Bioremediation of Refinery Wastewater by microbial consortium immobilized on agro-based residues.

Iheanacho G. C[1]., Ibiene A. A[1]. and Okerentugba P. O[1].

[1.]Department of Microbiology,
Faculty of Science,
University of Port Harcourt.
Corresponding author: iheanachocg@gmail.com

Abstract

Effluent discharge from petrochemical refineries are characterized by the presence of pollutants like phenol, metals, surface active substances, high BOD_5, COD and oil and grease. Bacterial immobilization has proven to be an effective way of maintaining a high bacterial biomass on contaminated sites. In this study, the suitability of three agro-waste materials: Corncob (CC), Palm Kernel Shell (PKS) and Cocopeat (CP) for bacterial immobilization were tested. BET MultiPoint surface area analysis (CC: 450.02m^2/g, PKS: 418.33 m^2/g, CP: 684.36 m^2/g) as well as micropore volume (CC: 0.163cc/g, PKS: 0.147 cc/g, CP: 0.186 cc/g) indicates their suitability for bacterial adhesion. Bacterial isolates from hydrocarbon polluted soil and water were screened for phenol and hydrocarbon utilization. Three isolates were selected and identified as *Pseudomonas aeruginosa* (MN294989), *Bacillus tequilensis* (MN294990) *and Micrococcus* sp. using the 16S rRNA approach. Scanning Electron Microscopy (SEM) was used to ascertain the immobilization of the consortium on the agro-base carriers. The ability of the immobilized consortium to treat Port Harcourt refinery wastewater was studied in comparison with the free form of these bacteria. After 15 days of a laboratory scale bioremediation set up, agro-waste immobilized consortium showed a reduction in phenol (99%), BOD_5 (96%), COD (92%) oil and grease (93%). This is a corresponding 5%, 6%, 10% and 29% higher efficiency as observed unimmobilized consortium. This study suggests that these agro-based residues could serve as suitable bio-carriers for an enhanced bioremediation of refinery wastewater.

Key words: wastewater, bioremediation, immobilization, biocarriers, agro-based residues

Introduction

Oil exploration in the Niger Delta has led to a lot of environmental hazards. One of such is the discharge of untreated or poorly treated refinery effluents into receiving water bodies in the region. With increasing global energy demand, the exploration and processing of petroleum is expected to increase considerably in the years to come (Aljuboury, Palaniandy, & Feroz, 2017). Refining of petroleum just like other process industries require huge volumes of water which must be returned back into the environment. This makes industrial wastewater treatment an important area of

environmental engineering (Aljuboury et al., 2017; Hasan, Mohd, Wan, & Aziz, 2010). Coelho et al. (2006) reported that the volume of refinery effluent generated during crude oil processing is about 0.4 – 1.6 times the amount of crude oil processed. With the volume of crude oil refined worldwide on daily basis, one can only imagine the quantity of wastewater which would eventually be discharged into the environment.

Pollutants in refinery effluents pose serious environmental hazards. Due to ineffectiveness of purification systems, wastewaters may become hazardous leading to the accumulation of toxic products in receiving water bodies (Deka, Devi, & G.Bhattacharyya, 2013). The composition of these effluents depends on the type of oil being processed, the plant configuration and operational procedures (Hasan et al., 2010; Ishak, Malakahmad, & Isa, 2012). Ishak et al., (2012) reported that the major constituents of refinery wastewater in general are dissolved and dispersed oil (mixture of hydrocarbons – benzene, toluene, ethylbenzene, xylenes, polyaromatic hydrocarbons and phenols) and dissolved formation minerals (anions and cations including heavy metals). Deka et al., (2013) pointed out that although some effluents contain considerable amount of nutrients which may be beneficial to plants, the major environmental problem emanates from the acidic nature of effluents, high contents of heavy metals and sulphates as well as presence of organic contaminants like polycyclic aromatic hydrocarbons in the soil.

