Desalination and Water Treatment
www.deswater.com
doi: 10.5004/dwt.2018.21795

102 (2018) 349–358
January

Kinetic modeling and metabolite identification of dimethyl phthalate biodegradation by *Bacillus* sp. KS1 isolated from municipal wastewater contaminated soil

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Received 9 June 2017; Accepted 9 December 2017

ABSTRACT

Phthalate esters (PEs) have engrossed attention and intensified environmental concern owing to their widespread applications. In the present study, dimethyl phthalate (DMP), a low molecular weight PE, degrading aerobic bacterium was isolated from soil contaminated with municipal wastewater. Based on morphological, biochemical characteristics and 16S rRNA sequencing, the isolate was identified as *Bacillus* sp. KS1. Complete degradation up to 1,400 mg/L of DMP was observed within 168 h. At low concentrations (25–100 mg/L) of DMP, degradation data fitted well with the first-order kinetics, and the half-life was observed as 3 h. Growth kinetic study of the high concentrations of DMP (0–1,400 mg/L) was performed by using various kinetic models where Haldane model was found to fit well with the experimental data (R^2 = 0.9210). Analysis of DMP degradation residual by high-performance liquid chromatography and electrospray ionization-mass spectrometry revealed the presence of phthalic acid as the DMP degradation metabolite. The results obtained in the present study will be significant for exploring an application of *Bacillus* sp. KS1 for bioremediation of PEs.

Keywords: Dimethyl phthalate; Municipal wastewater; Biodegradation; 16S rRNA sequencing; *Bacillus* sp.; Haldane model; Phthalic acid

1. Introduction

Phthalate esters (PEs) are alkyl esters of phthalic acid (PA), mainly used in plastic products to enhance their properties along with wide applications in the variety of products such as pharmaceutical products, detergents, toys, packaging, adhesives, cosmetics, paints, medical devices, and pesticides [1]. Dimethyl phthalate (DMP) is a low molecular weight PE used in plastics, solid rocket propellants, insect repellents, lacquers, rubbers, cosmetics, etc. DMP is not chemically bound to the plastic product matrix, and it can leach into the environment through municipal and industrial wastewater, sewage sludge, and various consumer products [2]. Because of their low water solubility, DMP has become widespread in the environment and found in natural waters, soils, sediments, and air [3]. Exposure to DMP may occur from food, drinking water, polyvinylchloride bags, etc. [4]. Acute exposure to DMP, via inhalation in humans and animals, results in irritation of the eyes, nose, and throat. It is a subchronic toxicant and an endocrine disrupting chemical that decreases fertility in males and females [5]. U.S. Environmental Protection Agency listed DMP as a hazardous environmental priority pollutant in-group D [4].

The chemical structure of PEs restricts natural remediation processes. On the other hand, biodegradation is a critical

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process in which microorganisms utilize PEs as part of their metabolism for energy and growth, and it affects their environmental fate [3]. The widespread usages and low solubility in water ensure the presence of PEs in significant amount in the municipal wastewater discharge area [6]. The microbial communities existing at the sites contaminated with municipal wastewater might be able to tolerate efficiently various concentrations of PEs. Bacteria isolated from different environment exhibits dissimilarity in degradation efficiency and the tolerance potentiality toward the same substrate. Hence, soil at these sites can be a possible source of isolating highly efficient PEs degrading bacteria.

Evaluation of the biodegradation kinetics of substrate is imperative to understand the degradation capacities of the microorganisms and for the operation of biological reactors. The choice of an appropriate degradation kinetics model has a crucial role in degradation study [7]. At a relatively low concentration and without inhibition, first-order kinetic model and Monod kinetics can describe well the PEs degradation. If the substrate causes inhibition at high concentrations, there is a necessity to identify inhibition coefficient. Haldane model, Aiba model, Yano model, Edward model, etc. are mainly reported as inhibition kinetic models [8]. Among them, Haldane and Aiba models are extensively used for the kinetic study of inhibiting substrate. Wen et al. [9] studied the biodegradation kinetics of dibutyl phthalate (DBP), by *Arthrobacter* sp. C21, in batch mode, using first-order kinetics and Haldane model. Xu et al. [10] studied the biodegradation kinetics of *n*-butyl benzyl phthalate by *Pseudomonas fluorescens* B-1. They have fitted biodegradation of *n*-butyl benzyl phthalate to first-order kinetics. However, there is no report available on the growth kinetic study of bacteria showing DMP degradation using kinetic models such as Monod, Haldane, and Aiba models.

The objectives of the work are to isolate, identify, and characterize DMP degrading bacterial strain, to explore the performance of bacterial strain on degradation of DMP through kinetic study and to obtain a mechanism for DMP degradation with identification of metabolites.

2. Materials and methods

2.1. Chemicals and preparation of medium

The standard chemicals including DMP and PA with >99% purity were obtained from Sigma-Aldrich Chemicals (India). The solvent *n*-hexane and methanol used for extraction were of high-performance liquid chromatography (HPLC) grade (>99.5% purity) and purchased from the Fischer Scientific (India). The rest of the chemicals and reagents used in this research were obtained from Fischer Scientific and Merck (India). Nutrient agar and nutrient broth were used for enrichment of bacterial strains. The mineral salt medium (MSM) contained (g/L): $(NH_4)_2SO_4$ 4, KH_2PO_4 4, Na_2HPO_4 6, $MgSO_4.7H_2O$ 0.2, yeast extract 0.1, $CaCl_2.H_2O$ 0.01, $FeSO_4.7H_2O$ 0.01 (pH 7.01) [11]. All the media were sterilized by autoclaving at 121°C for 15 min.

