MATHEMATICAL MODELLING OF BIOPROCESSES

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THE ROLE OF THE MATHEMATICAL MODELING

What is a model?

A physical, mathematical, or otherwise logical representation of a system, entity, phenomenon, or process

- (physical/concrete) something that a copy can be based on because it is a good example of its type
  - (small size) an aircraft, a building, a city, a planet, a solar system etc.,
  - (actual size) a car, a mold, a piece of clothing, an organism etc.

- (conceptual/abstract) a set of ideas and numbers that describe the past, present, or future state of something
  - (ideas) an educational model, a behavioral model, an economic model, a management model etc.
  - (simulations) 2D/3D graphical models
  - (numbers/equations) mathematical models

The biotechnological processes are complex interactions between:

- physical processes,
- chemical processes,
- biological processes.

These interactions are expressed in engineering terms using techniques such as:

- mass and energy balance,
- growth kinetics of the microorganisms,
- transport processes,
- stoichiometric equations etc.

A mathematical model associates these terms in a unified package that can be used for:

- predicting the dynamics of the bioprocess,
- estimation of some unmeasurable variables,
- process control,
- designing of the bioreactors,
- simulation of the process (education),
- management of the bioprocess,
- research etc.

"A good mathematical model is compromise between accuracy, applicability and clarity"

The 4 rules of the mathematical modeling of bioprocesses:

- a mathematical model can only approximate a real bioprocess which is natively complex and partially known in many cases. Thus, the mathematical models must satisfy some previously well-defined objectives.

Objective definition

- the mathematical modeling is a procedure that is developed continuously. A mathematical model is an abstraction that reduces the problem to its fundamental characteristics.

Start from simple concepts

- the mathematical modeling is an "art", but represents, at the same time, an important action to understand the bioprocess.

Use the model to understand the bioprocess

- the mathematical models must be both realistic and robust

The models exist to be used

THE CLASSIFICATION OF THE MATHEMATICAL MODELS

Empirical vs. Mechanistic:

- Empirical models are based on data and observation,
- Mechanistic models are based on hypotheses.

Deterministic vs. Probabilistic (Stochastic):

- Deterministic models are models where all parameters have defined values,
- Probabilistic models are models where the parameters can fluctuate randomly (e.g., models for the size and speed of gas bubbles in a reactor)

Structured vs. Unstructured:

- Structured models are related to cell material description using multiple chemical components,
- Unstructured models are based on the monitoring of cell and nutrient concentration and describe the process as an average of the species under ideal conditions.

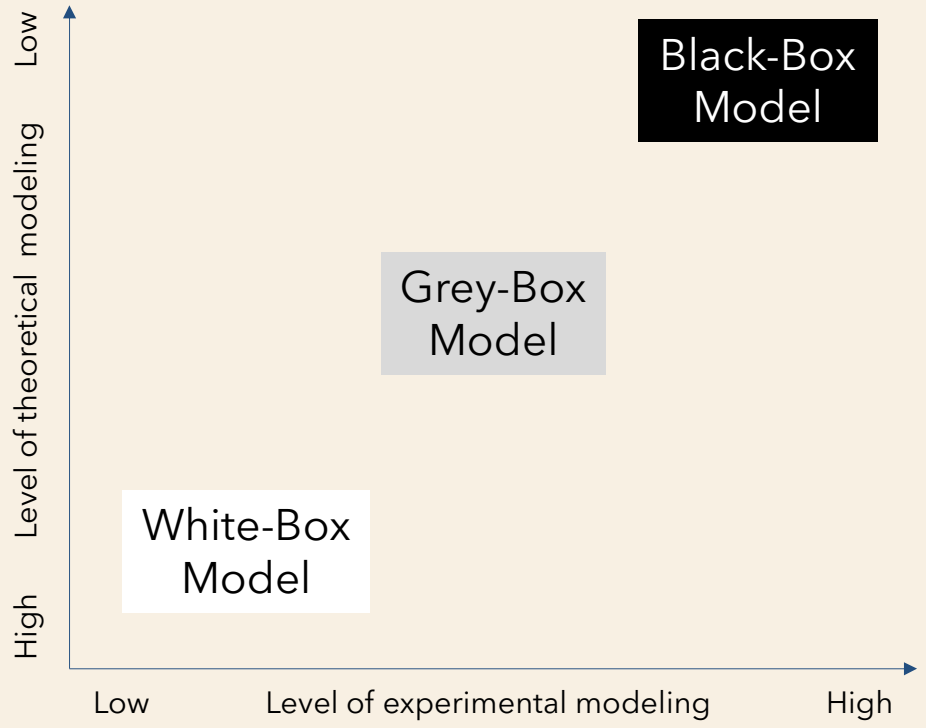
Dynamic vs. Steady-State:

- Dynamic models are complex models, formed of a set of differential equations and a set of algebraic equations that can be solved numerically,
- Steady-state models are formed of a set of algebraic equations that can be solved analytically.

Linear vs. Nonlinear: a model is defined as linear if all the operators in a mathematical model exhibit linearity. A model is nonlinear otherwise.

White-Box vs. Grey-Box vs. Black-Box:

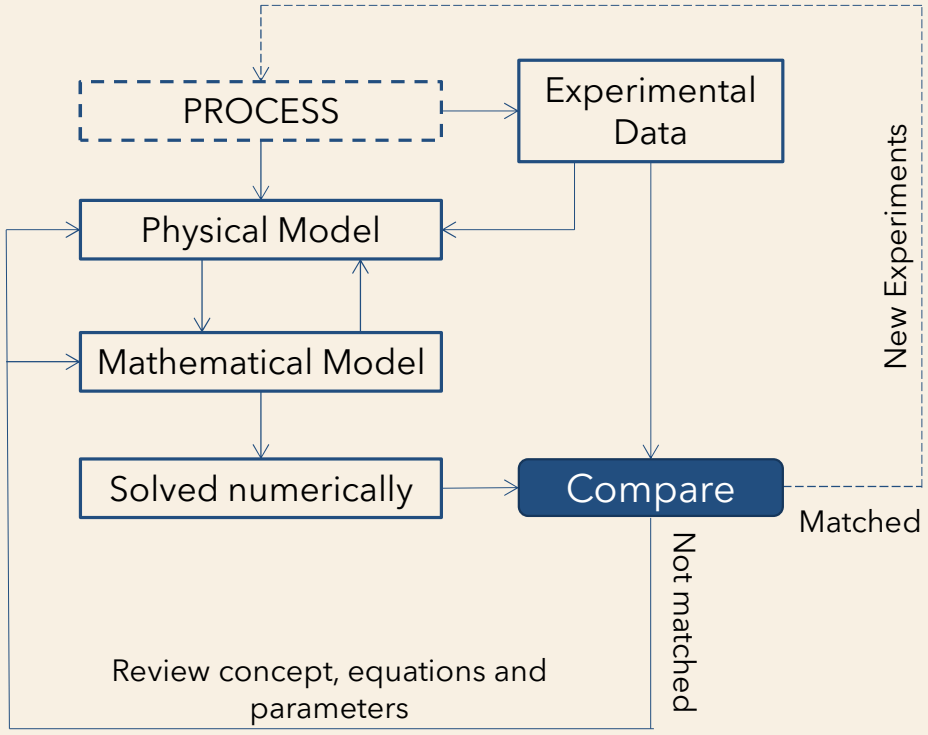
- White-box models are sets a linear/non-linear differential equations with known parameters;
- Grey-box models are sets a linear/non-linear differential equations with unknown parameters; transfer functions; space state models;
- Black-box models are based on artificial neural networks; fuzzy logic



