Modeling of biofilm in the water distribution system
Tai-Lee Hu, Chenfang Lin and Wei-Yu Chen

ABSTRACT
In order to understand the growth of biofilm and to serve as the basis of cleaning the water distribution pipeline, this study used a multi-attachment dynamic model to simulate the growth of microorganisms' attachment to the pipe wall. The model had considered attachment, detachment, and propagating factors. The attachment and detachment factors are divided into "cell to cell" and "cell to substratum". Factors for biofilm growth included two possibilities, which was "lateral growth" and "vertical growth". Data set of the biofilm biomass from a laboratory scale water distribution system was measured. The data and the model simulation curves were compared so as to justify the performance of the model.

The results show that several sets of parameters could be identified. From the simulation of biofilm biomass in the pipeline, the microbial growth related with the incubation time. Due to nutrients being restricted, the biomass of biofilm in the water distribution system did not continue to grow and reached a maximum at about Day 40. From the simulation results, it was suggested the time of cleaning the water distribution pipeline be shortened to one or two months.

The model was applied to simulate the tap water biofilm in the pipeline of Kaohsiung city, the second largest in Taiwan with 1.5 million population. The results revealed various levels of risks and the proportionality between the biomass in the water and the growth rate of biofilm. However, most households had the purifying facility of a reverse osmosis system. From the simulation, the facility proved its effectiveness for preventing the intervention of bacteria from the biofilm formation in the distribution pipelines.

Key words | biofilm formation model, water distribution system

INTRODUCTION
Biofilms may develop and accumulate due to contamination in the water distribution system. The formation and development of biofilms in the water distribution system will result in corrosion of pipelines, deterioration of water, the persistence of anti-disinfection and increasing in the outbreaks of waterborne diseases (Schoenen 2002).

The study of biofilm development in pipelines is of great interest to drinking water companies in concern of the effects on both the aesthetics and the public's health (Percival et al. 2000). Not until the 1970s did mathematically based modeling of biofilms begin to study the formation of biofilms. The early efforts focused mainly on substrate flux from the bulk liquid into the biofilm, and the substrate gradients are in one dimension. In the 1980s, stratified dynamic models of multisubstrate/multispecies biofilms were developed (Kreft et al. 2001). Since the 1990s to today, the models developed provide information for the factors controlling the formation of two- and
three-dimensional biofilm morphologies (Wanner et al. 2006). These biofilm models mostly were applied to describe the nutrient removal in reactors used for wastewater treatment such as rotary biological contactors (RBC). The mathematical biofilm models suitable for the wastewater treatment process may not be applicable to describe the biofilm formation in the oligotrophic environment of water distribution systems.

The purpose of this study was the development and validation of a mathematical biofilm model. In the mean time, model parameters of adsorption, detachment and propagation were studied. A multi-attachment concept was used instead of using conventional adsorption formula such as the Freundlich, Langumir or BET. The biofilm biomass of a laboratory-scale water distribution system was measured and used to verify the model. We also applied this model to predict the biofilms formation from influent with different bacterial counts. This study intended to develop and verify a dynamic model to serve as the basis for understanding and making suggestions to clean up water distribution pipelines.

**BIOFILM FORMATION MODEL**

The mathematical biofilm formation model was established based on the three basic mechanisms: attachment, growth and detachment. It is slightly modified from Bouwer (1987) by combining desorption into detachment mechanism. A multi-attachment concept was used to simulate the growth of microorganisms attach to the substratum. In addition, the parameters of the attachment and detachment factors are further divided into “cell to cell” and “cell to substratum”. Factors for biofilm growth have two possibilities, which are “lateral growth” and “vertical growth”. In the oligotrophic environment of water distribution system, the parameters related to substrate concentration (Hermanowicz 2001) are not included.

**Equations for biofilms formation**

Unattached site ($S_0$) on the substratum

\[
\frac{dS_0}{dt} = k_{a1} \times C \times \theta_V \times S_0 - k_{d1} \times C \times \theta_V \times S_0 - k_{d2} \times C \times \theta_V \times S_1
\]

Attachment site with one layer ($S_1$)

\[
\frac{dS_1}{dt} = k_{a1} \times C \times \theta_V \times S_0 + k_{a2} \sum_{i=1}^{n} S_i - k_{a2} \times C \times \theta_V \times S_1
\]

\[
- k_{d1} \times S_1 + S_0 \times \theta_V \times S_1 - S_1 \times \theta_V
\]

(2)

