Interaction of cyanobacteria and aerobic heterotrophic bacteria in crude oil biodegradation in the Niger Delta region of Nigeria

Ejileugha C, Okerentugba Phillip Oritsegbubemi, Okonko Iheanyi Omezuruike
Department of Microbiology, University of Port Harcourt, Port Harcourt, Nigeria
E-mail: phillip.okerentugba@uniport.edu.ng, Tel: +2348033087332

ABSTRACT: The interaction of cyanobacteria and bacteria in crude oil biodegradation in the Niger Delta region of Nigeria was examined using cyanobacterial and bacterial consortia. The bacterial consortium consist of *Bacillus licheniformis*, *Bacillus megaterium* 2, *Bacillus subtilis*, *Enterobacter asburiae*, *Staphylococcus aureus*, *Corynebacterium kutscheri*, *Corynebacterium ulcerans*, *Staphylococcus saprophyticus*, *Aeromonas hydrophila* group 2, *Acinetobacter baumannii*, *Serratia ficaria*, and *Kocuria varians*. The cyanobacteria consortium consists of *Anabaena cicadae*, *Pseudonabaena minima*, *Laptolyngbya* sp., *Oscillatoriales cyanobacterium*, *Microcoleus* sp., *Mycrocystis holsatica*, *Mycrocystis elabens*, *Phormidium faveolaurum*, *Phormidium* sp., and *Synechococcus* sp. The cyanobacteria and bacteria were used for biodegradation in sterile brackish water treated with Escravos light crude oil and monitored throughout the experimental period of 30 days. The pH in all the treatment setups (except for the control) decreased progressively with increase in microbial growth measured by optical density (OD) at 620 nm. The OD increased from 0.076 to 0.789 in setup A while the pH decreased from 6.88 to 5.34, the pH decreased from 6.90 to 5.70 for setup B while the OD increased from 0.069 to 0.896, for setup C the OD increased from 0.084 to 0.941 while the pH decreased from 6.93 to 5.64. The highest pH decline was observed in setup A while the highest OD increase was observed in setup C. Studies using Gas Chromatographic analyses showed that on the 30th day, 79.9 % of TPH were lost in setup A, 57.5% for setup B, 96.0 V for setup C, and 0.38% for setup D. The highest TPH lost was observed in setup C having both cyanobacteria and bacteria consortium, followed by setup A having only bacteria consortium and setup B having only cyanobacteria consortium to setup D which is the control with no organism. This study has shown the potential benefit of combining bacteria and cyanobacteria in bioremediation of crude oil polluted sites.

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1. INTRODUCTION

Environmental pollution by crude oil is the major environmental issue facing Nigeria especially areas of the Niger Delta region (Ezeonu et al., 2012). Oil spills in the Niger Delta region of Nigeria have made it one of the world's most environmentally devastated areas (Onwurah 1999, 2000, 2002; Ezeonu et al., 2012). Crude oil is the major energy source for daily life and the industry. As a result, oil spills constitute a major increasing public health problem and concern owing to the environmental and ecological impact on open sea and coastal areas. Chemical pollution by petroleum hydrocarbons can cause serious damage to aquatic as well as to foreshore marine life. Major changes in diversity, profusion and action of autochthonous populations has been reported due to oil spills (Llirós et al., 2008). Run-off from land, industrial/municipal wastes as well as routine ship maintenance like natural seeps, bilge cleaning, offshore oil production and tanker accidents are the main sources of marine hydrocarbon pollution (US Coast Guard, 1990; NRC, 1995; Ibraheem, 2010; Zohra et al., 2011).

Among the most severe ecological glitches facing Nigeria, pollution of surface water with crude oil and other petroleum hydrocarbons is one of them (Okoh et al., 2001; UNEP, 2011; Ichor *et al*., 2014). Petroleum hydrocarbons are foremost pollutants of the aquatic environs (Ibiene *et al*., 2011). Biodegradation is a capable and hopeful method for handling such polluted settings (Ibiene *et al*., 2011). All the exploration, production, transportation and refining of oil, handling of refined product and management of oily waste activities in the petroleum industries pose serious threats to human (Kuhad and Gupta, 2009). The possibility of preventing oil spills and complete remediation of contaminated systems is a major environmental concern.

