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Growth Kinetics of Aerobic Heterotrophic Bacteria and Cyanobacteria During Biodegradation of Total Petroleum Hydrocarbon in Bodo Creek

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Abstract

Laboratory studies on resident aerobic heterotrophic bacteria and cyanobacteria isolated from crude oil contaminated Bodo creek characterized by moderate salinity was investigated for total petroleum hydrocarbon degradation using GC-FID (Agilent 6890 model). Monod's empirical expression to determine the specific growth rate based on the effect of the limiting substrate –total petroleum hydrocarbons. The growth of aerobic heterotrophic bacteria and cyanobacteria increased with decrease in TPH and its percentage loss in all the treatments monitored did not vary significantly with time using a one-way analysis of variance (ANOVA) ($p>0.05$). The values for the maximum specific growth rate (μm) and the saturation constant (K_s) for the treatment were; aerobic heterotrophic bacteria, AHB μm = 0.02823day^{-1}, K_s = 4229.61 mg/L; cyanobacteria, CB, μm = 0.01468 day^{-1} K_s = 2130.21 mg/L, consortium of aerobic heterotrophic bacteria and cyanobacteria, AHB + CB, μm = 0.06085day^{-1}, K_s = 1716.51 mg/L and the control, μm = 0.074033 day^{-1}, K_s = 6200.71. The result obtained throughout the study implies that the consortium of aerobic heterotrophic bacteria and cyanobacteria had higher affinity for TPH having the lowest K_s value of 1716.51 mg/L compared to the other treatment options.

Keywords: Cyanobacteria; Aerobic; Heterotrophic bacteria; Specific growth rate; Degradation

Introduction

Bodo creek is located in Gokana LGA of Rivers State located in the oil producing Niger Delta region of Nigeria is polluted by petroleum hydrocarbons as a result of crude oil exploration activities and spillages due to community restiveness, crude oil pipeline vandalism and accidents. It is characterized by brackish water which has some percentages of salinity that could stress most known living organisms [1]. According to UNEP, (2011) petroleum hydrocarbons has polluted the surface water sources, soil, land areas, sediments and swampy areas throughout the creeks in Ogoniland, which Bodo creek is not an exception.

Earlier research reports on the capabilities of certain microbes to mineralize hydrocarbon components into environmentally friendly substances such as carbon dioxide and water, the ability of bacteria to break down hydrocarbons has stirred up extensive research interest and attention in modern day research in this regard [2]. Biodegradation of petroleum hydrocarbons by microorganisms is a key environmentally friendly removal process of hydrocarbons and is controlled by the hydrocarbon physic chemistry, environmental factors, bioavailability and the presence of catabolically active microbes [3]. The interaction between hydrocarbons, the environment, and microorganisms is complex and greatly determines its fate based on their chemical nature and the capability of the available and metabolically active microbial community to carry out degradation provided optimal environmental conditions which can affect microbial activities are present. The presence of metabolic inhibitors, a viable and active population of hydrocarbon utilizing microorganism in the environment can also determine the fate of hydrocarbons [1,4].

The fate of petroleum hydrocarbons in marine ecosystems has been extensively studied previously. Reports revealed that crude oil and petroleum distillate products introduced into the marine aquatic environment are subjected to different physical, chemical and biological changes [5,6]. Caruso et al. [7] posited that hydrocarbon-degrading microorganisms usually exist in very low abundance in marine environment but petroleum hydrocarbon pollution may however stimulate the growth of such organisms and also cause changes in the microbial community structure in the petroleum hydrocarbon contaminated area. Ichor et al. [1,8] reported on biodegradation of petroleum hydrocarbons by aerobic heterotrophic bacteria, cyanobacteria consortium isolated from Bodo creek respectively. Chikere and Ekwuabu [9] however isolated and characterized hydrocarbon utilizing bacteria from petroleum hydrocarbon contaminated soil and surface water from Bodo creek.

