ISSN = 1980-993X – doi:10.4136/1980-993X
www.ambi-agua.net
E-mail: ambi-agua@agro.unitau.br
Tel.: (12) 3625-421

RECEIVED 11 Mar 2011 20 Apr 2011 ACCEPTED

Inhibition of β-galactosidase and α-glucosidase synthesis in petroleum refinery effluent bacteria by phenolic compounds

(doi:10.4136/ambi-agua.172)

Christian O. Nweke[1]; Gideon C. Okpokwasili[2]

[1]Department of Microbiology, Federal University of Technology, P. M. B. 1526, Owerri, Nigeria
E-mail: xrisokey@yahoo.com
[2]Department of Microbiology, University of Port Harcourt, P. M. B. 5323, Port Harcourt, Nigeria
E-mail: gidsilman@yahoo.com

ABSTRACT

Inhibition of α-glucosidase (EC 3.2.1.20) and β-galactosidase (EC 3.2.1.23) biosynthesis by phenolic compounds (phenol, 2-chlorophenol, 4-chlorophenol, 4-bromophenol and 3,5-dimethylphenol) in *Escherichia coli*, *Bacillus* and *Pseudomonas* species isolated from petroleum refinery wastewater was assessed. At sufficient concentrations, phenols inhibited the induction of α-glucosidase and β-galactosidase. The patterns of these toxic effects can be mathematically described with logistic and sigmoid dose-response models. The median inhibitory concentrations (IC_{50}) varied among the phenols, the bacteria and enzymes. Quantitative structure–activity relationship (QSAR) models based on the logarithm of the octanol–water partition coefficient ($\log_{10}K_{ow}$) were developed for each bacterium. The correlation coefficients varied between 0.84and 0.99 for the enzymes. The test results indicated α-glucosidase and β-galactosidase biosynthesis as important microbial indices for evaluation of toxicity of phenolic compounds.

Keywords: Toxicity; phenolic compounds; QSAR; wastewater bacteria.

Inibição de β-galactosidase e síntese de α-glicosidase em bactérias de efluente de refinaria de petróleo por compostos fenólicos

RESUMO

A inibição da biossíntese da α-glicosidase (CE 3.2.1.20) e da β-galactosidase (3.2.1.23) por compostos fenólicos (fenol, 2-clorofenol, 4-clorofenol, 4-bromofenol e 3,5-dimetilfenol) nas espécies *Escherichia coli, Bacillus* e *Pseudomonas* isoladas de efluentes de refinarias de petróleo foi avaliada. Em concentrações suficientes, os fenóis inibiram a indução da α-glicosidase e β-galactosidase. O comportamento desses efeitos tóxicos pode ser descrito matematicamente com modelos de logística e sigmóide dose-resposta. A mediana da concentração inibitória (IC50) variou entre os fenóis, as bactérias e enzimas. Modelos da relação quantitativa de estrutura-atividade (QSAR) baseados no logaritmo do coeficiente da partição octanol-água ($\log_{10}K_{ow}$) foram desenvolvidos para cada bactéria. Os coeficientes de correlação variaram entre 0.84 e 0,99 para as enzimas. Os resultados dos testes indicaram a biossíntese da α-glicosidase e da β-galactosidase como importantes índices microbianos para avaliação da toxicidade de compostos fenólicos.

Palavras-chave: Toxidade; compostos fenólicos; QSAR; bactéria de resísuduo líquido.

1. INTRODUCTION

Phenolic compounds are common constituents in effluents from many industrial sources, including oil refineries and other petrochemical industries. Phenolic wastewaters have been treated using biological processes in which the compounds are digested by acclimated microbial consortium. Numerous microorganisms have been reported to degrade phenol and its derivatives. However, most phenol-degrading bacteria suffer from substrate inhibition, whereby growth and biodegradation rates are inhibited at high phenol concentration due to toxicity (Goudar et al., 2000; Oboirien et al., 2005; Okpokwasili and Nweke, 2006; Kumar et al., 2005; Saravanan et al., 2008; Agarry et al., 2008; Agarry and Solomon, 2008).

Bacteria are important group of microorganisms because of their unique role, for efficient functioning of natural ecosystems. The determination of the toxicity of chemicals to bacteria is an important criterion for the evaluation of pollution risk of chemicals in the environment. In this regard, rapid and sensitive bacterial toxicity tests for assessment of toxicity are developed. Bacterial test systems include the estimation of respiration activity (Anderson et al., 1988; Broecker and Zahn, 1977; Brown et al., 1981; Dutka et al., 1983; King and Painter, 1986), measurement of growth inhibition of bacteria (Strotmann et al., 1994), estimation of dehydrogenase activity (Strotman et al., 1993; Okolo et al., 2007), β - galactosidase activity (Katayama-Hirayama, 1986), intracellular ATP level (Pill et al., 1991), bioluminescence (Choi and Gu, 2001), nitrification inhibition (Strotmann and Eglsäer,1995; Gendig et al., 2003; Juliastuti et al., 2003), inhibition of periplasmic nitrate reductase (Okolo et al., 2007), inhibition of enzyme biosynthesis (Dutton et al., 1990; Odokuma and Okpokwasili, 2003; Nweke and Okpokwasili, 2011).

Bioassays involving inhibition of enzyme activity and enzyme biosynthesis are simple, rapid, cost-effective and require small volume of samples. Inhibition of biosynthesis and activity of α-glucosidase and β-galactosidase in bacteria have been used to assess toxicity of inorganic and organic toxicants (Barnhart and Vestal, 1983; Dutton et al., 1988; Dutton et al., 1990; Guven et al., 2003; Bitton and Koopman, 1994; Codina et al., 1994, Nweke and Okpokwasili, 2011). It was previously reported that bioassay based on α-glucosidase and β-galactosidase activity is insensitive to heavy metals (Barnhart and Vestal, 1983; Dutton et al., 1988, 1990). In addition, β-galactosidase biosynthesis was shown to be more sensitive to organic toxicants than β-galactosidase activity (Dutton et al., 1988).

Although inhibition of α-glucosidase and β-galactosidase biosynthesis have been used to assess toxicity of organic toxicants, they have not been extensively used to assess toxicity of phenolic compounds. Also, not much work has been done to assess toxicity of phenolic compounds on bacterial strains derived from petroleum refinery wastewater. The aim of this study was to compare the inhibitory effects of phenolic compounds on the biosynthesis of inducible enzymes, α-glucosidase and β-galactosidase, in petroleum refinery effluent bacteria. The information obtained should have bearing on the development of ecotoxicity assay using bacteria.

2. MATERIALS AND METHODS

2.1. Test chemicals and reagents

The phenolic compounds, 2-chlorophenol, 4-chlorophenol, and phenol were obtained from Sigma, USA. Others including 4-bromophenol and 3,5-dimethylphenol were obtained from Fluka Rieldel-de Haën. The enzyme substrates *p*-nitrophenyl-β-D-galactopyranoside and *p*-nitrophenyl-α-D-glucopyranoside were obtained from Sigma (USA). The Z-buffer for the enzyme assays contained the following components: $Na_2HPO_4.7H_2O$, 16 $g.L^{-1}$; NaH_2PO_4, 5.6 $g.L^{-1}$; $MgSO_4$, 0.12 $g.L^{-1}$; KCl, 0.754 $g.L^{-1}$ and β-mercaptoethanol, 2.7 $ml.L^{-1}$.

