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Physicochemical Characteristics of Fungal Xylanases and their Potential for Biobleaching of Kraft and Non-wood Pulps

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Authors' contributions

This work was carried out in collaboration among all authors. Authors ACFK and CH designed the study and wrote the first draft of the manuscript. Authors SSM and NFSS managed the literature searches. Author MKK managed the analyses of the study. All authors read and approved the final manuscript.

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ABSTRACT

Xylanases are enzymes with a wide variety of biotechnological applications, such as in the bioconversion of lignocellulosic materials, improvement of feed digestibility, and bleaching kraft pulps to increase pulp brightness. Many studies have been conducted and published over the years on cellulose pulp bleaching due to the need to search for more sustainable tools and thus reduce environmental pollution. Thus, in this review, we focus on analyzing the biochemical properties of xylanases produced by mesophilic and thermophilic fungi that have been used in the bleaching processes of kraft and non-wood pulps. *Eucalyptus* kraft pulp is still one of the most used raw materials in the production of pulp and paper, while straw and bagasse are alternative sources of non-wood pulps. Thermophilic fungal xylanases show optimum enzymatic activity at high temperatures and a shorter treatment period when compared to mesophilic xylanases in the

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bleaching step. However, mesophilic fungal xylanases exhibit a longer period of enzymatic treatment but achieve a satisfactory brightness and kappa number. Hence, these approaches will contribute to future applications of these xylanolytic enzymes in pulp and paper industries.

Keywords: Brightness; cellulose; fungus; kraft; xylanase.

1. INTRODUCTION

The pulp and paper industry is one of the most productive sectors in the world, stimulated by growing global demand. In Brazil, 7.84 million hectares of trees were planted in 2016, 34% of which belong to companies in the pulp and paper industry [1]. The industry is still growing – global paper production hit 400 million tons per year in 2014 – but it is traditionally known to contribute to environmental pollution, mainly due to its discharge of effluent [2]. The most significant environmental impact of the pulp and paper industry is caused by wastewater elimination [3] since it requires 75,000 to 227,000 liters of water per ton of paper produced.

Environmental regulation has increasingly restricted the use of chlorine compounds for the bleaching of kraft pulp since the effluents produced by the industry contain a variety of organic and inorganic contaminants that mostly originate from tannins, lignin, resins, and chlorine compounds [4,5]. Chlorinated organic compounds generated during the bleaching of pulp have been identified as toxic, mutagenic, and bioaccumulative. In addition, these chlorine compounds, even at low concentrations, may cause serious biological disorders [6,7]. The pulp and paper industries have adapted to restrictions by using sustainability strategies that minimise their environmental impact. Elemental chlorine-free (ECF) bleaching methods and enzymatic treatments have gained popularity because of environmental concerns about the chlorinated compounds that are generated during the manufacture of cellulose.

In this context, numerous studies have been conducted by many researchers over the years to implement the use of enzymes as xylanases in kraft pulp pre-bleaching processes in order to use increasingly sustainable technologies. Thus, we focus on searching and analysing published data on the physicochemical characteristics of xylanases produced by various species of mesophilic and thermophilic fungi that have been tested in the bleaching processes of kraft and non-wood pulps in order to assist in the future application of these xylanolytic enzymes in pulp bleaching processes.

2. USE OF FUNGAL XYLANASE IN KRAFT PULP BLEACHING

In accordance with their commitment to expanding its range of chlorine-free products, the paper industry has adopted enzymes as new, versatile tools. Viikari et al. [8] were the first to describe the use of enzymatic treatment to replace chlorine bleaching for the removal of lignin from wood pulp. Since the kraft bleaching process generates approximately 2 to 4 kg of organochlorine per tonne of pulp [9], the use of enzymes is a way to reduce the amount of chlorine dioxide required for pulp bleaching. Among the enzymes used, the xylanases are the most popular in the paper industry, where they are utilised as bleach boosting agents [10], reducing the chlorinated chemical volume by up to 25%. Consequently, a proportional reduction in the generation of chlorinated effluent has occurred [10]. Furthermore, when kraft and non-wood pulps were implemented with xylanase in the pre-bleaching stage, the use of chlorine-based chemicals in the subsequent stage was reduced by 20 to 25%, and consequently the levels of pollutants eliminated were reduced [11].

