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IMPROVING BIOREMEDIATION OF WATER CONTAMINATED WITH CRUDE OIL USING NATURAL BIOCARRIERS

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Abstract -Natural biocarriers, including soursop peel, cassava mash, maize bran, and Aspergillus niger fungus, were used to treat crude oil-contaminated water. The process water was analyzed for bioremediation parameters, including pH, dissolved oxygen (DO), chemical oxygen demand (COD), total petroleum hydrocarbons (TPH), and total microbial population (TMP). The chemical composition of the biocarriers was assessed, revealing the presence of essential nutrients that support microbial activity. The effects of pH, temperature, time, and dosage on TPH removal efficiency were also examined. The physicochemical analysis of treated water showed consistency with WHO standards. The biocarriers contained nutrients such as carbon, potassium, nitrogen, protein, and cellulose, which facilitated microbial biodegradation. Fourier Transform Infrared (FTIR) spectroscopy revealed functional groups like alkanes, aromatic rings, carbonyls, and amines, which promoted microbial growth. Over time, DO, pH, and TMP increased, while BOD and TPH decreased. TPH removal efficiency was influenced by process parameters, increasing from 44.36% to a peak of 80.30% for maize bran before declining to 68.20%. Cassava mash showed a similar trend, reaching 74.36% before dropping to 62.30%, while soursop peel peaked at 61.23% and later decreased to 52.10%. The biocarrier effectiveness followed the order: **maize bran > cassava mash > soursop peel**

Keywords: **Natural biocarriers, Bioremediation, Aspergillus Niger, Crude oil, and oil-contaminated water**

1 Introduction

Every day, more and more people around the world are looking to buy crude oil. Crude oil is a main ingredient in many petrochemical products and is also utilized as a fuel for mechanically transporting equipment. Oil and other petroleum hydrocarbons are very harmful to ecosystems since they are pollutants. The likelihood of an oil leak along the shores of offshore is rising in tandem with the expansion of both the transportation sector and offshore oil development. Natural occurrences, such as seepages, and some human activity, like discharge during tank loading and pipeline failure, are two potential sources of hydrocarbon pollution in marine and sea environments (Chen et al., 2017). According to Li, M., Liu, x. (2022) in Kvenvolden & Cooper (2003), the annual volume of natural crude oil seepage was estimated to be above 60,000 metric tons, with a potential uncertainty of 200,000 metric tons. The primary source of water and soil contamination, according to Kumar, A., and Kumar, S. (2020), is the release of hydrocarbons into the environment. This release might be inadvertent or caused by human activity. A growing nation like Nigeria has frequent and severe oil spills due to factors such as pipeline and oil facility neglect, bunkering, vandalism, sabotage, militancy, corrosion, and the leakage of old and outdated pipelines (Nwankwo, C.N., and Nwosu, U.L. 2020). Between 1976 and 2021, 6,817 oil spills were recorded in Nigeria, according to UNDP

(2006), and 4,200 spills were recorded by NOSDRA (2021). These spills resulted in the loss of almost four million barrels of oil, with more than 75% of that amount never being recovered. Soil erosion, brownish vegetation, barren farmland/aquatic farming, a depleted natural ecosystem, and negative impacts on human health and the economy are just a few of the many ecological and economic concerns linked with oil spillage and pollution (Robert, K-H & Broman, G. 2017)). According to research (Tormoehlem et al., 2014; Kumar, V, & Kumar, S.2018), acute hydrocarbon exposure can cause a variety of diseases, such as acidosis, dermatitis, and pneumonitis. There are significant risks to human and environmental health from crude oil spills. Ghosal et al. (2016) listed solvent extraction, incineration, and ultraviolet oxidation as some of the physical and chemical hydrocarbon treatment procedures that have been studied or used. Thus, most of these strategies come with a host of issues related to ecological consequences and removal efficiency; yet, the biological approach has shown to be highly promising for cleaning or removing hydrocarbons. Both bioremediation, which makes use of microbial populations to eliminate polluted environments, and phytoremediation, which employs plants for decontamination purposes, were among the numerous approaches detailed. Bioremediation has shown to be an efficient, safe, environmentally beneficial, and less expensive method of decontaminating hydrocarbon-polluted environments. The process relies on microbes' capacity to break down hydrocarbon waste into harmless byproducts like CO_2, biomass, and carbon monoxide (Kumar, A. and Kumar, S. (2020). Many bioremediation techniques, including bio-augmentation (BA) and bio-stimulation (BS), have been implemented. In bioremediation (BA), previously identified oil-degrading microorganisms are introduced to the ecosystem to increase their population size, whereas in biostimulation (BS), nutrients are introduced to the site to encourage the oil-degrading metabolisms of the native microbial populations. Nevertheless, the objective of this research is to enhance the bioremediation effectiveness of crude oil-contaminated water by the utilization of microcosm studies of the fungus Aspergillus Niger, soursop peel, cassava mash, and maize bran.

