Application of Microbial Enzymes in Dissolving Pulp Production

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Abstract

The uprising demand in dissolving pulp, a special chemical pulp, during the last decade has fascinated the researchers to develop a modern biotechnology which could either improve its existing processes or facilitate novel processes of production in eco-friendly manner. These include the use of different microbial enzymes or the microorganisms themselves in various bioprocesses such as biopulping and/or biobleaching of sulphite pulp or bioconversion of kraft pulp to dissolving pulp. The hydrolytic enzymes specifically xylanases and cellulases have been used as the process tools rendering the benefit of eco-friendly and economic bioprocess. Special emphasis is paid to convert kraft pulp, originating from both wood and nonwood, into dissolving pulp by using xylanases and cellulases to selectively reduce hemicelluloses and improve pulp reactivity, respectively. The viscose process being a major consumer of dissolving pulp has drawn more attention. Extensive research work has been conducted to achieve high pulp reactivity as well as accessibility towards solvent and reagent for reducing the carbon disulphide (CS_2) consumption in viscose process. Here, the various characteristic properties of dissolving pulp and its end use with various processes, including

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existing and novel, for its production are reviewed. Microbial enzymes, namely, xylanases and cellulases, for their immense potential as process tools are briefly discussed along with their mode of action.

Keywords
Dissolving pulp • Xylanases • Cellulases • Kraft pulp • Reactivity

8.1 Introduction

Nowadays, the increasing demand for textile fibres due to ever-growing population is largely covered by petroleum-based synthetic fibres. However, due to continuous depletion of fossil resources and other environmental concerns, there is tremendously growing interest in the utilisation of renewable resources for production of various products as a sustainable approach. For decades, the forest industry is mainly focused on the production of pulp for paper and board; however, a small niche is contributed towards the production of dissolving pulp. Dissolving pulp is a special chemical pulp and a key material to manufacture rayon, cellophane, cellulose acetate and other cellulose derivatives. Rayon is a main product of regenerated cellulose and a natural man-made fibre used in textile industry. More than three fourth of dissolving pulp is consumed in viscose process for the production of cellulosic fibres including viscose rayon staple, acetate staple, filament yarn, etc. (Bajpai 2012). The uprising demand in dissolving pulp during the last decade has fascinated the researchers to develop a modern biotechnology which could either improve its existing processes or facilitate novel processes of production in eco-friendly manner. To attain this purpose, the use of microbial enzymes in the production of dissolving pulp has become an active area of research.

Enzymes are the special catalysts that find a vast number of applications in various industries apart from playing a vital role in metabolic reactions. Except a small group of catalytic RNA molecules, all enzymes are proteins in their primary composition. They display a wide variety at supramolecular level due to different folding patterns. The folding involves both covalent and non-covalent interactions within the polypeptide chain and is directed by its amino acid sequence. It is because of this structural distinctiveness that they exhibit the unique specificity for their substrate and are able to speed up the reactions at a good pace without being consumed. Moreover, they are eco-friendly and infallible in their performance at their optimum conditions. They are considered as the main tools for the application of basic biotechnological techniques (genetic engineering and cell fusion), the target of therapeutics drugs and the indispensible intermediate in all bioprocesses (fermentation and cell culture) (Vitolo 2009).

Development of industrial processes for the production of rayon for textiles and cellophane film for packaging led to the development of the dissolving pulp industry in 1911. Until then, the pulp industry was concerned only with the manufacture of fibres for the paper industry; chemical derivatives of cellulose were produced from purified rags or cotton linters. Development of dissolving pulp from wood made available a relatively cheap and pure source of cellulose as a raw material for the expanding chemical industry (McGinnis and Shafizadeh 1979).

8.1.1 Dissolving Pulp

Dissolving pulp is a special chemical pulp consisting of 90–98 % cellulose with very little amounts of hemicelluloses, extractives, minerals and residual lignin. It exhibits uniform molecular weight distribution and high brightness. Dissolving pulp is a low yield pulp in comparison to other chemical pulps. In chemical pulping, raw material (generally wood) is cooked in the presence of acid or alkali at controlled conditions of

temperature, pressure and time to selectively remove lignin and other impurities, thereby separating the fibres (in the form of pulp). The digested raw material turns into a brown-coloured pulp due to reprecipitation of lignin and is further bleached to get the desired pulp properties. In general the resulting pulp containing both cellulose and hemicellulose is utilised for papermaking. Hemicellulose is retained in paper grade pulp for better yield and strength, while in dissolving grade pulp, its higher content causes detrimental effects in end product quality. Therefore, the high content of cellulose is desirable. Moreover, cellulose molecule properties are important, while fibre properties are not directly of interest. As shown in Fig. 8.1, dissolving pulp is manufactured either by the kraft process using a pre-hydrolysis step to remove hemicelluloses or by an acid sulphite process from both hardwoods and softwoods. Currently there are mainly two types of processes to produce dissolving pulp at mill scale. The acid sulphite process, being a major type, is covering nearly 65 % of production, whereas pre-hydrolysis kraft process contributes approximately 25 % of the total production (Sixta 2006; Bajpai 2012).

The acid sulphite process removes the major polymeric constituents other than cellulose such as hemicelluloses and lignin in single step, which later encumbers in the recovery process of hemicelluloses from the spent liquor. Still the recovery rate of the inorganic cooking chemicals is higher along with the advantage of totally chlorine-free (TCF) bleaching. The usage of raw material in the process is restricted to a narrow range of wood species because of its limited ability to dissolve extractives. Besides, more pollution is generated due to high BOD (biochemical oxygen demand) of sulphite mill effluents and high sulphur dioxide losses (Gustafsson et al. 2011). Also the pulp so produced shows broad molecular weight distribution.

The other process utilises alkaline cooking conditions after a pre-hydrolysis stage in which the hemicelluloses are withdrawn from the wood chips. Pre-hydrolysis is technically a pre-extraction stage in which the low DP (degree of polymerisation) carbohydrates specifically hemicelluloses undergo auto-hydrolysis caused by the release of acetic acid. In addition to the benefits of high pulp quality and high cooking capacity, the PHK (pre-hydrolysis kraft) cooking also has

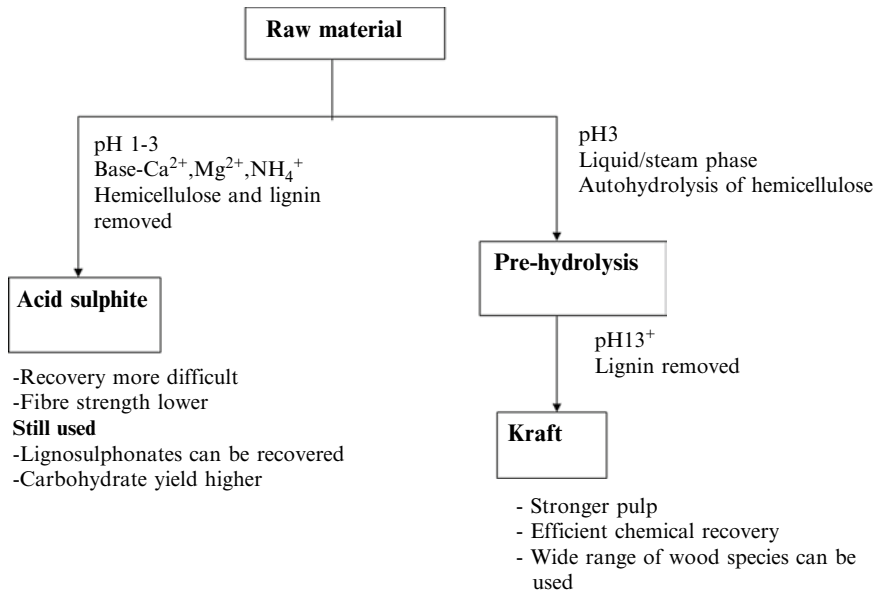

Fig. 8.1 Pulping processes for the production of dissolving pulp

a disadvantage of the formation of pitch-like compounds which hampers the drainage process.

Dissolving pulps have higher production costs in comparison to common kraft pulps as a consequence of costs of wood, capital and chemicals required for a given pulp yield. The lower yield of dissolving pulp than kraft pulp is attributed to retention of hemicelluloses in the pulping process of the latter. Low dissolving pulp yield requires more equipment to be employed to increase production rate. Moreover, this speciality pulp requires inventories and storage spaces prior to its delivery to specific customers with particular requirements (Hillman 2006).

Besides the high costs of dissolving pulp production, this industry is also confronted by the stringent environmental laws that have triggered interest in using enzymes for (1) advancement of paper grade pulps into dissolving grade pulp by selectively reducing the hemicellulose content and subsequently activating the pulps, (2) reactivity improvement of dissolving pulp for better end product quality and (3) investigation of other biotechniques, e.g. biopulping, biobleaching etc., in order to facilitate or improve the performance of conventional processes for dissolving grade pulp production.

