fives
Industry can do it

BATCHREACTOR APPLICATION EXAMPLE

WHITE BIOTECHNOLOGY

SIMULATION OF BATCH POLY-β-HYDROXYBUTYRIC ACID (PHB) 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. This white biotechnology example deals with the production of Poly-β-Hydroxybutyric acid (PHB) by the microorganism *Alcaligenes eutrophus.* The mathematical modeling of the reaction mechanisms uses specific equations (Monod and sigmoidal terms) which are not available in standard chemical reaction libraries.

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

CORRESPONDING BATCHREACTOR FILE	*BATCHREA_EX_EN - PHB.pbpr*

Reader is reminded that this use case is only an example and should not be used for other purposes. Although this example is based on actual case it may not be considered as typical nor are the data used always the most accurate available. Fives ProSim shall have no responsibility or liability for damages arising out of or related to the use of the results of calculations based on this example.

Energy

Fives ProSim

Siège social : Immeuble Stratège A - 51 rue Ampère - 31670 Labège - FRANCE

Tél. : +33 (0)5 62 88 24 30

S.A.S. au capital de 147 800 € - 350 476 487 R.C.S. Toulouse - Siret 350 476 487 00037 - APE 5829C - N° TVA FR 10 350 476 487

www.fivesgroup.com / www.fives-prosim.com

TABLE OF CONTENTS

Copyright © 2025 Fives ProSim, Labège, France – All rights reserved

1. INTRODUCTION

This example is taken from [HEU80] and deals with the production of Poly-β-Hydroxybutyric Acid (PHB), a biodegradable polymer, by the bacterium *Alcaligenes eutrophus.* The model developed by Heinzle and Lafferty [HEI80] describes the batch culture of these microorganisms and it considers that the growth and storage of PHB, which is used as an energy stock by the bacterium, are functions of the limiting substrates (NH_4^+), the residual biomass and the product concentrations. The influence of gas transfer is eliminated by the maintenance of the dissolved gas concentrations. In the growth stage there is enough substrate to allow the protein synthesis (residual biomass). When the substrate achieves a sufficient low concentration the protein production stops and the PHB production increases (storage stage).

2. REACTION MECHANISM

The reaction mechanisms for the PHB synthesis are:

- ✓ Growth stage:

$$\alpha CO_2 + \beta O_2 + \gamma H_2 + \delta Substrate \longrightarrow \varepsilon Residual\ Biomass + PHB + \theta H_2O + \mu H_2SO_4$$

For example,

$$\alpha CO_2 + \beta O_2 + \gamma H_2 + \delta (NH_4)_2SO_4 \longrightarrow \varepsilon C_{4,09}H_{7,13}O_{1,89}N_{0,76} + C_4H_6O_2 + \theta H_2O + \mu H_2SO_4 \quad \text{(R1)}$$

- ✓ Storage stage:

$$\rho CO_2 + \tau O_2 + \varphi H_2 \longrightarrow PHB + \omega H_2O$$

For example,

$$\rho CO_2 + \tau O_2 + \varphi H_2 \longrightarrow C_4H_6O_2 + \omega H_2O \quad \text{(R2)}$$

The stoichiometric coefficients of the reactions are found through a mass balance of each chemical element. The Excel solver is used for the calculation. This step is necessary because the stoichiometric reactions are not available on the used bibliography. It is important to notice that PHB is a polymer and its number of monomers is unknown. In [ISH91], this component is modeled by its monomer. The same hypothesis is adopted for the simulation. The stoichiometric coefficients used are presented on the table below.

	CO_2	O_2	H_2	Substrate $(NH_4)_2SO_4$	Residual biomass	PHB	H_2O	H_2SO_4
(R1)	-42.95238	-3.678021	-99.35604	-3.619048	9.5238095	1	73.26080	3.619048
(R2)	-4	-12	-33	-	-	1	30	-

Copyright © 2025 Fives ProSim, Labège, France – All rights reserved

3. COMPONENTS

Components which are taken into account in the simulation are:

Name	CAS number(1)
Carbon dioxide(*)	124-38-9
Oxygen(*)	7782-44-7
Nitrogen(*)	7727-37-9
Hydrogen(*)	1333-74-0
Water(*)	7732-18-5
Sulfuric acid(*)	7664-93-9
Ammonium sulfate(*)	7783-20-2
PHB	55001-01-9
Residual biomass	55001-02-0

Compounds with an asterisk are taken from the standard database of Simulis Thermodynamics, thermodynamics server used in BatchReactor. The thermophysical parameters stored in this database are the DIPPR recommended values [ROW2015]. The nitrogen is added because, at the beginning, the head space of the reactor is filled with air (79% N_2, 21% O_2). Carbon dioxide, oxygen, nitrogen and hydrogen vapor pressures were changed in order to well represent their solubility in water, Henry's law parameters were obtained from [FOG91].

The PHB and residual biomass compounds were created by the function "Add a new compound" of Simulis Thermodynamics. Their chemical formulas are taken from [ISH91]. The properties specified are:

- ✓ CAS number(*) : Arbitrary number
- ✓ Chemical formula : From [ISH91]
- ✓ Molecular weight : From [ISH91]
- ✓ Physical state at 25°C : Solid
- ✓ Physical state in aqueous solution at 25°C : Not soluble
- ✓ Enthalpy of formation for ideal gas at 25°C : 0 J/mol
- ✓ Vapor and liquid mass specific heat: : Same as water
- ✓ Vapor pressure : Parameters chosen to avoid the vaporization

$$Ln(P^0) = -30 \qquad \text{(Equation 101)}$$

- ✓ Vaporization enthalpy : 0 J/mol
- ✓ Liquid density : Same as water

The above properties, except for the CAS number(1), are also applied to the ammonium sulfate.

For all compounds in the liquid phase (sulfuric acid, ammonium sulfate, PHB and residual biomass) the liquid density is assumed to be equal to the density of water.

(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

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

4. THERMODYNAMIC MODEL

Reactions occurred at ambient temperature (30°C) and atmospheric pressure, so the gas phase was assumed to follow the Ideal Gas law.

The liquid phase contains insoluble solids (residual biomass, PHB and ammonium sulfate). These solids have been represented as non-volatile liquids (see § 3). They should be excluded from the liquid phase for the vapor-liquid equilibrium. If not, they modify the real compositions of the liquid phase and so the vapor-liquid equilibrium constants of volatile components (water, carbon dioxide, oxygen, nitrogen, hydrogen). Thus, the “Solids excluded from liquid phase” model has been selected to calculate the liquid fugacity.

Henry’s laws from [FOG91] were used to model the solubilities of the gases (carbon dioxide, oxygen, nitrogen and hydrogen) into water.

