Archives of Microbiology (2021) 203:5763–5782
https://doi.org/10.1007/s00203-021-02520-2

ORIGINAL PAPER

GCMS and FTIR spectral analysis of aqueous methylparathion biotransformation by the microbial mpd strains of *Pseudomonas aeruginosa* and *Fusarium spp*

Usharani Krishnaswamy[1,2]

Received: 4 October 2020 / Revised: 8 August 2021 / Accepted: 9 August 2021 / Published online: 12 September 2021

Abstract

This study aimed to find out the effective biotreatment processes of aqueous methyl parathion in stipulations of its biotransformation by potential indigenous mpd strains of *Pseudomonas aeruginosa* and *Fusarium spp*. Microbial biotransformation study of methyl parathion in contaminated synthetic wastewater at optimum conditions by *P. aeruginosa* mpd-5 (at temperature 33 °C, pH 7, under the aerobic condition with inoculum density of 10^8cells/mL) and by *Fusarium spp* mpd-1 (at temperature 30 °C, pH 7, under the aerobic condition with inoculum density of 25 mgL^{-1} dry biomass) was carried out. The major biodegradation compounds formed during the biotreatment process were analyzed by spectral studies using GCMS and FTIR. GCMS analysis of bacterial transformed compounds was p-nitrophenol, dimethylaminophenol, and glycyl-L-proline of 2-(3-methylpiperidino-4-5, 6-benzothiazin-4-one) and 2, 5-piperazinodione 3, 6-bis (2-methylpropyl). And the fungal transformed compounds were observed to be p-nitrophenol, phenol,2,4-Bis(1,1-Dimethylethyl), beta-L-arabinopyranoside-methyl, Cyclobutanethiol, 3-2(4)-thiophenone, dihydro-5-(1-methylethyl), Benzene,1,3-bis(1,1-dimethyl ethyl), Butanoic acid, 2-methyl methyl ester, and L-(+)-Ascorbic acid. The FTIR spectra of the molecule and the products of methyl parathion are observed to be changed in structures. It can be concluded from the aforementioned results and discussions that *P. aeruginosa* mpd-5 and *Fusarium spp* mpd-1 can be used in the biotreatment of pesticide wastewater having the high methyl parathion concentration and is possible by the indigenous microbial strains; they utilize as phosphate and carbon source of energy. Hence the strains can be used as a whole microbial cell, or its bioactive metabolites can be applied for the biotreatment of pesticide wastewater and potentially degrade methylparathion.

Keywords Aqueous methylparthion · GC–MS · FTIR · *Pseudomonas aeruginosa* mpd · *Fusarium spp* mpd

Introduction

The organophosphorus pesticides (OPs) are the collection of extremely toxic agricultural chemicals extensively utilized in plant protection. The universal members of the family of organophosphates (methyl parathion, Malathion, dimethoate, phosphamidon, phorate, fenitrothion, and monocrotophos) studies were relatively administered, and few works have reported on the removal of high concentration of pesticides from water. The organophosphate insecticide methyl parathion (MP) was found to be a frequently used insecticide in India. Chiron et al. (2000) stated that these types of pesticides pose an elevated threat for aquatic systems. The consume water directive sets an allowed contaminant level of 0.1 mg/L for one pesticide and 0.5 mg/L for the entire sum of pesticides (Council directive 1998). Ku et al. (1998) and also Ku and Lin (2002) were reported the handling and removal of OP pesticides from water; relatively few works have reported on the removal of pesticides at high concentrations of pesticides from wastewater. The industries manufacturing these pesticides release wastewater in water bodies or land. According to reference and literature survey, the pesticide pollution due to wastewater from formulating or manufacturing pesticide plants released the effluent up to

Communicated by Erko Stackebrandt.

✉ Usharani Krishnaswamy
usharaniks2003@yahoo.com

[1] Department of Environmental Sciences, Environmental Microbiology Division, Bharathiar University, Coimbatore, TN, India

[2] Department of Environmental Science, PSG College of Arts and Science, Coimbatore, TN, India

1000 ppm (Chiron et al. 2000). The major cause of pesticide water pollution releases into the environment was maximum 1000 ppm, so the maximum concentration of pesticide used in the study was about 1000 mg/L. Based on the literature survey (Chiron et al. 2000), the maximum concentration released into the environment was up to 1000 mg/L, so the synthetic wastewater concentration was fixed to 1000 mg/L. Wastewater from agricultural industries and pesticide formulating or manufacturing plants were reported to have pesticide contamination levels as high as 500 mgL^{-1} (Chiron et al. 2000).

Bioremediation way of removing or detoxifying, degrade, mineralize or transform more toxic xenobiotics to a less toxic and their field application in polluted sites using microbial process has proven effective and also reliable due to its eco-friendly nature. The competent and valuable treatment methods for the microbial removal of these pesticides at a high level are essential in practice. The use of microorganisms either present as native or introduced artificially to degrade the pollutants. Therefore, the process is referred to as bioremediation (Pointing 2001) and minimizes the pollutant levels to undetectable, non-toxic, or acceptable levels. The metabolism of microbes is perhaps the foremost important pesticide biodegradative process in soils (Kearney 1998).

Bioremediation technology has advantages compared to other physicochemical remediation methods, because it is often cost-effective and can achieve the entire degradation of organic pollutants without destruction of the sites material and its living beings, indigenous flora and fauna (Timmis and Pieper 1999). The achievement of microbial degradative competencies by native microbes at contaminated sites particularly when multiple biodegradation traits are requisite as is the case of sites contaminated with more than one organic compound (Ang et al. 2005). The aim of this study was to find out the effective biotreatment processes of aqueous methylparathion in the stipulations of its biotransformation and proposed pathway by competent potential indigenous strains of bacterium (*Pseudomonas aeruginosa* mpd5) and fungus (*Fusarium spp* mpd1). The efficient MP tolerant strains (*Pseudomonas aeruginosa* mpd5 and *Fusarium spp* mpd1) are screened for their potential in the laboratory and used to remove aqueous MP by GCMS and FTIR spectral analytical studies.

Materials and methods

Methyl parathion (analytical grade, 98.5% pure) was obtained from Merck India Ltd., and commercial grade MP named Devithion™ (50% EC) was purchased from Devidayal Agro Chemicals (Mumbai, India). Solvents such as dichloromethane, diethyl ether, n-hexane, methanol (chromatographic grade) and other chemicals (analytical grade) were purchased from Merck India Ltd. Deionized double distilled water was used for the preparation of all solutions. Temperature controlled incubator shaker, centrifuge, pH meter and pH buffers were also used.

Minimum inhibitory concentration (MIC) test

The screening of potential microbial strains using MIC test or plate assay. A potential bacterial strain (mpd-5) and fungal strain (mpd-1) are isolated from pesticide exposed and acclimatized agricultural soil. The original enrichment cultures were carried out in a synthetic wastewater containing mineral salts medium amended with the methylparathion (DevithionTM 50% EC) as the sole source of carbon and energy. The methylparathion concentration used in this study was 0.1%, pH was adjusted using 1 N NaOH and 1 N HCl (ELICO-L1127, India). The organisms were subsequently grown on nutrient agar medium (bacterial strain mpd-5) plates and Czapek's-Dox agar medium (fungal strain mpd-1) plates to obtain single colonies. A pure monoculture of methylparathion-degrading individual strains (mpd-5 and mpd-1) are isolated by series of replating on MSM with methylparathion agar plates. The Minimum inhibitory concentration (MIC) assay with plate screening method was carried out to screen methylparathion resistant bacteria using methylparathion MSM with methylparathion agar plates.

The inhibition growth zone was measured using an electronic micrometer and recorded and the inhibition percentage of bacterial and fungal growth was calculated using the below equations:

$$\%\text{Growth} = Ømp/Øb \times 100\% \text{ and } \%\text{CI} = 100\% - \text{Growth},$$

where Ømp represents the diameter (cm) of growth of the microorganism in the treatments exposed to methylparathion at each assessment. Øb is the diameter of the negative control microorganism growth in each evaluation and % CI is the percentage of inhibitory growth.

The growth rate of the microorganism inhibition was calculated with the use of the below equation:

$$V = \varphi C/t,$$

where "*V*" is the speed of growth, ØC represents the diameter (cm) of the microorganism growth (cm) and *t* is the incubation time (days).

Based on the MIC test, the potential bacterial culture was identified based on their morphological characters and biochemical tests as given in Bergey's Manual of Determinative Bacteriology (Buchanan and Gibbons 1974; Holt et al. 1994). The methylparathion-degrading strains (mpd-5) was further isolated and identified as *Pseudomonas* species by Pseudomonas isolation agar medium, pigment production by Kings medium and confirmation of the strain was carried out

using selective medium (Centrimide Agar-CA). The identification and characterization of the isolated prominent bacterial stains were performed based on their morphological characters and biochemical tests (Buchanan and Gibbons 1974; Holt et al. 1994). And the potential fungal culture was identified based on their morphological characters using lacto phenol cotton blue staining. The identification and characterization of the isolated prominent fungal stains were performed (Clements and Shear 1957 and Booth 1977. Furthermore, the strains were confirmed as *Pseudomonas aeruginosa* and *Fusarium spp* from GeNei (Pvt) Ltd. Services, India.

Molecular characterization of bacterial strain mpd-5 and fungal strain mpd-1

The isolated strain was phenotypically characterized by microscopy, along with different biochemical tests (Holt et al. 1994). All tests were accomplished using exponentially grown cultures, except for the spore test, which essential cultures from the early stationary phase. The genotypic characterization was done by DNA extraction, 16S rRNA gene sequencing and phylogenetic analysis. The Genomic DNA from the selected isolate strain was extracted from pure cultures (2–5 ml), previously grown in 20 ml of LuriaBertani broth (incubated at 37 °C at 200 rpm for 24 h) using bacterial genomic DNA isolation kit RKT09 (Chromous Biotech Pvt. Ltd., India) and visualized on 0.8%(w/v) agarose gel. The PCR amplification of 16S rRNA gene was executed using Thermal cycler (ABI 2720) in 100 µl reaction volume containing 2.5 mM each of four dNTP, 10·PCR buffer, 3U of Taq DNA polymerase, 10 ng template DNA and 400 ng each of primer (F) 50-AGA GTR TGATCM TYG CTW AC-30primer (R) 50-CGY TAM CTTWTT ACG RCT-30. The program for amplification was performed at an initial denaturation at 94 °C for 5 min, followed by 35cycles of 94 °C for 30 s, 55 °C for 30 s and 72 °C for 1.30 min with a final extension performed at 72 °C for 10 min. The sequencing was done according to manufacturer's protocol using Big Dye Terminator Cycle Sequencing Kit (v3.1, Applied Biosystems) and analysed in an Applied Biosystems Analyzer. The sequence of 16SrDNA (1461 bp) was aligned using the BLASTn program (Atschul et al. 1997; Stackebrandt and Goebel 1994; Bhattacharya et al. 2003) to identify the most similar sequence. Using consensus primers, the ~ 1.5 kb 16S rDNA fragment was amplified using Taq DNA polymerase and the PCR product was bi-directionally sequenced using the forward, reverse and an internal primer. Sequence data was aligned and analysed for finding the closest homologs for the bacteria. The sequence of isolate mpd-5 was submitted to the GeneBank and received the accession number KT948687. It was tentatively identified as *P. aeruginosa* as it shared 98% similarity with the closest known species in the database. The 16S rDNA sequences of different strains of *Pseudomonas aeruginosa* and its phylogenetically related species and genera were downloaded from Genbank database (http://www.ncbi.nlm.nih.gov/entrez) and aligned to construct a neighbor-joining phylogenetic tree using Clustal W algorithm with the help of MEGA software version4.1 (Tamura et al. 2007).

