fives
Industry can do it

BATCHREACTOR APPLICATION EXAMPLE

WHITE BIOTECHNOLOGY

SIMULATION OF FRESH SPIRULINA PRODUCTION WITHIN A PHOTOBIOREACTOR WITH USER DEFINED KINETICS

EXAMPLE PURPOSE
The main interest of this example is to illustrate how to model photobioreactors 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 fresh spirulina that can be produced if the favorable operating conditions are brought together (water, carbon dioxide, nutrients, temperature, pH and also light). The mathematical modeling of the bioreaction mechanisms is based on specific equations which are not available in standard chemical reaction libraries.

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

CORRESPONDING BATCHREACTOR FILE	*BATCHREA_EX_EN - Photobioreactor.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.

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

1. INTRODUCTION

This example deals with the production of fresh spirulina. This cyanobacteria has a size of about 200 micrometers with a spiral shape and a green color. Composed of various valuable vitamins, minerals and proteins, it has shown a major interest in the food industry. The metabolism corresponding to the production of spirulina involves specificities related to photosynthesis and can be modeled by taking into account the substrates uptake and the availability of light energy.

The goal of this example is to illustrate how to use BatchReactor to model this kind of process. The kinetic parameters presented in this document are used for this purpose and do not necessarily come from literature.

2. REACTION MECHANISM

With the appropriate cultivation conditions, the reaction mechanism for the production of spirulina can be represented as follows:

$$\alpha\ CO_2 + \beta\ H_2O + \delta\ NaNO_3 + \varepsilon\ CaSO_4 + \theta\ H_3PO_4 \rightarrow\ Spirulina + \mu\ O_2$$

Spirulina is modeled as $C_{1000}H_{1609}N_{173}O_{747}P_{71}S_6Ca_6Na_{173}$, leading to the following equation of bioreaction:

$$1000\ CO_2 + 698\ H_2O + 173\ NaNO_3 + 6\ CaSO_4 + 71\ H_3PO_4 \rightarrow\ C_{1000}H_{1609}N_{173}O_{747}P_{71}S_6Ca_6Na_{173} + 1389\ O_2$$

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
Sodium nitrate(*)	7631-99-4
Calcium sulfate(*)	7778-18-9
Water(*)	7732-18-5
Phosphoric acid(*)	7664-38-2
Spirulina	1111-11-1

Compounds with an asterisk are taken from the standard database of Simulis Thermodynamics, calculation server for thermophysical properties and phase equilibria calculations used in BatchReactor. The thermophysical parameters stored in this database are the DIPPR recommended values [ROW19].

The spirulina was created by selecting the Simulis Thermodynamics feature: “Add a new compound”. The physical properties are the following:

- ✓ CAS number[1] : 1111-11-1 (arbitrary number)
- ✓ Chemical formula : $C_{1000}H_{1609}N_{173}O_{747}P_{71}S_6Ca_6Na_{173}$
- ✓ Molecular weight : 34616.588 g/mol
- ✓ 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

[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 ACS. CAS Registry Numbers® have not been verified by ACS and may be inaccurate.*

- ✓ 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 sodium nitrate, calcium sulfate and phosphoric acid are also considered as non-volatile, and their liquid density is set to be the same as water. Carbon dioxide, oxygen and nitrogen vapor pressures were changed in order to well represent their solubility in water, Henry's law parameters were obtained from [FOG91]

4. THERMODYNAMIC MODEL

The bioreaction taking place at ambient temperature and atmospheric pressure, it is assumed that the gas phase follows the ideal gas law. The liquid phase contains insoluble solids. 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 would modify the real composition of the liquid phase as well as the vapor-liquid equilibrium constants of volatile components (water, carbon dioxide, oxygen). Therefore, 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 and nitrogen) into water.

5. REACTION MATHEMATICAL MODEL

The goal is to model the growth rate of spirulina which can be described using the general equation:

$$r_X = \frac{dC_X}{dt} = (\mu - k_d).C_X \qquad (1)$$

With:

r_X	Specific rate of formation of spirulina
C_X	Concentration of spirulina (corresponding to the biomass)
μ	Specific growth rate
k_d	Depletion rate

The specific growth rate of spirulina results from the sum of two different contributions:

$$\mu = \mu_{Substrate} \times \mu_{Light} \qquad (2)$$

✓ Substrate consumption:

The metabolism requires the uptake of different substrates, which can be modeled using the Monod law:

$$\mu_{Substrate} = \mu_{max} \prod \frac{C_S}{K_S + C_S} \quad (3)$$

With:

C_S	Concentration of substrate
K_S	Saturation constant
μ_{max}	Maximum specific growth rate

Four substrates are taken into account (sources of C, N, S and P), leading to the following equation:

$$\mu_{Substrate} = \mu_{max} \times \frac{C_{CO_2}}{K_{S,CO_2} + C_{CO_2}} \times \frac{C_{NaNO_3}}{K_{S,NaNO_3} + C_{NaNO_3}} \times \frac{C_{CaSO_4}}{K_{S,CaSO_4} + C_{CaSO_4}} \times \frac{C_{H_3PO_4}}{K_{S,H_3PO_4} + C_{H_3PO_4}} \quad (4)$$

