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DECOLORIZATION OF REACTIVE DYESBY CONSORTIUMS OF BACTERIA AND FUNGI

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Abstract: Reactive dyes are synthetic organic compounds widely used in several industries including textile, paper, printing and cosmetics, which have inverse effects on human life and environment. Reactive Black 5 (RB5) and Remazol Brilliant Blue R (RBBR) are type of reactive dye group that can decolorize by microorganisms. Three species of bacteria Brevibacillus panacihumi ZBI, Lysinibacillus fusiformis ZB2 and Enterococcus faecalis ZL and three species of fungi fungus Candida sp. S1, Meyerzoma sp. S7 and Rhizoctonia zeae SOL3 were used to decolorize dyes in individual and co-culture. Among the selected bacteria, Enterococcus faecalis ZL shows the highest decolorization rate of RB5 and RBBR reached to 77% and 58% respectively, in 4 days. Meanwhile, Rhizoctonia zeae SOL3 shows the highest decolorization ability among the selected fungi. The presence of glucose has a positive effect on decolorization by fungi and bacteria. In co-culture of Enterococcus faecalis ZL and Rhizoctonia zeae SOL3, the decolorization ability has decreased. This result suggests that fungi have adverse effect on the removal of dyes by bacteria.

Keywords: *Reactive dye, decolorization, Enterococcus faecalis ZL, Rhizoctonia zeae SOL3, co-culture.*

1.0 Introduction

Reactive dyes are synthetic organic compounds widely used several industries including textile, paper, printing and cosmetics, due to its low production cost and the variations in colour compared to natural dyes. The presence of a very small amount of dyes (even <1 mg/l for some dyes) is highly visible and effect on the ecosystem (Pandey *et al.*, 2007). The major group of reactive dyes is the azo group, then followed by anthraquinone dyes (Rodríguez-Couto, 2011). The structures of these synthetic dyes are developed to resist fading upon exposure to sweat, temperature, water and light. Due to the color, biorecalcitrant and potential toxicity to human and animals (Levine, 1991), treatment of

wastewater dyes has a major concern. Several methods such as adsorption (Tahir and Abdul Majid, 2013), photo degradation (Kusic *et al*., 2013), and biological method (Solís *et al*., 2012) are used to treat dying wastewater. However, biodecolorization of dye has received great attention in recent years due to its efficient application, especially by bacteria (Han *et al*., 2012) and fungi (Xian-Chun *et al*., 2007). It considered as a promising treatments due to its environmentally friendly and cost effectiveness (Novotný *et al*., 2009). The decolorization capability of reactive dye by bacteria and fungi has been investigated, such as *Brevibacillus panacihumi* (Mohd Ramlan *et al*., 2012), *Bascillus* (Wang *et al*., 2013), *Pleurotus eryngii* (Hadibarata *et al*., 2013), *Aspergillus aculeatus* (Perlatti *et al*., 2012), *Candida Olephila* (Lucas et al., 2006), *Candida rugopelliculosa* (Liu *et al*., 2011). However, it is difficult to maintain a pure single culture in the large-scale application of dye decolorization, which may have effects on its removal. Therefore, the existence of co-culture of bacteria and fungi may have positive or negative effect on the decolorization process, biomass growth and metabolic activities (Tarkka *et al*., 2009). The degradation in nature is a consequence of the sequential breakdown by fungi and bacteria, when the fungi performing the initial oxidation step (Sack and Fritsche, 1997).

Reactive Black 5 (RB5) and Remazol Brilliant Blue R (RBBR) represent an important class of toxic and recalcitrant organic pollutants. RB5 is a reactive di-azo dye, while RBBR is an anthraquinone dye; both were used in this research as representative of reactive dyes. The objective of this research was to investigate the capability of bacteria and fungi species in the decolorization of RB5 and RBBR. The decolorization of reactive dyes by these bacteria and fungi are not widely discussed in the previous studies. Furthermore, this work investigates the effect of co-culture of bacteria and fungus in the decolorization process.

