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Inhibition of dehydrogenase activity in petroleum refinery wastewater bacteria by phenolic compounds

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ABSTRACT

The toxicity of phenol, 2-nitrophenol, 4-nitrophenol, 2,4-dinitrophenol, 2-chlorophenol, 4-chlorophenol, 4-bromophenol and 3,5-dimethylphenol on *Pseudomonas*, *Bacillus* and *Escherichia* species isolated from petroleum refinery wastewater was assessed via inhibition of dehydrogenase enzyme activity. At low concentrations, 2-nitrophenol, 2-chlorophenol, 4-chlorophenol, 4-bromophenol and 3,5-dimethylphenol stimulated dehydrogenase activity and at sufficient concentrations, phenolic compounds inhibited dehydrogenase activities. Generally, phenol is less toxic than substituted phenols. Estimations of the degree of inhibition/stimulation of dehydrogenase activities showed significant dose-dependent responses that are describable by logistic functions. The toxicity thresholds varied significantly ($P < 0.05$) among the bacterial strains and phenolic compounds. The median inhibitory concentrations (IC_{50}s) ranged from 4.118 ± 0.097 mg.L^{-1} for 4-nitrophenol against *Pseudomonas* sp. DAF1 to 1407.997 ± 7.091 mg.L^{-1} for phenol against *Bacillus* sp. DISK1. This study suggested that the organisms have moderate sensitivity to phenols and have the potential to be used as indicators for assessment of chemical toxicity. They could also be used as catalysts for degradation of phenols in effluents.

Keywords: Dehydrogenase activity; phenolic compounds; petroleum refinery effluent.

Inibição da atividade de desidrogenase em bactérias em efluentes de refinaria de petróleo por compostos fenólicos

RESUMO

A toxicidade do fenol, 2-nitrofenol, 4-nitrofenol, 2,4-dinitrofenol, 2-clorofenol, 4-clorofenol, 4-bromofenol e 3,5-dimetilfenol para espécies de *Pseudomonas*, *Bacillus* e *Escherichia* isoladas de efluentes de refinaria de petróleo foi avaliada por meio da inibição da atividade da enzima desidrogenase. Em baixas concentrações, o 2-nitrofenol, 2-clorofenol, 4-clorofenol, 4-bromofenol e 3,5-dimetilfenol estimularam a atividade da desidrogenase e em concentrações suficientes, compostos fenólicos inibiram as atividades da desidrogenase. Geralmente, o fenol é menos tóxico do que os fenóis substituintes. As estimativas do grau de inibição ou estimulação das atividades da desidrogenase mostraram dependência significativa da dosagem descrita pelas funções de logística. Os limiares de toxicidade variaram significativamente ($P < 0,05$) entre as cepas de bactérias e compostos fenólicos. A concentração inibitória média (IC_{50}s) variou de $4,118 \pm 0,097$ mg.L^{-1} de 4-nitrofenol contra *Pseudomonas* sp. DAF1 de $1407,997 \pm 7,091$ mg.L^{-1} de fenol contra *Bacillus* sp. DISK1. Este estudo sugere que os organismos têm sensibilidade moderada a fenóis e têm potencial para serem utilizados como indicadores para a avaliação da toxicidade química. Eles também poderiam ser utilizados como catalisadores para a degradação de fenóis em efluentes.

Palavras-chave: atividade de desidrogenase; compostos fenólicos; efluentes de refinaria de petróleo.

1. INTRODUCTION

Phenolic compounds from myriads of petrochemical industries are among the pollutants most ubiquitously distributed in industrial effluents. Due to its wide distribution and injurious effects on humans, phenols are considered important environmental pollutants and their removal is of obvious interest. One of the most efficient approaches to phenol removal is biodegradation.

Wide ranges of microorganisms including bacteria, fungi and algae have been reported to degrade phenolic compounds. However, due to their toxicity, microbial degradation of phenolic compounds is usually inhibited at high concentrations (Goudar et al., 2000; Choi and Gu, 2001; Goudar and Delvin, 2001; Acuña-Argüelles et al., 2003; Oboirien et al., 2005; Okpokwasili and Nweke, 2006). In order to evaluate pollution risk of phenolic compounds in wastewater, it is important to assess their toxicity. Rapid and sensitive bioassays have been developed for assessment of toxicity of phenolic compounds. The estimation of respiratory activity is one of the most usually used laboratory screening tests (King, 1984; King and Dutka, 1986; King and Painter, 1986; Cenci et al., 1987; Strotmann et al., 1993; Dalzell et al., 2002; Okolo et al., 2007). In this assay, rates of oxygen uptake and reduction of redox indicators are followed polarographically and spectrophotometrically respectively. In the later approach, activities of dehydrogenase enzymes are determined via reduction of redox indicators to coloured forms whose intensity is measured in a spectrophotometer.

