Pesticide Biochemistry and Physiology 116 (2014) 24–31

Contents lists available at ScienceDirect

Pesticide Biochemistry and Physiology

journal homepage: www.elsevier.com/locate/pest

Biomineralization and formulation of endosulfan degrading bacterial and fungal consortiums

Jayanthi Abraham *, Sivagnanam Silambarasan

Microbial Biotechnology Laboratory, School of Biosciences and Technology, VIT University, Vellore, Tamil Nadu 632014, India

ARTICLE INFO

Article history:
Received 4 January 2014
Accepted 15 September 2014
Available online 30 September 2014

Keywords:
Biodegradation
Endosulfan
Bacterial consortium
Fungal consortium formulation

ABSTRACT

Microbial degradation offers an effective approach to remove toxicants and in this study, a microbial consortium consisting of bacterial strains and fungal strains were originally obtained from endosulfan contaminated agricultural soils. Identification of the bacterial isolates by 16S rRNA sequences revealed the isolates to be *Halophilic* bacterium JAS4, *Klebsiella pneumoniae* JAS8, *Enterobacter asburiae* JAS5, and *Enterobacter cloacae* JAS7, whereas the fungal isolates were identified by 18S rRNA sequences and the isolates were *Botryosphaeria laricina* JAS6, *Aspergillus tamarii* JAS9 and *Lasiodiplodia* sp. JAS12. The biodegradation of endosulfan was monitored by using HPLC and FTIR analysis. The bacterial and fungal consortium could degrade 1000 mg l^{-1} of endosulfan efficiently in aqueous medium and in soil. The infrared spectrum of endosulfan degraded samples in the aqueous medium by bacterial and fungal consortium showed bands at 1400 and 950 cm^{-1} which are the characteristics of COOH group and acid dimer band respectively. In the present investigation, low cost solid materials such as sawdust, soil, fly ash, molasses and nutrients were used for the formulation of microbial consortium and to achieve greater multiplication and survival of the microbial strains.

1. Introduction

Endosulfan is a cyclodiene insecticide that exhibits a relatively broad spectrum activity. Endosulfan is a mixture of two stereoisomers, α and β-endosulfan, in a ratio of 7:3. It is used extensively throughout the world to control the insect pests of a wide range of crops and most importantly, it has been used for the control of *Helicoverpa* sp. in cultivation of cotton [1]. This insecticide has been used extensively for over 30 years on a variety of vegetables, fruits, cereals and cotton as well as on trees, vines and ornamental plants [2]. Endosulfan is extremely toxic to fish and other aquatic organisms and affects the central nervous system, kidney, liver, blood and parathyroid gland; it has reproductive, teratogenic, and mutagenic effects. Endosulfan (α- and β-endosulfan) once introduced into the environment is converted to endosulfan sulfate in the soil and remains as a significant residue. Endosulfan sulfate is the only breakdown product considered toxic [3].

Endosulfan has been banned in India since 2011 due to the toxic effects incurred by it in Kasaragod district of Kerala. Before 2011, in India it was mainly used in rice, cotton and tea plantation. Degradation of endosulfan through biological method is receiving serious attention as compared to other existing methods such as incineration and landfill. Earlier studies on the microbial degradation of endosulfan have revealed various intermediates of metabolism including endosulfan sulfate, endosulfan diol, endosulfan ether, endosulfan lactone, endosulfan hydroxyether and endosulfan dialdehyde [4,5]. The biodegradation of endosulfan in soil and water environments by indigenous microbes has been reported such as *Achromobacter xylosoxidans* strain C8B [6], *Bordetella* sp. B9 [7], *Klebsiella oxytoca* [5], *Bacillus* sp. [8], *Pandoraea* sp. [9], *Micrococcus* sp. [10], *Aspergillus niger* [11], *Aspergillus terreus* and *Cladosporium oxysporum* [12], *Fusarium ventricosum* [9], *Mucor thermohyalospora* [13], *Phanerochaete chrysosporium* [14], *Trichoderma harzianum* [15].

