A Systematic Review on Enzymatic Refining of Recycled Fibers: A Potential to be Unlocked

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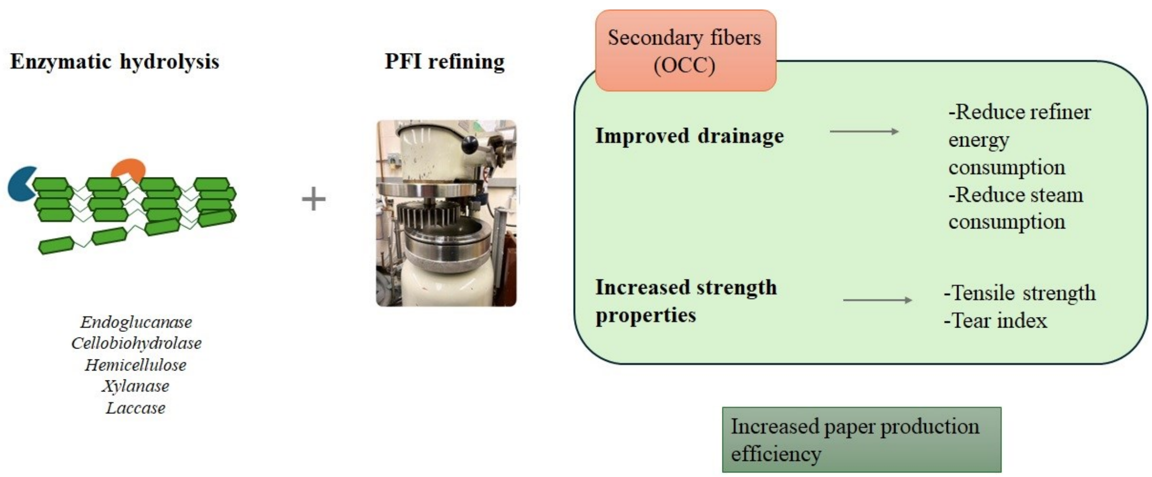
GRAPHICAL ABSTRACT



A Systematic Review on Enzymatic Refining of Recycled Fibers: A Potential to be Unlocked

Chiara Rossi ,* and Aina Solé

Enzymatic refining offers a sustainable alternative to mechanical refining for enhancing the quality of recycled paper fibers. This review examines (a) the benefits and limitations of enzymatic refining and (b) the most commonly used enzymes and their effectiveness. Studies from 2008 to 2023 were systematically analyzed using PRISMA screening to assess enzyme types, energy savings, and paper property improvements. Findings indicate that enzymatic refining reduces energy consumption by up to 20% while improving fiber bonding and drainage. Cellulases and hemicellulases are the most effective enzymes, enhancing mechanical strength and reducing water use. However, enzymatic refining alone is often insufficient, requiring additional mechanical refining for optimal results. Industrial adoption of enzymatic refining remains limited due to challenges in process integration and reaction optimization. This study highlights the role of this kind of refining in advancing circular economy goals and emphasizes future research needs, including enzyme formulation optimization and the development of scalable, one-step refining solutions.

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Keywords: Systematic review; Enzymes; Refining; Recycled paper; Bioenergy; Cellulases

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INTRODUCTION

Recycling is a necessity and an important part of sustainability in the modern world. In 2021, 71.4% of paper was recycled in Europe. One of the materials that are more linked to recycling processes is paper, since it is a material originally manufactured from textile wastes (Čabalová *et al.* 2011). The most recent data indicates a willingness of the entire society in contributing to sustain and maintain this achievement. These results have been possible owing to the sharing of better practices and the development of cross-sectional alliances promoted by the paper industry. A good example of this strategy is the “4ever green” initiative, a cross-industry alliance promoted by Confederation of European Paper Industries (CEPI) with the aim to represent the entire lifecycle of the paper-based materials. The goal is to reach a 90% recycling rate of fiber-based products by 2030 (Home - 4evergreen, 2025).

The use of recycled paper allows for significant environmental benefits, since it involves a remarkable reduction in carbon dioxide emissions ranging from 20% to 50% (SuFu | Tech-Enhanced Climate Consultancy, 2025) in comparison to paper derived from virgin fibers. On the other hand, in the last decade, the paper industry has faced increased scrutiny due to growing concerns about its water usage and the need for sustainable practices, both relative to recycled and non-recycled paper production. Nevertheless, papermaking based on secondary fibers generally reduces the need for water resources (TAPPI 2022; Naithani and Kathirvelu 2010).

Despite the benefits of paper recycling, technical challenges hinder the performance and competitiveness of recycled paper. Research has focused on understanding the loss of swelling capabilities and flexibilities in secondary fibers, which primarily attributed to hornification—a process identified in 1992 that stiffens the polymer structure of cellulosic materials upon drying or water removal (Čabalová *et al.* 2011). This phenomenon reduces fiber flexibility substantially, a key factor in paper strength.

Refining is a key process in recycling that enhances the binding ability of both virgin and secondary fibers, helping to counteract performance losses, though it cannot fully reverse recycling effects. Enzymes as refining agents can further improve the strength properties of recycled paper while reducing energy consumption, making the process more sustainable and efficient (Bajpai *et al.* 2023).

The papermaking process, particularly mechanical refining, is highly energy-intensive, with energy consumption accounting for 18% to 25% of total manufacturing costs in the global pulp and paper industry (Bajpai 2011; Verma *et al.* 2016). This presents both economic and environmental challenges, especially amid rising energy prices and growing pressure to reduce carbon emissions. Enzymatic refining has emerged as a promising alternative or complementary technology, with the potential to reduce energy demands while improving the functional properties of recycled fibers. These benefits make enzymes an attractive tool in the transition toward more sustainable and efficient paper production systems.

Cellulases and hemicellulases are widely used in secondary pulps to enhance drainability by reducing the levels of gel-like polysaccharides on fiber surfaces without increasing fines (Verma *et al*. 2010). While enzymatic hydrolysis can improve drainability, a balance is needed to retain enough fines for proper interfiber bonding. Studies by Verma *et al*. (2010) have shown that applying enzymes before mechanical refining enhances fiber recovery, with cellulase-5 significantly improving refining efficiency (Tang *et al*. 2012). Additionally, commercial enzymatic mixtures such as Fiberzyme LBR® have been reported to reduce energy consumption by 24% during refining (Torres *et al*. 2012).

