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Short Communication

To study the effects of various physicochemical parameters on bioremediation of p-nitrophenol (PNP)

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Abstract

Bioremediation has become one of the most rapidly developing fields for reducing concentration and toxicity of the pollutants. Current studies focuses on the harmful effects of p-nitrophenol, microbial features in biodegradation, biological solution to the pollution and effect of various physico-chemical factors on biodegradation. Information collected from different reviews is duly incorporated in the chapter. Decontamination of nitro-aromatic compounds to less harmful or nontoxic products is achieved with various methods like land farming, bioventig, bioaugmentation, biostimulation etc. The world-wide annual production of nitro-aromatics is about 108 tons, while p-nitrophenol alone contributes 20 million kg per annum. Microorganisms like fungi, bacteria are playing an important role in transforming para-nitrophenols and in the associated recycling of the nitrogen. Potential of biological solutions for remediation of nitro-aromatics has been reviewed by considering the central role of microbes in nutrient cycling through their vast enzymatic capacity to transform anthropogenic waste from diverse sources into non-toxic products. In this chapter, effects of various physicochemical parameters on microbial degradation of p-nitrophenol are reviewed.

Keywords: P-nitophenol, bioremediation, xenobiotics, nitroaromatics, bioaugmentation.

Introduction

Bioremediation is the degradation of xenobiotic compounds in its simpler forms it meanscomplex harmful compound into less harmful simpler compound. Some microbes are capable to obtain energy through biodegradation pathway of pollutants like nitro-aromatic compounds. On the contrary, nitro-aromatic compounds are released into the biosphere exclusively from the anthropogenic sources[1,2]. Bioremediation technology has been used at the number of sites in worldwide with success.

p-nitrophenol (PNP) is toxic to plant, animal and human health. It is a high priority pollutant and poses significant health and environmental risk due to its mutagenic and carcinogen activity. Nitrophenols and other nitro-aromatic compounds are generally considered to be highly resistant to microbial degradation. The purification of wastewater contaminated with these pollutants is very difficult since they are resistant to the conventional treatment techniques. Although several investigators have used physical and chemical methods such as volatilization, photodegradation, photo-catalysis and advanced oxidation to treat the wastewater containing nitrophenol, microbial biodegradation may have promising application for the removal of nitrophenol pollutants.

Several bacterial strains are found to eligible for PNP deghradation by using PNP as a sole source of carbon and energy like Arthrobacter[3,4] Bacillus[5], Microbial degradation of *p*-nitrophenol has been described for several genera including *Flavobacterium, Pseudomonas, Moraxella, Arthrobacter* and *Bacillus*. *Bacillus sphaericus*, isolated from an agricultural soil by selective enrichment, transform *p*-nitrophenol[6]. A strain of *Pseudomonas putida* was found to degrade *p*-nitrophenol as a sole source of carbon, nitrogen and energy[7]. *Pseudomonas* sp and *Rhodococcus opacus* can utilize *p*-nitrophenol as a sole source of carbon and energy[8]. *Arthrobacter aurescens* and *Nocardia* were also reported to use the hydroquinone route to degrade *p*-nitrophenol. The hydroquinone pathway for *p*-nitrophenol is apparently quite common in a variety of Gram-positive and Gram-negative bacteria[9]. Filamentous soil fungi like *Aspergillus terries, Cladosporium cladosporioides, Fusarium oxysporum, Gliocladium* roseum, removal of Cadmium[10]. The fungus *Penicillium frequentans* has been found to effectively remove *Penicillium* spp. and *Trichoderma koningii* isolated from industrially polluted sediments for the phenanthrene in soil[11]. The control and optimization of bioremediation processes is a complex system of many factors. These include microbial population, availability of the contaminants to microbes, the environmental factors like type of soil, temperature, pH, oxygen requirement, electron acceptor and nutritional sources.

Materials and methods

Chemicals: PNP was 99.9 of purity obtained from Merck. All the other reagents used were analytical grade.

