Ann. For. Res. 65(1): 8612-8625, 2022
ISSN: 18448135, 20652445

ANNALS OF FOREST RESEARCH
https://www.e-afr.org/

EXTRACTION AND OPTIMIZATION LIGNINOLYTIC ENZYMES LACCASE, LIGNIN PEROXIDASE AND MANGANESE PEROXIDASE FROM TERMITE

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ABSTRACT
This study was carried out in the Nutrition Laboratory / College of Agriculture - University of Baghdad. The aim of the study was to extract degrading enzymes from termites and to determine the activity of the enzymes Laccase (Lac), Lignin peroxidase (Lip) and Manganese peroxidase (Mnp)from termites. The effect of pH on enzyme activity was studied, at pH (3, 4, 5, 6, 7 and 8) were used to determine the optimal pH. The effect of temperature at the optimum pH for each enzyme was also studied, and different temperatures were chosen (25, 30, 35 , 40 and 45) to determine the best temperature for enzyme action. The best activity of the enzymes Lac, Lip and Mnp was at pH = 5, 7 and 4, respectively, and the optimum temperature was at 35, 40 and 35 ºC, respectively, for the aforementioned enzymes.
Keywords: Laccase; lignin peroxidase; Manganese peroxidase; Termite

1.Introduction
Enzymes are specialized proteins that act as biological catalysts for chemical reactions in living organisms. The action of enzymes is very important for life, as they provide energy, remove waste, and enzymes help organisms carry out their biological processes for their continuity (Hauer, 2020), and at present, many enzymes are produced by microorganisms, including bacteria, fungi and protozoa to another, such as Laccase was isolated from Bacillus subtilis ZHR (Hassan et al., 2018). Termites are of scientific and industrial importance due to their ability to degrade cellulosic biomass, the most abundant biopolymer on Earth that termites can feed on plant matter at different levels of water, including soil, wood, litter, lichen, etc. (Sánchez et al. .2021). Microorganisms are found in the soil (Hassan et al., 2018), in addition to termites, as well as in ruminants in the rumen in particular, which have the ability to break down. Lignocellulose is converted into sugars, which can then be used as a source of energy and carbon to produce various products. At present, research is focused on relying on bacteria to convert lignocelluloses into useful products (Chukwuma et al. 2021). Due to their ability and versatility, these organisms are able to grow on lignocellulosic substances and thus produce a wide range of enzymes that could be of scientific or industrial interest (Chaurasia et al., 2015). In addition, these enzymes are able to degrade phenolic compounds (Jasim et al., 2019). Termites have the ability to break down nucleocellulose. This

ability is based on its partnership with a diverse community of gut bacteria, prokaryotes, and eukaryotes, and has an important role in breaking down plant fibers and fermenting products into acetate and variable amounts of methane, using hydrogen as the central intermediate. In addition, depending on the enzymes secreted by these organisms are mostly peroxides (KUMAR et al,2020). Peroxides enzymes degrade lignocelluloses (Aziz et al .2018), and they are enzymes (fungal or bacterial) that are naturally secreted outside the cell associated with lignin degradation (Falade et al., 2017) Termites rely on biosynthetic capabilities to revive their gut as a food source. The high efficiency of the intestinal microorganism makes termites a promising model for the industrial conversion of knoscellulose into biological products and bioenergy production(KUMAR et al ,2020).Wood digestion by termites is much more efficient than less coarse forage grasses by ruminants (Charles et al. 2021). Currently, biological treatments (with enzymes) of some agricultural wastes are very necessary in order to decompose lignocellulose into lignin, cellulose and hemicellulose and to improve the crude protein content (Bennett et al., 2021) (Almaamory ,2016)(Yanti et al .2021). Any improvement in the nutritional value of feeds with a high content of feed and a high digestibility may lead to an increase in animal productivity in experiments studying the effect of adding enzymes to farm animal feed, and it also improves overall health. overweight. and improving the rumen environment (Almaamory et al., 2021). In this study, the objective was to identify Lac, Lip and Mnp enzymes in termites.

2.Materials and methods

Chemicals such as Guaiacol from BDH Chemicals Ltd pool England were used as the basis and efficient material for the production of the enzyme Laccase and Manganese peroxidase, and 3,4-Dimethoxybenzyl alcohol from (MACKLIN) was used as the base and efficient material for the production of the enzyme Lignin peroxidase.

