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Effect of Commercial Grade Methyl Parathion on the Survival of *Pseudomonas* at Varying Temperature and pH

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ABSTRACT

The wide-spread use of methyl parathion as an agricultural insecticide has been causing an increased level of contamination in soil, vegetation and groundwater reservoirs and as well as adverse effects on human, flora, fauna and ecosystems. Therefore, eco-friendly and cost-effective bioremediation system are needed to remove these pollutants from the contaminated sites and mitigate its hazardous. For this purpose, a lab scale study was conducted to investigate the effect of high concentrations of commercial grade methyl parathion (50 EC) on growth of indigenous soil *Pseudomonas* IES-Ps-1 under different environmental conditions such as pH and temperature. To determine the tolerance limit, *Pseudomonas* IES-Ps-1 was grown in nutrient broth supplemented with methyl parathion (0, 200, 400, 600, 800, 1000 and 1200 mg/l). The detailed pH (6, 7, 9) and temperature (25, 30 and 35 °C) studies were conducted using higher methyl parathion (400 and 800 mg/l). No growth was observed at 1000 and 1200 mg/l of pesticide after 96 hrs of incubation compared to other concentrations of 200, 400, 600 and 800 mg/l. The maximum growth for both 400 and 800 mg/l of pesticide was observed at pH 7 and 30 °C. The maximum removal of *total organic carbon* and chemical oxygen demand for 400 mg/l of pesticide were found 50 and 52 % while those for 800 mg/l were 46 and 49 % respectively. Hence, this study concluded that indigenous soil bacterium *Pseudomonas* IES-Ps-1 could serve as an efficient candidate at 30 °C and pH 7 in development of a bioremediation system for the removal of toxic effects of methyl parathion like pesticides from contaminated sites.

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INTRODUCTION

Aethyl-parathion is among the most widely used organophosphates-biased insecticide because its broad spectrum, specificity, and high efficiency toward insects and pests (Mulla *et al.*, 2020). However, improper, continuous, and ignorant use can lead to critical environmental pollution and life-threatening health problems due to the high toxicity of methamidophos to non-target organisms (Lin *et al.*, 2020). Therefore, methyl parathion is classified as Category Ia (extremely toxic) by

the World Health Organization as Toxicity Category I (most toxic) insecticide by the United States Environmental Protection Agency (Kumar *et al.*, 2020). Improvement in the quality of life requires efforts to combat such pesticide wastes by identifying cost-effective and nature-friendly remediation techniques. Many physicochemical methods have been developed and applied for the remediation of acephate and methamidophos, but they are thought to be too expensive to use on a large scale. For detoxification of pesticide-

contaminated soils and water, currently, bioremediation is recognized as a cost-effective, environment-friendly technique compared to other conventional methods such as land filling, incineration, and excavation (Vidali, 2001). Biodegradation is defined as the process by which organic matter is biologically broken down under controlled conditions to an innocuous state, or to levels below concentration limits established by regulatory authorities (Mueller *et al.*, 1996). The philosophy of the process is to enhance the rate of the natural microbial degradation of contaminants by supplementing these microorganisms with nutrients, carbon sources or electron donors. Indigenous microorganisms or enriched culture of microorganisms with specific characteristics are used in process that allows them to degrade the desired contaminant at a quicker rate. Ideally, bioremediation results in the complete mineralization of contaminants to water and carbon dioxide without the buildup of intermediates (Frazar, 2000). Worldwide, much more efforts are being made in identifying and characterizing potential microorganism for biodegradation to produce non-hazardous end products. Microbial degradation of methyl parathion has been studied extensively and its degradation pathway is well elicited (Kumar *et al.*, 2020). Several bacterial strains are reported to utilize methyl parathion as the sole carbon and energy source. These include *Flavobacterium* sp. (Misra *et al.*, 1992), *Pseudomonas putida* (Rani and Lalithakumari, 1994) *Pseudomonas* sp. A3 (Ramanathan and Lalithakumari, 1999) and *Ochrobactrum* sp. B2 (Qiu *et al.*, 2007). Keeping in mind that, microbial degradation is considered to be a better and effective method to establish the bioremediation system for the contaminated sites using indigenous soil bacterium *Pseudomonas* IES-Ps-1. Therefore, it was aimed to determine the concentration limit of methyl parathion that support the growth of isolated microorganism under different pH and temperature conditions.