Attachment site with $i$ layers $S_i$ ($i = 2 \sim (n - 1)$)

\[
\frac{dS_i}{dt} = k_{a1} \times C \times \theta_V \times S_{i-1} + k_{a2} \sum_{j=1}^{n} S_j - k_{a2} \times C \times S_i
\]

\[
\times \theta_V \times S_i - k_{d1} \times S_i + S_{i-1} \times \theta_V \times S_{i-1} + S_{i-1} \times \theta_V
\]

\[
- S_{i-1} \times \theta_V
\]

(3)

Attachment site with $n$ layers ($S_n$)

\[
\frac{dS_n}{dt} = k_{a1} \times C \times \theta_V \times S_{n-1} - k_{d1} \times S_n + S_{n-1} \times \theta_V \times S_{n-1}
\]

\[
+ S_{n-1} \times \theta_V
\]

(4)

The model parameters are shown in Table 1.

**Model assumptions**

1. The average radius of a bacterial cell is 1.5 \(\mu\)m.
2. The propagation parameter is divided into lateral growth \(g_l\) and vertical growth \(g_v\), and the ration of growth rate for \(g_l\) to \(g_v\) is 1: 2 $i$, where $i$ indicating the layer number of cell attached to substratum.

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Units</th>
<th>Parameter</th>
</tr>
</thead>
<tbody>
<tr>
<td>$C$</td>
<td>cell/ml</td>
<td>Influent cell concentration</td>
</tr>
<tr>
<td>$g_l$</td>
<td>cm$^2$/site.day</td>
<td>Lateral growth constant</td>
</tr>
<tr>
<td>$g_v$</td>
<td>day$^{-1}$</td>
<td>Vertical growth constant</td>
</tr>
<tr>
<td>$k_{a1}$</td>
<td>cm$^3$/cell.day</td>
<td>Attachment constant of cell-substratum</td>
</tr>
<tr>
<td>$k_{a2}$</td>
<td>cm$^3$/cell.day</td>
<td>Attachment constant of cell-cell</td>
</tr>
<tr>
<td>$k_{d1}$</td>
<td>day$^{-1}$</td>
<td>Detachment constant of cell-substratum</td>
</tr>
<tr>
<td>$k_{d2}$</td>
<td>day$^{-1}$</td>
<td>Detachment constant of cell-cell</td>
</tr>
<tr>
<td>$r$</td>
<td>(\mu)m</td>
<td>Radius of bacteria</td>
</tr>
<tr>
<td>$S_0$</td>
<td>site/cm$^2$</td>
<td>Unattached site on substratum</td>
</tr>
<tr>
<td>$S_1$</td>
<td>site/cm$^2$</td>
<td>Attachment site on layer 1</td>
</tr>
<tr>
<td>$S_i$</td>
<td>site/cm$^2$</td>
<td>Attachment site on layer $i$</td>
</tr>
<tr>
<td>$S_{n-1}$</td>
<td>site/cm$^2$</td>
<td>Attachment site on layer $n - 1$</td>
</tr>
<tr>
<td>$S_n$</td>
<td>site/cm$^2$</td>
<td>Attachment site on layer $n$</td>
</tr>
<tr>
<td>$t$</td>
<td>day</td>
<td>Time</td>
</tr>
<tr>
<td>$\theta_V$</td>
<td>ml/cm$^3$</td>
<td>Water content in the distribution system</td>
</tr>
</tbody>
</table>
3. There are many sites \( n \) in square shape on the substratum for bacteria attaching. \( S_0 \) indicates the number of substratum site without cell attachment, \( S_1 \) represents the sites on the substratum occupied by one cell, and \( n \leq S_1 \) represents all the sites are occupied. \( S_2 \) denotes number of sites of cells attached to \( S_1 \), and in this model the sites of cells attached to \( n \) layer is denoted \( S_0 \).

The Runge-Kutta Method of order 4 (RK-4), with high computing accuracy and widely used in numerical analysis was used (Harier et al. 1989). The numerical accuracy was checked by varying time interval in the calculation. At least four digits of accuracy was generated. The experimental data of biofilms was measured from a laboratory scale water distribution system (Hu et al. 2006).

### RESULTS AND DISCUSSIONS

#### Parameters identification and data fitting

There are several conditions for model simulation. First, the influent cell concentration is 100 CFU/ml, which is the maximal restriction for drinking water. Second, there is \( 1.11 \times 10^7 \) site for bacterial cell attaching to surface area of \( 1 \text{ cm}^2 \) on the substratum under the assumption of bacterial size with radius \( 1.5 \mu \text{m} \). Third, the water content \( \left( \mu_\text{v} \right) \) in the distribution system is 1. Finally, since the pipeline in the laboratory scale water distribution system is sterilized with 75% ethanol (Hu et al. 2006), the initial values for \( S_1 \), \( S \), and \( S_n \) is set to 0. Parameters identification was done by try and error to fit data from laboratory study (Table 2). The biofilms in the water distribution system was studied and measured in May, July and August 2005. In addition to biomass, water temperature was also monitored. 

