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UTILIZATION OF XYLANASE ENZYMES DERIVED FROM CASSAVA IN THE ECO-FRIENDLY BIOBLEACHING OF PULP

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ABSTRACT

The demand for paper has been increasing over time, leading to the pulp and paper industry becoming one of the largest contributors to global carbon emissions due to the chlorine-based bleaching process, particularly in Indonesia. An alternative to minimize chlorine usage involves using xylanase enzymes as part of the bleaching sequence. Xylanase can be produced from agricultural waste, including cassava residue, which contains a significant concentration of xylanase, approximately 21.3%. However, it still needs to be utilized in Indonesia. Therefore, this study aims to explore the production of xylanase enzymes from cassava residue and assess its effectiveness in the biobleaching process of pulp. The research methodology includes the production phase of xylanase enzymes by *Aspergillus niger*, chelating, bleaching sequence, bleaching, kappa number, and chemical saving assay. In the production of xylanase, the study determined that xylanase exhibits optimal activity under specific conditions, notably at a pH of 6 and a temperature of 60°C. Under these parameters, the enzymatic activity reached a level of 0.4986 U/mg protein. During the bleaching sequence, xylanase was used with doses of 0.3, 0.5, 0.7, 0.8, and 1 L/T pulp at 40, 45, 50, 55, 60, 65, and 70°C for 60 minutes. Following this process, bleaching was conducted at 65°C for 70 minutes, extraction at 80°C for 90 minutes, and a second bleaching phase. Subsequently, a kappa number test was performed, revealing the best kappa value at 60°C with a xylanase dose of 0.5 L/T pulp, reducing from an initial kappa number of 9 to 4.04. Additionally, under these conditions and dosage, xylanase enzymes could save approximately 23.67% in chlorine usage.

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INTRODUCTION

The global demand for paper has consistently risen over time, driven by continuous advancements in science and technology. The pulp and paper sector is a significant global industry, with 2020 production figures reaching 177.7 million tons from wood-derived pulp and 11.2 million tons from non-wood fiber sources. Additionally, approximately 229 million tons of waste paper were recycled, yielding around 400 million tons of diverse paper products. Given its substantial scale, this industry boasts a wide-ranging and swiftly evolving technological infrastructure. Environmental advocates closely monitor its operations due to its heavy reliance on forest and water resources, aiming to prevent environmental degradation and pollution [1]. The world's paper requirement reaches about 200 million

tons annually and continues to grow by roughly 3.5% annually. This increasing demand can lead to several environmental issues. The adverse effects arise from using wood as the primary raw material for pulp and the pulping process, which involves using hard-to-degrade chemicals in bleaching [2].

Several pulp and paper industries in Indonesia continue to use chlorine-based chemicals in the pulp bleaching process. This preference stems from chlorine's reactive nature, its effectiveness in producing high-quality and bright pulp, and its relative cost-effectiveness. However, chlorine's toxic, mutagenic, persistent, and bio-accumulative properties pose significant environmental issues. It is estimated that chloroform formation during pulp bleaching ranges from 2.07-5.34 g/ton of pulp, with approximately 30% of this chloroform being non-degradable by activated sludge and about 97% evaporating into the air [3]. Furthermore, the bleaching process generates chlorinated organic compounds or Adsorbable Organic Halides (AOX), including chlorinated resin acids, phenolates, and dioxins. These compounds are non-biodegradable, contaminating the food chain through bioaccumulation, with dioxins known for their extreme toxicity and carcinogenicity [4].

An alternative approach to reduce chlorine use is the implementation of bio-bleaching techniques using xylanase. Xylanase can modify the structure of xylan and glucomannan in pulp fibers, enhancing the delignification efficiency of chemical bleaching agents. The use of xylanase represents an alternative to reduce the impact caused by the use of chlorine in the Kraft pulp bleaching process [3]. Moreover, using biotechnology using enzymes in the pulp and paper industry can provide a simple, cost-effective, and environmentally friendly method for improving pulp and paper quality, eliminating waste, and reducing traditional processes [5].

Xylanase is typically used with bleaching chemicals before the bleaching process. Xylanase reduces the consumption of bleaching chemicals, thereby diminishing the release of organochlorine compounds into the environment. Xylanase can decrease the consumption of bleaching chemicals by 20% to achieve the same degree of brightness as controls without xylanase [3]. Pre-bleaching of kraft pulp using xylanase has provided numerous benefits to pulp mills, such as improved environmental performance, reduced bleaching costs, increased productivity, and enhanced pulp properties. This technology has been well-received worldwide [6]. Xylanase can be isolated from various sources, including fungi, bacteria, and yeast. Among these, fungi, particularly the *Aspergillus* species, are frequently chosen for their superior ability to produce higher amounts of xylanase in growth mediums. This efficiency makes them a focal point in research and application. This enzyme is also found in agricultural waste, such as cassava residue [5].