Microbial immobilization

Microbial immobilization is a natural phenomenon around the world (Bayat, Hassanshahian, & Cappello, 2015). Some advantages of bacterial immobilization in bioremediation are: provision of high biomass, high metabolic activities, improving genetic stability, resistance to toxic chemicals, elimination of cell washout problems etc. (Bayat et al., 2015; Cláudia et al., 2013; Liu, Guo, Liao, & Wang, 2012)

Materials and Methods

Bacteria source and consortium

The bacterial consortium used in this study were isolated from crude oil polluted water in Bodo creeks Gokana local government area of Rivers state. The water samples were enriched with Bushnell Haas media (BHM), prepared according to manufacturer's specification. A measure of 3.2g of the salt was dissolved in a litre of distilled water, pH of the media was adjusted using 1.0M HCl to pH 7.2. About 98ml of BHM was dispensed in a 250ml conical flask to create room for adequate headspace, 1%

Bonny Light crude oil (BLCO) was introduced to the media and sterilization was monitored at 121^{o}C for 15 minutes at 15 psi. Upon cooling, the 1% crude polluted pond water samples was added to the sterile set up and incubated in an orbital shaker incubator (Stuart, Germany S150) at 170 r.p.m at 37^{o}C for seven days (Ekwuabu, Chikere, & Akaranta, 2016; Xue et al., 2017). Further enrichments were conducted to obtain the selected strains, which were sub-cultured separately in nutrient broth medium for 24 hours. After incubation, the cells were harvested by centrifugation, washed with normal saline (0.85% NaCl) and re-suspended in fresh normal saline. Equal volumes of the suspension containing the different bacterial strains were mixed to form the consortium used in the study.

The selected strains were identified as 100% *Pseudomonas aeruginosa* (MN294989), 99% *Bacillus tequilensis* (MN294990) *and* 63% *Micrococcus* sp. using the 16S rRNA approach.

Biocarriers

Raw materials used as biocarriers (corncob, palm kernel shell and cocopeat) were all obtained from Nchia market, in Eleme local government area of Rivers state. These agro-based residues were first air- dried, grind mechanically and sieved to obtain homogenous particle size of 0.3-0.5mm. They were washed sequentially with ethanol and distilled water several times to prevent the impurities from affecting the growth and immobilization of the bacteria. The powder were dispensed in vials and sterilized by autoclaving at 120˚C, 1 atm for 15 min and kept at room temperature until use.

Surface area and pore distribution of biocarriers

The surface area and pore size distribution of the biocarriers were analyzed using the Brunauer-Emmet-Teller (BET) theory. This is based on the adsorption of gas molecules on solid surfaces. Prior to analysis, the samples were left in a desiccator at low temperature to ensure that they have as little remaining water vapor as possible. The mass of the samples are recorded. The sample was measured into the sample tube and the mass recorded and then de-gassed by attaching the tube to a vacuum pump and heating the tube to release water vapor (usually ~100–110 °C). The sample is allowed to degas for a minimum of 1 hour before starting the analysis. The analysis was performed by the BET analysis instrument, according to specifications. The out-gassed samples are immersed in a liquid nitrogen bath while the instrument performs the nitrogen adsorption tests.

Scanning electron microscopy

The scanning electron microscopy (SEM) was performed to examine the physical structure of the samples as well as the adsorption of the microbial cells on them. This was done using SEM model Phenom ProX, by Phenom World Einhoven, The Netherlands. Sample which was sputter coated by quorum technologies model Q150R 5nm of gold was placed on double adhesive which was on a sample stub. Thereafter it was taken to the chamber of SEM machine where it was viewed via NaVCaM for focusing and little adjustment. This was then transferred to SEM mode where focusing was automatically adjusted. The morphologies of different magnification were recorded.

Immobilization of bacterial consortium

About 0.5 g of the sterilized agro-based biocarriers were aseptically transferred into 500 mL of Bushnell Hass broth in separate one litre Erlenmeyer flasks. Five milliliter of the bacterial consortium was inoculated into the separate flasks containing the different biocarriers. After three days of incubation, the powder was harvested, washed by sterile 75% normal saline, and air-dried.

To determine the population of the bacteria consortium that immobilized on the biocarriers. They were washed with saline solution by centrifugation at 4000 rpm for 10 minutes to remove microorganisms that were not immobilized on the biocarriers. The microorganisms immobilized on the biocarriers were harvested by centrifugation at 8000 rpm for 10 min. The number of bacteria was approximately 5×10^7 CFU/ml in the experiment and was determined using the dilution plate method.

Wastewater sample collection

The wastewater samples were collected from the raw wastewater and treated wastewater reservoirs in Port Harcourt Refinery Eleme, Nigeria, and transferred to the laboratory immediately for analysis. All the collected samples were preserved and processed in accordance with standard guidelines. The samples were analysed for pH, Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand **(**COD), total dissolved solids, oil and grease, phenol, sulphide and some selected heavy metals.

