ENHANCEMENT THE BIODEGRADATION OF NITROGEN POLYAROMATIC HETEROCYCLIC COMPOUNDS BY ASSEMBLING MAGNETIC NANO-SORBENTS ON MICROBIAL CELL

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Abstract
Microbial cells of Gram +ve *Bacillus clausii* BS1 were coated by magnetic MFe_3O_4 nanoparticles (MNPs), which have good adsorption capacity towards carbazole (CAR) 95 µmol/g. The transmission electron microscope (TEM) analysis showed that the MNPs were efficiently assembled and adsorbed on the microbial cells. The coated cells not only showed higher biodegradation capabilities towards CAR, but could be also reused for four successive cycles, characterized by high storage and operational stabilities and have the advantage of magnetic separation.

Keywords: Petroleum, Pollution, Bacteria, Biotechnologies, Mediterranean Sea

Introduction
The persistent organic pollutants; nitrogen polyaromatic heterocyclic compounds (NPAHs), including CAR and its derivatives are released into the environment from oil spills, wastes and effluents of several industrial activities; oil drilling, refining and storage, coal tar processing, chemical manufacturing and wood preservation. CAR is known to be mutagenic, toxic and readily undergoes radical chemistry generating the genotoxic hydroxynitrocarbazole [1]. Microbial degradation can be safely applied to remediate these pollutants. However, one of the major drawbacks of bioremediation is being a lengthy process. Not only this, but the stability, reusability and separation of the applied microorganisms after the process completion are also drawbacks that should be solved. This work aims to solve these problems by applying the large surface to volume ratio and low toxic MFe_3O_4 NPs.

Experimental work
The previously prepared MFe_3O_4 NPs [2] were used to decorate a Gram +ve *Bacillus clausii* BS1 previously isolated for its ability to degrade CAR [1]. A comparative kinetic study was performed to follow up the biodegradation (BD) of 1000 ppm CAR in batch aqueous systems over a period of 7 days at room temperature and 150 rpm, using free and coated cells. High Performance Liquid Chromatographic (HPLC) analysis was used to quantitatively estimate the CAR-BD.

Results and discussion
The MFe_3O_4 NPs (9 nm) were efficiently assembled on the surface of the microbial cell (0.43 µm x 1.96 µm) (Figure 1), because of the large specific surface area of the prepared MNPs (≈110.5 m^2/g) and its high surface energy [2,3]. Due to the superparamagnetic properties of Fe_3O_4 [2], the MFe_3O_4-coated cells were easily separated by applying external magnetic field (Figure 2), recycled and reused for four successive cycles without losing its BD-efficiency. The MNPs expressed non-toxic effect on the *B. clausii* BS1. Approximately complete removal of CAR was achieved and the CAR-BD rate was doubled using coated cells. CAR-BD process followed the first order kinetic model ($R^2 > 0.92$), with rate constant (k = 0.0221 and 0.0107 h^{-1}) and half-life ($t_{1/2}$ = 31.36 and 64.78 h) for coated and free cells batch processes, respectively. The coated cells could be stored for 30 days at 4°C without losing its CAR-BD activity. Not only this, but it could be also used over 672 h without losing its CAR-BD efficiency. While the free cells could be used only once, lost half of its activity after storage for 30 days at 4°C and could be only separated by centrifugation or filtration. The inhibition effect of the toxic intermediates; anthranilic acid (ANA) and catechol (CAT) was also studied. The free cells were highly affected by ANA and CAT. But, the coated cells retained its maximum CAR-BD efficiency up to 370 ppm of ANA and CAT, which were further faster degraded to cis,cis muconate, that would enter into the bacterial TCA cycle.

Conclusion
The new technique of applying a magnetized-nano-biocatalyst for the biodegradation process is very promising as it has the advantages of magnetic separation, easy recovery, high storage and operational stabilities, can be reused for successive times, overcome the toxicity and inhibition effects of the byproducts, appears not to experience a mass transfer problem, and increases the rate of biodegradation of the recalcitrant high molecular weight NPAHs.

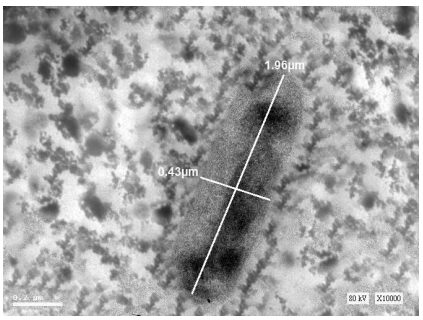

Fig. 1. TEM image of MFe_3O_4 coated *B. clausii* BS1.

Fig. 2. Collecting coated cells suspension by application of external magnetic field.

References
1 - Zakaria B.S., Nassar H.N., El-Gendy N.Sh. and El-Temtamy S.A. and Sherif S.M. 2015. Denitrogenation of carbazole by a novel strain *Bacillus clausii* BS1 isolated from Egyptian coke (Accepted in press).
2 - Zaki T., Saed D., Aman D., Younis S.A. and Moustafa Y.M. 2013. Synthesis and characterization of MFe_3O_4 sulfur nanoadsorbents. *J. Sol-Gel Sci. Technol.*, 65: 269–276.
3 - Saed D., Nassar H.N., El-Gendy N.Sh., Zaki T., Moustafa Y.M. and Badr I.H.A. 2014. Enhancement of pyrene biodegradation by assembling MFe_3O_4 nano-sorbents on the surface of microbial cells. *Energ. Source. Part A.*, 36: 1931–1937.