Results from the multi-species Benchmark Problem 3 (BM3) using two-dimensional models

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Abstract In addition to the one-dimensional solutions of a multi-species benchmark problem (BM3) presented earlier (Rittmann *et al*., 2004), we offer solutions using two-dimensional (2-D) models. Both 2-D models (called here DN and CP) used numerical solutions to BM3 based on a similar mathematical framework of the one-dimensional AQUASIM-built models submitted by Wanner (model W) and Morgenroth (model M1), described in detail elsewhere (Rittmann *et al*., 2004). The CP model used differential equations to simulate substrate gradients and biomass growth and a particle-based approach to describe biomass division and biofilm growth. The DN model simulated substrate and biomass using a cellular automaton approach. For several conditions stipulated in BM3, the multidimensional models provided very similar results to the 1-D models in terms of bulk substrate concentrations and fluxes into the biofilm. The similarity can be attributed to the definition of BM3, which restricted the problem to a flat biofilm in contact with a completely mixed liquid phase, and therefore, without any salient characteristics to be captured in a multidimensional domain. On the other hand, the models predicted significantly different accumulations of the different types of biomass, likely reflecting differences in the way biomass spread within the biofilm is simulated.

Keywords Benchmark problems; biofilm; cellular automaton; individual-based model; mathematical modeling; multidimensional; multispecies

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Introduction

The Benchmark Problem 3 (BM3), created by the Biofilm Modeling Task Group to evaluate biofilm modeling approaches in a multi-species situation, was solved using one-dimensional (1D) and two-dimensional (2D) models. The problem describes a flat biofilm in contact with a completely mixed liquid compartment, in which three limiting substrates (organic substrate, ammonia nitrogen, and oxygen) are consumed by a biofilm that contains aerobic heterotrophs, aerobic autotrophic nitrifiers, and inert or inactive biomass (Rittmann *et al*., 2004). A total of eleven solutions to the problem were submitted by members of the Task Group. Nine of these solutions simulated the problem in a 1D domain, while two of the solutions simulated the biofilm in a 2D domain. The 2D solutions were submitted by Picioreanu (model CP, Picioreanu *et al*., 2004) and Noguera (model DN). A detailed description of the 1D solutions is provided in Rittmann *et al*. (2004). In this manuscript, the 2D modeling approaches are described, and the results for the standard and special cases of BM3 are compared to the solutions provided by Wanner (model W) and Morgenroth (model M1), which were the two 1D models that employed full numerical solutions (Rittmann *et al*., 2004).

Models description

The CP and DN models were based on the same general mathematical framework as described in Rittmann *et al*. (2004) for the 1D multi-species and multi-substrate models. In 2D, the governing differential equations for soluble substrates within the biofilm are:

$$D_s\left(\frac{\partial^2 C_s}{\partial x^2}+\frac{\partial^2 C_s}{\partial y^2}\right)+R_s=0 \qquad s=S,N \text{ and } O_2 \tag{1}$$

$$C_s(L_F,y)=C_s(L_x,y)=C_{s,b} \tag{2}$$

$$\frac{\partial C_s(0,y)}{\partial x}=0 \tag{3}$$

$$C_s(x,0)=C_s(x,L_y) \tag{4}$$

where R_s is the net reaction rate of substrate s, as a function of the concentrations of organic substrate (C_S), ammonia-nitrogen (C_N), and oxygen (C_{O2}), and the concentrations of heterotrophic (X_H) and autotrophic (X_N) biomass. The boundary conditions described the substrate concentrations at the biofilm-liquid interface (at $x = L_F$) as equal to the bulk concentrations (at $x = L_X$, with $L_X = L_F$ at steady state) (Eq. (2)), the gradient of substrates at the impermeable substratum equal to zero (Eq. (3)), and lateral periodicity at the other edges of the 2D domain (Eq. (4)). Eq. (2) defines a boundary condition without a concentration boundary layer, as specified in the definition of BM3 (Rittmann *et al*., 2004).

