Bio-deinking of Mixed Office Waste Paper by *Penicillium citrinum* NCIM-1398 and its Comparative Study with Conventional Chemical Deinking

Pallavi Biswas,* Amit K. Bharti, Ashish Kadam, and Dharm Dutt

Penicillium citrinum NCIM-1398 is a co-producer of endo β-1,4glucanase, xylanase, and amylase enzymes. The synergistic effect of the enzymes present in the cocktail was found to be advantageous in deinking white mixed office waste (MOW) paper. Enzymatic deinking of MOW reduced the dependence on chemicals by up to 50% during chemical deinking and improved optical properties compared with the chemical deinking process. The chemi-enzymatic approach improved pulp brightness by 12.6% compared with MOW pulp. The ERIC (Effective residual ink concentration) value was reduced by up to 39.2%, whereas the strength properties of tear index, tensile index, and burst index achieved gains of 3.2%, 9.9%, and 6.2%, respectively, compared with the control. Whereas chemi-enzymatic treatment showed a reduction of 67.1% in COD and 61.8% in BOD, and there was an increase of 15.4% in total solid of effluents respectively, compared with the respective controls. Scanning electron microscopy (SEM), Fourier transform infrared (FTIR) spectroscopy, and X-ray diffraction (XRD) studies revealed that the pulp was modified by enzymatic and chemi-enzymatic treatments, which facilitate ink removal during deinking of pulp.

Keywords: White mixed office waste; Penicillium citrinum; Enzyme cocktail; Bio-deinking efficiency; Fiber surface modification

Contact information: Department of Paper Technology, Indian Institute of Technology Roorkee, Saharanpur campus, Saharanpur, Uttar Pradesh 247 001, India; * Corresponding author: pallavibiswas1@gmail.com

INTRODUCTION

With increasing population, major demand-drivers such as rising income levels, increasing literacy rate, requirement of better quality packaging of fast moving consumer goods (FMCG) products marketed through organized retail, and increasing ready-to-eat foods are causing escalation in paper consumption (Singh 2017). These factors ultimately have led to a rise in per capita paper consumption in India over 13 kg, which is well below the global average of 57 kg for financial year 2018. Credit analysis and research (CARE) ratings project that the overall paper demand growing at a compound annual growth rate CAGR) of 6.6% is likely to touch 18.5 million tons in 2018-19 (Sabnavis et al. 2018). The rapidly increasing paper demand in turn is raising consumption of forest resources, but with diminishing forest resources, paper mills are facing a scarcity of cellulosic raw materials (Pletka et al. 2000). To overcome the limited availability of wood, agro-based raw materials and waste paper are generally used as substitutes for paper manufacturing. However, the supply of agro-based raw material remains insufficient to achieve paper industry's demand due to its use as fodder (wheat and rice straws) for cattle and for power generation (sugarcane bagasse) (Ashna 2016). In this scenario, wastepaper is attractive because it is easily available, cheap, and a large source of valuable paper fibers. Every metric ton of recycled fiber saves trees plus the related energy and water required for paper making. To meet the increased demand of cellulosic

raw materials for paper making, more than 50% of India's recovered fiber is imported. At present the domestic recovery rate of waste paper is estimated at 30%, which is significantly below the recovery rate in developed countries (Bhalerao 2018). India alone imports about 4.0 million tons of waste paper every year (Phukan 2015). To reduce the dependency on imported waste paper fiber, efficient recovery strategies need to be adopted.

Various types of waste paper with different fiber compositions are used to manufacture paper. In papermaking there is a distinct tendency toward the use of white mixed office waste (MOW) mainly from photocopiers, print-outs, envelopes, ledgers, receipts, letterheads, hand written papers, *etc.*; MOW is a large source of valuable paper fibers that can be used to manufacture high-quality paper and sanitary tissues. Therefore, mixed office waste (MOW) paper was chosen for the present deinking studies.

