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Magnetite nanoparticle aided immobilization of *Pseudomonas* sp. GBS.5 for carbazole degradation

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Abstract

Pseudomonas sp. GBS.5 is a newly isolated biosurfactant producing and carbazole degrading bacterium. In the present study, this bacterium was coated with magnetite nanoparticles, synthesized using co-precipitation method. Scanning electron microscopy (SEM) studies confirmed the coating of the bacterial surface with these nanoparticles. Degradation activity of the coated cells obtained was 1.4 ppm/min as compared to 0.32 ppm/min for free cells and could be reused for five different cycles. These results indicate that magnetite nanoparticle can be efficiently used for the immobilization of biosurfactant producing bacteria involved in the degradation of polyaromatic compounds.

Keywords: Carbazole, Immobilization, Magnetite nanoparticles, *Pseudomonas* sp.

Introduction

Carbazole is a polyaromatic hydrocarbon (PAH) that is carcinogenic and mutagenic in nature (Jha et al 2002). Industrialization and increasing use of fossil fuel has led to the increase in concentration of carbazole in the soil. Chemical and physical methods for the removal of such contaminants are expensive and have limited effectiveness. Bioremediation is an emerging technology and several microorganisms are reported for the removal of these compounds. For commercial application, the recycle of the biocatalyst is an important factor determining the effectiveness and the cost of the process. Entrapment is the traditional method for the immobilization and separation of cells. The development of an immobilization process for carbazole degradation was initiated by Wang et al (2007) by entrapping magnetite nanoparticles along with the *sphingomonas* sp. cells inside the gellan gum gel beads. The separation and reusability of the cells was achieved but had mass transfer limitation. To overcome this limitation, Li et al (2013) proposed the coating of *Sphingomonas* sp. cells with magnetite nanoparticles. The degradation activity observed was comparable to free cells along with the ability to be reused.

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PAH are hydrophobic in nature and thus the biodegradation studies are primarily carried out in the presence of organic solvents and surfactants. *Pseudomonads* are the best known bacteria capable of utilizing hydrocarbons and producing biosurfactants at the same time. Several species of *Pseudomonas* are also reported for the degradation of carbazole. However, GBS.5 is the only *Pseudomonas* species reported to produce biosurfactant during carbazole degradation (Singh et al 2013). Since biosurfactant plays a key role in increasing the bioavailability of PAH compounds, this study aims at increasing the degradation activity of immobilized cells by coating the biosurfactant producing *Pseudomonas* sp. GBS.5 cells with magnetite nanoparticles. The degradation activity of coated cells was compared with the free cells and the reusability of the cells was also studied.

Materials and methods

Chemicals

Carbazole was purchased from Acros Organics (United States). Solvents used (acetonitrile, acetone, ethyl acetate) were of HPLC grade from Qualigens. Other materials were of analytical grade and available commercially.

Strain and cultivation condition

The bacterial strain *Pseudomonas* sp. GBS.5 was isolated in our laboratory as mentioned by Singh et al (2013). Briefly, the soil sample collected from a dye industry in Ahmedabad (23.03° N, 72.58° E) was incubated in the BSM media containing 3 mM carbazole. The media (1 liter) contained 2.44 g of KH_2PO_4; 5.57 g of Na_2HPO_4; 2 g of Na_2SO_4; 2 g of KCl; 0.2 g of $MgSO_4$; 0.001 g of $FeCl_3.6H_2O$; 0.02 g of $MnCl_2.4H_2O$; 0.003 g of $CaCl_2.2H_2O$. After 4 days of incubation 5% of the media was transferred to the fresh media. This procedure was repeated four times. The isolate obtained was selected based on the maximum carbazole degradation activity. Biodegradation of carbazole by free cells was studied by incubating 0.12 gm (dry cell weight) in 50 ml media. Cultures were incubated at 30 °C at 180 rpm.

