Enzymatic Deinking Of Office Waste Paper:An Overview

Pathak Puneet, Bhardwaj Nishi K., Singh Ajay K.

ABSTRACT

The utilization of the post consumer paper product in the production of new paper products is increasing all over the world in recent years. The use of enzymes in ecofriendly deinking of the recovered paper is one of the potential enzymatic application in the pulp and paper industry. Carbohydrate hydrolyzing enzymes (cellulases and xylanases) and lipases can deink office waste papers by enzymatic liberation of the ink particles from the fibre surface or hydrolysis of the ink carrier or coating layer. Enzymes have optimal deinking activity when presoaked before pulping at mostly acidic pH and medium consistency pulping. Enzymatic deinked pulp shows improved drainage, better physical properties, increased brightness, lower residual ink, reduced chemical consumption, lower COD as compared to chemically recycled pulps. Enzymes are supposed to retard redeposition of ink particles onto the fibres. Use of cellulases and hemicellulases, in excessive amount may cause the depolymerization resulting in fibre loss and high BOD in effluents. Accordingly, although the applications of the enzymes have been reported recently at mill scale but more research is needed to optimize process parameters and to tailor the enzymes through genetic engineering techniques according to the interest of the papermakers.

Keywords:- cellulases, xylanases, deinking efficiency, mixed office waste, residual ink

Introduction

The pulp and paper manufacturing industry is one of the largest wood consumers today. Along with increasing world economic growth, a substantial increase in paper consumption is expected. This means that more trees will be harvested and more solid waste will be created as paper products are consumed and disposed off because of the environmental and economic concerns associated with the consumption of our forest resources. The paper industry could well experience a limited raw material resource with concurrent reduction of industry growth. Therefore, "recycling of paper" as a solution to this problem is attracting more and more attention since it is an effective way to preserve forest resources and save energy and landfill space. One of the important processes in recycling of paper is the removal of the printing ink, also called deinking, from the used paper to obtain brighter pulp. Deinking involves the ink particles dislodgement from the fibre surface and the separation of the dispersed ink from fibre suspensions by washing or flotation (1,2). The efficiency of this method depends on the technique and printing conditions, kind of ink and kind of printing substrate. Papermakers are focusing on recycling as an economic necessity. Due to lack of any organized sector for the waste paper collection in India, imported paper waste comprising mainly Old Magazine (OMG) and Mixed Office Waste (MOW), constitutes the main ingredient used for preparing recycled paper pulp. New deinking mills established in response to this projected need are already competing for the cleanest and most homogeneous postconsumer paper source, e.g., sorted white ledger and soon will have to dip deeper into the postconsumer paper stream, e.g., unsorted (MOW -The most difficult raw material for deinking), to remain competitive (2,3). Indeed, photocopiers and laser printers physically bind the ink (thermosetting toners consisting of non-dispersible synthetic polymers) to the fibres as a result of high heat, making it difficult and expensive to remove by conventional chemical methods (4,5). Most of the conventional deinking techniques require large amounts of chemical agents, such as sodium hydroxide, sodium carbonate, diethylenetriaminepentacetic acid, sodium silicate, hydrogen peroxide and surfactants resulting in a costly wastewater treatment to meet the environmental regulations (2,6). Alternatively, enzyme usage has been reported to be a potentially efficient and less polluting solution to overcome this disposal problem (2,7).

Indian Institute of Technology, Roorkee,
Department of Paper Technology, Saharanpur Campus,
Saharanpur-247001, U.P, India

ENZYMES USED IN DEINKING:-

Enzymatic treatment is a recent process which gives better performance to reach desired deinked pulp properties. The study of enzyme application in the deinking is performed by many scientists. Several enzymes such as cellulases, hemicellulases, Pectinase, lipase, esterase, -amylase and lignolytic enzymes have been used for deinking of various recycled fibres. But the main enzymes used for deinking are cellulases and hemicellulases. Also many patents for the use of enzymes in deinking have been granted or applied for (1).

