EFFECT OF CRUDE OIL ON THE GROWTH OF MICROALGAE

BY

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FACULTY OF LIFE SCIENCE

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BENIN CITY

APRIL, 2024

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AN UNDERGRADUATE DISSERTATION SUBMITTED TO THE DEPARTMENT OF ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY, FACULTY OF LIFE SCIENCES, UNIVERSITY OF BENIN, BENIN CITY, EDO STATE, NIGERIA; IN PARTIAL FULFILMENT OF THE REQUIREMENTS FOR AWARD OF BACHELOR OF SCIENCE (B.Sc) DEGREE IN ENVIRONMENTAL MANAGEMENT AND TOXICOLOGY.

APRIL,2024

CERTIFICATION

This is to certify that this research titled EFFECT OF CRUDE OIL ON THE GROWTH OF MICROALGAE was carried out by **ASENOGUAN DAYO OGHOSA** and presented to the Department of Environmental Management and Toxicology, Faculty of Life Sciences, University of Benin, Benin City; in partial fulfillment of the requirements for the award of Bachelor of Science (B.Sc) in Environmental Management and Toxicology. It was conducted under suitable conditions, was carefully supervised and subsequently approved as having met the requirements for the award of Bachelor of Science degree in Environmental Management and Toxicology.

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DR J.U OGBEBOR **Date**

Project Supervisor

……. ……..

PROF A.A. ENUNEKU **Date**

Head of Department

DECLARATION

I ASENOGUAN DAYO OGHOSA declare that EFFECT OF CRUDE OIL ON THE GROWTH OF MICROALGAE is my own work and that all sources that I have used or quoted have been acknowledged by means of complete references and that this work has not been submitted before for any other degree at any other University.

Name of Student

DAYO OGHOSA

Date……..

DEDICATION

This project is dedicated to God Almighty, the source of wisdom, knowledge, and understanding for his love, strength, favor, grace, and mercy to work on this project. Also, to my parents Mr and Mrs Patrick Asenoguan for their unconditional love, care and support throughout the period of this project. I am forever indebted to you.

ACKNOWLEDGEMENT

All thanks to God for his love. My sincere appreciation to my parents for their unflinching support.

Also, I want to in a very profound way appreciate my project supervisor Dr J.U OGBEBOR whose fatherly and friendly mentorship is unparalleled, you are the best any generation could have asked for. I want to appreciate my siblings Collins, Faith, Charles, and Gerald, I say a very big thank you.

My sincere appreciations goes to Mr and Pharm Mrs Pius Okoto for their unmatched support.

To my friends Jennifer Agbori, Fred and Larry Okpeaye, Osayi Okungbowa, Hope Ikiyor, Miracle Erayamen, Favour, Blessed, Theresa, and Oyinkasola Talabi, who has made this project is big success.

I say a resounding thank you to Perfect, Chris, Rex, Temi, Kelvin, Egbon Happy, Wesley, Bishop Justice, Nympha, and Ope, and to that one special person Dorothy.

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ABSTRACT

This study investigated the effect of water soluble fractions of crude oil on the growth of *Monoraphidium contortum* and *Dimorphococcus lunatus* over a 14 day period. The test algae were grown in concentrations of CHU 10 nutrient media mixed with varying concentrations of WSF of crude oil (5%,10%, 25%, 50%, 75% 100%) which were prepared in triplicates. The growth response was measured using a visible spectrophotometer at two day intervals over a 14 day period. Physicochemical parameters (pH, EC, and TDS) were assessed on day 14 of the experiment and were compared to the stock concentration before exposure to the test microalgae. The results showed that there was growth stimulation for all concentrations of 0%, 5%,10%, 25%, 50%,75%, and 100% from day 0 to day 2, and a lag phase from day 2 to day 4 for 10% and 100%. The highest growth was recorded at 5% concentration on day 14 with an absorbance value of 0.035, followed closely by 100%, 50%, and 75% concentrations, while 10% WSF showed the least growth on day 14 with an absorbance value of 0.02. Statistically, the growth response of the microalgae to the WSF concentrations did not differ significantly ($p > 0.005$). Generally the percentage yield was higher in *M.contortum* compared to *D.lunatus.*

The physicochemical properties of *Dimorphococcus lunatus* and *Monoraphidium contortum* showed that when the quantity of WSF was reduced, both TDS and electrical conductivity rose. On the other hand, the pH revealed that it was somewhat more alkaline with a lower WSF concentration, suggesting acidity with a higher WSF. To summarize, *Dimorphococcus lunatus* exhibited a strong inhibitory reaction, and *Monoraphidium contortum* is better suited for bioremediation of crude oil in contaminated water.

CHAPTER ONE

INTRODUCTION

The modern global economy depends on fossil fuels—more especially, coal and petroleum—to produce energy. In 2020, the demand for liquid fuels hit all-time highs, surpassing 100 million barrels per day, while the use of petroleum climbed by 0.9 million barrels per day. World markets and stock exchanges are governed by the use of oil. Because of this, the process of extracting and refining crude oil is still very demanding. This extensive extraction leads to a number of issues that are crucial for the contamination of soil and water in the ecosystem. (Mota *et al.*, 2022)

According to Zakaria *et al.* (2000), petroleum hydrocarbons are multi-component homogeneous mixtures with complicated molecular structures made up of alkanes, alkenes, aromatic hydrocarbons, and heterocyclic aromatic hydrocarbons. All of these substances are hazardous to marine plankton, as demonstrated by (Huang *et al*., 2011).

Hydrocarbons derived from crude oil are responsible for the production of the greatest class of environmental contaminants globally. Hazardous aromatic organic chemicals, such as polyaromatic hydrocarbons (PAHs), aromatic molecules that are scarcely broken down by nature, chlorophenols, and cresols, which are poisons derived from hydrocarbons, escape into the environment during processing operations in the hydrocarbon oil sector. However, difficulties with oil contamination during storage and transportation have been made worse by crude oil spills. Bilali, Strassner and Hassen (2021).

The International Tanker Owners Pollution Federation (ITOPF) (2013) estimates that between 1970 and 2013, more than 5.74 million tonnes of crude oil were spilled into ocean waters, of which more than 90% was directly caused by human activity. Large-scale marine

oil spill incidents have drawn a lot of attention because of the devastation they cause to the ecosystem.

Minor oil spills and contamination have become serious dangers to environmental and public health due to the growth of offshore oil exploration and development as well as maritime transportation. Per the China State Oceanic Administration's 2014 assessment on marine environmental quality, petroleum hydrocarbons have surpassed nutrients (phosphate and nitrate) as the primary contaminants in the China Sea.

In addition, there have been numerous incidents involving the transportation of petroleum over the years that have resulted in environmental issues. Oil spills primarily affect aquatic areas, where they have a significant effect on the health of the flora and fauna. (Mota *et al.*, 2022).

Through the tainting of water sources, the release of poisonous fumes, and the irreversible contamination of aquatic and terrestrial environments, oil spills destroy ecosystems and the atmosphere Krivolapov (2019). For instance, the disastrous British Petroleum oil spill in 2010 that resulted from the explosion of the Transocean Deepwater Horizon rig, and the Conocophillips oil spill in 2011 that occurred in the Bohai Sea, China, all contributed to and continue to contribute to the loss of habitat and species diversity, the destruction of the halobios ecological balance, and the extinction of endemic species (Ndimele *et al*., 2010)

According to Cada (2009), ecosystems that are in close proximity to a crude oil spill are directly killed by the pollution. The spill has a negative effect on consumers who are not directly touched since it depletes their essential supplies of food, energy, and water. This depletion causes a chain reaction of deaths throughout the food web, turning otherwise thriving ecosystems into dead zones.

Various approaches have been suggested for the treatment of water contaminated by emulsified oils. Adsorbents have been utilized to adsorb emulsified oils at concentrations of

60 parts per million or less. Examples of these include vermiculite and organoclays. Compared to activated carbon, organoclay was shown to remove material at a rate seven times faster. But oils, greases, and other naturally occurring organic materials block the adsorbent's pores, greatly decreasing its efficiency (Da Silva *et al.*, 2003). Furthermore, the saturated adsorbents are regarded as secondary pollutants during treatment and need to be recycled or released in a safe manner. It has also been recommended to use chemical de-emulsifiers to break up the emulsions and use sedimentation to separate the oils. Kilpatrick (2012).

