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# HIGH RESISTANCE OF CRUDE AND NONCOMMERCIAL PEROXIDASE TO EXTREME TEMPERATURE AND pH CONDITIONS

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## 1. INTRODUCTION

*Trichoderma* extracellular enzymes are rarely mentioned in the literature due to the difficulty in offering favorable conditions for their expression in fermentation, often involving costly processes (JUN et al., 2019). The *Trichoderma* species are classified as Soft-Rot Fungi by Mäkelä, Hildén, and Kuuskeri (2020), who reported many fungal peroxidases (POD) being applied as biocatalysts to industrial reactions, but little is known regarding their characteristics. Regardless of the source, the enzymatic response is influenced by reaction thermodynamics and requires studies on varying pH, temperature, and availability of substrate and cosubstrate − relevant factors in understanding the catalytic route and reactional conditions (NELSON; COX, 2012). These variables were investigated in the present work for *Trichoderma* POD as the most promising storage strategy to maintain enzymatic activity. This work is the first to report the production of guaiacol peroxidase by the fungus *T. koningiopsis*, isolated from weeds from soybean and corn cultivation areas, in a fermentation where the medium was fresh microalgal biomass grown in swine wastewater digestate. This innovative approach places the study in the context of a circular bioeconomy.

## 2. MATERIALS AND METHODS

The crude peroxidase extract was produced by *T. koningiopsis* MK860714 under submerged fermentation supplemented with *Chlorella* spp. biomass (MICHELON et al., 2016). Fermentation was carried out for 72 h on an orbital shaker at 120 rpm and 28 °C, with a medium composed of 10 g of fresh microalgal biomass (89% humidity) and 90 mL of distilled water. After fermentation, the content was filtered, the liquid permeate was centrifuged, and the supernatant corresponded to the crude peroxidase extract.

The reaction conditions pH and temperature and the influence of POD:substrate and POD:cosubstrate ratios on enzymatic activity were evaluated by the Plackett-Burman (PB) experimental design. The enzymatic activity was quantified using sodium phosphate buffer (5 mmol $L^{-1}$), the substrate guaiacol (93 mmol $L^{-1}$), and the cosubstrate hydrogen peroxide − $H_2O_2$ (75 mmol $L^{-1}$). The unit of POD specific activity was defined as the enzyme amount capable of causing a 0.001 increase in the absorbance unit (at 470 nm) per minute per milligram of the total protein (quantified by the Bradford method).

Then, the crude extract was subjected to stress conditions to explore its resistance: boiling temperature (100 °C) in an open and closed flask, extremally acidic (pH 1.1) and alkaline (pH 10.2)

medium, and replacement of the cosubstrate source for sodium percarbonate salt ($Na_2CO_3 \cdot 1.5H_2O_2$). Furthermore, crude POD samples were stored at temperatures between −10 and 28 °C, and residual activity (RA) was monitored for up to 170 days. The data underwent statistical analysis (ANOVA and Tukey's test) using the software Statistica 8.0 and Protimiza Experimental Design.

## 3. RESULTS, DISCUSSION, AND CONCLUSIONS

The results of the Plackett-Burman design indicated enzyme stability even under adverse reaction conditions. When subjected to a pH range of 4.0-8.0 and temperatures of 20-80 °C, the enzymatic activity was not significantly affected at a 95% confidence level, maintaining a range from 5733 to 7700 U $mg^{-1}$. On the contrary, the POD:substrate and POD:cosubstrate ratios negatively affected the enzymatic response with statistical significance; POD activity improved at the minimum ratios studied (1 mL of POD for 0.5 mL of guaiacol and $H_2O_2$).

In sequence, two assays were conducted at 100 °C: controlling evaporation, which had a statistically significant decrease in enzymatic activity (76% of RA), and not controlling evaporation, with a statistically significant increase (296% of RA). That is, the enzymatic extract was concentrated by water evaporation. None of the reaction and stress conditions led to enzyme denaturation. Even after submitting the enzymatic extract to boiling temperature and highly acidic or alkaline pH, the lowest and the maximum activity obtained were 203 U $mg^{-1}$ and 965 U $mg^{-1}$, respectively.

Another important finding of this work was the POD response under the substitution of aqueous $H_2O_2$ by sodium percarbonate salt, aiming to use a more stable, safe, and easy transport and store solid reagent. The substitution did not significantly affect enzymatic activity, indicating that in solution, the salt released $H_2O_2$, an essential cosubstrate for peroxidase, at an adequate rate.

The storage study reinforced the high POD resistance under varied conditions. For the storage condition between 18-28 °C, there was no significant difference in activity until the 60$^{th}$ day, reaching 105% of RA, but a significant drop was observed on the 170$^{th}$ day. For storage at 4 °C, the RA value was maintained at 106% up to the 170$^{th}$ day. The extract stored at −10 °C initiated a significant and current decrease in enzymatic activity after 15 days, reaching 63% of RA in 90 days.

Crude and noncommercial POD showed potential for insertion in advanced bioxidative reactions due to its good stability when subjected to extreme conditions. These tests made it possible to determine that the enzymatic extract can be kept in the refrigerator for up to 170 days without sudden drops in peroxidase activity. In addition, keeping the extract at room temperature proved to be an interesting option for up to 60 days, but it still requires studies for more extended periods.

## 4. REFERENCES

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