Characterization of phenol-degrading fungi isolated from industrial waste water in Malaysia

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Abstract. Microorganisms have the ability to degrade phenol. However, in Malaysia, there are lack of study on indigenous microorganisms (fungi) that have the ability to degrade phenol. A total of 141 phenol-degrading fungi isolates were isolated from soil and water samples collected from various industrial areas located in Malaysia. The fungi isolate N12 P6C3 was chosen based on its high efficiency in degrading phenol. The fungi isolate N12 P6C3 isolated from a heavy metal factory, Dungun, Terengganu was able to degrade 700 mg/L of phenol within 6 days and the mycelium growth had increased to 0.25 g. The phylogenetic tree based on the ITS sequence analysis confirmed that the fungal identity was closely related to *Penicillium janthinellum* strain ATCC 4845. The optimum conditions of this fungus to degrade phenol was attained at temperature of 35°C, ammonium sulphate at 3 g/L, 0.05 g/L of sodium chloride, and pH 6. The ability of *P. janthinellum* strain N12 P6C3 in the degradation of phenol may provide additional knowledge on locally isolated phenol-degrading fungi which could contribute towards phenol waste management in Malaysia.

Keywords: biodegradation, fungi, phenol

INTRODUCTION

Environmental pollution is one of the critical problems the world is facing today. Rapid industrial growth, urbanization, as well as increasing of hazardous and toxic waste discharged, eventually leads to the increasing of xenobiotic levels in the environment (Gami *et al.*, 2014; Javed and Usmani, 2015). Based on reports by Agency for Toxic Substances and Disease Registry, phenol is one of the major industrial chemicals found in waste effluent which can be present in the environment through natural or chemical processes (ATSDR, 2008).

Phenol is an aromatic compound that is widely distributed as environmental pollutants due to their existence in the waste effluents of many industrial processes including petroleum refineries, pharmaceuticals, paper manufacture, textiles, plastics, dyes, and phenolic resin industries (Santos and Linardi, 2004; Leitão, 2009; Wang *et al.*, 2010). Phenols can also naturally present in decomposition of organic materials or in coal (Basha *et al.*, 2010). Excessive level of phenolic compounds in the environment leads to phenol's accumulation, affecting groundwater and

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soil quality (Michalowicz and Duda, 2007; Panagos *et al.*, 2013).

Distributions of phenol provide a great impact to the environment and human beings. Even at low concentrations, phenol can be toxic and lethal to aquatic organisms (Rittmann and McCarty, 2001). Phenol is rapidly absorbed by human body through inhalation, ingestion, and dermal contact (HPA, 2007). Exposure to phenol can cause irritation to the eye, severe skin damage, serious gastrointestinal damage, cardiovascular disease, and even death (ATSDR, 2008). Therefore, priority and great concern have been placed in these issues, toward the exposure of phenol and the removal of phenol effluent from the ecosystems (Barlow *et al.*, 2007).

Various treatment techniques have been developed for phenol removal including chemical, biological, and physical methods. Solvent extraction, chemical oxidation, adsorption, and incineration have been widely applied, but all these methods are very costly and most probably will produce hazardous by products (Basha *et al.*, 2010; Bui *et al.*, 2012; Thappu *et al.*, 2012). This is why biological approach, biodegradation is preferred to degrade phenol since it is environmentally friendly and cost effective (Wang *et al.*, 2010).

A number of microorganisms including bacteria and fungi have the ability to utilize phenol as their carbon source for growth (Chen *et al.*, 2006). Most of the phenol-degrading microorganisms reported were bacteria including *Acinetobacter* spp., *Pseudomonas* spp., *Bacillus* spp., and *Rhodococcus* spp. (Ahmad *et al.*, 2011; Sridevi *et al.*, 2011; Mahiuddin *et al.*, 2012; Aravindhan *et al.*, 2014; Hasan and Jabeen, 2015). Besides bacteria, fungi also have been reported as phenol degrader. Some of examples are *Aspergillus fumigatus*, *Fusarium flocciferum*, *Aspergillus awamori*, *Penicillium chrysogenum*, *Trichosporium cutaneum*, and *Candida tropicalis* (Mendonça *et al.*, 2004; Stoilova *et al.*, 2006; Leitão *et al.*, 2007; Tuah *et al.*, 2009; Ravikumar *et al.*, 2011).