Recently, oil spills have been contained by the application of solidifiers like poly iso-butylene (PIB), also referred to as "Elastol." This substance solidifies or gelatinizes the oil on the water's surface, preventing it from leaking or spreading. Despite being non-toxic and frequently present in food, PIB may have the potential to entangle or suffocate aquatic animals due to the presence of gelatin. Other chemical techniques that are typically more costly include the use of sorbents, dispersants, herders, and elasticity agents. Moreover, it was shown that chemically spread oil was more dangerous than naturally dispersed oil, and as a result, the ecosystem may suffer. (Tamis *et al.*, 2011)

Recent research has demonstrated that β-cyclodextrin-coated nanocomposite superparamagnetic iron-oxide nanoparticles (SPION) have a high oil recovery rate (Kumar *et al.*, 2015). After separating from the emulsion, the oil enters sponge pores and stays inside the core. Notwithstanding the promising outcomes, the composite is costly and necessitates regeneration, much like traditional adsorption methods. (Kiran *et al.*, 2009)

Because of this, environmentally friendly methods of removing and degrading oil have been pursued in recent years, with a focus on reducing the usage of chemical dispersants, which are themselves hazardous (Mota *et al.*, 2022). One of such methods is bioremediation.

Stewart (2012) stated that crude oil spills occur all throughout the world, and their repercussions are disastrous. Although beneficial, the current techniques for cleaning up oil spills have shortcomings. To guarantee effectiveness and success, an additional therapeutic approach must be used in addition to the current ones. Algal bioremediation is a promising and novel treatment; nonetheless, the specifics of algae. Certain bacterial strains have been studied as de-emulsifiers, including Bacillus subtilis and Ochrobactrum anthropi strain RIPI5-1.the single microbe. Research has demonstrated that bioremediation can save between 50 and 70 percent of the expenses associated with traditional physical and chemical methods.

In the aquatic environment are ecologically important. They are unicellular, photosynthetic microorganisms that play a crucial role in the functioning, diversity, and productivity of aquatic ecosystems (Naselli-Flores *et al*., 2022). Specifically, about half (49%) of the global net primary production in freshwater and marine environments is produced by phytoplankton, which is adapted to live in suspension in water masses (Friend *et al*., 2009). Moreover, microalgae exhibit a high diversity and include species highly distant from an evolutionary point of view (DeVargas *et al*., 2015). Despite their small size, which ranges between 0.2 and 200m, they can contribute to climate change mitigation through carbon fixation (Ratnapuram *et al*., 2018)

The use of natural processes to eliminate contaminants and dangerous compounds from the environment is known as bioremediation. Because it is a self-sustaining cycle, algae bioremediation is unique. Algae absorb and use oxygen from its surrounding environment to oxidize pollutants into less toxic compounds. carbon dioxide and water vapor are among these metabolites. Algae use photosynthesis, which needs CO_2 and H_2O for growth. The cycle is then repeated since photosynthesis releases oxygen which algae can use for more oxidation of pollutants. With the help of this promising approach, CO_2 may be captured and recycled into biomass, which can then be used to create value-added goods like bioenergy.

Given their capacity to metabolize different contaminants using them as carbon sources in an environmentally friendly manner, releasing oxygen into the atmosphere and removing CO_2, microalgae can be a great option.

Aims and objectives

The aim of this study was to ascertain the effect of water soluble fractions of crude oil on the growth and survival of *Monoraphidium concortum* and *Dimorphococcus lunatus.*

The specific objectives of the study were to

1. expose *Monoraphidium concortum* and *Dimorphococcus lunatus*. To varying concentrations of water soluble fractions of crude oil.
2. access the effects of the various water soluble fraction concentration on the growth and survival of *Monoraphidium concortum* and *Dimorphococcus lunatus*
3. determine the inhibitory or stimulatory response of the test algae to different concentrations of water soluble fractions of crude oil.
4. determine the percentage (%) yield for the test algae from day 0 to day 14, at different concentrations of water soluble fractions of crude oil.

CHAPTER TWO

LITERATURE REVIEW

Review of relevant literature on crude oil presented below in chronological order. (Essien, *et al*., 2005) in their study carried out in Nigeria showed that the direct impact of a recent oil spill on the abundance of microalgae in the coastal shore of the Qua Iboe Estuary was investigated as part of a concerted effort to apply epipsammic microalgae indices as a biological indicator of crude oil pollution and natural remediation in a tropical estuarine environment. There was a noticeable detrimental impact of contamination on the sandy beach soil's levels of nutritional salts (CO_2, Cl, and SO_2), acidity, and salinity. After contamination, the beach soil's Biological Index of Pollution (BIP) increased from the previous somewhat polluted level (18%) to 75, 88, 45, and 41% at sample distances of 5.5, 9.5, 11.5, and 15 m from the pollution control barrier. These correlated with an increase in microalgae density as the barrier's distance was increased. Thus suggesting that microalgal cells near the barrier were more severely affected by oil contamination. Overall, the epipsammic microalgae's capacity for regeneration was reduced, with a distance-dependent effect.

To ascertain its impact on the survival rate, Arimoro and Adamu (2008) assessed the acute toxicity of crude oil's WSF on *Chironomus* and mosquito larvae. During the 48-hour exposure in a static bioassay medium, the larvae were exposed to acute concentrations of WSF of crude oil (20.0, 10.0, 5.0, and 0.0 ml L^{-1}). It was discovered that mortality rose with exposure duration and intensity. For mosquito and *Chironomus* larvae, the 48-hour MLC was 5.75 and 6.61 ml L^{-1} respectively. For the other concentrations, the 48-hour MLT was 18.20 hours for 20 milliliters L^{-1}, 28.20 hours for 10 milliliters L^{-1}, 48 hours for 5 milliliters L^{-1}, and 26.80 and 41.80 hours for 20 and 10 milliliters L^{-1}, respectively. This research indicated that mosquito larvae were more sensitive to the WSF of crude oil than *Chironomus* larvae.

In their paper, Chao *et al*., (2012) examined the acute toxicity of four fuel oils including F120, F180, F380 and No.-20 to microalgae. This was evaluated by exposing the marine microalgae *Chlorella spp*. (Chlorophyta) and *Skeletonema costatum* (Bacillariophyta) in the fuel oil water accommodated fractions (WAF). The bio assay showed that F180 WAF was the most toxic to both microalgae. The 96 h EC50 value of F180 WAF for *Skeletonema costatum* and *Chlorella* spp. was 9.41 and 13.63 mg/L expressed in concentration of total petroleum hydrocarbons, respectively. WAFs of F120, F180 and F380 were more toxic to *Skeletonema costatum* than to *Chlorella* sp. In contrast, No.-20 WAF did not show significant toxicity for both *Skeletonema costatum* and *Chlorella* sp.

Pi *et al.* (2015) used a microbial consortium and specially made devices measuring 0.6 m (L), 0.3 m (W), and 0.4m (H), to replicate bioremediation on the oil spill-polluted marine intertidal zone. Following bioremediation, GC-MS analysis of crude oil revealed that the removal efficiency of n-alkanes and polycyclic aromatic hydrocarbon homologues was greater than 58% and 41%, respectively. Furthermore, the concentration-dependent acute toxicity of crude oil varied for three microalgae: *Dicrateria sp*, *Skeletonema costatum*, and *Phaeodactylum tricornutum.* The cell densities in the treated seawater increased over the course of 96 hours, from $4.0x10^5$, $1.0x10^5$, and $2.5x10^5$ cells per milliliter to $1.7x10^6$, $8.5x10^5$, and $2.5x10^6$ cells per milliliter, respectively. This showed that the bioremediation process significantly improved the quality of seawater contaminated by crude oil.

