Title: Symmetric and asymmetric cell division and modeling of interacting cell populations in the colonic crypt.

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Abstract

Mathematical modeling can be used to describe the behavior of cells within the colonic crypt. The colon is made up of nearly 10 million crypts which are responsible for producing the epithelial cells within the colon. Symmetric and asymmetric stem cells and cycling cells produce the cells within the crypt and when this behavior becomes dysregulated it can lead to the development of colorectal cancer. This model aims to make a simple spatial and time dependent model to describe the behavior of two types of cells within the colon. Both analytic and numerical solutions are presented for a range of initial conditions and time points. The model is then expanded for stochastic analysis to further examine the spatial relationships among the cell types.

Introduction

The colon, or large intestine, is the largest part of the digestive system and is responsible for removing water and electrolytes from food and the final stage of the chemical digestive process. Approximately 10 billion epithelial cells are replaced daily in the colon and this renewal is driven by stem cells located in the bottom of invaginations within the colon called colonic crypts [1]. The crypts are bullet shaped and divided into three regions comprising three different types of cells. Stem cells are found in the bottom third of the crypt and are self-renewing cells capable of both symmetric and asymmetric cell division. Cycling, or proliferative, cells produced by stem cells make up the middle third of the crypt and divide repeatedly until reaching their terminally differentiated state. The terminally differentiated cells are in the final, upper third of the crypt. Stem cells typically make up the smallest proportion (<1%) of the total number of cells in a crypt, but experiments show this proportion varies based on characteristics of the crypt [2]. Figure 1 depicts an immunostained set of colonic crypts and how this structure is translated to a representative schematic of a single crypt [3].

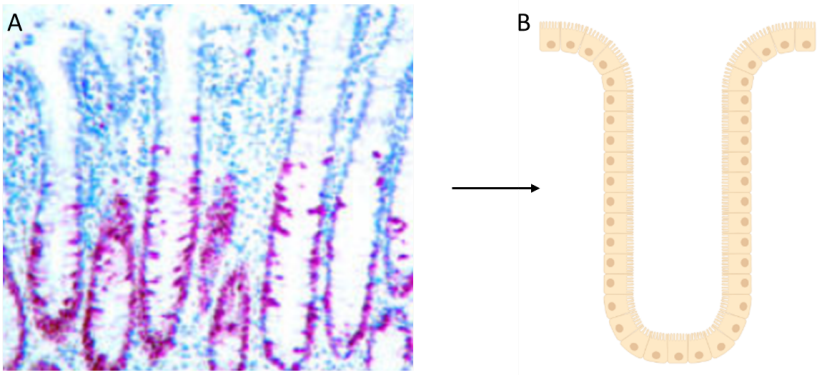

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Figure 1: Panel A is an image of an immunostained crypt of a healthy person, the red cells indicate stem cells are predominant in the base of the crypt as expected [3]; Panel B is a schematic representation of a single crypt used for modeling purposes.

Unregulated proliferation of stem cells is an accepted marker of colorectal cancer (CRC) [4]. CRC cases are 7.9% of all new yearly cancer cases in the US and 8.6% of yearly cancer deaths [5]. Adenomas (adenomatous polyps) are a well-confirmed risk factor for the development of CRC [4]. The severity of adenomas can vary greatly, but if they are not identified and/or left untreated, the unregulated stem cell growth of the adenoma can lead to an invasive, cancerous tumor [4]. Adenomas are diagnosed through imaging tests or through additional testing such as biopsy. In general, the earlier a precancerous adenoma is identified, the better the outcome is for the patient. Improving the ability of clinicians to identify and diagnose developing adenomas can translate directly to improved and less invasive care for patients. Mathematical modeling of the colonic crypt is emerging as a method for predicting the behavior of the varying types of cells found within the crypt.

