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Biodegradation Potentials of Microorganisms Isolated from Eleme Petrochemical Industrial Effluent

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Abstract
The biodegradation potentials of microorganisms isolated from different sampling points of Eleme petrochemical industrial effluent were analyzed. Eleme Petrochemical effluents take various forms which include; processed wastewater (raw effluent); clarified water (waste water undergoing treatment); retention pond gate (treated waste water) and receiving river. Biodegradation potentials of microorganisms isolated from the effluents were determined by shake flask degradation and screening test. The physicochemical analysis revealed that effluents from Eleme Petrochemical Company Limited generally contained low concentrations of physicochemical pollutants except for total suspended solids (78.48 ± 0.01mg/l) and oil and grease (25.80 ± 0.02mg/l) contents which were higher than FEPA recommended limits. The isolated microorganisms identified to the genus level included Pseudomonas, Bacillus, Serratia, Micrococcus, Escherichia, Klebsiella, Vibrio, Proteus, Achromobacter, Citrobacter, Flavobacterium, Aspergillus, Penicillium, Fusarium, Monilia and Trichoderma. Pseudomonas had the highest frequency of occurrence (10.33%) among bacteria while Aspergillus had the highest frequency of occurrence (19.58%) among fungi. Microbiological enumeration revealed that process waste water had the highest counts for total heterotrophic bacterial (THBC) (9.0×10^3cfu/ml) and total fungal counts (TFC) (5.0×10^3cfu/ml) while retention pond gate contained the highest counts for total hydrocarbon utilizing bacterial (THUB) (5.7×10^3cfu/ml). Pseudomonas, Serratia, Bacillus, Aspergillus, Penicillium and Fusarium were the most dominant microorganisms capable of utilizing petrochemical industrial effluent. The consortium of microorganisms demonstrated the highest efficacy to utilize petrochemical industrial effluent. Biodegradation of petrochemical industrial effluents by these microorganisms was manifested in the reduction of physicochemical parameters such as BOD (consortium of Pseudomonas and Bacillus sp, 96%) and COD (consortium of Aspergillus and Penicillium, 89%).

Key words: Biodegradation, Petrochemical, Effluents, Pollutants, Microorganisms, Waste water.

Introduction
Petrochemical effluents are wastewaters generated from petrochemical units as a result of many of the activities ranging from process operations such as vapour condensation, cooling tower blow down and storm water runoff. Effluents emanating from petrochemical industry also include wastewaters generated from the activities that take place in the offices and in the homes of staff living within the premises of the industrial complex. Petrochemical plants generate solid waste and sludge, some of which may be considered hazardous because of the presence of toxic organics and heavy metals (1). Accidental discharges as a result of abnormal operation, especially from polyethylene and ethylene oxide-glycol plants in a petrochemical complex, can be a major environmental hazard, releasing large quantities of pollutants and products into the environment (2, 3).The presence of objectionable conditions such as offensive odour and accumulation of debris have been reported to decrease the proper value and recreational uses of water. As earlier stated, wastewaters released by petrochemical industries are characterized by the presence of large quantities of polycyclic and aromatic hydrocarbons, phenols, metal derivatives, surface active substances, sulphides, naphthalenic acids and other chemicals (4). Due to the ineffectiveness of purification systems, wastewater may become seriously dangerous leading to the accumulation of toxic products in the receiving water bodies with potentially serious consequences on the ecosystem.

The Eleme Petrochemical Plant located in the oil rich-Niger Delta in Port Harcourt, Rivers State, Nigeria generates large quantities of effluents daily. The Eleme Petrochemical Company processes natural gas and liquids which occur in association with crude oil, made up of paraffins and olefins that can be combined to form desired petrochemicals. These products that serve as raw materials in the downstream industries for the production of plastics and other related products come out as crystalline thermophilic resins in the form of pellets. Additives such as antioxidants, stabilizers and others are added to polymers just before pelletizing to improve its resistance and quality. These activities generate different forms of wastes, which eventually end up