With the advent of enzyme technology in dissolving pulp industry, a number of bioprocesses have been developed, the implementation of which can result in:

1. Savings in chemicals for pulping and bleaching
2. Improvement in the quality of dissolving pulp
3. Improvement in the effluent quality
4. Improvement in resource utilisation with less impact on the environment

Although the enzyme technology for dissolving pulp production is new, it is gaining momentum all over the world, with a major aim of developing a new bioprocess for the production of dissolving pulp, the implementation of which would lead to positive changes in the dissolving pulp properties as well as mill effluent quality in an environmentally friendly and cost-effective way.

8.1.1.1 Dissolving Pulp and Cellulose

Dissolving pulp is predominantly cellulosic in its chemical composition. Depending upon the process of production, it exhibits wide changeability in performance. Although cellulose is the only desired constituent, it would be of no use if the form of native cellulose is modified. Here, the molecular properties of cellulose chiefly determine the end use and quality of product. The plants originally produce cellulose I (native cellulose) during wood formation, and both celluloses I and II have been found in pulp. Other polymorphs of crystalline cellulose are celluloses III and IV (Christoffersson-Elg 2005). The polymorphism is most typical of crystals of organic compounds whose molecule contains groups capable of hydrogen bonding (Bernstein 2002). The basic chemical structure of cellulose exhibits β-1, 4-linked D-glucopyranose rings arranged so that glucosidic oxygens point in opposite directions giving rise to cellobiose as repeating unit (Fig. 8.2). This rotation of the adjacent monomer units in the cellulose chain results in intramolecular hydrogen bonding between the ring oxygen O(5) atom of one glycosyl unit and the hydrogen atom of C-3 hydroxyl group of the preceding ring and similarly between the hydroxyl group on C(2) and the primary hydroxyl group on C(6).

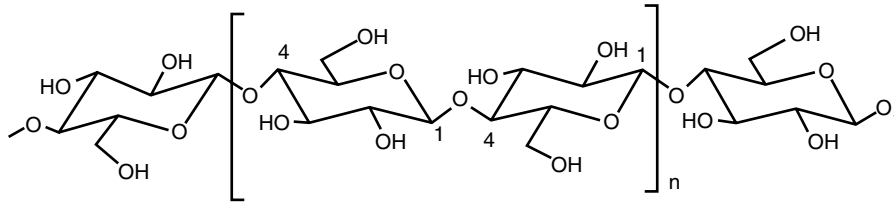

Fig. 8.2 Primary structure of cellulose molecule showing β-1, 4-linked D-glucopyranose residues

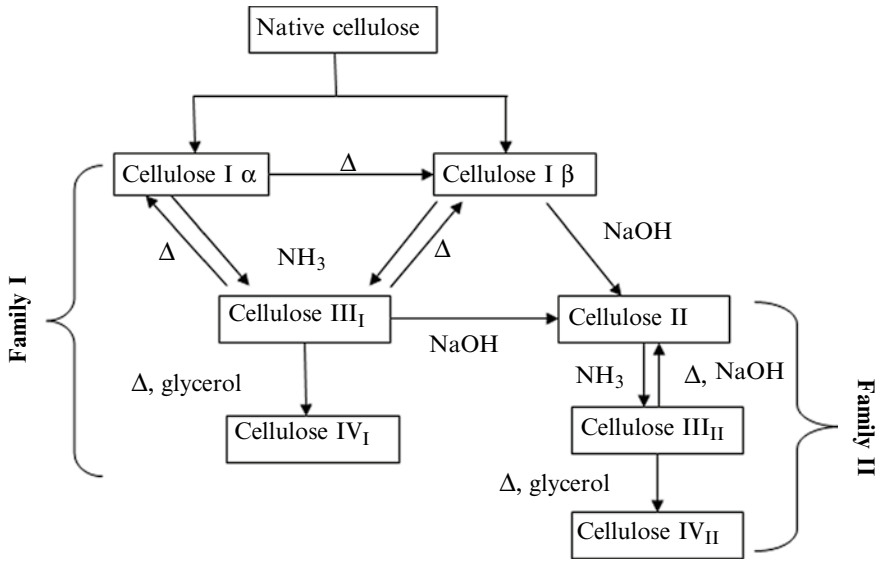

Fig. 8.3 Cellulose polymorphism

In cellulose I, the intermolecular hydrogen bonding between hydroxyl groups on C(6) and C(3′) in parallel chains results in the formation of cellulose sheets, whereas in cellulose II, the chains of cellulose are oriented antiparallel to each other and with different hydrogen bond patterns. Moreover, it carries an extra hydrogen bond per anhydroglucose unit (Henriksson and Lennholm 2009).

As the monomers bear three hydroxyl groups, their hydrogen bonding ability plays a major role in directing crystalline packing and in governing important physical properties (Dufresne 2012). More crystallinity and less amorphous regions in cellulose render poor accessibility to the solvents or reactants for any modification to occur. An efficient dissolution of cellulose in viscose process or its derivatisation hence require the availability of hydroxyl groups and the further access of the reactants to avail these hydroxyl groups for respective reactions to take place. In viscose process, regenerated cellulose filaments are obtained in an acid bath by spinning cellulose dissolved in CS_2 and alkali. Cellulose II is formed when pulp is immersed in strong alkali (18 % NaOH) solution during mercerisation and also when cellulose is regenerated in the acidic bath in the viscose manufacturing process. Cellulose III_{II} and cellulose IV_{II} are formed upon the subsequent treatment of cellulose II with liquid ammonia and with glycerol at high temperature (Henriksson and Lennholm 2009). As shown in Fig. 8.3, this constitutes family II which is converted from family I in an irreversible reaction since the former is thermodynamically more stable (Isogai et al. 1989).

8.1.1.2 Derivatives and End Products

The end products of dissolving pulp either require its dissolution in a suitable solvent or undergo various derivatisation reactions such as esterification, nitration, etherification, etc. Various end-use products derived from dissolving pulp and the corresponding manufacturing processes have been listed in Table 8.1. In the preparation of cellulose derivatives, e.g. cellulose acetate, basic reaction introduces an acetyl functional group $COCH_3$ on cellulose upon the addition of acetic anhydride in the presence of a small amount of catalyst (perchloric acid or sulphuric acid). Esterification reaction involves the addition of ester functional group COO on cellulose by condensation of a carboxylic acid group COOH and alcohol group OH (Dufresne 2012).

The dissolution and subsequent regeneration of cellulose in viscose process results in regener-

Table 8.1 Dissolving pulp end-use product and demand

Manufacturing process	Derivatives	End-use product	Demand by end use (million tons)
Xanthation	*Viscose rayon*		3.381
	Tyre cord	Tyre and belting reinforcement	
	Nonwovens	Filters, surgical packs, drapes, gown	
	Regular staple	Apparel	
	Continuous filament	Apparel	
	High-wet-modulus staple	Apparel, furnishing, parachute cord	
	Cellophane	Packaging	
	Industrial yarn	Heat ablative, brake	
	Miscellaneous	Sponges, sausage casing	
Acetylation	*Acetate*		0.735
	Tow	Filter cigarettes	
	Filament	Apparel, furnishing	
	Plastic mouldings	Film, sheet, extruded articles	
Ethering	*Cellulose ethers*		0.588
	Carboxymethyl cellulose	Detergents, cosmetics, food, textile and paper sizes, well drilling muds	
	Hydroxyethyl cellulose	Latex paints, oil well drilling muds, emulsion polymerisation	
	Methyl cellulose	Food, paints, pharmaceuticals	
	Ethyl cellulose	Coating and inks	
	Hydroxylpropyl cellulose	Foods and pharmaceuticals	
	Carboxymethyl hydroxyethyl cellulose	Liquid detergents	
Nitration	*Nitrates*		0.147
	Lacquers		
	Explosives		
	Celluloid		
	Film		
Others			0.049
Zinc chloride	Vulcan fibre	Lamination, insulation, tight machining	
Latex	Artificial leather	Fabrics, clothing	
Resin	Laminating and impregnation papers		
			Total = 4.9[a] million tons in 2010

[a]Including 1.1 million tons cotton linter pulp. Based on data from Hinck et al. (1985) and Floe (2011)

ated cellulose (mainly rayon). The most common solvent to dissolve cellulose is CS_2, which is very toxic, volatile and malodorous. Depending upon the conversion process used, there is a wide range of end products of dissolving pulp in various industries such as textile industry, pharmaceutical industry and food industry. In textile production, it has a unique advantage of complete recyclability. Cellulose nitrates containing higher N-content have been used extensively for military purposes. In addition, it can be used as a raw material of numerous food additives such as E-additives 460–469 except E462. Moreover a high-quality dissolving pulp finds its application in the manufacture of non-discolouring frames, cellophane and plastics as cellulose acetate

