ProSim

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 show how user can describe his own kinetics models using the advanced mode available in Simulis Reactions, the chemical reactions server used in BatchReactor software. 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 such as Simulis Reactions.

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CORRESPONDING BATCHREACTOR FILE	*BATCHREA_E01_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. 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.

TABLE OF CONTENTS

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	-

3. COMPONENTS

Components which are taken into account in the simulation are:

Name	CAS number
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	
Residual biomass	

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, 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.

4. THERMODYNAMIC MODEL

Reactions occurred at ambient temperature (30°C) and atmospheric pressure, so the gas phase was assumed to follow the Perfect 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 and are the two reactions implemented in the simulation.

- ✓ Growth associated term:

$$r_{P,1} = Y_{P/R} \times r_R \qquad \text{(R1)}$$

Where

$$\frac{dR}{dt} = r_R = \mu \times R$$

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

- ✓ Storage associated term:

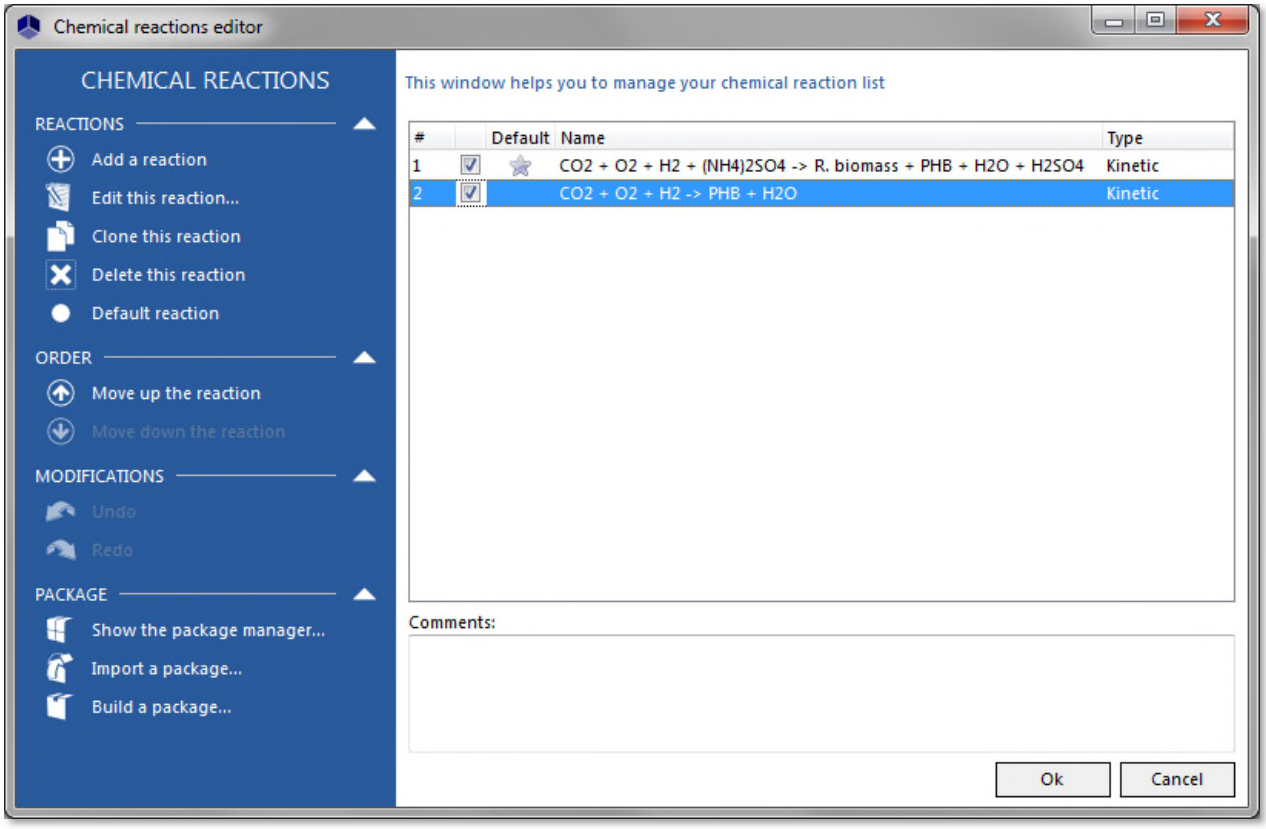
$$r_{P,2} = \frac{K_1}{K_1 + S}(-k_1 P + k_2 R) \qquad \text{(R2)}$$

