In the modeling part, we are mainly working on simulating the function of the fermentation system at different levels and serving suggestions for future implementation.

We first introduce the pathway of our project.

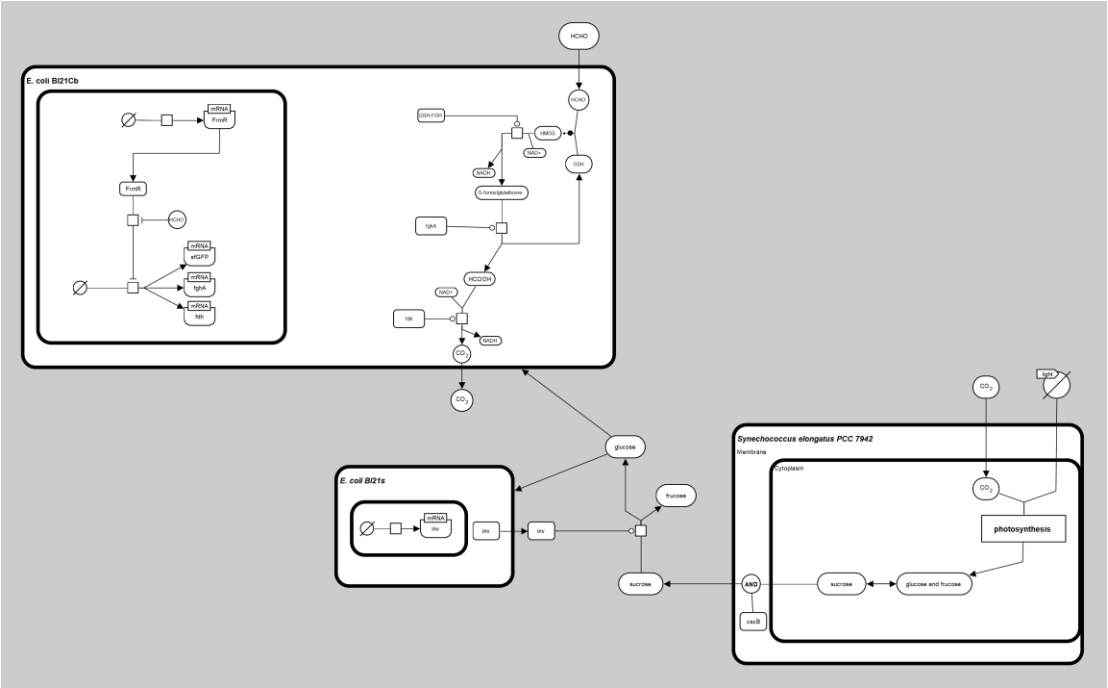


Figure 1 The biochemical pathway of the project. *Escherichia coli Bl21* and *Synechococcus PCC 7942* are two selected engineering microorganisms. *E. coli Bl21Cb* is responsible for the degradation of formaldehyde, *Synechococcus PCC 7942* for sucrose production, and E. coli Bl21s for extracellular sucrose hydrolysis for both *E. coli* to digest. The figure follows the SBGN standard (Novère et al., 2009).

The work consists of two parts, flux balance analysis (FBA) and diffusion model of alginate beads considering bacterial metabolism, specifically, the photosynthesis of *Synechococcus PCC 7942,* the formaldehyde degradation function, and sucrose hydrolysis by two types of *E. coli*.

1. Flux Balance Analysis (FBA)

The first part is about flux balance analysis, an approach for studying a genome-scale metabolic network, containing almost all known metabolic reactions, genes, and related enzymes for an organism (Orth, 2010). The reason we chose this approach as the simulation part is not only because of the constructed published microorganism models, but also through dynamic FBA and some well-developed toolboxes such as COBRA (Heirendt et al., 2019) and COMETs (Dukovski et al., 2021), we can track the changes in metabolite concentrations in the presence of complex reactions within microbial bodies, and even simulate the co-cultivation of blue algae and e. coli.

