

RESUMO

A utilização de enzimas nos processos de biorremediação de efluentes vem sendo cada vez mais estudada. A peroxidase é uma enzima do grupo oxirredutase, capaz de oxidar uma variedade de substratos orgânicos e inorgânicos usando peróxidos como co-substrato. As peroxidases comerciais apresentam alto custo, o que ainda inviabiliza sua utilização no tratamento de efluentes. Nesse contexto, o presente trabalho buscou estudar uma fonte alternativa de peroxidase para aplicação na descoloração de efluentes têxteis. A peroxidase das folhas de cedro (*Cedrela fissilis*) foi caracterizada, apresentando condições máximas de atividade na faixa de temperatura de 50°C a 62°C e pH 5, utilizando o guaiacol como substrato. Para o substrato ácido gálico, as condições máximas de atividade foram na faixa de temperatura 45°C a 50°C e faixa de pH de 6 a 6,4. Os valores de K_M da enzima quando utilizado o guaiacol e o ácido gálico como substratos foram de 18,7 mM e 31 mM, respectivamente. A temperatura de inativação da enzima foi de 85°C. A atividade enzimática não sofreu influência na presença dos íons metálicos Na^+ , K^+ , Mg^{2+} , Ca^{+2} e Zn^{2+} , mas diminuiu na presença do íon de Cu^{2+} . A purificação parcial utilizando acetona 65% (v.v⁻¹) apresentou um fator de purificação de 18,7. As atividades específicas do extrato enzimático bruto e parcialmente purificado foram de 6,78 U.mg⁻¹ e 126,69 U.mg⁻¹, respectivamente. Dentre as formas de armazenamento da enzima estudados, o ultra-freezer apresentou melhor resultado, onde manteve cerca de 90% de atividade relativa após um mês de armazenamento na temperatura de -82 °C. Em geladeira, a atividade relativa se manteve estatisticamente igual ao armazenamento em ultra-freezer até a terceira semana, sendo inferior após um mês. No freezer convencional (-20 °C), a atividade relativa diminuiu para aproximadamente 50%, em três semanas. A aplicação do extrato parcialmente purificado no corante têxtil azul celeste removeu 82,65% da coloração quando submetido às condições ótimas de reação. As soluções corantes tratadas não apresentaram fitotoxicidade em sementes de *L. sativa* e houve diminuição de citotoxicidade em células de sangue humano. O extrato parcialmente purificado da peroxidase extraída das folhas de cedro apresentou ser uma alternativa no tratamento de efluentes contendo corantes têxteis.

Palavras-chave: Otimização. Condições enzimáticas. Corantes. Toxicidade.

ABSTRACT

The use of enzymes in wastewater bioremediation has been increasingly studied. Peroxidase is an enzyme of the oxidoreductase group, capable of oxidizing a variety of organic and inorganic substrates using peroxides as co-substrate. The commercial peroxidase presents high cost, which still makes its use unviable in the treatment of wastewaters. In this context, the present study sought to study an alternative source of peroxidase for application in textile wastewater. The peroxidase of cedar leaves (*Cedrela fissilis*) was characterized, presenting maximum temperature conditions between 50 °C and 62 °C and pH 5, using guaiacol as substrate. For the substrate gallic acid, maximum temperature conditions were between 45 °C and 50 °C and pH ranges from 6 to 6.4. The K_M values for guaiacol and gallic acid were 18.7 mM and 31 mM, respectively. The required temperature found to inactivate the enzyme was 85 °C. The enzymatic activity was not influenced in the presence of Na^+ , K^+ , Mg^{2+} , Ca^{+2} and Zn^{2+} , but decreased in the presence of Cu^{2+} . Partial purification using 65% (v/v) acetone had a purification factor of 18.7. The specific activities of crude and partially purified enzyme extract were 6.78 U. mg^{-1} and 126.69 U. mg^{-1} , respectively. Among the storage methods analyzed, the ultra-freezer presented the best result, where it maintained about 90% of relative activity after one month of storage at -82 °C. In the refrigerator, relative activity maintained statistically equal to the ultra-freezer until the third week, becoming lower after the forth. In the conventional freezer (-20 °C), relative activity fell to almost 50% in three weeks. Application of the partially purified extract in Brilliant Sky Blue dye presented 82.65% of decoloration when subjected to optimum conditions. The treated dye solutions did not present phytotoxicity in *L. sativa* seeds and decreased cytotoxicity in human blood cells. The partially purified extract of the peroxidase extracted from cedar leaves presented as an alternative in the treatment of effluents containing textile dyes.

