OPTIMIZATION OF SOYBEAN (*GLYCINE MAX*) HULLS ACID HYDROLYSIS TO PRODUCE SUGARS FOR MICROBIAL CULTIVATION

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ABSTRACT - The soybean hull is approximately 5% of raw soybean. This paper proposes the use of this residual hull as a substrate for acid hydrolysis reactions in order to obtain sugars. For this, variations of pressure, temperature and quantities of acid were made. The particle size was also tested using natural hull and ground hull. It was observed that under the pressure of 1.1 atm and the temperature of 120 °C with 0.20 g of sulfuric acid, the ground hull led to the best sugar production, standing out from the others, with a production of 24.93 g/L.

1. INTRODUCTION

Brazil stands out as a world producer of soybeans, being the second largest producer of the grain with an estimated production of 113.923 million tons (EMBRAPA, 2017). For each ton of harvested soybeans to be processed, approximately 50 kg of hulls is obtained, which represents approximately 5% of the initial mass (BLASI et al., 2000). The soybean hulls are rich in lignocellulosic material, and the chemical composition of this material comes mainly from glucose (39.7%), xylose (19.6%) and proteins (13.1%) (CASSALES, 2010). These two first compounds are monosaccharide carbon sources which can be used by microorganisms such as yeast. Lignocellulosic residues are mainly composed of alfa cellulose, hemicellulose and lignin, which are compounds resistant to direct bioconversion and must undergo a previous treatment: the hydrolysis. For this treatment, usually physical, chemical, biological procedures or even a combination of them are done. The most common hydrolysates are acid and enzymatic.

In acid hydrolysis, two routes may be used: concentrated or dilute acid. The acid attack produces hexoses (mainly glucose), pentoses (mainly xylose), lignin, among other products depending on the type of raw material used. Hydrolysis conditions, such as temperature, acid concentration and pressure, should be tested in order to verify the best combination in the hydrolysate preparation (CASSALES, 2010).

In this paper, chemical hydrolysis was carried out under different temperatures, pressure and acid concentrations, verifying the best hydrolysis condition for the ground soybean hulls and for the soybean hulls without grind, in order to obtain sugars for microbial culture.
2. MATERIAL AND METHODS

2.1. Material

The hulls were donated by a company from Rio Grande do Sul. The acid used for the hydrolysis was sulfuric acid (Synth, 98%).

2.2 Methods

Determination of moisture, ash, lipids and proteins in the hull: These determinations were made following the methodology described by AOAC (2000), in triplicate.

Grinding: In the experiment, it was used without grinding and ground hulls. The last one was obtained in a knife mill (MC090/FA, Marconi) to get granulometry less or equal to 1 mm.

Hydrolysis: The hydrolysis occurred in an autoclave (AV 30, Phoenix Luferco). The amount of hulls without ground, ground hulls, pressure, temperature and amount of acid were listed in Figure 1. For all cases, distilled water was used to reach the final volume of 10 mL. The flasks used for the hydrolysis were threaded test tubes.

Microscopy: The visualization of the smooth and rough surface before and after the hydrolysis was done in a scanning electron microscope (JSM - 6610LV, Jeol), with different magnifications.

Total reducing sugars: This determination was made following the methodology used by Miller (1959). The method is based on the reaction between 3,5-dinitrosalicylic acid (DNS) and the reducing sugars present in the sample.

3. RESULTS AND DISCUSSION

In Table 1 are detailed the results of moisture, ash, lipid and protein tests.

Table 1 – Composition of soybean hulls

| Soybean hulls | Moisture (%) | Ash (%) | Lipid (%) | Protein (%) |
|---------------|--------------|---------|-----------|-------------|
|               | 11.73 ± 0.04 | 4.25 ± 0.08 | 2.56 ± 0.03 | 6.94 ± 1.00 |
The results for the measurements of the total reducing sugars (TRS) obtained after chemical hydrolysis are detailed in Table 2, for hulls without grinding and the ground hulls. In the Table 2 are also detailed the Tukey test for homogeneous groups at a significance level of 5%.

