Microbial enzymes in paper and pulp industries for bioleaching application

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Abstract

In the present world, the demand of paper recycling process is quite high. Presently many industries rely on chemical based process for biobleaching of paper pulp which is toxic. Hence, we aimed at replacing the chemical process with suitable microbial enzymes in biobleaching and pulp modification application. This chapter reviews the various microbial enzymes, its mechanisms and quality enhancement of recycled paper. This also shed light on the challenges in usage of microbial enzymes in scale up process in paper industries. The biological deinking is a process which is an eco-friendly method that removes ink entrapped in the paper pulp. During enzymatic deinking there is reduction of heavy metal content also. This is highly significant process in the sustainability aspect.

Keywords: Deinking; Pulp; Brightness; Enzymes; Eco-friendly

Introduction

The growing demand for paper imposes a severe thrust on the environment especially the forest resources. However, because of depleting forest resources pressure has increased in the search for new solutions to meet the growing demands for paper. To resolve this issue, the recycling of waste paper is gaining an increased importance in modern society. Thereby, waste paper becomes an important fiber source in paper and pulp industries throughout the world [1]. Recycling of paper is considered as an alternate solution to reduce the stress exercised on the environment [2]. Similarly, in some places like in the Province of Quebec, recycling of old paper becomes mandatory for the paper and pulp industries. Moreover, there was an increased usage of recycled fibers due to social pressure for sustainable development, increasing landfill cost and other regulatory actions imposed by the United States [3].

Microbial enzymes are extensively studied for the various applications. Many have identified the bio-beaching potential and pulp characteristics modification properties of certain microbial enzymes such as cellulase, xylanase, pectinase, mannase, laccase and lipase. However, their mode of action in bio-bleaching is to facilitate the residual ink removal by fiber breakage. Though there have been many reports on the bio-bleaching applications of such enzymes, they have not been used for application. Paper and pulp industries still go for chemical deinking methods in spite of their negative effects on the environment.

The biological deinking method using microbial enzyme is quite promising and rather eco-friendly but highly challenging when compared to chemical deinking. However, the combinational usage of chemical and biological deinking method reduces the chemical consumption. But, the toxicity of the resul-
tient effluent is to be resolved and only biological method can achieve this.

Conventional deinking methods

During the paper recycling process, the major issue is the residual ink entrapped in the paper which reduces the brightness of the paper. There was an increased utilization of secondary fibers over the last decade and deinking process is the crucial step involved in the same [4]. The current deinking process depends upon the usage of many environmental hazardous chemicals namely NaOH, Na$_2$SiO$_3$, Na$_2$CO$_3$, H$_2$O$_2$, chelating agents and surfactants [5, 6]. The chemical based deinking methods produce toxic effluents which increase the COD values of the water and hence resulting in costly waste water treatment [5]. Chemical deinking mainly uses chlorine based chemicals. There is a demand for chlorine free paper and paper products since the chemical deinking retains the residual chlorine in paper and paper products [7, 8].

Microbial enzyme assisted deinking

Enzymes are used as alternate agents to overcome the problems of the polluting technologies [9]. In order to overcome these disadvantages, enzymatic deinking has attracted a great deal of attention due to its high efficiency and low environmental impact [10-12]. Bio-bleaching using enzymes could replace the use of chlorine and chlorine compounds in the bleaching process [13]. [14] Reported on the application of white-rot fungi to degrade residual lignin in the pulp by lignolytic enzymes like manganese peroxide and laccase or by hemicellulolytic enzymes such as xylanase.

The deinking process employing the microbial enzymes mainly act upon the paper pulp fibers and make the xylan to break and expose lignin. Lignin is the main cause for yellowing due to oxidation process. The cellulase and xylanase enzyme act upon pulp fibers to break them to smaller fractions and facilitate the ink residues adhering on the fiber surface to release out. The fiber surface smoothing or polishing is the main reason the deinking process. The efficiency of enzymes also depends upon the type of paper, the type of printing ink used etc. The deinking process of inkjet-printed waste paper and flexographic printing waste vary due to the nature of the ink residues. Flexographic ink usually requires more complex enzyme cocktails with inclusion of lipase.

