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DEPARTMENT OF CIVIL AND CONSTRUCTION ENGINEERING

Effectiveness of microorganisms in breaking down of hydrocarbons found in effluent discharges from petroleum stations

MSc Research Thesis

By

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DECLARATION

This MSc Thesis is my original work and has not been presented for a degree in any other University or learning institution

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PLAGIARISM STATEMENT

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DEDICATION

To my family

Anne, Andrew & Gabrielle Wangaki

ABSTRACT

The study aimed at establishing the potential of using aerobic microbial degradation to treat and reduce volume of hydrocarbon in effluent discharges from petrol stations wastes. Microbial degradation of hydrocarbons (HC) has been considered the most promising technology and it is natural, less toxic and relatively cost-effective having been used to clean oil spills in rivers, oceans and in tank farms.

The objectives were realised through subjecting two different samples of 1.5 litres each from different service stations interceptors to physiochemical laboratory tests, chromatography analysis and a heterotrophic bacteria count. The samples were further split into two so that one sample remained as collected from site while the other one had nitrates and phosphates (N/P) added to it. The tests were to establish the changes in the physiochemical parameters, to confirm the changes that took place by characterising the samples and lastly to study the behaviour and growth of the bacteria over 40 days respectively. Addition of nitrates and phosphates was to confirm the impact of nutrients on the HC breakdown as well as bacterial growth.

The changes in the physiochemical parameters (COD, TDS, Nitrates and Phosphates) showed reduction in hydrocarbons. COD tests were critical to establish reduction of hydrocarbons (organic compounds) as COD quantities reduced by 35% and 30% in the TPS and Oilibya Samples with N/P respectively. The chromatography analysis also showed quantities of the various hydrocarbons that constituted the effluent reducing over time and in some cases completely eliminated. Addition of N/P saw faster growth of the microorganism population i.e. by 284% in the Oilibya Sample with N/P as compared to 5.8% in sample without N/P and 307% in the TPS sample with N/P compared to 8.5% in sample without. The study recommends a further study on identification of the responsible HC utilizing bacteria and use of the same on site in the interceptors.

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LIST OF ACRONYMS AND ABBREVIATIONS

ASTM	American Society of Testing Materials
BOD	Biological Oxygen Demand
BS	Biosurfactants
COD	Chemical Oxygen Demand
DO	Dissolved Oxygen
DoE	Design of Experiments
EMCA	Environmental Management Coordination Act
GC-FID	Gas chromatograph-flame ionization detector
GCMS	Gas Chromatography Mass Spectrometry
HC	Hydrocarbons
HUB	Hydrocarbon utilizing bacteria
LC	Light Crude oil
MAH	Monocyclic Aromatic Hydrocarbon
NBS	National Bureau of Standards
NEMA	National Environment Management Authority
NIST	National Institute of Standards and Technology
N/P	Nitrogen & Phosphorous
OS	Oil Sludge
PAH	Polycyclic Aromatic hydrocarbon
PCA	Plate count agar
ST	Surface Tension
TBH	Total heterotrophic bacterial
TCA	Tricarboxylic acid cycle (TCA cycle)
TDS	Total Dissolved Solids
TOC	Total organic carbon
TPH	Total petroleum hydrocarbons
TPS	Total Petrol Station on Limuru Road

1.0 INTRODUCTION

1.1 Background

One of the major environmental problems today is hydrocarbon contamination resulting from the activities related to the petroleum industry, which keeps on growing by the day as population increases. There are frequent accidental releases of petroleum products to the environment making a major environmental concern. Petroleum products are all hydrocarbons and their components have been known to belong to the family of carcinogens and neurotoxic organic pollutants which are challenging to breakdown. Currently accepted disposal methods of incineration or burial in secure landfills can become prohibitively expensive depending on the quantities involved. Mechanical and chemical methods which are generally used to remove hydrocarbons from contaminated sites have limited effectiveness and also prove to be expensive. (Das and Chandran, 2010)

Petroleum-based products are the major source of energy for industry all over the world and dominate many activities in our daily lives. Accidental spills, leaks and accidents occur regularly during the exploration, production, refining, transport, and storage of petroleum and petroleum products. A good example in Kenya is the Sachangwan oil tanker incident which spilled a lot of oil and resulted to loss of many lives. By 2003, the amount of natural crude oil seepage was estimated to be 600,000 metric tons per year globally with a range of uncertainty of 200,000 metric tons per year (Kvenvolden and Cooper, 2003).

With regulatory agencies like National Environmental Management Authority (NEMA) becoming more concerned with the release of petroleum products into our environment, there is a growing need to develop more effective and less expensive technologies to remediate the petroleum-contaminated effluent from petroleum sites to acceptable standards. NEMA through the Environmental Management and Coordination Act 1999 (EMCA) under water quality regulations 2006 (Legal notice No. 121) stipulates that; All firms or

persons discharging effluent into the aquatic environment are required to submit quarterly discharge monitoring records to NEMA based on prescribed procedures of sampling and analysis.

There are numerous technologies available to remediate petroleum-contaminated sites to acceptable standards. The selection of a suitable method for the remediation of a contaminated effluent depends on such factors as site characteristics, hazardous waste characteristics, regulatory guidelines and cost.

Bioremediation is considered the most promising technology for the treatment of these contaminated sites since it has proven to be cost-effective and leads to complete mineralization. Bioremediation operates on biodegradation, which may refer to complete mineralization of organic contaminants into carbon dioxide, water, inorganic compounds and cell protein.

Alternatively it can also be described as use of biological processes to degrade, break down, transform, and/or essentially remove contaminants or impairments of water quality. Bioremediation is a natural process which relies on micro-organisms such as bacteria and fungi to alter contaminants as these organisms carry out their normal life functions. These organisms through metabolic processes are capable of using chemical contaminants as an energy source, rendering the contaminants harmless or less toxic products in most cases. (Donlon and Bauder, 2006)

The use of micro-organisms to detoxify or remove pollutants owing to their diverse metabolic capabilities is an evolving method for the removal and degradation of many environmental pollutants including the products of the petroleum industry (Medina-Bellver et al, 2005). In addition, bioremediation technology is believed to be natural, less toxic and relatively cost-effective (April et al, 2005). Biodegradation by natural populations of micro-organisms represents one of the primary mechanisms by which petroleum and other hydrocarbon pollutants can be removed from the environment (Das and Chandran, 2011) and is cheaper than other remediation technologies (Leahy and Colwell, 1990).

While employing bioremediation it is important to identify the microorganisms which have the ability to degrade the hydrocarbons present in the water or soil, so that in case of a large spill these can be stimulated further in order to clean-up the area. Nutrients are very important ingredients for successful biodegradation of hydrocarbon pollutants and growth of microorganism population, especially nitrogen, phosphorus, and in some cases iron (Das and Chandran, 2011). Identification of such strains can ensure better efficiency of remediation as these strains will be well adapted to grow in the respective environment. It has been discovered that a number of micro-organisms have the capability to degrade the hydrocarbons from the oil spills. These include strains of bacteria, fungi, yeast, algae etc. (Kazuya, 2001)

1.2 Problem Statement

Spilled petroleum hydrocarbons are one of the main environmental pollutants that are harmful and not easy to remediate. Their abundance and persistence in several polluted environmental areas have been reported by Mohammed et al, in 2004. Oil spillage may be caused due to accidental leaks during various activities like exploration, refining, storage, fuelling of vehicles in service stations and transportation. The causes are usually numerous but the consequences remain the same. In the case of service stations the spillages are meant to be captured by gulley traps and directed through drainage to the oil interceptor before being released in to the storm water drains. We also have spillages during cleaning of vehicles in carwashes and servicing of vehicles in garages. The nature of the oil whether heavy or light can affect the clean-up procedures.

The Kenya Standard KS 1969:2006 Sec. 5.5.3 has given conditions of having impermeable concrete surface around fuelling pumps, under the canopy where most pumps are erected as well as remote fuelling pumps which are usually away from the canopy. This is to ensure the spilled fuel is well captured and does not seep into the ground. All the drains from where there is a potential spill are directed towards the interceptor for separation of water and oil before the water is released into the storm water drains. Interceptors are good and

effective in most service stations however they have limitations which need to be mitigated through enhancing their effectiveness. Interceptors are meant to be cleaned often by skimming the floating oil as well as removal of the sludge. The scooped oil should be stored for collection by authorised used oil dealers who are meant to dispose the oil in the right manner. Unfortunately very few station operators are keen on frequent cleaning of the interceptors, hence they cumulate hydrocarbons to the extent that they release it to the storm water drains especially in the rainy season when they overflow due to cumulated sludge and dirt. Use of micro-organisms to clean oil spills by breaking down hydrocarbons has been widely used in cleaning of oceans but never in oil interceptors hence the need of the study.

1.3 Objectives

The main objective of this study was to confirm the potential of using aerobic microbial degradation to treat and reduce volume of hydrocarbon in effluent discharges from petrol stations

1.3.1 Specific objectives

The study was guided by the following specific objectives;

- Establish the performance of native bacterial species in treating and reducing hydrocarbon wastes
- Determine the impact of addition of phosphorous and nitrogen in to the effluent by observing the rate of hydrocarbons degradation as well as bacterial growth.
- To characterize the effluent discharge in service station interceptors at various intervals of the breakdown

1.4 Significance of the study

Microbial degradation of hydrocarbons is a natural, effective and affordable way of cleaning petroleum pollutants in the environment. This method has been applied in many situations to clean up petroleum spills in the ocean, sea

and land. The current method of cleaning effluents from Petrol stations in Kenya is by use of interceptors. If microbial degradation is established to be effective in interceptors then it can go a long way in ensuring we have an affordable solution. This will lead to fewer hydrocarbons being released into our storm water drains hence less pollution of the natural waters from our petrol stations.

2.0 LITERATURE REVIEW

2.1. Hydrocarbons

A hydrocarbon is an organic compound composed of two elements which are hydrogen and carbon. A large part of the composition of petroleum products is made up of hydrocarbons of varying lengths. The smallest hydrocarbon, methane, is composed of a single carbon atom and four hydrogen atoms. However, hydrocarbons can literally consist of hundreds or thousands of individual atoms that are linked together in any number of ways, including chains, circles, and other complex shapes.

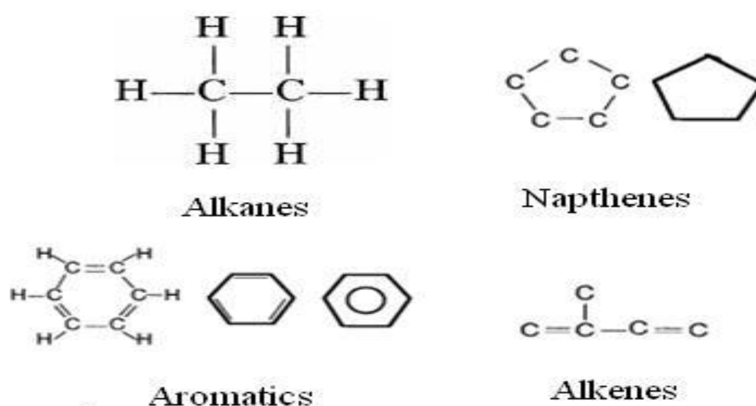


Figure 2.1: Molecular structures of hydrocarbons

Petroleum Hydrocarbons are naturally occurring, flammable organic compounds in the crude oil found in geologic formations beneath the Earth's surface. Crude Oil is explored and refined to make various petroleum products which all contain hydrocarbons. Hydrocarbons contained in crude, refined and used oils are categorized on the basis of molecular composition as alkanes, naphthenes, aromatics, and alkenes (Scholz et. al, 1999)

2.1.1 Alkanes

Most of the compounds in crude oil are hydrocarbons. This means they only contain hydrogen and carbon atoms, joined together by covalent bonds. Remember that a covalent bond is a shared pair of electrons. Alkanes are a type of hydrocarbon. The number of hydrogen atoms in an alkane is double the number of carbon atoms, plus two. For example, the molecular formula of methane is CH4. For ethane, it is C2H6.

All alkanes are saturated, which means they only contain single bonds between all carbon atoms. Alkanes are the basis of petroleum fuels and are found in linear and branched forms. Examples include methane, ethane, propane and butane.

2.1.2 Naphthenes

Naphthenes are also called Cycloalkanes and are generally stable and relatively insoluble in water. They have a chemical formula of CnH2n. Naphthenes are similar to alkanes, however they are characterized by the presence of one or more rings of carbon atoms in the chemical structure.

2.1.3 Aromatics

Aromatics are the hydrocarbons that contain alternating double and single bonds between carbon atoms. They are considered to be the most acutely toxic component of crude oil, and are also associated with carcinogenic and chronic effects. With the low weight aromatics being soluble in water, this increases the potential of exposure to aquatic resources. The term ‘aromatic’ was coined before the physical mechanism determining aromaticity and was derived from the fact that many of the compounds have a sweet scent in 1855 by Hofmann as edited by Lutz in his book of modern Arylation methods (Ackermann, 2009) Mostly they have a number of rings which ranges from one to six. Aromatics with two or more rings are known as polycyclic aromatic hydrocarbons.

2.1.4 Alkenes

Alkenes are also called Olefins or Isoparaffins and are not generally found in crude oils, but are common in refined products, such as gasoline (Garapati, 2012). They have a chemical formula of CnH(2n-2) and fall under unsaturated hydrocarbons. Alkenes are characterized by branched or unbranched chains of carbon atoms, similar to alkenes excluding for the presence of double-bonded carbon atoms. These are mixed with alkanes in petroleum and contribute more carbon dioxide per pound than do saturated hydrocarbons Aromatic compounds are often drawn as cyclic alkenes, but their structure and properties are different and they are not considered to be alkenes.

2.2 Hydrocarbon Properties and Characteristics

Hydrocarbons are organic compounds made of carbon atoms bound to each other forming the main structure but with hydrogen atoms attached to the remaining sites on carbon. The carbon backbone can be straight or normal, branched, or cyclic (Olah and Molnar, 1995). Compounds containing only carbon and hydrogen are referred to as parent compounds. Compounds containing substitutions of other elements or smaller carbon and hydrogen groups onto the original carbon backbone in place of the hydrogen are called derivative compounds (McMurry, 1988).

Hydrocarbons are classified based on the chemical structure of parent compounds or their physical properties. The chemical structure classifications include alkanes, cycloalkanes, alkenes, cycloalkenes, and alkynes. Alkanes, or paraffin, contain single carbon to carbon bonds in a straight backbone structure. Cycloalkanes, or naphthenes, contain single carbon to carbon in a ring structure. The single carbon to carbon bond is characteristic of saturated hydrocarbons. Alkenes, or olefins, contain double carbon to carbon bonds in a normal backbone. Cycloalkenes, or aromatics, contain double bonds in a cyclic structure. Alkynes, also referred to as olefins, contain a triple carbon to carbon bonds in their backbone. The double and triple carbon to carbon bond

characterizes unsaturated hydrocarbons (McMurry, 1988; Ratledge, 1978; Olah and Molnar, 1995).