Table 2 - TRS produced on the respective reaction conditions

| Pressure and Temperature Condition - Ground soybeans hulls | 0.5 atm and 111 °C | 1.1 atm and 120 °C | 1.5 atm and 127 °C |
|----------------------------------------------------------|-------------------|-------------------|-------------------|
| H₂SO₄ (g)                                               | 0.13              | 0.17              | 0.20              |
| TRS (g/L)                                               | 15.67C            | 16.52B            | 16.92A,c          |
|                                                        | ± 0.01            | ± 0.01            | ≤ 0.01            |

| Pressure and Temperature Condition – Soybean hulls without grinding | 0.5 atm and 111 °C | 1.1 atm and 120 °C | 1.5 atm and 127 °C |
|--------------------------------------------------------------------|-------------------|-------------------|-------------------|
| H₂SO₄ (g)                                                          | 0.13              | 0.17              | 0.20              |
| TRS (g/L)                                                          | 0.25A,ab          | 0.17B             | 0.18B             |
| ± 0.01                                                             | < 0.01            | < 0.01            | ≤ 0.01            |

Equal capital letters on the same line represent that there is no statistical difference at the significance level of 5% on Tukey’s test between different acids quantities under the same conditions of temperature e pressure. Equal lower-case letters in the same line represent that there is no statistical difference at the significance level of 5% on Tukey’s test between the best quantity of acid under specific conditions of temperature and pressure.

From Table 2, for the ground soybean hulls, the statistical analysis showed that at 1.1 atm and 120 °C using 0.20 g of acid, the production of TRS was higher than the others. For this same condition of temperature e pressure, and at 0.5 atm and 111 °C, the use of 0.20 g of sulfuric acid showed to be the best choice, but at 1.5 atm and 127 °C the effect of acid quantity was the opposite, where 0.13 g was the most productive quantity of acid tested. The statistical analysis also showed that for the soybean hulls without grinding, the best condition was 1.5 atm and 127 °C when was used 0.20 g of sulfuric acid. Analyzing each condition of temperature and pressure tested, for the condition of 0.5 atm and 111 °C and 1.1 atm and 120 °C, the use of 0.13 g of acid resulted in the best values, although at 1.5 atm and 127 °C, the use of 0.20 g was the best for the production of reducing sugars from the soybeans hulls without grinding. For the soybean hull without grinding, all values were shown to be lower in comparison with ground hulls. When comparing the values obtained for the two types of the soybean hulls, it was clear that the use of ground soybean hulls resulted on values much higher than those obtained without grinding, indicating that the high contact surface plays an important role in the production of sugars by hydrolysis.

In Figure 2, scanning electron microscopies of the soybeans hulls can be seen before and after undergoing hydrolysis. Microscopies were made for all experimental assays (Figure 1), but only those representing one of the best hydrolysis conditions (1.1 atm and 120 °C) were recorded in Figure 2. The chemical attack under pressure and temperature changed the completely smooth morphology (Figure 2a) to a rough and leaked surface (Figure 2b).
Figure 2 - Scanning electron microscopy with 3000x magnification. (a) microscopy of the ground soybeans hulls prior to hydrolysis and (b) after hydrolysis.

4. CONCLUSION

The pressure, temperature and concentration of the acid in the acid hydrolysis influence the release of reducing sugars, and the soybeans hulls morphology changes after the reactions.

The contact surface exerts a strong influence, being the soybean hulls with particles size less or equal to 1 mm more efficient in the liberation of reducing sugars during the hydrolysis process, when compared to the soybeans hulls with granulometry of more than 1 mm. Between the tested conditions, the best production of TRS (24.93 g/L) was obtained at 1.1 atm and 120 °C with the addition of 0.20 g of acid.

5. ACKNOWLEDGMENT

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