The most widely used conventional deinking technique involves various chemicals such as sodium hydroxide, sodium silicate, hydrogen peroxide, chelating agents, and surfactants (Bajpai and Bajpai 1998; Bajpai 1999). NaOH is used as alkaline agent that facilitates swelling of fibre and thus, results in an increase of inter-fibre bonding. In conventional chemical deinking, the rise in brightness may be due to the presence of H_2O_2 (Jeffries *et al.* 1994). It was noticed that non-ionic surface-active agents such as poly-oxyethylene sorbitan mono-oleate (Tween 80) could increase the activity of hydrolase enzyme on cellulosic fibres and thus facilitate detachment of ink from fibres. The same surfactant also helped in ink agglomeration and in the formation of intermediate size agglomerate with fines and dust. The agglomerate formed with fines, dust, or with ink is of a particular size that goes out with air bubbles, forming foam. This results in an increased deinkability factor. Sodium silicate improves whiteness of paper by reducing the amount of ink in recycled paper. Silicate ions polymerize with ink particles and thus suspend easily. In addition, silicate acts as hydrogen peroxide stabilizer because it can form complex with metals ions; and it stabilizes pH due to its buffering action (Ma et al. 2011). Chelating agents prevent hydrogen peroxide from decomposing by forming soluble complexes with heavy metal ions (Bajpai 2013). Prolonged pulping time can have several adverse effects. It lowers the flotation removal efficiency and reduces the size of ink particles. The lower sized particles that are generated due to excessive mechanical disintegration resulting from higher pulping time might get re-deposited on the fibre surface. Marchildon et al. (1989) in their study stated that an optimum combination of deinking chemicals and pulping temperature facilitated the formation of big flocks of ink. Magnin et al. (2002) mentioned that de-inkability was affected by temperature above 65 °C and there would be reversal of ink dissociation back into the system. High pH favours cellulose fiber swelling, which reduces the ink adhesion to fiber (Goovaerts et al. 2002). The relatively high consistency of 10% was chosen because it prompts fiber-fiber attrition, favouring ink detachment (Pathak et al. 2011).

The enzymatic approach of deinking is more efficient and effective for the removal of ink compared with conventional chemical deinking of MOW paper (Singh *et al.* 2019). The most popular and widely accepted mechanism suggests that enzymatic hydrolysis along with shear forces remove the surface layer of cellulose from fibers. Cellulase and hemicellulase preparations have been found to remove fines and microfibrils, which resulted in increased freeness of pulp and thus facilitated the removal of ink during washing or flotation (Prasad 1993; Jeffries *et al.* 1994; Heise *et al.* 1996). The enzymatic approach reduces the requirement of chemicals and energy, and it minimizes waste for paper recycling (Lucia and Hubbe 2010; Joshi *et al.* 2015; Meena *et al.* 2018).

The aim of this study was to describe the bio-deinking of white MOW paper using endo β -1,4-glucanase, xylanase, and amylase present in a crude enzyme extract from newly isolated *P. citrinum* NCIM-1398. Not much literature has been reported on bio-deinking of MOW paper using *Penicillium citrinum*. The present study also aimed to reduce the quantity of chemicals required for conventional chemical deinking, without compromising the optical and physical properties of paper.

EXPERIMENTAL

White MOW Paper Collection and Characterization

White MOW paper was collected from district offices of the Food Corporation of India, Saharanpur, and the Indian Institute of Technology, Roorkee, Saharanpur, India. The MOW paper was typically generated as office waste containing white ground-wood free papers such as computer printouts, paper from photocopiers (coated with toners and laser printing), envelopes, hand-written papers, letterhead sheets, ledgers, and receipts. Prohibited materials and out throws were 2.7% of the total MOW paper. The MOW paper contained 14.4% ash (TAPPI T 211 om-02 2002) and 3.9% moisture content (TAPPI T 208 wd-98 1994).

Waste Paper Pulping

The manually collected MOW paper was torn into small pieces of 1.5 to 2.0 cm² and immersed in warm water at 50 °C for 30 min. The soaked paper bits were transferred to a hydrapulper (Lab hydra pulper, Universal Engg. Corporation, Saharanpur, India) of 20 L capacity, and tap water was used to maintain 10% consistency. Pulping was performed for 20 min at 60 °C using 650 rpm, and the pH was adjusted to 7.2 \pm 0.2. A Tween-80 surfactant dose of 0.05% was charged for pulping.