Cyanobacteria have been observed in association with aerobic heterotrophic bacteria in microbial mats (Abed and Koster, 2005; Abed, 2010). The interaction of cyanobacteria with associated aerobic heterotrophic bacteria had been utilized in several processes like agriculture and cleanup processes (bioremediation). Cyanobacteria have been observed to colonized oil polluted sites in association with aerobic heterotrophic bacteria. This led to the quest to understand their major role in petroleum hydrocarbon degradation together with any possible positive interaction between the cyanobacteria and

aerobic heterotrophic bacteria that may lead to or enhance the cleanup of petroleum hydrocarbon polluted sites (Abed and Koster, 2005; Abed, 2010).

There have been reports of biodegradation and bioaccumulation of petroleum hydrocarbon by some cyanobacterial species. Cyanobacteria have been reported to bioaccumulate petroleum hydrocarbon and the ability of cyanobacteria to degrade petroleum hydrocarbon have also been reported (Al-Hasan *et al.*, 1994, 1998, 2001; Cerniglia *et al.*, 1980; Cohen, 2002; Grotzschel *et al.*, 2002, and Lliros *et al.*, 2008).

Cyanobactria have been used in waste water treatment processes and in bioremediation using microbial mats. Although cyanobacteria are known to be photosynthetic, the heterotrophic nature reported among some species puts them in the list of potential hydrocarbon degraders. They are known to be attached with aerobic heterotrophic bacteria making them potential immobilizing agents on which bacteria can attach and carry out their degradation processes. The use of cyanobacteria as immobilizing agents is considered to be cost effective compared to the use of other artificial immobilizers.

The use of cyanobacteria in the cleanup of petroleum hydrocarbon polluted sites is regarded as a stimulation and augmentation process through their reported ability to release organic exudates coupled with their reported biodegradation potential. Thus, the objective of this study is to examine the interaction of cyanobacteria and bacteria in crude oil biodegradation in the Niger Delta region of Nigeria using cyanobacterial and bacterial consortia.

2. MATERIALS AND METHODS

2.1. Bacterial and cyanobacterial consortia: Identified and characterized cyanobacteria and bacteria consortia isolated from petroleum hydrocarbon contaminated Bodo creek in Gokana Local government area of Rivers state, Nigeria were collected and used in this study. The bacterial and cyanobacterial consortia were obtained from the Environmental Microbiology laboratory, Department of microbiology, University of Port Harcourt. Nigeria.

2.2. Cyanobacterial consortium: The cyanobacterial consortium consists of *Microcoleus* sp., *Anabaena cicadae*, *Pseudonabaena minima*, *Oscillatoriales cyanobacterium*, *Mycrocystis holsatica*, *Mycrocystis elabens*, *Phormidium faveolaurum*, *Phormidium* sp., *Synechococcus* sp., *Laptolyngbya* sp.

2.3. Culture medium: The consortium was cultured in BG-11 medium. The culture was incubated at room temperature near closed transparent glass window to allow rays of sunlight to reach culture. BG-11 medium used in this study is made up of the following composition: $NaNO_3$ (1.5 g), Na_2CO_3 (0.02 g), $MgSO_4.7H_2O$ (0.075 g), K_2HPO_4 (0.04 g), $CaCl_2.2H_2O$ (0,036 g), Citric acid (0.06 g), Ferric ammonium citrate (0.06 g), EDTA (0.01 g), Agar (for solid medium), trace metal mix 1.0 ml (H_3BO_3: 2.86 g, $MnCl_2.4H_2O$; 1.81 g, $ZnSO_4.7H_2O$; 0.222 g, $Na_2MoO_4.2H_2O$; 0.39 g, $CuSO_4.5H_2O$; 0.079 g, $Co(NO_3)_2.6H_2O$; 0.494 g, Distilled Water; 1000 ml), distilled water (1.0 L), pH 7.1 (Rajeshwari et al., 2012).

