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Evaluation of optimum conditions for improved cell growth and MNP enzyme activity in some selected edible mushrooms

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Abstract

This study was carried out to investigate and optimize conditions for enhanced cell growth and manganese peroxidase (MnP) enzyme activity in selected mushroom isolates. Single parameter optimization of different process variables was carried out for cell growth/enzyme production. The isolates used were *Pleurotus porrigens, Gerronema chrysophyllum* and *Lepiota procera.* The influence of temperature, pH, nitrogen sources, and metal ions were examined to determine their effects on both cell proliferation and MnP production of the isolates. Results showed that the optimum growth temperature for MnP enzyme activity for *P. porrigens* was 35^{o}C. It was also revealed that all the isolates showed optimum growth rate at pH 5. *P. porrigens* showed the highest growth rate (OD 15.2). Peptone was the best nitrogen source, with *P. porrigens* having the highest growth rate (OD 0.184). Both Mn^{2+} and Cu^{2+} stimulated growth rate in all the isolates, with Mn $^{2+}$ (2Mm) stimulating the highest growth rate (OD 22.8) in *P. porrigens.* Hg^{2+}, Fe^{3+}, Zn^{2+} and Pb^{2+} were inhibitory to growth rate. All the isolates showed that the optimization of culture conditions enhanced growth rate of the isolates.

Keywords: Optimization; Manganese Peroxidase; Mushroom; Growth Rate; Peptone

1. Introduction

The advancement of cell multiplication and enzyme activity is crucial in various fields, including biotechnology, pharmaceuticals, and biofuel production. One important enzyme that has gained significant attention is manganese peroxidase (MnP), which is a heme-containing glycoprotein produced by many white-rot fungi. MnP plays an essential role in lignin degradation and has potential applications in various industrial processes such as pulp bleaching, wastewater treatment, and organic synthesis. It plays a crucial role in the degradation of organic pollutants and has attracted considerable attention due to its wide range of applications in different industries. MnP catalyzes the oxidative breakdown of complex organic compounds, making it valuable in treating wastewater contaminated with recalcitrant substances like polycyclic aromatic hydrocarbons (PAHs), polychlorinated biphenyls (PCBs), dyes, pentachlorophenol, and heavy metals [1]. The capacity of MnP to remove toxic components enables better co-cultivation of energy crops and facilitates fermentation processes [2]. MnP exhibits potent antioxidant properties, rendering it useful in the food sector for preventing lipid peroxidation and extending product shelf life [3]. Furthermore, MnPs have been shown to decolorize various food-derived pigments, offering a potentially greener alternative to traditional bleaching agents [4]. However, the optimal conditions for improving cell growth and MnP enzyme activity are not well established.

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Several factors contribute to the enhancement of MnP yield and performance, including nutrient availability, physical growth conditions, and genetic regulation [5]. For instance, alterations in carbon and nitrogen sources affect not only microbial proliferation but also MnP expression levels [6,7].

Recent studies have focused on optimizing cultural conditions to enhance MnP enzyme activity and cell growth. For instance, Zhang et al. [8]. investigated the effect of different carbon sources on MnP production by *Phanerochaete chrysosporium* and found that glucose and xylose significantly enhanced MnP activity compared to other carbon sources. Similarly, Gao et al. [9]. examined the impact of nitrogen sources on the growth and MnP activity of *Irpex lacteus* and reported that ammonium sulfate was the most favorable nitrogen source for both cell growth and MnP production. Moreover, Li et al. [10] studied the influence of temperature and pH on the activity and stability of MnP from *Ceriporiopsis subvermispor*a and determined that the optimum temperature and pH were 50°C and 4.0, respectively.

Despite these findings, there is still a need to evaluate the optimum conditions for advancing cell growth and MnP enzyme activity further. Therefore, this study was conducted to further evaluate the optimum conditions for improved cell growth and MnP enzyme activity in some selected mushrooms.

