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Enzymatic Deinking of Old News Paper(ONP) by Cellulases Produced by Various Fungal Strains

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The continuously growing paper manufacturing industry imposes a severe demand on green plants that forms the basic raw materials .Shortage of forest based raw materials and problem in processing agro residues are the major constrains in growth of production of paper industry. Waste paper is the single largest component of the solid waste stream and has a great effect on the environment. Recycling of paper not only saves energy and forest resourses for pulping and paper making but also reduce the cost of waste disposal. Consumption of recovered paper is estimated to be 4-5 million tones in india only. India is currently using both imported & domestic waste paper. Conventional deinking technology requires a large amount of chemical agents such as sodium hydroxide, sodium carbonate, sodium silicate, hydrogen peroxide. This will result in the increase of COD level and the concentration of chemicals in the effluent water. Ultimately, these will result in a high detrimental impact to the environment which subsequently will require a costly wastewater treatment processes to meet the environmental regulations. Consequently, experts paid more and more attention to new deinking technologies and the research of biodeinking technology has opened up a new avenue for paper recycling. Therefore, an enzymatic deinking of waste papers has becomes as an environmental friendly approach. The present paper discusses the effect of cellulases (crude) produced by different fungal strains on enzymatic deinking of old news paper in terms of their brightness and tensile strength.

Key words: Endoglucanases, Cellulases, FPase, ONP,Brightness, Tensile strength.

The critical major barriers in the profitable conversion of this relatively abundant and inexpensive raw material into quality products are the ink and contaminants removal[1]. This requires an effective, efficient and more economical deinking technology for waste paper recycling. During the past few decades, a number of enzymes, including cellulase, xylanase, pectinase and lipase, have been evaluated for their potential to replace hazardous chemicals in deinking recycled paper. Conservation of wood products, toxic emissions from traditional paper making methods and new process technologies have made paper recycling a viable industry[2]. Deinking is one of the most important steps in waste paper recycling and a variety of techniques including conventional froth flotation and washing are being used currently to deink secondary fibers. Conventional chemical deinking uses large quantities of chemicals, resulting a high pollution load which required costly water treatment systems. In order to overcome these problems, enzymatic deinking

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becomes a better option, due to its high efficiency and low environmental impacts. Enzyme based deinking offers a potential means for reducing chemicals used in conventional deinking process thus reducing the load on waste water treatment system[3].Therefore, enzyme assisted deinking has received more attention as potentially environmentally sound approaches. Enzymatic treatment is a recent process which is supposed to give better deinked pulp properties. Several enzymes such as cellulases, hemicellulasses, Pectinase,lipase, easterse, a-amylase and lignolytic enzymes have been used for deinking of various recycled fibers[4]. There are three different approches for the use of enzymes in deinking: Hemicellulases, cellulases and lignolytic enzymes can be attacked on fiber surface.These enzymes are considered to alter fiber surfaces by chemical bond modification in the vicinity of ink particles, there by ink particle on the surface gets released by washing and flotation. Amylases hydrolysed starch based coatings whereas vegetable oil based inks can be degraded by lipase. This review has emphasized more on deinking with cellulases. Many cellulases from different microorganism have been discovered, re-engineered and studied[4,5]. Cellulases consist of three basic components. They are endo-β-glucanase (E.C. 3.2.1.4), exo-β-glucanase (E.C. 3.2.1.91) and β-glucosidase (E.C. 3.2.1.21). Each particular component performs its particular function.

* Endo-β-4 glucanase randomly cuts the cellulose chain into glucose and cello-oligosaccharides.
* Exo-β- 4 glucanase attacks the non reducing end of cellulose chain with cellobiose as the primary product.
* β- glucosidase hydrolyzes cellobiose into glucose.

Most commercial cellulases used for deinking are a mixture of several components, therefore the deinking effect of commercial cellulase on recycled paper has not been optimized. Investigation of the contributions of each monocomponent to the deinking process could lead to a better choice of an enzyme preparation for the deinking[6,7].Enzyme partially hydrolyze and depolymerize cellulose molecule at fiber surfaces, thereby weakning bonds between fiber and freeing them from one another. Ink particles simply are dislodged as fibers separate during pulping. Cellulases peel fibrils from fiber surfaces, thereby freeing ink particles for dispersal in suspension. This peeling mechanism has also been implicated as the pulp freeness increases after enzymatic treatment of secondary fibers[5].The present paper discusses the comparative studies of chemical and enzymatic deinking by utilizing the cellulases from different fungal sources.