In the industry, cassava residue can be a raw material for pulp production. It contains 21.3% hemicellulose, primarily xylan, making it a potential source for xylanase enzyme production as a bleaching agent in bio-bleaching techniques [3]. The use of other agricultural waste as a source of

xylanase for the bio-bleaching process includes corn cobs with a xylan content of 12.9%, husks with a xylan content of 6.3%, and peanut shells with a xylan content of 6.3%. In the cassava dregs we examined, we found that the xylan content was 8%. Thus, cassava dregs are quite effective and efficient for bio-bleaching pulp [7]. Indonesia, known for its large-scale cassava production, reports an annual output of about 2.6 million tons, which continues to rise significantly [8]. However, the rapid decomposition of cassava industry waste leads to environmental pollution due to its potent smell [9]. Currently, cassava residues have not been utilized optimally, leading to environmental pollution concerns when discarded into rivers. Remarkably, these residues contain significant amounts of xylan, which can be hydrolyzed to produce xylooligosaccharides (XOS), a valuable functional food with considerable economic potential. Its market price remains high due to the utilization of commercial substrates [4]. Furthermore, according to Government Regulation No. 101 of 2014 concerning Hazardous and Toxic Waste (B3) Management, B3 waste is defined as the residue of a B3 business or activity which, due to its nature, concentration, or amount, either directly or indirectly, can pollute or damage the environment, or endanger the environment, health, and survival of humans and other living creatures [10]. Therefore, this research aims to explore the process of producing xylanase enzymes from cassava pulp and assess their effectiveness in the bio-bleaching process of pulp, ultimately addressing waste issues in both the agricultural and pulp and paper industries.

METHODS

1. Sample Preparation

The sample preparation involved thoroughly cleaning the cassava residue by rinsing it repeatedly under running water to separate the starch from the pulp. Chemically, cassava residue comprises 1.57 g of protein, 1.06 g of fat, 21.10 g of fiber, and 1.10 g of ash. The fiber in lignocellulosic material contains cellulose (36.6%), hemicellulose (21.3%), and lignin (17.3%). Additionally, the water content of cassava residue is determined by the weight difference before and after oven drying at 105℃. After drying, the cassava residue is ground into a fine powder [11].

2. Delignification and Xylan Extraction

Xylan extraction is influenced by delignification treatment using sodium hypochlorite (NaOCl) solvent ranging from 0.5% to 7.5%. The lowest yield was observed with a NaOCl concentration of 7.5%, where a high concentration leads to the dissolution of hemicellulose in the material. In contrast, at a concentration of 0.5%, only a small portion of the hemicellulose is dissolved [12].

A 10-gram sample was soaked in a 0.5% NaOCl solution at 28℃ for approximately 5 hours, followed by filtration. The resultant solid was then immersed in a NaOH solution with varying concentrations (3%, 6%, 9%, 12%, and 15%) for 24 hours at 28℃, followed by filtration. Increasing the NaOH concentration enhances lignin hydrolysis in pulp [13]. The supernatant from

the NaOH treatment was acidified to lower its pH and then neutralized by the gradual addition of HCl solution, which was administered dropwise.

3. Enzyme Production of Xylanase

A 5-gram sample was autoclaved at 121℃ for 15 minutes. An aseptic transfer of an *Aspergillus niger* isolate was then introduced into 10 mL of sterile distilled water. This mixture was added to an Erlenmeyer flask containing the sample and a small amount of sterile distilled water as a moistening agent. The inoculation process continued for 5 days at 30℃. The enzyme formed was extracted using 50 mL of distilled water and centrifuged at 3000 rpm for 30 minutes. The activity of the resulting enzyme extract was evaluated through an activity assay to assess its effectiveness.

4. Xylanase Activity Assay

Xylanase activity determination involved mixing 0.5 mL of crude xylanase enzyme extract with 0.5 mL of a 0.25% xylan substrate and 0.5 mL of acetate buffer. The mixture was then incubated at 60℃ for 55 minutes. After incubation, the reaction was halted by adding 1 mL of DNS reagent and heating to 100℃ for 15 minutes before rapid cooling under running water. The assay's conditions are critical for the efficiency of xylanase in the pre-bleaching process [10].

5. Protein Assay

This protein assay utilized UV-Vis Spectrophotometry following the Biuret method, based on the interaction between cupric ions and protein in an alkaline solution, resulting in an absorbance peak at 545 nm [14]. For this assay, 2 mL of crude enzyme extract was mixed with 8 mL of Biuret reagent and 2 mL of acetate buffer, then incubated at 50℃ for 30 minutes. The absorbance at 545 nm was measured, and the protein concentration was calculated by plotting absorbance values against a standard casein curve using a regression equation.

6. Chelating

The chelating process aims to remove metal ions from the pulp, enhancing the efficacy of the bleaching process. This method involves mixing 20 grams of dry pulp with 10 mL of a 5 g/L sulfuric acid solution, 2 kg of EDTA per ton of pulp, and sufficient distilled water to achieve a 10% consistency. After thorough stirring for homogeneity, the mixture is heated in a water bath at 70℃ for one hour [15][16].

7. Bio-bleaching of Pulp Using Xylanase Enzyme

The pulp bleaching process uses the Elemental chlorine-free (ECF) method, which incorporates xylanase enzymes and chlorine dioxide (ClO_2) as the principal bleaching agents. Prior research indicates that enzymatic pretreatment of pulp, characterized by a higher kappa number, demonstrates superior bleaching performance and results in more significant savings in chlorine dioxide usage compared to pulp with a lower kappa number [17].