Bioremediation using immobilized mixed culture

The efficacy of the immobilized consortium in bioremediation of refinery wastewater was determined in microcosm trials. Batch cultures were performed using one litre conical flasks containing 500ml refinery wastewater autoclaved at 121^{o}C for 15 minutes at 15 psi. On cooling, 0.5g of the immobilized consortium is introduced separately in each of the conical flasks. The control had the sterilized raw wastewater only. The different treatment options and the content of each representative flask is shown

in table 1. The samples were analyzed for pH, Biological Oxygen Demand (BOD_5), Chemical Oxygen Demand **(**COD), total dissolved solids, oil and grease, phenol, sulphide and some selected heavy metals on every five days intervals

Table 1: Definitions of the different treatment options

S/N	Treatments	Code	Description
1	Immobilized consortium on Corncob	CC	Immobilized consortium + sterile raw wastewater
2	Immobilized consortium on Palm kennel shell	PKS	Immobilized consortium + sterile raw wastewater
3	Immobilized consortium on Cocopeat	CP	Immobilized consortium + sterile raw wastewater
4	Free form of bacterial consortium	FB	Free form of consortium + sterile raw wastewater
5	Control	NB	Sterile raw wastewater

Results

Table 2: Surface properties and proximate analysis of the agro-based biocarriers

	Parameters							
Biocarriers	BET surface area (m^2/g)	Micropore volume (cc/g)	Ash	Crude fat	Crude lipid	Moisture	Crude protein	Carbohydrate
Palm kernel shell	418.3	0.148	18.5±0.42	52.7±0.91	0.2±0.01	1.6±0.06	0.3±0.03	26.6±0.28
Corncob	450.0	0.163	2.5±0.08	32.7±0.92	4.8±0.13	5.9±0.21	4.4±0.21	48.5±0.14
Cocopeat	684.4	0.186	2.4±0.07	32.5±0.14	2.2±0.04	10.1±0.07	0.5±0.03	52.3±0.4

Data presented as Mean of duplicates ± Standard deviation; Similar superscripts in a column imply there was no significant difference, those with different superscripts are significant at p-value <0.05

Table 3: Result for screening of potential phenol degrading strains

S. No	Isolates	Phenol concentrations (mg/l)					
		10	20	30	40	50	100

1	RW3 (*Bacillus tequilensis*)	+++	+++	+++	++	++	+
2	JW4 (*Pseudomonas aeruginosa*)	++	++	+	+	+	-
3	GW5 (*Micrococcus* sp.)	+++	+++	++	++	++	-

