Biofilm modeling with AQUASIM

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Abstract AQUASIM is a computer program for the identification and simulation of aquatic systems. The program includes a one-dimensional multisubstrate and multispecies biofilm model and represents a suitable tool for biofilm simulation. The program can be used to calculate substrate removal in biofilm reactors for any user specified microbial system. One-dimensional spatial profiles of substrates and microbial species in the biofilm can be predicted. The program also calculates the development of the biofilm thickness and of the substrates and microbial species in the biofilm and in the bulk fluid over time. Detachment and attachment of microbial cells at the biofilm surface and in the biofilm interior can be considered, and simulations of sloughing events can be performed. Furthermore, AQUASIM allows pseudo two-dimensional modeling of plug flow biofilm reactors by a series of biofilm reactor compartments. The most significant limitation of the model is that it only considers spatial gradients of substrates and microbial species in the biofilm in the direction perpendicular to the substratum.

Keywords AQUASIM; biofilm; detachment; model; multispecies; multisubstrate; one-dimensional; simulation; software

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Introduction

The objective of the Task Group of the International Water Association (IWA) on Biofilm Modeling was to test various biofilm models to determine their suitability for solving typical biofilm problems (Noguera and Morgenroth, 2004). These tests included the solution of three benchmark (BM) problems, the first investigating a simple monospecies biofilm (Morgenroth *et al*., 2004), the second examining the significance of modeling flow in the bulk fluid (Eberl *et al*., 2004), and the third evaluating microbial competition in the biofilm (Rittmann *et al*., 2004). The models tested included analytical (Perez *et al*., 2003), pseudo-analytical, and one- to multi-dimensional numerical solutions (Eberl *et al*., 2003). This paper provides an overview of the one-dimensional multisubstrate and multispecies biofilm model (Wanner and Reichert, 1996; Reichert and Wanner, 1997) used in the test and an introduction to the implementation of this model in AQUASIM. The computer program AQUASIM is a tool for the identification and simulation of aquatic systems (Reichert, 1998a, b). The program also performs parameter estimation and sensitivity analyses. Besides the biofilm model, AQUASIM also contains models for lakes, rivers, soil columns, completely mixed and advective-diffusive reactors.

Features of the biofilm model implemented in AQUASIM

The biofilm reactor compartment

For biofilm modeling and simulation, AQUASIM offers a biofilm reactor compartment consisting of three zones: "bulk fluid," "biofilm solid matrix," and "biofilm pore water" (Figure 1). For all three zones, AQUASIM calculates the development over time of microbial species and substrates, as well as the biofilm thickness. In the biofilm, spatial gradients perpendicular to the substratum are calculated for microbial species and substrates. The bulk fluid is assumed to be completely mixed, and a liquid boundary layer between the

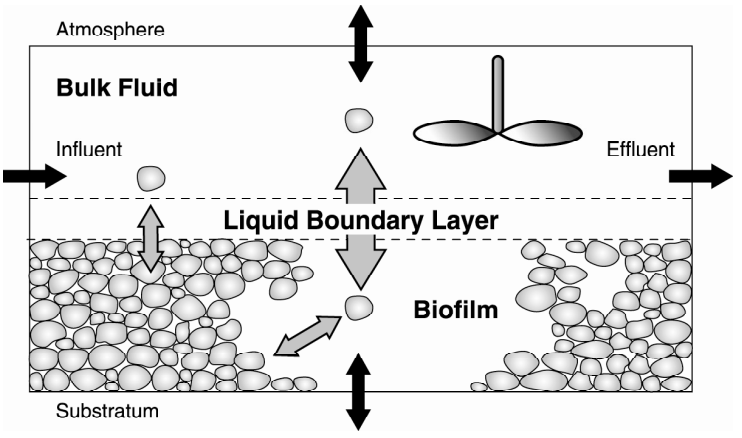

Figure 1 Setup of the AQUASIM biofilm reactor compartment. Solid arrows indicate possible mass fluxes across compartment boundaries, and shaded arrows indicate mass fluxes within the compartment

biofilm and the bulk fluid can be considered. The AQUASIM biofilm reactor compartment can be connected to other compartments. Solid arrows in Figure 1 indicate possible mass fluxes across the compartment boundaries. These fluxes include influent, effluent, exchange between the bulk fluid and the atmosphere, and transport across a permeable substratum. Shaded arrows indicate mass fluxes between the various zones in the compartment. These fluxes account for detachment and attachment of microbial cells in the biofilm and at the biofilm surface and diffusion of soluble and suspended particulate compounds through the liquid boundary layer.