2.1. living organisms (termites)

Termites were obtained from Diyala orchards and then washed with distilled water and homogenized in a sodium acetate buffer solution at a concentration of 0.01 M and pH = 5. The solution contained 1 mmol of diethylene tetraacetic EDTA, then the insects were mashed with an electric blender. The centrifugation process was carried out at a speed of 10000 cycle/min for 10 minutes the filtrate is taken. The enzyme is extracted if it is filtered using cotton, followed by a second filtration step using filter paper, and the extract that represents the crude enzyme extract that determines its size, effectiveness and protein concentration in it. And keep in the refrigerator until use.

2.2 Protein estimation

The protein concentration was estimated according to the Lowry method modified by Cooper (1977) (Al-Ani, 2005).

2.3 Estimation of the efficacy of the extracted enzymes

2.3.1. Determination of the activity of Laccase enzyme:

The determination of the activity of the Laccase enzyme was carried out using the Guaiacoi Assay Method described by (Desai and Nityanand, 2011) as the basic and efficient material for the production of the enzyme. The reaction mixture was prepared.

1- ml of a solution of Guaiacol at a concentration of 0.002 M, which was prepared by adding 0.2482 g / liter of Guaiacol in a liter of distilled water.

2- Add 3 ml of Sodium Acetate with pH 5 at a concentration of 0.01 M.

3- ml of enzyme filtrate.

4- As for the control treatment (Blank), the reaction mixture is made of the same components above, with the replacement of the termite's intestinal enzyme filtrate with sterile distilled water. the mixture was incubated at 30 °C for 15 min, and then the enzymatic activity was measured by a spectrophotometer with a wavelength of 450 nm. Quaicol per minute Under ideal conditions, the enzymatic activity in each unit/ml was calculated using the following law (Vantamuri. Kaliwal, 2016) and the extinction value used = 6740 M-1Cm (vantamuri, Kaliwal, 2019):

$A=(A \times V)/(t \times e\times v)$

E.A represents the enzyme activity in (U/Ml).

A represents the absorbance at the wavelength nm

V represents the total volume of the reaction mixture in (ml).

t represents the incubation time of the reaction mixture in minutes (min).

e is the depreciation factor.

v is the volume of the leachate (ml).

2.3.2. Determination of the activity of lignin peroxidase enzyme

1- The activity of Lignin peroxidase enzyme was estimated by using the reaction substance 3,4-Dimethoxybenzyl alcohol (Astuti et al., 2021).

2- Where 0.1 ml of the reaction material was used at a concentration of 0.008M, it was prepared by adding 1.3455 g/L of distilled water.

3- 0.05 ml of H_2O_2 at a concentration of (0.005) molar was added, 0.2 ml of Acetat buffer solution was added at pH 3, 0.4 ml of distilled water was added with the addition of 0.2 ml of enzyme filtration solution. The solutions were shaken well, and then the enzymatic activity was measured by a spectrophotometer at a wavelength of 310 nm with an interval. Time 0 and 30 minutes.

4- As for the control treatment (Blank), the reaction mixture is made from the same components above, with the replacement of the termites intestinal enzyme filtrate with sterile distilled water.

5- The enzyme activity was estimated according to the previous equation and the extinction value used = 9300 M-1Cm (Peng et al., 2014).

2.3.3. Determination of the activity of Manganese peroxidase enzyme

1- The activity of Manganese peroxidase enzyme was estimated using Guaiaco as a base material described by (Astuti et al., 2021).

2- 0.1 ml of N-Lactate at a concentration of (0.05)M was used, which was prepared by adding 4.5 gm of lactate solution to NaOH at an amount of 2 g/litre distilled at pH = 5.
3- Add 0.1 ml of Guaiacol at a concentration of (0.04) M.
4- 0.2M of MnSO4 was added at a concentration of (0.0019) M, which was prepared by adding 0.16902 g/L of distilled water.
5- 0.1 ml of H2O2 at a concentration of (0.001) M.
6- Mix with 0.2 (ml) of the enzyme filter with the addition of 0.3 (ml) of distilled water. The solutions were shaken well, and then the enzymatic activity was measured by a spectrophotometer at a wavelength of 465 nm with an interval of 0 and 30 min. The enzyme activity was estimated according to the previous equation and the extinction value used = 12100 m-1 cm (Patrick et al. 2010).
As for the control treatment (Blank), it is a reaction mixture of the same components above, taking into account the replacement of the termites' intestinal enzyme filtrate with sterile distilled water.