Keywords: Optimization. Enzyme conditions. Color solutions. Toxicity.

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ADEWALE, I. O.; ADEKUNLE, A. T. Biochemical properties of peroxidase from white and red cultivars of kolanut (*Cola nitida*). **Biocatalysis and Agricultural Biotechnology**, v. 14, n. October 2017, p. 1–9, 2018. Elsevier Ltd. Disponível em: <<https://doi.org/10.1016/j.bcab.2018.01.013>>..

AGNETA, R.; MÖLLERS, C.; RIVELLI, A. R. Horseradish (*Armoracia rusticana*), a neglected medical and condiment species with a relevant glucosinolate profile: a review. **Genetic Resources and Crop Evolution**, p. 1923–1943, 2013.

AGUELMOUS, A.; EL FELS, L.; SOUABI, S.; et al. Petroleum sludge bioremediation and its toxicity removal by landfill in gunder semi-arid conditions. **Ecotoxicology and Environmental Safety**, v. 166, n. September, p. 482–487, 2018.

AL-ANSARI, M. M.; SAMAR, M.; SAHA, B.; et al. Soybean peroxidase applications in wastewater treatment. In: J. E. Maxwell (Org.); **Soybeans: Cultivation, Uses and Nutrition**. p.189–221, 2011. Nova Science Publishers, Inc.

AL-BAGMI, M. S.; KHAN, M. S.; ISMAEL, M. A.; et al. An efficient methodology for the purification of date palm peroxidase: Stability comparison with horseradish peroxidase (HRP). **Saudi Journal of Biological Sciences**, v. 26, n. 2, p. 301–307, 2018. King Saud University. Disponível em: <<https://doi.org/10.1016/j.sjbs.2018.04.002>>..

ALI, M.; HUSAIN, Q. Guar gum blended alginate/agarose hydrogel as a promising support for the entrapment of peroxidase: Stability and reusability studies for the treatment of textile effluent. **International Journal of Biological Macromolecules**, v. 116, p. 463–471, 2018. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.ijbiomac.2018.05.037>>..

ALI, M.; HUSAIN, Q.; SULTANA, S.; AHMAD, M. Immobilization of peroxidase on polypyrrole-cellulose-graphene oxide nanocomposite via non-covalent interactions for the degradation of Reactive Blue 4 dye. **Chemosphere**, v. 202, p. 198–207, 2018. Elsevier Ltd. Disponível em: <<https://doi.org/10.1016/j.chemosphere.2018.03.073>>..

ALMAGUER, M. A.; CARPIO, R. R.; ALVES, T. L. M.; BASSIN, J. P. Experimental study and kinetic modelling of the enzymatic degradation of the azo dye Crystal Ponceau 6R by turnip (*Brassica rapa*) peroxidase. **Journal of Environmental Chemical Engineering**, v. 6, n. 1, p. 610–615, 2018. Elsevier. Disponível em: <<https://doi.org/10.1016/j.jece.2017.12.039>>..

ALMULAIKY, Y. Q.; AL-HARBI, S. A. A novel peroxidase from Arabian balsam (*Commiphora gileadensis*) stems: Its purification, characterization and immobilization on a carboxymethylcellulose/Fe₃O₄ magnetic hybrid material. **International Journal of Biological Macromolecules**, v. 133, p. 767–774, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.ijbiomac.2019.04.119>>..

ALTUNKAYA, A.; GÖKMEN, V. Purification and Characterization of Polyphenol Oxidase , Peroxidase and Lipoxygenase from Freshly Cut Lettuce (*L. sativa*). **Food Technology and Biotechnology**, v. 49, n. 2, p. 249–256, 2011.

AURIOL, M.; FILALI-MEKNASSI, Y.; TYAGI, R. D.; ADAMS, C. D. Oxidation of natural and synthetic hormones by the horseradish peroxidase enzyme in wastewater. **Chemosphere**, v. 68, n. 10, p. 1830–1837, 2007.

AZEVEDO, A. M.; MARTINS, V. C.; PRAZERES, D. M. F.; et al. Horseradish peroxidase: A valuable tool in biotechnology. **Biotechnology Annual Review**, v. 9, p. 199–247, 2003.

BAS, D.; BOYACI, I. H. Modeling and optimization I: Usability of response surface methodology. **Journal of Food Engineering**, v. 78, n. 3, p. 836–845, 2007.

BASHA, S. A.; RAO, U. J. P. Purification and characterization of peroxidase from sprouted green gram (*Vigna radiata*) roots and removal of phenol and p-chlorophenol by immobilized peroxidase. **Journal of the Science of Food and Agriculture**, v. 97, n. 10, p. 3249–3260, 2017.

