Chapter 12
Microbial Enzymes and Their Application in Pulp and Paper Industry

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12.1 Introduction

Industrial utilization of wastepapers in the production of new one is increasing globally. Currently, pulp and paper industry is one of the largest consumer of wood. Based on their demands due to global economic growth, more trees will be harvested and waste will be consumed and disposed in the environment (Pathak et al. 2011). Chemical agents such as sodium hydroxide, hydrogen peroxide, sodium carbonate, diethylenetriaminepentaacetic acid, sodium silicate, and surfactants are used in large quantities by paper industries as conventional methods of deinking wastepaper which lead to expensive wastewater treatment to meet environmental regulations (Pathak et al. 2011; Saxena and Chauhan 2016). Enzymes such as lipase, xylanase, pectinase, cellulase, hemicellulase, amylase, and esterase are used as substitute to chemical conventional methods of deinking wastepapers (Yadav et al. 2015, 2016). These enzymes are reported to be environmentally friendly as compared to conventional method. It was realized several decades ago that microbial enzymes might be useful in processing of papers since it is composed of natural polymers such as cellulose, hemicellulose, and lignin. Microbial enzymes have been commercially used in pulp and paper industry only in the previous decade, while microorganisms are presently used in other industrial processing steps, though long been used in the treatment of wastewater. This is due to the fact that wood and pulps which act as substrates are difficult to degrade. In addition, most research now focuses on lignin biodegradation since it is lignin that is

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removed from wood in chemical pulping and pulp in bleaching. Lignin likely evolved in part as a deterrent to microbial degradation, and it continues to be an impediment to biotechnological processing of wood and pulps. During the last decade, the number of possible applications of enzymes in paper and pulp industries in which many are of commercial quantity has grown rapidly. These include xylanase for enzymatic bleaching, lipase for pitch removal, as well as cellulase and hemicellulase for freshness enhancement (Kirk et al. 1996).

Globally, our lives are somewhere governed by little inscriptions laid on paper in one way or the other. The major domains of public sector are reliant on paper and paper products directly or indirectly. The history of paper dates back almost 2000 years, while the manufacturing process happened to be reported in China when inventors imprint their writings on crafted cloth sheets. The knowledge of papermaking then became popularized through westward and eventually reached India around 605 AD. A wide variety of raw materials used in the papermaking process include cellulose originated from agriculture waste, forests, and wastepaper, noncellulosic coal, talcum powder, etc. However, raw materials like wood logs and its waste, bamboo, wastepaper, bagasse, and agricultural residues like bran of wheat, rice straw, grasses, and seaweed are majorly used in the process. The basic product coming out during paper processing is the cellulosic pulp which is used in papermaking and as animal feedstock. Pulp being a lignocellulosic fibrous material separates the cellulosic fibers from wood logs, fibers, wastepaper, and rags. Two steps are involved in pulp production: wood pulping and pulp bleaching (Fig. 12.1). Both processes require high-energy input and reagents which leads to the production of significant amount of gaseous, liquid, and solid wastes that need to be treated. Biotechnological approaches provide substantial solution of the aforementioned problem. Lignin degradation by white-rot fungi has been intensively studied for biotechnical applications such as biopulping, biobleaching, and treatment of pulp mill effluents. Also, lignolytic enzymes and hemicellulases provide a prospective way to decrease the usage of hazardous bleaching reagents and to make an improvement in the quality of bleached pulp (Tavares et al. 2014). The application of

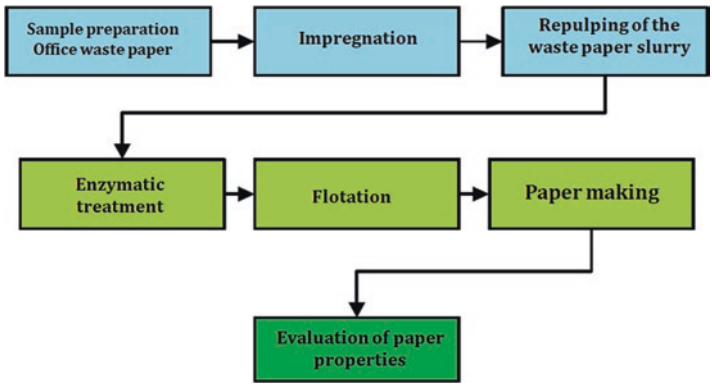

Fig. 12.1 The stages of enzymatic deinking process

microbial enzymes in paper and pulp industries has increased rapidly which lead to the decrease of adverse effect on ecosystem due to the increased awareness of sustainability issues (Yadav et al. 2019a, b). Utilization of these microbial enzymes also leads to the decrease in energy consumption, processing time, and amount of chemicals in deinking wastepaper. They are equally used to aid deinking and bleach in paper and pulp industry as well as waste treatment by increasing biological oxygen demand (BOD) and chemical oxygen demand (COD).

It is difficult to deink laser-printed office papers by employing the conventional method of deinking. Due to higher demand of laser printers and copy machines every year, there has been an increase in the amount of nonimpact printed papers entering the recycled papers. It is challenging for the inventors to remove ink from these papers. The reason is primarily due to the strong adherence of the toner particles to the paper surface. The photocopier printers are indulged in using thermosetting toners made up of synthetic polymers as ink. Due to applying high amount of heat during inking, the ink particles become physically bonded to the paper making it difficult as well as expensive to remove by conventional method.