✓ Light energy:

The availability of light energy is an essential operating parameter in the microalgae metabolism. This energy can either come from a natural or an artificial source. In BatchReactor, it is assumed that operating parameters (temperature, pressure, concentrations…) are homogeneously distributed inside the reactor. Therefore, the mean value of light intensity is considered. The impact of light intensity on the growth rate is described with the following equation:

$$\mu_{Light} = \frac{I}{K_{S,I} + I + \frac{I^2}{K_{I,I}}} \quad (5)$$

With:

I	Mean light intensity
$K_{S,I}$	Saturation constant
$K_{I,I}$	Inhibition constant

Since the light intensity inside the photobioreactor will decrease as the concentration of spirulina will increase, tabulated data are provided to represent the evolution of light intensity in function of spirulina concentration:

Concentration of spirulina (g/l)	Mean light intensity (W/m²)
0 – 0.1	220
0.1 – 0.15	132
0.15 – 0.2	95
0.2 – 0.3	62
0.3 – 0.5	37
0.5 – 0.7	27
0.7 – 0.9	21
0.9 – 1.5	13

Finally, the overall rate of bioreaction is brought down to the following equation:

$$r_X = (\mu - k_d).C_X$$

$$r_X = \left(\mu_{max} \times \frac{C_{CO_2}}{K_{S,CO_2} + C_{CO_2}} \times \frac{C_{NaNO_3}}{K_{S,NaNO_3} + C_{NaNO_3}} \times \frac{C_{CaSO_4}}{K_{S,CaSO_4} + C_{CaSO_4}} \times \frac{C_{H_3PO_4}}{K_{S,H_3PO_4} + C_{H_3PO_4}} \times \frac{I}{K_{S,I} + I + \frac{I^2}{K_{I,I}}} - k_d \right).C_X \quad (6)$$

The kinetic parameters that are used in this example are presented in the following table:

μ_{max} (s^{-1})	k_d (s^{-1})	K_{S,CO_2} (g/l)	$K_{S,NaNO_3}$(g/l)	$K_{S,CaSO_4}$(g/l)	K_{S,H_3PO_4}(g/l)	$K_{S,I}$ (W/m²)	$K_{I,I}$(W/m²)
2.5 10^{-5}	5.6 10^{-7}	0.011643	0.0053	0.00025	0.00027	20	3500

6. Reaction model implementation using Simulis Reactions

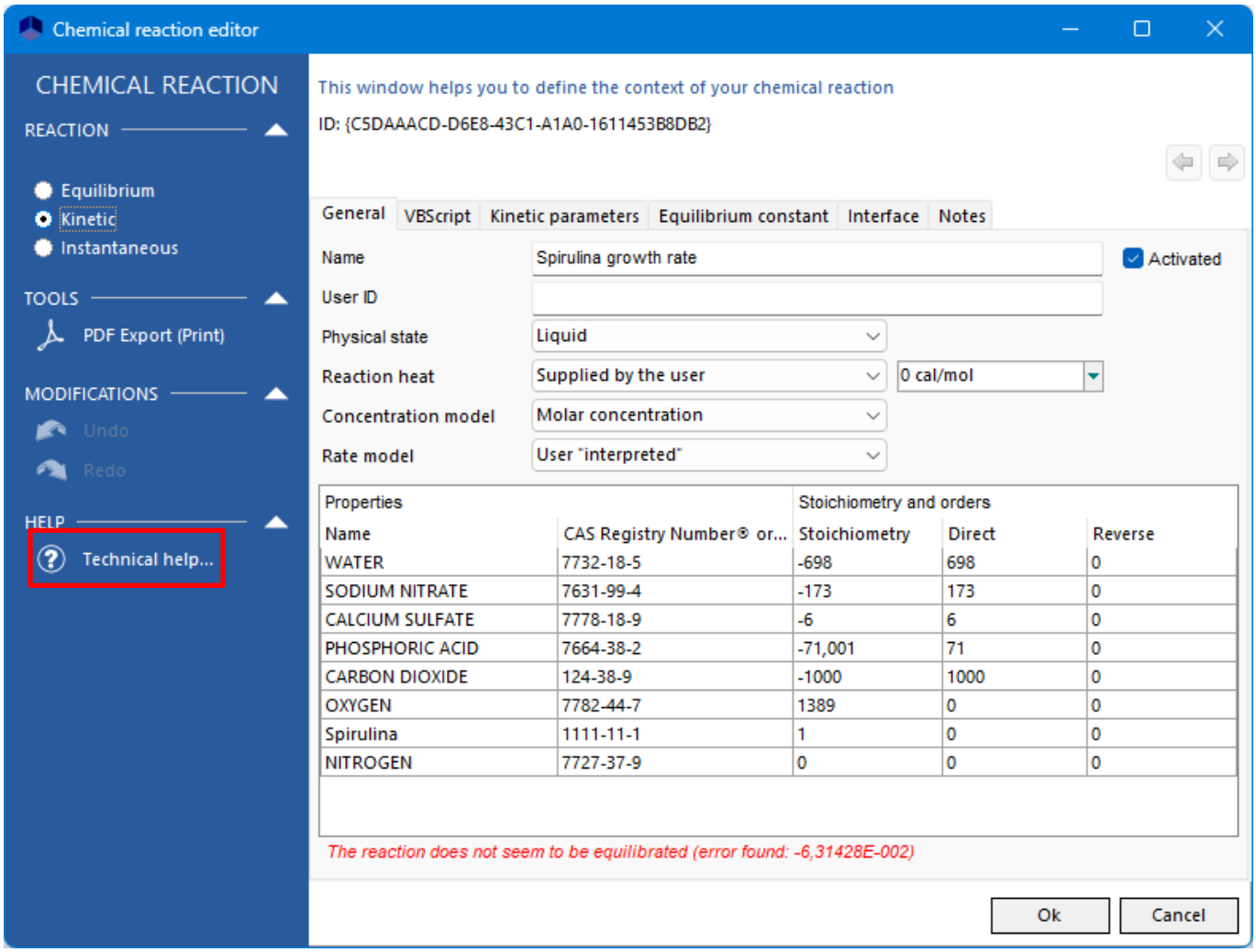
User “interpreted” kinetic rate model is used to implement the mathematical model related to this bioreaction. This feature enables the user to write his own custom code for the kinetic model, using VBScript (Microsoft Visual Basic Scripting Edition), which is an interpreted language: it does not require 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*”.