2.2. Soil sample collection

Soil samples for isolation of bacteria were collected from municipal wastewater discharge site at Koel River, Rourkela (Odisha). The top layer of soil was removed up to 1–2 cm, and the sterile scoops were used for collection of soil samples. Four subsamples were taken from each point and mixed in sterile plastic bags. Soil samples were stored at ambient temperature during traveling and at 4°C before use.

2.3. Enrichment and acclimatization of DMP degrading bacterial strain

The method exercised for an enrichment of bacterial strain was similar to Patil and Jena [12] with slight modification. Soil sample (5 g) was added into the 100 mL nutrient broth containing 30 mg/L of a mixture of phthalates, that is, an equal amount of mixture of phthalates (DMP, diethyl phthalate [DEP], and DBP) and was incubated in 250 mL flasks at 30°C and 140 rpm for 48 h. After incubation, soil particles were allowed to settle, and 5 mL of particle free sample was inoculated in 100 mL MSM supplemented with 30 mg/L mixture of phthalates and incubated at 30°C for 48 h at 140 rpm. The same procedure was repeated for 2 months with increasing phthalates concentration up to 300 mg/L to get acclimatized microbial consortium. The acclimatized samples were diluted and transferred to sterile Petri plates containing MSM with the mixture of phthalates as a carbon source. After incubation, morphologically distinct colonies were selected for a further screening study.

2.4. Screening of DMP degrading bacteria

All isolates obtained after enrichment were independently inoculated in MSM containing 100 mg/L of DMP as a sole source of carbon and incubated at 30°C and 140 rpm for 48 h. The same procedure was repeated for individual strains with an increment of DMP by 50 mg/L to get dominant bacterium. The DMP degradation capability of each bacterium was confirmed by observing turbidity. Among them, the strain (KS1) showed tolerance at high concentrations of DMP, was selected as a subject of the present study. The isolate was purified by repeated streaking on MSM. It was subcultured at an interval of every 15 d and stored at 4°C.

2.5. Morphological and biochemical characterization

The strain KS1 was examined for colony morphology: size, shape, color, margin, opacity, elevation, and textures. Motility test, Gram staining, catalase test, oxidase test, and other biochemical tests were performed for morphological and biochemical characterization. For analyzing cell shape, scanning electron microscopy (SEM) was performed using scanning electron microscope (JEOL, Japan). In SEM analysis, the microorganism was fixed to glass slides with the help of glutaraldehyde fixation method under subsequent drying with increasing concentration of ethanol. The magnification of the microscope was 5,000×.

2.6. Sequencing of 16S rRNA and phylogenetic analysis

The strain KS1 was cultured in LB medium for 24 h, and its genomic DNA was extracted by Qiagen DNA extract kit (Qiagen, India). The 16S rRNA gene fragment was amplified from the genomic DNA by polymerase chain reaction (PCR) using 16S rRNA gene universal

primers: 8F 5′-AGAGTTTGATCCTGGCTCAG-3′ and 1492R 5′-ACGGCTACCTTGTTACGACTT-3′. The purified PCR product was subjected to automated DNA sequencing on ABI 3730xl Genetic Analyzer (Applied Biosystems, USA). The similarity of 16S rDNA gene sequence was determined by Basic Local Alignment Search Tool (BLAST) with the nucleotide database of National Centre for Biotechnology Information Databases (NCBI). Based on the maximum identity score and query coverage, the best highly matching 10 sequences were selected and aligned using multiple alignment software program ClustalW (MEGA4 tool). Phylogenetic analysis was exercised in MEGA software version 4 using the neighbor-joining method. The obtained 16s rRNA gene sequence was registered in nucleotide database of NCBI with GenBank accession number KY214478.

2.7. Batch DMP biodegradation

Degradation of DMP was performed at various DMP concentrations in liquid culture. For each experiment, the freshly prepared inoculum was prepared in nutrient broth, and was incubated for 24 h at 28.27°C and 140 rpm. The cells were harvested, washed with phosphate buffer (pH 7.0), and subsequently inoculated in the 250 ml Erlenmeyer flask containing 100 mL of MSM containing initial low and high DMP concentrations of 25–100 and 250–14,00 mg/L, respectively. The flasks were incubated at 30°C and 140 rpm until complete degradation of DMP. Each experiment was conducted in triplicate under the same operating conditions, and average values of percentage degradation of three independent experiments were reported. The reaction mixture containing all media components except bacterial inoculums were used as a control. The culture samples were withdrawn at intervals (6 h) and centrifuged at 8,000 rpm for 10 min. The pellet was taken for estimation of biomass, and the supernatant was used for estimation of DMP. For biomass determination, the pellet suspended in distilled water was analyzed against a reference (distilled water) at 600 nm using a UV–visible spectrophotometer (Jasco V-530, Japan).