Enzyme Production and Application

Isolation, identification, and enzyme production

A co-producer of endo β -1,4-glucanase, xylanase, and amylase enzyme was isolated from Ichhapur, West Bengal, India. The strain was identified as *Penicillium citrinum* NCIM (National Collection of Industrial Microorganisms) Pune, Maharashtra, India and submitted with accession number NCIM-1398. Enzyme production was carried out using solid state fermentation (SSF) of wheat bran by *P. citrinum* using nutrient salt solution (NSS) at initial pH 5.0 and temperature 30 °C for 5 days. The NSS was composed of 4.0 g of NH₄Cl, 1.5 g of KH₂PO₄, 0.5 g of KCl, 0.5 g of MgSO₄, and 1.0 g of ammonium sulphate dissolved per litre of distilled water, supplemented with 0.04 mL/L of trace element solution containing 20 µg/L MnSO₄•7H₂SO₄, 180 µg/L ZnSO₄•7H₂SO₄, and 200 µg/L FeSO₄•7H₂SO₄ (Singh *et al.* 2014).

Deinking of MOW

Deinking of MOW pulp was carried out using chemical, enzymatic, and chemienzymatic processes. During chemical deinking, MOW paper was pulped in hydrapulper, where MOW was charged with a chemical dose of 2% sodium hydroxide, 1% H_2O_2 , 0.05% surfactant (Tween 80), 2.5% sodium meta silicate, and 0.5% diethylene triamine penta acetic acid (DTPA). The pulping conditions of temperature, time, and pH were maintained at 65 ± 2 °C, 20 min, and 7.2 ± 0.2, respectively (Carrasco *et al.* 1999; Pelach *et al.* 2001). During enzymatic bio-deinking, MOW paper was pulped and then

treated with a crude enzyme extract obtained from *P. citrinum* NCIM-1398. The enzyme dose of 6 IU/g, pulp consistency of 12%, pH 5.2 \pm 0.2, temperature 55 \pm 2°C, and reaction time of 60 min were maintained. The MOW was bio-deinked using a commercial cellulase (cellulase ex. Aspergillus niger 95382) obtained from Sisco Research Laboratories Pvt. Ltd. (SRL), Mumbai, Maharashtra, India. The MOW paper was pulped and then treated with an enzyme dose of 6 IU/g. The pulp consistency of 10%, pH 5.2 \pm 0.2, temperature of 55 \pm 2 °C, and reaction time of 60 min was maintained. During chemi-enzymatic deinking, MOW pulp obtained after enzymatic treatment was charged with different doses of chemicals, i.e., 100, 50, and 25%. The pulping conditions, as well as the enzymatic and chemical deinking conditions are listed in Table 1. The pulp obtained after treatment was transferred to a Weverk laboratory flat stationary screen (300 mesh wire bottom) for dislodgement of hydrolyzed chemicals. Thereafter, the pulp was subjected to 10 min of ink flotation in a flotation cell, where pulp consistency of 1%, temperature of 35 ± 2 °C, and pH 7.2 ± 0.2 were maintained. The pulp was washed with lukewarm water. The obtained pulp was evaluated for deinking efficacy. Primary pulp pads were prepared (TAPPI T 218 sp-02 2002) to evaluate physical properties. The pulp brightness was determined according to TAPPI T452 om-02 1998). Similarly, the effective residual ink concentration (ERIC) was determined by infra-red reflectance measurement. The pulp was de-fibered in a Weverk disintegrator for 15 min and then evaluated by TAPPI T 227 om-99 1999. Pulp viscosity was determined using TAPPI T 230 om-04 2013. Laboratory handsheets were prepared on a British sheet former of 60 g/m² using TAPPI T 205 sp-02 2006 to evaluate physical strength properties of enzymatically treated pulp. Sheets were conditioned at $65 \pm 2\%$ relative humidity and 27 ± 1 °C temperature (IS 1060 (Part 1) 1966). Conditioned sheets were then subjected for evaluation of tensile index (TAPPI T494 om-01 2006), burst index (TAPPI T 403 om-97 1997), double fold (TAPPI T 423 cm-98 2007), and tear index (TAPPI T 414 om-98 2004). Likewise, to evaluate dirt count laboratory-made handsheets were prepared (TAPPI T 213 om-01 2001). Deinkability factors such as deinkability based on ERIC $(D_{\rm E})$ and deinkability based on brightness $(D_{\rm B})$ were evaluated (Dutt *et al.* 2012). The filtrate after ink flotation and chemical/ enzymatic treatment were collected and mixed equally. The combined effluent obtained was then subjected for the analysis of BOD (IS 3025-44 2006), COD (IS 3025 (Part 58) 2006), and the total solids (IS 3025 (Part 15) 2003). A Thermo reactor CR2010 was used to analysed COD by close reflux titrimetric method.