BAUMER, J. D.; VALÉRIO, A.; GUELLI, S. M. A.; et al. Toxicity of enzymatically decolored textile dyes solution by horseradish peroxidase. **Journal of Hazardous Materials**, v. 360, n. August, p. 82–88, 2018. Elsevier. Disponível em: <<https://doi.org/10.1016/j.jhazmat.2018.07.102>>..

BELTRAME, T. C. **Caracterização de Efluente Têxtil e Proposta de Tratamento**, 2000. Universidade Federal do Rio Grande do Norte.

BILAL, M.; RASHEED, T.; IQBAL, H. M. N.; YAN, Y. Peroxidases-assisted removal of environmentally-related hazardous pollutants with reference to the reaction mechanisms of industrial dyes. **Science of the Total Environment**, v. 644, p. 1–13, 2018.

BOWNIK, A. Daphnia swimming behaviour as a biomarker in toxicity assessment: A review. **Science of the Total Environment**, v. 601–602, p. 194–205, 2017. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.scitotenv.2017.05.199>>..

BRADFORD, M. M. A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. **Analytical Biochemistry**, v. 72, n. 1–2, p. 248–254, 1976.

BUCHANAN, I. D.; NICELL, J. A. Model development for horseradish peroxidase catalyzed removal of aqueous phenol. **Biotechnology and Bioengineering**, p. 251–261, 1997.

BURNETTE, F. S. Peroxidase and Its Relationship To Food Flavor and Quality: a Review. **Journal of Food Science**, v. 42, n. 1, p. 1–6, 1977.

CHANG, J.; LIN, C. Decolorization kinetics of a recombinant Escherichia coli strain harboring azo-dye-decolorizing determinants from Rhodococcus sp . **Biotechnology Letters**, v. 23, n. 8, p. 631–636, 2001.

CHIONG, T.; LAU, S. Y.; LEK, Z. H.; KOH, B. Y.; DANQUAH, M. K. Enzymatic treatment of methyl orange dye in synthetic wastewater by plant-based peroxidase enzymes. **Journal of Environmental Chemical Engineering**, v. 4, n. 2, p. 2500–2509, 2016. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.jece.2016.04.030>>..

COSTA, M. C.; MOTA, F. S. B.; DOS SANTOS, A. B.; MENDONÇA, G. L. F.; DO NASCIMENTO, R. F. Effect of dye structure and redox mediators on anaerobic azo and anthraquinone dye reduction. **Química Nova**, v. 35, n. 3, p. 482–486, 2012.

COULET, P. R.; BLUM, L. J. Bioluminescence/chemiluminescence based sensors. **TrAC Trends in Analytical Chemistry**, v. 11, n. 2, p. 57–61, 1992. Disponível em: <[https://doi.org/10.1016/0165-9936\(92\)80078-K](https://doi.org/10.1016/0165-9936(92)80078-K)>..

DELÉE, W.; O'NEILL, C.; HAWKES, F. R.; PINHEIRO, H. M. Anaerobic Treatment of Textile Effluents : a Review. **Journal of Chemical Technology and Biotechnology**, v. 73, p. 323–335, 1998.

DEMARCHE, P.; JUNGHANNS, C.; NAIR, R. R.; AGATHOS, S. N. Harnessing the power of enzymes for environmental stewardship. **Biotechnology Advances**, v. 30, n. 5, p. 933–953, 2012. Elsevier Inc. Disponível em: <<http://dx.doi.org/10.1016/j.biotechadv.2011.05.013>>..

DEVA, A. N.; ARUN, C.; ARTHANAREESWARAN, G.; SIVASHANMUGAM, P. Extraction of peroxidase from waste *Brassica oleracea* used for the treatment of aqueous phenol in synthetic waste water. **Journal of Environmental Chemical Engineering**, v. 2, n. 2, p. 1148–1154, 2014. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.jece.2014.04.014>>..

DORNELAS, M. C.; PINHEIRO, A.; RODRIGUEZ, M. The tropical cedar tree (*Cedrela fissilis* Vell ., Meliaceae) homolog of the *Arabidopsis LEAFY* gene is expressed in reproductive tissues and can complement *Arabidopsis* leafy mutants. **Planta**, p. 306–314, 2006.

DRAGANA, R.; NIKOLA, G.; ŽELJKO, D.; GORDANA, A.; OLGICA, N. Separation of peroxidases from *Miscanthus x giganteus*, their partial characterisation and application for degradation of dyes. **Plant Physiology and Biochemistry**, v. 120, n. September, p. 179–185, 2017.