Biomass growth is modeled as a function of the location within the biofilm and obeys the general mass balances presented in Eqs (5) to (7), where all the variable names are as defined in Rittmann *et al*. (2004):

Heterotrophic biomass

$$\frac{dX_H}{dt}=\mu_{\max,H}\frac{C_S}{K_S+C_S}\frac{C_{O2}}{K_{O2,H}+C_{O2}}X_H-b_{ina,H}X_H-b_{res,H}X_H\frac{C_{O2}}{K_{O2,H}+C_{O2}} \tag{5}$$

Autotrophic biomass

$$\frac{dX_N}{dt}=\mu_{\max,N}\frac{C_N}{K_N+C_N}\frac{C_{O2}}{K_{O2,N}+C_{O2}}X_N-b_{ina,N}X_N-b_{res,N}X_N\frac{C_{O2}}{K_{O2,N}+C_{O2}} \tag{6}$$

Inert biomass

$$\frac{dX_I}{dt}=b_{ina,H}X_H-b_{ina,N}X_N \tag{7}$$

A significant difference between the 2D and 1D models is the simulation of biomass spreading within the biofilm. While the 1D models employed the concept of a velocity at which different types of biomass move away from the substratum (Rittmann *et al*., 2004), the 2D models used a discretized representation of biomass as "microbial particles". These particles grow in mass according to the mass balances shown above, but are limited to a maximum density. When this maximum density is reached, the microbial particles divide and the "newborn" microbial particle is placed in a random location next to the "mother" particle. With this discrete rule, biofilm growth occurs when newborn particles shove other particles away. Biomass detachment is a consequence of this shoving effect. When a microbial particle is pushed across the imposed biofilm thickness limit, the particle is removed from the biofilm.

The reactor's bulk-liquid volume was modeled as completely mixed, according to the general mass balance represented by Eq. (8), in which L_x and L_y are the dimensions of simulated biofilm in directions perpendicular and parallel to the substratum, respectively, and A_f and V represent the total area of biofilm and the reactor volume, respectively. Both

2D models were solved in a dynamic manner, with an initial condition defined by randomly seeding a small number of heterotrophic and autotrophic "microbial particles" at the substratum, and allowing the system to reach steady state.

$$\frac{dC_{s,b}}{dt} = \frac{Q}{V}\left(C_{s,in} - C_{s,b}\right) + \frac{A_f}{VL_y}\int_0^{L_x}\int_0^{L_y} R_s(x,y)dydx \qquad s = S, N \text{ and } O_2 \tag{8}$$

The CP model is a hybrid differential-discrete particle-based model derived from the cellular-automaton approach of Picioreanu *et al*. (1998) and the individual-based approach by Kreft *et al*. (2001), described in detail in Picioreanu *et al*. (2004). Substrate gradients and biomass growth are simulated on a continuum, using Eqs (1) to (7). Biomass is distributed in spherical microbial particles that push each other when growing. A microbial particle could contain biomass of any type (i.e., heterotrophic, autotrophic, or inert), but was restricted to a maximum concentration of $C_{X,max}$ = 10,000 g/m^3, which corresponds to the maximum biomass density specified for BM3 (Rittmann *et al*., 2004). In a particle division event, the "newborn" particle randomly receives between 40 and 60% of the biomass contained in the "mother" particle.

The DN model is a fully-discretized cellular automaton model based on Pizarro *et al*. (2001) and Noguera *et al*. (2004). In this model, differential equations are not used to describe substrate concentration and transport nor biomass growth. Rather, stochastic discrete rules about local "food particle" and "microbial particle" movement and fate simulate mass transport, substrate utilization, and microbial growth and distribution within the biofilm. Substrate diffusion is implemented using the concept of random walks of food particles (Chopard and Droz, 1991; Pizarro *et al*., 2001), while substrate utilization and microbial growth are represented by a discrete version of Monod kinetics (Noguera *et al*., 2004). Microbial particles are defined as being of three different types (i.e., heterotrophic, autotrophic, or inert) and having a concentration equal to the maximum biomass density within the biofilm. Decay events convert heterotrophic and autotrophic particles into inactive particles, while a division event creates a "newborn" particle with exactly the same characteristics as the "mother" particle.

Results and discussion

The results of the 2D models (CP and DN models) are compared to the results from the 1D models that used fully numerical solutions (W and M1 models). Table 1 summarizes the key output parameters for the standard case. As a measurement of similarity in the output parameters, the last two rows in the table indicate average values and the relative variation for each parameter. The latter was calculated as the standard deviation divided by the average, and expressed as a percentage. For parameters with relative variations greater than 20%, entries significantly larger than the mean (greater than 1.2 times the average) are indicated in boldface, while those significantly lower than the mean (smaller than the average divided by 1.2) are indicated in italics font.