2.0 Materials and Methods

2.1 Dyes and Chemicals

RB5 and RBBR were supplied from Sigma-Aldrich/USA. The chemical structure of RB5 and RBBR are shown in Figure 1. Glucose grade AR 99.8% assay from QREC (Asia) SDN BHD. Chloramphenicol 98% purity from Acros Organic/USA. All other chemicals that have been used in experiments were of the highest purity available and of an analytical grade.

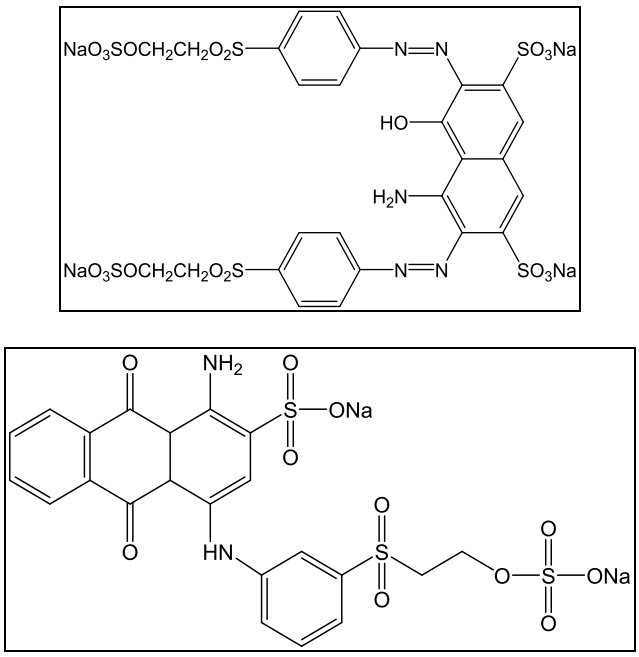


Figure 1: Chemical structure of RB5 (left) and RBBR (right), drawn by ChemDraw

2.2 Bacteria Collection and Cultivation

Brevibacillus panacihumi ZB1 and *Lysinibacillus fusiformis* ZB2 were isolated from textile effluent and were acclimatized in sterilized textile wastewater. *Enterococcus faecalis* ZL isolated from palm oil mill effluent (POME). Each bacterium was grown overnight in textile wastewater enriched with nutrient broth. The cultures were agitated in a rotary shaker at 37˚C, until the exponential growth phase was reached, before added into the reactor at ratio of 1:1:1:1 (Kee *et al*., 2014). The stock culture was transferred into 50 ml of nutrient broth and grown under aerobic conditions at 37°C for 24 h (Mohd Ramlan *et al*., 2012). Stock cultures for each type bacterium were maintained in the bead culture and stored at -80°C. Inoculum preparation was performed by transferring fresh pure colonies of the bacteria isolated from the nutrient agar plate into liquid medium using a sterile wire loop. The nutrient broth (NB) was used to cultivate the bacteria. The liquid medium was sterilized at 120°C and 16 bars for 45 minutes to prevent contamination by microorganism. One loop full of culture was inoculated into 50 ml falcon tube containing 10 ml of NB. The cultivated strains were then incubated for 24 h at room temperature in order to produce inoculum or master cell culture. Then, 10 ml of inoculum was used to inoculate in 100 ml of the same media and in the same condition as mention before to produce active cell's culture for the dye decolorization.

2.3 Fungi Collection and Cultivation

Fungal species; *Candida* sp S1 and *Meyerzoma* sp S7 were isolated from dead tree from tropical forest in Johor, Malaysia. *Rhizoctonia zeae* SOL3 was isolated from soil from industrial site in Johor, Malaysia. The identification was based of the comparison of sequences of the 18s rRNA gene sequence species with those found in databanks. The fungi were transferred from the stock cultures to malt extract agar (MEA) medium containing 200 mg/l chloramphenicol to prevent bacterial growth, and allowed to grow at 28°C in dark for 1-2 weeks prior to use in the decolorization of synthetic dyes.

