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Full Length Research Paper

Evaluation of microbial systems for bioremediation of petroleum refinery effluents in Nigeria

Ojumu, T.V.[1], Bello, O.O.[2], Sonibare, J.A.[2] and Solomon, B.O.[2]*

[1]Bioprocess Engineering Unit, University of Cape Town, Cape Town, South Africa.
[2]Department of Chemical Engineering, Obafemi Awolowo University, Ile-Ife, Nigeria.

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The potential of various organisms to catabolize and metabolize organic compounds has been recognized as potentially effective means of disposing of hazardous wastes. Phenolic compounds has long been recognized as one of the most recalcitrant and persistent substance in petroleum refinery effluents. This is a cause of some concern because of the high toxicity and of this compound. Bioremediation of phenolic compounds has been recognized as a potential solution for the disposal of phenolic compounds due to its scale ability, cost effectiveness and simplicity. The two species of *Pseudomonas*, *P. aeruginosa* and *P. fluorescence* were studied for their bioremediation potential on Refinery effluent with respect to phenol biodegradation in a batch reactor. Phenol was degraded completely by the two species. While *P. aeruginosa* completely mineralize phenol at the 60th hour of cultivation, only 75% (23 mg/l) of phenol was degraded by *P. fluorescence*; complete degradation was achieved at the 84th hour of fermentation. There was highly positive correlation between phenol biodegradation and the microbial growth. (r = +0.994 and r = +0.980 at P $\leq$0.05 for *P. aeruginosa* and *P. fluorescence*, respectively). The maximum specific growth rate (μ_{max}) and inhibitory constant (K_i); 0.019(h^{-1}) and 30.89 mg/l, and 0.011 (h^{-1}) and 33.43 mg/l were obtained from Haldane model for *P. aeruginosa* and *P. fluorescence*, respectively. The study revealed the high potency of these strains and the possibility of using them in bioremediation of petroleum refinery and petrochemical waste waters.

Key words: Bioremediation, phenol, biodegradation, *Pseudomonas*, refinery effluents.

INTRODUCTION

Petroleum refineries and petrochemical plants are the kingpins of the petroleum industry. The latter is one of the barons of the industrial sector globally and the kingpin in the Nigerian industrial sector. Optimum utilization and benefits from crude oil are derived by converting crude oil through processing in a refinery into a wide range of products such as petroleum fuels, lubricants, bitumen and waxes based on market demand. Refineries vary in complexity of processes used and normally require more than one for the production of the desired finished product.

The petrochemical industries derive its feedstock from refinery processes which are then converted into valuable products such as plastics and resins, synthetic rubber, materials for agriculture including agrochemicals and cleaning agents, which further serve as raw materials for downstream industries. While petroleum and refinery and petrochemical industries are most desirable for national development and improved quality of life, the unwholesome and environmentally unacceptable pollution effects of the wastes from these industries which have been reported world–wide (Ruiz-Rrdaz et al., 2001; Chang et al., 1998). Prominent among the pollutants in these wastes are phenols and its derivatives. They are distributed in various environmental sites as artificial or natural mono-aromatic compounds and pose a serious ecological problem due to widespread use, toxicity and occurrence throughout the environment (Fava et al.,

*Corresponding author. E-Mail: bsolomon@oauife.edu.ng. Tel. +234-034-244929.

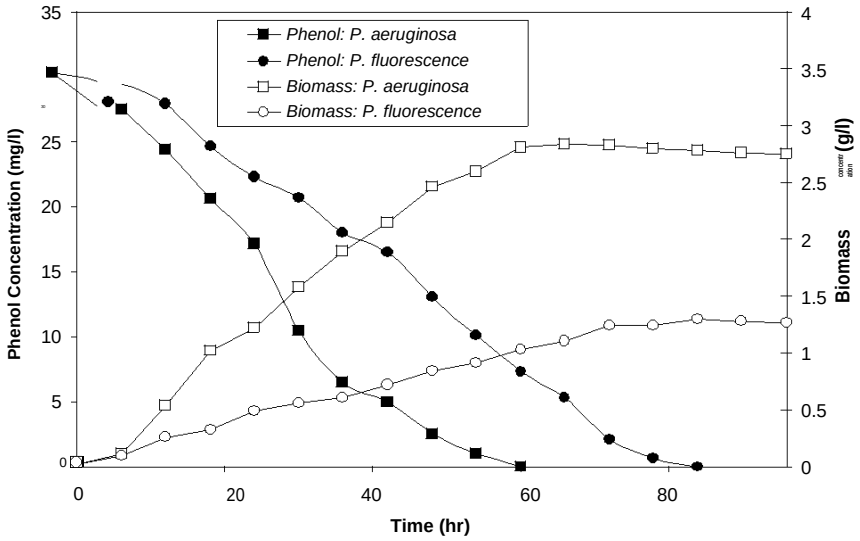

Figure 1. Time course plot of phenol concentration and biomass concentration during Biodegradation of Warri Refinery waste water by the two Pseudomonas species.