Previous studies have investigated the cell population behavior within the crypt. A model by Tomlinson and Bodmer showed how different rates of cell division, differentiation, and death can lead to exponential growth of stem cells within the colonic crypt [6]. This study inspired many other mathematical models such as the computational models by Johnston et al. [7] and Meineke et al. [8], and the deterministic models of Boman et al. [9] and Hardy et al. [10]. The models presented in this study aim to add a spatial dimension to previously existing simple models and track the change in each represented cell population to predict biological conditions where unregulated stem cell growth occurs.

Methods

I. Model Development and Analytic Solution While a biologically ideal colonic crypt model incorporates the three previously mentioned categories of cells, our model uses only stem cells (C), differentiated cells (D), and asymmetric division of stem cells. This simplifying assumption allows us to add a spatial dimension to the model and compute both an analytic and numerical solution.

Assumptions:

1. The crypt is one dimensional with height L as seen in Figure 2.
2. One C divides into one C and one D at some constant rate k1.
3. All the cells have the same size and are rigid.
4. Upon cell division, the creation of a new cell will push the above cells up.
5. We will assume there are no cell deaths within the crypt and the only way for cells to leave the crypt is for them to be pushed out at the top.

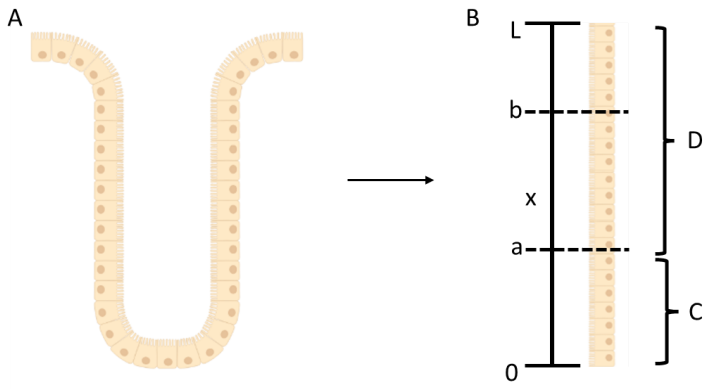

Figure 2: The schematic representation of the crypt was reduced to a single line of cells for simplicity in the mathematical model. The spatial variable x varies from zero to length L

We will be using a continuous model with respect to time and space and C(x, t) and D(x, t) will be the density of stem cells and differentiated cells, respectively, at position x and time t. To construct an appropriate spatial model, first consider an arbitrary interval [a,b] (Figure 2). The rate of change of stem cells and rate of change differentiated cells in [a,b] are

$$\frac{\partial}{\partial t}\int_a^b C\,dx \qquad\qquad \frac{\partial}{\partial t}\int_a^b D\,dx \tag{1}$$

respectively. By the conservation law, these rates of change will be equivalent to the flux in minus the flux out plus the cell production in [a,b]. Since k1 is the rate at which a C cell divides into two cells, the total production of cells in the crypt below position a, is

$$\int_0^a k_1 C\,dx \tag{2}$$

Therefore, the flux of stem cells into the interval [a,b] will be this total production of cells in [a,b] times the proportion of stem cells at position a. Similarly, the flux out of [a,b] of stem cells will be the total production of cells in [a,b] times the proportion of stem cells at x=b. Since upon division of cells the total number of stem cells does not change, the total production of stem cells will be zero on [a,b]. The same holds for differentiated cells, except the total production of differentiated cells on [a,b] is

$$\int_a^b k_1 C\,dx \tag{3}$$

In summary, we have expressions for the partial derivatives in (1). That is,

$$\begin{aligned}
\frac{\partial}{\partial t}\int_a^b C\,dx &= \int_0^a k_1 C\,dx\left[\frac{C}{C+D}\right]\Big|_{x=a} - \int_0^b k_1 C\,dx\left[\frac{C}{C+D}\right]\Big|_{x=b} \\
\frac{\partial}{\partial t}\int_a^b D\,dx &= \int_0^a k_1 C\,dx\left[\frac{D}{C+D}\right]|_{x=a} - \int_0^b k_1 C\,dx\left[\frac{D}{C+D}\right]|_{x=b} + \int_a^b k_1 C\,dx.
\end{aligned} \tag{4}$$

Based on our 3rd assumption, the total number of cells per unit length will be constant. That is, $C + D = \rho_{max}$ for some constant ρ_{max}, which is the total density of cells per unit length.

