



ISSN: 2523-5664 (Print)
ISSN: 2523-5672 (Online)
CODEN: WCMABD



REVIEW ARTICLE

THE POTENTIAL OF ECOENZYME AS BIOCATALYSTS : A REVIEW IN WASTEWATER TREATMENT

Pitriani^{a,b}, Agus Bintara Birawida^{c*}, M. Nasir^{d,b}

^aDoctoral Student at Environmental Health Department, Public Health Faculty, Hasanuddin University, Indonesia

^bEnvironmental Health Department, Public Health Faculty, Tadulako University, Indonesia

^cEnvironmental Health Department, Public Health Faculty, Hasanuddin University, Indonesia

^dDepartment of Epidemiology and Biostatistics, College of Public Health, University of Georgia University of Georgia, Athens, Georgia 30605, USA.

*Corresponding Author Email: agusbirawida@gmail.com

This is an open access journal distributed under the Creative Commons Attribution License CC BY 4.0, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited

ABSTRACT

Article History:

Received 11 October 2025
Revised 21 November 2025
Accepted 17 December 2025
Available online 01 January 2026

The literature focuses on the potential of coenzymes as biocatalysts in wastewater treatment. The strategy used is Systematic Literature Review as an instrument. Metadata was collected using Publish or Perish, and 3.151 articles were extracted from CrossCef, PubMed, OpenAlex, and Google Scholar during the period 2015-2025. After eliminating duplicate and anonymous articles, 1.898 articles remained, then bibliometric as data processor which is analyzed by VOSviewer software. This literature study presents a more accurate bibliographical objective for future researchers according to the themes discussed. The potential of coenzymes as biocatalysts in wastewater treatment lies in their enzyme content, such as protease, lipase, and amylase. These enzymes play an important role in the degradation of organic pollutants contained in wastewater. In addition, the organic acid and the presence of lactic acid bacteria have been found to inhibit the growth of pathogenic bacteria commonly found in wastewater, such as *Staphylococcus aureus* and *Escherichia coli*. Although they have the potential to be developed as biocatalysts, the application of coenzymes in conjunction with existing wastewater treatment technologies such as biofilters, constructed wetlands, and combinations with phytoremediation still face challenges, particularly related to enzyme stability in field conditions. Immobilized enzymes have been proven to be one of the promising methods to enhance enzyme stability. Further studies are needed in scale-up enzyme-based biocatalysts and integration with established systems.

KEYWORDS

Ecoenzyme, Biocatalyst, Wastewater, SLR, Bibliometric

1. INTRODUCTION

Wastewater management is an important part of water resource management; the main challenge in this regard is the discharge of wastewater itself. Discharging untreated wastewater into water bodies can cause serious environmental consequences like eutrophication and detrimental effects on aquatic life (Li et al., 2023 ; Singh et al., 2023). Humans' activities are the cause of increasing water pollution worldwide. There are many factors that become challenges in wastewater management. In many areas, gaps in regulations or inadequate enforcement led to non-compliance in wastewater treatment. This condition is aggravated by lacking wastewater monitoring, resulting in limited understanding the extent of the problem and inadequate action to address it (Santosa et al., 2023 ; Makanda et al., 2022). Wastewater can be produced in different activities, which have different characteristics. Biological treatment is the most widely used to remove organic pollutants

due to its cost-effectiveness and eco-friendliness, but these methods need to address technical limitations for obtaining the necessary removal of organic and inorganic pollutants (Zhao et al., 2019 ; Shahid et al., 2024). Biofilter becoming popular in wastewater treatment, it is cost-effective, simple, sustainable, and eco-friendly compared to conventional techniques the principle advantage of the biofilter its efficient use of space and energy (Ningsih et al., 2024 ; Said et al., 2024). The anaerobic biofilter works well in decomposing organic matter in oxygen-free conditions, thereby reducing the need for external energy. The aerobic stage can be performed with efficient aeration control; even passive aeration systems can be adopted to conserve energy (Putri and Julianti, 2025). However, biofilters have the disadvantage of taking a long time to build up a biofilm, as a key feature of biofilters is that the organisms are organized in biofilms (Xu et al., 2024). Biofilm is formed when bacteria or any other suitable microbe finds appropriate condition, attach to it, grow, multiply and they are immobilized in a matrix of extracellular polymeric substances (EPS)

Quick Response Code



Access this article online

Website:
www.watconman.org

DOI:
10.26480/wcm.04.2025.798.804

both environmental pollution and resource recovery objectives. Further, ecoenzyme utilization contributes to sustainable development goals by facilitating low-cost wastewater treatment solutions that can be adapted for either communal or household scale implementation. The environmental friendliness coupled with cost-effectiveness positions ecoenzymes as alternative for enhancing wastewater treatment processes (Sahil Salvi et al., 2024). Studies on the use of ecoenzymes have been widely conducted in wastewater treatment (Basri et al., 2022 ; Galintin et al., 2021 ; Santosa et al., 2023), natural fertilisers (Amadea et al., 2025), and cleaning agents. Ecoenzymes are already being used due to domestic wastewater (Bharvi S. Patel et al., 2021) and even industrial wastewater (Zhu et al., 2025 ; Werner et al., 2016). Trends of studies on the application of ecoenzymes invarious wastewater treatment processes are presented in table 1.