Cellulase

Cellulase is a major enzyme well characterized and studied with many industrial applications. Cellulose is the major sub- strate of the cellulase enzyme and degradation of cellulose requires a combination of different types of cellulase. Cellulase enzymes are classified into various types namely endoglucanase (E.C. 3.2.1.4) which cleaves cellulose internally where as exo-glucanase (E.C. 3.2.1.91) cleaves cellulose from free ends resulting in cellobiose. The carbohydrate binding domains are the key sites for substrate enzyme interaction. However, the paper pulp industries utilize cellulase with no carbohydrate binding sites for enzymatic bleaching.

Xylanase

Xylanase are hydrolyases, depolymerising the plant cell wall component-xylan, the second most abundant polysaccharide. There were many applications identified for the xylanase enzyme. However, the thrust area of application is identified in paper and pulp industry. Removal of lignin and its derivatives are linked to xylan and cellulose is tedious process in the paper bleaching. Xylanases are more suitable in the paper and pulp industry than lignin degrading system [15].

Figure 1: A typical deinking process adopted in a small scale paper recycling unit.

Enzymes in deinking and their mode of action

There are many enzymes employed for the deinking applications and the effectiveness depends on the source and the enzyme efficiency. Sometimes, fungal enzymes were found more potential for deinking application than bacterial counterparts. However, the bacterial derived enzymes have advantages for ease in cultivation, scale up and high possibility for protein engineering and its expression. For the paper and pulp industries, enzymes that can withstand the industrial conditions are essential for the deinking application. During the paper recycling process, there may be unintentional presence of many inhibitors of enzymes that includes heavy metals present in the printing ink or residual heavy metals, aromatic compounds, phenolic that must have formed during the degradative reactions. These enzyme inhibitors are often a threat to enzymatic deinking process especially on commercial basis. The researchers must identify suitable enzymes for that acts efficiently in the paper and pulp industrial conditions. The typical deinking process using microbial enzymes utilize ligno-cellulosic enzymes. Cellulase, xylanase, laccase and lignin peroxidase are some of the major enzymes. Other hydrolytic enzymes used for deinking include pectinase, mannanse, amylase and lipases.

There are many reports on hemi-cellulases which deal with the production, properties, mode of action, and applications of xylanases [16-21]. Xylanases are mostly belonging to either of the two structurally different glycosyl hydrolase groups. Xylanase produced by Trichoderma reesei from family 11 is a small, ellipsoidal enzyme with a diameter between 32 and 42 Å. It does not have any separate substrate-binding domain [22]. Some xylanases have been reported to contain either a xylan-binding domain [23] or a cellulose-binding domain [24, 25]. The significant difference among different xylanases characterized are the differences in the spectrum of end products produced by hydrolyzing different types of xylans, showing only. However, there is a lack of systematic studies on the substrate specific-
ity of xylanases belonging to different families on fiber-bound substrates.

**Lignin modifying enzymes**

Laccases are widely distributed group of multi copper enzymes present in higher plants and fungi. Ascomycetes, deuterozymes, basidiomyeteces and many white-rot fungi are the many sources for laccase. The ability of laccase to act on wide range of substrates has made them very important in many of the biotechnological applications. They have wide activity range of temperature and pH. They act upon both phenolic and non-phenolic lignin related compounds. Action of laccases on highly recalcitrant environmental pollutants enable its application in detoxification, de-colorization of industrial effluents and waste water treatment. They can also be used effectively in paper and pulp industries as well as textile industries [26].

Apart from laccase, there are many other lignin degrading or modifying enzymes. Fungal peroxidases are heme proteins which oxidize their substrates with hydrogen peroxide as electron acceptor in 2 one-electron steps, like all other peroxidases [27]. LiPs have high redox potentials and can oxidize non-phe- nolic lignin model compounds, aromatics ethers, and polycyclic aromatics. Manganese peroxidase is a heme protein which oxidizes Mn$^{2+}$ to Mn$^{3+}$. To stabilize the ion and to promote its release from the enzyme, chelation of Mn$^{3+}$ by organic acids is necessary. Laccases belong to the copper metalloenzymes and to the blue oxidase subgroup. Laccases contain four copper atoms per molecule.

