Journal of Research in Biology

An International Scientific Research Journal

Original Research

Biodegradation of phenol at low and high doses by bacterial strains indigenous to Okrika River in the Niger Delta of Nigeria

Authors:
Nwanyanwu CE [1*]
Abu GO[2].

Institution:
1.Department of Microbiology, Federal university of Technology, P.M.B.1526, Owerri, Nigeria.

2.Department of Microbiology, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.

Corresponding author:
Nwanyanwu CE.

Email:
cnwanyanwu2000@yahoo.com

Web Address:
http://jresearchbiology.com/documents/RA0318.pdf.

ABSTRACT:

Assessments on biodegradation at low and high doses of phenol by bacterial strains indigenous to Okrika River in Niger Delta of Nigeria were carried out. Growth at low dose of 0.01 µg/l phenol showed that highest and lowest cell density values of OD_{540nm} of 0.15 and 0.09 in *Pseudomonas* sp. SD1 and *Citrobacter* sp. RW1 while at 1.0 µg/l phenol concentration the highest cell density values of OD_{540nm} of 0.28 was observed in *Staphylococcus* sp. RW2. The highest specific growth rate of 0.019 h^{-1} at 500 mg/l of phenol was obtained for *Pseudomonas* sp. SD1 while *Citrobacter* sp. RW1 had the lowest specific growth rate of 0.014 h^{-1} at 500 mg/l of phenol. The specific phenol degradation rate ranges from 55.35 to 130.98 mg/(L.h.OD). The order of specific phenol consumption rate at 1000 mg/l by the organisms is: *Bacillus* sp. SD3>*Pseudomonas* sp. SD1>*Citrobacter* sp. RW1>*Staphylococcus* sp. RW2. *Citrobacter* sp. RW1 exhibited highest growth yield in 250 mg/l of phenol with the growth yield of 6.24 (x 10^{-4} A_{540} unit.l/mg). The results showed that the test organisms might be the most suitable bacterial strains for removal of phenols at low and high doses in phenolic polluted media.

Keywords:
Biodegradation, phenol, bacteria, Okrika River.

Article Citation:
Nwanyanwu CE and Abu GO.
Biodegradation of phenol at low and high doses by bacterial strains indigenous to Okrika River in the Niger Delta of Nigeria.
Journal of Research in Biology (2013) 3(3): 911-921

Dates:
Received: 26 Dec 2012 **Accepted:** 17 Jan 2013 **Published:** 06 May 2013

INTRODUCTION

Contamination of aquatic environment brought about by the discharge of wastewater resulting from anthropogenic activities clearly continues to be a major environmental issue. Effluents are very important sources of chemicals entering aquatic ecosystems. They may contain hundreds, or even thousands of chemicals, but only a few of them are responsible for effluent toxicity (Tisler *et al.,* 1999). High strength wastewaters have been reported to be associated with chemical processing industries. Wastewaters generated from these processing industries such as petrochemical, oil refineries, coke-processing plants, etc contain a large number of organic and inorganic pollutants at high concentrations that exhibit adverse effect on the environments when released (Papadimitriou *et al.,* 2009). The presence of high level of these contaminants formed the major pollutant in the water body as a result of continuous discharge of effluents by industries into the ecosystem. In water these pollutants of the discharged effluent sorbs onto particulate materials and if not degraded eventually end up in sediments. As an ultimate respiratory of most xenobiotic contaminants that enter water bodies, sediments act as both carrier and sources of contaminants in aquatic environment (Akan *et al.,* 2010). Thus, the contaminated sediments may represent a continual threat of recontamination of the aquatic environment as the adsorbed pollutants if not degraded, in turn lead to the exposure of aquatic life to organic pollutants such as phenol (Mort and Dean-Ross, 1994). On the other hand, the release of contaminants from sediments could increase the amount of toxic compounds in the waters making them more available to organisms and affecting their life cycles, reproduction, metabolism and physiology. Microorganisms being ubiquitous in nature exploit many carbon and energy sources in its niche for growth. Several species of micro-organisms inhabiting hostile ecological niche have been reported by Colwell and Walker (1977), Atlas (1981), Heinaru *et al.* (2000) and Polymenakou and Stephanou (2005). Microorganisms indigenous to aquatic environment are crucial for the biodegradation of organic matter and the cycling of nutrients, while these microorganisms are susceptible to toxic pollutants from industrial effluent discharges, especially petroleum refinery. Therefore, perturbations of aquatic microbial communities could have consequences for the higher trophic levels and for the overall aquatic environment.

The composition of effluents from petroleum refineries varies according to their origin, storage and treatments as these wastewaters are enriched with different pollutants. Phenol and its derivatives along with other organic and inorganic compounds is one of the most common contaminants present in refinery effluents (Jena *et al.,* 2005) which renders refinery effluents its toxic nature. Phenols as constituents of industrial effluents may remain in water body for much longer period if it is continually or consistently released into the aquatic environments from sources thereby increasing its elevation in the environment. The toxic nature of phenol and its derivatives to microbial cells is well documented (Kahru *et al.,* 2002; Keweloh *et al.,* 1990). Owing to toxic nature of phenol, its contact with microorganisms always results in the decrease of microbial enzyme activity (Nwanyanwu and Abu, 2011) as well as leading to death of organisms at higher concentration.

