

# A COUPLED AND MULTI-SCALE FLUID-STRUCTURE INTERACTION AND MASS TRANSFER MODEL FOR BIOFILM GROWTH SIMULATIONS

MIRELLA CORONEO, LENA YOSHIHARA AND WOLFGANG A. WALL

Institute for Computational Mechanics (LNM)  
Technische Universität München  
Boltzmannstr. 15, 85747 Garching b. München, Germany  
e-mail: {coroneo, yoshihara, wall}@lnm.mw.tum.de, web page: <http://www.lnm.mw.tum.de>

**Key words:** biofilm growth, mass transport, fluid-structure interaction, multi-scale model, finite element method

**Abstract.** The formation and development of different biofilm structures is known to be influenced by nutrients availability and flow conditions. For this reason an approach which takes in account the effect of local structure deformation and fluid flow on mass transfer is essential for the understanding of biofilm macro-scale dynamic. The objective of the present work is therefore to study the fluid-structure interaction (FSI) and the substrate transport and reaction of big growing biofilm aggregates, for which continuum models can be applied. For this purpose we propose a novel growth model for the simulation of biofilm structures development. It is based on a finite element approach, developed in our in-house research code, for the numerical simulation of a sequential one-way coupling of the FSI and the scalar transport models [1]. The biofilm growth is coupled to the other processes through a multi-scale approach and takes in account the effects of mass transfer and shear stresses. First numerical examples are run at the purpose to demonstrate the suitability of the growth model to catch the main features of growing biofilm structures. This type of approach can give an important contribution to the understanding of biofilm architectures living in a complex environment. It allows to study the development of complex and real-life biofilm structure shapes often seen in nature and industry, to understand the influence of operating conditions, and therefore can enable the control of biofilm behaviour.

## 1 INTRODUCTION

Biofilm architecture, mechanics and interaction with surrounding fluid have a profound influence on their behaviour and potential treatments [2]. For the purpose of being able to control their formation and development, it results then extremely important to understand the mutual interaction of all the involved mechanisms. There are, however,

still a lot of points which need to be properly investigated, mainly because of the many difficulties in carrying out experiments which could isolate each single mechanism.

In this context, biofilm modelling is arising as a mean of producing quantitative tools [3] and a way to study biofilm behaviour. From the mathematical point of view, biofilm models can be divided in three main categories: cellular automata, individual-based models and continuum models [4]. Among them the last one is suitable to study bigger biofilm aggregates. The assumptions of continuum models that the biomass concentration can be adequately described by one or more density fields and that each density field obeys to some conservation law [5] can be considered satisfied for big aggregates. Indeed, while constituted of micro-scale objects, biofilm structures interact with the surrounding fluid as macro-scale materials and can be studied as flexible structures located in a moving liquid flow. Modelling approaches available at present however result to be limited to fixed biofilm geometries, neglecting biofilm deformation and growth. Only few works account for shear-induced deformation of the biofilm structure [1, 6, 7, 8, 9], but none of them takes in account the effect of local structure deformation and fluid flow on both mass transfer and growth.

For the purpose of understanding the influence of operating conditions on biofilm growth and erosion and of being able to control their behaviour, we propose a novel multi-scale computational approach, developed in our in-house research code BACI, taking in account both the fluid-structure interaction and the transport processes [1], and the effect they have on biofilm growth. Biofilm interaction with the surrounding fluid is modelled through an Arbitrary Lagrangian-Eulerian (ALE) approach, while the mass transfer is calculated through the solution of the dynamic convection-diffusion-reaction equation, assuming a non-linear Monod kinetic. The growth model is coupled to the fluid-structure interaction and substrate transport taking in account the different phenomena time-scales and calculates the growth based on the local characteristics of the biofilm structures. In this way it is possible to properly predict the effect of local biofilm deformation and of fluid flow on the transport and reaction of nutrients, and the influence that transport processes and shear stresses have on biofilm growth.

