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Bioremediation of Methylene Blue from Synthetic Wastewater using Immobilized Cyanobacterial Consortium Beads

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***Abstract*- Treating wastewater to remove dye economically and ecofriendly is a need of the hour. Since methylene blue is being used by various industries and has various environmental problems and health hazards associated with it, therefore it has been selected for the present case. Present study aims at bioremediation of methylene blue from synthetic wastewater using a calcium alginate immobilized cyanobacterial consortium collected from local pond nearby the campus of CVRCE, Bhubaneshwar, India. Growth study of the strain has been done. Optimization of methylene blue removal has been done by varying its initial concentration in the range of 25-75 mg/L. Maximum removal of 93% has been observed in the study using the immobilized beads in a batch process.**

Keywords— Bioremediation; methylene blue; cyanobacterial consortium; calcium alginate beads

1. Introduction

Dye contamination of water bodies has become an acute problem in today's world. Its elimination from water bodies by applying ecofriendly and low cost methods is a requirement of the hour. Methylene blue (MB) is a very common dye and is used by various textile, timber and paper industry [1, 2]. It has different health hazards associated with it when come in human contact such as diarrhea, gastritis, vomiting, breathing problem etc [3]. Moreover, it also has adverse effect on aquatic ecosystem. Therefore proper treatment of dye wastewater before being discharged into waterbodies is mandatory. Though several techniques have been used for treating dye wastewater such as oxidation [4], adsorption [5], electrochemical techniques [6] etc, each of these methods have their own constrains. Under such circumstances biological method can be an alternative pathway for dye removal from wastewater. In the present study cyanobacterial [7] consortium collected from a local pond has been used for the removal study of the methylene blue from simulated wastewater. Immobilization of calcium alginate has been done using the standard protocol as described by [8, 9]. For immobilization purpose calcium alginate has been used as carrier matrix because of its ability to absorb MB partially.

2. Material and Methods

2.1. Study of the growth kinetics of cyanobacterial consortium

The micro-algal growth is highly influenced by factors such as concentration of nutrient, light intensity and temperature. The nutrients necessary for their growth are nitrogen, sulphur, carbon and phosphorus [10]. For studying the growth kinetic of the strains, they were grown in BG-11 media [11]. Inoculum of 10% of the test strain consortium was added to 300 mL of sterile BG-11 medium aseptically taken in 500 mL Erlenmeyer flasks. The flasks then were kept in algal incubator under the illumination of 2500 lux at 25°C temperature with 16 h: 8 h as day and night cycle for 14 days. The sample analysis were done with interval of two days. The collected sample is centrifuged at 5000 rpm for 15 minutes, twice for better results. The biomass obtained as the residue was washed with distilled water for residual salt removal and then dried at 60^{o}C overnight in an air oven. Finally the biomass obtained has been utilized for analyzing the growth of the consortium in terms of dry biomass concentration

2.2. Immobilization of cyanobacterium consortium using calcium alginate

The cyanobacterium consortium was immobilized by forming calcium alginate beads and was used for methylene blue removal from the synthetic wastewater. Immobilization was done following standard protocol was described by Kathiravan et al. [12,13]. For getting significant amount of biomass, the culture was primarily grown in BG-11 media under aseptic conditions for 14 days. The culture was then centrifuged at 5000 rpm for 10 mins. The residual biomass was then washed thoroughly with sterile distill water and then centrifuged again at the same rpm for 10 mins more. It was then added to 0.15 L of sodium alginate solution of 30 g/L concentration. The mixture was then added dropwise to a 2 (M) chilled and sterile $CaCl_2$ solution. The calcium alginate were then added into the $CaCl_2$ solution and left for hardening at around 4^{o}C for 24 hours. The beads were put in storage under water at around 4^{o}C. The final formed calcium alginate with immobilized calcium alginate beads is shown in Fig. 1.

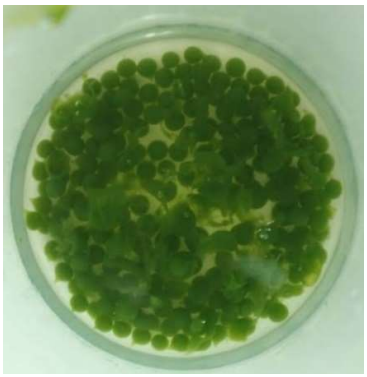

Figure 1. Cyanobacterial Consortium immobilized in Calcium Alginate beads

2.3 Study of removal of methylene blue from synthetic wastewater

Methylene Blue (MB) solutions of different concentrations (25 mg/L- 75 mg/L) were prepared from the sterile stock solution of (100 mg/L) by diluting it with sterile growth media (BG-11) solution under aseptic condition in a bio-safety cabinet (Lunar, India) (Fig. 2).The test strain calcium alginate beads were added to the prepared synthetic wastewater taken in 500 mL Erlenmeyer flask aseptically. To study the methylene blue removal kinetics, the culture was grown for 14 days. The strain culture was then grown in the algal incubator and shaker for 14 days at 900 rps and same conditions as mentioned above. Samples are withdrawn after every two days and was centrifuged at 6,000 rpm for 10 min to separate the beads. The supernatant was analyzed for residual MB concentration using a UV-Vis spectrophotometer (UV-VIS -2300, TECHCOM) at a wavelength of 660 nm.