Phenol-degrading microorganisms may utilize phenol in two pathways either through aerobic or anaerobic pathway (Haiyin *et al.*, 2007; Tuah *et al.*, 2009; Sarwade and Gawai, 2014). The absence or presence of oxygen is very important in determining the fate of phenol biodegradation pathway (Sridevi *et al.*, 2012). In aerobic condition,

oxygen is used by the enzyme phenol hydroxylase to produce catechol. Depending on the microorganisms involved, the resulting catechol can be degraded into two pathways either ortho or meta pathway. In the ortho pathway, the enzyme catechol 1, 2-dioxygenase transforms catechol to cis, cis muconate whereas in the meta pathway, the enzyme catechol 2, 3-dioxygenase convert catechol to 2-hydroxymuconic semialdehyde (van Schie and Young, 2000; Tuah *et al.*, 2009; Supriya and Neehar, 2014). The products for both pathways are further incorporated into the Krebs cycle (Soudi and Kolahchi, 2011).

In contrast to bacteria, fungi are able to adapt easily to the ecosystem and able to grow at extreme condition (Atagana, 2004; Stoilova *et al.*, 2008). However, in Malaysia, the investigation on phenol-degrading fungi is poorly reported. Therefore, this study was conducted to provide preliminary information on phenol-degrading fungi in Malaysia.

MATERIALS AND METHODS

Isolation and screening of phenol-degrading fungi. Water and soil samples were collected from various industrial areas located in Selangor, Kedah and Terengganu, Malaysia. The water and soil samples were collected from 10-20 cm from the soil and water surface. Aseptically the samples were transferred into sterile polypropylene tubes and stored at 4°C. The samples were diluted in 10 mL of sterile distilled water and were grown in Liquid Mineral Salt medium (LMS); made up of 0.5 g MgSO₄·7H₂O, 11.8 g KH₂PO₄, 2.3 g K₂HPO₄, 0.05 g CuSO₄, 0.05 g NaCl, 0.25 g NH₄Cl, 0.01 g MnSO₄, 0.1 g FeSO₄, and 0.01 g ZnSO₄ (Cai *et al.*, 2007). All the chemicals were dissolved in distilled water, pH to 6 and brought up to 1 L. Appropriate amount of phenol and 100 µg/mL of penicillin-streptomycin antibiotic were added to the autoclave's medium prior to inoculation. The cultures were incubated at room temperature for 3 days.

Sensitivity of phenol-degrading fungi. The ability of each isolate to utilize phenol was tested at different phenol concentrations. Concentration

of phenol was gradually increased from (mg/L) 100, 300, 500, and up to 700. Mycelium of the isolate was homogenized and incubated into Potato Dextrose Broth (PDB) for 24 h at 125 rpm under room temperature. After 24 h, 0.1 g of fungal mycelium was harvested and incubated into 50 mL LMS solution supplemented with various concentrations of phenol at 125 rpm under room temperature. All samples were periodically taken for dry weight measurement and phenol degradation rate analysis. Mycelia from LMS media were filtered and washed with sterile distilled water for several times. Mycelia obtained were then kept in a small pack of aluminium foil and dried at 60°C. The dry weight of the mycelia was measured by subtracting the initial weight of mycelia and aluminium foil. 4-amino antipyrine colorimetric method was used for phenol degradation rate analysis (APHA, 1998). All experiments were carried out in three replicates.

Identification of phenol-degrading fungi.

