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Hydrolysis of Lignocellulosic Biomass: Current Status of Processes and Technologies and Future Perspectives

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1. Introduction

Bioethanol can be produced from several different biomass feedstocks: sucrose rich feedstocks (e.g. sugar-cane), starchy materials (e.g. corn grain), and **lignocellulosic biomass.** This last category, including biomass such as corn stover and wheat straw, woody residues from forest thinning and paper, is promising especially in those countries with limited lands availability. In fact, residues are often widely available and do not compete with food production in terms of land destination. The process converting the biomass biopolymers to fermentable sugars is called **hydrolysis**. There are two major categories of methods employed. The first and older method uses acids as catalysts, while the second uses enzymes called **cellulases**. Feedstock **pretreatment** has been recognized as a necessary upstream process to remove lignin and enhance the porosity of the lignocellulosic materials prior to the enzymatic process (Zhu & Pan, 2010; Kumar et al., 2009).

Cellulases are proteins that have been conventionally divided into three major groups: **endoglucanase**, which attacks low cristallinity regions in the cellulose fibers by endoaction, creating free chain-ends; **exoglucanases** or cellobiohydrolases which hydrolyze the 1, 4-glycocidyl linkages to form cellobiose; and **β-glucosidase** which converts cello-oligosaccharides and disaccharide cellobiose into glucose residues. In addition to the three major groups of cellulose enzymes, there are also a number of other enzymes that attack hemicelluloses, such as **glucoronide**, **acetylesterase**, **xylanase**, **β-xylosidase**, **galactomannase** and **glucomannase**. These enzymes work together synergistically to attack cellulose and hemicellulose. Cellulases are produced by various **bacteria and fungi** that can have cellulolytic mechanisms significantly different.

The use of enzymes in the hydrolysis of cellulose is more effective than the use of inorganic catalysts, because enzymes are highly specific and can work at mild process conditions. In spite of these advantages, the use of **enzymes in industrial processes** is still limited by

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several factors: most enzymes are relatively unstable at high temperatures, the costs of enzyme isolation and purification are high and it is quite difficult to recover them from the reaction mixtures. Currently, extensive research is being carried out on cellulases with **improved thermostability**. These enzymes have high specific activity and increased flexibility. For these reasons they could work at low dosages and the higher working temperatures could speed up the hydrolysis reaction time. As consequence, the overall process costs could be reduced. Thermostable enzymes could play an important role in assisting the liquefaction of concentrated biomass suspensions necessary to achieve ethanol concentrations in the range 4-5 wt%.

The immobilization of enzymes has also been proposed to remove some limitations in the enzymatic process (Hong et al., 2008). The main advantage is an easier recovery and reuse of the catalysts for more reaction loops. Also, enzyme immobilization frequently results in improved thermostability or resistance to shear inactivation and so, in general, it can help to extend the enzymes lifetime.

This chapter contains an overview of the lignocellulosic hydrolysis process. Several process issues will be deepened: cellulase enzyme systems and hydrolysis mechanisms of cellulose; commercial mixtures; currents limits in the cellulose hydrolysis; innovative bioprocesses and improved biocatalysts.

2. Structure of lignocellulose biomass

Lignocellulosic biomass is typically nonedible plant material, including dedicated crops of wood and grass, and agro-forest residues. Lignocellulosics are mainly composed of **cellulose, hemicellulose, and lignin**.

Cellulose is a homopolysaccharide composed of β-D-pyranose units, linked by β-1, 4-glycosidic bonds. Cellobiose is the smallest repetitive unit and it is formed by two glucose monomers. The long-chain cellulose polymers are packed together **into microfibrils** by hydrogen and van der Waals bonds. Hemicellulose and lignin cover the microfibils (Fig.1). Hemicellulose is a mixture of polysaccharides, including pentoses, hexoses and uronic acids. Lignin is the most complex natural polymer consisting of a predominant building block of phenylpropane units. More specifically, p-coumaryl alcohol, coniferyl alcohol and sinapyl alcohol are the most commonly encountered alcohols (Harmesen et al., 2010). Lignocellulosic materials also contain small amounts of pectin, proteins, extractives (i.e. no- structural sugars, nitrogenous material, chlorophyll and waxes) and ash (Kumar et al., 2009).

The composition of the biomass constituents can vary greatly among various sources (Table 1). Accurate measurements of the biomass constituents, mainly lignin and carbohydrates, are of prime importance because they assist tailored process designs for the maximum recovery of energy and products from the raw materials.

Since 1900, researchers have developed several methods to measure the lignin and carbohydrates content of lignocellulosic biomass. Globally recognized Organizations, such as American Society for Testing and Materials (ASTM), Technical Association of the Pulp and Paper Industry (TAPPI) and National Renewable energy and Laboratory (NREL) have developed methods to determine the chemical composition of biomass, based on modifications of the two main procedures developed by Ritter (Ritter et al., 1932) and by Seaman (Saeman et al., 1954), (Table 2).

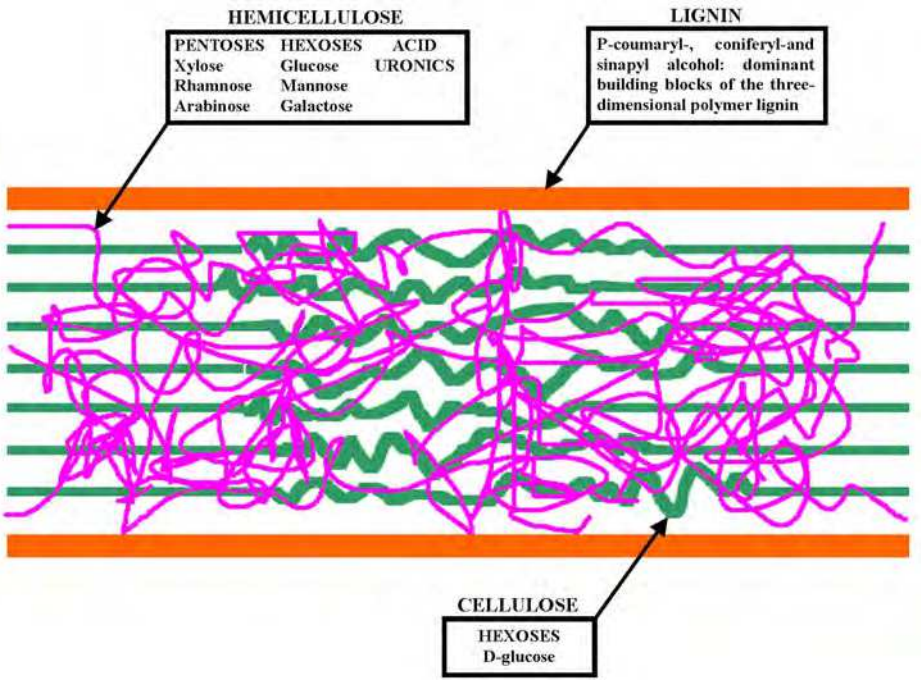

Fig. 1. Lignocellulosic materials: composition of major compounds (Kumar, 2009)