METHODS AND MATERIAL

Chemicals used

For growth studies, different concentrations of methyl parathion were added to nutrient broth medium to determine the utilization and biodegradation potential of IES-Ps-1. Different concentrations (0, 200, 400, 600, 800, 1000, and 1200 mg/l) of commercial-grade methyl parathion (50 EC) were used to replicate the actual field conditions instead of selecting the analytical grade pesticide.

Biodegradation test

Seed culture of Indigenous soil bacterium *Pseudomonas* IES-Ps-1 was prepared by growing bacterial strain in 100 ml nutrient broth having 150 mg/l of methyl parathion for 48 hrs at 30 °C on an orbital shaker at 120 rpm. A loopful of culture was streaked on nutrient agar slants, incubated for 24 hrs at 35 °C. The culture slants were washed with 10 ml of phosphate buffer having pH value of 7 and then used in biodegradation of methyl parathion in nutrient broth. After 24 hrs interval, aliquots of samples were removed to determine both the microbial growth and amount of organic matter. All the experiments were performed in triplicate. Tubes without culture were maintained as control and the results are reported as average of triplicate experiments.

Optical density

The growth of *Pseudomonas* was determined by measuring the optical density (OD) at various time intervals such as 0, 2, 4, 24, 26, 28, 48, 50, 52, 72, 74, 76, 94, 96 and 98 hrs for each concentration of pesticide at 600 nm using spectrophotometer (Spectronic Genesys 5).

COD and TOC

The removal of organic matter in combined medium of nutrient broth and methyl parathion was determined by measuring the total organic carbon (TOC) and biochemical oxygen demand (COD) for pesticide concentrations of 400 and 800 mg l⁻¹ as per methods described in standard methods (APHA, 2005).

Experimental setup

The growth of bacterial culture under various environmental conditions was performed in orbital shaker (Labcon Spo- MP8). In 500 ml conical flasks, 250 ml of nutrient broth with different concentrations of methyl parathion (200, 400, 600, 800, 1000 and 1200 mg/l) was inoculated with 10 ml of inoculums and incubated on orbital shaker at ambient temperature at 120 rpm. Control experiments with equal volumes of nutrient broth and pesticide but no inoculums were also conducted in parallel to determine bacterial growth.

RESULTS AND DISCUSSION

Effect of different concentrations of methyl parathion

In the present study bacterial growth for six different concentrations of methyl parathion (0, 200, 400, 600,

800, 1000 and 1200 mg/l) was determined by measuring OD₆₀₀. One set of experiments without addition of methyl parathion to the medium was conducted to obtain the plot of OD values indicating bacterial growth in nutrient broth alone. Among the different methyl parathion concentrations, *Pseudomonas* IES-Ps-1 showed a significant increase in OD for 200, 400, 600 and 800 mg/l, due to increase in bacterial population. The growth curves for these four concentrations have a lag phase of 48 hrs and a log phase of 80 hrs which were similar to growth curve exhibited by bacteria in nutrient broth alone. For the concentration of 1000 and 1200 mg/l, no significant increase in optical density was observed after 96 hrs of incubation.

Plotting the growth curves for different methyl parathion concentrations showed that pesticide has suppressed the bacterial growth and bacteria can tolerate only a certain concentration of it. These results indicated that the bacterial culture was able to grow in presence of methyl parathion up to concentration of 800 mg/l (Figure 1). It has previously been reported that pesticides exhibited high toxicity to growth of microorganisms beyond a certain value that limits the microbial growth and subsequent degradation by microbes (Labana *et al.*, 2005). The results obtained during these experiments are in agreement with previous studies, reporting decreased bacterial counts at high pesticide concentration (Kao *et al.*, 2005; Yuan *et al.*, 2000).

