Enhancing the Repulping Efficiency of Enzyme-Treated Wet-Strength and Hard-Sized Wastepaper

Haniya Mehmood,[a] Chul-Hwan Kim,[b,]* Hyeong-Hun Park,[a] Min-Sik Park,[a] Jae-Sang Lee,[c] Dong-Gi Lee,[d] and Kyung-Sik Shin [e]

Effects of mechanical refining parameters and enzymatic treatments were studied relative to the repulping efficiency of four wastepaper grades: kraft grocery bag (KGB), double-lined kraft (DLK), American old corrugated container (AOCC), and Korean old corrugated container (KOCC). Parameters including freeness, fiber length, fines, flake content, and specific energy consumption were examined under varied conditions. Optical microscopy confirmed that narrowing the mechanical disperser gap increased fiber disintegration intensity, reducing freeness and flake content but raising energy consumption. A synergistic effect was demonstrated between optimized mechanical refining and enzyme formulations (Cell E-B), specifically targeting wet-strength and hard-sized wastepaper. Instead of directly degrading chemical additives, the cellulase-based treatment facilitates fiber recovery by partially hydrolyzing cellulose at the fiber surface and disrupting fiber–additive interactions. This enzymatic action promotes fiber swelling and liberation, leading to further reductions in freeness and flake content while preserving fiber length. These findings provide comparative insights and actionable data to support the transition from laboratory findings to planned full-scale industrial manufacturing, offering pathways for energy savings and improved fiber utilization in the paper recycling industry.

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*Contact information: a: Department of Forest Products, Gyeongsang National University, Jinju 52828, Republic of Korea; b: Department of Forest Products, Institute of Agriculture and Life Sciences (IALS), Gyeongsang National University, Jinju 52828, Republic of Korea; c: Department of Pulp & Paper Chemical Engineering, Gyeongsang National University, Jinju 52828, Republic of Korea; d: Department of Environmental Materials Science, Gyeongsang National University, Jinju 52828, Republic of Korea; e: CEO, KNS Chemical Co., LTD, 5F, Dongin Building, 2, Sinam-ro, Jung-gu, Daegu 41972, Korea; sks5403@naver.com (S-K. Shin) *Corresponding author: jameskim@gnu.ac.kr*

INTRODUCTION

The global paper industry continues to face increasing pressure to strengthen its sustainability profile by improving recycling efficiency and reducing reliance on virgin fiber and chemical inputs. One of the significant obstacles in this direction is the growing volume of post-consumer wastepaper containing wet-strength resins and hard-sizing agents. These chemical additives are commonly used in food packaging, labels, and hygiene products to impart water resistance and structural integrity (Francolini *et al.* 2023; Christenson and Sjöstrand 2024; Małachowska 2024). This is especially true for materials treated with polyaminoamide-epichlorohydrin (PAE) resins and alkenyl succinic anhydride (ASA) or alkyl ketene dimer (AKD) sizing agents, which are notoriously difficult to repulp using conventional recycling processes due to the irreversible crosslinking and strong hydrophobic properties they confer on the fibers (Hubbe 2007; Christenson and Sjöstrand 2024). Their intense resistance to water-induced disintegration often results in low fiber recovery, reduced yield, and higher energy and chemical consumption during secondary fiber processing (Lee *et al.* 2021; Christenson and Sjöstrand 2024).

Specifically, wet-strength agents such as PAE form robust covalent amine and ester bonds with cellulose, while hard-sizing agents such as AKD rely on ester linkages. While specific enzymes could be tailored to target these chemical bonds directly, their industrial application is often limited by the heterogeneous nature of wastepaper streams. Instead, this study utilizes cellulase-based formulations to modify the underlying cellulose matrix. By inducing partial hydrolysis at the fiber surface, these enzymes weaken the structural "anchor points" of the resins, thereby facilitating the disruption of fiber-resin and fiber-sizing interactions during mechanical repulping.

In response to these challenges, enzymatic repulping has emerged as a green alternative. Enzymes such as cellulases, hemicellulases, esterases, and laccases have shown promise in selectively modifying fiber surfaces, loosening fiber bonding, and breaking down binder resins thus improving repulpability without the excessive fiber damage caused by alkaline or oxidative chemical treatments (Bajpai 1999; Pèlach *et al.* 2003; Bajul-Baradon *et al.* 2023; Pal *et al.* 2025). Enzyme-based processes can offer several advantages, including energy savings, reduced effluent load, and lowered chemical oxygen demand (COD) in wastewater (Singh *et al.* 2019; Bajul-Baradon *et al.* 2023; Rossi and Solé 2025). However, despite these considerable technical and environmental benefits, the widespread adoption of enzymes in the paper recycling and manufacturing industries remains relatively limited (Jahan *et al.* 2016; Rossi and Solé 2025). Several barriers hinder industrial deployment, including the following:

Substrate complexity and enzyme specificity

Wet-strength and hard-sized papers are chemically resistant, partly due to lignin-rich fibers, synthetic adhesives, and sizing agents that confer durability and hydrophobicity, making them resistant to enzymatic hydrolysis (Kirk and Jeffries 1996; Singh *et al*. 2016; Christenson and Sjöstrand 2024). Variability in waste-paper feedstock composition necessitates highly specialized enzyme formulations, constraining the universality of such solutions (Pathak and Bhardwaj 2010; Singh *et al.* 2016; Rossi and Solé 2025). Furthermore, enzymatic selectivity is critical. Overzealous action of cellulases can cause fiber shortening, loss of paper strength, and increased fines generation, negatively impacting product performance and marketability (Bajpai 1999; Demuner *et al.* 2011; Biricik and Atik 2012).

Process sensitivity

Enzymes generally require narrow operating windows (specific temperature, pH, and reaction time), which may not align with existing pulping lines and can require capital investment or operational modifications to mill processes (Bajpai 1999; Bajul-Baradon *et al.* 2023; Kenealy and Jeffries 2003). Additives in wastepaper (such as inks, synthetic polymers, and fillers) can also inhibit enzyme action, complicating process control and reliability (Min and Ramarao 2017; Fillat *et al.* 2018).

Economic considerations

Although biotechnological advances have reduced enzyme production costs, enzymes are often more expensive per ton of pulp compared to traditional chemicals in many markets, posing economic hurdles for cost-sensitive mills, especially those operating at a large scale (Kenealy and Jeffries 2003; Bajul-Baradon *et al.* 2023; Immerzeel and Fiskari 2023). Logistics, including cold-chain storage and regional supply, also factor into adoption decisions (Kenealy and Jeffries 2003; Haske-Cornelius *et al.* 2020; Immerzeel and Fiskari 2023).

Industrial inertia and skills gap

Resistance to change among mills with established chemical-based processes, a limited trained workforce, and insufficient integration models for enzyme-based operations slows the rate of adoption (Jain *et al*. 2001; Kenealy and Jeffries 2003; Mäki *et al.* 2021).

Knowledge gaps

Ongoing research is necessary for the development of more effective and robust industrial enzyme cocktails, improved specificity for fiber preservation, and enhanced performance across diverse conditions (Sharma *et al.* 2020; Kumar *et al.* 2022; Yang *et al.* 2025).

Given these limitations, the practical implementation of enzymatic repulping remains an underutilized but promising opportunity. Focused research on enzyme selection, dosage optimization, and process integration tailored to specific wastepaper streams could help overcome these practical roadblocks (Dixit *et al.* 2022; Kumar and Verma 2025; Gupta *et al.* 2025).