A biological process using enzymes had been evaluated which implied positive results in deinking process of different types of wastepaper. One of the major advantages of using enzymes is production of minimum treatment effluent, and it is also less harmful to the environment. The most important step involved in recycling process is removal of ink from the paper (Table 12.1). Several enzymes like cellulase, hemicellulase, α-amylase, lipase, xylanase, and other lignolytic enzymes are involved in the biological process of deinking. The enzymatic treatment is favorable because enzymes are eco-friendly in nature and during its processing ink detachment occurs without any discharge of harmful chemicals, thus rendering our environment green. Most of the cited studies reported deinking of mixed office waste consisting of photocopier paper using commercially available enzymes (Roushdy 2015). India generates approximately 36.5 million tons of municipal solid waste annually, of which 14.6 million tons consist of paper wastes. The Indian Agro and Recycled Paper Mills Association (IARPMA) estimated that India is among the countries with low recycling of waste of wastepapers (26%) as compared to countries like China (38%), Thailand (45%), and Germany (80%). Based on the shortage of raw materials, resources, and high demands being imposed on green plants, Indian paper industries are facing many challenges on daily basis. One of the attractive solution to these problems in India is the recycling of municipal office waste (MOW) papers but still very difficult to remove nonimpact ink. Deinking process which involves the removal of printed ink from used paper involves dislodgement of ink particles from fiber surface and the separation of dispersed ink from the fiber suspensions by washing and flotation (Tavares et al. 2014).

In chemical deinking process, industries used high quantity of chemicals such as chlorine, chlorine-based derivatives, sodium hydroxide, sodium carbonate, sodium silicate, hydrogen peroxide, hypochlorite, and chelating agents, which lead to hazardous effluent disposal problems (Vega et al. 2012; Pala et al. 2004). For these reasons, biological deinking by using microbial

Table 12.1 Microbial enzyme and their applications in pulp and paper industry

Microorganism	Enzymes	Application	References
Commercial enzymes	Cellulase	Ink removal, freshness, and reduction of drainage time	Pathak et al. (2011)
Trichoderma harzianum	Cellulase and xylanase	Improved drainage, high deinking efficiency, brightness, and reduction of drainage time	Pathak et al. (2014)
Commercial enzymes	Cellulase	Not good if specks surface of the deink paper are used	Tsatsis et al. (2017)
Commercial enzymes	Cellulase	Detach significant amount of ink from ONP/OMG	Zhang et al. (2008)
Aspergillus niger	Cellulase and hemicellulase	Enhanced optimum deinking efficiency	Lee et al. (2007)
Streptomyces sp. L22001	Xylanase	Biobleaching effect	Li et al. (2010)
Bacillus altitudinis	Xylanase	Potential for biodeinking and biobleaching	Adhyaru et al. (2017)
Bacillus sp. CKBxID	Xylanase	Deinking agent of recycled wastepaper	Maity et al. (2012)
Alkalothermotolerant	Xylanase and pectinase	Commercially viable with better paper quality	Singh et al. (2012)
Aspergillus niger	Xylanase	Deink old newspaper with improved brightness, removal of surface ink particles from ONP pulp	Desai and Iyer (2016)
Aspergillus nidulans	Xylanase	Reduction of ink and increased brightness of recycled paper	Taneja et al. (2002)
Commercial enzymes	Laccase and hemicellulase	Deink old newspaper	Xu et al. (2011)
Commercial enzymes	Cutinase and amylase	Increase brightness and ink removal	Wang et al. (2018)
Commercial enzymes	Amylase and cellulase	Improve brightness	Gil et al. (2013)

enzymes which act directly either on the fiber or on the ink film becomes more attractive. For example, hydrolysis of cellulose and hemicellulose brings detachment at fiber/ink interbonding regions and finally releases ink particles into the suspension when treated with cellulase/hemicellulase enzymes (Lee et al. 2007). Microbial enzymes can also detach small fibrils from the surface of the ink particles; hence, they can change the usual hydrophobicity of the particles, which brings about their separation in the flotation/washing step. These enzymatic technologies have been described as especially advantageous in deinking high-quality wastepapers, whose reuse is usually limited as the nonimpact inks (toners) polymerize onto the paper surface using thermoplastic binders during the high-temperature printing process. In the chemical deinking process, the toner particles usually remain large, flat, and rigid and separate very poorly from papers during the fiber/ink separation stages (Jiang and Ma 2000).

12.2 Enzyme Producing Microbes

Twelve bacterial culture has been isolated from alkaline sediments from Lonar Lake in Maharastra India. Based on 16S rRNA sequencing, biochemical characterization, and phylogenetic analysis, all the isolates were identified. For the first time in Lonar lake, bacteria such as haloalkaliphilic *Marinobacter excellens*, *Alkalimonas delamerensis*, *Roseinatronobacter monicus*, and *Rhodobaca bogoriensis* were identified (Borgave et al. 2012). In another research conducted by Kladwang et al. (2003), about 490 alkaline-tolerant fungi from a natural environment using petri dishes containing potato dextrose agar medium buffered at pH 11.0 were identified. Many of the alkaline tolerant fungi has been isolated from fifty one samples from different habitats of Thailand. This research indicated that a good source of alkaline enzyme production can be found from alkaline-tolerant fungi isolated from tree holes in alkaline and acidic environments. A bacterium *Bacillus pumilus* SV-205 produces xylanase in an optimized fermentation condition. A medium containing a mixture of yeast extract and peptone yields high xylanase as compared to other combinations. High pH stability over a range of 6–11 in 24 h is the major characteristic feature of this enzyme which can also maintain 65% activity after 2 h incubation at 60 °C (Nagar et al. 2012).