Lignocellulosic materials	Cellulose (%)	Hemicellulose (%)	Lignin (%)
Coastal bermudagrass	25	35.7	6.4
Corn Cobs	45	35	15
Cotton seed hairs	80-95	5-20	0
Grasses	25-40	35-50	10-30
Hardwoods steam	40-55	24-40	18-25
Leaves	15-20	80-85	0
Newspaper	40-55	25-40	18-30
Nut shells	25-30	25-30	30-40
Paper	85-99	0	0-15
Primary wastewater solids	8-15	NA	24-29
Softwoods stems	45-50	25-35	25-35
Solid cattle manure	1.6-4.7	1.4-3.3	2.7-5.7
Sorted refuse	60	20	20
Swine waste	6.0	28	NA
Switchgrass	45	31.4	12.0
Waste papers from chemical pulps	60-70	10-20	5-10
Wheat straw	30	50	15

Table 1. Composition of some common sources of biomass (Sun and Cheng, 2002)

TAPPI		ASTM		NREL
Method	**Title**	**Method**	**Title**	**Title**
T 13 os 54; Later T222 om-06	Lignin in Wood (original) Acid- Insoluble Lignin in Wood and Pulp (later)	D 1106-96 (2007)	Standard Test Method for Cromatographic Analysis of Chemically Refined Cellulose (1996)	Determination of Structural Carbohydrates and Lignin in Biomass
T249 cm-00	Carboydrate Composition of Extractive –Free Wood and Wood Pulp by Gas-Liquid Chromatography	ASTM D1915-63 (1989) withdrawn, replaced by D5896	Standard Test Method for Chromatographic Analysis of Chemically Refined Cellulose (1996)	
		AST D5896-96	Standard Test Method for Carbohydrate Distribution of Cellulosic Material	
		E1721	Standard Test Method for Determination of Acid-Insoluble Residue in Biomass	
		E1758	Determination of Carbohydrates in Biomass by High Performance Liquid Chromatography	

Table 2. Methods provided by globally recognized organizations for the chemical composition of biomass (Sluiter et al., 2010)

3. Products from lignocellulosic biomass

Lignocellulosic biomass is a potential source of several bio-based products according to the **biorefinery** approach. Currently, the products made from bioresources represent only a minor fraction of the chemical industry production. However, the interest in the bio-based products has increased because of the rapidly rising barrel costs and an increasing concern about the depletion of the fossil resources in the near future (Hatti-Kaul et al., 2007). The goal of the biorefinery approach is the generation of energy and chemicals from different biomass feedstocks, through the combination of different technologies (FitzPatrick et al., 2010).

The biorefinery scheme involves a multi-step biomass processing. The first step concerns the feedstock pretreatment through physical, biological, and chemical methods. The outputs from this step are **platform (macro) molecules or streams** that can be used for further processing (Cherubini & Ulgiati, 2010). Recently, a detailed report has been published by

DOE describing the value added chemicals that can be produced from biomass (Werpy, 2004). Figure 2 displays a general biorefinery scheme for the production of specialty polymers, fuel, or composite materials (FitzPatrick et al., 2010).
Besides ethanol, several other products can be obtained following the hydrolysis of the carbohydrates in the lignocellulosic materials. For instance, xylan/xylose contained in hemicelluloses can be thermally transformed into furans (2-furfuraldeyde, hydroxymethil furfural), short chain organic acids (formic, acetic, and propionic acids), and cheto compounds (hydroxy-1-propanone, hydroxy-1-butanone) (Güllü, 2010; Bozell & Petersen, 2010).

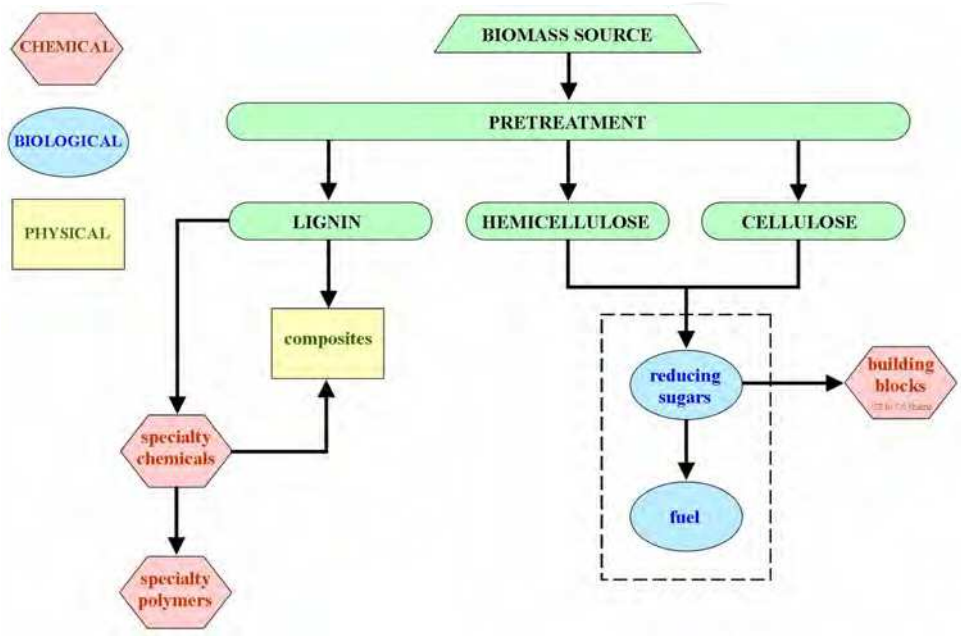

Fig. 2. Scheme of a lignocellulosic biorefinery. The shape of each step describes the type of process used, chemical, biological, and physical (legend) (FitzPatrick et al., 2010)

Furfural can be further processed to form some building blocks of innovative polymeric materials (i.e. 2, 5-furandicarboxylic acid). In addition, levulinic acid could be formed by the degradation of hydroxymethil furfural (Demirabas, 2008). Another product prepared either by fermentation or by catalytic hydrogenation of xylose is xylitol (Bozell & Petersen, 2010). Furthermore, through the chemical reduction of glucose it is possible to obtain several products, such as sorbitol (Bozell & Petersen, 2010). The residual lignin can be an intermediate product to be used for the synthesis of phenol, benzene, toluene, xylene, and other aromatics. Similarly to furfural, lignin could react to form some polymeric materials (i.e. polyurethanes) (Demirabas, 2008).

4. Production for ethanol from lignocellulosic biomass

Ethanol is the most common renewable fuel recognized as a potential alternative to petroleum-derived transportation fuels. It can be produced from lignocellulosic materials in

various ways characterized by common steps: hydrolysis of cellulose and hemicellulose to monomeric sugars, fermentation and product recovery (fig 3). The main differences lie in the hydrolysis phase, which can be performed by dilute acid, concentrated acid or enzymatically (Galbe & Zacchi, 2002).

4.1 Acid hydrolysis

The main advantage of the acid hydrolysis is that acids can penetrate lignin without any preliminary pretreatment of biomass, thus breaking down the cellulose and hemicellulose polymers to form individual sugar molecules. Several types of acids, concentrated or diluted, can be used, such as sulphurous, sulphuric, hydrocloric, hydrofluoric, phosphoric, nitric and formic acid (Galbe & Zacchi, 2002). Sulphuric and hydrochloric acids are the most commonly used catalysts for hydrolysis of lignocellulosic biomass (Lenihan et al., 2010).
The acid concentration used in the **concentrated acid hydrolysis** process is in the range of 10-30%. The process occurs at low temperatures, producing high hydrolysis yields of cellulose (i.e. 90% of theoretical glucose yield) (Iranmahboob et al., 2002).

