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# BATCHREACTOR APPLICATION EXAMPLE

# WHITE BIOTECHNOLOGY

# SIMULATION OF BATCH GLUCONIC ACID PRODUCTION WITH USER DEFINED KINETICS

| EXAMPLE PURPOSE |
|---|
| The main interest of this example is to illustrate how to model bioreactors using BatchReactor software. With the advanced mode available in Simulis Reactions, the user can import libraries of kinetic models that can be easily modified and adjusted to suit a wide range of bioreactions.<br>This white biotechnology example deals with the fermentation of glucose to gluconic acid, which involves the oxidation of the aldehyde group of the sugar to a carboxyl group. The mathematical modeling of the reaction mechanisms uses specific equations (Monod type) which are not available in standard chemical reaction libraries. |

| ACCESS | ☑ Free-Internet | ☐ Restricted to ProSim clients | ☐ Restricted | ☐ Confidential |
|---|---|---|---|---|

| CORRESPONDING BATCHREACTOR FILE | *BATCHREA_EX_EN - Gluconic acid.pbpr* |
|---|---|



Energy

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# 1. INTRODUCTION

This example is taken from [COK01] and deals with the fermentation of glucose to gluconic acid, which involves the oxidation of the aldehyde group of the sugar to a carboxyl group. The industrial production of gluconic acid is done by strains of *Aspergillus* and *Pseudomonas ovalis*. The enzyme that catalyzes the oxidation of glucose is a dehydrogenase, which is capable of transforming glucose to gluconolactone. gluconic acid is produced by the hydrolysis of the gluconolactone, which can be either an enzymatic or non-enzymatic process. The enzyme that is required for the hydrolysis step is gluconolactonase, although the presence of this enzyme in *Apsergillus* and *Pseudomonas* has not been revealed. Rai and Constantinide [RAI73] considered the hydrolysis stage as a non-enzymatic process. The byproduct of the reaction is decomposed to water and oxygen by the enzyme catalase, which is present in the living cells.

Gluconic acid is extensively used in the food, pharmaceutical and a wide range of products. In the textile industries, gluconic acid, glucono-$\delta$-lactone and ammonium gluconates are used in acid catalysts. Gluconates are incorporated in antibiotic complexes (e.g., tetracycline) to improve stability, lower the toxicity and increase antibiotic levels in the blood. Calcium gluconates are employed for treating calcium deficiencies in both humans and animals. The hydrogen peroxide produced in the glucose oxidase catalyzed reaction contains an antibacterial action. The addition of a catalase converts the hydrogen peroxide to water and oxygen.

## 2. REACTION MECHANISM

The reaction mechanisms in the fermentation of glucose to gluconic acid are [COK01]:

- Cell growth:

$$Glucose \xrightarrow{Cell} More\ cells$$

Namely,

$$C_6H_{12}O_6 \xrightarrow{Cell} 1000\ Cells \quad \text{(R1)}$$

- Glucose oxidation:

$$Glucose + O_2 \longrightarrow Gluconolactone + H_2O_2$$

Namely,

$$C_6H_{12}O_6 + O_2 \xrightarrow{Glucoseoxidase} C_6H_{10}O_6 + H_2O_2 \quad \text{(R2)}$$

- Gluconolactone hydrolysis:

$$Gluconolactone + H_2O \longrightarrow Gluconic\ acid$$

Namely,

$$C_6H_{10}O_6 + H_2O \longrightarrow C_5H_{11}O_5COOH \quad \text{(R3)}$$

- Hydrogen peroxide decomposition:

$$H_2O_2 \longrightarrow H_2O + \frac{1}{2}O_2 \quad \text{(R4)}$$

## 3. COMPONENTS

Components which are taken into account in the simulation are:

| Name | CAS number[1] |
|---|---|
| Water(*) | 7732-18-5 |
| Glucose(*) | 50-99-7 |
| Gluconolactone | 90-80-2 |
| Gluconic acid | 526-95-4 |
| Oxygen(*) | 7782-44-7 |
| Nitrogen(*) | 7727-37-9 |
| Hydrogen peroxide(*) | 7722-84-1 |
| Cell | 55000-00-5 |

Compounds with an asterisk are taken from the standard database of Simulis Thermodynamics, thermodynamics server used in BatchReactor. The thermophysical properties stored in this database are the DIPPR recommended values [ROW15]. Oxygen and nitrogen vapor pressures were changed in order to well represent their solubility in water, Henry's law parameters were obtained from [FOG91]. Oxygen and nitrogen liquid specific heat were set equal to their perfect gas specific heat.

Gluconolactone and gluconic acid compounds were created by cloning the glucose compound from the standard database. Only the name, chemical formula, molecular weight and CAS registry number[1] were changed.

Cell compound was created by cloning the water compound from the standard database. Only the name, the chemical formula (arbitrary set to CHON), molar weight, CAS registry number[1] and vapor pressure correlation (set as non-volatile) were changed.

For all components, molar volume correlation parameters were changed in order to have the same density as water.

## 4. THERMODYNAMIC MODEL

Most of the components are non-volatile in the reaction conditions (glucose, gluconolactone, gluconic acid). Reactions occurred at ambient temperature and atmospheric pressure. The liquid phase was thus been assimilated to an ideal solution and gas phase was assumed to follow the perfect gas law. For the enthalpy calculations, the liquid phase at 25°C and 1 atmosphere enthalpy basis is used.

[1]: CAS Registry Numbers® are the intellectual property of the American Chemical Society; and are used by Fives ProSim SAS with the express permission of CAS. CAS Registry Numbers® have not been verified by CAS and may be inaccurate

## 5. REACTION MATHEMATICAL MODEL

Rai and Constantinide [RAI73] developed a mathematical model for the fermentation of the bacterium *Pseudomonas ovalis*, which converts the glucose to gluconic acid. The following equations describe the dynamics of the logarithmic growth phase:

- Rate of cell growth:

$$\frac{dC_{Cell}}{dt} = b_1 \times \left(1 - \frac{C_{Cell}}{b_2}\right) \times C_{Cell} \quad \text{(R1)}$$

- Rate of gluconolactone formation:

$$\frac{dC_{Gluconolactone}}{dt} = b_3 \times \frac{C_{Glucose}}{b_4 + C_{Glucose}} \times C_{Cell} \quad \text{(R2)}$$

- Rate of gluconic acid formation:

$$\frac{dC_{Gluconic\ acid}}{dt} = b_5 \times C_{Gluconolactone} \quad \text{(R3)}$$

- Rate of hydrogen peroxide decomposition: (R4)

  Hydrogen peroxide decomposition reaction was assumed to be a fast reaction following Arrhenius law, with a reaction rate constant of $1.10^6$ $h^{-1}$.

