Brazilian Journal of Development

# Xylan solubilization and use as carbon source/inductor for microbial xylanase production

# Solubilização de Xylan e utilização como fonte/indutor de carbono para a produção de xilanase microbiana

DOI:10.34117/bjdv6n10-320

Recebimento dos originais: 10/09/2020
Aceitação para publicação: 15/10/2020

**Danilo Bueno**
Institute for Research in Bioenergy (IPBEN), São Paulo State University (UNESP), Rio Claro, SP, 13500-230, Brazil
e-mail: danilo.bueno1@unesp.br

**Michel Brienzo**
Institute for Research in Bioenergy (IPBEN), São Paulo State University (UNESP), Rio Claro, SP, 13500-230, Brazil
e-mail: michelbrienzo@unesp.br

**ABSTRACT**
Xylanases are enzymes that hydrolyze the main chain of xylan, producing xylooligosaccharides and xylose. These enzymes are of great importance for the pharmaceutical industries and also for the production of biofuels. For the production of microbial xylanases, xylan substrate is used, which can be obtained from different biomass such as sugarcane residue. However, the problem when using commercial xylanases for xylanase induction is its high cost. In this context, this study sought to use a methodology for extracting xylan from sugarcane in alkaline medium quickly and inexpensively, and also the application of this xylan as substrate for xylanase production, using for both fungi Aspergillus nidulans, Aspergillus tubingensis and Aspergillus versicolor. The cultures in the presence of 1% xylan for these microorganisms were compared with cultures containing 1% wheat bran, an important substrate for xylanase production. The results indicate that xylan extracted by this methodology, besides presenting some amount of lignin, contributed to a higher production of xylanases for A. nidulans, and in A. tubingensis and A. versicolor the production was close to the when wheat bran was used as an inducer.

**Keywords:** xylanases, sugarcane, biomass, biorefinery, biofuels.

**RESUMO**
As xilanases são enzimas que hidrolisam a cadeia principal do xilano, produzindo xiloligossacarídeos e xilose. Estas enzimas são de grande importância para as indústrias farmacêuticas e também para a produção de biocombustíveis. Para a produção de xilanases microbianas, é utilizado substrato de xilano, que pode ser obtido a partir de diferentes biomassas, tais como resíduos de cana-de-açúcar. No entanto, o problema na utilização de xilanases comerciais para a indução de xilanase é o seu elevado custo. Neste contexto, este estudo procurou utilizar uma metodologia de extracção rápida e barata de xilano da cana de açúcar em meio alcalino, e também a aplicação deste xilano como substrato para a produção de xilanase, utilizando para ambos os fungos Aspergillus nidulans, Aspergillus tubingensis e Aspergillus versicolor. As culturas na presença de 1% xylan para estes microrganismos foram comparadas com culturas contendo 1% de farelo de trigo, um importante substrato para a produção de xilanase. Os resultados indicam que o

Brazilian Journal of Development

xilano extraído por esta metodologia, além de apresentar alguma quantidade de lignina, contribuiu para uma maior produção de xilanases para A. nidulans, e em A. tubingensis e A. versicolor a produção foi próxima de quando o farelo de trigo foi utilizado como indutor.

**Palavras-chave**: xilanases, cana-de-açúcar, biomassa, biorefinaria, biocombustíveis.

## 1 INTRODUCTION

Xylanases are hydrolytic enzymes that cleave β-1,4 bonds of xylan, one of the components of the plant cell wall (Walia et al., 2017). Complete hydrolysis of xylan requires the joint action of enzymes that hydrolyze its main chain (xylanases and β-xylosidise) and accessory enzymes responsible for hydrolyzing its branches (α-arabinofuranosity, acetyl-xylan esterase, α-glucuronosity and feruloyl esterase) (Yang et al., 2017).

Xylanolitic enzymes can be induced by xylan, in the most of the time its best inducer (Fernández-Espinar et al., 1994), however some authors showed the inducing effect of xylan fragments (Aro et al., 2005) and its repression by readily assimilable sugars such as glucose, lactose and xylose (GASPAR et al., 1997). It is known that xylan is the second most abundant renewable polysaccharide, the first being cellulose (Melati et al., 2018; Walia et al., 2017).

Lignocellulosic substrates are used to obtain xylanolitic enzymes on a large scale and with low cost in submerged or solid cultures (MISHRA et al., 1990). The use of xylanases in industrial processes has increased over time. In industries, xylanases can be used in processes such as bakery, production of biofuels, and can also be used in the food industries in processes such as juice extraction and clarification (Lara et al., 2013).

During growth in xylan, several microbial species produce specific xylanases with little or no cellulase. However, when microorganisms are grown in a culture medium containing cellulose, cellulases are produced together with xylanases. One strategy used to obtain the cellulosic-free xylanolitic complex is to grow microorganisms in a culture medium containing xylan not contaminated with cellulose (Brienzo et al., 2011; BIELY et al., 1986).