This present study was conducted to ascertain the relationship between the growth rate of aerobic heterotrophic bacteria, cyanobacteria isolated from Bodo creek and their consortium to the concentration of total petroleum hydrocarbon during biodegradation using the Monod equation. The study also determined the percentage loss of TPH throughout the period monitored from different treatments studied.

Materials and Methods

Sampling

Ten samples each of crude oil polluted water and sediment were collected under aseptic conditions from Bodo creek in Ogoniland, Rivers State in the Niger Delta using appropriate water and sediment collection equipments. Sediment samples were collected with an Eckman grab and poured into sterile polyethylene bags whereas crude

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oil contaminated water were collected into sterile bottles. Samples were obtained at different points of each site and transported to the Environmental Microbiology Laboratory, University of Portharcourt at temperature of 4°C in ice pack.

Determination of physicochemical parameters of samples

Physicochemical parameters such as conductivity, phosphate, total organic carbon (TOC), pH, moisture content, nitrate, turbidity, salinity and temperature, were determined using methods described in APHA (2008) as reported in Ichor et al., [10].

Chromatographic analysis

Total petroleum hydrocarbons (TPH) and polycyclic aromatic hydrocarbons (PAHs) were extracted from the samples and quantified using GC-MS and GC-FID as described by Ichor et al. [8,10].

Enumeration of total heterotrophic bacteria count

The total aerobic heterotrophic bacteria count of the water/sediment samples was carried out using nutrient agar (Oxoid). The medium was prepared according to the manufacturer's instruction. Aliquot 0.1ml) of 10^3 dilution of samples was plated in duplicate using the spread plate techniques after which the plates were incubated in an inverted position at 37°C and counted after 24 hr of incubation as described by Ichor et al. [1].

Enumeration of hydrocarbon utilizing bacteria

The method of Hamamura et al. [11] was adopted for enumeration of Hydrocarbon utilizing bacteria (HUB). The 10^3 dilution of the sample suspensions were considered and plated on Bushnell-Haas agar (Sigma-Aldrich, USA). Petroleum hydrocarbons were supplied using the vapour phase transfer to hydrocarbon utilizers by placing sterile Whatman No.1 filter papers impregnated with 5 ml Bonny Light crude oil on the lids of the inverted plates and incubated for 14 days at room temperature.

Characterization of bacteria and cyanobacteria isolates and Petroleum hydrocarbon degradation test

Molecular characterization tests on bacteria and cyanobacteria isolates was done using universal primers for bacteria and cyanobacteria as described by Ichor et al. [1,8]. Biodegradation tests were conducted using the treatments of aerobic heterotrophic bacteria, cyanobacteria, consortium of aerobic heterotrophic bacteria and cyanobacteria, and the control as described by Ichor et al. [10].

Growth Kinetics of Bacteria and Cyanobacteria in Total Petroleum Hydrocarbon Biodegradation. In determining the specific growth rate based on the effect of the limiting substrate (TPH in petroleum hydrocarbon contaminated in the various treatments), Monods empirical expression $\mu = \frac{\mu_{max} S}{K_S + S}$ was applied.

Results

The total amount of TPH after contamination of water with crude oil showed that Aerobic Heterotrophic Bacteria, Cyanobacteria, consortium of Aerobic Heterotrophic Bacteria + Cyanobacteria and Control had TPH of 24091, 29882, 16267 and 6706 mg/L respectively for the first day of contamination [1,8]. The percentage loss of Total Petroleum Hydrocarbon for the respective treatment is as presented in Figures 1-4, and did not vary significantly with time using a one-way analysis of variance (ANOVA) ($p>0.05$).