In Barabanki district of Uttar Pradesh, India, George et al. (2001) isolate a novel alkalothermophilic actinomycete with optimum growth at pH 9 and 50 °C from self-heating compost. *Thermomonospora* sp. was able to produce 23 IU/ml carboxymethyl cellulase (CMCase) enzyme purified under fractional ammonium sulfate precipitation followed by cellulose affinity chromatography and Sephacryl S-200 gel filtration. The enzyme retained 100% activity at 50 °C for 72 h and had half-lives of 7 and 3 h at 60 and 70 °C, respectively. The enzyme activity was tested as an additive to laundry detergents based on its stability in the presence of commercial detergents viz. Ariel, Henko, and Surf Excel. During an investigation exploring possible sources of novel thermophilic species in natural products, a novel thermophilic and alkaliphilic actinomycete capable of producing alkaline cellulase from soil of a tropical rain forest in Yunnan province, China, was isolated and identified (Wu et al. 2018). The whole-cell hydrolysates were found to contain glucose and ribose. The organism was identified as Genus *Streptomyces* based on 16S rRNA gene sequence analysis. Organism formed a distinct phyletic line together with closely related type strain called *Streptomyces burgazadensis* ZIR7 T. Strain named *Streptomyces thermoalkaliphilus* represent a novel species in the genus Streptomyces based on its phenotypic, chemotaxonomic, and phylogenetic characteristics.

Bilanenko et al. (2005) reported an isolate representing the group Ascomycete from saline soda soils of Central Asia and Africa. The bacterium described as *Heleococcum alkalinum* sp. novel was isolated on alkaline agar with carboxymethyl cellulose (CMC) and was a dominant species in samples of soda soils with pH >10 and relatively high salinity. It shows an alkali-tolerant adaption by growing within the pH range of 6.7–10.8. This cellulolytic activity of an alkaliphilic obligate anaerobic bacterium, Z-7026, which was isolated from the microbial community of soda-lake

sediments belonging to the cluster III of *Clostridia* with low G + C content was investigated by Zvereva et al. (2006). The bacterium has the ability of growing in media with cellulose or cellobiose as the sole energy source. The maximum growth rate on cellobiose was found at a pH range of 8.5–9.0, while that of cellulase synthesis, assayed by using a novel fluorimetric approach, was observed at pH 8–8.5. In the laboratory, bacterium *Penicillium citrinum* (MTCC 6489) was previously isolated from soil producing an alkali-tolerant and thermostable cellulase (Dutta et al. 2008). The study reports the presence of alkali-stable cellulase from alkali-tolerant fungus Penicillium citrinum for the first time, which may have potential effectiveness as additives to laundry detergents.

According to Vyas and Lachke (2003), two extracellular alkali-stable 1,4-β-D-glucan-4 glucanohydrolase (EC3.2.1.4) fractions, namely, EndoA and EndoB, were separated from the culture filtrate of an alkalotolerant *Fusarium* strain. These enzymes are found to be suitable for deinking mixed office wastepapers leading to the increase in brightness with decrease in ink counts of the recycled paper. Probable mechanism of enzymatic deinking process was schematically presented based on the distinct properties of endoglucanases. Picart et al. (2007) reported one fungal strain from subtropical soils on the medium supplemented with rice straw exhibiting high cellulase activity. Using isoelectric focusing, zymography, and sodium dodecyl sulfate polyacrylamide gel electrophoresis, these new strains were identified as *Penicillium* sp. CR-316 and *Penicillium* sp. CR-313 which indicated that the strains secreted multiple enzymes that hydrolyze cellulose. Crude cellulose produced by *Penicillium* sp. CR-316 has potentials in industrial applications since it showed activity and stability at high temperature and produced a thermostable cellulase. Kalpana and Rajeswari (2015) has isolated *Streptomyces* from agricultural waste capable of producing enzymes for degrading xylan. *Streptomyces* sp are vital source of enzymes involved in lignocellulosic degradation. Isolate was reported to grow on different types of feedstuffs such as oat spelt xylan, sugarcane molasses, tomato pomace, rice bran, wheat bran, and sawdust under submerged fermentation conditions.

The xylanase activity in each production medium was confirmed by measuring the amount of reducing sugars liberated from the medium by the DNS method using crude extract which was found to have an application in deinking of newsprint. Nadagouda et al. (2016) isolate cellulase enzyme from *Trichoderma viride* GSG12 under solid-state fermentation technique using cheap and readily available agricultural waste material called rice bran. This indicates the possibility of using rice bran to produce cellulase using *Trichoderma viride* under solid-state fermentation. The finding shows that cellulase production can be influence by optimal pH, initial moisture level of the medium, incubation temperature, inoculum size, and incubation time. The optimum pH, initial moisture level, incubation temperature, and inoculums size were 5.5, 70%, 32 and 2×10^8 spores/flask, 120 h, respectively. Increased enzyme production was favored by supplement of lactose and corn-steep solid to the rice bran.

From various mangrove sites in the Philippines, conventional as well as analytical profile index (API) were used to characterize and phenotypically identify five

promising cellulase producing bacterial strains. The finding provide data regarding species of *Bacillus* producing cellulase enzyme and additional knowledge regarding the bacterial diversity of mangrove forests in the Philippines (Tabao and Monsalud 2014). Makky and Abdel-Ghany (2009) described old newspaper (ONP) waste as a carbon source for growing *Bacillus subtilis* where avicelase and carboxymethylcellulase (CMCase) enzymes are estimated in the culture filtrate. *Bacillus subtilis* CMCase has more activity at optimal temperature and pH than Avicelase. Deinking with these enzymes brings about an increase in brightness of the sheet effective removal of ink particles and also prevents redeposition onto the fiber surfaces. These findings indicate that enzymatic deinking can perform better than the conventional chemical method. *Bacillus halodurans* was purified to homogeneity to produce an extracellular haloalkaline cellulase by bioconversion of lignocellulosic waste by (Annamalai et al. 2013).