Table 8.2 Some important quality parameters of dissolving pulp

Chemical properties	Description
α-cellulose	Represents undamaged long-chain cellulose content; should be >90 %
Alkali solubility	Measures solubility of pulp (S10, S18, S21.5) in different concentrations of NaOH, represents the amount of hemicellulose and degraded cellulose present in the pulp
S10 (%)	Optimum swelling of pulp; both hemicellulose and degraded cellulose (up to <150 DP) are dissolved
S18 (%)	Hemicellulose (<50 DP) dissolved. Sometimes degraded cellulose of very low DP (usually after cellulase treatment) also dissolves. **Viscose process yield = 0.88(100-S18)** (Hinck et al. 1985)
S21.5 (%)	(100-S21.5) is proportional to viscose process yield
Reactivity (%)	Most critical parameter; methods include viscose filter value (Treiber 1987) and more common Fock method (Fock 1959); Fock reactivity should be >65%
Pentosan (%)	Represents major hemicellulose fraction of hardwoods; should be 0.5–10 %
Viscosity (ml/g)	Corresponds to the length of cellulose chain; gives a relative indication of the degradation (decrease in cellulose molecular weight)
Molecular weight distribution	Determined by gel permeation chromatography (GPC); should be uniform
Degree of polymerisation	Related to the molecular weight (M) by the formula **DP = M/162**, where 162 is the molecular weight of the anhydroglucose unit (Christoffersson-Elg 2005) and to intrinsic viscosity [η] for cellulose dissolved in cupriethylenediamine by $[\eta] = 1.33 * DP^{0.905}$ Mark–Houwink equation (Gustafsson et al. 2011)
Ash (%)	Should be <0.06 %
Extractives (%)	Should be <0.5 %
Residual lignin	Should be <0.2 %
Brightness (% ISO)	Should be >90 %
Yield (%)	30–35 %

(Mäntyranta 2013). Recently, dissolving pulps seem to be the preferred substrate for the manufacture of nanofibrillated cellulose (NFC), a future precursor of advanced materials (Sixta et al. 2013).

8.1.2 Dissolving Pulp Properties

Dissolving pulp is a high-quality pulp intended for the manufacture of various industrial products. Although the end-use product defines the quantitative constitution and the purity required, nevertheless there are certain fundamental requirements (Table 8.2) that must be fulfilled prior to use of dissolving pulp as a raw material.

8.1.2.1 Pulp Reactivity

The reactivity of pulp is certainly an important quality parameter of dissolving pulp since it signifies the processability or suitability of dissolving pulp to be used as raw material for the viscose and other derivatisation processes (Sixta 2006). The reactivity of a pulp directly affects the subsequent derivatisation reactions, such as acetylation, xanthation or nitration, and is mainly measured by the availability of C2 and C6 hydroxyl groups of the monomeric units of cellulose to the reactants (Sixta 2006). These hydroxyl groups are therefore mainly responsible for the reactions of cellulose. The hydroxyl group at the 6th position of the carbon atoms in anhydroglucose unit acts as a primary alcohol, whereas at the 2nd and 3rd positions, hydroxyl groups behave as secondary alcohols. From the esterification studies, it has been established that the relative reactivity is ten times more for the hydroxyl group at the 6th position than the other OH groups (Hebeish and Guthrie 1981; Dufresne 2012). Meanwhile, hydroxyl group at the 2nd position has been found to react two times faster than the hydroxyl group at the 3rd position (Dufresne 2012). Also, more amorphous

cellulose in a pulp may exhibit higher reactivity, but for a quality product, homogenous substitution along the cellulose molecule is desired Zhu et al. (2006). Furthermore, the secondary structure of cellulose is also important as it gives rise to different hydrogen bond patterns and thus reactivity. Since an increased number of hydrogen bonds are present in cellulose II, its reactivity towards different derivatisation reactions is poor. Moreover the ratio of the two allomorphs, Iα and Iβ, present in cellulose I also influences the pulp reactivity as Iα is metastable and thus more reactive than Iβ, while the latter prevails in higher plants (Dufresne 2012).

It also seems that fibre wall supramolecular structure of cellulose hinders the efficient removal of hemicelluloses resulting in some residual xylan in the pulp. Besides, the presence of residual hemicelluloses is thought to impede the subsequent conversion processes by competing with cellulose in the reactions involved. Therefore, a detailed description of the fibre surface is required in order to gain the information on residual hemicelluloses and fibrillation as well as fibril structure. This further helps in understanding the associated phenomenon of hornification, the accessible inner surface and the pore volume to define the term reactivity (Krässig 1993).

8.1.2.2 Alkali Solubility

Low molecular weight carbohydrates (hemicellulose and degraded cellulose) can be extracted from pulps with sodium hydroxide solutions, referred to as alkali solubility. Solubility of a pulp in alkali thus provides information on the degradation of cellulose and on loss or retention of hemicelluloses during pulping and bleaching processes. The solubility of pulp in 10 % NaOH at 25 °C and 1.5 % consistency (S10) represents optimum swelling of pulp that results in dissolution of not only the degraded cellulose fraction (up to <150 DP) but the hemicellulose fraction as well. S18 or solubility in 18 % NaOH exhibits somewhat reduced swelling power, solubilising essentially the shorter chemical residual hemicellulose fraction (<50 DP). However, this is not always true; especially for pulps with low DP, both the low molecular weight cellulose and the hemicelluloses are dissolved (Christoffersson-Elg 2005). α-cellulose content is also a significant value for dissolving pulp and a measure of the long-chain cellulose content. For viscose pulps, 0.88(100-S18) as well as resistance of pulps to 21.5 % sodium hydroxide solution R21.5 (100-S21.5) is proportional to viscose process yield (Hinck et al. 1985).

8.1.2.3 Pentosan Content

Pentosan content in pulp is the indication of the amount of hemicellulose in general, lost or retained during pulping and bleaching processes. Pentosan content in softwoods is about 7–10 % and in hardwoods about 19–25 %. This is so because hardwood hemicellulose consists mainly of pentosans, whereas softwood hemicellulose consists of both pentosans and hexosans. Since hemicellulose contributes to the strength of paper pulps, high pentosan content is desirable, whereas in dissolving pulps, particularly acetate pulps, pentosan content should be kept low.

8.1.2.4 Viscosity/Degree of Polymerisation/Molecular Weight

Viscosity corresponds to the length of the cellulose chains and can be measured in different solvents. The solution viscosity of a pulp gives a measurement of the degree of polymerisation of the cellulose. Such an analysis therefore is useful in determining the relative indication of the degradation (decrease in cellulose molecular weight) caused by pulping and/or bleaching process. Molecular weight distribution and polydispersity, measured by gel permeation chromatography (GPC), viscosity measurements or other methods, are important parameters for characterising the pulp. The degree of polymerisation (DP) is related to the molecular weight (M) by the formula DP = M/162, where 162 is the molecular weight of the anhydroglucose unit (Christoffersson-Elg 2005), and to intrinsic viscosity [η] for cellulose dissolved in cupriethylenediamine by the following Mark–Houwink equation (Gustafsson et al. 2011):

$$[\eta] = 1.33 \times DP^{0.905}$$

The molecular weight can be expressed in various ways, for example, the number average molecular weight (DP_n) or the weight average molecular weight (DP_w), depending on the method used. The polydispersity index, the ratio DP_n/DP_w, corresponds to the width of the molecular weight distribution.

8.1.2.5 Ash Content and Extractives

The inorganic impurities of the pulps, collectively described as the ash content, mainly consist of silicates, sulphates and carbonates, as well as metal ions combined to the acidic groups of the pulp. A common subdivision is acid-soluble and acid-insoluble ashes, the latter being mainly the silicates. These impurities affect the processing properties of the dissolving pulps. Some impurities, mainly iron, manganese and copper, catalytically accelerate the air oxidation and impair the brightness, brightness stability of pulps, rayon colour and aging of the alkali cellulose. The presence of carbonates and carbonate forming ions, such as calcium and magnesium, causes trouble in the pressing of the alkali cellulose, the filtration of viscose and the spinning of the rayon. Polyvalent metal ions cause viscosity anomalies and haze in the cellulose acetate prepared from impure acetate pulp. Extractives are those constituents that are soluble in neutral organic solvents, which can be both either lipophilic or hydrophilic. The compounds which are extracted by acetone are called acetone extractives (%). The content of extractives should be as low as possible, although they have positive effects on the viscose process. However, it is better to have low constant levels than uncontrolled varying levels in order to maintain constant viscose process parameters. For dissolving pulps to have good quality, they must not exceed 0.5 %.

8.1.2.6 Kappa Number and Brightness

Residual lignin in pulp is not easy to measure directly due to its complex and variable chemical structure. But the lignin content correlates well with the consumption of certain oxidants like potassium permanganate, and the amount consumed is expressed as kappa number (Gustafsson et al. 2011). For dissolving grade pulp, residual lignin should be below 1 %. Dissolving pulp exhibits high brightness and whiteness along with good dye ability.

