

Decolorization and Phenol Removal of Palm Oil Mill Effluent by Termite-Associated Yeast

P. Chaijak, M. Lertworapreecha, C. Sukkasem

Abstract—A huge of dark color palm oil mill effluent (POME) cannot pass the discharge standard. It has been identified as the major contributor to the pollution load into ground water. Here, lignin-degrading yeast isolated from a termite nest was tested to treat the POME. Its lignin-degrading and decolorizing ability was determined. The result illustrated that *Galactomyces* sp. was successfully grown in POME. The decolorizing test demonstrated that 40% of *Galactomyces* sp. could reduce the color of POME (50% v/v) about 74-75% in 5 days without nutrient supplement. The result suggested that *G. reessii* has a potential to apply for decolorizing the dark wastewater like POME and other industrial wastewaters.

Keywords—Decolorization, palm oil mill effluent, ligninolytic enzyme, yeast, termite.

I. INTRODUCTION

CURRENTLY, the decreasing amount of fossil fuel is a significant concern for the global energy situation. As a result, the oil industry has been looking for alternative sources of fuel such as from renewable energy sources, including biodiesel from crude palm oil. The crude oil from oil palm (*Elaeis guineensis*) is more interesting than other oil crops like soybean and rapeseed because oil palm provides a higher yield and consumes less energy than the other crops [11]. From the crude palm oil extraction process, about 0.6 tons of highly-colored POME is released from every ton of fresh fruit bunches [9]. The raw effluent palm oil mill is composed of 95-96% colloidal suspension in water, 4-5% total solids and about 0.6-0.7% oil [6]. The dark color of POME is caused by lignin and its degraded products under anaerobic treatment such as tannin [8].

The treatment of effluent by bacteria, both aerobic and anaerobic treatment systems, can reduce the biological oxygen demand (BOD), but cannot remove the dark brown color. Some physical and chemical methods have been used to address this issue, but at high cost per unit volume of wastewater [5]. Thus, biological decolorization could prove to be a more cost-effective method.

Biological catalysts such as ligninolytic enzymes from lignin-degrading microorganisms play significant roles in the decolorization of dark brown effluent through their enzyme systems, such as extracellular laccase, manganese dioxide and

xylanase [5]. Nowadays, the biological treatment of colored POME by lignin-degrading fungi such as *Aspergillus fumigatus* and *Curvularia clavata* is more interesting than other methods because of its low cost per unit volume of wastewater and non-environmental impact [3], [4], [9].

Insects are the hugely successful species on the world that can survive in diverse habitats. The termites associated microorganisms play important roles in biomass digestion, nitrogen cycle and carbon mineralization. Insects possess a dual lignocellulolytic system; for example, in lower termites, enzymes are contributed by both the insect and its gut microbes [2]. In this study, an evaluation was conducted on the ability of lignin-degrading yeast *Galactomyces* sp. isolated from the nest of termites for efficient decolorization of POME and examined the lignin degrading enzyme of an isolate. Furthermore, the effect of incubation time, culture concentration and effluent concentration on color removal efficiency for this organism was investigated.

II. EXPERIMENTAL DETAILS

A. Effluent Source

Highly-colored POME was collected from the oil palm industry in Trang Province, located in southern Thailand. The effluent was collected in sterile plastic bottles and filtered through filter paper Whatman number 1 to remove suspended particles. The filtered effluent samples were stored at 4 ± 1 °C until use.

B. Microorganism and Culture Condition

Galactomyces sp. (IFO 10823) was obtained from the laboratory at Thaksin University, Phatthalung Campus, Thailand. Cultures were grown in malt extract broth (malt extract 20 g/L, sucrose 20 g/L, peptone 6 g/L) supplemented with 10 g/L of rice bran under aerobic conditions at 30 °C with shaking at 200 rpm for 7 days. After that, the cultures were stored at 4 °C for further studies.

C. Determination of Lignin Degrading Activity

Galactomyces sp. was inoculated in a modified malt extract broth (20 g/L of malt extract, 20 g/L of dextrose, 6 g/L of peptone, 10 g/L of rice husk added with 3% of CaCO_3). Culture was grown at 30 °C with shaking at 200 rpm for 5 days and then measured for laccase activity. To prepare the crude enzyme, the culture media was centrifuged at 9,000 g for 5 min to remove suspended particles. The supernatant was filtered using filter paper and the filtered culture media was stored at 4 °C until use.

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Laccase activity was determined spectrophotometrically at 436 nm using an ABTS assay. The assay mixture contained 1 mM ABTS in 100 mM sodium acetate buffer (pH 3.6) mixed with 50 μ L aliquots of crude enzyme in a total volume of 1 mL [1]. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μ mole of oxidized ABTS per min.