2.2. Cell cultures

Bacterial strains used were *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli* isolated from petroleum refinery wastewater obtained from wastewater treatment system of Port Harcourt petroleum refinery, Port Harcourt, southeastern Nigeria. The method of sample collection, isolation and identification of the bacterial strains was as described by Nweke and Okpokwasili (2010). They were maintained in nutrient agar slants at 4°C. The cells were grown by inoculating 50 ml of sterile nutrient broth media (HIMEDIA) in 100ml Erlenmeyer flask. The cultures were incubated at room temperature (28 ± 2^{o}C) for 16 – 24 hours on rotary shaker operated at 150 rpm. Cells were harvested by centrifugation at 4000 rpm for 10 minutes. Harvested cells were washed twice in sterile deionized distilled water and resuspended in the same water. The cell suspensions were standardized in a spectrophotometer to an optical density of 0.6 at 420 nm. The standardized cell suspensions were used as inocula in the enzyme assays.

2.3. β-Galactosidase activity assay

β-Galactosidase activities were determined using *p*-nitrophenyl-β-D- galactopyranoside, which is hydrolyzed to yellow-coloured *p*-nitrophenol. Portions (0.1 ml) of standardized cell suspensions were inoculated into sterile triplicate 20 ml screw-capped test tubes containing 1.9 ml of Z-buffered (pH 7.0) nutrient broth-lactose medium (consisting of 0.4 ml of Z-buffer, 0.4 ml of nutrient broth and 0.1 ml of 0.4% w/v lactose) supplemented with a particular concentration of phenolic compound (0 – 4000 mg.L^{-1}). The controls consisted of inoculated medium without toxicant. The cultures were shake-incubated at room temperature for 1 h. Thereafter, 0.1 ml of 7% w/v sodium dodecyl sulphate (SDS) was added into each tube and shaken to solubilize the cells. Then, 0.1 ml of 0.4% w/v *p*-nitrophenyl-β-D-galactopyranoside was added and the reaction mixture incubated at room temperature for 24 hours. The reactions were stopped with 1 ml of cold 1 M Na_2CO_3 solution. The absorbances of *p*-nitrophenol solution produced were measured spectrophotometrically at 420 nM (λmax). The β-galactosidase activities were calculated relative to controls as shown in equation 1.

2.4. α-Glucosidase activity assay

α-Glucosidase activities were determined using *p*-nitrophenyl-α-D-glucopyranoside that is hydrolyzed to yellow-coloured *p*-nitrophenol. Portions (0.1 ml) of standardized cell suspensions were inoculated into sterile triplicate 20 ml screw-capped test tubes containing 1.9 ml of Z-buffered (pH 7.0) nutrient broth-lactose medium (consisting of 0.4 ml of Z-buffer, 0.4 ml of nutrient broth and 0.1 ml of 0.4% w/v maltose) supplemented with a particular concentration of phenolic compound (0 – 6000 mg.L^{-1}). The controls consisted of inoculated medium without toxicant. The cultures were shake-incubated at room temperature for 1 h. Thereafter, 0.1 ml of 0.4% w/v *p*-nitrophenyl-α-D-glucopyranoside was added and the reaction mixture incubated at room temperature for 24 h. The reactions were stopped by adding 1 ml of cold 1 M Na_2CO_3 solution. The absorbances of *p*-nitrophenol solution produced were measured spectrophotometrically at 420 nM (λmax). The α-glucosidase activities were calculated relative to controls as shown in equation 1.

2.5. Data analysis

The degree of inhibition was determined relative to control (100% enzyme activity) on the basis of measured absorbances as shown in equation 1. Differences at enzyme activity levels between the controls and other samples were taken as the effect of phenolic compounds on enzyme biosynthesis. At least three replicate tests were carried out on each toxicant. The data were plotted in terms of percent of enzyme activity in control test on *y*-axis versus concentration of phenolic compound on *x*-axis with means and standard deviations (n =3) shown as data points and bars respectively. The toxicity thresholds of the toxicants (IC_{20}, IC_{50}

and IC_{80}) were determined by fitting the experimental data (obtained from equation 1) into non-linear logistic (equation 2) and sigmoid dose-response (equation 3) models. All regressions were done iteratively using the data mean and standard deviations at 95% confidence limit. The toxicity thresholds were compared pairwise between the phenolic compound and between β-galactosidase and α-glucosidase using student's t-test. The linear regression analyses were done using Microsoft Excell 2003.

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

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

Where, C_A is the absorbance of *p*-nitrophenol in uninhibited control (without toxicant), T_A the absorbance of *p*-nitrophenol 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

$$\text{Enzyme activity (\% of control)} = \frac{a}{1+e^{b(x-c)}} \quad [3]$$

Where b is slope parameter indicating the inhibition rate and c is IC_{50}

2.6. Quantitative structure-activity relationship (QSAR) analysis

The correlation between IC_{50} values and physicochemical properties for some phenolic compounds were evaluated. Two parameters that are often employed in QSAR analysis were used; the logarithm of the octanol-water partition coefficient, $Log_{10}K_{ow}$, characterizing the lipophilicity and the dissociation constant of acid and pKa describing the electronic effect. The $Log_{10}K_{ow}$ and pKa values were obtained from Aptula et al. (2002). Linear regression was performed on the IC_{50} (y-axis) versus parameter (x-axis) plot. The predicted IC_{50} were generated from the linear models.

3. RESULTS AND DISCUSSION

The effects of phenolic compounds on the biosynthesis of two inducible enzyme, β-galactosidase and α-glucosidase are shown in Figures 1 – 3. The compounds tested included, phenol, 2-chlorophenol, 4-chlorophenol, 4-bromophenol and 3,5-dimethylphenol. In all the bacteria, phenolic compounds inhibited β-galactosidase and α-glucosidase biosynthesis in a dose-dependent manner. Generally, substituted phenols were more inhibitory than phenol. In terms of α-glucosidase biosynthesis, *Escherichia coli* tolerated 2-chlorophenol more than other bacteria. 3,5-Dimethylphenol was more inhibitory to α-glucosidase induction in *Pseudomonas* sp. RWW2 than in other bacteria. *Pseudomonas* sp. DAF1 and *Escherichia coli* were tolerant to toxicity of phenol than *Pseudomonas* sp. RWW2 and *Bacillus* sp. DISK1. Inhibition of β-galactosidase synthesis in *Pseudomonas* sp. DAF1 increased with increase in concentration at concentrations ranging from 0 – 5000 $mg.L^{-1}$ for phenol and 0 – 1000 $mg.L^{-1}$ for the other phenolic compounds. At 0 – 1000 $mg.L^{-1}$, 3,5-dimethylphenol and phenol progressively inhibited β-galactosidase synthesis in *Pseudomonas* sp. RWW2. On the other hand, 2-chlorophenol, 4-chlorophenol and 4-bromophenol progressively inhibited β-

galactosidase synthesis in this bacterium at concentrations up to 320 $mg.L^{-1}$ and total inhibition of β-galactosidase synthesis was observed at 320, 220, 200, 800 and 1000 $mg.L^{-1}$ of 2-chlorophenol, 4-chlorophenol, 4-bromophenol, 3,5-dimethylphenol and phenol respectively.