2. Materials and methods

2.1. Effect of pH on growth rate of mushroom isolates and their MnP activity

The effect of pH (4, 5, 6, 7, 8, 9 and 10) on the growth rate of the mushrooms from previous study [11] was determined. Hydrochloric acid (0.1M) and Sodium hydroxide (0.1M) were used to adjust the pH of the nutrient broth to the required values. Ten millimeter (10ml) of the adjusted medium were dispensed into test tubes and 8mm agar plugs from 72 hours-old cultures of the isolates were used to inoculate the tubes. Incubation was at room temperature for 7 days. The influence of pH on the growth rate of the mushroom isolates was measured by reading the optical density at 600nm. Also, the effect of pH on MnP activity at room temperature was studied using the assay procedure described by [12]. The following buffers were used to achieve the different pH ranges tested: Citrate phosphate, sodium phosphate and glycine-NaOH.

2.2. Effect of temperature on MnP activity of the mushroom isolates

The effect of temperature on MnP activity was determined in the range of 30^{o}C to 60^{o}C with 5^{o}C intervals. Eight millimeter agar plugs from test isolates were inoculated into 10ml nutrient broth in test tubes and incubated at room temperature for 7 days. After the incubation period, the samples were centrifuged and analyzed for MnP activity according to [12].

2.3. Effect of different nitrogen sources on the growth rate of the mushroom isolates and their MnP activities

The effect of both organic (urea, peptone and beef extract) and inorganic ($(NH_4)_2SO_4$, and NH_4NO_3) nitrogen sources on the growth rate and MnP production of the mushroom isolates were studied at various concentrations (1-5%). A minimal salt broth (MSB) was prepared without the nitrogen source and appropriate grams of the test nitrogen sources were weighed and added to the MSB. Agar plugs 8mm in diameter from 72 hours-old cultures of isolates were used to inoculate 10ml of the medium and test tubes were incubated at room temperature for 7 days. Effect of the nitrogen sources on the growth rate of the isolates was measured by reading the optical density at 600nm. Also, their effect on MnP production/activity was studied using the method described by [12].

2.4. Effect of metal ions on the growth rate of the mushroom isolates and their MnP activities

The effect of metal ions (Fe^{3+}, Cu^{2+}, Hg^{2+}, Pb^{2+}, Zn^{2+} and Mn^{2+}), using the salts of the metals; iron (II) sulphate 7-hydrate, copper (II) carbonate hydroxide, mercury (II) oxide red, lead (II) nitrate, zinc chloride and manganese sulphate on the growth rate and MnP production of the mushroom isolates were studied in the concentration range of 1-5Mm [13,14]. The appropriate grams of the salts were weighed and dissolved in prepared nutrient broth medium. The metal ion supplemented media were dispensed intro test tubes (10ml) and autoclaved. Eight millimeter agar plugs from 72 hours-old cultures of isolates were used as inocula and samples incubated at room temperature for 7 days. After the incubation period, the effect of metal ions on the growth rate of isolates was measured by reading the optical density at 600nm. Also, the effect of metal ions on MnP production/activity was determined by the method described by [12].

3. Results

3.1. Effect of pH on growth rate of the mushroom isolates

The effect of pH on the growth rate of the isolates is shown in Figure 1. All the isolates showed optimum growth rate at pH 5 and a decrease in growth rate with increasing pH values. *P. porrigens* had the highest growth peak at OD 15.2 while *L. procera* had the least at OD 7.9

3.2. Effect of pH on MnP activity of the mushroom isolates

The optimum pH for enzymes activity of MnP produced by the isolates was at pH 5, which coincided with the optimum pH for growth by isolates. *P. porrigens* showed the highest MnP activity (39.80U/ml), while *L. procera* had the least MnP activity (20.23U/ml) as shown in Figure 2.

3.3. Effect of temperature on MnP activity of the mushroom isolates

Figure 3 shows a graphical representation of the effect of temperature on enzyme activity of isolates. Optimum temperature for enzyme activity of *L. procera* was at 30°C (MnP: 20.8U/ml) while 35°C was the optimum temperature for enzyme activity of MnP produced by *P. porrigens* (MnP: 28.84U/ml). Further increase in temperature resulted in significant losses of activity in all isolates.

