A Novel Approach in Biodegradation of low concentrated water pollutant, Diethylhexyl phthalate: using Self-Aligned Facile Silver Nanoparticles

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Introduction

- Phthalate esters (PEs) are benzo-carboxylic acids used in the manufacture of PVC and other plastic products
- These are priority chemical pollutants-listed as potential endocrine disruptors
- PEs are not covalently bond to polymer matrixes and tend to leach readily into the environment
- Bacterial strains in the environment are efficient in utilising PEs
- Biodegradation rate of PEs decreases as the molecular weight of PEs increases
- Apart from molecular weight- concentration, chemical nature and efficiency of micro organisms are important factors affecting biodegradation of PEs
- Based upon the chemical properties of PEs: C_{10}, C_{11} and C_{13} esters are most hydrophobic and least soluble in water (< 0.001 mgL^{-1})
- Hydrophobicity and low concentration reduces the probability of PE contact to bacterial cells, in turn decreasing the rate of efficient degradation

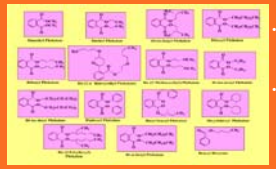

Fig. 1 Group of Phthalate esters

- To overcome this- improved cell surface and hydrophobic resistant bacterial cells are needed
- Silver nanoparticles being good catalysts for most chemical reactions, an attempt was made to biosynthesise self-aligned silver nanoparticles (SA-Ag-NPs) over bacterial cell surface
- Effect of SA-Ag-NPs in improving hydrophobicity and surface area for biodegradation was studied
- DEHP-a high molecular weight PE, commonly used plasticiser was selected for the study

Objectives

- Assessment of bacterial strain to hydrolyse and degrade DEHP at low concentration
- Biosynthesis of facile self-aligned silver nanoparticles (SA-Ag-NPs) onto bacterial cell surface
- Analysis of SA-Ag-NPs toxicity over bacterial growth and tendency to improve degradation of low concentrated pollutant, DEHP

Methodology

Isolation of DEHP utilising bacteria and optimisation of growth parameters → pH, temperature, time, substrate concentration

Synthesis of self-aligned silver nanoparticles (SA-Ag-NPs) → Absorbance at 420 nm, size, shape, assembly on bacterial cell surface, toxicity

Effect of DEHP degradation at various minimal concentration (1, 10, 50, 100, 500 and 1000 µg L^{-1}) with and without SA-Ag-NPs → Morphological changes, growth rate, total utilisation of low concentrated DEHP

Results and Discussion

- Bacterial strain used in the study was capable of utilising DEHP at the concentration of 1000 mg L^{-1} and the complete degradation occured within 144 h
- The nature of bacterial growth in MSM changed based on the concentration of DEHP available; from small granular pattern to aggregated cells floating over the medium.
- Changes in growth morphology may be due to stress, hydrophobicity, cellular toxicity, nature of interaction and availability of substrate

Synthesis of facile SA-Ag-NPs

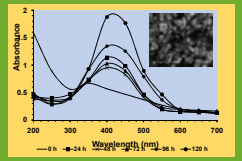

Fig. 2. UV-Visible spectra of SA-Ag-NPs; inset-SEM photograph of Ag-NPs

- Exitation of surface plasmons due Ag-NPs increased with the increase of time period upto 120 h

Fig. 3 Bacterial growth in MSM without and with SA-Ag-NPs

- After 72 h, bacterial growth without SA-Ag-NPs reveals the presence of DEHP onto the surface of the cells
- Bacterial cells with SA-Ag-NPs are less glossy revealing efficient utilisation of DEHP

Morphological changes in bacterial cells in response to DEHP

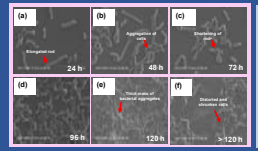

Fig. 4 without SA-Ag-NPs

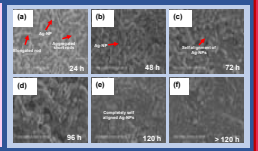

Fig. 5 with SA-Ag-NPs

- The minimal concentration 1 µg L^{-1} followed by 10, 50, 100 and 500 µg L^{-1} did not show any morphological changes.
- The cells were elongated, did attach to each other nor formed aggregates (Fig 4a).
- While at 1000 µg L^{-1} concentration- aggregation of cells, decrease in number of elongated cells, shortening of rods and and formation of thick mass of colonies occured (Fig. 4b, c, d and e)
- Beyond 120 h physical and morphological structure of bacterial cells distorted and shrunk (Fig. 4f).
- Aggregation of bacterial cells could be an adaptation response to DEHP, in order to decrease interaction with toxic substrate and metabolites formed during degradation.
- Similar morphological changes occured even among with SA-Ag-NPs trated bacterial cells (Fig. 5 a,b).
- After 72 h of incubation, showed self-alignment of Ag-NPs over the bacterial surface started (Fig. 5c, d).
- Figure 5 e and f shows completely self-aligned Ag-NPs of bacterial cells incubated for 120 h and above.