Microorganisms: The fungal stain used for microbial degradation of *p*-nitrophenol is *Fusarium oxysporum* NCIM 1008. The strain was maintained on mineral salt glucose (MSG) medium fortified with 50 ppm concentration of PNP at 4^0C.

Preparation of inoculums: Cells were pre-grown in MS medium supplemented with 1.0gL^{-1} yeast extract overnight at room temperature. PNP (50 ppm) was added in the growth medium for acclimation of the culture, wherever not mentioned.

Typical experimental set up for biodegradation studies: An aliquot of 100 ml MS medium (pre-sterile) was dispensed in 500 ml capacity Erlenmeyer flasks. The assay medium in each flask was supplemented with filter sterilized PNP (50ppm). The assay medium in all experimental sets were inoculated with pregrown fungal biomass of 0.5g (%) unless and otherwise mentioned. The flasks were kept on a rotary shaker (120rpm) at room temperature. The flasks were incubated at 30^0C upto 48 h and samples withdrawn intermittently for analysis. Control (without culture) was run simultaneously to detect auto-oxidation or volatilization of PNP, if any.

Effect of physicochemical parameters on PNP biodegradation: PNP degradation was carried out under varying conditions of nutrients, *p*H and temperature, at 50 ppm, using 0.5 g (%) biomass inoculum under aerobic conditions on a rotary shaker (120rpm, 30^0C, 48h). The results are reported as an average of three independent experiments.

Nutritional supplement: Glucose: MSG medium fortified with filter sterilized PNP at 50 ppm was used to study the effect of glucose as a secondary substrate (1, 5 and 10 gL^{-1}) on PNP degradation. Medium without glucose served as a control.

Nitrogen: Sodium nitrate (1, 5 and 10 gL^{-1}) was added to MS medium containing 50 ppm PNP to study the effect of nitrogen source on PNP degradation, medium exclusively with PNP served as control.

Physical parameters: Temperature: PNP degradation was studied using MS medium containing 50 ppm PNP as a function of temperature (25, 30, 37 and 45^0C) of incubation.

***p*H:** PNP degradation was studied as a function of pH 5.0, 5.5, 6.0 and 6.5 (acidic range) and 7.5, 8.0, and 9.0 (alkaline range). For this purpose, 0.1N HCl and 0.1N NaOH were used to set the *p*H in respective range.

Results and discussion

Effect of nutritional supplements and physicochemical parameters on PNP degradation: Supplemental substrates (viz. glucose) have shown enhancement in nitro-aromatics degradation[12,13]. Repression of growth due to nutrient supplements is rarely reported. On this background, degradation of PNP in the presence various concentrations of glucose as a carbon and sodium nitrate as a nitrogen source, and influence of temperature, *p*H and surfactants were examined by inoculating pre-grown mycelial growth of *Fusarium oxysporum* in MS medium containing PNP (50 ppm).

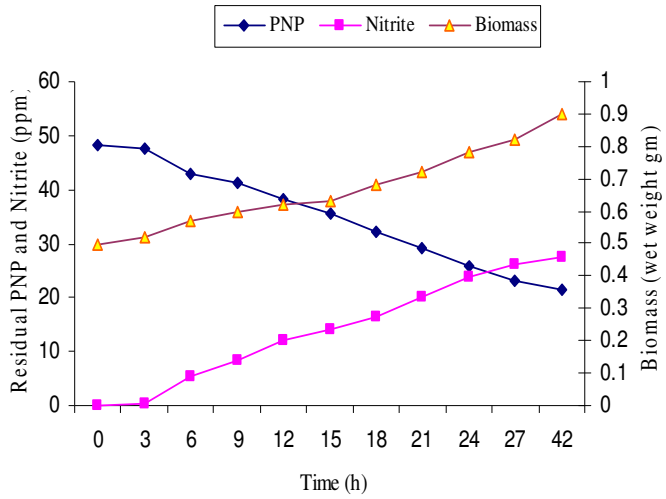

Figure-1: Typical biodegradation profile of *Fusarium oxysporum* NCIM 1008 in MS medium.

