BIOREMEDIATION POTENTIAL OF INDIGENOUS MICRO-ORGANISMS (*P. aeruginosa and P. fluorescens*) ON PHENOLIC WASTES IN REFINERY LIQUID EFFLUENTS

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ABSTRACT

In this study, a physico-chemical analysis of untreated and treated phenol-containing waste water samples collected from a Nigerian Refinery was carried out and the biodegradation of the constituent phenol using two Pseudomonas species were investigated in batch processes. The results of the physico-chemical analysis of the raw water and the treated water showed the phenolic content, BOD at 20^0C,sulphide content, Total suspended solids, Total dissolved solid, Phosphate content and Ammonia content for the raw water to be 28.4ppm, 94mg/L, 5.15ppm, 248ppm, 1196ppm, 10.36ppm, 2.16ppm and for the treated water to be 7.26ppm, 82mg/L, 2.84ppm, 116ppm, 1148ppm, 3.77ppm, 0.52ppm respectively. This results indicate that even though the installed treatment plant was very efficient for phenol removal by removing 74.44% of initial phenol concentration of the untreated waste water, the resultant treated water phenol concentration failed to comply with the Federal Environmental Protection Agency (FEPA) limit. Subsequently, the aerobic chemoheterotropic growth of both pseudomonas aeruginosa and pseudomonas fluorescens on a formulated basal medium using the wastewater samples was investigated. During the batch growth processes, the wastewater samples were supplemented with nutrients, the culture of organisms were added and the progress followed for 48hrs. Samples were withdrawn at regular intervals of 6 hrs and analyzed for biomass and phenol concentrations. The data obtained were used to estimate the pertinent growth parameters such as the average specific growth rates (μ_m), average energetic yield (η_m) and the doubling times (t_d).

The results of the biodegradation studies showed that pseudomonas aeruginosa successfully removed 82.64% of the phenol in the raw water while pseudomonas fluorescens correspondingly removed 49.01% after a residence period of 48 hours. The results also showed that the average energetic yield (η_m) values were 0.049 and 0.025 for pseudomonas aeruginosa and pseudomonas fluorescens respectively. The average specific growth rates (μ_m) of $0.032hr^{-1}$ and $0.015hr^{-1}$ and corresponding doubling times (t_d) of 21.7hr and 47.54hr were also obtained for pseudomonas aeruginosa and pseudomonas fluorescens respectively.

The results revealed that both organisms were able to degrade phenol in the waste water effluents, but with varied effectiveness and that pseudomonas aeruginosa is superior to pseudomonas fluorescens with the range of experimentation.

Keywords: Bioremediation, Physico-chemical, aerobic, Phenolic *Pseudomonas aeruginosa*, Chemoheterotrophic *Pseudomonas fluorescens.*

INTRODUCTION

The petroleum refineries and petrochemical plants have been the kingpin of the petroleum industry over the years worldwide. They are quite diversified and complex in the processes used in the conversion of the raw materials to finished valuable products (Christman et.al., 1993). In the refineries, crude oil are converted to wide range of products such as lubricants, petroleum fuels, bitumen and waxes while the petrochemical industries derive its feedstock from refinery processes which are then converted into valuable products such as plastics, resins, synthetic rubber etc. The petroleum refinery and petrochemical industries are most desirable for national development and improved quality of life. However, the various operations conducted at these refineries and petrochemical plants result in the generation of wastes which must be discharged (Christiman et. al., 1993). The unwholesome and environmentally unacceptable pollution effects of these wastes have been reported world-wide (Klein and Lee, 1978; Guieysse et. al., 2001). Prominent among the pollutants in these wastes are phenols and its compounds. In most refineries and petrochemical wastewater worldwide, phenol and its compounds seems to be one of the most difficult compounds to remove and this account for their distribution in various environmental sites as artificial or natural mono-aromatic compounds and thus pose a serious ecological problem due to its toxicity and persistent occurrence in the refinery environment (Prpich and Daugulis, 2005). High concentrations of organics in sediments have been traced to petroleum refinery effluents and

suspension of such may constitute air pollution problem. The use of phenolic components as biological indicator of air quality confirms this (Gutnick and Rosenberg, 1977). The inhalation and dermal exposure to phenol in air and water have been found to be highly irritating to the skin, eyes and mucous membranes in humans and its potential uptake through respiration have been confirmed (Eric, 2000). Moreover, phenol and its derivatives in wastewater that finds its way to water bodies pose a potent 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.