Amylases also improve secondary pulp treatment by removing fines and impurities, enhancing the mechanical strength of Old Corrugated Cardboard (OCC) fibers (Torres *et al*. 2012). At the microscopic level, enzymatic treatments primarily affect fiber surfaces. Xylanase, for example, causes a peeling effect, making treated fibers rougher compared to the smoother surface of untreated pulp because of the detachment of flakes and filaments from enzymatic hydrolysis (Salgueiro *et al.* 2016).

Enzymatic biotechnology enhances fiber swelling when applied before mechanical refining, leading to potential energy savings, and improved physical properties in recycled paper. Despite its benefits, large-scale enzymatic applications in papermaking have only been in use since 1986 (Demuner *et al*. 2011). For widespread adoption, enzymatic treatments must demonstrate consistent and significant industrial impact, balancing efficacy with cost-effectiveness.

The implementation of enzymatic treatments in pulp and paper faces technical challenges, including the need to control parameters such as pH and chemical additions, as well as the financial investment required for infrastructure modifications (Kenealy and Jeffries 2003). Adoption is easier when enzymes can be integrated into existing processes. However, a lack of comprehensive biochemical understanding and the variability in fiber response to enzymes—mechanical fibers being more resistant to cellulase than chemical fibers necessitate further research (Kenealy and Jeffries, 2003). Restoring bond strength in recycled fibers is essential, particularly when additives like calcium carbonate are present, making alkaline cellulases more effective (Kenealy and Jeffries 2003).

Initially, enzyme applications relied on discovering new microorganisms, but recombinant DNA technology now enables cloning and modifying enzymes for specific pH or temperature conditions. Techniques including random mutagenesis and active site modifications further enhance the enzymatic efficiency. Ongoing research continues to optimize enzymatic reactions, with advancements such as enzyme immobilization thereby improving stability under extreme conditions such as high temperatures or pH variations (Maghraby *et al*. 2023).

This review is intended to present an updated overview on the use of enzymes as agents that improve the strength properties of secondary fibers through the refining process. It focuses on two main research questions: (a) What are the advantages and limitations of enzymatic refining for recycled fibers? (b) Which are the most commonly used enzymes for this application and their differences in performance?

The answers to the above questions are clarified by taking notice of several aspects, such as the potential of enzymes in promoting energy savings. This distinction is particularly relevant because enzymes are expected to behave differently on secondary fibers than on virgin fibers, due to structural alterations such as hornification and increased heterogeneity resulting from the recycling process. Also, the characteristic distinction between enzymatic refining of recycled and virgin fibers were evaluated, exploring the challenges faced by the industry in implementing this technology to restore the strength properties of secondary fibers

METHODOLOGY

A systematic review was carried out on the basis of the PRISMA 2020 statement (Page *et al.* 2021). The following steps were used to guide the process.

Table 1. Key Words, Searched Databases, and Timeline of Investigation

Keywords	Database	Timeline of Investigation
[TIAB] "Recycled fibers" and beating enzymes [TIAB] Beating enzymes and "recycled paper" [TIAB] "Recycled paper" and enzymes and refining [TIAB] "Recycled pulp" and biorefining	Google Scholar	November 2022 to May 2023
"Recycled fibers" and beating enzymes “Beating enzymes” and "recycled paper" "Recycled paper" and enzymes and refining "Recycled pulp" and biorefining	Paperity Mendeley	

A methodical database search was performed. As shown in Table 1, the main interrogated databases were Google Scholar, Paperity, and Mendeley. It is worth mentioning that Google Scholar was interrogated through the keyword combinations listed in Table 1, carrying the Boolean operator TIAB (Title and Abstract), which allowed for a more stringent search based on the presence of the selected keywords in the title and abstract of the articles listed in the database.

Screening and Selection of Included Studies

A total of 1,575 articles were initially obtained based on title relevance. These were screened by abstract and alignment with study objectives, then organized and filtered using Rayyan. The inclusion criteria focused on relevance (enzymatic refining of recycled paper), publication period (2008 to 2023), peer-reviewed sources, and key challenges (technical, economic, and efficiency differences). Automated duplicate removal excluded 51 articles, and 1,167 were discarded due to irrelevance or language restrictions. No exclusions were made based on geographical origin. The selection process followed PRISMA 2020 guidelines, as depicted in Fig. 1.

