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Biodegradation of Para-Nitro Aniline from Soil Sample of Nanded District (MS), India

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ABSTRACT

Pesticides are used to control various pests and excess use may destroy the plants. They form one of the important groups of xenobiotics compounds. Parathion is one of the pesticides which is used for controlling foliar pests. it is hazardous to humans& animals also. During its use when it dropped in the soil. It undergoes microbial degradation and is converted into para-nitroaniline, which is also a hazard. The response of three bacterial strains showing tolerance to the para-nitroaniline i.e., Azotobacter, pseudomonas, and bacillus was selected and their degradation activity was studied by determining the susceptibility of the strain towards the para-nitroaniline and spectrophotometric analysis and observed MIC was 320 ppm and percent of degradationincreases with time. The MIC for Bacillus spp. is 320 ppm (31mm) Pseudomonas spp. is 160 ppm (12mm) and Azotobacter spp. 160 ppm (12 mm). After 96 hrs. of incubation, the percent degradation of Bacillus spp. is 42% Pseudomonas is 41% and Azotobacter is 40%.

I. INTRODUCTION

Nitro-aromatic compounds are used extensively in dyes. Pesticide, herbicide, plasticizers, explosive and solvent, for example, nitrophenol, nitro anilines are released into the environment as parathion (Sethunathan, N. and Yoshida, T., 1973), hydrolytic products of methyl parathion like phosphorous insecticides(Gupte, S.P. and Chaudhari, R.V., 1988), herbicides(De Steven, D., 1991) or industrial waste (Marvin-Sikkema, F.D., and De Bont, J.A.M., 1994;Spain, J.C.1995).Parathion is an organophosphorus pesticide that is used only for limited plants because of its high toxicity and risk, it is classified as Restricted use pesticide (RUP).It interferes with the activity of cholinesterase, an enzyme that is essential for the proper working of the nervous system of insects. Human parathion direct exposure to humans inhibits cholinesterase and produces incoordination, slurred speech, loss of reflexes, and paralysis of the body extremities and respiratory muscle death may be caused by respiratory failure or cardiac arrest.

Soil microorganisms degrade parathion by three different pathways into three different compounds. Parathion after its microbial degradation gets converted into Para- nitro aniline. At alkaline pH organism,*Pseudomonas*

alcaligens converts the parathion into Para- nitro aniline (Munnecke, D.M., 1980). Para-nitro aniline is the hazardous compound that is included in hazardous class 0.1 and CAS No.100-01-6. It is a yellow-colored compound that is less soluble in water, with high oxidizing and reducing capacities.

II. MATERIAL AND METHODS

The bacterial strain used to study the biodegradation of p-nitroaniline is*Pseudomonas* sp. *Azotobacter* sp. and *Bacillus* sp.These bacterial strains were obtained from the collected soil sample of NES Science College campus, Nanded (MS), India. The collected bacterial strains were then preserved on the media i.e., nitrogen-free mannitol broth, King's B media, and nutrient agar respectively. All media were sterilized at 15 IBS pressure for 15 min. (Atlas and Bartha., 2006).

Determination Of susceptibility:

The susceptibility of this bacterial species can be confirmed by determining the minimum inhibitory concentration (MIC) of P-nitroaniline for each bacterial strain.The MIC can be defined as the lowest P-nitroaniline concentration that inhibits the growth after incubation 37˚c for 18-24 hrs doubling the concentration of p-nitroaniline (10.20.40.80.160.320.ppm). The media used for the MIC is MSG mineral glucose media.MSG media plates were prepared and bacterial isolates were inoculated uniformly on the surface of agar plates.Dilutions of the PNA were prepared to range from 10 ppm to 320 ppm(1 mg in 1000 ml D/W= 1 ppm). Sterile filter paper disc impregnated with the dilution of PNA was applied on the surface of the agar plate.Plates were kept at 37˚c for 18 to 24 hrs.The result was recorded by the observing zone of inhibition surrounding the disc (Tani, K., Masuhara, M., Welikala, N., Yamaguchi, N., and Nasu, M., 1998).

