# MODELING BIOREMEDIATION OF A PETROLEUM CONTAMINATED SOIL ENHANCED WITH NPK FERTILIZER AND ANIMAL/PLANT DERIVED ORGANIC MANURE

*BY*

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Modelling bioremediation of a petroleum contaminated soil enhanced with NPK fertilizer and animal/plant derived organic manure. By Udoye, M. C .is licensed under a Creative Commons Attribution-NonCommercial-NoDerivatives 4.0 International License.

## CERTIFICATION

This is to certify that this research project **'Modeling Bioremediation of a Petroleum Contaminated Soil Enhanced with NPK Fertilizer and Animal/Plant Derived Organic Manure'**, was carried out by **UDOYE MAUREEN CHIAMAKA** with registration number **20074636558**, of the Department of Chemical Engineering, Federal University of Technology, Owerri.

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**DEDICATION**

This work is dedicated to our **Blessed Mother Mary**, the mediatrix of all graces.

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| Abbreviations | Meaning | Units |
|---|---|---|
| $C_s$ | Concentration of TPH degraded | mg/kg |
| $C_r$ | Residual Concentration of TPH left in the soil at each time interval | mg/kg |
| Ex-situ | Treament Away from the spot | |
| $F_A$ | NPK Fertilizer | |
| $F_B$ | Poultry Manure | |
| $F_C$ | (Poultry + cow + saw dust) manure | |
| IN-SITU | Treatment on-the-spot | |
| S | Substrate concentration [TPH] | mg/kg |
| $S_o$ | Initial Substrate concentration [TPH] | mg/kg |
| SVE | Soil Vapour Extraction | |
| SVOC | Semi – Volatile Organic Compounds | |
| TOC | Total Organic Carbon | |
| TOM | Total organic Matter | |
| TPH | Total Petroleum Hydrocarbon | mg/kg |
| $\mu$ | Specific growth rate of the microbes. | |
| WHO | World Health Organization | |
| $X_o$ | Initial microbial concentration. | |
| $Y_G$ | Yield coefficient. | |
| $\Upsilon$ | Inverse of the maximum microbial concentration. | |

**ABSTRACT**

In this study, the potential effects of animal derived organic manure (cow dung, poultry droppings), saw dust and NPK fertilizer on the bioremediation of petroleum hydrocarbon contaminated soil was investigated. The rate of biodegradation was studied for the period of 10 weeks under laboratory conditions. The biodegradation data were fitted to eight models, four of which are based on microbial growth rate and the other four based on order of reaction. Results obtained show that bioremediation with NPK fertilizer and poultry manure followed the logistic growth curve with a constant yield i.e (the ratio of microbial population increase per unit substrate consumed being constant). While treatment with blend of poultry+cow+saw dust occurred with the logistic growth curve with varying yield. It was observed that at optimum addition of NPK fertilizer and poultry manure, the process obeyed same trend as observed when a combination of poultry+cow+sawdust was applied. The result obtained showed a significant correlation coefficients well as higher degradation rate constant. It also revealed bioremediation as basically a first order process at low and moderate addition of biostimulants. NPK fertilizer and poultry manure obeyed first order rate model with ultimate contaminant greater than zero. It was also observed that application of NPK fertilizer and animal manure at an increased quantity without combinations offer similar effect with poultry+cow+saw dust i.e both followed second order rate model in which ultimate contaminant zero. Consequently, the result of the percentage degradation of hydrocarbon for the soil sample studied revealed that the rate of hydrocarbon biodegradation was in the following order (83.5%) > (72.6%) > (68.31%) for biotreatment with blend of poultry+cow+sawdust, poultry manure and NPK fertilizer respectively. The observations from the mathematical model, graphical and numerical fits results show that the proposed models employed in this work rather than the usual first order rate model were effective in predicting the bioremediation process.

**Key Words:** Modeling, bioremediation, polluted soil, organic manure, reaction rate order.

## CHAPTER ONE

# INTRODUCTION

### 1.1 Background of the Study

The pollution of soil and subsurface environment by petroleum product is a major concern in the industrial world (Rowell, 1977). This is as a result of frequent industrial activities, rapid industrialization and increasing demand for petroleum hydrocarbons and hydrocarbon derived products. Petroleum spills arise from vandalism, sabotage (people robbing the pipeline of its products for the purpose of making compensation claims as well as to get clean-up contracts) of oil facility sites and installations, corrosion of over aged oil facilities via uncontrolled spillage in oil refineries, and storage tanks that pose inevitable damage to our immediate environment. It is very important to realize that, the discharge of hydrocarbons into the environment by transportation via tankers and barges does not limit crude oil spillage only to oil producing states, but also to neighboring states that are prone to the risk of oil spill due to transportation accidents and ruptured pipeline network that runs across such areas. Oil spill pollution could also result from the sales and uses of petroleum products, pipeline overflow, breakage, and storage tank spill, (Obire, 1996). The contamination of soil by crude oil and petroleum products has become a serious problem that represents a global concern for the potential consequences on ecosystem and human health (Onwurah et al., 2007). This oil spills alters the physicochemical properties of the soil, making it impossible for the soil to produce at its optimal capacity as a result of hardening of the soil structure by the hydrocarbons.

Depending on the degree of contamination and remediation measures taken, such environment may remain unsuitable for crop growth for a very long time. The sustainability of soil is of an immense interest and concern to man because of the direct reliance of man's existence on soil. This, therefore serve as an essential reason why soil quality, fertility and productivity should be continually maintained and monitored.

Finding solutions to oil polluted sites (soil) has always been the subject of several studies (Leahy and Colwell, 1990). A wide range of remediation measures have been preferred with the aim of offering solution to the damages caused by crude oil on nature of the soil and its physiochemical characteristics. Over the decades, the biological methods of cleaning–up the environment have received much attention. This is because of its potential to reduce, detoxify and mineralize chemical pollution, restoring chemical balance at low cost. Bioremediation is defined as the use of living micro-organisms to breakdown or degrade petroleum hydrocarbon into harmless products such as $CO_2$ and $H_2O$. Bioremediation is characterized by lots advantages such as its cost effectiveness, environmental friendliness, simplicity in technology, conservation of soil texture and properties and its ability to produce harmless end products. This is contrary to other physical and chemical treatment methods whose limitations include; transfer of pollutants from one place/phase to another, being a complex technology and expensive to implement at full scale (Vidali, 2001). Due to the limitations of the physiochemical technologies stated above, great deal of literature has reported that bioremediation methods are alternative and/or supplements to these methods. The biostimulants involved in this study include; cow dung, poultry droppings, saw dust and inorganic fertilizers (NPK).

Nigeria is blessed with domestic birds and livestock such as fowl, ram, sheep, cow, goat, and etc. These livestock's produces waste "dungs", and are abound in the cattle markets (i.e., slaughter houses), which are avenue for such dungs, and are considered waste. These wastes are considered useless to the ordinary man. But research has shown that such wastes are useful material to modify the soil physical and chemical properties and also, to release nutrients for a longer period of time. These animal wastes are thus used as biostimulants to provide and maintain favourable conditions for the growth of the soil microorganisms (Allard, 1997). It is important to note that bioremediation is the controlled process of degradation in which microorganisms are used to remove environmental pollutants from soil,

water and other sediments (Pala, 2006). Biostimulation has been proven to be a promising bioremediation technique for the treatment of polluted soil (Rosenberg et al., 1992).

The wood processing business has become a big industry due to increasing demand. As a result, an appreciable amount of wood waste has been generated. These wastes, which were once put in the landfills, have increasingly been made into usable products. Existing wood waste comes from three major sources: 1) municipal solid waste; 2) construction and demolition waste; and 3) wood residues from primary timber processing mills. Sawmill residues that were once considered practically useless and, therefore, often discarded have increased in value due to a demand for these residues as salable products. These residues have been reused in many different ways, including pulp and paper production, nonstructural panels, strand board, domestic and industrial fuel. Despite the remarkable effort that has been made in recycling wood residues, a great amount of these materials are still being unused. For sawdust in particular, dumping in landfills, burning, or simply outdoor storage in piles are common types of waste management. As wood waste generation increases, disposal of this amount of sawdust by land fill requires a fairly large area which is not a wise or recommended solution in the long run. Burning a great amount of this waste will release increasing $CO_2$ gas and other pollutants into the atmosphere. Piling of sawdust can lead to a number of problems in the environment such as $CO_2$ emission due to microbial activity and other complex changes derived from successive activities of biochemical, microbiological, and organic chemical reactions .Therefore, it is necessary to search for an environmentally-oriented solution to this Problem. To date, several practical ways of utilizing sawdust in an environmentally sound manner are: as mulch, as bedding for livestock, in compost, and by direct application into the soil. Since sawdust is an additional plant residue and a rich carbonaceous substance, it holds promise in supplying humus to the soil, once applied in soils; can absorb petroleum metabolites and thus reduce soil toxicity, can enhance biodegradation, addition shows no appreciable negative impact on soil microorganism .For

the purpose of this study, using sawdust as a soil amendment was investigated. It does not only conserve but also improves soil properties most effectively.

Mathematical modeling is an important tool in analyzing and understanding environmental systems and process performance. Wherever many process of physical, chemical or biological nature interact with each other, mathematical models provide a rational frame work to formulate and integrate the knowledge that has been otherwise derived from (i) theoretical work (ii) fundamental (e.g laboratory investigations) and site specific experimental works. Nevertheless, when bioremediation strategies are applied, modeling often regards contaminant degradation (concentration), substrate consumption, and microbial growth rate/counts e.t.c.

In this study, bioremediation experiment on a petroleum contaminated soil was carried out; investigating the effect of inorganic fertilizer (NPK), poultry droppings and the mixture of poultry droppings, cow dung, and saw dust ash towards enhancing microbial biodegradation of hydrocarbon polluted soil. This work seeks to utilize relevant models representing the bioremediation of a petroleum contaminated soil under selected treatments including (poultry droppings, cow dung, saw dust and NPK fertilizer). These were ascertained, using the kinetic rate model and substrate dependent model.

### 1.2 Statement of the Problem

Prior to the period of oil boom, the Niger Deltans were predominantly farmers. They provided the nation with most of the agricultural produce like fish, palm oil etc but now most of these farm produces are being imported.

It is also an obvious fact that even in the case of oil spillages, the communities that were affected are after compensation by the oil company concerned rather than the clean-up of the affected area. Consequently, a lot of spilled sites are left unattended to, thus reducing the available fishing, farming and even building spaces. Again, there are cases of remediation attempts by the oil companies but most of them were inconclusive and abandoned. Based on

the above issues, it is imperative to find a remediation method that will be affordable, and at the same time available to the rural dwellers before oil spillage will turn to a norm in the Niger Delta region.

Quantification of risks and impacts associated with environmental management options, and design of remediation systems are also needed. To achieve this, a reliable predictive tool (usually in the form of numerical simulation models) is required. However, this work tries to assert the application of predictive models to achieve an efficient bioremediation of petroleum contaminated soil.

### 1.3 Objectives of the Study

The main objective of this research is to investigate the biodegradation potentials of (poultry droppings, cow dung, saw dust and NPK fertilizer) on petroleum contaminated soil and evaluate their effects using relevant models.

The specific objectives set out to achieve the aforementioned are as follows:

- To study and compare the effectiveness of NPK fertilizer and animal derived organic manure (poultry droppings, cow dung) and saw dust on the biodegradation of a crude oil contaminated soil.
- To determine the appropriate models to be employed in the bioremediation study.
- To evaluate the model fits and estimate relevant kinetic parameters.

### 1.4 Justification of the Study

It is particularly important to address oil polluted soil as soon as possible as the contamination can have the potential to damage soil resources and affect the health of those animals and humans that consume contaminated food.

Besides the varying rates of biodegradation, researchers have consistently documented a lag time after oil is spilled before indigenous microbes begin to break down the oil molecules (Thies and Rillig, 2009). This lag time is related to the initial toxicity of the volatile fractions of the oil, which evaporate in the first few days of a spill. Microbial populations must begin

to use oil and expand their population before measurable degradation takes place, a period usually lasting several days. This fact becomes very important when considering the appropriateness of bioremediation as a quick or first response technique. Soil pollution is widespread in Nigeria leading to varying forms of degradation and is associated with loss of bioresources especially plant materials. In reaction to this, it becomes imperative to use biological techniques in restoring and resisting further degradation.

This research will help to widen awareness and understanding towards maximizing the effort of the oil industries and private sectors in minimizing release of oil into the environment. It will also equip environmental scientist with adequate knowledge on how to handle soil pollution for a sustainable environment. This can be found useful found/ relevant in many areas such as;

- Environmental clean-up: use of plants and animal waste generated within the environment are converted to useful materials when applied to sediments as biostimulants. This reduces the rate of waste disposed to the environment which might pose subsequent hazards man and his environment.
- Industries where these biostimulants can be used as major soil treatment option can be established, this will help reduce incessant discard of waste and as well create job opportunity for both skilled and unskilled workers.

### 1.5 Scope of the Study

This research pays attention to the investigation of the biodegradation rate of a petroleum contaminated soil using NPK fertilizer, poultry dung, cow dung and saw dust. This was ascertained via two main techniques; establishing relevant models and using bar charts.

The following parameters were be investigated and evaluated;

- Soil pH
- Soil moisture content**.**
- Total organic carbon (TOC)
- Total organic matter
- Soil nutrients -nitrogen (N)

  - Phosphorous (P)

  -Potassium (K)
- Total petroleum Hydrocarbon (TPH)

## CHAPTER TWO

# LITERATURE REVIEW

### 2.1 Environmental Effects of Petroleum

Petroleum causes pollution at every stage, from mining and recovery to refining, transporting, and using it as fuel. Crude oil as it comes out of the ground is composed of many chemicals mixed together, and these must be separated into gasoline, kerosene, diesel fuel, heating oil, and heavier materials. These are just indication of the potential for refineries to leak chemicals into the air, soil, and groundwater; to cause accidental fires and breakages that produce more pollution; and to create sites that are heavily toxic for future generations.

### 2.2 Crude Oil

Crude oil is a fossil fuel; it is formed from decaying plants and animals living in ancient seas millions of years ago. It can also be defined as a naturally occurring, unrefined petroleum product composed of hydrocarbon deposits. Most places you can find crude oil were once sea beds. Crude oil varies in colours, from clear to tar-black, and in viscosity, from water to almost solid. Crude oil is such a useful starting point for so many different substances because they contain hydrocarbons. Hydrocarbons are molecules that contain hydrogen and carbon and come in various lengths and structures, from straight chains to branching chains to rings.

For instance;

- ✓ Hydrocarbons contain a lot of energy. Many of the things derived from crude oil like gasoline, diesel fuel, paraffin wax and so on take advantage of this energy.
- ✓ Hydrocarbons can take on many different forms. The smallest hydrocarbon is methane ($CH_4$), which is a gas that is lighter than air. Longer chains with five or more carbons are liquids. Very long chains are solids like wax or tar.

### 2.2.1 Major Classes of Hydrocarbons in Crude Oil

- ✓ Paraffins: generally formula $C_nH_{2n+2}$ (n is a whole number, usually from 1 to 20) straight – or branched-chain molecules can be gasses of liquids at room temperature depending upon the molecule examples: methane, ethane, propane, butane, isobutene, pentane and hexane.
- ✓ Aromatic: general formula $C_6H_5 – Y$ (Y is a longer, straight molecule that connects to the benzene ring) ringed structures with one or more rings contain six carbon atoms, with alternating double and single bonds between the carbons typically liquids examples: benzene, naphalene.
- ✓ Napthenes or Cycloalkanes: general formula $C_nH_{2n}$ (n is a whole number usually from 1 to 20) ringed structures with one or more rings contain only single bonds between the carbon atoms typically liquids at room temperature example: cylohexane, methyl cyclopentane.
- ✓ Other hydrocarbons alkenes general formula: $C_nH_{2n}$ (n is a whole number usually from 1 to 20) linear to branched chain molecules containing one carbon – carbon double-bond can be liquid or gas examples: ethylene, butane, isobutene Dienes and Alkynes general formula: $C_nH_{2n-2}$ (n is a whole number usually from 1 to 20) linear or branched chain molecules containing two carbon-carbon double-bonds can be liquid or gas examples: acetylene, butadienes, etc.

### 2.2.2 Impact of Hydrocarbon Contamination on the Environment and Human Health

Hydrocarbon spills in the form of petroleum products both on land and in water have been a problem since discovery of oil as a fuel source. They can have devastating effects on the biota of an environment. Oil spills and oil waste discharged into the sea from refineries, factories or shipping activities contain poisonous compounds that constitute potential danger to plants and animals. These poisons can pass through the food web of an area and may eventually be consumed by humans.

Environmental contamination by hydrocarbons and petroleum products constitute nuisance to the environment due to their persistent nature and tendency to spread into the ground and surface water. Environmental pollution by petrochemical products has attracted much attention in recent decades. The presence of various types of automobiles and machinery has caused an increase in the use of motor oil. Oil spillages into the environment have become one of the major pollution problems. Hydrocarbon contamination of the air, soil, freshwater (surface water and groundwater) especially by PAHs has drawn public concerns because many are toxic.

### 2.2.3 Examples of the Impact of Crude Oil in the Environment

- Toxic to humans/fauna/flora by ingestion, inhalation and transport across membrane structures.
- Groundwater contamination
- Physical impact, e.g. Soil structure denaturization, water ingress prevention, increased toxicity levels.
- Physical impact on biota, e.g. coating of avian plumage, blockage of invertebrate respiratory and feeding mechanisms, blockage of sunlight on water surface.
- Prevention of use of amenities.
- Consequential economic /social impacts

Humans can be exposed to hydrocarbon contamination by ingestion, inhalation and dermal contact; effects can be either acute and/or chronic. Acute effects arise from short-term exposure; effects include contact dermatitis, respiratory difficulties, anaphylactic shock.

Chronic effects build up over extended periods e.g. kidney damage, neurological conditions or carcinogenic effects. Also, there are risks such as fire, explosion and/or asphyxiation.

Some of the effects that have been observed on plants and animals, this include; yellowing and death of leaves, reduction of seedlings, and death of the plant. In general, the smaller the hydrocarbon molecule, the more toxic the oil to plants because the smaller the oil molecule

the easier it can penetrate into the plants. The oil molecule enters the plant more easily through stomata or at the point of contact (Baker, 1970). Three factors are related to phytotoxicity of oil:

- The properties of the oil
- The quantity spilled and
- The environmental conditions.

Once a spill has occurred, the oil must be able to penetrate and move within the plant to cause injury. Once inside the plant, the oil travels through intercellular spaces into the plant cells. Within the cell, the oil damages the plasma membrane and cell sap leaks into intercellular spaces (Baker, 1970). This leakage of cell sap causes the leaf to darken and lose turgor. Oils have also been found to affect transpiration, respiration and photosynthetic rates of plants. Transpiration rates were found to be consistently reduced because of the physical interference of oil on or in the leaf tissue. The effects of oil on respiration rates vary with each plant species. Oil also has been found to consistently reduce the rate of photosynthesis, primarily by its physical interference with gaseous exchange similar to respiration reduction. (Ana, 2000) found that when oil is spilled in natural water, shellfish and fin fish are killed by the smothering action of the oil. Fish kills have been known to occur and species of marine organisms differ widely in susceptibility. The ingested oil may interfere with fish nutrition, and gaseous exchange may be limited by the floating oil. The floating oil coats the gills of fishes thereby causing damage and also entangles and kills surface organisms. (Bouwer and Zehnder, 1995) observed some adverse effects of the NAOC Oshika oil spill on plankton, micro-invertebrates and tetragonicity in the shrimp Desmocarls. The investigators reported that eggs and larva of aquatic organisms are more sensitive to crude oil toxicity than adults, and that crustaceans are more susceptible than most other adults, and that crustaceans are more susceptible than most other groups of aquatic animals. The oil in the feathers of birds

affects their ability to swim, fly and maintain body warmth. In other words, they will succumb to the combined effect of poisoning, freezing and drowning.

### 2.2.4 Factors Affecting Hydrocarbon Concentration and Mobility

The persistence of the contaminant in the environment is dependent upon the initial composition and concentration of the hydrocarbon contamination and other environmental parameters in processes known collectively as Natural Attenuation. Natural Attenuation involves the physical processes, the biological action (biodegradation), and any combination of these processes such as Climatic conditions examples are the evaporation, flooding, Temperature, availability of moisture and oxygen etc.

## 2.3 Soil Contamination

Soil contamination has been recognized by the United Nations as a major problem throughout the world. Developing countries wanting to top into World Bank Funding must first demonstrate a desire to improve their environmental record, and this often involves cleaning up their contaminated soil sites and improving their drinking water sources in conjunction with or prior to receiving World Bank Funding. Much of the soil pollution in the world has been caused by hydrocarbon or oil-related substances called volatile organic compounds (VOCs). An organic compound is considered volatile if it produces a vapor or gas at room temperature and normal atmospheric pressure. Some of these fumes are dangerous to humans when inhaled in great quantities or over a long period of time, and also form harmful ozone. Contaminants in the soil can adversely affect the water table and pollute the drinking water so necessary for life.

### 2.3.1 Source of Soil Contaminants

The releases chemicals into the environment in some cases are deliberate and well regulated (e.g. industrial emissions) while in other cases they are accidental and largely unavoidable (e.g. oil/chemical spills). Many of these compounds are toxic to both terrestrial and aquatic environments. Environmental studies in Nigeria reveal that the development and production processes in the oil industries require an urgent need to plan, protect and prudently utilize the

environmental resources for a better environment for man. These indicate that subtle changes occur in the Nigeria aquatic and terrestrial ecosystems due to the activities of the oil industries. Most of the environmental changes as earlier mentioned occur from the release of crude oil into the environment.

The major sources of crude oil pollution include:

- Oil tanker road accidents
- Blow-outs from oil wells
- Leaks from cracks in pipelines
- Oil dumped into harbours resulting from tank washing
- Leakages into refinery cooling water/process leaks
- Human error during plant operation
- Equipment malfunction
- Sabotage
- Tank ruptures
- Well simulation or drilling activities

## 2.4 Oil Eating Microbes and their Life Span

- Microbes, which live on oil, can be collected from natural water and soil sources from all over the world. They are selected based on their particular affinity for eating up hydrocarbon-based compounds or products. Remediation time vary from several hours to several weeks depending on the type and concentration of the hydrocarbon. The lifespan of the microbes depends on the duration of degradation of the hydrocarbon. This is so because the microbes either die off or reduce significantly in population after the contaminants have completely broken down.
- Organisms for Bioremediation are listed in the table 2.1 below.

**Table 2.1 Common Organism for Bioremediation (Okpokwasili, 2005)**

| Types of Contaminant | Genus |
|---|---|

| | |
|---|---|
| Petroleum | Pseudomonas, proteus, Bacillus, Pencillum, Cunninghamella |
| Aromatic Rings | Pseudomonas, Achromobacter, Bacillus, ArthrobacterPencillum, Aspergillus, Fusarium , Phanerrocheate |
| Cadmium | Staphylococcus, Bacillus, Pseudomonas, Citrobacter, Klebsiella, Rhodococcus |
| Sulfur | Thiobacillus |
| Chromium | Alcaligenes, Pseudomonas |
| Copper | Escherichia, Pseudomonas |

### 2.4.2 Predominant Microorganisms Responsible for the Degradation of Petroleum Hydrocarbons

Bacteria and fungi make the most major contribution to the mineralization of oil pollutants (Abed et al., 2001). The bacteria most commonly encountered are the Gram-negative species of the alpha proteobacteria group, such as species of *Pseudomonas*, *Sphingomonas*, *Moraxella*, *Acinetobacter*, *Alcaligenes*, and *Proteus*. Other important groups are the low G+C Gram-positives, such as *Bacillus* and *Micrococcus*, and the high G+C Gram-positives, particularly the actinomycetes (Amund, 2000; Parales et al., 2003). *Pseudomonas* species are often isolated from hydrocarbon-contaminated sites and hydrocarbon-degrading cultures. Members of this genus have a broad affinity for hydrocarbons and can degrade selected alkanes, alycyclics, thiophenes and aromatics (Allen et al., 1997). Polycyclic aromatic hydrocarbons (PAHs) are among the most recalcitrant components of crude oil (Kanaly and Harayama, 2000). The isolated crude oil degraders belong to the genera *Micrococcus*, *Corynebacterium*, *Bacillus*, *Enterobacter*, *Pseudomonas*, *Alcaligenes*, *Flavobacterium*, *Moraxella*, *Aeromonas*, *Acinetobacter* and *Vibrio*. The flora reflects the normal heterotrophic bacteria present in soil, and native genera seem to be crude oil utilizers. Several other workers also reported on the above genera as hydrocarbon-degrading microorganisms (Atlas, 2006; Leahy and Colwell, 1990).

Table 2.2 below summarizes information on some commercially available bacterial and fungal strains used for petroleum hydrocarbon bioremediation. The bioremediation capacity

of bacteria has been investigated more extensively because they are (1) easier to culture, (2) more amenable to molecular biology techniques, (3) capable of metabolizing chlorinated organic, and (4) capable of mineralizing these chemicals and using them as carbon energy sources (Bouwer and Zehnder, 1993). Although capable of metabolizing some aromatic contaminants, fungi require a primary growth substrate, such as glucose or cellulose to co-oxidize these compounds. However, because fungi cannot further metabolize the products of co-oxidation, mixed cultures with bacteria are required for complete mineralization of the organic contaminant (Bouwer and Zehnder, 1993)

Table 2.2: Available bacterial and fungal strains used in bioremediation (Korda et al., 1997).

| **Name** | **Description** |
|---|---|
| **HYDROBAC** | |
| Pseudomonas, Rhodococcus, Arthrobacter | Biosurfactant-producing bacteria |
| P. oleovorans | Naphthalene-degrading bacteria |
| Acinetobacter calcoaceticus MM5 | Bacterial species |
| Pseudomonas fluorescens 2a | Bacterial species |
| Candida sp. | Fungus |
| Candida tropicalis VSB-637 and Mycococcus lactis | Bacterial and fungal species |
| Acinetobacter oleovorum subsp. paraphinicum | Bacterial and fungal species |
| VSB-576 and Candida guilliermondii subsp. paraphinicum VSB-638 (pair)a | |
| Trichoderma sp. AP-5 | Fungus |
| Rhodococcus erythropolis | Bacterial species |
| Bacillus sp. | Petroleum-degrading bacterium |
| BB-232 | Petroleum-degrading bacterium |
| Pseudomonas putida, and Geotrichum candidum | Mixed bacteria/fungi culture |

## 2.5 Remediation

Remediation of contaminated land is necessary when the results of risk assessment defined the land as harmful to the environment media; air, land and water. Harm is damage or destruction to receptors – humans, fauna, flora and natural environment.