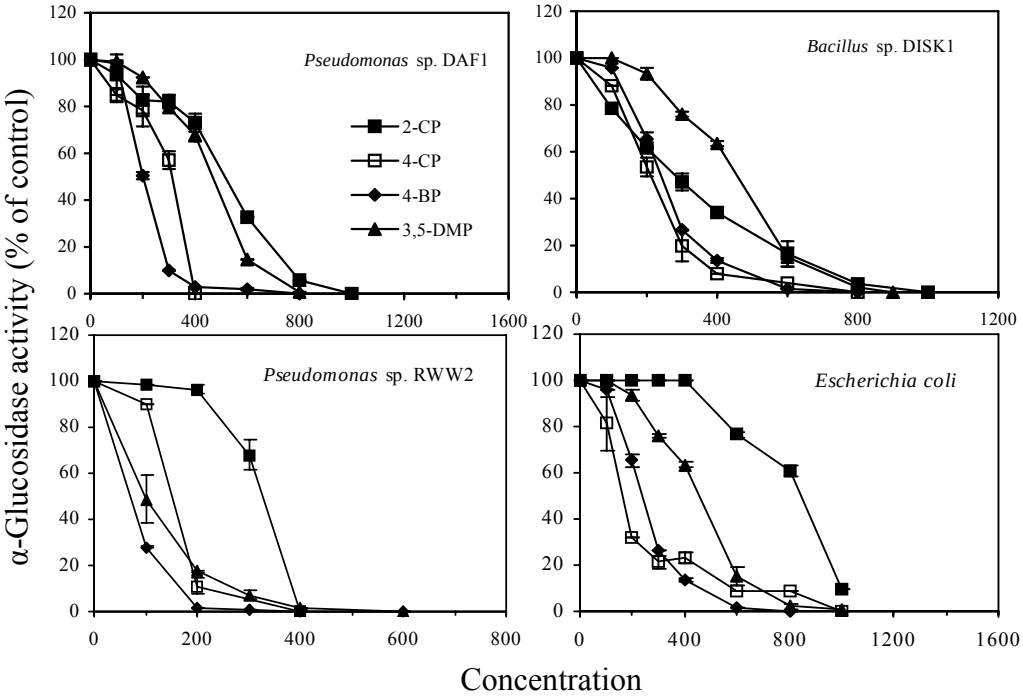

Figure 1. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP) and 3,5-dimethylphenol (3,5-DMP) on α-glucosidase activity of bacterial strains.

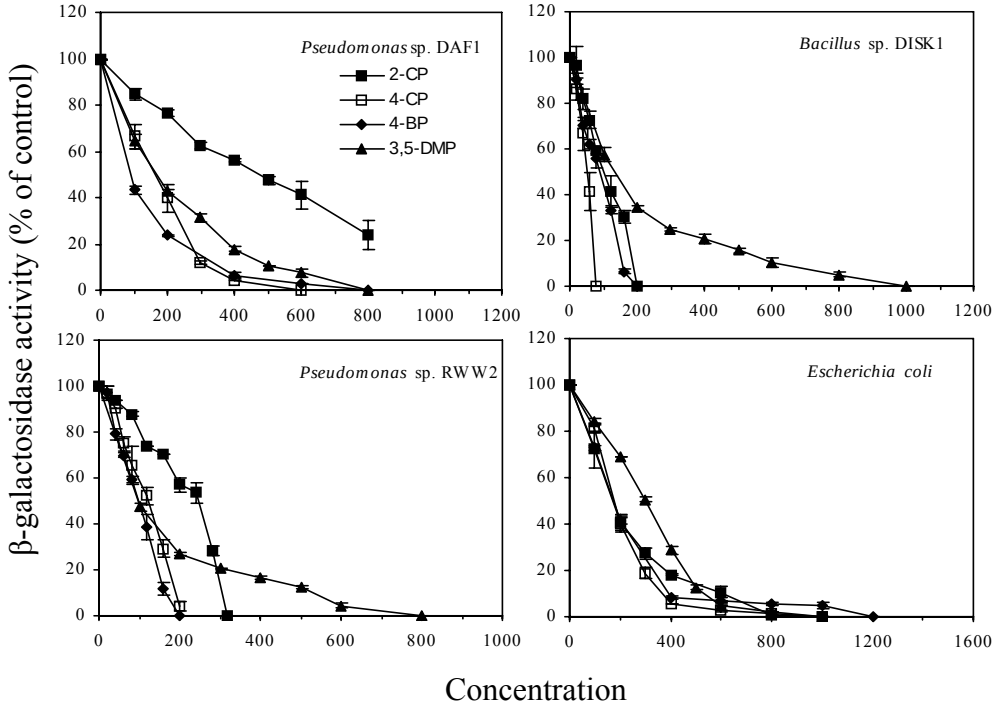

Figure 2. Effects of 2-chlorophenol (2-CP), 4-chlorophenol (4-CP), 4-bromophenol (4-BP) and 3,5-dimethylphenol (3,5-DMP) on β-galactosidase activity of bacterial strains.

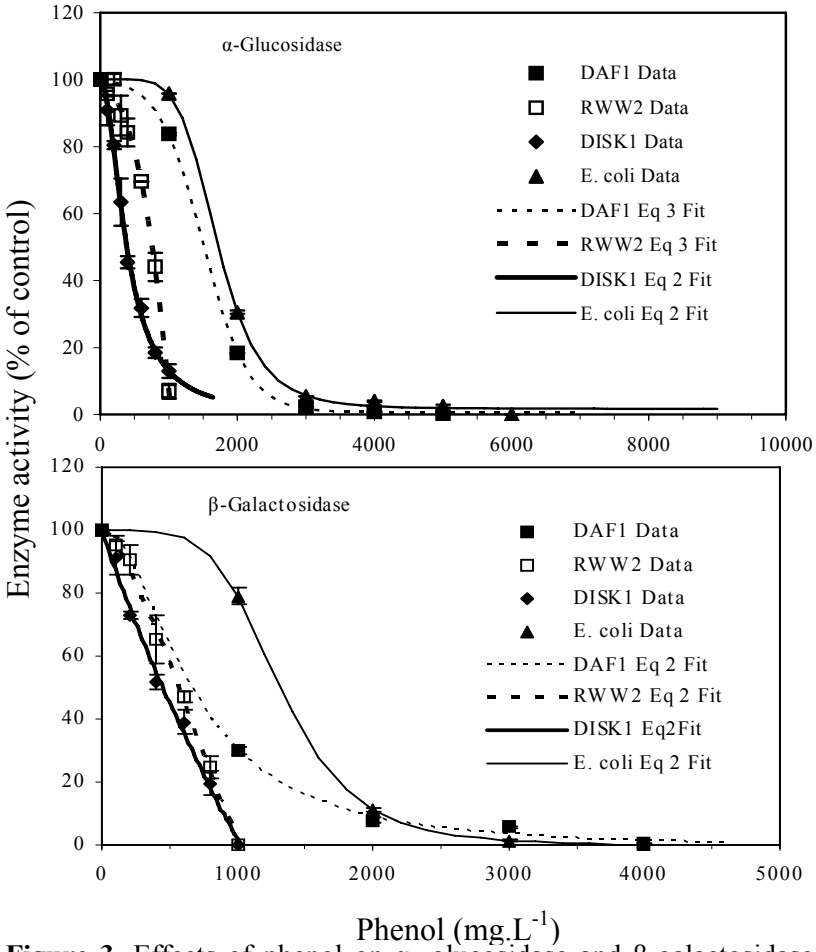

Figure 3. Effects of phenol on α- glucosidase and β-galactosidase activities of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli*. The continuous and dotted lines are predicted values determined from dose response models. Differences at enzyme activity levels between the controls and other samples were taken as phenol effect on enzyme biosynthesis.