1.1 Verification

First of all, we need to verify the model. We searched for the existing E. coli Bl21 growth experiment data (Christensen & Eriksen, 2002) to compare with the chosen model iB21_1374 (Monk et al., 2013). We adjusted the intake limitation of glucose intake of the model to restrict it from unlimited nutrient intake, and the simulation result is presented below.

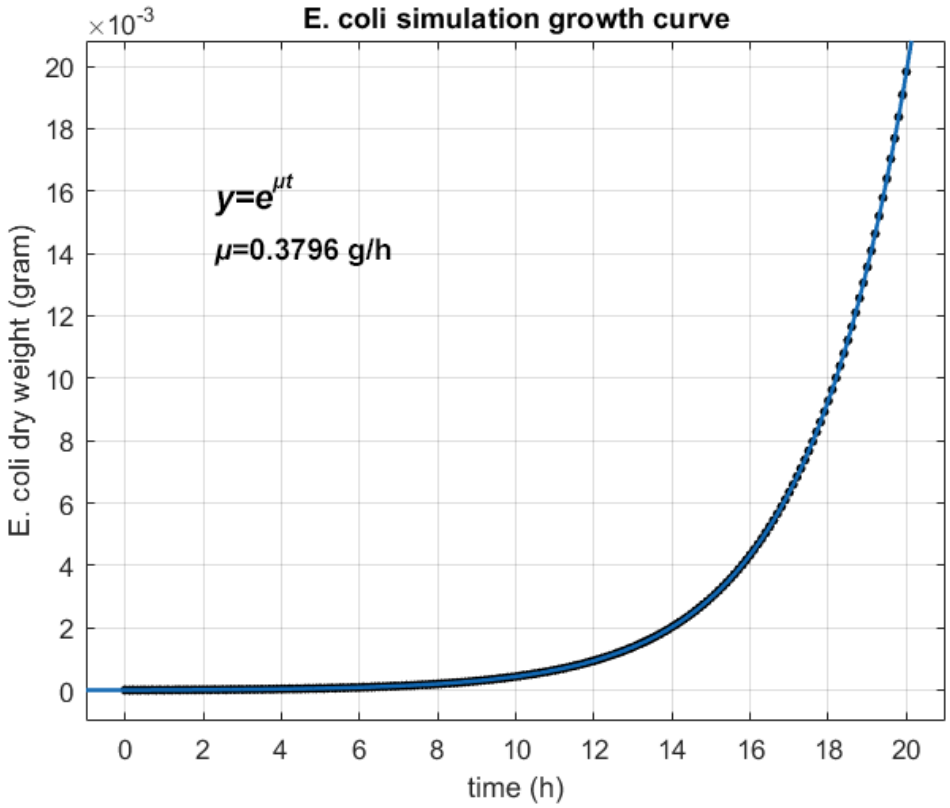
Table 1. Specific rates of growth and yield coefficients for growth of *E. coli* BL21 on glucose as carbon substrate.

Parameters	**Reference value** (Christensen & Eriksen, 2002)	**Simulation value**
μ(gDW h^{-1})	0.38±0.03	0.3796
$Y_{\mathrm{x/glc}}(\mathrm{gg}^{-1})$	0.41±0.01	0.5109
$Y_{\mathrm{NH_4/x}}(\mathrm{mmolg}^{-1})$	9.6±0.8	11.22

μ(gDW h^{-1}), specific growth rates estimated from gram in dry weight,
$Y_{x/glc}(\mathrm{gg}^{-1})$, yield of biomass per unit of glucose consumed.