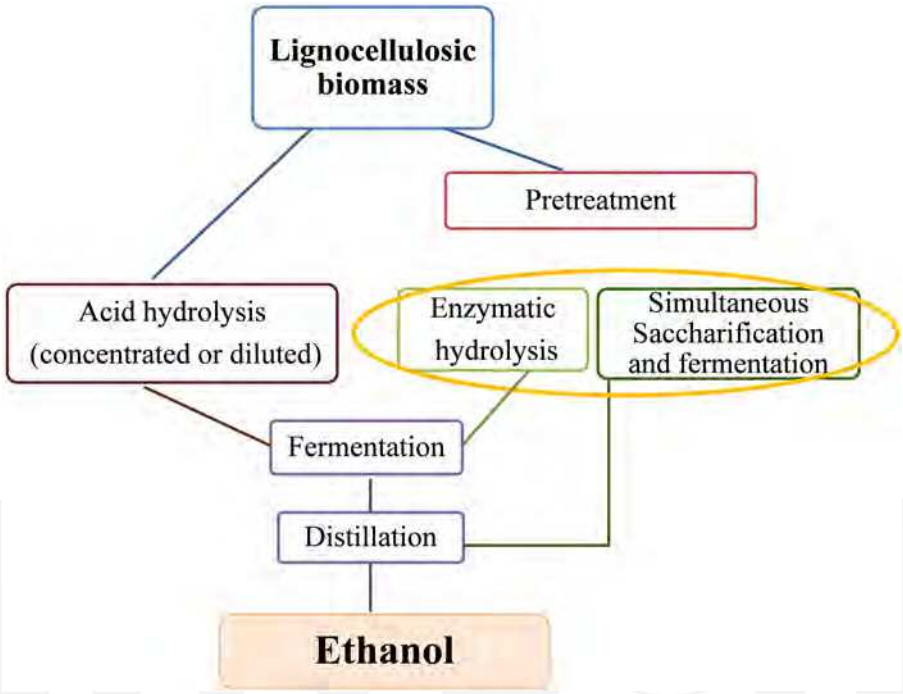

Fig. 3. Process for production ethanol from lignocellulosic biomass. The circle in the scheme indicates two alternative process routes: simultaneous hydrolysis and fermentation (SSF); separate hydrolysis and fermentation (SHF).

However, this process requires large amounts of acids causing corrosion problems to the equipments. The main advantage of the **dilute hydrolysis process** is the low amount of acid required (2-5%). However this process is carried out at high temperatures to achieve acceptable rates of cellulose conversion. The high temperature increases the rates of

hemicellulose sugars decomposition thus causing the formation of toxic compounds such as furfural and 5-hydroxymethyl-furfural (HMF). These compounds inhibit yeast cells and the subsequent fermentation stage, causing a lower ethanol production rate (Larsson et al., 1999; kootstra et al., 2009). In addition, these compounds lead to reduction of fermentable sugars (Kootstra et al., 2009). In addition, high temperatures increase the equipment corrosion (Jones & Semrau, 1984).

In 1999, the BC International (BCI) of United States has marketed a technology based on two-step dilute acid hydrolysis: the first hydrolysis stage at mild conditions (170-190°C) to hydrolyze hemicellulose; the second step at more severe conditions to hydrolyze cellulose 200-230°C (Wyman, 1999).

In 1991, the Swedish Ethanol Development Foundation developed the CASH process. This is a two-stage dilute acid process that provides the impregnation of biomass with sulphur dioxide followed by a second step in which diluted hydrochloric acid is used. In 1995, this foundation has focused researches on the conversion of softwoods using sulphuric acid (Galbe & Zacchi, 2002).

4.2 Pretreatment

A pretreatment step is necessary for the enzymatic hydrolysis process. It is able to remove the lignin layer and to decristallize cellullose so that the hydrolytic enzymes can easily access the biopolymers.The pretreatment is a critical step in the cellulosic bioethanol technology because it affects the quality and the cost of the carbohydrates containing streams (Balat et al., 2008). Pretreatments methods can be classified into different categories: physical, physiochemical, chemical, biological, electrical, or a combination of these (kumar et al., 2009), (Table 3).

On the whole, the final yield of the enzymatic process depends on the combination of several factors: biomass composition, type of pretreatment, dosage and efficiency of the hydrolytic enzymes (Alvira et al., 2010).

The use of enzymes in the hydrolysis of cellulose is more advantageous than use of chemicals, because enzymes are highly specific and can work at mild process conditions. Despite these advantages, the use of enzymes in industrial applications is still limited by several factors: the costs of enzymes isolation and purification are high; the specific activity of enzyme is low compared to the corresponding starch degrading enzymes. As consequence, the process yields increase at raising the enzymatic proteins dosage and the hydrolysis time (up to 4 days) while, on the contrary, decrease at raising the solids loadings. One typical index used to evaluate the performances of the cellulase preparations during the enzymatic hydrolysis is the **conversion rate** to say the obtained glucose concentration per time required to achieve it (g glucose/L/h/). Some authors reported conversion rates of softwoods substrates (5%w/v solids loading) in the range 0.3-1.2 g/L/h (Berlin et al., 2007). In general, compromise conditions are necessary between enzymes dosages and process time to contain the process costs.

In 2001, the cost to produce cellulase enzymes was 3-5$ per gallon of ethanol (0.8-1.32$/liter ethanol), (Novozymes and NREL)[1]. In order to reduce the cost of cellulases for bioethanol production, in 2000 the National Renewable Laboratory (NREL) of USA has started collaborations with Genencor Corporation and Novozymes. In particular, in 2004, Genencor has achieved an estimated cellulase cost in the range $0.10-0.20 per gallon of ethanol (0.03-

[1] News on: *Sci Focus Direct on Catalysts*, 2005

		Operating conditions	Advantages	Disadvantages
Physical	Chipping Grinding Milling	Room temperature Energy input < 30Kw per ton biomass	Reduces cellulose critallinity	Power consumption higher than inherent biomass energy
Physio-chemical	Steam pretreatment	160-260°C (0. 69-4.83MPa) for 5-15 min	Causes hemicellulose auto hydrolysis and lignin transformation; cost-effective for hardwoods and agricultural residues	Destruction of a portion of the xylan fraction; incomplete distruption of the lignin-carboydrate matrix; generation of inhibitory compounds; less effective for softwoods
	AFEX (Ammonia fiber explosion method)	90°C for 30 min.1-2kg ammonia /kg dry biomass	Increases accessible surface area, removes lignin and hemicellulose;	Do not modify lignin neither hydrolyzes hemicellulose;
	ARP (Ammonia recycle percolation method)	150-170°C for 14 min Fluid velocity 1cm/min	Increases accessible surface area, removes lignin and hemicellulose;	Do not modify lignin neither hydrolyzes hemicellulose;
	CO_2 explosion	4kg CO_2/kg fiber at 5.62 Mpa 160 bar for 90 min at 50 °C under supercritical carbon dioxide	Do not produce inhibitors for downstream processes. Increases accessible surface area, does not cause formation of inhibitory compounds	It is not suitable for biomass with high lignin content (such as woods and nut shells) Does not modify lignin neither hydrolyze hemicelluloses
	Ozonolysis	Room temperature	Reduce lignin content; does not produce toxic residues	Expensive for the ozone required;
	Wet oxidation	148-200°C for 30 min	Efficient removal of lignin; low formation of inhibitors; low energy demand	High cost of oxygen and alkaline catalyst
Chemical	Acid hydrolysis: dilute-acid pretreatment	Type I: T>160°, continuous-flow process for low solid loading 5-10%,)- Type II: T<160°C, batch process for high solid loadings (10-40%)	Hydrolyzes hemicellulose to xylose and other sugar; alters lignin structure	Equipment corrosion; formation of toxic substances