In this study, we assessed the toxicity of eight phenolic compounds to phenol-degrading bacterial strains isolated from petroleum refinery wastewater via reduction of 2,3,5-triphenyltetrazolium chloride (TTC) to red-coloured triphenyl formazan (TPF).

2. MATERIALS AND METHODS

2.1. Wastewater and bacterial strains

Pure cultures of bacteria were isolated from wastewater of Port Harcourt crude oil refinery. The untreated wastewater samples include the process wastewater derived from the refining process (PWW) and the raw wastewater (RWW) which is a combination of PWW and sewage that is channeled to the dissolved air floatation unit (DAF) for physical removal of oil droplet and then to the rotary biodisk (DISK) for biological treatment. The treated wastewater samples include treated wastewater, which is refinery effluent that has undergone both chemical and biological treatment to eliminate or reduce contents, and the observation pond wastewater (OPWW). Water samples were collected in sterile bottles, stored in a cooler and taken to the laboratory for microbiological analyses. The samples were analyzed within 6 h of collection to avoid deterioration of sample. The phenol-degrading bacteria were isolated on mineral salts agar supplemented with phenol as the only source of carbon and energy (Hill and Robinson, 1975). The phenol-degrading bacteria growing on the mineral salts-phenol agar were purified on nutrient agar (Lab M) and stored in nutrient agar slants at 4 ^{o}C. The isolates were characterized biochemically using standard microbiological methods. Identification to generic level followed the scheme of Holt et al. (1994). The phenol-degrading bacterial strains, *Pseudomonas* sp. DAF1 and *Pseudomonas* sp. RWW2 were isolated from the dissolved air floatation unit and the raw wastewater respectively. *Bacillus* sp. DISK1 and *Escherichia* sp. DISK2 were isolated from the rotary biodisk wastewater. The bacterial strains represent the preponderant morphotypes in their respective sources.

2.2. Reagents

The phenolic compounds, 2-nitrophenol, 4-nitrophenol, 2-chlorophenol, 4-chlorophenol, and phenol were obtained from Sigma, USA. Others including 4-bromophenol, 3,5-

dimethylphenol and 2,4-dinitrophenol were obtained from Fluka Rieldel-de Haën. The dehydrogenase enzyme substrate, 2,3,5-triphenyltetrazolium chloride was obtained from Sigma, USA.

2.3. Dehydrogenase assay

Dehydrogenase activity (DHA) was determined using 2,3,5-triphenyltetrazolium chloride (TTC) as the artificial electron acceptor, which is reduced to red-coloured triphenyl formazan (TPF). The assay was done in 3-ml volume of nutrient broth-glucose-TTC medium supplemented with varying concentrations of phenolic compounds in separate screw-capped test tubes. Portions (0.3 ml) of washed bacterial suspensions (A_{420} = 0.5) were inoculated into triplicate glass tubes containing 2.5 ml of phthalate-buffered (pH 7.0) nutrient broth glucose medium amended with each phenolic compound. Thereafter, 0.2 ml of 0.4% (w/v) TTC in deionized distilled water was added to each tube to obtain final concentrations of 20 - 200 $mg.L^{-1}$ (substituted phenols) and 200 – 2000 $mg.L^{-1}$ (phenol). The final concentrations of nutrient broth and glucose in the medium were 2 mg/ml each. The controls consisted of the isolates and the media without phenolic compound. The reaction mixtures were incubated under static conditions at room temperature (28 ± 2°C) for 24 h. The TPF produced was extracted in 4 ml of amyl alcohol and determined spectrophotometrically. The dehydrogenase activities as percent of control were computed.

2.4. Data analysis

Data were expressed as the mean and standard deviations. The effect of phenolic compounds on dehydrogenase activity was calculated relative to the control as shown in equation 1. To estimate the toxicity thresholds (IC_{20}, IC_{50} and IC_{80}), the data generated from equation 1 were fitted into logistic dose-response model (equation 2). For the responses with stimulation of dehydrogenase activity, data were fitted into asymmetric logistic dose-response model (equation 3). Curve fitting was done by iterative minimization of least squares using Levenberg-Marquardt algorithm of Table Curve 2D. All regression was done using the mean data and standard deviation. The toxicity thresholds for each bacterium and phenolic compound was compared pairwise using student's t-test with the levels of significance set at $P < 0.05$.

$$\text{DHA (\% of control)} = \frac{T_A}{C_A} \times 100 \quad [1]$$

$$\text{DHA (\% of control)} = \frac{a}{1 + \left(\frac{x}{b}\right)^c} \quad [2]$$

where C_A is the absorbance of triphenyl formazan produced in uninhibited control (without phenolic compound), T_A the absorbance of triphenyl formazan produced in inhibited test (with different concentrations of phenolic compound), x is the concentration of phenolic compound, a the uninhibited value of enzyme activity (100 %), b is IC_{50} and c is dimensionless toxicity parameter.

$$\text{DHA (\% of control)} = an(1+n)^{-d-1} d^{-d} (d+1)^{d+1} \quad n = \exp(-(x + c * \ln(d) - b)/c) \quad [3]$$

where a, b, c and d are model parameters.

