INTERNATIONAL JOURNAL OF RESEARCH AND TECHNOPRENEURIAL INNOVATIONS(IJRTI)

Volume 1 Issue 1 (April 2024)

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Laboratory Production and Testing of Biocompost from Water Hyacinth (*EichhorniaCrassipes*) for Bioremediation of Crude Oil-Impacted Agricultural Soil

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To cite this article:
Abu, G.O., Vincent-Akpu, I.F., Akwukwaegbu, R.N., Stanley. H.O., Udume, O.A. Laboratory Production and Testing of Biocompost from Water Hyacinth (*Eichhornia Crassipes*) for Bioremediation of Crude Oil-Impacted Agricultural Soil. *International Journal of Research and Technopreneurial Innovations* 2024; 1(1): 85-91

Keywords:
250 Litre metal bioreactor, water hyacinth compost, crude oil-impacted agricultural soil, bioremediation, bio-technopreneurial product

ABSTRACT
The water hyacinth (*Eichhornia crassipes*) is an invasive aquatic weed that poses challenges in water bodies. The goal was to produce a biofertilizer (compost) from water hyacinth using a locally fabricated metal bioreactor, and test the compost on the bioremediation of crude oil-impacted soil. Water hyacinth was hand-picked and loaded into canoes, during the day time, at the New Calabar River, Choba, Rivers State. Freshly harvested plants were sun and air dried at ambient outdoor conditions and then shredded using a standard cutter. The samples were fed into a 250L bioreactor at half capacity loading. The samples were irrigated to 60% v/w with a suspension of a consortium of microbes isolated from cow rumen liquid. The bioreactor, designed with baffles, was operated by mechanical turning to effect tumbling and mixing. The material was composted for sixty days. The resulting compost was ground into coarse powder using a motorized industrial grinding mill. A final grinding with a manual grinder was done to improve the solubility. The water hyacinth compost (WHC) finished product was used to effect bioremediation of crude oil-impacted agricultural soil under laboratory conditions. Results reveal 71% reduction in Total Petroleum Hydrocarbon TPH in the compost-treated set ups; this compares with 12% in the untreated set ups. There was an increase in the microbial load in the WHC-treated set ups where the microbial counts ranged from $3.3\pm3 \times10^7$ to $7.0\pm3 \times10^7$cfu/g. The procedure described here gave a microbial bio-technopreneurial produce that is useful in remediating hydrocarbon-impacted farm lands.

1. INTRODUCTION

In water bodies, controlling the invasive aquatic weed known as water hyacinth (*Eichhornia* *crassipes*) can be difficult. It has a dark green, rounded leaves which may grow to a height of 65 cm tall as floating waterweeds. Their leaves have a circumference of 5 to 10 cm. The youngest plants possess puffy, rounded, soft leaves branches, whereas older varieties possess a longer time, spongier leaf stalks. A straight stalk holds up a single increase of eight to fifteen flowers. The flowers measure 4-6 cm in length and 3.5–5 cm in width. They have a pale dark purple colour that has a more intense blue-purple at the middle.Fruit capsular structures can hold as many as 300 seeds and are 10–15 mm long, egg-shaped, 0.5–1.5 mm long and the broad root plant are fibrous.