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in the environment with or without treatment. So also are the wastewaters generated as a result of office or domestic activities in the company (domestic wastewater or sewage) (5). Here, petrochemical effluents take various forms which include; processed waste water (PWW) which refers to water that is intended to come into contact with hydrocarbons or treated chemicals at the petrochemical plant, clarified water (CW) which is a combination of PWW and sewage and retention pond gate valve (RPGV) which is the effluent that has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels (6).
Studies have revealed that although effluents from Eleme Petrochemical Company Limited generally contain relatively low concentrations of pollutants in the water and sediment, accumulation of these pollutants overtime can be fatal to aquatic and human life. Also, continued discharge of improperly treated effluent may further compound the environmental problems of communities living around this company. This therefore makes imperative the need for early resolution of the problem of treatment for Eleme petrochemical effluent (6). This dire need in recent times has found microorganisms quite instrumental. Microorganisms have been implicated in showing strong ability to biotreat petrochemical effluents. The potentials of microorganisms to catabolize and metabolize xenobiotic compounds have been recognized as potentially effective means of toxic and hazardous wastes disposal. Phenol and its derivatives have long been recognized as some of the most persistent chemicals in petroleum refinery/petroleum waste waters with high toxicity even at low concentrations. Two species of *Pseudomonas, P. aeruginosa* and *P. fluorescence* have been studied for their biodegradation potential on phenol present in refinery wastewater under a batch fermentation process. Phenol was successfully degraded by both species and there was high positive correlation between phenol biodegradation and microbial growth (7).
That microorganisms are ubiquitous in nature is a proven fact, so also is the age long fact that microorganisms not only cause disease in humans or deterioration of substances but are also useful in the manufacturing (food and pharmaceutical) industries and degradation or transformation of both organic and inorganic substances. In fact, the degradative ability of microorganisms is the reason why we are not all deep in hydrocarbons today. This study was therefore conducted to isolate and identify indigenous microorganisms present in Eleme Petrochemical industrial effluent and ascertain its potentials to degrade the effluent.

Materials and Methods

Description of the Study Area

Eleme Petrochemical Company Limited (EPCL) is situated in Eleme, Rivers state in the oil-rich Niger Delta area of Nigeria. It was established by the Federal Government of Nigeria in 1988.The major feedstock used in the company is delivered to it in liquid form via pipeline from the liquefied natural gas (LNG) plant located at Obiafu/Obrikom in Rivers state. The feedstock is free from methane, but composed of ethane, propane and butane with minor quantities of pentane and heavier hydrocarbons. The major products of the company are low density and linear low density polyethylene (LLDPE), polypropylene (PP), vinyl chloride monomer, butane and mixture of other olefins. Effluents are usually treated with sulphuric acid, caustic soda, alum, urea, Di-ammonium phosphate, anionic polyelectrolyte and calcium hypochlorite. Thereafter, the treated effluent is directly discharged into receiving river bodies.
The Eleme River in Eleme kingdom took its source at Oyigbo and flows down Agbonchia farm settlement, Njuru, Okerewa and Aluto at which point the petrochemical effluent is discharged into it before entering into tidal creek by NNPC housing estate Aleto and flows down to Okrika. The river passes through sparse vegetation and its course flows across many roads and as such receives storm water runoffs from roads too.

Sample Collection

Samples were collected once a month between July 2007 and March 2008. Water samples were collected with a 2litre plastic Hydrobios water sampler and transferred to a clean 2litre polyethylene containers and 250ml capacity borosilicate glass bottles. The effluent samples include the process wastewater (PWW) (untreated effluent),clarified water (CW), retention pond gate (RPG), which is the industrial effluent that has undergone both chemical and biological treatment to eliminate or reduce waste contents to acceptable levels and the receiving river (RR) of Eleme kingdom. These were collected in polyethylene containers and borosilicate bottles of the same capacity. They were rinsed several times with water or effluent samples at the point of collection. The samples were transported to the laboratory using ice packed coolers after appropriate labeling. The effluent samples for dissolved oxygen and biological oxygen demand were fixed on the site by adding 1.00 ml each of Winkles reagent solution and taken to the laboratory for other physicochemical and microbiological analyses.

