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Biodegradation of toxic organic compounds using a newly isolated *Bacillus* sp. CYR2

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Abstract

The objective of this study was to isolate a new bacterium and investigate its ability for degradation of various toxic organic compounds. Based on 16S rRNA gene sequence and phylogenetic analysis, the isolated strain was identified as *Bacillus* sp. CYR2. Degradation of various toxic compounds and growth of CYR2 strain were evaluated with 2% and 4% inoculum sizes. All the experiments were conducted for 6 days, flasks were incubated at 30 ^{O}C under 180 rpm. Among the 2% and 4% inoculum sizes, bacteria showed highest growth and toxic compounds degradation at 4% inoculum size. Especially, compared to 2% inoculum size, growth of the strain CYR2 at 4% inoculum size was increased by 15.3 folds with phenanthrene, 15.1 folds with 4-secondary-butylphenol, 13.6 folds with naphthalene, 9.1 folds with phenol, and 5.4 folds with 4-tertiary-butylphenol. Strain CYR2 at 4% inoculum size showed highest removal of phenol (84±5%), followed by 4-tertiary-butylphenol (66±3%), 4-secondary-butylphenol (63±5%) and 4-nonylphenol (57±6%). Compared with 2% inoculum size, degradation ability of strain CYR2 with 4% inoculum size was enhanced by 3.45 times with 4-tertiary-octylphenol, and 2.53 times with 4-tertiary-butylphenol. Our results indicated that the newly isolated *Bacillus* sp. CYR2 can be used for in situ bioremediation of phenol and alkylphenols contaminated water.

Keywords: *Bacillus* sp. CYR2; alkylphenols; phenol; naphthalene.

Abbreviations: MSM, mineral salt medium; OD, optical density; PAHs, poly-aromatic hydrocarbons; 4-BP, 4-butylphenol; 4-s-BP, 4-secondary-butylphenol; 4-t-BP, 4-tertiary-butylphenol; 4-t-OP, 4-tertiary-octylphenol; 4-NP, 4-nonylphenol.

1. INTRODUCTION

Phenol is a high volume chemical with worldwide production of more than 6 billion pounds, and ranks in the top 50 for chemicals produced in the United States. Manufacture of phenolic resins is the largest use of phenol. Phenol and its derivatives are widely distributed as environmental pollutants [1]. They are released as waste material by various industrial facilities such as oil refineries, coking plants, pharmaceuticals, pesticides, and leather manufactures [2]. Due to its high water solubility, phenol present in wastewaters easily reaches downstream water sources and may be harmful to life in aquatic environments [3]. Biodegradation of phenols, as well as many petroleum products by microorganisms, represents the primary way by which these compounds are removed from contaminated sites. Diverse microorganisms like bacteria, yeasts, algae, and filamentous fungi have the metabolic capability to degrade phenols [2].

Alkylphenols are prevalent groundwater contaminants, which are human carcinogens, mutagens, and toxins. Alkylphenols are widely used as raw material for alkylphenol ethoxylates (APEOs) and in the preparation of phenolic resins, polymers, antioxidants and have variable charge properties under different conditions [4-8]. Major part of APEOs is discharged into the aquatic environment through domestic sewage [9]. APEOs are rickety in aquatic environments with several days of half-life and degrade into alkylphenols [9]. Though, the degradation products alkylphenols are more stable with a half-life of some decades [9]. Due to the toxic nature of nonylphenol and octylphenol, the use of such compounds have firmly monitored in many countries. But up to now, alkylphenols are used in China and the Korean peninsula. Yellow Sea and the East China Sea are revealed as imperative sinks of pollutants [9]. 4-tertiary-octylphenol is the most effective alkylphenol, it causes damage to sexual and reproductive development systems [10]. Human beings are mostly exposed to 4-tertiary-octylphenol by taking polluted foods [10]. The US Environmental Protection Agency (USEPA) established standards about alkylphenols concentrations in water. Hence, the effluents

containing alkylphenols must be appropriately treated. Though, alkylphenols are persistent in the environment due to their resistance to usual degradation. Alkylphenols are categorized into three major groups based on the carbon number *i.e.,* short chain (1 to 2), medium chain (3 to 7) and long chain (8 to12) [11].