Initially, a bleaching sequence is performed by adding xylanase enzyme in varying doses of 0.3, 0.5, 0.7, 0.8, and 1 L/T pulp at 10% consistency. The bleaching

sequence involves treating the pulp for 60 minutes at varying temperatures of 40, 45, 50, 55, 60, 65, and 70°C in a water bath. The selection of temperature variations is based on the optimal conditions for enzyme production, which are active under harsh conditions at a pH of 9-10 and a temperature range of 65-80°C [18]. Subsequently, the pulp is filtered, and the bleaching process continues for 70 minutes at 65°C, followed by an extraction process using NaOH for 90 minutes at 80°C.

8. Kappa Number Test

The lignin content analysis is conducted through the kappa number test, which is applied to both pulps bleached with xylanase enzymes and pulp bleached without these enzymes. The common method for determining the kappa number is the titration method, where a lower kappa number indicates a reduced presence of lignin in the pulp. Moreover, the numerical relationship between Kappa number (KN) and the content of residual lignin is currently recommended by TAPPI as follows: Residual Lignin=KN×0.13

The test begins with a blank test to establish a baseline for calculating the kappa number. The procedure involves mixing 230 mL of distilled water, 25 mL of $KMnO_4$, 25 mL of H_2SO_4, and 2.5 grams of dry pulp, followed by stirring for 10 minutes. Then, 6 mL of KI is added and titrated with $Na_2S_2O_3$ solution until a color change from brown/red-brick to clear is observed. The Kappa number is then calculated using the following formula [19]:

$$Kappa\ Number = \frac{Va-Vb}{W} \times d \quad \textbf{(2)}$$

$$d = 10^{(0.00093 \times \frac{Va-Vb}{0.4-0.5})} \quad \textbf{(3)}$$

Where

Va = Volume of blank

Vb = Volume of titration with $Na_2S_2O_3$

W = Weight of pulp sample

The Kappa Number standards can be adjusted based on the Technical Association Pulp and Paper Industry (TAPPI) standards as follows [2]:

Table 1. The Kappa Number standards

Parameter	Decent	Sufficient	Less
Kappa Number	14 – 20	35 – 50	60 – 110
Lignin (%)	<5	3,12 – 4,45	>7
Yield of Pulp (%)	90 – 95	55 – 90	40 – 55

Source: Technical Association Pulp and Paper Industry (TAPPI)

9. Chemical Savings Assay

A chemical savings assay is necessary to assess the effectiveness of xylanase enzymes from cassava pulp in reducing the consumption of bleaching chemicals. The amount of remaining bleaching agents is determined for pulp that does not use xylanase in the bleaching sequence. Similarly, the residual bleaching chemicals are measured for pulp treated with xylanase during the bleaching sequence to determine how efficiently the xylanase reduces the need for these chemicals. The

concentration of chemicals in the sample is determined using the following formula [20]:

$$ClO_2 \frac{g}{L} = [S_2O_3] \times \frac{b \times 67,45}{a \times 2} \quad \textbf{(4)}$$

Where,

a : volume of sample (mL)

b : volume of $Na_2S_2O_3$ (mL)

RESULTS AND DISCUSSION

1. Characterization of Xylanase Enzyme

Before examining the effect of xylanase dosing on pulp quality, it is crucial to understand the characteristics of the xylanase enzyme to ascertain its optimal conditions for use. As outlined in the methods section, xylanase activity was determined using a UV-VIS spectrometer at a wavelength of 485 nm. The findings were analyzed and plotted on a regression curve derived from a standard sugar reduction curve. The data reveal how temperature and pH influence the activity of the xylanase enzyme:

Table 2. The Correlation Between Temperature and pH to the Activity of the Xylanase Enzyme.

pH	Enzyme Activity of Xylanase (U/mg of Protein)				
	40℃	**50**℃	**60**℃	**70**℃	**80**℃
4	0.2499	0.2451	0.2986	0.2948	0.2419
5	0.2588	0.3081	0.4725	0.3890	0.3351
6	0.2799	0.3385	0.4986	0.3457	0.3020
7	0.2498	0.2566	0.3372	0.2520	0.2210
8	0.2532	0.2804	0.2859	0.2298	0.2128