**Remediation approaches typically include:**

- Excavation
- Containment and
- Treatment-based technologies:
  - Physical processes
  - Biological methods
  - Natural attenuation
  - Chemical processes
  - Thermal processes e.t.c

Remediation may also be effected by use of a single, or a combination of approaches.

### 2.5.1 Biological Methods Utilized for the Contaminated Land Remediation Depend on One or More of the Four Basic Processes;

- Biodegradation
- Biological Transformation (Biotransformation)
- Biological Accumulation (bioaccumulation)
- Biological Mobilization

### 2.5.2 Biodegradation

This is a series of metabolic processes that affect the decomposition of organic compounds into smaller simpler chemical subunits, catalyzed largely through the action of microorganisms - bacteria and/or fungi. Since the organic compounds are converted into different forms, the process is also known as *bioconversion.* Plants also can cause biodegradation reactions (universally termed phytoremediation), but they are more suited for uptake and accumulation reactions. Inorganic contaminants (metal, non-metals, metal oxyanions, and radionuclides) cannot be biodegraded, but their environmental mobility can

be altered through oxidation-reduction, sorption, methylation and precipitation reactions mediated by microorganisms or plants.

### 2.5.3 Biotransformation

This is the conversion of a contaminant to a less toxic and/or less mobile form by the biodegradation process directly, or as a chloroalkanes into alkane and chloride ion; and the example of consequential decontamination of water-soluble heavy metals, by precipitation as virtual insoluble sulphide forms, the sulphide having been generated as a result of microbial reduction of sulphate.

### 2.5.4 Bioaccumulation

This is the accumulation of contaminants within the tissues of biological organisms; this mechanism may be exploited to concentrate contaminants into harvestable biomass.

### 2.5.5 Mobilization

This is the bioconversion of contaminants into more readily accessible varieties, such as water soluble forms or gases, which facilitates subsequent removal and recovery or destruction.

These processes are the basis for potential site cleanup technology; thus, bioremediation is the intentional use of biodegradation or contaminant accumulation processes to eliminate environmental pollutants from sites where they have been released.

## 2.6 Bioremediation

Bioremediation is concerned with the biological restoration and rehabilitation of historically contaminated sites and with the cleanup of areas contaminated in more recent times, either accidentally or incidentally, as a result of the production, storage, transport, and use of organic and inorganic chemicals.

Bioremediation is one of the most viable options for remediating soil contaminated with organic compounds that has been considered detrimental to environmental health. Bioremediation is a process defined as the use of microorganisms/plants to detoxify or remove organic and inorganic contaminants from the environment. It offers the possibility of

degrading, removing, altering, immobilizing, or otherwise detoxifying various chemicals from the environment through the action of bacteria , fungi (Gadd, 2001) and plants (Morel et al., 2002). The main advantage of bioremediation is its reduced cost compared to other techniques. Besides cost-effectiveness, it offers a permanent solution, which may lead to complete mineralization of the pollutant. Furthermore, it is a non-invasive technique, leaving the ecosystem intact. Bioremediation can deal with lower concentration of contaminants where the cleanup by physical or chemical methods would not be feasible. Bioremediation can be effective only where environmental conditions permit microbial growth and activity, its application often involves the manipulation of environmental parameters to allow microbial growth and degradation to proceed at a faster rate.

There are two techniques for utilizing bacteria to degrade petroleum in the aquatic and terrestrial environments. One of these methods is biostimulation which uses the indigenous bacteria, which are stimulated to grow by introducing nutrients into the soil or water environment and thereby enhancing the biodegradation process. The other method, bioaugmentation, involves culturing the bacteria independently and then adding them to the site.

### 2.6.1 Bioremediation Techniques

Bioremediation of a contaminated soil can be carried out in different ways;

**In-Situ Bioremediation**

In the case of in-situ techniques, soil and associated groundwater are treated in place without excavation (Blackburn et al., 1993). In situ bioremediation is a very site specific technology that involves establishing a hydrostatic gradient through the contaminated area by flooding it with water carrying nutrients and possibly organisms adapted to the contaminants. Water is continuously circulated through the site until it is determined to be clean.

The average time frame for an in situ bioremediation project can be in the order of two years depending on the levels of contamination and depth of contaminated soil due to the poor

mixing in this system, it becomes necessary to treat for long periods of time to ensure that all the pockets of contamination have been treated.

Examples of in situ techniques include;

Pump and treat, percolation (flooding), bioventing, air sparging, bioslurging and permeable reactive barriers, (Singh, 2006). In-situ-remediation ranges from partially closed (contained) systems to completely open ones such as oil spills, e.g. Exxon Valdez case.

**Bioventing**

Bioventing is the process of injecting air (i.e., aeration) to an unsaturated soil zone through the installation of a well(s) connected to associated pumps and blowers which with vapour extraction. A vacuum is applied at some depth in the contaminated soil which draws air down into the soil from holes drilled around the site and sweeps out any volatile organic compounds. The development and application of venting and bioventing for in situ removal of petroleum from soil have been shown to remediate approximately 800 kg of hydrocarbons by venting, and approximately 572 kg by biodegradation (Reddi and Inyang, 2000).

- This is similar to biostimulation. It is not suitable for removing halogenated gases that contribute to ozone layer damage.
- May not work well in clays or in highly layered subsurface environments because $O_2$ cannot be evenly distributed throughout the treatment area.

**Biosparging**

Air sparging or biosparging involves the injection of air into the saturated zone of a contaminated soil, at low flow rates (<5 $m^3$/h per point) ( Buckingham, 1981). This is used to increase the biological activity in soil and to promote aerobic biodegradation by increasing the $O_2$ supply via sparging of air or oxygen into the soil. In some instances air injections are replaced by pure oxygen to increase the degradation rates. However, in view of the high cost of this treatment in addition to the limitations in the amount of dissolved oxygen available for microorganisms, hydrogen peroxide ($H_2O_2$) is introduced as an alternative, and it is used on a

number of sites to supply more oxygen (Reddi and Inyang, 2000). In biosparging volatilization is typically less than that of the standard air sparging system.

This is used to increase the biological activity in soil by increasing the $O_2$ supply via sparging air or oxygen into the soil. In some instance, air injections are replaced by pure oxygen to increase the degradation rates. However, in view of the high cost of this treatment in addition to the limitations is the amount of dissolved oxygen available for microorganisms, hydrogen peroxide ($H_2O_2$) was introduced as an alternative, and it was used on a number of sites to supply more oxygen. The $H_2O_2$ put into the soil would supply ~ 0.5mg/l of oxygen from each mg/l of $H_2O_2$ added, but a disadvantage comes from its dangerous toxicity to microorganisms even at low concentrations.

### 2.6.2 On-site (Ex-situ) Bioremediation

In the case of ex-situ techniques, soil and groundwater are removed from their original locations for treatment. Examples of ex-situ techniques include land farming, irrigation, compost piles, engineered biopiles, and ex-situ slurry techniques (Sinkkonen, 2000).

Here, the contaminated soil is excavated/ removed from their original location and placed into a lined treatment cell. Thus, it is possible to sample the site in a more thorough and, therefore, representative manner. On-site treatment involves land treatment or land farming, where regular tilling of the soil increases aeration and the supplement area is lined and dammed to retain any contaminants that leak out. This process allows for better control of the system by enabling the engineering firm to dictate the depth of soil as well as the exposed surface area.

As a consequence of the depth and exposed surface area of the soil being determined, one is able to better control the temperature, nutrient concentration, moisture content and oxygen availability. Examples of ex-situ techniques include excavation, land farming, Irrigation, compost piles, engineered biopiles, and ex-situ slurry techniques.

#### 2.6.2.1 Excavation

Excavation (removal) is a fundamental remediation method involving the removal of contaminated soil/media, which can be shipped off-site for treatment and/or disposal, or treated on-site when contaminants are amenable to reliable remediation techniques. Excavation is generally utilized for localized contamination and point source and is also used for removal of underground structures that are out of compliance or have been identified as a potential or actual point source of contamination. The limiting factor for the use of excavation is often represented by the high unit cost for transportation and final off-site disposal. A common practice is to place the soil as a shallow layer within a bermed and lined treatment cell (or biocell), occasionally amend the soil with nutrients and water to stimulate biodegradation, and regularly till (aerate) the soil to mix and aid contaminant volatilization (Yeung, 1997).

#### 2.6.2.2 Land Farming

Land farming is land treatment of soil for degradation or transformation of contaminants by a combination of volatilization and biodegradation by indigenous microorganisms.

This is a process where contaminated soil is spread out over a large area, usually the top of a liner material, and tiled like farm soil. This tilling action allows the sun and rain to evaporate and wash the contaminants from the soil. Runoff water must be captured and treated in some cases (Singh, 2006). The advantages of this treatment method are its relative low cost and simplicity, whereas the disadvantages are the need for large soil bed areas and the atmosphere gets the contaminants in the form of vapor, thus adding to the global warning and acid rain problems in many instances.

#### 2.6.2.3 Soil Washing

This method uses water to flush out contaminants from soils. The process works by either dissolving or suspending contaminants in the wash solution and is often used in conjunction with other physical separation technologies. This method does not destroy or immobilize the contaminants; hence the concentrated soil must be disposed of carefully. Wash water requires treatment and air emissions can be a problem (Liebeg and Cutright, 1999).This technology is

fairly wide-spread in Europe but not so in North America. It appears to work for removing semi-volatile organic compounds (SVOC), fuels and some heavy metals, plus selected VOCs and pesticides. The drawbacks are the length of time, cost and air emission problems.

#### 2.6.2.4 Thermal Desorption

This system uses heat to separate as opposed to absorb) the contaminants from the soil. Early attempts at this technology were in the form of adapting crop dryers and later asphalt plants to perform this separation process. As the need to remediate contaminated sites increased in the 1980s and up to 1996 several companies began manufacturing equipment specific to desorbing hydrocarbon contaminants from the soil. These systems are based on the Rotary Kiln design where soil is conveyed into a cylindrical drum and heat applied either directly or indirectly to the drum as it is rotated (Thomas and Ward, 1989). By thus heating the soil, contaminants are vaporized and driven or sucked off where they can be destroyed in an afterburner or purged through a filtration system. Nicknamed “dirt-burners” approximately 144 of these systems were built during the late 1980s and early 1990s and a number are still in operation worldwide. The thorough-put capacity and speed of remediation made rotary.

#### 2.6.2.5 Compost piles

This consists of soil supplemented with composting material (i.e. wood chips, straw, manure, rice hulls, etc.) to improve its physical handling properties and its water- and air-holding capacities. Although compost piles are exposed to the atmosphere, the interior is often anaerobic due to the oxygen demand of the contaminants and amendments. Thus, air is drawn through the compost (by vacuum, although aerated piles have been used to enhance the drainage) to supply $O_2$ to the soil for promoting aerobic degradation of organic material and remove evaporated water. Compost piles are subjected to intermittent mixing using specially manufactured equipment that is capable of turning the pile over onto itself. Temperatures can increase to 60 – 70°C due to the exothermic nature of biodegradation, and mixing, aeration, and moisture addition help dissipate excess heat that could be inhibitory to biodegradation (Fontes et al., 1991). One advantage of composting is that it is more effective than other

solid-phase treatment systems for soils and sludge contaminated with viscous substances such as coal tar, creosote, or petroleum production and still bottoms. Soil treatment using composting systems is limited to biodegradable chemicals. The technology cannot treat metals and most other inorganic chemicals. Additionally, the technology cannot readily biodegrade halogenated chemicals. However the composting system effectively remediates soils that are heavily contaminated and cannot be treated by in-situ methods as well as wastes containing hazardous volatile constituents untreatable by land farming methods (Lees, 1995).

#### 2.6.2.6 Biopile

Biopile is a remediation technique that involves placing contaminated soils into piles or cells above ground, and stimulating aerobic or anaerobic microbial activity within soils through controlled aeration and/or addition of minerals and nutrients. Air is supplied to the biopile via a pipe-and-pump system, which either forces air into the pile (positive pressure) or draws air through the pile (negative pressure). Forcing air into the pile helps maintain constant temperature and aerobic conditions, while drawing air out of the pile can create anaerobic conditions. Although composting systems require large amounts of nutrients and bulking agents, fewer additives are needed for biopiles. Biopiles are normally operated at lower temperatures since less organic material is added. Biopiles have some potential limitations. For example, certain chemicals such as polychlorinated biphenyls (PCBs) and other hydrocarbons are resistant to biodegradation. In addition, high concentrations of toxic metals, such as lead, copper, and mercury, may limit biopiles treatment (Lees, 1995).

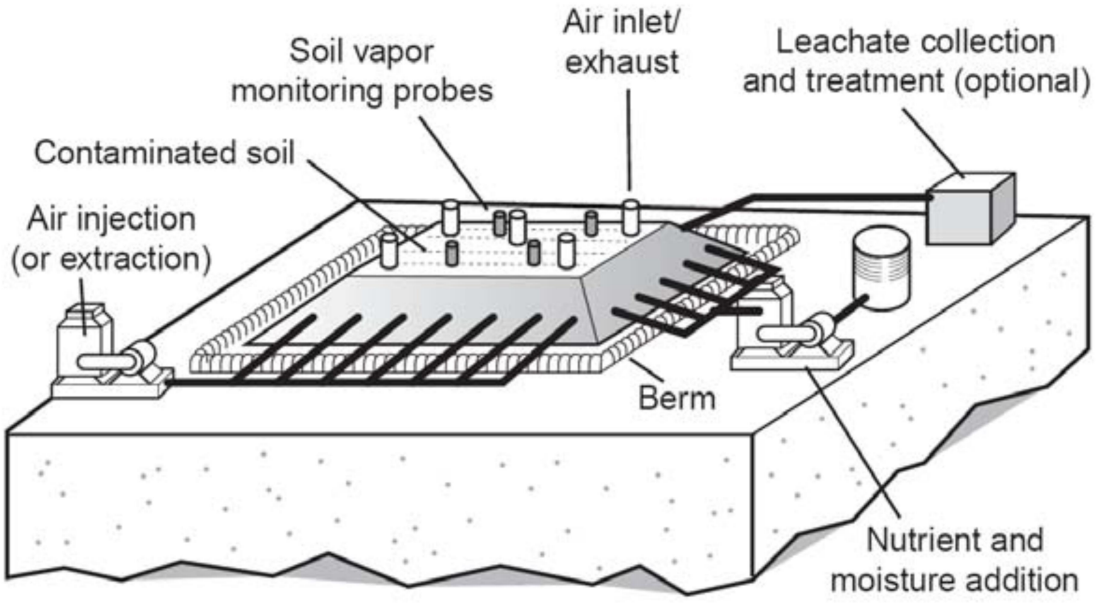


Figure 2.1: Typical biopile system

## 2.7 Biostimulation

Biostimulation is the modification of the environment by adding nutrients, such as nitrogen and phosphorus, as well as oxygen and other electron acceptors to stimulate the rate of biological degradation of contaminants by indigenous microorganisms. This alternative is also chosen when a natural microbial population exists at the site which has the potential to degrade the chemicals, but is actually lacking oxygen, nitrogen or other nutrients to degrade them. The missing component(s) can then be introduced into the system and the degradative activity of the microbial community can be induced. Most bioremediation systems employ some form of biostimulation. This process basically involves the stimulation of indigenous microorganisms to degrade contaminant. This involves addition of nutrients, either organic or inorganic, to enhance the activities of indigenous microbes. The microbial degradation of many pollutants in aquatic and soil environments is limited primarily by the availability of nutrients, such as nitrogen, phosphorus, and oxygen. The addition of nitrogen and phosphorus-containing substrates has been shown to stimulate the indigenous microbial populations (Liebeg and Cutright, 1999). Input of large quantities of carbon sources such as crude oil, used lubricating oil, diesel oil etc, tends to result in a rapid depletion of the

available pools of major inorganic nutrients such as nitrogen and phosphorous. Levels of Nitrogen and Phosphorous added to stimulate biodegradation of contaminated sites are often estimated from C/N ratios (Reddi and Inyang, 2000).

Biostimulation aims at enhancing the activities of indigenous microorganisms that are capable of degrading pollutant from soil environment, it is often been applied to the bioremediation of oil-contaminated soil. In some instances, manure, wood chips and straw may provide microbes with the sources of carbon as a fertilizer. The concept of biostimulation is that, by adding more nutrients; microorganisms replicate, increase in number and grow rapidly and thus increase the rate of biodegradation. Addition of inorganic nutrients, do act as fertilizer to stimulate biodegradation by autochthonous microorganisms in some cases; in other cases, it is the intentional stimulation of resident xenobiotic-degrading bacteria by use of electron acceptors, water, nutrient addition, or electron donors. Combinations of organic nutrients often are more effective than single nutrients. Biostimulation can also be achieved by the use of composting bioremediation technologies. Composting bioremediation strategy relies on mixing the primary ingredients of compost with the contaminated soil, such that as the compost matures, the pollutants are degraded by the active microflora within the mixture. Organic wastes like banana skin, spent mushroom compost and brewery spent grain in earlier studies were found to enhance the biodegradation of used lubricating oil within the period of three months (Abioye et al., 2009). Depending on the nature of the contaminated soil, some of these nutrients could become limiting, hence the additions of nutrients are necessary to enhance the biodegradation of oil pollutants. In a study using poultry manure as organic fertilizer in contaminated soil, biodegradation was reported to be enhanced in the presence of poultry manure alone, but with substrates or surfactants (Okolo et al, 2005). However, excessive nutrient concentrations can inhibit the biodegradation activity, and several authors have reported the negative effect of high NPK levels on the biodegradation of hydrocarbons more especially on the aromatics.

## 2.8 Bioaugmentation

Bioaugmentation is the soil amendment with non-indigenous allochthonous microorganisms or cultivated indigenous species or engineered microbes via inoculation, or use of bioproducts, (e.g. enzymes i.e. lipases, proteases, cellulases etc.), to enhance the biodegradation of contaminants (Buckingham, 1981). Most microbial inoculants or additives sold for use in bioaugmentation approaches have historically been blends or consortia of microorganisms, purportedly tailored for the types of compounds found in the target waste stream. Some commercial proprietary bioaugmentation products are branded by the names M-1000TM, (Micro-Bac International, Inc), Bac-Terra, (Microbe Technology Corporation), ABR (Augmented Bioreclamation) Microbial Blends, (SSybron Chemicals, Inc.), WST Bioblends, (Waste Stream Technology, Inc.), PetroKlenz, (Aqualogy BioRemedics), GT-1000, (Bio-Genesis Technologies). Information for these products is limited and originates mainly from the production/application companies. For example, Bac-Terra includes natural organic matter with a blend of microbial consortia capable of working in both aerobic and anaerobic environments, including psychrophilic, mesophilic, thermophilic, bacteriacultures for use at temperatures ranging from 28 to 240°C. Bac-Terra requires soil moisture greater than 20%. PetroKlenz is a dry powder containing specific cultured facultative anaerobes, naturally occurring microbes that were originally derived from soil and have been preserved through advanced drying techniques. The various strains are grown individually in pure culture and compounded together with powdered wetting agents, buffering agents, and other synergists that allow the organisms to readily adapt to the treatment environment. The organisms have been carefully matched to complement each other for the effective biodegradation of hydrocarbons. GT-1000 is a synergistic group of non-pathogenic microorganisms. According to the vendors most of these products have been used successfully in bench, pilot or full scale levels to demonstrate the ability to degrade pollutants

such as benzene, ethylene, toluene, and xylene (BETX), volatile aromatic hydrocarbons, oil and grease, coal tars, phenolic compounds, and chlorinated organic solvents.

Bioaugmentation is the soil amendment with non-indigenous allochthonous microorganisms or cultivated indigenous species or engineered microbes via inoculation, or use of bioproducts, (e.g. enzymes i.e. lipases, proteases, cellulases etc.), to enhance the biodegradation of contaminants (Reddi and Inyang, 2000). This process involves the introduction of pre-selected organisms to the site for the purpose of increasing the rate or extent, or both, of biodegradation of contaminants. The introduced microorganisms augment, but do not replace, the resident microbial population. Usually bacteria with necessary catalytic activities and other required characteristics are injected directly into the polluted site usually together with nutrients. Bioaugmentation can be necessary also in cases where bacteria with the required catalytic activity although present at the site degrade the pollutants incompletely and/or at a very slow rate. Successful microbial inoculation requires a range of factors: (1) the population must be capable of surviving and growing in the new environment; (2) the microorganisms must retain their degradative abilities under the new conditions; (3) the organisms must come in contact with the contaminants; and (4) the electron donors/acceptors and nutrients necessary for microbial growth and contaminant degradation must be made available to the population (Thomas and Ward, 1989). Once the microorganisms are injected into the aquifer, there must be some mechanism for dispersing them throughout the biostimulation zone before they attach to the solid matrix and carry out the degradation reaction of interest. Cell transport within porous media is highly dependent on the characteristics of both the solid media and the microbial cells. Experiments have shown that the conditions that best promote microbial transport in porous media include (in order of their importance) highly permeable media, ground water of low ionic strength, and small-diameter cells (Fontes et al., 1991). Unfortunately the microorganisms that are efficient in the laboratory conditions do not cope well in the real world. Under natural conditions,

laboratory strains face unfavorable nutritional and physicochemical conditions. They have to compete with already established indigenous communities and they have to withstand a variety of predators. Moreover, there is often a mismatch between the normal habitat of the introduced species and the ecological conditions in which they are placed. Finally, when biostimulation and bioaugmentation are used simultaneously, it is a common finding that the added nutrients favour mostly indigenous populations so that they overgrow the introduced species. It seems that in most cases biostimulation with nutrients is often more effective concerning biodegradation rates than bioaugmentation with inoculums (Margesin and Schinner, 2001). The limitation of distributing the exogenous microbial cultures in the subsurface and the question of long-term survivability of these lab-grown cultures under field conditions also discourage bioaugmentation. Bioaugmentation may play a prominent role in bioremediation when the release of genetically engineered organisms will be permitted (Fingerman and Nagabhushanam, 2005).

### 2.9 Monitored Natural Attenuation (MNA)

Natural attenuation, often called intrinsic remediation, intrinsic bioremediation, bioattenuation, or monitored natural attenuation (MNA), consists of unassisted and unenhanced physical, chemical, and biological processes (e.g. biodegradation, abiotic transformations, mechanical dispersion, dilution, evaporation, volatilization and adsorption) that act to limit the migration and reduce the mass, toxicity, mobility, volume, or concentration of contaminants in soil, sediment, or groundwater (Lees, 1995). Natural attenuation, as an in-situ technology, is very important because it is often technically infeasible to clean a contaminated site to regulatory cleanup levels for a variety of conditions including the presence of low-permeability soils, the inability to remove all the contaminants from the individual soil particles etc.

Natural attenuation is usually considered as the ‘baseline option’, and although it takes place without human intervention, the technology is not equal to a “do-nothing” or “no further

action" approach. The difference between the use of "natural attenuation" and "no further action" as a remedial strategy is that natural attenuation requires thorough documentation and extensive monitoring of the role of microorganisms and other additional site characterization and development of a groundwater monitoring phase for an acceptable period of time may be necessary. Natural attenuation, if properly demonstrated, increases the overall protection of the environment by either containment or destruction of contaminants. No further action, on the other hand, implies that no additional investigation is required regardless of whether the contaminants of concern are degrading or migrating. Natural attenuation also serves as (1) an interim measure until future technologies are developed, (2) a managerial tool for reducing site risks, and (3) a bridge from active engineering (i.e., pump-and-treat, vapour extraction, etc.) to no further action. No further action, however, may be preferable to natural attenuation in certain instances. Very low risk situations may be better served since it eliminates the need of continued monitoring and further documentation. Sites with low levels of contaminants or nondiscernible plumes may be better candidates for no further action. Furthermore, very minor releases of hydrocarbons to the subsurface may not be sufficient to support bioremediation (Alvarez and Illman 2006). A site-specific, cost–benefit analysis is required to determine if an active remediation system or MNA would be the most effective remediation option. MNA may be an appropriate cleanup option when the facility can demonstrate that the remedy is capable of achieving specific ground-water cleanup levels in a reasonable cleanup time frame. If MNA is chosen then there are several costs associated with the implementation of it. These costs include modelling contaminant degradation rates to determine if natural attenuation is a feasible remedial alternative, subsurface sampling and sample analysis (potentially extensive) for determining the extent of contamination and confirming contaminant degradation rates and cleanup status. Regular operation and maintenance (O & M) costs are required for monitoring to verify degradation rates and

maintain data on contaminant migration. In some cases, such long-term monitoring is more expensive than active remediation (Lees, 1995).

When natural attenuation is permitted as a remediation strategy, extensive monitoring is required. It is beyond the scope of this paragraph to report extensive strategies for MNA protocols. Interested readers can find information in the literature (Nielsen, 2005). While natural attenuation will not be a suitable remedy for all contaminated sites, it does offer the potential advantages of in-situ technologies:

• Generates less secondary wastes, reduced risk of human exposure during treatment, reduced potential for cross-media transfer of contamination.

• Operates in-situ with minimal site disturbance.

• Can be used in conjunction with other remediation technologies.

• Reduced need for on-site structures associated with cleanup.

• Potentially reduces overall remediation costs.

However, the potential limitations of natural attenuation include (Margesin and Schinner, 2001).

• It is well established as a remediation approach for only a few types of contaminants, (e.g. benzene, toluene, ethylbenzene, and xylene referred as BTEX, oxygenated hydrocarbons, low-molecular-weight alcohols, ketones, esters, and methylene chloride).

• Generally requires longer time frame for remediation, (for many years or decades).

• Requires more involved site characterization and monitoring.

• Toxicity and mobility of transformation products may be greater than that of the parent compound. Some compounds can form hazardous by-products that in some cases can persist in the environment.

• Changes in environmental or site conditions may allow contaminant migration.

• There is a potential for remobilization of previously stabilized metals and radionuclides.

