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ISOLATION OF TRINITROPHENOL (TNP) DEGRADERS AND ITS APPLICATION IN BIOREMEDIATION

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ABSTRACT

Trinitrophenol (picric acid) is a strongly acidic, toxic and highly explosive organic compound extensively used in biomedical studies, ammunitions, industries, pharmaceuticals, etc. The commonly used disposal methods use energy and monetary resources and add secondary pollutants into the environment. Therefore, rigorous research has been diverted to bioremediation which will exploit the inherent microbial capacity of metabolizing picric acid to non-toxic end products. In the current study a strain of Pseudomonas putida was isolated from natural environment, identified using MALDI-TOF and subjected to immobilization for its degradation studies. The calcium-alginate entrapped cells could degrade16.27% of trinitrophenol and copper chitin adsorbed cells metabolized 84.50% of the initial trinitrophenol concentration. Thus, these immobilized cells can be employed in various novel bioremediation strategies.

KEY WORDS

Trinitrophenol, Immobilization, Pseudomonas putida, Chitin

I. INTRODUCTION

Trinitrophenol (picric acid) is a widely used organic compound in the manufacture of dyes, explosives, pharmaceuticals and pesticides [1,2]. Due to its extensive use it is commonly found as a contaminant in soil, waste waters and industrial effluents [3,4]. Any solid waste containing picric acid is considered as 'hazardous waste' and must be managed according to the prescribed guidelines for hazardous waste management [5].

The physical and chemical methods used for treating trinitrophenol require expert handling, uses sophisticated instruments and require good amount of resources. Additionally, they add to secondary pollutants into nature and possess a threat to life and property in case of accidents [6]. Bioremediation is an attractive solution for treating trinitrophenol pollutants in soil and water as it implies the principles of green chemistry. Immobilization of cells on natural or artificial matrix allows the organisms to reduce competition with the indigenous flora, extends protection from predation, extremes of pH and decreased toxicity of the contaminants [7].

In the present study, attempts were made to determine the activity of previously isolated trinitrophenol degrading immobilized cells in calcium alginate beads and copper-chitin sorbents for treating synthetic trinitrophenol water.

II. LITERATURE SURVEY

Isolation and characterization of picric acid degrading micro-organisms, **Christian Behrend *et al***, 1999.

Christian Behrend *et al* studied the degradation pathway of picric acid by *Nocardiodes sp.* CB 22-2 Isolated from soil sample. The strain was able to mineralize 6mM of picric acid irrespective of the initial picric acid concentration. The degradation process led to a color change from yellow to orange-red of the medium and the metabolites formed were determined

by High Performance Liquid Chromatography. Picric acid, nitrite and H-TNP were the three major end products found at the end of the fermentation. The results revealed that during picric acid transformation, the formation of [H2]-Meisenheimer complexes of 2,4-dinitrophenol (H2-DNP) and picric acid indicates H2-DNP formation as a physiologically critical step in the picric acid metabolism [8].

Phenol degradation by immobilized cells of *Arthrobacter citreus*, **Chandrakant Karigar *et al***, 2006

Chandrakant Karigar *et al* isolated phenol utilizing *Arthrobacter citreus* by selective enrichment technique. The studied strain could metabolize up to 22mM of phenol concentration to catechol as the end product, above which it was inhibitory for cell growth. The results suggest that alginate and agar immobilized cells were able to continuously remove phenol for eight days as compared to the free cells which survived only for a day. This indicated that immobilization did not affect the degradation process and hence the above matrices can be used in biotechnology for treating phenolic effluents [9].

III. MATERIALS AND METHODS

In the previous study, 9 trinitrophenol degraders were isolated from soil on basal salt medium supplemented with trinitrophenol. The isolate W5-IIe demonstrating highest degradation ability, which was determined by High Performance Liquid Chromatography was further selected for immobilization studies.

- **Sequencing of the Isolate giving maximum Trinitrophenol Degradation:**
  The isolate giving highest trinitrophenol degradation was sequenced using matrix assisted laser desorption / ionization – time of flight (MALDI-TOF) at the National Centre for Cell Sciences Pune, Maharashtra, India.
- **Synthesis of Calcium Alginate Beads:**
  1ml of culture suspension was added to 6% Sodium alginate solution and mixed well. This mixture was added drop-wise to pre-chilled 4 % calcium chloride solution. The beads were refrigerated overnight for hardening. 25 beads were suspended in 20ml of basal salt medium with picric acid and the picric acid concentration in the medium was determined after every 1hour by UV spectrometry at wavelength of 354 nm.

