Evaluating trends in biofilm density using the UMCCA model
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Abstract

We present a series of modeling cases that illustrate the trends described by the unique features of the unified multiple-component cellular automaton (UMCCA) model for a heterogeneous, two-dimensional biofilm. The outputs of the UMCCA model show five general trends. (1) The concentration profiles for the two soluble microbial products are opposite the profile for original substrate. (2) The top of the biofilm is dominated by active biomass and EPS, while the bottom is dominated by residual inert biomass. Within the top layers, active biomass has a much higher concentration than EPS. (3) The top of all biofilm is quite “fluffy,” while the bottom is dense. (4) The peak of the composite density does not correspond to the peak of active biomass. (5) All biomass types show considerable local heterogeneity. The series of cases also indicate what conditions lead to particular characteristics observed in some biofilms. Biofilm clusters are promoted by substrate limitation, a high detachment rate, or strong consolidation. A high biofilm density is associated with an old biofilm, which is favored by a low substrate concentration, a high detachment rate, and strong consolidation. Old biofilms also can develop low-density pockets near the substratum, a possible cause of sloughing. Local heterogeneity is generally related to the same factors that cause a high density. We also solved the UMCCA model for conditions similar to the experiments of Bishop et al. (Water Sci. Technol. 31(1) (1995) 143), who measured the total biomass density in layers from the substratum. The model outputs captured all the major trends in the experimental data: the overall thickness and density of biofilms increase with time, and the total biomass density is 5–10 times greater near the substratum than near the top of the biofilm. Furthermore, the model indicates that the residual inert biomass becomes denser toward the substratum, a trend observed experimentally; the UMCCA model suggests that this trend is due to the combined effects of consolidation and inert biomass having a larger maximum density.
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Keywords: Biofilm; Clusters; Consolidation; Density; Detachment; EPS; Inert biomass; Modeling

1. Introduction

Biofilms are heterogeneous structures consisting of living cells, dead cells, and cell debris in a matrix of extracellular polysaccharide (EPS) attached to a surface (the substratum). Biofilm density is an important measure of biofilm heterogeneity, and it is one that has been measured experimentally by several workers (Masuda et al., 1991; Zhang and Bishop, 1994; Bishop et al., 1995; Hoehn and Ray, 1973; Kornegay and Andrews, 1968; Tomlinson and Snadon, 1966). Furthermore, Ohashi et al. (1999) found that the biofilm tensile strength correlated to the biofilm density, suggesting that density may be a predictor of the biofilm’s mechanical strength. Such an association is essential for the study of biofilm detachment, a determining factor for biofilm structure formation, as it often is the primary process that balances growth (van Loosdrecht et al., 1995, 1997).

In a companion paper (Laspidou and Rittmann, 2004), we present the unified multi-component cellular automaton (UMCCA) model, which predicts
quantitatively the development of the biofilm’s composite density for three biofilm components: active bacteria, inert or dead biomass, and EPS. The UMCCA model, a hybrid discrete-differential mathematical model, introduces the novel feature of biofilm consolidation. The fluid over the biofilm creates pressures and vibrations that cause the biofilm to consolidate, or pack itself to a higher density over time. Each biofilm compartment in the model output consolidates to a different degree that depends on the age of its biomass.

In this paper, we illustrate trends that the UMCCA model describes concerning how the composite density and the distribution of the biofilm components vary with time and location in the biofilm. Because the UMCCA model discriminates active biomass from other types of biomass and also computes a local composite density, its output can be compared to experimental observations of biofilm density (Bishop et al., 1995). Therefore, we solve the model for conditions simulating the relevant experiments of Bishop et al. (1995). Although the UMCCA model describes multiple components in an aerobic, heterotrophic biofilm, it does not include other types of active biomass. In other words, the model does not apply to biofilms that have major ecological diversity, such as layering of aerobic heterotrophs with fermenters or nitrifiers. Some of the experiments conducted by Bishop et al. (1995) involved very thick biofilms (> 1000 μm) in which their bottom layers surely were depleted of oxygen, creating an anaerobic sub-layer ideal for fermenters to develop. Therefore, we limit our comparisons to relatively thin biofilms, or biofilms that are aerobic throughout their thicknesses. For such conditions, we evaluate if the model can reproduce the experimental results of Bishop et al. (1995) and use the model results to gain insight into why biofilm density varies throughout its depth and over time.

2. Trends in the modeling results

A detailed description of the UMCCA model, as well as a solution strategy and the values of the parameters used in the model, were presented in the companion paper (Laspidou and Rittmann, 2004). In this paper, we present results at the end of a modeling run for six quantities: S, UAP, BAP, Xα, Xε, and EPS. We do not present oxygen results, because the same profile is used every time (Eq. (10) in Laspidou and Rittmann, 2004).

