# Horseradish and Potato Peroxidase Biobleaching of Mixed Office Waste Paper

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

Mixed office waste (MOW) pulp was biodeinked with crude enzyme extracted from *Penicillium citrinum* NCIM-1398. Crude enzyme dose was charged having activities of endo β-1,4-glucanase 6I U/g, xylanase 876.19 IU/g, and amylase 26.53 IU/g. The present study aimed to bleach the biodeinked MOW pulp with 3% $H_2O_2$ in the presence of a stabilizing agent *viz.* 0.1% ethylenediaminetetraacetic acid (EDTA) and 0.1% magnesium sulfate ($MgSO_4$), which improved the pulp brightness up to 4.2% and the ERIC value was reduced by 24.1%. The residual $H_2O_2$ left in the pulp slurry after bleaching was subjected to peroxidase treatment using enzyme dose 0.017 U/g at 20 °C for 3 h at 200 rpm. Horseradish peroxidase reduced residual $H_2O_2$ in the pulp slurry from 0.30 to 0.05 g/L and improved the brightness of pulp to 88.1%, while the ERIC value was reduced by 20.9%. Potato peroxidase reduced residual $H_2O_2$ from 0.30 to 0.04 g/L, reduced the ERIC value by 30.9%, and improved the brightness to 89.2%. Peroxidase treatment was not only observed to consume the residual hydrogen peroxide left after the bleaching stage but also may come up as eco-friendly technology to recycle MOW paper as writing printing grade paper.

*Keywords: Mixed office waste (MOW) paper; Hydrogen peroxide bleaching; Stabilizers; Biobleaching; Horseradish peroxidase; Potato peroxidase*

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## INTRODUCTION

Despite the continual focus on digitization, the demand for paper in India is expected to rise by 53% by the year 2020 (Jha 2014). Paper consumption is rising primarily due to increasing population and literacy rate, growing per capita income, modern retailing, growing consumerism, and the growing use of documentation. India's per capita utilization of paper is 13 kg, which is low compared to the global average per capita utilization of 57 kg. India's per capita utilization is estimated to rise 20 kg per person by 2020. Each 1 kg increment in per capita utilization results in the supplementary demand of > 1 kg per person in a year (Smevebqu 2018). To overcome the limited availability of wood, agro-residues, and non-woody materials, waste paper is generally used as an abundant source of cellulose fibers.

Among all recyclable paper grades, mixed office waste (MOW) paper is a significant source of valuable paper fibers that can be used for manufacturing writing- and printing-grade paper. White MOW paper mainly consists of photocopies, computer printouts, envelopes, receipts, ledgers, *etc*. Additionally, MOW paper either may contain wood-free fibers or lignin in a negligible amount, but a variety of dyes, chromophoric groups, and printing inks are present as contaminants. The chromophoric groups present in different dyes and printing ink molecules are responsible for imparting color to the pulp. Bleaching refers to the destruction of the light-absorption capacity of dye (Walsh 1993). Despite the availability of various efficient bleaching options, MOW paper is still considered one of the difficult grades of waste paper to recycle because of the presence of commonly used recalcitrant paper dyes such as the stilbene dye, direct yellow 11, and the methine dye Basazol 46L (Darlington 1992). MOW paper also shows poor bleachability with the commonly used chemical bleaching agents, including chlorine dioxide, oxygen, hydrogen peroxide, and sodium dithionite (Knutson *et al.* 2005). To overcome the poor bleachability of MOW pulp by chemical agents, a wide number of enzymes, including cellulase (Tiwari *et al.* 2018), xylanase (Kumar *et al.* 2017), laccase (Riva 2006; Singh and Arya 2019), and peroxidase (Archibald 1992), have been studied (Biswas *et al.* 2019b). The enzyme application in pulp and paper industry are limited due to their high cost, and it is hard to maintain their stability during the industrial process (Kumar *et al.* 2018b,c, 2019a; Lin *et al.* 2018). Synthetic dyes possess a complex structure, which requires high cost and high energy input during conventional biological wastewater treatment. In addition, toxic byproducts are produced during the chemical process (Kumar *et al.* 2018a), which results in environmental pollution. In comparison, a biobleaching process is a less intrusive, less expensive method (An *et al.* 2002; Biswas *et al.* 2019b) and it has been shown to be an eco-friendly technology compared to chemical processes (Biswas *et al.* 2019a; Kumar *et al.* 2019b). Therefore, microbial degradation of dyes is gaining popularity in the paper and textile industries.