Given this demand, it would be important to develop a low cost hemicellulose extraction methodology that could be used in the microorganism cultures for the production of xylanases. In this way, each laboratory could extract its hemicellulose for use in the cultivation/induction of xylanase production.

In this context, this study sought to evaluate a methodology for xylan extraction from sugarcane in alkaline medium quickly and cheaply, and also to apply this xylan as a substrate for

Brazilian Journal of Development

the xylanase production, using fungi *Aspergillus nidulans*, *Aspergillus tubingensis* and *Aspergillus versicolor*.

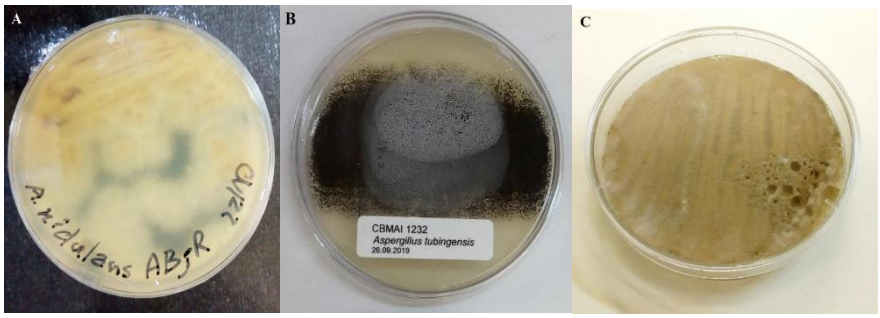
## 2 MATERIAL AND METHODS

The sugarcane bagasse was provided by São João Mill (Araras-SP). The bagasse was ground and then selected using a 20 mesh sieve (Figure 1).

Figure 1. Sugar cane bagasse before and after milling (20 mesh).


The alkaline extraction was performed with 6% $H_2O_2$ m/v at 25 °C overnight for 4 hours (Alves et al., 2020; Brienzo et al., 2009), calculating the yield of extracted xylan and quantifying the presence of insoluble lignin (Golveia et al., 2009). The solubility of the extracted xylan was determined by making a 1% xylan solution in 50 mmol/L sodium acetate buffer, pH 4.8, according to ALVES (2020). For the tests, the species of fungi *A. nidulans*, *A. tubingensis* and *A. versicolor* were cultivated (Figure 2).

Figure 2. *A. nidulans* (A), *A. tubingensis* (B) e *A. versicolor (C)*.

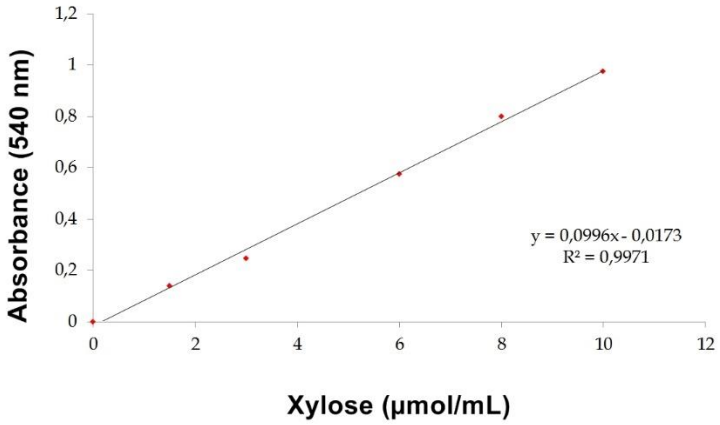



Cultures were made at 30 °C in Erlenmeyer flasks (125 ml), containing 9 ml of the medium, where 1 ml of the spore suspension (5x107 spores / ml) was inoculated for 5 days in Vogel's liquid medium (1965) containing 1 % of wheat bran (control) and 1% of xylan, in triplicates.

Brazilian Journal of Development

The quantification of reducing sugars was elaborated by the 3,5-dinitrosalicylic acid (DNS) method (MILLER, 1959), using as a parameter a raw xylose pattern with different concentrations (Figure 3).

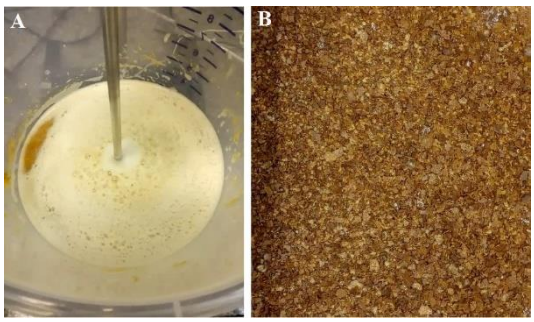
Figure 3. Standard curve.