Toxic nature of poly-aromatic hydrocarbons (PAHs) gain interest in placing efforts to eliminate PAHs from the environment [12]. PAHs are organic compounds that consist of two or more aromatic rings in their structure. The USEPA has identified 16 PAH compounds as the priority pollutants [12]. Duarte et al. (2017) used a culture-independent screening approach to analyse the aerobic microbial catabolome for PAHs degradation of a soil subjected to 12 years of in situ bioremediation [13]. A total of 422,750 fosmid clones were screened for key aromatic ring-cleavage activities using 2,3-dihydroxybiphenyl as substrate. High abundance of Rieske non-heme iron oxygenases were identified. Martinkosky et al. (2017) reported that earthworms accelerate bioremediation of crude oil in soils, including the degradation of the heaviest polyaromatic fractions [14]. Jeon et al., (2017), reported that PAHs and alkylphenols are extensively dispersed in sediments and have been recognized as major contaminants along the west coast of South Korea [15]. PAHs are emitted into the atmosphere mainly from anthropogenic sources such as vehicles and industrial facilities. Because of their mutagenicity, carcinogenicity, and toxic effects that are similar to those of dioxins, their concentrations have been evaluated in a variety of environmental matrices including sediments.

Wastewaters with high concentration of these organic compounds can be treated mainly by physicochemical processes such as Fenton's reagent, ozonation, UV, and H_2O_2 treatment. But these methods are costly and in-appropriate for large wastewater volumes [16]. Biological treatment is considering as alternative to physicochemical processes due to low associated costs [17]. Numerous aerobic bacteria are capable of using phenolic compounds as the sole carbon

and energy sources [18]. Biodegradation of phenol by *Pseudomonas putida* have been conducted by various authors due to its better removal efficiency [19]. Recently, consideration has been directed towards to find out new microorganisms which have high potential to remove toxic compounds. In this study, degradation pattern of nine different toxic compounds was evaluated using a newly isolated *Bacillus* sp. CYR2. Further, the effect of inoculum size on the growth of *Bacillus* sp. CY2, and degradation of various toxic compounds was assessed. Furthermore, the growth pattern of the strain CYR2 at 4% inoculum size was compared with acetate which is a non-toxic compound.

2.MATERIALS AND METHODS

2.1. *Bacillus* sp. CYR2 isolation

The bacteria were isolated from the soil collected at Muroran institute of Technology, Japan using enrichment techniques. Genomic DNA from the isolated bacteria was extracted using Achromopeptidase (Wako Pure Chemical Industries, Ltd., Osaka). The 16S rRNA gene fragment was amplified by polymerase chain reaction (PCR) with a pair of universal primers 9 F and 1510 R under standard conditions. The PCR-amplified product of 6 μL was subjected to agarose gel electrophoresis to estimate concentration. Sequencing reactions were performed using the Big Dye Terminator v3.1 cycle sequencing kit (Applied Biosystems); reactions were run in an Applied Biosystems 3130/3130XL genetic analyzers. Sequences were compared with those deposited in the GenBank nucleotide database using the BLAST program. These sequences were further aligned with the closest matches found in the GenBank database with the CLUSTALW function of Molecular Evolutionary Genetics Analysis package (MEGA) [20]. Neighbor-joining phylogenetic tree was constructed with the MEGA version 6.0. The 16S rRNA gene sequence of the isolate (*Bacillus* sp. CYR2) determined in this study was deposited in the DDBJ under accession no: LC171646.1.

2.2. Chemicals

Three different types of toxic organic compounds, *i.e.,* alkylphenols, mono-aromatic hydrocarbons, and PAHs were selected for degradation studies. Five types of alkylphenols [4-butylphenol (4-BP), 4-secondary-butylphenol (4-s-BP), 4-tertiary-butylphenol (4-t-BP), 4-tertiary-octylphenol (4-t-OP), and 4-nonylphenol (4-NP)]; two types of mono-aromatic hydrocarbons [phenol and 4-chlorophenol], and two types of PAHs [naphthalene and phenanthrene] were used. All chemicals used were of analytical grade and were purchased from Tokyo Chemical Industry (Tokyo, Japan).

2.3. Growth media

For the growth of *Bacillus* sp. CYR2, nutrient broth and mineral salt medium (MSM) were used. Nutrient broth was prepared by adding peptone (0.5%), yeast extract (0.3%), and NaCl (0.5%) in distilled water. The MSM contained 1.0 g $(NH_4)_2SO_4$, 1.0 g K_2HPO_4, 0.2 g NaH_2PO_4, 0.2 g $MgSO_4$ $7H_2O$, 0.05 g NaCl, 0.05 g $CaCl_2$, 8.3 mg $FeCl_3.6H_2O$, 1.4 mg $MnCl_2.4H_2O$, 1.17 mg Na_2MoO_4 $2H_2O$, and 1 mg $ZnCl_2$ per one liter of deionized water. The pH of the medium was adjusted to 7 by using 5 N NaOH solution and autoclaved before adding to the flasks.

