HORSERADISH PEROXIDASE-MEDIATED DOXORUBICIN DEGRADATION

Naionara A. Daronch[1], Maikon Kelbert[1], Karina Cesca[1], Débora de Oliveira[1] & Sergio Y. G. González[1]

[1] *Chemical Engineering/Technology Center/Department of Chemical Engineering and Food Engineering/Chemical Engineering Postgraduate Program, Federal University of Santa Catarina/UFSC, Florianópolis-SC, Brazil.*
** Corresponding author's email address: naionaradaronch@gmail.com*

ABSTRACT

The use of enzymes presents significant potential to detoxify pollutants through transformation and biodegradation, employing an environmentally friendly and cost-effective catalytic process. Biocatalysts are excellent alternatives to traditional chemical processes due to their high selectivity under mild conditions. Horseradish peroxidase (HRP), a heme-containing enzyme, catalyzes oxidation reactions of phenolic acids and amines in hydrogen peroxide (H_2O_2). Environmental pollutants, such as pharmaceuticals, are concerning due to their hazardous behavior and the persistence of their metabolites. This study evaluated the potential of HRP in degrading the anticancer drug doxorubicin (DOX) commonly found in wastewater. Experiments were conducted to investigate different reaction conditions, evaluating different HRP and H_2O_2 concentrations. DOX degradation was observed at all tested enzyme concentrations. The best condition was 0.1 μM of H_2O_2 and 3600 $U{\cdot}L^{-1}$ of HRP, leading to 100% degradation after 3 h and 30 min of reaction. Higher HRP concentrations resulted in faster degradation times but did not necessarily lead to complete DOX removal. Excessive H_2O_2 concentrations led to enzyme inactivation. The results indicated that for efficient DOX degradation, it is crucial to balance HRP and H_2O_2 concentrations, as insufficient and excessive peroxide can reduce degradation efficacy.

Keywords: Peroxidase. Enzyme. Emerging contaminant. Doxorubicin. Biocatalysis.

1 INTRODUCTION

Enzymes used in bioremediation hold significant potential for detoxifying pollutants by catalyzing the transformation and degradation of these compounds, being an environmentally friendly and cost-effective catalytic process[1]. Horseradish peroxidase (EC 1.11.1.7) or HRP is a heme-containing enzyme with iron (III) protoporphyrin and two calcium atoms in its structure[2]. These enzymes can catalyze an oxidation reaction of phenolic acids, aromatic phenols, and non-aromatic amines in hydrogen peroxide[3].

Environmental pollutants such as heavy metals, dyes, plastics, pesticides, polycyclic aromatic hydrocarbons (PAHs), and pharmaceuticals are of particular concern due to their hazardous behavior[4,5,6]. These compounds can primarily be derived from pharmaceuticals, personal care products, and endocrine-disrupting compounds[7]. In addition, the degradation of organic compounds may result in accumulating persistent metabolites[8].

The emerging contaminants (ECs) have been detected in surface water, groundwater, drinking water, and wastewater treatment plants (WWTPs)[7,9,10]. Environmental protection agencies such as the USEPA and Environment Canada have raised concerns about these pollutants[11,12]. Designed processes of WWTPs do not include an effective removal of these compounds, resulting in their discharge to receiving surface water, such as rivers, lakes, and coastal waters[12,13].

Particular attention should be given to anticancer drugs once they present cytotoxic, mutagenic, teratogenic, and carcinogenic effects even in trace levels ($ng{\cdot}L^{-1}$ to $mg{\cdot}L^{-1}$)[14]. The anthracycline doxorubicin (DOX) is extensively used in cancer therapy due to its ability to treat solid tumors and leukemias in adults and children[15]. However, despite the numerous benefits, DOX has poor oral bioavailability, and large amounts of the ingested doses are released into the environment through excretions as mixtures of the original structure and their metabolites[16]. In this sense, the milestone of this study was to evaluate the potential of HRP to degrade doxorubicin.

2 MATERIAL & METHODS

HRP activity was determined before each experiment to standardize the initial activities. The measurement assessed the transformation of ABTS to $ABTS^{+}$. The activity was measured at 25 ºC for 3 minutes and calculated using Equation 1.

$$U/L = \frac{= (\Delta abs \cdot V)}{(\varepsilon \cdot d \cdot v \cdot t)} \tag{1}$$

One unit of enzyme activity (expressed in $U{\cdot}L^{-1}$) is defined as the amount of enzyme necessary to catalyze 1 μmol of ABTS per min. Δabs is the variation of absorbance. V is the reactional volume (mL). ε is the molar extinction coefficient (for ABTS = 3.6×10^4 $M^{-1}{\cdot}cm^{-1}$). d is the cell path length (cm). v is the volume of the enzyme solution (mL), and t is the reaction time (min).

