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MODELING OF MASS TRANSFER DURING WOOD FERMENTATION PROCESSES TO PRODUCE BIOALCOHOL

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Abstract

The aim of this work is to model the bioethanol production by wood degradation, and to check if there is mass transfer limitations in the reactions steps from the wood particles to the microorganisms due to the transferring of molecules that are involved in. Cellulose and xylan have been considered the only reactive components of wood to get simple sugars for cell fermentation. Lignin affects only the mass transfer through the wood particles. The effect of mass transfer are studied by modeling the reactions steps of two processes that could have future develop in lignocellulosic bioethanol production: process 1, a thermal auto hydrolysis of xylan followed by *Simultaneous Saccharification and Co-Fermentation* (SSCF) step; process 2, with a SSCF single reaction step, including enzymatic hydrolysis of both xylan and cellulose.

The diffusive mass transfer of simple sugars produced by wood components degradation has been determined inside the wood and inside the microbial cell wall. A shrinking cores approach has been used for wood particles. The software used is Matlab®.

The study shows that the diffusion through the wood is limiting for both of the processes modeled: indeed the cellulose and xylan conversions, as well as the ethanol production, are much affected by wood particle dimensions.

Riassunto

Lo scopo del lavoro svolto in questa tesi è di studiare il *mass transfer* e i suoi effetti in due processi di produzione di bioetanolo da legno. Il legno è costituito da tre componenti principali: cellulosa, emicellulosa o xilano, e lignina. I componenti che possono essere idrolizzati per fornire i monosaccaridi fermentabili sono la cellulosa e lo xilano. Entrambi sono polissaccaridi: la cellulosa è costituita prevelavente la esosi (glucosio) mentre lo xilano da pentosi (xilosio e arabinosio). La lignina è anche un biopolimero che ha principalmente funzione strutturale. Essa è difficilmente degradabile ma comunque riduce la diffusività all'interno delle particelle di legno, e nello stesso tempo mantiene inalterato il loro diametro durante il decorso della reazione.

Il processo 1 è caratterizzato da due step di reazione. Nel primo, che viene condotto in un reattore PFR operante ad alta temperatura (170-230 °C), lo xilano subisce un processo di auto idrolisi termica che porta alla formazione di componenti monomerici come xilosio, arabinosio e acido acetico. Previo raffredamento a circa 40 °C la sospensione è inviata nel reattore discontinuo dello step di SSCF (*Simultaneous Saccharification and Co-Fermentation*). Qui i sistemi enzimatici e l'inoculo cellulare sono in grado di idrolizzare la cellulosa a glucosio (monomero) e cellobiosio (dimero) e, contemporaneamente, lo xilosio e il glucosio sono fermentanti a bioetanolo.

Nel processo 2 lo step di reazione è unico: previo riscaldamento a 40 °C la sospensione viene introdotta in un reattore batch in cui avviene la simultanea saccarificazione e cofermentazione (SSCF). Dopo l'aggiunta di appositi sistemi enzimatici e dell'inoculo cellulare, xilano e cellulosa sono contemporaneamente idrolizzati, e nello stesso tempo i microorganismi fermentano glucosio e xilosio per dare etanolo.

Da vari studi di letteratura risulta che gli organismi che sono stati impiegati per la fermentazione sono i più disparati: in questo lavoro si è fatto riferimento a batteri di *Escherichia Coli,* poiché in questo caso sono disponibili dati riguardanti il trasferimento di materia dei principali metaboliti dall'esterno all'inteno della cellula.

La modellazione dinamica ha riguardato principalmente due delle tre fasi della sospensione reattiva: le particelle legnose e le cellule batteriche. La fase liquida costituente il bulk di reazione è stata assunta come ben mescolata.

Il meccanismo di trasferimento di materia su cui ci si è concentrati è la diffusione. Nei modelli relativi ai due processi si sono implementate le equazioni di trasporto per il trasferimento degli zuccheri derivanti dall'idrolisi (nel legno) e degli zuccheri fermentabili attraverso la parete cellulare batterica.

Il legno è stato modellato con un approccio *shrinking core* modificato in cui due ho più strati a porosità e composizione diversa, a seconda del processo, si formano all'interno della particella legnosa durante la reazione. Tali strati sono caratterizzati da coefficienti di diffusione effettiva diversa. Le particelle sono state assunte sferiche e simmetriche, così come le cellule batteriche.
Per quanto riguarda il mass transfer all'interno delle cellule batteriche si è studiato il solo contributo relativo alla parete cellulare batterica. Infatti, secondo alcuni autori, è questo il trasferimento di materia limitante. Tale trasporto, per i metaboliti glucidici, è quasi esclusivamente passivo ovvero avviene secondo gradiente di concentrazione e senza consumo di energia dalle cellule attraverso apposite proteine intermembrana denominate porine.

I risultati ottenuti dalle simulazioni effettuate mostrano come entrambi i processi sono limitati dal trasferimento di xilosio e glucosio all'interno delle particelle legnose. Questo può essere notato osservando che le conversioni di cellulosa e xilano, e la produzione di etanolo sono fortemente limitate all'aumentare del diametro delle particelle legnose. Per questo i gradienti di concentrazione degli zuccheri semplici negli stati legnosi sono di circa 2/3 ordini di grandezza maggiori di quelli riscontrati nella parete cellulare.
Sul processo 2 è anche stato effettuato una analisi di sensitività che ha avuto l'obbiettivo di identificare quali parametri influenzano maggiormente i risultati relativi alle conversioni di cellulosa e xilano.

Una possibile limitazione dello studio è costituita dall'incertezza dei parametri usati, i quali sono stati trovati in letteratura per sistemi analoghi ma non sono stati confermati sperimentalmente per il sistema studiato. Infatti, la forte peculiarità dei materiali oggetto di studio (legno e cellule viventi) richiederebbe un approfondimento sperimentale che alla fine consentirebbe di eseguire l'ottimizzazione delle condizioni di processo per la produzione di bioetanolo.

Index

Introduction

The term “biofuel” is usually given to fuels that are in form of either liquid or gas and are derived from biomass or biological waste (Demirbas, 2007). The production of biofuels occur from different feedstock. Accordingly, they are known as first, second and third biofuel generation. The first generation is the first way that was developed, where biofuels are obteined from food crops. Hence this causes and have caused etic questions. The second generation is what this study is focused on. Indeed it produces biofuel from inedible cellulosic material like waste biomass, food industry waste and the object of this work, that is wood.

The third biofuel generation aims to produce biofuel from algae: it is a quite interesting way, because algae don’t compete with foodstocks.

Also the biofuel can be divided in different types: biogas, obtained from different forms of biomass with anaerobiosis conditions, in order to break the long organic chain in the most reduced form of carbon, methane, and CO_2; biodiesel is the most common biofuel used in UE because it can be used in current diesel engine. It is obtained by processing oils and fats; bioalcohols among which the most common is bioethanol. This work is focused on bioethanol production from fermentation of wood for land.

Specifically this study is focused on the evaluation of mass transfer resistance of different main substances involved in the wood fermentation of wood like glucose, cellobiose and so on.

The mechanism of degradation of carbohydrates to obtain bioethanol was modeled in two main steps: hydrolysis using either microorganisms or enzymes and fermentation with microorganisms.

Object of this work is to evaluate the role of mass transfer of some molecules involved in bioalcohol production, from wood. Two main different processes are studied: Simultaneous Saccharification and Co-Fermentation (SSCF) with thermal xylan pretreatment; SSCF single step process. Both mass transfer inside the wood and across the cell wall of microorganisms used were simulated by a dynamic model. A sensitivity study allowed to evaluate which parameters affect more the results obtained.

This work can be useful to predict the treatment time of different wooden feedstock with a characteristic pellet size, component composition, temperature, solid loading, and to evaluate the profiles of product concentrations, cell concentration and particle size along with treatment time and in the different phases, in view of an optimization of second generation bioethanol production process.

The thesis is made up by 6 chapter. The first one resumes the characteristic of wood and bacteria that are the base for the process studied, and for modeling; in the second one the

reader is informed about the theoretical method used to model the mass transfer through the heterogeneous system instead. Chapter three picks up all information found in literature about the bioalcohol production and further describes the two process that have been modeled in the subsequent two chapter. Then in chapter 4 approximations, algebraic and differential equations and solving methods of model for SSCF with xylan thermal pretreatment are presented and discussed, whereas. In the chapter 5 this is done for process with a single step SSCF. Finally, chapter 6 picks up the results about the base simulations of two process, data about xylan pretreatment and its influence on SSCF step, concentrations trend in wood particles, bulk and cell, same experimental validations of these results found in literature, data about sensitivity analysis and some theoretical consideration about the shrinking core models developed.

I would like to acknowledge Dr Norman Kirkby of University of Surrey, Guildford, the main promoter of this work. He with useful suggestion has made possible the realization of the present work during my stay in University of Surrey.
Also I would like to do a sincere acknowledgment to Prof. Alberto Bertucco of Università di Padova, for his helping to permit a correct writing of this thesis and some important suggesting about the wide potential of this work.

Chapter 1

Wood and bacteria characterization

To study and modeling wood and bacteria and in particular the mass transfer of some molecules involved in bioalcohol production processes, to know more knowledge about wood and bacteria is very important. This chapter is filled by composition and structure date about those. This searching was the first step of this project.

1.1 Wood characterization

The main components of wood are Cellulose, Xylan and Lignin. They are the only compounds that have been taken into account in the wood degradation and cell fermentation model. The chemical composition of wood was retrieved from literature. Some data are summarized in Table 1.1.

Table 1.1 *Chemical composition, weight fraction on dry basis of different type of wood and grass from Europe and America (Sola, et al., 2010).*

Components	Aspen (Pioppo tremulo) [% m/m]	Hybrid poplar (Pioppo) [% m/m]	Switchgrass (Erba di prateria alta) [% m/m]	Corn stover (Paglia del mais) [% m/m]
Cellulose	53.2	43.67	33.75	37.4
Xylan	19.09	15.63	22.13	21.1
Lignin	19.09	27.23	16.82	18.0
Arabinan	4.24	0.71	2.81	2.9
Mannan	2.12	2.27	0.19	1.6
Galactan	1.59	0.94	0.89	2.0
Ash	0.85	1.35	5.96	5.2
Extractives	0	3.39	15.55	4.7
Acetate	0	0	0	2.9
Protein	0	0	0	3.1
Soluble solids	0	4.81	1.89	1.1

The major component in the rigid cell walls of plants is cellulose. Cellulose is a linear polysaccharide polymer with many glucose monosaccharide units. The acetal linkage is of type *beta* which makes it different from starch. This peculiar difference in acetal linkages results in a major difference in digestibility in humans. Humans are unable to digest cellulose

because the appropriate enzymes to breakdown the beta acetal linkages are lacking. Indigestible cellulose is the fiber which aids in the smooth working of the intestinal tract. Cellulose mainly is a biopolymer having D-Glucose as monomer. The structure of cellulose consists of long polymer chains of glucose units connected by *beta acetal* bounds. The graphic Figure 1.1 shows a very small portion of a cellulose chain. All of the monomer units are beta-D-glucose, and all the beta acetal links connect C #1 of one glucose to C #4 of the next glucose (http://www.elmhurst.edu).

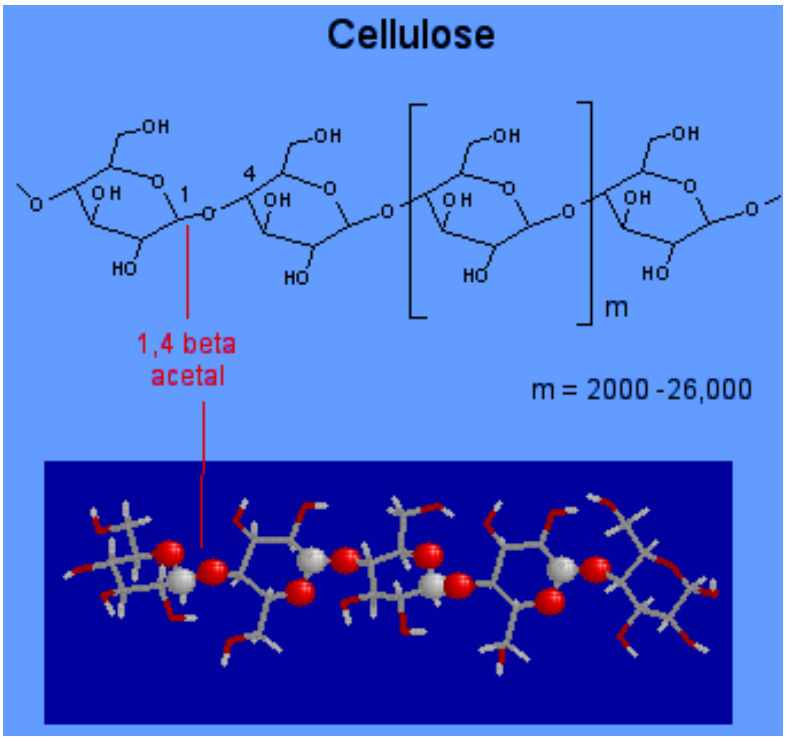


Figure 1.1 *General cellulose structure (http://www.elmhurst.edu)*

Xylan is a polymer made up of β-xylopyranose (xylose) units linked through (1→4)-glycosidic bonds. Arabinose, acetyl groups, and uronic acids are also present as lateral chains. However, in all the kinetic models thus far presented in the literature, xylan is loosely defined as the total amount of pentoses present in the product fractions (Sola, et al., 2010). A xylan general molecular structure is given in Figure 1.2.

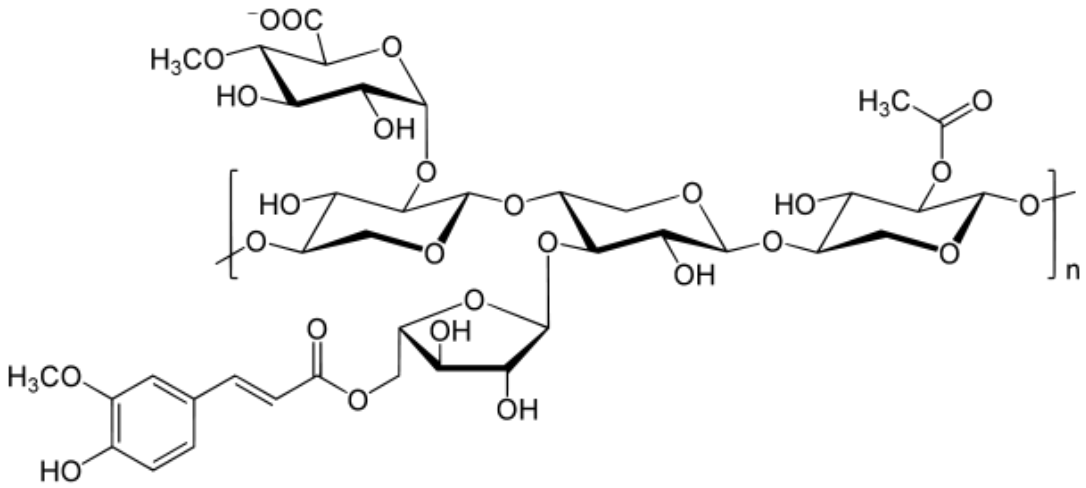


Figure 1.2 *Xylan general molecular structure.*

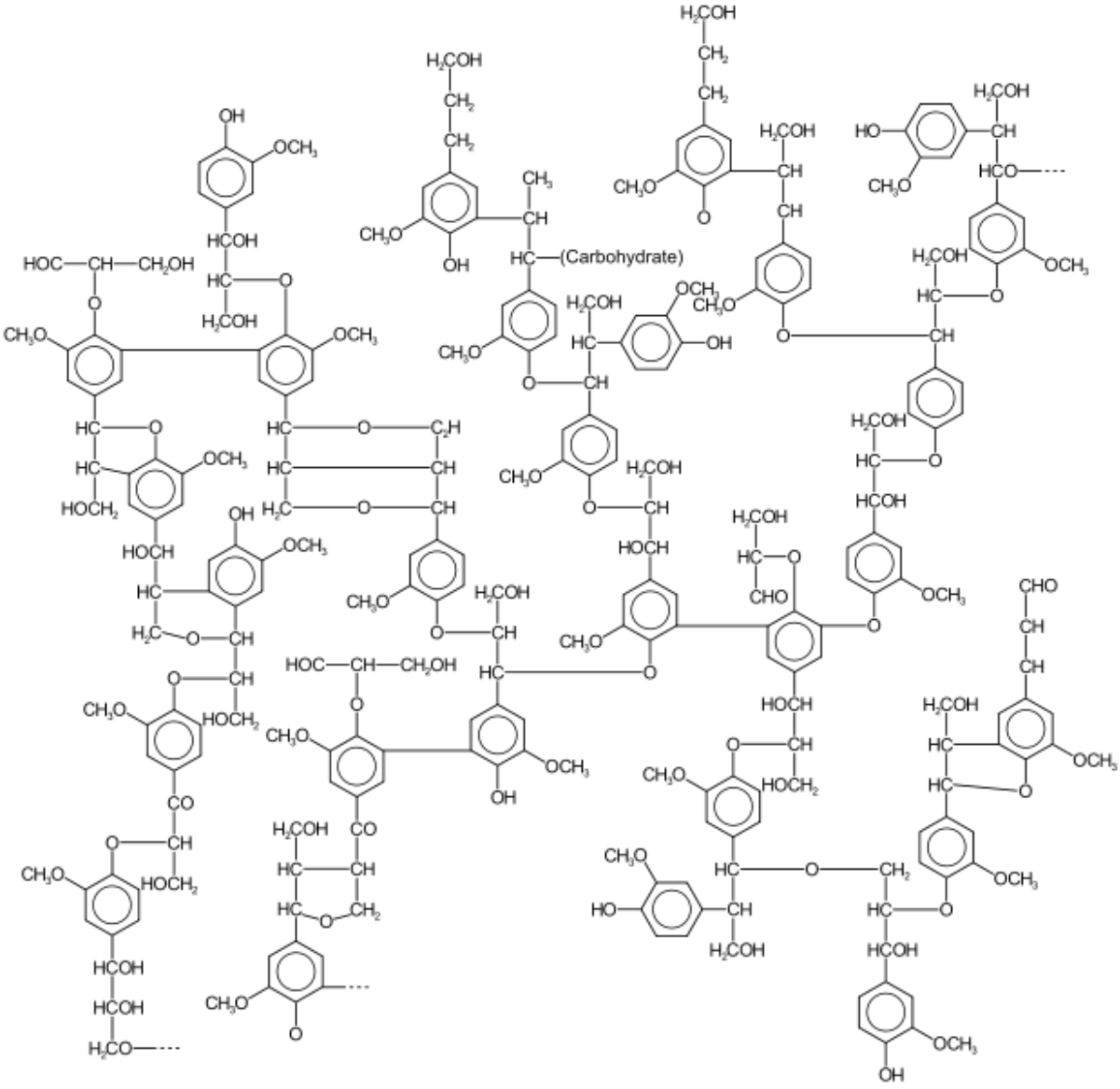


Figure 1.3 *Lignin general molecular structure.*

For modeling purposes it is some time assumed that xylan may be divided in two types: the first one (Xy1) is assumed to be more reactive and to have a higher content of arabinose and acetyl groups than the less reactive fraction, which is the type (Xy2) (Nabarlatz, et al., 2004).
Lignin is a complex chemical compound (Figure 1.3), most commonly derived from wood, and an integral part of the secondary cell walls of plants and some algae. As a biopolymer, lignin is unusual because of its heterogeneity and lack of a defined primary structure. Its most commonly noted function is the support through strengthening of wood in trees (Sola, et al., 2010).
Wood, microscopically, consists of bundles of large hollow tubes with doors across the tubes. These tubes are the walls of living cells, long since dead with only the skeleton remaining. Indeed hollow tubes are the skeletons of those cells (http://www.star-distributing.com/howtoguides/woodrots.html). The structural material of these tubes is lignin that gives the classic rigidity to wood. Lignin is difficult to be degraded by bacteria and fungi. The microscopical structure also consists of closed vessel that are impermeable to mixture transport through wood material.

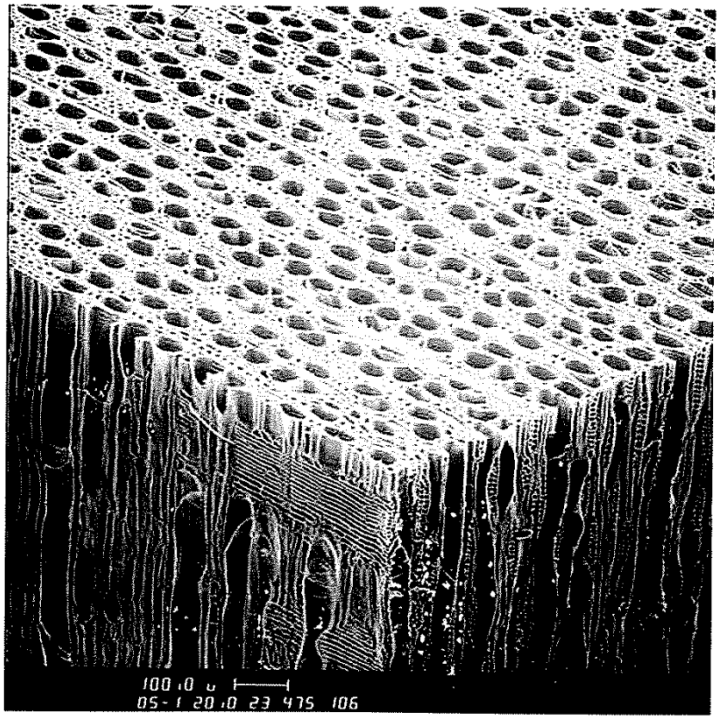


Figure 1.4 *A microscopic image of yellow poplar, showing the hollow tubes in the wood*

Density, pores size, porosity, void fraction depend by many factors as wood age and humidity. A wood particle will be modeled as a sphere inside which the composition of three main constituents is homogenous. Another assumption is to have all wood particles with the same initial diameter, shape and composition.

Table 1.2 *Typical values of wood particles used in base case simulations and other simulations.*

Property	Value
Density [mg/ml] (Gryc, et al., 2008)	726
Cellulose content [m/m] (Sola, et al., 2010)	0.52
Xylan content [m/m] (Sola, et al., 2010)	0.19
Lignin content [m/m]	0.29
Xylan (type 1) content [m/m] (Nabarlatz, et al., 2004)	0.80
Radius of wood pores/channel [cm] (Gryc, et al., 2008)	8e-4
Porosity [v/v] (Siau, 1984)	0.03

Typical values of woods properties useful for the present work were found in the literature and are summarized in Table 1.2.

1.2 Bacteria characterization

To study how fast is mass transfer through the cell outer membrane it is important to know the structure of the bacteria and is own membrane that substances must across to be metabolized inside the organelles able to metabolize. Except for some species, most bacteria have strong walls that give them shape and protect them from osmotic lysis. During this study a lot of simplifications were made about the metabolic reaction (see later) and about the resistance to mass transfer through a number of membrane that are not just outside the cytoplasmic space but also inside the cell arround each organelle. Only the mass transfer resistance generated by cells outer membrane was considered.

Bacteria may be either Gram-positive or Gram-negative. Gram-Positive ones are surrounded by a relatively thick, amorphous cell wall, made up mainly by peptidoglycan. It reacts with Gram reactants giving a positive sample and characterizing the cells as Gram-positive (Figure 1.6).

The organisms of interest for this work are Gram-negative ones. Their complicate cell envelope is resolved into an outer membrane, that contains mainly lipopolysaccharides, anchored to a thin peptidoglycan layer and underlying the cytoplasmic membrane. Between the peptidoglycan layer and the outer membrane there is a space called periplasmic space. According to the literature (Nikaido, 1981) the peptidoglycan layer have a negligible effect on mass transfer resistance.

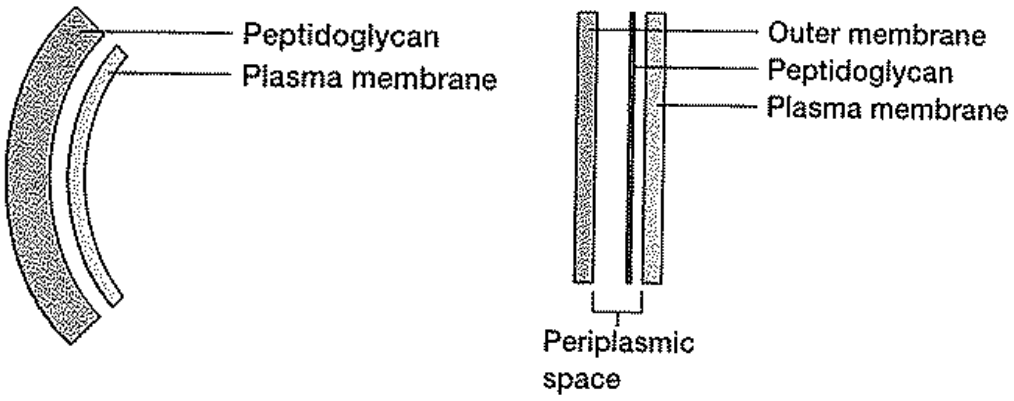


Figure 1.5 *Comparison among external structures of Gram Positive cell (a) and Gram Negative cell (b).*

Many authors, (Prescott, et al., 1996), (Nikaido, et al., 1981) and (Renkin, 1954) underlined the importance of the outer membrane proteins. It is because they are able to form pores and so these proteins are called "porins" (Figure 1.6 and Figure 1.7).

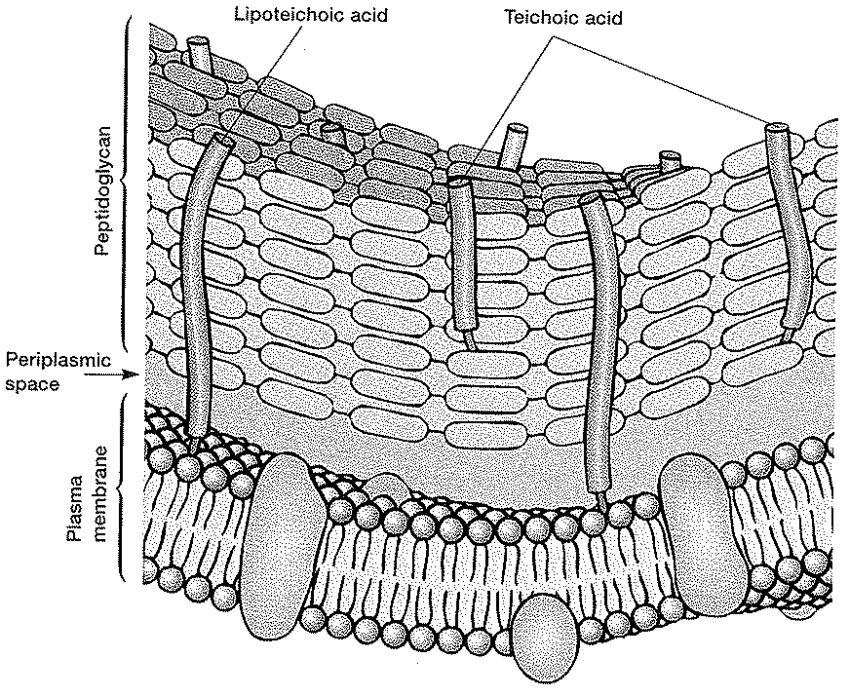


Figure 1.6 *Details of Gram Positive cell outer structure.*

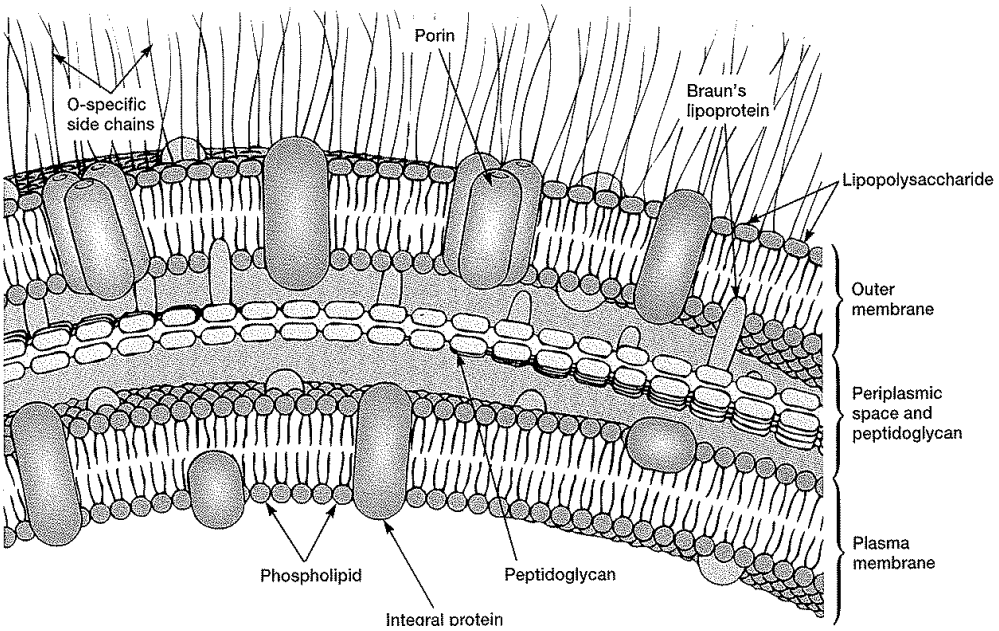


Figure 1.7 *Details of Gram Negative cell outer structure.*

These porins have a particular structure that is able to allow flow through the outer membrane. This is vital for cells, that can receive all metabolites from the outside environment and also can excrete substances like enzymes, CO_2 and so on.

Table 1.3 *Main components of bacteria membranes. Comparison between Gram Negative and Gram Positive bacteria .*

	Gram Positive	**Gram Negative**
Peptidoglycan	Present (Thick)	Present (Thin)
▪ % (wt.) of envelope wall	40-95 %	10-20 %
▪ Diaminoacid	Lysine or DAP	DAP
▪ Teichoic acid	Present in many	Absent
▪ Teichuronic acid	Present in some	Absent
▪ Lipopolysaccharides	Absent	Present
▪ Lipoprotein	Absent	Present
Outer Membrane	Absent	Present

The transfer through the membranes can be classified in three main types as: passive diffusion, facilitated diffusion and active transport.

In active transport a solute is moved against a concentration or electrochemical gradient: in doing so the transport proteins involved consume metabolic energy, usually ATP.

Facilitated diffusion works according to the same thermodynamic principle of transport along a gradient as passive diffusion. However, the transport is facilitated by the presence of

channel proteins, which facilitate the transport of, in this instance, water or certain hydrophilic ions and molecules.

Passive diffusion is a spontaneous phenomena that increases the entropy of a system and decreases the free energy. The transport process is influenced by the characteristics of the transport substance and the nature of the layer. Membrane proteins are not involved in passive diffusion. The diffusion velocity of a pure phospholipid membrane will depend mainly on concentration gradient and particles size.

Knowing transport mechanisms of substances through the cell membranes is very important to try to model this phenomena. An example is about the *E.Coli*, that is a Gram Negative bacteria where transportation occurs with active transport from periplasmic space to cytoplasm through cytoplasmic membrane and its linked proteins; otherwise, through outer membrane and specifically through porins of outer membrane, the transport of substances from outside to periplasmic space occurs by passive transport mechanism (Nikaido, et al., 1981).

Chapter 2

Modeling Mass Transfer: theoretical aspects

In this study it has been necessary to know as deep modeling process should be so to choose the more appropriate equations and physical expressions. The main object of this chapter is to identify the equations to describe mass transfer inside particles modeled as wood particles and bacteria cell. All transferring and reactions are phenomena occur in water.

2.1 Mass transfer inside wood

Diffusion mechanism via the Fick's Law was used to describe mass transfer inside wood pellet. Convection mass transfer mechanism was neglected, assuming it much faster than diffusion. The Fick's Law was written as:

$$\dot{m} = DA\frac{\mathrm{d}\,c}{\mathrm{d}\,r} \quad , \tag{2.1}$$

where:

$\dot{m}$ = Mass flow rate [mg/s];
D = Diffusion coefficient [cm^2/s];
A = Diffusion area [cm^2];
c = Concentration [mg/cm^3];
r = Spatial coordinate [cm].

D is a property to describe how fast one substance moves through the solvent. Many equations are available in literature to calculate D, considering the environment where the molecule is and where it should move.
Free diffusion occurring in free solvent, without any pores, is called also molecular diffusion, the coefficient D is also named D_{ab}, where "a" is the solute and "b" is the solvent. The equations most used to calculate it at infinite dilution (D_{ab}^{0}, in cm^2/s) is the Wilke-Chang (Modified Stoke-Einstein) equation (Kirkby, 2009):

$$D_{ab}^{0} = 7.4 \times 10^{-8} \frac{(\phi M_B)^{0.5}\, T}{\eta_B V_A^{0.6}} \quad , \tag{2.2}$$

where:

ϕ = Association factor of solvent [-] (for water it is 2.6 as in Kirkby, 2009);

M_B = Molecular weight of solvent [g/mol];

T = Temperature [K];

η_B = Viscosity of solvent [cP];

V_A = Molar volume of solute a at its normal boiling point [cm^3/mol].

In this case always $D_{ab} = D_{ab}^{0}$ has been considered. V_A has been estimated as a function of molecular weight of the solute, using a polynomial correlation whose parameters were found correlating with a polynomial about 200 data retrieved from Perry, et al., 2006. Data regression was done with Microsoft Excel (See Appendixes in Data about normal molar boiling volume) and the result is shown in Figure 2.1.

