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Decolourization of Turqoise Blue (Remazol Blue BB) Dye by Immobilized *Penicillium* sp. into Sodium-Alginate-Sulfate Beads

Nor Atikah Husna Ahmad Nasir[1], Noor Farazian Zafira Che Pa[1], Muhammad Akmal Roslani[1], Rohayu Ramli[1] and Nor Azimah Mohd Zain[2]

[1] *Faculty of Applied Sciences, Universiti Teknologi MARA Cawangan Perlis, Kampus Arau, 02600 Arau, Perlis, Malaysia*

[2] *Department of Biosciences, Faculty of Science, Universiti Teknologi Malaysia, 81310 Skudai, Johor*

Authors' Email Address: atikah1388@uitm.edu.my [1]

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ABSTRACT

Turqoise blue (Remazol Blue BB) is a type of common dye which is constantly discharged from industries into the water bodies without proper treatment. This dye could affect aquatic and human life due to its toxicity. Existing methods to overcome this issue are too expensive and not eco-friendly. Alternatively, this study was conducted by immobilizing Penicillium sp. into sodium-alginate-sulfate beads (IC) to decolorize the turquoise blue dye at 10 ppm. The percentage of dye decolourization, Chemical Oxygen Demand (COD) removal and laccase of IC and free cells were analysed throughout this study. IC successfully decolourized dyes up to 72.83%, meanwhile, free cells could only decolourized dyes up to 56.59%. In addition, COD removal by IC cell is 31.92% higher compared to free cell. For laccase activity, IC is higher compared to free cells up to 30%. Based on higher decolourization, enzymatic activity and COD removal, IC has a potential to be an alternative to decolourize dyes better than free cells.

Keywords: *immobilized cells, free cells, decolourization, dyes, laccase*

INTRODUCTION

It is reported about 10,000 types of dyes are produced worldwide with over $7x10^5$ ton were used. However, 10% of this amount were frequently being discharged into water bodies are not treated (Nasir et al., 2013). This prolonged habit will affect the environment and cause harm towards aquatic plants and animals (Chen et al., 2003). Worst, most of the synthetic dyes used contained chemicals such as chlorine, formaldehyde (CH_2O) and heavy metals (Jaishankar et al., 2014).

In industries, reactive dyes such as turquoise blue dye (Remazol Blue BB) is widely used (Joshi et al., 2013). This is due to the cost effectiveness in synthesis compared to natural dyes. However, this feature makes them to be first recognized when discharged into the water bodies. Therefore, the colour must be removed before being discharged into the environment (Joshi et al., 2013).

There are a few physical and chemical methods that had been implemented to overcome this situation. However, most of the suggested methods bring drawbacks such as costly and large amount of waste might be produced after the treatments (Coelho et al., 2015). Thus, bioremediation by using microorganisms seem a great alternative to overcome the problem. Bioremediation offers an environmentally friendly, safe and cost effective by absorbing and degrading the dyes without causing harm towards the ecosystem.

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Microorganism such as fungus is preferred to be used in the study due to their morphology which able them to degrade and absorb better than bacteria (Al-Fawwaz and Abdullah, 2016). In this study, the ability of white rot fungi, *Penicillium* sp. to degrade and absorb the dyes had been studied. This fungus can secrete an enzyme known as laccase which can rapidly increase the decolourization activity and able to be maintained under unfavorable conditions (Murugesan et al., 2007). Additionally, the laccase enzymes are very important to reduce the aromatic ring into non-toxic forms (Fu and Viraraghavan, 2001).
However, there is a limitation in term of reusability and stability present when microorganism is used for the bioremediation. Thus, the idea of this study is to immobilize *Penicillium* sp. into sodiumalginate-sulfate beads. Through cell immobilization, cells will be protected from high environment such as acidity and extreme temperature (Chen et al., 2003; Nasir et al., 2017). Additionally, the immobilization matrix will mimic the natural habitat by stimulating ligninolytic enzymes production, depending on the support matrix (Devi and Nagamani, 2018). Thus, the stability and reusability of the cells could be overcome, as well reducing the operational cost.

