PAPER • **OPEN ACCESS**

Laccase nanoparticle: Synthesis, characterization, entrapment in alginate beads and application in the biodegradation of Bisphenol A

To cite this article: N Ademakinwa Adedeji *et al* 2021 *IOP Conf. Ser.: Mater. Sci. Eng.* **1150** 012005

Laccase nanoparticle: Synthesis, characterization, entrapment in alginate beads and application in the biodegradation of Bisphenol A

Ademakinwa Adedeji N[1], Abidemi Ojo[2], Mariana Erasmus[2], Agunbiade Mayowa O.[3*]

[1]Department of Physical and Chemical Sciences, Elizade University, Nigeria
[2]Centre for Mineral Biogeochemistry, University of the Free State, Bloemfontein, 9301. South Africa
[3]Applied Microbiology and Health Biotechnology Institute, Cape Peninsula University of Technology, Cape Town, South Africa

E-mail: Agunbiadem@cput.ac.za

Abstract. Laccase nanoparticles (*LNP*) were synthesized by desolvation of the enzyme with ethanol and cross-linking with glutaraldehyde. *LNP* was characterized via scanning electron microscopy (*SEM*) and then entrapped in alginate beads for the biodegradation of bisphenol A (*BPA*). The stability of the immobilized *LNP* (*iLNP*) to heat inactivation was also investigated. The *iLNP* retained about 53±4.7% of its initial activity after 7 cycles of catalysis. The *iLNP* was stable to thermal inactivation at 40-70ºC as obtained from its kinetic and thermodynamic parameters. The *iLNP* removed 80 mg/L *BPA* from solutions with a biodegradation efficiency (*BE*) of 92% after 1 h. Repeated use of the *iLNP* in *BPA* removal resulted in a BE of 75% after 7 cycles of catalysis. *iLNP* serves as a novel biocatalyst in the bioremediation of pollutants such as *BPA,* and its thermostable characteristics make it useful for other biotechnological applications

1. Introduction

Bisphenol A is toxic due to its estrogenic effects on the female reproductive systems, thus leading to BPA being termed as an endocrine-disrupting chemical [1]. The adverse effects of prolonged exposure to BPA include breast cancers, reduction in male sperm counts, immune disruption, to name a few. The effects on aquatic life are also deleterious as the fertility in fish is reduced [2]. Several techniques have been suggested to remove phenols and their derivatives; these include chemical oxidation, solvent extraction and even electrochemical methods. These techniques are often deficient as they lead to the release of more toxic by-products to the environment, they are too costly, and they are not very efficient [3]. The use of the biological approach is more cost-effective as it is efficient and generates non-toxic by-products. Examples of biological approaches include using enzymes produced by microorganisms and plants in the biodegradation of these phenols [4]. Enzymes that play key roles in the biodegradation of these phenols are laccases. Laccases (benzenediol: oxygen oxidoreductase EC 1.10.3.2) are oxidoreductases that play a role in the oxidation of a

wide range of aromatics. Laccases have been employed in both free and immobilized form in the biodegradation of phenols and other pollutants such as polycyclic aromatic hydrocarbons, chlorophenols etc. A good review on how laccases are deployed in the biodegradation of pollutants such as BPA was provided by Bilal et al. [1]. Laccase for the biodegradation of phenols and derivatives are mostly obtained from the *Trametes* genus, and there is the need to explore other fungi capable of expressing these enzymes [5, 6]. As proposed in this study, we and other authors have noted that *Aureobasidium pullulans* are good producers of extracellular laccase [7, 8]. In reports where laccases (in free or immobilized forms) were used in the biodegradation of phenols, often, numerous setbacks are observed due to the relative poor catalysis and reduced reusability of the enzymes. Hence, there is the need for a novel and intuitive approach to immobilization of laccase for the biodegradation of these phenols. Therefore, we proposed using laccase nanoparticles entrapped in alginate beads. Enzyme nanoparticles are known to be more catalytically active, thermostable than free enzyme. In fact, enzyme nanoparticles are often used as biosensors because of their increased electrochemical properties [9]. This study is the first of its kind to explore the potential use of enzyme nanoparticles in the biodegradation of phenols.