Table 1 : Trends of Ecoenzymes Application in Various Wastewater Treatment		
References	Treatment/Sampel	Result
(Natt SK et al., 2025)	electroplating industry Wastewater	10% of ecoenzymes Fe (99.19%), P (63.02%), As (98.72%), S (60.79%)
(Sahil Salvi et al., 2024)	Domestic Wastewater	20% ecoenzyme reduce BOD (94.20%), COD (86.34%), total coliform (96.96%)
(Das et al., 2023)	textile wastewater treatment	Reduce BOD (80–85%), COD (81–85%), TDS (35–37%), TSS (55–60%)
(Galintin et al., 2021)	aquaculture sludge	effectively reduce TSS, VOC, COD, Ammonia, Phosphorus
(Sulistyah S et al., 2022)	Liquid waste	ecoenzyme at a concentration of 20% can kill <i>E. coli</i> (99.95%) and <i>P. aeruginosa</i> (99.90%)
(Shivalik Y et al., 2022)	Domestic Wastewater	mixing ecoenzymen with sample (1:1000) capable of removing COD, BOD, ammoniacal nitrogen, nitrate nitrogen, and nitrite nitrogen
(Amadea et al., 2025)	dairy wastewater	6% of ecozyme effectively reduce alkalinity, pH, COD, TDS, TSS, oil & grease
(Kumar A et al., 2020)	Domestic Wastewater (Greywater)	15% ecoenzyme can remove TDS, BOD ₅ , COD, nitrogen and phosphate
(Pachaiappan R et al., 2022)	metal based effluen	Reduce BOD (70), TS (32.5%), TDS (39.5%) and TSS (33.0%) after 10 weeks applications

3.3 Enzyme-based biocatalysts potential in wastewater treatment

The production process of ecoenzyme typically involves a batch fermentation approach, wherein organic wastes are fermented with a carbohydrate source such as brown sugar/molasses and water mostly in ratio of 1:3:10 (Hemalatha and Visantini, 2020 ; Varshini and Gayathri, 2023). The carbohydrate source may affect the quality of the ecoenzyme; brown sugar is recommended (Phanama et al., 2024). The sucrose content in palm sugar tends to be higher than that in granulated sugar, leading it to become a better energy source to bacteria in the ecoenzyme fermentation process (Laela and Titik, 2025). The fermentation process usually extends over a period of 90 days to allow for enzymatic activity development and metabolite maturation (Rukmini and Astuti Herawati, 2023 ; Karimah and Lusiani, 2024), this duration proposed to achieve the highest antioxidant activity (Putri et al., 2024) and had strong inhibition against *Staphylococcus aureus* dan *Escherichia coli* (Tallei et al., 2023).

Ecoenzymes comprise a broad spectrum of biochemical constituents, predominantly organic acids, hydrolytic enzymes, and mineral nutrients. Acetic acid is frequently identified as a major organic acid responsible for

antimicrobial properties, Meanwhile, the hydrolytic enzymes amylase, protease, and lipase serve to mediate the breakdown of carbohydrates, proteins, and lipids (Galintin et al., 2021 ; Arun and Sivashanmugam, 2015). These enzymes act synergistically as catalysts in the degradation of organic pollutants in wastewater, thereby increasing treatment efficiency (Feng et al., 2025). Ecoenzyme production processes and attribute potential as enzyme-based biocatalysts in wastewater treatment illustrated in figure 3.

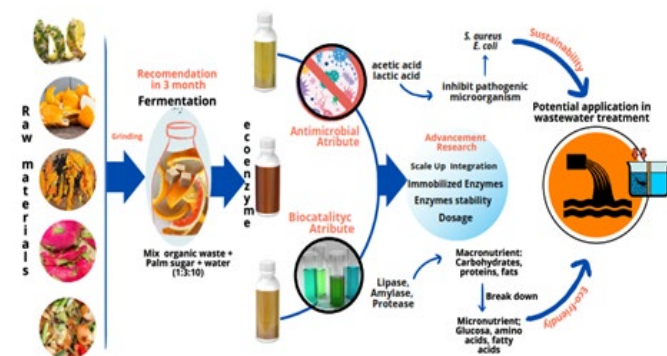


Figure 3 : Enzyme-based biocatalysts potential in wastewater treatment

Ecoenzymes contribute to wastewater treatment through enzymatic biodegradation of organic pollutants, hydrolytic enzymes break down complex molecules into simpler and more biodegradable forms. Amylases convert starches into simpler sugars, proteases degrade proteins into amino acids, and lipases hydrolyze fats into fatty acids and glycerol (Benny et al., 2023 ; Pandey et al., 2000). In the case of organic compounds, converted to simple inorganic molecules such as carbon dioxide and water through catabolism. Their broad substrate specificity enables the break down a variety of organic contaminants in domestic and industrial wastewater (Gumilar, 2023a). Ecoenzymes have an essential role in biochemical processes that occur in nature, including the decomposition of organic matter, nutrient cycling, and detoxification of dangerous substances. Ecoenzymes act in concert with native microbial consortia and enhance biodegradation efficiency through synergistic interactions. The presence of diverse enzymes and organic acids creates a conducive microenvironment that stimulates microbial metabolism and pollutant breakdown (Pasalari, 2024). The fermentation process not only generates a spectrum of enzymes but also enriches the solution with beneficial microorganisms including bacteria, fungi, yeast, and microalgae (Abdelfattah et al., 2023). Numerous microalgae species, including *Scenedesmus*, *Chlorella*, *Ankistrodesmus*, *Chlamydomonas*, *Ecoenzymes* also contain bioactive compounds and organic acids, which contribute to their antimicrobial activity. The presence of organic acids such as acetic acid and hydrolytic enzymes further enhances their ability to inhibit or kill pathogens. In the past few years, ecological enzymes have become increasingly popular due to their dual benefits in waste management and as natural antimicrobial agents in various fields of health, agriculture, and the environment. Ecological enzymes are rich in bioactive compounds such as flavonoids, tannins, and saponins, which contribute to their antimicrobial properties (Vidalia et al., 2023). Antimicrobial assays demonstrated that ecoenzymes effectively inhibit various pathogenic bacteria, especially by disrupting cell structure and inhibiting nucleic acid synthesis or interfering genetic and protein synthesis (Rachwał and Gustaw, 2025 ; Wan Mohd Zain et al., 2025). A few studies report that ecoenzymes effectively inhibit a range of pathogenic bacteria e.g., *Staphylococcus aureus*, *Streptococcus mutans*, *Enterococcus faecalis* and gram negative e.g., *Escherichia coli* (Sai et al., 2023). Ecoenzyme from pineapple, orange, banana stem, noni and quinine waste showed inhibition against both types of bacteria *Staphylococcus aureus* and *Escherichia coli* (Lubis et al., 2025). Ecoenzymes hold real promise as cost-effective, natural antimicrobial agents for health care, food preservation, agriculture, environmental management, and household sanitation (Basso and Serban, 2019). Evidence based enzymatic hydrolysis and antimicrobial *Phormidium*, *Chlamydomonas*, *Spirulina* and *Botryococcus* have demonstrated excellent capabilities for the bioremediation of nutrients, emerging pollutants and pathogens in wastewater treatment, this process helps to prevent eutrophication (Ahmad et al., 2021). These microbial consortium further contribute to the decomposition of organic matter through their metabolic activities and might secrete additional enzymes, enhancing the overall biodegradation process (Singh and Jain, 2024a ; Xia et al., 2025). This synergism enables the removal of persistent organic compounds that are often resistant in conventional treatment properties of ecoenzyme (Farma et al., 2023) illustrated in table 2.