The equation selected to fit the curve is

$$m = e^{\mu t} + m_0 \tag{1}$$



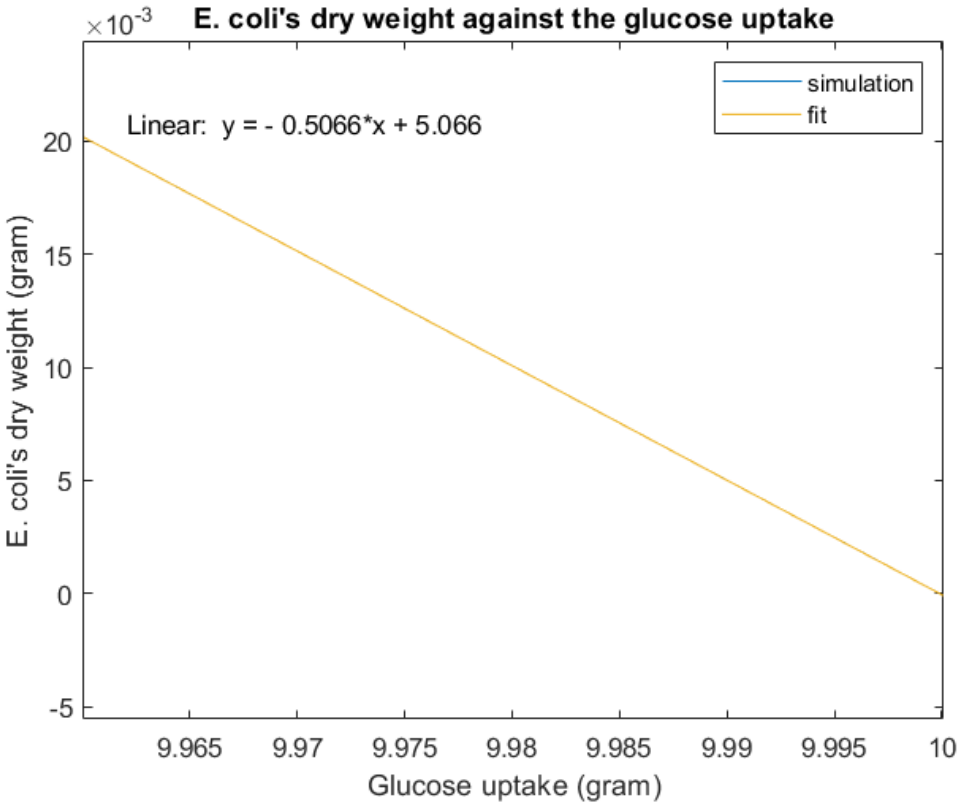


Figure 2 Fitted curve of E.coli's dry weight against

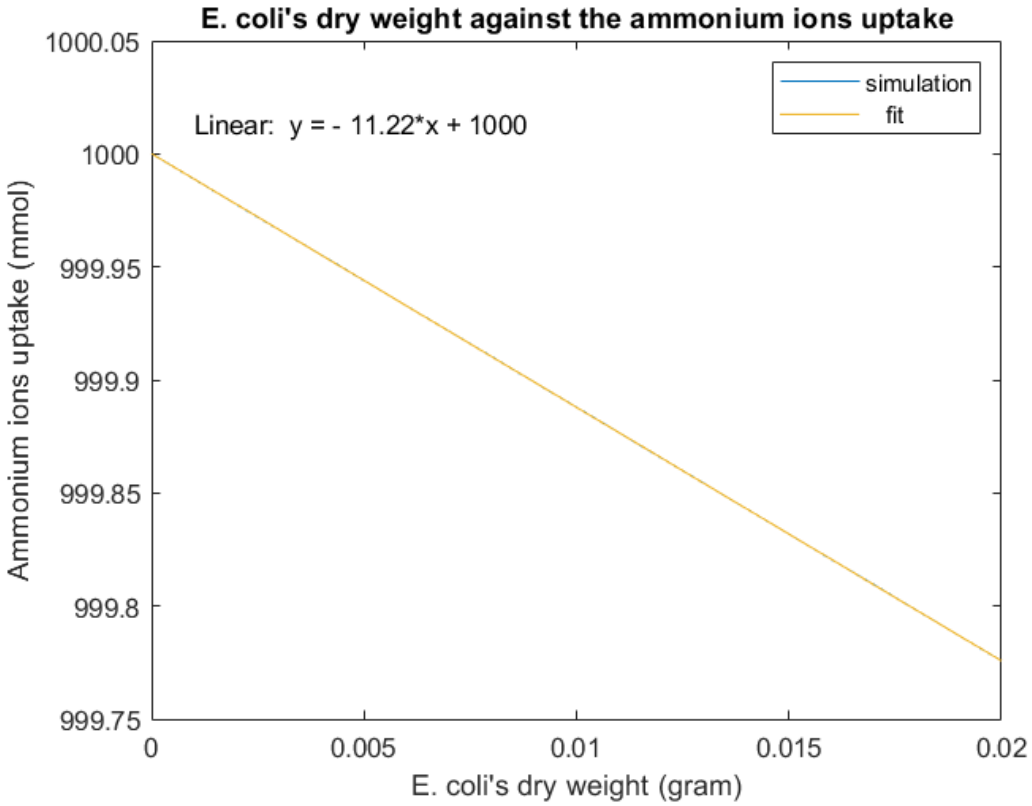


Figure 3

Notice that the blue line for the simulation result is covered by the fit line.

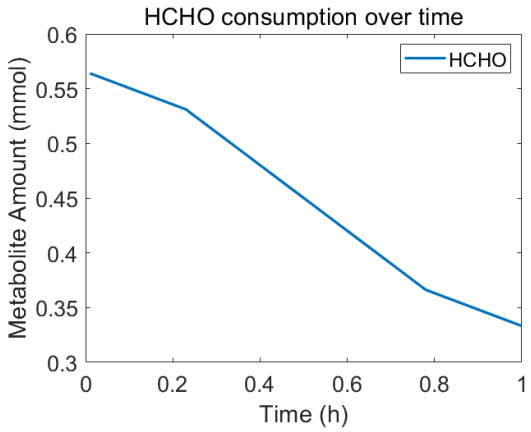
Although the result is not perfectly consistent with the reference value, the bias is acceptable and predictable, for the real case has many more hidden variables, such as temperature and thermodynamic process.

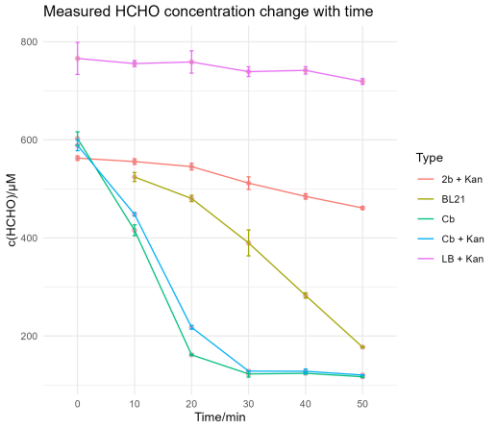
1.2 Modification

Then, we modify the model according to the metabolite pathway.

1.3 Dynamic-FBA

After that, we adjusted the parameters to fit the experiment data, and the simulation result of *E. coli*'s HCHO consumption is presented below.





~~1.4 Multi-species dFBA (dynamic FBA)~~

~~This part gives a general idea of how the coculture system works.~~

2. Diffusion Model

This model is aimed at giving an insight into how alginate beads affect the metabolite of the microorganism embedded. In our project, we make three types of beads, one with blue algae for sucrose production, one with e. coli for sucrose degradation, and one with e. coli for formaldehyde degradation. During the development, we realized that we could increase the accuracy by the embedding ODE system, which describes the formaldehyde degradation pathway.

The model can be separated into two parts: diffusion and metabolite. We first developed the diffusion model and started a rough simulation, then combined the metabolite part with the diffusion model.