The UMCCA model was run from the initial conditions specified in Laspidou and Rittmann (2004) until each compartment contained some biomass. This time varies depending on the parameters used. For the standard case, the elapsed time is 24.5 days. This means that the oldest biofilm compartment in the system is 24.5 days old, while the youngest biofilm compartment is a few minutes old. The age of each biofilm compartment, or bioage, defines the value of the consolidation ratio Uc, or the maximum packing density of each compartment (Eq. (9)) in Laspidou and Rittmann, 2004).

For all cases, we present results for every point in the grid by plotting the concentration in a shading format, giving each compartment a shade of gray that is proportional to the concentration; thus, a concentration of 100% corresponds to a black compartment, and 0% corresponds to a white one. The shading scale used every time is shown next to each graph. The shading plots show the nature of the heterogeneity of the different components.

For some cases, we present averages for each row of biofilm compartments. Therefore, we “collapse” the compartments shown in the shaded graphs to a single data point for each row by taking the average of each data point the same distance from the substratum. This presentation corresponds to what could be measured with “slices” of biofilm, such as can be obtained with a microtome (Zhang and Bishop, 1994; Bishop et al., 1995). For each datum, we also present an error bar that is 2 standard deviations long, with one standard deviation above and below the mean.

2.1. Case 1. Standard conditions

Fig. 1 shows how the six quantities vary on average throughout the thickness of the biofilm for the standard case. All variables on the abscissa are dimensionless, while the ordinate represents the biofilm width and ranges from 0 to 280 μm. Each graph shows 70 data points, one for the average of every row of the grid. An analysis of the observed trends shown in Fig. 1 for the standard case follows for original donor substrate, biomass species, and UAP and BAP.

To quantify the heterogeneity for the standard case, we performed a statistical analysis that is summarized in Table 1. We present the range of values (lowest and highest) for the whole biofilm and the total average, or the average of all values in every biofilm compartment. We computed average values and standard deviations for each row and each column. As a measure of heterogeneity, Table 1 presents the standard deviations (σ) of the row and column averages. Since some average values are much smaller than others, we also present the values of σ as percentages of the mean value, which makes the standard deviations directly comparable.

2.1.1. Original donor substrate

Fig. 1 shows that the substrate concentration decreases steadily with distance from the top surface, and it reaches approximately 0.2 for the bottom 100 μm of the biofilm. S = 0.2 corresponds to s = 100 mg/L, or 5 times Ks. The substrate concentrations in each row stay relatively constant, so that concentration is determined almost totally by distance from the sub-
stratum, not by lateral position. The error bars shown are very small, signifying the small variability of substrate concentration within each row. On the other hand, column variability is high (Table 1), since for every column, substrate concentration varies from about 1.0–0.2. Thus, lateral diffusion in the biofilm is sufficient to “level out” the substrate concentration, despite differences in utilization rate due to different densities of active biomass, which are illustrated below.

2.1.2. Biomass species: $X_a$, EPS, and $X_{res}$

Overall, active biomass is very low in the bottom rows of the biofilm column, peaks just above the middle rows, and drops down to lower values in the top rows. In the bottom rows, active biomass is close to zero, because 24.5 days is long enough for all the initially synthesized active biomass to decay almost completely and turn to residual inert biomass ($X_{res}$), which is relatively high. The very top of the biofilm also has a low concentration

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Table 1

<table>
<thead>
<tr>
<th>Variable</th>
<th>Range of Values</th>
<th>Total Average</th>
<th>Average $\sigma$ by row</th>
<th>% of average value</th>
<th>Average $\sigma$ by column</th>
<th>% of average value</th>
</tr>
</thead>
<tbody>
<tr>
<td>$S$</td>
<td>0.189–1.0</td>
<td>0.3479</td>
<td>0.0034</td>
<td>0.98%</td>
<td>0.2130</td>
<td>61.2%</td>
</tr>
<tr>
<td>$X_a$</td>
<td>0–0.7537</td>
<td>0.2430</td>
<td>0.0508</td>
<td>20.9%</td>
<td>0.2125</td>
<td>87.4%</td>
</tr>
<tr>
<td>$X_{res}$</td>
<td>0–0.4887</td>
<td>0.2299</td>
<td>0.0260</td>
<td>11.3%</td>
<td>0.1322</td>
<td>57.5%</td>
</tr>
<tr>
<td>EPS</td>
<td>0–0.1553</td>
<td>0.0652</td>
<td>0.0134</td>
<td>20.6%</td>
<td>0.0402</td>
<td>61.7%</td>
</tr>
<tr>
<td>UAP</td>
<td>0–0.0131</td>
<td>0.0109</td>
<td>$1.85 \times 10^{-5}$</td>
<td>0.17%</td>
<td>$3.85 \times 10^{-5}$</td>
<td>38.7%</td>
</tr>
<tr>
<td>BAP</td>
<td>$0–1.3 \times 10^{4}$</td>
<td>$9.94 \times 10^{-5}$</td>
<td>$2.50 \times 10^{-7}$</td>
<td>0.25%</td>
<td>$3.85 \times 10^{-5}$</td>
<td>38.7%</td>
</tr>
<tr>
<td>CompDen (g COD/L)</td>
<td>0–131.4</td>
<td>80.6</td>
<td>11.42</td>
<td>14.2%</td>
<td>23.54</td>
<td>29.2%</td>
</tr>
</tbody>
</table>