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}	$Y_{P/R}$	K_1 (g/l)	k_1 (h^{-1})	k_2 (h^{-1})
0.13	0.08	0.1	1.0	5	0.105	0.041	0.045	0.18

6. REACTION MODEL IMPLEMENTATION USING SIMULIS REACTIONS

The reactions (R1) and (R2) presented in the paragraphs 2 and 5 were described in Simulis Reactions, as shown in the next screen shot.



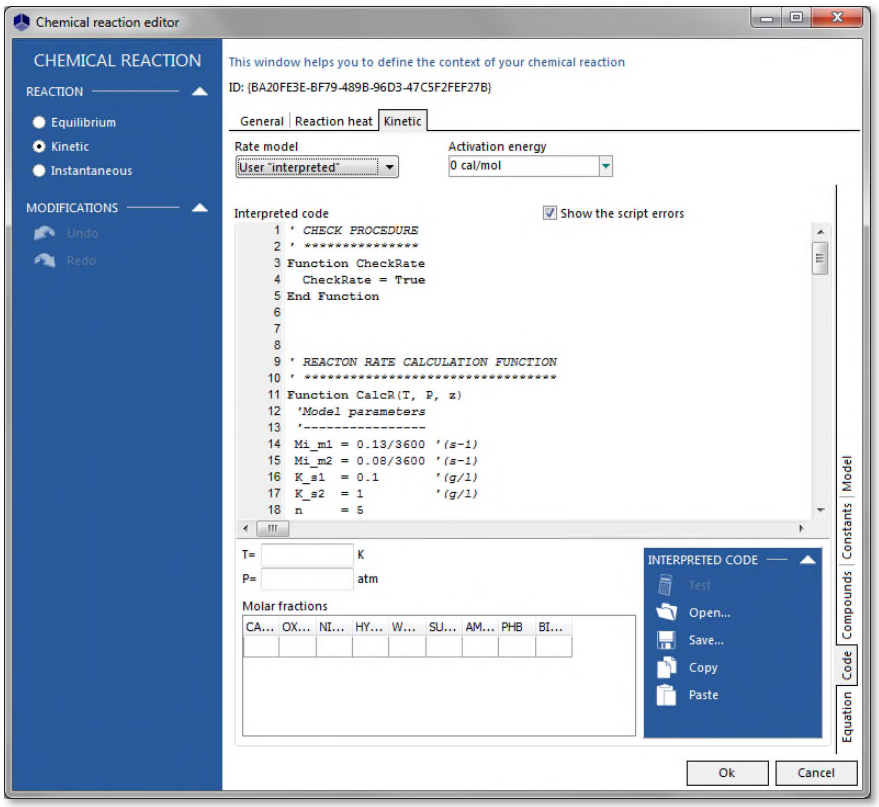
User “interpreted” kinetic rate model was used to implement the mathematical models presented by [HEI80] for the two reactions, as shown on the next screenshot. Thanks to this functionality of Simulis Reactions, user can write his own code for the kinetic model using VBScript (Microsoft Visual Basic Scripting Edition), which is an interpreted language: it doesn’t require any compilation before being executed and it requires the computer that has to run it has an interpreter (i.e. a program that can understand the whole instructions, e.g. *wscript.exe* in Windows environment). For more information about VBScript language, one can refer to:

http://msdn.microsoft.com/en-us/library/t0aew7h6(v=vs.84).aspx

http://en.wikipedia.org/wiki/VBScript

All reactions take place in the liquid phase.