The addition of xylanase during the bleaching step was one of the first reports on the efficient use of enzymes as a technological tool that could be added to an existing industrial plant without large investment. The xylanolytic system includes enzymes such as endo-1,4-β-xylanases (EC 3.2.1.8), β-xylosidases (EC 3.2.1.37), α-L-arabinofuranosidases (EC 3.2.1.55), α-D-glucuronidases (EC 3.2.1.139), acetyl xylan esterases (EC 3.1.1.72) and feruloyl esterases (EC 3.1.1.73) [12,4]. Endo-1,4-β-xylanases (E.C.3.2.1.8) are the main glycosyl hydrolases (GH) of the xylanolytic system and are commonly called "xylanases". The enzymes hydrolyse β-1,4 glycosidic bonds inside the xylan chain, producing xylooligosaccharides (XOS), and belong to the GH10 and GH11 families [13]. Xylan-1,4-β-xylosidases (E.C.3.2.1.37) act cooperatively with endo-1,4-β-xylanases,

Table 1. Physicochemical properties of fungal xylanases and their performance in pulp bleaching

Fungus	Xylanase (U/g pulp)	pH	Temperature (°C)	Treatment period (h)	Kappa number	Kappa efficiency (%)	Brightness (%)	Type of pulp	References
Aspergillus flavus	10	6.5	60	1	9.02	35.93	nr	*Eucalyptus* Kraft pulp	[15]
Aspergillus flavus	11	6.5	55	2	89	36.32	nr	*Eucalyptus* Cellulose pulp	[16]
Aspergillus flavus	10	6.5	55	2	2.56	18.34	nr	*Eucalyptus* Cellulose pulp	[17]
Aspergillus fumigatus	10	5.0 – 5.5	70	1	6.8	11.7	nr	*Eucalyptus* Cellulose pulp	[18]
Aspergillus japonicus	10	5,2	50	3	11.6	25.2	57.7	*Eucalyptus* Cellulose pulp	[19]
Aspergillus niger	10	6.5	55	2	10.34	25.93	nr	*Eucalyptus* Cellulose pulp	[16]
Aspergillus niger	35	5.5	55	2	7.4	14.9	59.6	*Eucalyptus* Cellulose pulp	[20]
Aspergillus niger	5	7	50	4	4.8	nr	70.8	Cellulose pulp	[21]
Aspergillus niger	60	nr	50	2	19.9	nr	41.8	Paper pulp	[22]
Aspergillus niveus	10	4.5 – 5.0	65	1	7.1	39.6	58.1	*Eucalyptu*s Cellulose Kraft	[23]
Aspergillus niveus	35	5.5	55	2	6.9	20.7	59.6	Cellulose pulp	[20]
Aspergillus ochraceus	35	5.5	55	2	6.9	20.7	58.9	Cellulose pulp	[20]
Aspergillus ochraceus	10	5	65	1	7.5	36.4	57.1	Cellulose pulp	[24]
Aspergillus oryzae NRRL 1808	51	6.0	60	nr	nr	nr	1.4	Eucalyptus pulp	[25]
Aspergillus sydowii SBS 45	25	8.2	40	5	14.32	nr	41	Kraft pulp	[26]
Aspergillus terricola	10	6.5	60	1	6.6	14.3	60.3	*Eucalyptus* Cellulose pulp	[24]
Penicillium corylophilum	5	7	50	4	4.9	nr	73	*Eucalyptu*s Cellulose Kraft	[21]
Penicillium crustosum	25	5.5	50	2	9.77	nr	nr	*Eucalyptu*s Cellulose Kraft	[27]
Penicillium janczewskii	2	5.5	50	1	<3	nr	nr	*Eucalyptus* Kraft pulp	[28]
Penicillium sp	20	nr	30	1	2.3	nr	0.9	Waste office paper	[29]
Thermomyces lanuginosus CBS 288.54	40	7.0 – 7.5	70 - 75	1	nr	nr	66.91	Wheat straw pulp	[30]
Thermomyces lanuginosus wild type	10	7	60	1.5	nr	8.6	2.63	Wheat straw pulp	[31]
Thermomyces lanuginosus mutant (M7)	10	8	65	1.5	2.29	18.6	nr	Wheat straw pulp	[31]
Thermomyces lanuginosus SSBP	50	6.5	50	3	9	nr	46.1	Bagasse pulp	[32]
Trichoderma asperellum	100	5.0	50	1	4.2	nr	4	Paper pulp	[33]
Trichoderma longibrachiatum	5	7	50	4	4.3	nr	71.6	Cellulose kraft pulp from *Eucalyptu*s	[21]
Trichoderma viride	10	6				nr	nr	Kraft pulp from *Eucalyptu*s	[34]

Nr: not related

converting the xylooligosaccharides from the non-reducing end until D-xylose units and filamentous fungal β-xylosidases have been described only for families 3, 43 and 54 [14,13]. However, many microorganisms, including filamentous fungi, yeast, and bacteria, are recognised producers of these xylanases [12].