DOX concentration was measured by a fluorescence spectrophotometer (SpectraMax® GeminiTM EM, Molecular devices®) for all the assays. The excitation and emission wavelengths were 480 and 598 nm[15]. Assays were carried in 96-well black plates (Corning incorporated Costar®), with 200 μL of total volume reaction. The DOX degradation was measured in intervals of 5 min for 12 h. All experiments were performed in 3 replicates.

Experiments were performed to investigate the effects of different reaction conditions on HRP-assisted degradation of DOX. Each reaction was prepared in 0.1 M phosphate buffer solution (PBS) with a pollutant concentration of 250 μg·L^{-1}, pH 7, and a temperature of 30 °C fixed in these experiments. The concentrations of HRP and H_2O_2 were evaluated: from 225 to 9000 U·L^{-1} and 0.001 to 10 μM, respectively.

3 RESULTS & DISCUSSION

Doxorubicin (DOX) degradation catalyzed by horseradish peroxidase (HRP) in the presence of H_2O_2 occurred at all enzyme concentrations tested. Various HRP and H_2O_2 concentrations were tested to find the optimal combination for DOX degradation. As shown in Fig. 1, complete degradation was first observed with the highest enzyme concentration. Increasing enzymatic activity decreased degradation time: with 0.1 μM of H_2O_2 and 3600 U·L^{-1} of HRP, 100% degradation occurred after 3.5 hours. However, using the same H_2O_2 concentration, assays with 1800 U·L^{-1} and 900 U·L^{-1} HRP reached complete degradation after 5 and 7.5 hours, respectively. At lower H_2O_2 concentrations, complete degradation occurred after 9 hours with 1800 U·L^{-1} HRP. At higher HRP concentrations, the reaction plateaued, possibly due to H_2O_2 consumption or metabolite inhibition. Furthermore, higher H_2O_2 concentrations (1 μM) did not improve degradation rates, indicating the inactivation of peroxidase by excess H_2O_2[18].