Table 2 : Enzymatic Hydrolysis and Antimicrobial Properties of Ecoenzyme

References	Fermentation Duration	Ecoenzyme Materials	Active Compounds/ Enzymatic Composition
(Viona S et al., 2025)	3 months	pomelo peels	octadecanoic acid, hexadecanoic acid, octadecenoic acid, and neophytadiene
(Ismail AY et al., 2024)	3 months	Mixture fruit peels and mixture vegetables	acetic acid, lactic acid, citric acid, oxalic acid
(Das et al., 2023)	3 months	banana, pineapple, and orange fruit peels	protease, amylase, and lipase
(Karimah and Lusiani, 2024)	3 months	orange and melon peels	pH 3.9-4.1 TDS 1.339-1405 ppm Acetic acid 0.81-1.08 % w/w Colour bright yellow
(Joshi, V.B et al., 2022)	3 months	garlic peels and onion peels and husks	Amylases enzymes Alcohol 6.9±0.04 %
(Santosa et al., 2023)	3 months	Papaya, mango, spinach and Chinese cabbage waste	pH 3.1-3.4 Alcohol 4.1-4.8% TDS 1.339-1405 ppm Acetic acid 0.81-1.08 % w/w Colour bright yellow
(Galintin et al., 2021)	3 moths	sisa nanas, jeruk, tomat, dan mangga	protease, amylase, and lipase
(Suliestyah S et al., 2022)	3 moths	papaya, dragon fruit, and orange peels	amylase and cellulase enzymes
(Hemalatha and Visantini, 2020)	3 moths	Citrus fruit peels	Antimicrobial
(Vama L and Cherekar M, 2020)	3 moths	Citrus fruit peels	flavonoids, alkaloids, quinones, cardenolides, and saponins protease, amylase, and lipase activity
(Gu S et al., 2021)	3 moths	Honeylocust Fruits	protease, amylase, lipase, cellulase
(Rahayu et al., 2021)	8-10 days	rambutan fruit skin, corn cobs, chayote skin + frangipani sandalwood extract	<i>the longer fermentation time, alcohol increased, while pH decreased.</i> <i>Antibacterial</i>
(Rasit N, Fern L and Ghani WA, 2019)	3 moths	orange, pineapple, tomato, and mango dregs	<i>possessed amylase, protease, and lipase activity</i>
(Arun and Sivashanmugam, 2015)	3 Days	pineapple and citrus peels	protease, amylase, lipase respectively 56.409, 44.039, 74.990 U/ml

4. CHALLENGES AND LIMITATIONS OF ECOENZYME BASED TREATMENTS

4.1 Variability in Result

Numerous studies have demonstrated the success of applying ecoenzymes in reducing organic pollutants in wastewater, but there are also studies showing that this evidence is inconsistent. Conventional wastewater treatment plants face challenges in removing contaminants such as CECs, HMs, and E. coli due to their small size and resistance, suggesting that ecoenzymes may also face similar limitations in their effectiveness (Matesun et al., 2024). In addition, extreme conditions in liquid waste can hinder effective treatment, thereby limiting the biodegradation of micropollutants.

4.2 Dosage Regulation

An evidence base exists to support the Ecoenzymes that are effective in liquid waste treatment. However, determining the best raw material variations and the appropriate dosage is still limited. Further research is needed to set the dosage of ecoenzymes carefully to balance the benefits with potential ecological impacts, noting that despite their effectiveness, challenges include ensuring optimal application to avoid adverse environmental impacts during liquid waste treatment (Yatma et al., 2024).

4.3 Physicochemical Properties and Stability

Ecoenzymes can lose their catalytic activity over time, particularly under varying environmental conditions as temperature, pH and complexity of wastewater compositions, which can limit their effectiveness in wastewater treatment (Ekeoma et al., 2023 ; Upadhyay et al., 2024). Enzymatic activities, especially those of amylase, lipase, and protease, exhibit specific optimum pH value, around 5 - 8, with peak activity reported in neutral to slightly alkaline conditions. Optimum activity of the amylase at pH 6.5, while the lowest was at pH 3.07, which indicated that the amylase activity was deficient at acidic condition and optimum pH (Galintin et al., 2021). The lowest amylase activity when tested at pH 5.88, enzyme activity has a maximum contained in a specific pH, deviations of this optimum pH may lead a decrease enzyme activity, at extreme pH levels, enzymes can be denatured, which changes their structure and impairs their ability to bind with substrates (Sharif, 2024). The rate of degradation decreases dramatically when the pH level is strongly acidic or alkaline (Al-Maqdi et al., 2021). Immobilisation of free enzymes is a promising approach to overcome these weaknesses (Gaur et al., 2025).