2.4. Growth curve and degradation studies

Bacillus sp. CYR2 was cultivated in MSM at 30 ^{O}C by supplementing with three different types of toxic organic compounds individually *i.e.,* alkylphenols, mono-aromatic hydrocarbons, and PAHs at 100 mg/l final concentration as the sole carbon and energy source. Stock solution (10 g/l) of various toxic compounds was prepared using methanol. Hexane was used as stock solution for preparation of PAHs, *i.e.,* naphthalene and phenanthrene. A loop of *Bacillus* sp. CYR2 strain was initially inoculated into 50 ml of nutrient broth in 100 ml flask, and kept in shaking incubator at 30 ^{O}C for overnight at 180 rpm under aerobic condition. For growth and

degradation experiments, 2 ml (2% wv^{-1}) of the overnight grown culture was inoculated into 100 ml of MSM with the targeted toxic compound. Further studies were conducted using 4% inoculum without changing the conditions to focus on the effect of inoculum size on growth of bacteria and degradation of toxic compounds. Furthermore, the growth of *Bacillus* sp. CYR2 with toxic compounds was compared with non-toxic compound like acetate at 4% inoculum size. All the experiments were conducted for 6 days. Samples were collected at different time intervals. Growth of the bacteria was monitored spectrometrically at 600 nm. Degradation of toxic compounds were analysed by using high pressure liquid chromatography (HPLC). Results reported are the averages of three replicates.

2.5. Analysis of toxic compounds degradation

The degradation patterns of toxic compounds were analyzed using HPLC (Shimadzu) with an SPD-10AV UV/Vis detector and Shim-pack VP-ODS column (4.5×150 mm diameter, particle size 5 lm; Shimadzu, Kyoto, Japan). Samples collected for HPLC analysis were acidified with phosphoric acid (10% wv^{-1}) to stop the biological reaction, extracted with an equal volume of 1:1 (v v^{-1}) ethyl acetate, shaken for 3 min, and centrifuged at 8000×g for 10 min. The organic layer was filtered and then analyzed directly by HPLC. Filtered and degassed mobile phase mixture composed of acetonitrile: water (8:2) was used as mobile phase at a flow rate of 1.0 ml/min. The column was maintained at a temperature of 40 ^{O}C in a thermostat chamber. Conditions maintained for HPLC analysis were described in Table 1. Degradation concentrations were calculated from the area of the curve obtained for 1 mM of the standards. The detection limit was 0.03 mg/l. Recovery of samples was 99.5% in percent.

3. RESULTS AND DISCUSSION

3.1. *Bacillus* sp. CYR2

The isolated *Bacillus* sp. CYR2 is aerobic, Gram-positive, and rod-shaped bacterium. The 16S rRNA gene sequence and phylogenetic analysis also showed that strain was closely related to *Bacillus* sp. cp-h1 EU558968.1 (100% sequence identity), *Bacillus* sp. I-103 AB531392.1 (99% sequence identity), *Bacillus thuringiensis* KF150502.1 (100% sequence identity), and *Bacillus cereus* EU741099.1 (100% sequence identity) (Figure 1). Many authors reported about efficiency of *Bacillus* group for degradation of various toxic compounds [21-24].

3.2. Effect of inoculum size on growth

Bacillus sp. CYR2 was cultivated in MSM medium at 30 ^{O}C by supplementing with five different types of alkylphenols (4-BP, 4-s-BP, 4-t-BP, 4-t-OP, and 4-NP). Growth study results with 2% inoculum denoted that all the compounds showed significant influence and there is a marked difference on the growth of bacteria. Strain CYR2 denoted good growth with 4-NP followed by 4-s-BP, 4-t-BP, and 4-t-OP (Figure 2). Bacteria with 4-BP were unable to show growth because of the inhibition caused by alkylphenols. Among the mono-aromatic hydrocarbons, good growth was observed with phenol. Bacteria showed lag phase up to 2nd day, and log phase from 2nd day to 4th day (Figure 2). After 4th day it entered the decline phase. Bacteria with 4-chlorophenol were unable to show growth. Strain CYR2 was unable to show growth with PAHs. It indicates that, at 2% inoculum size bacteria were unable to utilize naphthalene, and phenanthrene as carbon source for their growth (Figure 2).