"+++" = high growth, "++"= moderate growth, "+" = low growth", "-" = no growth

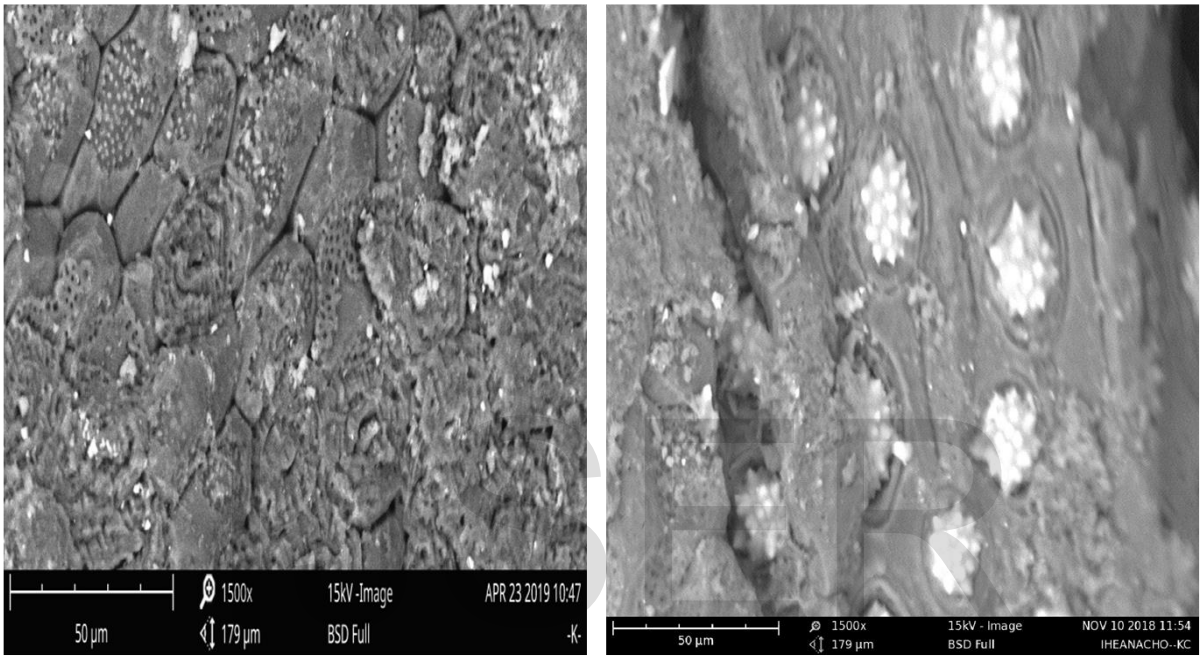

Figure 1: Scanning electron micrograph of palm kernel shell without the consortium (A), with the consortium (B)

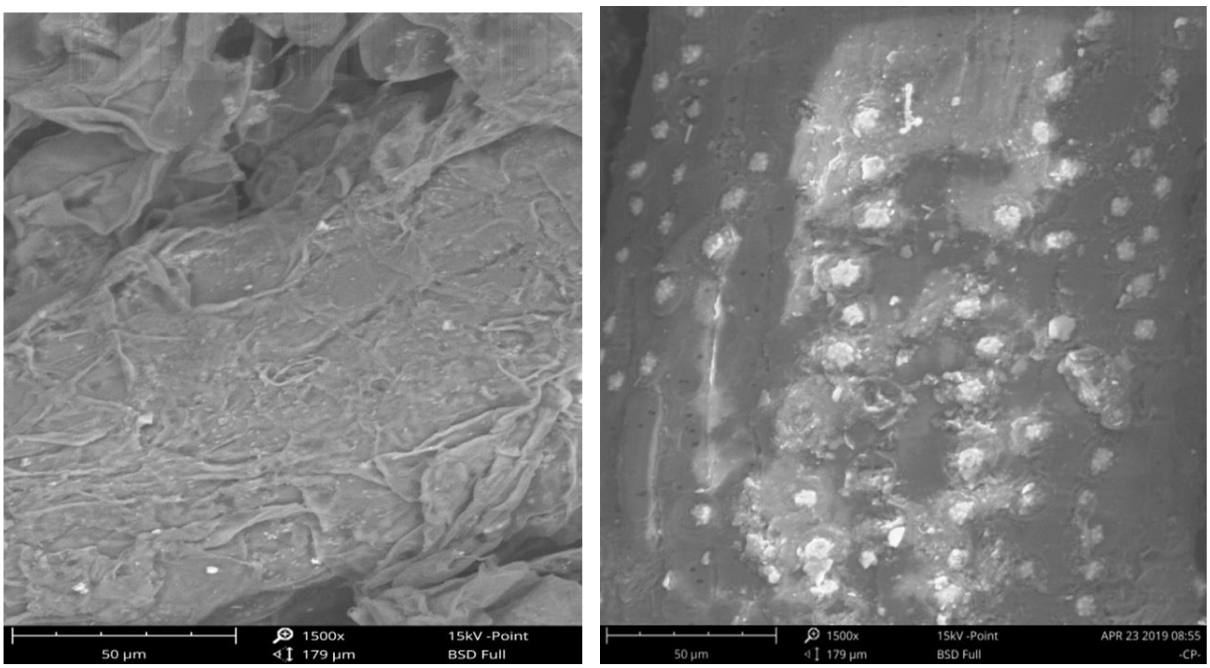

Figure 2: Scanning electron micrograph of cocopeat without the consortium (A), with the consortium (B)