Biodegradation of PNA:

All bacterial strains were grown in MSG medium 30˚c. cells were aseptically harvested, washed twice in a sterile mineral salt solution, resuspended in 100 ml of MSG supplemented with PNA. The same culture in MSG medium without fortifying with PNA was used as control. These cultures were taken in 500 ml Erlenmeyer flasks and incubated in dark at 30˚c on a rotary shaker (170 rpm). Their growth (biomass) and PNA consumption were monitored at regular intervals by spectrophotometry at OD 600 nm.Each bacterial inoculums (2gL-1) were taken in a 100 ml Erlenmeyer flask containing MSG medium (100 ml) fortified with 200 p-nitro anilines.After 24, 48, 72, and 96 hrs of incubation aliquots of broth were collected, centrifuged at 10,000 rpm for 10 min, and percent biodegradation in the supernatant was determined (Jain, R.K., Kapur, M., Labana, S., Lal, B., Sarma, P.M., Bhattacharya, D. and Thakur, I.S., 2005; Gisi, D., Stucki, G. and Hanselmann, K.W., 1997).

III. RESULTS AND DISCUSSION

Determination Of susceptibility:

The susceptibility of this bacterial species can be confirmed by determining the minimum inhibitory concentration (MIC) of P-nitroaniline for each bacterial strain as shown in Table 1.

Table 1: Minimum inhibitory concentration against PNA of *Azotobacter sp., Bacillus sp.*, and *Pseudomonassp.* at different concentrations.

The concentration of PNA (ppm)	Zone of inhibition(mm)		
	Azotobacter sp.	*Bacillus sp.*	*Pseudomonas sp.*
10	2	3	2
20	2	4	3
40	5	4	3
80	5	6	5
160	8	10	8
320	12	15	12

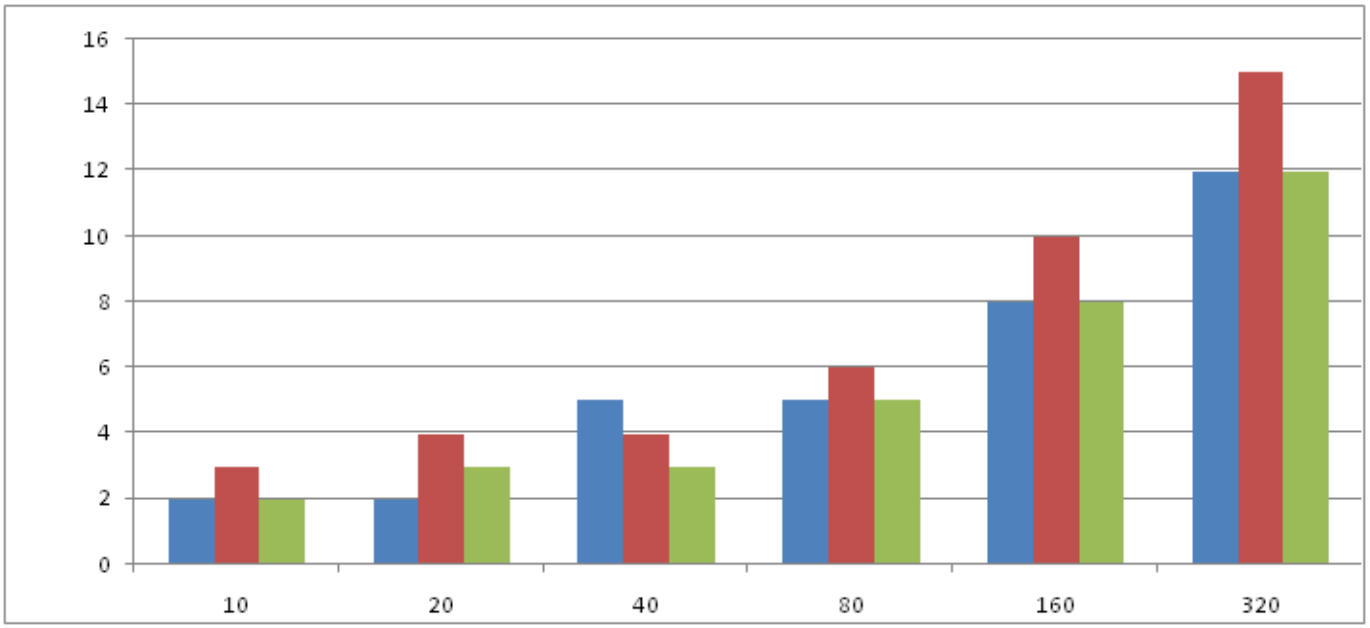

Fig.1: Minimum inhibitory concentration against PNA of *Azotobacter sp., Bacillus sp.*, and *Pseudomonas sp.*at different concentrations.