Cellulases:

Fungal cellulases and bacterial cellulases are components of large systems or complexes that hydrolyze -1, 4-glucosidic linkages in cellulose which produce water soluble sugars. Cellulases can be divided into three major classes. These are endoglucanases or endo-1,4--glucanase, cellobiohydrolase and glucosidase. Endoglucanases, attack randomly along the cellulose fibre, resulting in a rapid decrease in the chain length of CM-cellulose or H_3PO_4-swollen cellulose and yielding glucose, cellobiose, cellotriose and other higher oligomers (9). These three hydrolytic enzymes act synergistically. Endoglucanases (EG) hydrolyze internal bonds, producing oligomers with new chain ends. EGs act preferentially in the amorphous regions of the microfibrills. Cellobiohydrolases (CBHs) act processively on the existing chain ends and on those created by the endoglucanases, releasing cellobiose molecules. glucosidase cleaves the released cellobiose to two glucose molecules (9).

Hemicellulases:

Due to the complex structure of hemicelluloses, several different enzymes are needed for their enzymatic degradation or modification. The two main glycosyl hydrolases depolymerising the hemicellulose backbone are endo-1,4- L-D-xylanase and endo-1,4-L-D-mannanase (10). Since xylan is a complex component of the hemicelluloses, its complete hydrolysis requires the action of a complete enzyme system, which is usually composed of L-xylanase, L-xylosidase, and enzymes such as -L-arabinofuranosidase, -glucuronidase, acetylxylan esterase, and hydroxycinnamic acid esterases that cleave side chain residues from the xylan backbone. All these enzymes act cooperatively to convert xylan to its constituents (11). Xylanases attack randomly the backbone of xylan to produce both substituted and non-substituted shorter chain oligomers, xylobiose and xylose (6). Xylosidases are essential for the complete breakdown of xylan as they hydrolyse xylooligosaccharides to xylose. The enzymes arabinosidase, -glucuronidase and acetylxylan esterase act in synergy with the xylanases and xylosidases by releasing the substituents on the xylan backbone to achieve a total hydrolysis of xylan to monosaccharides (9).

-Mannanases catalyse the random hydrolysis of -D- 1,4-mannopyranosyl linkages within the main chain of mannan and various polysaccharides consisting mainly of glucomannan, galactomannan and galactoglucomannan (10). Other glycosyl hydrolases that are important for the degradation of mannan include mannosidases and galactosidases. Mannosidase catalyses the hydrolysis of terminal, non-reducing -D-mannose residues in mannan (10). -Galactosidases hydrolyse terminal, non-reducing -D-galactosides from galactose oligosaccharides, galactomannan and galactoglucomannan and are also capable of removing -1,6-bound galactosyl units from polymeric galactomannan..

APPLICATION OF THE ENZYMES AND THEIR EFFECTIVENESS:-

In 1991, Kim *et al.* showed that crude cellulases applied to pulps could facilitate the deinking process (12). A deinking efficiency of almost 73%, by the enzyme combination of cellulase and hemicellulase of *Aspergillus niger* was obtained for enzymatic deinking of laser printed waste papers on a laboratory scale (13). Selvam *et al*. (14) has studied white rot fungi *Fomes lividus, Thelephora sp. and Trametes versicolor* and their enzymes such as lignin peroxidase (LiP), manganese dependent peroxidase (MnP), laccase and mixture of these enzymes for deinking waste papers with respect to kappa no. and brightness of paper produced. Immobilized cellulase can produce superior deinking results than soluble cellulose (15). Morkbak *et al.* (16) explained that the deinking effect of the lipase was caused by a partial degradation of the binder of the soybean oil-based inks, thereby releasing the ink particles from the paper. First time, Gubitz *et al.* (3) combined magnetic deinking technique with the application of enzymes. At a deinking efficiency of 94% they measured a yield loss of only 2.8% after enzymaticmagnetic deinking, while flotation deinking was reported to reach yield losses of up to as much as 15%**.** The bacterial -amylase produced the greatest ink particle reduction on coated colored printed magazine (17). Viesturs *et al.* (18) suggested that, for alkaline papers, the majority of inks are localized on the paper coatings and fillers consisting mainly of $CaCO_3$. Enzyme treatment improved by stock acidification and dissolution of $CaCO_3$ prior to flotation resulted in effective detachment and dispersion of toner specks ensuring a high deinking effectiveness. The enzymatic treatment is sometimes related to the ink films fragmentation (12,19), thus being necessary to control the enzymatic action in order to maintain the ink size in a range suitable to the separation process.

