FUNGAL DEGRADATION OF VEGETABLE OIL (GROUNDNUT OIL) ON THE SURFACE OF CLAY

Supported by tetfund TERTIARY EDUCATION TRUST FUND

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Received: March 17, 2019 **Accepted:** June 20, 2019

Abstract: The study of fungal degradation of vegetable oil on the surface of the clay was carried out in Kogi State University, Anyigba, Kogi State. The soil sample was collected in Ankpa, Kogi State. The soil was contaminated to determine the presence of fungi. The physio-chemical properties of the soil were also determined. The fungi isolated were identified as *Aurebasidium sp*, *Paecilomycetes sp* and *Aspergillus sp*. The isolates were cultured on a potato dextrose agar to test their ability to grow. The vegetable oil biodegradation was determined by gravimetric analysis. From the analysis it was found that *Aspergillus sp* (79%) and *Paecilomycetes spp* (61%) used the oil efficiently than *Aurebasidium spp* having 59%. The results suggest that the consortium of the isolates could be used in remediating vegetable oil-contaminated soils.

Keywords: *Aurebasidium sp, Paecilomycetes sp, Aspergillus spp,* and consortium

Introduction

Vegetable oils are oils gotten from plants and fruits such as groundnut, palm nut, sunflower, soybean, coconut, rapeseed, canola, olive, castor and corn. Like mineral oils, vegetable oils can vary significantly and when released to the marine environment for instance will behave differently according to their individual characteristics. These characteristics depend on factors at the time of cultivation of the feed stock for example, climate; degree of processing; type and specific nature of the oil, sea state and weather conditions at the time of the spill. In many cases, the influence of vegetable oil characteristics and its behavior in the environment is not well-studied or understood. Consequently, the behavior and fate of specific vegetable oils is somewhat more difficult to predict than that of mineral oils. Vegetable oils comprised primarily of triacylglycerol's or fatty acids, which, in their fresh state, may be broken down by bacteria, fungi and yeast. This breakdown is as a result of lipases, a class of hydrolases primarily responsible for hydrolysis of acylglycerides. Many microorganisms such as bacteria, yeasts, molds and a few protozoa are known to secrete lipases for the digestion of lipid materials (Nagarajan, 2012). These organisms produce veritable development sources of lipases with different enzymological properties and specificities but molds are known to be more potent lipase producer (Choo *et al.*, 1998). Lipase producers have been isolated mainly from soil or spoiled food material that contains vegetable oil. Lipase production from a variety of bacteria, fungi and actinomycetes has been reported in several works (Kulkarni *et al.*, 2002). Vegetable oil spills are becoming more common and are potentially more challenging than hydrocarbon spills. One spill can spread fast between bodies of water and the land, coming in contact with humans, plants and animals. Microorganisms are therefore useful hi protecting the environment. It is significant to isolate microbes of high potential for the biodegradation of vegetable oil.

Vegetable oil like coconut oil consists of hydrocarbon called triglycerides. The triglyceride composition is unique for every plant's oil. Triglyceride molecules consist of glycerol bound to three fatty acid molecules. Different plant oils have different compositions and there is no natural oil that consists of just one triglyceride type. The different triglycerides have different properties and their coagulation point differ. That is why some triglycerides coagulate before others as they are cooled. The oxygen content is the most important difference in chemical composition between fossil oils and vegetable oils. Another important difference is that vegetable oils are polar compounds and have high lubricity feature for "boundary" lubrication of fuel injection pumps and injector nozzle units.

Biodegradation is the process by which organic substances are broken down by the enzymes produced by living organisms. The term is often used in relation to ecology, waste management and environmental remediation (bioremediation). Organic material can be degraded aerobically, with oxygen or anaerobically, without oxygen. A term related to biodegradation is biomineralisation, in which organic matter is changed into minerals (Diaz, 2008). By definition, biodegradation is the chemical transformation of a substance caused by organisms or their enzymes. There are two major types of biodegradation – Primary Biodegradation, which refers to the modification of a substance by microorganisms such that a change is caused in some specific measurable property of the compound (US Army Corps of Engineers, 1999). Thus, mineralization is the true aim. When this happens it is referred to as Ultimate or Complete Biodegradation; which is the degradation achieved when a substance is totally utilized by microorganisms resulting in the production of carbon dioxide, methane, water, mineral salts, and new microbial cellular constituents (US Army Corps of Engineers, 1999).