2.4 Culture Conditions

Static cultivation was carried out in 100 ml Erlenmeyer flasks with 20 ml liquid medium. The constituent of the liquid medium was as follows (g/l): 10 glucose as carbon source for fungi culture only, 10 yeast extracts as the nitrogen source. After autoclaving and addition of 200 mg/l chloramphenicol for fungi culture only, the medium was adjusted to pH 6 using 0.1 mM HCl. All media were supplemented with RB5 or RBBR at a final concentration of 50mg/l. The flasks were inoculated with one wort agar plug (10 mm diameter), cut from an actively growing part of a colony on a petri dish for fungi culture, whereas 1 mm of bacterial broth for bacteria culture. All prepared cultures were incubated at 28°C for 15 days.

2.5 Decolorization Assays

Decolorization of RB5 or RBBR in the liquid medium was measured in the culture filtrates after removing the mycelia or bacteria by filtration through filter paper and monitored spectrophotometrically by HACH DR5000 at the maximum wavelength of absorbance (596 nm for RB5 and 592 nm for RBBR). The systems without the fungus served as abiotic controls. The decolorization rate is measured as the difference between the control and the sample.

3.0 Results

3.1 Decolorization in Liquid Media

The maximum wavelengths were measured at 596 and 592 nm for RB5 and RBBR respectively. The pattern of RB5 and RBBR are shown in Figure 2.

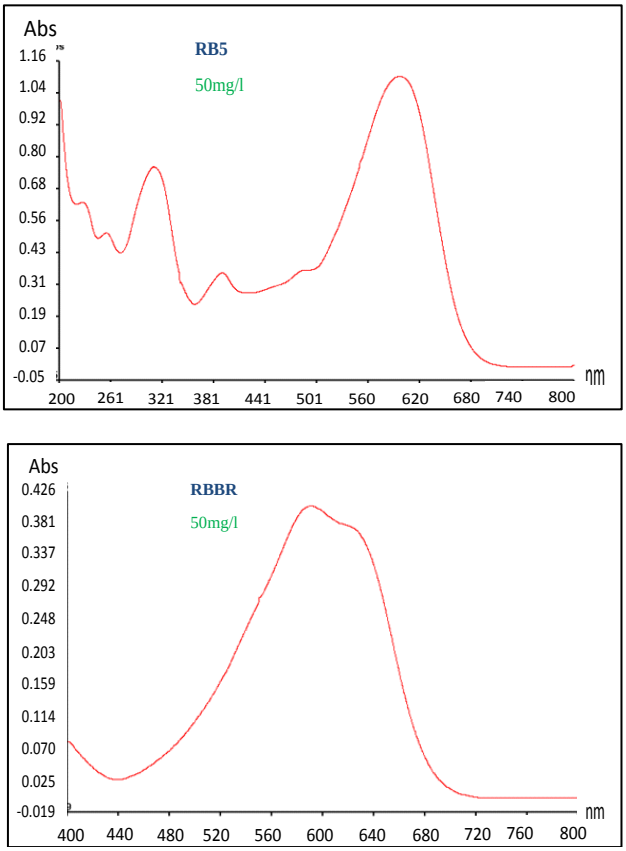


Figure 2: UV-spectrophotometer of RB5 (left) and RBBR (right)

3.2 Decolorization in Liquid Media by Fungi

The decolorization studies were carried out in liquid media containing RB5 or RBBR, in the presence of glucose and yeast extract, in addition to chloramphenicol. Uninoculated controls showed no color removal. The decolorization of dyes by *Candida* sp S1, *Meyerzoma* sp S7 and *Rhizoctonia zeae* SOL3 have commenced after day one. Among the selected fungi, *Rhizoctonia zeae* SOL3 shows the highest decolorization rate of RB5 and RBBR, which reached 87% and 45% respectively in 15 days. The pattern of decolorization of RB5 and RBBR by fungi is shown in Figure3.