The isolated fungal strain suspected to be *Fusarium spp*. based on their macro morphological characteristics and microscopic features were further subjected to molecular identification based on partial 18S rRNA, ITS1, 5.8S rRNA, ITS2 and partial 28S rRNA gene. The Fungal Genomic DNA was isolated from the culture using GeNeiTM Fungal Genomic DNA Extraction kit (#616112500011730). Using consensus primers, the ~ 650 bp; 18S rRNA, ITS1, 5.8S rRNA, ITS2 and 28S rRNA gene fragment was amplified using high-fidelity PCR Polymerase. The PCR product was cloned and sequenced using the forward, reverse and internal primers. The Sequence data was aligned and analyzed for finding the closest homologs for the yeast. The fungal strain were grown in Czapek's-Dox Broth (Sigma-Aldrich, India) at 25 °C in a shaker at 150 rpm for 6 days, and subsequently genomic DNA was extracted with the Master Pure Yeast DNA Purification Kit (Thermo Fisher Scientific) in accordance with manufacturer's instructions. The FSSC cultures were preferred using the FSSC specific PCR (He et al. 2011) and confirmed with an EcoRI digestion-based PCR–RFLP method (Homa et al. 2018). The amplification program was performed at an initial denaturation at 94 °C for 5 min followed by 35cycles of 94 °C for 30 s, 56 °C for 30 s and 72 °C for 1 min with a final extension achieved at 72 °C for 10 min. The FSSC—*Fusarium solani* species complex is a group currently estimated to contain at least 60 phylogenetically distinct species (Nalim et al. 2011; O'Donnell 2000; O'Donnell et al. 2008; Zhang et al. 2006). The identification at species level for FSSC positive isolates, the 5′ portions of translation elongation factor 1α (TEF1) coding region and introns were amplified (O'Donnell et al. 1998). After Sanger sequencing the TEF1 sequences were deposited in the GenBank (https://www.ncbi.nlm.nih.gov/nucleotide/) under the accession numbers listed in table and used as BLAST (Altschul et al. 1997), Fusarium MLST database (O'Donnell et al. 2010).

Biotreatment process

The methylparathion containing synthetic wastewater (1000 mgL^{-1}) was prepared using commercial MP (Devithion™ 50% EC). The stock solution of pure MP (98.5%) was prepared by dissolving 1 g in 100 mL methanol, made up to 1000 mL of distilled water, and was used as a reference for instrumental analysis. MP were dissolved in methanol, and 1 mL of a stock solution was dispensed into an empty

500 ml flask. Once the methanol had evaporated completely, 500 ml mineral salts solution was added, providing a MP concentration of 1000 mgL^{-1} and the solution shaken until the pesticide had dissolved. The synthetic wastewater consists of mineral salts medium amended with the MP (MSM gL^{-1}: Sodium nitrate 2; Magnesium sulphate 0.5; Potassium chloride 0.5; Ferrous sulphate 0.01; Calcium chloride 0.05; Peptone 5; Yeast extract 3; Glucose 10; Methylparathion 1 g; pH 7.0 ± 0.2). The pH was adjusted to 5, 7, and 9 for the bacterial strain and 4, 6.5, and 9 for fungal strain using 1 N NaOH and 1 N HCl by pH meter (ELICO-L1127, India). For degradation studies, the respective strain was inoculated into sterile shake-bottles containing 250 mL of MSM, 0.1% (w/v) methylparathion and incubated under aerobic conditions on a shaker (150 rpm/3 days for bacterial isolate mpd-5 and 120 rpm/7 days for fungal isolate sp mpd-1). The parameters include pH, culture temperature, time and agitation, were part of the experimental design. All experiments were performed in triplicate and the results are expressed as an average of three replicates (Usharani 2013; Usharani and Lakshmanaperumalsamy 2016).

Experimental procedure

The potential bacterial and fungal strain resistant to MP was selected based on the MIC test and used for biotransformation in the biotreatment process. The efficient MP utilizer or tolerant strains (bacterial isolate mpd-5 and fungal isolate sp mpd-1) are screened for their potential in the laboratory and were used for the removal of aqueous MP by the analysis of GCMS and FTIR spectral studies.

To determine the bacterial growth pattern and MP degradation efficiency, the mineral salts medium amended with and without MP in presence or absence of glucose were prepared, and selected bacterial isolate was inoculated and incubated for 24 h at 30 °C on a rotary shaker at 150 rpm. The cells were removed by centrifugation (10,000 rpm for 15 min) (CR22GII-Hitachi, Japan) and were transferred to sterile saline.

The bacterial cell concentration of each strain was adjusted to an optical density at 600 nm (OD_{600}) of 0.1 and used as inoculum. Bacterial isolate mpd-5 was added as inoculum by adjusting the cell concentration to 0.1 of OD_{600}. About 95 (mpd-5) × 10^8 $CFUmL^{-1}$ (1 mL of 0.1OD) of the cells were used as an inoculum (bioinoculant) due to its potential stability to the concentration of MP.

The optical density of the bacterial growth was found out. The rate of degradation of MP was studied in MSM-medium (250 ml in 500 ml Erlenmeyer flasks) amended with MP and without MP (1000 mgL^{-1}). The medium was inoculated with 1 ml of bacterial isolate mpd-5. Another flask of the same composition lacking any carbon source except MP (1000 mgL^{-1}) was inoculated to check the utilization of MP as the sole carbon and phosphorus source (Usharani 2013; Usharani and Lakshmanaperumalsamy 2016).

To determine the growth pattern and MP degradation efficiency and the mineral salts medium amended with and without MP in presence or absence of glucose were prepared, and the medium was inoculated with a mycelial mat on agar plugs (1 cm diameter) taken from the margins of actively growing cultures of the studied fungal strain (fungal isolate sp mpd-1) as inoculums into a series of flasks containing mineral salts medium (250 ml in 500 ml Erlenmeyer flasks) with and without MP (MP) (1000 mgL^{-1}). Another flask of the same composition lacking any carbon source except MP (1000 mgL^{-1}) was inoculated to check the utilization of MP as the sole carbon and phosphorus source. The flasks were incubated at 28 ± 2 °C on a rotary shaker at 120 rpm, one flask from each series was removed at 24 h intervals up to a period of 168 h. The fungal biomass from each flask was separated by filtration method using Whatman filter paper No.1 and washed with deionized water. The dry weight of fungal biomass was determined by drying for constant weight in an oven at 50 °C in pre-weighed aluminium foil cups. The growth in terms of dry biomass was expressed in gL^{-1} (Usharani 2013; Usharani and Muthukumar 2013).

Preparation of sample for residual analysis

For the biotreatment process, the treated samples were centrifuged at 10,000 rpm for 15 min (bacterial biotreatment) and 15,000 rpm for 30 min (fungal biotreatment) using a high-speed refrigerator centrifuge (CR22GII-Hitachi, Japan). The centrifuged samples were filtered through 0.2 µm sterile syringe nylon filters and then used for the analysis of residual MP and intermediate products using GC–MS and FTIR.

Analytical estimation

The MP removal efficiency of the biotreatment processes were analyzed in residual MP concentration of the wastewater before and after the treatment process. The samples were withdrawn at different time intervals after biotreatment (bacterial treatment from 0 to 168 h and fungal treatment from 0 to 264 h) were analyzed for residual MP. All experiments were performed in triplicates. The treated wastewater pH was adjusted and monitored using a pH meter (ELICO-L1127, India). The residual MP was analyzed using GC–MS (Perkin Elmer-Clarus 600, Germany).

GC–MS analysis of biotreatment process intermediates

After treatment, the samples were transferred to 250 mL separating funnel and extracted with an equal ratio of diethyl

ether and n-hexane (1:1) by liquid–liquid extraction method. The organic phase were pooled and evaporated with a rotary evaporator to about 5 mL, then purged to dryness with high purity Nitrogen. The residue was reconstituted in 1 mL methanol for qualitative analysis by gas chromatography–mass spectrometry (GC–MS). The intermediate analysis was carried out on GC–MS (Perkin Elmer-Clarus 600, Germany) equipped with a MS capillary column (60 m × 250 µm). Helium was employed as the carrier gas at a constant flow rate of 1 mL min^{-1}. The MS transfer temperature and the ion source temperature was 200 °C. The oven temperature program was set as the following: Initial temperature 50 °C, hold for 0 min, ramped to 260 °C at 10 °C min^{-1}, and held for 3 min. The scan mode was selected, and the range of m/z was from 50 to 450 Da. Split injections (20:1) were used, and the MS data acquisition was set at 3 min post-injection after the elution of the solvent peak. The injection volume was 5 µL. For these operational settings, the retention time for standard MP was 18.5 min (Liu et al. 2006; Moctezumaa et al. 2007; Usharani 2013).

FTIR analysis of biotreatment process intermediates

The surface functional groups present on the microbial pellets were analyzed through Fourier Transform Infrared Spectroscopy (FTIR) analysis (Perkin Elmer 783, Germany). FTIR spectra were obtained by scanning the spectrum in the range 450–4000 cm^{-1} at a resolution of 4 cm^{-1}. The complete dried samples were treated with spectral grade KBr for pelleting. The extractions were identified by comparing GC retention time (RT) and FTIR with those of external standards (Usharani 2013).

Kinetics of MP biodegradation in MSM

Kinetics analysis was carried out, because the microbial cells were utilized as an enzyme system for MP (MP) biodegradation (Dykaar and Kitanidis 1996). The Langmuir–Hinshelwood (L–H) kinetic model was used to describe the biotreatment process and biodegradation rate of MP by plotting the graph of ln (C_t/C_0) versus time, t, at different concentrations. The simulated kinetics curves meet the first-order kinetic model was

$$\ln\left(C_t/C_0\right) = -kt. \quad (1)$$

Determined using the following equation. The obtained result are considered, where C_0 is the initial concentration of MP, C_t is the concentration of MP at time t, and k is the reaction constant of the first-order reaction:

$$\ln\left(C_t/C_0\right) = -kt. \quad (1)$$

First-order integrated law

$$\ln\left[C_{MP}\right]_0/\left[C_{MP}\right]_t = -K_{MP}t. \quad (2)$$

Rearrange this equation: $\ln (CMP)_0/CMP)_t = -K_{MP}\,t$
[use log ln(x/y) = ln x–ln y];
$\ln [C_{MP}]_0 - \ln [C_{MP}]_t = -K_{MP}\,t$

$$\ln\left[C_{MP}\right]_0 = -K_{MP}t + \ln\left[C_{MP}\right]_t. \quad (3)$$

Linear Equation: similar to straight line equation

$$\left[\text{Equation of Straight Line}: \; Y = mX + C\right] \quad (4)$$

m = Slope; X = time = t; C = y intercept.
Compare the equations of straight line (Eq. 4) and linear equation (Eq. 3)
m = Slope; i.e., m = −k = −K and X = t;
A first-order reaction is an exponential decay (in terms of reactant):

$$\left[C_{MP}\right]_t = \left[C_{MP}\right]_0 e^{-Kt}. \quad (5)$$

The concentration of reactant $[C_{MP}]$ decreases exponentially over time:

$$\text{Rate constant, K; unit for K} = \text{day}^{-1} \quad (6)$$

$$\ln\left[C_{MP}\right]_0 = -K_{MP}t + \ln\left[C_{MP}\right]_t,$$

where C_0 or $[C_{MP}]_0$ is the initial concentration of MP (mg/L), C_t or $[C_{MP}]_t$ is the MP concentration after biotreatment at time, t, and k or K_{MP} is the first-order rate constant.

