CURRENT STUDIES IN COMPARATIVE EDUCATION, SCIENCE AND TECHNOLOGY, VOLUME 2, NUMBER 2, 2015, PP. 164-177

Comparison of Biodegradation Potential of Polycyclic Aromatic Hydrocarbons (PAHs) by Bacteria and Fungi of effluents from Forcados Terminal in Delta State

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ABSTRACT The biodegration potential of polyclic aromatic hydrocarbons (PAHS) of effluents from Forcados Terminal in Delta State by some bacterial and fungal isolates was carried out by incubating the isolates in a mineral salt broth amended with PAHs. Gas chromatographic method was used to determine the levels of PAHs left after 21 days incubation period. The bacteria isolated from effluents of ForcadosTerminal included *Pseudomonas* sp*, Klebsiella pneumoniae, Escherichia coli, Micrococcus* sp and *Staphylococcus* sp. *Pseudomonas* sp is the most commonly occurring while the fungi isolated from effluents of Forcados Terminal included *Aspergillus Penicillium*, Mucor, Rhizopus and *candida* species. *Aspergillus* sp is the most commonly occurring. Result of the 21-day biodegradation test by hydrocarbon utilizing bacterial and fungal isolates showed that there was a reduction in the original concentration of PAHs used for the test. Test results showed decrease in concentration of PAHs with increase in exposure time. Analysis after 21 days showed complete absence of PAHs. Both pure and mixed cultures of bacteria and fungi were able to biodegrade the recalcitrant PAHs and are found to be potential agents of bioremediation of environment impacted by PAHs. Comparing the biodegradation capability of bacteria and fungi to degrade PAHs, fungi had greater capability to degrade PAHs faster than bacteria

Keywords: Biodegradation, fungal cultures, bacteria cultures, polycyclic aromatic hydrocarbons, effluent

Introduction

The toxicity of petroleum depends on its chemical composition. The most toxic components of petroleum are typically found in the aromatic fraction.

Also, toxicity occurs when petroleum has entered the environment and its chemical composition begins to change. The change depends on properties such as how easily they dissolve in water and their oxidizabilities. The rate of change varies with environmental conditions (Chen *et al.,* 2002).When petroleum pollutes the environment; it affects aquatic species by altering essential elements of their habitat. For example, petroleum spillage in water does not quickly dilute, but tends to remain in a concentrated mass on the surface, which is only slowly changed and degraded. Thus, its most pronounced effects on aquatic organisms are on those who make use of the water surface or inhabit the shorelines (Chen *et al.,* 2002). Petroleum pollution also causes high mortality in marsh grass, mangrove, and plant communities. Algae may die off or grow more abundantly in response to petroleum, depending on conditions and on the concentration of the petroleum (Albers, 1998). Petroleum may also contribute to deep-sea pollution. Most petroleum pollution enters the deep sea from spills on land or rivers during transportion of petroleum. Polycyclic aromatic hydrocarbons (PAHs) are molecules made of two, three or more fused aromatic rings in various structural configurations, e.g. pyrene, fluoranthene, phenanthrene, etc (Karthikeyan and Bhandani, 2001). They are a class of compounds found throughout the environment in the air, in the soil and in the water. They are found naturally in crude oil, creosote, coal tar, and coal. They can also be made by incomplete combustion of hydrocarbons in coal, oil, gas, tobacco and during forest fires (Vila *et al*., 2001). Examples of some known PAHs include: naphthalene, 2-methylnaphthalene, acenaphthene, acenaphthylene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, benzo(a)anthracene, chrysene, benzanthracene, benzo(b)fluoranthene, benzo(k)fluoranthene, benzo(a)pyrene, dibenzo (a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene. Concern about PAHs initially focused on their ability to cause cancer, but more recently, concern has turned to their interference with hormone systems and their potential effects on reproduction as well as their ability to depress immune function (Chaloupka, 1993). The objectives of this study therefore, was to isolate and identify bacteria and fungi in the effluents of Forcados Terminal, to ascertain the level of pollution by enumerating the number of bacteria and fungi found in the effluents and to compare the biodegradation of PAHs by associated Bacteria and Fungi.

