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Wheat Straw Processing Effect Study on Their Enzymatic Hydrolysis by *Trichoderma viride* and *Aspergillus awamori* Cultures

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Abstract: The effect of wheat straw processing on the enzymatic hydrolysis of the straw by *Trichoderma viride* and *Aspergillus awamori* cultures is of importance for the bioconversion of renewable biomass. The study aimed to determine the optimal wheat straw processing method that would result in the highest yield of fermentable sugars from straw. A combination of physical and chemical methods was used to process wheat straw, including milling, steam explosion, and acid treatment. The enzymatic hydrolysis of the processed wheat straw was then evaluated using *T. viride* and *A. awamori* cultures. The steam explosion method was the most effective at breaking down wheat straw, resulting in the highest yield of fermentable sugars. Additionally, it was found that *T. viride* was more effective at enzymatic hydrolysis than *A. awamori*, producing a higher yield of glucose from the processed wheat straw. The findings of this study have implications for the development of more efficient and sustainable methods for producing biofuels and other valuable chemicals from agricultural waste materials like wheat straw.

Keywords: Depolymerization, Polysaccharides, Enzymes, Enzymatic Hydrolysis, Polymerization

Introduction

The bioconversion of renewable lignocellulosic biomass into biofuels and economically valuable products has seen widespread development in various countries. Lignocellulosic raw materials, which include agricultural waste and wood, hold significant potential as affordable and sustainable resources. They primarily consist of cellulose, hemicellulose, and lignin. Enzymatic conversion of cellulose-containing raw materials offers promise not only for creating environmentally friendly, low-waste technologies but also for reducing the environmental impact of industries that process plant-based raw materials and generate substantial waste, such as pulp-and-paper industries.

Enzymatic technologies have emerged as highly effective means for transforming a wide range of biological raw materials. The use of enzymes as biocatalysts has the potential to diversify the raw material base for the food industry and livestock feed production, enhance raw material processing, introduce novel food and feed products, and improve their digestibility and sensory qualities (Hoebler *et al*., 1989; Silverstein *et al*., 2007).

Additionally, shifting from conventional chemical methods to biotechnological approaches often becomes the sole option for developing low-waste and eco-friendly industries.

Wheat straw, a byproduct of global wheat cultivation, is underutilized. While a small portion is used for livestock feed, manure, and fuel, a significant amount is either discarded or burned, leading to both wasted fuel resources and environmental pollution (Yoshida *et al*., 2008; Yu *et al*., 2012). Extensive research has been conducted to expand the utilization of wheat straw, including its conversion into reducing sugars through hydrolysis and subsequent fermentation into valuable products like ethanol or other chemicals using suitable microorganisms.

This research addresses the prevalent issue of wheat straw underutilization, which often results in waste and environmental harm. Despite its widespread availability, only a fraction of wheat straw serves productive purposes like livestock feed, manure, or fuel, while the majority is disposed of or burned. To rectify this inefficiency, the study explores the conversion of wheat straw into valuable products via hydrolysis and fermentation processes. Specifically, it investigates the breakdown of wheat straw into reducing sugars through hydrolysis, followed by the use of appropriate microorganisms to ferment these sugars into desired products, such as ethanol or other chemicals. By assessing the feasibility of this approach, the research aims to increase the value of wheat straw, reduce waste, and contribute to both resource efficiency and environmental preservation.

Materials and Methods

As a raw material for enzymatic hydrolysis, we used (for comparison): Wheat straw. Enzymatic hydrolysis was carried out using *T. viride* and *A. awamori* cultures.

A preliminary treatment was carried out to reduce the degree of crystallinity of cellulose and increase the specific surface area of the substrate. The wheat straw was subjected to mechanochemical pretreatment and the optimal modes of pretreatment were established at a temperature of 98-100°C for 20-30 min. Chemical pretreatment was also used, where the wheat straw was cooked in a low concentration of alkaline solution (0.5-1.0%).

The enzymatic hydrolysis of wheat straw was then carried out using optimal modes of enzymatic hydrolysis, with a dosage of the enzyme preparation of 0.05 g/g of substrate, a temperature of 50°C, and a pH of 5.0.

In summary, wheat straw as the plant material was employed for both mechanochemical and chemical pretreatment to determine the optimal modes of enzymatic hydrolysis.

