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Decolourization of Methylene Blue by Resting Cells and Immobilized Cells of Rhodococcus Strain UCC 0003

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ABSTRACT

Methylene blue is extensively used in printing and batik industries in Malaysia. The increase in usage of coloured dyes which are toxic and long lasting in the natural environment can affect the water quality. Thus, there is a worthy technique which was developed as a cheaper way to achieve desired environment without hazards. The current study investigated the use of locally isolated Rhodococcus strain UCC 0003 for methylene blue dye decolourization. The decolourization of methylene blue dye was carried out in the two different modes namely resting cells and immobilized cells of Rhodococcus strain UCC 0003 in gellan gum matrix. The methylene blue removal using resting cells and immobilized cells after 24 hours of incubation resulted in 38 % and 73 % decolourization, respectively. Further characterization was proceeded with immobilized cells in gellan gum matrix to evaluate the potential of repeated use of the biocatalyst. The reusability of immobilized cells of Rhodococcus strain UCC 003 was carried out for 17 repeated cycles. The first cycle was initiated by adding 50 mL (0.5 g/L) of methylene blue solution in the Erlemeyer flask that contained 50 beads of gellan gum. The first 16 cycles resulted in complete decolourization within an hour incubation period for each cycle. Meanwhile at the 17th cycle, the decolourization efficiency dropped to 91%. This trend was repeated when fresh cells of Rhodococcus strain UCC 0003 immobilized in gellan gum were tested to decolourize 0.5 g/L of methylene blue. These findings clearly proposed that immobilized cells of Rhodococcus strain UCC 0003 in gellan gum matrix has huge prospects to remediate the industrial wastewater since the biocatalyst could be reused which optimized the cost and method is environmentally acceptable.

Keywords:
Decolourization; immobilization;
Methylene blue; resting cells;
Rhodococcus strain UCC 0003 strain;

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1. Introduction

Methylene blue is known as aniline-based dyes and heterocyclic chemical compound that is extensively used in the textile industry but later realized that it can be used as microscopy stains as well. In textile industry, methylene blue was a commonly used component for drying (or dying) cotton, wood and silk. Currently, there are various treatment processes such as coagulation, filtration, ozonation, oxidation process, membrane filtration, reverse osmosis and hydrogen peroxide which are applied to remove the colour from wastewater [8]. However, these processes are ineffective due to high cost and may produce secondary pollutants. So, researchers prefer biological treatment which is cost effective and eco-friendly method towards environment for textile wastewater bioremediation. Among various methods, biological method is a green technique to

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remove methylene blue from textile effluent with low cost and optimum operating time by applying biological materials such as fungi, algae, bacteria and yeast.

The genus Rhodococcus was recognised as one of the most potential microorganism besides Pseudomonads for remediation of pollutants as the strains have an extensive catabolic versatility, unique enzymatic capabilities and robust cellular physiology and which in turn, possess the ability to degrade large number of organic compounds, ranging from natural, xenobiotic to anthropogenic compounds with high toxicity and concentrations [4]. But, the application of microorganisms in biodegradation are challenging in term of reusability, long term usage and complexity in recovery [10]. Thus, immobilization is a method to improve long term stability under reaction condition and act as biocatalyst for biodegradation.

Biodegradation can be conducted via immobilization of growing cells, resting cells, partially purified enzymes or fully purified enzymes using numerous matrices such as hydrogel, calcium alginate, polyurethane foam, agar, cellulose triacetate gel and chitosan [1]. There are studies available on the use of immobilized cells to biodegrade methylene blue dye, however no works have been done on testing the capability of immobilized cells and resting cells of Rhodococcus strains to decolourize the methylene blue dye and also carry out reusability method using immobilized cells in gellan gum matrix for decolourization activity. Thus, the current study is an effort of using locally isolated immobilized Rhodococcus UCC 0003 strain for decolourization of methylene blue and the results suggests that the immobilized mode of strain will be able to provide better decolourization activity when applied in textile industry wastewater.

2. Materials and Methods

A. Chemicals

All the chemicals used in this study are of analar grade and commercially available in the markets. The chemicals were obtained either from Merck (Germany), Sigma (USA) or Fisher Scientific (Singapore).

B. Microorganisms

Locally isolated Rhodococcus UCC 0003 strain was used in this research. The isolate was obtained from Unisel Culture Collection Unit at Institute of Bio-IT Selangor in Universiti Selangor. The strain was kept in bead form in freezer at -80 ℃ and was resuscicated on nutrient agar plate.

