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TOPIC:

PRECIOUS GIFTS FROM FERMENTATIVE AND PHOTOSYNTHETIC MICROORGANISMS TO MANKIND

By
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An Inaugural Lecture Delivered at the University of Lagos Main Auditorium on Wednesday 2nd December, 2009

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PREAMBLE

Mr. Vice-Chancellor, Sir; the Deputy Vice-Chancellor (Management Services); the Deputy Vice-Chancellor (Academic and Research); the Registrar, other Principal Officers of this great University and other Universities; the Dean, Faculty of Science; Dean, School of Postgraduate Studies, other Deans here present; Members of the University Senate: my Colleagues, great Microbiology students of Faculty of Science and other Universities here present; Friends of Microbiology; Chaplains and Members of Chapel of Christ Our Light, Unilag and ST John's Anglican Church, Amumara Mbaise here present; Members of the Press, my Family members; distinguished Ladies and Gentlemen, indeed, it is a great honour to be given the opportunity to give an inaugural lecture during the 2009/2010 academic session. I remain very grateful to the University of Lagos where my development was perfected, to University of Ife, Ile-Ife now (Obafemi Awolowo University) where my development to become a scientist was initiated and above all to God Almighty for lifting me up to this great height, Professor of Industrial / Environmental Microbiology.

OBJECTIVE OF THIS LECTURE

An Inaugural Lecture is a big ceremony designed to admit formally to the office of a Professor an academic who has distinguished himself / herself, to showcase his / her activities in the University, community, country in particular and in the world at large through teaching, research and service to mankind. The title of this inaugural lecture is: "***Precious gifts from fermentative and photosynthetic microorganisms to mankind.***" In the course of this lecture, I will discuss precious products as gifts to mankind derived from fermentation, respiration and photosynthesis as important microbiological processes. Moreover, I will also highlight some of my contributions to add value to these precious gifts by microorganisms for the aesthetics and good management of man in his natural environment.

INTRODUCTION

Microbiology is the study of organisms and agents which cannot be seen by the unaided eye. Microorganisms are so small that they are measured in a unit known as micrometer (um) and 1um = 10^{-6}m or 0.000001m. Thus, the study of microbiology was delayed for lack of instruments to make microorganisms visible to the eye even though before their observation, some investigators had already suggested their existence and responsibility for many diseases. It was not until Anthony van Leeuwenhoek (1632 – 1723) developed his own magnifying lenses that the first groups of microorganisms namely protozoa and bacteria were observed and described. Today, Antony van Leeuwenhoek is accepted by scientists all over the world as the "Father of Microbiology" because of his discovery. His lenses could magnify objects about 50 to 300 times which aided human eye to see and describe microorganisms (Prescott *et al.*, 2002). There are five major groups of microorganisms namely bacteria, fungi, viruses, protozoa and algae, which are well studied in microbiology. The study of these groups of microorganisms is described as bacteriology, mycology, virology, protozoology, and algology or phycology, respectively. At this great University, these subject areas are covered in years one to three while the year four programme deals with applied aspects of microbiology to include Industrial Microbiology, Pharmaceutical Microbiology, Food Microbiology and Security, Environmental Microbiology, Agricultural Microbiology, Medical Microbiology, Soil Microbiology, Aquatic Microbiology, Microbial Genetics, etc. all of which have great impacts on the development and welfare of mankind.

DEFINITION OF FERMENTATION

What is fermentation?

Fermentation is a metabolic process with many meanings: any process involving a large scale cultivation of microorganisms either under aerobic or anaerobic condition is regarded as a fermentation process. Any biological process occurring in the absence of oxygen is a fermentation process. The most popular definition is that fermentation is a term used to describe the

production of alcoholic beverages. Yet many microbiologists regard fermentation as a growth process which is dependent on substrate level phosphorylation for energy interplay. Against this background, ***fermentation can be defined physiologically as a metabolic process in which carbohydrate and related organic compounds are oxidized with the release of energy in the absence of any external electron acceptors, the final electron acceptors being organic compounds produced directly from the breakdown of the carbohydrate or related compounds.*** Microorganisms and, indeed, all living things that obtain energy and metabolites required for sustenance of life through fermentation are known as ***fermentative organisms***. Thus, in fermentations, the substrate for energy and metabolite production is oxidized and degraded without any involvement of exogenous electron acceptors. The electron acceptors that come into play during the process are intermediates produced as the substrate is oxidized. In other words, fermentation is an energy yielding process in which carbohydrates and related organic compounds such as proteins, lipids etc. serve as both electron donors and electron acceptors(Jay, 1978).

HOW DOES FERMENTATION DIFFER FROM RESPIRATION?

An energy- yielding metabolism involving exogenous or external electron acceptors is known as respiration of which there are two main types namely aerobic and anaerobic respiration. Oxygen is the final electron acceptor in aerobic respiration whereas in an anaerobic respiration different exogenous electron acceptors other than oxygen such as nitrates, sulphates, carbonates, elemental sulphur, oxidized iron (Fe^{3+}), etc. can be used as the final electron acceptors. In fermentation none of these substances is involved as the final electron acceptor. Again, the energy interplay differs in respiration and in fermentation. In fermentation, energy is formed by substrate level phosphorylation. According to Prescott *et al.,* (2002), the amount of energy obtainable in fermentative microorganisms is quite small, about 2 ATP. This is because the electron acceptors involved in fermentation process exist at the same

oxidation number as the original nutrient with no overall net oxidation number of the nutrient by the end of the process. Consequently only a limited amount of energy as ATP (Adenosine triphosphate) is released during a fermentation process as no electron transport chain is involved. In contrast, the electron acceptors in both aerobic and anaerobic respirations have reduction potentials much more positive than the substrate being degraded resulting in release of more energy about 38 ATP along the electron transport system (chain) by the time one mole of glucose is broken down completely to give CO_2 and H_2O.

HOW DO FERMENTATIVE MICROORGANISMS OBTAIN THEIR NEEDS FROM NUTRIENTS OR FOODS SUPPLIED TO THEM?

Microorganisms grow in systems containing six basic factors namely: energy, carbon, nitrogen source, minerals elements and growth factors (vitamins) all solubilized in water. When these substances are present in water in the right proportions, they constitute microbial nutrients and the media in which they are present are known as microbiological media. There are different types of microbiological media available to microbiologists, to use for cultivation of microorganisms examples include general purpose media such as nutrient agar and potato dextrose agar; selective media such as MacConkey agar, differential media such as eosin methylene blue agar. When these media are formulated as directed by the manufacturers for various purposes, the six basic factors enumerated above are present in the right proportions. Thus, by fermentation and/or respiration processes, microorganisms utilize the substrates to meet their growth requirements. To achieve this, they use several metabolic pathways and cycles to breakdown the organic substrates present in media of which the most common ones include glycolysis and tricarboxylic acid (TCA) cycle. These metabolic processes, as important stages in both fermentation and respiration, consist of enzyme catalyzed reactions which are thermodynamically possible to produce useful metabolites and energy required for life processes. For

glycolysis, the starting organic compound as a source of energy and carbon could be glucose, fructose (carbohydrates), proteins, lipids or amino acids; these are converted to pyruvate whether oxygen is present or not. Subsequently, what happens to the pyruvate produced depends on a number of factors such as availability of oxygen or lack of it, nature of the microorganisms involved, obtainable physiological conditions etc. Mr. Vice-Chancellor Sir, many products which I consider as precious gifts to mankind can be derived from pyruvate when man explores the above listed number of factors. For TCA cycle, which is much more efficient in terms of energy generation, oxygen must be present as the final electron acceptors and the pyruvate is completely oxidized to carbon oxide and water. The reactions, energy interplay as ATP (Adenosine triphosphate), intermediate metabolites and products, enzymes, coenzymes etc derivable from microbial metabolism and which are precious gifts to mankind are illustrated in Figure 1.

Examples of precious gifts to mankind by fermentative microorganisms:

1. PRODUCTS FROM PYRUVATE

Many fermentative microorganisms can ferment pyruvate to numerous products which are very useful to mankind for various purposes. A few of the products from pyruvate and the key microorganisms associated with the fermentations are illustrated in Figure 2. Mr. Vice-Chancellor Sir, many microbiological and chemical industries are all over the world including Lagos, Nigeria, producing these products to meet the needs of mankind.

Figure 1: Precious products derivable from microbial metabolism.

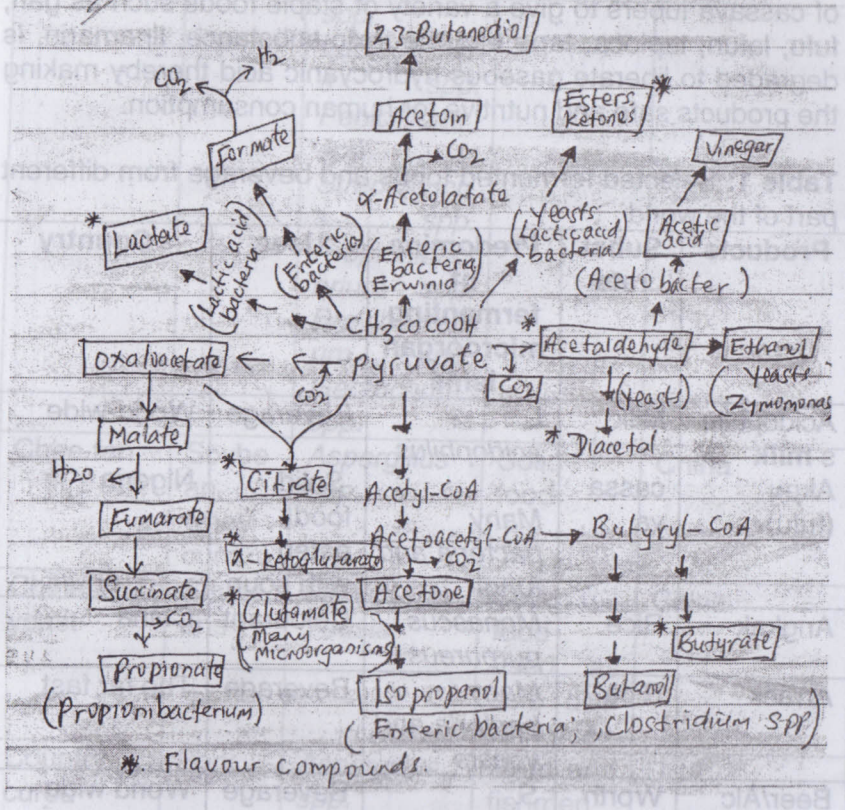


Figure 2: Some useful products produced by fermentation of pyruvate, many other organic compounds can be similarly fermented to give numerous useful products to mankind.

2. **Food/Beverage products from fermentation processes:** Many staple foods, beverages, condiments, starters and seasoning agents are produced by fermentation processes for the benefits of mankind. Table 1 gives examples of food, beverages and seasoning agents produced by fermentative microorganisms by different countries of the world. In these products, the microorganisms implicated modify and improve the original unfermented substrates nutritionally, physically and organoleptically. For example, unfermented fresh cassava tubers contain linamarin, in high concentrations, which is a

poisonous cyanogenic glucoside. Thus during the fermentations of cassava tubers to give a variety of staple foods such as gari, fufu, lafun, tapioca, tape etc, the toxic substance, linamarin, is degraded to liberate gaseous hydrocyanic acid thereby making the products safe and nutritive for human consumption.

Table 1: Selected fermented foods and beverage from different part of the world

Products	Substrate	Predominant fermenting microorganisms	Uses	Country
Acidophilus **milk** Akpu (fufu)	Milk cassava	*L. acidophilus* *Many bacteria and fungi*	Beverage. Solid food eaten with soup	World-wide Nigeria
Angkak	Rice	*Monascus purpureus*	Colourant	China
Arrack	Rice	*Many bacteria and yeasts*	Beverage	The far fast
Beer/Alc	Worth from cereals	*S. carlsbergensis S. uvarum S. cerevisiae*	Beverage	World wide
Binuburan bread	Rice wheat flour	*Yeasts S. cerevisiae*	Solid from beverage	World-wide
Bourbon whiskey	Maize, Rye	*S. cerevisiae*	Beverage	USA
Bulgarian buttermilk	Milk	*L. bulgaricus*	Beverage	Bulgaria
Cheese	Milk card	*Lactobacillus spp. Pencillium spp, Lactobacillus spp. Penicillium spp Streptocucus spp. Propionibacterium spp. Brevibacterium spp. Geotrichum spp.*	Solid food	World-wide
Chee-fan	Soybean whey curd	*Aspergillus spp.*	Solid food	China
Chinese yeast	Soybeans	*Mucor spp., Yeasts*	Solid food used as sole dish with rice	China
Country cured	Pork	*Aspergillus spp.*	Meat and fishmen food	USA
Cider	Fruits such as apples, oranges	*Saccharomyces spp.*	Beverage	World-wide
Cocoa beans	Cocao fruits (pods)	*Gestrichum spp Candida krusei*	Food	Africa S. American
Coffee beans	Coffee cheeries	*Erwinia dissolvens Saccharomy*	Food condiment	Congo, India

		ces spp		
Dawadawa	African locust bean	*Bacteria, moulds and yeasts*	Solid food eaten fresh	Nigeria W. Africa
Fish sauces	Small fish	*Bacillus spp.*	Food saurce	Asia
Garri	Cassava tubers	*Corynebacterium spp. Lactobacillus spp. Saccharomyces spp., Candida spp. Leuconostoc spp.*	Solid food products eaten with soup	Nigeria, Ghana
Idli	Rice	*Lactobacillus spp., Leuconostoc spp., Streptococcus spp. Torulopsis spp., Trichosporon spp.*	Spongy food served as a substitute for bread	India, Pakistan
Izushi	Rice, fish and vegetable	*Bacteria e.g. Lactobacillus spp.*	Solid food	Japan, Taiwan
Kanga-Kpouwai	Maize	*Bacteria and yeasts*	Solid food eaten as vegetable	New-Zealand
Kefir	Milk	*Streptococcus lactis., Lactobacillus bulgoricus., Torula spp.*	Beverage	Asia

Ketjap	Soybeans	*Aspergillus oryzae.*	Syrup used as seasoning	Indonesia
Kenkey	Maize	*Aspergillus spp. Penicllium spp. Lactobacillus spp. Yeasts*	Solid food product	W. Africa
Kimichi	Cabbage	*Lactic acid bacteria*	Solid food eaten as vegetable	Korea
Kumiss	Raw mare's milk	*Lactobacillus spp. Torula spp.*	Beverage	Russia
Lao-Chao	Rice	*Rhizopus oryzae. R. chinensis., Chlamydomucor spp. Saccharomycopsis spp*	Soft juice food product eaten in combination with shrimps, perwinkles, eggs etc	China, Taiwan, Japan
Lafun	Cassava tuber	*Many bacteria and fungi*	Solid food eaten with stew/soup	Nigeria, Ghana