Effect of glucose supplement: Addition of supplemental substrates has shown profound effect on the microbial degradation of most of the nitro-aromatics[13]. In the present work (1 and 50gL^{-1}) was added as second carbon source in addition to PNP in MS medium. It showed that: i. glucose at 1 to 5gL^{-1} exerted lower degradation rate of PNP and ii. glucose at 10gL^{-1} enhanced PNP degradation (36 ppm) (Figure-2).

Effect of nitrogen supplements: To study the effect of sodium nitrate as a nitrogen source various concentrations (1, 5 and 10 gL^{-1}), was separately supplemented to MS-medium containing PNP (50 ppm) at *p*H 7.0, 37^0C (120 rpm).

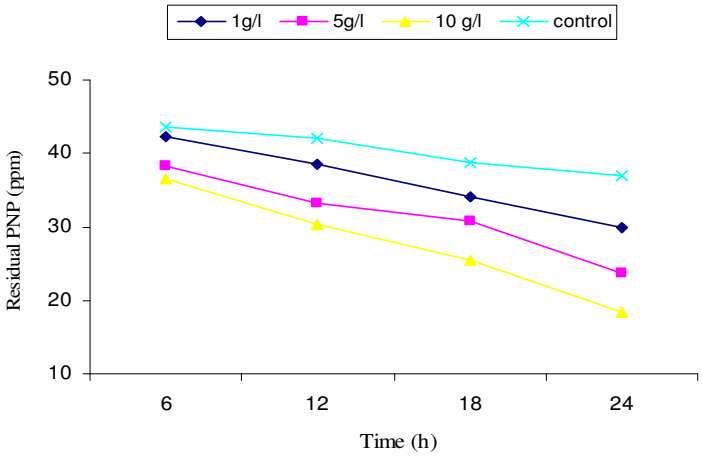

Figure-2 Effect of glucose supplement (1, 5 and 10gL^{-1}) on PNP degradation by *Fusarium oxysporum* NCIM 1008 in MS medium containing PNP (50 ppm) at pH 7.0, 37^0C (120 rpm).

Containing 50 ppm PNP in order to find the effect on PNP degradation by *F*usarium *oxysporum*. It revealed that addition of sodium nitrate (5 and 10gL^{-1}) did not have any remarkable effect on PNP degradation while sodium nitrate (1gL^{-1}) supplementation slightly stimulated PNP biodegradation rate by *Fusarium oxysporum* (Figure-2). Overall, the addition of sodium nitrate was not preferentially preferred by *F. oxysporum* in the presence of PNP in the MS medium.

Effect of temperature: To study the effect of temperature on PNP degradation, MS medium containing 50 ppm PNP was inoculated with 0.5g (%) biomass of *Fusarium oxysporum* and incubated on a rotary shaker (120 rpm) at various temperatures (27, 37 and 45^{0}C). It appears from Figure-3 that i. maximum PNP degradation at 27^{0}C, ii. at 37 and 45^{0}C delayed degradation of PNP suggesting that PNP metabolism by *Fusarium oxysporum* require temperature below 27^{0}C.

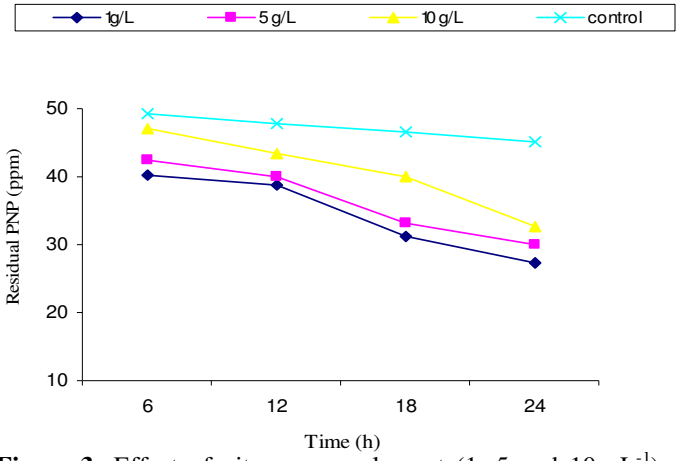

Figure-3: Effect of nitrogen supplement (1, 5 and 10 gL^{-1})on PNP degradation by *Fusarium oxysporum* NCIM 1008 in MS medium containing PNP (50 ppm) at pH 7.0, 37^{0}C (120 rpm).