2. Methodology

2.1. Microorganism and conditions

Aureobasidium pullulans was maintained on malt extract agar at 4ºC. Sub-culturing was done every 72 h. Production of mycelia for extraction of intracellular laccase was done as described by Ademakinwa et al. [8]. The culture supernatant was centrifuged at 4500 x g for 15 min, and the mycelia obtained served as the source of intracellular laccase. Mycelia homogenization for extraction of the intracellular enzyme was carried out using techniques described by Ademakinwa and Agboola [10]

2.2. Extraction of intracellular laccase and assay

Mycelia obtained in section 2.1 above was homogenized in an equal volume of very chilled 50 mM phosphate buffer pH 6.5. The homogenate was centrifuged at 13500 x g for 20 min to obtain a clear supernatant that serves as the intracellular laccase. Laccase was assayed for using ABTS as substrates respectively [7]

2.3. Preparation, characterization and immobilization of laccase nanoparticles

Laccase nanoparticles (LNP) were prepared according to the methods described by Jahkar and Singh et al. [9]. Briefly, laccase was desolvated using ethanol and cross-linked using glutaraldehyde. The laccase nanoparticles were purified by centrifugation at 10000 x g for 10 min. The LNP was stored at 4ºC for further use. The LNP was characterized using scanning electron microscopy [9]. Immobilization of the nanoparticles on the alginate complex was carried out as described by the methods of Ademakinwa and Agboola [12] and Bezbaurah et al. (13) with minor modifications.

2.4. Thermodynamic and kinetic properties of laccase nanoparticles entrapped in alginate support

The thermal inactivation process was determined according to the methods described by Ademakinwa et al. [14], Ademakinwa and Agboola [10] and Ademakinwa et al. [12] after the determination of the temperature profile.

2.5. Biodegradation of phenols and bisphenol A in model wastewater

The biodegradation of bisphenol A was performed according to the methods of Asagdol et al. [11]. The reaction mixture contained 0.1 g of immobilized LNP and phenol or BPA solution (final concentration of 80 mg/L) followed by incubation at 35°C and stirring at 50 rpm for 40 min. Samples were withdrawn every 10 min and analyzed for residual concentrations of phenolic pollutants.

3. Results and Discussion

Fig.1 shows the SEM images for the laccase nanoparticles. The immobilized laccase nanoparticles (iLNP) were retained in the alginate beads, and they oxidized ABTS. The iLNP were used for several rounds of ABTS catalysis and the iLNP retained about 53% of residual activity after 7 catalytic cycles (Fig. 2). A gradual loss in enzyme activity might be associated with enzyme leakage from the beads [16]. Table 1 summarizes the kinetic and thermodynamic properties of the free laccase and iLNP. The heat denaturation process generally followed first-order reaction kinetics. This observation has been noted in

Figure1. Scanning electron microscopy images of Laccase Nanoparticles (A) x100 (B) x 500

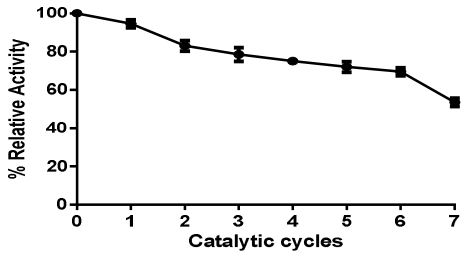

Figure 2. *iLNP* catalysis of ABTS oxidation. The experiment was performed in triplicate, and error bars indicate deviation from mean

enzymes produced by this fungus [10, 12, 14, 15]. It can be observed that generally, the half-life ($t_{1/2}$) values decreased as the temperature was increased from 50 to 80ºC.

Table 1. The kinetic and thermodynamic parameters for free laccase and *LNP* thermal inactivation process

T (K)	k_d (h^{-1})	$t_{1/2}$ (h)	ΔH_d^* (kJ/mol)	ΔG_d^* (kJ/mol)	ΔS_d^* J/mol/K
40	#0.031	#22.0	#37.8	#88.3	#-161.7
	*0.010	*69.0	*42.4	*89.9	*-151.2
50	#0.070	#11.0	#37.5	#88.8	#-161.3
	*0.011	*63.0	*42.3	*92.2	*-154.4
60	#0.170	#4.0	#37.3	#86.9	#-156.8
	*0.019	*37.0	*42.2	*93.4	*-153.4
70	#0.180	#4.0	#37.1	#89.8	#-155.2
	*0.0200	*35.0	*42.1	*94.9	*-152.5
80	#0.200	#4.0	#37.1	#90.3	#-155.1
	*0.030	*23.0	*42.1	*95.3	*-151.0

#- Free laccase

*- Immobilized Laccase Nanoparticles (*iLNP*)

The result showed that the denaturation of the iLNP and the free enzyme increased as the temperature increased. Meanwhile, k_d, which is the inactivation constant decreased as the temperature increased both in the free laccase and iLNP. Comparatively, the values obtained for the kinetic parameters indicated that the iLNP was thermally stable to thermal inactivation than the free enzyme. From the values obtained for thermodynamic parameters (for the free laccase and laccase iLNP) such as $\Delta H_d^*, \Delta S_d^* \text{ and } \Delta G_d^*$, it can be concluded that the iLNP showed better resistance to heat inactivation and hence it could be useful for other biotechnological applications. For example, the entropic values were negative; this implies that there was resistance to disorderliness as the temperature increased. And overall, this indicated thermostability to thermal denaturation. The iLNP was able to efficiently biodegrade BPA with a biodegradation efficiency of 76% after 2 h. The biodegradation efficiency of the iLNP decreased to 56% after the third catalytic cycle. The decrease might be associated with leakage of the LNP from the beads [16].