2.4. Determination of optimal conditions for the effectiveness of enzymes

The effect of pH and temperature was studied in order to determine the optimal conditions for the work of these enzymes.

2.4.1. Determine the optimum pH

The effect of pH on enzyme activity was studied, as pH levels (3, 4, 5, 6, 7 and 8) were used to determine the optimal pH for the activity of each of the three enzymes.

2.4.2. optimum temperature

The effect of heat was studied at the pH for each enzyme and different temperatures (25, 30, 35, 40 and 45) were chosen to determine the best temperature for enzyme action.

3. Results and discussion

3.1. pH

The pH directly affects the activity of exogenous enzymes and the metabolism of the producing organisms. We note in Figure (1) that the best activity of the enzyme Laccase at pH 5, which was 0.023 units/ml, was mentioned (Yang et al. 2020) that the original activity of the Laccase activity (Lac 37 II) is stable, and more than 80% is maintained, at pH 4 and 5 after incubation for 36 hours when produced from Trametes trogii S0301, a white mold fungus, as shown in the results of Yin et al (2019), that The enzyme retained about 60% of the original activity at a pH level of 5.0 to 8.0. In addition to the maximum activity observed at pH 5.0 (Sharma and Leong, 2021), the enzyme produced by bacteria was found to be more stable at pH 5.0 and relatively stable towards acidic or alkaline conditions, since most Lacs exhibit an ideal pH at numbers between 3- 5.5 on phenol substrates because it rapidly loses its activity at pH higher than 7 (Xu et al., 1998).

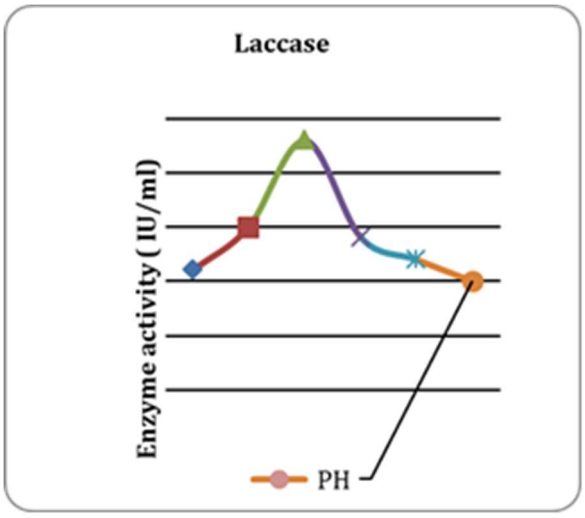

Figure 1 Laccase activity at different pH values. This figure isfor pH optima of enzyme. Enzyme activity was assayed atdifferent pH. The optimum pH of enzyme was found at 5. Otherassay conditions such as incubation period, substrate concentra-tion, was kept constant.

We note in Figure (2) that the maximum activity of lignin peroxidase is pH = 7, which amounted to 0.011 units/ml, while the activity of lignin peroxidase decreased at pH 8. 6.5 Extracted from the intestines of termites.

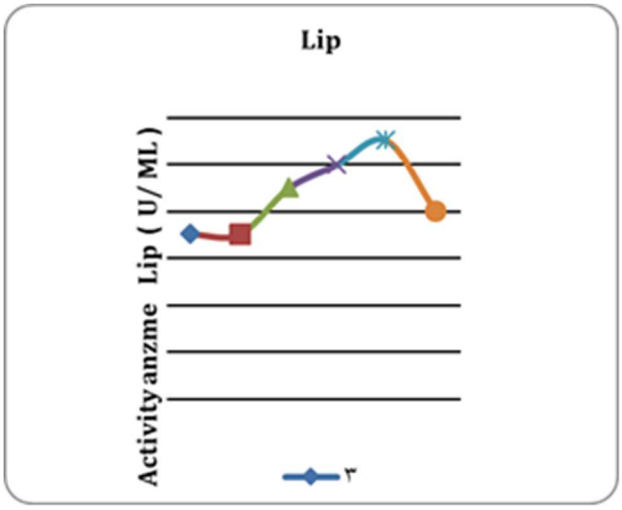

Figure2 lignin peroxidase activity at different pH values. This figure is for pH optima of enzyme. Enzyme activity was assayed atdifferent pH. The optimum pH of enzyme was found at 7. Otherassay conditions such as incubation period, substrate concentra-tion, was kept constant.