The enzyme has retained up to 80% activity at 80 °C, 12% and 35% temperature, pH and NaCl with optimum of 60 °C, 9.0 and 30% respectively. When detergents and organic solvents such as n-hexane, acetone and acetonitrile are present, the enzyme was found to be stable. This indicates that a purified cellulase produce from *Bacillus halodurans* utilizing ligncellulosic biomass could be of great potentials in industrial process. Maitan-Alfenas et al. (2016) used *Aspergillus nidulans* to isolate and characterize xylanase in *Pichia pastoris*. At 60 °C and 7.5 as well as 50 °C and 6.0 temperature and pH, respectively, this enzyme has its optimum activity which is completely inhibited by SDS and $HgCl_2$. Another important bacterium capable of producing an extracellular and thermostable xylanase enzyme is *Bacillus pumilus* ASH when grown on solid-state fermentation (SSF). When wheat bran is moistened with deionized water at a substrate to moisture ratio 1:2.5 (w/v), higher xylanase activity is obtained with optimum production temperature of 37 °C. The enzyme activity was slightly lower in solid-state fermentation (SSF) than in submerged fermentation technique, but the ability of the organism to produce such a high level of xylanase at room temperature, with deionized water and with no addition of any mineral salts in SSF, could lead to substantial reduction in the overall cost of enzyme production.

12.3 Cellulase and Its Applications

Microbial cellulases have been focused as the important biocatalysts being multiplex in nature and bearing extensive applications. Cellulase and hemicellulase enzymes are both synthesized by fungi and bacteria as seen in Table 12.2. These microorganisms can be aerobic, anaerobic, mesophilic and thermophilic. Among them, the most commonly studied fungal genera are *Thermomonospora*, *Trichoderma*, and *Aspergillus*. Fungal and bacterial cellulases are structurally similar (Fig. 12.2). Fungal cellulase having two domains: a catalytic domain and a cellulose binding module. Commercially, cellulase enzymes have been accessible for 30 years or more, and these enzymes have been used for study purpose as well as

Table 12.2 Microbial enzymes and their sources

Sample	Location	Organism	Enzyme	pH	Temp (°C)	References
Soil	Pulp and paper industries, India	*Bacillus subtilis*	Cellulase	4.0	60	Pala et al. (2004)
Soil	Macuya rain forest, Pucallpa, Peru	*Aspergillus* sp. LM-HP32 and *Penicillium* sp. LM-HP33 and 37	Cellulase	4.8–9.4	28	Vega et al. (2012)
Soil	Iguazú rainfalls, Argentina	*Penicillium* sp. CR-313 and CR-316	Cellulase	4.5	65	Picart et al. (2007)
Wastepaper	USM Campus, Penang, Malaysia	*Aspergillus niger*	Cellulase, hemicellulose	6.0	50	Lee et al. (2013)
Agricultural waste	Cairo, Egypt	*Bacillus thuringiensis* MAM-29 and MAM-38	Cellulase, xylanase	3–7.6	60–80	Abo-State et al. (2013)
Waste photocopy paper	Medellin, Colombia	NA	Cellulase, amylase	7.0	40	Gil et al. (2013)
Wild herbivore, rain deer	Wayanad, Kerala, India	*Escherichia coli* SD5	Cellulase, xylanase	NA	37–39	Kumar et al. (2018)
Soil, compost, animal waste slurry	Jeju Island, South Korea	*Bacillus subtilis* C5–16 and S52–2	CMCase, avicelase, xylanase	5.0	50	Kim et al. (2012)
Wastepaper	NA	NA	Cutinase, amylase	9–11	50	Wang et al. (2018)
Water	Lonar Lake, Buldhana, Maharashtra, India	Many haloalkalihpilic bacteria	Lipase, amylase, caseinase, cellulose	10.5	23	Kanekar et al. (2008)
Soil	Vellore, Tamil Nadu, India	*Streptomyces* sp.	Xylanase	7.5	37	Kalpana and Rajeswari (2015)
Old newsprint, magazine, inkjet, Xerox	Chandigarh, Punjab, India	*Bacillus halodurans* FNP135	Xylanase	8–9.5	65	Virk et al. (2013)
Soil	Ambala Cantt, Haryana, India	*Bacillus pumilus*	Xylanase	6–11	60	Nagar et al. (2012)
Soil	Tianshan Xinjiang, China	*Streptomyces rameus* L2001	Xylanase	5–8	70	Li et al. (2010)

(continued)

Table 12.2 (continued)

Sample	Location	Organism	Enzyme	pH	Temp (°C)	References
Industrial effluents	Shreyans Paper Industry, Ahmedgarh, Punjab, India	*Aspergillus nidulans* KK-99	Xylanase	8–8.5	55	Taneja et al. (2002)
Compost pit	BREC Sadra, Gujarat, India	*Bacillus altitudinis* DHN8	Xylanase	8.0	45–55	Adhyaru et al. (2017)
Wastepaper	Chandigarh, Punjab, India	*Bacillus halodurans*	Xylanase and laccase	8–9.5	65	Virk et al. (2013)
Soil	Effluents of paper industries, India	*Bacillus pumilus* AJK10414	Xylanase, pectinase	8.5	55	Singh et al. (2012)