8.1.3 Microbial Enzymes

Microbes have always been exploited as a major source of industrial enzymes. They have been used along the centuries in industrial processes as tannery, brewing, bakery, dairy, etc., but their potential use in the pulp and paper industry was realised in the late 1980s, and thereafter, it has shown a rapid growth. This growth has been triggered by various factors that include:

1. Better understanding and improved knowledge of the interactions between enzymes and the pulp constituents
2. An increased requirement of the industry to adopt eco-friendly approach
3. Improved production technology and process economics for the relevant enzymes

The enzymes can be obtained from any living form such as plants (e.g. papain, ficin, bromelain), animals (pepsin, trypsin and chimosin) and microorganisms (keratinases, pectinases and amylases, among others), but the tendency of using microbial enzymes on an industrial scale is prominent. Since they render plentiful supply, less sophisticated equipment/conditions for production and easy control on all the phases of production, they are economical than their counterparts (Vitolo 2009). Also the commercial processes are well standardised for the production. There exist more than 3000 different known enzymes of which commercially used enzymes are 150–170 only (Binod et al. 2013). Moreover, a diverse range of enzymes similar to those of plants and animals can be synthesised by genetically manipulating microbial strains through RDT (recombinant DNA technology).

Potential benefits of using microbial enzymes in pulp and paper industry have been realised for the past 30 years. The enzymes were first employed at mill scale during the 1980s, shortly after the discovery and validation of the xylanase-aided

bleaching concept. Since then, the industrial enzymes with a wide pH and temperature ranges suitable for target processes have been developed rapidly. In addition, novel enzymatic and microbial applications have been investigated for improving the processing of wood fibres as well as for improving the sustainability of the pulping, bleaching and papermaking processes (Viikari 2002). The integration of hemicellulases and cellulases or microbial strains into processing of dissolving pulp production along with some chemical treatments for the attainment of better quality product in a cost-effective manner as well as reducing the environmental load could be of great benefit to the industry.

Enzymes are known to modify a number of pulp and paper properties. Of the different enzymes and their applications, cellulases have been used for deinking (Heitmann et al. 1992; Pathak et al. 2011; Prasad et al. 1993) and dewatering of recycled fibre (Verma et al. 2013), xylanases have been implemented for biobleaching and also to improve the mechanical properties of pulps and the refining process (Bhardwaj et al. 1996; Buchert et al. 1998; Cadena et al. 2010; Gil et al. 2009), lipases have been used for pitch control (Fischer et al. 1993) and laccases or the laccase-mediator system have been used in the bleaching process (Ibarra et al. 2006). Microorganisms have also been investigated for the biotechniques such as biopulping and biobleaching to produce dissolving pulp (Christov et al. 1998; Christov 1999; Ferraz et al. 1998 and Mosai et al. 1999).

The use of enzymes in pulp and paper industry is still being explored, either directly acting upon the pulp to selectively alter the properties for intended purpose or modification of materials used in the papermaking such as starch, nanocellulose, etc. One of the enzyme applications includes the reactivity improvement of dissolving pulp by using cellulases (Cao and Tan 2002, 2006; Engström et al. 2006; Henriksson et al. 2005; Kvarnlöf et al. 2005; Rahkamo et al. 1996, 1998). Furthermore, endoglucanases have been applied for surface treatments in the textile and laundry industries. Cellobiohydrolases are used to decrease the energy consumed during mechanical pulping, due to their good defibrillation properties (Teeri 1998).

On the other hand, the most widely used application of xylanases is in the pre-bleaching of kraft pulp to reduce the use of bleaching chemicals and the associated environmental problems along with an improved quality of product (Allison et al. 1993; Bajpai et al. 1994; Bim and Franco 2000; Senior and Hamilton 1991, 1992; Yang et al. 1992; Zhan et al. 2000). Studies have also shown their applicability in desizing and scouring of fabric (Csiszar et al. 2001; Dhiman et al. 2008a; Losonczi et al. 2005), increasing water retention, reducing beating times in virgin pulps, restoring bonding and increasing freeness in recycled fibres (Dhiman et al. 2008b). Xylan-degrading enzyme systems also have considerable potential in other biotechnological applications including the bioconversion of lignocelluloses and agricultural wastes into fermentative products, biobleaching in paper manufacture, liquefaction of coffee mucilage, alteration of the rheological and organoleptic properties of musts and wines, extraction of pigments and flavours, protoplast formation and production of modified hemicelluloses for industrial use (Coughlan 1992). Recently, xylanases have been used alone or in combination with alkaline extraction for dissolving grade pulp production as demonstrated by Bajpai and Bajpai (2001), Gehmayr et al. (2011), Jackson et al. (1998) and Köpcke (2010).

8.2 Mode of Action

8.2.1 Xylanases

Xylanases (EC3.2.1.8) are a group of hydrolytic enzymes known to cleave the 1, 4-β-D xylosidic bonds in xylan (Collins et al. 2005). Xylan is a major part of hemicelluloses in hardwood. Due to its high molecular mass, the penetration of xylan into the cell wall is not feasible, whereas its low molecular mass fragments can readily enter inside the cell and regulate the enzyme biosynthesis. These short fragments are actually the products of constitutively produced enzymes that are present

in the small amounts in the cell. The liberated molecules released by the action of these enzymes are consisting of xylose, glucose and their heterodisaccharides and positional isomers together with fragmented xylan of different DP such as xylobiose, xylotriose and xylooligosaccharides (Motta et al. 2013). For the complete hemicellulose degradation, a complement of about 24 enzymes is required (Shrinivasan 1992; Battan et al. 2007). Hemicellulases are typically produced as multiple isozymes, i.e. enzymes virtually with the same catalytic action but display different kinetic parameters or different regulatory properties. On the basis of amino acid or nucleic acid sequence of their catalytic modules, they are classified into two groups, glycosidic hydrolases (GHs) or carbohydrate esterases (CEs), and further grouped into various families according to their primary sequence homology (Henrissat and Bairoch 1996; Rabinovich et al. 2002a, b; Battan et al. 2007). Moreover, the information provided by Carbohydrate-Active Enzyme (CAZy) (2014) database (http://www.cazy.org) on sequence annotations of enzymes related to carbohydrates and glycoconjugates metabolism has associated the xylanases with GH families 5, 7, 8, 9, 10, 11, 12, 16, 26, 30, 43, 44, 51 and 62. Among them, the families 5, 7, 8, 10, 11 and 43 possess a distinct catalytic domain exhibiting endo-1, 4-β-xylanase activity, whereas 16, 51 and 52 families seem to be consisting of two catalytic domains with bifunctional enzymes. Other families, namely, 9, 12, 26, 30 and 44, may exhibit side activity of xylanase (Motta et al. 2013).

Of several enzymes with different functions which participate in the hydrolysis of xylan to its monomers, the most significant are the endo-β-1, 4- xylanases (Sunna and Antranikian 1997). These enzymes degrade xylan to short-chain xylooligosaccharides of varying lengths. On the basis of their hydrophobic cluster analysis of the catalytic domain and sequence homology, these enzymes are chiefly grouped into families 10 and 11 in the numerical classification of glycosyl hydrolases (Battan et al. 2007). Although there have been extensive study done to understand the catalytic properties of the members of the families 10 and 11 and several models have been proposed to explain the mechanism of their action, little is known about the remaining families 5, 7, 8 and 43. Xylanases are hydrolytic enzymes and the bond cleavage takes place by either the retention or the inversion of the anomeric carbon of the reducing sugar monomer of the substrate. The families that undergo retention of the anomeric configuration are 5, 7, 10 and 11. The catalytic mechanism which is also a double-displacement mechanism requires the involvement of two glutamate residues resulting in the formation of a covalent glycosyl-enzyme intermediate for subsequent hydrolysis. In the first step, the carboxylate group of one glutamate residue acts as a general acid by donating a H^+ to O-4′ that links C-1 of the incipient reducing sugar and C-4′ of the departing glycosyl fragment, while the second carboxylate group carries out a nucleophilic attack leading to C_1–$O_{4'}$ bond cleavage and departure of aglycone. Thus, α-glycosyl-enzyme intermediate is formed. Meanwhile, a water molecule near the glycosidic oxygen side is dissociated by the first carboxylate group (now acts as a general base) and resulting hydroxyl ion reacts with the anomeric carbon leading to a second substitution. In this manner the β-configuration at the anomeric centre is retained. Also, xylanases that undergo retaining mechanism are believed to effect hydrolysis via boat transition state (Fig. 8.4a), rather than the half chair that appears to be used by glucosidases and cellulases. Considering the hypothesis that the bulky C5-hydroxymethyl substituent is placed in an unfavourable axial position in a boat conformation would explain the absolute xylan specificity of family 11, while the broad specificity of family 10 enzymes for cellulose/xylan is believed to be attributed by the half chair transition state (Rye and Withers 2000).

