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Methylene blue decolorization fungi from crude oil contaminated soils

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Abstract. *Permana I, Awaluddin A, Saryono. 2019. Methylene blue decolorization fungi from crude oil-contaminated soils. Biodiversitas 20: 2693-2697.* One of the world problems is the treatment to remove dyes in textile wastewater. Nowadays, the most effective and compatible method to the environment is decolorization by microorganisms. Therefore, the objective of this study is to find out the best fungi to decolorize synthetic dye methylene blue (MB). Total eight fungi that isolated from crude oil-contaminated soil were analyzed to study the effect of various parameters such as pH, the concentration of dyes, contact times and agitation on their rate of reaction. The initial screening showed that *Penicillium* sp. FTM7 had greater potential in biodegradation the MB. The optimum decolorization of MB by *Penicillium* sp. was found after 8 days incubation with agitation 150 rpm and concentration of MB is 40 ppm and unadjusted pH (95.45%). The decolorization of MB was found to follow first-order reactions.

Keywords: Biodegradation, fungi, methylene blue

INTRODUCTION

Due to rapid industrialization and urbanization, a lot of chemicals including dyes are manufactured and are being used in almost every day. Industries worldwide have witnessed tremendous growth over the years in the use of synthetic dyes. Synthetic dyes are widely used by several industries such as textiles, leather, cosmetics, paper, printing materials and plastics. About 280,000 tons of textile dyes are discharged in industrial effluents every year. There are 20-30% unfixed reactive dyes applied, with an average concentration of about 2,000 ppm. The release of these pollutants into the environment is undesirable due to the serious environmental problems linked with the dyes and their breakdown products. The most contaminants applied in the industry is azo dyes. These dyes are complex chemical structure confers on them the ability to remain recalcitrant to degradation in water and soil, even possess carcinogenic properties due to their azo bond (-N=N-). It has also had a negative impact to water environment like depletion of dissolved oxygen (DO), also cause an adverse impact on total organic carbon (TOC), biological oxygen demand (BOD) and chemical oxygen demand (COD) (Kumar et al. 2016). Among of dyes, methylene blue (MB) includes the dye classification of thiazine. MB is cationic dye as it forms positively charged molecules when dissolved in water. In another application MB also used as medicine. Some diseases that use MB in treatment are Duck hepatitis B and Psoriasis. Also, there is negative effect occurs when contact with MB. It may cause increased heart rate, nausea, Heinz body formation, headache and gastritis. MB resistant to degraded by environmental activities and cause unpleasant sight to the water bodies due to their intense color (Dahri et al. 2015).

Dyes in the environment are always difficult to degrade or decolorize by many known chemical and physical methods. Currently, the physical and chemical treatments are available and applicable to remove dyes from wastewater. Such as adsorption, coagulation, and membrane process, electrochemical methods, advanced oxidation processes (photochemical and photo-catalytic process). Unfortunately, several of them have disadvantages such as use more energy, expensive cost, limited applicability, cause secondary pollution problems in the form of sludge after treatment and toxic reagent also employed (Yang et al. 2016). Among these methods, degradation decolorization by a biological method is more interesting due to low energy cost, inexpensive, specificity, producing less amount of sludge, ease of control and environmental friendly process (Shanmugam and Ulaganathan 2017).

Many studies reported had great results due to the degradation of synthetic dye by biological methods. Research on the fungal degradation of dye has been performed in recent years. Several fungi with the capability to degrade dyes have been reported. For example, *Peyronellaea prosopidis* was able to degrade Scarlet RR dye (Bankole et al. 2018), *Aspergillus niger* had maximum degradation of the dye basic fuchsin, *Phanerochaete chrysosporium* had maximum degradation of the dye Nigrosin (Rani et al. 2014), *Coriolopsis* sp. even had ability to degrade 4 kinds of azo dyes (ponceau 2R, orange G, direct blue and biebrich scarlet) (Cheng et al. 2016), *Aspergillus flavus* and *Penicillium canescens* were the best active fungal species for degradation of direct blue dye (Hefnawy et al. 2017), *Aspergillus terreus* had ability to degrade the Methylene blue and Congo Red (Ramamurthy et al. 2013), *Trichoderma harzianum* was able to degrade triphenylmethane Cresol Red (Nor et al. 2015), *Pleurotus ostreatus* was able to degrade MB in liquid and solid media (Menezes et al. 2017). Base on the previous studies it was reported that fungi isolated from soil contaminated by

crude oil had great potential for degradation of crude oil waste. Generally, these fungi had extracellular enzyme (i.e., laccase, lignin peroxidase and manganese peroxidase) that able to degrade such big and complex aromatic compound in crude oil (Sari 2017). White-rot fungus had reported that to have ability degrade dyes in textile effluent and the enzymes that play important roles in the biodegradation process are laccase, manganese peroxidase and lignin peroxidase (Mustafa et al. 2017).

