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Degrading of Nitrobodies Using Microorganisms

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Abstract:- *In this study biodegradation of p-nitrophenol (PNP) by soil microorganisms is evaluated. In biological degradation, performance of pure culture and mixed culture was investigated. It has been observed that the cultivable flora of soil sample was 4x10 ^{7}cfu/gm which could utilize PNP 100 ppm as energy source. The organisms were isolated ,identified and screened on the basis of maximum PNP degradation with least time at room temperature. Five bacterial isolates were selected on the basis of their ability to tolerate as well as degrade PNP 1000ppm. Isolate PD07 could be able to grow and reach absorbance 1 up to within 17 hrs at room temperature. Growth profile of the this isolate showed maximum growth in presence of Nutrient broth and PNP. As minimal media (without nitrogen and carbon source) was used for whole study it has been concluded that this organism utilizes PNP as carbon and nitrogen source.*

Keywords : Nitrobodies, Microorganisms

Introduction:-

Paranitrophenol is considered for the study as nitrobody. Its degradation by microorganisms may help in utilization of such microorganisms in degradation of many explosive compositions which basically are nitrobodies.

Several microorganisms including bacteria, fungi and actinomycetes are able to conversion or transforming nitroaromatics. It has been observed that several bacteria like *Flavobacterium* spp,*Pseudomonas* spp, *Moraxella* spp, *Arthrobacter* spp and *Bacillus* spp participate in PNP degradation (Mitra and Vaidyanathan, 1984; Hanne *et al.*, 1993; Spain, 1995).

The aim of the present investigation is to isolate PNP degrading soil bacteria from PNP contaminated soil. To screen the isolated organisms for higher PNP tolerance as well as fast growing capacity at room temperature. Further studies have been conducted to check the growth of selected isolats in presence of different combinations of media ingredients and PNP.

Materials and methods :-

Sample collection :- Soil samples in and around nitrobody manufacturer are collected from different places and stored at –20º C in refrigerator immediately after collection.

Determination of total viable count (colony forming units):-Plating for bacterial colonies was used as a culture dependent approach to determine the number of bacteria (inoculum size) present in one gram of sample and for isolation of PNP-degrading bacteria . Serial dilution of 1 gram of soil sample was carried out with the help of sterile saline. 0.1 ml sample aliquot of each dilution was inoculated into the minimal salt media (K2HPO4, 0.75 gm/lit; KH2PO4, 0.2 gm/lit; MgSO4 _ 7H2O, 0.09 gm/lit and FeSO4 _ 7H2O, 0.06, Agar agar powder 15 gm/lit) containing 0.1 mg % of PNP. After incubation at room temperature for 2 days bacterial colonies were counted.

Colonies becoming visible after 2 days of incubation at room temperature. They were picked up.To ensure purity they were restreaked for several times. The pure culture was then inoculated into MSM containing 100 ppm PNP. No other carbon and nitrogen source was added in to the medium. The isolated bacteria which was able to turn the culture (which was initially yellow) to colourless was selected for further studies.

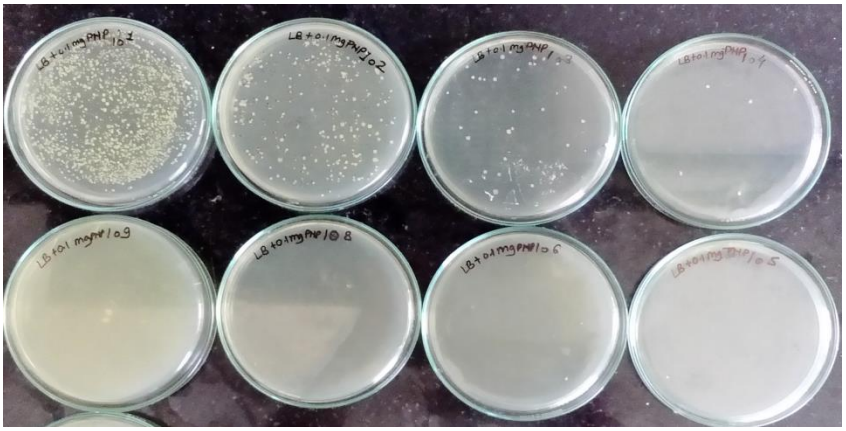
Biodegradation studies by total soil bacteria:- 0.1 ml aliquots of 10^{1} 10^{2}, 10^{3}, 10^{4}, 10^{5}, 10^{6} 10^{7}, 10^{8}, 10^{9}, 10^{10} dilutions(which are previously discussed) are inoculated into the 100 ml minimal salt broth with 100 ppm PNP. These flasks were incubated at room temperature and observed intervally for complete colour decolourization(yellow to colorless).