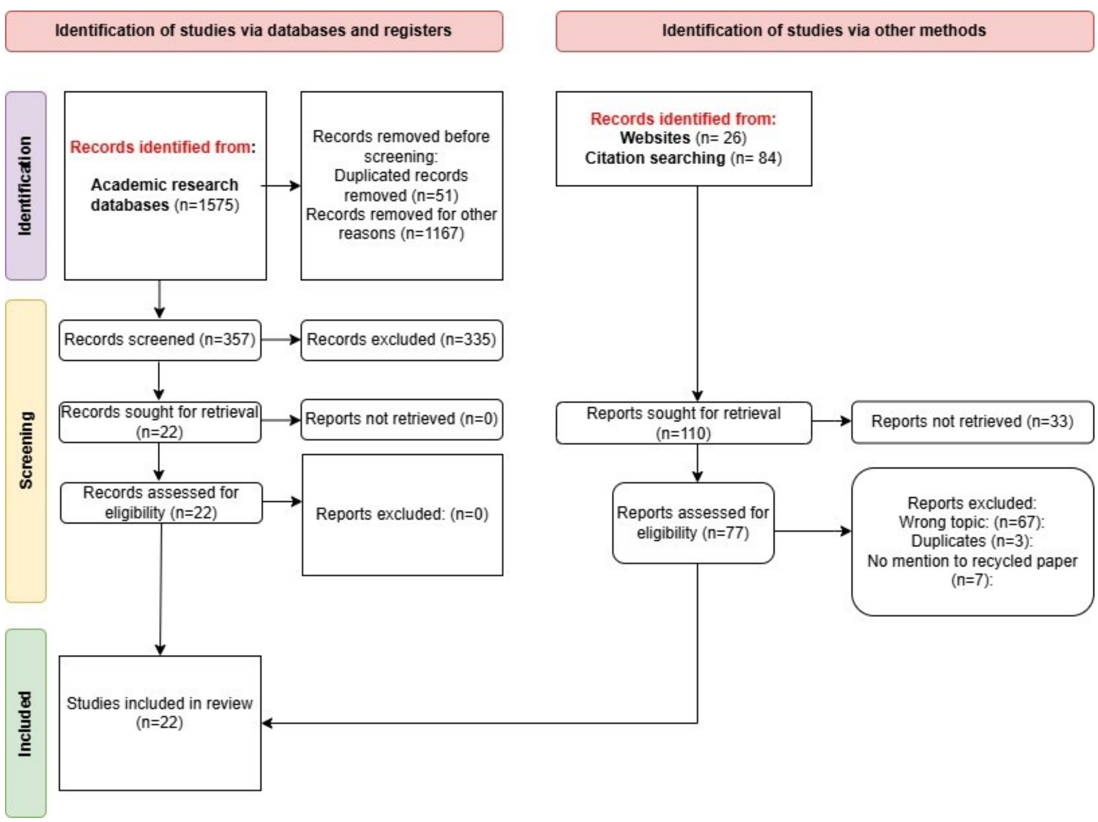

Fig. 1. The diagram summarizes the steps followed for the selection of literature sources. It includes two methods of identification: by databases and registers or *via* other methods. The whole set of references underwent a screening phase to decide whether to include it or not in the review.

Thus, a total of 1218 works were removed previously to the screening process (Identification section in Fig. 1).

Passing to the screening phase, the selection of relevant literature was conducted through the evaluation and reading of title and abstracts to obtain the body of full articles assessed for eligibility (357 papers were evaluated, see Fig. 1). Articles with abstracts/titles irrelevant to topics such as focus on recycled paper and application of the refining process to secondary fibers, were excluded. Other articles were excluded as the information reported being redundant in comparison to other sources (336 of 357). No article was excluded from this point forward, since all of them were relevant to the objective of the present review.

An additional search was conducted through identification *via* other methods (websites and citation searching, see the right section of Fig. 1). The 110 records identified by this method were all excluded from the screening step: 67 for dealing with the wrong topic, 3 for being duplicated, and 7 for containing no mention to recycled paper. At the end of the screening step, 22 articles were deemed strictly relevant to the target topic. Each record was screened independently by the two authors.

Quality Assessment and Data Collection

The quality assessment of the 22 articles selected at the end of the literature search was conducted following the indications of the QualSyst appraisal tool (Kmet *et al.* 2004). The QualSyst tool provides standardized checklists for assessing the quality of both quantitative and qualitative research studies. Each checklist comprises specific items that guide reviewers in evaluating the methodological rigor and credibility of studies. Below is Table 2 summarizing these items along with their explanations:

Table 2. Main Items Evaluated during the Quality Assessment Process of Selected Studies

Item	Quantitative Checklist Explanation	Qualitative Checklist Explanation
Research Question	Clearly stated	
Study Design	Appropriate for the research question	
Context	Clearly linked to existing knowledge	
Sampling Strategy	Detailed method	Sampling strategy of relevant literature justified
Measurement and bias control	Outcomes well defined and resistant to measurement bias	Data collection methods systematic and clearly described
Sample size / variance	Sample size appropriate. Reported variance	Not applicable
Analysis and Interpretation	Analytic methods justified Results reported in sufficient detail	Systematic data analysis
Credibility and bias control	Controlled confounding	Credibility of sources verified
Results	Reported in sufficient detail	Findings from existing literature reported in detail
Conclusions	Supported by results	Supported by findings from existing literature

The evaluation of sources was driven by applying the two different checklists proposed by the authors, differentiating qualitative from quantitative research. This procedure was applied to the full-text screening, independently by the authors. Inconsistencies were discussed until consensus was obtained. According to the proposed protocol, the minimal punctuation for a paper to be of acceptable quality was 0.55. The articles which received a punctuation of less than 0.55 from any of the authors, were excluded.

Thus, 20 articles were finally included in the review. The remaining 2 were excluded, since the average score obtained were 0.50 and 0.32, respectively. Figure 2 summarizes the quality assessment protocol.

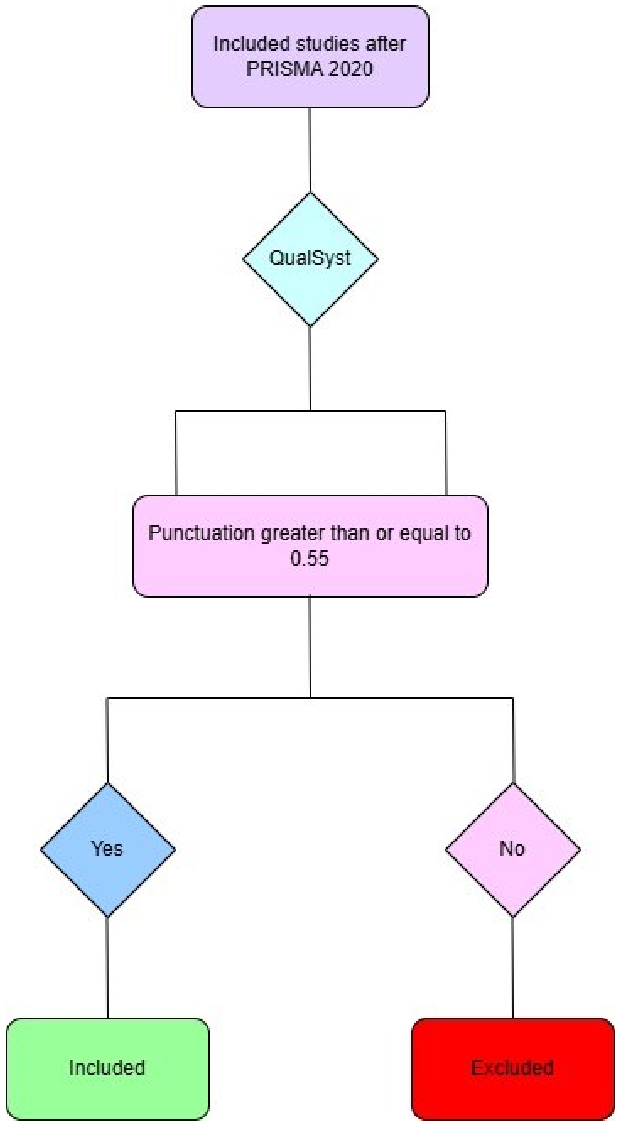

Fig. 2. The chart illustrates the quality assessment process applied to the studies selected according to PRISMA 2020. The sources were classified into quantitative and qualitative before being evaluated independently by each author.

