and unloading cycle. Figure 7 shows the deformation and recovery properties of the biofilm (46 % EPS). In this case, the fluid velocity was accelerated at 20 $m/s^2$ and reached the maximum value (0.06 m/s) at 3 ms. The maximal deformation angle was captured at the same time (Figure 7B), approximately 25.3 degree (0.44 rads). After the fluid flow was stopped, the biofilm started to recovery. As seen in figure 7C, the biofilm had not returned to the original shape after the full relaxation time, about 6 times of the duration of flow induced biofilm deformation. The results were not surprising for our simulation, as the interactions between the biofilm particles were modelled as spring-dashpot based viscoelastic models. It matches observations of real world biofilms, as this kind of residual strain was also be observed in (Klapper et al., 2002), and such a residual deformation is due to the viscous nature of biofilm (Jafari et al., 2018).

To understand the viscoelastic deformation of biofilms at different loading rates and stresses, additional simulations were performed by accelerating the fluid at 30 $m/s^2$ and 40 $m/s^2$ for 3ms, with the corresponding peak fluid velocities of 0.09 m/s and 0.12 m/s, respectively. Figure 8A shows the fluid-induced shear stress on biofilms overtime. It is evident that the higher flow acceleration resulted in higher shear stress imposed on biofilms (Figure 8A). This can lead to larger deformation (or shear strain) and deformation rate of biofilms (or strain rate), as seen in Figure 8B.

After the flow was removed at 3 ms, the fluid induced stress in the biofilm decreased rapidly (3-4 ms, Figure 8A), and some of the deformation (8 % - 10 %) was immediately recovered attributable to a time-independent elastic response. Afterwards (4-20 ms, Figure 8A), the stress decay slowed down dramatically and almost reached a plateau at the end of the recovery. A residual deformation (or strain) during biofilm relaxation was captured in each deformation – recovery test and increased with the maximum fluid velocity. Such a strain rate dependent recovery was due to the nature of viscoelastic models adopted within the biofilms and is a common characteristic for viscoelastic materials (Capurro & Barberis, 2014; Chen, Bader, Lee, & Knight, 2011).

Figure 8C shows the corresponding stress-strain curve of biofilms during biofilm deformation (0 – 3 ms) and recovery process (3 – 20 ms). At the lowest shear stress and shear rate, the stress could recover to zero within the allotted 17ms of relaxation time while a full recovery could not be achieved for higher shear stresses with higher strain rates. The hysteresis loop in the curve represents the dissipation of energy during the biofilm deformation process. The area of hysteresis loop increased with the maximum fluid velocity, which suggested more energy dissipation. The calculated apparent shear moduli of biofilms (Equation (1)) determined at small deformation (strain < 0.1), were 10.75 ± 1.28 Pa, 12.41 ± 1.24 Pa and 15.21 ± 1.94 Pa at given fluid velocities (0.06, 0.09 and 0.12 m/s), respectively. The results indicate that the lower loading rate leads to smaller shear modulus, which is due to the viscous effect of biofilms.

To study the EPS effect on biofilm mechanics, we have focused on biofilms containing higher EPS amounts (40 %, 46 % and 51 %) subjected to the ramping fluid velocity at a constant acceleration of 40 $m/s^2$. Biofilms with lowers EPS amount were not considered here since they could easily detach, thus the stress-strain curve would not be captured during deformation.

Figure 9A and 9B show the fluid induced stress and strain changes overtime. For biofilms with 40 % and 46 % EPS, the fluid induced stresses on the biofilm were similar, but the peak shear stress on the 51 % EPS biofilm (Figure 9A) was higher. The different stress profiles could be attributed to the different height profiles of the biofilms. Since the fluid flow was applied along the top wall in the simulation domain, the velocity varied with the height of simulation box (figure S7). Therefore, it is important to note that although the inlet fluid condition was set as the same, the biofilms would be subjected to different fluid shear force if their height varied due to growth.

The deformation of the biofilm with 40 % EPS was greater compared to biofilm with 46 % EPS, and both experienced similar shear stresses. This suggests that higher EPS resulted in the better resistance to the external fluid shear force. However, the shear strain of the 51 % EPS biofilm was lowest although the maximum stress was almost 16 % higher than for its counterpart biofilms. Taken together, the results suggest that biofilms with higher EPS might be stiffer, which agrees with what was found in (Gloag, Fabbri, Wozniak, & Stoodley, 2020). At very small strain (<0.1 in Figure 9C), the stress-strain curve was linear and the apparent shear moduli could be determined, as 12.82 ± 2.03 Pa, 15.21 ± 1.94 Pa and 17.18 ± 3.3 Pa for biofilms with 40 % EPS, 46 % EPS and 51 % EPS, respectively. In general, all these biofilms exhibited some strain stiffening effect followed by strain softening at larger strains, which is possibly due to the viscoelastic properties of biofilms and the change in the microstructure of biofilms during the deformation (Figure 9C). This is also consistent with some experimental measurements of biofilms at a wide range of strains (Jana et al., 2020). ANOVA test was used for statistical analysis ($\alpha = 0.05$). The result ($p < 0.05$) suggests that the change of shear modulus with EPS amount has a statistically significant difference.

After the fluid was stopped, the stress on the biofilms decayed exponentially and the deformation recovered slowly which is a common feature for viscoelastic materials (Chen &

Lu, 2012). The overall biofilm deformation recovery was 16 %, 20 % and 22% for biofilms with 40 %, 46 % and 51 % EPS within the simulation period, respectively. The result suggests that the abundant presence of EPS in the deformed biofilms make a significant contribution to their recovery, as the bacterial cell is much more loosely associated with bacterial cell than EPS particle. Similar results were also verified in the experimental work, which released that the EPS is required to induce bacterial rearrangement during stress relaxation (Peterson, Busscher, Sharma, & Van Der Mei, 2014).