However, detailed studies on long-term enzyme stability under variable operational conditions remain limited. Understanding these aspects is imperative for developing robust ecoenzyme formulations that retain catalytic performance during storage and application

4.4 Scale-Up and Integration with Established Systems

The application of ecoenzymes in wastewater treatment faces limitations due to studies being primarily conducted under laboratory conditions rather than in real environmental settings, this requires a permanent solutions to effectively address wastewater pollution challenges are at risk of inefficiency in varying environmental conditions, require specific substrates, and pose challenges in scaling up applications, which may hinder widespread adoption (Rath et al., 2024 ; Çaloğlu et al., 2024). Furthermore, the economic viability of large-scale enzyme production and its application have remained a concern (Swarupa et al., 2024). Overcoming these challenges is crucial to improving the efficiency and practicality of enzyme-based biotechnology approaches in wastewater treatment. Furthermore, the long-term ecological impact of integrating these bio-enzymes into existing treatment systems needs to be analyzed.

4.5 Strain-Specific Limitations

The limitations of ecological enzymes can vary significantly between different strains, which affects their effectiveness in bioremediation and other applications. Specific variations in enzyme production, activity, and adaptability to environmental conditions are critical factors that influence the overall effectiveness of these enzymes. In fact, not all ecological enzyme formulations are equally effective against all pathogens. *Eucalyptus*-based enzymes do not show inhibitory effects against *Pseudomonas aeruginosa*, indicating that effectiveness may depend on the raw materials, fermentation process, and target organism (Taqiyyuddin et al., 2024).

Strain-specific limitations may hinder the effectiveness of ecological enzymes, these limitations also provide opportunities for targeted biotechnology applications. Understanding these boundaries can lead to the further elaboration of an efficient bioremediation strategy tailored to specific environmental conditions and pollutant types.

5. INTEGRATION WITH ADVANCED BIOTECHNOLOGICAL APPROACHES

The emerging trends and future perspectives of ecoenzymes highlight their potential as sustainable solutions for waste management and

industrial applications, include exploring their efficacy and effectiveness in waste management and product development (Winarsih et al., 2023). There is a growing body of research focused on the efficacy and effectiveness of it, with bibliometric analyses indicating a rise in publications on this topic. Bioenzyme immobilization as a key trend to enhancing stability and reusability (Padrosa et al., 2022). The combination of raw materials with exceptional selectivity and catalytic properties in coenzymes produce must keep going to find a best quality of it. While ecoenzymes offer promising solutions for sustainable development, challenges remain in scaling up production and integrating them into existing industrial processes. Continued research and collaboration across scientific disciplines are essential to overcome these barriers and fully realize the potential of it in promoting environmental sustainability and economic growth.

6. CONCLUSION

This study conducted a bibliometric analysis to investigate the potential coenzyme as biocatalyst in wastewater treatment. In total, 1,898 articles obtained from CrossRef, PubMed, OpenAlex, and Google Scholar database were examined, using bibliometric analysis methods. This study found that coenzymes have promising potential as biocatalysts in wastewater. This potential stems from the content of enzymes such as lipase, amylase, and cellulase, which play an important role in accelerating the degradation of complex organic materials into simpler and more easily degradable forms. In addition, the content of various organic acids and the presence of lactic acid bacteria isolated from various coenzyme samples can enhance their antimicrobial attributes. Numerous studies have demonstrated the success of applying eco-enzymes in reducing organic pollutants in wastewater which highlights the ability of coenzymes to reduce BOD, COD, oil and grease. Although their effectiveness can vary based on wastewater conditions and dose usage. However, behind this success lie obstacles that must be overcome to maximize the use of coenzymes in wastewater treatment. The application of coenzymes in wastewater treatment faces limitations due to studies being primarily conducted under laboratory conditions rather than in real environmental settings. Concerns in field applications lie in enzyme stability and the activity under operational conditions such as temperature and pH. Future research trends tend to be directed towards efforts to utilize bioenzymes in broader applications, efforts to increase enzyme stability are important to support the scale up application.

There are limitations in the studies we reviewed, including the studies focus on short treatment durations, limiting understanding of the long-term performance and stability of it in field applications. In addition, there is a lack of comprehensive analysis of the long-term impact of their application on various types and conditions of domestic wastewater. The limited scope of current research calls for further investigation into the long-term effectiveness and wider environmental impact of their application. In addition, further research is needed to optimize its production process and explore their application in various wastewater treatment systems, including biofilters, constructed wetlands, and combinations with phytoremediation.

ACKNOWLEDGEMENT

The team of authors would like to express their gratitude to the Center for Financing and Assessment of Higher Education, which is part of the Secretariat General of the Ministry of Higher Education, Science, and Technology of the Republic of Indonesia, as the funder of this research.