		Operating conditions	Advantages	Disadvantages
	Alkaline hydrolysis	Low temperature; Long time high. Concentration of the base; For soybean straw: ammonia liquor (10%) for 24 h at room temperature	Removes hemicelluloses and lignin; increases accessible surface area	Residual salts in biomass
	Organosolv	150-200 °C with or without addition of catalysts (oxalic, salicylic, acetylsalicylic acid)	Hydrolyzes lignin and hemicelluloses	High costs due to the solvents recovery
Biological		Several fungi (brown-, white- and soft-rot fungi	Degrades lignin and hemicelluloses; low energy requirements	Slow hydrolysis rates
Electrical	Pulsed electrical field in the range of 5-20 kV/cm,	~2000 pulses of 8 kV/cm	Ambient conditions; disrupts plant cells; simple equipment	Process needs more research

Table 3. Methods for biomass lignocellulosic pretreatment (Kumar et al., 2009)

0.05$/liter ethanol) in NREL´s cost model (Genencor, 2004)[2]. Similarly, collaboration between Novozymes and NREL has yielded a cost reduction in the range $0.10-0.18 per gallon of ethanol (0.03-0.047$/liter ethanol), a 30-fold reduction since 2001 (Mathew et al., 2008).

Unlike the acid hydrolysis, the enzymatic hydrolysis, still has not reached the industrial scale. Only few plants are available worldwide to investigate the process (pretreatment and bioconversion) at demo scale. More recently, the steam explosion pretreatment, investigated for several years in Italy at the ENEA research Center of Trisaia (De Bari et al., 2002, 2007), is now going to be developed at industrial scale thanks to investments from the Italian Mossi & Ghisolfi Group.

5. Enzymatic hydrolysis: Cellulases

5.1 Cellulolytic capability of organisms: Difference in the cellulose-degrading strategy

Different strategies for the cellulose degradation are used by the cellulase-producing microorganisms: aerobic bacteria and fungi secrete soluble extracellular enzymes known as *non complexed cellulase system*; anaerobic cellulolytic microorganisms produce *complexed cellulase systems*, called *cellulosomes* (Sun et al., 2002). A third strategy was proposed to explain the cellulose-degrading action of two recently discovered bacteria: the aerobic *Cytophaga hutchinsonii* and the anaerobic *Fibrobacter succinogenes* (Ilmén et al., 1997).

[2] Genencor, relations, 21 October 2004, avaible from: http:/genencor.com/cms/connect/genencor/media_relations/news/archive/2004/gen_211004_en.htm

- *Non-complexed cellulase system*. One of the most fully investigated non-*complexed cellulase system* is the *Trichoderma reesei* model. *T. reesei* (teleomorph *Hypocrea jecorina*) is a saprobic fungus, known as an efficient producer of extracellular enzymes (Bayer et al., 1998). Its non-complexed cellulase system includes two cellobiohydrolases, at least seven endoglucanases, and several β-glucosidases. However, in *T. reesei* cellulases, the amount of ß-glucosidase is lower than that needed for the efficient hydrolysis of cellulose into glucose. As a result, the major product of hydrolysis is cellobiose. This is a dimer of glucose with strong inhibition toward endo- and exoglucanases so that the accumulation of cellobiose significantly slows down the hydrolysis process (Gilkes et al., 1991). By adding ß-glucosidase to cellulases from either external sources, or by using co-culture systems, the inhibitory effect of cellobiose can be significantly reduced (Ting et al., 2009).

  It has been observed that the mechanism of cellulose enzymatic hydrolysis by *T.reesei* involves three simultaneous processes (Ting et al., 2009):

  1. Chemical and physical changes in the cellulose solid phase. The chemical stage includes changes in the degree of polymerization, while the physical changes regard all the modifications in the accessible surface area. The enzymes specific function involved in this step is the *endoglucanase*.
  2. Primary hydrolysis. This process is slow and involves the release of soluble intermediates from the cellulose surface. The activity involved in this step is the *cellobiohydrolase.*
  3. Secondary hydrolysis. This process involves the further hydrolysis of the soluble fractions to lower molecular weight intermediates, and ultimately to glucose. This step is much faster than the primary hydrolysis and *β-glucosidases* play a role for the secondary hydrolysis.

- *Complexed cellulase system.* Cellulosomes are produced mainly by anaerobic bacteria, but their presence have also been described in a few anaerobic fungi from species such as *Neocallimastix*, *Piromyces*, and *Orpinomyces* (Tatsumi et al., 2006; Watanabe & Tokuda, 2010). In the domain Bacteria, organisms possessing cellulosomes are only found in the phylum *Firmicutes*, class *Clostridia*, order *Clostridiales* and in the *Lachnospiraceae* and *Clostridiaceae* families. In this latter family, bacteria with cellulosomes are found in various clusters of the genus Clostridium (McCarter & Whiters, 1994; Wilson, 2008).

  Cellulosomes are protuberances produced on the cell wall of the cellulolytic bacteria grown on cellulosic materials. These protuberances are stable enzyme complexes tightly bound to the bacteria cell wall but flexible enough to bind strongly to cellulose (Lentig & Warmoeskerken, 2001). A cellulosome contains two types of subunits: *non-catalytic subunits*, called *scaffoldins*, and *enzymatic subunits*. The scaffoldin is a functional unit of cellusome, which contain multiple copies of *cohesins* that interact selectively with domains of the *enzymatic subunits*, CBD (cellulose binding domains) and CBM (carbohydrates binding modules). These have complementary cohesins, called dockerins, which are specific for each bacterial species (Fig. 4) (Gilligan & Reese, 1954; Lynd et al., 2002; Arai et al., 2006;).

  For the bacterial cell, the biosynthesis of a cellulosome enables a specific adhesion to the substrate of interest without competition with other microorganisms. The cellulosome allows several advantages: (1) synergism of the cellulases; (2) absence of unspecific adsorption (McCarter & Whiters, 1994; Zhang & Lynd, 2004). Thanks to its intrinsic Lego-like architecture, cellulosomes may provide great potential in the biofuel industry.

The concept of cellulosome was firstly discovered in the thermophilic cellulolytic and anaerobic bacterium, *Clostridium thermocellum* (Wyman, 1996). It consists of a large number of proteins, including several cellulases and hemicellulases. Other enzymes that can be included in the cellulosome are lichenases.