Lebanon-bologna	Beef	*Pedicoccus cerevisiae*	Meat product eaten fresh like sausage	U. S. A.
Magon	Maize	*Lactobacillus spp., Leuconostoc spp*	Beverage product	Bantus of S. Africa
Meitauza	Soybean-cake	*Actinomucor elegans*	Solid food product fried or cooked with vegetables	Taiwan
Meju	Soybeans	*Rhizopus spp*	Solid paste used as a seasoning agent	Korea
Mescal	Century plant	*Yeasts*	Beverage product	Mexico
Minchin	Wheat gluten	*Fusarium spp, Aspergillus spp Cladosporium spp, Penicillium spp, Rhizopus spp., Trichotheciu m spp*	Solid paste used as food condiment	China
Miso	Cereal (rich	*Lactobacillus spp.*	Soup base or	Japan, China

	barley, Soybean)	*Leuconostoc spp., Saccharomyces rouxii., Aspergillus spp.*	seasoning agent	
Ogi (Akamu)	Maize	*Lactic acid bacteria such as Lactobacillus plantarum, Streptococcus lactis, Leuconostoc spp; Fungi Cephalosporium spp., Aspergillus spp., Penicillium spp, Candida mycoderma, Saccharomyces cerevisiae, Rhodotorula rubra*	Very important staple food product	W. African Countries
(Soy-Ogi)	Maize, Soybean	*Lactic acid bacteria such as Lactobacillus plantarum, Streptococcus lactis, Leuconostoc spp; Fungi Cephalosporium spp,*	Ogi enriched with protein to produce a weaning food product	Nigeria, Ghana

		Aspergillus spp., Penicillium spp, Candida mycoderma Saccharomyces cerevisiae, Rhodotorula rubra		
Oncom	Peanut, Press-cake	*Rhizopus spp Aspergillus spp, Neurospora sitophila*	Solid food product fried and served as a substitute for meat	Indonesia
Palm wine	Palm sap	*Lactic acid bacteria, moulds and yeasts*	Beverage product	Nigeria, Ghana
Peijeum	Cassava	*Moulds and yeast*	Solid food product	Indonesia
Oo	Rice	*Yeasts and moulds*	Beverage product	Thailand
Pickles	Cucumbers	*Pediococius cerevisiae, Lactobacillus spp*	Jepehable product	World-wide
Poi	Taro corns	*Candida spp Lactobacillus spp Leuconostoc spp*	Semi solid food product served with meak and	Hawalt

			seafood	
Sauerkraut	Cabbage	*Lactic acid bacteria, some fungi*	Solid food product	World-wide
Sake	Rice	*Saccharomyces spp*	Beverage product	Japan
Scotch whiskey	Barley other cereals	*S. cerevisiae*	Beverage product	Scotland
Shoyu	Cereals such as wheat soybeans	*Lactobacillus spp. Leuconostoc spp, Aspergillus soyae Saccharomyces rouxii*	Liquid product used for seasoning cereals, fish, meat and vegetable before consumption	Japan, China, Philippines Taiwan
Sufu	Soybean whey curd	*Mucor subtilissimus., Mucor spp.*	Solid food product eaten like cheese	Taiwan
Tape	Cassava tubers rice	*Consortium of microorganisms comprising moulds, yeasts and a few bacteria.*	Soft solid food product eaten as fresh staple	Indonesia
Temp	Soybeans	*Rhizopus spp.*	Solid food fried in oil and eaten as	Indonesia, Taiwan

			meat substitute, rich in proteins	
Vinegar	Fermented liquor e.g. wine	*Acetobacter spp*		World-wide
Wines	Fruits such as grapes, oranges	*Saccharomyces cerevisiae var ellipsoideus*	Beverage	World-wide

3. **Gaseous products:** Gases such as carbon IV oxide, hydrogen sulphide, hydrogen, mercaptan are examples of gases produced as a result of fermentation. Some of these products are very useful industrially. For example, evolution of CO_2 by fermentative microorganisms such as *Saccharomyces cerevisiae* is indispensable in baking industries. Bread, rolls, sweet goods such as Danish pastry, crackers, doughnuts, bagels, pretzels are among the products in which *S. cerevisiae* and recently *Saccharomyces carlsbergensis* (By-products of brewing industries) are used as the leavening agents.

4. **Microbial Transformations:** By fermentation process many microorganisms are used to carry out bioconversions of substrates to produce more useful products. Steroids are complex chemical substances derived from saturated cyclopentanophenanthrene nucleus that have many medical applications. They are used for the treatment of many diseases such as arthritis, rheumatism, anemia, allergy etc. Some selected fungi and actinomycetes are used to effect chemical changes in steroidal substances extracted from plants and animals transforming them by fermentation to produce more actively therapeutic agents. The chemical synthesis of the

steroidal products is possible but exceedingly very difficult and costly. Figure 3 shows progesterone (a steroid) transformed to give a variety of useful products by fungi and some mould-like bacteria such as *Streptomyces sp.* The reactions effected by fermentative microorganisms used include either oxidation e.g. hydroxylation, epoxidation, dehydrogenation; or reduction e.g. hydrogenation of a double bond or carbonyl group. For example transformation of progesterone to 6B II-Dihydroxyprogesterone by *Rhizopus nigricans* is a hydroxylation process while conversion of cortisone to prednisone is a dehydrogenation process.

Figure 3: Progeste-rone conversion

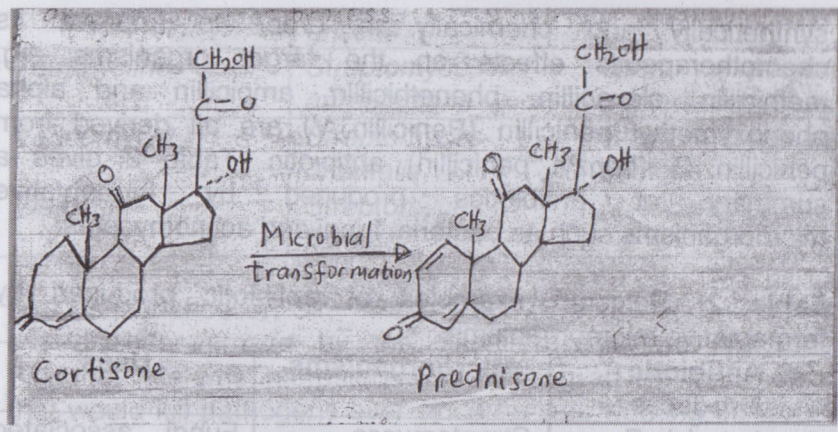


Figure 4: Bioconversion of cortisone to prednisone by microbial transformations

Thus, prednisone is a precious product by selected fermentative microorganisms with increased anti-inflamatory action. It is used for the treatment of allergy. The key to a specific transformation to give these precious products resides in the utilization of selected microbial strains having the desired enzymes constitution in conjunction with suitable substrates.

5. **Antibiotic production:** Antibiotics are metabolic chemotherapeutic agents produced by organisms which are detrimental or inhibitory to other microorganisms in very small concentrations. Generally antibiotics produced by fermentation processes exert their chemotherapeutic effects on pathogenic microorganisms by inhibiting cell wall formation, damaging cell membrane, inhibiting metabolism of DNA or RNA and by interfering with protein synthesis. Soil ecosystem is a convenient and vast reservoir of diverse microorganisms potentially capable of providing the basic products or materials for the microbiological studies used in the search for new antibiotics. Although there are some synthetic and semi-synthetic antibiotics, most of the antibiotics used in the treatment of many diseases of microbial origin are produced as a result of fermentations by selected microorganisms. Then substituents can be added to the basic microbial product

synthetically and chemically in order to improve its chemotherapeutic effects on the target organisms e.g. methicillin, cloxacillin, phenethicillin, ampicillin and alpha phenoxymethyl penicillin (Penicillin V) are all derived from penicillin G (benzyl penicillin) antibiotic. Table 2 gives a summary of antibiotics produced by fermentative microorganisms such as bacteria, fungi and actinomycetes.

Table 2: Selected examples of antibiotic produced by fermentative microorganisms

Antibiotic Products	Microorganisms	Uses
Amphotericin B	*Streptomyces nodosus*	Fungi associated with mycoses
Bactitracin	*Bacillus licheniformis*	Gram positive bacteria
Chloramphenicol	*Streptomyces venezuelae*	Broad spectrum
Cycloheximide	*Streptomyces griseus*	Fungi (plants)
Erythromycin	*Streptomyces erythraeus*	Gram positive bacteria
Fumagillin	*Aspergillus fumigatus*	Amebas
Grigseofulvin	*Penicillium griseof vlvum, P. patulum*	*Microsporum trichophytum*
Kanamycin	*Streptomyces kanamyceticus*	*Mycobacterium tuberculosis*
Neomycin	*Streptomyces fradiae*	Broad spectrum
Novobiocin	*Streptomyces spp*	Gram positive bacteria
Nystatin	*Streptomyces noursei*	Intestinal *Candida*
Penicillin	*Penicillium chrysogenum*	Gram positive bacteria
Polymyxin	*Bacillus polymyxa*	Gram negative bacteria
Streptomycin	*Streptomyces griseus*	Broad spectrum
Tetracycline	*Streptomyces aureofaciens*	Gram positive bacteria
Vancomycin	*Streptomyces orientalis*	Gram positive bacteria
Viomycin	*Streptomyces floridae*	*M. tuberculosis*

Mr. Vice-Chancellor, Sir, Plate 1 shows something, similar to the discovery made by the Scottish physican, Alexander Fleming. He was interested in developing an efficient method that would kill pathogens ever since he was working on wound infections during the First World War. He cultured *Staphylococcus aureus* in a Petri-dish but *Penicillium notatum* accidentally landed on the surface of the culture as a contaminant(Prescott *et al.*, 2002). Later, Fleming observed that the contaminant was growing at one edge and that the *Staphylococcus aureus* (which was the real culture being studied) surrounding it had been destroyed just as was observed for Plate 1 during my investigation to develop suitable methods for the bioremediation of crude petroleum polluted environments (Nwachukwu, 1998a; 2000a; 20001). Alexander Fleming was amazed at his observation and rather than discarding the contaminated plate, he concluded that his mould contaminant was producing a diffusible substance lethal to *Staphylococcus aureus*. This was the origin of the first antibiotic and Alexander Fleming with his colleagues namely Florey and Chain received the Nobel Prize in 1945 for the discovery and production of the first antibiotic known as penicillin G (benzyl penicillin). Mr. Vice-Chancellor, Sir, the studies of the culture in Plate 1 could not be continued because of the lack of the enabling environment most especially power outage. Consequently, the culture became profusely contaminated during the course of the primary screening of the isolate which was identified as *Streptomyces* species.

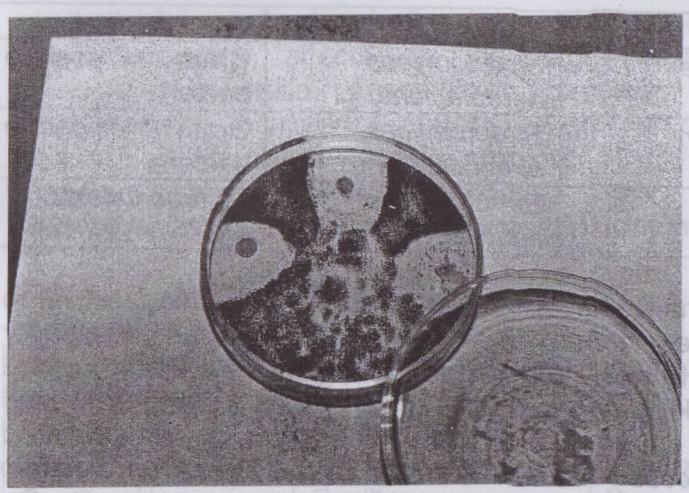


PLATE I: Culture of *Aspergillus niger* being inhibited by *Streptomyces* spp.

6. Useful Products of Photosynthesis to Mankind

All life on the planet, earth, is largely dependent on photosynthesis. During the process which takes place in microscopic green algae and higher plants, carbon dioxide and water react to form simple sugars that provide the building blocks for the synthesis of complex carbohydrates, proteins, fats and nucleic acids with the liberation of oxygen in the presence of sunlight energy trapped by chlorophyll in plants. The simple summary equation for the process is given as:

$$CO_2 + H_2O + \text{Light Energy} \xrightarrow[\text{Chlorophyll (Plants)}]{\text{Enzymes}} \text{Sugars} + O_2 \text{ -------- (1)}$$

-Complex carbohydrates
-Proteins
-Fat
- Nucleic acids

Also for comparison, the simple summary equation for aerobic respiration is:

$$\text{Sugars} + O_2 \xrightarrow[\text{Animals, Plants}]{\text{Enzymes}} CO_2 + H_2O + ATP \text{ -----(2)}$$

Thus, the products of photosynthesis in equation 1 fuel the activities of living things as summarized in equation 2. For

example, the oxygen liberated during photosynthesis is used for aerobic respiration and therefore essential to most organisms in the biosphere on the planet, earth, where you and I inhabit. In other words, over time, the oxygen gas released during photosynthesis has dramatically changed the earth's biosphere and enabled the evolution of aerobic respiration in animals and other organisms. Furthermore, photosynthesis is the primary source of our comfort by providing foods, beverages, fibres, clothing, paper, wood for furniture and building, energy for transportation and so on. The automobile which we run today depends on modern products formed by processing the products of ancient photosynthesis that became oil, gas and coal a long time ago in the earth's crust.

If oxygen were to be prepared by chemists by the action of heat on potassium trioxochlorate (iv) [$KClO_3$] with manganese IV oxide (MnO_2) as the catalyst, by oxidation of hydrogen peroxide (H_2O_2), by thermal decomposition of mercury (ii) oxide (HgO) and lead(iv) oxide.(PbO) or by reaction of sodium peroxide (H_2O_2) with water [H_2O] which all take place under very stiff conditions, no doubt, oxygen would only be available for the rich and not for the poor. Indeed, chemists are aware of these facts and consequently made more incursions through seasoned researches into how to prepare oxygen cheaply to meet the needs of mankind especially patients in hospitals who could not access the free oxygen in the natural environments. Thus, oxygen can be prepared industrially by the electrolysis of water or by the fractional distillation of liquefied air containing about 21% v/v of oxygen originating mainly from photosynthesis. Again, provision of oxygen by these methods cannot be adequate to meet the needs of mankind in his environment. Moreover, these methods are only possible in places where a cheap source of electricity is available and, in Nigeria, additional reaction unit operations must be put in place to first of all purify the air before liquefaction because of the heavy contaminants present in Nigerian air.

Mr. Vice-Chancellor, Sir, if I state that, today, virtually all life after creation depends on photosynthesis and its products, I am

not wrong. Thus, the products of photosynthesis are the most precious gifts by photosynthetic microorganisms and higher green plants to mankind inhabiting this planet, earth.

My Contributions to Knowledge

Mr. Vice-Chancellor, Sir, as a member of staff of this great University and servant of God Almighty, God has helped me to conduct researches in four key areas making outputs which are beneficial to mankind. The four areas which we have made very useful contributions to knowledge include:

a. Food production and security.
b. Environmental impact assessment of recalcitrant molecules.
c. Bioremediation of environments polluted with recalcitrant molecules.
d. Manufacturing industries.