The adverse consequences of crude oil spills were not given much thought prior to the early I960s.The 1989 Exxon Valdez petroleum spill on the coast of Alaska and the subsequent 1991 huge spill of crude oil following the Gulf War, however, garnered the worldwide interest.As a result, there was a rise in ecological research and the implementation of various national and international control measures, including the 1991 Oil Spill Actof United States of America (USA). Risks associated with environmental and crude oil pollution persisted despite all these control devices. Massive unintentional spills of oil release a substantial amount of pollutants into the environment worldwide [1]. Apart from the aforementioned catastrophic events, various environmental issues can also arise as a result of the existence of hazardous substances in small amounts to minimal continuous flows, offshore exploration, tank cleaning, and various connected operations, such as the 2023 spill in Eleme LGA, Rivers state (Business Day, June 19, 2023; Ignatius Chukwu). Restoration of the surroundings affected by these contaminants is essential because the harmful substances found in petroleum products include a wide range of hydrocarbons, nitrogen-sulfur-oxygen (NSO) compounds, and metals. These pollutants can have both acute and long-term effects on biodiversity [2]. Furthermore, because of higher exposure and ensuing absorption of hazardous petroleum-based substances to agricultural produce, soil that has been contaminated may present risks for human residence as well as the development of agricultural steeds [3]. Because of the numerous catastrophes caused by crude oil exploration and development activities, which have destroyed agricultural land and other ecosystem services, this study is pertinent in the Nigerian Niger Delta area. Oil-contaminated places are being cleaned up using techniques for remediation like soil washing, vapour extraction, thermal desorption, incineration, and solidification; nevertheless, these methods are labor-intensive, unsettling and fairly costly [4]. Biochemical bioremediation processes are technologies based on the science of biodegradation (in case of organic pollutants), which involve natural introduction of microorganisms - augmentation or the addition of nutrients or fertilizers to increase or boost the population of microorganisms - biostimulation. The microbes have thus, been investigated as a potential remediation agent. The contamination phenomena caused by hazardous or toxic crude oil have had an effect on the natural world.When oil from production facilities spills onto the ground, the greasy fractions slowly seep into the soil and are broken down by soil-dwelling microorganisms, while the lighter petroleum parts vanish.As a result, over time, those native soil microbes facilitate the biological breakdown of the petroleum product through a natural attenuation process[5]. Thus, the three ways of biochemical bioremediation currently in use are biological enhancement, biostimulation, and natural attenuation. For better results, these methods depend on the fundamental principles of raising soil organic matter contents and lowering soil pH. The addition of nutrients like phosphates and nitrates promotes the growth of hydrocarbonoclastic microbes, this is also known as biostimulation which is essential in boosting the effectiveness of bioremediation. Petroleum-based compounds (which are essentially organic), are an important cause of pollution in both aquatic and terrestrial ecosystems, in spite of the fact that hydrocarbons from petroleum have greatly benefited human economic growth and development.Compared to other chemical and physical techniques, bioremediation is thought to be safe, economical, and an ecologically conscious cleanup technology because it usually results in a full mineralization of the hydrocarbons and does not result in additional pollution.

Bioremediation has been described as "hope for the Nigerian oil pollution"[6].

The purpose of this study is to produce a microbial technopreneurial biocompost from water hyacinth that is adjudged to be environmentally friendly to be used in the recovery of crude oil impacted farmlands and would promote a cost-effective removal of the plant from water bodies where it has become an economic menace.

2. MATERIALS AND METHODS

2.1 *Collection and Preparation of Raw Materials*

The water hyacinth hydrophyte (Plate 1) grows wildly on water bodies in the Niger Delta.

Plate 1. Water hyacinth

Water hyacinth was harvested from the New Calabar River in Rivers State, by hand picking the plants and loading into canoes, during the day time. The freshly harvested plants were dipped into the surrounding water to remove all loosely attached materials from the leaves, stems and the roots, then air and sun-dried at ambient temperatures. The dried plants were shredded into pieces using a standard cutter.

The Bioreactor

The 250 liter, cylindrical bioreactor was fabricated from galvanized tin plates. It is fitted with baffles attached to a central pipe that conveys air into the chamber. The baffles create a mixing effect. The cylindrical structure was rotated using a pulley system with a chain, giving a mechanical turning, tumbling and mixing effect (Plate 2). It is housed in a rectangular structure with a corrugated roof that provides shed from direct sun and rain. The shed is located within the perimeter fence of the University of Port Harcourt Ecological Green House Facility, which is run by the Department of Plant Science and Biotechnology (PSB).

Plate 2. The 250L bioreactor Actual, LEFT and Computer aided design (CAD), RIGHT.

Operation of the Composting Bioreactor

The shredded material was fed into the 250L metal bioreactor operated at half the total capacity. The shredded material inside the bioreactor was irrigated to 60% (v/w) with a suspension of a consortium of microbes isolated from cow rumen liquid. Composting was carried out for sixty days, with intermittent mechanical turning using the chain and pulley. The resulting compost was ground into coarse powder using a small, motorized industrial grinding mill. A final grinding with a mechanical manual grinder was done to improve the solubility of the finished product.

