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Determination of Carbofuran by spectroscopic method using Azo coupling reaction during the biodegradation of Carbofuran.

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ABSTRACT

Carbofuran a type of organophosphorous pesticide widely used in agriculture field and found in soil, water samples. A sensitive spectroscopic method was developed using 4-methyl aniline and sodium nitrate in concentrated HCl. Carbofuran in alkaline medium allow to react with 4-methyl aniline and sodium nitrate mixture and form diazonium salt which was measured at 470 nm spectrophotometrically. Amount of carbofuran measured from carbofuran degrading mixture was maximum up to 93% by *Enterobacter cloacae* after 72 hours.

Key words: Carbofuran, 4-methyl aniline, diazotization.

INTRODUCTION

Carbofuran (2,3-dihydro-2,2,-dimethyl-7-benzofuran 7-methyl carbamate) belongs to furadan family and carbamate group of pesticides having highly pest control ability so used widely in field of agriculture¹. Also control soil-dwelling process, root worm, land mosquito etc. in agriculture field. Bioremediation technology offers potential tool to treat soil pesticide pollution without need for excavation & required little energy input also an economical method of decontamination to solve hazardous waste problem of pesticide contaminated soil ^{2,3}.

Experimental

Acetanilide and 4-amino acetanilide were dissolved in 25ml of 1:1 HCl/HNO₃ and 2 ml of bromine at 38⁰C. After stirring for 3 hours, precipitate was filtered, washed with distilled water and dried at 40⁰C^{4,5}.

Synthesis of P-aminoazobenzene(p-tolueneazoaniline):

4-methyl aniline (p-toluene) was mixed with 1.5 ml concentrated HCl and 0.5% of 2.5 ml of NaNO₂ at 0-5⁰ C to form the azo solution. Then the resulting azo solution was treated with Aniline at 0-5⁰ C of pH 7-9 to get the aminoazo dye. The precipitates were washed with distilled water and dried. The synthesis of p-aminoazobenzene is shown in Scheme 1.

Carbofuran Degradation:

Carbofuran was organophosphorous pesticide which is used to control the pest in agriculture. This carbofuran have various negative impacts on human sand other lives so it has to be removed from environment & microorganism proved to have great role to remediate it from environment. In present study Mineral Salt Medium(MSM) along with carbofuran (250ppm) was used to study biodegradation of carbofuran⁷⁻¹². Microorganisms were inoculated in the medium, incubated at room temperature for upto 72 hours. After incubation time was over, remaining amount of carbofuran in the medium was analyzed using above spectroscopic method⁶.

In first reaction the diazonium salt was prepared using p-toluidine , NaNO₂ & HCl which results in formation of p-toluidine diazonium chloride. In second reaction product of first reaction *i.e* p-toluidine diazonium chloride was allowed to react with carbofuran which results in formation of diazonium salt of carbofuran which was yellow colour compound.

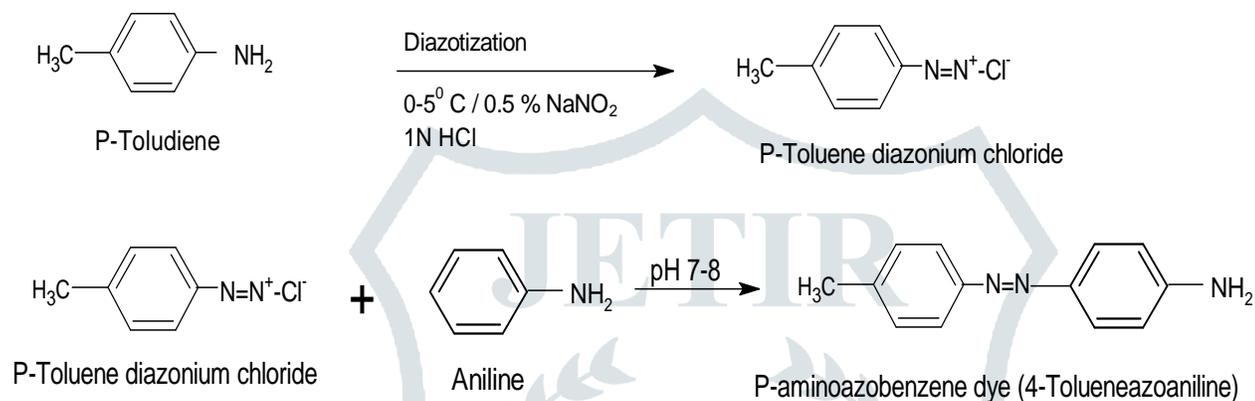
For Standard: Aliquots of 1ml, 2ml ...10ml were taken followed by addition of 1ml of 2% NaOH. Tubes were incubated for 15 minutes at room temperature. After incubation period was over, 1ml of diazotized mixture was added. Tubes were kept in ice (0⁰C - 5⁰C & shaken thoroughly for 15 minutes. After 15 minutes, absorbance was measured at 440nm against reagent blank.