2. Materials and Methods

2.1 Materials

Soursop peel (SP), maize bran (MB), and cassava mash (CM) were the primary natural biocarriers utilized in this study. Biocarriers were sourced from ripe soursop, maize bran, and cassava processing waste, respectively. Reagents and chemicals were acquired from Conraws Chemical and Scientific Equipment Limited, located at N0 2 Presidential Road, Enugu, while Bonny light crude oil was procured from shell SPDC Port Harcourt. The University of Nigeria Nsukka (UNN) Microbiology Laboratory donated the aspergillus niger microbes utilized in this study after they isolated, screened, and grew them.).

2.2 Methods

2.2.1 Preparation of natural biocarriers (cassava mash, soursop peel, and maize bran)

The cassava tubers were prepared by peeling, washing, and chopping them into little pieces. After four days of fermentation in a plastic container filled with clean water, the cassava was passed through a sieve to separate the filtrate from the residue, which is called cassava mash. The process of fermenting maize seeds in clean water for three days, grinding them with a grinder, and then sieving the residue (maize bran) was described by (Ani and Chukwuma, 2020). Meanwhile, fresh soursop fruit was allowed to ripen, its peels were removed, and the mixture was left to dry in the open air with maize bran and cassava mash for two weeks. After that, it was ground with a mortar and passed through a 5mm sieve to ensure homogeneity.

2.2.3 Physicochemical analysis of processed water

The physicochemical characteristics or parameters of processed water were determined by comparing pre-and post-contamination analyses of water samples. Among these, you

can find the following: turbidity, temperature, conductivity, TDS, TSS, and total dissolved solids. factors such as total phosphorus, alkalinity, total hardness, sulphates, nitrates, iron, lead, cadmium, manganese, arsenic, mercury, copper, zinc, 100% TPH, DO, BOD, COD, and so on.

2.2.4 Determination of %TPH

The gravity-based method EPA 1664 of ASTM D7066 was used to determine the percentage of hydrocarbon (TPH). 200 milliliters of hexane were added to 100 milliliters of pollutant oil sample taken from the bioremediation setup, and the mixture was shaken violently for two minutes to facilitate phase separation. Once the phases had separated, the organic phase containing TPH was collected and spun at 300 rpm for 10 minutes in a centrifuge. The supernatant was then discarded. A rotary evaporator or oven set to 60 °C was used to dry the filtrate, which was collected from the remaining organic phase following centrifugation. The weight of the remaining residue was then reordered.

The % TPH was calculated using the formula (EPA1664 of D7066):

$$\text{initial TPH conc} = \frac{\text{Weight of residue}}{\text{volum of sample}} \text{ x } \frac{100}{1}$$

$$\text{finial} = \frac{\text{TPH con= Weight of residue after bioreme}}{\text{volum of sample}} \text{ x } \frac{100}{1}$$

$$\%\, TPH\ removed = \frac{TPH_{initial} - TPH_{finial}}{TPH_{intial}}\ x\ \frac{100}{1} \quad (1)$$

$$Or\ \%\, TPH = \frac{W_1 - W_2}{W_1}\ x\ \frac{100}{1}$$

Where W_1 = $TPH_{initial}$ (mg/l) or (g/ml), W_2 = TPH_{finial} (mg/l) or (g/ml)

2.2.5 Determination of chemical oxygen demand (COD)

COD was calculated using AOAC 1999 method 973.4. The following steps were taken: a clean cylinder was used to measure one liter of oil-contaminated water; 2–5 milliliters of this sample was transferred to the COD digestion vessel; 2 milliliters of COD reagent and 1-2 milliliters of sulfuric acid were added; mixed thoroughly. Following two hours of heating to a temperature of 150°C ± 2°C, the organic matter was allowed to digest and oxidize in the digestion vessel. When the digestion vessel cooled down to room temperature, the absorbance of the digested sample was measured using a COD meter. This is the formula that was used to determine the COD in the water sample:

$$\text{COD}\left(\frac{\text{mg}}{\text{L}}\right) = (\text{absorbance of sample} - \text{absorbance of blank}) \text{ x } \left(\frac{\text{COD standard solution conc}}{\text{absorbance of COD standard solution}}\right) \text{(AOAC 1999)} \quad (2)$$

2.2.6 Determination of pH

The pH of the water was determined using the standard test technique outlined in ASTM D1293-95 (2013). After collecting the water sample, it was agitated to make sure it was stable. Then, it was immersed with the electrode of a pocket pH meter. After waiting for the sample to stabilize for 1-2 minutes, the pH values were recorded appropriately.