The physical property classifications vary and include: gases, gasoline, kerosene, heating oil, mineral oil, waxes, resins, and asphalthenes. Gases include compounds with one to six carbons and have a boiling point below 30°C. Gasoline includes compounds with four to twelve carbons and has a boiling point from 30°C to 200°C. Kerosene and heating oil include compounds with eleven to fifteen carbons and have a boiling point from 200°C to 300°C. Mineral oil and waxes include fifteen to twenty five carbons and have a boiling point from 300°C to 400°C. The asphalthenes contain more than twenty-five carbons and have a boiling point above 400°C (Olah and Molnar, 1995; Prince 1993). The physical property classification above is derived from the crude petroleum oil separation processes. Most hydrocarbon compounds used by the industry are derived from crude petroleum oil, coal, and natural gas.

2.3 Hydrocarbons of Crude oil

Oils hydrocarbons are further categorized into three broad groups, according to their molecular weight. To easily understand we can generally group them in three categories namely light weight, medium weight, and heavy weight components (Scholz et. al., 1999). Crude oils compose of various combinations of these three categories as illustrated with the following general characteristics.

2.3.1 Light Weight Components

These have carbon atoms ranging from C1 to C10 which have smaller molecules with few numbers of atoms. They are characterized by high volatility, readily dissolve and evaporate and leave little or no residue because of their short residence time. They are highly flammable and readily inhaled, and therefore are of concern for human health and safety. Many of these components e.g. benzene and toluene are more bio available to animals by primary exposure route (respiratory system).

2.3.2 Medium Weight Components

These have carbon atoms ranging from C11 to C22 which have complex molecules. They have low rate of evaporation and dissolve very slowly taking several days with some residue remaining. They are not as bio available as lower weight components; they are less likely to affect aquatic animals. Their primary exposure area is the respiratory system and they are readily absorbed through skin.

2.3.3 Heavy Weight Components

These are components with more than 23 carbon atoms. They have the longest residence time with very little loss due to evaporation or dissolution. They can cause chronic effect through smothering as residue in the water column and sediments like tarballs etc. Their primary exposure route is direct topical contact. Some heavy weight components contain carcinogens that are absorbed through the skin. Their risk of exposure is increased due to long residence time enhancing probability of contact and adsorption of the oil components. (Garapati, 2012)

2.4 Microbial Degradation of Hydrocarbons

2.4.1 Biological remediation

Bioremediation is the biological process of transformation or mineralization of organic compounds introduced into the environment to less toxic or innocuous forms (Hazen, 1997, Brigmon et al, 2002). It describes several technologies and practices that take advantage of natural systems and processes to clean up pollution. Biodegradation greatly depends on the composition, state, and concentration of the oil or hydrocarbons, (Jahangeer and Kumar, 2013)

Bioremediation technologies can be broadly classified as ex-situ or in-situ (Iwamoto, 2001). Ex-situ technologies involve those treatment modalities which use the physical removal of the contaminant material to another area for

treatment. Good examples of ex situ treatment techniques are bioreactors, land farming, composting, and some forms of solid-phase treatment.

In contrast, in-situ techniques involve treatment of the contaminated material in place. Bioventing for the treatment of contaminated soils and biostimulation of indigenous aquifer microorganism are good examples of these treatment techniques. If biological treatment of a hazardous waste is contemplated, care is required to ensure that other components in the waste do not poison the organism. Various types of bioremediation practices are biostimulation, bioaugmentation, intrinsic treatment and phytoremediation.

Micro-organisms are responsible for biodegradation of hydrocarbons in the environment as the natural way of cleaning nature. This process is highly dependable on the composition of the hydrocarbons being broken down and nutrients available responsible for stimulating the performance and growth of the micro-organisms. Hydrocarbons are natural compounds produced by animals, plants, and bacteria (Ratledge, 1978). Therefore, it is not surprising that hydrocarbons can be biologically degraded by a variety of organisms.

Bioremediation has emerged as one of the most promising alternative treatment options for oil removal since its successful application after the 1989 Exxon Valdez spill (Swannell. et al., 1996; Venosa, 2003). Bioremediation is also defined as "the act of adding materials to contaminated environments to cause an acceleration of the natural biodegradation processes" (U.S. Congress, 1991).

2.4.2 Biodegradation Mechanisms

In most cases biodegradation reduces the toxicity and the migration potential of hydrocarbons (Field et al. 1991). The degrading organisms utilize hydrocarbons as carbon and hydrogen sources. Most hydrocarbons used as energy sources are degraded aerobically to produce carbon dioxide and water. This degradation process is referred to as a catabolic process while degradation to inorganic compounds is called mineralization. However, some hydrocarbons are not mineralized but transformed into simpler compounds

(Ferrari et al. 1996). The hydrocarbons used as a carbon source are degraded to smaller compounds and incorporated into the cell materials. This degradation process is a combination of catabolic and anabolic processes (Brock et al. 1994). Cometabolism is another mode of degradation, which is observed in the degradation of hydrocarbons. In cometabolism the hydrocarbon is transformed, but the organism does not gain any energy or nutrients (Field et al., 1991; Juhasz et al., 1996).

The specific degradation mechanisms are determined by the compound structure. Linear alkanes degrade through;

- oxidation in which the backbone is into two carbons at a time and the resulting acetyl
- Acetyl CoA is mineralized in the TCA cycle. Some cyclic alkanes degrade through cometabolism (Juhasz et al., 1996). Aromatic compounds are generally degraded via a dioxygenase enzyme, which converts the compound to a catechol followed by ring fission in the ortho or meta positions (Prince, 1993). Factors that determine the degradation mechanism as well as its rate and extent are: the chemical structure of hydrocarbons, the presence and capabilities of organisms, the presence of nutrients, the presence of electron acceptor, and the physical availability of the substrate hydrocarbon to appropriate organisms (Huesemann, 1995; Brock et al. 1994).

2.5 Conditions that influence Microbial Degradation

2.5.1 Temperature

Temperature is a key determinant of the rate of hydrocarbon degradation whether it is in fresh water or marine. The warmer the temperature the faster the degradation because the heat generated within the water body further encourages the breakdown of the spilled petroleum through natural processes such as evaporation. This leaves the oil-degrading microbes with a smaller size of

hydrocarbon pollutant to clean up (The American Academy of Microbiology, 2011).

2.5.2 Nutrients

Oil-eating microbes require nutrients for optimal growth and development just like any other organism nutritional needs. These nutrients are available in the natural environment but occur in low quantities. When there is an oil spill, the petroleum hydrocarbon provides carbon nutrients for the oil-eating bacteria to utilize, but the rate of degradation depends on the availability of other nutrients. The two most required and limiting nutrients observed are nitrogen and phosphorus which are incorporated into the cellular biomass and stimulate hydrocarbon metabolism (Prince, 1997; McKew et al., 2007; Calvo et al., 2009). Other nutrients include sulphur and potassium (Evans et al., 2004).

2.6 Challenges of Microbial Degradation of Hydrocarbons

Lighter fractions of petroleum are more soluble in water while heavier ones are not; aromatics are much heavier than alkanes. Benzene, the lightest MAH has a solubility rate of 1780g/m3 whilst naphthalene, the lightest PAH has a solubility of 31g/m3 (Parker et al., 1971; Clark and MacLoed, 1977). Although alkanes are the most biodegradable petroleum hydrocarbons, those with 5-10 carbon atoms are toxic to most microorganisms by disrupting their lipid membranes (Bartha, 1986). Petroleum hydrocarbons with 20-40 carbon atoms are hydrophobic at room temperature, explaining their slow biodegradation (Bartha and Atlas, 1977). Some bacteria, remarkably, produce waxes after degrading crude oil (Ishige et al., 2003). According to Van Hamme et al. (2003), the susceptibility of crude oil components to microbial degradation are in the following order: alkanes >light aromatics (MAHs such as benzene) >cycloalkanes > heavy aromatics (PAHs such as Phenanthrene)> asphalthenes. Resins are easily degraded naturally because they are light polar molecules (Spiecker et al., 2003).

Asphalthenes are highly resistant to biodegradation due to their heavy and viscous nature (The American Academy of Microbiology, 2011). Asphalthenes are very complex chemical structures made up of sulphur (0.3 - 10.3%), nitrogen (0.6 - 3.3%), oxygen (0.3 - 4.8%) and trace amounts of metals such as iron, nickel and vanadium (Tavassoli et al., 2012). Asphalthenes have the highest molecular weight of all hydrocarbon compounds in crude oil with values ranging from 600 to 3x105 g/mol and from 1000 to 2x106 g/mol (Speight and Moschopedis, 1981; Kawanaka et al., 1989; Flores and Mestahoward, 2001). This chemical complexity has rendered asphalthenes resistant to microbial attack. So far very few studies have been carried out to enhance the potential of biodegradation of asphalthenes.

2.7 Functioning of an interceptor

Oil interceptors also known are oil separators can be fitted to surface water drainage systems to protect the environment from pollution by oils. They separate the oil from the water, and then retain the oil safely until it is removed. Interceptors are mainly used for treating stormwater runoff from areas where hydrocarbon products are handled (e.g. petrol stations, airports, storage terminals) or where small spills routinely fall on paved surfaces exposed to rain. Treatment should be as close to the source of the hydrocarbon products as possible to retain the oil in a floatable, non-emulsified form. Oil separators are not usually applicable for general urban runoff, because by the time the oil reaches the device it has emulsified or coated sediment in the runoff and is too difficult to separate.

The objective of oil and water separators is to treat most of the flow (90 to 95%) from the catchment to an acceptable degree (15 mg/l oil and grease) and to remove free floating oil, so as not to produce a discharge that causes an ongoing or recurring visible sheen in the stormwater discharge or in the receiving water.

Oil and water separators are not primarily designed to remove suspended sediment. Sites that generate both TSS and hydrocarbons will need separate treatment systems. They are installed to contain oil leaks from vehicles and plant from accidental spillages.

Oil separators need to be correctly designed, installed and maintained to be effective. Therefore, emphasis must be given to proper application, design, operation and maintenance and prevention of plugging. Other treatment systems, such as sand filters and bioremediation may be considered for the removal of insoluble oil and TPH. Most petrol stations in Kenya use Interceptors as the device for cleaning effluents from the service station. (Auckland Regional Council Technical Publication, 2003)

Trapped gulley pots provide adequate protection for car parks that are too small to justify the installation of a separator (interceptor) but they must be properly operated and maintained by frequent removal of the petroleum hydrocarbons (BS EN 858-1:2002).

2.7.1 Interceptor Operation and Maintenance

The oil which collects in the separator must be removed before the oil layer exceeds 3 mm depth. Oil may be removed after each storm in cases where it is important to remove the oil layer, but more generally oil removal may be on a regular (for example, bimonthly) basis.

Sludge deposits should be removed when the thickness exceeds 150 mm.
Sludge that collects at the base of the separator must be removed. Such sludge may be allowed to collect for a month before it is removed. Solids may be pumped out as slurry. Lastly Since an oil layer may sit in the tank for some time, consideration should be given to appropriate venting for safety reasons.

2.8 Hydrocarbon Petroleum Waste cleaning techniques

Oil is presently a nonrenewable energy source in everyday life. This fossil fuel is used for a variety of purposes especially in running of a variety of

machinery. Unfortunately, oil spills into the environment are due to avoidable and unavoidable circumstances such as earthquakes, intentional spills from war and dumping. Several techniques can be used to clean up oil spills and to prevent further destruction by this hazardous constituent. Cleanup and recovery of spilled oil is difficult and depends upon many factors, including the type of oil spilled, the climatic conditions of spilled site which includes temperature of the water, tidal intensity and the types of shorelines and beaches involved. Generally spilled oil can be cleaned in three methods namely physical, chemical and Natural methods (Garapati, 2012).

2.8.1 Physical remediation

Physical treatment methods are mainly used to facilitate the solid-liquid separation in order to reduce the volume of hazardous wastes. Several physical processes including sedimentation, flotation, filtration, evaporation, clarification, centrifugation, distillation, reverse osmosis etc. are used in hazardous waste management. The various physical treatment technologies available for different applications are carbon adsorption, air stripping, filtration, centrifuging, distillation, evaporation, solidification and encapsulation. (Riser-Roberts, 1998). These methods are not very effective with liquid products, hence not commonly used where petroleum products are involved but are good for reduction.

2.8.2 Chemical remediation

Chemical treatment methods convert contaminants into less toxic forms or destroy them completely. Most commonly used chemical treatment technologies available are coagulation, hydrolysis, neutralization, oxidation/reduction, precipitation, fixation, ion exchange and coal agglomeration. The only limitation of chemical remediation is that they may harm the environment more with the remnants of the process.

2.9 Microbial Degradation use in restoring the Environment

Actually, the bacteria, fungi and other microorganisms that consume hydrocarbons do not work that fast. They can take weeks to months to years to degrade oil, depending on the type of oil that is being broken down. It is unfortunate that the microbes' speed is limited not by the availability of oil or even its droplet size. That is why chemical dispersants have been used to break up the oil into microbe-friendly globules. Availability of various nutrients, such as nitrogen and phosphorus that wash into the ocean via rivers carrying sediments from the continents results into stimulation of faster degradation.

Bioremediation boosting microbial activity by ensuring a steady supply of such nutrients is quite difficult, this is clear in the case of gusher in the Gulf of Mexico. That's one main reason we don't see bioremediation in the open ocean. The good news is, eventually microbes devour the entire oil spill no matter where it ends up except the heaviest, nastiest stuff: asphalthenes and other big-chain hydrocarbons that go on to form the millions of tar balls dotting the world's oceans. After all, roads would not be coated in the stuff if a slick bio film of bacteria was going to form on them. (Biello, 2010)

The low solubility and adsorption of high molecular weight hydrocarbons limit their availability to microorganisms. The addition of biosurfactants enhances the solubility and removal of these contaminants, improving oil biodegradations rates (Scott and Jones, 2000)

3.0 METHODOLOGY

3.1 Study Area and Sampling

3.1.1 Study Area

The study was carried out on samples collected from two Service Stations interceptors in Nairobi. The stations were TOTAL petrol station on Limuru Road (TPS) as well as Baba Dogo Oilibya Service Station on Outering Road in Baba Dogo area. The main reason why the particular stations were chosen was to have a contrast in the composition of the samples, by virtue of having one station that is regularly cleaned and another one that is not cleaned regularly hence make more comparisons while undertaking the tests. The samples were picked from the first chamber of each interceptor.

3.1.2 Sampling

One sample of 1.5 litre effluent polluted water and sediments from each of the interceptors of the two service stations were collected. The effluent was collected in clean two-litre sterile bottles. Samples collected from the different stations were not bulked for provision of making many comparisons. The two different samples were transported to the laboratory and stored at 4°C to ensure that no reaction would take place before the actual experiment was started.

Each Sample from each station was divided in two so that there was one sample being tested as a natural sample while the other sample had Nitrogen and phosphorus (Bacteria growth enhancing nutrients) added to it.