Fig. 1. Average values and error bars for $S$, $X_a$, EPS, $X_{res}$, UAP and BAP throughout the biofilm thickness for the standard case (time is 24.5 days). Each error bar is 2 standard deviations long, with one standard deviation above and below the mean.
of active biomass, but for the opposite reason: the biofilm is very young and has not had enough time to synthesize and consolidate biomass that completely fills in the top compartments; thus, the active biomass and the inert biomass are close to zero at the top.

The active biomass has its peak concentration from about 220 to 250 μm from the substratum, where substantial synthesis has had time to occur, but decay to inert biomass has not yet become dominant. Inert biomass can be produced only after active biomass is synthesized and has had time to decay. Thus, \( X_{\text{res}} \) has its highest concentration below where the active biomass peaks, and \( X_{\text{res}} \) declines steadily from about 120 μm to the top surface of the biofilm.

Since the active biomass moves in a stochastic manner within the CA algorithm, the biomass concentrations between two adjacent compartments may vary drastically. Unlike for \( S \), the plots for \( X_a \) and \( X_{\text{res}} \) show significant small-scale variability in both directions. For example, the density of active biomass at the top of the biofilm is quite irregular, making the standard deviation in those rows high (Table 1), as indicated by the large error bars in Fig. 1. Furthermore, active and inert biomass have high-density and low-density pockets throughout the zones in which their average density is significant.

Although the average active biomass concentration is low in the top and bottom rows, the variability (error bars) is high only in the top rows. This is true because, in the top rows, some compartments are relatively full, but most are almost completely unoccupied, resulting in a low mean value with a high standard deviation. In the bottom rows, the mean value is close to zero, because most compartments have active-biomass concentrations close to zero, due to extensive decay; thus the standard deviation is close to zero as well.

Similar to active biomass, EPS peaks from about 140 to 240 μm from the substratum, although EPS values are lower than active biomass throughout the biofilm. The biofilm is “old” near the bottom of the biofilm, and EPS has decayed to BAP. EPS is at a low concentration in the top layers, since its formation depends on the presence of active biomass, which is very sparse at the top of the biofilm. EPS shows small-scale heterogeneity similar to that of active biomass. The standard deviation by row is low in the bottom rows and gets higher for the top rows, where many of the compartments are nearly unoccupied.

2.1.3. UAP and BAP

Besides \( S \), the other two soluble substrates are UAP and BAP. UAP, which are formed directly during substrate utilization, are consumed by active biomass as an electron-donor substrate and also diffuse out of the biofilm through the top surface. Like \( S \), UAP have little heterogeneity across their rows (very small average \( \sigma \) by row, shown in Table 1, and small error bars in Fig. 1). The UAP concentration is low throughout the biofilm.

Because BAP are formed from the hydrolysis of EPS, they are generated mainly in the 140–240 μm layers, where EPS has an important concentration. The BAP concentration is stable below about 100 μm, since active biomass is low there and not able to consume BAP. Like UAP, the BAP concentration declines toward the top surface because of its consumption by active biomass and diffusion to the bulk liquid above the biofilm. The BAP concentrations also have little heterogeneity across the rows.

2.1.4. Biofilm composite density

The UMDCA model computes the composite density of the solid components for every compartment. Fig. 2a shows the composite density as a shading plot for the standard case. The top, where the biofilm is young and irregular, has small composite densities, since all three solid species have low values. With little of any type of biomass and an irregular surface, the top of the biofilm takes on a “fluffy” nature. The highest composite density, near the middle of the biofilm (100–180 μm), is the result of active biomass, EPS, and inert biomass having significant densities together. The highest composite density does not correspond to the same location as active biomass has its highest density, but is somewhat deeper in the biofilm, where inert biomass has had time to accumulate. The bottom part of the biofilm has a moderate composite density, composed almost totally of inert biomass. The composite density shows small-scale heterogeneity (Fig. 2a). Its average standard deviation by row (as a percentage) lies among those of EPS, \( X_a \), and \( X_{\text{res}} \), while the standard deviation by column is lower than the other solid species (Table 1), since combining all 3 solid species (\( X_a \), EPS, and \( X_{\text{res}} \)) “evens out” their differences.

