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Mechanistic aspects of enhanced kinetics in sonoenzymatic processes using three simultaneous approaches

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Abstract

Enzymes are highly efficient bio-catalysts made up of large bio-macromolecules composed of amino acids.[1,2] Enzyme mediated processes are winning the competition against other catalytic processes due to its cost-effectiveness, low energy intake, nontoxic, and eco-friendly nature.[3,4] Significant research has been carried out to enhance the kinetics of biocatalytic processes by using microwaves,[5] supercritical fluid,[6] ionic liquids,[7] applications of modifiers,[8] and ultrasound.[2,4,9] However, very little is known about the mechanism of enhanced kinetics.[2] Herein, we propose a methodology that includes three simultaneous approach to unravel the mechanistic aspect of enhanced kinetics with the application of sonication. This amalgamated approach can be represented as vertices of a solution triangle that include (1) estimation of secondary structural changes in enzymes, (2) computational techniques such as molecular docking, and (3) mathematical modelling of enzymatic reactions. It is known from the previous literature that ultrasound irradiation has impacts on the secondary structure composition of enzymes which can be estimated via techniques such as deconvolion of FT-IR spectra, CD spectroscopy, and Fluorescence spectroscopy. To explain the impacts of structural changes on reaction kinetics, one can perform molecular docking analysis that reveals the molecular mechanism of enzymatic reactions and product formation. Furthermore, mechanistic investigation by using mathematical models can elucidate the thermodynamic and macroscopic effects of ultrasound on faster reaction kinetics. As a proof of concept, we have published one research article entitled *"Mechanistic Investigations in Sonoenzymatic Synthesis of N-Butyl Levulinate."* in the journal Process Biochemistry. In that study, we have reported the mechanistic accounts of sonoenzymatic synthesis of n-butyl levulinate using Novozym 435. Mechanistic investigation was performed using Ping-Pong Bi-Bi kinetics model, molecular docking simulations, and secondary structural analysis using FTIR that revealed interesting influence of sonication on reaction system.

Keywords: Ultrasound, Molecular docking, Density functional theory, Kinetic Mechanism, Secondary structure

Reference

(1) Al-Maqtari, Q. A.; AL-Ansi, W.; Mahdi, A. A. Microbial Enzymes Produced by Fermentation and Their Applications in the Food Industry - A Review. **2019**, *8* (1), 21.
(2) Kumar, K.; Roy, K.; Moholkar, V. S. Mechanistic Investigations in Sonoenzymatic Synthesis of N-Butyl Levulinate. *Process Biochemistry* **2021**, *111*, 147–158. https://doi.org/10.1016/j.procbio.2021.09.005.
(3) Ortiz, C.; Ferreira, M. L.; Barbosa, O.; dos Santos, J. C. S.; Rodrigues, R. C.; Berenguer-Murcia, Á.; Briand, L. E.; Fernandez-Lafuente, R. Novozym 435: The "Perfect" Lipase Immobilized Biocatalyst? *Catal. Sci. Technol.* **2019**, *9* (10), 2380–2420. https://doi.org/10.1039/C9CY00415G.
(4) Malani, R. S.; Umriwad, S. B.; Kumar, K.; Goyal, A.; Moholkar, V. S. Ultrasound–Assisted Enzymatic Biodiesel Production Using Blended Feedstock of Non–Edible Oils: Kinetic Analysis. *Energy Conversion and Management* **2019**, *188*, 142–150. https://doi.org/10.1016/j.enconman.2019.03.052.
(5) Shinde, S. D.; Yadav, G. D. Insight into Microwave-Assisted Lipase Catalyzed Synthesis of Geranyl Cinnamate: Optimization and Kinetic Modeling. *Appl Biochem Biotechnol* **2015**, *175* (4), 2035–2049. https://doi.org/10.1007/s12010-014-1367-3.
(6) Habulin, M.; Šabeder, S.; Sampedro, M. A.; Knez, Ž. Enzymatic Synthesis of Citronellol Laurate in Organic Media and in Supercritical Carbon Dioxide. *Biochemical Engineering Journal* **2008**, *42* (1), 6–12. https://doi.org/10.1016/j.bej.2008.05.012.
(7) Zheng, M.-M.; Wang, L.; Huang, F.-H.; Guo, P.-M.; Wei, F.; Deng, Q.-C.; Zheng, C.; Wan, C.-Y. Ultrasound Irradiation Promoted Lipase-Catalyzed Synthesis of Flavonoid Esters with Unsaturated Fatty Acids. *Journal of Molecular Catalysis B: Enzymatic* **2013**, *95*, 82–88. https://doi.org/10.1016/j.molcatb.2013.05.028.
(8) Jia, R.; Hu, Y.; Liu, L.; Jiang, L.; Huang, H. Chemical Modification for Improving Activity and Stability of Lipase B from Candida Antarctica with Imidazolium-Functional Ionic Liquids. *Org. Biomol. Chem.* **2013**, *11* (41), 7192. https://doi.org/10.1039/c3ob41076e.
(9) Malani, R. S.; Shinde, V.; Ayachit, S.; Goyal, A.; Moholkar, V. S. Ultrasound–Assisted Biodiesel Production Using Heterogeneous Base Catalyst and Mixed Non–Edible Oils. *Ultrasonics Sonochemistry* **2019**, *52*, 232–243. https://doi.org/10.1016/j.ultsonch.2018.11.021.