3.0. RESULT AND DISCUSSION

The effects of phenolic compounds on dehydrogenase activities of the wastewater bacteria are shown in Figures 1 – 5. Generally, 3,5-dimethylphenol is less toxic to the bacterial strains than the other phenolic compounds. In *Pseudomonas* sp. DAF1, 2-

chlorophenol and 4-chlorophenol stimulated dehydrogenase activity at 20 mg.L^{-1}. Also, there was slight stimulation of dehydrogenase activity by 20 and 40 mg.L^{-1} of 2-nitrophenol. Thereafter, these phenolic compounds inhibited dehydrogenase activity. Other phenols progressively inhibited dehydrogenase activity with 4-nitrophenol and 2-chlorophenol reaching 100 % inhibition at 120 mg.L^{-1}. At 200 mg.L^{-1}, 4-bromophenol, 2,4-dinitrophenol, 4-chlorophenol, 2-nitrophenol and 3,5-dimethylphenol inhibited dehydrogenase activity by 88.604 ± 2.467, 71.429 ± 4.762, 88.889 ± 11.111, 90.741 ± 1.234 and 47.293 ± 2.467 % respectively. In *Pseudomonas* sp. RWW2, 3,5-dimethylphenol stimulated dehydrogenase activity at 40 mg.L^{-1} and was less toxic than other phenolic compounds. Similarly, slight stimulation of dehydrogenase activity by 4-chlorophenol and 4-bromophenol occurred at 20 mg.L^{-1}, and thereafter dehydrogenase activity was progressively inhibited until 100 % inhibition occurred at 180 mg.L^{-1}. 4-Nitrophenol and 2-chlorophenol are comparably more toxic than other phenolic compounds, and 100 % inhibition of dehydrogenase activity occurred at 100 and 180 mg.L^{-1} respectively. Although the inhibition of dehydrogenase activity was not sharp at low concentrations of 2-nitrophenol, total inhibition of dehydrogenase activity occurred at relatively lower concentration of 140 mg.L^{-1}. In *Bacillus* sp. DISK1, 3,5-dimethylphenol stimulated dehydrogenase activity at concentration ranging from 20 to 120 mg.L^{-1}. On the other hand, 2-nitrophenol, 4-chlorophenol, 4-bromophenol, 4-nitrophenol and 2-chlorophenol progressively inhibited dehydrogenase activity in *Bacillus* species from 20 mg.L^{-1}. 4-Nitrophenol appeared to be most toxic to *Bacillus* sp. DISK1 inhibiting dehydrogenase activity by 97.545 ± 0.640 % at 120 mg.L^{-1}. In *Escherichia* sp. DISK2, 4-chlorophenol, 4-bromophenol, 2-nitrophenol, 2-chlorophenol and 3,5-dimethylphenol stimulated dehydrogenase activity at low concentrations and thereafter inhibited it. At 40 mg.L^{-1}, 2-nitrophenol sharply inhibited dehydrogenase activity. 4-Nitrophenol was most toxic to *Escherichia* species, inhibiting dehydrogenase activity sharply at 20 mg.L^{-1} and reaching total inhibition at 120 mg.L^{-1}.

Generally, phenol was less toxic to the organisms than the substituted phenols. In *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2 and *Escherichia* sp. DISK2, phenol inhibited dehydrogenase activity with successive increase in the concentration of phenol. Total inhibition of dehydrogenase activity occurred at 1200 and 1400 mg.L^{-1} in *Escherichia* sp. DISK2 and *Pseudomonas* sp. DAF1 respectively. At concentrations ranging from 200 to 900 mg.L^{-1}, phenol stimulated dehydrogenase activity in *Bacillus* sp. DISK1 and thereafter inhibited it until total inhibition at 2000 mg.L^{-1}.