Pseudomonas sp. DAF1 was more tolerant than *Pseudomonas* sp. RWW2 to inhibition of β-galactosidase synthesis by phenolic compounds. In *Pseudomonas* sp. DAF1, 4-chlorophenol, 4-bromophenol and 3,5-dimethylphenol completely inhibited β-galactosidase synthesis at 600, 800 and 800 $mg.L^{-1}$ respectively, and at 800 $mg.L^{-1}$, 2-chlorophenol inhibited β-galactosidase synthesis by 76.078 ± 6.479%. Like in other bacteria, inhibition of β-galactosidase synthesis in *Bacillus* sp. DISK1 by phenolic compounds is dose-dependent. However, the extent of inhibition differs from the other bacteria. *Bacillus* sp. DISK1 is more sensitive to 4-chlorophenol and total inhibition of β-galactosidase biosynthesis was observed at 80 $mg.L^{-1}$. On the other hand, 2-chlorophenol, 4-chlorophenol and 3,5-dimethylphenol completely inhibited β-galactosidase synthesis at 200, 200 and 1000 $mg.L^{-1}$ respectively. Similar responses to the toxicity of 3,5-dimethylphenol were shown by *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2 and *Bacillus* sp. DISK1. Inhibition of β-galactosidase by 3,5-dimethylphenol was sharp at lower concentrations but became less pronounced with successive increase in concentration at higher concentrations. With the exception of 2-chlorophenol, the inhibition pattern of β-galactosidase biosynthesis in *Escherichia coli* is similar to that of *Pseudomonas* sp. DAF1.

Generally, phenol was less inhibitory to enzyme biosynthesis than the substituted phenol. Higher concentrations (up to 4000 $mg.L^{-1}$) of phenol were required to inhibit β-galactosidase and α-glucosidase biosynthesis in *Escherichia coli* and *Pseudomonas* sp. DAF1. Substituted phenols are known to be more toxic than phenol. For instance, using a number of microbial

indices, chlorinated phenol was reported to be more toxic than phenol (Cenci et al., 1987). The sensitivity of these bacteria to inhibitory effect of phenolic compounds corroborates previous reports that biosynthesis of β-galactosidase was more sensitive to organic pollutants than β-galactosidase activity (Dutton et al., 1988; Reinhartz et al., 1987). Cenci et al. (1987) also reported that phenol and some chlorophenols did not inhibit β-galactosidase activity of *Escherichia coli* even at high concentration. Similar insensitivity of β-galactosidase activity to phenol has also been confirmed through a number of investigations (Bitton et al., 1992a, 1992b; Bitton and Koopman, 1994). Similarly, Barnhart and Vestal (1983) reported that *in vitro* α-glucosidase activity was relatively insensitive to pentachlorophenol, phenol and sodium dodecylsulphate. Therefore, enzyme biosynthesis is more reliable than enzyme activity as bacterial index for assessment of toxicity of organic compounds. The inhibition of β-galactosidase and α-glucosidase biosynthesis reported in this study is consistent with the report of Dutton et al. (1990).

The dose-dependent responses of all the bacterial strains are describable by logistic and sigmoid dose-response models with r^2 values ranging from 0.9791 to 0.9999. The toxicity thresholds of the phenolic compounds estimated from the models are shown in Table 1. Also, the significant difference ($P < 0.05$) of the thresholds between β-galactosidase and α-glucosidase enzymes were shown.

On the basis of the toxicity threshold, the toxicity of the phenolic compounds varies among themselves, the bacteria and enzyme. Generally, the thresholds of β-galactosidase vary significantly with that of α-glucosidase for a particular bacterium and phenolic compound. The median inhibitory concentration (IC_{50}) of phenol ranged from 387.862 ± 26.468 $mg.L^{-1}$ with *Bacillus* sp. DISK1 to 1724.492 ± 6.853 $mg.L^{-1}$ with *Escherichia coli* for α-glucosidase biosynthesis and from 450.031 ± 37.615 $mg.L^{-1}$ with *Bacillus* species to 1312.851 ± 32.237 $mg.L^{-1}$ with *Escherichia coli* for β-galactosidase. The values were consistent with toxicity thresholds reported by Dutton et al. (1990).

Quantitative structure activity relationship (QSAR) models based on K_{ow} was established for each bacterium. The octanol-water partitioning coefficient (K_{ow}) is a physicochemical descriptor that is widely used in QSARs. It indicates the ability of a chemical to partition between the aqueous phase and cell membrane. The IC_{50}s were plotted on *y*-axis versus $\log_{10}K_{ow}$ on the *x*-axis as shown in Figures 4 and 5. The $\log_{10}K_{ow}$-dependent QSAR models have the following general form:

$$IC_{50}(\text{mg/l}) = a\ \log_{10} K_{ow} + b \qquad [4]$$

A linear regression of IC_{50} ($mg.L^{-1}$) with $\log 10K_{ow}$ yields a straight line, with the slope and intercept corresponding to the constants a and b in equation 4 respectively. The octanol-water partitioning coefficient (Kow) predicted inhibition of β-galactosidase synthesis by 3,5-dimethylphenol better in Pseudomonas sp. RWW2 than in the other bacteria. As shown in Figures 4 and 5, the correlation coefficients (r^2) ranged from 0.9218 to 0.9889 for α-glucosidase and from 0.8440 to 0.9722 for β-galactosidase. The high regression coefficients indicate that the phenols possibly have the same mode of action against the induction of the enzymes, and that Kow is a strong determinant of toxicity of the phenolic compounds. The octanol-water partitioning coefficient have been adjudged the most important physicochemical parameter related to biological activity (Dearden, 1985). The r^2 values obtained in this study are comparable to those reported in QSAR analyses involving other organisms. Ren and Frymier (2002) reported r^2 values ranging from 0.69 to 0.99 when modelling toxicity of organic chemicals against bioluminescent bacteria. Schultz et al. (1998) obtained r^2 values of 0.87 when modelling the toxicity of nonpolar narcotic chemicals to bioluminescent *Vibrio fischeri*, and r^2 values of 0.69 when modelling the toxicity of phenols to *V. fischeri*. Similarly, Gül and Öztürk (1998) reported r^2 values of 0.98 and 0.54 in a $\log_{10}IC_{50}$ versus $\log_{10}K_{ow}$ relationship for aliphatic and aromatic alcohols respectively against

dehydrogenase activity of *Pseudomonas putida*. Using the models generated, the predicted IC_{50} were estimated. The relationship between the predicted IC_{50} and the experimental IC_{50} are shown in Figures 6 and 7. The values were close to unity as indicated by their closeness to the line $y = x$ in Figures 6 and 7.

Table 1. Threshold concentrations of phenolic compounds for inhibition of α- glucosidase and β-galactosidase biosynthesis in wastewater bacteria.