THE GENERAL PROCEDURE FOR MODELING

The steps of mathematical modeling:

- Proper definition of the problem,
- The existing concepts must be expressed in mathematical terms,
- When a model is formulated, it must be solved numerically,
- The prediction capacity of the model must be verified and, if necessary, it must be reviewed.



THE MATHEMATICAL MODELING OF BIOPROCESSES THROUGH DYNAMIC MASS BALANCE EQUATIONS

1. INTRODUCTION
2. DEVELOPMENT OF MASS BALANCE EQUATIONS
3. THE GENERAL DYNAMIC MODEL
4. MODELING OF A MICROBIAL GROWTH PROCESS.

DYNAMIC MASS BALANCE EQUATIONS

PRINCIPLE OF MASS CONSERVATION: The mass of an isolated system is nether created nor destroyed by chemical reactions or physical transformations.

MASS [Kg]

Mass balance is simple way to quantify the mass of the system at a certain moment in time:

$$\begin{pmatrix}\textbf{The mass of}\\ \textbf{the system at } t+\Delta t\end{pmatrix} = \begin{pmatrix}\textbf{The mass of}\\ \textbf{the system at } t\end{pmatrix} + \begin{pmatrix}\textbf{The mass that enters}\\ \textbf{the system at } t+\Delta t\end{pmatrix} - \begin{pmatrix}\textbf{The mass that leaves}\\ \textbf{the system at } t+\Delta t\end{pmatrix}$$

Disadvantage: we must know the beginning and the end of the balance period

MASS FLOW [Kg/h]

In engineering we prefer to express the mass that is transported through the system as mass flow:

$$\frac{\begin{pmatrix}\textbf{The mass of}\\ \textbf{the system at } t+\Delta t\end{pmatrix} - \begin{pmatrix}\textbf{The mass of}\\ \textbf{the system at } t\end{pmatrix}}{\Delta t} = \frac{\begin{pmatrix}\textbf{The mass that enters}\\ \textbf{the system at } t+\Delta t\end{pmatrix}}{\Delta t} - \frac{\begin{pmatrix}\textbf{The mass that leaves}\\ \textbf{the system at } t+\Delta t\end{pmatrix}}{\Delta t}$$

$$\begin{pmatrix}\textbf{Mass accumulation rate}\\ \textbf{in the system}\end{pmatrix} = \begin{pmatrix}\textbf{Input}\\ \textbf{mass flow rate}\end{pmatrix} - \begin{pmatrix}\textbf{Output}\\ \textbf{mass flow rate}\end{pmatrix}$$

If no mass is accumulating in the system, then:

$$\begin{pmatrix}\textbf{Input}\\ \textbf{mass flow rate}\end{pmatrix} = \begin{pmatrix}\textbf{Output}\\ \textbf{mass flow rate}\end{pmatrix}$$

The mass balance for elements:

$$\begin{pmatrix}\textbf{Mass accumulation flow rate}\\ \textbf{of carbon in the system}\end{pmatrix} = \begin{pmatrix}\textbf{Input mass flow rate}\\ \textbf{of carbon in the system}\end{pmatrix} - \begin{pmatrix}\textbf{Output mass flow rate}\\ \textbf{of cobon from the system}\end{pmatrix}$$

The mass balance for a component:

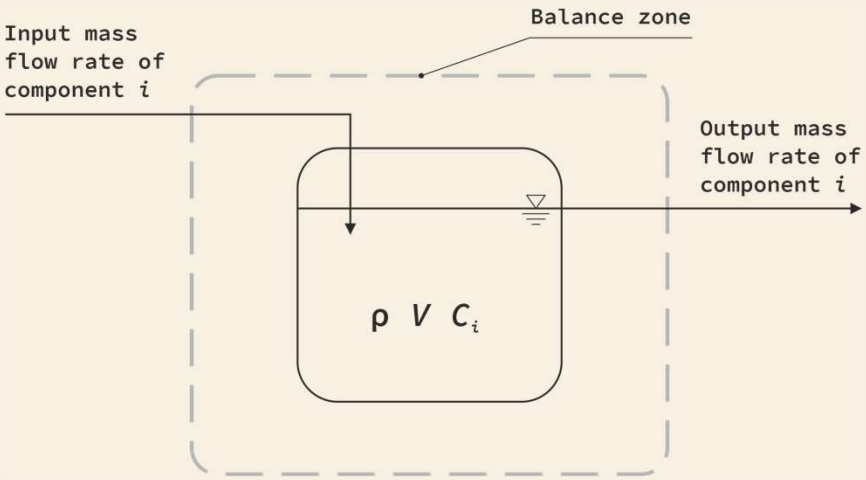
$$\begin{pmatrix}\textbf{Mass accumulation rate}\\ \textbf{of a component}\\ \textbf{in the system}\end{pmatrix} = \begin{pmatrix}\textbf{Input mass flow rate}\\ \textbf{of a component}\\ \textbf{in the system}\end{pmatrix} - \begin{pmatrix}\textbf{Output mass flow rate}\\ \textbf{of a component}\\ \textbf{from the system}\end{pmatrix} + \begin{pmatrix}\textbf{Production rate}\\ \textbf{of a component}\\ \textbf{through reaction}\end{pmatrix}$$