2.4. Hydrocarbon utilizing bacterial consortium: The hydrocarbon utilizing bacterial consortium consists of *Bacillus licheniformis*, *Bacillus megaterium* 2, *Bacillus subtilis*, *Enterobacter asburiae*, *Staphylococcus aureus*, *Corynebacterium kutscheri*, *Corynebacterium ulcerans*, *Staphylococcus saprophyticus*, *Aeromonas hydrophila* group 2, *Acinetobacter baumannii*, *Serratia ficaria*, and *Kocuria varians.* The consortium was cultured in nutrient broth and incubated at 35 ± 2°C prior to the biodegradation experiment.

2.5. Identification: The bacterial strains capable of degrading crude oil were gram stained and different standard physiological, morphological and biochemical methods were carried out using API 20 kits.

2.6. Biodegradation with bacteria isolates: The level of crude oil degradation by the microbial consortia was tested using brackish water collected from an unpolluted water body. The brackish water was dispensed 100 ml into four different sterile 250 ml Erlenmeyer flasks labeled A to D. The four flasks and the Escravos light crude oil (obtained from Chevron Nigeria Limited) to be used for the test were autoclaved at 121°C for 15 minutes, cooled to room temperature, and 10% v/v of sterile Escravos light crude oil was introduced into the flasks respectively. To prevent any fungal, bacterial and cyanobacterial activity, 0.25 g/l each of nystatin, streptomycin, $CuSO_4$ was added to respective flasks. To SETUP A 1.0 ml of culture suspension of the bacteria consortium was inoculated; to SETUP B 1.0 ml of culture suspension of the cyanobacteria consortium was inoculated; to SETUP C 1.0 ml each of culture suspension of bacteria and cyanobacteria consortia were inoculated as described below. SETUP A: Brackish water + crude oil + Bacteria consortium + Nystatin + $CuSO_4$; SETUP B: Brackish water + crude oil + Cyanobacteria consortium + Streptomycin + Nystatin; SETUP C: Brackish water + Crude oil + Bacteria consortium + Cyanobacteria consortium + Nystatin; SETUP D (CONTROL): Brackish water + Crude oil + Streptomycin + $CuSO_4$ + Nystatin. After

inoculation of the flasks with bacteria and cyanobacteria consortia from a broth culture of the bacteria and cyanobacteria consortia, the setups were incubated at room temperature near a transparent glass window to allow rays of sunlight to reach the setups and were monitored throughout the experimental period of 30 days. pH and optical density (at 620 nm) were measured in the four flasks using a pH meter calibrated with buffers (pH 4, 7 and 9) and P7 PG spectrophotometer respectively.

2.7. Chromatographic Analyses: At specific time intervals, samples were taken from the biodegradation setups and the residual crude oil was extracted and quantified using Gas Chromatography equipped with mass spectrometer. GC-MS QP2010 Ultra Shimadzu was used for the analysis with the following parameter setting. Column oven temperature 40^{o}C, injection temperature 290^{o}C, splitless injection mode, helium gas source, temperature program 40^{o}C held for 1 minute, ramped at 10^{o}C/min 300^{o}C held for 2 minutes. Ion source temperature 230^{o}C, interface temperature 290^{o}C, scanned from 30-400 m/z. Column type Rtx 5MS 30m × 0.25 mm.

2.8. Data Analysis: The data generated were subjected to analysis of variance (ANOVA) at $p \leq 0.05$ (Steel and Torrie, 1980) using SPSS software (version 20.0, Chicago, USA). A correlation analysis was used to compare values of the experimental treatment and control group. A comparison was also considered statistically significant if the p value was ≤ 0.05.

3. Results and Discussion

The changes in pH and optical density in biodegradation setups including the control (setup D) is represented in Fig. 1 and Fig. 2 below. The pH in the setups except for setup D (control) decreased progressively in relation to a progressive increase in optical density. Similar result on pH and optical density changes during biodegradation was reported by Adebusoye *et al.* (2006). The decrease in pH in the set-ups may be as a result of the metabolites released by the microbes as they carryout biodegradation and other metabolic activities. Previous studies have demonstrated that the pH range optimal for biodegradation of hydrocarbons is 6-7 (Chikere and Ekwuabu, 2014). The optical density is a measure of microbial population while the pH is a measure of changes in metabolites released in the course of the biodegradation process. The release of acidic metabolites causes a decrease in pH making the medium more acidic while the release of more basic metabolites makes the medium more alkaline in nature. However, Shin et al. (2004) in their study showed that pH had no significant effect on bacterial growth.