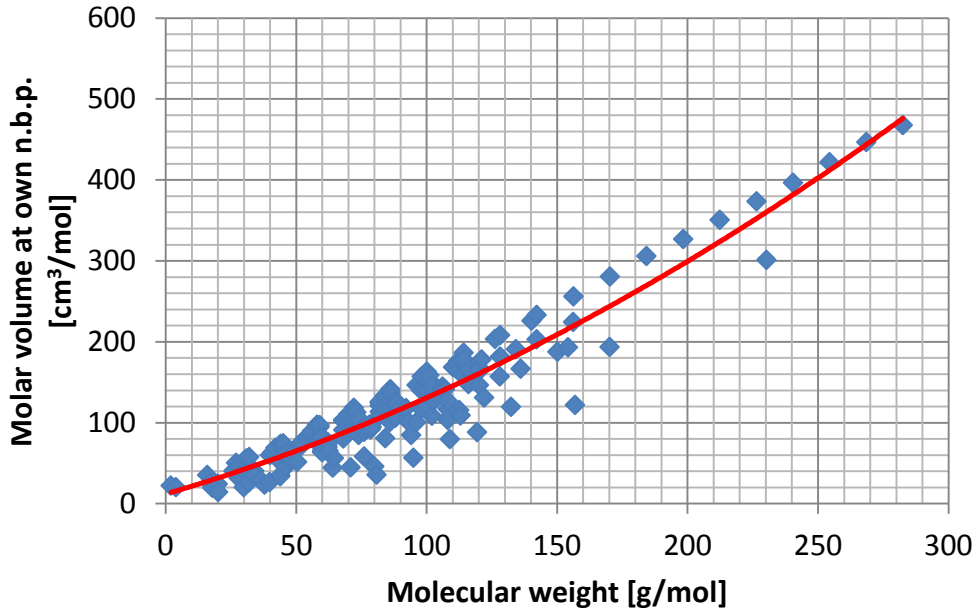


Figure 2.1 *Correlation of molar volume of solvent at its boiling point [cm^3/mol] with molecular weight [g/mol] . The red line correlates about 200 data from (Perry, et al., 2006)*

The V_A function represented in Figure 2.1 is given by:

$$V_A = 2.486 \times 10^{-3} M^2 + 9.408 \times 10^{-1} M + 11.93 \ . \tag{2.3}$$

The viscosity of solvent (only water is used in the process studied) η_B is calculate by (Perry, et al., 2006).

$$\eta_B = \exp\left(-2.471 \times 10^1 + \frac{4.209e3}{T} + 4.527 \times 10^{-2}\, T + 3.376 \times 10^{-5}\, T^2\right) \quad (2.4)$$

For mass transfer within small pores, such as in the cell wall, the Knudsen diffusivity should be taken in account.

In practice, Knudsen diffusion applies only to gases because the mean free path for molecules in the liquid state is very small, typically close to the diameter of the molecule itself. The typical coefficient of Knudsen flow is given by

$$D_K = 9700 r_e \sqrt{\frac{T}{M}} \quad , \quad (2.5)$$

where:

D_K = Knudsen diffusivity [cm^2/s];

r_e = Average pore radius [cm];

M_B = Molecular weight of solute [g/mol].

The pore diffusivity, D_p, is usually obtained with the approximation (2.6) when Knudsen diffusivity occurs (Kirkby, 2009).

$$\frac{1}{D_p} = \frac{1}{D_{ab}} + \frac{1}{D_K} \quad , \quad (2.6)$$

otherwise,

$$D_p = D_{ab} \quad . \quad (2.7)$$

However the pore diffusivity applies to the pores and not to the whole pellet so it is necessary introducing a correction with void fraction and tortuosity. The last one is the factor to account for the extra path that the molecule has to take to get to the center of the pellet by the route forced on it by pores. This factor relies on the pore geometry and connectivity and it is expected it will be characteristic of a given material. Therefore tortuosity could be considered as a parameter of a model because it is not easy to be measured for real system. So effective diffusivity D_e [cm^2/s] is obtained from following equation:

$$D_e = D_p \frac{\varepsilon_p}{tor} \quad , \quad (2.8)$$

where:

ε_p= Void fraction of pellet [-];

tor = Tortuosity [-].

D_e will be used to represent the mass transfer resistance inside the wood pellet and is calculated for each wood portion, typically for different layers formed during the degradation of wood's components, because they have different void fraction. The diffusion coefficient depends also on the component considered. These values are stored in a matrix $\mathbf{D_e}(i,j)$ in which *i* is the diffusing component and *j* is the wood section.

2.1.1 Molecular weight calculation

The diffusion coefficient calculation needs to know the molecular weight of each molecule involved. A Matlab function was developed to calculate molecular weight of all molecules. It was adapted to Matlab programming language using a previous work developed in Fortran (Gevorgyan, 2010).

It is able to calculate the molecular weight of molecules starting from them chemical formula. For each element the atomic weight is stored in function `Periodic_Table.m`. The function `mol_weight_improved.m` is able to identify all atoms present in a molecule and how many atoms of each type are in the molecule. These functions are described in Appendixes (Molecular weight calculation: main functions).

2.2 Mass transfer inside cells

In this paragraph only the mass transfer through the cell wall will be considered. All other mass transfer limitations within the cell have been neglected, because they are driven by active mechanisms.

The pores dimension was seen to be comparable with molecules diameter only within the Bacteria cell wall. Instead of Knudsen theory, another approximation developed for living cell and membranes was used, the Renkin equation (Renkin, 1954) . It considers, the effect of pores and molecular dimension and corrects the permeability coefficient of solutes through porins channels of the outer cell wall membrane. The permeability coefficient is calculated according to the work of (Nikaido, et al., 1981):

$$P = D_{ab}\,\frac{cor}{thi}\,\frac{a_0}{A_{cell}}\ ; \tag{2.9}$$

$$cor = (1 - ronR)^2 \times (1 - 2.104\,ronR + 2.09\,ronR^3 - 0.95\,ronR^5) \tag{2.10}$$

$$ronR = \frac{hr}{pore_{rad}} \tag{2.11}$$

Where:

P = Permeability [cm/s];

cor = Permeability correction [-];

thi = Cell wall thickness [cm];
a_0 = Total pores crossing surface per cell [cm^2];
A_{cell} = Cell surface [cm^2/cell];
hr = Hydrated radius of compound [nm];
$pore_{rad}$ = Hydrated pore radius [nm].

Similarly as for tortuosity, it is not simple to calculate a_0 and $pore_{rad}$. Because during cell growth the conformation of cell wall can change. So, in the models developed in this work, they will be considered as parameters.

2.2.1 Accurate method

More complicate but more accurate methods was found in the literature. They consider not only the diffusion transport through a membrane but also other mechanisms that involve not only the solute but also the solvent.
All mechanisms that are involved in membrane transport are:

- Filtration
- Osmotic Transport
- Ultrafiltration
- Diffusion

The filtration and osmotic transport are responsible of solvent transport, the last two for solute transport.
Two simple equations to describe all mechanisms are the Kedem and Katchalsky equations (Suchanek, 2006):

$$J_v = L_p \Delta P - L_{pD} \Delta \Pi \quad ; \tag{2.12}$$

$$J_D = -L_{Dp} \Delta P + L_D \Delta \Pi \quad . \tag{2.13}$$

Where J_v and J_D are respectively solvent flux and solute flux, L_p, L_{pD}, L_{Dp} and L_D denote coefficients of filtration, osmotic transport, ultrafiltration and diffusion, respectively; ΔP and $\Delta \Pi$ denote pressure differences (mechanical and osmotic pressure).

Also other methods more accurate for solution with one solvent and more than one components are developed (Suchanek, 2006).

Chapter 3

Processes

In this chapter the processes that will be modeled are presented. Two different process are considered: first, a two steps reaction process in which the first one is the thermal degradation of xylan, and the second one is the enzymatic hydrolysis step of cellulose and the sugars fermentation; second, a simultaneous saccharification cofermentation (SSCF) process in which degradation of xylan and cellulose occurs in the same step.

3.1 Literature review

Owing to the rise of environmental concerns and to the periodical crises in oil exporting countries, followed by threat of permanent increase of the oil price, bioethanol has became a viable and realistic alternative in fuel market. Corn and wheat are the common raw materials to obtain bioethanol in USA and Europe. However, in the last years, the experimental research has focused on lignocellulosic material like wood. The process that uses this type of feed is called "Second generation process". Lignocellulosic substrates, differently from starchy ones, that need only a fast cooking step as pretreatment, require more complex steps before hydrolysis. Only in this way the amount of sugars recovered can be maximized without use to many enzymes and too long saccharification time. On the other hand, the pretreatment is expensive and may be relevant in determining the overall production costs of the process. Among the pretreatment types suitable to achieve the best target, liquid hot water (LHW), steam explosion, dilute acid, lime and ammonia processing are the most suitable ones (Franceschin, et al., 2009).

In the present work these pretreatments step will be neglected. However, the mass transfer limitation inside the lignocellulosic material and that is needed to transfer the metabolites into cell for the ethanol fermentation, will be discussed.

The process so can be divided in two main steps: the degradation of carbohydrates (hemicellulose or xylan and cellulose), by the hydrolysis, in order to converted that into simple sugars, pentose or hexose, of one or two monomeric unit; the fermentation of these sugars to alcohols. Fermentation is an anaerobic biological process in which sugar are converted to alcohol by the action of microorganisms, usually yeast or bacteria.

The typical reaction that occurs during fermentation and is catalyzed by Zymase, an enzyme produced mainly by yeast, is (Demirbas, 2007):

$$C_6H_{12}O_6 \rightarrow 2\ C_2H_5OH + 2\ CO_2 \quad , \tag{3.1}$$

Cellulose is a pure organic polymer, consisting solely of units of anhydrous glucose held together in a giant straight chain molecule. Cellulose must be hydrolyzed to glucose before fermentation to ethanol. Cellulose is insoluble in most solvents and has a low accessibility to acid and enzymatic hydrolysis. Hemicelluloses are derived mainly from chains of pentose sugars and act as the cement material holding together the cellulose micelles and fiber (Demirbas, 2007).

3.1.1 Two steps process

In this case the process is divided in two different steps, hydrolysis and fermentation. The most commonly applied methods to hydrolyze sugar polymers can be classified in two groups: chemical hydrolysis (dilute and concentrated acid hydrolysis) and enzymatic hydrolysis (Demirbas, 2007).
In the first one an acid is used to catalyze the hydrolysis reaction, in the second one the degradation is catalyzed by enzymes, like gluco-amylase, that convert the starch into glucose and/or fructose. The main disadvantages of the acid process are: using anticorrosion equipment and the need to recover a high percent of sugar from the acid reaction bath, to have on economically viable process. Two basic types of acid hydrolysis processes are commonly used: with dilute acid and concentrated acid. The main advantage of dilute acid process is fast rate of reaction, which facilitates continuous processing. Concentrated sulfuric or hydrochloric acids are used for hydrolysis of lignocellulosic materials. The concentrated acid process uses relatively mild temperatures, and the only pressures involved are those created by pumping materials from vessel to vessel. Reaction times are typically much longer than for dilute acid. This process provides a complete and rapid conversion of cellulose to glucose and hemicelluloses to 5-carbon sugars with little degradation. The critical factors needed to make this process economically viable are to optimize sugar recovery and to reduce the cost to recover and recycle the acid.
The enzymatic way has that aim too, but the reaction occurs through one or more enzymatic systems that are able to degrade complex polymer like cellulose and hemicellulose.

Another way to hydrolyze the hemicellulose content of a lignocellulosic material is thermal degradation. The work by (Nabarlatz, et al., 2004) provides a kinetic model to describe the production of simple sugars for cosmetic use from xylan. This model was be extend to our case. In this work thermal hydrolysis of xylan will be modeled as the initial step.

3.1.2 One step processes (SSF and SSCF)

The current cost of conversion is a major bottleneck for commercial application. Among the strategies to lower processing costs are decreasing the enzymes loading, consolidating process steps, and using all sugars present in the biomass. Therefore the processes that have only one real reaction step there are particularly interesting. In this case hydrolysis and fermentation occur at same time. Usually the hydrolysis occurs by enzymatic way. In fact the mild conditions required are suitable also for organisms that are responsible of fermentation reactions (Zheng, et al., 2009).

These processes can be divided in two categories: Simultaneous Saccharification and Fermentation (SSF) and Simultaneous Saccharification and Co-Fermentation (SSCF). SSF process is able to convert only cellulose to ethanol. Different studies on kinetic reactions rate have different assumption on mechanism of cellulose degradation, enzymes adsorption on substrate and so on. Both batch reactor (Zheng, et al., 2009) and CSTRs series reactors (Quiroga, et al., 2009) have been investigated so for.

In SSCF hydrolysis of xylan and cellulose occurs simultaneously and fermentation of both hexose and pentose sugars as well. However there are only a few published studies of converting both cellulose and hemicellulose to ethanol via SSCF (Zhang, et al., 2009).

In this work a model for SSCF will be developed.

3.1.3 Organisms suitable for bioethanol production

Nowadays in literature works information can be found about bacteria able to make simultaneously both steps, hydrolysis and fermentation. Also, have been found bacteria able to carry out enzymes production for hydrolysis, and also organisms that are able to degrade lignin content of lignocellulosic material.

Saccharomyces Cerevisiae RWB22 is genetically modified derivative of *S. cerevisiae CEN. PK*. This yeast is able to perform a SSCF with specific enzymes for hydrolysis. Xylose utilization in this strain was achieved by integrating the xylose isomerase from *Piromyces Sp E2*, over expression of the native pentose phosphate pathway and direct evolution for growth on xylose (Zhang, et al., 2009);

Caldicellulosiruptor Saccharolyticus was described as a highly thermophilic, obligately cellulolityc bacterium. It is able to degrade as well different plant materials including starch, cellulose, hemicellulose and pectine. It produces a large amount of hydrogen during the fermentation (4 mol of H_2 per 1 mol of glucose) and it is also capable of fermenting C5 and C6 simultaneously but the organisms prefer to consume glucose over xylose. The H_2 yield was found to be dependent on the formation of lactate and ranged between 50% and 94% of

theoretical obtainable amount. Stripping gas is generally used to improve the yields of hydrogen in fermentation process (Khan, et al., 2010).

Clostridium thermocellum produces mainly ethanol, hydrogen and carbon dioxide, that can be found by testing the fermentative powers of the thermophilic bacterium on various sugars and cellulose. The optimum temperature of this anaerobic organisms is 60°C. When ethanol was added to the bacterium, it continued to grow for 1-2 hours before entering in a phase of arrested growth, the duration of which was dependent of the age of inocula. In this phase the cultures grow at an exponential rate that was a function of ethanol concentration. They also found that the optimum growth temperature of the organism decreased as the concentration of ethanol increased. Also *C. Thermocellum* synthesis and secretes a highly effective cellulase system which has the ability to saccharify completely various forms of cellulose (Khan, et al., 2010).

Trichoderma Reesei is capable of synthesising cellulase under conditions of high catabolite repression. Some results shown that both the glucose-rich hydrolysates and the pentose extracts can be used as a source of carbon for cellulase production by *T. Reesei* (Khan, et al., 2010). A commercial process was developed over the previous decade on converting cellulosic and lignocellulosic material into ethanol. A pilot plant, with a 1 tonne/day feedstock input, based on the utilisation of the *T. Reesei* fungal enzyme systems has been operated successfully at a pulp and paper mill. Successive improvements in techniques and operating conditions led to a production of ethanol with conversions of 80-90% of theoretical maximum. Their results showed that ethanol inhibits the cellulase progressively and linearly up to concentration of 65 g/L (Wald, et al., 1983).

The actinomycete strain *Streptomyces griseus B1,* isolated from soil, when grown on cellulose powder as submerged culture produces high levels of all the three components i.e. filter paper lyase (FPase), CMCellulase and bglucosidase of the cellulolytic enzyme system (Arora, et al., 2005). This organism has been used in solid-state fermentation of lignocellulosics in an attractive process for developing countries, as it requires low capital and infrastructure and is practical for complex substrates including agricultural, forestry and food-processing residues. It is ables to degrade lignin. It is thought that native lignin degradation by actinomycetes is associated with primary growth and their main activity is lignin depolymerization and solubilization rather than mineralization. Further developments in high value applications for lignins and other byproducts could go a long way towards offsetting the high cost of cellulose hydrolysis.

Escherichia Coli can be used for fermenting of sugar C5 and C6 but it is not able to produce the enzimatic system for hydrolysis of cellulose or xylan. It is a gram negative bacteria and more details about the moleculus transport inside this cell was found in a work by Nikaido, et al., 1981. Based on *E.Coli* some pilot plant facilities were depeloped, simultaneously with the development of process design for the first commercial installations of this novel thecnology (Katzen, et al., 1994).

The processes described in next section are based on *E. Coli*, because on these are the only information available about mass transfer inside the cells.

3.2 Process with xylan pretreatment – Process 1

With reference to Figure 3.1, in the first process, the raw material is collected, milled to suitable size and washed. Water is added to have a slurry with the solid loading fraction desired. The heart of this process is made up by two main reaction steps. The first one is a pretreatment step in which the wood is treated to hydrolyze the xylan content to simple sugar. The xylose, arabinose and acetic acid are assumed to be the only monomer of the xylan fraction and so the only sugar in final water solution. Xylan degradation is a autohydrolysis carried out by high temperature reaction (170°C – 230 °C). This reaction could be performed in different reactor types (Franceschin, et al., 2009) like batch reactor, continuous reactor (PFR) and semi batch reactor. In this work only the PFR has been simulated. The possibility that the monomers are degraded in to furfural is neglected by process simulation. Indeed furfural amount can be maintained quite low with particular process shrewdness (Franceschin, et al., 2009) or with a good pH control during reaction step (Katzen, et al., 1994). After the pretreatment step the reaction bath is cooled down to 40 °C and the hydrolyzed fraction and the residue solid are mixed together and undergo an enzymatic hydrolysis that occurs in a batch reactor. The enzymes used (cellulase system and β-glucosidase) are added to the reactor. This step is a SSCF for cellulose only. The bacteria used for fermentation are the *E. Coli CM 6*, simple Gram Negative cell, used also in the work of (Katzen, et al., 1994). It is assumed that only xylose and glucose content can be fermented by cells. The two reactions are both isothermal.

Then by a separation section is needed the main product (ethanol) is separated from by-products: waste water is sent to liquid effluent treatment, whereas wood residue (lignin and other biopolymer not react in the previous step) can be useful for animal feeding. Bacteria cells should be recycled in SSCF reactor to provide the bacteria inoculum in next reaction batch. The same holds for the enzymes system necessary to hydrolysis.

A block flow diagram of the process is shown in Figure 3.1.

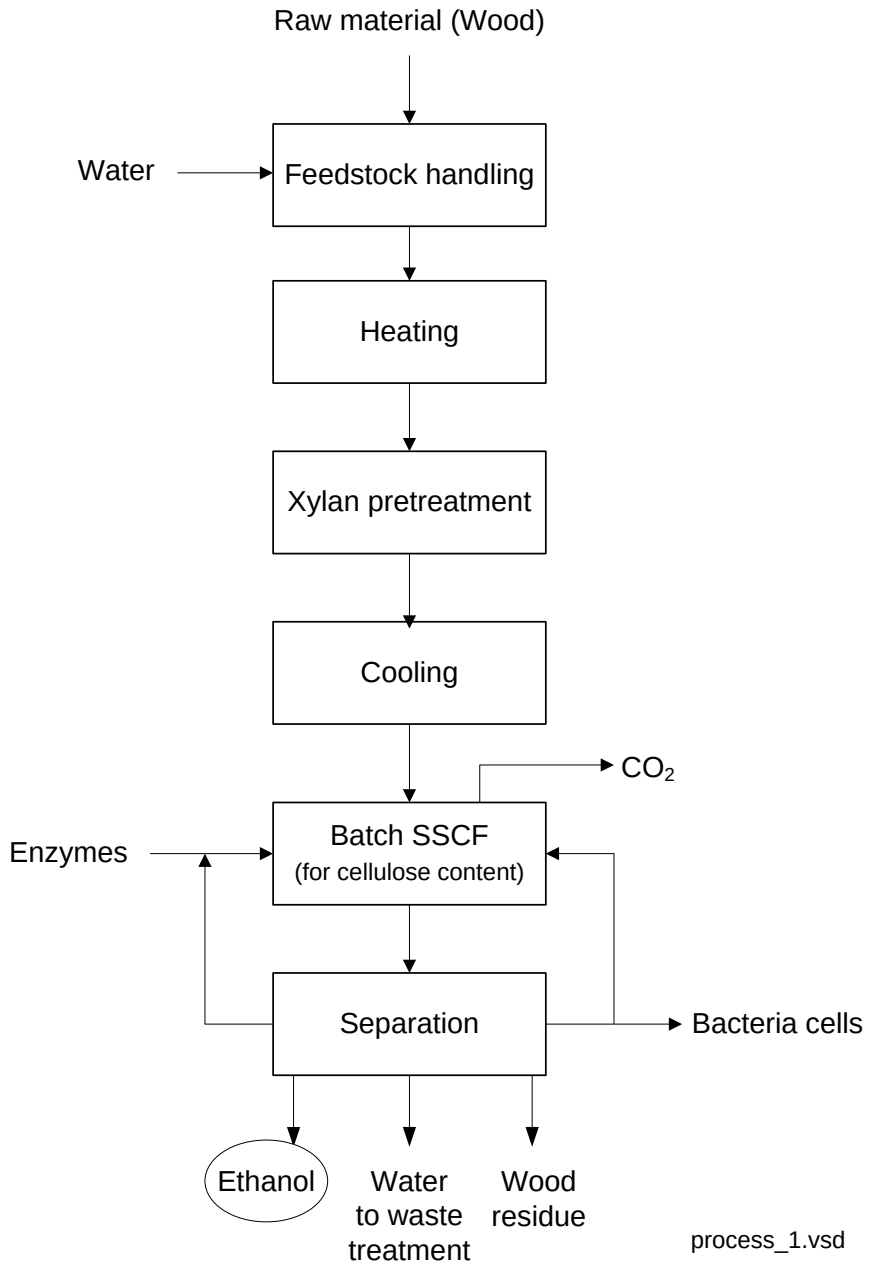


Figure 3.1 *Process with xylan pretreatment. Block flow diagram.*

3.3 Process single step SSCF – Process 2

The main differences from the process described in the previous section is the absence of a pretreatment stage. Indeed, in this case after the feedstock handling the slurry is delivered to batch SSCF reactor. According with Zhang, et al. (2009) the degradation of cellulose and xylan could occurs simultaneously thanks to the same enzymes mentioned in § 3.2, but using *S. Cerevisiae* instead of *E. Coli*.

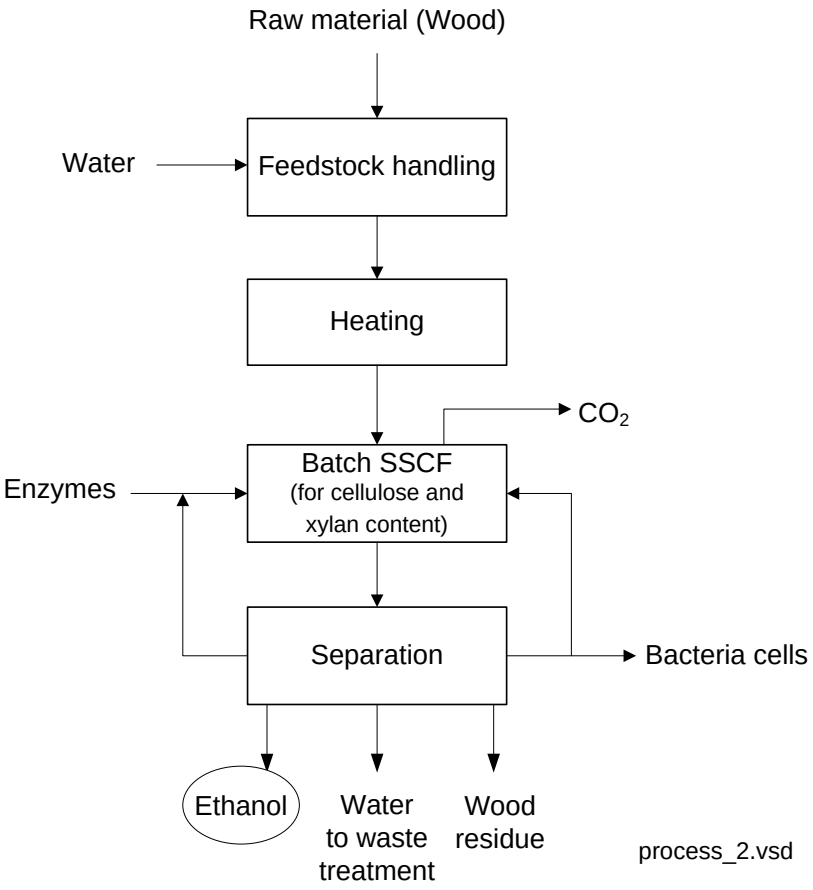


Figure 3.2 *Process single step SSCF. Block flow diagram.*

The process block flow diagram is in Figure 3.2. In the model developed possible mass transfer limitation were studied with respect to *E.Coli* which is able to degrade glucose and xylose, using the enzymes cited above. In the same way also the cooler step is not useful anymore. It should be substituted by a single heating step in which the reaction bulk is heated to about 40 °C.

Finally also the final separation step remain the same.

Chapter 4

Process 1 - Dynamic Mathematic Model

This chapter presents the model developed for process 1, where a thermal pretreatment is made to degrade the xylan content. The work involves two reactions steps: pretreatment and SSCF reactor. The equations of the dynamic mathematical model are reported, and discussed, together with the assumption done. Also the solving method is described.

4.1 Xylan pretreatment

Xylan autohydrolysis occurs in this step. The degradation is very affected by temperature and residence time in the reactor. An isothermal PFR is modeled. The kinetic used to describe these reactions are taken from the work of Nabarlatz, et al.(2004). These authors validate their work with experimental data obtained in a batch reactor system at temperature from 150 °C to 190 °C. Xylan is assumed to be divided in two fractions, xylan 1 (Xy1) and xylan 2 (Xy2), according with the different reactivity, and the amount of each fraction, was determined experimentally for native corncobs. Xy1 is considered more reactive and its fraction (w_{Xy1}) is a model parameter because, it cannot be measured analytically. Furthermore both xylan types are degraded in water soluble xylan oligomers (XO) that react to give the final products xylose (P3), arabinose (ARA) and acetic acid (ACE). During reaction it is assumed that the wood particle has a uniform temperature equal to temperature of dispersant water. In this way the xylan autohydrolysis and XO reactions are uniform in all the particles and in the reaction bulk. This process step changes particle density, porosity and mass fraction of lignin, cellulose and xylan. This affects the next enzymatic reaction step that will be discussed later in this chapter.

4.1.1 Vector of results

To explain the shape and the equations that will be used in the mass balance equations, focusing on the vector of results of dynamic model is important.

Table 4.1 *Result vector of pretreatment reaction model. Meaning of each component.*

x(1)	=	Xylan 1 (Xy1) mass over total initial mass of xylan [-];	**x**(5)	=	Acetic acid (ace) mass in xylan oligomers (XO) over total initial mass of xylan [-];
x(2)	=	Xylan 2 (Xy2) mass over total initial mass of xylan [-];	**x**(6)	=	P3 concentration in reaction bulk [mg/ml];
x(3)	=	Xylose (P3) mass in xylan oligomers (XO) over total initial mass of xylan [-];	**x**(7)	=	ARA concentration in reaction bulk [mg/ml];
x(4)	=	Arabinose (ARA) mass in xylan oligomers (XO) over total initial mass of xylan [-];	**x**(8)	=	ACE concentration in reaction bulk [mg/ml];

The vector **x** contains all internal variables of this model: they are 8 and are listed in Table ***4.1***. These variables appear in all model equations presented in the next sections.

4.1.2 Variables and parameters

Vector **x** variables are time dependent, so to each one a differential equation is associated, which will be described in the next section.

There are also external variables and model parameters. The last ones are kinetic constants and physical properties that are unknown, which must be determined fitting the model with experimental or literature data. In the model developed, 17 parameters were identified. Their value were determined by Nabarlatz, et al. (2004) fitting to experimental data obtained with corncob as biomass. In this work is assumed they hold also for wood. They were stored in a vector called **param$_{pre}$**, shown in Table 4.2.

Table 4.2 *Each component of vector* **param$_{pre}$**. *The meaning of each parameters was be discussed in previous sections. New symbols used are:* k_{i0} *= Arrhenius constant for each kinetic constant* k_i *[1/h];* Ea_i *= Activation energy for each reaction as* k_i *are [J/mol];* x_{Xy1-P3}, $x_{Xy1-ARA}$, $x_{Xy1-ACE}$ *for Xy1 and* x_{Xy2-P3}, $x_{Xy2-ARA}$, $x_{Xy2-ACE}$ *for Xy2 are the fraction of P3, ARA, ACE in each xylan fraction.*

Vector Index	Parameter	Vector Index	Parameter	Vector Index	Parameter	Vector Index	Parameter
1	$k_{1,0}$	6	Ea_1	11	x_{Xy1-P3}	16	$x_{Xy2-ACE}$
2	$k_{2,0}$	7	Ea_2	12	$x_{Xy1-ARA}$	17	w_{Xy1}
3	$k_{3,0}$	8	Ea_3	13	$x_{Xy1-ACE}$		
4	$k_{4,0}$	9	Ea_4	14	x_{Xy2-P3}		
5	$k_{5,0}$	10	Ea_5	15	$x_{Xy2-ARA}$		

Table 4.3 *External variables used in the model. They define reactor and wood state. New declared variables are: T_{pre} = Pretreatment temperature [°C]; ρ_{tot} = Density of slurry in before pretreatment [mg/ml]; m_{wood} = Massive wood flow rate [Kg/h]; d_{PFR} = PFR diameter [cm]; L_{max} = PFR length of one tube [m]; Nt = Number of tube in one PFR; sl = Initial solid loading before pretreatment [g solid/g solution]; w_{Xy} = Xylan mass fraction before pretreatment [-];*

State and Reactor Variables	Wood Properties
T_{pre}	sl
ρ_{tot}	w_{Xy}
m_{wood}	
d_{PFR}	
L_{max}	
Nt	

The so called, external variables, are physical properties of substrates involved in the process, and state variables like temperature or reactor length and diameter. They are listed in Table 4.3: the values of these 8 variables are kept constant during the simulations.

4.1.3 Mass balances

In this section the differential equations representing the reactions in pretreatment process step are listed according with Matlab® script. The reactor is a PFR having Nt tubes each with a length of L [m].

The independent variable in all ODEs is not the time but the time coordinate τ [h]. The equivalence space-time is given by:

$$\tau = \frac{l}{\bar{v}} \quad . \tag{4.1}$$

Where:

$\bar{v}$ = Spatial velocity of slurry in tube reactor[m/h]; it is constant throughout the reactor;

l = Spatial reactor coordinate [m].

The production rates of the substances involved in this kinetic model are:

$$r_{Xy1} = -k_1\,\mathbf{x}(1) \quad , \tag{4.2}$$

$$r_{Xy2} = -k_2\,\mathbf{x}(2) \quad , \tag{4.3}$$

$$r_{P3\,in\,XO} = k_1\,x_{Xy1-\mathrm{P3}}\,\mathbf{x}(1) + k_2\,x_{Xy2-\mathrm{P3}}\,\mathbf{x}(2) - k_3\,\mathbf{x}(3) \quad , \tag{4.4}$$

$$r_{ARA\ in\ XO} = k_1\, x_{Xy1-\mathrm{ARA}}\, \mathbf{x}(1) + k_2\, x_{Xy2-\mathrm{ARA}}\, \mathbf{x}(2) - k_4\, \mathbf{x}(4) \quad , \tag{4.5}$$

$$r_{ACE\ in\ XO} = k_1\, x_{Xy1-\mathrm{ACE}}\, \mathbf{x}(1) + k_2\, x_{Xy2-\mathrm{ACE}}\, \mathbf{x}(2) - k_5\, \mathbf{x}(5) \quad , \tag{4.6}$$

$$r_{P3} = k_3\, \mathbf{x}(3)\, c_{Xy,in} \quad , \tag{4.7}$$

$$r_{ARA} = k_4\, \mathbf{x}(4)\, c_{Xy,in} \quad , \tag{4.8}$$

$$r_{ACE} = k_5\, \mathbf{x}(5)\, c_{Xy,in} \quad . \tag{4.9}$$

Where r_i are the mass production rate respectively of Xy1, Xy2, P3 in XO, ARA in XO, ACE in XO, P3, ARA, ACE. Production rates from (4.2) to (4.6) are expressed in [1/h], instead (4.7), (4.8) and (4.9) are expressed in [mg/ml/h].
k_1, k_2, k_3, k_4, k_5 are kinetic constant [1/h], generally k_i, that are temperature dependent according with the following equation:

$$k_i = k_{i0} \exp\left(\frac{-Ea_i}{R\, T_{pre}}\right) \quad , \tag{4.10}$$

where new symbols used are:

R = Universal gas constant [J/K/mol];
T_{pre} = Pretreatment constant temperature [K];
$c_{Xy,in}$ = Initial xylan concentration in reaction bulk [mg/ml].

Below the model equations are written. First two equations are the mass balances, on Xy1 and Xy2:

$$\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,\tau} = r_{Xy1} \quad , \tag{4.11}$$

$$\frac{\mathrm{d}\,\mathbf{x}(2)}{\mathrm{d}\,\tau} = r_{Xy2} \quad . \tag{4.12}$$

Next are the mass balances of P3, ARA and ACE in the xylan oligomers:

$$\frac{\mathrm{d}\,\mathbf{x}(3)}{\mathrm{d}\,\tau} = r_{P3\ in\ XO} \quad , \tag{4.13}$$

$$\frac{\mathrm{d}\,\mathbf{x}(4)}{\mathrm{d}\,\tau} = r_{ARA\ in\ XO} \quad , \tag{4.14}$$

$$\frac{\mathrm{d}\,\mathbf{x}(5)}{\mathrm{d}\,\tau} = r_{ACE\ in\ XO} \quad , \tag{4.15}$$

Finally the last three balances refer to P3, ARA, ACE respectively in the bulk:

$$\frac{\mathrm{d}\,\mathbf{x}(6)}{\mathrm{d}\,\tau} = r_{P3} \quad , \tag{4.16}$$

$$\frac{\mathrm{d}\,\mathbf{x}(7)}{\mathrm{d}\,\tau} = r_{ARA} \quad , \tag{4.17}$$

$$\frac{\mathrm{d}\,\mathbf{x}(8)}{\mathrm{d}\,\tau} = r_{ACE} \quad , \tag{4.18}$$

4.2 SSCF step

After thermal degradation of xylan the slurry is now cooled down to about 40 °C that is the optimum work temperature of enzyme systems (Zheng, et al., 2009). The enzyme system is fed to the SSCF reactor. The concentration of xylose, arabinose and acetic acid depends on reaction intensity in previous step. Arabinose and acetic acid are assumed not be metabolized by cells, so they in concentrations do not change during fermentation reaction.

4.2.1 Vector of results

This vector contains values of internal variables as results of the differential equations. The 13 components of vector **x** are listed in Table 4.4.