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



The VBS code for the reaction (R1) is the following one:

```
' CHECK PROCEDURE
Function CheckRate
  CheckRate = True
End Function


' REACTON RATE CALCULATION FUNCTION
Function CalcR(T, P, z)
 ' Model parameters
 Mi_m1 = 0.13/3600 '(s-1)
 Mi_m2 = 0.08/3600 '(s-1)
 K_s1  = 0.1       '(g/l)
 K_s2  = 1         '(g/l)
 n     = 5
 Y_PR  = 0.105
 ' Calculation of the molar volume
 Vml = ThermoCalculator.PCalcVml(T,P,z)
 ' Units conversion
 ' Molar volume
 Set Repository = CreateObject("CverStarDustRepository.StarDust_CVER_Repository")
 Set Quantity   = Repository.QuantityByName("Molar volume")
```



```
 Vml            = Quantity.Convert(Vml,"cm3/mol","l/mol")
 'Molar mass
 Set MwQty = Repository.QuantityByName("Molar mass")
 ' Calculation of the concentrations
 CASN_AmmoniumSulfate = "7783-20-2"
 CASN_ResidualBiomass = "55001-02-0"
 CASN_PHB             = "55001-01-9"
 For i=1 To ThermoCalculator.Compounds.Count
   With ThermoCalculator.Compounds.Items(i-1)
     If (.CasRegistryNumber = CASN_AmmoniumSulfate) Then
         ipos_AmmoniumSulfate = i-1
         Mw_AmmoniumSulfate   = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
         C_AmmoniumSulfate    = z(ipos_AmmoniumSulfate)*Mw_AmmoniumSulfate/Vml
         S                    = C_AmmoniumSulfate '(g/L)
     ElseIf (.CasRegistryNumber = CASN_ResidualBiomass) Then
         ipos_ResidualBiomass = i-1
         Mw_ResidualBiomass   = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
         C_ResidualBiomass    = z(ipos_ResidualBiomass)*Mw_ResidualBiomass/Vml
         R                    = C_ResidualBiomass '(g/L)
     ElseIf (.CasRegistryNumber = CASN_PHB) Then
         Mw_PHB = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
     End If
   End With
 Next
 ' Calculation of the reaction rate
 RateR = ((Mi_m1*S)/(K_s1 + S) + (Mi_m2*(S/K_s2)^n)/(1 + (S/K_s2)^n))*R
 Rate  = Y_PR*RateR  '(g PHB/L.s)
 CalcR = Rate/Mw_PHB '(mol PHB/L.s)
End Function


' CALCULATION PROCEDURE
' T: Variant - Temperature (K)
' P: Variant - Pressure (atm)
' z: Variant - Molar fractions
' --- Results ---
' Rate:    Variant - rate in mol/l/s
' dRatedT: Variant - rate derivative with the respect to temperature in mol/l/s/K
' dRatedP: Variant - rate derivative with the respect to pressure in mol/l/s/atm
' dRatedN: Variant - rate derivative with the respect to number of moles in mol/l/s
' Err:     Variant - Error code
```



```
Sub CalcRate(T, P, z, Rate, dRatedT, dRatedP, dRatedN, Err)
  ' Reaction rate
  Rate = CalcR(T, P, z)
  ' Temperature derivative
  dT      = 0.1
  T1      = T + dT
  Rate1   = CalcR(T1, P, z)
  dRatedT = (Rate1-Rate)/dT
  ' Pressure derivative
  dP = 0.1
  P1 = P + dP
  Rate1 = CalcR(T, P1, z)
  dRatedP = (Rate1-Rate)/dP
  ' Compositions derivatives
  NC = ThermoCalculator.Compounds.Count
  Dim z1()
  ReDim z1(NC-1)
  For i=0 To NC-1
    For j=0 To NC-1
      z1(j) = z(j)
    Next
    dz = z1(i)*5e-6
    If (dz < 1e-8) Then
      dz = 1e-8
    End If
    z1(i) = z1(i) + dz
    Tot = 0
    For j=0 To NC-1
      Tot = Tot + z1(j)
    Next
    For j=0 To NC-1
      z1(j) = z1(j) / Tot
    Next
    Rate1      = CalcR(T, P, z1)
    dRatedN(i) = (Rate1-Rate)/dz
  Next
End Sub
```