The data from Table 2 is then visualized in Figure 1

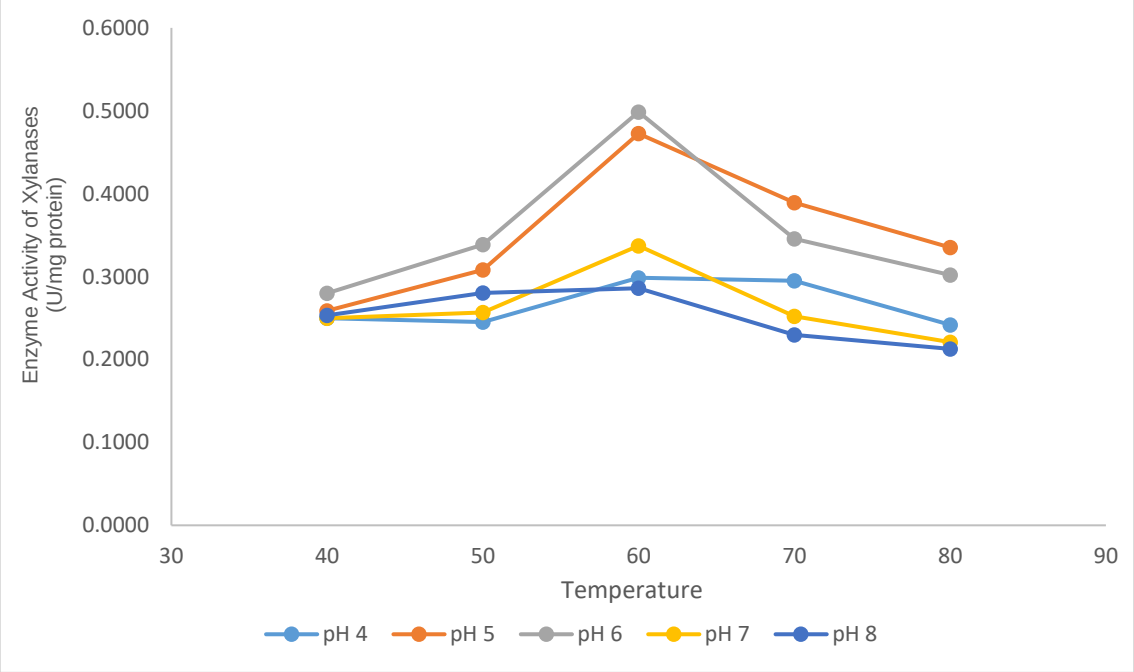

Figure 1. The Correlation Between Temperature and pH to the Activity of the Xylanase Enzyme

As indicated in Figure 1a, experiments demonstrated that xylanase derived from the fermentation of cassava residue using *Aspergillus niger* exhibited optimal activity at a pH of 6 and a temperature of 60℃, achieving an activity level of 0.4986 U/mg protein. However, when the temperature ranged from 70-80℃, xylanase activity diminished due to denaturation, which resulted in conformational changes in the enzyme. This alteration impaired the substrate's ability to bind effectively to the enzyme's active site. The enzyme operates most effectively at a pH of 6; denaturation occurs in environments above 8. Variations in pH values influence xylanase activity because they alter the catalytic site's conformation, where the ionic nature of the carboxyl and amino groups is particularly susceptible to changes in pH.

The protein concentration of the xylanase enzyme was quantified following a specific protocol. The crude enzyme extract was initially mixed with Biuret reagent and acetate buffer. The mixture was then incubated for 30 minutes at 50℃. Subsequently, the solution's absorbance was measured using a UV-VIS spectrometer at the peak absorption wavelength of casein (545 nm). This measurement facilitated the generation of a regression equation aligned with the standard casein curve. The data gathered elucidates the impact of pH on the protein concentration in the xylanase enzyme, providing insights into the enzyme's stability and activity under varying pH conditions

Table 3. The Correlation between pH and protein concentration in the xylanase enzyme.

pH	Protein concentration (mg/mL)
4	9.533
5	9.500
6	**10.300**
7	10.289
8	10.022

Table 3 shows a graph delineating the correlation between pH and protein concentration in the xylanase enzyme.

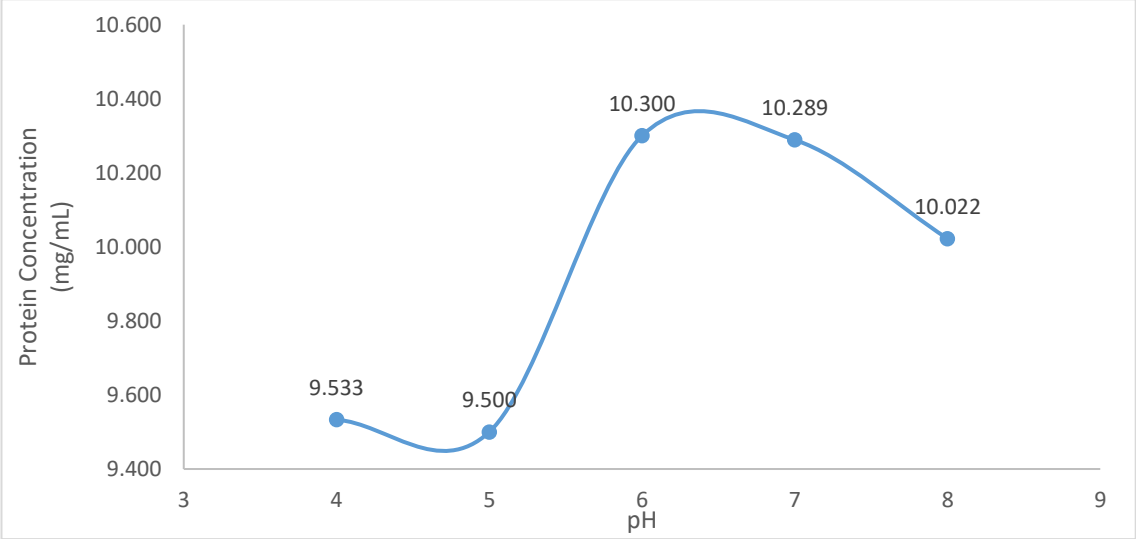

Figure 2. The Correlation between pH and protein concentration in the xylanase enzyme

According to Figure 2, optimal enzyme activity for xylanase occurs at a pH of 6, with a protein concentration of 10.3 mg/mL. This optimal condition supports the activity of *Aspergillus niger*, which utilizes carbohydrates for growth, thereby increasing its protein concentration. The observed decrease in protein levels in xylanase could be attributed to its consumption by *Aspergillus niger* as a nutrient source for its growth.