## 3 RESULTS AND DISCUSSION

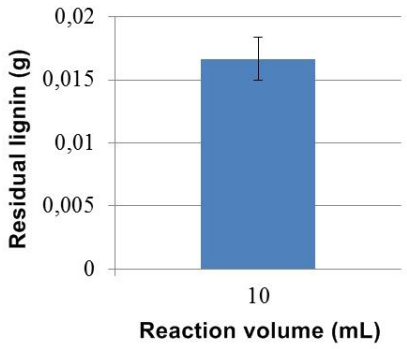
A total of 50g of xylan extracted in alkaline medium was obtained under mechanical agitation (Figure 4). The extraction yield was around 50%, containing an average of only 5% insoluble lignin (Figure 5).

Figure 4. Xylan extraction. (A) Mechanical agitation. (B) xylan extracted.



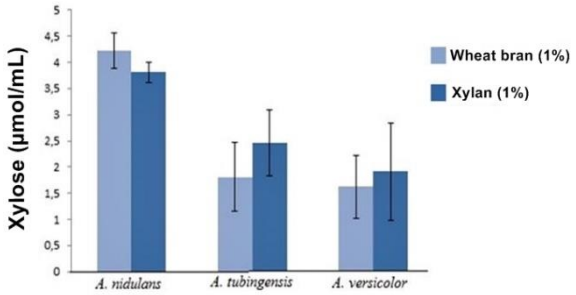
Brazilian Journal of Development

Figure 5. Determination of insoluble lignin by chemical characterization.




Both wheat bran and xylan (extracted from sugarcane bagasse) at a concentration of 1% in VOGEL medium (1965) induced the production of xylanases in *A. nidulans*, *A. tubingensis* and *A. versicolor*, which could be found via enzymatic hydrolysis by reacting the crude extract of each culture at 50ºC for 5 minutes in the presence of 1% xylan (Figure 6), producing different concentrations of xylose. For *A. nidulans*, xylan presented itself as a slightly better substrate for inducing the production of xylanases, whereas for *A. tubingensis* and *A. versicolor*, the average of wheat bran was higher than that of xylan, but the results are statistically similar.

Figure 6. Xylose production via enzymatic hydrolysis.

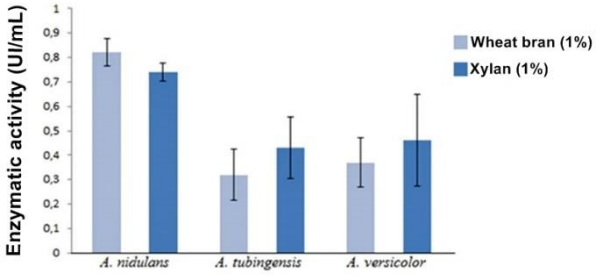



After xylose production determination by enzymatic hydrolysis of fungi grown in wheat bran and xylan, it was observed that the enzymatic activity of xylanases produced by each microorganism in the presence of the two substrates. In the presence of wheat bran, *A. nidulans* xylanases showed an average enzyme activity of 0.82 IU/mL whereas in the presence of wheat bran, this average value was equivalent to 0.74 IU/mL (Figure 7). The observed results are statistically different, but with values very closed to xylan.

Brazilian Journal of Development

For *A. tubingensis*, the production of xylanases in wheat bran was equivalent to an average of 0.32 IU/mL, and in xylan it was 0.43 IU/mL. For *A. versicolor*, the activity of xylanases in the presence of wheat bran averaged 0.37 IU/mL, while in the presence of xylan the value obtained was on average 0.46 IU/mL. Although xylan apparently induced a greater production of xylanases, statistically the values are very close. The results show that both wheat bran and xylan are good inducers of xylanases in culture medium for these microorganisms.

Figure 7. Determination of enzymatic activity.




Several agricultural and organic wastes have been used as a carbon source for inducing the xylanase production (Bhardwaj et al., 2019), such as wheat bran (Kumar et al., 2018), sugarcane bagasse (Suleman et al., 2016), wood pulp (Kalpana & Rajeswari, 2015), rice straw (Bhardwaj et al., 2017), among others.

The use of commercial xylans to induce the production of xylanases in microorganisms is not an economically viable alternative (Rosmine et al., 2019). However, there is a need to develop new methodologies for extracting and applying xylan in a culture medium in an easy, fast and low cost way. This study presents itself as an alternative to solve this problem, because even containing residual lignin in its composition, the substrate developed here was efficient for inducing the production of xylanases.

## 4 CONCLUSION

Both wheat bran and xylan at a concentration of 1% were good substrates for inducing the xylanase production in the microorganisms evaluated here, and potentially in many other microorganisms. This evaluated methodology can help to reduce the cost and time required for the production of xylanases using this xylan as a substrate, taking into account its yield and its low amount of insoluble. lignin.

