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HIDRÓLISE E QUANTIFICAÇÃO DA GLICOSEDE *ERAGROSTIS PLANA NEES*[1]

HYDROLYSIS AND QUANTIFICATION OF GLUCOSE FROM ERAGROSTIS PLANA NEES

Bianca M. Dolianitis[2], Viviane D. Frescura[3], Jefferson F. Silva[4], Norton M. Silveira[5], Estêvão S. L. Cunha[6], Iuri p. Bitencourte[7], Renan O. Ferreira[8], Giovani L. Zabot[9], Marcus v. Tres[10] e Janessa A. Zappe[11]

RESUMO

O capim-annoni é uma planta invasora de difícil controle, originária da África do Sul e introduzida acidentalmente no Brasil. A espécie invade o Brasil e outros países como Estados Unidos e Uruguai. O estado com maior invasão é o Rio Grande do Sul, o que é preocupante devido a presença do bioma Pampa. O método mais eficaz para controle dessa invasora é o uso de herbicidas, porém, após aplicação a biomassa da espécie permanece na área em processo de decomposição liberando substâncias alelopáticas que interferem no desenvolvimento de espécies nativas. Por isso, a importância de fornecer alternativas para uso dessa biomassa. Assim, o estudo tem como objetivo padronizar o método de hidrólise com água subcrítica da biomassa de capim-annoni e quantificar a glicose nos hidrolisados. Para isso foram coletadas plantas de capim-annoni em estádio vegetativo, realizando-se a secagem e moagem desse material. No processo de hidrólise foram avaliadas as variáveis tempo, temperatura e razão água/sólido. Após a hidrólise realizou- se a quantificação de glicose nos hidrolisados utilizando o método DNS (Ácido 3,5-dinitrosalicílico) e quantificação em espectrofotômetro. A melhor condição de hidrólise para obtenção da glicose ocorreu a temperatura de 220 ºC na maior razão água/sólido no tempo de 2 minutos. Na temperatura de 180 ºC na menor razão água/sólido nos tempos de 8 e 10 minutos também se obteve elevada quantidade de glicose. O processo de hidrólise com água subcrítica foi eficiente para obtenção de glicose na biomassa estudada.

Palavras-chave: Planta invasora; Água subcrítica; Açúcar fermentescível; Capim-annoni.

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2 Universidade Federal de Santa Maria - UFSM. E-mail: biancadolianitis@hotmail.com. ORCID: https://orcid.org/0000-0003-1818-9529
3 Universidade Federal de Santa Maria - UFSM. E-mail: viviane.frescura@ufsm.br. ORCID: https://orcid.org/0000-0003-1520-6979
4 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0002-8421-3817
5 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0001-6549-648X
6 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0003-2565-6017
7 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0002-8083-5096
8 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0002-5576-1304
9 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0001-7933-6161
10 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0001-5252-0735
11 Universidade Federal de Santa Maria - UFSM. ORCID: https://orcid.org/0000-0002-8880-4729

ABSTRACT

Annoni grass is an invasive and difficult to control plant that originated in South Africa and was accidentally introduced to Brazil. The species invades Brazil and other countries such as the United States and Uruguay. The state with the largest invasion is Rio Grande do Sul, which is worrisome due to the presence of the Pampas biome. The most effective method to control this invader is the use of herbicides, but after application, the biomass of the species remains in the area in the process of decomposition, releasing allelopathic substances that interfere with the development of native species. Therefore, it is important to provide alternatives for the use of this biomass. Thus, the study aims to standardize the method of subcritical water hydrolysis of biomass of capim-annoni and quantify the glucose in the hydrolysates. For this purpose, Annoni grass plants were collected in their vegetative stage, and the material was dried and ground. In the hydrolysis process, the variables time, temperature and water/solid ratio were evaluated. After hydrolysis, the glucose in the hydrolysates was quantified using the DNS (dinitrosalic acid) method and quantification in spectrophotometer. The best hydrolysis condition to obtain glucose occurred at a temperature of 220 ºC in the highest water/solid ratio for 2 minutes. A high amount of glucose was also obtained at the temperature of 180 ºC in the lowest water/solid ratio in the times of 8 and 10 minutes. The process of hydrolysis with subcritical water was efficient to obtain glucose in the studied biomass.