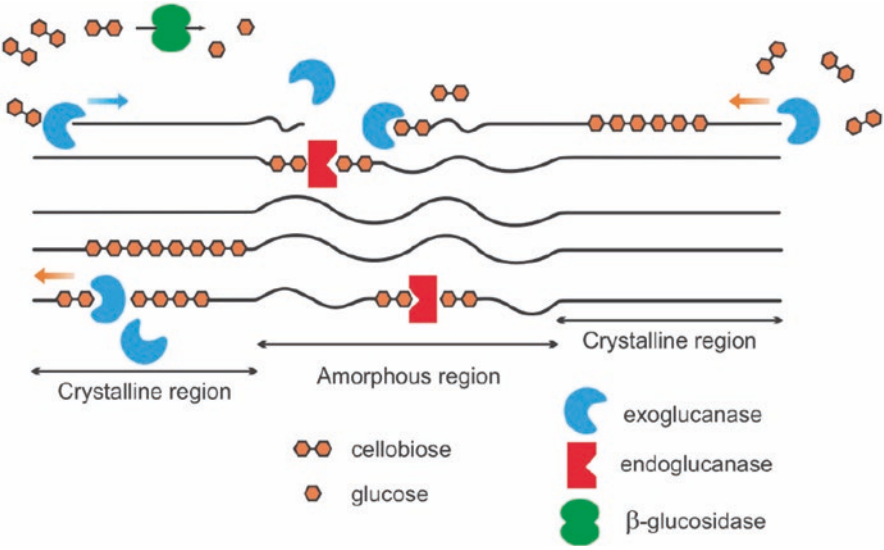

Fig. 12.2 A simplified schematic representation of the enzymatic action of cellulase, involving exoglucanase, endoglucanase, and β-glucosidase

industrial researchers. The different applications of cellulase enzyme in this industry have reached a considerable increase in the last decade. The conventional method of the woody raw material using refining and grinding happens to pulp fines with high content, bulks, and stiff. However, the enzymatic pulping process using cellulase leads to decrease in the utilization in the energy during refinement and improving the strength content of handsheet.

In Peru, a soil from an undisturbed forest was investigated for fungi capable of producing alkaline cellulase. At different $_{p}$H value, plate clearing assay and carboxymethyl

cellulase as substrate, 11 of 50 morphological colonies were selected. These 11 fungal strains produced cellulase of high alkaline PH values in a liquid culture media. The best producers of cellulase in highest productivities are the *Penicillium* sp. LM-HP33, *Penicillium* sp.LM-HP37 as well as *Aspergillius* sp. LM-HP32. These fugal strains are found to be suitable production of alkaline cellulase (Vega et al. 2012). Pathak et al. (2011), conducted a study on deinking photocopier papers using chemicals and commercial cellulase enzyme where they optimized all the parameters of deinking experiment for hydro pulping. Ink removal efficiency and freshness were improved by 24.6% and 12.6%, respectively, along with reduction of drainage time of 11.5% as compared to chemical deinking.

As compared to fungi, bacteria have a high rate of cellulase enzyme production rate due to its advantage of high bacterial growth rate. The most important parameters for successful production of cellulase enzyme are the screening of the organism, optimization of fermentation conditions and selection of substrates. Using carboxymethyl cellulase as substrate, (Ariffin et al. 2006) produced enzyme cellulase from local isolate of *Bacillus pumilus* EB3.This enzyme screened from this bacterium was purified using ion exchange chromatography using anion exchanger for cellulase characterization. Rawat and Tewari (2012) isolated and identified microorganism which hydrolyzed carboxymethyl cellulose (CMC) as *Bacillus subtilis* strain LFS3. Gel filtration chromatography, ion exchange, and sodium sulfate precipitation are the methods used to isolate and screen enzyme carboxymethylcellulase with overall recovery of 15%. Optimum temperature and pH for active profile of this enzyme was 60 °C and 4.0 respectively. A fungi called *Coprinopsis cinerea* was found to have the ability of producing a crude cellulase and xylanase enzymes with potentials of deinking photocopier wastepaper deink photocopier wastepapers as reported by Pathak et al. (2014). In their view to achieve maximun and possible efficiency without affecting paper and its strength propertis, enzyme dose, point of enzyme addition, pulp consistency, and reaction time were investigated which also confirmed the potential of crude enzyme of *C. cinerea* for deinking of photocopier wastepapers.

Effects on the use of cellulase for deinking of office wastepaper were investigated by Tsatsis et al. (2017). Better results were achieved by the use of enzyme in deinking experiment as compared to those in which enzymes are inactive. It was discovered that enzyme application has disadvantage if specks surface of the deink paper sheets was uses as compared conventional deinking. Based on their finding, more research is needed in formulations of enzyme with better performance under alkaline conditions as well as the types of paper printed in different photocopier and laser printers.

Abo-State et al. (2013) isolated *Bacillus* strains from agricultural waste and identified as *Bacillus thuringenesis* which have the ability to produce cellulase and xylanase based on their pH and temperature. Stability at different temperatures (60–80 °C) at separate duration was also investigated. Zhang et al. (2008) evaluate three commercial cellulase enzymes for their application on deinking artificially aged old newspaper (ONP) mixed with fresh old magazine (OMG) in a ratio of 7:3. At the start of repulping, these enzymes were added followed by incubation for 3 h.

Despite the fact that cellulase enzymes were able to remove a significant amount of ink from ONP/PMG, they have low efficiency than using conventional methods of either sulfite or alkaline deinking chemistry. None of the three cellulase enzymes tested were able to separately deink aged ONP/OMG, and a poor deinkability was also observed by using either sulfite or alkaline chemistry. However, the research indicates a significant increase of deinking when a combination of enzyme and sulfite is applied which provide a potential strategy of achieving effective deinking of old newspapers at neutral pH.