This study evaluated the effectiveness of enzyme-assisted repulping in enhancing fiber recovery from wet-strength and hard-sized wastepaper under controlled bench-scale conditions. By optimizing treatment protocols to improve pulp quality, yield, and process efficiency, this research established the foundational parameters necessary for industrial upscaling. While enzymatic potential is often demonstrated only at the laboratory level, the successful outcomes of this study are currently being utilized to transition toward planned full-scale manufacturing. Ultimately, this work provides actionable insights to bridge the gap between laboratory findings and industrial practicality, supporting the sustainable transformation of the paper recycling industry.

EXPERIMENTAL

Materials

Four types of wastepaper, kraft grocery bag (KGB), double-lined kraft paper (DLK), American old corrugated container (AOCC), and Korean old corrugated container (KOCC) were supplied by Jeonju Paper Co., Ltd. (Jeonju, Korea). All samples were cut into 2 to 3 cm^2 pieces and stored in sealed polyethylene bags at 23 ± 2 °C and 50 ± 5% RH until use. These specific wastepaper types are regularly used at Jeonju Paper and have posed ongoing challenges regarding repulping efficiency during industrial operations. In particular, the presence of strong wet-strength and sizing additives in these waste grades has led to incomplete fiber dispersion and reduced pulp yield, hindering the papermaking process.

While the precise chemical history of the collected wastepaper grades (KGB, DLK, AOCC, and KOCC) was not provided by the suppliers, they are assumed to follow standard industrial chemical treatments. Based on common papermaking practices, it is highly likely that polyamidoamine-epichlorohydrin (PAE) was the primary wet-strength agent, given its widespread use in packaging and bag grades. Similarly, hydrophobic sizing is expected to have been achieved using alkyl ketene dimer (AKD) or alkenyl succinic anhydride (ASA). These agents form covalent linkages with cellulose, primarily ester bonds, which contribute to the repulping resistance observed in this study.

Methods

The chemical composition of each wastepaper was determined using ISO standard methods. Lignin content was measured according to ISO 21436 (2019), α-cellulose content was determined following TAPPI T 429 cm-23 (2023), and ash content was measured according to ISO 1762 (2019).

Fiber morphology, including arithmetic mean fiber length, length-weighted mean fiber length, and fines content, was analyzed using an FQA-360 analyzer (OpTest, Canada) in accordance with ISO 16065-2 (2014). Additionally, microscopic observations of fiber morphology and structural changes were conducted at 100× magnification using an Olympus BX51 microscope (BX51, OLYMPUS, Tokyo, Japan).

Mechanical and water-repellent properties were evaluated using ISO 1924-2 (2008) for tensile strength and ISO 3781 (2011) for wet tensile strength. The contact angle and Stöckigt sizing degree were measured using a custom-built integrated system developed by the research team, which enables simultaneous measurement based on automatic color recognition, as described by Song *et al.* (2006).

Enzyme Treatment

Enzymatic treatments were conducted to enhance the repulping efficiency of wastepaper materials. Two types of cellulase-based enzyme formulations, both supplied by KNS Chemical Co., Ltd. (Daegu, Korea) were used. A general cellulase preparation (Cell E-A) and a specialized formulation (Cell E-B), designed for effective action on wastepaper containing wet-strength resins and hard sizing agents.

For each experiment, the enzyme was added to the pulp slurry at a dosage of 1% based on the pulp's oven-dry (OD) weight. The enzymatic activity of both formulations was 10,000 U/mL. Treatments were performed under controlled conditions at specified temperatures and pH values, with reaction times exceeding 30 min. Throughout the reaction, the pulp slurry was continuously agitated to ensure even contact between the fibers and the enzyme solution.

Following enzymatic treatment, the pulp was further processed for repulping and subsequent analytical evaluation. These conditions were selected to assess the effects of enzyme type, temperature, and pH on the repulping efficiency of wastepaper materials containing wet-strength and hard-sizing additives. The specific conditions for each treatment are summarized in Table 1.

Table 1. Enzyme Types and Process Conditions Used for Wastepaper Repulping

Enzyme Type	Activity (U/mL)	Dosage (%, on OD pulp weight)	Temp. (°C)	pH	Reaction Time (min)	Note
Cell E-A	10,000	1	40	7, 9	>> 30	General cellulase
Cell E-B	10,000	1	40	7, 9	>> 30	Wet-strength specific enzyme

Repulping

Repulping of the enzyme-treated wastepaper samples was conducted using a laboratory Hollander beater (Daeil Machinery Co., Daejeon, Korea), with the pulp slurry prepared at a stock consistency of approximately 0.7%. Repulping experiments were performed at stock temperatures of 40 and 60 °C, and at pH values of 7 and 9, to assess the influence of these parameters on fiber dispersion. Before adding pulp and water, the gap between the bed plate and the beater roll was set according to the manufacturer's specifications; after loading and applying a 2.5 kg weight to the roll, the gap was readjusted to ensure consistent mechanical action throughout the disintegration process.

Repulping was carried out for a standardized disintegration period with continuous agitation. During the process, power consumption was monitored in real time using a smart energy meter (PM-P230, DawonDNS, Seoul, Korea) to evaluate the impact of enzymatic treatment on energy demand. All data (n = 5 per condition) were analyzed by one-way ANOVA followed by Tukey's HSD ($\alpha = 0.05$). To directly compare enzyme treatments with the untreated control at 40 °C and pH 9, prespecified two-sided Student's t-tests with Holm adjustment for multiplicity were applied. Normality (Shapiro–Wilk) and homogeneity of variances (Levene) were checked; when assumptions were violated, Welch's ANOVA and Holm-adjusted Welch t-tests were used.

Fiber Analysis

Upon completion, repulped samples were withdrawn for further analysis. Freeness was measured based on ISO 5267-1(1999) (Canadian Standard Freeness method) to determine pulp drainage characteristics. Flake content was quantified according to ISO 5263-1 (2004), employing a Somerville screen (Daeil Machinery Co., Daejeon, Korea) for the automatic screening and determination of the percentage of incompletely defibered particles in each sample.

The mean fiber length and fines content of the repulped pulp were analyzed using a fiber quality analyzer (FQA-360, OpTest Equipment Inc., Hawkesbury, Canada), in accordance with ISO 16065-2 (2014), providing quantitative information on the fiber length distribution and the fraction of fines.

Additionally, microscopic observations of fiber morphology and structural changes were conducted at 100× magnification using an Olympus BX51 microscope (BX51, OLYMPUS, Tokyo, Japan). Dimensional changes in fiber width and length before and after enzymatic treatment were quantified using Axiovision imaging software (Ver. 4.8, Carl Zeiss, Germany), allowing precise measurement of fiber swelling and modification.

These combined assessments were used to evaluate the effectiveness of the enzymatic and repulping treatments with respect to fiber dispersion, drainage, reduction of flake content, and overall pulp quality.

RESULTS AND DISCUSSION

Physicochemical Characteristics of Wastepaper Grades

The physicochemical characteristics of the four wastepaper types, including lignin content, α-cellulose content, ash content, length-weighted mean fiber length, and fines content, are summarized in Table 2. KGB had the highest lignin content (17.9 ± 1.0%), KOCC was notable for its high α-cellulose content (66.6 ± 0.2%) and significantly lower fines content (21.6 ± 0.4%), and length-weighted mean fiber length, a key indicator of pulp quality, ranged from 0.9 to 1.7 mm across the four grades.