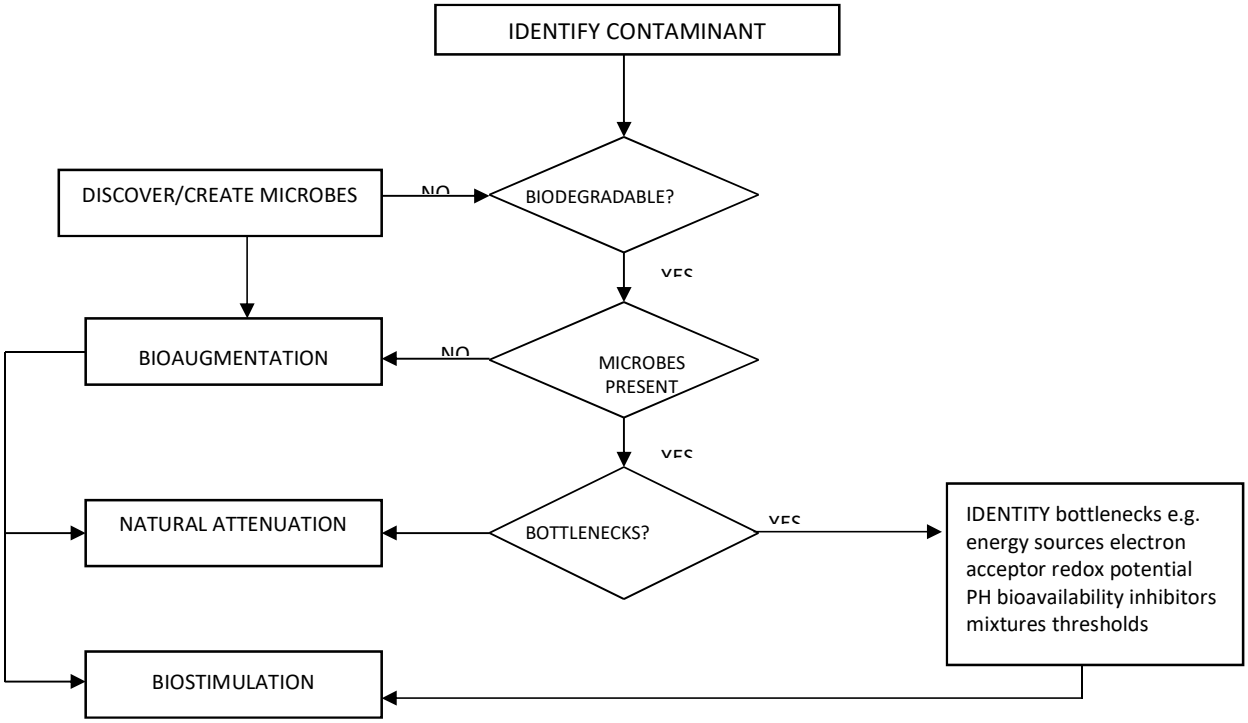


Figure 2.2: Routes to bioremediation, adapted from Spain (2001)

Many compounds have been shown in the laboratory to be readily biodegraded, yet still persist in the environment. Spain (2001) suggests that this may be addressed by manipulating conditions of bacterial communities. A number of factors (Table 2.3) can limit biodegradtion.

Table 2.3: Factors limiting biodegradation (Vidali, 2001)

| **Factors** | **EXAMPLE/ Conditions Required for Microbial Growth** |
|---|---|
| Soil moisture | Water required for microbial metabolism and movement. In excess, can reduce oxygen availability |
| Soil type | Availability of nutrients<br>Sorption of contaminants |
| Aeration | Oxygen required for aerobic metabolism<br>May be needed as a substrate for oxygenases |

| | |
|---|---|
| Redox potential | Terminal electron acceptors for microbial respiration. |
| PH | Affects microbial metabolism<br>Solubility and sorption of contaminants |
| Temperate | Metabolic rates<br>Contaminant solubility, sorption, viscosity, volatilization |
| Availability of contaminant | Low solubility<br>Uptake problems<br>Sorption to soil/sediment<br>Restricted movement of microbes<br>Low concentration of contaminants |
| Metabolic constraints | Lack of appropriate enzymes<br>Requirements for cometabolites<br>Preferential metabolism of alternate sources<br>Toxicity of contaminant |
| Predation of bacterial by protozoa | May reduce bacterial numbers to unsustainable levels |

**2.10 Advantages of Bioremediation**

1. Bioremediation is a natural process and is therefore perceived by the public as an acceptable waste treatment process for contaminated materials such as soil. Microbes that is able to degrade the contaminant increase in numbers when the contaminant is present. When the contaminant is degraded, the biodegradative population declines. The residues for the treatment are usually harmless produces and include carbon dioxide, water and biomass.
2. Theoretically, bioremediation is useful for the complete destruction of a wide variety of contaminants. Many compounds that are legally considered to be hazardous can be transformed to harmless products. This eliminates the chance of future liability associated with treatment and disposal of contaminated material.
3. Instead of transferring contaminants from one environmental medium to another, for example, from land to water or air, the complete destruction of target pollutants is possible.

4. Bioremediation can often be carried out on site, often without causing a major disruption of normal activities. This also eliminates the need to transport quantities of waste off site and the potential threats to human health and the environment that can arise during transportation.

**5.** Bioremediation can prove less expensive than other technologies that are used to clean-up of hazardous waste.

### 2.11 Disadvantages of Bioremediation

Bioremediation, although considered a bone in the midst of present day environmental situations, can also be considered problematic because, while additives are added to enhance the functioning of one particular bacterium, fungi or any other microorganisms, it may be disruptive to other organisms inhabiting that same environment when done in situ (Vidali, 2001). Even if genetically modified microorganisms are released into the environment after a certain point of time it becomes difficult to remove them. Bioremediation generally, can take several months for the remediation to achieve acceptable levels. Bioremediation is limited to those cosmpounds that are biodegradable. Not all compounds are susceptible to rapid and complete degradation.

1. There are some conditions that the products of biodegradation may be more persistent or toxic than the compound.
2. Biological processes are often highly specific. Important site factors required for success include the presence of metabolically capable microbial populations, suitable environmental growth conditions, and appropriate levels of nutrients and contaminants.

**3.** It is difficult to extrapolate from bench and pilot-scale studies to full-scale field operations**.**

### 2.12 Phytoremediation of Hydrocarbon

Phytoremediation is a remediation method that utilizes plants to remove, contain or detoxify environmental contaminants. Phytoremedation appears attractive because in contrast to most other remediation technologies, it is not invasive and, in principle, delivers intact, biologically active soil. The most common plants species used in phytoremediation of organic and inorganic compounds include willows, poplar and different types of grasses. On-site phytoremediation of petroleum hydrocarbons and heavy metals can be enhanced by employing a combination of common agronomic practices (e.g. fertilizer application, tillage and irrigation), this is because available nutrient reserves can be quickly depleted as the microbial community begins to degrade the contaminants.

### 2.13 Phisicochemical, Hydrological and Microbiological Factors that Controls Bioremdiation of Contaminants

To understand the different technologies applied in bioremediation of petroleum contamination, it is necessary to be introduced to the physicochemical, hydrological and microbiological factors that control bioremediation of the contaminant. Therefore, this section outlines the different factors affecting the biodegradation of the petroleum hydrocarbons.

Numerous factors are known to affect both the kinetics and the extent of hydrocarbon removal from the environment. These include the following:

#### 2.13.1 Chemical Composition and Hydrocarbon Concentration

Susceptibility of hydrocarbons to microbial degradation has been shown to be in the following order: n-alkanes > branched alkanes> low-molecular-weight aromatics> cyclic alkenes (Perry, 1984).

Alkanes are usually the easiest hydrocarbons to be degraded by their conversion to alcohol via mixed function oxygenase activity. The simpler aliphatics and monocyclic aromatics are readily degradable, but more complex compounds such as PAHs are not easily degraded and may persist for some time. The persistence will be increased if the compound is also toxic or its breakdown products are toxic to the soil microflora. High-molecular-weight aromatics,

resins, and asphaltenes have been shown to feature a slow rate of biodegradation. High concentration of hydrocarbons in water means heavy undispersed oil slicks causing a limited supply of nutrients and oxygen, and thus resulting in the inhibition of biodegradation. The lowest rates of degradation of crude oil were observed in protected bays, while the highest rates happened in the areas of greatest wave action. Oil sludge contaminating the soil at high concentrations also inhibits microorganisms and their action. This indicates that the quantity of crude oil spilled in soil influences the rate and total extent of disappearance of the contaminant in the soil environment.

### 2.13.2 Physical State

One of the factors that limit biodegradation of oil pollutants in the environment is their limited availability to microorganisms. Availability of the compound for degradation within the soil plays a crucial factor in the determination of the rate of hydrocarbon degradation. Soil, freshwater lakes and marine hydrocarbon-utilizing bacteria have been demonstrated to synthesize and release biosurfactants which greatly enhance their effectiveness in handling uptake of hydrocarbons. Therefore, to overcome this problem surfactants have been added to contaminated soils and sea water to improve access to the hydrocarbons with different chemical dispersant formulations having been studied as means of increasing the surface area thus enhancing breakdown of hydrocarbon pollutants. The chemical formulation of the dispersant (i.e. its concentration and the dispersant/oil application ratio) have been shown to determine its effectiveness in enhancing the biodegradation of oil slicks (Leahy and Colwell, 1990). However, some sources indicated that not all dispersants enhance biodegradation. Soil structure, its porosity and composition, and the solubility of the compound itself will affect availability. Soil particle size distribution also affects microbial growth, so that a soil with an open structure will encourage aeration and thus the rate of degradation will be affected likewise. In addition to that, infiltration of oil into the soil would prevent evaporative losses of volatile hydrocarbons, which can be toxic to microorganisms. Particulate matter can

reduce, by absorption, the effective toxicity of the components of oil, but absorption and adsorption of hydrocarbons to humid substances probably contribute to the formation of persistent residues (Leahy and Colwell, 1990).

## 2.14 Physical Factors

### 2.14.1 Temperature

Soil temperature is one of the most important factors controlling microbiological activity and the rate of organic matter decomposition. Temperatures of both air and soil affect the rate of biological degradation processes in the soil, as well as the soil moisture content. Generally, an increase in temperature increases the rate of degradation of organic compounds in soil. This rate usually doubles for every $10^0$ increase in temperature. Biological activity has an optimum temperature, beyond which biological activity often rapidly decreases, thus displaying a growth curve that skewed to the right. Microbial utilization of hydrocarbons has been shown to occur at temperatures ranging from $2^0$ to $70^0$C. Most soil, especially those in cold climates, contain pyschropholic microorganisms that grow best at temperatures below $20^0$C and are effective at temperatures below $0^0$C. Soils in hot environments usually support many thermophilic microorganisms that are effective at temperatures above $60^0$C. However, most soil microorganisms are mesophiles and exhibit maximum growth in the range of $20^0$C to $35^0$C. The majority of hydrocarbon utilizes are most active in this range. Temperatures in the thermophilic range (50 to $60^0$C) were shown to greatly accelerate decomposition of organic matter in general. At these temperatures, *actinomycetes* will be naturally predominant over fungi and bacteria. Therefore, in certain situations, composting may offer potential for maximizing the biodegradation rate of waste industrials chemicals.

Temperature has a considerable influence on petroleum biodegradation by its effect on the composition of the microbial community and its rate of hydrocarbon metabolism, and on the physical nature and chemical composition of the oil (Atlas,2006).

In some cases the decrease in evaporation of toxic components at lower temperatures was associated with inhibited degradation. (Atlas, 2006) found that the optimum temperature for biodegradation of mineral oil hydrocarbons under temperate climates is in the range of 20-30$^{o}$C.

At low temperatures, rate of biodegradation of oil is discouraged as a result of the decreased rate of enzymatic activities (Atlas, 2006).

### 2.14.2 Pressure

The importance of pressure is confined to the deep-ocean environment where the oil that reaches there will be degraded very slowly by microbial populations.

### 2.14.3 Soil Moisture

Biodegradation of waste chemicals in the soil requires water for microbial growth and for diffusion of nutrients and by - products during the breakdown process. A typical soil is about 50% solid matter. Water entering the soil fills the pore spaces until they are full. Soils with large pores, such as sands, lose water rapidly. Soil with a mixture of pore sizes, such as loamy soils, hold more water at saturation and lose water more slowly. The density and texture of the soil determine the water – holding capacity, which in turn affects the available oxygen, redox potential, and microbial activity.

The amount of water hold in a soil between field capacity and the permanent wilting point for plants is known as available water. This is the water available for plants and a similar quantity may be required for optimum soil microbial and chemical reactions. Generally, with decreasing water potentials, fewer organisms are able to grow and reproduce; and bacterial activity is usually greatest at high water potentials (wet conditions). Some fungi can tolerate dry soil and do not grow well if the soil is wet. Bacteria may be antagonistic to fungi under moisture conditions, at low potentials, bacteria are less active, allowing fungi to predominate.

### 2.14.4 Soil pH

Biological activity in the soil is greatly affected by the pH, through the availability of nutrients and toxicants and the tolerance of organisms to pH variations. Some microorganisms can survive within a wide pH range, while others can tolerate only small variations. The optimum pH for rapid decomposition of waste and residues is usually in the range of 6.5 to 8.5. Bacteria and actinomycets have pH optima near 7.0. A soil pH of 7.8 should be close to the optimum The pH can influence the solubility or availability of micro (especially phosphorus) and micronutrients, the mobility of potentially toxic materials, and the reactivity of minerals (e.g., iron or calcium)(McLean, 1982) . Bacteria on the other hand, grow better at a neutral or slightly basic pH. The pH of different soil types can vary. A calcareous (containing calcium carbonate) soil can range from pH 7 to 8.3. A soil (high in sodium carbonate) can go as high as pH 8.5 to 10. Saline soils tend to be around pH 7. The soil pH may need to be lowered by adjusting with sulfur or other acid – forming compounds, or raised by adding crushed limestone or lime products to bring it between pH 5.5 and 8.5 to encourage microbial activity. The overall biodegradation rate of hydrocarbons is generally higher under slightly alkaline conditions. So appropriate monitoring and adjustments should be made to keep such systems in the pH range of 7.0 -7.5. The pH of the soil is an important factor for anthracene and pyrene degradation activity of introduced bacteria *(Sphingomonaspaucimobilis BA 2 and BP 9).* A shift of the pH from 5.2 to 7.0 enhanced anthracene degradation by *S. paucimobilis* strain BA 2. However, a pH of 5.2 may not lead to total inhibition of activity.

### 2.14.5 Oxygen Supply

The degree to which the soil pore space is filled with water affects the exchange of gases through the soil. Microbial respiration, plant root respiration, and the respiration of other organisms remove oxygen from the soil and replace it with carbon dioxide. Gases slowly diffuse into the soil from the air above, and gases in the soil slowly diffuse into air. However, the oxygen concentration in surface unsaturated soil may be only half that in air,

while carbon dioxide concentrations may be many times that of air. As the soil becomes saturated, the diffusion of gases through the soil is severally restricted. In saturated soil, oxygen can be consumed faster than it can be replaced, and the soil becomes anaerobic facultative anaerobes, which use alternative electron acceptors, such as nitrate (denitrifies), and strict anaerobic organisms become the dominant species. While many soils bacteria can grow under anaerobic conditions, though less actively, most fungi and *actomycetes* do not grow at all. As the oxygen is depleted from soils, the reactions become anaerobic with the production of malodorous compounds, such as amines, mercaptans, and $H_2S$. These can be phytotoxic, and if the soil is heavily over-loaded, the soil may remain anaerobic for sometimes. However, if the oxygen balance is maintained, relative to the amount of contaminants and the soil conditions, rapid aerobic decomposition will occur, and the end products will be inorganic carbon, nitrogen, and sulfur compounds.

In most petroleum-contaminated soils, sediments, and water, oxygen usually is the limiting requirement for hydrocarbon biodegradation because the bioremediation methods for the reclamation of these contaminated sites are mainly based on aerobic processes. The availability of oxygen in soils, sediments, and aquifers is often limiting and dependent on the type of soil and whether the soil is waterlogged. Oxygen concentration has been identified as the rate-limiting variable in the biodegradation of petroleum hydrocarbons in soil.

### 2.14.6 Soil Texture and Structure

Microorganisms have been shown to be present, often in large numbers, in the entire vertical profiles of sediments in wells several hundred feet deep. The vertical distribution of microorganisms in a soil profile differs greatly as a function of soil type. In different soil types, biomass and activity decline with increasing depth; however, the magnitude and pattern of this decline differed for each soil type. The type of soil will also influence the mobility of microorganisms through the subsurface. Bacteria generally do not move large distances in fine-textured soil (less than a few meters, for example), but they can travel much larger

distances in course-textured or fractured materials. The composition of soil influences infiltration rate and permeability, water-holding capacity, and adsorption capacity for waste components. These, in turn, have an effect on the biodegradability of the contaminating wastes and the ability of microorganism to metabolize the compounds.

### 2.14.7 Organic Matter

Organic material is very important in the soil matrix. The presence of organic materials may have many effects on soil properties, including degree of structure, water-holding capacity, bulk density, mobilization of nutrients (hindering degradation of organic wastes), reduction in soil erosion potential, and soil temperature. Soil contains organic material in varying stages of decomposition. Around 65 to 75% of this material usually consists of humid substances which have very large surface areas and high cation exchange capacities. The remainder of the organic material consists of polysaccharides and proteins, such as carbohydrates, protein, peptides, amino acids, fats, waxes, alkanes, and low molecular weight organic acids, which are rapidly decomposed by the soil microorganisms. Organic matter can also contribute to nitrogen, phosphorus, sulfur, Zinc, and boron, all of which add to the nutrient status of the soil. If biodegradable organic materials are added to the soil in order to raise the carbon/ nitrogen ratio higher than about 20:1, mineral nitrogen in the soil will be immobilized into microbial biomass, and the decomposition process will be slowed considerable. Phosphorous is similarly immobilized when carbon is in excess. If the soil must be managed to decompose organic matter during the treatment of hazardous waste-contaminated soil, nitrogen and phosphorus may be required to bring the C.N.P ratio close to that of the bacterial biomass. However, C.N. ratio should be used cautiously, since they do not indicate the availability of the carbon or nitrogen to microorganism.

### 2.14.8 Nutrients

Microbial degradation of hazardous compounds required the presence of certain nutrients for optimum biological growth. Feeding nutrient solutions containing inorganic nutrients, such as soluble nitrogen, phosphorus, and sulfur compound, to natural soil bacteria often enhances

the ability of the microorganisms to degrade organic molecules into carbondioxide and water. Without added nutrients, aromatic hydrocarbons were noted to be more readily attacked than saturated aliphatic hydrocarbons by the microbes (Dibble and Bartha, 1979). Addition of nitrogen or phosphorus stimulated degradation of saturated hydrocarbons more than of aromatic hydrocarbons. These nutrients may be present but may not be readily available or may not supply all that is required. Three of the major nutrients, nitrogen, phosphorus, and potassium, can be supplied with common inorganic fertilizer. Sufficient nitrogen and phosphorus should be applied to ensure that these nutrients do not limit microbial activity. The main danger at hazardous waste sites may be in over-loading the soil with elements that may have been present in the waste or already in the soil, e.g., Phosphorus Plugging, causing toxicity and leaching problem.

### 2.15 Biological Factors

The rate of petroleum hydrocarbon biodegradation in the environment is determined by the populations of indigenous hydrocarbon degrading microorganisms, the physiological capabilities of those populations, plus other various abiotic factors that may influence the growth of the hydrocarbon-degraders (Atlas 2006; Leahy and Colwell, 1990). Leahy and Colwell (1990) reviewed this subject and concluded that hydrocarbon biodegradation depends on the composition of the microbial community and its adaptive response to the presence of hydrocarbons. Among all microorganisms, bacteria and fungi are the principal agents in hydrocarbon biodegradation, with bacteria assuming a dominant role in the marine ecosystems and fungi becoming more important in freshwater and terrestrial environments. Hydrocarbon-utilizing bacteria and fungi are readily isolated from soil and the introduction of oil or oily wastes into soil caused appreciable increases in the numbers of both groups.

Microbial communities with a history of being previously exposed to hydrocarbon contamination exhibit a higher potential of biodegradation than communities with no history of such exposure. The process of getting organisms to be adapted to hydrocarbon pollutants

includes selective enrichment. Such treatment encourages the hydrocarbon-utilizing microorganisms and the build-up of their proportion in the heterotrophic community.

### 2.16 Role of Modeling Bioremediation Processes

Bioremediation is a cost–effective contaminant clean–up method compared to other methods such as landfill disposals or incineration. Site contamination clean-up projects are faced with such question as:

- What are the expected average and maximum contaminant concentration levels?
- What is the time–scale for clean-up?
- What is the sensitivity of say, the duration of the remediation process to changes in physical, biological and chemical conditions?
- What is the probability of failure of the proposed remediation scheme?

The role of modeling in a remediation investigation is more than that of gaining and increased qualitative understanding of the biological system.

Model scale not only affects parameter values but can impact apparent firms of consistence relations. The strength of models based on reasonable mechanistic assumptions is that they may be used for predicting and optimization either for changes in the scale of the process (lab or field scale).

### 2.17 Review of Bioremediation Model Equations

When an organic waste (petroleum hydrocarbon) is discharged into the environment, the content of the effluent undergoes biochemical degradation (Ijah and Antal, 2003). The rate of biodegradation is influence by the concentration of the substrate and product inhibition. Biodegradation is generally classified as aerobic and anaerobic processes. Bioremediation process results in the production of new biomass; carbon dioxide and water.

$$(\text{Soil} + \text{Crude oil})_{\text{mixed}} + \text{Microorganism} \longrightarrow \text{carbon dioxide} + \text{water} \qquad 2.1$$

There are many models of varying complexity that seek to describe bioremediation process. The monod equations is one of such expressions. It is particularly significant in

relating limiting nutrient concentration to population growth rate. The monod equation is stated as follows:

$$\mu = \mu_{max} \{S\}/(Ks + \{S\}) \quad 2.2$$

Where S = Concentration of limiting nutrient

$\mu$ = specific growth rate coefficient

$\mu_{max}$ = Maximum specific growth rate

$k_s$ = half saturation coefficient

In certain cases , these microbial process does not follow the classical model of substrate – limiting biomass growth and product formation by monod. Therefore a logistic equation / approach , (a substrate independent model is used as an alternative empirical function . In many system example in polysaccharide fermentation by micro-organism, cell growth can be characterized by logistic equation (Willey and Sons 2003). The logistic equation can be described as follows:

$$\frac{dx}{dt} = \mu_m \left(1 - \frac{x}{x_m}\right) x \quad 2.3$$

where $\mu_m$ = maximum specific growth rate ($h^{-1}$)

$x_m$ = maximum alternatives biomass concentration (g/l)

Integrating equation using $x = x_0$ at $t = 0$, gives a sigmoid variation of x as a function of t which may represent both an exponential and stationary phase equation.

Other equations such as the Luedeking-Piret equation can be used to describe the kinetics based on product formation .According to the model, product formation rate ($r_p$) depends on both the instantaneous biomass concentration and the growth rate in a linear manner. It is stated as:

$$r_p = \frac{dp}{dt} = \alpha \frac{dx}{dt} + \beta x \quad 2.4$$

where $\propto$ and $\beta$ are product formation constant

Oyoh and Osoka (2007) based on certain assumptions were able to develop some models which they fitted to experimental data from NPK fertilizer enhanced bioremediation. The models include:

- If Microbial growth is exponential and yield is constant (Model 1):

$$S = S_o + \frac{x_0}{Y_G}(1 - e^{\mu t}) \quad 2.5$$

- If Microbial growth is exponential and yield is not constant (Model 2):

$$S_o = S_o\,(e^{\mu t})^{\frac{1}{Y_G}} \quad 2.6$$

- If microbial growth is Logistic growth with constant yield (Model 3)

$$S = S_O + \frac{x_0}{Y_G}\left(1 - \frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right) \quad 2.7$$

- If microbial growth is Logistic growth with yield not constant (Model 4):

$$S_o = S_o\,(\frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})})^{\frac{1}{Y_G}} \quad 2.8$$

Where, $S =$ substrate concentration TPH, (mg/kg)

$So$ = Initial substrate concentration (initial TPH)

$x_0$ = Initial microbial concentration

$Y_G$ = Yield coefficient

μ = Specific growth rate of the microbes

$\gamma$ = Inverse of the maximum microbial concentration.

t = Time (weeks)

This is similar to the semi- empirical equation of the logistics equation used for microbial growth rate dx/dt adding a term that takes into account the population history.This equation is given:

$$\frac{dx}{dt} = Kx(1 - \beta x) + \text{Ko}\int_o^t (t)\,dt \quad 2.9$$

$$\frac{dc}{dt} = \frac{1}{T}\,kx(1 - \beta x) \quad 2.10$$

Where the adjustable parameter K, $K_O$, β and $\gamma$ can be estimated through a non- linear regression techniques $\gamma$ = Yield factor.

## 2.18 Review of Related Works

The research on bioremediation potential has been the subject of several studies. Numerous applications exist where bioremediation has been practiced at laboratory/site level. Some pertinent examples include; the study carried out by Asuka (2014), on the efficiency of plantain peels and guinea corn shaft for the bioremediation of a crude oil polluted soil. The study was carried out for the period of 56 days. The bioremediation data fitted well to a first order reaction rate model and the result reveals that reduction in total petroleum hydrocarbon was highest in guinea corn shaft amended samples compared to the plantain peel amended sample.

Zahad (2011), in his study on the application of carbon- nitrogen supplements from plants and animal sources in in-situ bioremediation of diesel oil exploits the potential effects of saw dust, yam peel and a mixture of cow dung and goat dung used alone or in combination to biostimulate authochthonous microflora for hydrocarbon degradation. The study was carried out for a period of 42days under laboratory conditions and the rate of biodegradation fitted well to a first order kinetic model. Their study confirms that the use of a combination of saw dust+yam peel+cow dung+goat dung+pig dung offered the highest biodegradation efficiency of (97.7%) followed by the mixture of cow dung+goat dung +pig dung (90.5%) , the use of NPK fertilizer (82.3%) then the use of saw dust (69.2%) and yam peel (63.8%).

Similarly, Ofoegbu et al (2015) in their study on the bioremediation of crude oil contaminated soil using organic and inorganic fertilizer made use of a first order kinetic rate model. The experiment was carried out using inorganic NPK fertilizer, cow dung, palm kernel husk ash applied singly and in combination. The degree of bioremediation was observed for a remediation period of 40 days under laboratory conditions. The results obtained showed that a higher biodegradation rate constant exist for amendments with the (cow dung + NPK fertilizer) and the order or remediation from the most treated is (cow dung, + NPK fertilizer)>(cow dung, + palm kernel husk ash)>NPK fertilizer>cow dung used singly.

The percentage degradation of 71.8%, 63.54% and 54% was recorded for biotreatment with (cow dung, + NPK fertilizer),(cow dung, + palm kernel husk ash),NPK fertilizer and cow dung used singly respectively.