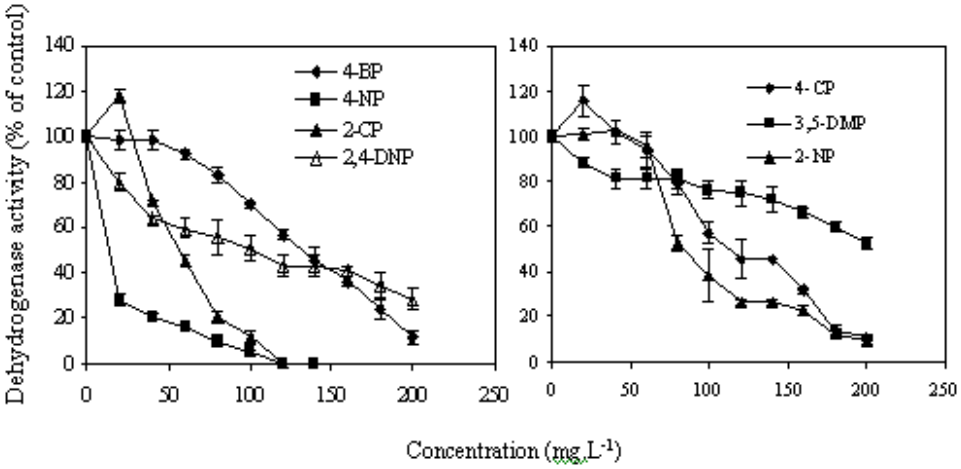

Figure 1. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 3,5-dimethylphenol (3,5-DMP) on dehydrogenase activity of *Pseudomonas* sp. DAF1.

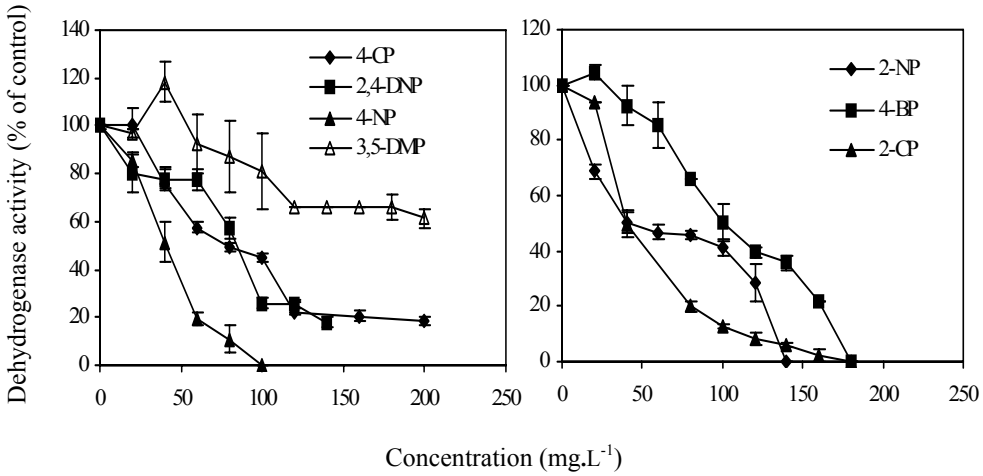

Figure 2. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 3,5-dimethylphenol (3,5-DMP) on dehydrogenase activity of *Pseudomonas* sp. RWW2.

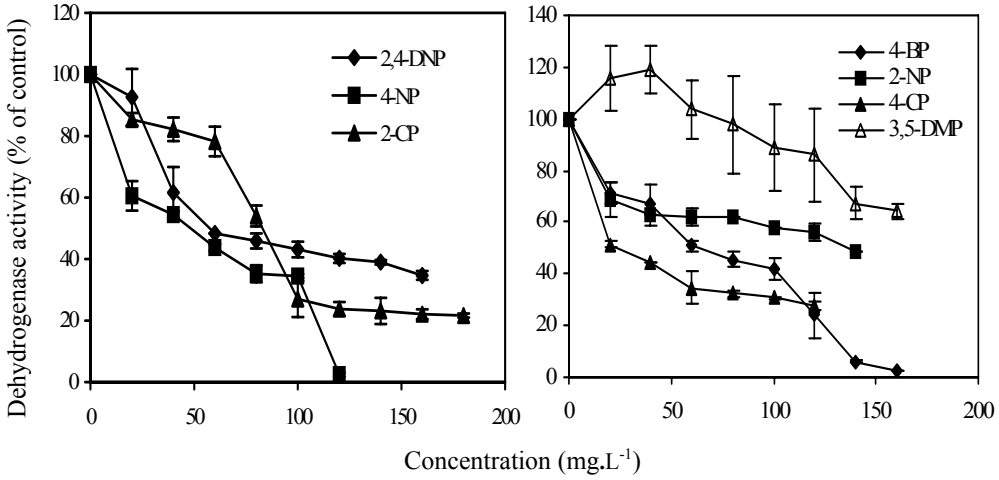

Figure 3. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 3,5-dimethylphenol (3,5-DMP) on dehydrogenase activity of *Bacillus* sp. DISK1.