METHODOLOGY

Inoculum Preparation

Penicillium sp. were inoculated on (PDA) Potato Dextrose Agar and incubated at 37˚C until mycelia growth formed. Then, spores were harvested after 7 days of incubation by using 1 % (v/v) Tween 80. Spores concentration was determined at 6.3 x 10^8 spores/ml using haemocytometer.

Immobilization of *Penicillium* sp.

10 ml of spore suspension from the inoculum preparation were mixed with sodium alginate (4 % w/v) solution for 20 minutes. Then, the mixture was added into 50 ml of 100 mM calcium chloride (2 % w/v) by using syringe to form alginate beads. The beads were stirred around 30-50 minutes before stored at 24 hours, 4 ˚C in sterilized distilled water (Rajendran et al., 2012).

Germination of Immobilized Fungal Spores

2% of malt extract was used to germinate growth of immobilized spores as a starting culture. The beads were incubated at 37˚C until the growing of biomass enters idiophasic growth. After 4 days, the growth medium was replaced aseptically with the turquoise blue dye colour solution (at 10 ppm) and nitrogen limited medium (1.5% glucose, 0.04% w/v malt extract,174 µM $MnSO_4.H_2O$, 0.0004% $MgSO_4.7H_2O$, 20 Mm 3,3-dimethylglutrate) (Nasir et al., 2017).

Colour Removal

Colour removal is measured based on American Dyes Manufacturer's Institute (ADMI) removal using spectrophotometer at 340 nm. The rate of decolourization is calculated as:

$$\text{Percentage of decolourization} = \frac{(A0-At)}{A0} \; X \; 100$$

A0 - initial absorbance
At - Absorbance at time "t"

Laccase Activity

One-unit enzyme activity is defined as the amount of enzyme required to reduce 1 µm of substrate min-1 (Kadam et al., 2011). Laccase test was performed for both immobilized cells and free cells at room temperature. 0.2 ml samples, 1.7 ml of sodium acetate buffer (20 mM, pH4), and 0.3 ml o-tolidine (1 mM stock) were mixed before absorbance was recorded at 436 nm.

$$(U/mL) = \frac{DA_{436}/min \times 4 \times V_t \times dilution\ factor}{A_0\ (e \times V_s)}$$

V_t - final volume of reaction mixture (mL)
V_s - sample volume (mL)
E - micromolar extinction co-efficient of tetraguaicol (cm^2/micromol) 4
- derives from unit definition and principle

Chemical Oxygen Demand (COD) Removal

Samples were centrifuged at 4000 rpm, 4 ˚C for 15 minutes before mixing them with 0.1 g of mercury sulfate and 2 ml of COD reagent (Nasir et al., 2013). When the vial cooled, spectrophotometer was used to record the absorbance using the following equation:

$$\text{Percentage of COD removal} = \frac{(A0-At)}{A0}\ X\ 100$$

A0- initial absorbance
At- Absorbance at time "t"

Statistical Analysis

The independent t-test was conducted to study if there were significant difference in immobilized and free cell towards the percentage of ADMI removal, laccase enzyme activity and the percentage of COD removal.

RESULTS AND DISCUSSION

Immobilisation of *Penicillium* sp. in Sodium-Alginate-Sulfate Beads

Figure 1 shows the immobilized *Penicillium* sp. into sodium-alginate-sulfate beads for the decolourization. Sodium-alginate-sulfate beads were used for fungal entrapment because it brings many benefits such as non-toxicity, eco-friendly, cost effective and best performance in terms of thermo stability, reusability and efficiency (Bilal and Asgher, 2015).

Figure 1: *Penicillium* sp. in sodium-alginate-sulfate beads

Colour Removal

Figure 2 shows colour removal between immobilized and free cell in six days in incubation. From the result, the reading of percentage was increased from day to day. Immobilized cell recorded the highest percentage of colour removal compared to the free cell in day 1 at 43.11% and 34.09% respectively. In day 2, there were slight increment of the colour removal percentage for both immobilized and free cell at 55.36% and 42.48% respectively. At this stage, it is believed the *Penicillium* sp. had adapted to the medium and started to secrete enzyme such as laccase for the decolourization (Rani et al., 2014).