3.4. Effect of different nitrogen sources on the growth rate of the mushroom isolates

Isolates were able to grow in MSB without addition of nitrogen but grew better with added nitrogen sources. Organic sources of nitrogen were superior to inorganic sources. Increase in rate of supplementation of nitrogen sources beyond 2% appeared to inhibit the growth of isolates. Peptone (2%) stimulated the highest growth in *P. porrigens* (OD 0.184) as shown in Table 1

3.5. Effect of different nitrogen sources/concentration on MnP activities of the mushroom isolates

The effect of nitrogen sources on MnP production/activities of isolates in shown in Table 2. Similar to their effect on growth rate, organic nitrogen sources stimulated more MnP production/activity than the inorganic sources. Inhibition in MnP production/activity was observed beyond 2%. The highest MnP production/activity was observed at 2% Peptone in *P. porrigens* (41.66U/ml).

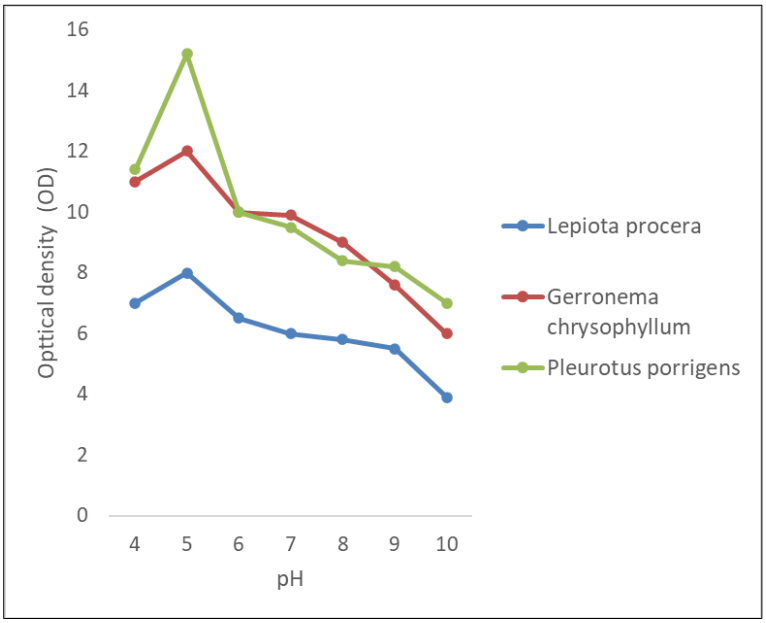

Figure 1 Effect of pH on growth rate of the mushroom isolates

3.6. Effect of different metal ions on the growth rate of the mushroom isolates

Mn^{2+} and Cu^{2+} stimulated the growth of isolates but increase in concentration beyond 2mM was inhibitory to the growth of isolates. This is shown in Table 3. Fe^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} were inhibitory to the growth of isolates and increase in concentration of these metal ions led to increased inhibition of growth rates. The highest inhibition was observed with Fe^{3+}

3.7. Effect of different metal ions/concentrations on MnP activities of the mushroom isolates

Similar to the effect of metal ions on growth rate of isolate, Mn^{2+} and Cu^{2+} stimulated MnP production/activity, but became inhibitory beyond 2mM, Mn^{2+} (2mM) stimulated the highest MnP production/activity in *P. porrigens* (34.41U/ml). Fe^{2+}, Hg^{2+}, Pb^{2+} and Zn^{2+} were inhibitory to MnP production/activity as shown in Table 4.