3.2 Physiochemical parameters of the effluent samples

Physicochemical parameters such as Temperature, pH, Chemical Oxygen demand (COD), Biological Oxygen Demand (BOD), Nitrate, Phosphate and TDS (Total Dissolved Solids) for the samples were determined. The tests were carried out at 10-day intervals for a period of 40 days on all the four samples

i.e. TPS sample without N/P (Nitrogen Phosphorous), TPS sample with N/P, Oilibya sample without N/P and Oilibya sample with N/P

3.2.1 Determination of pH

The pH of a solution refers to its hydrogen ion (H+) activity and is expressed as the logarithm of the reciprocal of the hydrogen ion activity in moles per litre at given temperature.
pH= -log (H+): The negative logarithm to base ten of hydrogen ion concentration in a solution. Measurement of pH was guided by ASTM (D1293 AND D5464)

3.2.2 Chemical Oxygen Demand

COD is widely used to characterize the organic strength of wastewater and pollution of natural waters. It is also used to indirectly measure the amount of organic compounds in water.

It is an important rapidly measured parameter for stream and petroleum service stations effluents. It is also a rapid measure of the chemically oxidisable matter present in the effluent sample.

The dichromate reflux method (Standard methods for examination of water and wastewater; methods 5220C) was selected for the COD determination because it has advantages over other oxidants in oxidisability, applicability to a wide variety of samples and case manipulation

3.2.3 Biological Oxygen Demand

This is a parameter used to define the strength of the organic waste. It is applied in measuring waste loadings to treatment plants and in evaluating the efficiency of such treatment system as well as determining the relative oxygen requirements of treated effluents and polluted waters.

It is the amount of oxygen utilized by a mixed population of microorganisms in aerobic oxidation (organic matter in a sample of wastewater) at a

temperature of 20^oC. The BOD testing was undertaken using the standard dilution method.

BOD_5= DO 1st day-DO 5th day X Dilution factor

3.2.4 Total Dissolved Solids

This is the portion of the effluent sample that passes through the filter paper after evaporating and its subsequent drying in an oven at a defined temperature (usually 100-105^oC). This depends on physical and chemical nature of the material and suspension, the pore size of the filter, the area and thickness of the filter mat, and the amount and physical nature of materials deposited in the effluent.

The Total Dissolved Solids were determined using ASTM D5907 for determination of filterable matter, total dissolved solids (TDS), and non-filterable matter, total suspended solids (TSS)

3.2.5 Determination of Dissolved Nitrogen (KJELDAHL Nitrogen)

Analytically organic nitrogen and ammonia can be referred together as total nitrogen. Organic nitrogen is defined functionally as organically bound nitrogen in the oxidation tri negative state. This includes natural materials as proteins and peptides, nucleic acids and urea. The Nitrates were determined using ASTM D3867

3.2.6 Determination of Dissolved Phosphorous

The Phosphates were determined using Ascorbic Acid procedure

3.3 Chromatographic analysis

Residual total petroleum hydrocarbons (TPH) were extracted from the samples and quantified using GC-MS (Gas chromatography–mass spectrometry).

3.3.1 Extraction

10ml of oil was transferred into disposable centrifuge tube and extracted with 20ml Hexane 0.6g anhydrous magnesium sulphate and 1.5g of sodium chloride was added. The tubes were then hand-shaken vigorously for the components to mix. They were then centrifuged at 10000rpm for 5min.

3.3.2 Solid-phase Dispersion (SPD) clean-up

The Hexane extract was cleaned up using two solid phase dispersive steps. The extract (10ml) was transferred to a second tube containing 330mg of C18 and 1.2g anhydrous magnesium sulfate. The tube was vortexed and centrifuge at 10000rpm for 5min. The extract (200µl) was transferred to a clean vial and further diluted with 2ml of hexane. 10µl of the solution was transferred to a smaller vial and 1µl of internal standard added.

3.3.3 Determination of composition of the petroleum hydrocarbons

GC-MS analysis of the samples was performed with Agilent 7595 Instrument. Chromatographic separation were achieved using a fused silica capillary column (Hewlett Packard ,50M x 0.32mm ID) coated with carbowax 20M (0.3µM film thickness) with Helium as a carrier gas. All the GCMS analysis was made in the split less mode with Helium as carrier gas. The Oven temperature was programmed from 60°C for 7M, to 120°C at 5°C per min, then to 180°C at 10°C per min then finally to 220°c at 10°C per minute where it was maintained for 10 min. Constituent of the analytes were identified by analysis of their mass spectra, direct comparison with these with those in the Wiley National Bureau of Standards (NBS) and National Institute of Standards and Technology (NIST) databases.

3.4 Enumeration of total heterotrophic bacteria

Total heterotrophic bacterial (THB) counts were determined using spread plate method on plate count agar (PCA) using nutrient Agar**.** From each sample 1 ml was homogenized in 9 ml of 0.85% sterilized distilled water.

3.4.1 Isolation of hydrocarbon utilizing bacteria

Isolation of oil degrading bacteria was carried out under aerobic condition with kerosene as sole source of carbon. 500µl of 10^{-3} serial diluted petrol station effluent was inoculated in liquid MSM and 100 µl of the same was inoculated on solid MSM. The mineral salt media (MSM) was made up of the following components (g/L): 0.2 MgSO4, 0.02 CaCl2, 1.0 KH2PO4, 1.0 K2HPO4, 1.0 NH4NO3, and 0.05 FeCl3, and the pH was adjusted to pH 7.4 containing 1% kerosene oil by spread method according to Liu (Liu et al.,1995) who used crude oil as a source of carbon. Culture plates were incubated for 10 days at temperature 28°C. After incubation period growth of microbial colonies were observed on the petri dishes.

In the morphological characterization study, grams positive bacterial cells retained purple stain. Gram negative bacterial cells retained pink safranin colour. It is the dark purple crystal violet stain retained by the thick layer of peptidoglycan which forms the outer layer of the gram positive cell. In gram negative bacteria, the thin peptidoglycan layer in the periplasm does not retain the dark stain, and the pink safranin counter stain stains the peptidoglycan layer. Both the gram positive and gram negative bacteria were rod shaped Bacillus cells.

3.4.2 Purification and characterization of Heterotrophic bacteria

Discreet colonies of different Hydrocarbon utilizing Bacteria were randomly picked using a sterile inoculating wire loop and sub cultured for purification by streaking on nutrient agar plates and incubated for 28°C for 24 h. To monitor cell numbers and biodegradation, 1 ml of effluent was removed from each container at the set times and re suspended in 10 ml of sterile saline in sterile centrifuge tubes. 0.1 ml of mixture was sampled for CFU (Colony Forming Units) counts.

The study was conducted at a temperature of 28°C and monitoring was performed on Days 0, 10, 20, 30, and 40. The bacterial species indigenous to the effluent samples were isolated by spread plate technique using 0.1 ml

aliquots of appropriate dilution onto nutrient agar plates. Individual cultures were identified morphologically using the taxonomic scheme of Bergey's Manual of Determinative Bacteriology (Holt et al., 1994). Just like in the other experiments, this was repeated after adding nitrogen and phosphorous to compare the counts.

4.0 RESULTS AND DISCUSSION

4.1 Introduction

All experiments undertaken were directed towards establishing the effectiveness of microorganisms in breaking down hydrocarbons. This was established by observing;

- The physiochemical changes that were taking place in the effluent samples, comparing the rate of changes in samples with and without additional nutrients of N/P
- Looking at the hydrocarbon characteristics over time using a chromatographic machine
- Lastly, monitoring growth of microorganisms in all the samples.

It was expected that the samples would show physiochemical changes reflecting the activities by the micro-organisms. This was mainly to be reflected through reduction in the hydrocarbon quantities and an increase in the number of bacteria. It was also expected that the changes in the hydrocarbon composition as well as increase in micro-organisms number will be faster where the bacterial growth nutrients of Nitrogen and phosphorous had been added. All the results and observations are discussed in this chapter with a view of achieving the objectives of the study.

4.2 Effects of biodegradation on the physicochemical parameters of the effluent samples

4.2.1 pH Test Results

pH stands for power of Hydrogen and is a measure of the molar concentration of hydrogen ions in a solution which as such is a measure of the acidity or basicity of a solution. The results of the pH tests are reflected in Figure 4.1.

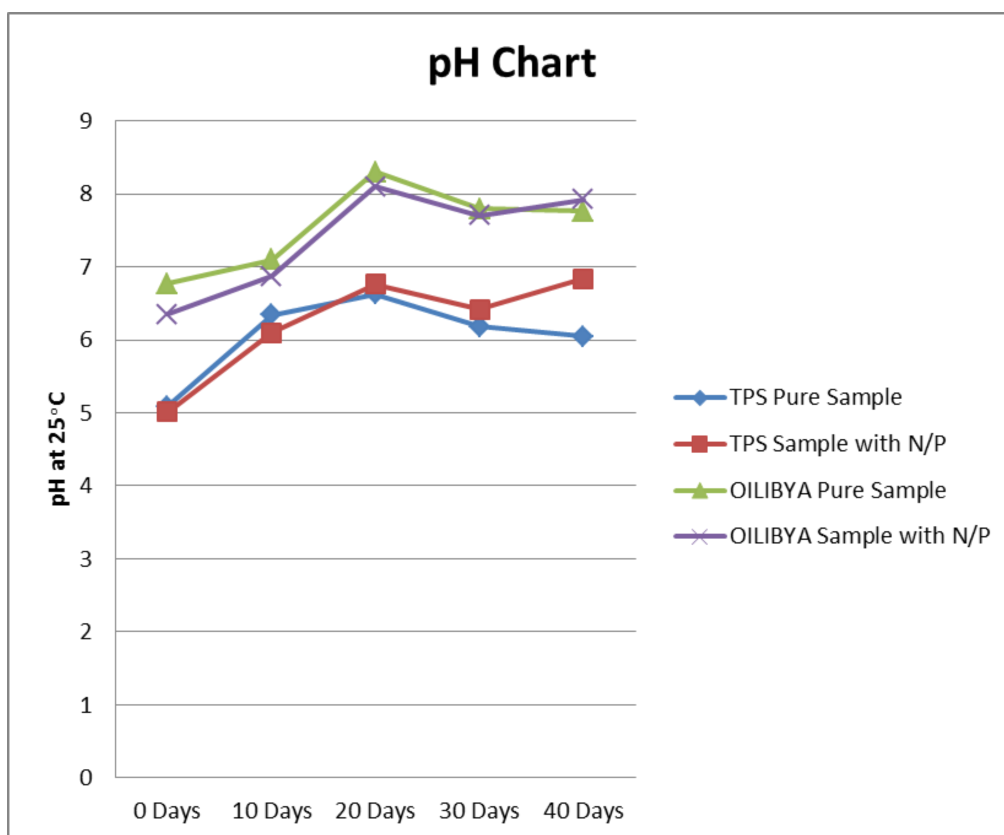


Figure 4.1 Chart Showing pH tests results

From Day zero to the 20th day, the TPS samples which looked more contaminated with and without N/P (Nitrogen and Phosphorous) moved from being moderately acidic towards being neutral i.e. 5.09 and 5.01 to 6.63 and 6.76 respectively. This was attributed to breaking down of the hydrocarbons into the constituent elements i.e. hydrogen and carbon. The hydrogen readily combined with oxygen to form water while carbon combined with oxygen to form carbon dioxide. Water is good in diluting many solutions. On day 40 all the pH in all the four samples was between 6 and 8.5 which was considered good pH.

$$\mathbf{HC+2O_2 \rightarrow CO_2+H_2O+Other\ Products} \quad \textbf{(4.1)}$$

Looking at the Oilibya Samples with and without N/P, there was a similar trend whereby both samples were diluted by water slightly from being acidic i.e. 6.77 and 6.35 to 8.3 and 8.1 respectively.

4.2.2 Chemical Oxygen Demand

The results of COD tests are as shown in figure 4.2.

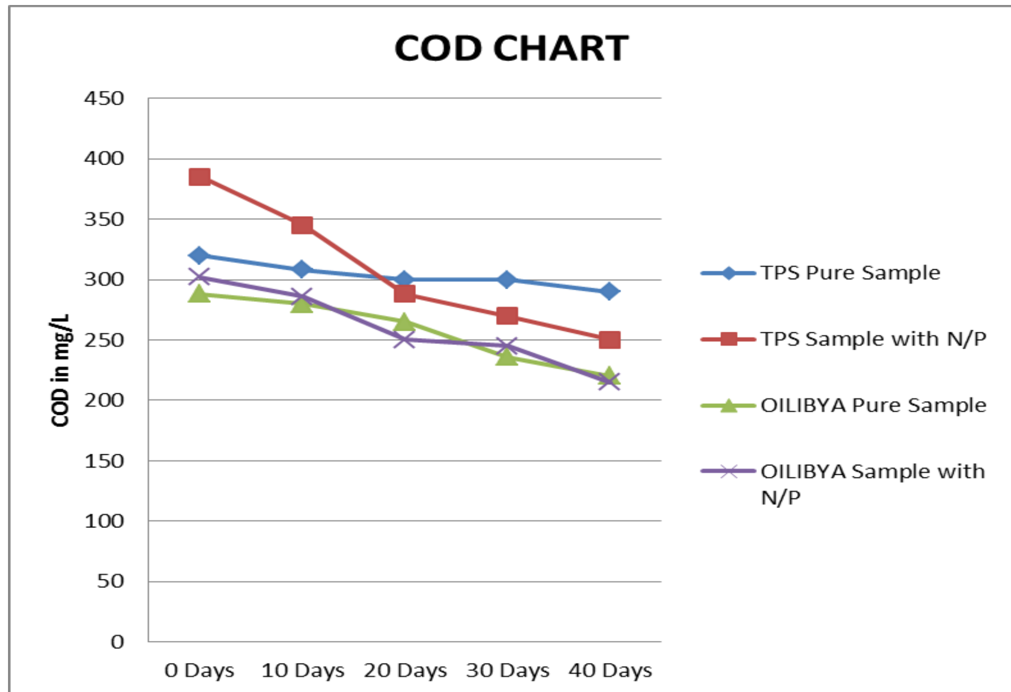


Figure 4.2 Chart showing COD test results from the experiments

COD is indirectly used to measure the amount of organic compounds; in this case the hydrocarbons are the organic compounds. COD continuously dropped in all the four samples in the 40 days of the experiment as shown in the Figure 4.2. This meant that the reactions taking place continuously utilized the available oxygen hence affecting the COD. It is key to note that COD dropped by 35% in the TPS sample with N/P and by 29% in the Oilibya Sample with NP compared to TPS sample without N/P at 9% and Oilibya sample without N/P at 24%. These result clearly indicated that the nutrients played a major role in increased activities by the microorganisms. It can also be clearly deduced that the changes in COD drastically slowed down after twenty days, meaning the HC breakdown hit the peak at between 20 to 30 days then slowed down. This can be attributed to the population of bacteria reaching its maximum population whereby all nutrients are exhausted hence the population could not further increase in number. The COD is above the NEMA standards

for discharge of effluent to the environment which advices 50, but most likely the water that will leave the interceptor will be cleaner now that we picked our sample from the first interceptor compartment.

