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Research article

Bacterial assisted degradation of chlorpyrifos: The key role of environmental conditions, trace metals and organic solvents

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ABSTRACT

Wastewater from pesticide industries, agricultural or surface runoff containing pesticides and their residues has adverse environmental impacts. Present study demonstrates effect of petrochemicals and trace metals on chlorpyrifos (CP) biotransformation often released in wastewater of agrochemical industry. Biodegradation was investigated using bacterial strain *Pseudomonas kilonensis* SRK1 isolated from wastewater spiked with CP. Optimal environmental conditions for CP removal were CFU (306×10^{6}), pH (8); initial CP concentration (150 mg/L) and glucose as additional carbon source. Among various organic solvents (petrochemicals) used in this study toluene has stimulatory effect on CP degradation process using SRK1, contrary to this benzene and phenol negatively inhibited degradation process. Application of metal ions (Cu (II), Fe (II) Zn (II) at low concentration (1 mg/L) took part in biochemical reaction and positively stimulated CP degradation process. Metal ions at high concentrations have inhibitory effect on degradation process. A first order growth model was shown to fit the data. It could be concluded that both type and concentration of metal ions and petrochemicals can affect CP degradation process.

1. Introduction

Chlorpyrifos (O,O-Diethyl O-3,5,6-trichloropyridine-2-yl phosphorothioate) an organophosphate (OPP) insecticide which is in use for more than sixty years for controlling crop pests. Use of OPPs has played an important role in a remarkable increase in agricultural productivity. CP in introduced into the environment through agricultural and industrial runoff. CP and its metabolites are detected in environmental samples globally (Chishti et al., 2013). CP poisoning can causes nausea, abnormal functioning of nervous system, sudden irregular movement of the body, paralysis and may cause death of insects, humans and other mammals (Yadav et al., 2014); hence its removal from the contaminated environment has attained immense attention.

Pesticide industries in Pakistan release large volume of wastewater into nearby water bodies without sufficient treatment. This wastewater contains pesticides, their residues, organic solvents and many other harmful chemicals. In order to avoid environmental contamination, this wastewater should be treated. Microbial biotreatment is an important pathway for elimination of toxic organic compounds such as pesticides (Chishti et al., 2013). Bacterial species having organophosphorous hydrolase enzyme can use OPPs as a source of carbon and energy (Singh and Walker, 2006). Several studies have been carried out for removal of OPPs from wastewater using bacterial strains. Recently we reported biodegradation of organophosphate pesticide chlorpyrifos using *Psychrobacter alimentarius* (Khalid and Hashmi, 2015).

Wastewater from pesticide industries contains variety of organic and inorganic compounds such as trace metals and petrochemicals (organic solvents). Research on pesticides mostly focuses on organic dimensions but there is a dearth in literature regarding inorganic dimensions. Inorganics such as metal ions are present in commercial formulations but unfortunately these are not reflected in theoretical structures and labels. Pesticides with trace metals in their chemical structures have been detected in groundwater samples globally (Kolpin et al., 2000a, b). Presence of trace metals may affect biodegradation reaction as they take part in biochemical reactions, effecting bioavailability (Shomar, 2006, Mahmood et al., 2015). Organic compounds such as benzene, toluene, phenols also called petrochemicals are used as solvents for making liquid pesticide formulations. Whether or not these organic solvents take

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part in metabolic reactions, needs investigation. However there are lacunae in literature on this aspect, hence experimental evidence is needed to demonstrate their influence on biodegradation.

Environmental and nutritional factors play important role in substrate utilization, production and release of enzymes and formation of metabolites. Understanding role of these factors is important for designing a sustainable biotreatment facility (Chen et al., 2006). Current study was planned to isolate and identify bacterial specie capable of degrading CP. Attempts were made to find out effect of environmental parameters such as inoculum density, pH, initial CP concentration and nutrients availability on CP biodegradation and kinetic properties. Hydrolyzing metabolites 3, 5, 6-trichloro-2-pyridinol (TCP) and tricholoromethoxypyridine (TMP) were also detected. Concentration dependent effect of organics (petrochemicals) and inorganics (trace metals) on biodegradation of CP was also investigated. Data aggregation on this prospect could shape a premise for better plans and biological operation.

2. Materials and methods

2.1. Sample collection, isolation and taxonomic characterization

Wastewater samples for isolation of bacteria were collected in sterilized containers from wastewater drain of NUST Islamabad Campus, Pakistan. GC/HPLC grade standards of CP, TCP and TMP were procured from Sigma Aldrich Corporation. Mineral salt medium (MSM) as described earlier in Khalid and Hashmi (2015) was used.10 ml wastewater was added to MSM supplemented with CP and put into incubation chamber at 37 °C for 7days. Several fold dilutions were spread on MSM agar and single colonies were obtained. Several biochemical tests (oxidase, catalase, and growth on differential medium, analytical profile index kit) and morphological tests were performed for identification of chlorpyrifos degrading strains using standard procedures.