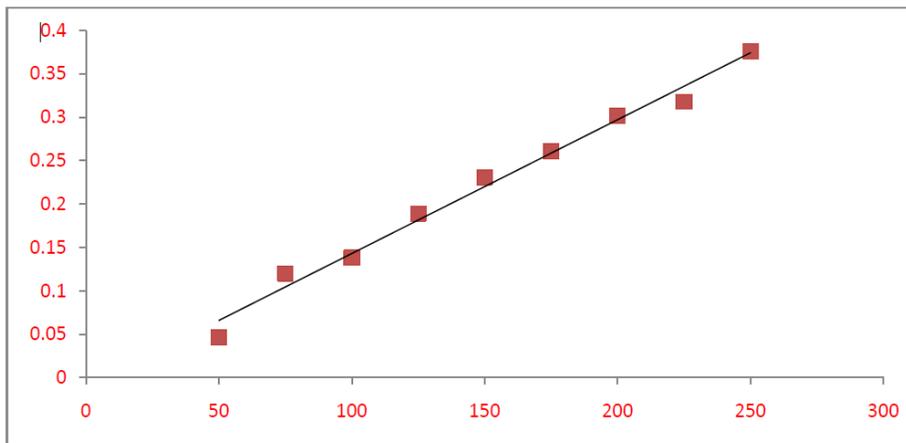
For Test: 1ml of sample was inoculated with 2% NaOH for 15 minutes at room temperature, followed by the addition of 1ml of diazotized mixture, which was kept in ice (0⁰C-5⁰C) & shaken thoroughly for 15 minutes. After 15 minutes, absorbance was measured at 440nm against blank reagent^{4,5}.

Instrumentation:

The spectral recording in the UV-VIS region for the coloured compound was carried out by HITACHI U 2001 spectrophotometer. Elemental analysis was carried out by Perkin- Elmer 240 C elemental analyser. An Analab pH meter with combined calomel-glass electrode was used for all pH measurements.

Scheme- 1



Graph 1: Standard graph of Carbofuran**Table No.1: Biodegradation study of carbofuran**

Organism	Carbofuran concentration measured
N-1	90%
N-2	92%
N-3	87%
N-4	84%
N-5	45%
N-6	87%
N-7	90%
N-8	87%
N-9	92%
N-10	93%
N-11	90%
N-12	58%
N-13	91%
N-14	71%
N-15	60%

Measurement of carbofuran concentration in test sample (Biodegradation study):

MSM medium containing carbofuran was inoculated with respected organism to study biodegradation process^{7,8,9}. After 72 hours, the remaining amount of carbofuran concentration was measured by modified method. Highest carbofuran degradation was observed by bacteria N-10 upto 93% carbofuran degradation with remaining carbofuran concentration of 17.5ppm. later this organism was identified as *Enterobacter cloacae* by 16s rRNA technique.

CONCLUSION

By modified method, carbofuran can able to combine with diazo-compound results in a formation of yellow colour. Colour was directly proposional to carbofuran concentration. Maximum degradation was observed by isolate N-10 i.e. *Enterobacter cloacae* after 72 hours. Method proved to be easy and sensitive for carbofuran estimation.

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