2.2.7 Determination of dissolved oxygen

The Winkler method (2005) was used to measure the dissolved oxygen. To oxidize manganese oxide, which reacts with potassium iodide to release iodine (I_2), 20 ml of polluted samples were collected and placed in a BOD bottle together with manganese (ii) sulfate (M_2SO_4) and alkaline potassium iodide (KI). Afterward, a solution of Sodium thiosulfate ($Na_2S_2O_3$) was added to dissolve the precipitate and release iodine into the solution. The color was then titrated with sulfuric acid (H_2SO_4) until it became pale yellow. A few drops of starch indicator were then added to turn the color blue. The titration was continued until the color disappeared.

DO concentration was calculated by the formula:

$$DO\ (mg/l) = \frac{Vthiosulfate \times N \times 8}{Vol.\ of\ sample} \text{ (APHA 2020) (3)}$$

where: $V_{thiosulfate}$ =Volume of sodium thiosulfate used

N = Normality of thiosulphatution

V_{sample} = Volume of the water sample

2.2.8 Determination of BOD

Eaton et al. (2005) technique was used to estimate BOD. To dilute the oil-contaminated sample water, it was collected, sealed in a BOD bottle so that oxygen couldn't dissolve or escape, and then mixed with aerated dilution

water. To prevent photosynthesis, incubate the sealed BOD bottle in a dark place at 20–C for 5 days. Starting at the beginning of the incubation period and again after 5 days, the initial dissolved oxygen (DO) was determined using the Winkler DO method to track the amount of oxygen eaten. To determine BOD, the difference between the starting and ending DO concentrations was used.

$BOD_5 = DO_{inital} - DO_{final}$ (APHA 2020) (4)

2.3 Chemical characterization of biocarriers

The official method of analytical chemistry (AOAC 1990) was used to determine the total percentage of nitrogen content (%TON), total percentage of organic carbon (% TOC), and total percentage of phosphorous content (%P)

Fourier transform infrared (FTIR) analysis of the biocarriers.

The types of chemical bonds (functional groups) in a molecule were determined by creating an infra-red absorption spectrum, which is similar to a molecule's "fingerprint," using an FTIR-8400S Fourier Transform Spectrophotometer, manufactured by Shimadzu and installed at the springboard research facility in Awka.

2.4 Bioremediation study

Screening and isolation of microorganisms for bioremediation study. The microbial consortium's concentration (the number of cells in 1 ml of a suspension) was determined using Thom's chamber method (Amenaghawon et al., 2013). At the microbiology lab of the University of Nigeria Nsukka, the fungus Aspergillus Niger was cultivated, screened, and isolated to serve as the oil-degrading microorganisms in this study. Up to their use, the microbes were kept in a fridge at 4OC.

2.4.1 Preparation of crude oil for bioremediation experiment

Bonny light crude oil (BLCO) was spilled upon treated potable water to create the artificially polluted water used in this investigation. An oil refining company in Port-Harcourt River State was the source of the oil recovery. At 410C, the crude oil sample had a specific gravity of 0.85, a sulfur concentration of 0.15 wt %, a viscosity of 3.28 cp, and an API gravity of 35.2%. A mixture of 320 milliliters of BLCO crude oil and 1280 milliliters of portable water was artificially produced in four plastic containers at a ratio of one to four (Anih et al., 2019). There were four plastic containers (A, B, C, and D) into which these were introduced; the control container (A) contained crude oil, water, and microorganisms; the second container (B) contained the same, plus cassava mash; the third container (C) contained the same, plus soursop peels; and the fourth container (D) contained the same, plus maize bran. Inoculated into the several vessels for the bioremediation study procedures were 50 grams of biocarrier and 10 milliliters of Aspergillus Niger microorganisms at a concentration of 1x106 CFU/mL.

2.4.2 Bioremediation experiment

Bioremediation of water polluted with crude oil was conducted according to the protocol laid out by (Anih et al 2019). The plastic containers A, B, C, and D were mixed and agitated twice a day using an orbital mixer H-Z 200 model to bring the oil-contaminated water, microorganisms, and nutrients (biocarriers) into closer contact with one another. After that, on day zero and then every seven days for 35 days, we measured bioremediation indicating parameters in the polluted water, such as biochemical oxygen demand (BOD), pH, dissolved oxygen (DO), cell population (TMC), and total hydrocarbon (TPH).

2.5 Effect of bioremediation parameters

2.5.1 Effect of pH

A constant 10g of biocarriers, 1g of crude oil, 100ml of water, and 10ml of 1x10-6 CFU/mL of microorganisms were used to study the impact of pH on the bioremediation ability and the percentage crude oil (%TPH) elimination. While the amount of oil extracted was measured and all process parameters remained constant, the pH was adjusted from, 5.0, 6.0, 7.0, 8.0, and 9.0.

2 .5.2 Effect of temperature

Keeping all other parameters constant and monitoring the percentage of oil removed, we tested the effect of temperature by varying the initial concentration of an oil-water mixture that had been prepared by mixing 1g of crude oil with 100ml of water, maintaining a constant

10g of biocarriers, and 10ml of 1x10-6CFU/mL of microorganism. The temperatures used were 25°C, 27°C, 29°C, 31°C, and 33°C.