Members of GH families 8 and 43 operate through inversion mechanism in which the catalytic residues are perhaps glutamate and aspartate that lead to single-displacement reaction. As its name suggests, the reaction catalysed by these inverting enzymes occurs in single step in which one carboxylate protonates the substrate and the second triggers a nucleophilic water molecule which in turn attacks the anomeric carbon. As a

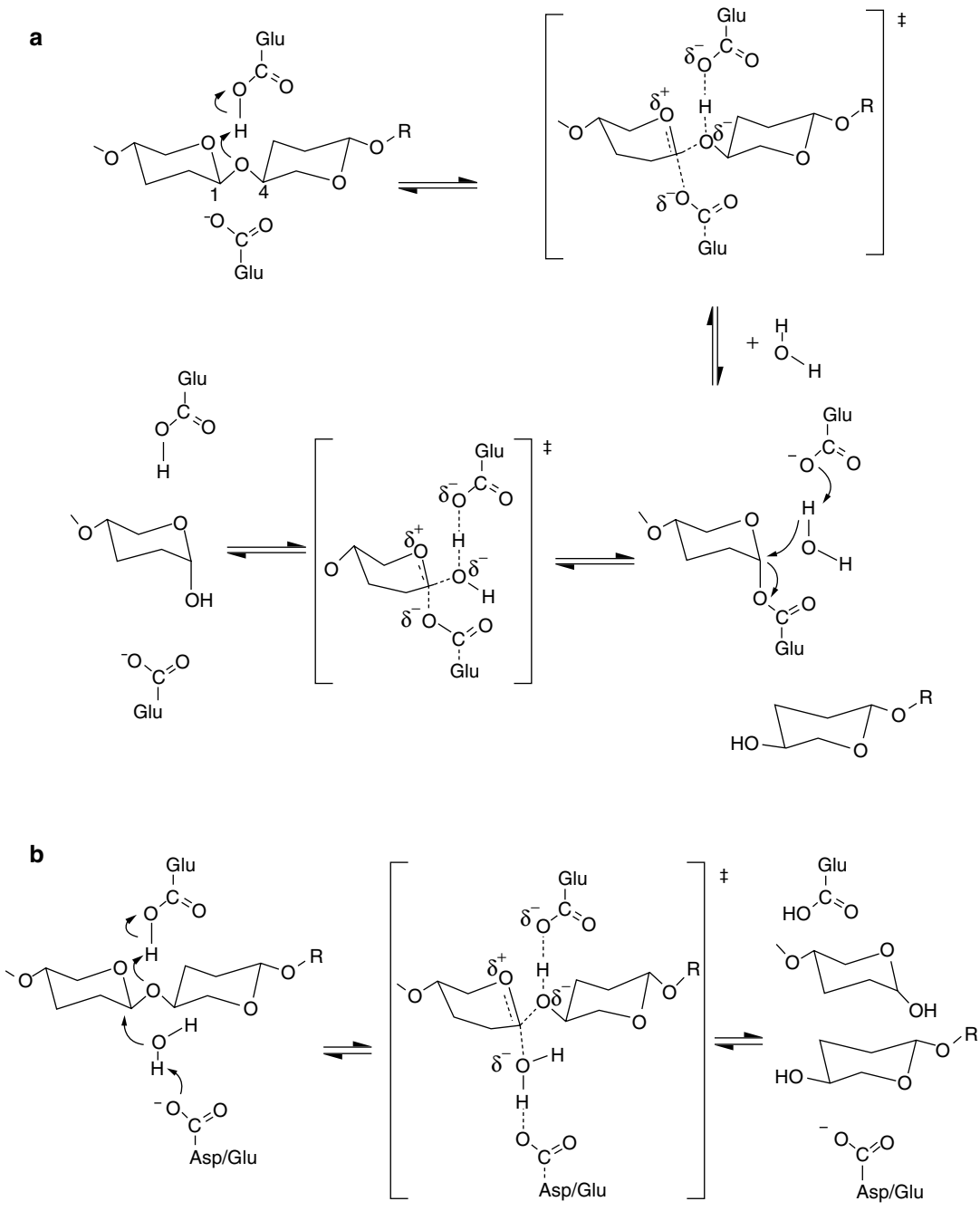

Fig. 8.4 Action mechanisms of xylanases (**a**) retention and (**b**) inversion (Based on Collins et al. 2005; figures were prepared with CS ChemDraw Ultra version 8.3)

consequence of these reactions, the aglycone is departed and the configuration at the anomeric centre is inverted simultaneously (Collins et al. 2005; Coughlan 1992; Paës et al. 2012). GH11 family is considered to be the family of true xylanases since its xylanases exploit D-xylose-containing compounds as their sole substrates. Moreover, their reaction products can be utilised

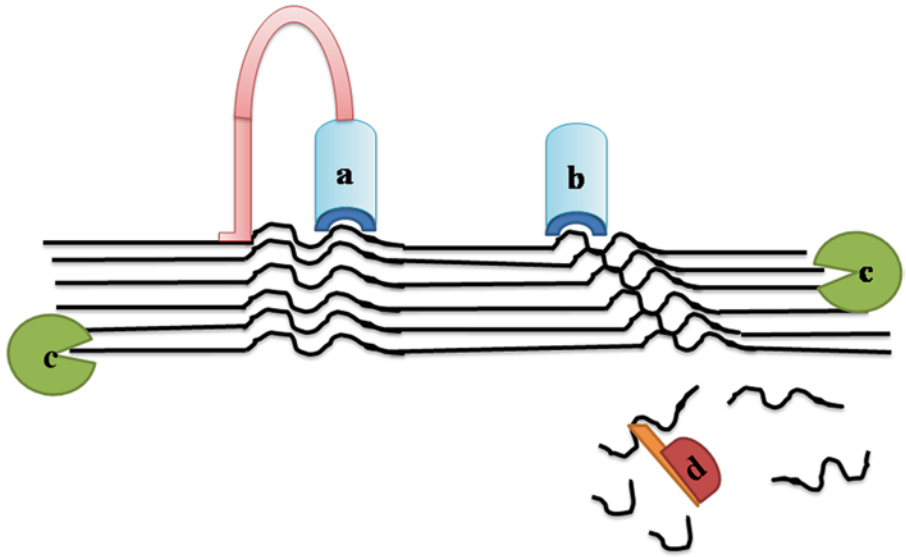

Fig. 8.5 Mode of action of different groups of cellulases. (**a**) Endoglucanase with cellulose-binding domain, (**b**) endoglucanase without cellulose-binding domain, (**c**) cellobiohydrolase, (**d**) glucosidase

as substrates by the enzymes of family 10. The sites where the xylose residues bind to xylanases are known as subsites and the bond cleavage occurs between the sugar residues at the −1 (non-reducing) and the +1 (reducing) ends of the polysaccharide substrate (Davies et al. 1997; Motta et al. 2013). In enzyme assays where arabinoxylan was used as a substrate, family 11 products were found to have arabinose residues substituted at the +2 subsite, while the family 10 products have arabinose residues substituted at the +1 subsite. Therefore, GH 10 enzymes are able to hydrolyse xylose linkages closer to the side-chain residues, whereas the GH 11 xylanases preferentially cleave the unsubstituted regions of the arabinoxylan backbone (Motta et al. 2013).

8.2.2 Cellulases

Recently, cellulases have been studied extensively for their various applications in pulp and paper industry. Especially monocomponent endoglucanases have been found to play a significant role in the enhancement of the cellulose reactivity and accessibility of dissolving pulps (Engström et al. 2006; Henriksson et al. 2005; Kvarnlöf et al. 2005; Köpcke 2010). The cellulase treatment results in the formation of additional openings/surface areas in the fibre structure via the possible action of 'etching'. As a result, the pore volume of pulp fibres increases, which lead to the increase in the accessibility to xanthation (Miao et al. 2014). Due to their synergistic action, cellulases have traditionally been considered detrimental to pulp and paper properties particularly with regard to yield and strength properties. However, the use of monocomponent cellulases or defined cellulase mixtures alone or together with hemicellulases has been shown to have potential in pulp and paper applications (Suurnäkki et al. 2000).

Cellulases are the hydrolytic enzymes that cleave the 1, 4-β-D-glucosidic linkages of the cellulose chain. The prerequisite for hydrolysis is the binding of cellulases to its substrate (Zhang and Lynd 2004). Further, all the three major types of cellulases must act together synergistically for the maximum degradation of cellulose. These groups are endoglucanases (EC. 3.2.1.4), glucosidases (EC. 3.2.1.21) and cellobiohydrolases or exo-1, 4-β-glucanases (EC. 3.2.1.91) as illustrated in Fig. 8.5. The reaction products of these enzymes may vary depending on whether the

enzymes are used alone or in combination. Among all, the key component for the hydrolysis of cellulose is endo-1, 4-β-glucanases that generate new chain ends after randomly cleaving the polysaccharide chain, preferably amorphous regions. The cascade of hydrolysis then involves cellobiohydrolases to act upon the ends of polymeric chain to generate cellobiose, which in turn is substrate for β-glucosidases, ultimately leading to the end product glucose (Lynd et al. 2002. The degree of cellulose degradation depends upon the three primary parameters: the crystallinity, the specific surface area and the degree of polymerisation of the cellulose chain (Eremeeva et al. 2001; Mansfield et al. 1999).