A large number of microbial genera possess the capability to degrade organic pollutants. Among the bacterial genera implicated in the degradation of phenol include *Pseudomonas, Bacillus, Corynebacterium* species etc. The ability of organisms to degradation phenol and other toxicants is related to adaptation of the microorganisms to the compound of concern and adaptation is associated with synthesis of new enzymes capable of transformation of the toxicant to harmless substances (Jaromir and Wirgiliusz, 2007). The resultant effect of biodegradation of phenol and other organic compounds is growth as the organic pollutants are used

as the source of carbon and energy.

This research assessed the growth and utilization of phenol at low and high doses by bacterial strains indigenous to Okrika River in the Niger Delta of Nigeria.

MATERIALS AND METHODS

Chemical reagents

All chemical reagents used in the study were of analytical grade and were obtained from sigma chemical company, St Louis Missouri, USA, BDH chemicals, Poole, England and HACH chemical company.

Sample collection and analysis

The Okrika River is a small tidal river that empties into Bonny estuary in Niger Delta of Nigeria. The River is highly polluted as a result of effluent discharges from Port Harcourt petroleum refinery industry sited along its bank (IAIA09 Conference Proceeding, 2009). Sediment and water samples were collected from the river as described by Nweke *et al.,* (2007) and the samples analyzed within few hours of collection. The results of the physicochemical analysis of the samples are as shown in Table 1.

Isolation and identification of bacterial strains

The bacterial strains used in this work were isolated from the samples by spreading one tenth of decimally diluted sediment suspension and water samples on mineral salt agar-phenol (2.5 mM) medium and the isolated organisms identified as described elsewhere (Nwanyanwu *et al.,* 2012). The isolates were designated according to their sources (RW for River water, SD for sediment) and were then maintained on nutrient agar slants.

Preparation of inoculum

The bacterial strains used for the assay were grown in 100 ml of sterile nutrient broth media for 48 h. The turbid culture medium were harvested, washed and suspended in deionized distilled water then followed by standardization of the suspensions spectrophotometrically to an optical density of 0.4 at 540 nm and used as inocula.

Assay for isolates growth in very low concentrations of phenol

The ability of the isolates to grow and utilize phenol at low concentrations (0-1.0 µg/l) was assessed in sterile Bushnell Haas (BH) mineral salt broth medium. The assay was carried out as described by Nwanyanwu *et al.,* (2012) with little modification. The medium without agar was used instead for the assay. After inoculation of the flasks, growth profile of the organisms was monitored by the optical density (OD_{540nm}) on daily basis.

Growth and biodegradation of phenol at high concentration

Degradation of phenol at high concentration by the organisms was carried out in sterile BH medium contained Erlenmeyer flasks. The flasks were supplemented with aliquot of sterile phenol (2000 mg/l) to bring the final phenol concentrations in the flasks to 250, 500, 750 and 1000 mg/l. The flasks after inoculation with the test organisms were incubated at 30^{o}C in an incubator. At predetermined time, samples were withdrawn to determine cell growth and phenol concentration. Controls, one without phenol and another without cells in BH medium were set up. At predetermined time, samples were removed and used to measure for cell growth (optical density, OD_{540nm}) and

Table 1: Physicochemical characteristics of Okrika River

Parameter/unit	Sample source	
	Water	Sediment
pH	8.90	6.90
Elect. conduc. (µscm^{-1})	364	615
Oil and grease (mg/l)	16.0	103.0
BOD (mg/l)	8.16	-
COD (mg/l)	84.0	-
PO_4 (mg/l)	0.15	0.90
SO_4 (mg/l)	118	117
Phenol (mg/l)	6.1	15.5
Zn (mg/l)	0.03	3.48
Cu (mg/l)	<0.01	0.06
Pb (mg/l)	<0.01	<0.01

Table 2: Yield factor (Y) of biomass after growth at different initial phenol concentrations

Bacteria	Yield factor, Y(x 10^{-4} A_{540} units[a]. l/mg)			
	Phenol concentration (mg/l)			
	250	500	750	1000
Citrobacter sp. RW1	6.24	4.46	2.69	3.11
Staphylococcus sp.RW2	4.96	3.80	3.28	3.00
Pseudomonas sp. SD1	4.96	3.80	3.28	3.00
Bacillus sp. SD3	3.28	4.46	2.69	3.11
[a] A_{540} units = optical density at 540 nm				

phenol residue (4-amino antipyrine) in cell free samples.

Analytical methods

Cell growth was determined spectrophotometrically while phenol was analyzed by photometric method using 4-aminoantipyrine as the colouring agent and measuring the absorbance at 500 nm (Folsom *et al.,* 1990).