The governing equations of the fluid-structure interaction (FSI) and mass transport subproblems are summarized in sections 2 and 3, respectively. Afterwards, the growth model is presented in section 4, while in section 5 a description of the multi-scale algorithm is reported. Some selected numerical examples are then presented in section 6 before conclusions and outlook, provided in section 7.

## 2 FLUID-STRUCTURE INTERACTION

Biofilm interaction with the surrounding fluid is modelled through an arbitrary Lagrangian-Eulerian (ALE) approach. As a consequence, the FSI problem domain consists of three fields: the fluid  $\Omega^F$  and the structure  $\Omega^S$  domains, sharing a common interface  $\Gamma$ , and a third, non-physical mesh field produced by the ALE approach  $\Omega^{G,F}$ , on which a different equation has to be solved.

Biofilms are well-known to be viscoelastic materials, i.e. they behave elastically, when subjected to external forces over short periods of time, and in a viscoelastic way, when external forces are applied at longer time periods. Since the fluid-structure interaction is considered only at very small time scales, the structural motion of the biofilm is safely described by an elastic material model. In the biofilm domain the following non-linear structural elastodynamic equation is solved at the purpose to obtain the displacement field  $\mathbf{d}^S$

$$\rho^S \frac{d^2 \mathbf{d}^S}{dt^2} = \nabla \cdot (\mathbf{F} \cdot \mathbf{S}) + \rho^S \mathbf{b}^S \quad \text{in } \Omega^S \times (0, T), \quad (1)$$

where  $\rho^S$  denotes the structural density,  $\Omega^S$  is the undeformed domain while  $\mathbf{b}^S$  represent the external body forces. The internal forces are expressed in terms of the second Piola-Kirchhoff stress tensor  $\mathbf{S}$  and the deformation gradient  $\mathbf{F}$ . At boundaries Dirichlet or Neumann conditions are imposed and the initial boundary value problem is completed by imposing appropriate initial conditions. In case of displacement-based finite element (FE) formulation, equation (1) is multiplied by the virtual displacements and then integrated by parts. The resulting weak form is the starting point for the spatial discretization.

In the deforming fluid domain, the fluid flow is considered to be laminar, since biofilm structures usually grow attached to surfaces and are considered to be in the hydrodynamic boundary layer. As a result, in the fluid domain the following incompressible time-dependent ALE version of Navier-Stokes equations are solved for both pressure  $p^F$  and velocity  $\mathbf{u}^F$  fields

$$\rho^F \frac{\partial \mathbf{u}^F}{\partial t} + \rho^F (\mathbf{c}^F \cdot \nabla) \mathbf{u}^F - 2\mu \nabla \cdot \boldsymbol{\varepsilon}(\mathbf{u}^F) + \nabla p^F = \rho^F \mathbf{b}^F \quad \text{in } \Omega^F \times (0, T), \quad (2)$$

$$\nabla \cdot \mathbf{u}^F = 0 \quad \text{in } \Omega^F \times (0, T). \quad (3)$$

In the momentum equation (2),  $\boldsymbol{\varepsilon}(\mathbf{u}^F)$  denotes the strain rate tensor of the fluid,  $\mu$  is its dynamic viscosity,  $\mathbf{b}^F$  a prescribed body force, and  $\mathbf{c}^F$  the fluid ALE convective velocity, representing the fluid velocity  $\mathbf{u}^F$  relative to the arbitrarily moving fluid domain

$$\mathbf{c}^F = \mathbf{u}^F - \mathbf{u}^{G,F}. \quad (4)$$

The fluid grid velocity  $\mathbf{u}^{G,F}$  is defined by

$$\mathbf{u}^{G,F} = \frac{\partial \boldsymbol{\varphi}}{\partial t} \quad \text{in } \Omega^F \times (0, T), \quad (5)$$

where  $\boldsymbol{\varphi}$  represents a unique, arbitrary mapping for the deformation of the fluid domain  $\mathbf{d}^{G,F}$

$$\mathbf{d}^{G,F}(\mathbf{x}, t) = \boldsymbol{\varphi} \left( \mathbf{d}_r^{G,F}, \mathbf{x}, t \right) \quad \text{for } (\mathbf{x}, t) \in \Omega^F \times (0, T), \quad (6)$$

while  $\mathbf{d}_\Gamma^{\text{G,F}}$  represents the mesh interface displacement, later related to the structure interface displacement  $\mathbf{d}_\Gamma^{\text{S}}$ . Also for the fluid domain Dirichlet and Neumann boundary conditions are imposed together with a divergence-free initial velocity field. For the fluid field the weak form is obtained by multiplying equations (2) and (3) with test functions for the velocity and pressure and then integrating by parts.