2.8. Analysis of DMP biodegradation

For analysis of DMP degradation metabolites, samples from biodegradation experiments carried at 500 mg/L of DMP were centrifuged at 8,000 rpm for 10 min to remove biomass. The supernatant was further extracted using *n*-hexane, and subsequently evaporated to dryness at 30°C. The dried samples were mixed with methanol and filtered through a syringe filter (pore size 0.22 μm). The samples were analyzed by Agilent HPLC 1200 Infinity series (Agilent Technologies, Hewlett-Packard, California), equipped with a Quaternary pump (G1311-90015), a diode array detector (DAD) (G4212-90013), and an auto sampler (G1329-90015). An Eclipse plus C18 column (5 μm particle size, 250 mm × 4.6 mm i.d.) was used with a mobile phase consisting of methanol/water (80:20, v/v) at a flow rate of 1.0 mL/min and an injection volume of 20 μL was used. Limit of quantitation was 1 μg/L and limit of detection was 0.02–0.03 μg/L. DAD was set at 275 nm (primary wavelength) and 280 nm (secondary wavelength). Calibration plot for DMP was prepared for the desired concentration range (10–1,500 mg/L). The degradation samples (1 mL) were also subjected to electrospray ionization-mass spectrometry (ESI-MS; Perkin Elmer, USA). The ESI-MS spectra were recorded in the positive electron ionization mode. The drying gas temperature was 300°C with capillary exit voltage of 100 V. The mass range was 120–400 μm and pulse counting was 100 μs with coarse resolution 10.9.

2.9. DMP biodegradation kinetics

The relationship between the specific growth rate (μ) and substrate concentration (*S*) are essential to be quantified to design and operate efficient biological waste treatment [12]. The first-order kinetic model was frequently used to define biodegradation process at low substrate concentrations [13]. In the present study, first-order kinetic model was employed to fit the experimental data obtained from DMP degradation at a low concentration range (25–100 mg/L). The first-order kinetic equation can be represented as:

$$\ln C = -Kt + A \tag{1}$$

Specific growth rate of the culture at different substrate concentrations was calculated as per the following relationship:

$$\mu = \frac{1}{X}\frac{dX}{dt} \tag{2}$$

where μ is specific growth rate (h^{-1}), *X* is biomass concentration (mg/L), and *t* is time (h). Similarly, experimental data for DMP degradation in the concentration range (0–1,400 mg/L) were fitted to Monod, Haldane, and Aiba models. To characterize non-inhibition characteristics of DMP biodegradation, Monod model was used, and it is represented as follows:

$$\mu = \frac{\mu_{max} S}{K_s + S} \tag{3}$$

where μ_{max} is maximum specific growth rate (h^{-1}), K_s is half-saturation coefficient (mg/L). DMP acts as inhibitory compound at high concentrations. Haldane model is mathematically simple and one of the best models to describe the DMP degradation behavior [9]. Hence, Haldane model was used for the study of growth kinetics of isolated strain in DMP degradation. The Haldane model equation relates microbial specific growth rate (μ) and limiting substrate concentration (*S*) as follows:

$$\mu = \frac{\mu_{max} S}{K_s + S + \frac{S^2}{K_i}} \tag{4}$$

where K_i is the substrate inhibition constant (mg/L). Aiba et al. [14] proposed a model for the inhibitory kinetics of process, Edwards [15] further modified it, and it is represented as follows:

$$\mu = \frac{\mu_{max} S}{K_s + S} \exp\frac{-S}{K_i} \tag{5}$$

The biokinetic parameters μ_{max}, K_i, and K_s for the isolate were estimated by fitting their respective experimental growth data to kinetic models. These models except first-order kinetics were solved by the use of a non-linear regression method using computer software (MATLAB V 8.5.1).

3. Results and discussion

3.1. Isolation of DMP degrading bacterial strain

After enrichment of soil sample, seven isolates were obtained. One strain showed promising DMP degradation efficiency on subsequent screening of these strains. It was considered as the subject of the current study and was designated as a strain KS1.

3.2. Identification and characterization of DMP degrading bacteria

Morphological characteristics of strain KS1 were observed by spread plate technique. Fig. 1 shows the colony morphology and SEM image of the strain KS1. The colony morphology of the strain was irregular, undulate, and slight yellowish on nutrient agar plates. The morphological examination under the SEM showed rod-shaped bacillus. Morphological and biochemical characteristics are enlisted in Table 1. The isolate was identified as a gram-positive motile, aerobic bacterium. The positive results were observed for catalase, fructose and lactose fermentation, oxidase, citrate, and methyl red tests. It showed negative results for nitrate reduction, indole, urease, glucose fermentation, gelatin liquefaction, Voges–Proskauer, and starch hydrolysis tests.

For molecular characterization, 16S rDNA was sequenced, and BLAST was performed with GenBank database to compare with existing sequences. The phylogenetic tree showed that isolate belongs to the *Bacillus* genus, as it was close to *Bacillus* sp. EB435 (GenBank Accession Number: FJ785493.1) (Fig. 2).

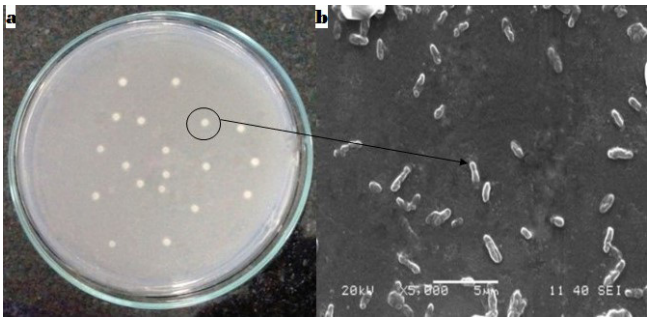

Fig. 1. Colonies of the bacterial isolate cultured on a nutrient agar plate (a) and magnified image (5,000×) under SEM (b) of *Bacillus* sp. KS1.