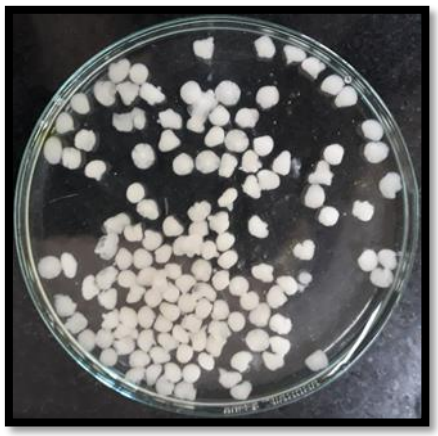

Fig – 1: Calcium – alginate beads

- **Synthesis of Copper Chitin Sorbent:**
  0.5 gm of dry chitin was added to 100ml of 1% copper sulfate solution. The mixture was incubated at 0-4^{O}C for 2 days. The solid phase was separated and dried at 50-60^{O}C for 24 hours. A culture suspension of 1.0 O. D was passed through the sorbent column and allowed to stay in contact for 24 hours. The picric acid degradation was analyzed by UV spectrometry at 354 nm.

Fig – 2: Copper-chitin sorbent

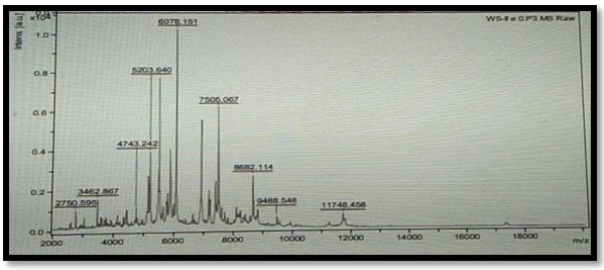

Fig-3: MALDI TOF Analysis

- **Analysis of Trinitrophenol Degradation:**
  The determination of trinitrophenol degradation by the immobilized cells was done by UV spectrometry at 354 nm at intervals of 1 hour.

III. RESULT AND DISCUSSION

Sequencing of Isolate degrading maximum trinitrophenol

From the 9 isolated colonies utilizing trinitrophenol as the sole carbon source, the isolate W5-IIe could breakdown trinitrophenol into hydride meisenheimer complex and other compounds yet to be identified. The isolate W5-IIe was given for sequencing by MALDI-TOF. The isolate was identified as a member of genus *Pseudomonas*.

Laboratory report- MALDI TOF analysis from National Centre for Cell Sciences

The MALDI-TOF sequence analysis of the isolate degrading maximum trinitrophenol was identified as *Pseudomonas putida* B400 UFL as it showed a high score value (1.753) to *Pseudomonas putida* B400 UFL. In a similar study, a bacterial strain able to metabolize 2,4,6 TNP was isolated from an activated biomass of effluent treatment plant (ETP) belonging to the strain *Arthrobacter* HPC1223 and was identified by 16S ribosomal gene sequencing [10]. In another study, researchers have isolated a gram-positive, catalase positive and strictly aerobic rod-shaped bacterium *Rhodococcus sp.* NJUST16. The 16S rDNA sequence of the isolate was closely related to the *Rhodococcus sp.* NCIMB12038 and *Rhodococcus koreensis* having a sequence identity greater than 99% [11]. In a different study researcher have isolated four picric acid degraders from a industrial waste treatment facility which were close relatives of *Nocardioides simplex* (ATCC 6946) which were then identified based on their small subunit (16S) rRNA gene sequences. The cultures showed 65% radioactivity in the form of released $14CO_2$ when grown on a medium containing 14C-UL-picric acid [12].

The Std. TNP curve was used to evaluate the % TNP degradation obtained by calcium-alginate and copper-chitin immobilized cells.

Table – 1: Std. TNP curve recorded at 354 nm

SR. No.	Concentration (Std. TNP)	O.D. (At 354 nm)
1.	0.05	1.434
2.	0.10	1.936
3.	0.15	2.185
4.	0.20	2.600
5.	0.25	3.000
6.	0.30	3.000
7.	0.35	3.000
8.	0.40	3.000
9.	0.45	3.000