Bacteria/Toxicant	Toxicity thresholds ($mg.L^{-1}$)		
	IC_{20}	IC_{50}	IC_{80}
		α- glucosidase	
Pseudomonas sp. DAF1			
2-Chlorophenol	316.587 ± 44.120 b,c,e **	510.206 ± 10.502 b,c,d,e *	682.736 ± 6.029 b,c,d,e **
4-Chlorophenol	194.348 ± 30.259 a,d,e **	311.066 ± 14.952 a,c,d,e **	371.946 ± 4.476 a,c,d,e **
4-Bromophenol	156.839 ± 3.585 a,d,e **	200.569 ± 2.337 a,b,d,e **	257.578 ± 0.881 a,b,d,e **
3,5-Dimethylphenol	324.364 ± 8.313 b,c,e **	453.466 ± 6.771 a,b,c,e **	578.932 ± 3.958 a,b,c,e **
Phenol	1077.214 ± 8.313 a,b,c,d **	1515.887 ± 4.520 a,b,c,d **	1966.339 ± 0.642 a,b,c,d **
Pseudomonas sp. RWW2			
2-Chlorophenol	276.103 ± 14.509 b,c,d,e **	326.394 ± 7.692 b,c,d,e **	367.635 ± 3.078 b,c,d,e **
4-Chlorophenol	112.712 ± 0.573 a,c,d,e **	143.406 ± 4.195 a,c,e **	182.980 ± 9.981 a,c,e *
4-Bromophenol	38.596 ± 1.477 a,b,e **	73.139 ± 0.528 a,b,e **	112.801 ± 1.344 a,b,d,e **
3,5-Dimethylphenol	38.422 ± 15.481 a,b,e *	96.618 ± 21.515 a,e *	187.368 ± 14.035 a,c,e **
Phenol	478.775 ± 45.488 a,b,c,d **	757.961 ± 20.098 a,b,c,d **	936.006 ± 11.414 a,b,c,d **
Bacillus sp. DISK1			
2-Chlorophenol	99.473 ± 2.565 b,c,d,e **	271.105 ± 19.175 b,c,d,e **	558.626 ±37.686 b,c,e **
4-Chlorophenol	134.421 ± 3.155 a,c,d,e **	204.578 ± 11.141 a,c,d,e **	306.326 ± 23.620 a,d,e **
4-Bromophenol	162.756 ± 5.839 a,b,d **	234.964 ± 4.954 a,b,d,e **	337.664 ± 2.473 a,d,e **
3,5-Dimethylphenol	303.104 ± 4.939 a,b,c,e **	440.241 ± 10.532 a,b,c **	579.313 ± 19.532 b,c,e **
Phenol	191.416 ± 26.137 a,b,d *	387.862 ± 26.468 a,b,c *	779.184 ± 45.821 a,b,c,d *
Escherichia coli			
2-Chlorophenol	634.982 ± 16.437 b,c,d,e **	836.175 ± 9.685 b,c,d,e **	965.745 ± 3.222 b,c,d,e **
4-Chlorophenol	94.856 ± 21.675 a,c,d,e *	161.187 ± 14.096 a,c,d,e *	302.438 ± 8.382 a,c,d,e **
4-Bromophenol	162.756 ± 5.839 a,b,d,e **	234.964 ± 4.954 a,b,d,e **	337.664 ± 2.473 a,b,d,e **
3,5-Dimethylphenol	303.774 ± 5.851 a,b,c,e **	440.402 ± 10.895 a,b,c,e **	579.353 ± 19.560 a,b,c,e **
Phenol	1348.403 ± 5.581 a,b,c,d **	1724.492 ± 6.853 a,b,c,d **	2222.688 ± 9.469 a,b,c,d **
		β- galactosidase	
Pseudomonas sp. DAF1			
2-Chlorophenol	160.798 ± 16.087 b,c,d,e **	468.424 ± 30.312 b,c,d,e *	806.047 ± 34.854 b,c,d,e **
4-Chlorophenol	67.240 ± 14.596 a,c,e **	154.676 ± 19.042 a,c,e **	267.526 ± 13.756 a,c,d,e **
4-Bromophenol	28.904 ± 2.586 a,b,d,e **	82.985 ± 4.781 a,b,d,e **	219.806 ± 6.767 a,b,d,e **
3,5-Dimethylphenol	50.880 ± 7.932 a,c,e **	161.279 ± 11.581 a,c,e **	390.342 ± 11.311 a,b,c,e **
Phenol	323.299 ± 12.597 a,b,c,d **	653.448 ± 0.625 a,b,c,d **	1298.329 ± 42.081 a,b,c,d **
Pseudomonas sp. RWW2			
2-Chlorophenol	123.198 ± 8.049 b,c,d,e **	228.988 ± 7.415 b,c,d,e **	292.399 ± 2.929 b,c,d,e **
4-Chlorophenol	57.274 ± 8.659 a,d,e **	118.120 ± 9.443 a,c,d,e **	173.682 ± 5.979 a,c,d,e *
4-Bromophenol	44.676 ± 2.724 a,d,e **	94.414 ± 5.884 a,b,e **	150.418 ± 6.111 a,b,d,e **
3,5-Dimethylphenol	14.408 ± 2.426 a,b,c,e *	83.482 ± 6.356 a,b,e *	311.217 ± 9.134 a,b,c,e **
Phenol	285.813 ± 57.893 a,b,c,d **	564.407 ± 41.753 a,b,c,d **	827.755 ± 16.975 a,b,c,d **
Bacillus sp. DISK1			
2-Chlorophenol	45.609 ± 9.798 e **	105.847 ± 9.214 b,c,e **	166.424 ± 4.617 b,c,d,e **
4-Chlorophenol	28.451 ± 6.868 e **	53.438 ± 5.712 a,c,d,e **	70.760 ± 2.421 a,c,d,e **
4-Bromophenol	32.414 ± 3.903 e **	82.696 ± 4.830 a,b,d,e **	143.191 ± 3.157 a,b,d,e **
3,5-Dimethylphenol	32.624 ± 4.584 e **	125.283 ± 10.270 b,c,e **	382.735 ± 19.384 a,b,c,e **
Phenol	163.780 ± 27.901 a,b,c,d *	450.031 ± 37.615 a,b,c,d *	778.131 ± 27.742 a,b,c,d *
Escherichia coli			
2-Chlorophenol	73. 611 ± 18.945 d,e **	169.641 ± 21.668 d,e **	368.240 ± 14.977 b,c,d,e **
4-Chlorophenol	103.465 ± 4.706 c,e *	171.614 ± 7.942 d,e *	280.343 ± 10.939 a,d,e **
4-Bromophenol	86.197 ± 2.506 b,d,e **	161.977 ± 3.893 d,e **	306.320 ± 7.452 a,d,e **
3,5-Dimethylphenol	144.915 ± 3.198 a,b,c,e **	291.119 ± 5.125 a,b,c,e **	453.126 ± 8.348 a,b,c,e **
Phenol	987.639 ± 32.181 a,b,c,d **	1312.851 ± 32.237 a,b,c,d **	1741.889 ± 30.200 a,b,c,d **

At $p < 0.05$ (within each threshold, bacterial strain and enzyme)
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 Phenol
** = threshold of α- glucosidase is significantly different from that of β- galactosidase
* = threshold of α- glucosidase is not significantly different from that of β- galactosidase

There was poor correlation between pKa (the negative logarithm of the first ionization constant) and the IC_{50} (plots are not shown). This indicates that the toxicity of the phenols is independent on their ionization constants. Ren and Frymier (2002) have made similar observation in a QSAR analysis of organic chemicals against *Vibrio fischeri*.