<table>
<thead>
<tr>
<th>Identified values of parameters</th>
<th>May</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
<td>( k_{a1} )</td>
<td>( 9 \times 10^{-9} )</td>
<td>( 1.27 \times 10^{-8} )</td>
<td>( 1.2 \times 10^{-8} )</td>
</tr>
<tr>
<td>( k_{a2} )</td>
<td>( 1.2 \times 10^{-8} )</td>
<td>( 1.2 \times 10^{-8} )</td>
<td>( 1.2 \times 10^{-8} )</td>
</tr>
<tr>
<td>( k_{d1} )</td>
<td>2.23</td>
<td>2.2</td>
<td>2.2</td>
</tr>
<tr>
<td>( k_{d2} )</td>
<td>0.4</td>
<td>0.53</td>
<td>0.51</td>
</tr>
<tr>
<td>( g_l )</td>
<td>( 6.6 \times 10^{-8} )</td>
<td>( 6.6 \times 10^{-8} )</td>
<td>( 6.6 \times 10^{-8} )</td>
</tr>
<tr>
<td>( g_v )</td>
<td>0.25</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

Figure 1 | Simulated dynamics of biomass in water distribution system.

Figure 2 | Simulation of average biofilm thickness.

Figure 3 | Simulation of increasing time span on the verse time accumulation of biomass.

Table 2  
<table>
<thead>
<tr>
<th>Parameters</th>
<th>May</th>
<th>July</th>
<th>August</th>
</tr>
</thead>
<tbody>
<tr>
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<td>( 1.27 \times 10^{-8} )</td>
<td>( 1.2 \times 10^{-8} )</td>
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</tr>
<tr>
<td>( g_v )</td>
<td>0.25</td>
<td>0.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>
The detachment constant of cell-substratum \(k_{d1}\) is 2.2 (day\(^{-1}\)), and the detachment constant of cell-cell \(k_{d2}\) is 0.4–0.53 (day\(^{-1}\)). The lateral growth constant \(g_l\) is 6.6 \(\times\) 10\(^{-8}\) (cm\(^2\)/site.day), and the vertical growth constant \(g_v\) is 0.25–0.3 (day\(^{-1}\)).

Simulation results of the dynamic biofilm formation fitted the experimental data (Figure 1). The coefficient of determination \(R^2\) of F-test showed significant correlation \((R^2 > 0.987)\). Single cell successively detaching from the biofilm was also simulated, results showed no obvious difference with the simulations of sloughing detachment.

Figure 2 shows the simulation results of biofilm thickness to the incubation time. The average biofilm thickness increased with time, in July and August where the average water temperature (31.5°C) were higher than in May, the thickness of biofilm could reach 250 \(\mu\)m. While in May the average water temperature was 29°C, after incubation for 28 days, the thickness of biofilm reached 200 \(\mu\)m.

If the incubation time was expanded, the simulation showed that at day 40, the biofilm biomass of July and August reached plateau, and the biomass would no longer increased, however, the biofilm biomass of May would increase with incubation time until at day 80 (Figure 3). In the oligotrophic environment of water distribution system, nutrients is the main factor controlling the growth of microorganisms, besides that water temperature and velocity are also affecting the biofilm formation (Percival et al. 2000). From the results of Figure 4, the unoccupied site \(S_0\) on substratum decreased with increasing time span. There were less \(S_0\) in May compared with that of July and August reflected the slower growth rate of biofilms in May.

This model could simulate the growth of biofilms. Figure 5 indicates that the biomass in layers one, \(i\) to \(n\) is \(S_i > S_1 > S_n\), in which \(S_i\) represented \(S_2–S_9\) (May) and \(S_2–S_8\) (July and August). Maximal layer \((S_n)\) for biomass attachment of May, July and August were \(S_{10}\), \(S_9\) and \(S_9\), it showed that in May the growth rate of biofilm formation was slow, the cells stacked higher than those having faster growth rate of July and August. The biomass distribution in layers of \(S_i\) or \((S_2–S_{n-1})\) also reflected biofilm biomass decreased with increasing stacks. The shear forces by the water velocity in the distribution system would result in the detachment of biofilm, and usually it starts from the bulk boundary (Percival et al. 2000). The numerical accuracy was verified using analysis of time interval \((\Delta T)\) on the effect of biofilm biomass and confirmed all the numerical values are within \(10^{-5}\) of accuracy.