REFERENCES

- Abdelfattah, A., Ali, S.S., Ramadan, H., El-Aswar, E.I., Eltawab, R., Ho, S.-H., Elsamahy, T., Li, S., El-Sheekh, M.M., Schagerl, M., Kornaros, M., Sun, J., 2023. Microalgae-based wastewater treatment: Mechanisms, challenges, recent advances, and future prospects. *Environmental Science and Ecotechnology* 13, 100205. <https://doi.org/10.1016/j.ese.2022.100205>
- Ahmad, I., Abdullah, N., Koji, I., Yuzir, A., Mohamad, S.E., 2021. Potential of Microalgae in Bioremediation of Wastewater. *Bull. Chem. React. Eng. Catal.* 16, Pp. 413–429. <https://doi.org/10.9767/bcrec.16.2.10616.413-429>
- Al-Maqdi, K.A., Elmerhi, N., Athamneh, K., Bilal, M., Alzamly, A., Ashraf, S.S., Shah, I., 2021. Challenges and Recent Advances in Enzyme-Mediated Wastewater Remediation—A Review. *Nanomaterials* 11, 3124. <https://doi.org/10.3390/nano11113124>
- Amadea, B., Budiastuti, M.T.S., Pranoto, 2025. Effect of Eco-Enzyme on Communal Domestic Wastewater Treatment Plant Effluents. *IOP Conf. Ser.: Earth Environ. Sci.* 1489, 012022. <https://doi.org/10.1088/1755-1315/1489/1/012022>
- Anggraini, W., 2025. The Role of Microbial Enzymes in Organic Waste Bioconversion: A Biochemical and Renewable Energy Perspective. *Jurnal Pijar MIPA* 20, Pp. 835–841. <https://doi.org/10.29303/jpm.v20i5.9343>
- Arun, C., Sivashanmugam, P., 2015. Investigation of biocatalytic potential of garbage enzyme and its influence on stabilization of industrial waste activated sludge. *Process Safety and Environmental Protection* 94, Pp. 471–478. <https://doi.org/10.1016/j.psep.2014.10.008>
- Basri, H.F., Muda, K., Omoregie, A.I., Imran, M.J., Jefri, M., Nor, M.N., Jabar, S.N.S.A., Pausi, F.M., Hong, C.Y., 2022. Eco-Enzyme on Water and Wastewater Treatment: A Review. *International Conference on Environment and Sustainability, Johor Baru, Malaysia.*
- Basso, A., Serban, S., 2019. Industrial applications of immobilized enzymes—A review. *Molecular Catalysis* 479, 110607. <https://doi.org/10.1016/j.mcat.2019.110607>
- Benny, N., Shams, R., Dash, K.K., Pandey, V.K., Bashir, O., 2023. Recent trends in utilization of citrus fruits in production of eco-enzyme. *Journal of Agriculture and Food Research* 13. <https://doi.org/10.1016/j.jafr.2023.100657>
- Bharvi S. Patel, Bhanu R. Solanki, Archana U. Mankad, 2021. Effect of eco-enzymes prepared from selected organic waste on domestic waste water treatment. *World J. Adv. Res. Rev.* 10, Pp. 323–333. <https://doi.org/10.30574/wjarr.2021.10.1.0159>
- Çaloğlu, B., Laçın, K., Binay, B., Hung, Y.T., 2024. Microbial Enzymes for Wastewater Treatment, in: Wang, L.K., Sung Wang, M.-H., Hung, Y.-T. (Eds.), *Waste Treatment in the Biotechnology, Agricultural and Food Industries, Handbook of Environmental Engineering*. Springer International Publishing, Cham, Pp. 65–132. https://doi.org/10.1007/978-3-031-44768-6_3
- Das, B., Satyam, Patra, S., 2023. Some recent innovations related to enzyme immobilization. *Biocatalyst Immobilization* Pp. 149–163. <https://doi.org/10.1016/b978-0-323-91317-1.00014-1>
- Ekeoma, B.C., Ekeoma, L.N., Yusuf, M., Haruna, A., Ikeogu, C.K., Merican, Z.M.A., Kamyab, H., Pham, C.Q., Vo, D.-V.N., Chelliapan, S., 2023. Recent advances in the biocatalytic mitigation of emerging pollutants: A comprehensive review. *Journal of Biotechnology* 369, Pp. 14–34. <https://doi.org/10.1016/j.jbiotec.2023.05.003>
- Farma, S.A., Luzik, N.D., Sakina, S., Putri, I.L.E., Advinda, L., Anhar, A., 2023. The potential of local orange peel-derived eco-enzymes in producing indole acetic acid. *actabioina* 6, 135. <https://doi.org/10.32889/actabioina.135>
- Feng, S., Guo, W., Ding, A., Parsa, S.M., Pan, J., Cheng, D., Tung, T.V., Ngo, H.H., 2025. Enzyme sources in wastewater treatment: Their influence on enzymatic bioremediation and large-scale applications. *Chemical Engineering Journal* 510, 161891. <https://doi.org/10.1016/j.cej.2025.161891>
- Galintin, O., Rasit, N., Hamzah, S., 2021. Production and Characterization of Eco Enzyme Produced from Fruit and Vegetable Wastes and its Influence on the Aquaculture Sludge. *Biointerface Research in Applied Chemistry* 11. <https://doi.org/10.33263/BRIAC113.1020510214>
- Gaur, N., Yadav, M., Singh, R., Sheikh, A., Shrivastava, V., Nghiem, X.-H., 2025. Enzyme-Assisted Wastewater Treatment in the Pharmaceutical Industry, in: Grewal, A.S., Deswal, G., Srivastav, A.L. (Eds.), *Advances in Environmental Engineering and Green Technologies*. IGI Global, Pp. 399–424. <https://doi.org/10.4018/979-8-3693-8487-9.ch013>
- Gu, S., Xu, D., Zhou, F., Chen, C., Liu, C., Tian, M., Jiang, A., 2021. The Garbage Enzyme with Chinese Hoencylost Fruits Showed Better Properties and Application than When Using the Garbage Enzyme Alone. *Foods* 10, 2656. <https://doi.org/10.3390/foods10112656>
- Gumilar, G.G., 2023a. Ecoenzyme Production, Characteristics, and Applications: A Review. *Jurnal Kartika Kimia* 6. <https://doi.org/10.26874/jkk.v6i1.186>