4.2.3 Biological Oxygen Demand

Biological Oxygen Demand refers to the amount of oxygen that would be consumed if all organics in one litre of water were oxidized by Bacteria and Protozoa. In this case Bacteria are responsible for decomposing organic waste.

The BOD test results are as shown in figure 4.3.

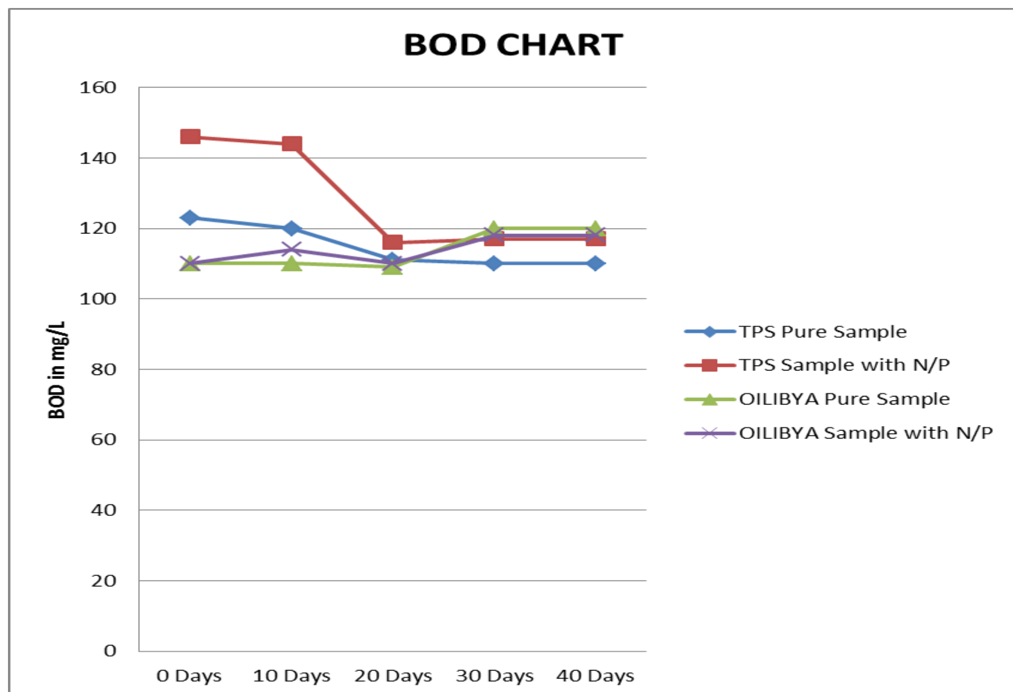


Figure 4.3 Chart showing results of the BOD experiments

Looking at the BOD graph in figure 4.3 it shows that BOD in TPS samples was reducing. The TPS sample without N/P had a slight reduction for twenty days then no more reduction. There was a faster and notable reduction in the sample with N/P followed by stagnation after twenty days.

On the other hand the Oilibya Samples showed slight growth in the BOD in the Oilibya samples for twenty days, and then the growth stagnated. BOD determination involves dilution of the effluent samples, to reduce the

concentrations of toxic or inhibitory substances. Removal of degradable organics generally does not proceed uniformly. Readily metabolised substrates are rapidly removed followed by slower degradation of the biomass. Because of the complexities involved BOD determinations are difficult to interpret and make comparisons among different wastes difficult to make. (Droste, 2005)

Very few effluents usually have sufficient biological populations to perform BOD testing without providing an acclimated seed. This is a mixed culture of bacteria and protozoa adapted to biodegrading specific effluents with a low number of nitrifying bacteria. Insufficient populations may have contributed to the little differences in BOD changes. (Hammer & Jr. Hammer, 2005)

The remaining BOD can be removed or accounted for through coagulation, which can be introduced in the second chamber to ensure NEMA standard for effluent discharge to the environment (third schedule) of 30 is met.

4.2.4 Total Dissolved Solids

The results of tests on TDS are as shown in figure 4.4.

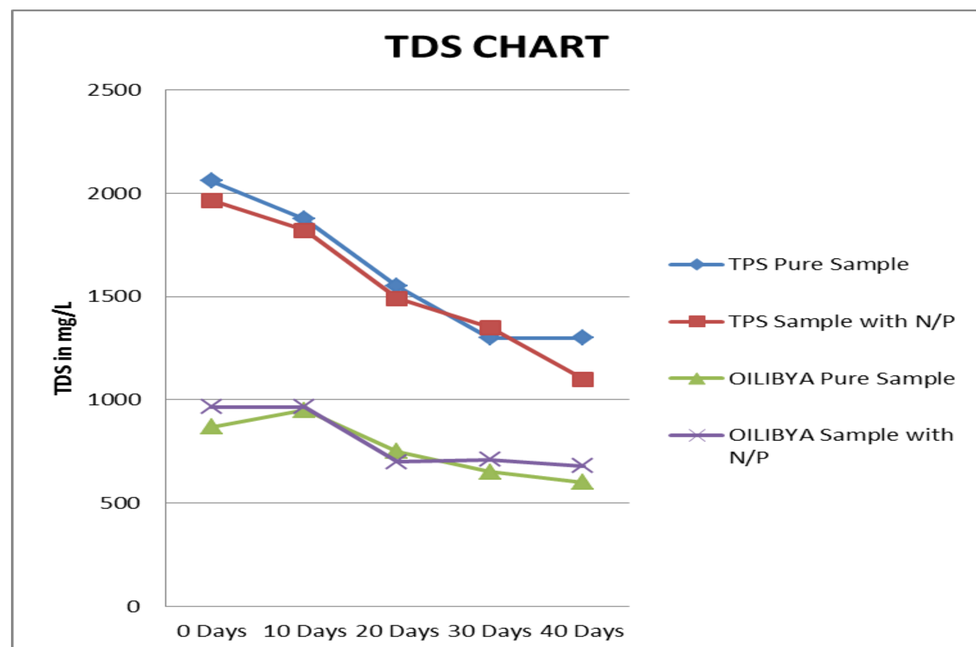


Figure 4.4 Chart showing changes in the Total Suspended Solids

The Total Dissolved Solids (TDS) in the TPS samples were high compared to Oilibya. The dissolved solids in the pure TPS sample reduced for 30 days then stagnated, meaning the dissolved solids must have been consumed. All the other three samples i.e. TPS with N/P, Oilibya with N/P and Oilibya without N/P continued to have the TDS reduce all through the 40 days. This is attributed to the fact that N/P enhances growth of Bacteria. The growth in population of bacteria increases the likelihood of consumption of TDS in the samples. Oilibya samples looked cleaner providing a good environment for continuous consumption of TDS for a longer period.

4.2.5 Nitrates

The results of Nitrates experiments are as shown in figure 4.5.

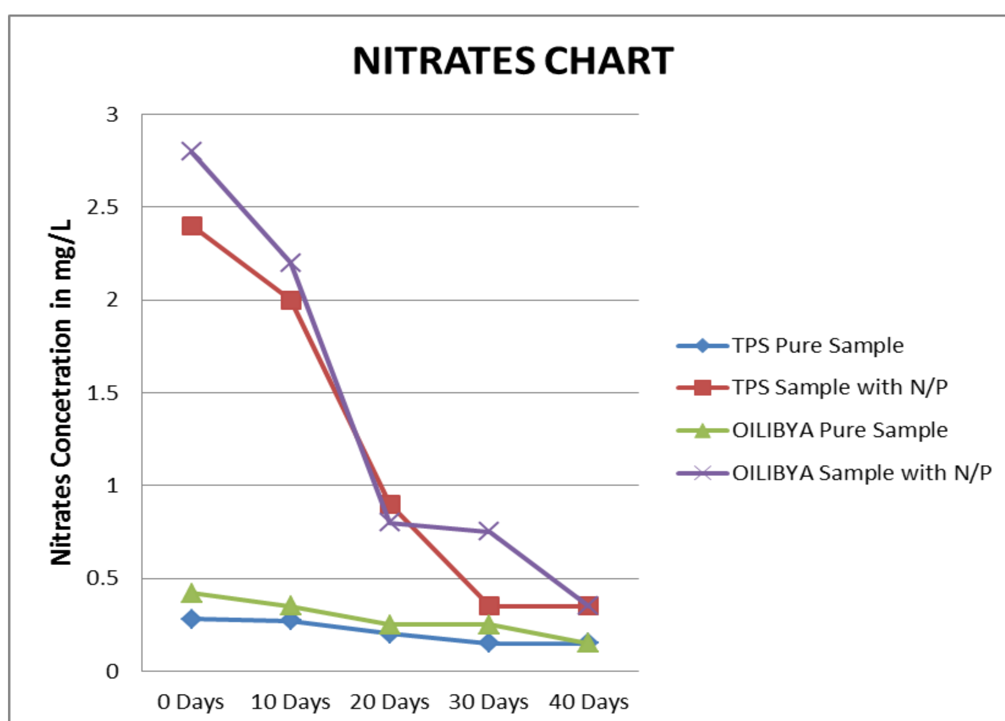


Figure 4.5 Chart showing changes in Nitrates

Nitrogen available in TPS samples seemed to have been consumed in the first twenty days, by 46% in the sample without N/P and 85% in sample with N/P. In the case of Oilibya there was further reduction of nitrogen after twenty days

i.e. 88% and 64% in sample with and sample without N/P respectively. This was attributed to the fact that the sample had a more conducive environment to the microorganisms allowing for more reactions to further take place. Figure 4.4 above illustrates nitrate reduction trends.

4.2.6 Phosphates

The phosphorous available in TPS and Oilibya pure samples seemed to have been fully consumed in the first twenty days. Reducing by 73% and 51% in TPS samples with and without N/P respectively, while the Oilibya samples reduced by 79% & 65% in the sample with and without N/P respectively. In the case of the samples with N/P there was evidence of further reduction of phosphorous beyond 40 days showing that additional phosphates might have needed more time to be exhausted beyond the experiment time. This was attributed to the fact that the sample was less contaminated, freely allowing for more reactions due to the conducive environment. The results of the phosphates tests are as shown in figure 4.6.

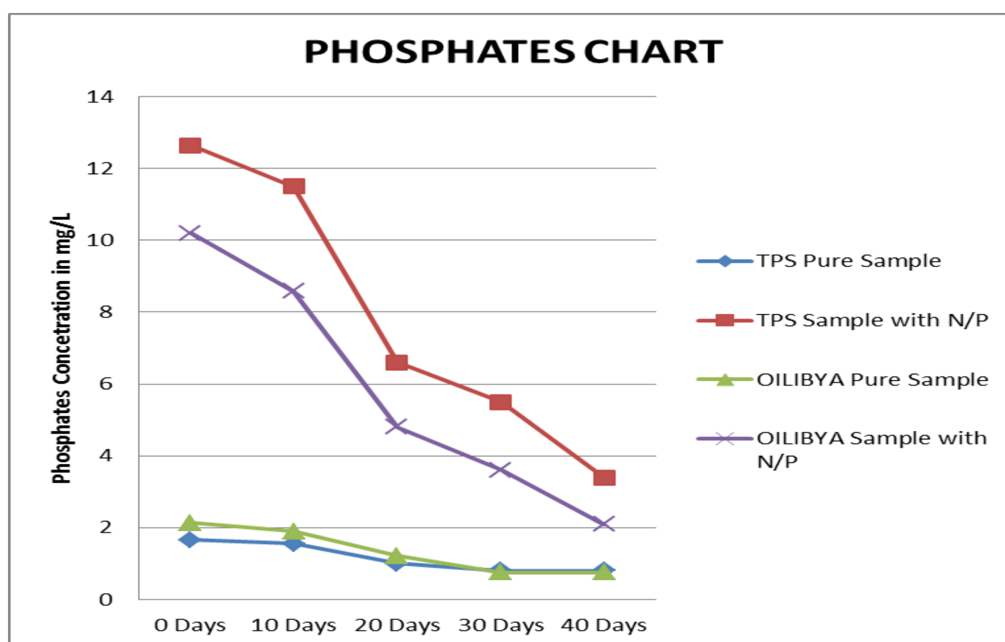


Figure 4.6 Chart showing reduction in Phosphorous

4.3 Chromatographic analysis results

The results of chromatography tests on the initial TPS sample are as presented in the table 4.1

Table 4.1 Composition of Initial TPS sample

Peak number	Retention time(min)	Compound name	Conc(ng/µl)
1.	11.8	Decane	0.82
2.	13.82	Dodecane	2.46
3.	21.5	Naphthalene	1.32
4.	22.5	2-Methyl naphthalene	0.30
5.	30.93	Eicosane	2.86
6.	32.5	1-Chloro-octadecane	4.63
7.	33.5	Fluoranthene	12.68
8.	34.0	Pyrene	2.51
9.	34.5	Tetracosene	2.62
10.	36.3	Hexacosane	4.82
11.	38.3	Triacontane	0.28

The results of the chromatography tests on the initial TPS sample without N/P after 20 days are as presented in the table 4.2

Table 4.2 TPS Sample without N/P after 20 Days

Peak number	Retention time(min)	Compound name	Conc(ng/µl)
1.	11.8	Decane	0.82
2.	13.82	Dodecane	2.46
3.	21.5	Naphthalene	1.32
4.	22.5	2-Methyl naphthalene	0.30
5.	30.93	Eicosane	2.86
6.	32.5	1-Chloro-octadecane	17.53
7.	33.5	Fluoranthene	760.1
8.	34.0	Pyrene	12.62
9.	34.5	Tetracosene	12.51
10.	36.3	Hexacosane	17.43
11.	38.3	Triacontane	0.28

The results of the chromatography tests on the initial TPS sample with N/P after 20 days are as presented in the table 4.3

Table 4.3 TPS Sample with N/P after 20 Days

Peak number	Retention time(min)	Compound name	Conc(ng/µl)
1.	11.8	Decane	0.74
2.	13.82	Dodecane	2.40
3.	21.5	Naphthalene	1.29
4.	22.5	2-Methyl naphthalene	0.30
5.	30.93	Eicosane	2.72
6.	32.5	1-Chloro-octadecane	17.53
7.	33.5	Fluoranthene	760.2
8.	34.0	Pyrene	12.62
9.	34.5	Tetracosene	12.49
10.	36.3	Hexacosane	17.41
11.	38.3	Triacontane	0.25

The results of the chromatography tests on the initial TPS sample without N/P after 40 days are as presented in the table 4.4

Table 4.4 TPS Sample without N/P after 40 Days

Peak number	Retention time(min)	Compound name	Conc(ng/µl)
1.	13.1	Decane	0.41
2.	14.8	Dodecane	1.82
3.	24.8	Naphthalene	0.83
4.	31.2	2-Methyl naphthalene	0.32
5.	32.93	Eicosane	1.68
6.	33.1	1-Chloro-octadecane	0.81
7.	33.8	Fluoranthene	0.42

The results of the chromatography tests on the initial TPS sample with N/P after 40 days are as presented in the table 4.5

Table 4.5 TPS Sample with N/P after 40 Days

Peak number	Retention time(min)	Compound name	Conc(ng/µl)
1.	14.8	Dodecane	0.38
2.	21.5	Naphthalene	0.29
3.	22.5	2-Methyl naphthalene	0.31
4.	40.0	Eicosane	1.32
5.	35.5	1-Chloro-octadecane	0.26