Wang *et al*. (2015) through single-species and customized community experiments, determined the ecotoxicological effects of a mixture of petroleum hydrocarbons, and evaluated on densities of two algae species (*Platymonas helgolandica* var. *tsingtaoensis* and *Isochrysis galbana*) and one rotifer species (*Brachionus plicatilis*). Test concentrations were from 0 to 100 mg L^{-1}, and within a month, five to seven treatments were evaluated in triplicate. In single species toxicity tests, densities significantly decreased at petroleum

hydrocarbon concentrations greater than 1.0 mg L^{-1}. The customized community's algae equilibrium densities, on the other hand, exhibited a distinct pattern that peaked at 20.0 mg L^{-1} and increased with concentration. There was a difference between the NOEC obtained from single-species toxic tests (0.25 mg L^{-1}) and the community-based NOEC (1.0 mg L^{-1}). This showed that because of ecological interactions, the effects of ecotoxicology on plankton as a community differ significantly from those of toxicity tests conducted on individual species.

In a study by Uba, (2019) on the 'Effects of Aromatic Hydrocarbons and Marine Sediments from Niger Delta on the Growth of Microalga *Phaeodactylum tricornutum*" Three sediments and potassium dichromate were added to long cells containing 25 mL of the algae-toxicant dilutions for fifteen treatments and the control. The treatments were incubated for three days at 20 ± 2°C. and included xylene, anthracene, and pyrene at concentrations of 0.0 mg/L, 1.0 mg/L, 1.8 mg/L, 3.2 mg/L, 5.6 mg/L, and 18.0 mg/L. The median effective concentration (ErC50) of each of the fifteen treatments and the control ($K_2Cr_2O_7$), denoted as C0, C1, C2, C3, C4, and C5, on the growth of microalga *Phaeodactylum tricornutum*, was ascertained. The sand samples from the three regions under investigation were subjected to a physicochemical analysis and a marine microalga toxicity test in a laboratory setting. The results showed that the sediment samples from the three sampling sites had higher concentrations of heavy metals, aromatic compounds, and other physicochemical characteristics than the water samples. There was also a very strong significant positive linear connection between sample concentrations and algal number (p=0.05). Therefore, the toxicity results (> 1 mg /L <EC50 ≤ 10 mg /L) in this study are deemed scientifically relevant in the ecotoxicological risk assessment of the Niger Delta, Nigeria, since they are consistent with other toxicity values for this category of toxicants

Santoso *et al*. (2020) studied how the cell density of the microalgae *Scenedesmus vacuolatus* is affected by saltwater contaminated with hydrocarbons. This showed the optimal treatment to lower total petroleum hydrocarbons (TPH) levels as well as the impact of hydrocarbon-polluted seawater concentration on the density of *Scenedesmus vacuolatus* microalgae cells. The hydrocarbon-polluted saltwater utilized in this study's treatment was amplified in Jakarta's KaliAdem port. A mixture of 25% hydrocarbon-polluted seawater (A), 50% (B), 75% (C), and 100% (D). Walnemedium is controlled using sterile seawater that isn't from the Kali Adem port. The *Scenedesmus vacuolatus* sample had the highest average density, according to the data. This is evident from the log phase length of *Scenedesmus vacuolatus* and the average cell density at the cells, which was in the control peak period of 29.48 x105cells/mL. TPH measurements revealed decreases in TPH across all treatments. With a drop percentage of 70.62%, therapy B is the most effective in lowering TPH levels.

Masifwa *et al*. (2022) studied the concentration of oil and grease and the measurement of its effects on algae, invertebrates, and fish were inferred. Water samples were taken in April and September of 2012 to 2018 at the upstream and downstream transects and in the reservoir, and they were analyzed for oil and grease using standard procedures. The environmental compliance was compared to the 10 mg/l NEMA discharge standard and the ≤ 0.1 mg/l PAH effluent discharge standard. Throughout the sampling period, average concentrations of oil and grease were below 10 mg/l at all sites. Of the 14 data sets for each transect, only three were along the upstream transect and two at each of the downstream transect and the reservoir were compliant. The authors reported that grease and oil had a relatively high quantity (> 0.1 µg/l) in comparison to total polycyclic aromatic hydrocarbons, which is regarded dangerous to most aquatic animals. They also reported that the project area's varied activities, suggested that the sources of oil and grease were similarly varied.

Ezenweani and Kadiri (2023) utilized tropical marine algae for bioremediation of petroleum fuels specifically, the investigation looked at the growth of *Nannochloropsis oculata* and *Porphyridium cruentum* at 0%, 10%, 20%, 30%, 40%, 50%, 75%, and 100% of WSF of kerosene, diesel, and gasoline in order to determine the productivity and bioremediation potential of these two microalgae in the Water Soluble Fraction (WSF) of petroleum fuels. For fourteen days, growth was observed optically every two days using a 721 Visible Spectrophotometer. The author reported that in all the fuel examined, the minimum growth for both species was recorded at 100%. *Porphyridium cruentum* reached its maximum growth at 10% in all fuels, while *Nannochloropsis oculata* reached its maximum growth at 30% in gasoline and kerosene and at 50% in diesel. All fuels significantly inhibited *Porphyridium cruentum,* but at lower fuel concentrations, *Nannochloropsis oculata* was stimulated. With removal efficiencies of 84.58%, 65.51%, and 70.77% for kerosene, diesel, and gasoline, respectively, *Nannochloropsis oculata* outperformed *Porphyridium cruentum*, which had removal efficiencies of 58.94%, 46.64%, and 56.67%.

To assess the capacity of *Chlorella vulgaris* for bioremediation of sediment contaminated by crude oil, Ugboma *et al*. (2023) conducted a research which made use of three (3) flat rubber experimental designs, *Chlorella vulgaris* was added to the setup, excluding controls 1 and 2 (Us and Cs). Standard analytical methods were used to determine the sediment profile parameters, such as temperature, pH, nitrogen, phosphorus, potassium, electrical conductivity, moisture content, total organic carbon, soil organic matter, and total hydrocarbon content (THC), prior to contamination. Throughout the experiment, parameters such as temperature, pH, nitrogen, phosphorus, potassium, and total hydrocarbon content (THC) were monitored. Also, microalgae and hydrocarbon-utilizing algae (HUA) were observed using conventional techniques in microbiology. The percentage quantity of THC reduction from the first day of

monitoring was used to estimate the bioremediation. Results revealed the amount of hydrocarbon removed and percentage bioremediation efficiency after 56 days of monitoring with different treatment on the set up was given in a decreasing order as follows: (initial contamination value of 10525mg/kg) Cs+Chl (7700mg/kg; 73.15%) > control (Cs) contaminated without amendment of organisms (6345mg/kg 60.28%) > and Us uncontaminated sediment 1969.96 mg/kg. The total hydrocarbon content (THC) of the treated setup decreased from (10525mg/kg initial contamination value) at the start of bioremediation to Cs+Chl (7700mg/kg: 73.15%) at the end of bioremediation. Throughout the monitoring, the following set ups had the highest counts of microalgae (log10cfu/g): day 0 Us (7.65), day 14 Cs (7.63), day 28 Us (7.70), day 42 Cs+Chl (7.60), and day 56 Cs (7.30). The count showed a high on day 28 (7.70) and a fall on day 56 (7.30). For each setup during the monitoring, the maximum hydrocarbon utilizing algae (Log10cfu/g) count were as follows: day 0 Cs+Chl (5.23) day 14 Cs+Chl (5.30) day 28 Cs (5.23) day 42 Cs+Chl (4.77) > day 56 Cs (5.07). Day 14 saw the highest total, while day 42 saw a decline. The study's findings demonstrated that *Chlorella vulgaris* can break down hydrocarbon components. Hydrocarbons were used more quickly in the *Chlorella vulgaris* setup than in the control.