In the previous reaction rates, $C_i$ is the concentration of the compound "i" (g/L) and $t$ is the time (h).

All parameters taken from [COK01] are presented in the following table.

| $b_1$ ($h^{-1}$) | $b_2$ (g/l) | $b_3$ ($h^{-1}$) | $b_4$ (g/l) | $b_5$ ($h^{-1}$) |
|---|---|---|---|---|
| 0.949 | 3.439 | 18.72 | 37.51 | 1.169 |

# 6. REACTION MODEL IMPLEMENTATION USING SIMULIS REACTIONS

Except for the hydrogen peroxide decomposition, the user “interpreted” kinetic rate model was used to implement mathematical models presented by Rai and Constantinide [RAI73]. This feature allows the user to write his own code for the kinetic models using VBScript (Microsoft Visual Basic Scripting Edition), which is an interpreted language: it does not require any compilation before being executed and only requires for the computer to include an interpreter (e.g. *wscript.exe* in Windows environment).

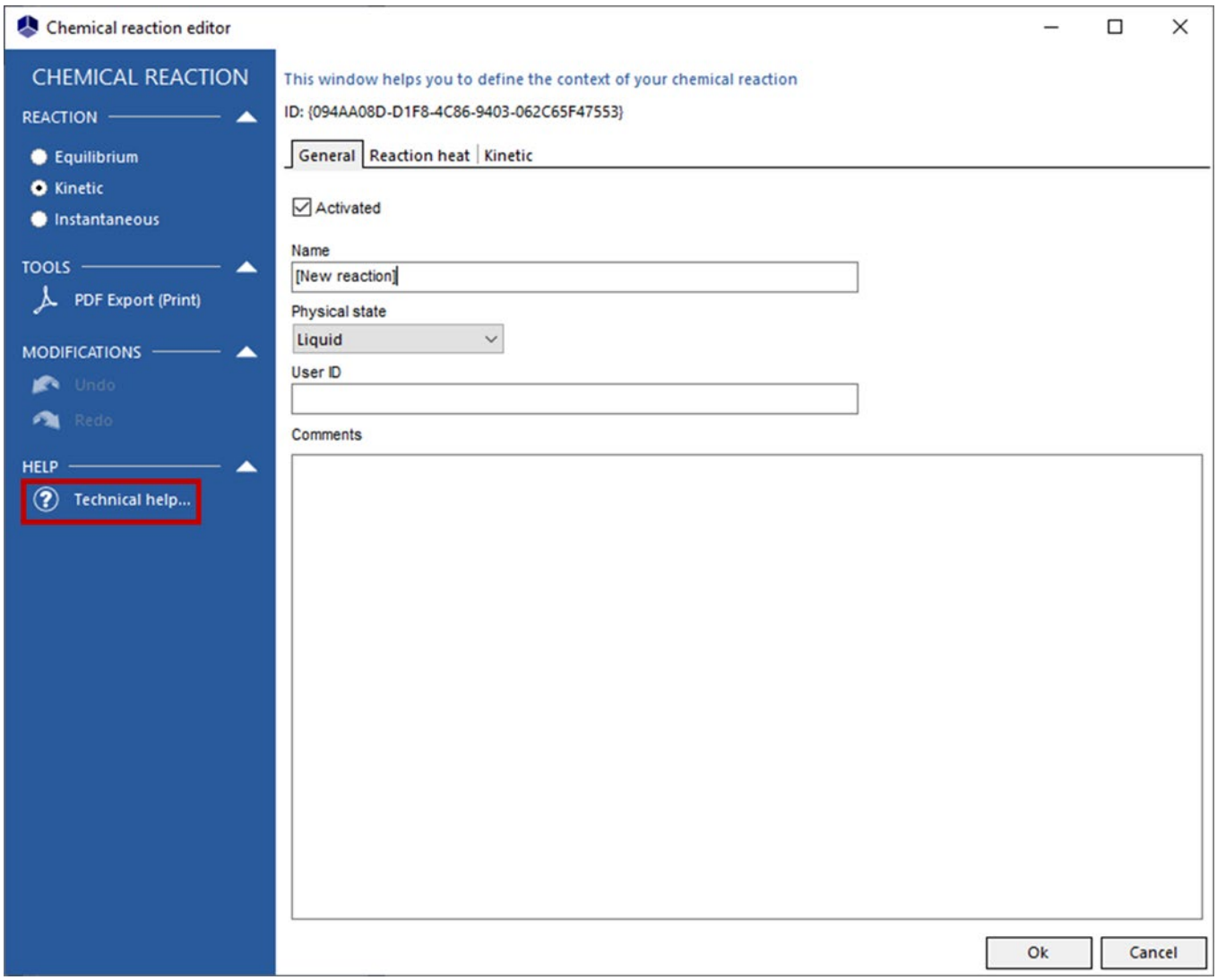
**A library of VBScripts for bioreaction kinetic models is provided with BatchReactor. For a step-by-step application example about the use of these scripts, please consult “*Getting started with BatchReactor – use case 2*”.**

All reactions take place in the liquid phase.

The heat of reaction of each reaction is assumed to be 0.

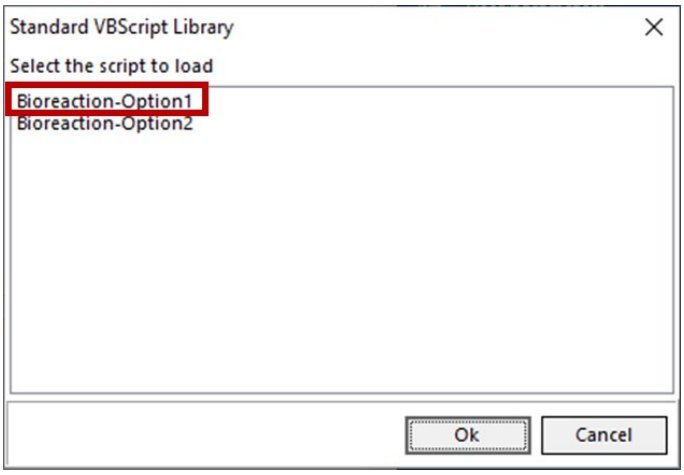
## 6.1. Modeling of the reaction 1

To get information about the suitable kinetic model to use along with the parameters to provide, access the “technical help”, available in the “help tab” of the “chemical reactions editor”:



The global reaction rate of the reaction 1 ($r_{G,1}$) can be modelled using the VBScript called “Bioreaction-option1”, available in the VBScript library:




$$Option\ 1\text{:}\ r_{G,1} = \left(\alpha . \mu_{max} \prod_{i=1}^{NLS} r(C_{Si}) + \beta\right) . C_{Cell}$$

$$knowing\ that\ r_{G,1} = \frac{r_{cell,1}}{\nu_{Cell,1}}$$

A single elementary kinetic term is required and its index is selected from the table 2 provided in the technical help:

$$\frac{dC_{Cell}}{dt} = b_1 \times \left(1 - \frac{C_{Cell}}{b_2}\right) \times C_{Cell}$$

$\mu_{max}$ ← Elementary kinetic model #9 (Luong): $1 - \left(\frac{C_I}{K_I}\right)^N$

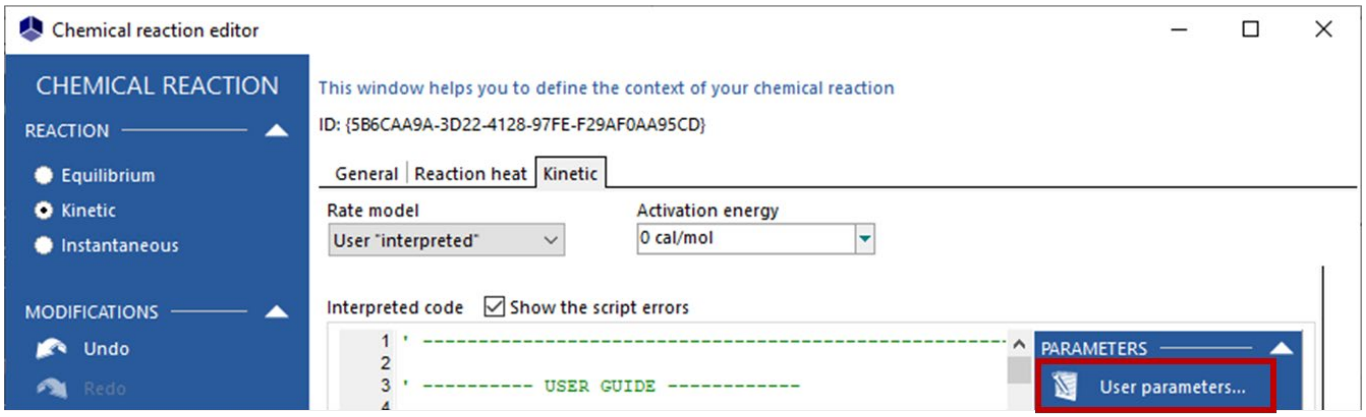
Here are the parameters to provide, where the "Biomass" compound refers to the "Cells" in this application example:

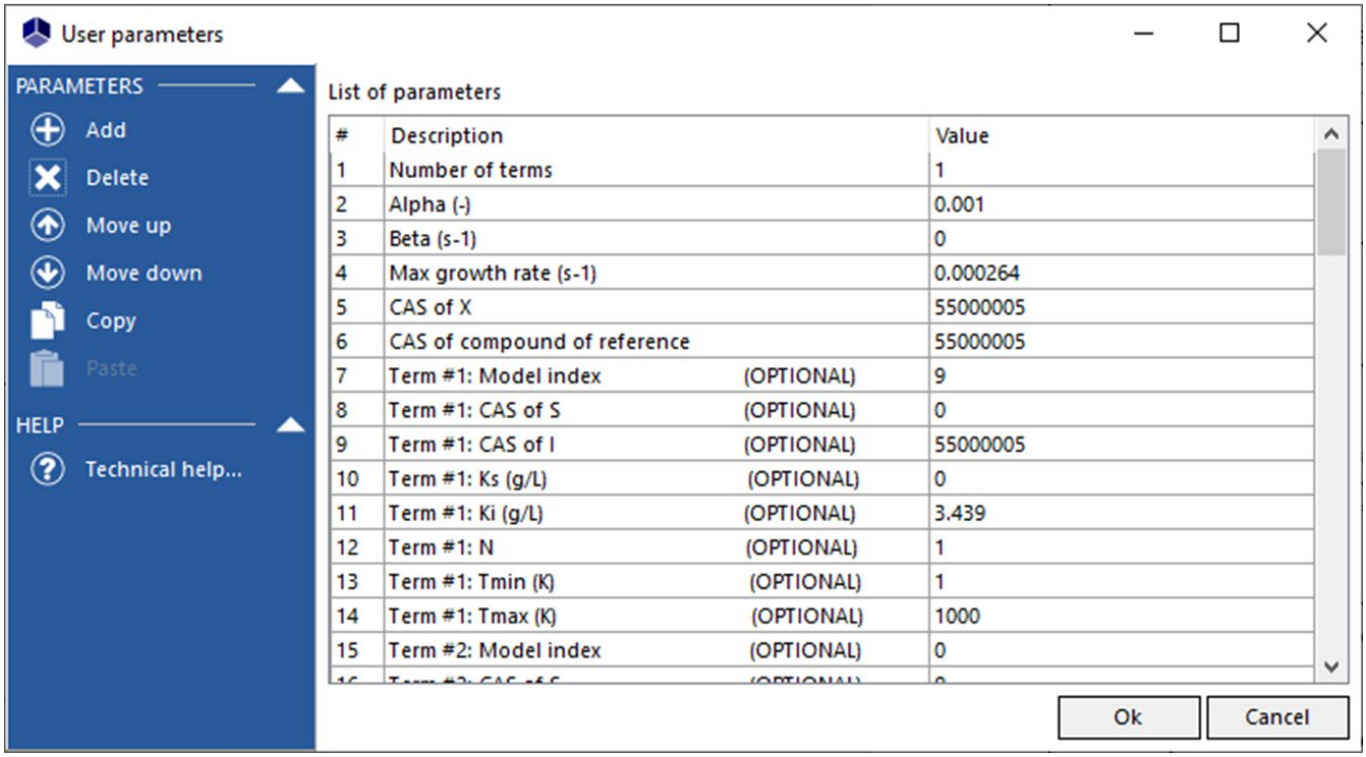
| **Model parameters** | **Reaction $R_1$** |
|---|---|
| Number of elementary terms | 1 |
| $\alpha$ ("*Alpha*") | $\frac{1}{\nu_{Cell,1}} = \frac{1}{1000} = 1.10^{-3}$ |
| $\beta$ ("*Beta*") | 0 |
| $\mu_{max}$ ("*Max growth rate*") | $\mu_{max} = b_1 = 0.949/3600 = 2.64.10^{-4}$ $s^{-1}$ |
| Selection of the "Biomass" compound ("*CAS of X*") | CAS number(*): 55000-00-5 |
| Selection of the compound of reference ("*CAS of Reference*") | CAS number(*): 55000-00-5 |
| Parameters of Term #1 | $Model\ index = 9$: $1 - \left(\frac{C_I}{K_I}\right)^N$<br>With:<br>Selection of the inhibitor ("*CAS of I*"): 55000-00-5<br>$K_I = b_2 = 3.439$ g/L<br>N = 1 |

