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International Conference on Chemical, Metallurgy and Environmental Engineering

June 3-4, 2015 Istanbul (Turkey)

EFFECTS OF MALATHION ON THE MICROBIAL FLORA IN INDIGENOUS ACTIVATED SLUDGE

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Abstract- Malathion S-[1, 2- di (ethoxycarbonyl) ethyl] dimethyl phosphorothiolothionate; CAS No 121-75-5; C10 H19 O6 PS2] is one of the most widely used organophosphate insecticides throughout the world. It is commonly used to control mosquitos and a variety of insects that attack fruits and vegetables. Removal of this pesticide can be attained by physical-chemical and biological processes. Several studies have examined the degradation of malathion by microbes such as bacteria, but little information is available on its effects on bacteria and fungi in mixed cultures. Therefore, in this study, the identification and enumeration of dominant aerobic, heterotrophic flora of a mixed culture of indigenous activated sludge were achieved before and after the addition of malathion. The results showed that malathion affected the composition of the microbial flora of the activated sludge used. Indeed, the findings revealed that *Pseudomonas aeruginosa*, yeasts and molds counts increased after addition of malathion. On the other hand, total coliforms, faecal coliforms, *Escherichia coli* and faecal streptococci numbers were not affected as a consequence of the addition of the insecticide. This feature might, in the future, lead the way to predict biodegradation efficiencies of the pesticide in detoxification processes.

Keywords: activated sludge; malathion; microbial flora; *Pseudomonas aeruginosa*.

Introduction

Malathion (S-1, 2- bis (ethoxycarbonyl) ethyl O,O-dimethyl phosphorodithioate]; CAS No 121-75-5; C_{10} H_{19} O_6 PS_2] is one of the most widely used organophosphate insecticides throughout the world , this is due to its proven effectiveness in the control of insects. Indeed, malathion is commonly used to control mosquitoes and a variety of insects that attack fruits, vegetables landscaping plants and shrubs (Toft 2004). Suspended growth systems comprise aggregates of microorganisms generally growing as flocs in intimate contact with the wastewater they are treating. The aggregates or flocs are responsible for the removal of polluting material and comprise a wide range of microbial species (Horan 2003). Several studies have examined the degradation malathion by microorganisms (Goda et al. 2010; Singh et al. 2012). Little information is available concerning the effect of malathion on microorganisms. Therefore, the aim of this study is to investigate the effect of malathion on dominant aerobic, heterotrophic flora of indigenous activated sludge.

Materials and methods

Culture medium

Technical malathion having purity around 95% was purchased from Alphyte company which is located in Algiers. The pesticide was added to mineral salt medium to the required concentration (60 mg/L)

The composition of the mineral salt medium used was as follows (mg/L): $MgSO_4 \cdot 7H_2O$ (400 mg), $(NH_4)_2SO_4$ (7000 mg), $FeSO_4$ (13.66 mg), $MnCl_2 \cdot 4H_2O$ (3.14 mg), $ZnSO_4 \cdot 7H_2O$ (4.4 mg), $CuSO_4 \cdot 5H_2O$ (0.79 mg) and $CaCl_2$ (55.43 mg).

The final pH value was adjusted to 6 using Sorensen buffer solution.

The medium was sterilized by autoclaving at 121ºC for 15 min before use. All chemicals used in this study were analytical grade**.**

Microorganisms

In the present study, an indigenous mixed activated sludge culture, collected from the aeration basin of wastewater treatment plant located in Beni-Messous-Algiers, was used. The capacity of processing of the plant is 50.4 mega liters of wastewater per day (MLD) (250000

population equivalent). The operating sludge age was 10 days and the biomass concentration was about 5000 mg/L.

Dominant microbial flora study

The total aerobic mesophilic flora was enumerated on plate count agar, after incubation at 30°C for 72 hr according to ISO 6222 (1999).

According to ISO 9308-2 (1990), the lactose broth was used as the medium for the identification and the enumeration of total coliforms after incubation at 37°C for 48 hr. They are provisionally identified by the production of acid and gas from the fermentation of lactose. Faecal coliforms (heat-tolerant coliforms) were isolated and enumerated on Brilliant Green Lactose Bile Broth after incubation at 44°C for 48hr and examination for gas formation in the Durham tubes. For the detection and the enumeration of *Escherichia coli*, the Mackenzie test was carried out.

According to ISO 7899 (1984), the Roth broth was used as the medium for the presumptive test for the identification and the enumeration of faecal streptococci after incubation at 37°C for 48hr. The Bile Aesculin Azide agar was used as the medium for the confirmatory test.

Cetrimide agar was used for the identification of *Pseudomonas aeruginosa* based on pyocyanin production according to ISO 8360-1 (1988). The colonies that produce a green-blue color and fluoresce under UV light are considered presumptive *Pseudomonas aeruginosa.*

For the detection and the enumeration of *Staphylococcus aureus*, Baird Parker Agar was used after incubation at 37°C for 48 hr (ISO 6888-1 1999).

For the identification and enumeration of Sulfito reductor clostridia, the method used was that according to ISO 15213 (2003). The sample was heated in a water bath to 80°C for 10 min and cooled rapidly to kill vegetative cells. The spores of *Clostridium* were counted on Iron Sulfite Meat-Liver Agar after incubation at 46°C for 24 hr.

Yeasts and molds were detected and counted using Oxytetracycline Glucose Agar according to ISO 7954 (1987).

Results and discussion

The results of the dominant microbial flora study before and after the addition of the pesticide tested at 60mg/L showed that malathion affected the composition of the microbial flora of the activated sludge used. The data revealed that *Pseudomonas aeruginosa* and yeasts and molds counts increased after addition of malathion. This could be explained by the fact that *Pseudomonas aeruginosa* and yeasts and molds could hydrolyze the pesticide and, therefore could use it as their sole source of carbon and energy.

The reduction of the *Staphylococcus aureus* and Sulfito reductor clostridia numbers from 16,000 (c.f.u/ml) and 1800 (c.f.u/ml) to 0 respectively, could be explained by a possible bactericidal effect exerted by the pesticide on these bacteria.

On the other hand, it should be pointed out that total coliforms, faecal coliforms, *Escherichia coli* and faecal streptococci numbers were not affected when the pesticide was added. This might mean that these bacteria could not carry the necessary enzymes required to degrade the organophosphorus pesticide or that the pesticide could exert a bacteriostatic effect on these bacteria.

Conclusions

In this study, indigenous activated sludge was sampled for microbiological analysis before and after addition of malathion. Data revealed that the pesticide has been found to alter the microbial composition of activated sludge tested. In fact, in the presence of malathion, Faecal streptococci count decreased, which may be due to an inhibitory effect exerted by the pesticide. In addition, malathion seemed to exert a bacteriocidal effect on *Staphylococcus aureus* and Sulfito reductor clostridia. It might be interesting to determine the contribution of each species in the degradation of the pesticide. This feature may be capitalized on in future efforts to predict biodegradation efficiencies of the pesticide in detoxification processes.

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