Microbiological Analysis

*Determination of total heterotrophic microbial count***:** This was performed in triplicates by plating out aliquot 0.1ml of the samples on nutrient agar plates containing Fushin (antifungal antibiotic), using the spread plate techniques. Plates were enumerated after 48h of incubation at 37^0C. Isolation and enumeration of fungal isolates were equally performed in triplicate by plating out aliquot 0.1ml of the effluent sample in 20ml of molten potato dextrose agar (PDA) containing two drops of streptomycin (5µg/ml) using the spread techniques. Plates were enumerated after 3-7 days of incubation at room temperature as described by (8).

Isolation and Characterization of Bacterial Isolates: Distinct colonies from mixed cultures in the Petri dishes were picked and transferred aseptically into sterile nutrient broth. The nutrient broth cultures were incubated at 37^{o}C for 24h. The broth cultures were streaked on sterile nutrient agar plates and incubated at 37^{o}C for 24h. Pure colonies were picked and transferred aseptically to nutrient agar slants, incubated for 24h at 37^{o}C and stored in the refrigerator at 4^{o}C for further characterization.

Pure cultures were presumptively identified on the basis of their morphological and biochemical characteristics by means of schemes of (9, 10, 11). The following tests were performed in duplicates using standard microbiological techniques; colonial morphology, cellular morphology, Gram reaction, motility, oxidase, catalase, urease activity, indole production, nitrate reduction, citrate utilization, carbohydrate metabolism (Hugh and Leifson's test), acid and gas production from various sugars, starch hydrolysis, methyl red test, Voges-Proskaeur test and coagulase test.

Isolation and Characterization of Fungal Isolates: Distinct hyphae from the mixed cultures in Petri dishes were isolated and transf were then stored in refrigerator at 4^{0}C. Microscopic examination was carried out to determine the shape and size of spores and mycelium. A drop of lactophenol cotton blue stain was placed on a clean slide and using a straight sterile needle, a small portion of the fungus was transferred into lactophenol cotton blue stain on a wet slide. A cover slip was then placed over the suspension and pressed. The wet mount preparation was observed under a light microscope and the shape, cell arrangement and structural characteristics were recorded. Identification was according to (12).

Screening and Utilization of Petrochemical Industrial Effluent by Isolated Microorganisms: Bacterial isolates were tested for utilization of petrochemical effluent using the modified mineral salt medium (13).The composition of the medium was as follows: NaCl (10.00g), $MgSO_4.\ 7H_2O$ (0.42g), KCl (0.29g), KH_2PO_4 (0.83g), $NaHPO_4$ (1.25g), $NaNO_3$ (0.42g), deionised water (1L), pH 7.2.

The medium was dispensed in 9mls quantities in four test tubes to which 1ml of petrochemical effluent was added to each tube. After capping, all test tubes were sterilized at 121^{o}C for 15 minutes and allowed to cool. On cooling, the tubes were inoculated with two drops of cell suspension of an isolate in sterile mineral salts medium. The cell suspension was prepared by suspending a loopful of bacterial and fungal isolates from nutrient and potato dextrose agar plates respectively, into 2ml mineral salt medium. One of the tubes which served as control remained uninoculated. All the test tubes were incubated at room temperature (28 - 30^{0}C) for 14 days after which each tube was scored for turbidity which indicated utilization of petrochemical industrial effluent.

Shake Flask Degradation Test: Organisms which showed the highest turbidity in the screening test were selected for shake-flask degradation tests. Two bacteria (*Pseudomonas* sp. and *Bacillus* sp.) and two fungi (*Trichoderma* sp. and *Aspergillus* sp.) were selected for this test. Effluent degradation by fungal isolates was assayed using Zapek-Dox medium (Rajamohon and Karthikeyan, 2004). One hundred and fifty millilitres of this medium were placed in four 250ml Erlenmeyer flasks and 10mls effluent added. These were sterilized at 121^{0}C for 15 minutes using an autoclave. Fungal inoculants for shake flask experiments were prepared by placing a loopful of isolates in 2ml each of Zapek-Dox medium. A third inoculant was prepared by placing one loopful each of both isolates in 2ml of medium to obtain fungal consortium for the degradation studies. One millilitre of each of the three inoculants was placed in three of the Erlenmeyer flasks containing sterile effluent and medium. A fourth flask remained uninoculated and served as control. Effluent degradation by bacterial isolates was carried out using modified mineral salts medium (13) earlier described for the screening test. One hundred and fifty milliliters of this medium was dispensed into four Erlenmeyer flasks and 10ml of effluent added. Bacterial inoculants for shake-flask experiment were prepared by suspending a loopful of each isolate (*Pseudomonas* sp. and *Bacillus* sp.) in 2ml of mineral salt medium. A third inoculum of bacterial consortium was prepared by mixing one loopful of each of both bacterial isolates in 2ml of medium, 1ml each of the three inoculants was added to three of the Erlenmeyer flasks and the fourth flask was uninoculated and served as control. All flasks were incubated at room temperature on a rotary shaker operated at 120 rpm for 20 days.