Figure 4.6 Showing Reduction of Hydrocarbons in TPS sample without N/P

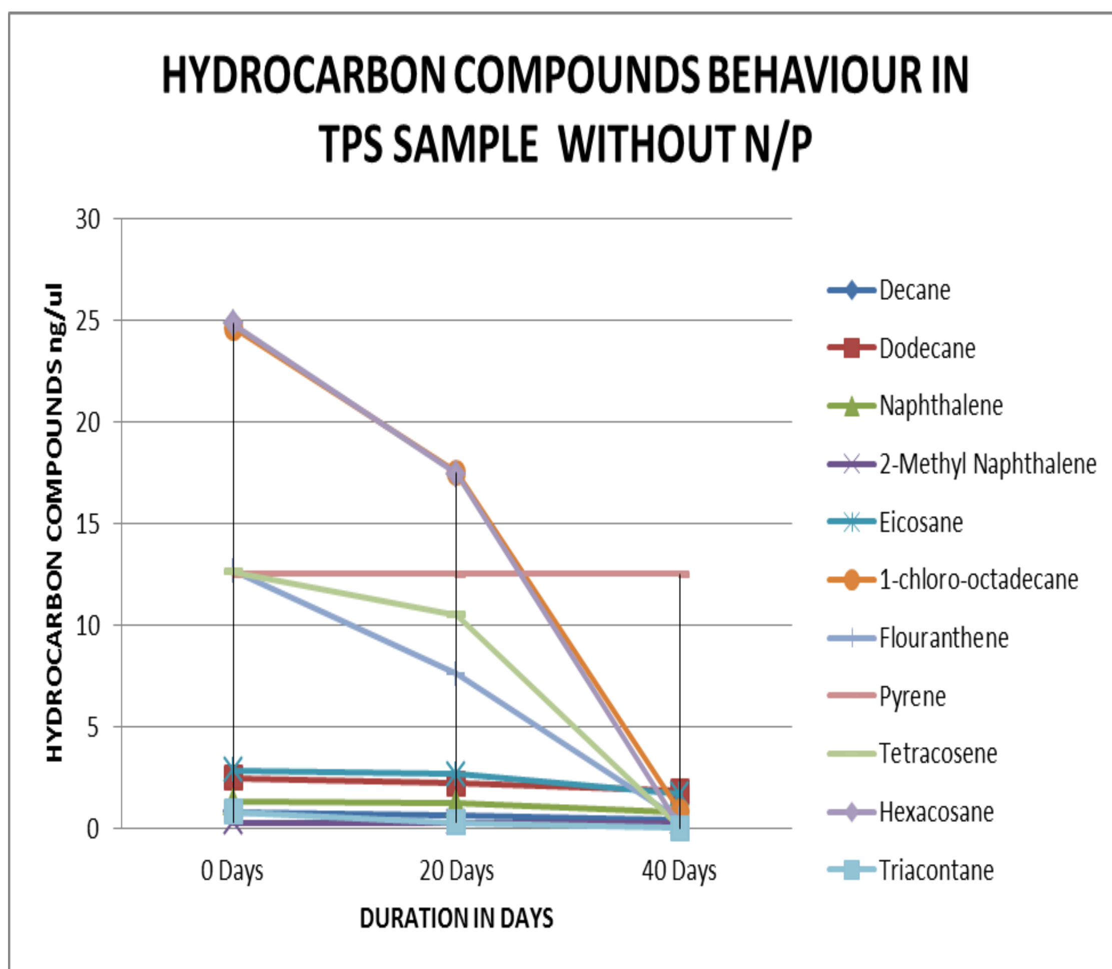
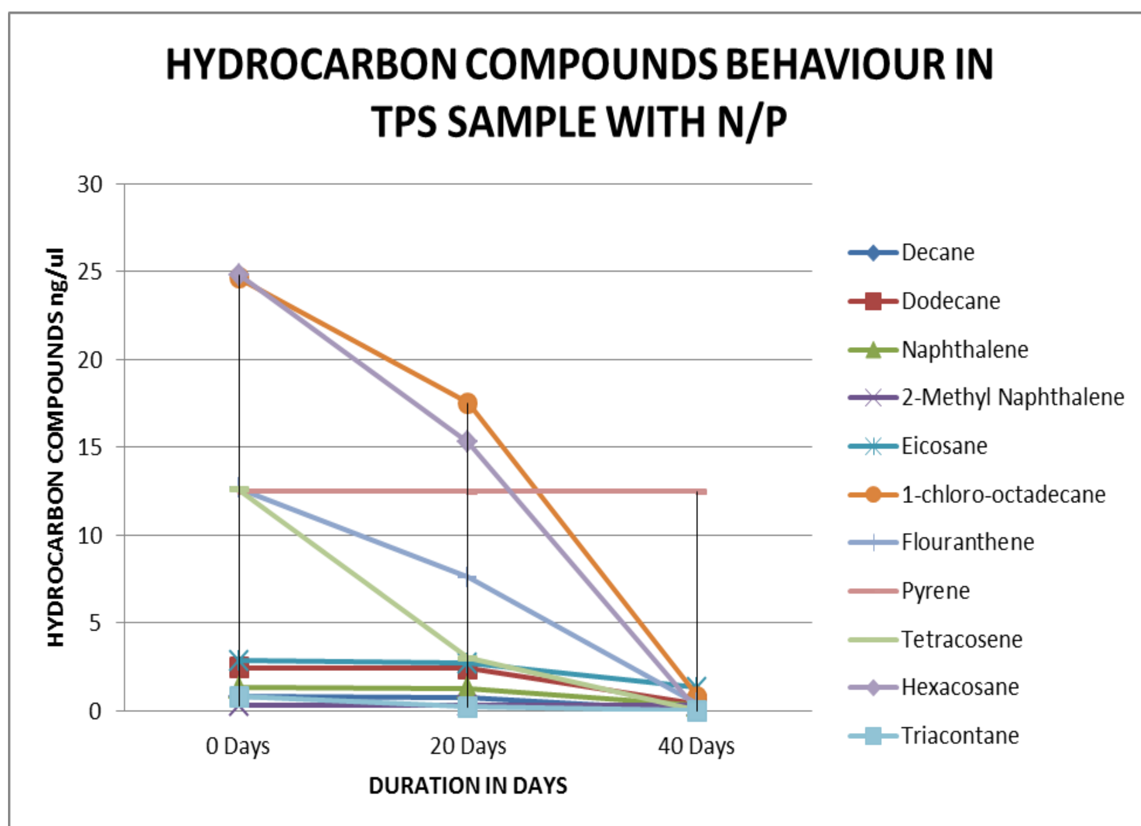


Figure 4.7 Showing Reduction of Hydrocarbons in TPS sample with N/P



Looking at both figures 4.6 and graph 4.7 all the constituent hydrocarbons are reducing. The reduction becomes significant after twenty days except for pyrene which remains the same. This clearly indicates that the hydrocarbons are being broken down by the bacteria and in the case where the N/P has been added the reactions are much faster further emphasizing the importance of nutrients. There is complete elimination of constituent hydrocarbons like tetracosane, hexacosane and triacontane.4.4 Isolation, Enumeration and characterization of bacteria

4.4.1 Purification and characterization of heterotrophic bacteria

The study adapted taxonomic classification of bacteria using physical characteristics and properties to classify. In the morphological characterization study, Grams staining bacterial cells which retained purple stain are called gram positive while the bacterial cells that retained pink safranin colour are

called gram negative. The dark purple crystal violet stain retained by the thick layer of peptidoglycan, forms the outer layer of the gram positive cell. In gram negative bacteria, the thin peptidoglycan layer in the periplasm does not retain the dark stain, and the pink safranin counter stain stains the peptidoglycan layer. Both the gram positive and gram negative bacteria were rod shaped Bacillus cells.

Table 4.6: Bacteria physical characteristics classification

Colony Characteristics	**General Observations**	
Gram reaction	+ve	-ve
Colony configuration	Circular	Circular
Colony margin	Entire	Wavy
Colony elevation	Flat	Flat
Colony surface	Smooth	Smooth
Colony texture	Moist	Slimy
Cell shape	Rod	Rod
Spore formation	+ve	-ve

Of all the heterotrophic bacteria that were established to exist there was none whose elevation was raised. They were all flat and circular, none was wavy.

4.4.2 Microbial monitoring and Enumeration of Heterotrophic bacteria

The naturally occurring bacteria in the effluent numbered $237x10^6$ cfu/ml for the samples collected from Oilibya petrol station and $217x\ 10^6$ cfu/ml for the samples collected from TOTAL petrol station respectively. This enumeration was done on nutrient agar.

The addition of nitrogen and phosphorus suspensions into the samples resulted in the growth of bacteria population to $513x10^7$ and $486x10^7$ cfu/ml for the samples collected from Oilibya and TOTAL petrol station respectively after 10 days. This was a significant increase in population growth considering that

for the samples where N/P were not added the population stood at 824×10^6 and 698×10^6 for Oilibya and TOTAL petrol stations respectively.

The bacterial count in the enriched samples increased to 776×10^8 and 668×10^8 CFU/ml for the Oilibya and TPS respectively by the 20th day into the study. There was a decrease in the population on the 30th day of the study. The populations stood at 135×10^8 and 348×10^8 CFU/ml for the Oilibya and TPS respectively.

Many microbial strains are capable of degrading only specific components of oil sludge. However, oil sludge is a complex mixture of different petroleum hydrocarbon (Mac Naughton et al., 1999). Single bacterial species have limited capacities to degrade all the fractions of hydrocarbons present (Rojas, 2007). Hence, a mixture of different bacterial species that can degrade a broad range of the hydrocarbon constituents such as present in oil sludge would show better potential. The results were as shown below in figures 4.8 and 4.9;

Figure 4.8 Bacterial count for the samples without nitrogen and phosphorus suspension added in 10^6

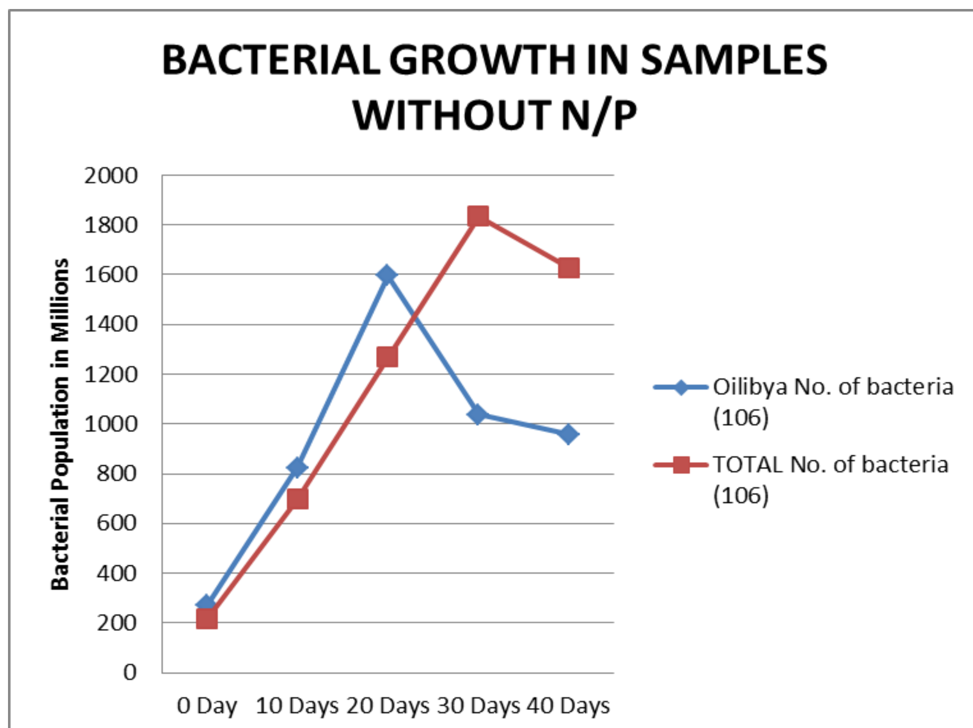
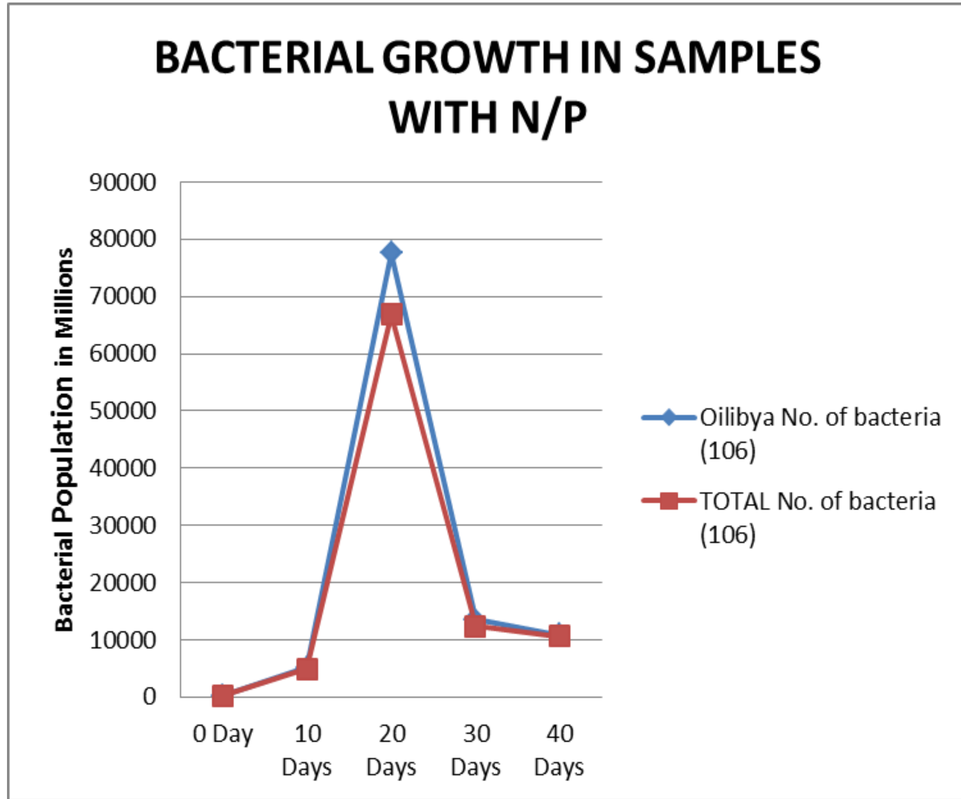


Figure 4.9 Bacterial count for the samples with nitrogen and phosphorus suspension added in 10^6



5.0 CONCLUSIONS AND RECCOMENDATIONS

5.1 Conclusion

There were faster reactions and increased populations of the bacteria in samples where nitrates and phosphates were added. Nitrates were consumed fast and exhausted by the 30th day, clearly indicating higher requirement of the same. Phosphates were not exhausted by the 40th day; there still remained huge amounts of phosphorous within the samples. All the same there was increased reduction of HC in the samples with phosphorous a clear indication that phosphorous is required to enhance the biodegradation process, but in limited quantities.