1995). Inhalation and dermal exposure to phenol in air is highly irritating to the skin, eyes and mucous membranes in humans (Calabrese and Kenyon, 1991; HSDB, 1991; RTES, 1993). Its potential uptake through respiration had been confirmed Ren (2003). Phenol in wastewater that finds its way to water meant for domestic purpose could pose a great danger to health. Acute (short- term) animal tests, such as the LD_{50} tests in rats, mice and rabbits, have shown phenol to have high acute toxicity from oral exposure (Calabrese and Kenyon, 1991).

High concentrations of hydrocarbons in sediment have been traced to petroleum refinery effluents have been traced to petroleum refinery effluent (Morel and Koffi, 1995) and suspension of such could constitute air pollution problem (Edwards et al., 2001). The use of phenolic components as biological indicator of air quality confirms this (Pasqualini et al., 2003).

The toxicity of phenols and phenolic compounds often results in the reduction of wastewater biotreatment even at relatively low concentrations (Hinteregger et al., 1992; Abd-El-Haleem et al., 2003). Although physico-chemical methods has been employed for removal of phenols and it compounds (Kobayshi and Rittman, 1982), biological methods is preferred as the former is costly and often produce other undesirable products which are toxic, requiring further processing steps (Collins and daugulis, 1997; Thavasi and Jayalakshmi, 2003).

Biodegradation of phenols and phenolics compounds had been actively studied (Ruiz-Rrdaz et al., 2001; Chang et al., 1998; Fava et al., 1995; Abd-El-Haleem et al., 2003; Dean-Ross, 1989; Solomon et al., 1994; Ahmed et al., 1995; Alleman et al., 1995; Collins and daugulis, 1997; Fulthorpe and Allen, 1995; Lin et al., 1990; Morris and Lester, 1994; Ryu et al., 2000; Wang et al., 1996). Studies have shown that in phenol-contaminated sites, bacteria can adapt and degrade over time to low phenol concentrations, but the lag phase increases by increasing phenol concentration (Dean-Ross, 1989; Collins and daugulis, 1997). In fact Collins and Daugulis (1997) obtained an excellent result by using a biphasic reactor for biodegradation of phenol using *Pseudomonas species*, with a fed batch system degrading 28 g phenol in about 165 h with reduced lag phases.

This paper therefore describes the biodegradation potentials of two different indigenous *Pseudomonas* species; *P. aeruginosa* and *P. flourescence* on Nigerian Refinery and Petrochemical effluents with the hope of isolating and using the organism for the bioremediation wastewaters. The results from these studies would be useful for the prediction of the bioremediation mechanisms of these microbial systems.

MATERIALS AND METHODS

Microorganisms and refinery effluent samples

Indigenous *P. aeruginosa* and *P. fluorescence* collected from the stock culture of the Microbiology Department, Obafemi Awolowo University, Ile-Ife, Nigeria were used throughout this work. Liquid effluent samples were collected from a Nigerian Refinery wastewater.

Culture medium

In order to meet the nutritional requirement of the microorganisms for proper growth, the wastewater samples were supplemented with mineral salts medium containing the following constituents:

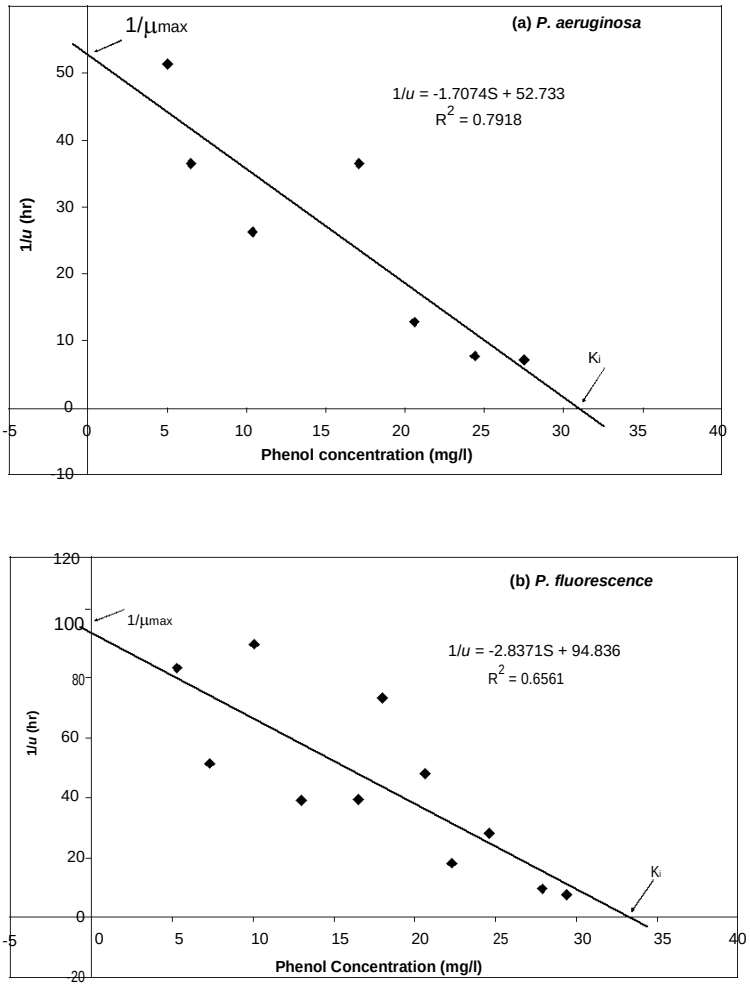

Figure 2. Haldane Model for substrate inhibition for the batch of the *Pseudomonas* species in Warri Refinery waste water.

