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MICROBIAL CONSORTIA AS A UNIQUE APPROACH FOR DEGRADATION AND BIOREMEDIATION OF DIMETHOATE IN EGYPTIAN CONTAMINATED WATER

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ABSTRACT

A unique approach of the degradation of organophosphorus pesticide dimethoate in aqueous media with microbial consortia was studied. These effective microorganisms were capable of utilizing dimethoate as sole carbon and energy source and could rapidly utilized dimethoate beyond (100 $mg^{-1}L$) dimethoate and showed prolific growth in a mineral salts medium. The concentration of the pesticide in the solution decreased exponentially with the exposure time. The microbial consortia could tolerate 120 mg mL^{-1} of technical grade dimethoate. In this study, several factors influencing dimethoate degradation were investigated. The growth rate μ (h^{-1}) of these effective microorganisms was ca. 0.925. Complete disappearance of dimethoate was detected after 3 days of incubation. Gas Liquid Chromatography (GLC) analyses revealed the complete mineralization of dimethoate. However, the precise conditions for surface targeting or pesticide degradation were not fully understood. Change in pH of culture broth to acidic range supported the biological transformation. Optimal growth conditions were 8.5 and 27°C, pH and temperature, respectively. Two isolates from these microbial consortia lost their ability to utilize the dimethoate. The intermediate compounds were also metabolized, further resulting in complete mineralization of dimethoate. The microbial consortia could prove to play a valuable role for the bioremediation of dimethoate contaminated water.

INTRODUCTION

During recent years, owing to the widespread use of pesticides in agriculture, the amounts of these compounds in aqueous media have increased significantly. Presently, several hundred pesticides of various chemical natures are used worldwide for agricultural and non agricultural purposes. Indeed, pesticides constitute major pollutants of the aquatic environment, and their presence is of great concern because of their potential toxicity towards animals and humans. The prevalence of such materials into the environment has increased interest in studying microbes involved in their biodegradation (Zhuang et al., 2003). 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 organophosphates decomposition such as hydrolysis, photolytic oxidation, microbial transformations and other biological processes have been reported recently (Zhang, 2002). 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, planthoppers and whiteflies on ornamental plants, alfalfa, apples, corn, cotton, grapefruit, grapes, lemons, melons, oranges, pears, pecans, safflower, sorghum, soybeans, tangerines, tobacco, tomatoes, watermelons, wheat and other vegetables. It is also used as a residual wall spray in farm buildings for house flies. It has been administered to livestock for control of botflies (Hayes et al. 1990). This compound acts by interfering with the activities of cholinesterase, an enzyme that is essential for the proper working of the nervous systems of both humans and insects and is possibly carcinogenic. A Dimethoate is highly toxic to fish and to aquatic invertebrates. It undergoes rapid degradation in the environment and in sewage treatment plants (Cheminova 1991). Because dimethoate is highly soluble in water and it adsorbs only very weakly to soil particles. In water, dimethoate is not expected to adsorb to sediments or suspended particles, nor to bioaccumulate in aquatic organisms. It is subject to significant hydrolysis, especially in alkaline waters.

The biotransformation 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. Biotic degradation, or metabolic processes, are known to play a vital role in this respect. They contribute not only to the disappearance of the original form of pesticides, but also change their physicochemical properties, and thus affect their transport and distribution behavior 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. The technology of Eeffective Mmicroorganisms 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 organisms commonly found in all ecosystem, namely (lactic acid

bacteria, yeast, actinomyces, and photosynthetic bacteria). These were blended in a molasses or sugar medium and maintained at low pH under ambient condition. Thus, it may be possible to take advantage of EM to bioremediate environmental pollution by OPs.

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. More than 100 000 kg are used annually in Canada.1 Dimethoate has a vapour pressure of 1.1 × 10-3 Pa at 25°C; its solubility in water at 21°C is 25 g/L.2 Reported log octanol–water partition coefficients are 0.78 and 0.79.3 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 biodegradation. The half-life of dimethoate in soil ranges from four to 16 days.4 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 hours to eight weeks.4 Dimethoate is degraded in the environment to another more toxic pesticide, omethoate; the proportion of omethoate in the total residue reaches about 50% after five weeks.6

MATERIALS AND METHODS

Chemicals

The organophosphate insecticide dimethoate [dimethyl S- (N-methylcarbamoylmethyl) phosphorothiolothionate], is produced by Kafr El-Zyat pesticides and chemicals Co. (kz), was employed in this investigation. Samples have been prepared in deionised water using ethylacetate. Dimethoate was use in emulsifiable concentrate (EC).