1. Conclusions arising from Physiochemical sample test findings

COD is indirectly used to measure the amount of organic compounds; in this case the hydrocarbons are the organic compounds. COD dropped by 35% in the TPS sample with N/P (from 385 to 250) and by 29% (from 302 to 215) in the Oilibya Sample with NP. The TPS sample without N/P reduced by 9% and Oilibya sample without N/P reduced by 24%. The break down was accelerated in the samples in which N/P was added, indicating the nutrients had a positive impact on the rate of breakdown.

From the experiment results, dosing of effluents in service stations with Nitrates and Phosphates at the rate of 50ml of 50ppm concentrate of nitrates per litre of effluent and 50ml of 100ppm concentrate of phosphorous per litre of effluent will ensure a breakdown of hydrocarbons by 30% efficiency.

Having picked our sample from the first compartment which is usually the most contaminated since the cleanup continues in the other two chambers, use of aerobic degradation will ensure the water being released into the storm water is much less contaminated since 30% more of HC are removed in first chamber .

This reduction will ensure the frequency of scooping off the oil in the first chamber reduces to once in three weeks as opposed to weekly. This will greatly reduce collection costs to almost once a month and the interceptor cleaning time.

2. Conclusions arising from Chromatographic analysis

When quantifying the residual total petroleum hydrocarbons (TPH) extracted from the samples using GC-MS – Gas chromatography–mass spectrometry, there was significant reduction in most constituent hydrocarbons as shown in Graph 4.6 and 4.7. In some cases there was complete elimination of various hydrocarbon components after 40 days e.g. Tetracosene and Hexacosane in the aromatic hydrocarbons where N/P was added.

3. Conclusions arising from Enumeration, Isolation and Characterization of Bacteria

The experiments done established the presence of more than ten different varieties of bacteria, whose population steadily grew up to the 30th day; thereafter the population started going down meaning it had reached its optimum and could not grow further due to limited source of food and nutrients. The addition of nitrogen and phosphorous increased the proliferation of biodegrading bacteria, resulting in an increase in degradation rates.

The bacteria can be cultured and introduced in the interceptors to ensure breakdown of hydrocarbons is enhanced. Dosing with N/P in the proportions of 50ml of 50ppm concentrate of nitrates per litre of effluent and 50ml of 100ppm concentrate of phosphorous per litre of effluent respectively will hasten the process by increasing population by more than 1000 times as shown in earlier discussion on enumeration of heterotrophic bacteria.

5.2 Operational and Engineering Significance

Environmental management is a key requirement in Engineering, any development should not negatively affect the environment hence all risks and

hazards that can result to pollution should be mitigated. An oil separator also referred to as an interceptor is key in ensuring that oil is removed from the waste water from a petroleum service station before being released into the storm water drain. However with challenges of poor design, maintenance and operation of the interceptor which includes need to frequently clean, use of microorganisms to reduce hydrocarbons in effluent plays a major role in enhancing interceptor efficiency.

5.3 Recommendations

1. So as to realize the full benefit of this study it is important to do further studies of identifying the hydrocarbon utilizing bacterial species responsible for the breakdown then culture the same to grow the numbers for use in the interceptors of petrol stations to see how well they decompose hydrocarbon contaminated effluents in situ.

2. The study can also be expanded to identify the role of other microorganisms which might be responsible like fungi and algae.

3. The further studies will require DNA isolation and extraction, Polymerase Chain Reactions, sequencing and eventually blasting using a sequencer. Biochemical reactions will also be applied to fully conclude the study.

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APPENDIX 1: PHOTOS OF BACTERIAL TYPES

