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Physical parameters such as pH and temperature influencing dimethoate biodegradation by *Pseudomonas sutzeri*

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ABSTRACT

Current study is based on biodegradation of organophosphorus pesticide dimethoate in liquid MSM medium with *P. sutzeri*. It was found to be capable of utilizing dimethoate as sole carbon and energy source and could rapidly utilized dimethoate beyond 100 ppm and showed maximum growth in MSM. The concentration of the dimethoate in the solution decreased exponentially with the increased exposure time. *P. sutzeri* could tolerate dimethoate from 100 ppm to 900ppm. In current study, several factors influencing dimethoate degradation were investigated. Complete disappearance of dimethoate was detected within 72 h of incubation. UV-Visible spectroscopic analysis (660 nm) revealed the complete mineralization of dimethoate. The optimal pH and temperature growth conditions were 8.5 and 30°C, respectively. The microbial consortia could prove to play a valuable role for the bioremediation of dimethoate contaminated field soil.

Keywords: Dimethoate, *P. sutzeri*, biodegradation, pesticide, pollution

INTRODUCTION

During recent years, owing to the tremendous use of pesticides and insecticides in agriculture soil, the amounts of residues of these chemicals in soil and water reservoirs have increased significantly. Hundreds of different types of pesticides and insecticides of various chemical natures are used worldwide for agricultural and non agricultural purposes[1]. Most commonly pesticides and insectides constitute major pollutants of the aquatic and soil environment, and their presence and persistence is of great concern because of their potential toxicity towards animals including humans[7]. The persistence of such hazardous chemicals into the environment has increased interest in researchers in studying microbes involved in their biodegradation. Microbial activities immensely influence the degradation of various soil pollutants including pesticides, insecticides and herbicides. Hydrolytic enzymes produced during microbial activities degrade the pesticides[3]. Biodegradation has found more efficient and more cost effective compared to chemical and photodegradation of such compounds in the environment. Organophosphorus compounds (OPs) used in agriculture as pesticides represent an attempt to maximise insecticide activity and minimize environmental persistence. They have replaced organochlorine compounds which persist and accumulate in the environment. This group of pesticides has been used in large quantities throughout the world since the first introduction of a synthetic insecticide, dimethoate, for use in crop protection for long time. Different pathways of organophosphate decomposition such as hydrolysis, photolytic oxidation, microbial transformations and other biological processes have been reported recently. Problems of contamination resulting from surplus pesticides and wastewater from pesticide factories have become obvious.

Dimethoate is an insecticide used to kill mites and insects systemically and on contact. It used against a wide range of insects, including aphids, thrips, plant hoppers and whiteflies on ornamental plants, grape, pomegranate alfalfa, apples, corn, cotton, melons, orange, sorghum, soybeans, tomatoes, and many more vegetables. Dimethoate is highly toxic to fish and to aquatic invertebrates. It undergoes rapid degradation in the environment and in sewage treatment plants[2]. Because dimethoate is highly soluble in water and it adsorbs only very weakly to soil particles. It subject to significant hydrolysis, especially in alkaline waters. The bioconversion of pesticides in the environment results from physicochemical reactions as well as from the activity of cellular

or extracellular components of the Effective Microorganisms. In the environment, pesticides are exposed to various degradative forces. Biodegradation is known to play a vital role in this respect[8]. It contribute not only in the disappearance of the original form of pesticides, but also change their physicochemical properties, and thus affect their transport and distribution behaviour among various compartments in the environments. Most forms of living organisms are capable of directly interacting with pesticides and some of them are capable of metabolizing even very recalcitrant compounds[4]. The technology of effective microorganisms commonly termed EM Technology was developed by Dr. Teruo Higa in 1970's at the University of Ryukyus, Okinawa, Japan. This technology includes three principles types of organis-ms commonly found in all ecosystem, namely (lactic acid bacteria, yeast, actinomyces, and photosynthetic bacteria). Thus, it may be possible to take advantage of EM to bioremediate environmental pollution by OPs[1].