Results from each of the included studies were independently selected and summarized by the authors on the basis of their relevance to each of the subparagraphs in the Results section of the review. Consequently, the authors jointly decided which results to include in each subsection.

Most of the reported results are qualitative, while the quantitative ones are presented in terms of percentage increase or decrease (*e.g.*, for the mechanical strength properties following an enzymatic treatment or the variations in energy consumption).

The data were reported directly from the sources without being subjected to any statistical processing or meta-analysis. The qualitative data derived from the comparison of the effects of enzymatic refining on virgin and recycled fibers have been presented in table format to facilitate visual comparison between the two applications.

On the other hand, the distribution of the selected sources over the 15-year period considered has been illustrated with a pie chart to highlight the percentage distribution of the studies across specific time intervals. It is worth mentioning that, in addition to the sources selected according to the PRISMA 2020 protocol criteria, 15 additional sources were included in the "References" section to provide contextual information (*e.g.*, the data about recycling trends in the Introduction). These works have been selected based on compliance with the following criteria: accuracy, authority, objectivity, currency, and coverage. This review was not registered, and a protocol was not prepared.

Overview of the Main Features of Selected Studies

Table 3 summarizes the key characteristics of the studies included in this systematic review, following the selection process based on the PRISMA 2020 guidelines (Page *et al.* 2021) and quality assessment protocol (Kmet *et al.* 2004).

Table 3. Summary of the Main Features of Selected Sources

Author(s)	Year of Publication	Raw Material Used	Enzyme(s) Used	Main Results
Verma *et al.*	2010	Secondary fibers (OCC)	Endoglucanase and Cellobiohydrolase	Improved drainage
Tang *et al*.	2012	Secondary fibers (OCC)	Single-component cellulase Blend of cellulases	Reduced energy consumption. Increased breaking length. Increased bursting index.
Torres *et al.*	2012	Secondary fibers	Endoglucanase Cellulase Hemicellulase	Improvement of fiber interactions. Energy reduction, Better paper properties.
Maximino *et al.*	2011	Secondary fibers (OCC, kraft liners, office paper)	Cellulases and Hemicellulases	Improved drainage
Biricik	2012	Long fiber fraction of recycled fibers (OCC)	Commercial cellulases	Increased short-span compression. Increased bursting strength.
Chen *et al.*	2012	Secondary fibers (OCC)	Laccase	Improved pulp swelling and bonding.
Si-yang *et al.*	2010	Secondary fibers (OCC)	Laccase with natural mediators	Improved fiber smoothness. Increased kappa number.

Author(s)	Year of Publication	Raw Material Used	Enzyme(s) Used	Main Results
Covarrubias and Denowski	2017	Secondary fibers	Blend of cellulases	Improved burst strength.
Singh *et al.*	2011	Secondary fibers	Cellulase, hemicellulase	Improved drainage and fiber quality.
Liu *et al.*	2012	Secondary fibers (mixed office waste)	Endoglucanase	Improved drainage
Anisa *et al.*	2022	Secondary fibers	Biocatalyst from *Carica papaya*	Increased whiteness. Reduced freeness.
Piyush Kumar *et al.*	2017	Secondary fibers	Endoglucanase from *Pycnoporus sanguineus*	Improved drainage and tensile strength.
da Silva *et al*.	2013	Secondary fibers (post-consumer cardboard scrap)	Cellulases, hemicellulases	Improved tensile and ring crush strength
Salgueiro *et al.*	2016	Bleached recycled pulp (hardwood fibers)	Endo-xylanase from *Thermomyces lanuginosus*	Improved tensile and tear strength
Delgado *et al.*	2015	Deinked secondary fibers (Old newspapers Old magazines)	Endoglucanases	Enhancement of mechanical properties without drainage reduction.
Maximino *et al.*	2013	Secondary fibers	Cellulases and hemicellulases	Improved drainability and fiber strength.
Hossein *et al.*	2015	Secondary fibers (Mixed office wastepaper)	Cellulase from *Trichoderma reesei*	Improved strength properties and brightness.
Ghosh *et al.*	2018	Recycled fibers	Cellulases and hemicellulases	Increased fiber bonding, strength, and drainage.
Bajpai	2011	Recycled and virgin fibers	Cellulases, hemicellulases, amylases, lipases	Energy savings, better drainage, and process improvements.
Verma *et al.*	2016	Secondary fibers	Endoglucanase	Improved drainage

Table 3 provides an overview of essential study features, including author details, publication year, raw material used in the study, and key findings. This summary facilitates a comprehensive comparison of the included studies and highlights their contributions to the overall synthesis of evidence.

RESULTS

This section provides an overview on the application of enzymes to the refining of secondary pulps, based on its advantages and disadvantages. Also, it relates the main enzymatic classes used for this purpose with results of case studies reported in literature, focusing both on the effects of enzymatic treatment on strength properties of recycled paper and drainage capacities of secondary pulps. In addition, it explores the evolution of biorefining, considering its combination with other treatments, such as the addition of cellulose nanofibrils to the paper pulp to enhance its mechanical resistance. Finally, general considerations on the potential of enzymes in reducing energy consumption in the recycling of paper are introduced, in order to reflect on the contribution of enzymatic refining to the circular economy, which involves maximizing the value of materials and minimizing waste.

The application of enzymes to the refining process of secondary fibers is aimed to modify the fiber quality on a molecular level, improving the effectiveness of mechanical refining on a macro level. Several years of research in the field have proved that, with each recycle, the quality of paper pulp deteriorates due to unwanted changes in the fiber properties and the establishment of a higher drainage resistances compared to virgin fibers (Torres *et al.* 2012),

As already depicted in the introduction, enzymes can be useful to partially restore the properties that make cellulosic materials suitable for paper production, such as improving its mechanical strength and drainability (Liu *et al.* 2012). However, a successful enzyme application is necessarily based on a careful selection of the right enzymes for a mill's specific furnish, process conditions, and water chemistry (Singh and Bhardwaj 2011; Jevtović *et al.* 2024).

In spite of controlling these factors, the application of enzymatic refining as a complement of the traditional mechanical process, still could be challenging. An interesting analysis of the advantages and disadvantages in using enzymes for the refining of paper pulp was conducted by Torres *et al*. (2012). Table 4 summarizes their considerations, showing, respectively, the advantages and disadvantages of enzymatic refining on virgin and recycled fibers.

