# A Multispecies Biofilm Growth Model

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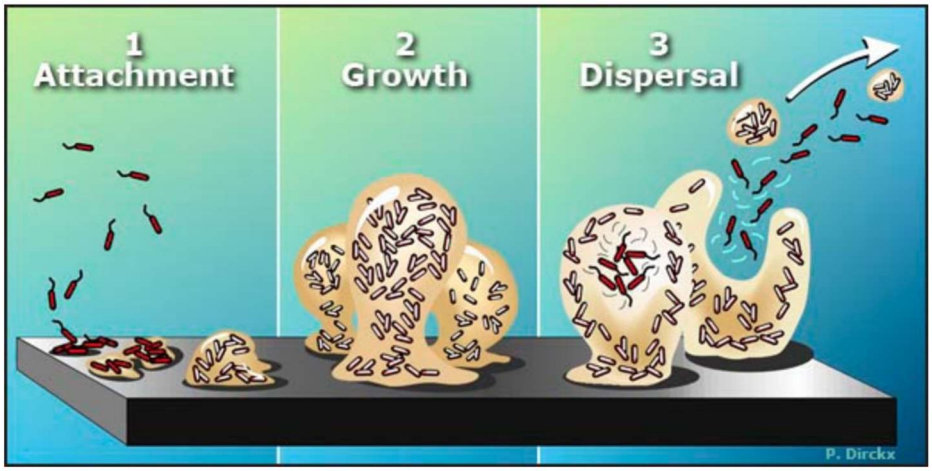
## Introduction

A biofilm is a structured collection of bacteria that adheres to organic or inorganic surfaces. The development of biofilms is relevant to many fields including wastewater management, industrial processes, natural aquatic ecology, and medicine. In a typical formation of biofilm, bacteria compete in a bulk liquid for nutrients, called substrates. The bacteria reproduce until they attach to a surface where it then forms a protective biofilm. This protective layer allows tolerance to antibiotics and other threats. As substrate diffuses into the biofilm, it grows in thickness until it reaches the dispersion stage. In this stage, the biofilm releases bacteria into the bulk liquid to colonize additional surfaces. Applications of biofilms are widespread, for example, most water treatment plants now use biofilms for secondary filtration (Mancl, 2009).

**Figure 1** The Biofilm Life Cycle




**Note. From Dirckx, P. A Brief Introduction to Biofilms**

The Fundamentals of Biofilm Engineering (EBIO 566) course taught by Dr. Phil Stewart in Chemical and Biological Engineering needs a new program to numerically model biofilm growth and dispersion in a continuously stirred tank reactor. The existing software is old, difficult to use, and the source code is no longer available making it impossible to update or modify. The goal of

this project is to develop a new program to model biofilm growth in the presence of multiple substrates. Such a model would need to both accurately predict biofilm thickness and be easy to use for those unfamiliar with coding languages. A successful model would need to account for microbial growth, the effects of multiple substrates and biofilms on growth rates, the diffusion of substrates into the biofilm, the dispersion of biomass into the bulk liquid, and concentrations of substrates in the bulk liquid.

## Background

A working program to model single species biofilm growth in the presence of a single substrate has already been developed using the equations outlined in the methods section. It was based on the methods used in the Biofilm Accumulation Model (BAM), a historical model for biofilm growth that was developed at the Center for Biofilm Engineering over 30 years ago. BAM stems from the BIOSIM model which was grounded in the works of Oskar Wanner and Willi Gujer.

In my studies, I have been drawn to coding and numerical modelling and therefore have taken several numerical solutions courses. Most notably, I completed a graduate level numerical solutions course with Dr. Mark Owkes that used MATLAB extensively and covered all the methods utilized in this project. This is an opportunity to explore this field and learn about modelling techniques and my background positions me well to ensure success on this project.

## Methods

There are three main parameters that are needed to model biofilm thickness. These parameters need to be expanded for multiple substrates and biofilm species and packaged into a GUI. The first two are the rate of change of biomass concentration and the change in substrate concentration in the tank, how fast the proportions of bacteria and substrate suspended in the

tank liquid are changing. These effect the growth rate and can be represented by the following equations with initial conditions of $x(t = 0) = x_0$ and $S(t = 0) = S_0$ (Stewart, 1994)

$$\frac{dx}{dt} = \mu(S)x - \frac{Qx}{V} + \frac{v_{det}AX_b}{V} \qquad \frac{dS}{dt} = -\frac{\mu(S)x}{Y_{xs}} + \frac{QS_{in}}{V} - \frac{QS}{V} - \frac{AB_{flux}}{V}$$