**Lipases**

Lipases (E.C. 3.1.1.3) are a class of carboxylesterase which catalyze the hydrolysis of long chain acylglycerols to glycerol, free fatty acids and mono- and di-glycerides. These enzymes are widely found in the animal and plant kingdoms, as well as in molds and bacteria. In addition to their natural function of hydrolyzing carboxylic ester bonds, lipase can also catalyze esterification, interesterification, and transesterification reactions in non-aqueous media. This versatility makes lipase a good candidate for the application in food, detergent, pharmaceutical, leather, textile, cosmetics and paper industries [28].

Application of lipase in wastepaper deinking can increase brightness, intensity and the pulping rate. It can also decrease chemical consumption, prolong machinery life, reduction in pollution, save energy, time and production cost. The addition of lipase from *Pseudomonas sp.* KWI-56 to a deinking composition for ethylene oxide-propylene oxide oxide adds to paper and reduces residual ink spots [29]. Lipase has also been used in enzymatic pitch control in a large scale paper making process since 1990s [30].

**Enzymatic deinking and paper characteristics**

The enzymatic treatment of paper pulp results in various changes including its morphological, chemical and physical changes. These changes also vary by its degree with the enzymes used. In the following section, the effect of various enzymes on its morphological as well as chemical characteristics are described. Moreover, the deinking which is measured by the change in brightness level is also addressed.

**Cellulase-Hemicellulase systems in biobleaching**

When cellulases/hemicellulases are used, the release of ink particles into suspension is generally attributed to the cellulose hydrolysis on the fiber/ink inter bonding regions, which facilitates ink detachment. Furthermore, these enzymes can remove small fibrils from the surface of the ink particles thus altering the relative hydrophobicity of the particles, which facilitates their separation in the flotation or washing step. However, it was found that the application of enzymatic deinking causes increased brightness and increased dirt removal but there was a reduction in mechanical properties of hand sheets [31]. [32] studied the application of endoglucanases and endo-xylanases in deinking of mixed office wastepaper. When crude enzyme of *Trichoderma viride* was used, an increase of ink removal by 24% was observed. Both enzymes contributed to the enhancement of paper strength properties and were found effective for deinking of mixed office wastepaper.

Recycling of photocopier paper is a major problem due to the difficulty in removal of non-impact ink. Commercial cellulase enzyme was used along with deinking chemicals to improve the deinking efficiency. Cellulase assisted deinking could enhance ink removal efficiency by 24.6% and freeness by 21.6% with a reduction in drainage time of 11.5% when compared to the results from chemical deinking. Physical properties like burst index, and tensile strength were improved by 15.3% and 2.7% respectively and brightness by 2.1% [33]. In 2010, Valls et al., [34] proved the efficiency of xylanase in bleaching of eucalyptus kraft pulp. However, the study was performed using xylanase combination with hydrogen peroxide and chlorine dioxide. The xylanase did not significantly bleach the pulp but there was an increase in hexenuronic acid (HexA) removal by 10% after the chlorine dioxide treatment. Among the two xylanases studied, one exhibited an increased delignification of 9% and brightness of 3% ISO.

Maity et al., [35] used xylanase produced by *Bacillus sp.* to deink the laser jet paper waste. The deinking of laser printed waste was performed using the purified enzyme mixture. After the enzymatic deinking using the bacterial xylanase, the physical properties of the pulp like brightness and ERIC values were enhanced, whereas the pulp opacity was much reduced when compared to the control. Woldesenbet et al., [36] produced xylanase enzyme using *Bacillus halodurans* FNP 135 for the bio-bleaching of eucalyptus kraft pulp. Microwave irradiation enhanced xylanase mediated bio-bleaching which increased brightness of 1%, decreased kappa number by 14.3% and 20% reduction in chlorine consumption. In 2013, Woldesenbet et al., [37] used alkalophilic bacterial xylanase along with man- nase and laccase-mediator system. 40.7% reduction in kappa number and 38.3% hexenuronic acid content was achieved with increased brightness of 31.5%. There was an increase in burst factor by 20.6%, tear factor by 20.2%, breaking length by 39%, and pulp viscosity by 12.1%. 30% reduction in chlorine and 44.4% of H$_2$O$_2$ consumption was observed when triple enzyme treatment was used.