Thus, the physicochemical properties of fungal xylanases, as well as results obtained on the bleaching of pulps from studies carried out by several researchers are compiled in a table including treatment conditions for the bio-bleaching of cellulose pulp and *Eucalyptus* kraft pulp, treatment period, kappa number and brightness (Table 1).

3. DISCUSSION

The bleaching of cellulose pulp, *Eucalyptus* kraft, or non-wood pulp has been supplemented with xylanases from both mesophilic and thermophilic fungi. *Eucalyptus* pulp is one of the most commonly used pulps in paper production, while wheat straw represents a common source of alternative raw material or non-wood pulp. Xylanases produced by thermophilic fungi such as *Aspergillus fumigatus* and *Thermomyces lanuginosus* show optimum enzymatic activity at high temperatures (70–75ºC), but the treatment period with xylanase is shorter (0.5 h), an important factor in reducing cost in the bleaching process. In contrast, mesophilic fungal xylanases show optimum activity in the range of 50–55°C and acidic pH (5.0–6.5), require a longer enzyme treatment period (2–4 h) to achieve a satisfactory brightness and have a lower kappa number. According to the properties of the enzyme, a neutral or alkaline pH also improves pulp bleaching, with a 70.8% increase in brightness at pH 7.0 [21]. Similarly, xylanases with an optimum pH of 7.0 from the fungi *Penicillium corylophilum* and *Trichoderma longibrachiatum* produced an increase in brightness of 73% and 71.6%, respectively [21].

Other hemicellulases, such as ferulic acid esterases and galactosidases, have also been reported to help in the bleaching of cellulose pulp with xylanases [4]. While lipases are generally applied during the processing of the paper, xylanase and laccase are more commonly used in the bleaching and delignification processes [35]. The benefits of enzymatic treatment include a reduction of operating costs and organic pollutants, improvement in the brightness and properties of the pulp, reduction of the kappa number, and increase in fiber yield [36]. In addition, bio-bleaching of non-wood kraft pulps (rice straw, wheat straw, bagasse, etc.) treated with *T. lanuginosus* SSBP xylanase released chromophores and organic halogens, reduced sugars, and decreased the kappa number [37].

Several hypotheses have been proposed to explain the role of cellulase-free xylanase in the bleaching of paper pulp: the treatment of cellulose pulp with cellulase-xylanase helps and improves the infiltration of bleaching chemicals, breaking down the xylan structure, and consequently removes the lignin-based chromophores from the cellulose fiber [8,38,39, 40]. Some studies have shown that pre-bleaching with xylanase is an innovative and environmentally friendly, as well as inexpensive, method that can decrease the amount of chemicals needed to achieve a given brightness in the later stages of chemical treatment. Therefore, pre-treatment with xylanases increases the efficiency of the chemical extraction of lignin from pulp and further minimises the need for chlorine dioxide (ClO_2) [41,42]. Thus, a significant number of pulp and paper industries in Europe, North America, South America, and Japan have used enzymes in the kraft bleaching processes. North America has already improved the processing of 2.5 million tonnes of pulp using xylanase in the pre-bleaching steps [43].

4. CONCLUSION

Eucalyptus kraft pulp is still the most reported among pulp and paper production, while wheat straw represents a source of alternative raw material or non-wood pulp. The bleaching of *Eucalyptus* kraft or non-wood pulps by both mesophilic and thermophilic fungal xylanases has been studied. Thermophilic fungal xylanases have shown optimum enzymatic activity at high temperatures, an advantage because they require a shorter treatment period, which reduces operational cost. On the other hand, mesophilic fungal xylanases are effective in achieving satisfactory results in brightness and a lower kappa number, but they require a longer treatment time compared to thermophilic fungal xylanase. Thus, these approaches will contribute to future applications of these xylanolytic enzymes in pulp and paper industries.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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