Manganese peroxidase activity was estimated at 415 nm using ABTS as a substrate by spectrophotometry [7]. One unit of enzyme activity was defined as the amount of enzyme required to produce 1 μ mole of oxidized ABTS per min.

D. Screening of the Growing in POME

The ability of *Galactomyces* sp. to grow in POME was determined with POME broth (1 g/L of malt extract, 1 g/L of dextrose, 0.3/L g of peptone, and 10% of POME). The culture was inoculated into POME agar and incubated at 30 °C for 5 days, with cell growth monitored by spectrophotometry at 600 nm.

E. Color Measurement

Color concentration of the phenolic compound [7] was measured using a UV/vis spectrophotometry (Shimadzu UV mini 1240) at 765 nm. The color removal efficiency R (%) after the process was calculated using (1):

$$R(\%) = \frac{C_0 - C}{C} \times 100 \quad (1)$$

where C_0 and C are the absorbance of POME wastewater before and after the process, respectively.

F. Decolorization in Raw POME

For raw POME preparation, the filtered POME was diluted with sterile distilled water and then stored at 4 °C until use.

For inoculum preparation, *Galactomyces* sp. was grown for 7 days at 30 °C in a 250 mL Erlenmeyer flask containing 100 mL of malt extract broth. After 7 days of growth, the inoculum was transferred into a 50 mL plastic tube containing non-culture filtered POME with various incubation times (1, 2, 3, 4 and 5 days), then incubated at 30°C with shaking at 200 rpm in order to study the effect of time.

The effect of wastewater concentration was studied by various POME concentrations (50, 60, 70, 80, 90 and 100% v/v) incubated at 30 °C for 5 days with shaking at 200 rpm.

The effect of culture concentration was determined by various culture concentrations (10, 20, 30, 40 and 50% w/v) with 100% v/v of POME and incubation at 30 °C for 5 days with shaking at 200 rpm.

III. RESULT AND DISCUSSION

A. Lignin Degrading Activity

Ligninolytic microorganisms have high potential for the decolorization of industrial wastewater through the function of extracellular enzymes such as laccases and manganese peroxidases [5]. In this study, *Galactomyces* sp. was cultured in enzyme production media containing with rice husk as natural inducer for ligninolytic enzyme production for 5 days

at 30 °C with shaking. The data of the enzyme activity showed that *Galactomyces* sp. could produce extracellular laccase and manganese peroxidase at 84.78 U/mL and 60.0 U/mL, respectively (Fig. 1). On the other hand, *G. geotrichum* could produce manganese peroxidase at 136.25 U/mL and laccase at 0.135 U/mL, but this method was performed over 16 days [10]. Thus, the result showed *Galactomyces* sp. is suitable inoculum for extracellular laccase and manganese peroxidase production from simple media using rice husk as natural inducer.

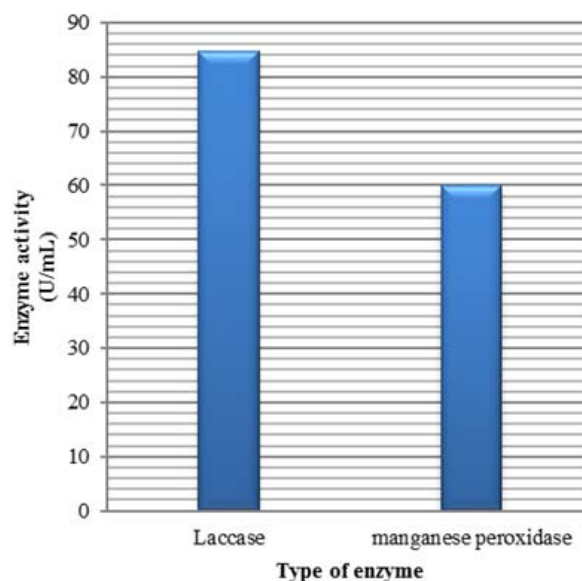


Fig. 1 Ligninolytic enzyme activity of *Galactomyces* sp. in modified malt extract broth

B. Growth in POME Broth

In primary screening for the growth ability of *Galactomyces* sp. in POME, the culture was transferred into the POME broth after incubation. The result showed that the culture could grow in POME broth (Fig. 2). Indirect cell counting by spectrophotometry was used for the monitoring of cell growth, which showed an increase of 8.17×10^6 cells/mL after 5 days of culturing.



Fig. 2 *G. reessii* grown in POME broth at 30 °C

C. Decolorization of Effluent

The data of incubation times have shown that dark brown color in POME decreased at 75.06% (Fig. 3) after inoculating

the culture in POME and incubation for 4 days, higher than decolorization by *Aspergillus fumigatus* [3]. Moreover, the data showed that *Galactomyces* sp. can grow in raw POME without culture media. No previous study reported about decolorization of POME by *Galactomyces* sp.