Accordingly, the objective of this study is to evaluate the biodegradation of Methylene Blue by fungi that isolated from soil contaminated by crude oil.

MATERIALS AND METHODS

Materials

Potato Dextrose Agar (PDA; 20% potato, 2% dextrose and 2 % agar) medium was used to grow the fungal cultures. Potato Dextrose Broth (PDB; 20% potato, 2% dextrose) used for liquid fungal culture (Saryono et al. 2016). Dye-containing PDA was prepared with PDA medium and the addition of MB solution to reach concentration 1,000 mg/L. The chemicals used were NaOH 0.5N, HCl 0.5 N, aquadest (neutral pH), ethanol 70%, and methylene blue dye that was purchased from Merck and used without further purification.

Microorganisms

The fungi used in this study were isolated previous study by Sari (2017). Pure culture of each fungus isolated was maintained at Biochemical Laboratory UR. Eight fungi namely *Fusarium* sp. (FTM1), *Penicillium* sp. (FTM2), *Trichoderma* sp. (FTM3), *Penicillium* sp. (FTM4), *Aspergillus* sp. (FTM5), *Aspergillus* sp. (FTM6), *Penicillium* sp. (FTM7), *Aspergillus* sp. (FTM8). Slants of these fungi were in the test tubes with PDA medium and kept for further use.

Procedures

Calibration of dye solution

The dye solutions were initially calibrated for concentration in terms of absorbance units. Each of the standard dye solution (100 mg/L) was diluted with distilled water to concentrations of 0, 1.0, 2.0, 4.0, 6.0, 8.0 and 10.0 mg/L, respectively. Each concentration of solutions was measured for its absorbance value at its corresponding λ_{max}. The absorbance values versus concentrations were then plotted.

Initial screening

A mycelium disc from the fungi strains that grown on PDA medium plates after 2 days at room temperature was inoculated into the center of the petri dish that filled by dye-containing PDA medium. The plates were incubated at room temperature for 7 days. The ability of the fungi to degrade the MB was determined by the outside diameter of clear zone in PDA medium (mm). The fungi strain giving maximum outside diameter of the clear zone was selected and used for further experiments.

Experimental method

The fungi that showed a decolorized zone of strain on the solid media were taken out for next testing in liquid media. Degradation of MB dye was carried out under 150 rpm agitation condition with 100 mL culture of fungi pre-grown on PDB. The dye (1,000 mg/L) was added into each 250 mL Erlenmeyer flask containing 100 mL of individual pre-grown cultures of fungi and further incubated until degradation was observed in 0 until 8 days. About 5 mL aliquots of the culture supernatant were withdrawn at regular time intervals during the process of degradation. A 3,000 rpm centrifugation for 15 minutes was carried out to separate the fungi cell mass (Bankole et al. 2018). The degradation was monitored by measuring the change in absorbance maximum of the dye (λ_{max} methylene blue was 655 nm) using a spectrophotometer Thermo Scientific Genesys 10S UV-Vis. The percentage of degradation was calculated using the following formula:

$$\%\ Degradation = \frac{Initial\ absorbance - final\ absorbance}{Initial\ absorbance} \times 100$$

The effect of various physicochemical conditions such as pH (4.0, 6.0, 8.0 and 10.0), contact time (2, 4, 6 and 8 days), agitation (0, 100 and 150 rpm) and MB initial concentration (20, 40, 60, 80 and 100 ppm) by the isolate were also studied.

RESULTS AND DISCUSSION

Initial screening

The biodegradation capability of eight fungi that isolated from crude oil-contaminated soil was assessed on dye-containing PDA plates with synthetic dyes inoculated at room temperature for one week. The dye biodegradation results on agar plates showed that three fungi strains had high abilities to decolorize MB. These fungi were FTM2, FTM6 and FTM7 had potential to decolorize MB (Figure 1).