- *Third cellulose-degrading strategy*. The third strategy was recently proposed to explain the cellulose-degrading behavior of two recently sequenced bacteria: *Cytophaga hutchinsonii* and *Fibrobacter succinogenes* (Ilmén, 1997). *C. hutchinsonii* is an abundant aerobic cellulolytic soil bacterium (Fägerstam & Petterson, 1984), while *F. succinogenes* is an anaerobic rumen bacterium which was isolated by the Rockville, (Maryland), and San

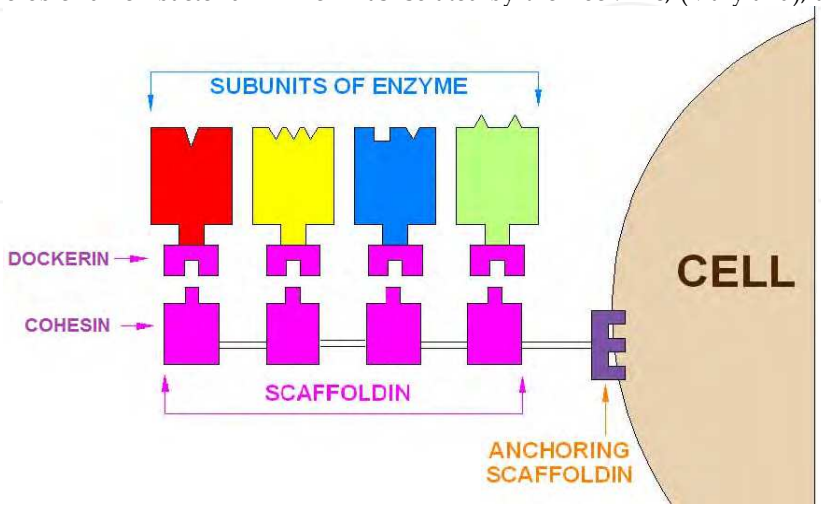

Fig. 4. Schematic representation of a cellulosoma

Diego (California) Institute of Genomic Research (TIGR) (Mansfield et al., 1998). In the aerobic *C. hutchinsonii* no genes were found to code for CBM and in the anaerobic *F. succinogenes* no genes were identified to encode dockerin and scaffoldin. Thus, a third cellulose degrading mechanism was proposed. It includes the binding of individual cellulose molecules by outer membrane proteins of the microrganisms followed by the transport into the periplasmic space where they are degraded by endoglucanases (Ilmén, 1997).

5.2 Characteristics of the commercial hydrolytic enzymes

Most cellulase enzymes are relatively unstable at high temperatures. The maximum activity for most fungal cellulases and β-glucosidase occurs at 50±5°C and a pH 4.5- 5 (Taherzadeh & Karimi, 2007; Galbe & Zacchi, 2002). Usually, they lose about 60% of their activity in the temperature range 50–60 °C and almost completely lose activity at 80°C (Gautam et al., 2010). However, the enzymes activity depends on the hydrolysis duration and on the source of the enzymes (Tengborg et al., 2001). In general, cellulases are quite difficult to use for prolonged operations.

As mentioned before, the enzyme production costs mainly depend on the productivity of the enzymes-producing microbial strain. Filamentous fungi are the major source of cellulases and mutant strains of *Trichoderma* (*T. viride, T. reesei, T. longibrachiatum*) have long

been considered to be the most productive (Gusakov et al., 2007; Galbe & Zacchi, 2002). Preparations of cellulases from a single organism may not be highly efficient for the hydrolysis of different feedstocks. For example, *Thrichoderma reesei* produces endoglucanases and exoglucanases in large quantities, but its β-glucosidase activity is low, resulting in an inefficient biomass hydrolysis. For this reason, the goal of the enzymes producing companies has been to form cellulases cocktails by enzymes assembly (multienzyme mixtures) or to construct engineered microrganisms to express the desired mixtures (Mathew et al., 2008). Enzyme mixtures often derive from the co-fermentation of several micro-organisms (Ahamed & Vermette, 2008; Kabel et al., 2005; Berlin et al., 2007), (Table 4). All the commercial cellulases listed in table 4 have an optimal condition at 50°C and pH of 4.0-5.0. More recently, some enzymes producers have marked new mixtures able to work in a higher temperature ranging from 50 to 60°C (Table5).

In 2010, new enzymes were produced by two leading companies, Novozymes and Genencor, supported by the USA Department of Energy (DOE). Genencor has launched four new blends: **Accelerase®1500, Accelerase®XP, Accelerase®XC and Accelerase®BG**. Accelerase®1500 is a cellulases complex (exoglucanase, endoglucanase, hemi-cellulase and β-glucosidase) produced from a genetically modified strain of *T. reesei.* All the other Accelerase are accessory enzymes complexes: Accelerase®XP enhances both xylan and glucan conversion; Accelerase®XC contains hemicellulose and cellulase activities; Accelerase® BG is a β-glucosidase enzyme. In February 2010, Genencor has developed an enzyme complex known as **Accellerase®Duet** which is produced with a genetically modified strain of *T. reesei* and that contains not only exoglucanase, endoglucanase, β-glucosidase, but includes also xylanase. This product is capable of hydrolyzing lignocellulosic biomass into fermentable monosaccharides such as glucose and xylose (Genencos, 2010)[3]. Similarly, Novozymes has produced and commercialized two new enzymatic mixtures: **cellic Ctec**, and **cellic Htec**. **Cellic CTec** is used in combination with Cellic HTec and this mixture is capable to work with a wide variety of pretreated feedstocks, such as sugarcane bagasse, corn cob, corn fiber, and wood pulp, for the conversion of the carbohydrates in these materials into simple sugars (Novozyme, 2010)[4].

In order to meet the future challenges, innovative bioprocesses for the production of new generation of enzymes are needed. As already described, conventional cellulases work within a range of temperature around 50°C and they are typically inactivated at temperatures above 60-70 °C due to disorganization of their three dimensional structures followed by an irreversible denaturation (Viikari et al., 2007). Some opportunities of process improvement derive from the use of thermostable enzymes.

5.3 Enzymes for the cellulose liquefaction: Thermophilic enzymes

The thermophilic microrganisms can be grouped in thermophiles (growth up to 60 °C), extreme thermophiles (65-80 °C) and hyperthermophiles (85-110 °C). The unique stability of the enzymes produced by these microrganisms at elevated temperatures, extreme pH and high pressure (up to 1000 bar) makes them a valuable resource for the industrial

[3] Genencor, products, 14 January 2010, avaible from: http:// www.genencor.com/ wps/ wcm/ connect/ genencor/ genencor/ products and services/ business development/ biorefineries/ products/ accellerase product line en.htm

[4] Novozyme, brochure, 29 January 2010, Viable from: http:// www.bioenergy. novozymes.com/ files/ documents/ Final%20Cellic%20Product%20Brochure_ 29Jan2010.pdf

Commercial mixture	FPU (U/ml)[a]	Cellobiase (U/ml)[b]	Proteins (U/ml)[c]	Source	Supplier
Bio-feed beta L	<5	12	8	*T. longibrachiatum T. reesei*	Novozymes (Bagsvaerd, Denmark)
Cellubrix (Celluclast)	56	136	43	*T. longibrachiatum A. niger*	Novozymes
Cellulase 2000L	10	nd	7	*T. longibrachiatum T. reesei*	Rodhia –Danisco (Vinay, France)
Cellulyve 50L	24	nd	34	*T. longibrachiatum T. reesei*	Lyven (Colombelles France)
Energex L	<5	19	28	*T. longibrachiatum T. reesei*	Novozymes
GC220	116	215	64	*T. longibrachiatum T. reesei*	Genencor-Danisco (Rochester, USA)
GC440	<5	70	29	*T. longibrachiatum T. reesei*	Genencor
GC880	<5	86	43	*T. longibrachiatum T. reesei*	Genencor
Novozymes 188	<5	1,116	57	*A.niger*	Novozymes
Rohament CL	51	28	44	*T. longibrachiatum T. reesei*	Rhom-AB Enzymes (Rajamäki, Finland)
Spezyme CP	49	nd	41	*T. longibrachiatum T. reesei*	Genencor
Ultraflo L	<5	20	18	*T. longibrachiatum T. reesei*	Novozymes
Viscozyme L	<5	23	27	*T. longibrachiatum T. reesei*	Novozymes
Viscostar 150L	33	111	40	*T. longibrachiatum T. reesei*	Dyadic (Jupiter, Usa)

A) One FPU (filter paper unit) is the amount of enzyme that forms 1 μmol of reducing sugars/min during the hydrolysis reaction of filter paper Whatman No.1
B) One CBU (cellobiase unit) corresponds to the amount of enzyme which forms 2 μmol of glucose/min from cellobiose

Table 4. Commercial cellulases

Commercial mixture	B-glucosidase activity(U/ml)[a]	pH	Temperature (°C)	Source	Supplier
Biocellulase A	32	5	55	*A. niger*	Quest Intl. (Sarasota, Fl)
Cellulase AP 30 K	60	4.5	60	*A. niger*	Amano Enzyme Inc.