MATERIAL AND METHODS

Enzyme Production

All the chemicals and reagents were used to perform experimental work are of Himedia and Sigma aldrich make. All the standard fungal strains(*Trichoderma reesei* NCIM1186, *Trichoderma viride* NCIM1195,*Neurospora crassa* NCIM1021 and *Aspergillus niger*

were procured from National Chemical Laboratory (NCL) Pune, India.The procured fungal stock was kept at 4^0C in 20% (v/v) glycerol. All the cultures were grown on PDA slants at 28^0C for 4-5 days irrespective to *Neurospora* culture, which was grown on M_2 slants at 28^0C for 4-5 days. Slants were maintained at 4^0C and subcultured at monthly intervals. For the study of growth and production separate set of batch experiments have been performed. First set of experiment was carried out(for getting culture solution) in a 250 ml Erlenmeyer flasks containing 150 ml of M_2 broth in which 5 loopfull cultures of filamentous mycelia were added and shaken at 180 rpm at 30^0 C in an incubator shaker for 3-4 days. While other set of batch experiments were carried out in 250 ml Erlenmeyer flasks containing sieved wheat bran as the raw material for the growth and production of organisms impregnated with following production media in (g/l)Urea,0.3;$(NH_4)_2SO_4$,1.4; KH_2PO_4, 2.0; $MgSO_4.7H_2O$,0.3;Peptone,1.0; Tween80,0.2;$FeSO_4.7H_2O$, 0.005; $MnSO_4.7H_2O$, 0.0016; $ZnSO_4.7H_2O$; 0.0014; $CaCl_2$ 0.4,$CoCl_2.6H_2O$, 0.02. In all the experiment initial pH 5.0 was maintained. Wheat bran bed soaked with basal salt production media were autoclaved and then inoculated with specific volume (0.56 g/l cell dry weight) of PD and M_2 broth culture solution of respective strains. All the production flasks were placed in incubator for 6 days. Extraction of Enzyme were performed by using distilled water. Distilled

water was added to fermented samples (in a 1:5 proportion) in erlenmeyer flasks and the extraction was done after shaking at 180 rpm for 1h. The samples were then filtered and the extract obtained was centrifuged at 6000 rpm by centrifuge and further resulting supernatant was stored and used as enzyme source for deinking.

Deinking Experiments

Old news papers were individually torn in approximate one inch square for the preparation of pulp .In all the experiments pulping in the hydrapulper was carried out using 525 gm air dried (AD) mass at 6 % cy with approximately 6% moisture content at 65^0C.For chemical deinking chemicals were added on the basis of oven dry weight as follows NaOH:2%, Sodium silicate: 2.5%, H_2O_2:1%, DTPA:0.5%, EDTA:0.2%with Tween – 80 surfactant. Retention time in hydrapulper was about 15 min. Pulping experiments were carried out in laboratory helicon pulper supplied by universal engineering corporation, Saharanpur. It has provision for controlling rotor speed and temperature at varying conditions. It was adapted to operate at rotor speed ranging from 0-650 rpm. After the completion of pulping process slurry from the hydrapulper was sent to the second stage of flotation deinking in case of chemical deinking, whereas in enzymatic deinking pulp was treated with different dosages of crude enzymes produced by various fungal strains with Tween-80 surfactant for 3 h contact time. Afterthat this enzyme treated pulp with 1% cy were sent to flotation cell for further processing. In the flotation stage, the deinking experiments were performed in a laboratory flotation cell supplied by Universal engineering corporation Saharanpur. In flotation process about 100 g oven dried (OD) pulp of the repulped stock from the hydrapulper was diluted to 1% cy about 10 liter diluted stock was then sent in the batch flotation cell .The agitation speed was fixed at 2000 rpm. Retention time in flotation cell was about 10 min. The optical and strength properties were measured from handsheets with a basis weight of 60 g/m^3 prepared after flotation on the British standard handsheet machine according to TAPPI standard method T-205.Brightness was measured on both side of sheet and reported as an average on two.