In general, enzymes consist of structurally and functionally discrete units called domains that can fold independently of other regions of the same polypeptide chain or of a larger aggregate. Cellulases, in most cases, are composed of two domains. One is a catalytic domain, designed for the specific cleavage of 1, 4-β-D-glucosidic bonds of cellulose, while the other is cellulose-binding domain required to bring the catalytic domain in the proximity of substrate by binding to the cellulose chain. The endoglucanases are consisting of cleft-shaped catalytic domain, whereas the exoglucanases are comprised of tunnel-shaped catalytic domain. The two domains are connected to each other with the help of an inter-domain linker (Rabinovich et al. 2002a, b). The main factors that affect the hydrolytic efficiency of these enzymes involve their size and structure since a large enzyme complex would only be able to modify the surface of the substrate (Mansfield et al. 1999; Zhang and Lynd 2004).

8.3 Biopulping

Biopulping is the treatment of wood chips and other lignocellulosic materials with natural wood decay fungi or other isolated microorganisms since their enzymes such as xylanases, pectinases, cellulases, hemicellulases, ligninases and their combination prior to pulping process improve the pulp properties (Kirk and Jeffries 1996). Biopulping is preferred because:

- It reduces the chemical and energy utilisation.
- It reduces the pollutants.
- It increases the yield and strength properties of pulp.

The first experiment on fungal pretreatment of eucalyptus wood for biosulphite pulping was carried out using *Ceriporiopsis subvermispora* L-14807 SS-3 (Ferraz et al. 1998). The resulting pulp showed improved characteristics especially in terms of kappa number (reduced by 31 %) and pentosan content (reduced by 36 %) at the same level of pulp yield. This could be translated into increased pulp yield over the control at a given kappa number (Ferraz et al. 1998). Christov et al. 1998 investigated five different strains of *C. subvermispora* for biosulphite pulping of eucalyptus in conjunction with various bleaching sequences. Prior to the acid sulphite pulping, the wood chips were subjected to fungal treatment for 2 weeks. Table 8.3 presents the various properties such as alkali solubility, α-cellulose, viscosity, etc., of the pulp so produced. The resulting pulps showed reduction in both brightness and yield over the control. Although kappa number was reduced by

Table 8.3 Biosulphite pulping of eucalyptus wood using five strains of *C. subvermispora*[*]

Strains	S10 (%,w/w)	S18 (%,w/w)	α-cellulose (%,w/w)	Kappa number	Brightness (%, ISO)	Viscosity (cP)	Yield (%,w/w)
Control	9.2	6.8	90.7	8.9	51.5	48.5	47.4
SS-1	10.2	6.9	90.0	8.0	47.7	46.1	40.8
SS-3	9.2	6.7	90.6	8.3	49.3	47.5	47.1
SS-4	9.8	6.8	90.5	8.7	49.4	47.8	45.2
SS-5	9.4	6.7	90.7	8.9	48.6	47.1	46.9
SS-10	9.6	6.9	90.3	8.7	46.8	47.5	45.6

*Data adopted from Christov et al. (1998)

10 %, it did not correlate with the corresponding brightness of the pulp samples.

Several other strains of white-rot fungi were also investigated by many researchers for their potential use in biosulphite pulping of *Eucalyptus grandis*, and the pulp characteristics in terms of kappa number, yield and brightness were evaluated (Christov et al. 1998; Christov 1999; Ferraz et al. 1998; Mosai et al. 1999). Mosai et al. (1999) evaluated the biopulping efficiency of ten different white-rot fungi strains on *Eucalyptus grandis* in terms of pulp yield, kappa number, brightness, alkali solubility (S10 and S18) and viscosity when subjected to different pretreatment time. Fungal pretreatment of eucalyptus wood chips with *C. subvermispora* SS-3 for 10 days without additional carbon source resulted in substantial decrease in kappa number (by 29 %) and increase in brightness (by 12 %) (Mosai et al. 1999). As shown in Table 8.4, the resulting pulps had lower pulp yield compared to control. Reduction in kappa number (24 %) and simultaneous increase in brightness (6 points) in comparison to control were only observed in *C. subvermispora* SS-3, whereas in other treated pulp, yield loss and apparent increase in lignin occurred (Christov 1999; Mosai et al. 1999). Scott et al. (1996) studied the effect of biosulphite pulping of pine when treated with *C. subvermispora* SS-3 for 2 weeks and observed the reduction in kappa number by 21 % over control. Also, the pretreatment of spruce with *C. subvermispora* CZ –3 for 2 weeks resulted in reduced kappa number by 22 % compared to control (Fischer et al. 1994).

Biopulping of eucalyptus wood with fungal strain SS-3 followed by bleaching resulted in a pulp of higher brightness and pulp yield comparable to control. However, the fungal strain SS-5 produced biopulp resulting in higher pulp yield with the same brightness as control (Table 8.5). Therefore, the pretreatment of eucalyptus wood with the *C. subvermispora* strains increased by 1 percentage point either the final pulp yield (SS-5) or pulp brightness (SS-3) relative to the controls (Christov et al. 1998; Christov 1999;

Table 8.4 Effect of biosulphite pulping of eucalyptus with selected strains of white-rot fungi[a]

Fungal strain	Yield (%)	Kappa number	Brightness (%)
Control	47.0	8.6	51.6
Phanerochaete sordida YK-624	46.9	10.7	45.6
Trametes versicolor 52 J	46.5	9.5	46.0
Laetiporus sulphureus SCC- 180	46.0	10.6	44.5
Phanerochaete chrysosporium BKMF-1767	45.8	10.0	49.0
Ceriporiopsis subvermispora SS-3	45.3	6.5	57.3
Ceriporiopsis subvermispora SS-5	44.4	8.6	49.9
Stereum hirsutum SCC-74	44.0	12.0	35.4
Trametes versicolor ATCC 20869	43.7	12.5	46.0
Sclerotinia sclerotiorum UFHRF	42.9	12.1	42.9

[a]Wood chips were pretreated with fungi for 10 days. Based on data from Christov (1999) and Mosai et al. (1999)

Table 8.5 Effects of biosulphite pulping of *Eucalyptus grandis* with two strains of *Ceriporiopsis subvermispora* on properties of unbleached and bleached dissolving pulp

	Unbleached dissolving pulp			Bleached dissolving pulp	
Fungal strain[a]	Kappa number	Yield (%)	Brightness (%)	Yield (%)	Brightness (%)
Control	8.6	47.0	51.6	40.8	93.1
Ceriporiopsis subvermispora SS-3	6.5	45.3	57.3	40.4	94.2
Ceriporiopsis subvermispora SS-5	8.6	44.4	49.9	41.8	93.1

[a]Wood chips were pretreated with fungi for 10 days. Based on data from Christov (1999)

Mosai et al. 1999). This could be translated into savings of chemicals or energy at a given pulp yield. The increased pulp yields thus indicate the better selectivity of the bleaching process resulting from the biopulping.

8.4 Biobleaching

Biobleaching of pulps is performed with either hemicellulolytic enzymes, in particular xylanases or lignin-degrading fungi or their enzymes, namely, ligninases and laccases. The action of xylanases on pulp results in the hydrolysis of hemicelluloses as a consequence of which the bleaching chemicals get an easier access to lignin, whereas the white-rot fungi and their lignin-olytic enzymes depolymerise the lignin in pulp by a direct action on it. The main objective, however, in both cases is to increase delignification so as the amount of bleaching chemicals specifically chlorine and chlorine-containing compounds is reduced in the subsequent bleaching of pulp. Besides, biobleaching is considered as one of the preferred processes due to a number of benefits it offers over conventional chemical bleaching. One of the major advantages is the reduced adsorbable organic halide (AOX) levels in the discharged effluents resulting from the reduced requirement of chlorine during bleaching along with improved pulp quality, brightness gain and post colour (PC) number reduction. Most of the research on biobleaching for the production of dissolving pulp has been carried out using either white-rot fungi or xylanases. Ligninolytic enzymes are not yet studied for dissolving pulp production although they are extensively being explored for the production of paper grade pulp. Biobleaching with xylanases has been investigated for both kraft and sulphite pulps, whereas white-rot fungi are studied for sulphite pulp only.

