OPTIMIZATION OF SUBMERGED FERMENTATION CONDITIONS FOR GIBBERELLIC ACID PRODUCTION

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ABSTRACT

Gibberellic acid is a plant hormone commonly used as a biostimulant in plants, due to its various beneficial effects that favor crop productivity. In a scenario of increased agronomic efficiency of plants, the use of this metabolite tends to be a possible strategy to be adopted by farmers. In this context, optimizing GA3 production becomes a plan with great market potential. Therefore, this work aimed to optimize the temperature and pH conditions for the production of gibberellic acid by two fungi: Aspergillus niger and Fusarium fujikuroi. A two-level, three-factor factorial design was used, totaling eight assays, which were performed in triplicates. The results pointed to a better yield in relation to the substrate for the Aspergillus fungus, with values greater than 13 mg product/g of substrate, while for Fusarium the values did not exceed 9.2 mg product/g of substrate. In relation to productivity, the Aspergillus fungus also performed better than Fusarium, with 1.8 mg/L.h and 1.69 mg/L.h. The highest concentrations of acid were obtained at a temperature of 28ºC and a pH close to 5.

Keywords: *Aspergillus niger*, *Fusarium fujikuroi*, biofertilizers, biostimulants.

1 INTRODUCTION

Gibberellins are a group of plant hormones, like others, that play an important role in promoting plant growth and development. They are crucial in several physiological processes, such as: flowering, seed germination and stem development. There are more than 100 types of gibberellins identified, however, only 4 are the most studied, namely GA_1, GA_3, GA_4 and GA_7 [1, 2].

Several studies have sought to optimize the production of GA_3, whether by solid state fermentation or submerged fermentation. The main variables adopted in the experiments are: variation in substrate composition: glucose concentration, presence of additives, different sources of nitrogen and carbon, the presence of micronutrients, whether isolated or in mixture with others, as well as complete replacement by substrates that are by-products of industry in general. Associated with this, studies have evaluated the ideal conditions for fermentation, such as: temperature, pH and aeration [3, 4, 5].

The main microorganisms used are *Fusarium fujikuroi* and *Aspergillus niger*, however, there are studies that present the use of bacteria, such as those of the genus *Pseudomonas sp*. that some species have the capacity to produce the phytohormone, either in a fermentative environment or by inducing the plant itself to produce it. Some advances in gibberellic acid production research bring to light bioengineering techniques for mutating some fungi, mainly from the *Fusarium* genus, and the results obtained are promising, as they considerably increased the concentration of GA_3 [4].

2 MATERIAL & METHODS

Two fungal strains donated by Embrapa Agroenergia to the Biotechnological Processes Center at the Federal University of Uberlândia were selected. After preliminary tests, the strains were subjected to a 2^3 factorial design, with the variables and their respective levels: fungi Insumicro 175 (*Fusarium fujikuroi*) (+1) and LGB-034-2015 (*Aspergillus niger*) (-1), temperatures 26ºC (-1) and 28ºC (+ 1) and pH 4.5 (-1) and 5.5 (+1), according to Table 1. The responses for this design were: gibberellic acid concentration (ppm), residual glucose concentration (g/L), pH, initial and final biomass (g/L). All treatments were carried out in triplicate, totaling 24 batches.

Table 1 Factorial design 2^3.

Run	Fungi	Temperature	pH
1	Insumicro 175	26	4,5
2	LGB-034-2015	26	4,5
3	Insumicro 175	28	4,5
4	LGB-034-2015	28	4,5
5	Insumicro 175	26	5,5
6	LGB-034-2015	26	5,5
7	Insumicro 175	28	5,5
8	LGB-034-2015	28	5,5

The fungi were subjected to submerged fermentation tests, as shown in Figure 1, using a 500 mL Erlenmeyer flask with a reaction volume of 300 mL, with 20 mL aliquots being collected every 48h.

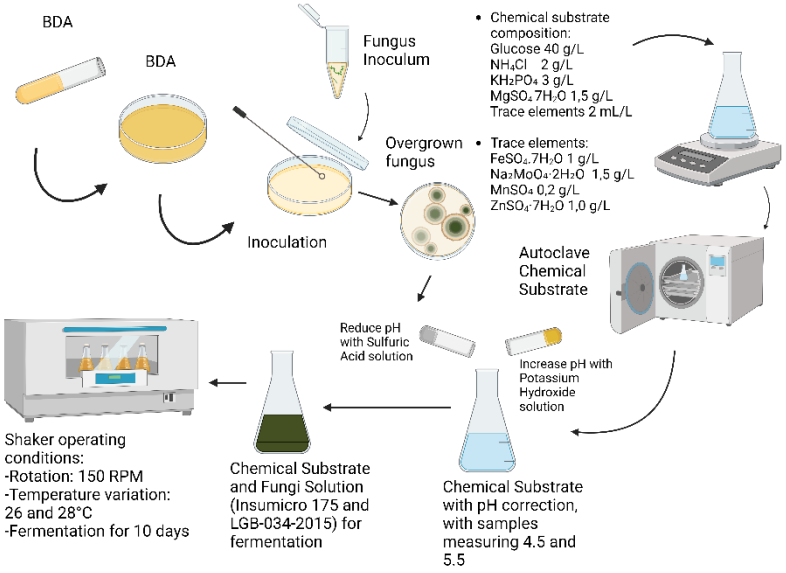

Figure 1 Fermentation process for fatorial design.

After the sampling stage, filtration was carried out to separate the biomass and a 10 mL aliquot of the fermented product was collected, which was subjected to the clarification process to precipitate macromolecules using 0.5 mL of a 30% Zinc Acetate solution and 0.5 mL of a 15% Potassium Ferrocyanide solution. Subsequently, the mixture was centrifuged and 1 mL of the supernatant was pipetted and dispensed into a 10 mL volumetric flask, 1 mL of ethanol was added and the volume was completed with a 30% HCl solution. The solution was kept at rest for 75 min and then read on a spectrophotometer at 254 nm, previously calibrated [6].