Table 1 Summary of key output parameters for the standard case

Model	$C_S g_{CODS}/m^3$	$C_N g_N/m^3$	$J_S g_{CODS}/m^2 d$	$J_N g_N/m^2 d$	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	5.14	1.50	4.95	0.89	1.81	0.72	**2.60**
DN	5.14	1.74	4.96	0.85	**2.88**	0.68	*1.44*
W	5.39	1.59	4.92	0.88	1.88	0.79	2.33
M1	4.84	1.45	5.03	0.91	2.02	0.83	2.15
Average	5.13	1.57	4.97	0.88	2.15	0.76	2.13
Relative variation	4.4%	8.1%	0.9%	2.8%	23.1%	9.0%	23.3%

According to Table 1, the four models had an excellent agreement in the prediction of bulk substrate concentrations and fluxes into the biofilm, which are the two main parameters of interest in a macroscopic analysis of biofilm activity. The most significant deviations among the model output parameters were the higher accumulation of heterotrophic biomass in the DN model, and the higher accumulation of inert biomass in the CP model. The DN model also resulted in a significantly lower accumulation of inert biomass. However, these differences in biomass accumulation did not significantly influence the calculation of fluxes or bulk concentrations, likely due to the depletion of organic substrate in the deeper regions of the biofilm, which contributed to the very low activity of a large fraction of the heterotrophic biomass. Furthermore, the variation in predictions between the 2D and 1D models shown in Table 1 was not as large as the variations observed with the other 1D models (Rittmann *et al*., 2004).

The same trends of higher heterotrophic biomass accumulation in the DN model and higher accumulation of inerts in the CP model, but insignificant differences in the predictions of substrate concentrations and fluxes, were observed in the simulations of the special case with a high N:COD ratio (Table 2). Notably, all the models agreed in the accumulation of nitrifiers, which was the bacterial group enhanced in this special case.

The models also agreed in the relative distribution of biomass within the biofilm, as depicted in Figure 1, which compares output profiles from the CP and M1 models. For this special case, inert biomass accumulated in the deeper region of the biofilm, while the majority of heterotrophs were located in the outer layers of the biofilm. The biomass distribution predicted with the DN model was slightly different as shown in Figure 2. With this model, there was a significantly higher survival of heterotrophs in the deeper layers of the biofilm and the distribution of nitrifiers and inactive biomass was even throughout the biofilm. The differences in biomass distribution with this model are likely due to the rules used for biomass spreading.

When the special case of low N:COD ratio was simulated, the DN model predicted a significantly lower accumulation of nitrifiers compared to the other models (Table 3), suggesting that the dynamics of biofilm growth in this model did not offer the same level of "protection" to this slow-growing population as the other models did. Nevertheless, the trend of similar predictions of bulk substrate concentrations and fluxes was maintained in this special case. Furthermore, the prediction in nitrifier accumulation was not as extreme as observed with the other 1D models, for which the nitrifier population disappeared (Rittmann *et al*., 2004).

When the production of inerts was accentuated by increasing the inactivation rate, the models exhibited the highest degree of variation in the output parameters (Table 4). This was the only simulation in which the predictions of substrate concentrations had a relative variation greater than 20%, with the CP model providing a significantly higher nitrogen concentration, in agreement with its prediction of a significantly lower nitrifier population. In contrast, the M1 model predicted a significantly lower nitrogen concentration, but this

Table 2 Summary of key output parameters for the special case of high influent N:COD ratio

Model	C_S g_{CODS}/m^3	C_N g_N/m^3	J_S g_{CODS}/m^2d	J_N g_N/m^2d	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	5.45	18.15	4.90	2.35	1.71	1.07	**2.42**
DN	5.56	20.26	4.89	1.89	**2.92**	1.10	*0.98*
W	5.86	18.93	4.83	2.21	1.73	1.07	2.20
M1	5.35	17.03	4.93	2.59	1.83	1.24	1.93
Average	5.56	18.59	4.89	2.26	2.05	1.12	1.88
Relative variation	4.0%	7.3%	0.9%	12.9%	28.5%	7.3%	33.7%