Biodegradation of MB by fungi

The effect of various physicochemical conditions like contact time, initial concentration, agitation and pH on degradation of MB was studied in detail. Effect of contact time on the removal of dye MB is shown in Figure 3. The dye removal process begins at 0 days. Generally, degradation of MB by all fungi sharply increased by 0 days and it gradually increased until it reached 94.20% at 8 days. Increasing the initial concentration provides more driving force to overcome the mass transfer resistance between the solid phase and the liquid phase (Dahri et al. 2015). Figure 4. shown the effect of agitation towards ability of FTM7 to degrade MB. It is clear that fungi FTM7 had maximum 94.20% dye degradation ability at 150 rpm, 81.21% at 100 rpm and in static condition was 64.59%. Effects of MB initial concentration toward degradation of MB by fungi showed by Figure 5 which indicates that there is no significant different percentage of dyes degradation with the increasing initial concentration of dyes. Otherwise, these fungi able to achieve 95.45% degradation of MB at

40 ppm. The biodegradation of MB by fungi FTM7 was affected by changes of pH as shown in Figure 6. The range of pH studies used for MB was 4.0-10.0. It has shown the most significant degradation MB obtained in pH 6.0.

Kinetic study

Kinetics is an important parameter to evaluate the performance of fungi to degrade MB. Generally, the kinetic study determined by linear regressions of degradation time versus MB concentration depends on kinds of order reaction. MB concentration can be obtained from the linear equation in calibration graphic (Figure 2). When looking at the linearity coefficient (R^2) for three models of order reaction (Table 1), it can be concluded that the first-order reaction had R^2 bigger than the others. It is assumed that the degradation rate of MB was effected by MB concentration.