Results

Minimum inhibitory concentration (MIC) test

The MIC assay with plate screening method was carried out to screen methylparathion resistant bacteria using methylparathion MSM with methylparathion agar plates. Among the isolates, mpd-5 were screened and identified as predominant methylparathion utilizers based on the MIC test, as shown in Fig. 1. The identification and characterization of the isolated prominent bacterial stains were performed based on their morphological characters and biochemical tests. The MIC of methylparathion (MP) in MSM media and the time taken for the appearance of first visible colony on the agar plate was worked out for the bacterial isolates. The delay in appearance of growth coincided with the concentration of pesticide. The MIC of bacterial isolates were in the range of 100–1000 mgL^{-1} of MP and the growth of isolates was observed within 24–96 h of incubation. The hydrolysis zone resulting from

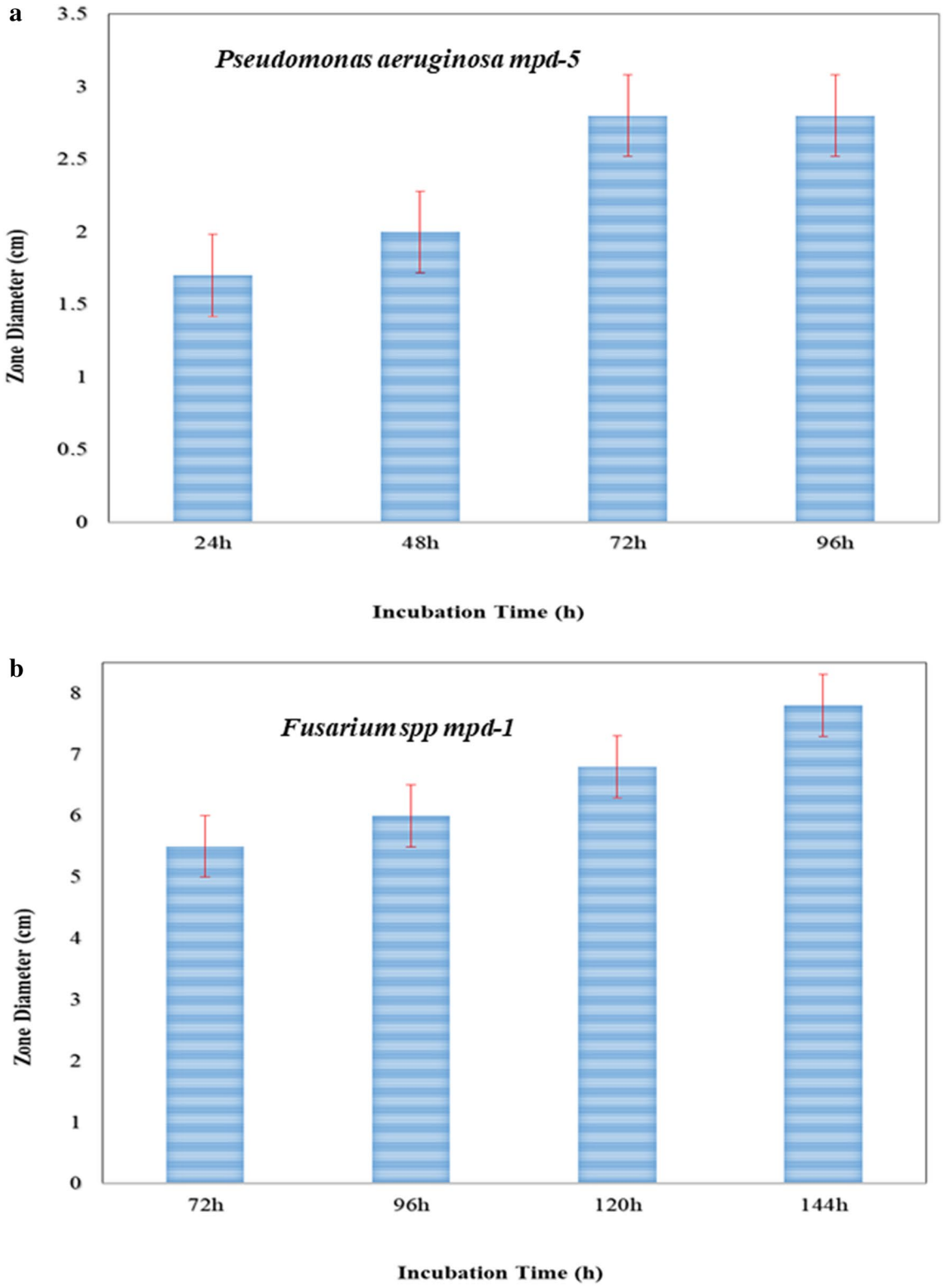


Fig. 1 Screening of potential methylparathion degrading **a** *Pseudomonas aeruginosa* and **b** *Fusarium spp* mpd strains based on the minimum inhibitory concentration (MIC) test

Table 1 First-order rate constants for the degradation of methylparathion (MP) bioreduction by Biotreatment process in batch scale system under aerobic condition

Temperature (°C)	Inoculum density	Initial MP concentration	Agitation	pH	First order K_{MP}	MP R^2
(a) Bacteria *Pseudomonas aeruginosa* mpd-5 (95% removal)	Cells/ml	(mgL^{-1})	Rpm		(day^{-1})	
33	10^8	1000	150	5	0.0130	0.95
33	10^8	1000	150	6	0.0214	0.95
33	10^8	1000	150	7	0.0294	0.97
33	10^8	1000	150	8	0.0241	0.94
33	10^8	1000	150	9	0.0144	0.85
(b) Fungi *Fusarium spp* mpd-1 (93% removal)	(mgL^{-1})	(mgL^{-1})	rpm	pH	First order K_{MP} (day^{-1})	MP R^2
30	25	1000	120	5	0.0081	0.62
30	25	1000	120	6	0.0108	0.68
30	25	1000	120	7	0.0129	0.73
30	25	1000	120	8	0.0124	0.73
30	25	1000	120	9	0.0100	0.70

methylparathion degrading were observed in mpd-5 strain and the strain with a higher ratio of the clearing hydrolysis zone diameter to colony size was selected for further analysis. The bacterial strain mpd-5 showed highest value of MIC (1000 mgL^{-1}) and the growth appeared after 96 h of incubation at the temperature of 37 °C (Fig. 1a) and identified as *Pseudomonas spp* (Holt et al. 1994). Among the fungal isolates, the strain mpd-1 was screened and identified as predominant methylparathion utilizers based on the MIC test and inhibition zone was observed after 72 h of incubation, as shown in the figure. The isolated fungal strains were then identified according to Clements and Shear (1957) and Booth (1977). Four potential fungal strains were observed on mineral salts agar medium enriched with methylparathion were subjected to morphological studies. The fungal isolate mpd-1 was identified as *Fusarium*, based on microscopic examination of morphological characteristics using lactophenol cotton blue staining. One of the fungal strains, the mpd-1 stain that grew most rapidly and luxuriously shows the highest methylparathion-hydrolyzing capability was selected, and further identified and confirmed as *Fusarium spp* (Fig. 1b). The results from Table 1 showed the first-order rate constants for the degradation of MP bioreduction by biotreatment process in batch scale system under aerobic condition. All experiments were performed in triplicates and their kinetic study was carried out which meet the first-order kinetic model, from the experimental data the standard error and error bar was added as per the observed results. From Fig. 2a, b, it had been shown that a linear relationship was obtained with $k = 0.0294$ day^{-1} and $R^2 = 0.97$ for bacteria (mpd-5) and with $k = 0.0129$ day^{-1} and 0.73 for fungi (mpd-1).

Molecular characterization of bacterial strain mpd-5 and fungal strain mpd-1

The bacterial strain showed yellowish white colour, mucoid colonies with smooth, regular margins and fruity odour on Pseudomonas isolation agar and Centrimide Agar plates at 37 °C for 24 h. The colonies fluoresced under UV light, the phenotypic characterization revealed that the isolated strain was motile, small rod (0.6 × 1.7 µm) and stained Gram negative. It was grown at the temperature ranging from 25 °C to 40 °C but maximum growth was recorded at 37 °C. The positive reactions were recorded for, indole test, methyl red, Voges–Proskauer, citrate utilization, catalase activity, oxidase activity, gelatine hydrolysis, amylase, phosphatase, H_2S production, urease activity and lysine decarboxylase enzyme. The bacterial strain was also able to utilize glucose, galactose, fructose, lactose, mannitol as sole carbon sources and the negative reaction was recorded for casein hydrolysis.

The genotypic characterization based on the nucleotides homology and phylogenetic analysis the sample, studied bacteria (NAL Bacteria) was detected to be *Pseudomonas aeruginosa* (GenBank Accession Number: KT948687). The nearest homolog was found to be *Pseudomonas citronellolis* (Accession No: AF530074). The information about other close homologs for the microbe can be found from the Alignment View table. The 16S rRNA gene sequence of

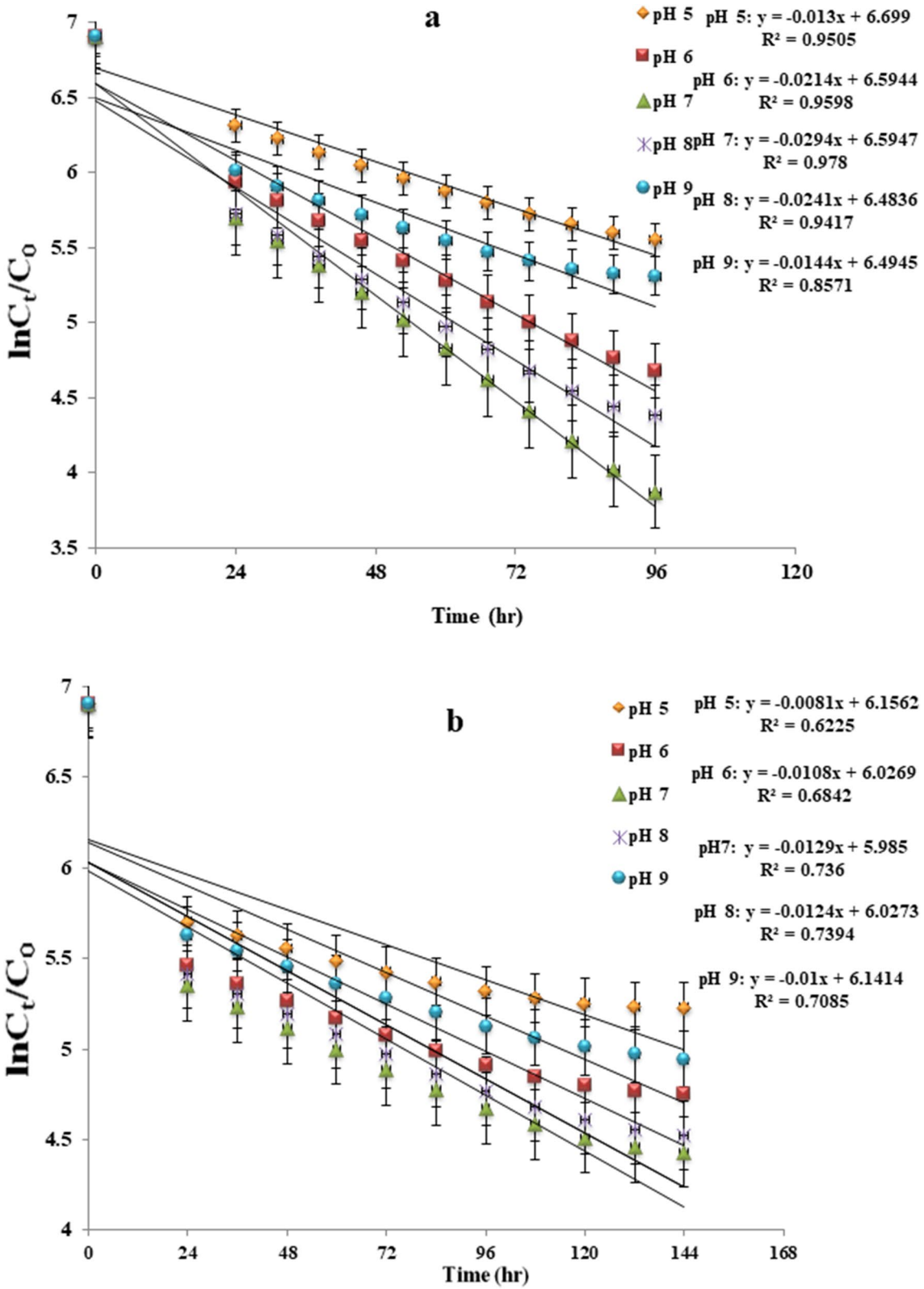


Fig. 2 Kinetic analysis of residual methylparathion from biotreated samples with optimized conditions by **a** *Pseudomonas aeruginosa* mpd-5 (temperature 33 °C, 150 rpm, inoculum density10^8 cells/ml) and **b** *Fusarium spp* mpd-1 (temperature 30 °C, 120 rpm, 25 mgL^{-1} dry biomass) at various time intervals and pH