Determination of Physicochemical Parameters

Some physicochemical parameters of the effluents were determined. They include; pH, temperature, total dissolved solids, total suspended solids, turbidity, conductivity, potassium, nitrate, chemical oxygen demand., dissolved oxygen, biochemical oxygen demand, copper, lead, sulphate ions, total hydrocarbon content, phosphate, phenol, sodium, calcium, magnesium, chlorine, ammonia, zinc, nickel, vanadium, oil and grease, chromium, salinity, iron, cadmium and cobalt. The methods used were adapted from standards for the examination of water and wastewater. pH was read using equitronics digital pH meter mode EQ - 610 at 30^{0}C. Conductivity was determined using a conductivity meter after meter had been calibrated and stabilized at 0.0µs/cm. Total dissolved solids(TDS) was determined using a weigh balance Setra BL-4105. Chemical oxygen demand (COD) was determined by the open reflux method (14). Nitrate level was determined using phenol disulphonic acid method and absorbance was read at 410nm. Genesys 20 thermospectronic spectrophotometer was used for turbidity reading. Atomic absorption spectrophotometer was used for nickel, iron, lead and cadmium.

Results and Discussion

Results of physicochemical parameters from the study (Table 1) showed that the major pollutants in Eleme Petrochemical effluent were total suspended solid (TSS) (78.48±0.01mg/l) from retention pond gate and oil and grease (25.80±0.02mg/l) from process wastewater (PWW) which were higher than FEPA regulatory standards for discharge of petrochemical industrial effluent. Comparison of the different sampling points of Eleme Petrochemical industrial effluent depitched that process wastewater contained the highest number of total dissolved solids (TDS) (294.00±0.14mg/l) and total hydrocarbon content (THC) (0.88±0.00mg/l). Heavy metals such as lead (Pb) and iron (Fe) were found in low concentration and were not considered as health hazard. Table 2 presented the diversity of the isolated and identified microorganisms from the study. The prevalence of Gram negative bacteria was observed with Gram positive bacteria (*Bacillus*, *Micrococcus*, *Staphylococcus* and *Lactobacillus*) making up only 21.68% of the identified bacteria. *Pseudomonas* was the most predominant genus of bacteria isolated from the study with a frequency occurrence of 10.33%. The dominance of *Pseudomonas* in petrochemical industrial effluent has been reported (15). *Aspergillus*, *Penicillium* and *Fusarium* species were the predominant fungi isolated. The association of *Mucor*, *Cladosporium*, *Aspergillus* and *Penicillium* species with degradation of petroleum products has also been reported (16).

Table 1: The Physicochemical Characteristics of Process Wastewater (PWW), Clarifier (CW) and Retention Pond Gate (RPG)