Data Analysis

Specific growth rate

The specific growth rate (μ) for each concentration of phenol was calculated from the slope of linear logarithmic plots of optical density against time as expressed in equation 1 (Gokulakrishnan and Gummadi, 2006):

$$\mu = \frac{In(X_2/X_1)}{t_2 - t_1} \qquad 1$$

Specific degradation rate

The specific degradation rate (Q_s) was determined through the relationship of equation 2 (Loh and Wang, 1998):

$$Q_s = \frac{d[Ph]/dt}{X} \qquad 2$$

Where: [Ph] denotes phenol concentration (mg/l), t denotes incubation time (h) and X denotes cell concentration (optical density, $OD_{540\,nm}$).

Yield factor

Yield factor (Y) of the biomass was calculated using equation 3 (Bajaj *et al.,* 2009):

$$Y = -\frac{dX}{dS} \qquad 3$$

Where dX is the change in cell biomass related to the change in substrate concentration dS. X was replaced with the OD at 540 nm.

RESULTS AND DISCUSSION

The phenol content of Okrika River water and sediment were 6.1 and 15.5 mg/l while oil and grease of the River water and sediment were 16.0 and 103.0 mg/l respectively (Table 1). This level of oil and grease as well as phenol in the River water and sediment were much higher than the previously reported levels of 10.56 and 15.23 mg/l (oil and grease) and 5.13 and 16.0 mg/l (phenol) (Otokunefor and Obiukwu, 2005). This indicated that these compounds have accumulated in Okrika River over time and pose the major pollutants of the river.

Figure 1 shows the growth of the test organisms in low concentration of phenol amended mineral salt medium. All the organisms showed progressive growth in low phenol concentration medium. Highest growth of the organisms was observed in phenol concentration of 1.0 μg/l followed by 0.1 μg/l. The least growth was observed in 0.01 μg/l. Among the test organisms, *Staphylococcus* sp. RW2 showed the highest growth in 0.1 and 1.0 μg/l of phenol with optical density (OD) values of 0.23 and 0.28 respectively while *Citrobacter* sp. RW1 showed the least growth in all the low concentrations (0.01, 0.1 and 1.0 μg/l) of phenol amended medium with OD values of 0.09, 0.11 and 0.13 respectively. Growth of microorganisms especially bacterial species at phenol concentration as low as microgram per litre have been reported by many authors. Chesney *et al.,* (1985) have reported growth of water microorganism in water sample supplemented with 0.001 to 1.0 μg/ml of phenol. Also Goldstein *et al.,* (1985) have reported the growth of *Pseudomonas* sp. in a