In order to calculate the fluid ALE convective velocity  $\mathbf{c}^{\text{F}}$  appearing in equation (2), it is necessary to define the mapping  $\varphi$  introduced in equation (6). For this purpose, the boundary of the fluid ALE mesh is coupled to the Lagrangian mesh of the structures and to an Eulerian mesh at the in- and outflow portions. Within the domain, it is allowed to deform arbitrarily and, in the present study, it was chosen to treat the fluid ALE field as a quasi-elastostatic pseudo-structure [10]. The ALE equation of motion results then to be

$$\nabla \cdot \boldsymbol{\sigma}^{\text{G,F}} = \mathbf{0} \quad \text{in} \quad \Omega^{\text{G,F}} \times (0, T) \quad (7)$$

with  $\boldsymbol{\sigma}^{\text{G,F}}$  defined as in [1]. Due to the continuous position change of the fluid-structure interaction surface,  $\Gamma$ , and to the ALE formulation, the mesh will be continuously deformed and this deformation is controlled by kinematic and dynamic constraints imposed at the interface  $\Gamma$ . First of all, an equilibrium of forces has to be fulfilled, resulting in equal surface tractions at the fluid and structure surface; second, the fluid grid velocity  $\mathbf{u}_\Gamma^{\text{G,F}}$  and the fluid velocity  $\mathbf{u}_\Gamma^{\text{F}}$  have to match at the interface. In addition, the fluid velocity,  $\mathbf{u}_\Gamma^{\text{F}}$ , is imposed to be equal to the structure deformation rate, since a mass flow across  $\Gamma$  as well as a relative tangential movement of fluid and structure at  $\Gamma$  are prohibited. For insights in the formulation of the weak forms and of their discretizations refer to Yoshihara et al. [1].

### 3 SCALAR TRANSPORT

The scalar transport has to be solved on the fluid and on the solid domain, which also in this case share a common mass transfer interface  $\Gamma$ . For the calculation of the scalar field, the water-substrate solution is considered diluted and in the fluid domain  $\Omega^{\text{F}}$  the following convection-diffusion equation is solved for  $\Phi^{\text{F}}$

$$\frac{\partial \Phi^{\text{F}}}{\partial t} + \mathbf{c}^{\text{F}} \cdot \nabla \Phi^{\text{F}} - \nabla \cdot (D^{\text{F}} \nabla \Phi^{\text{F}}) = 0 \quad \text{in} \quad \Omega^{\text{F}} \times (0, T). \quad (8)$$

In the previous equation  $D^{\text{F}}$  is the fluid diffusion coefficient and  $\mathbf{c}^{\text{F}}$  is the ALE convective velocity, which has already been introduced in equation (4).

On the other hand, in the solid domain  $\Omega^{\text{S}}$  the following diffusion-reaction equation in the conservative form is solved

$$\frac{\partial \Phi^{\text{S}}}{\partial t} + \Phi^{\text{S}} (\nabla \cdot \mathbf{u}^{\text{S}}) - \nabla \cdot (D^{\text{S}} \nabla \Phi^{\text{S}}) + R^{\text{S}} = 0 \quad \text{in} \quad \Omega^{\text{S}} \times (0, T). \quad (9)$$

Here,  $D^{\text{S}}$  is the solid diffusion coefficient,  $\mathbf{u}^{\text{S}}$  denotes the solid velocity, while  $R^{\text{S}}$  represents the reaction rate term. The microbial growth rates in an aqueous environment is usually

related to the concentration of a limiting nutrient through the Monod kinetic and the substrate consumption  $R^S$  reads

$$R^S = k \frac{\Phi^S}{K + \Phi^S}, \quad (10)$$

where  $k$  represents the reaction rate constant and  $K$  the half-saturation concentration of substrate.