5. REACTION MATHEMATICAL MODEL

[HEI80] developed a mathematical model for the production of PHB by the bacterium *Alcaligenes eutrophus*. The evolution of the PHB concentration is modeled as the sum of two contributions:

$$\frac{dP}{dt} = r_P = r_{P,1} + r_{P,2}$$

These two contributions are described below.

- ✓ Growth associated term:

$$r_{P,1} = Y_{P/X} \times r_{X,1} = \frac{\nu_P}{\nu_X} \times r_{X,1} \qquad \text{(R1)}$$

Where

$$r_{X,1} = \mu \times X$$

$$\mu = \mu_1 + \mu_2 = \mu_{m,1} \frac{S}{K_{S,1} + S} + \mu_{m,2} \frac{(S/K_{S,2})^{n_{Hill}}}{1 + (S/K_{S,2})^{n_{Hill}}}$$

- ✓ Storage associated term:

$$r_{P,2} = \frac{K_1}{K_1 + S}(-k_1 P + k_2 X) \qquad \text{(R2)}$$

All parameters taken from [HEI80] are presented in the following table.

$\mu_{m,1}$ (h^{-1})	$\mu_{m,2}$ (h^{-1})	$K_{S,1}$ (g/l)	$K_{S,2}$ (g/l)	n_{Hill}	K_1 (g/l)	k_1 (h^{-1})	k_2 (h^{-1})
0.13	0.08	0.1	1.0	5	0.041	0.045	0.18

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

6. REACTION MODEL IMPLEMENTATION USING SIMULIS REACTIONS

User “interpreted” kinetic rate model was used to implement the mathematical models presented by [HEI80] for both reactions. 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).

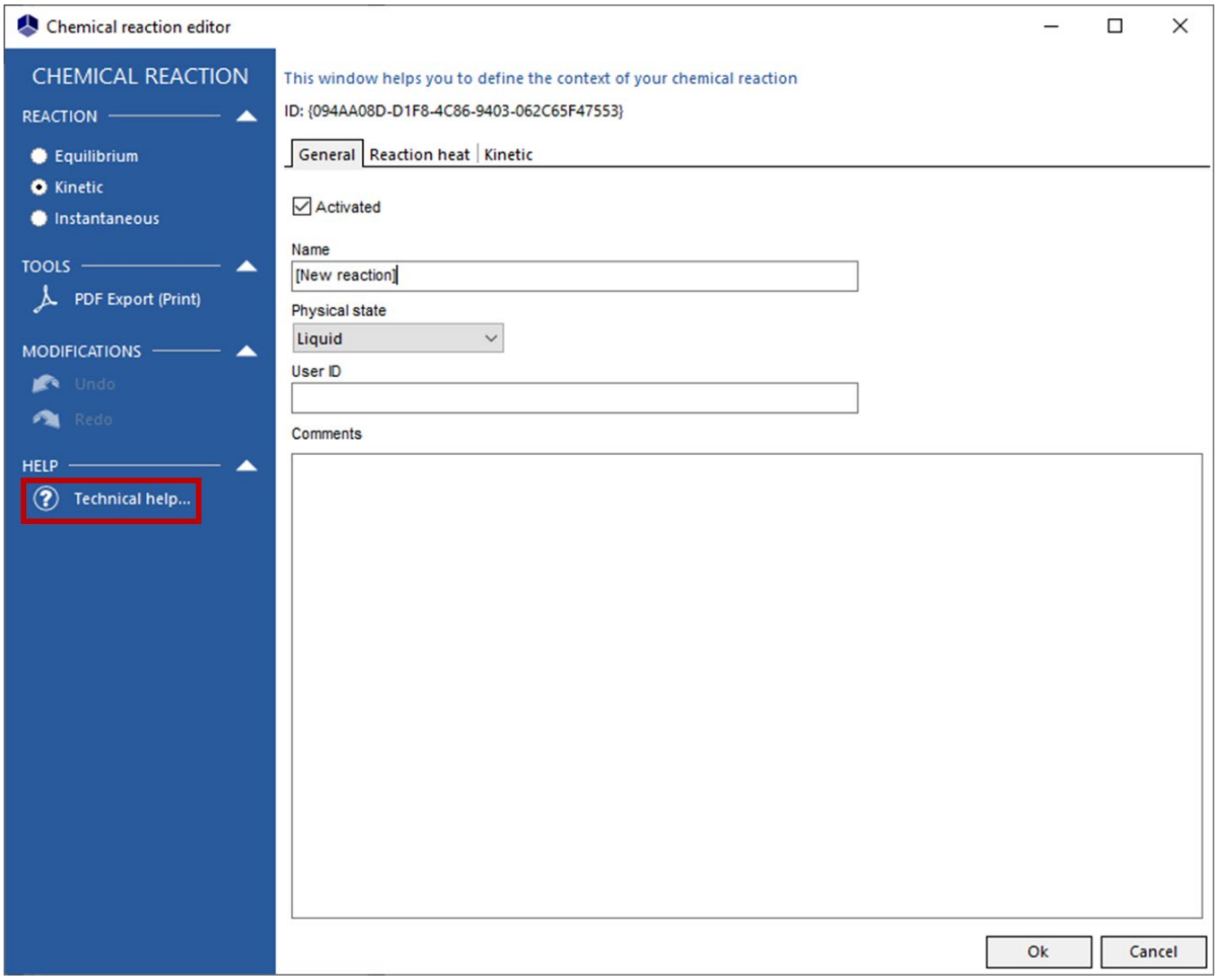
A library of VBScripts for bioreaction kinetic models is provided with BatchReactor. For a step-by-step application example about the use of these scripts, please consult “*Getting started with BatchReactor – use case 2*”.

All reactions take place in the liquid phase.