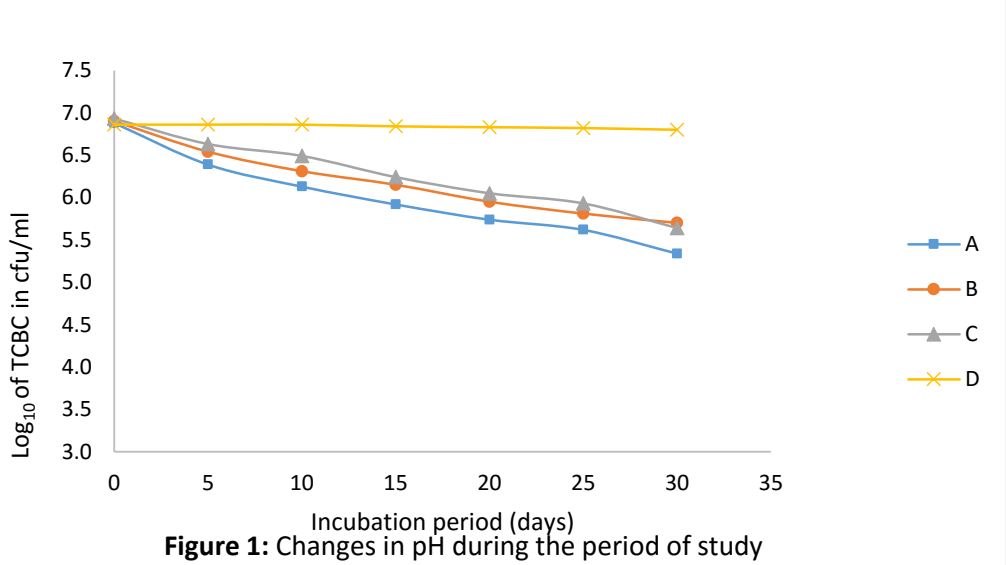

Figure 1: Changes in pH during the period of study

Setup A: Brackish water + Bacteria consortium + Crude oil
Setup B: Brackish water + Cyanobacteria consortium + Crude oil
Setup C: Brackish water + Bacteria consortium + Cyanobacteria Consortium + Crude oil
Setup D (Control): Brackish water + Crude oil

The change in TPH values in the biodegradation setups on day 0, day 15 and day 30 are represented in Figures 3-6. From the Gas Chromatographic results, setup C had the highest percentage biodegradation of 96.0 % while setup B had the lowest percentage biodegradation

of 57.5 %. The change in TPH value in setup D is negligible and may be attributed to other factors other than biodegradation. Visual gradual decrease in crude oil accompanied the increase in population of microbial communities in this study. The observation is in accordance with the report of Ichor *et al.* (2014). The removal rate of TPH obtained correlates with the values of Optical density obtained in the setups. The setup with the highest OD increase which is a measure of microbial proliferation also had the highest TPH loss which can be attributed to the metabolic activities of the multiplying microbes which led to the depletion and degradation of the TPH in the setup. The combination of cyanobacteria and bacteria consortia in setup C is the principal reason for the high TPH loss when compared to other setups with either cyanobacteria or bacteria consortium. The interaction of cyanobacteria and bacteria led to the high TPH loss which was observed. The models in Figures 3-6 can help to determine the percentage TPH loss and time interval at various point not determined from the experimental data. The x represents time interval while the Y represents the TPH loss. So at any given time, from the model the TPH loss can be determined. The model can also assist in determining at what time the organisms are likely to completely remove the TPH from the polluted or contaminated sites.