C. Preparation of Starter Culture

The nutrient broth was prepared by dissolving 8.0 g of nutrient broth with 1.0 L of deionized water. Then, the media was sterilised by autoclaving at 121℃, 15 psi for 20 minutes. This will be followed by inoculating starter culture of the Rhodococcus strain UCC 0003 from nutrient agar plate into 50 mL nutrient broth medium in a 250 mL of Erlenmeyer flask in triplicate. The culture was incubated in an incubator shaker at temperature of 30℃ and agitation of 160 rpm for 24 hours. After 72 hours, the growth of starter culture was observed by measuring its optical density reading at wavelength of 600 nm (OD600) by using ultraviolet visual (UV-Vis) spectrophotometer using Biospectrophotometer Biomate 3, Thermo Scientific (USA) model with distilled water as blank. The OD600 value of starter culture was maintained at 1.2 to 1.3 prior to inoculation into the production medium [10].

D. Preparation of Resting Cells

Seventy-two hours grown culture of Rhodococcus strain UCC 0003 in nutrient broth was collected in 50 mL of falcon tubes. All the cells in production medium was harvested by centrifugation at 4℃, 16 000 × g for 30 minutes in falcon tubes and proceeded with washing with phosphate buffer (pH 7) twice. At last, the collected Rhodococcus strain UCC 0003 cells were stored in phosphate buffer and refrigerated at 4℃ until further use as resting cells and for the preparation of immobilized cells.

E. Preparation of Immobilized Cells

The preparation of immobilized cells of Rhodococcus strain UCC 0003 in gellan gum followed the procedures established by Maniyam *et al*., [10], with minor changes. Resting cells amounting to 25 mL was used for immobilization. The control has been prepared without adding the resting cells.

F. Decolourization of methylene blue by Resting cells and Immobilized cells of Rhodococcus strain UCC 0003in gellan gum matrix.

An amount of 25 mL of methylene blue solution was added into 250 mL Erlenmeyer flask and added with 25 mL of resting cells and 50 gellan gum beads (corresponding to 25 mL of resting cells). The flasks were prepared in triplicates for each mode of inoculum. Then, the flasks were incubated at 30℃ for 24 hours under static condition.

G. Reusability in Gellan Gum Matrix

After 30 minutes of incubation in the biodegradation medium containing phosphate buffer and 25 mM KCN, the immobilized beads containing of Rhodococcus strain UCC 0003 were recovered by filtration through a sieve. The beads were washed with sterilized 0.9% (w/v) sodium chloride solution and soaked with 0.3 M calcium chloride solution for 2 hours. Then, the beads were removed from calcium chloride solution and washed repeatedly for 3 times with sodium chloride solution again. The washed beads subsequently added to fresh decolourized solution and biodegradation of methylene blue was carried out as described above for 30 minutes. The process was repeated for several batches until efficient removal of methylene blue dye deteriorated.

H. Analytical Methods
I. Decolourization Assay

Approximately 1 mL of sample was centrifuged at 16 000 × g for 30 minutes at 4 C at 0 hour and 24 hours of incubation period, respectively. Then, the supernatant was collected to record the percentage of decolourization. The decolourizing activity was evaluated in terms of decolourization percentage by calculating the decrease in absorbance for methylene blue wavelength at 620 nm. Decolourization activity (%) was calculated as [(A-B)/A] × 100%.

A= initial absorbance

B= observed absorbance

3. Results and Discussion

A. Decolourization of Methylene Blue Dye by Resting Cells and immobilized cells in Gellan gum matrix of Rhodococcus strain UCC 0003

The decolourization of 0.5 g/L of methylene blue solution in two different modes was shown Table 1.

Table 1
Decolourization of methylene blue by resting cells and immobilized cells of Rhodococcus strain UCC 0003 n gellan gum matrix

Matrices	Methylene Blue removal (%)
Resting Cells	38
Gellan Gum	73

Table 1 shows the efficient removal of methylene blue dye by resting cells and immobilized cells of *Rhodococcus* strain UCC 0003, respectively. The gellan gum matrix recorded the highest percentage in removal of methylene blue dye significantly ($p < 0.05$) when compared to resting cells which is decolourized at 38%. Meanwhile, the complete decolourization efficiency was noted using immobilized cells in gellan gum matrix is 73% which 35% higher than resting cells on methylene blue removal. These results serve that immobilized cells of *Rhodococcus* strain UCC 0003 in gellan gum exhibited higher capability in decolourizing methylene blue compared to resting cells. The cells embedded in gellan gum showed greater catalytic activity stability in comparison to resting cells as proof for higher percentage of methylene blue removal. Gellan gum acts as the best entrapmet method with properties such as having high binding capacity, tolerance towards high acidic conditions, resistant to temperature and the matrix provides chemical and mechanical strength [5]. This finding is extremely significant for proving the fact that methylene blue removal can be efficiently carried out by immobilized cells of *Rhodococcus* strain UCC 0003.

