Evaluation of Lignocellulatic Activity of Enzymes from Microwave-irradiated *Pleurotus sajor-caju* Cultivated with Wheat Straw

Aisha M. H. Al-Rajhi,[a,*] Abdulrahman S. Bazaid,[b,c] Ahmed M. Abdulfattah,[d] T. M. Abdelghany,[e] Abdel-Rahman M. Shater,[f] and Samy Selim [g,*]

The production of lignocellulytic enzymes by microwave-radiated *Pleurotus sajor-caju* was assayed. Wheat straw was employed as substrate to *P. sajor-caju* for production of laccase, manganese peroxidase (MnPase), filter-paperase (FPase), carboxmethyl cellulase (CMCase), and cellulase (as evaluated using microcrystalline cellulose). *P. sajor-caju* exposed to 10 s of microwave radiation (MR) showed maximum growth with colony radius of 7.17 ± 0.45 cm, while with increasing the exposure time up to 50 s the growth decreased up to 2.67 ± 0.22 cm. Moreover, it failed to grow at 80 s of exposure time. Cellulase, MnPase, FPase, CMCase, and laccase activities were induced to 37 ± .0.54, 49 ± 2.36, 189 ± 2.12, 0.37 ± 0.06, and 1.58 ± 0.03 U/mL compared to that at control 31 ± 0.25, 46 ± 1.25, 177 ± 1.65, 0.28 ± 0.03, and 1.37 ± 0.12 U/mL, respectively as a result of *P. sajor-caju* exposure to 10 s of MR. As the exposure time increased, these enzymes activity decreased. Different levels of moisture with surfactant (polysorbate 80) were applied to optimize the enzymes activities at 10 s of exposure time. The optimum activities 3.15 ± 0.23, 0.62 ± 0.06, 269 ± 5.36, 65 ± 1.63, and 48 ± 0.98 U/mL were recorded for cellulase, MnPase, FPase, CMCase, and laccase, respectively at 70% of moisture and 0.15 mL/L of polysorbate 80.

DOI: 10.15376/biores.19.4.8882-8893

Keywords: Lignocellulatic Enzymes; Pleurotus sajor-caju; Wheat Straw; Surfactant

Contact information: a: Department of Biology, College of Science, Princess Nourah bint Abdulrahman University, P.O. Box 84428, Riyadh 11671, Saudi Arabia; b: Department of Medical Laboratory Science, College of Applied Medical Sciences, University of Ha'il, Hail 55476, Saudi Arabia; c: Medical and Diagnostic Research Center, University of Ha'il, Hail 55473, Saudi Arabia; d: Department of Medical Laboratory Sciences, Faculty of Applied Medical Sciences, King Abdulaziz University, Jeddah 21589, Saudi Arabia; e: Botany and Microbiology Department, Faculty of Science, Al-Azhar University, Cairo 11725, Egypt; f: Biology Department, College of Science, Jazan University, Jazan 82817, Saudi Arabia; g: Department of Clinical Laboratory Sciences, College of Applied Medical Sciences, Jouf University, Sakaka, Saudi Arabia;
** Corresponding authors: amoalrajhi@pnu.edu.sa* (A.M.H.A.), *sabdulsalam@ju.edu.sa* (S.S.)

INTRODUCTION

Lignocellulose is a major constituent of woody and decayed plant materials. Various extracellular enzymes generated from white rot fungi (WRF), such cellobiose dehydrogenase (CDHase), lignin peroxidase (LiPase), laccase, and manganese peroxidase (MnPase), are associated with the breakdown of lignin and its derivatives (Reyes *et al*. 2021; Al-Rajhi *et al*. 2022a). Each year, a large amount of straw is generated from wheat cultivation. While some is utilized as feed for animals or as a substrate to cultivate edible mushrooms, the remainder can be regarded as a huge underutilized resource of energy

(Devi *et al.* 2024). Globally, a significant amount of lignocellulosic remains from agriculture (such as wheat straw) and wood (such as wood chips), and different wastes of industry have accumulated as a result of the growing agro-industrial activities. The ecosystem is contaminated by these substances (Elgueta and Diez 2010; Sánchez and Montoya 2020). It is crucial to use lignocellulose residues to increase WRF's ability to produce extracellular phenoloxidase enzymes (Omoni *et al*. 2022; Abiola-Olagunju *et al.* 2024). Maintaining sustainable social development may depend on making full use of lignocellulosic resources. Enzymatic hydrolysis of these wastes to produce valuable compounds and solvents is the most promising method (Shankar *et al*. 2024). The past 20 years have seen a great deal of study in this field. Two sub-processes make up the conversion: first, lignocellulosic materials are hydrolysed to produce fermentable sugars, and then those sugars are fermented to produce the desired products (Singh *et al*. 2024).