Maju-Oyovwikowhe and Osayande, (2023) for a duration of 28 days, subjected 50 fingerlings of *Oreochromis niloticus* to a water soluble fraction of Australian crude oil bioconcentration in a laboratory setting. The water soluble fraction (WSF) of crude oil was used in an initial acute toxicity test at various concentrations (25 ml/L, 50 ml/L, 75 ml/L, 100 ml/L, and a control). The LC50 was 28.18 ml/L, and the LT50 for 100 ml/L, 75 ml/L, 50 ml/L, and 25 ml/L, respectively, were 35.5 hours, 63.1 hours, 79.4 hours, and 100 hours. The bioconcentration was calculated on days 4, 7, 14, 21, and 28 using two values (4.4 ml/L and 2.2 ml/L) derived from the LC50. As the exposure period and bioconcentration factor (BCF)

increased, so did the hydrocarbon concentration in the fish tissue and test fluid.Between the two crude oil WSF concentrations, there was a significant difference (F= 6.26 ≥ P=0.0370.05). This suggests that WSF of crude oil bioconcentrates in aquatic species' tissues and may have a negative impact on fish eaters' health. As a result, the author recommended that suitable steps should be taken to ensure effective wastewater treatment technology and prevent oil spills into the Niger Delta's aquatic ecosystem.

Hamouda *et al*. (2023) carried out studies to determine whether green algae *Chlorella vulgaris*, blue-green algae *Synechococcus* sp., and their consortium could grow and break down hydrocarbons like kerosene at various concentrations (0, 0.5, 1, and 1.5%) and whether algal biomasses could be used to make biofuel. The optical density (O.D.) at 600 nm, dry weight, and pigment concentrations like chlorophyll a, b, and carotenoid were used to estimate the algal growth. FT-IR analysis was used to assess the amount of kerosene degradation both before and after the algae and consortium were cultivated. By using GC-MS spectroscopy, the components of the methanol extract were identified. The results show that after ten days of cultivation, the algal consortium from O.D. showed the best growth with 1.5% kerosene, whereas *C. vulgaris* showed the highest dry weight. The algae and consortium were shown to have great efficacy in degrading kerosene by FT-IR. Following a 15-day cultivation period with 1% K, the highest percentage of lipids (32%) was produced by *C. vulgaris*. Undecane was detected in large proportions in C. vulgaris (19.9%), *Synechococcus sp* (82.16%), and the algae consortium (79.51%), according to the GC-MS profile of the methanol extract of the two algae and the consortium. Fatty acid methyl ester was also present in moderate amounts in *Synechococcus sp*. Overall, their findings show that a group of algae can both make biofuels like biodiesel and petroleum-based fuels and absorb and eliminate kerosene from water.

Das and Deka, (2019) performed phycoremediation of hydrocarbons in the water. This was accomplished by separating a native algae species from formation water, identifying it through morphological analysis, and using 18S ribosomal RNA to confirm that the isolate was *Chlorella vulgaris* with a remediation rate of 98.63% petroleum hydrocarbons present in formation water after 14 days of incubation, the algal isolate demonstrated a high biomass productivity of 1.76 gm L^{-1} d^{-1} (specific growth rate: 0.21 d^{-1}, initial inoculum: 1500 mg L^{-1}), suggesting an effective hydrocarbon remediation process. In addition, the hydrocarbon remediation process reduced the formation water's Chemical Oxygen Demand (COD) load by 75% and eliminated all sulfate, allowing it to be safely disposed of or used again as oil well injection water. The current procedure gets over the limitations of external growth, nutrient addition, or dilution that occur with traditional biological treatment, producing a solution for oil field formation water remediation that is both practically relevant and reasonably priced.

CHAPTER THREE

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the University of Benin, Benin city, Edo State, Nigeria. the tests were carried out in the Limnology and Phycology Laboratory of the department of Plant Biology and Biotechnology.

3.2 Test Microalgae

The test microalga were *Monoraphidium concortum* and *Dimorphococcus lunatus.*

3.3 Collection of Test Microalgae

Water samples from algal blooms of various microalgae used for the studies were collected from ditches around medical street, Uselu, Benin city,(Lat long 6.367234706878662°, 5.626601696014404°) and a fish pond at Airport road, Benin city, (Lat, long 6.290207469178526°, 5.586585166076196°)

3.4 Isolation of Pure Culture of Microalgae

Culture of test microalgae were obtained by isolating desired alga and inoculating into a growth medium. Unialgal cultures were obtained after a series of subcultures and the resulting cultures were exposed to microscopic inspection to confirm the algal species in the samples prior to use.

3.5 Botany of Test Algae

3.5.1 *Monoraphidium* is a genus of green algae in the family Selenastraceae. *Monoraphidium* is found free-floating or attached to surfaces in water, or in soils. It is one of the most common types of phytoplankton in freshwater habitats, and has a cosmopolitan distribution

Monoraphidium consists of single cells, which are 2-182 by 1-8 micrometers. The cell is straight to lunate to sigmoid or helically shaped. Cells contain a single nucleus, a single parietal chloroplast and a single pyrenoid lacking a starch sheath (or no pyrenoid at all). Reproduction occurs asexually by autospores.

Taxonomy Classification of *Monoraphidium contortum*

Kingdom: Protista

Division: Chlorophyta

Class: Chlorophyceae

Order: Chlorellales

Family: Scenedesmaceae

Genus: *Monoraphidium*

Species: *Monoraphidium contortum*

3.5.2 ***Dimorphococcus*** is a genus of fresh water green algae in the family Scenedesmaceae. It is found as a component of the phytoplankton of freshwater ponds, lakes, and peat bogs. It is widespread, but usually not very common. *Dimorphococcus* is usually found in small colonies of multiples of four cells, surrounded by a gelatinous mass. Groups of four cells are further attached to each other via mucilaginous strands, which are the remnants of the mother cell wall. Cells are kidney-shaped to heart-shaped, 10–25 μm long and 3–8(–15) μm wide.

Each cell is uninucleate (containing one nucleus) and has one parietal chloroplast each with one or more pyrenoids.

Taxonomy Classification of *Dimorphococcus lunatus*

Kingdom: Protista

Domain: Viridiplantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Scenedesmaceae