In recent years, market demand for industrial chlorine-free bleached pulp has surged. Environmentally friendly bio-bleaching, alongside applications such as pitch removal in paper waste, can be effectively conducted using potent biocatalysts like xylanase and laccase. Historically, the integration of enzymes in pulp and paper technology was not deemed economically or technically feasible due to the scarcity of biocatalysis. However, scientific institutions and enzyme manufacturers' ongoing research efforts have fostered biocatalysis's development, offering substantial benefits to the pulp and paper industry [21].

The adoption of enzymes as an alternative, cost-saving method has emerged as an economical and straightforward strategy to diminish the use of chlorine and other bleaching chemicals. Enzymatic technology, particularly as a pre-bleaching step, has been implemented in several production facilities due to its successful scaling to industrial levels. The principal advantages driving the adoption of biocatalysis in pulp bleaching plants are its economic and environmental benefits [22]. These developments underscore the growing importance of sustainable practices in the industry, emphasizing the critical role of enzymatic processes in enhancing the ecological footprint of pulp and paper manufacturing.

2. Kappa Number Test

The bleaching process is stratified into several stages, from chelating to extracting metals from the pulp to optimize the bleaching outcome. This is followed by a bleaching sequence in which the pulp is treated with xylanase at predetermined doses and temperature variations to determine the most effective conditions and dosage. Integrating xylanase in the bleaching process has brought forth enhancements in various aspects of the pulp and paper industry, reducing production costs, improving product quality, and decreasing environmental pollution. Commonly used bleaching chemicals include hydrogen peroxide (H_2O_2) and chlorine. Chlorine, as stipulated by Minister of Health Regulation no. 472/Menkes/Per/V/1996 is recognized as a hazardous substance due to its toxicity and irritative properties. Chlorine usage in materials can generate highly toxic by-products, resulting in dangerous absorbable organic halides (AOX), which pose risks of toxic compounds and potential damage to human organs.

The role of xylanase in the initial processing of hardwood kraft pulp using the Elemental Chlorine Free (ECF) or Totally Chlorine Free (TCF) approaches is critical to reducing the usage of chlorine-based chemicals. Xylanase has been shown to

decrease bleaching costs by up to 89%, enhance the brightness of bleached pulp, and provide an environmentally friendly alternative to traditional bleach. It possesses properties that do not damage fabric fibers and do not adversely impact human health or the environment [23].

Following the initial treatments, bleaching is conducted using chlorine dioxide (ClO_2) with the $D_0/E/D_1$ method at 65℃ for 70 minutes. This study also investigates the impact of temperature and xylanase dosage on the lignin content of the pulp. The kappa number is calculated to ascertain the lignin content. Importantly, before conducting the kappa test on the bleached pulp, it is essential to determine the unbleached pulp's kappa number. This preliminary step is crucial to establish the baseline for how significantly the xylanase enzyme alters the pulp's kappa number. The initial kappa number recorded for the unbleached pulp is 9. Below are the kappa number results for each variation in xylanase dosage and temperature, illustrating the enzymatic impact on the lignin content within the pulp

Table 4. The correlation of temperature and xylanase dosage to the kappa number of pulp after the bleaching sequence.

Xylanase (L/T of Pulp)	**Kappa Number**						
	40℃	**45℃**	**50℃**	**55℃**	**60℃**	**65℃**	**70℃**
0.3	7.12	6.56	6.06	5.69	5.56	7.1	7.16
0.5	6.12	5.91	5.72	5.54	5.44	6.24	6.44
0.7	6.72	6.2	6.32	6.29	6.28	6.84	6.92
0.8	6.83	6.46	6.24	6.3	6.34	7.12	7.24
1	6.91	6.7	6.3	6.52	6.56	7.24	7.44

The reduction in Kappa Number following the bleaching sequence is attributable to applying xylanase. This enzyme specifically targets hemicellulosic xylan, acting at the interface between cellulose and lignin, thereby facilitating the removal of lignin-associated hemicelluloses while maintaining the integrity of the pulp fibers. Additionally, the pre-treatment of pulp with xylanase aids in mitigating the partial disruption of lignin and carbohydrate bonds within the pulp fibers. This enhances the accessibility of subsequent bleaching chemicals to the pulp, leading to more effective bleaching [24].