DNA extraction was done as described by Raeder and Broda with some modification (Raeder and Broda, 1985). The extracted genomic DNA was amplified using Internal Transcribed Spacer (ITS) primers, ITS1 (5'-TCCGTAGGTGAACCTGCGG-3') and ITS4 (5'-TCCTCCGCTTATTGATATGC-3') (White *et al.*, 1990; Pryce *et al.*, 2006). The PCR reaction mixture consisted of 25 µL of 2X *Taq* master mix (Vivantis Technologies), 19 µL of free nuclease water, 2 µL of 0.5 µM of each primer, and 2 µL of DNA template. The reaction was performed in a thermal cycler under the following temperature: denaturation at 94°C for 2 min, 30 cycles of denaturation at 94°C for 1 min, annealing at 49°C for 30 s, and primer extension at 72°C for 25 s, with the penultimate step of 72°C for 10 s and lastly cooling at 4°C. DNA sequence was analyzed using Basic Local Alignment Search Tool (BLAST) (www.ncbi.nlm.gov). The sequence was aligned using CLUSTAL W in Molecular Evolutionary Genetics Analysis version 6 (MEGA6). Phylogenetic tree was constructed using Neighbour-Joining method with bootstrap value replication of 1000.

Fungal growth and phenol degradation activity of the selected isolates. To study the physiological factors affecting the growth and

phenol degradation of the selected isolate, different temperature (10, 15, 20, 25, 30, 35, 40, 45, 50, and 55°C), nitrogen source (ammonium nitrate, ammonium sulphate, ammonium chloride, sodium nitrate, serine, isoleucine, valine, and phenylalanine), concentration of nitrogen source (0, 0.25, 0.5, 1, 2, 3, 4, and 5 g/L), sodium chloride (0, 0.05, 0.1, 0.2, 0.3, 0.4, 0.6, 0.8, and 1 g/L) and pH range of 4.0 – 9.0 were investigated. Fungal isolate (0.1 g) was inoculated in 50 mL LMS and incubated on an orbital shaker at 125 rpm for 2 days. The degradation of phenol was detected using 4-AAP colorimetric method and the fungal growth was measured by measuring the dry weight of the mycelium.

Statistical analysis. All data obtained were subjected to statistical analysis. The SPSS 17.0 software was used for the analysis. Comparison among the groups was performed using one-way analysis of variance (ANOVA) with post hoc analysis by Tukey's test (Haynes, 2013).

RESULTS AND DISCUSSION

Isolation and screening for phenol-degrading fungi.

A total of 141 phenol-degrading fungi were isolated from soil and water samples collected from various industrial areas located in Malaysia. Most samples were obtained from charcoal factories, heavy metal waste sites, drains, sludges, agricultural sites, and oil contaminated areas. Sixteen fungal isolates were selected for further investigation based on their ability to degrade phenol (Table 1). All isolates were grown on LMS agar plate supplemented with phenol as the sole carbon source. All 16 isolates of phenol-degrading fungi were assessed daily for phenol degradation (100 mg/L phenol) in LMS. Among the 16 isolates, 6 isolates (N7 P1C1, N5 P2C2, N3 P2C2, DD P1C2, N12 P6C3 and N12 P4C1) were able to degrade 100% phenol within 2 days (Figure 1a). Isolate N4 P2C1 consumes phenol completely in 4 days while isolates 3 LP2, I LP1, N15 P2C1 and N2 P9C1 required 6 days to completely degrade 100 mg/L of phenol. Isolates 4 P1C1 and N3 P2C1 only able to degrade less than 30% of phenol at the end of incubation period (day 8). Growth rate of each fungal isolates was recorded

(Figure 1b). The mycelia cell increased as the phenol concentration started to decrease. This proved phenol was being utilized as carbon source for cellular growth (Wang *et al.*, 2010).

Figure 1. (a) Phenol degradation of 16 fungal isolates grown in LMS solution supplemented with 100 mg/L of phenol for 8 days at 125 rpm under room temperature and (b) dry weight of 16 fungal isolates within 8 days. Each point represents the mean of triplicate $\pm$ SD.

