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Biodecolourisation of Methyl Red Dye by Bacterial-Fungal Consortium

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The textile industry is known to use massive amount of dyes to colour fabrics and subsequently the treatment of textile effluent is one of the greatest challenge. The aim of this research is to investigate the ability of the bacterial-fungal consortium to decolourise methyl red dye. The synergy effects of microbial consortium offer considerable advantages over single culture in mineralisation of complex dye compounds. The consortia were developed by using isolated white rot fungi and strain of Bacillus subtilis. The sample of white rot fungi were isolated from rotten wood and then were observed under microscope to determine its morphology. The individual cultures of white rot fungi designated as WRF D1, WRF E1, WRF F1, WRF G1 and WRF H1 and Bacillus subtilis were tested on the ability of decolourising 50 mg/L of methyl red. The results of the study were compared with the performance of microbial consortium of white rot fungi and Bacillus subtilis. Based on the observation, the strain of isolated fungi exhibited similar morphological characteristics of white rot fungi with the filamentous shape and the presence of basidiospores. The preliminary study demonstrated the ability of the isolated white rot fungi to produce ligninolytic enzyme by formation of brown ring on PDA plate containing $MnCl_2$. The decolourisation of methyl red by the individual cultures was in the range of 35 - 40 %. The result revealed that consortium of white rot fungi and Bacillus subtilis significantly enhanced the decolourisation of methyl red dye with more than 80 % of dye removal. The highest percentage of decolourisation (82.16 %) was exhibited by the consortium of WRF E1 and Bacillus subtilis after 72 h in shaking conditions (120 rpm, 30 ºC and pH 5). The data collected from this research provided convincing evidence on the potential of bacterial-fungal consortium for dye decolourisation.

1. Introduction

In the recent years, extensive researches have been conducted on biological methods as an eco-friendly alternative for decolourisation of synthetic dye. Biodecolourisation by using fungi, bacteria and yeast are alternative indeed inexpensive method in comparison to the existing physico-chemical approach of dye removal (Su and Lin, 2013).
Bacteria and fungi have proven to be capable to remove dye compounds by means of adsorption or biodegradation by oxidation and reductive enzymes (Su and Lin, 2013). Fungi especially white rot fungi are known as efficient ligninolytic organisms. They are able to degrade various types of dyes including azo, heterocyclic, reactive and polymeric (Kumaran and Dharani, 2011). The extent of dye degradation by white rot fungi depends on the complexity of dye, nitrogen availability and enzymatic activity (Singh, 2006). The capability of white rot fungi is associated with the lignin degrading system that consists of various extracellular enzymes such as laccases (Lac), manganese peroxidase (MnP) and lignin peroxidase (LiP) (Prasongsuk et al., 2009). In addition to enzymatic degradation, adsorption of dye by white rot fungi may proceed via binding with the hyphal structures, physical desorption to the solution and enzymatic degradation by living hyphal structures (Singh, 2006). The ability of bacteria to decolourise dye is connected to intracellular and extracellular oxidoreductive enzymes namely laccase, azoreductase and

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NADH-DCIP-reductase (Kadam et al., 2011). Either in aerobic or anaerobic condition, the reduction of the azo bond (-N=N-) chromophore group is the primary step in the degradation of azo dyes by bacteria in which the reduction might be achieved by the enzymes (Singh et al., 2015).
In spite of their promising ability, both bacteria and fungi have suffered certain limitations such as incomplete degradation of dye. A mixed microbial system has been reported to be more efficient in decolourisation of azo dye in comparison with pure culture (Khehra et al., 2005). A richer metabolic network of microbial consortium can be exploited to overcome the problem of incomplete mineralisation. The synergy effects of the consortium enable the complete mineralisation as the product formed by one microorganism is used by another microorganism and converted to a nontoxic form. The decolourisation by microbial consortium is possibly enhanced as different microorganism may attack at the different positions of the dye molecules (Lade et al., 2012).
Most of the previous studies focused on the development of bacterial consortium as well as the fungal consortium. There is limited information on the consortium of white rot fungi and bacteria even though this type of fungi has exceptional ligninolytic capability. Only individual culture of white rot fungi has been reported for its biodecolourisation ability includes Phanerocheate chrysosporium and Pleurotus sajor-caju (Kumaran and Dharani 2011). The consortium of white rot fungi and bacteria is believed to be able to overcome the typical problems of biodecolourisation process which are partial degradation, long degradation time and formation of toxic metabolites (Lade et al., 2012). This study attempted to develop microbial consortia of white rot fungi and bacteria for biodecolourisation of azo dye. The white rot fungi were first isolated and then combined with B. subtilis to enhance the efficiency of decolourisation of methyl red dye in aerobic batch cultivation system.