Table 4.4 *Result vector of model. Description of each component.*

x(1)	=	Total particle core volume for all particles [ml];	**x**(8)	=	P2 concentration in the periplasmic space [mg/ml];
x(2)	=	Cellobiose (P1) concentration in the reaction bulk [mg/ml];	**x**(9)	=	P3 (Xylose) concentration in the periplasmic space [mg/ml];
x(3)	=	Glucose (P2) concentration in the reaction bulk [mg/ml];	**x**(10)	=	P4 (Ethanol) concentration in the reaction bulk [mg/ml];
x(4)	=	Total concentration of cellulase complex system (EG/CBH) [mg/ml];	**x**(11)	=	P3 concentration in the reaction bulk [mg/ml];
x(5)	=	Total concentration of β-glucosidase [mg/ml];	**x**(12)	=	Cell concentration in reaction bulk [mg/ml];
x(6)	=	P1 concentration in particle core [mg/ml];	**x**(13)	=	Total periplasmic volume for all cells [ml]
x(7)	=	P2 concentration in the particle core [mg/ml];			

Note that cellobiose **x**(6) and glucose **x**(7) concentration in particle core are referred to the particle core volume. In the same way **x**(8) and **x**(9) are concentrations referred to the particle periplasmic space volume. Differently, cellulose concentration, that derives from **x**(1), is

referred to the total volume of reaction according to reaction rates expression founded on literature (§ 4.2.2.1).

4.2.2 Wood model

The model developed about degradation of wood and specifically in this section for cellulose, is a modified single shrinking core model for a batch reactor, where the diameter of wood particles is constant during the reaction because the lignin is not degraded. Inside the reacting particle we can distinguish two layers:

- the deepest one is a spherical portion; it will be called "core particle" in which composition, density and porosity after pretreatment are constant during all reaction coordinate; note that the core particle porosity is also equal to the initial porosity after pretreatment (4.22);
- the outer one, called "layer 1", and it is made up by lignin and residue xylan content not degraded during the pretreatment.

A sketch is shown in Figure 4.1.

The porosity of these two particle portions is different. The porosities are calculated taking in account the component currently present in each one.

$$\rho_C = \rho_{Xy} = \frac{\rho}{(1-\varepsilon_{in})} \quad , \tag{4.19}$$

After pretreatment the mass fractions of three woods components is modified and they are calculated by

$$w_{C,pre} = \frac{c_{C,in}}{c_{C,in}+c_{Xy,pre}+c_{L,in}} \quad , \tag{4.20}$$

$$w_{L,pre} = \frac{c_{L,in}}{c_{C,in}+c_{Xy,pre}+c_{L,in}} \quad . \tag{4.21}$$

Also the wood particle density is different and it is calculated by:

$$\rho_{pre} = \left(c_{C,in} + c_{Xy,pre} + c_{L,in}\right)\frac{V_{tot}}{V_{par}} \quad . \tag{4.22}$$

From these properties it is possible calculate the porosity of the two layers:

$$\varepsilon_{cor} = \varepsilon_{in} + \frac{\rho_{pre}}{\rho_{Xy}}\frac{c_{Xy,in}-c_{Xy,pre}}{c_{C,in}+c_{Xy,pre}+c_{L,in}} \quad , \tag{4.23}$$

$$\varepsilon_{l1} = \varepsilon_{cor} + \rho_{pre}\frac{w_{C,pre}}{\rho_C} \tag{4.24}$$

Where:

ρ = Initial raw wood particle density [mg/ml];
ρ_{pre} = After pretreatment wood particle density;
ρ_C = Cellulose density [mg/ml];
ρ_{Xy} = Xylan density [mg/ml];
ε_{in} = Initial porosity of raw wood [-];
ε_{cor} = Core porosity of wood particle in SSCF reactor [-];
ε_{l1} = Layer 1 porosity of wood particle in SSCF reactor [-];
$w_{C,pre}$ = After pretreatment mass fraction of cellulose in wood particle [-];
$w_{Xy,pre}$ = After pretreatment mass fraction of xylans in wood particle [-];
$c_{C,in}$ = Initial concentration of cellulose [mg/ml];
$c_{L,in}$ = Initial concentration of lignin [mg/ml];
$c_{Xy,pre}$ = After pretreatment concentration of xylan [mg/ml].

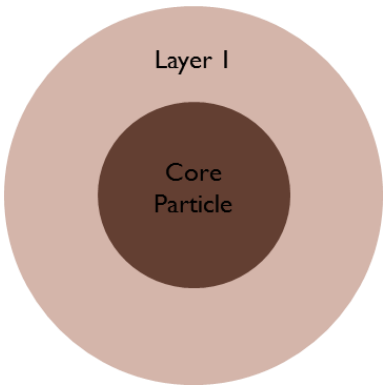


Figure 4.1 *Stylized wood particle with inside layers that are considered by model.*

Another assumption is that the core size depends on the amount of cellulose only. From (4.23) and (4.24) it can be noted that the porosity of core and layer 1 are calculated assuming that the amount of xylan is that residue after pretreatment. In layer 1 also the cellulose amount is zero. As initial condition, it is assumed that layer 1 is absent.
The effective diffusivity D_e for each substance according to the method described in chapter 2 is used. That was done for layer 1 and for substances P1, P2 that diffuse through it. Each D_e value was stored in vector $\mathbf{D_e}(i)$, which index *i* identifies the substances.

4.2.2.1 Cellulose reaction rate model

In this part reactions and assumptions made to model the main component of wood particle (cellulose) are reported. The considerations in this section are based on the work of (Zheng, et al., 2009) which has been modified to be used in a shrinking core system. We took also advantage of an older paper (Wald, et al., 1983) to give a mathematical description of

enzymatic hydrolysis kinetic of cellulose. The reaction involved, (4.25) and (4.27), are heterogeneous reactions. Reaction (4.26) is a homogenous reaction, it involves molecules that are degradation products of cellulose, but not directly cellulose, and it will be discussed in § 4.2.3.

$$\text{Cellulose} + 0.056\ H_2O \xrightarrow{R_3} 1.056\ \text{Cellobiose} \tag{4.25}$$

$$\text{Cellobiose} + 0.111\ H_2O \xrightarrow{R_4} 1.111\ \text{Glucose} \tag{4.26}$$

$$\text{Cellulose} + 0.053\ H_2O \xrightarrow{R_5} 1.053\ \text{Glucose} \tag{4.27}$$

In previous equations, R_3, R_4, R_5 are, respectively, the reactions rates expressed in [mg/ml/h]. The reaction coefficients are mass stoichiometric coefficients. These enzymatic hydrolysis reactions involve two enzymes: cellulase system (EG/CBH) in reactions R_4 and R_5, that will be called E1 and β-glucosidase in reaction R_3, that will be called E2. The reaction rates are respectively expressed from (4.28), (4.29) and (4.30):

$$R_3 = \frac{k_{1r} \times E_{1bC} \times SR}{V_{tot}} \times \frac{\rho \times \mathbf{x}(1) \times w_C}{1 + \frac{\mathbf{x}(6)}{K_{1IG_2}} + \frac{\mathbf{x}(7)}{K_{1IG}}} \quad , \tag{4.28}$$

$$R_4 = k_{3r} \times E_{2f} \times \frac{\mathbf{x}(2)}{K_{3M}\left(1 + \frac{\mathbf{x}(3)}{K_{3IG}}\right) + \mathbf{x}(2)} \quad , \tag{4.29}$$

$$R_5 = \frac{k_{2r} \times E_{1bC} \times SR}{V_{tot}} \times \frac{\rho \times \mathbf{x}(1) \times w_C}{1 + \frac{\mathbf{x}(6)}{K_{2IG_2}} + \frac{\mathbf{x}(7)}{K_{2IG}}} \quad . \tag{4.30}$$

Where new symbols used above are:

k_{ir} = Reaction rate constant [mg/ml/h] (i=3 for (4.25); i=4 for (4.26);
E_{1bC} = Bound concentration of E1 on cellulose content [mg protein/ml];
SR = Substrate reactivity [-];
V_{tot} = Total volume of reaction bath [ml];
K_{iIG_2} = Inhibition constant of cellobiose on enzymes [mg/ml] (i=1,2 and 3 as in R_i);
K_{iIG} = Inhibition constant of glucose on enzymes [mg/ml] (i=1,2 and 3 as in R_i);
E_{2f} = Concentration of free E2 with substrate [mg protein/ml];
K_{3M} = Cellobiose saturation constant [mg/ml].

Reactions where cellulose is involved occur only in the core. Indeed the denominator term in equations (4.28) and (4.30) have the concentration of P1 and P2 in the core, which refer to the total core volume.

In order to represent inhibitions of glucose and cellobiose on cellulose enzyme system, a competitive inhibition pattern was adopted; the reaction rate R_3 and R_5 are first order equations about concentration of cellulose, but they depend also by concentration of E1 adsorbed on cellulose (E_{1bC}); the effect of crystallinity degree of cellulose is neglected.

4.2.2.2 The enzyme adsorption equations

The model of the cellulose hydrolysis considers that enzymes are adsorbed on cellulose according with a Langmuir mechanism. The negative effect of lignin is evaluated to estimate the effective concentration of E1 useful for reaction. In fact lignin is implicated as a competitive adsorbent for E1 reducing its amount available to catalyze cellulose hydrolysis. The Langmuir Isotherm equations for E1 are:

$$\frac{\mathbf{y}(1)}{S} = \frac{E_{1max} \times K_{1ad} \times E_{1f}}{1 + K_{1ad} \times E_{1f}} \quad , \tag{4.31}$$

$$\frac{\mathbf{y}(2)}{L} = \frac{E_{1max} \times K_{1ad} \times E_{1f}}{1 + K_{1ad} \times E_{1f}} \quad . \tag{4.32}$$

The mass balances equations for enzyme are:

$$E_{1bC} = \mathbf{y}(1) - \mathbf{y}(2) \times \lambda \quad , \tag{4.33}$$

$$E_{1f} = \mathbf{x}(4) - \mathbf{y}(1) \quad . \tag{4.34}$$

New symbol are:

$\mathbf{y}(i)$ = Vector of the enzymes adsorption variables [mg protein/ml] (i=1 bounded concentration of E1 on substrate, i=2 bounded concentration of E1 on lignin;

E_{1max} = Maximum mass of E1 that can be adsorbed onto a unit mass of substrate [mg protein/g substrate];

K_{1ad} = Dissociation constant for E1 adsorption/desorption reaction with substrate [ml/mg protein];

E_{1f} = Concentration of free E1 in solution with substrate [mg protein/ml];

S = Substrate (Cellulose and lignin content) concentration at a given time [g/ml];

L = Lignin content concentration [g/ml];

E_{1bC} = Bound concentration of E1 on cellulose content in substrate [mg protein/ml];

λ = ratio of lignin content exposed to enzymes to the total amount of lignin content contained in substrate [-].

It also possible to neglect competitive lignin adsorption. In this case equation (4.32) is not considered by computation.

It is assumed that λ, parameter in (4.33), is equal to 1. It means that the cellulose does not block the adsorption of enzymes on lignin content.
Equations (4.31) and (4.32) are simplified respect to the original absorption model reported in (Zheng, et al., 2009). In fact to simplify the calculation, the same parameter values of E_{1max}, K_{1ad} and E_{1f} are used in both equations. This means to have a content of enzyme bounded with either total substrate (cellulose and lignin content) or lignin that is depending from either substrate or lignin content only.

4.2.3 Bulk model

The density of the aqueous slurry, ρ_{tot}, was assumed to be always 1000 mg/ml like pure water. Its contribution to mass transfer was always neglected, assuming perfet mixing in the bulk. Here, some important reactions occur like homogenous enzymatic reaction (4.26) with reaction rate (4.29). The concentration of enzyme available to catalyze that reaction is calculated by:

$$\frac{\mathbf{y}(3)}{L} = \frac{E_{2maxL} \times K_{2adL} \times E_{2f}}{1 + K_{2adL} \times E_{2f}} \quad , \tag{4.35}$$

$$E_{2f} = \mathbf{x}(5) - \mathbf{y}(3) \quad . \tag{4.36}$$

Where:

$\mathbf{y}(3)$ = Bounded concentration of E2 onto lignin content [mg/ml];
E_{2maxL} = Maximum mass of E2 that can be adsorbed onto a unit mass of lignin [mg protein/g lignin];
K_{2adL} = Dissociation constant for E2 adsorption/desorption reaction with lignin [ml/mg protein];
E_{2f}= Concentration of free E2 in solution with substrate [mg protein/ml];

Note that equation (4.38) is not solved if it is chosen not to consider the lignin's not in productive adsorption. In that case the $E_{2f} = \mathbf{x}(5)$.
The model includes also the degradation of both enzymes, which was hypothesized to be a first order reaction:

$$R_1 = k_{dE1} \times \mathbf{x}(4) \quad , \tag{4.37}$$

$$R_2 = k_{dE2} \times \mathbf{x}(5) \quad . \tag{4.38}$$

Where:

R_1 = degradation rate of E1 [mg/ml/h];

R_2 = degradation rate of E2 [mg/ml/h];
k_{dE1} = reaction constant for R_1 [1/h];
k_{dE2} = reaction constant for R_2 [1/h].

In the results presented, the reaction constants k_{dE1} and k_{dE2} are always set to 1e-8 1/h to make all results independent of enzyme degradation.

4.2.4 Cell model

About cell model the information owned by (Nikaido, et al., 1981) was used. Many assumptions were needed of the reaction volume The data in the cited paper refers to *Escherichia Coli* strain *CM6*, a bacteria of Gram Negative type, whose typical characteristics are reported in § 1.
Have also assumed that single bacteria's cell shape, cell volume and mass transfer properties do not change with processing time. The main idea is to have a cell made up by an outer cell wall, a periplasmic space between cell wall, and a cytoplasmic space in which all reactions occur. According to (Prescott, et al., 1996) the periplasmic space has a volume that is about the 30 % of the whole cell volume. The mass transfer from bulk to periplasmic space occur as described in § 2 through porins of cell wall, the main responsible of metabolites transport inside the cell. The transportation of substances from periplasmic space to cytoplasmic space occur through active transportation carried with specific energy consumer systems on the cytoplasmic membrane. So, according to (Nikaido, et al., 1981) the transfer rate of each compound from periplasmic space to cytoplasmic space is equal to the consumption rate of that metabolite by the cell. These reactions follow Michaelis-Menten kinetic:

$$R_6 = \frac{v_{max,1} \times \mathbf{x}(12) \times \mathbf{x}(8)}{K_{M,1} + \mathbf{x}(8)} \quad , \tag{4.39}$$

$$R_7 = \frac{v_{max,2} \times \mathbf{x}(12) \times \mathbf{x}(9)}{K_{M,2} + \mathbf{x}(9)} \quad . \tag{4.40}$$

Where:

R_i = Cell consumption rates [mg/ml/h] (i = 6 for P2; i = 7 for P3);
$K_{M,i}$= Michaelis-Menten constant [mg/ml] (i as in R_i);

$v_{max,i}$ is the maximum substrate consumption rate, measured in [mg substrate/h/mg cell] (i as in R_i). It is calculated by:

$$v_{max,i} = \frac{cell_{mg,i}}{eta_i} \times 3600 \quad . \tag{4.41}$$

Where:

$cell_{mg,i}$ = Maximum growth rate [1/s] (i as in R_i);

eta_i = Growth yield [g cells/g substrate] (i as in R_i);

It can be noted that the concentrations of substrates in (4.39) and (4.40) are the concentration of each substance in the periplasmic space and are referred to the periplasmic space volume. It was not possible to find all necessary parameters from the literature: $cell_{mg,2}$ was hypothesized equal to $cell_{mg}$ for arabinose found on (Nikaido, et al., 1981), this because that compounds are the same molecular formula; $K_{M,2}$ instead was hypothesized equal to $K_{M,1}$, having no other data.

The number of cells changes during time and the cell concentration has effect on the reaction rate expressed by (4.39) and (4.40). To obtain cell growth rate the following reaction was used:

$$eta_1 \text{ Cellulose} + eta_2 \text{ Xylose} \rightarrow \text{Cell} \tag{4.42}$$

A simple death mechanism was also hypothesized to be expressed by:

$$R_8 = k_{dcell} \times \mathbf{x}(12) \quad , \tag{4.43}$$

where kd_{cell} is well called cell death constant [1/h].
So the total cell production rate, $\mathbf{rr}(8)$, in [mg/ml] is expressed by:

$$\mathbf{rr}(8) = eta_2 \times R_6 + eta_2 \times R_7 - R_8 \quad . \tag{4.44}$$

The model calculates the ethanol (P4) production from glucose and xylose fermentation. No ethanol transport limitation will be considered. In fact it is assumed that the ethanol concentration is homogeneous in all reactor.
The production rate of ethanol, rr_7, is expressed by equation (4.45).

$$rr_7 = eta_3 \times R_6 + eta_4 \times R_7 \tag{4.45}$$

Where eta_3 and eta_4 are the ethanol production yield respectively for glucose and xylose [g ethanol/g substrate].

4.2.5 Mass balances

The SSCF model mass balances are the base for the final solving structure. These balances refer to a batch process in which particle are described through a double shrinking core model.

The object of this work is to represent and predict the mass transfer possible limitations due to internal wood mass transfer and diffusion through bacteria cell walls. All equations including mass transfer term are partial differential equations (PDE) with a simple space variable. Symmetry has been assumed for bath, wood and bacteria.

To represent all reactions in a compact way, they were stored in a vector/matrix system. Vector **R** contains all reaction rates discussed in previous paragraphs:

$$\mathbf{R} = (R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8) \tag{4.46}$$

The stoichiometric coefficients for all substances involved in the system and for all reactions to which those substances participate is called **aa** and component **aa**(*i*, *j*) is referred to substance *i* in the reaction *j*.

Table 4.5 specifies each substance and own index *i*.

Table 4.5 *Substances corresponding with index i in stoichiometric coefficients matrix.*

i	1	2	3	4	5	6	7	8	9
Substance	E1	E2	Cellulose	P1	P2	P3	P4	Cell	Xylan

The matrix **aa** is given by

$$\mathbf{aa} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 \\ 0 & 0 & 1.056 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1.053 & 1.1116 & -1 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & eta_3 & eta_4 & 0 \\ 0 & 0 & 0 & 0 & 0 & eta_1 & eta_2 & -1 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \end{pmatrix} \tag{4.47}$$

$$\mathbf{rr} = \mathbf{aa} \cdot \mathbf{R}^{\mathrm{T}} \tag{4.48}$$

From this array the vector of production rates, **rr**, can be calculated. This vector has as many components as the rows are in matrix **aa**. Each **rr** component is the production rate expressed

in [mg/ml/h] for all component involved in the system as in Table 4.5. It is given by equation (4.48).

4.2.6 Variables and parameters

The components of the vector of results are also called internal variables of model . These variables are time dependent and are associated each one to a differential equation (§ 4.2.1).

Table 4.6 *Each component of vector* **param***. The meaning of each parameters was be discussed in previous sections.*

Vector Index	Parameter	Vector Index	Parameter	Vector Index	Parameter	Vector Index	Parameter
1	k_{1r}	9	K_{3IG}	17	eta_2	25	τ
2	K_{1IG2}	10	K_{1ad}	18	eta_3	26	a_0
3	K_{1IG}	11	K_{2adL}	19	eta_4	27	$pore_{rad}$
4	k_{2r}	12	E_{1max}	20	$cell_{mg,1}$	28	k_{dE1}
5	K_{2IG2}	13	E_{1maxL}	21	$cell_{mg,2}$	29	k_{dE2}
6	K_{2IG}	14	λ	22	$K_{M,1}$	30	k_{dcell}
7	k_{3r}	15	SR	23	$K_{M,2}$		
8	K_{3M}	16	eta_1	24	$wood_{pore}$		

The model contain 30 parameters overall. Their values were retrieved from the literature and were stored in a vector called **param**; see Table 4.6 for details.
The 23 external variables, listed in Table 4.7, are physical properties of substrates involved in process, state variable like temperature or reactor properties like reaction volume. They are always assigned before running the simulation (Except $\mathbf{x}_{pre}$).

Table 4.7 *External variables used in the model. They define reactor state, wood, cell and enzymes. New declared variables are: vf_{ps} = Periplasmic volume fraction of total cell volume [-];w_L = Lignin mass fraction before pretreatment [-]; A_{spec} = Cell external surface over cell mass [-]; thi = Cell wall thickness [cm]; A = Cell outer surface [cm^2]; hr_i = Hydrated radius (i = 1 for P1; i = 2 for P2; i = 3 for P3).*

State and Reactor Variables	Wood Properties	Cell Properties	Enzymes Properties	Bulk properties
T	d_{par}	$c_{cell,in}$	K_{FPU}	$\mathbf{x}_{pre}$
V_{tot}	sl	A_{spec}	K_{CBU}	
ρ_{tot}	ρ	vf_{ps}	FPU	
	w_C	thi	CBU	
	w_{Xy}	A		
	w_L	hr_1		
	ε_{in}	hr_2		
		hr_3		

$\mathbf{x}_{pre}$ is the matrix output of the pretreatment simulated (§ 4.1). Only some pretreatment results of slurry are important as input to the SSCF model (total amount of xylan do not react and xylose concentration in slurry): they are saved in last row of $\mathbf{x}_{pre}$.
For all simulations w_L is calculated as $1 - w_C - w_{Xy}$.
K_{FPU} [FPU/mg enzyme] and K_{CBU} [CBU/mg enzyme] are two variables to convert the E1 and E2 activities, respectively *FPU* [FPU/g-cellulose] and *CBU* [CBU/g-cellulose], into the initial concentration of two enzymes. In the referred paper for the enzymatic kinetic of cellulose degradation the concentrations of these two enzymes system is given in terms of activity: filter paper unit (FPU) for E1 and cellobiose unit (CBU) for E2 (Zheng, et al., 2009). These two ways to measure enzyme concentration depends on different methods to determine that concentration (Ghose, 1987). Indeed the enzymatic activity depends also on the conditions to which the enzyme work (Zheng , et al., 2007). In our case, K_{FPU} and K_{CBU} are calculated from the data reported by (Zheng, et al., 2009).

4.2.7 Balance equations

The model equations are listed below. For the model solution variables the same symbols as in Table 4.4 are used. A first equation allows to calculate the core volume $\mathbf{x}(1)$ ($\sum_{particles} core\ volume$) at any time:

$$\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{rr}(3)\,\frac{V_{tot}}{\rho \times w_C} \quad . \tag{4.49}$$

The next two mass balances refers to P1 and P2 bulk concentration as object ($\mathbf{x}(2)$ and $\mathbf{x}(3)$). These two equations have at least two terms on the right hand side: the first one is the diffusion term in wood layer-1; the second one is the reaction that respectively occur in bulk. Only in equation (4.51) there is a third term describing the flow rate from the bulk to the cells. We remember that only P2 is assumed be metabolized by cells.

$$\frac{\mathrm{d}\,\mathbf{x}(2)}{\mathrm{d}\,t} = \left(\frac{\mathrm{d}\,c_{P1,l1}}{\mathrm{d}\,r}\right)_{r_{par}} \mathbf{D_e}(1)\,\frac{4\pi r_{par}^2}{V_{tot}} N + \mathbf{aa}(4,4) R_4 \quad ; \tag{4.50}$$

$$\frac{\mathrm{d}\,\mathbf{x}(3)}{\mathrm{d}\,t} = \left(\frac{\mathrm{d}\,c_{P2,l1}}{\mathrm{d}\,r}\right)_{r_{par}} \mathbf{D_e}(2)\,\frac{4\pi r_{par}^2}{V_{tot}} N + \mathbf{aa}(5,4) R_4 - \\ + P_2 A_{spec} \mathbf{x}(12)\big(\mathbf{x}(3) - \mathbf{x}(8)\big) \quad . \tag{4.51}$$

Two more balances calculate the concentrations of total enzymes in system respectively for E1 and E2:

$$\frac{\mathrm{d}\,\mathbf{x}(4)}{\mathrm{d}\,t} = \mathbf{rr}(1) \quad , \tag{4.52}$$

$$\frac{\mathrm{d}\,\mathbf{x}(5)}{\mathrm{d}\,t} = \mathbf{rr}(2) \quad . \tag{4.53}$$

These mass balances are used to calculate the concentrations in particles core, respectively for P1 and P2. It is important to note that the balances consider also the volume core variation along with time:

$$\mathbf{x}(1)\frac{\mathrm{d}\,\mathbf{x}(6)}{\mathrm{d}\,t} + \mathbf{x}(6)\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{aa}(4,3)R_3V_{tot} - \left(\frac{\mathrm{d}\,c_{P1,l1}}{\mathrm{d}\,r}\right)_{r_{cor}} \mathbf{D_e}(1)4\pi r_{cor}^2 N \quad , \tag{4.54}$$

$$\mathbf{x}(1)\frac{\mathrm{d}\,\mathbf{x}(7)}{\mathrm{d}\,t} + \mathbf{x}(7)\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{aa}(5,5)R_5V_{tot} - \left(\frac{\mathrm{d}\,c_{P2,l1}}{\mathrm{d}\,r}\right)_{r_{cor}} \mathbf{D_e}(2)4\pi r_{cor}^2 N \quad . \tag{4.55}$$

Next is a balance to calculate the concentration of P2 in the periplasmic space (**x**(8)). It should be noted that the periplasmic volume changes along with time.

$$\mathbf{x}(13)\frac{\mathrm{d}\,\mathbf{x}(8)}{\mathrm{d}\,t} + \mathbf{x}(8)\frac{\mathrm{d}\,\mathbf{x}(13)}{\mathrm{d}\,t} = \left(\mathbf{aa}(5,5)R_5 + P_2A_{spec}\mathbf{x}(12)\big(\mathbf{x}(3) - \mathbf{x}(8)\big)\right)V_{tot} \tag{4.56}$$

A similar balance for P3 in the periplasmic space yelds:

$$\mathbf{x}(13)\frac{\mathrm{d}\,\mathbf{x}(9)}{\mathrm{d}\,t} + \mathbf{x}(9)\frac{\mathrm{d}\,\mathbf{x}(13)}{\mathrm{d}\,t} = \tag{4.57}$$

$$\left(\mathbf{aa}(6,7)R_7 + P_3A_{spec}\mathbf{x}(12)\big(\mathbf{x}(11) - \mathbf{x}(9)\big)\right)V_{tot}$$

The mass balance on P4 refers to the total reaction volume:

$$\frac{\mathrm{d}\,\mathbf{x}(10)}{\mathrm{d}\,t} = \mathbf{rr}(7) \quad . \tag{4.58}$$

The next one is a balance on P3 in the bulk:

$$\frac{\mathrm{d}\,\mathbf{x}(11)}{\mathrm{d}\,t} = -P_3A_{spec}\mathbf{x}(12)\big(\mathbf{x}(11) - \mathbf{x}(9)\big) \quad . \tag{4.59}$$

The cell mass balance in the system is given by:

$$\frac{\mathrm{d}\,\mathbf{x}(12)}{\mathrm{d}\,t} = flg_{cell}\,\mathbf{rr}(8) \quad . \tag{4.60}$$

Eventual, the total periplasmic volume balance is written, which considers all the cells in the system:

$$\frac{\mathrm{d}\,\mathbf{x}(13)}{\mathrm{d}\,t} = flg_{cell}\,\mathbf{rr}(8)\,\frac{V_{tot}}{m_{cell}}\,V_{cell}\,vf_{ps} \quad . \tag{4.61}$$

To solve the resulting differential equation system it is necessary to know the profiles of P1, P2 in layer 1 ($c_{P1,l1}$ and $c_{P2,l1}$) to calculate $\left(\frac{\mathrm{d}\,c_{i,l1}}{\mathrm{d}\,r}\right)_{r_{cor}}$.

The concentrations of P1, **x**(6), and P2, **x**(7) are constant with the core radius. That assumption is quite strong because it means that the heterogeneous reactions occur with same rate throughout the core where the substances produced are well mixed.

The equation to calculate the profile P1 and P2 concentration in layer 1, $c_{i,l1}$, is.

$$\frac{\mathrm{d}\,c_{i,l1}}{\mathrm{d}\,t} = \mathbf{D_e}(i)\,\frac{\mathrm{d}^2\,c_{i,l1}}{\mathrm{d}\,r^2} \qquad r = [\forall r: r_{cor} \le r \le r_{par}] \quad , \tag{4.62}$$

Where in a same equation, *i* is P1 or P2.

Boundary conditions at any *t* for equation (4.62):

$$c_{P1,l1}(r = r_{cor}) = \mathbf{x}(6) \quad , \tag{4.63}$$

$$c_{P2,l1}(r = r_{cor}) = \mathbf{x}(7) \quad , \tag{4.64}$$

$$c_{P1,l1}(r = r_{par}) = \mathbf{x}(2) \quad , \tag{4.65}$$

$$c_{P2,l1}(r = r_{par}) = \mathbf{x}(3) \quad , \tag{4.66}$$

New symbols used in the previous equations are:

r_{cor} = Radius of core particle wood [cm];

r_{l1} = Radius of sphere that is made up core particle wood and layer 1 [cm];

V_{par} = Sum of initial volume for all wood particle in the system;

$c_{E1,in}$ = E1 initial concentration in the system [mg/ml];

$c_{E2,in}$ = E2 initial concentration in the system [mg/ml];

$V_{ps,in}$ = Periplasmic space initial volume [ml];

V_{cell} = Single cell volume [cm^3];

flg_{cell} = Flag that can be 1 or 0, it is 1 simulation consider the cell growth, otherwise it does not;

$c_{P3,pre}$ = concentration of xylose in reaction bulk after pretreatment, taken from $\mathbf{x_{pre}}$ [mg/ml].

Table 4.8 *Initial conditions (IC) for all internal variables in mass balance equations from (4.49) to (4.62).*

Variable	IC	Variable	IC	Variable	IC
$\mathbf{x}(1)$	V_{par}	$\mathbf{x}(6)$	0	$\mathbf{x}(11)$	$c_{P3,pre}$
$\mathbf{x}(2)$	0	$\mathbf{x}(7)$	0	$\mathbf{x}(12)$	$c_{cell,in}$
$\mathbf{x}(3)$	0	$\mathbf{x}(8)$	0	$\mathbf{x}(13)$	$V_{ps,in}$
$\mathbf{x}(4)$	$c_{E1,in}$	$\mathbf{x}(9)$	0	$c_{P1,l1}$	0
$\mathbf{x}(5)$	$c_{E2,in}$	$\mathbf{x}(10)$	0	$c_{P2,l1}$	0

Finally an initial condition is needed for each equation from (4.49) to (4.62), in summary 14 conditions (See Table 4.8). We remember 5 out of the 21 internal variables change also with r.

4.2.8 Solving method

The numerical methods used to solve the model are listed. The first approximation was made about the true concentration profile of P1 and P2 in the wood layers. This assumption avoids two partial differential equations (4.62). Solving a so complicated model is quite useless in the presence of the other model assumption. So a linearization of the profiles concentration in each layer was made. This means making the following approximation:

$$\frac{\mathrm{d}\, c_{P1,l1}}{\mathrm{d}\, r} = \frac{\text{P1 concentration in core} - \text{P1 concentration in bulk}}{r_{par} - r_{cor}} = \frac{\mathbf{x}(6) - \mathbf{x}(2)}{r_{par} - r_{cor}} \quad , \tag{4.67}$$

$$\frac{\mathrm{d}\, c_{P2,l1}}{\mathrm{d}\, r} = \frac{\text{P2 concentration in core} - \text{P2 concentration in bulk}}{r_{par} - r_{cor}} = \frac{\mathbf{x}(7) - \mathbf{x}(3)}{r_{par} - r_{cor}} \quad , \tag{4.68}$$

These two equations can substituted all gradient terms inside equations from (4.49) to (4.61). This makes useless the PDEs in (4.62), as well as their initial and boundary conditions.
In summary the model has 13 internal variables, listed in Table 4.4, 13 ODEs (ordinary differential equations) and 23 external variables, listed in Table 4.7, that have to be known to run the model.

The equation system is implemented on Matlab® 7.10.0 that work with a double precision. The solving function is `ode23t`. Its algorithm is an implementation of the trapezoidal rule using a "free" interpolant (Shampine, et al., 1999). The function have the syntax below:

```
[T,Y] = ode23t(odefun,tspan,y0,options)
```

Whose attribute are:

- `odefun` - A function handle that evaluates the right side of the differential equations. All solvers solve systems of equations in the form `x'=f(t,x)` or problems that involve a mass matrix, `M(t,x)x'= f(t,x)`;
- `tspan` - A vector specifying the interval of integration, `[t0,tf]`. The solver imposes the initial conditions at `tspan(1)`, and integrates from `tspan(1)` to `tspan(end)`. To obtain solutions at specific times (all increasing or all decreasing), use `tspan = [t0,t1,...,tf]`;
- `x0` - A vector of initial conditions;
- `options` - Structure of optional parameters that change the default integration properties. This is the fourth input argument. To create options the `odeset` function can be used.

As it can be noted some model equations have more than one differential term. That is implemented in Matlab using a mass matrix, `M(t,x)x'= f(t,x)`.

The code is reported in Appendixes (Process 1: main functions).

Chapter 5

Process 2 - Dynamic Mathematic Model

This chapter presents the model developed for process 2, where there are no wood pretreatments and some wood handling. Only the SSCF reaction step is modeled. The equations of dynamic mathematical model are written for the case when the reaction hydrolysis rate of cellulose is faster than xylan one.
In case the xylan consumption rate is higher or equal than cellulose consumption rate the equations differ in some points. To know more details about this case refer to Appendixes (Process 2: main functions).

5.1.1 Vector of results

To explain the equations used in the modelling is important focus on the vector of results, reported in Table 5.1.

Table 5.1 *Result vector of model. Meaning of each component.*

x(1)	=	Total particle core volume for all particles [ml];	**x**(10)	=	P3 concentration in core particle and layer 1 [mg/ml];
x(2)	=	Cellobiose (P1) concentration in the reaction bulk [mg/ml];	**x**(11)	=	Total layer 1volume for all particles [ml]
x(3)	=	Glucose (P2) concentration in the reaction bulk [mg/ml];	**x**(12)	=	P2 concentration in the periplasmic space [mg/ml]**;**
x(4)	=	Total concentration of cellulase complex system (EG/CBH) [mg/ml];	**x**(13)	=	P1 concentration in layer 1 [mg/ml];
x(5)	=	Total concentration of β-glucosidase [mg/ml];	**x**(14)	=	P2 concentration in layer 1 [mg/ml];
x(6)	=	P1 concentration in particle core [mg/ml];	**x**(15)	=	P4 concentration in the reaction bulk [mg/ml];
x(7)	=	P2 concentration in the particle core [mg/ml];	**x**(16)	=	P3 concentration in the reaction bulk [mg/ml];
x(8)	=	P2 concentration in the periplasmic space [mg/ml];	**x**(17)	=	Cell concentration in the reactor [mg/ml];
x(9)	=	Xy concentration in reactor [mg/ml];	**x**(18)	=	Total periplasmic space volume for all cells [mg/ml];

The vector of results **x** has 18 components. Specifically **x**(6) and **x**(7) are concentrations referred to the particle core volume. In the same way **x**(13) and **x**(14) are concentrations referred to layer 1 volume, and **x**(8) and **x**(12) are referred to the periplasmic space volume. Differently, **x**(9) and cellulose concentration calculated from **x**(1), are referred to total volume according to reaction rates expressions found in literature (§ 4.2.2.1 and § 5.2.2). Also the total volume of reaction is assumed to be constant along the reactions.