(*): CAS Registry Numbers® are the intellectual property of the American Chemical Society; and are used by Fives ProSim SAS with the express permission of CAS. CAS Registry Numbers® have not been verified by CAS and may be inaccurate

The model parameters are specified as follows:

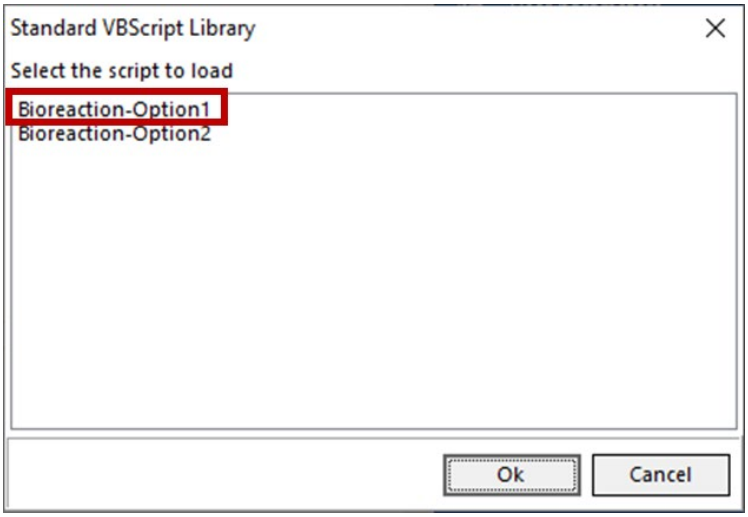



| # | Description | | Value |
|---|---|---|---|
| 1 | Number of terms | | 1 |
| 2 | Alpha (-) | | 0.001 |
| 3 | Beta (s-1) | | 0 |
| 4 | Max growth rate (s-1) | | 0.000264 |
| 5 | CAS of X | | 55000005 |
| 6 | CAS of compound of reference | | 55000005 |
| 7 | Term #1: Model index | (OPTIONAL) | 9 |
| 8 | Term #1: CAS of S | (OPTIONAL) | 0 |
| 9 | Term #1: CAS of I | (OPTIONAL) | 55000005 |
| 10 | Term #1: Ks (g/L) | (OPTIONAL) | 0 |
| 11 | Term #1: Ki (g/L) | (OPTIONAL) | 3.439 |
| 12 | Term #1: N | (OPTIONAL) | 1 |
| 13 | Term #1: Tmin (K) | (OPTIONAL) | 1 |
| 14 | Term #1: Tmax (K) | (OPTIONAL) | 1000 |
| 15 | Term #2: Model index | (OPTIONAL) | 0 |

## 6.2. Modeling of the reaction 2

The global reaction rate of the reaction 2 ($r_{G,2}$) can be modelled using the VBScript called “Bioreaction-option1”, available in the VBScript library:




$$Option\ 1:\ r_{G,2} = \left(\alpha.\mu_{max}\prod_{i=1}^{NLS} r(C_{Si}) + \beta\right).C_{Cell}$$

$$knowing\ that\ r_{G,2} = \frac{r_{Gluconolactone,2}}{\nu_{Gluconolactone,2}}$$

A single elementary kinetic term is required and its index is selected from the table 2 provided in the technical help:

$$\frac{dC_{Gluconolactone}}{dt} = b_3 \times \frac{C_{Glucose}}{b_4 + C_{Glucose}} \times C_{Cell}$$