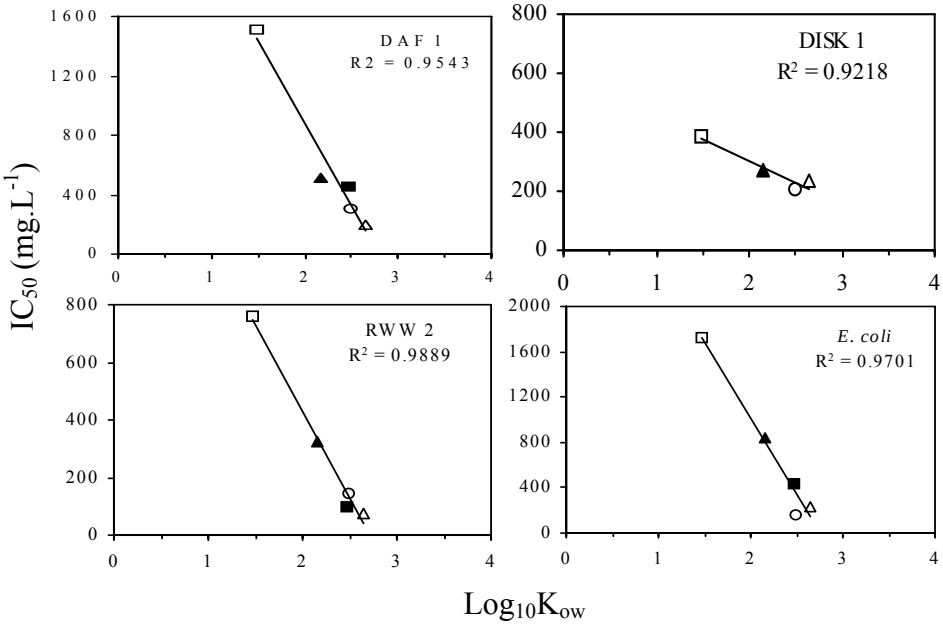

Figure 4. Acute toxicity of five phenols against Log K_{ow} for α-glucosidase activity of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli*. The linear regression relationships are based on: phenol □; 2-chlorophenol ▲; 3,5-dimethylphenol ■; 4-bromophenol Δ and 4-chlorophenol ○.

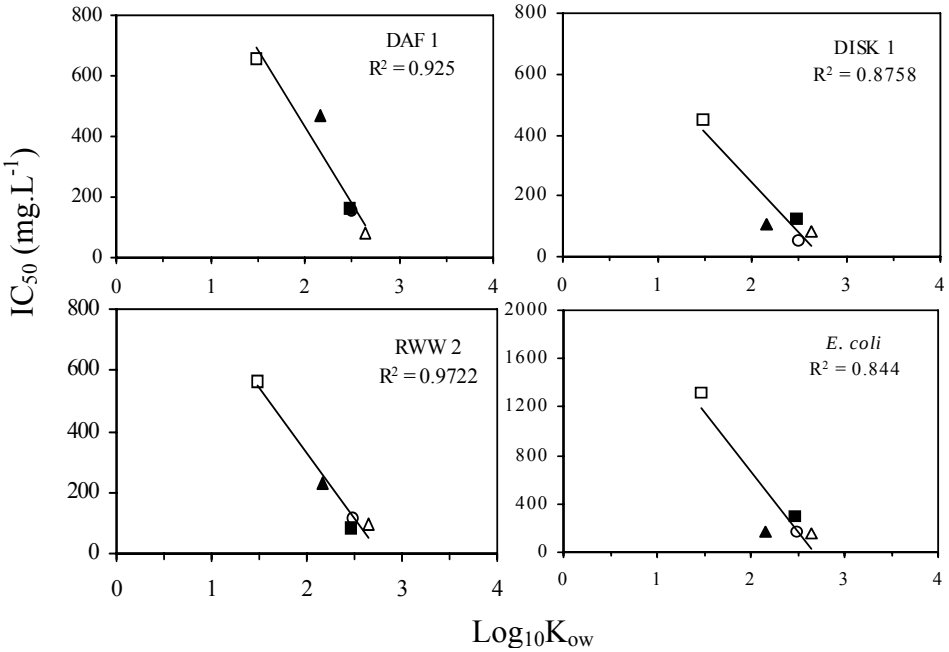

Figure 5. Acute toxicity of five phenols against $Log_{10}K_{ow}$ for β-galactosidase activity of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli*. The linear regression relationships are based on: phenol □; 2-chlorophenol ▲; 3,5-dimethylphenol ■; 4-bromophenol Δ and 4-chlorophenol ○.

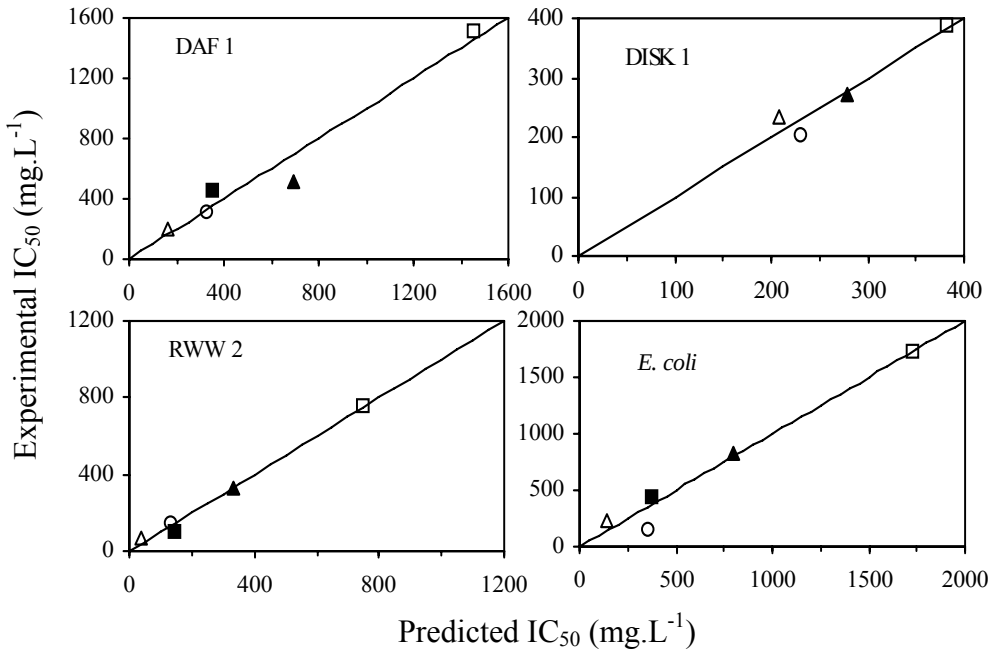

Figure 6. Relationship between experimental and predicted toxicity of five phenols for α-glucosidase activity of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli*. Data represents: phenol □; 2-chlorophenol ▲; 3,5-dimethylphenol ■; 4-bromophenol Δ and 4-chlorophenol○.

