

Production of substrates for determination of xylanase activity**Produção de substratos para determinação da actividade da xilanase**

DOI:10.34117/bjdv6n10-318

Recebimento dos originais: 10/09/2020

Aceitação para publicação: 15/10/2020

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ABSTRACT

The hemicelulose is a molecule heterogeneous and, therefore, its extraction from different biomass is a challenge. In this context this study proposed an hemicelulose extraction from sugarcane biomass using alkaline pretreatment to determination of xylanase activities and evaluation of affinity between substrates and enzymes. The results suggest that the xylan from sugarcane leaves showed greater affinity with the enzymes, but the xylan from bagasse was with more efficient and pure extraction.

Keywords: hemicelulose; biorrefinering; biomass; xylanolytic enzymes.

RESUMO

A hemicelulose é uma molécula heterogênea e, portanto, a sua extração de biomassa diferente é um desafio. Neste contexto, este estudo propôs uma extração de hemicelulose a partir de biomassa de cana de açúcar utilizando pré-tratamento alcalino para determinação das actividades da xilanase e avaliação da afinidade entre substratos e enzimas. Os resultados sugerem que o xilano das folhas da cana de açúcar mostrou maior afinidade com as enzimas, mas o xilano do bagaço estava com uma extração mais eficiente e pura.

Palavras-chave: hemicelulose; biorrefinamento; biomassa; enzimas xilanolíticas.

1 INTRODUCTION

There is a need for production of xylan for different applications. Xylan became widely used by industry with the development of bioproducts. There are several studies involving this macromolecule, for example production of furfuraldehyde through hydrolysis and xylose

dehydration, xylitol production by hydrogenation of xylose, in addition to the production of prebiotics from xylooligosaccharides as well as production of hemicellulose films. The xylan still may have laboratory use, being widely used as substrate for determination of enzymatic activity (Bailey et al., 1992).

However, this substrate is no longer marketed by some companies, creating the demand for the development of a methodology hemicellulose extraction with conditions appropriate for determining xylanase enzymatic activity.

There are some bioenergy and biorefinery studies where hemicelluloses of different biomasses, highlighting materials such as sugarcane straw and bagasse (Alves et al., 2020; Brienzo et al., 2009; Rocha et al., 2017), soft and hard woods (Wang et al., 2015) and grain biomass like soy and corn (Corredor et al., 2008; Bernal et al., 2014). These works take advantage of the portion of hemicellulose based on its solubility or its ability to convert to a molecule of greater value. In many of these conversion occurs the use of enzymes that act in xylan chains, xylanases. To carry out these conversions efficiently is need to know the relationship that the enzyme has with its substrate. Xylan is a versatile raw material, in addition to activities of determination of the enzymatic activity. As macromolecule, hemicellulose can be used as an additive in paper production, in its hydrolyzed form can be used in the form of oligosaccharides in therapeutic activities and under monomer form can be used for production xylitol (Freitas et al., 2019). Due to its versatility it is of interest seek various sources of extraction of hemicellulose.

Although it is possible to extract hemicellulose from several lignocellulosic biomass. The biggest challenge is to find methods and biomasses that provide the extraction of hemicellulose with high purity and high yield (Brienzo et al., 2016). Identifying a suitable substrate further research may emerge in order to optimize and improve methods for extracting products from added value. In this context, the project proposes the evaluation of different lignocellulosic biomasses as a source of hemicellulose extraction and test some characteristics of interest of these obtained substrates.

2 MATERIAL AND METHODS

2.1 HEMICELLULOSE EXTRACTION

Biomass was treated individually with a solution of tetracetic acid ethylenediamine in concentration 0.2% (m/v) for 1 hour at 90 °C. This process removed metals present in the biomass that can react with H₂O₂. After removal of metals 10 g of biomass was added in a vial containing 200 mL of 6% H₂O₂ (m/v) solution at a pH of 11.6. That mixture was stirred for 4 hours and finally filtered using filter paper to separate the solid material, and hemicellulose in the liquid fraction.

After filtered the mixture had its pH adjusted to 6 using 6 mol/L HCl. After adjustment, this material is heated in oven at 45 °C to concentrate.

2.2 DETERMINATION OF ENZYMATIC ACTIVITY

The xylan extracted from each biomass was evaluated in the xylanase activity according to BAILEY et al. (1992), using commercial enzymes Cellic HTEC and Celluclast. Quantification of reducing sugars released from xylan according to the 3,5-dinitrosalicylic acid method (Miller, 1959).

2.3 AFFINITY BETWEEN ENZYME AND SUBSTRATE

The affinity between enzymes and different xylans was obtained by the method created by Neilands & Sumpf (1955), calculating the values of Km, Michaelis constant, and Vmax, maximum value of initial speed, thus building the graphs of enzymatic kinetics.

2.4 DETERMINATION OF INSOLUBLE LIGNIN

About 300 mg of biomass was be added to 1.5 mL of 72% H₂SO₄ (m/m) and the reaction will take place at 45 °C for the time of 7 minutes (Brienzo et al., 2009). The reaction is interrupted by the addition of 45 mL of distilled water. This mixture is autoclaved at 121 °C for 30 minutes (Gouveia et al., 2009). The content will be filtered through a number 4 porous plate filter previously tared. The solid residue on the plate is washed with distilled water and dried in an oven 105 °C to constant weight for determination of insoluble lignin.