2.5.3 Effect of time

A bioremediation experiment was conducted with 1 gram of crude oil, 100 milliliters of water, 10 grams of biocarriers, and 10 milliliters of a microbe with a concentration of 1x10-6 CFU/mL at intervals of 7, 14, 21, 28, and 35 days. Then the percentage of oil that was removed was measured.

2.5.4 Effect of dosage

The effect of dosage was investigated by increasing the dosage of the crude oil from 1g, 3g, 5g, 7g, and 9g in 100ml of water and 10g of biocarriers and 10ml of $1x10^{-6}$CFU/mL of microorganism at constant time, PH and temperature. After, the percentage of crude oil removed was calculated.

3. Results and Discussions

Table 1: Result of physiochemical analysis of processed water before and after contamination with crude oil.

Parameters analyzed	WHO	Result Before Analysis	Result After Analysis
pH	6.5-8.5	7.14	7.14
Iron (mg/l)	0.3	0.108	0.108
Lead (mg/l)	0.01	0.0	0.0
Manganese (mg/l)	0.05	0.046	0.046
Cadmium (mg/l)	0.003	0.00	0.00
Zinc (mg/l)	5-15	0.012	0.012
Copper (mg/l)	0.05-1.5	0.0	0.0
Arsenic (mg/l)	0.01-0.05	0.0	0.0
BOD (mg/l)	5	ND	14.32
COD (mg/l)	10	ND	16.02
DO (mg/l)	5	7.2	3.16
TPH (mg/l)	-	ND	1000

(World Health Organization (2017); NIS- SON: 554-2015)

3.1: Physiochemical analysis of processed water before and after contamination with crude oil.

Table1 shows the results of the Physiochemical analysis of processed water before and after contamination with crude oil. The processed water's physical, chemical, and biological properties were all within the recommended ranges set by the World Health Organization's (WHO) drinking water standards. The results of the biological analysis showed a significant change after crude oil contamination. The processed water did not contain BOD, COD, or TPH before contamination, but after contamination, the levels of these contaminants were 14.32 mg/l of BOD, 16.02 mg/l of COD, 7.2 mg/l of DO, and 1000 mg/l of TPH.

Table 2: The results of characterization analysis of Biocarriers

Composition	Soursop Peel Biocarrier	Cassava Mash Biocarrier	Maize Bran Biocarrier
Nitrogen (%)	5.24	5.56	5.82
Total organic Carbon (%)	75.10	75.42	78.61
Phosphorus (%)	18.652	19.748	19.898
Potassium (ppm)	1.023	1.114	2.103
Sodium (ppm)	1.224	1.234	1.832
Calcium (ppm)	1.408	1.447	2.832

Iron (ppm)	0.376	0.387	0.4217
Cellulose (%)	9.153	15.663	17.795
Protein (%)	6.300	8.92	9.102
Fiber (%)	5.791	8.391	10.552
Carbohydrate (mg/l)	124.324	155.598	100.436
Lignin (%)	9.617	5.718	4.056

3.2: Characterization analysis of biocarriers

The results of the characterization analysis in Table 2 above revealed that the total carbon content ranges from 78.615 % -75.10%; Nitrogen content 5.24% - 5.82%; Phosphorous from 18.652%-19.898%; Potassium content from 1.023 ppm-2.103 ppm; Calcium contents 1.408 ppm-2.832ppm; protein contents 6.30%-9.102%; cellulose content 9.155%-17.795%; fiber content 5.791% – 10.552% for the three biocarriers (soursop peel, cassava mash and maize bran), while carbohydrate contents were 124.324mg/l, 155.598 mg/l and 100.436 m/l for soursop peel, cassava mash, and maize bran biocarriers. These indicates that the biocarriers (soursop peel, cassava mash, and maize bran) contained enough micro and macronutrients that are capable of providing needed supports for proper cell functioning like energy source, protein and amino acid synthesis (Schmidt et al., 2011), in bioremediation of crude oil-contaminated water, in contrast to the study by Udume et al. (2023) on biostimulation of petroleum-contaminated soil using organic and inorganic amendments, and to ensure the correct functioning of cell wall membranes and energy metabolism to microbial development. According to Singh, J.S. Kumar, A., & Singh, D. P. (2020) in Paul and Clark (1989), microorganisms need a specific ratio of carbon to nitrogen and phosphorus to grow unchecked in a microcosm study of bioremediation. This ratio is 30% carbon to 5% nitrogen and 1% phosphorus.