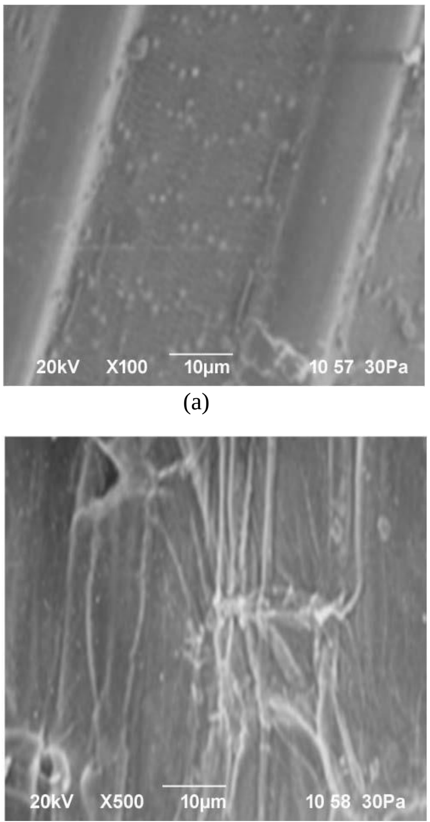
Results

For effective use of Cellulose-Containing raw materials (CC), pretreatment is necessary to reduce the degree of crystallinity of cellulose and increase the specific surface of the substrate. Pretreatment methods include physical, chemical, physico-chemical, biological, and various combinations thereof. The choice of a pretreatment method for a specific biomass (or mix of materials) is influenced by several factors such as carbohydrate preservation and digestibility (Lynd *et al*., 2002; Hu and Ragauskas, 2011; 2012 Zaldivar *et al*., 2001; Bychkov *et al*., 2015). The concentration of Reducing Substances (RS) was determined using a dinitrosalicylic reagent and the concentration of Reducing Sugars (RS) was determined by the Shomodi-Nelson method (Nelson, 1944). The solid residue of the wheat straw samples after enzymatic hydrolysis was investigated by IR spectroscopy to determine the degree of crystallinity of cellulose (Andersen, 2007).

Table 1 shows the values of cellulose crystallinity indices of plant raw materials after enzymatic hydrolysis and Table 2 shows the influence of water prehydrolysis and boiling time of wheat straw in an alkaline solution on the yield and quality of the semi-finished product. From Tables 1-2, it can be seen that processing of cellulose-containing raw materials, i.e., prehydrolysis in water and acid, can be used to obtain hemicellulose, but with this mode of pulping, it is possible to get cellulose without pressure and at a lower temperature (98-110°C). Table 3 shows the effect of prehydrolysis and boiling time of wheat straw in nitric acid on the yield and quality of cellulose.

During enzymatic hydrolysis, the degree of crystallinity of cellulose-containing residues of plant material samples increases. This is because predominantly amorphous areas of cellulose microfibrils are primarily subjected to enzymatic hydrolysis. Highly ordered areas cannot be hydrolyzed under these conditions (Carvalho *et al*., 2013; Olsson, 2014; Kadic, 2017).

A distinctive feature of wheat straw is the presence of a grease layer on the surface of straw stalks, which performs a protective function (Fig. 1a-b). This feature of the raw material makes delignification difficult and necessitates the use of additional stages in the processing of straw into fibrous products.



(a)

(b)

Fig. 1: Wheat straw from the outer (a) and from the inner (b) sides ×100 (a); ×500 (b)

Table 1: Values of cellulose crystallinity indices of plant raw materials after enzymatic hydrolysis

	Crystallinity index		
Duration of fermentation	Wheat straw	Guza-pay a	Rice hulls
2	0.37	0.52	0.38
3	0.41	0.53	0.47
4	0.42	0.54	0.48
6	0.44	0.55	0.49
24	0.45	0.56	0.51

Table 2: The influence of water prehydrolysis and boiling time of wheat straw in an alkaline solution on the yield and quality of the semi-finished product

			Pulp indicators		
Time preguided in water min.	Boiling time in 2% NaOH solution, in hour	Output, %	Containing α-cellulose, %	Containing hemicellulose %	Degree of polymerization
30	1.0	35.51	77.84	12.32	1080
Without damping	1.0	33.02	75.59	16.14	1140
30	1.5	38.58	79.72	10.41	1020
Without damping	1.5	34.23	78.37	15.23	1100
30	2.5	42.45	81.17	9.00	980
Without damping	2.5	35.01	78.98	13.92	1020
30	3.0	44.24	83.55	8.14	950
Without damping	3.0	38.86	79.84	11.98	970

*The statistical error of the experiment is ±0.01 with a confidence probability of 0.97 or 97%

Table 3: The effect of prehydrolysis and the boiling time of wheat straw in nitric acid on the yield and quality of cellulose

				Indicators cellulose		
Time (min) preguided in 2% HNO_3	Boiling time (min) in 2% solution of HNO_3	Boiling time (hour) in 2% solution of NaOH,	Output, in %	Content of α-cellulose, in %	Content of hemicellulose, %	Degree of polymerization
5	20	0.5	38.92	79.92	11.98	820
Without damping	20	0.5	35.14	77.08	14.50	850
5	20	1.0	40.04	80.33	10.08	800
Without damping	20	1.0	36.21	79.95	12.98	840
5	20	1.5	44.72	80.90	8.52	750
Without damping	20	1.5	38.50	79.12	10.21	800
5	20	2.0	46.07	84.86	7.76	740
Without damping	20	2.0	40.87	81.25	8.89	780

*The statistical error of the experiment is ±0.01 with a confidence probability of 0.97 or 97%

To reduce the consumption of chemical solutions for delignification and increase the yield of cellulose, pretreatment of the fibrous product in a twin-screw apparatus is used before the stage of boiling, so-called thermomechanical treatment. A high yield of cellulose is obtained by combining two-stage mechanical treatment, including chopping straw in a straw chopper and processing chop in an auger apparatus (Palonen, 2004; Toda *et al*., 2005; Palkovits *et al*., 2010).