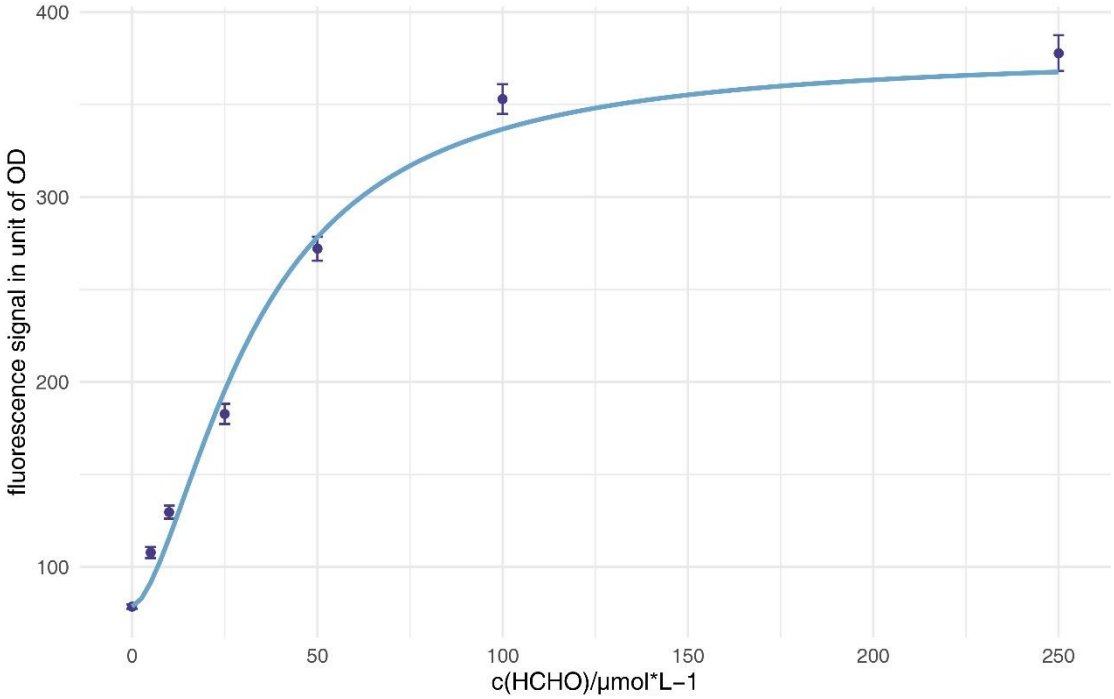
2.1 Metabolite function

Before we start, notice that one bead will only have one function, and we will not simulate multiple beads together for now.

2.1.1 Formaldehyde detection

The detection is based on the FrmR operon regulation on GFP, and the outcome is that when formaldehyde is present, the beads will give green fluorescence under UV light.

Based on the research by Woolston et al. (2018), we chose Hill's equation to fit the experiment data, and the result is presented below.



Fitted equation:

$$S = S_{min} + (S_{max} - S_{min}) \times \frac{[F]^n}{K_m^n + [F]^n} \quad (2)$$

Where S represents the fluorescence signal, and [F] represents the formaldehyde concentration. The result met our expectations.

Parameters	Estimate	Std. error	P
K_m	32.7494	2.5972	5.57e-05 ***
n	1.6467	0.1996	4.27e-04 ***

2.1.2 Formaldehyde degradation

The reason we use the ODE system to model this pathway again, after FBA, is that it is more convenient to build the ODE system into the simulation process.

(Wait to be integrated)

2.1.3 Photosynthesis

We describe photosynthesis with the Michaelis-Menten equation and obtain the parameters through curve fitting with data from the wet lab.