DEVELOPMENT OF MASS BALANCE EQUATIONS

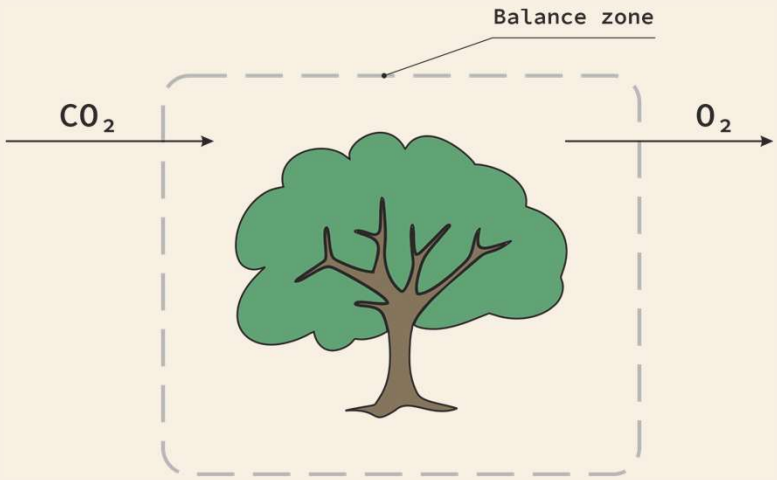
A dynamic mass balance equation is a mathematical expression through which there are described the dynamics of numerous chemical or biological components that are transported throughout the system

1. SELECTION OF BALANCE ZONE. A balance zone is selected according to the defined objective. It can contain:

- very small volumes (e.g., an atom, a molecule, a cell etc.)
- very large volumes (e.g., a lake, a continent, a planet, a solar system etc.)



Balance zone of a reactor



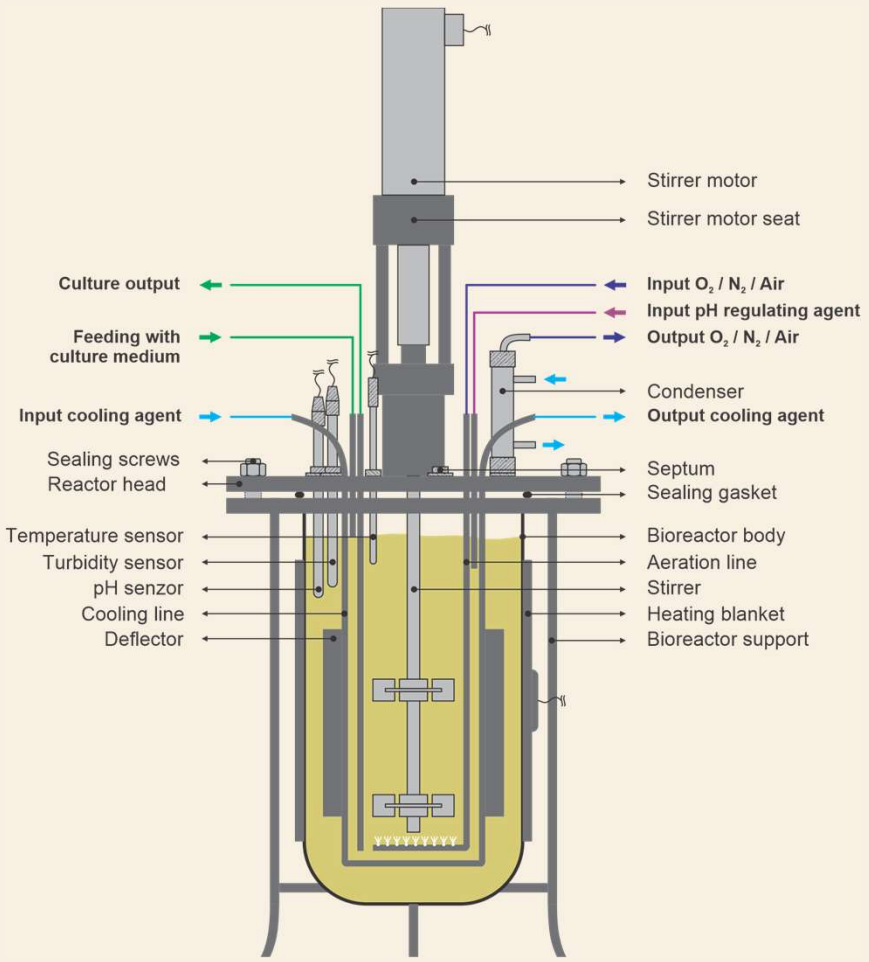
Balance zone of a tree

In bioprocesses the balance zone is usually selected around a bioreactor or around a plant with few tanks, but it can be also reduced to a gas bubble, a microorganism etc.

!!! The mass of a component inside the selected balance zone must be constant (for a certain point in time). Otherwise, the system can be divided into sub-systems that will be modeled individually.

After selecting the balance zone, the components that will be modeled must be identified, for example:

- biomass – a species, a class or the entire mass or organisms in a bioreactor,
- substrates – carbon source (glucose, polycarbohydrates, bicarbonate), nutrients (N and P), sulfur etc.
- products – enzymes, organic acids, alcohols etc.
- dissolved gases – CO_2, O_2,
- chemical species – NH_3, NH_4^+, CO_2, HCO_3^-, CO_3^{2-} etc.
- molar fractions of the gases – N_2, O_2 and CO_2.



The components of a laboratory bioreactor

FUNDAMENTAL MODEL REACTORS

The modeling of the bioprocesses must be reduced to simple concepts in order to describe easily the dynamics of the state variables of the process. The state variables are components that characterize the process and that can be measured or estimated.

According to the distribution of a component in a bioreactor, this can be:

- **homogenous – continuously stirred reactor tank (CSTR).** This reactor is a system with concentrated parameters and is described through ordinary differential equations where the derivation variable is the time, *t*.