Christov et al. (1996) reported that biopulping of eucalyptus wood chips in conjunction with bleaching/biobleaching of pulp to produce dissolving pulp results in a brightness gain over the control in most instances. The properties of biosulphite pulp produced by the pretreatment with SS-3 and SS-5 strains of *C. subvermispora* were evaluated with three different bleaching sequences: $OD_1E_OD_2H$, $OD_1E_OD_2P$ and X $OD_1E_OD_2P$ (O, oxygen; D_1 and D_2, chlorine dioxide; E_O, sodium hydroxide with reinforced oxygen; H, sodium hypochlorite; and P, hydrogen peroxide) (Christov et al. 1998). Biobleaching effect due to xylanase was only observed in fungus pretreated samples, not in control as shown in Table 8.6 (Ferraz et al. 1998). Brightness gain due to xylanase treatment was found maximum

Table 8.6 Effects of bleaching/biobleaching[a] of sulphite pulp produced by biopulping of wood chips with two different strains of *Ceriporiopsis subvermispora*

Fungal strain[b]	Bleaching sequence	S10 (%, w/w)	S18 (%, w/w)	α-cellulose (%, w/w)	Brightness (%, ISO)	Yield loss (%)
Control	$OD_1E_0D_2H$	7.9	4.7	92.8	93.1	14.1
	$OD_1E_0D_2P$	7.7	4.5	92.7	93.2	13.2
	X-$OD_1E_0D_2P$	8.1	4.5	92.5	93.2	13.4
SS-3	$OD_1E_0D_2H$	7.8	4.8	92.8	94.2	14.3
	$OD_1E_0D_2P$	8.3	5.4	92.6	92.9	14.0
	X-$OD_1E_0D_2P$	8.1	4.5	92.7	95.5	14.1
SS-5	$OD_1E_0D_2H$	7.9	4.7	92.9	93.1	11.6
	$OD_1E_0D_2P$	8.5	4.6	92.2	93.6	10.3
	X-$OD_1E_0D_2P$	8.4	4.5	92.3	94.0	10.4

[a]Xylanase pretreatment(X):4 IU/g pulp using Cartazyme HS-10; Charges at D_1 and D_2: 0.9 and 0.6 % as active Cl; P: 0.6 % H_2O_2; 0.4 % NaOH; 100 °C. Based on data from Ferraz et al. (1998)

[b]Fungal pretreatment of wood chips: 2 weeks

in SS-3 pretreated sample, whereas the yield loss resulting from bleaching of the biopulped samples was found minimum in SS-5 pretreated sample. The yield of unbleached pulp produced after biosulphite pulping with strain SS-5 was lower than that of control (Table 8.5); however, it was the highest after (bio)bleaching (Table 8.6). Therefore, the fungal pretreatment of wood chips not only increases the extent of lignin removal during pulping and bleaching, resulting in higher brightness of the dissolving pulp, but also improves the selectivity of the bleaching process, thus increasing the final pulp yield.

Biobleachability of industrial sulphite dissolving pulp was also studied by Christov et al. 1996 using white-rot fungi and xylanase from *A. pullulans*. It was reported that two strains of *C. subvermispora* (L-14807 SS-3 and CZ-3) effectively bleached the sulphite pulp. Both the strains were capable to remove lignin (as kappa number) with strain SS-3 by 85 % and strain CZ-3 by 88 %, in particular (Table 8.6). Also, the strain SS-3 significantly contributed to pentosan removal from pulp (13 %) than strain CZ-3 (5 %). The brightness of fungus-treated samples increased by 42 % (strain SS-3) and 47 % (strain CZ-3), respectively, as compared with the control samples. In addition, the α-cellulose content was reduced by 5 (SS-3) and 4 (CZ-3) points. In comparison the xylanase pretreatment lowered the pentosan content by 13 % and improved the α-cellulose content by nearly a point without significantly affecting the brightness and kappa number (Table 8.7).

Bajpai and Bajpai (2001) developed a bleaching process for dissolving grade pulp in which treatment with xylanase enzyme preceded the beaching sequence CEHED (C, chlorine; E, extraction with sodium hydroxide; H, hypochlorite; and D, chlorine dioxide). The unbleached pulp was produced involving a mild prehydrolysis step to retain relatively high pentosan content in the pulp that resulted in higher unbleached pulp yield at the same kappa number. Then after, the enzyme-treated pulp was bleached that led to the reduced requirement of bleaching chemicals. Further, improved brightness and bleach pulp yield were attained at desired levels of pentosan. The pulp reactivity and rayon yield were also found to be higher for the enzyme-treated pulp.

Enzyme treatment using commercial laccase from *Trametes villosa* with different natural and synthetic mediators has also been investigated on unbleached sulphite pulp for its biobleaching potential (Quintana et al. 2013). The studies reported the laccase–violuric acid (L–VA) system as the most effective enzyme mediator combination among others to obtain improved quality dissolving pulp. At first the laccase-mediator treatment reduced the ISO brightness and kappa number in all the samples due to delignification and formation of chromophore groups, but after P (P-hydrogen peroxide) stage, laccase–violuric acid treatment increased brightness (28 %) and reduced kappa number (87 %) while viscosity was reduced to 2.5 % of the control. Also, the effluent generated after laccase–violuric acid treatment was found to have least toxicity. An extended TCF biobleaching with L–VA stage was also assessed and could be translated in saving H_2O_2 consumption and reaction time.

Table 8.7 Treatment of sulphite pulp with *Ceriporiopsis subvermispora* (L-14807 SS-3 and CZ-3) and *A. pullulans* xylanase (X)[a]

Pulp treatment	Kappa number	α-cellulose (%, w/w)	Pentosan (%)	Brightness (%, ISO)
Control	6.7	89.9	3.9	56.0
SS-3	1.0	84.9	3.4	79.4
CZ-3	0.8	86.0	3.7	82.2
Xylanase	6.5	90.7	3.4	56.5

[a]Fungal treatment time: 2 weeks; X: 15 IU xylanase/g pulp: 3 h; 55 °C; pH 4.7; 9 % pulp consistency. Based on data from Ferraz et al. (1998)

8.5 Bioconversion of Paper Grade Pulp to Dissolving Pulp

The kraft process is known to be the world's major pulping method and is likely to remain so into the foreseeable future. During its evolution over a period of a century, it has become highly refined. Currently about 70 % of the world's annual pulp output of approximately 100 million tonnes is produced by the kraft process. Although some disadvantages are associated with it, still it is by far the most cost-effective, versatile and efficient wood delignification method available. Because of this fact and the large amount of capital already invested in kraft pulping, it is unlikely that the process will be replaced in the near future (Thakur et al. 2012). On the other hand, demands for dissolving pulp and fibres have boomed worldwide within the last decade, and thus, nowadays much effort is put into the production of dissolving pulps from kraft pulps besides the dissolving pulp production from the commonly applied technologies such as the acid sulphite process and the alkaline pre-hydrolysis kraft process (Sixta 2006).

With a view on the worldwide pulp production capacity of about 150×10^6 t per year, the production of dissolving pulps represents only a niche market of totally 2.5 % of the global chemical pulp production (Puls et al. 2006). This market situation and the high price difference between both pulp categories raises the question of why paper grade pulps cannot be converted into dissolving pulps in an economically feasible process by the selective reduction of hemicelluloses from kraft pulp and subsequently activating it. In this context, several studies have been conducted focusing on the development of economic and efficient process steps to upgrade paper grade pulp into dissolving pulp. Recently it has been found that treating the paper grade pulps with enzymes can give them a higher reactivity, enabling these pulps to be utilised as an economic alternative to dissolving grade pulps for viscose production (Gehmayr and Sixta 2011; Jackson et al. 1998; Köpcke et al. 2008; Wang et al. 2014). Also enzyme treatment and alkali extraction subjected to sisal pulps indicated that such pulps could also be used as dissolving pulps (Ibarra et al. 2010).

Cellulases are the most extensively used enzymes for the improvement of pulp reactivity and accessibility (Engström et al. 2006; Gehmayr and Sixta 2011; Gehmayr et al. 2011; Henriksson et al. 2005; Ibarra et al. 2010; Köpcke et al. 2008; Köpcke 2010; Kvarnlöf et al. 2007; Miao et al. 2014; Östberg 2012; Rahkamo et al. 1996, 1998; Wang et al. 2014; Wollboldt et al. 2010) of both dissolving grade pulp and paper grade pulp. Miao et al. (2014) reported that cellulase treatment at the dose as low as 2 u/g (based on the dry weight of pulp) subjected to hardwood kraft-based dissolving pulp resulted in increased Fock reactivity from 47.67 % to 79.9 %.

Upgradation of kraft paper pulps to dissolving pulps can also be achieved by a post-extraction of xylan by using an approximately 2 M sodium hydroxide solution at temperatures slightly above room temperature (cold caustic extraction or CCE; Jayme and Schenck (1949), Wallis and Wearne (1990)). Anyhow, pulp treatment at raised alkali concentration leads to the modification of cellulose I to cellulose II. Moreover, the residual xylan after CCE is characterised by rather high molecular weight and a resistance to dissolution in alkali (Gehmayr and Sixta 2011; Wollboldt et al. 2010). Both contribute to significant decrease in pulp reactivity, hence necessitating the gentle method of hemicelluloses extraction and reactivity enhancement. A number of treatments based on enzymatic treatments, hypochlorite treatments, alkali extraction and alkaline peroxide treatment either in conjunction or alone have been investigated in order to attain the commercial dissolving pulp quality in terms of cellulose reactivity, hemicelluloses content, pulp viscosity and molecular weight distribution. The treatment types, sequences and process parameters were further optimised for different raw materials used.