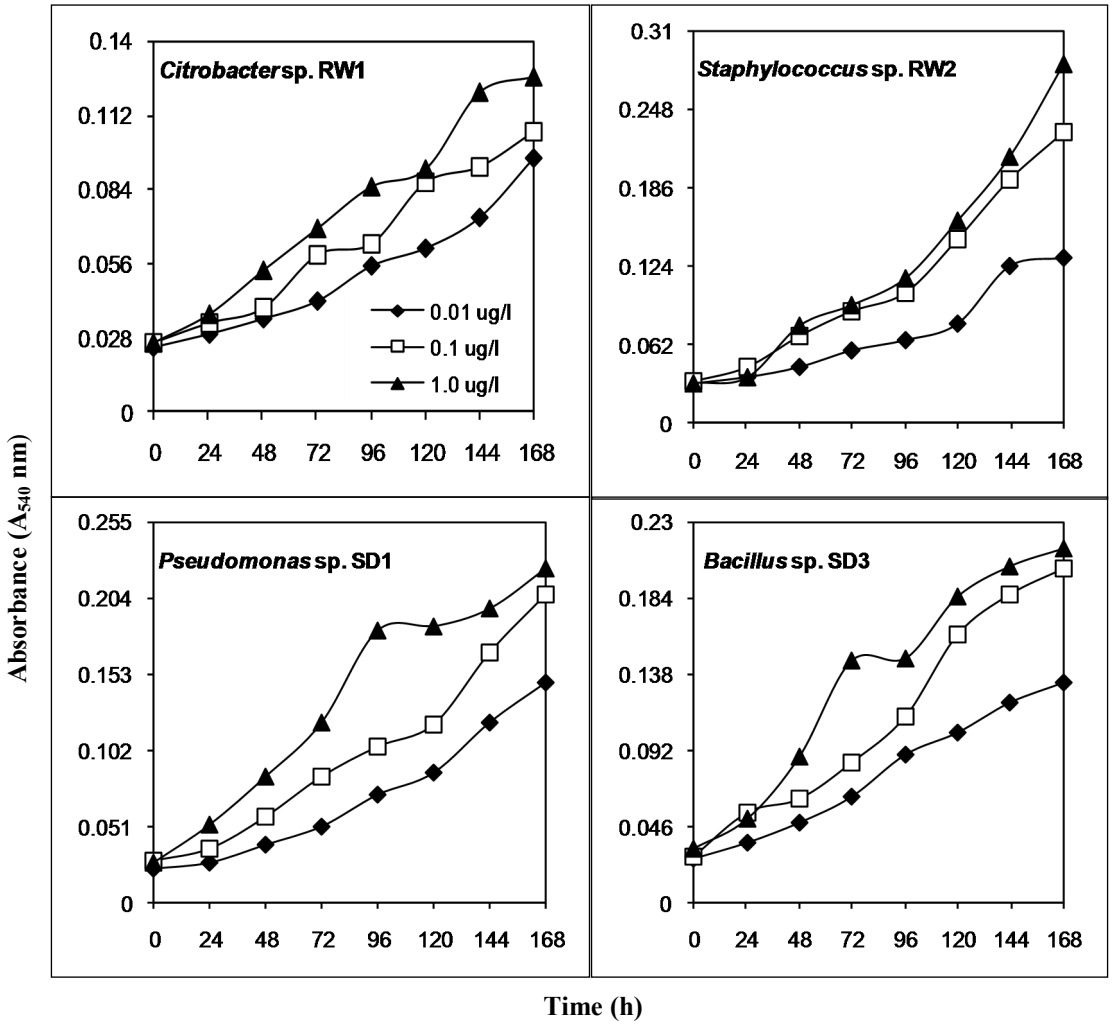

Figure 1: Growth profile of the bacteria in mineral salt medium fortified with phenol concentrations

medium amended with 1.0 and 10.0 µg/l concentration of 2, 4-dichlorophenol. Pahm and Alexander (1993) found that *Pseudomonas* sp. K, *Flavobacterium* sp. M4, *Flavobacterium* sp. M1 and *Pseudomonas* sp. SP3 grown in p-nitrophenol (PNP) of concentration of 0.1 µg/l reached a total viable count of 10^5 and 10^6 cells/ml.

Figures 2 and 3 showed typical profiles of cell growth and biodegradation of phenol at high concentrations by bacterial strains of Okrika River ranging from 250 to 1000 mg/l. The lag phase of the organisms in phenol fortified medium was short. The short in lag phase period depends on the pre-exposure of the organism. Phenol was completely utilized by the isolates within 180 h of incubation. Phenol concentrations of 500, 750 and 1000 mg/l was degraded completely within 96, 132 and 156 h by *Pseudomonas* sp. SD1 while same concentrations of phenol was degraded completely within 108, 144 and 180 h by other test organisms. Time-dependent degradation of organic compounds has been reported to be linked with concentration of the organic compound as observed by many authors (Colwell and Walker, 1977; Kotresha and Vidyasagar, 2008). This may be due to changes in the transport mechanism of the substrate across the cell membrane in response to high phenol concentration hence diminished capacity to catabolize phenol. This is in line with the reports of Gilbert and Brown (1978), Keweloh *et al.,* (1990), Collins and Daugulis (1997) and