## 4 Conclusions

A CFD-DEM coupled model developed here has enabled us to predict biofilm deformation and detachment under varied hydrodynamic conditions. When the biofilm was exposed to a steady fluid shear force (inlet fluid velocity was kept as constant), the detachment rate increased with inlet fluid velocity (i.e. shear stress). When the inlet flow velocity was below 0.1 m/s, the biofilm deformed along the fluid direction with sparse erosion. Biofilm sloughing occurred when the inlet flow velocity increased to 0.2 m/s. When the inlet fluid velocity reached 0.3 and 0.4 m/s, the detachment events were dominated by sloughing and the detached biofilm removed in a rolling motion.

At a given inlet fluid velocity of 0.3 m/s, the detachment rate coefficient decreased with EPS amount. For the biofilm with low proportional EPS (less than 32 %), the biofilm easily detached from the surface and dispersion of individual cells was observed. In these cases, the limited amount of EPS was incapable of protecting the biofilm bacteria from shear stress. Biofilm detachment frequency decreased with the increase of EPS amount. The biofilms appeared to be stiffer at higher loading rate, which is a typical characteristic for viscoelastic materials. Such viscoelastic features of biofilms also led to the hysteresis loop (energy dissipation) in the stress strain curves of the biofilms. Furthermore, we also found that higher

EPS led to higher apparent shear modulus for the biofilm modelled here. The nonlinear stress-strain characteristics of the simulations were consistent with experimental findings.

**Conflict of Interest:**

There are no conflicts to declare.

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**Author Contributions**

Y.X, P.G.J., P.S. and JC designed the research. Y.X performed the simulation work, acquired the data, and did data analysis. JC, PGJ, and BL contributed to data analysis. YX, PGJ and JC prepared the original draft. JC and PGJ provided the overall guidance of the work. All the authors contributed to the writing of the manuscript, revised it, and approved the final version.

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## Table

Table 1 The mechanical and physical parameters of the biofilm used in our simulations.

| Numerical simulation parameters | | |
|---|---|---|
| Density of particles | $10^3$ kg $m^{-3}$ | (Xia et al., 2021) |
| Normal and tangential elastic constants | $10^3$ kg $m^{-1}$ $s^{-2}$ | (Böl, Ehret, Bolea Albero, Hellriegel, & Krull, 2013) |
| Normal damping constants | $10^{13}$ $m^{-1}$ $s^{-1}$ | Chosen |
| Tangential damping constants | 10 $m^{-1}$ $s^{-1}$ | Chosen |
| Parameters for cohesive model | | |
| Particle interaction | Cohesive strength | |
| bacteria-EPS | $1.6 \times 10^{-18}$ J | Chosen |
| bacteria - particle wall | $2.3 \times 10^{-21}$ J | (Lower, 2005) |
| EPS - particle wall | $2.3 \times 10^{-18}$ J | Chosen |
| EPS - EPS | $5 \times 10^{-18}$ J | (Fang et al., 2000) |
| bacteria - HET bacteria | $1.6 \times 10^{-21}$ J | (Bos, Van der Mei, & Busscher, 1999) |

## Figure Legends:

Figure 1. Representation of pre-grown biofilm (with 51% EPS) in the channel. Bacterial cells are represented by blue particles while the grey particles are EPS agents, and red particles are a layer of the growth surface wall.

Figure 2. Biofilm deformation and detachment at inlet flow velocity in the range of 0.1 to 0.4m/s. t = 14 ms.

Figure 3. Biofilm deformation and detachment at inlet flow velocity in the range of 0.1 to 0.4m/s. t = 40 ms.

Figure 4. Biofilm deformation and detachment at time of 14 ms with the inlet fluid velocity of 0.3 m/s. The amount of EPS within the biofilm increased from (A) 20 %, (B) 32 %, (C) 40 % to (D) 51 %.

Figure 5. Biofilm deformation and detachment at time of 40 ms with the inlet fluid velocity of 0.3 m/s. The amount of EPS within the biofilm increased from (A) 20 %, (B) 32 %, (C) 40 % to (D) 51 %.

Figure 6. (A) The effect of fluid velocity on biofilm detachment rate coefficient for a typical biofilm with 46 % EPS. (B) The effect of EPS amount on biofilm detachment rate for a given inlet flow velocity of 0.3 m/s. The error bars show the standard deviation calculated from three replicated.

Figure 7. (A) The original shape of a biofilm with 46 % EPS, (B) maximum biofilm deformation in flow and (c) biofilm recovery 17ms after flow stopped.

Figure 8. The fluid induced (A) stress and (B) strain on biofilms changed with time and the corresponding averaged (C) stress-strain curve (standard deviation did not give here for the high resolution). Where the flow was applied on the biofilm with 46 % EPS for 3 ms, accelerated at 20 $m/s^2$, 30 $m/s^2$ and 40 $m/s^2$, to reach the peak velocities of 0.06 m/s, 0.09 m/s and 0.12 m/s.

Figure 9. The fluid induced (A) stress and (B) strain on biofilms changed with time and the corresponding averaged (C) stress-strain curve when the flow was applied and terminated (standard deviation did not give here for the high resolution). The biofilms with 40 %, 46 % and 51 % EPS were selected. The fluid velocity was applied at an acceleration of 40 $m/s^2$.