In the AQUASIM dialog box "Edit Biofilm Reactor Compartment" (Figure 2), the properties of the biofilm system to be modeled are specified. The reactor type is chosen to be "confined" if the volume of the biofilm plus the bulk fluid is constant, as is the case in a closed reactor, and to be "unconfined" if the biofilm can grow freely, as may be the case in a trickling filter. The pore volume can be specified to contain only a liquid phase and dissolved substances, or it can also contain suspended solids. The biofilm matrix can be

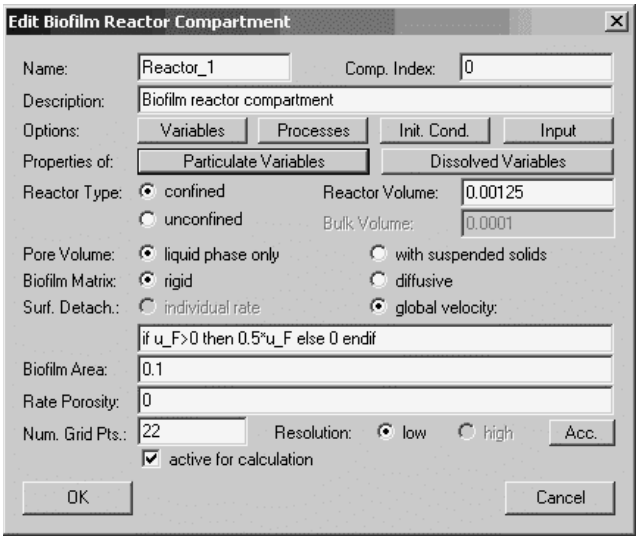

Figure 2 The AQUASIM dialog box "Edit Biofilm Reactor Compartment" is used to specify the properties of the biofilm system

assumed to be rigid, i.e., to change its volume due to microbial growth and decay only, or it can be assumed to be diffusive, which means that microbial cells can move within the biofilm matrix also by diffusion. Detachment at the biofilm surface can be described by rates, which are properties of individual microbial species and are specified via the button "Particulate Variables." Otherwise, it can be described by a global velocity, which means that all species are detached at the same rate. In Figure 2, the detachment velocity is assumed to be global and equal to $0.5*u_F$, where u_F is the velocity by which the biofilm surface would be displaced as a result of the production and decay of microbial mass in the biofilm. Thus, it is assumed here that half of the net production of biomass is detached into the bulk fluid. The biofilm area is a constant for a flat biofilm and is a function of the distance from the substratum for a spherical or cylindrical biofilm geometry. The porosity, i.e., the fraction of the pore water volume of the biofilm, is usually assumed to be constant. If it varies with time or space, this change can be modeled by a rate of porosity.

The option "Variables" serves to activate or inactivate variables, which denote concentrations of substrates and microbial species. For each activated variable, AQUASIM automatically calculates mass balance equations for the substrates and microbial species in both the biofilm and the bulk fluid. The option "Processes" serves to activate or inactivate processes. Only activated processes are included in the calculations, while the value of the rates of inactivated processes is set to zero. This feature makes it possible to easily modify a model and to readily test alternative models. In AQUASIM, the term "Processes" refers to biotic or abiotic conversion reactions. These have to be specified by the user, while the equations describing transport processes are intrinsic parts of AQUASIM. In Figure 3, it is exemplified how the user can implement conversion reactions in AQUASIM. The example shows the rate law and the stoichiometric coefficients of the process "heterotrophic growth." The options "Initial Conditions" and "Input" in Figure 2 serve to provide initial and influent values for the microbial species and substrates, as well as for the water flow rate.