Lignocellulolytic enzymes serve numerous roles in biotechnological processes in the chemical, fuel, brewery, food, wine, textile, and pulp, dyes degradation and paper industries (Xu *et al.* 2023; Pham *et al.* 2024). In fungi, extracellular enzymes are constitutively produced in different amounts, and they are affected by many typical fermentation factors such as medium constituents, carbon/nitrogen ratio, temperature, pH, and aeration level (Abdel Ghany *et al.* 2018; Al Abboud *et al.* 2022; Bakri *et al*. 2022; An *et al.* 2023; Aza and Camarero 2023), besides metal ions, particularly Cu^{2+} (Akpinar and Ozturk 2017; Abdel Ghany *et al.* 2020; Al-Rajhi *et al*. 2022b). The occurrence of Mn^{2+} is known to induce the production of MnPase in many WRF, but Mn^{2+} lowers LiPase titers (Li *et al.* 2022). The content of moisture is a critical agent on enzymes production (Sosa-Martínez *et al.* 2023). A reduction in enzyme yield can be caused by higher moisture levels due to steric hindrance of the producer strain. The bioavailability of less soluble substrates for the fungi can be increased by surfactants, especially polysorbate-80 (Sun *et al*. 2018). Many investigators have mentioned that some natural inductive compounds such as phenols and flavonoids are present in lignocellulosic wastes including rice bran, oak sawdust, wheat straw, and wheat bran; these substrates can be used to induce the fungal lignocellulatic enzymes secretion (Wang *et al*. 2015; Wang *et al*. 2019; Hermosilla *et al*. 2020). Also, olive leaves contain hydroxytyrosol and oleuropein which act as inducers for laccase production by white rot fungi (Yuan *et al*. 2015).

Electromagnetic radiation includes several forms, including microwave radiation (MR). During MR, the target samples are heated by the thermal energy generated from electromagnetic energy, and subsequently interacts with the ingredients of the target samples (de la Hoz *et al*. 2005). Fungal growth is regulated and inhibited *via* numerous chemical, physical, and biological methods. However, MR is one of the greatest physical techniques to manage of fungal pathogens and decaying wood fungi (Ahmed and Amein 2023). According to Poonia *et al.* (2021) the viability of treated white and brown rot fungi namely *Trametes versicolor* and *Rhodonia placenta* by microwave radiation decreased the dependency on exposure time with complete inhibition at 180 s. In another study, Mahdi *et al.* (2021) mentioned that the exposed bacteria and fungi to radiation of microwave for 45 s were completely killed. Poonia *et al*. (2021) utilized MR to repress the growth of *Serpula lacrymans* (brown rot fungus), which is important in European indoor rot inside thick wooden beams (Poonia *et al.* 2021). Several properties such as rapid reaction time and low reactivity with applied biomass were associated with MR. The authors are proposing the hypothesis in this work that MR at low contact time may enhance the activity of used fungus to degrade wheat straw with lignocellulotic enzymes production. Therefore, the main purposes of this investigation were to evaluate the influence of microwave

radiation on the white rot fungus *Pleurotus sajor-caju*, in addition to polysorbate 80 to enable their efficient hydrolysis of lignocellulosics biomass from wheat straw to produce lignocellulotic enzymes.

EXPERIMENTAL

Fungus and Wheat Straw Used

Pleurotus sajor-caju (obtained from Professor Abdelghany TM, Al Azhar University, Egypt) was cultivated for 15 days at 28 °C on the surface of Malt agar in petri dishes. Mycelium Agar plugs containing active mycelia (6 mm in diameter) were cut and used for fungus propagation. Dried wheat straw (WS) was collected from agriculture areas in Saudi Arabia and then cut into 2 to 3 mm lengths.

Fungal Growth and Production of Lignocellulytic Enzymes at Different Times of Microwave Radiation

The cultivated *P. sajor-caju* on malt agar plates at 8 days was exposed to a household microwave oven (Type Zanussi, 230 V, 50 Hz, 2450 MHz, 1100 W, China) for 10, 30, 50, or 80 sec. From each plate, a disc (6 mm) of exposed fungus to microwave ray was used to inoculate a uniform malt agar media and incubated at 28 °C. The developed colony of the fungus was measured after 8 days. The well growth mycelium colonies on malt agar plates were plugged (9 mm in diameter) from the growing colonies and applied to inoculate on wheat straw as a substrate. The flasks containing the fungus inoculum were incubated for 14 days at 28 °C, and then laccase, MnPase, CMCase, FPase, cellulase (evaluated based on microcrysalline cellulose degradation), and extracellular protein were determined.

Enzymes Preparation

In a 500 mL conical flask, 10 grams of the wheat straw waste were added. After adding different levels (50 to 80 %) of moistening agent besides different concentration (0.05 to 0.2 mL/L) of polysorbate 80 (Tween 80) to the substrate, it was autoclaved for 30 min at 121 °C. Two 6 mm fungus discs were utilized to inoculate every flask. For 15 days, they were incubated at 28 °C. Following the incubation period, each flask received 100 mL of distilled water, and the flasks were shaken for 60 min at 200 rpm. Using muslin cloth on a glass funnel, the entire contents of the flask were filtered into a dry, clean flask. The filtrates underwent a 10-min cooling centrifugation at 80,000 rpm. Crude enzymes were made from the supernatant solutions. The crude enzymes were directly applied in some experiments, while other quantity of enzymes was kept for short times in a refrigerator at 4 °C.

Tests for Cellulase Activity

Before describing various tests for enzyme activity, it is important to clarify that three of them, namely the “CMCase”, “FPase”, and “MCCase” tests, are all different means of determining the activity of one class of enzymes, the cellulase class. The CMCase test employs a soluble derivative of cellulose, whereas the “MCCase” test employs crystalline cellulose. The “FBase” can be regarded as intermediate in character, since the cellulose fibers within the filter paper will contain both crystalline and non-crystalline cellulose domains.