Six fungal isolates were further analyzed for phenol tolerance at different phenol concentrations (300, 500, and 700 mg/L). At 300 mg/L of phenol, all isolates required 3 days to completely degrade the phenol except for isolate N3 P2C2 which needed 4 days (Figure 2a). While at 500 mg/L phenol, only two isolates (N12 P6C3 and DD P1C2) were able to degrade 100% phenol within 4 days of incubation time (Figure 2b). As the phenol concentration increased to 700 mg/L, only isolate N12 P6C3 was able to completely degrade 700 mg/L of phenol within 6 days ($p < 0.05$) compared to other isolates (Figure 2c). While isolates DD P1C2 and N7 P1C1 required 7 days to completely degrade 700 mg/L of phenol. The least potent fungi were isolates N5 P2C2, N12 P4C1, and N3 P2C2 which degraded 2 - 20% of phenol at the end of incubation time. The mycelia dry weight had increased to 0.25 g for 700 mg/L of phenol concentration (Figure 3). Isolate

N12 P6C3 was further analysed due to its efficiency in degrading phenol compared to other isolates. The results show that the higher phenol concentration, the more time required to completely degrade phenol and also a longer lag phase was observed (Figure 2) (Stoilova *et al.*, 2006; Wang *et al.*, 2010; Supriya and Neehar, 2014).

Figure 2. Phenol degradation of 6 fungal isolates: (—■—) N12 P6C3, (—●—) DD P1C2, (—◆—) N7 P1C1, (—▲—) N5 P2C2, (—■—) N12 P4C1, and (—×—) N3 P2C2 incubated in LMS supplemented with various phenol concentration: (a) 300 mg/L, (b) 500 mg/L and (c) 700 mg/L. Values shown are mean $\pm$ SD, $n=3$.

Figure 3. Dry weight of 6 fungal isolates: (—■—) N12 P6C3, (—●—) DD P1C2, (—◆—) N7 P1C1, (—▲—) N5 P2C2, (—□—) N12 P4C1, and (—*—) N3 P2C2 incubated in LMS supplemented with various phenol concentration: (a) 300 mg/L, (b) 500 mg/L and (c) 700 mg/L. Values shown are the mean $\pm$ SD, $n=3$.

Optimization of conditions for growth and phenol degradation of *Penicillium janthinellum* strain N12 P6C3. The effect of temperature on the growth of *Penicillium janthinellum* strain N12 P6C3 in the presence of 300 mg/L phenol was studied at different temperatures ranging from 10 to 60°C. The mycelial growth and the phenol degradation gradually increased as the temperature increased until it reached an optimum temperature, 35°C ($p<0.05$) (Figure 6). The mycelial growth and

phenol degradation ability of the fungus decrease at 40°C and ceased at 60°C. Supriya and Neehar (2014) reported similar observation in *Aspergillus niger* where the optimum temperature for the degradation of phenol was recorded at 35°C and the growth decreases drastically at low (25°C) and high (60°C) temperature. The growth and phenol degradation rate decrease at low temperature due to the delayed activity in cold conditions. While at high temperature, the enzyme responsible for phenol degradation has been denatured, thus exhibit lower growth rate (El- Naas *et al.*, 2009).

Figure 4. Microscopic image of isolate N12 P6C3 under light microscope with 1000X magnification.

Ammonium sulphate appeared to be the best nitrogen source for *P. janthinellum* strain N12 P6C3 with 73.4% phenol degradation (Figure 7a). Ammonium sulphate gave the highest phenol degradation activity and fungal growth compared to other nitrogen sources ($p<0.05$), followed by ammonium chloride and ammonium nitrate. The contribution of nitrogen sources leads to faster phenol degradation rate and increases in cell biomass. It is probable that the simultaneous metabolization of these nutrients with phenol allows the cells to overcome the inhibition effect of fungal growth caused by phenol (Neumann *et al.*, 2004; Khleifat, 2006). Figure 7b shows the effects of different concentration of ammonium sulphate ranges from 0-5 g/L on phenol degradation and fungal growth. The optimum concentration of ammonium sulphate for the highest phenol degradation activity was recorded at 3 g/L with 89.9% degradation ($p<0.05$).

Table 1. Growth of fungal isolates on LMS supplemented with 100 mg/L of phenol for 3 days at room temperature.