A. FOOD PRODUCTION AND SECURITY

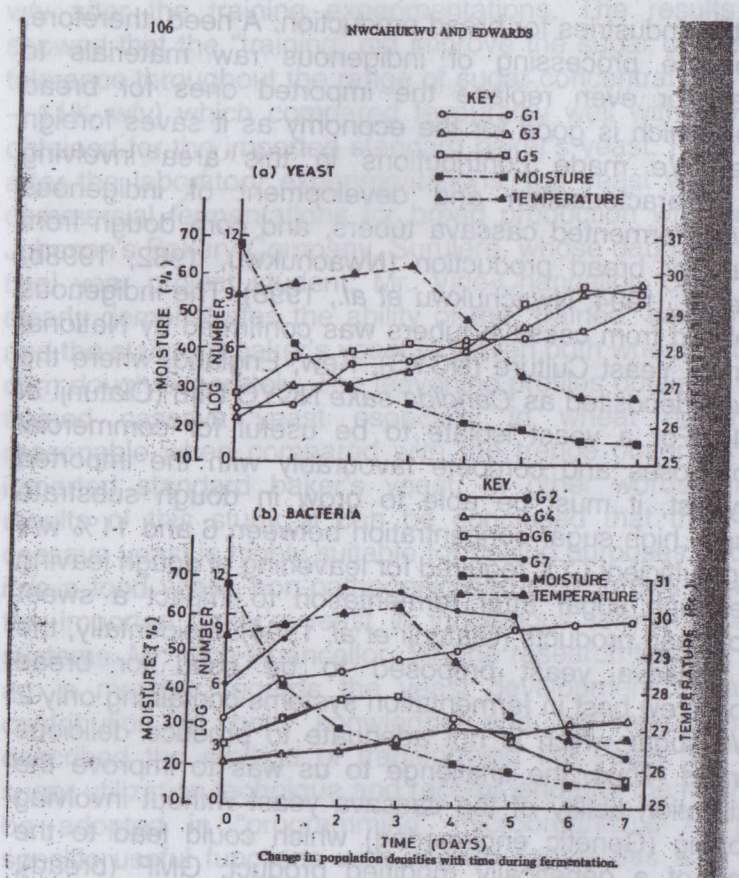
Food can be defined as any nutritious substance that people or animals eat or drink or that plants absorb in order to maintain life and growth. Of the three basic needs of mankind namely food, shelter and clothing, food is most important. Food, microorganisms, and mankind have had a long and interesting association that developed long before the beginning of recorded history. Food microbiology is an aspect of microbiology dealing with microorganisms in food, their portal of entry into the food, their ability to multiply in it and produce toxins or survive in it to infect mankind, and with methods of analyzing foods to detect them (Sharpe, 1980). Thus, the objectives of persons/individuals concerned with foods differ. On the regulatory side are those whose aims and objectives are to protect the public from food borne microbial hazards thereby ensuring that foods sold to mankind are of acceptable quality (i.e. Food Security). On the other side are those whose main interest is to ensure the maximum commercial exploitation of materials to produce foods without running into problems of either the regulatory agencies or adverse publicity (i.e. Food Production/ Technology). Mr. Vice-Chancellor, Sir, I belong to both sides. This is because the probable behaviours of microorganisms are of great interest to both sides. Thus,

microorganisms and their metabolic processes including fermentation and respiration play vital roles in food production and security.

(i) Precious Fermented Gifts From Cassava Tubers

Fermented cassava tubers form some of the staple foods of people of the rain forest belt of West Africa. Incidentally fresh cassava tubers are highly toxic containing linamarin with very high content of poisonous cyanogenic glucoside. My first major contribution to knowledge was the study of cassava tubers fermentations to produce fufu, gari, lafun and pupuru which are staple foods among West African countries. Figure. 5 gives a summary of the mean changes in the populations densities of the microorganisms during the fermentation of cassava tubers for gari production. The most important property which colonizing species of cassava tubers have always demonstrated is ability to carry out natural fermentation of cassava tubers to give desired food products. Furthermore, fufu (Edwards and Nwachukwu, 1986), lafun (Nwachukwu, 1982; Nwachukwu and Edwards, 1987) and pupuru (Aboaba *et al*, 1988) are staple food products produced as a result of natural fermentations of cassava tubers and the varieties of the microorganisms associated with the fermentation have been described. Collard and Levi (1959) and Okafor (1977) made a similar observation although they identified mainly one bacterium, *Corynebacterium manihot* and one fungus, *Geotrichum candidum* as the major indigenous organisms associated with the fermentation. Indeed, our study of cassava fermentations for the production of these food products added more value to the previous works carried out by earlier workers in that a detailed account of the microbial types and their population distributions over the fermentation period was established. Moreover, the differences in the colonizing species and their population densities in fresh cassava tubers as well as those associated with the utensils used for the fermentations and not necessarily only those naturally present in the cassava tubers were observed to be responsible for some of the differences in the organoleptic properties of the fermented cassava products. Thus, we are able to differentiate Ijebu gari

from Benin gari after drinking them even with sugar, nuts or fish to improve the nutritional value of the product. This is because the metabolic pathways employed by the different organisms for the transformations of the cassava substrate may not follow the same sequence of reactions and hence lead to differences in the organoleptic properties of the products. Most importantly, the fermentative microorganisms implicated all have the ability to detoxify cassava tubers through the hydrolysis of linamarin liberating the poisonous gaseous hydrocyanic acid thereby making the products safe for human and animal consumption as staple foods. Again, fresh cassava tuber is highly perishable, losing its economic value within a few days after its harvest by a farmer. Thus, the transformations of the tubers by the fermentation processes to give a variety of products have helped to improve the shelf life of the fermented products to more than six months. It is important to note that the shelf life of the products has further been increased to about 12 months through the improved indigenous technology for gari production as handled and described by Federal Institute of Industrial Research Oshodi (FIIRO).



Change in population densities with time during fermentation.

Figure 5: Mean changes in population densities of organisms during cassava fermentation for gari production: G1, *C. tropicalis*; G3, *P. onychis*; G5, *S. cerevisiae*; G2, *Leuconostoc* sp; G4, *Lactobacillus* sp; G6, *Alcaligenes sp;* G7, *Corynebacterium* sp.

(ii) **Precious Fermented Gifts From Wheat Flour**

Bread is another staple food product in many homes worldwide. In Nigeria and many other countries of the world, the basic raw materials namely wheat flour and baker's yeast (*Saccharomyces cerevisiae*) are imported to meet the needs of

fermentation industries for bread production. A need, therefore, exists for the processing of indigenous raw materials to supplement or even replace the imported ones for bread production which is good for the economy as it saves foreign exchange. We made contributions in this area involving isolation, characterization and development of indigenous yeasts from fermented cassava tubers, and corn dough from corn flour, for bread production (Nwachukwu, 1982; 1998b; Olatunji *et al.,* 1994; Nwachukwu *et al.,* 1998). The indigenous yeast isolated from cassava tubers was confirmed by National Collection of Yeast Culture (NCYC), Kew, England, where the culture was deposited as *Candida sake* NCYC DII6 (Olatunji *et al.,* 1994). For a yeast isolate to be useful for commercial baking purposes and compete favourably with the imported baker's yeast, it must be able to grow in dough substrate containing a high sugar concentration between 6 and 11% w/v producing sufficient CO_2 required for leavening of dough leaving behind enough sugar after fermentation to impact a sweet flavour to bread products (Olatunji *et al,* 1994). Incidentally, the isolated cassava yeast proposed to be used for bread production grew best in fermentation systems containing only 2 – 5% w/v sugar which is not adequate to produce delicious sweet bread. Thus, the challenge to us was to improve the sugar utilization ability of the cassava yeast without involving gene cloning (Genetic engineering) which could lead to the production of a genetically modified product, GMP (bread), which may not be widely acceptable considering the hazards associated with GMP. Thus, we adopted a natural method to solve this problem through a series of experimentations which involved growing the indigenous cassava yeast, *Candida sake* NCYC D116, in fermentation systems containing high sugar concentration gradients ranging from 3% w/v to 11% w/v for seven days with repetitions of the processes to confirm the reliability and reproducibility of the results obtained and comparing the results with those of the imported standard baker's yeast, *Saccharomyces cerevisiae*. Table 3 shows the results obtained with granulated market sugar. Thus, the optimum sugar concentration supporting the maximum biomass yield of *C. sake* (NCYC D116) was shifted from 5% w/v to 7%

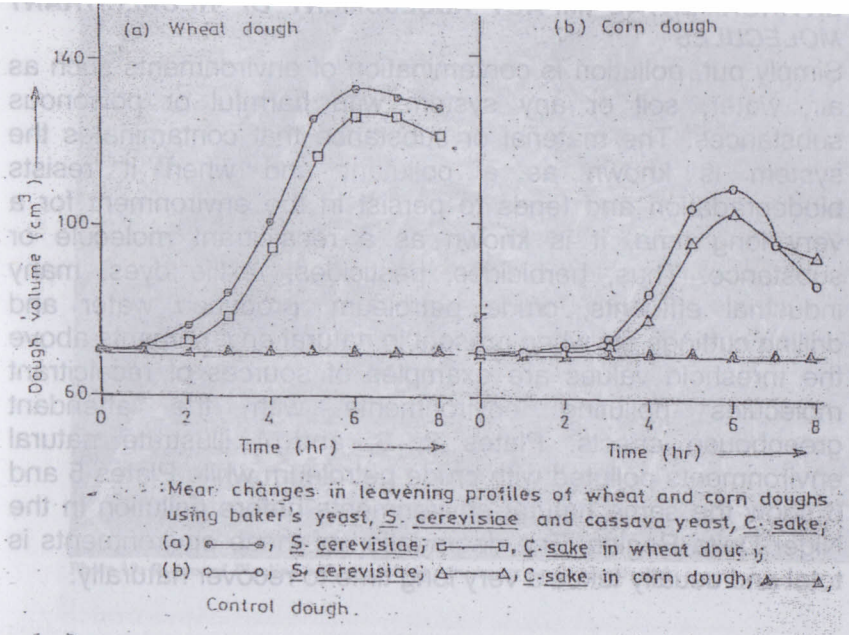
w/v after the training experimentations. The results further showed that the "training" did improve the sugar concentration tolerance throughout the range of sugar concentrations used (5 – 11% w/v) which compared favourably well with the results obtained for the imported standard baker's yeast. Furthermore, after the laboratory experimentations, the yeast was tried for commercial fermentations for bread production with one Mrs. Johnson's Bakery Company, Surulere, whose daughter was my final year project student. Mr. Vice-Chancellor, Sir, Figure 6 clearly demonstrates the ability of the "trained" cassava yeast and the standard baker's yeast to leaven both wheat dough and corn dough separately. The leavening profiles obtained with the trained cassava yeast especially in wheat dough were reasonable when compared with the trends observed for the imported standard baker's yeast. In other words, from the results of this study, it can be concluded that the "trained" cassava yeast is highly suitable for baking purposes. Moreover, it is a food yeast, non-pathogenic and even grows faster than the imported baker's yeast in indigenous fermentation broth systems. Mr. Vice-Chancellor, Sir, the research team of which I am a member regards the yeast development as a useful contribution to both knowledge and commerce and we described the method of transforming the yeast as "step-up sugar utilization technique and recommend that the method can be adopted in "programming" microorganisms to perform specific useful functions with desired capabilities and with no associated environmental hazards. Indeed, this is in line with our Lord's injunction to parents "***Train up a child in the way he should go and when he is old, he will not depart from it***" (Proverbs *22:6*). The cassava yeast was trained up the way it should go and when this was realized, it did not depart from achieving the desired purpose of leavening wheat dough for bread production. However, both the bakers yeast and the "trained" cassava yeast performed poorly in leavening corn dough. This observation was probably due to the low gluten content of corn flour (Vickery and Vickery, 1979). Gluten is an important protein constituent required in baking flours. It forms an elastic envelop or bag which traps carbon dioxide produced by yeasts so that the dough rises homogeneously as the gas

tries to escape (Nwachukwu, 1982). The gluten content of wheat flour is about 12%, that of the corn is below 6% w/w (Vickery and Vickery, 1979). As the gluten level of corn flour is very low, the dough was rather porous forming no "elastic bag". Consequently the gas produced by the yeasts escaped freely with attendant low leavening profiles and poor consistency of the dough. These observations clearly indicate that corn flour is not suitable for baking purposes without any enrichment with other substrates.

Table 3: Mean changes in biomass (mg/l) of yeast types grown for 7 days on granulated market sugar at 30°C. in troths

Yeast types	Biomass (Dry weight, mg/l) % (w/v) granulated market sugar						
	5%	6%	7%	8%	9%	10%	11%
Candida sake NCYC D116 (Prototype strain)	890.0 ± 9.0	870.0±13.0	840.0±12.0	830.0±8.0	410.0±10.0	380.0±10.0	250.0±6.0
Candida sake NCYC D 116 (Trained strain)	890.0±9.0	960.0±11.0	1030.0±8.0	940.0±6.0	790.0±7.0	530.0±7.0	360.0±6.0
Saccharomyces cerevisiae (standard Baker's yeast)	890.0±10.0	970.0±9.0	980.0±10.0	860.0±9.0	580.0±8.0	450.0±8.0	390.0±8.0

Initial biomass in each case at day 1 = 230.0 ± 5.0mg/l
Source: Olatunji *et al.*, (1994).



Mean changes in leavening profiles of wheat and corn doughs using baker's yeast, S. cerevisiae and cassava yeast, C. sake; (a) o——o, S. cerevisiae; □——□, C. sake in wheat dough; (b) o——o, S. cerevisiae; △——△, C. sake in corn dough, ▲——▲, Control dough.

Figure 6: Mean changes in the leavening profiles of wheat and corn doughs using baker's yeast and cassava yeast, *C. Sake*

ENVIRONMENTAL IMPACT ASSESSMENT OF RECALCITRANT MOLECULES

Simply put, pollution is contamination of environments such as air, water, soil or any system with harmful or poisonous substances. The material or substance that contaminates the system is known as a pollutant and when it resists biodegradation and tends to persist in the environment for a very long time, it is known as a recalcitrant molecule or substance. Thus, herbicides, pesticides, textile dyes, many industrial effluents, crude petroleum, produced water and drilling cuttings etc when present in natural environments above the threshold values are examples of sources of recalcitrant molecules polluting environments with the attendant greenhouse effects. Plates 2, 3, and 4 illustrate natural environments polluted with crude petroleum while Plates 5 and 6 show the same natural environments before pollution in the Niger Delta Region. The degradation of these environments is total and usually takes a very long time to recover naturally.