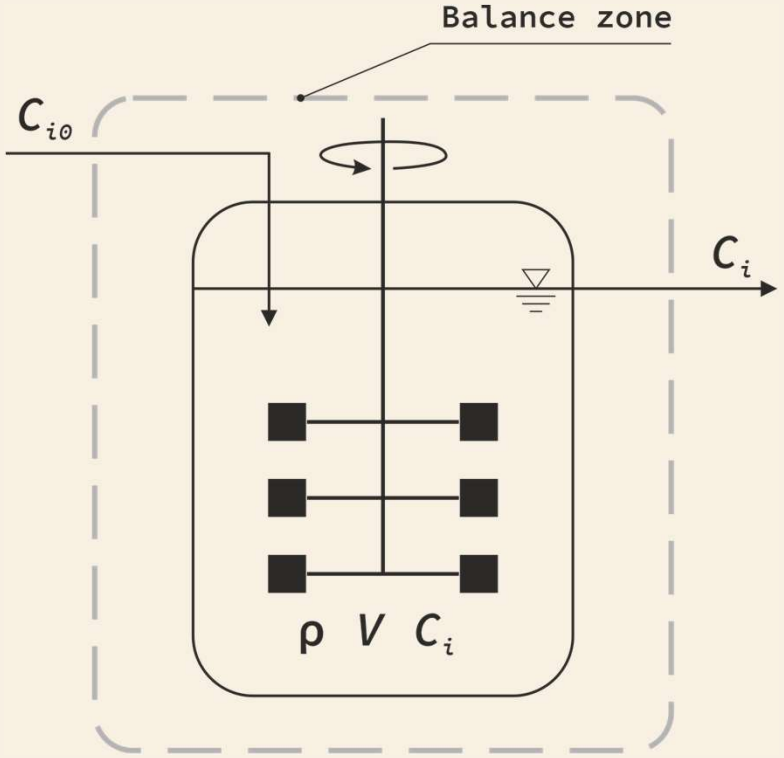
- **heterogenous – plug-flow reactors (PFR).** This reactor is a system with distributed parameters and is described through partial differential equations where, besides the time, another derivation variable exists (a space coordinate such as length).

THE CONTINUOUSLY STIRRED TANK REACTOR (CSTR)

In a CSTR the distribution of the modeled component is uniform in any point of its volume.

There are 3 types of CSTRs:

- discontinuous (batch)
- semi-continuous (fed-batch)
- continuous



Balance zone around a CSTR

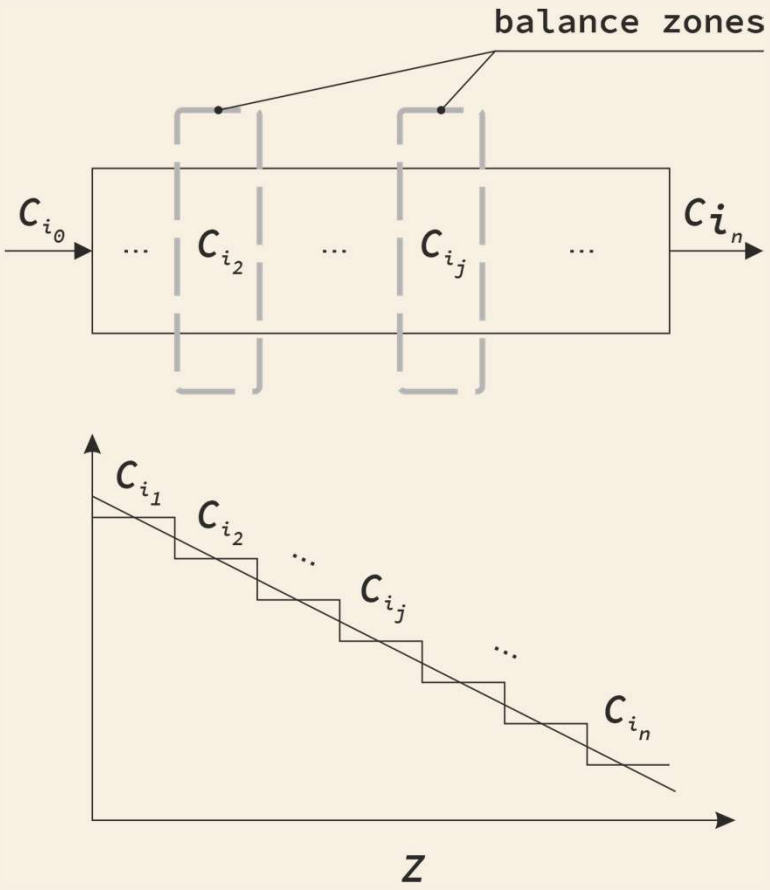
THE PLUG-FLOW REACTOR (PFR)

In a PFR the distribution of the modeled component is NOT uniform in any point of its volume, but variates on one dimension (high or length)

A PFR can also be discontinuous (batch), semi-continuous (fed-batch) or continuous.

In a PFR the concentration of the components is considered constant on one dimension (ox or oy) and varies across the other dimension.

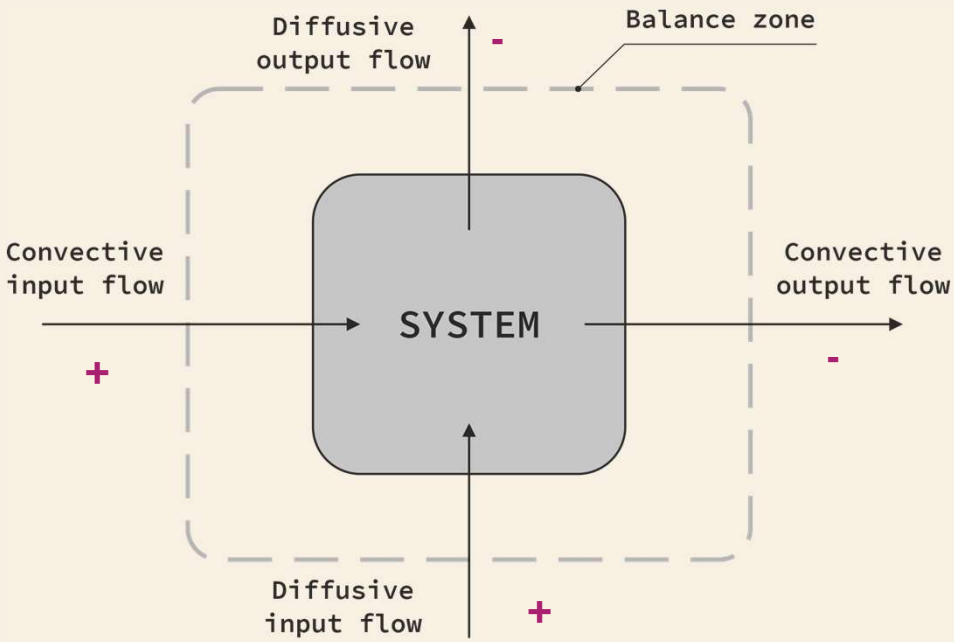
A PFR can be divided in *n* zones which can be modeled through *n* ordinary differential equations.



Balance zones of a PFR and the approximative concentration gradient

2. IDENTIFICATION OF TRANSPORT FLOWS. After the selection of the balance zone, the second step is the identification of the transport flows across the system bounds. These flows are:

- input/output convective flows – liquids that transport dissolved or colloidal components
- input/output diffusive flows – gases bubbled into the reactor. They have 2 components: the bubbled gas flow rate and the gas-liquid mass transfer rate.