2.2. Case 2. Higher detachment \( (b_{\text{det}}) \)

We performed a second run by increasing the detachment rate to a much higher value, 0.88/day, which could be created by a high-turbulence environment. This run terminated in 30 days. The rapid loss of active biomass from the top surface slows the net accumulation of biomass and causes the termination time to be longer for case 2 than for the standard case.

Fig. 2b shows the shading plot of the composite density for case 2. As compared with the standard case, the composite density in case 2 is lower overall (a mean value of 66.5 g COD\(_{\text{m}}\)/L), has a wider range of values (0–147 g COD\(_{\text{m}}\)/L), and has a greater variability by column (\( \sigma = 52 \% \) of the mean) and by row (\( \sigma = 51 \% \) of the mean). The biofilm has a rough front made of individual mushroom-type clusters, rather than being smooth and
filling up all the physical space. The channels tend to be wider at the bottom surface than at the top of the clusters. The insides of the clusters show considerable local heterogeneity.

A shading plot of $S$ (not shown) illustrates that $S$ roughly follows the shape of the biofilm clusters, with lighter areas inside the clusters—due to consumption of substrate—and darker areas where there is little biomass growth. The clusters that protrude highest have a higher $S$ value at their outer surface, and this gives them a slight growth advantage that leads to uneven clusters when the detachment is strong. Biomass at the bottom of the clusters sees a low substrate concentration and cannot grow to fill in the channels that develop at the bottom. Thus, once a channel is established at the bottom of the biofilm, it is not easily filled in by synthesis of excess biomass near the bottom of the biofilm.

Active biomass is lower near the top surface for case 2, compared to the standard case, and the top of the biofilm is quite fluffy. The higher detachment rate, which occurs only at the top surface of any biofilm cluster, mainly removes active biomass there, and this keeps the concentration of active biomass low near the top surfaces. In addition, two large biofilm clusters do not extend above 170–200 μm, resulting in a large void in the system’s physical space. Unique to case 2 is that void spaces also are found lower in the grid, making the variability high throughout the biofilm depth. Although the bottom of the biofilm is dominated by residual inert biomass, overall, the biofilm for case 2 has a lower proportion of residual inert biomass, because the loss of active biomass is shifted from decay to detachment, which removes all types of biomass.

### 2.3. Case 3. Reduced consolidation

In this run, we reduce the consolidation feature of the UMCCCA model by altering the $B$ and $\eta$ values of the consolidation model. The new values are $B = 0.5$ (from 0.982) and $\eta = 0.000315/h$ (from 0.0315/h). By using a much smaller $\eta$ value, we make consolidation occur very slowly, and the consolidation ratio $U_c$ remains close to 1-$B$. Thus, case 3 demonstrates the impact of consolidation by nearly removing its effect.

Case 3 terminated in $t = 14.5$ days. The composite-density shading plot for this case is shown in Fig. 2c. Compared to the standard case, case 3 has a lower mean value (42 g COD$_{e}$/L), a smaller range (0–64.5 g COD$_{e}$/L) and average $\sigma$ by row (6.4 g COD$_{e}$/L), and a lower $\sigma$ by column (10.3 g COD$_{e}$/L). Without consolidation, the biofilm is less dense overall. It has a relatively uniform density in the vertical direction and much less local heterogeneity than the other cases. The lower average density means that the same biofilm mass takes up more volume, which accounts for the relatively shorter termination time. In addition, no separate clusters develop.

Our analysis shows that all quantities, except for the substrate, are lower than the standard case. Due to the reduced consolidation, all biomass types are less dense in...
the biofilm. As a result, substrate utilization is lower, and substrate concentrations in every row of the physical space are higher for case 3. Row heterogeneity for the soluble species is minimal, since the biofilm is uniform without some clusters extending more than others. Although the biomass components show the same general trends as in the other cases ($X_s$ and EPS mainly in the top half, with $X_{res}$ mainly in the bottom half), the different types of biomass overlap considerably more. Thus, consolidation accentuates the formation of a low-activity bottom zone and a high-activity upper zone.