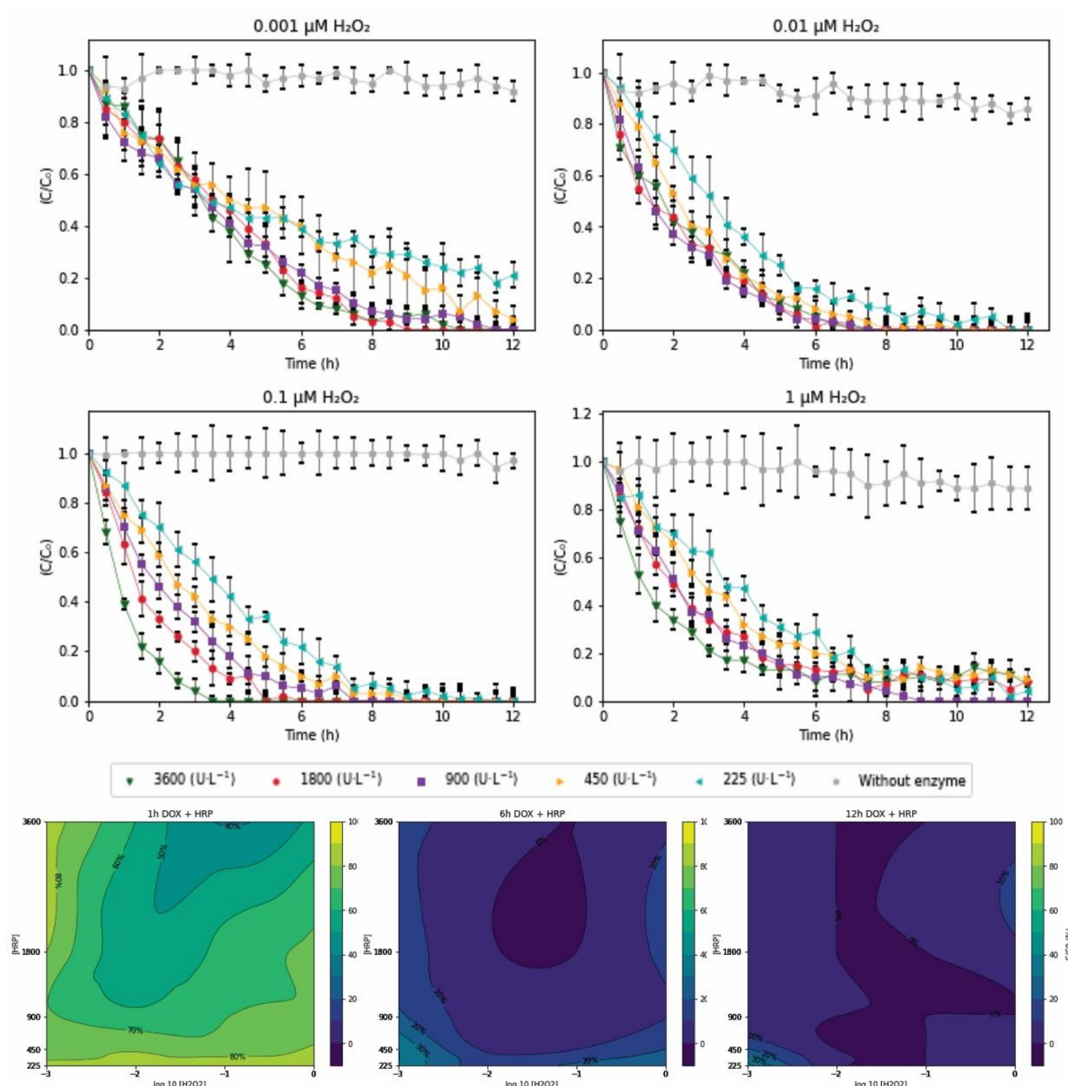

Figure 1 Variations of horseradish peroxidase (HRP) and H_2O_2 concentrations to degrade doxorubicin (DOX) at 250 μg·L^{-1} concentration. All degradations were carried out at pH 7 and 30 °C.

Table 1 shows the initial specific degradation rates for all HRP concentrations tested. Increased HRP activity decreased the particular rate at all H_2O_2 concentrations. An outlier was observed with 7200 U·L^{-1} HRP and 0.01 μM H_2O_2, where the degradation rate doubled compared to the assay with 5400 U·L^{-1}. This inverse relationship between enzyme activity and degradation rate is due to enzyme-substrate (ES) complex formation. This behavior occurs when the enzyme-to-substrate concentration ratio is high. More enzyme units than substrate molecules are available for ES-complex formation. In the case of HRP, there is also a

relationship with the H_2O_2 as the electron donor. Excessive H_2O_2 inactivates the enzyme, which spontaneously reverts to its native form[18].

Comparing the same enzymatic activity with different H_2O_2 concentrations (Table 1) shows that at lower activities (225 and 450 $U \cdot L^{-1}$), lower H_2O_2 concentrations yield higher degradation rates. For activities from 900 to 3600 $U \cdot L^{-1}$, the highest rates occur at 0.01 and 0.1 µM H_2O_2. The highest rates were achieved using 0.1-10 µM H_2O_2 and 5400 $U \cdot L^{-1}$. Moreover, the highest rates were observed for 7200 and 9000 $U \cdot L^{-1}$ with 1 and 10 µM H_2O_2.

Table 1 Comparison between the specific initial degradation rates of the peroxidase from Horseradish to degrade doxorubicin at concentrations of 250 $\mu g.L^{-1}$ with different concentrations of H_2O_2 (0.001 to 10 µM), at pH 7, 30 °C.

HRP concentrations ($U \cdot L^{-1}$)	Inicial specific degradation rate $\mu g_{DOX} \cdot (U.h)^{-1}$ [H_2O_2]				
	0.001 µM	0.01 µM	0.1 µM	1 µM	10 µM
225	0.223 ± 0.066	0.189 ± 0.026	0.227 ± 0.021	0.199 ± 0.011	-
450	0.121 ± 0.019	0.118 ± 0.0052	0.128 ± 0.017	0.100 ± 0.009	-
900	0.041 ± 0.005	0.095 ± 0.0039	0.064 ± 0.0115	0.065 ± 0.005	-
1800	0.018 ± 0.008	0.055 ± 0.0014	0.049 ± 0.0043	0.044 ± 0.004	-
3600	0.005 ± 0.002	0.0272 ± 0.0012	0.044 ± 0.0018	0.034 ± 0.0008	-
5400	0.0009 ± 0,00004	0.0011± 0.0006	0.012 ± 0.0006	0.009 ± 0.0023	0.02 ± 0.0001
7200	0.0005 ± 0.00013	0.0023 ± 0.0001	0.004 ± 0.00002	0.005 ± 0.0058	0.01 ± 0.0003
9000	0.0004 ± 0,0002	0.0001 ± 0.0002	0.0017 ± 0.0002	0.002 ± 0.0003	0.008±0.0043