Scanning electron microscopy (SEM)

Pulp samples were studied for morphological changes on the fiber surface using the pulp before and after the enzymatic or chemi-enzymatic treatments. A Tescan MIRA3 scanning electron microscope (Libusina TR21., 62300, BRNO, Czech Republic) was used. Oven-dried pulp fiber mats were examined using the gold shadowing technique. A magnification range of 700X to 65kX was used to collect electron micrographs.

Fourier transformation infrared (FTIR) spectroscopy

The FTIR studies of air-dried pulp samples were carried out on a Perkin-Elmer Spectrum-2 (Singapore, Singapore). Air-dried pulp samples were investigated for complex and intermolecular interactions, polysaccharides, and structural changes that occurred during pulp treatment. At room temperature, FTIR spectra were recorded over a frequency range of 500 to 4000 cm⁻¹ at 1 cm⁻¹ resolution and 16 scans per sample. Spectrum software was used for data analysis.

X-Ray diffraction analysis

X-Ray diffraction (XRD) is a fast analytical technique used to identify the total area of phase of crystalline material. In this study, the complex cellulose crystallinity of deinked pulp and control fiber samples was determined using powder XRD (Rigaku Ultima IV, Tokyo, Japan) using nickel filtered Cu/K α radiation and copper as a target wave length (λ) of 1.54 Å. A goniometer scanning rate of 2°/min was used. The range of scanning angles was $2\theta = 5$ to 90° (Bragg angle). A peak resolution program was used to calculate the crystallinity index (Crl) (Segal method 1959).

Statistical analysis

All experiments were carried out in triplicate, and experimental results were represented as the mean \pm standard deviation of values.

RESULTS AND DISCUSSION

Effect of Enzyme Treatment on MOW Pulp Bio-deinking

Deinking trials were conducted to compare conventional chemical, as well as the enzymatic and chemi-enzymatic deinking (Tables 1 and 2). Chemical deinking reduced the ERIC value by 41.0% and increased deinkability based on ERIC (D_E) by 41.0%, whereas brightness improved by 13.0% and deinkability based on brightness $(D_{\rm B})$ by 57.7%. The viscosity of chemically deinked pulp increased from 373 to 480 cm³/g, tear index from 5.38 to 6.02 mNm²/g, tensile index from 28.6 to 32.4 Nm/g, and burst index from 1.94 to 3.81 kPam²/g compared with MOW pulp after pulping. Deinking includes saponification, pulp swelling, and detachment of printing ink. During chemical deinking, NaOH is used as the alkaline to facilitate swelling of fiber and thus to increase the interfiber bonding. It consequently increases the surface area, flexibility, and conformability of the fiber. The increased surface area increases the hydrophobicity of the fiber, which ultimately hinders water drainage. Zever et al. (1994) reported that sodium hydroxide (NaOH) turns reprocessed paper yellow and reduces the strength properties of reprocessed paper. Pathak et al. (2011) reported that when photocopier waste paper was chemically deinked, the pulp attained a brightness of 80.4%, which was 2.1% higher than enzymatically deinked pulp. The tear index of chemically deinked pulp was 7.11 m.N.m²/g, and the tensile index and burst index were 28.5 N·m/g and 1.37 kPa·m²/g, respectively. Ink removal, washing, and flotation also facilitate the removal of fines and fillers from the pulp. Filler removal increases inter-fiber bonding, which leads to improved paper strength. In contrast, fines removal increases permeability of the paper and makes it less dense, which can lead to reduced paper strength (Deng 2000). Pathak et al. (2014) also reported that for 100% photocopied papers, enzymatic deinking is better than chemical deinking because it results in in higher deinking efficiency (25.4%), brightness (5.2%), burst index (23.9%), folding endurance (15.9%), and freeness (19.6%).