The convection-diffusion transport of solute in the liquid domain, solved on spatial coordinates, is coupled to the diffusion-reaction transport equation in the biofilm domain, solved on material coordinates, with the constraint of equal concentrations and fluxes at the fluid-structure interface. The initial boundary value problem is completed through the imposition of Dirichlet and Neumann conditions at the boundaries of the domain and with appropriate initial concentration fields. Also in this case the weak form is obtained multiplying equations (8) and (9) with the virtual concentrations and integrating by parts. For insights in the formulation of the weak forms and of their discretizations refer also in this case to Yoshihara et al. [1].

#### 4 THE GROWTH MODEL

The formation and development of different biofilm structures are known to be influenced by nutrients availability and flow conditions [11]. For this reason, a reliable modelling of biofilm growth has to take in account both variables connected to mass transfer as well as to flow conditions. The present novel biofilm model uses stresses and mass fluxes at the interface resulting from the FSI and mass transfer step to calculate the local amount of growth and erosion. The growth is calculated in term of displacement perpendicular to the interface in the following way

$$\tilde{\mathbf{d}}^S = K_1 J^S - K_2 \sigma^S \quad \text{on } \Gamma \times (0, T). \quad (11)$$

Here  $K_1$  and  $K_2$  are constants, while  $J^S$  and  $\sigma^S$  are the mass flux through the interface and the shear stress at the interface, respectively. The latter two variables can be evaluated at the end of the calculation of the coupled FSI and mass transfer model, if it reaches a stationary solution, or as an average over a fixed period of time, if the problem reaches a periodic steady state solution, as it is the case for flapping streamers [9].

In order to permit biofilm to grow and the structure mesh to be appropriately deformed, in this step an ALE approach is applied also to the structure. For this purpose, the interface displacement due to growth calculated from equation (11) is applied as a Dirichlet boundary condition on the grid structure interface

$$\mathbf{d}^{G,S} = \tilde{\mathbf{d}}^S \quad \text{on } \Gamma \times (0, T), \quad (12)$$

while all the other boundaries of the structure ALE mesh are considered fixed. Within the domain, the ALE field is treated also in this case as a quasi-elastostatic pseudo-structure

[10] and an equation of motion similar to equation (7) is solved on the structure ALE mesh

$$\nabla \cdot \boldsymbol{\sigma}^{\text{G,S}} = \mathbf{0} \quad \text{in} \quad \Omega^{\text{G,S}} \times (0, T). \quad (13)$$

## 5 MULTI-SCALE COUPLED ALGORITHM

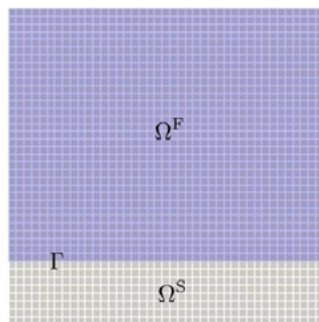
Monolithic schemes were found to be the most stable and efficient approach to model complex biological problems involving the coupling of incompressible flows to soft structures [12, 13]. For this reason the fully coupled non-linear FSI problem is solved monolithically. Moreover, since in biofilm applications the fluid flow and the structural deformation are not influenced by mass transport processes, a one-way coupling of fluid-structure interaction and transport models has been applied, as reported in [1]. For what concern the growth model, since the different involved phenomena happen in different time-scales, a multi-scale algorithm was applied, consisting of (i) an inner timeloop solving FSI and scalar transport at fluid-dynamic time-scale, (ii) an outer timeloop solving only the biofilm growth at biological time-scale.