Table 1
Morphological and biochemical characteristics of isolate

Characteristics	Strain KS1
Size	1–2 mm
Shape	Irregular
Color	Slight yellowish
Margin	Undulate
Opacity	Opaque
Elevation	Convex
Textures	Viscous
Grams nature	Gram positive
Motility	Motile
Oxygen requirement	Aerobic
Catalase test	+
Oxidase test	+
Nitrate reduction	–
Indole test	–
Glucose fermentation	–
Fructose fermentation	+
Lactose fermentation	+
Urease test	–
Citrate test	+
Gelatin liquefaction	+
Starch hydrolysis	–
Methyl red test	+
Voges–Proskauer test	–

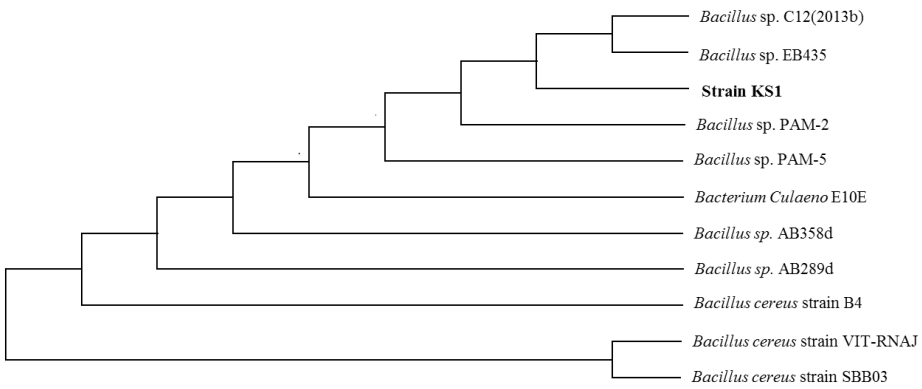

Fig. 2. Phylogenetic tree for *Bacillus* sp. KS1.

3.3. DMP biodegradation

The growth and degradation profile for strain KS1 were studied at various initial DMP concentrations at optimized conditions. Fig. 3(a) shows growth profile of strain KS1 at initial low DMP concentrations (25–100 mg/L). Isolate with no lag phase was observed which indicates non-inhibition of DMP at these concentrations. Fig. 3(b) shows degradation profile for low concentrations (25–100 mg/L) where complete degradation of the DMP was documented without any inhibition at these concentrations. Lu et al. [16] isolated an aerobic bacterial strain *Rhodococcus ruber* capable of utilizing DMP and observed that isolate could partially degrade up to 200 mg/L of DMP within 6 d without a lag phase.

Fig. 4(a) represents growth profile of strain KS1 at initial high DMP concentrations (250–1,400 mg/L). A short lag phase was observed at 250 mg/L and thereafter a prominent lag phase was noted whose duration was increased with increase in concentration. At high concentration (1,400 mg/L) of DMP, a lag phase of 36 h duration was observed that could be attributed to an inhibitory effect of DMP. Fig. 4(b) shows degradation profile for high concentrations (250–1,400 mg/L). It showed complete degradation of 250, 500, 750, 1,000, 1,250, and 1,400 mg/L of DMP within 36, 60, 96, 132, 156, and 168 h, respectively. At 250 mg/L of DMP, high degradation rate was observed

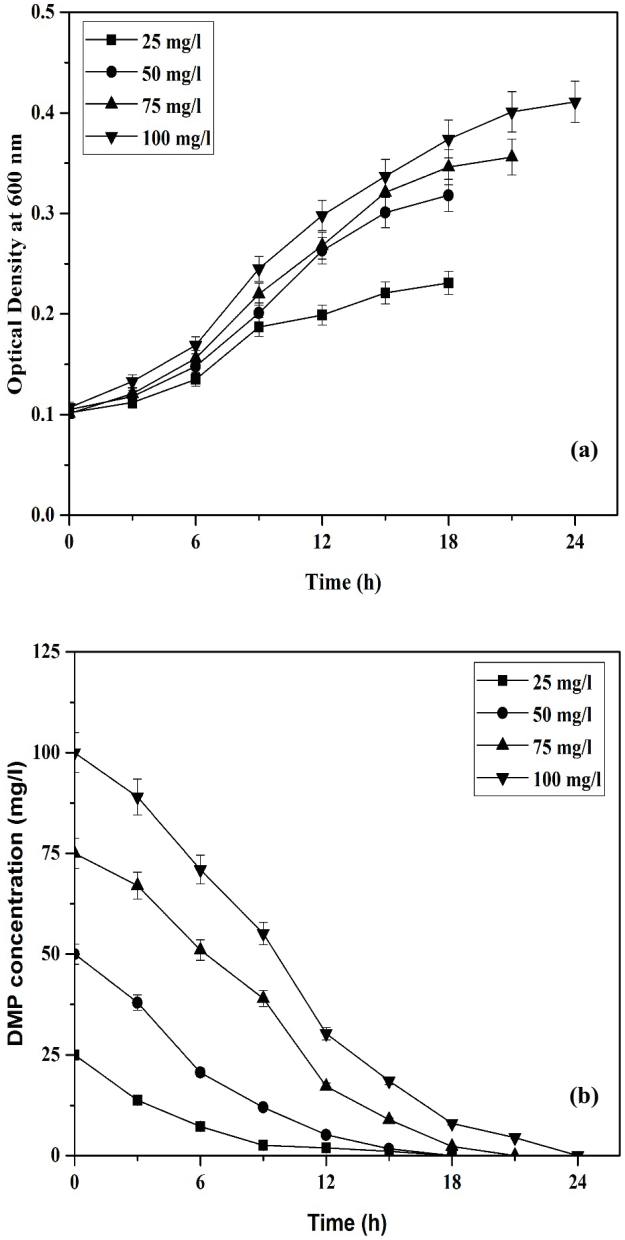

Fig. 3. Growth profile (a) and DMP degradation profile (b) for *Bacillus* sp. KS1 at various initial low DMP concentrations.