Role of surfactant in enzymatic deinking:

Numerous studies have reported on deinking using enzymes with surfactants. Understanding the interaction and the relationships between enzymes and surfactants will be very helpful when using enzymes for the deinking. In subsequent studies, some researchers (2,4,17,19,20) showed that enzymatic deinking is most effective when enzymes are used during high consistency fiberization in the presence of non-ionic surfactants. Kaya *et al.* (21) showed that the surfactants could be usefed as both accelerators and inhibitors. The activity of the enzymes increased with nonionic and cationic surfactants. From circular dichorishm (CD) analyses and studies, the molecular structure of the enzymes was changed by nonionic and some anionic surfactants. As the concentration of the different anionic and some cationic surfactants increased, the activity of the enzymes decreased to different degrees.Treimanis *et al.* (19) have treated the laser-printed wastepaper samples with a commercial cellulase, Celluclast 1.5L, and lipase, Resinase A2X, and reported enhanced removal of toners by promoting the toner dispersion in smaller particles. During the flotation deinking, the addition of an appropriate surfactant, such as hydrocarbon oil proved to be a necessary factor to prevent the redeposition of microink particles on the fibre surfaces and promote the separation of highly dispersed toner particles from the fibre network (18). Elegir, *et al.* (17) used a non-ionic surfactant, along with a cellulase and commercial -amylase mixture which assisted deinking process of xerographic office wastepaper. The area coverage of the residual toner particles, measured by image analyzer, was decreased by 96%. Deinking of electrostatic wastepaper with commercial cellulolytic enzymes and surfactant in neutral pH, results into higher freeness, strength properties and lower residual ink (22). Although the use of surfactant improves deinking efficiency but it may alter the fibre's surface properties, reducing the strength of the inter-fibre bonds and decreasing the paper strength due to the adsorption of surfactant onto the fibre's surface (17,23). Operating cost and environmental effect can be minimized if the enzymatic deinking didn't use surfactants and alkaline deinking chemicals, such as the deinking of the alkaline sized nonimpact-printed paper didn't require the surfactant because calcium carbonate generates adequate froth during floatation (2).

Enzymatic deinking with various mono components of enzymes:

Elegir *et al.* (17) reported that among cellulases mono-component endoglucanases were more efficient than crude cellulose for deinking of mixed office waste. Extracellular endoxylanase and endoglucanase enzymes obtained from xylan-grown *Aspergillus terreus* CCMI 498 and cellulose-grown *Trichoderma viride* CCMI 84 showed better strength and

Table 1
Quality control tests for recycled paper handsheets made from enzyme deinked pulp

QC Test	Blank	Control	Test Run
Brightness (%)	68.6	70.6	74.0
Opacity (%)	83.1	86.6	84.2
Ink specks/m2			
>220 m	412	262	173
160 m	**576**	464	180
80–160 m	472	178	56
10–80 m	584	298	63
Breaking length (m)	2295	3228	3300
Burst index (gscm/gsm)	17.14	21.4	22.8
Tear index	72.52	73.0	69.2