Materials and Methods

Materials

Glassware: Conical flask, petri-dishes, beakers, test-tubes and hot air oven

Media: PDA (Potato Dextrose Agar)

Sterilization of media and glass wares

All glass wares were sterilized at 160^0C for 1 h in a hot air oven

Sample collection and preparation

Clay soil sample was collected from Ankpa, Kogi state. And the groundnut oil used was gotten from Kano state, Nigeria.

Sample preparation

Clay soil sample was collected from Ankpa, Kogi State. The soil was contaminated with groundnut oil (150:50 w/w) and allowed to stand for 3 days; this was done to allow the indigenous fungi to acclimatize.

Preparation of media

The media used was potato Dextrose Agar and it was prepared according to the manufacturer specification. 40 g of the powder was dissolved into 1000 ml of distilled water. The mixture was thoroughly shaken and allowed to boil. It was sterilized by autoclaving at 121°C for 15 min. After the sterilization, it was allowed to cool before dispensing into petri-dishes.

Isolation and identification of fungal from groundnut oil contaminated clay soil

1 g of oil- contaminated soil was weighed and added to 9ml of sterile distilled water to make 1:10 dilution and it was shaken properly to give homogeneous mixture. Serial dilution of the oil-contaminated soil sample was prepared as 10^{-1}, 10^{-2}, 10^{-3}, 10^{-4}, 10^{-5}, 10^{-6}, 10^{-7} 10^{-8}. 1 ml was taken from dilution 10^{-3}, 10^{-5} and 10^{-7}, respectively into separate sterilized petri dishes containing potato dextrose agar media supplemented with streptomycin to inhibit bacteria growth.

All the plates were incubated for 5 days. All the plates were incubated for 5 days. Morphological appearances of the fungal growths on the plates were observed and recorded. Distinct colonies were sub-cultured to obtain pure fungi isolates. They were maintained on potato dextrose agar plates and stored in refrigerator for further study.

All the fungal growth on culture plates were observed based on morphological appearances or characteristics and microscopic examination using a light microscope (Bennett, 2010).

Preparation of inoculum

A pure culture of each of the isolates was grown for 5 days on potato dextrose agar supplemented with streptomycin to inhibit bacteria growth. One disk of agar and mycelium of each isolates were aseptically introduced into the petri dish containing PDA using a sterile cork borer.

Microbial biodegradation test

The mineral salt medium (MSM) was modified from (Bharati p et al.2012). The composition of the medium was: K_2HPO_4 : 1.0 g, NH_4NO_3 : 1.0 g, $MgSO_4.7H_2O$: 0.2 g, $CaCl_2.2H_2O$: 0.1 g, NaCl : 0.1 g, $FeCl_3.6H_2O$: 0.01 g, pH : 7.0 and D/W : 1000 ml

The medium was autoclaved at 121^0C for 15 min, 9 ml of MSM plus 1 ml of groundnut oil was prepared in four test tubes. Three (3) test tubes contained an agar plug of the isolated fungi species and the forth was left as control. They were vigorously shaken and the absorbance was taken at 520 nm. Subsequently, the absorbance was checked at 3-day intervals to identify the isolates with the highest oil utilization rates. The fungi isolates with the highest utilization rates were subsequently used for the degradation studies.

Biodegradation potentials of the isolates

The rates and total extent of groundnut oil utilization by fungal isolates were determined using the gravimetric analysis method of Ijah and Antai (2003). 100 ml of MSM medium was poured into four conical flasks each respectively and 2 ml of oil was added to the medium, three (3) conical flask contained the isolated fungi species and the forth flask left as control. The flasks were shaken at 120RPM for 2 h daily for 25 days. After 25 days the residual oil in each flask was extracted using diethyl ether. The amount of degraded groundnut oil was calculated using the formula:

Amount of vegetable oil degraded =

$$\frac{weight\ of\ vegetable oil\ (control) - weight\ of\ vegetable(\text{deg}raded)}{weight\ of\ vegetable oil\ (control)}\ x\ 100$$

Physicochemical analysis of oil contaminated clay sample

P^H determination

Oil contaminated clay soil pH was determined according to IITA (1979). 20 g of air dried clay (passed through 2 mm sieve) was weighed into a 50 ml beaker. 20 ml of distilled water was added and allowed to stand for 30 min. and stirred occasionally with a glass rod. The pH meter was calibrated with buffer of pH 7.0 before use. The electrode of the pH meter was inserted into the partly settled suspension and the reading on the pH meter was noted and recorded accordingly.