The VBS code for the reaction (R2) is the following one:

```
' CHECK PROCEDURE
Function CheckRate
  CheckRate = True
End Function

' REACTON RATE CALCULATION FUNCTION
Function CalcR(T, P, z)
 ' Model parameters
 K1  = 0.041      '(g/L)
 k_1 = 0.045/3600 '(s-1)
 k_2 = 0.18/3600  '(s-1)
 ' Calculation of the molar volume
 Vml = ThermoCalculator.PCalcVml(T,P,z)
 ' Units conversion
 ' Molar volume
 Set Repository = CreateObject("CverStarDustRepository.StarDust_CVER_Repository")
 Set Quantity   = Repository.QuantityByName("Molar volume")
 Vml            = Quantity.Convert(Vml,"cm3/mol","l/mol")
 ' Molar mass
 Set MwQty = Repository.QuantityByName("Molar mass")
 ' Calculation of the concentrations
 CASN_AmmoniumSulfate = "7783-20-2"
 CASN_ResidualBiomass = "55001-02-0"
 CASN_PHB             = "55001-01-9"
 For i=1 To ThermoCalculator.Compounds.Count
   With ThermoCalculator.Compounds.Items(i-1)
     If (.CasRegistryNumber = CASN_AmmoniumSulfate) Then
         ipos_AmmoniumSulfate = i-1
         Mw_AmmoniumSulfate   = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
         C_AmmoniumSulfate    = z(ipos_AmmoniumSulfate)*Mw_AmmoniumSulfate/Vml
         S                    = C_AmmoniumSulfate '(g/L)
     ElseIf (.CasRegistryNumber = CASN_ResidualBiomass) Then
         ipos_ResidualBiomass = i-1
         Mw_ResidualBiomass   = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
         C_ResidualBiomass    = z(ipos_ResidualBiomass)*Mw_ResidualBiomass/Vml
         R                    = C_ResidualBiomass '(g/L)
```



```
    ElseIf (.CasRegistryNumber = CASN_PHB) Then
        ipos_PHB = i-1
        Mw_PHB   = MwQty.Convert(.Mw.Value,.Mw.UnitName,"g/mol")
        C_PHB    = z(ipos_PHB)*Mw_PHB/Vml
        P        = C_PHB '(g/L)
    End If
  End With
 Next
 ' Calculation of the reaction rate
 Rate  = K1*(-k_1*P+k_2*R)/(K1+S) '(g PHB/L.s)
 CalcR = Rate/Mw_PHB              '(mol PHB/L.s)
End Function

' CALCULATION PROCEDURE
' T: Variant - Temperature (K)
' P: Variant - Pressure (atm)
' z: Variant - Molar fractions
' --- Results ---
' Rate:    Variant - rate in mol/l/s
' dRatedT: Variant - rate derivative with the respect to temperature in mol/l/s/K
' dRatedP: Variant - rate derivative with the respect to pressure in mol/l/s/atm
' dRatedN: Variant - rate derivative with the respect to number of moles in mol/l/s
' Err:     Variant - Error code

Sub CalcRate(T, P, z, Rate, dRatedT, dRatedP, dRatedN, Err)
  ' Reaction rate
  Rate = CalcR(T, P, z)
  ' Temperature derivative
  dT      = 0.1
  T1      = T + dT
  Rate1   = CalcR(T1, P, z)
  dRatedT = (Rate1-Rate)/dT
  ' Pressure derivative
  dP = 0.1
  P1 = P + dP
  Rate1 = CalcR(T, P1, z)
  dRatedP = (Rate1-Rate)/dP
  ' Compositions derivatives
  NC = ThermoCalculator.Compounds.Count
  Dim z1()
  ReDim z1(NC-1)
```



```
  For i=0 To NC-1
    For j=0 To NC-1
      z1(j) = z(j)
    Next
    dz = z1(i)*5e-6
    If (dz < 1e-8) Then
      dz = 1e-8
    End If
    z1(i) = z1(i) + dz
    Tot = 0
    For j=0 To NC-1
      Tot = Tot + z1(j)
    Next
    For j=0 To NC-1
      z1(j) = z1(j) / Tot
    Next
    Rate1      = CalcR(T, P, z1)
    dRatedN(i) = (Rate1-Rate)/dz
  Next
End Sub
```


