NIGER DELTA UNIVERSITY
WILBERFORCE ISLAND

31ST INAUGURAL LECTURE

TITLED

UBIQUITOUS MICROBES; THE VIRTUOUS, THE INIQUITOUS AND THE INNOCUOUS

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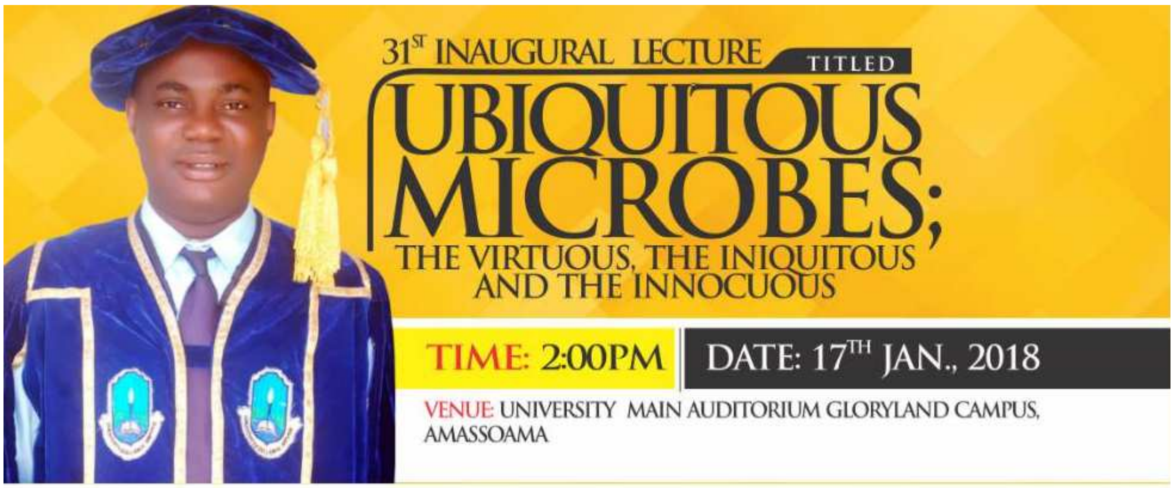

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PART 1: INTRODUCTION

BACKGROUND

There seems to be two contrasting opinions concerning the purpose of inaugural lecture. One school of thought claimed that Inaugural Lecture is an opportunity for newly-promoted or appointed Professors to inform colleagues in the University and the public, about their research activities and update colleagues on their present and future research directions. Another school of thought claimed that inaugural lecture is a debt every academician owes the university before retirement. This second position partially explained why in some universities, some academicians are not in hurry to deliver their inaugural lectures. Hence, they choose to deliver it just before retirement. But as the name implies, inaugural lecture ought to be delivered at the beginning of an academic career so that the university will know your research focus, which could create opportunity for research collaboration. However, some universities including UNIPORT have started giving valedictory lecture, which is a lecture given just before retirement. In addition, UNIPORT have started doing special valedictory senate for retiring professors. Hence, inaugural lecture ought to be given immediately somebody is promoted to the rank of a professor, while valedictory lecture should be given prior to retirement. I therefore align with the first school of thought for inaugural lecture and the second school of thought for valedictory lecture. That is why I am thanking God, the Vice Chancellor, the Deputy Vice Chancellor, the Registrar, the Bursar and Librarian, Provost, Deans, HODs and the entire university community for creating the avenue for me to deliver my inaugural lecture. I am therefore open for research collaboration with colleagues.

Microbial ecology is the study of microbes and how they interact with the environment (physical, chemical and biological). In general, microbes are found practically everywhere and are therefore regarded as ubiquitous. They could be found in the air, surface and underground water, sediments and soil. Microbes are found in many geological formations including soil at various strata, rocks, other surface and underground formations such as oil and gas reservoir. microbes are also found within and outside tissues of plants, animals and humans. Microbes are also found in outer space. Areas devoid of microbes are usually regarded as being sterile. Sterile environments are not common naturally. For instance, medical personnel routinely create aseptic conditions through sterilization to permit safe surgery. Without the control of microbes, medical operations could be fatal. Though, microbes are the smallest living creatures, they exert a profound effect on humans, the world and even in outer space.

The microbial world is quite interesting comprising of microscopic lifeforms including viruses, bacteria, fungi, algae and protozoa. They are mostly invisible to the naked eyes, but they have phenomenal influence on humans and the entire ecosystem. It appears that nature compensated microbes with metabolic diversity for their small sizes. Mister Vice Chancellor sir, this lecture shall focus on various environments microbes are found, their activities whether virtuous (good), iniquitous (bad) or innocuous (harmless) and the potential for sustainably harnessing microbial technology in different economic sectors, even beyond petroleum. I attempt to proffer sustainable microbiology solutions to the health, environmental and energy challenges of Bayelsa State and Nigeria. Welcome on board, as we journey through the microbial world.

MICROBIAL ECOLOGY AND DIVERSITY

Like humans, the majority of microbes thrive in average environmental conditions of pH (6-8), temperature (25-30^oC), pressure (760 mm Hg), aerobic and tolerate moderate salt. On the contrary some microbes, broadly classified as extremophyles are adapted to grow and proliferate in extreme environments of high and low temperatures, high acid and alkaline environments, high pressures and salinity, dry conditions, anaerobic and microaerophilic environment. Information on selected extremophiles are presented in Table 1. Vice Chancellor sir, my PhD research was focused on using extremophilic bacteria for the remediation of heavy metal polluted soils in the Niger Delta (**Ohimain** 2001).

Table 1: Types and examples of extremophyles and implications of their activities

Parameter	Type of extremophyle	Extreme environmental conditions	Examples	Implications of extremophilic activities
pH	Acidophyle	Adapted to live under acidic conditions, pH 2-6	Acidithiobacillus thioxidans & A. thioxidans, used in biomining	Could still cause spoilage of food preserved with vinegar.
	Alkalinophyle	Adapted to live under alkaline conditions, pH>8	Natronobacterium gregoryi	
Temperature	Psychrophyle	Adapted to live under cold conditions, -4 to 10^oC	Polaromonas vacuolata	Could still cause food spoilage even inside the fridge
	Thermophile	Adapted to live under hot conditions, 42 - 68^oC	Bacillus stearothermophilus	Could still cause food spoilage after warming
	Hyper-thermophyle	Adapted to live under very hot conditions, 65-114 ^oC	Thermococcus celer, pyrolobus fumarri	Could still cause food spoilage after boiling
Pressure	Barophyle	Adapted to live under high pressure conditions	Moritella yoryonosii	Can cause corrosion even under high pressures
Salinity	Halophyle	Adapted to live under high salt conditions	Halobacterium salinarum, Vibrio fischeri	Could still cause food spoilage under high salt concentration
Oxygen	Anaerobe	Adapted to live in the absence of oxygen	Clostridium botulinum, Cl. tetani	Could contaminate canned food
	Microaerophilic	Adapted to live with little air	Facultative anaerobes	Inside confined spaces

Microbes are the most diverse and populated living things or earth. Microbes have large species diversity and genetic diversity and exhibit a high level of habitat diversity. There are total population of microbes as represented by bacteria, archaea, yeast, fungi, viruses, algae and parasites are too numerous to count. A recent study estimated the population of bacteria alone to be five million trillion trillion (5×10^{30}) and with a species diversity of 10^7- 10^9. Microbes are evolving resulting in different stains, serogroups, sequence types and clonal complexes of a particular species. The population of microbes in 1.0g of agricultural soil is more than the 7 billion human population on earth. The reasons why microbes are ubiquitous is because they easily colonize and adapt to diverse environment due to their large population, fast growth and reproduction, exhibit broad environmental tolerance, high biodiversity, and display physiological and metabolic diversity. Unlike plants that uses CO_2 to synthesize food substances autotrophically, which animals consume heterotrophically, microbes can exhibit autotrophy, heterotrophy or mixotrophy, which is a combination of both. Microbes can also derive their nutrients from a variety of substances or elements including hydrogen, CO_2, heavy metals, and nitrogen, sulphur and phosphorus compounds. Unlike other organisms, which respire using only oxygen as terminal electron acceptors, microbes respire using a variety of electron acceptors such as oxygen, CO_2, carbohydrates, heavy metals and nitrogen, sulphur and phosphorus compounds. Because of their diverse metabolism, microbes are the agents of global biogeochemical cycles of carbon, nitrogen, sulphur, iron, manganese and other heavy metals. Microbes also interact widely, among themselves and with plants, animals and humans. These diverse interactions of microbes with the biotic and abiotic environment can be exploited in diverse economic sectors.

HUMAN-MICROBIAL INTERACTIONS

There is probably no better example to demonstrate the good, bad and harmless nature of microbes than their interactions with humans. Interest in, and knowledge about, the human microbiota has recently exploded. Microbial interactions with humans are mostly beneficial, some commensal, though with others out rightly harmful. Microbes are involved in virtually all activities including feeding and defecated, sweating, kissing, sex, good health and sickness. Without microbes, medical doctors, pharmacist, nurses and other health workers will have little work to do. Microbes constantly put medical professionals on their feet.

The human body is a large reservoir of microbial cells. Human physiology, nutrition and protection from diseases is dependent on relationships with microbes. These highly diverse communities of microbes live in and on us in staggering numbers. The total community of microbe in the human body is called microbiome. There are more microbial cells in human body than human cells. There are about 100 trillion (10^{14}) bacteria cells in adult human beings, which is greater than 30 trillion eukaryotic cells in humans. Most authors generally report that there about ten times microbial cells than human cells even in humans. There are about 1-2kg of microbes in the gut of humans. Through cultural means, about 400-500 bacteria species have been isolated from the human gut. But with the advances in molecular biology techniques of Polymerase Chain Reactions (PCR), over 3,500 species of bacteria have been detected in human gut. It is generally estimated that $<1\%$ of the microbes in the gut can be detected through cultural means. Hence, there is a wide gap in knowledge about gut microbes, which is an opportunity for research. It is now recognized that microbes play vital roles in human health, participating in many important physiological functions such as digestion and metabolism of foods, and immune responses and inflammation.

Normal flora

The human body provides many unique environments or niches for different kinds of microbes to live. In a healthy animal, the internal tissues and organs including the blood, brain, muscle, kidney, liver are normally sterile i.e. free of microbes or microbe-free. But the surface tissues and linings such as the skin, mucous membranes such as the nostrils and the gut that are constantly in contact with the environment, are readily colonized by various microbial species, mostly bacteria and a few fungal species. The mixture of organisms regularly found at any anatomical site is variously referred to as the normal flora, normal microbiota or indigenous microbiota, while the total microbe DNA is referred to as microbiome. These are the microbes that colonize the human body during birth or shortly thereafter, remaining throughout life. Normal flora can be found in many sites of the human body including the skin (especially the moist areas, such as the groin and between the toes), respiratory tract (particularly the nose), urinary tract, and the digestive tract (primarily the mouth and the colon). Normal flora may be categorized into two types: resident microflora, which are always present and transient microflora, which are only present for short period of time, but could cause disease when the resident microbes are disturbed.

Different microbes occupy and are established at different anatomical sites of the human body. The microbes have evolved and become adapted to the human anatomy and physiological conditions at those sites and microbes have be shown to influence the physiology at the site they occupy. Figure 1 present a list of the predominant normal flora at various sites of the human body. The growth of microbe in a particular site could depend on several physicochemical and physiological factors including pH, oxygen, temperature, moisture, presence of food and inhibitory substances.

There are about five types of associations between microbes and humans. They include

1. Mutualism, which is a beneficial relationship where both microbe and host benefit. The good bugs are at work, please don’t disturb
2. Commensalism, causing no immediate harm or no benefit to host, but could be beneficial to the microbe. The harmless bugs are just watching
3. Parasitism, where the organism is benefitting, but causing harm to the host. The bad bugs
4. Pathogenic, where the microbe causes disease on the host. The very bad bugs
5. Opportunistic pathogens, mostly transient organisms or even resident commensals can also cause disease when the host immune system is compromised, or the normal flora is disturbed or when microbes are established in a different anatomical site. These are the not-always bad bugs depending on the circumstance

The beneficial effects of normal flora in the human body can be summarized as follows:

1. They synthesize Vitamins K and B12, which are utilized by the hosts host,
2. They prevent colonization by pathogens by competing for attachment sites or for essential nutrients.
3. The normal flora may antagonize other bacteria through the production of substances (e.g. fatty acids, peroxides & bacteriocins) which inhibit or kill potentially harmful microbes.
4. They stimulate the development of certain tissues, i.e., the caecum.
5. The normal flora stimulates the production of natural antibodies thus boosting the immune system of the host

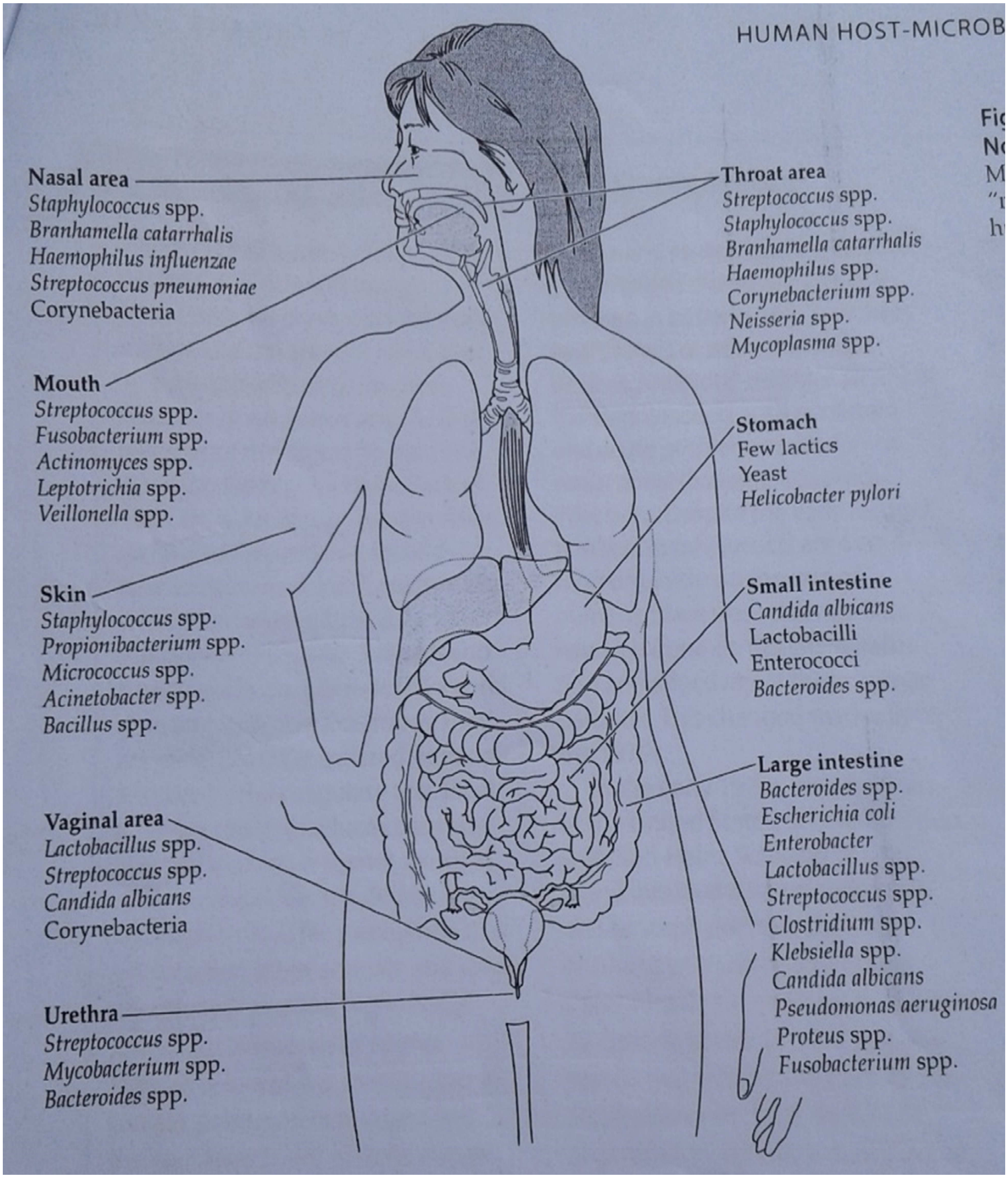

Figure 1: Some predominant microbial flora of the human body
(Source: Perry et al, 2002-Microbial Life)

Lactobacillus and Bifidobacteria are among the most beneficial microbes to man. They produce lactic acid, which aid food digestion and discourage bad microbes.

Likewise, the harmful effects of the human normal flora include

1. Bacterial synergism between a member of the normal flora and potential pathogens helping them to establish and cause infection.
2. They compete for nutrients with the host. They steal our food
3. They produce minute amounts of bacterial toxins such as. endotoxin.
4. They could become opportunistic pathogens.
5. They can be transmitted to susceptible hosts, where they could cause disease e.g. Neisseria meningitidis.
6. Farting, mouth and body odours could be linked to the metabolic activities of some normal flora

Normal Flora of the Human Body Systems

Microbes are found everywhere in the human body in virtually all the systems such as the oral cavity, nasal cavity, GIT, skin and in the genitals (Figure 1).

Normal flora of the Oral cavity

The oral cavity is quite conducive for microbes because of the abundance of food. It is estimated that about 500-600 different kinds of bacteria with a population of about 100 million (10^8) cells thrive on the mucus and food remnants in the mouth. But microbes must overcome the inhibitory effect of lysozyme and lactopereoxidase in saliva and the mechanical shearing actions of the mouth and teeth. Notwithstanding, some are adapted to these conditions and become established in the mouth. The mouth is populated by bacteria such as Actinomyces, Bacteroides, Bifidobacterium, Eubacterium, Fusobacterium, Lactobacillus, Leptotrichia, Peptococcus, Peptostreptococcus, Propionibacterium, and Veillonella; and fungi including Candida, Cladosporium, Aspergillus, Fusarium, Alternaria, Penicillium, and Cryptococcus. Hence, a simple lovely or harmless kiss can tranmit millions of these microbes. These supposedly harmless normal flora has been linked to some problems in the oral cavity. *Streptococcus mutans* is the primary bacterium involved in plaque formation and initiation of dental caries, while Lactobacilli probably contribute to acid formation that also leads to dental caries.

Normal flora of the upper respiratory tract

The upper respiratory tracts of humans consist of mouth, throat and nasopharynx, while the lower respiratory tract, which is usually sterile except under disease conditions, is made up of trachea, bronchi and the lung. The nasal, which is moist and in constant contact with the environment during respiration, is quite receptive to microbial colonization. *Streptococcus pneumoniae, H influenzae* and *Neisseria minigitidis*, which are present in the upper respiratory tract of humans, are opportunistic pathogens linked to pneumonia, flu and meningitis in humans. Other microbes found in the upper respiratory tract include Staphylococcus, Corynebacteria and Branhamella catarrhalis. Fungi include *Candida*, *Saccharomyces* and *Aspergillus.*

Normal flora of the skin

The human skin is not particularly conducive for microbial growth, because the skin surface is relatively dry, slightly acidic, with the presence of lysozymes, and the source of nutrition are limited to secretion from sebaceous glands and dead cells. This hostile environment, which prevents the growth of many microbes, but a few have adapted to live on the skin such as *Propionibacterium acnes* and *Staphylococcus epidermidis*. Even *S. epidermidis* despite being a commensal, can cause life threatening disease in hospital patients when invasive medical devices such as catheters are used. Under this scenario, *S. epidermidis* would be considered an opportunistic pathogen, since it remains benign until provided with specific conditions that allow it to cause disease. Whereas, its relative, *Staphylococcus aureus*, which also thrives on the skin and other places in the body have been linked to several diseases, hence it is a pathogen. Methicillin Resistant *Staphylococcus aureus* (MRSA) have been widely reported. The skin is not homogenous, but have several micro-niches such as the armpit, scalp, palm, finger nails, groins, feet and toes, where different microbes inhabit.

Normal flora of the Gastrointestinal tract (GIT)

The gastrointestinal tract is quite broad consisting of different micro-niches including the stomach, different sections of the small intestine (duodenum, jejunum and ileum) and the large intestine/colon, that inhabits different species of microbes.

The stomach is quite acidic, with a pH of 1-2 and is therefore hostile to most microbes. But few species such as Lactobacillus and yeast are adapted to survive in the stomach. Helicobacter pylori, which is sometimes listed among the stomach microflora, have been implicated in peptic ulcer.

The small intestine is acidic and has high flow rates and short resident times (3-5 hours), hence few microbes are adapted there. Such as *Helicobacter pylori*, which is also known to cause gastric ulcers and duodenal cancer. The large intestine is anaerobic with food residing for long times (24-48 hours). The colon with pH of 5.7 to 6.7, anaerobic and temperature of 37-37.2 ℃ provides the optimal growth condition for many microbes particularly anaerobes. The colon has a population density of 10^{11}-10^{12} cells/ml^2 with over 99% are anaerobes and contains 3.3 million unique microbial genes with over 1000 species.

Good microbes prevent bad microbes from establishing in the gut through several interference mechanisms such as competition for food and attachment sites, and inhibition through the production of metabolites such as bacteriocins, acids and other toxic products. Several microbial-related activities take place in the colon including enzyme activities, microbial fermentation and synthesis of vitamins (K and B) and short chain fatty acids such as acetate, butyrate, propionate and the neurotransmitter serotonin.

The colon appears to be the most diverse anatomical site in the body. *Bacteroides fragilis, Bacteroides melaninogenicus, Bacteroides oralis, Enterococcus faecalis*, *Escherichia coli, Enterobacter sp., Klebsiella sp., Bifidobacterium bifidum, Staphylococcus aureus, Lactobacillus, Clostridium perfringens, Proteus mirabilis, Clostridium septicum* and *Pseudomonas aeruginosa.* About 95 – 99% belong to the genera *Bacteroides, Bifidobacterium, Eubacterium,* *Peptostreptococcus* and *Clostridium*, which can be broadly divided into *Firmicutes* and *Bacteriodetes*. These gut bacteria play key roles in regulating gut metabolism and are therefore critical in understanding metabolism dysfunctions.

Although, the normal flora can inhibit pathogens, many of them can cause disease in humans. Anaerobes in the intestinal tract are the primary agents of intra-abdominal abscesses and peritonitis. Bowel perforations produced by appendicitis, cancer, infarction, surgery, or gunshot wounds almost always seed the peritoneal cavity and adjacent organs with the normal flora, which is often fatal. Anaerobes can also cause problems within the gastrointestinal lumen. For instance, *Bacteroides* have been implicated in the initiation colitis and colon cancer. Treatment with antibiotics may allow certain anaerobic species to become predominant and cause disease. For instance, *Clostridium difficile*, which can remain viable in a patient undergoing antimicrobial therapy, may produce pseudomembranous colitis. Faecal indicators such as E. coli, Enterococcus faecalis and Clostridium perfringes have also been implicated in infections. Some strains of *E. coli* are pathogens that cause intestinal infections, urinary tract infections and neonatal meningitis. *Enterococcus faecalis* has emerged as a significant, antibiotic-resistant, nosocomial pathogen.

Normal flora of the Genito-urinary tract

There is a succession of different types of microbes in the vagina with respect to the stage of development. The type of bacterial flora found in the vagina depends on the age, pH, and hormonal levels of the host. The vagina becomes colonized soon after birth with Corynebacteria, staphylococci, streptococci, *E. coli* and *Lactobacillus acidophilus*. *Lactobacillus* spp. predominate in female infants, when the vaginal pH is approximately 5 especially during the first month of life. Glycogen secretion appears to cease from about 1 month of age to puberty. During this time, the pH increases to about 7, other microbes such as diphtheroid, *S. epidermidis*, streptococci, and *E. coli* predominate. At puberty, glycogen secretion resumes, the pH drops, and women acquire an adult flora in which *L. acidophilus,* Corynebacteria, peptostreptococci, staphylococci, streptococci, and Bacteroides become predominant.

During reproductive life, i.e. from puberty to menopause (i.e. during child-bearing years), the vaginal epithelium contains glycogen due to the actions of circulating oestrogens. *Lactobacillus acidophilus* predominates, being able to metabolize the glycogen to lactic acid and production of hydrogen peroxide, which inhibit colonization by many other microbes especially pathogens. This is a typical example of the protective effect of the normal bacterial flora for their human host. However, acid tolerant species like Streptococcus and the yeast, *Candia albicans*, still strive well in the vagina, but are not predominating and unable to cause diseases. However, the use of antibiotics can cause a significant decrease in Lactobacillus and Streptococcus, which could result in the dominance and invasion of Candida into the vagina tissues, resulting in opportunistic infections (candidiasis/vaginitis). After menopause, pH again rises, less glycogen is secreted, and the flora returns to that found in prepubescent females.

Urinary tract infections (UTI) often result when bacteria enters the urinary tract through the urethra, multiply and colonize the urinary tract and cause disease, when the host defence system is unable to wade off the infection. UTI are mostly caused by *Escherichia coli*, which is a normal microbial flora of the GIT. In instances where the infection is found in the urethra, resulting from the spread of *Escherichia coli* from the anus to the urethra, it is called urethritis. Studies have shown that the normal vaginal microflora, *Gardnerella vaginalis*, plays a significant role and is commonly associated with bacterial vaginosis when it persists in the vagina and overwhelms the healthy *Lactobacillus* population, especially in young, sexually active females. vaginosis, which is characterized by a reduction in the numbers of Lactobacilli and an increase in other bacteria causing a shift from Gram positive to predominantly Gram-negative bacteria including: *Gardnerella vaginalis*, *Bacteroides* spp, *Mobiluncus* spp, anaerobic Streptococci, *Mycoplasma hominis* and *Ureaplasma urealyticum*.