the isolate had more identity with the type strain of *Pseudomonas aeruginosa*, available in the NCBI GeneBank. The bacterial sequence of isolate mpd-5 was submitted to the GeneBank and received the accession number KT948687. It was tentatively identified as *P. aeruginosa* as it shared 96.8% similarity to the type strain GQ254065 and 98% to DSM 50,071. The phylogenetic analysis of bacterial strain mpd-5 sequence homology with *P. aeruginosa*, was characterized as a phylogenetic tree (Fig. 3).

Based on nucleotides homology and phylogenetic analysis the Microbe (Sample: NLA Fungi) was detected to be *Fusarium spp* (GenBank Accession No: KT948688). The genotypic characterization based on the nucleotides homology and phylogenetic analysis the sample, studied bacteria (NAL Bacteria) was detected to be *Fusarium spp* (GenBank Accession Number: KT948688). The nearest homolog was found to be *Fusarium solani* (Accession No: DQ236758) and *Fusarium falciforme*. The information about other close homologs for the microbe can be found from the Alignment View table. The gene sequence of the isolate had more identity with the type strain of *Fusarium spp*, available in the NCBI GeneBank. The fungal sequence of isolate mpd-1 was submitted to the GeneBank and received the accession number KT948688. It was tentatively identified as *Fusarium spp* as it shared 86% similarity to the type strain DQ236758 and 61% to GU170653. The phylogenetic analysis of fungal strain mpd-1 sequence homology with *Fusarium spp*, was characterized as a phylogenetic tree (Fig. 4).

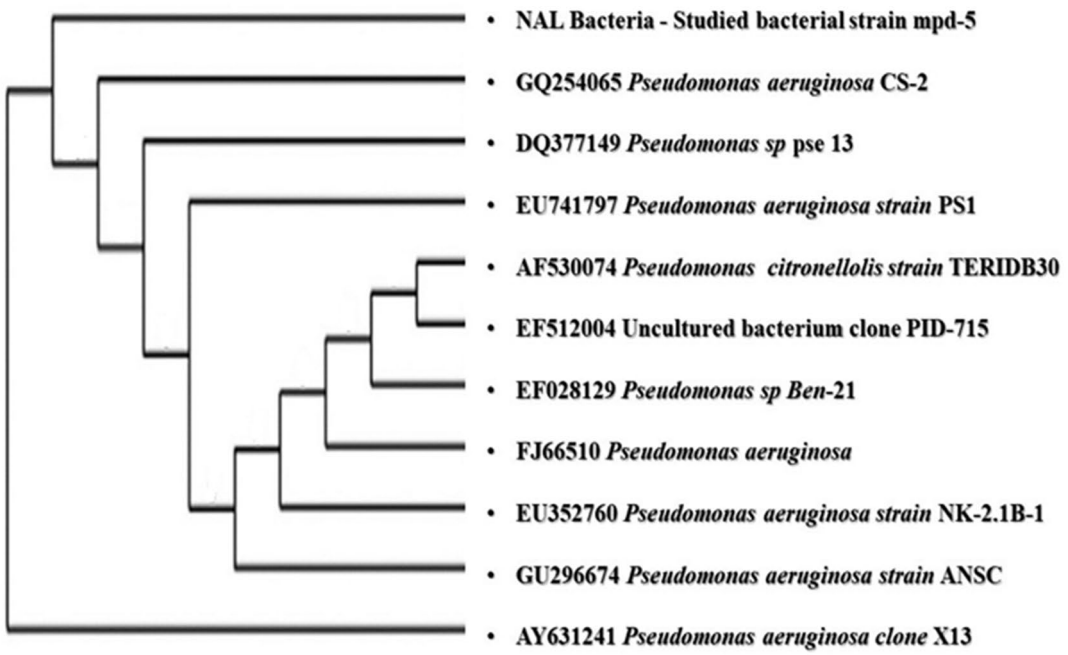
GC–MS analysis of microbial biotransformation

The *Pseudomonas aeruginosa* mpd-5 strain observed the experimental data approached from the in vitro assays with methylparathion wastewater and a pure microbial monoculture strains within the optimized conditions, the utmost removal (95%) of MP with bio-inoculum density of 10^8cells/ml for 3 days (at pH7, 150 rpm) and 93% of MP removal was recorded by the *Fusarium spp* mpd-1 strain with bio-inoculum density 25 mgL^{-1} (dry biomass–mycelium) for 6 days (pH7, 120 rpm) under the aerobic condition in batch scale biotreatment process (Usharani 2013; Usharani and Lakshmanaperumalsamy 2016).

The bacterial biotreated samples were analyzed by GC–MS for the identification of the residual MP and their intermediates. The GC spectrum profile of MP biodegradation by *Pseudomonas aeruginosa* mpd-5 after 3 days is shown in Fig. 5. The fungal biotreated samples were analyzed by GC–MS for the identification of the residual MP and their intermediates. The GC spectrum profile of MP biodegradation by *Fusarium spp* mpd1 after 3 days is shown in Fig. 6. The retention time of MP ($C_8H_{10}O_5NSP$-263 MW) standard in GC analysis was observed to be 18.5 min. The compounds having the individual retention times of 11.21, 12.42, 14.64, 17.47, and 18.5 min in GC were identified as intermediates of MP by NIST library explore and by assessment with the real standards as phenol-4-amino (aminophenol), phenol,3-(dimethylamino), phenol, 4-nitro (p-nitrophenol), glycyl-L-proline and also the parent MP, respectively. The MP characteristic fragment ions having a mass of m/z 263, 125, 109, 93, 79, 63 was observed. The fragment ion of m/z 125 was formed by the loss of p-nitrophenol from the molecular ion of m/z 263. The fragment ion having the mass of m/z 109 was created by the loss of the chemical group (NO) from the nitrobenzene group. Further fragmentation by the loss of an oxygen atom gives $[C_6H_5O]+$ at m/z 93. The loss of a methyl group and a methoxyl group produces a mass of m/z 79 which was within the thiophosphoric moiety, followed by the loss of another oxygen atom to grant the relative molecular mass of m/z 63 ions. The fragment ions with m/z 139, 109, 93, 81, and 65 within the MS spectrum are characteristic fragment ions of 4-nitrophenol ($C_6H_5O_3N$-139 MW), which was observed as a major peak. The fragment ions with m/z 136, 121, 94, 65 and 154, 111, 98, 83, 70, 55 within the MS spectrum are all characteristic fragment ions of 3-dimethylaminophenol and Glycyl-L-Proline, respectively. The fragment ions with m/z 136, 108, 98, 55 and 211, 170, 113, 86, 55 within the MS spectrum are all characteristic fragment ions of 2-(3-methylpiperidino-4-5,6-benzothiazin-4-one) and a couple of 2,5-piperazinodione3,6-bis (2methylpropyl), respectively (Figs. 6, 7 and Table 2). The proposed reaction pathway for the biodegradation of MP by the strain of *Pseudomonas aeruginosa* mpd5 was at initial pH 7, as shown in Fig. 7 and Table 2a, and also the biodegradation by *Fusarium spp* mpd1 strain was at initial pH 6.5 is shown in Fig. 8 and Table 2b.

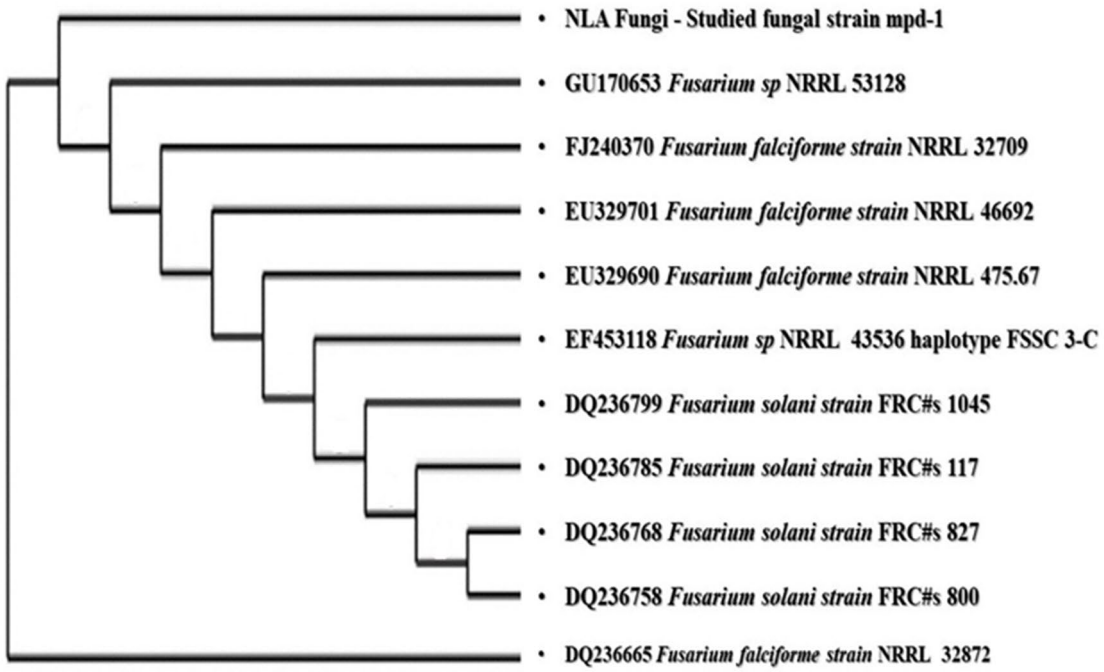
FTIR analysis of biotreatment process

The FTIR spectroscopy gives more insight into structural changes of the MP. The FTIR spectra of the molecule and products are depicted in Fig. 9. It can be seen that some structural changes might have occurred during the bacterial and fungal biotreatment processes in the treated sample. The appearance of the band at 2955 cm^{-1} indicates the presence of CH for CH_3. The band at 837 cm^{-1} indicates the presence of CH in the outer plane of the aromatic ring and a band at 1641 cm^{-1} for C=C (Usharani 2013). The presence of a band at about 1346 cm^{-1} is thought to belong to the aromatic NO_2 group. The P–OCH_3 group has a closer band located at 1047 cm^{-1}. The phosphate group arising at 576 and 1036 cm^{-1} can be assigned to P–O stretching and bending vibrations, respectively, and at 1036 cm^{-1} and 767 cm^{-1} to P–O–C and P = S stretching, respectively.