Strain CYR2 at 4% inoculum size showed significant growth with all the alkylphenols except 4-BP (Figure 3). Among all the alkylphenols, bacteria grown with 4-t-BP showed highest growth followed by 4-s-BP, 4-NP, and 4-t-OP. Bacterial growth with 4-s-BP at 4% inoculum size was 15.1 times higher than the 2% inoculum. The growth of strain CYR2 was 5.4 times higher with 4-t-BP, 2.59 times higher with 4-NP, 1.34 times higher with 4-t-OP respectively, when growth was compared with non-toxic compound like acetate. Contrary to this, 4-BP

showed 2.46 times lower growth than acetate. We have found some interesting results in the growth curve study, up to 3rd day bacteria grown with 4-NP, 4-t-OP, and acetate showed higher growth than 4-s-BP and 4-t-BP, after that bacteria grown with 4-s-BP and 4-t-BP showed higher growth than 4-NP, 4-t-OP, and acetate. It indicates that up to 3rd day the compounds 4-s-BP and 4-t-BP showed toxic effect on bacterial growth, after 3rd day bacteria get optimization. In the case of 4-BP, it showed toxic effect up to 6th day, so there is no bacterial growth.

Strain CYR2 incubated with phenol showed rapid growth at 4% inoculum size, it showed short lag phase and within 1 day it entered in to log phase (Figure 3). Bacteria at 6th day showed highest growth, it was 9.1 times higher when compared with 2% inoculum. The growth of strain CYR2 was 5.6 times higher with phenol, when growth was compared with acetate. Contrary to this, bacteria grown with 4-chlorophenol showed lower growth than acetate in initial days (from 0th day to 3rd day), after that growth was almost equal to acetate (up to 6th day). Growth study results clearly indicated that inoculum size had significant influence on the growth of bacteria. At 4% inoculum size bacteria had potential application and it can be used for the degradation of phenol contaminated water.

With 4% inoculum size also strain CYR2 does not showed significant growth with naphthalene, and phenanthrene (Figure 3). Strain CYR2 with naphthalene exhibited good growth up to 6th day. Bacteria supplemented with phenanthrene denoted growth from 0th day to 5th day, after that it showed slight decrement. Growth study results clearly indicated that the strain CYR2 has low degradation capacity of naphthalene, but it does not have the capacity to degrade phenanthrene.

3.3. Effect of inoculum size on degradation of toxic compounds

Bacillus is a member of the phylum Firmicutes. Many reports were available about degradation of various toxic compounds using the bacteria belongs to *Bacillus* group. Bacterial

strains such as *B. pumilus*, and *B. subtilis* were able to grow and utilize PAH compounds as carbon source [21]. Kuang et al. (2013) used *B. fusiformis* as biocatalyst and achieved 62% of phenol removal within 7 h at 200 mg/l concentration [22]. Fayidh et al. (2015) reported 43% and 94% removal of phenol at 200 mg/l concentration using *Bacillus* sp. and *B. cereus* respectively [23]. Bioremediation of crude oil using anaerobic mixed culture was done by Devi et al. (2011), and they reported the presence of bacteria belongs to *Bacillus* group in the mixed culture [24].

Among all the compounds, bacteria at 2% inoculum size showed highest degradation of 4-NP (48±5%), followed by 4-s-BP (32±4%), 4-t-BP (26±3%), and 4-t-OP (11±2%) at 6^{th} day of operation (Table 2). Bacteria were unable to degrade 4-BP up to 6^{th} day. It indicates that, at 2% inoculum size bacteria cannot degrade the alkylphenols due to their toxic nature, so further studies were carried out using 4% inoculum without changing the conditions. Strain CYR2 at 4% inoculum size showed improvement in the degradation of alkylphenols. Bacteria incubated with 4-t-BP showed highest degradation. The degradation was 2.53 times higher than the 2% inoculum. Ogata et al. reported 100% degradation of 4-t-BP using *Sphingobium fuliginis* within 3 days, but they used lower concentrations of 4-t-BP than the present study [25]. Compared with 2% inoculum size, the strain CYR2 showed 1.96 times higher removal of 4-s-BP at 4% inoculum. The degradation efficiency of *Bacillus* sp. CYR2 with 4-s-BP was coordinated well with existing literature reports. Hahn et al. reported 42% degradation of 4-s-BP within 7 days at 250 mg/l using *Mycobacterium neoaurum* [26]. Ogata et al. reported 100% degradation of 4-s-BP using the bacteria *Sphingobium fuliginis* within 3 days. The strain CYR2 showed 57±6% removal of 4-NP at 4% inoculum. The degradation efficiency of *Bacillus* sp. CYR2 with 4-NP was almost similar with the other bacteria according to previous reports. Ogata et al. reported 50% degradation of 4-NP at the concentration of 110 mg/l using the bacteria *Sphingobium fuliginis* [25]. Wang et al. reported highest degradation *i.e.,* 89% and 80% of 4-