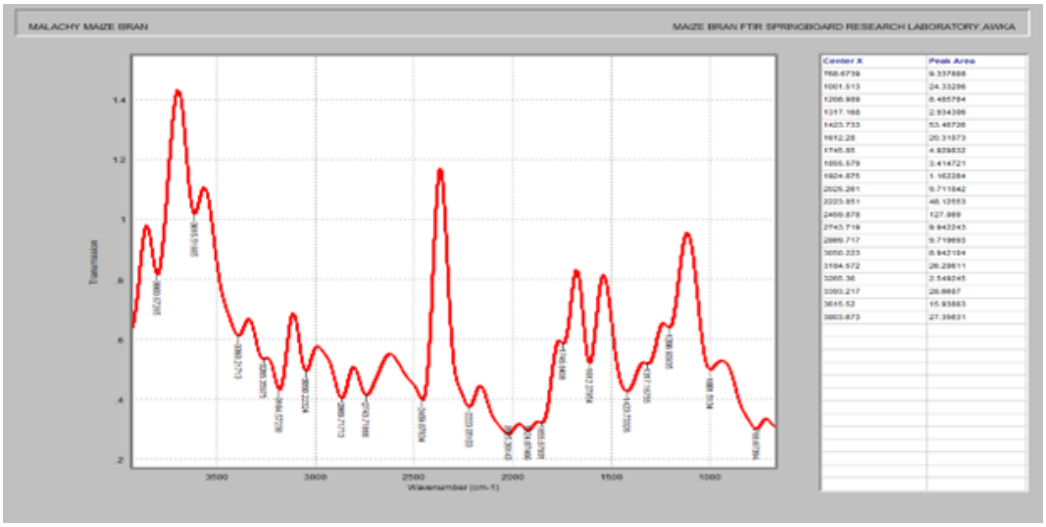

Figure 1: FTIR Results for Maize Bran

3.3 FTIR result analysis for maize bra biocarrier.

The FTIR spectra result for maize bran biocarrier was presented in Figure 1. Based on the result of the analysis, the Maize bran biocarrier' wavelength/ frequency stretched from 768.67394cm^{-1} to 3803.672cm^{-1} showing the presence of Chlorinated aliphatic compound, aromatic ether/esters, cycloalkanes, alkane, carboxylic group, ketone group, aliphatic ketone, aromatic nitrile, cyanide, phenol, methyl, alcohol, and aromatic phenol, the FTIR spectrum suggests that the maize bran contains compounds that may be contributed to its potential in supporting microbial growth (Kumar et al 2019).

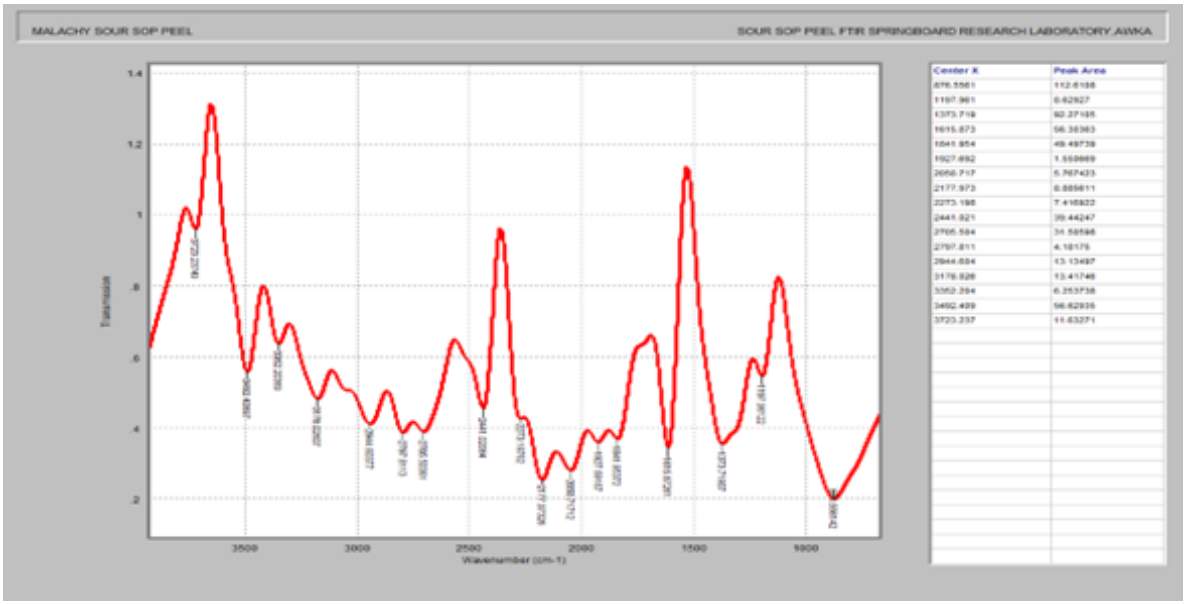

Figure 2: FTIR result of Soursop peel biocarriers

3.4 FTIR result analysis for soursop peel biocarrier

The result of the FTIR for Soursop peel is shown in Figure 2. From the figure, the wave number of the biocarrier stretched from 876.5561cm^{-1} to 3723.23749cm^{-1}, indicating the presence of functional groups. This wide spectral range suggest that the biocarrier contains a complex mixture of organic compound including alkane, aromatic ring, carbonyl group, cyclics esters, ether, ketone, amides group, nitriles, alkayne, and amine groups.The specific absorption peaks within this range can be attributed to the streching and bending viberations of various molecular bonds, providing valuable information on the chemical composition (Kumar et al 2019).