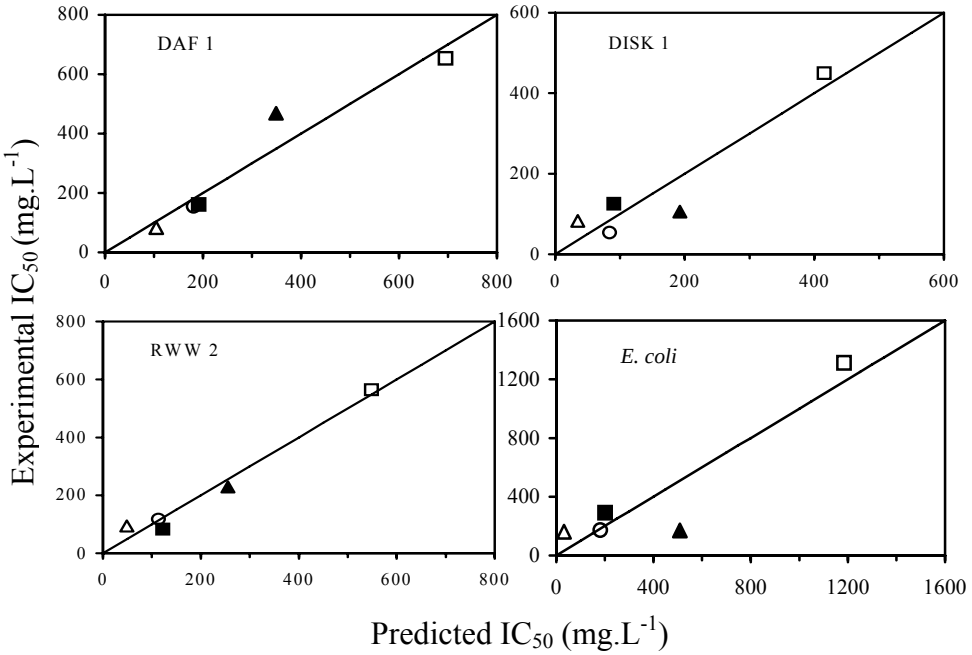

Figure 7. Relationship between experimental and predicted toxicity of five phenols for β-galactosisidase activity of *Pseudomonas* sp. DAF1, *Pseudomonas* sp. RWW2, *Bacillus* sp. DISK1 and *Escherichia coli*. Data represents: phenol □; 2-chlorophenol ▲; 3,5-dimethylphenol ■; 4-bromophenol Δ and 4-chlorophenol○.

4. CONCLUSION

This study has shown that induction of α-glucosidase and β-galactosidase enzymes in *Escherichia coli*, *Bacillus* and *Pseudomonas* species could be inhibited by phenolic compounds to levels dependent on octanol–water partition coefficient. Thus, the organisms may serve as convenient indices for assessment of toxicity of environmental pollutants and by extrapolation, the risk assessment of industrial wastes.

5. REFERENCES

AGARRY, S. E.; SOLOMON, B. O. Kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescence*. **Int. J. Environ. Sci. Tech.**, v. 5, p. 223 – 232, 2008.

AGARRY, S. E.; SOLOMON, B. O.; LAYOKUN, S. K. Substrate inhibition kinetics of phenol degradation by binary mixed culture of *Pseudomonas aeruginosa* and *Pseudomonas fluorescence* from steady state and wash-out data. **Afr. J. Biotechnol.,** v. 7, n. 21, p. 3927 – 3933, 2008.

ANDERSON, K.; KOOPMAN, B.; BITTON, G. Evaluation of INT-dehydrogenase assay for heavy-metal inhibition of activated sludge. **Water Res.,** v. 22, p. 349 – 353, 1988. http://dx.doi.org/10.1016/S0043-1354(88)90220-5

APTULA, A. O.; NETZEVA, T. I.; VALKOVA, I. V.; CRONIN, M. T. D.; SCHULTZ, T. W.; KÜHNE, R.; SCHÜÜRMANN, G. Multivariate discrimination between modes of toxic action of phenols. **Quant. Struct.-Act. Relat.,** v. 21, p. 12 – 22, 2002. http://dx.doi.org/10.1002/1521-3838(200205)21:1%3C12::AID-QSAR12%3E3.0.CO;2-M

BARNHART, C. L.; VESTAL, J. R. Effect of environmental toxicants on metabolic activity of natural microbial communities. **Appl. Environ. Microbiol.,** v. 46, p. 970 – 977, 1983.

BITTON, G.; KOOPMAN, B. Evaluation of a microplate assay specific for heavy metal toxicity. **Arch. Environ. Contam. Toxicol.,** v. 27, p. 25 – 28, 1994. http://dx.doi.org/10.1007/BF00203883

BITTON, G.; KOOPMAN, B.; AGAMI, O. MetPAD™: a bioassay for rapid assessment of heavy metal toxicity in wastewater. **Water Environ. Res.,** v. 64, p. 834 – 836, 1992a.

BITTON, G.; CAMPBELL, M.; KOOPMAN, B. MetPAD™: a bioassay kit for the specific determination of heavy metal toxicity in sediments from hazardous waste sites. **Environ. Toxicol. Water Qual.,** v. 7, p. 323 – 328, 1992b. http://dx.doi.org/10.1002/tox.2530070403

BROECKER, B.; ZAHN, R. The performance of activated sludge plants compared with the results of various bacterial toxicity tests – A study with 3,5-dichlorophenol. **Water Res.,** v. 11, p. 165 – 172, 1977. http://dx.doi.org/10.1016/0043-1354(77)90122-1

BROWN, D.; HITZ, H. R.; SCHAFER, L. The assessment of the possible inhibitory effect of dyestuff on aerobic wastewater bacteria- Experience with a screening test. **Chemosphere,** v. 10, p. 245 – 261, 1981. http://dx.doi.org/10.1016/0045-6535(81)90025-4

CENCI, G.; CALDINI, G.; MOROZZI, G. Chlorinated phenol toxicity by bacterial and biochemical tests. **Bull. Environ. Contam. Toxicol.,** v. 38, p. 868 – 875, 1987.

CHOI, S. H.; GU, M. B. Phenolic toxicity: detection and classification through the use of a recombinant bioluminescent *Escherichia coli*. **Environ. Toxicol. Chem.,** v. 20, p. 248 – 255, 2001.

CODINA, J. C.; PEREZ-GARCIA, A.; DE VICENTE, A. Detection of a heavy metal toxicity and genotoxicity in wastewater by microbial assay. **Water Sci. Tech.,** v. 30, p. 145 – 151, 1994.

DEARDEN, J. C. Partitioning and lipophilicity in quantitative structure-activity relationships. **Environ. Health Perspect.,** v. 61, p. 203 – 228, 1985. http://dx.doi.org/10.2307/3430073

DUTKA, B. J.; NYHOLM, N.; PETERSEN, J. Comparison of several microbiological toxicity screening tests. **Water Res.,** v. 17, p. 1363 – 1368, 1983. http://dx.doi.org/10.1016/0043-1354(83)90265-8

DUTTON, R. J.; BITTON, G.; KOOPMAN, B. Enzyme biosynthesis versus enzyme activity as a basis for microbial toxicity testing. **Tox. Assess.,** v. 3, p. 245 – 253, 1988. http://dx.doi.org/10.1002/tox.2540030302

DUTTON, R. J.; BITTON, G.; KOOPMAN, B.; AGAMI, O. Effect of environmental toxicants on enzyme biosynthesis: a comparison of β-galactosidase, α-glucosidase and tryptophanase. **Arch. Environ. Contam. Toxicol.,** v. 19, p. 395 – 398, 1990.