Family: Microthamnaceae

Genus: *Dimorphococcus*

Species: *Dimorphococcus lunatus*

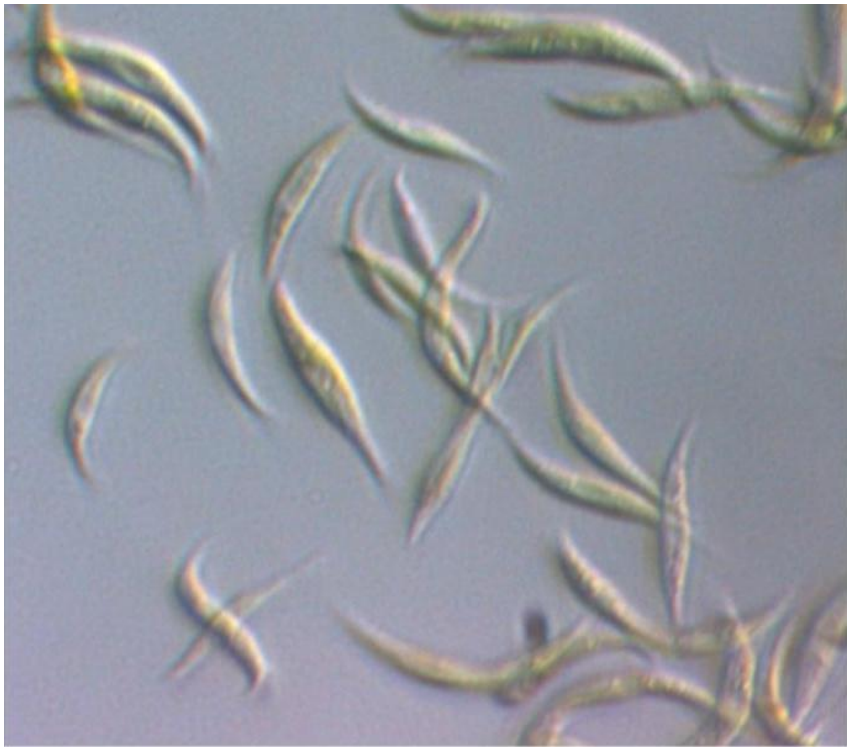

Plate 3.1: A microscopic image of *Monoraphidium concortum*

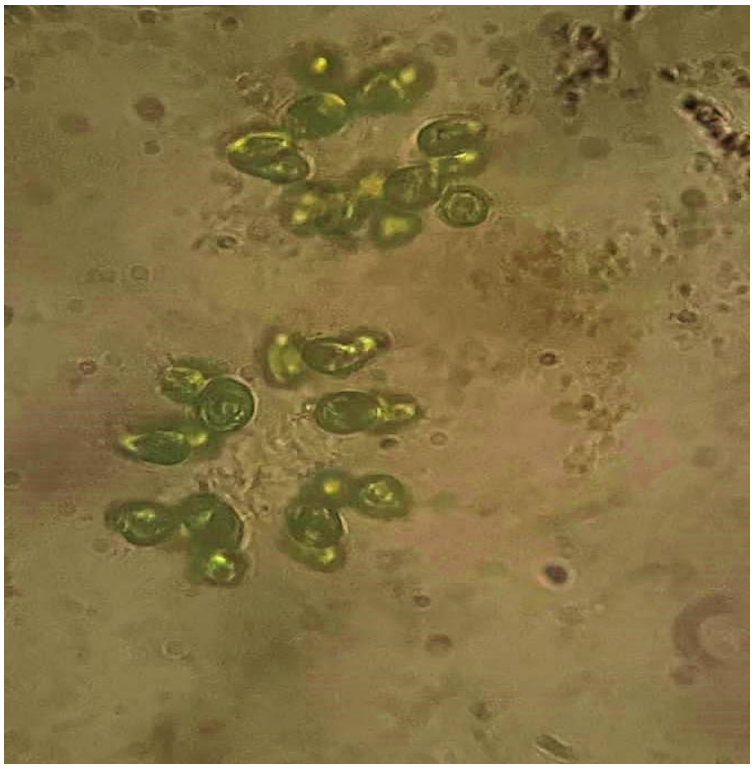

Plate 3.2: A microscopic image of *Dimorphococcus lunatus*

3.6 Preparation of Culture Media

The micro algae species were grown in an artificial medium, Chu modified number 10 medium was used in mixture with the water soluble fraction (WSF) of crude oil. The composition of the modified medium is shown in the table below:

3.8 Culture vessels

The study made use of brand new plastic culture bottles, each with a capacity of 250ml and a height of 6cm. Upon purchase, these brand-new plastic bottles were thoroughly washed using clean tap water, acid washed and rinsed several times with distilled water. Following this procedure, the bottles were left to air dry before they are used. They were then systematically labeled to correspond with the test algae species and the specific water soluble fraction (WSF) concentrations being studied.

3.9 Crude oil

The crude oil was gotten from Oredo OML field of the Nigeria Petroleum Development Company, Benin City, Edo State. The

3.10 Preparation of Water Soluble Fraction(WSF)of crude oil

The process of Ezenweani and Kadiri (2023) was employed in order to get the water soluble fractions of crude oil in 100% stock solution. One (1) part crude oil and nine (9) parts distilled water were combined to create the stock solution. Using a magnetic stirrer hotplate, the mixture was swirled for a period of 24 hours. The aqueous phase was separated and regarded as 100% of the water soluble fractions of crude oil after the solution was left to stand in a separating funnel for an additional 24 hours.

3.11 Preparation of Different Concentrations of Treatment.

Different concentrations of treatment samples were prepar**ed** by adding the volume of stock solution and growth medium as shown in the table below.

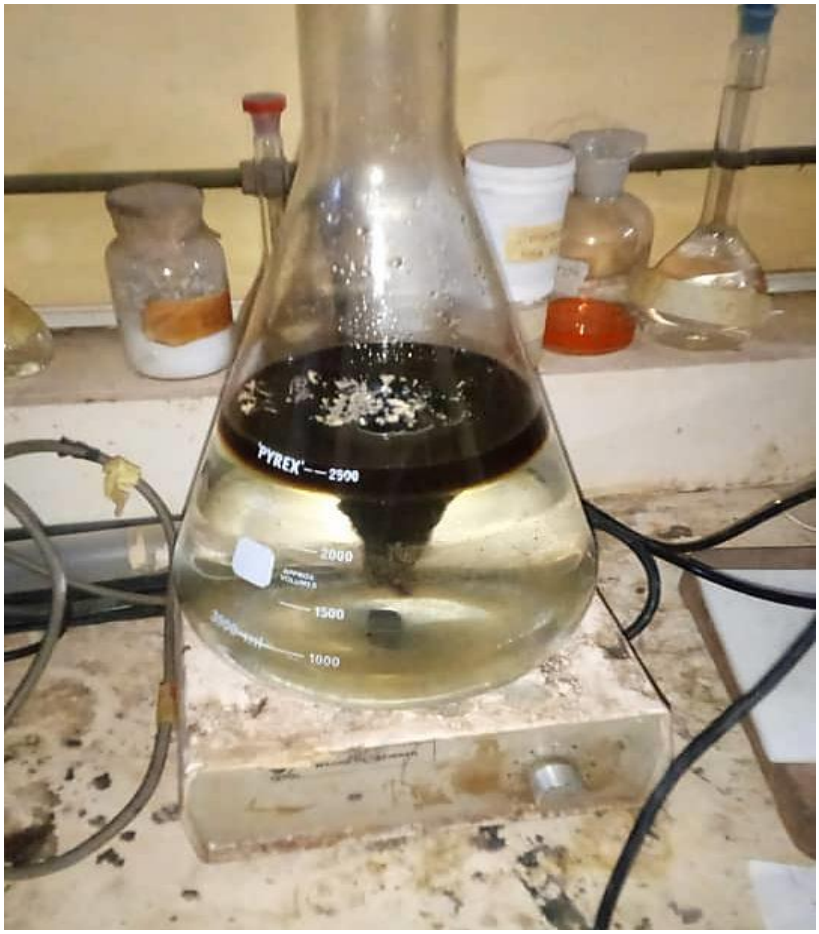

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Plate 3.4: *Monoraphidium contortum* set-up at day 1

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Plate 3.7: *Dimorphococcus lunatus* set-up at day 14

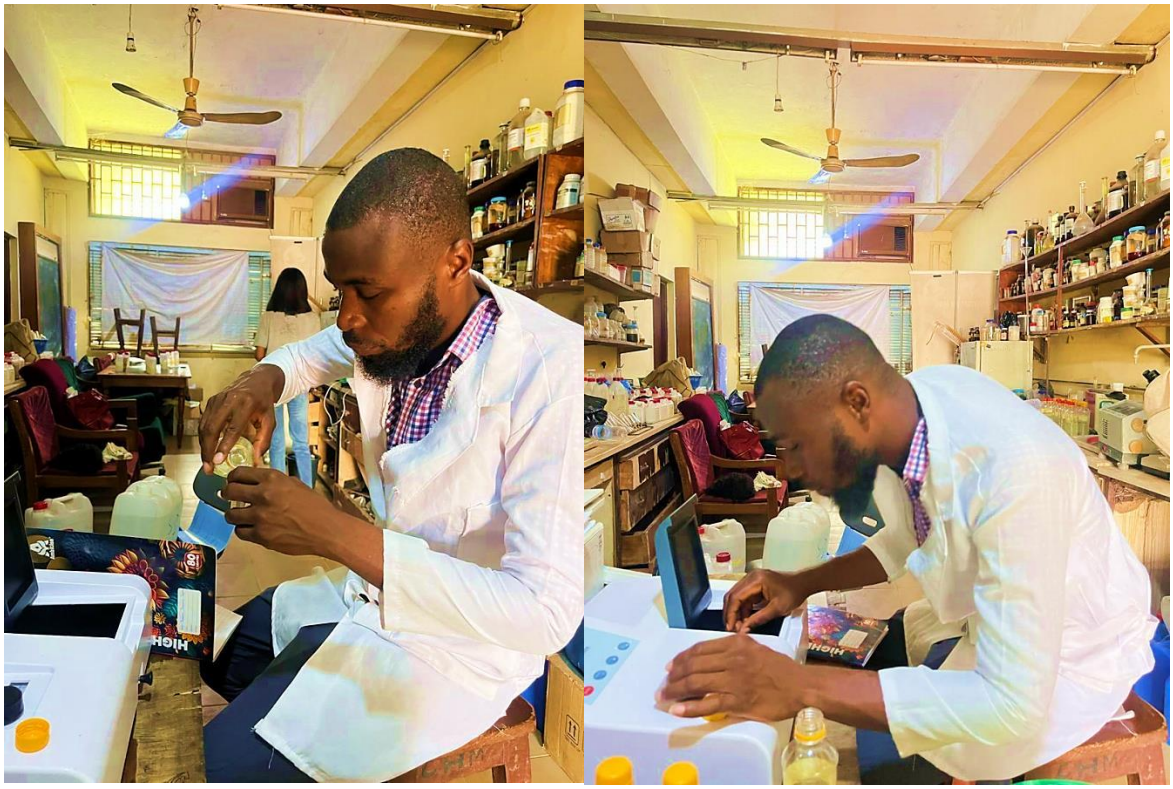

Plate 3.8: Lab work using the UV Spectrophotometer

Percentage inhibition

The formula below was used for the determination of percentage inhibition

$$Percentage\ inhibition\ (\%) = 100 - \frac{measured\ biomass}{Theoritical\ biomass} \times \frac{100}{1}$$