Materials and Methods

Effluent samples for bacteria and fungi analysis were collected using the method of Adesemoye *et al* (2006). Sterile 1 litre sample bottles were used to asceptically draw part of the effluent water. The effluent samples were collected from four different locations: Barge Jetty A, Barge Jetty B, Saver Pit 1 and Saver Pit 2. Control samples were collected 500m away from the sam-

pling points. After collection, the samples were placed in a cooler containing ice blocks and transported immediately to the laboratory for analysis. 10ml of each effluent samples was added to 90ml of sterile distilled water to get an aliquot. Subsequent serial dilutions were made by adding 1.0ml of the last dilution to 9ml of fresh diluents. Finally, 0.1ml of appropriate dilutions (10^{-2} and 10^{-3}) of bacteria and fungi respectively were inoculated unto sterile solidified agar in petridish and evenly spread out with a sterile glass spreader. Cultures were prepared in duplicates on dry media, and incubated at room temperature. Total heterotrophic bacterial and fungal counts of the effluents was determined. The spread plate technique as described by Prescott *et al*. (2005) was adopted. The plates were incubated at room temperature for about 3-5 days after which the colonies were counted and the mean of the counts recorded accordingly. Different bacteria and fungal colonies were subcultured on sterile Nutrient Agar and Potato Dextrose Agar respectively to get pure cultures. Pure cultures of bacteria and Fungi were subcultured on Nutrient Agar and Potato Agar slants which were then stored in the refrigerator for further use. Polycyclic Aromatic Hydrocarbon contents of the samples were determined by gas chromatographic (GC) analysis. A Schimadzu GC-17A gas chromatograph equiped with flame ionization detector (FID) was used.

Isolation of Hydrocarbon Utilizing Bacteria and Fungi from Forcados Terminal Effluents

The population of hydrocarbon utilizing bacteria and fungi was determined by inoculating 0.1ml aliquot of the 10^{-2} and 10^{-3} diluted samples for bacteria and fungi respectively onto mineral salt agar media using the spread plate technique as described by Odokuma (2003). The vapour phase transfer method phase method was adopted. It employed the use of sterile filter paper discs soaked in filter sterilized crude oil which served as the only carbon source in the mineral salt agar. The sterile crude oil-soaked filter papers were then aseptically transfered to the inside cover of the inoculated petri dishes and incubated for 5days at room temperature. After the incubation period, mean of the colonies for the triplicate plates were calculated and recorded accordingly

Adaptation of Polycyclic Aromatic Hydrocarbon Degrading Isolates

Bacteria and fungal isolates were adapted for polycyclic aromatic hydrocarbon utilization and degradation using mineral salt broth with polycyclic aromatic hydrocarbon as the sole carbon source. Incubation was at 30°C and aerated at 100 strokes per minute (Wang, 1984) for 30 minutes each day for

10 days. A loopful of the adapted culture medium was transferred onto mineral salt agar plates as described by Odokuma (2003). The media contains polycyclic aromatic hydrocarbon as the only carbon source. The plates were incubated at 30°C for 5 days after which discrete colonies that developed were transferred onto Nutrient Agar and Potato Agar plates and then incubated at 30°C for 24 hours after which they were stored in the refrigerator for further use.

Preparation of PAHs Standard Solution

An ampoule of polycyclic aromatic hydrocarbon (Sigma, USA) containing 1mg each of naphthalene, acenaphthylene, acenaphthene, fluorene, phenanthrene, anthracene, fluoranthene, pyrene, 1,2 – benzanthracene, chrysene, benzo(b)fluoranthene, benzo(k) fluoranthene, benzo(a)pyrene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthracene and benzo(g,h,i)perylene was asceptically mixed with 99ml of sterile normal saline (diluent) making 100ml of stock solution containing 10mg/l of individual polycyclic aromatic hydrocarbon constituent. From the stock solution 0.3ml each of this stock solution (containing 0.03mg/l of each PAH) was added to each experimental flask that contained 99.7ml mineral salt broth and 0.1ml of the microorganisms in the biodegradation experiments.