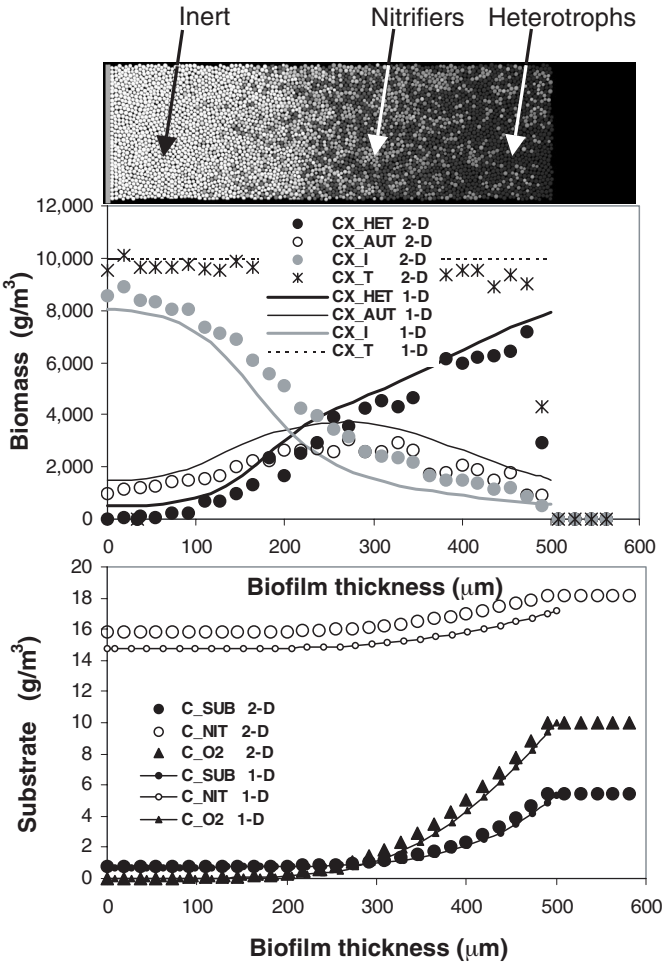

Figure 1 Biomass distribution in the biofilm at steady state (120 days) for the Special Case of High Influent N:COD. Lines in the graph are results of the 1D M1 model and symbols are average profiles computed with the CP particle-based 2D model. Top figure shows the 2D biomass distribution, with heterotrophic and nitrifying particles. The whiter the color of the particles, the more inerts they contain (e.g., at biofilm depth)

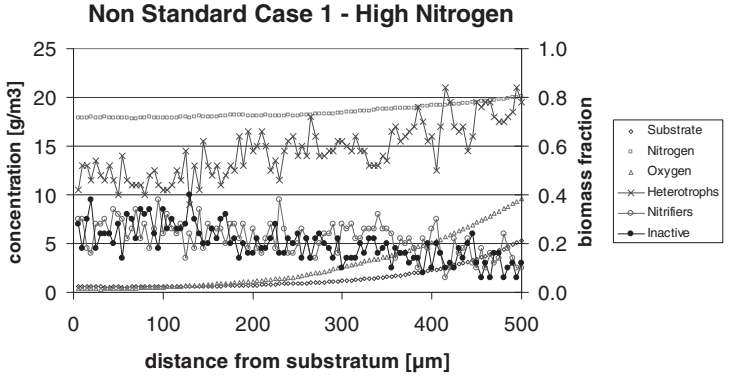

Figure 2 Results of biomass distribution and substrate profiles within the biofilm from the DN model for the special case of high influent N:COD

Table 3 Summary of key output parameters for the special case of low influent N:COD ratio

Model	C_S g_{CODS}/m^3	C_N g_N/m^3	J_S g_{CODS}/m^2d	J_N g_N/m^2d	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	4.39	0.44	5.16	0.15	2.11	0.23	2.73
DN	4.98	0.48	4.96	0.12	2.96	*0.13*	1.91
W	5.19	0.48	4.96	0.14	2.00	0.21	2.80
M1	4.66	0.45	5.07	0.15	2.14	0.21	2.65
Average	4.81	0.46	5.04	0.14	2.30	0.20	2.52
Relative variation	7.3%	4.5%	1.9%	10.1%	19.2%	22.7%	16.4%

Table 4 Summary of key output parameters for the special case of large production of inerts

Model	$C_S g_{CODS}/m^3$	$C_N g_N/m^3$	$J_S g_{CODS}/m^2d$	$J_N g_N/m^2d$	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	4.61	**3.03**	5.10	0.60	**2.80**	*0.24*	2.03
DN	5.54	1.97	4.92	0.81	2.54	0.49	1.97
W	5.71	1.72	4.86	0.86	*1.74*	**0.69**	2.57
M1	5.14	*1.56*	4.97	0.89	1.87	**0.72**	2.41
Average	5.25	2.07	4.96	0.79	2.24	0.54	2.25
Relative variation	9.3%	32%	2.1%	16.6%	22.9%	41.4%	13.0%

prediction was also in agreement with having the highest accumulation in the nitrifier population. The W model resulted in the highest accumulation of inerts and a significantly lower accumulation of heterotrophic biomass.

The best agreement among the models was observed in the special case in which the detachment rate was accentuated and a thin biofilm was simulated (Table 5). The only significant difference was the accumulation of a small amount of inactive biomass in the DN model, which is likely the result of a few "inert microbial particles" that remained embedded within the biofilm matrix.