Percentage Yield

This was computed according to the formula below

$$Percentage\ yield\ (\%) = \frac{actual\ yield}{Theoritical\ yield} \times \frac{100}{1}$$

Cumulative Growth

This was computed according to the formula below

$$\text{Cumulative growth rate} = \left(\frac{Ending\ value}{Beginning\ value}\right)^{\frac{1}{n}} - 1$$

Where n = number of periods the growth rate is measured.

Statistical Analysis

The means and standard errors of the data were computed using Microsoft Excel. To determine statistically significant differences between the different treatments, ANOVA was employed using Microsoft Excel 2016.

PHYSICOCHEMICAL PARAMETERS

Total Dissolved Solid (TDS) (mg/L)

A HACH CO150 TDS/Conductivity/Salinity meter was used to measure the total dissolved solid. Following a complete homogenization of the samples, the probe was dipped into the cultures within the 250ml culture vesicle. The readings were taken once the value had stabilized.

Electrical conductivity (µs/cm)

Using a HACH CO150 TDS/Conductivity/Salinity meter, conductivity values were measured. The 250 ml plastic bottles containing the water samples were firmly shaken once more. After that, the probe was quickly plunged into the water samples, and the relevant readings were noted.

Hydrogen Ion Concentration (pH)

This was obtained using a HANNAH field pH meter. The pH meter was dipped into the culture samples and the appropriate values were taken.

CHAPTER FOUR

MATERIALS AND METHODS

3.1 Study Area

This study was carried out in the University of Benin, Benin city, Edo State, Nigeria. the tests were carried out in the Limnology and Phycology Laboratory of the department of Plant Biology and Biotechnology.

3.2 Test Microalgae

The test microalga were *Monoraphidium concortum* and *Dimorphococcus lunatus.*

3.3 Collection of Test Microalgae

Water samples from algal blooms of various microalgae used for the studies were collected from ditches around medical street, Uselu, Benin city,(Lat long 6.367234706878662°, 5.626601696014404°) and a fish pond at Airport road, Benin city, (Lat, long 6.290207469178526°, 5.586585166076196°)

3.4 Isolation of Pure Culture of Microalgae

Culture of test microalgae were obtained by isolating desired alga and inoculating into a growth medium. Unialgal cultures were obtained after a series of subcultures and the resulting cultures were exposed to microscopic inspection to confirm the algal species in the samples prior to use.

3.5 Botany of Test Algae

3.5.1 *Monoraphidium* is a genus of green algae in the family Selenastraceae. *Monoraphidium* is found free-floating or attached to surfaces in water, or in soils. It is one of the most common types of phytoplankton in freshwater habitats, and has a cosmopolitan distribution

Monoraphidium consists of single cells, which are 2-182 by 1-8 micrometers. The cell is straight to lunate to sigmoid or helically shaped. Cells contain a single nucleus, a single parietal chloroplast and a single pyrenoid lacking a starch sheath (or no pyrenoid at all). Reproduction occurs asexually by autospores.

Taxonomy Classification of *Monoraphidium contortum*

Kingdom: Protista

Division: Chlorophyta

Class: Chlorophyceae

Order: Chlorellales

Family: Scenedesmaceae

Genus: *Monoraphidium*

Species: *Monoraphidium contortum*

3.5.2 *Dimorphococcus* is a genus of fresh water green algae in the family Scenedesmaceae. It is found as a component of the phytoplankton of freshwater ponds, lakes, and peat bogs. It is widespread, but usually not very common. *Dimorphococcus* is usually found in small colonies of multiples of four cells, surrounded by a gelatinous mass. Groups of four cells are further attached to each other via mucilaginous strands, which are the remnants of the mother cell wall. Cells are kidney-shaped to heart-shaped, 10–25 μm long and 3–8(–15) μm wide. Each cell is uninucleate (containing one nucleus) and has one parietal chloroplast each with one or more pyrenoids.

Taxonomy Classification of *Dimorphococcus lunatus*

Kingdom: Protista

Domain: Viridiplantae

Division: Chlorophyta

Class: Chlorophyceae

Order: Scenedesmaceae

Family: Microthamnaceae

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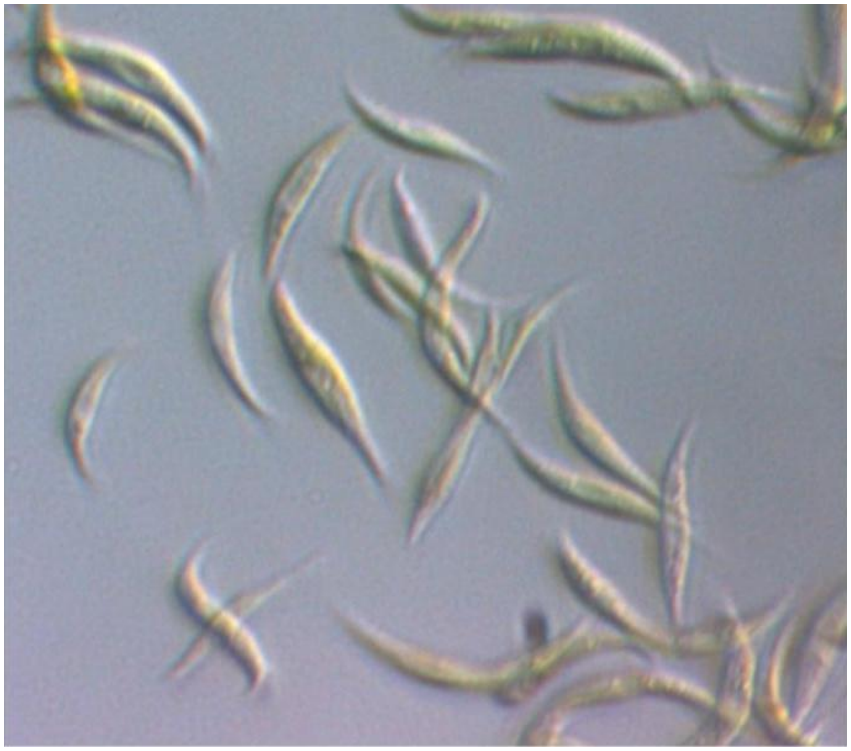