Where $x$ is the biomass concentration, $t$ is time, $S$ is substrate concentration, $v_{det}$ is detachment velocity, μ is the biofilm growth rate, $Q$ is flowrate into the tank, $V$ is the tank volume, $A$ is the wetted surface area on the biofilm, $X_b$ is the biofilm biomass density, $Y_{xs}$ is the change in substrate due to biofilm growth, $S_{in}$ is intake substrate concentration, $B_{flux}$ is substrate consumed due to growth. These parameters must be expanded to vectors to account for more than one biofilm species and substrate. $S_{b,j}$, the concentration of the jth substrate in the biofilm is the final parameter needed to calculate biofilm thickness (Stewart, 1994)

$$\frac{d^2S_{b,j}}{dz^2} = \frac{\mu_j(S_b)X_b}{Y_{xs}D_e}$$

Where $dz$ is change with respect to distance. This equation is a simplification resulting from assuming a pseudo steady state condition. Since the timescale of diffusion in the biofilm is much faster than the relevant timescale for the system, this is a valid measure that avoids a very small timescale requirement.

The growth rate of a biofilm is dependent on substrate concentration and determines all other parameters. There are several models used to determine growth rate, the model selected depends on biofilm species and substrate. The growth rate equations are (Stewart, 1994)

$$\mu = \mu_{max}\frac{S}{K_m + S} \qquad \mu = \mu_{max}\frac{S_x}{K_{m,x} + S_x}\frac{S_y}{K_{m,y} + S_y} \qquad \mu = \mu_{max}\frac{S_x}{K_{m,x} + S_x}\frac{1}{1 + \frac{S_y}{K_{i,y}}}$$

Where $K_m$ and $K_i$ are the Monod half saturation coefficient and the inhibition coefficient, a measure how a specific substrate helps or harms the biofilm. The equations from left to right are the Monod Growth Rate, The Double Monod Growth Rate, and the Inhibition Model. They are used to model single, double, and inhibitive substrates (Bailey, 2021).

The model assumes a well-mixed tank with biomass and substrate concentrations that vary with respect to time in the tank and with respect to time and space within the biofilm. Since the tank concentrations vary only with time, they can be solve simultaneously using a Runge-Kutta scheme (Chapra & Chanal, 2020). The scheme uses the slope at three intermediate points to improve the approximation at each step and an error estimate to optimize time step size. Since the equation for substrate concentration in the biofilm $S_b$ is dependent on both time and space, it must be discretized by the direct finite-difference method leading to the equation (Stewart, 1994)

$$\frac{S_{b,j,i-1} - 2S_{b,j,i} + S_{b,j,i+1}}{dz^2} = \frac{\mu_j(S_b)X_b}{Y_{xs}D_e}$$

The dependance on the growth rate μ introduces a nonlinearity that prevents solving in one step, meaning the equation must be solved iteratively. The discretized equations lead to a system of $N_s$ x $N_z$ equations for the $N_s$ x $N_z$ unknowns $S_{b,j,i}$. Where $N_s$ and $N_z$ are number of substrates and number of spatial grid points in the biofilm respectively. These can be linearized and organized into a matrix and solved iteratively by matrix inversion (Chapra & Chanal, 2020). Once the

substrate concentration throughout the biofilm has been found, the change in biofilm thickness can be computed using the following first order differential equation (Stewart, 1994)

$$\frac{dL_f}{dt} = \bar{\mu}(S_b)L_f - k_{det}L_f^2$$

Euler's method can be used to compute the solution at the next time step (Chapra & Chanal, 2020). The biofilm thickness will need to be packaged into a Graphics User Interface (GUI) to make it readable so it can be used as a teaching aid. This will be done using native MATLAB GUI features. With these an interface can be created that will allow a user to intuitively enter substrate and biofilm properties and clearly display model results. When the new code is complete, it will be used to analyze a multispecies biofilm system as an illustration of the utility of the model. This work will have potential for publication as original research.

## Timeline

The timeline will include three benchmarks for success, each being a significant improvement on existing software. First the code will be update for multiple substrates by the middle of Fall Semester 2021. Then, multiple biofilms will be added by early Spring Semester 2022. Lastly, a GUI will be implemented by the end of Spring 2022.

## Collaboration with Faculty Sponsor

Work will be done under Dr. Mark Owkes as a faculty sponsor. Dr. Owkes' research focuses on improving numerical solutions to multiphase fluid flows. He also teaches several MATLAB based numerical solutions courses. Weekly meetings will be held with Dr. Owkes as well as Dr. Phil Stewart to discuss progress and steps forward.

## References Cited

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