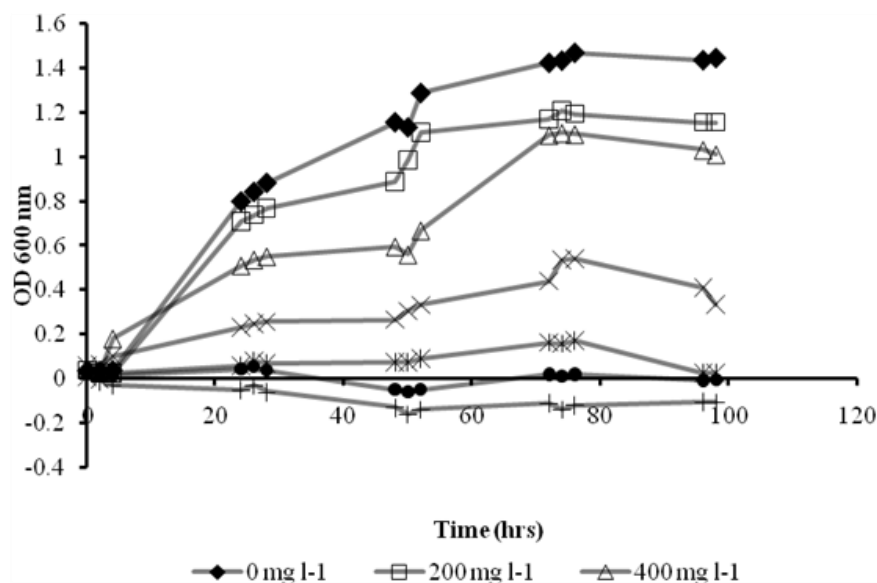


Figure 1. Growth of *Pseudomonas* in nutrient broth with different concentrations of methyl parathion.

Effect of different temperatures

The experiments were carried out to determine the optimal temperature for bacterial growth at pH 7. The results showed that bacterial growth can occur over a wide range of temperatures. However, the greatest growth of *Pseudomonas* IES-Ps-1 was observed at 30 °C compared to 25 °C and 35 °C for both 400 and 800 mg/l methyl parathion. The initial value of COD for combined media of nutrient broth and 400 mg/l of methyl parathion was 441.0 mg/l which decreased to 220.0 mg/l under the controlled temperature (30 °C), showing a decrease (50 %) in organic matter content. For TOC, the initial value was 180.0 mg/l, which decreased to 86.0 mg/l, showing a decrease of 52.0 % in the organic content (Figure 2 and

3). Similarly, in the case of 800 mg/l, the initial value of COD at 30 °C was 481.0 mg/l which decreased to 257.0 mg/l and showed a decrease of 46.0 % in COD content. For TOC, the initial value was 213.0 mg/l, which decreased to 107.0 mg/l and showed a decrease of 50.0 % in TOC content (Figure 4 and 5). Although both COD and TOC do measure pesticide removal qualitatively, however a part of organic matter removal was due to pesticide degradation (Geetha *et al.*, 2008; Krishna and Philip, 2008).

Microorganisms are poikilothermic as their temperature varies with the external environment and influence the enzyme-catalyzed reactions thereby affecting the growth. Studies show that *Pseudomonas* is a mesophilic microbe

that requires a certain range of temperatures to obtain maximum growth and to perform its metabolic activities (Prescott *et al.*, 2003). The studies of Kao *et al.*, (2005) reported that maximum growth of *P. mendocina* and breakdown of pentachloro phenol (PCP) was achieved at 30 °C. There are several similar studies which have

reported the 30 °C as optimum temperature for the growth of different *Pseudomonas* species in the presence of different contaminants such as benzene, toluene, endosulfan, and nitrobenzene. (Arshad *et al.*, 2008; Chang *et al.*, 1997; Nishino and Spain, 1993).