Balance zone and the input/output flows

3. MATHEMATICAL FORMULATION. Each term of the general mass balance equation must be expressed mathematically.

$$(\text{Accumulation}) = (\text{Input}) - (\text{Output}) + (\text{Production})$$

The term associated to the accumulation rate. The mass of an open system or of a component in the system can vary in time, their accumulation rate being expressed mathematically through a time derivative:

$$\begin{pmatrix} Mass\ accumulation\ rate\ of\ a \\ component\ in\ the\ system \end{pmatrix} = \left(\frac{dM_i}{dt}\right)$$

In engineering the concentration of a component is preferred. For this the reference volume is required:

$$\frac{dM_i}{dt} = \frac{d(V\ C_i)}{dt}$$

For a gaseous component, the ideal gas law can be used:

$$p_i V = n_i RT$$

The concentration of the gas can be expressed as:

$$C_i^m = \frac{n_i}{V} = \frac{p_i}{RT} = \frac{y_i P}{RT}$$

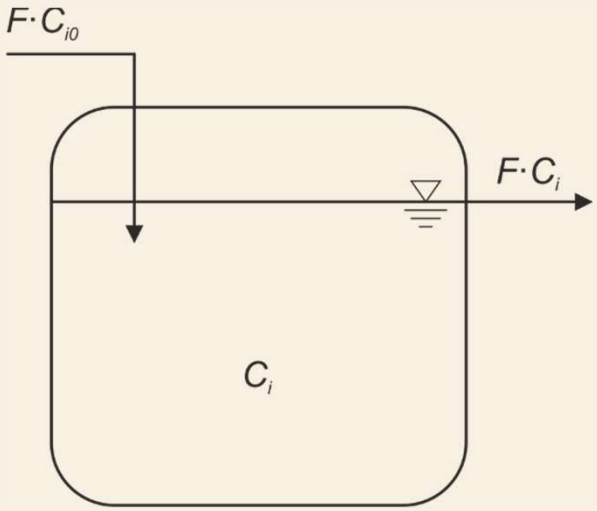
Thus, the term associated to the accumulation rate of a gaseous component can be expressed in molar concentration of molar fraction:

$$\frac{dn_i}{dt} = \frac{d(V\ C_i^m)}{dt} = \frac{d\left(\frac{p_i V}{RT}\right)}{dt} = \frac{d\left(\frac{y_i PV}{RT}\right)}{dt}$$

The term associated to the convective transport flows. Mass flows are the product between the volumetric flows and density:

$$(Convective\ mass\ flow) = (Volumetric\ flow)\left(\frac{\text{Mass}}{\text{Volume}}\right)$$

$$M_i = FC_i$$



The terms associated with the convective flows in a CSTR

Diffusive flows. Example for O_2

$$N_{O_2} = k_L a\left(C^*_{O_2} - C_{O_2}\right)$$

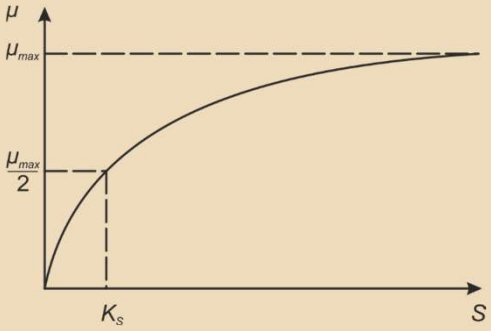
The term associated to the production rate. Expresses the production or consumption of a component through a chemical or biological reaction:

$$\begin{pmatrix} Mass\ production\ rate \\ of\ component\ i \end{pmatrix} = \begin{pmatrix} Volumetric\ production\ rate \\ of\ component\ i \end{pmatrix} \begin{pmatrix} Volume\ of\ the \\ system \end{pmatrix}$$

$$R_i = r_i V$$

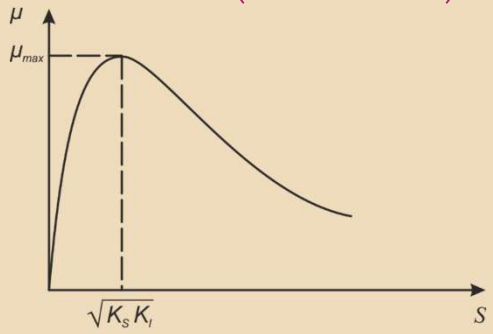
Kinetic models

$$r_x = \mu X = (\mu_g - \mu_d)X = \mu_{max}\left(\frac{S}{K_s + S}\right)X - \mu_d X$$



Substrate limitation kinetics (Monod)

$$r_x = \mu X = \mu_{max}\left(\frac{S}{K_s + S + \frac{S^2}{K_I}}\right)X - \mu_d X$$



Substrate inhibition kinetics

THE GENERAL DYNAMIC MODEL

The mass accumulation rate of any component of a system, expressed through mass balance, associated two types of terms:

- conversion terms (describe the kinetics of the chemical and biological reactions, and the conversion yields),
- transport terms (describe the transition of the mass through the system, in liquid and/or gaseous form, and the gas-liquid mass transfer phenomena)

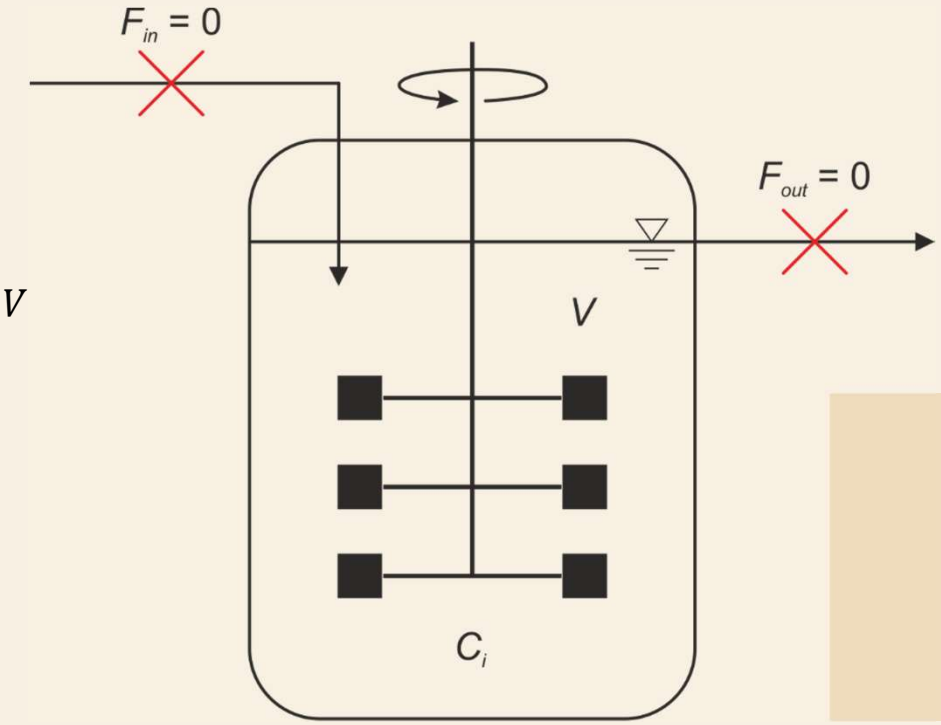
A series of hypotheses must be formulated:

- the process takes place in a CSTR,
- only the convective flows are considered,
- there are *n* input convective flows.

$$\frac{dVC_i}{dt} = r_i V + \sum_{j=1}^{n} F_{in,j} C_{i,0} - F_{out} C_i$$

The general dynamic model for discontinuous processes (batch). A discontinuous process has no transport flows over its bounds, $F_{in} = 0$ and $F_{out} = 0$:

$$\frac{d(VC_i)}{dt} = + F_{in}C_{i,0} + r_iV - F_{out}C_i$$



Batch CSTR

$$V\frac{dC_i}{dt} + C_i\frac{dV}{dt} = r_iV$$

$$\frac{dV}{dt} = 0$$

$$\frac{dC_i}{dt} = r_i$$

The general dynamic model for semi-continuous processes (fed-batch). A semi-continuous process has no output flow $F_{out} = 0$ while the reactor is filled up to a maximum volume:

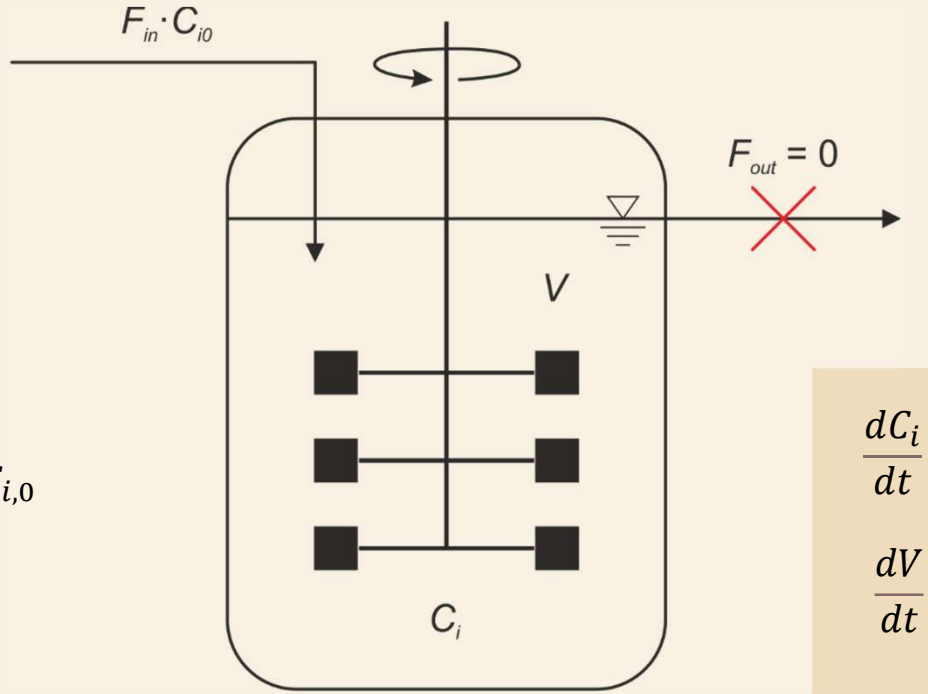
$$\frac{d(VC_i)}{dt} = + F_{in}C_{i,0} + r_iV - F_{out}C_i$$

$$\frac{dV}{dt} = F \qquad F_{in} = F$$

$$\frac{d(VC_i)}{dt} = r_iV + FC_{i,0}$$

$$V\frac{dC_i}{dt} + C_i\frac{dV}{dt} = r_iV + FC_{i,0}$$

$$\frac{dC_i}{dt} = r_i + \frac{F}{V}C_{i,0} - \frac{F}{V}C_i$$



Fed-batch CSTR

$$\frac{dC_i}{dt} = r_i + DC_{i,0} - DC_i$$

$$\frac{dV}{dt} = F \quad \text{with} \quad D = \frac{F}{V}$$

The general dynamic model for continuous processes. In a continuous process the fresh culture medium is continuously added, thus $F_{in} = F_{out} = F$:

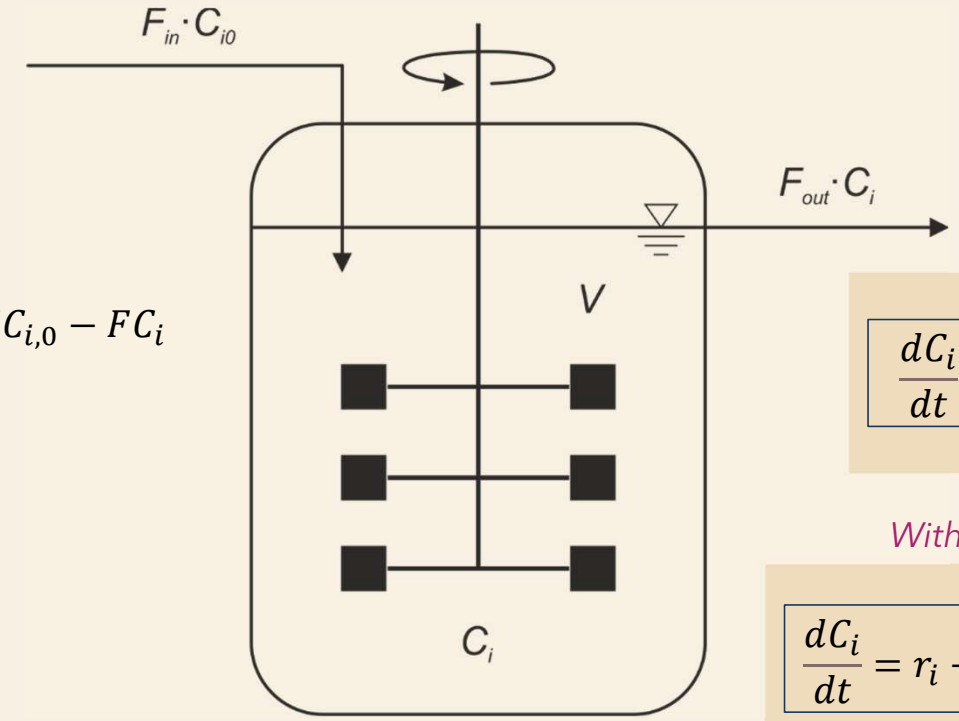
$$\frac{d(VC_i)}{dt} = + F_{in}C_{i,0} + r_iV - F_{out}C_i$$