Photo 19: Ruptured Pipeline with oil gushing out; mangrove forest affected

Photo 20: Ruptured Pipeline with oil gushing out: mangrove forest affected cont'd

Photo 05: Spilled oil along pipeline in Mangrove Forest of Niger Delta

Photo 06: Spilled oil from Vandalized Pipeline in the Swamp

Photo 11: Ruptured Pipeline clamped for repairs

Photo 12: Freshly Polluted Mangrove Forest

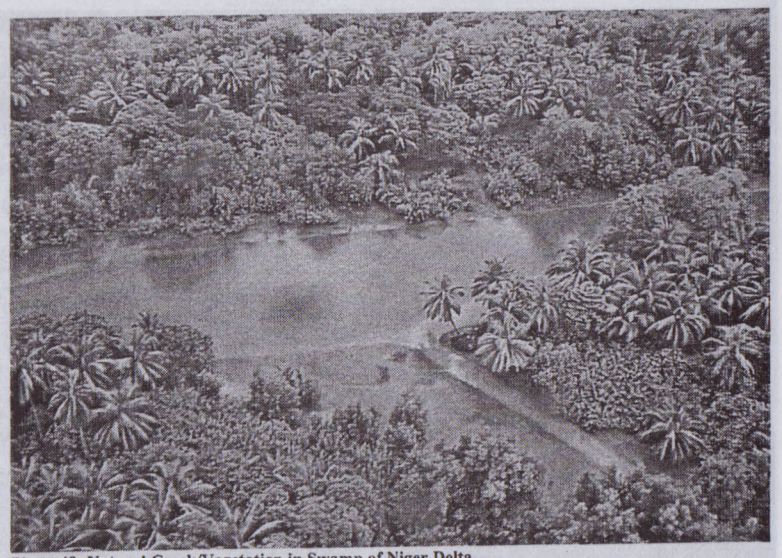


Photo 48: Natural Creek/Vegetation in Swamp of Niger Delta

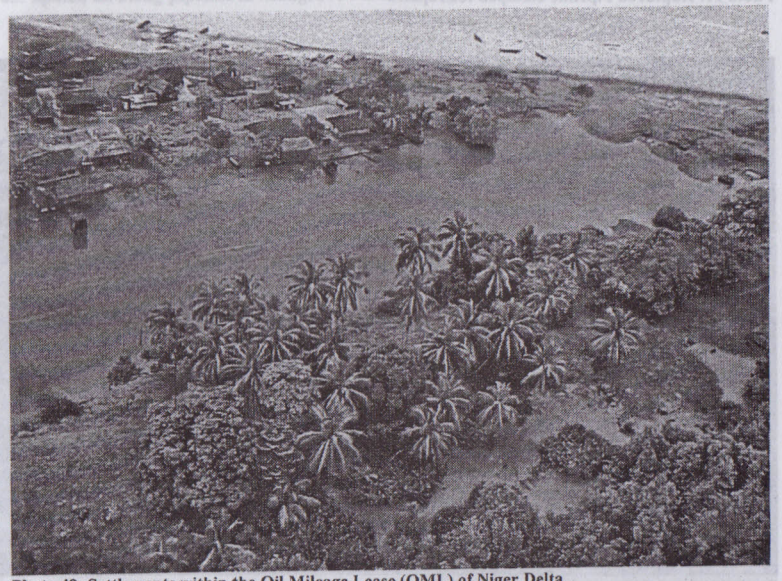


Photo 49: Settlements within the Oil Mileage Lease (OML) of Niger Delta

Photo 55: New Settlement within the OML of Niger Delta under construction

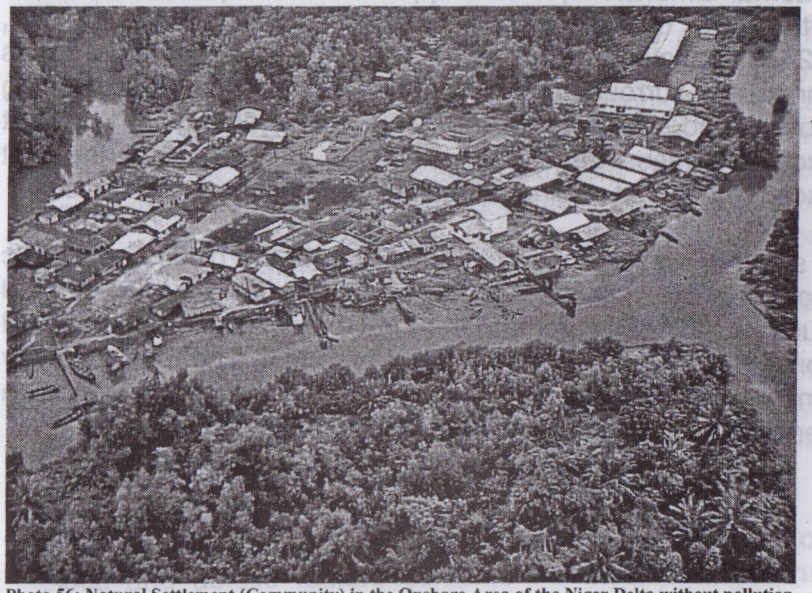


Photo 56: Natural Settlement (Community) in the Onshore Area of the Niger Delta without pollution

Mr. Vice-Chancellor, Sir, as researchers our objectives in this area include the following:

a. To carry out environmental impact assessments (EIA) of pollutants.

b. To predict and audit environments for safety and sustainability.

c. To develop appropriate cost-effective technologies for a rapid elimination of pollutants from environments.

d. To develop a biological index (BI) which can be adopted by polluters and decision makers to pay commensurate compensation to members of the community upon pollution of their environments.

In line with these objectives, I first of all investigated the impacts of crude oil pollution and biodegradation in a pristine agricultural soil here in the Botanical Garden of the University of Lagos. The permission to use the Garden for the study was granted by the then Vice-Chancellor through the then Head of the Department of Biological Sciences, Late Professor R.E. Ugborogbho. In the Garden, a plot of land measuring 2m x 2m (internal dimensions) was mapped out. The plot was flooded with 250L of crude oil (specific gravity, 0.84±.10; pH, 5.24±0.03; colour, dark brown). The plot was separated from its immediate surroundings by making a trough around it using blocks and mortar covered with nylon sheets to prevent excessive mass transfer of materials into or out of the plot except those that originated mainly from the air above the soil.

A summary of the mean changes in both the biotic and abiotic factors in the polluted soil is given in figure 7 while figure 8 illustrates the gas chromatogram (GC) profiles of the residual oil concentration (ROC)over times in the plot. The total recovery time of the polluted plot in terms of the microbial population densities was given by the population model dynamics as:

$$\frac{\text{Log}N_t - \text{log}N_o}{\text{Log2}} = \frac{t}{T} = Z \qquad (3)$$

$$T = \frac{0.693}{K} \qquad (4)$$

$$\frac{dc}{dt} = \frac{D^*d^2C}{dx^2} - \frac{V^*dc}{dx} - K^*C \qquad .....(5)$$

Where Nt =
Final Population density at time, t

No = Initial Population density at time, 0.
T = Mean generation time (hrs, days, weeks, or months).
T = Exponential phase growth time (hrs, days, weeks or months)
Z = Number of generations the populations went through to reach the final population density, N_t at time, t
C = Concentration of pollutant (mgIL)
X = Distance of migration (cm)
V* = Velocity (cm/s) = V/R
K* = K/R
R = 1 + B K/N = retardation factor (dimensionless)
V = Average insterstitial pore-water velocity (cm/day)
K = degradation rate coefficient (day^{-1})
B = bulk density of pollutant (g/cm^3)
N = Volumetric water content (dimensionless) for unsaturated zone
D* = dispersion coefficient (cm^2/day)

The above differential equations describe the pollutant mass balance in a representative milieu or receiving ecosystem volume. As in the polluted plot, the equations show that the change in the concentration of pollutants with time at any distance X is equal to the algebraic sum of the dispersive transport, the convective transport and the degradation or decay rate. The above mathematical models can also be applied in predicting movement of any saturated zones. The numerical solutions to the equations could be carried out over a temporal and spatial discretized domain through a finite difference or finite element mathematical techniques or analytically by seeking exact solutions for simplified environmental conditions or by probability method as described by Onwurah (2000).

In this study, the initial decrease in the values obtained for the biotic and abiotic factors confirms the toxic and destructive impacts of crude oil and petroleum products to soil inhabitants (Figure 7). Soil samples were collected and analysed over a period of 129 days and when the data obtained were fitted into

observed that it would take about 329 days before the ecological balance, diversity and fertility of the agricultural land could be restored. Both the total heterotrophs (TH) and total hydrocarbon utilizers (THCU) correlated highly but negatively with the changes in ROC [r = - 0.91 for TH and – 0.89 for THCU) indicating that the increase in TH and THCU was associated with a highly significant reduction in the concentration of the oil pollutant over the period. The predominant heterotrophs identified from the soil samples were species of *Clostridium*, *Bacillus, Lactobacillus, Pseudomonas, Acinetobacter, Corynebacterium* (Bacteria); *Aspergillus, Penicillium, Candida* and *Rhodotorula* (Fungi). Incidentally, most of these organisms, with the exception of *Clostridium* spp, grew well in minimal salt medium containing crude petroleum as the sole carbon and energy source, under aerobic incubation at 37°C and were, therefore, identified as hydrocarbon utilizers (HCU) (Nwachukwu, 2000a).

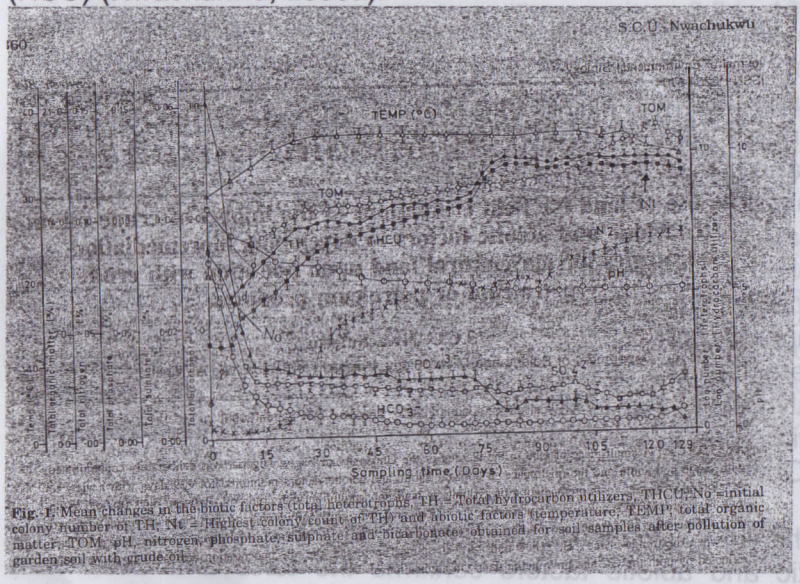


Fig. 1: Mean changes in the biotic factors (total heterotrophs, TH = Total hydrocarbon utilizers, THCU, No = initial colony number of TH, Nt = Highest colony count of TH) and abiotic factors (temperature, TEMP, total organic matter, TOM, pH, nitrogen, phosphate, sulphate and bicarbonate) obtained for soil samples after pollution of garden soil with crude oil.

Figure 7: Changes in oil polluted environment

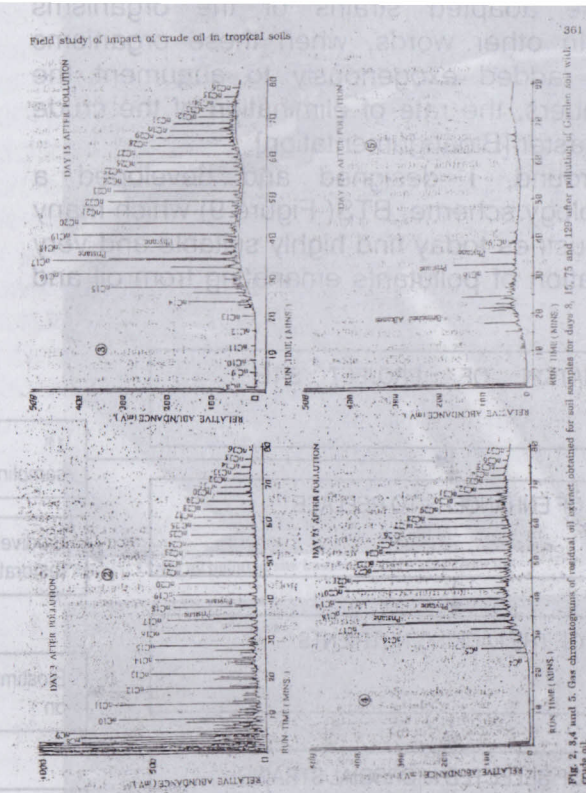


Figure 8: Gas Chromatogram of residual oil in oil-impacted soil.

No doubt, this study helped us in our further researches into bioremediation of oil polluted environments and from the results of the study the following useful deductions were made:

1. Inorganic nutrient ions such as SO_4^{2-}, PO_4^{3-} and NO_3^- may become limiting in crude oil polluted soil environments which could lead to a reduction in the growth and multiplications of biotic factors.
2. There is, therefore, the need to monitor the environmental variables that influence biodegradation and adjust same appropriately to enhance the growth of biotic factors that will eventually eliminate the oil pollutants (Biostimulation).
3. The crude oil pollutant present in environments serves as the carbon and energy source for the growth of the biotic

factors such as the adapted strains of the organisms enumerated above. In other words, when these organisms known as HCU are added exogenously to augument the authochthonous members, the rate of elimination of the crude oil pollutant could be faster [Bioaugumentation].

Against this background, I designed and developed a bioremediation technology scheme, BTS(Figure 9) which many organisations and industries today find highly suitable and very efficient in the elimination of pollutants emanating from oil and gas industries.

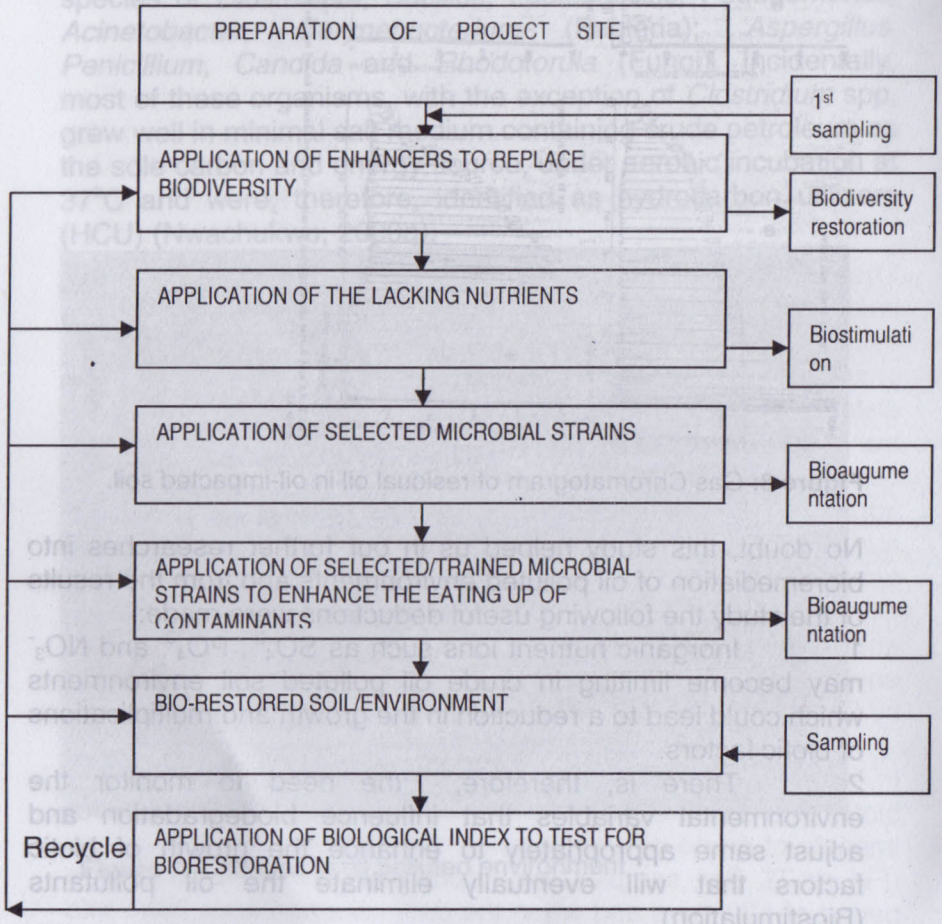


Figure 9: Designed Flow Chart for Bioremediation Programmes for Environments Impacted with Recalcitrant Molecules.