Biodegradation Experiment

The method used in this study is applicable for the determination of biodegradation rates of organic compounds in an activated sludge process. The test method is designed to evaluate the ability of an aerobic biological reaction system to degrade or destroy specific components such as PAHs in waste streams. This method was also used by Okoro (2008) and Nwachukwu (2010). Preparation of inoculum. Two bacterial isolates, *Pseudomonas sp.* and *Klebsiella sp.*, were subcultured separately on sterile nutrient agar medium followed by incubation at 37°C for 24 hours. A loopful of each was then inoculated into a sterile nutrient broth medium separately for pure cultures and combined for mixed cultures, followed by incubation at 37°C for 24 hours.

The cells were then harvested by centrifuging at 2000 rpm for 30 minutes, after which the cells were individually re-suspended in sterile physiological normal saline and further washed by centrifuging at 2000 rpm for another 30 minutes to obtain neat cells which were suspended in sterile physiological normal saline and further diluted with sterile physiological normal saline to a low density cell suspension of 0.2 absorbance containing 1×10^3 cfu/ml and 1×10^4 cfu/ml of bacteria and fungi respectively. 0.1ml from this dilution

which served as inocula was added to the 3 sets of experimental flasks as shown below.

Composition of PAHs Biodegradation Experiment Flasks (Bacteria)

- 99.7ml mineral salt broth +0. 3ml (0.03mg/l) solution of PAH + 0.1ml culture of *pseudomonas* sp.
- 99.7ml mineral salt broth +0.3ml (0.03mg/l) solution of PAH + 0.1ml culture of *Klebsiella sp.,*
- 99. 7ml mineral salt broth + 0.3ml (0.03mg/l) solution of PAH + 0.1ml mixed culture of bacteria *(Pseudomonas sp.,* and *Klebsiella sp.,)*
- 99.7ml mineral salt broth + 0.3ml (0.03mg/l) solution of PAH + 0.1ml sterile Nutrient broth (control)

Composition of PAHs Biodegradation Experiment Flask (Fungi)

- 99.7ml mineral salt broth +0.3ml (0.03mg/l) solution of PAH + 0.1ml culture of *Aspergillus* sp.
- 99.7ml mineral salt broth + 0.3ml (0.03mg/l) solution of PAH + 0.1ml culture of *Penicillium* sp.
- 99.7ml mineral salt broth + 0.3ml (0.03mg/l) solution of PAH + 0.1ml mixed culture of fungi *(Aspergillus* sp*., & Penicillium* sp)
- 99.7ml mineral salt broth + 0.3ml (0.03mg/l) solution of PAH + 0.1ml Nutrient broth(Control)

Total Heterotrophic Bacterial Counts

The total heterotrophic bacterial (THB) counts ranged between 2.1 x 10^3- 9.5 x 10^3 cfu/ml with a mean value of 4.5 x 10^3 cfu/ml. The highest bacterial count of 9.5 x 10^3 cfu/ml was recorded in July at Saver Pit 1 while the lowest count of 2.1 x 10^3 cfu/ml was recorded in January for Barge Jetty B. Comparison of mean spatial variations in total bacteria counts at the sampling locations and their controls showed that minimum and maximum mean values 3.7 x 10^3 cfu/ml and 4.6 x 10^3 cfu/ml occurred at Saver Pit 1 control and Saver Pit 2 respectively. The microbial loads of all the effluent samples were low. Microorganisms are said to be ubiquitous and are known for essential functions which include decomposition of organic materials, bioaccumulation of chemicals and biogeochemical cycling of elements. Their presence, abundance and growth in the environment are greatly influenced by factors such as pH, temperature, Inocula size, availability of nutrients and salinity. In this study, it was observed that effluent samples from Saver Pits 1 had the highest total bacterial count. The results revealed that effluents from Saver

Pit 1 had higher total heterotrophic bacterial count than Saver Pit 2, Barge Jetty A and Barge Jetty B. The increased bacterial load may be due to the activities of the sewage compartment of the Forcados Terminal that can contaminate the Saver Pits.