5.2 Wood model

Wood is assumed to be made up of three components: cellulose, hemicellulose and lignin. These three components have a different consumption rates, as from literature. Lignin is not degraded while cellulose and xylan undergo hydrolysis. So the model developed about enzymatic degradation of wood is a modified double shrinking core model for a batch reactor, in which the diameter of wood particles is constant along with the reaction because the lignin does not react. Inside the reacting particle there are layers:

- the deepest one is a spherical portion: the "core particle" in which initial composition, density and porosity are constant during all reaction time; the core particle porosity is also equal to the initial porosity (5.2);
- the medium one called "layer 1" and it has the initial composition of wood particles but without the cellulose that is in the core particle only;
- the outer one is "layer 2", that is made up by lignin only.

A sketch is reported in Figure 5.1. If cellulose reacts slower than xylan, then layer-1 is made up by lignin and cellulose. However the particle core has still raw wood inside. If xylan degradation rate is equal to the cellulose degradation rate, the only layer formed during reaction is made up just by lignin and the core particle has the raw wood composition.
Also the porosity of these three particle shells has to be calculated. It is constant for each layer and is calculated taking in account the component present in each one of them.

$$\rho_C = \rho_{Xy} = \frac{\rho}{(1-\varepsilon_{in})} \quad , \tag{5.1}$$

$$\varepsilon_{cor} = \varepsilon_{in} \quad , \tag{5.2}$$

$$\varepsilon_{l1} = \varepsilon_{cor} + \rho \frac{w_C}{\rho_C} \quad , \tag{5.3}$$

$$\varepsilon_{l2} = \varepsilon_{l1} + \rho \frac{w_{Xy}}{\rho_{Xy}} \quad . \tag{5.4}$$

Where:

ρ = Wood particle density [mg/ml];

ρ_C = Cellulose density [mg/ml];
ρ_{Xy} = Xylan density [mg/ml];
ε_{in} = Initial porosity [-];
ε_{cor} = Core porosity [-];
ε_{l1} = Layer 1 porosity [-];
ε_{l2} = Layer 2 porosity [-];
w_C = Initial mass fraction of cellulose in wood particle [-];
w_{Xy} = Initial mass fraction of xylans in wood particle [-].

It was assumed that the densities of all solid wood particle components are the same. They are estimated by (5.1). Another assumption is that the xylan layer size depends on the amount of xylan only. From equation (5.4) can be noted that porosity of layer 2 is calculated thinking that the amount of xylan is zero. Instead, layer 1 is considered to have a porosity that does not take in account the cellulose amount.

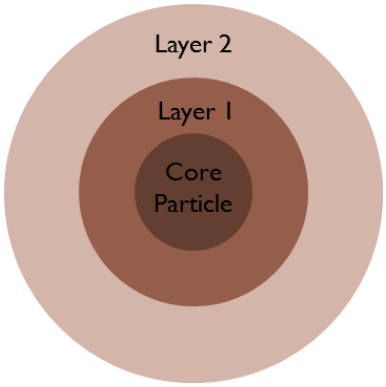


Figure 5.1 *Stylized wood particle with inside layers that are considered by model.*

The different porosity of each layer is useful to calculate the effective diffusivity D_e as described in § 2. This was done for the different layers and for substances P1, P2 and P3. All D_e values are stored in a matrix $\mathbf{D_e}(i,j)$ in which index i identifies the substance and index j identifies the layer.

5.2.1 Cellulose reaction rate model

The considerations about cellulose hydrolysis are similar to those in § 4.2.2.1. The reaction involved are showed below.

$$\text{Cellulose} + 0.056\ H_2O \xrightarrow{R_3} 1.056\ \text{Cellobiose} \qquad (5.5)$$

$$\text{Cellobiose} + 0.111\ H_2O \xrightarrow{R_4} 1.111\ \text{Glucose} \quad (5.6)$$

$$\text{Cellulose} + 0.053\ H_2O \xrightarrow{R_5} 1.053\ \text{Glucose} \quad (5.7)$$

Where R_3, R_4, R_5 are respectively the reactions rate expressed in [mg/ml/h] of equations (4.25), (4.26) and (4.27).
Also the details about enzymes adsorption are those listed in § 4.2.2.2.

5.2.2 Xylan reaction rate model

The reactions equations and assumptions done to describe the hydrolysis of xylans in SSCF reactor are now presented. This section is based on the kinetic reported by (Zhang, et al., 2009). Next considerations involve just the heterogeneous reaction that from xylan brings to xylose (P3). Another hypothesis is that the reactions occur with same enzymes that degrade the cellulose. The reaction is:

$$\text{Xy} \xrightarrow{R_9} 1.136\ \text{Xylose} \quad , \quad (5.8)$$

where R_9 [mg/ml/h] is assumed to be:

$$R_9 = \frac{c_{Xy}}{c_C} \times (R_3 + R_5) \times k_{Xy} \quad , \quad (5.9)$$

In eq. (5.9):

R_3 and R_5 are given by equations (4.28) and (4.30);
c_{Xy}= Concentration of total xylan based on total volume at time *t* [mg/ml];
c_C= Concentration of total cellulose based on total volume at time *t* [mg/ml];
k_{Xy}= Xylan kinetic constant [-].

The original value found in the paper of (Zhang, et al., 2009) for R_9 was $w_{Xy}/w_C \times (R_3 + R_5)$. That term was proposed for a fermentation where a yeast is the biocatalyst and comes from hypothesis verified experimentally for that case, that the conversion of xylan and cellulose is the same at any time. That maybe a strong hypothesis for other degrader method so the second term, k_{Xy}, and c_{Xy}/c_C instead w_{Xy}/w_C were inserted in the kinetic expression. This change is able:

- to consider different case that may be realistic (k_{Xy}). This term makes the xylan volumetric consumption rate faster than the cellulose one if it is bigger than 1. Otherwise cellulose is consumed faster than lignin if k_{Xy} is less than 1. Finally with $k_{Xy} = 1$, core particle correspond with layer 1: layer 2 is now renamed layer 1.

- c_{Xy}/c_C, to consider the time changing of both concentrations. That ratio is constant and equal to w_{Xy}/w_C only if $k_{Xy} = 1$.

5.3 Bulk model

The same considerations presented in § 4.2.3 hold as in this case.

5.4 Cell model

About cell model the information by (Nikaido, et al., 1981) was used. The assumptions made in § 4.2.4 are still valid for this model.

$$R_6 = \frac{v_{max,1} \times \mathbf{x}(17) \times \mathbf{x}(8)}{K_{M,1} + \mathbf{x}(8)} \quad , \tag{5.10}$$

$$R_7 = \frac{v_{max,2} \times \mathbf{x}(17) \times \mathbf{x}(12)}{K_{M,2} + \mathbf{x}(12)} \quad . \tag{5.11}$$

$$R_8 = kd_{cell} \times \mathbf{x}(17) \quad , \tag{5.12}$$

We note that equations, (5.10), (5.11) and (5.12) have different variables inside because the vector of results of this model (§ 5.1.1) is different from the one for the SSFC (§ 4.2.1) previous process considered.

5.5 Mass balances

The model mass balances that are the base for the final solving structure are here reported. These balances are based on a batch process, with solid wood particles described through a double shrinking core model.

The object of this work is to predict the possible mass transfer limitations due to internal wood mass transfer and to bacteria cell walls diffusion. All equations including mass transfer term are partial differential equations (PDE) with only one space variable, as sphere cell symmetry was assumed for all wood particles and bacteria.

As in § 4.2.5 the vector **R** stores all reaction rates discussed in previous paragraphs, for this process:

$$\mathbf{R} = (R_1, R_2, R_3, R_4, R_5, R_6, R_7, R_8, R_9) \tag{5.13}$$

The stoichiometric coefficients matrix, **aa** has components **aa**(*i*, *j*) which refer referred to component *i* in the reaction *j*. Correspondence between index *i* and the substances are contained in Table 4.5.

$$\mathbf{aa} = \begin{pmatrix} -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & -1 & 0 & 0 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & -1 & 0 & -1 & 0 & 0 & 0 & 0 \\ 0 & 0 & 1.056 & -1 & 0 & 0 & 0 & 0 & 0 \\ 0 & 0 & 0 & 1.053 & 1.1116 & -1 & 0 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & -1 & 1.136 & 0 \\ 0 & 0 & 0 & 0 & 0 & eta_3 & eta_4 & 0 & 0 \\ 0 & 0 & 0 & 0 & 0 & 0 & 0 & -1 & 0 \\ 0 & 0 & 0 & 0 & 0 & eta_1 & eta_2 & 0 & -1 \end{pmatrix} \tag{5.14}$$

From those arrays the vector of production rates, **rr**, can be calculated from (5.15).

$$\mathbf{rr} = \mathbf{aa} \cdot \mathbf{R}^{\mathrm{T}} \tag{5.15}$$

rr components are the productions rate expressed in [mg/ml/h] for all components involved in the system.

5.5.1 Variables and parameters

In the model developed 31 parameters were be identified. Their values were retrieved from the literature and stored in a vector called **param**. Each component is associated to a parameter like Table 4.6 shows; another parameter, kd_{cell}, is added and is used in xylan degradation reaction rate (See § 5.2.2).
The so named, external variables are instead stored in Table 4.7.

Table 5.2 *External variables used in the model. They define reactor state, wood, cell and enzymes.*

State and Reactor Variables	Wood Properties	Cell Properties	Enzymes Properties
T	d_{par}	$c_{cell,in}$	K_{FPU}
V_{tot}	sl	A_{spec}	K_{CBU}
ρ_{tot}	ρ	vf_{ps}	FPU
	w_C	thi	CBU
	w_{Xy}	A	
	ε_{in}	hr_1	
		hr_2	
		hr_3	

There are 21 variables always constant during the simulation with model developed.

5.5.2 Balance equations

The rigorous equations of the model are listed below. Only equations that differ from those in § 4.2.7 will be commented. The others will be just listed below. For the model solution variables the same symbols as in Table 5.1 will be used.

$$\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{rr}(3)\frac{V_{tot}}{\rho \times w_C} \quad , \tag{5.16}$$

$$\frac{\mathrm{d}\,\mathbf{x}(2)}{\mathrm{d}\,t} = \left(\frac{\mathrm{d}\,c_{P1,l2}}{\mathrm{d}\,r}\right)_{r_{par}} \mathbf{D_e}(1,2)\frac{4\pi r_{par}^2}{V_{tot}}N + \mathbf{aa}(4,4)R_4 \quad , \tag{5.17}$$

$$\frac{\mathrm{d}\,\mathbf{x}(3)}{\mathrm{d}\,t} = \left(\frac{\mathrm{d}\,c_{P2,l2}}{\mathrm{d}\,r}\right)_{r_{par}} \mathbf{D_e}(2,2)\frac{4\pi r_{par}^2}{V_{tot}}N + \mathbf{aa}(5,4)R_4 - \\ + P_2 A_{spec}\mathbf{x}(17)\big(\mathbf{x}(3) - \mathbf{x}(8)\big) \quad , \tag{5.18}$$

$$\frac{\mathrm{d}\,\mathbf{x}(4)}{\mathrm{d}\,t} = \mathbf{rr}(1) \quad , \tag{5.19}$$

$$\frac{\mathrm{d}\,\mathbf{x}(5)}{\mathrm{d}\,t} = \mathbf{rr}(2) \quad , \tag{5.20}$$

$$\mathbf{x}(1)\frac{\mathrm{d}\,\mathbf{x}(6)}{\mathrm{d}\,t} + \mathbf{x}(6)\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{aa}(4,3)R_3 V_{tot} - \left(\frac{\mathrm{d}\,c_{P1,l1}}{\mathrm{d}\,r}\right)_{r_{cor}} \mathbf{D_e}(1,1)4\pi r_{cor}^2 N \quad , \tag{5.21}$$

$$\mathbf{x}(1)\frac{\mathrm{d}\,\mathbf{x}(7)}{\mathrm{d}\,t} + \mathbf{x}(7)\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} = \mathbf{aa}(5,5)R_5 V_{tot} - \left(\frac{\mathrm{d}\,c_{P2,l1}}{\mathrm{d}\,r}\right)_{r_{cor}} \mathbf{D_e}(1,2)4\pi r_{cor}^2 N \quad , \tag{5.22}$$

$$\mathbf{x}(18)\frac{\mathrm{d}\,\mathbf{x}(8)}{\mathrm{d}\,t} + \mathbf{x}(8)\frac{\mathrm{d}\,\mathbf{x}(18)}{\mathrm{d}\,t} = \Big(\mathbf{aa}(5,5)R_5 + P_2 A_{spec}\mathbf{x}(17)\big(\mathbf{x}(3) - \mathbf{x}(8)\big)\Big)V_{tot} \tag{5.23}$$

Mass balance of xylan in the reaction system. **x**(9) is the concentration of xylan in total reaction volume.

$$\frac{\mathrm{d}\,\mathbf{x}(9)}{\mathrm{d}\,t} = \mathbf{rr}(9) \quad . \tag{5.24}$$

Next balance is made on total mass amount of P3 a in sphere that includes particle core and layer-1. The left and side of equation considers the core and layer 1 variations along time. P3 concentration in above said volume, **x**(10), is given by:

$$(\mathbf{x}(11) + \mathbf{x}(1))\frac{\mathrm{d}\,\mathbf{x}(10)}{\mathrm{d}\,t} + \mathbf{x}(10)\left(\frac{\mathrm{d}\,\mathbf{x}(1)}{\mathrm{d}\,t} + \frac{\mathrm{d}\,\mathbf{x}(11)}{\mathrm{d}\,t}\right) = \mathbf{aa}(6,8)R_9 V_{tot} - \\ \left(\frac{\mathrm{d}\,c_{P3,l2}}{\mathrm{d}\,r}\right)_{r_{l1}} \mathbf{D_e}(3,2)4\pi r_{par}^2 N \quad . \tag{5.25}$$

Another balance is needed to know the total layer-1 volume **x**(11) at any time ($\sum_{particles} layer\ 1\ volume$):

$$\frac{\mathrm{d}\,\mathbf{x}(11)}{\mathrm{d}\,t} = \mathbf{rr}(8)\frac{V_{tot}}{\rho \times w_{Xy}} - \mathbf{rr}(3)\frac{V_{tot}}{\rho \times w_C} \quad , \tag{5.26}$$

and the concentration of P3 in the periplasmic space:

$$\mathbf{x}(18)\frac{\mathrm{d}\,\mathbf{x}(12)}{\mathrm{d}\,t} + \mathbf{x}(12)\frac{\mathrm{d}\,\mathbf{x}(18)}{\mathrm{d}\,t} = \tag{5.27}$$

$$\Big(\mathbf{aa}(6,7)R_7 + P_3 A_{spec}\mathbf{x}(17)\big(\mathbf{x}(16) - \mathbf{x}(12)\big)\Big)V_{tot} \quad .$$

Mass balance of P4 in total reaction volume is:

$$\frac{\mathrm{d}\,\mathbf{x}(15)}{\mathrm{d}\,t} = \mathbf{rr}(7) \quad . \tag{5.28}$$

The next one is a balance on P3 in the reaction bulk. Differently from equation (4.59) that have the same purpose in § 4.2.7 in this case also the flow term of mass transfer from wood particle to bulk is accounted for (It is the first term on the right side of equation below):

$$\frac{\mathrm{d}\,\mathbf{x}(16)}{\mathrm{d}\,t} = \left(\frac{\mathrm{d}\,c_{P3,l2}}{\mathrm{d}\,r}\right)_{r_{par}} \mathbf{D_e}(3,2)\frac{4\pi r_{par}^2}{V_{tot}}N - P_3 A_{spec}\mathbf{x}(17)\big(\mathbf{x}(16) - \mathbf{x}(12)\big) \quad . \tag{5.29}$$

Cell mass balance and total periplasmic volume balance in the system are respectively:

$$\frac{\mathrm{d}\,\mathbf{x}(17)}{\mathrm{d}\,t} = flg_{cell}\,\mathbf{rr}(8) \quad , \tag{5.30}$$

$$\frac{\mathrm{d}\,\mathbf{x}(18)}{\mathrm{d}\,t} = flg_{cell}\,\mathbf{rr}(8)\frac{V_{tot}}{m_{cell}}V_{cell}\,vf_{ps} \quad . \tag{5.31}$$

To solve the differential equation system above it is needed to know the profiles of P1, P2, P3 in the two wood particle layers that are formed during reaction. The concentrations of P1, **x**(6), and P2, **x**(7), are uniform in all core particle radius and it is as well for P3 up r_{l1}, **x**(10). That assumption is quite strong because it means that the heterogeneous reactions occur with same rate throughout the sections where the substances produced are well mixed.
The equations below calculate the profile of P1 and P2 concentration in layer 1, $c_{i,l1}$, and in layer 2, $c_{i,l2}$, that are generated by diffusion resistances.

$$\frac{\mathrm{d}\,c_{i,l1}}{\mathrm{d}\,t} = \mathbf{D_e}(i,1)\frac{\mathrm{d}^2\,c_{i,l1}}{\mathrm{d}\,r^2} \quad r = [\forall r: r_{cor} \le r \le r_{l1}] \quad , \tag{5.32}$$

$$\frac{\mathrm{d}\, c_{i,l2}}{\mathrm{d}\, t} = \mathbf{D_e}(i,2)\frac{\mathrm{d}^2\, c_{i,l2}}{\mathrm{d}\, r^2} \quad r = [\forall r: r_{l1} < r \leq r_{par}] \quad . \tag{5.33}$$

Where in a same equation, *i* can be P1 or P2.
Boundary conditions at any *t* are needed to solve equations (5.32) and (5.33):

$$c_{P1,l1}(r = r_{cor}) = \mathbf{x}(6) \quad , \tag{5.34}$$

$$c_{P2,l1}(r = r_{cor}) = \mathbf{x}(7) \quad , \tag{5.35}$$

$$c_{P1,l2}(r = r_{par}) = \mathbf{x}(2) \quad , \tag{5.36}$$

$$c_{P2,l2}(r = r_{par}) = \mathbf{x}(3) \quad , \tag{5.37}$$

$$c_{i,l1}(r = r_{l1}) = \; c_{i,l2}(r = r_{l1} + \mathrm{d}\, r) \quad , \tag{5.38}$$

$$\mathbf{D_e}(i,1)\left(\frac{\mathrm{d}\, c_{i,l1}}{\mathrm{d}\, r}\right)_{r_{l1}} = \mathbf{D_e}(i,2)\left(\frac{\mathrm{d}\, c_{i,l2}}{\mathrm{d}\, r}\right)_{r_{l1}+\mathrm{d}\, r} \quad . \tag{5.39}$$

For P3, that diffuses only in layer 2, the equation is:

$$\frac{\mathrm{d}\, c_{P3,l2}}{\mathrm{d}\, t} = \mathbf{D_e}(2,3)\frac{\mathrm{d}^2\, c_{P3,l2}}{\mathrm{d}\, r^2} \quad r = [\forall r: r_{l1} \leq r \leq r_{par}] \quad , \tag{5.40}$$

and the boundary condition for that are:

$$c_{P3,l2}(r = r_{par}) = \mathbf{x}(16) \quad , \tag{5.41}$$

$$c_{P3,l2}(r = r_{l1}) = \mathbf{x}(10) \quad . \tag{5.42}$$

Finally initial conditions are needed for each equation from (5.16) to (5.33) and for equation (5.40). They are 21 equations with 21 internal variables that change during time (5 of them change also with *r*). In Table 5.3, the 21 initial conditions are listed.

Table 5.3 *Initial conditions (IC) for all internal variables in mass balance equations from (5.16) to (5.33) and for (5.40).*

Variable	IC	Variable	IC	Variable	IC
$\mathbf{x}(1)$	V_{par}	$\mathbf{x}(8)$	0	$\mathbf{x}(17)$	$c_{cell,in}$
$\mathbf{x}(2)$	0	$\mathbf{x}(9)$	$c_{Xy,in}$	$\mathbf{x}(18)$	$V_{ps,in}$
$\mathbf{x}(3)$	0	$\mathbf{x}(10)$	0	$c_{P1,l1}$	0
$\mathbf{x}(4)$	$c_{E1,in}$	$\mathbf{x}(11)$	V_{par}	$c_{P1,l2}$	0
$\mathbf{x}(5)$	$c_{E2,in}$	$\mathbf{x}(12)$	0	$c_{P2,l1}$	0
$\mathbf{x}(6)$	0	$\mathbf{x}(15)$	0	$c_{P2,l2}$	0
$\mathbf{x}(7)$	0	$\mathbf{x}(16)$	0	$c_{P3,l2}$	0

5.6 Solving method

About the numerical methods used to solve the model, a first approximation was made about the true concentrations profile of P1, P2, P3 in the wood layers. This allows to avoid to solve (5.30), (5.31) and (5.40), that are 5 partial differential equations. A linearization of the profiles concentration in each layer was made:

$$\frac{\mathrm{d}\, c_{P1,l1}}{\mathrm{d}\, r} = \frac{\text{P1 concentration in core} - \text{P1 concentration in layer 1}}{r_{l1} - r_{cor}} = \frac{\mathbf{x}(6) - \mathbf{x}(13)}{r_{l1} - r_{cor}} \quad , \tag{5.43}$$

$$\frac{\mathrm{d}\, c_{P2,l1}}{\mathrm{d}\, r} = \frac{\text{P2 concentration in core} - \text{P2 concentration in layer 1}}{r_{l1} - r_{cor}} = \frac{\mathbf{x}(7) - \mathbf{x}(14)}{r_{l1} - r_{cor}} \quad , \tag{5.44}$$

$$\frac{\mathrm{d}\, c_{P1,l2}}{\mathrm{d}\, r} = \frac{\text{P1 concentration in layer 1} - \text{P2 concentration in layer 2}}{r_{par} - r_{l1}} = \frac{\mathbf{x}(13) - c_{P1,l2}}{r_{par} - r_{l1}} \quad , \tag{5.45}$$

$$\frac{\mathrm{d}\, c_{P2,l2}}{\mathrm{d}\, r} = \frac{\text{P2 concentration in layer 1} - \text{P2 concentration in layer 2}}{r_{par} - r_{l1}} = \frac{\mathbf{x}(14) - c_{P2,l2}}{r_{par} - r_{l1}} \quad , \tag{5.46}$$

$$\frac{\mathrm{d}\, c_{P3,l2}}{\mathrm{d}\, r} = \frac{\text{P3 concentration in layer 1} - \text{P3 concentration in layer 2}}{r_{par} - r_{l1}} = \frac{\mathbf{x}(10) - c_{P3,l2}}{r_{par} - r_{l1}} \quad . \tag{5.47}$$

These five equations can substitute all gradient terms inside equations from (5.16) to (5.31), making the PDEs (5.32), (5.33) and (5.40) useless and so in the same way also their initial and boundary conditions, as well. Anyway this method needs to know the concentrations of transferring substances in layer 1($\mathbf{x}(13)$ and $\mathbf{x}(14)$), and in layer 2 ($c_{P1,l2}$, $c_{P2,l2}$ and $c_{P3,l2}$). These variables are calculated when also the following equations are added to the model.

$$\mathbf{x}(11)\frac{\mathrm{d}\,\mathbf{x}(13)}{\mathrm{d}\, t} + \mathbf{x}(13)\frac{\mathrm{d}\,\mathbf{x}(11)}{\mathrm{d}\, t} = \left(\left(\frac{\mathbf{x}(6) - \mathbf{x}(13)}{r_{l1} - r_{cor}}\right)\mathbf{D_e}(1,1)4\pi r_{cor}^2 - \left(\frac{\mathbf{x}(13) - \mathbf{x}(2)}{r_{par} - r_{l1}}\right)\mathbf{D_e}(1,2)4\pi r_{l1}^2\right)N \quad , \tag{5.48}$$

$$\mathbf{x}(11)\frac{\mathrm{d}\,\mathbf{x}(14)}{\mathrm{d}\, t} + \mathbf{x}(14)\frac{\mathrm{d}\,\mathbf{x}(11)}{\mathrm{d}\, t} = \left(\left(\frac{\mathbf{x}(7) - \mathbf{x}(14)}{r_{l1} - r_{cor}}\right)\mathbf{D_e}(2,1)4\pi r_{cor}^2 - \left(\frac{\mathbf{x}(14) - \mathbf{x}(3)}{r_{par} - r_{l1}}\right)\mathbf{D_e}(2,2)4\pi r_{l1}^2\right)N \quad , \tag{5.49}$$

$$c_{P1,l2} = \mathbf{x}(2) \quad , \tag{5.50}$$

$$c_{P2,l2} = \mathbf{x}(3) \quad , \tag{5.51}$$

$$c_{P3,l2} = \mathbf{x}(16) \quad . \tag{5.52}$$

Equations (5.48) and (5.49) allows to determine the concentrations $\mathbf{x}(13)$ and $\mathbf{x}(14)$. With the last equalities the (5.45), (5.46) and (5.47) become respectively:

$$\frac{\mathbf{x}(13)-\mathbf{x}(2)}{r_{par}-r_{l1}} \quad , \quad \frac{\mathbf{x}(14)-\mathbf{x}(3)}{r_{par}-r_{l1}} \quad , \quad \frac{\mathbf{x}(10)-\mathbf{x}(16)}{r_{par}-r_{l1}} \quad .$$

Summarizing, the model finally have 18 internal variables overall, listed in Table 5.1, 18 ODEs and 21 external variables, listed in Table 5.2, that have to be fixed to get the model working.

This equation system is implemented on Matlab® 7.10.0 and the solving function is `ode23t` as described in § 4.2.8.

The whole code is reported in Appendixes (Process 2: main functions).

Chapter 6

Results

In this chapter the results of simulation for process 1 and process 2, described in previous chapters, will be discussed and confronted with literature data, when available. Importance will be given to possible limitations due to mass transfer of molecules involved in the processes. At the end a sensitivity analysis will be presented with respect to the parameters of SSCF model in the case of process 2 (single reaction step).

6.1 Preliminary simulation

First of all the kinetic of cellulose degradation kinetic used in this work was compared with the experimental data by Zheng, et al. (2009). Degradation of other wood components are neglected and no glucose cell consumption is accounted for. As some modification to the original kinetic model was made, there are some differences between experimental data and calculated values. These modifications are due to some untestable kinetic symbols used in the paper for enzymes adsorption kinetic in the work. The results are plotted in Figure 6.1 and the conditions of both simulation and experimental data are listed in Table 6.1.

Table 6.1 *External variables fixed for simulations of original kinetic for cellulose degradation found on (Zheng, et al., 2009).*

External Variables	Value
ρ_{tot}	1000 mg/ml
sl	0.08
w_C	0.53
w_L	0.38
K_{FPU}	1.666
K_{CBU}	7.5
FPU	150 FPU/g
CBU	150 CBU/g

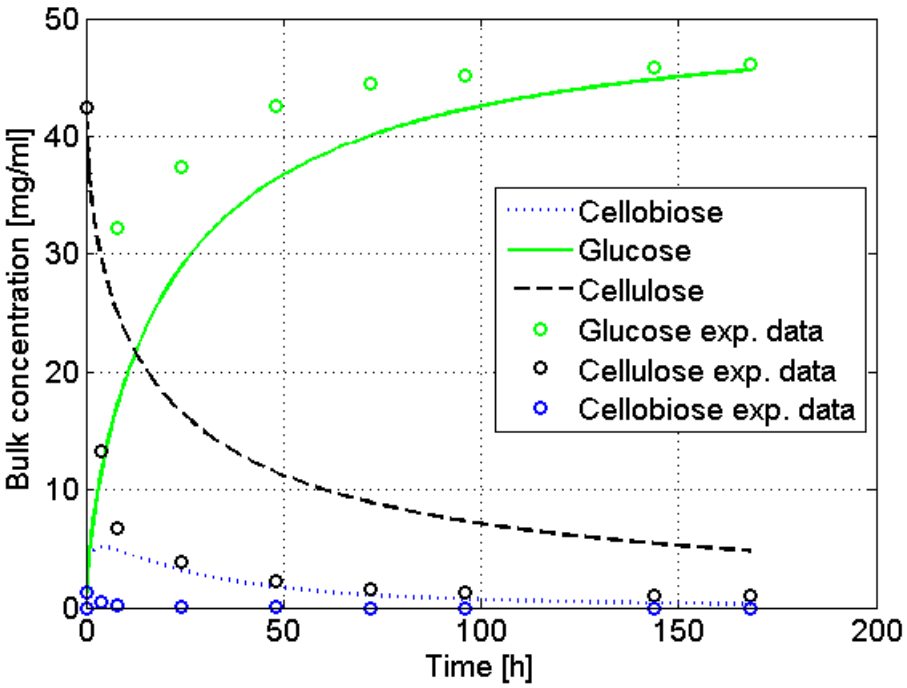


Figure 6.1 *Bulk concentrations during reaction time. According with curve color the points 'o' are the experimental values of those concentrations.*

6.2 Process 1 simulations

We discuss now about the thermal degradation of xylan in pretreatment step of process 1 with two reaction steps (§ 3.1.1). Of course the role of temperature is very important to drive the conversion of xylan in this step.

Choosing a target conversion (X*) of 95%, Figure 6.2 shows the length of reactor tubes necessary to achieve it at different temperatures. Details between 200 °C and 230 °C of Figure 6.2 are shown in Figure 6.3. It can be noted that the length of reactor tubes to reach the conversion of 95% at 200 °C or more is two orders of magnitude less than the pretreatment reactor operating around 170 °C.

The values assigned to external variables to obtain the previous figures are resumed in Table 6.2. Another variable fixed is the xylan conversion at the reactor outlet. So the model has two freedom degrees, T_{pre} and L_{max}. The parameters about the pretreatment are listed in Table 6.3. They are used for all simulations presented in this paragraph.

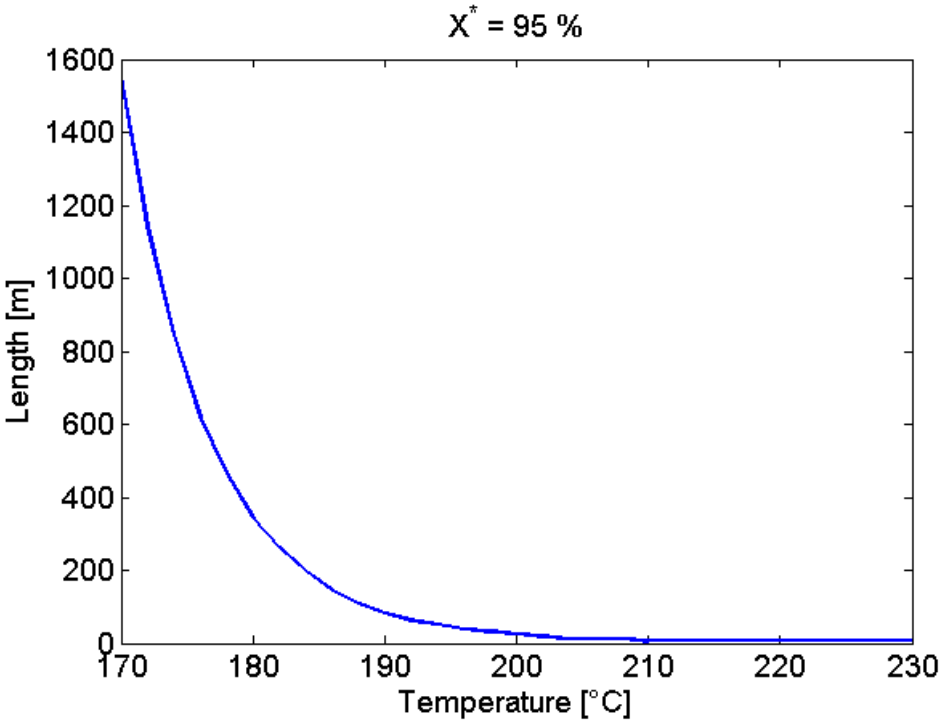


Figure 6.2 *Length necessary to reach the star conversion of 95% at different reactor work temperature.*

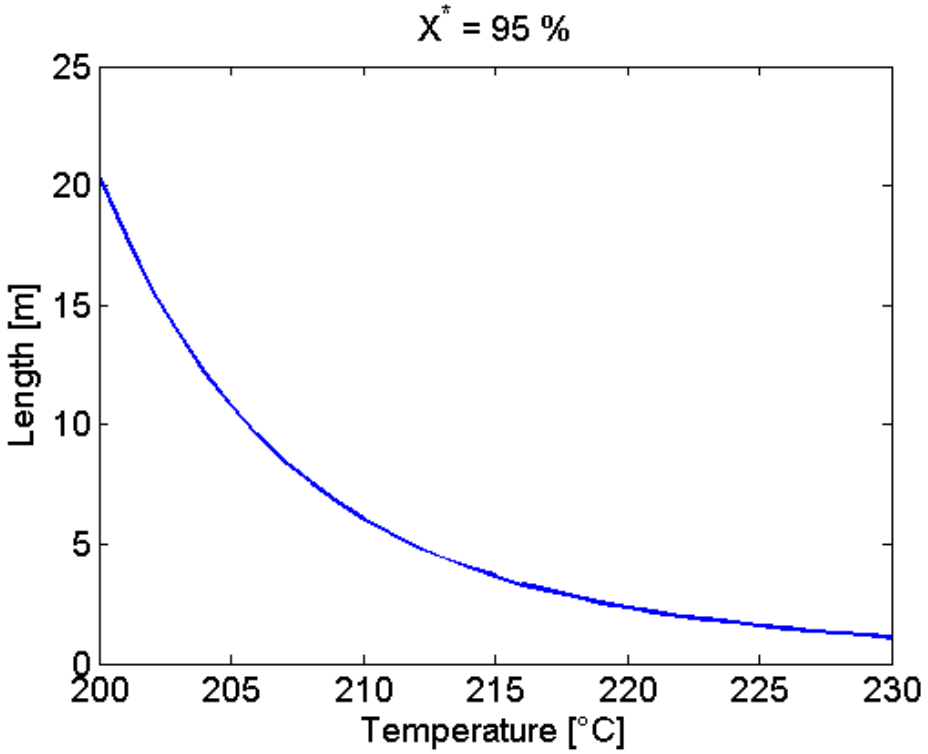


Figure 6.3 *Details of Figure 6.2 for temperature in the reactor between 200 and 230 °C.*

Table 6.2 *Values of variables to obtain data reported in Figure 6.2 and Figure 6.3.*

T_{pre}	ρ_{tot}	m_{wood}	d_{PFR}	L_{max}	Nt	sl	w_{Xy}
free	1000	1000	5	free	100	0.08	0.19
	mg/ml	Kg/h	cm				

Table 6.3 *Parameters values for the simulation of the pretreatment step.*

$k_{1,0}$	$k_{2,0}$	$k_{3,0}$	$k_{4,0}$	$k_{5,0}$	Ea_1	Ea_2	Ea_3	Ea_4
31.52	61.41	27.55	25.08	14.18	127.3	251.7	119.0	106.2
h^{-1}	h^{-1}	h^{-1}	h^{-1}	h^{-1}	KJ/mol	KJ/mol	KJ/mol	KJ/mol
Ea_5	x_{Xy1-P3}	$x_{Xy1-ARA}$	$x_{Xy1-ACE}$	x_{Xy2-P3}	$x_{Xy2-ARA}$	$x_{Xy2-ACE}$	w_{Xy1}	
65.1	0.779	0.101	0.120	0.895	0.044	0.061	0.8	
KJ/mol	-	-	-	-	-	-	-	

Figure 6.4 shows the different concentrations trend of xylose (P3), main product of xylan hydrolysis, in the bulk of the PFR. Its concentration and residence time depend on the position in the reactor. Four curves are shown in Figure 6.4 according with different reactor working temperatures (expressed in °C). Of course at the highest temperature the maximum concentration permitted is reached before in the reactor. Figure 6.5 shows the xylan conversion inside the tube reactor and those curves show similar information to Figure 6.4. The light blue line in both figures corresponds to the simulation at the highest temperature. The maximum conversion is achieved for a length much less than 10 m, whereas the maximum concentration of xylose (P3) is achieved only at more than 10 m. This happens because xylan undergoes just the degradation into xylan oligomers, introducing a delay in P3 production that can be noted at all temperature curves.