Due to its electron-donating ability to substrates, the reducing potential of peroxidase has made it useful in both paper and textile industries (Bansal and Kanwar 2013). Moreover, various other biotechnological applications of peroxidases include the decomposition of pollutants, sewage treatment, biosensors, and removal of peroxides from materials such as industrial wastes. Peroxidases catalyze the oxidation reactions in different inorganic and organic compounds (Hamid and Rehman 2009). The enzymatic reaction is as given below,

$$\text{Peroxidase} + H_2O_2 \rightarrow \text{Compound I} + H_2O \quad (1)$$

$$\text{Compound I} + RH \rightarrow \text{Compound II} + R^\circ \quad (2)$$

$$\text{Compound II} + RH \rightarrow \text{Peroxidase} + R^\circ + H_2O \quad (3)$$

and the overall reaction is,

$$2RH + H_2O_2 \rightarrow 2R^\circ + 2H_2O \quad (4)$$

where RH is a peroxidase substrate, and R° is a free-radical product derived from it.

Distinct intermediate enzyme forms are produced during the catalytic reaction of peroxidase (Wong and Yu 1999). In the initial step, an unstable intermediate compound I (CoI) is produced *via* oxidation of a native ferric enzyme by hydrogen peroxide. It has a heme structure of FeIV=O porphyrin π-cation radical, and there is a consequent reduction of peroxide to water. A free radical is produced by CoI when it oxidizes an electron donor substrate to give compound II (CoII) (same oxyferryl structure but protonated). The CoII is further reduced by a second substrate molecule regenerating the iron (III) state and producing another free radical. Knutson *et al.* (2005) reported enzymatic biobleaching of two recalcitrant paper dyes with horseradish and soybean peroxidase. Crude peroxidase enzymes obtained from different vegetable sources are found to be highly unstable, while potato peroxidase is observed to be more stable over a wide range of temperatures (Suha *et al.* 2013) and can maintain its activity in the pH range of 4 to 8 (Kurnik *et al*. 2015).

The aim of the present study was to stabilize hydrogen peroxide with magnesium sulfate and ethylenediamine tetraacetic acid for bleaching of biodeinked MOW pulp (deinked with crude enzymes obtained from *Penicillium citrinum* NCIM-1398). Hydrogen peroxide ($H_2O_2$) is commonly used for pulp bleaching. However, under bleaching conditions, $H_2O_2$ is very unstable, especially in the presence of transition metals such as Fe, Mn, and Cu (Sun *et al.*1999; Zhang *et al*. 2010). The decomposition of $H_2O_2$ leads to carbohydrates degradation and generates reducing groups responsible for brightness loss, low fiber strength and increased bleaching costs (Allison 1983). To overcome the effect of transition metal ions during bleaching, some stabilizers such as magnesium sulfate, DTPA, and EDTA are commonly used (Singh 1979; Zhao *et al*. 2018). Stabilizers form stable and water-soluble complexes with transition metal ions. The transition metal complexes can then be removed from the pulp during pressing and washing stages (Finnegin *et al.*1998; Wekesa *et al.* 2011). Residual $H_2O_2$ present in $H_2O_2$-bleached MOW pulp was used to catalyze the oxidation of recalcitrant organic compounds by peroxidase enzymes extracted from horseradish and freshly peeled potato.

## EXPERIMENTAL

### Materials and Methods

*Hydrogen peroxide bleaching*

Manually torn MOW paper with a size in between of 1.5 to 2.0 $cm^2$ was soaked in water at 50 °C and kept for 30 min. MOW paper was pulped in a hydra pulper at 65±2 °C, for 20 min and pH 7.2±0.2. Then the MOW pulp was treated with crude enzyme obtained from *Penicillium citrinum* NCIM-1398. Crude enzyme dose was charged having activities of endo β-1,4-glucanase 6IU/g, xylanase 876.19 IU/g, and *amylase* 26.53IU/g. Pulp consistency of 12%, pH 5.5±2, temperature 55±2 °C, and reaction time of 60 min were maintained.

The MOW pulp biodeinked with crude enzyme, obtained from *P. citrinum* NCIM-1398 (crude enzyme extracted in Biotechnology Laboratory of Department of Paper Technology, Indian Institute of Technology Roorkee Saharanpur Campus, Saharanpur, India), was treated with $H_2O_2$ with or without the presence of chelating agents such as ethylenediaminetetraacetic acid (EDTA) and magnesium sulfate ($MgSO_4$). The brightness of biodeinked MOW paper pulp was determined according to TAPPI T452 om-02 (1998) and the ash content according to TAPPI T211 om-02 (2002). In the first set of experiments, MOW biodeinked pulp was bleached with 3% $H_2O_2$ at 90 ± 2 °C, with 10% consistency, pH 11.8 ± 0.2, and a reaction time of 19 min (Table 2). Furthermore, biodeinked pulp was treated with (a) 3% $H_2O_2$ and 0.1% EDTA, (b) 3% $H_2O_2$ with 0.1% $MgSO_4$, and (c) 3% $H_2O_2$ with 0.1% EDTA and 0.1% $MgSO_4$, all maintaining the same reaction conditions. Finally, residual $H_2O_2$ was determined as per the method given by Hunt and Lee (1995). Bleached pulp was then squeezed, washed with tap water, and maintained at neutral pH. Primarily pulp pads were prepared according to TAPPI T218 sp-02 (2002) for the evaluation of physical properties.