Carboxymethyl cellulase (CMCase) test and protein quantity detection

As described in Wang *et al*. (2008), 1 mL of pH 5.0 sodium acetate buffer was mixed with 1 mg of carboxymethyl cellulose. An aliquot of 1 mL of the supernatant (enzyme) was placed in a clean, dry tube along with 1 mL of 1 percent CMCase in acetate buffer. For thirty min, the tube was kept at 62 °C, after which the released reducing sugar was recorded using the Miller (1959) DNS method, with the absorbance being measured at 540 nm. The blank was one mL of distilled water rather than one mL of supernatant (enzyme). Using the glucose standard curve, the concentration of the resulting reducing sugar was ascertained. The micromole of glucose released/mL of culture filtrate/min is one unit of CMCase. The detected quantity of soluble proteins in the supernatant containing the fungus filtrate of medium growth was performed according to protocol of Lowry (1951).

Filter-paperase (FPase) test

An aliquot of 1 mL of the enzyme-containing supernatant (pH 4.8) and 2 mL of 0.1M citrate buffer pH 4.8, containing 0.05 g of filter paper (Whatman No. 1) (Gadgil *et al.* 1995). For 60 min, the tube was incubated at 50 °C. The micromole of glucose released/mL of filtrate per/min is equivalent to one unit of FPase.

Microcrystalline cellulose (MCC)-ase test

Avicell MCC (2.0 g) was mixed with 100 mL of sodium phosphate buffer (SPB) with pH 6.6 according to method of Li and Gao (1997). An aliquot of one mL of the enzyme-containing supernatant was combined with 1 mL of 2% MCC in SPB in a clean, dry tube. For two hours, the reaction mixture was incubated at 40 °C. One micromole of glucose liberated/mL of filtrate/min was defined as one unit of MCCase.

Laccase Test

Laccase activity was tested according to Garzillo *et al*. (2001). Two mM 2,2 azino-bis 3-ethyl benzo-thiazoline-6 sulforicacide (ABTS) was mixed with McIlvaine buffer (pH 5) in a 1 mL reaction mixture. One hundred microliters of centrifuged extracellular supernatants were added to the assay mixture. Through observation of the absorbance at 420 nm at 30 °C, the enzymatic activity was calculated in IU.

Manganase Peroxidase (MnPase) Test

In a one cm quartz cuvette, MnPase activity was measured. In a McIlvaine buffer (pH 5.0), the 1 mL reaction mixture contained one mM Mn^{2+} and two mM ABTS. Initiating the peroxidase activity, 0.4 mM H_2O_2 was added to the assay mixture after 100 µL of centrifuged extra cellular fluids (supernatants) were added (Garzillo *et al.* 2001). Using a spectrophotometer (JENWAY, Model 6300, EU) at 30 °C, the absorbance change at 420 nm (ABTS), =36 $mM^{-1}cm^{-1}$, was monitored to estimate the enzymatic activity in IU.

Statistical Examination

Standard deviation (± SD) was estimated from the calculation of the mean three replicates. Subsequently, the Tukey-Kramer honestly significant difference (HSD) test was employed. The level of significance was 5%.

RESULTS AND DISCUSSION

The production of lignocellulytic enzymes on WS as a cheap, renewable substrate has become well known. In this work, WS was employed as substrate for production of value-added enzymes using the fungus *P. sajor-caju*. In a previous study (Biswas *et al*. 2019) it was stated that WS is a suitable substrate for MnPase and lipase production as well as other enzymes. Table 1 shows the effect of MR on growth of *P. sajor-caju*. The outcomes of fungus growth reflected that as the fungus was exposed to different times of MR, the enzyme production increased up to 7.17 ± 0.45 mm at 10 s, while after this exposure time the growth decreased to 4.53 ± 0.32 and 2.67 ± 0.22 mm at 30 and 50 s. Furthermore, the fungus failed to grow at exposure time 80 s. All of these results were compared with untreated fungus, where the colony growth was 6.66 ± 0.28 mm with HSD at 5% of 0.816 among all growths at the different exposure times. The obtained findings were in agreement with outcomes of Poonia *et al*. (2021), where the growth of brown and white rot fungi namely *Rhodonia placenta* and *Trametes versicolor*, respectively, decreased based to the applied exposure time of MW. Górny *et al*. (2007) mentioned that the influence of microwave radiation on fungal and bacterial viability depended on the time of exposure and power density of radiation.

Table 1. Effect of Different Exposure Times of Microwave Radiation on the Growth of *P. sajor-caj*

Time (s)	Growth Diameter (cm)
Control	6.66 ± 0.28
10	7.17 ± 0.45
30	4.53 ± 0.32
50	2.67 ± 0.22
80	0 ± 0.0
HSD at 5%	0.816

± Standard deviation of average 3 repetitions of results

The effect of microwave radiation as a physical effect on development of fungi and their lignocellulatic enzymes activity was selected due to several advantages such as non-contact and volumetric heating, rapid reaction period, and little reactant consumption (Bichot *et al*. 2022). According to other studies, the generated electromagnetic waves from microwaves penetrate easily and rapidly inside the applicable biomass or substrate (Beneroso *et al.* 2017; Bundhoo 2018). Enzymes activity of *P. sajor-caju* was evaluated under different times of exposure to MR (Table 2). Exposure to short time 10 s of microwave irradiation induced all the examined enzymes namely MCCase, MnPase, FPase, CMCase, and laccase, where the activities were 37 ± .0.54, 49 ± 2.36, 189 ± 2.12, 0.37 ± 0.06, and 1.58 ± 0.03 U/mL compared to that at control 31 ± 0.25, 46 ± 1.25, 177 ± 1.65, 0.28 ± 0.03, and 1.37 ± 0.12 U/mL, respectively. MR at 30 and 50 s of MR repress the enzymes production by *P. sajor-caju*. Since at 50 s, the values of activities were 19 ± 0.65, 27 ± 0.65, 124 ± 3.25**,** 0.21 ± 0.05**,** and 0.78 ± 0.09 U/mL for MCCase, MnPase, FPase, CMCase, and laccase, respectively. The effect of MR was studied by Zhu *et al.* (2006) on the enzymatic hydrolysis of rice straw. Their results showed that the level of initial hydrolysis was greatly speeded up, but the yield then declined slightly. Improving the enzymatic saccharification of grain stillage by *Phanerochaete chrysosporium* was reported by pretreated *via* microwave-assisted hydrothermal treatment (Ren *et al*. 2020).