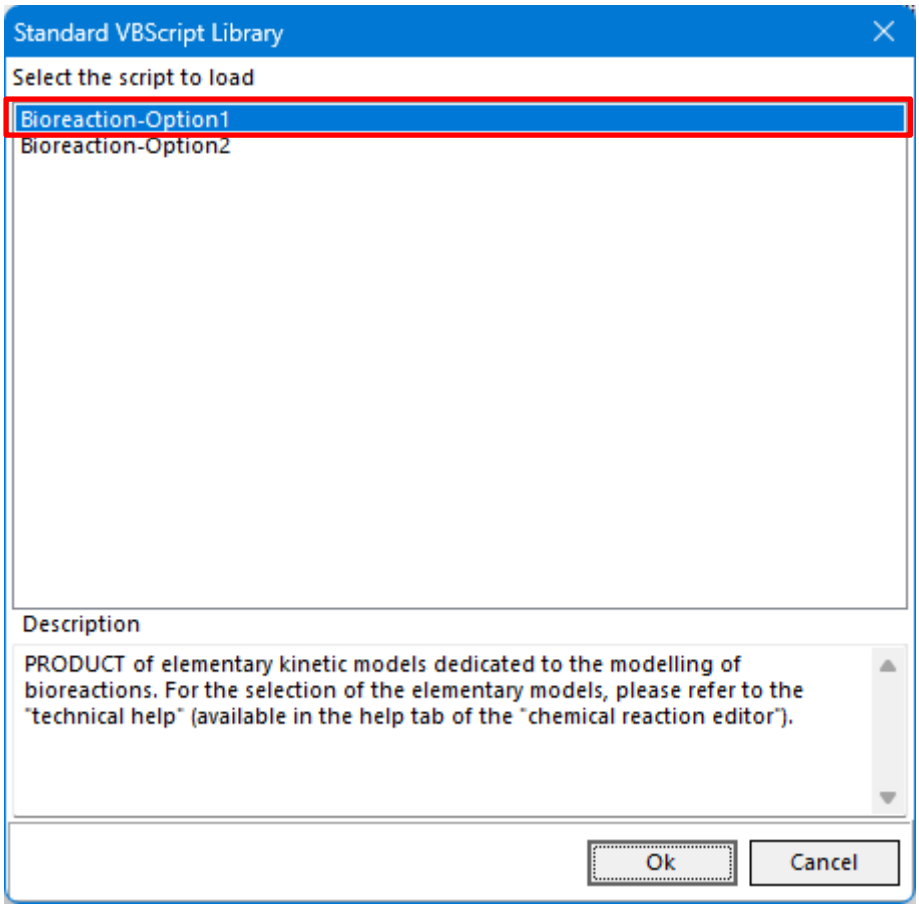
All reactions take place in the liquid phase.

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

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 can be modelled using the VBScript called “Bioreaction-option1”, available in the VBScript library:



This option corresponds to the following expression of the reaction rate:

$$r_G = \left(\alpha.\mu_{max}\prod_{i=1}^{NLS} r(C_{Si}) + \beta\right).C_X \quad (7)$$

With:

r_G	Global rate of bioreaction
μ_{max}	Maximum growth rate
α	Growth-related coefficient
β	Non-growth-related coefficient
$\prod_{i=1}^{NLS} r(C_{Si})$	Product of elementary kinetic terms that can be selected from a library (as referred in the technical help)

This expression is convenient because it is general enough to model a wide range of bioreactions. It can be used to represent the equation (6) presented above (with $\alpha = 1$):