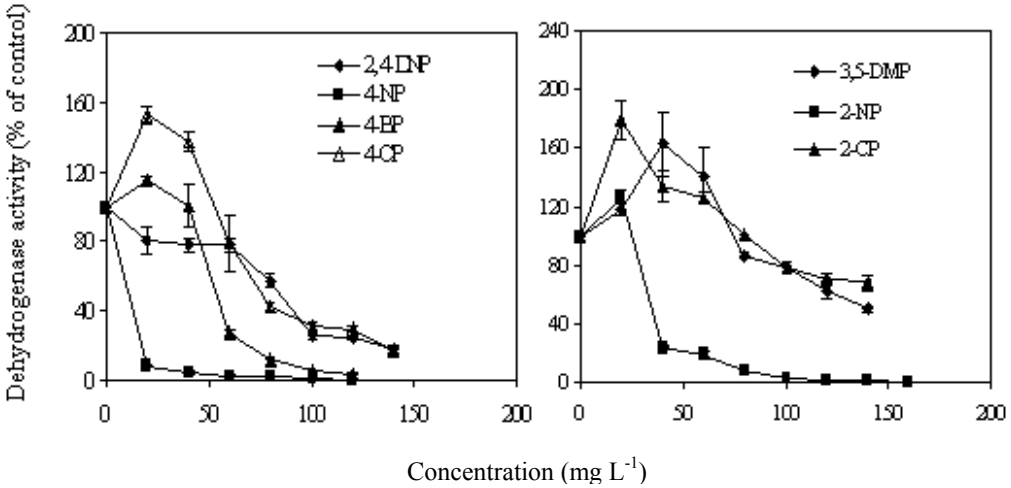

Figure 4. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP), 2-nitrophenol (2-NP), 4-nitrophenol (4-NP), 2,4-dinitrophenol (2,4-DNP) and 3,5-dimethylphenol (3,5-DMP) on dehydrogenase activity of *Escherichia* sp. DISK2.

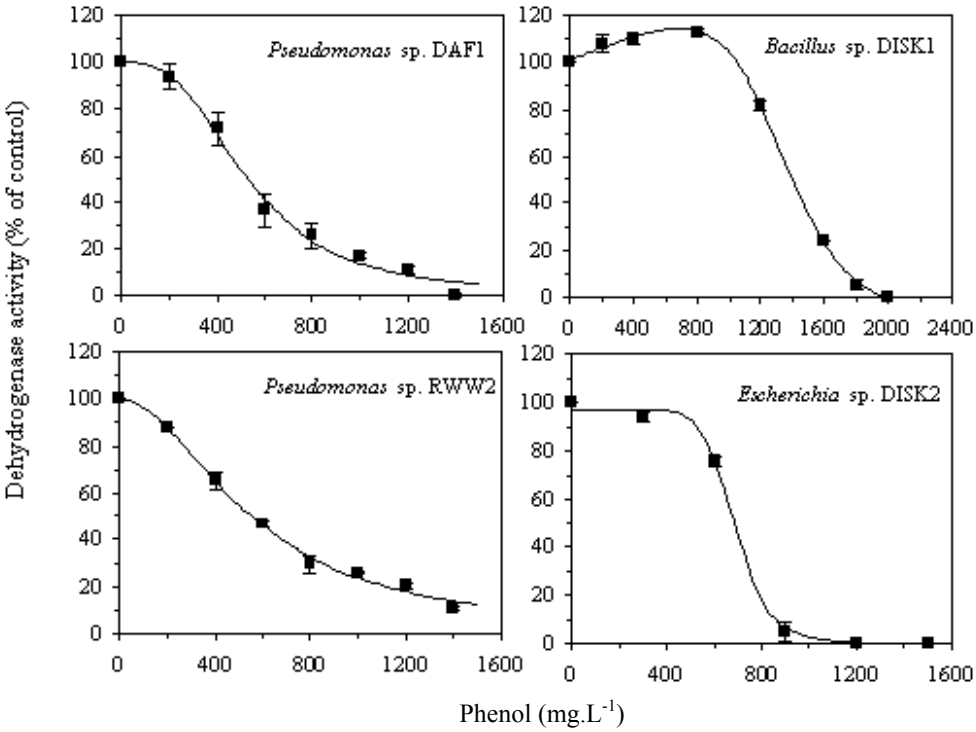

Figure 5. Effects of phenol on dehydrogenase activity in the bacterial strains. The data points indicates experimental data and the solid lines indicates values predicted from the models. Bars indicate ± 1SD, some of which are within data points.