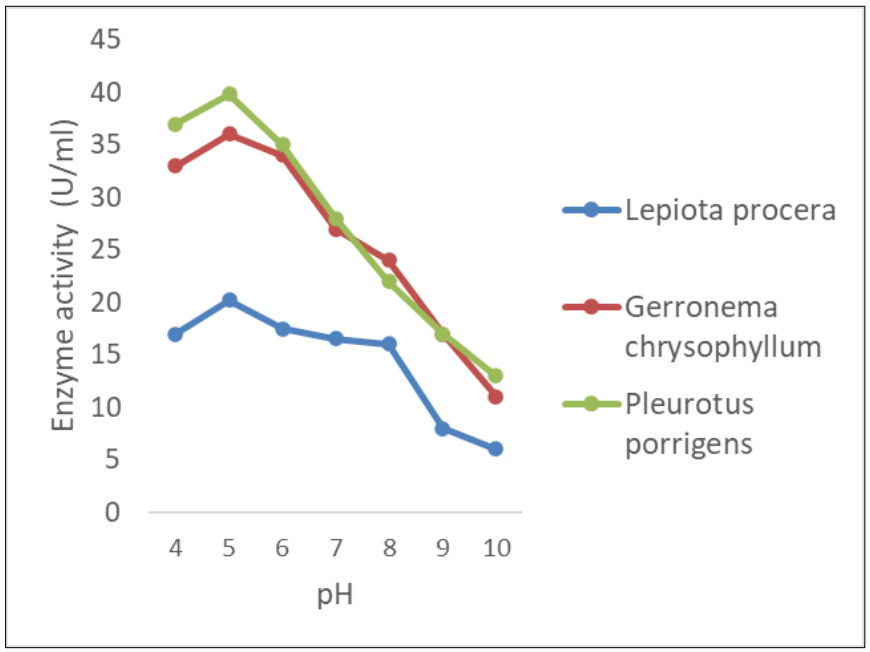

Figure 2 Effect of pH on MnP activity of the mushroom isolates

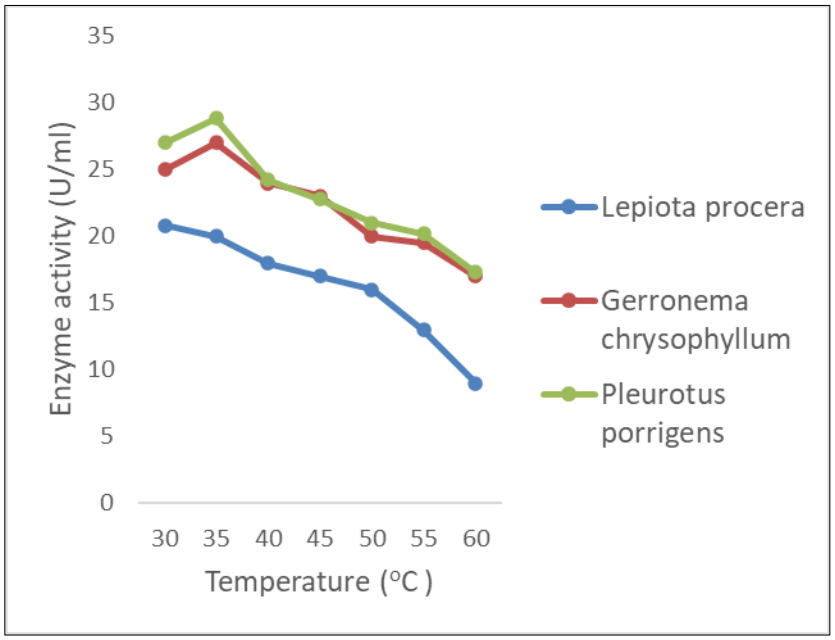

Figure 3 Effect of temperature on MnP activity of the mushroom isolates

Table 1 Effect of different nitrogen source on the growth rate of isolates in minimal salt broth (MSB)

Isolates	**Nitrogen Sources(%)**	**Control None**	1	2	3	4	5
			Enzyme activity (U/ml)				**(No unit)**
Gerronema chrysophyllum		0.096					
	Urea		0.114	0.168	0.118	0.091	0.086
	Peptone		0.111	0.177	0.140	0.090	0.082
	Beef Extract		0.105	0.162	0.091	0.084	0.071
	$(NH_4)_2SO_4$		0.109	0.100	0.062	0.037	0.032
	NH_4NO_3		0.133	0.107	0.067	0.054	0.053
Pluerotus porrigens		0.087					
	Urea		0.125	0.173	0.110	0.083	0.072
	Peptone		0.122	0.184	0.161	0.100	0.070
	Beef Extract		0.108	0.171	0.144	0.082	0.080
	$(NH_4)_2SO_4$		0.099	0.111	0.056	0.048	0.040
	NH_4NO_3		0.121	0.092	0.073	0.061	0.044
Lepiota procera		0.051					
	Urea		0.108	0.071	0.061	0.050	0.046
	Peptone		0.056	0.125	0.088	0.069	0.047
	Beef Extract		0.063	0.101	0.073	0.059	0.044
	$(NH_4)_2SO_4$		0.057	0.063	0.052	0.038	0.031
	NH_4NO_3		0.059	0.066	0.042	0.039	0.030