$$F_{in} = F_{out} = F$$

$$V\frac{dC_i}{dt} + C_i\frac{dV}{dt} = r_iV + FC_{i,0} - FC_i$$

$$\frac{dV}{dt} = 0$$

$$\frac{dC_i}{dt} = r_i + \frac{F}{V}C_{i,0} - \frac{F}{V}C_i$$



Continuous CSTR

$$\frac{dC_i}{dt} = r_i + DC_{i,0} - DC_i$$

With gaseous components:

$$\frac{dC_i}{dt} = r_i + DC_{i,0} - DC_i + N_i$$

THE MODEL OF A MICROBIAL GROWTH PROCESS

Autocatalytic process

$$S + X = nX$$

Differential equations

$$\frac{dX}{dt} = r_x - DX$$

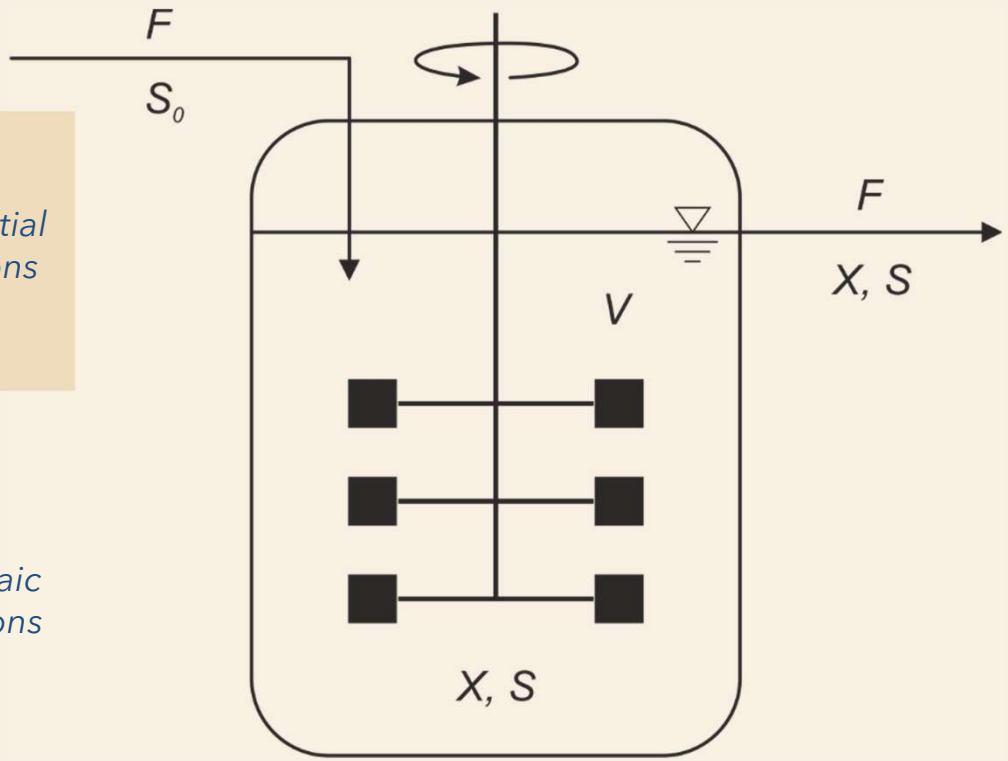
$$\frac{dS}{dt} = r_s + DS_0 - DS$$

Algebraic equations

$$r_x = r_{xg} - r_{xd} = \mu X - \mu_s X$$

$$r_x = \mu_{max} \frac{S}{K_S + S} X - \mu_s X$$

$$r_s = -\frac{1}{Y} r_{xg}$$



Microbial growth in a continuous CSTR

The function file with the mathematical model

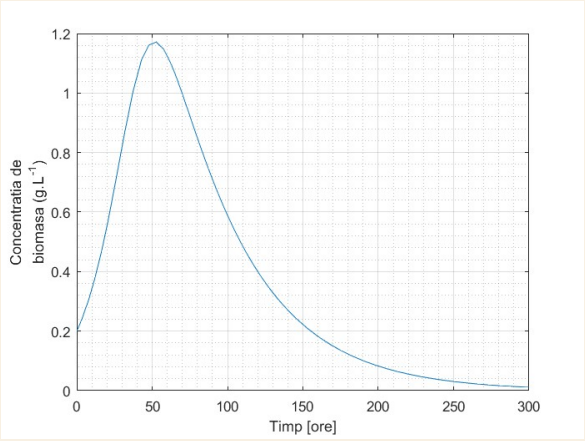
```
function dx = procbio(~,x)

global mumax mus KS Y Sin D

% Specific growth rate [h-1]
mu = mumax*(x(2)/(KS+x(2)));
% Volumetric growth rate [g.L-1.h-1]
rxg = mu*x(1);
% Volumetric decay rate [g.L-1.h-1]
rxd = mus*x(1);
% Global volumetric growth rate[g.L-1.h-1]
rx = rxg - rxd;

% __State variables
dx(1) = rx - D*x(1);                  % Biomass [g.L-1]
dx(2) = -1/Y*rxg + D*Sin - D*x(2);    % Substrate [g.L-1]

dx = dx';

end
```