Table 5. Commercial cellulases able to work at temperature ranging from 50 to 60°C.

bioprocesses that run at harsh conditions (Demain et al., 2005). Of special interest is the thermoactivity and thermostability of these enzymes in the presence of high concentrations of organic solvents, detergents and alcohols. On the whole, thermophilic enzymes have an increased resistance to many denaturing conditions such as the use of detergents which can be often the unique efficient mean to obviate the irreversible adsorption of cellulases on the substrates. Furthermore, the utilization of high operation temperatures, which cause a decrease in viscosity and an increase in the diffusion coefficients of substrates, have a significant influence on the cellulose solubilization. It is worth noting that, differently from the mesophilic enzymes, most thermophilic cellulases did not show inhibition at high level of reaction products (e.g. cellobiose and glucose). As consequence, higher reaction rates and higher process yields are expected (Bergquist et al., 2004). The high process temperature also reduces any contamination of the fermentation medium.

Several cellulose degrading enzymes from various thermophilic organisms have been investigated. These include cellulases mainly isolated from anaerobic bacteria such as *Anaerocellum thermophilum* (Zverlov et al., 1998), *Clostridium thermocellum* (Romaniec et al., 1992), *Clostridium stercorarium* (Bronnenmeier et al., 1991; Bronnenmeier & Staudenbauer, 1990) and *Caldocellum saccharolyticum* (Te'o V et l., 1995), *Pyrococcus furiosus* (Ma & Adams, 1994), *Pyrococcus horikoshi* (Rahman et al.,1998), *Rhodothermus* strains (Hreggvidsson et al., 1996), *Thermotoga sp.*, (Ruttersmith et al., 1991), *Thermotoga marittima* (Bronnenmeier et al., 1995), *Thermotoga neapolitana* (Bok et al., 1998).

Xylanase have been detected in *Acidothermus cellulolyticus* in different *Thermus*, *Bacillus*, *Geobacillus*, *Alicyclobacillus* and *Sulfolobales* species (Sakon et al., 1996).

Although many cellulolytic anaerobic bacteria such as *Clostridium thermocellum* produce cellulases with high specific activity, they do not produce high enzymes quantities. Since the anaerobes show limited growth, most researches on thermostable cellulases production have been addressed to aerobic species. Several mesophilic or moderately thermophilic fungal strains are also known to produce enzymes stable and active at high temperatures. These enzymes are produced from species such as *Chaetomium thermophila* (Venturi et al., 2002), *Talaromyces emersonii* (Grassick et al., 2004), *Thermoascus aurantiacus* (Parry et al., 2002). They may be stable at temperatures around 70 °C for prolonged periods. Table 6 summarizes some of thermostable enzymes isolated from Archea, Bacteria and Fungi.

During the last decade several efforts have been devoted to develop different mixtures of selected thermostable enzymes. In 2007, mixtures of thermostable enzymes, including cellulases from *Thermoascus auranticus*, *Thrichoderma reseei*, *Acremonium thermophilum* and *Thermoascus auranticus*, have been produced by ROAL, Finland (Viikari et al., 2007). Multienzyme mixtures were also reconstituted using purified *Chrysosporium lucknowense* enzymes (Gusakov et al., 2005).

Despite the noticeable advantages of thermostable enzymes, cultivation of thermophiles and hyperthermophyles requires special and expensive media, and it is hampered by the low specific growth rates and product inhibition (Krahe et al., 1996; Schiraldi et al., 2002;Turner et al., 2007). Large scale commercial production of thermostable enzymes still remains a challenge also dependent on the optimization of their production from mesophilic microorganisms.

6. Immobilization of enzymes

Thanks to the latest breakthroughs in the research for improving the enzymes, nowadays most enzymes are produced for a commercially acceptable price. Nonetheless, the industrial

Archea					
Enzymes	**Organism**	**pH optimum**	**T optimum (°C)**	**Stability (half life)**	***Refs.***
β-glucosidase	*Pyrococcus furiousus*	5	102	13h at 110°C	*Ma & Adams, 1994*
	Pyrococcus horikoshi	6	100	15h at 90°C	*Rahman et al., 1998*
Endoglucanase	*Pyrococcus furiousus*	6	100	40h at 95°C	*Bergquist et al., 2004*
	Pyrococcus horikoshi	6-6.5	100	19h at 100°C	*Bergquist et al., 2004*
Bacteria					
Enzymes	**Organism**	**pH optimum**	**T optimum (°C)**	**Stability (half life)**	**Refs.**
Endoglucanase	*Acidothermus cellulolyticus*	5.0	83	Inactivated at 110°C	*Sakon J. et al. 1996*
	Anaerocellum thermophilum	5-6	95-100	40min at 100°C	*Zverliv et al., 1998*
	Clostridium stercorarium	6-6.5	90	Stable for several days	*Bronnenmeier K et al., 1991*
	Clostridium thermocellum	6.6	70	33% of activity remained after 50h at 60°C	*Bergquist et al., 2004*
	Clostridium thermocellum	7.0	70	50% of activity remained after 48h at 60°C	*Romaniec et al. 1992*
	Rhodothermus marinus	7.0	95	50% of activity remained after 3.5h at 100°C, 80% after 16h at 90°C	*Bergquist et al., 2004*
	Thermotoga marittima	6.0-7.5	95	2h at 95°C	*Bronnenmeier K, et al., 1995*
	Thermotoga neapolitana	6.0	95	>240min at 100°C	*Bok JD et al., 1995*
Exoglucanase	*Clostridium stercorarium*	5-6	75	3 days at 70°C	*Bronnenmeier K et al., 1990*
Fungal					
Enzymes	**Organism**	**pH optimum**	**T optimum (°C)**	**Stability (half life)**	**Refs.**

Endoglucanase	*Chaetomium termphilum*	4.0	60	60min at 60°C	*Venturi L. Et al., 2002*
	Thermoascus aurantiacus	4.5	75	98h at 70°C and 41h at 75°C	*Parry N., 2002*
Exoglucanase (CBH IA)	*Talaromyces emersonii*	3.6	78	34 min at 80°C	*Grassik A., 2004*

Table 6. Thermostable cellulases

utilization of cellulases could be even more convenient by improving their stability in long-term operations and by developing methods/processes for the downstream recovery and reuse. These objectives can be achieved by the **immobilization of the enzymes** (Cao, 2005).
The main advantages of the enzyme immobilization are:

1. more convenient handling of enzymes
2. easy separation from the product
3. minimal or no protein contamination of the product
4. possible recovery and reuse of enzymes
5. enhanced stability under storage and operational conditions (e.g. towards denaturation by heat or organic solvents or by autolysis) (Sheldon, 2007).