2.1.4 Sucrose hydrolysis

This could be summarized as a Michaelis-Menten equation.

$$v = \frac{V_{max} \times c_{sucrose}}{K_m + c_{sucrose}}$$

2.2 Diffusion model

2.2.1 Mathematics

The diffusion model is based on Fick's second law

$$\frac{\partial c}{\partial t} = \frac{\partial^2 c}{\partial x^2} D \tag{3}$$

In three-dimension space, the formula can be rewrote with Laplacian operator

$$\frac{\partial c}{\partial t} = \nabla^2 cD \tag{4}$$

Where

$$\nabla^2 = \frac{\partial^2}{\partial x^2} + \frac{\partial^2}{\partial y^2} + \frac{\partial^2}{\partial z^2} \tag{5}$$

Which means for a point, the change of concentration within unit time equals the point's divergence times original concentration times the diffusion coefficient of the substance in a specific matter, in this case, the alginate is the matter.

The diffusion coefficient is related to the type of substance and the concentration of the medium, the values are given below, all at 0.2% of alginate.

Diffusion coefficient	**Value($mm^2 * s^{-1}$)**	**Reference**
$D_{glucose\_water}$	6.7	(McDonald, 1956)
$D_{sucrose\_water}$	5.2	(Cussler, 1984)
$D_{sucrose\_gel}$	1.73	(Pu & Yang, 1988)
$D_{sucrose\_gel}$	1.73	(Pu & Yang, 1988)
D_f		Formaldehyde

This formula tells us how a substance diffuses into another quantitively, and more importantly, based on former work, we could set up a simulation with the finite element method (FEM), which is suitable for solving partial differential equations and visualization.

2.2.2 Finite Element Method (FEM)

We will briefly introduce how it works, to learn more details, please refer to some professional resources.

FEM is a numerical method to approximate the solution of continuous, boundary-initial-value problems described by partial differential equations as discrete models (Tekkaya & Soyarslan, 2014), which is commonly applied in practice in the engineering field.

Why did we choose FEM? Sometimes obtaining an analysis solution for the model is unnecessary, and we can tolerate the bias, then, instead of solving the model directly, we split the space into thousands even millions of nodes to discretization the space, and update the state of nodes from initial state step by step to discretization the time to simplify the problem.

With this idea, we develop the following simulation algorithm.