Determination of moisture content

Moisture content of the oil contaminated clay soil was determined using the method of IITA (1979). An empty container was weighed (W_0) and 2 g of clay content was added and weighed again (W_1). The Samples were dried in hot air oven at 105 - 110°C for 24 h. until constant weight is achieved (W_2). Both the container and the dried samples were reweighed again. The moisture content was calculated as:

$$\%\ moisture\ oil = \frac{W_2 - W_0}{W_1 - W_2}\ x\ 100$$

Soil particle size determination

50 g of air dried soil sample which passed through a 2 mm sieve was weighed into 100 ml beaker, and 50 ml of 5% sodium hex metaphosphate was added to the beaker, the mixture was stirred for 3 min with glass rod, the mixture was then transferred into 1 L cylinder and distilled water was added to make to the mark. The top of the cylinder was covered with hand and inverted for several times until all the particles are set on motion, and the hydrometer was inserted into the suspension, after 40 seconds, the hydrometer and the thermometer readings were taken as H1 and T1, respectively. After the next 3 hours the new hydrometer and thermometer readings was taken as H2 and T2, respectively. the first reading measure the % sand in suspension while second readings measures the % clay and % silt is determine by subtracting sum of % sand and clay from 100%.

Sand = 100.0–Hl+0.3 (T1 -20)-2.0)2

Clay= H2 +0.3 (T2 – 20) - 2.00

Soil = 100.0 - (% sand + % clay)

Organic carbon determination

One (1 g) of finely grinded soil sample was weighed in duplicate into 250 ml conical flask. 10 ml of 0.16M $K_2Cr_2O_7$ was added, followed by 20 ml H_2SO_4 rapidly. The solution was swirled vigorously for 1 minute and was allowed to stand for 30 minutes. 7-8 drop of diphenylamine indicator was added and titrated against 0.5N Iron (II) sulphate. As the end point is approached, the solution shall give a greenish coloration at the end point; the ferrous sulphate was added in drops until the color change from green to brownish red. The blank titration was made in the same manner but without the soil and result shall be calculated as shown below:

%ORGANIC CARBON = organic matter

ORGANIC MATTER = %Organic Carbon x 1.724

Determination of the nitrogen content of the clay sample

1 g of 0.5 mm sieved soil sample was weighed into 250 ml micro kjeldahl boiling tube, 5 ml of concentrated H_2SO_4 and 1 g mixture of catalyst was added to each sample. The tube and it content was heated on the digestion stand until the solution become clear. The flask was removed from the digestion chamber and allowed to cool and 10 ml of distilled water was added to the content. The blank digestion was carried out the same way. Micro kjeldahl distillation apparatus was setup. 10 ml of 2% boric acid containg 5 drops of bromocresol green/methyl red indicator was added to clean dry 50 ml conical flask. The boric acid in flask traps down free ammonia vapour liberated from the digestion setup. The flask was placed so that the tip of the condenser tube is below the surface of boric acid solution. The digest was transfer to reaction chamber and 20 ml of 40% NaOH was added and the joint of the tube was closed and distillation commences and 50 ml distillate was collected inside the receiving flask. The distillate was titrated with 0.1N HCL. The blank distillation and titration was carried out the same way.

$$\%nitrogen = \frac{(S-B)\ x\ 0.1\ x\ 14.01}{wt.\ of\ \ sample\ in\ gram}\ x\ 100$$

Crude protein = %N x 6.25. Where; S = sample titre value, B = blank titre value

Determination of phosphorus content of the clay sample

The method of IITA (1979) was used. In this method, 2 g of soil, 1 teaspoon of carbon black and 40 ml of the extracting solution were all put into a 125 ml Erlenmeyer flask. The flask was shaken for 30 min, on a mechanical shaker. The suspension was filtered through the Whatman No. 40 paper. More carbon was be added to obtain a clear filtrate, thereafter 2 ml of the clear supernatant was dispensed in a 20 ml test tube and 5 ml of distilled water plus 2 ml of ammonium molybdate was added. The contents were mixed properly and 1 ml of dilute stannous chloride solution was added and mixed again. After 5 min the percentage transmittance on the electrophotometer at 660 nm wavelength was measured and the reading was recorded using the formula:

$$P\ (mg/kg) = \frac{\text{Re}\ading\ x\ 0.61\ x\ dilution\ \ factor}{atomic\ weight\ of\ \ phosphorus}$$