The properties of the microbial species considered are specified via the button "Particulate Variables." Figure 4 shows the dialog box in which these properties can be selected. The density, defined as cell mass per unit cell volume, is the only property that must be specified at all times. AQUASIM is set up such that additional features of the model are omitted if their parameters have a value of zero. These features include attachment of cells to the biofilm surface and to the solid matrix within the biofilm, individual detachment of cells from the biofilm surface or solid matrix, and cell diffusion in the pore

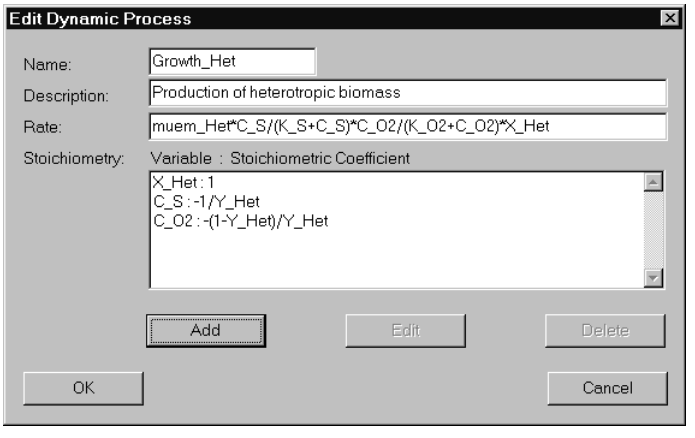

Figure 3 The AQUASIM dialog box "Edit Dynamic Process" is used to specify the rate laws and stoichiometric coefficients of biotic and abiotic conversion processes

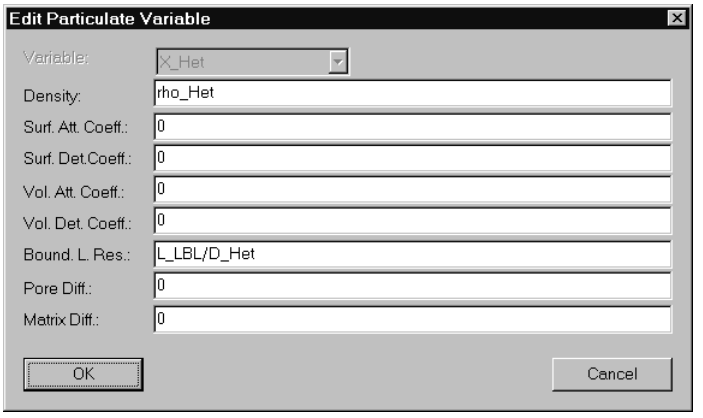

Figure 4 The AQUASIM dialog box "Edit Particulate Variable" is used to specify the properties of microbial species

Figure 5 The AQUASIM dialog box "Edit Dissolved Variable" is used to specify the properties of dissolved substrates

water and in the solid matrix. Furthermore, the implementation of the model considers a liquid boundary layer at the biofilm surface that is omitted if the value of its resistance is set to zero. The button "Dissolved Variables" leads to a dialog box in which the properties of the dissolved substrates can be specified (Figure 5). The diffusivity of the substrate in the pore water of the biofilm must be specified, while the boundary layer resistance can be set to zero.

Applications of the model

Substrate removal

AQUASIM can be used to model substrate removal in a biofilm reactor. Based on the kinetics of Benchmark 3 (BM3) (Rittmann *et al*., 2004), the reactor substrate outflow can be calculated as a function of the substrate inflow and the development of the biofilm in the reactor. The example in Figure 6 shows the substrate outflow decreasing during the first days because of biofilm growth. Then, after about three days, biofilm growth and biomass detachment reach an equilibrium, and the substrate outflow remains constant. Figure 6 is an original plot as it is produced by AQUASIM.

Biofilm growth, microbial composition and detachment

AQUASIM can model biofilm growth as a result of the production of microbial mass in the biofilm. In Figure 7, an example of the development of the thickness and microbial composition of the biofilm is shown. Again, this example is based on the kinetics of BM3 (Rittmann *et al*., 2004). The inserts in Figure 7 display the relative abundance of autotrophic and heterotrophic microbial species and also inert mass in the biofilm. In the beginning, the fast growing heterotrophic organisms dominate throughout the biofilm. After fifteen