DUARTE-VÁZQUEZ, M. A.; GARCÍA-ALMENDÁREZ, B. E.; REGAALADO, C.; WHITAKER, J. R. Purification and Properties of a Neutral Peroxidase Isozyme from Turnip (*Brassica napus* L . Var . Purple Top White Globe) Roots. **Journal of Agricultural and Food Chemistry**, v. 49, n. 9, p. 4450–4456, 2001. Disponível em: <<https://doi.org/10.1021/jf010043e>>..

DUARTE BAUMER, J.; VALÉRIO, A.; DE SOUZA, S. M. A. G. U.; et al. Toxicity of enzymatically decolored textile dyes solution by horseradish peroxidase. **Journal of Hazardous Materials**, v. 360, n. May, p. 82–88, 2018. Elsevier. Disponível em: <<https://doi.org/10.1016/j.jhazmat.2018.07.102>>..

DYER, J. C.; MIGNONE, N. A. **Handbook of industrial residues**. 1º ed. Ridge, NJ, USA: Noyes Publications, 1983.

ELSAYED, A. M.; HEGAZY, U. M.; HEGAZY, G. A.; et al. Purification and biochemical characterization of peroxidase isoenzymes from *Ficus carica* latex. **Biocatalysis and Agricultural Biotechnology**, v. 16, p. 1–9, 2018. Elsevier Ltd. Disponível em: <<https://doi.org/10.1016/j.bcab.2018.07.009>>..

FALADE, A. O.; MABINYA, L. V; OKOH, A. I.; NWODO, U. U. International Journal of Biological Macromolecules Biochemical and molecular characterization of a novel dye-decolourizing peroxidase from *Raoultella ornithinolytica* OKOH-1. **International Journal of Biological Macromolecules**, v. 121, p. 454–462, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.ijbiomac.2018.10.045>>..

FUKUI, M.; YAMABE, N.; ZHU, B. T. Resveratrol attenuates the anticancer efficacy of paclitaxel in human breast cancer cells in vitro and in vivo. **European Journal of Cancer**, v. 46, n. 10, p. 1882–1891, 2010. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.ejca.2010.02.004>>..

HALPIN, B.; JEN, J.; MONDY, N. Purification and Characterization of Peroxidase Isoenzymes from Green Peas (*Pisum sativum*). **Journal of Food Science**, v. 54, n. 3, p. 644–649, 1989.

HAMID, M.; KHALIL-UR-REHMAN. Potential applications of peroxidases. **Food Chemistry**, v. 115, n. 4, p. 1177–1186, 2009. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.foodchem.2009.02.035>>..

HAO, O. J.; KIM, H.; CHIANG, P. Technology Decolorization of Wastewater Decolorization of Wastewater. **Critical Reviews in Environmental Science and Technology**, v. 30, n. 4, p. 449–505, 2010.

HAQ, I.; RAJ, A. Biodegradation of Azure-B dye by *Serratia liquefaciens* and its validation by phytotoxicity, genotoxicity and cytotoxicity studies. **Chemosphere**, v. 196, p. 58–68, 2018. Elsevier Ltd. Disponível em: <<https://doi.org/10.1016/j.chemosphere.2017.12.153>>..

HIRAGA, S.; SASAKI, K.; ITO, H.; OHASHI, Y.; MATSUI, H. A large family of class III plant peroxidases. **Plant and Cell Physiology**, v. 42, n. 5, p. 462–468, 2001.

HOSU, O.; LETTIERI, M.; PAPARA, N.; et al. Colorimetric multienzymatic smart sensors for hydrogen peroxide, glucose and catechol screening analysis. **Talanta**, v. 204, p. 525–532, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.talanta.2019.06.041>>..

JAEGER, N.; MORAES, J. P.; KLAUCK, C. R.; et al. Cytotoxicity assays to evaluate tannery effluents treated by photoelectrooxidation. **Brazilian Journal of Biology**, v. 75, n. 4, p. 53–61, 2015.

JUN, L. Y.; YON, L. S.; MUBARAK, N. M.; et al. An overview of immobilized enzyme technologies for dye and phenolic removal from wastewater. **Journal of Environmental Chemical Engineering**, v. 7, n. 2, p. 102961, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.jece.2019.102961>>..

KAMEL, M. Y.; SALEH, N. A.; GHAZY, A. M. Gallic acid oxidation by turnip peroxidase. **Phytochemistry**, v. 16, n. 5, p. 521–524, 1977.