Hence, in each fluid dynamic time step, at first the non-linear equations governing the fluid flow and the structure displacement, with the mentioned boundary conditions, are solved monolithically till when residuals meet a problem-specific tolerance. Subsequently, the local deformations and velocities obtained from the FSI calculation are transferred to the mass transport subproblem and the dynamic convection-diffusion-reaction equations for the mass transport are solved on the deformed fluid and solid domains till when also in this case residuals meet a problem-specific tolerance. These steps are repeated till when a stationary or a periodic steady state condition for the flow and concentration fields is reached. Afterwards, information regarding the mass flux through the interface and the shear stresses on it are transferred to the growth and erosion subproblem. At this point, the correct amount of growth is calculated for a longer time step and applied to the structure domain.

## 6 NUMERICAL EXAMPLES

The proposed simulations are based on the implementation of the algorithm discussed above in our in-house research code BACI [14]. The numerical solution of the field equations is obtained discretizing in space through a finite element method and in time through implicit time integration schemes. The resulting set of non-linear algebraic equations is then solved using a Newton-type method. A one-step- $\theta$  time integration scheme with  $\theta = 0.66$  is used for time discretization of fluid, structure and transport equations, while for space discretization trilinear, hexahedral finite elements are used. The presented examples are based on three-dimensional models and discretizations, although a pseudo two-dimensional deformation and flow state is enforced by specific boundary conditions.

The presented approach for coupling FSI and mass transport has been already successfully applied to the simulation of flapping biofilm streamers [9, 1] for flow conditions



**Figure 1:** Domains definition.

similar to those experimentally investigated by Stoodley et al. [15]. Those simulations have demonstrated the importance and the suitability of the proposed coupling of FSI and mass transport for simulating convective and diffusive mass transport on coupled and deformable fluid and solid domains under real operating and material conditions. For these reasons, the numerical examples proposed in the present study focus only on the suitability of the multi-scale growth model to simulate simple growing biofilm structures, and in particular on the possibility of the proposed model to catch the effect of operating conditions on biofilm growth, giving minor importance to real material parameters.

The presented examples reproduce a section of a fluid channel, where a uniform biofilm layer is present on the wall. The fluid domain  $\Omega^F$  (dimensions  $10\text{mm} \times 8\text{mm} \times 0.2\text{mm}$ ) is bound by a biofilm structure (dimensions  $10\text{mm} \times 2\text{mm} \times 0.2\text{mm}$ ) at the bottom, as reported in Figure 1. The interface between the biofilm and the fluid is denoted by  $\Gamma$  and represents the interface for FSI, mass transfer and biofilm growth. For the purpose of highlighting the effect of operating conditions on biofilm growth, different boundary conditions are applied to the same domains, while material parameters are taken constant. In all the presented cases, only the interface  $\Gamma$  is allowed to move in any direction, while all the other boundaries are kept fixed. When not specified also a zero velocity field is applied at the fluid boundaries and a zero-flux conditions at the solid and fluid boundaries. As initial condition always a zero velocity field is prescribed. Differences between the three presented cases are reported in the following.

- (a) In the first simulation a constant unitary concentration is applied at the top of the fluid domain and as initial condition a linear concentration gradient in the vertical direction is prescribed on all the domain.
- (b) In the second simulation, a cosine profile with a unitary maximum value at its center is applied at the top boundary of the fluid domain

$$\Phi^F(x, y, z) = 0.5 + 0.5 \cos(0.2\pi x)$$

with  $-5\text{mm} \leq x \leq 5\text{mm}$  and  $-0.1\text{mm} \leq z \leq 0.1\text{mm}$ . While as initial condition the following function is prescribed

$$\Phi^F(x, y, z) = [0.5 + 0.5 \cos(0.2\pi x)] [1 + \cos(0.05\pi y + 1.25\pi)]$$

with  $-5\text{mm} \leq x, y \leq 5\text{mm}$  and  $-0.1\text{mm} \leq z \leq 0.1\text{mm}$ .

- (c) Finally, the third simulation is similar to the second one except for the fact that in this case also a non-zero velocity boundary was applied at the left fluid boundary. A linear velocity gradient in the vertical direction with maximum value of  $0.1 \frac{\text{mm}}{\text{ms}}$  is imposed to the velocity  $x$ -component

$$\mathbf{v}_x^F(x, y, z) = 0.0375 + 0.0125y$$

with  $-5\text{mm} \leq y \leq 5\text{mm}$  and  $-0.1\text{mm} \leq z \leq 0.1\text{mm}$ . Consequently also flow through the right fluid boundary is allowed and a slip boundary condition is prescribed at the top boundary.