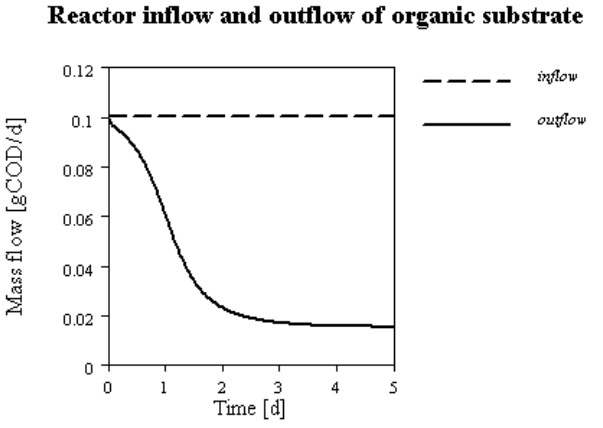

Figure 6 Typical AQUASIM plot showing the development in time of the reactor inflow and outflow of organic substrate

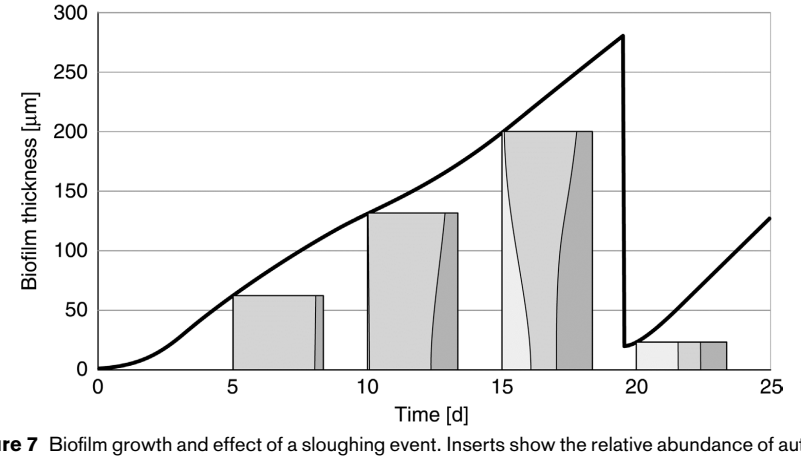

Figure 7 Biofilm growth and effect of a sloughing event. Inserts show the relative abundance of autotrophic (light gray) and heterotrophic (medium gray) microbial species and of inerts (dark gray) in the biofilm between the substratum (bottom) and the biofilm surface (top)

days, as the biofilm gets thicker, the slow growing autotrophic microorganisms are more abundant in the biofilm depth, while the heterotrophic organisms still dominate near the biofilm surface. At 19.5 days, there is a sloughing event, by which most of the biomass is detached to the bulk fluid. In the remaining biomass, the autotrophic organisms are dominant.

In AQUASIM, a sloughing event can be modeled as

$$\begin{aligned} u_{de} &= 0.5 * u_F && \text{for } t \le 19.5 \text{ days} \\ u_{de} &= 500 * u_F && \text{for } 19.51 \le t \le 19.52 \text{ days} \\ u_{de} &= 0.5 * u_F && \text{for } t \ge 19.53 \text{ days} \end{aligned} \qquad (1)$$

where u_{de} is the global velocity of surface detachment (Figure 2) and u_F is the velocity by which the biofilm surface is displaced as a result of the production and decay of microbial mass in the biofilm. For most of the time, the detachment velocity u_{de} is smaller than the production velocity u_F, and the biofilm is growing. However, between 19.51 and 19.52 days, u_{de} has a value much larger than u_F, leading to an increased detachment of biomass to the bulk fluid and a rapid decrease of the biofilm thickness. Between 19.5 and 19.51 days

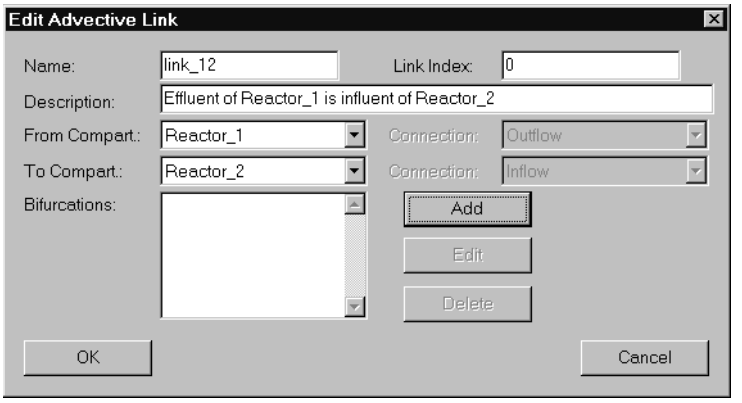

Figure 8 The AQUASIM dialog box "Edit Advective Link" is used to model water flow and advective substance transport from one compartment to another

and again between 19.52 and 19.53 days, the value of u_{de} is linearly interpolated. There are many other possibilities to model sloughing in AQUASIM, one of them is to define a base thickness above which all biofilm is removed during a sloughing event (Morgenroth and Wilderer, 2000; Horn *et al.*, 2003).

