



Effect of C and N Supplements on the Bioremediation of p-Nitrophenol

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ABSTRACT

Microbial degradation is a major determinant of the fate of pollutants in the environment. p-Nitrophenol (PNP) is an EPA-listed priority pollutant with a wide environmental distribution, but little is known about the microorganisms that degrade it in the environment. The effect of different organic nutrient supplements like nitrogen and carbon on the total PNP degradation in polluted soil was studied. In this study the *Pseudomonas aeruginosa* NP1 was isolated from polluted site used for biodegradation studies. MSM medium used for degradation. study. Nutritional supplements like carbon (glucose, sucrose, fructose, dextrose, lactose, maltose) and nitrogen supplements (ammonium sulphate, ammonium acetate, sodium nitrate, urea, yeast extract) were evaluated for PNP bioremediation. This study has proved that the PNP bioremediation was hasten by addition of supplementary carbon and nitrogen sources in the growth medium to flourish microbial growth. This study may be useful in optimizing biodegradation processes by adding required nutritional supplements in the medium in required concentrations.

Keywords: Bioremediation, Nutritional supplements, MSM, PNP, *Pseudomonas aeruginosa*

INTRODUCTION

Nitro-aromatic or nitro-phenolic compounds, including paranitrophenols, are widely distributed in the nature. Among this group p-nitrophenol or 4-nitrophenol (also called paranitrophenol and abbreviated as PNP)

is an important member of the nitrophenol group. This chemical is a manufactured item that does not occur naturally in the environment (ATSDR, USA, 1992). p-nitrophenol has various applications in the agriculture, dyes, engineering polymers and pharmaceuticals. p-nitrophenol is used as fungicide for leather, for the production of parathion and for organic synthesis (Montgomery and Welkon, 1980). It was found that various factors affect the rate of biodegradation of organic pollutants (Gerhard and Markus, 1995; Osman et al., 2020; Jiayi et al., 2005).

Among other xenobiotics, organophosphorus (OP) compounds are of great concern considering their environmental impact and threat to human health. These synthetic chemicals are potent cholinesterase inhibitors once intended for military use, but at the present time, widely employed as insecticides. Occupational exposure to the OP pesticides in agricultural industries and self-poisoning with OP compounds causes significant health problems (Grillo et al., 1989; Eddleston et al., 2005; Calvert et al., 2005).

The carbon, nitrogen and phosphorus ratio in an oil contaminated soil is high requirement to the oil biodegradation (Benyahia et al., 2007).

Deficiencies of the micronutrients like nitrogen and phosphorus affect the rate of biodegradation because they are required for active metabolism of the microorganisms, so the applications of these micronutrients can help to stimulate the biodegradation in the soil (Kovacheva et al., 2001).

When there is organic pollution in the soil, the concentration of carbon increases while the concentration of nitrogen and phosphorus decreases. So in such cases to increase the rate of biodegradation of hydrocarbon, nitrogen will be added in the form of ammonia while phosphorus added in form of phosphates (Koshikawa et al., 2001).

The hydrocarbon biodegradation could have accelerated by addition of sufficient nitrogen, phosphorus and other nutrients contained in fertilizer in aqueous environments and sediments (Margesin et al., 2006). However, studies have shown that excess of nitrogen can suppress biodegradation process due to its toxic effects. (Kovacheva et al., 2001).

In present study, the effect of carbon and nitrogen supplements on the bioremediation of PNP by *Pseudomonas aeruginosa* (NP1) was evaluated.

MATERIALS AND METHODS

Culture Medium

The MSM (minimal salt medium) containing (g/L) of NaH_2PO_4 , 0.75; $\text{Na}_2\text{HPO}_4 \cdot 2\text{H}_2\text{O}$, 2.5; NH_4NO_3 , 0.25; $\text{MgSO}_4 \cdot 7\text{H}_2\text{O}$, 0.2; Ca(NO)_3 0.1. The pH was adjusted to 7, was autoclaved for 20 min.

Effect of carbon sources

To study the effect of carbon sources on the biodegradation of p-Nitrophenol by using *Pseudomonas aeruginosa* (NP1) Glucose, Lactose, Maltose, Dextrose and Sucrose were separately added each of 5 g/L concentrations in 100 ml MS medium containing 500 mg/L PNP concentration in Erlenmeyer flask inoculated with 0.5 g % biomass of *Pseudomonas aeruginosa* (NP1) and kept under incubation at 37°C for 6-7 days on rotary shaker at 120 rpm. The growth was monitored at 620 nm absorbance and by CFU per ml.