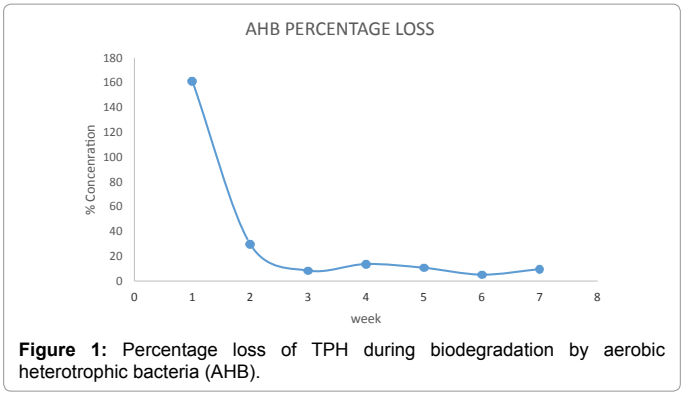

Figure 1: Percentage loss of TPH during biodegradation by aerobic heterotrophic bacteria (AHB).

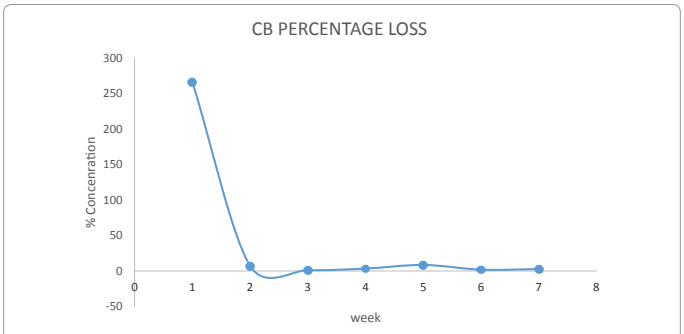

Figure 2: Percentage loss of TPH during biodegradation by cyanobacteria (CB).

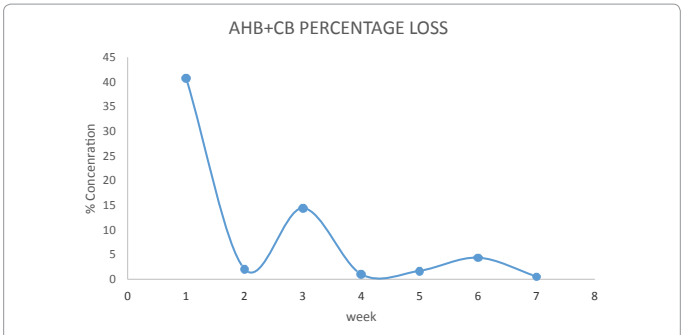

Figure 3: Percentage loss of TPH during biodegradation by a consortium of aerobic heterotrophic bacteria and cyanobacteria (AHB + CB).

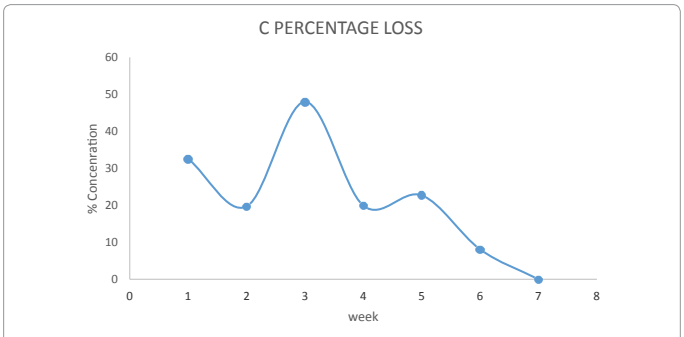

Figure 4: Percentage loss of TPH during biodegradation by the control (C).

To estimate the unknown parameter μm and K_s, nonlinear regression analysis (Excel 2010) was applied.

μ = Specific growth rate.

μm = Maximum specific growth rate.