*Extraction of peroxidase*

Fresh potato (*Solanum tuberosum*) was collected from a local market (Saharanpur, India), and washed. The washed potatoes were milled and homogenized at pH 5 with ice-cold ten mM of sodium phosphate buffer. The milled potato sample was mixed with sodium phosphate buffer at a ratio of 1:1 (w/v). The crude enzyme extract was then filtered through cheesecloth and centrifuged at 8000 rpm for 10 min to remove traces of fibrous particles and cell debris. The supernatant was stored at 4 °C and used as a stock solution for further experiments. All chemicals used for the experiments were of reagent grade. Commercial horseradish peroxidase (Peroxidase horseradish RZ 3.0 (HRP Type 1), 250 U/mg) was purchased from Sisco Research Laboratories Pvt., Ltd. (SRL), Mumbai, Maharashtra, India.

*Enzyme assays*

Peroxidase activity was measured spectrophotometrically using the Marangoni *et al.* (1995) method. A total of 10 µL of enzyme solution was mixed with 2 mL of 100 mM citrate-phosphate buffer solution that contained 18.2 mM guaiacol and 4.4 mM $H_2O_2$ as substrates. The samples were incubated at 25 °C and pH 5.5. The change in absorbance at 470 nm was spectrophotometrically monitored. The result was calculated by the Bach *et al.* (2013) method. One unit of peroxidase activity represents the amount of enzyme catalyzing the oxidation of 1 µmol of guaiacol in 1 min at 25 ± 2 °C. The activity at each time interval and temperature was expressed as a percentage of the total activity.

*Biobleaching of pulp*

MOW pulp slurry bleached with 3% $H_2O_2$ in combination with both stabilizers 0.1% EDTA and 0.1% $MgSO_4$ was used to conduct further biobleaching experiment. The $H_2O_2$ bleached pulp slurry (containing residual $H_2O_2$) was used for conducting further biobleaching experiments using horseradish peroxidase and potato peroxidase. A total of 0.017 U/g of horseradish peroxidase was added in 250 mL of pulp slurry with 10% consistency. Similarly, for another set of experiments, 0.017 U/g of potato peroxidase was added to 250 mL of pulp slurry with 10% consistency. The control contained only 250 mL of pulp slurry of 10% consistency. All three reaction mixtures were kept on a shaker at a speed of 200 rpm, at 20 ± 3 °C for 3 h. After that, residual $H_2O_2$ was determined as per the method given by Hunt and Lee (1995). Then, the pulp was squeezed, washed thoroughly with tap water, and maintained at neutral pH. Pulp pads were prepared and evaluated for various optical parameters as per TAPPI T218 sp-02 (2002). Pulp brightness was determined as per TAPPI T452 om-02 (1998). Effective residual ink concentration (ERIC) was determined using an infrared reflectance measurement (L&W Elrepho SE 070A; L&W, Kista, Sweden). Likewise, to evaluate the dirt count, handmade sheets were prepared according to TAPPI T213 om-01 (2001). Deinkability factors, such as $D_E$ (Deinkability based on ERIC) and $D_B$ (Deinkability based on brightness) were calculated according to Dutt *et al.* 2012.

*Statistical analysis*

All experiments were carried out in triplicate, and experimental results were presented as a mean of ± standard deviation of three identical values.

## RESULTS AND DISCUSSION

### Hydrogen Peroxide Bleaching

MOW pulp deinked with crude enzyme obtained from *P. citrinum* NCIM-1398 improved pulp brightness by 9.5%, compared to MOW paper after pulping with an ash content of 1.88% (Table 1). The $H_2O_2$ bleaching showed an increase in brightness of 2.0% (Fig. 1), deinkability based on brightness ($D_B$) of 10.8%, and deinkability based in ERIC ($D_E$) of 8.8% (Fig. 2). Viscosity rose from 380 to 391cm$^3$/g, while the ERIC value was reduced by 11.6%, dirt count by 5.6%, and the post color number was reduced from 0.87 to 0.73. Residual $H_2O_2$ left in the filtrate was 0.19 g/L (Table 2).