Table 4. Advantages and Disadvantages of Enzymatic Refining for Virgin and Recycled Fibers

Advantages of Enzymatic Refining	
Virgin fibers	Recycled fibers
Good effectiveness on both soft and hard wood	Degradation of water-retention colloids
Low activity on cellulose	
Preferential action on lignin and hemicellulose	
Time and energy saving	
Improvement in physical properties of fibers	
Less pulping chemicals	Improved swelling

Disadvantages of Enzymatic Refining	
Virgin fibers	Recycled fibers
Selection of the suitable strain for enzyme production	
Limited selectivity of enzymes toward lignin in virgin fibers	Enzymatic and mechanical refining combination needed for optimal results
Risk of over-degradation of cellulose	Limited treatment does not contribute to interfiber binding
Decrease of pulp brightness	Optimal fibrillation harder to achieve
Long time required to delignify cell walls	Lower energy saving than for virgin fibers
Optimization of reaction parameters	

Noticeably, enzymes are used in other stages of fiber processing other than refining, such as biopulping for virgin fibers and the upgradation of deinking of secondary fibers. In biopulping, where wood chips are treated with white-rot fungi prior to mechanical pulping, the fast growth rate of fungi as a source of enzymes makes the process applicable to decrease the high energetic demand in refining (Torres *et al.* 2012). In the case of the deinking of recovered paper enzymes, it helps to reduce the use of chemicals such as NaOH, silicates, and surfactants (Torres *et al.* 2012).

In addition to the differences in strengths and weaknesses regarding the application of refining enzymes to the two types of fibers, this study detected a relevant scarcity in the production of scientific literature about the application of the enzymatic technology to the refining of secondary fibers. The data presented in Fig. 4 highlight this phenomenon, suggesting a resistance in its broadening for the treatment of recycled pulps.

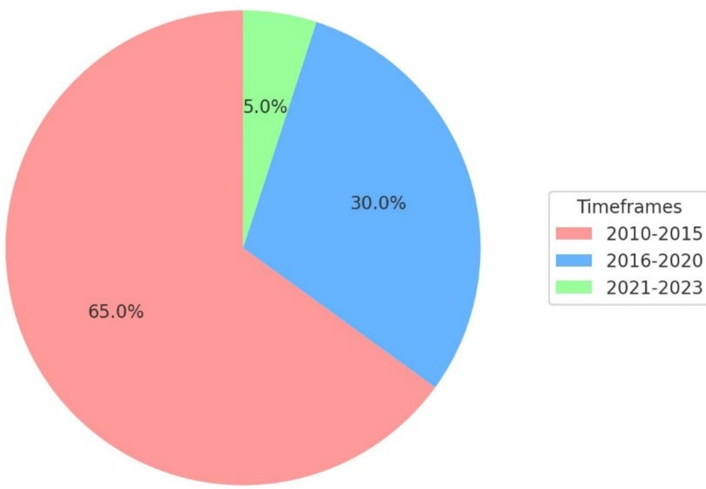

Fig. 3. The graph illustrates the distribution of the selected sources over a period of 15 years, highlighting the progressively higher lack of relevant literature on the topic over time.

Enzymatic Classes Used in Biorefining: Effects on the Strength Properties of Recycled Paper

Cellulases and hemicellulases

Cellulases and hemicellulases are key enzymes in plant biomass degradation and the carbon cycle, as they break down cellulose and hemicelluloses into soluble sugars. While cellulases degrade cellulose, hemicellulases target compounds such as xylans and glucomannans through a modular enzymatic action, where carbohydrate-binding modules facilitate interaction with insoluble polysaccharides (Wilson 2009; Yi 2021)

In the paper industry, these enzymes improve pulp drainability without compromising the mechanical properties of the paper. Pommier *et al.* (1990) demonstrated that a mixture of cellulases and hemicellulases can optimize this process (Maximino *et al.* 2011). Maximino *et al.* (2011) investigated the enzymatic treatment of secondary fibers using Pergalase 40, a commercial blend of cellulases and hemicellulases derived from *Trichoderma longibrachiatum*. Their study, conducted on recycled industrial pulp, found that treatments with 5.2 IU/g enzyme dosage, 5% pulp concentration, and 30 min of treatment time, followed by refining at 2000 revolutions in a

PFI mill, resulted in improved drainability and energy savings of approximately 36% (Maximino *et al.* 2011).

Tang *et al.* (2012) focused on the effect of cellulase enzyme treatment on energy consumption and the mechanical properties of old corrugated cardboard (OCC). Their study compared different cellulase formulations, concluding that Novozyme Fibercare D was the most effective for reducing energy consumption in refining, while Fibercare U showed better strengthening effects on paper sheets. These findings highlight the importance of selecting the appropriate enzyme for specific raw materials to achieve the best balance between strength development and energy efficiency (Biricik and Atik 2012)

Laccases

Laccases, a class of copper-containing oxidoreductase enzymes, are emerging as valuable tools in industrial applications due to their ability to oxidize a broad range of compounds (Weiss *et al*. 2023; Yadav *et al.* 2023). When combined with a mediator, they can effectively oxidize non-phenolic lignin compounds, making them useful in the treatment of recycled paper fibers. The choice of mediator significantly impacts the final paper properties (Castilla-Marroquin *et al.* 2023).

Chen *et al.* (2012) demonstrated that using 1-hydroxybenzotriazole (HBT) as a mediator with commercial OCC pulp led to the highest paper strength, increasing the carboxyl group content by 21% compared to untreated pulp. This enhanced fiber swelling, resulting in improved mechanical properties. Fourier-transform infrared spectroscopy analysis suggested that new carboxylic acid groups were formed during Laccase-HBT treatment. The study also tested alternative mediators, ABTS and VA, finding that VA improved strength properties to a lesser extent than HBT, while ABTS showed no significant benefit (Chen *et al.* 2012).