S = Concentration of growth limiting substrate in solution utilized by microorganisms (in this case petroleum hydrocarbons)

K_s = Saturation constant which has value equal to the substrate concentration when μ = 1/2μm

The value of unknown parameters as determined for sample AHB were μm = 0.02823day^{-1}, K_s = 4229.61 mg/L, For CB, μm = 0.01468 day^{-1} K_s = 2130.21 mg/L, AHB + CB, μm = 0.06085day^{-1}, K_s = 1716.51 mg/L, and the control, μm = 0.074033 day^{-1}, K_s = 6200.71 (Figures 5-7).

Figure 8 shows the evolutionary relationships of the isolated aerobic heterotrophic bacteria during the biodegradation experiment. The evolutionary history was inferred using the Neighbour-Joining method [12]. The optimal tree is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (500 replicates) are shown next to the branches [13]. The tree is drawn to scale, with branch lengths in the same units as those of the evolutionary distances used to infer the phylogenetic tree. The evolutionary distances were computed using the Jukes-Cantor method [14]. Evolutionary analyses were conducted in MEGA6 [15].

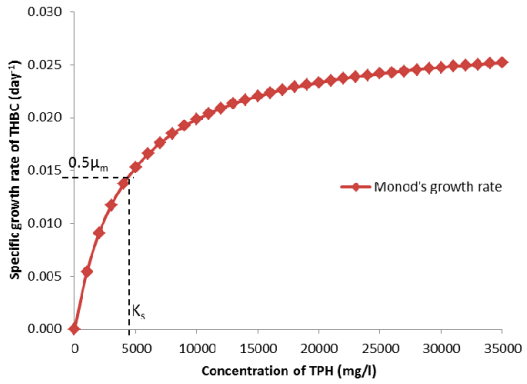

Figure 5: Specific growth rate of bacteria in treatment AHB during TPH biodegradation.

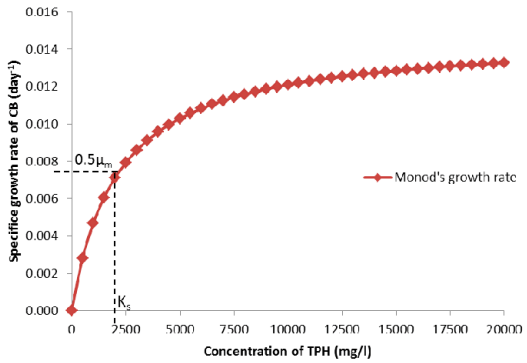

Figure 6: Specific growth rate of cyanobacteria in CB treatment option during TPH biodegradation.

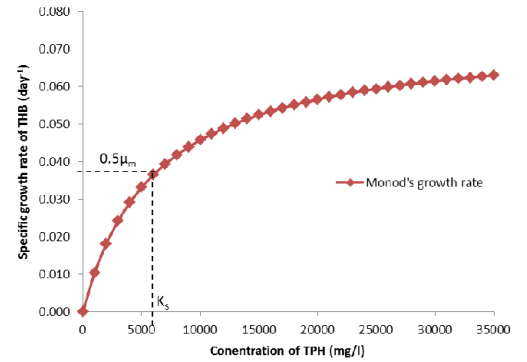

Figure 7: Specific growth rate of bacteria in crude oil contaminated control during TPH biodegradation.

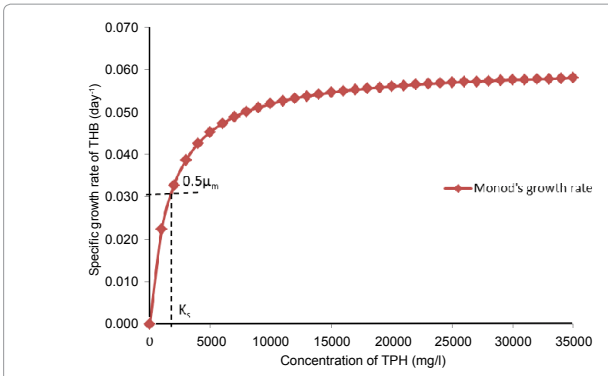

Figure 8: Specific growth rate of bacteria in AHB+CB treatment during TPH biodegradation.