### 2.4. Case 4. Low oxygen

In this run, we reduce the maximum oxygen concentration $O_2_{max}$ from 9.2 mg DO/L to 2.5 mg DO/L. This run terminated in 175 days, which is much longer than the standard run, because active biomass and EPS growth is slowed down significantly due to the low oxygen concentration. As a result, the biofilm grows very slowly, while accumulation of residual dead biomass proceeds even in the absence of oxygen, making the biofilm aged, dense, and almost 100% inert. The composite-density shading plot for this case is shown in Fig. 2d.

Ohashi and Harada (1994, 1996) evaluated biofilm adhesion strength by taking photographs of the transparent PVC-made biofilm/substratum interface. They found that, for biofilm that was aged for a long time—over 65 days—cavities began to appear, and the size and the number of cavities steadily increased with increasing cultivation time. Both studies by Ohashi and Harada (1994, 1996) suggest that cavity formation is a crucial factor for deterioration of adhesion strength and coincides with the abrupt deteriorations in tensile strength. They also confirmed experimentally that the adhesion strength was strongly affected by the extent of biofilm maturity, and that biofilms have a tendency to immediately lose their adhesion strength as they became aged, with the most weakened location toward biofilm depth. Ohashi and Harada (1994, 1996) indicate that there is a definite need of clarification of the mechanisms of cavity formation, a need that the UMCCA model can fulfill.

As shown in Fig. 2d, the UMCCA model suggests that, especially under low-oxygen conditions (bottom row of biofilm has 0.49 mg DO/L), cavities form at the base of an old biofilm. As a result, UMCCA results suggest that the integrity of the base of the biofilm is compromised when low dissolved-oxygen concentration leads to a biofilm with cavities at its base. Such loss of biofilm integrity in its base can be extended to possibly provide an explanation for biofilm sloughing, a phenomenon not well understood so far (Rittmann and McCarty, 2001).

### 2.5. Case 5. Lower $s_{max}$

We performed a final run in which we reduced $s_{max}$ to a lower value, 100 mg/L, which is approximately equal to the substrate concentration in the bottom layers of the biofilm for the standard case. The run terminated after 221 days. The lower substrate concentration caused a slower growth rate and, hence, a longer time to have some biomass in each compartment. We show the shading plot of the composite density in Fig. 2e. Unlike the relatively smooth biofilm of the standard case (Fig. 2a), the biofilm grown with the lower $s_{max}$ value is rough, with projecting biofilm clusters and empty channels between the clusters. To compare with the standard case, the composite density in case 5 is overall higher (mean value of 90.7 g COD/L versus 80.6 g COD/L for case 1), has a wider range of values (0–215 g COD/L versus 0–131 g COD/L), and has a greater variability by column ($\sigma = 85.4\%$ of the mean versus 29.2%) and by row ($\sigma = 49.7\%$ of the mean versus 14.2%).

Of high significance is that the variability of original substrate is greater for depths higher than 150 μm, where most of the growth is concentrated in one mushroom cluster. A shading plot of the original donor substrate is shown in Fig. 2f. The substrate-concentration profiles roughly follow the shape of the clusters shown in Fig. 2e—being lightly colored inside all clusters, but darker above the clusters. When compared to the standard case, original substrate is lower for a major part of the biofilm depth, approaching zero for the inner 120 μm of biofilm. About half of the biofilm has an $S$ value less than $K_s$. Although substrate concentration tracks the cluster geometry in a general way, it is not the same for each cluster. The substrate concentration at the top of the largest cluster is significantly higher (very dark shading corresponding to approximately 90%, with 100% at the very top of the grid) than it is at the top of the shorter clusters (shading corresponding to approximately 30%). This non-uniform pattern for substrate explains why the large cluster preferentially develops. Once it protrudes above the top of the other clusters (a random process related to surface inoculation and the CA algorithm), it is exposed to a higher substrate concentration than the other clusters, and this gives it a growth-rate advantage. This cluster effect is not important for the standard case, because $s_{max}$ is enough greater than $K_s$ that the biomass growth rate was not sensitive to differences in $s_{max}$.

A similar cluster effect for low $S$ was predicted by the model of Picioreanu et al. (1998), who defined the $G$ group as the ratio of maximum biomass growth rate to the maximum substrate transport rate. A high $G$ is a “transport-limited regime,” and a low $G$ is a “growth-limited regime.” $G$ increases as $s_{max}$ decreases, as long as $s_{max}$ is not in the saturation, or zero-order, region of the Monod relationship. Picioreanu et al. (1998) observed...
that, when the substrate-transport rate became more limiting (i.e., higher $G$), the biofilm surface became more irregular. Comparing our case 5 to the standard case, $G$ is larger for case 5, and the advantage of a cluster protruding is enhanced.