Keywords: *Invasive plant; Subcritical water; Fermentable sugar; Annoni grass.*

1 INTRODUCTION

Annoni grass is an invasive and difficult to control plant that originated in South Africa and was accidentally introduced to Brazil. Currently, the invasion of the species occurs in several states in Brazil and even in other countries (MEDEIROS and FOCHT, 2007).

Among the states invaded by the species, the state with the highest level of invasion of capim-annoni is Rio Grande do Sul. One of the reasons for the high level of invasion of the species in this state is due to the presence of the Pampa biome, which is mainly composed of field vegetation, one of the main invasive environments of the species. In this biome, the vegetation cover is used as natural pasture or occupied by agricultural activities, mainly rice cultivation (IBGE, 2018). Thus, the presence of Annoni grass in this biome hinders the development of other species, causing environmental and socioeconomic damage.

In an attempt to control this invasive species, many institutions are studying control strategies using different techniques. However, the most efficient technique for its control is the use of herbicides. But with the use of herbicides, the dry biomass remains in the area previously infested by the grass and may cause allelopathy on the germination of native species and species with interesting nutritional characteristics for agricultural activity, as observed by Ferreira *et al.* (2008), who studied the allelopathic effect of grass-annoni on the germination of seeds of *Paspalum notatum* Flüggé. P. regnelli Mez, *Megathyrsus maximus* B. K. Simon and S. W. Jacobs, *Setaria sphacelata* (Schumach) Staff and C. E. Hubb ex Chipp and observed that the allelopathic effect of Annoni grass is manifested in the decomposition process of plant tissues. Agricultural species such as *Oryza sativa* (rice), *Avena sativa* (white oats), and *Trifolium resupinatum* (Persian clover) were found to be sensitive to allelopathy from Annoni grass in a study conducted by Quatrin *et al.* (2025), where the aqueous extract from the dry aerial part was applied to crop seeds, causing a significant reduction in the germination percentage, initial length, and dry mass of the species. In isolated cases, annoni grass may also have an allelopathic effect that stimulates the germination and development of species such as Vachellia caven (Molina) Seigler & Ebinger, whose seeds, when exposed to an aqueous extract of annoni grass, showed stimulation of germination and initial development (MENDES *et al*., 2024). Therefore, it is important that this biomass has another purpose than to remain in the environment where other species are found, native or with nutritional quality for agricultural activity. To propose another purpose for this biomass, it is necessary to obtain information about its characteristics. And the subcritical water hydrolysis method may be an alternative, as it is considered a promising method for obtaining compounds from biomass. The variables involved in this process, such as temperature and pressure, vary from 150 ºC to 374 ºC and from 5 to 22 MPa (SARKER *et al*., 2021). The method has the advantages of being environmentally friendly, generating no solvent waste, having a short reaction time, being easy to operate, and being capable of dissociating cellulose and hemicellulose (CAMARENA-BONONAD *et al*., 2024).

Therefore, the present work aims to standardize the method of subcritical water hydrolysis of biomass of Annoni grass and to quantify the glucose present in the hydrolysates.

2 MATERIAL AND METHODS

The collection of leaves and stems of Annoni grass was conducted in the municipality of Cachoeira do Sul, in the districts of Cordilheira (lat: 30 11 33.1332 S, long: 52 51 4.0) and Capané (lat: 30 5 18.7656 S, long: 52 55 47.4384 W), when the plants were in the vegetative stage.