**Table 1.** Properties of MOW Pulp after Pulping and Biodeinking

| Serial No. | MOW Pulp | Brightness (%) | Ash Content (%) |
|---|---|---|---|
| 1. | After Pulping | 73.66 ± 1.15 | 14.29 ± 0.14 |
| 2. | After Biodeinking ( *P. citrinum* NCIM-1398) | 83.20 ± 1.16 | 1.88 ± 0.09 |

It is reported that during bleaching, $H_2O_2$ is activated and decomposed into water and hydrogen peroxide anion ($HOO^-$) (Galbács and Csányi 1983). The $HOO^-$ provides nascent oxygen (O), which oxidizes organic compounds including chromophores. The $HOO^-$ reacts nucleophilically with chromophores and thus intensifies the bleaching boosting effect of hydrogen peroxide. Metal ions catalyze the degradation of $H_2O_2$ (Galbács and Csányi 1983; Evans and Upton 1985), which in turn diminish $HOO^-$ concentration, and thus adversely affect the bleaching efficiency. Chauveheid *et al.* (1998) reported that bleaching of MOW paper with hydrogen peroxide revealed a gain of 7.1% in brightness. A similar result was also reported by Lunabba *et al.* (1998) that higher brightness was achieved due to decolonization of the chromophores present in lignin and dyes present in MOW pulp.

During $H_2O_2$ treatment, where EDTA was used as a stabilizer, biodeinked pulp showed an increase in brightness by 2.96%, deinkability based on brightness increased by ($D_B$) 16.0%, deinkability based on ERIC ($D_E$) increased by 13.0%, and the viscosity increased from 380 to 391cm$^3$/g. The ERIC value was reduced by 17.2%, dirt count by 12.4%, and the post color number reduced from 0.87 to 0.67. Residual $H_2O_2$ left in the filtrate was 0.24 g/L. In comparison, $H_2O_2$ treatment in the presence of $MgSO_4$ showed an increase in brightness by 3.4%, deinkability based on brightness ($D_B$) increased by 18.5%, deinkability based on ERIC ($D_E$) increased by15.1%, and viscosity was increased from 380 to 447cm$^3$/g. The ERIC value was reduced by 20.0%, dirt count was reduced by 12.4%, and the post color number reduced from 0.87 to 0.67. Residual $H_2O_2$ left in the filtrate was 0.26 g/L.

In contrast, enzymatically biodeinked pulp treated with $H_2O_2$ in the presence of EDTA and $MgSO_4$ exhibited an increase in brightness by 4.2%, deinkability based on brightness ($D_B$) increased by 22.4%, deinkability based on ERIC ($D_E$) increased by 18.3%, and viscosity was increased from 380 to 597cm$^3$/g. The ERIC value and dirt count were reduced by 24.1% and 33.6%, respectively, whereas post color number was reduced from 0.87 to 0.46 (Table 2). Thus, enzymatic treatment of pulp was found to be useful in terms of reduced bleach chemical consumption, improved pulp brightness, reduced effluent toxicity, and lower pollution load.

The addition of $MgSO_4$ and EDTA during $H_2O_2$ bleaching showed the maximum bleach-boosting effect. Because MOW contained transition metals, the addition of $MgSO_4$ and EDTA during $H_2O_2$ bleaching hindered the $H_2O_2$ decomposition by forming the complex compounds with transition metals. Kopania *et al.* (2008) reported a 5% increase in pulp brightness during the peroxidase bleaching of MOW paper with $MgSO_4$ and EDTA. Brogdon *et al.* (1998) also reported enhanced brightness at high-temperature peroxide bleaching of MOW using formulated bleach stabilizers.

**Table 2.** Effect of EDTA and $MgSO_4$ During $H_2O_2$ Bleaching of Biodeinked Pulp of White MOW Paper

| Particulars | Control | Effect of Chelating Agent (EDTA), Carbohydrate Stabiliser ($MgSO_4$), on $H_2O_2$ Bleaching of MOW Pulp | | | |
|---|---|---|---|---|---|
| | | $H_2O_2$ | $H_2O_2$ + EDTA | $H_2O_2$ + $MgSO_4$ | $H_2O_2$ + EDTA + $MgSO_4$ |
| Brightness (%) | 83.20 ± 1.05 | 85.19 ± 1.52 | 86.16 ± 1.40 | 86.63 ± 0.90 | 87.35 ± 1.64 |
| % Increase/Decrease | 00 | + 1.99 | + 2.96 | + 3.43 | 4.15 |
| Deinkability ($D_B$) (%) | 51.54 ± 0.60 | 62.29 ± 0.73 | 67.53 ± 0.78 | 70.07 ± 0.81 | 73.96 ± 0.94 |
| % Increase/Decrease | 00 | + 10.75 | + 15.99 | + 18.53 | + 22.42 |
| Dirt count ($mm^2/m^2$) | 1528.53 ± 13.65 | 1443.41 ± 11.58 | 1338.63 ± 12.45 | 1131.05 ± 10.74 | 1014.63 ± 9.73 |
| % Increase/Decrease | 00 | - 5.56 | - 12.42 | - 26.00 | - 33.62 |
| Viscosity ($cm^3/g$) | 380 ± 7.46 | 391 ± 7.46 | 397 ± 7.46 | 447 ± 7.46 | 579 ± 7.46 |
| Post Colour Number | 0.87 ± 0.06 | 0.73 ± 0.04 | 0.67 ± 0.04 | 0.54 ± 0.03 | 0.46 ± 0.03 |
| ERIC (ppm) | 172.19 ± 1.77 | 152.23 ± 1.96 | 142.54 ± 1.70 | 137.81 ± 1.61 | 130.64 ± 1.22 |
| % Increase/Decrease | 00 | - 11.59 | - 17.21 | - 19.96 | - 24.13 |
| Deinkability ($D_E$) (%) | 41.98 ± 0.38 | 50.76 ± 0.58 | 55.03 ± 0.67 | 57.11 ± 0.71 | 60.27 ± 0.74 |
| % Increase/Decrease | 00 | + 8.78 | + 13.05 | + 15.13 | + 18.29 |
| Residual $H_2O_2$ (g/L) | NA | 0.19 ± 0.084 | 0.24 ± 0.09 | 0.26 ± 0.10 | 0.30 ± 0.11 |