Handsheets were made after enzymatic treatment to the mixed office waste paper pulp. The treatment was carried out in the presence of surfactant (0.1%) concentration at 10% consistency in the pulper for 30 min. The enzyme dose of 50 IU was selected for treating 100 g of pulp sample. Each treatment was followed by 10 min floatation run at 1% consistency. Control run were taken under similar conditions replacing active enzyme by same volume of heat denatured enzyme preparation. Blank samples were the pulp sample not treated with enzyme and also not processed by flotation.
***Based on Vyas *et al.* (2003)**

Table -2
Quantification of fibre degradation and deinking efficiency after enzymatic and chemical treatment (MOW and PHOT)

Assay	Mixed office wastepaper					Photocopied wastepaper				
	% Solubilisa-tion	Ink area (ppm)	Deinking efficiency (%)[c]	Ink particles count	Ink particles median size (μm^2)	% Solubilisa - tion	Ink area (ppm)	Deinking efficiency (%)[c]	Ink particles count	Ink particles median size (μm^2)
Enzymatic deinking										
Control ENZ		3109		850	822		7625		455	3141
Xylanase Cd	3.1	2625	16	422	836	3.5	6381	16	352	2567
Viscozyme L	1.7	3174	0	872	895	n.t.	n.t.	n.t.	n.t.	n.t.
Celluclast	1.1	2471	21	457	806	1.3	7953	0	437	2896
Buzyme 2523	1.0	2302	26	310	689	n.t.	n.t.	n.t.	n.t.	n.t.
Pentopan mono	2.5	2200	29	214	940	2.8	6696	12	328	3292
AXC 2.3	2.3	1789	42	164	896	2.6	5911	22	294	3045
Novozym 342	0.8	2313	25	369	1179	n.t.	n.t.	n.t.	n.t.	n.t.
T. viride CCMI 84[a]	1.1	2372	24	325	1037	n.t.	n.t.	n.t.	n.t.	n.t.
A. terreus CCMI. 498[a]	0.8	2850	8	583	867	n.t.	n.t.	n.t.	n.t.	n.t.
IOGEN celulase	0.5	1910	39	290	1403	n.t.	n.t.	n.t.	n.t.	n.t.
SAFISYM CP.	1.3	2432	22	317	1597	n.t.	n.t.	n.t.	n.t.	n.t.
Chemical deinking										
Control CHEM		2839		745	1233		6337		381	2000
Chemical deinking	0		31	262	1042	0	4006	37	208	1806
Non-treated pulp[b]		5331		1351	746		14976		861	2119

n.t.: not tested.
a Marques et al. (2003) (24).
b The same batch of non-treated pulp was used in both enzymatic and chemical assays.
c Deinking efficiency expressed as percentage of the respective control.
* based on Pala *et al.* (2004) (23).

ink removal properties for mixed office waste paper deinking (24). Vyas *et al.* (25) separated two extracellular alkali-stable 1,4-α-D-glucan-4-glucanohydrolase (EC 3.2.1.4) fractions, i.e., Endo A and Endo B from the culture filtrate of an alkali-tolerant *Fusarium* strain. The enzyme treatment resulted in the increase in brightness with the decrease in ink counts of the recycled paper **(table 1 and 2).** Based on the distinct properties of endoglucanases, a probable mechanism of enzymatic deinking process is presented schematically **(fig.-1)**. Furthermore, although enzymes favour ink removal, their action (in higher dose) significantly affects the paper strength properties (26). Gubitz *et al.* (3) treated laser-printed wastepaper individually and with combinations of purified endoglucanases from *Gloeophyllum sepiarium* and *Gloeophyllum trabeum* and found that pure endoglucanases were responsible for most of the success in deinking (94%) with increased freeness, slightly decreased intrinsic fibre strength and unaffected or even marginally improved hand sheet's strength.