Though there are many works pertaining to degradation of endosulfan with single isolate, not much work has been done with bacterial or fungal consortium. Hence, in the present study, a bacterial consortium consisting of *Halophilic* bacterium JAS4, *Klebsiella pneumoniae* JAS8, *Enterobacter asburiae* JAS5, *Enterobacter cloacae* JAS7 and fungal consortium consisting of *Botryosphaeria laricina* JAS6, *Aspergillus tamarii* JAS9, *Lasiodiplodia* sp. JAS12 were prepared from isolates obtained from endosulfan contaminated agricultural soil. Degradation by the consortium would be incomplete if it was not applicable in a formulation mode; hence a simple and cost effective formulation was prepared with fly ash, soil and molasses.

* Corresponding author. Fax: +91 416 2243092.
E-mail address: abraham.jay7@gmail.com (J. Abraham).

http://dx.doi.org/10.1016/j.pestbp.2014.09.006
0048-3575/

2. Materials and methods

2.1. Chemicals

Analytical standards of α-endosulfan (99% purity), β-endosulfan (99% purity) and endosulfan sulfate (99% purity) were purchased from Sigma Aldrich (St. Louis, MO, USA). Technical grade endosulfan, a 35% emulsifiable concentrate used in this study, was obtained from Hindustan Insecticides Limited, Kerala, India. Chromatographic grade acetonitrile and ethyl acetate were purchased from SD Fine Chem Limited (India).

2.2. Bacterial and fungal strains

The bacterial strains *Halophilic* bacterium JAS4, *Klebsiella pneumoniae* JAS8, *Enterobacter asburiae* JAS5, *Enterobacter cloacae* JAS7 and fungal strains *Botryosphaeria laricina* JAS6, *Aspergillus tamarii* JAS9 and *Lasiodiplodia* sp. JAS12 were isolated from endosulfan treated agricultural soils. The 16S rRNA gene sequence of *Halophilic* bacterium JAS4, *Klebsiella pneumoniae* JAS8, *Enterobacter asburiae* JAS5 and *Enterobacter cloacae* JAS7 were submitted to NCBI GenBank with accession numbers KC509575, KC509582, KC509576 and KC509581, respectively. The GenBank accession number for the 18S rRNA sequence of *Botryosphaeria laricina* JAS6, *Aspergillus tamarii* JAS9 and *Lasiodiplodia* sp. JAS12 were KC509580, KC509583 and KC583445, respectively.

2.3. Degradation of endosulfan and its metabolites by bacterial and fungal consortium in the aqueous medium

The degradation studies were performed in 250 ml Erlenmeyer flasks containing 100 ml of sterile MSM (contained in g l^{-1}: Na_2HPO_4 5.8, KH_2PO_4 3, NaCl 0.5, NH_4Cl 1, $MgSO_4$ 0.25 and pH 6.8–7.0) and M1 medium (contained in g l^{-1}: $NaNO_3$ 2, KCl 0.5, $MgSO_4{\cdot}7H_2O$ 0.5, $FeCl_3$ 10 mg, $BaCl_2$ 0.2, $CaCl_2$ 0.05 and pH 6.8) for bacterial and fungal consortium respectively spiked with 1000 mg l^{-1} of endosulfan. One ml of bacterial (3×10^6 cells ml^{-1}) and fungal consortium (10^8 spore's ml^{-1}) was transferred to the MSM and M1 medium respectively and non-inoculated flask was maintained as control. All the flasks were incubated at 30 ± 2 °C on a rotary shaker at 120 rpm. The samples were periodically removed aseptically for residual analyses to determine pesticide concentration by high performance liquid chromatography (HPLC).