The usual first order kinetic rate model was also found to fit well to experimental data in a study carried out by Agarry and Lukman (2013) on the Application of Carbon-Nitrogen Supplementation from Plant and Animal Sources in In-situ Soil Bioremediation of Diesel Oil: Experimental Analysis and Kinetic Modeling. The model revealed that the combination of sawdust, yam peel, cow dung, goat dung and poultry dung elicited higher diesel oil biodegradation with biodegradation rate constant of $0.089day^{-1}$ and half-life of 7.79 days.

## CHAPTER THREE

# RESEARCH METHODOLOGY

### 3.1 Experimentation

#### 3.1.1 Sample/Material Collection

This chapter deals with the experimental procedures, nature and sources of data, analytical methods and model techniques employed in this study.

#### 3.1.2 Description of Study Region

The soil samples used for this study were collected from petroleum polluted site of Agbada flow station. Agbada flow station is located at Mkpokwu manifold, Kpokwudi Community of Rivers State. The oil spill was reported to have occurred in January 2012 while the soil samples were collected from the same site in May 2012 when clean-up exercise has not commenced.

#### 3.1.3 Materials Used for the Bioremediation Study

The following materials were utilized in the course of this study, they include:

- Petroleum contaminated soil
- Inorganic Fertilizer (NPK)
- Cow Dung (CD)
- Poultry dropping (PD)
- Saw dust (SD)

#### 3.1.4 Soil Sample/Manure Collection

Soil samples used for this study were collected with a shovel at a depth of (0-15cm) from a petroleum spilled site of Agbada filling station. The uncontaminated soil sample was collected from an unpolluted site close to the spilled site.

The poultry manure was collected from a local poultry farm situated at Umuchichi of Osisioma Ngwa North L.G.A. in Abia State while the cow dung were obtained from a slaughter market located at Ogbor Hill in Obi Ngwa L.G.A. of Abia State. The NPK fertilizer was purchased from a standard Agrochemical shop at Eke- Akpara Market, Aba while the

sawdust for the treatment come from a timber market, the sawdust was mixed with different types of wood sawdust and it was dry and thin.

### 3.1.5 Soil Sample/Manure Preparation

The soil samples were sun-dried for three weeks after which it was grounded into powdered form then sieved with a 2mm mesh sieve. The sieved soil samples were then used for laboratory analysis.

The cow dung and the poultry droppings were also sun dried for three week after which they were grounded into powdered form. The ground cow dung and the poultry droppings were passed through a 2 mm standard mesh sieve thereafter, some samples were sent to the laboratory for the determination of its minerals contents such as carbon, nitrogen and phosphorus, etc. This was carried out to ascertain the remediating properties of the organic manure used. The saw dust was also sun dried for three weeks. At the expiration of the third week, dried saw dust was passed through a 2mm standard mesh sieve and thereafter, some sample were taken for analysis of some mineral constituents, e.g., carbon, nitrogen, phosphorus and nitrate, etc., as presented in Table 4.1.

### 3.1.6 Experimental Procedure

The bioremediation study took place from the month of May to July 2012. The treatment was subdivided into three options. Each of the treatment options 1-3 constitutes five (5) replicate treatments. The only common proportion of all was the petroleum contaminated soil of 100g. The target was to find out how different ratios of NPK fertilizer, poultry manure and a combination of (poultry manure + cow manure + saw dust) would affect the degradation of a petroleum contaminated soil. The objective of the variation in the treatment levels was to investigate the most appropriate quantity of each treatment option that will give the best remediating result

**The set-up of treatment is as follows:**

Option 1: The five constituent replicates in this option received 10g, 20g, 30g, 40g and 50g of 20:10:10 NPK fertilizer which was applied at two-week interval during the ten week study period.

Option 2: The five constituent replicates in this option is treated with poultry manure. Each of the replicates in this option received 10g, 20g, 30g, 40g and 50g of poultry manure which were applied at two weeks intervals during the ten-week remediation study.

Option 3: The five replicates in this option had the application of a blend of (poultry + cow) manure and saw dust mixed in equal ratio. 10g, 20g, 30g, 40g and 50g of the mixed manure was applied to each replicates at two-week intervals for a period of ten weeks.

It is worthy of mention that several studies have demonstrated the necessity of nutrients and oxygen in bioremediation of oil-contaminated soil, hence all the replicates were supplied with nutrients as stated above with little watering and exposed to oxygen by milled tilling in order to facilitate degradation.

Table 3.1: Shows how the experiment was divided into three treatment options; each treatment had 100grams of contaminated soil plus different proportions of NPK fertilizer, poultry manure and a combination of (poultry manure + cow manure + saw dust).

Table 3.1: Experimental Design for the Bioremediation Study

| **Options** | **Treatment/biostimulants** |
|---|---|
| Option 1 | Contaminated soil + NPK fertilizer ($F_A$) |
| Option 2 | Contaminated soil + poultry manure ($F_B$) |
| Option 3 | Contaminated soil + (poultry + cow) manure + saw dust ($F_c$) |

Table 3.2: Experimental Set-up

| | | Treatment options | | |
|---|---|---|---|---|
| Treatment numbers | Contaminated soil (g) | NPK fertilizer(g) | Poultry manure (g) | Poultry manure+Cow manure+Sawdust(g) |
| 1 | 100 | 10 | 10 | 10 |
| 2 | 100 | 20 | 20 | 20 |
| 3 | 100 | 30 | 30 | 30 |
| 4 | 100 | 40 | 40 | 40 |
| 5 | 100 | 50 | 50 | 50 |

The experimental layout is shown in Table 3.3 below;

Table 3.3: Experimental Layout

| **Treatment Options** | | | |
|---|---|---|---|
| **Treatment number** | **NPK fertilizer (g)** | **Poultry manure (g)** | **Poultry+cow+saw dust manure (g)** |
| 1 | $F_{A1}$ | $F_{B1}$ | $F_{C1}$ |
| 2 | $F_{A2}$ | $F_{B2}$ | $F_{C2}$ |
| 3 | $F_{A3}$ | $F_{B3}$ | $F_{C3}$ |
| 4 | $F_{A4}$ | $F_{B4}$ | $F_{C4}$ |
| 5 | $F_{A5}$ | $F_{B5}$ | $F_{C5}$ |

Where:

$F_A$ - NPK (20:10:10) fertilizer

$F_B$ - Poultry manure

$F_C$ - (Poultry + cow+ saw dust) manure

$F_{A1}$ - 100g of contaminated with 10g of NPK (20:10:10) fertilizer

$F_{A2}$ - 100g of contaminated with 20g of NPK (20:10:10) fertilizer

$F_{A3}$ - 100g of contaminated with 30g of NPK (20:10:10) fertilizer

$F_{A4}$ - 100g of contaminated with 40g of NPK (20:10:10) fertilizer

$F_{A5}$ - 100g of contaminated with 50g of NPK (20:10:10) fertilizer

$F_{B1}$ - 100g of contaminated with 10g of poultry manure

$F_{B2}$ - 100g of contaminated with 20g of poultry manure

$F_{B3}$ - 100g of contaminated with 30g of poultry manure

$F_{B4}$ - 100g of contaminated with 40g of poultry manure

$F_{B5}$ - 100g of contaminated with 50g of poultry manure

$F_{C1}$ - 100g of contaminated with 10g of (poultry + cow) manure + saw dust

$F_{C2}$ - 100g of contaminated with 20g of (poultry + cow) manure + saw dust

$F_{C3}$ - 100g of contaminated with 30g of (poultry + cow) manure + saw dust

$F_{C4}$ - 100g of contaminated with 40g of (poultry + cow) manure + saw dust

$F_{C5}$ - 100g of contaminated with 50g of (poultry + cow) manure + saw dust

This research work was conducted for 10 weeks, during which samples were taken to the laboratory for analysis at two weeks interval.

The pictures below show that the treatments were already mixed and placed according to the treatment numbers and the irreplicates.

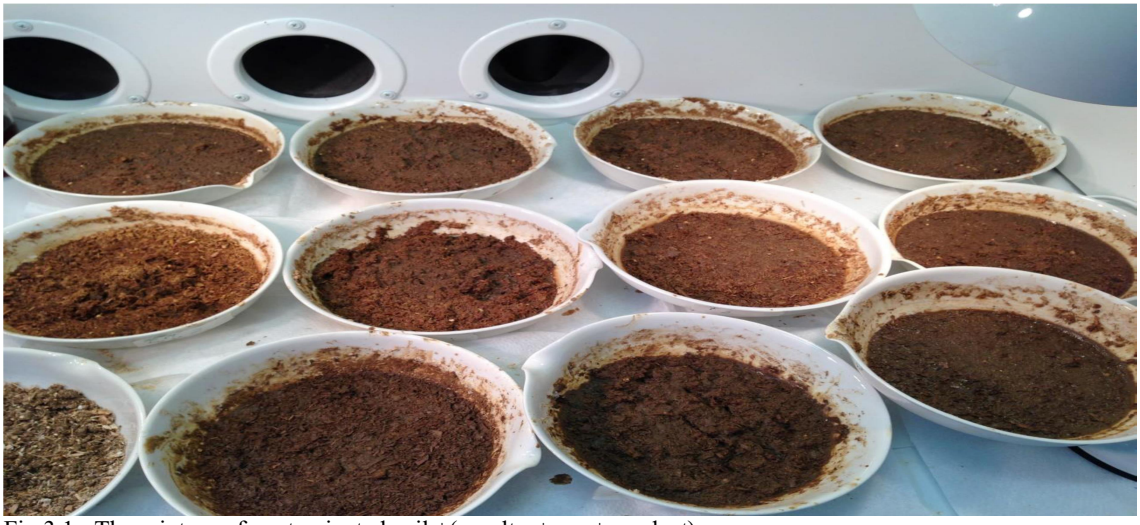

Fig 3.1: The mixture of contaminated soil +( poultry+cow+sawdust) manure

### 3.1.7 Soil Characterization/Physicochemical Analysis

The soil samples and the various biostimulants were characterized for some physical and chemical properties such as soil pH, Total Organic Carbon (TOC), Total Organic Matter (TOM), total Nitrogen (N), total phosphorous (P), total potassium, moisture content, (K), Total Petroleum Hydrocarbon (TPH), etc according to the standard method adopted by the Research and Development Center (RDC) of National Nigeria Petroleum Company (NNPC). The physicochemical parameters are discussed below.

### 3.1.8 Soil pH

Soil pH is an indication of the relative acidity alkalinity of the soil and is an important parameter that affects microbial population/development and therefore the rate at which the entire process proceed. Microbes thrive within a fairly narrow pH range.

For this study soil pH was analyzed using the Orion pH meter model 470 (Anderson, 1989).The pH of the soil was analyzed once in twice ;( at the beginning of the experiment and the end of the experiment) for a period of ten weeks, and it was recorded as variation of pH with time (week). 20g of the air-dried (sieved soil) and the biostimulants were placed into different glass beaker and 50ml of distilled water was added to it. The mixture was allowed to stand for 2 minutes while being stirred gently and occasionally with a glass rod. The electrodes were rinsed with deionized water and wiped dry with a clean tissue or filter paper after each reading while the pH meter was calibrated with pH 7.0 and 4.0 buffers before use. The clean electrode of the Orion pH meter model 407 (Anderson and Lyrem 1989) was inserted into the Suspension and the pH value measured and recorded.

### 3.1.9 Soil Moisture Content

The soil moisture content is an indication of the amount of water contained in the soil. Soil moisture content ranges from 50 – 80% field capacity but varies for Laboratory capacity based on the quantity of soil used and the environmental condition.

In this study a representative samples of moist soil (20.0g) was placed in a clean and dry crucible of a known mass ($W_1$) with the lid securely in position. A weighing balance was then used to determine the “mass of the container and moist soil” ($W_2$), After the lid of the

crucible is removed and placed in an oven maintained at 110 $\pm$ 5°c for four (4) hours to obtain a constant weight of the (crucible + soil). This is allowed to cool in a desiccators and the mass determined and recorded as (%moisture content).

The moisture content of the soil (% moisture content) is calculated as follows:

$$\%M = \left(\frac{W_3 - W_1}{W_2 - W_3}\right) \times 100 \quad 3.1$$

Where; W = moisture content of the soil (%)

$W_1$ = constant (known) weight of crucible (kg)

$W_2$ = Weight of crucible + moist soil (kg)

$W_3$ = Weight of crucible + air-dried soil

### 3.1.10 Organic Carbon

Ten gram (10g) of each of the soil samples were introduced into a 250ml conical flask. 10ml of chronic acid mixture was added to the soil samples in the flask. The flask was heated on a digestion rack for approximately 30mins. The mixture was then allowed to cool just warm and then diluted to 100ml with distilled water. 5ml of indicator solution was added and a tick bluish colour developed. This was then titrated with 0.4ml Ferrous Ammonium Sulphate Solution until a greenish colour is developed. The volume of 0.4ml Ferrous Ammonium Sulphate Solution gave the titre value and the total organic carbon determined using:

$$\%TOC = \left(\frac{N(T-B)}{W}\right) \times 0.0039 \times 100 \times f \quad 3.2$$

Basis: Air-dry basis

Where N = Concentration or Normality of $K_2CrO_7$.

T = Volume of titre (ml)

B = Blank reading

W = Weight of soil sample used

f = 1.33 (correction) factor

### 3.1.11 Phosphorus (P)

Ten gram (10g) of the soil sample was diluted with 10ml distilled water in a 50ml volumetric flask and the solution pipetted into a clean dry test tube. Phenolphthalein indicator solution (0.05ml) was added, and allowed to give a bluish colour. 8ml of combined reagents was

added and the mixture thoroughly mixed. The mixture was allowed to stand for at least 10 minutes. The absorbencies of the samples were taken thereafter at a 700nm wavelength using reagents blank as the reference solution. Phosphorus concentration in the soil was extrapolated from the standard curve of the spectrophotometer and calculated using the relationship.

$$mg\text{P}/1000of\ sample\ =\ \frac{200\times100\ concentration\ from\ graph}{1000} \qquad 3.3$$

### 3.1.12 Potassium (K)

Soil samples were measured at 760nm wavelength. A calibration curve was prepared from the standard range by setting the top standard to a suitable scale deflection and the *ppm* standard to zero. The sample solution was aspirated into the flame under the same condition as the standards. The top zero and intermediate standards were checked frequently. The burner and the atomizer were flushed frequently with water, particularly at the end of each run. The calibration curve was then used to determine potassium (ppmK), in the sample solution. Blank determination using distilled water was carried out in the same way.

Thus, potassium concentration was calculated using;

$$P\ =\frac{C(ppm)\times solution\ volume\ (mc)}{10^4\ samples\ weigh\ (g)} \qquad 3.4$$

### 3.1.13 Nitrate (N)

Here, the Brucine method was used. Calorimetric procedure was applied to measure the yellow nitro derivatives formed between the reactions of nitrate ions with Brucine in the presence of a strong acid solution such as $H_2SO4$. The colour that developed was measured at 470nm wavelength and nitrogen concentration extrapolated from a standard nitrate curve. One gram (1g) of each of the soil sample were weighed, 10ml of distilled water was added to it. 1ml of the solution was pipetted into a clean test tube and then 0.5ml of 2.5% brucine solution in acetic acid was added to the tube.

After this, 2ml of concentrated $H_2SO4$ was added and thoroughly mixed. The tube was then allowed to stand for about 15-30 minutes. The colour developed was measured at 470nm wavelength using distilled water as a blank. The nitrogen concentration in the sample was extrapolated from standard nitrate graph, prepared from, bitrates stock solution of 0.1*mg/ml.* Concentration of nitrogen was then determined using;

$$Nmg/l \ = \frac{N(mg) \times 100}{Aliquot\ volume(m^2)} \qquad 3.5$$

### 3.1.14 Total Petroleum Hydrocarbon Determination

The method employed was the photometric method adopted from Shell Manual of American Petroleum Institute (1980). 20g of each soil sample was mixed with 10ml of carbon tetra chloride solution. This mixture was stirred and separated using a separation funnel into a glass capped container. Clean tap water was added and shaken vigorously until all silt materials in the soil were displayed. The mixture was allowed to stand out and the carbon tetrachloride phase decanted into a clean conical flask. Enough $Na_2S0_4$, (Anhydrous), was added and shaken vigorously to remove all traces of water that may still have been present in the mixture. The resultant clear solution (the absorbance) was analyzed spectro-phometrically at 420nm wavelength using carbon tetrachloride solution as a blank. Hydrocarbon (oil and grease) concentrations in the samples were extrapolated from the standard curve and the total petroleum hydrocarbon (TPH) calculated using the relationship;

$$\%TPH \ = \frac{Concentration\ from\ graph \times \text{T.V.S.E}}{Weight\ of\ sample\ (mg)} \qquad 3.6$$

Where

T.V.S.E. = Total volume of solvent extract (10ml).

TPH = Total petroleum hydrocarbon (mg/kg)

### 3.2 Model Formulation

First order Kinetics is commonly used to describe biodegradation in environment fate model because mathematically the expression can be incorporated easily into models (Greene et al, 2000) . In the same trend, many researchers grasp at first order kinetics because of the ease in presenting and analyzing the data, the simplicity in plotting the logarithm of the chemical remaining versus time as a straight line and the ease in predicting future concentrations.

In a different focus , first order rate model may not be suitable . In this case different models can be formulated to suit bioremediation process and this can be achieved based on several reasonable assumptions.

In this study, the bioremediation can be generally represented as an nth reaction rate order . Thus;

$$\frac{ds}{dt} = -k(s - s_{\infty})^n \qquad 3.7$$

where, S = substrate (contaminant) concentration at any time

S∞ = the ultimate substrate (contaminant) Concentration

K = the reaction rate constant ($week^{-1}$).

t = time (weeks)

n = the order of the reaction (Osoka & onyelucheya, 2010)

**MODEL 1:**

If the reaction order is zero order, equation 3.7 becomes;

$$\frac{ds}{dt} = -k(s - s_{\infty})^0 \qquad 3.8$$

integrating within the limits of $s(t = o) = s_{o,}$ and $s(t) = s$ , we have

$$\int_{s_0}^{s} ds = -k \int_{o}^{t} dt \qquad 3.9$$

$$s - s_0 = -kt$$

therefore;

$$s = s_0 - kt \quad (3.10)$$

Where $s_0$ is the initial substrate (contaminant) concentration.

**MODEL 2:**

If the reaction is first order, equation 3.7 becomes;

$$\frac{ds}{dt} = -k(s - s_\infty) \quad (3.11)$$

Integrating under similar limits as in model 1 above,

$$\frac{ds}{dt} = -k \int_o^t dt$$

$$\ln(s - s_\infty)^s = -k\,t$$

$$\ln\left(\frac{s - s_\infty}{s_0 - s}\right) = -kt \quad (3.12)$$

assuming bioremediation eventually remove all (contaminant) such that the ultimate (contaminant) concentration becomes zero that is ,$s_{\infty=0}$, equation 3.12 becomes

$$\frac{ds}{dt} = \mathrm{e}^{-\mathrm{kt}}$$

$$s = s_0\, e^{-kt} \quad (3.13)$$

**MODEL 3:**

If the ultimate contaminant concentration is not zero, i.e ($s_{\infty\neq0}$)

$$s - s_\infty = (s_0 - s)e^{-k}$$

$$s = s_\infty + (s_0 - s_\infty)e^{-kt} \quad (3.14)$$

**MODEL 4:**

If the reaction is second order, equation 3.7 becomes:

$$\frac{ds}{(s - s_\infty)^2} = -k\,dt \quad (3.15)$$

Integrating within the same limits as in model 1 equation 3.15 becomes:

$$\frac{s_0 - s}{s - s_\infty} = (s - s_\infty)kt \quad 3.16$$

If the ultimate contaminant concentration is zero ($s_\infty = 0$), then;

$$\frac{s_0 - s}{s} = s_0 kt$$

$$\frac{s_0}{s} = 1 + s_0 kt$$

$$s = \frac{s_0}{1 + s_o kt} \quad 3.17$$

Table 3.4: Models Based on Order of Reaction

| **S/N** | Models | **Order** | **Model equation** |
|---|---|---|---|
| 1 | Model 1 | 0 | $s = s_0 - kt$ |
| 2 | Model 2 | 1 ($S_\infty=0$) | $s = s_0\, e^{-kt}$ |
| 3 | Model 3 | 1($S_\infty>0$) | $s = s_\infty + (s_0 - s_\infty)e^{-kt}$ |
| 4 | Model 4 | 2($s_\infty = 0$) | $s = \frac{s_0}{1 + s_o kt}$ |

## CHAPTER FOUR

# RESULTS AND DISCUSSION

### 4.1 Result Presentation

This chapter deals with experimental data analysis and process modeling of experimental data. The analysis includes the interpretation of experimental results and evaluation of the logistic and kinetic model fits.

In this research, laboratory investigations were used to assess the rate of degradation of petroleum contaminated soil medium. The soil parameters that were used to characterize the effect of the various amendments used in this study are presented in table 4.1.

More so the experimental data were fitted to selected mathematical models in order to obtain the appropriate rate model for the degradation of the substrate through the remediation strategies employed in this work. The curve fitting tool of MatLab was used in fitting the data. The model fit results are given below in figure 4.1 - 4.30.

Table 4.1: Results of Nutrient Analysis /Soil Physicochemical Properties before and after remediation.

| BEFORE REMEDIATION | | | | | | AFTER REMEDIATION |
|---|---|---|---|---|---|---|
| **Parameters** | **NPK Fertilizer $F_A$** | **Poultry Manure $F_B$** | **Poultry+ cow+sawdust $F_C$** | **Uncontaminated soil** | **Contaminated Soil sample** | **Contaminated Soil sample** |
| $P^H$ | 6.5 | 7.13 | 7.25 | 6.33 | 4.70 | 8.36 |
| Nitrogen | 0.5 | 0.33 | 0.42 | 0.13 | 0.19 | 0.28 |
| Phosphorous | 1.01 | 0.32 | 0.36 | 6.10 | 3.42 | 5.14 |
| Organic Carbon (%) | 21.3 | 22.2 | 26.2 | 1.34 | 4.03 | 5.35 |
| Organic matter (%) | 4.30 | 6.21 | 9.18 | 3.08 | 4.33 | 5.83 |
| Organic C/N Ratio | | | | 14.6:1 | 25.81 | 34.66 |
| THC (mg/kg) | / | / | | 3.14 | 1980 | |
| % sand | / | / | | 83.31 | 83.10 | |
| % Silt | / | / | | 1.22 | 1.44 | |
| % clay | / | / | | 15.47 | 15.49 | |

### 4.1.1 Results for the Change in Total Petroleum Hydrocarbon (TPH) with time for 10-week bioremediation for the various bio-treatments

Below are the results of the TPH changes for the bioremediation with fertilizer ($F_A$), poultry ($F_B$) and mixture of poultry+cow dung+saw dust ($F_C$), respectively.

Table 4.2: Change in Total petroleum Hydrocarbon (TPH) with time for 10-week bioremediation with NPK fertilizer ($F_A$)

| **Duration/Time (Week)** | **Quantity of Fertilizer ($F_A$) (g)** | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| Week 0 | 1980 | 1980 | 1980 | 1980 | 1980 |
| Week 2 | 1742 | 1502 | 1485 | 1278 | 1193 |
| Week 4 | 1202 | 1114 | 804 | 820 | 714 |
| Week 6 | 960 | 814 | 516 | 590 | 512 |
| Week 8 | 713 | 603 | 401 | 360 | 304 |
| Week 10 | 515 | 446 | 394 | 335 | 298 |

Table 4.3: Change in Total Petroleum Hydrocarbon (TPH) with time for a 10-week bioremediation with poultry manure, ($F_B$)

| **Duration/Week (Time)** | **Quantity of Poultry Manure ($F_B$) (g)** | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| Week 0 | 1980 | 1980 | 1980 | 1980 | 1980 |
| Week 2 | 1563 | 1322 | 1201 | 1155 | 1097 |
| Week 4 | 1021 | 920 | 866 | 733 | 614 |
| Week 6 | 874 | 776 | 603 | 596 | 418 |
| Week 8 | 632 | 593 | 541 | 471 | 206 |
| Week 10 | 545 | 401 | 321 | 302 | 264 |

Table 4.4: Change in Total Petroleum Hydrocarbon (TPH) with time for 10-week bioremediation with (poultry + cow + saw dust) manure ($F_C$)

| **Duration/Time (week)** | **Quantity of (poultry + cow + saw dust) ($F_C$) (g)** | | | | |
|---|---|---|---|---|---|
| | 10 | 20 | 30 | 40 | 50 |
| Week 0 | 1980 | 1980 | 1980 | 1980 | 1980 |
| Week 2 | 1221 | 1173 | 1196 | 1142 | 1063 |
| Week 4 | 916 | 862 | 939 | 714 | 681 |
| Week 6 | 789 | 564 | 743 | 482 | 462 |
| Week 8 | 507 | 406 | 439 | 358 | 213 |
| Week 10 | 426 | 339 | 303 | 296 | 202 |

### 4.1.2 MatLab Comparative Curve Fitting Results

#### 4.1.2.1 Graphical Fit Results for Models Based on Microbial Growth

The graphical fit results for models based on microbial growth are given in figures 4.1-4.15 and the corresponding numerical fits results shown in Tables 4.5 - 4.10

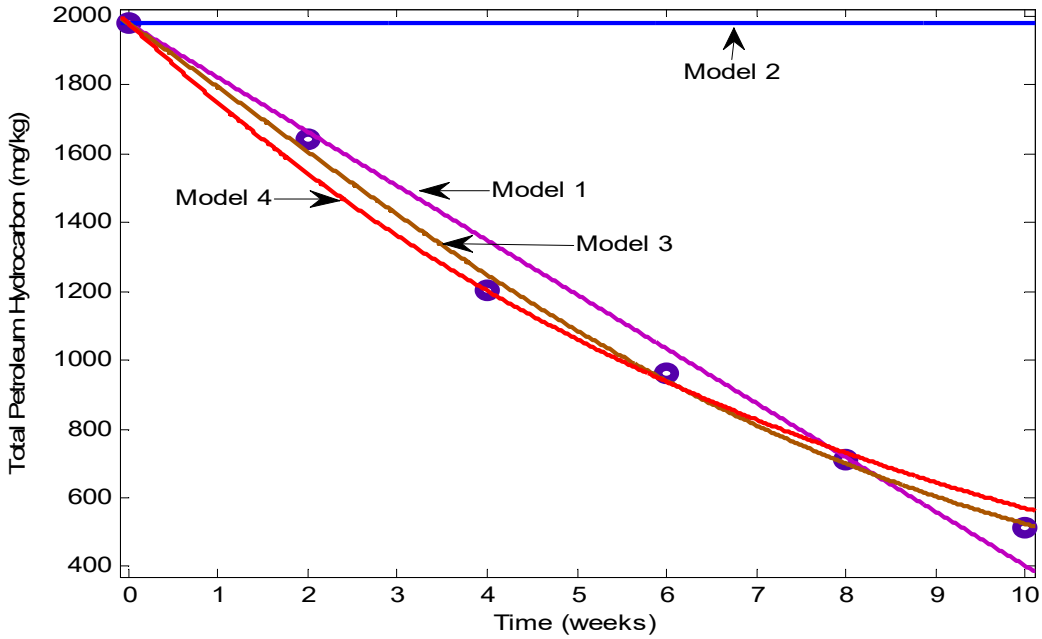


Figure 4.1: Total Petroleum Hydrocarbon versus Time for 10g of NPK fertilizer

where;

- Model 1: $S = S_o + \frac{x_0}{Y_G}(1 - e^{\mu t})$ -- If Microbial growth is exponential and yield is constant.