3 RESULTS AND DISCUSSION

3.1 HEMICELLULOSE EXTRACTION AND CHEMICAL CHARACTERIZATION

Hemicellulose extractions was elaborated in triplicates, as well ass chemical characterization to determinate the amount of insoluble lignin, and the results obtained were summarized in the table 1.

Table 1. Hemicellulose extraction and insoluble lignin content

Biomass	Hemicellulose recovered (g/100g)	Insoluble lignin (%)
Sugarcane bagasse	10.53 %	13.7%
Sugarcane leaves	9.40 %	18%

Using the aforementioned extraction method, the amount of average extracted hemicellulose is about 10% of the biomass, that is, for each quantity of biomass treated, 10% of that mass will be converted into hemicellulose.

The amounts of cellulose, lignin and hemicellulose in sugar cane vary depending on the plant age, degree of fertilization, variety species and plant tissue analyzed. Exist certain dispersion of results, where there are studies presenting hemicellulose data composing only 8% of the mass (GOUVEA, 2009), while that other studies this amount can reach 33% (PURCHASE, 1995). Therefore, to validate the effectiveness of the method in extracting hemicellulose from biomass chemical analysis tests can be done to quantify the hemicellulose that makes up the biomass used.

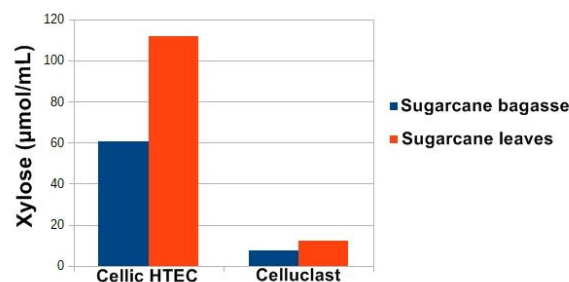
The amount of insoluble lignin extracted from the hemicellulose was expressive. In the case of bagasse, 13.7% of the total mass extracted is insoluble lignin residual, in the case of the leaves this amount was 18%. Lignin is a molecule that is difficult to break down.

As lignin biomass, along with hemicellulose, make up a matrix that protects cellulose from action chemical, physical and biological. Residual lignin present in the extracted hemicellulose may have an similar to what it does as biomass, preventing the full action of xylanolytic enzymes hemicellulose, resulting in media with lower affinity and lower reaction speeds

3.2 DETERMINATION OF ENZYMATIC ACTIVITY

Similar to extraction, the determination of enzymatic action was also carried out in triplicate form. Below, figure 1 represents the converted values of xylose obtained at from the enzymatic hydrolysis reaction.

Figure 1. Enzymatic Hydrolysis



It can be noted the discrepant relationship between results obtained from the different enzymes cockatiels. The Cellic HTEC enzyme releases a greater amount of xylose reacting the same amount

of material than Celluclast. This difference can be linked to the type and purpose of the different cocktails enzymatic. The HTEC was more effective to hydrolyze the hemicellulose.

3.3 AFFINITY BETWEEN ENZYME AND SUBSTRATE

The results of affinity test between Cellic HTEC and Celluclast enzymes with sugarcane bagasse and leaves substrates were summarized in the tables 2 and 3. The results showed that in both uses of cocktails, the sugarcane leaves is superior to sugarcane bagasse. This is evident since the K_m values for the cane leaves are about 4 times smaller. This identifies that hemicellulose of sugarcane leaves has greater affinity to enzymes than sugarcane bagasse.

As for maximum speed, the Cellic HTEC enzyme presents similar speeds, being the substrate with sugarcane leaves hemicellulose faster. When we use the Celluclast enzyme at the maximum speed of substrate with bagasse hemicellulose becomes about 3 times faster than the substrate with leaves.

Table 2. Estimated K_m and V_{max} results for sugarcane bagasse.

Enzymes	K_m ($\mu\text{mol/mL}$)	V_{max} ($\mu\text{mol/min}\cdot\text{mL}$)
Cellic HTEC	222,989	47,196
Celluclast	198,129	19,400

Table 3. Estimated K_m and V_{max} results for sugarcane leaves.

Enzymes	K_m ($\mu\text{mol/mL}$)	V_{max} ($\mu\text{mol/min}\cdot\text{mL}$)
Cellic HTEC	46,048	55,404
Celluclast	38,927	6,9341

4 CONCLUSION

According to the data presented, it is possible to conclude that each biomass has its advantage. The sugarcane bagasse showed a better extraction, and the quantities extracted and their degree of purity were slightly larger than the leaf. As for the affinity and speed, the hemicellulose of the sugarcane leaves showed an affinity much higher than that of the sugarcane bagasse, however the speed may vary quite according to the enzyme cocktail used. Therefore, it is possible to conclude that both biomasses have potential for extraction and use. The feature that will adjusting one biomass as superior to the other will be the purpose of its use.

ACKNOWLEDGMENTS

This study was grant by processes number 2018/07648-0, 2019/12997-6 and 2017/22401-8, São Paulo Research Foundation (FAPESP).

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