Screening of isolates :-The primary screening was carried out on the basis of capability of the isolated organism to grow in presence of PNP (100 ppm).A total of 30 isolates of PNP degrading ability were selected. The medium turns yellow to colourless is the indictor of PNP degradation. Such bacteria was selected.Secondary screening was on the basis of rate of tolerance as well as utilization of PNP (100 ppm,200 ppm,300 ppm,400 ppm, 500 ppm, 800ppm and 1000 ppm) as carbon and nitrogen source. To check the viability of resistant bacteria, minimal salt medium supplemented with PNP was used. Five bacterial PNP degrading isolates were selected for further studies. Another required characteristic for PNP degrading isolates was to grow fast at room temperature. Single isolate PD07 was selected for further studies.

Growth profile of the isolate in presence of different media ingredients and PNP:- Six types of different combinations were used namely i) yeast extract with 100 ppm PNP ,ii) peptone with 100 ppm PNP ,iii)Glucose with 100 ppm PNP, iv)Nutrient broth with with 100 ppm PNP , v) Nutrient broth with with 50 ppm PNP , vi)Nutrient broth with with 25 ppm PNP , to study growth profile of the isolates in presence of growth promoters and PNP.

Briefly, minimal salt broth with typical growth ingredient and PNP was inoculated with isolated organism. Further it was incubated at room temperature. MSM broth without and with PNP was used as a control. By measuring the absorbance with a spectrophotometer at 620 nm at hourly intervals for 30 hrs bacterial growth was monitored. The absorbance values obtained were plotted against the incubation time, and growth pattern of isolated strain was studied.

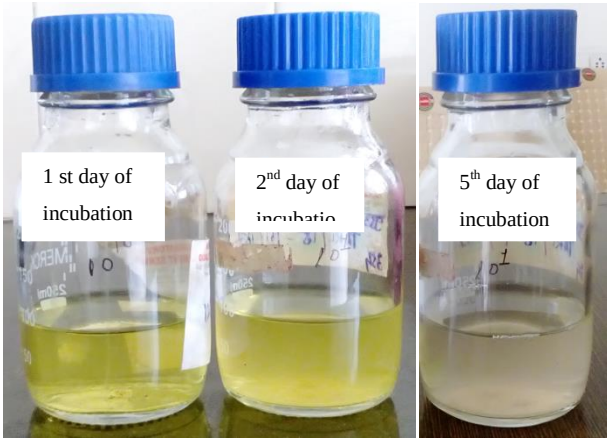
Results and Discussions:-



Photograph 01 Plates with viable colonies after incubation of 2 days at room temperature

Inoculated with serially diluted soil sample

It is regularly essential to determine how many live bacteria are actually in a soil sample, especially when measuring growth rates or determining the tolerance of bacteria to particular chemical . This involves the serial dilution of bacteria samples and plating them on suitable growth media. The colonies we see growing on the plate are considered to have started from one viable bacterial unit. The typical experiment was run to check the cultivable bacteria in soil which could tolerate and utilize 100 ppm PNP as the sole carbon and nitrogen source .It has been observed that the cultivable flora of soil sample was $4x10^{7}$cfu/gm which could also utilize PNP as energy source.(Photograph 01)



Photograph 02 Minimal broth with 100 ppm PNP inoculated with 10^{1}diluted sample for first, second and fifth day incubation at room temperature

Incubation of soil dilutions in MSM broth with PNP can completely utilize PNP as carbon and nitrogen source. The color of broth changes from yellow to colorless. It has been observed that minimal salt broth containing 100 ppm PNP with 0.1 ml of 10^{1}dilution of soil sample was degrade complete PNP within five days of incubation at room temperature. The color was changed from yellow to colorless indicating complete utilization of PNP. .(Photograph 02)

After serially diluted sample was inoculated on Minimal agar with 100 ppm PNP, 30 different isolates utilizing PNP as the sole carbon and nitrogen source were obtained. Further five isolates were selected on the basis of tolerance to 1000 ppm PNP. An isolate (named PD07) showing the highest PNP tolerance and degradation activity with less time was selected for further studies. It has been observed that this isolate is fast grower among all isolated organisms.(O.D. 1 at 620 nm was reached within 17 hrs at RT)

Its cells were straight rod-shaped,gram-negative, strictly aerobic, motile with polar fllagella. Colonies of strain PD07 on LB agar plates were circular, smooth,glossy, convex, pale yellow and 1.3–1.8 mm in diameter after 2 days' incubation at room temperature.