Through rearranging (Appendix 1), we can convert this into a system of PDEs.

$$\begin{cases} \dfrac{\partial C}{\partial t} + \dfrac{\partial}{\partial x}\left[\dfrac{C(x)}{\rho_{\max}}\displaystyle\int_0^x k_1 C(z,t)\,dz\right] = 0 \\ \dfrac{\partial D}{\partial t} + \dfrac{\partial}{\partial x}\left[\dfrac{D(x)}{\rho_{\max}}\displaystyle\int_0^x k_1 C(z,t)\,dz\right] = k_1 C \quad x \in [0,L], t > 0. \end{cases} \tag{5}$$

This model is a nonlocal PDE and so we will introduce new variables to convert to a local PDE before attempting to solve. Let,

$$S(x,t) = \int_0^x C(z,t)\,dz \qquad T(x,t) = \int_0^x D(z,t)\,dz. \tag{6}$$

By substituting (6) into (5) and rearranging (Appendix 1), we get the local PDE,

$$\begin{cases} \dfrac{\partial S}{\partial t} + \dfrac{k_1}{\rho_{\max}} S \dfrac{\partial S}{\partial x} = 0 \\ \dfrac{\partial T}{\partial t} + \dfrac{k_1}{\rho_{\max}} S \dfrac{\partial T}{\partial x} = k_1 S \quad x \in [0,L], t > 0. \end{cases} \tag{7}$$

Since these PDEs have similar forms to the inviscid Burgers' equation [11], we know that we can solve this system analytically when given an initial condition for S and T by applying the characteristic method.

II. Numerical Solution and Variation of Conditions While we can solve these PDES analytically for a given initial condition, we will also determine a method to calculate them numerically. Using the method of characteristics, we converted the system of PDEs into a system of ODEs which are described below.

$$\begin{aligned} \frac{dS}{ds} &= 0 \\ \frac{dt}{ds} &= 1 \\ \frac{dx}{ds} &= \frac{k_1}{\rho_{\max}} S \end{aligned} \tag{8}$$

Simplifying this system of ODEs using the method of characteristics leads to -

$$S = S(x_0, 0) \tag{9}$$

$$t = s$$
$$x = \frac{k_1}{\rho_{max}} S(x_0, 0)s + x_0 \tag{10}$$

We assumed the following initial condition for the distribution of S(x,0) i.e. the initial value of S.

$$S(x,0) = \begin{cases} \frac{\rho_{max} X}{10} & 0 \le x \le L/10 \\ \frac{\rho_{max} L}{100} & L/10 \le x \le L \end{cases} \tag{11}$$

This model was used for obtaining a numerical solution. For this, we start at an initial time point and an initial x_0 value. We use this x_0 value to obtain the value of x, which is used to obtain the values of S and T. This is then iteratively done for a number of times and x_0 values.

We also derived exact solutions for these initial conditions -

$$S(x_0, 0) = \begin{cases} \frac{\rho_{max} x}{k_1 t + 10} & for \;\; 0 \le x \le L k_1 t/100 + L/10 \\ \frac{\rho_{max} L}{100} & for \;\; L k_1 t/100 + L/10 \le x \le L \end{cases} \tag{12}$$

to get the values of S, and compared these solutions to those from the numerical method.

III. Stochastic Model We attempt to introduce a stochastic component with probability of interaction between C, P, and D cells. We assume that C cells divide both symmetrically and asymmetrically at rate k1. The probability of asymmetric division is p while the probability of symmetric division as $1 - p$. P cells also divide asymmetrically and symmetrically, but at rate k2 with respective probabilities of q and $1 - q$. D cells can be assumed to be at rate k3, but this parameter is set to zero to indicate that these cells are terminally differentiated and do not undergo division.