NP at 100 mg/l using *Sphingobium* sp.WZ2, and *Rhizobium* sp. WZ1 respectively [27]. But they incubated both the bacteria for longer time than our experimental study, i.e., *Sphingobium* sp. WZ2 for 13 days, and *Rhizobium* sp. WZ1 for 17 days. The strain CYR2 showed 3.45 times higher removal of 4-t-OP at 4% inoculum, compared with 2% inoculum. Tuan et al. reported 91% degradation of 4-t-OP at 30 mg/l using *Acinetobacter* strain OP5 within 8 days [28]. Ogata et al. reported 50% degradation of 4-t-OP using the bacteria *Sphingobium fuliginis* within 3 days at 103 mg/l [25]. Toyama et al. reported 100% degradation of 4-t-OP using *Stenotrophomonas* strain IT-1 within 5 days at 1000 mg/l [29]. Among all the alkylphenols, 4-BP showed higher toxic effect on growth of bacteria up to 6^{th} day, so the degradation was very low (4±2%). Chang et al. (2016) isolated a fungal strain *Penicillium* sp. CHY-2 from the Antarctic soil and reported that, the strain CHY-2 effectively degraded the alkyl phenols such as 4-BP (98%), 4-s-BP (97%), 4-t-BP (100%), 4-NP (71%), 4-t-OP (70%),4-CP (60%), and phenol (68%) at 15 °C within one week [30]. Reddy et al., (2015) converted five different types of alkylphenols into poly-3-hydroxybutyrate (PHB) using the *Bacillus* sp. CYR1 [31]. They reported that strain CYR1 showed good removal of compounds in the presence of tween-80 within 3 days, but it took 6 days without addition of tween-80. Strain CYR1 showed 29% of PHB production with 4-nonylphenol.

Degradation studies with 2% inoculum denoted that 61±5% removal of phenol, and 0% removal of 4-chlorophenol. With 4% inoculum, strain CYR2 showed higher degradation of phenol (84±5%), it was 1.37 times higher than the degradation at 2% inoculum size. There are many reports on the degradation of phenol using different types of bacteria. Bacteria *Rhodococcus ruber* could effectively degrade phenol within 1.5 days [32]. Maza-Márquez and his co-workers reported 93% degradation of phenol within 6 days using the bacteria *Raoultella terrigena* [33]. Bonfa et al. reported 100% degradation of phenol within 5 days at 280 mg/l using *Modicisalibacter tunisiensis* [34]. Strain CYR2 showed lower degradation (16±3%) of

4-chlorophenol with 4% inoculum also. These results clearly indicating that the strain CYR2 has good degradation capacity of phenol, but it does not have the capacity to degrade 4-chlorophenol. At 4% inoculum size bacteria had potential application and it can be used for the degradation of phenol contaminated water.

Two ring PAH compound naphthalene, and three ring PAH compound phenanthrene were used for degradation studies. Studies with 2% inoculum size showed 6±2% removal of naphthalene and 0% removal of phenanthrene. With 4% inoculum also strain CYR2 did not showed rapid degradation of naphthalene (29±5%) and phenanthrene (12±3%). Experimental results clearly indicated that the strain CYR2 has low degradation capacity of PAHs compounds. Bacteria showed lower growth and degradation with PAHs due to their complex nature and presence of more than one phenyl rings in their structure. There are many reports on the degradation of PAHs using different types of bacteria. Studies reported about degradation and conversion of PAHs into the bioplastics like polyhydroxybutyrate (PHB) using *Bacillus* sp. CYR1 [31] and *Cupriavidus* sp. CY-1 [35]. Qi et al. reported 78% and 86% degradation of naphthalene and phenanthrene using the bacteria *Pseudomonas aeruginosa* at 10 and 15 mg/l concentrations respectively [36]. *Pseudomonas* USTB-RU showed 86% degradation of phenanthrene within 8 days at 100 mg/l concentration [37]. Patel et al. reported 100% degradation of phenanthrene within 5 days using *Pseudoxanthomonas* DMVP2 at 300 mg/l concentration [17].