Photo 27: Crude oil polluted site boomed (yellow) for clean up

Photo 28: Freshly crude oil polluted site; surveillance, assessment, booming, and clean up in top gear

BIOREMEDIATION OF ENVIRONMENTS POLLUTED WITH CRUDE PETROLEUM

Bioremediation can be defined as the technique or process by which microorganisms are stimulated to rapidly degrade and mineralize hazardous recalcitrant contaminants to safe concentrations or threshold values naturally present in

environments such as aquatic, air, soil, waste or sludge environments. Thus, bioremediation is a technique that enhances the natural rate of biodegradation of pollutants through reactions carried out by selected microorganisms. For example, the eventual focus of a bioremediation programme of contaminated land is to decommission the soil for farmers' use for food and livestock productions to meet the needs of mankind.

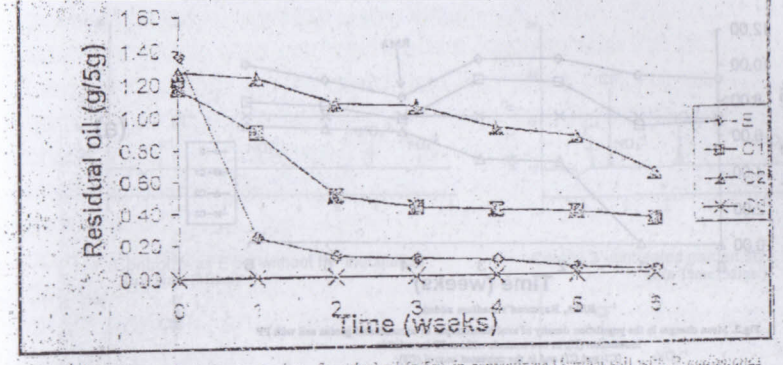
Mr. Vice-Chancellor, Sir, I have been a member of a number of research teams here in Nigeria and in United Kingdom involved in bioremediation programmes of aquatic and soil ecosystems polluted with crude petroleum and petroleum products and we made some useful contributions in this area as described below:

(i) **Application of Spent Brewing Yeast for Bioremediation of Petroleum Impacted Soil:** In-situ bioremediation of petroleum oil polluted agricultural soil was investigated using brewing-spent yeast, *Saccharomyces cerevisiae*, cultured in molasses broth system (Aribike *et al.* 2001). Five stations namely A, B, C, D and E (each, 1m x 1m in dimension) were prepared on a piece of land polluted with 400ml crude petroleum as observed in the Niger Delta Region of Nigeria and demarcated with concrete blocks to check mass transfer as previously described (Nwachukwu, 2000a; 2000b). Stations A and B were inoculated with 250ml of spent brewing yeast (i.e. Bioaugumentation) propagated in molasses, C was inoculated with 250ml of sterilized molasses (9.70 x 10 cfulml) (i.e. Biostimulation), D was neither inoculated with spent brewing yeast nor with sterilized molasses (i.e. Surrogate Control) while E was left bare with no treatment (i.e Overall Control). Furthermore, 20 biovents or holes were made in A to facilitate aeration. Changes in both the biotic and abiotic factors were determined over time in days. For biokinetic analysis, the cell population data for stations A,B,C and D were subjected to microbial growth kinetic analysis using the population growth dynamic models already described above. From the results, approximately 90% oil removal in A, 58% in B, 36% in C and 33% in D, were achieved within 33 days. However, kinetic

analysis of the substrate concentration versus time data for stations A and B showed that the biodegradation process could not be adequately represented by first-order law (Aribike *et al.*, 2001). Thus, in this study both bioaugumentation (A and B with additional bioventing factor in A) and biostimulation (C) played some synergistic roles in the biodegradation and elimination of the oil pollutant when compared with D which was not treated. In other words, from the results of this study, spent-brewing yeast is highly suitable as a bioaugumentation agent and molasses as a biostimulation agent for bioremediation and enhanced recovery of oil polluted environments. The yeast, *Saccharomyces cerevisiae* used as a bioaugementation agent during this study, is a food yeast, non-pathogenic, very cheap and abundant in brewery effluents and also grows very well with very high yield in inexpensive substrates such as molasses, a by-product of sugar industry. Moreover, the yeast is a good hydrocarbon utilizer and also a good biosurfactant producer with no reported environmental hazards (Aribike *et al.*, 2001).

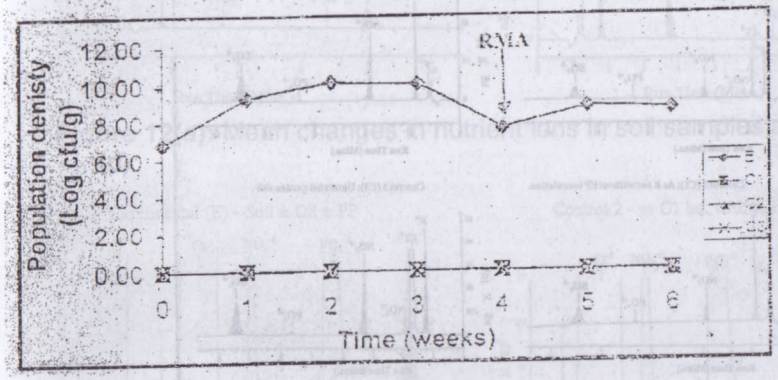
(ii) **Importance of Nutrient ion in Bioremediation**: Crude petroleum and petroleum products are insoluble in water (i.e. hydrophobic phase). Incidentally nutrients (namely carbon, energy and nitrogen sources, mineral elements, growth factors) are available and utilizable by microorganisms only in solubilised phase with water as the solvent (i.e. hydrophilic phase). In crude oil polluted environments, the crude oil serves as the abundant source of carbon and energy and when the other growth requirements are absent or limiting, the crude oil with its solubility problems persists in the environment for a very long time even upon inoculation with hydrocarbon utilizing microorganisms. In other words, microorganisms that biodegrade crude oil pollutant must have adaptive characteristics to solve solubility problems and the six basic nutrients including mineral elements as nutrient ions as enumerated above, must be available. Many researchers, involved in hydrocarbon biodegradation study, have alluded to this fact (Atlas, 1981; 1991; Amund and Igiri, 1990; Amund, 2000). With the collaboration of my friends at the School of Environment and Life Sources, University of Salford, UK, and at Manchester University, UK, we handled a research work titled

"Inorganic nutrient utilisation by "adapted" Pseudomonas putida strain used in the bioremediation of agricultural soil polluted with crude petroleum" under the financial support by the Royal Society, London (Nwachukwu, *et al,* 1999; 2001). In this study, garden soil was polluted with crude oil and subjected to bioremediation programmes using *Pseudomonas putida(PP).* The strain used was isolated from my previous studies (Nwachukwu, 2000a) and adapted to utilize high concentrations (17.5% v/v) of n-hexadecane [CH_3 $(CH_2)_{14}$ CH_3] through a training treatment which we described as complex hydrocarbon utilization technique (Nwachukwu *et al,* 1999). The mean changes in ROC and the population densities of *Pseudomonas putida* and those of the total heterotrophs(TH) are shown in Figures 10 and 11. The ion chromatograms describing the levels of inorganic nutrient ions present in the soil samples are shown in Figures 12 and 13. It was observed that the levels of all the inorganic nutrient ions tested decreased much more rapidly in the experimental samples E inoculated with PP than observed in the control samples (C1, C2, C3) which were not inoculated. The trend was particularly strong for $P0_4^{3-}$, $N0_3^{-1}$ and $S0_4^{2-}$. Eventually, these nutrients were depleted to levels where they became limiting at day 28(Week 4) (Fig.11, 12, 13) particularly in E inoculated with PP. Consequently, the organisms stopped growing and when Raymond's medium containing these nutrient ions was added to the garden soil, the nutritional balance was restored and active growth of both PP and TH resumed with a proportional decrease in the oil levels. Thus, microorganisms require phosphorus as phospholipids in synthesizing cell membranes, as nucleotides to replicate nucleic acids and as pyrophosphate for sugar phosphorylation during metabolism. Also, microorganisms exploit $N0_3^{-1}$ and $S0_4^{2}$ sources to meet their protein and nucleic acid requirements. These observations emphasise the importance in all bioremediation programmes of monitoring routinely the environmental variables known to influence the biodegradation of pollutants and to adjust same appropriately correcting any imbalances, and hence facilitate the recovery rate of the contaminated environment (Nwachukwu *et al.*, 2001)



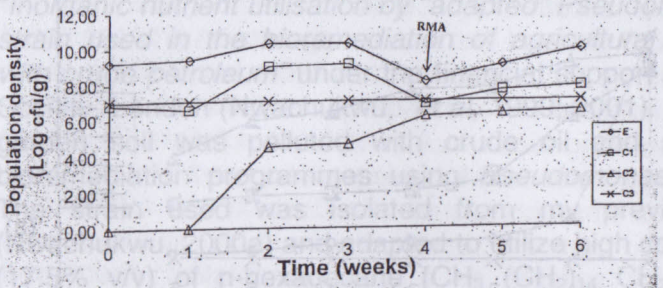
(a)

Fig. 1. Mean changes in the concentration of residual oil (g/5g), in contaminated garden soil with *P. putida* (PP) inoculation (E), and without PP inoculation (C1, C2) and incubated for two (C3).



(b)

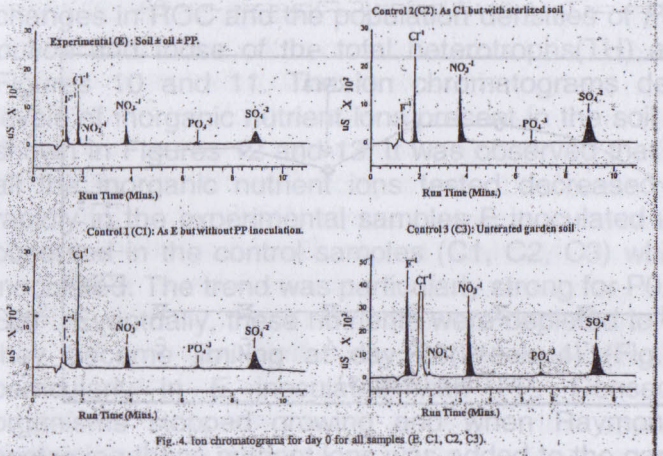
Figure 10: Mean changes in residual oil concentration (ROC) (a) and population density (b) in oil impacted soils



(a)

RMA, Raymond's medium added.

Fig.3. Mean changes in the population density of total heterotrophs in contaminated garden soil with PP inoculation (E), in two controls without PP inoculation (C1 and C2) and in the untreated control (C3).



(b)

Fig. 4. Ion chromatograms for day 0 for all samples (E, C1, C2, C3).

Figure 11: Mean changes in population densities (a) and nutrient ions (b) in soil samples at day O.

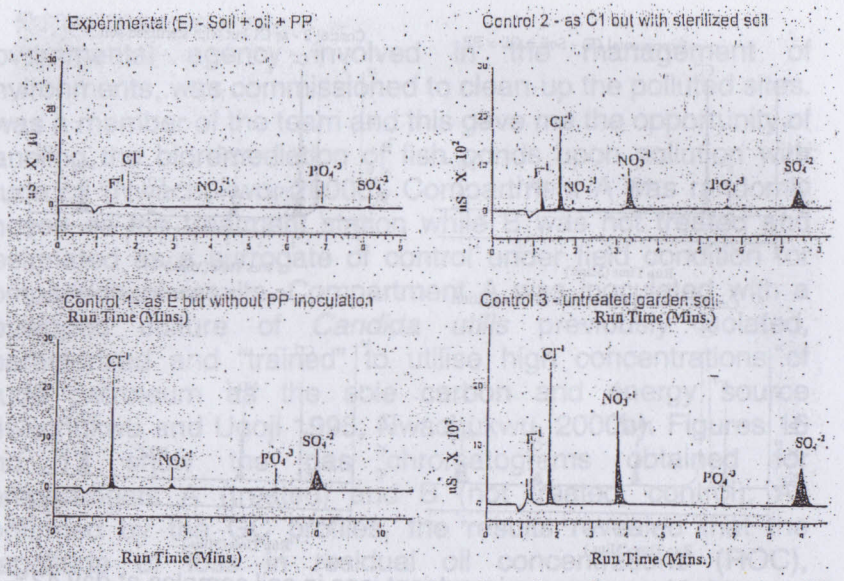


Figure 12(a): Mean changes in nutrient ions in soil samples at day 28.

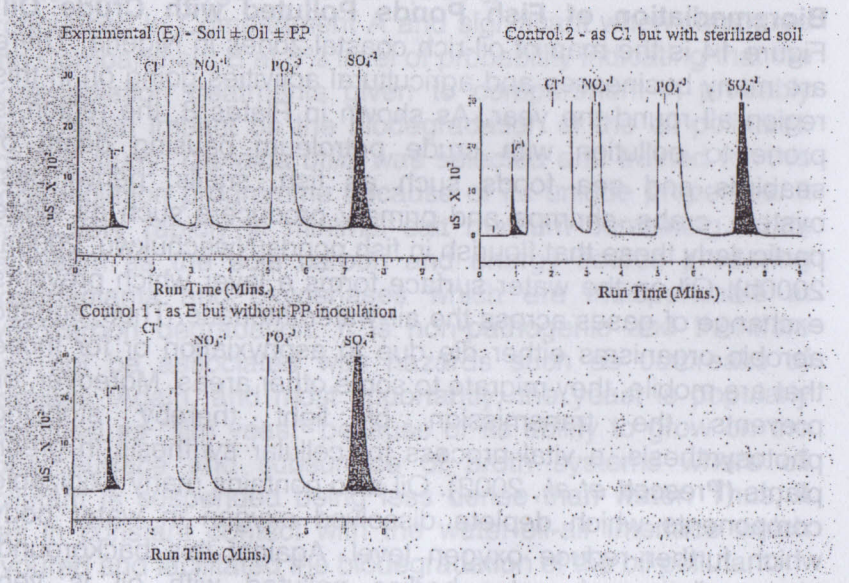


Figure 12(b): Mean changes in nutrient ions in soil samples immediately after adding Raymond's medium at day 28.