Similarly, deinking of old newspaper pulp with combination of xylanase and laccase enzyme was investigated. Compared to the individual enzyme treatment, the combination of xylanase and laccase achieved a higher reduction of ERIC values to 65.8%. There was an increase in brightness to 21.6%, breaking length (16.5%), burst factor (4.2%), tear factor (6.9%), viscosity (13%) and cellulose crystallinity (10.3%) along with a decrease in kappa number (22%) and chemical consumption (50%) which were observed [38].
Laccase in deinking

Laccase removes the surface lignin in the presence of some low molar mass chemical mediators [39]. Hence, this is found to be promising for the deinking of old newspaper which mainly contains lignin rich mechanical pulp [11]. Xu et al., [40] observed that deinking of old newspaper with laccase-violuric acid system (LVS) gave 20% and 13% higher tensile and tear strength respectively. The brightness of the LVS - deinked pulp was 4.2% ISO higher than that of the control after being bleached with H$_2$O$_2$; however, the brightness was only 52% ISO.

Lund and Felby [41] found an improvement of wet tensile strength when unbleached kraft pulp was treated with laccase or laccase-mediator system (LMS). Old newspaper could effectively deinked by combining hemicellulase with LMS. In another research, the brightness of the hemicellulase/LMS-deinked pulp after H$_2$O$_2$ bleaching was higher than that of the LMS-deinked and hemicellulase-deinked pulp. Decrease of ERIC value indicates that more ink particles have been detached from the fibers. There was a synergetic deinking effect between these two enzymes [42]. Virk et al., [43] observed the bio-bleaching efficacy of laccase produced by Rheinheimera sp. There was an increase in brightness by 2.9% and reduction of kappa number by 20% of eucalyptus kraft pulp bleaching. The xylanase ABTS-laccase system resulted in 15, 13 and 10.9% increase in burst factor, tear factor and viscosity with a 20% reduction in elemental chlorine and hypochlorite consumption.

Lipase in deinking

Celine et al., [3] evaluated the potential of lipase and laccase in deinking and bleaching process. Treatment using lipase enzyme at pH values from 8.5 to 10 had a slight impact on pulp brightness. At pH 9, an increase in brightness of 0.9% was obtained. A slight reduction in the effective residual ink concentration (ERIC) value was observed after the flotation step.

Figure 2: The deinking of paper pulp during pulping process in a beater machine. Fungal enzymes produced in solid state fermentation extracted and added to beater just after the initial pulping.

Figure 3: Application of various enzymes used in the paper and pulp industries and their functions.

Figure 4: Representation of catabolite repression during the enzyme addition into the paper pulp. The sugar moieties released during hydrolysis can be utilised for bioenergy production or even as carbon source for further enzyme production.

Point of enzyme introduction in deinking

As aforementioned, the enzymes involved in the deinking process are largely produced from both bacterial and fungal sources. However, there point of introduction into the deinking process is critical in achieving the deinking efficiency. The deinking process may even be initiated before the pulping process. Sometimes, the deinking cocktail can be added along with the paper waste during pulping process into a hydrapupler or a beater. Otherwise, it could be introduced to paper pulp after the pulping process just before flotation. The result of deinking may tend to vary according to the point of addition of these enzymes. Another option is to use some enzymes like cellulase and xylanase in combination in the pulping process and addition of lipase like enzymes later on just before flotation.

Figure 5: Schematic representation of point of addition of enzymes during the paper pulp deinking process.

Strategy -1

<table>
<thead>
<tr>
<th>Waste paper</th>
<th>Pulping</th>
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<td>Cellulase/Xylanase/Laccase/Pectinase + Lipase</td>
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Strategy -2

<table>
<thead>
<tr>
<th>Flotation</th>
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<tr>
<td>Lipase</td>
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</table>

Paper making
Characteristics modification of paper pulp

Ligno-cellulosic enzymes had been used for pulp modification by many previous researchers. In our studies, cellulase, xylanase and laccase were used for pulp modification [44-48]. The 5% paper pulp treated with 10% (v/w) crude cellulase enzyme produced by *T. viride* VKF3 and was found to be optimum for the beneficial pulp modification during recycling process [45]. 5% pulp consistency with 15% enzyme dosage could reduce the lignin content to the maximum level when treated with laccase produced from *F. equiseti* VKF2 [47]. Low cellulase enzyme dosage from *E. coli* SDS was found suitable for pulp modification [48].