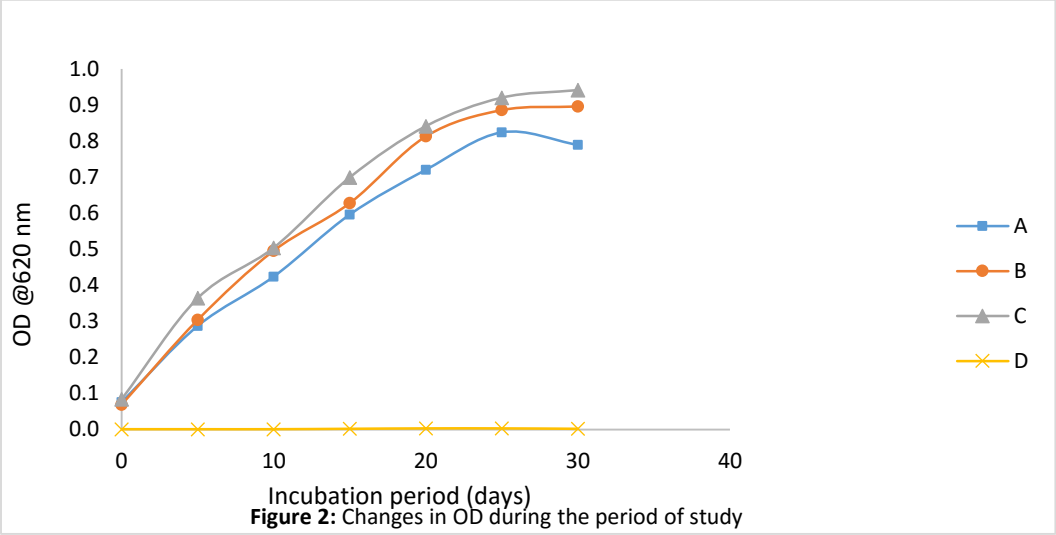

Figure 2: Changes in OD during the period of study

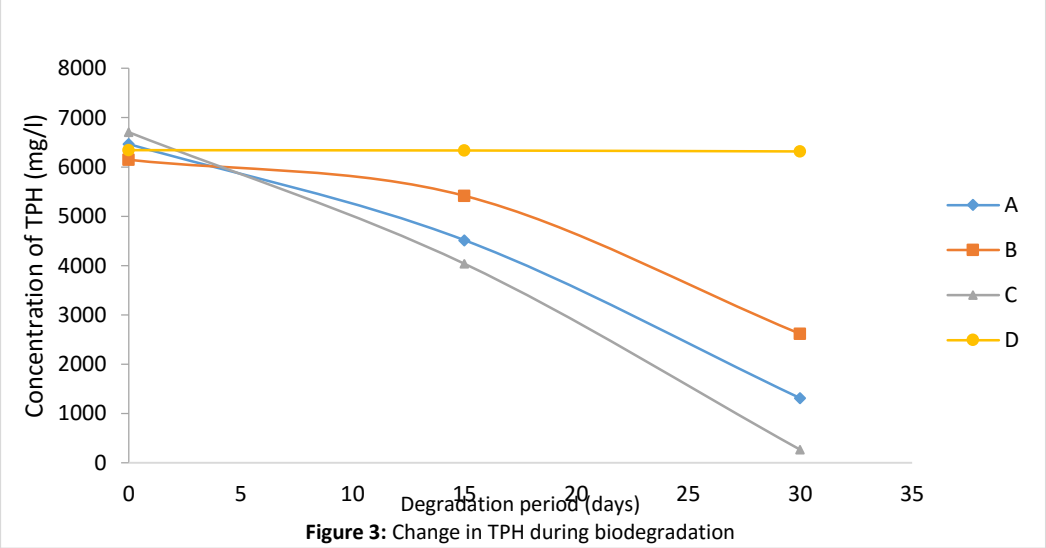

Figure 3: Change in TPH during biodegradation

Setup A: Brackish water + Bacteria consortium + Crude oil
Setup B: Brackish water + Cyanobacteria consortium + Crude oil
Setup C: Brackish water + Bacteria consortium + Cyanobacteria Consortium + Crude oil
Setup D (Control): Brackish water + Crude oil