Note: ± refers to standard deviation, NA = Not Applicable;
**Reaction conditions:**
Temperature (°C) = 90 ± 2
Reaction time (min) = 19
Consistency (%) = 10
$H_2O_2$ dose (%) = 3.0
EDTA dose (%) = 0.1
$MgSO_4$ dose (%) = 0.1
pH = 11.8 ± 2

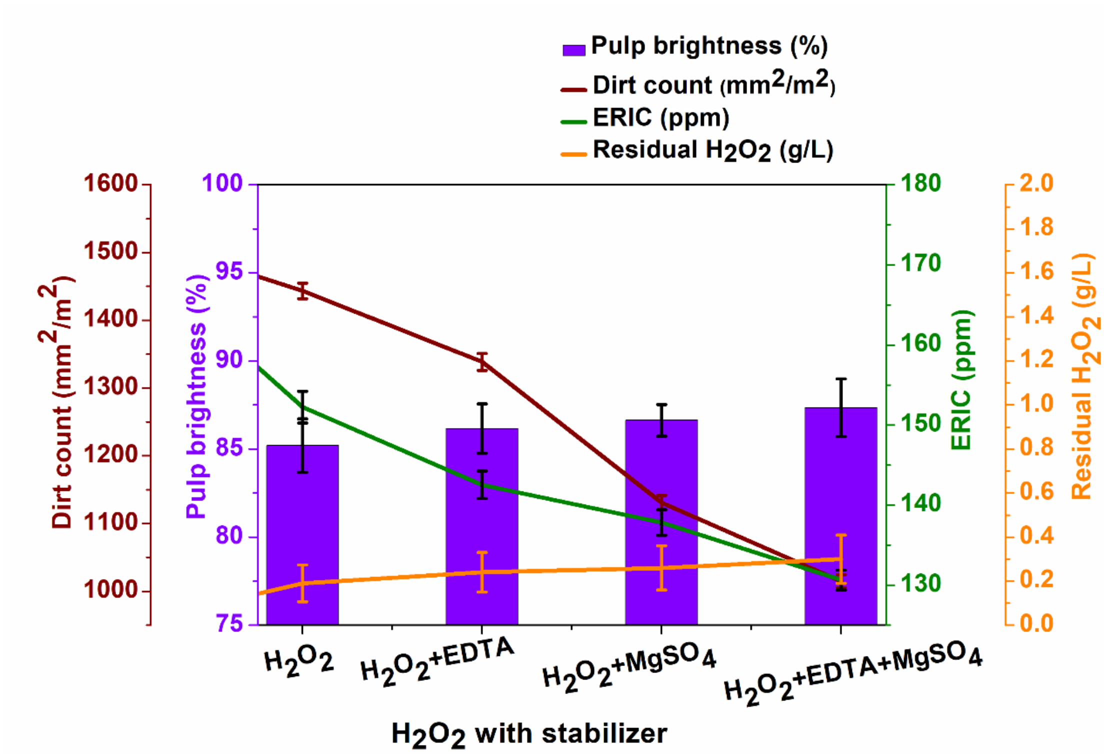


**Fig. 1.** Effect of EDTA and $MgSO_4$ on Brightness, ERIC (ppm), Residual $H_2O_2$, and Dirt count ($mm^2/m^2$)

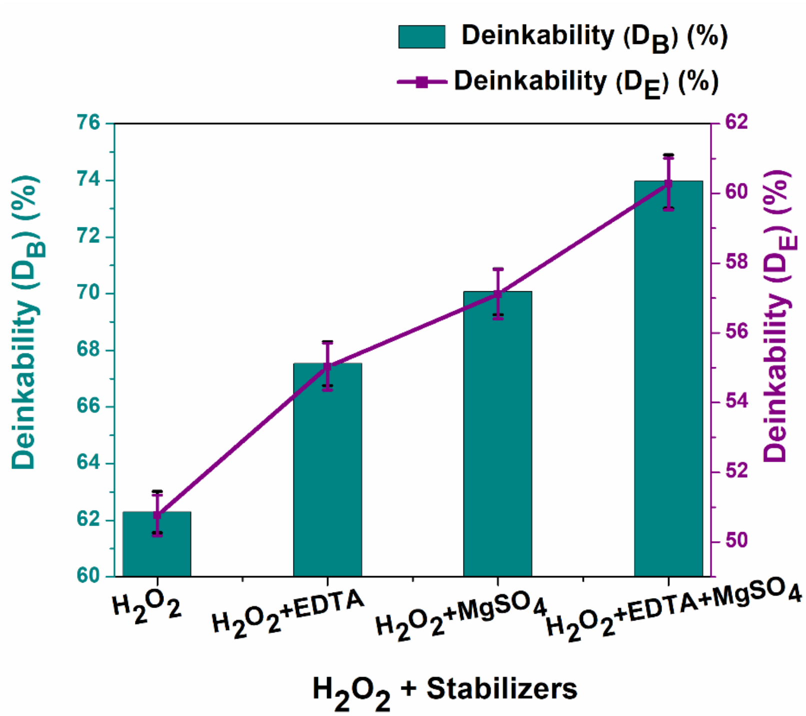


**Fig. 2.** Effect of EDTA and $MgSO_4$ on deinkability based on brightness($D_B$)% and deinkability based on ERIC ($D_E$)%

## Biobleaching

Potato peroxidases and horseradish peroxidase were separately added to the slurry of $H_2O_2$-bleached MOW pulp. Horseradish peroxidase treatment improved the brightness of $H_2O_2$-bleached MOW pulp to 88.1%, *i.e.*, a 0.79% gain in brightness compared to its respective control. Similarly, potato peroxidase treatment improved the pulp brightness of $H_2O_2$-bleached MOW pulp to 89.2%,which was 1.84% higher brightness compared to its respective control (Table 3) (Fig. 3). The pulp brightness of 89.2% obtained after potato peroxidase treatment was approximately 2% less compared to the brightness of photocopy paper from Century Paper Mills Ltd. (Gazipur, New Delhi, India), *i.e.