Table 2. Effect of Different Times of MR on Cellulytic Enzymes and Extracellular Protein Produced by *P. sajor-caju* by Solid State Fermentation of Wheat Straw (± Standard deviation of average 3 repetitions of results)

Time (s)	Enzyme Activity (U/mL)					Protein (µg/mL)
	MCCase	FPase	CMCase	MnPase	Laccase	
Control	31 ± 0.25	46 ± 1.25	177 ± 1.65	0.28 ± 0.03	1.37 ± 0.12	181 ± 3.25
10	37 ± .0.54	49 ± 2.36	189 ± 2.12	0.37 ± 0.06	1.58 ± 0.03	197 ± 2.36
30	30 ± 0.36	36 ± 1.56	167 ± 1.32	0.29 ± 0.04	1.32 ± 0.21	152 ± 4.25
50	19 ± 0.65	27 ± 0.65	124 ± 3.25	0.21 ± 0.05	0.78 ± 0.09	102 ± 2.52
HSD at 5%	4.25	6.45	9.19	0.06	0.36	11.43

Table 3. Production of Lignocellulytic Enzymes on Wheat Straw Amended with *P. sajor-caju*

Treatments		**Enzymes Productivity (U/mL)**					**Proteins (µg/mL)**
Moisture (%)	**Polysorbate 80 (mL/L)**	**Laccase**	**MnPase**	**CMCase**	**FPase**	**MCCase**	
50	0.05	0.85 ± 0.04	0.23 ± 0.06	128 ± 3.25	27 ± 1.32	18 ± 0.96	179 ± 1.96
	0.10	1.41 ± 0.09	0.27 ± 0.03	162 ± 2.36	34 ± 1.65	24 ± 1.32	196 ± 1.25
	0.15	0.55 ± 0.06	0.16 ± 0.04	125 ± 3.25	24 ± 2.32	17 ± 0.36	122 ± 2.54
	0.20	0.53 ± 0.02	0.15 ± 0.01	120 ± 4.12	21 ± 1.03	14 ± 0.21	118 ± 1.54
HSD at 5%		0.15	0.07	6.21	1.36	2.12	8.25
60	0.05	1.37 ± 0.06	0.28 ± 0.05	178 ± 4.25	47 ± 1.25	33 ± 1.01	182 ± 2.85
	0.10	1.05 ± 0.09	0.33 ± 0.03	196 ± 2.54	52 ± 0.9	34 ± 0.65	185 ± 3.52
	0.15	2.06 ± 0.12	0.48 ± 0.04	215 ± 3.26	64 ± 2.10	39 ± 0.25	232 ± 5.14
	0.20	1.03 ± 0.11	0.46 ± 0.05	190 ± 4.65	62 ± 1.36	34 ± 0.69	216 ± 4.58
HSD at 5%		0.32	0.08	14.54	3.54	1.54	7.5
70	0.05	2.93 ± 0.21	0.38 ± 0.03	181 ± 2.23	49 ± 1.36	40 ± 2.54	183 ± 2.54
	0.10	1.96 ± 0.13	0.59 ± 0.02	184 ± 1.32	57 ± 0.89	44 ± 1.32	196 ± 4.36
	0.15	3.15 ± 0.23	0.62 ± 0.06	269 ± 5.36	65 ± 1.63	48 ± 0.98	221 ± 3.54
	0.20	1.94 ± 0.12	0.48 ± 0.03	198 ± 2.54	63 ± 0.78	39 ± 2.21	165 ± 1.36
HSD at 5%		0.28	0.09	6.55	5.32	4.12	8.25
80	0.05	0.86 ± 0.08	0.25 ± 0.02	113 ± 2.54	22 ± 1.21	15 ± 0.65	135 ± 2.10
	0.10	1.32 ± 0.12	0.36 ± 0.05	150 ± 3.14	27 ± 1.13	22 ± 0.36	155 ± 1.21
	0.15	0.57 ± 0.06	0.29 ± 0.03	122 ± 4.21	20 ± 0.69	16 ± 0.21	136 ± 1.13
	0.20	0.55 ± 0.07	0.24 ± 0.04	111 ± 1.32	18 ± 1.12	14 ± 1.02	87 ± 1.42
HSD at 5%		0.241	0.06	6.54	2.54	2.21	15.65