Preparation of magnetite nanoparticles

Magnetite nanoparticles were prepared by coprecipitation method. $FeCl_3.6H_2O$ and $FeSO_4.7H_2O$ were mixed in the ratio of 1:3 in 200 ml of water under nitrogen gas. $NH_3.H_2O$ and oleic acid was added to the above mixture and the solution was kept at 85 °C for 30 min. The surface of the nanoparticles so formed was then modified with $NH_3.H_2O$. The nanoparticles were separated from organic solvent either by centrifugation or magnetic decantation and suspended in aqueous solution.

Coating of bacterial cells with magnetite nanoparticles

Coating of bacterial cells was performed by mixing nanoparticles and bacterial cells in the ratio of 1:10 (DCW). The mixture was incubated at room temperature for ten minutes. Uncoated cells were separated by magnetic decantation.

Analytical methods

Bacterial cells coated with magnetite nanoparticles were characterized using Scanning Electron Microscopy (SEM). Cells (coated and non-coated) were harvested during exponential phase and the pellet was washed to remove any media component. Fixation of the cells was carried using 2.5% glutaraldehyde. The cells were gold coated and viewed using a Scanning Electron Microscope Zeiss EVO40 at 10K X magnification. Quantification of carbazole was performed using High Performance Liquid Chromatography (HPLC, Waters associates, Milford, MA). After extraction with ethyl acetate, separation was achieved with a C8 column (3.3 µm pore size; Waters RP 8; 150 x 4.6 mm). CAR detection was performed at 233.7 with a photodiode array detector (PDA 2996; Waters).

Results and Discussion

Characteristics of bacterial cells coated with magnetite nanoparticles

The nanoparticles synthesized using coprecipitation method is reported to exist in monodisperse state in aqueous phase as the surface of the nanoparticles is hydrophilic. These cells were easily redispersed when the magnetic field was removed. This way the separation of the cells from the media becomes easier. Cells immobilized via entrapment require special techniques like centrifugation, affinity chromatography, or designing of special bioreactors for the separation of the entrapped cells from the media. These separation methods are either costly or time consuming. However, the cells coated with magnetite nanoparticles can be easily separated by the use of magnetic field. Wang et al. 2007 has reported the separation of cells by using magnetic field by entrapping magnetite nanoparticles inside gellan gum gel beads.

Fig. 1 shows the SEM image of the *Pseudomonas* sp. cells coated with magnetite nanoparticles. It can be seen in the figure that the nanoparticles are coated over the bacterial surface.

Biodegradation of carbazole by immobilized cells

In order to characterize degradation activity of magnetite nanoparticles coated *Pseudomonas* sp. cells, time course of carbazole degradation by free cells and coated cells was studied. Fig. 2 shows the time course of carbazole degradation by free cells and coated cells. Both coated and free cells showed complete utilization of carbazole in 4 h. However, the coated cells showed 90 % of carbazole utilization in first 2 hrs whereas free cells were able to utilize only 60 % carbazole during the same time. The degradation rate of carbazole for coated cells and uncoated cells was 1.4 ppm/min and 0.32 ppm/min, respectively. This increase in degradation activity of coated cells could be attributed to the increase in membrane permeability due to the coating of bacterial surface with magnetite nanoparticles. From an industrial perspective, coated cells will take half the time as compared to free cells to complete the task, greatly reducing cost and labor.

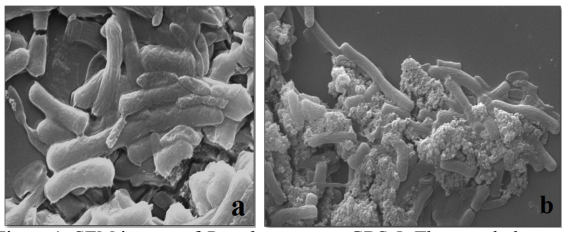

Figure 1: SEM images of *Pseudomonas* sp. GBS.5: The morphology of the cells was observed under the Scanning Electron Microscope (Zeiss EVO40) at 10K magnification. (a) Free cells, (b) cells coated with magnetite nanoparticles.