Table 2 Effect of different nitrogen sources/concentrations on MnP activities of isolates in minimal salt broth (MSB)

Isolates	**Nitrogen Sources(%)**	**None**	**Enzyme activity (U/ml)**				
			1	**2**	**3**	**4**	**5**
Gerronema chrysophyllum		22.12					
	Urea		28.65	33.01	29.71	20.88	20.11
	Peptone		27.62	31.25	29.41	20.39	18.74
	Beef Extract		26.56	25.34	24.81	20.42	18.61
	$(NH_4)_2SO_4$		24.21	23.18	19.23	16.46	12.87
	NH_4NO_3		25.84	24.14	20.07	18.53	17.21
Pluerotus porrigens		23.64					
	Urea		34.41	39.45	26.94	22.31	21.76
	Peptone		38.48	41.66	26.14	24.07	20.12
	Beef Extract		30.54	27.16	25.48	21.09	17.45
	$(NH_4)_2SO_4$		26.31	24.58	21.26	18.55	14.71
	NH_4NO_3		27.41	25.78	24.55	21.81	19.24
Lepiota procera		14.37					
	Urea		21.08	20.16	17.90	13.36	13.10
	Peptone		18.19	22.86	20.44	16.18	12.41
	Beef Extract		17.38	19.75	16.42	14.04	11.56

	$(NH_4)_2SO_4$		16.46	18.73	15.52	10.80	8.87
	NH_4NO_3		16.87	20.12	18.34	13.47	10.88

Table 3 Effect of different metal ions on the growth rate of isolates in nutrient broth

Isolates	**Metals (mM)**	**None**	**Conc. of metal ions**				
			1	**2**	**3**	**4**	**5**
Gerronema chrysophyllum		0.082					
	HgO		0.050	0.063	0.053	0.047	0.048
	$Fe_2(SO_4)_3$		0.058	0.064	0.051	0.043	0.040
	$CH_2Cu_2O_5$		0.100	0.141	0.106	0.089	0.080
	$MnSO_4$		0.122	0.160	0.104	0.085	0.071
	$Pb(NO_3)_2$		0.071	0.063	0.060	0.055	0.051
	$ZnCl_2$		0.075	0.081	0.068	0.061	0.053
Pluerotus porrigens		0.076					
	HgO		0.056	0.061	0.046	0.042	0.035
	$Fe_2(SO_4)_3$		0.060	0.051	0.048	0.041	0.038
	$CH_2Cu_2O_5$		0.093	0.132	0.099	0.081	0.068
	$MnSO_4$		0.184	0.228	0.136	0.090	0.073
	$Pb(NO_3)_2$		0.062	0.066	0.057	0.054	0.049
	$ZnCl_2$		0.064	0.080	0.072	0.051	0.040
Lepiota procera		0.054					
	HgO		0.050	0.052	0.046	0.040	0.034
	$Fe_2(SO_4)_3$		0.040	0.046	0.037	0.034	0.028
	$CH_2Cu_2O_5$		0.072	0.087	0.066	0.059	0.038
	$MnSO_4$		0.090	0.109	0.081	0.061	0.042
	$Pb(NO_3)_2$		0.050	0.048	0.041	0.036	0.033
	$ZnCl_2$		0.049	0.053	0.051	0.044	0.032

Table 4 Effect of different metal ions/concentrations on MnP activities of isolates in nutrient broth

Isolates	**Metals (mM)**	**None**	**1**	**2**	**3**	**4**	**5**
			Enzyme activity (U/ml)				
Gerronema chrysophyllum		23.73					
	HgO		15.32	17.11	14.37	11.72	8.51
	$Fe_2(SO_4)_3$		17.71	19.66	16.24	15.10	13.44
	$CH_2Cu_2O_5$		24.96	25.91	25.43	24.39	22.69
	$MnSO_4$		26.00	27.51	25.34	24.49	17.77
	$Pb(NO_3)_2$		20.49	21.81	18.43	17.89	15.77
	$ZnCl_2$		21.65	22.45	20.33	17.77	15.13
Pluerotus porrigens		25.81					
	HgO		16.26	18.72	17.02	12.39	10.40
	$Fe_2(SO_4)_3$		19.95	18.42	16.73	15.89	13.19
	$CH_2Cu_2O_5$		26.66	28.46	27.89	27.51	22.03