$\mu_{max}$

Elementary kinetic model #1 (Monod): $\frac{C_S}{K_S + C_S}$

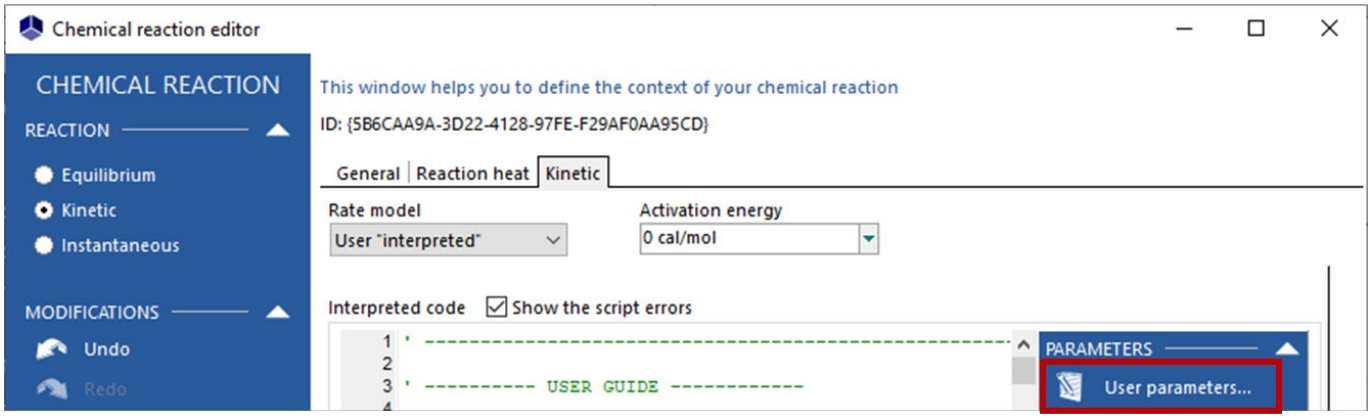
 

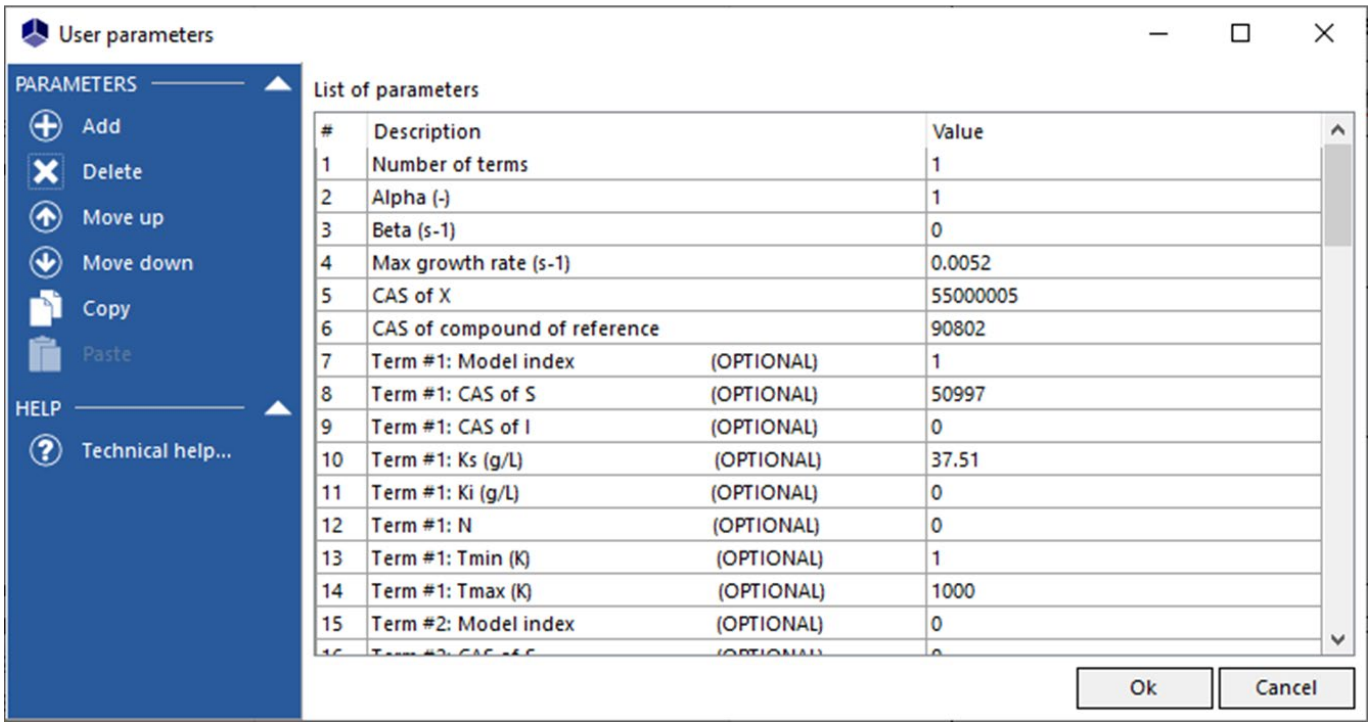
Here are the parameters to provide, where the “Biomass” compound refers to the “Cells” in this application example:

| **Model parameters** | **Reaction $R_2$** |
|---|---|
| Number of elementary terms | 1 |
| $\alpha$<br>("*Alpha*") | $\frac{1}{\nu_{Gluconolactone,2}} = 1$ |
| $\beta$<br>("*Beta*") | 0 |
| $\mu_{max}$<br>(“*Max growth rate*”) | $\mu_{max} = b_3 = 18.72/3600 = 5.2.10^{-3}\ s^{-1}$ |
| Selection of the “Biomass” compound<br>(“*CAS of X*”) | CAS number(*): 55000-00-5 |
| Selection of the compound of reference<br>(“*CAS of Reference*”) | CAS number(*): 90-80-2 |
| Parameters of Term #1 | $Model\ index = 1: \frac{C_S}{K_S + C_S}$<br>With:<br>Selection of the substrate (“*CAS of S*”): 50-99-7<br>$K_S = b_4 = 37.51$ g/L |

(*): CAS Registry Numbers® are the intellectual property of the American Chemical Society; and are used by Fives ProSim SAS with the express permission of CAS. CAS Registry Numbers® have not been verified by CAS and may be inaccurate

The model parameters are specified as follows:

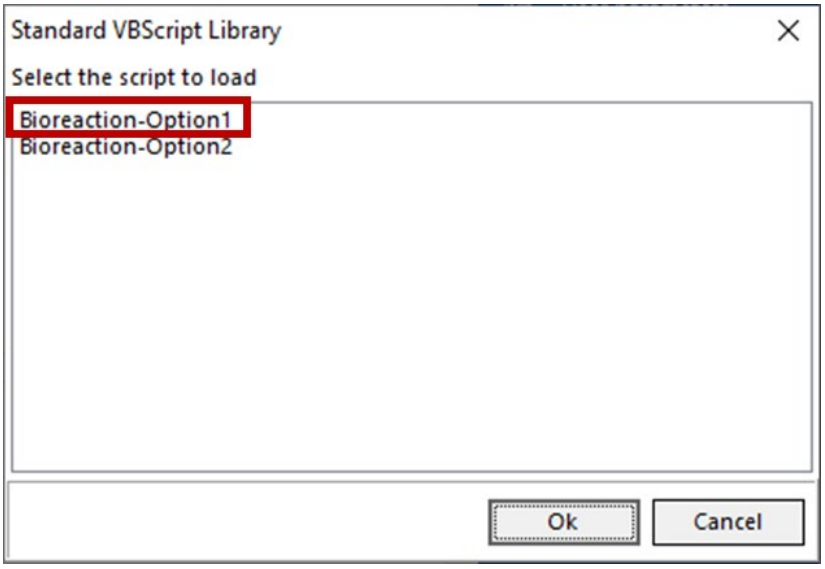



| # | Description | | Value |
|---|---|---|---|
| 1 | Number of terms | | 1 |
| 2 | Alpha (-) | | 1 |
| 3 | Beta (s-1) | | 0 |
| 4 | Max growth rate (s-1) | | 0.0052 |
| 5 | CAS of X | | 55000005 |
| 6 | CAS of compound of reference | | 90802 |
| 7 | Term #1: Model index | (OPTIONAL) | 1 |
| 8 | Term #1: CAS of S | (OPTIONAL) | 50997 |
| 9 | Term #1: CAS of I | (OPTIONAL) | 0 |
| 10 | Term #1: Ks (g/L) | (OPTIONAL) | 37.51 |
| 11 | Term #1: Ki (g/L) | (OPTIONAL) | 0 |
| 12 | Term #1: N | (OPTIONAL) | 0 |
| 13 | Term #1: Tmin (K) | (OPTIONAL) | 1 |
| 14 | Term #1: Tmax (K) | (OPTIONAL) | 1000 |
| 15 | Term #2: Model index | (OPTIONAL) | 0 |