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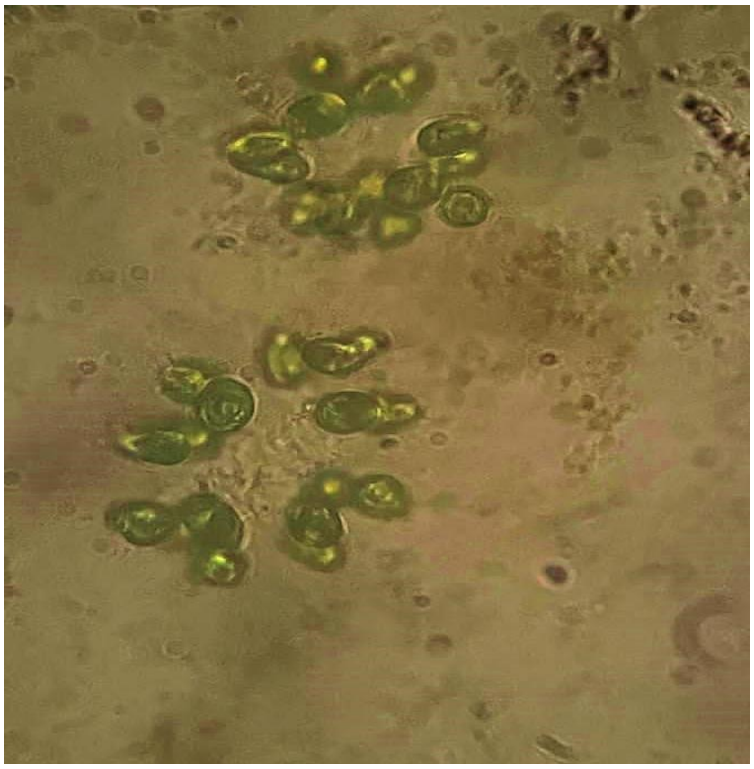

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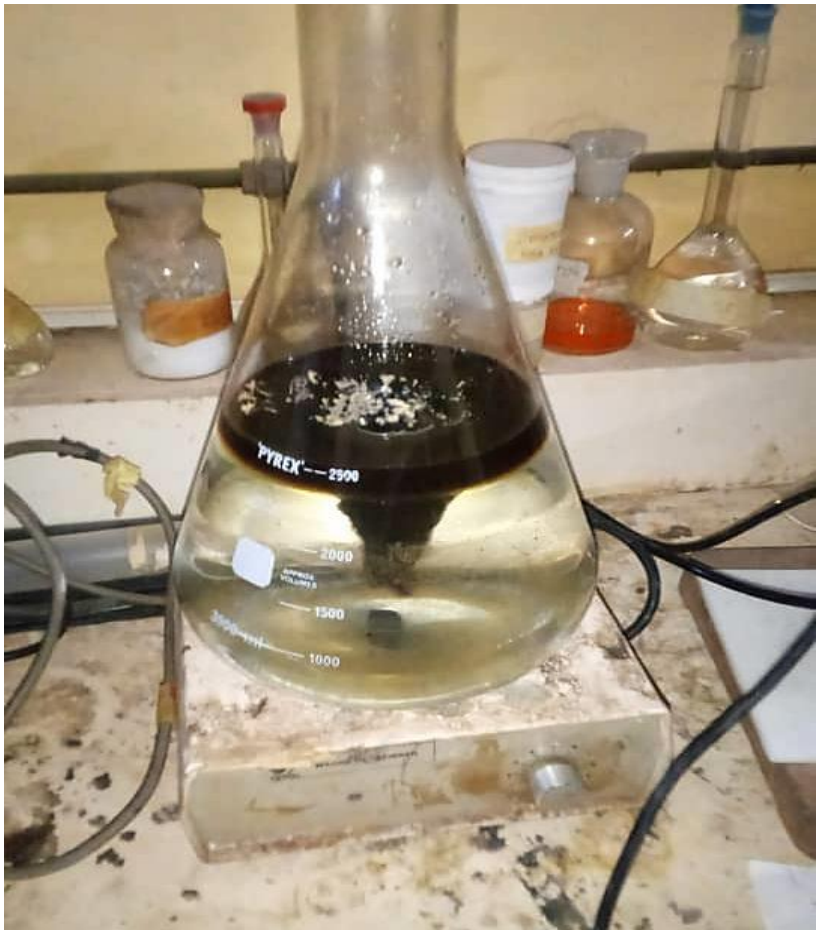

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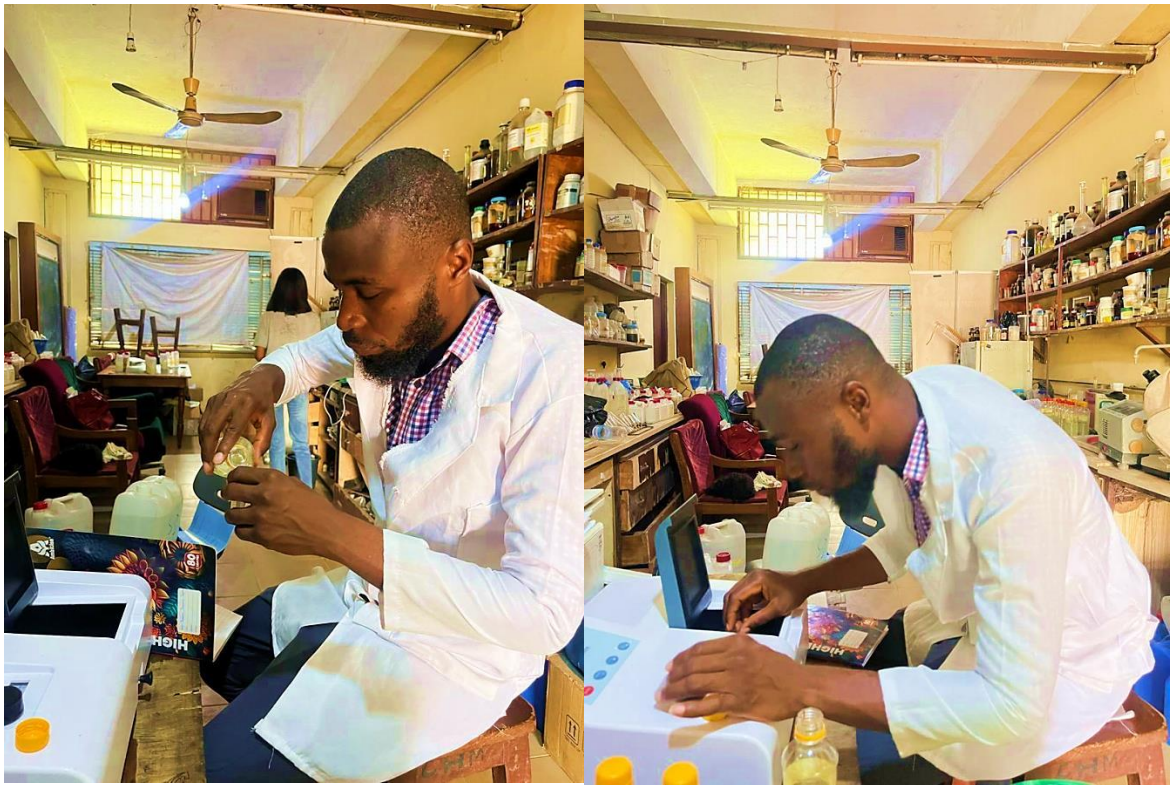

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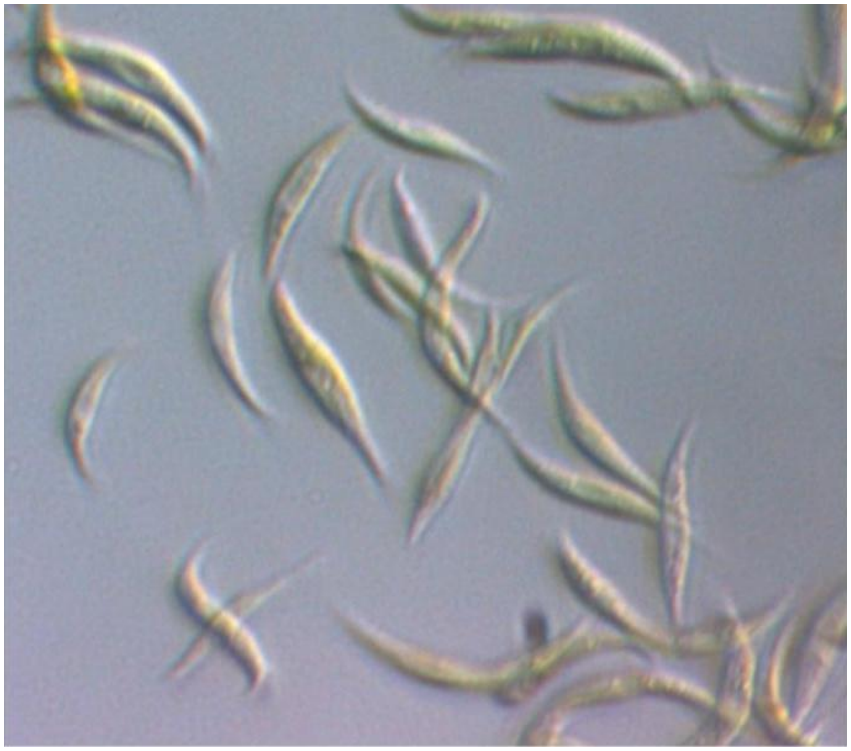