Plate A1: Gram positive circular bacteria

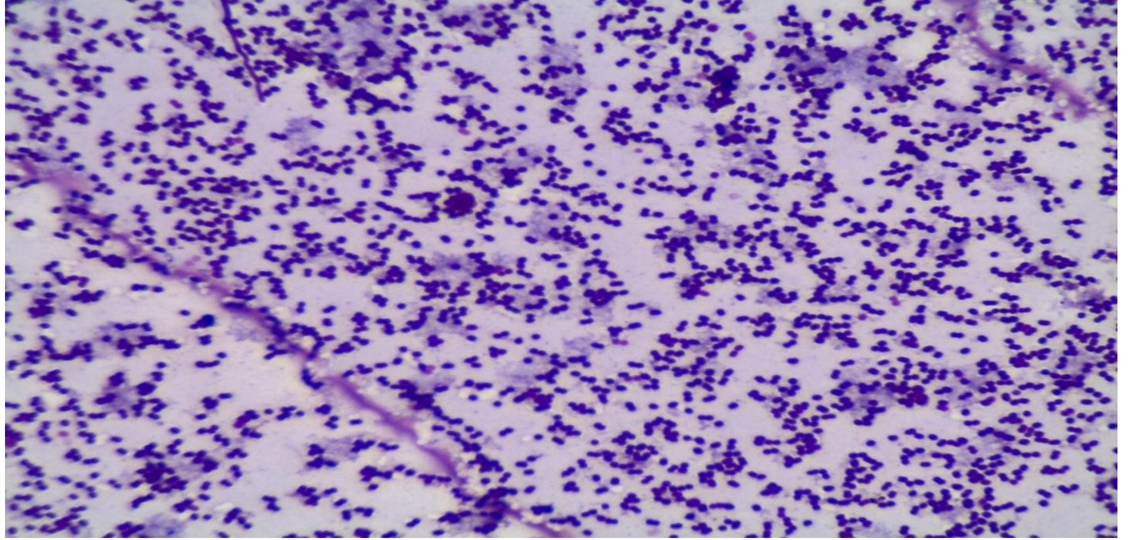


Plate A1: Gram Negative Moist Bacteria

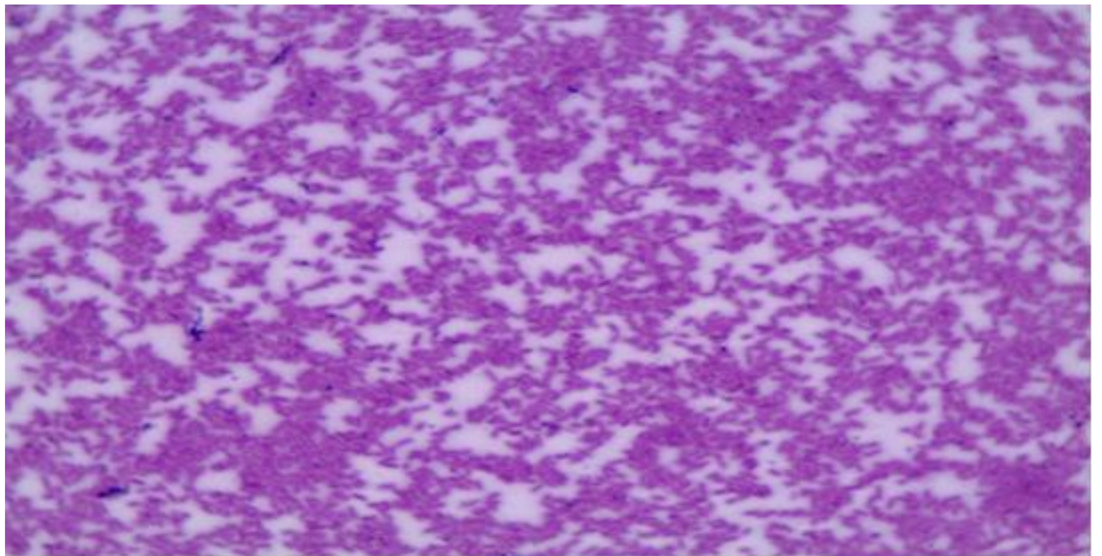


Plate A1: Gram positive entire bacteria

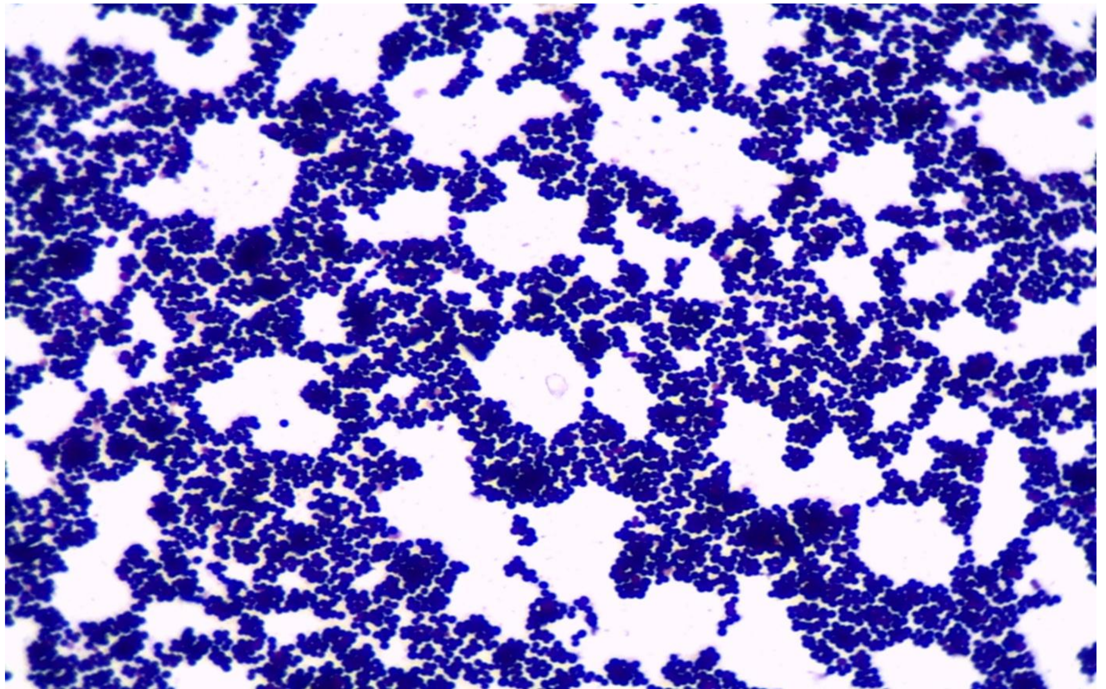


Plate A1: Gram positive entire bacteria

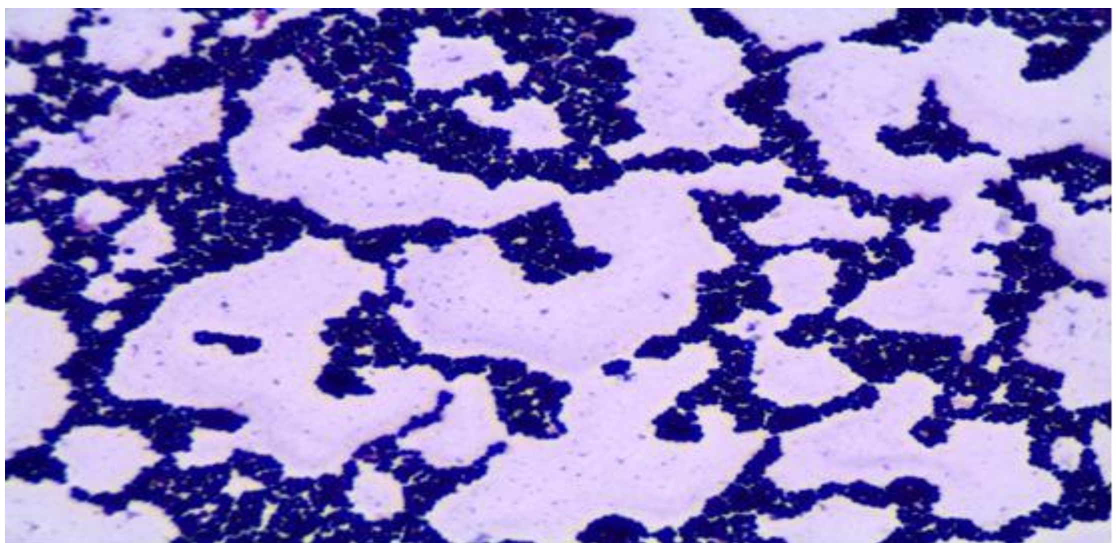


Plate A1: Gram positive entire bacteria

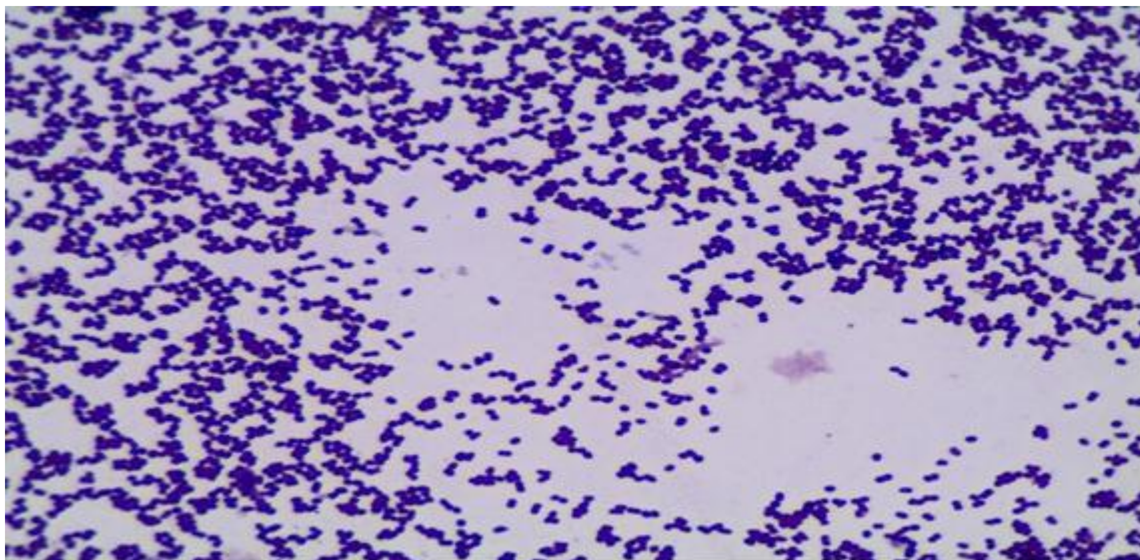


Plate A1: Gram negative rod bacteria

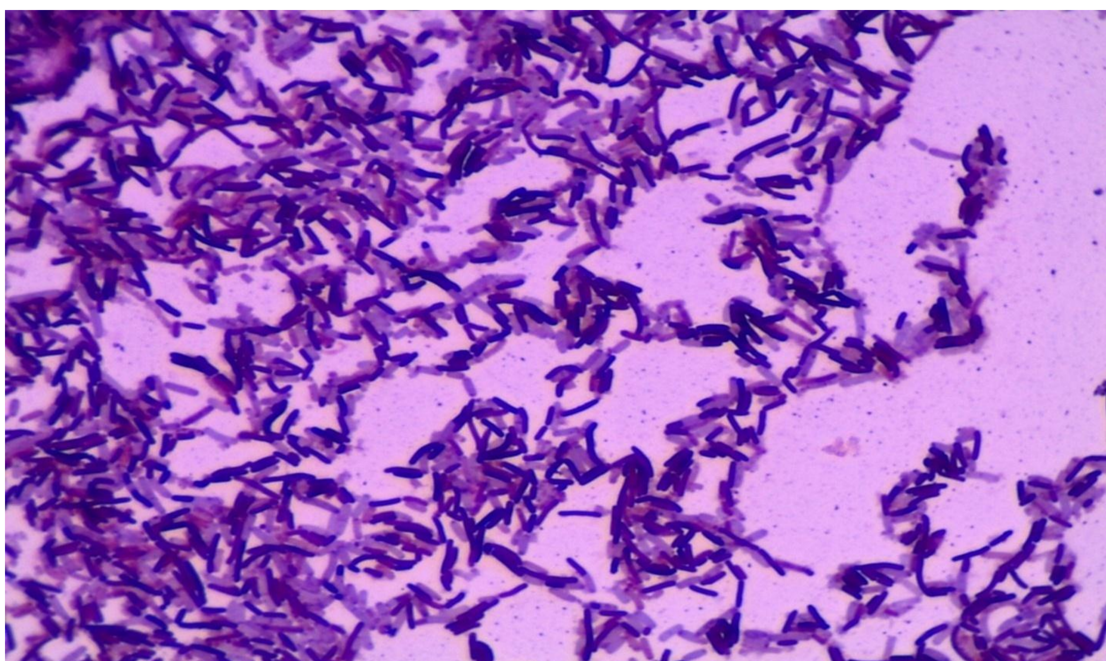


Plate A1: Gram negative smooth bacteria

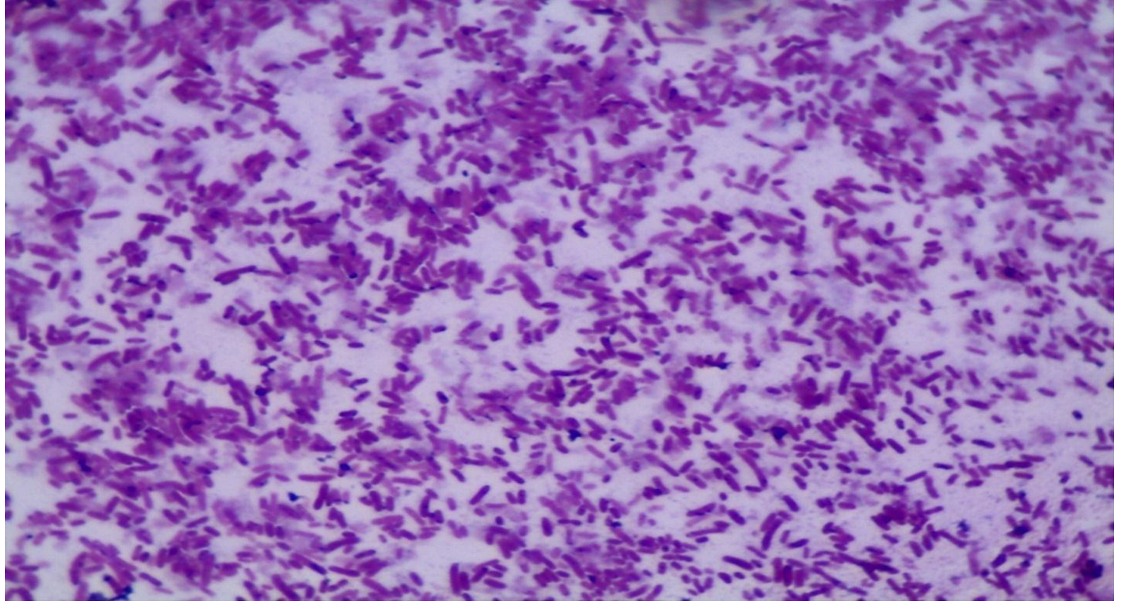


Plate A1: Gram negative rod bacteria

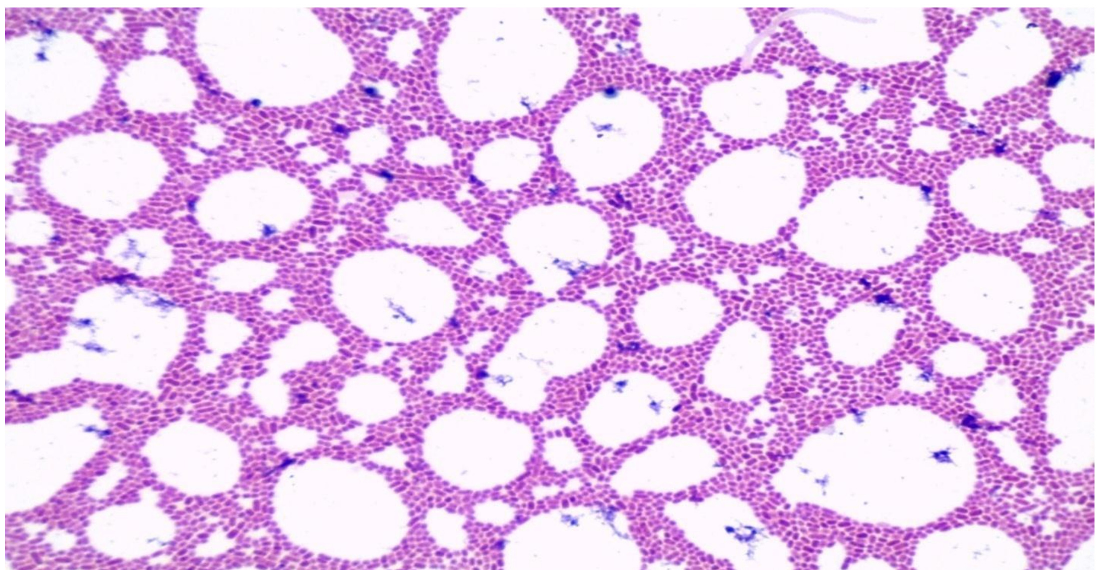


Plate A1: Gram positive entire bacteria

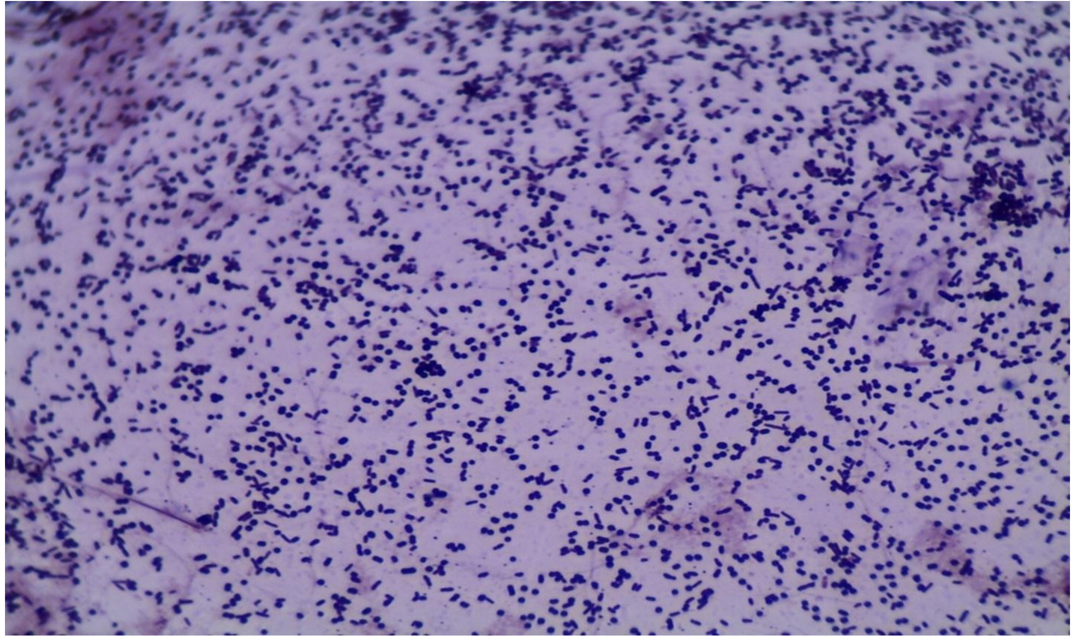


Plate A1: Gram Positive circular bacteria

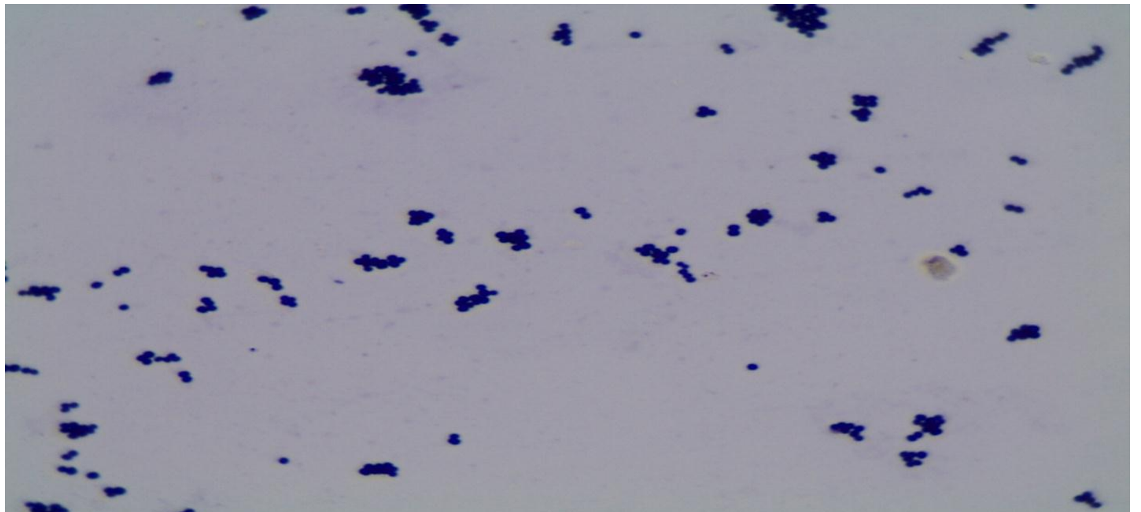
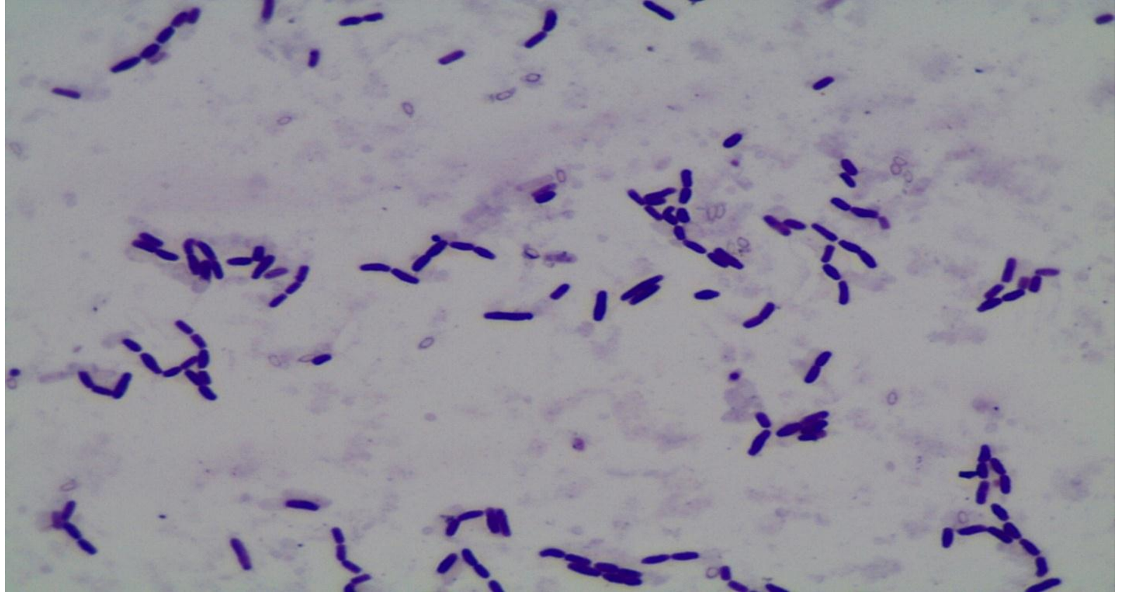


Plate A1: Gram Positive rod shaped bacteria



APPENDIX 2: SAMPLES PHOTOS

Plate A2: 1 Initial Samples that were refrigerated

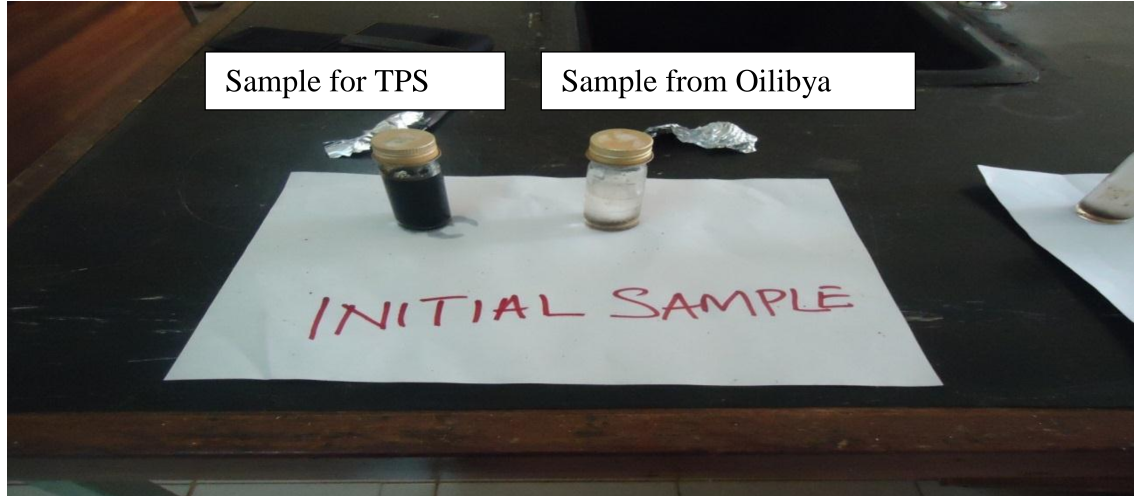


Plate A2: 2 Sample parts refrigerated after 10 days.