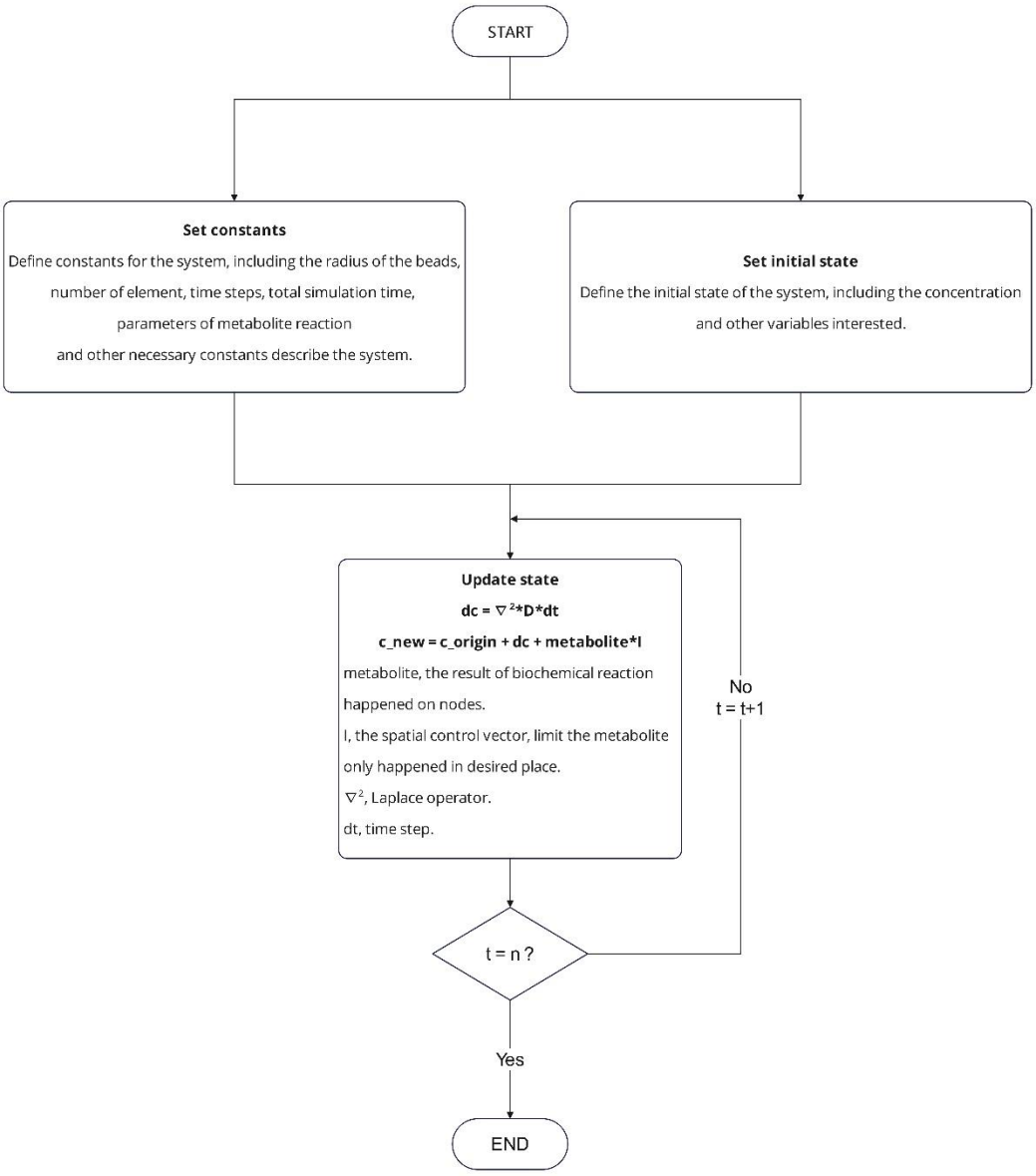


Figure 4 The numeric simulation algorithm based on diffusion model and FEM.

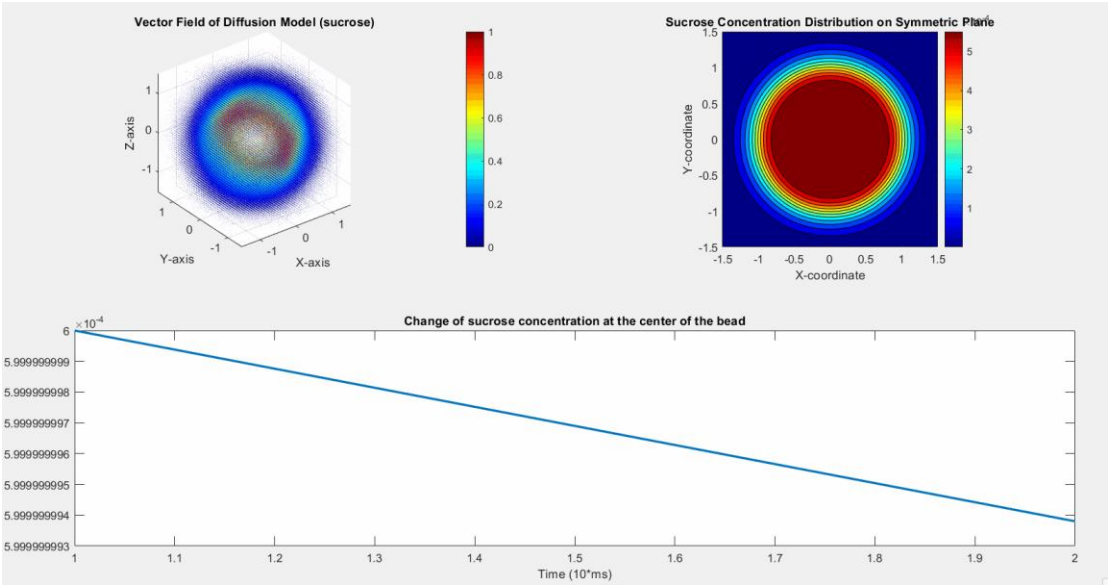
With the simulation, we are able to get insight on how to build and improve the system and prevent some possible traps in advance.