Table 2. Physico-chemical Characteristics of Raw Materials

Raw Materials	Lignin (%)	α-cellulose (%)	Ash (%)	Arithmetic mean fiber length (mm)	Length-weighted mean fiber length (mm)	Fines (%)
KGB	17.9±1.0	58.8±1.4	0.6±0.1	0.6±0.1	1.7±0.1	51.7±3.0
DLK	10.2±0.8	53.8±1.0	0.3±0.0	0.5±0.0	1.4±0.0	51.8±7.0
AOCC	10.1±0.6	51.8±2.5	0.5±0.1	0.6±0.0	1.58±0.0	46.0±3.1
KOCC	12.3±0.4	66.6±0.2	0.9±0.1	0.6±0.0	0.9±0.0	21.6±0.4

KGB exhibited the highest lignin content at 17.9%, consistent with its origin from kraft grocery bags, which typically undergo limited bleaching and retain a higher proportion of residual lignin than other recycled paper grades. In contrast, KOCC exhibits the highest α-cellulose content at 66.6%, indicating a higher proportion of purified cellulosic fibers, likely due to repeated recycling and processing steps that progressively remove lignin and hemicellulose. The ash content is relatively low across all grades, ranging from 0.3% in DLK to 0.9% in KOCC, suggesting a limited presence of mineral fillers and coating materials.

In terms of fiber properties, the arithmetic mean fiber length was approximately 0.5 to 0.6 mm for all samples, but the length-weighted mean fiber length varied more substantially, with KGB having the longest fibers (1.7 mm) and KOCC the shortest (0.9 mm), reflecting differences in the proportion of long, less-damaged fibers present in KGB compared to the more extensively recycled and fragmented fibers in KOCC. Fines content was highest in KGB and DLK, 51 to 52%, while KOCC had the lowest fines content at approximately 22%. These compositional differences are expected to significantly influence the enzymatic susceptibility and repulping behavior of each grade, as higher lignin content can sterically hinder enzyme access to cellulosic substrates, while higher fines content may indicate prior mechanical damage that could affect fiber liberation efficiency (Kirk and Jeffries 1996; Bajpai 1999).

Table 3 presents the mechanical and water-repellent characteristics of the wastepapers. Tensile strength ranged from 3.1 ± 0.5 kN/m for KOCC up to 11.9 ± 0.9 kN/m for AOCC, with AOCC benefiting from a higher proportion of longer and less-degraded fibers compared to KOCC (Nazhad 2005).

Table 3. Physical Characteristics of Different Wastepaper Grades

Raw Materials	Tensile Strength (kN/m)	Wet Tensile Strength (kN/m)	Contact Angle (°)	Stöckigt Sizing Degree (s)
KGB	4.1±0.8	0.9±0.1	106.5±5	436.6±21.2
DLK	5.0±1.2	0.3±0.0	71.9±2.3	600.0±15.1
AOCC	11.9±0.9	2.2±0.1	104.2±0.8	600.0±10.0
KOCC	3.1±0.5	0.0±0.0	113.8±0.4	244.1±7.5

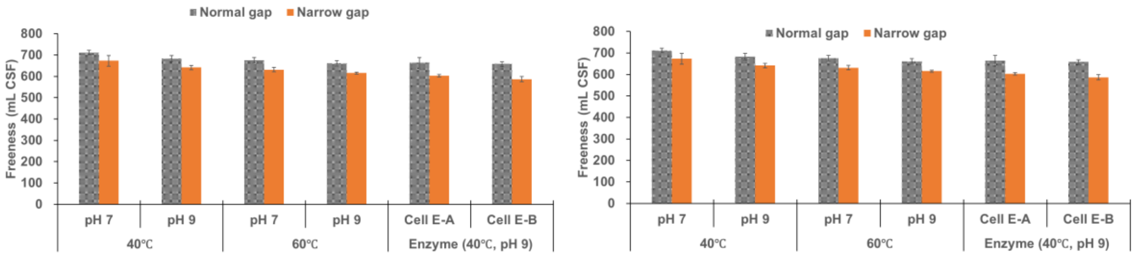
Wet tensile strength was highest for AOCC (2.2 ± 0.1 kN/m), indicating better fiber bonding under wet conditions and the presence of wet-strength resins, and lowest in KOCC, where it is essentially zero (0.0 ± 0.0 kN/m). The wet-to-dry tensile strength ratio for AOCC was approximately 18.5%, which is characteristic of paper products containing polyamide-epichlorohydrin (PAE) or similar wet-strength agents that form covalent cross-links with cellulose.

Contact angle measurements, indicating surface hydrophobicity, varied with KOCC having the highest value (~114°). This was likely due to the presence of hydrophobic contaminants, residual sizing agents, or surface-enriched lignin (Hubbe *et al.* 2007). DLK had the lowest contact angle (~72°), indicating a more hydrophilic surface with reduced effective sizing. The Stöckigt sizing degree, a measure of sizing effectiveness, was highest for DLK and AOCC at 600 seconds, suggesting strong sizing treatment with alkyl ketene dimer (AKD) or alkenyl succinic anhydride (ASA), while KOCC had the lowest sizing degree (244 seconds) and KGB had an intermediate value (437 seconds).

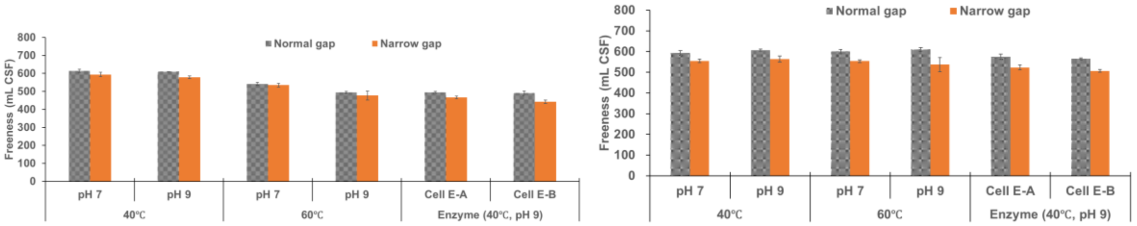
These data revealed significant differences in chemical composition, fiber morphology, strength properties, and water-repellent characteristics among the four wastepaper grades, which are expected to influence their enzymatic susceptibility and repulping efficiency. Specifically, grades with higher wet strength (AOCC, KGB) and sizing degrees (DLK, AOCC) are hypothesized to benefit more from enzymatic pre-treatment targeting these chemical barriers.

Effect of Enzymatic Treatment on Freeness

Figure 1 displays the freeness results for KGB, DLK, AOCC, and KOCC respectively after repulping under various refining and enzyme treatment conditions. Across all wastepaper grades, the use of a narrow gap consistently resulted in lower freeness values (200 to 350 mL CSF) compared to a normal gap (300 to 500 mL CSF), indicating more effective fiber swelling, fibrillation, and breakdown due to the intensified mechanical action.



(a) KGB (b) DLK



(c) AOCC (d) KOCC

Fig. 1. Effect of mechanical gap setting and enzyme treatment on the freeness of various wastepaper grades: (a) KGB, (b) DLK, (c) AOCC, and (d) KOCC

Alkaline conditions (pH 9) and elevated temperature (60 °C) further reduced freeness compared to neutral pH (7) and lower temperature (40 °C). This pH-temperature effect can be attributed to enhanced fiber swelling under alkaline conditions, which facilitates water retention and reduces drainage rate, as well as to the temperature-dependent increase in fiber flexibility and the hydrolysis of hemicelluloses at elevated temperatures.