Finally, when the oxygen sensitivity of the nitrifiers was tested by increasing the oxygen half-saturation constant (Table 6), the 2D models were consistently different from the 1D models in the prediction of biomass distribution, although the variations in predictions of bulk substrate concentrations and fluxes were again insignificant. In the 2D models, the lower rate of nitrogen utilization that resulted from an increase in the half-saturation constant effectively reduced the amount of nitrifier biomass in the biofilm (compared to Table 1). The opposite result was observed for the 1D models, which predicted an increase in the accumulation of nitrifiers. As discussed in Rittmann *et al.* (2004), the counter-intuitive increase in nitrifier population in the 1D models could be due to the mechanism used to simulate biomass growth, which created a protected region for slow-growing bacteria in the deepest regions of the biofilm. The 2D models, with their mechanisms of random

Table 5 Summary of key output parameters for the special case of high detachment leading to a thin biofilm (20 μm)

Model	$C_S g_{CODS}/m^3$	$C_N g_N/m^3$	$J_S g_{CODS}/m^2d$	$J_N g_N/m^2d$	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	22.19	6.00	1.55	0.00	0.20	0.00	0.00
DN	21.89	6.00	1.34	0.00	0.20	0.00	**0.003**
W	22.23	6.00	1.55	0.00	0.20	0.00	0.00
M1	22.23	6.00	1.55	0.00	0.20	0.00	0.00
Average	22.14	6.00	1.50	0.00	0.20	0.00	0.00
Relative variation	0.7%	0.0%	7.0%		0.0%		200.0%

Table 6 Summary of key output parameters for the special case of high oxygen sensitivity of nitrifiers

Model	$C_S g_{CODS}/m^3$	$C_N g_N/m^3$	$J_S g_{CODS}/m^2$–d	$J_N g_N/m^2$–d	Heterotrophs g_{CODX}/m^2	Nitrifiers g_{CODX}/m^2	Inerts g_{CODX}/m^2
CP	5.98	1.74	4.82	0.85	2.74	*0.52*	1.84
DN	5.11	2.40	4.99	0.69	**2.88**	*0.65*	1.47
W	5.41	1.87	4.92	0.83	*1.84*	**1.21**	1.95
M1	4.86	1.68	5.03	0.86	1.98	**1.21**	1.81
Average	5.34	1.92	4.94	0.81	2.36	0.90	1.77
Relative variation	9.0%	17%	1.9%	9.8%	22.3%	40.6%	11.7%

placement of "newborn" microbial particles and the shoving of microbial particles in the internal regions of the biofilm, did not result in an overprotection of the slow-growing nitrifiers.

Conclusions

The general trend observed in the comparison of two 2D models with two 1D models was that all the models produced similar predictions of bulk substrate concentrations and fluxes into the biofilm, even though, in some cases, the models resulted in significantly different accumulations of the different types of biomass. The similarity in output parameters for substrate concentration and fluxes is likely the result of having all the models being based on a similar theoretical and mathematical framework, with the obvious difference that substrate concentrations in the 2D models were calculated using mathematical descriptions that represented the 2D domain, while the 1D models had simpler 1D mathematical descriptions. Nevertheless, since the specifications of BM3 restricted the system to a flat biofilm in a completely mixed reactor, the problem was intrinsically a 1D problem and there were no prominent characteristics to be captured by the multidimensional models.

On the other hand, there were significant variations in the predicted distribution of the different types of biomass. In this regard, the only identifiable trend when comparing the 1D to the 2D models was the apparent overprotection of nitrifiers in the 1D models, especially when this population was affected by a large accumulation of inerts (Table 4) or a lower rate of oxygen utilization (Table 6). This trend likely reflects the most significant difference between 1D and 2D models, which is the mechanism used to distribute the biomass within the biofilm. While the 1D models used the concept of a continuum field of biomass that moved away from the substratum (Rittmann *et al*., 2004), the 2D models used the concept of microbial particles and cellular automaton rules to place newborn microbial particles close to the mother particles, with the final distribution of biomass within the biofilm being a consequence of the self-organization of the particles and the shoving of old particles by new particles. Finally, using the 1-D models as reference, the CP 2-D model generally leads to an overestimation of minority species, whereas the DN model tends to overestimate the majority species. However, there is no experimental proof that one of these models is better at reflecting reality than the other, and therefore, the significance of different mechanisms used to model microbial distributions in a multispecies biofilm is, in our opinion, a research direction that needs further investigation.

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