- Haque, S., Singh, R., Harakeh, S., Teklemariam, A.D., Alharthy, S.A., Tripathi, S.C., Singh, R.P., Aly Hassan, A., Srivastava, N., Gupta, V.K., 2022. Enzyme-based biocatalysis for the treatment of organic pollutants and bioenergy production. *Current Opinion in Green and Sustainable Chemistry* 38, 100709. <https://doi.org/10.1016/j.cogsc.2022.100709>
- Hasan, H.A., Rahim, N.F.M., Alias, J., Ahmad, J., Said, N.S.M., Ramli, N.N., Buhari, J., Abdullah, S.R.S., Othman, A.R., Jusoh, H.H.W., Juahir, H., Kurniawan, S.B., 2024. A Review on the Roles of Extracellular Polymeric Substances (EPSs) in Wastewater Treatment: Source, Mechanism Study, Bioproducts, Limitations, and Future Challenges. *Water* 16, 2812. <https://doi.org/10.3390/w16192812>
- Hemalatha, M., Visantini, P., 2020. Potential use of eco-enzyme for the treatment of metal based effluent. *IOP Conf. Ser.: Mater. Sci. Eng.* 716, 012016. <https://doi.org/10.1088/1757-899X/716/1/012016>
- Ismail AY, Nainggolan MF, Nauli AP. Characterizing the Chemical Composition of Eco-Enzymes Derived from Vegetable and Fruit Sources. *IJDNE [Internet]*. 2024 Jun 25 [cited 2025 Sep 3];19(3). Available from: <https://www.iieta.org/journals/ijдне/paper/10.18280/ijдне.190334>
- Joshi, V.B., Pathak, A., Murkute, S., Das, B., Khan, Z., 2022. Extraction, purification and characterization of amylase, protease, lipase of halophilic bacterial isolates from Bordi, India. *International Journal of Applied Biology* 6.
- Karimah, M., Lusiani, C.E., 2024. Analysis Of Eco Enzyme Characteristics With Variation Of "Tape" Yeast Concentrations. *Distilat: J. Tekn. Sep* 10, Pp. 233–244. <https://doi.org/10.33795/distilat.v10i1.4195>
- Kumar A, Sadhya HK, Ahmad E, Dulawat S. Application of Bio-Enzyme in Wastewater (Greywater) Treatment. *International Research Journal of Engineering and Technology (IRJET)*. 2020;7(5).
- Laela Nur Dayanti, Titik Suryani, 2025. Quality of Kefir Combination Between Sweet Corn Milk and Skim Milk on Variations of Sugar Types and Fermentation Duration. *Quagga* 17, Pp. 9–15. <https://doi.org/10.25134/quagga.v17i1.334>
- Li, Y., Lu, H., Liu, Y., Dai, P., Zhu, H., Zhang, B., Peng, S., Fan, Y., Yang, Q., 2023. Media coalescence filtration as a novel method for pretreatment of refinery wastewater. *Journal of Environmental Chemical Engineering* 11, 109107. <https://doi.org/10.1016/j.jece.2022.109107>
- Low Chin Wen, Regina Leong Zhi Ling, Swee-Sen Teo, 2021. Effective Microorganisms in Producing Eco enzyme from Food Waste for Wastewater Treatment. *Applied Microbiology: Theory and Technology* 28–36. <https://doi.org/10.37256/amtt.212021726>
- Lubis, N., Damayanti, R., Wardani, S., 2025. Antimicrobial Activity of Eco-enzymes with Various Dilutions As A Natural Disinfectant. *jppipa, pendidikan ipa, fisika, biologi, kimia* 11, 506–513. <https://doi.org/10.29303/jppipa.v11i5.11052>
- Makanda, K., Nzama, S., Kanyerere, T., 2022. Assessing the Role of Water Resources Protection Practice for Sustainable Water Resources Management: A Review. *Water* 14, 3153. <https://doi.org/10.3390/w14193153>
- Matesun, J., Petrik, L., Musvoto, E., Ayinde, W., Ikumi, D., 2024. Limitations of wastewater treatment plants in removing trace anthropogenic biomarkers and future directions: A review. *Ecotoxicology and Environmental Safety* 281, 116610. <https://doi.org/10.1016/j.ecoenv.2024.116610>
- Natt SK, Priya Katyal, Phutela UG, Chandel S. Bioremediation of electroplating industrial wastewater using bioenzymes generated from citrus. *ECJ*. 2025 Feb 1;26(1): Pp. 1–8.
- Ningsih, L.M., Hasanudin, U., Roubik, H., 2024. Acclimatisation Process of Biogas Production from Tofu Industrial Wastewater Using Biofilter in Anaerobic Baffled Reactor (ABR). *Renewable Energy* 121519–121519. <https://doi.org/10.1016/j.renene.2024.121519>
- Padrosa, D.R., Benítez-Mateos, A.I., Paradisi, F., 2022. Back to the future: taking enzymes to the next level of sustainability. *The Biochemist* 44, Pp. 19–22. https://doi.org/10.1042/bio_2022_109