The most significant reductions in freeness occurred with Cell-B treatments, which is formulated to partially hydrolyze cellulose and thus indirectly disrupting fiber–resin and fiber–sizing interactions during mechanical repulping leading to potential wet-strength and sizing-agent degradation. This effect was especially marked for KGB and AOCC, where freeness decreased by 25 to 40% compared to mechanical controls. Table 3 reveals that these grades had the highest wet tensile strengths and sizing degrees (KGB: 0.9 kN/m, 437 s; AOCC: 2.2 kN/m, 600 s), suggesting a high content of wet-strength resins and sizing additives that hinder fiber dispersion and water drainage during standard mechanical treatment.

The mechanisms shown in Figs. 2 and 3 help explain these results at the level of fiber-surface interactions. Figure 2 illustrates that endo-cellulases (EC 3.2.1.4) cleave β-1,4-glycosidic bonds within the amorphous regions of cellulose chains (Bajpai 1999), creating new chain ends and enabling fiber loosening, internal fibrillation, and swelling. Exo-cellulases (cellobiohydrolases, EC 3.2.1.91) subsequently trim cellulose chains from the reducing and non-reducing ends, releasing cellobiose units and facilitating further fiber liberation with minimal fiber shortening. This synergistic endo-exo action increases the specific surface area of fibers and exposes more hydroxyl groups, enhancing water retention capacity and thus reducing freeness (Kirk and Jeffries 1996; Bhat 2000).

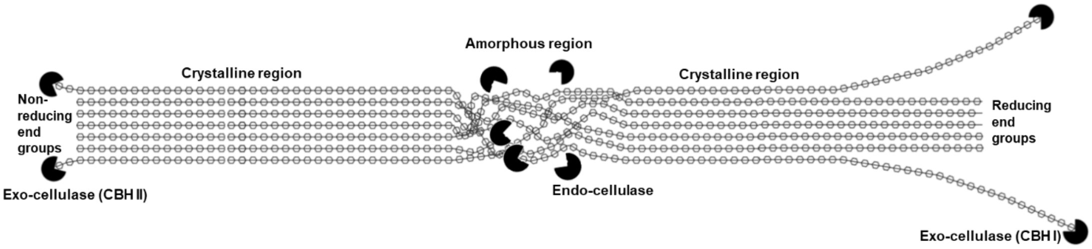


Fig. 2. Mechanisms of endo- and exo-cellulase action on cellulose microfibrils: enzymatic cleavage sites and fiber structure by Cell E-B during repulping

Figure 3 illustrates how the cellulase-based formulation mainly acts by modifying the fiber surface (swelling and limited fibrillation), thereby weakening the anchoring points at the fiber–additive interface and promoting fiber release during mechanical treatment, as reflected by lower freeness values.

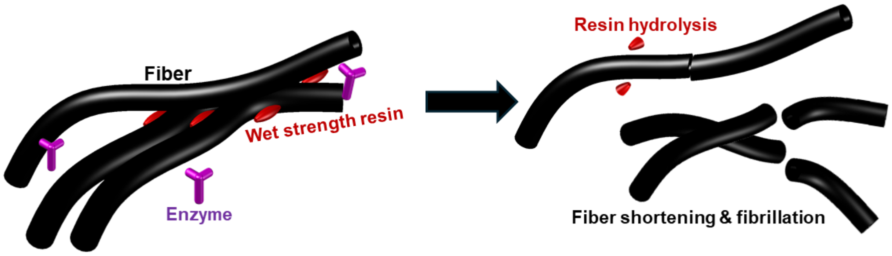


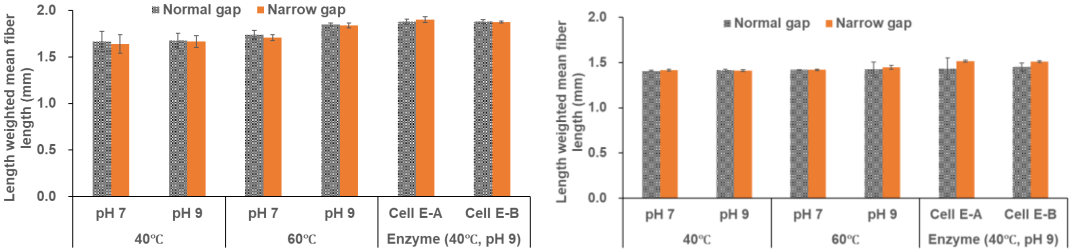
Fig. 3. Schematic of cellulase-mediated repulping: The enzyme modifies the fiber surface *via* partial cellulose hydrolysis, weakening the anchoring points of wet-strength and sizing agents to facilitate fiber liberation during disintegration.

No direct hydrolysis of amide/ester linkages was demonstrated in this study. While esterases may directly target ester linkages in AKD/ASA systems, the present work focuses on mill-compatible cellulase-based surface modification; follow-up experiments will evaluate an esterase alone and in combination with cellulase to assess selectivity and potential synergy. This enzymatic effect was particularly pronounced in grades with high wet-strength content (KGB and AOCC), where Cell E-B treatment reduced freeness by up to ~150 mL CSF compared with the corresponding mechanical controls under the same conditions. In contrast, DLK and KOCC, which had lower wet tensile strengths (0.3 and 0.0 kN/m, respectively), showed less dramatic responses to enzymatic treatment, with freeness reductions of only ~50–80 mL CSF.

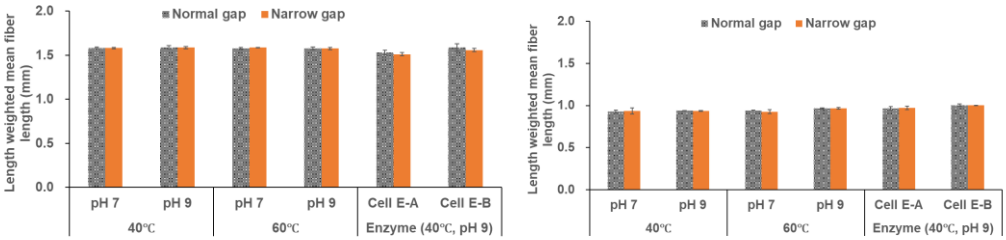
These findings are consistent with previous studies (Jahan *et al.* 2016; Bajul-Baradon *et al*. 2023), which reported substantial freeness reductions in recycled pulps containing wet-strength additives following cellulase treatment. The present study extends those observations by demonstrating grade-dependent responses and by providing mechanistic insights based on microscopic and process-level analyses. In summary, the largest freeness reductions after narrow-gap refining and especially after cellulase treatment (notably with Cell E-B) occurred in wastepapers with higher wet-strength and sizing content, highlighting the role of **controlled cellulase-mediated fiber-surface modification** in overcoming chemical and physical barriers to efficient fiber liberation and water retention (Kirk and Jeffries 1996; Bajpai 1999).

Effect of Enzymatic Treatment on Fiber Length

Figure 4 presents the length-weighted mean fiber length of four wastepaper grades (KGB, DLK, AOCC, and KOCC) after repulping under different mechanical gap settings and with or without enzyme treatment.



(a) KGB (b) DLK



(c) AOCC (d) KOCC

Fig. 4. Effect of mechanical gap setting and enzyme treatment on the mean fiber length of various wastepaper grades: (a) KGB, (b) DLK, (c) AOCC, and (d) KOCC

In all cases, the narrow gap led to a moderate decrease in mean fiber length (5 to 12%) compared to the normal gap, as stronger mechanical shear forces cause more fiber cutting and breakage. For instance, KGB fiber length decreased from 1.65 mm (normal gap) to 1.48 mm (narrow gap) under control conditions at pH 7, 40 °C.