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

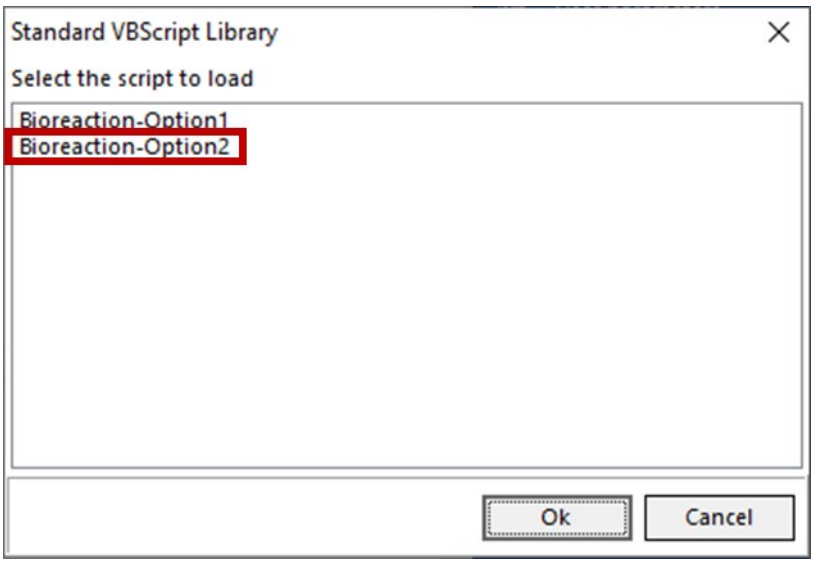
6.1. Modeling of the first reaction

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”:



Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

The global reaction rate of the reaction 1 ($r_{G,1}$) can be modelled using the VBScript called “Bioreaction-option2”, available in the VBScript library:

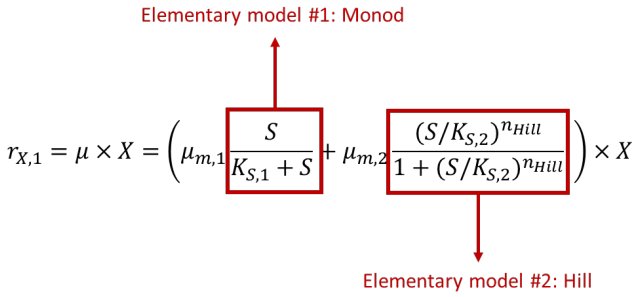


$$r_{G,1} = \left(\alpha . \sum_{i=1}^{NLS} \mu_{max} . r(S_i) + \beta \right) . X$$

$$knowing\ that\ r_{G,1} = \frac{r_{X,1}}{\nu_{X,1}} = \frac{\mu \times X}{\nu_{X,1}}$$

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

Two elementary terms are required and their indexes are selected from the table 2 provided in the technical help:



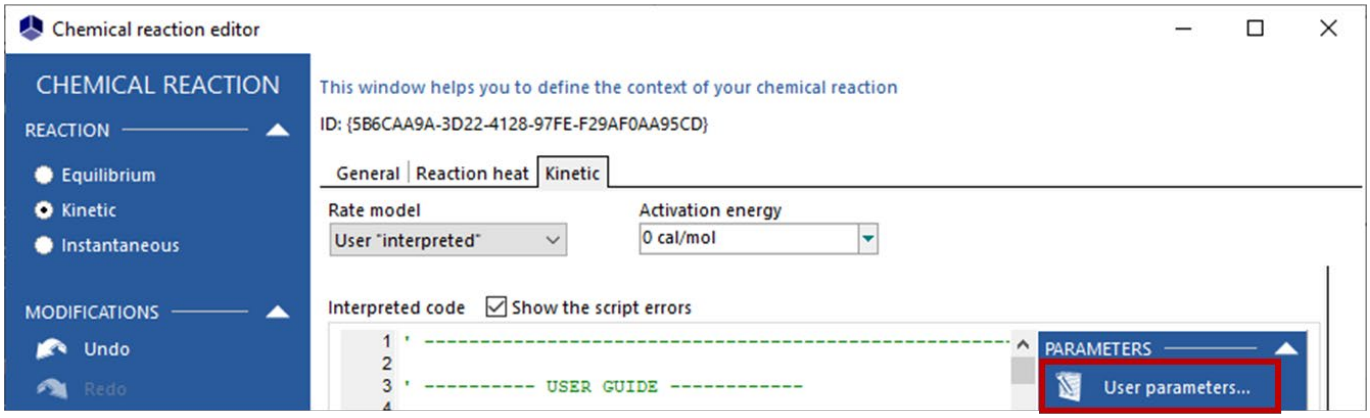
Here are the parameters to provide:

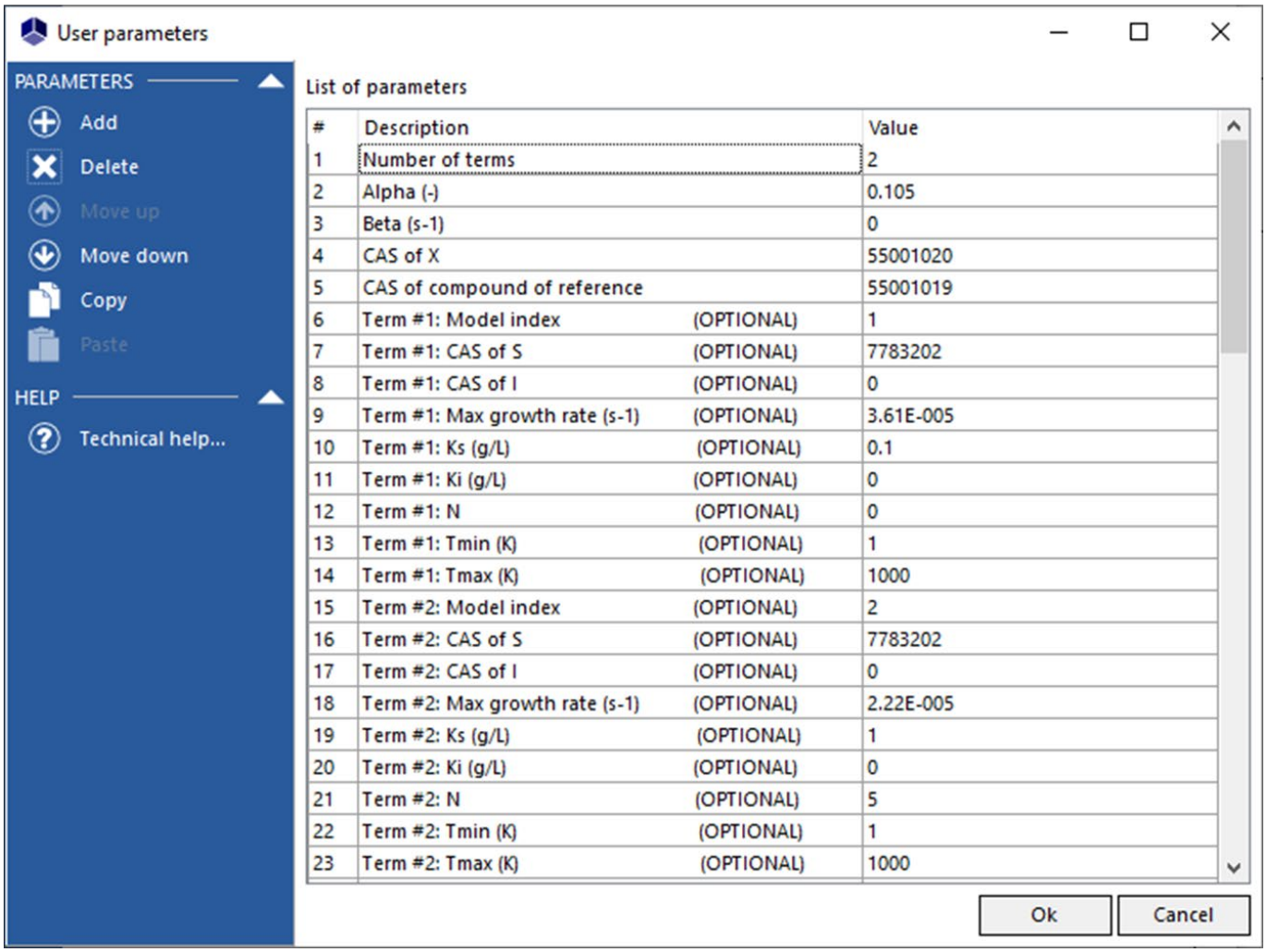
Model parameters	**R_1**
Number of elementary terms	2
α ("*Alpha*")	$\frac{1}{\nu_{X,1}} = \frac{1}{9.52} = 0.105$
β ("*Beta*")	0
Selection of the "Biomass" compound ("*CAS of X*")	CAS number(*): 55001-02-0
Selection of the compound of reference ("*CAS of Reference*")	CAS number(*): 55001-01-9
Parameters of Term #1	$Model\ index = 1: \frac{C_S}{K_S + C_S}$ With: Selection of the substrate ("*CAS of* S"): 7783-20-2 $K_S = K_{S,1} = 0.1$ g/L $\mu_{max,1} = \mu_{m,1} = 0.13/3600 = 3.61.10^{-5}$ s^{-1}
Parameters of Term #2	$Model\ index = 2: \frac{{C_S}^N}{{K_S}^N + {C_S}^N}$ With: Selection of the substrate ("*CAS of* S"): 7783-20-2 $K_S = K_{S,2} = 1$g/L $N = n_{Hill} = 5$ $\mu_{max,2} = \mu_{m,2} = 0.08/3600 = 2.22.10^{-5}$ s^{-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