## 6.3. Modeling of the reaction 3

The global reaction rate of the reaction 3 ($r_{G,3}$) can be modelled using the VBScript called “Bioreaction-option1”, available in the VBScript library:




$$Option\ 1:\ r_{G,3} = \left(\alpha . \mu_{max} \prod_{i=1}^{NLS} r(C_{Si}) + \beta\right) . C_{Cell}$$

$$knowing\ that\ r_{G,3} = \frac{r_{Gluconic\ acid,3}}{\nu_{Gluconic\ acid,3}}$$

A single elementary kinetic term is required and its index is selected from the table 2 provided in the technical help:

$$\frac{dC_{Gluconic\ acid}}{dt} = b_5 \times C_{Gluconolactone}$$

$\mu_{max}$

Elementary kinetic model #12 (1st order): $\frac{C_s}{C_X}$

Here are the parameters to provide, where the “Biomass” compound refers to the “Cells” in this application example:

| **Model parameters** | **Reaction $R_3$** |
|---|---|
| Number of elementary terms | 1 |
| $\alpha$ ("*Alpha*") | $\frac{1}{\nu_{Gluconic\ acid,3}} = 1$ |
| $\beta$ ("*Beta*") | 0 |
| $\mu_{max}$ (“*Max growth rate*”) | $\mu_{max} = b_5 = 1.169/3600 = 3.25.10^{-4}\ s^{-1}$ |
| Selection of the “Biomass” compound (“*CAS of X*”) | CAS number(*): 55000-00-5 |
| Selection of the compound of reference (“*CAS of Reference*”) | CAS number(*): 526-95-4 |
| Parameters of Term #1 | $Model\ index = 12: \frac{C_S}{C_X}$<br>With:<br>Selection of the substrate (“*CAS of S*”): 90-80-2<br>Note: in this case, it is not necessary to select the biomass since it was previously done |

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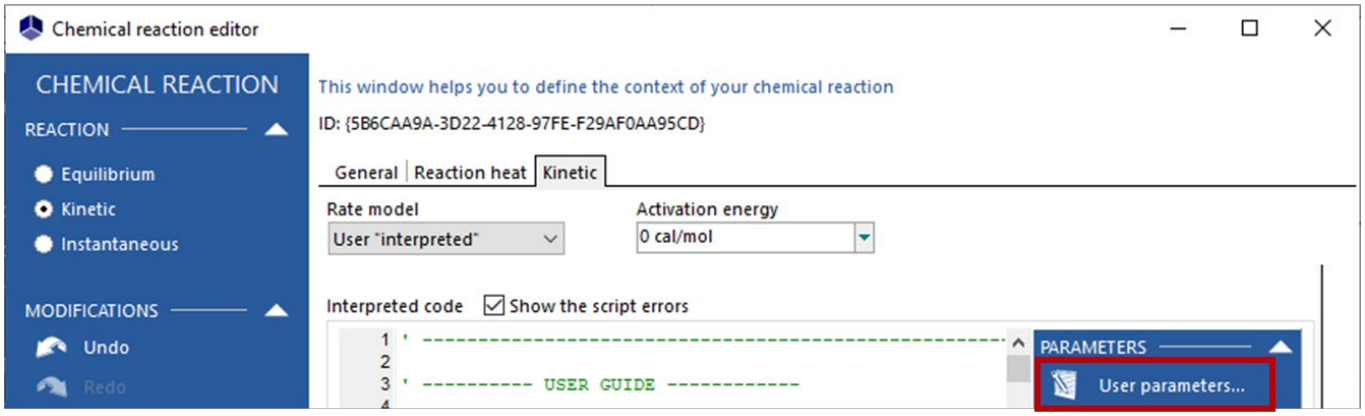
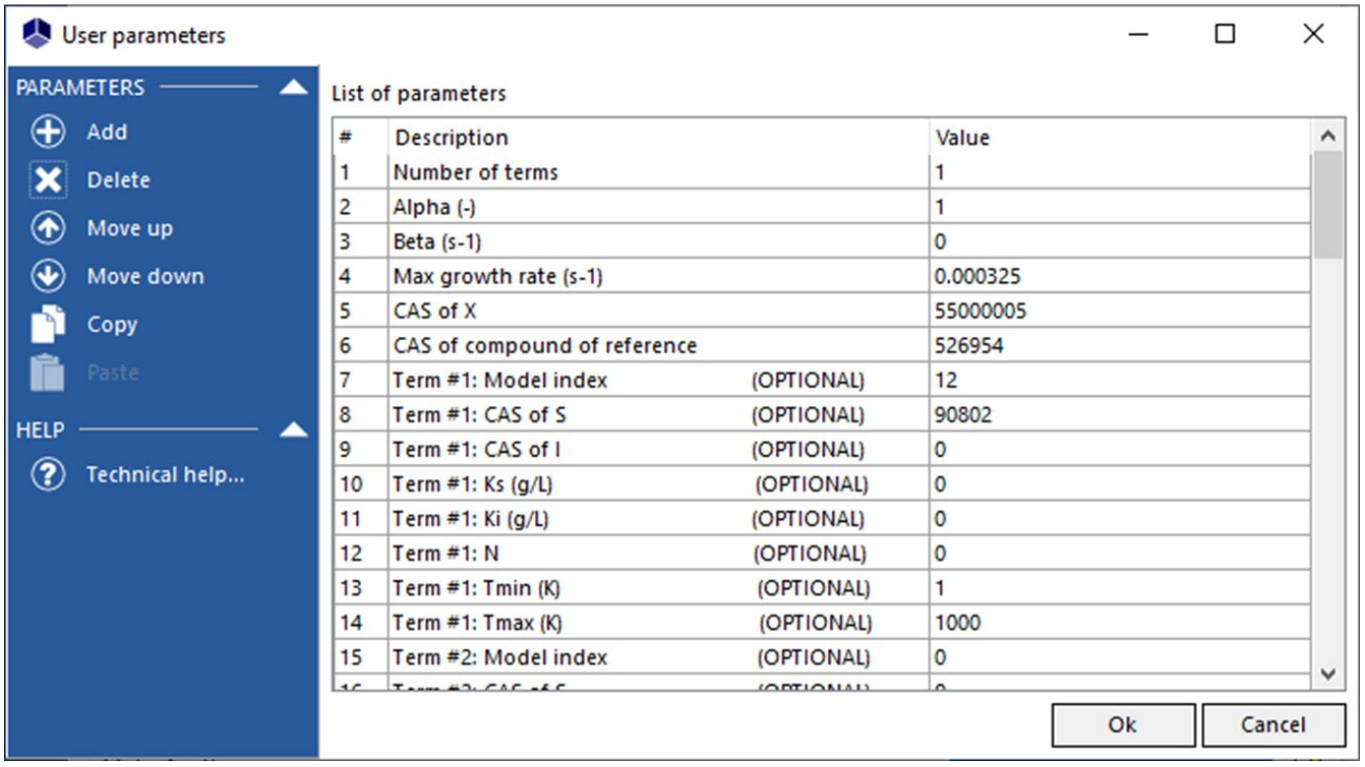
The model parameters are specified as follows:

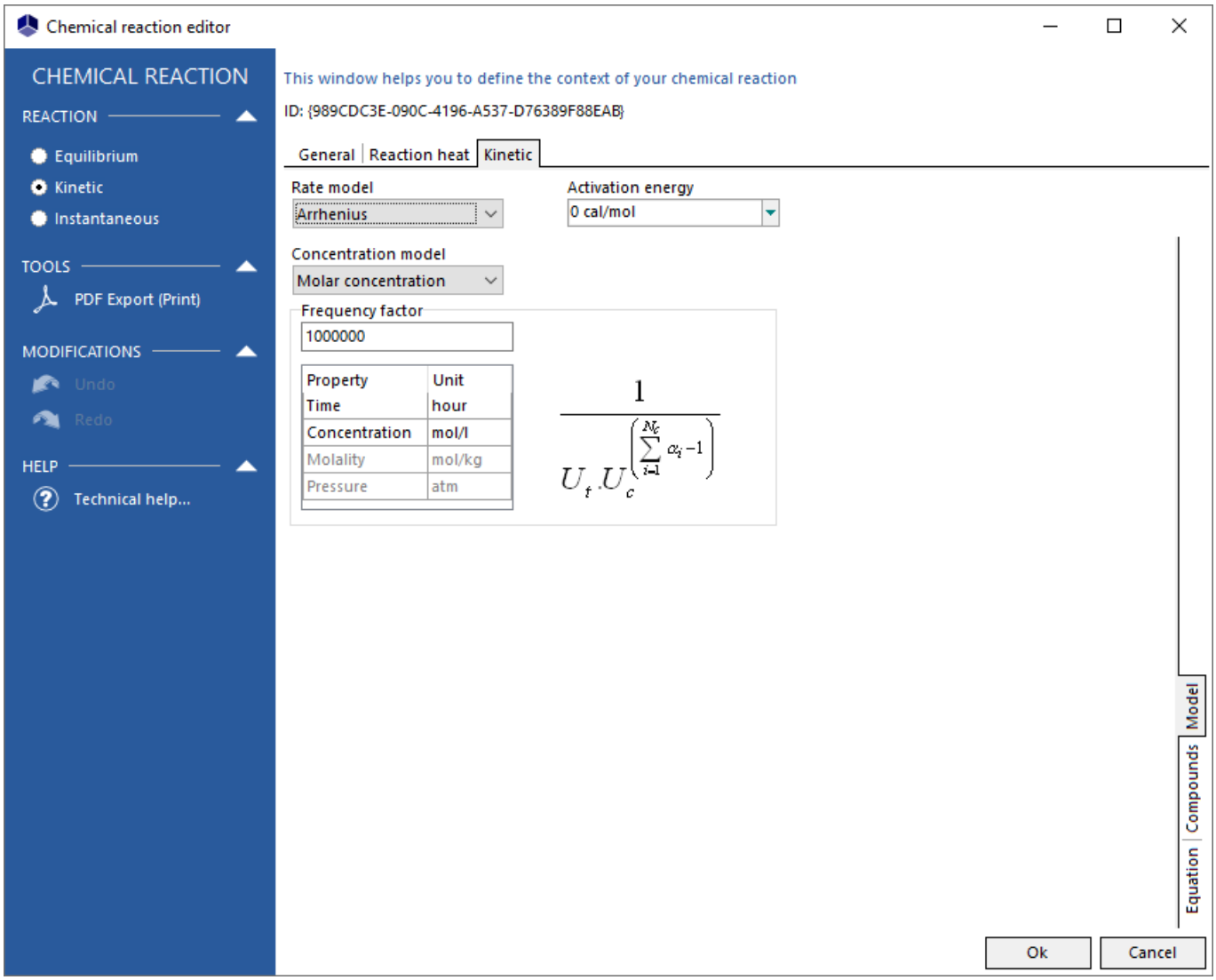


## 6.4. Modeling of the reaction 4

Since the reaction 4 is modeled with the Arrhenius law, the standard chemical rate model can be used (with a reaction rate constant of $1.10^6$ $h^{-1}$):



# 7. SIMULATION

## 7.1. Process description

The reactor used for the gluconic acid production is described in the table below.

| Reactor | |
|---|---|
| Type | Closed vapor-liquid |
| Global volume (vapor + liquid) | 5.5 $m^3$ |
| Head space (initial) | Nitrogen |

The initial conditions are presented in the following table:

| Initial conditions | |
|---|---|
| **Temperature** | 25°C |
| **Pressure** | 1 atm |
| **Initial load (kg)** | |
| **Glucose** | 50 |
| **Cell** | 0.5 |
| **Oxygen** | 1.2 |
| **Water** | 950 |
| **Nitrogen** | 3.3 |
| **Other compounds** | 0 |