- Model 2: $S_o = S_o\,(e^{\mu t})^{\frac{1}{Y_G}}$ -- If Microbial growth is exponential with varying yield.

- Model 3: $S = S_O + \frac{x_0}{Y_G}\left(1 - \frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right)$ -- If microbial growth is Logistic growth with constant yield.

- Model 4: $S = S_O \left(\frac{e^{\mu t}}{1-\gamma x_0(1-e^{\mu t})}\right)$-- If microbial growth is Logistic growth with varying yield.

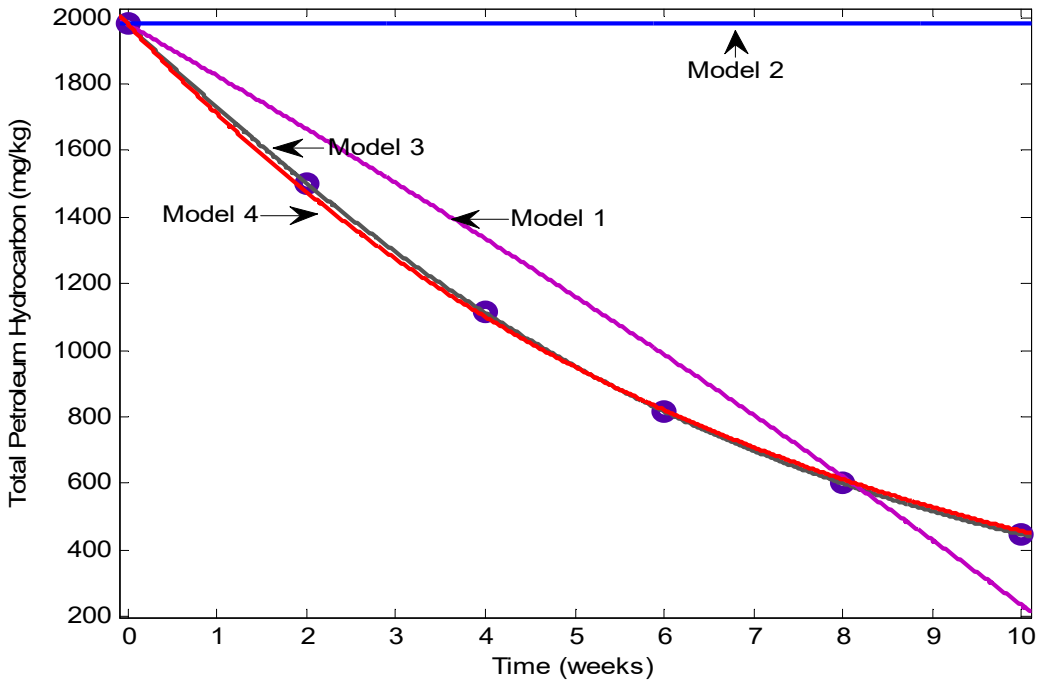


Figure 4.2: Total Petroleum Hydrocarbon versus Time for 20g of NPK fertilizer

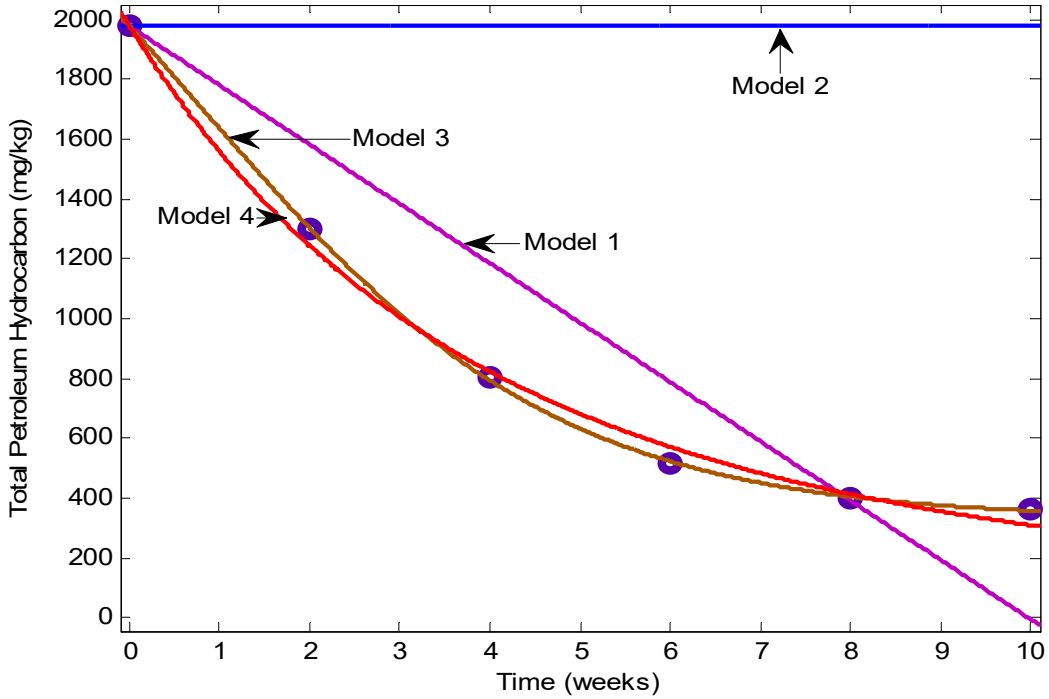


Figure 4.3: Total Petroleum Hydrocarbon versus Time for 30g of NPK fertilizer

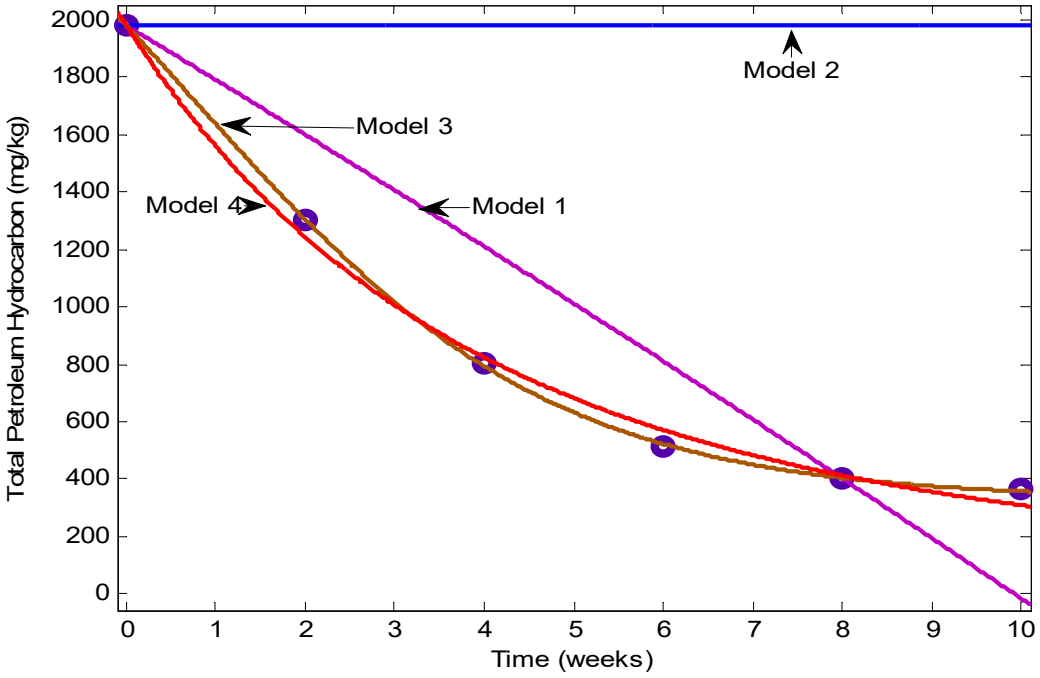


Figure 4.4: Total Petroleum Hydrocarbon versus Time for 40g of NPK fertilizer

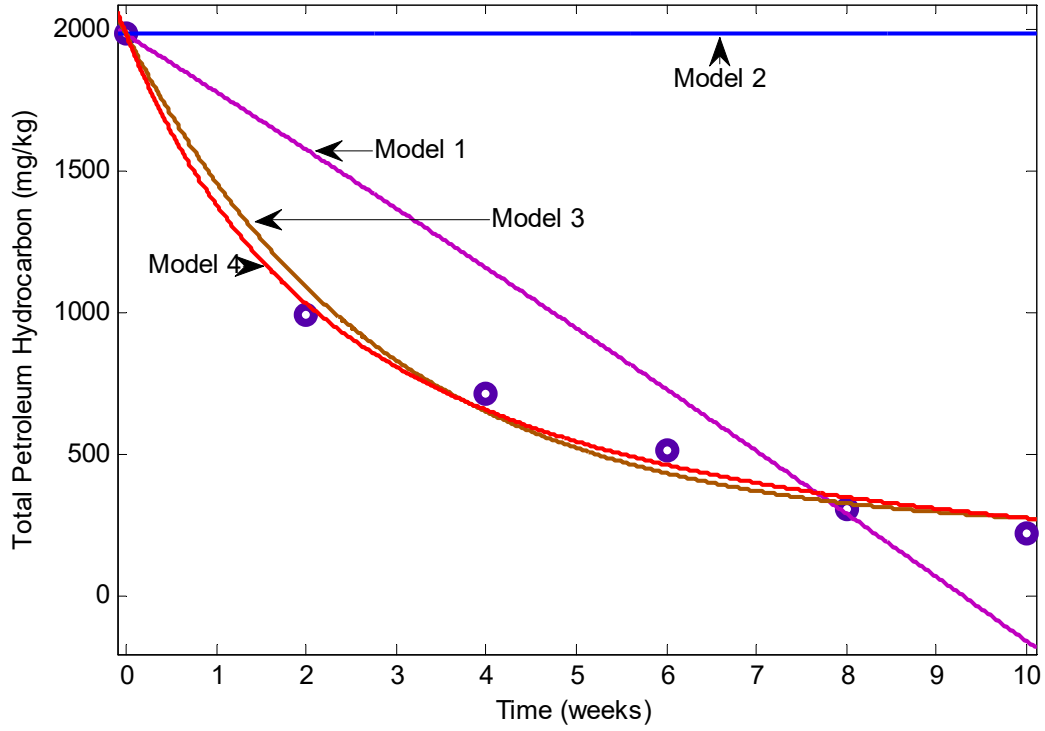


Figure 4.5: Total Petroleum Hydrocarbon versus Time for 50g of NPK fertilizer

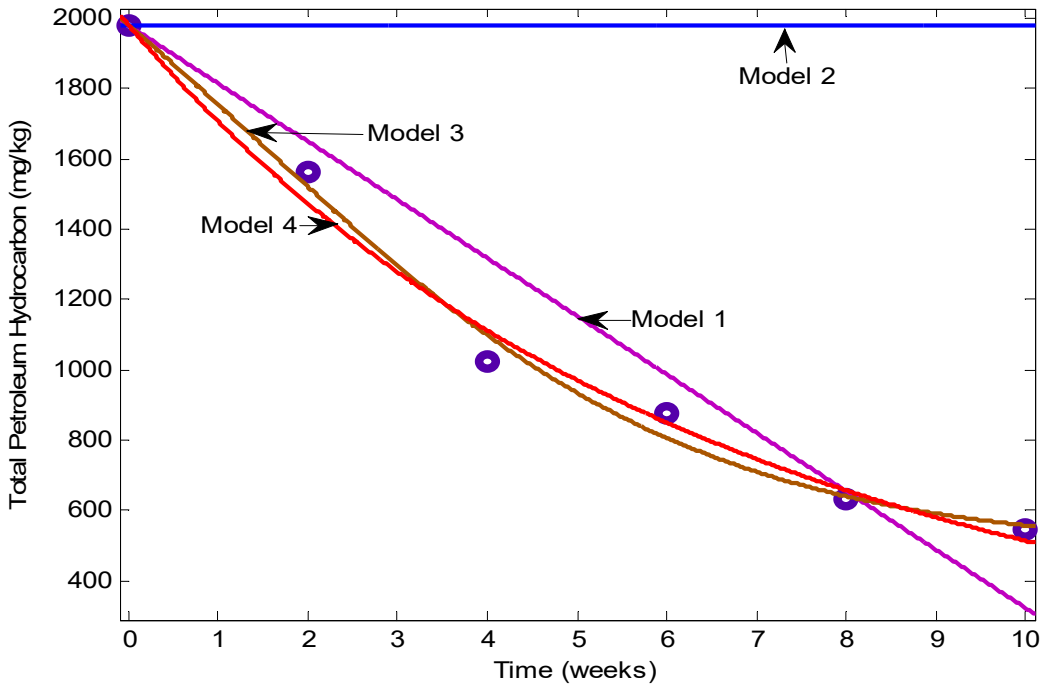


Figure 4.6: Total Petroleum Hydrocarbon versus Time for 10g of poultry manure

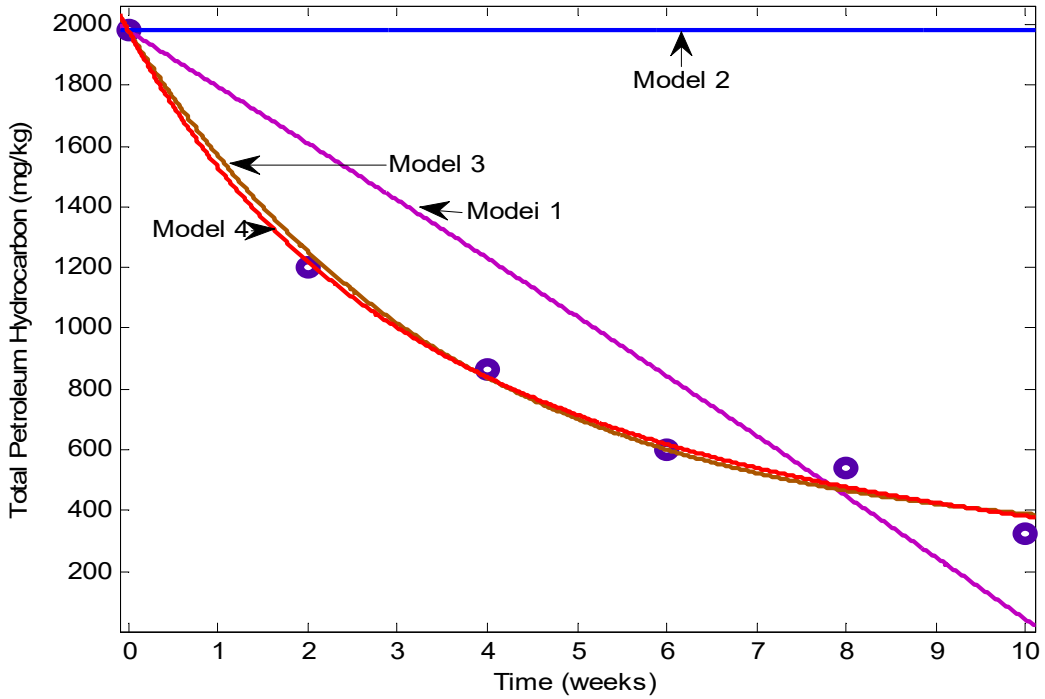


Figure 4.7: Total Petroleum Hydrocarbon versus Time for 20g of poultry manure

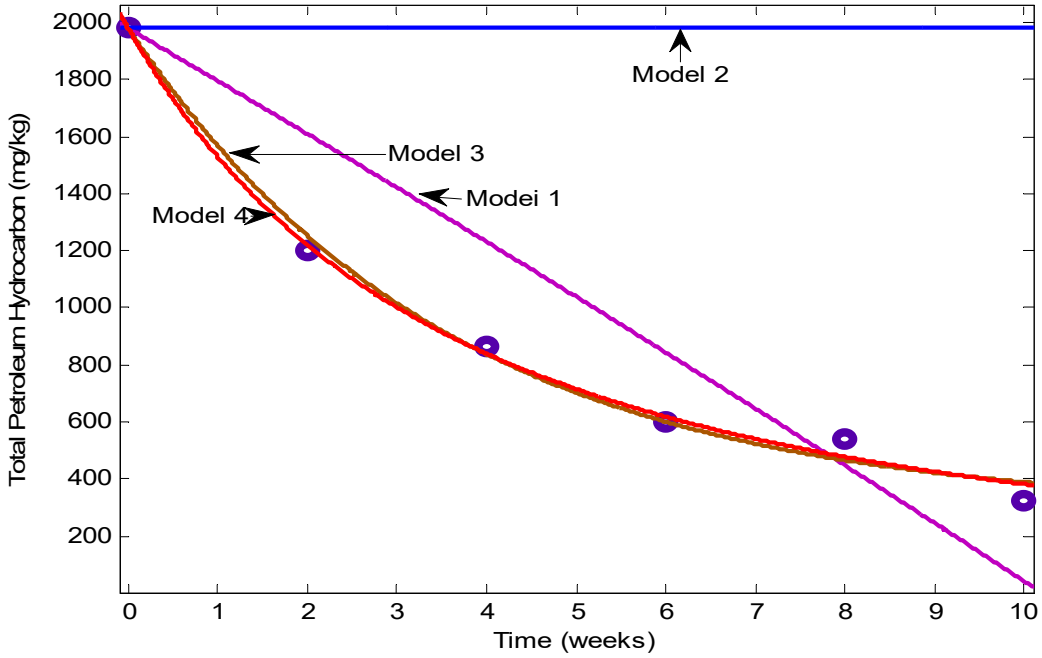


Figure 4.8: Total Petroleum Hydrocarbon versus Time for 30g of poultry manure

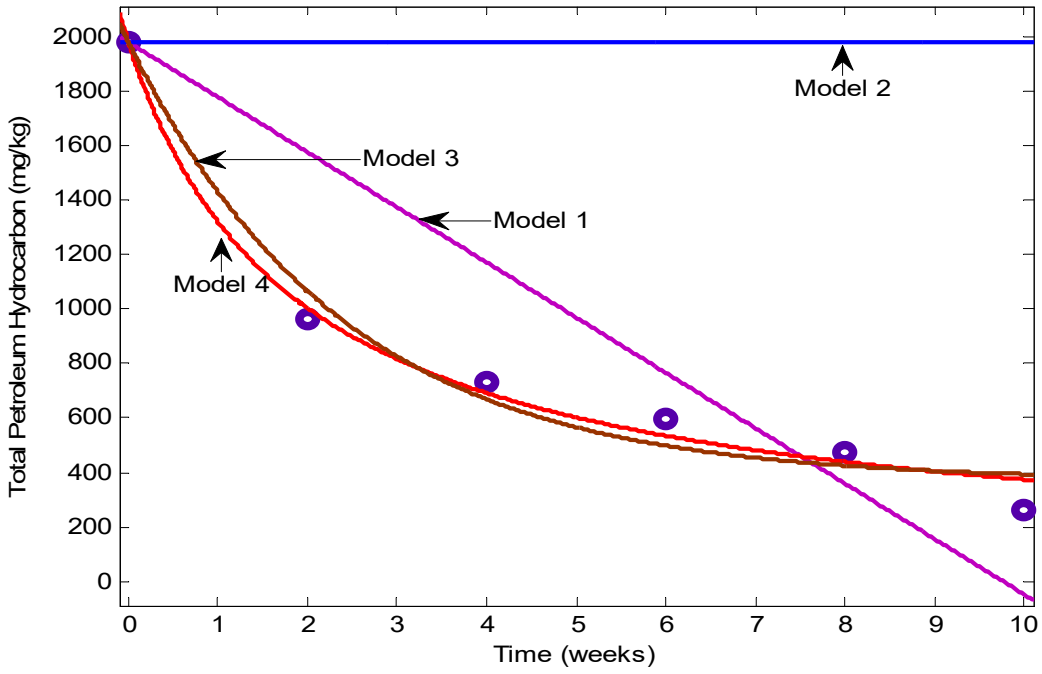


Figure 4.9: Total Petroleum Hydrocarbon versus Time for 40g of poultry manure

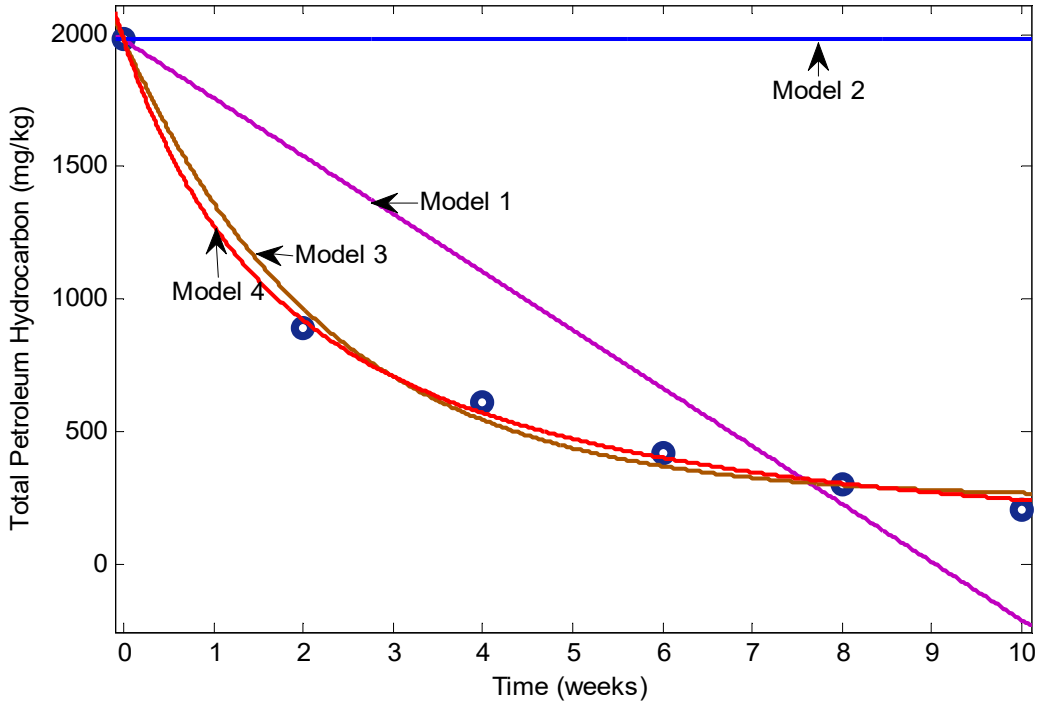


Figure 4.10: Total Petroleum Hydrocarbon versus Time for 50g of poultry manure

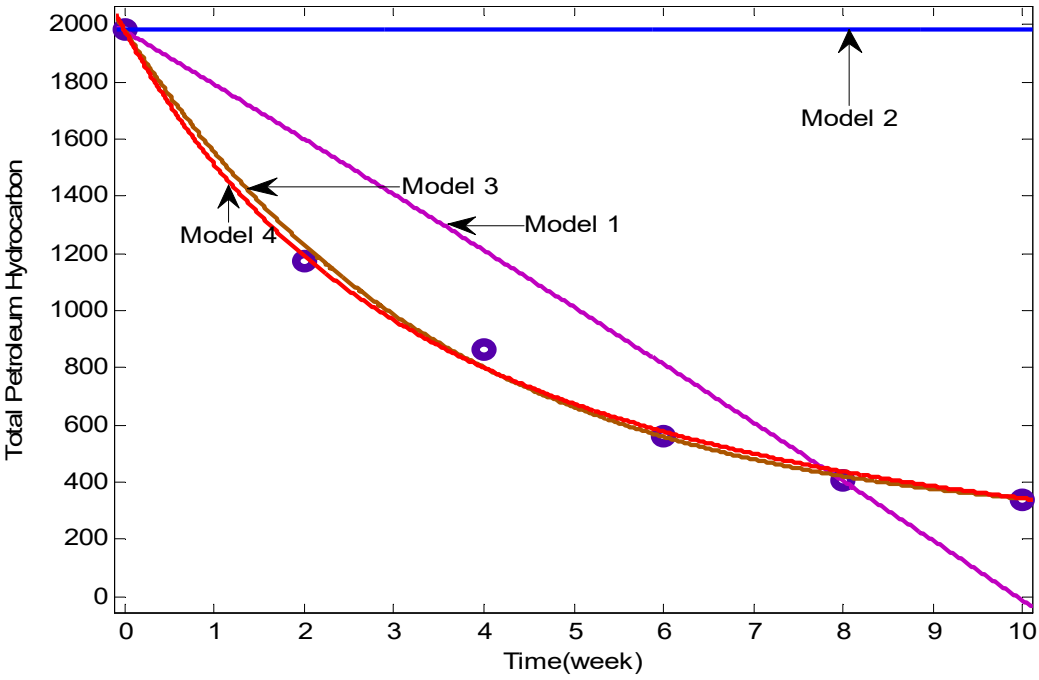


Figure 4.11: Total Petroleum Hydrocarbon versus Time for 10g of (poultry+cow+sawdust) manure