Source and enrichment of the Effective Microorganisms

Effective Microorganisms (EM) were got from Ministry of State for environmental affairs – Alexandria branch. The enrichment and propagation were carried out in sterilized 250 ml Erlenmeyer flasks containing 600 ml distilled warm water, with supplemented with 100 ml molasses, 100 ml natural vinegar, 100 ml ethyl alcohol and 100 ml EM liquid concentrate. The cultivation was carried out in sterilized 100 ml flasks containing 18 ml MSM for each, 1 ml EM supplemented with serial concentrations of dimethoate estimated by 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800, 2000 ppm (w/v) was used. The pH value of the culture solution was adjusted to 7.0 with NaOH.

Determination of the growth rate (μ) h^{-1}

In time interval of 6 hrs, 10 µl from each flask were taken from each flask. The growth rate (μ) h^{-1} was calculated according to the growth curve. The growth of the microbial consortia was determined by spectrophotometer (Uvicon 860, Kontron, Germany) at 570 nm. The growth rate (μ) h^{-1} was determined according to Herbert and Burill, 1997.

$$\text{Growth rate } \mu\ (h^{-1}) = \ln 2.\left(\frac{\log x_2 - \log x_1}{T_2 - T_1}\right)$$

Where: x_1 and x_2 are growth values at time t_1 and t_2, respectively

Determination of pH and temperature optima

Different flasks containing media were adjusted to different range of pH (4-9). The flasks were incubated on a rotary water bath shaker at room temperature and 200 rpm. In time interval of 6 hrs, 10 µl, a sample was taken to determine consortia growth. Depending on the optimal pH; the temperature values were adjusted to 10, 20 and 30°C with previously mentioned procedures and conditions.

Analysis and determination of the dimethoate residues by Gas Liquid

This method provides procedures for the Gas Liquid Chromatography (GLC) determination of organophosphorus compounds (OPs). HP 6890 series (GC) with FID detector was used for analyses. GLC conditions were as follows: the initial temperature was 80°C; temperature increased initially by 15 min^{-1} up to 280°C; injector port temperature was 250°C, detector temperature was 250°C. The method of extraction was adapted from the literature (Parrilla and Martinez 1997). A volume of 0.5 L of the sample was extracted with 3 portions of 50 mL of dichloromethane. Water was removed from the combined organic extract by addition of anhydrous sodium sulfate. Solvent evaporation was performed in a rotary evaporator until ± 3 mL, then this volume was transferred into a small vial. A volume of 1 mL of methanol/ water 6:4 (v/v) was added and this solution was filtered through a 0.45 µm membrane before injectioninto the chromatograph.Strotmann and Rschenthaler (1987). Retention time for dimethoate was 20.5 min.

Isolation and characterization of the bacterial strains from the microbial consortia

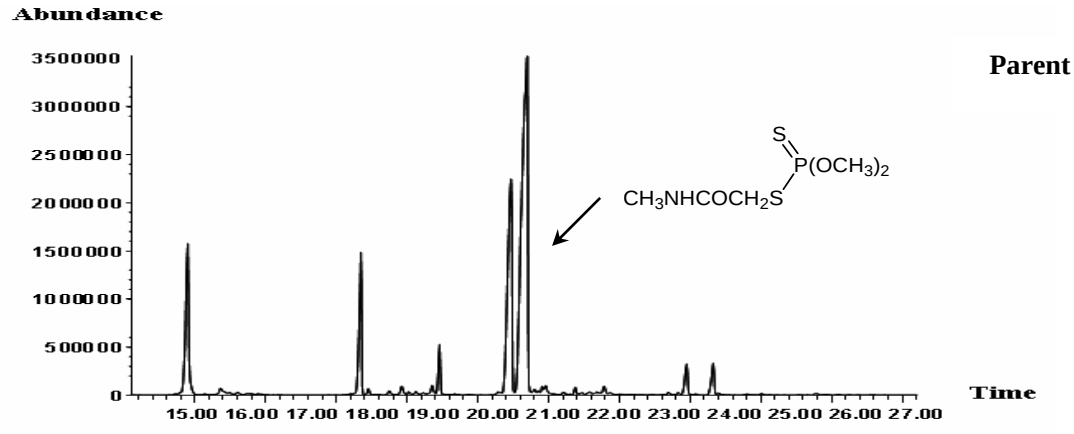
Isolation and characterization of the degrading bacteria were carried out by streak method technique Kiyohara et al., 1982. After the incubation period at 25°C, the single colonies were picked and cultivated again in liquid mineral salt

