1. **Introduction:** Provide a statement of the objective(s) and the anticipated significance of the work to your field of study. What problems will be investigated? What hypothesis will be tested? We suggest that the introduction begin with a brief description of the project in general terms before the more technical aspects of the project are discussed. **INBRE Applicants - be sure clearly identify what contributions this project makes to the fields of biomedical or public health research.**

**Introduction**

A biofilm is a structured collection of bacteria that adheres itself to organic or inorganic surfaces. The development of biofilms is relevant to many fields including wastewater management, aquatic habitat study, and medicine. In a typical formation of biofilm, bacteria compete in a bulk liquid for nutrients, called substrates. The bacteria grow biomass until it attaches itself to a surface where it then forms a protective biofilm. This protective layer allows resistance 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.

A picture containing graphical user interface

Description automatically generated

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. The existing model 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 that can model the growth biofilms 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 affects of multiple substrates 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.

1. **Background:** Provide a brief review of the work that has been done in the project area together with complete references in appropriate professional style. This section should also include any personal information about you that would indicate to the reviewers your qualifications for successfully completing this project, including a statement of how the project will contribute to your academic and career goals.

**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. This work was based on the methods used in the Biofilm Accumulation Model (BAM), a model for biofilm growth that was developed at the Center for Biofilm Engineering. BAM stems from the BIOSIM model which utilized the works of Oskar Wanner and Willi Gujer.

I have taken several numerical solutions courses in my mechanical engineering degree. Most notably, I recently completed a graduate level numerical solutions course with Dr. Mark Owkes that used Matlab extensively and went over all the methods utilized in this project. Throughout my time at Montana State, I have been drawn to coding and numerical modelling as it relates to engineering. This is an opportunity to explore this field and learn about modelling techniques.

1. **Methods:** Provide a detailed description of the research methods that you will use in the project. This should include a justification for the specific approach that you will use. For example, how do the methods answer the questions that have been posed, test the hypothesis, or lead to the desired goal?

**Methods**

The rate of change in biomass and substrate concentration can be modeled by the following equations and initial conditions of x(t=0)=x0 and S(t=0)=S0.

These two differential equations can be packaged into a single function. Once this is done, they can be solved using a Runge-Kutta numerical method based on the intrinsic Matlab function ODE23. The Runge-Kutta scheme calculates the slope at three intermediate points between iterations to improve the accuracy of the estimation.

There are several equations that can be used to model biofilm growth as a function of substrate concentration. The model selected depends on biofilm species and substrate type and calculates the growth rate to be used in several calculations. The first equation is the Monod Growth Rate.

The Double Monod Growth Rate models growth as a function of two substrates ‘x’ and ‘y’.

The final equation is the Inhibition Model.

The diffusion of substrates into the biofilm can be represented by

Where the first term on the right-hand side represents substrate diffusion into the biofilm and the second represents substrate consumed for biofilm growth. This can be simplified to the following equation by assuming a pseudo steady state condition for the substrate concentration.

This equation can be discretized by the direct finite-difference method leading to the equation

A wall and no-flux boundary condition is implemented at the bottom of the biofilm using the following equation.

The diffusion of substrate into the biofilm is controlled by the diffusion of substrate through the bulk liquid leading to the following flux-matching boundary condition.

The derivative can be discretized using a finite-difference operator to yield the following.

This can be simplified to

The equations described lead to a system of Ns x Nz equations for the Ns x Nz unknowns Sb,j,i. These can be packaged into a matrix and solved iteratively by matrix inversion.

The flux term describes the growth of biomass in the biofilm and can be described as

Using the following first order differential equation, the biofilm thickness can be computed.

Where the first term on the right-hand side represents the growth velocity and the second term represents the detachment velocity. Euler’s method can be used to compute the solution at the next time step. Where the superscripts n and n+1 represent information at time steps n and n+1.

1. **Timeline:** Provide dates for the initiation and completion of each phase of the project. Attempt to lay out a reasonable schedule taking into consideration all phases of the research and final deliverables.
2. **Collaboration with Faculty Sponsor:** Provide a description of the way you and your faculty sponsor will collaborate on the project. The faculty sponsor should play a significant role in responding to your ideas, providing advice for new directions and resources, discussing the implications of the results, and helping you prepare for your public presentation. Will there be regularly scheduled meetings between you and your sponsor? Explain how the project relates to the ongoing work of your sponsor, if this is the case.

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.

1. **References Cited (include in an additional page within the project proposal):** Include a list of any literature that you have cited in the proposal. Nearly all good science and engineering proposals cite papers reporting related results, describing the methods to be used or providing background information. Please note-the review panel rarely recommends funding for proposals without adequate references.

Dirckx, P. (2010). *The Biofilm Life Cycle* [Illustration]. A Brief Introduction to Biofilms. https://www.cs.montana.edu/webworks/projects/stevesbook/contents/chapters/chapter001/section002/green/page001.html

Stewart, P. S. (1994). Biofilm accumulation model that predicts antibiotic resistance of Pseudomonas aeruginosa biofilms. *Antimicrobial Agents and Chemotherapy*, *38*(5), 1052–1058. https://doi.org/10.1128/aac.38.5.1052

Wanner, O., & Gujer, W. (1986). A multispecies biofilm model. *Biotechnology and Bioengineering*, *28*(3), 314–328. https://doi.org/10.1002/bit.260280304

1. **Report on Previous Research Experience (please save and upload this as a separate document):** If you have done any previous research as an undergraduate you must include a 1-2 page (double-spaced) summary of your research results or creative products.*Please note-if you have received funding from USP or INBRE your proposal will not be considered unless you complete this section.*