2. Methodology

2.1 Isolation of white rot fungi

Rotten wood samples were collected from the backyard of Downstream Laboratory, UniKL MICET. One cm^2 of fungal fruiting bodies were taken by using sterilised cutter and were aseptically cultured on a 39.5 g/L Potato Dextrose agar (PDA; pH 5) containing 0.5 g/L chloramphenicol. The plates were incubated at 30 °C for 4 d. One cm diameter of mycelium plug with actively growing mycelium was cut by using sterilised cutter and was sub-cultured until single colony was obtained. The characteristics and morphology of the isolates were observed using microscope.

2.2 Detection of ligninolytic enzyme activities of white rot fungi

The isolated white rot fungi denoted as WRF D1, WRF E1, WRF F1, WRF G1 and WRF H1 were grown on PDA (39.0 g/L) plate containing $MnCl_2$ (0.1 g/L) to screen the ability of the isolates to produce ligninolytic enzymes. One cm diameter of mycelium was cultured on the plate and incubated at 30 °C for 7 d. The occurrence of dark brown colour around the fungal colonies indicated a positive result of the presence of ligninolytic enzymes activities (Prasongsuk et al., 2009).

2.3 Decolourisation of methyl red dye

The isolates and B. subtilis were tested for the ability to decolourise methyl red dye. The individual cultures were inoculated separately into Erlenmeyer flask containing 100 mL of 50 mg/L methyl red dye that was supplemented with essential nutrients. The cultures were incubated at 30 °C with the speed of 120 rpm for 2 d. Flasks without culture served as control for dye decolourisation study.
Determination of the ability of microbial consortia to degrade dye were proceeded by combining two pieces of 1 cm diameter of mycelium of white rot fungi with 10 mL of B. subtilis liquid culture. The bacterial-fungal consortium (D1, E1, F1, G1 and H1) was then inoculated separately into Erlenmeyer flask containing 100 mL of 50 mg/L methyl red dye and was supplemented with essential nutrients (Glucose 1 g/L; Yeast Extract 0.5 g/L, peptone 5 g/L, NaCl 5 g/L; $(NH_4)_2SO_4$ 10 g/L; K_2HPO_4 5 g/L and $MgSO_4.7H_2O$ 0.5 g/L) at pH 5. The consortia were grown in an incubator shaker at 30 °C, 120 rpm for 3 d. 10 mL of samples were collected daily and the absorbance was measured by using UV-VIS spectrophotometer (526 nm).

3. Result and discussion

3.1 Isolation of white rot fungi

Five strains of white rot fungi were successfully isolated on PDA media and were labelled as WRF D1, WRF E1, WRF F1, WRF G1 and WRF H1 as shown in Figure 1. The similarity between the strains was observed as all the isolates have cotton-like surface and diversely grown on wide area. WRF F1 and WRF H1 showed an irregular edge of the colonies while other strains exhibited even smooth edge colonies. The morphology of the isolates was similar with Phanerocheate chrysosporium studied by Susanti et al. (2015) where the fungal mycelium formed from a single colony of mycelium that covering the surface of PDA plate. The study observed yellowish white pigmentation with cotton and sticky structures for the upper colonies and white pigmented for the bottom colonies.