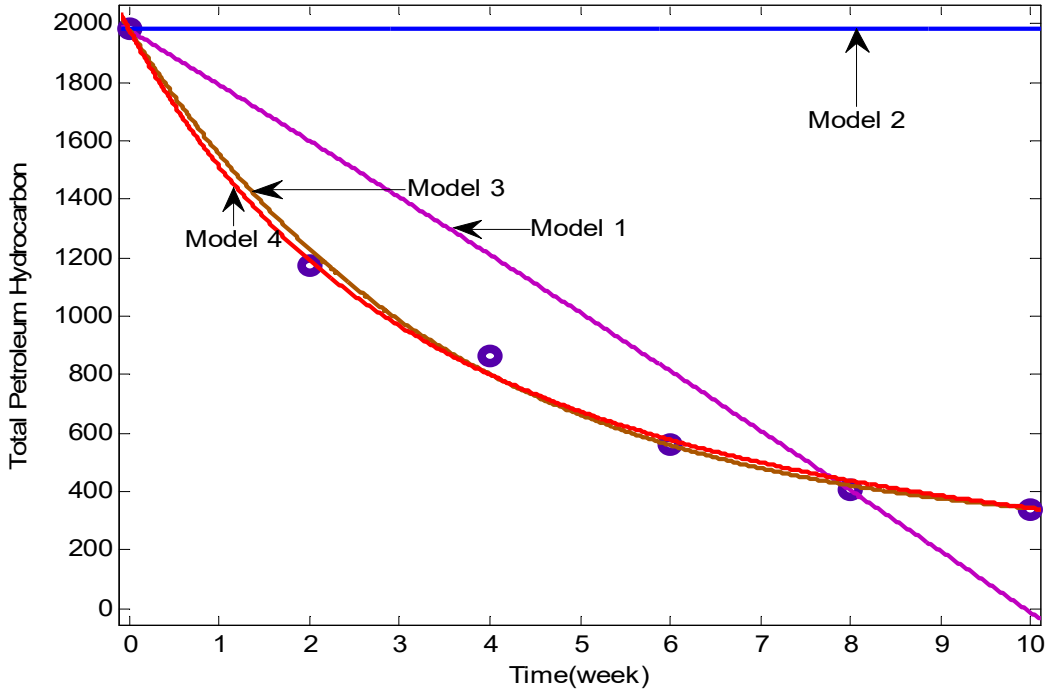


Figure 4.12: Total Petroleum Hydrocarbon versus Time for 20g of (poultry+cow+sawdust) manure

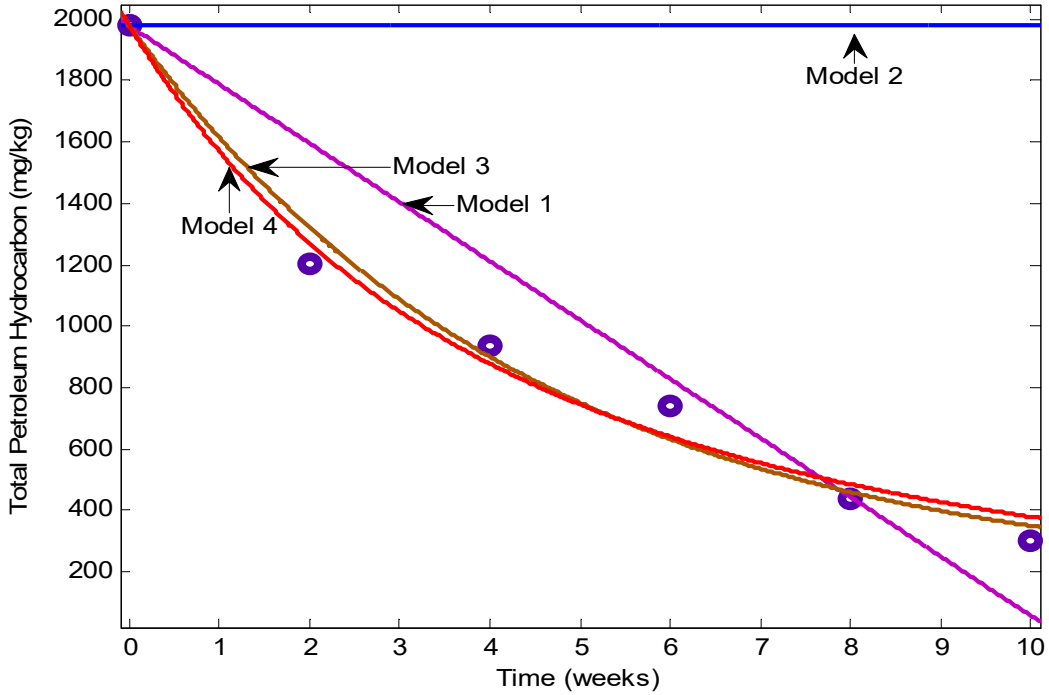


Figure 4.13: Total Petroleum Hydrocarbon versus Time for 30g of (poultry+cow+saw dust) manure

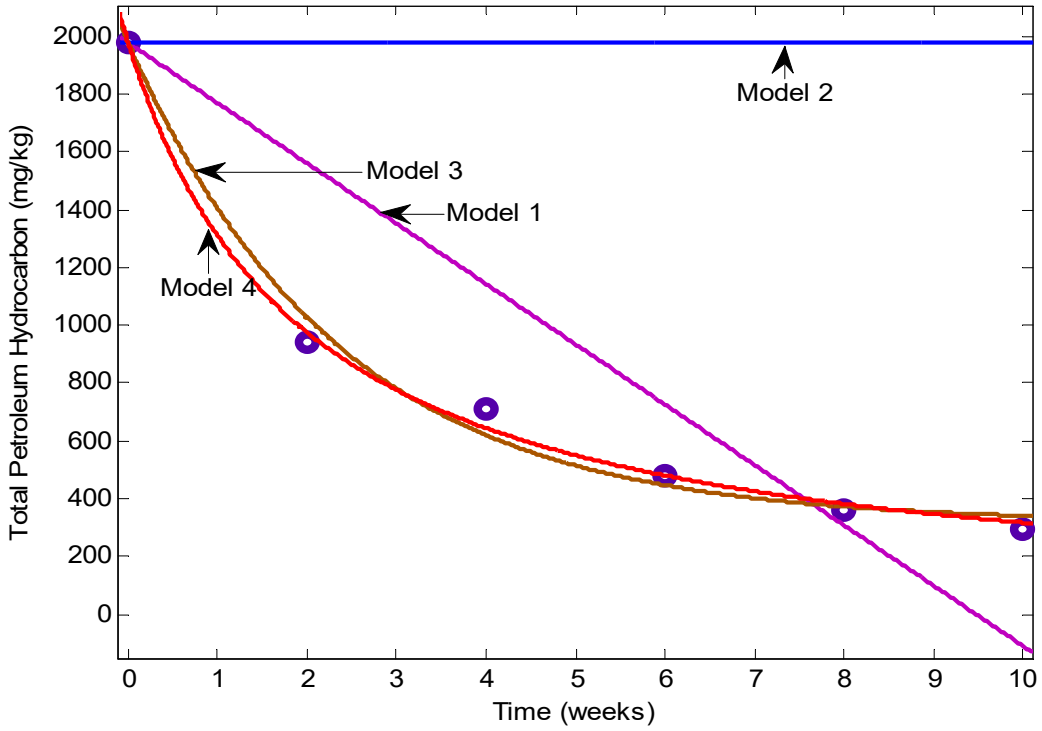


Figure 4.14: Total Petroleum Hydrocarbon versus Time for 40g of (poultry+cow+saw dust) manure

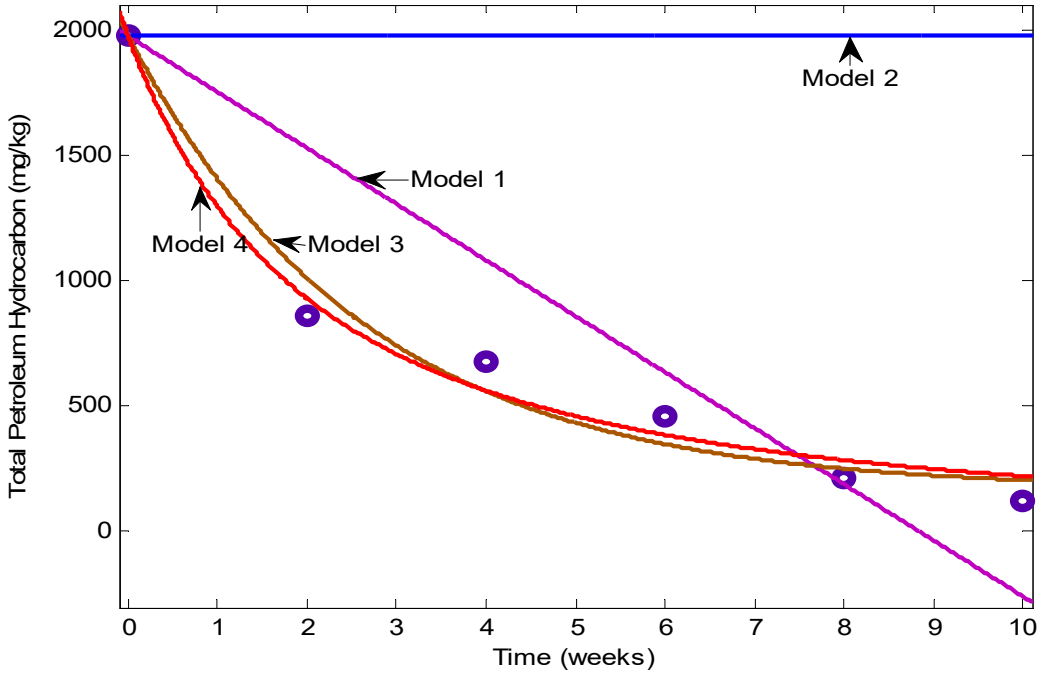


Figure 4.15: Total Petroleum Hydrocarbon versus Time for 50g of (poultry+cow+saw dust) manure

#### 4.1.2.2 Graphical Fit Results for Models Based on Order of Reaction

The graphical fit results for models based on order of reaction are represented in figures 4.16-4.30 below:

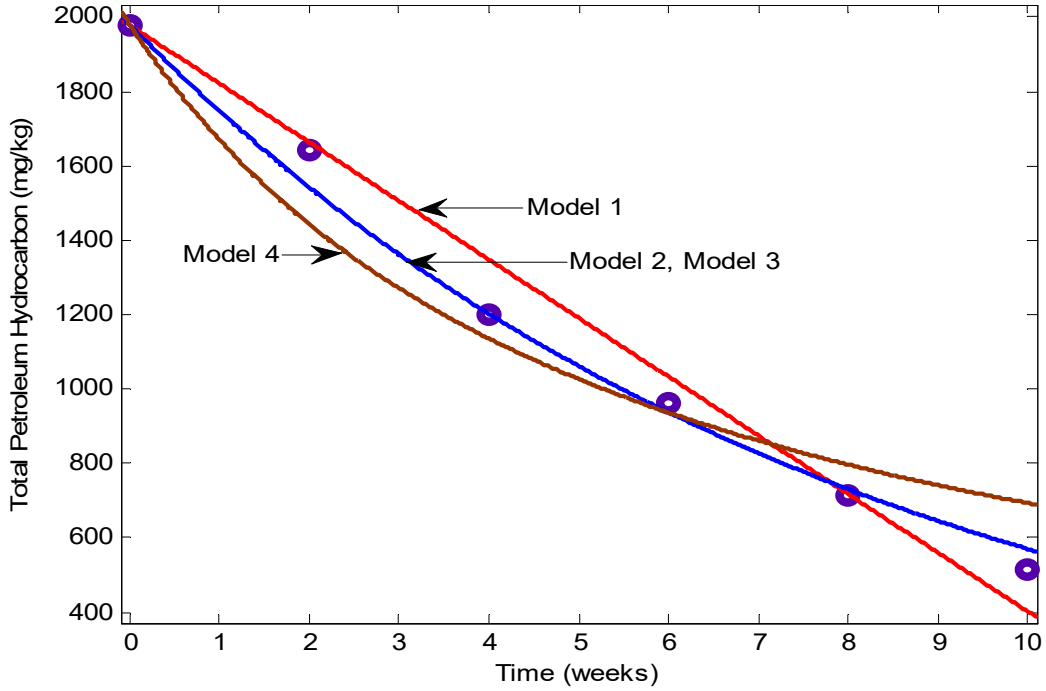


Figure 4.16: Total Petroleum Hydrocarbon versus Time for 10g of NPK fertilizer

*where*;

| **Models** | | **Order** | **Model equation** |
|---|---|---|---|
| Model 1 | | 0 | $s = s_0 - kt$ |
| Model 2 | (S∞=0) | 1 | $s = s_0\, e^{-kt}$ |
| Model 3 | (S∞>0) | 2 | $s = s_\infty + (s_0 - s_\infty)e^{-kt}$ |
| Model 4 | (s∞ = 0) | 2 | $s = \frac{s_0}{1+s_0 kt}$ |