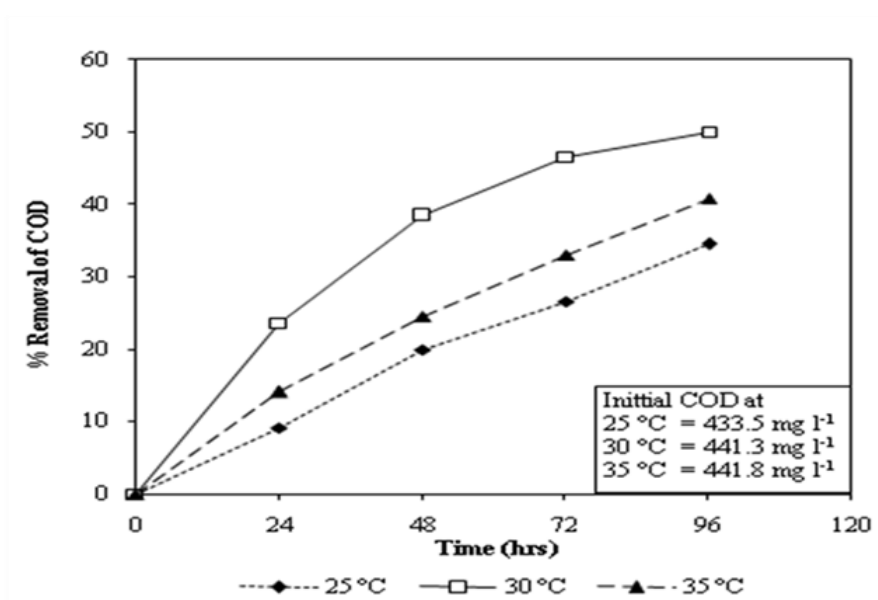


Figure 2. Effect of temperature on % reduction of COD in 400 mg-1 methyl parathion.

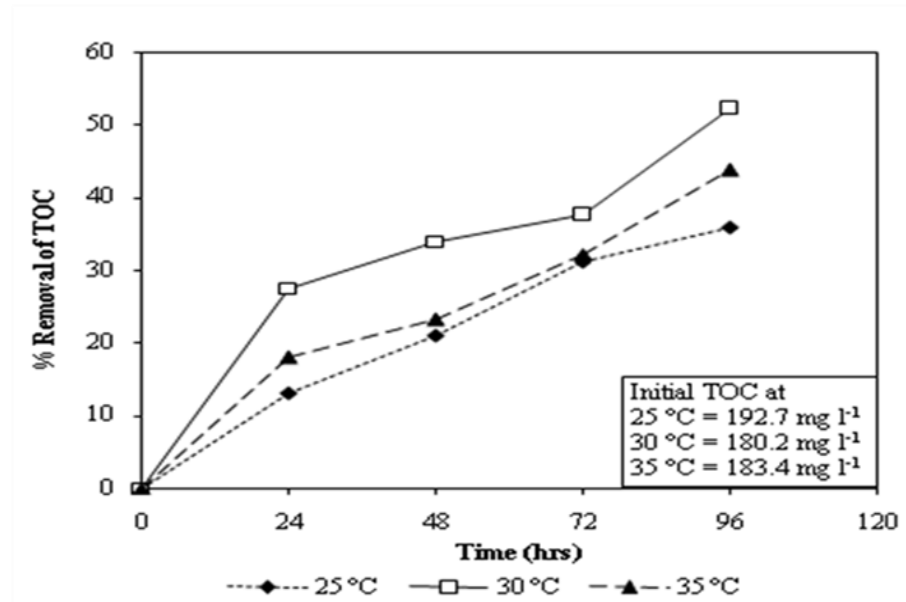


Figure 3. Effect of temperature on % reduction of TOC in 400 mg-1 methyl parathion.