Effluent Parameters	**PWW**	**CW**	**RPG**	**FEPA Effluent Limitation guideline (1991) (mg/l)**
pH	11.11±0.01	8.46±0.02	5.80±0.01	6-9
Temperature (^{o}C)	29.80±0.14	27.00±0.01	30.0±0.01	30.00
Total dissolved solids(TDS, mg/l)	294.00±0.14	50.00±0.01	60.60±0.01	2000
Total suspended solids(TSS, mg/l)	77.48±0.01	15.90±0.01	78.48±0.01	30
Turbidity(NTU)	9.38±0.02	5.80±0.02	5.10±0.02	300
Conductivity(µs/cm)	109.00±0.01	205.00±0.01	609.20±0.01	1000
Potassium (mg/l)	3.10±0.01	3.38±0.01	6.60±0.01	NI
Nitrate (mg/l)	0.95±0.01	0.05±0.01	0.60±0.01	1.0
Chemical oxygen demand (COD, mg/l)	28.60±0.01	30.10±0.01	28.55±0.01	40
Dissolved oxygen (DO, mg/l)	7.15±0.01	13.40±0.01	10.40±0.00	40
Biochemical oxygen demand (BOD)	3.04±0.01	4.03±0.00	6.15±0.01	10
Copper (mg/l)	0.015±0.01	<0.01	0.01±0.00	1.5
Lead (mg/l)	0.034±0.01	<0.01	<0.01	0.5
Sulphate ions (mg/l)	3.68±0.00	2.40±0.00	3.55±0.00	50
Total hydrocarbon content (THC)	0.88±0.00	ND	ND	10
Phosphate (mg/l)	0.06±0.01	4.44±0.00	1.55±0.01	5.0
Phenol (mg/l)	59.55±0.59	3.45±0.01	1.31±0.01	0.2
Sodium (mg/l)	16.62±0.61	2.28±0.03	15.82±0.47	200
Calcium (mg/l)	10.43±0.06	1.00±0.01	7.21±0.01	100
Magnesium (mg/l)	5.04±0.08	1.02±0.04	5.21±0.01	100
Chlorine (mg/l)	51.79±0.01	55.65±0.01	6.21±0.1	600
Ammonia (mg/l)	0.40±0.01	0.01±0.01	0.31±0.02	NI
Zinc (mg/l)	0.02±0.01	0.01±0.01	0.01±0.01	1.0
Nickel (mg/l)	<0.01	<0.01	<0.01	1.0
Vanadium (mg/l)	0.06±0.00	0.00±0.00	0.03±0.00	NI
Oil and grease (mg/l)	25.80±0.02	11.20±0.01	13.62±0.02	10.00
Chromium (mg/l)	0.01±0.00	0.00±0.00	0.01±0.00	0.5
Salinity (g/l)	0.21±0.02	0.17±0.01	0.13±0.04	NI
Iron (mg/l)	6.50±0.03	4.10±0.01	5.08±0.00	20
Cadmium (mg/l)	<0.01	<0.01	<0.01	1.0
Cobalt (mg/l)	0.10±0.00	ND	0.40±0.00	0.5

Values represent the Means± Standard Deviation of seven samples collected over a period of seven months
NI : Not indicated

Table 2: Microbial isolates from petrochemical industrial effluent and their frequencies of isolation

Bacteria genera	**Frequency of isolation (%)**	**Fungi**	**Frequency of isolation (%)**
Pseudomonas	10.33		
Bacillus	10.00	*Aspergillus*	19.58
Serratia	9.08	*Penicillium*	19.58
Micrococcus	8.23	*Fusarium*	19.58
		Rhizopus	10.55
Escherichia	8.23	*Mucor*	10.55
Klebsiella	8.23	*Geotrichum*	9.55
Vibrio	6.20	*Monilia*	9.55
Proteus	6.20	*Cladosporium*	5.23
Achromobacter	3.89	*Trichoderma*	5.22
Citrobacter	3.89		
Flavobacterium	3.89		
Acinetobacter	3.89		
Alcaligenes	3.86		
Enterococcus	3.67		
Enterobacter	3.66		
Staphylococcus	2.45		
Aeromonas	2.34		
Azotobacter	1.10		
Lactobacillus	1.00		
Salmonella	1.00		