s∞ = ultimate contaminant concentration

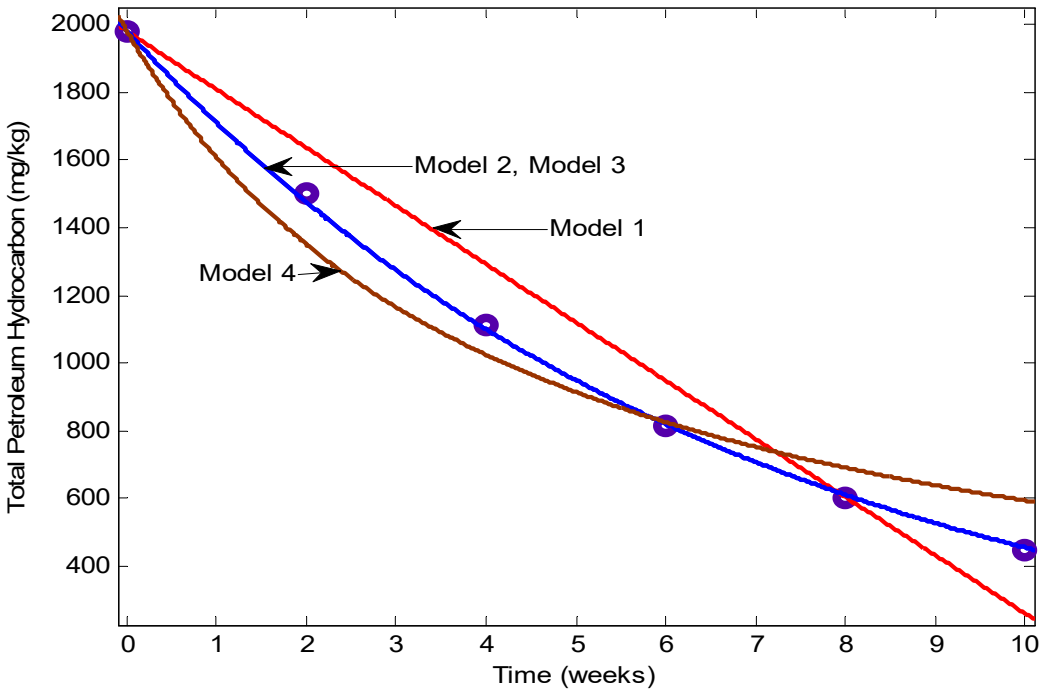


Figure 4.17: Total Petroleum Hydrocarbon versus Time for 20g of NPK fertilizer

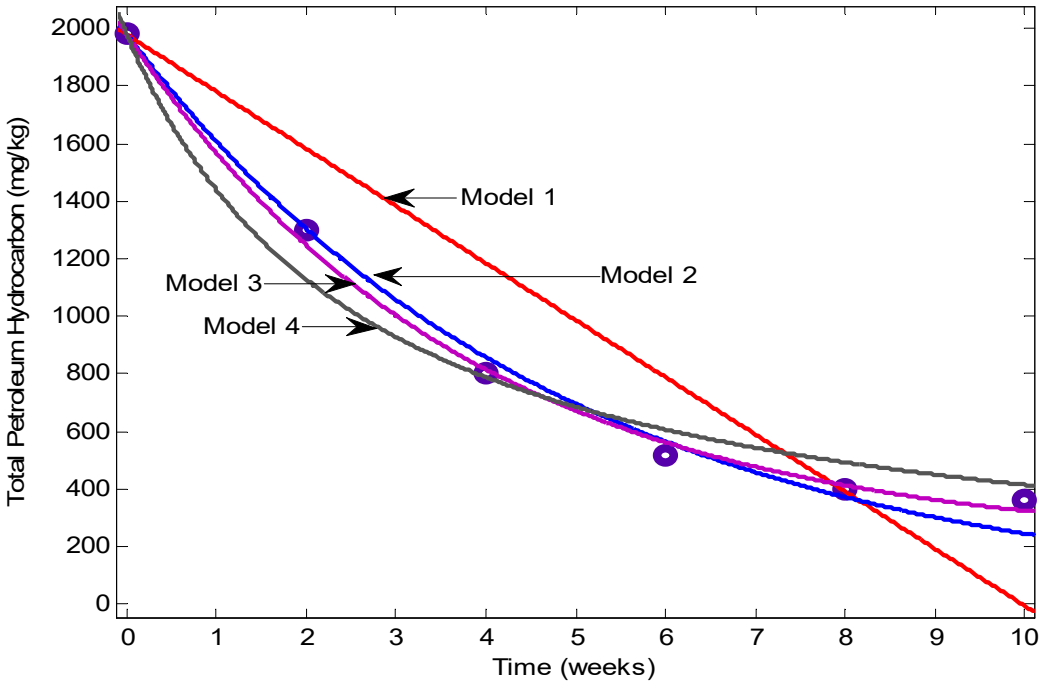


Figure 4.18: Total Petroleum Hydrocarbon versus Time for 30g of NPK fertilizer

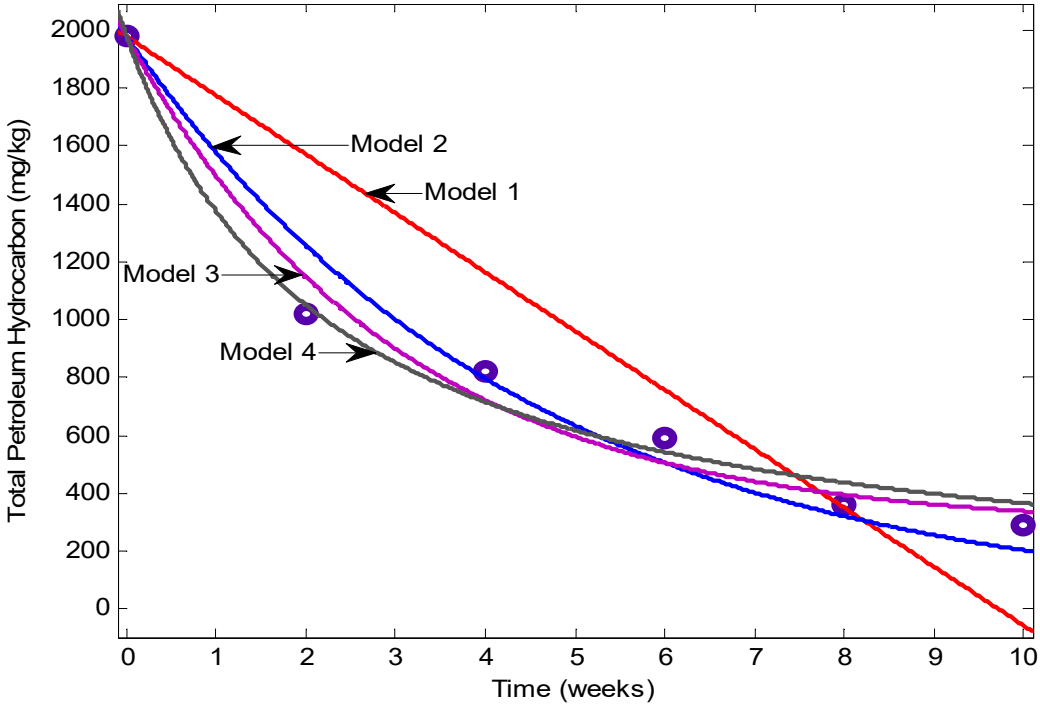


Figure 4.19: Total Petroleum Hydrocarbon versus Time for 40g of NPK fertilizer

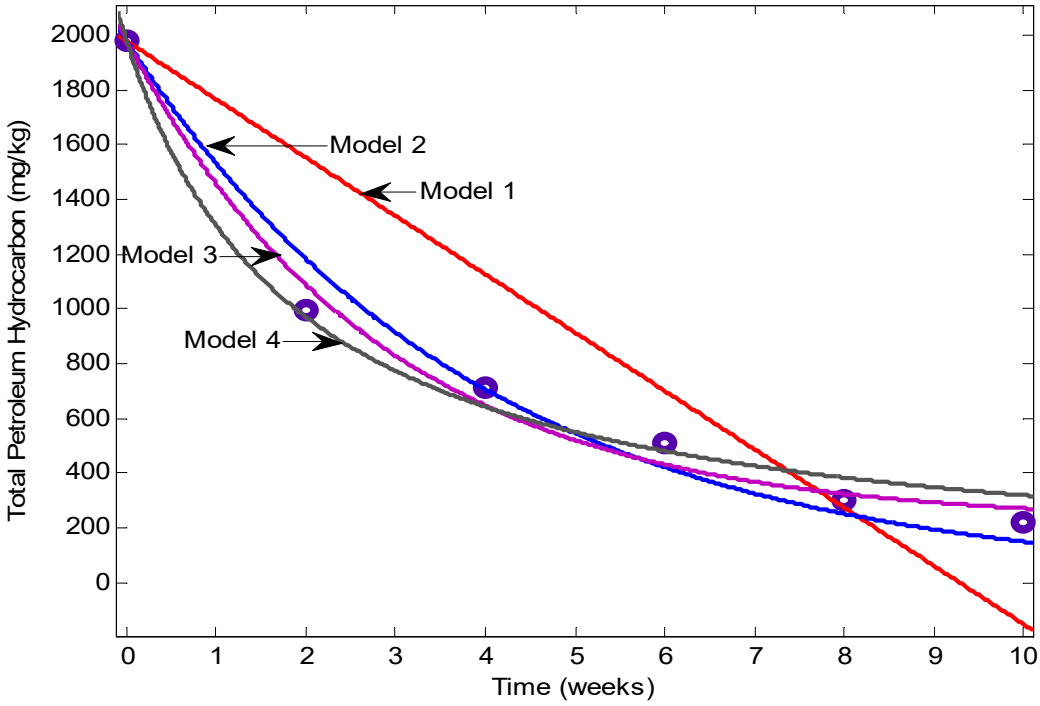


Figure 4.20: Total Petroleum Hydrocarbon versus Time for 50g of NPK fertilizer

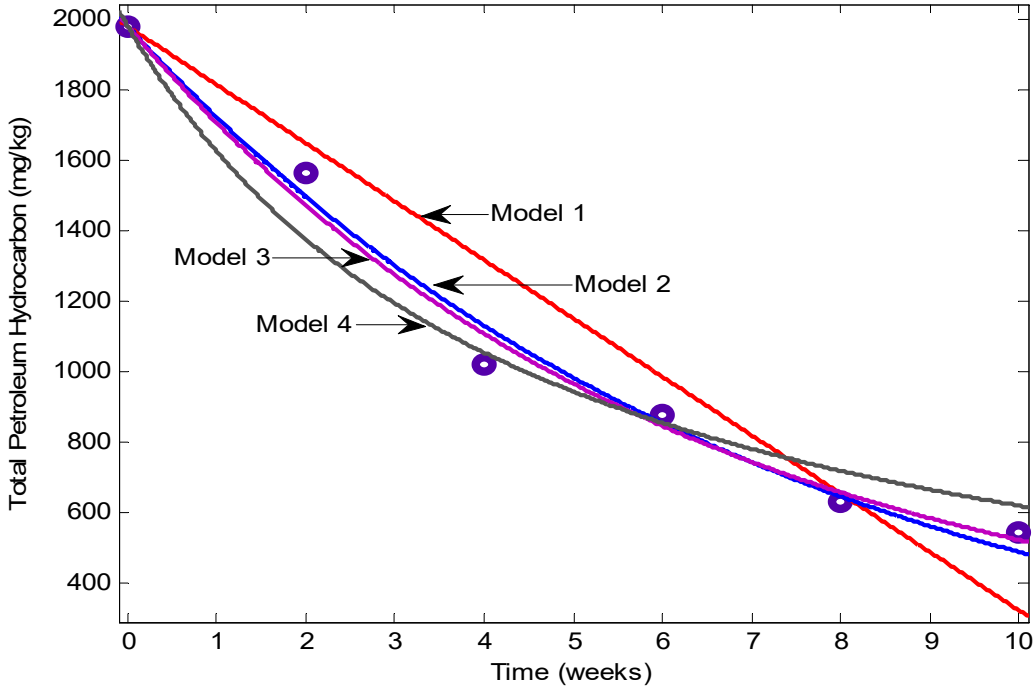


Figure 4.21: Total Petroleum Hydrocarbon versus Time for 10g of poultry manure

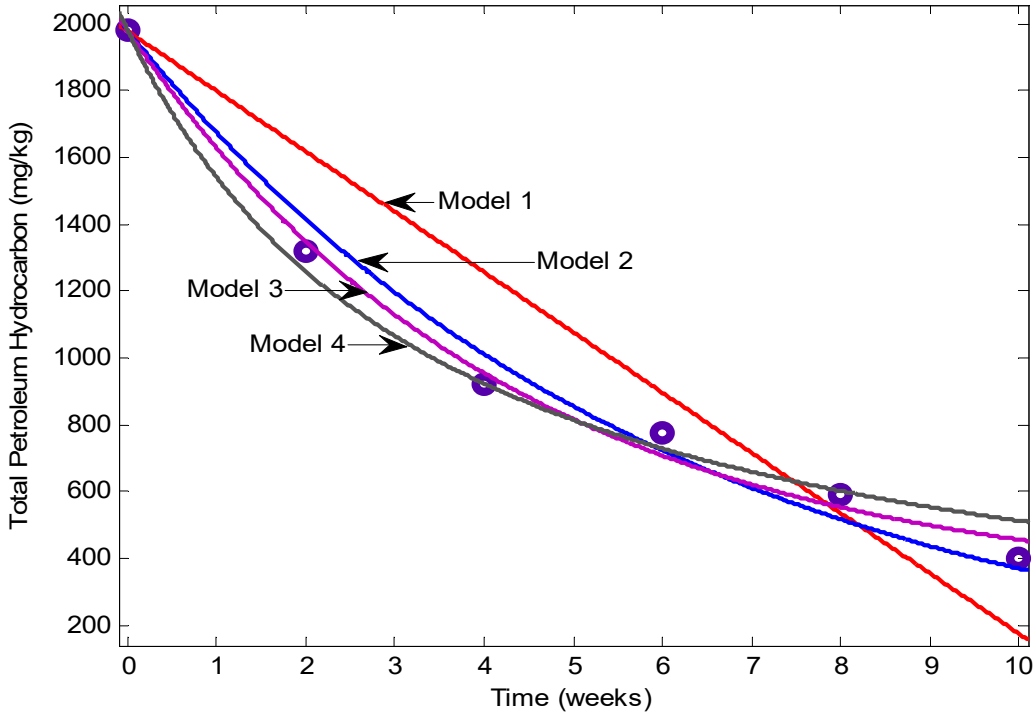


Figure 4.22: Total Petroleum Hydrocarbon versus Time for 20g of poultry manure

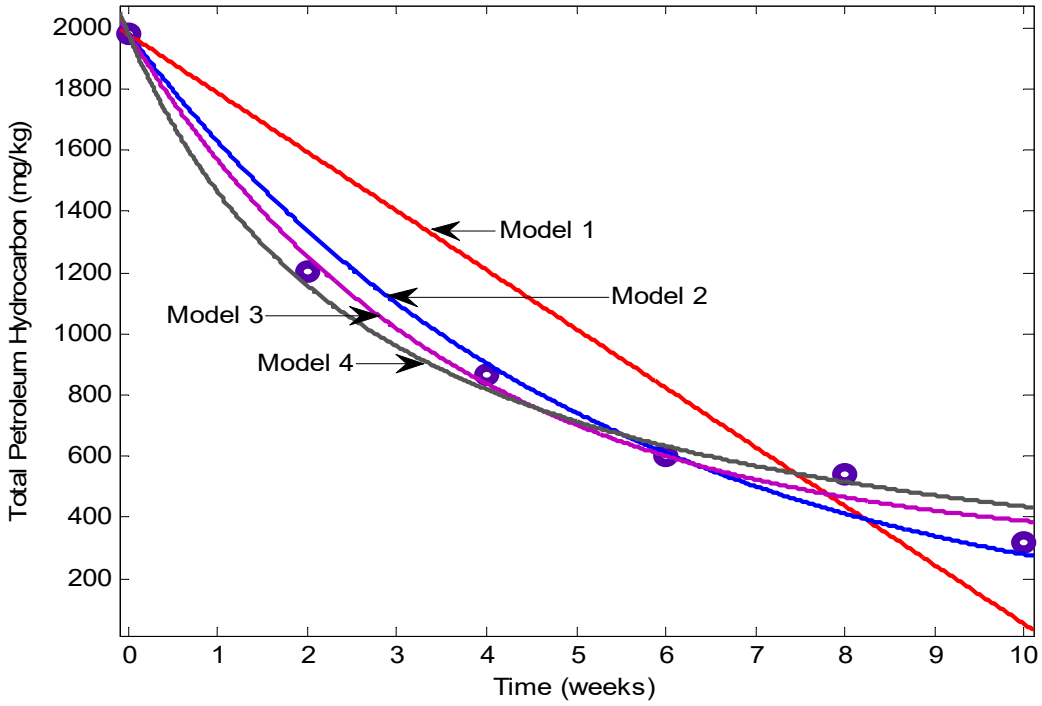


Figure 4.23: Total Petroleum Hydrocarbon versus Time for 30g of poultry manure

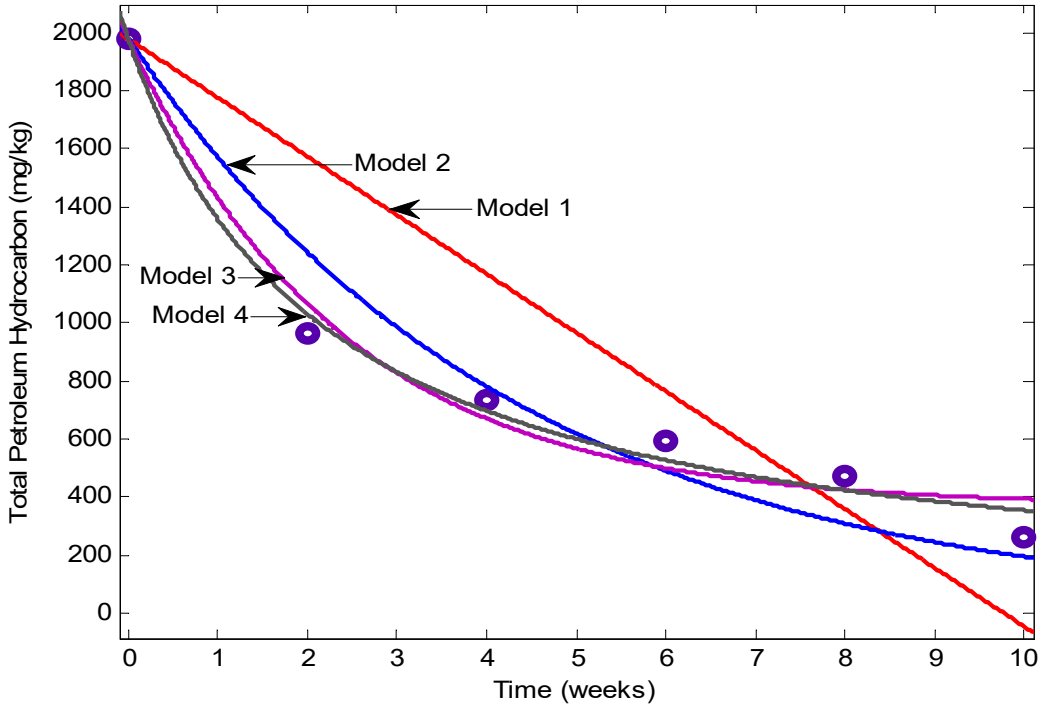


Figure 4.24: Total Petroleum Hydrocarbon versus Time for 40g of poultry manure

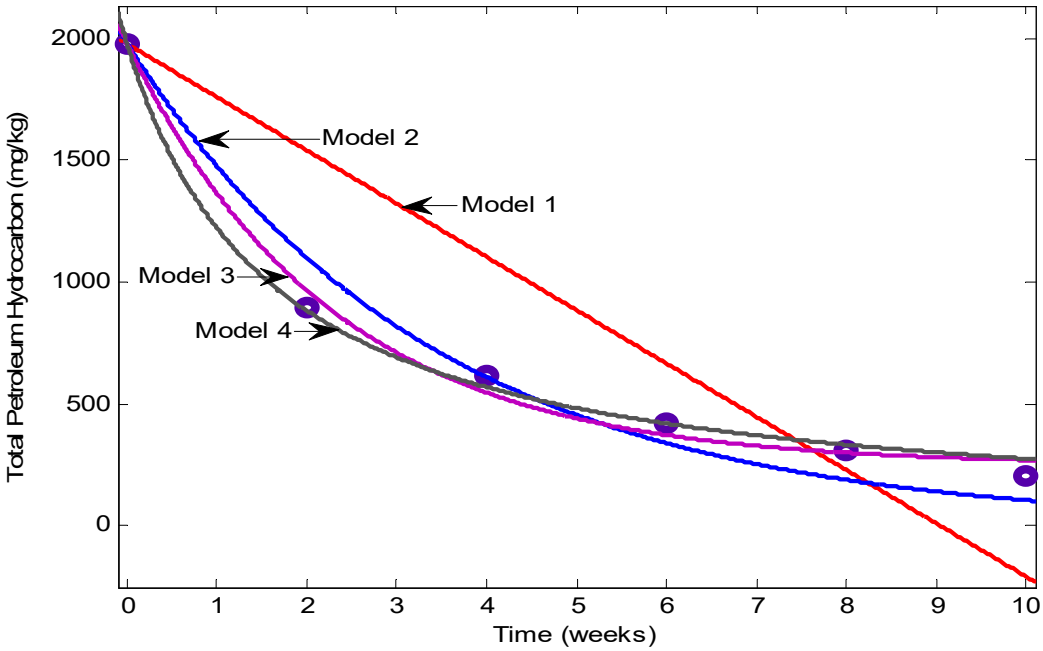


Figure 4.25: Total Petroleum Hydrocarbon versus Time for 50g of poultry manure

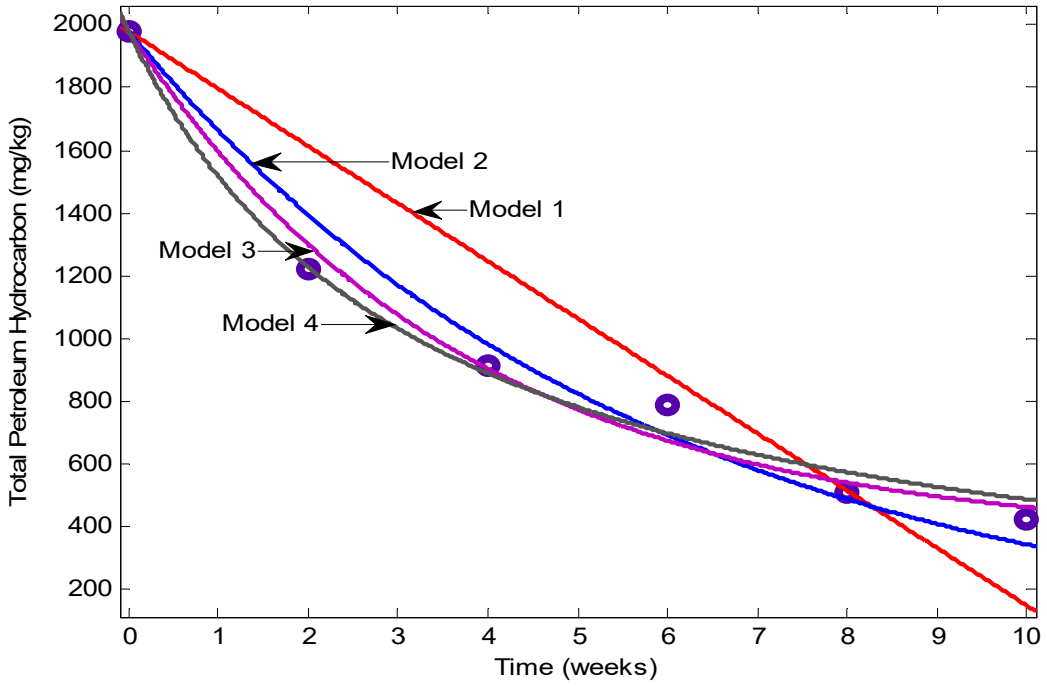


Figure 4.26: Total Petroleum Hydrocarbon versus Time for 10g of (poultry+cow+saw dust)

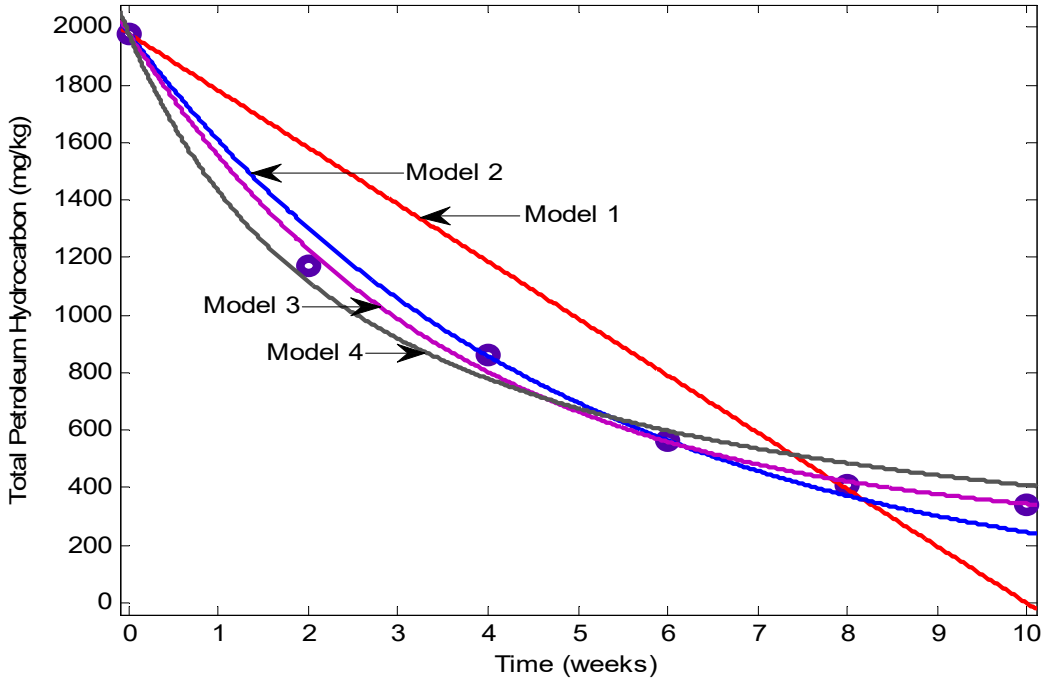


Figure 4.27: Total Petroleum Hydrocarbon versus Time for 20g of (poultry+cow+sawdust) manure.

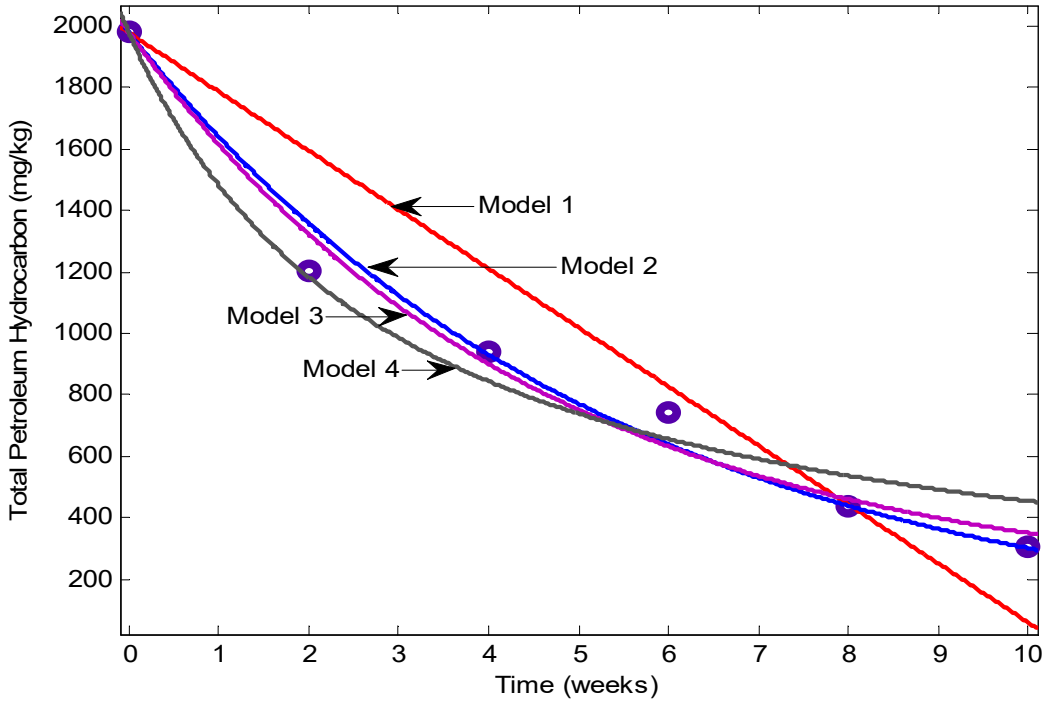


Figure 4.28: Total Petroleum Hydrocarbon versus Time for 30g of (poultry+cow+saw dust) manure

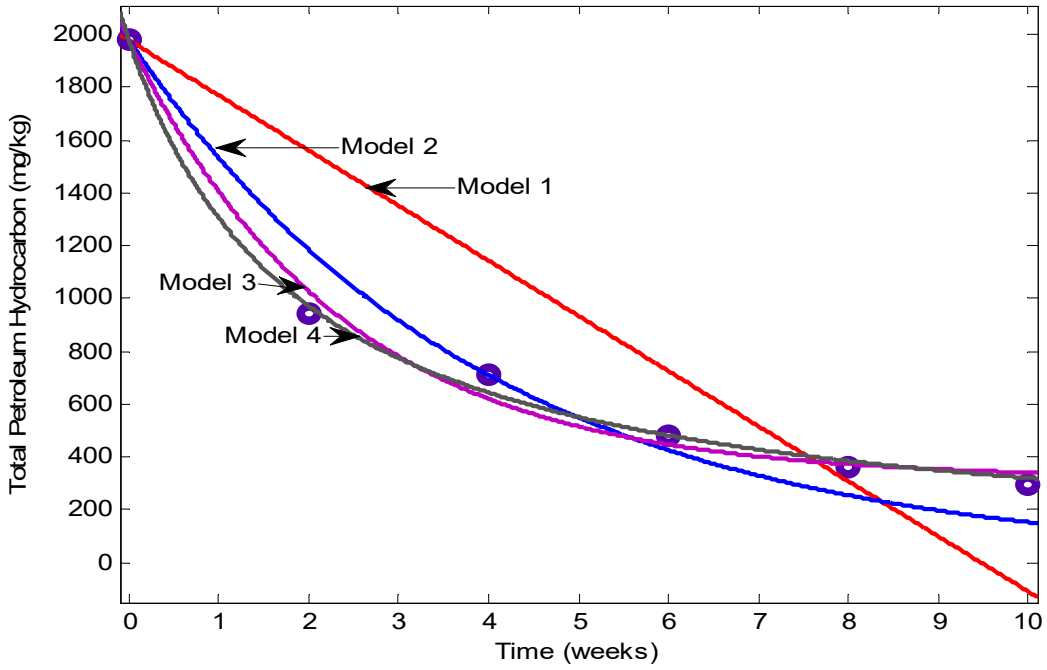


Figure 4.29: Total Petroleum Hydrocarbon versus Time for 40g of (poultry+cow+saw dust) manure

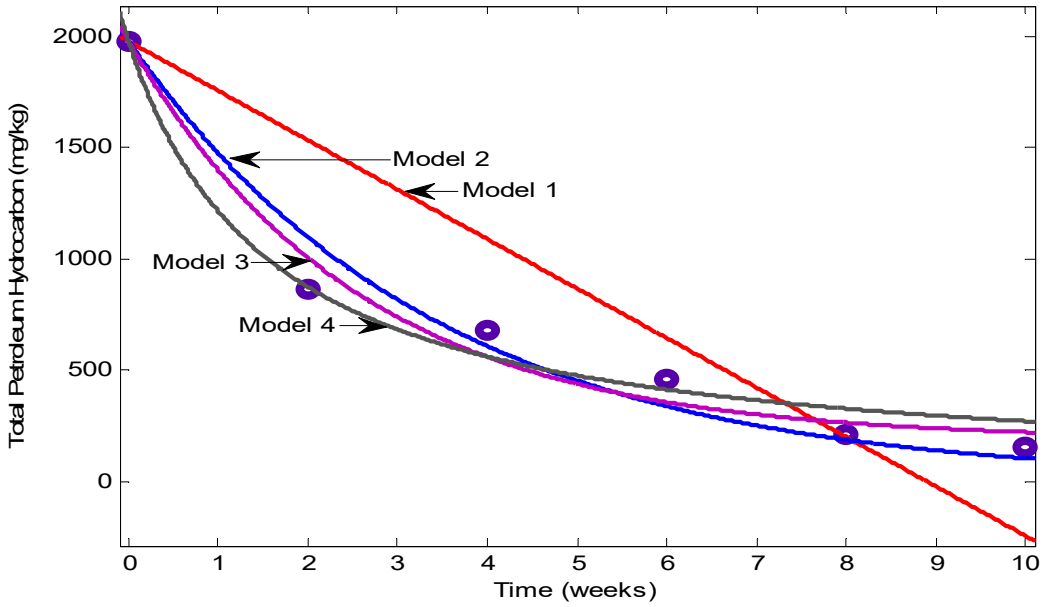


Figure 4.30: Total Petroleum Hydrocarbon versus Time for 50g of (poultry+cow+sawdust) manure

### 4.1.3 Numerical Fit Results for the Models Based on Biomass Growth

Table 4.5: Parameter Values and Numerical Fit Results for Treatment with NPK fertilizer, ($F_A$)

| Quantity of fertilizer (g) | $S_o$ (mg/kg) | $Y_G$ | $X_o/Y_G$ | $\mu$ | $\gamma X_0$ | $R^2$ | RMSE | SSE |
|---|---|---|---|---|---|---|---|---|
| 10-$E_1$ | 1980 | | 0.000000172 | 0.00000915 | | 0.6532 | 100.4 | 0.000028 |
| $E_2$ | | 71.87 | | 0.0000222 | | -2.529 | 1050 | 0.00000551 |
| $E_3$ | | | 0.00001320 | 0.2408 | 0.4239 | 0.9764 | 36.81 | 4.0644 |
| $E_4$ | | 0.002009 | | 0.00000034 | 67.76 | 0.9913 | 67.4 | 1.3638 |
| 20-$E_1$ | 1980 | | 0.00000191 | 0.02584 | | 0.9106 | 194.3 | 0.0000051 |
| $E_2$ | | 69.36 | | 0.00000000176 | | -2.899 | 1148 | 0.0000066 |
| $E_3$ | | | 0.000001468 | 0.02584 | 0.6614 | 0.9963 | 2.428 | 1.7695 |
| $E_4$ | | 0.001702 | | 0.2117 | 46.91 | 0.9954 | 19.08 | 1.0922 |
| 30-$E_1$ | 1980 | | 0.00000175 | 0.002044 | | 0.8864 | 153.6 | 0.000037 |
| $E_2$ | | 74.02 | | 0.00000003435 | | -2.628 | 1226 | 0.0000042 |
| $E_3$ | | | 0.0001899 | 0.491 | 0.4152 | 0.9985 | 6.378 | 0.106 |
| $E_4$ | | 0.00164 | | 0.05957 | 1.001 | 0.9952 | 56.79 | 0.9677 |
| 40-$E_1$ | 1980 | | 0.00000136 | 0.002135 | | 0.8895 | 132.4 | 0.0000056 |
| $E_2$ | | 62.05 | | 0.000000422 | | -2.224 | 1250 | 0.0000044 |
| $E_3$ | | | | 0.491 | 0.4152 | 0.9997 | 5.344 | 0.164 |
| $E_4$ | | 0.001522 | 0.0001682 | 0.05957 | 1.001 | 0.9920 | 47.79 | 0.877 |
| 50- $E_1$ | 1980 | 60.13 | 0.00000132 | 0.003231 | | 0.9254 | 108.8 | 0.00000047 |
| $E_2$ | | | | 0.00000063 | | -2.662 | 1299 | 0.00000533 |
| $E_3$ | | | 0.0001437 | 0.356 | 0.991 | 1 | 5.067 | 0.116 |
| $E_4$ | | 0.001361 | | 0.0001261 | 2.130 | 0.9962 | 43.82 | 0.646 |

$E_{1=}$ Biomass Growth model equation 1, $E_{2=}$ Biomass Growth model equation 2, $E_{3=}$ Biomass Growth model equation 3, $E_{4=}$ Biomass Growth model equation

Table 4.6: Parameter Values and Numerical Fit Results for Treatment with poultry manure, ($F_B$)

| Quantity of poultry manure (g) | $S_o$ (mg/kg) | $Y_G$ | $X_o/Y_G$ | $\mu$ | $YX_0$ | $R^2$ | RMSE | SSE |
|---|---|---|---|---|---|---|---|---|
| 10-$E_1$ | 1980 | | 0.0000485 | 0.0000482 | | 0.7162 | 25.16 | 0.0000017 |
| $E_2$ | | 74.2 | | 0.00000212 | | -3.316 | 12.66 | 0.0000007233 |
| $E_3$ | | | 0.00000144 | 0.4023 | 0.3586 | 0.9919 | 44.97 | 1.215 |
| $E_4$ | | 0.4516 | | 0.00005.305 | 473.6 | 0.9804 | 78.5 | 5.766 |
| 20-$E_1$ | 1980 | | 0.0000147 | 0.00109 | | 0.7214 | 27.47 | 0.00000262 |
| $E_2$ | | 72.08 | | 0.0000224 | | -3.512 | 12.18 | 0.00001136 |
| $E_3$ | | | 0.00000164 | 0.236 | 0.9899 | 0.9934 | 61.54 | 0.1336 |
| $E_4$ | | 0.614 | | 0.0001525 | 747.9 | 0.9952 | 51.46 | 7.994 |
| 30-$E_1$ | 1980 | | 0.00001568 | 0.01165 | | 0.6917 | 23.37 | 0.0000446 |
| $E_2$ | | 65.71 | | 0.0000004131 | | -3.713 | 11.09 | 0.000000856 |
| $E_3$ | | | 0.00000172 | 0.2826 | 0.9002 | 0.9924 | 57.89 | 1.3834 |
| $E_4$ | | 0.597 | | 0.0002017 | 711.2 | 0.9950 | 54.96 | 5.4061 |
| 40-$E_1$ | 1980 | | 0.0000171 | 0.00117 | | 0.6204 | 22.96 | 0.000000972 |
| $E_2$ | | 62.82 | | 0.00000000344 | | -4.243 | 10.95 | 0.00007042 |
| $E_3$ | | | 0.00000133 | 0.4226 | 0.980 | 0.9817 | 32.29 | 0.000428 |
| $E_4$ | | 0.507 | | 0.0002357 | 307.1 | 0.9969 | 49.5 | 2.0082 |
| 50-$E_1$ | 1980 | | 0.0000000174 | 0.0001269 | | 0.5833 | 1.994 | 0.0000000901 |
| $E_2$ | | 63.01 | | 0.00000000354 | | -4.306 | 10.31 | 0.0000000114 |
| $E_3$ | | | 0.000000189 | 0.4617 | 0.991 | 0.9985 | 28.25 | 0.0000611 |
| $E_4$ | | 0.456 | | 0.0003074 | 191.1 | 0.9996 | 37.47 | 2.004211 |

Table 4.7: Parameter Values and Numerical Fit Results for Treatment with (poultry+cow+saw dust) manure, ($F_C$)

| Quantity of poultry+Cow+sawdust | $S_o$ (mg/kg) | $Y_G$ | $Xo/Y_G$ | $\mu$ | $YX_0$ | $R^2$ | RMSE | SSE |
|---|---|---|---|---|---|---|---|---|
| 10-$E_1$ | 1980 | | 0.0000156 | 0.00012106 | | 0.5564 | 310 | 0.00000384 |
| $E_2$ | | 62.06 | | 0.00000000499 | | -4.306 | 12.42 | 0.0000000774 |
| $E_3$ | | | 0.0000975 | 0.211 | 0.9983 | 0.9865 | 85.65 | 0.0000161 |
| $E_4$ | | 0.784 | | 0.0000382 | 1553 | 0.991 | 69.44 | 1.04069 |
| | | | | 0.0001387 | | | | |
| 20-$E_1$ | 1980 | | 0.0000156 | 0.001196 | | 0.7433 | 350.2 | 0.0000049o4 |
| $E_2$ | | 73.29 | | 0.00001863 | | -3.745 | 13.47 | 0.0009077 |
| $E_3$ | | | 0.00000171 | 0.2844 | 0.9963 | 0.9963 | 48.75 | 0.000007076 |
| $E_4$ | | 0.784 | | 0.0001588 | 999.1 | 0.9972 | 42.08 | 5.312 |
| 30-$E_1$ | 1980 | | 0.00000705 | 0.000112 | | 0.8375 | 380.4 | 0.00000115 |
| $E_2$ | | 72.22 | | 0.0000222 | | -3.557 | 87.73 | 0.000000027 |
| $E_3$ | | | | 0.2246 | 0.9927 | 0.9831 | 101.9 | 0.00000614 |
| $E_4$ | | 0.4479 | 0.00000248 | 0.00164 | 667 | 0.9853 | 95.02 | 5.305 |
| 40-$E_1$ | 1980 | | 0.0000011724 | 0.000133 | | 0.4965 | 446.3 | 0.000007968 |
| $E_2$ | | 62.89 | | 0.00000000307 | | -4.274 | 14.42 | 0.0000000103 |
| $E_3$ | | | 0.00000337 | 0.4263 | 0.9951 | 0.9901 | 480.0 | 0.0000559 |
| $E_4$ | | 0.6728 | | 0.0001963 | 2519 | 0.9965 | 48.26 | 3.988 |
| 50- $E_1$ | 1980 | | 0.0000013 | 0.0001152 | | 0.5537 | 407.4 | 0.00000773 |
| $E_2$ | | 58.37 | | 0.000000311 | | -0.412 | 33.54 | 0.0000000158 |
| $E_3$ | | o.6163 | 0.000001437 | 0.484 | 0.9875 | 0.9895 | 138.6 | 0.00000157 |
| $E_4$ | | | | 0.0002509 | 1.268 | 1 | 23.15 | 3.686 |

### 4.1.4 Numerical Fit Results for the Best Models Based on Order of Reaction

Below are the numerical fit results for the bioremediation with fertilizer ($F_A$), poultry ($F_B$) and mixture of poultry+cow dung+saw dust ($F_C$), respectively.