FT-IR analysis of the enzyme treated paper pulp was performed (Figure 6). O-H stretching was detected due to hydrocarbon chromophores during fungal cellulase + xylanase treatment. There was disappearance of this C-H stretching during fungal lipase and laccase treatment. Some common groups detected were C=S stretching vibrations, O-H bending and C-O stretching vibrations due to phenols in paper pulp. There was C-Cl stretching vibration in treated pulp. N-H bending vibrations and CH symmetric bending are due to primary and secondary amines disappeared during enzymatic deinking. There was only C=S stretching vibrations detected in lipase treated pulp whereas S=O stretching vibrations of sulfonyl chlorides and N-H stretching vibrations due to secondary bonded amides was detected with laccase enzyme treatment. These observations were supported by Xu et al. [42] who obtained bands indicated the presence of cellulose (at 3335 and 1000-1200 cm⁻¹), hemicellulose (at 1058, 1315, 898-895 and 810 cm⁻¹) and high lignin content (at 1510-1508, 1605 and 1652 cm⁻¹) in the pulp during FT-IR analysis. Xu et al. [42] also observed a reduction in relative intensity at 1375-1372 cm⁻¹ due to the lowering of Phen-OH of lignin during combinatorial enzyme treatment during deinking which was in agreement with the present study.

![Figure 6: FT-IR spectrum of paper pulp showing the chemical modification of the paper pulp during enzymatic deinking process.](image)

From the SEM analysis of enzyme treated pulp, breakage of fibers was confirmed which attributed to release of adhering ink particles. This was in agreement with the decrease in fiber coarseness during enzymatic deinking. General changes observed were development of cracks, pores and peeling which facilitated the release of high amount of lignin molecules and the ink particles entrapped. The results were in hand with the previous reports [49]. During XRD analysis of the deinked recycled paper, only a negligible reduction in crystallinity index was observed which was supported by the results of Park et al., [50]. Fibrillations were observed in the SEM analysis of enzyme treated pulps and crystallinity index calculated from XRD analysis was found to be lowered when compared to the untreated control. Efrati et al., [51] also reported the effects of cellulase from *Trichoderma* on deinked pulp on fiber morphology, crystallinity and strength of pulp.

**Brightness and physical property changes in deinking of waste papers**

It was found that the enzyme facilitated a better reduction of kappa number and Hexenuronic acid (Hex A). Maximum Δ brightness (10%) was observed when 5% paper pulp was treated with 10% fungal cellulase for 1 hr [47]. The fungal xylanase achieved maximum Δ brightness (%) when treated with high enzyme dosages of 20 and 30% with 4 hrs of incubation time. Fungal lipase and laccase also contributed towards the increase in paper brightness. Laccase exhibited a Δ brightness of 15% following 4 h of enzymatic treatment [47]. However, it was observed that the cellulase and xylanase were more efficient in deinking of newspaper pulp which was in agreement with the report of Paik and Park, [52]. Ghataora et al., [53] also observed a maximum chromophore release, reducing sugar release and increase in brightness of pulp 2.04 ISO units when treated with alkaline xylanase produced by *Malbranchea* sp. It was found that hemicellulose in combination with laccase mediator system efficiently reduced lignin content from the fiber surface. The effective deinking through ink removal may be attributed to the detachment of ink through synergistic delignification effect [42].

Similar to the present work, Dutt et al., [54] tested the biodeinking efficacy of cellulase, xylanase, amylase and lipase which resulted in improved brightness and a reduction in ERIC and dirt counts by 68.18 and 88.04%, respectively. Dutt et al., [55] also used xylanase and cellulase produced by *Coprinus cinereus* AT-1 MTCC 9695 deinking of sorted old paper waste which resulted in an improved brightness of 3.07%. These results supported the present observations. Xu et al., [49] also supported the efficiency of the enzyme combinations in deinking process.

In the individual enzyme treatment, there was a reduction of kappa number, increased Hex A release and improvement in brightness which was supported by the report of Savitha et al., [56]. It was observed that xylanase enzyme pretreatment could reduce kappa number from 19 to 15 and increase brightness by 2 ISO units. Similar results were also observed by Palaniswamy, [57] and Angayarkanni et al. [58]. Valls et al., [34] reported that, Hex A release by xylanase was due to the increased dissolution of xylans during the enzymatic treatment of pulps containing these acids. The enzymatic deinking in the present study was performed at room temperature and there was sufficient improvement in brightness. The process performance at room temperature is advantageous as it saves energy consumption during process and reduces the process cost.
• Enzymatic deinking utilizes microbial enzymes which are easy to synthesize
• The process is cost effective
• This results in lowering of energy consumption
• This enhance many physical properties like burst index and tensile strength.