The influence of various parameters of the wheat straw delignification process (Shimizu *et al*., 2009; Yang *et al*., 2011) on the quality of the obtained fibrous products was studied. At variations in the concentration of sodium hydroxide, hydronic module, and the duration of delignification, the yield of fibrous products varies from 33.4-69.4% by weight of absolutely dry straw.

The use of the above mechanical preparation makes it possible to obtain positive results in obtaining high-quality cellulose for chemical processing. High-yield cellulose requires milder regimes: The prehydrolysis of wheat straw is carried out in an aqueous medium at a temperature of 98-100°C for 20-30 min. As a chemical treatment, boiling in a low concentration of an alkaline solution (0.5-1.0%) is expressed, while the yield of cellulose from a wheat straw may be 55-65%. Modes of obtaining cellulose high yield of wheat straw and physical-chemical properties of the samples are given in Tables 2-3.

Discussion

The results indicate that pretreatment is necessary to obtain high-quality cellulose for chemical processing. Mechanical pretreatment before boiling and pulping can significantly increase the yield of cellulose from wheat straw. Prehydrolysis in water and acid can be used to obtain hemicellulose, but the mode of pulping affects the yield and quality of cellulose. The physicochemical properties of the obtained cellulose are influenced by the pretreatment method, including the degree of crystallinity and specific surface area. The physical, chemical, physico-chemical, biological, and various combinations thereof of pretreatment methods should be carefully considered based on the specific biomass and downstream processes.

Table 4: The content of monosaccharides in samples of enzymatic hydrolysates of wheat straw and Guza-paya

	Content, % by weight of dry substance, in enzymatic hydrolyzate obtained from lignocellulosic material	
Monosahar	Wheat straw	Guza-paya
Rhamnose	-	-
Arabinose	-	-
Xylose	5.4	8.0
Mannose	2.2	1.7
Glucose	75.8	53.5
Galactose	0.6	0.5
Total monosaccharides	84.0	63.7

*The statistical error of the experiment is ±0.01 with a confidence probability of 0.97 or 97%

The content of hemicellulose in wheat cellulose significantly depended on the duration of boiling (Huber *et al*., 2006; Onda *et al*., 2008). In the range of boiling times from 1.5 and 3.0 h, the hemicellulose content during boiling varies from 25.4-23.5%. As a result of this treatment, the yield of the fibrous product and the degree of polymerization decreased from 1300-970.

The monosaccharide composition of the resulting enzymatic hydrolysates is presented in Table 4. Glucose is the main monosaccharide of enzymatic hydrolysates from both types of raw materials. In terms of reducing substances, its share is equal to 90.2% for LCM enzymatic hydrolyzate of wheat straw and 84.0% for guza-paya LCM of enzymatic hydrolysate.

Conclusion

A technology has been developed for processing plant biomass. It is based on a combination of thermochemical pretreatment of raw materials and enzymatic hydrolysis. This technology ensures the selectivity of enzymatic catalysis in comparison with acidic catalysis. It provides selective hydrolysis of polysaccharide glycosidic bonds and the absence of secondary transformations of the resulting monosaccharides. This allows them to get close to theoretically possible. The process can be carried out at relatively low temperatures. The suitability of wheat straw for the process of depolymerization of natural polysaccharides and the synthesis of sugars on this basis has been determined. Studies have established the optimal modes of mechanochemical pretreatment of wheat straw (temperature 98-100°C within 20-30 min) and chemical (cooking in a low concentration of an alkaline solution (0.5-1.0%)) while the yield of cellulose from a wheat straw may be 55-65%. The optimal modes of enzymatic hydrolysis of wheat straw (the dosage of the enzyme preparation is 0.05 g/g of the substrate, temperature 50°C, pH 5.0) enable us to obtain a glucose yield of 63.97% from the raw materials.

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Author's Contributions

Makhatov Zhaksylyk and Serzhan Mombekov: Participated in all experiments, coordinated the data analysis, and contributed to the written of the manuscript.

Bakhytzhan Kedelbayev and Assel Kozhamzharova: Designed the research planned and organized the study.

Ethics

This article is original and contains unpublished material. The corresponding author confirms that all of the other authors have read and approved the manuscript and no ethical issues are involved.

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