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

The model parameters are specified as follows:





#	Description		Value
1	Number of terms		2
2	Alpha (-)		0.105
3	Beta (s-1)		0
4	CAS of X		55001020
5	CAS of compound of reference		55001019
6	Term #1: Model index	(OPTIONAL)	1
7	Term #1: CAS of S	(OPTIONAL)	7783202
8	Term #1: CAS of I	(OPTIONAL)	0
9	Term #1: Max growth rate (s-1)	(OPTIONAL)	3.61E-005
10	Term #1: Ks (g/L)	(OPTIONAL)	0.1
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)	2
16	Term #2: CAS of S	(OPTIONAL)	7783202
17	Term #2: CAS of I	(OPTIONAL)	0
18	Term #2: Max growth rate (s-1)	(OPTIONAL)	2.22E-005
19	Term #2: Ks (g/L)	(OPTIONAL)	1
20	Term #2: Ki (g/L)	(OPTIONAL)	0
21	Term #2: N	(OPTIONAL)	5
22	Term #2: Tmin (K)	(OPTIONAL)	1
23	Term #2: Tmax (K)	(OPTIONAL)	1000

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

6.2. Modeling of the second reaction

The specific rate of reaction corresponding to the formation of PHB (P) in the second reaction can be written as follows:

$$r_{P,2} = \frac{K_1}{K_1 + S}(-k_1 P + k_2 X) = k_2 \frac{K_1}{K_1 + S} X - k_1 \frac{K_1}{K_1 + S} P$$

A way to use the library of bioreaction kinetic models is to decompose this reaction into 2 reactions with opposite directions:

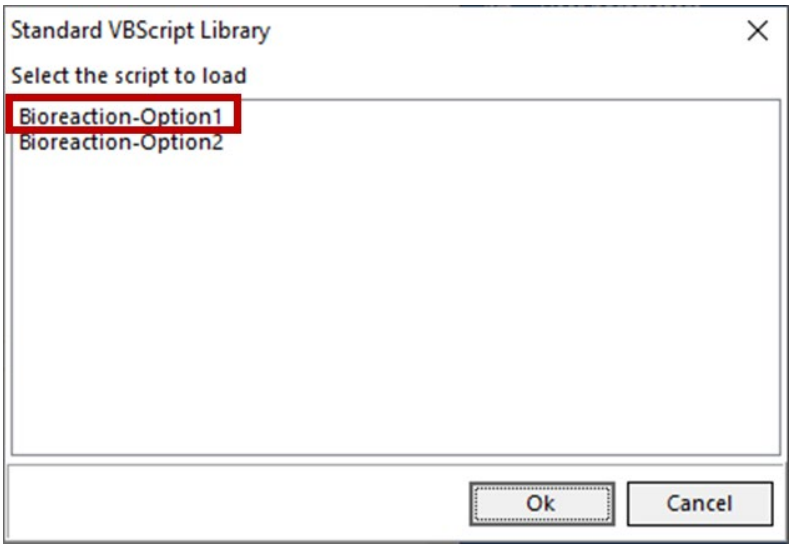
$$R_{2a}: \quad 4CO_2 + 12O_2 + 33H_2 \longrightarrow 1PHB + 30H_2O$$

$$with \ r_{P,2a} = k2 \frac{K1}{K1 + S} X$$

$$R_{2b}: \quad 1PHB + 30H_2O \longrightarrow 4CO_2 + 12O_2 + 33H_2$$

$$with \ r_{P,2b} = -k1 \frac{K1}{K1 + S} P$$

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



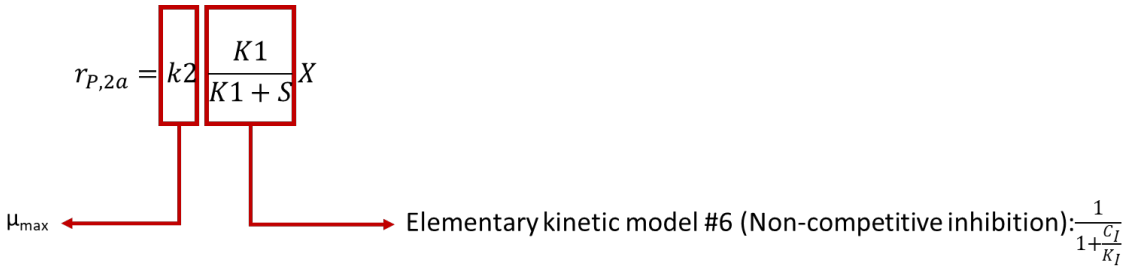
$$r_G = \left(\alpha . \mu_{max} \prod_{i=1}^{NLS} r(S_i) + \beta \right) . X$$