Alignment View	ID	Alignment results	Sequence Description
	NAL Bacteria	1.00	Studied Sample
	AF530074	0.91	*Pseudomonas citronellolis* strain TERIDB30
	GQ254065	0.98	*Pseudomonas aeruginosa* strain CS-2
	GU296674	0.98	*Pseudomonas aeruginosa* strain ANSC
	EU741797	0.94	*Pseudomonas aeruginosa* strain PS1
	EF512004	0.96	*Uncultured bacterium* clone P1D1-715
	AY631241	0.98	*Pseudomonas aeruginosa* clone X13
	DQ377149	0.98	*Pseudomonas sp.* pse13
	FJ665510	0.98	*Pseudomonas aeruginosa*
	EU352760	0.98	*Pseudomonas aeruginosa* strain NK 2.1B-1
	EF028129	0.98	*Pseudomonas sp.* Ben-21

Fig. 3 Phylogenetic tree showed the genetic relationship of *Pseudomonas aeruginosa* with taxonomically similar strains, species, and genus based on 16S rDNA sequences



Alignment View	ID	Alignment results	Sequence Description
	NLA-FUNGI	1.00	Studied Sample
	GU170653	0.61	*Fusarium sp.* NRRL 53128
	FJ240370	0.67	*Fusarium falciforme* strain NRRL 32709
	EU329701	0.67	*Fusarium falciforme* strain NRRL 46692
	EU329690	0.67	*Fusarium falciforme* strain CBS 475.67
	EF453118	0.61	*Fusarium sp.* NRRL 43536 haplotype FSSC 3-c
	DQ236799	0.86	*Fusarium solani* strain FRC#s1045
	DQ236785	0.86	*Fusarium solani* strain FRC#s1177
	DQ236768	0.86	*Fusarium solani* strain FRC#s827
	DQ236758	0.86	*Fusarium solani* strain FRC#s800
	DQ236665	0.86	*Fusarium falciforme* strain NRRL32872

Fig. 4 Phylogenetic tree showed the genetic relationship of *Fusarium spp* with taxonomically similar strains, species, and genus based on partial 18S rRNA, ITS1, 5.8S rRNA, ITS2 and partial 28S rRNA gene

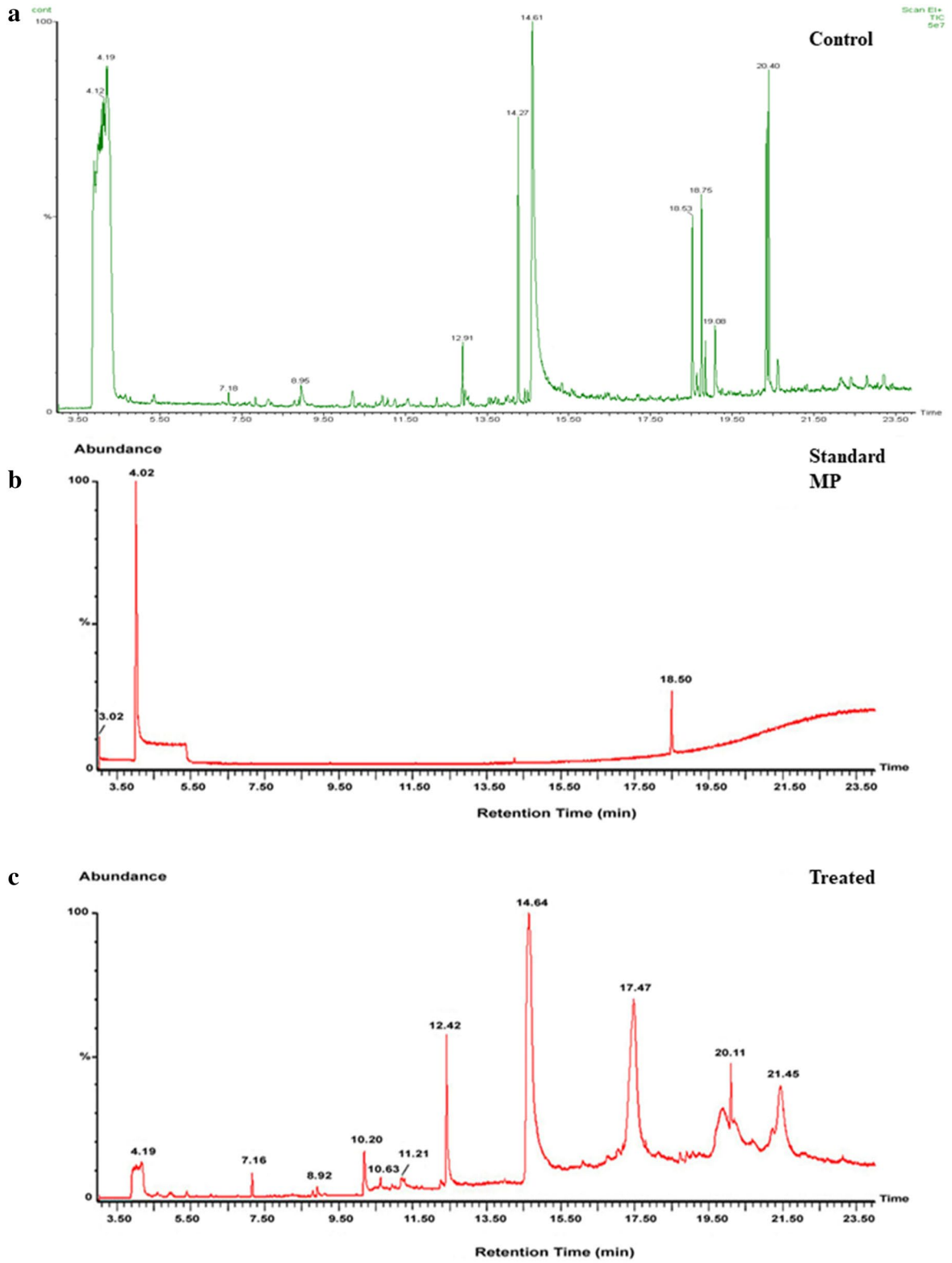


Fig. 5 GC Spectrum of methylparathion standard and biotreated sample by *Pseudomonas aeruginosa* (mpd5) (**a**) Control (**b**) Standard and (**c**) Treatedfor 72h

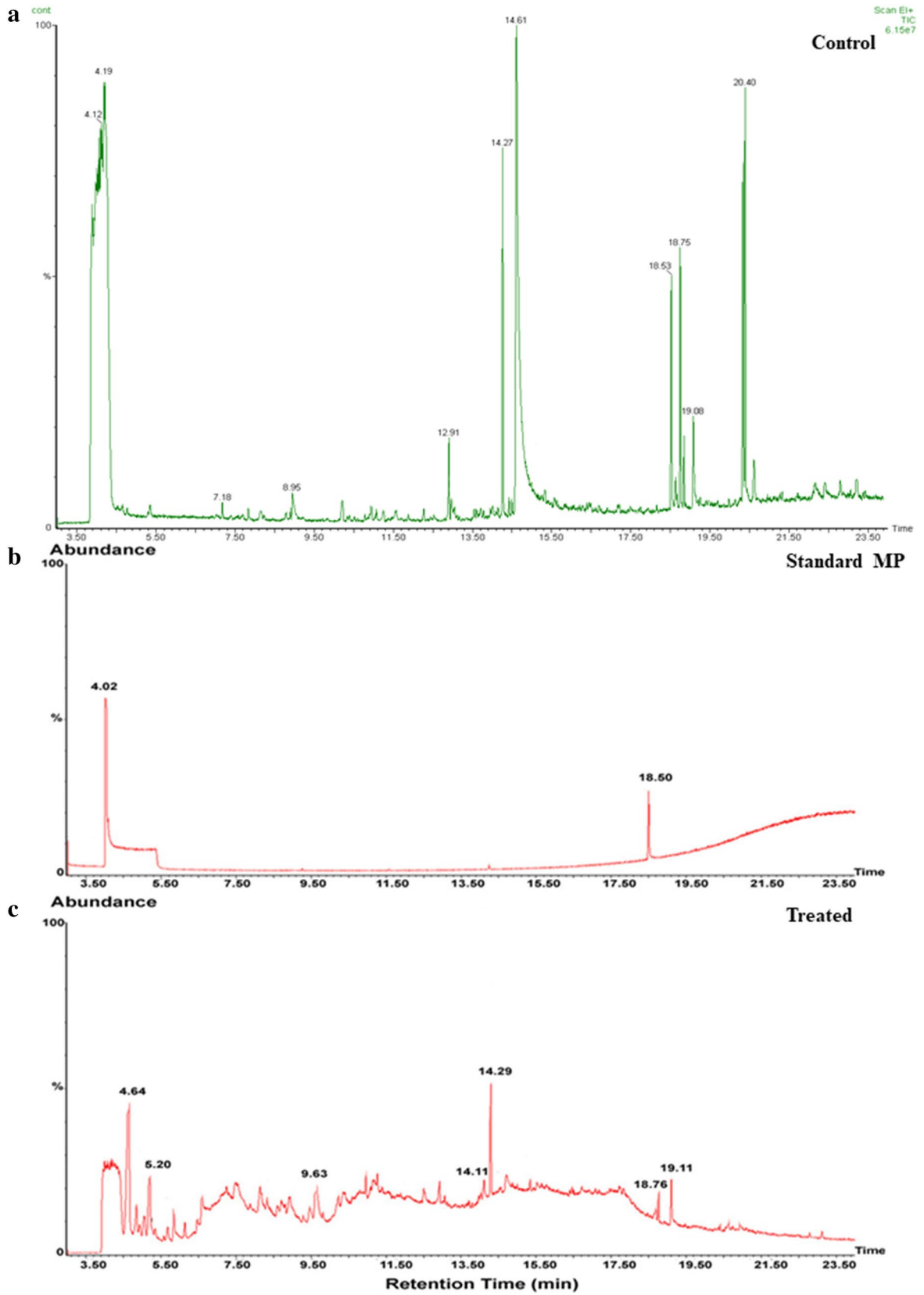


Fig. 6 GC Spectrum of methylparathion standard and biotreated sample by *Fusarium spp* (mpd1) (**a**) Control (**b**) Standard and (**c**) Treated for 120h