The main methods of enzyme immobilization can be classified into four classes: support binding (carrier), entrapment, encapsulation and cross-linking.
Support binding is based on fixing the enzyme to the external or internal surface of a substrate, by physical (adsorption), ionic or covalent bonding. **Adsorption** is a simple and inexpensive method of immobilization, and does not modify the enzyme chemical structure. However, it does not produce strong bonds between enzyme and substrate and this could cause a progressive lost of the enzyme from the support. **Ionic-binding** determines a strong bond between enzyme and support. The supports may be functionalized with a variety of chemical groups to achieve the ionic interaction, including quaternary ammonium, diethylaminoethyl and carboxymethyl derivates (Brady & Joordan, 2009). **Covalent binding** is the most widely used method of immobilization. Here the amino group of lysine is typically used as point of covalent attachment (Brady & Joordan, 2009). Lysine is a very common amino-acid in proteins, often localized on the surface of proteins. It has a good reactivity and provides acceptable bonds stability (Krenkova & Forest, 2004). Supports containing *epoxy groups* are widely used in the immobilization by covalent binding. These can react with lysine and with many other nucleophilic groups on the protein surface (e.g. Cys, Hys, and Tyr). Epoxy groups also react, in a slower way, with carboxylic groups (Mateo et al., 2007). The support used in this immobilization method is typically a prefabricated carrier, such as synthetic resins, biopolymers, inorganic polymers such as silica or zeolites.
Entrapment is based on inclusion of the enzyme in a polymer network (i.e. organic polymer, silica sol-gel). Unlike the previous methods, entrapment requires the synthesis of the polymeric network in the presence of the enzyme (Sheldon, 2007). This method has the advantage of protecting the enzyme from direct contact with the environment, reducing the effects of mechanical sheer and hydrophobic solvents. However, low amount of enzymes can be immobilized (Lalonde & Margolin, 2002).
Encapsulation is a method similar to entrapment, but, in this case, the enzyme is enclosed in a membrane that acts as a physical barrier around it (Cao L., 2005). The disadvantage is that entrapping or encapsulating matrix offer a certain resistance to the substrates diffusion.

Cross-linking results in the formation of enzyme aggregates by using bifunctional reagent, like glutaraldehyde, able to bind enzymes to each other without resorting to any support. In 1996, cross-linked enzyme crystals (CLEC; St. Clair and Navia 1992) were commercialized by Altus Biologics (Margolin, 1996). However the CLEC formation requires laborious and expensive processes of protein purification and it is applicable only to crystallisable enzymes. In addition, only one kind of enzyme can be used in the CLEC formation (Brady & Joordan, 2009). In 2001 a less-expensive method, known as CLEA (cross linked enzyme aggregates) was developed in Sheldon´s Laboratory and commercialized by CLEA Technologies (Netherlands), (Sheldon et al., 2005). Recently a new method has been developed, especially suitable for lipase immobilization. It is defined Spherezymes and it is based on the formation of a water-in-oil emulsion, in which lipases and surfactant are dissolved. Following the addition of a bifunctional cross-linker, permanent spherical particles of enzyme are generated (Brady & Joordan, 2009).
The most interesting immobilization procedures are in the area of covalent binding. Supports containing epoxy groups are widely used in the immobilization by covalent binding because these generate intense multipoint covalent attachment with different nucleophiles present on the surface of the enzyme molecules (Mateo et al., 2007). One limitation of the epoxy supports is the slow reaction of immobilization. To overcome this problem, Mateo and coworkers have designed epoxy supports able to ensure a mild physical adsorption of the enzymes followed by a very fast intramolecular covalent binding with the material epoxy groups. These supports were used to immobilize and stabilize enzymes such as glutaryl acylase (Mateo et al., 2001), β-galactosidase from *Thermus sp.* (Pessela et al., 2003), and peroxidase (Abad et al., 2002). Epoxy supports, known as **Sepabeads®** are marketed by Resindion s.r.l. and quickly have begun to supersede another commercial support, known as Eupergit. This last is a microporous, epoxy-activated acrylic beads with a diameter of 100-250μ, used for a wide variety of different enzymes (Boller et al., 2002).

6.1 Immobilization of cellulases

In literature, only few papers are available on the cellulases immobilization. This is due to the fact that cellulose is not soluble and some immobilization techniques, such as enzymes entrapment, impede the interaction enzyme-substrate. Immobilization of cellulases via covalent bonds appears to be the most suitable technique. Besides the enzyme stabilization, the covalent-immobilization allows the use of supported enzymes for several cycles of reactions (Brady & Joordan, 2009; Li et al., 2007; Mateo et al., 2007; Dourado et al., 2002; Yuan et al., 1999).
In 1999, Yuan and coworkers, immobilized cellulases onto acrylamide grafted acrylonitrile copolymer membranes (PAN) by means of glutaraldehyde. They showed that the enzyme stability was increased after the immobilization process. Also, the activity of the immobilized cellulases was higher than the free cellulases at pH 3 - 5 and at temperatures above 45 °C (Yuan et al., 1999).
In 2002, cellulases from *T. reesei* were immobilized on Eudragit L-100 by researchers of the University of Minho (Portugal). They used the commercial mixture Celluclast® 1.5L supplied by Novozymes (Denmark). This method allowed to improve the stability of the enzymes without significant loss of its specific activity. The adsorption of cellulases on Eudragit lowered the enthalpy of denaturation, but affected only slightly the denaturation temperature (Dourado et al., 2002).

In 2006, Li and coworkers, immobilized cellulase enzymes by means of liposomes. These are phospholipid vesicle, ranging in size from 25 nm to 1μm. In this method, glutaraldehyde-activated liposome bound to the enzyme thus forming the liposome-cellulase complex. Following this step, the complex was immobilized on chitosan-gel. The immobilized enzyme by the liposome molecules showed efficiency higher by 10% compared to the enzyme immobilized in chitosan-gel without liposome. The immobilized cellulase-liposome complex showed a loss of activity of 20% with respect to the original value after six cycles of reaction. Therefore, liposome-binding cellulase appeared to prevent or limit the enzyme deactivation (Li et al., 2007).
In recent investigations, two commercial cellulase enzymes (Celluclast 1.5 and Novozym 188) were immobilized on epoxy Sepabeads® support (Resindion s.rl.). The preliminary data showed that 60% of loaded Celluclast proteins were adsorbed by the support and that more than 90% of these proteins remained stably linked even after repeated washings (Verardi et al., 2011).

7. Process strategies for the hydrolysis and fermentation of lignocellulosics

After the pretreatment step, the bioconversion of lignocellulosic materials includes the biopolymers hydrolysis and the sugar fermentation. These two steps can be performed separately (SHF, separate hydrolysis and fermentation) or simultaneously (SSF, simultaneous saccharification and fermentation). SSF technology is generally considered more advantageous than SHF technology, for several reasons:

- reduced number of the process steps (Koon Ong, 2004)
- reduced end product inhibition because of the rapid conversion of glucose into ethanol by yeast (Viikari et al., 2007)
- reduced contamination by unwanted microorganisms thanks to the presence of ethanol (Elumalai & Thangavelu, 2010).