Succession of human microflora

The normal flora of the body changes with age i.e. from birth, through infants, childhood, teenage, young adult and the elderly. The normal flora in humans usually develops in an orderly sequence, or succession, after birth, leading to stable populations of bacteria that become established and constitute the normal adult flora. Body environmental factors determine the composition of the normal flora includes pH, temperature, redox potential and oxygen, water, osmotic pressure and nutrient levels. Other factors such as peristalsis, saliva, lysozyme secretion and antibodies also play roles in the establishment of microflora in an anatomical site.

Prior to birth, the foetus is sterile, but infant begins to contact organisms as it passes through the birth canal. During passage through the vagina, they pick up some of the normal flora of the virginal particularly Lactobacilli and Bifidobacteria. But children delivered via caesarean section remains sterile at the point of birth. But during clean-up and subsequent handling they could contact some microbes. The initial microbes colonizing the gut vary with the food source of the infant. Breast-fed infants soon pick up Lactic acid bacteria (mostly Lactobacillus and Bifidobacteria from the skin of their mum and their normal flora develop differently from bottle-fed infants. For instance, *Bifidobacterium bifidum*, which account for over 90% of the total intestinal bacteria, is the predominant bacterial species in the intestine of breast-fed infants, where it presumably prevents colonization by potential pathogens through the production of lactic acid. It appears that human milk contains a growth factor that support the growth of Bifidobacteria. But enterobacteria dominate the guts of bottle-fed infants with no Bifidobacteria. However, when breast-fed infants are switched to a diet of cow's milk or solid food, Bifidobacteria are progressively joined by enterobacteria, Bacteroides, enterococci and clostridia. Hence, the journey of man and microbes starts immediately during delivery, continues throughout the entire lifespan of man, and contribute ultimately to the death of man and continue thereafter to its decomposition and mineralization.

Modulation of the gut microbes for health benefits

The gut microbiota is a very complex ecosystem which interacts extensively with the host, influencing multiple metabolic and physiological functions. In fact, the normal flora consists of communities of microbes that function as microbial ecosystems in human bodies. There are evidence indicating that richer and more diverse community of microbes in the gut of humans and other animals lower the risk of disease. Maintaining a balance human microbiome is crucial. But when the balance is tipped or disrupted, the upset has been linked to changes in immunity and the development of chronic diseases.

Substances that can be detrimental to gut microbiome include uncontrolled use of antibiotics, diets, artificial sweeteners, strong alcoholic beverages, phytochemicals, tissue damage, medical procedures and the introduction of new pathogens. For instance, antibiotic treatment targeting one species of microbes can lead to increased growth of another, and may also inadvertently deplete helpful bacteria, which can upset the balance of species. Unhealthy or imbalanced microbiome composition, which is often referred to as dysbiosis can lead to inflammation. Disruption of the gut microbiota might contribute to a variety of conditions including childhood asthma, obesity, colitis and colon cancer.

The fascinating role of gut microbiota on metabolic disease opens new avenues in the treatment of obesity, insulin resistance and type 2 diabetes. Substances that influence gut microbes include human diet i.e. food, antibiotics, probiotics, prebiotics, synbiotics pharmabiotics, nutraceuticals and supplements. Prebiotics are non-digestible food components that promote the growth of beneficial gut microbes, which include fibre diet, yogurt, cheese, palm wine and many locally fermented beverages. Fibre containing inulin can boost the gut microbiome. Inulin is found in garlic, wheat and barley. Regular intake of prebiotics increases the fermentation of gut bacteria, reducing appetite and caloric intake and increasing glucose tolerance. Thus, reducing obesity and diabetes. Inulin helps encourage the gut microbiome to produce butyrate, an acid that feeds cells in the colon and keeps inflammation in under control. Studies have also suggested that diets high in inulin lead to increases in health-promoting Bifidobacteria, which break down carbohydrates to short-chain fatty acids (acetate, propionate & butyrate), which may in turn decrease the risk of cancer, digestive and heart disease.

Probiotics are viable microorganisms that can be delivered orally and incorporated into intestinal microbiome for health-related benefits or therapeutic purposes. They can be used to restore balance of the gut microbiota. The term "pharmabiotics" is used in various ways, to mean: pharmaceutical formulations (standardized manufacturing that can obtain regulatory approval as a drug) or probiotics, prebiotics, or synbiotics; or probiotics that have been genetically engineered or otherwise optimized for best performance (shelf life, survival in the anatomical site) and the natural products of gut flora metabolism (vitamins). FAO/WHO (2012) released a guideline for the evaluation of probiotics in food. Some probiotic products are now in the market (Plate 1 Table 2).

Table 2: Medicinal uses of probiotics

Disease	Strain	Products (manufacturer)
Eczema	*Escherichia coli*	-
	Bifidobacterium bifidum	Oasis Health Break with Probiotics, A Lassonde Inc, Canada
	Bifidobacterium lactis	Natrel pro, Agropur, Division Natrel, Canada
	Lactococcus lactis	-
Diarrhea	*Saccharomyces boulardii lyo*	Florastor, Medical Futures Inc, Canada
Improve transit time (regularity)	*Bifidobacterium animalis*	Activia yogurt, Danone, Canada
Pouchitis and Ulcerative colitis	Some strains of *Bifidobacterium* and *Streptococcus*	VSL#3, VSL Pharmaceuticals Inc, Canada
Anti-inflammatory and anti-infective effects	*Lactobacillus rhamnosus*	Fem-Dophilus, Jarrow Formulas, USA
	Lactobacillus reuteri	Fem-Dophilus, Jarrow Formulas, USA

Food allergies	*Escherichia coli*	-
Immunity	*Bacillus circulans*	-
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada
Antibiotic effect removal	*Enterococcus mundtii*	-
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada
	Lactobacillus brevis	-
	Lactobacillus strains	-
	B*ifidobacterium* strains	-
Gastroenteritis Therapeutics	*Lactobacillus casei*	-
Hyperpermeability	*Lactobacillus plantarum* species	TuZen, Ferring Pharmaceuticals, Canada
Vaginal candidiasis (thrush)/ Urinary tract infection	*Lactobacillus rhamnosus*	Bacid, Aventis Group, Canada
	Lactobacillus reuteri	Fem-Dophilus, Jarrow Formulas, USA
Lactose Intolerance	*Lactobacillus acidophulus*	Yoptimal immuni+, Yoplait, Canada
Non-steroidal anti-inflammatory drug	*Escherichia coli* strain	-
Intestinal dysbiosis	*Lactobacillus johnsonii*	-
	Lactobacillus strain	-
	Lactobacillus	-
Irritable/inflammatory bowel syndrome	*Bifidobacterium infantis*	Align, Proter & Gamble, USA
	Escherichia coli	-
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada
Radiation-induced diarrhea	*Lactobacillus casei*	-
Crohn's disease	*Escherichia coli* strain Nissle	-
Prevention of colon cancer	*Enterococcus faecium*	-
	lactic acid bacteria	-
Ulcerative colitis	*Lactobacillus acidophilus*	Yoptimal immuni+, Yoplait, Canada
	Escherichia coli Nissle	-
	Bifidobacterium	-
Peptic ulcer disease	*Lactobacillus acidophulus*	Yoptimal immuni+, Yoplait, Canada
Prevention of atopy	*Lactobacillus rhamnosus*	Bacid, Aventis Group, Canada
Hypercholesterolemia and cardiovascular diseases	*Enterococcus faecium*	-
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada
	Propionibacterium freudenreichii	-
	Lactobacillus plantarum	TuZen, Ferring Pharmaceuticals, Canada

Plate 1: Some probiotic products in the market

Microbes in space

Microbes in space behave differently. Hence, much work is needed to determine the characteristics of bacteria growing in a weightless atmosphere. There is growing concern about enhanced bacterial growth and resistance in outer space. For instance. In the 1960s, NASA discovered that *E. coli* and *Salmonella* grow twice as fast when orbiting in a space module. A simultaneous study compared *E. coli* growth on a space station with those on Earth. results show that the bacteria grew 13 times faster in orbit and that dozens of *E. coli* genes were more active in space than on Earth. Soviet scientists in the 1970s found that bacteria developed increased resistance to five antibiotics while in orbit. Similarly, in 2006, Cheyl Nickerson from Arizona State University found that bacteria became more lethal to lab animals in space than on Earth. In November 2017, Russian cosmonauts discovered novel bacteria on a swab collected from outside the International Space Station. The cramped interior within Space Stations (Plate 2) creates a concern, especially when astronauts experience an altered microbiome after extended periods of time in space. The different behaviour of microbes in zero gravity environment opens opportunity for research collaboration between microbiologist and space engineers. Besides, the presence of microbes in any planet is the first indication of life on the planet.

Plate 2: The overcrowded interior within the International Space (Source: Microbyte).

Contact and Transmission of Microbes

Generally, microbes, because they are ubiquitous, they can be transmitted through various media including air, soil, water, food and beverages. There are many common practice today that aid the transmission of microbes, whether good, bad or harmless:

1. Hand shaking
2. Kissing
3. Nose picking
4. Ear swabbing
5. Religion practices such as Holy Communion, anointing services, Hajj pilgrimage
6. Eating fruits or snacks without hand washing
7. Taking drugs without hand washing
8. Sharing microphones & other mouth instruments
9. Door knob
10. ATM
11. Sharing pen or other writing materials
12. Phone/Handset
13. Computer keyboards
14. Vehicle steering
15. Cloths exchange (choir robes, academic gowns, coverall, vests, aprons)

16. Sweeping
17. Cleaning
18. Sneezing
19. Buying and selling (the concentration of microbes in the Naira increase with decreasing denominations)
20. Tooth picking
21. Beddings especially in hotels and hospitals
22. Normal sexual activities (kissing and intercourse)
23. Abnormal sexual activities (gay, anal, oral)
24. Polygamy, infidelity & multiple sexual relationships
25. Sexual perversion (homosexual, bisexual, heterosexual or their combination)

Few Examples
1. A young nurse was infected with and killed by tetanus after attending to a patient infected with the disease. The nurse was on her monthly period and got infected through changing of pad and because the tetanus bacteria is an anaerobe it blossomed and killed her
2. Ebola virus could be transmitted by a simple hand shake
3. The three children of a couple who were HIV negative, became HIV positive linked to the house help that was sharing toothbrush with the kids
4. Neisseria miningitidis, monkey virus and SARS virus are air-borne and can be transmitted to somebody by simply doing nothing

Ladies and gentlemen, at the 23rd NDU inaugural Lecture, Prof Ogoina, lectured on microbes as associates, adversaries and adjuvants. There is therefore the need research collaboration to take medical microbiology to the next level.

PART 2: SECTORAL ASPECTS OF APPLIED MICROBIOLOGY (MY CONTRIBUTIONS)

The good, bad and harmless nature of microbes could manifest in different sectors of the economy. The good microbes are true agents of sustainable development, because their activities have social, economic and environmental dimensions. People fail to realize that the alcoholic beverages used in social engagements are of microbial origin produced via fermentation. The production and distribution of these beverages generate employment and income. But microbial fermentation generates carbon dioxide, which is a threat to environmental sustainability. Fortunately, there are other microbes such as algae and cyanobacteria that utilizes carbon dioxide for the production of carbohydrates, oils and other macromolecules. Thus, effectively closing the carbon dioxide loop. This section therefore present selected sectors where microbial processes and products feature prominently. It also presents potential areas of research collaboration with other disciplines, which is one of the importance of inaugural lecture as opposed to valedictory lecture. This section also contains local, regional, national and global issues where microbes features prominently.

WETLANDS DREDGING, SAND FILLING AND GEOMICROBIOLOGY

Wetlands are ecosystems characterized by hydrophytic vegetation, hydric soils and hydrology, which are also used as indicators for wetlands delineation (**Ohimain** and Akinnibosun, 2007, 2008, 2010). The Niger Delta region is located in a wetland of over 76,000 sq km with a mangrove forest (Plate 3) of 11,134 sq km, which is the largest in Africa and the fourth largest in the world.

Dredging and sand filling are common activities in many coastal areas including the Niger Delta and Bayelsa State in particular. Dredging is mostly done to create navigable accesses to oil and gas locations and coastal communities, while sand filling is mostly done to reclaim land from wetlands or water courses such as rivers for community expansion purposes. For instance, the construction of an airport in Wilberforce Island and Oil and Gas facilities resulted in the extensive dredging of the Nun River to obtain sand for filling. Large scale sand filling projects are ongoing in several cities/towns including Yenagoa, Warri, Port Harcourt, Lagos, Abonema, Buguma etc.

Plate 3: Healthy *Concocarpus erectus* with *Rhizophora racemosa* mangrove (in the background, with prop roots evident) in the Niger Delta

In the tropics, most hydrocarbon prospects are found in coastal wetlands especially in the Gulf of Mexico, West Africa, South America and South-East Asia. Most of these wetlands are dominated by mangrove and freshwater vegetation. Generally, mangrove wetlands are spawning grounds for coastal and marine fisheries and provide feeding and nesting habitats for migratory species. Wetlands are also important in shoreline protection. However, oil exploration in mangrove areas is challenged by access difficulties, which the oil companies often overcome by carrying out dredging. Dredging in these wetlands is often carried out to create safe navigable accesses for oil and gas exploitation. During dredging, sediments and soils are removed from the right of way, placed along canal banks mostly upon fringing mangroves and abandoned (Plate 4). This often results in the killing of the mangroves and other biological entities that depend on it including fish. Several hectares of mangroves all over the tropical world fringing most of the creeks where oil exploration related dredging has taken place have been killed likewise. The role of mangrove ecosystems in protecting the shoreline and adjoining hinterland in this era of climate change and rising sea levels could be threatened by dredging (**Ohimain** 2004, 2009).

Plate 4: Impacted *Rhizophora racemosa* mangrove forest in the Niger Delta

The environmental impacts of dredging and spoil disposal are many and can be overwhelming, including water pollution (**Ohimain** et al, 2008a), heavy metal pollution (**Ohimain** et al., 2008b), killing of benthic invertebrates (**Ohimain** et al 2005), zooplankton (**Ohimain** et al 2002) and fisheries (**Ohimain** 2004, 2010a), alteration of algae causing algal blooms (**Ohimain** et al, 2003), alteration of topography and hydrology (**Ohimain** et al., 2010a) causing change in vegetation leading to invasion by alien species (**Ohimain** et al 2004) and corrosion of steel, concrete and other civil engineering infrastructure (**Ohimain** 2008a, 2009). I have authored 30 papers on mangrove alone.

There is a possible microbial role in the weathering of materials that is linked to acidification and the environmental impacts. Worldwide, coastal mangroves are known to contain reduced iron sulphide called pyrite. These pyrites when undisturbed under water cover are innocuous, but their disturbance often results in severe acidification and ecosystem damage (**Ohimain**, 2004). The microbial reduction of sulphate is the origin of pyrite in coastal plain. In many coastal regions all over the world, clayey sediments are deposited over extensive areas under brackish to saline conditions. Such tidal marshes are normally covered by dense mangrove forests. Abundance of organic matter from mangrove trees, soil saturated with tidal water containing sulphate under reducing conditions creates suitable conditions for the formation of metal sulphides mediated by sulphate reducing bacteria. If iron is available from ferric oxides or iron- bearing silicates, much of the sulphide produced is fixed as pyrite (cubic FeS_2). The greater part of the alkalinity (HCO_3) formed during sulphate reaction is moved to the sea by tidal action leaving a potentially acid residue as pyrite. The formation of pyrite may be represented by the following overall equation and presented in Figure 2 diagrammatically:

$Fe_2O_{3(s)} + 4SO_4^{2-}{}_{(aq)} + 8CH_2O + 1/2O_{2(aq)} \longrightarrow FeS_{2(s)} + 8HCO^-_{3(aq)} + 4H_2O$ microbe catalyzed

Iron III Oxide from the sediment	sulphate ions from seawaters	organic matter	dissolved oxygen	pyrite	carbonate

Following the exposure and abandonment of sulphidic dredged materials, acidic drainage results from the interactions of sulphide minerals (pyrite) with oxygen, water, and bacteria (*Acidithiobacillus* sp). The overall stoichiometric reaction describing the oxidation of pyrite is summarized as follows:

$FeS_2(s) + 3.75\ O_2 + 3.5\ H_2O = Fe(OH)_3(s) + 2\ SO_4^{2-} + 4\ H^+$ microbe catalyzed

This reaction leads to the production of sulphuric acid causing the pH of the drainage to be low, often <3. At such a low pH, heavy metals are released and mobilized in the drainage. This low pH could lead to severe acidification with attendant consequences including heavy metal pollution, vegetation dieback, reduced plant/animal productivity, corrosion of steel, concrete, and other engineering structures, degradation of surface and ground water quality, mortality of estuarine biota especially fishes and bioaccumulation of pollutant (**Ohimain** 2004, 2008a, 2008b, 2011). Figure 3 shows the process of acid formation following the dredging and abandonment of pyritic sediments. Figure 2.3 shows ecosystem damage following the dredging of an oil flow line access canal in the Niger Delta. The pH of this site was <3. Both the long and short-term impacts of acidification on fisheries are presented in Table 3.

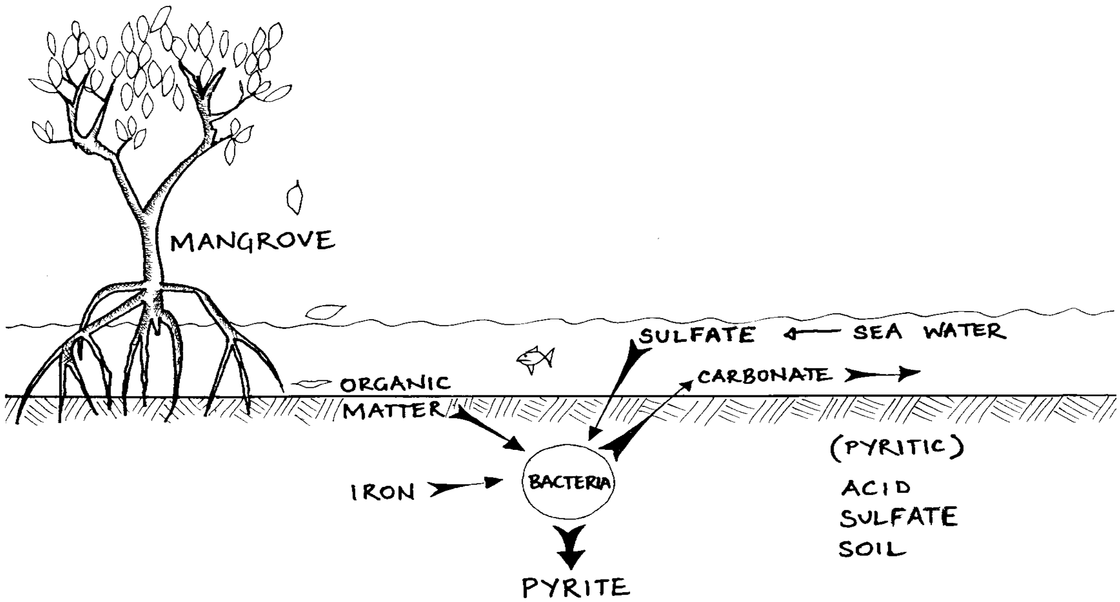

Figure 2: Biogenesis of pyrite formation in coastal mangrove soils (Smith *et al.,* 1995)

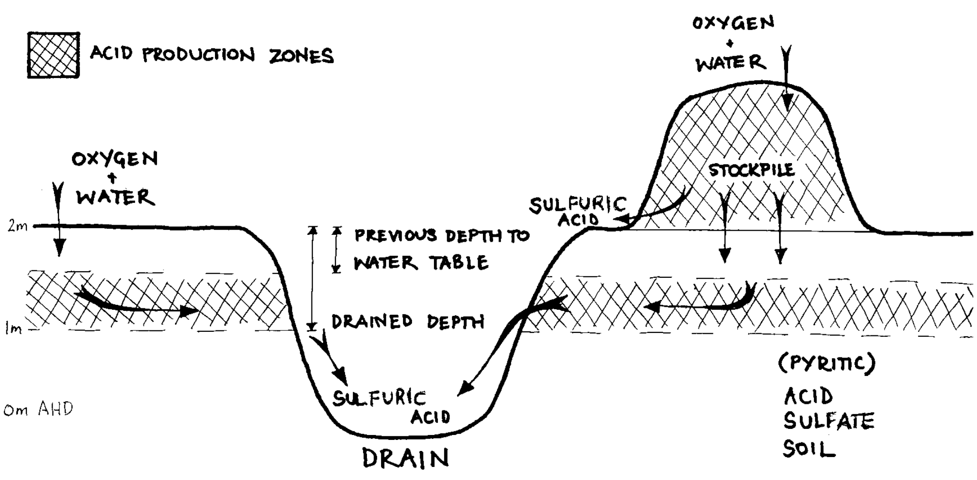

Fig 3: Microbial oxidation of pyrite causing the release of sulphuric acid (Smith *et al.,* 1995)

Table 3: Short term and long-term impacts of acid water on fisheries

Short-term effects	**Long-term effects**
• Fish kills	• Loss of habitat
• Fish disease	• Persistent iron coating
• Mass mortalities of microscopic organism	• Alterations to water plant communities
• Increased light penetration due to lower water turbidity	• Invasion by acid-tolerant water plants
• Loss of acid-sensitive crustaceans	• Reduced spawning success due to stress
• Destruction of fish eggs	• Chemical migration barriers
• Oyster mortality	• Reduced food resources
	• Dominance by acid-tolerant plankton species
	• Growth abnormalities
	• Reduced growth rates
	• Changes in food chain and web
	• Damaged and undeveloped eggs
	• Reduced recruitment
	• Higher water temperatures due to increased light penetration
	• Increase availability of toxic elements
	• Reduced availability of nutrients
	• Poor growth of oysters and other bivalves

Ohimain et al 2014

Roles of microbes in mangrove restoration

Several attempts have been made to restore mangroves upon dredged spoils in the Niger Delta, but they mostly failed. There are a few we did that succeeded in the mangrove swamps of Escravos and Benin River (Plate 5). The ‘gardening approach’ to mangrove restoration always fails because it has not addressed the underlining cause of the problem. We successfully restored mangrove swamps by restoring the hydrology, using microbial techniques for the removal and of acidity and heavy metals in the dredged spoil. There are several methods of mangrove restoration including direct planting, nursery techniques and enhanced natural restoration (**Ohimain** et al 2016a).

Mangrove forests are highly productive ecosystems but low in nutrients particularly nitrogen and phosphorus. Hence, phosphate solubilizing and nitrogen fixing bacteria play major role in mangrove restoration. Diazotrophs have been identified as key organisms that provide nitrogen to these environments. Studies showed that mangroves grew better in the presence of N_2-fixers indicating a potentially mutualistic relationship. Examples of N_2-fixing microbes isolated from mangrove roots or sediments are *Marinobacterium mangrovicola*, *Listonella anguillarum*, *Vibrio campbelli*, *Azotobacter*, *Azospirillum*, *Microcoleus* sp, *Derxia*, *Desulfuromonas*, *Sphingomonas*, and *Pseudomonas*. Other roles of in mangrove ecosystems include nutrient cycling, mitigation of high acidity and iron.

Plate 5: Re-established mangroves in the Niger Delta

Microbial transformation of heavy metals

Microbes transform many heavy metals in nature such as iron, mercury, vanadium, chromium, copper, manganese, lead, arsenic by changing their valence state, which could result alteration of their mobility, reactivity and toxicity. For instance, the microbial transformation of mercury, results in the volatilization and uptake of mercury by fish (Fig 4). Upon consumption of contaminated fish, resulted in human death. This incident happened in Mina Mata Bay in Japan.

The activities of these same microbes producing acids and transforming metals causing pollution have been used beneficially in South Africa, Eastern Europe and other places for the extraction and beneficiation of metals especially from low grade ores in a process called biological mining. Therefore, whether a microbe is considered beneficial or detrimental is dependent on the applications. For instance, these same microbes transforming heavy metals have also been used for the treatment of heavy contaminated wastes (**Ohimain** 2001) including radioactive elements. Unlike hydrocarbons, heavy metals cannot be biodegraded. But they can be bioremediated by microbial transformation processes. Microbial mechanisms for the remediation of heavy metals includes biosorption (**Ohimain** 2001), bioleaching (**Ohimain** et al 2008b, 2008, 2008c, 2009a, 2011) and bio-precipitation (**Ohimain** 2010a). Microbes tolerant to mercury have been isolated from Min Mata Bay, with potentials for transforming mercury in a bioremediation perspective. Staphylococcus aureus and Bacillus sp were found to transform and detoxicate mercury compounds through mercury reduction and volatilization.

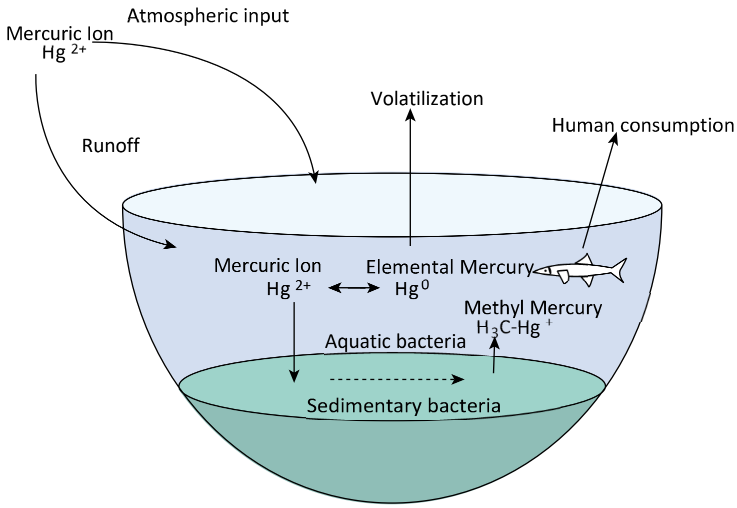

Fig. 4: Microbial transformation of mercury in aquatic ecosystem
Source: American Society of Microbiology

PETROLEUM MICROBIOLOGY

Microbes have diverse applications in the petroleum industry and the energy sector.