A. Reusability of Immobilized Cells in Gellan Gum Matrix of Rhodococcus Strain UCC 0003

One of the significant features of immobilized cells is that the cell can be recycled or reused for several times. Therefore, this work was conducted to evaluate the potential of reusing the immobilized cells of *Rhodococcus* strain UCC 0003 for decolourization of methylene blue in order to consider the encapsulated cells in gellan gum as a desired industrial biocatalyst. Figure 1 demonstrates the reusability of the immobilized cells of *Rhodococcus* strain UCC 0003. The methylene blue dye decolourization activity was measured in the system containing the immobilized cells exposed to 25mM KCN. The control sets contained the immobilized beads without the resting cells.

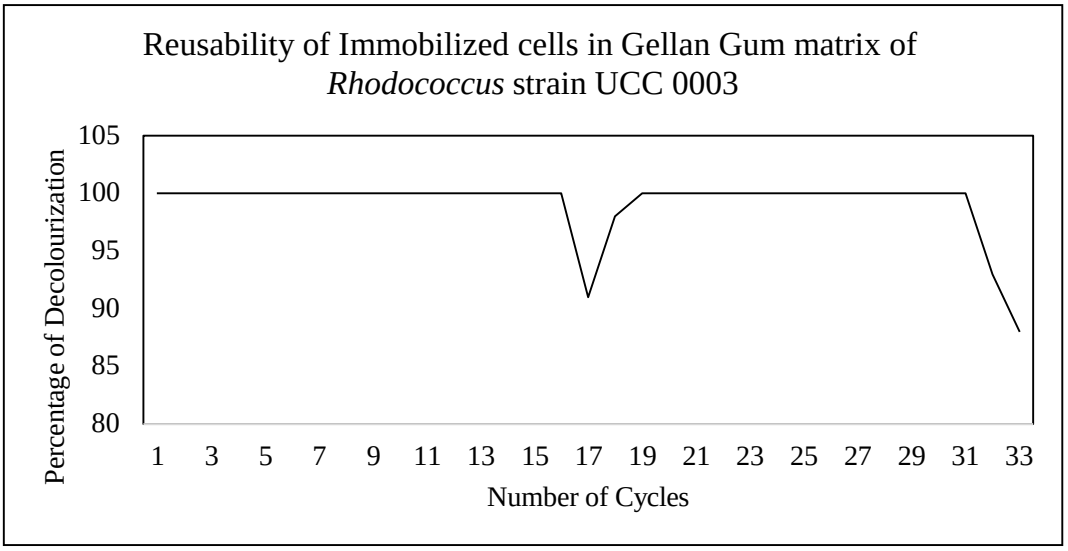

Fig. 1. The reusability of the immobilized cells of *Rhodococcus* strain UCC 0003 in gellan gum matrix for methylene blue dye decolourization activity

The performance of reusability of the immobilized cells was observed for 33 successive cycles of methylene blue dye decolourization as shown in Figure1. Interestingly, there is very minimal loss of methylene blue observed in the control system confirming that methylene blue removal was due to decolourization act by *Rhodococcus* strain UCC 0003. The immobilized cells successfully exhibited 0.5 g/L methylene blue decolourization activity with 100% colorant removal without any marked activity loss when used at least for sixteen times as batch studies. Batch seventeen observed decrease in percentage of decolourization to 91% and the percentage of methylene blue removal increased to 100 % at eighteenth cycle due to the use of fresh immobilized cells. The immobilized cells managed to retain methylene blue decolourizing ability with 100% dye removal until batch 31. Cycle 32 witnessed declined percentage of decolourization to 93% and further reduced to 88% at batch 33. Thus, the findings collected in the current study indicated that the immobilized cells of *Rhodococcus* strain UCC 0003 can be employed as a significant biocatalyst since the immobilized cells can be easily recovered from the reaction solution unlike free cells which obviously reduces the cost of treatment for decolourization of methylene blue. In addition, these biocatalysts could be reused for 16 cycles without any loss of activity indicating the practicality if utilizing this local isolate for actual treatment of textile wastewater.

4. Conclusion

Bacteria is one of the better options for decolourization of methylene blue and act as a biocatalyst due to higher mass production and biodegradation. In this study, the locally isolated *Rhodococcus* strain UCC 0003 was used to decolourize the methylene blue dye. This strain presented 73% decolourization activity using immobilized cells in gellan gum compared to resting cells. Furthermore, the performance of reusability of the immobilized cells was carried out to decolourize 0.5 g/L of methylene blue solution. The first 16 cycles showed complete decolourization activity which proved to be advantageous economically. These findings provided sufficient data for implementing the protocols for real application in treating textiles industry wastewater especially from the growing batik industries in Malaysia. Further characterization will be carried out with HPLC and FTIR studies to determine the biodegradation mechanism performed by *Rhodococcus* strain UCC 0003.

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