For Figure 6.4 and Figure 6.5 the same fixed external variable have been used as in Table 6.2. Other data for these simulations are length (L_{max}) and temperature of reactor (T_{pre}).

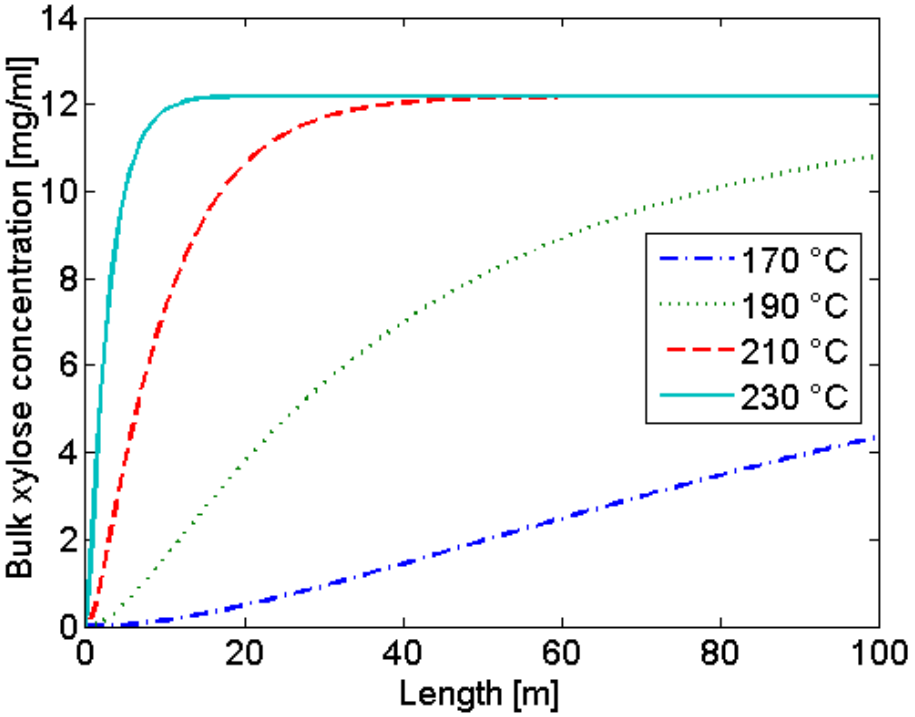


Figure 6.4 *Bulk xylose concentration in reactor at different tube length. Each curve is referred to a different temperature as shown in the legend inside the figure. Temperatures are in °C.*

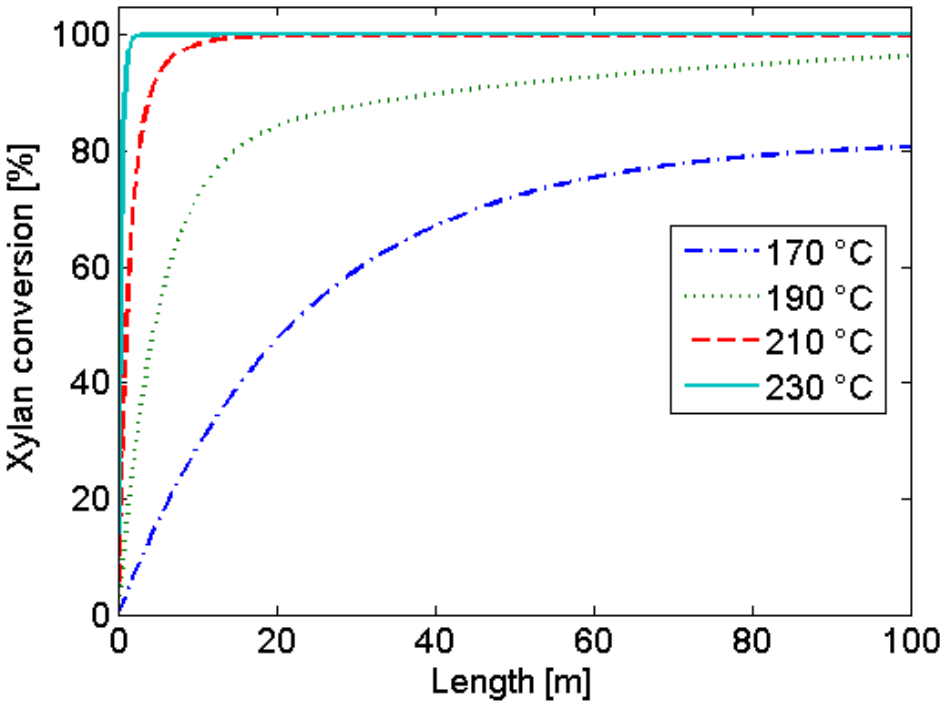


Figure 6.5 *Xylan conversion in the reactor at different tube length. Each curve is referred to a different temperature as shown in legend inside the figure. The temperatures are °C.*

Figure 6.6 shows the exit conversion of xylan in a reactor 100 m long. Clearly, at temperature larger than 200°C it not useful to work with a longer reactor because the conversion is already 100 %. Otherwise the conversion of xylan will not be complete.

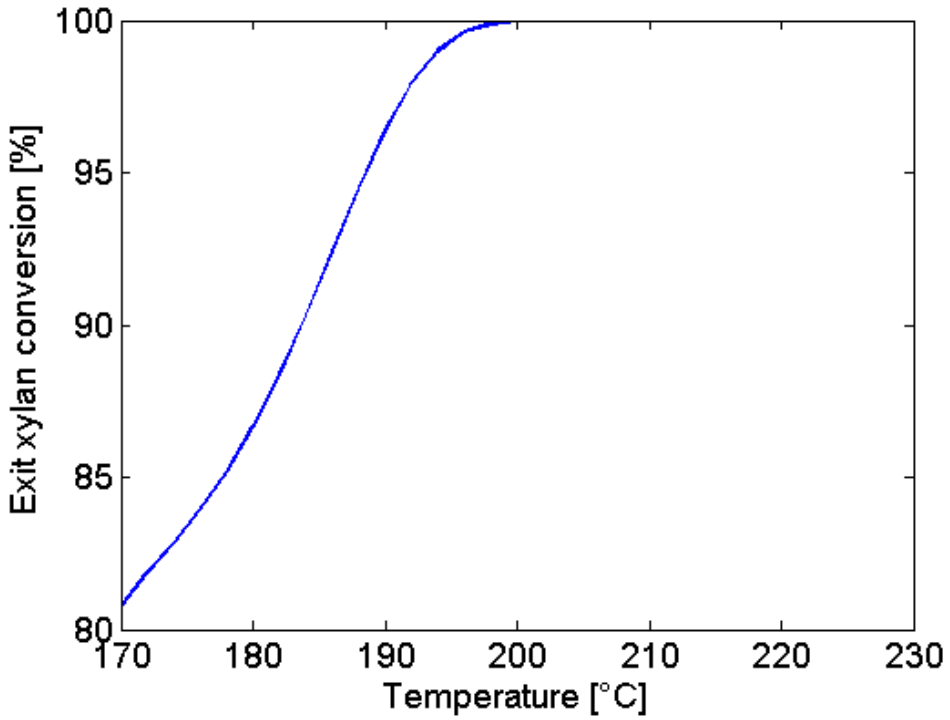


Figure 6.6 *Xylan conversion on reactor exit versus working temperature of reactor. The length of reactor used is 100 m.*

The scripts used to obtain these previous pictures are reported in Appendixes (Pretreatment).

Process 1 is now considered with both reaction steps and the results of simulation of a base case are shown below. The variables set for this base case are listed in Table 6.4 for the pretreatment and in Table 6.5 for SSCF step. The parameters used for simulation of the SSFC step are listed in Table 6.6.

Table 6.4 *External variables values in the pretreatment step for the base simulations of process 1*

T_{pre}	ρ_{tot}	m_{wood}	d_{PFR}	L_{max}	Nt	sl	w_{Xy}
170	1000	1000	5	100	100	0.08	0.19
°C	mg/ml	Kg/h	cm	m	-	-	-

Table 6.5 *External variables fixed for SSCF step for case base simulation of process 1*

T	V_{tot}	d_{par}	ρ	w_C	w_L	ε_{in}	$c_{cell,in}$	A_{spec}	vf_{ps}
40	125e3	0.1	726	0.53	1-w_C-w_{Xy}	0.03	0.1	131	0.3
°C	ml	cm	mg/ml	-		-	mg/ml	cm²/mg	-
thi	A	hr_1	hr_2	hr_3	K_{FPU}	K_{CBU}	FPU	CBU	
8e-7	3e-8	NaN	0.42	0.38	1.666	7.5	150	150	
cm	cm²/cell		nm	nm	FPU/mg	CBU/mg	FPU/g	FPU/g	

Table 6.6 *Parameters values for simulation of SSCF step.*

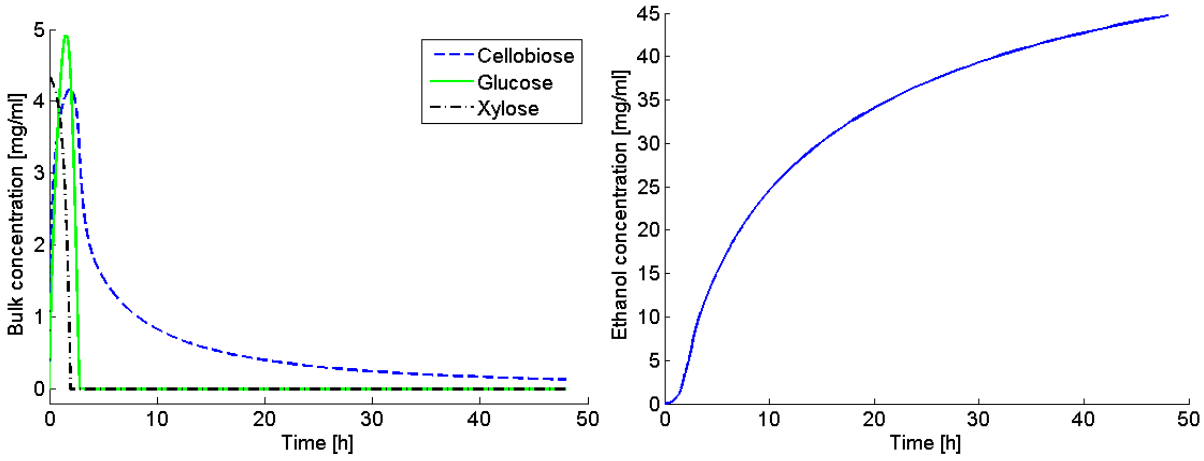
k_{1r}	K_{1IG2}	K_{1IG}	k_{2r}	K_{2IG2}	K_{2IG}	k_{3r}	K_{3M}	K_{3IG}	K_{1ad}
16.5	0.04	0.1	7.1	132.5	0.01	267.6	25.5	2.1	0.6
ml/mg/h	mg/ml	mg/ml	ml/mg/h	mg/ml	mg/ml	h⁻¹	mg/ml	mg/ml	ml/mg
K_{2adL}	E_{1max}	E_{1maxL}	λ	SR	eta_1	eta_2	eta_3	eta_4	$cell_{mg,1}$
0.75	42.55	173.5	1	1.007	0.52	0.41	0.42	0.42	2.88e-4
ml/mg	mg/g	mg/g	-	-	-	-	-	-	s⁻¹
$cell_{mg,2}$	$K_{M,1}$	$K_{M,2}$	$wood_{pore}$	τ	a_0	$pore_{rad}$	k_{dE1}	k_{dE2}	k_{dcell}
2.50e-4	6	6	8e-4	3	1.13e-9	0.58	1e-8	1e-8	0.05
s⁻¹	μM	μM	cm	-	cm²	nm	h⁻¹	h⁻¹	h⁻¹

The concentrations of main products in the reaction bulk are plotted in Figure 6.7.

These results can be verified by comparison with Tang, et al. (2005) work. These authors showed that working with a glucose concentration of 5%, a slurry with an ethanol concentration of about 40 mg/ml is produced, very close to the simulated results. The hydrolysis step is an acid treatment of biomass and the fermentation step occurs in another reactor with *Saccharomyces Cerevisiae* strain *KF-7* at 35 °C for 48 h. The result is comparable because assuming an instantaneous complete conversion of cellulose content in glucose (The true conversion of cellulose in glucose and cellobiose is about 98 %). The initial concentration of glucose in fermenting slurry is about 5 %:

$$1.053 \times sl \times wC = 1.053 \times 0.08 \times 0.53 = 0.045 \Longrightarrow 4.5\ \% \simeq 5\ \%$$

In the work of Tang, et al. (2005) the wood particle dimension has no influence in ethanol production because the cellulose hydrolysis does not occurs simultaneously to fermentation, in the same mode as in the SSCF process.



(a) *(b)*

Figure 6.7 *Bulk concentrations [mg/ml] of main products from wood degradation in SSCF reactor during time: (a) Simple sugars concentration like showed in legend; (b) Ethanol.*

Figure 6.8 shows the cells concentration during reaction in SSCF reactor. At time equal to 0 a production cell delay can be noted. This figure has the typical shape of a kinetic for the cell growth (§ 4.2.4).

The particle core diameter profile is shown in Figure 6.9. The final core diameter at 48 h is 0.28 mm, corresponding to a cellulose conversion of 97.7 % (Table 6.7).

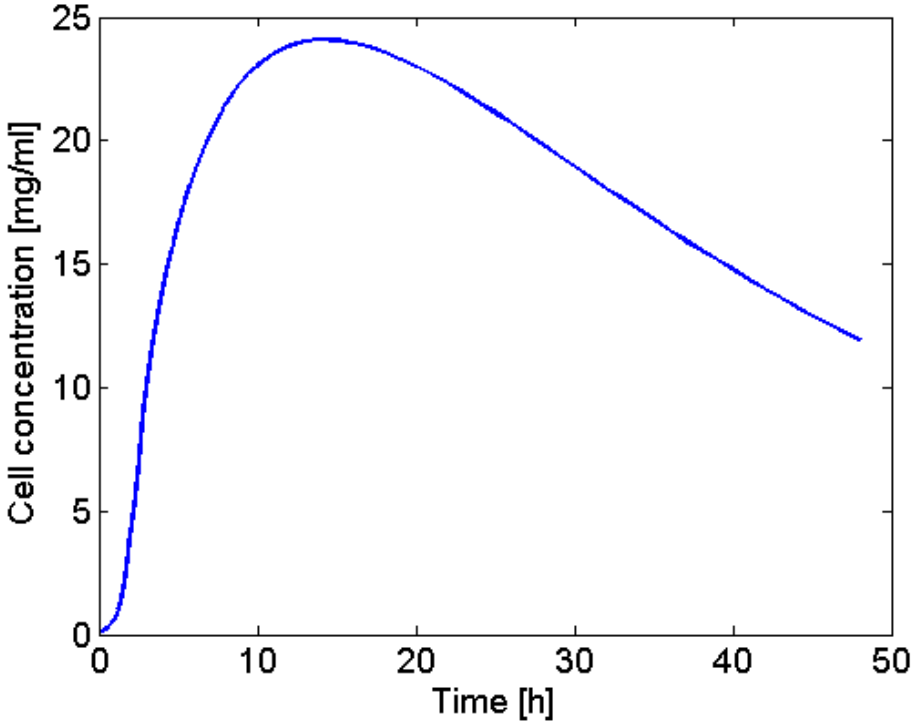


Figure 6.8 *Cells concentrations in SSCF reactor during reaction time.*

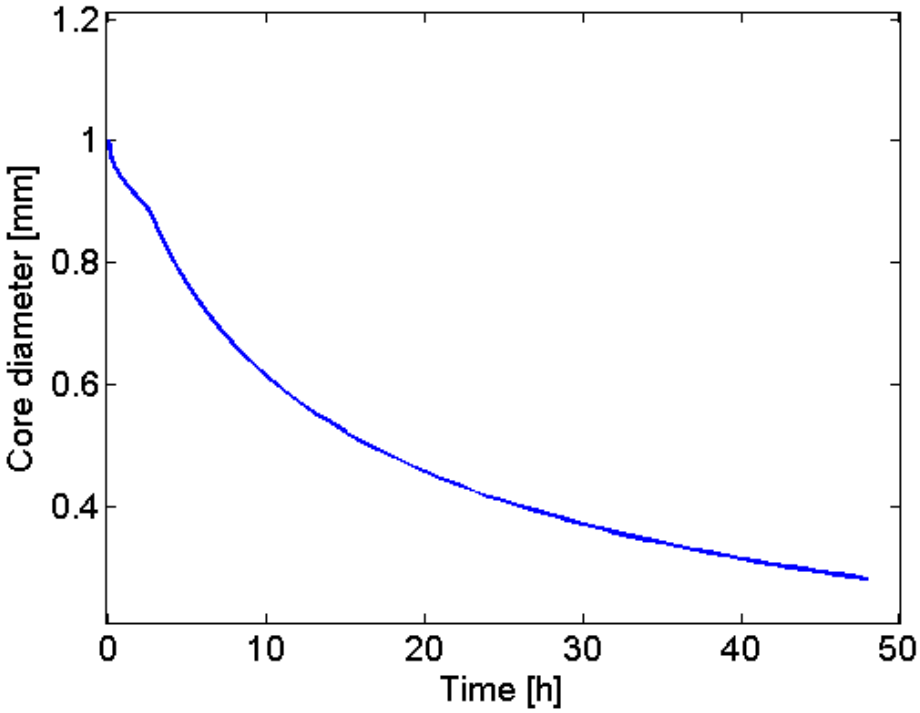


Figure 6.9 *Cores diameter trend during the reaction time.*

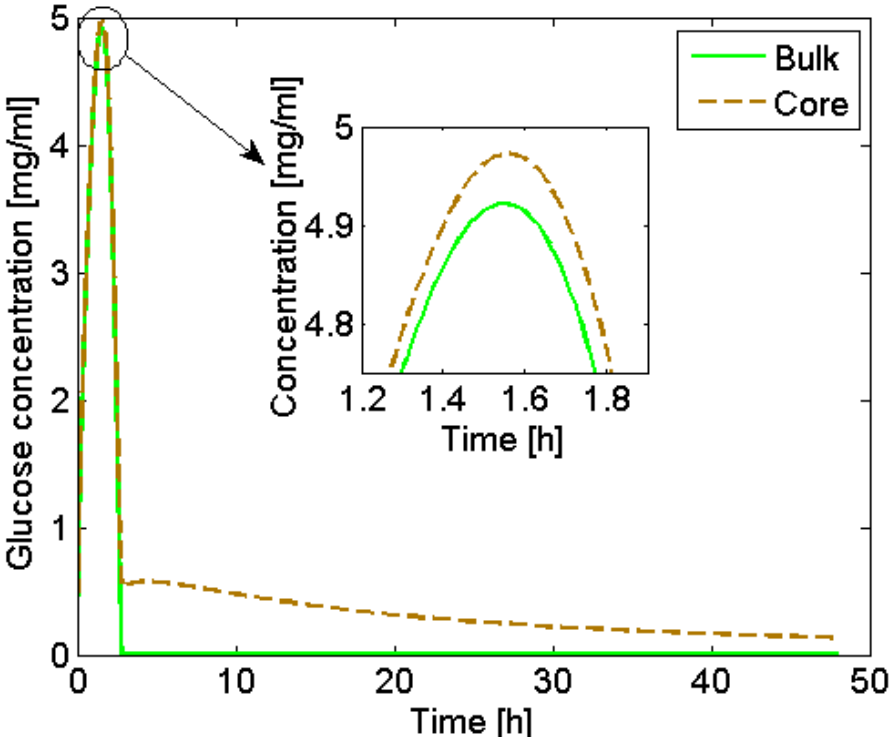


Figure 6.10 *Glucose concentration in wood particle core and in bulk during reaction time. A detail is also shown at initial times. Particle diameters is 1mm*

So there is flux of simple sugars from the core, where the hydrolysis reaction occurs, to the bulk: a concentration gradient of that sugar is necessary. Figure 6.10 shows that for glucose

profile. It can be noted that glucose increases in the initial hours because the cell content is still low. When cells grow, the glucose in the bulk goes down to zero, and the only limit to glucose consumption is the mass transfer out of the core. Indeed the cells are responsible of glucose consumption from bulk.

Figure 6.11 shows the concentration of glucose at fixed times in different points. Some considerations can be done:

- Figure 6.11 (a) shows that the glucose concentration gradient in layer 1 after 10 h is higher than after 48 h;
- The bulk glucose concentration is three, after 10 h, or four, after 48 h, magnitude order lower than it is in particle core. The lowest concentrations in Figure 6.11(a) match with the highest concentrations in Figure 6.11(b) according with times of reference;
- The concentration gradient in layer 1 is larger by about two magnitude orders (from 10^{-1} mg/ml to 10^{-3} mg/ml). Instead, in the cell wall it is about one magnitude order (from 10^{-3} mg/ml to 10^{-4} mg/ml). This shows that the only limiting mass transfer occurs in wood particles, through layer 1.

Similar considerations can be done for cellobiose mass transfer, even though it is not transferred into the cells.

The mass transfer resistance remains low also reducing the hydrolysis rate, for example decreasing the enzyme concentrations.

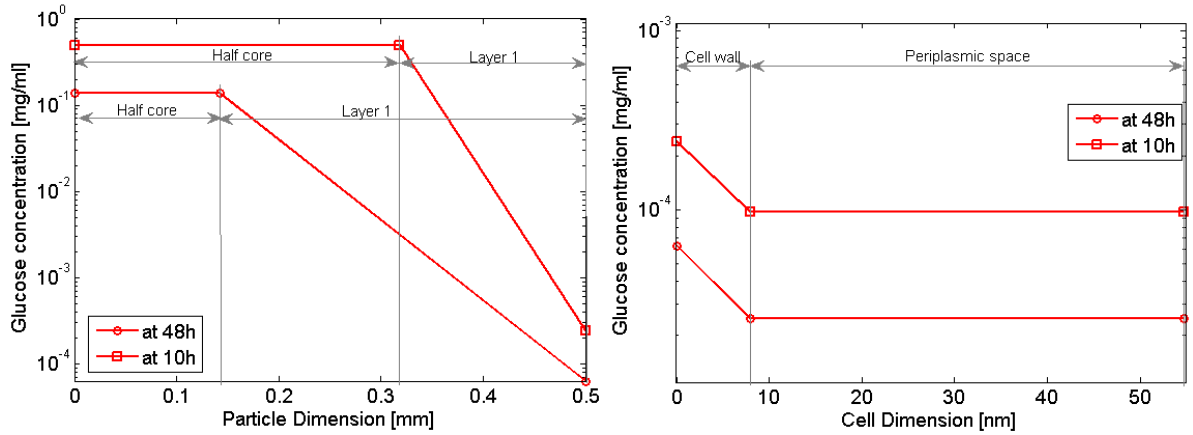


Figure 6.11 *Profiles about the concentration of glucose at 48h and 10h in the SSCF reactor: (a) Concentrations in wood particles; (b) Concentrations in cells.*

The importance of simple sugars mass transfer resistance inside the wood particles can be noted watching Figure 6.12. It shows that the time to get 95 % of cellulose conversion (Time*) is a quite linear function of wood particles diameter. This is so because a higher concentration of glucose and cellobiose in the particles core inhibits cellulose hydrolysis. This consideration is confirmed by Figure 6.13, where the time trend of core and bulk

concentration of glucose for a simulation of the SSCF reactor, with an initial particles core diameter of 1.1 cm and at the conditions of Table 6.4 and Table 6.5. The differences between those results and these of the base case in Figure 6.10 are evident.

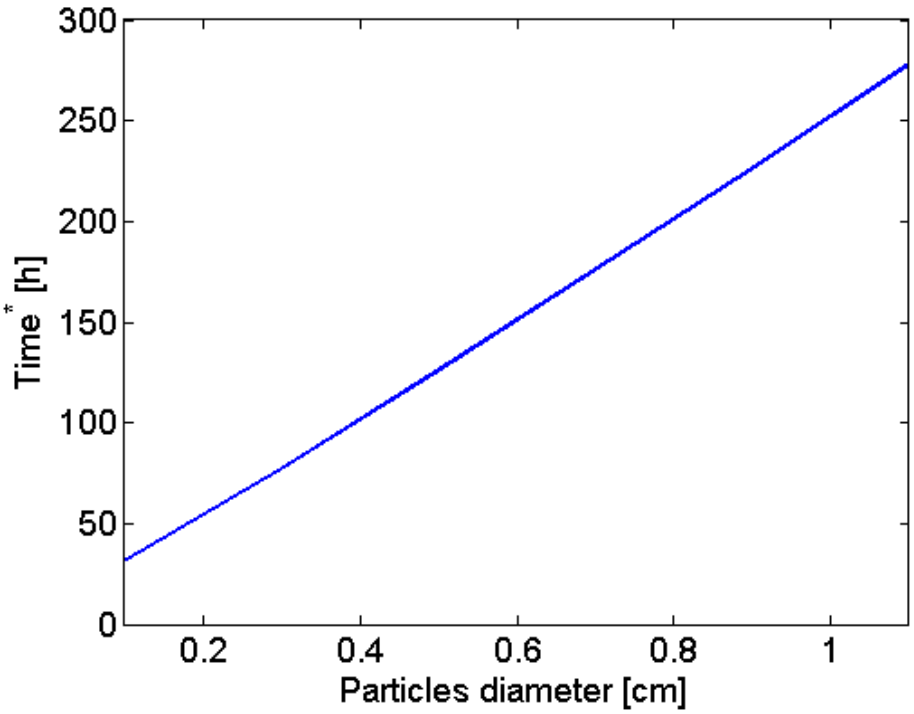


Figure 6.12 *Time needed to get a cellulose conversion of 95 % (Time*) versus the initial particle diameter.*

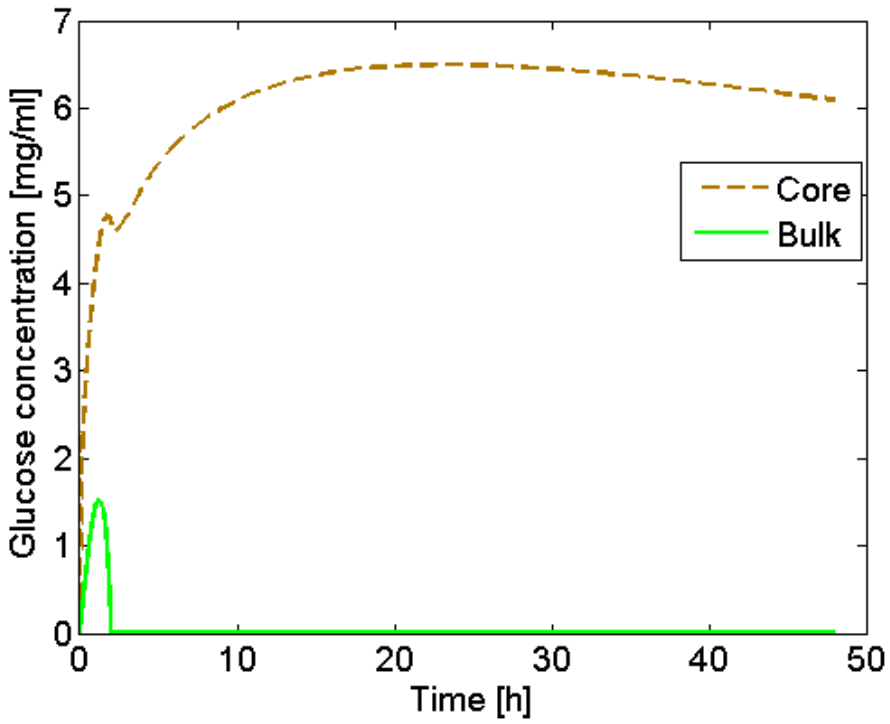


Figure 6.13 *Glucose concentration in wood particle core and in bulk during reaction time. Particles diameter is 1.1 cm.*

Table 6.7 *Cellulose conversion at end of SSCF step. Two simulation are performed with wood particles having diameter of 0.1 cm and of 1.1 cm and at different pretreatment temperature.*

Pretreatment Temperature [°C]	Conversion [%] (d_{par} = 0.1 cm)	Conversion [%] (d_{par} = 1.1 cm)
130	97.3	67.4
150	97.5	68.6
170	97.7	69.7
190	97.8	70.1
210	97.9	70.2
230	97.9	70.2

The xylan pretreatment have influence also on the SSCF step, in fact:

- the conversion of cellulose increases if the xylan content is less because so the diffusion coefficient is higher and the simple sugars concentrations in core, which inhibit cellulose conversion, decrease. This effect is shown in Table 6.7 where different final cellulose conversion from SSCF reactor are listed at different work temperature for two particle sizes. The larger one negatively affects simple sugars diffusion;
- higher temperature in the pretreatment step supplies a higher initial concentration of xylose in the SSCF step causing a higher concentration of cells that are able to produce a larger final concentration of ethanol (Figure 6.14).

From Table 6.7 it can be noted that conversion is influenced by pretreatment temperature only with the larger particle diameter. However, above 210 °C, the xylan conversion is about always 100 %, as noted in ***Figure 6.6***.

Figure 6.14 shows the influence of different pretreatment temperature on ethanol production. At 230 °C the xylose concentration in the slurry is higher, so SSCF reactor produce more ethanol than at 130 °C.

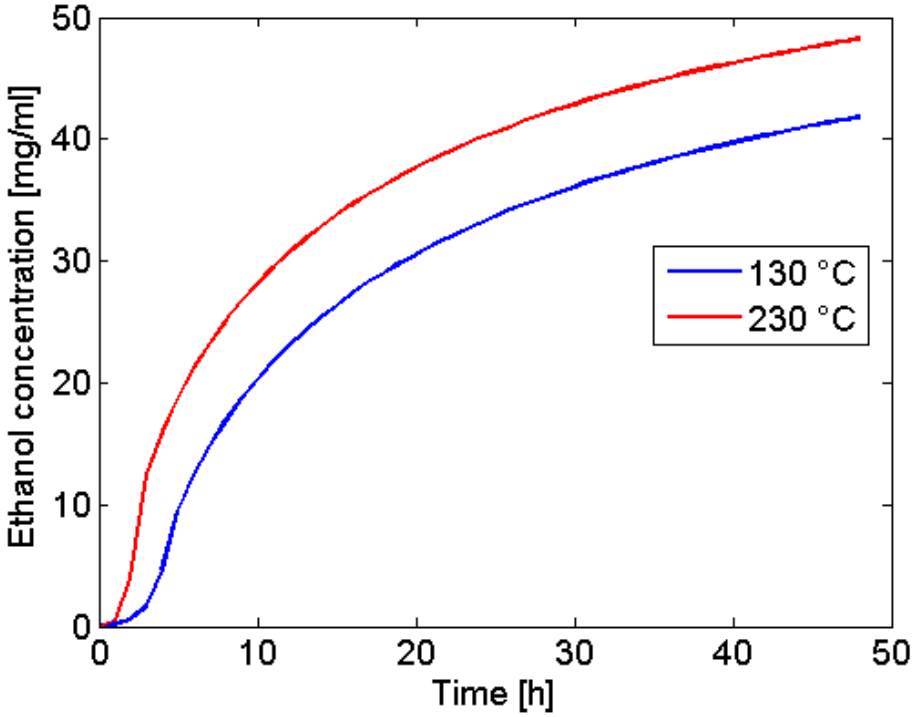


Figure 6.14 *Time production of ethanol in SSCF reactor. The curves are referred to a different pretreatment temperature as it is showed in legend.*

6.3 Process 2 simulation

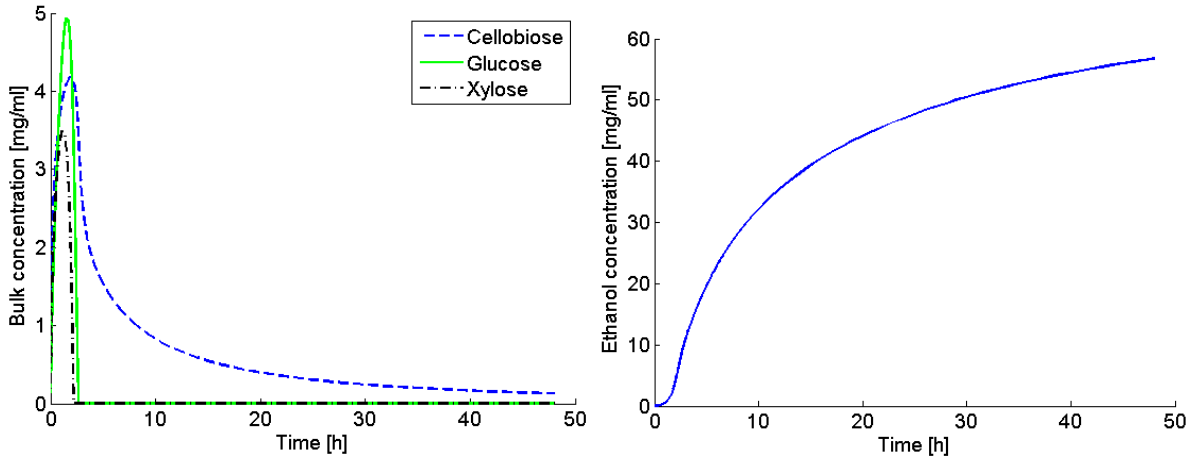
The results here reported are about the case when xylan has a reaction rate faster than cellulose. So the size of wood particle core is determined by xylan reaction rate; the layer 1 contains cellulose, not xylan, and its size is determined by the cellulose consumption rate. Considerations about mass transfer are similar to those presented in § 5.