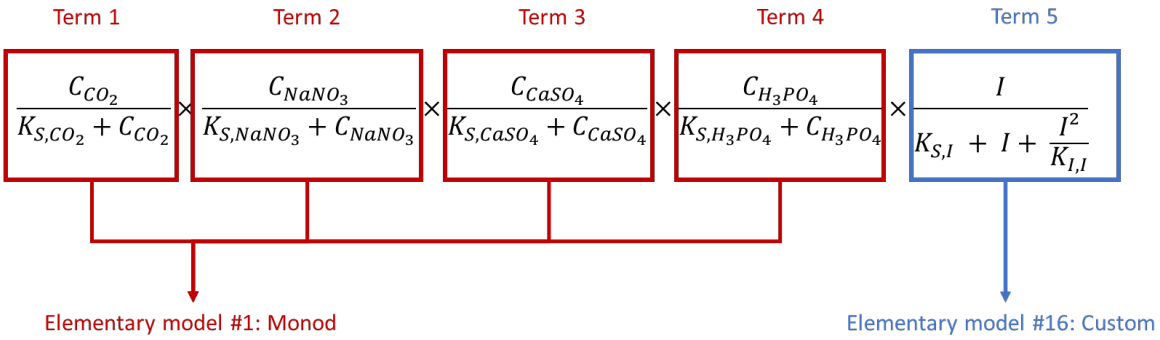
$$r_G = \left(\alpha.\mu_{max}\prod_{i=1}^{NLS} r(C_{Si}) + \beta\right).C_X$$

$$\left(\mu_{max} \times \frac{C_{CO_2}}{K_{S,CO_2} + C_{CO_2}} \times \frac{C_{NaNO_3}}{K_{S,NaNO_3} + C_{NaNO_3}} \times \frac{C_{CaSO_4}}{K_{S,CaSO_4} + C_{CaSO_4}} \times \frac{C_{H_3PO_4}}{K_{S,H_3PO_4} + C_{H_3PO_4}} \times \frac{I}{K_{S,I} + I + \frac{I^2}{K_{I,I}}} - k_d\right).C_X$$

In this example, five elementary kinetic terms are required:

- The ones tied to the substrates uptake are represented by the Monod law, which is available in the library of elementary kinetic terms (elementary model #1).

- The one tied to the contribution of light energy is not available as a standard model but it can be directly coded by the user with the selection of the “custom elementary model” (elementary model #16).



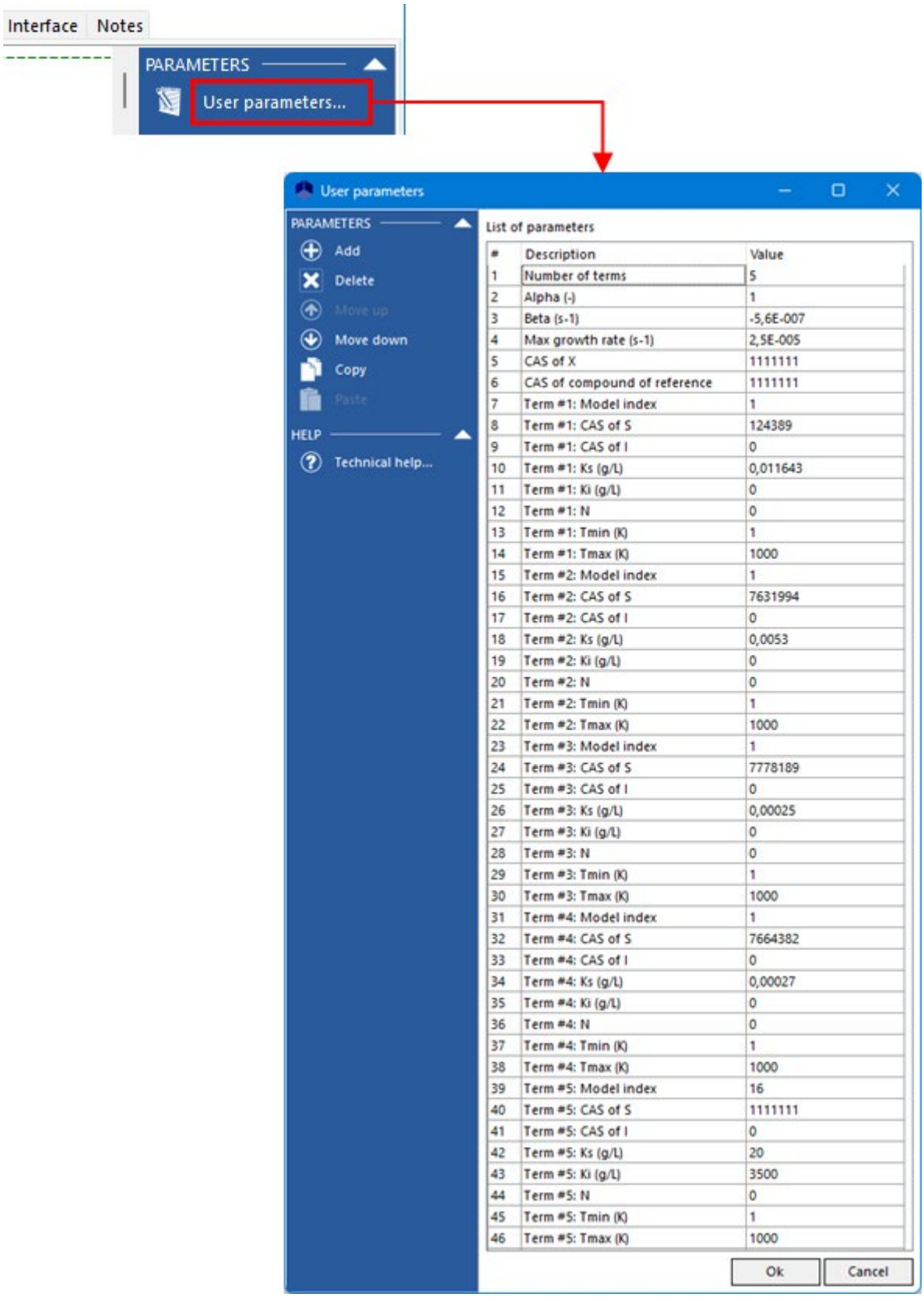
Here are the general parameters to provide:

Model parameter	Value
Number of elementary terms	5
α ("*Alpha*")	1
β ("*Beta*")	$-k_d$ = -5.6 10^{-7}
μ_{max} ("*Max growth rate*")	μ_{max} = 2.5 10^{-5} s^{-1}
Selection of the "Biomass" compound ("*CAS of X*")	CAS number: 1111-11-1
Selection of the compound of reference ("*CAS of Reference*")	CAS number: 1111-11-1
Parameters of Term #1	$Model\ index = 1: \frac{C_S}{K_S + C_S}$ With: Selection of the substrate ("*CAS of* S"): 124-38-9 K_S = 0.011643 g/l
Parameters of Term #2	$Model\ index = 1: \frac{C_S}{K_S + C_S}$ With: Selection of the substrate ("*CAS of* S"): 7631-99-4 K_S = 0.0053 g/l
Parameters of Term #3	$Model\ index = 1: \frac{C_S}{K_S + C_S}$ With: Selection of the substrate ("*CAS of* S"): 7778-18-9 K_S = 0.00025 g/l
Parameters of Term #4	$Model\ index = 1: \frac{C_S}{K_S + C_S}$ With: Selection of the substrate ("*CAS of* S"): 7664-38-2 K_S = 0.00027 g/l
Parameters of Term #5	$Model\ index = 16: Custom$ With: Selection of the substrate ("*CAS of* S"): 1111-11-1 $K_S = K_{S,I}$ = 20 W/m² $K_I = K_{I,I}$ = 3500 W/m²

For the Term#5 corresponding to the contribution of light intensity, since it is a custom elementary term, the following piece of code is provided:

```
'The user may code below the custom elementary rate:
'-------- Beginning of custom code ------------
'Substrate Concentration:
S_Index = GetCompoundIndex(S(i-1))
With ThermoCalculator.Compounds.Items(S_Index)
    Mw_S = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
    CS = z(S_Index)*Mw_S/Vml 'in g/L
End with
If (CS <= 0.1) Then 'g/L
    Intensity = 220
ElseIf (CS > 0.1) And (CS <= 0.15) Then 'g/L
    Intensity = 132
ElseIf (CS > 0.15) And (CS <= 0.2) Then 'g/L
    Intensity=95
ElseIf (CS > 0.2) And (CS <= 0.3) Then 'g/L
    Intensity=62
ElseIf (CS > 0.3) And (CS <= 0.5) Then 'g/L
    Intensity=37
ElseIf (CS > 0.5) And (CS <= 0.7) Then 'g/L
    Intensity=27
ElseIf (CS > 0.7) And (CS <= 0.9) Then 'g/L
    Intensity=21
ElseIf (CS > 0.9) And (CS <= 1.5) Then 'g/L
    Intensity=13
End If
'Kinetic law
RE = Intensity/(Ks(i-1)+Intensity+Intensity^2/Ki(i-1))
'--------    End of custom code    ------------
```