2.4. Degradation of endosulfan and its metabolites by bacterial and fungal consortium in the soil

Two soil microcosm studies were carried out with bacterial and fungal consortium: (1) addition of pesticide, microbial consortium and nutrients (carbon, nitrogen and phosphorus) and (2) addition of pesticide and microbial consortium devoid of nutrients (control). Before using the soil for degradation studies, it was sterilized three fold by autoclaving for 30 min at 121 °C. 30 ml of solution containing consortium, nitrogen, phosphorus, glucose and 1000 mg kg^{-1} of endosulfan were added to 250 ml Erlenmeyer flask which contained 100 g of sterilized soil. The sources of carbon, nitrogen and phosphorus were glucose, ammonium sulfate and dipotassium hydrogen phosphate, respectively. The amounts of carbon, nitrogen and phosphorus were calculated using the relationship C/N/P (100:10:1) [16,17]. In order to study the insecticide dissipation rates under abiotic conditions, samples of soil, without microbial consortiums, were kept as controls and were maintained in triplicates. The flasks were incubated at 30 °C and soil samples were analyzed at one day interval regularly for the determination of endosulfan and its major metabolites. All the biodegradation experiments were performed in triplicates.

2.5. Analytical studies

Endosulfan and its major metabolites in the aqueous medium was extracted by the addition of equal volume of acetonitrile and thoroughly mixed for 1 h with rotary shaker and then centrifuged. 10 g of soil sample were collected from each treatment and extracted with 50 ml of acetonitrile, which was kept in a rotary shaker for 1 h at 200 rpm followed by centrifugation. The supernatant was decanted into a glass beaker and the organic solvent was concentrated in a water bath at 35 °C [18]. The extracted samples were analyzed by HPLC (Waters 1525 binary HPLC pump, Milford, USA) on a Symmetry C_{18} column (Waters 5 µm, 4.6 mm × 150 mm). The isocratic mobile phase comprised a mixture of acetonitrile:water (65:35, V:V), which was pumped through the column at a flow rate of 1 ml min^{-1}, duration of cycle 20 min, detector wavelength 214 nm at an injection volume 25 µl [6]. Infrared (IR) spectra of the parent compound (endosulfan) and sample after degradation of endosulfan with bacterial and fungal consortium were recorded in the frequency range of 4,000–500 cm^{-1} with a Fourier transform infrared (FTIR) spectrophotometer (8400 Shimadzu, Japan, with Hyper IR-1.7 software for Windows) with a helium-neon laser lamp as a source of IR radiation. Pressed pellets were prepared by grinding the extracted samples with potassium bromide in a mortar with 1:100 ratio and immediately analyzed in the region of 4,000–400 cm^{-1} at a resolution of 4 cm^{-1}.

2.6. Kinetic studies

Degradation of pesticide in aqueous medium and soil has been applied to the various kinetic models such as zero order, first order, pseudo first order, second order and pseudo second order to determine the rate constant (k). The times in which the pesticide concentration in MSM or soil was reduced by 50% (DT_{50} values) was calculated from the linear equation obtained from the regression between C_t–C_o (zero order model), $\ln(C_t/C_o)$ (first order model), ln C_t (pseudo first order model), $1/C$ (second order model), t/Ct (pseudo second order model) of the chemical data and time. Kinetic model equations were described by:

1 $C_t - C_o = kt$ (zero order model)
2 $C_t/C_o = e^{-kt}$ (first order model)
3 $\ln C_t = -kt + \ln C_o$ (pseudo first order)
4 $1/C = kt + 1/Co$ (second order kinetic)
5 $t/Ct = t/Ce + 1/kCe^2$ (pseudo second order)

Whereas C_o, Ce are the amount of pesticide in MSM or soil at time zero and C_t, C, Ct are the amount of pesticide in MSM or soil at time t. k and t are the rate constant (d^{-1}) and degradation time in days, respectively.

2.7. Estimation of carbon dioxide

The amount of carbon dioxide (CO_2) evolved during the degradation of endosulfan by bacterial and fungal consortium was analyzed. A series of 250 ml Erlenmeyer flasks containing 100 ml of MSM and M1 medium were taken separately and supplemented with 1000 mg l^{-1} of endosulfan. One ml of bacterial and fungal consortium was added to the MSM and M1 medium, respectively. After that the flasks were sealed with airtight jar containing glass beakers filled with standard sodium hydroxide solution to trap the evolved CO_2. The samples were taken at regular intervals up to 120 h. The dissolved CO_2 concentration in alkali solution was estimated by titrating with 1N hydrochloric acid in the presence of phenolphthalein and barium chloride [11].