RESULTS AND DISCUSSION

Separate sets of pulping and flotation experiments have been performed to study the chemical as well as enzymatic deinking. Cellulases produced by different fungal strains used separately to carried out enzymatic deinking. As literature reported that when cellulases and 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. Additionally, these enzymes can remove small fibrils from the surface of the ink particles thus altering the relative hydrophobicity of the particle,

Table 1. Comparative brightness and tensile strength of chemical and enzyme deinked old news paper

Deinking type	Cellulases from fungal strains	Enzyme dosages(ml)	Contact time (h)	Brightness (ISO%)	Tensile strength (Nm/g)
Chemical	-	-	-	55.0	37.19
Enzymatic	*Trichoderma reesei*	5	3	58.0	26.14
		10	3	58.2	25.76
		15	3	59.8	23.04
Enzymatic	*Neurospora crassa*	5	3	60.0	29.66
		10	3	61.5	26.96
		15	3	61.1	29.00
Enzymatic	*Aspergillus niger*	5	3	60.8	26.97
		10	3	60.5	26.21
		15	3	60.1	24.48
Enzymatic	*Trichoderma viridae*	5	3	59.1	31.45
		10	3	61.4	30.21
		15	3	61.8	29.45

which facilitates their separation in flotation/ washing steps[7,8].

Literature suggests that application of cellulase in deinking increases brightness and decreases the ERIC (Effective residual ink content) value of ONP/OMG after flotation when added in low charge prior to repulping, at pH 5. The breaking length, burst index, tear index, tensile index also decreased by various degrees depending on the enzyme charges and contact time[9]. Chemical deinked pulp handsheet showed good strength property but lesser improvement in brightness as viewed by Table 1.

It is also reported that the main effect of cellulase is the hydrolysis and superficial degradation of cellulose that implies ink removal from fibers. Understanding the mechanism of enzymes towards the fibers is essential to minimize the related negative impact on the strength of the paper and its quality[10].

The role of cellulases in deinking is still not clear. According to Jefferis et al cellulases having high filter paper degrading activities are effective in deinking[11]. Whereas other researchers have suggested that the filter paper degrading activities of cellulase complex from *Trichodermma pseudokoningi* S 28 had detrimental effect on the paper strength[12]. It has been observed from Table 1that enzymes produced from different fungal strains impact differently on the ONP brightness and tensile strength. As viewed from Table 1 that no significant improvement in the brightness has been observed on secondary fibers treated with *Trichoderma reesei* but on the other hand significant decrement in the tensile strength of the pulp was observed. This might be due to the presence of more FPase activity in comparison to the endoglucanase, which affect more frequently on the strength property. When examined the optical and strength properties of *Neurospora crasa* treated deinked pulp,we found that significant improvement in the brightness has been occurred,whereas strength property was less affected this may be due to that *Neurospora* cellulases having more endoglucanase compared to FPase which ultimately improve their brightness with less affecting in strength property.

Literature showed that cellobiohydrolase (CBH) hydrolyze cellulose from the ends of cellulose chain because cellulose chain ends are limited so it is not much effective for removal of ink from the cellulose fiber and is also does not affect significantly the strength property of paper whereas endoglucanase have ability to hydrolyze the internal portion of cellulose chain so it is much more effective. Endoglucanase has positive effect on deinking, where as exoglucanase has a negative impacts on deinking efficiency[4,5,6]. Whereas *Aspergillus niger* producing cellulase treated pulp showed better increment in the brightness with good strength property probably due to the presence of more endoglucanase.*Trichoderma viridae* produced cellulose, treated pulp showed better result in paper properties.

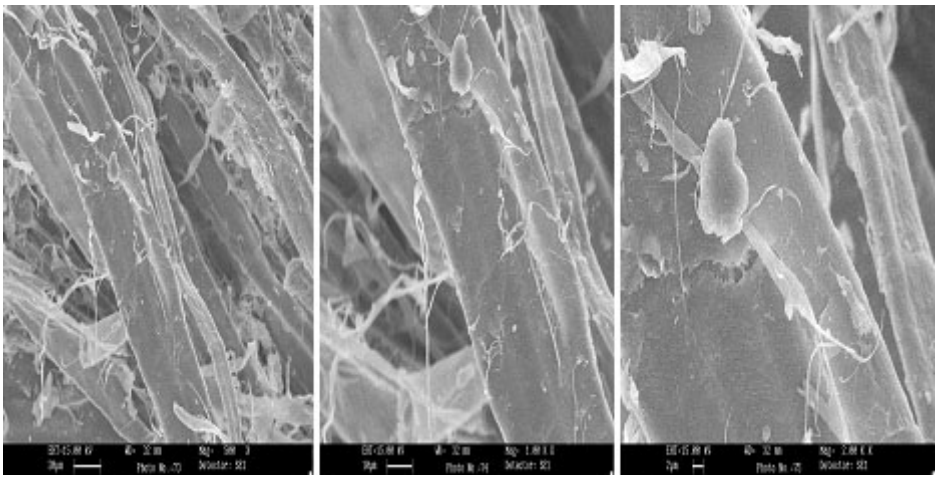
Handsheet obtained after *T.viridae* cellulase treatment, having improved brightness with less affected tensile strength certainly due to the presence of more endoglucanase as well as cellobiohydrolase activity. Decrement in the strength property has been observed in each enzyme deinked pulp, compared to chemical deinking,this can be overcome by reducing the contact time between enzyme and pulp. Marques et al have investigated the effect of glucanases secreted by *A.niger* CCMI 498 and *Trichoderma viridae* CCMI 84 on enzymatic deinking of MOW(mixed office waste),an increment of 24% in ink removal was observed by *T.Viridae*, compared with control due to the fact that *T.Viridae* contained 4-5 fold more endoglucanase activity than *A terreus*[13].Brightness of enzyme deinked as well as chemical deinked pulp can be compared by Fig 1.