	$MnSO_4$		31.29	34.41	27.13	26.47	24.77
	$Pb(NO_3)_2$		20.23	21.80	18.57	16.17	15.87
	$ZnCl_2$		24.39	23.19	21.84	16.64	16.07
Lepiota procera		16.55					
	HgO		10.87	13.14	8.79	8.23	6.71
	$Fe_2(SO_4)_3$		14.00	14.55	12.68	11.02	10.51
	$CH_2Cu_2O_5$		18.90	20.80	18.34	17.59	8.79
	$MnSO_4$		20.61	21.18	18.53	17.40	14.84
	$Pb(NO_3)_2$		15.04	14.75	13.33	11.71	11.00
	$ZnCl_2$		13.43	15.55	11.16	8.32	7.66

4. Discussion

The growth rate of organisms has a role to play on how rapid they can colonize/penetrate any substrate. Asamudo *et al.,* [15] reported that the faster the growth rate/mycelia thickness, the higher the rate of mechanical penetration and breaking down of substrate and this leads to higher bioremediation capabilities. The finding that fungal isolates exhibit optimal growth at a lower pH of 5 and display diminishing growth rates as pH increases relates with the study reported by Zhao et al [16] that observed that pH 4 favored faster mycelial growth of *Penicillium expansum* when compared to neutral or alkaline conditions. Li et al. [17] analyzed *Colletotrichum gloeosporioides*, reporting maximum proliferation in slightly acidified media mirroring the present observation with *P. porrigens* having the highest growth at OD 15.2 and *L. procera* at OD 7.9. Certain fungi thrive in low-pH conditions, suggesting that manipulating environmental acidity might prove beneficial in optimizing industrial fermentation procedures and managing unwanted microbial growth in various habitats.

dos Santos et al. [18] evaluated *Pleurotus ostreatus* performance in various pH ranges and observed increased MnP efficiency at pH 5 which relates with the findings of this study. Another study by Karimiyan et al. [19] investigated *Phlebia tremellosa*, demonstrating optimal MnP production at pH 5. Also, Baharlouei et al. [20] examined *Ceriporiopsis subvermispora* functioning, confirming increased MnP yields under acidic conditions matching the described pH 5 optima.

Arora & Gill [21] investigated *Irpex lacteus* subjected to varied temperature and reported high MnP activity at 30°C, Manavalan et al. [22] examined *Pycnoporus sanguineus* confirmed the temperature dependence of MnP yield, showing increased activity between 30-40°C. These findings relate with this study. MnP activity of fungal isolates responds differently based on temperature variations. Thermal stability profiles show increase corresponding to optimal MnP production. Appropriately adjusting temperature settings offers considerable promise for expanding the scope of commercial applications requiring efficient ligninolytic agents, such as biofuels, pulp, and paper industries, as well as organic pollutant remediation processes.

Bhatt et al. [23] demonstrated that organic nitrogen provisions greatly enhanced *Phlebia radiata* colonization. In alignment with the mentioned study, the growth of *P. porrigens* was high with peptone (2%; OD 0.184) as opposed to alternative nitrogen sources tested. Gao et al. [24] studied *Phlebia brevispora*, noting enhanced growth and MnP activity when supplied with peptone and yeast extract, whereas ammonium tartrate usage led to lower outcomes. Salihi et al. [25] worked with *Bjerkandera adusta* and determined that tryptone and beef extract fueled robust growth and MnP synthesis compared to ammonium sulfate and sodium nitrate, which stunted development and impaired enzyme creation. Organic nitrogen drive optimal fungal growth and MnP production in fungi, pointing to promising possibilities for biotechnological exploitation in sectors demanding oxidative enzymes, such as pulp bleaching, textile processing, and bioremediation technologies.