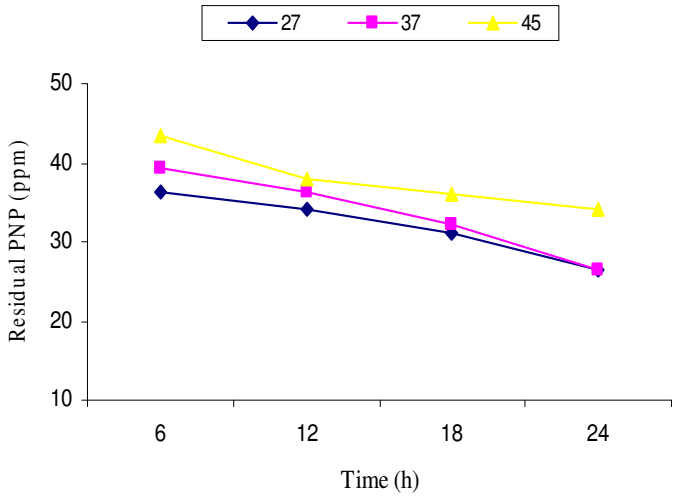

Figure-4: Effect of temperature on PNP biodegradation by *Fusarium oxysporum* NCIM 1008 on PNP degradation in MS medium containing PNP (50 ppm) at pH 7.0, (120rpm).

Effect of pH: The of pH of MS medium containing 50 ppm PNP are as pH 5.5,pH 6, pH 6.5, pH 7.5, pH 8 and pH 9 and inoculated with acclimated culture of *F. oxysporum* at 30^{0}C (Figure-5). At pH of 6.5, *F. oxysporum* degraded maximum amount of PNP (28.4 ppm), while at pH 5.5, 6.0 (acidic range) and 7.5, 8.0, and 9.0 (alkaline range) PNP degradation was reduced.

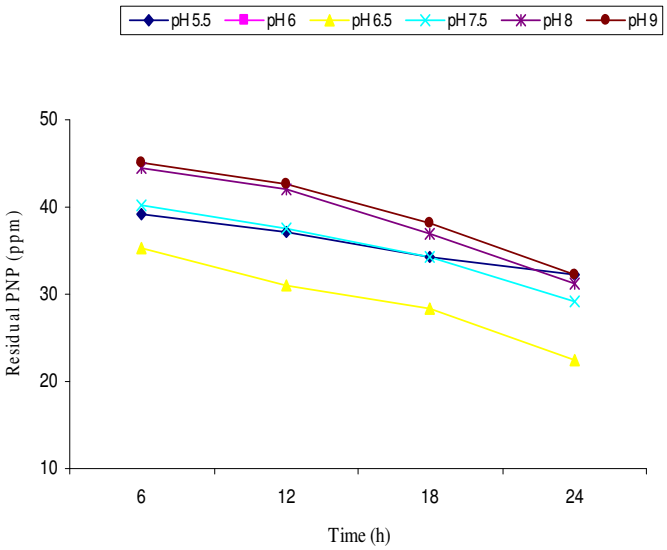

Figure-5 Effect of pH on PNP biodegradation by *Fusarium oxysporum* NCIM 1008 on PNP degradation in MS medium containing PNP (50 ppm) at 37^{0}C (120 rpm).

Conclusion

In this study it is found that strain *Fusarium oxysporum* has the degradation potential for PNP bioremediation under optimum conditions .It is suggested that the physicochemical parameters play crucial role in effective bioremediation processes. Various physicochemical parameters like pH, temperature. Effect of nutritional supplements like glucose, nitrogen is efficient in case of bioremediation studies.

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