Figure (3) shows that the optimum pH for the activity of Manganese peroxidase is pH = 4, which has a value of 0.07 units/ml. These results agreed with Huy et al. (2017) In an acidic medium around 4.0 MnP showed high activity, and the results also indicate that MnP is highly sensitive to

pH. The enzyme activity decreased rapidly when the pH was less than 5.0 and greater than 3.0, and the retained activity was 60% smaller, especially at pH 6 (Hariharan and Padma, 2013).

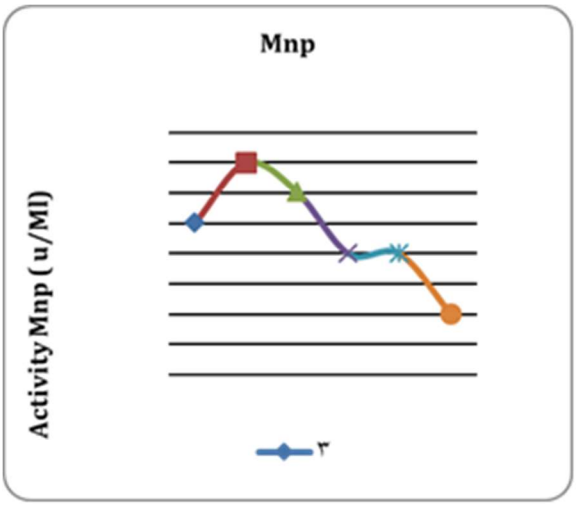

Figure3 Manganese peroxidase activity at different pH values. This figure isfor pH optima of enzyme. Enzyme activity was assayed atdifferent pH. The optimum pH of enzyme was found at 4. Otherassay conditions such as incubation period, substrate concentra-tion, was kept constant.

In addition to the foregoing, the presence of more than one type or image of the enzyme produced by the organism under study may cause such fluctuations, up and down, and the optimum pH for production varies according to the lignocellulase of the microorganism adopted in production (Al-Ani, 2005) and this fluctuation may also be due to The effect of pH on the solubility of salts as well as its influence on the ionic state of the reaction materials and then on their readiness for the microorganism (Berry, 1975).

3.2. temperature

Temperature influences the determination of the activity of different microorganisms, especially the growth and vital activities of organisms, and thus it is a basic means to control both the bio-building and demolition activities of these organisms. It has been observed that thermally active lacase enzymes are in heat-loving or heat-tolerant strains (Yang et al., 2020).), we note in the graph Figure (4) after determining the optimum pH of the Laccase enzyme that the optimum temperature for measuring the activity of the Laccase enzyme is at 35 ºC, as the effectiveness reached 0.016 units/ml and it was reduced at other temperatures, as the temperature difference returns Optimum Laccase enzyme to different strains and the nature of its presence (Narayanan et al., 2015). This is what Sura found when producing the Laccase enzyme from Trichoderma harzianum produced (Sura et al ,2022) .

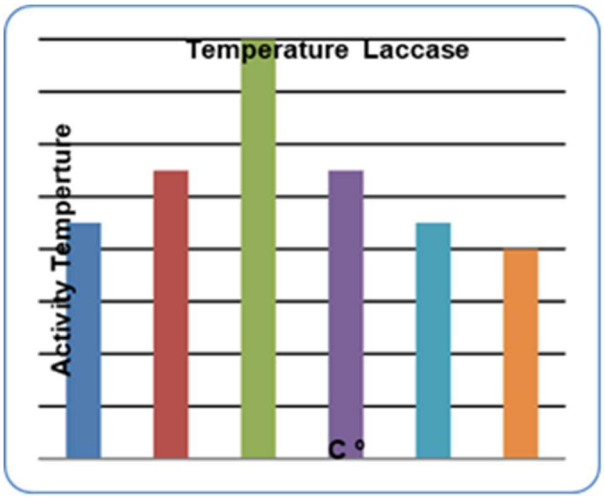

Figure 4 Laccase activity at different temperatures. The enzymeactivity was determined at different temperatures by keeping allother conditions of enzyme assay constant. The was measured at 35 °C