Rahman et al. [26] investigating the brown-rot Basidiomycota *Serpula lacrymans* recorded increased hyphal extension and MnP activity with 2 mM concentration of Mn^{2+} and Cu^{2+} while Pandey et al. [27] focusing on *Pleurotus ostreatus* recorded MnP activity with same concentration of Mn^{2+} and Cu^{2+}. This also aligns with the findings of this study. The presence of metals in some environments has been attributed to oil spillage. Their effects are often concentration dependent and also vary in their individual toxicity [13].

5. Conclusion

This study demonstrated that regulation of culture conditions offers great prospect for amplifying cell growth and MnP production, consequently expanding the applicability of this versatile enzyme in various industrial processes. Nevertheless, continued explorations should be pursued given the inherent complexity of biological systems and ever-evolving technological landscapes.

Compliance with ethical standards

Disclosure of conflict of interest

No conflict of interest to be disclosed.

References

[1] Wang X, Huang Z, Yang W, Chen Q, Shao Z, Li B and Lin H. (2019). Laccase-and manganese peroxidase-based technologies for the treatment of emerging micropollutants: Status quo and future directions. Science of the Total Environment, 670, 440–453.

[2] Kumar R, Gupta R, Verma P, Kumar V, Sharma A, Yadav S and Pruthvi D. (2018). Recent Advances in Lignin Valorization towards Sustainable Platform Chemicals via Catalytic Processes, An Overview. Industrial Crops and Products, 122, 456–468.

[3] Doyle TP, Cullen JM, O'Connell E, McDonnell A, Murphy P and Hill R. (2015). Antioxidant activities of crude extracts obtained from solid state cultivated *Pleurotus ostreatus* using agricultural residues under unoptimized conditions. Journal of Applied Phycology, 27(5), 2439–2447.

[4] Fernández-Francos I, Martínez ÁT, Gómez-Toribio N, Rodriguez-Delgado MA, Fernández-Lafuente R, del Río JC and Martín D. (2016). Comparison of Decolourizing Capacities of White Rot Fungi Isolated from Temperate Forest Soils Using Different Substrates. International Journal of Environmental Research and Public Health, 13(7), 680.

[5] You Y, Qiu Y, Xu H, He R, Zhang L, Wang Z and Xia Y. (2024). Effective and food-grade detoxification of multiple mycotoxins using yeast expressed manganese peroxidases. Food Bioscience, 59, 103886.

[6] Zhang Y, Jiang Z, Sun R, Su Y and Zheng Y. (2014). Medium optimization and purification of manganese peroxidase from *Irpex lacteus* CD2 grown on wheat bran. World Journal of Microbiology and Biotechnology, 30(5), 1447–1455.

[7] Raghuwanshi S, Prasher IB and Shehnaz. (2023). Optimal Growth and Ligninolytic Enzyme Production by *Geotrichum europaeum*. Proceedings of the National Academy of Sciences, India Section B: Biological Sciences, *93*(2), 451-459.).

[8] Zhang Y, Sun T, Huang F, Luo Z, and Qiu Y. (2021). Optimization of Carbon Source for Enhanced Production of Manganese Peroxidase by *Phanerochaete chrysosporium* Using Response Surface Methodology. International Biodeterioration & Biodegradation, 160, 105202.

[9] Gao X, Wang J and Chen Y. (2020). Effects of Different Nitrogen Sources on Cell Growth and Lignin Peroxidase Activity of *Irpex lacteus* CD2. BioResources, 15(3), 6789-6799.

[10] Li W, Yang H, Yan R and Jiang J. (2021). Purification and Characterization of Manganese Peroxidase From *Ceriporiopsis subvermispora* and Its Application in Dye Degradation. Journal of Chemistry, 2021, 1-11.

[11] Eze OJ, Anakwenze VN and Ogbo FC. (2019). Manganese Peroxidase (MnP) activity of some Nigerian edible mushrooms. The Bioscientist, 7(1):28-43

[12] Patricia LO, Marta CTD, Alexandre, NP and Lucias RD. (2009) Purification and Partial Characterization of Manganese Peroxidase from *Bacillus pumilus* and *Paenibacillus,* Brazilian Journal of Microbiology. 40 4:

[13] Ogbo EM and Okhuoya JA (2011). Bioavailability of some Heavy metals in Crude Oil contaminated soils remediated with *Pleurotus tuber-regium* (Fr.) Singer. Asian Journal of Biological Sciences. 4 1: 323 – 327.