The Origin of Petroleum

There are numerous theories on the origin of petroleum. Two of the most accepted theories are those of biogenic and abiogenic origin. Argument have been advanced for each theory, though conflicting, both theories have been generally accepted. In the biogenic (organic) theory, petroleum is said to originate from dead organic matter from plant, animal and microbial remains. These organic materials both from land and marine origin are transported to the marine sediment, where the action of microorganisms leads to their partial decomposition. In the sediments, these organic precursors mix with the mud, silt and sand. After several years of deposition and with increasing overburden, the organic precursor becomes anaerobic. Through physical, biological and chemical transformation the organic precursors are transformed to oil and gas in sedimentary rocks, which are often called source rocks. Through earth movement and physical disturbances such as earthquakes and tectonics, oil migrate from porous formation until their movement is stopped by impermeable cap rock overlying the porous and permeable rocks, which are also called reservoir. In the abiotic (inorganic) theory, oil is deposited in the earth crust from materials incorporated into the mantle at the time of the earth formation. Out gassing process transported the oil into porous and permeable sedimentary rocks. Chemical analysis of varied organic sediments including coal and petroleum revealed that they derive much of their organic matter from microbial lipids called bacteriohopanetetrol (Ourisson et al., 1984). Also, the presence of microorganisms in the subsurface oil field environment and deep oil reservoirs supports the biotic origin of petroleum, which reinforces the importance of microbiology in petroleum engineering (**Ohimain,** 2010). But the recent discovery of the fungi *Gliocladium roseum*, that directly synthesize diesel-like compounds tend to challenge the biotic and abiotic theories concerning the origin of petroleum

Petroleum reservoir microbiology

The presence of microorganism in the subsurface oil field environment and deep oil reservoirs, have been well documented. But what is however not clear is whether the bacteria found in the reservoirs are indigenous or inadvertently introduced during well drilling. Notwithstanding, the roles of reservoir microorganisms are becoming clearer. Though, the proximity of the oil-water interface has been shown to affect the composition of oil or gas, the activities of microbes in the reservoir often alter mineralogical, hydrological, and geological condition of the reservoir including its rocks and fluids properties (Bubela, 1989; Donaldson *et al.,* 1989). The most significant geological changes are:

- Precipitation of dissolved minerals especially carbonates;
- Change of permeability due to precipitation in pore throats;
- Change in the fluid properties
- Production of methane gas to pressurize the reservoir
- Change of porosity, either increase or decrease, depending on the equilibria of dissolve salts and products of organic acids.
- Change of wettability (possibly)

There are both positive and negative influences of bacteria in oil reservoirs. Studies have shown that reservoir microbes could be stimulated to enhance oil production. *In-situ* microbial growth apparently resulted in chemical and petrophysical changes within the reservoir that lead to positive microbial enhancement of oil recovery.

Biotechnological Applications in the Petroleum Industry

Several problems encountered in the petroleum industry during drilling and production operations could be solved using biotechnological techniques. Such challenges include poor recovery; environmental pollution, sour crude, production of oil from shale; sewage and waste management especially waste drilling mud and cuttings. Specific microbiological techniques for tackling these challenges are discussed below.

Microbial Enhanced Oil Recovery

Typically, not all the petroleum existing underground is recoverable. Less than 50% of heavy crude oil and nearly 80% of light oil is recovered using conventional methods even under favourable circumstances. The Conventional oil production occurs in three stages: primary, secondary and tertiary. In the Primary stage the oil extraction results due to the natural reservoir pressure, which exist within the formation. When this internal driving force diminishes to a point where oil production declines then secondary recovery method are applied which involves the use of gas injection or water flooding of the oil reservoirs. When secondary recovery is no longer economical, tertiary recovery methods involving the use of chemical or thermal energy could be considered. The recovery method through the use of chemical energy involves chemical flooding and polymer flooding which includes the use of alkaline water floods, carbon dioxide flooding and polymer flooding. Thermal recovery on the other hand includes in situ combustion and injection of steam. But, all these recovery methods suffer from technological problems and many other constraints (Bubela, 1989). Hence, attention is now focused on the use of microbes for enhanced oil recovery.

Microbial enhanced oil recovery (MEOR) or microbial improved oil recovery (MIOR) in a broad sense can be described as the application of the microorganisms and their metabolic products to increase the production of oil from reservoirs of marginal productivity. MEOR is a secondary / tertiary oil recovery process whereby microorganisms are used to extract oil from wells through several mechanisms including:

- Increasing the solubility of oil in crude oil formations by bio-surfactants produced by oil-degrading microbes;
- Controlling the viscosity of flood solutions during secondary oil recovery by polymers produced by microbes;
- Reducing the viscosity of the oil by microbes that can metabolize long chain alkanes (e.g. waxes).
- Re-pressurizing and redirecting the oil flow in the reservoir by carbon dioxide or hydrocarbon gases produced by microbes *in-situ* in the reservoir

There are several marginal wells in the Niger Delta, which were acquired by marginal field operators, some which are indigenous companies. Many of these marginal fields were initially suspended because of several reasons such as the presence of heavy crude oil, depleted or low reservoir pressures and high water cut. Microbial enhanced oil recovery process could be useful in the production of these marginal wells.

Microbial technology utilized for enhanced oil recovery are basically two, either the injection of nutrients only that would stimulate indigenous reservoir microbes or the introduction of microbes along with nutrients into the reservoir. Bacteria provide a cheap, less energy intensive and environmentally acceptable alternate to the several oilfield chemicals used in EOR. Microbes are able to grow on low cost substrates such as molasses; brewery, oil palm and cassava processing wastes and their small size help them penetrate deep into the porous formation where they can act *in situ*.

There are a variety of natural products that bacteria produce which can lead to increase recovery rates. Bio-surfactants allow oil to travel more freely though the reservoir by reducing oil-water interfacial tension releasing the capillary entrapped oil. Organic acids dissolve carbonate rocks, widening fissures and channels. Gases such as CO_2, H_2, N_2, and CH_4 can be used to re-pressurize the reservoir, and reduce oil viscosity. Microbial biomass and production of *in situ* biopolymers can be used to selectively plug areas of high permeability, thus diverting the water floods to sweep the fresh areas of the reservoir, resulting in the mobilization of oil to the well bore.

Reservoir microorganisms can synthesize a large variety of biochemical products from crude oil constituents when provided with essential nutrients and favourable environmental conditions. The range of metabolic products from microbial attack of petroleum is very broad, depending on environmental conditions (pressure, temperature, salinity, pH, and the presence or absence of oxygen), supporting nutrients available for cell metabolism (nitrogen, phosphorus, etc.) and the specific bacteria interacting with the petroleum. Generally, microbial metabolic products may be gases (methane, hydrogen, carbon dioxide, hydrogen sulphide), carboxylic acids (formic, acetic, valeric), solvents (alcohols, aldehydes, ketones), polymers (proteins, polysaccharides), surface-active compounds (poly-anionic lipids) and many other compounds ranging from simple to very complex macromolecules (Donaldson *et al.,* 1989). Table 4 presents a list of substances that are produced by microbes, which enhances oil recovery.

For microbes to be used for in-situ enhanced oil recovery (EOR), they must be able to withstand extreme conditions (temperature, pressure, and salinity) in the reservoirs. Mixed cultures of bacteria from various locations that adapted to reservoir conditions includes *Pseudomonas, Escherichia, Arthrobacter, Mycobacterium, Micrococcus, Peptococcus, Bacillus,* and *Clostridium.*

Table 4: Microbial products and activities useful in enhanced oil recovery

Products or activity	Examples	Relevance in oil recovery
Gases	Methane, carbon dioxide,	Reservoir re-pressurization, oil viscosity reduction
Acids	Acetic acid, butyric acid, lactic acid	Increase in porosity and permeability
Solvents	Ethanol, methanol, butanol, acetone	Oil viscosity reduction, wettability alteration
Polymers	Polysaccharides, proteins, Xanthan gum	Mobility control, permeability rectification
Bio-emulsifiers	Hetero-polysaccharides, proteins	Oil emulsification, wax and paraffin control
Bio-surfactants	Glycolipids, lipopeptides	Interfacial tension reduction, emulsification, wettability alteration
Hydrogen metabolism	Cleave long chain hydrocarbons, production of alkyl succinic acid	paraffin control, viscosity reduction, methane production, reservoir re-pressurization
Biomass production	Microbial cells	Selective reservoir plugging, water flooding

Adapted from **Ohimain** (2010b)

Microbial Assisted Wellbore Cleaning

Another application of microbial technology is the production of metabolites that are useful in well bore cleaning to enhance oil production. Typically, acidizing processes using mineral acids such HCl are routinely used for well bore cleaning. Microbial processes are now increasingly being considered for well bore cleaning processes because they are cheap, renewable and cause less impacts on the environment. Well bore cleaning processes involve the use of drilling mud degrading or hydrocarbon degrading or scale removing or acid producing bacteria to remove drilling muds, heavy oil, scales and other materials in the well bore.

Microbial synthesis of acids, solvents and gases helps in well bore cleaning in the following ways:

- These acids (organic and inorganic) react with various minerals, especially carbonates, and loosen clay particles in inorganic deposits.
- Microbially produced solvents dissolve or swell the precipitated organic deposits, improving mobility of the oil.
- Gases formed by microbes include methane, carbon dioxide and hydrogen sulphide.

Well Stimulation
A number of oil wells have been suspended for decades because they have reached their economic limit of production. This suspension would allow well bore pressure build up over time. However, the wells could be stimulated within a short time of about 2 weeks through the injection of microbial cultures and fermentable substrates such as molasses and cassava processing wastes. The injected nutrients and microbes will cause fermentation in the well resulting in the production of gases, which will re-pressurize the well

Microbial Repair of Formation Damage Caused by Hydraulic Fracturing
Formation damage cause by hydraulic fracturing is one of the many problems of the petroleum industry. Oil and Gas well stimulation involves fracture initiation and propagation from the well bore, which is often cased, perforated and inclined with respect to the *in-situ* principal stress direction. Consequently, fractures generally are complex, three-dimensional, non-planar features that change shapes and orientation as they propagate away from the wellbore. As propagation continues, fracture interaction and coalescence occur, often producing classical bi-wing shape.

When properly performed, fracture stimulation can create a network of highly permeable flow channels that effectively increase drainage of the production zones. However, the full potential of fracture stimulation is sometimes not realized because gelling agents used in the fracture fluids cause formation damage by decreasing permeability and blocking flow from producing zone. Well treatments were designed to repair the formation damage caused by the polymer, restore flow and enhance the effectiveness of the fracture stimulations. Wells treated with microbes and their products, which removed polymer damage and restored oil flow. Microbial repair of fractures is cheaper, less technical and sustainable method of fracture repair. A new technology developed by Micro-Bac Inc. uses a polymer product to repair costly fracture jobs that have failed or had poor results.

Microbial desulphurization and de-nitrogenization for the production of cleaner oil
One of the contaminant of crude oil is sulphur. Around the world, environmental regulations require its removal from oil products, in order to reduce sulphur pollution in the atmosphere. The conventional method for removing sulphur, nitrogen and other impurities from oil involves the use of hydrogen. In hydrodesulphurization (the process of using hydrogen to remove sulphur from oil), the oil, a catalyst, and hydrogen are processed at high pressure and temperatures. During this process, the sulphur binds with the hydrogen, and is eventually separated from the oil. This method is expensive and energy intensive. An alternative is to this is the use of bacteria which are specifically able to remove sulphur from fossil fuels. Sulphate/Sulphur reducing bacteria are capable of desulphurization of crude oil. Field trials were successfully conducted for the control of hydrogen sulphide and nitrogen by the use of the bacteria, *Acidithiobacillus denitrificans* (McInerney et al, 1996).

Bacterial Engineering for Sustainable Oil Production from Shale and Sandstones
Researchers have been able to genetically engineer *Pseudomonas* and *Bacillus* strains of hydrocarbon degrading bacteria to develop a cost-effective, environmentally sensitive oil extraction process. The goal is to modify the bacteria to simply store oil, rather than digest it. In this case, the bacteria would be used to recover oil from a largely untapped source. North America

has significant underground deposits of what is known as oil shale. This is sedimentary rock containing an organic material called kerogen, which decomposes to yield oil when heated. Recovery of oil from shales and sandstone by the USA is partly responsible for the declining oil prices at the international market.

Microbiological Prospecting of Petroleum Hydrocarbons.

Oil exploration involves series of studies to understand the subsurface conditions/events leading up to oil accumulation, which include sedimentation, movement of petroleum constituent from the source rock into reservoir rock and trapping of the oil. Modern petroleum exploration involves the detection of both source and reservoir rocks, traps and the drilling exploration wells for testing and lab analysis. Seismic and geochemical exploration for oil is quite expensive, highly technical and time consuming, hence the consideration for geomicrobiological method or prospecting for oil. In the 1940s-70s, microbiological methods were used to prospect for crude oil by detecting anomalous population of hydrocarbon utilizing microbes.

Microbial Activities Detrimental to the Petroleum Industry

Activities of microorganisms in oil wells are not always beneficial. Some microbes have been associated with certain deleterious activities. In considering the detrimental activities of microorganisms with respect to the petroleum industry, one of the first and major aspects is microbial corrosion of metals, particularly steel and iron. Corrosion of iron and steel by whatever cause is of interest and concern because of the vast amount of metal/steel equipment employed in petroleum industrial operations. Furthermore, corrosion of pipelines, casings, tubings and storage facilities has resulted in loss of products and pollution of the environment. Other microbes are involved in the deterioration of drilling muds, additives and other petroleum products. Yet, others are involved in the formation of hydrogen sulphide, which is toxic and also associated with the precipitation of metal sulphides, plugging of reservoir and contamination of fuel. Adequate knowledge of the biology of the microbes involved, their mode of action, their nutritional requirements and general physiological /environmental requirements, may be necessary in controlling the noxious activities of these organisms in the petroleum industry. Details of the detrimental activities of microbes in oil field environment is presented below;

Bio-Corrosion

The subject of corrosion is a very broad inter-disciplinary study involving many aspects of Chemistry, Metallurgy, Civil and Chemical Engineering, now microbiological corrosion is gaining prominence. Corrosion is one of the major causes of oil spillage in the Niger Delta and similar oil provinces in the world. Structures which have been attacked include well casings, pipelines (exterior and interior), pumps, fuel holding tanks, and other subsurface equipment. With the exception of cast iron, corrosion of steel and other metals by microorganisms is generally the pitting type, often leading to perforation. The sulphide stress cracking of certain steels by microbiologically produced hydrogen sulphide is well documented. Microbial aspects of corrosion could occur by any or a combination of the mechanisms listed in Table 5.

Table 5: Mechanisms of microbial corrosion of metals in the oil and gas industry

Mechanism of corrosion	Corrosion process	Relevance to the oil industry
The production of corrosive metabolic products;	Inorganic and organic acids are among the most corrosive products produced by microbes. Oxidation of pyrite by *Acidithiobacillus* sp produces sulphuric acid under aerobic conditions. Extreme acidity of pH<3 is common	Acids cause corrosion of steel and concrete platforms
	Production of H_2S by sulphate reducing bacteria, *Desulfovibrio* under anaerobic condition	corrosion of underground metallic structures, primarily iron and steel, and the internal corrosion of oil well casings and tubings, pipelines and flow lines, and storage tanks
The production of differential aeration and concentration cells;	Microbial growth and activities on metal surfaces forming tubercle cause variations in the oxygen or ion concentration on the metal surface generating electric currents, which play an important role in the corrosion of many metals. *Gallionela*, *Crenothrix* and *Leptothrix* are among the principal bacteria genera associated with pitting corrosion.	pitting corrosion of the internal surfaces of oil and water pipes
Depolarization of cathodic processes;	By utilizing the hydrogen protection on metal surfaces, sulphate-reducing bacteria depolarize the metal and cause it to corrode.	Corrosion of pipelines and flow lines
Disruption of natural and protective films	Microorganisms on the metal surface may prevent natural oxide film formation from taking place or removing these films once they have formed. Protective films of iron sulfide formed on steel could be detached by sulfate-reducing bacteria.	Various applied protective organic coatings used to coat buried pipes (polyester, waxy, or asphalt coatings) have been observed to become attacked by soil organisms.
The breakdown of corrosion inhibitors.	Microorganisms breakdown corrosion inhibitors and predispose the metal to corrosion. Nitrite and nitrate that are used to inhibit corrosion are attacked by *Pseudomonas* sp.	Corrosion of cooling tower during in petroleum refinery

Source: **Ohimain** 2010b

Corrosion in aqueous environments could be regarded as an electrochemical process. A metal in solution tends to ionize.

$$M \longrightarrow M^{z+} + Ze \qquad (1)$$

Removal of electrons by one or more mechanisms causes the reaction to go to the right, thus leading to corrosion. Two principal mechanisms for the removal of electron are:

$$2H^{+} + 2e \longrightarrow 2H \longrightarrow H_2 \qquad (2)$$

$$H_2O + \frac{1}{2} O_2 + 2e \longrightarrow 2\,(OH)^{-} \qquad (3)$$

The oxidizing agents (also referred to as depolarizing agents) involved in these mechanisms are Hydrogen ions and Oxygen, respectively. If a plentiful supply of an oxidizing agent were present, the corrosion process would be expected to go unimpeded, were it not for the formation of films of oxidation products which will slow down one or more of the above reactions (1, 2 & 3). Biocorrosion mainly involved the stimulation of these electrochemical processes.

Microbial souring of oil and sulphide toxicity

The production of hydrogen sulphide in the reservoir by sulphate reducing bacteria has been associated with the souring of oil and gas reserves. This reduces the quality, price and acceptability of the petroleum product. Hydrogen sulphide production by bacteria may occur in the reservoir and after production during storage and transportation of oil containing water.

Biogenically produced hydrogen sulphide is highly toxic when exposed to workers. Hydrogen sulphide is extremely toxic if inhaled. It easily escapes from contaminated waters and may accumulate under poorly ventilated conditions. Fortunately, another group of microbes such as *Acidithiobacillus* sp are able to remove microbes from crude oil.

Microbial Reservoir Plugging (formation damage)

Water flooding of petroleum reservoirs is a common, effective means for the secondary recovery of oil and microbiology is an integral part of this application. Floodwaters employed in secondary oil recovery vary depending upon the availability and formation water disposal requirement. Floodwaters may consist of recycled water, water supplied from other sources including river water, and or both. Saline produced water may be recycled for the dual purpose of re-pressuring the oil reservoir and circumventing the problem of disposal of the salt water without polluting the recipient environment. Uncontrolled uses of floodwater often lead to the contamination of the reservoir by microbes. The proliferation of these noxious microbes (bad bugs) results in formation damage causing reduction in the permeability of reservoir rock in various ways:

- The formation of microbial films clogs the liners and reservoir pore spaces (plugging due to biofilm formation)
- Bacteria (especially dead forms) also cause particulate plugging without necessarily forming films
- The precipitation of ferrous sulphide by SRB results in the plugging of the well
- The bacterial reduction of sulphate in formation can lead to the precipitation of calcite

- The activities of iron bacteria (*Lepthothrix, Crenothix, Sphaerotis, Gallionella* and *Thiobacillus*) in oil wells results in the precipitation of ferric hydroxide and ferric sulphate, which could cause plugging in addition to the bacteria themselves.

(Donaldson *et al.,* 1989)

Decomposition of drilling fluids and additives

Petroleum drilling, which is done using drilling rigs, is carried out to gain access to the oil in the reservoir. In drilling processes, turning the bit is accompanied by the all-important function of the drilling fluid/muds in effectively removing the cuttings produced. The viscosity and other properties of the mud is often adjusted to perform this function under variable conditions of rock composition, temperature, pressure and reservoir fluids including gas, oil and water. Oil well drilling and the application of drilling fluids in the drilling process are now highly developed technologies. The microbiology of drilling fluids is a relatively small aspect of the overall technology involved, but even today the actual economic importance of microbial activity in modifying the composition of drilling fluids has not been fully appreciated.

Different kinds of drilling mud are in used today. They could be generally classified as water-based, oil-based and pseudo- or synthetic based mud. A drilling fluid perform at least five functions, namely; (1) bringing the borehole cuttings to the surface, (2) cooling and lubricating the drill bit, (3) walling up and stabilizing the borehole, (4) counteracting oil, gas and water pressures and (5) reducing friction between the drill pipe and the borehole. Organic additives such as corn starch, natural gums, carboxymethyl cellulose (CMC) or chrome lignosulfonates are added to the drilling fluids to prevent or control water loss into porous formations which otherwise would be excessive due to the higher-pressure differential exerted by the column of drilling fluid in the borehole. Organic additives in drilling fluids have various other purposes including; dispersant (viscosity) effects and their emulsifying effects. Both natural and synthetic organic materials are often added to drilling muds. Unfortunately, several microbes isolated from oil field environment have the capability of degrading most of these additives. Microbial degradation of such organic additives is detrimental to their desired properties and the drilling programme may become risky and unnecessarily expensive. Several fungi and bacteria have been isolated which degrade CMC, starch, lignosulfonates and natural gum. In the Niger Delta, Benka-Coker and Olumagin (1995, 1996) isolated drilling mud utilizing bacteria. While these bacteria have the advantage of treating drilling wastes biologically, they could also cause the breakdown of drilling mud. The microbial degradation of drilling mud or its constituents can compromise the integrity of the mud, which is very risk and can result in well-blowout.

Microbial contamination deterioration of petroleum products

Microbial utilization of hydrocarbons is beneficial in the areas of petroleum prospecting, microbial enhanced oil recovery, and microbiological disposal of petroleum wastes. The ability of microbes to utilize petroleum, however, has its detrimental aspects, particularly in with respect to the deterioration of certain manufactured fuel and lubricants. Microbes have been reported to cause serious damage to petrol, fuel oils, kerosene, jet-aircraft oil, lubricants and other products. The attack of jet fuel tanks by the fungus *Cladosporium* has caused serious problems to the aviation industry. Also, the microbial attack on crude oil pipelines and storage facilities has been implicated in the loss of products and damage to the environment.

At least four microbial mechanisms exist for the deterioration of fuels, particularly gasoline and jet –aircraft fuels.

1. Microbial growth upon the fuel hydrocarbon components. This occurs to a great extent with the higher molecular weight hydrocarbons of kerosene (jet aircraft) fuels and results in formation of a microbial sludge.
2. Microbial growth upon organic additives in fuels with consequent microbial sludge production.
3. Production of hydrogen sulphide in associated waters by sulphate-reducing bacteria, which react with fuel components results in highly corrosive organic sulphides.
4. Microbial deterioration of organic storage tank linings resulting in both microbial sludge production and eventual serious metal corrosion of the tanks.

Oil spill clean-up and bioremediation

Oil spills are mostly caused by faulty equipment/mechanical failure, corrosion, sabotage and negligence. Of recent artisanal refineries is becoming the major cause of oil spillage in the Niger Delta. Depending on the terrain/environment involved, mechanical (booms, scooping, burning etc) and chemical (emulsifiers, surfactants, sinking agents) methods are mostly used for spill clean-up. These methods have the inherent drawback of being not able to clean up the spills to the recommended 50ppm residual oil levels (Department of Petroleum Resources, 2002). Hence, biological means offers the only possibility of reducing residual oil below the recommended limits. This could be achieved using one of the following microbial methods:

(1) Enrichment of natural organisms within the oil spill site (intrinsic bioremediation)
(2) The application of genetically modified microorganisms (Engineered bioremediation)
(3) Application of microbial products (mostly enzymes).

Hydrocarbon degrading microbes (bacteria, fungi, yeast and algae) are ubiquitous and are widely distributed in marine, fresh water and soil habitats and could therefore catalyse the degradation of oil spills. Biodegradation can be defined as the biologically catalysed reduction in the complexity of chemicals (mostly organic). In the most common bioremediation applications, microorganisms that occur naturally in contaminated soil or waters are encouraged to accelerate the degradation of organic contaminants such as petroleum hydrocarbons by the manipulation of environmental conditions, e.g., oxygen supply, nutrient concentrations and moisture content. Microbial bioremediations offer the key to the clean-up of Ogoni and other oil polluted sites in the Niger Delta. The United Nations have already set aside $1 billion, which the Federal Government is expected to match, for the clean-up of Ogoni. Microbes are the agents that can be used to clean-up the Niger Delta sustainably.