4. CONCLUSION

The ability of newly isolated *Bacillus* sp. CYR2 to utilize different toxic organic compounds was studied. Strain CYR2 was effective in the degradation of phenol, and some of the alkylphenols when inoculum size was increased from 2 to 4%. It showed lower degradation of 4-chlorophenol, naphthalene, and phenanthrene. These results suggested that the

biodegradation patterns of toxic organic compounds using a newly isolated *Bacillus* sp. CYR2 were strongly affected by inoculum size. However, further research is needed to study the effect of co-substrate addition on degradation process, to find out the metabolites formed during the degradation process, and genes involved in the bioremediation process.

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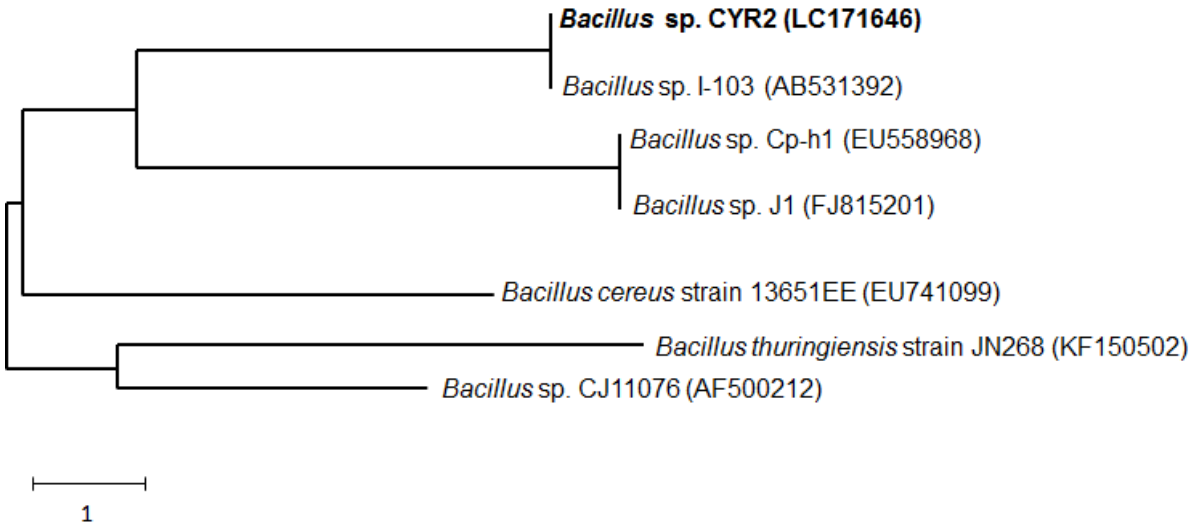
Lists of Figures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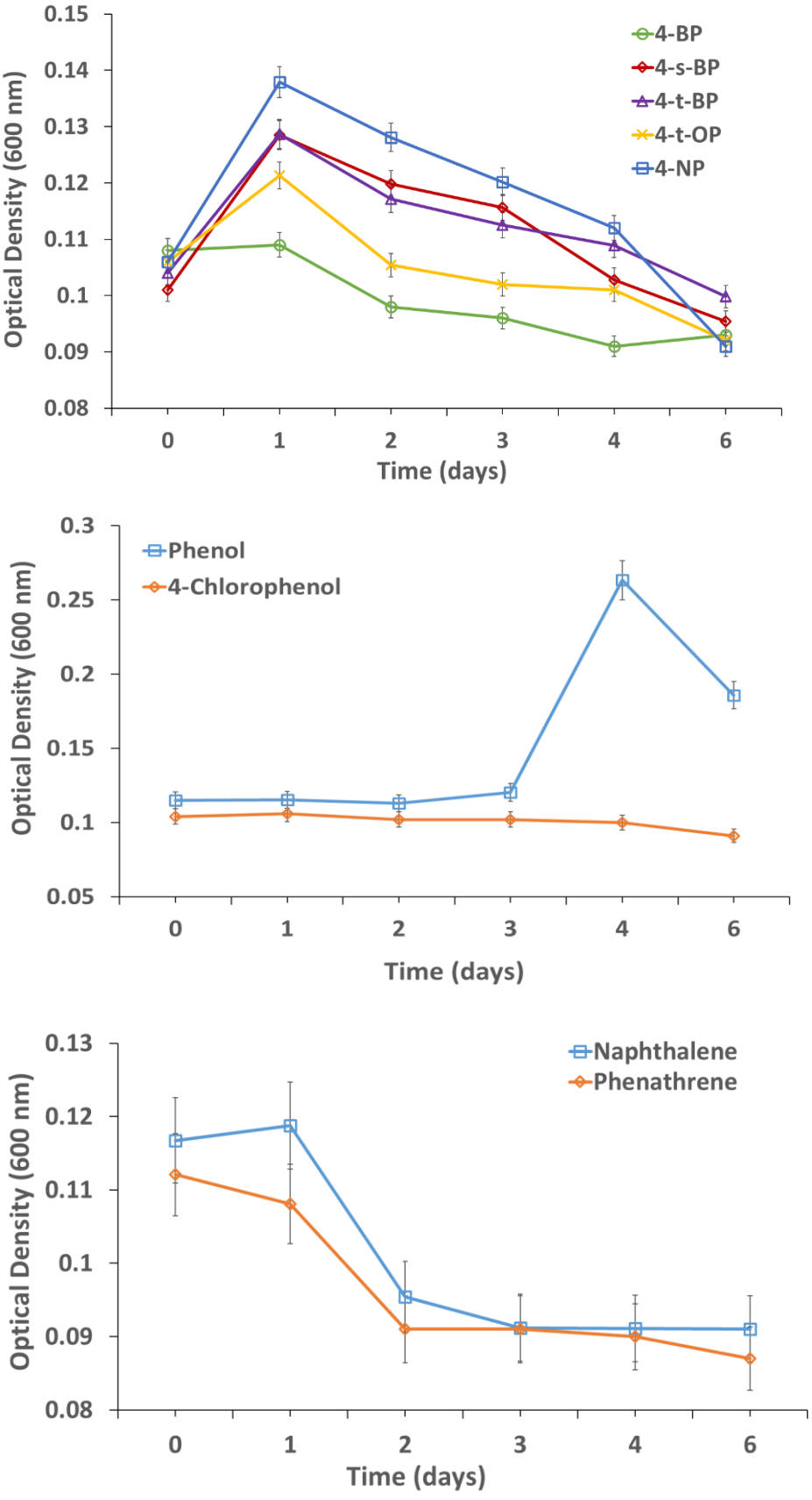
1