Meanwhile in day 3, the immobilized cell recorded the percentage of colour removal at 63.53% and free cell recorded at 47.40%. Followed by day 4 where the percentage of the colour removal were recorded at 68.77% and 53.11% for immobilized and free cell respectively. The percentage were increased in day 5 at 71.87% for immobilized cell and 57.54% for free cell. Overall, the immobilized cells successfully decolourized the turquoise blue dye at 72.83%, meanwhile, free cells could only decolourized the dye up to 59.69% recorded in day 6.

From the result, the discolouration percentage by immobilized cells is approximately 13% higher than the free cells since the highest rate of colour removal recorded in day 6 at 72.83% and 59.69% for immobilized and free cell respectively. The colour removal might be attributed to the absorption and degradation enzymes secreted such as laccase (Nasir et. al., 2013; Rani et al., 2014). However, the independent t-test shows that there is no significant difference in immobilized and free cell towards the percentage of ADMI removal since $p>0.05$.

The higher decolourization by immobilized fungi might also be attributed to the bio-absorption on sodium-alginate-sulfate beads. In this state, sodium-alginate-sulfate beads act both as a support and absorbent for the dye absorption and this is proven by the increasing adsorption of immobilized beads (Gao et al., 2016).

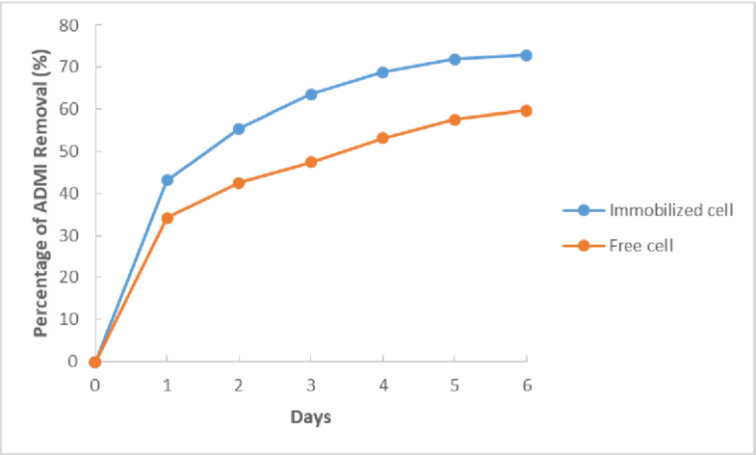

Figure 2: Turqoise blue (colour removal) between immobilized and free cell

Laccase activity

Figure 3 shows laccase activity of immobilized and free cells of *Penicillium* sp. in the degradation and biosorption of turquoise blue dye. From the result, the enzyme activity of immobilized cell recorded at 0.45 U/mL while, the free cell recorded at 0.29 U/mL. In day 2, it was recorded some increment in the activity of enzyme where immobilized and free cell recorded at 0.57 U/mL and 0.37 U/mL respectively. The enzyme activity recorded the increment in day 3 where both immobilized and free cell recorded at 0.68 and 0.41 U/mL respectively. In day 4 the immobilized cell recorded the increment of the enzyme activity at 0.73 U/mL meanwhile, there is a slightly reduction recorded in free cell at 0.39 U/mL. In day 5, the enzyme activity recorded at 0.79 U/mL (immobilized cell) and 0.48 U/mL (free cell). The highest laccase value is 0.82 U/ml for immobilized cells and 0.52 U/ml for free cells which was recorded in day 6. It is observed that the enzyme activity is more efficient in immobilized compared to free cells. This shows that the enzyme activity by immobilized cell is approximately 0.3 U/mL higher than the free cell.

Akpor, 2018 mentioned immobilized cell could offer advantages over free cells. The higher rate of immobilized cell might be attributed to the sodium-alginate-sulfate-beads that prevent the mechanical stress from the environment towards cells, which leads to higher enzymatic activities. In addition, the rate of laccase secreted by immobilized and free cell of *Penicillium* sp. steadily increased from day one. This analysis suggests an involvement of laccase in the dye decolourization via biodegradation. Laccase has long been associated with the ability of *Penicillium* sp. to degrade several organic pollutants (Senthivelan et al., 2019). However, the independent t-test result shows that there is no significant difference in immobilized and free cell towards the laccase enzyme activity since $p>0.05$.