Nigerian Petroleum industry and energy challenges

Petroleum, which is located in the Niger Delta region, is the main stay of Nigeria accounting for over 90% of her earnings and 80% of her export. Hence, the Nigerian economy is essentially driven by petroleum. Nigeria is the sixth largest producer and eighth largest exporter of petroleum. The country has a proven reserve of 36.22 billion barrel of crude oil and 187 trillion scf of natural gas. The country produces about 2.6 million bbl of crude oil daily. About 22.3% is refined locally for the production of liquid transport fuels (gasoline, diesel and aviation kerosene), cooking fuels (kerosene and LPG). Petroleum accounts for 83.88% of the commercial primary energy consumption in Nigeria (Sambo, 2009). Nigeria consumes 30-33 million litres of petrol daily, which cannot be met by domestic refining. There are three petroleum refineries in Nigeria located in Port Harcourt, Warri and Kaduna. Nigeria has a total installed refining capacity of 445,000 bbl/day. These refineries operate at very low capacity utilization. For a period of twelve years (1997-2008) the refineries were operated at 0 – 34.95%, 17.84 – 52.17% and 0.00 – 63.39% for Kaduna, Port Harcourt and Warri refineries respectively. Apart from poor refining capacity, incidences of pipeline vandalism have increased during this period, which have affected crude oil supply to the refineries. Also, Nigerian crude oil reserves are located in the Niger Delta region, which is sometimes plagued by ethnic militias attacking oil infrastructures and kidnapping oil industry personnel. Due to all these constraints, the nation is unable to meet up with domestic demand of petroleum products; hence, the bulk of these products distributed in Nigeria is now being imported. Fuel scarcity is a common occurrence especially during festive periods. Power is also unstable resulting in the use of generators (Plate 6) and other innovations (Plate 7)

Due to poor logistics and instability of crude oil prices in the foreign markets, the supply of refined products to Nigeria is very unstable causing frequent scarcities. Primarily, Nigeria wants to reduce her dependence on foreign nations for her refined products by stimulating the local production of biofuels. By substituting gasoline with ethanol, biogas with LPG and diesel with biodiesel will help Nigeria reduce refine product importation, thereby saving foreign exchange, mitigate unreliable foreign supplies and conserve her oil reserves, which is diminishing. Thus, diversifying and increasing the renewable energy share of the country's energy source.

The environmental impacts of oil exploration in the Niger Delta are many including air pollution caused by gas flaring, seismic lines that have become permanent, and habitat destruction via oil spill (Plates 8). Recent activities of indigenous people in illegal oil bunkering and artisanal refining is becoming a threat to the environment (Plate 9). Also, globally, the transportation sector along is responsible for 70-90% of total carbon monoxide emissions, 25% of particulates, 40-50% of VOC, 30% of atmospheric lead, 15% of CO_2, 45% of nitrogen oxides, 5% of sulphur oxides and 70% ot total noise (Akpoghomeh 2017)

Plate 6: Power challenges in Nigeria

Plate 7: Power instability innovations

Plate 8: Petroleum industry environmental challenges

Plate 9: Environmental impacts of artisanal refining

Air Pollution and Climate Change; the microbial dimension

Two major challenges linked to the use of fossil fuels especially coal and petroleum for power generation and fuels for automobiles are air pollution and climate change. Air pollution is a major problem in many urban centres all over the world. About 40,000 premature deaths linked to toxic air occur every year in the UK. Higher incidences have been reported in China and India. Even in Port Harcourt, mystery soot has been persistent in much of 2017 until the rain washout. Hence, the current push for alternative energy and electric cars with zero emissions.

Humans are the major cause and casualty of climate change. The increased use of fossil fuel, which generate excess carbon dioxide, methane and nitrous oxide and many synthetic chemicals, which destroy the protective ozone layer, is the cause of global warming. Heat radiations from the sunlight passes through the ozone layer as short waves, but reflected back as long waves, which become trapped by GHG in the atmosphere, thus resulting in global warming and climate change. Global warming has been linked to several environmental problems including heat waves, polar ice melting and increased natural disasters such as tsunamis, flooding and hurricanes. It has also caused the expansion in the range and spread of tropical disease vectors, crop failures, desertification etc. There is a high degree of correlation between rising temperature and increasing incidence of tropical diseases. Many tropical diseases and their vectors are also expanding their ranges into temperate countries as a result of climate change.

Like humans, microbes are major causes of climate change and are affected by it. Through the global biogeochemical cycling and transformation of elements, microbes produce the major greenhouse gases (GH), which are carbon dioxide, methane and nitrous oxide (Table 6). Carbon and nitrogen cycles (Fig 5.) are particularly relevant to GHG production and utilization. During the breakdown and decomposition of organic wastes, microbes produce carbon dioxide aerobically and methane anaerobically. Microbes in the rumen of ruminants, rice swamp, and wetlands also produce carbon dioxide and methane. Some microbes such as the methanogens, synthesize methane from carbon dioxide. Microbes fixes atmospheric nitrogen into the soil as ammonia, which are oxidized in a two-step reaction, first to nitrite and to nitrate by microbes such as Nitrosomonas and Nitrobacter respectively and in the process nitrous oxides are produced. Nitrous oxides are also produced during the denitrification of nitrate to ammonia by *Psuedomonas denitrificans.*

Despite microbes being major contributors of GHGs, they also hold promise for the control of the gases. Microbes such as algae and cyanobacteria through photosynthesis converts nitrogen to carbohydrates, proteins and lipids/oils. The carbohydrates are food substances and also used for the production of biofuel. Algae produce oil, which is also used as biofuels. Microbial production and utilization of methane, can be used for the control of the gas. On a global scale, microbes fixed large volume of carbon as organic matter in the soil and dissolved organic matter (DOM) in the seas. Also, the control of microbial nitrification and denitrification processes can be used control nitrous oxide emissions. Some microbes that have been studied and promoted for their role in the control of GHGs include *Silicibacter pomeroyi, Emiliana, Nitrosomonas europaea, Prochlorococcus, Roseobacter denitrificans* and *Mycobacterium vanbaalenii*.

Table 6: Major greenhouse gases

Species	**Chemical formula**	**Lifetime (years)**	**Global Warming Potential (Time Horizon)**		
			20 years	**100 years**	**500 years**
CO_2	CO_2	variable §	1	1	1
Methane	CH_4	12±3	56	21	6.5
Nitrous oxide	N_2O	120	280	310	170

Source: United Nations Framework Convention on Climate Change

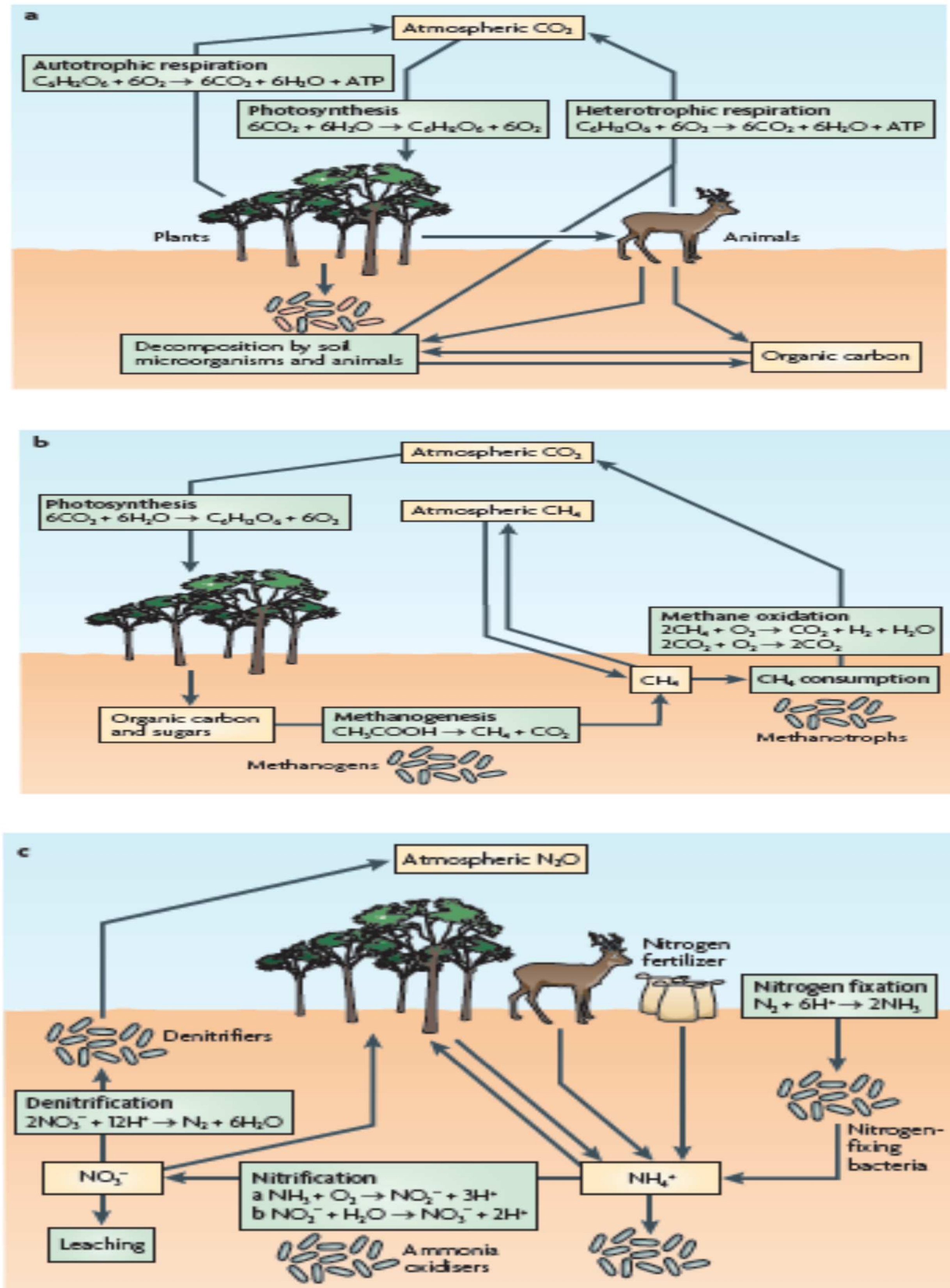

Fig 5. The role of microbes in the formation and utilization of GHG (Source: Signh et al 2010)

ALTERNATIVE ENERGY/NIGERIA BEYOND OIL/BIOENERGY & BIOFUEL

The case for bioenergy

From independence in 1960, agriculture used to be the mainstay of Nigeria, until the early 1970s when it was neglected in preference for the petroleum sector. Though, the petroleum industry has brought much wealth to the Nigeria nation, but it has failed to provide socio-economic benefits to the people. The three-immediate previous government of Nigeria then planned to use the petroleum industry as a spring board to boost the agricultural sector. The plan was to transform Nigeria automotive fuel sector from pure fossil fuel to biofuel/fossil fuel mix by linking the petroleum sector with the agricultural sector, which could result in the enhancement of the agricultural sector. By incorporating biofuel into Nigeria's liquid fuel mix could create a demand for feedstock, which could boost the agricultural sector by creating opportunities for business and employment. Unlike the petroleum resources, which is physically located in the Niger Delta region, agricultural resources are widespread, with every part of the country being able to produce different feedstock depending on their soil and agro-climate. With biofuel, political disturbance in a section of the country will not threaten the fuel situation in the entire country as it is presently. Because of the huge demand for feedstocks, opportunities for the biofuel industry will not be narrow as in the oil industry but will also be widespread. Unlike the oil industry that employ only few and highly skilled people mostly expatriates in the narrow field of petroleum engineering, the biofuel industry is capable of employing people from diverse backgrounds including the educated, non-educated, semi-educated, technical staff etc. including those in the rural and urban sector. Several authors have documented the rippling and multiplier effects of biofuels to include employment, poverty reduction, wealth creation and rural development. Besides, petroleum is a non-renewable energy resource, which is depleting and therefore needed to be conserved. The use of biofuel will also increase the renewable energy share of Nigeria's energy mix. In addition, biofuel will reduce the reliance of Nigeria on foreign refined products. Thus, enhancing Nigeria's Energy security. Biofuels offer several technical and environmental benefits over conventional fossil fuels, which make them attractive for the liquid transportation fuel. The benefits include reduction in greenhouse gas emissions and diversification of fuel sector. Besides biofuels are biodegradable, renewable and sustainable (Fig 6). Biomass fuels generate more employment than any other fuel sources of equivalent energy content. The sugarcane industry in Brazil generates about 1.3 million direct jobs, of which 54% are directly related to ethanol production. Bioenergy could increase income for farmers, possibilities to expand agriculture and create more employment. I have authored/co-authored 65 papers in the energy sector, of which 32 are on biofuels alone.

Biofuel Conversion Technology

There are several methods /pathways/technologies for the conversion of biomass to energy namely, biochemical, thermo-chemical and biophysical (Fig. 7). Prior to the conversion of biomass waste to biofuel, it will require pre-treatment.

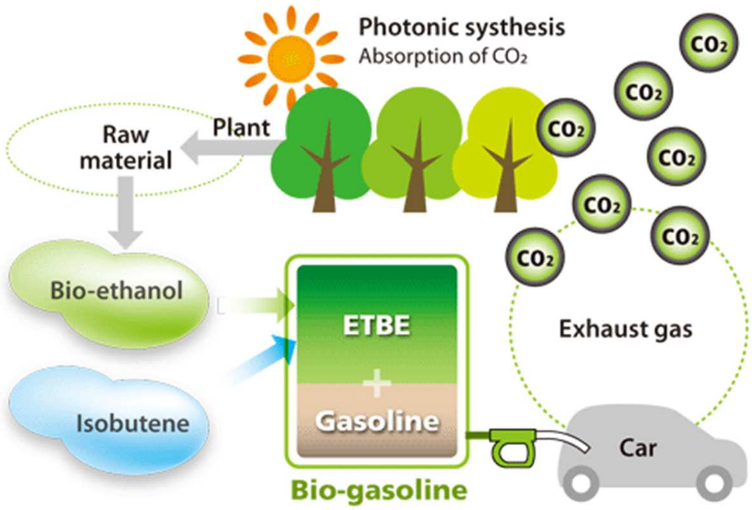

Figure 6: Environmental sustainability of biomass fuel (Source: Toyo Engineering Corporation)

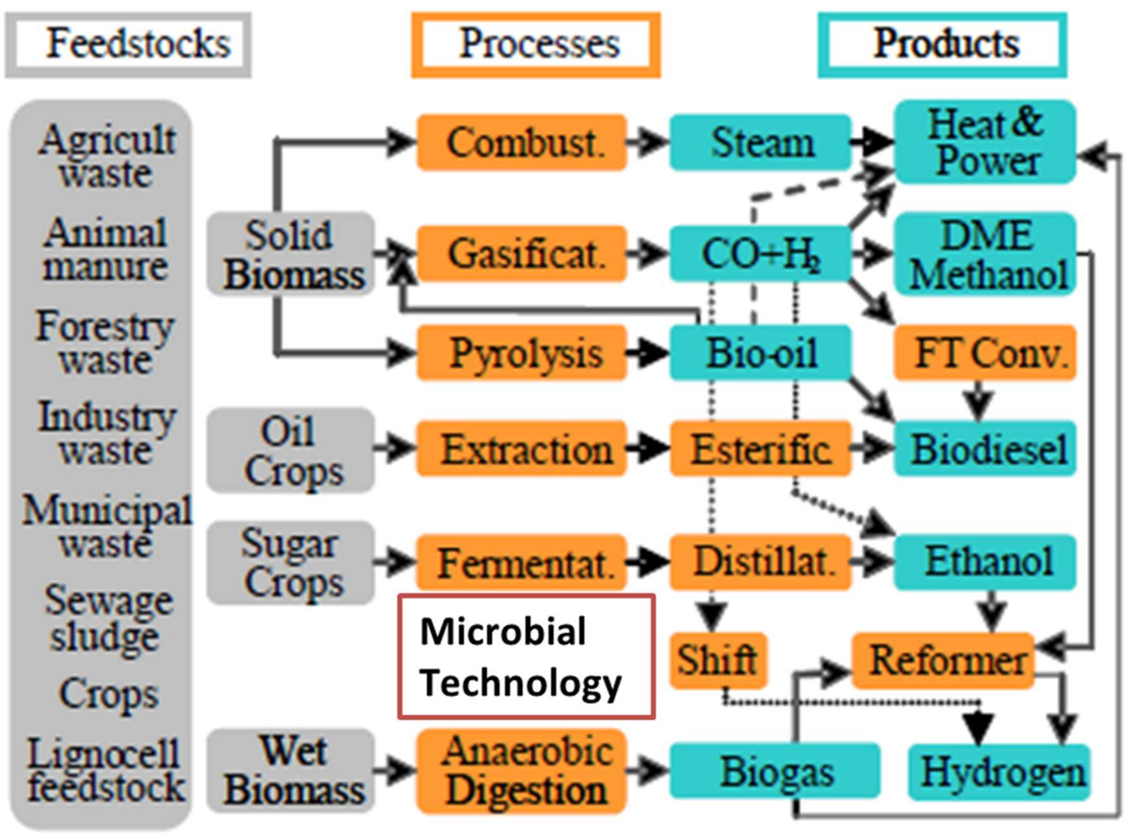

Fig. 7. processes for the conversion of waste biomass to various energy carriers
Source: Modified from **OECD/IEA 2007**

Biomass pre-treatment
Methods for the pre-treatment of the biomass prior to conversion to useful energy forms can be categorized into three, physical, chemical and biological. Physical pre-treatment mostly involves the separation and sorting of the biomass into fairly homogenous sub-groups, while chemical pre-treatment involves the use of reagents such as acids and alkaline for the hydrolysis of complex carbohydrates to simple sugars, prior to fermentation. Sulphuric acid at high or low concentration under high temperature or low temperature, high pressure or low pressure is commonly used. Biological pre-treatment involves enzymatic breakdown of complex carbohydrate to simple sugars by the use of commercially available enzymes prior to fermentation. Most enzymes used in the industry are of microbial origin. Typically, physical pre-treatment precedes chemical or enzymes pre-treatment before conversion to useful energy carriers.

Biochemical biomass conversion
Proven and commercially available biomass biochemical conversion technologies include anaerobic digestion (AD), fermentation and esterification.

Fermentation (microbial)
Ethanol, which is the most widely used biofuel, is produced through microbial fermentation. Ethanol can be produced from biomass containing sugar, starch or cellulose. Ethanol is produced directly from sugar containing biomass such as molasses through fermentation. Fermentation is an anaerobic biological process in which sugars are converted by the action of microorganisms, usually yeast, *Saccharomyces cerevisiae* or bacteria, *Zymomonas mobilis*. However, starch containing feedstock such as cassava, potato, yam, maize and cellulose containing wastes such as wood sawdust, rice husk, and wheat bran etc will require pre- treatment using enzymes, acid or alkaline for the hydrolysis of starch and cellulose to simple sugars prior to fermentation. These technologies are conventional and are available in Nigeria.

Anaerobic digestion (microbial)
Anaerobic digestion is the microbial decomposition of biomass in the absence of oxygen to produce biogas consisting of methane, carbon dioxide and traces of other gasses including NOx, SO_2. Anaerobic digestion is also known as bio-gasification. Biogas is most commonly produced by using animal dung (Plate 10) mixed and /or with other wastes such as crop residue, which are mixed with water in airtight digesters. The biogas produced after 4 – 8 weeks of anaerobic digestion can be used directly for cooking and space heating, used as fuel in internal combustion engines to vehicles and gas turbine to generate electricity.

Esterification and Trans-esterification
Biodiesel is a methyl or ethyl esters of fatty acids. Biodiesel is produced from vegetable oil such as oil palm, Soya beans, groundnut, coconut oil etc. But because of competition with food sources, emphasis is now placed on the production of biodiesel from non-food feedstock include waste or used vegetable oil or other non- edible oils such as raffia palm oil, rubber seed oil. Trans esterification reaction can be catalysed by alkalis, acids or enzymes. Biodiesel is commonly produced from Trans-esterification of triglyceride to methyl esters with methanol using sodium or potassium hydroxide dissolved in methanol as catalyst. Biodiesel is commonly used as fuels for vehicles, heating and cooking.

Plate 10: Cow dung; source of microbes for rechargeable battery and biomass for biogas Whether to graze or not?

Thermo Chemical Conversion

Thermo-chemical conversion is also referred to as biomass to liquid (BTL). Two commonly cited technologies of biomass thermo-chemical conversion processes are pyrolysis and gasification, though few authors classified direct combustion as thermo-chemical

Pyrolysis of biomass

Pyrolysis is the thermo-chemical processes that convert biomass into liquid (bio-oil or bio-crude) charcoal (biosolid) and non-condensable gasses, acetic acid, acetone and methanol by heating the biomass to about 750k in the absence of air (Demirbas, 2001). The liquid and gas products of pyrolysis can be used in engine and turbine for power generation. Pyrolysis processes can be adjusted to favour charcoal, oil, gas and methanol production with a 95.5% fuel – to – feed efficiency (Balat et al., 2009; Demirbas et al., 2009). There are different types of pyrolysis including conventional, fast and flash pyrolysis.

Biomass gasification

Gasification is a form of pyrolysis, which are performed at high temperatures with little amount of air. The resulting gas, which is known as syngas or producer gas, consists mainly of CO and H_2 with traces of CH_4, N_2, and CO_2 (Demirbas 2001). Syngas have very many applications; it can be burned to produce process heat and steam or used in gas turbines to produce electricity. High efficiencies of above 50% biomass electricity conversion is achievable using integrated gasification combined cycle (IGCC) gas turbine systems, where waste gases from the gas turbine are recovered to produce steam for use in a steam turbine (Dermirbas, 2001). Electricity can be generated from syngas. Using the Fischer-Tropsch synthesis (FTS) a range of chemicals can be produced from biomass including ethanol, diesel, gasoline, DME (dimethyl ether), methanol, and ethanol (Fig. 8).

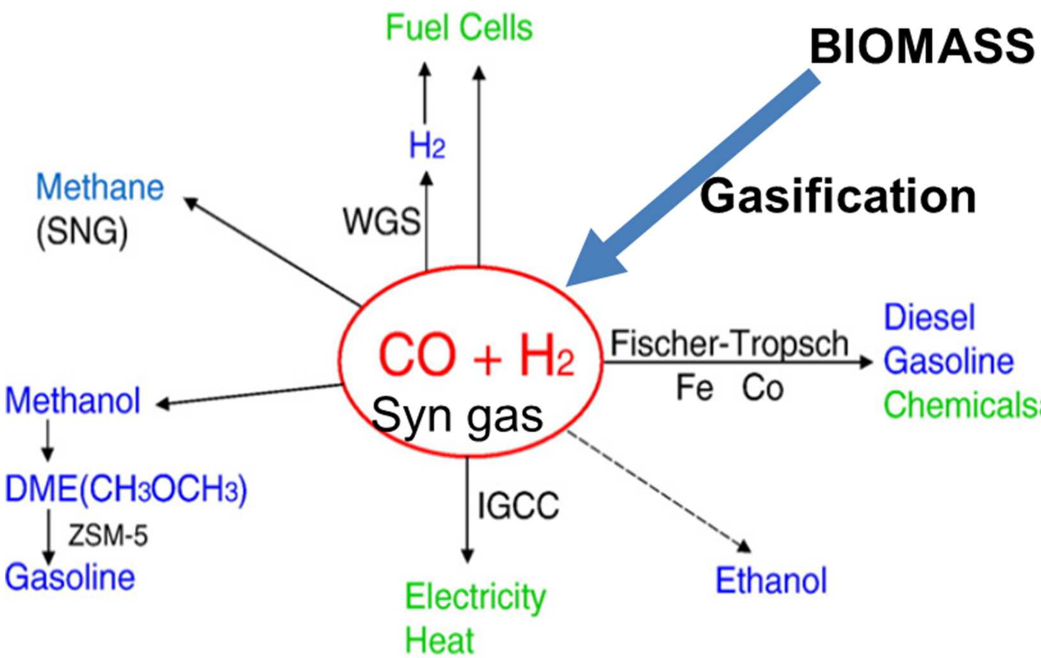

Fig 8: Commercially available fuel products from syngas produced through biomass gasification (modified from Zhang, 2010). WGS= water gas shift; IGCC= integrated gasification combined cycle

Direct biomass combustion

Direct combustion is the main process of biomass conversion in Africa. Biomass waste such as wood saw dust, crop residues, food processing waste etc are often combusted directly to produce heat and/ or steam for cooking, space heating and industrial processes, electricity generation etc.

Physical conversion/densification

Agro processing and wood waste such as saw dust, rice husk, maize cobs etc are compressed physically to increase their density to form briquettes. These briquettes burn with smokeless blue flames and are therefore excellent cooking fuel.

Biomass feed stock

There are several available feedstocks that have been converted to various biofuel carrier including sugar crops, starchy crops and waste biomass (Table 7). nominal volume of ethanol production from common feedstock under Nigerian conditions is presented in Table 8. Technologies for the conversion of waste biomass to energy is shown in Fig 8. most of the commonly used biofuel feedstock are available in Nigeria. Some of the feedstocks, which are food crops such as cassava, sugarcane, maize and sorghum could conflict with human and animal nutrition. Hence, attention is now focused on second-generation biofuels produced from wastes especially agro-processing wastes. Hence, such fuel is providing the twin benefits of converting wastes to useful substance i.e. wastes to wealth (WTW). There is also other second-generation biofuel produced from under-utilized or purposely grown energy crops such as Jatropha, raffia palm. Third-generation biofuels are now being produced from microalgae in bioreactors. Microalgae yield the largest volume of oil per hectare than any oil crop.

Table 7: Feedstock for the production of biofuel

Feedstock type	feedstock	biofuel
Sugar crop	Sugarcane, sugar beets, sweet sorghum	Ethanol
Starch crop	Cassava, maize, sorghum	Ethanol
Waste biomass (cellulose)	Corn cobs, plantain peelings, cassava peelings, rice husks, oil palm processing solid wastes,	Ethanol, biodiesel, biogas, electricity, briquettes, bio-oil
Waste biomass (animal wastes)	Cow dung, goat dung, sheep dung, camel dung, sewage	Biogas
Other wastes	municipal solid wastes, saw dusts, plastic wastes	Ethanol, biodiesel, biogas, electricity, briquettes, bio-oil

Table 8: Nominal ratios of feedstock conversion to ethanol (global estimates)

Feedstock	Yield (Tonnes/ha/yr)	Conversion efficiency (litres/tonne)	Ethanol yield (litres/ha/yr)
Cassava	12	180	2160
Sugarcane	65	70	4550
Sweet sorghum*	35	80	2800

* Under Nigerian climatic conditions, sweet sorghum could be cultivated and harvested twice annually, which could bring the total yield to70 tonnes/ha/year corresponding to ethanol yield 5600 litres/ha/yr. Source: **Ohimain** (2010c)

Several types of wastes are used for the production of biofuel including biomass, waste engine oil, municipal solid wastes and even plastic wastes. In order to achieve high biomass to energy (BTE) conversion efficiencies, it is important for the biomass waste streams to be fairly homogenous. Waste streams from industrial process such as breweries, wineries, distilleries, flour mills etc generate fairly homogenous waste streams such as barely wastes, bagasse, molasses, wheat bran, cassava peeling, saw dust etc. These wastes might not require physical pre-treatment but may require chemical or biological pre-treatment depending on the route of biomass conversion. are used as well.