Pseudo two-dimensional modeling

A plug flow biofilm reactor can be modeled in AQUASIM by a series of connected biofilm reactor compartments. The dialog box in Figure 8 shows an advective link, which is used to model advective mass fluxes between two reactor compartments. The effect of this link is that the effluent from Reactor 1 automatically becomes the influent to Reactor 2. Figure 9 shows the biofilm in a plug flow reactor, which is modeled by 5 AQUASIM biofilm reactor compartments in series. In each compartment, the biofilm develops independently, and there are gradients of the substrate concentrations and the biofilm composition in the direction of flow. The biofilm that develops in the first compartment consists of a layer of inerts near the substratum, a layer of heterotrophic organisms near the biofilm surface, and a layer of autotrophic organisms between them. In the other compartments, the distribution of the microbial species is more or less uniform.

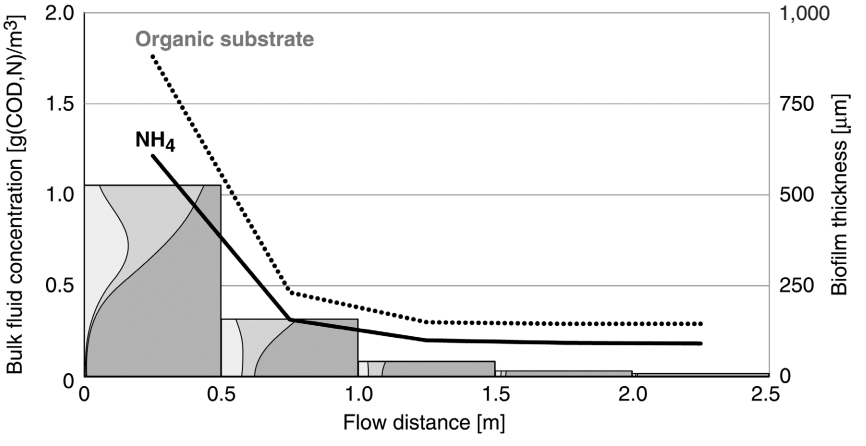

Figure 9 Pseudo two-dimensional modeling by a series of five biofilm reactor compartments. In the reactor inflow the concentration of organic substrate is 10 gCOD/m^3 and of ammonia 6 gN/m^3. Inserts show the relative abundance of autotrophic (light gray) and heterotrophic (medium gray) microbial species and of inerts (dark gray) in the biofilm between the substratum (bottom) and the biofilm surface (top)

In this biofilm reactor model, mass transfer in the flow direction occurs only between the bulk fluid zones of the compartments, while transport in the biofilm occurs only in the direction perpendicular to the substratum. Thus, the model is pseudo two-dimensional. The kinetics for this example were also taken from BM3 (Rittmann *et al*., 2004).

Limitations of the model

Like any model, the one-dimensional multisubstrate and multispecies model is based on simplifying assumptions. A list of these assumptions can be found in a recently published review on biofilm modeling (Wanner, 2002). Certainly, the most significant restriction is the assumption that the biofilm is one-dimensional in space. This is clearly contradictory to experimental observations, which show that some biofilms consist of three-dimensional, mushroom like structures. In the one-dimensional model the concentrations of substrates and microbial species are averaged over planes parallel to the substratum and spatial gradients are considered in the direction perpendicular to the substratum only. The benchmark tests have revealed that for practical purposes this is not a severe restriction; for the major microbial constituents of the biofilm solid matrix the one-dimensional model still yields accurate results. Only for the spatial distribution of specialists, i.e., of microbial species with very small cell numbers in the biofilm, might the one-dimensional model yield results that are not accurate.

Mathematical treatment

The mass balance equations, boundary conditions, and additional expressions needed for simulations with the one-dimensional multisubstrate and multispecies biofilm model are readily available (Wanner and Reichert, 1996; Reichert and Wanner, 1997). However, biofilm simulation with this model requires the solution of the moving boundary problem created by biofilm growth and the solution of the stiff differential equation system of the mass balances for substrates and microbial species. These mathematical problems have been solved by the integration routines and numerical algorithms implemented in AQUASIM.