± Standard deviation of average 3 repetitions of results

The exposed fungus culture to 10 s of MR with cultivated certain optimum condition was studied for the optimization of enzymes production (laccase, MnPase, FPase, MCCase, and extracellular protein). The variables used were volume of moisting agent polysorbate 80 was added as surfactant to help in secretion of enzymes on solid state fermentation. There has been some investigation showing that lignocellulytic enzymes secretions by *Pleurotus ostreatus* depend on the producer of fungi, substrate composition, and cultivation condition (da Silva *et al*. 2019; Zhang *et al*. 2023). The maximum activities of studied enzymes were noted at 0.10 mL/L of polysorbate 80 and moisture 50%, at 0.15 mL/L of polysorbate 80, and moisture 60% and 70%. At 80% moisture, the maximum activities of all studied enzymes were recorded at 0.10 mL/L of polysorbate 80. Generally, 70% of moisture and 0.15 mL/L of polysorbate 80 represented the optimum conditions for enzymes activities (3.15 ± 0.23, 0.62 ± 0.06, 269 ± 5.36, 65 ± 1.63, and 48 ± 0.98 U/mL for MCCase, MnPase, FPase, CMCase, and laccase, respectively). The outcomes of Zhang *et al*. (2023) designate that it was probable to reach important activities of some enzymes, namely LiP and cellulase utilizing *Pleurotus ostreatus* with lignocellulosic biomass fermentation under solid state condition by aiding exogenous inducers of surfactants. Generally, according to published reports, the microwave-irradiated biomasses were affected by several associated parameters of microwave irradiation; from these parameters the hydration condition and the polarity of the reaction substrate which subsequently effect the enzymatic productivity (Chen *et al.* 2020). Moreover based on another study, microwave heating appears to possess an influence on the stereo-selectivity of enzymes (Mazumder *et al.* 2004). A direct microwave energy absorption by the enzyme polar substrates result in a greater reactivity of the chemical functional groups included in the enzymatic reaction. Other mechanisms associated with change of enzyme activity caused my microwaves were reported (Habinshuti *et al*. 2020; Deng *et al.* 2022). For instance, the activity of thermally unstable enzymes was affected *via* treatment by microwave because of heat denaturation. Treatment by microwave may cause some protein sites to become more susceptible to enzymatic hydrolysis due to molecular rearrangement and unfolding of proteins. In tables (2 and 3), there is a relation between the activity of enzymes and the detection amount of proteins, where the high activity of enzymes at all treatments was accompanied by a high detected amount of protein and *vice versa*.

In the present paper, polysorbate 80 at a specific concentration increased the enzymes activity. According to Shrestha *et al*. (2023), surfactant giving an appropriate membrane composition for enzymes to join with the substrate can be expected to increase their activity. Polysorbate 80 modifies the structure of fungal cell membranes to encourage the excretion of ligninolytic enzymes (Rodrigues *et al*. 2008). However, at high concentrations of polysorbate 80 above 0.15 mL/L, the activity of enzymes was decreased, maybe due to that surfactant at these concentrations affecting the permeability of cell membrane, leading to blockage the enzymes secretion as mentioned previously (Ahlawat *et al*. 2009). Laccase yield from *Pleurotus sajor-caju* was increased from 33.5 to 50 times when growth medium amended with 7.5% mL/v of polysorbate 80 compared to its yield in medium without polysorbate 80 (Teodoro *et al*. 2018). He *et al*. (2023) documented that the addition of polysorbate 80 promoted the increasing in temperature during the composting process that a companied by accelerate the lignocellulose degradation and decline the phytotoxicity as well as increase the lignocellulytic enzymes. Further investigations are required to determine the effect of suitable conditions such as pH, temperature, fermentation process, and some metals in combination with the effect of MR

on the lignocellulatic enzymes activity as well as the application of lignocellulatic enzymes on the industrial scale.

CONCLUSIONS

1. The investigation concluded that low exposure time (10 s) of microwave radiation has better potential of application to induce lignocellulotic enzymes of *P. sajor-caju* using WS biomass.
2. Moisture level of 70% and 0.15 mL/L of polysorbate 80 considered the optimum conditions for the secretions of lignocellulotic enzymes.

ACKNOWLEDGMENTS

The authors would like to acknowledge Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

FUNDING

This research was funded by Princess Nourah bint Abdulrahman University Researchers Supporting Project number (PNURSP2024R217), Princess Nourah bint Abdulrahman University, Riyadh, Saudi Arabia.

REFERENCES CITED

Abdel Ghany, T. M., Bakri, M. M., Al-Rajhi, A. M. H., Al Abboud, M. A., Alawlaqi, M. M., and Shater, A. R. M. (2020). "Impact of copper and its nanoparticles on growth, ultrastructure, and laccase production of *Aspergillus niger* using corn cobs wastes," *BioResources* 15(2), 3289-3306. DOI: 10.15376/biores.15.2.3289-3306

Abdel Ghany, T. M., Ganash, M., Bakri, M. M., and Al-Rajhi, A. M. H. (2018). "Molecular characterization of *Trichoderma asperellum* and lignocellulolytic activity on barley straw treated with silver nanoparticles," *BioResources* 13(1), 1729-1744. DOI: 10.15376/biores.13.1.1729-1744

Abiola-Olagunju, O., Mako, A. A., Ettu, R. O., and Afolabi, B. O. (2024). "Improving nutritional quality of some fibrous agricultural wastes using white rot fungi (*Pleurotus plumonarius*)," in: *Proc. 49th Conf., Nig. Soc. for Anim. Prod. Nigerian Journal of Animal Production*, pp. 798-801.