The Barge Jetties A and B had lower bacterial counts. This could be a result of less sewage contamination around the jetties. The boats only anchor there before and after conveying people to the terminal. Generally, bacterial counts from effluents of Barge Jetties and Saver Pits of Forcados Terminals were higher than those of the control which signifies partial contamination of the test samples by the activities of the oil industries that discharge into Forcados Terminal.

Total Heterotrophic Fungal Counts

The total heterotrophic fungal counts ranged between 1.0×10^4 and 8.5×10^4 cfu/ml with a mean value of 3.2×10^4. The highest fungal count of 8.5×10^4 cfu/ml was recorded in July at Saver Pit 1 while the lowest count of 1.0×10^4 was recorded in May at Barge Jetty A.

The microbial loads of all the effluent samples were low. Microorganisms are said to be ubiquitous and are known for essential functions which include decomposition of organic materials, bioaccumulation of chemicals and biogeochemical cycling of elements. Their presence, abundance and growth in the environment are greatly influenced by factors such as pH, temperature, Inocula size, availability of nutrients and salinity. In this study, it was observed that effluent samples from Saver Pit 1 had the highest total fungal counts. The result revealed that effluents from Saver Pit 1 had higher total heterotrophic fungi count than Saver Pit 2, Barge Jetty A and Barge Jetty B. The increased fungal load may be due to the activities of the sewage compartment of the Forcados Terminal that can contaminate the Saver Pits.
The Barge Jetties A and B had lower fungal counts. This could be a result of less sewage contamination around the jetties. The boats only anchor there before and after conveying people to the terminal.

Generally, counts from effluents of Barge Jetties and Saver Pits of Forcados Terminals are higher than those of the control which signifies partial contamination of the test samples by the activities of the oil industries that discharge into Forcados Terminal.

However, fungal counts in test and control effluent samples were not significantly raised. This is as a result of Forcados Terminal being capable of treating the effluents before discharge into the storage tank that settles in the Saver Pits and Barge Jetties.

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by a Pure Culture of *Pseudomonas* sp.

The results of the biodegradation of the PAHs by a pure culture of Pseudomonas sp. after 7, 14, and 21 days were as follows. The results after 7 days showed the absence of naphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo(a) anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h) anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene and benzo(k) fluoranthene, while fluoranthene had a value of 0.0025mg/L. On the 14th day, fluoranthene had a value of 0.0012mg/L. Analysis after 21 days showed complete absence of all the PAHs.

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by a Pure Culture of *Klebsiella* sp.

The results of the biodegradation of the PAHs by a pure culture of *Klebsiella* sp. after 7, 14, and 21 days were as follows. The results after 7 days showed the absence of naphthalene, 2-methylnaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo (a) anthracene, chrysene, benzo (b) fluoranthene, benzo (a) pyrene, dibenzo (a,h) anthracene, benzo (g,h,i) perylene, indeno(1,2,3-cd)pyrene and benzo(k)fluoranthene, while fluoranthene had a value of 0.0025mg/l and acenaphthalene had a value of 0.0043mg/l. On the 14th day, fluoranthene had a value of 0.0015mg/l, while phenanthrene had a value of 0.0087mg/l. Analysis after 21 days showed complete absence of all the PAHs.