Conclusions

Mathematical models are suitable tools for the analysis of biofilm processes and for the prediction of the behavior of biofilm systems. The IWA Task Group on Biofilm Modeling (Noguera and Morgenroth, 2004) has tested various biofilm models to determine their suitability for solving typical biofilm problems (Eberl *et al*., 2004; Morgenroth *et al*., 2004; Rittmann *et al*., 2004). These tests have shown that simple analytical models can be used for many practical applications (Perez *et al*., 2003), while more complex two- or three-dimensional models are recommended for the correct reproduction of the structure and development of biofilms (Eberl *et al*., 2003). The one-dimensional multisubstrate and multispecies model (Wanner and Reichert, 1996; Reichert and Wanner, 1997) is positioned between the two groups of models mentioned above. This model is suited best to model situations in which competition between the microbial constituents of the biofilm matrix is significant. The model is implemented in the computer program AQUASIM (*www.aquasim.eawag.ch*), which is an efficient tool for biofilm simulation and parameter estimation.

References

Eberl, H.J., van Loosdrecht, M.C.M., Morgenroth, E., Noguera, D.R., Perez, J., Picioreanu, C., Rittmann, B.E., Schwarz, A.O. and Wanner, O. (2004). Modelling a spatially heterogeneous biofilm and the bulk fluid: selected results from the benchmark problem (BM2). *Wat. Sci. Tech.*, **49**(11–12), 155–162 (this issue).

Eberl, H.J., Picioreanu, C. and Noguera, D.R. (2003). Two- and three-dimensional approaches to biofilm modeling. IWA Conference on *Biofilm Systems V*, Cape Town, South Africa, September 2003.

Horn, H., Reiff, H. and Morgenroth, E. (2003). Simulation of growth and detachment in biofilm systems under defined hydrodynamic conditions. *Biotechnol. Bioeng.*, **81**(5), 607–617.

Morgenroth, E. and Wilderer, P.A. (2000). Influence of detachment mechanisms on competition in biofilms. *Wat. Res.*, **34**(2), 417–426.

Morgenroth, E., Eberl, H.J., van Loosdrecht, M.C.M., Noguera, D.R., Pizarro, G.E., Picioreanu, C., Rittmann, B.E., Schwarz, A.O. and Wanner, O. (2004). Comparing biofilm models for a single species biofiilm system. *Wat. Sci. Tech.*, **49**(11–12), 145–154 (this issue).

Noguera, D.R. and Morgenroth, E. (2004). Introduction to the IWA task group on biofilm modeling. *Wat. Sci. Tech.*, **49**(11–12), 131–136 (this issue).

Perez, J., Picioreanu, C. and van Loosdrecht, M.C.M. (2003). Modeling biofilm processes based on analytical solution of reaction-diffusion equations. *Preprint, IWA Conference on Biofilm Systems V, Cape Town, South Africa, September 2003*.

Reichert, P. and Wanner, O. (1997). Movement of solids in biofilms: significance of liquid phase transport. *Wat. Sci. Tech.*, **36**(1), 321–328.

Reichert, P. (1998a). *AQUASIM 2.0 – User Manual*. Swiss Federal Institute for Environmental Science and Technology (EAWAG), Duebendorf, Switzerland.

Reichert, P. (1998b). *AQUASIM 2.0 – Tutorial*. Swiss Federal Institute for Environmental Science and Technology (EAWAG), Duebendorf, Switzerland.

Rittmann, B.E., Schwarz, A.O., Eberl, H.J., Morgenroth, E., Perez, J., van Loosdrecht, M.C.M. and Wanner, O. (2004). Results from the multi-species benchmark problem (BM3) using one-dimensional models. *Wat. Sci. Tech.*, **49**(11–12), 163–168 (this issue).

Wanner, O. and Reichert, P. (1996). Mathematical modeling of mixed-culture biofilms. *Biotechnol. Bioeng.*, **49**, 172–184.

Wanner, O. (2002). Modeling of Biofilms. In: *Encyclopedia of Environmental Microbiology*, G. Bitton (ed.), John Wiley & Sons, New York, pp. 2083–2094.