Results

I. Analytic Solution Our methods allowed us to create the local PDE model in (5) which can be solved given an initial condition analytically by using the characteristic method. For a particular example, if we consider that the initial distribution of stem cells is uniform and covers 10% of the total number of cells along the crypt, we have the corresponding initial conditions for S and T as

$$S(x,0) = \frac{\rho_{\max}}{10}x \qquad\qquad T(x,0) = \frac{9\rho_{\max}}{10}x. \tag{13}$$

Using the characteristic method (Appendix 2), we get the exact solution,

$$S(x,t) = \frac{\rho_{\max}}{k_1 t + 10}x \qquad\qquad T(x,t) = \rho_{\max} x\left(1 - \frac{1}{k_1 t + 10}\right). \tag{14}$$

Note that S(x,t) represents the total number of stem cells below x at time t and T(x, t) represents the total number of stem cells below x at time t. As t approaches infinity, S(x, t) approaches 0 and T(x, t) approaches ρ_{max} for each x in the interval [0,L]. While this model is a good starting point, since this asymptotic behavior is not what we would hope to see in a healthy crypt, restricting our model to consider only stem cell asymmetric division in this manner is not sufficient to capture what we would expect biologically.

II. Numerical Method: Below is a graph showing the characteristic curves for various values of x and t -

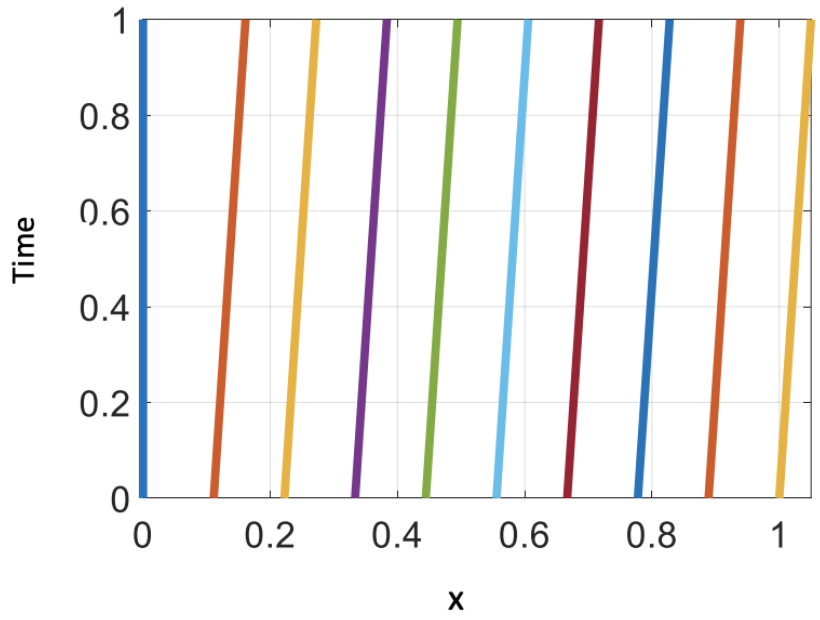

Figure 3: This graph shows the characteristic curves used to solve the system of equations.

We can notice that there is a vertical characteristic coming up from x = 0. Because of the fact that S is the integral of C from 0 to x, S(0,0) will always vanish, it forces the characteristic through that point to be vertical. Also note that there is a dividing characteristic coming out of x = 0.1 on the x-axis. Between the vertical characteristic curve coming up from x = 0 and this special characteristic, the slope of the characteristics decreases smoothly as their x-intercept increases. All the characteristic curves to

the right of the dividing characteristic are parallel. Also, the characteristic curves intersect the right side of the domain. This corresponds to the fact that information about the solution is propagated along the characteristic curves and in terms of what we are trying to model, the fact that the cells get pushed in the positive x direction every time a cell divides.

Next is a plot of S and T values against x, plotted for several values of time.