Through enzymatic hydrolysis followed by fermentation, Ballesteros et al. (2010) produced 160L of ethanol per ton of MSW, Obileku et al (2010) produced cellulosic ethanol from wood saw dust after sulphuric acid and caustic soda pre- treatment (hydrolysis). Shi et al (2009) estimated the global production of ethanol from MSW and found that up to 82.9 billion litres of waste paper derived cellulose ethanol can be produced worldwide replacing 5.3b litres of gasoline consumption, with accompanying GHG emission reduction of between 29.2 % and 86.1%. Demirbas (2004) produced gasoline-range hydrocarbons from municipal plastic wastes.

Central role of microbes in biofuels

Microbes play a central role in the bioenergy sector in several ways; as feedstock, bio-catalyst/ enzymes, bio-batteries, microbial fuel cells (MFC), bio-solar panels/antenna and direct synthesis of biofuels. Also, biogenic theory suggests that activities of microbes in sedimentary basins led to the formation of petroleum in source rocks from where they migrate to reservoir rocks. Microbes can convert light, water and CO_2 to variety of fuels, pharmaceuticals and chemicals (Fig. 9).

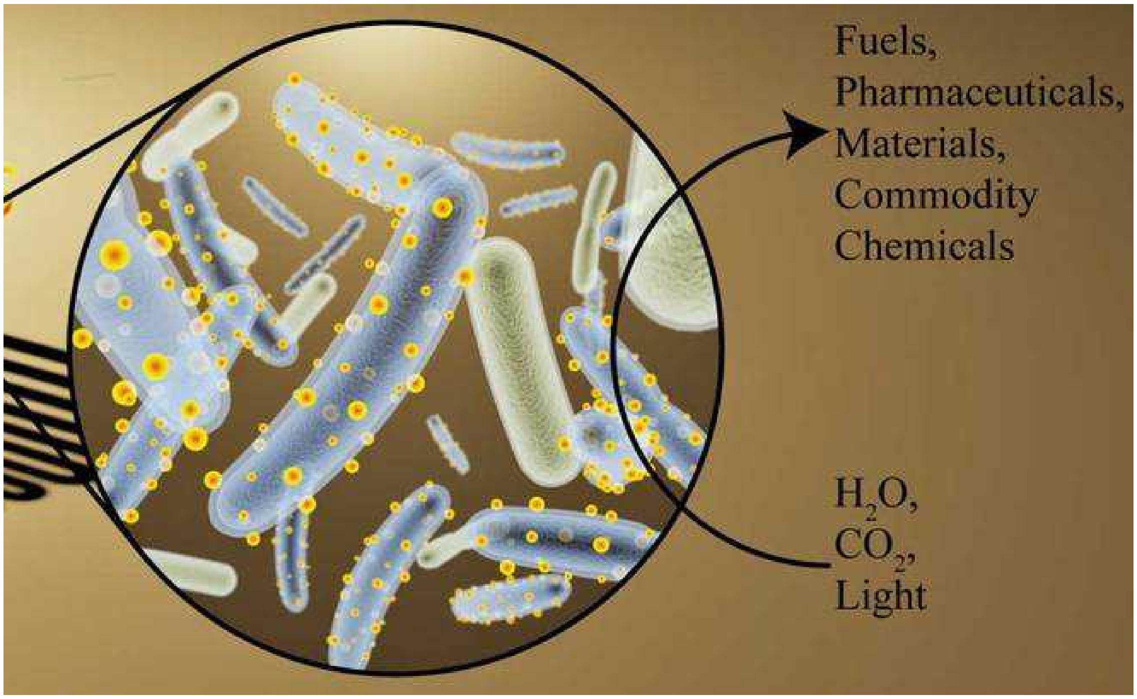

Figure 9: Microbial utilization of light and CO2 for the production of fuel, pharmaceuticals, materials and commodity chemicals (source: Kelsey K. Sakimoto)

Microbes as feedstocks for biofuels

Two types of biofuels that have been commercialized are bioethanol and biodiesel. While bioethanol is mostly produced from sugar crops (sugar cane and sugar beets) and starch crops (mostly cassava), biodiesel is produced from oil crops particularly oil palm, rapeseed and soya bean. The biofuel produced from these food crops could conflict with food uses. Hence, attention have been focused on alternative feedstocks. Microbes such as algae and Cyanobacteria have emerged as a viable alternative for the production of carbohydrates and oils. Like plants, green algae produce mostly carbohydrates, while others produce oil. Microalgae are sunlight-driven cell factories that convert carbon dioxide to potential biofuels, foods, feeds and green chemicals (Plate 11). Algae produce by far greater yield of oil than oil crops (Table 9). Hence, the most productive oil crop i.e. oil palm, will occupy x20 of land mass to produce the same volume of oil as microalgae. Algae are quite prolific in the production of oil (Table 10). Apart from biodiesel and ethanol (Fig. 10), other biofuels that can be produced from algae include biogas/methane and biohydrogen. For example, ExxonMobil in partnership with a biotech company called Synthetic Genomics is developing the microalgae *Nannochloropsis gaditana* for bioenergy and other industrial applications. Here in NDU, my MSc student, Saturday Ukpabi extracted and characterized oil from three species of algae (Table 11).

Table 9: Oil yield of oil crop compared to microalgae

	L/Ha/year	**gallons/acre/year**	
Crop	**Oil yield (Chisti 2007)**	**Oil yield (oilgae 2017)**	**Oil yield (Extension, 2017)**
Corn	172	18	18
Soybeans	446	48	48
Safflower		83	
Sunflower		102	
Canola	1190	127	127
Jatropha	1892		202
Coconut	2689		287
Oil Palm	5950	635	636
Microalgae	58,700-136,900	5000-15000	6283-14641

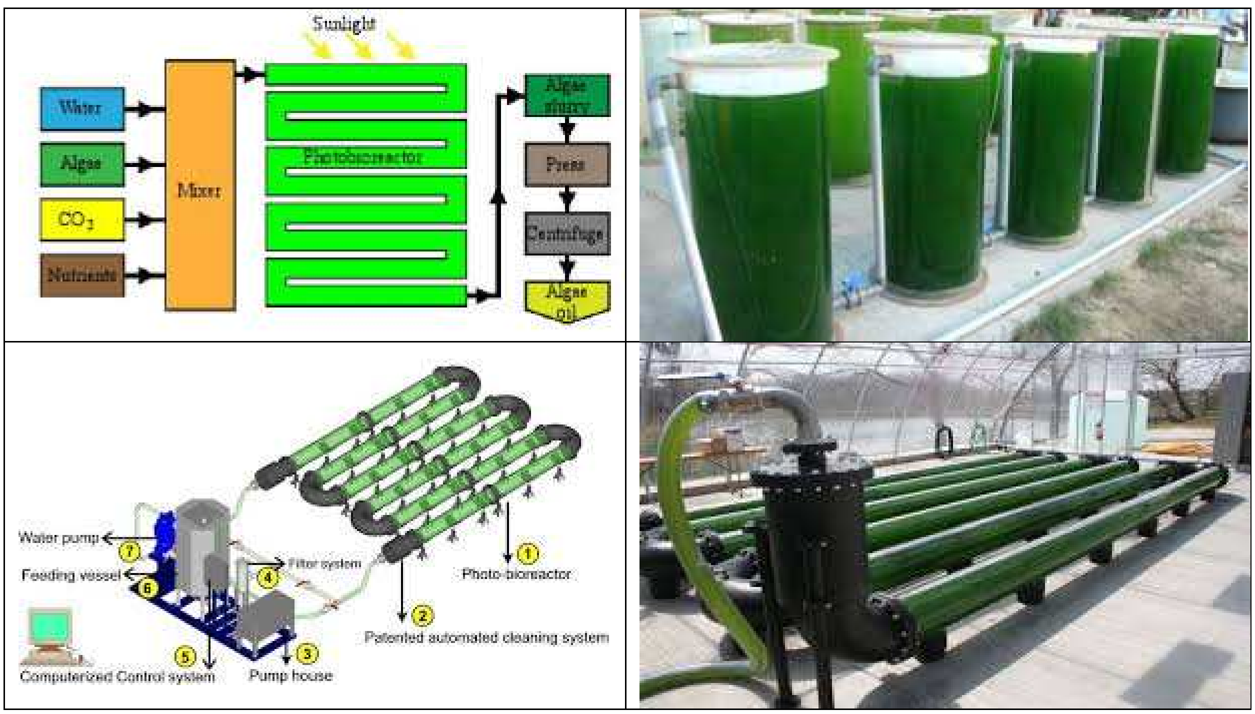

Plate 11: Algal oil production systems

Table 10: Oil content of selected microalgal species:

	Oil content (% dry weight)		
Microalgal species	Oilgae	**Chisti 2007**	**Araujo 2011**
Ankistrodesmus	28-40		
Botryococcus braunii	29-75	25-75	
Chaetoceros gradlis			15.50-60.28
Chaetoceros mulleri			11.67-25,25
Chlorella sp.	29	28-32	
Chlorella vulgaris			16.60 – 52.49
Chlorella protothecoides	15-55		
Cyclotella	42		
Crypthecodinium cohnii		20	
Cylindrotheca sp.		16-37	
Dunaliella tertiolecta	36-42		
Dunaliella primolecta		23	
Dunaliella sp.			12.00 – 30.12
Hantzschia	66		
Isochrysis sp.		25-33	6.50 - 21.25
Monallanthus salina		>20	
Nannochloris	31 (6-63)	20-35	
Nannochloropsis	46 (31-68)	31-68	
Nannochloropsis oculata			22.75 – 23.00
Neochloris oleobundans		35-54	
Nitzschia sp	28-50	45-47	
Phaeodactylum tricornutum	31	20-30	
Scenedesmus	45		
Schizochytrium sp		50-77	
Stichococcus	33 (9-59)		
Tetraselmis suecica	15-32	15-23	
Tetraselmis chui			17.25 – 23.50
Tetraselmis sp			1.00 – 8.00
Tetraselmis tetrathele			29.18 – 30.25
Thalassiosira pseudonana	(21-31)		
Thalassiosira welssflogii			6.25 - 13.21
Crpthecodinium cohnii	20		
Neochloris oleoabundans	35-54		
Schiochytrium	50-77		

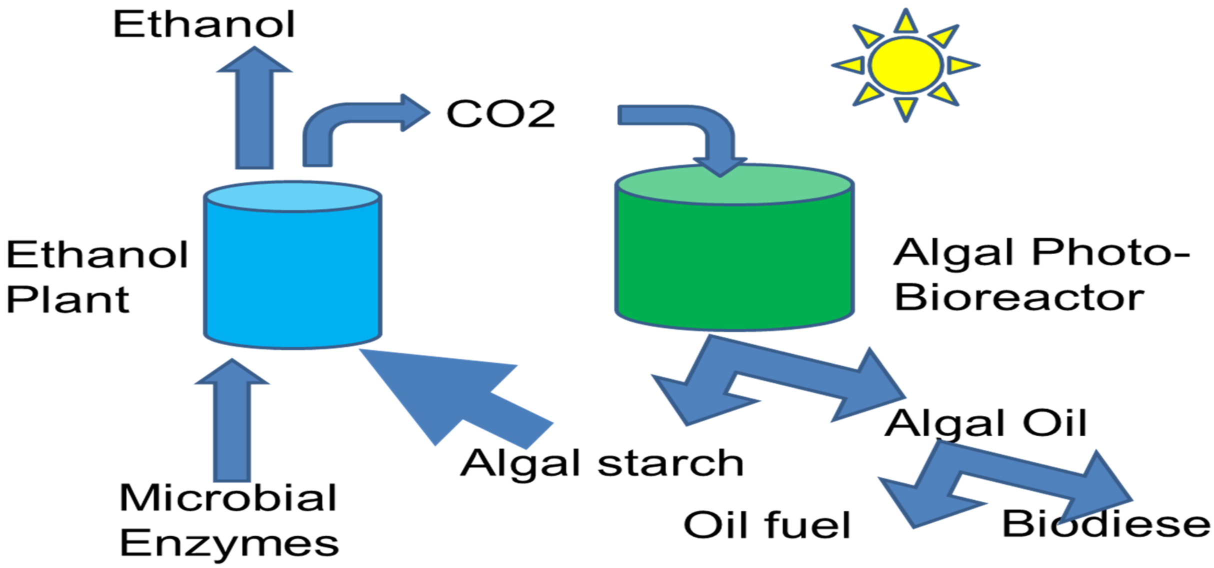

Figure 10: Algal biofuels and bioenergy

Table 11. Physicochemical properties of algal oil

Oil parameters	*Chlorella* sp.	*Desmid* sp.	*Chara* sp.
Density	1.105gm/ml	1.102g/ml	1.016
Viscosity	6.68mm/s	6.66	6.00
Acid value	1.8578	1.8632	1.8126
Saponification	176.68	176.61	176.00
Free fatty acid	0.88gm	0.89	0.79
Peroxide	3.06meq/kg	3.09	2.90
Iodine value	0.21mg	0.25	0.23
Moisture content	46.20 ±0.20	45.30±0.26	44.25±0.20
Impurity value	0.807%	0.707	0.806

Microbes as source of enzymes/catalyst for biofuel conversion

Plate 12 present an art work showing ethanol production in Bayelsa State. Different carbohydrate sources are used for the production of ethanol in different countries. Europe uses sugar beets, USA uses corn, Brazil uses sugar cane and Thailand uses cassava. Apart from sugar beets, all the other feedstocks are available in Nigeria. Production of ethanol from carbohydrates require the use of enzymes, most of which are of microbial origin. Enzymes such as amylases and cellulases, which are produced by many microbes, first convert starch and cellulose to respectively glucose, which are further converted to ethanol by yeast (Saccharomyces cerevisiae) or bacteria (Zymomonas mobilis). Other enzymes such as lipases are used for biodiesel production. The conversion of wood to cellulosic ethanol require the use of a cocktail of several enzymes. More efficient catalysts are being developed using nanotechnology.


Plate 12: Indigenous ethanol production art works in NDU

Hybrid microbe-solar systems
Plants are exceptional sunlight harvesters. But they store only about 1% of the energy they absorb, producing sugars and other organic molecules they use cell building. Like plants, purple bacteria is equipped with light harvesting complex/antenna complex called bacteriochlorophylls and carotenoids. Using genetic engineering, scientists have boosted light absorption efficiencies with light-absorbing microbes. Recently, scientists have developed a hybrid microbial- solar panels, that uses a combination of catalysts and microbes to convert 10% of the captured solar energy into liquid fuels and other commodity chemicals.

In a recent study, a bio-solar panel was designed using Cyanobacteria in a 3x 3 pattern (Wei et al 2016). Cyanobacteria are ubiquitous in terrestrial and aquatic habitats worldwide. The panel continuously generated electricity from photosynthesis and respiratory activities of the bacteria in 12-hour day-night cycles over 60 total hours.

Moorella thermoacetica a bacterium that resides at the bottom of very still swamps, naturally produces acetic acid from carbon dioxide, without photosynthesis. But the scientists made a hybrid organism called *M. thermoacetica-CdS* by feeding the bacteria with cadmium and cysteine, an amino acid, resulting in the production of cadmium sulphide nanoparticles. Those nanoparticles that are essentially semiconductor nanocrystals which covered the bacteria surface, thereby becoming solar panels. These nanocrystals are much more efficient than chlorophyll and can be grown at a fraction of the cost of manufactured solar panels. *M. thermoacetica* typically reduce CO_2 to acetic acid without photosynthesis. But the new bacteria-nanoparticle hybrid called *M. thermoacetica*-CdS, can use the light-absorbing Cd-S particles to convert CO_2 and water to acetic acid at efficiencies above 80%. Acetic acid is a useful reagent for the production fuels, drugs, plastics, vinegar and other chemicals.

Renewable energy storage

One of the major challenges in using electrical energy is the efficiency in its storage. As solar and wind power grow in use, researchers have begun looking for ways to store the excess energy such systems produce. Current methods, such as chemical batteries, hydraulic pumping, and water splitting, suffer from low energy density or incompatibility with current transportation infrastructure. Batteries are currently very expensive. But energy-rich chemicals, which can be chemical tanks and transported through pipeline like petroleum is a more viable option especially in the short-term. Li et al (2012) reported a method to store electrical energy as chemical energy in higher alcohols, which can be used as liquid transportation fuels. They genetically engineered a lithoautotrophic microorganism, *Ralstonia eutropha* H16, to produce iso-butanol and 3-methyl-1-butanol in an electro-bioreactor using CO_2 as the sole carbon source and electricity as the sole energy input (Plate 13). The process integrates electrochemical formate production and biological CO_2 fixation and higher alcohol synthesis, opening the possibility of electricity-driven bioconversion of CO_2 to commercial chemicals called electrofuels. In place of electricity input, Torella et al (2015) efficiently produced iso-propanol from CO_2 using a hybrid microbial-solar water splitting catalyst system. Also, note that wind energy and PV water splitting are methods used for hydrogen production for fuel use. Note that hydrogen vehicles emit only steam and no pollutants.

Solar or wind energy conversion to fuels could offer electricity storage that would have the energy density comparable to gasoline. The method provides a way to store electrical energy in a form that can be readily used as a transportation fuel. The hybrid bioelectric system would also offer a more efficient way of turning sunlight to fuel rather than growing plants and converting them into sugars or oils, which are into converted to biofuel. This technology have been used for the direct production of solar diesel, solar petrol, solar ethanol and other solar fuels by microbes.

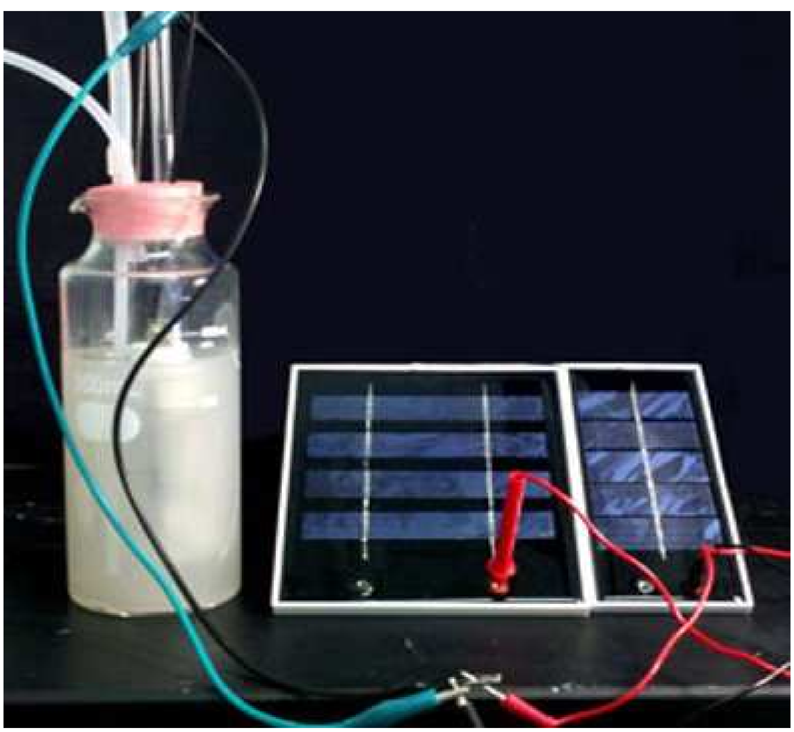

Plate 13: Hybrid microbial-solar system (source: Han Li et al 2012)

Microbial fuel cells (MFC)

Microbial fuel cell (MFC) is a device that converts chemical energy to electrical energy by the activities of microorganisms. These electrochemical cells are constructed using either a bioanode usually a microbe and/or a biocathode, which are sometimes separated by a membrane. Oxidation takes place in the anode during the degradation of organic compounds or transformation of inorganic substances resulting in the generation of electrons, which are transported (with or without mediator) to the cathode where reduction takes place, resulting in electricity generation. MFC have been applied commercially in the treatment of different types of wastes.

The U.S. Naval Research Laboratory (NRL) patented a self-assembling, self-repairing, and self-contained hybrid microbial photo-electrochemical solar cell driven entirely by sunlight and microorganisms. A solar microbial fuel cell (SMFC) is a non-semiconductor-based system, which employs microorganisms to generate electric power by photosynthetically replenishing reactants of a sealed microbial fuel cell using sunlight. The Self-sustaining, solar-driven bioelectricity generation microbial fuel cell was designed using co-culture of photosynthetic bacteria (that will use CO_2 to generate glucose releasing oxygen) and heterotrophic (that will degrade glucose releasing energy and CO_2). This interdependency results in long-term electricity generation from sunlight without replenishment of the microbial fuel cell reactants (glucose, oxygen, CO_2). SMFC combines energy storage with power delivery. For instance, when there is abundant sunlight, photosynthesis will result in generation of glucose and oxygen, some of which can be used immediately to generate power, and the remainder accumulated to be used later when there is no Sun. This is ideally done without the need for capacitor or battery.

Another self-sustaining configuration is the benthic microbial fuel cell (BMFC), which generates electrical power by oxidizing organic matter (fuel) residing in sediment pore water with oxygen (oxidant) in overlying water and consists of an anode imbedded in the marine sediment connected by an external electrical circuit to a cathode positioned in overlying water (Fig. 11). MFC technology have been used to for the generation of electricity from the treatment of urine produced during a festival at the University of West England.

Mr. Vice Chancellor sir, one of my PG student, Richard Agedah, worked and generated biogas and electricity from MFC using cow dung.

Microbial rechargeable batteries

Bio-electrochemical systems (Fig. 12) hold potential for both conversion of electricity into chemicals through microbial electrosynthesis (MES) and the provision of electrical power by oxidation of organics using microbial fuel cells (MFCs). By coupling both processes into a single unit, results in the production of the first microbial rechargeable batteries. The microbial rechargeable battery is a metabolic unit comprising of a charging cell, which perform microbial electrosynthesis (anabolism) and discharging cell, which is basically a microbial fuel cell that generates electrons from the catabolism of organic compounds. Hence, the battery consists of two microbial processes i.e. microbial electrosynthesis and microbial fuel cells combined into a single device to create the first microbial rechargeable battery.