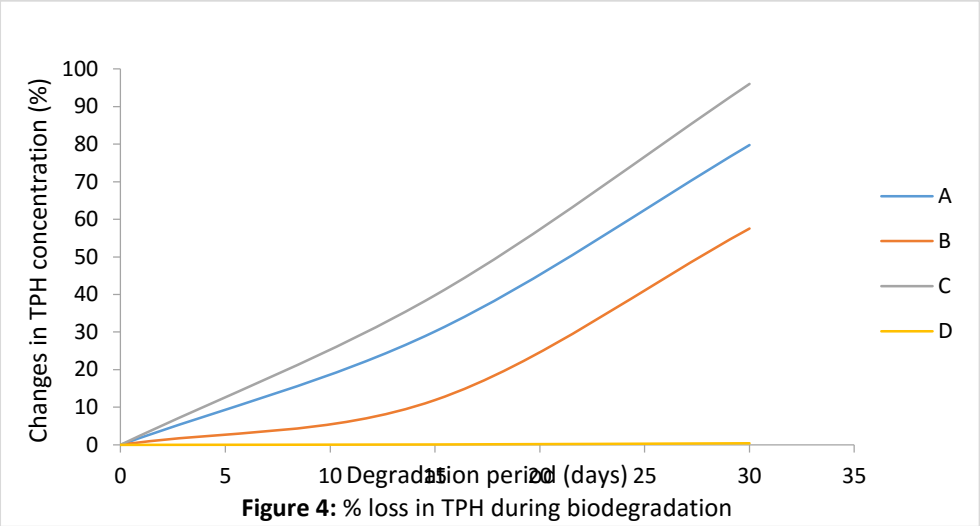

Figure 4: % loss in TPH during biodegradation

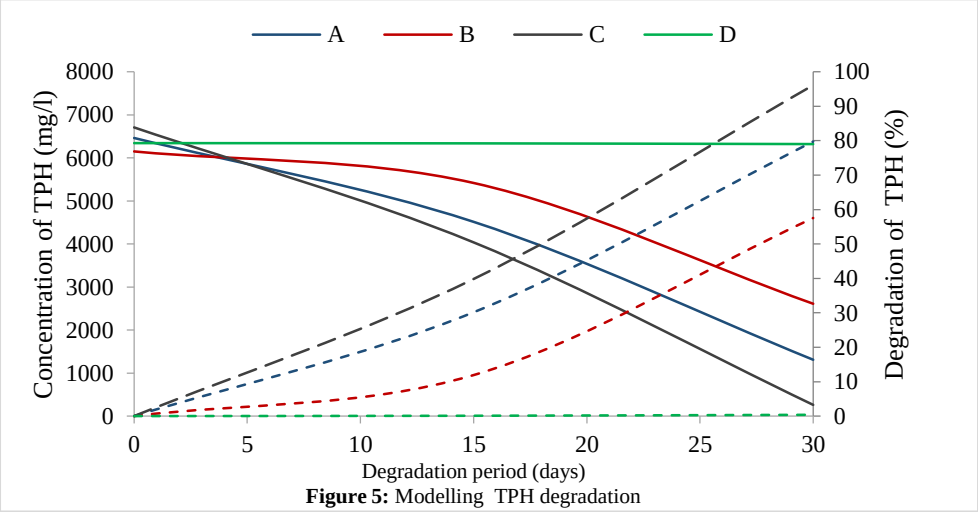

Figure 5: Modelling TPH degradation

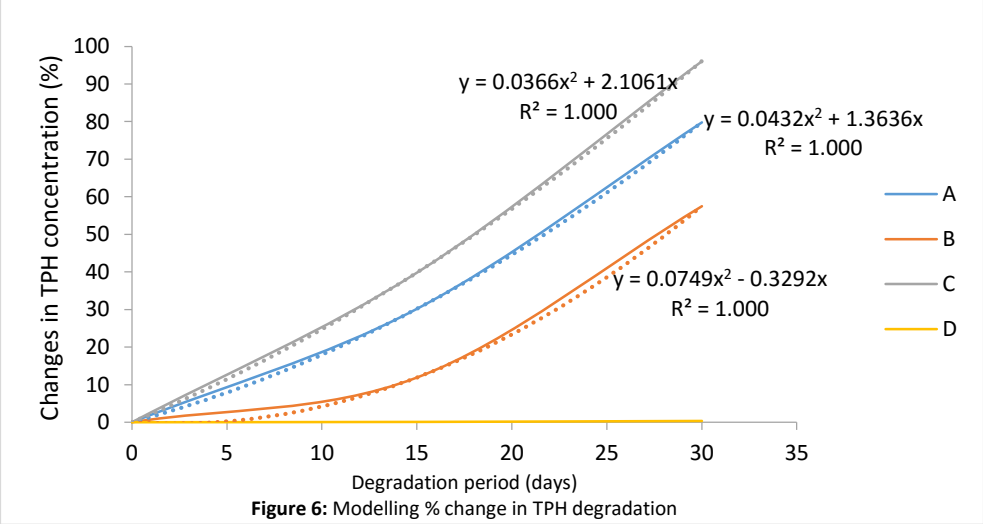

Figure 6: Modelling % change in TPH degradation

Setup A: Brackish water + Bacteria consortium + Crude oil
Setup B: Brackish water + Cyanobacteria consortium + Crude oil
Setup C: Brackish water + Bacteria consortium + Cyanobacteria Consortium + Crude oil
Setup D (Control): Brackish water + Crude oil

Biodegradation using cyanobacterial and bacterial consortium is an effective method for the cleansing of crude oil polluted water (Gouda et al., 2007; Adebusoye et al., 2010; Chekroud et al., 2011; Singh et al., 2014). Biodegradation of crude oil by cyanobacteria and aerobic heterotrophic bacteria has been previously reported (Abed, 2010). Abed and Koster (2005) examined degradation of hydrocarbons by cyanobacteria and other aerobic heterotrophic bacteria and found out that they were chiefly responsible. Similar observations have been also reported previously in aquatic setting (Jones et al., 1983; Bartha and Bossert, 1984; Rahman et al., 2003; Leahy and Colwell, 1990; Adebusoye et al., 2007; Yakimov et al., 2007; Brooijmans et al., 2009; Uzoigwe and Okpokwasili, 2012; Luiz and Raquel, 2012; Ichor et al., 2014).

The findings of this present study have obviously established the aptitude of the consortia of cyanobacteria and other aerobic heterotrophic bacteria to breakdown total petroleum hydrocarbon (TPH) (Ichor et al., 2014). This is also in consonance with Diaz et al. (2002) who observed bacterial consortium shows good stability in immobilised systems. Previous studies have reported on biodegradation of petroleum hydrocarbons in both terrestrial and marine environment under toxic conditions (Leahy and Colwell, 1990; Van Hamme et al., 2003; Perez-Pantola et al., 2010; Luiz and Raquel, 2012; Ichor et al., 2014).