The enzymatic deinking trial was conducted to deink MOW pulp using crude enzyme extract from *P. citrinum* NCIM-1398. Enzymatically deinked pulp revealed an ERIC value reduced by 26.4% and increased deinkability based on ERIC (D_E) by 27.3%, whereas, brightness was improved by 9.6% and deinkability based on brightness (D_B) by 39.2%.

Table 1. Comparison of Chemical, Chemi-enz	ymatic and Enzymatic Deink	ng by F	P. citrinum NCIM-1398
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	Deinking process									
Particulars		Commercial		P. citrinum NCIM-1398						
	Blank	Endo β-1,4-	(C)							
		glucanase		E	C 100%+E	C50%+E	C25%+E			
		enzyme								
	Result after pulping									
Brightness, %	73.60±1.5									
ERIC, ppm	269.54±2.36									
	Results after ink flotation									
Total pulp yield, %	83.70±1.38	81.12±1.40	78.70±1.7	78.65±1.08	74.77±1.72	75.24±0.84	76.29±1.40			
Brightness (ISO), %	75.92±1.57	81.63±1.66	86.64±1.65	83.19±1.84	88.15±1.48	86.18±1.10	84.07±1.64			
% Increase/Decrease	+2.32	+8.03	+13.04	+9.59	+14 55	+12.58	+10 47			
(Result after pulping)	12.02	10.00	110.01	10.00		112.00				
Deinkability (D _B), %	12.49±0.21	43.24±0.58	70.22±0.69	51.64±0.47	78.35±0.72	67.74±0.65	56.38±0.51			
% Increase/Decrease		+30.75	+57.73	+39.15	+65.86	+55.25	+43.89			
ERIC, ppm	236.37±1.93	189.24±1.67	139.32±1.28	173.85±1.43	124.04±1.37	143.79±1.20	164.84±1.69			
% Increase/Decrease		-19.93	-41.05	-26.45	-47.52	-39.16	-30.26			
Deinkability (D _E), %	14.47±0.23	35.04±0.46	56.82±0.62	41.75±0.53	63.49±0.77	54.87±0.62	45.68±0.67			
% Increase/Decrease		+20.57	+42.35	+27.28	+49.02	+40.40	+32.21			
Dirt count, mm ² /m ²	18834.46±28	1901.75±16	1209.30±18	1456.03±11	1134.45±14	1254.82±13	1315.34±16			
% Increase/Decrease		-89.90	-93.57	-92.26	-93.97	-93.33	-93.01			
CSF (ml)	680±3.00	721±4	745±4	740±4	757±5	750±5	745±6			
Pulp viscosity, cm ³ /g	372.56±4.81	415.64±5.37	480.32±6.02	415.34±5.21	385.16±5.29	391.53±5.85	402.83±6.21			
	Effluent characteristics									
Total solids, ppm	1.56	1.68	1.74	1.68	1.87	1.80	1.82			
COD, ppm	140	212	262	206	251	234	218			
BOD _{5 days} , ppm	55	80	101	78	99	89	83			
	Mechanical strength properties									
Tensile index, Nm/g	28.56±0.87	33.23±0.85	32.39±0.78	31.82±0.91	31.14±0.50	31.40±1.41	31.64±1.13			
Tear index mNm ² /g	5.38±0.30	6.45±0.50	6.02±0.43	5.78±0.43	5.46±0.28	5.55±0.17	5.62±0.33			
Burst index, kPam ² /g	1.94±0.34	3.07±0.09	3.81±0.13	2.12±0.20	2.02±0.12	2.06±0.15	2.09±0.14			
Double fold, number	8	7	8	7	7	6	6			

± refers standard deviation, all chemical and enzyme doses based on oven dry raw material basis, Blank = no chemicals are added during pulping, C = chemical deinking, E= enzymatic deinking.