Table 6.8 *External variables values for SSCF single step process (base case).*

T	V_{tot}	d_{par}	ρ	ρ_{tot}	sl	w_C	w_{Xy}	w_L	ε_{in}	$c_{cell,in}$
40	125e3	0.1	726	1000	0.08	0.53	0.19	1-w_C-w_{Xy}	0.03	0.1
°C	ml	cm	mg/ml	mg/ml	-	-	-		-	mg/ml
A_{spec}	vf_{ps}	thi	A	hr_1	hr_2	hr_3	K_{FPU}	K_{CBU}	FPU	CBU
131	0.3	8e-7	3e-8	NaN	0.42	0.38	1.666	7.5	150	150
cm²/mg	-	cm	cm²/cell		nm	nm	FPU/mg	CBU/mg	FPU/g	FPU/g

For the base case simulation the external variables are listed in Table 6.8. The parameters values for this simulation are the same of Table 6.6, except for parameter is k_{Xy} that has been chosen equal to 1.3. This parameter is very important with respect to xylan reaction rate (§ 5.2.2), and its value is kept constant also for other simulations.

The concentrations of main products in the bulk are plotted in Figure 6.15. The processing time in the SSCF reactor is of 48 h.



(a) *(b)*

Figure 6.15 *Bulk concentrations [mg/ml] of main products from wood degradation in SSCF reactor along with time: (a) Simple sugars concentrations; (b) Ethanol concentration.*

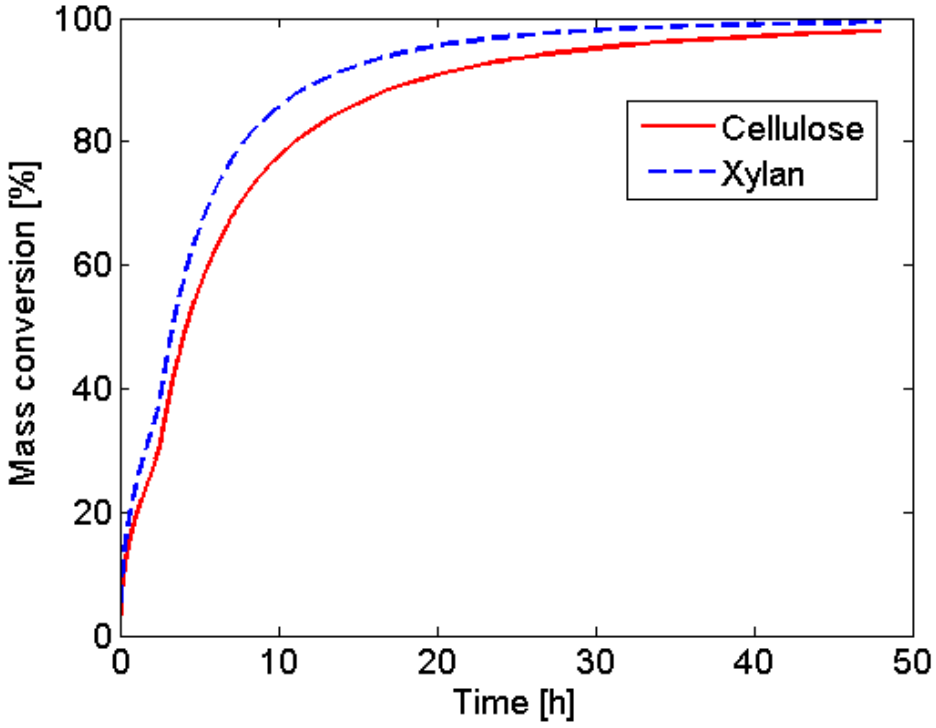


Figure 6.16 *Mass conversion along with reaction time in SSCF reactor, with respect to cellulose and xylan.*

Of course in this process the xylose concentration at starting time is zero. However, the final concentration of ethanol in the bulk (Figure 6.15-b) is higher than in process 1 (Figure 6.7-b) at a given processing time because in process 2 the xylan degradation mechanism produce

only xylose, that can be converted to ethanol. In process 1 arabinose and acetic acid are produced from xylan as well.
Final conversion is 97.9 % for cellulose and 99.3 % for xylan (Figure 6.16).

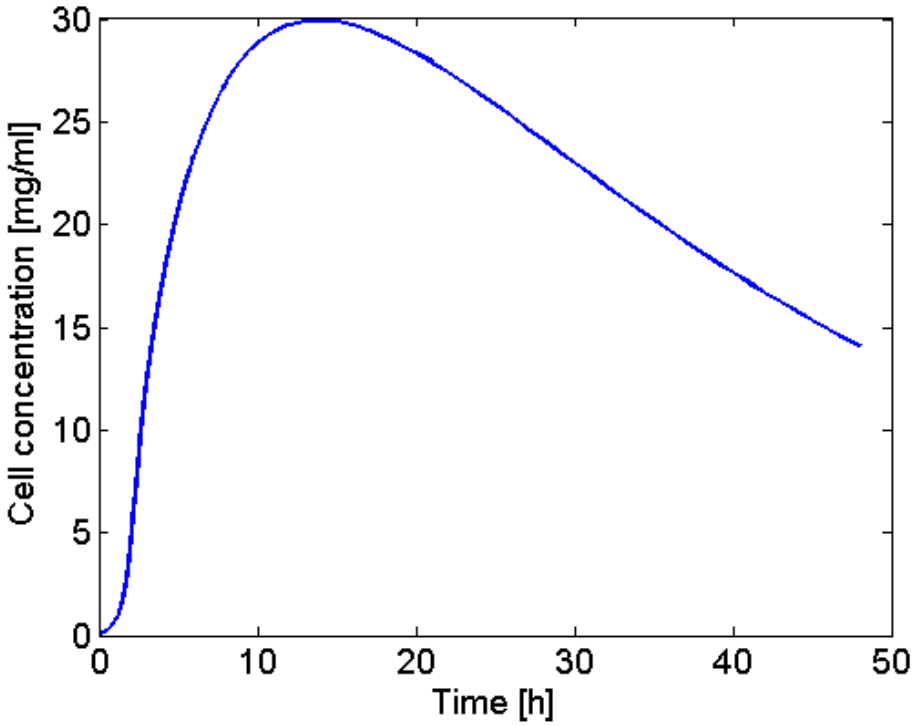


Figure 6.17 *Cells concentrations in SSCF reactor during reaction time.*

The cell concentration in the reactor is shown in Figure 6.17. Compared with Figure 6.8, in this case it is higher because more simple sugars are available for cellular growth and for ethanol production.
In Process 2 three reacting regions are formed inside each wood particle: core, layer 1 and layer 2. The diameters profiles of core and layer 1 are shown in Figure 6.18 as function of time.
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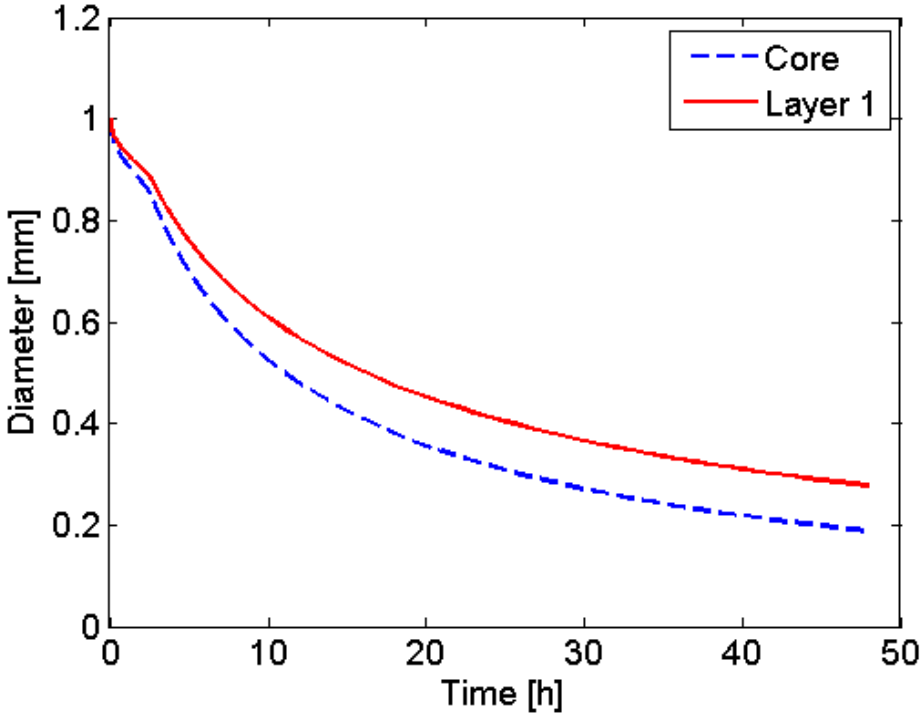

Figure 6.18 *Cores and layers 1 diameters trend during the reaction time.*

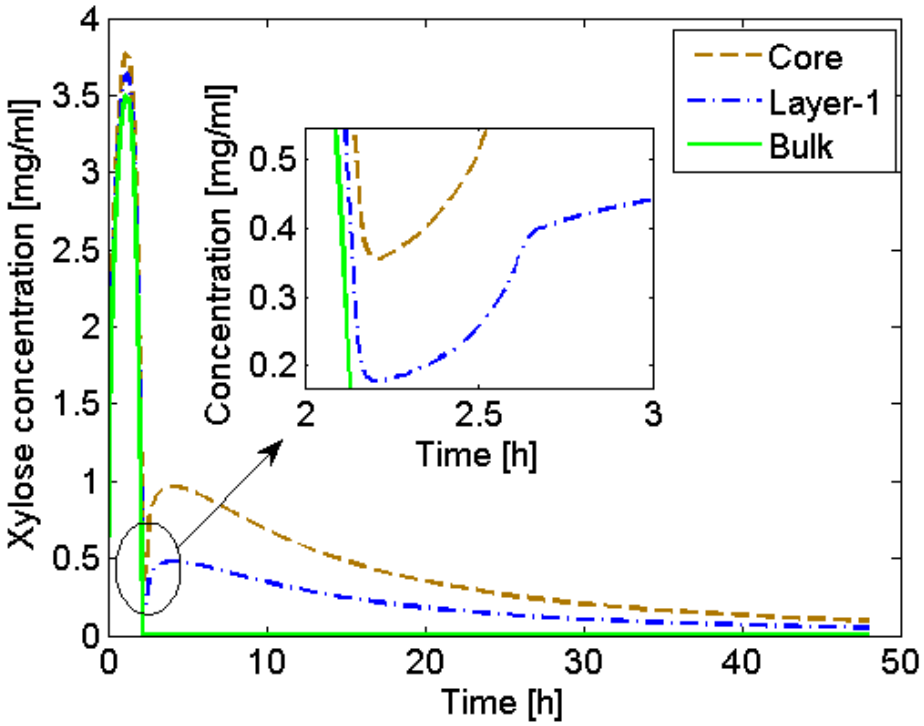

Figure 6.19 *Concentration of xylose inside core, layer 1 and bulk as is explained in figure legend. Also a details of that concentrations is showed.*

In Figure 6.19 the profiles of xylose concentration in the core, layer 1 and bulk are plotted. In fact xylose, the product of degradation of xylan, is transferred from core, where it is

produced, to the bulk, and then toward the cell. During all the processing time there are always differences between these concentrations, that permit diffusion.

Figure 6.20 shows the concentrations of xylose at fixed times in the different points. It has been chosen to represent the concentration of xylose because this is the only product that moves through all layers (glucose and cellobiose move only through layer 2). Also for this process the important resistance is in wood and specifically in layer 2. This is so because the void fraction of layer 2 is bigger than the one of layer 1, and so the diffusion coefficient of xylose in layer 2 is higher.

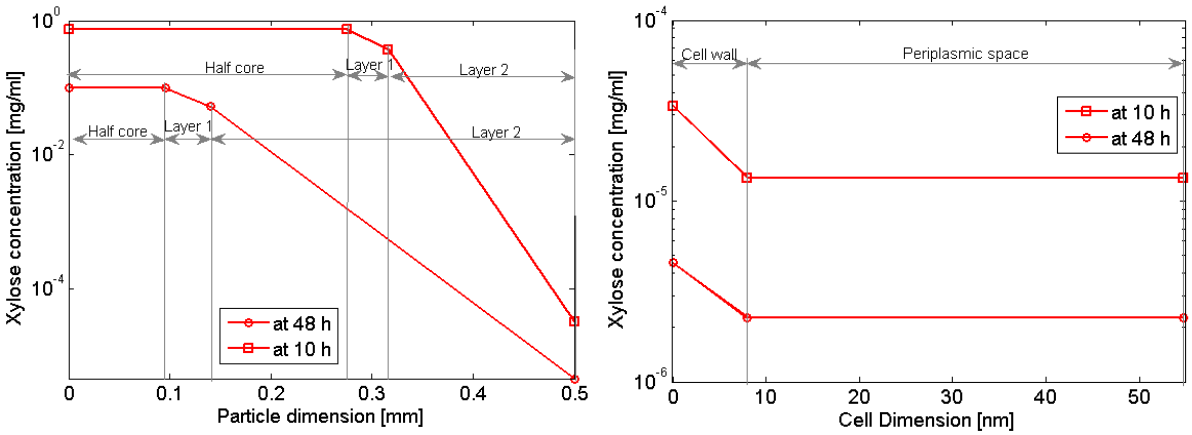

Figure 6.20 *Profiles about the concentration of xylose at 48h and 10h in the SSCF reactor: (a) Concentrations in wood particles; (b) Concentrations in cells.*

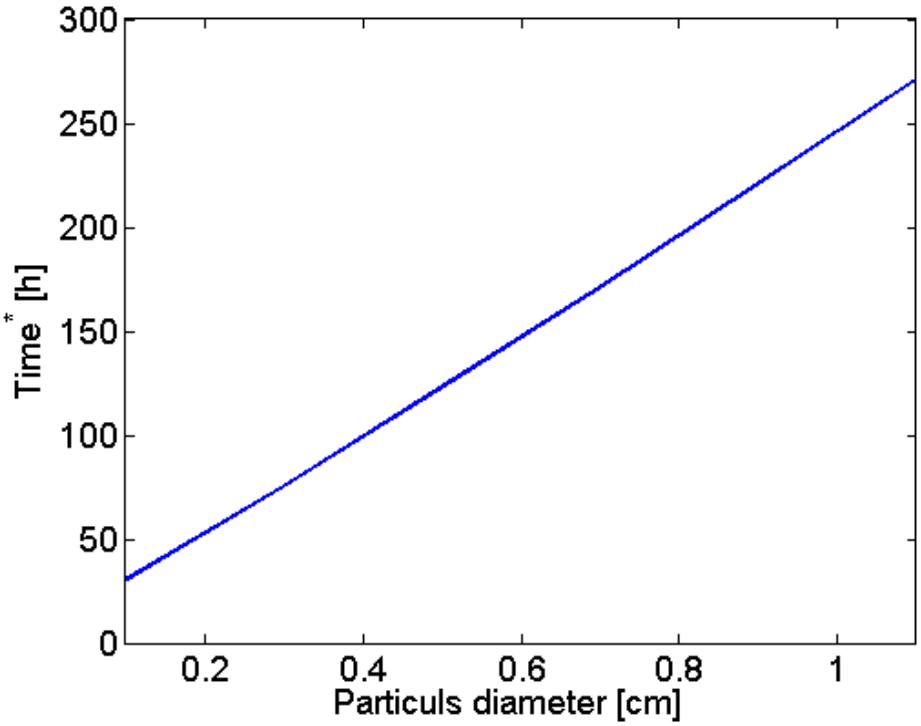

Figure 6.21 *Necessary time to get a cellulose conversion of 95 % (Time*) versus the initial particles diameter.*

As in Figure 6.12, Figure 6.21 shows that the time to get 95 % of cellulose conversion (Time*) is a quite linear function of wood particles diameter and that the mass transfer limitation inside the wood particles becomes more important increasing the initial diameter of the particles.

6.4 Sensitivity analysis

It is useful to study the sensitivity of the solution to the value of parameters. Such information is useful for parameters estimation (to find the best set of parameters for a model) and to decide if a parameter needs to be measured more accurately (Perry, 1999).
A sensitivity analysis was performed on the process 2 base case when the volumetric consumption rates of the cellulose and xylan are equal. The sensitivity values are calculated by the expression:

$$\Delta_{i,j} = \left| \frac{x_i(t^*,\ (1+\delta)\times param_j) - x_i(t^*,\ param_j)}{\delta_j \times param_j} \right| \quad . \tag{6.1}$$

Another method that gives dimensionless results is expressed by:

$$\Lambda_{i,j} = \left| \frac{x_i(t^*,\ (1+\delta)\times param_i) - x_i(t^*,\ param_j)}{x_i(t^*,\ param_j)} \times \frac{1}{\delta} \right| \times 100 \quad . \tag{6.2}$$

Where $\Delta_{i,j}$ is the sensitivity value of state variable x_i to parameter j ["variable dimension"/"parameter dimension"], $\Lambda_{i,j}$ the dimensionless variation of state variable x_i as a function of parameter j [%]. They were calculated with respect to cellulose and xylan conversions (X). The perturbation δ can be positive or negative but in the following results only δ = 0.02 was used. The results with δ = -0.02 are similar. t^* for the shown results is time [h] to get the base case conversion (cellulose or xylan conversion) to 95 %.

In Table 6.9 the sensitivity analysis results are shown about the sensitivity of cellulose conversion to parameters variation of 0.02. Similarly, Table 6.10 shows the sensitivity value for xylan conversion with same parameters perturbation. The parameters which have higher influence in the model results are λ and *SR*, respectively the fraction of lignin available for competitive adsorption of the enzymes involved in cellulose degradation, and the substrate reactivity. Both of these parameters are in the kinetics that describes the consumption rate of cellulose. The only important difference from sensitivity analysis results in Table 6.10 is that k_{Xy} is much more influent ($\Lambda_{K_{Xy}} = 15.42\%$) on xylan than it is on cellulose conversion. Indeed this is the parameter that makes it different the volumetric xylan reaction rate from volumetric cellulose reaction rate, when it is not equal to 1.

Table 6.9 *Sensitivity Δ for all parameters of base case of process 2 when volumetric reaction rate of cellulose is equal to volumetric reaction rate of xylan. The results are for parameters perturbation of* 0.02. *The column "Where" shows the model section of which the parameter is. Λ_j from (6.3) is shown for same variables.*

Parameter	Where	Δ_j for cellulose conversion		Λ_j for cellulose conversion [%]
k_{1r}	cellulose	0.16	conv%/(ml/mg/h)	2.74
K_{1IG2}	cellulose	58.12	conv%/(mg/ml)	2.45
K_{1IG}	cellulose	2.15	conv%/(mg/ml)	0.23
k_{2r}	cellulose	0.17	conv%/(ml/mg/h)	1.30
K_{2IG2}	cellulose	0.00	conv%/(mg/ml)	0.00
K_{2IG}	cellulose	120.06	conv%/(mg/ml)	1.26
k_{3r}	cellulose	0.00	conv%/h^{-1}	0.88
K_{3M}	cellulose	0.03	conv%/(mg/ml)	0.87
K_{3IG}	cellulose	0.00	conv%/(mg/ml)	0.00
K_{1ad}	enzymes	2.07	conv%/(ml/mg)	1.30
K_{2adL}	enzymes	0.77	conv%/(ml/mg)	0.60
E_{1max}	enzymes	0.08	conv%/(mg/g)	3.64
E_{1maxL}	enzymes	0.00	conv%/(mg/g)	0.72
λ	cellulose	18.96	conv%	19.94
SR	cellulose	3.79	conv%	4.02
eta_1	cell	0.33	conv%	0.18
eta_2	cell	0.12	conv%	0.05
eta_3	cell	0.00	conv%	0.00
eta_4	cell	0.00	conv%	0.00
$cell_{mg,1}$	cell	1.52	conv%/ms^{-1}	0.46
$cell_{mg,2}$	cell	0.62	conv%/ms^{-1}	0.16
$K_{M,1}$	cell	0.00	conv%/μM	0.00
$K_{M,2}$	cell	0.00	conv%/μM	0.00
$wood_{pore}$	wood	0.00	conv%/cm	0.00
τ	wood	0.96	conv%	3.02
a_0	celll	0.00	conv%/μm^2	0.00
$pore_{rad}$	cell	0.00	conv%/nm	0.00
k_{dE1}	enzymes	0.13	conv%/s^{-1}	0.00
k_{dE2}	enzymes	0.13	conv%/s^{-1}	0.00
k_{Xy}	xylan	0.30	conv%	0.31
k_{dcell}	cell	0.27	conv%/h^{-1}	0.01

Table 6.10 *Sensitivity Δ_j for all parameters of base case of process 2 when volumetric reaction rate of cellulose is equal to volumetric reaction rate of xylan. The results are for parameters perturbation of* 0.02. *The column "Where" shows the model section of which the parameter is. Λ_j from (6.3) is shown for same variables.*

Parameter	Where	Δ_j for xylan conversion		Λ_j for xylan conversion [%]
k_{1r}	cellulose	0.16	conv%/(ml/mg/h)	2.74
K_{1IG2}	cellulose	58.12	conv%/(mg/ml)	2.45
K_{1IG}	cellulose	2.15	conv%/(mg/ml)	0.23
k_{2r}	cellulose	0.17	conv%/(ml/mg/h)	1.30
K_{2IG2}	cellulose	0.00	conv%/(mg/ml)	0.00
K_{2IG}	cellulose	120.06	conv%/(mg/ml)	1.26
k_{3r}	cellulose	0.00	conv%/h^{-1}	0.88
K_{3M}	cellulose	0.03	conv%/(mg/ml)	0.87
K_{3IG}	cellulose	0.00	conv%/(mg/ml)	0.00
K_{1ad}	enzymes	2.07	conv%/(ml/mg)	1.30
K_{2adL}	enzymes	0.77	conv%/(ml/mg)	0.60
E_{1max}	enzymes	0.08	conv%/(mg/g)	3.64
E_{1maxL}	enzymes	0.00	conv%/(mg/g)	0.72
λ	cellulose	18.96	conv%	19.94
SR	cellulose	3.79	conv%	4.02
eta_1	cell	0.33	conv%	0.18
eta_2	cell	0.12	conv%	0.05
eta_3	cell	0.00	conv%	0.00
eta_4	cell	0.00	conv%	0.00
$cell_{mg,1}$	cell	1.52	conv%/ms^{-1}	0.46
$cell_{mg,2}$	cell	0.62	conv%/ms^{-1}	0.16
$K_{M,1}$	cell	0.00	conv%/µM	0.00
$K_{M,2}$	cell	0.00	conv%/µM	0.00
$wood_{pore}$	wood	0.00	conv%/cm	0.00
τ	wood	0.96	conv%	3.02
a_0	celll	0.00	conv%/μm^2	0.00
$pore_{rad}$	cell	0.00	conv%/nm	0.00
k_{dE1}	enzymes	0.13	conv%/s^{-1}	0.00
k_{dE2}	enzymes	0.13	conv%/s^{-1}	0.00
k_{Xy}	xylan	14.66	conv%	15.42
k_{dcell}	cell	0.27	conv%/h^{-1}	0.01

6.5 Theoretical consideration

In shrinking core models it can be identified a finite time when the reagent has reacted completely. This can be derived from theory: (Kirkby, 2011) and (Schmidt, 1998).
The following balance can be written for a substance that is consumed with the typical reaction mechanisms modeled by a shrinking core model: a superficial reaction. A simplified model according to the typical spherical shrinking core theory can be written assuming substance A as the cellulose, reagent E as the enzymes and *r* as the core radius:

$$\frac{\mathrm{d}\, c_A}{\mathrm{d}\, t} = -k\, c_E\, \frac{4\,\pi\, r^2}{V} \quad , \tag{6.3}$$

Where:

c_A = Concentration of reagent A referred to total bulk volume [mg/cm^3];
k = Generical kinetic constant [1/h];
c_E = Superficial concentration of enzyme E adsorbed on core surface [mg/cm^2];
V = Bulk volume [cm^3].

Usually c_E can be assumed constant during the reaction time. So it can be written:

$$\frac{\mathrm{d}\, r}{\mathrm{d}\, t} = -\frac{k}{\rho}\, c_E \quad , \tag{6.4}$$

Where: ρ = Core density, usually constant during reaction time [mg/cm^3].

Integrating (6.4) from initial core radius at time 0 ($r(t{=}0) = r_0$) to its final one at time *t* ($r(t) = r$) it is obtained:

$$\frac{r - r_0}{t} = -\frac{k}{\rho}\, c_E \quad , \tag{6.5}$$

So a time star t^* where $r = 0$ exists:

$$t^* = \frac{r_0\, \rho}{c_E\, k} \quad , \tag{6.6}$$

On the other hand, the shrinking core models developed in the present work can be represented by:

$$\frac{\mathrm{d}\, c_A}{\mathrm{d}\, t} = -k\, c_E\, c_A \quad . \tag{6.7}$$

$$c_E = k_a\, c_A \tag{6.8}$$

Where c_E is the enzyme content adsorbed on cellulose of core [mg] and k_a is the enzyme adsorption constant [cm^3/mg].

Substituting (6.8) in (6.7):

$$\frac{\mathrm{d}\, c_A}{c_A^2} = -k\, k_a\, \mathrm{d}\, t \quad . \tag{6.9}$$

Integrating from c_A at time 0 ($c_A(t=0) = c_{A,0}$) to c_A at time t ($c_A(t) = c_A$), the following equation can be obtained:

$$\frac{1}{c_A} - \frac{1}{c_{A,0}} = -k\, k_a\, t \quad , \tag{6.10}$$

and

$$c_A = \frac{1}{k k_a t + \frac{1}{c_{A,0}}} \quad . \tag{6.11}$$

From (6.11) it can be noted that:

$$c_A = 0 \;\Leftrightarrow t = \infty \quad . \tag{6.12}$$

This last expression explains because conversion of cellulose and xylan, have an asymptotic trend to 100 %.

Conclusions

The simulation of the production of lignocellulosic bioethanol has been addressed. The heterogeneous systems modeled are made by three phases: wood particles, the reaction bulk and the living cells. The aim was to study the presence of mass transfer limitations due to molecules diffusion through wood and towards reaction bulk and from bulk towards the cell periplasmic space. Wood particles were assumed to be spherical and made up by three component, cellulose, xylan and lignin. Only cellulose and xylan are hydrolysable polysaccharides to get simple sugars fermentable by cells. The lignin does not react and it has just the effect to reduce the void fraction of wood: so, it affects negatively the values of diffusion coefficients of hydrolyzed sugars that are moving outside the wood particle. *Escherichia Coli* cells were used as strain for this modeling work.
Two processes have been studied:

- Process 1 has two reactions steps: the first one is the thermal auto hydrolysis of xylan in a plug flow reactor and the second one is a Simultaneous Saccharification and Co-Fermentation (SSCF) in a batch reactor, where only the cellulose content is hydrolyzed (enzymatically), and xylose from xylan and glucose from cellulose are fermented by living cells.
- Process 2 has a single SSCF reaction step: it is performed in a batch reactor where both cellulose and xylan are hydrolyzed simultaneously and resulting sugars are fermented.

A shrinking core approach has been used for wood particles modeling. The mass transfer through the cell wall has been modeled as well. Diffusion has been the only mass transfer mechanisms considered. The reaction bulk has been always assumed to be well mixed.

The results obtained show that mass transfer, that occurs inside the wood particles, limits the SSCF reaction steps in both processes. Specifically, it can be noted that processing larger wood particles affects negatively the xylan and cellulose conversions in the same way for ethanol production. The SSCF step is so controlled by diffusion of simple sugars through wood particles and the diffusion through cells wall has no limitations.

The main limitation of this work is the reliability of the values of the models parameters. They have been retrieved from the literature in cases that involve the materials modeled, but the peculiarity of components (wood and living cells) addressed in this thesis would need an experimental validation of the whole model and its parameters. A sensitivity analysis has been

performed to determine the parameters that influence more xylan and cellulose conversions calculated by model.

This approach, and specifically this work, is ready to be applied for lignocellulosic bioethanol production optimization to reduce processing time and so to decrease production cost of bioethanol.

Nomenclature

$\mathbf{D_e}(i,j)$	=	Matrix of effective diffusion coefficients in which index *i* identify the substances (P1, P2 or P3) and index *j* identify the layer (1 or 2) [cm^2/h]
rr	=	Vector of production rate for component as in Table 4.5 [mg/ml/h]
A_{cell}	=	Cell surface [cm^2/cell]
A_{spec}	=	Cell external surface over cell mass [cm^2/mg]
D_K	=	Knudsen diffusivity [cm^2/s]
D_{ab}	=	Molecular diffusion coefficient [cm^2/s]
D_{ab}^0	=	Molecular diffusion coefficient at infinite dilution [cm^2/s]
D_e	=	Effective diffusivity [cm^2/s]
D_p	=	Pore diffusivity [cm^2/s]
E_{1bC}	=	Bound concentration of cellulase on cellulose content [mg protein/ml]
E_{1bC}	=	Bound concentration of E1 on cellulose content in substrate [mg protein /ml]
E_{1f}	=	Concentration of free E1 in solution with substrate [mg protein/ml]
E_{1max}	=	Maximum mass of E1 that can be adsorbed onto a unit mass of substrate [mg protein/g substrate]
E_{2maxL}	=	Maximum mass of E2 that can be adsorbed onto a unit mass of lignin [mg protein/g lignin]
E_{if}	=	Concentration of free enzyme with substrate [mg protein/ml] (*i* = 1 for E1; *i* = 2 for E2)
K_{1ad}	=	Dissociation constant for E1 adsorption/desorption reaction with substrate [ml/mg protein]
K_{1ad}	=	Dissociation constant for E1 adsorption/desorption reaction with substrate (ml/mg protein)
K_{2adL}	=	Dissociation constant for E2 adsorption/desorption reaction with lignin [ml/mg protein]
K_{3M}	=	Cellobiose saturation constant [mg/ml]
K_{CBU}	=	Conversion variable for E2 [CBU/mg enzyme]
K_{FPU}	=	Conversion variable for E1 [FPU/mg enzyme]
$K_{M,i}$	=	Michaelis-Menten constant [mg/ml] (*i* as in $v_{max,i}$)
K_{iIG_2}	=	Inhibition constant of cellobiose on enzymes [mg/ml] (*i* = 1, 2 and 3 as in R_i)
K_{iIG}	=	Inhibition constant of glucose on enzymes [mg/ml] (*i*=1,2 and 3 as in R_i)
M_B	=	Molecular weight of solvent [g/mol]
R_i	=	Reaction rate [mg/ml/h]
T_{pre}	=	Pretreatment constant temperature [K]
V_A	=	Molar volume of solute a at its normal boiling point [cm^3/mol]
V_{cell}	=	Single cell volume [cm^3]

V_{par}	=	Sum of initial volume for all wood particle in the system
$V_{ps,in}$	=	Periplasmic space initial volume [ml]
V_{tot}	=	Total volume of reaction bath [ml]
a_0	=	Total pores crossing surface per cell [cm^2]
$c_{C,in}$	=	Initial concentration of cellulose [mg/ml];
$c_{E1,in}$	=	E1 initial concentration in the system [mg/ml]
$c_{E2,in}$	=	E2 initial concentration in the system [mg/ml]
$c_{L,in}$	=	Initial concentration of lignin [mg/ml];
$c_{Xy,in}$	=	Initial xylan concentration in reaction bulk [mg/ml]
$c_{Xy,pre}$	=	After pretreatment concentration of xylan [mg/ml].
$c_{cell,in}$	=	Initial cell concentration [mg/ml/h]
eta_i	=	Growth yield [g cells/g substrate] (i=1 and 2 as in $v_{max,i}$); Ethanol production yield [g ethanol/g substrate] (i=3 for P2; i=4 for P3)
k_{Xy}	=	Xylan kinetic constant [-].
k_i	=	Kinetic constant in xylan pretreatment [1/h]
k_{i0}	=	Arrhenius constant for each kinetic constant k_i [1/h]
k_{ir}	=	Reaction rate constant [mg/ml/h] (i = 3 for (4.25); i = 4 for (4.26); i = 5 for (4.27))
r_{cor}	=	Radius of core particle wood [cm]
r_e	=	Average pore radius [cm]
r_{l1}	=	Radius of sphere that is made up core particle wood and layer 1 [cm]
$\bar{v}$	=	Spatial velocity of slurry in each tube reactor [m/h]
$v_{max,i}$	=	$v_{max,i}$ is the maximum substrate consumption rate measured in [mg substrate/h/mg cell] (i = 1 for P2; i = 2 for P3)
$w_{C,pre}$	=	After pretreatment mass fraction of cellulose in wood particle [-];
w_C	=	Initial mass fraction of cellulose in wood particle [-]
$w_{Xy,pre}$	=	After pretreatment mass fraction of xylans in wood particle [-];
w_{Xy}	=	Initial mass fraction of xylans in wood particle [-]
$wood_{pore}$	=	Radius of wood pore [cm]
hr_i	=	Hydrated radius (i = 1 for P1; i = 2 for P2; i = 3 for P3) [nm]
hr	=	Hydrated radius of compound [nm]
L_{max}	=	Total PFR length for pretreatment step [m]
l	=	Spatial coordinate in the reactor [m]
R	=	Vector of reaction rates [mg/ml/h]
SR	=	Substrate reactivity [-]
T	=	Temperature in SSCF step [K]
x	=	Vector of results calculated from model
$\mathbf{y}(i)$	=	Vector of the enzymes adsorption variables [mg protein/ml] (i=1 bounded concentration of E1 on substrate, i=2 bounded concentration of E1 on lignin, i=3 bounded concentration of

		E2 on lignin)
A	=	Cell outer surface [cm^2]
CBU	=	E2 initial activity [CBU/g cellulose]
Ea_i	=	Activation energy for each reaction as k_i are [J/mol]
FPU	=	E1 initial activity [FPU/g cellulose]
L	=	Lignin content concentration [g/ml]
R	=	Universal gas constant [J/K/mol]
S	=	Substrate (lignin and cellulose content) concentration at a given time [g/ml]
$cell_{mg,i}$	=	Maximum growth rate [1/s] (i as in $v_{max,i}$)
cor	=	Permeability correction [-]
flg_{cell}	=	Flag that can be 1 or 0, it is 1 simulation consider the cell growth, otherwise it does not
kd_{cell}	=	Cell death constant [1/h]
$pore_{rad}$	=	Hydrated pore radius [nm]
sl	=	Initial solid loading in the reactor [g solid/g solution]
thi	=	Cell wall thickness [cm]
thi	=	Cell wall thickness [cm]
tor	=	Tortuosity [-]
vf_{ps}	=	Periplasmic volume fraction of the total cell volume [-]
$\boldsymbol{aa}$	=	Stoichiometric coefficients matrix [-]
Nt	=	Number of tube in PFR
t^*	=	Time to get the base case conversion (cellulose or xylan conversion) to 95%
r_i	=	Mass production rate respectively of Xy1, Xy2, P3 in XO, ARA in XO, ACE in XO (in [1/h]), P3, ARA, ACE (in [mg/ml/h]).