For running these simulations a multi-scale approach is applied and 100 steps with a time step of 1 ms are used in the inner timeloop and were found to be sufficient to reach a steady-state solution. After that a growth step is calculated based on the last calculated values and with a biological time step of 1000 ms. In Figure 2 the substrate distribution in the fluid domain for the three simulations and at different biological time steps is reported together with the biofilm structure grid. Results highlight the influence of the boundary conditions on the final biofilm shape. As a matter of fact in simulation (a) the structure growth is uniform, while in simulation (b) the presence of a non-uniform nutrients source produces higher growth of the initial flat biofilm shape in the center of the biofilm structure. Finally, in case (c) the presence of the velocity field and also its effect on the substrate distribution produces a non-symmetric final biofilm shape.

## 7 CONCLUSION AND OUTLOOK

To enable the investigation of the influence of operating conditions on biofilm growth, we have developed an advanced computational model for the multi-scale and coupled numerical simulation of fluid-structure interaction and mass transfer of moving and growing biofilm structures. For this purpose, the one-way coupling of the fully coupled nonlinear FSI problem and nonlinear multi-field mass transport model is encapsulated in a multi-scale approach. In this way it is possible to calculate FSI and mass transfer at hydrodynamic time scale and biofilm growth at biological time scales. The new methodology presented in this paper enables the mutual coupling of (i) flow and solid deformation, (ii) transport processes on deformed fluid and solid domains, (iii) as well as growth, based on mass transport and interfacial stresses. Hence, our approach can provide insights into how local deformations influence transport processes in both fluid and solid fields and how both fluid dynamic and mass transfer influence biofilm growth.



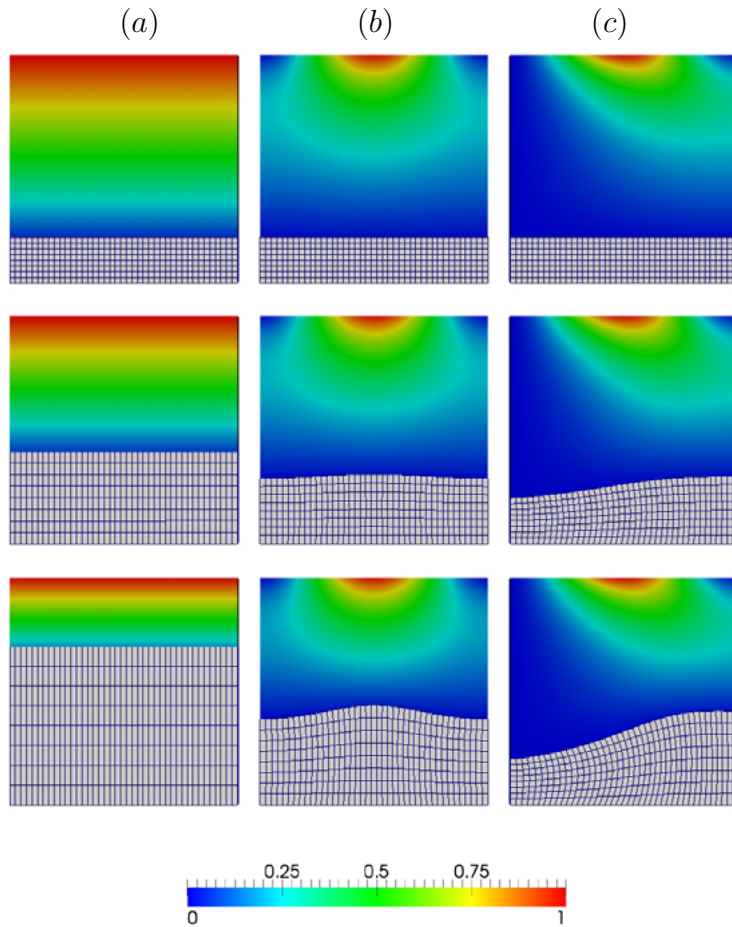


Figure 2: Concentration distribution for the three different simulations after 0, 30 and 60 biological time steps.