No	Location	Isolates	Diameter of mycelia (cm)
1	Charcoal factory, Dungun, Terengganu	N7 P1C1	2.633±0.115 ^e
2	Charcoal factory, Dungun, Terengganu	N5 P2C2	2.193±0.210 ^{de}
3	Dungun, Terengganu	N3 P2C2	2.200±0.100 ^{de}
4	Bangi, Selangor	DD P1C2	2.733±0.058 ^e
5	Awie Metal, Dungun, Terengganu	N12 P6C3	2.667±0.208 ^e
6	Awie Metal, Dungun, Terengganu	N12 P4C1	2.167±0.321 ^{de}
7	Charcoal factory, Dungun, Terengganu	N7 P3C1	1.567±0.208 ^{bc}
8	Serdang, Selangor	°4 P1C1	0.867±0.208 ^a
9	Klang River	3 LP2	1.533±0.153 ^{bc}
10	Pendang, Kedah	U3 P1C3	1.167±0.208 ^{ab}
11	Shah Alam, Selangor	1 LP1	1.200±0.200 ^{ab}
12	Charcoal factory, Dungun, Terengganu	N4 P2C1	1.667±0.153 ^{bcd}
13	Awie Metal, Dungun, Terengganu	N15 P2C1	0.933±0.231 ^a
14	Dungun, Terengganu	N3 P2C1	1.833±0.153 ^{cd}
15	Dungun, Terengganu	N2 P9C1	1.700±0.200 ^{bcd}
16	Awie Metal, Dungun Terengganu	N12 P7C1	1.333±0.115 ^{abc}

Note: Values are mean of triplicate ± standard deviation.

^{a-e}Mean values with different superscripts are significantly different ($p < 0.05$).

Figure 5. A phylogenetic tree (neighbor-joining method) showing genetic relationship between fungal isolate N12 P6C3 and other related reference fungi based on the ITS sequence analysis. Species name are followed by the accession numbers of their ITS region sequences. *Fusarium oxysporum* strain ATCC 52434 is the outgroup.

Figure 6. The effect of temperatures on the growth and phenol degradation of *Penicillium janthinellum* strain N12 P6C3. The isolate was grown in LMS supplemented with 300 mg/L phenol incubated in orbital shaker (125 rpm) for 2 days. Phenol degradation rate was determined using 4-AAP assay. Data represents mean $\pm$ SD, $n=3$.

Figure 7. Phenol degradation activity and growth of *Penicillium janthinellum* strain N12 P6C3. (a) Phenol degradation activity and growth in various nitrogen sources; (b) phenol degradation activity and growth in various ammonium sulphate concentrations. Data represents mean $\pm$ SD, $n=3$.

The effect of various concentration of NaCl on growth of strain N12 P6C3 was observed at 0 to 1 g/L (Figure 8). The results show *P. janthinellum* strain N12 P6C3 grew best at 0.05 g/L NaCl and

was able to degrade 88% of phenol after 2 days of incubation compared to other concentrations ($p<0.05$). The reduction of growth and phenol degradation were recorded at high NaCl concentrations. This indicates *P. janthinellum* strain N12 P6C3 requires low amount of NaCl for their optimum growth. High NaCl concentration could affect the degradation of phenol due to osmotic stress to the fungal growth which eventually decreases the phenol degradation rates (Annadurai *et al.*, 2008).

Figure 8. The effect of sodium chloride concentration on phenol degradation activity and growth of *Penicillium janthinellum* strain N12 P6C3. Data represents mean $\pm$ SD, $n=3$.

The effect of pH on the growth and degradation of phenol by *P. janthinellum* strain N12 P6C3 was studied using an overlapping buffer system consisting of acetate, phosphate, and Tris-HCl buffer at the pH ranging from 4.0-9.0 (Ahmad *et al.*, 2011). Overlapping buffer system was employed to cancel out the different effect of buffers on fungal growth and phenol degradation (Ahmad, 2012). The results (Figure 9) show the fungus able to grow at pH ranging from 5.5-6.5 ($p<0.05$) in phosphate buffer. The fungal growth increased with the increased of pH and peaked at pH 6. The highest degradation rate was achieved at pH 6 with 88.7% phenol degradation ($p<0.05$), indicating *P. janthinellum* strain N12 P6C3 prefer slightly acidic condition. The growth of this fungus and phenol degradation rate was dramatically decreased at pH using acetate and Tris-HCl buffers. Extremely low or high pH usually leads to loss of activity for most of the microbial enzyme. Enzymes produced by microorganisms are significantly affected by the change in pH as it is a crucial factor in the stability of the enzyme and can affect the solubility of

enzymatic compounds (Banerjee and Ghoshal, 2010).

of sodium chloride, and pH 6 using phosphate buffer system.