As depicted in Table 4, a graph illustrates the correlation between temperature and xylanase dosage and the kappa number of pulp following the bleaching sequence. This graphical representation helps elucidate the optimal conditions under which xylanase exerts the most significant effect on reducing the kappa number, thereby optimizing the bleaching process while minimizing chemical use and environmental impact

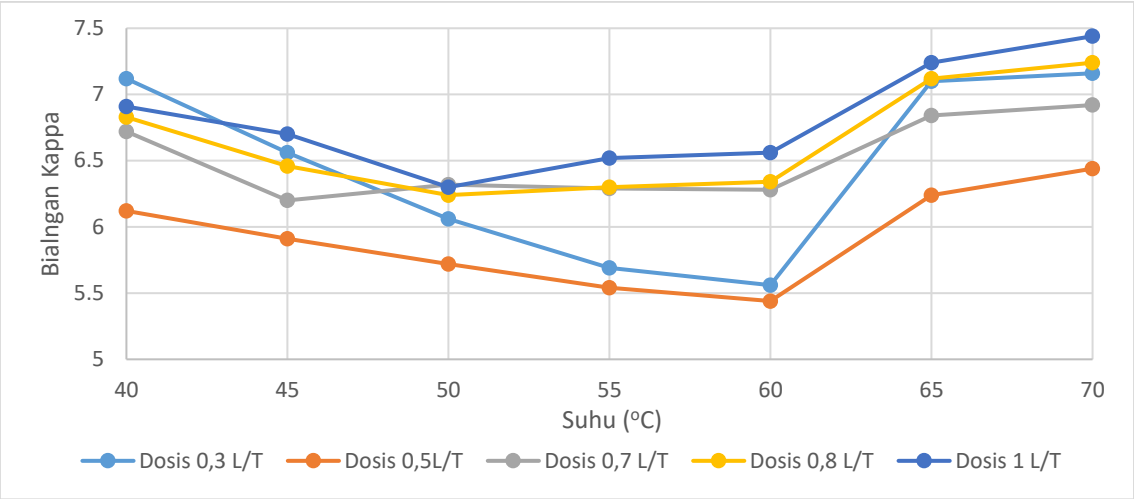

Figure 3. The Correlation of Temperature and Xylanase Dosage to the Kappa Number of Pulp after the Bleaching Sequence.

The data below illustrate the kappa numbers achieved for each variation of xylanase dosage and temperature following the complete bleaching process

Table 5. The Correlation of Temperature and Xylanase Dosage to the Kappa Number of Pulp after the Entire Bleaching Process is Concluded

Xylanase (L/T of Pulp)	**Kappa Number**						
	40℃	**45℃**	**50℃**	**55℃**	**60℃**	**65℃**	**70℃**
0.3	4.62	4.56	4.28	4.21	4.12	5.24	5.44
0.5	4.44	4.3	4.24	4.15	4.04	5.12	5.24
0.7	4.7	4.5	4.36	4.22	4.2	5.4	5.5
0.8	4.74	4.63	4.59	4.4	4.32	5.62	5.68
1	4.8	4.75	4.6	4.54	4.4	5.7	5.72

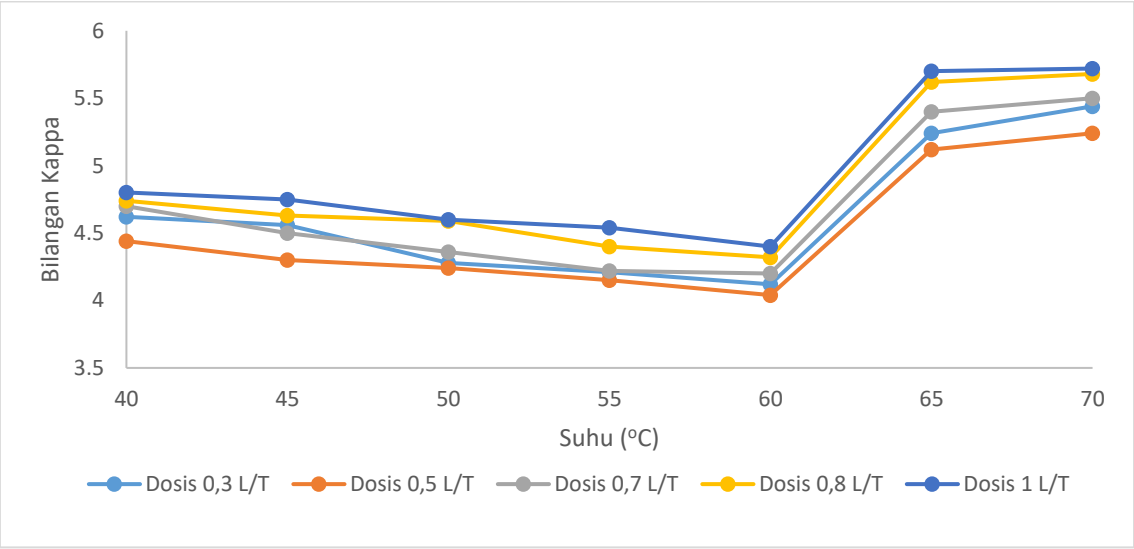

Figure 4. The Correlation of Temperature and Xylanase Dosage to the Kappa Number of Pulp after the Entire Bleaching Process is Concluded

According to Figure 4, the bleaching process, conducted under varying temperature and xylanase dosage conditions, shows that a temperature setting of 60℃ results in the lowest kappa number recorded at 5.44 after the bleaching sequences. This indicates the successful degradation of lignin by the xylanase enzyme. After the complete bleaching process utilizing xylanase produced from cassava residue, the kappa number of the pulp is further reduced to 4.04. In comparison, the use of xylanase from rice straw for pulp bio-bleaching reduced the kappa number from 8.01 to 6.71 [25], and the application of beechwood-derived xylanase significantly lowered the kappa number from 7.2 to 1.98 [26]. However, using cassava residue is more cost-effective and simpler than employing beechwood, making cassava a viable source of xylanase for bleaching efficacy.