Colony counts revealed the total heterotrophic bacteria and fungi and hydrocarbon utilizing bacteria and fungi present in the various samples analyzed. It was revealed that process wastewater (PWW) contained the highest number of total heterotrophic bacterial counts (9.0×10^3cfu/ml), clarifier wastewater (CW) had the highest total fungi (5.0×10^3cfu/ml) and hydrocarbon utilizing fungal counts (6.0×10^3cfu/ml) and retention pond gate had the highest hydrocarbon utilizing bacterial count (5.7×10^3 CFU/ml).The least counts for THBC, TFC, HUB and HUF were observed in CW (1.3×10^3 CFU/ml), receiving river (RR) (1.2×10^3CFU/ml) and PWW (1.1×10^3;1.4×10^3) respectively (Table 3). This implies that petrochemical industrial effluent is very prone to microbial degradation as they did harbour microorganisms that showed great potentials in utilizing hydrocarbon (HC) as their primary carbon and energy source. This corresponds with the earlier report of Ajuzie and Atuanya (17). However, the presence of indigenous microbes is not a direct indication that the indigenous microbes primarily utilize HC in the effluent as their carbon and energy sources, rather may utilize HC as their secondary carbon and energy sources. This aligned with the report of Okpokwasili and Okorie (18).

Table 3: Bacterial and fungal counts from various sampling points of Eleme petrochemical industrial effluent between July 2007 and March 2008.

Sample	**THBC (cfu/ml)**	**TFC(cfu/ml)**	**HUB (cfu/ml)**	**HUF (cfu/ml)**
A	9.0×10^3	5.0×10^3	1.1×10^3	1.4×10^3
B	1.3×10^3	4.0×10^3	1.5×10^3	6.0×10^3
C	4.9×10^3	2.0×10^3	5.7×10^3	2.5×10^3
D	8.0×10^3	1.2×10^3	2.6×10^3	5.5×10^4

A: Process wastewater (PWW)
B: Clarified water (CW)
C: Retention pond gate (RPG)
D: Receiving River (RR)
THBC: Total heterotrophic bacterial count
TFC: Total fungal count
HUB: Hydrocarbon utilizing bacteria
HUF: Hydrocarbon utilizing fungi

Table 4 : Chemical oxygen demand (COD) values of PWW treated with bacteria and fungi over a 20-day period at 4days interval (mg/l)

Organisms	**Day4**	**Day8**	**Day12**	**Day16**	**Day20**	**COD reduction**
Bacteria genera						
Pseudomonas	172 ± 17	140 ± 15	98 ± 7.0	72 ± 4.0	50 ± 3.0	86%
Bacillus	212 ± 5.0	156 ± 2.5	141 ± 1.0	80 ± 0.6	60 ± 0.4	82%
Serratia	118 ± 9.0	79 ± 7.0	77 ± 6.0	71 ± 5.0	68 ± 4.1	52%
Pseudomonas and Bacillus	208±5.0	160±2.0	155±1.1	60±1.0	50±0.1	96%
Bacterial control	180±6.0	180±6.0	180±6.0	180±6.0	180±6.0	-
Fungi genera						
Aspergillus	152 ± 5.0	145 ± 4.1	90 ± 3.0	60 ± 2.0	20 ± 0.6	88%
Penicillium	160 ± 7.0	152 ± 6.5	140 ± 5.1	122 ± 4.3	90 ± 3.0	38%
Aspergillus and *Penicillium*	120 ± 7.0	80 ± 6.0	78 ± 5.0	60 ± 4.0	12 ± 0.6	89%
Fungal control	150 ± 4.0	150 ± 4.0	150 ± 4.0	150 ± 4.0	150 ± 4.0	-

Data represent Means± Standard Deviation of three replicates

Table 5: BOD_5 values of effluent with bacteria and fungi over a 20-day period at 4days interval (mg/l)

	Day4	**Day8**	**Day12**	**Day16**	**Day20**	**COD reduction**
Bacterial genera						
Pseudomonas	40 ± 5.0	21 ± 4.5	20 ± 2.0	18 ± 1.0	15 ± 0.8	80%
Bacillus	40 ± 5.0	36 ± 3.0	23 ± 2.0	19 ± 0.5	18.8 ± 0.3	40%
Pseudomonas and Bacillus	48 ± 50	40 ± 4.0	39 ± 2.0	1 3 ± 1.8	2.0 ± 0.4	90%
Bacterial control	23 ± 1.0	23 ± 1.0	23 ± 1.0	23 ± 1.0	23 ± 1.0	-
Fungal genera						
Aspergillus	40 ± 3.0	36 ± 2.0	23 ± 1.2	15 ± 0.2	16 ± 0.2	86%
Penicillium	40 ± 3.0	37 ± 4.0	33 ± 2.0	30 ± 0.7	22 ± 2.0	41%
Aspergillus and *Penicillium*	37 ± 5.0	20 ± 2.0	17 ± 2.0	15 ± 4.0	4 ± 0.5	87%
Fungal control	36±0.5	36± 0.5	36±0.5	36± 0.5	36±0.5	-