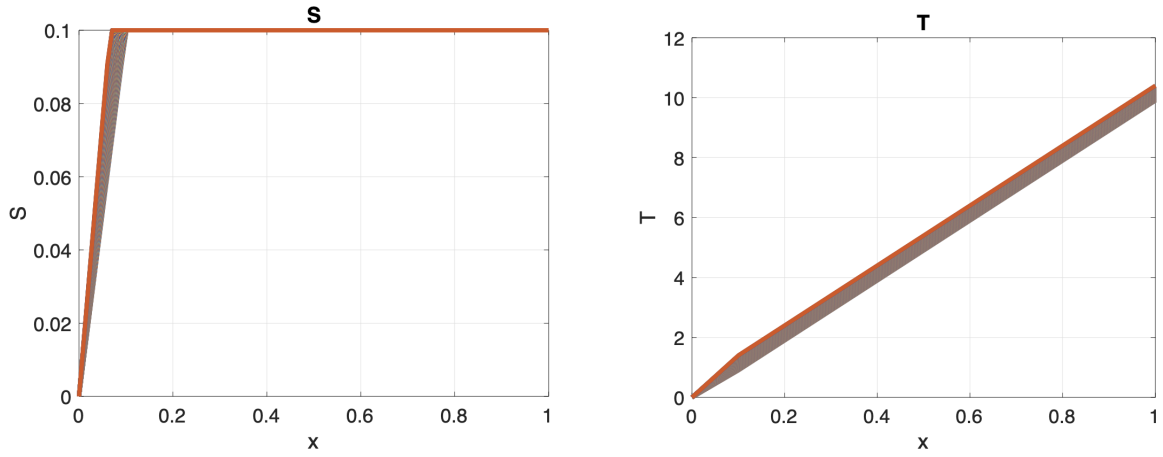

Figure 4: Plots of stem cell population (left) and differentiated cells (right) over time.

III. Stochastic Model: We assume that the total number of cells is fixed, $N = 80$. The cells interact from bottom to up as the time variable changes. The cells get pushed up and out of the system, but the new cells can be generated. At any time during the experiment, the total numbers of cells are always 80. The interesting cases happen at the top, where imagine situation can happens as follow:

1. When C is divided into C and P, now we have 2 types of new cells. Which cell gets to stay in the system and which cell gets pushed out of the system? We assigned a probability of $1/2$ (equally) to either case. That is, there is a 50% chance that P will stay and C gets pushed out. There is also another 50% chance that C will stay in the system and P gets pushed out. We implemented this probability in the code by introducing a random number picked between 0 and 1, then comparing it to 0.5.
2. A similar situation can happen at the top for the P divided into P and D. A similar 50% probability is assigned in that situation. That is, there is a 50% chance that P stays in the system and D gets pushed out, while there is another 50% chance that D stays in the system and P gets pushed out.

Discussion

While the two cell model is a good starting point, from our analysis of the analytic solutions we produced, this model may not encompass enough information to sufficiently represent the biological results we would expect. The value of this model is its simplicity, which ideally assists in creating a model which better illustrates the biology.

The animated version of a modeled crypt over a time period from $t = 0$ to $t = 500$ is illustrated in the presentation. The animated version shows what we expect to have a reduction in the number of C cells over time. The figure of stochastic model is shown in figure below to illustrate the result:

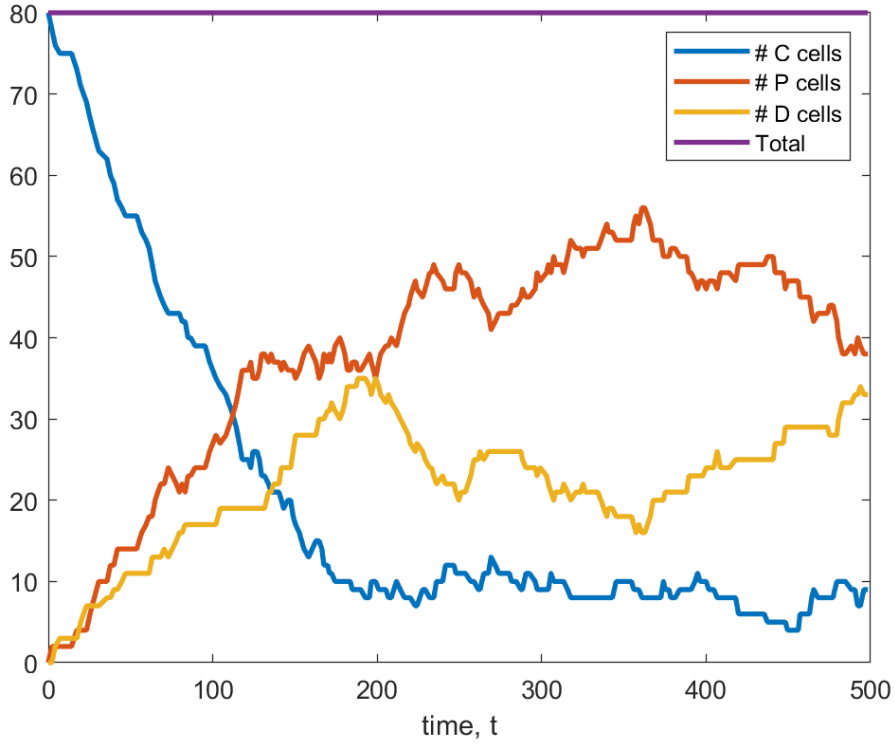

Figure 5: Stochastic model results depicting the population of each type of cell over time. The total number of cells (indicated by the purple line) remains constant over time.

We expect that a smooth out process will somewhat give us a stable solution to the system. A horizontal smooth out with moving velocity was tried, but unfortunately did not seem to produce such a stable solution (Figure 6).

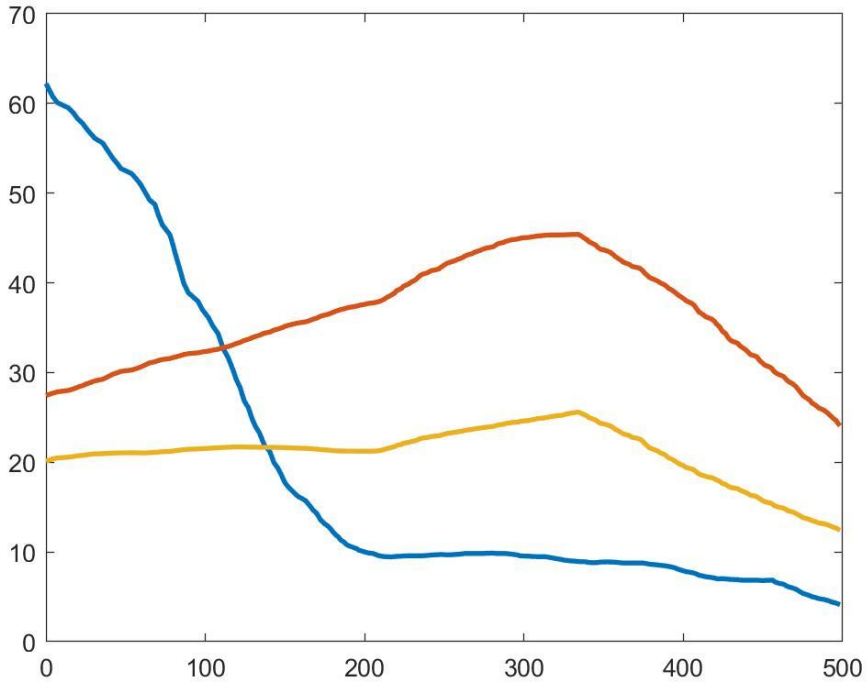

Figure 6: Result of smoothening process which did not lead to a stable solution for the model.

It would be beneficial if we can average out based on different illustrations and vertically. That is, suppose you have m illustratives of C. We would like to find a uniform t so that we can distribute or trajectory these C into a smooth version of C by averaging them out.