Enzymatic deinking has added advantages over conventional deinking viz. Reduced alkali usage, improving fiber brightness, and greater strength property. Moreover, enzymatic deinking process also prevents alkaline yellowing and reduces environmental pollution. However, excessive use of enzymes can be degradable as it might lead to significant hydrolysis. With the aim of increasing the rate of production, cellulase has been pursued by several mills in improving the drainage. These enzymes are also used in the production of easily biodegradable stationary objects including paper towels and sanitary paper (Kuhad et al. 2011). Laccase mediator system was used in a study conducted to identify the similarity on the application of cellulase/hemicellulase for deinking printed fibers from newspapers and magazines. In this regard, commercially available endoglucanase and endoxylanase activities and a commercial laccase were evaluated in the presence of synthetic or natural mediators. They concluded that factors to be considered for the application of enzymatic deinking processes in addition to enzymes include ink types, printing methods, and fiber/ink separation process (Ibarra et al. 2012). Lee et al. (2007) also developed a laboratory procedure for enzymatic deinking of wastepapers using cellulase and hemicellulase enzymes produced from *Aspergillus niger*. Using an optimized floatation system of 6.0 and 45 °C pH and temperature, respectively, an optimum deinking efficiency with these enzymes was enhanced to 95%. The deinked papers are found to have similar properties with commercial papers indicating the effectiveness of the developed enzymatic process.

12.4 Xylanase and Its Applications

Xylanase comprises the hydrolytic enzymes which are capable of breaking the β-1,4 backbone of the multiplex plant cell wall polysaccharide. Xylan is the second largest polysaccharide after cellulose (Yadav et al. 2017, 2018). A diverse array of microorganisms like bacteria, actinomycetes, yeast and fungi are involved in the hydrolysis of hemicellulose as indicated in Table 12.2. Wood is processed for debarking, chipping and screening. Then a chip undergoes steaming process so that the microorganisms become lesser in number. After this, the chips are allowed to cool down and inoculated with biopulping fungus. The biopulping process is cost effective and technologically feasible. The main advantage is the decrease in consumption of energy as well as the increase in mill consumption. The processes also lead to enhancement in the properties such as strength of the paper, and reduced

environmental impacts (Khonzue et al. 2011). From the past studies, it has been concluded that pre bleaching method with xylanase enzyme is cheaper and eco-friendly. It also decreases the significant amount of chemicals which are indulged in order to get brightness in chemically bleaching process. In conventional method of paper making process, the manufacturers use hazardous chemicals which impart negative impact to the environment.

A high amount of xylanase enzyme was produced from *Bacillus pumilus* SV-205 using optimized fermentation conditions. The bacterium secretes maximum amount of cellulase free xylanase in combination with yeast and peptone which also enhanced highest xylanase production that differ from other combinations. The enzyme maintained a thermal stability of 65% activity after incubation at 60 °C for 2 h (Nagar et al. 2012). Li et al. (2010) isolate xylanase with biobleaching potentials on wheat straw pulp from *Streptomyces* L2001 with stable optimum temperature of 70 °C and pH of 5.3. High production of xylanase from another bacterium called *Bacillus altitudinis* DHN8 followed by its purification and application was presented by Adhyaru et al. (2017). Using response surface technology, enzyme yield was improved by optimizing submerged fermentation conditions which includes incubation time, temperature, agitation speed, sorghum straw, inoculum size, and gelanin. This leads to twofold increase in activity based on the abovementioned optimized conditions as compared to activity in one factor at a time optimization. The research indicates a potential use of *Bacillus altitudinis* for biodeinking and biobleaching.

For pollution free environment, the recently employed technique is the recycling of civic paper waste by enzyme based technology. In these regards, a newly isolated bacterium for recycling of laser jet paper waste was isolated for its potential ability to purify xylanase enzyme by Maity et al. (2012).This potent xylanase producing bacterium from microbial consortia of termite gut was identified as *Bacillus sp* CKBx1D based on 16S rRNA sequence. Response surface methodology was the technique used to optimize all operational parameters, while purified enzyme mixture was used for laser printed paper waste deinking potentials. This deinking potential was detected from the enzyme at a pH of 6.8 with 72 h continuous shaking at constant temperature of 35 °C. Hence, the bacterial isolate and its xylanase enzymatic system could efficiently be used in recycling paper waste as deinking agent. Using cheap agricultural residue, pectinase and xylanase enzymes were isolated for the first time from alkalo thermotolerant bacterial strain with potentials of deinking capabilities. The enzymes may also play important role in making enzymatic deinking an eco-friendly having 50% decrease of chemicals, commercially viable with better paper quality (Singh et al. 2012). According to (Gessesse and Mamo 1999), overall cost of xylanase enzyme production from *Bacillus sp.* AR-009 can be greatly reduce using solid-state fermentation technique. This bacterium has the ability to produce dry bacterial bran xylanase activity when grown in solid-state fermentation and produced a high bacterial bran xylanase activity with wheat bran as substrate. The ability of the organism to produce high xylanase activity at alkaline pH and lower wheat bran to moisture ratio could have a potential advantage in minimizing the risk of contamination. In addition, the cost of downstream processing during

product upgrade and that of waste treatment steps can be greatly reduce since the enzyme can be produce with a least quantity of liquid.

Dutta et al. (2007) studied the culture filter of *Penicillium citrinum* grown on wheat bran bed in solid-state fermentation to purify an extracellular xylanase enzyme. Moderately thermostable xylanase showed optimum activity at 50 °C at pH 8.5. Purification and characterization of this novel endoglucanase free alkaliphilic xylanase from the alkali tolerant fungus *P. citrinum* were discovered for the first time which may have potential implications in paper and pulp industries. Desai and Iyer (2016) isolate cellulase free xylanase enzyme from fungi for deinking of Old News Paper (ONP) pulp. *Aspergillus niger* strain DX-23 from the total 16 fungal producing enzyme isolates had a maximum xylanase of 48.9 ± 0.02 Uml^{-1}. The enzyme deinked ONP pulp efficiently with improved brightness after optimization and subsequent H_2O_2 treatment as compared to untreated pulp. According to (Taneja et al. 2002), an alkaline thermostable xylanase was produced by alkalophilic fungi called *Aspergillus nidulans* KK-99 in an alkaline medium consisting of wheat bran, KNO_3, pH 10.0 and temperature of 37 °C. this partially purified enzyme was stable at a pH range of 4.0–9.5 and temperature of 55 °C and reach optimum activity at a pH 8.0 and same temperature of 55 °C where decrease of ink and brightness of recycled paper was achieved by this enzyme treatment. An investigation in to xylano pectinolytic enzymes for deinking of school wastepaper was conducted by Shatalov and Pereira (2008). This biodeinking in combination with conventional deinking approach leads to an increase in BOD and COD values of effluent as compare to use of only conventional deinking method. The usage of xylanase enzymes in deinking process has some limitations depending on different parameters. These comprise different factors like narrow pH ranges, thermal instability, no proper end product, and cost of production of enzyme.