- Pachaiappan R, Cornejo P L, Rajendran R, Manavalan K, Rajan VF, Awad F. A review on biofiltration techniques: recent advancements in the removal of volatile organic compounds and heavy metals in the treatment of polluted water. *BioEngineered*. 2022;13(4):8432–77.
- Pandey, A., Soccol, C.R., Mitchell, D., 2000. New developments in solid state fermentation: I-bioprocesses and products. *Process Biochemistry* 35, Pp. 1153–1169. [https://doi.org/10.1016/S0032-9592\(00\)00152-7](https://doi.org/10.1016/S0032-9592(00)00152-7)
- Pasalari, H., 2024. A systematic review on garbage enzymes and their applications in environmental processes. *Ecotoxicology and Environmental Safety*.
- Patel, R., Trivedi, U., Patel, K.C., 2022. Lipases: An Efficient Biocatalyst for Biotechnological Applications. *Journal of microbiology, biotechnology and food sciences* 11. <https://doi.org/10.55251/jmbfs.2498>
- Phanama, A., Ismail, D., Witono, J.A., Edwar, I.M., 2024. The effect of sugar types in the production of eco-enzyme based on pineapple and mango peel waste on the quality of petroleum processing wastewater. Presented at the The 5th International Conference On Buildings, Construction, And Environmental Engineering: BCEE5, Amman, Jordan, p. 070015. <https://doi.org/10.1063/5.0236916>
- Pitriani, P., 2015. The Effectiveness of EM4 Addition into Biofilter to Reduce of BOD, COD and MPN Coliform of Hospital Wastewater. *International Journal of Pharmtech Research* 8, 702–708.
- Putri, A.A.A.R., Permatananda, P.A.N.K., Pandit, I.G.S., Lestari, A., 2024. Antioxidant activity of ecoenzyme derived from Siamese orange peel (*Citrus nobilis*) at different fermentation duration. *Acta Biochimica Indonesiana* 7, 173–173. <https://doi.org/10.32889/actabioina.173>
- Putri, R.A., Julianti, S., 2025. Optimalisasi Sistem Pengolahan Air Limbah Domestik Berbasis Biofilter Anaerob-Aerob di Permukiman Padat Penduduk. *J. Sci. Tech. Alpha* 1, 42–47. <https://doi.org/10.70716/alpha.v1i2.173>
- Rachwał, K., Gustaw, K., 2025. Plant-Derived Phytobiotics as Emerging Alternatives to Antibiotics Against Foodborne Pathogens. *Applied Sciences* 15, 6774. <https://doi.org/10.3390/app15126774>
- Rahayu MR, Nengah M, Yohanes Parlindungan Situmeang. Acceleration of Production Natural Disinfectants from the Combination of Eco-Enzyme Domestic Organic Waste and Frangipani Flowers (*Plumeria alba*). *SEAS: Sust Envir Agric Sci*. 2021 Apr 30;5(1):15–21.
- Rath, P., Bhardwaj, L.K., Chaturvedi, M., Bhardwaj, A., 2024. Significance of Enzymatic Actions in Biomass Waste Management: Challenges and Future Scope, in: Srivastav, A.L., Bhardwaj, A.K., Kumar, M. (Eds.), *Valorization of Biomass Wastes for Environmental Sustainability*. Springer Nature Switzerland, Cham, pp. 223–237. https://doi.org/10.1007/978-3-031-52485-1_12
- Rasit N, Fern L, Ghani WA. Production And Characterization Of Eco Enzyme Produced From Tomato and Orange Wastes and Its Influence on The Aquaculture Sludge. *International Journal of Civil Engineering and Technology (IJCIET)*. 2019;10(3).
- Rukmini, P., Astuti Herawati, D., 2023. Eco-enzyme from Organic Waste (Fruit and Rhizome Waste) Fermentation: Eco-Enzyme Dari Fermentasi Sampah Organik (Sampah Buah Dan Rimpang). *J.Kireka* 4, 23–29. <https://doi.org/10.31001/jkireka.v4i1.62>
- Sahil Salvi, Ranjeet Sabale, Sudarshan Bobade, Arun Dhawale, 2024. Innovative Use of Eco-Enzymes for Domestic Wastewater Purification. *J. Environ. Nanotechnol.* 13, 435–439. <https://doi.org/10.13074/jent.2024.09.242771>
- Sai, S., V.M.J, A., K, M., Veronica, A., A, S., 2023. Treasure from trash – Is Ecoenzyme the new panacea in conservative dentistry and endodontics. *Journal of Conservative Dentistry* 26, 176–181. https://doi.org/10.4103/jcd.jcd_473_22
- Said, N.I., Hernaningsih, T., Widayat, W., Yudo, S., Septian, A., Setiyono, Rifai, A., Setiadi, I., Sulaeman, O., Wahyono, H.D., Hartaja, D.R.K., Darmawangsa, M.R., Ikhsan, I.N., 2024. Domestic wastewater treatment with anaerobic-aerobic biofilters using plastic honeycomb media. *IOP Conf. Ser.: Earth Environ. Sci.* 1388, 012058. <https://doi.org/10.1088/1755-1315/1388/1/012058>
- Santosa, S., Hassan, M.S., Kasim, A.H., 2023. Quality of an ecoenzyme and