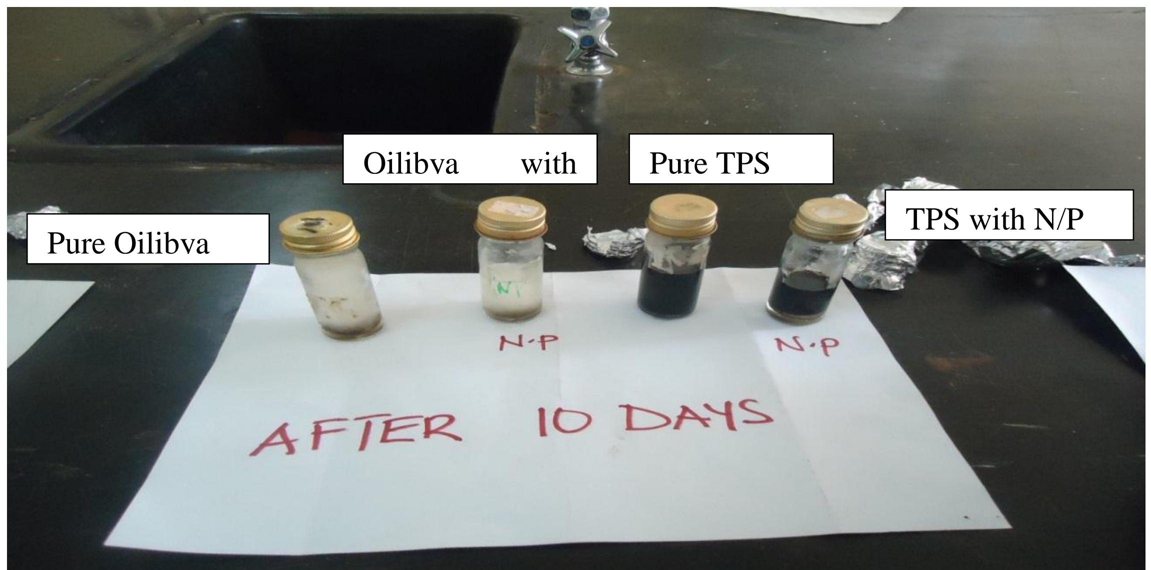


Plate A2: 3 Sample Parts refrigerated after 20 days

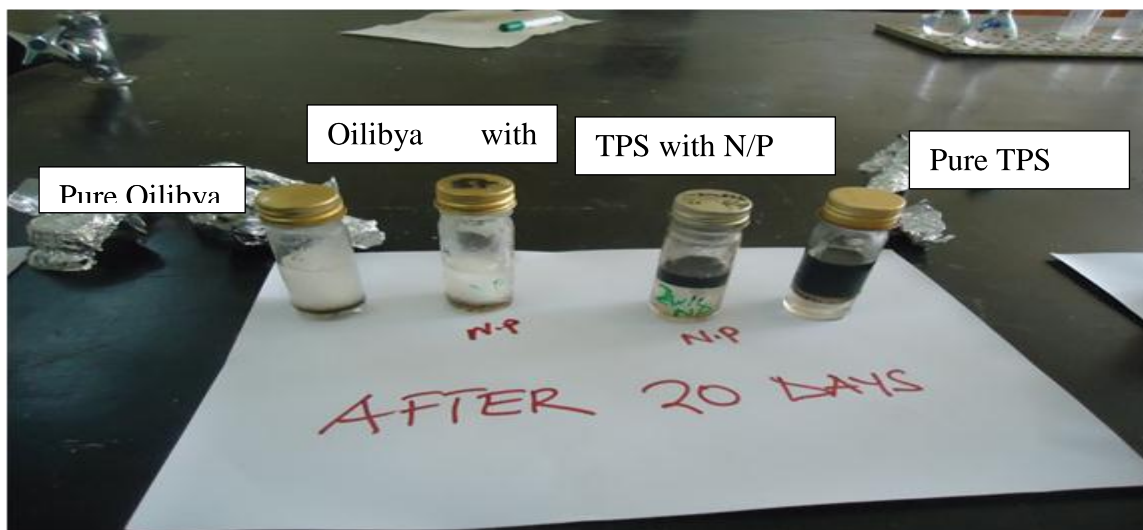


Plate A2: 4 Sample Parts refrigerated after 30 days

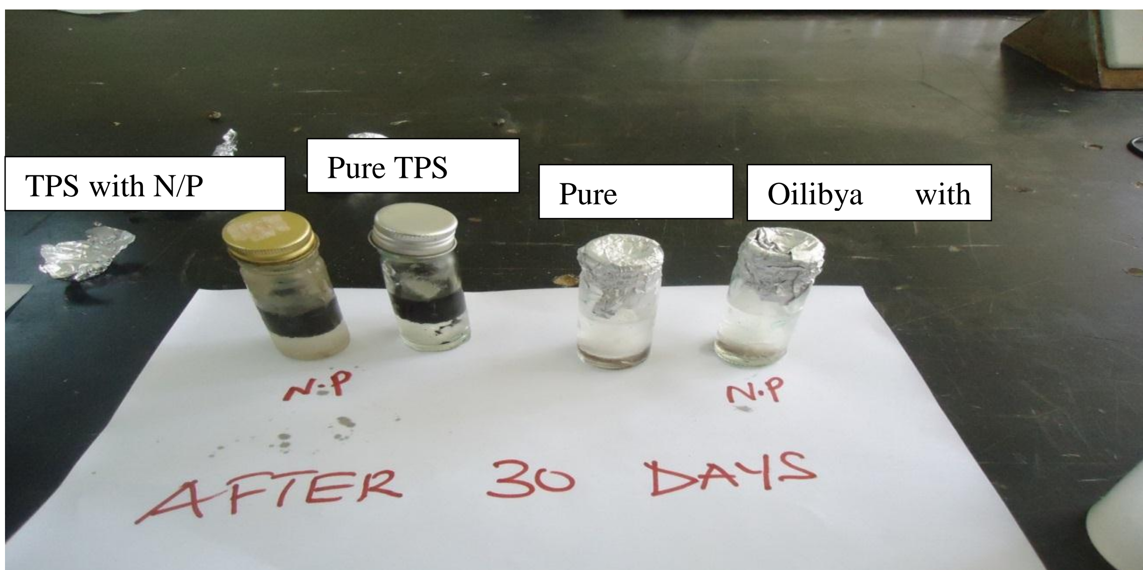


Plate A2: 5 Oilibya Samples tested for microorganisms after 10 days

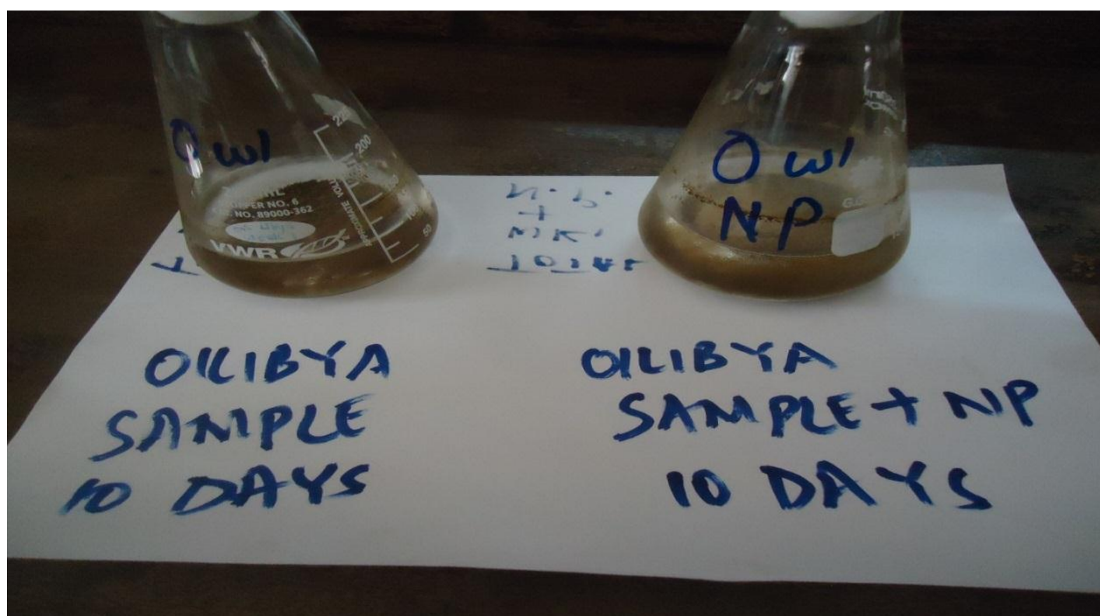


Plate A2: 6 TPS Samples tested for micro organisms after 10 days

Plate A2: 7 TPS Samples tested for micro-organisms after 30 days

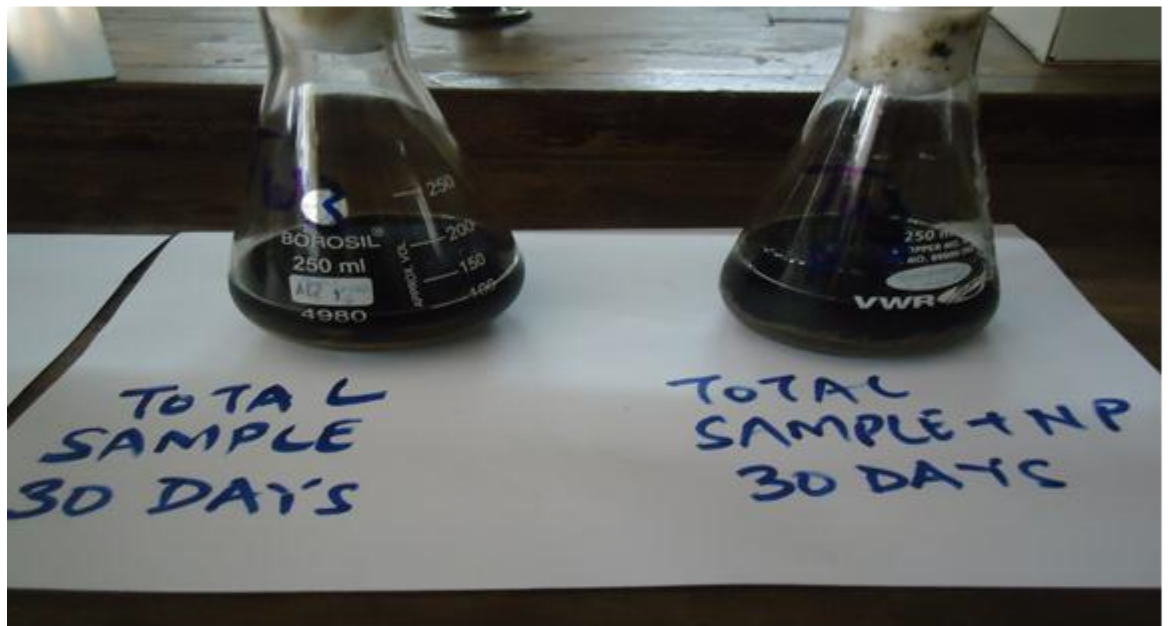


Plate A2: 8 All Samples being incubated in a shaker

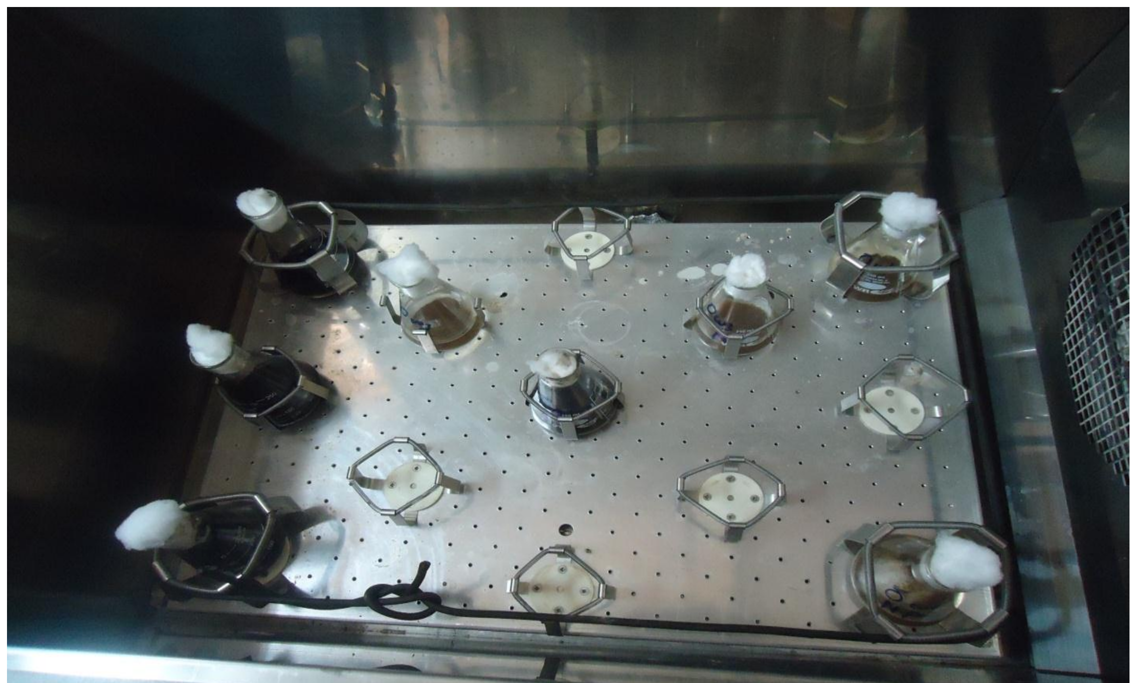


Plate A2: 9 Bacteria Growing on an Agar Plate

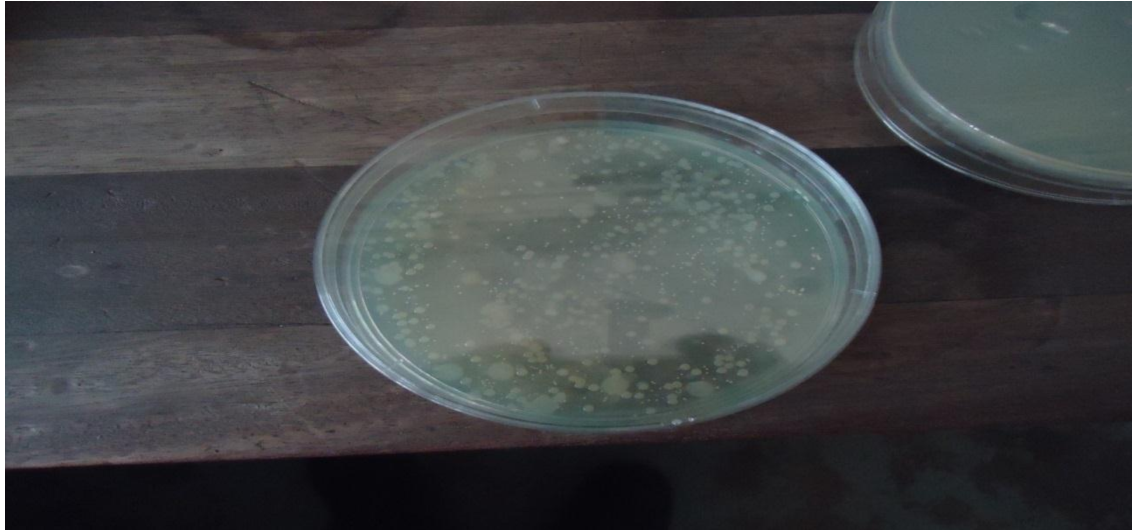


Plate A2: 10 Different Species of Bacteria growing on an Agar Plate