KHARATMOL, P. P.; PANDIT, A. B. Extraction , partial purification and characterization of acidic peroxidase from cabbage leaves (*Brasicca olearacea* var . *capitata*). **Protein & Peptide Letters**, v. 15, n. 4, p. 320–326, 2008.

KIM, Y. H.; YOO, Y. J. Peroxidase production from carrot hairy root cell culture. **Enzyme and Microbial Technology**, v. 18, n. 7, p. 531–535, 1996.

KLIBANOV, A. M.; BERMAN, Z.; ALBERTI, B. N. Preparative Hydroxylation of Aromatic Compounds Catalyzed by Peroxidase. **Journal of the American Chemical Society**, v. 103, p. 6263–6264, 1981.

KÖKSAL, E.; GULCIN, I. Purification and Characterization of Peroxidase from Cauliflower (*Brassica oleracea L. var. botrytis*) Buds. **Protein & Peptide Letters**, v. 15, n. 4, p. 320–326, 2008. Disponível em: <<https://doi.org/10.2174/092986608784246506>>.

KUMAR, P.; KAMLE, M.; SINGH, J. Biochemical characterization of *Santalum album* (Chandan) leaf peroxidase. **Physiology and Molecular Biology of Plants**, v. 17, n. 2, p. 153–159, 2011.

KUMAR, R.; SINGH, K. A.; SINGH, V. K.; JAGANNADHAM, M. V. Biochemical characterization of a peroxidase isolated from Caribbean plant: *Euphorbia cotinifolia*. **Process Biochemistry**, v. 46, n. 6, p. 1350–1357, 2011. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.procbio.2011.03.003>>..

KUMAR, S.; PATRA, P.; RANJAN, C.; RAUT, S. Pilot-scale evaluation of bio-decolorization and biodegradation of reactive textile wastewater : An impact on its use in irrigation of wheat crop. **Water Resources and Industry**, v. 21, n. May 2018, p. 100106, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.wri.2019.100106>>..

LAFI, R.; GZARA, L.; LAJIMI, R. H.; HAFIANE, A. Treatment of textile wastewater by a hybrid ultrafiltration/electrodialysisprocess. **Chemical Engineering and Processing - Process Intensification**, v. 132, n. February, p. 105–113, 2018.

LEE, M. Y.; KIM, S. S. Characteristics of six isoperoxidases from Korean radish root. **Phytochemistry**, v. 35, n. 2, p. 287–290, 1994.

LEON, J. C.; ALPEEVA, I. S.; CHUBAR, T. A.; et al. Purification and substrate specificity of peroxidase from sweet potato tubers. **Plant Science**, v. 163, n. 5, p. 1011–1019, 2002.

LIERS, C.; ARANDA, E.; STRITTMATTER, E.; et al. Phenol oxidation by DyP-type peroxidases in comparison to fungal and plant peroxidases. **Journal of Molecular Catalysis B: Enzymatic**, v. 103, p. 41–46, 2014. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.molcatb.2013.09.025>>..

LIMA, D. R. S.; TONUCCI, M. C.; LIBÂNIO, M.; AQUINO, S. F. DE. Fármacos e desreguladores endócrinos em águas brasileiras: ocorrência e técnicas de remoção. **Engenharia Sanitária e Ambiental**, v. 22, n. 6, p. 1043–1054, 2018.

LIU, J.; WANG, T.; JI, L. Enhanced dye decolorization efficiency by citraconic anhydride-modified horseradish peroxidase. **Journal of Molecular Catalysis B: Enzymatic**, v. 41, n. 3–4, p. 81–86, 2006.

LORENZI, H. **Árvores Brasileiras: manual de identificação e cultivo de plantas arbóreas nativas do Brasil**. 2º ed. Nova Odessa, SP: Editora Plantarum, 1998.

LYU, J.; PARK, J.; KUMAR, L.; et al. Testing the toxicity of metals, phenol, effluents, and receiving waters by root elongation in *Lactuca sativa* L. **Ecotoxicology and Environmental Safety**, v. 149, p. 225–232, 2018. Elsevier Inc. Disponível em: <<https://doi.org/10.1016/j.ecoenv.2017.11.006>>..

MACIEL, H. P. F.; GOUVÊA, C. M. C. P.; TOYAMA, M.; et al. Extraction, purification and biochemical characterization of a peroxidase from *Copaifera langsdorffii* leaves. **Química Nova**, v. 30, n. 5, p. 1067–1071, 2007.

MARTIN, R. E.; FLICK, G. J.; HEBARD, C. E.; WARD, D. R. Chemistry and Biochemistry of Marine Food Products. **Molecular Nutrition & Food Research**, v. 27, n. 10, p. 994, 1983.