Natural mediators such as gallic acid, syringyl alcohol, and ferulic acid have also been studied in laccase-mediator systems. The OCC fibers treated with laccase alone gained auto-adhesion capacity and a rougher surface texture compared to untreated fibers (Si-Yang *et al.* 2010). Additionally, wet strength improved significantly: laccase-treated pulp increased wet ring crush strength by 23.75%, while the inclusion of natural mediators further enhanced strength by 29.9% (gallic acid), 46.0% (syringyl alcohol), and 50.6% (ferulic acid) (Si-Yang *et al.* 2010). These findings highlight the importance of selecting the appropriate laccase-mediator combination to optimize mechanical properties in recycled fiber treatments.

Enzymatic mixtures

In addition to studies focused on specific enzyme families, several investigations have explored the effects of commercial enzyme mixtures on different types of secondary fibers. Verma *et al.* (2016) demonstrated a two-stage deinked wastepaper treated with four enzymatic mixtures, each varying in endoglucanase and exoglucanase activities. Despite differences in composition, all enzyme products increased the tensile index of paper sheets with higher enzyme dosages. However, the extent of improvement depended on the dominant enzyme in the mixture. The highest increase (16%) was observed with enzyme EG-D, an engineered blend primarily composed of endoglucanases with cellobiohydrolase side activity, which also showed the best improvement in pulp drainage. Conversely, Mixture A had almost no effect on tensile strength, like Mixture B, both of which contained a multi-component preparation with higher cellobiohydrolase activity. Mixture C, which combined both endoglucanase and cellobiohydrolase activities, negatively affected pulp fiber length (Verma *et al.* 2016).

Commercial enzyme mixtures continue to evolve as research advances in optimizing formulations. One example is Buckman's Maxymize 3504, a fiber modification product designed for recycled paper. When tested on OCC pulp, it enabled similar strength properties with reduced refining and increased machine speed compared to an earlier generation Buckman product (Covarrubias and Denowski 2017).

Effect of Enzymes on the Drainage Capacity of Secondary Pulps

Recycled fibers affect pulp drainage due to their structural characteristics, such as high fibrillation, short fiber length, and the presence of fines and impurities. Compared to virgin fibers, they have lower drainage capacity, making them denser and more resistant to water removal. Enzymes such as cellulases, hemicellulases, mannanases, and amylases can enhance drainage and improve production rates (Singh Rashmi 2011).

A key enzymatic action on fibers is the "peeling effect," where enzymes remove fibrils and fiber bundles, reducing the fibers' hydrophilicity and making them easier to drain. Proper control of enzymatic reactions is essential to prevent excessive fiber shortening. The peeling effect also increases the fibers' specific surface area, improving the efficiency of subsequent processes, such as additive incorporation or further enzymatic treatments, enhancing pulp quality and paper formation (Singh Rashmi 2011; Liu *et al.* 2012)

A study in 2022 demonstrated that enzymes from *Carica papaya* latex alter pulp fiber morphology, leading to surface erosion and increased water turbidity. The treated fibers developed structures resembling micro stalagmites, which function as water storage sites. The use of this bioactivator reduced pulp freeness and decreased the average water content by up to 2.89%. These effects align with those observed in enzymatic cellulose treatments, where breaking down larger molecules reduces viscosity, improving water flow, and drainage (Anisa *et al.* 2022),

Endoglucanases offer another enzymatic approach for improving pulp drainage. They specifically target the amorphous regions of cellulose, cleaving β-1,4-glycosidic bonds and producing new chain ends for further hydrolysis by cellobiohydrolases. Endoglucanases enhance pulp freeness without negatively affecting fiber properties (Nagl *et al.* 2021). Verma *et al*. (2010) investigated an alkali-stable endoglucanase from *Pycnoporus sanguineus* NFCCI-3628, which significantly improved pulp drainability by 9% to 14% and enhanced paper properties such as tensile index and smoothness. The study focused on selectively hydrolyzing ultra-fines to optimize drainage rates and paper production efficiency (Verma *et al.* 2010).

A related study published in 2017 further confirmed that the alkali-stable endoglucanase from *Pycnoporus sanguineus* improved drainability by 9% to 14%. The enzyme also enhanced paper properties, likely due to the hydrolysis of lower molecular weight carbohydrates and partial degradation of cellulose's crystalline structure, facilitating additional hydrogen bonding between cellulose and water molecules (Kumar *et al.* 2021).

Effects of Combined Treatments on the Properties of Secondary Fibers

Significant results have been reported when combining enzymatic treatments with non-enzymatic methods to enhance the properties of recycled paper. One study investigated the impact of refining and ultrasonication on pulps derived from post-consumer cardboard scrap before enzymatic treatment with cellulase, hemicellulase, or a mixture of both. The PFI mill refinings (0, 400, 800, and 1200 revolutions) and ultrasound (0, 10, 20, and 30 min) were applied prior to enzymatic treatments (da Silva *et al.* 2013). The combination of mechanical refining with enzymatic treatments resulted in higher drainage resistance compared to untreated pulp, while ultrasonication with enzymatic treatments had a milder effect. The PFI mill refining combined with enzymes significantly improved interfiber bonding properties, such as tensile index and ring crush strength. In contrast, ultrasonication with enzymatic treatments enhanced properties related to fiber strength, such as tear index and bulk (da Silva *et al.* 2013).

Another successful approach involved an endoxylanase from *Thermomyces lanuginosus*, applied after an ultra-high hydrostatic pressure (UHP) pre-treatment at 300–600 MPa for 10 min. The UHP pre-treatment improved enzymatic action by increasing fiber accessibility, enhancing xylan hydrolysis by 10 to 20%. This led to up to a 30% increase in strength properties, particularly tensile strength and burst resistance (Salgueiro *et al.* 2016).

Additionally, the combination of biorefining with cellulose nanofibers (CNF) addition positively affected final paper performance. A study examined the application of endo-β-1,4-glucanase on fibers derived from a blend of recovered materials, followed by CNF addition (0–3 wt%). Increasing CNF content improved all mechanical properties of the paper. With 3 wt% CNF, breaking length increased by 21%, while Young's modulus and Scott Bond improved by 55%. These enhancements made the paper suitable for high-performance applications (Delgado-Aguilar *et al.* 2015).