Values represent the Means± Standard Deviation of three replicates

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) values for effluent treated with bacteria and fungi over a 20-day period using shake flask degradation test are depicted in tables 4 and 5 respectively. The results indicated there was greater efficacy of microbial consortium to degrade petrochemical effluents than individual species. *Pseudomonas* sp. had the highest COD reduction among the individual bacterial isolates. Improved pollutants remediation using bacterial and fungal consortium have previously been reported by (19). Microorganisms present in activated sludge from a petrochemical industrial have been used in treatment system to biodegrade hydrocarbon-contaminated waste water (20).

Table 6: Turbidity evaluation of major genera of petrochemical industrial effluent utilizing bacteria and fungi

Microbial isolates	**Degree of Utilization**
Bacterial genera	
Pseudomonas	+++
Bacillus	+++
Serratia	+++
Proteus	++
Achromobacter	+
Citrobacter	+
Fungal genera	
Aspergillus	+++
Penicillium	+++
Rhizopus	++
Trichoderma	+

Key: +++heavy growth, ++moderate growth,+ scanty growth

Table 7: Effect of effluent on test organisms

	Pseudomonas	***Bacillus***	***Serratia***
	24h LD_{50}(%)	24h LD_{50}(%)	24h LD_{50}(%)
PWW	>10	>10	>10
RPG	>10	>10	>10

PWW – Process wastewater
RPG- Retention pond gate

Chemical oxygen demand (COD) and biochemical oxygen demand (BOD_5) values for effluent treated with bacteria and fungi over a 20-day period using shake flask degradation test are depicted in tables 4 and 5 respectively. The results indicated there was greater efficacy of microbial consortium to degrade petrochemical effluents than individual species. *Pseudomonas* sp. had the highest COD reduction among the individual bacterial isolates. Improved pollutants remediation using bacterial and fungal consortium have previously been reported by (19). Microorganisms present in activated sludge from a petrochemical industrial have been used in treatment system to biodegrade hydrocarbon-contaminated waste water (20). The degree of utilization of effluent by microbial isolates in mineral salt medium and degree of utilization using optical density and the effect of effluent on test organisms are depicted in Tables 6 and 7. Results revealed that *Pseudomonas*, *Bacillus* and *Serratia* had the highest turbidity (+++), followed by *Proteus, Achromobacter* and *Citrobacte*r (++, ++, +) respectively. Amongst fungi, *Aspergillus* and *Penicillium* had the highest degree of utilization (+++), followed by *Rhizopus* and *Trichoderma* (+) respectively (Table 6). Several fungal and bacterial genera have been reported to degrade hydrocarbons (21, 22). The LD_{50} analysis done using three different predominant isolates (Table 7)

revealed that petrochemical industrial effluent was less toxic to less than 10% (>10) of *Pseudomonas, Bacillus* and *Serratia* population. This further proves that these microorganisms have the potentials to grow favorably in petrochemical industrial effluents.

Conclusion

Evidence available from this investigation indicated that the effluents from Eleme Petrochemical Company Limited generally contain low concentrations of physicochemical pollutants except total suspended solids and oil and grease contents which were higher than FEPA recommended limits. *Pseudomonas, Bacillus, Serratia, Aspergillus, Penicllium* and *Fusarium* were the most dominant microorganisms capable of utilizing petrochemical industrial effluent. Since this study has unveiled the potentials of indigenous microorganisms in petrochemical effluent biodegradation, it becomes very vital that further leaps be taken from this study in a bid to exploring newer, more effective, less costly and better satisfactory methods of petrochemical wastewater management.

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