4. Conclusion

Laccase nanoparticles synthesized via de-solvation with ethanol, cross-linking with glutaraldehyde and immobilized by entrapment in alginate displayed unique properties such as resistance to thermal inactivation. The LNP was able to efficiently biodegrade BPA resulting in a biodegradation efficiency of 76%. The heat resistant properties of the iLNP suggest that it can be a novel biocatalyst for numerous industrial applications.

References

[1] Bilal M, Iqbal HMN, Barceló D.2019. Mitigation of bisphenol A using an array of laccase-based robust bio-catalytic cues – A review. Science of The Total Environment. doi:10.1016/ j.scitotenv. 2019.06.403
[2] Chhaya U, Gupte A. 2013. Possible role of laccase from Fusarium incarnatum UC-14 in bioremediation of Bisphenol A using reverse micelles system. Journal of Hazardous Material, 254–255:149–156.
[3] Modaressi K, Taylor KE, Bewtra JK, Biswas N. 2005. Laccase-catalyzed removal of bisphenol-A from water: protective effect of PEG on enzyme activity Water Research. 39:4309–4316.
[4] Inoue M, Masuda Y, Okada F, Sakurai A, Takahashi I, Sakakibara. M 2008. Degradation of bisphenol A using sonochemical reactions. Water Research. 42:1379–1386.
[5] Roy S, Ishita D, Munjal M, Karthic L, Kumar G, Kumar S, Rao KVB. 2014. Isolation and characterization of tyrosinase produced by marine actinobacteria and its application in the removal of phenol from the environment. Frontiers in Biology 9(4):301-316.
[6] Ryan D, Leukes W, Burton S. 2007. Improving the bioremediation of phenolic wastewaters by Trametes versicolor. Bioresource Technology, 98:579–587.
[7] Rich JO, Leathers TD, Anderson AM, Bischoff KM and Manitchotpisit P. 2013. Laccases from Aureobasidium pullulans. Enzyme and Microbial Technology, 53(1), 33–37. doi:10.1016/j.enzmictec. 2013.03.015
[8] Ademakinwa AN, Agboola FK. 2016. Biochemical Characterization and Kinetics of a Purified Yellow Laccase from Aureobasidium pullulans (De Bary) Isolated from Soil Containing Decayed Plant Matter. Journal of Genetic Engineering and Biotechnology 14(1):143-151.
[9] Jakhar S, Pundir S. 2018. Preparation, characterization and application of urease nanoparticles for construction of an improved potentiometric biosensor. Biosensor and Bioelectronics 100: 242-250

[10] Ademakinwa AN, Agboola FK. 2020. Some biochemical, catalytic, thermodynamic and kinetic properties of purified fructosyltransferase from wild and improved mutant-type Aureobasidium pullulans NAC8. Biocatalysis and Biotransformation, 1–12. doi:10.1080/10242422.2019.1671376
[11] Asagdol Z, Forootanfar H, Rezaei S, Mahvi AH, Faramarzi MA. (2013). Removal of phenol and bisphenol-A catalysed by laccase in aqueous solution. Journal of Environmental Health and Engineering 12:93 (1-5)
[12] Ademakinwa AN, Agboola FK. (2019). Kinetic and Thermodynamic Investigations of Cell-Wall Degrading Enzymes Produced by Aureobasidium pullulans via Induction with Orange Peels: Application in Lycopene Extraction. Preparative Biochemistry and Biotechnology. doi: 10.1080/10826068.2019.1650375
[13] Bezbaruah, A. N., Krajangpan, S., Chisholm, B. J., Khan, E. Elorza Bermudez, J. J. (2009). Entrapment of iron nanoparticles in calcium alginate beads for groundwater remediation applications. Journal of Hazardous Materials, 166(2-3), 1339–1343. doi:10.1016/j.jhazmat.2008.12.054
[14] Ademakinwa AN, Agunbiade MO and Fagbohun O. 2020. Biodegradation of cyanide in cassava wastewater using a novel thermodynamically-stable immobilized rhodanese. Preparative Biochemistry & Biotechnology, 1–11. doi:10.1080/10826068.2020.1846053
[15] Ademakinwa AN, Agunbiade MO, Ayinla ZA, Agboola FK (2019). Optimization of Aqueous Two-Phase Partitioning of Aureobasidium pullulans α-Amylase via Response Surface Methodology and Investigation of its Thermodynamic and Kinetic Properties. International Journal of Biological Macromolecules doi: https://doi.org/10.1016/j.ijbiomac.2019.08.159
[16] Lassouane F, Aït-Amar H, Amrani S, Rodriguez-Couto A. 2019. A promising laccase immobilization approach for Bisphenol A removal from aqueous solutions. Bioresour. Technol. doi:10.1016/j.biortech.2018.09.129