Figure 9. The effect of pH on phenol degradation activity and growth of *Penicillium janthinellum* strain N12 P6C3 using three overlapping buffers (—●—) acetate, (—■—) phosphate and (—▲—) tris-HCl buffers. (a) Phenol degradation activity; (b) fungal growth. Data represents mean $\pm$ SD, $n=3$.

CONCLUSION

A total of 141 phenol-degrading fungi were isolated from phenol-contaminated sites and tested for their ability to degrade phenol. Based on the results, strains N12 P6C3 was selected as the best fungal isolate for further investigations. Strain N12 P6C3 was identified as *Penicillium janthinellum*. Various parameters including temperature, nitrogen source, salinity, pH, and phenol concentration affecting phenol degradation activity of *P. janthinellum* strain N12 P6C3 were studied. The optimum conditions for phenol degradation were achieved at temperature of 35°C, ammonium sulphate at 3 g/L, 0.05 g/L

REFERENCES

- Agency for Toxic Substances and Disease Registry (ATSDR). 2008. Toxicological profile for phenol. Public Health Service, U.S. Department of Health and Human Services. Atlanta, GA.
- Ahmad, S. A. 2012. Biodegradation of phenol by locally isolated *Acinetobacter* sp. strain AQ5NOL 1 and purification of phenol hydroxylase. PhD Thesis, Universiti Putra Malaysia.
- Ahmad, S. A., Syed, M. A., Arif, N. M., Shukor, M. Y. A., & Shamaan, N. A. 2011. Isolation, identification and characterization of elevated phenol degrading *Acinetobacter* sp. strain AQ5NOL 1. *Australian Journal of Basic and Applied Sciences* 5(8): 1035-1045.
- American Public Health Association (APHA). 1998. Standard Methods for the Examination of Water Wastewater. 20th Edition. Method 5530. pp 540-544.
- Annadurai, G., Ling, L. Y., & Lee, J. F. 2008. Statistical optimization of medium components and growth conditions by response surface methodology to enhance phenol degradation by *Pseudomonas putida*. *Journal of Hazardous Materials* 151: 171-178.
- Aravindhnan, R., Naveen, N., Anang, G., Raghavarao, J., & Unnainair, B. 2014. Kinetics of biodegradation of phenol and a polyphenolic compound by a mixed culture containing *Pseudomonas aeruginosa* and *Bacillus subtilis*. *Applied Ecology and Environmental Research* 12(3): 615-625.
- Atagana, H. I. 2004. Bioremediation of creosote-contaminated soil in South Africa by land farming. *Journal of Applied Microbiology* 96: 510-520.
- Bandh, S. A., Kamili, A. N., Ganai, B. A., & Saleem, S. 2011. Isolation, identification and seasonal distribution of *Penicillium* and *Aspergillus* species in Dal Lake, Kashmir. *International Journal of current Research* 3(10): 38-42.
- Banerjee, A. & Ghoshal, A. K. 2010. Isolation and characterization of hyper phenol tolerant *Bacillus* sp. from oil refinery and exploration sites. *Journal of Hazardous Materials* 176: 85-91.
- Barlow, J., Johnson, J. A. P., Belcher, S. M., & Fenton, S. E. 2007. Early life exposure to phenols and breast cancer risk in later years: Fact sheet on phenols. *Breast Cancer and the Environment Research Centre Community Outreach and Translation Cores*. pp 1-20.
- Basha, K. M., Rajendran, A., & Thangavelu, V. 2010. Recent advances in the biodegradation of phenol: A review. *Asian Journal Experiment Biology Science* 1(2): 21-234.
- Bui, H. B., Nguyen, L. T., & Dang, L. D. 2012. Biodegradation of phenol by native bacteria isolated from dioxin contaminated soils. *Journal Bioremediation and Biodegradation* 3: 168.
- Cai, W., Li, J., & Zhang, Z. 2007. The characteristics and mechanisms of phenol biodegradation by *Fusarium* sp. *Journal of Hazardous Materials* 148: 38-42.
- Chen, K. N., Chen, M. J., & Lin, C. W. 2006. Optimal combination of the encapsulating materials for probiotic microcapsules and its experimental verification (R1). *Journal of Food Engineering* 76: 313-3.
- El-Naas, M. H., Muhtaseb, S. A. A., & Makhlof, S. 2009. Biodegradation of phenol by *Pseudomonas putida* immobilized in polyvinyl alcohol (PVA) gel. *Journal of Hazardous Materials* 164: 720-725.
- Gami, A. A., Shukor, M. Y., Khalil, K. A., Dahalan, F. A., Khalid,