However, when enzyme treatments (Cell E-A and Cell E-B, 40 °C, pH 9) were applied, the mean fiber length was generally maintained at a similar or slightly higher level (within 3 to 5% difference) than that observed for mechanical disintegration alone under the same gap condition. This preservation of fiber length was statistically significant for the KGB and AOCC grades. This trend was most apparent in KGB and AOCC grades, where enzyme treatment combined with narrow-gap refining resulted in mean fiber lengths of 1.52 and 1.48 mm, respectively, compared with 1.45 mm and 1.41 mm for mechanical controls.

These results indicate that enzymatic repulping primarily enhanced fiber separation by weakening interfibrillar bonds and indirectly hydrolyzing wet strength agent cross-links and sizing agent deposits, especially those formed by polyamide-epichlorohydrin resins or AKD/ASA sizing chemicals rather than by inducing mechanical cutting or peeling of fiber walls. The enzyme's mode of action involves targeted cleavage of amide and ester bonds at the resin-fiber interface (as illustrated in Fig. 3), facilitating fiber liberation without the need for excessive mechanical force that would otherwise cause fiber shortening.

As a result, enzyme-treated grades tended to retain longer mean fiber lengths, which is beneficial for preserving pulp strength properties in recycled products. Longer fibers contribute more effectively to interfiber bonding and tensile strength development during sheet formation, making this preservation of fiber length a critical advantage for maintaining recycled pulp quality.

The fiber length preservation effect was most pronounced in AOCC (mean fiber length maintained at 1.48 mm with Cell E-B *vs*. 1.41 mm in the control, narrow gap), which correlates with its high wet-strength content (Table 3). In contrast, KOCC, which lacks wet-strength additives, showed minimal differences between enzymatic and mechanical treatments (0.87 *vs.* 0.85 mm), suggesting that the enzyme's benefit is specific to its ability to target chemical bonding agents rather than affecting inherent fiber properties.

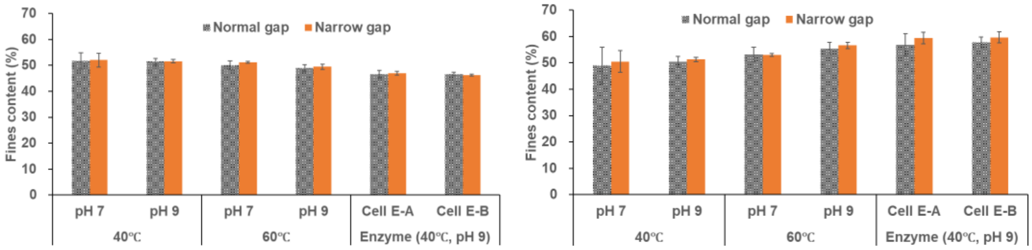
These findings align with reports by Pelach *et al.* (2003) and Haske-Cornelius *et al.* (2010), who observed that enzymatic treatments preserve or enhance fiber length by promoting fiber liberation through biochemical rather than mechanical means. The present study provides quantitative evidence supporting this mechanism across multiple wastepaper grades with varying chemical compositions.

Overall, Fig. 4 demonstrates that enzymatic treatment, especially when tailored for wet strength and sizing agents, can effectively aid fiber liberation without substantial loss of fiber length, supporting better quality in recycled pulp applications even when combined with intensive mechanical conditions. This finding has important practical implications for recycled paper mills seeking to maintain pulp strength while improving repulping efficiency and reducing energy consumption.

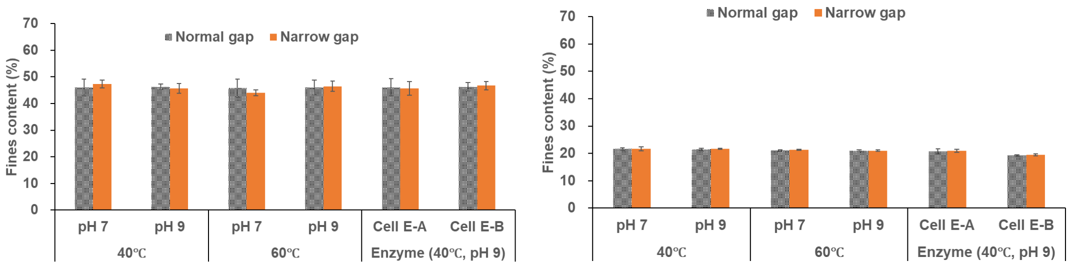
Effect of Enzymatic Treatment on Fines Content

Figure 5 presents the arithmetic mean fines content (percentage of particles < 0.2 mm) after repulping for four wastepaper types (KGB, DLK, AOCC, KOCC) under various mechanical and enzymatic conditions. While the general expectation, based on prior research (Jeffries and Viikari 1996; Monte *et al.* 2009), is that enzymatic treatments, especially those targeting wet-strength and sizing agents, can increase fines production due to enhanced fiber liberation and disintegration, the data here suggest a more nuanced, grade-specific response.

For KGB, AOCC, and KOCC, enzyme-treated samples actually showed a slight to moderate decrease in fines content (2 to 8% reduction) compared to the corresponding mechanical controls, particularly under the normal gap condition. For example, KGB showed fines content of 48.2% with Cell E-B treatment compared to 51.5% for mechanical control (normal gap, 40 °C, pH 9). This outcome contrasts with the substantial increase in fines typically reported when enzymes promote extensive fiber breakdown through aggressive hydrolysis.



(a) KGB (b) DLK



(c) AOCC (d) KOCC

Fig. 5. Effect of mechanical gap setting and enzyme treatment on the arithmetic mean fines contents of various wastepaper grades: (a) KGB, (b) DLK, (c) AOCC, and (d) KOCC

The decline in fines content after enzymatic repulping in these grades may be attributed to the enzyme's ability to selectively weaken interfibrillar and interfiber bonds formed by wet strength resins and sizing agents, resulting in improved fiber dispersion and separation at the fiber-to-fiber level without excessive mechanical cutting, peeling, or over-fibrillation of individual fiber walls. This selectivity facilitates clean fiber separation while minimizing secondary fiber damage, fragmentation, and fines generation which is a desirable outcome for maintaining pulp quality and strength potential.

Additionally, enzymatic cleavage of wet-strength resin crosslinks may allow fibers to separate as intact units rather than being torn apart mechanically, reducing the generation of fiber fragments and primary fines. The enzyme essentially "unlocks" the chemical bonds between fibers, allowing gentler mechanical separation (Jahan 2016).

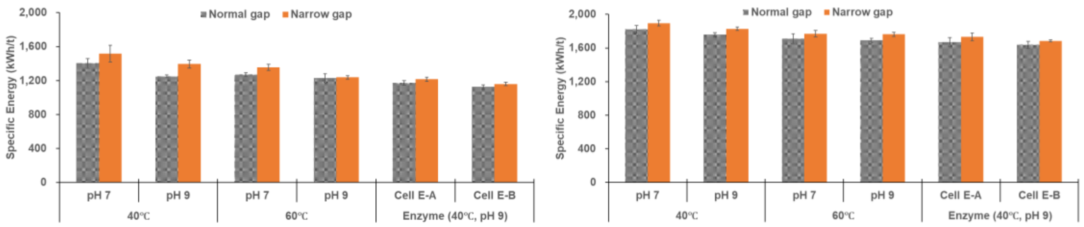
DLK, on the other hand, departed from this trend. Enzyme treatments led to a clear increase in fines content (from 50.8% to 56.3% with Cell E-B, narrow gap, 60 °C, pH 9), consistent with the expected effect of enzymatic hydrolysis on substrates rich in wet strength or sizing agents. This difference likely reflects specific compositional or structural properties of the DLK pulp including its low contact angle (72°), high sizing degree (600 s), and moderate wet strength (0.3 kN/m) that make it more susceptible to both enzymatic and mechanical generation of fines. The distinct response of DLK stems from superior enzyme accessibility, prior fiber degradation, and enhanced peeling effects through sizing-enzyme interactions.