Jackson et al. (1998) reported that high-quality recovered paper sources rich in hardwood content after given an initial cold alkali extraction, a xylanase treatment and a final cold alkali extraction produced dissolving pulp with reduced

hemicelluloses content, acceptable viscosity and alkali solubility. Henriksson et al. (2005) and Engström et al. (2006) studied the treatment effect of monocomponent endoglucanase and found that the reactivity of softwood sulphite pulp as determined by the Fock method (Fock 1959) was significantly increased with relatively low amounts of enzyme used, and the yield loss and decrease in viscosity were moderate.

Kvarnlöf et al. (2007) studied two different approaches, one chemical and one enzymatic, to enhance the pulp reactivity so as the carbon disulphide charge in the viscose process is reduced, thereby reducing the cost and indirectly the environmental impact. Different cellulases including either mixture or monocomponent endoglucanase were employed for enzymatic treatment of hardwood dissolving pulp and softwood dissolving and paper grade pulp. Kraft pulp has been shown an increase in reactivity of approximately 45 % units after being treated with enzyme. Finally, treating several cellulosic materials with enzymes showed that the reactivity of a paper grade pulp was increased from 40 % to about 80 %. Treating non-dissolving pulps with enzymes is therefore of great interest in the industrial manufacture of viscose. The work also demonstrated the reduced carbon disulphide consumption due to improved pulp reactivity in the viscose process while maintaining the good quality viscose dope. The best enzyme preparation among others was found to be the monocomponent endoglucanase, Carezyme, whose treatment was further optimised relating to viscose dope preparation. Moreover, the spent enzyme filtrate could be re-used several times without losing its efficiency under the industrially desired conditions of temperature, enzyme dose and reaction time, thus lowering the production cost. In the chemical method, pressurised oxygen was used after the mercerisation step resulting in the consumption of a carbon disulphide charge of 20 % instead of 30 % in the conventional viscose process. For enzyme-treated pulp to reach the lowered carbon disulphide charge, the dewatering and the sulphidation stage was modified accordingly. Also, a twofold increase in the reactivity of paper grade pulp was observed after the enzymatic treatment indicating that enzymes could be of great use in the upgradation of paper grade pulp to dissolving grade pulp.

Ibarra et al. (2010) and Köpcke (2010) evaluated different commercial monocomponent endoglucanases either with or without cellulose-binding domain (CBD) for their effect on the reactivity and accessibility of hardwood and softwood dissolving grade pulps for viscose production. It was observed that endoglucanase with a CBD notably enhanced the cellulose reactivity according to the Fock method by simultaneously decreasing the viscosity. This may be attributed to the proximity of catalytic domain with the cellulose chain for the required course of time after CBD has bound to the cellulose. Moreover, an inverting hydrolysis mechanism of endoglucanase with CBD had been the most effective among the different assayed enzymes to result in increased reactivity of both pulps (Ibarra et al. 2010). At the same time, more marked decrease in viscosity was observed for softwood dissolving pulp, whereas the effects of the different endoglucanase treatments were more pronounced on never-dried dissolving pulps.

Köpcke (2010) undertook research study to convert different wood and nonwood paper pulps to dissolving pulp. Commercial dried elemental chlorine-free (ECF) bleached kraft pulps from birch and eucalypt were studied along with several dried ECF bleached soda/anthraquinone pulps from flax, hemp, sisal, abaca and jute. Two alkali extraction steps followed by an endoglucanase treatment was found to be the most suitable sequence for birch, whereas for eucalyptus and sisal pulps, a combination of a xylanase treatment followed by an alkali extraction step prior to an endoglucanase treatment provided the best results. The xylanase treatment and alkali extraction steps reduced the hemicelluloses content, reaching values to those of a commercial dissolving grade pulp. The molecular weight distribution was found to be more uniform in converted dissolving pulps than that of a commercial dissolving pulp since only cellulose I was present in the former. Moreover the endoglucanase treatment resulted in improved cellulose reactivity of pulp and fulfilled the requirements of a commercial

dissolving pulp for viscose production. However, the pulp viscosity exhibited values lower than those of commercial dissolving pulp but suitable for the production of viscose. Pretreatment of dissolving pulp with monocomponent xylanase and monocomponent cellulase has also been evaluated to improve the following dissolution of pulp in NaOH/ZnO (Ambjörnsson et al. 2014). The treated pulp showed increased solubility from 29 % to 81 % in NaOH/ZnO solution after subjecting to 16.7 AXU/g of xylanase dose followed by alkali extraction (with 7 % NaOH) and 10 ECU/g endoglucanase dose. Surprisingly the DP_{η} was as high as 1074.

Gehmayr and Sixta (2011) introduced Hem-Extra Process in which commercial oxygen delignified *E. globules* kraft pulp subjected to xylanase and CCE treatment prior to TCF bleaching followed by endoglucanase treatment resulted in the production of novel high-purity dissolving pulp. It was found that xylanase pretreatment and subsequent CCE at reduced alkalinity were sufficient to reach the desired residual xylan content of the pulp. Moreover, the enzymatic degradation by endoglucanase exhibited a direct correlation between the cellulose II content and the chain cleavage of the pulps. The final average degree of polymerisation was also adjusted by endoglucanase treatment. On the other hand, Fock reactivity and filter value both showed an increase after xylanase pretreatment because of the lower degree of fibre hornification, whereas the Fock reactivity was higher for enzyme-treated pulps when compared to an acidly hydrolysed pulp. It was also found that a substantial increase in yield at a given R18 content, ranging between 4 % and 8 % on oven-dried wood basis, could be achieved by the new Hem-Extra-pulps as compared to a conventional pre-hydrolysis kraft pulp.

Östberg (2012) investigated the effect of enzyme pretreatment, subjected to kraft and dissolving pulp, on carbon disulphide consumption for high-viscosity viscose preparation. It was found that the Fock reactivity of dissolving pulp when treated with Fibre Care R increased in all instances if compared to the reference pulp sample, but a higher enzyme charge or long treatment time could reduce the reactivity. On the other hand, the limiting viscosity number was reduced, necessitating relatively low enzyme charges combined with a relatively short treatment time to produce viscose grades intended for high-strength products. Moreover, xylanase pretreatment alone showed no change either in Fock reactivity or the limiting viscosity number of the dissolving pulp. No correlation between the gamma number of the viscose and the Fock reactivity of pulp was observed; rather the gamma number either was reduced or remained unchanged upon enzyme treatment except for the conventional softwood kraft pulp where it increased. It indicates that gamma number measures the xanthate groups in both the cellulose and hemicelluloses that undergo a certain degree of substitution in the viscose process, thus a poor indicator if the pulp also contains a high content of hemicelluloses.

Wang et al. (2014) introduced alkaline peroxide treatment after enzymatic treatment with EG-rich industrial cellulase to convert bleached softwood paper grade pulp to dissolving pulp for viscose production. With the enzyme dose of 300 IU/g bone dry pulp, 9 wt% NaOH and 1 wt% H_2O_2, the treated pulp exhibited 68.7 % of reactivity, 92.1 % of α-cellulose content and 506.9 mL/g of pulp viscosity. Furthermore, the reducing sugar in the spent liquor derived from the stage of enzymatic treatment can be recovered for the production of value-added products based on the concept of biorefinery.

8.6 Conclusions and Future Prospects

Enzyme technology has been contributing a vast number of new techniques and processes to pulp and paper industry and can provide new horizons in the future as well. One of its applications is producing dissolving pulp by using advanced techniques and employing microorganisms and their enzymes to lessen the environmental load and improve the product quality in a cost-effective manner. This includes the use of microorganisms/enzymes in biopulping and biobleaching for their selectivity that has been shown to result in either

brightness gain or improved yield with reduced amount of pollutant generated. Also, the enzymes can be effectively used for reactivity improvement of dissolving pulp or techno-economic conversion of paper grade pulp to dissolve grade pulp. Various studies have shown that the upgradation of kraft pulp to a better quality dissolving pulp in terms of reactivity, brightness, yield, molecular weight with acceptable viscosity and hemicelluloses content could be achieved by enzymatic treatments and alkali extraction. Moreover, producing dissolving pulp from kraft pulp offers a pulp with higher market value along with the production of value-added bioproducts such as hemicelluloses. Hemicelluloses being one of the main biorefinery feedstocks can be further utilised to produce biopolymers, thermoplastic xylan derivatives, bioethanol/biofuels and immunomodulators or as dietary fibre depending upon the extent of degradation. Therefore, exploring more biotechnological applications and further implementing them in pulp and paper industry can be a solution for reducing environmental impact and producing better quality product simultaneously.

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