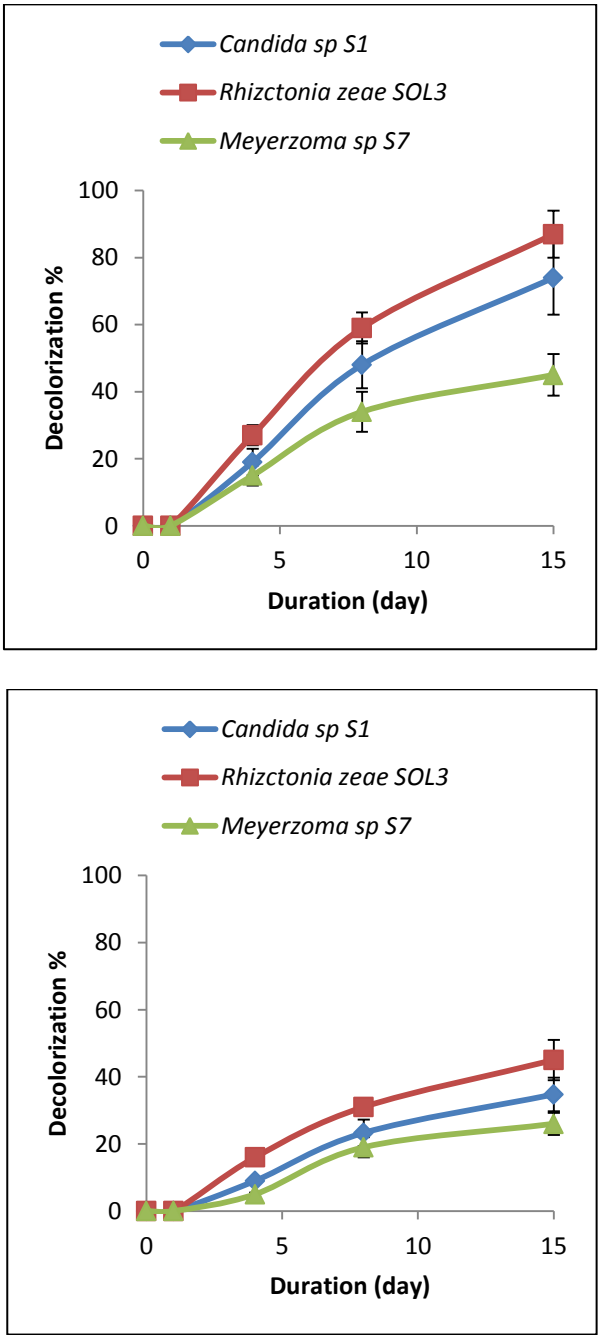


Figure 3: The decolorizationof RB5 (left) and RBBR (right) by fungi; *Candida* sp S1, *Meyerzoma sp* S7 and *Rhizoctonia zeae* SOL3, the bars represents error in duplicate samples

In all fungi samples used, the decolorization has commenced from day one. This is due to the fungi species need time to grow and decolorize. The highest decolorization was achieved by *Rhizoctonia zeae* SOL3 followed by *Candida* sp S1 then *Meyerzoma* sp S7. This result shows that fungi species are different in terms of their ability to decolorize dyes (Andersson and Henrysson, 1996; Machado *et al*., 2006). The decolorization rate of RB5 was higher than RBBR by the three fungi species investigated; this may due to the fused aromatic structure of RBBR is more stable than RB5 (Banat *et al*., 1996). According to Novotny *et al*., (2001), the anthraquinone dyes are more resistant to degradation especially when compared to azo dyes. The decolorization of dyes by fungi are relied on the presence of glucose as cometabolim (Korniłłowicz-Kowalska and Rybczyńska, 2012). The decolorization of RB5 by *Candida Olephila* was only occurred in presence of glucose (Lucas *et al*., 2006).

3.3 Decolorization in Liquid Media by Bacteria

The decolorization studies were carried out in liquid media containing RB5 or RBBR, in the presence of yeast extract only. Uninoculated controls showed no color removal. The decolorization of dyes by *Lysinibacillus fusiformis* ZB2, *Brevibacillus panacihumi* ZB1 and *Enterococcus faecalis* ZL have commenced from day one. Among the selected bacteria, *Enterococcus faecalis* ZL shows the highest decolorization rate of RB5 and RBBR, which reached 77 % and 59% respectively in 4 days. The pattern of decolorization of RB5 and RBBR by bacteria is shown in Figure 4.