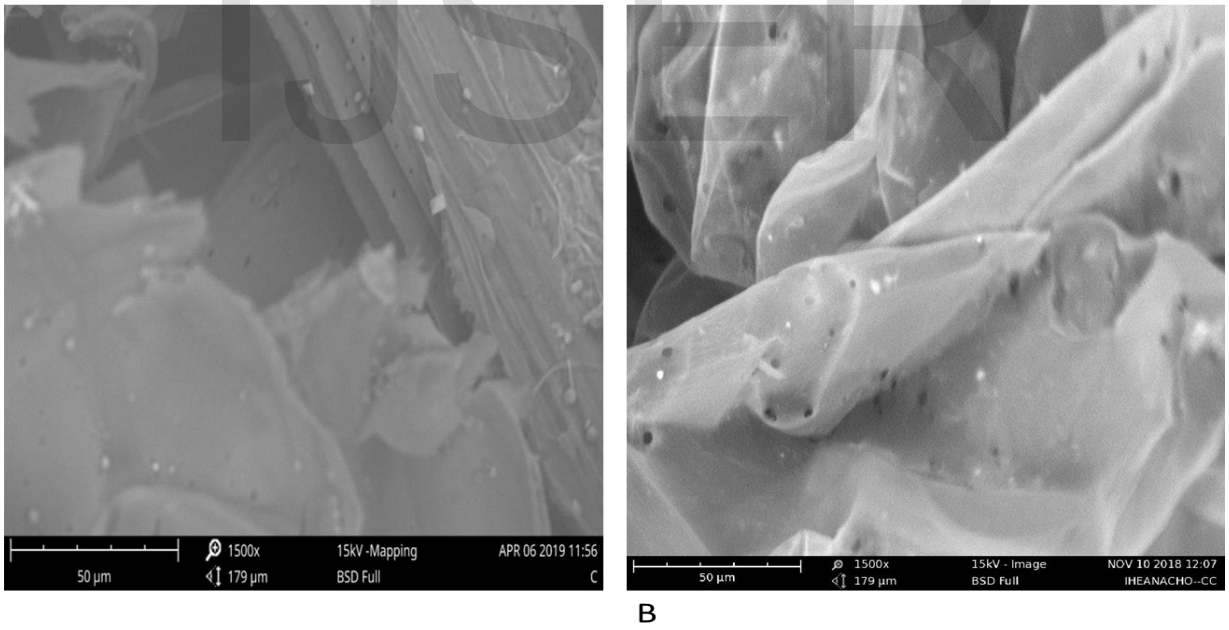

Figure 3: Scanning electron micrograph of corncob without the consortium (A), with the consortium (B)

Table 4: Comparison of refinery wastewater effluents with the different treatment options and discharge standard of DPR/EGASPIN

parameters	DPR specifica-tion	Raw wastewater	Treated wastewater	Corncob	Palm kernel shell	Cocopeat	Unimmobilized consortium
pH	-	7.9±0.20	6.4±0.18	7.1±0.14	6.9±0.11	6.9±0.11	6.9±0.10
Conductivity	1400	140.2±6.20	168.2±5.4	171.7±2.4	155.3±1.13	154.4±0.99	158.1±4.25
Salinity	NA	28.1±0.30	18.4±1.2	14.1±0.14	16.4±0.57	14.7±0.21	14.9±0.16
TDS	<2000	464.6±18.5	248.4±14.2	230.0±6.40	260.0±10.8	212.4±16.40	250.0±12.8
Phenol	0.5	96.8±6.54	1.2±0.60	0.5±0.03	0.47±0.04	0.6±0.04	5.62±1.20
BOD_5	10	146.8±8.24	18.6±0.51	5.5±0.42	7.6±0.07	5.6±0.21	14.8±0.46
COD	40	269.4±12.50	38.4±1.2	28.3±0.57	32.3±0.28	21.3±0.42	48.6±2.51
Oil and Grease	10	48.5±0.5	8.6±0.21	7.5±0.42	3.3±0.14	8.6±0.28	17.6±1.32
Ammonia-nitrogen	0.2	0.91±0.21	0.62±0.11	0.4±0.04	0.18±0.00	0.27±0.01	0.21±0.10
Phosphate	0.2	1.8±0.21	2.20±0.42	0.82±0.03	0.36±0.00	0.48±0.00	0.26±0.00
Iron	1.0	0.52±0.20	0.25±0.01	0.12±0.01	0.20±0.01	0.27±0.01	0.22±0.01
Zinc	1.0	0.06±0.00	0.02±0.01	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00

Data presented as Mean ± Standard deviation; Similar superscripts in a column imply there was no significant difference, those with different superscripts are significant at p-value <0.05