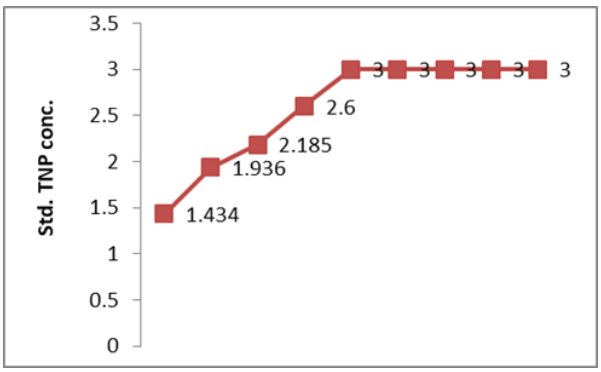

Fig – 4: Std. TNP curve

Table -2: TNP degradation analysis of calcium – alginate immobilized W5-IIe

Time (In Hour)	O.D (at 354 nm)	Conc. Of TNP (from std. TNP curve)	% Degradation after 24 hr
0	2.473	0.215	
1	1.964	0.195	
2	1.646	0.195	
3	1.873	0.190	16.27%
4	1.855	0.185	
5	1.837	0.180	
24	1.798	0.18	

Trinitrophenol degradation by calcium alginate encapsulated cells

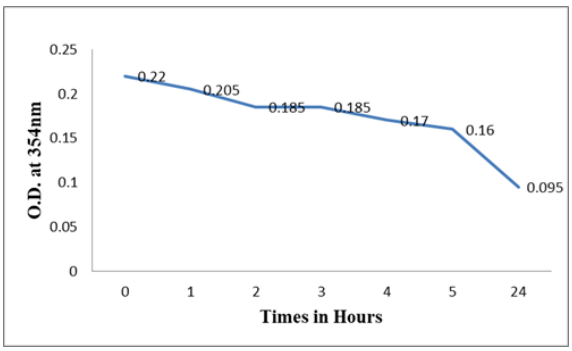

Fig – 5: Graph of TNP degradation by calcium-alginate immobilized cells

Trinitrophenol degradation by copper – chitin adsorbed cells

Table – 3: TNP degradation analysis of copper-chitin sorbent

Time (In Hour)	O.D (at 354 nm)	Conc. Of TNP (from std. TNP curve)	% Degradation after 24 hr
0	2.594	0.220	
1	2.063	0.205	
2	1.860	0.185	
3	1.841	0.185	84.50%
4	1.730	0.170	
5	1.645	0.160	
24	1.010	0.095	

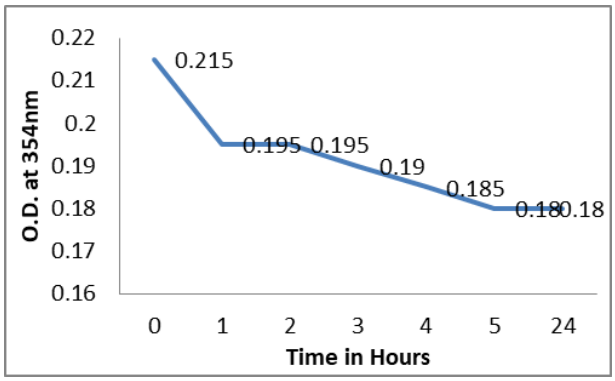

Fig – 6: Graph of TNP degradation by copper-chitin immobilized cells

The cells entrapped into calcium – alginate beads were able to degrade 16.27% of trinitrophenol as compared to 84.50% of degradation obtained by copper-chitin adsorbed cells. The lower degradation rates obtained in Calcium alginate encapsulated cells may be due to the incapability of the large TNP molecules to enter into the beads or its reactivity with the surface of the beads. In a study of oil palm residue immobilized *Methylobacterium sp.* NP3 and *Acinetobacter sp.* PK1, researchers observed that organisms could effectively remove up to 5,000 mg/L phenol in a carbon free mineral medium (CFMM) as compared to the suspended bacteria [13]. A different study quoted 0.100-0.200 mg/ ml of phenol degradation by *Pseudomonas pictorum* at 30^{O}C and a pH of 7.0 by chitin immobilized cells. The cells also showed 80-90% adsorption to the surface of immobilization matrix [14]. Although many *Pseudomonas* strains have been studied for phenol degradation not much work has been reported for degradation of trinitrophenol by immobilized cells.

IV. CONCLUSION

Trinitrophenol degradation was carried out by using calcium alginate encapsulated and copper- chitin adsorbed *Psuedomonas putida B400 UFL*. Thus, immobilized cells can be used for bioremidiating trinitrophenol contaminated waste water, industrial effluents and soil in biological aerobic filters or anaerobic bioreactors. Such technologies provide an eco-friendly and sustainable solution to the existing physical or chemical treatment methods.

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