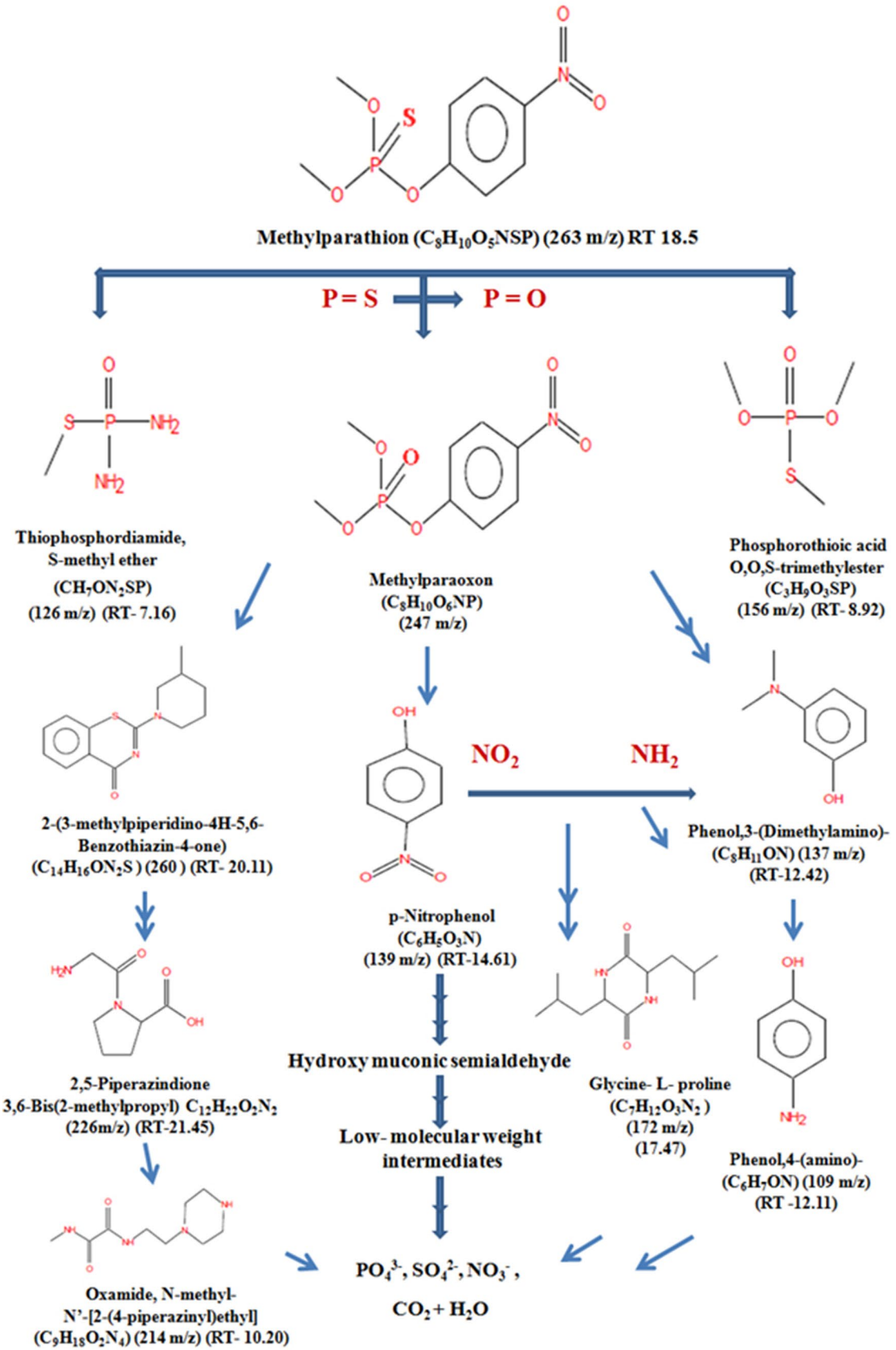


Fig. 7 Possible degradation pathway of aqueous methylparathion by *Pseudomonas aeruginosa* mpd5

Table 2 GC–MS analysis of Intermediates of methylparathiom biotreatment by *Pseudomonas aeruginosa* mpd-5 and *Fusarium spp* mpd-1

(a) Bacteria-*Pseudomonas aeruginosa* mpd-5 compound name	Formula	Molecular weight	Retention time (RT)
Methylparathion	$C_8H_{10}O_5NSP$	263	18.50
Thiophosphordiamide, S-methyl ether	CH_7ON_2SP	126	07.16
Phosphorothioic acid, O,O,S-Trimethyl ester	$C_3H_9O_3SP$	156	08.92
Oxamide, N-methyl-N'-[2-(4-piperazinyl)ethyl]	$C_9H_{18}O_2N_4$	214	10.20
Phenol-4-amino	C_6H_7ON	109	11.21
Phenol,3-(Dimethylamino)-	$C_8H_{11}ON$	137	12.42
Phenol, 4-nitro	$C_6H_5O_3N$	139	14.64
Glycyl-L-Proline	$C_7H_{12}O_3N_2$	172	17.47
2-(3-methylpiperidino-4H-5,6-Benzothiazin-4-one)	$C_{14}H_{16}ON_2S$	260	20.11
2,5-Piperazindione3,6-Bis(2-methylpropyl)-	$C_{12}H_{22}O_2N_2$	226	21.45
(b) Fungi-*Fusarium spp* mpd-1 compound name	Formula	Molecular weight	Retention time (RT)
Methylparathion	$C_8H_{10}O_5NSP$	263	18.50
Beta-L-arabinopyranoside, methyl	$C_6H_{12}O_5$	164	04.64
Cyclobutanethiol	C_4H_8S	88	05.20
3–2(4)-thiophenone,dihydro-5-(1-methylethyl)-	$C_7H_{12}OS$	144	09.63
Benzene,1,3-bis(1,1-dimethylethyl)	$C_{14}H_{22}O$	206	10.96
Lactose	$C_{12}H_{22}O_{11}$	342	14.11
Phenol,2,4-Bis(1,1-Dimethylethyl)-	$C_{14}H_{22}O$	206	14.29
o-nitrophenylbeta D-galactopyranoside	$C_{12}H_{15}O_8N$	301	14.70
Butanoic acid, 2-methyl methyl ester	$C_7H_{14}O_2$	139	18.69
L-(+)-Ascorbic acid	$C_{38}H_{68}O_8$	652	19.11

Discussion

Minimum inhibitory concentration (MIC) test

The pesticide exposed contaminants and pesticide acclimatized soils with prospective and effective microbes can leads to the reduction or modification of the microorganisms. Though, some microbes persist and increase their population and this may indicate the ability of the microbes to progress and adapt to such conditions. The screened strains were observed to be more resistant on nutrient agar medium enriched with methylparathion. For large scale culture of such bacterial isolates, the potential strain was used for biotreatment and bioremediation purpose, it is essential to determine the optimum growth, temperature and pH. These isolated strains of bacteria are highly adapted to the existing environmental conditions and thus could be effectively utilized for bioremediation and metabolic detoxification of methylparathion. The enrichment culture techniques are capable of degrading OPs using bacteria, these included *Enterobacter spp* (Singh et al. 2004), *Pseudomonas putida* (Rani and Lalithakumari 1994; Karpouzas et al. 2000a, b), *Arthrobacter spp* (Misra et al. 1992) and *Flavobacterium spp* (Sethunathan and Yoshida 1973). Tchelet et al. (1993) isolated *Pseudomonas spp* and *Xanthomonas spp* which were able to hydrolyse parathion and can further metabolize p-nitrophenol. The isolated *Pseudomonas putida* that could hydrolyze methyl parathion and utilize p-nitrophenol as a source of energy (Rani and Lalithakumari 1994). Chaudhry et al. (1988) reported that *Pseudomonas spp* can co-metabolically degrade methyl parathion. Zhongli et al. (2002) also isolated *Pseudomonas spp* (A3) which can utilize p-nitrophenol as sole source of carbon and nitrogen. The strain of *Pseudomonas spp* (WBC) was isolated from polluted soils around a pesticide factory which was capable of degrading methylparathion and could exploited it as sole source of carbon and nitrogen (Yali et al. 2002).

GC–MS analysis of intermediates in biotreatment process

The kinetic analysis was carried out, because the microbial cells were utilized as an enzyme system for MP biodegradation. Dykaar and Kitanidis (1996) studied similar kinetic analysis in macro transport of a biologically reacting solute through porous media. The Langmuir–Hinshelwood (L–H) kinetic model was used to express the biotreatment process

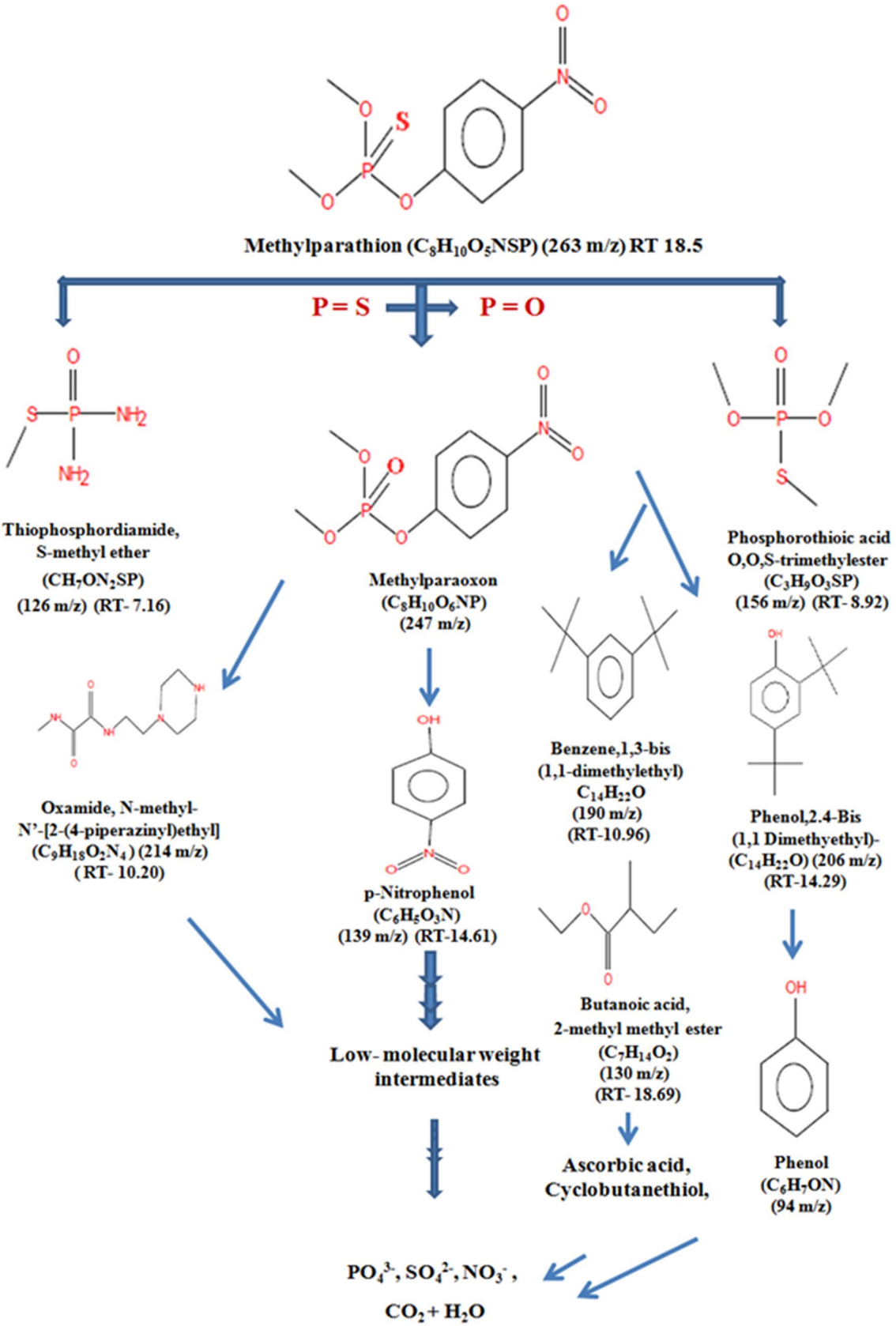


Fig. 8 Possible degradation pathway of aqueousmethylparathion by *Fusarium spp* mpd1