MATTO, M.; HUSAIN, Q. Decolorization of direct dyes by immobilized turnip peroxidase in batch and continuous processes. **Ecotoxicology and Environmental Safety**, v. 72, n. 3, p. 965–971, 2009.

MAZZEO, D. E. C.; LEVY, C. E.; ANGELIS, D. DE F. DE; MARIN-MORALES, M. A. BTEX biodegradation by bacteria from effluents of petroleum refinery. **Science of the Total Environment**, v. 408, p. 4334–4340, 2010.

MEUNIER, B. N- and O-demethylations catalyzed by peroxidases. In: J. Everse; K. E. Everse; M. B. Grisham (Orgs.); **Peroxidases in Chemistry and Biology**. 2º ed, p.201–217, 1991. CRC Press.

MIHOS, F. C.; SILVA, L. M. C.; SALGADO, A. M.; PEREIRA, P. R. PURIFICAÇÃO PARCIAL DA ENZIMA PEROXIDASE EXTRAIDA DO FEIJÃO (*Phaseolus vulgaris* L.) VISANDO APLICAÇÃO EM BIOSSENSOR AMPEROMÉTRICO PARA DETECÇÃO DE AGROTÓXICOS. XX Congresso Brasileiro de Engenharia Química. *Anais...* p.1–8, 2014. Florianópolis/SC.

MIRANDA-MANDUJANO, E.; MOELLER-CHÁVEZ, G.; ROSAS-VILLEGAS, O.; BUITRÓN, G.; GARZÓN-ZÚÑIGA, M. A. Decolourization of Direct Blue 2 by peroxidases obtained from an industrial soybean waste. **Water SA**, v. 44, n. 2, p. 204–210, 2018.

MIZOBUTSI, G. P.; FINGER, F. L.; RIBEIRO, R. A.; et al. Effect of pH and temperature on peroxidase and polyphenoloxidase activities of litchi pericarp. **Scientia Agricola**, v. 67, n. 2, p. 213–217, 2010.

MOHAMED, S. A.; ABDEL-ATY, A. M.; HAMED, M. B.; EL-BADRY, M. O. *Ficus sycomorus* latex: A thermostable peroxidase. **African Journal of Biotechnology**, v. 10, n. 76, p. 17532–17543, 2011.

MURUGESAN, K.; NAM, I.; KIM, Y.; CHANG, Y. Decolorization of reactive dyes by a thermostable laccase produced by *Ganoderma lucidum* in solid state culture. **Enzyme and Microbial Technology**, v. 40, n. 7, p. 1662–1672, 2007.

NAKANE, P. K.; KAWAOI, A. *Journal of Histochemistry & Cytochemistry. Journal of Histochemistry and Cytochemistry*, p. 1084–1091, 1974.

NELLIS, S. C.; CORREIA, A. F. K.; SPOTO, M. H. F. Extração e quantificação de carotenoides em minitomate desidratado (Sweet Grape) através da aplicação de diferentes solventes. **Brazilian Journal of Food Technology**, v. 20, p. 1–5, 2017. Disponível em: <<http://dx.doi.org/10.1590/1981-6723.15616>>..

NELSON, D. L.; COX, M. M. **Lehninger principles of biochemistry**. 6º ed. New York: W. H. Freeman and Company, 2013.

OECD. **Test No. 202: Daphnia sp. Acute Immobilisation Test**. OECD Publishing, 2004.

OLIVEIRA, G. A. R. DE; LEME, D. M.; LAPUENTE, J. DE; et al. A test battery for assessing the ecotoxic effects of textile dyes. **Chemico-Biological Interactions**, v. 291, p. 171–179, 2018. Elsevier. Disponível em: <<https://doi.org/10.1016/j.cbi.2018.06.026>>.

.

OLIVEIRA, L. A. DE; MAGALHÃES, H. P. DE. Quantitative evaluation of acidity tolerance of root nodule bacteria. **Revista de Microbiologia**, v. 30, n. 3, p. 203–208, 1999. Disponível em: <<http://dx.doi.org/10.1590/S0001-37141999000300004>>..

OZTEKIN, A.; ALMAZ, Z.; GERNI, S.; et al. Purification of peroxidase enzyme from radish species in fast and high yield with affinity chromatography technique. **Journal of Chromatography B**, v. 1114–1115, p. 86–92, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.jchromb.2019.03.035>>..