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by a Mixed Culture of Bacteria (*Pseudomonas sp*. and *Klebsiella sp.)*

The result of the biodegradation of the PAHs by a mixed culture of *Pseudomonas sp*. and *Klebsiella sp*., after 7, 14 and 21 days were as follows.The results after 7 days showed the absence of naphthalene, 2-methylnaphthalene, *benzo (*acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene, indeno(1,2,3-cd) and benzo(k) fluoranthene, while fluoranthene had a value of 0.0030mg/l and benzo(a) anthracene anthracene had a value of 0.0016mg/L. On the 14th day, fluoranthene had a value of 0.0015mg/l. Analysis after 21 days showed complete absence of all the PAHs

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by a Pure Culture of (*Aspergillus* sp.)

The result of biodegradation of individual polycyclic aromatic hydrocarbons (PAHs) by a pure culture of *Aspergillus sp*. after 7 days, 14 days, and 21 days. The results after 7 days showed the absence of naphthalene, 2-methylnaphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, chrysene, fluoranthene, benzo(a) pyrene, benzo(b)fluoranthene, benzo(g,h,i)perylene, indeno(1,2,3-cd)pyrene, dibenzo(a,h)anthrancence and benzo(k)fluoranthene, while fluoranthene had a value of 0.0021mg/L. On the 14th day, fluoranthene had a value of 0.0012mg/L. Analysis after 21 days showed complete absence of all the PAHs.

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by *a* Pure Culture of *(Penicillium sp.)*

The result of biodegradation of individual PAHs by a pure culture of *Penicillum sp*. after 7 days, 14 days, and 21 days.The results after 7 days showed the absence of naphthalene, 2- methylnaphthalene, acenaphthalene, acenaphthene, fluorene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo(b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and benzo(k)fluoranthene, indeno(1,2,3-cd)-pyrene, while fluoranthene had a value of 0.0022mg/L. On the 14th day, fluoranthene had a value of 0.0016mg/L. Analysis after 21 days showed complete absence of all the PAHs.

Biodegradation of Individual Polycyclic Aromatic Hydrocarbon by a Mixed Culture of Fungi (*Aspergillus sp. and Penicillium* sp.)

The result of biodegradation of the individual PAHs by a mixed culture of fungi sp after 7, 14 and 21 days. The results after 7 days showed the absence of naphthalene, methylnaphthalene, fluorene, phenanthrene, anthracene, pyrene, benzo(a)anthracene, chrysene, benzo (b)fluoranthene, benzo(a)pyrene, dibenzo(a,h)anthracene, benzo(g,h,i)perylene and benzo(k)fluoranthene, while fluoranthene had a value of 0.0028mg/L and acenaphthalene had 0.0048mg/L. On the 14th day, fluoranthene had a value of 0.0015mg/L. Analysis after 21 days showed complete absence of all the PAHs.

Comparism of Biodegradation of Polycyclic Aromatic Hydrocarbons (PAHs) by Pure and Mixed Cultures of Bacteria and Fungal Isolates

Biodegradation is the breaking down of organic matter into nutrients that can be used by other organisms. In this study, it was observed that the levels of PAHs in Barge Jetties and Saver Pits of Forcados Terminal were within 0.001-0.05mg/l which is higher than the Environmental Protection Agency limit of 0.0004mg/l. Pure cultures of fungi and bacteria (*Aspergillus sp, penicillium sp pseudomonas sp* and *klebsiella sp)* were used for the biodegradation tests as well as mixed cultures of fungi *(Aspergillus sp* and *penicillium sp),* mixed cultures of bacteria *(pseudomonas sp* and *klebsiella* sp) and mixed cultures of bacteria and fungi (*Aspergillus sp, penicillium* sp*, pseudomonas* sp and *klebsiella* sp*.)*.