Bioremediation Set Up: Biocell Construction and Preparation

Biocells, measuring (L)50cm x (B)50cm and 15cm depth were constructed from disused timber and underlain with medium thickness, solvent resistant polyethylene liners/sheets (Plate 3 Biocells). Agricultural soil sample (topsoil 0-15cm deep) was collected from a farm at the University of Port Harcourt, according to standard procedures [7]. The soil sample was analyzed for physicochemical properties that included: nitrogen, total organic carbon (TOC), ash content, phosphate, potassium, nitrate, sodium, calcium, magnesium, organic matter and pH. The physicochemical analyses were done on the soil using standard methods [8].

Plate 3. Biocells each 50cm x 50cm and 15cm deep under treatment.

Fifteen kilograms (15kg) of the agricultural soil were weighed out and mixed with 5ml of (Bonny Light) crude oil sample, the mixture of the soil and crude oil was thoroughly homogenized to ensure even distribution of the crude oil. Aliquots of the soil and crude oil mixture were sent to the laboratory to determine the loading of the crude oil (ppm) in the soil, according to DPR (NUPRC) standards. The agricultural soil was spiked crude oil for 10 minutes and later amended with varying quantities of water hyacinth compost (WHC) representing different treatments in biocells. A set of three biocells containing 15kg agricultural soil each received 50g of WHC/kg of soil, another set of three biocells each received 100g of WHC/kg of soil, another set of three biocells each received 200g WHC/kg of soil and another set of three biocells each received 400g WHC/kg of soil. The biocells were turned every three days for proper mixing; the biocells were also irrigated with 1litre of tap water per cell, every three days. The treatment was carried out for 42 days. Samples were collected at seven days intervals for a period of 42 days.

The parameters assayed were pH, electrical conductivity, nitrate, total organic carbon (TOC), phosphate, iron, cadmium, lead, potassium, copper, nickel and TPH. Microbiological analysis were done to determine the total heterotrophic bacterial count over time,

2.2. Equations

Bioremediation is driven by nutrient limitation following the Monod kinetic model:

$$\mu = \frac{\mu max.\ S}{Ks + S} \qquad (1)$$

Where μ = specific growth rate (g/g.h), h^{-1}
μmax = maximum specific growth rate
S = limiting substrate concentration
Ks = substrate utilization constant (Similar to Km)

3. RESULTS AND DISCUSSION

3.1. Products from Water hyacinth Plant.

The bioreactor was effective, and biocompost produced had a dark-brown coloration. Both the ground and the unground compost were useful. The final mechanical hand grinding gave a product that was completely soluble in water. This is significant because, it means the final product can be used as powder/solid and as liquid forms. For applications in soil, either the solid or the liquid would be beneficial. For applications in polluted aquatic environment/ecosystems, the liquid form would be beneficial (unpublished data).

3.2 Outcomes of Testing of the Water Hyacinth Compost

The results of the background values of soil parameters used in the bioremediation set up are shown in Table 1. The water hyacinth contained low level of total petroleum hydrocarbon (TPH); it has low levels of toxic heavy metals. This makes the plant a good raw material for the production of bioremediation supplements. The uncontaminated soil has low level of TPH; it is low in toxic heavy metals. This makes the soil a good raw material for bioremediation testing. The contaminated soil at Day 0 has high level of TPH. This shows that the procedure for spiking the agricultural soil was appropriate. The TPH values (22,395.072 mg/kg) met the DPR (NUPRC) Intervention value of 5,000mg/kg). This shows the stringency and validity of the bioremediation experiment. The contaminated soil plus WHC at Day 0 showed reduced level of TPH. The reduction in the TPH values in the set up containing the WHC, on Day 0, is attributed to the natural attenuation process of physical dilution. There are about eight natural attenuation processes; dilution (v/v, w/w) is one of them. Clean, uncontaminated soil can be mixed with contaminated soil to effect dilution. The addition of the WHC at 100g per kilogram of clean soil led to the reduction of the TPH on Day 0. This is remediation by enhanced natural attenuation (RENA). The electrical conductivity values are appropriate for bioremediation set up. The nutrient regime of nitrates and phosphates in the WHC is suitable for bioremediation

The results of the bioremediation testing are shown in Table 2. There was a reduction in TPH with time. The reduction is more drastic in the WHC-treated set up than in the control. There are natural attenuation processes that can bring about such reductions, in a typical Niger Delta set up like this; they include volatilization/evaporation, dilution, photooxidation and biodegradation [9]. The Niger Delta region has ample moisture, temperatures favor mesophilic microorganisms like bacteria and fungi, there is adequate solar radiation and there is a rich population of microbiota. The increase in the total heterotrophic bacterial population (Table 2) and the decrease in the TPH concentration (Table 2) over the period of observation suggest that bioremediation occurred as a result of biostimulation.