PANDEY, V. P.; DWIVEDI, U. N. Enzymatic Purification and characterization of peroxidase from Leucaena leucocephala , a tree legume. **Journal of Molecular Catalysis. B, Enzymatic**, v. 68, n. 2, p. 168–173, 2011. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.molcatb.2010.10.006>>..

PANDEY, V. P.; RANI, J.; JAISWAL, N.; et al. Chitosan immobilized novel peroxidase from Azadirachta indica: Characterization and application. **International Journal of Biological Macromolecules**, v. 104, p. 1713–1720, 2017. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.ijbiomac.2017.02.047>>..

PEREIRA, P. C. G.; REIMÃO, R. V.; PAVESI, T.; et al. Lethal and sub-lethal evaluation of Indigo Carmine dye and byproducts after TiO₂ photocatalysis in the immune system of Eisenia andrei earthworms. **Ecotoxicology and Environmental Safety**, v. 143, n. November 2016, p. 275–282, 2017. Elsevier Inc. Disponível em: <<http://dx.doi.org/10.1016/j.ecoenv.2017.05.043>>..

ROBINSON, C. D.; BROWN, E.; CRAFT, J. A.; et al. Effects of sewage effluent and ethynodiol upon molecular markers of oestrogenic exposure, maturation and reproductive success in the sand goby (*Pomatoschistus minutus*, Pallas). **Aquatic Toxicology**, v. 62, n. 2, p. 119–134, 2003.

SALGAONKAR, M.; NADAR, S. S.; RATHOD, V. K. Biomineralization of orange peel peroxidase within metal organic frameworks (OPP-MOFs) for dye degradation. **Journal of Environmental Chemical Engineering**, v. 7, n. 2, p. 102969, 2019. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.jece.2019.102969>>..

SAMPLINER, D.; MILLER, A. Ethnobotany of Horseradish (*Armoracia rusticana*, Brassicaceae) and Its Wild Relatives (*Armoracia* spp.): Reproductive Biology and Local Uses in Their Native Ranges. **Economic Botany**, v. 63, n. 3, p. 303–313, 2010.

ŠEKULJICA, N. C.; T, N. C. P.; T, A. B. S.; et al. Decolorization of Anthraquinonic Dyes from Textile Effluent Using Horseradish Peroxidase : Optimization and Kinetic Study. , v. 2015, 2015.

SHANK, L. P.; KIJJANAPANICH, P.; PHUTRAKUL, S. Characterization of Partially Purified Peroxidase from Fingerroot (*Boesenbergia Rotunda* (L .) Mansf .). **Journal of Medical and Bioengineering**, v. 4, n. 3, p. 170–177, 2015. Disponível em: <<https://www.doi.org/10.12720/jomb.4.3.170-177>>..

SILVA, M. C.; CORRÉA, A. D.; TORRES, J. A.; AMORIM, M. T. S. P. Descoloração de corantes industriais e efluentes têxteis simulados por peroxidase de nabo (*Brassica campestris*). **Química Nova**, v. 35, n. 5, p. 889–894, 2012.

SILVA, M. C.; TORRES, J. A.; VASCONCELOS DE SÁ, L. R.; et al. The use of soybean peroxidase in the decolourization of Remazol Brilliant Blue R and toxicological evaluation of its degradation products. **Journal of Molecular Catalysis B: Enzymatic**, v. 89, p. 122–129, 2013. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.molcatb.2013.01.004>>..

SINGH, R. L.; SINGH, P. K.; SINGH, R. P. International Biodeterioration & Biodegradation Enzymatic decolorization and degradation of azo dyes e A review. **International Biodeterioration & Biodegradation**, v. 104, p. 21–31, 2015. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.ibiod.2015.04.027>>..

SINGH, S.; MISHRA, R.; SHARMA, R. S.; MISHRA, V. Phenol remediation by peroxidase from an invasive mesquite: Turning an environmental wound into wisdom. **Journal of Hazardous Materials**, v. 334, p. 201–211, 2017. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.jhazmat.2017.04.007>>..

SIQUEIRA-SILVA, A. I.; PEREIRA, E. G.; MODOLO, L. V.; LEMOS-FILHO, J. P.; PAIVA, E. A. S. Impact of cement dust pollution on *Cedrela fissilis* Vell. (Meliaceae): A potential bioindicator species. **Chemosphere**, v. 158, p. 56–65, 2016. Elsevier Ltd. Disponível em: <<http://dx.doi.org/10.1016/j.chemosphere.2016.05.047>>..

SLOKAR, Y. M.; MARECHAL, A. M. LE. Methods of decoloration of textile wastewaters. **Dyes and Pigments**, v. 37, n. 4, p. 335–356, 1998.