media for at least 5 days. This procedure was repeated until getting identical colonies. The isolated strains were characterized and identified by Deutsche Sammlung von Mikroorganismen und Zellkulturen (DSMZ), Brauenschweig, Germany, according to the fatty acids analysis and 16s rDNA sequence.

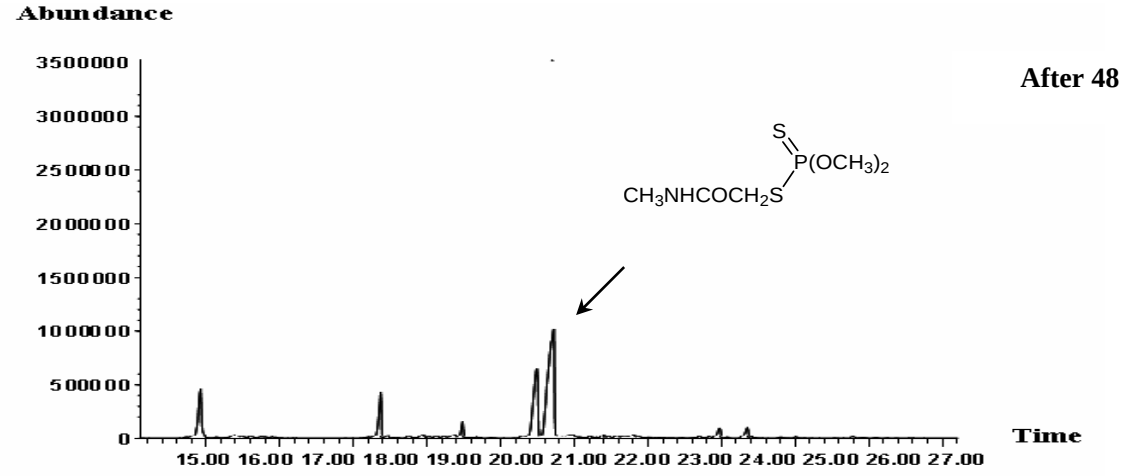
RESULTS AND DISCUSSION

Aerobic growth of microbial consortia and degradation of dimethoate

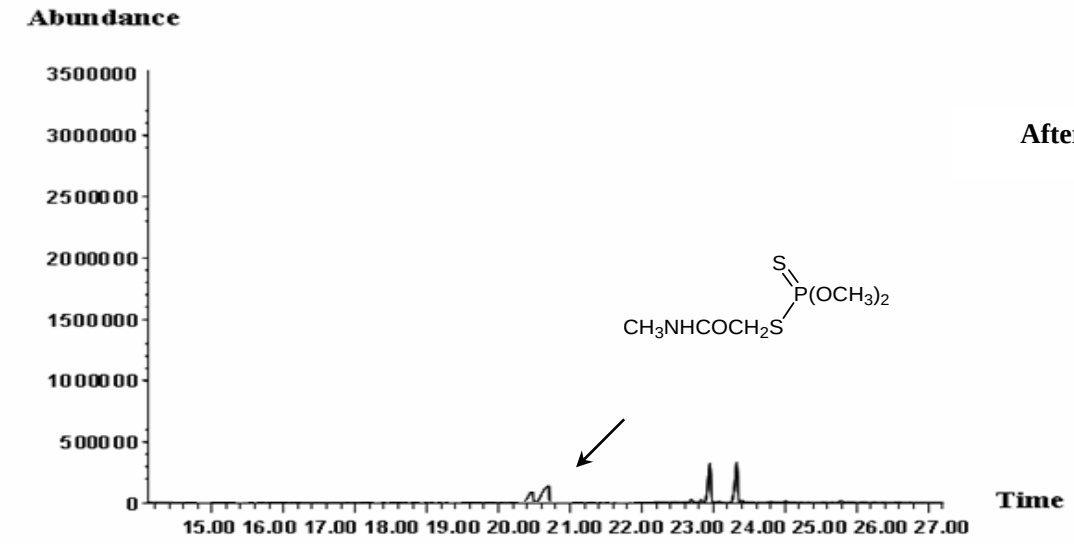
The microbial consortia was enriched and cultivated on dimethoate containing media. The microbial consortia grew well by utilizing dimthoate, as was evident from the increase in the optical density at 570 nm (Fig. 1); and the simultaneous loss of dimthoate from the culture was observed by GLC analyses (Fig. 2). When the microbial consortia were grown in medium with dimethoate as the only carbon source, the milky color of the medium was appeared.