Discussion

Growth of aerobic heterotrophic bacteria and cyanobacteria isolated from Bodo creek implicated during degradation of total petroleum hydrocarbon was monitored. The result showed increased growth of the isolates with a corresponding decrease of the substrate which implies utilization of petroleum hydrocarbons in all the treatment options and the control. Ichor et al. [10] previously reported on the capability of a consortium of aerobic heterotrophic bacteria from the area under study to effectively biodegrade petroleum hydrocarbons. The studies of Chikere and Ekwuabu [9] revealed the presence of indigenous bacteria with inherent capability to utilize petroleum hydrocarbons in oil contaminated sites (Figure 9).

Previous research findings revealed a negative correlation between the growth of aerobic heterotrophic bacteria, cyanobacteria isolates from Bodo creek and loss of total petroleum hydrocarbons. This signifies that as the growth of bacteria and cyanobacteria increased, there was a corresponding decrease in the quantity of TPH [8,10].

The significance of monods saturation constant K_s is that it gives an idea of the microbe's affinity for the substrate, by implication the lower the K_s value, the higher the affinity of the microbes for the substrate. The result obtained throughout the study implies that the consortium of aerobic heterotrophic bacteria and cyanobacteria had higher affinity for TPH having the lowest K_s value of compared to the other treatment options. The determination of specific growth rate using monods in this present study shows that the consortium of AHB + CB has more affinity for total petroleum hydrocarbon as shown by the monods saturation constant, K_s (1716.51 mg/L) which is the lowest K_s value obtained compared to other treatments. The study provides evidence that the consortium of these microorganisms has a great influence on the microbes involved and on petroleum hydrocarbon biodegradation process. It is however apparent here that oxidization of petroleum hydrocarbon components is more effective by mixed cultures compared to single cultures of microorganisms.

Previous researchers reported on the important role cyanobacteria plays in the biodegradation of organic including hydrocarbons and