[14] Daniela C and Miroslav O. (2013). Effect of Potential Inductors on Laccase Production by White-Rot fungus *Geripopriopsis subvermispora.* Journal of Microbiology, Biotechnology and Food sciences. 3 2:84 – 87

[15] Asamudo NU, Daba AS and Ezeronye OU. (2005). Bioremediation of textile effluent using *Phanerochaete chrysosporium*. African Journal of Biotechnology, 4(13):1548-1553

[16] Zhao Y, Huang X, Du J and Jiang Y. (2021). Optimization of Fermentation Parameters for Production of Penicillium Expansum Mycoprotein Using Response Surface Methodology. Biotechnology journal, 16(9), 2100182.

[17] Li Y, Wang Q, Cheng Y, Yang J, Wu W and Sun S. (2021). Effect of initial medium pH on mycelial growth and spore production of *Colletotrichum gloeosporioides*. BioControl, 66(3), 507-516.

[18] dos Santos LRF, Soares LA, da Silva Pereira R, do Prado RM and Melo Filho MAB. (2020). Use of response surface methodology to optimize the decolourisation of synthetic textile dye solutions by *Pleurotus ostreatus*. Process Safety and Environment Protection, 137, 426-433.

[19] Karimiyan H, Alidadi SE, Mohammadi S, Pourakbar M, Hashemian Z and Azizi M. (2021). Characterization of phloroglucinol red color removal from water by phlebia tremellosa fungus. Desalination and Water Treatment, 200, 201-207.

[20] Baharlouei A, Nazari S, Taherirad N, Ahmadikouchaksaraie A, Soltanimehr H and Khodaparast Z. (2020). Enhanced degradation of bisphenol A by combined application of ultrasound irradiation and *Ceriporiopsis subvermispora* isolated from petroleum polluted soil. Environmental Technology & Innovation, 18, 100957.

[21] Arora P and Gill B. (2020). Role of laccase, manganese peroxidase and versatile peroxidase in lignin depolymerization: Present scenario and future prospects. International Biodeterioration & Biodegradation, 149, 105018.

[22] Manavalan P, Ramalingam C, Kumar R, Balaraman S, Palvannan T, Jayabalan R, . . . Venkappayya B. (2021). Insights into extracellular thermostable xylanase and cellulase production by newly isolated *Pycnoporus sanguineus* strain JS-2017: Statistical optimization and characterization. Applied Microbiology and Biotechnology, 105(5), 2143-2159.

[23] Bhatt SS, Mahajan S, Patil PP, Shete RB, Naresh K, Shukla AK and Agarkar GS. (2021). Deciphering the molecular mechanism involved in plant polysaccharide deconstruction by white rot fungus *Phlebia radiata* grown under defined carbon and nitrogen source combinations. Scientific Reports, 11(1), 1-16.

[24] Gao Y, Ren W, Jin Y, Tan J and Yu Q. (2021). Efficient degradation of polycyclic aromatic hydrocarbon mixtures by *Phlebia brevispora* pretreated with microwave radiation. Ecotoxicology and Environmental Safety, 218, 112259.

[25] Salihi L, Öztürk M and Demirci B. (2020). Bioprospecting of endophytic fungi inhabiting medicinal plants collected from Turkey: antagonistic, antibacterial, and antioxidant properties. Turkish Journal of Biology, 44, 1593-1602.

[26] Rahman MA, Yamada R, Ohga A, Sakai K and Furuta T. (2021). Effects of heavy metals on decomposition and mineralization of wood by *Serpula lacrymans*. Science of the Total Environment, 780: 146642.

[27] Pandey A, Rawat M, Kotwal R and Shahi SK. (2021). Degradation of trichloroethylene by ligninolytic enzymes produced by Pleurotus ostreatus. SpringerPlus, 10(1): 1-11.