- potential of its residues as composting bioactivator. *Journal of Natural Resources and Environmental Management* 13, 417–424. <http://dx.doi.org/10.29244/jpsl.13.3.417-424>
- Shahid, F., Aman, A., Qader, S.A.U., 2024. Synthesis and characterization of cross-linked aggregates of dextranase (CLAD) for improved stability and recycling efficiency of the biocatalyst. *Biocatalysis and Agricultural Biotechnology* 58, 103151–103151. <https://doi.org/10.1016/j.bcab.2024.103151>
- Sharif, N., 2024. Effects of pH on Enzyme Activity in Pakistan. *JCHEM* 3, Pp. 49–59. <https://doi.org/10.47672/jchem.2512>
- Shivalik Y, Goyal A. Treatment of Domestic Waste Water Using Organic Bio-Enzymes Extracted from Seasonal Citrus Fruits. *IJRASET*. 2022 Aug 31;10(8):1023–6.
- Singh, A., Jain, R., 2024. Treatment of Domestic Wastewater Using Bio-Enzymes: A Review. *Arch. Curr. Res. Int.* 24, Pp. 147–154. <https://doi.org/10.9734/acri/2024/v24i121004>
- Singh, B.J., Chakraborty, A., Sehgal, R., 2023. A systematic review of industrial wastewater management: Evaluating challenges and enablers. *Journal of Environmental Management* 348, 119230. <https://doi.org/10.1016/j.jenvman.2023.119230>
- Suliestyah S, Aryanto R, Palit C, Yulianti R, Suudi BC, Meitdwitri A. Eco enzyme production from fruit peel waste and its application as an anti-bacterial and TSS reducing agent. *irjeis*. 2022 Oct 9;8(6):270–5.
- Swarupa, V., Varghese, R., Gaonkar, B., Roopashree, R., Subbulakshmi, G., 2024. Biocatalysts in Remediation of Wastewater, in: *Application of Engineering Principles and Practices In Biotechnology*. Apple Academic Press, New York, Pp. 27–40. <https://doi.org/10.1201/9781003439929-3>
- Tallei, T.E., Fatimawali, Niode, N.J., Alsaihati, W.M., Salaki, C.L., Alissa, M., Kamagi, M., Rabaan, A.A., 2023. Antibacterial and Antioxidant Activity of Ecoenzyme Solution Prepared from Papaya, Pineapple, and Kasturi Orange Fruits: Experimental and Molecular Docking Studies. *Journal of Food Processing and Preservation* 2023, Pp. 1–11. <https://doi.org/10.1155/2023/5826420>
- Taqiyuddin, Z., Retnowati, W., Indiatuti, D.N., 2024. Antibacterial effects of *Melaleuca leucadendra* Ecoenzyme on *Pseudomonas aeruginosa*. *World Journal of Advanced Research and Reviews* 21, Pp. 410–415. <https://doi.org/10.30574/wjarr.2024.21.1.0010>
- Upadhyay, A.K., Hasan, K.N., Chakraborty, A., Priyadarshini, M., 2024. Novel Trends of Biotechnology in Wastewater Treatment, in: Bhat, R.A., Dar, G.H., Tonelli, F.M.P., Hamid, S. (Eds.), *Aquatic Contamination*. Wiley, Pp. 95–111. <https://doi.org/10.1002/9781119989318.ch6>
- Vama L, Cherekar M. Production, Extraction And Uses Of Eco-Enzyme Using Citrus Fruit Waste: Wealth From Waste. *Asian Jr of Microbiol Biotech Env Sc.* 2020;22(2):346–51.
- Varshini, B., Gayathri, V., 2023. Role of Eco-Enzymes in Sustainable Development. *Nat. Env. Poll. Tech* 22, 1299–1310. <https://doi.org/10.46488/NEPT.2023.v22i03.017>
- Vidalia, C., Angelina, E., Hans, J., Field, L.H., Santo, N.C., Rukmini, E., 2023. Eco-enzyme as disinfectant: a systematic literature review. *IJPMS* 12, 1171. <https://doi.org/10.11591/ijpms.v12i3.22131>
- Viona Setiawan J, Retno Rosariastuti M, Sri Budiastuti Mt. Characterization of Bioactive Compounds from Eco-enzyme Produced from Organic Waste. *IOP Conf Ser: Earth Environ Sci.* 2025 Apr 1;1489(1):012003.
- Wan Mohd Zain, W.Z., Ghazali, A.H., Ariffin, S.A., Mohammad, A., Ismail, N.S.A., Windayani, N., 2025. Phytochemical composition, antioxidant potential, and antimicrobial applications of ecoenzymes: A comprehensive review. *APJMBB* Pp. 32–41. [https://doi.org/10.35118/apjmbb.2025.033.2\(Special\).04](https://doi.org/10.35118/apjmbb.2025.033.2(Special).04)
- Werner, N., Dreyer, M., Wagner, W., Papon, N., Rupp, S., Zibek, S., 2016. *Candida guilliermondii* as a potential biocatalyst for the production of long-chain α,ω -dicarboxylic acids. *Biotechnology Letters* 39, 429–438. <https://doi.org/10.1007/s10529-016-2264-3>
- Winarsih, Kohar, A.W., Ratih, Y.W., Aziz, A.Z., Nugroho, W.A., 2023. Trends in research publication topics related to eco enzymes with bibliometric analysis. *E3S Web Conf.* 450, 01006. <https://doi.org/10.1051/e3sconf/202345001006>
- Xia, Z., Ng, H.Y., Bae, S., 2025. Synergistic microalgal-bacterial interactions enhance nitrogen removal in membrane-aerated biofilm photoreactors treating aquaculture wastewater under salt stress: Insights from metagenomic analysis. *Water Research* 283, 123878. <https://doi.org/10.1016/j.watres.2025.123878>
- Xu, S., Li, Z., Yu, S., Chen, Z., Xu, J., Qiu, S., Ge, S., 2024. Microalgal–Bacteria Biofilm in Wastewater Treatment: Advantages, Principles, and Establishment. *MDPI: Water* 16. <https://www.mdpi.com/2073-4441/16/18/2561#>
- Yatma MA, Hamzani S, Zubaidah T, 2024. The Effect of Eco Enzyme on Improving River Water Quality Based on Turbidity, Temperature, pH, and TDS Parameters. *GHEP* 1, Pp. 164–168. <https://doi.org/10.61848/ghep.v1i3.71>
- Zhang, S., Chen, H., Xia, Y., Zhao, J., Liu, N., Li, W., 2015. Re-acclimation performance and microbial characteristics of a thermophilic biofilter for NO_x removal from flue gas. *Applied Microbiology and Biotechnology* 99, Pp. 6879–6887. <https://doi.org/10.1007/s00253-015-6585-2>
- Zhao, Y., Liu, D., Huang, W., Yang, Y., Ji, M., Nghiem, L.D., Trinh, L.T.T., Tran, N.H., 2019. Insights into biofilm carriers for biological wastewater treatment processes: Current state-of-the-art, challenges, and opportunities. *Biosource Technology* 288. <https://doi.org/10.1016/j.biortech.2019.121619>
- Zhu, X., Jiang, L., Tian, Y., Wang, L., Pan, Y., Li, W., Li, A., 2025. Strategy for repurposing waste anion exchange resins to construct a biofilter for removing dissolved organic matter: performance and mechanism. *Water research* 281, 123687. <https://doi.org/10.1016/j.watres.2025.123687>