SOLTIS, P. S.; SOLTIS, D. E. **Isozymes in Plant Biology**. Baltimore: Timber Press, 1990.

SOUZA, S. M. A. G. U. DE; FORGIARINI, E.; SOUZA, A. A. U. DE. Toxicity of textile dyes and their degradation by the enzyme horseradish peroxidase (HRP). **Journal of Hazardous Materials**, v. 147, p. 1073–1078, 2007.

STEEVENSZ, A.; MADUR, S.; AL-ANSARI, M. M.; et al. A simple lab-scale extraction of soybean hull peroxidase shows wide variation among cultivars. **Industrial Crops & Products**, v. 48, p. 13–18, 2013. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.indcrop.2013.03.030>>..

TAO, Y. M.; WANG, S.; LUO, H. L.; YAN, W. W. Peroxidase from jackfruit: Purification, characterization and thermal inactivation. **International Journal of Biological Macromolecules**, v. 114, p. 898–905, 2018. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.ijbiomac.2018.04.007>>..

TEREFE, N. S.; DELON, A.; VERSTEEG, C. Thermal and high pressure inactivation kinetics of blueberry peroxidase. **Food Chemistry**, v. 232, p. 820–826, 2017. Disponível em: <<http://dx.doi.org/10.1016/j.foodchem.2017.04.081>>..

TIJSSEN, P.; KURSTAK, E. Highly efficient and simple methods for the preparation of peroxidase and active peroxidase-antibody conjugates for enzyme immunoassays. **Analytical Biochemistry**, v. 136, n. 2, p. 451–457, 1984. Disponível em: <[https://doi.org/10.1016/0003-2697\(84\)90243-4](https://doi.org/10.1016/0003-2697(84)90243-4)>..

TREICHEL, H.; MARQUES, C. T.; GOLUNSKI, S. M.; et al. Descoloração de efluente pela ação da enzima peroxidase extraída de farelo de arroz. XXV Congresso Brasileiro de Ciência e Tecnologia de Alimentos: Alimentação: a árvore que sustenta a vida. **Anais...** . p.1–6, 2016. Gramado/RS.

VEITCH, N. C. Horseradish peroxidase: a modern view of a classic enzyme. **Phytochemistry**, v. 65, n. 3, p. 249–259, 2004.

VEITCH, N. C.; SMITH, A. T. Horseradish peroxidase. **Advances in Inorganic Chemistry**, v. 51, p. 107–162, 2000. Disponível em: <[https://doi.org/10.1016/S0898-8838\(00\)51002-2](https://doi.org/10.1016/S0898-8838(00)51002-2)>..

WAGNER, M.; NICELL, J. A. Peroxidase-catalyzed removal of phenols from a petroleum refinery wastewater. **Water Science & Technology**, v. 43, n. 2, p. 253–260, 2001.

WEISBURGER, J. H. Comments on the history and importance of aromatic and heterocyclic amines in public health. **Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis**, v. 507, p. 9–20, 2002. Disponível em: <[https://doi.org/10.1016/S0027-5107\(02\)00147-1](https://doi.org/10.1016/S0027-5107(02)00147-1)>..

WELINDER, K. G. COVALENT STRUCTURE OF THE GLYCOPROTEIN HORSERADISH PEROXIDASE (EC 1.11.1.7). **FEBS Letters**, v. 72, n. 1, p. 19–23, 1976.

WIJETUNGA, S.; LI, X.; JIAN, C. Effect of organic load on decolourization of textile wastewater containing acid dyes in upflow anaerobic sludge blanket reactor. **Journal of Hazardous Materials**, v. 177, p. 792–798, 2010. Elsevier B.V. Disponível em: <<http://dx.doi.org/10.1016/j.jhazmat.2009.12.103>>..

ZHANG, H.; ZHANG, J.; ZHANG, X.; GENG, A. Purification and characterization of a novel manganese peroxidase from white-rot fungus Cerrena unicolor BBP6 and its application in dye decolorization and denim bleaching. **Process Biochemistry**, v. 66, p. 222–229, 2017. Elsevier. Disponível em: <<http://dx.doi.org/10.1016/j.procbio.2017.12.011>>..

ZHU, N.; ZOU, Y.; HUANG, M.; et al. A sensitive, colorimetric immunosensor based on Cu-MOFs and HRP for detection of dibutyl phthalate in environmental and food samples. **Talanta**, v. 186, p. 104–109, 2018. Elsevier B.V. Disponível em: <<https://doi.org/10.1016/j.talanta.2018.04.023>>..