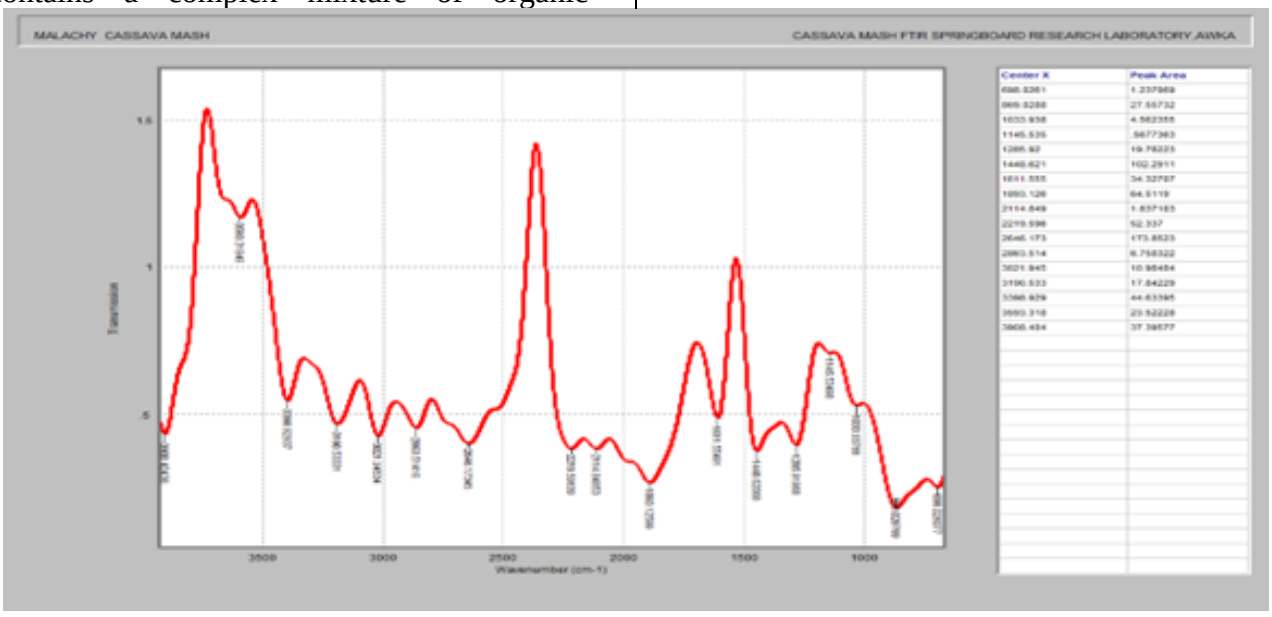

Figure 3: FTIR result for cassava mash biocarrier

3.5. FTIR result analysis for cassava mash biocarrier

Fourier transform spectra of the cassava mash biocarriers are presented in Figure 3. It was observed that the wave number of cassava mash biocarrier stretched from 698.0261cm^{-1} to 3908.404cm^{-1}, which shows the presence of Hydroxyl groups, chlorinated aliphatic, aliphatic phosphate, aliphatic ether, aromatic ether, alkanes, carboxylic group, esters, ketone, and nitriles groups; which revealed that cassava mash biocarrier contains a diverse range of organic compounds that contributed to its potential in aiding microbial growth (Kumar et al 2019).