Factors That Influence the Efficiencies of Enzymatic Refining of Secondary Fibers

One of the key factors influencing the enzymatic pretreatment of recycled fibers is the timing of enzyme application within the process sequence—specifically, whether the enzyme is added before or after mechanical refining—as this significantly affects the resulting paper properties. A study by Maximino *et al.* (2013) investigated this effect on unbleached industrial pulp, both with and without refining. The recycled pulp composition included 56.5% OCC, 37.5% kraft liner papers, and 6% printing and writing papers. Statistical analysis revealed that variations in pulp consistency and enzyme (Pergalase 40) dosage had opposite effects on drainability, depending on whether the enzyme was applied before or after mechanical refining. When applied to unrefined pulp, the enzyme improved drainability, particularly under conditions of 10% pulp consistency and 5.2 IU/g enzyme dosage (Maximino *et al.* 2013).

However, when enzymatic treatment was followed by mechanical refining in a PFI mill, the pulp exhibited increased apparent sheet density, tensile index, and fines content. Conversely, applying the enzyme after industrial refining did not improve drainability or bonding. Refining this pulp further in a PFI mill even reduced the tensile index compared to untreated pulp. It was hypothesized that, in this case, the enzyme negatively affected fiber surfaces, weakening inter-fiber bonding. This suggests that enzymatic treatment enhances drainability and tensile strength when applied before industrial-scale mechanical refining (Maximino *et al.* 2013).

Supporting this, a 2015 study by Hossein *et al.* (2015) explored different sequences of enzymatic and mechanical treatments. Their study used a recycled fiber blend of 70% waste office paper and 30% book paper, repulped under controlled conditions (30 min, 10% consistency, 40 °C). Two treatment methods were compared:

enzymatic treatment followed by refining before deinking (ERD method) and refining followed by enzymatic treatment before deinking (RED method). It was expected that the RED method would generate more fines due to primary refining, which would then be removed *via* enzymatic hydrolysis, improving drainage. These results confirmed not only improved drainage but also enhanced strength and optical properties, highlighting the complex effects of different fines on paper properties. However, applying refining after enzymatic treatment (ERD method) negatively impacted fiber properties and flotation efficiency for ink removal.

These findings emphasize the importance of the sequence in which enzymatic and mechanical refinings are applied to optimize paper properties (Hossein *et al.* 2015). Further research by Ghosh *et al.* (2018) examined how pH affects the enzymatic refining of OCC pulp using the commercial enzyme mixture “Enzyme A”. The study tested whether increasing pH from 7.5 to 10 during enzymatic refining would improve final paper properties. Using a disc refiner to achieve realistic conditions, they found that enzyme treatment improved fiber reswelling and increased paper strength at a given refining energy. However, raising the pH to 10 had no significant effect compared to treatment at pH 7.5. This indicates that while many factors influence the enzymatic refining, pH adjustments do not always yield benefits and must be carefully considered based on pulp composition (Ghosh *et al.* 2018).

Overall, despite the process variability, extensive literature supports the application of enzymes in industrial refining of recycled pulp, as their effects are generally positive. However, enzymatic reactions must be carefully monitored and adapted to specific processes and fiber compositions to ensure optimal results.

Potential of Enzymes in Reducing Energy Consumption in Recycled Paper Manufacturing

Energy consumption accounts for 18% to 25% of manufacturing costs in the global pulp and paper industry, making it a crucial concern due to rising energy prices and diminishing fossil fuel reserves (Bajpai 2011; Verma *et al.* 2016). Refining is essential for optimizing paper-making properties, particularly for recycled fibers, as it helps counteract the weakening effects of repeated recycling cycles (Verma *et al.* 2016; Kumar *et al.* 2021).

The use of enzymes in refining recycled fibers offers a promising strategy for reducing energy consumption and improving the sustainability of the paper manufacturing process. Several case studies, as follows, highlight the impact of enzymatic treatments on energy efficiency.

A 4 kWh/T reduction in refiner load was observed when comparing Buckman’s Maximyze 3504 to a second-generation enzymatic mixture from the same company (Covarrubias and Denowski 2017). A study on cellulase/hemicellulase-assisted refining of bleached and unbleached mixed pulp reported a 15 to 20% reduction in refining energy, with greater effectiveness in bleached pulp (Yi 2021).

FiberCare® D, an endoglucanase-rich cellulase enzyme developed by Novozymes, has been shown to enhance the runnability of recycled raw materials and reduce steam consumption for drying when applied after mechanical refining (Kumar *et al.* 2021). Researchers at La Cellulose du Pin demonstrated that a cellulase-hemicellulase mixture improved the freeness of recycled pulps, enhancing drainage and allowing for higher paper machine speeds, leading to notable energy and cost savings (Bajpai 2011).

Despite these benefits, enzymatic refining of recycled pulp requires careful control of reaction parameters. Pulp consistency significantly affects energy administration; for example, at 45 ºC, enzymatic treatment at 5% pulp consistency requires 110% more heat energy than at 10% pulp consistency, impacting implementation costs (Maximino *et al.* 2011).

Although enzymatic refining effectively reduces energy consumption for recycled fibers, its impact is even more pronounced in virgin fiber refining. A study on northern bleached softwood kraft pulp found that cellulase pretreatment, particularly with cellulose-binding domains (CBDs), reduced refining energy by 13% to 24%. In contrast, cellulases without CBDs were less effective in fiber adsorption and hydrolysis. These energy savings surpass those reported by Kumar *et al.* (2021) for recycled pulp, where the maximum refining energy reduction observed was 20% (Hossein *et al.*, 2015; Kumar *et al.*, 2021). These findings highlight the substantial potential of enzyme-assisted refining in improving energy efficiency, with notable cost benefits depending on fiber type and process conditions.

DISCUSSION

This study has reviewed the state of art of enzymatic refining with a special focus on secondary fibers, with the aim to identify gaps in past research and understand both the potential and limitations of this type of technology. As mentioned in the results section, the scarcity of literature dealing with the application of enzymes to recycled pulps is striking. The lower impact that enzymes play on modification of secondary fibers in comparison to virgin fibers were identified; while trying to uncover transverse aspects of the selected studies in an attempt to explain this phenomenon.

As demonstrated by Torres *et al.* (2012) (see Table 1), enzymatic refining of secondary pulps must be combined with mechanical treatments in order to be effective. In spite of this, the energy savings achieved with the combination of enzymatic and mechanical refinings are still noticeably lower than with virgin fibers. These limitations are corroborated by other studies mentioned in this paper. Indeed, the mechanical properties of the final paper produced starting from a mixture of post-consumer pulps in the work of Maximino *et al.* (2011) were significantly improved only with the combination of enzymatic treatment and mechanical refining in a PFI mill at 2000 rev/min. Also, the positive effect on energy consumption was detected exclusively when applying both technologies. Similar findings are described for the experimentation with several enzymes from the Novozyme Fibercare family; regardless of the difference in performance, all of them required a co-treatment of pulp in a PFI mill, showing that each enzyme was able to reduce the number of revolution/min at same extend, without being able to be used as a one-step treatment for the restoring of fiber properties.