Ahlawat, S., Dhiman, S. S., Battan, B., Mandhan, R. P., and Sharma, J. (2009). "Pectinase production by *Bacillus subtilis* and its potential application in biopreparation of cotton and micropoly fabric," *Process Biochemistry* 44(5), 521-526. DOI: 10.1016/j.procbio.2009.01.003

Ahmed, H. A., and Amein, K. A. (2023). "Influence of peanut seed exposure to microwave and X-ray radiations on *Sclerotium* stem rot and effects on yield improvement, and RAPD-PCR mutagenic analysis of peanut plants," *Egyptian Journal of Phytopathology* 51(2), 14-29. DOI: 10.21608/ejp.2023.221669.1098

Akpinar, M., and Ozturk Urek, R. (2017). "Induction of fungal laccase production under solid state bioprocessing of new agroindustrial waste and its application on dye decolorization," *3 Biotech* **7**, 98. DOI: 10.1007/s13205-017-0742-5

Al Abboud, M. A., Al-Rajhi, A. M. H., Shater, A.-R. M., Alawlaqi, M. M., Mashraqi, A., Selim, S., Al Jaouni, S. K., and Abdelghany, T. M. (2022). "Halostability and thermostability of chitinase produced by fungi isolated from salt marsh soil in subtropical region of Saudi Arabia," *BioResources* 17(3), 4763-4780. DOI: 10.15376/biores.17.3.4763-4780

Al-Rajhi, A. M. H., Asmaa, A. A., Reham, Y., and Abdel Ghany, T. M. (2022a). "Induction of hydrolytic enzyme production and antibiosis *via* a culture of dual fungal species isolated from soil rich with the residues of woody plants in Saudi Arabia," *BioResources* 17(2), 1729-1744. DOI: 10.15376/biores.17.2.2358-2371

Al-Rajhi, A. M. H., Yahya, R., Alawlaqi, M. M., Fareid, M. A., Amin, B. H., and Abdelghany, T. M. (2022b). "Copper oxide nanoparticles as fungistat to inhibit mycotoxins and hydrolytic enzyme production by *Fusarium incarnatum* isolated from garlic biomass," *BioResources* 17(2), 3042-3056. DOI: 10.15376/biores.17.2.3042-3056

An, Q., Zhou, Z. G. W., Guo, S., Chen, Z., Yuan, Y. N., Sun, X. Q., and Han, M. L. (2023). "Laccase produced by *Coriolopsis trogii* and *Cerrena unicolor* with the mixed of metal ions and lignocellulosic materials," *BioResources* 18(2), 3895-3908. DOI: 10.15376/biores.18.2.3895-3908

Aza, P., and Camarero, S. (2023). "Fungal laccases: Fundamentals, engineering and classification update," *Biomolecules* 13(12), article 1716. DOI: 10.3390/biom131217160

Bakri, M. M., Al-Rajhi, A. M. H., Abada, E., Salem, O. M. A., Shater, A.-R., Mahmoud, M. S., and Abdel Ghany, T. M. (2022). "Mycostimulator of chitinolytic activity: Thermodynamic studies and its activity against human and food-borne microbial pathogens," *BioResources* 17(3), 4378-4394. DOI: 10.15376/biores.17.3.4378-4394

Beneroso, D., Monti, T., Kostas, E. T., and Robinson, J. (2017). "Microwave pyrolysis of biomass for bio-oil production: scalable processing concepts," *Chemical Engineering Journal* 316, 481-498. DOI: 10.1016/j.cej.2017.01.130

Bichot, A., Raouche, S., Faulds, C. B., Méchin, V., Bernet, N., Delgenès, J. P., and García-Bernet, D. (2022). Effects of successive microwave and enzymatic treatments on the release of p-hydroxycinnamic acids from two types of grass biomass," *Biochemical Engineering Journal* 182, article 108434. DOI: 10.1016/j.bej.2022.108434

Biswas, P., Bharti, A. K., Kadam, A., and Dutt, D. (2019). "Wheat bran as substrate for enzyme production and its application in the bio-deinking of mixed office waste (MOW) paper," *BioResources* 14(3), 5788-5806. DOI: 10.15376/biores.14.3.5788-5806

Bundhoo, Z. M. (2018). "Microwave-assisted conversion of biomass and waste materials to biofuels," *Renewable and Sustainable Energy Reviews* 82, 1149-1177. DOI: 10.1016/j.rser.2017.09.066

Chen, R., Wei, Q., Wei, X., Liu, Y., Zhang, X., Chen, X., Yin, X., and Xie, T. (2020). "Stable and efficient immobilization of bi-enzymatic NADPH cofactor recycling system under consecutive microwave irradiation," *PLoS One* 15(11):e0242564. DOI: 10.1371/journal.pone.0242564.

da Silva, I. F., da Luz, J. M. R., Oliveira, S. F., de Queiroz, J. H., and Kasuya, M. C. M.

(2019). "High-yield cellulase and LiP production after SSF of agricultural wastes by *Pleurotus ostreatus* using different surfactants," *Biocatalysis and Agricultural Biotechnology* 22, article 101428. DOI: 10.1016/j.bcab.2019.101428

de la Hoz, A., Díaz-Ortiz, A, and Moreno, A. (2005). "Microwaves in organic synthesis. Thermal and non-thermal microwave effects," *Chem. Soc. Rev*. 34(2), 164-178. DOI: 10.1039/b411438h.

Deng, X., Huang, H., Huang, S., Yang, M., Wu, J., Ci, Z, He, Y., Wu, Z., Han, L., and Zhang, D. (2022). "Insight into the incredible effects of microwave heating: Driving changes in the structure, properties and functions of macromolecular nutrients in novel food. *Front Nutr*. 13(9), article 941527. DOI: 10.3389/fnut.2022.941527.