In order to differentiate the importance among those six parameters used in this study, the relative sensitivity factor (RSF) was compared. From the results of Table 3, the important order of these parameters is \(k_{d1} > g_l > g_v > g_e > k_{d2} > k_{a1} > k_{a2}\). The sequences for May, July and August are the same. It indicated that the most important factor affect the biofilm biomass is the detachment of cell
from the substratum, i.e. the pipeline wall; the least important factor is the attachment of the cell to cell in the biofilm. It implied that we can control the drinking water quality by backwashing or disinfection to remove the biofilms from the pipeline wall.

The lateral growth ($g_l$) of biofilms is more important than the vertical growth ($g_v$) which is for the stacks of biofilm. This characteristics coincided with what Characklis & Marshall (1990) described, in which the development of biofilms in water distribution system is in the thin and spread form.

**Simulation application**

This model was applied to simulate and predict the biofilms formation of tap water and reverse osmosis treated water samples. The total count of tap water from Kaohsiung city, the second largest with 1.5 million populations in Taiwan, is 160 CFU/ml–840 CFU/ml, which is two to eight folds higher than the regulation, and the residual chlorine in the tap water is also lower than the regulation of 2 mg/l (Lo & Lin 2003). Figure 6 shows the biofilm biomass increasing with the influent total count. After 35 days the water passes through the distribution system, the biofilm biomass will reach maximum, and the total biofilm biomass is 80–650 CFU/cm². The simulations of the reverse osmosis treated water give similar results (Figure 7). The simulation of vertical propagation of biofilm formation with influents of 0.01–840 CFU/ml resulted that after 40 days the number of biofilm stacks in different water quality levels were 0, 2, 12, 12, 13 and 14, respectively (Figure 8). Theoretically, the lower bacterial count of the influent in

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Attachment ($k_{a1}$)</th>
<th>Attachment ($k_{a2}$)</th>
<th>Detachment ($k_{d1}$)</th>
<th>Detachment ($k_{d2}$)</th>
<th>Propagation ($g_l$)</th>
<th>Propagation ($g_v$)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Identified value</td>
<td>$9 \times 10^{-9}$</td>
<td>$1.2 \times 10^{-8}$</td>
<td>2.2</td>
<td>0.4</td>
<td>$6.6 \times 10^{-8}$</td>
<td>0.25</td>
</tr>
<tr>
<td>RSF</td>
<td>1.0</td>
<td>$1.6 \times 10^{-5}$</td>
<td>-5.1</td>
<td>-3.1</td>
<td>3.8</td>
<td>3.4</td>
</tr>
<tr>
<td>Order of importance</td>
<td>5</td>
<td>6</td>
<td>1</td>
<td>4</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>

* data of May.

![Fig 6](https://example.com/fig6.png)  
**Figure 6** | Effect of influent bacterial count on the formation of biofilm in the water distribution system.

![Fig 7](https://example.com/fig7.png)  
**Figure 7** | Simulation of biofilm formation in reverse osmosis water samples with two levels of bacterial counts.

![Fig 8](https://example.com/fig8.png)  
**Figure 8** | Effect of influent bacterial count on the stacks of biofilm.
the water distribution, the less is the stacks of biofilms in the system. The simulations indicated that the water samples with higher than 100 CFU/ml the regulation standard, all have only 12 stacks in the biofilm. This result again confirmed the parameters' sensitivity analysis of $g_i > g_v$.

**CONCLUSIONS**

1. A multi-attachment dynamic biofilm model was established. Attachment, detachment and propagating factors are considered. The attachment and detachment factors are divided into “cell to cell” and “cell to substratum” for consideration. Factors for biofilm spreading had two possibilities, “lateral growth” and “vertical growth”. This model also describes the stacking of bacterial cells in the biofilms.

2. The results of parameter’s sensitivity analysis showed the detachment parameter ($k_{d1}$) is the most important factor among the biofilm formation. The analysis provided the water supply company with help in making decision for cleaning up the pipeline.

3. The simulation results showed that at about Day 40, the biofilm biomass reached a constant, and the time for the biomass to reach maximum is unrelated to the water temperature and the influent biomass in our experiments.

4. The model was applied to simulate the tap water biofilm in the pipeline with high level of bacterial counts. The results revealed various levels of risks and the proportionality between the biomass in the water and the growth rate of biofilm.

**REFERENCES**


Hu, T.-L., Ko, F. N. & Lin, C. 2006 Detection of *Legionella* sp. and *Mycobacterium* sp. in biofilms from a model distribution system using polymerase chain reaction, 106th General Meeting, American Society for Microbiology.