- A., & Ahmad, S. A. 2014. Phenol and phenolic compounds toxicity. *Journal of Environmental Microbiology and Toxicology* 2(1): 11-23.
- Haiyin, X., Thomas, M., Chen, S., Cimeglia, C. E., & Ceh, H. 2007. Anaerobic metabolism of 1-amino-2-naphthol-based azo dyes (Sudan dyes) by human intestinal microflora. *Applied Environmental Microbiology* 73: 7759-7762.
- Hasan, S. A., & Jabeen, S. 2015. Degradation kinetics and pathway of phenol by *Pseudomonas* and *Bacillus* species. *Biotechnology and Biotechnology Equipment* 29(1): 45-53.
- Haynes, W. 2013. Tukey's Test. In: Dubitzky, W., Wolkenhauer, O., Cho, K.H., Yokota, H. (eds) Encyclopedia of Systems Biology. Springer, New York, NY.
- Health Protection Agency (HPA). 2007. Phenol: General information. Health Protection Agency. London.
- Javed, M., & Usmani, N. 2015. Stress response of biomolecules (carbohydrate, protein and lipid profiles) in fish *Channa punctatus* inhabiting river polluted by Thermal Power Plant effluent. *Saudi Journal of Biological Sciences* 22: 237-242.
- Khleifat, K. M. 2006. Biodegradation of phenol by *Ewingella americana*: Effect of carbon starvation and some growth conditions. *Process Biochemistry* 41: 2010-2016.
- Leitão, A. L. 2009. Potential of *Penicillium* species in the bioremediation field. *International Journal of Environmental Research and Public Health* 6(4): 1393-1417.
- Leitão, A. L., Duarte, M. P., & Oliveira, J. S. 2007. Degradation of phenol by a halotolerant strain of *Penicillium chrysogenum*. *International Biodeterioration and Biodegradation* 59: 220-225.
- Mahiuddin, Md., Fakhrudin, A. N. M., & Abdullah-Al-Mahin. 2012. Degradation of phenol via meta cleavage pathway by *Pseudomonas fluorescens* PU1. *International Scholarly Research Network* 2012: 1-6.
- Mendonça, E., Martins, A., & Anselmo, A. M. 2004. Biodegradation of natural phenolic compounds as single and mixed substrates by *Fusarium flocciferum*. *Electronic Journal of Biotechnology* 7(1): 30-37.
- Michalowicz, J. & Duda, W. 2007. Phenols – sources and toxicity: Review. *Polish Journal of Environment Study* 16(3): 347-362.
- Neumann, G., Teras, R., Manson, L., Kivisaar M., Schayer, F., & Heipieper, H. J. 2004. Simultaneous degradation of atrazine and phenol by *Pseudomonas* sp. strain ADP: Effect of toxicity and adaptation. *Applied and Environmental Microbiology* 70(4): 1907-1912.
- Panagos, P., Liedekerke, M. V., Yigini, Y., & Montanarella, L. 2013. Contaminated sites in europe: Review of the current situation based on data collected through a european network. *Journal of Environmental and Public Health* 2013: 1-11.
- Pryce, T. M., Palladino, S., Price, D. M., Gardam, D. J., Campbell, P. B., Christiansen, K. J., & Murray, R. J. 2006. Rapid identification of fungal pathogens in BacT/ALERT, BACTEC and BBL MGIT media using polymerase chain reaction and DNA sequencing of the internal transcribed spacer regions. *Diagnostic Microbiology and Infectious Disease* 54: 289-297.
- Raeder, U. & Broda, P. 1985. Rapid preparation of DNA from filamentous fungi. *Letters in Applied Microbiology* 1: 17-20.