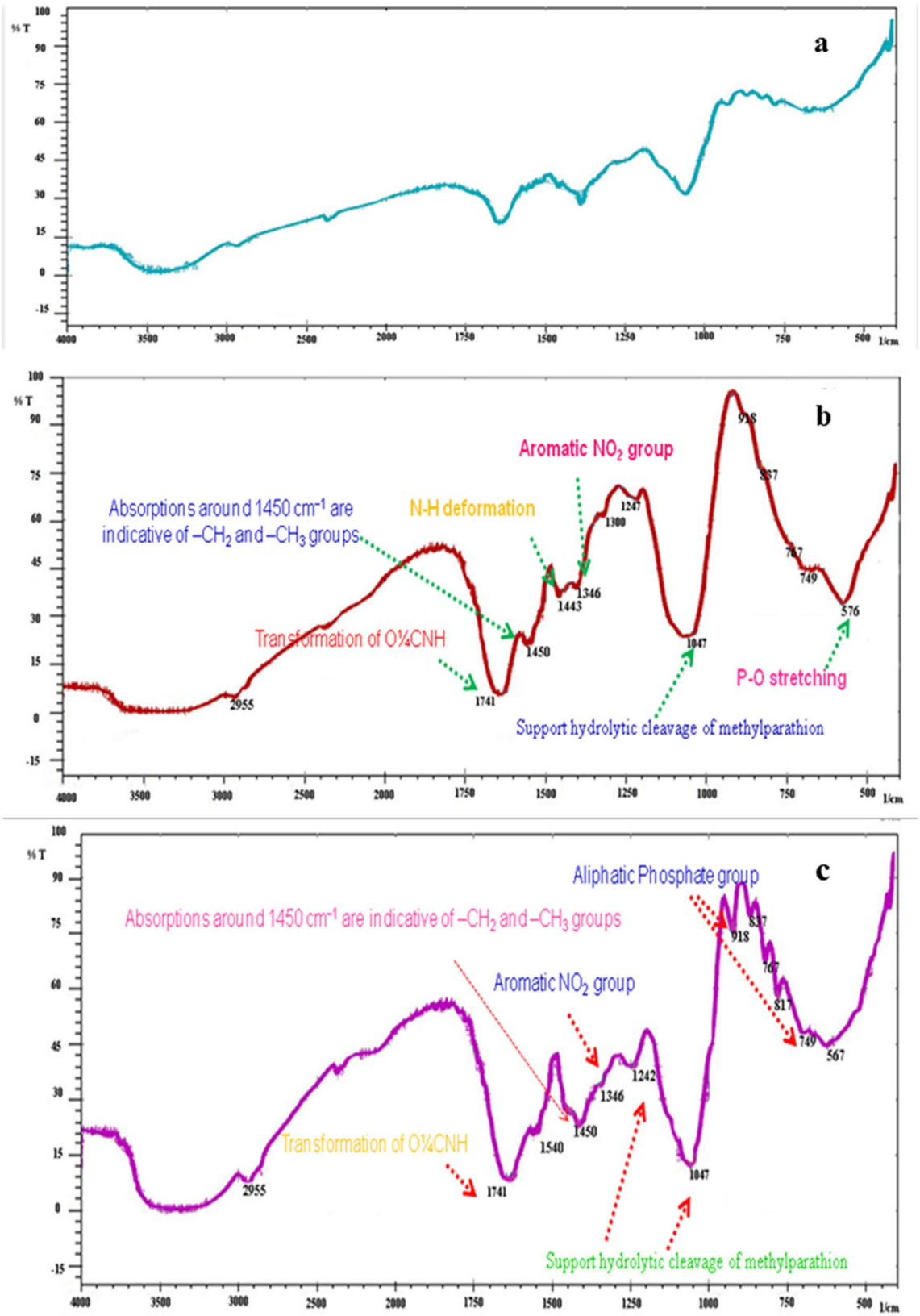


Fig. 9 FTIR spectra of the methanol extracted methylparathion before treatment (**a**) Control and after biodegradation (**b**) by *Pseudomonas aeruginosa* mpd5 treated at 72h and (**c**) by *Fusarium spp* mpd1 treated at 120h

and biodegradation rate of MP by plotting the graph of ln (C_t/C_0) versus time, *t*, at different concentrations (Eq. 1), and the first-order rate constant was determined from the slope of the straight line.

The intermediate product of MP conversion to a more toxic analog of methyl paraoxon was not recorded. The metabolites were not quantified during this study. Para nitrophenol and dimethylaminophenol were found as the major intermediates produced during the biotreatment process of methylparathion by *Pseudomonas aeruginosa* mpd5. The bacteria *Pseudomonas putida* utilized MP as sole a source of carbon and phosphorus. The microbial catalytic enzyme produced by the bacteria, such as organophosphorus acid anhydrase, which hydrolyzed methylparathion to p-nitrophenol, and furthermore, it converted to hydroquinone and 1,2,4-benzenetriol. The ring compound of 1,2,4-benzenetriol was cleaved by the enzyme benzenetriol oxygenase, which was converted to maleylacetate as mentioned by Rani and Lalithakumari (1994). The enzyme phosphotriesterase may be involved in the biodegradation of organophosphates, and during the breakdown of methyl parathion, nitrite was released as a catabolic by-product was observed by Ramanathan and Lalithakumari (1999). Therefore, it was stated that para-nitrophenol was the chief metabolite of parathion and MP biodegradation. The low down molecular weight intermediate compounds formed account for the enhancement biodegradability. These microbial strains degrading xenobiotic chemicals have elaborate enzyme systems and degraded the organophosphates by the enzymes that include phosphatases, esterases, hydrolases, and oxygenases. The previous study reported that the biodegradation of OPs by the enzyme organophosphorus hydrolase (Richins et al. 1997). Singh et al. (2004) also reported that the higher phosphotriesterase activity was observed in *Enterobacter* strain (B-14), which involved in the hydrolyzing chlorpyriphos (35 mgL^{-1}) within 24 h. The enzyme phosphotriesterase was responsible for the monocrotophos biodegradation by the two bacterial isolates *Pseudomonas aeruginosa,* and *Clostridium michiganense* sub sp insidiosum, and the plasmid-borne gene was responsible for the production of phosphotriesterase (Singh and Subhas 2002). In addition, the cell-free extracts of bacteria are involved in the biodegradation, it was noted that *Flavobacterium balustinum* was able to hydrolyze a variety of OPs, namely, fenitrothion, quinalphos, and monocrotophos (Sureshkumar et al. 1998).

During the breakdown of MP, nitrite was liberated as a catabolic byproduct (Ramanathan and Lalithakumari 1999). The utilization of organophosphates by a wild type strain of *Penicillium notatum* was reported by Bujacz et al. (1995). The results stated that the fungus might play a big role in the biodegradation of organophosphonates. The enzymatic activities of the fungus are dependable for the degradation of methylparathion. The hydrolysis of parathion, diazinon, fensulfothion and coumaphos by the enzyme phosphotriesterase (Dumas et al. 1989). Marinho et al. (2011) reported that the presence of a glucose concentration (0.5 mgL^{-1}) helped the removal of the pollutant. The enzymatic systems in fungi include glucose oxidase, catalase, lactanase, cytochrome P450 monooxygenase, and ligninolytic enzymes (Witteveen 1993; Prenafeta Boldu 2002). The cytochrome P450 monooxygenase is coupled to NADPH reductase, which works as a source of electrons for oxidation reactions. The enzyme catalyses the aromatic ring's epoxidation, producing arene oxides that are formed through the epoxide hydrolase trans-dihydrodiols or are rearranged non enzymatically to form phenols (Marinho et al. 2011) and the enzymatic system must encourage the partial degradation of methyl parathion. They established similarities with cytochrome P450 action, but, unlike cytochrome P450, the chloroperoxidase could not cleave the oxone structures.

FTIR analysis of biotreatment process

The intense bands at 1242 cm^{-1} and 1047 cm^{-1} support the hydrolytic cleavage of MP by *Pseudomonas aeruginosa* mpd-5 and *Fusarium spp* mpd-1. Bhalerao and Puranik (2009) reported similar trends in the biodegradation of monocrotophos by *Aspergillus oryzae*, the reduction in the band intensity of aliphatic phosphate groups (918 cm^{-1} and 749 cm^{-1}). The FTIR spectrophotometric determination of malathion in pesticide formulation was analysed by Quintás et al. (2004).

Complete degradation of a chemical in the medium to carbon dioxide and water involves many different types of reactions. The key termination drawn from the liquid culture degradation study is the isolated cultures were potent and able to degrade methyl parathion. The isolated cultures have the potential to apply in biotreatment of MP contaminated wastes (Usharani 2013; Usharani and Lakshmanaperumalsamy 2016). These data showed very high efficiency of MP degradation by a potential bacterial and fungal strain over a short time period. The results indicate the promise of the bacterial and fungal isolate in developing a biotreatment or bioremediation for organophosphate contaminated aqueous or solid wastes. These results also suggest that the need for further studies on inoculation of bacterial and fungal cultures in organophosphate contaminated aqueous or solid wastes for restoring the microbial activity.

While these processes when applied in the real time wastewater matrix in presences of other organics (mixed contaminated xenobiotics) may influence the rate of reaction either positively or negatively. It is quite natural, the process was aimed to developed and optimized only for the single contaminants removal, due to the presence of various organics type or kind of mixed contaminants (Xenobiotics)

in the wastewater matrix; it may slow down the process rate when compared to the laboratory pilot study.

Conclusions

The biotreatment process may involve, the biodegradation strategies exhibited by potential bacterial and fungal strains include the bioprocess of cometabolism, biotransformation of a molecule coincidental to the normal metabolic functions of the microbe. The catabolism and the utilization of the molecule as a nourishment or power source, also by extracellular enzymes (phosphatases, laccases and amidases) liberated into the treatment system, which can act on the molecule as a substrate. Three basic types of reactions may occur degradation, conjugation, and rearrangements, all of which can be microbially mediated. Bacterial biotransformation of MP contaminated synthetic wastewater by *Pseudomonas aeruginosa* mpd-5 was optimum at temperature 33 °C and pH 7 under aerobic conditions an inoculum density of 108 cells/mL. The major compounds formed during the degradation of MP were p-nitrophenol, dimethylaminophenol, glycyl-L-proline of 2-(3-methylpiperidino-4-5,6-benzothiazin-4-one), and 2,5-piperazinodione 3,6-bis(2methylpropyl). Fungal biotransformation of MP contaminated synthetic wastewater by *Fusarium spp* mpd-1 was found to be optimum at temperature 30 °C and pH 7 and under the aerobic condition with an inoculum density of 25 mgL^{-1} dry biomass. The major compounds formed during the degradation of MP were p-nitrophenol, phenol,2,4-Bis(1,1-Dimethylethyl), beta-L-arabinopyranoside-methyl, cyclobutanethiol, 3-2(4)-thiophenone, dihydro-5-(1-methylethyl), benzene,1,3-bis(1,1-dimethylethyl), butanoic acid, 2-methyl methyl ester, and L-(+)-ascorbic acid. The efficient microbial removal of MP by the bacteria and fungi may occur due to the co-metabolic activity. The MP in the synthetic wastewater may influence the growth of the MP degraders and utilize as a phosphate and carbon source of energy. Therefore, the synthetic wastewater containing MP supports bacteria's growth and thus removed the MP at a higher level. It is clear that MP removal by bacterial and fungal strain proved to be an effective biotreatment for MP contaminated wastewater. Thus, MP removal at a high concentration from wastewater is possible by microbial strains. They may use the contaminants as nutrients and as an energy source, or it may be utilized by co-metabolism. It can be concluded from the aforementioned results and discussions that the strains of *P. aeruginosa* mpd-5 and Fusarium *spp* mpd-1 can be used as microbial agents for the biotreatment of pesticide wastewater and having the potential to degrade methyl parathion. Hence the potential bacterial and fungal strains could be used in the biotreatment process for wastewater and bioremediation of MP contaminated environments.