3.6. Effect of process parameters on %TPH removal using biocarriers

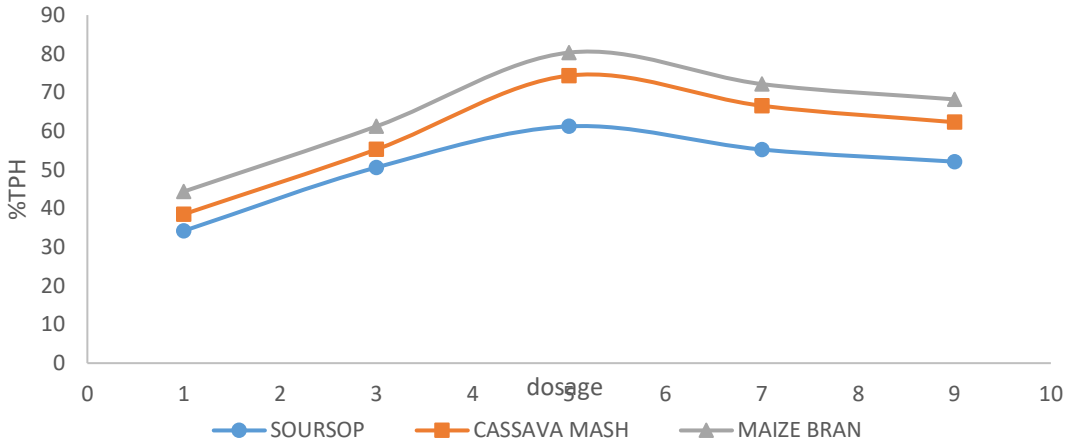

Figure 4: Effect of dosage on %TPH removal

The effect of biocarrier dosages on %TPH removal is shown in Figure 4. The result shows that the total percentage of TPH removed increased with an increase in the dosage of all the biocarriers (maize bran, cassava mash, and soursop peel). The reason for this is due to the presence of micro-macro chemical components present in them. TPH decreased as a result of eutrophication (limited oxygen), which reduced microbial growth. This is in agreement with Zhang et al 2019, Prince et al 2019, and Wang et al 2020). However, in comparison, the %TPH removal increased in the order of **maize bran > cassava mash> soursop peel**.

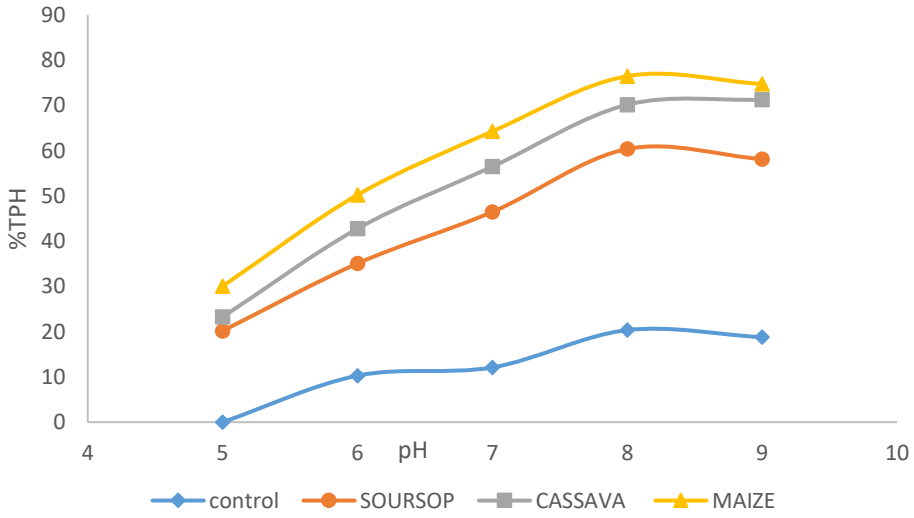

Figure 5: Effect of pH on TPH removal

Figure 5 shows the impact of pH on the %TPH removal. Soursop biocarrier achieved a maximum TPH removal of 55.34 % at pH 8.0, cassava mash biocarrier was 60.35% at pH 8, and maize bran was 70.12 % at the same pH 8. From the figure, it can be seen that as the pH increased, the percentage of TPH removed decreased. This is because proteins denaturize and cellular metabolism changes when the pH rises over optimal, causing the cytoplasm to become acidic or basic (Dahalan et al 2013).

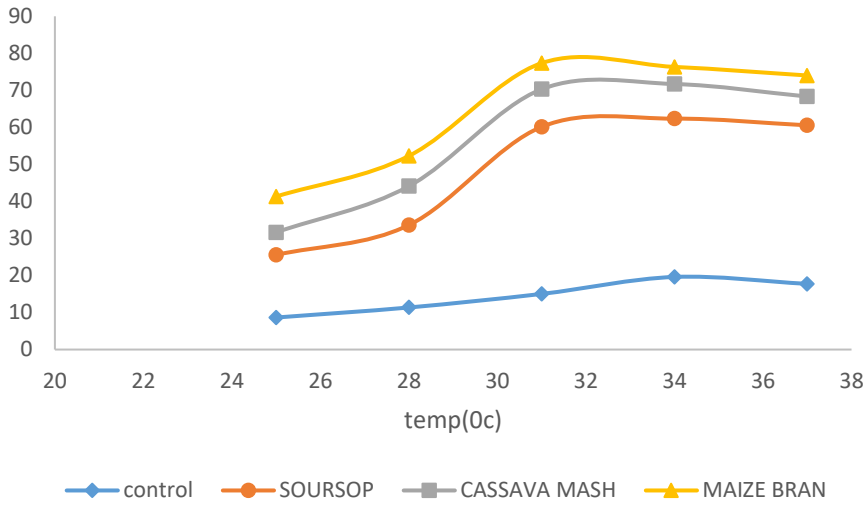

Figure 6: Effect of temperature on TPH removal

Figure 6 shows the influence of temperature on the elimination of TPH from oil-contaminated water. All of the biocarriers show that the percentage of TPH removed increases with increasing temperature. At its optimal temperature of 31 °C, the soursop peel biocarrier removes 62.16% of the TPH, while the cassava mash and maize bran biocarriers remove 71.71% and 76.33% of the TPH, respectively. As illustrated in Figure 6, the percentage of TPH removed decreased as the temperature increased. The reason is, air emission, denaturation of proteins, and reduced sorption of TPH occur at temperatures higher than the optimal (31^{O}C) (Wang et al and Him et al 2018).