After collection, leaves and stems were separated and roots were discarded. The leaves and stems were placed in brown paper bags to be dried in an oven at 55 ºC. The samples remained in the oven until their mass remained constant. After drying, the biomass of Annoni grass was ground in a knife mill and kept under refrigeration for the subsequent hydrolysis process.

The hydrolysis unit of the Laboratory of Agroindustrial Process Engineering (LAPE) of UFSM was used for the hydrolysis process. In the process of hydrolysis with subcritical water, the variables time (1, 2, 3, 4, 6, 8, 10 min), temperature (180 and 220 ºC) and water/solid ratio (5.5 and 11 g water/g biomass) were evaluated.

After hydrolysis, the flasks containing the hydrolyzed material were kept refrigerated for subsequent glucose determination.

The determination of the amount of glucose in the hydrolysates was carried out according to the protocol for the determination of total sugars by the method of Maldone; Carvalho; Ferreira (2013),the DNS (dinitrosalic acid) method.

For the determination of the glucose standard curve, the standard solution of 1 g/L glucose was diluted with distilled water in test tubes. After preparation, the solution was added to the spectrophotometer.

After the determination of the glucose standard curve, samples of hydrolysates of Annoni grass biomass were prepared. First, all the samples were filtered.

Due to the lack of a glucose quantification assay with Annoni grass biomass, dilution tests were performed until a final sugar concentration between 0.1 and 1.0 g/L was found. After each sample was diluted in test tubes, 1 mL of DNS was added to each tube. This step was performed in the absence of ambient light. The diluted DNS solution was heated in a water bath at approximately 100 °C for five minutes and then cooled in an ice bath for another five minutes. Samples were prepared in duplicate.

After the water bath and ice bath, 16 mL of double potassium sodium tartrate was added, and finally the absorbance was read in a spectrophotometer to quantify the glucose content of each sample.

To analyze the data obtained, the treatments were arranged in a factorial arrangement (2x2x7), where the factors were the temperatures of 180 and 220 °C, the ratios between biomass and water used in the hydrolysis of 5 and 10, and the hydrolysis times of 1, 2, 3, 4, 6, 8 and 10 minutes. The experimental design used was completely randomized and the analyses were performed in duplicate. The data obtained were subjected to an analysis of variance and the means were compared using the Scott-Knott test with a 5% probability of error.

3 RESULTS AND DISCUSSION

It is observed in Table 1 that when the lowest ratio of biomass to water was used (S/F=5), the highest concentrations of glucose were obtained in the times of 8 and 10 minutes for the temperature of 180 °C and 10 minutes at the temperature of 220 °C, while for the S/F=10 at 180 °C the hydrolysis time did not interfere with the amount of glucose, and at 220 °C the best result was obtained in the time of 2 minutes, followed by the times of 3 and 8 minutes.

Table 1 - Glucose (⟦g.L⟧^(-1)) in our biomass hydrolysate of *Eragrostis plana* Nees (grass-annoni) at the different temperatures, biomass to water ratios and hydrolysis times.

		Time						
S/F	**Temperature**	**1 min**	**2 min**	**3 min**	**4 min**	**6 min**	**8 min**	**10 min**
5	**180 °C**	7.58 Ba	5.08 Bb	7.01 Ba	4.10 Bb	7.81 Ba	21.22 Aa	16.88 Ab
	220 °C	6.53 Ba	13.51 Ba	10.35 Ba	9.55 Ba	10.00 Ba	7.38 Bb	25.75 Aa
	180 °C	7.24 Aa	3.66 Ab	3.81 Ab	6.58 Ab	4.75 Aa	4.01 Ab	3.38 Aa
10	**220 °C**	6.85 Ea	31.74 Aa	24.20 Ba	12.15 Da	9.25 Ea	17.34 Ca	6.94 Ea

(1) Means followed by the same lowercase letter in the columns within each S/F and same uppercase letter in the line do not differ by the Scott-Knott test, at a 5% probability of error.