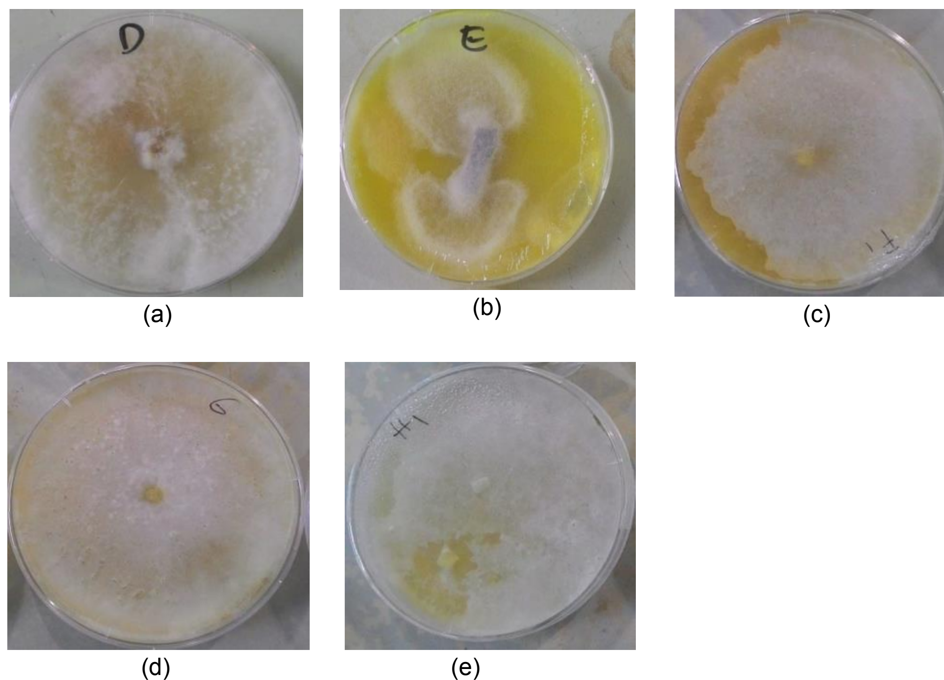

Figure 1: Isolated white rot fungi; (a) WRF D1, (b) WRF E1, (c) WRF F1, (d) WRF G1 and (e) WRF H1

As shown in the Figure 2, the hypae of WRF D1 and WRF E1 displayed unbranched smooth-walled form, with the presence of unbranched eclipse sporangiospores. The number of spores in WRF D1, WRF E1 and WRF H1 are in a lesser amount as compared to WRF F1 and WRF G1. As observed, WRF F1, WRF G1 and WRF H1 have septate with branched hypae whereas WRF H1 and WRF E1 are similar with thick walled hypae. The sporangiospores and filamentous structure of the locally isolated white rot fungi similar to the microscopic characteristics of P. chrysosporium based on the study by Susanti et al. (2015).

3.2 Determination of ligninolytic enzyme activities of isolated white rot fungi

The result found that only isolated WRF E1 exhibited a positive result of ligninolytic enzymes activities with the appearance of dark brown colour which indicated the presence of oxidising product (MnO_2) that was caused by MnP enzyme (Prasongsuk et al., 2009). The finding is supported by Namoolnoy et al. (2011) that reported the positive result for MnP is caused by 3, 3-dimethoxybenzidine that was converted from reduced form (colourless) into oxidised form (brown colour). Prasongsuk et al. (2009) reported only P. chrysosporium gave a positive result on the $MnCl_2$ agar medium by showing brown flecks of MnO_2 after 6 d and the brown colour became darker after 7 d and 8 d of incubation.

3.3 Decolourisation of methyl red dye

The ability of individual cultures of isolated white fungi WRF D1, WRF E1, WRF F1, WRF G1 and WRF H1 and B. subtilis to decolourise methyl red dye was initially investigated and the result obtained is illustrated in Figure 3. WRF G1 showed the highest percentage of decolourisation (40.10 %) with degradation capacity of 0.54 mg/L/h. B. subtilis and other isolates resulted in the range of 35 to 39 % of decolourisation of 50 mg/L of methyl red dye.
Even though most of the white rot fungi displayed the negative result on MnP activitiy, some degree of colour reduction was observed due to the fact that dye removal might be facilitated by adsorption mechanism. This is explained by Ghasemi et al. (2010) that found decolourisation of several types of dyes by P. chrysosporium were not only associated with degradation by MnP and LiP extracellular enzymes but also through adsorption mechanism. Other study by Singh et al. (2006) found that P. chrysosporium successfully degraded Direct Red (50 ppm) in which enzyme LiP responsible for 100 % of degradation of the dye within 24 h.