Stimulation of dehydrogenase activity in some bacteria by phenolic compounds could indicate the use of the phenol as a growth substrate. *Pseudomonas*, *Bacillus* and *Escherichia* species have been reported to degrade phenol and other substituted phenols (Dapaah and Hill, 1992; Hollender et al., 1994; Monserrate and Häggblom, 19997; Jain et al., 1994). Similar stimulation of dehydrogenase activity in a soil *Acinetobacter* species by 4-nitrophenol and 2,4-dinitrophenol was reported by Okolo et al. (2007). The progressive inhibition of dehydrogenase activity with increasing concentration of phenols is in line with the well documented inhibitory nature of phenols at high concentrations for organisms which can use phenols as growth substrates (Acuña-Argüelles et al., 2003; Ruiz-Ordaz et al., 1998). The substituted phenols inhibited dehydrogenase activity more than phenol. This greater toxicity of substituted phenols have been reported. For instance, Cenci et al. (1987) reported that chlorophenols inhibited dehydrogenase activity in bacteria more than phenol. In a similar dehydrogenase activity assay using *Pseudomonas putida*, nitrophenols and chlorophenols was reported to be more toxic than phenol (Gül and Öztürk, 1998). In a bioluminescence assay, phenol was reported to be less toxic than 4-bromophenol, chlorophenols and nitrophenols (Ren and Frymier, 2002).

The dose-response patterns of the organisms are describable by logistic functions with high coefficient of regression ($R^2 > 0.9$). The toxicity threshold concentrations are shown in Table 1. The median inhibitory concentrations (IC_{50}s) ranged from 4.118 ± 0.097 $mg.L^{-1}$ for 4-nitrophenol against *Pseudomonas* sp. DAF1 to 1407.997 ± 7.091 $mg.L^{-1}$ for phenol against *Bacillus* sp. DISK1. The IC_{50} of phenol varied significantly ($P < 0.05$) with that of other phenolic compounds. Also, the statistical analysis indicated that the toxicity thresholds varied significantly among the phenolic compounds and bacteria. Variable toxicity thresholds estimated from inhibition of dehydrogenase activity have been reported. These are shown in Table 2. The IC_{50} of phenol against *Escherichia coli* reported by Cenci et al. (1987) is comparable with those reported in this study. However, the toxicity thresholds in this study were lower than the values reported by Gül and Öztürk, 1998, Abbondanzi et al. (2003)

reported lower IC_{50} of 210 $mg.L^{-1}$ phenol for *Pseudomonas fluorescens*. Based on 5-day oxygen consumption during biodegradation of peptone by mixed bacterial culture, Tišler and Zagorc-Končan (1995) reported phenol IC_{50} of 487 $mg.L^{-1}$. The artificial electron acceptor, 2,3,5-triphenyltetrazolium chloride (TTC) has been widely used as a measure of microbial growth (Tengerdy et al., 1967; Ghaly and Ben-Hassan, 1993). Abbondanzi et al. (2003) has also suggested good correlation of TTC-dehydrogenase activity with microbial growth. Thus, the toxicity thresholds obtained from growth inhibition data were compared with that obtained in this study. In this regard, the growth inhibition IC_{50}s reported for phenol by Dutka and Kwan (1981) are comparable with the values reported in this study (Table 2).

Table 1. Toxicity threshold concentrations of phenolic compounds for inhibition of dehydrogenase activity in the bacterial strains.