- Ravikumar, S., Parimala, P. S., & Gokulakrishnan, R. 2011. Biodegradation of phenolic compounds by using halotolerant microbes. *International Journal of Plant, Animal and Environmental Sciences* 1(2): 38-45.
- Rittmann, B. E. & McCarty, P. L. 2001. Environmental Biotechnology: Principles and Applications, New York.
- Santos, V. L. & Linardi, R. 2004. Biodegradation of phenol by a filamentous fungi isolated from industrial effluents – identification and degradation potential. *Process Biochemistry* 39: 1001-1006.
- Sarwade, V. D. & Gawai, K. R. 2014. Biodegradation of phenol by alkaliphilic *Bacillus badii* D1. *Journal of Environmental Science, Toxicology and Food technology* 8(5): 28-35.
- Soudi, M. R. & Kolahchi, N. 2011. Bioremediation potential of a phenol degrading bacterium, *Rhodococcus erythropolis* SKO-1. *Progress in Biological Sciences* 1: 31-40.
- Sridevi, V., Chandana Lakshmi, M. V. V., Adimadhyam, S. V. N., & Mdicherla, N. R. 2011. Statistical optimization of process variables by Response Surface Methodology to enhance phenol degradation by *Pseudomonas putida* (NCIM 2012). *Advances in Bioscience and Biotechnology* 2: 175-181.
- Sridevi, V., Chandana Lakshmi, M. V. V., Manasa, M., & Sravani, M. 2012. Metabolic pathways for the biodegradation of phenol. *International Journal of Engineering Science and Advanced Technology* 2(3): 695-705.
- Stoilova, I., Krastanov, A., & Bui, H. 2008. Biodegradation of mixed phenolic compounds by a microbial association of *Aspergillus awamori* and *Thermoascus aurantiacus*. *Electronic Journal of Environmental, Agricultural and Food Chemistry* 7: 2625-2633.
- Stoilova, I., Krastanov, A., Stanchev, V., Daniel, D., Gerginova, M., & Alexieva, Z. 2006. Biodegradation of high amounts of phenol, catechol, 2,4-dichlorophenol and 2,6-dimethoxyphenol by *Aspergillus awamori* cells. *Enzyme and Microbial Technology* 39: 1036-1041.
- Supriya, C. H. & Neehar, D. 2014. Biodegradation of phenol by *Aspergillus niger*. *Journal of Pharmacy* 4(7): 11-17.
- Thappu, B., Ajay Kumar, K. C., & Ghimire, A. 2012. A review on biodegradation of petroleum hydrocarbon contaminated soil. *Kathmandu University Journal of Science, Engineering and Technology* 8(1): 164-170.
- Tuah, P. M., Rashid, N. A. A., & Salleh M. M. 2009. Degradation pathway of phenol through ortho-cleavage by *Candida tropicalis* RETL-Cr1. *Borneo Science* 1-8.
- Van Schie, P. M. & Young, L. Y. 2000. Biodegradation of phenol: Mechanisms and applications. *Bioremediation Journal* 4: 1-18.
- Visagie, C. M., Houbaken, J., Frisvad, J. C., Hong, S. B., Klaassen, C. H. W., Perrone, G., Seifert, K. A., Varga, J., Yaguchi, T., & Samson, R. A. 2014. Identification and nomenclature of the genus *Penicillium*. *Studies in Mycology* 78: 343-31.
- Wang, L., Li, Y., Yu, P., Xie, Z., Luo, Y., & Lin, Y. 2010. Biodegradation of phenol at high concentration by a novel fungal strain *Paecilomyces variotii* JH6. *Journal of Hazardous Materials* 183: 366-371.
- White, T. J., Burns, T., Lee, S., & Taylor, J. 1990. Amplification and direct sequencing of fungal ribosomal RNA genes for phylogenetics. In: Innis, M.A., Gelfand, D.H., Sninsky, J. J. and White, T.J. (Eds.). *PCR Protocols: A Guide to Methods and Application*. Academic Press. San Diego, CA. pp 315-322.