**ACKNOWLEDGMENT**

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

**REFERENCES**

Alves, R.C., Melati, R.B., Casagrande, G.M.S., Contiero, J., Pagnocca, F.C., Brienzo, M. Sieving process selects sugarcane bagasse with lower recalcitrance to xylan solubilization. Chemical Technology and Biotechnology, p. 1-8, 2020.

Aro, N., Pakula, T., Pentillä, M. Transcriptional regulation of plant cell wall degradation by filamentous fungi. FEMS Microbiology Reviews, 29, 719-739, 2005.

Bailey, M.J., Biely, P., Poutanen, K. Interlaboratory testing of methodos for assay of xylanase activity. J. Biotech., v. 23, n. 3, p. 257-270, 1992.

Bhardwaj, N., Chanda, K., Kumar, B. Statistical optimization of nutritional and physical parameters for xylanase production from newly isolated Aspergillus oryzae LC1 and its application in the hydrolysis of lignocellulosic agro-residues. BioResources 12, 8519–8538, 2017.

Bhardwaj, N., Kumar, B., Verma, P. A detailed overview of xylanases: an emerging biomolecule for current and future prospective. Bioresources and Bioprocessing, 6, 1-36, 2019.

Biely, P., MacKenzie C.R., Puls, J., Schneider, H. Cooperativity of esterases and xylanases in the enzymatic degradation of acetyl xylan. Bio/Technology, 4, 731-733, 1986.

Brienzo, M., Siqueira, A.F., Milagresm A.M.F. Search for optimum conditions of sugarcane bagasse hemicellulose extraction. Bioch. Eng. J., v. 46, p. 199-204, 2009.

Brienzo, M., Monte, J.R., Milagres, A.M.F. Induction of cellulase and hemicellulase activities of Thermoascus aurantiacus by xylan hydrolyzed products. World Journal of Microbiology and Biotechnology, v. 28, p. 13-119, 2012.

Fernández-Espinar, M., Piñaga, F., Graaff, L., Visser, J., Ramón, D., Vallés, S. Purification, characterization and regulation of the synthesis of an *Aspergillus nidulans* acidic xylanase. Biochemical Engineering, 42, 555-562, 1994.

Gaspar, A., Cosson, T., Roque, C., Thonart. Study on the production of a xylanolytic complex from Penicillium canescens 10-10c. Applied Biochemistry and Biotechnology, v.67, p.45- 67, 1997.

Golveia, E.R., Nascimento, R.T., Souto-Maior, A.M., Rocha, G.J.M. Validação de metodologia para a caracterização química de bagaço de cana-de-açúcar. Química Nova, v. 32, p. 1-4, 2009.

Kalpana, V.N., Rajeswari, V.D. Production of xylanase from various lignocellulosic waste materials by Streptomyces sp. and its potential role in deinking of newsprint. Asian Journal of Biochemistry, 10, 222–229, 2015.

Kumar, B.A., Amit, K., Alok, K., Dharm, D. Wheat bran fermentation for the production of cellulase and xylanase by Aspergillus niger NFCCI 4113. Research Journal of Biotechnology, 13, 11-18, 2018.

Lara, C.A. Xilanases de leveduras e fungos leveduriformes e sua aplicação em processos de produção de bioetanol lignocelulósico e panificação. Tese (Doutorado) - UFMG, Belo Horizonte, 2013.

Melati, R.B., Shimizu, F.L., Oliveira, G., Pagnocca, F.C., Souza, W., Sant'Anna, C., Brienzo, M. Key factors affecting the recalcitrance and conversion process of biomass. Bioen. Res., v. 12, p. 1-20, 2018.

Mishra, C., Forrester, I.T., Kelley, B.D., Burgess, R.R., Leatham, G.F. Characterization of a major xylanase purified from Lentinula edodes cultures grown on a commercial solid lignocellulosic substrate. Applied Microbiology Biotechnology, v.33, n. 2, p. 226-232, 1990.

Rosmine, E., Sainjan, N.C.E., Silvester, R., Varghese, S.A. Utilisation of agrowaste xylan for the production of industrially important enzyme xylanase in deinking of newsprint. International Journal of Current Microbiology Applied Science, 8, 2061-2076, 2019.

Suleman, M., Bukhari, I.H., Aujla, M.I., Faiz, A.H. Production and characterization of xylanase from Aspergillus niger using wheat bran, corn cobs, and sugar cane bagasse as carbon sources with diferent concentrations. Journal of Bioresource Management, 3, 1-9, 2016.

Vogel, P., Rhee, V., Koelensmid, B. A Rapid Screening Test for Aflatoxin-synthesizing Aspergilli of the *flavus-oryzae* Group. Journal of Applied Bacteriology, v. 28, p. 213-220, 1965.