Nutrient limitation can occur among different nutrients such as nitrates (supplying nitrogen) and phosphates (supplying phosphorus). This would be reflected in the C: N, C: P ratios; the favourable regimes/ratios for bioremediation are 100:1 and 10:1respectively. In this way the pollutant (crude oil or petroleum hydrocarbon) is removed by microbes, thereby detoxifying the impacted environment.

Other studies [8] have shown that bioremediation by biostimulation is more effective than other modes of remediation of hydrocarbon-impacted environmental media in the Niger Delta.

Table 1. Background values of physicochemical parameters of Agricultural soil used in the Bioremediation Testing

Parameters	Uncontaminated soil	Contaminated soil(Day 0)	Water hyacinth	Contaminated + 100gWHC/kg soil (Day 0)
pH	6.80	5.20	7.4	4.97
Total organic carbon (TOC)%	11.78	1.50	9.87	2.70

Lead (mg/kg)	0.90	1.90	2.35	1.40
Total petroleum Hydrocarbon(TPH) mg/kg	243.073	22395.072	150.879	9864.978
Nitrogen %	7.90	9.56	2.87	10.67
Ash Content %	85.60	89.50	7.65	91.02
Electrical conductivity (µs/cm)	116.12	126.00	134.5	111.00
Nitrate (NO_3) mg/kg	1.28	1.28	5.60	1.20
Phosphate (PO_4) mg/kg	3.10	1.30	95.37	3.00
Potassium (mg/kg)	0.90	0.50	4.68	1.20
Zinc	0.02	0,03	0.03	0.04

Table 2. *Bioremediation of crude oil-contaminated soil with water hyacinth compost*

Duration of treatment in days Effect of Treatment (mg/kg)				
Biocell Treatment	**Day 0,**	**Day 7**	**Day 21**	**Day 42**
Soil+Crude oil + 100gWHC/kg of soil	9,865	5,447	4,414	2,833
soil+Crude oil (Control)	22,395	21,585	20,769	1 9,800
Microbial Load (THBC) cfu/g for WHC-treated cell	$3.3\pm3x10^7$	$4.3\pm5x10^7$	$5.0\pm10x10^7$	7. $7.0\pm3x10^7$

4. CONCLUSION

The long term strategy or goal of bioremediation technology is to develop a cost effective and environmentally friendly approach. The water hyacinth biocompost apart from being cost effective is also environmentally friendly; it is low in toxic substances. The production process of the biocompost already has shown potential for scale-up. There is also potential for automation. The results of the bioremediation experiments have shown that the biocompost produced is effective in supplying limiting nutrients necessary for the bioremediation of crude oil contaminated soil. The decrease in soil total petroleum hydrocarbon (TPH), is accompanied by increase in the total viable count (TVC) of the total heterotrophic bacterial count (in cfu/g) of the impacted soil. The effect of time played a noticeable role in aiding the remediation of the crude oil-impacted soil. These environmental-friendly remedial actions are geared towards sustainable development in the Niger Delta. This study has shown a procedure for converting the menacing *Eichhornia crassipes*plant into biocompost as a suitable organic amendment for bioremediation and conditioning of hydrocarbon-impacted soil and the opportunity to derive economic and environmental benefits from water hyacinth, thereby minimizing any negative impacts and potential reduction of eco-toxicity arising from the continued use of inorganic fertilizers. The microbial biocompost from water hyacinth is a technopreneurial product; it has potential for scale-up and large-scale production through automation that can make use of artificial intelligence (AI), as the trending hallmark of innovation

Acknowledgements

Funding: This work was supported by Tertiary Education Trust Fund (TETFUND), Nigeria [NRF/SETI/NRT/00031]

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