We note in Figure (5) that the activity of lignin peroxidase produced by the organisms in the insect is affected by temperature. The optimum temperature was at 35-40 °C and with an efficiency of 0.012 units/ml for both degrees, while the enzyme activity decreased at other temperatures and this agrees with the results of (Asgher et al., 2007) who stated that the ideal temperature for the lignin peroxidase enzyme model is 40 Celsius. Use veratril alcohol as a substrate. The results of bacterial and fungal isolates from the gut of termites showed that they could be potential sources of lignin peroxidase. This microorganism can also be used as a potential source for commercial enzyme production for application in various industries such as food, paper production, cosmetic production, biotechnology and other chemical industries (Egwim et al., 2015).

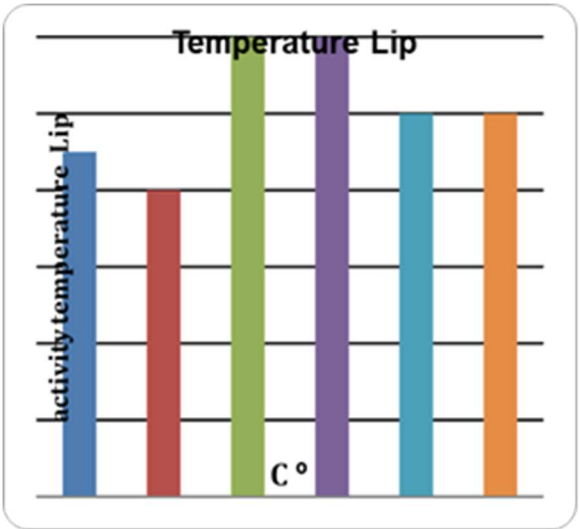

Figure 5 lignin peroxidase activity at different temperatures. The enzymeactivity was determined at different temperatures by keeping allother conditions of enzyme assay constant. The was measured at 35 °C

Figure (6) shows that the optimum temperature for measuring the activity of the manganese peroxidase enzyme is at 35 ºC, as the activity was 0.012 units/ml compared with the rest of the temperatures. These results agreed with Huy et al. (2017) that MnP activity increased significantly when the temperature was raised from 20 °C and reached its highest activity at 35 °C. The enzyme activity decreased with increasing temperature. The retained MnP activity was 30% when the reaction temperature was 50 °C.

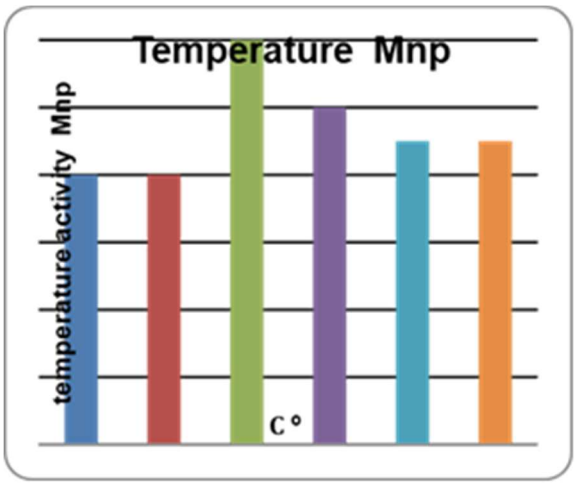

Figure 6 manganese peroxidase activity at different temperatures. The enzymeactivity was determined at different temperatures by keeping allother conditions of enzyme assay constant. The was measured at 35 °C .

The results show that microorganisms can withstand and coexist with all difficult environmental conditions, such as salinity, temperature, pressure difference, increase or decrease in pH, that is, according to the conditions of the environment in which the organism lives.

Conclusions

Most of the organisms have developed their ways of living to suit their living conditions, such as termites adapted to the microorganisms that inhabit the intestines of this insect, so it is necessary to study and detect the microbes of this insect because it has interesting enzymes that degrade biomass lignocellulose, which can be used to enhance the ability of decomposition Aqueous fetolytic enzymes. And because of the development of biotechnology, which can be harnessed to uncover untapped opportunities.

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