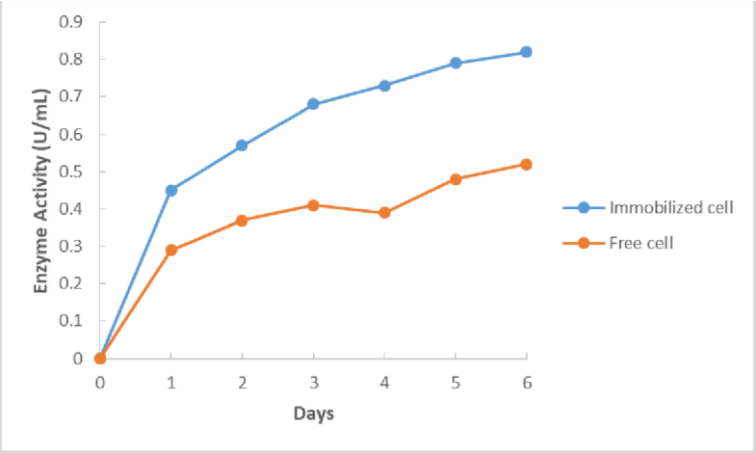

Figure 3: Laccase activity of immobilized and free cell

COD removal

Figure 4 shows the percentage of COD removal between immobilized and free cell within six days. COD removal signifies the quantity of oxygen required to oxidize the chemical present in liquid sample especially waste water. High COD value shows that the effluents have also high oxygen demanding wastes which causes the depletion of Dissolved Oxygen (DO) which is a fundamental requirement for aquatic life (Suryawan et al., 2018). Based on figure above, the rate of COD removal for both immobilized and free cell is increased from day one to day six of incubation. The highest removal was achieved by immobilized cells which is 62.17% meanwhile free cell is 30.25%.

The reduction in COD value indicates a corresponding reduction in the concentration of pollutants. Entrapment of *Penicillium* sp. in sodium-sulfate-alginate beads could prevent the by-product produced from degradation from contributing to high COD reading (Wijetunga et al., 2010). However, the independent t-test result shows that there is no significant difference in immobilized and free cell towards the percentage of COD removal since $p>0.05$.

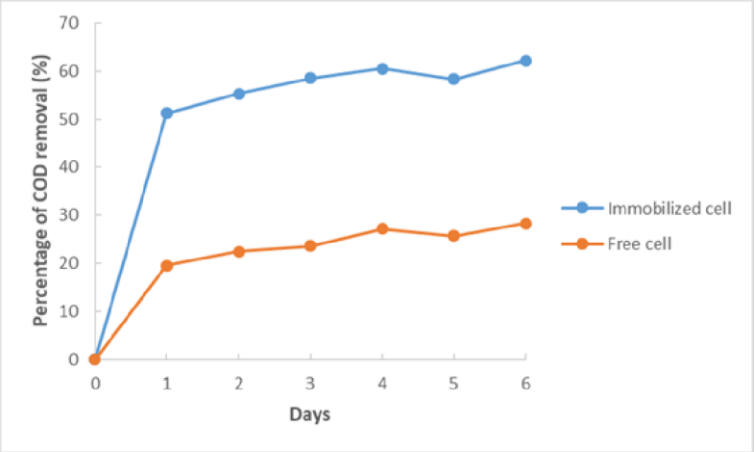

Figure 4: Percentage of COD removal of immobilized and free cell

CONCLUSION

Penicillium sp. was successfully immobilized in sodium-alginate-sulfate-beads to be used as decolourizers. Apparently, immobilization has to some extent improved the cells ability in the decolourization of textile effluents since immobilization cells can decolourize at 72.8% compared to free cells which is 56.59%. Meanwhile, the enzymatic activity also recorded higher result in immobilized cell compared to the free cell (which is 30% higher). Moreover, the decreasing rate of COD removal represents the ability of immobilized cells to degrade and decolourize the dyes. In short, immobilized cells proved the sodium-alginate-sulfate-beads could be used to help and support the process of decolourization of dyes.

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