$(NH_4)SO_4$, 4 gl^{-1}; KH_2PO_4, 2 gl^{-1}; NaCl, 1.5 gl^{-1}; $CaCl_2$, 1.2 gl^{-1}; $NaHPO_4$, 0.8 gl^{-1}; $MgSO_4.7H_2O$, 0.25 gl^{-1}; $FeSO_4$, 1 gl^{-1}; $CoCl_2$, 1 gl^{-1}; and molybdenum power, 1 gl^{-1}.

Inocula development

Colonies were transferred from the agar plates containing *P. aeruginosa* and *P. fluorescence* to 100 ml medium (containing both the mineral medium and the wastewater samples in ratio 4:1) in 250 ml Erlenmeyer flasks. These flasks were cultivated for 24 h on the New Brunswick Gyratory Shaker G25-R at 120 rpm and 30 ^{o}C. Between 5-10% (v/v) of these grown cultures were used to inoculate fresh flasks and these were also cultivated at the same conditions as stated above for 24 h.

Batch fermentation studies

All fermentation experiments were carried out on the New Brunswick microferm twin fermentor designed for mass cultivation of micro organisms in batch fermentation and continuous culture. The working volume was 4 l. All cultivations were carried out at 30^{o}C. Aeration was effected with compressed air at a flow of 1 vvm and the stirrer speed was set at 400 rpm. Samples were withdrawn every 6 h for analyses and each fermentation was for a period of 96 h.

Analytical methods

Dry Biomass Concentration

In estimating the dry biomass concentration, 10 ml of each sample taken were centrifuged and the supernatants were withdrawn leaving behind wet biomass. These were washed with equal volume of distilled water and these were carefully transferred to preweighed filter papers and they were then dried to constant weight in oven at 105^{o}C for 24 h. Cooling was effected in a desiccator followed by reweighing.

Phenol Concentration

Phenols in the culture supernatants were determined quantitatively by a colorimetric method using 4-aminoantipyrine as colour

reagent. The procedures of Greenberg et al. (1992) were employed.

RESULTS AND DISCUSSION

Figure 1 shows the biodegradation potentials of both indigenous *P. aeruginosa* and *P. fluorescence* in degrading phenol present in the effluent collected from Warri Refinery under batch conditions. Both *P. aeruginosa* and *P. fluorescence* were able to degrade the phenol but with different effectiveness. *P. aeruginosa* was able to remove phenol from the effluent completely at the 60 h of cultivation. Although only 73.1% phenol was removed by *P. fluorescence* from the effluent at this same time, complete mineralization of phenol was achieved at about the 84 h of fermentation.

The figure also shows the biomass yield of the *Pseudomonas* species during these fermentation studies. A maximum of 2.75 mg/l and 1.26 mg/l biomass concentration were obtained for *P. aeruginosa* and *P. fluorescence*, respectively. Our results showed that both bacteria strains have the potential to degrade phenol, there exist strong highly positive correlation between phenol degraded and biomass growth of the order of r = +0.994 and r = +0.980 at 95% confidence level. Typical of Pseudomonas cultivation on phenol and phenolic compounds is an initial lag phase but our results did not show any lag phase in all the batch fermentation experiments conducted. This could be explained by the low level of phenol (<100mg/l) present both in the effluent samples used. Thomas et al. (2002) reported an increasing lag phase period during phenol biodegradation with concentration ranging from 100 to 1000 mg/l. No lag phase was reported for 100 mg/l phenol as also observed by Collins and Daugulis (1997). Comparison of the two strains of bacteria employed in this work showed that *P. aeruginosa* was more effective in degrading phenol than *P. fluorescence*. Significant amount of phenol remained in the effluent sample after 60 h fermentation in the case of *P. fluorescence.* This is evident in the values of the kinetic parameter obtained for the two species using the Haldane model (Haldane, 1930; Jones et al., 1973) (Figure 2); $K_{i\,(P.aeruginosa)}$= 30.8 mg/l, $\mu_{max\,(P.aeruginosa)}$ = 0.019 h^{-1} and $K_{i\,(P.fluorescence)}$= 33.5 mg/l, $\mu_{max\,(P.fluorescence)}$ = 0.011 h^{-1}. It can be concluded from the aforementioned that the toxicity of refinery and petrochemical effluent can be reduced using *Pseudomonas* species and that the biodegradation of refinery and petrochemical effluent correlated with the ability of the microbial species to bioremediation. This could result in reducing its environmental pollution potential including air pollution which is a confirmed way of its contamination thus reducing the atmospheric impact (Luttrell, 2003). However, there is need to study the synergy between the species, if any, by the utilization of their mixed culture for the analysis of the bioreactor system.

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