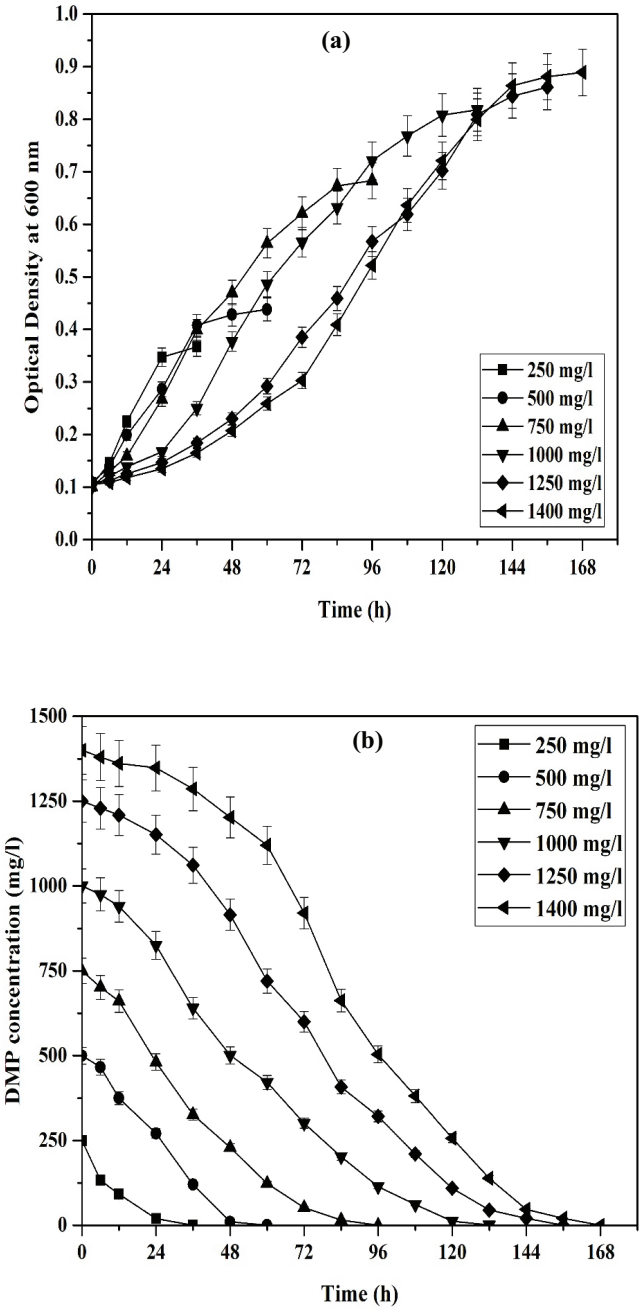

Fig. 4. Growth profile (a) and DMP degradation profile (b) for *Bacillus* sp. KS1 at various initial high DMP concentrations.

that indicated no inhibition at this concentration. Prasad and Suresh [17] investigated biodegradation of DMP by a bacterial strain *Variovorax* sp. isolated from garbage-dumped soil. They observed that isolate degrade >99% of 300 mg/L of DMP within 30 h with no inhibition. It was observed that degradation rate was increased after the lag phase for each concentration studied. In addition, as concentration was increased, degradation rate decreased indicating that the initial concentration of DMP affects the biodegradability of DMP. Zeng et al. [13] studied the biodegradation of DMP by *P. fluorescens* FS1 and observed that the biodegradation rate decreases and inhibition effect increase significantly with increasing concentration of substrate. Jin et al. [18] also observed similar trend for the biodegradation of one of the commonly used phthalates (DBP) by *Gordonia* sp. strain QH-11. As the lag period was increased from 6.44 to 17.51 h, the DBP concentrations augmented from 100 to 750 mg/L. It is evident from these reports that cell growth is inhibited by dialkyl phthalates at high concentration, and this demonstrates that DMP is likely to inhibit microbial growth at high concentrations.