Dimethoate is an organophosphorus insecticide and acaricide used for the control of houseflies, as well as a wide range of insects and mites on a variety of fruit, vegetable, field and forestry crops. Its solubility in water at 21°C is 25 g/L. Dimethoate released to the environment does not adsorb onto the soil and is subject to considerable leaching. It is also lost from the soil through evaporation and biodegrade-ation. The half-life of dimethoate in soil ranges from four to 16 days. It is relatively stable in aqueous media at pH 2 to 7.5 Reported half-lives for dimethoate in raw river water range from 18 h to eight weeks. Dimethoate is degraded in the environment to another more toxic compounds such as omethoate; the proportion of omethoate in the total residue reaches about 50% after five weeks.

MATERIALS AND METHODS

Chemicals

The organophosphate insecticide dimethoate [dimethyl S-(N-methylca-rbamoylmethyl) phosphorothiolothion- ate], is manufactured by Indichem ,Vatwo, Ahemadabad, India, was employed in this investigation. Samp-les have been prepared in deionised water using ethyl acetate. Dimethoate was used in emulsifiable concentrate.

Source and enrichment of the Effective Microorganisms

Effective Microorganisms (EM) were isolated from Grape field soil, Nashik, Maharashtra, India[1]. The enrichment and propagation were carried out in sterilized 250 ml Erlenmeyer flasks containing 50 ml Minimal Salt Medium (MSM)with following salts ZnSO4: 0.01mg; CaCl2.2H2O:10mg; MgSO4. 7H2O:0.5g; K2HPO4:0.5g; (NH4)2SO4: 0.5g and FeCl3.H2O:10 mg per litre in D H2O , supplemented with 100 ppm dimethoate and 1 ml EM inoculums of O.D. 1.0. The pH value of the culture solution was adjusted to 7.0 with 1.0 N NaOH or 1.0 N HCl.

Isolation and characterization of the bacterial strains from the micro-bial consortia

Isolation of dimethoate degrading microbes was carried out by grinding sampled (air dried) soil in fine form. Then making its suspension in sterile saline(0.85 %) and inoculating in in the MSM agar plates containing dimethoate as a sole source of carbon. Mixed culture obtained then used for getting pure culture on sterile MSM agar plate by steak plate method Characterization and identification of confirmed degraders was carried out by streak method technique and morphological and biochemical methods (Bergey's Bacteriology). After the incubation period at 30°C, the single colonies were picked and grown again in liquid mineral salt media for at least 5 days. This procedure was repeated until getting identical colonies. The isolated strains were characterized and identified by biochemical methods[2].

Determination of pH and temperature optima

Three 250 ml Erlenmeyer flasks containing 50 ml MSM medium were adjusted to different range of pH (5,7,9). The flasks were incubated on a rotary shaker at room temperature and 100 rpm. In time interval of 12 h, 2 ml, sample was taken to determine bacterial growth[9]. Depending on the optimal pH; the temperature values were adjusted to 25, 30 and 32°C with previously mentioned procedures and conditions [7,10].

RESULTS AND DISCUSSION

Aerobic growth of Pseudom-onas sutzeri and degradation of dimethoate

The microorganisms were enriched and cultivated on dimethoate containing MSM media. The microorganism grew well by utilizing dimethoate, as was evident from the increase in the optical density at 660 nm; and the simultaneous loss of dimethoate from the culture was observed by calculating percent biodegradation (Fig. 1 and 2). When the microorganisms were grown in medium with dimethoate as the only carbon source, the milky colour of the medium was appeared. The disappearance of the dimethoate was monitored by spectr-ophotometric analysis. The mineralization of dimethoate by P. sutzeri was very promising and the concentration of dimethoate almost vanished and utilized as a sole of carbon and energy source after 36 h[4,5,6,8]. The optimal pH for the growth was determined as 9.0 with optimal growth temperature of 30°C. The biodegradation of the dimethoate was promising at these optimal conditions as shown in Fig.1 and 2. Pseudomonas sutzeri could be well grown at the optimal pH and temperature[10].

Dimethoate Concentration (ppm) O. D. 660 (nm)

When the bacteria grow at optimal pH and temperature the transport of the substrates will be ideal through the membrane, hence the growth rate increased. It can be observed that growth over the higher temperatures the transport of the substrates is impaired.