	***Aspergillus* sp.**	***Penicillum* sp.**	**Mixed Culture of Fungi**	***Pseudomonas* sp.**	***Klebsiella* sp.**	**Mixed Culture of Bacteria**	**Mixed Culture of Bacteria & Fungi**
Day 1	0.03mg/l	0.03mg/l	0.03mg/l	0.03mg/l	0.03mg/l	0.03mg/l	0.003mg/l
Day 7	0.0021mg/l	0.0022mg/l	0.0028 mg/l	0.0025mg/l	0.0025 mg/l	0.0030mg/l	0.0043mg/l
Day l4	0.0012 mg/l	0.0016 mg/l	0.0015 mg/l	0.0012mg/l	0.0015 mg/l	0.0015 mg/l	0.0022 mg/l
Day 21	0	0	0	0	0	0	0

Throughout the biodegradation tests, the concentrations of the PAHs reduced between 7 days and 14 days and disappeared completely after 21 days in the experimental and controls. This may be due to abiotic factors such as pH, temperature, salinity, inoculum size and possibly the volume of the effluent. The two fungal isolates *Aspergillus* sp *and Penicillium* sp and the two bacteria isolates *Pseudomonas* sp and *Klebsiella* sp used in this study were predominant fungal and bacteria isolates found in effluents of Forcados Terminal. This agrees with Okoro (1999) who observed that the two fungal isolates used in his study, *Aspergillus* sp and *Penicillium* sp were the predominant fungal isolates found in produced water effluent from Chevron's Escravos tank farm.

Both the fungal isolates (*Aspergillus* sp *and penicillium* sp*)* and bacteria isolates (*pseudomonas* sp and *klebsiella* sp) form part of the microflora of the effluents of Forcados Terminal and therefore can tolerate the pH range (4.86 -8.09), slightly low biochemical oxygen demand (BOD_5) and high salinity of the effluent water. According to Okoro and Amund (2002), when degradation is carried out by mixed cultures of bacteria and fungi, fungal cultures are usually outgrown by their bacteria counterparts. Since the bacteria are usually fast degraders, the degradation potential of fungi are not usually observable until the time that the population density of the bacteria species has dropped significantly. However, comparing the degradation efficiency of bacteria and fungi from the effluents of Forcados Terminal on the PAHs, it is evident that fungi have a greater capacity and enzymatic capability to degrade the recalcitrant PAHs than bacteria. This can be as a result of the fact that fungal species dominate in the Forcados Terminal produced water (the total number of species of fungi isolated were 493 as compared to the 153 total number of bacteria species isolated). The pure cultures of *Aspergillus* sp *and Pencillium* sp used in this study showed greater capacity in the degradation of PAHs than the pure cultures of bacteria *(Pseudomonas* sp *and Klebsiella* sp). This is in agreement with the results of Okoro and Amund (2002).

The mixed cultures of fungi *Aspergillus sp and Pencillium* sp were found to have a greater capacity and enzymatic capability to degrade PAHs than the mixed cultures of bacteria (Table 4.11). The study also revealed that single pure cultures of fungi (especially *Aspergillus* sp) had a greater capacity to degrade PAHs than mixed cultures of fungi or mixed cultures of bacteria and fungi, therefore proving *Aspergillus* sp an excellent degrader. This trend of results is in agreement with the result of Okoro (2008) who observed that fungi have a greater capacity and enzymatic capability to degrade PAHs than bacteria, the pure culture of *Aspergillus* sp used in his study was the fastest degrader of PAHs. The enzymatic capability of *Aspergillus* sp is greater when used singly than when combined, it is possible that when combined with other fungi which might try to out-grow them, their activity reduces. Pure cultures of bacteria (*Pseudomonas* sp or *Klebsiella* sp*)* were faster degraders when used singly than when mixed together (Table 4.11). This might be due to the fact that only few organisms have the full enzymatic capability to mineralize hydrocarbons to carbondioxide and water and one of the bacteria used had a greater capacity and enzymatic capability to degrade PAHs than when combined. The result of this study is however contrary with the earlier observation and reports by Walker and Colwell (1974). They observed that mixed cultures performed as much as twice in biodegradation of hydrocarbons than single cultures. This is possible due to fact that they must have combined species of bacteria that have great capacity and enzymatic capability to degrade PAHs.