Chemical deinking (C)		Enzymatic deinking (E)		Chemical +Enzyme deinking (C+E)		
*Pulping conditions:	0)	* Pulping conditions:	/	* Pulping conditions	9(012)	
Pulping time, min	= 20	Pulping time, min	= 20	Pulping time, min	= 20	
Surfactant (Tween 80), %	= 0.05	Surfactant (Tween 80), %	= 0.05	Surfactant (Tween 80), %	= 0.05	
NaOH, % (as such)	= 2	Enzymatic treatment (E+T)	NaOH dosing		
		Endo β-1,4-glucanase, I	U/a =6	100%	=2	
			0	50%	= 1	
				25%	= 0.5	
H ₂ O ₂ , %	= 1	Xylanase, IU/g		H ₂ O ₂ dosing		
		=876.19		100%	=1	
				50%	= 0.5	
		Amylase, IU/g =26.53		25%	= 0.25	
Sodium meta	= 2.5	Surfactant (Tween 80),	= 0.05	Sodium meta silicate dosing		
silicate		%		100%	= 2.5	
				50%	= 1.25	
				25%	= 0.63	
DTPA,%	= 0.5	pН	=	DTPA dosing		
			5.2±0.2	100%	= 0.5	
				50%	= 0.25	
				25%	= 0.13	
Temperature, °C	= 65±2	Consistency, %	= 12	Temperature, °C	= 65±2	
nН	= 7 2 +0 2	Temperature °C	= 55+2	рН	= 7 2	
E	0.2	porataro, o	00	E	±0.2	
		Reaction time, min	= 60	Enzyme conditions is same mentioned in (E+T)	as	
*Flotation conditions: Flotation time, min = 10, consistency, % = 1, temperature, °C = 35±2 and pH =7.2±0.2						

Table 2. Conditions of Chemical, Chemi-enzymatic and Enzymatic Deinking of Mow pulp

The viscosity of deinked pulp increased from 372.6 to 415.3 cm³/g, tear index from 5.38 to 5.78 mNm²/g, tensile index from 28.6 to 31.8 Nm/g, and burst index from 1.94 to 2.12 kPa·m²/g compared with MOW pulp after pulping. Lee *et al.* (2000) explained that the basic function of cellulase is hydrolysis and superficial degradation of cellulose, which facilitates ink removal. Thus, the binding of cellulase on pulp fiber might result in fiber surface alterations that facilitate ink removal during pulping. However, xylanase has a tendency to adsorb at the polymeric toner surface. Toner particles interact with the xylanase hydrocarbon chain *via* hydrophobic surfaces. The hydrophilic portions of xylanase extend from the toner surface and support the dispersion power of enzyme onto the toner particles, which facilitates the dispersion of the toner particles during washing of pulp (Yehia and Reheem 2012).

Starch is widely used as a sizing agent and a wet end additive in MOW paper; its degradation is likely to help cellulase-assisted deinking of MOW paper. The application of crude enzyme cocktail was advantageous in the deinking of white MOW paper, maybe due to the synergistic effect of β -1,4-glucanase, xylanase, and amylase obtained from *P. citrinum* NCIM-1398. Dutt *et al.* (2012) deinked sorted office waste paper (SOP) with a mixture of cellulase, xylanase, amylase, and lipase at a dosage of 6, 3, 1.5, and 6 IU/g of oven dried pulp, respectively, and observed improvement in brightness by 13.3% (ISO), D_B by 37.8%, and D_E by 83.0%, whereas, a reduction in ERIC by 68.2% and dirt counts by 88.0% was observed as compared to respective control. Xu *et al.* (2009) also showed that the enzymatically deinked pulp having cellulase and xylanase in combination gave a higher brightness, lower ERIC, and improved physical properties.

Chemically deinked pulp had 3.45% higher brightness value than enzymatically deinked pulp. The rise in brightness may be due to the presence of H_2O_2 in conventional chemical deinking (Jeffries *et al.* 1994). Chemi-enzymatically treated pulp was found to have the efficacy to reduce the chemical demand (requirement of sodium silicate, sodium hydroxide, and hydrogen peroxide) with a 50% reduction compared with conventional chemical deinking to obtain the approximately similar optical properties and strength properties as of chemically deinked MOW pulp (Table 1). Singh *et al.* (2012) reported a 50% reduction in chemical dose with the gain of 4.85% in viscosity, 3.06% in tear index, 9.04% tensile, and 5.82% burst index with respect to the control. There was a reduction in COD (68.0%) and BOD (55.0%) and an increase in total solids (13.3%) compared with conventional chemical consumption was recorded during chemienzymatic de-inking of old newspaper compared with chemical deinking.