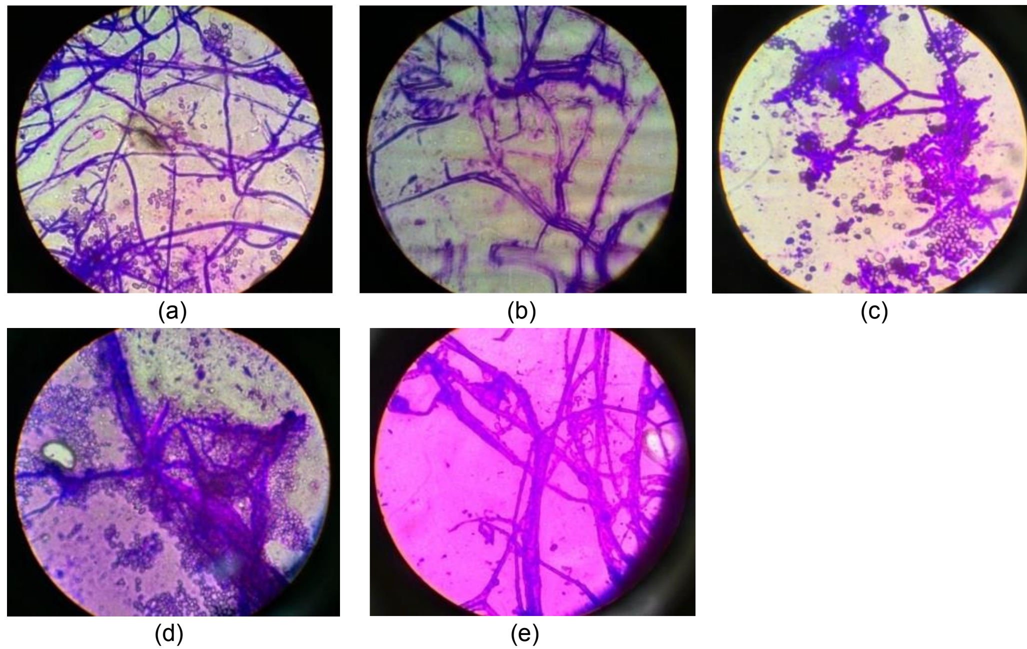

Figure 2: Morphology of isolated white rot fungi under compound microscope (100×); (a) WRF D1, (b) WRF E1, (c) WRF F1, (d) WRF G1 and (e) WRF H1

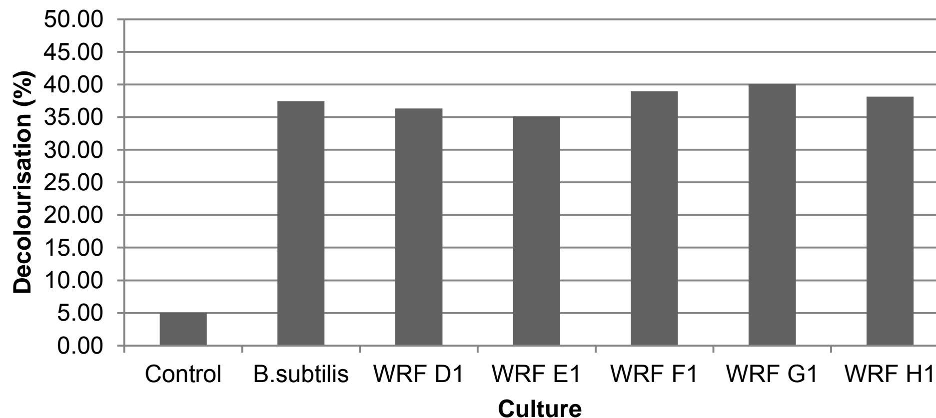

Figure 3: Decolourisation of methyl red by individual isolates and B. subtilis

The study was continued with determination of the ability of the consortia to degrade methyl red dye. The consortium of WRF E1 and B. subtilis resulted in the highest percentage of decolourisation (82.16 %) after 72 h in aerobic batch cultivation. Other consortia resulted in slightly lower decolourisation efficiency as portrayed in Figure 4. The dye reduction was rapid within the first 24 h for all consortia with highest degradation capacity of 0.89 mg/L/h obtained by consortium E1 before becoming nearly constant. The greater performance of consortium E1 in comparison to other consortia is corresponding to the positive result on MnP activity.