Effect of nitrogen sources

For the study of the effect of different nitrogen sources, Ammonium Sulphate, Ammonium Acetate, Sodium Nitrate, Urea and Yeast Extract were added each of 5 g/L concentration in 100 ml MS medium containing 500 mg/L of PNP in Erlenmeyer flask inoculated with 0.5 g % biomass of *Pseudomonas aeruginosa* (NP1). These flasks were incubated at 37°C on rotary shaker at 120 rpm for 6-7 days. The growth was monitored at 620 nm absorbance and by CFU per ml.

RESULTS AND DISCUSSION

Effect of Carbon Sources

During the biodegradation process the microorganisms are utilizing PNP as a carbon source for their metabolism. Addition of supplementary carbon sources in the medium in lower concentrations increases the speed of metabolism and bioremediation. Here supplementary carbon sources enhance speed of biodegradation. The experiment has showed increases in growth of the bacteria as compared to control. Addition of different carbon sources like glucose, lactose, maltose, dextrose, sucrose, fructose was responsible for growth of the *Pseudomonas aeruginosa* in the form of CFU/ml and hence speedup biodegradation process. (Fig1).

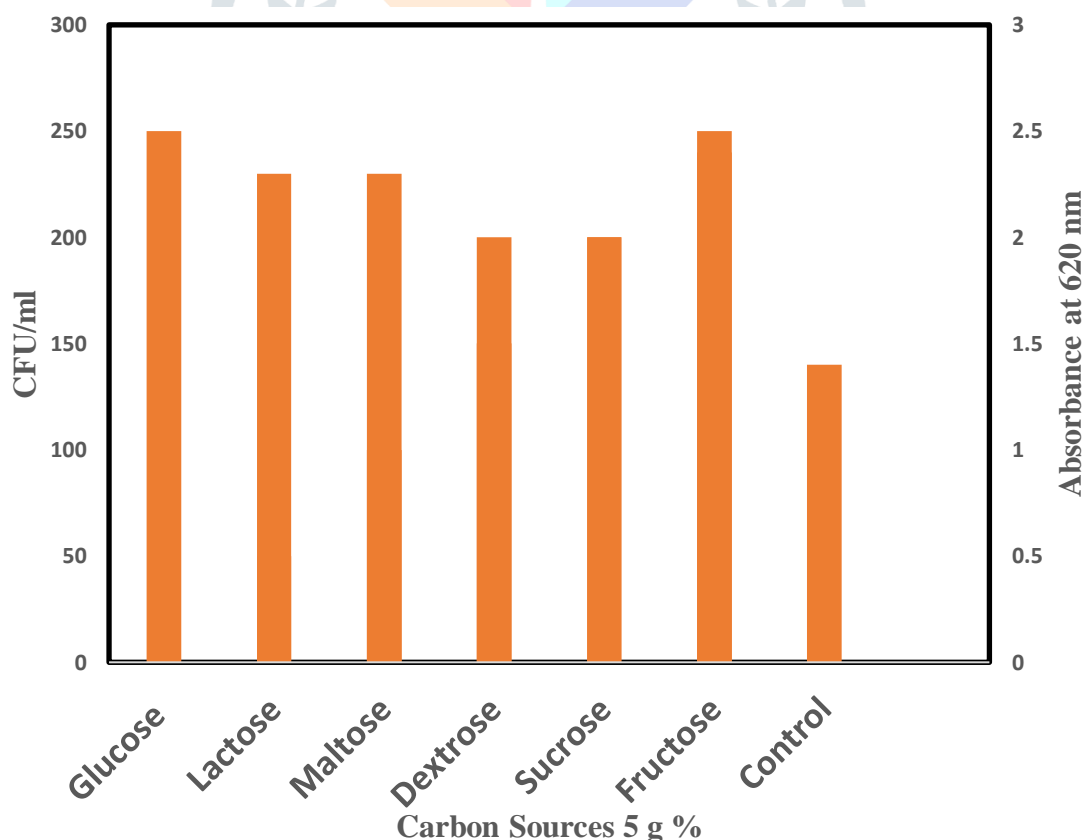


Fig 2: Effect of Carbon Supplements on PNP Degradation by *Pseudomonas aeruginosa* NP1 in MS Medium fortified with 500 mg/L PNP

Effect of Nitrogen Supplements

It has been studied that ammonium sulphate is the principal nitrogen source for bioremediation (King et al., 1992). Among nitrogen sources ammonium sulphate has showed maximum growth as compared to other hence it enhances the biodegradation process. Ammonium acetate and sodium nitrate also showed increase in growth of bacteria. Addition of urea showed very minor increase in growth. (Fig.2).