While Cell E-B generally minimized fines generation in KGB, AOCC, and KOCC compared to Cell E-A, it increased fines in DLK. This indicates that the wet-strength-specific formulation in Cell E-B interacts uniquely with pulp substrates based on their chemical compositions.

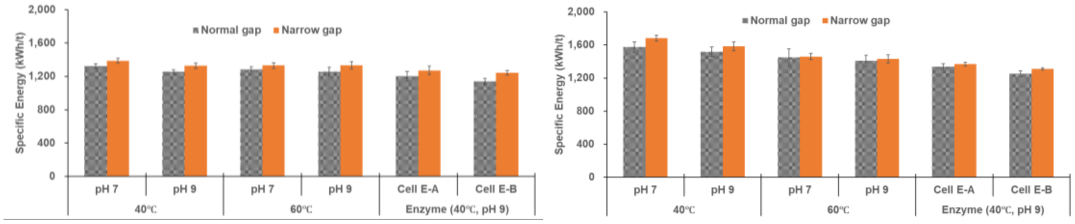
Enzyme application can optimize recycled pulp processing by promoting fiber liberation without excessive damage, effectively controlling fines in most grades. However, the divergent results for DLK highlight the need for grade-specific optimization of dosage and conditions to prevent localized over-refining or fines accumulation.

Energy Consumption During Repulping

Figure 6 shows the specific energy consumption (kWh/t) during repulping for four types of wastepaper grades, namely KGB, DLK, AOCC, and KOCC, under different mechanical gap settings (normal and narrow), pH values (7 and 9), temperatures (40 °C and 60 °C), and enzyme treatments (Cell E-A and Cell E-B at 40 °C and pH 9).



(a) KGB **(b)** DLK



(c) AOCC **(d)** KOCC

Fig. 6. Values are mean ± standard deviation (n = 5). For each furnish, statistical comparisons were performed separately within each mechanical gap setting using one-way ANOVA followed by Tukey's HSD test ($p < 0.05$). Additional pairwise comparisons of cellulase treatments against the untreated control at 40 °C and pH 9 were conducted using t-tests with Holm correction.

Across all grades, narrow-gap refining consistently required more energy than normal-gap refining, although the magnitude of the increase depended on furnish type and operating condition. Based on the replicate data, the narrow gap increased specific energy consumption by approximately 3.4 to 11.4% relative to the corresponding normal-gap condition. For example, in KGB at 40 °C and pH 9, the specific energy increased from 1214 to 1353 kWh/t when the gap was changed from normal to narrow. These results indicate that mechanical gap setting is an important contributor to repulping energy demand, but its effect is substrate-dependent rather than uniform across all grades.

Increasing the temperature from 40 °C to 60 °C generally reduced specific energy consumption, with the magnitude of reduction varying among substrates and pH conditions. Depending on grade and gap setting, the temperature increase decreased the specific energy by about 1.0 to 12.9%, consistent with improved fiber flexibility, increased water penetration, and facilitated fiber separation at elevated temperatures (Smook 2016). Likewise, alkaline conditions (pH 9) lowered the specific energy relative to pH 7, with reductions of approximately 3.0 to 13.6% at 40 °C, consistent with the known effects of alkali on fiber swelling (Bajpai 1999; Kenealy and Jeffries 2003) and weakening of inter-fiber hydrogen bonding.

Enzymatic pre-treatment further reduced the specific energy requirement relative to the corresponding untreated control at 40 °C and pH 9. Under normal-gap conditions, Cell E-A reduced energy consumption by 3.7 to 13.0%, whereas Cell E-B achieved 7.7 to 16.3% reductions. Under narrow-gap conditions, the corresponding reductions were 3.9 to 16.2% for Cell E-A and 7.5 to 19.4% for Cell E-B. Statistical testing confirmed that these decreases were significant across all furnishes and for both gap settings. In pairwise, two-sided t-tests versus the untreated control with Holm adjustment, all enzyme-versus-control contrasts remained significant (adjusted $p < 0.05$; see Table 4). Consistent with these results, Tukey's HSD from the omnibus ANOVA showed significant differences of the cellulase-treated means from the untreated control within each furnish/gap set. Cell E-B consistently yielded the lowest mean specific energy and was lower than Cell E-A in every furnish/gap combination.

Table 4. Cellulase Treatment versus Mechanical-Only Control at 40 °C and pH 9 (n = 5; Holm-adjusted t-tests, unit: kWh/t)

Grade	Gap	Control (40 °C, pH 9) mean ± SD	Cell E-A mean ± SD	Reduction (%)	adj. p	Cell E-B mean ± SD	Reduction (%)	adj. p
KGB	Normal	1214.0 ± 16.0	1147.0 ± 22.5	5.52	0.0009	1102.0 ± 14.4	9.23	<0.001
KGB	Narrow	1353.0 ± 21.4	1200.0 ± 20.0	11.31	<0.001	1150.0 ± 23.5	15.00	<0.001
DLK	Normal	1708.0 ± 20.2	1604.0 ± 14.3	6.09	<0.001	1553.0 ± 16.0	9.07	<0.001
DLK	Narrow	1813.0 ± 17.5	1711.0 ± 20.7	5.63	<0.001	1660.0 ± 20.6	8.44	<0.001
AOCC	Normal	1254.0 ± 16.7	1208.0 ± 20.2	3.67	0.0047	1158.0 ± 20.2	7.66	<0.001
AOCC	Narrow	1305.0 ± 15.8	1254.0 ± 14.3	3.91	0.0014	1207.0 ± 21.7	7.51	<0.001
KOCC	Normal	1503.0 ± 19.2	1308.0 ± 20.2	12.97	<0.001	1258.0 ± 20.2	16.30	<0.001
KOCC	Narrow	1622.0 ± 19.9	1359.0 ± 19.2	16.21	<0.001	1308.0 ± 20.2	19.36	<0.001

The energy savings can be attributable to enzymatic pre-weakening of fiber-to-fiber bonding and partial hydrolysis of fiber surface components, which reduce the mechanical force required for fiber separation. In particular, the wet-strength-targeted formulation Cell E-B appears to have been more effective than Cell E-A in lowering the resistance to defibration. This interpretation is consistent with the proposed role of enzyme-assisted weakening of wet-strength-related bonding barriers (Malachowska 2024), as well as the reduction in mechanical work expected when interfiber bonding is disrupted prior to refining (Hubbe et al. 2007). In addition, partial hydrolysis of cellulose by endo- and exo-cellulases (Fig. 2) may increase fiber flexibility and decrease fiber stiffness, thereby facilitating repulping at lower energy input.