Devi, A., Singh, A., and Kothari, R. (2024). "Fungi based valorization of wheat straw and rice straw for cellulase and xylanase production," *Sustainable Chemistry for the Environment* 5, article 100077. DOI: 10.1016/j.scenv.2024.100077

Gadgil, N. J., Daginawala, H. F., Chakakrabarti, T., and Khanna, P. (1995). "Enhanced cellulose production by mutant of *Trichoderma reesei*," *Enzyme Microbial Technol*. 17(10), 942-946. DOI: 10.1016/0141-0229(94)00131-A

Garzillo, A. M., Colao, M. C., Buonocore, V., Oliva, R., Falcigno, L., Saviano, M., and Sannia, G. (2001). "Structural and kinetic characterization of native laccases from *Pleurotus ostreatus*, *Rigidoporus lignosus*, and *Trametes trogii*," *Journal of Protein Chemistry* 20, 191-201. DOI: 10.1023/A:1010954812955

Górny, R. L., Mainelis, G., Wlazlo, A., Niesler, A., Lis, D. O., Marzec, S., and Kasznia-Kocot, J. (2007). "Viability of fungal and actinomycetal spores after microwave radiation of building materials," *Annals of Agricultural and Environmental Medicine* 14(2), 313-324

Habinshuti, I., Mu, T.-H., and Zhang, M. (2020). "Ultrasound microwave-assisted enzymatic production and characterisation of antioxidant peptides from sweet potato protein," *Ultrason Sonochem* 69, article 105262. DOI: 10.1016/j.ultsonch.2020.105262

He, X., Duan, X., Gao, W., Zhang, Z., Gao, Y., Diao, H., and Luo, J. (2023). "Evaluation of the effect of tween-80 and its additive amount on the maturity of cassava residue compost," *Journal of Environmental Chemical Engineering* 11(5), article 110791. DOI: 10.1016/j.jece.2023.110791

Hermosilla, E., Schalchli, H., and Diez, M. C. (2020). "Biodegradation inducers to enhance wheat straw pretreatment by *Gloeophyllum trabeum* to second-generation ethanol production," *Environ Sci. Pollut. Res.* 27, 8467-8480. DOI: 10.1007/s11356-019-07460-5

Li, Q., Wang, J., Wang, Z., Zhang, W., Zhan, H., Xiao, T., and Zheng, Y. (2023). "Surfactants double the biodegradation rate of persistent polycyclic aromatic hydrocarbons (PAHs) by a white-rot fungus *Phanerochaete sordida*," *Environmental Earth Sciences* 82(12), article 285. DOI: 10.1007/s12665-023-10970-8

Li, S., Liu, Q., Liu, J., Sun, K., Yang, W., Si, Y., and Gao, Y. (2022). "Inhibition mechanisms of Fe2+/Fe3+ and Mn2+ on fungal laccase-enabled bisphenol a polyreaction," *Chemosphere* 307, article 135685. DOI: 10.1016/j.chemosphere.2022.135685

Li., X., and Gao, P. (1997). "Isolation and partial properties of cellulose decomposing strain of *Cytophaga* sp. LX-7 from soil," *J.Applied Microbiology* 82(1), 73-80. DOI:10.1111/j.1365-2672.1997.tb03299.x

Lowry, O. H., Rosebrough, N. J., Farr, A. L., and Randall, R. J. (1951). "Protein

measurement with the Folin phenol reagent," *J. Biol. Chem.* 193(1), 265-275.

Mahdi, W. M., Al-Badri, K. S. L., and Alqaisi, M. R. M. (2021). "Effect of microwave radiation on bacteria, fungi and some growth characteristics of cowpea *Vigna unguiculata* L.," *Gesunde Pflanzen* 73, 161-167. DOI:10.1007/s10343-020-00534-2

Mazumder, S., Laskar, D. D., Prajapati, D., and Roy, M. K. (2004). "Microwave induced enzyme-catalyzed chemoselective reduction of organic azides," *Chem Biodivers.* 1(6), 925-929. DOI: 10.1002/cbdv.200490074

Miller, G. L. (1959). "Use of dinitrosalicylic acid reagent for determination of reducing sugar," *Anal. Chem*. 31(3), 426-428. DOI: 10.1021/ac60147a030

Omoni, V. T., Ibeto, C. N., Lag-Brotons, A. J., Bankole, P. O., and Semple, K. T. (2022). "Impact of lignocellulosic waste-immobilised white-rot fungi on enhancing the development of 14C-phenanthrene catabolism in soil," *Science of The Total Environment* 811, article 152243.

Pham, L. T. M., Deng, K., Choudhary, H., Northen, T. R., Singer, S. W., Adams, P. D., Simmons, B. A., and Sale, K. L. (2024). "An engineered laccase from *Fomitiporia mediterranea* accelerates lignocellulose degradation," *Biomolecules* 14(3), article 324. DOI: 10.3390/biom14030324

Poonia, P. K., Deepa, S. R., Kumar, M., and Kumar, A. (2021). "Viability of wood decaying fungal mycelium after microwave radiation of bamboo culm," *Maderas. Cienciay Tecnologí* 23(4),1-6. DOI: 10.4067/s0718-221x2021000100404

Ren, H., Sun, W., Wang, Z., Fu, S., Zheng, Y., Song, B., and Peng, Z. (2020). "Enhancing the enzymatic saccharification of grain stillage by combining microwave-assisted hydrothermal irradiation and fungal pretreatment," *ACS Omega* 5(22), 12603-12614.