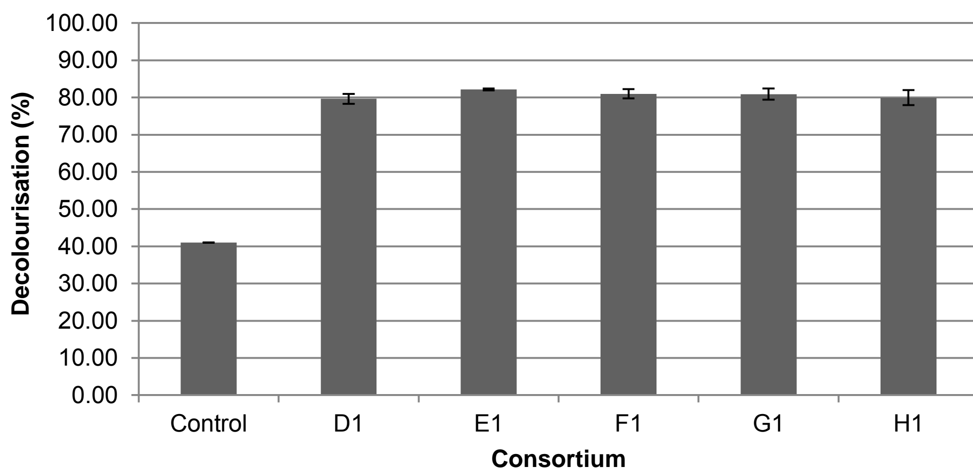

Figure 4: Decolourisation of methyl red by consortia of WRF and B. subtilis

The result obtained proves that the consortia of Bacillus sp. and white rot fungi are able to improve the dye removal rate in comparison with individual cultures. The finding is in agreement with Su and Lin (2013) that found consortium of A. niger and Bacillus sp. decolourised more than 90 % of 50 mg/L of Reactive red 120 meanwhile individual culture of A. niger and Bacillus sp. only decolourised 50 % and 30 % of dye. This is supported by Cui et al. (2012) that discovered that bacterial consortia significantly enhanced decolourisation of methyl red both in aerobic and anaerobic conditions. The study also reported the bacterial consortia have demonstrated non-specificity of azo bond reduction as the consortia efficiently decolourised several types of azo dyes including Methyl red, Congo red and Orange 1 that have different chemical structures. A study by Khehra et al. (2005) observed that bacterial consortium of Bacillus cereus (BN-7), Pseudomonas putida (BN-4), Pseudomonas fluorescence (BN-5) and Stenotrophomonas acidaminiphila (BN-3) has successfully decolourised Acid Red 88 dye in 24 h whereas individual pure culture took more than 60 h to achieve similar result. Su and Lin (2013) reported that decolourisation of azo dye by a consortium of fungal-bacterial are achieved by cleavage of the azo bond by azo reductase of bacteria, biosorption by cells and consecutive biodegradation by extracellular enzymes of fungi. The study found that aromatic amines, the intermediate product of degradation by bacteria are degraded completely in co-culture of fungi and bacteria. Gou et al. (2009) reported that degradation of intermediate inclusive aromatic amines by bacteria decrease the inhibition of fungi and consequently resulted in a superior performance of fungal bacterial consortia. The complementary interactions among the Bacillus and white rot fungal resulted in greater decolourisation efficiency of methyl red dye.

4. Conclusion

The study has successfully developed microbial consortia system that consists of isolated white rot fungi and B. subtilis for decolourisation of azo dye. The outcome of this study demonstrated that the developed consortia have considerably enhanced the decolourisation with WRF E1 and co culture of B. subtilis successfully decolourized 82 % of methyl red dye. The finding of this study suggested the synergism of bacterial-fungal consortium has a great potential towards large scale application of dye wastewater treatment.

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