Parent compound



After 48 hrs.



After 72 hrs.

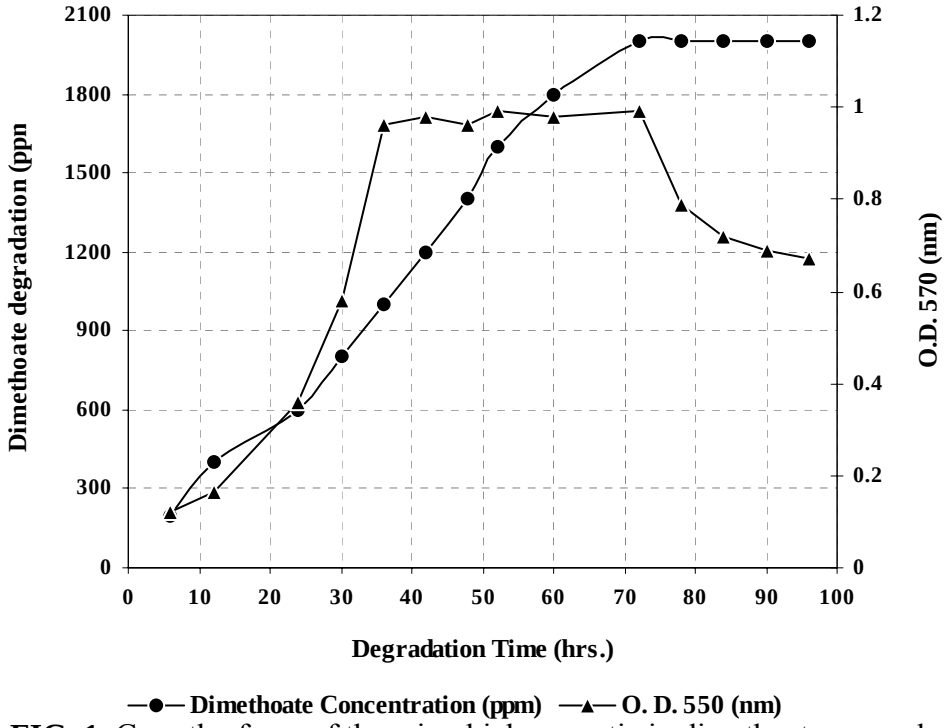
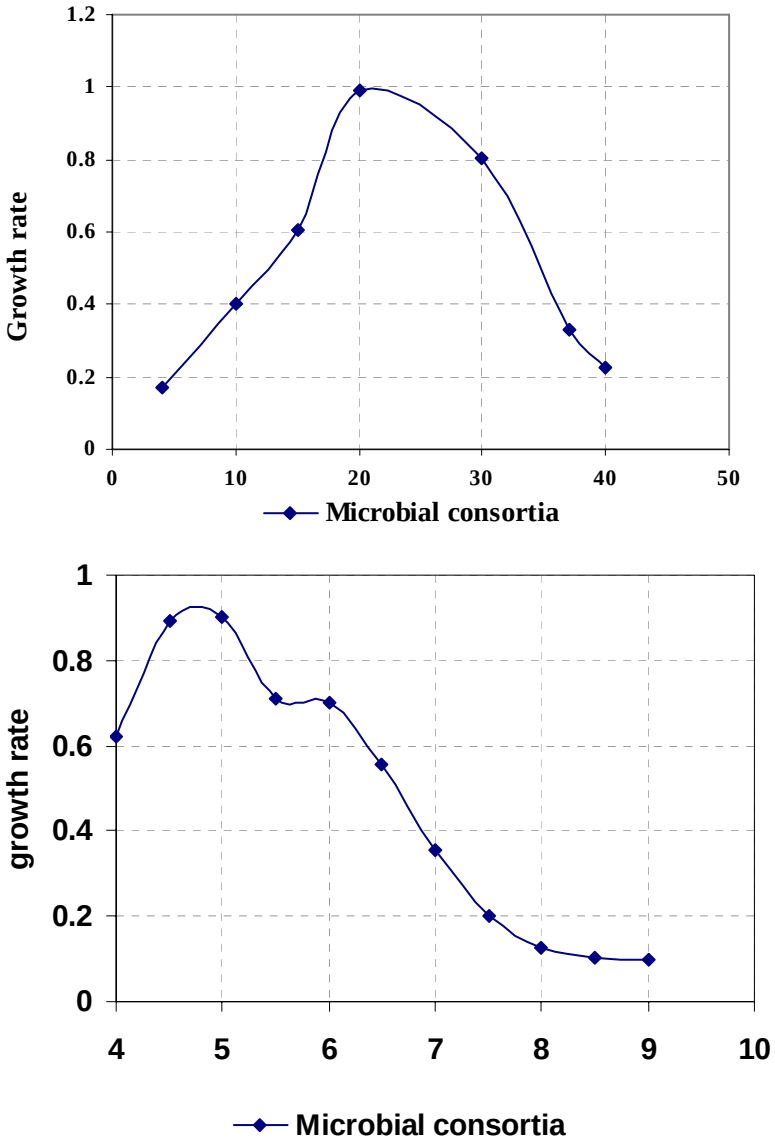