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

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

- For the reaction R_{2a}:

One elementary kinetic term is required, corresponding to the model index 6 (Non-competitive inhibition) of the table 2, which is provided in the technical help:



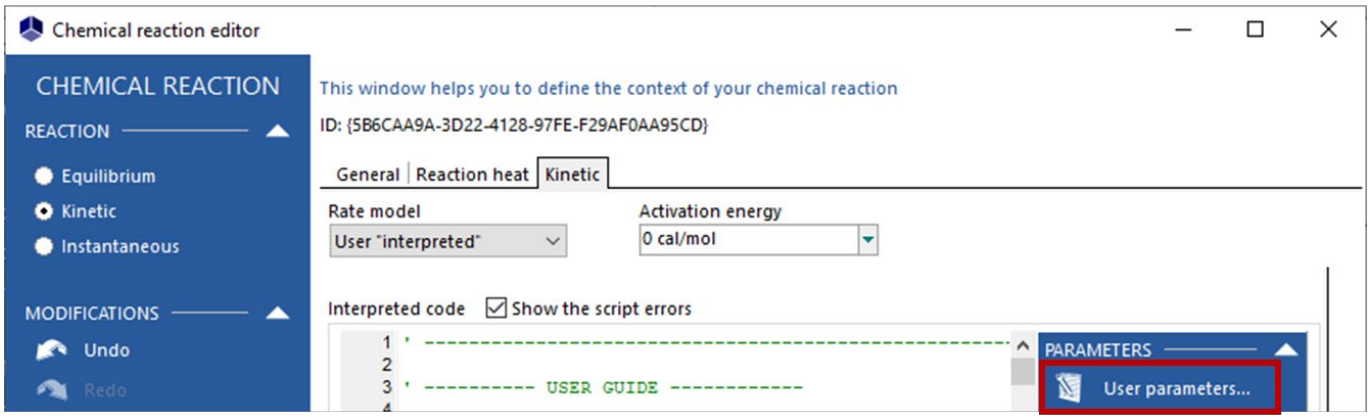
Here are the parameters to provide:

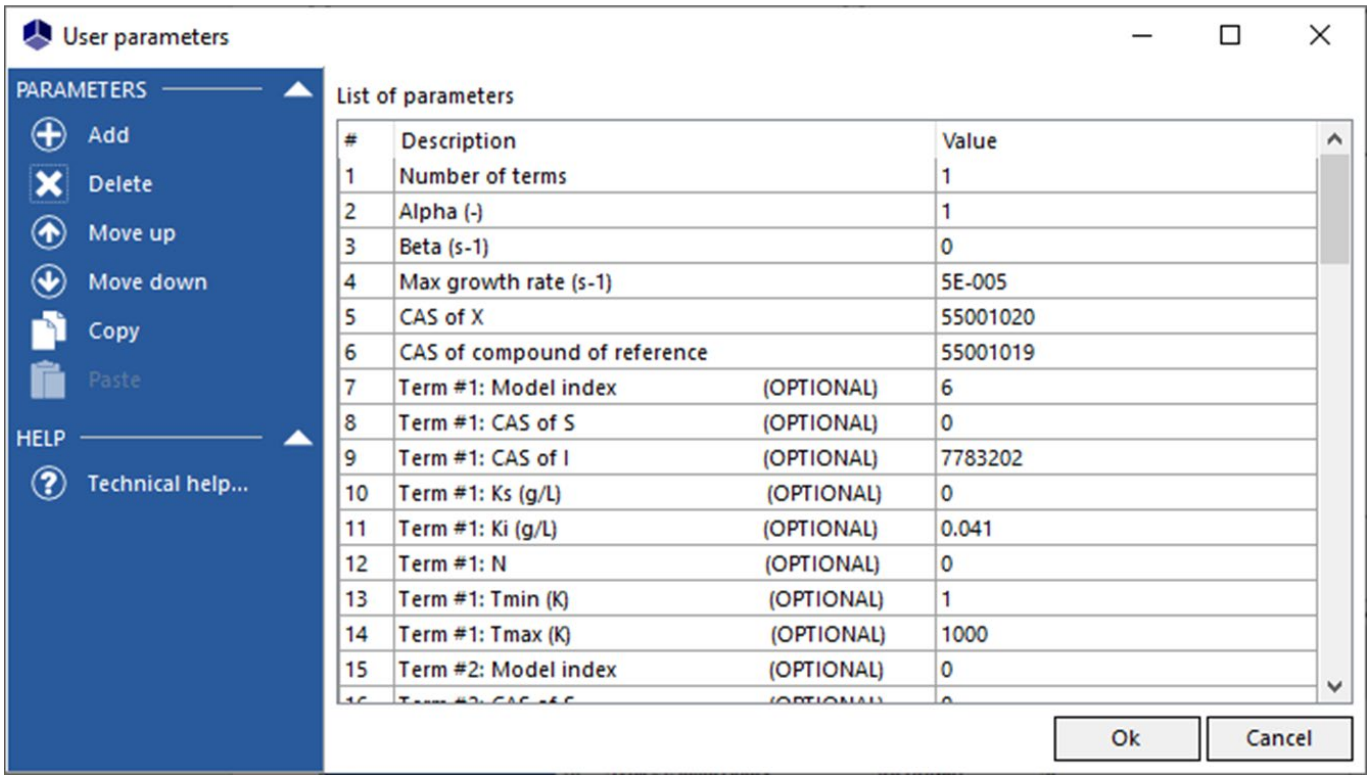
Model parameters	**R_{2a}**
Number of elementary terms	1
α ("*Alpha*")	$\frac{1}{\nu_{P,2a}} = 1$
β ("*Beta*")	0
μ_{max} ("*Max growth rate*")	$\mu_{max} = k_2 = 0.18/3600 = 5.10^{-5}\ s^{-1}$
Selection of the "Biomass" compound ("*CAS of X*")	CAS number(*): 55001-02-0
Selection of the compound of reference ("*CAS of Reference*")	CAS number(*): 55001-01-9
Parameters of Term #1	$Model\ index = 6: \frac{1}{1+\frac{C_I}{K_I}}$ Selection of the inhibitor ("*CAS of I*"): 7783-20-2 $K_I = K_1 = 0.041$ 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

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

The model parameters are specified as follows:

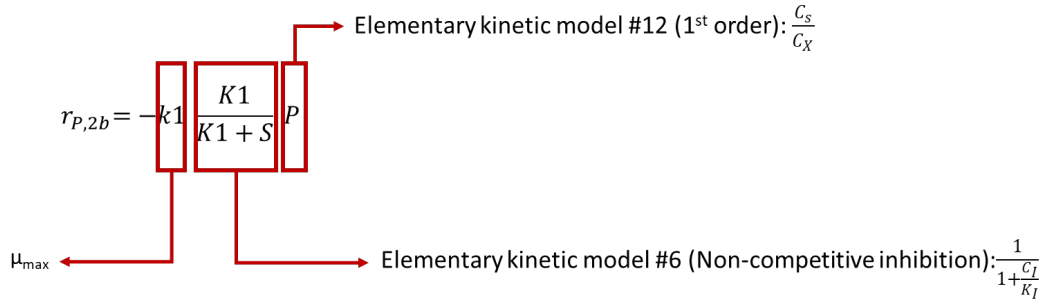




Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

- For the reaction R_{2b}:

Two elementary kinetic terms are required, corresponding to the model index 6 (Non-competitive inhibition) and 12 (1st order) of the table 2, which is provided in the technical help:



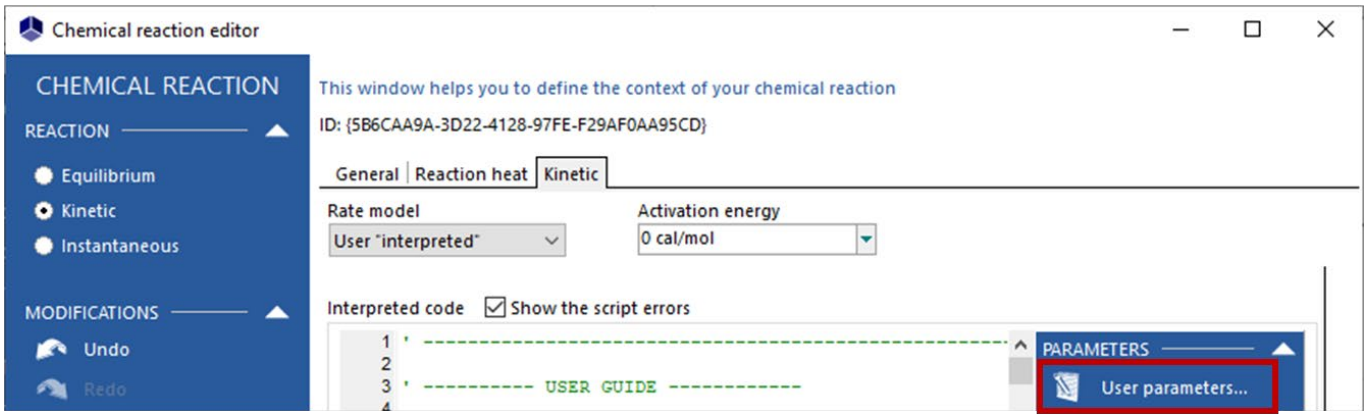
Here are the model parameters:

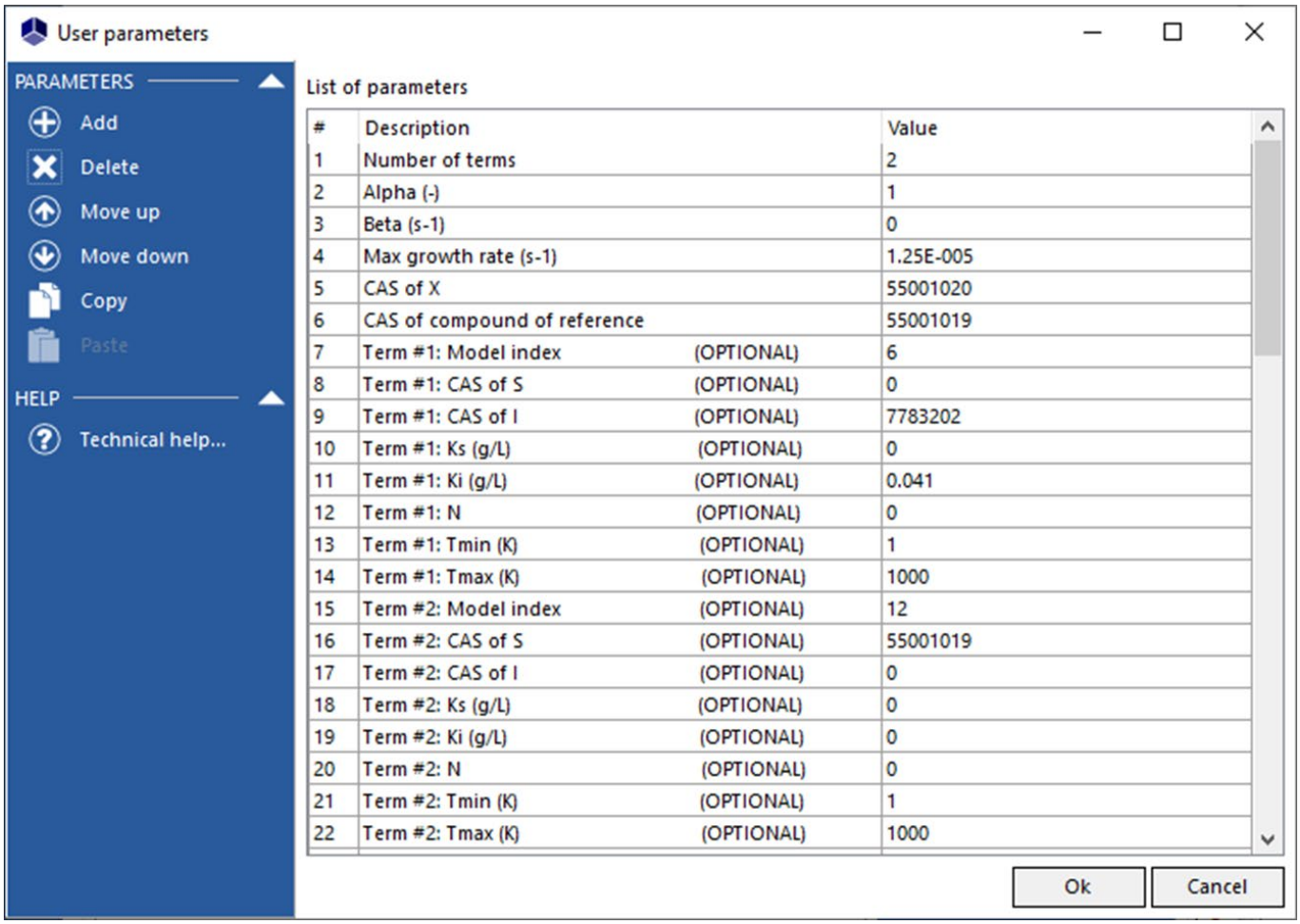
Model parameters	**R_{2b}**
Number of elementary terms	2
α ("*Alpha*")	$-\frac{1}{\nu_{P,2b}} = 1$
β ("*Beta*")	0
μ_{max} ("*Max growth rate*")	$\mu_{max} = k_1 = 0.045/3600 = 1.25.10^{-5}$ s^{-1}
Selection of the "Biomass" compound ("*CAS of X*")	CAS number(*): 55001-02-0
Selection of the compound of reference ("*CAS of Reference*")	CAS number(*): 55001-01-9
Parameters of Term #1	$Model\ index = 6: \frac{1}{1+\frac{C_I}{K_I}}$ Selection of the inhibitor ("*CAS of I*"): 7783-20-2 $K_I = K_1 = 0.041$ g/L
Parameters of Term #2	$Model\ index = 12: \frac{C_S}{C_X}$ With: Selection of the substrate ("*CAS of* S"): 55001-01-9