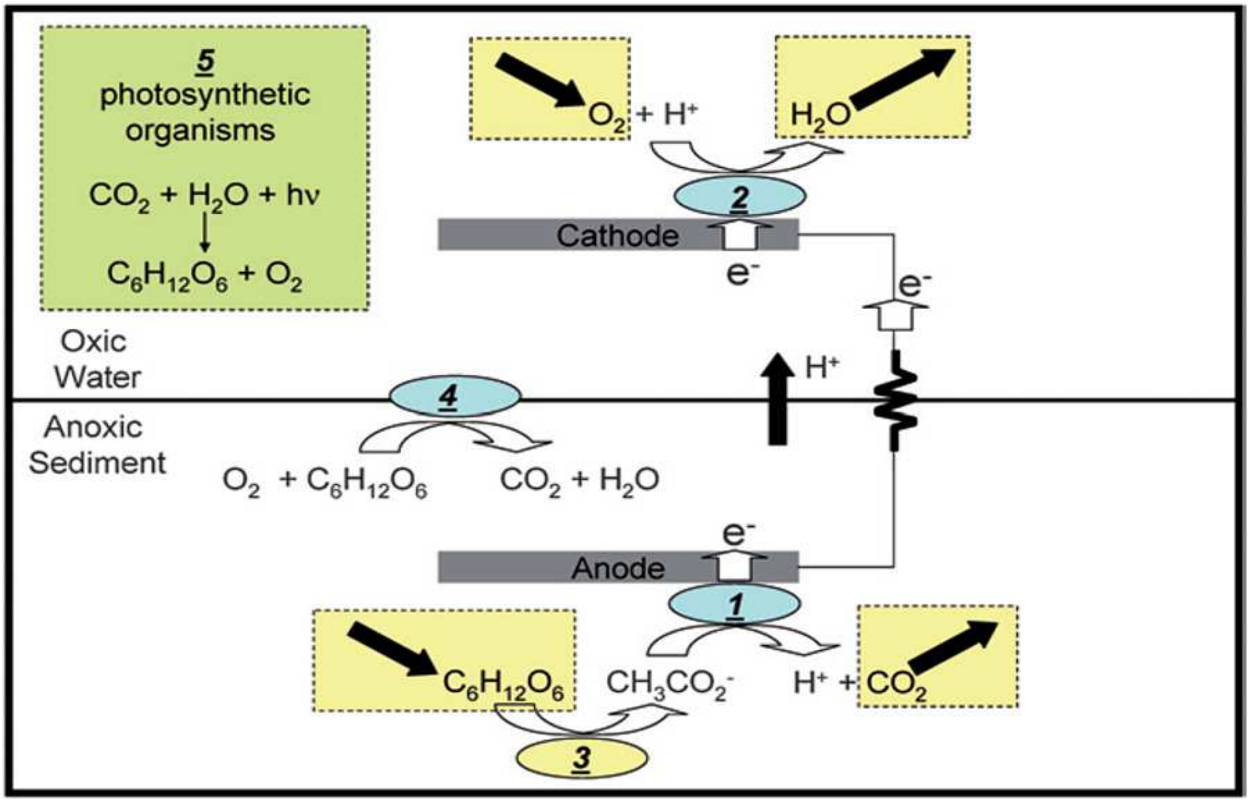

Fig 11. Schematic depiction of simplified mechanism of power generation by the benthic microbial fuel cell (BMFC): (1) biofilm catalyzed anode reaction; (2) biofilm catalyzed cathode reaction; (3) fermentative reaction, (4) the microbial oxygen barrier and (5) photosynthesis for the microbial photo-electrochemical solar microbial fuel cell (SMFC). (Source U.S. Naval Research Laboratory)

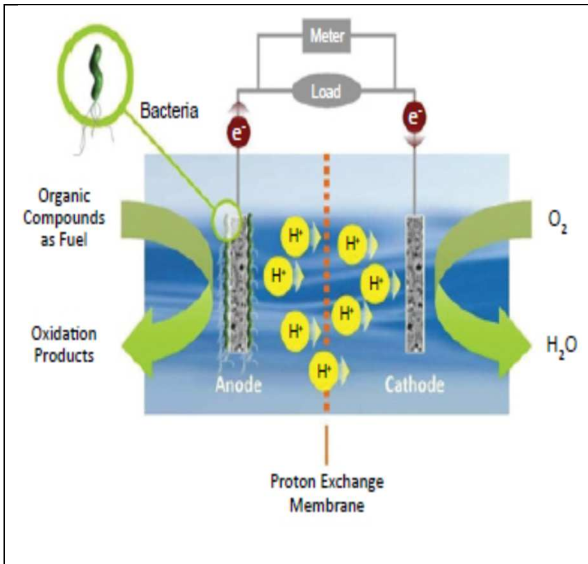	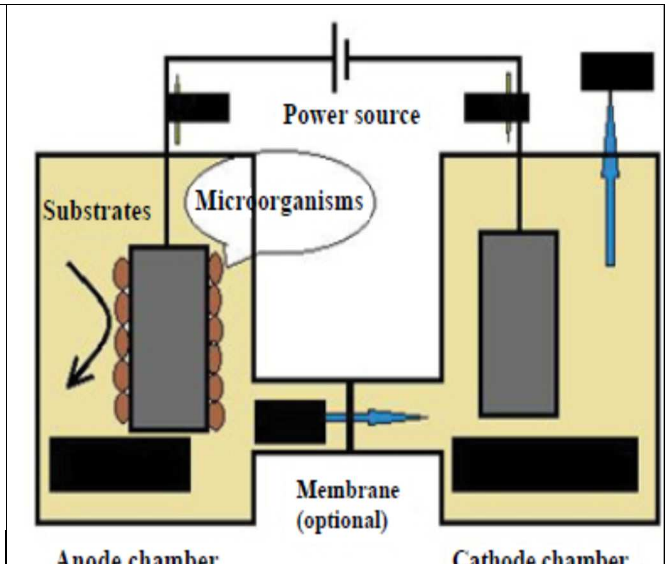
Microbial fuel cell	Microbial electrosynthesis

Fig 12: Microbial electro-chemical systems (source: Parkash 2016)

The battery's bio-electrolyte, which contained microorganisms (isolated from cow dung), that can naturally convert hydrogen and carbon dioxide into small organic compounds like acetate. On the other hand, microbial fuel cell produces electricity by breaking down small organic compounds. By coupling the anabolic production and catabolic degradation of acetate by microbes, a microbial rechargeable battery was produced (Fig. 13). To charge the battery, microorganisms from the cow dung in the cathode are supplied with electrons from the degradation of the organic compounds at the anode, which they combine with carbon dioxide from the environment to produce acetate. To discharge the battery, different microorganisms in the anode consume the acetate to produce electrons. The researchers demonstrated that the charge can be stored for several hours, and the battery can be repeatedly charged and discharged over the course of two weeks (Molenaar, et al. 2016). In the future, the microbial rechargeable battery could provide a way to store energy captured by renewable energy sources, such as solar and wind. These energy sources would provide the electrons that are originally supplied to the microorganisms in the cathode. Overall, the battery could offer an inexpensive, clean, renewable alternative to existing batteries.

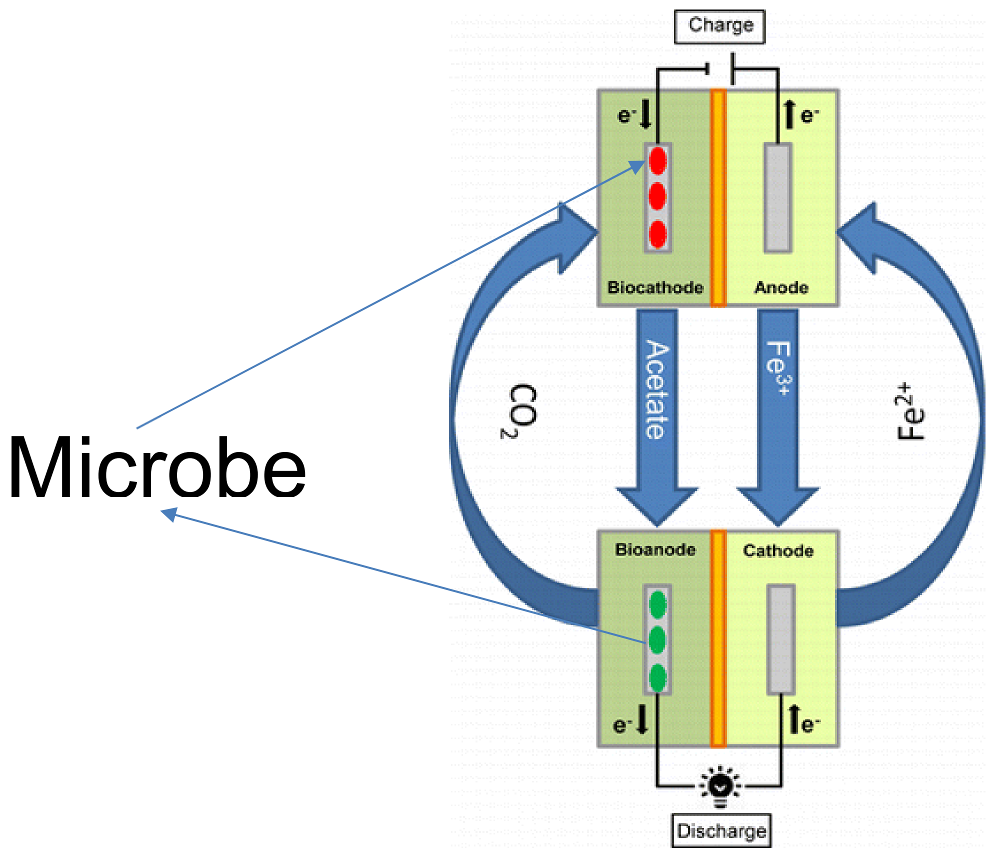

Figure 13: Microbial batteries (modified from Molenaar, et al. 2016)

Myco-diesel

A newfound fungus, *Gliocladium roseum*, living in rainforest of Patagonia in South America. The fungus which grows inside the Ulmo tree, synthesizes diesel compounds from cellulose. This is the only organism that has ever been shown to produce diesel directly. Plant cell walls are made of cellulose, lignin and hemicellulose, which end up as wastes. Over 400 million tons of plant waste are generated annually, mostly from stalks, sawdust, woodchip, pods, peelings, shells etc. These wastes, which are currently being considered for the production of cellulosic ethanol via microbial degradation, saccharification and fermentation processes, can now be used to produce diesel directly using the fungus *G. roseum*. Again, converting wastes to wealth.

Biofuel Policy and Incentives

The transition to biofuel-based economy requires policy reforms. Biofuel can only come to fruition when the policy climate is favourable. Nigeria and virtually all other countries of the world have biofuel policies. Nigeria entered the biofuel race with the aim of gradually reducing the dependence on foreign nations for the supply of refined petroleum products, while creating a robust domestic biofuel economy. Therefore, in compliance to the August 2005 Government directive on automotive biofuel, on the 24th of July 2007, the Nigerian Biofuel Policy and Incentives (Table 12) was released which mandated the Nigerian National Petroleum Corporation (NNPC) to incorporate biofuels into the nation's transportation fuel mix (NNPC 2007). The policy mandated the NNPC to blend gasoline and diesel with bioethanol and biodiesel up to 10% and 20%, thus forming E10 and B20 blends respectively (**Ohimain** 2010c, 2013a, 2013b, 2013c, 2013d, 2013e, 2014d). The policy created an immediate demand of 1.3 billion litres and 480 million litres of bioethanol and biodiesel respectively, which could increase to 2.0 billion litres and 900 million litres respectively by 2020. The domestic production of biofuel is unable to meet this huge demand; hence, the country started importation of bioethanol until when domestic production can meet the current demand. Apart from creating the demand for biofuels, the policy also contained other incentives including the granting of pioneer status to companies that responded to the call for domestic ethanol production (**Ohimain**, 2012). Benefits accruable to pioneering biofuel companies include long-term preferential loans, tax waivers (withholding and VAT), import duty waivers and insurance coverage (**Ohimain**, 2015a, 2015b).

One country that has successfully transformed into a biofuel economy is Brazil. Brazil commenced the ProAlcool Program in 1975 in response to the oil crisis of the early 1970s. The Government of Brazil released policies, incentives and created a favourable environment for the establishment of a stable biofuel economy. The policy mandated all gasoline sold in Brazil should contain 22-25% ethanol. The policy also encouraged the use of 100% hydrous ethanol in vehicles. In 2005, Brazil had over 324 operational ethanol plants of various sizes. By 2008, the number of ethanol plants in Brazil has risen to 378 and over 400 by 2011. The current annual ethanol production in Brazil alone as now reached 30 billion litres. Brazil has successfully integrated her agricultural sector with the energy sector, producing ethanol mostly from sugarcane and generating power from the waste bagasse. Historically, sugarcane has been one of the most important agricultural activities in Brazil since 1532, occupying about 8 million ha in 2008. The entrance of Brazil into the biofuel sector boosted the agricultural sector. In 2008, Brazil produced 558.14 million tonnes of sugarcane, which was processed to 22.2 million m^3 of ethanol, 308 million tonnes of refined sugar and 664.12 MWh of cogenerated electricity (Ometto et al., 2009). The country now has the longest history of fuel ethanol usage in the automobile industry. Brazil is now ranked as the largest sugarcane producing country in the world, followed by India, China and Thailand in decreasing order. This feat can be replicated in Nigeria because we have similar agro-climatic conditions with Brazil.

Table 12: Nigerian Biofuel policy implementation plan and targets

Implementation phase	Action/activity	Duration
Phase 1: seeding the market	1. This will involve the blending of up to 10% of fuel ethanol with gasoline to achieve E-10 blend. 2. This phase will commence with a seeding of the market through importation of fuel ethanol until such a time that sufficient capacity and capability would have been developed in the country for large scale production of biofuel feedstock and establishment of biofuel plant. 3. The seeding phase is expected to commence with initial penetration of selected cities during the first 3 years of the programme, while a national roll-out is expected within 5-10 years.	5-10 years (2007-2016)
Phase 2: biofuel production programme	1. This phase will commence concurrently with the seeding programme. This will be the core of the agricultural integration programme and will entail the establishment of plantations and the construction of biofuel distilleries and plants. 2. Based on current demand for gasoline in the country, at 10% blend ratio with fuel ethanol, about 1.3 billion litres will be required for the country, this is estimated to increase to about 2 billion litres by 2020. It is also estimated that market demand for bio-diesel will be about 900 million litres by 2020 as compared to current market possibility of about 480 million litres for a 20% blend of bio-diesel. 3. The Biofuel Production programme aspires to achieve 100% domestic production of biofuels consumed in the country by 2020. 4. Investment in domestic production of biofuels will be private sector driven, with the government through its various agencies providing an environment conducive to players in the industry.	2007 onwards

Source: derived from NNPC (2007)

Transition of Nigeria to biofuel economy: Progress Thus Far (2007-2017)

Following the release of the Nigerian Biofuel Policy and Incentives (2007), the government quickly created demand for biofuels particularly ethanol. Ethanol was introduced into the transportation and household cooking fuel, which created a huge demand of 5.13 billion litres of ethanol annually (Table 13), whereas, the installed capacity of ethanol production then was 133.9 million litres per annum (Table 14), which is about 2.6 % of the country's ethanol demand when operated at full capacity. In line with the Nigerian Biofuel policy, the Government started the importation of ethanol from other countries mostly Brazil and Cuba, until domestic production can meet the huge demand. This is expected to boost the agricultural and industrial sectors, stabilize and secure the nations fuel and create massive employment opportunities.

With the favourable policy and economic climate, about 20 bioethanol factories started construction (Table 15) with additional 14 projects in the offing (**Ohimain**, 2010c). Cassava was promoted as feedstock for conversion to ethanol gel fuel to replace kerosene as household cooking in a project tagged cassakero. The cassakero project was targeted at the installation of 10,000 small scale bio-ethanol refineries in the 36 states of Nigeria including the Federal Capital Territory (FCT), over the next four years from December 2009 to December 2013 to produce the daily ethanol cooking fuel requirement for 4 million families (**Ohimain** 2012). Construction of the ethanol plants, which started in haste, is now slow due to regime change. The previous regime of President Obasanjo (1999-2007) focused on revitalization of the agricultural/bioenergy sector, but subsequent regimes have paid less attention on bioenergy sector. With this set back, most of the 20 first generation ethanol projects announced in the wake of biofuel policy have slowed down and some abandoned. Of the 20 new projects, only one is operational. The NNPC, which have completed feasibility studies for the construction of 2 cassava-based ethanol and 3 sugarcane-based ethanol projects have slowed down or abandoned. Nigeria therefore missed a golden opportunity to transit to biofuel and green fuel economy.

Table 13: Current Ethanol Demand in Nigeria

Use	Substitution	Annual consumption (billion litres)
Transportation	E10 gasoline blend	1.30
Household cooking and lighting	Paraffin (replacement with ethanol-based cooking gel fuel)	3.75
Manufacturing sector	Industrial ethanol demand (wines, chemicals, raw materials, solvents, pharmaceuticals etc)	0.09
Total		5.14

Table 14: Current ethanol production in Nigeria

Name of Company	**Plant location**	**Feedstock**	**Installed Capacity Million L/year**
Alconi/Nosak*	Lagos	Crude Ethanol (imported)	43.8
UNIKEM**	Lagos	Crude ethanol (imported)	65.7
Intercontinental Distilleries	Ota-Idiroko	Crude Ethanol (imported)	9.1
Dura clean (Formerly NIYAMCO)	Bacita	Molasses/cassava	4.4
Allied Atlantic Distilleries Ltd (AADL)	Sango-Ota	Cassava	10.9
Total			**133.9**

*Alconi Nigeria announced a new ethanol plant for Calabar, Cross River State
UNIKEM recently acquired a second plant which brought their total ethanol production capacity to 400,000 litres/ day Source: modified from **Ohimain (2010c)

Table 15: Bioethanol projects under construction following the release of the Nigerian Biofuel Policy and Incentives

Pioneers	Project	Cost	Location	Project summary, ethanol production / year
NNPC	Automotive biofuel project (Kwali Sugarcane ethanol project)	$80 - 100 million	Abuja, FCT	120 million litres, 10-15MW (electricity)
	Automotive biofuel project	$306 million	Agasha Guma, Benue State	75 million litres, 116,810 metric tonnes (sugar), 59MW (electricity)
	Automotive biofuel project	$306 million	Bukuru, Benue state	75 million litres, 116,810 metric tonnes (sugar), 59MW (electricity)
	Automotive biofuel project	$306 million	Kupto, Gombe state	75 million litres, 116,810 metric tonnes (sugar), 59MW (electricity)
	Automotive biofuel project	$125 million	Ebenebe, Anambra State	40 – 60 million litres
	Automotive biofuel project	$125 million	Okeluse, Ondo State	40 – 60 million litres
Global Biofuels Ltd	Ethanol refinery and sorghum farm	$70m	Arigidi Akoko, Ondo State	84 million litres Bio-refinery + farm
	Ethanol refinery and sorghum farm	$92m	Illemso, Ekiti state	30.8 million litres Bio-refinery + farm
Ethanig (via Starcrest Nigeria Energy)	Sugar cane ethanol project	$300 m	Katsina Ala, Benue State	100 million litres, sugar and electricity
	Sugar cane ethanol project	$300 m	Kebbi State	100 million litres, sugar and electricity
Dangote Industries Ltd	Savannah sugar company	$167m	Numan, Adamawa State	Expansion to produce 100 million litres, one billion tonnes sugar, 0.1 million

				tonnes fertilizer & 300MW electricity
Kwara Casplex Ltd/ Kwara State Govt	Cassava ethanol project	$90 m	Kwara state	38.86 million litres
Ekiti state Govt + Private	Oke-Ayedun cassava ethanol project	$18 m	Oke-Ayedun, Ekiti state	38.1 million litres Bio-refinery + farm
Ekiti state Govt/ CrowNet Green Energy	ethanol plant	$122 m	Iyemero, Ekiti state	65 million litres, (100 tonnes of starch and 50 tons of CO_2 /day)
Taraba state	Cassava ethanol plant	$115 million	Taraba state	72 million litres, 0.36 Million tons of cassava flour and 57 Mgy of liquid fertilizer , 1600 MW
Niger State and others	Niger State Govt. Ethanol Plant	$90m	Niger State	27 million litres, Bio-refinery + farm
NA	Cassava bio-ethanol project	$ 138m	Niger Delta region	58 million litres/year Bio-refinery + farm
NA	Bioethanol from sugarcane / molasses	$85 m	Niger Delta region	60 million litres
Private + Ogun State Govt.	Cassava industrialization project	$16.4 m	Ogun state	3 million litres
Private sector	National Cassakero cooking fuel programme	$ 1 billion	36 states + Abuja	1.44 billion litres

Source: Modified from **Ohimain** (2010c, 2013a)

Diversity and commercialization of biofuels

Due to the twin problems of air pollution especially in urban centres and greenhouse gas emissions causing global climate change, attention is gradually shifting away from fossil fuel such as crude oil and coal into biofuels. Attention is now focused on low carbon and zero emission equipment and automobiles. Several biofuels have been commercialized such as bioethanol and methanol, bio-oil and biodiesel, biogas, while biohydrogen and microbial fuel cells are in the experimental stage (Table 16). Ethanol produced from biomass (bioethanol) can be used as automotive fuel in various forms; low level blend with gasoline (≤ 20%), high level blends for flex vehicle (≥ 85%) and neat (100% hydrous ethanol). Note that low level blend of ethanol with gasoline will not require any engine modification. Typically, 1.2 litres of hydrated ethanol can replace 1 litre of gasoline in the neat (pure) alcohol vehicles. The advantages of ethanol fuel over gasoline have long been recognized to include higher compression ratios, higher heat of vaporization, the possibility of using leaner air fuel mixtures and greater power is obtained per unit with ethanol fuels, hence requiring smaller engine sizes. In addition, ethanol fuels generate lesser emissions compared to gasoline and are generally considered as carbon-neutral. Apart from biofuels, alcohols particularly ethanol have many industrial uses including the production of beverages, solvents, flavours and colourants, thermometers, scents, pharmaceuticals and many other chemical syntheses including esterification and transesterification reactions for the production of biodiesel.

Table 16: Alternative fuel from microbial and other bio-based sources

Energy carrier	Feed stock	Conversion technology	Applications	Stage of development
Bioethanol	Sugarcane, sweet sorghum, raffia and oil palm, cassava	Microbial fermentation	Transportation fuel, cooking, heating	Commercialized
Biomethanol	Sugarcane, sweet sorghum, raffia and oil palm, cassava	Microbial fermentation, thermochemical	Transportation fuel, cooking, heating	Commercialized
Microbial oils	Algal oil	Direct use	Fuel for diesel vehicles and generators	Commercialized
Biodiesel	Algal oils, raffia and oil palm,	Esterification/ trans-esterification	Fuel for diesel vehicles and generators	Commercialized
Bio-crude oil	Biomass including plant and animal remains	Pyrolysis	Cooking and transportation fuel, power generation	Commercialized
Biogas	Sewage, cow dung	Gasification, microbial fermentation, methanization	Cooking and transportation fuel, power generation	Commercialized
Microbial fuel cells	Cow dung, waste water		Power little devices	Commercialized
Biogasoline	Biomass	Pyrolysis, Hydro-cracking	Vehicle fuel	Commercialized
Butanol		microbial fermentation	Vehicle fuel	Commercialized
Hydrogen		microbial fermentation	Vehicle fuel	Commercialized
Myco-diesel	biomass	Gliocladium roseum synthesis	Vehicle fuel	Experimental

Electric and Solar Energy Deployment

Alternative energy has come to stay. There has been a phenomenal increase in the renewable energy share of the energy mix in most countries. Solar energy is increasing being tapped for electricity, rechargeable appliances are increasingly being used such as lamps, touch lights, AC, fridge, and even electric fans. Other solar appliances that have been commercialized include solar traffic and street lights (Plate 14), solar thermal heaters (Plate 15) and portable mobile electric box (Plate 16). Energy saving bulbs are increasingly being used. Electric and hybrid fuel cars are also on the increase. Even in car racing sports, formula-e is poised to replace formula-1. The implication of this alternative energy development is that fossil fuel is increasingly being displaced for environmental protection but could hurt the economy of oil producing countries including Nigeria. Some countries such as Britain and France have placed a ban on the production of new 100% petrol and diesel powered internal combustion engines (ICE) by 2040, while Norway and India have set goals for switching to electric or hybrid cars by 2025 and 2030 respectively. Many auto makers have produced electric or hybrid vehicles including Tesla, Volvo, Toyota, BMW, Volkswagen, Mercedes Benz etc. Britain have already installed 12,000 public charging points. Britain have offered a plug-in-grant of £ 4,500 as a direct incentive to buy electric cars. Volvo planned to stop the production of internal combustion engine by 2025, while France planned to stop oil exploration by 2030.

In Nigeria and elsewhere, tricycle (keke) based on 100% solar have been developed (Plate 17). Various configurations of cars have emerged including battery electric vehicles (BEV) (Plate 18), solar car (Plate 19), fuel cell cars based on hydrogen fuel and various hybrids such as fuel cell-electric vehicle (FCEV) (Plate 20), EV-ICE hybrid. Features of some of the commonest EV are presented in Table 17. Aeroplanes have been tested on electric, solar and biofuel. The solar impulse plane (Plate 21) travelled round the world based on solar without using a drop of fossil fuel. Electric trains (Plate 22) have replaced coal power trains in most developed countriesThe Japanese company Genepax has unveiled a car that runs exclusively on water based on water electrolysis and fuel cell technology (Plate 23). With all these developments, the fate of our crude oil is uncertain. We have to look for alternative uses for our oil especially in the petrochemical sector.

Plate 14: Solar traffic lights and street lights

Plate 15. Solar thermal heaters

Plate 16: Portable and mobile electricity from MTN-Lumos

Table 17; Features of emerging 100% electric vehicle (EV) or plug-in or battery-powered cars

Automaker	Model	Driving range*		Power	Price	
		(Miles)	(Km)	(HP)	(US $)	(Million N)**
Nissan	Leaf^	150	241.40	147	30,000	12
Tesla	Model 3	220			35,000	14
Tesla	Model S				~100,000	40
Tesla	Model X				~100,000	40
Tesla	Roadster				~100,000	40
General Motors	Bolt EV	238	383.02	200	35,000	14
Volkswagen	ID Buzz	270	434.52	369		

^With a fuel-efficient Nissan Sunny (x hp), I spend an average of N10,000 per week on fuel to work, which translated to N520,000 per annum. hence, the cost of fuel alone for 23 years could buy brand new Nissan Leaf. Other advantages include zero emissions and low maintenance/ servicing costs (~ 10% of ICE)

-Disadvantage: no car charging points/filling stations in Nigeria yet

* distance covered by a single charge

** At $1 = N400 exchange rate

Plate 17: Solar powered tricycle

Plate 18: Battery electric vehicles

Plate 19: Solar charged electric vehicles

Plate 20: Fuel cell hydrogen cars

Plate 21: Solar powered aeroplane (solar impulse)

Plate 22: Train run by electricity

Plate 23: Water powered car (a reality or conspiracy)

BIOMATERIALS
The biomass feedstocks that we have worked with for biofuel, green chemicals and other purposes are raffia palm, oil palm, cassava and elephant grass, which are all abundant in Wilberforce Island and elsewhere in the Niger Delta.

Oil palm
Mr. Vice Chancellor sir, my research group have worked extensively on oil palm and have produced 29 papers on this crop alone. Our studies covered environmental impacts of oil palm processing, material mass balance and the bioenergy that could be potentially derived from oil palm processing wastes. We also studied the microbiology of palm oil commonly sold in Rivers and Bayelsa states.

Microbes are involved in several stages in the oil palm value chain. They colonize the fruit right from the tree even before harvest. Upon harvest, oleophilic microbes immediately start work on the full fruit bunch (FFB). When the FFB is heaped prior to slicing, fermentation starts, which result in the weakening of the fruitlet base and spikes, thus enhancing separation of fruits from the bunch. Beside separation, fermentation also soften the fruits, which could require less energy for subsequent boiling stage. However, prolong fermentation of the fruit could result in increased free fatty acid (FFA) in the final oil, which is detrimental. After, boiling, digestion follow, and the first grade of oil is extracted. The resulting palm press fibre (PPF) is heaped and allowed to undergo further microbial fermentation steps resulting in the extraction of second and third grade oils, which are sometime also consumed, but are mostly used for non-food processes such as the production of soap. Microbes also contaminate all the different grades of palm oil produced resulting in increasing the concentration of FFA.