Figure 2. Methylene blue solution with immobilized cyanobacterial beads

3. Result and Discussion

3.1 Study of the growth kinetics of cyanobacterial consortium

The change in dry biomass concentration with time is shown in Figure 3. From the figure it is observed that the consortium is in lag phase till second day. From third day onwards the growth has increased intensely till day ten indicating log phase or growth phase, after which the consortium enters into stationary phase till fourteen day.

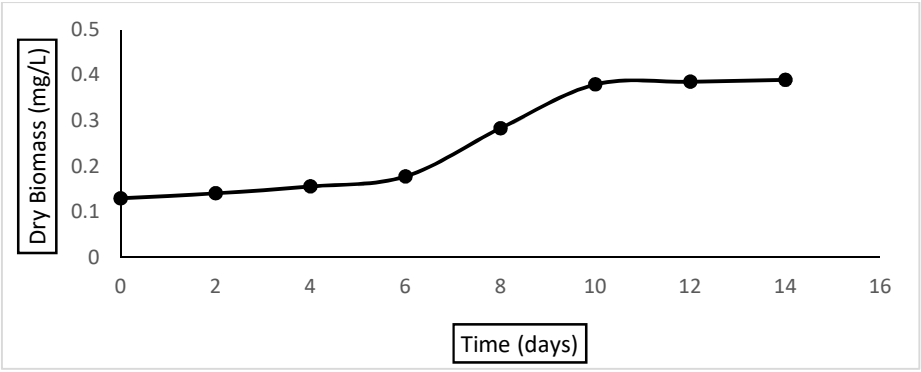

Figure 3. Growth study of cyanobacterial consortium in BG-11 media

3.2 Utilization of calcium alginate immobilized cyanobacterial consortium for methylene blue removal in batch study

Removal of Methylene Blue has been studied using immobilized cyanobacterial consortium beads. The initial concentration of MB has been varied as 25 mg/L, 50 mg/L and 75 mg/L. The beads dosage was kept constant of 20 g/L with a shaking speed of 900 rps (Fig. 4). From the graph of the figure 2. It is seen that the maximum removal of 93% is obtained with initial MB concentration of 25 mg/L and minimum removal of 74.4% is obtained with initial MB concentration of 75 mg/L. It is observed that the percentage removal decreases with the increase in initial concentration. It may be because of the exertion of the bioremediation capacity of the immobilized cyanobacterium beads with increased initial MB concentration [14]. Moreover it is also observed that the removal capacity by the strain is reduced after day 10. It may be because the strain enters into its stationary phase as observed during its growth study.

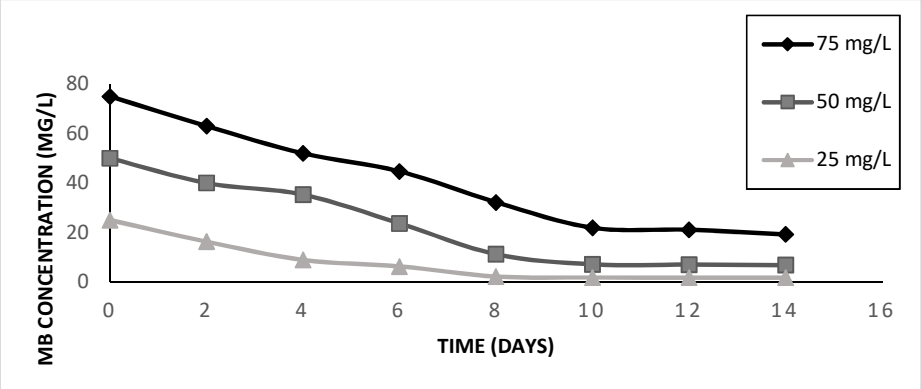

Figure 4. Decrease in MB concentration with time for different initial MB concentration.

4. Conclusion

Methylene Blue being a very harmful and extensively used dye in textile industry, paper and pulp industry etc. has been chosen for removal study in the present case. Removal of MB has been studied in batch process using cyanobacterial consortium immobilized in calcium alginate. Removal as high as 93% has been obtained during the kinetic study of removal in batch process. Use of calcium as support matrix for immobilization has enhanced the removal process due to its capability to absorb MB partially. Thus utilization of calcium alginate immobilized cyanobacterial consortium for removal of methylene blue can be seen as a viable option for greener world.

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