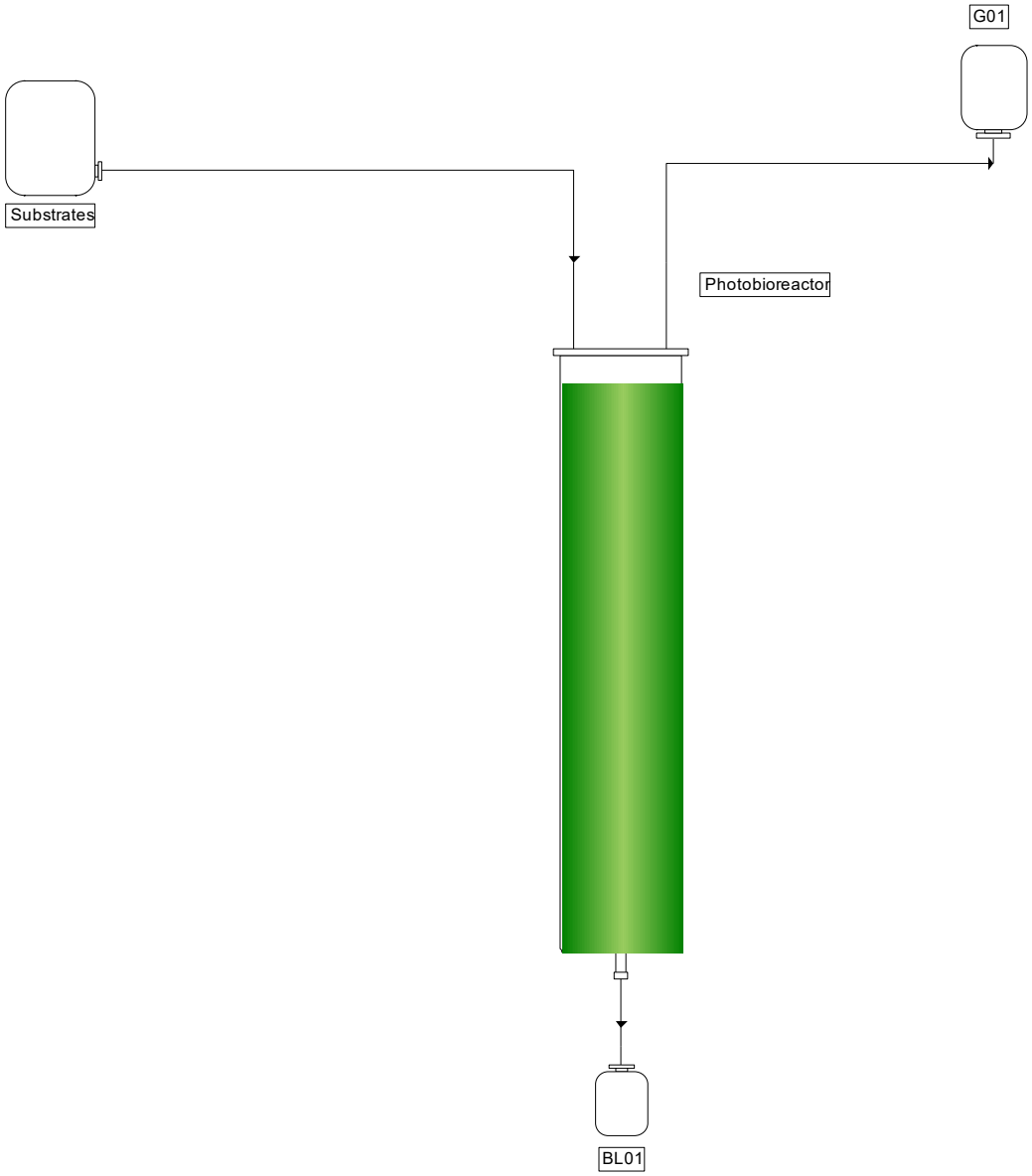

The model parameters are specified as follows:



7. SIMULATION

7.1. Configuration of the flowsheet

The flowsheet is presented on the right part of the main configuration screen



The general topology for the modeling of the photobioreactor is presented in the following table:

Reactor	
Type	Open vapor-liquid
Alarms on the volume	Minimum: 1 ml Maximum: 300 l
Alarms on the temperature	Minimum: 0°C Maximum: 200°C
Side streams	
Number of vapor side stream	1
Number of liquid side stream	1

The initial conditions are presented in the following table:

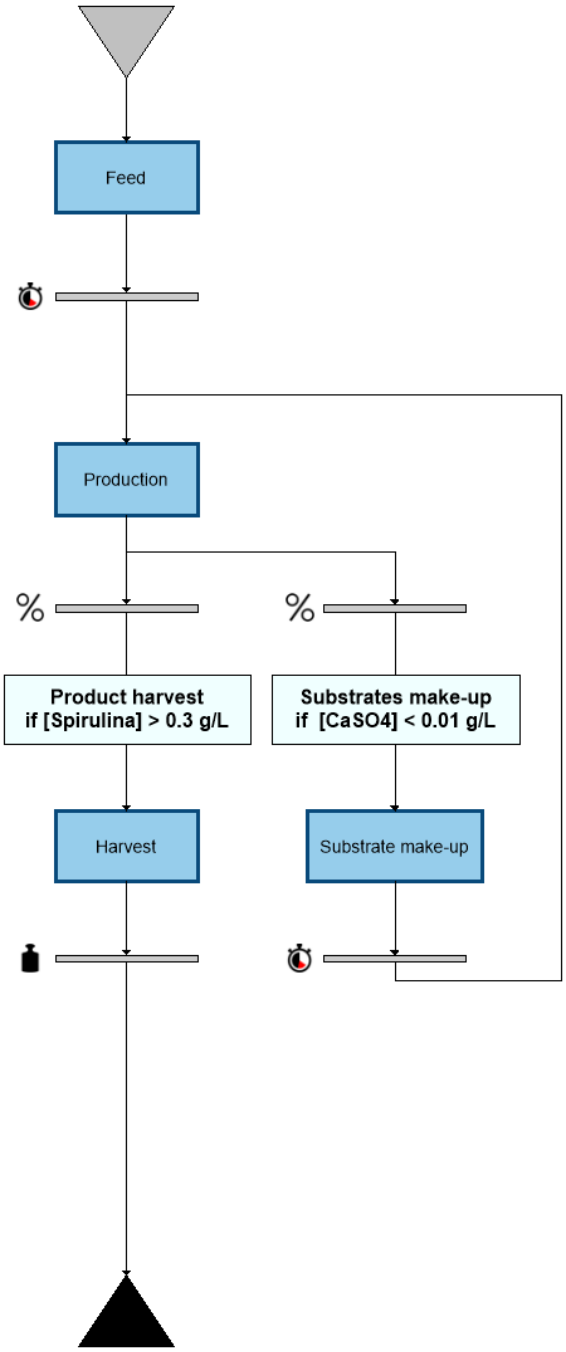
Initial conditions	
Temperature	25°C
Pressure	1 atm
Initial loads	
Water	240 kg
Sodium Nitrate	0
Calcium Sulfate	0
Phosphoric Acid	0
Carbon dioxide	0
Oxygen	0
Spirulina	30 g
Nitrogen	0