For molecular identification SRK1 was grown on nutrient agar plates and biomass was washed. Biomass was used for DNA extraction through instance matrix (biorad USA). Amplification was performed using universal primers 27F and 1492 R. Montage PCR cleanup kit (Millipore) was used for purification of amplification products. Sequencing was carried out at Macrogen, South Korea for this Big dye terminator cycle sequencing kit (Applied Biosystems, USA) and Applied Biosystems model 3730XL automated DNA sequencing system (Applied BioSystems, USA) was used. To get genus and specie names sequence comparison was made with already reported sequences at NCBI. The phylogenetic tree was constructed using software MEGA4.

2.2. Effect of environmental and nutritional factors on growth of SRK1

Effect of variable environmental conditions on growth of SRK1 was investigated. 100 ml of sterilized MSM amended with CP (100 mg/L) was added to 250 ml volumetric flask and inoculated with bacterial strains SRK1 differing in inoculum density (OD_{600} 0.5,1, 1.5, 2). Under same environmental conditions growth was monitored under variable pH (3, 4, 5, 6, 7, 8, 9, 10) in MSM and nutrient broth. Effect of initial substrate concentration on bacterial growth was investigated in MSM with CP (50, 100, 150, 200, 250, 300 mg/L). Glucose, sucrose and nutrient broth (1 g/L) was added as additional carbon source in MSM supplemented with CP for investigating their effect on growth of SRK1. 1–5 mg/L of trace metals (Cu(II), Hg(II), Fe(II), Mn(II), and Zn(II) were added in MSM individually in order to check their effect on growth of SRK1. Effect of petrochemicals on bacterial growth was monitored by supplementing MSM with 10 and 100 mg/L of benzene, toluene and phenol. Conditions for all experiments were inoculum density 1.5, pH 8, CP 100 mg/L and incubation time 48 h (unless mentioned). Optical density (growth) was measured using single beam spectrophotometer using cuvette of 1 cm path length.

2.3. Biodegradation of chlorpyrifos

2.3.1. Experimental setup and operation

Bench scale bioreactors previously described in Khalid and Hashmi (2015) were used for biodegradation study. Briefly four bench scale bioreactor of 10 L, 40 cm and 24 cm working volume, length, internal diameter working volume were used in this study. Bioreactors were stirred magnetically for uniform mixing of biomass. Air pumps along with porous grit stone diffusors were used for air supply. For feeding medium inlet valves and for withdrawal of sample outlet valves were installed. For avoiding contamination cotton plugs were utilized. Batch system worked at room temperature. As seeding inoculum biomass developed from CP degrading strain SRK1 was used. Initial pH of medium was set to 8 (unless mentioned). Later the temperature and pH of system was not controlled, yet their values were checked. There were three replications of all experiments. Sample were collected, filtered, extracted and analyzed periodically for remaining CP concentration using GC-ECD (Shimadzu 2010).

2.3.2. Kinetics of CP biodegradation

To determine zero order and first order rate constant following algorithms were used (Eqs. (1) and (2))

$$\ln C = a + kt \tag{1}$$

$$C = b + k_0 t \tag{2}$$

where k_o and k are zero order and first order rate constant respectively. Substrate concentration and its degradation duration are represented by t and C (Yang et al. 2014, Khalid and Hashmi, 2015). Half life ($t_{1/2}$) is the time required to reduce 50% of initial concentration. Half-life ($t_{1/2}$) of CP in batch system was calculated using Eq. (3) (Yang et al., 2014)

$$t_{1/2} = \frac{0.693}{k_1} \tag{3}$$

In *C* was plotted against time t and straight line regression equation was obtained. Slope of regression equation gives value for rate constant "k". R^2, first order rate constant k (h^{-1}) and $t_{1/2}$ (days) are presented in Table 1.

2.3.3. Effect of environmental factors on biodegradation

The effect of various environmental factors on SRK1 degradation ability was investigated. Runs were made in bioreactor with MSM amended with CP (150 mg/L) inoculated with SRK1 at inoculum density at OD_{600} (0.5, 1, 1.5, 2), pH (3–10). Effect of additional carbon source on CP biodegradation was investigated using MSM with CP (150 mg/L) by supplementing it with 1 g/L of glucose, sucrose and nutrient broth separately. Effect of initial concentration on biodegradation was studied in MSM with initial CP (50–300 mg/L). For all experiments in coulum density 1.5, pH 8 and CP concentration was 150 mg/L (unless mentioned). Medium without inoculum served as control. Optimum values acquired for one parameter was utilized in further tests. Sample were collected, filtered, extracted and analyzed periodically for remaining CP concentration using GC-ECD (Shimadzu 2010).