GENDIG, C.; DOMOGALA, G.; AGNOLI, F.; PAGGA U.; STROTMANN, U. J. Evaluation and further development of the activated sludge respiration inhibition test. **Chemosphere,** v. 52, p. 143 – 149, 2003. http://dx.doi.org/10.1016/S0045-6535(03)00111-5

GOUDAR, C. T.; GANJI, S. H.; PUJAR, B. G.; STREVETT, K. A. Substrate inhibition kinetics of phenol biodegradation. **Wat. Environ. Res.,** v. 72, p. 50 – 55, 2000. http://dx.doi.org/10.2175/106143000X137103

GÜL, S.; ÖZTÜRK, D. Determination of the structure-toxicity relationship of amphiprotic compounds by means of the inhibition of the dehydrogenase activity of *Pseudomonas putida*. **Turkish J. Chem.,** v. 22, p. 341– 349, 1998.

GUVEN, K.; TOGRUL, S.; UYAR, F.; OZANT, S.; DE POMERAI, D. I. A comparative study of bioassays based on enzyme biosynthesis in *Escherichia coli* and *Bacillus subtilis* exposed to heavy metals and pesticides. **Enzyme Microbial Technol**., v. 32, p. 658 – 664, 2003. http://dx.doi.org/10.1016/S0141-0229(03)00025-5

JULIASTUTI, S. R.; BAEYENS, J.; CREEMERS, C. Inhibition of nitrification by heavy metals and organic compounds: The ISO 9509 test. **Environ. Eng. Sci.,** v. 20, n. 2, p. 79-90, 2003. http://dx.doi.org/10.1089/109287503763336511

KATAYAMA-HIRAYAMA, K. Inhibition of activities of β-galactosidase and dehydrogenases of activated sludge by heavy metals. **Water Res.,** v. 20, p. 491 – 494, 1986. http://dx.doi.org/10.1016/0043-1354(86)90198-3

KING, E. F.; PAINTER, H. A. Inhibition of respiration of activated sludge: variability and reproducibility of results. **Toxic. Assess.,** v. 1, p. 27 – 39, 1986. http://dx.doi.org/10.1002/tox.2540010104

KUMAR, A.; KUMAR, S.; KUMAR, S. Biodegradation kinetics of phenol and catechol using *Pseudomonas putida* MTCC 1194. **Biochem Eng J.,** v. 22, p. 151 – 159, 2005. http://dx.doi.org/10.1016/j.bej.2004.09.006

NWEKE, C. O.; OKPOKWASILI, G. C., Influence of exposure time on phenol toxicity to refinery wastewater bacteria. **J. Environ. Chem. Ecotoxicol.,** v. 2, n. 2, p. 20 – 27, 2010.

NWEKE, C. O.; OKPOKWASILI, G. C. Inhibition of β-galactosidase and α-glucosidase synthesis in petroleum refinery effluent bacteria by zinc and cadmium. **J. Environ. Chem. Ecotoxicol.,** v. 3, n. 3, p. 68 – 74, 2011.

OBOIRIEN, B. O.; AMIGUN, B.; OJUMU, T. V.; OGUNKUNLE, O. A.; ADETUNJI, O. A.; BELIKU, E.; SOLOMON, B. O. Substrate inhibition kinetics of phenol degradation by *Pseudomonas aeruginosa* and *Pseudomonas fluorescence*. **Biotechnol.,** v. 4, n. 1, p. 56 – 61, 2005.

ODOKUMA, L. O.; OKPOKWASILI, G. C. Bacterial enzyme biosynthesis: a tool for ecotoxicity assay. **Global J. Pure and Appl. Sciences,** v. 9, n. 3, p. 311 – 318, 2003.

OKOLO, J. C.; NWEKE, C. O.; NWABUEZE, R. N.; DIKE, C. U.; NWANYANWU, C. E. Toxicity of phenolic compounds to oxidoreductases of *Acinetobacter* species isolated from a tropical soil. **Scientific Res. Essay,** v. 2, n. 7, p. 244 – 250, 2007.

OKPOKWASILI, G. C.; NWEKE, C. O. Microbial growth and substrate utilization kinetics. **African J. Biotechnol.,** v. 5, n. 4, p. 305 – 317, 2006.

PILL, K. G.; KUPILLAS, G. E.; PICARDAL, F. W.; ARNOLD, R. G. Estimating the toxicity of chlorinated organic compounds using a multiparameter bacterial bioassay. **Environ. Toxicol. Water Qual.,** v. 6, p. 271 – 291, 1991. http://dx.doi.org/10.1002/tox.2530060302

REINHARTZ, A.; LAMPERT, I.; HERZBERG, M; FISH, F. A new short-term, sensitive bacterial assay kit for the detection of toxicants. **Toxicol. Assess.,** v. 2, p. 193 – 206, 1987. http://dx.doi.org/10.1002/tox.2540020207

REN, S.; FRYMIER, P. D. Estimating the toxicities of organic chemicals to bioluminescent bacteria and activated sludge. **Water Research,** v. 36, p. 4406 – 4414, 2002. http://dx.doi.org/10.1016/S0043-1354(02)00153-7

SARAVANAN, P.; PAKSHIRAJAN, K.; SAHA, P. Growth kinetics of an indigenous mixed microbial consortium during phenol degradation in a batch reactor. **Bioresource Technol.,** v. 99, p. 205 – 209, 2008. http://dx.doi.org/10.1016/j.biortech.2006.11.045

SCHULTZ, T. W.; SINKS, G. D.; BEARDEN, A. P. QSAR in aquatic toxicology: a mechanism of action approach comparing toxic potency to *Pimephales promelas*, *Tetrahymena pyriformis* and *Vibrio fischeri*. In: DEVILLERS, J. (Ed). **Comparative QSAR.** London: Taylor and Francis, 1998. p. 51 – 109.

STROTMANN, U. J.; BUTZ, B.; BIAS, W. R. The dehydrogenase assay with resazurin: preactical performance as a monitoring system and pH-dependent toxicity of phenolic compounds. **Ecotoxicol. Environ. Saf.,** v. 25, p. 79 – 89. 1993. http://dx.doi.org/10.1006/eesa.1993.1009

STROTMANN, U. J.; EGLSÄER, H. The toxicity of substituted phenols in the nitrification inhibition test and luminescent bacteria test. **Ecotoxicol. Environ. Saf.,** v. 30, p. 269 – 273, 1995. http://dx.doi.org/10.1006/eesa.1995.1030

STROTMANN, U. J.; EGLSÄER, H., PAGGA, U. Development and evaluation of a growth inhibition test with sewage bacteria for assessing bacterial toxicity of chemical compounds. **Chemosphere,** v. 28, p. 755 – 766, 1994. http://dx.doi.org/10.1016/0045-6535(94)90229-1