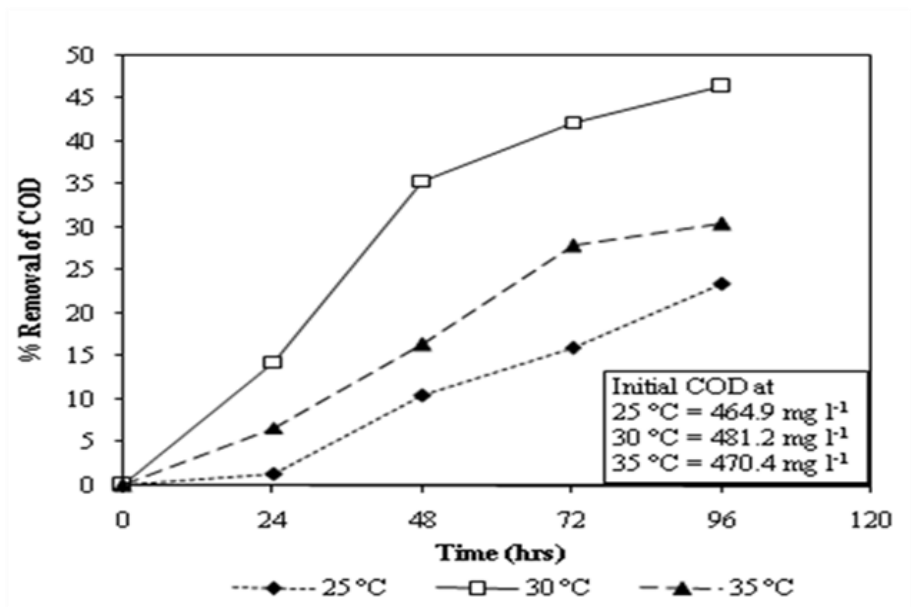


Figure 4. Effect of temperature on % reduction of COD in 800 mg-1 methyl parathion.

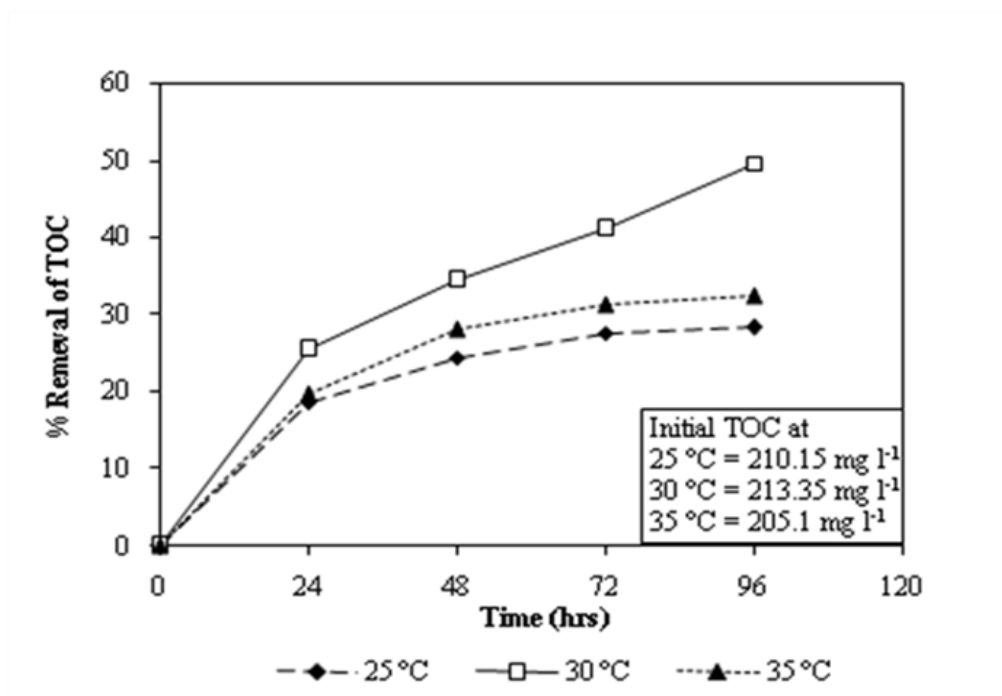


Figure 5. Effect of temperature on % reduction of TOC in 800 mg-1 methyl parathion.

Effect of different pH

The optimum pH for the growth of *Pseudomonas* IES-Ps-1 and removal of organic matter was found neutral for both 400 and 800 mg/l methyl parathion concentrations. The initial value of COD for combined media of nutrient broth and 400 mg/l of methyl parathion was 444.0 mg/l which decreased to 234.0 mg/l under the controlled pH 7,

showing a decrease of 47% in organic matter content. For TOC, the initial value was 235.0 mg/l, which decreased to 119.0 mg/l, showing a decrease of 49 % in the organic content (Figure 6 and 7). Similarly, in the case of 800 mg/l, the initial value of COD at pH 7 was 490.0 mg/l which decreased to 288.0 mg/l and showed a decrease of 41 % in COD content. For TOC, the initial value was 220.0

mg/l, which decreased to 124.0 mg/l and showed a decrease of 44 % in TOC content (Figure 8 and 9). Changes in pH can harm microorganisms by disrupting the plasma membrane or by inhibiting the enzyme activity. Therefore, it is needed to determine pH preferences for achieving optimum microbial growth (Prescott *et al.*, 2003). Results of bacterial growth and

organic matter removal at varying pH were found in agreement with previous reports on growth of *Pseudomonas* in presence of 2, 4-dichlorophenol, benzene, toluene, naphthalene at pH 7 (Elkarmi *et al.*, 2008; Chang *et al.*, 1992; Kao *et al.*, 2005; Pathak *et al.*, 2008).