Biodegradation of DEHP at various concentration

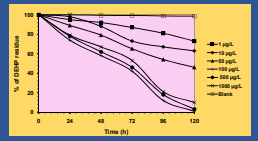

Fig.6 without SA-Ag-NPs

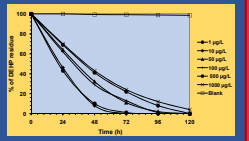

Fig. 7 with SA-Ag-NPs

- After 72 h, the residues of DEHP drastically started to decrease; the % of DEHP remained in the medium without SA-Ag-NPs were almost 73, 1 and 10 % for 1, 100 and 1000 µg L^{-1} (Fig. 6)
- The inoculated bacterial cells as inoculum had cell count of 10^4 CFU mL^{-1}; while, at the end of experiment (after 120 h)- CFU mL^{-1} were 12, 35, 46, 89, 98, 109 x 10^6 at various concentrations of DEHP: 1, 10, 50, 100, 500 and 1000 µg L^{-1}
- With SA-Ag-NPs, complete utilisation of 1 and 10 µg L^{-1} DEHP occured within 72 h (Fig. 7)
- Presence of SA-Ag-NPs has improved viable bacterial cell count even at low concentrations (1, 10 and 50 µg L-); CFU mL^{-1} were 32, 35, 52, 91, 108, 126 x 10^6 at various concentrations of DEHP: 1, 10, 50, 100, 500 and 1000 µg L^{-1}

Growth rate and kinetic parameters in DEHP degradation

Various concentration of DEHP amended in MSM (µg L^{-1})	Without SA-Ag-NPs			With SA-Ag-NPs		
	Growth rate (h^{-1})	Total DEHP degradation (%)	DEHP utilisation rate (h^{-1})	Growth rate (h^{-1})	Total DEHP degradation (%)	DEHP utilisation rate (h^{-1})
MSM	0.003 ± 0.001	-	-	0.006 ± 0.003	-	-
1	0.012 ± 0.001	27 ± 3	0.012 ± 0.008	0.044 ± 0.008	99 ± 1	0.178 ± 0.001
10	0.018 ± 0.005	37 ± 4	0.069 ± 0.005	0.042 ± 0.012	99 ± 1	0.175 ± 0.007
50	0.023 ± 0.009	54 ± 1	0.097 ± 0.012	0.038 ± 0.005	97 ± 2	0.169 ± 0.013
100	0.024 ± 0.012	99 ± 2	0.167 ± 0.003	0.037 ± 0.001	96 ± 3	0.157 ± 0.006
500	0.025 ± 0.008	97 ±3	0.161 ± 0.004	0.035 ± 0.012	96 ±3	0.152 ± 0.010
1000	0.036 ± 0.003	90 ± 4	0.153 ± 0.010	0.032 ± 0.009	95 ± 2	0.148 ± 0.004

- Growth rate of bacterial cells at 100 µg L^{-1} of DEHP showed twice increase in cell density when compared to low concentartion of 1 µg L^{-1} (Table 1)
- Total degradation rate was also observed to be directly proportional as it was thrice increase in degradation rate at 100 µg L^{-1} than 1 µg L^{-1}
- In presence of SA-ag-NPs, total degradation of DEHP at low concentration of 1 µg L^{-1} was three time higher than degradation without SA-Ag-NPS(Table 1)
- In both the cases, growth of bacterial cells showed shorter period of lag phase and followed Monod first order kinetics

Conclusion

- SA-Ag-NPs proved to have promising role in bioremediating low concentrated pollutant, DEHP from surface water and wastewater
- Ag-NPs tends to improve hydrophobicity, surface area and interaction between compound and bacteria; in turn facilitating enhanced utilisation of DEHP by bacterial cells.
- This study could also serve the purpose of bioremediating other low concentrated toxic water pollutants rather than PEs by efficiently synthesising SA-Ag-NPs on bacterial cell surface.