However, the optimum temperature for the enzymatic hydrolysis is typically higher than that of fermentation. Therefore, in SHF process, the temperature for the enzymatic hydrolysis can be optimized independently from the fermentation temperature, whereas a compromise must be found in SSF process (Olofsson et al., 2008). Another obstacle of the SSF process is the difficulty to carry out continuous fermentation by recirculating and reusing the yeast due to the presence of the solid residues from the hydrolysis.
High solids loadings are usually required to obtain high ethanol levels in the fermentation broths (**high gravity fermentation**). In particular, solids loadings of pretreated biomass up to 30% (w/w) could be necessary to reach an ethanol concentration of 4-5 wt% that is considered a threshold level for a sustainable distillation process. However, increasing the amount of the solids content in a bioreactor, the hydrolytic performances of the enzymes mixture tends to worsen. In particular, the high initial substrate consistency causes a **viscosity increase** (Sassner et al., 2006) that is an obstacle toward the homogeneous and effective distribution of the enzymes in the bioreactor. This problem could be partly overcome by using thermostable enzymes. In particular, the hydrolysis could be carried out in two steps: a former step at elevated temperatures with thermostable hydrolytic enzymes producing the liquefaction of biomass (SHF); the latter step, aimed at completing the biomass saccharification, could be carried out at milder temperatures by using the SSF approach (Olofsson et al., 2008).

8. Innovative bioreactor geometries and process strategies

A major requirement in cost-efficient lignocellulosics-to-ethanol process is to employ reactor systems yielding the maximal conversion of the cellulose with the minimal enzyme dosage. As consequence, one of the most important parameter for the design and operation of bioreactors for lignocellulosic conversion is the effective use of the biocatalysts to obtain high specific rates of cellulose conversion (namely the yield of glucose obtained per amount of enzymes). The maximization of the product concentration, i.e. the amount of glucose obtained per liquid volume, is also an important parameter as well as the optimization of the volumetric productivity, in this case the rate of glucose formation per reactor volume.
When the hydrolysis is carried out with high dry matter contents, hence high cellulose levels, the product concentration will drive up. For this reason, some recent researches have been finalized into attempting the enzymatic biomass conversion at high-solids loads (Jørgensen et al., 2007; Tolan, 2002). The most important problem of high solid loadings is related to the fact that the viscosity of the reaction mixture is very high and the rheology of the mixture has to be well studied: normal stress might become very significant during bioconversion. In particular, mixing and mass transfer limitations, and, presumably increased inhibition by intermediates come into play. Various fed-batch strategies have been attempted with the scope of supplying the substrate without reaching excessive viscosities and unproductive enzyme binding to the substrate (Rosgaard et al., 2007a; Rudolf et al., 2005).
As said, the currently employed cellulolytic enzyme systems, that include the widely studied *T. reesei* enzymes, are significantly inhibited by the hydrolysis products cellobiose and glucose. This inhibition retards the overall conversion rate of lignocellulosics-to glucose (Gan et al., 2002; Katz and Reese, 1968). Product inhibition is particularly significant during processing at high substrate loadings mainly because the glucose concentration is higher than that obtained in diluted biomass suspensions. (Kristensen et al., 2009; Rosgaard et al., 2007a). As consequence, both the conversion rate and the glucose yields achievable in batch processing of lignocellulose are reduced (Rosgaard et al., 2007b; Tengborg et al., 2001).
General criteria in the bioreactor design and in the selection of the operating conditions could be: use of reactors or reaction regimes that allow a rapid reduction of the glucose concentration; running of the reactions at low to medium substrate concentrations in order to maintain higher conversion rates and hence obtain higher volumetric productivity of the reactor (Andrić et al. 2010, a).
The integration of the bioreactor with a separation unit (reaction–separation hybrids) has shown promising results with product inhibited or equilibrium limited enzyme-catalyzed conversions, because it is possible to remove the products as they are formed (Ahmed et al., 2001; Gan et al., 2002). In this regard, membrane (bio) reactors could be a viable process configuration. Unlike the SSF approach in which the glucose consumption is carried out by the microrganisms simultaneously available in the hydrolyzate, the use of membrane bioreactors would accomplish the same function without any compromise in the reaction temperature. A membrane (bio-) reactor is a multifunction reactor that combines the reaction with a separation, namely in this case product removal by membrane separation, in one integrated unit, i.e. in-situ removal, or alternatively in two or more separate units. The membrane bioreactors hitherto used for the separation in enzymatic processes have been mainly ultra- and nanofiltration (Pinelo et al., 2009). However, the use of this technology is limited by the bank-up of unreacted lignocellulosics (lignin and particularly recalcitrant cellulose) in large-scale and/or continuous processing (Andrić et al. 2010, b). Already in the past, some authors

improved the efficiency of the continuous stirred tank bioreactor (CSTR) by incorporating separation membranes in the reactor design. In particular, Henley et al. (1980) incorporated an UF membrane (UF) or hollow-fiber cartridge (HFC) into the CSTR-UF and CSTR-HFC system, respectively (Henley et Al., 1980). Ishihara et al. (1991) accomplished a semi-continuous hydrolysis reaction by using a continuously stirred reservoir tank, connected to a suction filter unit for the removal of the lignin-rich residue and an ultra-filtration membrane unit (tubular module), through which the filtrate was pumped in order to separate the hydrolysis products from cellulases. The concentration of the lignocellulosic substrate in the reactor was maintained almost constant by the addition of fresh substrate at appropriate intervals. The filter and ultrafiltration units were operated intermittently, while the enzymes were added at the start, recovered in the UF module, and recycled back into the reactor (Ishihara et al., 1991). More recently, Yang et al. (2006) designed the removal of reducing sugars during the cellulose enzymatic hydrolysis through a system consisting in a tubular reactor, in which the substrate was retained with a porous filter at the bottom and buffer entered at the top through a distributor. The hollow-fiber ultrafiltration module with polysulfone membrane enabled the permeation and the separation of the sugars. To keep the volume constant in the tubular reactor, all the remaining buffer was recycled back from the UF membrane and the make-up buffer was continuously supplied from the reservoir (Yang et al., 2006). In some applications an additional microfiltration unit has exceptionally been used to retain the unconverted lignin-rich solid fraction due to the presence of tightly bound enzymes (Knutsen and Davis, 2004) or has been employed to remove the unconverted substrate from the reactor. These set-ups result in slightly complex process layouts for the hydrolysis.
It is evident that the optimization of the reactor designs will permit to overcome both the rheological and inhibition limit of the bioconversion and maximize the enzymatic conversion. Therefore, the reactor design become strong relevant for large-scale processing of cellulosic biomass (Lynd et al., 2008; Wyman, 2008).

9. Conclusion

In this chapter an overview of the current knowledge on the hydrolysis of lignocellulosics for bioethanol production has been presented. In the last years several important breakthroughs have been made either on the biochemical and technological sides. This is confirmed by several industrial initiatives spread over the world. Among these, in recent days, the first brick of the lignocellulosic bioethanol demo plant (40 kton/y) has been layed in Northern Italy by the Mossi and Ghisolfi Group. Some cooperation agreements were strengthen with Novozymes for improving the efficiency of the hydrolysis step. This event represents an important stage for all the Europe making the production of lignocellulosic ethanol closer to the industrialization and opening the way to new lignocellulosic biorefineries.

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11. References

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Recent studies have shown strong evidence of human activity impact on the climate of the planet. Higher temperatures and intensification of extreme weather events such as hurricanes are among the consequences. This scenario opens up several possibilities for what is now called "green" or low carbon economy. We are talking about creating new businesses and industries geared to develop products and services with low consumption of natural resources and reduced greenhouse gases emission. Within this category of business, biofuels is a highlight and the central theme of this book. The first section presents some research results for first generation ethanol production from starch and sugar raw materials. Chapters in the second section present results on some efforts around the world to develop an efficient technology for producing second-generation ethanol from different types of lignocellulosic materials. While these production technologies are being developed, different uses for ethanol could also be studied. The chapter in the third section points to the use of hydrogen in fuel cells, where this hydrogen could be produced from ethanol.

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