Microbial breakdown is the foremost and eventual regular mechanism by which crude oil pollutants can be cleaned from the environment (Jones et al., 1983; Amund and Nwokoye, 1993; Lal and Khanna, 1996; Ichor et al., 2014). Microbial degradation has been proven to be more efficiently completed by a consortium of bacteria which out performs single species isolated even from the consortium and can occur under toxic and non-toxic conditions (Wang et al., 2008; Ichor et al., 2014).

The findings of this present study also corroborates the previous reports on the isolation of hydrocarbon degraders from environment (van Beilen and Funhoff, 2007; Wang et al., 2008; Ron and Rosenberg, 2010; Ichor et al., 2014). In corroboration with the findings of this present study, Latha and Kalaivani (2012) and Ichor et al. (2014) in their studies, reported an association between augmented crude oil breakdown to the proliferation in bacterial cell number which signifies that the isolates were accountable for the breakdown.

4. Conclusion

The findings from this study showed in accordance with previous researches that aerobic heterotrophic bacteria have the ability to grow and multiply using crude oil as a sole carbon source (Abed and Koster, 2005; Abed, 2010). This result is in accordance with previous finding by Abed and Koster (2005), Adebusoye *et al.* (2006); Nwaogu *et al.* (2008) and Ichor *et al.* (2014). This shows they can be very useful in bioremediation of petroleum hydrocarbon polluted sites. The cyanobacteria were also observed in this study to degrade crude oil. Similar results about hydrocarbon degradation by cyanobacteria were reported by Al-Hassan *et al.* (1994, 2001) and Grotzschel *et al.* (2002). There are also reports of heterotrophic growth in cyanobacteria which is attributed to the breakdown of crude oil by cyanobacteria. The setup with both cyanobacteria and bacteria had the highest TPH loss so we conclude that the combination of cyanobacteria and bacteria in bioremediation processes is more effective than the use of cyanobacteria or bacteria alone. Hence, the interaction of cyanobacteria and bacteria in crude oil biodegradation is effective for the cleanup of polluted sites.

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