To determine the glucose content, the Liquiform Labtest Kit adapted to the expected concentration in the medium was used. The technique is compared to a standard provided in the Kit, where, after treating the sample, a spectrophotometer reading is performed at 502 nm. The pH reading was performed with the aid of a pH meter from the manufacturer Hanna Instruments.

Using data obtained from biomass and gibberellic acid concentration analyses, several kinetic parameters were calculated to evaluate fermentation performance, thus facilitating the understanding of the kinetics of the fermentation process [7]. To calculate the parameters, the maximum GA_3 production value, the time in which this concentration was obtained, as well as the biomass final value and the initial value at time 0 were considered.

3 RESULTS & DISCUSSION

Table 2 presents the kinetic parameters calculated using data obtained from the kinetic graphs of GA3 production and glucose consumption, facilitating the understanding of the influence of pH and temperature factors on the dynamics of acid production by the fungi evaluated. Regarding the productivity of the fungus in relation to the product, it is observed that at lower pH, this index tends to be lower, and that when there is an increase in temperature, it is also increased, so that the maximum value found was of 1.8 $mg.L^{-1}.h^{-1}$ in run 4. Some authors found similar productivity values in submerged fermentation with the fungus *Fusarium fujikuroi*, varying between 0.94 and 2.84 $mg.L^{-1}.h^{-1}$ [8]. Regarding the productivity of the fungus in relation to biomass, the values tend to be higher for higher pH and lower temperatures. In this index, the fungus of the genus *Aspergillus*, regardless of the condition, presented higher values than the genus *Fusarium*.

Table 2 Productivity of each fungus in relation to the product (P_P - mg/L.h), biomass (P_X - g/L.h), specific speed of product formation (μ_p - g product/g cell.h), substrate consumption (μ_s - g substrate/g cell), cell growth (μ_x - h^{-1}), biomass yield ($Y_{X/S}$ – g cells/g substrate) and product yield ($Y_{P/S}$ – mg product/g substrate).

Corr.	Fungo	Temp. (ºC)	pH	P_P	P_X	μ_p	μ_s	μ_x	($Y_{X/S}$)	($Y_{P/S}$)
1	Insumicro 175	26	4,5	1,25	0,058	0,131	0,019	0,006	0,327	6,20
2	LGB-034	26	4,5	1,24	0,090	0,089	0,011	0,010	0,565	7,82
3	Insumicro 175	28	4,5	1,66	0,038	0,215	0,019	0,004	0,259	7,29
4	LGB-034	28	4,5	1,80	0,067	0,146	0,011	0,007	0,476	13,66
5	Insumicro 175	26	5,5	1,64	0,061	0,168	0,019	0,006	0,336	9,18
6	LGB-034	26	5,5	1,01	0,094	0,070	0,011	0,010	0,591	6,35
7	Insumicro 175	28	5,5	1,69	0,032	0,239	0,022	0,003	0,208	6,99
8	LGB-034	28	5,5	1,79	0,072	0,138	0,012	0,008	0,477	12,06

For the specific speed of product formation, Insumicro 175 presented higher values compared to LGB-035-2014, regardless of the fermentation condition. Temperature was also an important factor for this speed, as at a temperature of 28ºC, regardless of the fungus, the formation speed was higher than at a temperature of 26ºC. Evaluating the influence of pH for the fungus of the genus *Fusarium*, the increase in this factor favored the speed of formation indicating greater efficiency in this condition, while for *Aspergillus* there was a reduction. The substrate consumption rate was higher, regardless of the condition, for Insumicro 175, reaching a value of 0.022 g substrate/g cell, twice as high as achieved by LGB-034-2015. In relation to pH and temperature, there was little influence on this index, since it was only at pH 5.5 and a temperature of 28ºC that the values were higher than the other conditions evaluated.

In terms of product yield, runs 4 and 8 were those that obtained the best result, being 13.66 and 12.06 mg product.g substrate^{-1}, respectively. Others authors obtained yields, for different types of submerged fermentation bioreactors, between 2.36 and 3.17 mg product.g substrate^{-1}, being lower than those obtained in this work [8]. The fungus LGB-034-2015, the only difference being the increase in pH value, confirming a slight tendency for the fungus of the genus *Aspergillus* to perform better at lower pH, produced both. For the Insumicro 175 strain, the highest product yield value achieved was 9.18 mg product.g substrate^{-1}, at 26 ºC and pH 5.5. This can be explained because the substrate consumption profile of the two microorganisms is different. The mild sugar consumption profile presented by *Aspergillus* means that the product yield per substrate quantity is greater, which may point to a positive economic effect on the industrial production of the acid. The high rate of substrate consumption presented by *Fusarium* indicates that this strain should produce more GA3 to be economically similar to LGB-034-2015. In relation to biomass yield, regardless of the condition studied, *Aspergillus* was superior to *Fusarium*, however, this factor can also interfere with industrial economic issues, since the fermentation product is the metabolite and not the biomass of the fungus, which is a possible byproduct of the fermentation process.

4 CONCLUSION

Through the results obtained, it is concluded that the fungus of the genus *Fusarium fujikuroi* has better productivity performance at pH 5.5 and a temperature of 28ºC. For the fungus *Aspergillus niger*, the best condition is at pH 4.5 and a temperature of 28ºC. The fungus of the genus *Aspergillus* presents a higher yield in relation to the substrate when compared to *Fusarium*, which, due to the accelerated consumption of substrate, presents a higher yield at a temperature of 26ºC.

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