A further common point among the literature analyzed here, is the importance of controlling a long series of factors when treating secondary fibers enzymatically. Among them, the application point of a particular enzyme in the production flow could be crucial for the outcoming of the refining. For instance, the best results in terms of drainability and tensile index were obtained when the enzyme Pergalase 40 was applied to a secondary pulp followed by mechanical refining in a PFI mill, while changing the order of treatment resulted in no improvement of drainability or interfiber bonding.

However, as already mentioned, mechanical refining is not the only non-enzymatic action applied to secondary pulps in combination with enzymes. For instance, the combination of enzymes with PFI mill refining or ultrasonication targets different fiber characteristics: the former tends to be more effective in enhancing interfiber bonding, while the latter primarily increases the intrinsic strength of individual fibers. It is important to note, however, that both PFI mills and ultrasonication are primarily laboratory-scale tools used to simulate industrial refining processes. While the PFI mill has been shown to reasonably replicate the effects of industrial refiners—particularly disc refiners commonly used in paper mills—differences still exist in energy transfer, fiber interactions, and process scalability. This methodological distinction partly explains the observed scarcity of experimental data on enzymatic refining in industrial contexts. Many published studies originate from academic institutions using lab-scale equipment, while industrial-scale data—often generated within paper companies—is typically not disclosed due to confidentiality and competitive considerations. As enzyme-based processes are increasingly implemented at scale, less information becomes publicly available. Nonetheless, systematic reviews like the present one remain crucial, as they consolidate available knowledge and support the broader adoption of enzymatic treatments by identifying transferable insights and research gaps.

Based on these trends in the application of enzymes to secondary fibers among the different sources consulted, one can speculate that the progressive reduction of experimental works or reviews published about the enzymatic refining of secondary fibers could be mainly explained by the following considerations:

- To the best of our knowledge, enzymatic refining is not extensively applicable as a one-step treatment for the refining of secondary fibers at industrial scale. It still needs to be combined with other non-enzymatic methods. Some of them are based on physics such as mechanical refining and ultrasounds; others are chemical, like the addition of small amounts of CNF to the pulp suspensions as reinforcing agents.
- A large set of factors must be taken under control when applying enzymes to secondary pulps. Among them, pH value is fundamental for the action of the enzyme on fibers, especially to achieve a satisfactory level of fibrillation, interfiber bonding, and final strength in the resulting paper. Heterogeneity of the raw material is a further aspect to be considered when investigating the optimization of reaction parameters.
- The advantage in energy savings is much more evident for virgin fibers than for recycled. For instance, the enzymatic refining of chemical bleached pulps with xylanase has been related to a greater reduction in energy demand. Considering that recycled paper is often not bleached to reduce the use of hazardous chemicals and water, the reduction in energy supply is not comparable with virgin, bleached paper.
- There is no general consensus on the best enzyme to obtain a certain effect on paper fibers. Different enzymes are often used in combination, such as cellulases and hemicellulases, in order to achieve specific properties in the final paper.

However, in light of the reviewed literature, the authors conclude that the most effective enzyme preparations for enzyme-based refining of recycled fibers are those composed of cellulases and hemicellulases, particularly in commercial blends tailored for pulp treatment. Among these, endoglucanase-rich formulations such as Novozyme's FiberCare D and Buckman's Maximyze 3504 have shown superior performance in enhancing drainage and mechanical properties while enabling energy savings of up to 24%.

These enzymes act selectively on fiber surfaces, facilitating fibrillation and fiber bonding without excessive fines generation. Hemicellulases, especially xylanases, contribute to the degradation of hemicellulosic compounds that hinder drainage, further optimizing pulp processability. Laccases, particularly in combination with appropriate mediators like HBT or natural compounds such as syringyl alcohol, also exhibit promise in improving fiber bonding and wet strength through oxidative mechanisms.

The most efficient outcomes have consistently been reported when enzymatic treatments are applied prior to mechanical refining, underscoring the synergistic effect of enzyme-mechanical co-treatments. Therefore, the recommended approach for industrial application involves tailored enzyme blends—primarily based on endoglucanases, hemicellulases, and selectively used laccases—applied in combination with mechanical refining under optimized process conditions to ensure both fiber quality enhancement and energy efficiency.

CONCLUSIONS AND PERSPECTIVES

In conclusion, the use of enzymes in the refining of recycled paper offers several significant advantages.

1. Enzymes can enhance the efficiency of the pulping process leading to higher-quality fibers and improved pulp yield. This biotechnological approach reduces the need for harsh chemicals, making the process more environmentally friendly.
2. The use of enzymes in processing recycled pulp also may be cost-effective, if the right combination enzyme-furnish is found and a good optimization of the reaction parameters is achieved. Additionally, enzymatic treatments can help to minimize energy consumption and water usage, aligning with sustainable practices in the paper industry.
3. Overall, integrating enzymes into recycled paper refining represents a promising strategy for advancing both environmental sustainability and product quality in the paper manufacturing process. A further advantage of enzymatic refining is its milder effect on fibers compared to mechanical treatment, achieving the softening and selective modification of fiber components. However, further research is needed to reduce the current limitations of biorefining, such as the need for continuous optimization of process parameters, its dependence on auxiliary mechanical treatments, and the relatively limited benefit in terms of energy savings for recycled fibers. In addition, attention must be paid to the potential for fiber damage caused by excessive enzymatic activity—particularly in cases of overdosing—which may lead to over-hydrolysis, fiber weakening, and a decline in tensile strength. Another important consideration for industrial implementation is the removal or inactivation of residual enzymes after treatment, to prevent unintended downstream effects and ensure process stability. Addressing these

technical challenges through targeted research and industrial trials will be key to fully unlocking the potential of enzymatic refining in large-scale applications.

4. Promoting the investigation on molecular modeling could be a starting point to revive the interest of the scientific community on this topic, hopefully through the generation of improved enzymes that could function as a one-step treatment to refine recycled fibers in a greener way.

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