Conclusions

Overall this model is a reasonable starting point to begin to understand cell population behavior and dynamics. Future versions of the model could be expanded to include all three types of cells located in the crypt or a different geometrical representation of the crypt. Experimental results could be used to validate an appropriate range of reaction/division rates in order to narrow down reasonable initial conditions. Alternatively, reaction rates could be modeled as functions dependent on local populations of cells to introduce an extracellular signaling component to the model. Our results indicate the wide variability in cell populations dependent on initial conditions. Some experimental results have shown that stem cell populations can reach up to 60% of the cells even in healthy cells which indicates that local conditions heavily influence the behavior of each cell type within the crypt.

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Appendix

1 Converting (7) to a PDE

By the conservation law, we can equate the total change in stem cells (C) on a given interval $[a, b]$ with the in flux of stem cells in minus the in flux of stem cells out plus the production of stem cells within $[a, b]$. Similarly, we can create an equality for the total change in differentiated cells (D). These equalities are what we concluded in (7), with justification within the body of the report.

Below (7), we concluding that $C + D = \rho_{\max}$ and so (7) is

$$\frac{\partial}{\partial t}\int_a^b C\,dx = \int_0^a k_1 C\,dx\left[\frac{C}{\rho_{\max}}\right]\Big|_{x=a} - \int_0^b k_1 C\,dx\left[\frac{C}{\rho_{\max}}\right]\Big|_{x=b} \tag{A1}$$

$$\frac{\partial}{\partial t}\int_a^b D\,dx = \int_0^a k_1 C\,dx\left[\frac{D}{\rho_{\max}}\right]\Big|_{x=a} - \int_0^b k_1 C\,dx\left[\frac{D}{\rho_{\max}}\right]\Big|_{x=b} + \int_a^b k_1 C\,dx. \tag{A2}$$

We will now rewrite these as partial differential equations. First note that the first two terms in (A1) can be viewed as a function evaluated at a minus it evaluated at b. This is similar to the first two terms in (A2). That is, letting $f_C(x,t) = \dfrac{C(x,t)}{\rho_{\max}}\displaystyle\int_0^x k_1 C\,dz$ and $f_D(x,t) = \dfrac{D(x,t)}{\rho_{\max}}\displaystyle\int_0^x k_1 C\,dz$ and applying the Fundamental Theorem of Calculus, (A1) and (A2) become

$$\frac{\partial}{\partial t}\int_a^b C\,dx = -\int_a^b \frac{\partial f_C}{\partial x}(x,t)\,dx \frac{\partial}{\partial t}\int_a^b D\,dx = -\int_a^b \frac{\partial f_D}{\partial x}(x,t)\,dx + \int_a^b k_1 C\,dx.$$

Rearranging, we have

$$\int_a^b \left(\frac{\partial C}{\partial t} + \frac{\partial f_C}{\partial x}\right)\,dx = 0$$

$$\int_a^b \left(\frac{\partial D}{\partial t} + \frac{\partial f_D}{\partial x} - k_1 C\right)\,dx = 0$$

Since $[a, b]$ is an arbitrary interval and the integrands are continuous functions, we can conclude that

$$\begin{cases} \dfrac{\partial C}{\partial t} + \dfrac{\partial f_C}{\partial x} = 0 \\ \dfrac{\partial D}{\partial t} + \dfrac{\partial f_D}{\partial x} - k_1 C = 0 \quad x \in [0, L], t > 0. \end{cases} \tag{A3}$$

By the definitions of f_C and f_D, (A3) is a nonlocal system. Therefore before solving, we will convert this to a local model by substitution. Integrating the equations in (A3), we have

$$\int_0^x \frac{\partial C}{\partial t}\,dz + \int_0^x \frac{\partial f_C}{\partial x}\,dz = 0$$

$$\int_0^x \frac{\partial D}{\partial t}\,dz + \int_0^x \frac{\partial f_D}{\partial x}\,dz = \int_0^x k_1 C\,dz.$$