The data of culture concentration have found that the maximal removal efficiency was shown when the wastewater was inoculated by 40% of culture concentration (Fig. 4).

The study of the effect of wastewater concentration showed that the maximal activity of decolorization in POME showed at 50% wastewater concentration (Fig. 5). Another study reported that the decolorization of toxic dye by *G. geotrichum* (MTCC 1360) showed increasing the culture concentration decreased the time period required for treatment, whereas increasing the concentration of highly-colored wastewater increased the time required for treatment [12].

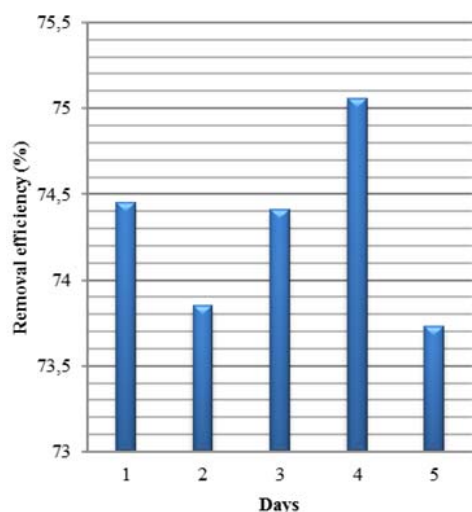
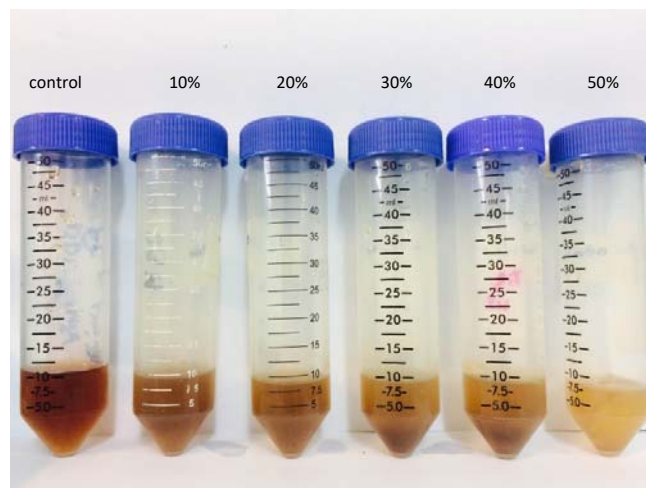


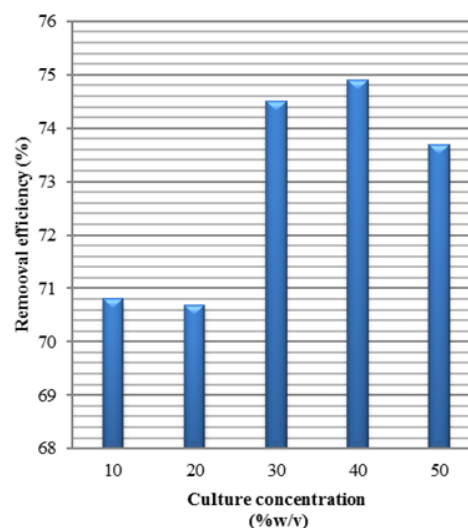
Fig. 3 Effect of incubation times for the removal efficiency (%) of phenolic compound in POME by *Galactomyces* sp. at 30 °C

IV. CONCLUSION

This study has revealed significant knowledge regarding the ligninolytic activity of termite-associated yeast and their roles in decolorization of POME. The results indicate that *Galactomyces* sp. has the potential to be an efficient strain for the decolorization of POME based on extracellular enzyme such as laccase and manganese peroxidase, which might be responsible for its effluent decolorization ability. Research on the effect of incubation time indicated 4 days as the most suitable period for POME treatment. Further, study of the effect of culture and wastewater concentration showed that an increasing culture concentration decreased the period of time required for treatment, whereas an increasing of wastewater concentration increased the time required for treatment. The knowledge gained from these studies could be exploited for the development of enzyme system and the wastewater treatment conditions for effective decolorization of POME.