Commercial enzyme formulations for enzymatic deinking

There was numerous research carried out in understand the efficiency of enzyme mediated deinking. As a result of these continuous efforts, presently there are many industries came forward with commercial products for the deinking process. These products are either individual enzymes with superior properties withstanding the paper pulp industrial conditions or a combination of enzymes that is added as a cocktail. Some of these products claim to improve fiber strength, lower energy consumption, dewatering and improve processability. Products like MetZyme® BRILA™ has extraordinary pH and temperature capabilities allowing for adoption to most recycled / deinked pulp processes. POVON™ another product claims the increases the strength properties of chemical pulp without breaking the fibre. Removal of lignin and a selective partial hydrolysis of the precipitated hemicellulose will be achieved by treatment of kraft pulp with MetZyme® POVON™ prior to bleaching. This results in increased quantities of lignin can be washed from the pulp, and the pulp is predisposed to bleaching chemicals.

Demand projections for paper and pulp related enzymes

As per the Pulp & Paper Enzymes Market Size – Industry Share Report 2017-2024, it was found that cellulase market will hike upto 3.5 kilo tons by 2024. Increased usage of cellulase enzyme for fiber modification, improving machine run ability, reducing risk of unwanted deposits and drain refining recycled waste cardboard will fuel the product demand. Reduced use of harsh chemicals including, caustic soda owing to emergence of cellulase will support product demand. Amylase paper and pulp enzymes market was valued over USD 45 million in 2016. The product demand in this segment will be driven by its wide scale usage in various applications including, deinking, coating of starch, cleaning and drainage improvement particularly in cardboard industry. Similarly, xylanases will be worth over USD 35 million by 2024. In the gesture of chemical usage reduction for bleaching of kraft pulps to attain a specified brightness, there will be an increased xylanases demand. Novozymes, Dupont and AB Enzymes capturing around 60% of the overall industry share at global level. This is followed by other enzyme producers like Buckman Laboratories and Enzymatic Deinking Technologies accounts for 20% of the market share. Anthem Cellutions, Biotech, Rossari Biotech, MetGen, Krishna Speciality Chemicals, Nature Bioscience, KPS Bio, Enzyme Solutions and Iogen Corporation are the other prominent industry players. Moreover, a 6% demand will raise for lipase by 2024.

Effluent characteristics of deinking plant

Physicochemical parameters were analyzed for the resultant effluents after the enzymatic deinking process. Devi et al., characterized wastewater from a South India Paper Mill, Karamataka, India which is using recycled waste paper as a raw material. Heavy metals like iron, lead, copper, cadmium, nickel, and zinc were found in less quantity in raw effluent and were almost completely removed after treatment. It was found that the en-
zymatic deinking process reduces COD more than the conventional alkaline deinking method. This finding is supported by the report of Yang et al. Wastewater effluents from enzymatic deinking were reported to be 20e30% lower in COD than wastewater from chemical deinking processes. Another report indicated that the COD load after enzyme treatment was 50% lower than for conventional chemical deinking. In 2018 (a), Nathan et al., [46] also observed that laccase along with ligno-cellulosic enzymes results in an effluent free of heavy metals. Fig 10 depicts the elementar analysis of solid sludge resulted from an enzyme mediated deinking. Table 4 enlist the composition of the solid sludge. There is a reduction in C/N ration during enzymatic deinking that lower the organic load. This opens a new approach of bio-mediation in paper industries.

![Figure 10: Elemenat analysis of solid sludge resulted from biological deinking showing high amount of organic matter which could be utilized for fertilizing soil.](image)

### Challenges of biological deinking

While discussing the challenges of biological deinking, the major points come to our mind are the outweighing advantage and ease of chemical deinking methods. Though environmentally harmful, the chemical deinking is still practiced in many industries. Following are the major challenges of biological deinking.