Due to the lower substrate concentration throughout much of the biofilm, active biomass and EPS are lower throughout the biofilm for case 5. Active biomass and EPS dip at about the 150-μm biofilm depth, because almost all biomass at that depth is in one “mushroom”-shaped cluster. The large cluster has little active biomass (and EPS) at that depth, because its active biomass is much closer to its top surface. The very top of the mushroom cluster is fluffy, being irregular with small $X_a$, EPS, and $X_{rev}$. Residual inert biomass is very high in the bottom rows, since the biofilm in the bottom rows is very old (221 days) and, hence, fully consolidated (i.e. $U_s = 1$). A plateau from about 150 to 200 μm occurs with the transition to all the biomass being in the one mushroom-like cluster above 150 μm.

When compared with the standard case, we see much greater variability in all quantities. Row heterogeneity also is very high for active biomass, EPS, and composite density. The ranges are high whenever $X_a$ and EPS have a high concentration, and they are especially high above 150 μm, where compartments range from nearly unoccupied to significantly filled inside the large cluster.

3. Comparison to experimental data

Bishop et al. (1995) used laboratory-scale rotating drum biofilm reactors with working volumes of 954 ml to develop their biofilms. The outer cylinder of each reactor had removable strips that were wrapped with polyolefin shrink film, offering a surface for the growth of biofilms that could be sampled easily. Influent COD concentrations were 350 and 700 mg/L. The effluent COD concentrations were usually higher than 30 mg/L, with bulk DO in the range of 0.5–2 mg/L. They used a microtome to slice the biofilm into layer samples with a thickness of 10–20 μm. This enabled them to measure the density of the biofilm for different distances from the substratum. We compare the results represented by the model with the experimental data of Bishop et al. (1995) by computing the average composite density in layers of different distance from the substratum.

To simulate the experimental conditions, we ran the UMCCA model using the set of parameters in Table 2. The microbial kinetic parameters, also used in the standard case, are appropriate, since they are typical values of heterotrophic biofilms. Three variables differ from the standard case. The bulk substrate concentrations $s_{max}$ and $o_{2, max}$ were reduced to 30 mg COD/L and 2.0 mg DO/L, respectively, to match the experimental conditions in Bishop et al. (1995). The decay rate ($b$) was set at 0.2/day, a value typical for aerobic heterotrophs in environmental biotechnology (Rittmann and McCarty, 2001).

We employed the same initial and boundary conditions, cellular automaton (CA) algorithm, and grid (150 compartments wide and 70 compartments deep) as in Laspidou and Rittmann (2004). We started by inoculating a random selection of half of the 150 compartments with active biomass that has a dimensionless concentration between 0.5 and 1.0. The biofilm grew and expanded in a stochastic manner according to the CA algorithm, and we present results for different thicknesses and times when the biofilm remains fully aerobic.

### Table 2

| Parameter values used in the UMCCA model to simulate the experiments of Bishop et al. (1995) |
|---------------------------------|---------------------------------|
| $D_a$                           | $1.38 \times 10^{-4}$ m$^2$/day |
| $s_{max}$                       | 350 mg/L                        |
| $u_{max}$                       | 500 mg COD$_p$/L                |
| $b_{max}$                       | 50 mg COD$_p$/L                 |
| $X_{a, max}$                    | 70 g COD$_x$/L                  |
| $X_{eps}$                       | 200 g COD$_x$/L                 |
| $2s_{max}$                      | 220 g COD$_x$/L                 |
| $o_{2, max}$                    | 2.0 mg$_{o_2}$/L                |
| $Y_s$                           | 0.34 mg$_s$/mg$_s$              |
| $Y_p$                           | 0.45 mg$_p$/mg$_p$              |
| $b$                             | 0.2/day                         |
| $b_{det}$                       | 0.15/day                        |
| $f_4$                           | 0.8                             |
| $\dot{q}_s$                     | 28.5 mg COD$_s$/mgCOD$_x$/day   |
| $K_s$                           | 20 mg COD$_x$/L                 |
| $\dot{q}_{UAP}$                 | 1.8 mg COD$_x$/mgCOD$_x$/day    |
| $K_{UAP}$                       | 100 mg COD$_p$/L                |
| $\dot{q}_{RAP}$                 | 0.1 mg COD$_x$/mgCOD$_x$/day    |
| $K_{RAP}$                       | 85 mg COD$_p$/L                 |
| $k_1$                           | 0.05 mg COD$_p$/mgCOD$_s$       |
| $k_{EPS}$                       | 0.18 mg COD$_p$/mgCOD$_s$       |
| $k_{hyd}$                       | 0.17/day                        |
| $\lambda$                      | 0.005/m                        |
| $\eta$                          | 0.0315/hr                       |
| $B$                             | 0.9820                          |
31.4, 40.9, 54.0, and 73.7 days. The value of 0 in the abscissa indicates the biofilm surface, while a value of 1.0 indicates the bottom of the biofilm, next to the substratum. Since these biofilms are relatively thin, they are not limited by oxygen. Fig. 4 shows the DO concentration at the substratum for each biofilm thickness. The lowest substratum DO concentration is 0.5 mg/L for a thickness of slightly over 280 µm.