*, 92.2% (virgin chemical pulp) (Table 4). The post color number of potato peroxidase treated pulp was reduced from 0.76 to 0.46, whereas ash content was reduced from 14.29% to 1.88% compared to the respective control. Horseradish and potato peroxidase improved the $D_B$ by 4.27% and 9.94%, respectively and $D_E$ by 9.07% and 13.37%, respectively, in $H_2O_2$-bleached MOW pulp compared to the respective control (Fig. 4). In contrast, dirt counts were reduced 6.22% and 10.62%, respectively, and ERIC values 20.9% and 30.9%, respectively, in $H_2O_2$-bleached MOW pulp compared to the respective control (Table 3).

**Table 3.** Effect of Horseradish Peroxidase and Potato Peroxidase on $H_2O_2$-bleached Slurry of White MOW Pulp

| Particulars | Effect of Horseradish Peroxidase and Potato Peroxidase on $H_2O_2$-bleached MOW Pulp | | |
|---|---|---|---|
| | Control | Horseradish Peroxidase | Potato Peroxidase |
| Brightness (%) | 87.35 ± 0.73 | 88.14 ± 0.65 | 89.19 ± 0.78 |
| % Increase/Decrease | 00 | + 0.79 | + 1.84 |
| Deinkability ($D_B$) (%) | 73.96 ± 0.62 | 78.23 ± 0.71 | 83.90 ± 0.77 |
| % Increase/Decrease | 00 | + 4.27 | + 9.94 |
| Dirt count ($mm^2/m^2$) | 1038.97 ± 9.13 | 974.33 ± 8.06 | 928.56 ± 7.31 |
| % Increase/Decrease | 00 | - 6.22 | - 10.62 |
| Viscosity ($cm^3/g$) | 392 ± 6.32 | 365 ± 5.48 | 337 ± 5.36 |
| Post Colour Number | 0.61 ± 0.4 | 0.57 ± 0.3 | 0.46 ± 0.3 |
| Eric (ppm) | 98.44 ± 0.89 | 77.83 ± 1.56 | 68.05 ± 1.02 |
| % Increase/Decrease | 00 | - 20.93 | - 30.87 |
| Deinkability ($D_E$) (%) | 74.44 ± 0.81 | 83.51 ± 0.97 | 87.81 ± 0.89 |
| % Increase/Decrease | 00 | + 9.07 | + 13.37 |
| Residual $H_2O_2$ (g/L) | 0.30 ± 0.06 | 0.05 ± 0.01 | 0.04 ± 0.01 |