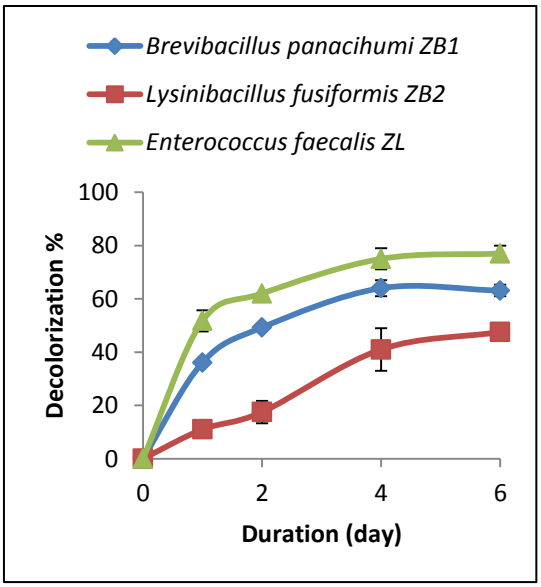


Figure 4: Decolorization of RB5 (left) and RBBR (right) by bacteria, the bars represents error in duplicate samples

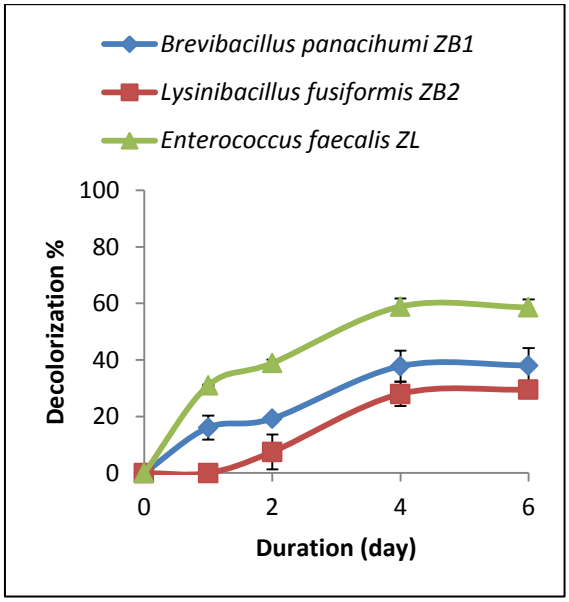
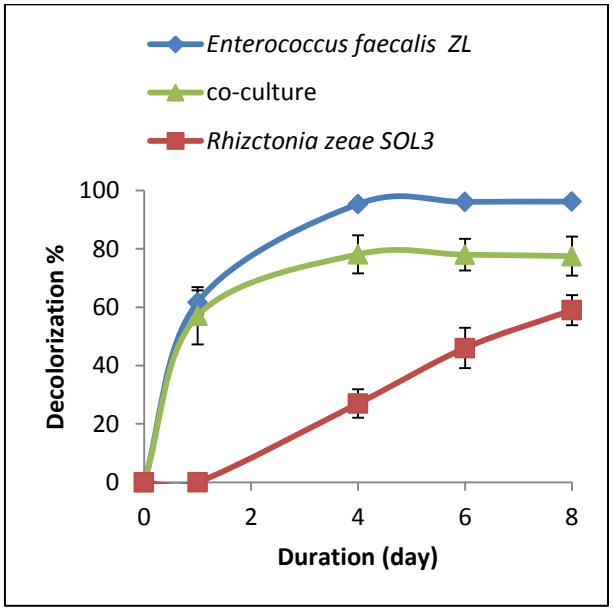


Figure 4 (con't): Decolorization of RB5 (left) and RBBR (right) by bacteria, the bars represents error in duplicate samples

The decolorizationof RB5 and RBBR by bacteria as a sole source of carbon and energy has increased with time. However, increasing the incubation time more than 4 days would not enhance the decolorization rate. The decolorization of RBBR was less than RB5 by all bacteria investigated (*Brevibacillus panacihumi* ZB1, *Lysinibacillus fusiformis* ZB2 and *Enterococcus faecalis* ZL). This is due to the fused aromatic structure of RBBR is more toxic than RB5 to microorganisms. The toxicity and genotoxicity were not detectable when decolorized RB5 under aerobic conditions by bacteria (Gottlieb *et al*., 2003).