Determination of potassium

The flame photometer was set for K by inserting appropriate filter (usually of 768 wavelengths). The instrument shall was set to 100% transmittance by feeding 10 ppm K solution and all the standard solutions were ran and a standard curve was prepared by plotting transmittance readings against concentration of standard K solution. The soil extract was run and was calculated as follows:

K (mg/kg) =

$$\frac{milliequivalent\ per\ 100g\ oven\ dry\ weight\ of\ \ soil}{conc.\ of\ \ K\ in\ the\ extract\ \ from\ s\tan dard\ curve}$$

Determination of sodium

The flame photometer was set for Na by inserting appropriate filter (usually 58 wavelengths). The instrument was set to 100% transmittance by feeding 25 ppm Na solution. All the standard solutions were run and a standard curve was prepared by plotting transmittance readings against concentration of standard Na solution. The clay extract was run again and calculation was made, using the formula:

$$\text{Na} = \frac{milliequivalent\ per\ 100g\ oven\ dry\ weight\ of\ \ soil}{conc.\ of\ \ Na\ in\ the\ extract\ \ from\ s\tan dard\ curve}$$

Determination of Magnesium

Two soil aliquots of Mg standard solution was pipetted into two titration flasks and distilled water was added to each to make total volume of 100 ml. Twenty milliliters of buffer solution was added to get a pH of 10. This was followed by the addition of 10 drops each of (KON, NH_2) H, HCI. $K_4Fe(ON)6$ and triethanolamine. Ten drops of indicator were added and the solution was titrated against EDTA from a red to permanent blue colour. The blank was run with mg standard solution. The Mg was calculated per millilitre of EDTA as follows:

$$\text{Mg/Ml EDTA} = \frac{0.1216\ x\ 5ml\ \ Mg\ \ s\tan dard\ solution}{net\ ml\ \ EDTA\ to\ the\ end\ \ po\text{int}}$$

Determination of calcium

Two 5 ml aliquots of the extract were put in two titration flasks and distilled water was added to get a volume of about 150 ml. Ten drops each of KON, NH_2OH, HCl and triethanolamine and 4 ml of 10% NaOH was added. The solution was titrated with EDTA to a purple end point. The pH was raised to 12 to precipitate Mg as Mg (OH) 2. The blank was run with 0.5 ml of NH_4OH solution and the net ml of EDTA was calculated by subtracting the titre from that needed for the extract. From the calibration of EDTA, Ca is calculated as:

Net ml of EDTA-Net ml for Ca

Results and Discussion

Table 1 shows the fungi identified from groundnut oil contaminated clay soil. The pure culture of these isolates was maintained on potato dextrose agar medium (PDA). The morphological identification of all the isolates were carried out and identified as *Aurebasidium spp*, *Paecilomycetes spp* and *Aspergillus spp*.

Table 1: Fungi species isolated from the groundnut oil contaminated with soil sample

Isolate code	Macroscopy	Microscopy	Organism
Go1	Rapid growth, initially the colony appeared pinkish but add shade of brown as it ages	Young colonies appear yeast like consisting of unicellular budding cells	*Aurebasidium spp*
G02	The colonies are flat, powdery the reverse is dirty white	Septate hyaline hyphae, conidiosphore, philiades	*Paecilomyces spp*
G03	The surface growth was velvety, the reverse is white to tan to pale yellowish	Hyphae are septate with smooth walled conidiosphore	*Aspergillus spp*

They were identified using the method used by Micheli (1729) to identify *Aspergillus* species under the microscope as reported by Bennett (2010) in his work was used.

The physiochemical characteristics of groundnut oil contaminated clay soil are present in Table 2. Result of the moisture content shows that the moisture content of vegetable oil contaminated clay soil had low moisture content of 2.11%. This shows the clay soil used was dry. The moisture content of the oil free soil is lower than that of the contaminated site. This observation is due to the permeability and infiltration of water in the oil polluted site (Edema *et al*., 2010). The pH of the groundnut oil contaminated clay soil was 5.6 which is slightly acidity. Soil pH affects the behavior of soil microbes, encouraging or inhibiting the growth of pathogens and affecting how well helpful microbes are able to breakdown organic matters. The organic carbon of the oil contaminated site increased due to the oil spill compare to the control (oil free). Organic carbon serves as energy for the metabolism of the vegetable oil from soil. Nitrogen content of the oil contaminated site is higher than that of the oil free site. The reason could be attributed to higher organic matter content in the groundnut oil contaminated site. The phosphorus analyzed showed that the oil free clay soil sample had higher phosphorus content than that of the vegetable oil polluted clay sample. There was significance difference in the phosphorus content of the sample analyzed.