Note: ± refers to standard deviation, enzyme activity of extracted potato peroxidase = 0.30 U/mL, and enzyme activity of commercial horseradish peroxidase = 250 U/mg

**Reaction conditions:**

Temperature (°C) = 20 ± 3
Test sample = 250 mL of $H_2O_2$-bleached pulp (slurry containing residual $H_2O_2$)
Consistency (%) = 10
Orbital shaker speed = 200 rpm
Peroxidase enzyme dose = 0.01713 U/g
Reaction time (min) = 3 h

The filtrate of peroxidase-bleached pulp contained a negligible amount of residual $H_2O_2$, which indicated that most of the residual $H_2O_2$ had been used by peroxidases for

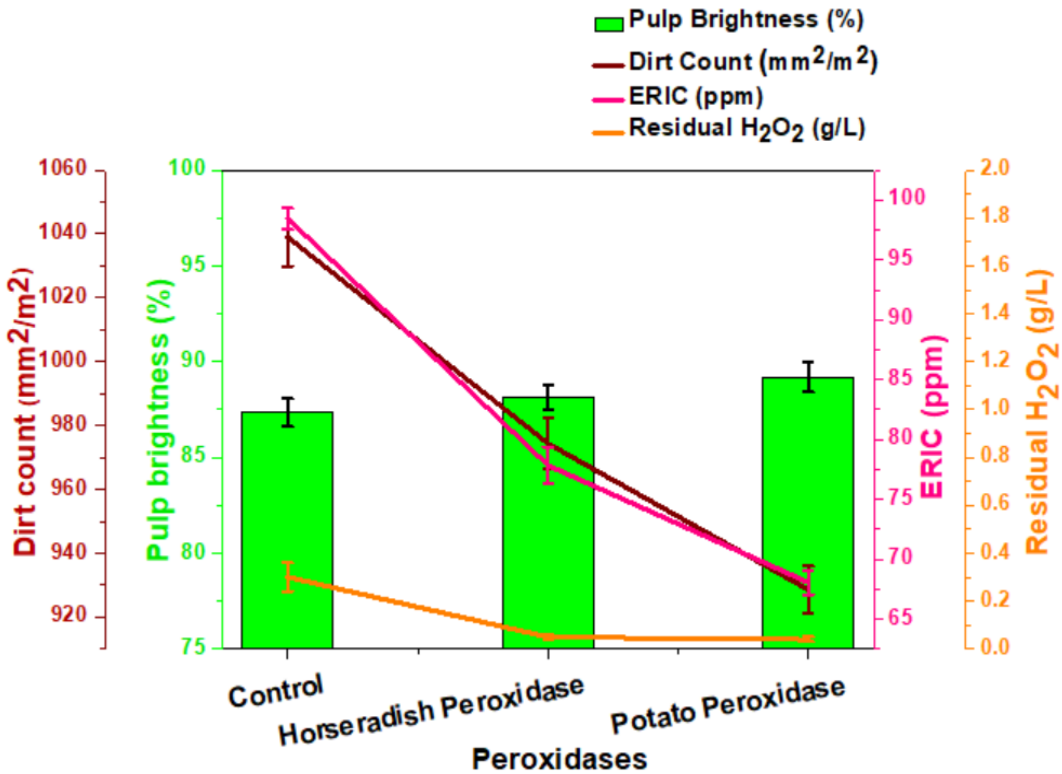


**Fig. 3.** Effect of horseradish peroxidase and potato peroxidase on brightness, ERIC (ppm), residual $H_2O_2$ and dirt count ($mm^2/m^2$)

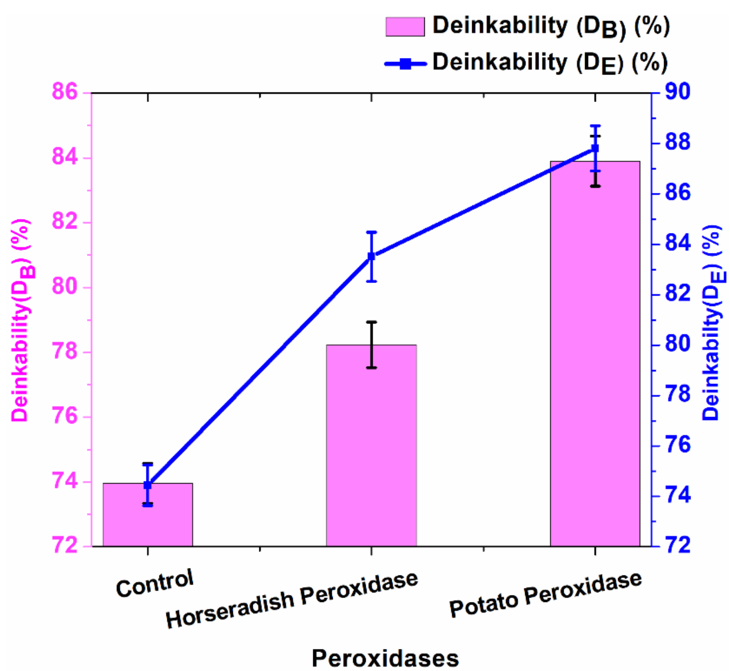


**Fig. 4.** Effect of horseradish peroxidase and potato peroxidase on deinkability based on brightness ($D_B$)% and deinkability based on ERIC ($D_E$)%.