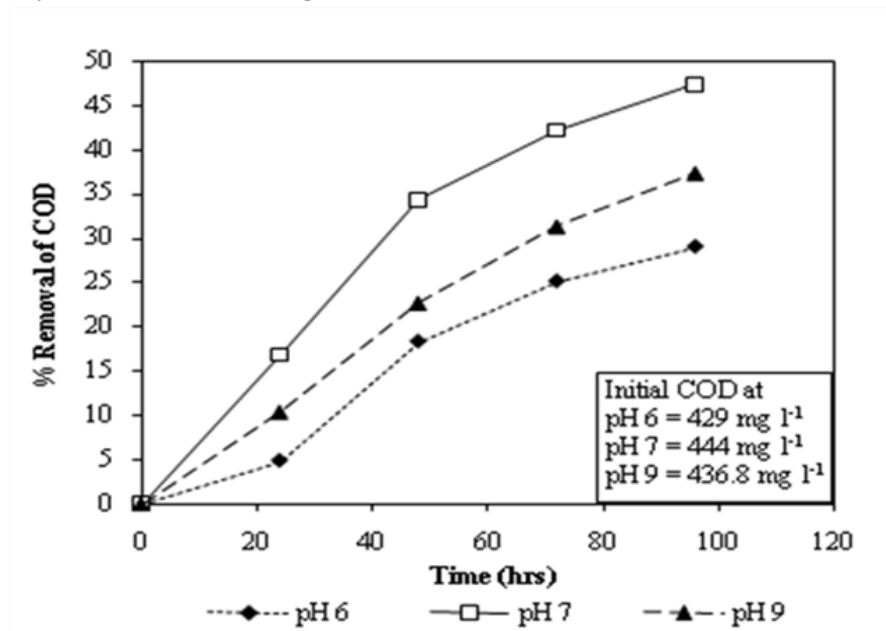


Figure 6. Effect of pH on % reduction of COD in 400 mg-1 methyl parathion.

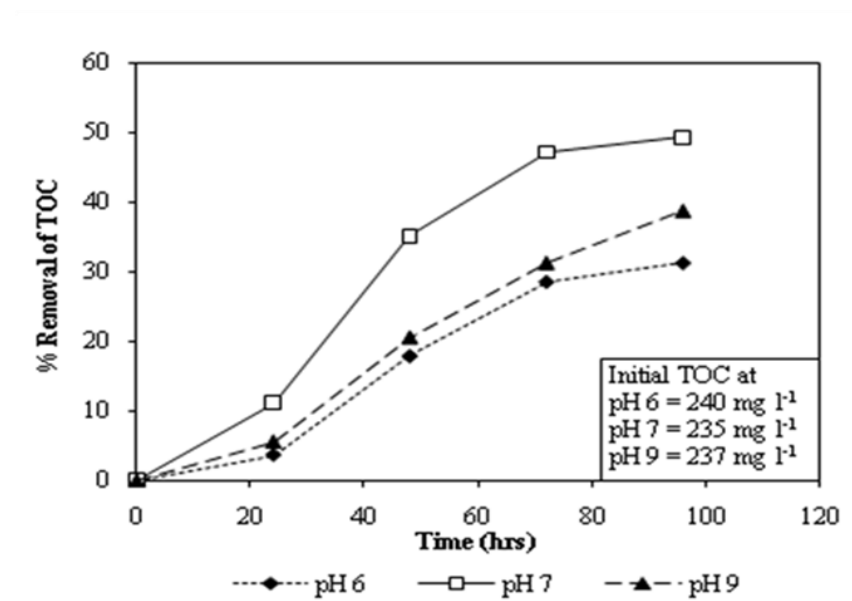


Figure 7. Effect of pH on % reduction of TOC in 400 mg-1 methyl parathion.