Fig. 1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0.12
0.11
0.1
0.09
0.08
0
1
2
3
4
6
Time (days)
4-BP
4-s-BP
4-t-BP
4-t-OP
4-NP
Optical Density (600 nm)
0.3
0.25
0.2
0.15
0.1
0.05
Phenol
4-Chlorophenol
Time (days)
Optical Density (600 nm)
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0.12
0.11
0.1
0.09
0.08
Naphthalene
Phenathrene
Time (days)

Fig. 2

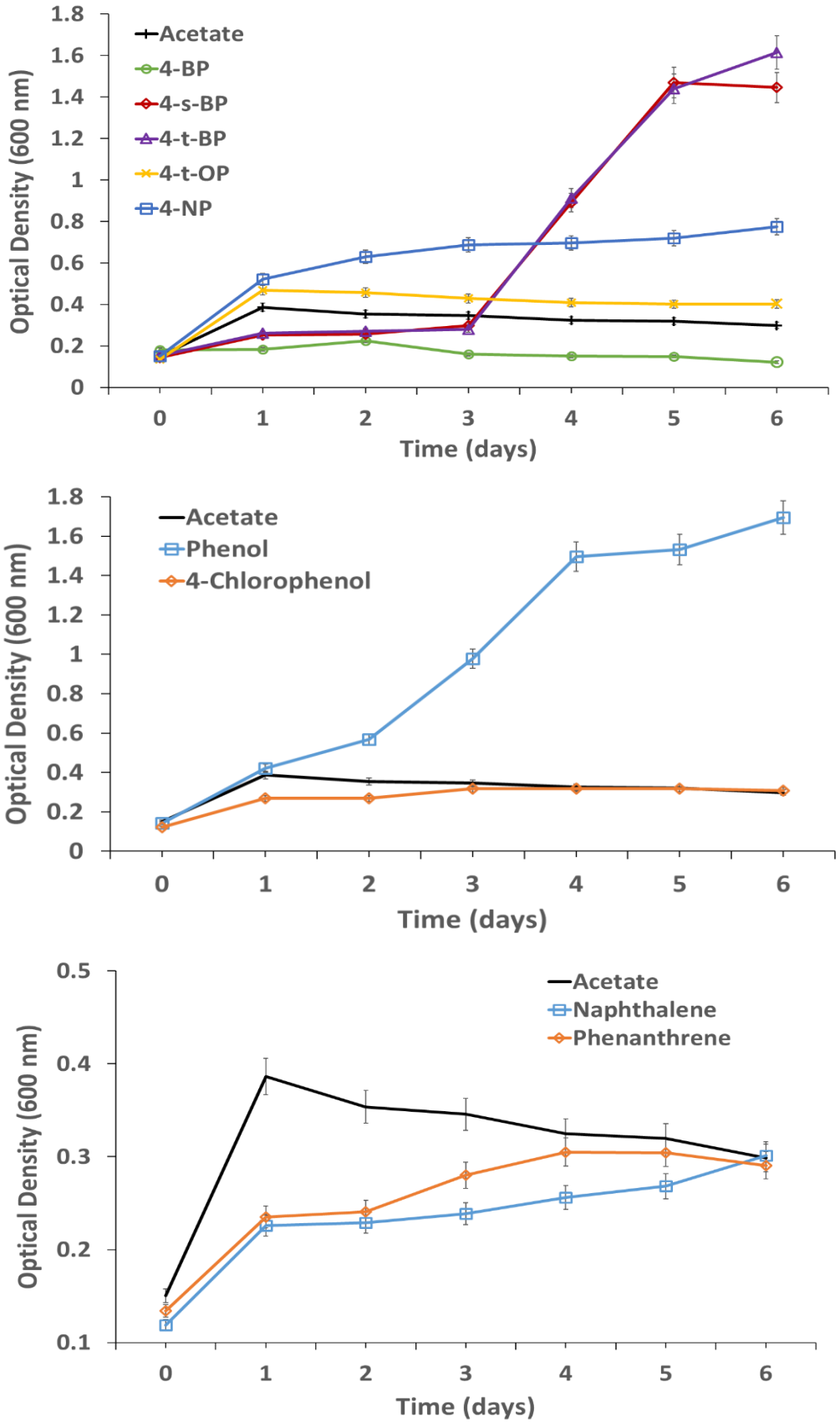
Optical Density (600 nm)
Time (days)
Acetate
4-BP
4-s-BP
4-t-BP
4-t-OP
4-NP
Acetate
Phenol
4-Chlorophenol
Acetate
Naphthalene
Phenanthrene

Fig. 3

Table list

Table 1. Conditions maintained for analysis of various toxic compounds using HPLC.

4-BP[1]: 4-butylphenol; 4-s-BP[2]: 4-secondary-butylphenol; 4-t-BP[3]: 4-tertiary-butylphenol; 4-t-OP[4]: 4-tertiary-octylphenol; 4-NP[5]: 4-nonylphenol.

Table 2. Alkylphenols, mono and poly-aromatic hydrocarbons degradation using *Bacillus* sp. CYR2 at 2% and 4% inoculum sizes.