catalyzing the oxidation of dyes (Fig. 3). Wong and Yu (1999) mentioned that peroxidases catalyzed the oxidation of a wide variety of synthetic dyes and was used extensively in the dyeing and printing industries in the presence of $H_2O_2$ or other peroxides. Horseradish peroxidase was well established to degrade recalcitrant organic compounds, such as azo dyes, phenol, and substituted phenols, through a free radical polymerization mechanism (Bhunia *et al.* 2001). Kurnik *et al.* (2015) reported the potential application of potato pulp peroxidases for the removal of phenol from synthetic and industrial wastewater. Similarly, Gimeno *et al.* (2005) reported degradation of certain recalcitrant organic aromatic compounds including phenols and aromatic amines by horseradish peroxidase. Tatsumi *et al.* (1996) reported that horseradish peroxidase degrades phenol and substituted phenols by a free radical polymerization mechanism. Bhunia *et al.* (2001) reported effective degradation of an industrially important azo dye, such as Remazol, by horseradish peroxidase.

**Table 4.** Comparison of Fresh Unused Photocopy Paper (Century Paper Mill) with Biodeinked and Biobleached MOW Pulp

| **Particulars** | **MOW after Pulping** | **MOW after Biodeinking (*P. citrinum* NCIM-1398) + Biobleaching(Potato Peroxidase)** | **Unused A4 Photocopy - Century Star (Century Paper Mill)** |
|---|---|---|---|
| Brightness (%) | 73.66 ± 1.15 | 89.19 ± 0.78 | 92.17 ± 0.69 |
| % Increase/Decrease | 00 | + 15.53 | + 18.51 |
| Post Colour Number | 0.87 ± 0.06 | 0.46 ± 0.03 | 0.76 ± 0.07 |
| Ash Content (%) | 14.29 ± 0.14 | 1.88 ± 0.09 | 14.29 ± 0.24 |

**Reaction conditions**

**Biodeinking**
Endo β-1,4-glucanase, IU/g =6
Xylanase, IU/g =876.19
Amylase, IU/g =26.53
Sufactant(Tween80) ,% =0.05
pH =5.2±0.2
Temperature (°C) =20 ± 3
Consistency (%) =12
Reaction time (min =60

**Biobleaching**
Temperature (°C) = 20 ± 3
Test sample = 250 mL of $H_2O_2$-bleached pulp (slurry containing residual $H_2O_2$)
Consistency (%) = 10
Orbital shaker speed = 200 rpm
Peroxidase enzyme dose = 0.01713 U/g
Reaction time (min) = 180 (3 h)

Knutson *et al.* (2005) mentioned that soybean peroxidase was effective in the decolorization of paper dye Basazol 46L and Direct Yellow 11 paper dye. Peralta *et al.* (1998) reported that the pulp and paper, and textile industry effluent were decolorized over 50% after 4 h of horseradish peroxidase treatment. Thus, enzymatic treatment of pulp using biobleaching have been found useful in terms of reduced consumption of bleaching chemical, improved pulp and paper quality, improved brightness, reduced effluent toxicity, and pollution load.

## CONCLUSIONS

1. The use of $H_2O_2$ stabilizing agents intensified the bleaching effect during hydrogen peroxide bleaching and hence improved pulp brightness 4.15%.
2. Potato peroxidase and horseradish peroxidase treatment not only removed excessive residual $H_2O_2$ present in pulp slurry but also improved pulp brightness. Potato peroxidase was more efficient for MOW biobleaching compared to horseradish peroxidase in terms of gaining higher brightness, as well as improved $D_B$ and $D_E$, whereas dirt counts and ERIC values decreased.
3. The pulp brightness of 89.2% obtained after potato peroxidase treatment was approximately 2 percentage points less compared to copy paper of Century Paper Mills Ltd., *i.e.*, 92.2%.
4. Biobleaching of MOW paper not only improved pulp brightness but also improved other properties without the release of harmful chemicals as seen in conventional chemical process of bleaching. Thus, biobleaching with potato peroxidase prove itself as cleaner technology to reduce environmental pollution and may support the development of cost-effective and eco-friendly technology for paper recycling.

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