1. Enzyme based deinking depends on the efficiency of enzyme under various environmental conditions. For example, an enzyme may have its optimum activity at mesophilic condition and hence may show less deinking efficiency at psychrophilic and thermophilic conditions.
2. The enzyme must withstand inhibitory actions of various additives in paper and pulp industries.
3. The enzyme production must be easy, scalable, reproducible process and economically viable.
4. Pure enzymes are rather costly and may increase the process cost of enzymatic deinking. Hence, the partial purified enzyme or crude enzyme itself should be able to perform deinking process.
5. Enzymatic deinking should achieve superior recycled paper quality in terms of paper brightness as well as its mechanical properties.
6. The source of enzyme should be non-pathogenic and the enzyme as such should not have any deleterious effect even if exposed by the handlers.
7. The enzyme cocktails are found more efficient than individual enzymes in deinking process. However, the formulation when made using enzyme combinations, they should not have any antagonistic effect.
8. Enzyme should not undergo inhibition due to mechanisms like catabolite repression. During cellulose and xylan degradation, sugar moieties will be released into the pulp solution which in turn may lead to catabolite feedback mechanism.
9. Unlike other industrial applications, in paper and pulp industries, use of immobilized enzyme may become a difficult task which reduces the chance of the enzyme recovery and reuse.
10. Organic load will be more during the enzymatic deinking and may result in a high BOD. This effluent needs proper treatment before release.

These above challenges need to be addressed by future researchers in the field of enzymatic deinking for making it commercially viable and implementable. These above challenges are critical to be addressed for making the deinking process truly eco-friendly through enzymatic process.

### Future directions in deinking research

Enzyme based deinking has a great prospect in the future. The waste management is a great issue in the modern days and effective recycling of paper waste will reduce a great proportion of solid waste. This will help in lowering of greenhouse gas emission as well as the menace caused by solid waste. The enzyme assisted deinking is rather safer for environment and is simpler. Implementing the biological deinking can make the paper recycling a complete eco-friendly process. As mentioned above, many challenges in implementing enzyme based deinking have been identified and need to be address well in the further studies. Safety and viability are the key factors for commercialization of this technology. Researchers should further identify the research gaps and try resolving them to make the paper recycling process a successful one through enzymatic deinking process.

### Acknowledgement

Authors are thankful to Department of Science and Technology, Government of India and Tamil Nadu State Council for Science and Technology for providing the grant to facilitate the research [Sanction order no. DST/SSTP/TN/2K 10/126(G)]. Authors thank Management and Department of Botany and Microbiology, Lady Doak College, Madurai for providing the facilities and supporting the work. NVK acknowledge the financial support of Science and Engineering Board (SERB), Government of India through National Post-Doctoral fellowship [PDF/2016/000438].
Table 1: Examples of enzyme systems used in deinking process with its characteristics modifications.

<table>
<thead>
<tr>
<th>Enzyme Used</th>
<th>Remarks</th>
<th>References</th>
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<td>Cellulase/ Hemicellulase</td>
<td>Increased brightness and dirt removal reduction in mechanical properties</td>
<td>Raje and Vivek, 2011</td>
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<tr>
<td>Endoglucanases Endoxylanases</td>
<td>Increase of ink removal by 24% enhancement of paper strength properties for deinking of mixed office wastepaper.</td>
<td>Marques et al., 2003</td>
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<tr>
<td>Cellulase</td>
<td>Enhance ink removal efficiency by 24.6% and freeness by 21.6% with a reduction in drainage time of 11.5% Burst index and tensile strength - improved by 15.3% and 2.7% Brightness by 2.1%</td>
<td>Pathak et al., 2011</td>
</tr>
<tr>
<td>Cellulase and Xylanase</td>
<td>Δ brightness of approximately 10% were achieved Enzyme facilitated a better reduction of Kappa number and Hexenuronic acid (Hex A)</td>
<td>Nathan et al., 2018</td>
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<td>Laccase</td>
<td>Δ brightness of 15% following 4 h of enzymatic treatment. laccase facilitated the maximum hexenuronic acid release with Kappa number reduction.</td>
<td>Nathan et al., 2018</td>
</tr>
<tr>
<td>cellulase, xylanase and laccase</td>
<td>Enhance the paper brightness to about 32.86% whereas combination- al effect could enhance only about 28.67%. Release of chromophores from phenolic and hydrophobic compounds released during the deinking process.</td>
<td>Nathan et al., 2018</td>
</tr>
<tr>
<td>Xylanase</td>
<td>Δ brightness of 11% following 4 h of enzymatic treatment. Strength properties enhanced during deinking.</td>
<td>Nathan et al., 2017</td>
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Table 2: Cellulose crystallinity changes in paper pulp during enzymatic deinking process.