Table 4.8: Model Rate Constants and Numerical Fit Results for NPK fertilizer, ($F_A$)

| Quantity of Fertilizer (g) | $R^2$ | RMSE | K | SSE |
|---|---|---|---|---|
| 10- $M_1$ | -2.3752 | 15.33 | 0.0000018 | 3.31 |
| $M_2$ | 0.8574 | 106 | 0.0121 | 2.53 |
| $M_3$ | 0.9433 | 58.19 | 0.0225 | 1.35 |
| $M_4$ | 0.9669 | 128.7 | 0.3335 | 4.28 |
| 20- $M_1$ | -2.6643 | 18.08 | 0.0000001175 | 2.83 |
| $M_2$ | 0.8843 | 118 | 0.0131 | 4.12 |
| $M_3$ | 0.9771 | 65.19 | 0.0235 | 1.06 |
| $M_4$ | 0.9898 | 128.7 | 0.0353 | 6.08 |
| 30- $M_1$ | -2.2442 | 21.75 | 0.0001902 | 4.14 |
| $M_2$ | 0.8865 | 143 | 0.0146 | 3.66 |
| $M_3$ | 0.9967 | 44.6 | 0.0242 | 6.72 |
| $M_4$ | 0.9758 | 110.3 | 0.0272 | 4.88 |
| 40-$M_1$ | -0.3422 | 22.67 | 0.0002226 | 1.72 |
| $M_2$ | 0.9311 | 158 | 0.0151 | 3.55 |
| $M_3$ | 0.9810 | 95.69 | 0.2441 | 3.66 |
| $M_4$ | 0.9865 | 72.17 | 0.3354 | 2.63 |
| 50- $M_1$ | -0.2843 | 20.06 | 0.0002618 | 3.18 |
| $M_2$ | 0.9552 | 103 | 0.01553 | 2.13 |
| $M_3$ | 0.9973 | 75.57 | 0.0252 | 2.28 |
| $M_4$ | 0.9939 | 67.19 | 0.3533 | 2.25 |

*Where*; $M_1$=Reaction rate model equation, $M_2$=Reaction rate model equation, $M_3$=Reaction rate model equation 3, $M_4$=Reaction rate model equation

Table 4.9: Model Rate Constants and Numerical Fit Results for poultry manure, ($F_B$)

| Quantity of poultry manure ( g) | $R^2$ | RMSE | K | SSE |
|---|---|---|---|---|
| 10- $M_1$ | -3.2331 | 14.91 | 0.0001108 | 1.26 |
| $M_2$ | 0.8884 | 98.11 | 0.01165 | 1.08 |
| $M_3$ | 0.9888 | 66.51 | 0.02311 | 0.16 |
| $M_4$ | 0.9685 | 99.53 | 0.2653 | 0.00011 |
| 20- $M_1$ | -3.4443 | 23.06 | 0.000144 | 3.03 |
| $M_2$ | 0.9127 | 100.29 | 0.0135 | 1.21 |
| $M_3$ | 0.9932 | 64.41 | 0.I338 | 0.23 |
| $M_4$ | 0.9886 | 78.62 | 0.2816 | 0.00014 |
| 30- $M_1$ | -3.5452 | 20.55 | 0.000179 | 2.66 |
| $M_2$ | 0.9093 | 118.4 | 0.0138 | 2.34 |
| $M_3$ | 0.9924 | 58.56 | 0.1503 | 0.28 |
| $M_4$ | 0.9897 | 61.15 | 0.2031 | 0.00018 |
| 40 $M_1$ | -3.33335 | 44.14 | 0.000234 | 2.32 |
| $M_2$ | 0.9514 | 56.08 | 0.01388 | 0.53 |
| $M_3$ | 0.9771 | 103.1 | 0.1654 | 0.4183 |
| $M_4$ | 0.9887 | 64.79 | 0.3335 | 0.00023 |
| 50- $M_1$ | -4.1614 | 39.83 | 0.000044 | 1.17 |
| $M_2$ | 0.8896 | 45.55 | 0.0156 | 0.88 |
| $M_3$ | 0.9968 | 67.36 | 0.1562 | 0.16 |
| $M_4$ | 0.9868 | 97.77 | 0.6823 | 0.000121 |

Table 4.10: Model Rate Constants and Numerical Fit Results for (poultry+cow+saw dust) manure, ($F_C$)

| Quantity of (poultry+cow+saw dust) | $R^2$ | RMSE | K | SSE |
|---|---|---|---|---|
| 10- $M_1$ | -2.4633 | 19.05 | 0.0001545 | 3.83 |
| $M_2$ | 0.8434 | 51.21 | 0.0132 | 1.16 |
| $M_3$ | 0.9831 | 74.13 | 0.2331 | 2.99 |
| $M_4$ | 0.9894 | 58.81 | 0.2454 | 1.72 |
| 20- $M_1$ | -2.3744 | 13.33 | 0.0001945 | 4.05 |
| $M_2$ | 0.8551 | 46.07 | 0.0133 | 1.55 |
| $M_3$ | 0.9954 | 41.83 | 0.0421 | 2.09 |
| $M_4$ | 0.98885 | 66.34 | 0.3144 | 1.20 |
| 30- $M_1$ | -2.4623 | 18.43 | 0.0001696 | 3.51 |
| $M_2$ | 0.9165 | 56.81 | 0.03512 | 0.81 |
| $M_3$ | 0.9963 | 41.13 | 0.1 6232 | 3.10 |
| $M_4$ | 0.9885 | 61.34 | 0.33553 | 1.90 |
| 40- $M_1$ | -3.3251 | 13.04 | 0.0002615 | 2.91 |
| $M_2$ | 0.9556 | 37.51 | 0.0532 | 1.33 |
| $M_3$ | 0.9832 | 39.81 | 0.1835 | 1.94 |
| $M_4$ | 0.9734 | 37.62 | 0.3473 | 1.70 |
| 50- $M_1$ | -2.3733 | 11.93 | 0.0003189 | 2.03 |
| $M_2$ | 0.9185 | 30.05 | 0.1663 | 0.56 |
| $M_3$ | 0.9765 | 24.9 | 0.2914 | 1.28 |
| $M_4$ | 0.9997 | 52.69 | 0.4655 | 1.29 |

The percentage degradation of petroleum for the various bio-treatment measures is shown in the figures below;

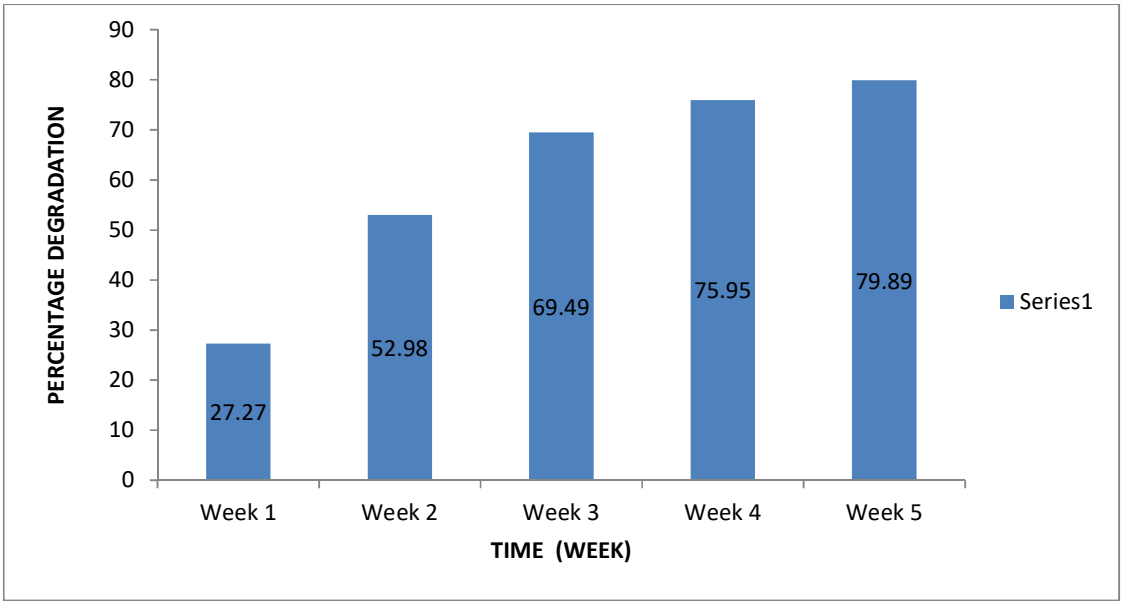


Figure 4.31a: Percentage degradation of petroleum hydrocarbon using NPK fertilizer ($F_A$)

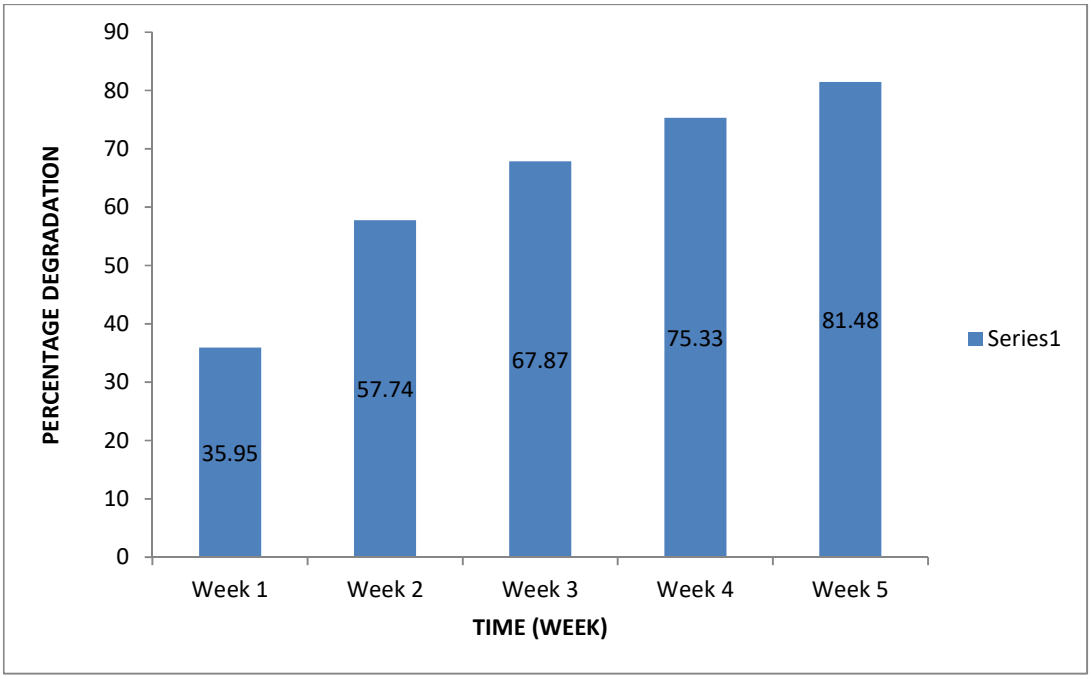


Figure 4.31b: Percentage degradation of petroleum hydrocarbon using poultry manure ($F_B$)

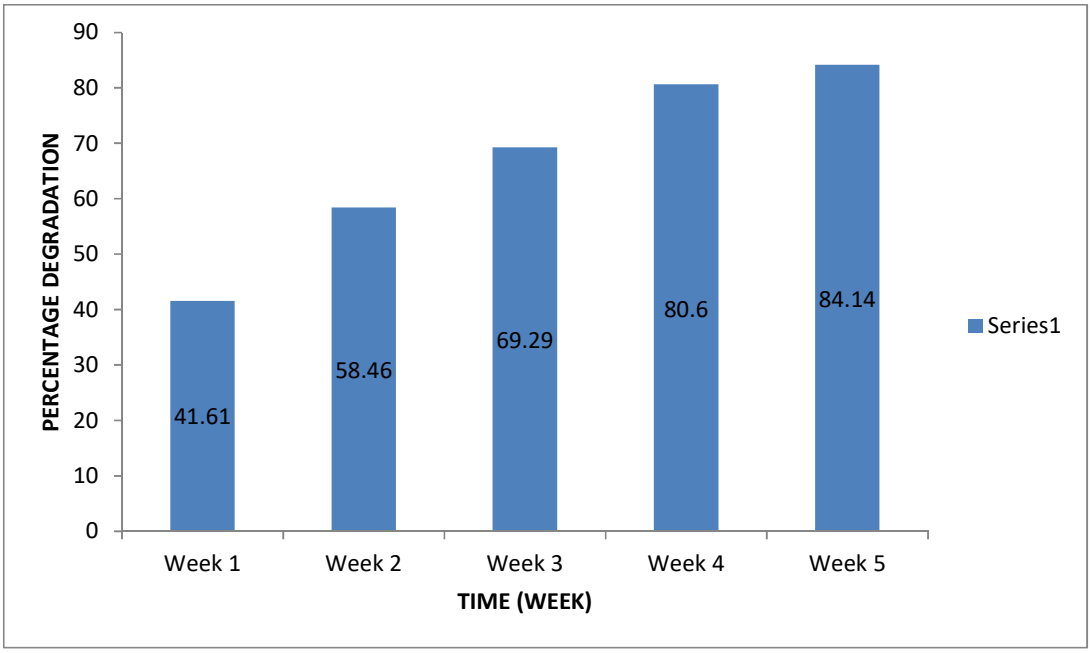


Figure 4.31c: Percentage degradation of petroleum hydrocarbon using (poultry+cow+saw dust) manure ($F_C$)

## 4.2 Result Discussion and Analysis

The soil parameters that were used to characterize the effect of the various amendments used in this study are shown in table 4.1. The initial values of these parameters represent the baseline or starting point for any bioremediation process. Some of the soil parameters were altered after treatment, such parameters include; the soil pH (4.70-8.36), organic carbon (4.33-5.83), organic matter (4.33-5.83) were observed before and after the remediation actions. Increase in some soil parameters before bioremediation could be to the fact that the contaminated soil contains varying proportions of organic carbon while increase in some parameters after bioremediation could be connected to the fact that the nutrient supplements contain some proportions of organic matter, nitrogenous substances e.t.c. Furthermore, at the end of 10 weeks, 79.89%, 81.48% and 84.14% degradation was achieved using NPK fertilizer, poultry manure and poultry+cow+saw dust manure respectively. The result obtained from this investigation shows that the combination of poultry+cow+saw dust manure offers the highest percentage degradation. The removal rates with the later perhaps were feasible due to the presence of sawdust as bulking agent (porous media) that could allow desorption processes as well as biodegradation.

The experimental data was fitted according to the model developed by Oyoh and Osoka (2007) using a curve fitting tool. Also from the numerical fit result of table 4.2-4.4, it can be deduced that; The specific growth rate (μ) increases with increase in amount of nutrient applied. The specific growth rate (μ) increase was higher at the optimum load of poultry+cow+saw dust manure. $YX_0$, defines the initial microbial concentration to the final, thus the lower its value the higher the degradation rate. This was observed to decrease steadily as the quantity of manure applied increases. $Xo/Y_G$ is the ratio of the initial microbial concentration to the yield coeeficient. This value was observed to increase with decrease in yield coefficient. It was also observed to increase with increase in amount of manure applied.

Increase in $X_O/Y_G$ was more pronounced at the application of ( poultry+cow+saw dust) manure though at higher quantity.

The graphs of the kinetic pattern for total hydrocarbon content reduction for the various bio-stimulants employed in this study are shown in Figures 4.16-4.30. In rate modeling and analysis, it is very important to have a realistic measure of reaction rate constant .The higher the rate constants (k) and the correlation coefficients ($R^2$), the higher the rate of the biodegradation process.

The values of model rate constants k, coefficients of determination $R^2$ and other parameters as estimated from the model fits are represented in table 4.5-4.7.

The table reveals a positive correlation coefficient $R^2$ for the reduction in total hydrocarbon content, with high rate constants. From the result obtained, the biodegradation rate constant (k) was higher for the combination of (poultry+cow+saw dust) manure.

It was observed from fig. 4.16-4.30 that 10g, 20g and 30g of both NPK fertilizer and poultry manure fitted well to first order rate model in which the ultimate contaminant concentration is not zero i.e ($S\infty \neq 0$) but quickly changes trend on the addition of 40g and 50g. Thus as the quantity of fertilizer and poultry manure addition increases the second order rate model is obeyed i.e a case in which ultimate contaminant concentration is zero, ($S\infty = 0$). A different trend was observed in treatment with a blend of (poultry+cow+saw dust) manure in which the rate model equation 4 (second order rate model with $S\infty = 0$) is obeyed. It simply mreans that the application of NPK fertilizer and poultry manure at an increased quantity offer similar effect with (poultry+cow+saw dust) manure. This implies that rather than combining biostimulants, poultry manure or NPK fertilizer applied singly at a higher quantity can be used to obtain the same effect when poultry+cow+saw dust is supplied.

## CHAPTER FIVE

# CONCLUSION AND RECOMMENDATIONS

### 5.1 Conclusion

Based on the results obtained from these experiments, the following conclusions can be drawn; Remediation of the oil contaminated soil at the end of ten weeks revealed a positive correlation coefficient ($R^2$) and high reaction rate constants (k) for the various biotreatments. Petroleum removal effeciency in tems of TPH can reach a maximum of 83.6% for (cow dung+poultry droppings+saw dust) over a period of 10 weeks within the range of experimental conditions investigated in this study. A lesser degree of enhancement was obtained when nutrients were added singly. The above result indicates that the biostimulants employed in this study offer significant reduction in the hydrocarbon content (i.e., used singly/or in combination).

This study demonstrates that at optimum load of fertilizer and poultry manure (singly), the rate of microbial growth increases as the level substrate consumed increases. This accord with the result obtained when a combination of (poultry+cow+saw dust) is employed. It was observed that the treatment measures employed in this work followed a first order kinetic rate model with the ultimate contaminant concentration not being zero i.e ($S\infty \neq 0$ ) when biostimulants is applied in smaller quantities. But increase in amount of treatment tends to change the reaction towards the second order kinetic rate model with the ultimate concentration assumed zero i.e ($S\infty = 0$). Therefore, both growth curve model and the kinetic model approach employed in this work provided a good description of an effective bioremediation process.

The technology for bioremediation that was employed in this study is a simple, effective, inexpensive and environmentally friendly approach, whose biostimulant is readily available, cheap and is compatible to the environment

These observations indicate that the mixture of saw dust, cow dung,and poultry dung (animal source waste) used alone and/or in combination enhanced biodegradation in soil. Similar observations have been reported for the use of plant and animal-derived organic waste (Liu et al., 2010) in the bioremediation of soil contaminated with petroleum hydrocarbons.( Liu et al. 2010) used organic manure made up of rice straw and pig dung to biostimulate the degradation of an oily sludge and obtained a TPH reduction of 58.2% in a remediation period of 360 days, while( Agarry et al) in their investigation on kinetic model and half life study of Bonny light crude oil amended with crop residue and animal derived organic manure confirms that the use of crop residue and animal derived organic manure improved the rate of biodegradation of hydrocarbon in a crude oil contaminated soil. A maximum Total Petroleum Hydrocarbon removal of 96.6% and 94.86% was obtained for the use of pig dung and cassava peels as biostimulants.

## 5.2 Recommendations

Based on the results obtained in this study, I recommend that further studies should be carried out on the use of other materials such as stimulants from plant sources in bioremediation study. However, extensive development on this technique to achieve zero residual total petroleum hydrocarbons (TPH) is recommended. Therefore research should be made on how to improve and overcome limitations that hinder bioremediation of petroleum hydrocarbons.

Research need to relate these model parameters with the potential effects of certain environmental and climatic factors such as temperature changes, leaching and flooding e.t.c on achieving an effective bioremediation of a petroleum contaminated soil. Thus additional research is recommended on how to translate success in the laboratory in order to succeed in this field of study.

Finally, development of sound environment and economical clean-up procedures is essential and must be highly encouraged.

## 5.3 Contribution to Knowledge

Findings from this research have helped reveal the optimal conditions needed to achieve an efficient degradation of a petroleum contaminated soil. It has obviously enhanced understanding on the key factors and measures that can be employed to successfully address environmental problems and subsequently enhance biodegradation rate of a petroleum contaminated soil. It has also broadened the idea on the best approach for this purpose.

This study provides background information to prioritize research and development, thus can be used to size a pilot plant for a field scale bioremediation purpose.

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## APPENDIX A

**Tables A.1: Concentration of total petroleum Hydrocarbon (TPH) in the soil at time (week), $C_r$ and the concentration of (TPH) degraded ($C_d$) for 10-week period of bioremediation with ($F_A$, $F_B$, $F_C$).**

| Time(Week) | $C_r$ | | | $C_d = (C_o - C_r)$ *($C_o$=1980mg/kg)* | | |
|---|---|---|---|---|---|---|
| | $F_A$ | $F_B$ | $F_C$ | $F_A$ | $F_B$ | $F_C$ |
| Week 0 | 1430.2 | 1370.4 | 1210 | 549.8 | 609.6 | 770 |
| Week 2 | 1291.4 | 1181.8 | 1040 | 688.6 | 804.2 | 940 |
| Week 4 | 930.8 | 830.8 | 820 | 1049.2 | 798.2 | 1160 |
| Week 6 | 878.4 | 652.4 | 598 | 1101.6 | 1327.6 | 1383 |
| Week 8 | 500.2 | 488.6 | 388.2 | 1479.8 | 1497.4 | 1591.8 |
| Week 10 | 374.8 | 337.80 | 233 | 1605.2 | 1648.2 | 1747 |

**Table A.2: Percentage degradation of Petroleum Hydrocarbon at the end of 10week**

| Biostimulation treatment | %degradation $\left(\frac{C_o - C_r}{C_r}\right) \times 100$ |
|---|---|
| NPK fertilizer | 79.89 |
| Poultry manure | 81.48 |
| (Poultry+cow+saw dust) manure | 84.14 |

$C_o$= initial Total Petroleum Hydrocarbon, ($C_o$ =1980mg/kg), $C_r$= Residual TPH concentration

## APPENDIX B

### Soil Outlook at Different Stages of the Experiment

At the beginning of the experiment the contaminated soil was like mud soil, all wet and more like clay soil. Picture number B.1 was taken after one week of the experiment. The difference is that, the soil before the treatment was dark muddy and one could smell the HC from far way. After the tenth day of the experiment as shown in picture number B.2, the contaminated soil was much lose and was changing to lighter brown color.

**Fig B.1: Soil outlook after the 7th day of the experiment**

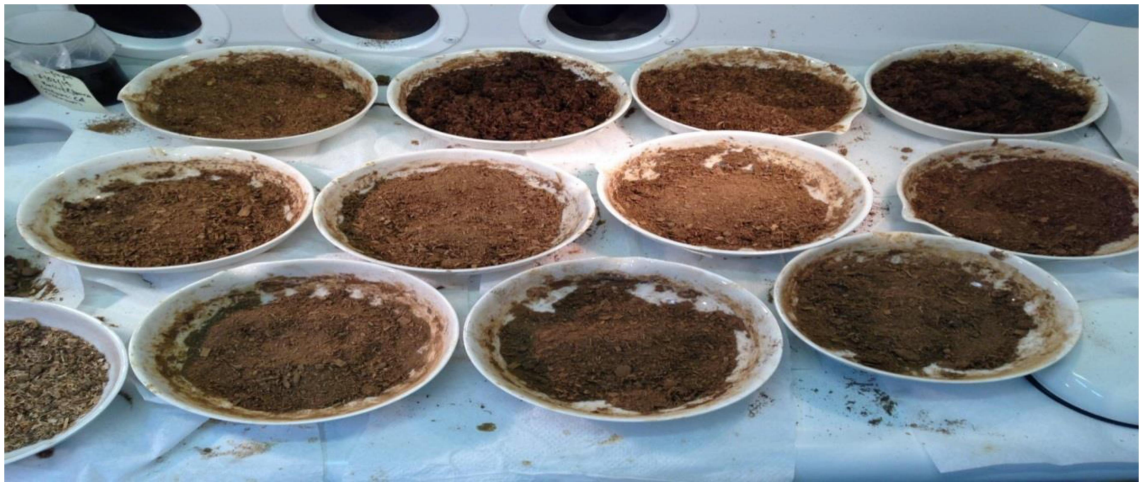

**Fig B.2: Soil outlook after the fifth week of the experiment**

At this stage of the experiment, after the fifth week the hydrocarbons in the soil are starting to become lose. This means that the percentage of hydrocarbon contamination is decreasing on the application of amendments.

**Fig B.3: picture taken after the tenth week of the experiment**

The picture number B.3 was taken after the tenth week. One can observe that the soil is very lose and the treatments are dryer. If we have to compare to the initial contaminated soil, picture B.3 looks much better and treated. The picture number B.3 was taken in the last days

of the experiment, although it look very loose but not totally remediated, one can still smell a little bit of hydrocarbon in the soil. In this picture number B.3 not all the sample was dry, the reason is because of some of the sample did not get enough temperature. This can be a reason for the variations in the rate of degradation observed in the different amendments.

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