A feed stream including the substrates is fed to the photobioreactor when necessary. The characteristics of this stream are provided hereafter:

Temperature	37°C
Pressure	1 atm
Partial flowrates (g/s)	
Water	28.57
Sodium Nitrate	33.41
Calcium Sulfate	1.86
Phosphoric Acid	15.81
Carbon dioxide	100
Oxygen	0
Spirulina	0
Nitrogen	0

7.2. Configuration of the operating scenario

The operating scenario is presented on the left part of the main configuration screen:



The operating scenario consist of the following steps:

- **Feed step**: the feed stream (with the characteristics presented above) is active during 20 s. It enables to provide the substrates and heat up the reactor.

Type		Specified reactor temperature
Operating conditions	**Temperature**	Temperature slope: from 25°C to 37°C
	Pressure	1 atm
	Feed	Open
Stop event	**Step duration**	20 s

- Production step: this is the main step during which the bioreaction occurs. There are 2 possible events leading to the following step:

- If the target spirulina concentration of 0.3 g/l is reached, the scenario moves on to the next step corresponding to the “harvest step”.
- If the concentration of Calcium sulfate (limiting substrate) goes under 0.01 g/l, the scenario moves on to the “make-up step”.

Type		Specified reactor temperature
Operating conditions	**Temperature**	37 °C
	Pressure	1 atm
Stop event	**Spirulina concentration**	> 0.3 g/l
	Calcium sulfate concentration	< 0.01 g/l

- Make-up step: when this step is reached, a make-up of substrates is provided to ensure the maintain of the bioreaction until the target spirulina concentration is obtained.

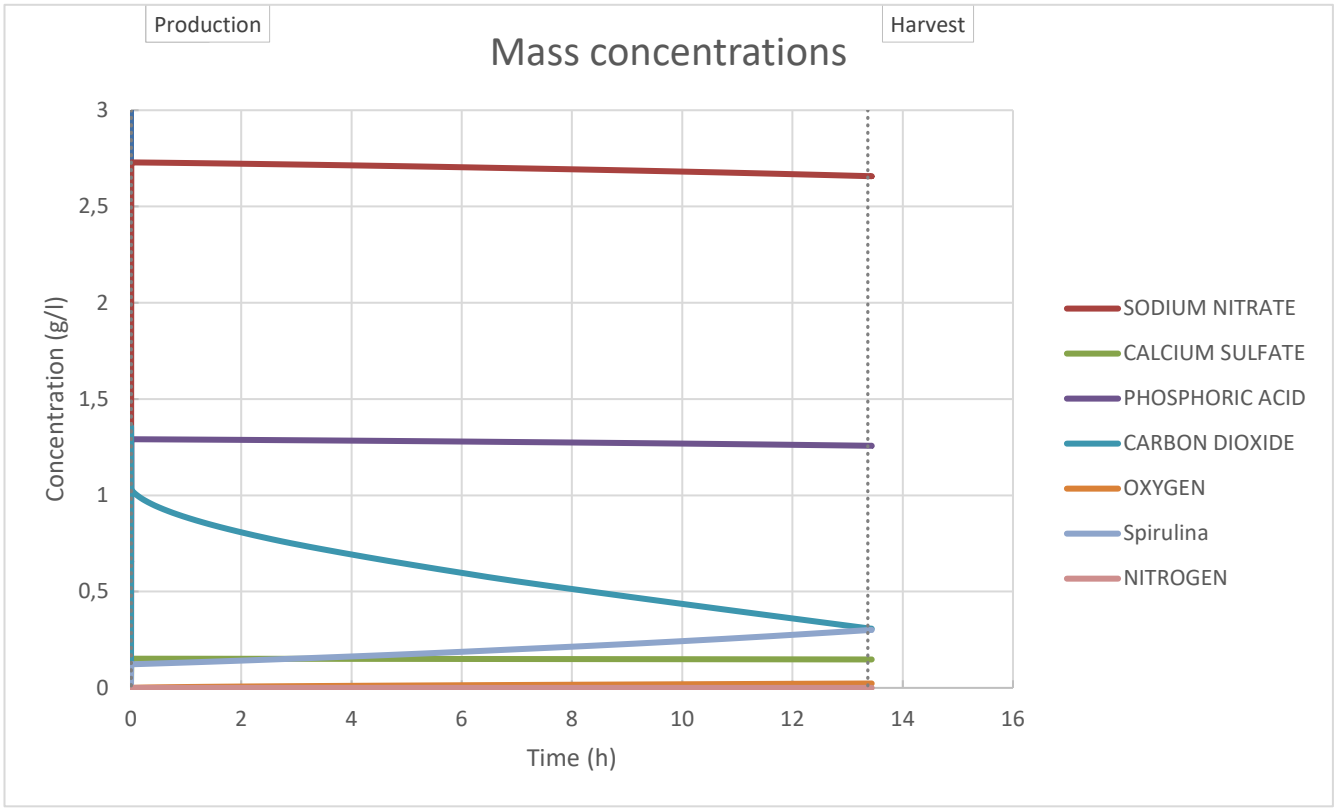
Type		Specified reactor temperature
Operating conditions	**Temperature**	37 °C
	Pressure	1 atm
	Feed	Open
Stop event	**Step duration**	20 s