<table>
<thead>
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<th>Samples</th>
<th>Crystallinity Indices</th>
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<td>IV</td>
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</table>

Table 3: Commercially available enzyme formulation for deinking

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Applications</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aquazym®, BAN (Bacterial Amylase Novo), Fungamyl®</td>
<td>Amylases for low-temperature modification of starch.</td>
</tr>
<tr>
<td>Novozym® 342</td>
<td>A cellulase preparation used for deinking of Mixed Office Waste.</td>
</tr>
<tr>
<td>Pulpzyme™ HC</td>
<td>A xylanase preparation for reducing the need of bleaching chemicals when bleaching kraft pulp</td>
</tr>
<tr>
<td>Resinase® A 2X</td>
<td>A preparation used to eliminate pitch/resin-related problems.</td>
</tr>
<tr>
<td>Enzymk® Enzymatic Deinking Technologies, LLC</td>
<td>Maximize ink detachment such that all plant equipment can do a better job in removal and issues such as brightness “reversion” in dispersion are minimized.</td>
</tr>
<tr>
<td>MetZyme® BRILA™</td>
<td>Pulp and paper enzymes for deinked pulp applications</td>
</tr>
<tr>
<td>MetZyme® POVON™</td>
<td>Highly complex enzymatic solution tailored to match the demanding and variable conditions of chemical and special pulping. The main application is improvement of fibre strength properties and bleachability.</td>
</tr>
<tr>
<td>ArrowPulp, Anthem Cellu- tions (India) Ltd.</td>
<td>Thermostable Xylanases for pre-bleaching of pulp. Bleach boosting enzyme.</td>
</tr>
<tr>
<td>ArrowDeink, Anthem Cellu- tions (India) Ltd.</td>
<td>Enzyme for an eco-friendly deinking. Low chemical load</td>
</tr>
<tr>
<td>ArrowRefine, Anthem Cellu- tions (India) Ltd.</td>
<td>Enzyme for pulp refining. Saves energy</td>
</tr>
<tr>
<td>ArrowFibre, Anthem Cellu- tions (India) Ltd.</td>
<td>Enzyme for drainage control</td>
</tr>
<tr>
<td>ArrowSize, Anthem Cellu- tions (India) Ltd.</td>
<td>Amylase for starch cooking</td>
</tr>
<tr>
<td>SEBrite PR 810 L / PR 811 S, Advanced Enzymes, India</td>
<td>Unique blend of a bio-degradable polymeric surfactant &amp; cellulase for deinking of recycled paper.</td>
</tr>
<tr>
<td>SEBrite DI L / DI Plus S, Advanced Enzymes, India</td>
<td>Unique cellulase (liquid / powder) preparation for deinking of recycled paper</td>
</tr>
<tr>
<td>SEBrite Bleach L, Advanced Enzymes, India</td>
<td>Thermostable alkaline xylanase (liquid) preparation for bleach-boosting of virgin pulp.</td>
</tr>
</tbody>
</table>

Table 4: Commercially available enzyme formulation for deinking

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Content %</th>
<th>Peak Area</th>
<th>C/N Ratio</th>
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<tbody>
<tr>
<td>A. Paper pulp Untreated</td>
<td></td>
<td></td>
<td></td>
<td>18.91</td>
</tr>
<tr>
<td>N</td>
<td>0.781</td>
<td>2079</td>
<td></td>
<td></td>
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<tr>
<td>C</td>
<td>14.78</td>
<td>21032</td>
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<tr>
<td>S</td>
<td>1.348</td>
<td>927</td>
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<tr>
<td>H</td>
<td>0.275</td>
<td>736</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B. Pulp residues in deinking effluent</td>
<td></td>
<td></td>
<td>10.58</td>
<td></td>
</tr>
<tr>
<td>N</td>
<td>0.633</td>
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<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>S</td>
<td>1.674</td>
<td>2798</td>
<td></td>
<td></td>
</tr>
<tr>
<td>H</td>
<td>3.452</td>
<td>38267</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
References