Environmental applications of the microbial activities in bioremediation

It was found that it was possible to apply the microbial activities and/or their biocatalysts, for the remediation of natural and soil water containing millimolar concentration of toxic, persistent aromatic

pesticides. It is expected that pesticides will be transformed into biodegradable compounds and mineralized into O2, H2O and CO2, by using these micro-organisms for an appropriate duration. This recent tools in biotechnology methods can be considered very efficient and much pH and temperature dependence of dimethoate biodegradation by Pseudomonas sutzeri.

Biological techniques are more efficient than chemical ones for improving the quality of soil, water and water resources and eliminating aromatic pesticide residues dissolved or dispersed in water and soil. As a conclusion microbial processes in the various kinds of aerobic and anaerobic systems for treating industrial, agricultural and municipal wastes are very important because these systems represent the hot spots of discharge of many chemicals into environment[9,10].

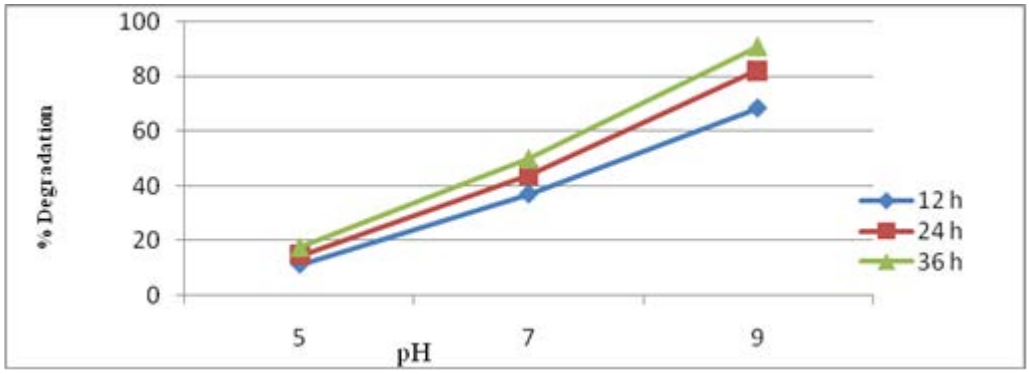

FIG.1. Pseudomonas sutzeri grown in MSM at diff. pH with dimethoate as a sole source of carbon and energy for pH optima.

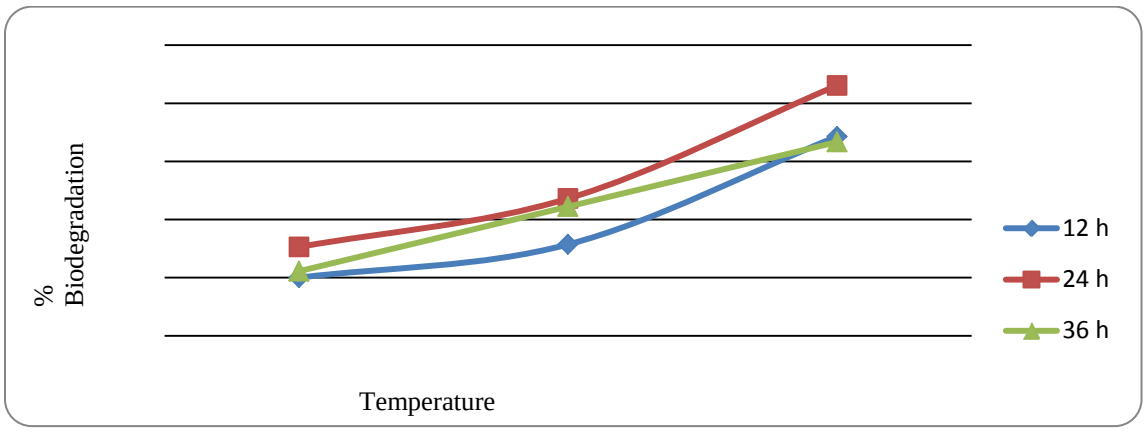

FIG.2. Growth of Pseudomonas sutzeri in MSM with dimethoate as a sole source of car-bon and energy for temperature optima.

Soil microorganisms as Pseudomonas sutzeri play a vital role in the in situ biodegradation of organophosphates and aromatic pesticides in environments, where ambient temperatures often present. The effective and stable biodegradation capacity of these microorganisms in utilizing and degrading these compounds reflected their potential in biotechnological application at room temperature bioremediation of organophosphate compounds contaminated sites.

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