The average values produced by the UMCCA model match the trends of the measured density distribution very well (Fig. 3). The first set of data (*) (Bishop et al., 1995) was obtained from biofilms of thicknesses between 52 and 130 µm, and the experimental values follow the model outputs for the thicknesses in that range (64 and 96 µm). The other data set (■), which was obtained with biofilms 130–240-µm thick, lies above the first data set and matches well to the modeling result for 164 µm. The modeling curve for the highest thickness (280 µm) lies above the second data set, as we would expect, since that thickness is outside the data range.

For all curves and data sets, average biofilm densities in the bottom layers are 5–10 times higher than those in the top layers. This happens because the bottom layers have biofilm that is much “older” than the ones in the top. A large bioage leads to the bottom layers having a consolidation ratio \( U_c \) much larger than the younger top layers, and, as a result, being packed more densely. In addition, the bottom layers have more time to accumulate inert biomass, which has a higher maximum density, which also contributes to the higher composite density near the substratum. The top layers, which are “young” biofilm, have a small consolidation factor, are mostly active biomass and EPS, and remain loosely packed and fluffy.

The model-described shapes of the 5 biofilms are shown in Fig. 5, which is a shading plot of the composite density of the biofilm. Fig. 5 shows significant “gaps” in the thin biofilms, including channels that extend to the substratum and are up to 20 µm wide. The variability is high because, due to the low \( s_{\text{max}} \), the young biofilm is not smooth, but is rather patchy, with an irregular surface. When compared to the thinner biofilms, the thicker biofilms are less patchy and have higher density at the bottom. Older, thicker biofilms have more time to fill-up the empty gaps between the microbial clusters, making the biofilm smoother and exhibiting less variability. The combination of filling in the channels and also having the clusters consolidate with more inert biomass accounts for the continually increasing average composite density with biofilm age.

### 3.2. Density of active biomass plus EPS

We also calculated the density of active biomass plus EPS for the 5 biofilm thicknesses in Figs. 3 and 5. It was calculated using the dimensionless quantities active biomass \( X_a \) and EPS and the corresponding maximum concentrations:

\[
\text{Density of active biomass plus EPS} = x_{a,\text{max}} \times X_a + \text{eps}_{\text{max}} \times \text{EPS}.
\]

Active biomass and EPS constitute the biomass components that actively growing biofilm produces, and with the modeling cases we show that the concentrations of active biomass and EPS are strongly correlated for all types of conditions.

Parts (a) through (e) of Fig. 6 show the density of active biomass plus EPS for the 5 biofilm thicknesses. We repeat the total biomass results (Fig. 3) for direct comparison. The residual inert biomass is the difference between the two lines. Although total biofilm density increases dramatically deeper into each biofilm, the
density of active biomass plus EPS rises slowly toward the middle of the biofilm. For the older, thicker biofilms, active biomass plus EPS declines toward the substratum, giving a broad peak of actively growing biomass in the middle of the biofilm and more towards the outer surface. In other words, the dramatic increase in the total biofilm density deep in the biofilm is mainly due to residual inert biomass.

Zhang and Bishop (1994) presented data on the density of “viable” and total biomass throughout the biofilm depth for biofilm thicknesses that range from 720 to 2130 μm. To find the “viable” biomass within the biofilm, they measured lipid phosphate concentrations within the biofilm using phospholipids as a predictor of viable biomass. Since lipid phosphate can occur inside living cells and in its EPS, we interpret that the “viable” biomass measured by Zhang and Bishop (1994) is most like active biomass plus EPS in our model. We present the Zhang and Bishop (1994) results for “viable” and total biomass in panel (f) of Fig. 6.

Since the biofilms assayed by Zhang and Bishop (1994) were much thicker than the ones included in our modeling study here, they surely had an anaerobic sub-layer through much of the bottom of the biofilm. This anaerobic sub-layer supported the metabolism and accumulation of fermenting bacteria, something that the UMCCA model does not include. Therefore, we cannot directly compare the density values in this study with those of Zhang and Bishop (1994). However, the general trends that the UMCCA model describes for active biomass plus EPS are similar to those observed for “viable” biomass by Zhang and Bishop (1994). In particular, the total biomass density rises towards the bottom of the biofilm, while the “viable” biomass plateaus about the middle of the biofilm. Consequently, residual inert biomass accumulates and consolidates near the substratum.