3.4 Decolorization in Liquid Media by Co-culture of Bacteria and Fungi

The decolorization studies were carried out in liquid media containing RB5 or RBBR, in the presence of yeast glucose and yeast extract. The decolorization of dyes has investigated by *Enterococcus faecalis* ZL, *Rhizoctoniazeae* SOL3 and co-culture of *Enterococcus faecalis* ZL and *Rhizoctoniazeae* SOL3 in 8 days of incubation. The decolorization rate by *Enterococcus faecalis* ZL reached 95%for RB5 and RBBR for 69% which are higher than the decolorization by the same bacteria without glucose. The pattern of decolorization of RB5 and RBBR by bacteria is shown in Figure 5.



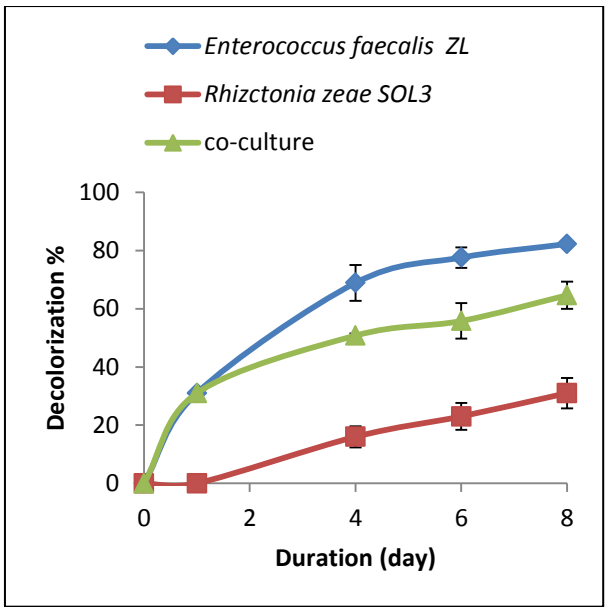


Figure 5: Decolorization of RB5 (left) and RBBR (right) by consortium of bacteria and fungus, the bars represents error in duplicate samples

The decolorization of dyes in the presence of glucose by *Enterococcus faecalis* ZL has enhanced compared to the decolorization without glucose additive. According to Kumar *et al*., (2009), glucose could enhance the growth requirements of the microbes and therefore increase the decolorization rate of reactive azo dye (Kumar *et al*., 2009). In co-culture of fungus and bacteria, the decolorization rate has decreased. This decreasing commence after one day of incubation, when the fungus starts its growth. Some interactions between microorganisms are antagonistic, due to pH changes or competition for carbon source. This causes indirectly inhibition of microbial potential and degradation ability of both fungi and bacteria (Thion *et al*., 2012). Bisnoi and co-workers found out that the degradation of polycylic aromatic hydrocarbon by *Phanerochaete chrysosporium* was lower in non-sterile soil, probably due to the effect of competition with the native microflora (Bishnoi *et al*., 2008). Our result is contrary to Qu *et al*., (2010) who found that the decolorization of reactive dark-blue K-R by consortium of both synergism of bacterial strain *Exiguobacterium* and fungal strain *Penicillium*, was better than fungus or bacteria alone (Qu *et al*., 2010).

4.0 Conclusions

The decolorization of RB5 and RBBR dyes is higher in bacteria compared to fungus, due to adoption capability of bacteria. In this research, all the selected bacteria could utilize dyes as a sole source of carbon and energy unlike fungi, which need glucose as an additional source of carbon. However, the addition of glucose to the bacterial culture would improve its decolorization ability. The removal of RBBR in the liquid medium was less than RB5 by all selected microorganisms (fungi or bacteria), due to the chemical structure of RBBR are more stable than of RB5 (Banat *et al*., 1996; Novotny *et al*., 2001). In co-culture of *Enterococcus faecalis* ZL and *Rhizoctonia zeae* SOL3, the decolorization rate has decreased due to the adverse interaction between microorganisms. This is may be attributed to the starvation and accumulation of toxic products by other organisms present in the culture.

5.0 Acknowledgments

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