2.3 Result

All plots are refreshed every 10ms simulation time.

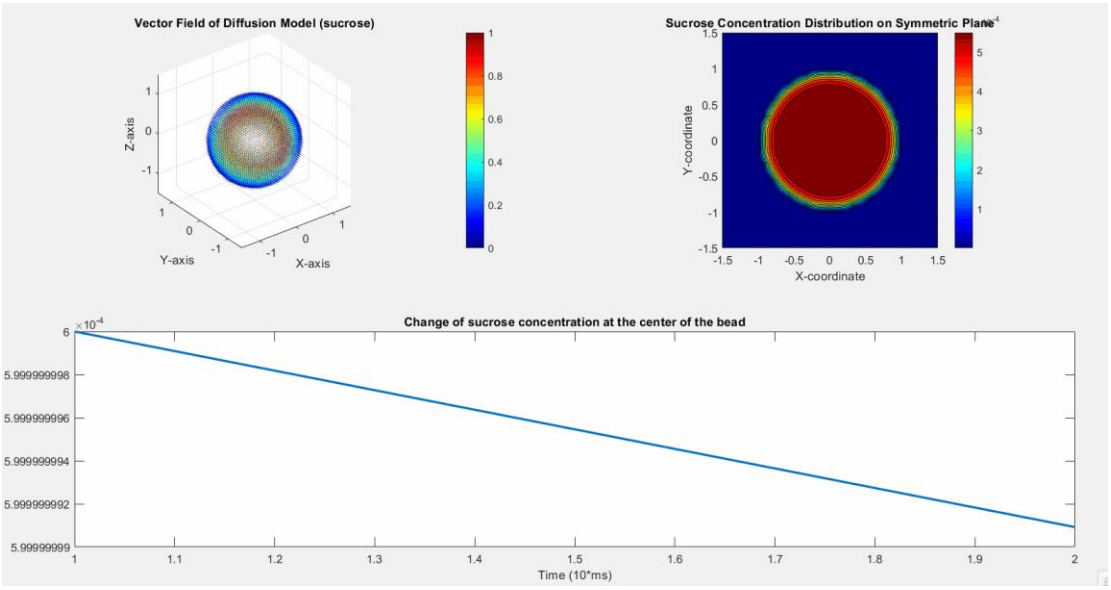
2.3.1 Simple beads

In this case, we do not care about the metabolite term and set it to zero.



When this result was obtained, it immediately came to our mind that, if the beads are static, the system's efficiency will be significantly affected due to the limit of substance's diffusion near the beads. This motivates the installation of a pump in our hardware.

To prove our thought, we refresh the outside concentration with 0 in each cycle to simulate the pump.



The result showed that at 300ms, the concentration at the center is lower than when not

refreshed.

2.3.2 Consider sucrose hydrolysis

The metabolite term is based on the sucroase Michaelis-Menten equation:

2.4 Discussion

We developed a model describing an “alginate bead with microorganism embedded”, and set up a mathematical simulation with the finite element method. The result showed that the system will reach homeostasis after a sufficient time, and the diffusion rate equals the consumption/production rate.

There are still many things to be done in the future. The cell growth is limited due to the volume of the beads, and the distribution inside the beads is uneven. There are still bricks left aside, thus we upload our commented MATLAB code and serve it as a rough framework for teams that wants to give FEM a try.

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