Physico- chemical methods have been employed for the treatment or removal of phenol and its derivatives in industries but have inherent drawbacks due to the tendency of the formation of secondary toxic materials and have proven to be costly (Bandyopadhyay et al., 1998). Thus, biological method of removal or treatment has turned out to be a favourable alternative for phenol degradation, being cost effective and does not produce toxic residues (Chandry, 1994; Tharasi and Jayalakshmi, 2003; Collins and Daugulis, 1997).

Biodegradation of phenols and its compounds have been actively studied (Solomon et al., 1994; Kotturi et al., 1995; Oboirien et al., 2005), but different microorganisms were used by these researchers for the degradation of phenols.

This study investigates the biodegradation potential of two different local strains of pseudomonas species; *pseudomonas aeruginosa* and *pseudomonas fluorescens* on Nigerian Refinery and Petrochemical effluents with the view of isolating and developing the organisms for the bioremediation of phenolic waste.

MATERIALS AND METHODS

Microorganisms and Refinery Effluent Samples

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

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; $(NH_4)_2SO_4$, 4g/l; KH_2PO_4,2g/l, NaCl,1.5g/l; $CaCl_2$, 1.2g/l, $NaHPO_4$, 0.8g/l; $MgSO_4.7H_2O$, 0.25g/l; $FeSO_4$,1g/l; $CoCl_2$,1g/l; and molybdenum powder,1g/l.

Inocula Development

Colonies were transferred from the agar plates containing *Pseudomonas.aeruginosa* and *Pseudomonas fluorescens* to 100ml medium (containing both the mineral medium and the wastewater samples in ratio 4:1) in 250ml Erlenmeyer flasks. These flasks were cultivated for 24hr on the New Brunswick Gyratory Shaker G25-R at 120rpm and 30°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 24hr.

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 4litres. All cultivations were carried out at 30°C. Aeration was effected with compressed air at a flow of 1vvm and the stirrer speed was set at 400 rpm . Samples were withdrawn every 6hr for analyses and each fermentation was for a period of 48hr.

Analytical Methods

Dry Biomass Concentration.

In estimating the dry biomass concentration, 10ml 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 then carefully transferred to preweighed filter papers. They were then dried to constant weight in an oven at 105 °C for 24hr. Cooling was effected in a dessicator followed by reweighing.

Phenol Concentration

Phenol 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 DISCUSSIONS

Physico-chemical Analysis

Table 1 shows the results of the physico- chemical analysis of both the untreated and treated wastewater. The values obtained were compared with the wastewater effluent limitation values specified by the Federal Environmental Protection Agency (FEPA, 1991). The table also shows the waste-treatment plant operating efficiency (α) and the degree of compliance with FEPA specifications (β).

Bioremediation Studies

Tables 2 and 3 show the results of biodegradation of phenol present in the wastewater effluent collected from a Nigerian refinery using pure cultures of *Pseudomonas aeruginosa* and *Pseudomonas fluorescens*. Tables 4 and 5 show the result of calculated kinetic parameters for the organisms. Table 6 shows the result of the average kinetic parameters for the organisms.

Table 1: Physico-chemical Characterisation of the Refinery Liquid Effluent Samples

Measured Parameters	Wastewater Samples Raw water	Treated Water	Treatment Plant Efficiency (α)%	Environmental Agency Limitation	Degree of Compliance with Agency Limitation β
Temperature(°c)	28.4	26.6	-	30o	-
PH	9.96	8.90	-	6-9	-
Conductivity(ms/cm)	3.56	3.86	-	-	-
Phenol(ppm)	28.4	7.26	74.44	0.5	14.52
Oil and grease(ppm)	277.5	178.5	35.67	10	17.85
BOD at 20Oc(mg/l)	94	82	12.76	10	8.2
Sulphide(ppm)	5.15	2.84	44.85	0.2	14.2
Total suspended solid(TSS)(ppm)	248	116	53.22	30	3.87
Total dissolved solid TDS (ppm)	1196	1148	4.01	2000	-
Phosphate (ppm)	10.36	3.77	63.6	5	-
Ammmonia (ppm)	2.16	0.52	75.93	0.2	2.6

$\alpha = \frac{\text{Parameter Value in Raw Water- Parameter Value in Treated Water x 100}}{\text{Parameter Value in Raw water}}$