Most cellulases comprise modular multidomain proteins containing at least three separate structural elements of different functions i.e., a catalytic domain (CD), a cellulose binding domain (CBD), and an interdomain linker. During hydrolysis, cellulose is "captured" and brought close to the catalytic site, in the proper orientation, by the cellulose binding domain and it is then catalyzed by the catalytic domain. It is reported by Li *et al.* (27) that Cellulose Binding Domain (CBD) alone has a negative impact on deinking efficiency. He prepared and purified a fusion protein containing a cellulose binding domain (CBD) from *Cellulomonas fimi* endoglucanase for deinking of a mixed office paper (MOP). Endoglucanases lacking a CBD could result in superior deinking effects and strength properties (28).

Enzymatic deinking in Mill trials:

Knudsen *et al.* (29) achieved dirt speck

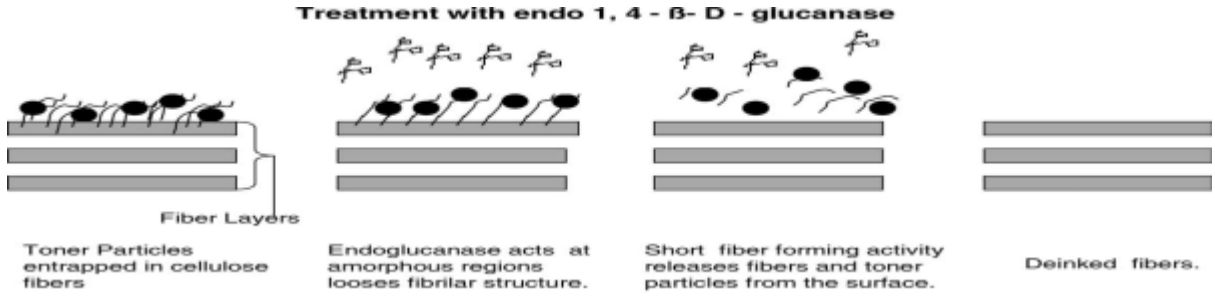

Fig-1. Schematic diagram showing probable mechanism of biodeinking by alkaline active endo 1,4-β-d-glucanase preparation from alkalotolerant Fusarium **sp.**

***Based on Vyas** et al. **(2003) (25).**

reduction of 35% along with a brightness increase of 2.2% for the final product (writing and printing paper was prepared from low grade office and printhouse waste at Stora Dalum Deinking Plant). Stickies were reduced by ~ >50% (indexed area from 100 to 46) and the mill throughput was increased by ~ 8 tons/day (from 215.5 to 223.7 tons/day). The conversion was at least cost-neutral. Magnin *et al.* (26) using a mixture of thermoxylanase, mannanase, lipase and amylase observed lowering in specks contamination in the floated pulp to 88% lower with respect to that observed with alkaline treatment. Based on these promising results, full-scale enzymatic deinking was carried out at a French mill. Increased brightness by 4 to 6 points, lower residual ink content, maintained mechanical strength and no runnability problem on paper machine, were obtained with the enzymatic deinking as compared to the mill chemical deinking. A full scale, enzymatic deinking experiment was carried out by Saari (30) at the Moulin Vieux Mill in France, a mill which produces deinked paper (DIP) from printed coated woodfree papers.

With respect to the enzymes, which allow different deinking efficiencies, it was not possible to establish a key activity (or activities) for ink removal. In fact, although the cellulolytic and/or xylanolytic activities are frequently related to effective deinking trials, it is difficult to establish the relative contribution of each one to the deinking process (2,5,12). The major contribution is given by the endoglucanases and xylanases. The wide range of results is probably due to the use of different type of paper samples, printing ink and enzymes obtained from different sources (20).

MECHANISMS OF ENZYMATIC DEINKING:

Different mechanisms for ink removal by enzymes have been proposed. The explanation for the performance has to be credited to the specific activity of that enzyme because enzyme cleaves specific bonds between sugars units.