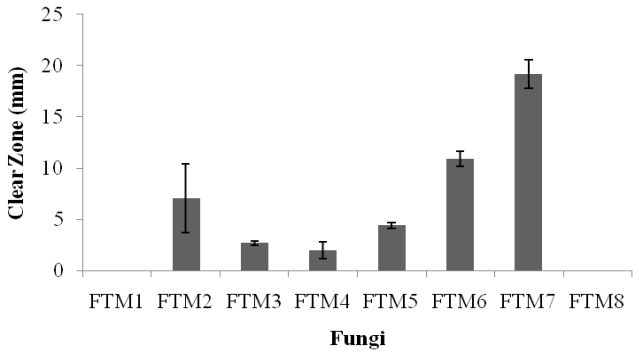

Figure 1. Initial screening MB degradation

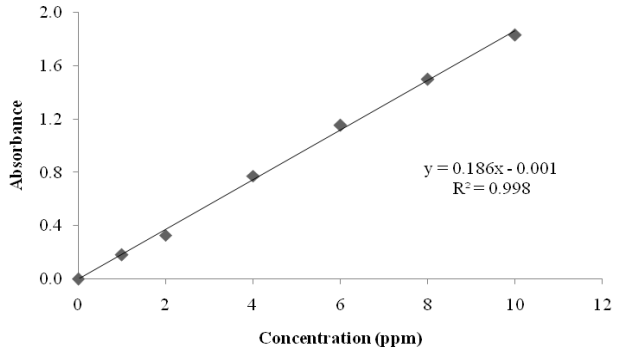

Figure 2. MB calibration graphic

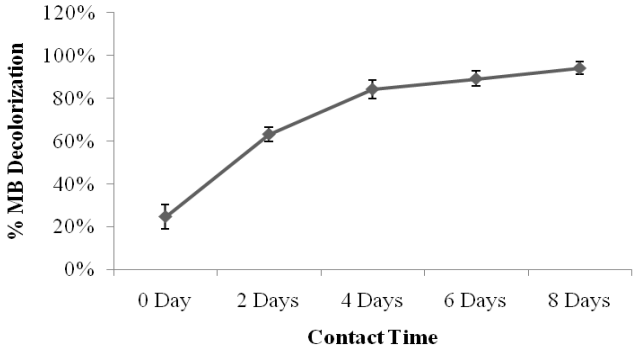

Figure 3. Effect of contact times

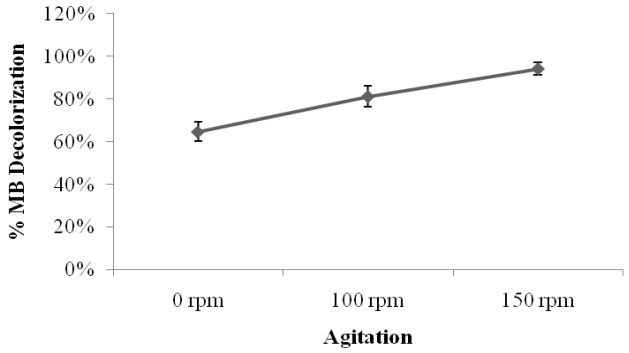

Figure 4. Effect of agitation

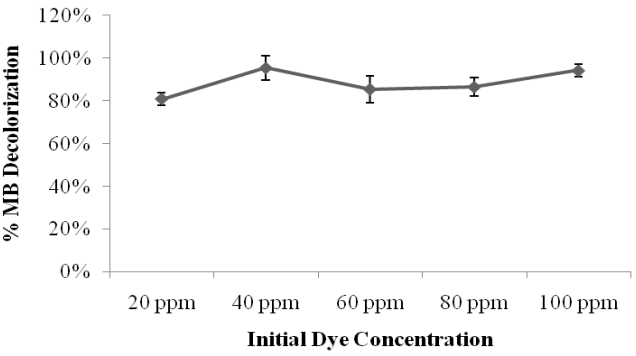

Figure 5. Effect of MB Initial Concentration

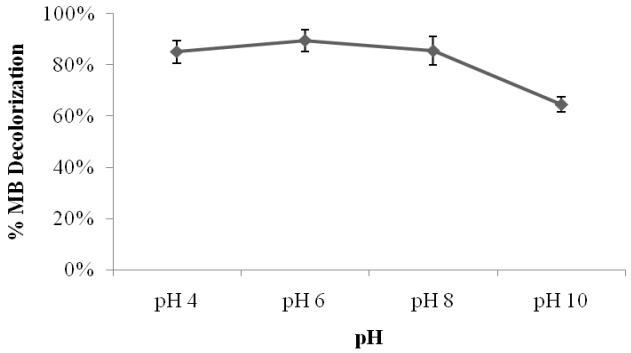

Figure 6. Effect of pH

Table 1. Means of R^2 MB degradation for various parameters and conditions.

Fungi	Linearity Coefficient (R^2)		
	Zero Order	First Order	Second Order
FTM7	0.899	0.903	0.794

Discussion

The previous study reported that there are eight fungi had been isolated from crude oil-contaminated soil. These fungi also had great potential to degrade big and complex aromatic compound in crude oil. These fungi were *Fusarium* sp. (FTM1), *Penicillium* sp. (FTM2), *Trichoderma* sp. (FTM3), *Penicillium* sp. (FTM4), *Aspergillus* sp. (FTM5), *Aspergillus* sp. (FTM6), *Penicillium* sp. (FTM7), *Aspergillus* sp. (FTM8) (Sari 2017). The initial screening of fungi for dye biodegradation via solid medium was identified by a clear zone surrounding the fungal colonies. It was used to determine the greatest potential of fungi to degrade MB. The highest dye degradation was detected in fungi FTM7 that of remaining 7 fungi. The clear zone that appears in around the colonies indicated that fungi FTM7 is able to degrade MB. The clear zone also signs that the biosorption degradation mechanism used by fungi (Park et al. 2006). These results also indicated that these fungi have enzymatic system for breaking organic complex structures. The dry weight biomass of this fungus with MB dye after treatment may have been due to a reaction of MB with enzymes secreted by the fungal mycelia. The previous study also reported that *Penicillium funigulosum* showed higher dyes removal activity towards MB (AI-Jawhari 2015). Afterward, fungi FTM7 selected for further studies.

Effect of contact time. Dye degradation time is a crucial parameter to be considered as it can determine the energy and cost of the removal process. In this study, the effect of contact time was investigated. The highest dye degradation was observed at contact time eight days. Longer time is taken to remove the dye also increases the cost of energy to supplied (Chen et al. 2015). This results reported that the capacity of MB degradation by fungi increased with an increase in contact time. However, the previous study reported that the fungal strain isolated from soils contaminated with dyes shown higher dyes degradation within 120 hours. The fungi were identified as *Penicillium oxalicum* SAR-3 based on 18S and internal transcribed spacer (ITS) rDNA gene sequence analysis (Saroj et al. 2014). The previous study also found that the enzyme laccase that isolated from fungus *Aspergillus oryzae* able to degrade 53% of MB after 30 minutes incubation (Forootanfar et al. 2012). Another study reported that consortium fungal-bacterial *Aspergillus ochraceus* NCIM-1146 and *Pseudomonas* sp. SUK1 was also able to degrade azo dye rubine GFL 95% in 30 hours (Lade et al. 2012). Nonetheless, these results need to improve degradation time due to the efficiency and effectiveness of time and energy. The different method even reported that dyes degradation by using AOP method only need 120 minutes to oxidize methylene blue into benign products such as CO_2 and H_2O (Zulfa et al. 2018).