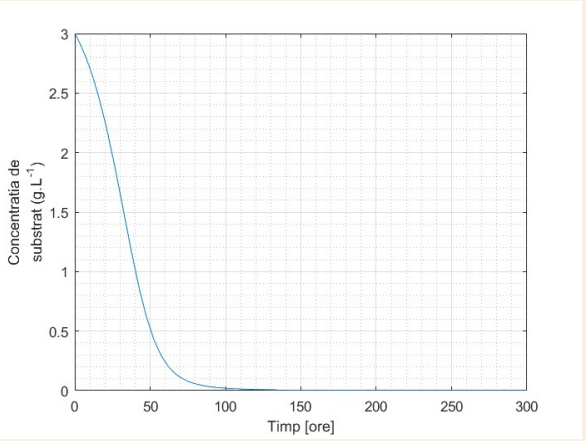
The script file with the integration function

```
clear; close all

global mumax mus KS Y Sin D

                % __Model parameters
mumax = 0.15;   % Maximul specific growth rate[h-1]
mus = 0.02;     % Specific decay rate[h-1]
KS = 0.1;       % Semi-saturation constant [g.L-1]
Y = 0.67;       % Conversion yield [-]

D = 0;          % Dilution [h-1]

x0 = [0.1 3];   % Initial conditions
Sin = 3;        % Input substrate concentration [g.L-1]

t0 = 0;         % initial time [h]
tfin = 300;     % final time [h]

[t,x] = ode45(@procbio,[t0 tfin],x0);

subplot(2,1,1); plot(t,x(:,1)); grid; grid minor
subplot(2,1,2); plot(t,x(:,2)); grid; grid minor
```

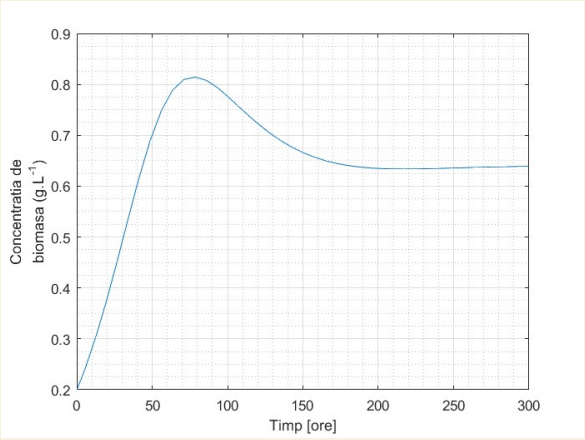
Simulation results

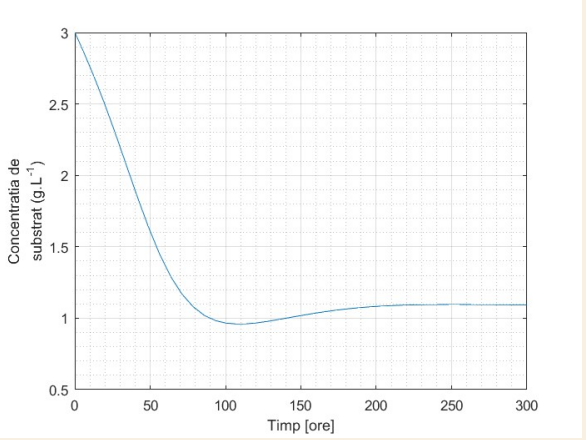
Batch: D = 0

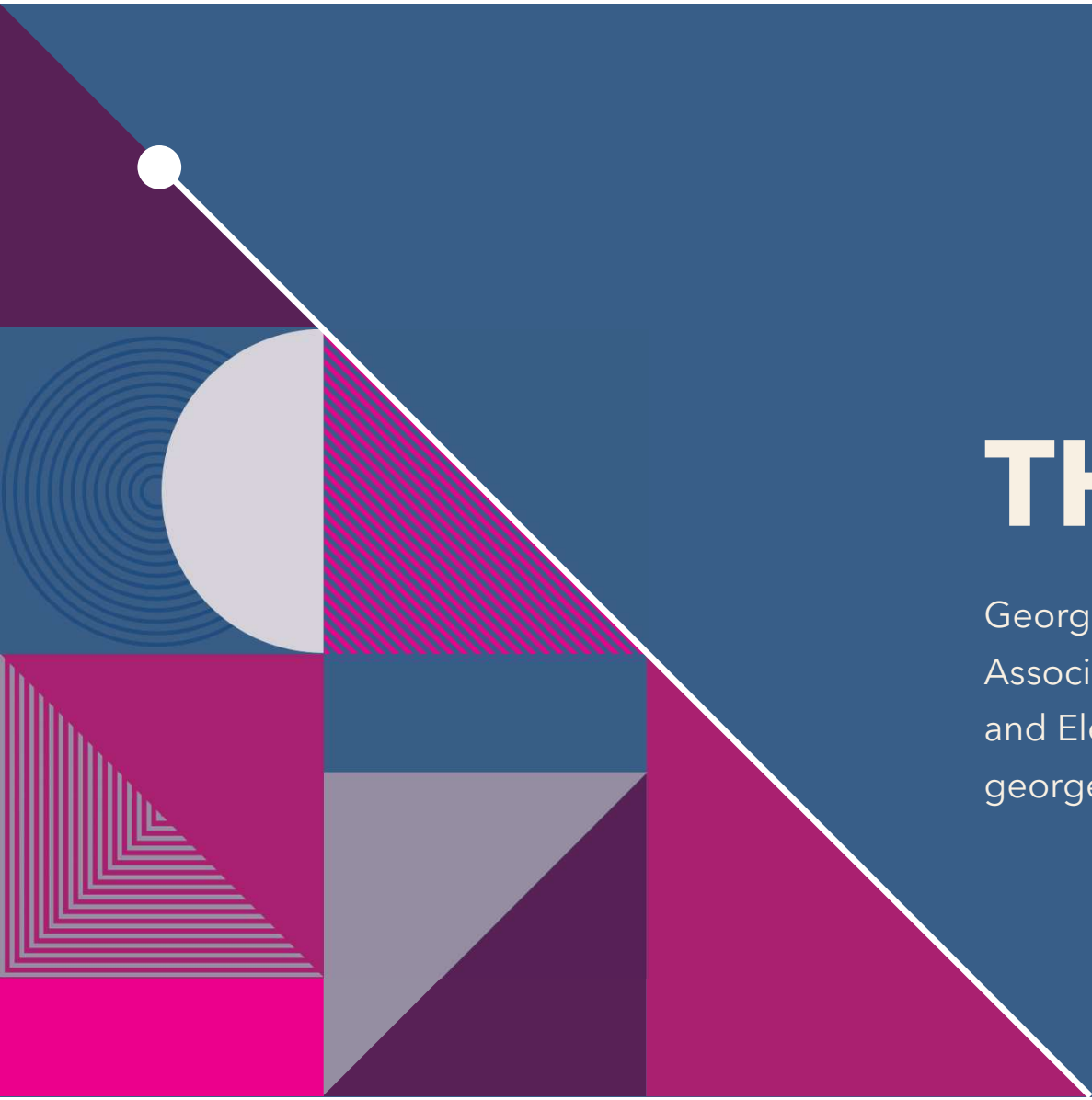




Continuous: D > 0







THANK YOU

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