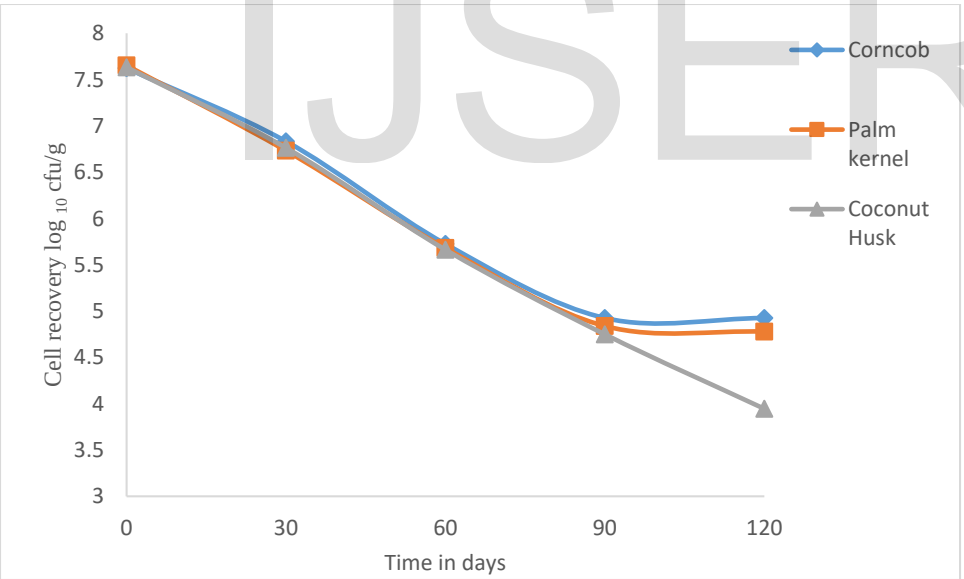

Figure 4: variation in total recoverable heterotrophic bacteria counts during storage period

Discussion

Figures 1 to 3 show the scanning electron micrographs of the adsorbed consortium on the agro-based biocarriers. The properties of surfaces affects bacterial adsorption on them. The distribution of porosity and the relative large surface area of the biocarriers are beneficial to bacteria attachment. The micrographs shows strong attachment of the consortium on the pores and crevices of the agro-wastes. Cell immobilization through adsorption brings about a direct contact between nutrients and immobilized cells (Lin, Liu, Chen, Wang, & Hu, 2014). Udawatte C & Sotheeswaran S, (2015) suggested that nutrient absorbed from carrier substrates could be an advantage to efficient colonization.

The proximate analysis of the biocarriers are shown in table 2. The analyzed parameters are within the range of previous analysis of the individual agro-based wastes. Ibrahim M., Ahmeed S. I , Mohammed A. M., Isah Y. M, (2017) analyzed the ash, moisture, volatile solids and fixed carbon for corncob (2.67%, 5.0%, 91.8%, and 5.57%) and groundnut shell (6.3%, 17.5%, 86.20% and 7.50%). Musa, Kalejaiye, Ismaila, & Oyerinde, (2010) got value 4.6% and 7.6% for ash contents and moisture contents of groundnut. Values of 10.23%, 3.24%, 1.42% and 85.11% have been reported for moisture, ash, fixed carbon and volatile matter (Onochie et al., 2017). Ash contents is generally a measure of quality for the assessment of the functional properties of food and high ash content suggests high mineral composition (Ooi, Iqbal, & Ismail, 2012). The high ash content of PKS and CP could be a possible reason for the high colonization of the surfaces.

The results for screening of potential phenol degrading strains as shown in table 3 indicated the ability of the isolates to thrive in concentrations of 50mg/100ml. All the isolates produced visible colonies after five days of incubation at 170 r.p.m at 37^{o}C. At concentration of 100mg/ml, only the *Bacillus tequilensis* produced three visible colonies after 72 hours of incubation.

Results of the physicochemical parameters of the Port Harcourt refinery raw wastewater effluents before and after treatment with the agro-waste immobilized bacterial consortium are presented in table 4. The major source of concern in effluent discharge into the environment include the presence of polycyclic and aromatic hydrocarbons especially phenol, metal derivatives, high COD and BOD_5. The concentrations of physical parameters like pH, conductivity, salinity, and total dissolved solids in the raw waste water are within the DPR/EGASPIN stipulated limits.