4 CONCLUSION

This study demonstrated that horseradish peroxidase (HRP) can effectively degrade the anticancer drug doxorubicin (DOX) in aqueous environments, highlighting the potential of enzymatic bioremediation for pharmaceutical pollutants. Optimal degradation was achieved by balancing HRP and hydrogen peroxide (H_2O_2) concentrations, as insufficient and excessive H_2O_2 negatively impacted the degradation process. These findings underscore the importance of fine-tuning enzyme and substrate concentrations to maximize pollutant removal efficiency in environmental applications.

REFERENCES

1 DING, Y., CUI, K., LIU, X., XIE, Q. GUO, Z., CHEN, Y., 2022. J. Haz. Mat. 431 (193). 128544.
2 ZDARTA, J., MEYER, A. S., PINELO, M., 2018. Adv. Col. and Interf. Scien. 258. 1–20.
3 BILAL, M., RASHEED, T., IQBAL, H. M. N., HU, H., WANG, W., ZHANG, X., 2018. Intern. J. Biolog. Macrom. 113. 983-990.
4 CAROLIN, C. F., KUMAR, P. S., JOSHIBA, J., MADHESH, P., RAMAMURTHY, R., 2021. J. Haz. Mat. 408. 124943.
5 PERINI, B. L. B., DARONCH, N. A., BITENCOURT, R. L., SCHNEIDER, A. L. S., ANDRADE, C. J., OLIVEIRA, D., 2021. J. Pol. Environ. 29. 2200-2213.
6 SARAVANAN, A., JUMAR, P. S., VO, D. V. N., JEEVANANTHAM, S., KARISHMA, S., YAASHIKAA, R., 2021. J. Haz. Mat. 419. 126451.
7 GOGOI, A., MAZUMDER, P., TYAGI, V. P., CHAMINDA, G. G. T., AN, A. K., KUMAR, M., 2018. Ground. Sus. Develop. 6. 169-180.
8 SØRENSEN, S. R., HOLTZE, M. S., SIMONSEN, A., AAMAND, J., 2007. Appl. Environ. Microb. 73 (2). 399-406.
9 SAMARAS, V. G., STASINAKIS, A. S., MAMAIS, D., THOMAIDIS, N. S., LEKKAS, T. D., 2013. J. Haz. Mat. 244. 259-267.
10 YANG, G. C. C., YEN, C. H., WANG, C. L., 2014. J. Haz. Mat. 277. 53-61.
11 BEAMAN, J., EIGNOR, D., HUFF, L. 2008. Aquatic life criteria for contaminants of emerging concern: General challenges and recommendations. United States of Environmental Protection Agency.
12 PEREIRA, C. F. S., MARANHO, L. A., CORTEZ, F. S., PUSCEDDU, F. H., SANTOS, A. R., RIBEIRO, D. A., CESAR, A., GUIMARAES, L.L., 2016. Scien. Tot. Environ. 548. 148-154.
13 PETRIE, B., BARDEN, R., KASPRZYK-HORDERN, B., 2014. Wat. Res. 72. 3-27.
14 PEREIRA, C. S., KELBERT, M., DARONCH, N. A., MICHELS, C., OLIVEIRA, D., SOARES, H. M., 2020. Appl. Microb. Biotech. 104. 23-31.
15 KELBERT, M., PEREIRA, C. S., DARONCH, N. A., CESCA, K., MICHELS, C., OLIVEIRA, D., SOARES, H. M., 2021. J. Haz. Mat. 409. 124520.
16 WENG, X., MA, L., GUO, M., SU, Y., DHARMARAJAN, R. CHEN, Z., 2018. Chem. Eng. J. 353. 482-489.
17 LI, J., PENG, J., ZHANG, Y., SHI, H., MAO, L., GAO, S. 2016. J. Haz. Mat. 310. 152-160.
18 KOHRI, M., 2014. Pol. Synth. React. 46. 373-380.

ACKNOWLEDGEMENTS

The authors acknowledge CAPES (Coordination for the Improvement of Higher Education Personnel) – Finance Code 001 and CNPq (National Council for Scientific and Technological Development) for scholarships and funding sources.