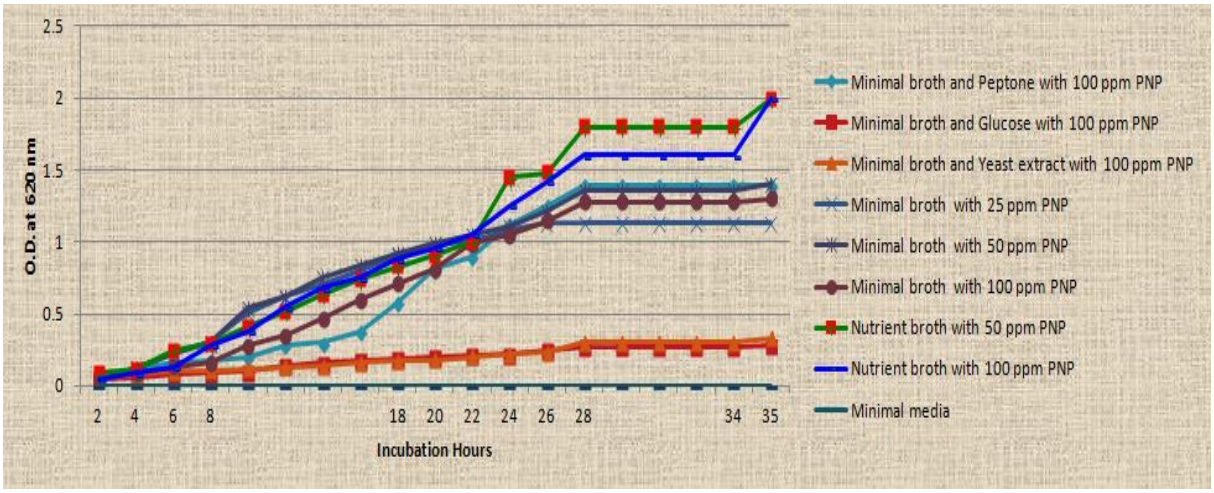


Fig No. 1.0 Growth profile of selected isolate in medium with different combinations i) Minimal broth and Peptone with 100 ppm PNP, ii) Minimal broth and Glucose with 100 ppm PNP iii) Minimal broth and Glucose with 100 ppm PNP iv) Minimal broth and Yeast extract with 100 ppm PNP v) Minimal broth with 25 ppm PNP,vi) Minimal broth with 50 ppm PNP,vi) Minimal broth with 100 ppm PNP,vii) Nutrient broth with 50 ppm PNP ,viii) Nutrient broth with 100 ppm PNP ,ix)Minimal broth

Several microorganisms capable of degrading the PNP are isolated but the rate of degradation is affected by the concentration of the PNP (Roldan, M. 1998 ; Gemini, V.2005; Kulkarni M. and Chaudhari A, 2006). Several authors give evidence of a significant biodegradation of PNP but in low concentrations (50-150 mg/L). In our studies the maximum PNP degradation in the form of growth was observed with concentration 25ppm hence support the results described by Qiu X.*et.al* and Pakala S.*et.al.* (Qiu X.*et.al.*2007; Pakala S.*et.al.*,2007).However there was no further growth observed after 24 hours as complete utilization of PNP was occur and there was no alternative carbon and nitrogen source for the growth of isolate .(Fig. No. 1.0) It was observed that Nutrient broth with 50 ppm PNP showed maximum growth .While comparing degradation of PNP in minimal broth and nutrient broth , the absorbance with nutrient broth was reaches up to 2 (620 nm).It indicates that nutrient broth support the growth of isolated soil bacteria. In present studies we used three different combinations to check the growth profile namely , peptone &PNP, glucose & PNP and yeast extract &PNP. The maximum growth of selected isolate was observed with peptone (0.5%) and 100 ppm PNP. Several studies show that biodegradation with pure microbial strain is realized in only a few hours. *Pseudomonas aeruginosa* HS-D38 mineralizes the PNP in an initial concentration of 500 mg/L in 24 h (Zheng Y. *et.al*.,2009). Our results support these findings as maximum PNP utilized as energy source by isolated organism PD07 in minimal media within 24 hrs indicating the color changes from yellow to colorless.

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