CHARACTERIZATION OF ENZYME EXTRACT OBTAINED FROM MICROALGAE PRODUCED IN WASTEWATER

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ABSTRACT

Reducing the generation of solid and chemical waste and more sustainable practices aimed at agriculture are among the global goals for achieving the 2030 agenda. Because of this, enzymatic extracts obtained from fermentations with fungi and microalgae are an excellent choice for their use in agriculture and treating industrial effluents. Therefore, this study aimed to characterize the extract obtained from fermentation in an Airlift bioreactor using microalgae and *Trichoderma koningiopsis* as the fermentative medium. The presence of the enzymes amylase, cellulase, peroxidase, laccase, lipase, and protease and their behavior in reaction media at different pH and temperature ranges were evaluated. As a result, if obtained, high peroxidase (52.083 U/mL) and protease (97.500 U/mL) activity and an increase in amylase activity (40.52 U/mL) were observed when having an alkaline reaction medium and high reaction temperature. The results demonstrate the possibility of using the extract obtained for different environmental applications, contributing to achieving global goals and the reduction of chemical compounds harmful to the environment.

.**Keywords:** *Trichoderma koningiopsis*. Peroxidase. Activities. Fermentation. Reaction medium.

1 INTRODUCTION

The establishment of the 2030 Agenda, along with the Objectives of Sustainable Development, has led to the search for the use of environmentally friendly processes that reduce the generation of solid and chemical waste, intending to ensure sustainable production and consumption standards and guarantee sustainable food production systems and that do not harm ecosystems.

Microalgae are considered promising organisms for their use in the most diverse sectors, from agriculture to effluent treatment, due to their rich composition of amino acids, bioactive lipids, and other components [1,2,3]. Furthermore, the microalgae can synthesize several enzymes, and microorganisms such as *Trichoderma* can produce an enzymatic pool, enabling fermented microalgal extract in several areas of environmental interest.

That said, the objective of this study is to evaluate the presence of different enzymes in the microalgal extract fermented with *Trichoderma koningiopsis* and the behavior of the reaction medium at various pH and temperature ranges.

2 MATERIAL & METHODS

The microalgae used in this study were supplied by Embrapa Swine and Poultry (Concórdia, SC, Brazil), and the microorganism used was the *Trichoderma koningiopsis* (GenBank MK860714), isolated from weed *Digitaria ciliares* [4].

The fermentative process occurred in Airlift Bioreactor, the fermentative medium composed of 200g of microalgae, 400 mL of antifoam, 1400 mL of distilled water, and 10^7 cells per mL of inoculum of *Trichoderma koningiopsis.*

The microalgae extract obtained after 96 hours of fermentation was evaluated for the enzymes in its composition. Therefore, cellulase, amylase, peroxidity, laccase, lipase, and protease activities were determined [5-12]. Afterward, the behavior of enzymatic activities was evaluated based on the change in pH and temperature of the reaction medium to determine enzymatic activity. Experimental designs were carried out for this evaluation using a central rotational composite design 2^2.

3 RESULTS & DISCUSSION

When evaluating the presence of the enzymes cellulase, amylase, peroxidase, laccase, lipase, and protease, only cellulase, peroxidase, and protease activities were obtained, being equal to 0.691 U/mL, 52.083 U/mL, and 97.500 U/ml, respectively.

However, to confirm whether the enzymes amylase, laccase, and lipase are present and their absence may be linked to the pH and temperature of the reaction medium, experimental planning was carried out for all enzymes, and the results are described in Table 1.

Table 1 Results of pH and temperature changes from enzymatic reactions.

Essay	pH	Temperature (ºC)	Amylase (U/mL)	Cellulase (U/mL)	Lipase (U/mL)	Laccase (U/mL)	Peroxidase (U/mL)	Protease (U/mL)
1	4 (-1)	20 (-1)	13.09	0.05	0.29	0.02	8.17	0.00
2	10 (1)	20 (-1)	0.00	0.15	0.79	0.01	8.83	2.89
3	4 (-1)	80 (1)	0.00	0.61	0.00	0.00	5.00	2.33
4	10 (1)	80 (1)	40.52	1.28	0.25	0.01	3.00	2.00
5	2.76 (-1.41)	50 (0)	2.91	0.00	0.89	0.00	7.67	0.56
6	11.24 (+1.41)	50 (0)	3.30	0.19	1.22	0.01	6.33	4.67
7	7 (0)	7.57 (-1.41)	11.15	0.24	0.00	0.00	1.50	4.00
8	7 (0)	92.43 (+1.41)	3.39	0.10	0.00	0.04	6.83	0.00
9	7 (0)	0 (50)	8.53	0.12	0.75	0.00	5.83	0.11
10	7 (0)	0 (50)	0.00	0.12	0.00	0.00	5.83	0.00
11	7 (0)	0 (50)	11.73	0.24	0.29	0.00	6.17	5.44
Traditional methodology			0.00	0.691	0.00	0.00	52.083	97.500

By changing the pH and temperature of the reaction medium, amylase (40.52 U/mL) and lipase (1.22 U/mL) activities were obtained. Cellulase resulted in a higher activity value, equal to 1.28 U/mL. The amylolytic activity obtained in test 4 may be due to its affinity for more alkaline pH levels; however, this is a new behavior since most fungal amylases have maximum activity in pH ranges between 4 and 6 and at temperatures between 30 and 70°C, most of which are produced by fungi from other genera and not *Trichoderma* sp.[13]. Cellulase and lipase activities have increased but are still considered low values. Peroxidase obtained its highest activity using the traditional methodology, as did protease, which resulted in activities equal to 52.083 U/mL and 97.500 U/mL, respectively.

The presence of these three enzymes in the fermented extract makes it possible to use them in obtaining agricultural bio inputs, treating effluents, and biorefinery products. The high presence of peroxidase mainly highlights its use as a bioherbicide. For example, how this enzyme acts when applied to plants increases the concentration of peroxide radicals and hydrogen superoxide, thus damaging the structure of weeds[1]. Furthermore, due to its redox potential, peroxidases in this extract make it possible to use it to remove dyes from effluents from the textile industry[2].

4 CONCLUSION

The enzymatic characterization of microalgae extract ferment by *Trichoderma koningiopsis* demonstrated that the change in pH and temperature causes the enzyme present in the extract to become active, as was the case with amylase. In addition to its presence, the activity values obtained for peroxidase and protease also make the use of this extract promising for the various environmental and economic sectors, enabling the reduction of waste generation and sustainable agriculture practices, since due to the presence of peroxidase it has potential for bioherbicidal action on specific plants.

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