Greek letters

ε_{l1}	=	Layer 1 porosity of wood particle in SSCF reactor [-];
ε_{l2}	=	Layer 2 porosity of wood particle in SSCF reactor [-]
ε_{cor}	=	Core porosity of wood particle in SSCF reactor [-];
ε_{in}	=	Initial porosity of raw wood [-];
ε_p	=	Void fraction of pellet [-]
η_B	=	Viscosity of solvent [cP]
ρ_C	=	Cellulose density [mg/ml]
ρ_{Xy}	=	Xylan density [mg/ml]
ρ_{pre}	=	After pretreatment wood particle density;
ρ_{tot}	=	Density of slurry in reactor [mg/ml]
λ	=	Ratio of lignin content exposed to enzymes to the total amount of lignin content contained in substrate [-]
ρ	=	Initial raw wood particle density [mg/ml];

τ	=	Time coordinate in PFR [h]
ϕ	=	Association factor of solvent [-]
$\Delta_{i,j}$	=	Sensitivity value of state variable x_i to parameter j ["variable dimension"/"parameter dimension"]
$\Lambda_{i,j}$	=	Dimensionless variation of state variable x_i to parameter j [%]
δ_j	=	Parameter perturbation [-]

Acronyms

ACE	=	Acetic acid
ARA	=	Arabinose
E1	=	Endogluconase /cellobiohydrolase (Cellulase complex system) (EG/CBH)
E2	=	β-glucosidase
P1	=	Cellobiose
P2	=	Glucose
P3	=	Xylose
P4	=	Ethanol
PFR	=	Plug flow reactor
SSCF	=	Simultaneous Saccharification and Co-Fermentation
SSF	=	Simultaneous Saccharification and Fermentation
Xy	=	Xylan
Xy1	=	Xylan type 1
Xy2	=	Xylan type 2

Appendixes

Molecular weight calculation: main functions

Code to perform the calculation of molecular weight of a list of compound given with their molecular formula.

```
function [mol_weight_all]=mol_weight_improved(compounds)
% Function to calculate the molecular weigth given a compound list
% Input:
%   - compound=list of compound file name (in this work: 'albert1.dat').
% Output:
%   - mol_weight_all=vector with molecular weight of compounds [g/mol].

Ch_Els=Periodic_Table;
fid1=fopen(compounds,'r');
fid2=fopen('error_report.txt','w');
fid3=fopen('mol_weight_report.txt','w');
a_str=textscan(fid1,'%s');
fclose(fid1);
A=a_str{1};
n_comp=length(A);
fprintf(fid2, 'FORMULA NUMBER \t\t ERROR TYPE\n');
fprintf(fid3, 'FORMULA \t\t\t   MW\n');
mol_weight_all=[];
for j=1:n_comp
    a=A{j};
    num=length(a);
    i=1;
    mol_weight=0;
    while i<=num
        symbol=a(i);
        if i<num
           index=a(i+1);
           flag1=isstrprop(index,'alpha'); % flag1=1 if it is a letter
           if flag1==1
              flag2 = isstrprop(index, 'lower'); %flag2=1 if it is a
lowercase
               if flag2==1
                   symbol=a(i:i+1);
                   i=i+1;
                   if i<num
                      tird=a(i+1);
                      flag1=isstrprop(tird, 'alpha'); % flag1=1 if it is a
letter
                      flag2 = isstrprop(tird, 'lower'); %flag2=1 if it is a
lowercase
                      if flag1==0
                          index=tird;
                          i=i+1;
                      elseif flag2==1
                          fprintf(fid2, '%3.0d\t\t\t\t\t  Element %s... is
not been listed. It is made by 3 characters;\n',j,symbol);
                          i=i+1;
```

```
                        mol_weight=NaN;
                        break
                    end
                end

            end
        else
            i=i+1;
        end
    else
        flag1=1;
    end
    mass=get_mass(symbol,Ch_Els,j,fid2);

    %%%%%% Index and total amount of an element calculation
    if flag1==0
        index=a(i); % one character index
        if i<num
            forth=a(i+1);
            flag1=isstrprop(forth, 'alpha'); % flag1=1 if it is a
letter
            if flag1==0
                index=a(i:i+1); % two character index
                i=i+1;
                if i<num
                    fifth=a(i+1);
                    flag1=isstrprop(fifth, 'alpha'); % flag1=1 if it is
a letter
                    if flag1==0
                        index=a(i-1:i+1); % three character index
                        i=i+1;
                    end
                end
            end
        end
        index=str2num(index);
        mass=mass*index;
    end
    mol_weight=mol_weight+mass;
    i=i+1;
  end
  mol_weight_all=[mol_weight_all; mol_weight];
  fprintf(fid3, '%18s\t %9.3f\n',a,mol_weight);
end

function mass=get_mass(symbol,Ch_Els,j,fid2)
% Function to calculate the molecular weight given dues to an atom in the
% molecule
% Input:
%   - symbol : string of atom symbol;
%   - Ch_Els : structure with name of element, symbol and atomic weight
[g/mol];
%   - fid2 : reference to error_report file [g/mol].
% Output:
%   - mass: sum of atomic of that atom in the molecule;
%   - vector with molecular weight of compounds [g/mol].

y=length(Ch_Els);
for i=1:y
```

```
    if symbol==Ch_Els{i}{3}
       mass=Ch_Els{i}{2};
       break
    elseif i==y
        mass=NaN;
        fprintf(fid2, '%3.0d\t\t\t\t\t Element %s is not been
listed;\n',j,symbol);
    end
end
```

Data about normal molar boiling volume

Data retrieved (229 values) from (Perry, et al., 2006) to determine an approximate polynomial of normal boiling molar volume (V_a) function of molecular weight (M).

M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]
2.016	22.270	59.111	81.615	84.161	115.570	107.155	141.252
4.003	20.087	60.053	65.439	86.090	100.673	108.140	105.367
16.043	35.178	60.053	63.142	86.134	117.537	108.140	117.144
17.031	25.196	60.096	81.228	86.134	112.819	108.140	103.410
18.015	19.362	60.096	81.615	86.134	117.537	108.140	126.999
20.006	24.097	60.096	81.615	86.134	126.604	108.966	79.294
20.180	14.322	62.068	70.043	86.177	141.252	110.199	168.351
26.038	40.408	62.136	75.819	86.177	135.305	112.215	175.960
27.026	50.202	62.136	73.506	86.177	138.476	112.215	171.954
28.010	33.690	62.499	65.439	88.106	108.894	112.215	163.953
28.014	31.463	64.065	44.164	88.106	108.894	112.558	115.570
28.054	47.556	64.514	56.275	88.106	106.151	112.986	109.286
30.006	20.087	68.075	80.454	88.106	107.326	112.986	108.894
30.026	41.158	68.119	103.410	88.106	106.934	114.188	160.359
30.070	52.855	68.119	102.628	88.150	123.053	114.188	158.363
31.057	55.894	68.119	103.410	88.150	123.053	114.231	186.397
31.999	25.930	68.119	103.019	88.150	123.053	114.231	173.957
32.042	41.909	68.119	90.927	88.150	123.841	114.231	177.965
32.045	57.417	69.106	103.802	88.150	124.631	116.160	153.181
34.033	40.408	70.134	110.463	88.150	123.841	116.160	147.609
34.082	35.178	70.134	109.286	88.150	126.604	116.203	165.952
36.461	28.875	70.134	109.286	88.150	123.841	119.377	88.206
37.997	23.365	70.134	95.599	90.189	115.177	120.151	146.416
39.948	26.297	70.905	44.540	90.189	115.177	120.194	167.951
40.065	59.704	72.107	95.989	90.189	115.177	120.194	163.953
41.053	63.142	72.107	99.501	90.189	120.293	120.194	163.554
42.081	68.891	72.150	118.324	92.141	117.931	120.194	165.152
44.010	33.690	72.150	113.998	93.128	100.673	121.182	177.965
44.013	34.806	73.095	97.549	94.113	84.713	122123	130.951
44.053	55.894	73.095	99.501	94.939	56.655	126.242	203.313
44.053	51.338	73.138	112.819	96.104	100.283	128.174	157.167

M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]	M [g/mol]	V_a [ml/mol]
44.097	73.506	74.079	85.877	96.172	146.416	128.214	181.577
45.041	59.322	74.079	84.713	98.145	116.750	128.258	208.158
45.084	65.822	74.079	85.489	98.188	157.167	132.450	119.506
45.084	74.277	74.123	103.019	98.188	141.649	134.221	190.821
46.026	44.917	74.123	100.673	98.188	139.269	136.150	166.351
46.069	61.231	74.123	103.019	100.161	121.082	140.269	225.967
46.069	62.377	74.123	103.019	100.161	139.665	142.241	202.910
48.060	59.704	74.123	103.019	100.161	139.665	142.285	232.866
48.109	52.476	74.123	104.976	100.161	139.665	150.177	187.603
50.488	51.338	76.095	88.594	100.161	140.459	154.211	192.833
52.036	71.582	76.143	58.179	100.161	139.665	156.268	224.346
52.076	75.433	76.163	94.430	100.204	163.154	156.312	256.062
54.092	81.228	76.163	94.430	100.204	150.394	157.010	121.870
54.092	81.228	78.114	97.159	101.192	147.609	170.211	193.236
54.092	81.615	78.541	91.705	101.192	152.384	170.338	280.584
55.079	84.713	78.541	91.705	101.192	158.762	184.365	305.616
56.108	89.371	79.101	94.430	102.090	108.502	198.392	326.617
56.108	86.265	80.064	45.670	102.133	128.184	212.419	350.576
56.108	87.818	80.912	35.550	102.133	130.161	226.446	373.365
56.108	88.206	82.145	121.082	102.133	131.743	230.309	301.095
58.080	75.048	82.145	124.631	102.177	144.429	240.473	396.218
58.080	77.363	82.145	125.815	103.123	127.789	254.500	421.634
58.123	94.820	82.145	113.605	104.152	132.930	268.527	446.703
58.123	97.159	82.145	108.894	106.167	141.649	282.553	467.643
59.068	79.294	84.142	80.841	106.167	142.840		
59.111	94.430	84.161	133.721	106.167	144.429		
59.111	96.769	84.161	119.900	106.167	142.046		

Pore diffusion coefficient calculation

Functions to calculate the pore diffusion coefficient. No Knudsen diffusivity is taken in account for this model.

```
function [Dp,Dk,Dab,M] = two_layer_diffusion_coef(compounds, T, re)
%DIFFUSION_COEF To calculate the total diffusion coefficient of each
%compounds
    % Input:  compounds: list of compound file name (es: 'albert1.dat');
    %         T=temperature in [K];
    %         re= averege pore radius [cm].
    %         ep= void fractions [-]
    %         tau= tortuosity [-]
    % Output: Dp=pore diffusion coefficient [cm^2/s];
    %         Dk=Knudsen diffusivity [cm^2/s]
    %         Dab=free diffusivity coefficient [cm^2/s]
    %         M=molecular weights of compounds list [g/mol]

[M]=mol_weight_improved(compounds);
Dk=9700*re*sqrt(T./M);

Va=2.486e-3.*M.^2 + 9.408e-01.*M + 1.193e+01; % from "fitting_Vb.xlsx"

% Viscosity of solvent water. Equation valid between 0 and 370 °C
A=-2.471e1;
B=4.209e3;
C=4.527e-2;
D=-3.376e-5;
eta=exp(A+B/T+C*T+D*T^2);

Dab=(7.4e-8*(2.6*18)^0.5*T)./(eta.*Va.^0.6);

Dp=(1./Dab).^-1; % No 1/Dk contribution because it is not for liquid but
just for gas diffusion
```

Process 1: main functions

Pretreatment

Functions for pretreatment step: for references about input variable see section § 4.1.

```
function
[t,x]=C_pretreatment(param_pre,T,wXy,sl,ro_tot,L_max,m_wood,d_PFR,Nt)
% Main function to simulate the xylan pretreatment step
% Input:
%   See thesis
% Output:
%   - t=vector of time [h];
%   - x= matrix of result with all internal variable along with the time
for pretreatment (=x_pre).

cXy_in=sl*ro_tot*wXy;      % xylan initial concentration [mg/ml]
v=m_wood/sl/ro_tot/(Nt*pi/4*(d_PFR/100)^2)/3600;  %slurry velocity [m/s]

v=v*3600;  %m/h
t_max=L_max/v; %h

wXy1=param_pre(17);

x0=[wXy1,1-wXy1,0,0,0,0,0,0];
tspan=0:0.01:t_max; % evaluation range time [h]
[t,x] = ode23t(@MB_eq,tspan,x0,[],T,cXy_in,param_pre);

function dx=MB_eq(t,x,T,cXy_in,param_pre)
% Kinetic constant about xylan autodegradation (TEMP. DEPENDENCE)
ki0=exp(param_pre(1:5)); %1/h
Eai=param_pre(6:10); %[KJ/mol]
ki=ki0.*exp(-Eai*1000./(8.31.*(273+T)));
ki=ki*60; %1/h
k1=ki(1);
k2=ki(2);
k3=ki(3);
k4=ki(4);
k5=ki(5);
x_Xy1=param_pre(11:13);  % 1=Xylose 2=Arabinose 3=Acetate
x_Xy2=param_pre(14:16);
```

SSCF step

Functions to solve base case of SSCF reactor: for references about input variable see section § 4.2

```
function
[tx]=B_SSCF_step(param,x_pre,T,Vtot,ro_tot,d_par,sl,ro,wC,wXy,wL,wood_pore,
ep_in,tau,ccell_in,Aspec,vf_ps,thi,a0,A,hr,pore_rad,K_FPU,K_CBU,FPU,CBU)
% Main function to simulate the SSCF reactor of process 1.
% Input:
%   See thesis
% Output:
```

```
%   - tx= matrix of result where the first column is time vector (h) and
other are all internal variable along with the time.

flg=input('With Lignin not productive adsorption (y or n) = ','s');
flg_cell=input('Simulation with cell growth (y=1 or n=0) = ');
t_plot=input('Glucose normalized concentration profile plotted at time [h]
= ');


%% CALCULATED PROPERTIES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% Calculated properties for wood
r_par=d_par/2;                              % initial particle radius [cm]
cC_in=sl*ro_tot*wC;                         % cellulose initial
concentration [mg/ml]
cL_in=sl*ro_tot*wL;
cXy_in=sl*ro_tot*wXy;      % xylane initial concentration [mg/ml]
cE1_in=(FPU/K_FPU)*cC_in/1000;              % enzyme_1 initial
concentration [mg/ml]
cE2_in=(CBU/K_CBU)*cC_in/1000;              % enzyme_2 initial
concentration [mg/ml]
N=(1/cC_in*wC*ro*4/3*pi*r_par^3*1/Vtot)^-1;  % particles number [-]
Vpar=4/3*pi*r_par^3*N;                      % initial volume particle [ml]

%% Pretreatment
cXy_pre=(x_pre(end,1)+x_pre(end,2))*cXy_in;
wC_pre=cC_in/(cC_in+cXy_pre+cL_in);
wL_pre=cL_in/(cC_in+cXy_pre+cL_in);
ro_pre=(cC_in+cXy_pre+cL_in)*Vtot/Vpar;

%% Calculated void fraction for the 3 layers
ro_C=ro/(1-ep_in);         % cellulose density [mg/ml]
ro_Xy=ro_C;                % xylanes density [mg/ml]
ep_cor=ep_in+ro_pre*(cXy_in-cXy_pre)/(cXy_pre+cL_in+cC_in)/ro_Xy;
% void franction of core [-]
ep_l1=ep_cor+ro_pre*wC_pre/ro_C;  % void franction of layer-1 [-]
ep=ep_l1;

% Compounds properties
[Dpv,mm,Dabv,Mv] = two_layer_diffusion_coef('albert1.dat', 273+T,
wood_pore);
Dp=[Dpv(97) Dpv(110) Dpv(401)]*3600;
Dab=[Dabv(97), Dabv(110),Dabv(401)]*3600;  % Free Diffusion [cm^2/h]
MW=[Mv(110) Mv(401)];                % Molecular weight [g/mol]
De=Dp'*ep./tau;  % Effective Diffusion Coefficent in layer-1 e layer-2 for
Cellobiose and Glucose [cm^2/h]

% Calculated properties for compounds
ronR=hr/pore_rad; % radius of solute / radius of pore
cor=(1-ronR).^2.*(1-2.104*ronR+2.09*ronR.^3-0.95*ronR.^5); % permeability
correction [-]
P=Dab.*cor/thi*a0/A; % compounds permeability through cell wall [cm/h]

% Calculated properties for cells
mcell=1/Aspec*A;           % cell mass [mg]
r_cell=sqrt(A/4/pi);       % cell radius [cm]
Vcell=4/3*pi*r_cell^3;     % cell volume [cm^3]
Vps_in=ccell_in*Vtot/mcell*Vcell*vf_ps; % periplasmic volume [cm^3]
```

```
%% %%%%%%%%%%%%%%% SOLVER %%%%%%%%%%%%%%%%%%%%%%
cP3_pre=x_pre(end,6);
x0=[Vpar, 1e-10, 1e-10, cE1_in, cE2_in, 1e-10, 1e-10, 1e-10, 1e-10, 1e-10,
cP3_pre, ccell_in,Vps_in];
t_fr=1
tspan=0:t_fr:t_plot; % evaluation range time [h]
options = odeset('Mass',@massfunction,'RelTol',1e-9);
[t,x] =
ode23t(@MB_eq,tspan,x0,options,Vtot,N,ro_pre,wC_pre,wL_pre,cXy_pre,De,r_par
,Vpar,flg,flg_cell,P,Aspec,Vcell,vf_ps,mcell,MW,param);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%% Plotting
Xc=(1-x(:,1).*ro_pre.*wC_pre./Vtot./cC_in)*100;
tx=[t,x,Xc];

plotting_xy_pretreatment; % script with plotting instruction


function
dx=MB_eq(t,x,Vtot,N,ro_pre,wC_pre,wL_pre,cXy_pre,De,r_par,Vpar,flg,flg_cell
,P,Aspec,Vcell,vf_ps,mcell,MW,param)

%%% Kinetic constant about cellulose enzyme degradation (NO TEMP.
DEPENDENCE, at 50°C)
k1r=param(1);      % ml/mg/h
K1IG2=param(2);    % mg/ml
K1IG=param(3);     % mg/ml
k2r=param(4);      % ml/mg/h
K2IG2=param(5);    % mg/ml
K2IG=param(6);     % mg/ml
k3r=param(7);      % h^-1
K3M=param(8);      % mg/ml
K3IG=param(9);     % mg/ml
K1ad=param(10);    % ml/mg
K2adL=param(11);   % ml/mg
E1max=param(12);   % mg/g
E2maxL=param(13); % mg/g
lamda=param(14); % ratio of lignin content exposed to enzymes
SR=param(15);  % Not Costant in (Zheng 2009)
kdE1=param(28); %[1/h]
kdE2=param(29); %[1/h]

%Yeld fractions 1:cell/P2; 2:cell/xylose; 3:P4/P2; 4: P4/P3
eta=param(16:19);

% Cell metabolic constant (NO TEMP. DEPENDENCE, at 37°C)
cell_mg=param(20:21);          % maximum growth rate [1/s] %Hy:
xylose=arabinose of Nikaido 1981
KM_uM=param(22:23);            % Micaelis Menten constant [microM] %Hy:
xylose=glucose
kd_cell=param(30);             % Death constant [1/h] % invented
vmax=cell_mg./eta(1:2).*3600; % Maximum substrate consumption rate [mg
substrate/h/mg cell]
KM=KM_uM.*MW/1e6;              % mg/ml

% There are 30 parameters
```

```
%%%%%%%%%%%%%%%%%% Instruction to solve LI equations for enzyme couple
%%%%%%%%%%%%%%%%%%%%%%%%
%%% Initial condition
% y0 1: E1 substrate bounded concentration [mg/ml]
%    2: E1 lignin bounded concentration [mg/ml]
%    3: E2 lignin bounded concentration [mg/ml]
if flg=='y'
    % With "Lignin's not productive adsorption"
    y0=[0,0,0];
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    y0=0;
end
options = optimset('Display','off','TolFun',1e-10);  % Turn off display
y=fsolve(@LI_eq,y0,options,x,E1max,E2maxL,K1ad,K2adL,wC_pre,wL_pre,cXy_pre,
Vpar,Vtot,ro_pre,flg);
if flg=='y'
    % With "Lignin's not productive adsorption"
    E1bC=y(1)-y(2)*lamda; % E1 cellulose bounded concentration [mg/ml]
    E2f=x(5)-y(3);       % E2 free concentration [mg/ml]
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    E1bC=y(1);
    E2f=x(5);
end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%

% Stechiometrich Coefficent Matrix
aa=[-1   0   0       0       0        0        0         0;   % E1
     0  -1   0       0       0        0        0         0;   % E2
     0   0  -1       0      -1        0        0         0;   % Cellulose
     0   0   1.056  -1       0        0        0         0;   % P1
     0   0   0       1.053   1.1116  -1        0         0;   % P2
     0   0   0       0       0        0       -1         0;   % P3
     0   0   0       0       0        eta(3)   eta(4)    0;   % P4=etanohol
     0   0   0       0       0        eta(1)   eta(2)   -1;   % Cells
     0   0   0       0       0        0        0         0]; % Xylan

r_cor=(x(1)/N*3/4/pi)^(1/3);    % core radius at time t [cm]
dr_l1=r_par-r_cor;              % layer-1 radius - core radius at time t
[cm]

% Numerical improvements
if dr_l1<=0
   dr_l1=1e-15;
end

% Reaction rates [mg/ml/h]
R(1)=kdE1*x(4);
R(2)=kdE2*x(5);
R(3)=k1r*E1bC*SR*ro_pre*wC_pre*x(1)/Vtot/(1+x(6)/K1IG2+x(7)/K1IG);
R(4)=k3r*E2f*x(2)/(K3M*(1+x(3)/K3IG)+x(2));        %%% E2fL=x(5)
R(5)=k2r*E1bC*SR*ro_pre*wC_pre*x(1)/Vtot/(1+x(6)/K2IG2+x(7)/K2IG);
R(6)=vmax(1)*x(8)*x(12)/(KM(1)+x(8));
R(7)=vmax(2)*x(9)*x(12)/(KM(2)+x(9));
R(8)=kd_cell*x(12);

%Production rate of each compound [mg/ml/h]
```

```
rr=aa*R';

% Mass balances
dx(1)=rr(3)*Vtot/ro_pre/wC_pre; % total volume of core [ml]
dx(2)=(x(6)-x(2))/dr_l1*De(1)*4*pi*r_par^2/Vtot*N+aa(4,4)*R(4);
% c_P1 in bulk [mg/ml]
dx(3)=(x(7)-x(3))/dr_l1*De(2)*4*pi*r_par^2/Vtot*N+aa(5,4)*R(4)-
P(2)*Aspec*x(12)*(x(3)-x(8)); % c_P2 in bulk [mg/ml]
dx(4)=rr(1);    % c_E1 in system [mg/ml]
dx(5)=rr(2);    % c_E2 in system [mg/ml]
dx(6)=aa(4,3)*R(3)*Vtot-(x(6)-x(2))/dr_l1*De(1)*4*pi*r_cor^2*N;  % c_P1 in
core [mg/ml]
dx(7)=aa(5,5)*R(5)*Vtot-(x(7)-x(3))/dr_l1*De(2)*4*pi*r_cor^2*N;  % c_P2 in
core [mg/ml]
dx(8)=(aa(5,6)*R(6)+P(2)*Aspec*x(12)*(x(3)-x(8)))*Vtot;            % c_P2
in ps
dx(9)=(P(3)*Aspec*x(12)*(x(11)-x(9))+aa(6,7)*R(7))*Vtot;           % c_P3
in ps [mg/ml]
dx(10)=rr(7);                                                      % c_P4
in bulk [mg/ml]
dx(11)=-P(3)*Aspec*x(12)*(x(11)-x(9));                             % c_P3
in bulk [mg/ml]
dx(12)=flg_cell*rr(8);                          % c_cell [mg/ml]
dx(13)=flg_cell*rr(8)*Vtot/mcell*Vcell*vf_ps; % Vps_tot [ml]
dx=dx';

function
red=LI_eq(y,x,E1max,E2maxL,K1ad,K2adL,wC_pre,wL_pre,cXy_pre,Vpar,Vtot,ro_pr
e,flg)

L=Vpar*ro_pre*wL_pre/Vtot/1000; % total lignin amount [g]
S=L+(x(1)*wC_pre*ro_pre/Vtot)/1000;   % total substrate amount at time t
[g]
E1f=x(4)-y(1);
if flg=='y'
    %%% With "Lignin's non productive adsorption"
    E2f=x(5)-y(3);
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(2)=y(2)/L-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(3)=y(3)/L-E2maxL*K2adL*E2f/(1+K2adL*E2f);
elseif flg=='n'
    %%% Without "Lignin's non productive adsorption"
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
end

function
M=massfunction(t,x,Vtot,N,ro_pre,wC_pre,wL_pre,cXy_pre,De,r_par,Vpar,flg,fl
g_cell,P,Aspec,Vcell,vf_ps,mcell,MW,param)
% Mass matrix of ode equations
M=[ 1    0 0 0 0 0    0    0 0 0 0 0 0 ;
    0    1 0 0 0 0    0    0 0 0 0 0 0 ;
    0    0 1 0 0 0    0    0 0 0 0 0 0 ;
    0    0 0 1 0 0    0    0 0 0 0 0 0 ;
    0    0 0 0 1 0    0    0 0 0 0 0 0 ;
    x(6) 0 0 0 0 x(1) 0    0 0 0 0 0 0 ;
    x(7) 0 0 0 0 0    x(1) 0 0 0 0 0 0 ;
    0    0 0 0 0 0    0    x(13) 0 0 0 0 x(8);
    0    0 0 0 0 0    0    0 x(13) 0 0 0 x(9);
    0    0 0 0 0 0    0    0 0     1 0 0 0;
```

```
0     0 0 0 0 0     0     0 0      0 1 0 0;
0     0 0 0 0 0     0     0 0      0 0 1 0;
0     0 0 0 0 0     0     0 0      0 0 0 1];
```

Process 2: main functions

If k_{Xy} less than 1

This functions work when $k_{Xy} < 1$. This the case described in § 5.

```
function
[tx]=xyl_less_cell(param,T,Vtot,ro_tot,d_par,sl,ro,wC,wXy,wL,wood_pore,ep_i
n,tau,ccell_in,Aspec,vf_ps,thi,a0,A,hr,pore_rad,K_FPU,K_CBU,FPU,CBU)
% Main function to simulate the SSCF reactor of process 2.
% Input:
%   See thesis
% Output:
%   - tx= matrix of result where the first column is time vector (h) and
other are all internal variable along with the time.
flg=input('With Lignin not productive adsorption (y or n) = ','s');
flg_cell=input('Simulation with cell growth (y=1 or n=0) = ');
t_plot=input('Glucose normalized concentration profile plotted at time [h]
= ');

%% CALCULATED PROPERTIES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% My parameter for Xylan hydrolysis
k_Xy=param(30); % [-] % invented

% Calculated properties for wood
r_par=d_par/2;                                % initial particle radius [cm]
cC_in=sl*ro_tot*wC;                           % cellulose initial
concentration [mg/ml]
cXy_in=sl*ro_tot*wXy;                         % xylane initial concentration
[mg/ml]
cE1_in=(FPU/K_FPU)*cC_in/1000;                % enzyme_1 initial
concentration [mg/ml]
cE2_in=(CBU/K_CBU)*cC_in/1000;                % enzyme_2 initial
concentration [mg/ml]
N=(1/cC_in*wC*ro*4/3*pi*r_par^3*1/Vtot)^-1;   % particles number [-]
Vpar=4/3*pi*r_par^3*N;                        % initial volume particle [ml]

% Calculated void fraction for the 3 layers
ro_C=ro/(1-ep_in);          % cellulose density [mg/ml]
ro_Xy=ro_C;                 % xylanes density [mg/ml]
ep_cor=ep_in;               % void franction of core [-]
ep_l1=ep_cor+ro*wC/ro_C;    % void franction of layer-1 [-]
ep_l2=ep_l1+ro*wXy/ro_Xy; % void franction of layer-1 [-]
ep=[ep_l1,ep_l2];

% Compounds properties
[Dpv,mm,Dabv,Mv] = two_layer_diffusion_coef('albert1.dat', 273+T,
wood_pore);
Dp=[Dpv(97) Dpv(110) Dpv(401)]*3600;
Dab=[Dabv(97), Dabv(110),Dabv(401)]*3600;  % Free Diffusion [cm^2/h]
MW=[Mv(110) Mv(401)];                  % Molecular weight [g/mol]
De=Dp'*ep./tau;  % Effective Diffusion Coefficients in layer-1 e layer-2
for Cellobiose and Glucose [cm^2/h]

% Calculated properties for compounds
ronR=hr/pore_rad; % radius of solute / radius of pore
```

```
cor=(1-ronR).^2.*(1-2.104*ronR+2.09*ronR.^3-0.95*ronR.^5); % permeability
correction [-]
P=Dab.*cor/thi*a0/A; % compounds permeability through cell wall [cm/h]

% Calculated properties for cells
mcell=1/Aspec*A;            % cell mass [mg]
r_cell=sqrt(A/4/pi);        % cell radius [cm]
Vcell=4/3*pi*r_cell^3;      % cell volume [cm^3]
Vps_in=ccell_in*Vtot/mcell*Vcell*vf_ps;

%% %%%%%%%%%%%%%%% SOLVER %%%%%%%%%%%%%%%%%%%%%%
x0=[Vpar, 0, 0, cE1_in, cE2_in, 0, 0, 0, cXy_in, 0, 1e-10,
0,0,0,0,0,ccell_in,Vps_in];
t_fr=1
tspan=0:t_fr:t_plot; % evaluation range time [h]
options = odeset('Mass',@massfunction,'RelTol',1e-9);
[t,x] =
ode23t(@MB_eq,tspan,x0,options,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg
,flg_cell,P,Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%% Plotting
Xc=(1-x(:,1).*ro.*wC./Vtot./cC_in)*100;
Xx=(1-x(:,9)*Vtot/(Vpar*ro*wXy))*100;
tx=[t,x,Xc,Xx];

plotting_xyl_less_cell; % script with plotting instruction

function
dx=MB_eq(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,Aspec,
Vcell,vf_ps,mcell,MW,param,k_Xy)

%%% Kinetic constant about cellulose enzyme degradation (NO TEMP.
DEPENDENCE, at 50°C)
k1r=param(1);     % ml/mg/h
K1IG2=param(2);   % mg/ml
K1IG=param(3);    % mg/ml
k2r=param(4);     % ml/mg/h
K2IG2=param(5);   % mg/ml
K2IG=param(6);    % mg/ml
k3r=param(7);     % h^-1
K3M=param(8);     % mg/ml
K3IG=param(9);    % mg/ml
K1ad=param(10);   % ml/mg
K2adL=param(11);  % ml/mg
E1max=param(12);  % mg/g
E2maxL=param(13); % mg/g
lamda=param(14); % ratio of lignin content exposed to enzymes
SR=param(15);  % Not Constant in (Zheng 2009)
kdE1=param(28);
kdE2=param(29);

%Yeld fraction 1:cell/P2; 2:cell/xylose; 3:P4/P2; 4: P4/P3
eta=param(16:19);

% Cell metabolic constant (NO TEMP. DEPENDENCE, at 37°C)
cell_mg=param(20:21);         % maximum growth rate [1/s] %Hy:
xylose=arabinose of Nikaido 1981
```

```
KM_uM=param(22:23);           % Micaelis Menten constant [microM] %Hy:
xylose=glucose
kd_cell=param(31);            % Death constant [1/s] % invented
vmax=cell_mg./eta(1:2).*3600; % Maximum substrate consumption rate [mg
substrate/h/mg cell]
KM=KM_uM.*MW/1e6;             % mg/ml

% There are 31 parameters

%%%%%%%%%%%% Function to solve LI equations for enzyme couple %%%%%%%%%%
%%% Initial condition
% y0 1: E1 substrate bounded concentration [mg/ml]
%    2: E1 lignin bounded concentration [mg/ml]
%    3: E2 lignin bounded concentration [mg/ml]
if flg=='y'
    % With "Lignin's not productive adsorption"
    y0=[0,0,0];
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    y0=0;
end
options = optimset('Display','off','TolFun',1e-10);  % Turn off display
y=fsolve(@LI_eq,y0,options,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg
);
if flg=='y'
    % With "Lignin's not productive adsorption"
    E1bC=y(1)-y(2)*lamda; % E1 cellulose bounded concentration [mg/ml]
    E2f=x(5)-y(3);       % E2 free concentration [mg/ml]
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    E1bC=y(1);
    E2f=x(5);
end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Stechiometrich Coefficent Matrix
aa=[-1  0  0      0      0       0       0       0     0;  % E1
     0 -1  0      0      0       0       0       0     0;  % E2
     0  0 -1      0     -1       0       0       0     0;  % Cellulose
     0  0  1.056 -1      0       0       0       0     0;  % P1
     0  0  0      1.053  1.1116 -1       0       0     0;  % P2
     0  0  0      0      0       0      -1       1.136 0;  % P3
     0  0  0      0      0       eta(3)  eta(4)  0     0;  % P4=etanohol
     0  0  0      0      0       eta(1)  eta(2)  -1    0;  % Cells
     0  0  0      0      0       0       0       0    -1]; % Xylan


r_cor=(x(1)/N*3/4/pi)^(1/3);         % core radius at time t [cm]
r_l1=((x(11)+x(1))/N*3/4/pi)^(1/3); % layer-1 radius at time t [cm] {from
ammout of xylane-2}
dr_l1=r_l1-r_cor;                    % layer-1 radius - core radius at time
t [cm]
dr_l2=r_par-r_l1;                    % particle radius - layer-1 radius [cm]

% Numerical improvements
if dr_l1<=0
   dr_l1=1e-15;
end
if dr_l2<=0
```

```
   dr_l2=1e-15;
end

% Reaction rates [mg/ml/h]
R(1)=kdE1*x(4);
R(2)=kdE2*x(5);
R(3)=k1r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K1IG2+x(7)/K1IG);
R(4)=k3r*E2f*x(2)/(K3M*(1+x(3)/K3IG)+x(2));       %%% E2fL=x(5)
R(5)=k2r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K2IG2+x(7)/K2IG);
R(6)=vmax(1)*x(8)*x(17)/(KM(1)+x(8));
R(7)=vmax(2)*x(12)*x(17)/(KM(2)+x(12));
R(8)=kd_cell*x(17);
R(9)=x(9)/(x(1)*ro*wC/Vtot)*(R(3)+R(5))*k_Xy;

%Production rate of each compound [mg/ml/h]
rr=aa*R';

% Mass balances
dx(1)=rr(3)*Vtot/ro/wC; % total volume of core [ml]
dx(2)=(x(13)-x(2))/dr_l2*De(1,2)*4*pi*r_par^2/Vtot*N+aa(4,4)*R(4); % c_P1
in bulk [mg/ml]
dx(3)=(x(14)-x(3))/dr_l2*De(2,2)*4*pi*r_par^2/Vtot*N+aa(5,4)*R(4)-
P(2)*Aspec*x(17)*(x(3)-x(8)); % c_P2 in bulk [mg/ml]
dx(4)=rr(1);    % c_E1 in system [mg/ml]
dx(5)=rr(2);    % c_E2 in system [mg/ml]
dx(6)=aa(4,3)*R(3)*Vtot-(x(6)-x(13))/dr_l1*De(1,1)*4*pi*r_cor^2*N;   % c_P1
in core [mg/ml]
dx(7)=aa(5,5)*R(5)*Vtot-(x(7)-x(14))/dr_l1*De(2,1)*4*pi*r_cor^2*N;   % c_P2
in core [mg/ml]
dx(8)=(aa(5,6)*R(6)+P(2)*Aspec*x(17)*(x(3)-x(8)))*Vtot;              % c_P2
in ps
dx(9)=rr(9);                                                        %
c_Xylan [mg/ml]
dx(10)=aa(6,8)*R(9)*Vtot-(x(10)-x(16))/dr_l2*De(3,2)*4*pi*r_par^2*N; % c_P3
in sphere with l1 [mg/ml]
dx(11)=rr(9)*Vtot/ro/wXy-rr(3)*Vtot/ro/wC;                          %
total volume of layer-1 [ml]
dx(12)=(P(3)*Aspec*x(17)*(x(16)-x(12))+aa(6,7)*R(7))*Vtot;           % c_P3
in ps [mg/ml]
dx(13)=((x(6)-x(13))/dr_l1*De(1,1)*4*pi*r_cor^2-(x(13)-
x(2))/dr_l2*De(1,2)*4*pi*r_l1^2)*N; % c_P1 in l1 [mg/ml]
dx(14)=((x(7)-x(14))/dr_l1*De(2,1)*4*pi*r_cor^2-(x(14)-
x(3))/dr_l2*De(2,2)*4*pi*r_l1^2)*N; % c_P2 in l1 [mg/ml]
dx(15)=rr(7);
% c_P4 in bulk [mg/ml]
dx(16)=(x(10)-x(16))/dr_l2*De(3,2)*N*4*pi*r_par^2/Vtot-
P(3)*Aspec*x(17)*(x(16)-x(12)); % c_P3 in bulk [mg/ml]
dx(17)=flg_cell*rr(8);                          % c_cell [mg/ml]
dx(18)=flg_cell*rr(8)*Vtot/mcell*Vcell*vf_ps; % Vps_tot [ml]
dx=dx';

function red=LI_eq(y,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg)

L=Vpar*ro*wL/Vtot/1000; % total lignin amount [g]
S=L+(x(1)*wC*ro/Vtot)/1000;   % total substrate amount at time t [g]
E1f=x(4)-y(1);
if flg=='y'
    %%% With "Lignin's not productive adsorption"
    E2f=x(5)-y(3);
```

```
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(2)=y(2)/L-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(3)=y(3)/L-E2maxL*K2adL*E2f/(1+K2adL*E2f);
elseif flg=='n'
    %%% Without "Lignin's not productive adsorption"
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
end

function
M=massfunction(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,
Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy)
% Mass matrix of ode equations
M=[ 1    0 0 0 0 0    0    0 0 0 0 0 0 0 0 0 0;
    0    1 0 0 0 0    0    0 0 0 0 0 0 0 0 0 0;
    0    0 1 0 0 0    0    0 0 0 0 0 0 0 0 0 0;
    0    0 0 1 0 0    0    0 0 0 0 0 0 0 0 0 0;
    0    0 0 0 1 0    0    0 0 0 0 0 0 0 0 0 0;
    x(6) 0 0 0 0 x(1) 0    0 0 0 0 0 0 0 0 0 0;
    x(7) 0 0 0 0 0    x(1) 0 0 0 0 0 0 0 0 0 0;
    0    0 0 0 0 0    0    x(18) 0 0 0 0 0 0 0 x(8) 0;
    0    0 0 0 0 0    0    0 1 0 0 0 0 0 0 0 0 0;
    x(10) 0 0 0 0 0    0    0 0 x(11)+x(1) x(10) 0 0 0 0 0 0 0;
    0    0 0 0 0 0    0    0 0 0 1 0 0 0 0 0 0 0;
    0    0 0 0 0 0    0    0 0 0 0 x(18) 0 0 0 0 x(12) 0;
    0    0 0 0 0 0    0    0 0 0 x(13) 0 x(11) 0  0  0 0 0;
    0    0 0 0 0 0    0    0 0 0 x(14) 0 0     x(11) 0 0 0 0;
    0    0 0 0 0 0    0    0 0 0 0     0 0     0     1 0 0 0;
    0    0 0 0 0 0    0    0 0 0 0     0 0     0     0 1 0 0;
    0    0 0 0 0 0    0    0 0 0 0     0 0     0     0 0 1 0;
    0    0 0 0 0 0    0    0 0 0 0     0 0     0     0 0 0 1];
```