Biological treatment using xylanase enzyme has proved to be helpful in both reducing the costs and also improved the quality of the fiber. Xylans are highly available to hydrolytic enzymes as they are not having a complex structure. Therefore, its specific activity becomes two to three times higher as compared to crystalline cellulose form (Shatalov and Pereira 2008). In order to obtain whiter and brighter pulpy matter to process better quality paper, it becomes necessary to separate the residual lignin with the use of bleaching method (Azeri et al. 2010). The most important advantages of biobleaching include: (a) Reduction in the use of the bleaching chemicals, (b) Enhanced quality of the paper and pulp, (c) Improving whiteness and brightness of the pulp, and (d) Decrease effluent toxicity and pollution load.

12.5 Laccase and Its Applications

Laccase is the oldest and widely studied enzymatic system discovered for the first time in 1883 by Yoshida from the exudates of Japanese lacquer tree called *Rhus vernicifera*. In 1985, Bertrand discovered it as a metal containing oxidase, while in

1896, it was demonstrated by both Bertrand and Laborde to be present in fungi. The redox potential is found to be higher in fungal laccases rather than bacterial or plant laccases up to 800 mV. Therefore, these are found to be beneficial in lignin degradation or in the eradication of phenolic toxins arising during lignin degradation. Laccase is found to be present in higher plants and fungi such as basidiomycetes, white root fungi and ascomycetes. White rot basidiomycetes are responsible for efficient degradation of lignin and the enzymes involved in lignin degradation includes lignin peroxidases, manganese dependent peroxidases and laccases. Also, fungal laccases have been involved in cellular processes in many ways including sporulation, plant pathogenesis and other specificity. Woody chips when pretreated with lignolytic fungus leads to increase in the strength of the pulp while decrement in the requirement of energy for the mechanical pulping process.

The most widely studied application of laccase in this industry is bleaching of kraft pulp. It was studied that when SL4, a lignolytic fungal strain, was applied, it reduced 25% usage of chlorine during bleaching of kraft pulp and produced 1.8 unit brightness (Kaur and Nigam 2014). *C. albidus* responsible for producing laccase helps in the reduction of lignin content found in the eucalyptus wood and happen to be useful in the process of biopulping. Fungal laccases also efficiently remove toxic effluents coming from the pulp mills which contain a significant number of phenolic compounds and chlorolignins. Laccase mediated biobleaching process is a friendly way to improve pulp and paper production.

The ink removal capability of laccase and xylanase enzymes from an alkalophilic bacteria on recycled old newspaper (ONP) in combination with physical deinking method of sonication and microwaving were investigated. Parameters such as PH, enzyme dose as well as treatment time of these enzymes were optimized statistically using Response Surface Methodology in which any mediator supplementation for deinking was not required. Optimization of these deinking parameters were conducted statistically using response surface methodology in which for the first time laccase did not need any mediator supplementation (Virk et al. 2013). An enzyme showing alkaliphilic laccase activity was purified from the culture supernatant of *Myrothecium verrucaria* 24G-4 by (Sulistyaningdyah et al. 2004). The enzyme was very stable in alkaline conditions with an optimum pH of 9.0 and was able to remove synthetic dyes under the same conditions which confirm the function of this enzyme in alkaline environment.

12.6 Amylase and Their Applications

Amylases hydrolyze starch molecules and give diverse array of products including dextrin and glucose units (Fig. 17.2). Microbial amylase enzymes are available in market in large number and they are replacing chemicals involving hydrolysis of starch. The first commercially produced microbial enzyme was amylase which was of fungal origin in 1894 and used as therapeutic aid to cure digestive disorder. Nearly 25% of the global enzyme comprises amylase. Enzymatic pretreatment of

wastepaper pulp was conducted for deinking process at neutral conditions using amylase and cellulase enzymes and ethoxylated fatty acids as surfactant. At a temperature of 40 °C, this enzymatic deinking process was conducted by flotation consistency of 0.8% within 6 min (Gil et al. 2013). In a future work, effectiveness of combine enzymes in removal of waste photocopy paper and other wastepapers mixtures is expected to be discussed.