Mixed cultures of bacteria and fungi together *(Aspergillus* sp*, Penicillium* sp*, Pseudomonas* sp *and Klebsiella* sp*)* were found to have a slower capacity and enzymatic capability in the degradation of PAHs than individual pure cultures of bacteria or fungi. This is in agreement with Okoro and Amund (2002), when degradation is carried out by mixed cultures of bacteria and fungi, fungal cultures are usually outgrown by their bacteria counterparts thereby reducing their degradation ability.

The order of rates of biodegradation observed in this study is as follows. *Aspergillus sp* >*Pencillium* sp > *Pseudomonas* sp > *Klebsiella sp* > mixed culture of fungi > mixed culture of bacteria > mixed culture of bacteria and fungi. Other researchers (e.g. Andrea *et al*. (2001), Cerniglia (1992), Gadd (2001) and Sutherland (2004)) have also reported that fungi are good PAHs degraders. It is apparent from this study that both pure and mixed cultures of fungi and bacteria (*Aspergillus* sp, *pencillum* sp, *Pseudomonas* sp and *Klebsiella* sp) were able to degrade the original level of PAHs present in the effluent samples of Forcados Terminal to an insignificant concentration after 21 days and that pure cultures of fungi particularly (*Aspergillus* sp) were able to degrade PAHs faster

Limitation of study

Throughout the biodegradation tests, the concentrations of the PAHs reduced between 7 days and 14 days and disappeared completely after 21 days in the experimental and controls. This may be due to abiotic factors such as pH, temperature, salinity, inoculum size and possibly the volume of the effluent. Measures should be taken while carrying out this kind of experiment next time to eliminate the effect of the abiotic factors. Methods of other researchers should be subjected to futher experiment to eliminate experimental errors before adopting a standard method.

Conclusion

This research was intended to isolate and identify bacteria and fungi in the effluents of Forcados Terminal, to ascertain the level of pollution by enumerating the number of bacteria and fungi in the effluents and to compare the biodegradation potential of some bacteria and fungi to degrade PAHs. The conclusion from this research are as follows:

- Comparing the degradation efficiency of bacteria and fungi on PAHs, Fungi have a greater enzymatic capability to degrade the PAHs than bacteria.
- The pure cultures of the fungi *Aspergillus* sp and *Penicillium* sp used in this study showed greater capabilities in the degradation of PAHs than the mixed culture of fungi (*Aspergillus* sp and *Penicillium* sp).

- The pure cultures of bacteria *Pseudomonas* sp and *Klebsiella sp* used in this study showed greater capacity in the degradation of PAHs than the mixed cultures of the bacteria (*Pseudomonas* sp and *Penicillium* sp*)*.
- The mixed culture of the fungi (*Aspergillus* sp and *Pencillium* sp) were faster degraders of PAHs than the mixed culture of the bacteria (*Pseudomonas* sp and *Klebsiella* sp).
- The individual mixed culture of fungi or bacteria were faster degraders of PAHs than the mixed cultures of fungi and bacteria (*Aspergillus* sp + *Penicillium* sp + *Pseudomonas* sp + *Klebsiella* sp).
- The pure culture of *Aspergillus* sp used in this study proved to be an excellent degrader as it showed fastest capacity in the degradation of PAHs, followed by *Penicillium* sp*, Pseudomonas* sp, *Klebsiella* sp, mixed culture of fungi, mixed culture of bacteria and mixed cultures of bacteria and fungi.

Recommendation

Active research into the waste and pollution minimization strategies, waste avoidance technologies, cleaner production processes and zero PAHs emission concepts in Nigeria should be encouraged. Periodic monitoring should be given to the Forcados Treatment plants in Delta State in order to maintain their efficiency

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