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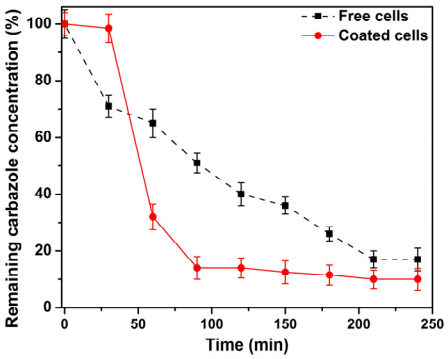

Figure 2: Time course of carbazole degradation by free and magnetite nanoparticles coated cells of strain GBS.5. The values are means of three independent replicates. SD was within the acceptable range.

The degradation activity of gellan gum immobilized cells was also less as compared to nanoparticles coated cells indicating that in addition to overcoming mass transfer limitation, this also helps in increasing the membrane permeability of coated cells.

The same cells coated with magnetite nanoparticles were tested repeatedly in a reaction mixture containing 100 ppm carbazole. Each cycle was carried out for 5 hrs. For reuse, cells were separated from the media by placing a permanent magnet at the side of the flask. After few minutes, supernatant was discarded and the coated cells were obtained. The cells were then resuspended in BSM supplemented with 100 ppm carbazole. BSM with magnetite nanoparticles was used as a negative control. All test and control experiments were conducted in triplicates. Fig. 3 represents the reusability of carbazole by the coated cells in different cycles. The degradation ability of the cells decreased gradually after few cycles. The degradation was found to be 90% for first cycle, 85% for second, 65% for third, 67.5% for fourth and 67% for fifth, each cycle being 4 h long. This decrease in degradation activity (Fig. 4) with each subsequent cycle can be attributed to loss of cells during each subsequent cycle or loss of degradation efficiency of the cells during reuse. Although, the carbazole degradation efficiency decreases in each subsequent cycle, still this approach is better as compared to free cells. Free cells when separated by centrifugation and reused in the next cycle showed only 10 % of degradation efficiency.

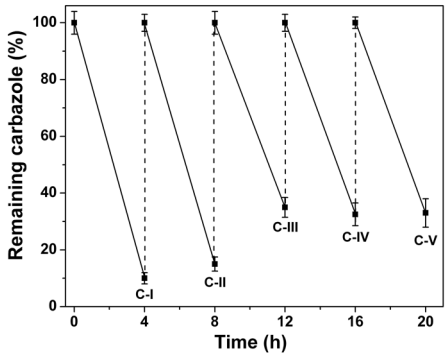

Figure 3: Reusability studies of carbazole degradation by GBS.5 cells coated with magnetite nanoparticles. The values are means of three independent replicates. SD was within the acceptable range.

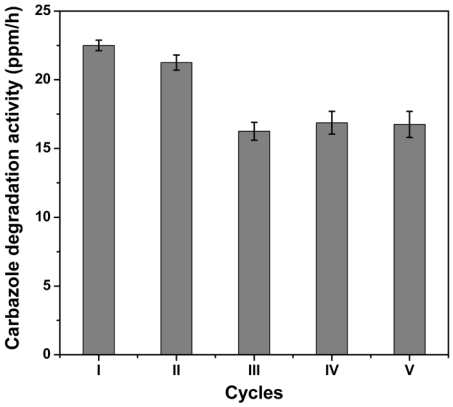

Figure 4: Carbazole biodegradation activities of *Pseudomonas* sp. GBS.5 cells coated with magnetite nanoparticles. The values are means of three independent replicates. SD was within the acceptable range.

Conclusion

In conclusion, method for immobilization of cells using magnetite nanoparticles was evaluated for the degradation of carbazole. Both the increase in degradation activity and reusability of coated cells as compared to free cells makes this technique a valuable tool for the immobilization studies.

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