(*): 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

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

The model parameters are specified as follows:





#	Description		Value
1	Number of terms		2
2	Alpha (-)		1
3	Beta (s-1)		0
4	Max growth rate (s-1)		1.25E-005
5	CAS of X		55001020
6	CAS of compound of reference		55001019
7	Term #1: Model index	(OPTIONAL)	6
8	Term #1: CAS of S	(OPTIONAL)	0
9	Term #1: CAS of I	(OPTIONAL)	7783202
10	Term #1: Ks (g/L)	(OPTIONAL)	0
11	Term #1: Ki (g/L)	(OPTIONAL)	0.041
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)	12
16	Term #2: CAS of S	(OPTIONAL)	55001019
17	Term #2: CAS of I	(OPTIONAL)	0
18	Term #2: Ks (g/L)	(OPTIONAL)	0
19	Term #2: Ki (g/L)	(OPTIONAL)	0
20	Term #2: N	(OPTIONAL)	0
21	Term #2: Tmin (K)	(OPTIONAL)	1
22	Term #2: Tmax (K)	(OPTIONAL)	1000

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

7. SIMULATION

7.1. Process description

The reactor used for the PHB production is described in the table below. A condenser is used in order to avoid the loss of water that evaporates.

Reactor	
Type	Closed vapor-liquid
Global volume (vapor + liquid)	10 l
Head space (initial)	Air
Condenser	
Type	Ideal sub-cooled
Temperature	0°C
Reflux ratio	1 (i.e. total reflux)

The initial conditions are presented in the following table:

Initial conditions	
Temperature	30°C
Pressure	1 atm
Initial load (kg)	
Water	7.97968
Ammonium sulfate	0.01840
PHB	0.00016
Residual biomass	0.00176
Other components	0
Total load	8

A continuous gas stream at ambient condition feeds the reactor in order to carry in the hydrogen, the carbon dioxide and the oxygen (6:2:1 molar respectively) required for the reactions. The characteristics of this feed are:

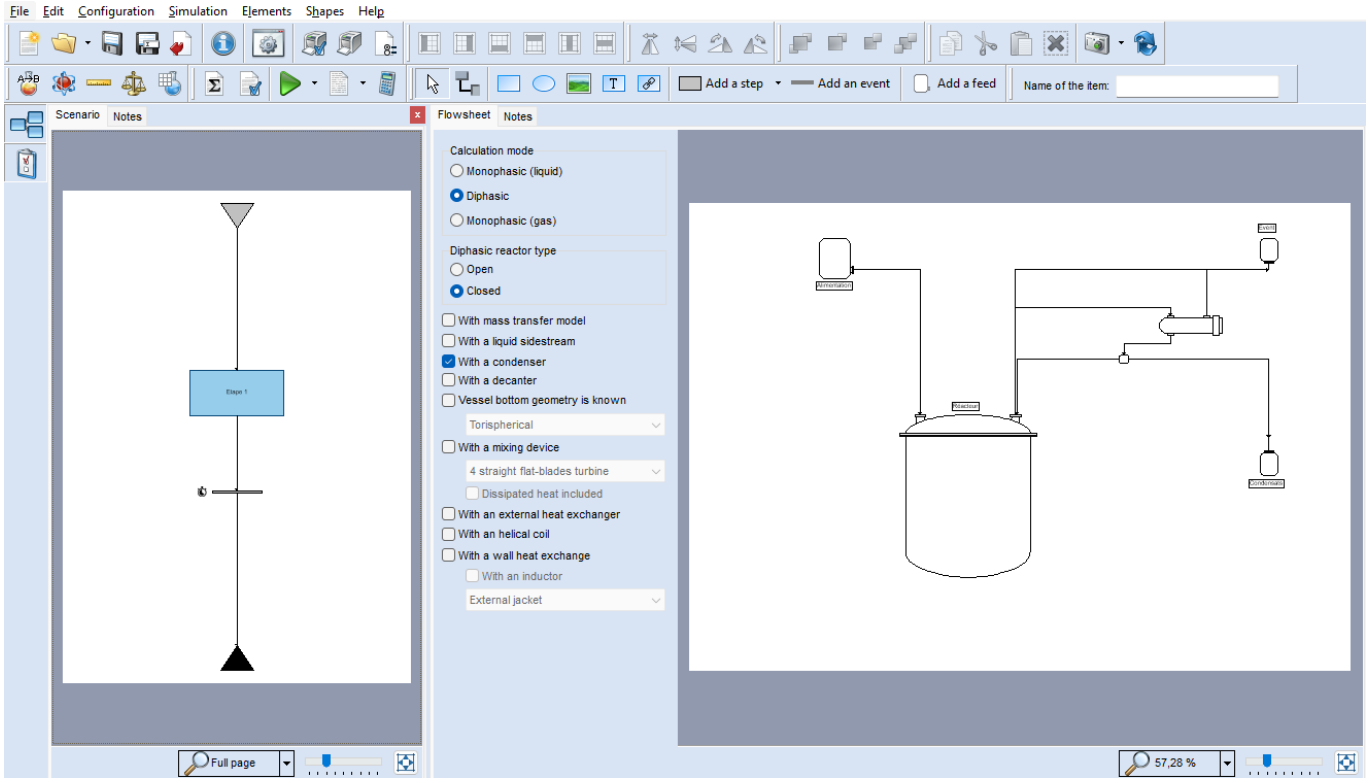
Temperature	30°C
Pressure	1 atm
Total flow rate	15 l/min
Mole fractions	
Carbon dioxide	0.11
Oxygen	0.22
Hydrogen	0.67
Other components	0