From a process-energy standpoint, the cellulase pretreatment therefore provided a clear and statistically significant advantage, although the magnitude of the benefit depended on the furnish. The strongest reductions were observed for KOCC and KGB, especially under narrow-gap refining, while AOCC showed a smaller but still significant response. Accordingly, combining enzymatic pretreatment with moderate mechanical refining offers a practical route to lowering repulping energy demand while maintaining effective fiber liberation. These findings support the potential of cellulase-assisted repulping as an operationally and environmentally beneficial strategy for difficult recycled fiber streams.

Flake Contents

Microscopic observations in Fig. 7 visually illustrate the presence of flakes (aggregates of incompletely dispersed fibers) left after repulping AOCC. The dense, dark and irregularly shaped regions represents compact fiber bundles that had not been fully separated. These flake structures contrast clearly with the surrounding well-dispersed fibers, which appear as thinner, more elongated, and individually distinguishable elements. The flakes are characterized by their larger size, non-uniform morphology, and reduced light transmission, giving them a more opaque appearance compared to the more translucent, individualized fibers. In some regions, partially disintegrated flakes can also be observed, where fiber edges begin to loosen, indicating intermediate stages of defibrillation.

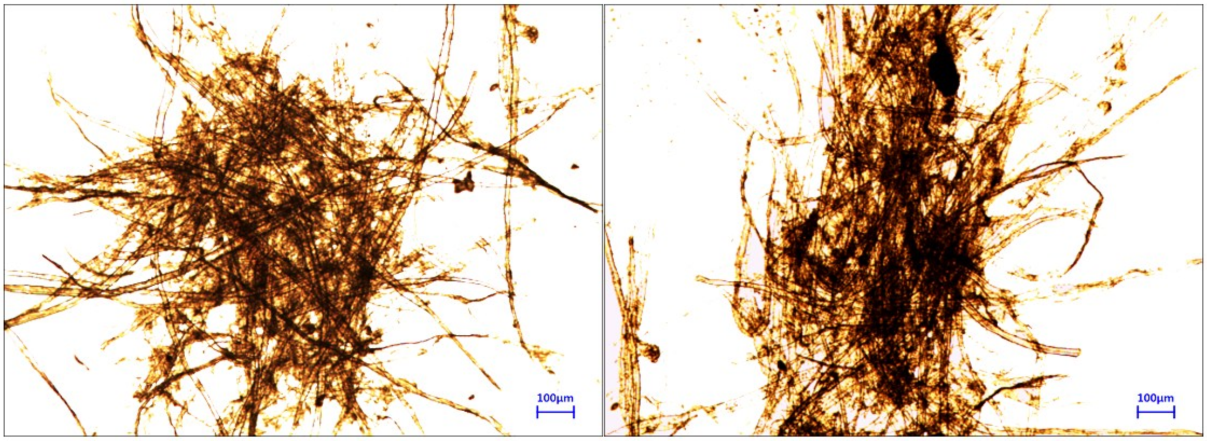
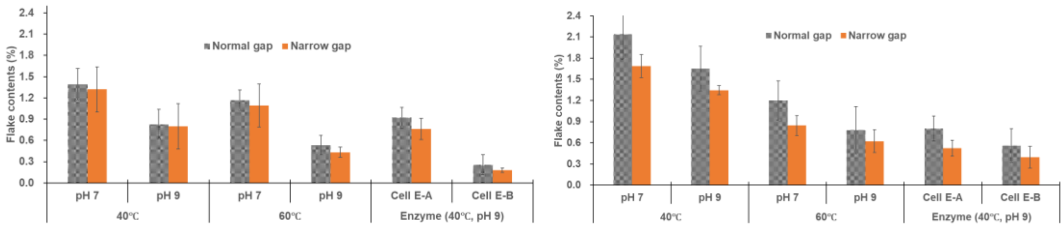


Fig. 7. Microscopic images of AOCC pulp after refining showing residual, incompletely dispersed fiber flakes and highlighted partially disintegrated regions

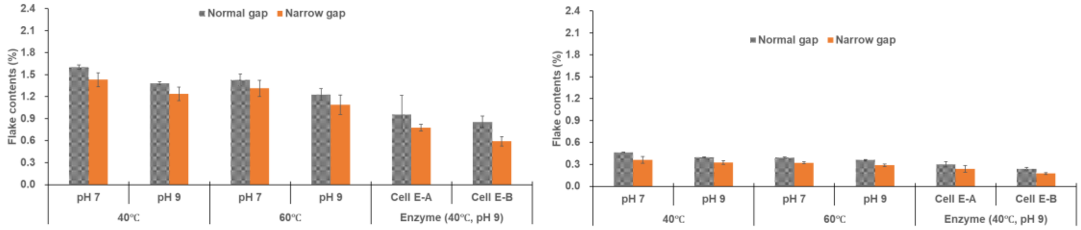
These flakes serve as a direct visual indicator of repulping efficiency. A higher concentration of flakes signifies insufficient fiber liberation, which can lead to significant operational challenges. From an industrial perspective, the persistence of these aggregates often results in "fish-eyes" or spots on the final paper web, severely compromising the product's aesthetic and structural uniformity. Furthermore, excessive flakes can cause frequent wet-end breaks on the paper machine and increase the load on subsequent screening and cleaning stages, leading to higher reject rates and material loss.

By minimizing flake content, mills can achieve a more homogenous pulp suspension, which ensures better formation and improved strength of paper.

Figure 8 presents the flake content (% by weight) of four wastepaper grades (KGB, DLK, AOCC, and KOCC) after repulping under various conditions, including mechanical gap settings (normal and narrow), pH levels (7 and 9), temperatures (40 °C and 60 °C), and enzyme treatments (Cell E-A and Cell E-B at 40 °C, pH 9).



(a) KGB **(b)** DLK



(c) AOCC **(d)** KOCC

Fig. 8. Effect of mechanical gap setting and enzyme treatment on flake contents of various wastepaper grades: (a) KGB, (b) DLK, (c) AOCC, and (d) KOCC

Across all wastepaper grades, narrow gap settings consistently reduced flake content (Smook 2016) compared to normal gap conditions. For instance, in AOCC, flake content decreased from approximately 1.6% under normal gap conditions (40 °C, pH 7) to about 1.4% under narrow gap conditions (approximately 12.5% reduction). This is attributed to the intensified mechanical shear forces that promote fiber bundle disintegration and more effectively break the interfibrillar bonds held together by wet-strength resins and sizing agents. However, as confirmed in Fig. 6, narrow gap settings increase energy consumption by 30 to 45%, necessitating a balance between flake reduction and energy efficiency.

Alkaline conditions (pH 9) and elevated temperature (60 °C) further reduced flake content compared to neutral pH (7) and lower temperature (40 °C) (Nazhad 2005). This occurs because alkaline conditions promote fiber swelling, weakening hydrogen bonds between fibers, while higher temperatures increase fiber flexibility and facilitate water penetration, thereby enhancing fiber separation. These results align with the trend of reduced freeness observed in Fig. 1, suggesting that increased fiber swelling and internal fibrillation improve fiber bundle separation.

The most significant reduction in flake content occurred with enzymatic treatment, particularly with the wet-strength specific formulation Cell E-B. In grades with high wet-strength content, such as KGB and AOCC, Cell E-B treatment reduced flake content by 40 to 50% compared to mechanical controls. For example, in AOCC under narrow-gap conditions (60°C, pH 9), flake content decreased from approximately 0.9% in the mechanical control to about 0.5% after Cell E-B treatment (approximately 44% reduction). This dramatic reduction is attributed to the selective hydrolysis by Cell E-B enzyme of covalent bonds formed by polyamide-epichlorohydrin (PAE) resins and alkyl ketene dimer (AKD)/alkenyl succinic anhydride (ASA) sizing agents, chemical bonds that cannot be efficiently dismantled by mechanical treatment alone.