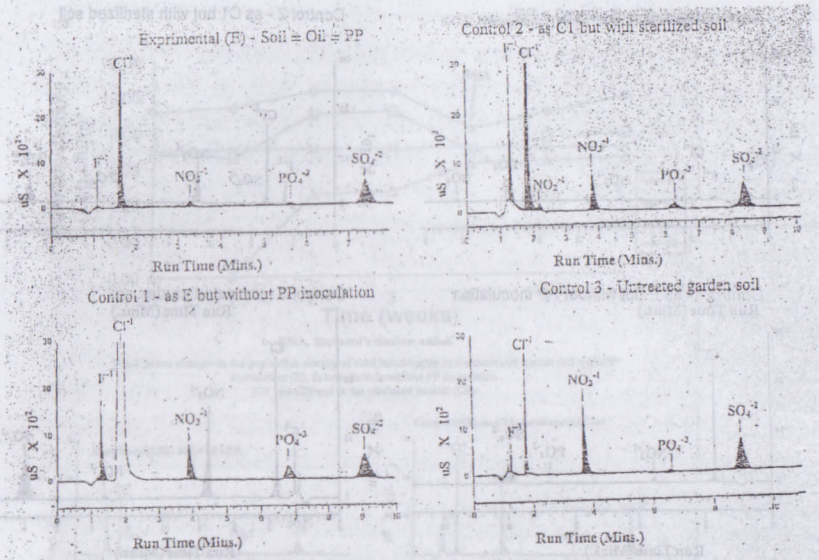
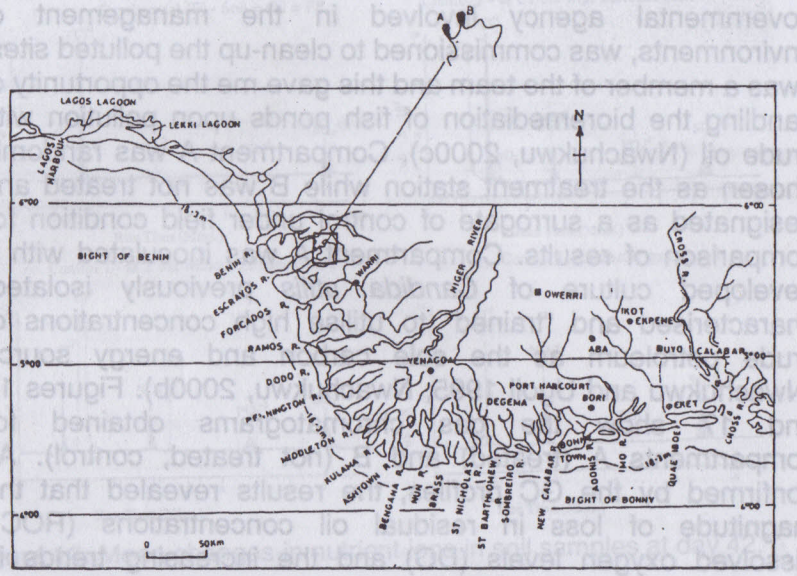


Figure 13: Mean changes in nutrient ions in soil samples at day 42.

Bioremediation of Fish Ponds Polluted with Crude Oil: Figure 14 is the map of oil-rich coastal zones in Nigeria. There are many businesses and agricultural activities going on in this region all round the year. As shown in Plates 8, the region is prone to pollution with crude petroleum causing death of seabirds and sea foods such as fish, snails, periwinkles, oysters, crabs, shrimps and primary producers such as algae particularly those that flourish in fish ponds(Nwachukwu, 2000a; 2000b). Oil on the water surface forms a layer which prevents exchange of gases across the air-water interface. Thus, aquatic aerobic organisms either die due to asphyxiation or for those that are mobile, they migrate to some other areas. Moreover, oil prevents the transmission of light thereby stopping photosynthesis, a vital process for cellular synthesis in green plants (Prescott *et al.*, 2002). Oil also contains many oxidizable components which deplete dissolved oxygen in water body which further reduce oxygen level. Against this background, bioremediation of water bodies polluted with oil is very important. In figure 15 are shown water compartments (Fish ponds) A and B along the Creeks of River Escravos grossly polluted with oil on July 21, 1996. Environ 2000, a non-

governmental agency involved in the management of environments, was commissioned to clean-up the polluted sites. I was a member of the team and this gave me the opportunity of handling the bioremediation of fish ponds upon pollution with crude oil (Nwachukwu, 2000c). Compartment A was randomly chosen as the treatment station while B was not treated and designated as a surrogate of control under field condition for comparison of results. Compartment A was inoculated with a developed culture of *Candida utilis* previously isolated, characterised and "trained" to utilise high concentrations of crude petroleum as the sole carbon and energy source (Nwachukwu and Ugoji 1995; Nwachukwu, 2000b). Figures 16 and 17 show the gas chromatograms obtained for compartments A (treated) and B (not treated, control). As confirmed by the GC profiles, the results revealed that the magnitude of loss in residual oil concentrations (ROC), dissolved oxygen levels (DO) and the increasing trends in biochemical oxygen demand(BOD_5) were much more pronounced in Compartment A and significant when compared with Compartment B at 5% level of probability indicating that the bioremediation treatments given to compartment A probably had a great impact on the biodegradation of the oil pollutant. For this study, *Candida utilis* was selected and trained for this bioremediation programme because of its unique properties. It grows very rapidly in minimal salt medium containing crude petroleum as the sole carbon and energy source producing biosurfactants and oxygenases which are indispensable in hydrocarbon degradation. It is non-pathogenic and therefore may not be associated with hazards such as outbreaks of diseases. Again, and most importantly, the yeast is popularly described as "film yeast" because of its ability to grow on the upper surface and subsurface of broth systems where oil pollutant is suspended being less dense than water. Thus, it was in constant contact with the water-oil-air interface which favoured and facilitated the biodegradation of the oil pollutant.



Figures 14: Map of Oil rich coastal zones in Nigeria

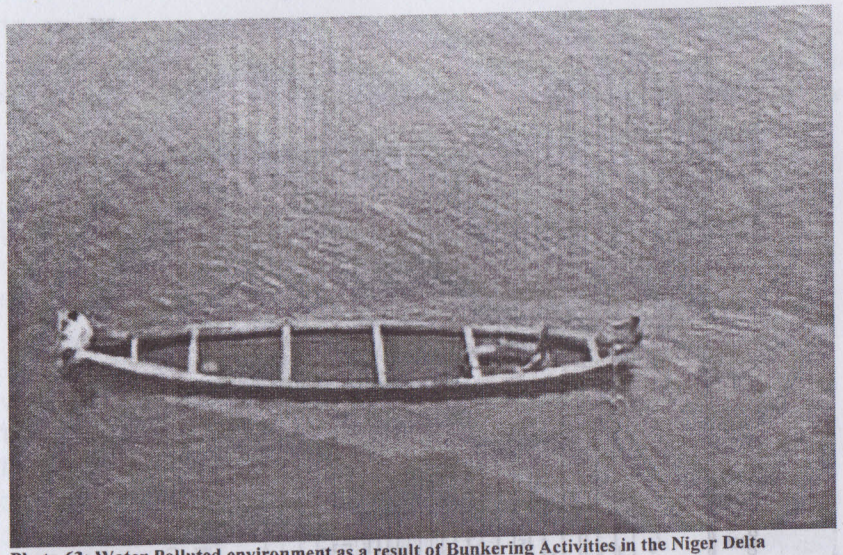


Photo 63: Water Polluted environment as a result of Bunkering Activities in the Niger Delta

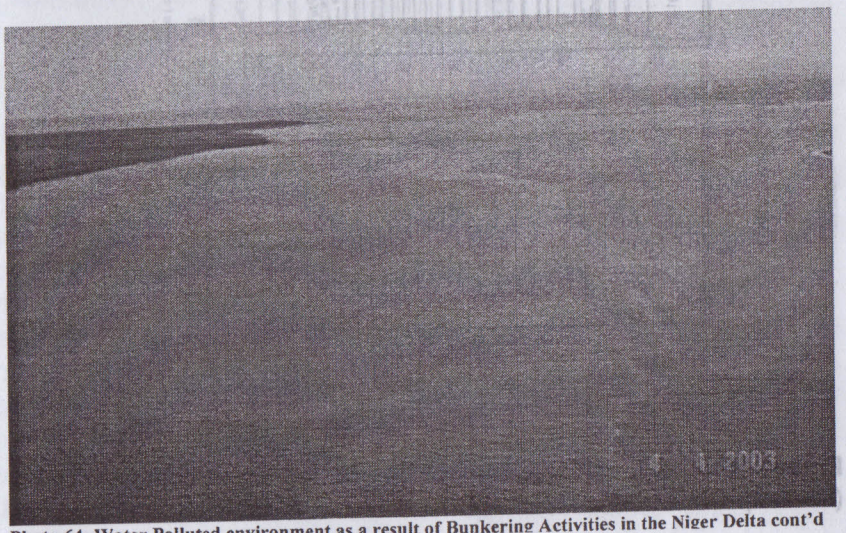


Photo 64: Water Polluted environment as a result of Bunkering Activities in the Niger Delta cont'd

Plate 8: Showing oil pollutant on the water surface in the Nigeria Delta Region

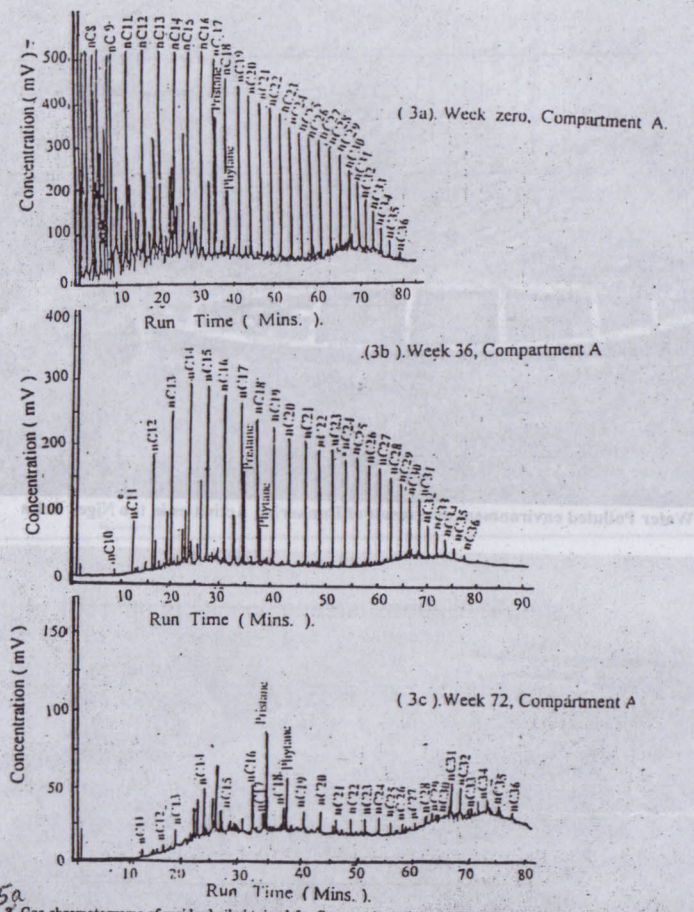


Fig. 3. Gas chromatograms of residual oil obtained for Compartment A at week 0 (Fig. 3a), week 36 (Fig. 3b) and week 72 (Fig. 3c).

Figure 16: Gas chromatograms of oil polluted water compartment A (treated)

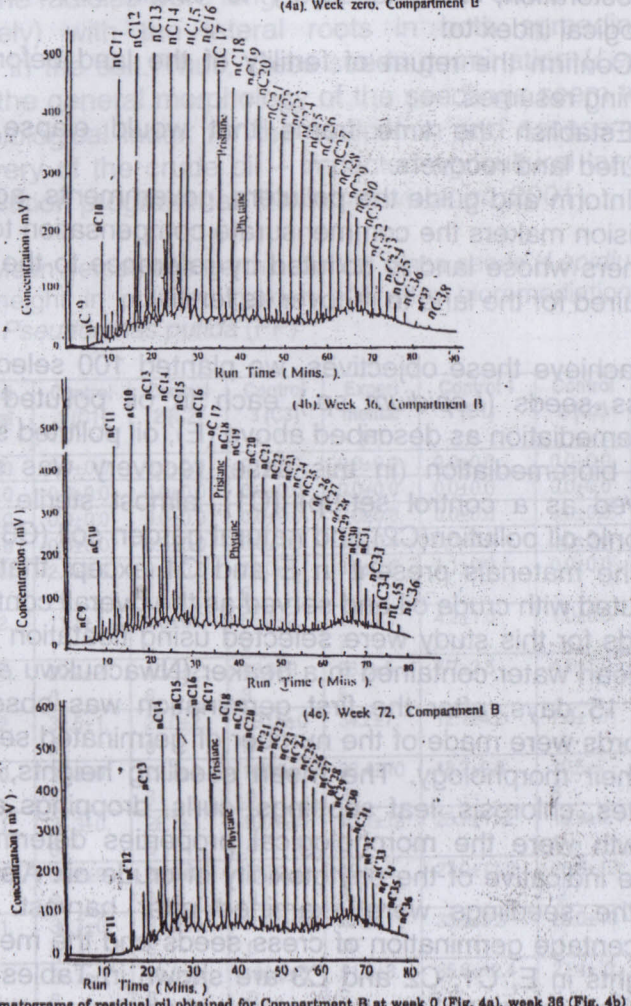


Fig. 4. Gas chromatograms of residual oil obtained for Compartment B at week 0 (Fig. 4a), week 36 (Fig. 4b) and week 72 (Fig. 4c).

Figure 17: Gas Chromatogram of oil polluted water compartment B (not treated, control)

(iii) **Seed Germination As Biological Index of Recovered Agricultural Soils Polluted with Crude Petroleum:** Thus, following the bioremediation treatment which brings about

biorestoration, it is necessary to obtain some indication or biological index to:

- Confirm the return of fertility of the land before intensive farming resumes.
- Establish the time frame that would elapse before oil polluted land recovers.
- Inform and guide the polluters, governments, agencies and decision makers the commensurate compensation to be paid to farmers whose land is polluted by reference to the time frame required for the land to recover its fertility.