We evaluated the material-mass balance of smallholder (Plate 24) and also semi-mechanized (Plate 25) oil palm processing in Niger Delta Nigeria. Measurement were made of the various processing fractions such as fresh fruit bunch (FFB) and all the processing intermediates/products including threshed fresh fruit (FF), palm pressed fibre (PPF), palm kernel shell (PKS), empty fruit bunch

(EFB), crude palm oil (CPO), chaff and nut. During the smallholder batch processing of Dura variety, the proportion of waste by- products computed in relation to the weight of the FFB (100%) are as follows; EFB (23.7 – 32.4%), chaff (0.8 – 2.4%), PKS (10.0 – 18.8%), and PPF (23.2 – 28.1%), while only 9.4 – 12.8% is converted to CPO (Fig 14). For the *Tenera* varieties, the compositions are as follows; EFB (25.7 - 28.2%), chaff (0.9 – 1.4%), PKS (6.8 – 7.5%), (19.1 – 20.3%) and CPO (26.0 – 28.2%). This result shows that *Tenera* produces more oil and less wastes compared to the *Dura* variety. The solid wastes fractions are used as energy sources during the processing of oil palm. Except the huge volume of wastes (71.8 – 90.6%) generated by oil palm processors is effectively utilized, the process will be unsustainable (**Ohimain** et al 2013f, **Ohimain** and Izah, 2014a: 2014b, 2015; Izah, **Ohimain** et al., 2016). Oil palm processing generates large volumes of effluent (**Ohimain** and Izah 2014), solid wastes and gaseous emissions (**Ohimain** et al 2013a; **Ohimain and Izah** 2013). Second grade oil and energy can be obtained from waste palm press fibre (Izah, **Ohimain** et al 2014).

With the knowledge of the abundance of waste biomass generated and unutilized during oil palm processing, we went further to study the energy self-sufficiency of oil palm processing. We evaluated the contribution of various energy sources to the smallholder processing of oil palm in Nigeria. The weight of the various solid wastes generated and utilized for boiling process were measured including EFB (empty fruit bunch), PPF (palm press fibre), PKS (palm kernel shell) and chaff, while the volume of diesel used for digestion was also measured. The processing of 1 tonne of FFB (fresh fruit bunch) in the mill yields 63.4 – 77.1 litres of CPO while the following waste by-products were generated from the FFB; 24 – 31 % EFB, 23 – 28 % PPF, 10 – 12 % PKS and 1.4 – 2.4 % chaff (Fig. 15). Out of the total biomass generated by the mills only 12.74 – 22.25% EFB, 24.43 – 33.38 % PPF, 2.71 – 6.71 % PKS and 15.12 – 49.04% chaff were utilized by the various mills for fruit boiling/sterilization, indicating that the majority of biomass wastes is unutilized in the mills. The volume of diesel utilized by the mills for digestion is quite low ranging from 0.6 – 0.8 litres. The gross calorific values of the waste biomass were determined to be; EFB 16.970 – 18.537 MJ/kg, PPF 16.472 – 21.037 MJ/kg and PKS 19.378 – 21.614MJ/kg. The total energy utilized by the mills for processing one tonne of FFB ranged from 2179.43– 3014.31 MJ. Out of these, biomass energy accounted for 98.22 – 98.75 %, while fossil fuel accounted for the remaining 1.25 – 1.78 %. The study concluded by suggesting innovative ways of substituting the <2% fossil fuel contribution with the direct use of pre-heated palm oil to fuel the digesters (**Ohimain** and Izah 2014). Various energy carriers can be produced from oil palm processing wastes including briquettes (**Ohimain** et al 2015), biogas (**Ohimain** and Izah 2014, 2017), bioethanol (Ohimain 2013), biodiesel (Izah and Ohimain, 2013, 2015; **Ohimain** and Izah 2014), biohydrogen (Izah and Ohimain, 2015) and electric power (Izah, **Ohimain** et al 2016).

We went further to investigate the energy self-sufficiency of a semi-mechanized oil palm processing. The study was designed to assess the energy requirement, biomass generation and the contribution of biomass in oil palm processing by a semi-mechanized palm oil processing mill in Nigeria. The energy required to extract oil from fresh fruit was measured at the Bayelsa Palm Mill, Elebele, Nigeria. The study assessed the energy requirement for processing 1 tonne of fresh fruit bunch (FFB). The total number of FFB that made up the 1 tonne was 74 bunches, each bunch with an average weight of 13.5 kg. The mass balance of FFB during processing is 26.0 % empty fruit bunch (EFB), 1.5 % chaff, 18.5% nut, 30.0 % palm press fibre (PPF), 14.0% moisture and 10.0% crude palm oil (CPO) (Fig. 16).

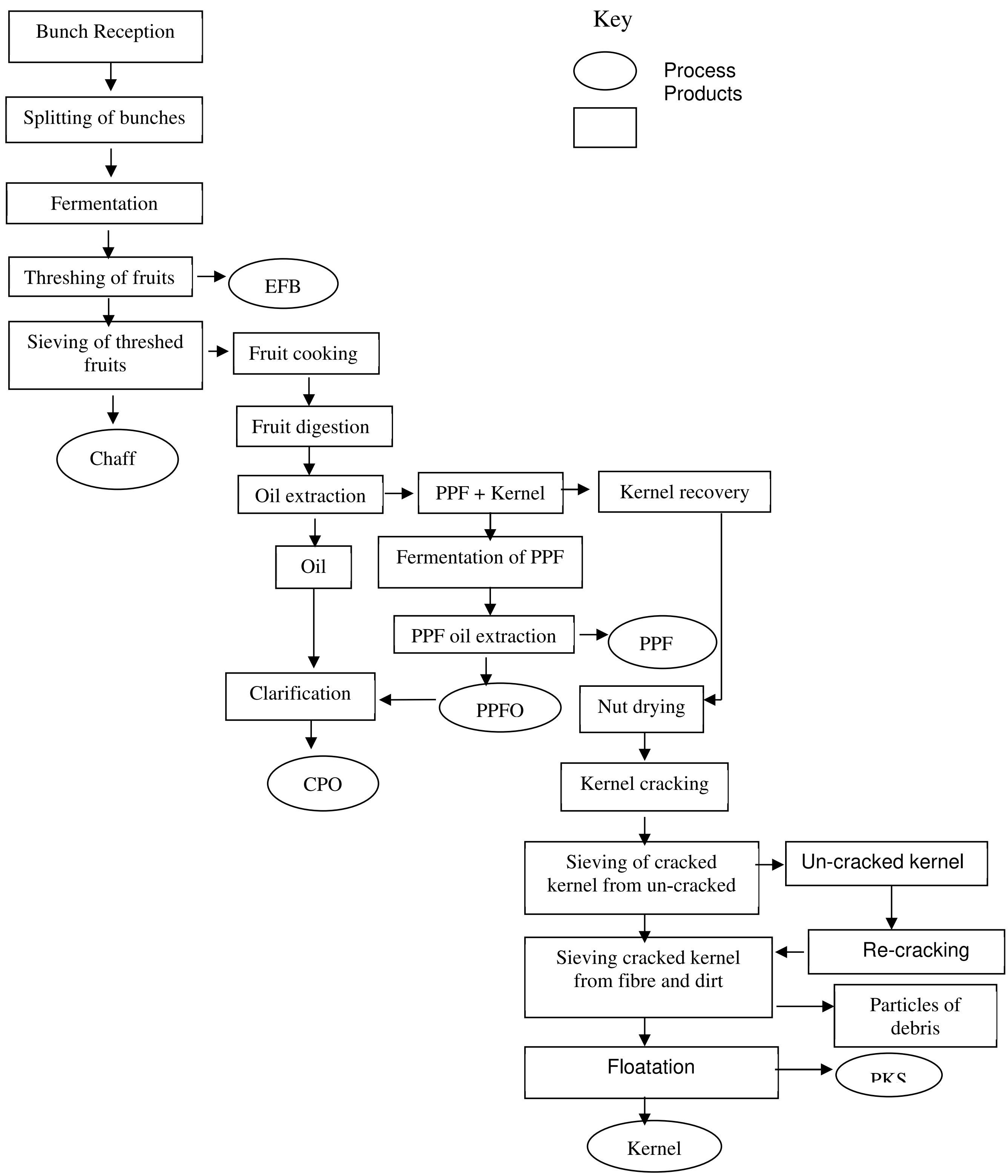

Fig. 14: A flow chart of small holder oil palm processing in the Niger Delta
(EFB = empty fruit bunch; PPF = palm press fibre; CPO = crude palm oil; PPFO = palm press fibre oil; palm kernel shell)

Plate 24: Pictorial show of oil palm processing by smallholders in Nigeria; [A] FFB reception, [B] Slicing of FFB, [C] Fermentation of sliced fruit, [D] Threshed fruit, [E] Sieving of threshed PF, [F] Boiling of PF in a barrel, [G] Digestion of boiled PF, [H] Oil extraction, [I] Fiber and nut [J]Fermentation of PPF, [K] Clarification drum [L] Storage of CPO

Plate 25: Semi-mechanized oil palm processing; [A] FFB reception [B] Stripper, [C] sterilization/boiling, [D] Digestion and oil extraction zone [E] Clarification zone [F] dryer , [G] Storage of CPO

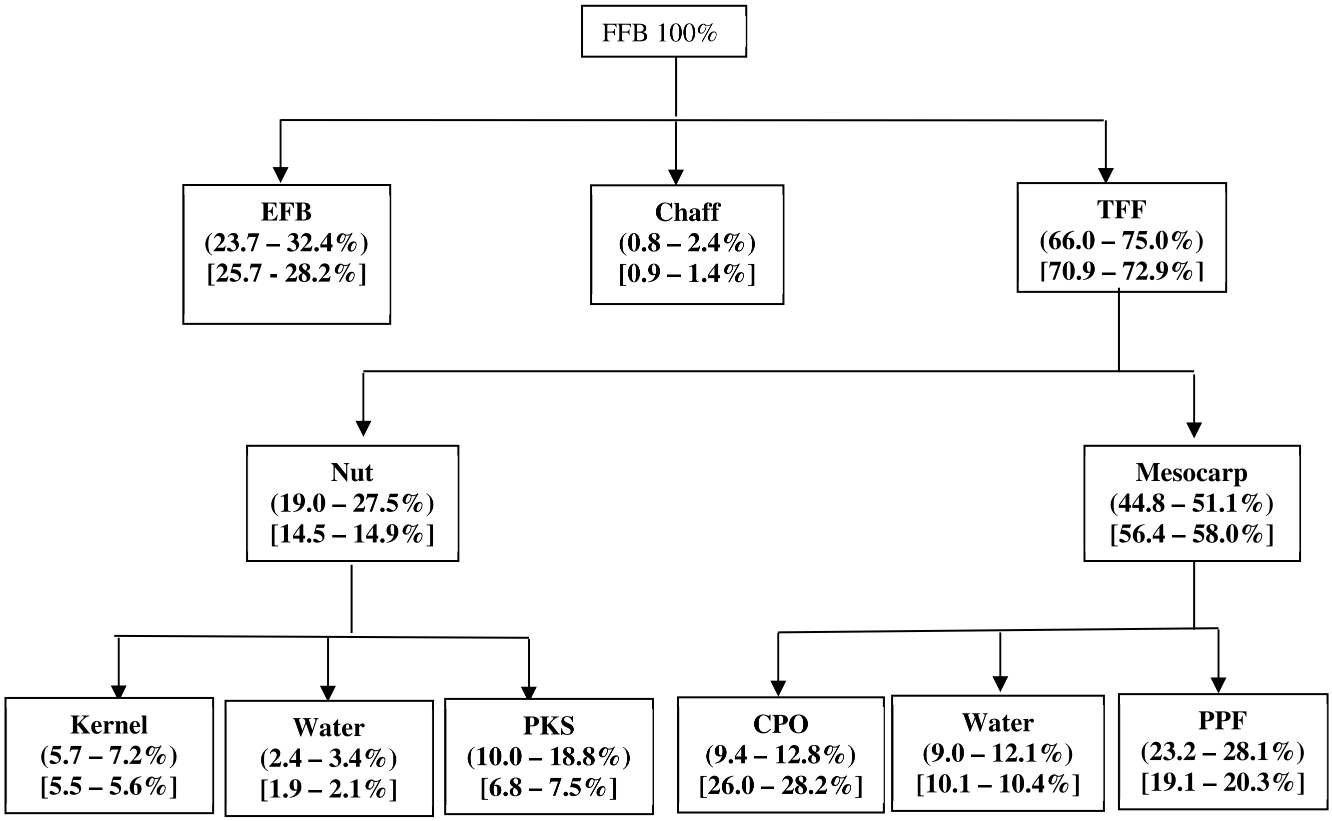

Fig. 15: Material-mass balance of FFB processed by small holders in Nigeria (**Ohimain** et al 2013f). Figures in bracket are the percentage of the different fraction of FFB: ( ) = *Dura* variety; [ ] = *Tenera* variety.

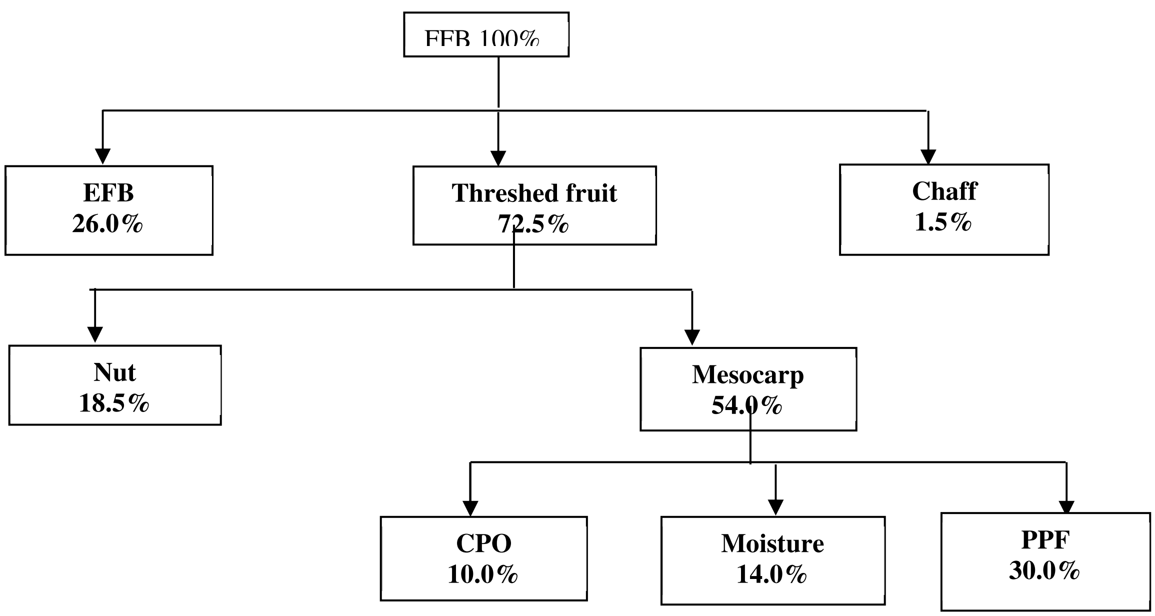

Figure 16: Material mass balance of semi-mechanized oil palm processing in Bayelsa state

The biomass generated during the processing of CPO from 1 tonne of FFB is 259.0 kg (EFB), 301.9 kg (PPF) and 14.8 kg (chaff). Of these, only 234.9 kg of EFB was utilized as fuel for boiling, while 1.85 litres of diesel were used to power generator for stripping, digestion/pressing and fibre separation. Majority of the biomass generated were un-utilized. About 40% of the potential energy in the biomass was utilized by the mills, while the rest was disposed by open combustion. The total energy utilized for the processing of FFB is 4291.39 MJ/ tonne. Of these, biomass fuel accounted for 97.5%, while fossil fuel supplied the remaining 2.5%. We conclude that the excess biomass can be converted into liquid fuel via pyrolysis and/or gasification and used for digestion purposes to attain 100% energy self-sufficiency to prevent air quality impacts associated with the open-air combustion of excess biomass (**Ohimain** and Izah 2014). Other potential biofuel that could be generated from oil palm waste biomass include solid (briquette), liquid (bioethanol, bio-methanol, bio-oil) and gaseous fuel (biogas, bio-hydrogen) and electricity.

Microbes found in palm oil mill effluent

We carried out a study to investigate the microorganisms associated with palm oil mill effluent (POME) produced by smallholder processors in Elele, Rivers State, Nigeria. The POME was subjected to standard microbiological analysis and biodegradation studies using crude palm oil as sole carbon source. The population of total heterotrophic bacteria (THB) ranged from 7.8×10^5 – 2.0×10^6 cfu/ml, the total fungi (TF) ranged from 3.2 – 5.7×10^4 cfu/ml. The hydrocarbon degrading bacteria (HDB) ranged from 6.5×10^5 - 2.0×10^6 cfu/ml and the hydrocarbon degrading fungi (HDF) ranged from 3.1 – 5.6×10^4 cfu/ml. Among the various mills, analysis of variance showed that there were significant differences ($P < 0.05$) in THB, THF and HDB but no significant difference ($P > 0.05$) in HDF. HDB and HDF accounted for 83.34 – 98.62% and 92.17 – 99.28% respectively of the heterotrophic population, indicating that majority of the microbes in the POME are hydrocarbon degraders. Microbial species identified in the POME include *Aspergillus niger*, *Aspergillus flavus*, *Fusarium*, *Mucor* sp., *Penicillin* sp., *Pseudomonas* sp., *Serratia* sp., *Staphylococcus* sp., *Corynebacterium* sp. We conclude that the POME contained microbial species capable of degrading hydrocarbons in the POME to prevent environmental impacts (**Ohimain** et al., 2012a, 2012b, 2012c, 2012d, **Ohimain** and Izah 2013, Izah and **Ohimain**, 2013).

The negative aspects of oil palm processing are many. Oil palm plantations have been used to displace and destroy many virgin forests and associated wildlife in southern Nigeria in especially Edo, Ondo, Bayelsa, Rivers, Cross River, Imo and Delta states. Oil palm processing generates several wastes that are not utilized but disposed into the environment without any form of treatment. Large volumes of solid wastes (EFB, chaff, kernel shell, PPF), gaseous emissions, foul odour and POME are freely discharged leading to environmental pollution.

Stagnancy in the Nigerian oil pal sector

In the 1950s-1960, Nigeria occupied the enviable position of being the largest producer of oil palm in the world. Accounting for over 50% of global production. In the 1970s, the Malaysians came to NIFOR to collect oil palm seedlings. Today, Indonesia and Malaysia are the largest oil palm producing nations in the world, with Nigeria occupying a distant fifth, with 2.9% (Table 18, Fig. 17). Nigeria lost her dominance in the oil palm sector. In the last two years, Nigeria production of oil palm has further dropped perhaps due to insecurity. Considering the vast application of oil palm in food (cooking oil, margarine, beverages/ palm wine, vitamin E and A), non-edible industrial uses (soap and several oleochemicals) and bioenergy applications (bioelectricity, biogas, bioethanol, bio-methanol, biodiesel, and briquettes). Due to the current high prices, Nigeria is now importing palm oil.

The country domestic production currently stands at 970,000 metric tons, while demand is 2.7million tons, leaving a deficit of 1.73million. The price of oil palm hit $718 per metric ton in November 2017 based on high demand by indigenous manufacturers. Despite the high exchange rate and its price, Nigeria imported 450,000 tons of crude palm oil valued at N116.3billion ($323.1 million) in 2017. Again, Nigeria have lost another golden opportunity for development of oil palm-based economy. Our research is now focused on the development of alternative edible vegetable oil, from raffia palm.

Table 18: Global oil palm production statistics

Rank	Country	1964 Production (1000 MT)	2017 Production (1000 MT)	2017 growth rate
1	Indonesia	157	36,000.00	5.88 %
2	Malaysia	151	21,000.00	12.00 %
3	Thailand	0	2,200.00	10.00 %
4	Colombia	2	1,320.00	15.18 %
5	Nigeria	537	970.00	0 %

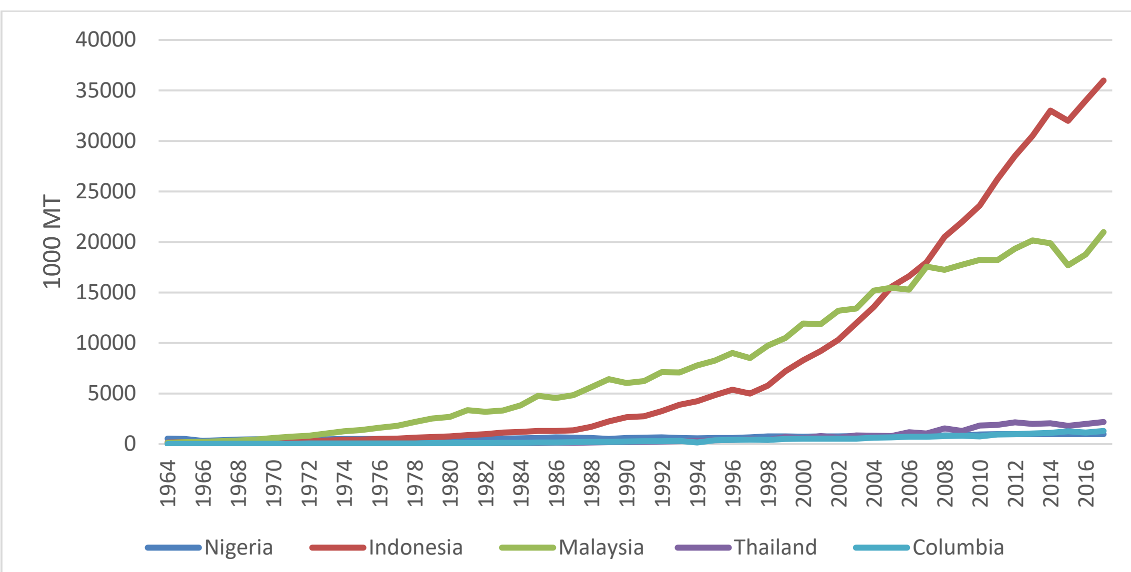

Figure 17: Major world oil palm producing nations, oil palm production from 1964-2017

Raffia palm

Raffia palm produces feedstock that are important to the emerging bioenergy sector including bioethanol, vegetable oil, biodiesel and other biomass that could be converted to electricity and other energy carriers using thermochemical conversion technology of pyrolysis, combustion and gasification. But raffia palm is hapazanthic (hapaxanthic) i.e. after a period of vegetative growth, it produces inflorescence and fruit only once and dies (Ndon, 2003). Despite the economic and ecologic importance and the hapazanthic nature of raffia palm, little efforts have been expended on the domestication of the plant. Due to the hapazanthic nature, potential biomass of raffia palm particularly the fruits are lost annually. Raffia palm is currently underutilized by indigenous people for building construction, production of brooms, basket, ropes and construction of fish, crab and turtle traps. Edible insect larva is obtained from the palm. Palm wine and locally fermented gin are the major uses of raffia palm (Plate 26).

Raffia palm (*Raphia* sp) occurs abundantly in the wild in the freshwater zone of the Niger Delta, occupying large expanse of land from the fresh water to the coastal mangrove, with a with of over 30km. We assessed the use, biomass productivity (Plate 27) and characterization of raffia palm oil from Bayelsa, Delta and Rivers. The wild palm has a biomass productivity of 933 trees/ha in Bayelsa and Delta states and 1066 trees/ha in Rivers. The oil was extracted using mechanical method (Fig. 18) and was characterized (Table 19). Results show that the relative density of the oil was 0.8700 – 0.9039, free fatty acid (FFA) content of 1.13% (Delta), 6.92% (Bayelsa) and 9.74% (Rivers), acidity value of 2.26% in Delta state, 13.94% in Bayelsa state and 19.48% in Rivers state ($P<0.05$), iodine value of 26.79% was recorded in the raffia palm oil from Rivers state, 28.60% from Bayelsa and 31.10% from Delta state. The peroxide value of the raffia oil was 5.58 mg KOH/g (Rivers state), 7.22 mg/KOH/g (Delta state) and 7.68mg/KOH/g (Bayelsa state) ($P<0.05$). The saponification number was 25.16 mg KOH/g in the oil from Bayelsa, 32.72 mg KOH/g from Delta and 213.18 mg KOH/g for Rivers state ($P<0.05$). The oil was characterized and was found to fairly fall within the SON/NIS standards for vegetable oil. In general, raffia palm oil resembles palm oil physically and chemically. Physically, it resembles oil palm in colour, odour and taste, but it is slightly bitter, due to the presence of saponin (Ndon 2003). Chemically, the predominant fatty acid in oil palm and raffia palm oil is palmitic acid and oleic acid. Now that palm oil is quite expensive, we are seeking research collaboration for the potability and safety of raffia palm oil as alternative vegetable oil. Raffia palm can also be used as biofuel feedstock for the production of soap, lubricating oil, biohydrogen, biodiesel, bioethanol and bio-methanol for transportation fuels, briquettes, biogas and biomass for cooking and power generation, and green chemicals including acetic acid and lactic acid produced via microbial fermentation. Raffia palm can be utilized as second-generation biofuel feed stock to mitigate food versus fuel conflicts.