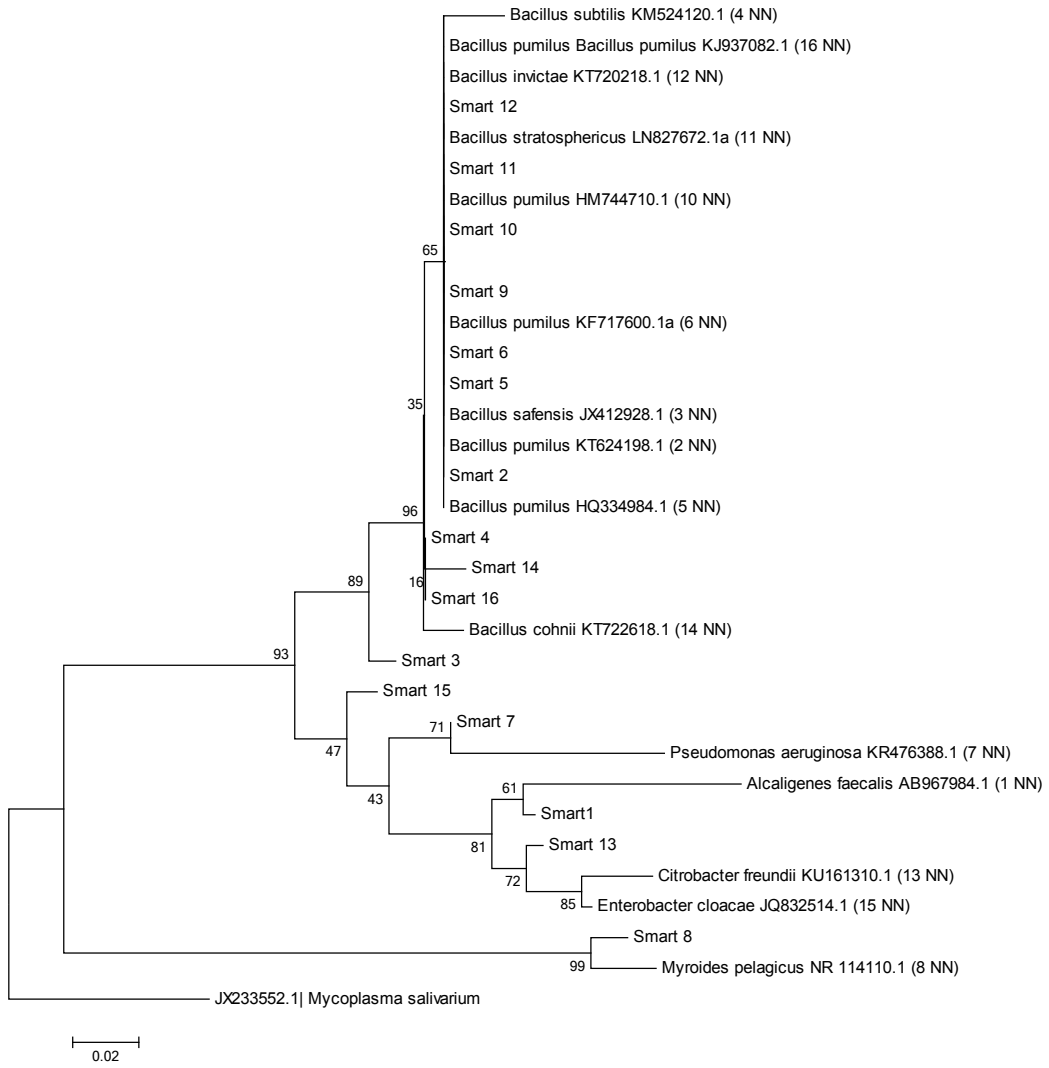

Figure 9: Phylogenetic tree showing the evolutionary history of the isolated aerobic heterotrophic bacteria.

evidence abound on the fact that microbial communities dominated by cyanobacteria are actively involved in oil degradation [16-18]. Previous studies demonstrated the significance of exudates from cyanobacteria in accelerating rates of hydrocarbon degradation. Glucose and lipids were shown to enhance degradation of alkanes in polluted soils from Kuwait [19]. The growth of cyanobacteria after oil spills into the Arabian gulf forming heavy thick mats gave the impression that cyanobacteria possess the potential to degrade oil components [17]. Cyanobacterial and associated bacterial have been reported to form a consortium that favours biodegradation and clean-up of polluted sites [18,20,21].

The studies of Abed [22] reported that cyanobacteria-aerobic heterotrophs association constitute a very efficient consortium for the degradation of hydrocarbons. Research findings of Abed [22], Abed and Koster [23] and several other researchers observed that heterotrophic bacteria was solely responsible for the observed degradation of petroleum hydrocarbons not cyanobacteria which plays an indirect role of by providing the associated bacteria oxygen, extracellular polymeric substances which emulsify hydrocarbons, fixed nitrogen, simple organics produced by photosynthesis and fermentation and also offers additional particle surfaces for attachment of bacteria and provides it with protection [22,24,25]. Our previous and present studies have however proven that both cyanobacteria and the associated aerobic heterotrophic bacteria effectively biodegraded total petroleum hydrocarbon which is congruent with our previous findings [8].

Conclusion

The aerobic heterotrophic bacteria-cyanobacteria consortia have shown a higher and stronger affinity for petroleum hydrocarbons which clearly points to the fact that the association is more efficient in degradation activities. Our study further demonstrated the capability of degradation and growth of cyanobacteria in the presence of cyanobacteria as a substrate and does not only contribute in stimulating the degradative activities of aerobic heterotrophic bacteria but also degrades. Further investigations in culture and field conditions, identification of the exudates from cyanobacteria, the metabolites produced and other factors responsible for the high affinity of the consortia to petroleum hydrocarbon should be undertaken.

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