If k_{Xy} more than 1

This functions work when $k_{Xy} > 1$. Results for this case are shown in § 6.3.

```
% Main function to simulate the SSCF reactor of process 2.
% Input:
%   See thesis
% Output:
%   - tx= matrix of result where the first column is time vector (h) and
other are all internal variable along with the time.

flg=input('With Lignin not productive adsorption (y or n) = ','s');
flg_cell=input('Simulation with cell growth (y=1 or n=0) = ');
t_plot=input('Glucose normalized concentration profile plotted at time [h]
= ');

%% CALCULATED PROPERTIES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% My parameter for Xylan hydrolysis
k_Xy=param(30); % [-] % invented

% Calculated properties for wood
r_par=d_par/2;                                  % initial particle radius [cm]
cC_in=sl*ro_tot*wC;                             % cellulose initial
concentration [mg/ml]
cXy_in=sl*ro_tot*wXy;                           % xylan initial concentration
[mg/ml]
```

```
cE1_in=(FPU/K_FPU)*cC_in/1000;                % enzyme_1 initial
concentration [mg/ml]
cE2_in=(CBU/K_CBU)*cC_in/1000;                % enzyme_2 initial
concentration [mg/ml]
N=(1/cC_in*wC*ro*4/3*pi*r_par^3*1/Vtot)^-1;  % particles number [-]
Vpar=4/3*pi*r_par^3*N;                        % initial volume particle [ml]

% Calculated void fraction for the 3 layers
ro_C=ro/(1-ep_in);         % cellulose density [mg/ml]
ro_Xy=ro_C;                % xylan density [mg/ml]
ep_cor=ep_in;              % void fraction of core [-]
ep_l1=ep_cor+ro*wXy/ro_Xy;  % void fraction of layer-1 [-]
ep_l2=ep_l1+ro*wC/ro_C;     % void fraction of layer-1 [-]
ep=[ep_l1,ep_l2];

% Compounds properties
[Dpv,mm,Dabv,Mv] = two_layer_diffusion_coef('albert1.dat', 273+T,
wood_pore);
Dp=[Dpv(97) Dpv(110) Dpv(401)]*3600;
Dab=[Dabv(97), Dabv(110),Dabv(401)]*3600;  % Free Diffusion [cm^2/h]
MW=[Mv(110) Mv(401)];                  % Molecular weight [g/mol]
De=Dp'*ep./tau;  % Effective Diffusion coefficients in layer-1 e layer-2
for Cellobiose and Glucose [cm^2/h]

% Calculated properties for compounds
ronR=hr/pore_rad; % radius of solute / radius of pore
cor=(1-ronR).^2.*(1-2.104*ronR+2.09*ronR.^3-0.95*ronR.^5); % permeability
correction [-]
P=Dab.*cor/thi*a0/A; % compounds permeability through cell wall [cm/h]

% Calculated properties for cells
mcell=1/Aspec*A;           % cell mass [mg]
r_cell=sqrt(A/4/pi);       % cell radius [cm]
Vcell=4/3*pi*r_cell^3;     % cell volume [cm^3]
Vps_in=ccell_in*Vtot/mcell*Vcell*vf_ps;

%% %%%%%%%%%%%%%%% SOLVER %%%%%%%%%%%%%%%%%%%%%%
x0=[Vpar, 0, 0, cE1_in, cE2_in, 0, 0, 0, cXy_in, 0, 1e-10,
0,0,0,0,0,ccell_in,Vps_in];
t_fr=0.01
tspan=0:t_fr:t_plot; % evaluation range time [h]
options = odeset('Mass',@massfunction,'RelTol',1e-9);
[t,x] =
ode23t(@MB_eq,tspan,x0,options,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg
,flg_cell,P,Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%% Plotting
Xc=(1-x(:,1).*ro.*wC./Vtot./cC_in)*100;
Xx=(1-x(:,9)*Vtot/(Vpar*ro*wXy))*100;
tx=[t,x,Xc,Xx];

plotting_cell_less_xyl; % script with plotting instruction

function
dx=MB_eq(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,Aspec,
Vcell,vf_ps,mcell,MW,param,k_Xy)
```

```
%%% Kinetic constant about cellulose enzyme degradation (NO TEMP.
DEPENDENCE, at 50°C)
k1r=param(1);      % ml/mg/h
K1IG2=param(2);    % mg/ml
K1IG=param(3);      % mg/ml
k2r=param(4);       % ml/mg/h
K2IG2=param(5);   % mg/ml
K2IG=param(6);     % mg/ml
k3r=param(7);     % h^-1
K3M=param(8);      % mg/ml
K3IG=param(9);      % mg/ml
K1ad=param(10);      % ml/mg
K2adL=param(11);    % ml/mg
E1max=param(12);   % mg/g
E2maxL=param(13); % mg/g
%%%%% ipotesis %%%%%%
lamda=param(14); % ratio of lignin content exposed to enzymes
SR=param(15);  % Not constant in (Zheng 2009)

kdE1=param(28);
kdE2=param(29);

%Yeld fraction 1:cell/P2; 2:cell/xylose; 3:P4/P2; 4: P4/P3
eta=param(16:19);

% Cell metabolic constant (NO TEMP. DEPENDENCE, at 37°C)
cell_mg=param(20:21);          % maximum growth rate [1/s] %Hy:
xylose=arabinose of Nikaido 1981
KM_uM=param(22:23);            % Micaelis Menten constant [microM] %Hy:
xylose=glucose
kd_cell=param(31);             % Death constant [1/s] % invented
vmax=cell_mg./eta(1:2).*3600; % Maximum substrate consumption rate [mg
substrate/h/mg cell]
KM=KM_uM.*MW/1e6;              % mg/ml

% There are 31 parameters

%%%%%%%%%% Function to solve LI equations for enzyme couple %%%%%%%
%%% Initial condition
% y0 1: E1 substrate bounded concentration [mg/ml]
%    2: E1 lignin bounded concentration [mg/ml]
%    3: E2 lignin bounded concentration [mg/ml]
if flg=='y'
    % With "Lignin's not productive adsorption"
    y0=[0,0,0];
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    y0=0;
end
options = optimset('Display','off','TolFun',1e-10);  % Turn off display
y=fsolve(@LI_eq,y0,options,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg
);
if flg=='y'
    % With "Lignin's not productive adsorption"
    E1bC=y(1)-y(2)*lamda; % E1 cellulose bounded concentration [mg/ml]
    E2f=x(5)-y(3);       % E2 free concentration [mg/ml]
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    E1bC=y(1);
```

```
    E2f=x(5);
end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

% Stechiometrich Coefficent Matrix
aa=[-1  0  0      0      0       0       0       0     0;  % E1
     0 -1  0      0      0       0       0       0     0;  % E2
     0  0 -1      0     -1       0       0       0     0;  % Cellulose
     0  0  1.056 -1      0       0       0       0     0;  % P1
     0  0  0      1.053  1.1116 -1       0       0     0;  % P2
     0  0  0      0      0       0      -1       1.136 0;  % P3
     0  0  0      0      0       eta(3)  eta(4)  0     0;  % P4=etanohol
     0  0  0      0      0       eta(1)  eta(2)  -1    0;  % Cells
     0  0  0      0      0       0       0       0    -1]; % Xylan

Vcor=(x(1)-x(11));
if Vcor<=0
    Vcor=1e-10;
end
r_cor=(Vcor/N*3/4/pi)^(1/3);        % core radius at time t [cm]
r_l1=(x(1)/N*3/4/pi)^(1/3); % layer-1 radius at time t [cm] {from amount of
xylane-2}
dr_l1=r_l1-r_cor;                   % layer-1 radius - core radius at time
t [cm]
dr_l2=r_par-r_l1;                   % particle radius - layer-1 radius [cm]

% Numerical improvements
if dr_l1<=0
   dr_l1=1e-15;
end
if dr_l2<=0
   dr_l2=1e-15;
end

% Reaction rates [mg/ml/h]
R(1)=kdE1*x(4);
R(2)=kdE2*x(5);
R(3)=k1r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K1IG2+x(7)/K1IG);
R(4)=k3r*E2f*x(2)/(K3M*(1+x(3)/K3IG)+x(2));       %%% E2fL=x(5)
R(5)=k2r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K2IG2+x(7)/K2IG);
R(6)=vmax(1)*x(8)*x(17)/(KM(1)+x(8));
R(7)=vmax(2)*x(12)*x(17)/(KM(2)+x(12));
R(8)=kd_cell*x(17);
R(9)=x(9)/(x(1)*ro*wC/Vtot)*(R(3)+R(5))*k_Xy;

%Production rate of each compound [mg/ml/h]
rr=aa*R';

% Mass balances
dx(1)=rr(3)*Vtot/ro/wC; % total volume up layer-1 [ml]
dx(2)=(x(6)-x(2))/dr_l2*De(1,2)*4*pi*r_par^2/Vtot*N+aa(4,4)*R(4); % c_P1 in
bulk [mg/ml]
dx(3)=(x(7)-x(3))/dr_l2*De(2,2)*4*pi*r_par^2/Vtot*N+aa(5,4)*R(4)-
P(2)*Aspec*x(17)*(x(3)-x(8)); % c_P2 in bulk [mg/ml]
dx(4)=rr(1);    % c_E1 in system [mg/ml]
dx(5)=rr(2);    % c_E2 in system [mg/ml]
dx(6)=aa(4,3)*R(3)*Vtot-(x(6)-x(2))/dr_l2*De(1,2)*4*pi*r_l1^2*N;   % c_P1
in core [mg/ml]
```

```
dx(7)=aa(5,5)*R(5)*Vtot-(x(7)-x(3))/dr_l2*De(2,2)*4*pi*r_l1^2*N;    % c_P2
in core [mg/ml]
dx(8)=(aa(5,6)*R(6)+P(2)*Aspec*x(17)*(x(3)-x(8)))*Vtot;              % c_P2
in ps
dx(9)=rr(9);                                                        %
c_Xylan [mg/ml]
dx(10)=aa(6,8)*R(9)*Vtot-(x(10)-x(13))/dr_l1*De(3,1)*4*pi*r_cor^2*N; % c_P3
in core [mg/ml]
dx(11)=rr(3)*Vtot/ro/wC-rr(9)*Vtot/ro/wXy;                          %
total volume of layer-1 [ml]
dx(12)=(P(3)*Aspec*x(17)*(x(16)-x(12))+aa(6,7)*R(7))*Vtot;           % c_P3
in ps [mg/ml]
dx(13)=((x(10)-x(13))/dr_l1*De(3,1)*4*pi*r_cor^2-(x(13)-
x(16))/dr_l2*De(3,2)*4*pi*r_l1^2)*N; % c_P3 in l1 [mg/ml]
dx(14)=1;
dx(15)=rr(7);
% c_P4 in bulk [mg/ml]
dx(16)=(x(13)-x(16))/dr_l2*De(3,2)*4*pi*r_par^2/Vtot*N-
P(3)*Aspec*x(17)*(x(16)-x(12)); % c_P3 in bulk [mg/ml]
dx(17)=flg_cell*rr(8);                          % c_cell [mg/ml]
dx(18)=flg_cell*rr(8)*Vtot/mcell*Vcell*vf_ps; % Vps_tot [ml]
dx=dx';

function red=LI_eq(y,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg)

L=Vpar*ro*wL/Vtot/1000; % total lignin ammount [g]
S=L+(x(1)*wC*ro/Vtot)/1000;   % total substate ammont at time t [g]
E1f=x(4)-y(1);
if flg=='y'
    %%% With "Lignin's not productive adsorption"
    E2f=x(5)-y(3);
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(2)=y(2)/L-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(3)=y(3)/L-E2maxL*K2adL*E2f/(1+K2adL*E2f);
elseif flg=='n'
    %%% Without "Lignin's not productive adsorption"
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
end

function
M=massfunction(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,
Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy)
% Mass matrix of ode equations
Vcor=(x(1)-x(11));

M=[ 1    0 0 0 0 0    0    0 0 0 0 0 0 0 0 0 0 0;
    0    1 0 0 0 0    0    0 0 0 0 0 0 0 0 0 0 0;
    0    0 1 0 0 0    0    0 0 0 0 0 0 0 0 0 0 0;
    0    0 0 1 0 0    0    0 0 0 0 0 0 0 0 0 0 0;
    0    0 0 0 1 0    0    0 0 0 0 0 0 0 0 0 0 0;
    x(6) 0 0 0 0 x(1) 0    0 0 0 0 0 0 0 0 0 0 0;
    x(7) 0 0 0 0 0    x(1) 0 0 0 0 0 0 0 0 0 0 0;
    0    0 0 0 0 0    0    x(18) 0 0 0 0 0 0 0 0 x(8) 0;
    0    0 0 0 0 0    0    0 1 0 0 0 0 0 0 0 0 0;
    x(10) 0 0 0 0 0   0    0 0 Vcor -x(10) 0 0 0 0 0 0 0;       %
    0    0 0 0 0 0    0    0 0 0 1 0 0 0 0 0 0 0;
    0    0 0 0 0 0    0    0 0 0 0 x(18) 0 0 0 0 x(12) 0;
    0    0 0 0 0 0    0    0 0 0 x(13) 0 x(11) 0  0  0 0 0;
    0    0 0 0 0 0    0    0 0 0 0 0 0     1 0 0 0 0;
```

```
    0    0 0 0 0 0    0    0 0 0 0    0 0    0    1 0 0 0;
    0    0 0 0 0 0    0    0 0 0 0    0 0    0    0 1 0 0;
    0    0 0 0 0 0    0    0 0 0 0    0 0    0    0 0 1 0;
    0    0 0 0 0 0    0    0 0 0 0    0 0    0    0 0 0 1];
```

If k_{Xy} equal to 1

This functions work when $k_{Xy} = 1$. Sensitivity analysis results for this case are shown in § 6.4.

```
function
[tx]=xyl_equal_cell(param,T,Vtot,ro_tot,d_par,sl,ro,wC,wXy,wL,wood_pore,ep_
in,tau,ccell_in,Aspec,vf_ps,thi,a0,A,hr,pore_rad,K_FPU,K_CBU,FPU,CBU)
% Main function to simulate the SSCF reactor of process 2.
% Input:
%   See thesis
% Output:
%   - tx= matrix of result where the first column is time vector (h) and
other are all internal variable along with the time.

flg=input('With Lignin not productive adsorption (y or n) = ','s');
flg_cell=input('Simulation with cell growth (y=1 or n=0) = ');
t_plot=input('Glucose normalized concentration profile plotted at time [h]
= ');

%% CALCULATED PROPERTIES %%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
% My parameter for Xylan hydrolysis
k_Xy=param(30); % [-] % invented

r_par=d_par/2;                                  % initial particle radius [cm]
cC_in=sl*ro_tot*wC;                             % cellulose initial
concentration [mg/ml]
cXy_in=sl*ro_tot*wXy;                           % xylan initial concentration
[mg/ml]
cE1_in=(FPU/K_FPU)*cC_in/1000;                  % enzyme_1 initial
concentration [mg/ml]
cE2_in=(CBU/K_CBU)*cC_in/1000;                  % enzyme_2 initial
concentration [mg/ml]
N=(1/cC_in*wC*ro*4/3*pi*r_par^3*1/Vtot)^-1;     % particles number [-]
Vpar=4/3*pi*r_par^3*N;                          % initial volume particle [ml]

% Calculated void fraction for the 3 layers
ro_C=ro/(1-ep_in);          % cellulose density [mg/ml]
ro_Xy=ro_C;                 % xylan density [mg/ml]
ep_cor=ep_in;               % void fraction of core [-]
ep_l1=ep_cor+ro*wC/ro_C;    % void fraction of layer-1 [-]
ep_l2=ep_l1+ro*wXy/ro_Xy; % void fraction of layer-1 [-]
ep=[ep_l1,ep_l2];

% Compounds properties
[Dpv,mm,Dabv,Mv] = two_layer_diffusion_coef('albert1.dat', 273+T,
wood_pore);
Dp=[Dpv(97) Dpv(110) Dpv(401)]*3600;
Dab=[Dabv(97), Dabv(110),Dabv(401)]*3600;  % Free Diffusion [cm^2/h]
MW=[Mv(110) Mv(401)];                   % Molecular weight [g/mol]
De=Dp'*ep./tau;  % Effective Diffusion coefficients in layer-1 e layer-2
for Cellobiose and Glucose [cm^2/h]
```

```
% Calculated properties for compounds
ronR=hr/pore_rad; % radius of solute / radius of pore
cor=(1-ronR).^2.*(1-2.104*ronR+2.09*ronR.^3-0.95*ronR.^5); % permeability
correction [-]
P=Dab.*cor/thi*a0/A; % compounds permeability through cell wall [cm/h]

% Calculated properties for cells
mcell=1/Aspec*A;            % cell mass [mg]
r_cell=sqrt(A/4/pi);        % cell radius [cm]
Vcell=4/3*pi*r_cell^3;      % cell volume [cm^3]
Vps_in=ccell_in*Vtot/mcell*Vcell*vf_ps;

%% %%%%%%%%%%%%%%% SOLVER %%%%%%%%%%%%%%%%%%%%%%
x0=[Vpar, 0, 0, cE1_in, cE2_in, 0, 0, 0, cXy_in, 0,0,0,0,ccell_in,Vps_in];
t_fr=1
tspan=0:t_fr:t_plot; % evaluation range time [h]
options = odeset('Mass',@massfunction,'RelTol',1e-9);
[t,x] =
ode23t(@MB_eq,tspan,x0,options,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg
,flg_cell,P,Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy);
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%

%% Plotting
Xc=(1-x(:,1).*ro.*wC./Vtot./cC_in)*100;
Xx=(1-x(:,9)*Vtot/(Vpar*ro*wXy))*100;
tx=[t,x,Xc,Xx];

plotting_xyl_equal_cell; % script with plotting instruction

function
dx=MB_eq(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,Aspec,
Vcell,vf_ps,mcell,MW,param,k_Xy)

%%% Kinetic constant about cellulose enzyme degradation (NO TEMP.
DEPENDENCE, at 50°C)
k1r=param(1);      % ml/mg/h
K1IG2=param(2);    % mg/ml
K1IG=param(3);     % mg/ml
k2r=param(4);      % ml/mg/h
K2IG2=param(5);    % mg/ml
K2IG=param(6);     % mg/ml
k3r=param(7);      % h^-1
K3M=param(8);      % mg/ml
K3IG=param(9);     % mg/ml
K1ad=param(10);    % ml/mg
K2adL=param(11);   % ml/mg
E1max=param(12);   % mg/g
E2maxL=param(13); % mg/g
lamda=param(14); % ratio of lignin content exposed to enzymes
SR=param(15);  % Not constant in (Zheng 2009)
kdE1=param(28);
kdE2=param(29);

%Yeld fraction 1:cell/P2; 2:cell/xylose; 3:P4/P2; 4: P4/P3
eta=param(16:19);

% Cell metabolic constant (NO TEMP. DEPENDENCE, at 37°C)
```

```
cell_mg=param(20:21);          % maximum growth rate [1/s] %Hy:
xylose=arabinose of Nikaido 1981
KM_uM=param(22:23);            % Micaelis Menten constant [microM] %Hy:
xylose=glucose
kd_cell=param(31);             % Death constant [1/s] % invented
vmax=cell_mg./eta(1:2).*3600; % Maximum substrate consumption rate [mg
substrate/h/mg cell]
KM=KM_uM.*MW/1e6;              % mg/ml

% There are 31 parameters

%%%%%%%%%%%%%%%%%% Function to solve LI equations for enzyme couple
%%%%%%%%%%%%%%%%%%%%%%%%
%%% Initial condition
% y0 1: E1 substrate bounded concentration [mg/ml]
%    2: E1 lignin bounded concentration [mg/ml]
%    3: E2 lignin bounded concentration [mg/ml]
if flg=='y'
    % With "Lignin's not productive adsorption"
    y0=[0,0,0];
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    y0=0;
end
options = optimset('Display','off','TolFun',1e-10);  % Turn off display
y=fsolve(@LI_eq,y0,options,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg
);
if flg=='y'
    % With "Lignin's not productive adsorption"
    E1bC=y(1)-y(2)*lamda; % E1 cellulose bounded concentration [mg/ml]
    E2f=x(5)-y(3);       % E2 free concentration [mg/ml]
elseif flg=='n'
    % Without "Lignin's not productive adsorption"
    E1bC=y(1);
    E2f=x(5);
end
%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%
%%%%%%%%%%%%%%%%%

% Stechiometrich Coefficent Matrix
aa=[-1   0   0       0       0        0        0        0      0;  % E1
     0  -1   0       0       0        0        0        0      0;  % E2
     0   0  -1       0      -1        0        0        0      0;  % Cellulose
     0   0   1.056  -1       0        0        0        0      0;  % P1
     0   0   0       1.053   1.1116  -1        0        0      0;  % P2
     0   0   0       0       0        0       -1        1.136  0;  % P3
     0   0   0       0       0        eta(3)   eta(4)   0      0;  % P4=etanohol
     0   0   0       0       0        eta(1)   eta(2)   -1     0;  % Cells
     0   0   0       0       0        0        0        0     -1]; % Xylan


r_cor=(x(1)/N*3/4/pi)^(1/3);         % core radius at time t [cm]
% r_l1=((x(11)+x(1))/N*3/4/pi)^(1/3); % layer-1 radius at time t [cm] {from
amount of xylane-2}
dr_l1=r_par-r_cor;                    % particle radius - core radius at
time t [cm]

% Numerical improvements
if dr_l1<=0
```

```
   dr_l1=1e-15;
end

% Reaction rates [mg/ml/h]
R(1)=kdE1*x(4);
R(2)=kdE2*x(5);
R(3)=k1r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K1IG2+x(7)/K1IG);
R(4)=k3r*E2f*x(2)/(K3M*(1+x(3)/K3IG)+x(2));       %%% E2fL=x(5)
R(5)=k2r*E1bC*SR*ro*wC*x(1)/Vtot/(1+x(6)/K2IG2+x(7)/K2IG);
R(6)=vmax(1)*x(8)*x(14)/(KM(1)+x(8));
R(7)=vmax(2)*x(11)*x(14)/(KM(2)+x(11));
R(8)=kd_cell*x(14);
R(9)=x(9)/(x(1)*ro*wC/Vtot)*(R(3)+R(5))*k_Xy;

%Production rate of each compound [mg/ml/h]
rr=aa*R';

% Mass balances
dx(1)=rr(3)*Vtot/ro/wC; % total volume of core [ml]
dx(2)=(x(6)-x(2))/dr_l1*De(1,2)*4*pi*r_par^2/Vtot*N+aa(4,4)*R(4); % c_P1 in
bulk [mg/ml]
dx(3)=(x(7)-x(3))/dr_l1*De(2,2)*4*pi*r_par^2/Vtot*N+aa(5,4)*R(4)-
P(2)*Aspec*x(14)*(x(3)-x(8)); % c_P2 in bulk [mg/ml]
dx(4)=rr(1);    % c_E1 in system [mg/ml]
dx(5)=rr(2);    % c_E2 in system [mg/ml]
dx(6)=aa(4,3)*R(3)*Vtot-(x(6)-x(2))/dr_l1*De(1,2)*4*pi*r_cor^2*N;   % c_P1
in core [mg/ml]
dx(7)=aa(5,5)*R(5)*Vtot-(x(7)-x(3))/dr_l1*De(2,2)*4*pi*r_cor^2*N;   % c_P2
in core [mg/ml]
dx(8)=(aa(5,6)*R(6)+P(2)*Aspec*x(14)*(x(3)-x(8)))*Vtot;              % c_P2
in ps
dx(9)=rr(9);                                                         %
c_Xylan [mg/ml]
dx(10)=aa(6,8)*R(9)*Vtot-(x(10)-x(13))/dr_l1*De(3,2)*4*pi*r_par^2*N; % c_P3
in core [mg/ml]
dx(11)=(P(3)*Aspec*x(14)*(x(13)-x(11))+aa(6,7)*R(7))*Vtot;           % c_P3
in ps [mg/ml]
dx(12)=rr(7);
% c_P4 in bulk [mg/ml]
dx(13)=(x(10)-x(13))/dr_l1*De(3,2)*N*4*pi*r_par^2/Vtot-
P(3)*Aspec*x(14)*(x(13)-x(11)); % c_P3 in bulk [mg/ml]
dx(14)=flg_cell*rr(8);                          % c_cell [mg/ml]
dx(15)=flg_cell*rr(8)*Vtot/mcell*Vcell*vf_ps; % Vps_tot [ml]
dx=dx';

function red=LI_eq(y,x,E1max,E2maxL,K1ad,K2adL,wC,wL,Vpar,Vtot,ro,flg)

L=Vpar*ro*wL/Vtot/1000; % total lignin amount [g]
S=L+(x(1)*wC*ro/Vtot)/1000;   % total substrate amount at time t [g]
E1f=x(4)-y(1);
if flg=='y'
    %%% With "Lignin's not productive adsorption"
    E2f=x(5)-y(3);
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(2)=y(2)/L-E1max*K1ad*E1f/(1+K1ad*E1f);
    red(3)=y(3)/L-E2maxL*K2adL*E2f/(1+K2adL*E2f);
elseif flg=='n'
    %%% Without "Lignin's not productive adsorption"
    red(1)=y(1)/S-E1max*K1ad*E1f/(1+K1ad*E1f);
```

```
end

function
M=massfunction(t,x,Vtot,N,ro,wC,wL,wXy,cXy_in,De,r_par,Vpar,flg,flg_cell,P,
Aspec,Vcell,vf_ps,mcell,MW,param,k_Xy)
% Mass matrix of ode equations
M=[ 1     0 0 0 0 0     0     0 0 0 0 0 0 0 0 ;
    0     1 0 0 0 0     0     0 0 0 0 0 0 0 0 ;
    0     0 1 0 0 0     0     0 0 0 0 0 0 0 0 ;
    0     0 0 1 0 0     0     0 0 0 0 0 0 0 0 ;
    0     0 0 0 1 0     0     0 0 0 0 0 0 0 0 ;
    x(6)  0 0 0 0 x(1)  0     0 0 0 0 0 0 0 0 ;
    x(7)  0 0 0 0 0     x(1)  0 0 0 0 0 0 0 0 ;
    0     0 0 0 0 0     0     x(15) 0 0 0 0 0 0 x(8);
    0     0 0 0 0 0     0     0 1 0 0 0 0 0 0 ;
    x(10) 0 0 0 0 0 0   0     0 0 x(1) 0 0 0 0 0 ;
    0      0 0 0 0 0 0  0     0 0 0 x(15) 0 0 0 x(11);
    0     0 0 0 0 0     0     0 0 0 0     1 0 0 0;
    0     0 0 0 0 0     0     0 0 0 0     0 1 0 0;
    0     0 0 0 0 0     0     0 0 0 0     0 0 1 0;
    0     0 0 0 0 0     0     0 0 0 0     0 0 0 1];
```

Sensitivity analysis

Script to run sensitivity analysis of process 2 with $k_{Xy} = 1$.

```
% Sensitivity analysis
 clear all
 clc

param_bc=[16.5 0.04 0.1  7.1   132.5    0.01    267.6   25.5 2.1   0.6 0.75
42.55 173.5 1 1.007 0.52 0.41 0.42 0.42  2.88e-4  2.50e-4...
6    6    8e-4 3     1.13e-9  0.58    1e-8 1e-8  1  0.05];

delta=0.02; %
l=length(param_bc);
[tx]=B_xyl_equal_cell(param_bc);
hh=1;
[m,n]=size(tx);

for i=1:m
    if tx(hh,17)>=95
        index_star_bc=[hh,hh];
        X_bc=[tx(hh,17),tx(hh,18)];
        break
    end
    hh=hh+1;
end

pm=[+1,-1];
sens=zeros(l,4);
ss=0;
TX={};
for i=1:l
    vet_sens=[];
    for j=1:2
        param=param_bc;
        param(i)=param_bc(i)*(1+delta*pm(j));
        k_Xy=param(30);
        if k_Xy<1-5e-4
           [tx]=B_xyl_less_cell(param);
        elseif k_Xy<=1+5e-4 && k_Xy>=1-5e-4
           [tx]=B_xyl_equal_cell(param);
        elseif k_Xy>1+5e-4
           [tx]=B_cell_less_xyl_new(param);
        end
        TX{i,j}=tx;
        [m,n]=size(tx);
        X_ts=[tx(index_star_bc(1),n-1),tx(index_star_bc(2),n)];
        vet_sens=[vet_sens,abs((X_ts-X_bc)./X_bc/delta)*100];
    end
    vet_sens
    ss=ss+1
    sens(i,:)=vet_sens;
    sens1(i,:)=[vet_sens(1:2)*X_bc(1)/100*delta./(param_bc(i)'*0.02),
vet_sens(3:4)*X_bc(2)/100*delta./(param_bc'*0.02)];
end
```

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