4-BP[1]: 4-butylphenol; 4-s-BP[2]: 4-secondary-butylphenol; 4-t-BP[3]: 4-tertiary-butylphenol; 4-t-OP[4]: 4-tertiary-octylphenol; 4-NP[5]: 4-nonylphenol. ND*: no degradation

Table 1

Type of compound		**Detector (nm)**	**Mobile phase (% ratio)**
Alkylphenols	4-BP[1]	277	Acetonitrile: water (8:2)
	4-s-BP[2]	277	Acetonitrile: water (8:2)
	4-t-BP[3]	277	Acetonitrile: water (8:2)
	4-t-OP[4]	277	Acetonitrile: water (8:2)
	4-NP[5]	277	Acetonitrile: water (8:2)
Mono-aromatic hydrocarbons	Phenol	277	Acetonitrile: water (8:2)
	4-chlorophenol	280	Acetonitrile: 1% Ethyl acetate (1:1)
Poly-aromatic hydrocarbons	Naphthalene	254	Acetonitrile: water (8:2)
	Phenanthrene	254	Acetonitrile: water (8:2)

Table 2:

S. No	Compound name	Degradation (%)	
		2% inoculum	4% inoculum
1	4-BP[1]	ND*	4±2
2	4-s-BP[2]	32±4	63±5
3	4-t-BP[3]	26±3	66±3
4	4-t-OP[4]	11±2	38±4
5	4-NP[5]	48±5	57±6
6	Phenol	61±5	84±5
7	4-chlorophenol	ND	16±3
8	Naphthalene	6±2	29±5
9	Phenanthrene	ND	12±3