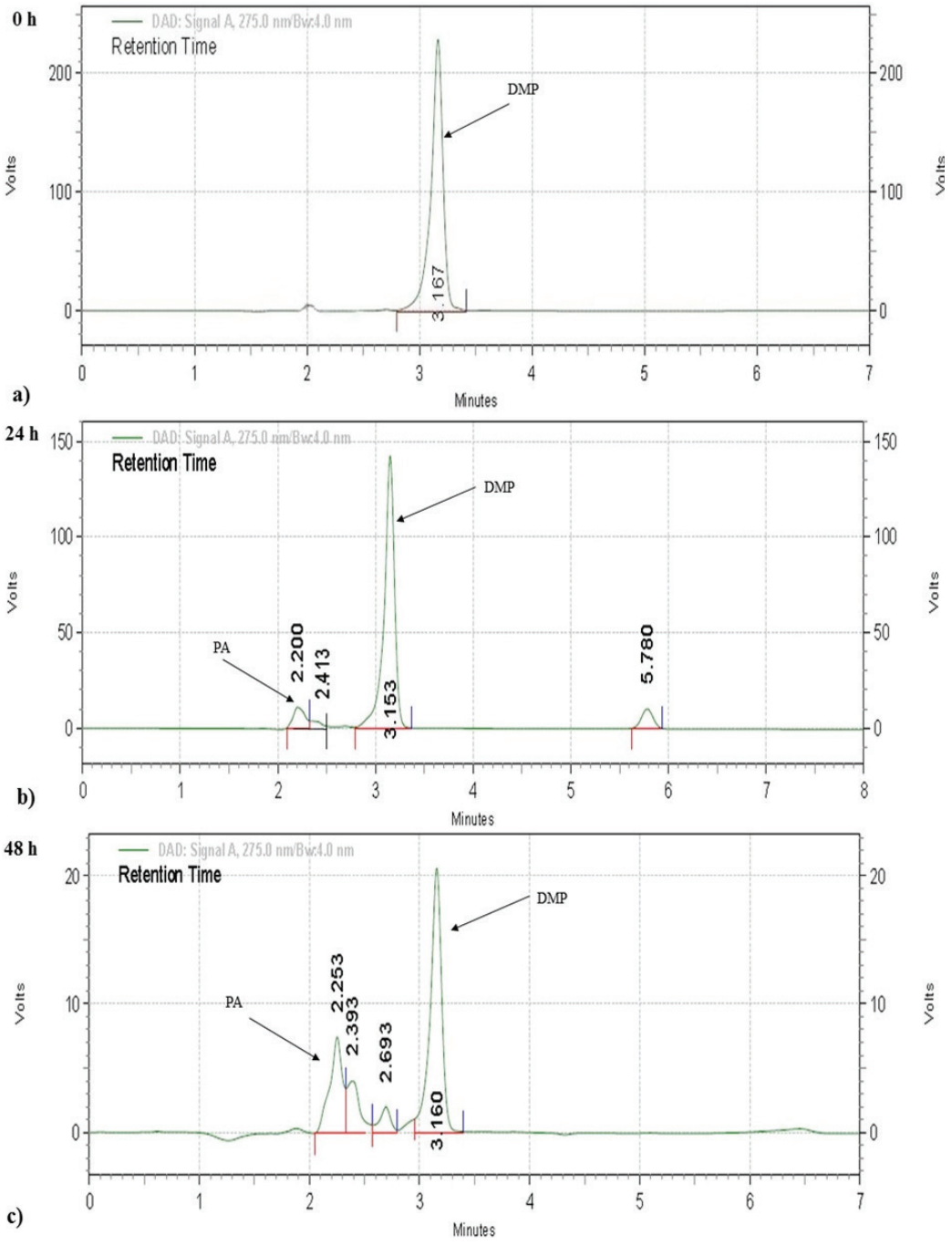

Fig. 5. HPLC chromatogram for detection of DMP and its degradation intermediates at various time intervals by *Bacillus* sp. KS1.

3.4. Metabolite identification of DMP biodegradation

HPLC analysis of standards of DMP and PA showed their retention time at 3.17 and 2.20 min, respectively. Fig. 5 shows HPLC analysis of degradation samples collected at 0, 24, and 48 h. Fig. 5(a) shows 0 h sample analysis, and it indicates the peak of DMP at 3.17 min retention time. Fig. 5(b) shows the peak for DMP and PA at 3.17 and 2.22 min, respectively, for degradation sample collected at 24 h. The analysis of a sample at 48 h shows that peaks for both the compounds with their reduced concentrations indicating their degradation (Fig. 5(c)). Fig. 6 shows the area under the observed peaks, Peak A (2.2 min), Peak B (2.39 min), Peak C (2.69 min), and Peak D (3.16 min) for 48 h sample. No peak of PA was detected in an uninoculated DMP containing MSM. Wang et al. [19] studied biodegradation of DMP by *Burkholderia cepacia* DA2. They have observed PA as a metabolite of DMP biodegradation using HPLC analysis.

ESI-MS spectra for 0, 24, and 48 h samples shown in Fig. 7. Fig. 7(a) shows analysis of 0 h sample and DMP along with the solvent peak was observed. Fig. 7(b) shows ESI-MS spectra for 24 h degradation sample. Based on the molar mass of the standard compound and ion paring (Na^+), the peak at *m*/*z* (mass to charge ratio) 217.08 was found to match with DMP (molar mass: 194.184 g/mol). PA was detected at *m*/*z* 165.06. Fig. 7(c) also shows peaks for DMP and PA. Gu et al. [20] studied biodegradation of DMP by *Sphingomonas yanoikuyae* DOS01 and reported that the biochemical pathway of DMP degradation involve desertification of DMP to intermediate MMP, and then hydrolysis to PA before the further aromatic ring cleavage. Pranaw et al. [21] investigated biodegradation of DMP by bacteria *Xenorhabdus indica* and observed that the DMP was hydrolyzed to PA. The mass spectrum and HPLC chromatogram of the intermediate product matched that of a standard sample of PA. Thus, HPLC and ESI-MS analyses indicate that *Bacillus* sp. KS1 hydrolyzed DMP to PA. In the present investigation, an occurrence of only PA was confirmed as a DMP degradation intermediate. Similar type of results has been reported earlier in the literature where only PA was found as a metabolite of PEs biodegradation [22].