Bacteria/Toxicant	Toxicity thresholds ($mg.L^{-1}$)		
	IC_{20}	IC_{50}	IC_{80}
Pseudomonas sp. DAF1			
2-Chlorophenol	38.754 ± 0.590 b,c,e,f,g,h	55.368 ± 1.368 b,c,d,e,f,g,h	80.469 ± 2.900 b,c,d,e,f,g,h
4-Chlorophenol	75.518 ± 4.798 a,f,g,h	115.824 ± 11.015 a,d,e,f,g,h	175.174 ± 20.024 a,d,e,f,g,h
4-Bromophenol	84.270 ± 5.382 a,d,e,f,g,h	133.873 ± 5.240 a,d,e,f,g,h	185.709 ± 6.747 a,d,e,f,h
3,5-Dimethylphenol	51.264 ± 18.446 c,f,g,h	203.318 ± 3.566 a,b,c,e,f,h	302.942 ± 2.311 a,b,c,e,f,h
2-Nitrophenol	67.269 ± 2.306 a,b,c,f,g,h	87.497 ± 6.725 a,b,c,d,f,h	138.099 ± 18.466 a,b,d,f,g,h
4-Nitrophenol	0.131 ± 0.060 a,b,c,d,e,g,h	4.118 ± 0.907 a,b,c,d,e,g,h	37.467 ± 3.041 a,b,c,d,e,g,h
2,4-Dinitrophenol	16.561 ± 3.953 a,b,c,d,e,f,h	97.644 ± 20.226 a,c,d,f,h	291.202 ± 38.167 a,b,c,e,f,h
Phenol	329.491 ± 48.950 a,b,c,d,e,f,g	527.881 ± 56.462 a,b,c,d,e,f,g	848.299 ± 58.103 a,b,c,d,e,f,g
Pseudomonas sp. RWW2			
2-Chlorophenol	25.458 ± 0.b,c b,c,d,e,g,h	42.723 ± 2.709 b,c,g,h	73.666 ± 6.630 b,c,e,g,h
4-Chlorophenol	37.345 ± 6.782 a,c,d,e,f,h	77.039 ± 4.130 a,c,e,f,g,h	189.952 ± 26.596 a,e,f,g,h
4-Bromophenol	62.167 ± 8.939 a,b,e,f,h	107.736 ± 5.371 a,b,e,f,g,h	154.537 ± 1.457 a,e,f,g,h
3,5-Dimethylphenol	89.436 ± 22.151 a,b,e,f,g,h	ND	ND
2-Nitrophenol	11.094 ± 1.990 a,b,c,d,f,g,h	44.217 ± 7.699 b,c,g,h	127.103 ± 3.541 a,b,c,f,h
4-Nitrophenol	23.963 ± 2.692 b,c,d,e,g,h	39.832 ± 4.020 b,c,g,h	62.192 ± 5.267 b,c,e,g,h
2,4-Dinitrophenol	45.721 ± 7.966 a,d,e,f,h	90.129 ± 4.064 a,b,c,e,f,h	133.346 ± 2.620 a,b,c,f,h
Phenol	271.252 ± 27.537 a,b,c,d,e,f,g	549.455 ± 34.050 a,b,c,e,f,g	1113.467 ± 45.815 a,b,c,e,f,g
Bacillus sp. DISK1			
2-Chlorophenol	59.196 ± 4.387 b,c,e,f,g,h	81.757 ± 2.713 b,e,f,g,h	ND
4-Chlorophenol	3.332 ± 0.898 a,c,d,e,f,g,h	26.555 ± 3.179 a,c,e,f,g,h	212.686 ± 8.087 c,f,h
4-Bromophenol	18.988 ± 6.241 a,b,h	66.309 ± 10.497 b,e,f,h	124.367 ± 6.646 b,f,h
3,5-Dimethylphenol	75.895 ± 36.871 b,e,f,h	ND	ND
2-Nitrophenol	10.569 ± 2.812 a,b,d,g,h	135.774 ± 6.850 a,b,c,f,g,h	ND
4-Nitrophenol	9.599 ± 2.370 a,b,d,g,h	43.706 ± 4.295 a,b,c,e,g,h	95.377 ± 1.763 b,c,h
2,4-Dinitrophenol	27.834 ± 6.659 a,b,e,f,h	55.785 ± 4.875 a,b,e,f,h	ND
Phenol	1222.503 ± 11.565 a,b,c,d,e,f,g	1407.997 ± 7.091 a,b,c,e,f,g	1625.283 ± 8.079 b,c,f
Escherichia sp. DISK2			
2-Chlorophenol	103.983 ± 4.478 b,c,d,e,f,g,h	ND	ND
4-Chlorophenol	59.611 ± 4.170 a,c,d,e,f,h	75.827 ± 7.013 c,e,f,h	ND
4-Bromophenol	45.526 ± 3.017 a,b,d,e,f,h	53.662 ± 1.504 b,e,f,g,h	64.762 ± 0.432 e,f,g,h
3,5-Dimethylphenol	86.651 ± 0.269 a,b,c,e,f,g,h	ND	ND
2-Nitrophenol	26.555 ± 0.537 a,b,c,d,f,g,h	32.538 ± 0.567 b,c,f,g,h	44.124 ± 0.902 c,f,g,h
4-Nitrophenol	2.042 ± 0.061 a,b,c,d,e,g,h	6.030 ± 0.049 b,c,e,f,g,h	13.383 ± 0.298 c,e,g,h
2,4-Dinitrophenol	51.236 ± 9.516 a,d,e,f,h	82.093 ± 3.633 c,e,f,h	131.881 ± 10.599 c,e,f,h
Phenol	583.487 ± 8.330 a,b,c,d,e,f,g	681.742 ± 4.789 b,c,e,f,g	790.242 ± 2.310 c,e,f,g

ND = Not determined
At $p < 0.05$ (within each threshold and bacterial strain)
a = significantly different from 2-Chlorophenol
b = significantly different from 4-Chlorophenol
c = significantly different from 4-Bromophenol
d = significantly different from 3,5-Dimethylphenol
e = significantly different from 2-Nitrohenol
f = significantly different from 4-Nitrohenol
g = significantly different from 2,4-Dinitrohenol
h = significantly different from Phenol

Table 2. Some reported toxicity threshold concentrations for phenolompcompounds.