The accumulation of an inert solid residual close to the substratum as biofilm ages is supported by other reports. Ohashi et al. (1999) found that the “live” cells, measured by a commercial kit (Live/Dead BacLight Viability Kit L-7007), were not uniformly distributed throughout the biofilm depth. In parallel with the trends described by the UMCCA model, the deep biofilm portion had a lower live-cell ratio compared to the outer surface.

Rittmann and Manem (1992) constructed a one-dimensional model for a multi-species biofilm and supported it with experimental data. They predicted that aerobic heterotrophs dominated near the outer surface of aerobic biofilms, nitrifiers were most important behind the heterotrophs, and inert biomass accumulated most near the attachment surface. The general pattern is the biomass types that are able to grow most rapidly accumulate nearest the biofilm’s outer surface, while slowly growing biomass accumulates nearer the substratum, where it is protected from detachment losses. Their experimental data for nitrification supported the layering concept: the nitrification rate was reduced when organic substrate was at a higher concentration and allowed heterotrophs to grow on top of the nitrifiers.

More recent studies exploiting in situ methods—particularly fluorescent in situ hybridization and micro-electrodes (Daims et al., 2001; Okabe et al., 1999; Okabe and Watanabe, 2002; Watanabe et al., 1995)—have provided strong experimental evidence for the layering predicted by the one-dimensional models. In particular, slower growing nitrifiers were found closer to the substratum than faster growing heterotrophs.

Cooke et al. (1999) included mineral precipitates with residual inert biomass as the “non-living” component that accumulates near the bottom of the biofilm in which
precipitation occurs. They found that the clogging of systems that collect landfill leachate was described well by having the active bacteria on the outside of the biofilm, with the non-living components accumulating near the substratum.

The UMCCA model represents patterns that correspond to the findings of all the experimental studies cited in this section. The slower-growing species—inert biomass, nitrifiers, or mineral precipitates—tend to accumulate away from the outer surface, which is the source of substrate and the location of detachment. The UMCCA model further includes the consolidation of these slower-growing species, which is consistent with the density measurements of Bishop et al. (1995).

4. Conclusions

All outputs of the UMCCA model showed five general trends. First, the concentration profiles for the two soluble microbial products are opposite the profile for original substrate, since they are produced in the biofilm and must diffuse out the top surface. Second, the top of the biofilm is dominated by active biomass and EPS, while the bottom is dominated by residual inert biomass. Within the top layers, active biomass has a much higher concentration than EPS. Third, the top of all biofilms is quite “fluffy,” since the newly synthesized biomass has not had time to fill in the top compartments and consolidate. Fourth, the peak of the composite density does not correspond to the peak of active biomass. Finally, the biomass concentration has considerable local heterogeneity in the vertical and lateral directions, even when the soluble species have generally flat profiles that vary only in the vertical direction.

All UMCCA model outputs showed the following particular effects for the set of parameters presented in this manuscript. Having the substrate concentration be near and below $K_s$ or having a low dissolved-oxygen concentration promotes the formation of the cluster-and-channel structure. Then, a cluster that protrudes above other clusters (due to random effects) experiences a higher substrate (or oxygen) concentration and gains a growth-rate advantage. Low substrate (or oxygen) concentration also slows the biofilm’s growth rate, giving the biofilm more time to consolidate to higher overall biomass density and become more inert. A high specific detachment rate also favors the
cluster-and-channel structure and maintains relatively open channels near the substratum. The high surface-detachment rate accentuates the growth-rate benefit for a protruding cluster. By itself, a high detachment rate keeps the biofilm less dense overall, but with a lower proportion of residual inert biomass. Consolidation shows two dramatic trends. It makes the biofilm denser overall, which slows its vertical expansion rate, and consolidation increases the local heterogeneity in all biomass types.

We also solved the UMCCA model for conditions relevant to the rotating-drum experiments of Bishop et al. (1995), who measured the total biomass density in layers from the substratum. We made direct comparisons for model-described thicknesses up to 280 μm, since these biofilms are fully aerobic and do not have an anaerobic layer of fermenting bacteria. The model outputs showed all the major trends in the experimental data of Bishop et al. (1995): The biofilms become thicker and denser overall with time, and the total biomass density is 5 to 10 times greater near the substratum than near the top of the biofilm. Residual inert biomass continually becomes denser toward the substratum; this is due to the combined effects of consolidation and inert biomass having a larger maximum density. On the other hand, active biomass and EPS do not increase in density toward the substratum, but have a broad peak near the middle and towards the outer surface of the biofilm. Thus, the UMCCA model describes the experimentally observed layering with inert biomass or other slow-growing species near the substratum.

References