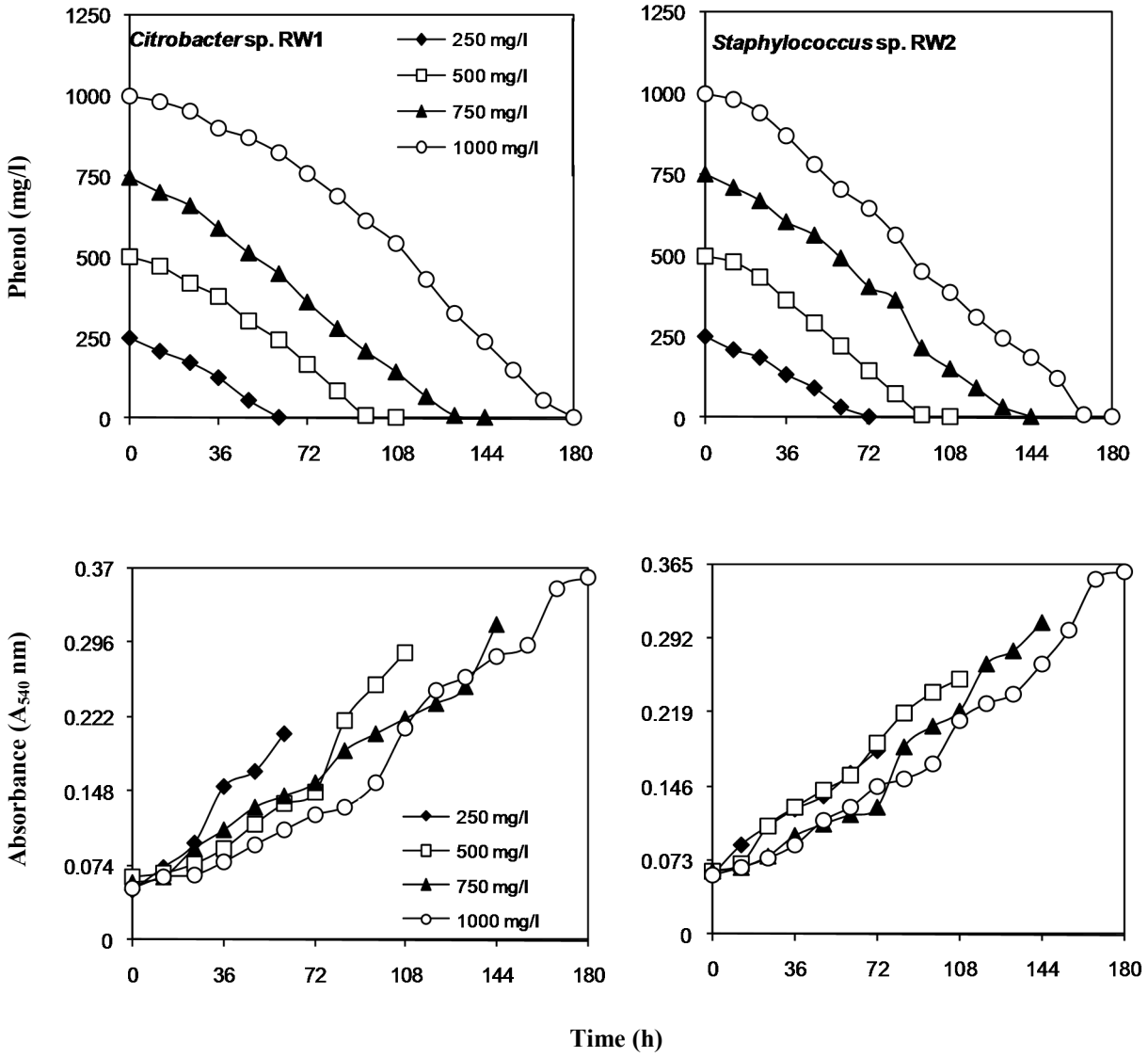

Figure 2: Biodegradation and cell growth profile of planktonic bacteria of Okrika River in high phenol concentrations

Nwanyanwu and Abu (2011) who observed the toxic effect of phenol at the membrane level, thereby disrupting the activity of enzymes in phenol-utilizing bacteria. Also, Joseph and Joseph (1999) and Ye and Shen (2004) reported that phenol toxicity depends on the sensitivity as well as source of organism.

The growth profiles of the pure cultures expressed as optical density and phenol residues at different initial concentrations are shown in figures 2 and 3. The cells gradually increase in number as the phenol residues of the medium progressively decreased. This may be due to high phenol concentration made available more carbon to the organism for growth. *Pseudomonas* sp. SD1 degraded 1000 mg/l of phenol in 160 h with a cell biomass (OD_{540nm}) of 0.363.

The dependence of specific growth rate on phenol concentration is shown in Figure 4. From this plot, the specific growth rate increased with increase in the initial phenol concentration upto 250 mg/l and then a progress decrease started with increase in phenol