Phenolic compound	Toxicity threshold	Response/Comments	Reference
Phenol 2-Chlorophenol 4-Chlorophenol 2,4-Dichlorophenol 4-Nitrophenol 2,4,5-Trichlorophenol Pentachlorophenol 2,4-Dinitrophenol 2,6-Dinitrophenol	**EC_{20} (mg.L^{-1})** 564.65 192.83 123.41 30.97 37.56 5.92 42.61 42.52 52.73	Toxicity of phenolic compounds at pH 7 obtained from the specific growth rate calculated from optical density data over an 80-min period during the exponential growth phase of a recombinant bioluminescent *Escherichia coli*	Choi and Gu (2001)
Phenol 2-Methylphenol 3-Methylphenol 4-Methylphenol 3-Nitrophenol 4-Nitrophenol 2,4-Dinitrophenol 2-Chlorophenol 3-Chlorophenol 4-Chlorophenol	**IC_{50} (mg.L^{-1})** 6309.57 2454.71 229.09 1621.81 1122.02 1202.26 102.33 2398.83 151.36 794.33	A 75 min toxicity determined using resazurin dehydrogenase activity of *Pseudomonas putida* DSM-50026 at pH 6.3	Gül and Öztürk (1998)
Phenol 2-Chlorophenol 4-Chlorophenol	**IC_{50} (mg.L^{-1})** 636.17 511.63 205.68	Toxicity thresholds of phenolic compounds against dehydrogenase activity of *Escherichia coli*	Cenci et al. (1987)
Phenol	**IC_{50} (mg.L^{-1})** 1600 880	A 18-h toxicity threshold from growth inhibition of *Aeromonas hydrophila* and *Pseudomonas fluorescens* respectively	Dutka and Kwan (1981)
Phenol	**EC_{50} (mg.L^{-1})** 244	A 6-h toxicity threshold estimated from inhibition of *Pseudomonas putida* growth	Slabbert (1986)
Phenol	**EC_{50} (mg.L^{-1})** 177	A 48-h toxicity assay via inhibition of TTC-dehydrogenase activity in *Pseudomonas fluorescens*	Amorós et al. (2007)
3.5-Dichlorophenol	**EC_{50} (mg.L^{-1})** 8.1 ± 2.4	A 6-h growth inhibition of sewage bacteria	Strotmann and Pagga (1996)
3,5-Dichlorophenol Pentachlorophenol	**EC_{50} (mg.L^{-1})** 34.2 32.7	Inhibition of respiration in unwashed cells of *lux*-marked *Pseudomonas fluorescens*	Brown et al. (1996)
Phenol	**EC_5 (mg.L^{-1})** 210	Toxicity determined using TTC dehydrogenase activity of *Pseudomonas fluorescens* ATCC13525	Abbondanzi et al. (2003)

Similarly, the toxicity thresholds obtained for substituted phenols through inhibition of respiration and growth were in some cases comparable with our TTC-dehydrogenase thresholds (see Tables 1 and 2). *Pseudomonas* and *Escherichia* species in this study seem to be of moderate sensitivity. *Bacillus* sp. DISK1 with IC_{50} of 1407.997 ± 7.091 mg.L^{-1} could be considered a resistant strain. Dehydrogenase activity in this bacterium was stimulated by phenol concentrations up to 800 mg.L^{-1}, and respiration still occur in the presence of 1800 mg.L^{-1} phenol. Thus, the *Bacillus* species have potential to be used in biotreatment of phenolic wastewater. *Staphylococcus*, *Corynebacterium*, *Bacillus* and *Proteus* species have been found to resist 10 mM (941.08 mg.L^{-1}) phenol (Ajaz et al., 2004).

4. CONCLUSION

The results of the *in vitro* toxicity assays indicate that increasing concentrations of phenolic compound are potentially toxic to phenol-degrading bacteria in petroleum refinery wastewater. However, the organisms tolerated low concentrations of phenols and stimulated dehydrogenase activities. Thus, they could be used as catalysts for degradation of phenolic compounds in effluents. Nevertheless, in order to achieve biological oxidation and mineralization of phenolic compounds in the wastewater, the concentrations of phenols must be finely adjusted to reduce toxicity in wastewater treatment plants.

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