Selected examples demonstrated the general suitability of the proposed model for modeling the fluid-structure interaction and the mass transfer of growing biofilm structures. Ongoing work is concerned with an appropriate calibration of the proposed growth model based on experimental data, while outlook will concern the application of the presented coupled and multi-scale approach to reproduce experimental biofilm development at different operating conditions and the utilization of the developed approach as a prediction tool also in conditions difficult to investigate experimentally.

## REFERENCES

- [1] Yoshihara L., Coroneo M., Comerford A., Bauer G., Klppel T., Wall W.A. A combined fluid-structure interaction and multi-field scalar transport model for simulating mass transport in biomechanics. *Journal of Computational Physics*, submitted (2013).

- [2] Stewart P.S. Biofilm accumulation model that predicts antibiotic resistance of *Pseudomonas aeruginosa* biofilms. *Antimicrobial agents and chemotherapy* (1994) **5**:1052–1058.
- [3] Duddu R., Chopp D.L., Moran B. A two-dimensional continuum model of biofilm growth incorporating fluid flow and shear stress based detachment. *Biotechnology and Bioengineering* (2009) **103**:92–104.
- [4] Duddu R., Bordas S., Chopp D., Moran B. A combined extended finite element and level set method for biofilm growth. *International Journal for Numerical Methods in Engineering* (2008) **74**:848–870.
- [5] Alpkvist E., Klapper I. A multidimensional multispecies continuum model for heterogeneous biofilm development. *Bulletin of mathematical biology* (2007) **69**:765–789
- [6] Alpkvist E., Klapper I. Description of mechanical response including detachment using a novel particle model of biofilm/flow interaction. *Water Science Technology* (2007) **55**:265–273.
- [7] Dillon R., Fauci L., Fogelson A. Modeling biofilm processes using the immersed boundary method. *Journal of Computational Physics* (1996) **129**:57–73.
- [8] Taherzadeh D., Picioreanu C., Küttler U., Simone A., Wall W.A., Horn H. Computational study of the drag and oscillatory movement of biofilm streamers in fast flows. *Biotechnology and Bioengineering* (2010) **105**:600–610.
- [9] Coroneo M., Yoshihara L., Bauer G., Wall W.A. A finite element approach for the coupled numerical simulation of fluid-structure interaction and mass transfer of moving biofilm structures, in J. Eberhardsteiner et al. (eds.). *European Congress on Computational Methods in Applied Sciences and Engineering, ECCOMAS 2012* (2012).
- [10] Wall W. A. Ramm, E. Fluid-structure interaction based upon a stabilized (ALE) finite element method, in S. R. Idelsohn, E. Onate, E. N. Dvorkin. *Computational Mechanics – New Trends and Applications* (1998).
- [11] Stoodley P., Dodds I., Boyle J., Lappin-Scott H. Influence of hydrodynamics and nutrients on biofilm structure. *Journal of Applied Microbiology* (1999) **85**:19S–28S.
- [12] Küttler U., Gee M., Förster Ch., Comerford A., Wall W. A. Coupling strategies for biomedical fluid-structure interaction problems. *International Journal for Numerical Methods in Biomedical Engineering* (2010) **26**:305–321.
- [13] Klöppel T., Wall W. A. A Novel Two-Layer, Coupled Finite Element Approach for the Nonlinear Elastic and Viscoelastic Behavior of Human Erythrocytes. *Biomechanics and Modeling in Mechanobiology* (2010) **10**:445–459 .

- [14] Wall W. A., Gee M. W. BACI - A multiphysics simulation environment. *Technische Universität München* (2010).
- [15] Stoodley P., Lewandowski Z., Boyle J., Lappin-Scott H. M. Oscillation characteristics of biofilm streamers in turbulent flowing water as related to drag and pressure drop. *Biotechnology and Bioengineering* (1998) **57**:536–544.