As illustrated in Fig. 3, Cell E-B As illustrated in Fig. 3, Cell E-B facilitates the release of fibers from the resin matrix indirectly. Rather than directly cleaving amide or ester linkages, the enzyme hydrolyzes the cellulose substrate, which disrupts the anchoring points of wet-strength and sizing agents. This structural weakening leads to the observed degradation of wet-strength and sizing effects during mechanical repulping. This enzymatic action facilitates fiber separation, increases fiber swelling, and dramatically reduces the proportion of incompletely defibrated particles. Additionally, the synergistic action of endo-cellulases and exo-cellulases presented in Fig. 2 cleaves β-1,4-glycosidic bonds in the amorphous regions of cellulose, inducing fiber loosening and internal fibrillation, which further promotes complete separation of fiber bundles.

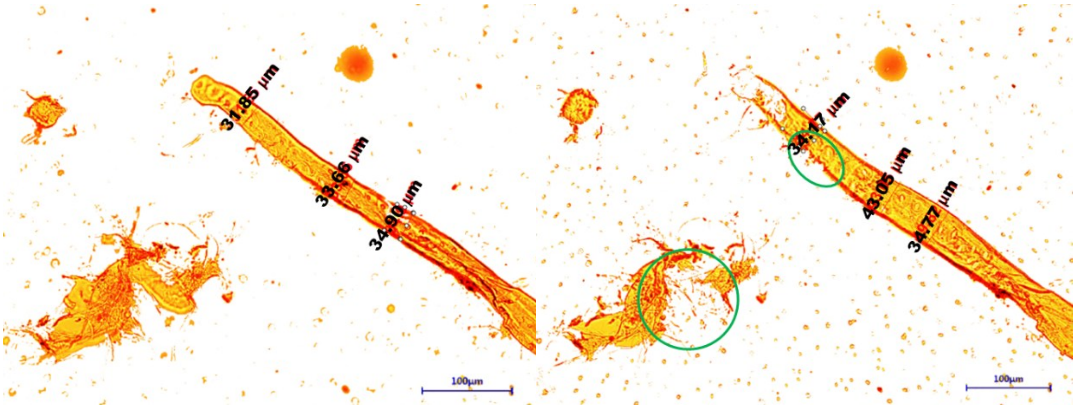
In contrast, KOCC, which has low or no wet strength, showed relatively limited flake reduction from enzymatic treatment (10 to 15% reduction). As shown in Table 3, KOCC’s wet tensile strength is 0.0 kN/m, indicating the absence of wet-strength resins and, therefore, a lack of the chemical substrate for Cell E-B’s hydrolytic activity (Jahan 2016; Bajul-Baradon *et al.* 2023). This demonstrates that the enzyme’s benefit is specific to its ability to target chemical bonding agents rather than inherent fiber properties.

Cell E-A (general cellulase) also showed some effectiveness in flake reduction (20 to 30% reduction), but was not as effective as Cell E-B. This confirms that specialized enzyme formulations are essential for maximizing efficiency in treating high wet-strength wastepaper. The combination of enzymatic treatment with appropriate mechanical refining conditions (*e.g.*, 60 °C, narrow gap, pH 9) achieved the lowest flake content, ensuring high-quality recycled pulp suitable for printing and writing paper applications.

The reduction in flake content extends beyond improving aesthetic quality, leading to enhanced printability, greater uniformity in paper structure, and improved strength properties of the final product. Therefore, enzymatic treatment, particularly the application of Cell E-B, provides a practical and environmentally friendly strategy that significantly enhances the recycling efficiency of wastepaper grades containing high levels of wet-strength resins and sizing agents.

Morphological Change of Fibers

The microscopic images in Fig. 9, captured at 100× magnification, visually illustrate the dimensional and surface morphological changes of representative pulp fibers before and after a 20-min treatment with Cell E-B enzyme. These observations were intended as qualitative support for the quantitative results (reduced freeness, lower energy consumption), rather than as standalone statistical evidence. In the image taken prior to enzymatic reaction (Fig. 9a), fiber widths were measured at approximately 32 to 35 μm, and the fiber walls appear smooth and largely intact, with little evidence of surface disruption. This represents the typical morphology of untreated fibers, suggesting that interfibrillar bonds maintained by wet-strength resins and sizing agents remain strongly intact.



(a) (b)

Fig. 9. Microscopic comparison of representative fiber morphology showing dimensional increase and surface fibrillation during Cell E-B enzymatic treatment for 20 min (a) untreated fiber and (b) treated fiber

Following 20 min of Cell E-B treatment (Fig. 9b), an overall trend of dimensional expansion was observed. In the analyzed fiber sections, widths increased to approximately 43 μm. This noted increase in width suggests fiber wall swelling and loosening induced by enzymatic activity, consistent with mechanisms described by Hubbe *et al.* (2007). These morphological changes correlate strongly with the freeness results in Fig. 1, the increased water-holding capacity and reduced drainage rate are physical manifestations of interlaminar delamination and the hydrolysis of amorphous cellulose regions within the fiber wall.

Beyond dimensional changes, the enzymatic treatment induced pronounced external fibrillation. As highlighted by the green circles in Fig. 9b, the fiber walls exhibited distinct disruption, fraying, and the emergence of fine fibrils, in contrast to the smooth surfaces of the untreated control. This phenomenon is driven by the synergistic action of endo- and exo-cellulases; endo-cellulases cleave β-1,4-glycosidic bonds to create new chain ends, while exo-cellulases subsequently trim these chains, leading to localized wall degradation and material removal (Bhat 2000).

These comprehensive structural modifications provide a unified mechanistic understanding of the multifaceted benefits reported in previous sections. The targeted swelling and surface disruption facilitate the liberation of individual fibers from tightly bound fiber bundles, thereby reducing the occurrence of unprocessed flakes (Fig. 8). Furthermore, by weakening the chemical and physical barriers of the representative fiber wall, the enzyme enables efficient defibration with preserved fiber length (Fig. 4) and controlled fines generation (Fig. 5), ultimately resulting in the significant energy savings documented in Fig. 6.

CONCLUSIONS

1. The comprehensive and combined evaluation of mechanical and enzymatic treatments on various recycled wastepaper grades revealed key insights into repulping process optimization. The refinement gap setting was found to strongly influence fiber properties, with narrower gaps promoting more intense fiber swelling and disintegration, as evidenced by lower freeness and reduced flake content, albeit at increased energy demands.
2. Enzyme application especially Cell E-B, a cellulase-based formulation tailored for causing swelling and limited fibrillation from partial hydrolysis of wastepapers containing wet-strength/sizing additives indirectly disturbing these bonds during mechanical pulping, enhanced repulping efficiency by modifying and weakening the fiber surface rather than directly degrading those additives, thereby improving fiber liberation with minimal fiber shortening or excess fines.
3. Microscopic observations supported these effects by showing representative fiber wall swelling for about 25 to 30% after enzymatic treatment. These synergistic mechanical and enzymatic effects contribute to more efficient fiber processing, lower energy consumption, and improved pulp quality.
4. Ultimately, tailoring enzyme formulations to the specific chemical composition of wastepaper, combined with optimized mechanical refining conditions, can substantially advance the sustainability and effectiveness of recycled fiber utilization in papermaking. The findings contribute to bridging the gap between laboratory-scale enzymatic potential and practical implementation in industrial recycling processes.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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