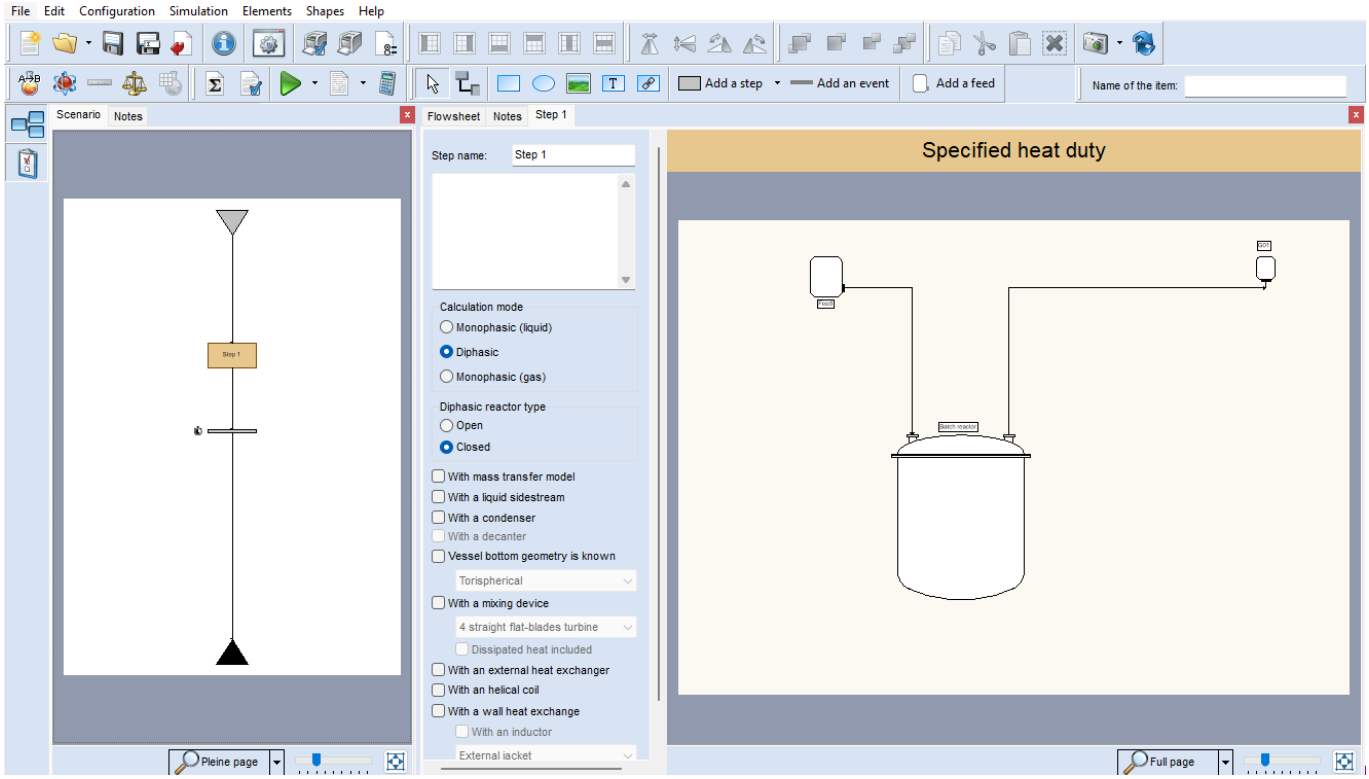
A continuous air stream at ambient condition feeds the reactor in order to carry in the oxygen required for the reactions. The characteristics of this feed are:

| | |
|---|---|
| **Temperature** | 25°C |
| **Pressure** | 1 atm |
| **Total flow rate** | 10 kg/h |
| **Mole fractions** | |
| **Oxygen** | 0.21 |
| **Nitrogen** | 0.79 |
| **Other components** | 0 |

The recipe consists in one adiabatic step with the following parameters:

| | |
|---|---|
| **Type** | Specified heat duty |
| **Heat duty** | 0 kcal/h |
| **Pressure** | 1 atm |
| **Step duration** | 10 h |

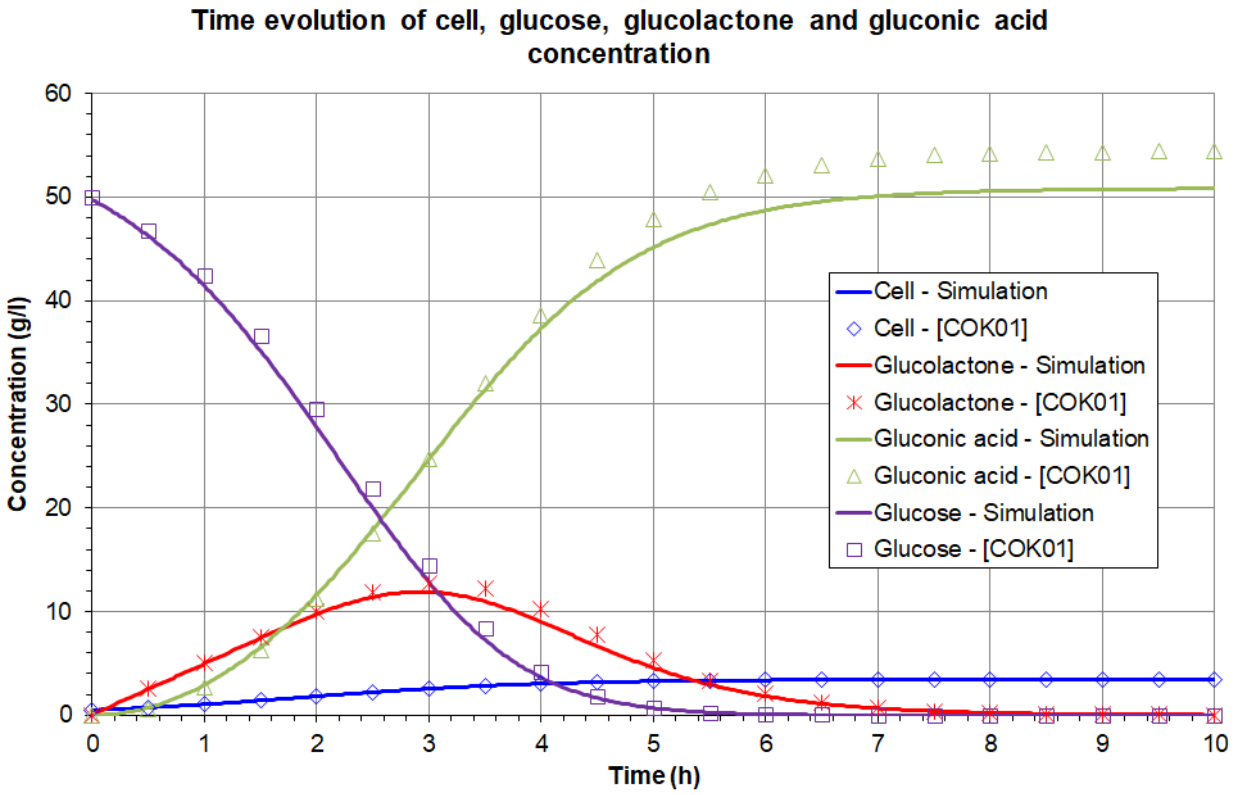
The scenario is presented on the left part of the following screen shot and the flowsheet on the right part.



## 7.2. Results

Next graph presents some simulation results obtained with BatchReactor software. The compounds concentrations vs. time curves show a good agreement with data provided by [COK01]. It has to be noticed that using BatchReactor software, all operating parameters can be monitored (liquid volume, gas phase compositions…). Moreover, the detailed modeling of the reactor (heating/cooling system, condenser, vessel geometry…) can be taken into account with BatchReactor.



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