The observed reduction in kappa number signifies significant lignin degradation within the pulp, enhancing its brightness. As the temperature increases beyond 60℃, the kappa number tends to rise due to diminished effectiveness of the xylanase enzyme, resulting in less efficient lignin degradation. The supplementation of enzymes boosts the performance of bleaching agents by disrupting the xylan matrix and facilitating the removal of entrapped lignin. Xylanase enzymes specifically cleave the bonds between cellulose and lignin, promoting xylan degradation and making lignin polymers more accessible for removal.

Optimal results are observed at a xylanase dosage of 0.5 L/T of pulp, potentially due to substrate depletion or a reduction in enzyme activity caused by the accumulation of xylose, which may act as an inhibitor in the bleaching process

Table 6. ANOVA Statistical analysis results to illustrate the reduction of Kappa Number

Source of Variation	*SS*	*df*	*MS*	*F*	*P-value*	*F crit*
Doses of Xylanase	0,0774	4	0,0194	77,1321	$2{,}42\ 10^{-13}$	2,776289
Temperature	0,0360	6	0,0060	23,9283	$5{,}11\ 10^{-9}$	2,508189
Error	0,0060	24	0,0002			
Total	0,1195	34				

Statistical analysis using ANOVA corroborates these findings, indicating a significant relationship between the doses and temperatures of xylanase applied. According to Table 6, the F-critical value is significantly lower than the F-value, confirming the statistical significance of the results and underscoring the efficacy of xylanase use in pulp bleaching processes.

3. Chemical Saving Assay

A chemical savings assay was conducted to evaluate the impact of temperature and xylanase enzyme dosage on the consumption of bleaching chemicals during the pulp bleaching process. This assay focused on measuring the concentration of chlorine dioxide (ClO_2) remaining in the filtrate from the bleached

pulp. More chemical bleach indicates reduced consumption, which benefits environmental sustainability.

In the pulp and paper industry, the pulping, bleaching, and washing stages typically produce effluents rich in organic and inorganic components such as lignin, hemicellulose, cellulose fragments, chlorophenols, fatty acids, inorganic salts, and chlorinated dioxins. The introduction of xylanase in these processes aims to reduce reliance on chlorine dioxide during bleaching, thus lowering effluent toxicity levels [24]. The xylanase enzyme facilitates the removal of the re-deposited xylan layer from the fiber surface, thereby creating more opportunities for the bleaching chemicals to penetrate the fiber. Moreover, xylanase reduces the concentration of hexenuronic acids in the pulp fibers, consequently diminishing the consumption of bleaching chemicals [27].

The table below presents the data on residual bleach in the filtrate at various temperatures and xylanase dosages. This information helps to quantify the environmental and chemical efficiency gains attributable to the enzymatic treatment in the bleaching process, reinforcing the value of integrating biocatalysts such as xylanase into traditional pulp and paper manufacturing workflows.

Table 7. Concentration of ClO_2 in the filtrate.

Xylanase (L/T of Pulp)	**[ClO_2] (g/L)**						
	40℃	**45**℃	**50**℃	**55**℃	**60**℃	**65**℃	**70**℃
0.3	0.43	0.40	0.40	0.38	0.43	0.40	0.38
0.5	0.64	0.67	0.69	0.69	0.71	0.67	0.65
0.7	0.62	0.6	0.64	0.65	0.64	0.65	0.64
0.8	0.48	0.5	0.48	0.43	0.48	0.48	0.43
1	0.51	0.51	0.53	0.56	0.50	0.51	0.50

Based on Table 7, the percentage of chemical savings obtained is as follows:

Table 8. Chemical Savings Percentage at Each Temperature and Dosage of Xylanase

Xylanase (L/T of Pulp)	**Chemical Savings (%)**						
	40℃	**45**℃	**50**℃	**55**℃	**60**℃	**65**℃	**70**℃
0.3	14.33	13.33	13.33	12.67	14.33	13.00	13.00
0.5	21.33	22.33	23.00	23.00	23.67	22.00	22.00
0.7	20.67	20.00	21.33	21.67	21.33	22.00	21.00
0.8	16.00	16.67	16.00	14.33	16.00	14.00	14.00
1	17.00	17.00	17.67	18.67	16.67	17.00	17.00