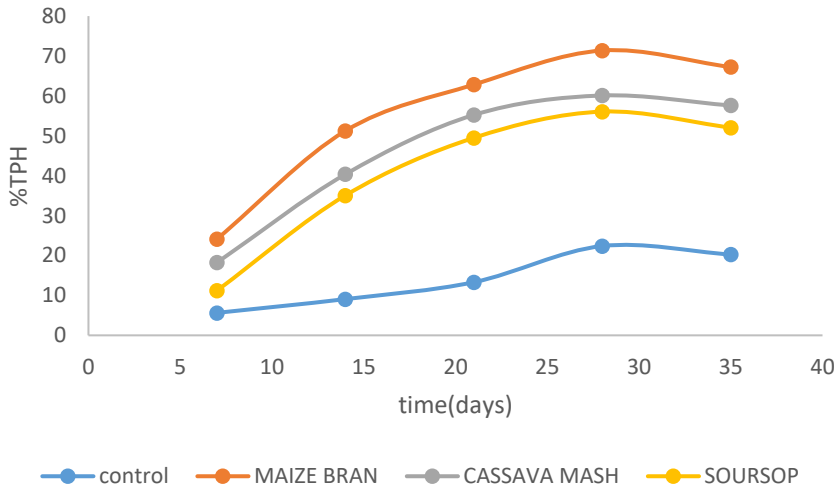

Figure 7: The effect of time on %TPH removal

Figure 7 shows the time-dependent effect of %TPH elimination by cassava mash, maize bran, and soursop. All of the biocarriers showed an increase in %TPH elimination with time, as indicated in the figure. At 35 days, the soursop biocarrier, cassava mash biocarrier, and maize bran biocarrier all eliminated a maximum of 56.06%, 60.11%, and 71.37% of TPH, respectively. The reason for variation in the %TPH removal with time for the three biocarriers is attributed to micro and macronutrient, which maize bran contained more than others and time-dependent by microorganisms for chemical oxidation, adaptation/enzyme production, volatilization, and increased bioavailability of TPH for effective separation of the oil and water phases during bioremediation (Hassanshahian et al 2020; Wang et al 2020 and Liu et al 2019).

4. Conclusion

This study examined the enhancement of bioremediation efficacy of biocarriers in crude oil-contaminated water utilizing soursop peel, cassava mash, and maize bran. The subsequent findings were drawn:

1. Physicochemical study of uncontaminated water indicates its suitability for ingestion, however, post-contamination with crude oil reveals significant fluctuations in BOD, DO, COD, and TPH levels. The fluctuation of these metrics signified a substantial pollution index.

2. The characterization results of biocarriers indicated the presence of chemical constituents abundant in micro and macronutrients, including nitrogen, carbon, phosphorus, potassium, sodium, calcium, iron, protein, carbohydrates, fiber, lignin, and cellulose, which enhanced microbial activities for biodegradation.

FTIR study of the biocarriers indicated that they predominantly comprise organic chemicals, including amino groups, hydroxyl groups, aliphatic groups, phenols, nitriles, aromatics, alcohols, ketones, carboxylic acids, esters, ethers, and peroxides, which facilitate microbial growth.

3. The influence of bioremediation measures such as dissolved oxygen (DO), biochemical oxygen demand (BOD), pH, and total petroleum hydrocarbons (TPH) indicates that DO, pH and total microbial count (TMC) rise over time, but BOD and TPH decrease. This suggests that biocarriers positively affect the treatment of oil-contaminated water.

4. The influence of process parameters: pH, time, temperature, and dosage on TPH removal demonstrated that efficiency increased with elevated parameters, achieving a maximum TPH removal of 80.30%. This efficiency subsequently decreased to 68.20% for maize bran, 74.30% to 62.30% for cassava mash, and 61.23% to 52.10% for soursop peel at dosages of 5g and 9g, pH levels of 8 and 9, temperatures of 31°C and 33°C, and durations of 28 to 35 days for maize bran, cassava mash, and soursop peel, respectively.

5. The influence of process parameters: time, temperature, dosage, and pH—on microbial growth rate increased from $2x10^6$ CFU/g to a peak of $54x10^6$ CFU/g for maize bran, from $15x10^6$ CFU/g to $42x10^6$ CFU/g for cassava mash, and from $11x10^6$ CFU/g to $32x10^6$ CFU/g for soursop peel, subsequently declining to $47x10^6$ CFU/g, $36x10^6$ CFU/g, and $25x10^6$ CFU/g for all biocarriers examined. Maize bran biocarrier has the most impact on microbial growth and %TPH removal.

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