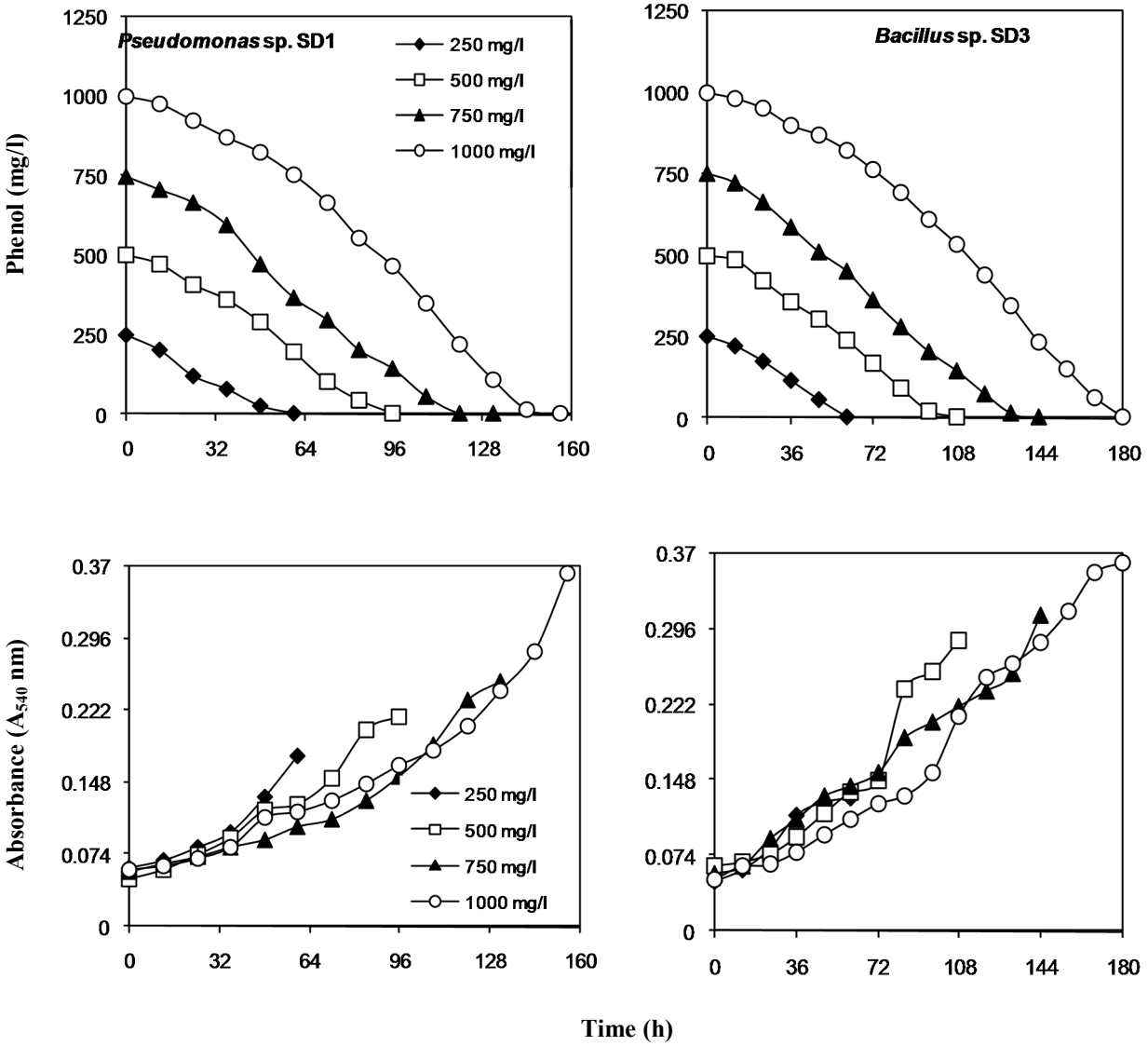

Figure 3: Biodegradation and cell growth profile of sediment bacteria of Okrika River in high phenol concentrations

concentration. In the present study, at 500 mg/l of phenol concentration, the specific growth rate of *Pseudomonas* sp. SD1 is increased (highest μ =0.017 h^{-1}). For concentration higher than 500 mg/l, the specific growth rate of *Pseudomonas* sp. SD1 decreases and became almost constant at 750 mg/l (μ = 0.011 h^{-1}) and 1000 mg/l (μ = 0.011 h^{-1}) of phenol. This is quite similar to the result obtained by Dey and Mukherjee (2010) who observed increase in specific growth rate (0.093 h^{-1}) of mixed microbial culture up to 300 mg/l of initial phenol concentration and then started decreasing to a constant (0.057 h^{-1}) at 600 and 700 mg/l of phenol. This trend suggested that the phenol is an inhibitory substrate. Thus the parameter has been found to be a strong function of initial phenol concentration. At 250 and 500 mg/l, the highest specific growth rate values of 0.026 and 0.017 h^{-1} were observed in *Citrobacter* sp. RW1 and *Pseudomonas* sp. SD1 respectively while the lowest specific growth rate of 0.016 and 0.014 h^{-1} at the same concentration of phenol was observed in *Pseudomonas* sp. SD1 and

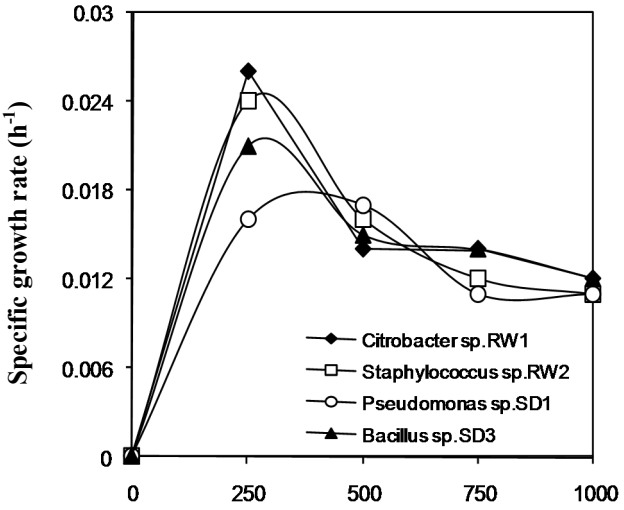

Figure 4: Specific growth rate of the organisms at different initial phenol concentrations

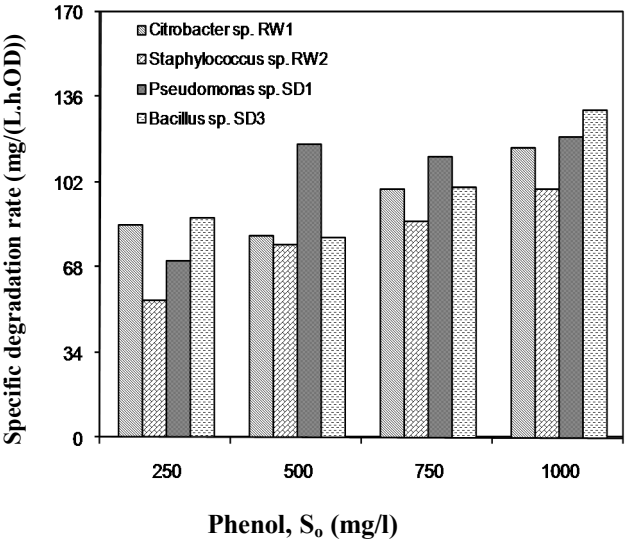

Figure 5: Specific degradation rate at different initial phenol concentrations by the bacterial strains

Citrobacter sp. RW1 respectively. However, the growth rates of the test organisms are similar to that of *Pseudomonas aeruginosa* and *Pseudomonas pseudomallei* degrading phenol in saline solutions (Afzal *et al*., 2007).