Reyes, C., Poulin, A., Nyström, G., Schwarze, F. W. M. R., and Ribera, J. (2021). "Enzyme activities of five white-rot fungi in the presence of nanocellulose," *Journal of Fungi* 7(3), 222. DOI: 10.3390/jof7030222

Sánchez, Ó. J., and Montoya, S. (2020). "Assessment of polysaccharide and biomass production from three white-rot fungi by solid-state fermentation using wood and agro-industrial residues: A kinetic approach," *Forests* 11(10), 1055. DOI: 10.3390/f11101055

Shankar, A., Saini, S., and Sharma, K. K. (2024). "Fungal-integrated second-generation lignocellulosic biorefinery: Utilization of agricultural biomass for co-production of lignocellulolytic enzymes, mushroom, fungal polysaccharides, and bioethanol," *Biomass Conversion and Biorefinery* 14(1), 1117-1131. DOI: 10.1007/s13399-022-02969-1

Shrestha, S., Chio, C., Khatiwada, J. R., Mokale, A. L., Chen, X., and Qin, W. (2023). "Optimization of cultural conditions for pectinase production by Streptomyces sp. and characterization of partially purified enzymes," *Microbial Physiology* 33(1), 12-26.

Sosa-Martínez, J. D., Montañez, J., Contreras-Esquivel, J. C., Balagurusamy, N., Gadi, S. K., and Morales-Oyervides, L. (2023). "Agroindustrial and food processing residues valorization for solid-state fermentation processes: A case for optimizing the co-production of hydrolytic enzymes," *Journal of Environmental Management* 347, article 119067. DOI: 10.1016/j.jenvman.2023.119067

Sun, F., Mukasekuru, M. R., Tan, L., Ren, J., Huang, Z., Ren, H., and Zhang, Z. (2018). "Optimization of on-site cellulase preparation for efficient hydrolysis of atmospheric glycerol organosolv pretreated wheat straw," *Journal of Chemical Technology and*

Biotechnology 93(8), 2083-2092. DOI: 10.1002/jctb.5605

Teodoro, T. S., Oliveira, F. D., Poffo, C., Braga, L. P., Arbigaus, A., Rampinelli, J. R., and Furlan, S. A. (2018). "The influence of Tween 80 on laccase production by *Pleurotus sajor-caju* and the efficiency of crude enzyme broth in the removal of bisphenol-A," *Arquivos do Instituto Biológico* 85, e1022017. DOI: 10.1590/1808-1657001022017

Tian, Q., Zhang, Y., Meng, D., Zhai, L., Shen, Y., You, C., and Liao, X. (2023). "Simultaneous removal of tetracycline and sulfamethoxazole by laccase-mediated oxidation and ferrate (VI) oxidation: The impact of mediators and metal ions," *Environ. Sci. Pollut. Res.* 30, 15708-15721. DOI: 10.1007/s11356-022-23232-0

Wang H., Peng L., Ding Z.Y., Wu J.Y., Shi G.Y. (2015). "Stimulated laccase production of *Pleurotus ferulae* JM301 fungus by *Rhodotorula mucilaginosa* yeast in co-culture,"*Process Biochem.* 50, 901-905. DOI: 10.1016/j.procbio.2015.03.004

Wang, F., Xu, L., Zhao, L., Ding, Z., Ma, H., and Terry N. (2019). "Fungal laccase production from lignocellulosic agricultural wastes by solid-state fermentation: A review," *Microorganisms* 7(12), article 665. DOI: 10.3390/microorganisms7120665

Wang, P., Hu, X., Cook, S., Begonia, M., Lee, K.S., and Hwang, H. M. (2008). "Effect of culture conditions on the production of ligninolytic enzymes by white rot fungi *Phanerochaete chrysosporium* (ATCC 20696) and separation of its lignin peroxidase," *World J. Microbiology and Biotechnol*. 24, 2205-2212. DOI: 10.1007/s11274-008-9731-5

Xu, C., Tong, S., Sun, L., and Gu, X. (2023). "Cellulase immobilization to enhance enzymatic hydrolysis of lignocellulosic biomass: An all-inclusive review," *Carbohydrate Polymers* 321, article 121319. DOI: 10.1016/j.carbpol.2023.121319

Yuan, J. J., Wang, C. Z., Ye, J. Z., Tao, R., and Zhang, Y. S. (2015). "Enzymatic hydrolysis of oleuropein from *Olea europea* (olive) leaf extract and antioxidant activities," *Molecules* 20(2), 2903-2921. DOI: 10.3390/molecules20022903

Zhang, J., Zhuo, X., Wang, Q., Ji, H., Chen, H., and Hao, H. (2023). "Effects of different nitrogen levels on lignocellulolytic enzyme production and gene expression under straw-state cultivation in *Stropharia rugosoannulata*," *International Journal of Molecular Sciences* 24(12), article 10089. DOI: 10.3390/ijms241210089

Zhu, S., Wu, Y., Yu, Z., Zhang, X., Li, H., and Gao, M. (2006). "The effect of microwave irradiation on enzymatic hydrolysis of rice straw," *Bioresource Technology* 97(15), 1964-1968. DOI: 10.1016/j.biortech.2005.08.008

Article submitted: August 1, 2024; Peer review completed: August 31, 2024; Revised version received: September 2, 2024; Accepted: September 8, 2024; Published: October 4, 2024.
DOI: 10.15376/biores.19.4.8882-8893