FIG. 1. Growth of one of the microbial consortia in dimethoate as a sole of carbon and energy source. The growth curve for the microbial consortia at O.D. 570 nm.

The disappearance of the dimethoate and the formation of the metabolites were monitored by GLC analysis. The mineralization of dimethoate by the microbial consortia was very promising and the concentration of dimethoate almost vanished and utilized as a sole of carbon and energy source after 3 days. The optimal pH for the growth was determined at 8.0 with optimal growth temperature of 27°C The assimilation and mineralization of the dimethoate was promising at these optimal conditions as shown in Fig. 5. It was clear that, the growth rate μ (h^{-1}) was ca. 0.925. *Pseudomonas amygdali* could be classified under the obligate psychrophiles organisms as previously mentioned, where the optimal temperature for the growth and the biodegradation was 20°C in accordance with Whyte et al., 1999.



pH and temperature dependence of dimethoate biodegradation by the microbial consortia

When the bacteria are grown in optimal temperature the transport of the substrates will be ideal through the membrane, hence the growth rate $\mu(d^{-1})$ increased. It can be observed that growth over the maximum growth temperature, the transport of the substrates is impaired. This fact can be simply explained that near the maximal activity the intracellular enzymes are being inactivated (Varanasi et al., 1981**).**

Gas chromatography

Further details were previously described The GLC parameters was performed according to the method of Stucki and Leisinger (1983) with a capillary gaschromatograph (gas chromatograph Shimadzu GC8, Varian CH7A) equipped with a fused silica capillary column (50 m) coated with SE 54 cross-linked film as stationary phase. Complete mass spectra in the appropriate range were recorded. Identification was done by comparing these spectra with reference spectra. Further conditions were previously described (Strotmann 1988). The dimethoate concentrations were quantified by gas chromatography mass spectrometry (GLC) analysis the substrate was actually degraded within the first 3 days.

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 water containing millimolar concentration of toxic, persistent aromatic pesticides. It is expected that pesticides will be transformed into biodegradable compounds and mineralized into H_2O and CO_2, by using these micro-organisms for an appropriate duration. This recent tools in biotechnology methods can be considered very efficient and much

cleaner techniques than chemical ones for improving the quality of water and water resources and eliminating aromatic pesticide traces dissolved or dispersed in water.

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 first point of discharge of many chemicals into environment. Cold-adapted microorganisms as *Pseudomonas amygdali* play a significant role in the in situ biodegradation of hydrocarbons and aromatic pesticides in cold environments, where ambient summer temperatures often coincide with their growth temperature range. The effective and stable degradation capacity of this isolate in utilizing and degrading these compounds reflected their potential in biotechnological application at low temperature bioremediation of aromatic contaminated sites.

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