Jeffries *et al.* (5) proposed that deinking may be caused not by enzymes but by additives used to enhance enzyme production and stabilization. Also, ink removal efficiency varied inversely with enzyme inhibition (31). Kim *et al* suggested that enzymes partially hydrolyze and depolymerize cellulose molecules at fibre surfaces, thereby weakening bonds between fibres and freeing them from one another. Ink particles simply are dislodged as fibres separate during pulping (12). Eom *et al* pointed out that enzymatic treatment weakens bonds, perhaps by increasing fibrillation or removing surface layers of individual fibres (27). Catalytic hydrolysis may not be essential; enzymes can remove ink under non-optimal conditions. Mere cellulase binding may disrupt fibre surfaces in a manner and to an extent sufficient to release ink during pulping (25). Hemicellulases facilitate deinking by breaking lignin-carbohydrate complexes and releasing lignin from fibre surfaces (19). Ink particles are dispersed with the lignin. Cellulase and hemicellulase treatment facilitated ink removal from newsprint, and was accompanied by release of lignin (31). Cellulases peel fibrils from fibre surfaces thereby freeing ink particles for dispersal in suspension (12). After enzymatic treatment of secondary fibre, pulp freeness increases due to peeling mechanism (13). Enzyme dosages and reaction times, however, seem too low to cause measurable cellulose degradation (5). Mechanical action is also important and prerequisite to proper enzymatic activity (20). But this mechanical action was also responsible for the distortion of cellulose chains at or near fibre surfaces, thereby increasing susceptibility to enzymatic attack. Assuming that fibre-fibre friction increases as the pulp consistency increases. Such an explanation appears trustworthy with earlier results that enzymatic deinking is more efficient at medium consistency than low consistency (5). Other research, however, disputes the importance of mechanical action (7). No improvement in brightness was observed when higher shear forces were applied at higher consistencies or for extended times. Enzymes can be denatured at higher shear forces caused by fibre-fibre friction (1).

Enzymatic effects may be indirect, that is, they remove microfibrils and fines, thereby improving freeness and facilitating washing or flotation (5). Putz *et al.* reported that fines content is not always reduced during enzymatic deinking (7). Ink particle hydrophobicity of the nonimpact-printed papers was increased after enzymatic treatment due to the removal of the fibrous material from ink particles, thereby facilitating separation during floatation (5). This promising hypothesis should be tested with a broad range of enzymes, paper grades, and inks. Zeyer *et al.* (20) presented a proof that only easily accessible cellulose chains are subject to enzymatic cleavage. For the removal of a significant amount of ink, mechanical action as surface friction on the fibre is able to improve this restriction by opening the outermost layers of the fibre to fully expose the cellulose chains. Lipases and esterases degrade carrier (vegetable oil) and thus disperse pigment in ink (16).

FACTORS AFFECTING ENZYMATIC DEINKING:-

It is necessary for the successful deinking that operating conditions must be optimized such as temperature, pH, reaction time, pulp consistency, enzyme dosage and mechanical action.

The activities of the cellulases and hemicellulases are mostly influenced by the pH. Enzymes have the optimum value in acidic pH, alkaline or in the neutral pH. Jeffries *et.al*. didn't adjust the pH slurry (initial pH 5.5-7.5), when worked with enzymes. But on the other hand an initial slurry of the alkaline sized nonimpact paper (pH 8.5) requires adjustment of the pH levels with the help of the acid because the enzymes work in the pH range of 5.5-7.5, thus acidic conditions may be beneficial in terms of cost, because lesser yellowing of the paper was reported in the acidic conditions (12). Higher dose and long reaction time both can reduce the viscosity of the pulp and damage the fibres. Enzymatic deinking is more efficient at medium consistency than low consistency (5). Enzymes have been used before pulping steps (12), during mixing (17) and during pulping (5). Putz et *al.* reported and clearly demonstrated that enzyme addition during initial mixing of paper and reaction medium was most effective (7). Longer presoaking times decreased brightness, presumably due to reduced ink particle size Longer presoaking times allowed finely dispersed ink particles to readhere to fibre surfaces or to penetrate into porous parts of fibers, thereby limiting effectiveness of flotation. Soaking after pulping, but before flotation, adversely affected deinking. This result was also attributed to readherence of ink particles to fibres. (12). Most of the enzymes work within the temperature range of the 40-65C. Above this temperature the enzyme will be denatured.