Acknowledgements The author K. Usharani wishes to thank the Department of Environmental Sciences, Bharathiar University and DRDO-BUCLS, TN, India for providing requirements and SRF. She wishes to extend her sincere gratitude to editor Prof. em. Dr. Erko Stackebrandt (DSMZ, Göttingen, Germany) for his critical comments and also thank anonymous reviewers for their kind efforts.

Declarations

Conflict of interest The author declare that there was no conflict of interest.

Ethical approval This article does not contain any studies with human participants or animals performed by the author.

References

Altschul SF, Maden TL, Schaffer AA, Zhang J, Zhang Z, Miller W, Lipman DJ (1997) Gapped BLAST and PSI-BLAST: a new generation of protein database search program. Nucleic Acids Res 25:3389–3402

Ang EL, Zhao H, Obbard JP (2005) Recent advances in the bioremediation of persistent organic pollutants via biomolecular engineering. Enzyme Microbial Technol 37:487–496

Bhalerao TS, Puranik PR (2009) Microbial degradation of monocrotophos by *Aspergillus oryzae*. Int Biodeter Biodegradation 63:503–508

Bhattacharya D, Sarma PM, Krishnan S, Mishra S, Lal B (2003) Evaluation of genetic diversity among *Pseudomonas citronellolis* strains isolated from oily sludge-contaminated sites. Appl Environ Microbiol 69(3):1435–1441. https://doi.org/10.1128/AEM.69.3.1435-1441.2003

Booth C (1977) Laboratory guide to the identification of the major species. Commonwealth Mycological Institute, Kew Surry, pp 130–153

Buchanan RE, Gibbons NE (1974) Bergey's manual of determinative bacteriology, 8th edn. Williams and Wilkins, Baltimore, pp 747–829

Bujacz B, Wieczorek P, Krzysko-Lupicka T, Golab Z, Lejczak B, Kavafarski P (1995) Organophosphonate utilization by the wild-type strain of *Penicillium notatum*. Appl Environ Microbiol 61:2905–2910

Chaudhry GR, Ali AN, Wheeler WB (1988) Isolation of a methyl parathion degrading Pseudomonas sp. that possess DNA homologous to the opd gene from a *Flavobacterium* sp. Appl Environ Microbiol 54:288–293

Chiron S, Fernández-Alba AR, Rodriguez A, Garcia-Calvo E (2000) Pesticide chemical oxidaion: state-of-the-art. Water Res 34:366–377. https:// doi. org/10.1016/S0043-1354(99)00173-6

Clements FE, Shear JL (1957) The genera of fungi. Honfer Publishing Co, New York

Council directive 98/83/EC of 3 November on the quality of water intended for human consumption. Official Journal of the European Communities, L 330/32 (1998)

Dumas DP, Caldwell SR, Wild JR, Raushel FM (1989) Purification and properties of phosphotriesterase from *Pseudomonas diminuta*. J Biol Chem 264:19659–19665

Dykaar BB, Kitanidis PK (1996) Macrotransport of a biologicaly reacting solute through porous media. Water Resour Res 32(2):307–320

He D, Hao J, Zhang B, Yang Y, Song W, Zhang Y, Yokoyama K, Wang L (2011) Pathogenic spectrum of fungal keratitis and

specific identification of *Fusarium solani*. Invest Ophthalmol vis Sci 52(5):2804–2808
Holt JG, Krieg NR, Sneath PHA et al (1994) Bergey's manual of determinative bacteriology, 9th edn. Williams & Wilkins, Baltimore
Homa M, Galgóczy L, Manikandan P et al (2018) South Indian isolates of the *Fusarium solani* species complex from clinical and environmental samples: identification, antifungal susceptibilities, and virulence. Front Microbiol 2018(9):1052. https://doi.org/10.3389/fmicb.2018.01052
Karpouzas D, Morgan J, Walker A (2000) Isolation and characterization of 23 carbofuran-degrading bacteria from soils from distant geographical areas. Lett Appl Microbiol 31:353–358
Karpouzas DG, Morgan JAW, Walker A (2000) Isolation and characterization of ethoprophos-degrading bacteria. FEMS Microbiol Ecol 33:209–218
Kearney P, Wauchope R (1998) Disposal options based on properties of pesticides in soil and water. In: Kearney P, Roberts T (eds) Pesticide remediation in soils and water. Wiley Series in agrochemicals and plant protection
Ku Y, Lin HS (2002) Decomposition of phorate in aqueous solution by photolytic ozonation. Water Res 36(16):4155–4159
Ku Y, Chang JL, Shen YS, Lin SY (1998) Decomposition of diazinon in aqueous solution by ozonation. Water Res 32:1957–1963
Liu J, Wang L, Zheng Li, Wang X, Lee FSC (2006) Analysis of bacteria degradation products of methyl parathion by liquid chromatography/electrospray time-of-flight mass spectrometry and gas chromatography/mass spectrometry. J Chromatogr A 1137(2):180–187
Marinho G, Rodrigues K, Araujo R, Pinheiro ZB, Marinho Silva GM (2011) Glucose effect on degradation kinetics of methyl parathion by filamentous fungi species *Aspergilus niger* AN400. Eng Sanit Ambient 16(3):225–230
Misra D, Bhuyan S, Adhya TK, Sethunathan N (1992) Accelerated degradation of methyl parathion, parathion and fenitrothion by suspensions from methylparathion and p-nitrophenol-treated soil. Soil Biol Biochem 24:1035–1042
Moctezumaa E, Leyva E, Palestino G, de Lasa H (2007) Photocatalytic degradation of methyl parathion: reaction pathways and intermediate reaction products. J Photochem Photobiol A Chem 186(1):71–84
Nalim FA, Samuels GJ, Wijesundera RL, Geiser DM (2011) New species from the *Fusarium solani* species complex derived from perithecia and soil in the old world tropics. Mycologia 103:1302–1330
O'Donnell K (2000) Molecular phylogenys of the *Nectria haematococca–Fusarium solani* species complex. Mycologia 92:919–938
O'Donnell K, Kistler HC, Cigelnik E, Ploetz RC (1998) Multiple evolutionary origins of the fungus causing Panama disease of banana: concordant evidence from nuclear and mitochondrial gene genealogies. Proc Natl Acad Sci USA 95:2044–2049. https://doi.org/10.1073/pnas.95.5.2044
O'Donnell K, Sutton DA, Fothergill A, McCarthy D, Rinaldi MG, Brandt ME et al (2008) Molecular phylogenetic diversity, multilocus haplotype nomenclature, and in vitro antifungal resistance within the *Fusarium solani* species complex. J Clin Microbiol 46:2477–2490. https://doi.org/10.1128/JCM.02371-07
O'Donnell K, Sutton DA, Rinaldi MG, Sarver BA, Balajee SA, Schroers HJ, Summerbell RC, Robert VA, Crous PW, Zhang N, Aoki T, Jung K, Park J, Lee YH, Kang S, Park B, Geiser DM (2010) Internet-accessible DNA sequence database for identifying fusaria from human and animal infections. J Clin Microbiol 48(10):3708–3718
Pointing S (2001) Feasibility of bioremediation by white rot fungi. Appl Microbiol Biotechnol 57:20–33
Prenafeta Boldu FX (2002) Growth of fungi on aromatic hydrocarbons: environmental technology perspectives. Thesis, Wageningen University, The Netherlands
Quintás G, Noé AM, Armenta S, Garrigues S, Guardia MD (2004) Fourier transforms infrared spectrometric determination of Malathion in pesticide formulations. Anal Chim Acta 502:213–220
Ramanathan MP, Lalithakumari D (1999) Complete mineralization of methylparathion by *Pseudomonas* sp A3. Appl Biotechnol 80(1):1–12
Rani NL, Lalithakumari D (1994) Degradation of methylparathion by *Pseudomonas putida*. Can J Microbiol 40:1000–1006
Richins R, Kaneva I, Mulchandani A, Chen W (1997) Biodegradation of organophosphorus pesticides using surface expressed organophosphorus hydrolase. Nat Biotechnol 15:984–987
Sethunathan N, Yoshida T (1973) A Flavobacterium sp. that degrades diazinon and parathion. Can J Microbiol 19:873–875
Singh DK, Subhas (2002) Biodegradation of monocrotophos by two bacterial isolates. In: National conference on soil contamination and biodiversity, Lucknow, 8–10 February, 37
Singh BK, Walker A, Morgan AW, Denis J, Wright DJ (2004) Biodegradation of chlorpyrifos by *Enterobacter* strain b-14 and its use in bioremediation of contaminated soils. Appl Environ Microbiol 70:4855–4863
Stackebrandt E, Goebel BM (1994) Taxonomic note: a place for DNA-DNA reassociation and 16S rRNA sequence analysis in the present species definition in bacteriology. Int J Syst Bacteriol 44:846–849
Sureshkumar C, Anuradha CM, Dayananda S (1998) Detoxification of organophosphorus pesticides by bacterial phosphotriesterase. In: 39 th AMI conference proceedings, 5–7 December
Tamura K, Dudley J, Nei M, Kumar S (2007) MEGA4: molecular evolutionary genetics analysis (MEGA) software version 4.0. Mol Biol Evol 24:1596–1599
Tchelet R, Levanon D, Mingelgrin U, Henis Y (1993) Parathion degradation by a *Pseudomonas* sp. and a Xanthomonas sp. and by their crude enzyme extracts as affected by some cations. Soil Biol Biochem 25:1665–1671
Timmis KN, Pieper DH (1999) Bacteria designed for bioremediation. Trends in Biotechnol 17:201–204
Usharani K, Lakshmanaperumalsamy P (2016) Box-Behnken experimental design mediated optimization of aqueous methyparathion biodegradation by *Pseudomonas aeruginosa* MPD strain. J Microbiol Biotechnol Food Sci 5(6):534–547. https://doi.org/10.15414/jmbfs.2016.5.6.534-547
Usharani K, Muthukumar M (2013) Optimization of aqueous methylparathion biodegradation by *Fusarium* sp in batch scale process using response surface methodology. Int J Environ Sci Technol 10:591–606. https://doi.org/10.1007/s13762-012-0144-5
Usharani K (2013) Biotreatment and ozonation process for the efficient removal of aqueous methylparathion an endocrine disruptor. PhD Thesis, Bharathiar University, Coimbatore
Witteveen Cor FB (1993) Gluconato formation and polyol metabolism in *Aspergillus niger*. Thesis Wageningen University, Wagenningen
Yali C, Ruifu Z, Jian H, Shunpeng L (2002) Study on *Pseudomonas* sp. WBC-3 capable of complete degradation of methyl parathion. Weishengwu Xuebao 42:490–497
Zhang N, O'Donnell K, Sutton DA, Nalim FA, Summerbell RC, Padhye AA et al (2006) Members of the *Fusarium solani* species complex that cause infections in both humans and plants are common in the environment. J Clin Microbiol 44:2186–2190. https://doi.org/10.1128/JCM.00120-06
Zhongli C, Shunpeng L, Guoping F (2002) Isolation and characterization of a p-nitrophenol degradation *Pseudomonas* sp. strain p3 and construction of a genetically engineered bacterium. Weishengwu Xuebao 42:19–26