To achieve these objectives, we planted 100 selected healthy cress seeds (*Lepidium* sp.) each in oil polluted soils after bioremediation as described above (E), oil polluted soils without any bioremediation (in this case, recovery was natural and served as a control set-up) (C1), almost sterile soil due to chronic oil pollution(C2) and natural garden soil (C3) containing all the materials present in E and C1 except that it was not polluted with crude oil and served as the overall control. Healthy seeds for this study were selected using floatation mechanism in clean water contained in a beaker (Nwachukwu *et al.*, 2001). For 15 days, after the first germination was observed, daily records were made of the number of germinated seedlings and of their morphology. The mean seedling heights, size of the leaves, chlorosis, leaf spottings, curls, droppings and stunted growth were the morphological properties determined which were indicative of the phytotoxicity of crude oil. Also, the roots of the seedlings were examined after harvest. The mean percentage germination of cress seeds and the mean seedling heights in E, C1, C2 and C3 are shown in Tables 4 to 6 and Plates 9 to 11. At day 15, when the study was completed, there was 98% mean germination in E, 100% in C3 and approximately 31% in both C1 and C2 with 63.8±6.9mm; 75.8±2.6mm, 34.0±11.4mm and 32.5mm as the corresponding seedlings heights respectively. For C1 and C2, most of the seedlings were stunted and had yellowing leaves (Chlorosis) indicating phytotoxicity due to oil impact. The radicles and the lateral roots of the seedlings in the soil were abnormally small (12.4±2.5mm) without spreading out for both C1 and C2. In E

and C3, the radicles were longer (22.4±2.2mm and 24.1±1.2mm respectively) with the lateral roots in both spreading out profusely in the soil. Thus, cress seeds germination (*Lepidium* sp.) and the general morphology of the seedlings seem to be a reliable biological index for the evaluation and assessment of the recovery of the crude oil – impacted agricultural land after bioremediation programmes (Nwachukwu *et al.*, 2001).

Table 4: Mean percentage germination of cress seeds (*Lepidium*) and seedling height in polluted garden soil after bioremediation using "adapted" *Pseudomnas putida* (PP)

Time (days)	Experimental (E)	Control 1 (C1)	Control 2 (C2)	Control 3 (C3)	Experimental (E)	Control 1 (C1)	Control 2 (C2)	Control 3 (C3)
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3	5.3±0.6	0.0±0.0	0.0±0.0	6.0±1.0	3.2±0.4	0.0±0.0	0.0±0.0	4.4±1.1
4	16.3±0.6	2.3±0.6	0.0±0.0	22.0±1.6	8.4±2.6	1.1±0.0	0.0±0.0	12.7±3.9
5	28.5±2.0	11.2±1.1	1.0±0.0	55.4±2.0	17.1±3.7	4.2±1.0	1.0±0.0	19.1±8.8
6	47.0±1.9	20.2±2.0	10.5±2.6	82.9±3.3	26.6±5.5	7/7±2.5	4.5±2.0	27.3±11.4
7	69.5±2.3	32.5±1.5	21.0±2.0	100.0±0.0	34.2±7.1	12.1±4.2	7.3±1.7	39.0±9.8
8	78.3±0.6	36.3±0.6	27.7±0.6	100.0±0.0	38.4±10.5	18.2±9.9	10.5±7.8	45.0±12.2
9	86.3±1.5	36.3±0.6	28.7±1.5	100.0±0.0	40.1±12.1	25.7±11.2	15.9±9.5	48.9±11.8
10	94.7±1.2	36.2±0.6	31.3±0.6	100.0±0.0	43.2±6.6	27.0±11.2	20.6±12.2	48.9±13.6
11	98.0±1.0	36.7±0.6	31.3±0.6	100.0±0.0	47.0±11.4	33.6±9.3	25.0±10.1	54.7±7.4
12	98.0±1.0	37.7±0.6	31.3±0.6	100.0±0.0	51.0±8.2	35.6±12.4	27.4±0.3	59.6±6.3
13	98.0±1.0	37.7±0.6	31.3±0.6	100.0±0.0	55.8±9.3	38.4±11.0	30.1±9.7	66.2±7.1
14	98.0±1.0	37.7±0.6	31.3±0.6	100.0±0.0	60.7±7.4	4.2±7.6	32.3±10.1	70.9±4.2
15	98.0±1.0	37.7±0.6	31.3±0.6	100.0±0.0	63.8±6.9	42.3±8.5	34.2±11.4	75.8±2.6

S. D. = Standard deviation

Table 5: Mean leaf and largest leaf breadth of cress seedling (*Lepidium sp*) planted in polluted garden soil after bioremediation using "adapted" *Pseudomnas putida* (PP)

Time (days)	Experimental (E)	Control 1 (C1)	Control 2 (C2)	Control 3 (C3)	Experimental (E)	Control 1 (C1)	Control 2 (C2)	Control 3 (C3)
0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
1	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
2	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
3	0.0±0.0	0.0±0.0	2.9±0.5	2.9±0.5	0.0±0.0	0.0±0.0	0.0±0.0	1.3±0.2
4	2.9±0.6	1.3±0.2	0.0±0.0	3.2±0.4	1.2±0.3	0.8±0.1	0.0±0.0	1.3±0.2
5	3.2±1.1	1.5±0.1	1.2±0.3	3.5±0.6	1.4±0.3	0.9±0.1	0.6±0.2	1.7±0.5
6	3.7±0.9	1.7±0.3	1.4±0.5	3.9±0.5	1.6±0.2	1.0±0.1	0.8±0.2	1.9±0.6
7	4.5±1.0	1.9±0.2	1.6±0.4	4.3±0.5	1.6±0.2	1.0±0.1	0.8±0.2	1.9±0.6
8	5.6±0.8	2.1±0.4	1.8±0.5	4.8±0.7	2.0±0.1	1.3±0.2	1.1±0.3	2.3±0.2
9	6.3±0.5	2.5±0.6	1.9±0.3	6.1±0.9	2.4±0.2	1.4±0.2	1.2±0.4	2.9±0.5
10	6.7±1.3	3.1±0.5	2.1±0.7	7.1±0.5	2.6±0.1	1.5±0.1	1.3±0.2	3.1±0.3
11	7.3±0.9	3.6±0.9	2.3±0.9	7.8±0.7	2.9±0.3	1.8±0.3	1.5±0.3	3.3±0.4
12	7.4±1.1	3.9±0.7	2.8±1.0	8.1±0.3	3.4±0.4	2.2±0.5	1.7±0.4	3.7±0.8
13	7.4±1.2	3.9±0.7	2.9±1.1	8.2±0.3	3.5±0.3	2.2±0.5	1.7±0.3	3.7±0.5
14	7.5±1.2	4.4±1.0	2.9±1.0	8.2±0.5	3.6±0.2	2.3±0.4	1.9±0.7	3.8±0.3
15	7.5±1.2	4.4±1.0	3.0±0.8	8.2±0.5	3.7±0.3	2.5±0.3	2.0±0.5	3.9±0.5

SD = Standard deviation

Table 6: Some qualitative morphological observations made of the cress seedling (*Lepidium sp*) planted in polluted garden soil after bioremediation using "adapted" *Pseudomonas putida* (PP)

Time (days)	Qualitative morphological properties of cress seedlings																							
	Stunted growth				Chlorosis of leaves				Chlorosis of stems				Leaf spotting/curls				Leaf droppings							
	E	C1	C2	C3	E	C1	C2	C3	E	C1	C2	C3	E	C1	C2	C3	E	C1	C2	C3				
0	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA	NA				
3	-	NA	NA	-	-	NA	NA	-	-	NA	NA	-	-	NA	NA	-	-	NA	NA	-				
6	-	+	+	-	-	++	++	-	-	-	+	-	-	-	+	-	-	-	-	-				
9	-	+	++	-	-	++	++	-	-	+	++	-	-	-	+	-	-	+	++	-				
12	-	++	+++	-	-	++	+++	-	-	+	++	-	-	+	++	-	-	+	++	-				
15	-	++	+++	-	-	++	+++	-	-	+	+++	-	-	+	++	-	++	++	+++	+				

E = Experimental: C1 = Control 1'; C2 = Control 2; C3 = Control 3; NA = Not applicable because no germination;
- = absent; + = present; ++ = present and conspicuous; +++ = present and highly conspicuous.

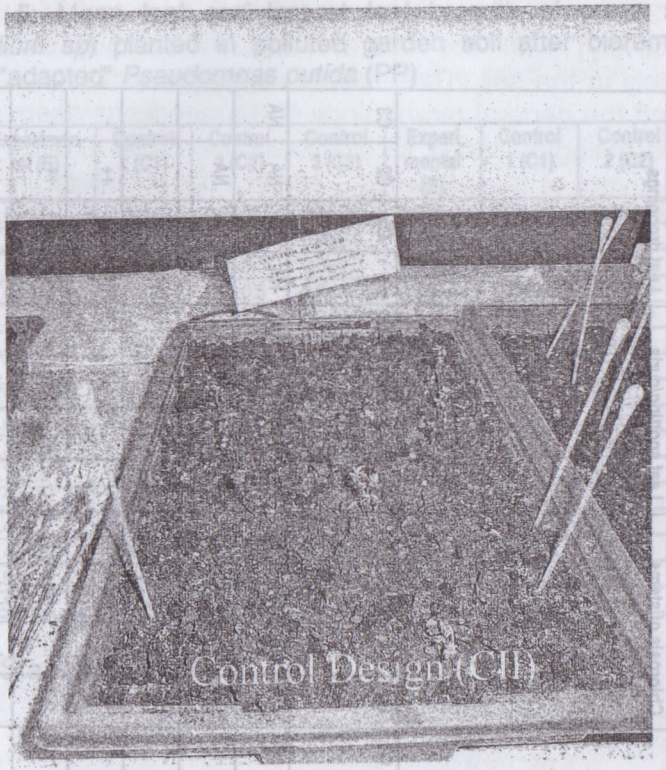


Plate 9: Seed Germination observed for Control 2, a similar result was observed for control 1.

Plate 10 : Seed Germination Observed for Experimental Design(E)

Plate 11: Seed Germination Observed for Control 3

MANUFACTURING INDUSTRIES

Manufacturing industries produce goods and services to meet the needs of mankind. The market for an industrialist or a manufacturer is usually very stiff as many people and organisations produce the same product to be sold in the same market. Thus, the competition for a good share of the market encourages quality control or quality assurance among industrialists. In industry, everything an industrialist does so that his products sell more than their counterparts in the same market is termed quality control. He embarks on a number of activities to achieve this. For example, he buys high quality raw materials, employs highly experienced and efficient personnel and gives them good training programme so that production

stages involving unit operations are rightly handled first time. He ensures that all equipment, installations and machinery (Checklists) in the factory are in good working conditions. Also, he mounts very efficient inspection team or services so that defective products are not passed on to customers. Equally, important is the fact that he does not ignore customers' complaints about finished products. This is what is meant by building in quality into products and the use of numbers or figures to build in quality into a product during its manufacturing and production is known as statistical quality control (SQC) (Tewell, 1990).

Mr. Vice-Chancellor, Sir, I have been involved also in researches which brought town and gown together when I made my expertise available to several industries and organisations by functioning as their consultant to develop quality control programmes for their products. Examples of such bodies and organisations include UNILAG Consult; Guinness Nigeria Plc, Ikeja; Leventis Foods, Lagos; Texaco, Apapa Lagos; NNPC, Lagos; Agbara Industrial Estate, Ile-Oluji, Cocoa Products Industries, Ondo; UAC Foods, Oregun, Lagos; CAPL Plc, Ikeja; ENVIRON 2000, Ikeja, etc.

(iv) **QUALITY CONTROL OF GREASE PRODUCTION;** At Texaco Nigeria Plc, I developed quality control charts for the production of grease. At Texaco Nigeria Plc, I conducted process capability studies (PCS) of a Grease Plant and from the PCS, standard control charts (CC) were developed to be used to monitor the percentage free alkali (FA) and to check the level of spoilage microorganisms (SM) in grease and grease products(Nwachukwu, 1998c). To achieve this, the percentage FA, species diversities, distribution patterns and population densities of SM present in 60 grease samples collected over 6 months period were analysed. No doubt, some people will be asking the question now in their mind "what has a Microbiologist got to do with grease production which is the exclusive responsibility of a Chemical Engineer?" For such people, grease is a hydrocarbon based product which is prone to microbial contamination. If quality control measures are not put

in place during its production to check the microbial load, the contaminants will "feed" on the grease which serves as their carbon and energy source. Consequently the grease looses its quality characteristics such as elasticity, density, viscosity, consistency and rather, becomes brittle with poor performance as a lubricant of engine components.

The PCS of the plant revealed that FA values varied between 1.00 and 1.12% (w/w) which were in line with standard specification limits of 0.00—1.30%. Also the microbial load (2.30×10^2 cfulg) enumerated for the grease samples were within the standard specification limits of $0.00 - 5.00 \times 10^2$ cfulg and were mainly *Bacillus* spp, *Alcaligenes* spp and *Lactobacillus* spp. For a mean chart ($\overline{X}$), the upper control limit (UCL) and lower control limit (LCL) were calculated and found to be 1.082 and 1.064% respectively with the central line (CL) value at 1.073%, the corresponding values for range chart (R-chart) were 0.120, 0.000 and 0.060%; and all the values were within the standard specification limits for grease products. When the mean and range values of the raw data generated from PCS were plotted in the boundaries defined by the above limits, a control chart with patterns characteristic of a process under good controls was obtained confirming uniformity and acceptability of the grease lots produced by this company. Thus, the developed chart from PCS is used up till today to monitor production by TEXACO Plc (Nwachukwu, 1998).

(v) MICROBIAL EVALUATION AND DETERIORATION OF PAINTS AND PAINT PRODUCTS

Paints are uniformly dispersed mixtures having a viscosity ranging from a thin liquid to a semi-solid paste. The various organic materials of paints represent a carbon source for practically all microorganisms and act as nutrients to stimulate microbial growth both in cans and on the dry paint film on surfaces. Consequently, paints and paint products are prone to microbial spoilage. A paint company in Ikeja commissioned my research team through the then Head of Department, Professor O.T. Ogundipe, to investigate the microbial quality of their paints with a view to improving the shelf-life(SL) of their

products. After solving their immediate problems bothering on microbial quality of raw materials and final products, I placed a Ph.D student to study in details at the molecular level the factors influencing paint deterioration including screening the microbial contaminants for their plasmids profiles encoding for the spoilage of the products. Figure 19 shows the microbial types and their population densities implicated in paints over a period of ten months immediately after production while Figures 17 and 18 illustrate the plasmids profiles of the microbial contaminants detected in finished products. From these data, the SL of the paint products were determined and the values ranged between 24 and 30 months when stored at room temperature. Again, spoilage genes detected in the contaminants were plasmids mediated. Thus, curing of the plasmids using sodium dodecyl sulphate (SDS) could improve the SL of the products. The values are not absolute and therefore subject to variations depending on the factors such as anaerobiosis/aerobiosis in the paint can, organic nature of paint components, storage temperature, relative humidity, microbial quality of packaging materials, hygiene level of the production environments and presence of plasmids in microbial contaminants encoding for spoilage factors.