ENVIRONMENTAL EFFECTS:-

Putz *et al*. (7) found the results that enzymatic treatment produced almost half the chemical oxygen demand (COD) loads as compared to that produced by conventional deinking. But some researchers have reported about 20-40% higher COD level in the process water than in the standard process (26, 30). Bobu (31) reported that although the process water of enzyme (Novozyme 342) deinking had higher COD, but it is more easily biodegradable than that resulting from chemical deinking. Amount of generated sludge was lower in enzyme deinking and had lower inorganic content, providing potential to reduce the costs of sludge treatment. Generally it was expected that enzymatic deinking lowers the environmental load due to less or no use of chemicals. Due to the some reported cases of increased COD, it is needed that researches should be going on to reduce and find out the reason of increased COD in such cases. For the proper controlling of the biological oxygen demand (BOD) levels more precise control over enzyme doses and retention time are required to minimize cellulose hydrolysis. Reduction in pulp yields and the accompanying release of reducing sugars might be expected from hydrolytic action of cellulases and hemicellulases. The dangers are fibre loss and heightened BOD in the effluents.

ADVANTAGES AND LIMITATIONS OF USING ENZYMES IN THE DEINKING:-

Enzymatically deinked pulp showed superior physical properties, higher brightness and lower residual ink, no alkaline yellowing, increment in pulp freeness, improved drainage and better machine runnability than chemically deinking recycled pulp. Also dewatering and dispersion steps as well as subsequent reflotation and washing may not be essential. Their elimination could save capital costs and reduce electrical energy consumption. Requirement of bleach chemicals may be lower for enzymatic deinking. In general, enzymatic deinking reduces chemical use thus reducing the load on waste water treatment.

Although there have been considerable advances in the application of biotechnology to paper recycling, enzymatic deinking processes still face problems that have limited their commercialization. Most of the commercially available enzyme products are too expensive to compete with conventional deinking chemicals. Enzymes are very sensitive to environmental fluctuations. Enzymatic processes usually have a relatively narrow operating range with regard to pH, temperature, and storage time, and operating conditions must be precisely controlled in order to maintain enzymatic activity. Enzymatic processes are generally slower and may be difficult to retrofit into existing pulp and paper mill operations (32).The enzymatic process effectiveness depends more critically on the furnish characteristics than the chemical one. Considering the wide variability on the industrial wastepaper supplies, this is probably the most important disadvantage of this methodology (23). Reduction in pulp yields and the accompanying release of reducing sugars might be expected from hydrolytic action of cellulases and hemicellulases (when used in higher dose).

FUTURE AREAS FOR RESEARCH IN ENZYMATIC DEINKING:

Future studies should consider evaluating the efficiencies of highly purified endoglucanases from different families in order to elucidate the mechanism of enzymatic deinking. The action of the cellulose-binding domains on the fibres is still unknown but it seems to be due to their adsorption to the fibre surface, which alters the fibre surface characteristics. Genetic engineering can be supposed to simplify production and lessen purification costs of enzymes.

Tailored Enzymes as Options for industries:

Over the last 15 years, research has enabled the tailoring of enzyme attributes to the needs of the particular plant. Thousands of potential enzymes structures are considered and tested with the help of large capacity computers to make enzymes as an increasingly valuable raw material to the pulp and paper industry. Tailored enzymes are rather complex, consisting of multiple components that perform singular and synergistic functions. So that multiple component formulations help in efficiency and effectiveness of the desired goal and provide a better fit for the specific attributes of each mill. (33)

CONCLUSION:

Although recent developments in genetic engineering have also broadened the optimum conditions of the enzymes, enabling them to work under some extreme mill conditions, but more research is needed to optimize process parameters and to tailor the enzymes through genetic engineering techniques according to the interest of the papermakers.

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