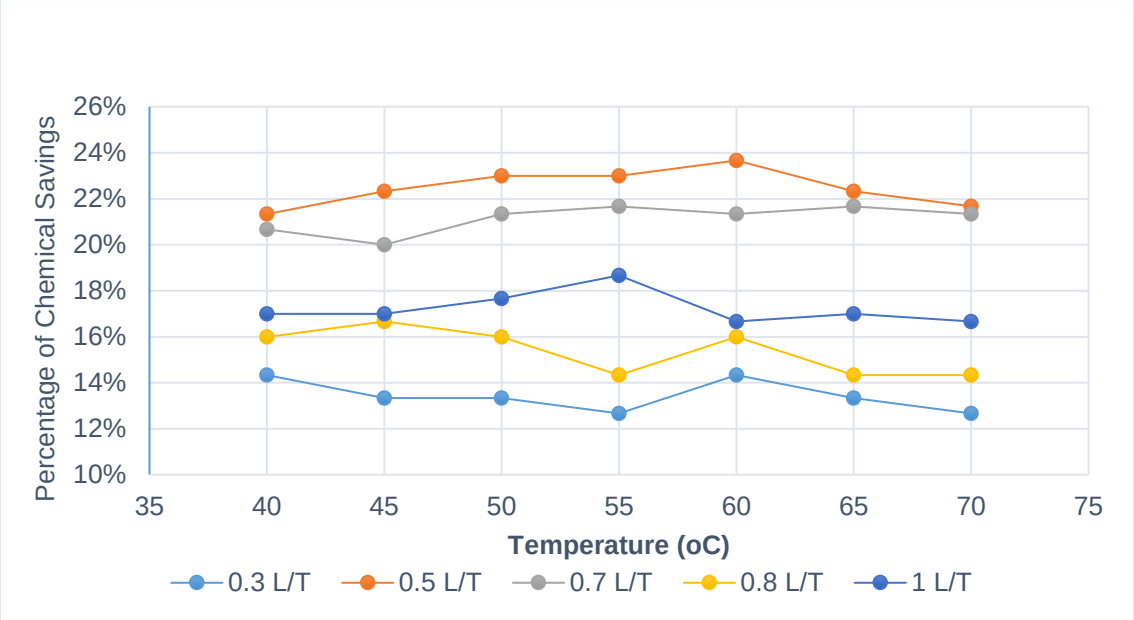

Figure 5. Chemical Savings Percentage at Each Temperature and Dosage of Xylanase

Figure 5 indicates that the highest residual bleach, measured at 0.71 g/L, is observed in the filtrate from pulp treated with 0.5 L/T of xylanase enzyme at 60℃. This condition results in a reduction of bleach consumption by approximately 23.67%. However, when the xylanase dosage exceeds 0.5 L/T of pulp, the concentration of chemicals in the filtrate decreases, reducing chemical savings. This decrease could be attributed to the depletion of substrate concentration or a reduction in enzyme activity caused by the formation of xylose, which acts as an inhibitor, thereby necessitating an increased use of bleaching chemicals compared to the optimal 0.5 L/T dosage.

The paper manufacturing process traditionally involves using large quantities of chemicals, which can lead to significant environmental challenges and hazardous waste production. The pulp and paper industries are increasingly exploring biotechnological methods as alternatives to chemical processes, particularly through enzymatic treatments. The application of xylanase as a bleaching agent by removing lignin not only aids in pulp fibrillation and enhances fiber freedom but also presents an environmentally friendly, cost-effective solution that reduces the need for traditional bleaching chemicals to achieve desired levels of brightness. This enzymatic pre-bleaching innovation has been demonstrated in several studies to improve paper quality, mass thickness, tensile strength, and tear indices and lower volatile organic compounds emissions [28].

Amid rising environmental concerns, more stringent environmental policies have been implemented. These policies encompass regulations that include performance standards, emission limit values, technology requirements, and incentive-based instruments such as taxes/levies and tradable emission

allowances. Environmental policies significantly influence the prospects of the pulp and paper industry; however, lengthy and inflexible licensing processes increase uncertainty and can impede sustainable technological advancements. Despite efforts to reduce emissions, achieving substantial reductions using current technologies remains challenging. Long-term steps toward sustainable industrial transformation necessitate developing and applying innovative, more environmentally friendly technologies, such as enzymatic applications. A regulatory approach that facilitates technological change and provides flexibility can be pivotal in fostering the development of new environmentally friendly technologies capable of achieving significant emission reductions [29].

CONCLUSION

Xylanase enzymes derived from cassava pulp using *Aspergillus niger* have demonstrated effectiveness in bio-bleaching pulp. The enzymes show optimal activity at 60℃ and a pH of 6, with an enzymatic activity of 0.4986 U/mg of protein and a protein concentration of 10.3 mg/mL at these conditions. In the bleaching sequence, using xylanase at 60℃ and dosage of 0.5 L/T of pulp resulted in the lowest kappa number, decreasing from 9 to 5.44, indicating effective lignin degradation. Further treatment with ClO_2 in the D0/E/D1 stages reduced the kappa number to 4.44, underscoring the conditions' efficacy. However, increasing the temperature beyond 60℃ and the enzyme dosage above 0.5 L/T of pulp resulted in a rise in kappa numbers due to diminished enzyme performance and potential inhibition effects from excessive enzyme presence. The optimal bleaching conditions also led to significant chemical savings of up to 23.67%. These findings highlight the potential of using cassava pulp-derived xylanase as an environmentally friendly bleaching agent in the pulp and paper industry. Further research should explore alternative microbial sources for producing xylanase to optimize the bleaching process, as the type of microorganism can influence enzyme efficiency and the required conditions for optimal activity

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