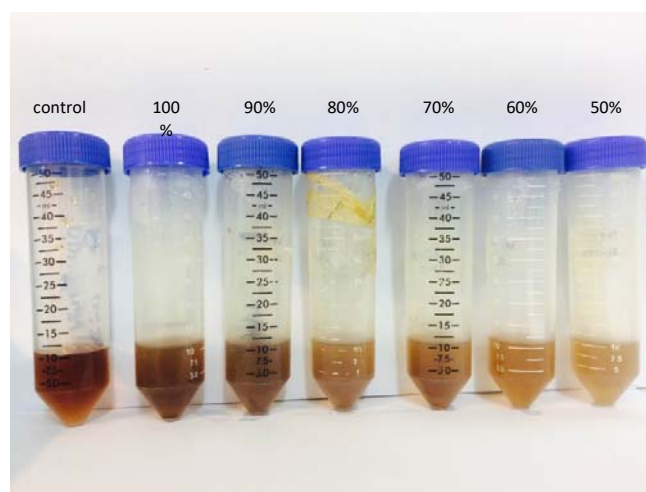


(a)

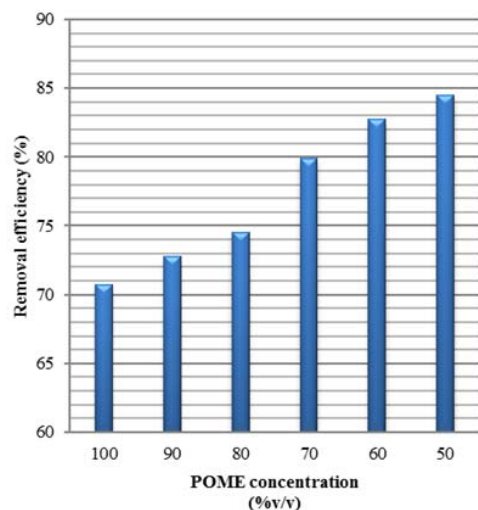


(b)

Fig. 4 (a) The color removal in POME at various culture concentrations; (b) The removal efficiency (%) of phenolic compound in POME by *Galactomyces* sp. at 30 °C



(a)



(b)

Fig. 5 (a) The color removal in POME at various wastewater concentrations; (b) The removal efficiency (%) of phenolic compound in POME by *Galactomyces* sp. at 30 °C

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REFERENCES

- [1] B. Bertrand, F. Martinez-Morales, R. Tinoco-Valencia, S. Rojas, L. Acosta-Urdapilleta, M.R. Trejo-Hernandez, 2015. Biochemical and molecular characterization of laccase isoforms produced by white-rot fungus *Trametes versicolor* under submerged culture conditions. *Journal of Molecular Catalysis B: enzymatic* 122, 339-347.
- [2] A. Brune, and M. Ohkuma, 2010. Role of the Termite Gut Microbiota in Symbiotic Digestion. In *Biology of Termites: a Modern Synthesis*, D.E. Bignell, Y. Roisin, and N. Lo, ed. (United State of America: Springer), 439-475.
- [3] C.H. Neoh, A. Yahya, R. Adnan, Z.A. Majid, and Z. Ibrahim, 2013. Optimization of decolorization of palm oil mill effluent (POME) by growing cultures of *Aspergillus fumigatus* using response surface methodology. *Environmental Science and Pollution Research* 20, 2912-2923.
- [4] C.H. Neoh, C.Y. Lam, C.K. Lim, A. Yahya, and Z. Ibrahim, 2014. Decolorization of palm oil mill effluent using growing cultures of *Curvularia clavata*. *Environmental Science and Pollution Research* 21, 4397-4408.
- [5] D.K. Sahoo, R. Gupta, 2005. Evaluation of ligninolytic microorganisms for efficient decolorization of a small pulp and paper mill effluent. *Process biochemistry* 40, 1573-1578.
- [6] J.C. Igwe, C.O. Onyegbado, and A.A. Abia, 2010. Studies on the kinetics and intraparticle diffusivities of BOD, colour and TSS reduction from palm oil mill effluent (POME) using boiler fly ash. *African Journal of Environmental Science and Technology* 4, 392-400.
- [7] M.H. Gold, J.K. Glenn, 1988. Manganese peroxidase of *Phanerochaete chrysosporium*. *Methods in Enzymology* 161, 254-264.
- [8] N. Oswal, P.M. Sarma, S.S. Zinjarde, and A. Pant, 2002. Palm oil mill effluent treatment by a tropical marine yeast. *Bioresource Technology* 85, 35-37.
- [9] R.R. Mohammed, 2013. Decolorisation of Biologically Treated Palm Oil Mill Effluent (POME) Using Adsorption Technique. *International Refereed Journal of Engineering and Science (IRJES)* 2, 1-11.
- [10] R. Varnaite, V. Raudoniene, 2008. Lignin degradation in plant remnants under liquid-phase fermentation conditions. *Biologija* 54, 183-186.
- [11] S. Mekhilef, S. Siga, and R. Saidur, 2011. A review on palm oil biodiesel as a source of renewable fuel. *Renewable and Sustainable Energy Reviews* 15, 1937-1949.
- [12] S.U. Jadhav, G.S. Ghodake, A.A. Telke, D.P. Tamboli and S.P. Govindwar, 2009. Degradation and Detoxification of Disperse Dye Scarlet RR by *Galactomyces geotrichum* MTCC 1360. *Journal of Microbiology and Biotechnology* 19, 409-415.