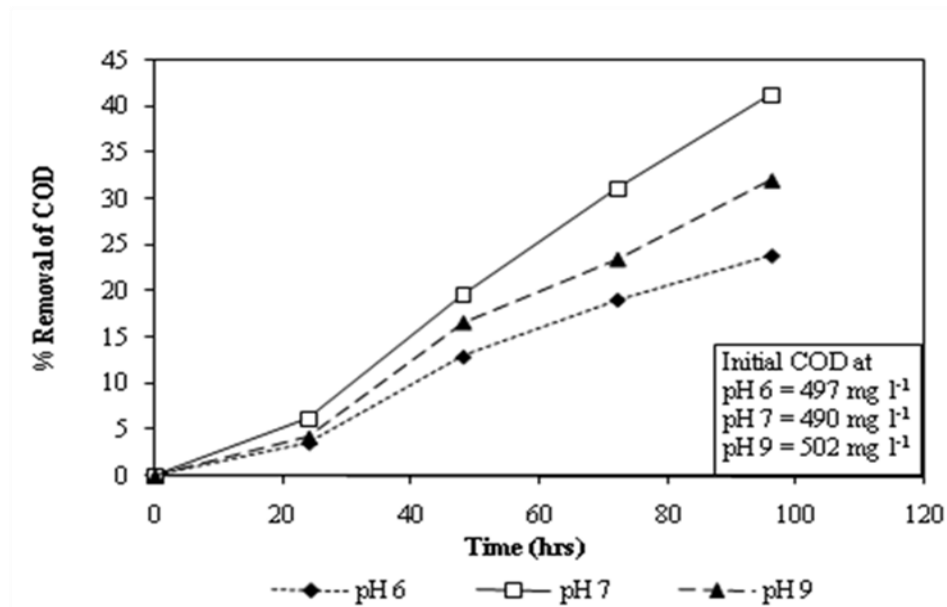


Figure 8. Effect of pH on % reduction of COD in 800 mg-1 methyl parathion.

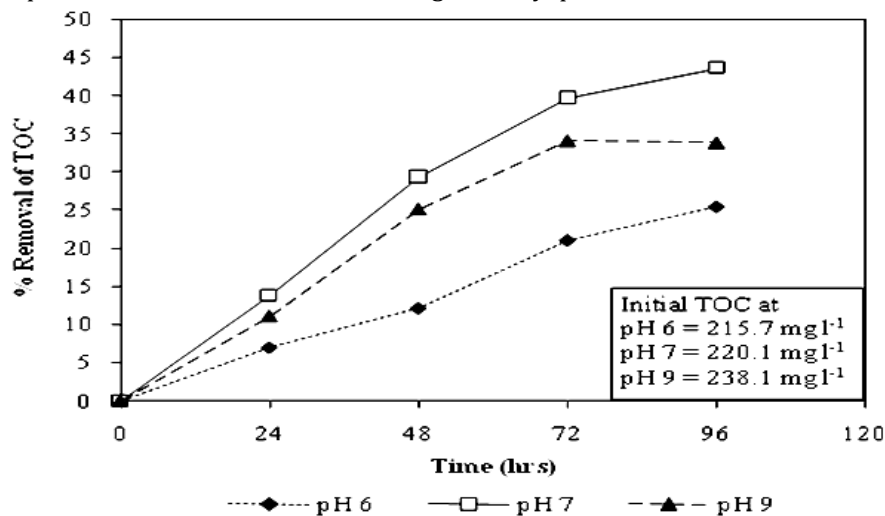


Figure 9. Effect of pH on % reduction of TOC in 800 mg-1 methyl parathion.

CONCLUSIONS

It was concluded that *Pseudomonas* IES-*Ps*-1 was able to grow effectively in higher concentrations of methyl parathion added to nutrient broth (up to 800 mg/l) at 30 °C and pH 7 resulted in significant removal of COD and TOC.

CONFLICT OF INTEREST

The authors declare that they have no conflicts of interest.

AUTHORS CONTRIBUTIONS

All the authors contributed equally to this work.

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