The highest glucose values obtained were 16.88; 21.22; 24.20; 25.75 and 31.74〖g.L^(-1), at 180 °C/ 10 minutes/ S/F=5, 180 °C/ 8 minutes/ S/F=5, 220 °C/ 3 minutes/S/F=10, 220 °C/ 10 minutes/ S/F=5 and 220 °C/ 2 minutes/ S/F=10, respectively. These results are higher than those found in the study by Abaide *et al.* (2019), where the highest concentration was 4.2 g/L using rice husk. Also, Draszewski *et al.* (2018), when studying the hydrolysis of rice straw, obtained a glucose concentration of 1.54 g.100 g 〖biomass〗^(-1). In the present study, both 180 °C and 220 °C provided high glucose concentrations. This must be related to the temperature at which lignocellulosic biomass is converted into fermentable sugars and bioproducts, which ranges from 50 to 280°C, since the temperatures studied are between these values (ABAIDE *et al.*, 2018).

This can be explained by the different composition and structure of the biomass used, as annoni grass is a grass that contains large amounts of cellulose and hemicellulose (Alfaya *et al.*, 2002), which are more sensitive to hydrolysis, and rice husks also contain hemicellulose and cellulose, but also has a large amount of silica and lignin (Abaide *et al.*, 2019), which means that less glucose is extracted. In other words, annoni grass is capable of releasing a greater amount of glucose due to its lower structural barrier, as it has a large amount of cellulose and hemicellulose and lower amounts of lignin and silica, unlike rice husks, which have higher lignin and silica contents, resulting in a more resistant structural barrier and thus releasing less glucose.

In the work of Draszewski *et al.* (2018), the temperature condition that provided the best results for sugar concentration was the one set at 220 °C. This temperature also provided high glucose concentrations in the present study. Abaide *et al.* (2018) using rice husk hydrolysis to obtain fermentable sugars, the highest value of reducing sugars found was at 220 °C using a biomass to water ratio of 5. Using annoni grass biomass, the highest concentration of glucose was also found at the temperature of 220 °C, but the biomass to water ratio was equal to 10. Abaide *et al.* (2019) once again using the rice husk, also by the highest concentration of glucose was obtained at the time of 3 minutes of hydrolysis at the temperature of 220 ºC when using the highest ratio of biomass and water. With the biomass of grass-annoni, the highest concentration of glucose was also obtained when used at the temperature of 220 ºC and higher ratio of biomass and water, but this concentration was obtained in the time of 2 minutes.

In the study with sugar cane bagasse performed by Prado *et al.* (2013), the highest concentration of glucose was obtained at a temperature of 251 ºC, where the concentration was 20.05 mg/g of bagasse at 30 minutes of hydrolysis, the highest concentration of glucose in this study was obtained at a different temperature and time from those found in the present study with biomass of grass-annoni, where the highest concentration occurred with only 10 minutes at a temperature of 220 ºC. In the present study the hydrolysis time used was less than that used by Prado *et al.* (2013), this is mainly due to the type of biomass used in the different studies.

Thus, it is possible to see that the hydrolysis process with subcritical water is an efficient method for obtaining fermentable sugars and bioproducts from different types of biomass and even residual biomass. It has an advantage over other methods in that it uses water as a solvent, which is considered a green solvent, contributing to the environment through sustainable processes.

4 CONCLUSION

From the results obtained it was observed that the hydrolysis with subcritical water was efficient for the extraction of glucose from the biomass of capim-annoni, recommending the use of a hydrolysis time of 2 minutes and temperature of 220 ºC for a mass ratio of biomass and water equal to 10. And in cases of limited amount of biomass, it is recommended to use half of the biomass at a temperature of 180 ºC and times of 8 or 10 minutes, or 220 ºC and time of 10 minutes.

Considering the results obtained for the glucose content in the biomass of Annoni grass, it can be proposed as an alternative for this biomass to be used in processes that use glucose, such as the production of biofuels. Since the concentration of glucose in this biomass is higher than that obtained from other biomasses used in these processes.

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