The COD of deinked pulp produced after enzymatic, and chemi-enzymatic treatment was found to increase by up to 206 and 234 ppm, respectively, compared to COD (140 ppm) of MOW after pulping (control). Similarly, the BOD value after enzymatic vs. chemi-enzymatic treatment, was also raised to 78 and 89 ppm, respectively, compared to BOD (55 ppm) of MOW after pulping. Effluents obtained after deinking also exhibited increased values for total solids (Table 1) as compared to MOW after pulping. On the other hand, values of COD, BOD, and total solids of deinked pulp obtained after enzymatic, and chemi-enzymatic treatment were found to be less in comparison of chemically deinking of MOW pulp that had COD, BOD, and total solids values of 262 ppm, 101 ppm, 1.74 ppm respectively (Table 1). Dutt et al. (2012) explained that the rise in COD, BOD, and total solids may be due to the removal of functional additives such as resins, sizing agent, starch, other chemicals, fines, fillers, and degraded cellulosic remains obtained due to alkaline peeling reactions. By contrast, due to the use of lesser amount of chemicals in enzymatic and chemi- enzymatic deinking as compared to conventional chemical deinking, the COD value of effluent of enzymatic deinking was found to be lower in comparison of chemical deinking. For the proper control of BOD levels, more precise control over enzyme doses and retention time are required to minimize cellulose hydrolysis (Qin et al. 2000; Magnin et al. 2002; Verma et al. 2011).

Scanning Electron Microscopy

The SEM images were analyzed to understand morphological modifications on the fiber surface of white MOW pulp during bio-deinking (Fig. 1). Figure A and B represents MOW just after pulping, where small ink particles were deposited on fiber surface, and a few ink particles entangled between fibers were clearly visible. Figure C represents pulp deinked with commercial cellulase. Slight trenches, cracks, and swollen fissures can be seen on the surface of MOW pulp fiber. Small grainy deposition was seen on the fibers, which may be starch or other additives present in MOW pulp. Figure D represents chemically deinked fibers. Some irregular particles or granules were found to have deposited on the fiber surface, compared to fiber surface of control. Those particles might be hemicellulose, cellulose, and starch-based additive. Figure E represents pulp treated with crude enzyme cocktail extracted from *P. citrinum* NCIM-1398. Peeling of fiber, cracks, swelling, and external fibrillation was clearly seen and may be due to the synergistic effects of endoglucanases, xylanase, and amylase present in crude enzyme.



Fig. 1. SEM photographs. (A) Ink particles entrapped in MOW pulp fibers having 1.80kx magnification; (B) MOW pulp fiber after pulping having 6.53kx magnification; (C) commercial endo β -1,4-glucanase deinked pulp having 1.70kx magnification; (D) chemically deinked MOW pulp having 2.72kx magnification; (E) MOW pulp deinked with crude enzyme extracted from *P. citrinum* NCIM-1398 having 881x magnification; (F) chemi enzymatically deinked pulp treated with crude enzyme obtained from *P. citrinum* NCIM-1398 and 50% chemical dose having 3.04kx magnification

The presence of endo β -1,4-glucanase and xylanase together, modified the fiber surfaces, making them rough and heterogeneous. Also, small microfibrils appeared on the surface as a result of producing cracks, peeling, swelling, and external fibrillation. Amylase facilitated decomposition of starch-based additives and thus helped in greater ink removal along with starch-based binders and additives (Dutt *et al.* 2012). Figure F represents chemi-enzymatically treated deinked pulp. It represents rough and heterogeneous and small microfibrils on the surface of pulp, which were the consequence of cracks, peeling, swelling, and external fibrillation. Similar results have been reported by Dutt *et al.* (2012) that the combined effect of enzyme and chemical facilitates better defibrillation of pulp fiber.