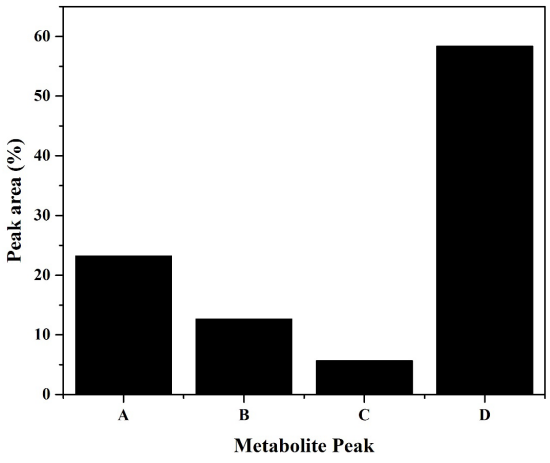

Fig. 6. Bar graph of peak area for HPLC analysis [Peak A (2.2 min), Peak B (2.39 min), Peak C (2.69 min), and Peak D (3.16 min)].

3.5. Biodegradation kinetic analysis

Study of growth of microbes and estimation of growth kinetic parameters has high importance in biodegradation study. In the present study, kinetic study for isolate was performed at low as well as high concentrations of DMP. At low concentrations of DMP, growth data was fitted with first-order kinetics. It was observed a half-life of 3 h (Table 2). Various researchers reported DMP biodegradation by pure strains of microorganisms with different capacities. Zeng et al. [13] observed that the first-order model fit with growth data under the experimental initial concentrations for the biodegradation kinetics of DMP, along with other phthalates such as DEP, di-*n*-butyl phthalate, di-isobutyl phthalate, di-*n*-octylphthalate, and di(2-ethylhexyl)phthalate by *P. fluorescens* FS1. They found that at the initial concentrations of DMP, less than 200 mg/L, the rate constants (*K*) were independent of initial concentrations. They also observed that half-life for DMP degradation varied from 6 to 10 h. Liang et al. [23] studied biodegradation of commonly used dialkyl phthalate, DBP and observed that complete degradation of 20 mg/L by *Acinetobacter lwoffii* in 5 d and of 100 mg/L by *Rhodococcus* in 2 d. Li et al. [24] also observed that, as the DBP concentration was less than 100 mg/L; the half-life of DBP was about 1.35 d (34 h) by *Serratia marcescens* C9. The half-life (3 h) for low DMP concentrations is quite lower as compared with available literature indicates *Bacillus* sp. KS1 is active and efficient in DMP degradation.

For high concentrations, the kinetic parameters were obtained for DMP degradation by isolated *Bacillus* sp. KS1 by fitting experimental growth data to kinetic model equations (Eqs. (2)–(5)). Fig. 8 shows the specific growth rate for various initial DMP concentrations. This figure indicates that the value of specific growth rate increases with the increase in initial DMP concentration to 341.8 mg/L, then it starts decreasing with further increase in the concentration. The maximum specific growth rate (μ_{max}), half-saturation coefficient (K_s), and substrate inhibition constant (K_i) were determined for the initial DMP concentration range of 0–1,400 mg/L, and tabulated in Table 3. The coefficient of correlation (R^2) was found to be 0.4914, 0.7427, and 0.9210 for Monod, Aiba–Edward, and Haldane models, respectively. It indicates that experimental data fits well for Haldane model as compared with other models. The K_s value refers to the minimum substrate concentration that microorganism can utilize for growth. The low μ_{max} value might be due to the higher DMP concentration used in the batch biodegradation study. Substrate inhibition constant (K_i) was estimated as 422.12 and 470.96 mg/L for Aiba–Edward and Haldane models, respectively. It indicates *Bacillus* sp. KS1 have high tolerance toward DMP. Zeng et al. [13] reported that DMP degradation rate decreases along with growth rate with increasing concentration. Previously, Wen et al. [9] also studied the kinetics of phthalates degradation by using Haldane model and reported the inhibitory effect of common phthalate (DBP) at high initial concentrations. There is no report available on the application of either of the Monod, Aiba–Edward, and Haldane models for biodegradation of DMP. Thus, the present study is the first to report the DMP degradation kinetics using these models, and Haldane model explained well the inhibition effect of DMP at high concentrations.