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

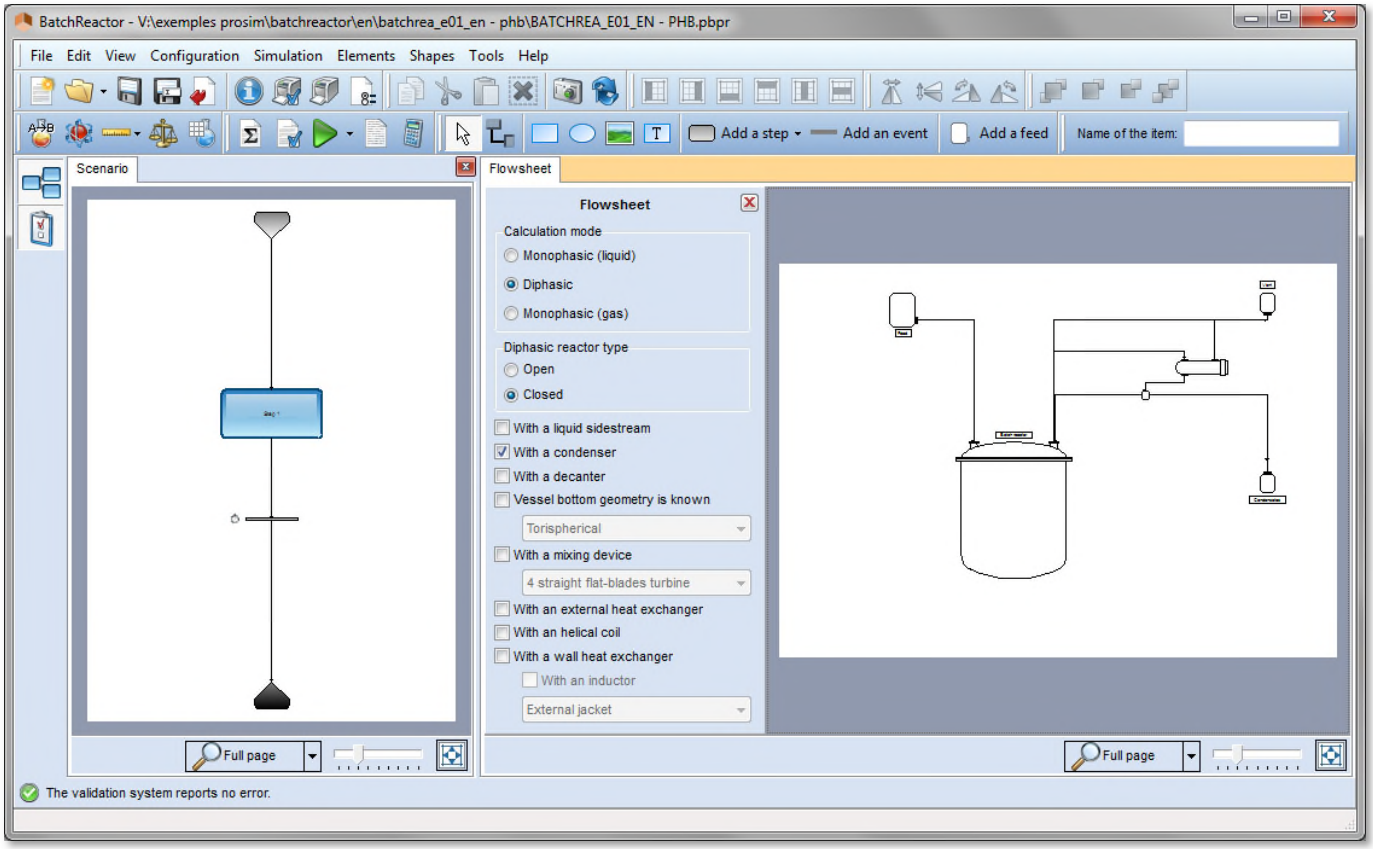
A continuous gas stream at ambient condition feeds the reactor in order to carry in the hydrogen, the carbon dioxide and the oxygen (6:2:1 molar respectively) required for the reactions. The characteristics of this feed are:

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

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

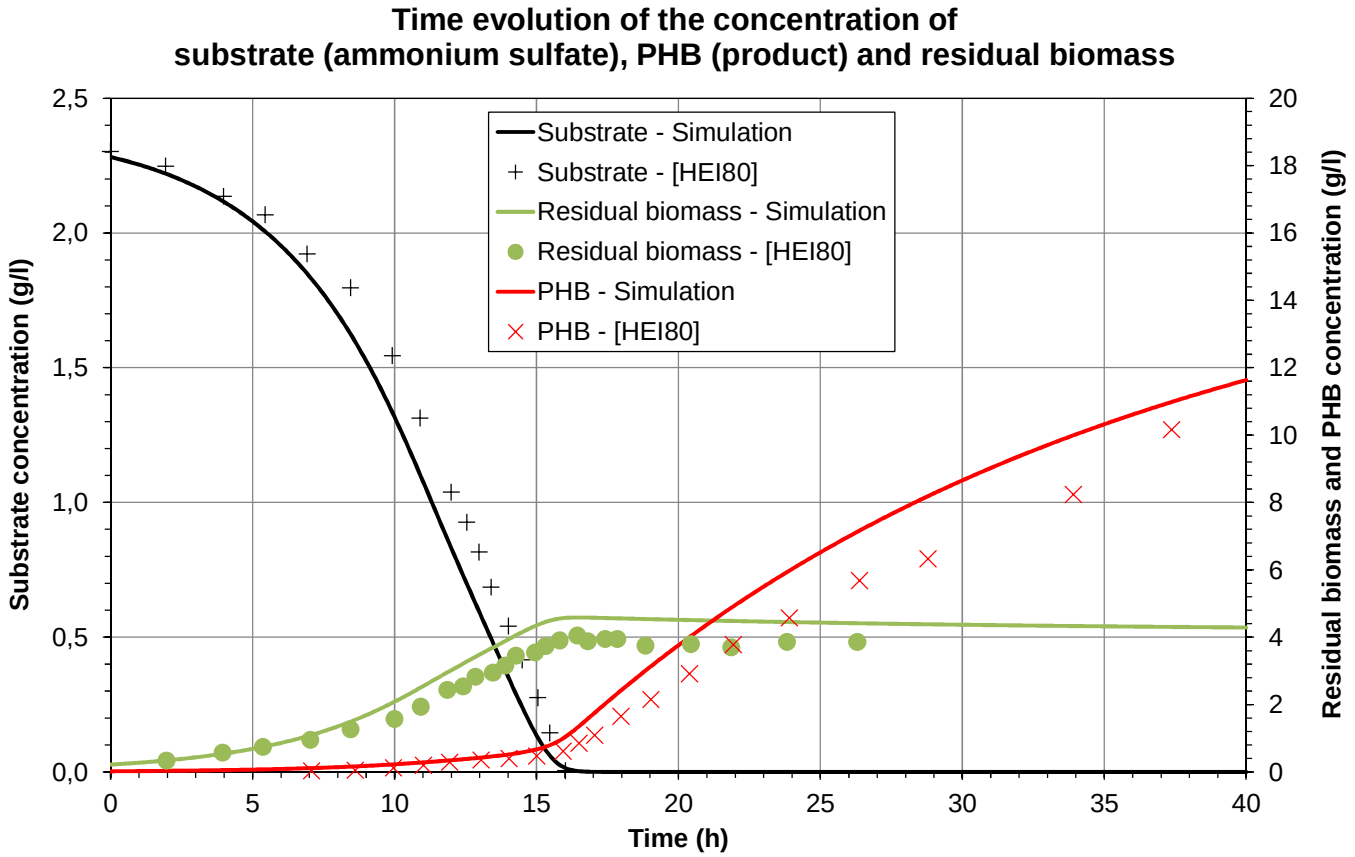
Type	Specified TR without thermal device
Temperature	30°C
Pressure	1 atm
Step duration	40 h

The scenario is presented on the left 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 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.



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)

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
R	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_R	Rate of synthesis of residual biomass	g/(l.h)
S	Concentration of substrate (ammonium sulfate)	g/l
t	Time	h
$Y_{P/R}$	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}