The role of endoglucanase in the overall process of cellulose degradation is to breakdown the cellulose fibers into the several amorphous sites and generate innumerous reducing ends of the chain. This action enhances the loosening of fibers, which ultimately helps in releasing the ink particles from MOW papers during flotation deinking process in the presence of surfactants[10]. Cellulases are widely reported to facilitate deinking of mixed office waste (MOW)[14]. Individual mono components of cellulase having a single EG (endoglucanase) effectively remove inks from MOP fiber. CBP (Cellulase Binding domain) have negative impact on deinking of MOP[15].

It has been observed from the SEM micrograph of Fig 2 and 3,that enzymatic deinked fiber shows higher brightness in comparison to chemical deinked fiber.

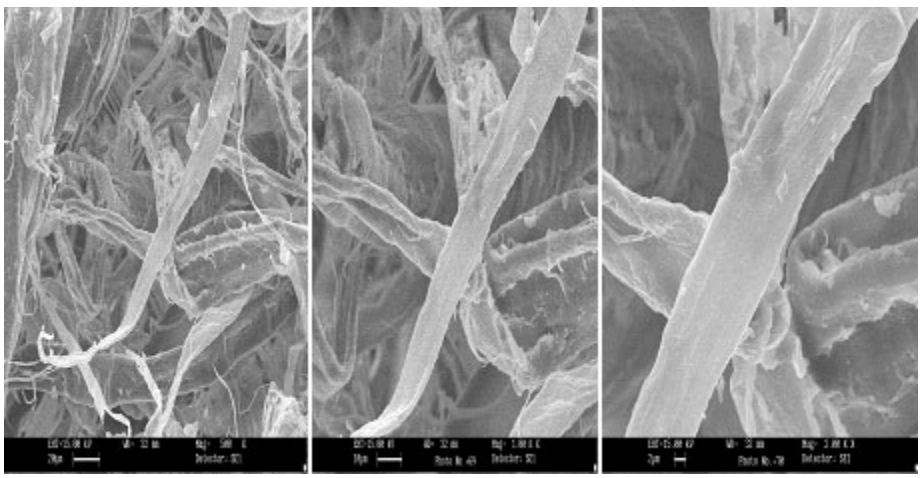
(1a) (1b)

Fig 1. Handsheet photograph of chemically deinked
(1a) old news paper and enzymatically deinked (1b) old news paper



(2a) (2b) (2c)

Fig. 2. Scanning electron micrograph of chemical deinked old newspaper pulp at 500X(2a), 1000X (2b) and 2000X (2c) respectively



(3a) (3b) (3c)

Fig. 3. Scanning electron micrograph of enzymatic deinked old news paper pulp at 500X (3a), 1000X (3b) and 2000X (3c) respectively

CONCLUSIONS

Compared with conventionally deinked pulp, we conclude from this study that cellulolytic deinked pulp will lead to improved optical properties such as brightness, lower residual ink contents. A proper combination of enzymes depending upon type of waste paper processed under proper operating conditions shall give better results. Selective enzyme with its specific components or suitable enzyme mixtures at their optimal conditions would be more effective in particular type of paper in deinking process. In order to maintain better strength properties with improved brightness, proper balance must be needed between the enzyme dosages and pulp. Contact time between the enzyme and pulp also plays a very active role in the enzymatic process, therefore it should be optimized. It has been concluded that cellulases from *Trichoderma viridae*, *Neurospora crassa* and *A. niger* are better strains for the enzymatic deinking in which *Trichoderma viridae* is much more suitable in terms of both optical and strength properties.

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