Powder XRD studies

X-ray diffractograms of chemically, enzymatically treated MOW pulp along with MOW after pulping stages are shown in Fig. 2. The highest intensity peak was found in the range of 20 to 28°. The chemical deinking of MOW resulted in 83.2% crystallinity, which was 1.7% higher than the crystallinity of MOW pulp. Chemicals used in deinking of pulp act nonspecifically on amorphous and non-amorphous region. They break hydrogen bonds and degrade mainly the non-crystalline portions of cellulose in the fiber, which increases the crystallinity (Cao and Tan 2002). The crystallinity of pulp after

treatment with commercial endo β -1,4-glucanase was 85.9%, which was 1.6% higher than MOW. Partial damage in the crystalline region of cellulose was due to surface fibrillation and formation of pores, which may be due to the effect of endo β -1,4glucanase. The amorphous region is more prone to endo β -1,4-glucanase attack than the crystalline region (Vyas and Lachke 2003). Crude enzyme-treated deinked pulp had 84.8% crystallinity, which was 4.6% higher than MOW. The increase in crystallinity was even higher than chemically treated pulp and commercial endo β -1,4-glucanase treated MOW pulp. This rise in crystallinity may be due to the synergistic effects of cellulase, xylanase, and amylase in the crude enzyme extract used for deinking.



Fig. 2. Combined graph representing XRD analysis of deinked pulp obtained after treatment with commercial enzyme, chemical and crude enzyme extracted from *P. citrinum* NCIM-1398 with respect of MOW after pulping.

Virk *et al.* (2013) reported similar results where the crystallinity of cellulose was increased by 10.3% during recycling of old newsprint.

Fourier transformation infrared spectroscopy

The FTIR studies confirmed the chemical structure of cellulose present in MOW pulp fibers. Major variations were observed at 3353 cm⁻¹, 2905 cm⁻¹, 1738 cm⁻¹, and 1050 cm⁻¹. The band depicted at 3352 cm⁻¹ reveals stretching of hydroxyl groups (-OH) (Virk et al. 2013). After enzymatic and chemical deinking, the wave number decreased compared with MOW. This shift of absorption band of enzymatically deinked pulp from 3352 to 3315 cm⁻¹ revealed the changes in intra- and intermolecular hydrogen bonding during chemical and enzymatic treatment of pulp fibers (Cao and Tan 2002). Another band at 2904.6 cm⁻¹ shifted to the lower wave number of 2881.3 cm⁻¹. This shift indicates aliphatic side chain degradation and corresponds to CH asymmetrical stretching vibrations in CH₃, CH₂, and CH in enzymatically treated pulp (Faix 1992). The band at 1729.9 cm⁻¹ disappeared in chemical and enzymatically deinked pulp. This band might be removed due to removal of vinyl ester resins during deinking. The presence of vinyl ester resins in binder facilitates binding of pigment to substrates such as cellulose, hemicellulose, and starch (Virk et al. 2013). The band at 1050 cm⁻¹ illustrates C=O stretching due to carbohydrate lignin linkage, which indicates the removal of residual lignin content in the pulp. Broad bands at 1400 and 1500 cm⁻¹ were characteristics of CaCO₃, a commonly used filler in paper. Virk *et al.* (2013) reported similar findings where a combination of xylanase and laccase was used for deinking of pulp.



Fig. 3. Combined graph representing FTIR analysis of deinked pulp obtained after treatment with commercial enzyme, chemical, and crude enzyme extracted from *P. citrinum* NCIM-1398 with respect of MOW after pulping.

CONCLUSIONS

- 1. The chemi-enzymatic approach of deinking reduced the use of toxic chemicals used in conventional chemical deinking by up to 50%, with the achievement of same brightness values and improved physical properties.
- 2. The present study revealed the reduction in COD, BOD, and total solid waste values of effluent of chemi-enzymatic treated pulp compared to chemical deinking. This reduction will lead to lower waste water treatment cost as well as reduces environmental pollution load. Thus, this is a step towards the development of cost effective, eco-friendly technology suitable for paper industries to recycle white MOW paper.

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