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

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

Type	Specified reactor temperature
Temperature	30°C
Pressure	1 atm
Step duration	40 h

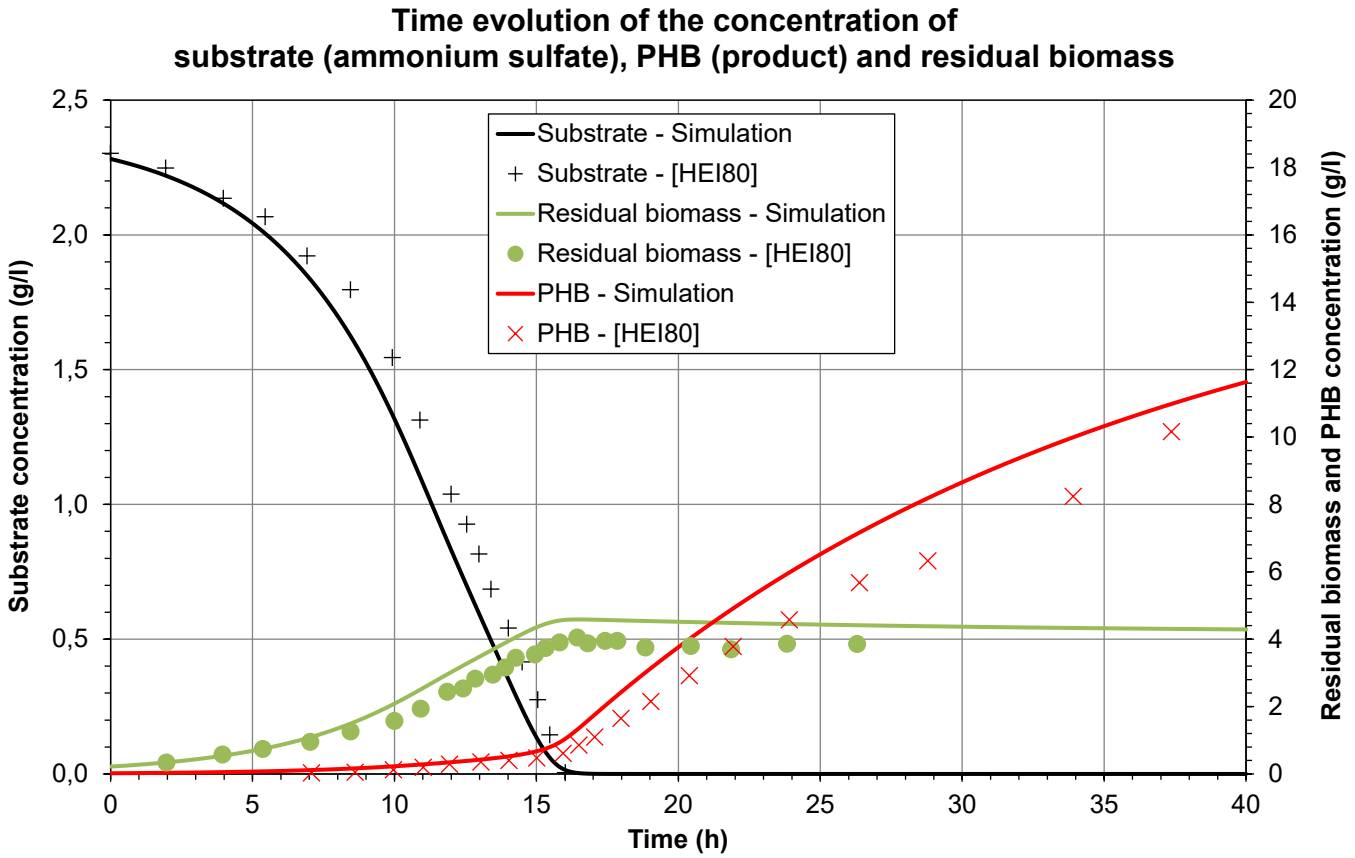
The scenario is presented on the left part of the following screen shot and the flowsheet on the right part.



Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

7.2. Results

Next graph presents some simulation results obtained with BatchReactor software. The compounds concentrations vs. time curves are in good agreement with data provided by [HEI80]. It has to be noted that using BatchReactor software, all batch 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.



Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

8. REFERENCES

[FOG91] FOGG P.G.T., GERRARD W., “Solubility of gases in liquids”, Wiley (1991)

[HEI80] HEINZLE E., LAFFERTY R.M., “A Kinetic Model for Growth and Synthesis of Poly-β-Hydroxybutyric Acid (PHB) in *Alcaligenes eutrophus* H16*”,* European J. Appl. Microbiol. Biotechnol. 11, 8-16 (1980)

[ISH91] ISHIZAKI A., TANAKA K., “Production of Poly-β-Hydroxybutyric Accid from Carbon Dioxide by *Alcaligenes eutrophus* ATCC 17697^{T}”, J. Ferment. Bioeng., 71, 254-257 (1991)

[ROW2015] ROWLEY R.L., WILDING W.V., OSCARSON J.L., GILES N.F., “DIPPR® Data Compilation of Pure Chemical Properties”, Design Institute for Physical Properties, AIChE, New York, NY (2015)

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés

9. NOMENCLATURE

k_1	Constant	h^{-1}
K_1	Inhibition constant	g/l
k_2	Constant	h^{-1}
$K_{S,1}$	Inhibition constant	g/l
$K_{S,2}$	Inhibition constant	g/l
n_{Hill}	Hill coefficient	(-)
P	Concentration of PHB	g/l
P^0	Vapor pressure of the pure component	Pa
X	Concentration of residual biomass	g/l
r_P	Rate of synthesis of PHB	g/(l.h)
$r_{P,1}$	Rate of the reaction (R1)	g/(l.h)
$r_{P,2}$	Rate of the reaction (R2)	g/(l.h)
r_X	Rate of synthesis of residual biomass	g/(l.h)
S	Concentration of substrate (ammonium sulfate)	g/l
t	Time	h
$Y_{P/X}$	Yield coefficient	$g_{PHB}/g_{substrate}$
μ	Specific rate of synthesis of residual biomass	h^{-1}
μ_1	Term 1 of the specific rate of synthesis of residual biomass	h^{-1}
μ_2	Term 2 of the specific rate of synthesis of residual biomass	h^{-1}
$\mu_{m,1}$	Maximum specific growth rate	h^{-1}
$\mu_{m,2}$	Maximum specific growth rate	h^{-1}

Copyright © 2025 Fives ProSim, Labège, France – Tous droits réservés