BOD_5 and COD provides information on the quantity of oxygen needed in the course of decomposition of organic materials in water. They are often used as an indicator of water quality. And their removal efficiencies are used to comparatively analyze a variety of wastewater treatment systems (Baharvand, Reza, & Daneshvar, 2019). The overall efficiency of treatment plants can be measured based on the chemical parameters of BOD and COD (Carducci & Verani, 2013). The BOD_5 and COD values in the raw wastewater were 146.8±8.24 and 269.4±12.50. After treatment with the different carrier-immobilized consortia, BOD_5 values of 5.5±0.42, 7.6±0.07, 5.6±0.21 and COD values of 28.3±0.57, 32.3±0.28 and 21.3±0.42 were obtained for CC, PKS and CP respectively. The unimmobilized consortium recorded BOD_5 and COD values of 14.8±0.46 and 48.6±2.51 respectively. These values indicate better performance for the immobilized consortium than the free form of the consortium.

A similarly trend was observed in the oil and grease reduction. Oil and grease values of 7.5±0.42, 3.3±0.14and 8.6±0.28 were obtained for CC, PKS and CP respectively, and were lower than the result of the unimmobilized consortium (17.6±1.32). Oil and grease are sticky in nature; they tend to aggregate and clog drain pipes and sewer lines, causing unpleasant odours and corroding sewer lines under anaerobic conditions (Aljuboury et al., 2017; Hasan et al., 2010). They also interfere with unit operations in municipal wastewater treatment plants because they float as a layer on top of the water.

When oil containing hydrocarbons are discharged into water body, they can cause depletion of dissolved oxygen due to transformation of organic components into inorganic compounds (Ajao, Yakubu, Umoh, & Ameh, 2013), and this has potential damaging effects on aquatic organisms.

The initial concentration of phenol in both the raw wastewater and the refinery treated wastewater were higher than the DPR discharge standard. After the 15 days laboratory treatment, an average of 99.4% phenol removal was noted in the three treatment options and 94.2% for the FB treatment option. However, only the corncob and the PKS-treatment options met the DPR effluent discharge standard.

Phenol degradation by microorganisms can involve the use of enzymes such as hydroxylase, monoxygenase and dioxygenase (Rehman and Ilyas, 2008). Aerobically, phenol is first converted (through oxygenation) to catechol, and subsequently degraded via the ortho or metha fission to intermediates of central metabolism. This ring fission process is catalyzed by either an ortho cleaving enzyme, catechol 1, 2- dioxygenase or by a meta cleaving 2, 3- dioxygenase enzyme (Sridevi, Lakshmi, Manasa, & Sravani, 2012) Compared to physical and chemical methods, biological treatment are preferable as it is relatively cheaper and reduces the challenges of by-products production. Phenol removal in refinery effluents has been a very challenging process in wastewater treatment. The chemical treatment with hydrogen peroxide prior to loading in the rotary bio disc is a very expensive procedure. The result shows that the selected consortium were able to degrade the phenol in the wastewater. The bacteria employed as a consortium in this study have previously been associated in hydrocarbon degradation. *Pseudomonas aeruginosa* and *Bacillus* sp. had been used as a consortium in phenol degradation (Poi, Aburto-Medina, Mok, Ball, & Shahsavari, 2017; Velmurugan & Arunachalam, 2009) while *Micrococcus luteus* had previously been mentioned as hydrocarbon degraders (Obuekwe & Al-Muttawa, 2001; Rehman and Ilyas 2008).

Bacterial recovery during storage is shown in figure 4. After storage for 120 days, viable counts of up to 10^4 were recorded from initial count of $10^{7.}$ The obtained bacterial counts may be lower than the actual counts as strongly adsorbed bacteria may be difficult to dislodge (Nuñal et al., 2014; Obuekwe & Al-Muttawa, 2001). Bacterial consortium immobilized on rice hull and cocopeat have been reported to be active after 6 months of storage (Nuñal et al., 2014). This result suggest that immobilized cells can be stored without losing their metabolic activities.

Conclusion

This work underscores the potential of agro-waste immobilized consortium of three hydrocarbon utilizing bacteria isolated from oil polluted environment in Bodo creeks, of Gokana local government area of Rivers state to effectively treat refinery raw wastewater. Bacteria immobilization on agro-waste materials could be introduced into refinery wastewater treatment protocols as a means of enhancing the treatment process.

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