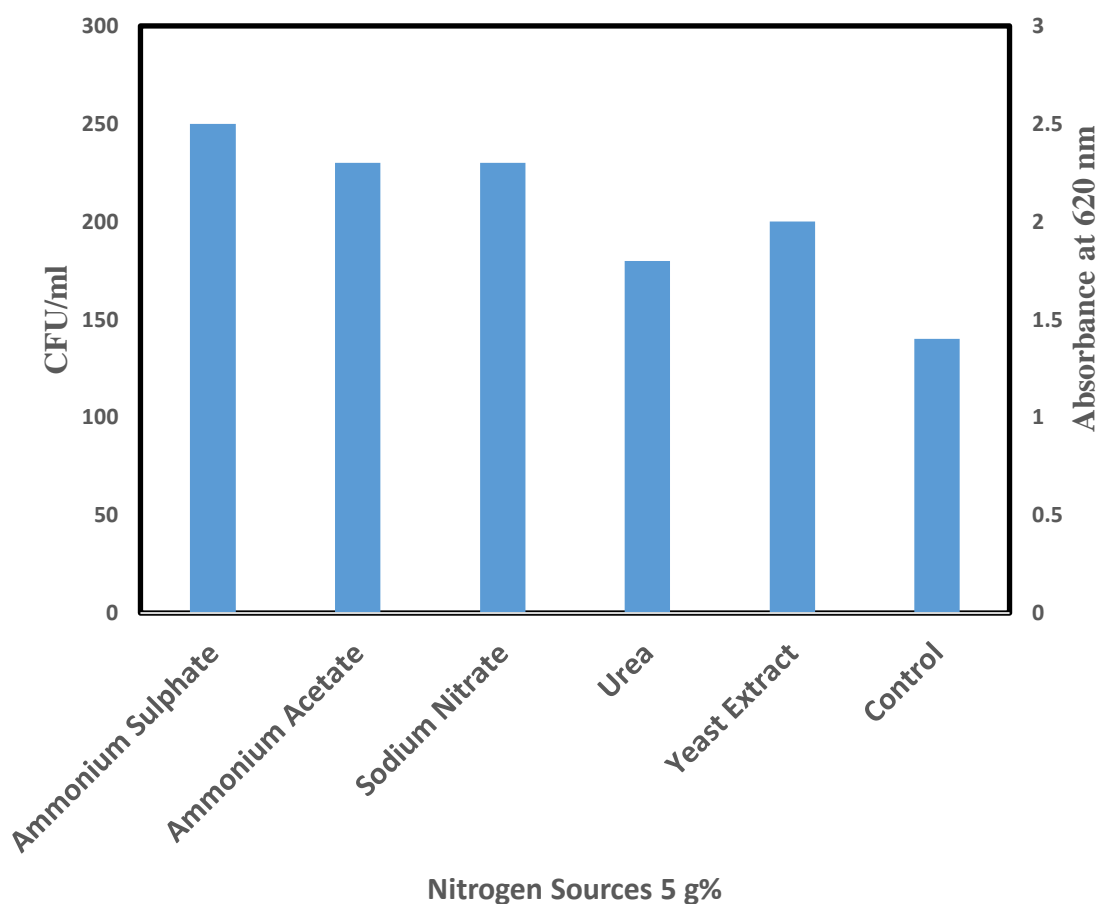


Fig 2: Effect of Nitrogen Supplements on PNP Degradation by *Pseudomonas aeruginosa* NP1 in MS Medium fortified with 500 mg/L PNP

CONCLUSION

This study has revealed that the PNP degradation by *Pseudomonas aeruginosa* NP1 has been made efficient by adding supplementary carbon and nitrogen sources in required concentrations. Here addition fructose, glucose, ammonium sulphate and sodium nitrate was showed maximum degradation. The rate of biodegradation of p-Nitrophenol can be increased by addition of organic wastes as a nutritional supplements in the pollutes site.

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