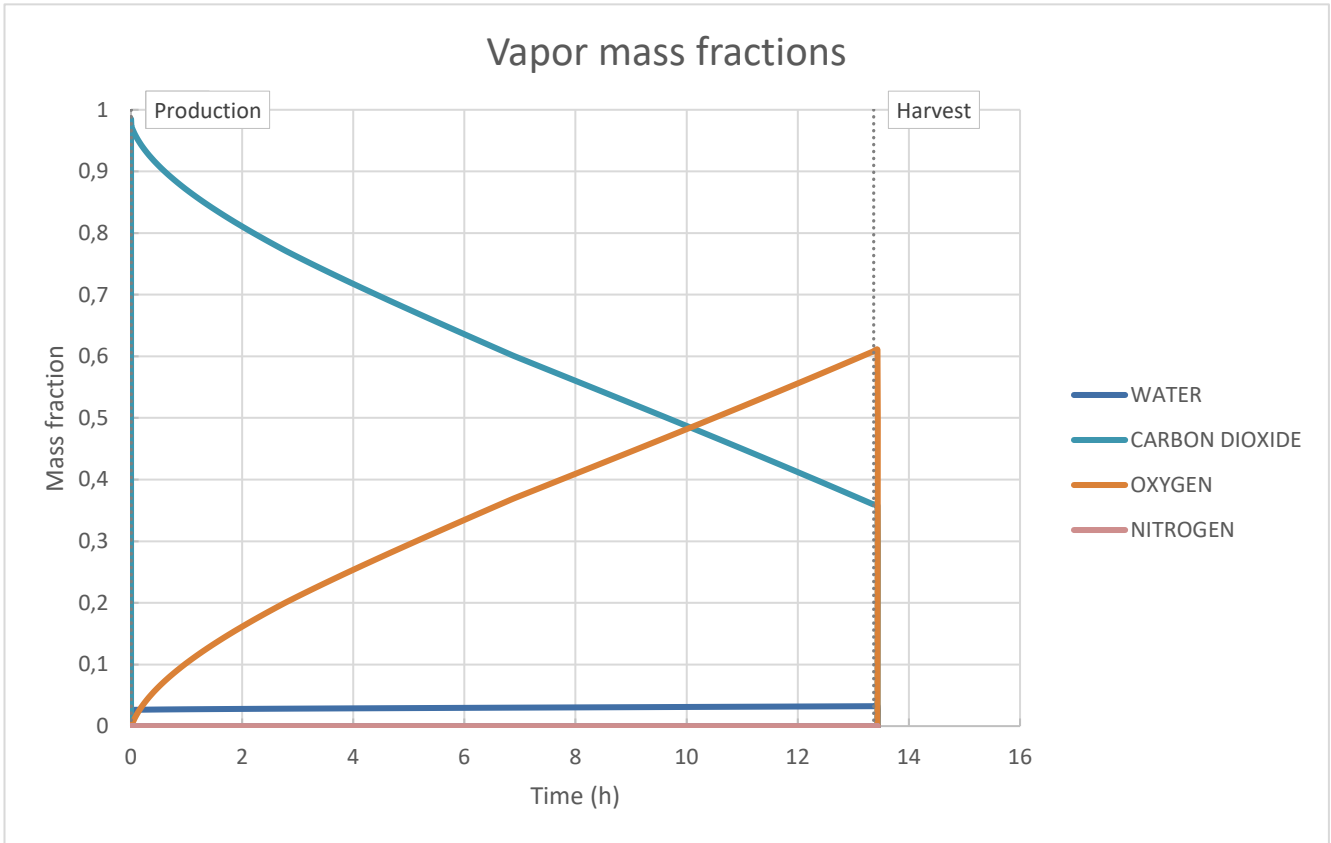
- **Harvest step:** when this step is reached, the reactor is discharged in order to collect the spirulina.

Type		Specified reactor temperature
Operating conditions	**Temperature**	37°C
	Pressure	1 atm
	Liquid sidestream flowrate	1 l/s
Stop event	**Total load**	< 2 ml

7.3. Results

The following graphs present some simulation results obtained with BatchReactor software.





It has to be noted that using BatchReactor software, all batch parameters can be monitored (liquid volume, heat duty, pressure, temperature…). Moreover, the detailed modeling of the reactor (heating/cooling system, condenser, vessel geometry…) can be taken into account.

BatchReactor software can then be used to:

- Optimize the process by tweaking the operating parameters (feed characteristics, pressure, temperature…), the equipment design and the operating scenario.

- Run safety analysis and simulate more complex scenarios.

- Scale-up from the lab scale to the industrial scale.

8. REFERENCES

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

[ROW19] 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 (2019)