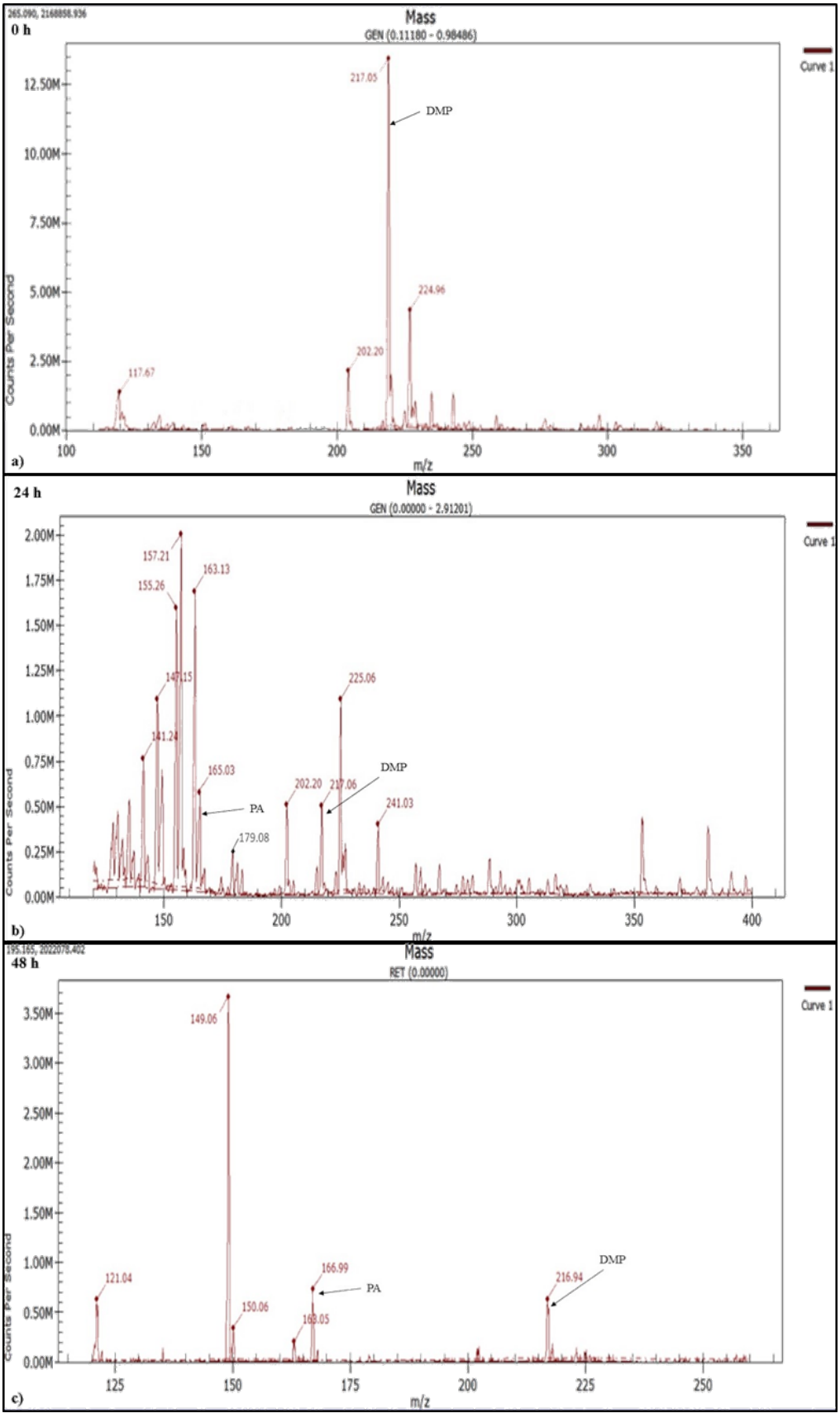

Fig. 7. ESI-MS spectra for detection DMP degradation metabolites at various time intervals by *Bacillus* sp. KS1.

Table 2
First-order kinetic equation for DMP biodegradation at low concentrations (<100 mg/L)

Initial DMP concentration (mg/L)	Kinetic equation	Half-life (h)	Correlation coefficient (R^2)
25	ln*C* = –0.1917 + 3.0736	3.615	0.9595
50	ln*C* = –0.2300 + 4.2433	3.01	0.9750
75	ln*C* = –0.2107 + 4.9558	3.2897	0.9149
100	ln*C* = –0.1822 + 5.2149	3.8043	0.9133

Table 3
Biokinetic constants

Strain	Model	μ_{max} (h^{-1})	K_s (mg/L)	K_i (mg/L)	Correlation coefficient (R^2)
Bacillus sp. KS1	Monod model	0.0307	20.02	–	0.4914
	Aiba–Edward model	0.0649	18.45	422.12	0.7427
	Haldane model	0.0602	29.43	470.96	0.9210

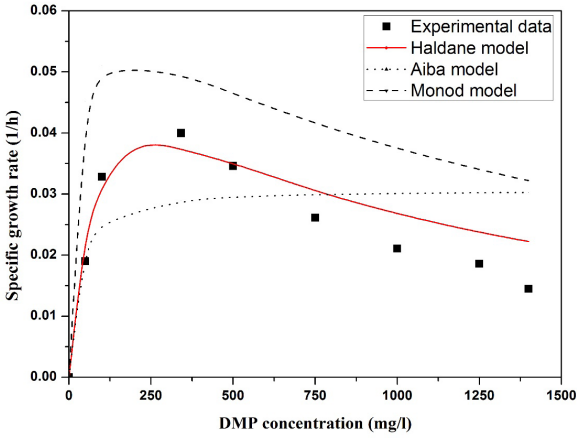

Fig. 8. Experimental and model predicted specific growth rates.

4. Conclusion

An efficient DMP degrading strain was isolated from enriched soil collected from municipal wastewater discharge site. The morphological and biochemical characteristics and 16s rDNA sequence indicated that the isolate belongs to the genus *Bacillus*. It utilized DMP as a sole source of carbon and showed complete degradation up to 1,400 mg/L of DMP concentrations. The degradation of low DMP concentration was observed to follow first-order kinetics. Haldane model fitted well for degradation profile at high concentrations, and strain KS1 exhibited high substrate inhibition constant. Thus, *Bacillus* sp. KS1 demonstrated better DMP degradation profile with high tolerance. Thus, it can be concluded that the evaluated biokinetic constants and observed degradation characteristics are essential to design a bioreactor treating DMP containing wastewater by *Bacillus* sp. KS1.

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