Amylases are distributed in plants, animals and microbes. However, amylases produced by microbes are replacing the chemical processing methodology indulged in paper industries due to cost effectiveness and technical advantages. Amylases classified into two types on the basis of catalysis, endoamylase and exoamylase. Endoamylases carry out hydrolysis in a random manner while exoamylases hydrolyze from the nonreducing end. Endoamylases results in oligosaccharides with branching of different chain length and exoamylases forms short end products. Main fungi involve in the production of industrially important enzymes are *Aspergillus niger*, *A. oryzae*, and *A. flavus.* Also these fungi are capable of producing large quantities of amylases which can be used commercially. *Aspergillus niger* having significant amount of hydrolytic capacities in the production of α-amylase (Sahni and Goel 2015)

Sakthivel et al. (2010) isolated and screened some bacteria that inhabit decaying vegetables for the production of amylase enzyme. *Enterococcus pseudoavium* is the only specie identified to have a relatively higher amylase activity of the bacterial species tested. Four days after growth, the organism deinked pulp completely when grown with paper pulp. When cultured with paper pulp, the bacterial culture immobilized in sodium alginate beads can decolorize this paper within 4 days. This shows the ability of extracellular amylase produced from *Enterococcus pseudoavium* to effectively deink and decolorize paper pulp within 4 days of incubation. The role of α-amylases in the paper industry as shown in Table 17.4 is to modify starch of coated mixed paper which comprises production of lower viscous, starch with high molecular weight, deinking, drainage and cleaning of paper. The coating tends to make the paper smoother and stronger, to improve the quality of writing. Since the viscosity of natural starch remains high enough for paper sizing and this can be changed by degradation of the polymer with the use of α-amylases in the batch or continuous culture. Starch being good sizing agent helps in giving fine finish of the paper, improvement of the paper quality, and reusability, besides behaving as a perfect paper coating (Singh et al. 2012).

12.7 Lipase and Its Applications

Lipases are the enzymes which hydrolyze long chain triglyceride and constitute as one of the most focal biocatalysts for biotechnological applications. It was first found to be present in pancreas by J. Eberle in 1834 and during 1856 by C.I Bernard. Under aqueous conditions, they are able to release fatty acids and glycerol by acting on carboxyl ester bonds present in triacylglycerol (Gupta 2004). These are serine

hydolases and are able to work independently without the use of any cofactor. Microbial lipases play an important part in the field of biotechnology due to its versatility and ease of mass production. It has got wide enzymatic properties. Both bacterial lipase and fungal lipase are widely used in different industries. Lipase producers include bacteria, fungi, yeast, and actinomycetes. Examples of fungi producing lipase are *Acinetobacter radioresistens*, *Aeromonas hydrophila*, *Aspergillus oryzae* (Andualema and Gessesse 2012).

Wood is the cheap source of paper pulp and pitch describes the hydrophobic contents of wood (triglycerides and waxes). These are appearing to create serious problem in the paper processing. The problems might be sticky deposits and holes or spots in the finish product. However, lipases remove the pitch from the pulpy matter during the process of paper making process. Around 90% of triglycerides present in the pitch get hydrolyzed into glycerol, monoglycerides, and fatty acids by lipase enzymes which are having less stickiness and more hydrophilic character (Jaeger and Reetz 1998). In Japan, a paper industry called Nippon Paper industries developed a method to control pitch that utilizes the *Candida rugosa* fungal lipases to hydrolyze up to 90% of the woody triglycerides present. Lipases have many advantages in general as its utility increase the rate of pulping, enhancing whiteness and intensity power, decrement in the usage of chemical, equipment with prolong life, reduction in the level of pollution in the wastewater, saving energy utility, and time and reduction in the composite cost. *Pseudomonas* sp. (KWI-56) has an added advantage as addition of its lipase to the deinking process leads to increase in the whiteness of the paper and reduce usage of residual inking. A thermophilic isolate of *Bacillus coagulans* BTS-3 can be used to screen alkaline lipase. The bacterium can be enhanced substantially when parameters like nitrogen source, carbon source, and initial pH of culture medium were consecutively optimized. Enzyme activity of culture medium was obtained in 48 h at 55 °C and pH 8.5 with refined mustard oil as carbon source and a combination of peptone and yeast extract as nitrogen sources. Maximum activity of the enzyme was achieved at 55 °C temperature and 8.5 pH and was also stable between the pH range of 8.0 and 10.5 at temperatures up to 70 °C. This purified lipase enzyme indicates a variable hydrolytic activity toward various 4-nitrophenyl esters (Kumar et al. 2005).

12.8 Other Microbial Enzymes and Their Applications

Cutinase and other microbial enzymes have also play a vital role in recycling of wastepaper (Table 17.2). For the first time, Wang et al. (2018) reported the effect of cutinase enzyme on the deinking of mixed office wastepaper (MOW). Combination of cutinase, amylase, and some complicated surfactants can be used to replace the conventional chemical deinking methods at neutral deinking process. When these enzymes are treated in combination of surfactants mixed with pulp and office wastepaper at a temperature of 50 °C for 30 min, the brightness, ink removal rate, tensile index, and tear index of the deink paper will increase significantly. Hemicellulase

was combined with laccase mediator system (LMS) by Xu et al. (2011) to deink old newsprint (ONP). The result indicates the effective residual ink concentration was lower as compared to pulp deink with hemicellulase or LMS individually. According to this study, an environmentally friendly and effective deinking method can be achieved when cutinase and amylase enzymes combine with cardanol polyoxyethylene ether and other surfactants.

12.9 Conclusion and Future Prospects

Industrial utilization of wastepapers in the production of new one is increasing globally. Currently, pulp and paper industry is one of the largest consumers of wood. Based on their demands due to global economic growth, more trees will be harvested, and waste will be consumed and disposed in the environment. With increasing demand of paper globally and high cost of conventional methods for deinking and recycling of wastepapers, attentions are now focused on the use of enzymes for eco-friendly deinking of wastepapers especially extremozymes such as cellulase, hemicellulase, xylanase, amylase, and laccase that are able to deink wastepapers at optimum environmental conditions. Combination of enzymatic and chemical methods can greatly increase the brightness of deink wastepaper, ink removal, and quality of recycled wastepapers. However, biological deinking method of wastepaper with the help of varied microbial enzymes comprises an eco-friendly way that can produce high-quality paper without relying upon the traditional usage of harmful chemicals. Thus, more efforts should be efficiently directed to still tap the wide underlying potential of these fungal lignolytic enzymes.

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