$\beta = \frac{\text{Parameter Value in Treated Water}}{\text{Parameter Value for Agency Limitation}}$

Table 2: Measured variables for the Batch Growth of *pseudomonas aeruginosa* on Rawwater

Fermentation time (hr)	Phenol concentration,s(ppm)	% Phenol removal	Biomass concantration,x(g/L)
0	28.478	-	0.59
8	26.318	7.58	0.80
16	21.020	26.19	1.03
24	17.566	38.32	1.28
32	13.158	53.80	1.62
40	9.034	68.28	2.02
48	4.944	82.64	2.45

Table 3: Measured variables for the Batch Growth of *Pseudomonas fluorescens* on Rawwater

Fermentation time(hr)	Phenol concentration,s(ppm)	% phenol removal	Biomass concentration,x(g/L)
0	28.478	-	0.24
8	26.052	8.52	0.37
16	24.292	14.70	0.48
24	22.636	20.51	0.57
32	20.878	26.69	0.63
40	17.606	38.18	0.68
48	15.824	44.43	0.73

Table 4: Calculated Kinetic Parameters for the Batch Growth of *Pseudomonas aeruginosa* on Rawwater

Time (hr)	μ (hr-1)	η
8	0.03125	0.04433
16	0.028	0.04469
24	0.0270	0.04514
32	0.0262	0.04712
40	0.0248	0.05296
48	0.0219	0.05955

Table 5: Calculated Kinetic Parameters for the Batch Growth of *Pseudomonas fluorescens* on Rawwatter

Time (hr)	μ (hr-1)	η
8	0.0439	0.0288
16	0.0286	0.0359
24	0.0197	0.0293
32	0.0119	0.0196
40	0.0092	0.0193
48	0.0086	0.0164

Table 6: Average Kinetic Parameters for the Batch Growth of *Pseudomonas* Species on different substrates

Organism/substrate	$\mu_m(hr^{-1})$	$t_d(hr)$	η_m
Pseudomonas aeruginosa on Raw water	0.032	21.7	0.049
Pseudomonas fluorescens on Rawwater	0.015	47.5	0.025

DISCUSSION OF RESULTS

From tables 2 and 3, it was observed that both *Pseudomonas aeruginosa* and *Pseudomonas fluorescens* were able to degrade phenol but with varied effectiveness. *Pseudomonas aeruginosa* was able to remove 82.6% (23.5mg/l) of the initial phenol concentration after 48hr of cultivation while *Pseudomonas fluorescens* correspondingly removed about 44.4% (12.7mg/l).

To show the growth kinetics of the organisms in different media, some kinetic parameters such as specific growth rate μ, and biomass energetic yield η, were calculated and presented in tables 4 and 5. From these tables, the average specific growth rates μ_m, and the average biomass energetic yield η_m values for *pseudomonas aeruginosa* and *pseudomonas fluorescens* were calculated and presented in table 6.

From the values presented in tables 4 and 5,it was observed that the for the growth of the organisms in both media, the specific growth rate decreased as the rate of substrate consumption and this showed that the growth rate of the organisms was a consequence of substrate consumption (Solomon et al.,1994).

To show the growth characteristics of the organisms in different media, the doubling times t_d of the organisms in each of the medium was also calculated. From the results, it could be seen that the growth of the organisms on Rawwater gave average specific growth rates (μ_m) of $0.032hr^{-1}$ and $0.015hr^{-1}$ and the corresponding doubling times (t_d) of 21.7hr and 47.54hr for *pseudomonas aeruginosa* and *pseudomonas fluorescens* respectively. From the literature (Layokun et al., 1987), it was explained that the growth of the microorganism correspond to the degradation of the substrate to release energy and the utilization of the energy to convert inorganic nitrogen to organic nitrogen (biomass), it therefore follows that the greater the growth rate, the faster the degradation of phenol. Also, the more the number of microorganisms, the faster the degradation process. Therefore, any medium that enables the microorganism to double its number within a shorter time would accomplish the degradation faster. These criteria therefore qualify the organism, *pseudomonas aeruginosa* as the more effective degrader of phenol constituent of the wastewater effluent of a Nigerian refinery out of the two organisms investigated.

CONCLUSION

It can be concluded from the aforementioned that the phenolic waste from petroleum refinery and petrochemical plants can be reduced or minimized by the use of these local strains of *pseudomonas* species for bioremediation.

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