Table 2: Physiochemical properties of oil polluted clay soil sample

Parameter	Control	Oil contaminated
P^H	7.20	5.65
Moisture %	1.80	2.11
%OC	2.23	1.24
%N	0.111	0.062
P (Mg/kg)	13.09	7.20
Na (Mg/kg)	1.25	0.97
K (Mg/kg)	4.40	1.08
Mg (Mg/kg)	4.58	1.72
Ca (Mg/kg)	6.76	4.14
Silt %	11.85	10.85
Clay %	47.28	48.28
Sandy %	40.87	40.87

%OC: Organic carbon, %N: Nitrogen, Mg: Magnesium, Ca: Calcium, K: Potassium

Table 3: Screening for degradation of vegetable oil by fungi

Samples	Day0 (nm)	Day3 (nm)	Day6 (nm)	Day 9 (nm)
Control	0.829	0.813	0.812	0.813
G. O (1)	1.370	1.093	1.426	1.596
G.O (2)	0.950	0.874	1.257	0.663
G. O	30.883	1.487	1.169	0.893

Table 4: Efficiency of the oil degradation

Isolates	Wt. of oil (control) (g)	Wt. of oil used (g)	Efficiency (%)
Aurebasidium spp	1.721	1.012	59
Paecilomyces spp	1.721	0.665	61
Aspergillus spp	1.721	0.354	79

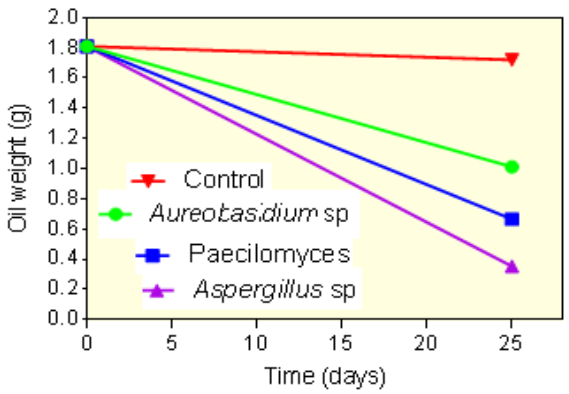

Fig. 1: Utilization rates of groundnut oil by some fungi species

Table 3 shows the lipolytic activity of the fungal isolated from oil contaminated clay soil. All the isolates were subjected for screening to check their lipolytic activity on a mineral salt medium by taking the absorbance at 520 nm using UV spectrophotometer. It was deduced that all the isolates are capable of utilizing groundnut oil on a contaminated site as their major source of carbon (Fig. 1). Table 4 shows the extent at which each fungi specie is able to utilize groundnut oils. It was deduced that *Aspergillus sp*, utilized the groundnut oil more effectively having the highest percentage of 79% compared to *Paecilomycetes* and *Aurebasidium spp* having 61 and 59%, respectively.

Conclusion

Discharge pollutants from refinery of vegetable oil (groundnut oil) consider one of the critical problems to the environment due to impact of which on the health and ecosystem. Currently the biological control to remove hazardous from environment is successful process due to it being a safe way to enhance a healthy environment in particular with low cost, technique and wide public acceptance to cleaning up contaminated sites. Based on previous studies, some fungi have ability to degradation groundnut oil isolated from contaminated clay soil. The data contained in this study shows that all the fungal species were capable of degrading the groundnut oil in varying degrees. The higher groundnut oil biodegradation efficiency was exhibited by *Aspergillus spp* compared with other species; nevertheless fungal species isolated from contaminated clay soil can be exploited in the bioremediation of vegetable oil (groundnut oil) to remove groundnut oil from contaminated environments.

Recommendation

Most of the research on fungal bioremediation has been conducted on laboratory scale and conditions, so further work is required to study these capacities taking into account the natural variables and their applicability in large-scale contaminated fields.

In addition, the screening of new fungal strains with interesting enzymatic activities is necessary for the degradation of the new pollutants from the increasing industry contamination. This microorganism screening, in combination with current biotechnologies such as genetic engineering, will pave the way to the future use of fungal whole cells and enzymes for bioremediation.

Conflict of Interest

Authors declare that there is no conflict of interest reported on this work.

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