The specific rate of phenol degradation of the organisms is depicted in figure 5. The specific degradation rate (Q_s), was estimated by correlating phenol concentration versus culture time using regression technique in Microsoft Excel to obtain the equation of best fit of the degradation curve. The correlation were differentiated with respect to time and then divided by the cell mass (Loh and Wang, 1998). The specific degradation (consumption) rate of a compound was suggested to be a measure of microbe performance. The highest specific consumption rate of phenol was observed in *Bacillus* sp. SD3 with specific degradation rate value of 130.98 mg/(L.h.OD) at 1000 mg/l while *Staphylococcus* sp. RW2 showed the least specific consumption rate of phenol with a specific degradation rate value of 99.83 mg/(L.h.OD) at the same concentration. The organisms in this work showed a robust decrease in specific degradation rate as the phenol concentration decreases. This is in line with the work of Cho *et al.,* (2000) who observed an increase in specific degradation rate as phenol concentration increases in their assessment of influence of phenol on biodegradation of *p*-nitrophenol by freely suspended and immobilized *Nocardioides* sp. NSP41. Agarry and Solomon (2008) also made similar reports in their work on kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescence.*

Table 2 shows the growth yield of the test organisms expressed as absorbance, A at 540nm unit litre of cells produced per mg of phenol substrate utilized. The growth yield varied among the test organisms ranging from 2. 69 to 6.24 (x $10^{-4}A_{540}$ units. l/mg). High growth yield were obtained at low concentration of toxicant (phenol) while low values of growth yield were obtained at high phenol concentration. At 250 mg/l highest and lowest growth yield were observed in *Citrobacter* sp. RW1 and *Bacilllus* sp. SD3 with cell yield coefficients of 6.24 and 3.28 (x $10^{-4}A_{540}$ units.l/mg) respectively. The higher value of Y observed in *Citrobacter* sp. RW1 indicate that phenol was degraded very efficiently by the organism. All the growth yields

reported here were lower than those reported by other authors. Yield coefficients of 0.14 and 0.16 have been reported (Bajaj *et al.,* 2009). The yield coefficients reported by Yoong *et al.,* (1997) are 0.16 and 0.27.

As *Citrobacter* sp. RW1*, Staphylococcus* sp. RW2*, Pseudomonas* sp. SD1and *Bacillus* sp. SD3 shown high specific phenol consumption rate, they have demonstrated strong potential to utilize and grow in phenol of low and high phenol concentrations of upto 1000 mg/l. This indicated that these strains have great potential for application in the treatment of phenolic wastewater and in the bioremediation of phenol impacted media.

REFERENCE

Afzal M, Iqbal S, Rauf S and Khalid ZM. 2007. Characteristics of phenol biodegradation in saline solutions by monocultures of *Pseudomonas aeruginosa* and *Pseudomonas pseudomallei*. *J. Hazard. Mat*. 149(1): 60 - 66.

Agarry SE and Solomon BO. 2008. Kinetics of batch microbial degradation of phenols by indigenous *Pseudomonas fluorescence. Int. J. Environ. Sci. Tech.* 5 (2): 223 – 232.

Akan JC, Abdulrahman FI, Sodipo OA, Ochanya AE and Askira YK. 2010. Heavy metals in sediments from River Ngada, Maiduguri Metropolis, Borno State, Nigeria. J. Environ. Chem. Ecotoxicol. 2(9): 131 - 140.

Atlas RM. 1981. Microbial degradation of petroleum hydrocarbons: an environmental perspective. *Microbiology Rev*. 45(1): 180 – 209.

Bajaj M., Gallert C and Winter J. 2009. Phenol degradation kinetics of an aerobic mixed culture. *Biochem. Eng. J.* 46(2): 205 – 209.

Chesney RH, Sollitti P, Rubin HE. 1985. Incorporation of Phenol Carbon at Trace Concentrations by Phenol-Mineralizing Microorganisms in Fresh Water. *Appl. Environ. Microbiol*. 49: 15 – 18.

Cho YG, Rhee SK, Lee ST. 2000. Influence of phenol on biodegradation of *p*-nitrophenol by freely suspended and immobilized *Nocardioides* sp. NSP41. *Biodegradation* 11(1)**:** 21 – 28.

Collins LD and Daugulis AJ. 1997. Characteristics and optimization of a two-phase partitioning bioreactor for the biodegradation of phenol. *Appl. Micobiol. Biotechnol*. 48(1):18 - 22.

Colwell RR and Walker J.D. 1977. Ecological Aspects of Microbial Degradation of Petroleum in the Marine Environment. *Crit. Rev. Microbiol.* **5(4):** 423 - 445.

Folsom BR, Chapman PJ, Pritchard PH. 1990. Phenol and trichloroethylene degradation by *Pseudomonas cepcia* GA: Kinetics and interaction between substrates. *Appl. Environ. Microbiol.* 56(5)**:** 1279 – 1285.