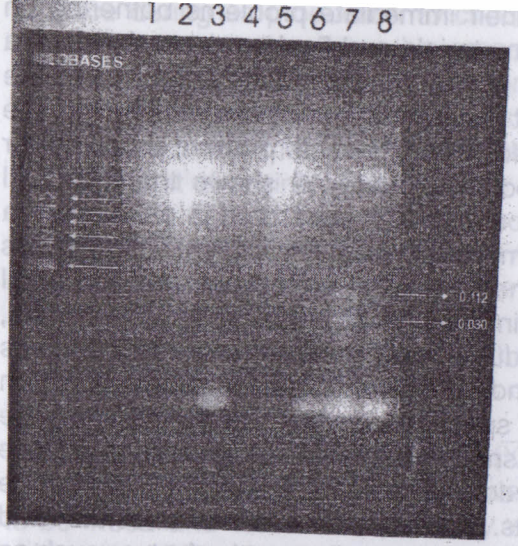


Figure 17: Agarose gel electrophoresis plate showing plasmid DNA isolated from a paint contaminant (pseudomonas aeruginosa)

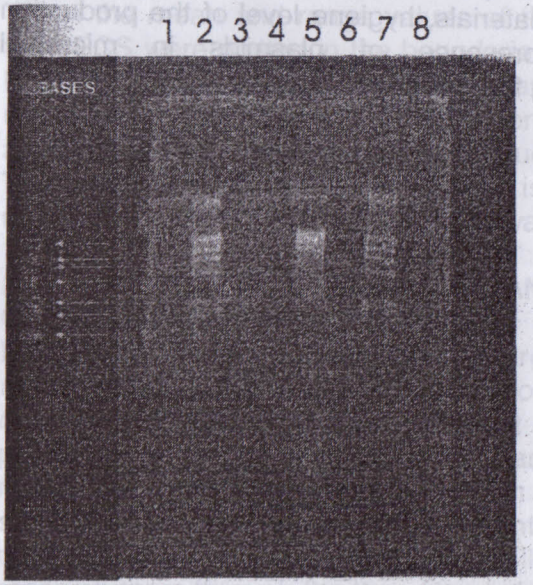


Figure 18: Agarose gel electrophoresis plate showing curved plasmids of pseudomonas aeruginosa, a paint contaminant

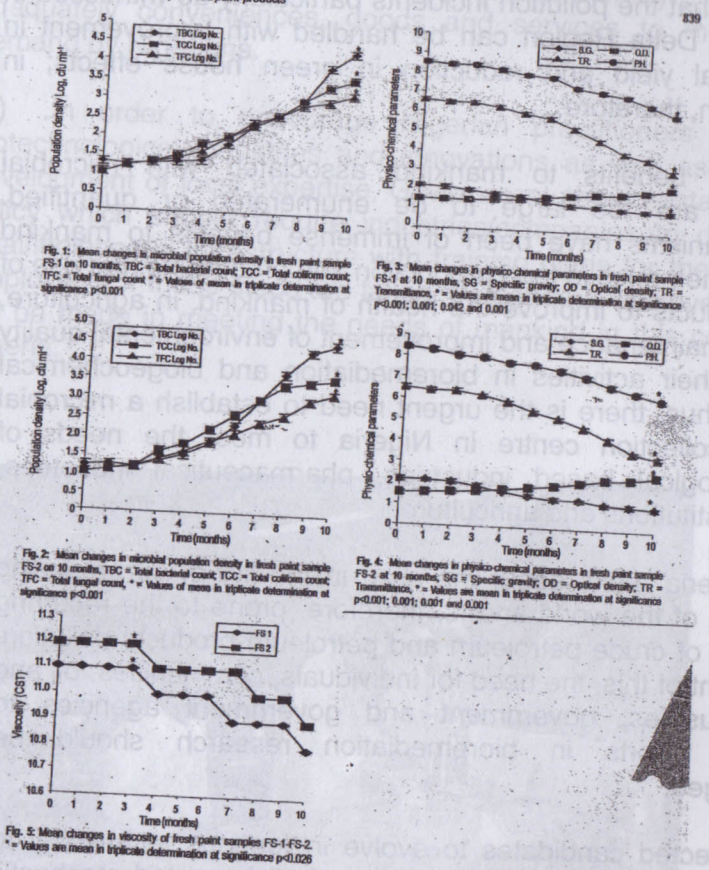


Fig. 1: Mean changes in microbial population density in fresh paint sample FS-1 on 10 months, TBC = Total bacterial count; TCC = Total coliform count; TFC = Total fungal count * = Values of mean in triplicate determination at significance p<0.001

Fig. 3: Mean changes in physico-chemical parameters in fresh paint sample FS-1 at 10 months, SG = Specific gravity; OD = Optical density; TR = Transmittance, * = Values are mean in triplicate determination at significance p<0.001; 0.001; 0.042 and 0.001

Fig. 2: Mean changes in microbial population density in fresh paint sample FS-2 on 10 months, TBC = Total bacterial count; TCC = Total coliform count; TFC = Total fungal count, * = Values of mean in triplicate determination at significance p<0.001

Fig. 4: Mean changes in physico-chemical parameters in fresh paint sample FS-2 at 10 months, SG = Specific gravity; OD = Optical density; TR = Transmittance, * = Values are mean in triplicate determination at significance p<0.001; 0.001; 0.001 and 0.001

Fig. 5: Mean changes in viscosity of fresh paint samples FS-1-FS-2. * = Values are mean in triplicate determination at significance p<0.026

Figure 19: Quality characteristics that determine the shelf life value of paint products.

SUMMARY AND CONCLUSION

I have taken the liberty of an inaugural lecture to discuss some of my feelings, thoughts and researches on how fermentation, respiration and photosynthesis as key biological processes are coordinated to meet the needs of mankind for his survival in a stochastic environment. With the new horizon in bioremediation

technology involving bioaugumentation and biostimulation principles as developed by our research team, there is hope for mankind that the pollution incidents particularly as witnessed in the Niger Delta Region can be handled with improvement in agricultural yield and reduction in green house effects; in conclusion, therefore:

(1) The benefits to mankind associated with microbial activities are too large to be enumerated or quantified. Microorganisms have been of immense benefits to mankind through their roles in food production and processing, the use of their products to improve the health of mankind, in agriculture, and the maintenance and improvement of environmental quality through their activities in bioremediation and biogeochemical cycles. Thus, there is the urgent need to establish a microbial culture collection centre in Nigeria to meet the needs of microbiological based industries, pharmaceutical industries, health institutions and agriculture.

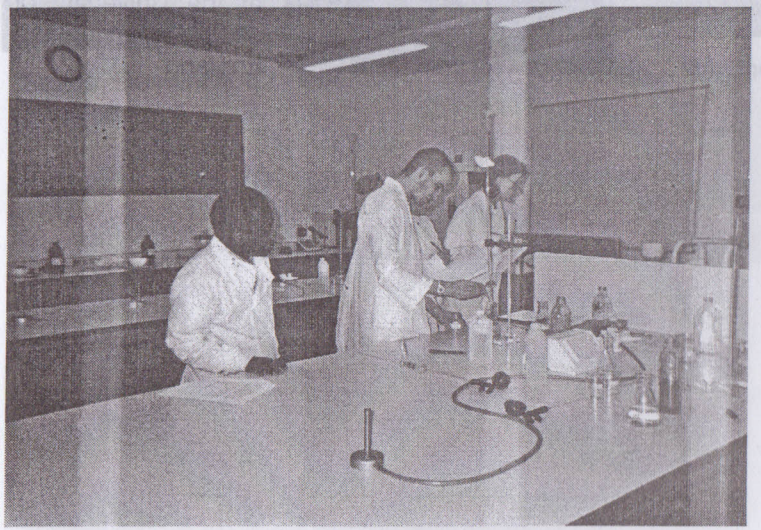
(2) Nigeria ranks about the sixth in the list of oil producing countries of the world and is, therefore, prone to the recurring incidents of crude petroleum and petroleum products pollution. In the light of this, the need for individuals, communities, oil and gas industries, government and government agencies to intensity efforts in bioremediation research should be encouraged.

(3) Selected candidates to evolve indigenous technology to handle bioremediation programmes of oil impacted stochastic environments should be seasoned researching scientists and engineers in the area with proven academic excellence, competence and sufficient intellectual merit.

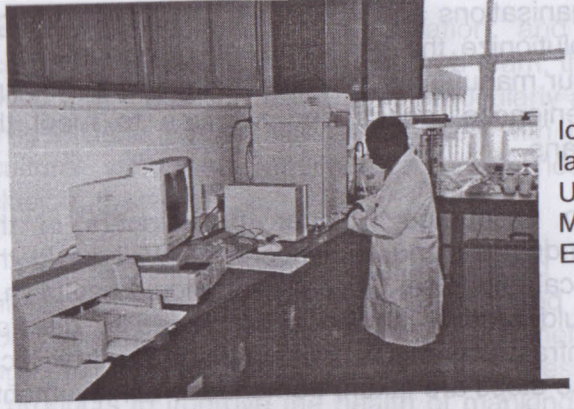
(4) The progress and support made in biotechnology today will contribute to the development of novel, economic and very efficient fermentation processes for the production of a variety of necessities required for the survival and sustainability of mankind and for the management of waste and toxic by-products generated by mankind. I strongly recommend that

governments, organisations and individuals should encourage, support and revolutionize the biotechnology developments in Nigeria to solve our manufacturing problems thereby producing adequately conveniences, goods and services to meet the demand by Nigerians.

(5) .In order to encourage Nigerian practitioners in the biotechnological industries and innovations as well as in the development of local expertise, Government should establish a policy which should require industries/companies to develop local/indigenous infrastructure with training plans for their local employees. If these steps are taken, tremendous achievements will be made in meeting the needs of mankind in this country, Nigeria.

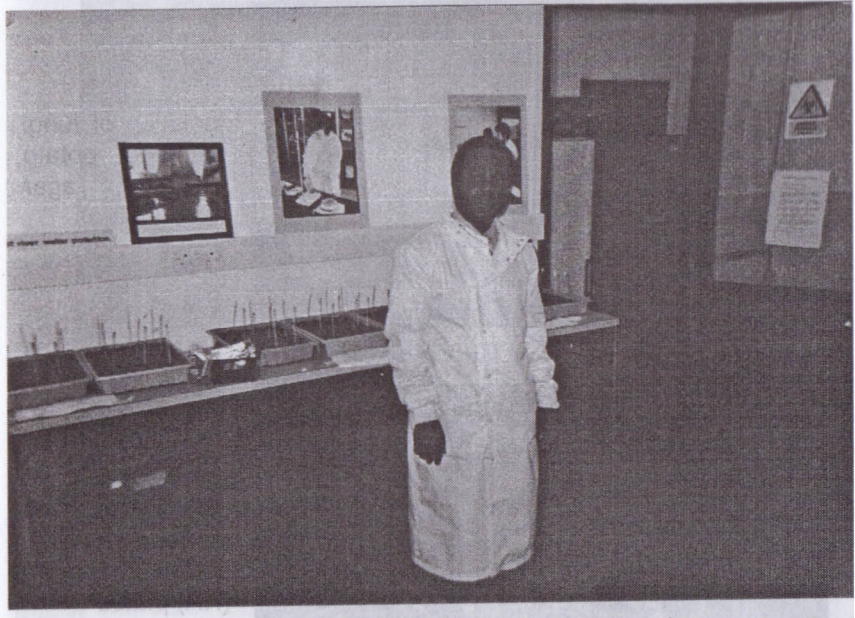


Members of the Bioremediation Team at the University of Salford, Manchester, England

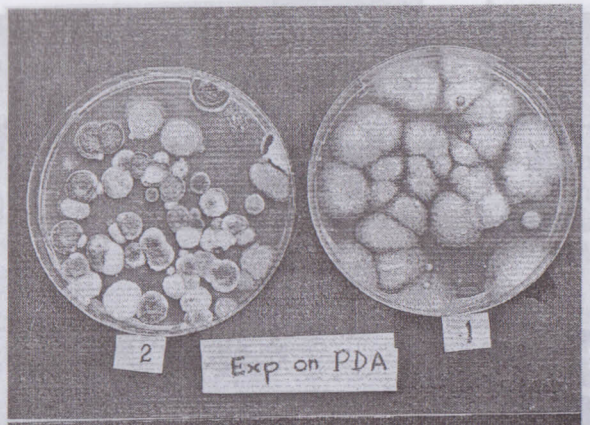


Ion Chromatography laboratory. University of Salford, Manchester, England

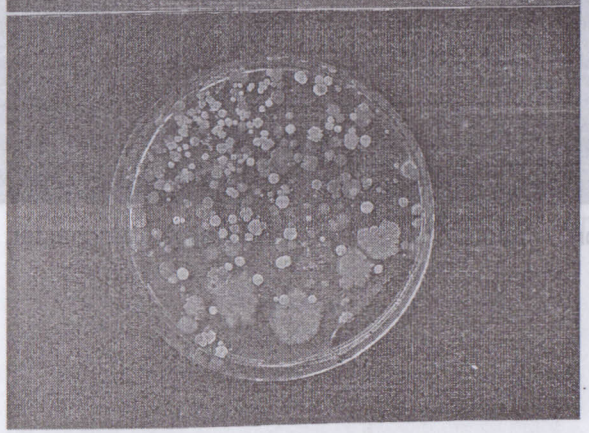
Dr. P. James (An Associate Professor), my host at the University of Salford, Manchester, England



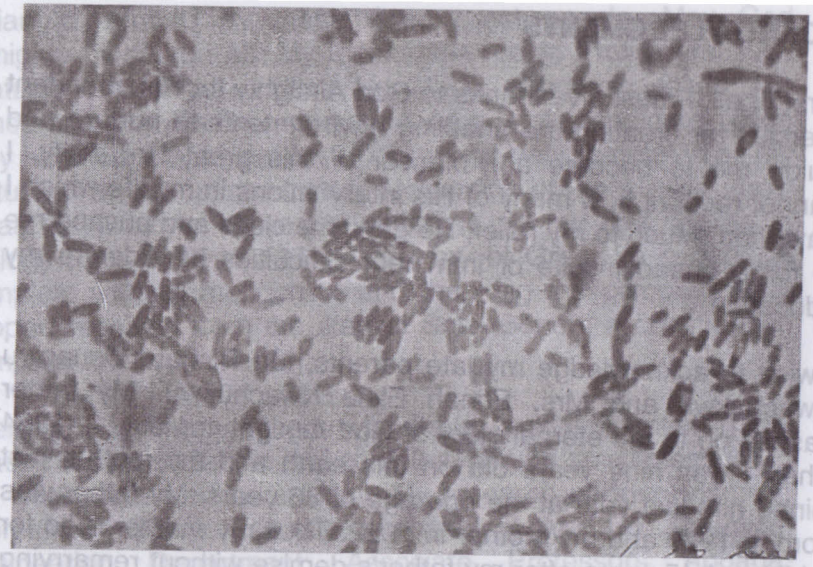
Seeds Germination, laboratory. Salford University, Manchester, England



Isolation of fungi using potato dextrose agar (PDA) plates



Isolation of bacteria using nutrient agar (NA) plates



Smear from NA plates, Gram stained and observed under the light microscope showing mainly Bacillus spp.

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Mr Vice-Chancellor, Sir; Distinguished Ladies and Gentlemen, I thank you all for your presence, patience and attention. Goodnight and May God Almighty bless you all.

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