Substituting in f_C and f_D and evaluating the second integral, we can conclude that

$$\frac{\partial}{\partial t}\int_0^x C(z,t)\,dz + \frac{k_1}{\rho_{\max}} C(x,t)\int_0^x C(z,t)\,dz = 0$$

$$\frac{\partial}{\partial t}\int_0^x D(z,t)\,dz + \frac{k_1}{\rho_{\max}} D(x,t)\int_0^x C(z,t)\,dz = k_1\int_0^x C(z,t)\,dz. \tag{A4}$$

Let

$$S(x,t) = \int_0^x C(z,t)\,dz \quad \text{and} \quad T(x,t) = \int_0^x D(z,t)\,dz.$$

and substituting into (A4), we have

$$\begin{cases} \dfrac{\partial S}{\partial t} + \dfrac{k_1}{\rho_{\max}} S\dfrac{\partial S}{\partial x} = 0 \\ \dfrac{\partial T}{\partial t} + \dfrac{k_1}{\rho_{\max}} S\dfrac{\partial T}{\partial x} = k_1 S \quad x \in [0,L], t > 0. \end{cases} \tag{A5}$$

2 Solving (A5) for a given initial condition

Let us consider the initial conditions for S and T as

$$S(x,0) = \frac{\rho_{\max}}{10}x \quad \text{and} \quad T(x,0) = \frac{9\rho_{\max}}{10}x.$$

The fact that $C + D = \rho_{max}$ and S, T are integrals of C and D, respectively, makes S and T satisfy

$$S(x,t) + T(x,t) = \rho_{max}x. \tag{A6}$$

We can solve the PDE of S in (A5), then use (A6) to extract the solution for T. Therefore, first we are going to solve the following for S

$$\begin{cases} \dfrac{\partial S}{\partial t} + \dfrac{k_1}{\rho_{\max}} S\dfrac{\partial S}{\partial x} = 0 \\ S(x,0) = \dfrac{\rho_{\max}}{10}x, \quad x \in [0,L], t > 0. \end{cases} \tag{A7}$$

Equation (A7) is similar to a fundamental PDE called inviscid Burgers' equation [11] so we can use the method of characteristic to solve it.

Method of characteristic for (A7)

By parametrizing the characteristic curves of (A7) with parameter s, we can reduce the PDE into ODE. Using chain rule we get

$$\frac{dS}{ds} = \frac{dt}{ds}\frac{\partial S}{\partial t} + \frac{dx}{ds}\frac{\partial S}{\partial x}. \tag{A8}$$

Assuming $\frac{dS}{ds} = 0$ and compare it to (A7), we get

$$\begin{cases} \frac{dS}{ds} = 0 \\ \frac{dt}{ds} = 1 \\ \frac{dx}{ds} = \frac{k_1}{\rho_{\max}} S \end{cases} \tag{A9}$$

We can set the initial condition of (A9) as $s = 0$ on the initial curve, $x = x_0$ a constant, and $S = S(x_0, 0)$. By integrating (A9) and using its initial condition, we get

$$\begin{cases} S = S(x_0, 0) = \frac{\rho_{\max}}{10} x_0 \\ t = s \\ x = \frac{k_1}{\rho_{\max}} S(x_0, 0)s + x_0 = \left(\frac{k_1}{10} s + 1\right) x_0 \end{cases} \tag{A10}$$

To get an explicit solution of S, we express x_0 in terms of x and t, and substitute it in the expression of S. Therefore, we have the expression of S as

$$S(x, t) = \frac{\rho_{\max}}{k_1 t + 10} x. \tag{A11}$$

Using the relation (A6) between S and T and the S solution (A11), we have

$$T(x, t) = \rho_{\max} x - \frac{\rho_{\max}}{k_1 t + 10} x = \rho_{\max} x \left(1 - \frac{1}{k_1 t + 10}\right)$$

By the definition of S and T, our solutions for C and D are the derivatives of S and T with respect to x, which are

$$\begin{cases} C(x, t) = \frac{\rho_{\max}}{k_1 t + 10} \\ D(x, t) = \rho_{\max} \left(1 - \frac{1}{k_1 t + 10}\right). \end{cases}$$