Gilbert P and Brown MRW. 1978. Influence of growth rate and nutrient limitation on the gross cellular composition of *Pseudomonas aeruginosa* and its resistance to 3- and 4- chlorophenol. *J. Bactriol.* 133(3): 1066 - 1072

Gokulakrishnan S and Gummadi SN. 2006. Kinetics of cell growth and caffeine utilization by *Pseudomonas* sp. GSC 1182. Proc. Biochem. 41: 1417 – 1421.

Goldstein RM, Mallory LM, Alexander M. 1985. Reasons for Possible Failure of Inoculation to enhance biodegradation. *Appl. Environ. Microbiol.* 50(4): 977 – 983.

Heinaru E, Truu J, Stottmeister U, Heinaru A. 2000. Three types of phenol and p-cresol catabolism in phenol and p-cresol-degrading bacteria isolated from River water continuously polluted with phenolic compounds. *FEMS Microbiol. Ecol.* 31(3): 195 – 205.

IAIA09 Conference Proceedings. 2009. Environmental Pollution: A case study of the impact of the Port Harcourt Oil Refinery Company (PHRC), Nigeria. Impact Assessment and Human Well-Being 29th Annual Conference of the International Association for Impact Assessment, 16-22 May 2009, Accra International Conference Center, Accra, Ghana (www.iaia.org)

Jaromir M and Wirgiliusz D. 2007. Phenols transformations in the environment and living organisms. *Curr. Topics in Bioph.* 30 (suppl. A): 24 - 36

Jena HM, Roy GK and Meikap BC. 2005. Development and comparative study of a semi-fluidized bed bioreactor for treatment of wastewater from process industries. Proc. Plant Eng. 23(1): 70 - 75

Joseph V and Joseph A. 1999. Acclimation of algal species following exposure to phenol. *Bull. Environ. Contam. Toxicol.* 62(1): 87 - 92.

Kahru A, Maloverjan A, Sillak H. and Pollumaa L. 2002. The toxicity and fate of phenolic pollutants in the contaminated soils associated with the oil-shale industry. *Environ Sci. Pollut Res*. 1: 27 – 33.

Keweloh H, Weyrauch G, Rehm HJ. 1990. Phenol-induced membrane changes in free and immobilized *Escherichia coli*. *Appl. Microbiol. Biotechnol.* 33(1): 66 - 71.

Kotresha D, Vidyasagar GM. 2008. Isolation and characterisation of phenol-degrading *Pseudomonas aeruginosa* MTCC 4996. *World J. Microbiol*. *Biotechnol*., 24(1): 541-547.

Loh KC and Wang SJ. 1998. Enhancement of biodegradation of phenol and a nongrowth substrate 4-chlorophenol by medium augmentation with conventional carbon sources. *Biodegradation* 8(5)**:** 329 – 338.

Mort SL and Dean-Ross D. 1994. Biodegradation of phenolic compounds by sulphate reducing bacteria from contaminated sediments. *Microb. Ecol.* 28: 67 – 77.

Nwanyanwu CE, Nweke CO, Orji JC. 2012. Growth responses of petroleum refinery effluent bacteria to phenol. J. Res. Biol. 3: 167 - 177

Nwanyanwu CE and Abu GO. 2011. Assessment of viability responses of refinery effluent bacteria after exposure to phenol stress. *J. Res. Biol.* 1(8): 594 - 602

Nweke co, Alisi CS, Okolo JC and Nwanyanwu CE. 2007. Toxicity of zinc to heterotrophic bacteria from a tropical river sediment. Appl. Ecol. Environ. Res. 5(1): 123-132

Otokunefor TV and Obiukwu C. 2005. Impact of refinery effluent on the physicochemical properties of a water body in the Niger Delta. *Appl. Ecol. Environ. Res.* 3 (1): 61 - 72.

Pahm MA and Alexander M. 1993. Selecting inocula for the biodegradation of organic compounds at low concentrations. *Microb. Ecol.* 25(3): 275 – 286.

Papadimitriou CA, Samaras P, Sakellaropoulos GP. 2009. Comparative study of phenol and cyanide containing wastewater in CSTR and SBR activated sludge reactors. Biores. Technol. 100: 31 – 37.

Polymenakou PN and Stephanou EG. 2005. Effect of temperature and additional carbon sources on phenol degradation by an indigenous soil *Pseudomonad*. *Biodegradation.* 16(5): 403 – 413

Tisler T, Zagorc-Koncan J, Ros M, Cotman M. 1999. Biodegradation and toxicity of wastewater from industry producing mineral fibres for thermal insulation. *Chemosphere* 38(6): 1347 – 1352.

Ye FX and Shen DS. 2004. Acclimation of anaerobic sludge degrading chlorophenols and the biodegradation kinetics during acclimation period. *Chemosphere*. 54

(10): 1573 – 1580.

Yoong ET, Lant PA, Greenfield PF. 1997. The influence of high phenol concentration on microbial growth. *Wat. Sci. Tech.* 36(2-3): 75 – 79.