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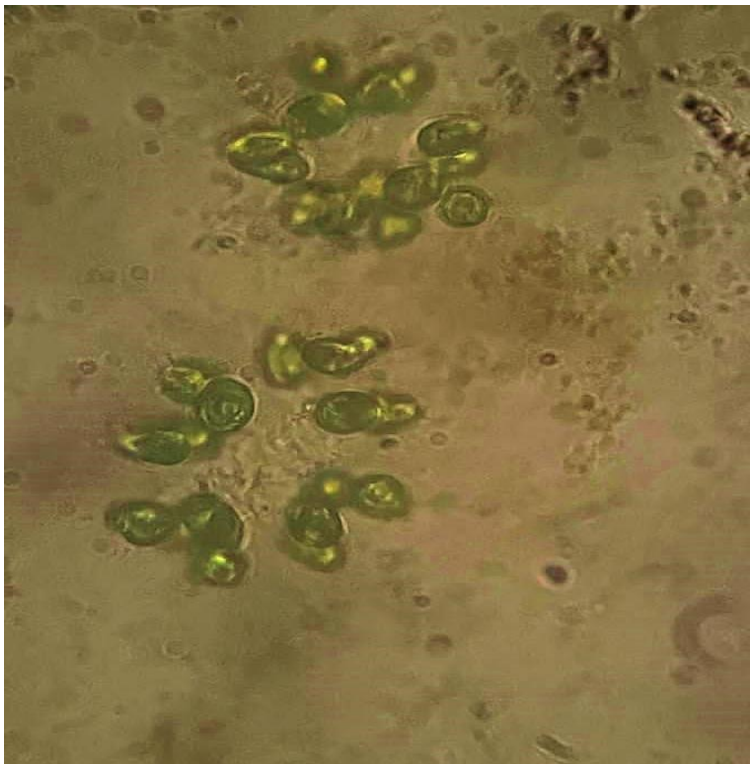

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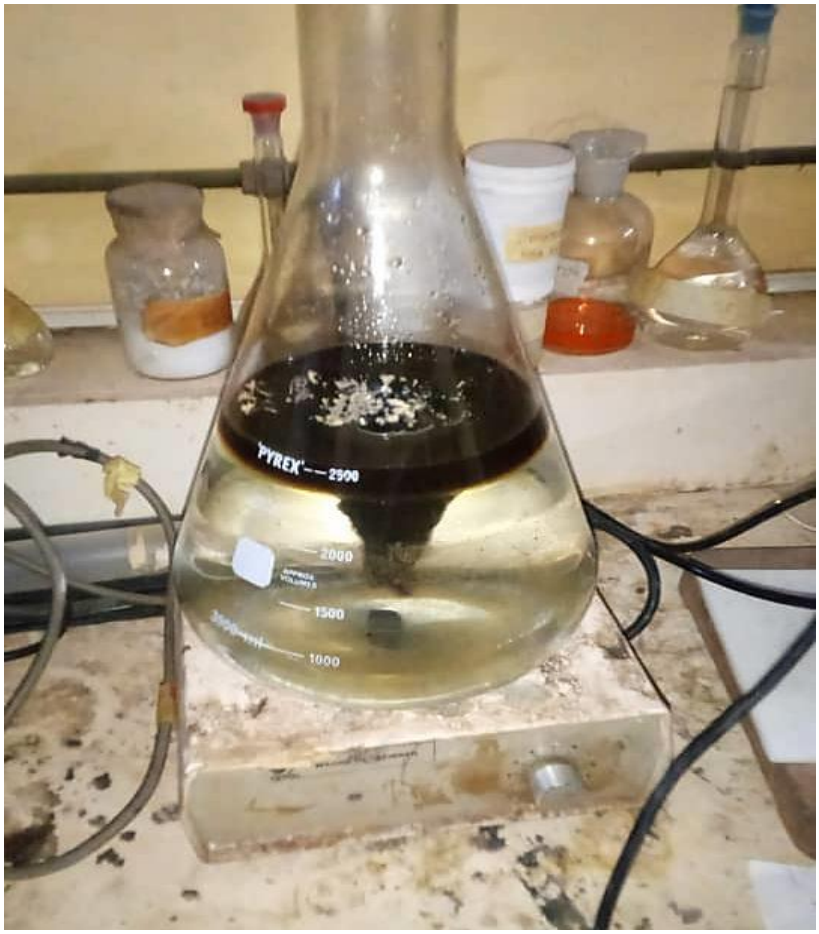

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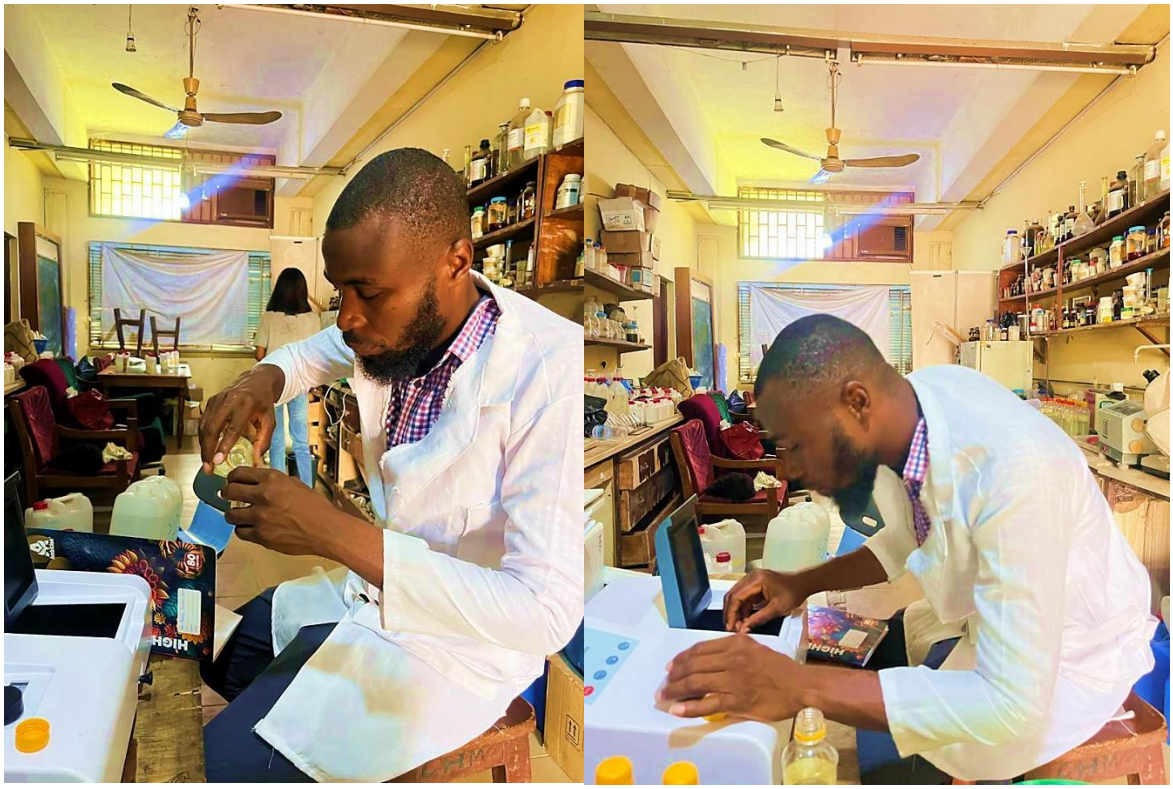

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