Biodegradation of PNA:

After 24, 48, 72, and 96 hrs of incubation aliquots of broth were collected, centrifuged at 10,000 rpm for 10 min and percent biodegradation in the supernatant was determined as shown in Table 2.

Table 2. Degradation of PNA (percent).

Time(hrs)	Percent Degradation of PNA		
	Azotobacter sp.	*Bacillus sp.*	*Pseudomonas sp.*
6	3	4	5
12	4	6	7
24	9	8	11
48	19	20	22
72	30	30	34
96	41	40	42

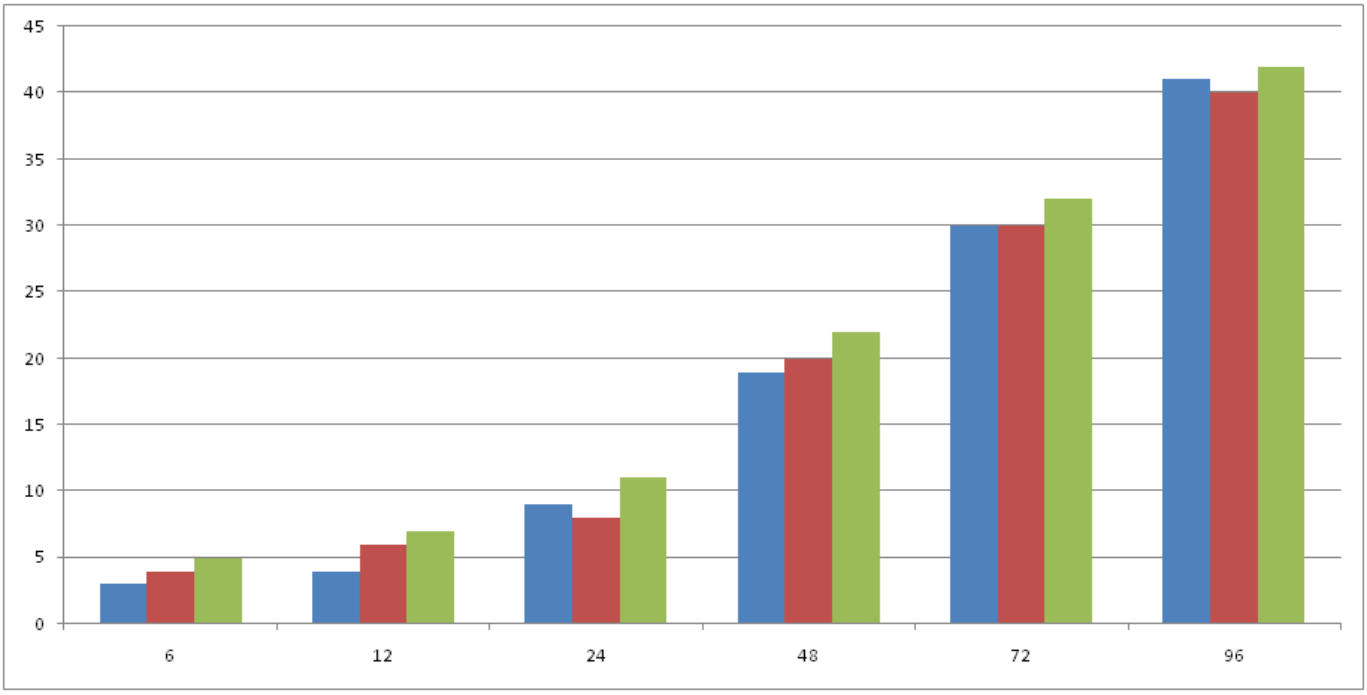

Fig.2: Degradation of PNA (percent).

IV. CONCLUSION

In the biodegradation of Para-nitro aniline. The methods applied are samples and from that, we can conclude that many bacterial strains like *Bacillus sp., Azotobacter sp.,*and*Pseudomonassp.*can able to degrade the Para-nitro aniline. From the all experiments that the performed *Bacillus* spp. can able to give rapid degradation than other species that are used i.e., 42%. As *Azotobacter*can fix nitrogen it can able to use nitrogen from PNA and hence it can be used in pesticide, fertilizer production. In the present project the experiment performed i.e., minimum inhibitory concentration checking and the percent biodegradation of Para-nitro aniline can be used as more suitable methods. The excess use of pesticides may affect the growth of seeds and hence to prevent this all these bacterial strains are used in fertilizers production.

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