Effect of agitation. The MB degradation by fungi FTM7 was evaluated at 0, 100 and 150 rpm. The results showed that the highest dye degradation was observed at agitation 150 rpm. Similar findings was observed in the previous study in which a novel white-rot fungi *Alternaria alternata* CMERI F6 reported to decolorized 99.99% of 600 mg/L congo red within 48 hours at 150 rpm (Chakraborty et al. 2013). It is due to aeration and agitation which is necessary to fulfill the microbial oxygen requirements during the cultivation and to enhance the oxygen gas-liquid mass transfer. This might affect the morphology of filamentous fungi and lead to an increased rate of enzyme synthesis (Irpex 2006). In contrast, another study reported that activities of *Aspergillus niger* and *Penicillium spp.* on the decolorization of the dye solution was more efficient in the static condition than the shaking condition (Sh 2008). Degradation performance of Scarlet R was 100% under static condition after certain incubation period with the consortium-GR (consisting of *Proteus vulgaris* NCIM-2027 and *Micrococcus glutamicus* NCIM-2168) (Saratale et al. 2009).

Effect of initial concentration. The concentration of dye can influence the efficiency of dye removal through a combination of factors including the toxicity of the dye at higher concentrations and the ability of the enzyme to recognize the dye efficiently at very low concentrations (Wanyonyi et al. 2019). The degradation percentage of methylene blue by fungi FTM7 was studied at different initial dye concentrations range from 20 to 100 mg/L. The results showed that the degradation rate affected by initial dye concentration slightly. A difference reported in a previous study revealed that the decolorization rate of dye decreased with increasing initial dye concentration (Daâssi et al. 2013). Similar results also reported that microorganisms also could decolorize reactive brilliant red 99% at concentration small than 200 mg/L. However, above this concentration, dye decolorization decreased about 20% at initial concentration was 1,000 mg/L (Chang et al. 2017). Decrease in degradation rate of *Lentinus crinitus* fungi was observed with the increase in initial dye concentration (Heyse et al. 2010). It happens due to high toxicity properties of dye towards microorganisms at high concentrations (Chen et al. 2015).

Effect of pH. It is important to study the effect of pH on degradation process, as transport of dye molecule into the cell is pH dependent and thought to be rate-limiting step for degradation of dyes The results showed that FTM7 had the highest degradation activity at pH 6.0, and the MB degradation decreases with increase in pH. It is similar to fungus *Sphingomonas paucimobilis* that had maximum dye degradation ability at pH 6-7, while condition moves to more acid and alkaline the decolorization efficiency decreases (Bunti et al. 2017). The previous study also reported that general fungi and yeast showed optimum degradation in acid or neutral conditions (Hazrat 2010). It is also similar for fungi *Phanerochaete chrysosporium* that able to degrade synthetic dye in acid conditions and had optimum decolorization at pH 5.0. In highly acid conditions, a decrease in decolorization rate may be due to a decrease in enzymatic activity when pH changed from optimum levels (Sharma et al. 2009).

Kinetic study. In the present study, the kinetics of MB degradation for different environmental conditions was modeled using zero, first and second-order kinetics models. Zero-order reaction plots were obtained by plotting dye concentration versus time, first-order reaction plot was obtained by plotting ln (concentration) against time and

second-order reaction model obtained by plotting 1/concentration versus time (Wanyonyi et al. 2019). The results showed that degradation of MB by fungi FTM7 was first-order reaction. Another study also reported that photodegradation methylene blue by TiO_2 catalyst followed first-order reaction (Sumerta et al. 2002).

In the end, the conclusion of the study was that Fungi FTM7 had the greatest potential to degrade methylene blue (MB) and the optimum degradation has been found at 8 days incubation period, agitation 150 rpm, the initial concentration of MB was 40 ppm and unadjusted pH condition.

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