Plate 26: Raffia palm art work from NDU

Table 19: Physicochemical characteristics of raffia palm oil

State	relative density	Saponification Number mg KOH/g	FFA, %	Acidity value, %	Peroxide value, mg **KOH/g**	Iodine value, %
Bayelsa	0.8770±0.01 b	25.16±0.01a	6.92±0.01 b	13.84±0.01 b	7.68±0.01 c	28.60±0.01 a
Delta	0.8700±0.01 a	32.72±0.01b	1.13±0.01 a	2.26±0.01a	7.22±0.00 b	31.10±0.01 c
Rivers	0.9039±0.02 c	213.18±0.01 c	9.74±0.01 c	19.48±0.01 c	5.58±0.01 a	26.79±0.01 b
Ndon 2003 (R. hookeri)	0.867	198	10.2		26.7	51
Ndon 2003 (R. vinifera)	0.860	201	4.7		19.7	63.8
Ndon 2003 (E. guinensis)	0.900	195-205	0.5-18.0		0 -5.0	54.9

Mean± SE, n=3. Along the column, means with the same alphabets are not significantly different (P>0.05) according to Duncan Multiple Range Test
Source: modified from Oduah and Ohimain 2015

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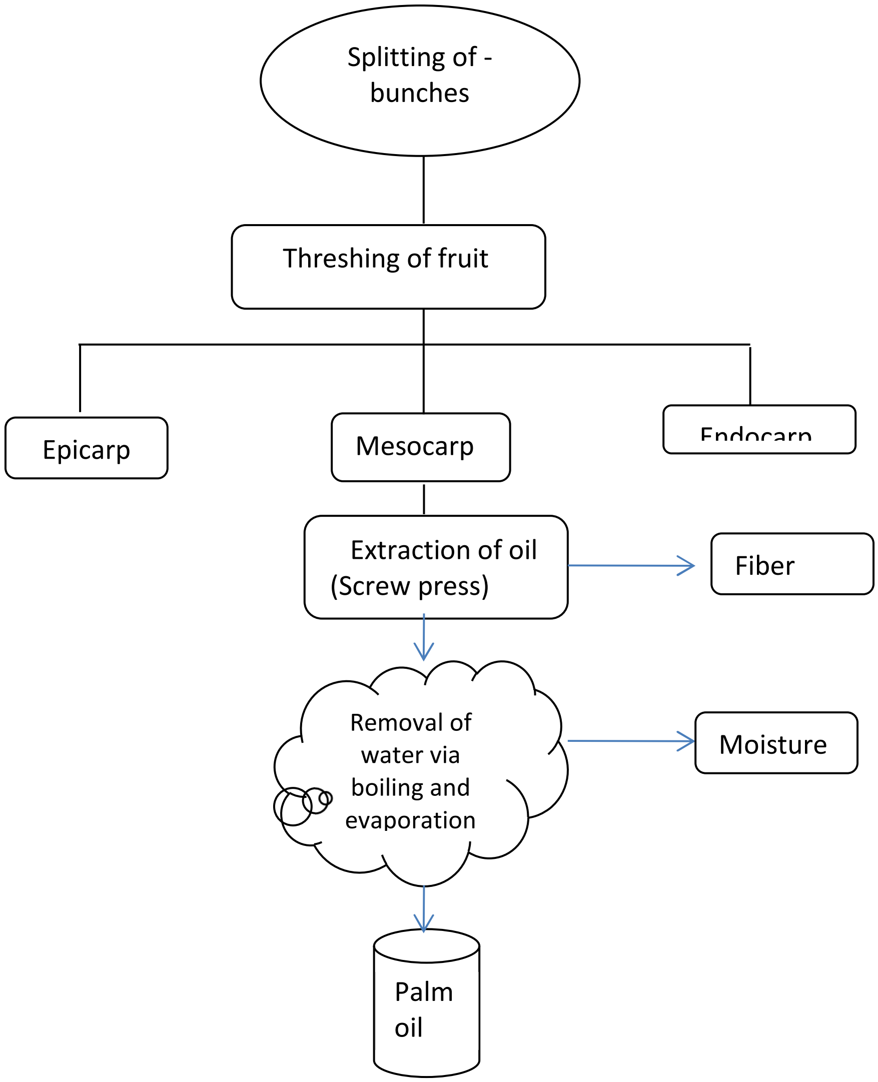


Figure 18: Raffia palm oil extraction

Raffia palm fruit bunch

Raffia palm sub-bunch

Empty fruit bunch

Terminal sub-bunch


Ripe raffia palm fruits

Raffia palm oil

Plate 27: Raffia palm fruit bunch and oil

Raffia palm used for fish stupefaction

The indigenous people of the Niger Delta region in Nigeria depend on fisheries as the main protein source. The mesocarp of raffia palm is used by the indigenous people to stupefy and catch fishes. The mechanism of action of the raffia palm mesocarp is not well understood. We investigated the effect of raffia palm mesocarp on the haematological properties of African catfish. Results show that increasing concentration of raffia palm led to decreasing red blood cells, platelets and haemoglobin levels while increasing white blood cells, eosinophils and monocytes. Water quality analysis results corroborated the haematological analysis. Alkalinity and pH increased, while dissolved oxygen decreased as the concentration of raffia palm fruit extract increases. We therefore concluded that the mode of action of raffia palm fruit mesocarp involved increased alkalinity and decreased oxygen tension in the water which temporarily stupefies fishes for easy catch (Ohimain et al, 2015). However, Ndon (2003) attributed the stupefaction of fish to the presence of saponin in the mesocarp of the palm

Raffia palm wine and methanol contamination

We also carried out a study on traditional fermentation and distillation of raffia palm sap for the production of bioethanol in Bayelsa State by indigenous/small holder processors (**Ohimain** et al 2012e). We found out that the process was catalysed spontaneously by the wild yeasts and other microbes. Distillation was carried out using rudimentary equipment (Plate 28). The raw fermented palm sap had ethanol concentration of 10.50 – 15.30%. Yeast population, pH, sugar, specific gravity and electrical conductivity differed significantly among the various sites and intermediates. Wood (105 – 155kg) was used as fuel to boil 280 – 480L of fermented palm sap producing 20L of 39 – 61.5% ethanol (Fig. 19). The smallholder processors are however challenged by the poor distillation apparatus and the lack of ethanol dehydration facilities. By further dehydration, the ethanol produced can be used as biofuel.

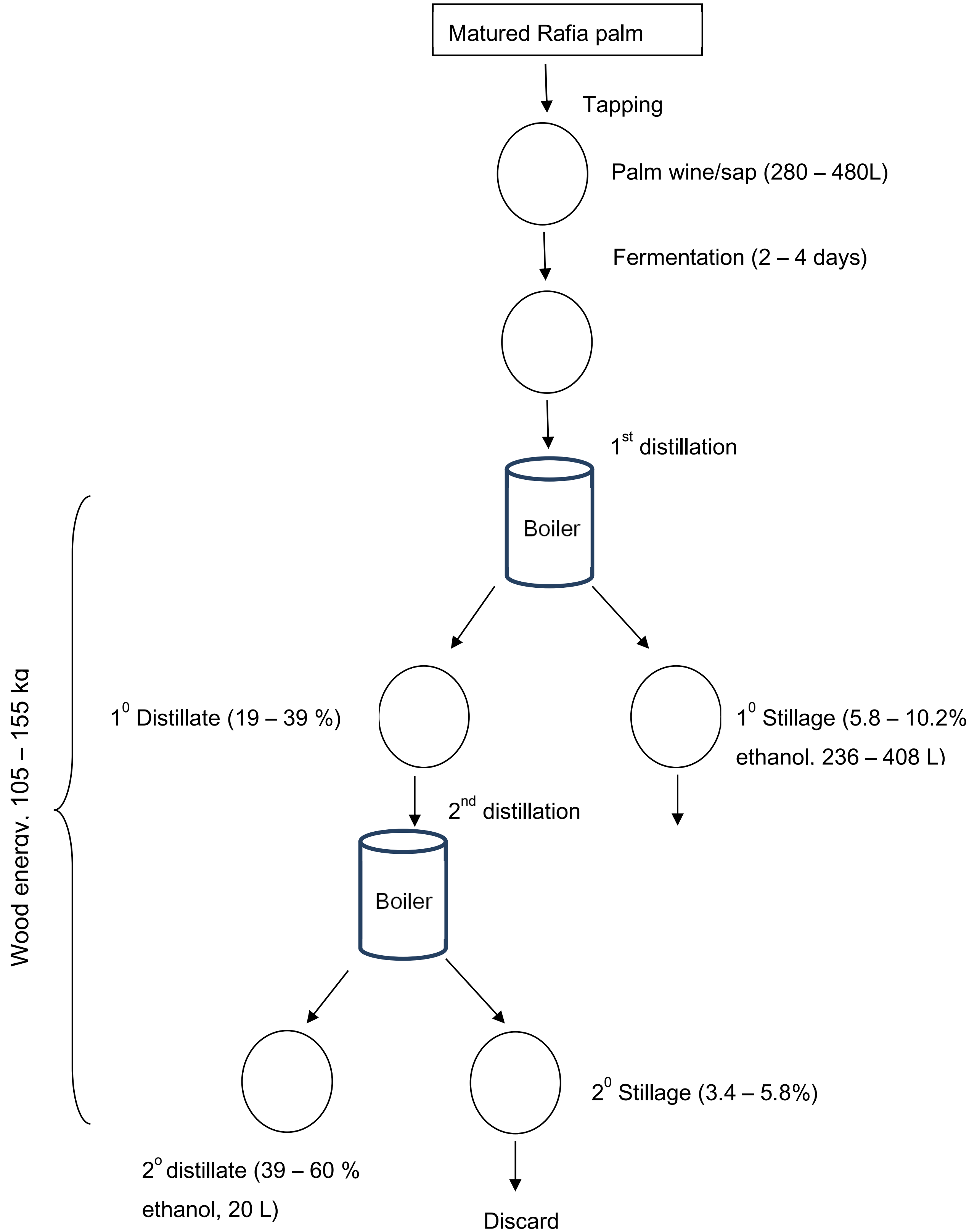


Fig 19: Batch process for the traditional fermentation and distillation of raffia palm sap for ethanol production

Fig. 28: Rudimentary ethanol distillation equipment used in the Niger Delta

Methanol contamination of traditionally fermented alcoholic beverages

Locally produced ethanol beverage called kaikai/ ogogoro/ apeteshi or illicit gin. Kaikai is produced mostly from the sap of raffia palm and oil palm and to a lesser extent from other palms such as date palm, nipa palm etc. Palm wine and locally produced alcoholic beverages is depended on spontenous fermentation by wild yeast and other microbes including bacteria such as *Lactobacillus* and *Zymomonas* species. However, spontaneous inoculation by wild yeast and other microbes, can result in the production of methanol, which can be fatal upon consumption.

In Nigeria, between April and June 2015, a total of 89 persons died following the consumption of Laboratory analysis carried out by WHO and NAFDAC (National Agency for Food, Drug Administration and Control) show that the beverage contains 16.3% methanol, while the blood methanol concentration of victims was found to be 1500 – 2000 mg/l. Victims exhibited symptoms of methanol poisoning including loss of consciousness, dizziness, weakness and breathing difficulties, blurred vision and blindness, weight loss, headache, abdominal pains, nausea, diarrhoea and vomiting. WHO (2014) reported that blood methanol concentration above 500mg/l is associated with severe toxicity, whereas concentration above 1500 – 2000 mg/l causes death in untreated victims. While investigation was ongoing on the source/origin of methanol in the beverage, the Federal Government of Nigeria (FGN) placed a ban on the production, sale, distribution and consumption of locally fermented beverage in Nigeria. Enforcement of the ban was heightened in the months (June – August 2015) following the incidence, but as of the time of writing enforcement has slacked.

Using gas chromatography, Young and Tarawou (2017) analyzed the alcohol contents of locally fermented gins produced in Bayelsa state and found that the ethanol content ranged from 35.4-60.2% and methanol concentration of 0.02-0.09%.

Incidence of methanol contamination of traditionally fermented beverages is increasing globally resulting in the death of several persons. The source of methanol contamination has not been clearly established in most countries. Several cases of methanol poisoning have been reported in India and elsewhere. For instance, in 2008, over 180 persons were killed in Bangalore and in 2009, 138 were killed in Gujarat, India. In 2015, 27 persons died in India after consuming toxic ethanol. In 2009, 25 persons died in Indonesia after consuming fermented palm wine containing methanol. About 130 persons died in some India villages in 2011 linked to poisonous ethanol consumption. In Czech Republic 127 persons were poisoned from contaminated alcohol, out of which 42 died. In 2014, the World Health Organization (WHO) alerted that there have been increasing out breaks of methanol poisoning in several countries including Kenya, Gambia, Libya, Uganda, India, Ecuador, Indonesia, Nicaragua, Pakistan, Turkey, Czech Republic, Estonia and Norway. The size of these outbreaks ranged from 20 to over 800 victims, with case fatality rates of over 30% in some cases (WHO 2014). Table 20 listed some traditional beverages that are prone to methanol contamination in different countries.

Table 20: Traditionally fermented alcoholic beverages prone to methanol contamination

Beverage	Feedstock	Fermenting organism	Countries	Alcohol content
Palm wine	silver date palm (*Phoenix sylvestris*), the palmyra, jaggery palm (*Caryota urens*), oil palm (*Elaeis guineense*) *Raffia palms*, *kithul* palms, or *nipa* palms. coconut palms *Borassus*	Yeast (Saccharomyces cerevisiae, Saccharomyces ludwigii, Candida parapsilosis, Candida fermentati, Pichia fermentans, Schizosaccharomyces romyces pombe, Schizosaccharomyces romyces bailli, Kluvyeromyces africanus, Hansenula auvarum, Kloeckera apiculata, Torulaspora delbrueckii) & Lactic Acid Bacteria (*Lactobacillus*, *Leuconostoc*, *Pediococcus*, *Lactococcus*, and *Streptococcus*), acetic acid bacteria (Acetobacter, Aerobacter)	Most African and Asian countries	
Local gin (ogogoro, kaikai, apetesi)	Palm wine	(Saccharomyces cerevisiae) & Bacteria (Lactobacillus)	Most African and Asian countries	40-60% Ethanol
Pito (local beer)	Sorghum or maize	Bacteria (Pediococcus halophilus, Lactobacillus) & yeast (Saccharomyces cerevisiae, Candida tropicalis, Schizosaccharomyces romyces pombe, Kluvyeromyces africanus, Hansenula anomala, Kloeckera apiculata, Torulaspora delbrueckii)	West Africa	2-3% Ethanol
Burukutu	Sorghum	Sacharomyces cerevisiae, Streptococcus, Lactobacillus, Aspegillus, Fusarium, Penicillium	Nigeria, Ghana	1.63% ethanol
Cachaca (banana pulp wine)	Banana	Sacharomyces cerevisiae	Brazil	Ethanol (5.34-7.84%), methanol (0.65-0.189%)
Cachaca	Sugarcane	Sacharomyces cerevisiae and wild yeasts (Pichia sp & Dekkera bruxelensis)	Brazil	Methanol (0-0.5%)
Noni	Morinda trifolia	Lactobacillus plantarum & L. casei	Thailand	853 mg/l methanol
Kwunu-zaki	Millet	Sacharomyces cerevisiae	Nigeria	?
Cocoa sap wine	Cocoa sap	Sacharomyces cerevisiae	Nigeria	?
Cholai	rice, sugar-cane, juice of date tree, molasses, and fruit juice (pineapple and jackfruits)	Sacharomyces cerevisiae	India	14.5% alcohol
Agave	Agave		Mexico	3.9-339g/l (ethanol), ND-1826 mg/l (methanol)
Plum wine	Plum		Romania	53-76% (ethanol), 554-4170 mg/l (ethanol)
Plum brandy	Plum		Macedonia	47-51% (ethanol), 564-999 mg/l (methanol)
Plum wine	Japanese Plum (Prunus salicina Linn)	Yeast	India	175mg/l Methanol

Source: **Ohimain** (2016b)

While there were speculations that unscrupulous vendors might have deliberately spiked the beverages with methanol, but it is more likely that the methanol might have been produced by contaminating microbes during traditional fermentation. A possible source of methanol in traditionally fermented alcoholic beverage is the fermenting microbes. The ethanol fermenting yeast *Saccharomyces cerevisiae* dominated traditional alcohol fermentation followed by *Lactobacillus*. The *Saccharomyces cerevisiae* have been used as catalysts for the production of ethanol for thousands of years. But recent studies have shown that there are different strains of *Saccharomyces cerevisiae* involved in traditional ethanol fermentation in Africa and Brazil. The big question is 'whether the traditional ethanol producing yeast have evolved into the production of methanol in addition'? We are open to further collaboration on this research.

Secondly, traditional fermentation occurs via spontaneous inoculation from the substrate and processing equipment (**Ohimain** et al. 2012b), hence a mixed culture carried out the fermentation. Therefore, contaminating microbes including other yeasts, fungi and bacteria could result in the production of several other products including methanol. And because methanol has a lower boiling point (65ºC) than that ethanol (78ºC), it could be further concentrated in the beverage during distillation. Even pure culture fermentation can result in the production of diverse products depending on the operating conditions. Hence, beverages produced via spontaneous fermentation by mixed culture under uncontrolled conditions, could produce greater variety of products. Some of these compounds are also very poisonous e.g. ethyl carbamate and some are even carcinogenic. Annan et al. (2003) listed 63 volatile compounds produced during the mixed culture fermentation of Ghanaian maize dough consisting of 20 alcohols, 22 carbonyls, 11 esters, 7 acids, 3 phenolic compounds and a furan.

Methanol production in traditional fermented beverages can be linked to the activities of pectinase producing microbes including yeast, fungi and bacteria. Pectins are a group of heterogeneous polysaccharides found in the intercellular regions and cell walls of most fruits and vegetables. During ripening, pectin in fruits is broken down by pectin methyl esterase (PME) resulting in the formation of methanol. Chaiyasut et al. (2013) compared pectin levels in fermented beverage containing *Morinda citrifolia* (9.89%) with that of other fruits including guava (4.36%), tomato (0.3%), apple (0.5%), carrot (0.8%) and cherries (0.4%). PME de-esterify pectin to low –methoxyl pectins resulting in the production of methanol

Plant cell wall degrading enzymes including pectinases are ubiquitous among pathogenic and saprophytic bacteria, fungi and fungi. Methanol is a major end product of pectin metabolism by microorganisms including Human colonic bacteria, *Erwinia carotovora*, anaerobic bacteria, particularly *Clostridium butyricum*, *C. therocellum*, *C. multifermentans* and *C. felsineum* and Pseudomonas. Dorokhov et al. (2015) listed at least 20 species of human colonic microbes capable of producing methanol endogenously.

Some strains of *Saccharomyces cerevisiae* that produces pectinolytic enzymes have been identified. Strains of *Saccharomyces cerevisiae* having PME activity could produce methanol during fermentation. During the production of sugarcane beverage called cachaca in Brazil, *Saccharomyces cerevisiae* produced no methanol while contaminating yeasts (*Pichia silvicola* and *P. anomata*) produced 0.5% methanol (Dato et al. 2005). Diverse yeast species are involved in the fermentation of palm wine including *Saccharomyces cerevisiae*, *Saccharomyces ludwigii*, *Schizosaccharomyces bailli*, *Candida parapsilosis*, *Pichia fermentans*, *Hanseniaspora uvarum* and *Candida fermentati* in addition to lactic acid bacteria and acetic acid bacteria (**Ohimain** 2016).

Cassava

Another biomass resource that we work on extensively is cassava. We started by assessing the wastes generated during cassava processing. We worked on the utilization of the wastes for the production of microbial enzymes for industrial use. We carried out energy analysis and environmental impacts of smallholder ethanol production from cassava feedstock for the replacement of kerosene household cooking fuel in Nigeria. We also worked on cassava bread

Nigeria is by far the largest cassava producing nation in the world, exceeding that of Brazil, Indonesia and Thailand. Nigeria cassava production has been increasing since 1960 when the country gain independence from Great Britain, reaching over 54 million tonnes in 2015. Several factors are responsible for the increased cassava production in Nigeria including the rising human population and favourable agro-climate for the crop, presidential cassava initiatives, increased research and development, increased business opportunities, greater access to loans and other farm inputs, increased infrastructure especially in rural areas and the renewed interest in the crop for the production of biofuels and as important industrial feedstock for the production of starch, beverages, flour, glucose syrup etc (**Ohimain** 2015a, 2015b, 2015c).

The Government of President Obasanjo selected cassava as a feedstock for industrialization. The Presidential Initiative on Cassava (PIC) identified some primary products from cassava that can be utilized industrially including starch, flour, chips and pellets. These products spurred cassava industrial revolution in Nigeria. Factories emerged which processed these primary products into high-end products including glue, modified starch, fuel and beverage ethanol, glucose syrup, food and animal feed. Also, cassava flour was partially substitute for imported wheat in the production of composite flour for the making of bread, biscuits, noodles and other confectioneries. Of the 32 million tonnes of cassava produced in 2001, 84% was consumed as food, while only 16% was utilized as industrial raw material. Among the several foods that cassava is processed into, gari is the most dominant. About 70% of cassava produced in Nigeria is processed into gari. Cassava processing to gari is dominated by the smallholders.

A flow chart for the traditional processing of raw cassava tubers to a toasted granule, gari is presented in Figures 20 and 21. Our study shows that for a given unit of raw cassava, gari yield is about 34% while generating 30%, 19.8% and 16.2% of solid, gaseous and liquid wastes respectively. Wastes are typically generated during the processing of agricultural feedstocks to products. Cassava processing to gari generates liquid effluents (whey), solids (mostly peelings and sieviates) and gaseous emissions. Cassava waste waters have high COD more than 32,000 mg/L, high BOD (16,000 mg/L), suspended solids (15,000 mg/L), low pH (3.8 – 4.2) and high cyanide content in the range of 10.4-274mg/L. Cassava processing wastes is quite toxic to fish and domestic animals. In other countries, especially in Brazil, China and Thailand with large-scale cassava bio-ethanol refineries, these by-products are processed to produce animal feed, fertilizer and biogas for electricity generation and ethanol via microbial processes. In Nigeria, where cassava is mostly processed by smallholders, the associated waste streams are not treated by disposed freely into the environment. Fermentation odours are common in major cassava processing communities in Nigeria like Okada, Ibillo, Omotosho, Ologbo, Ijebu and Mosogar.

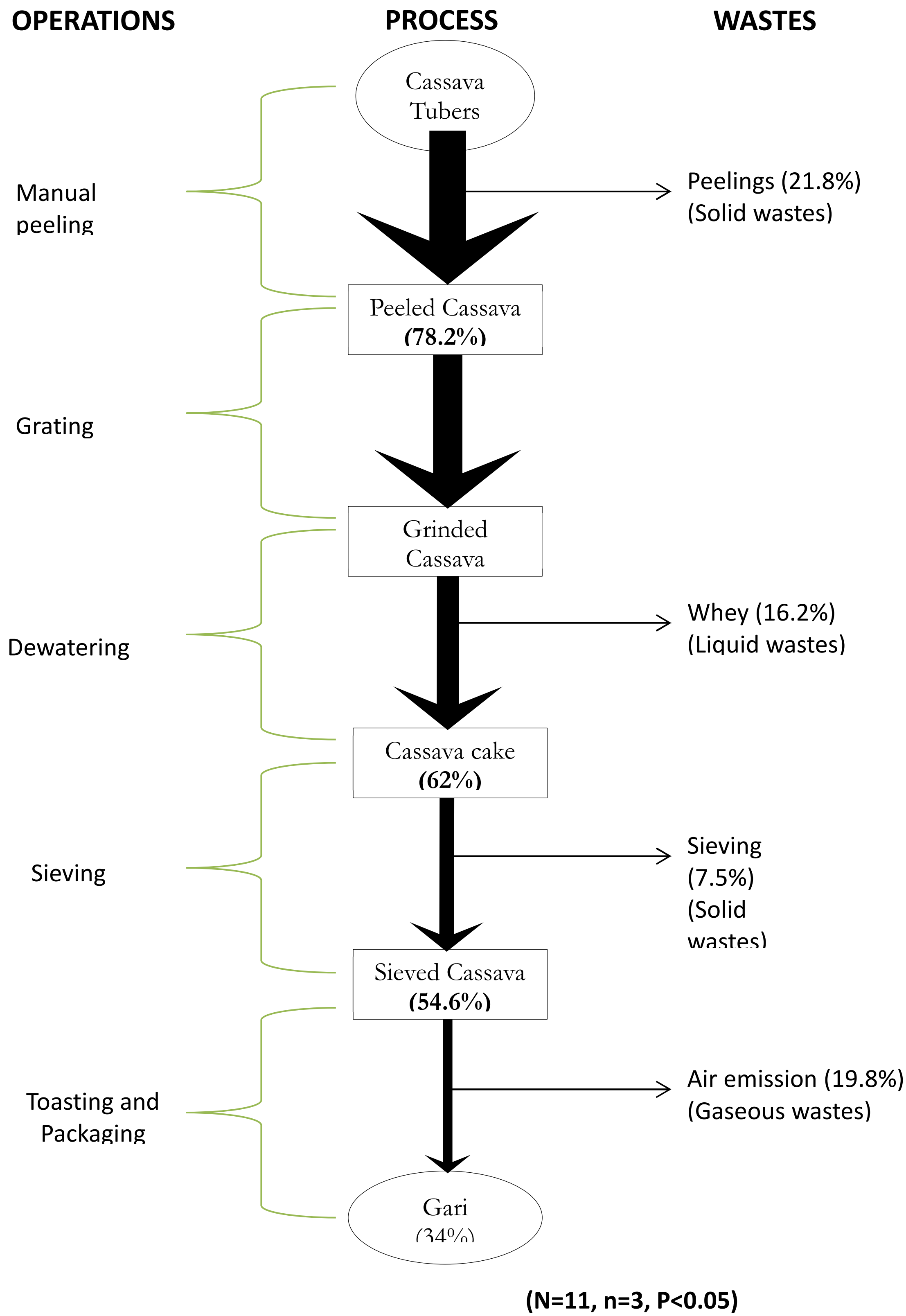
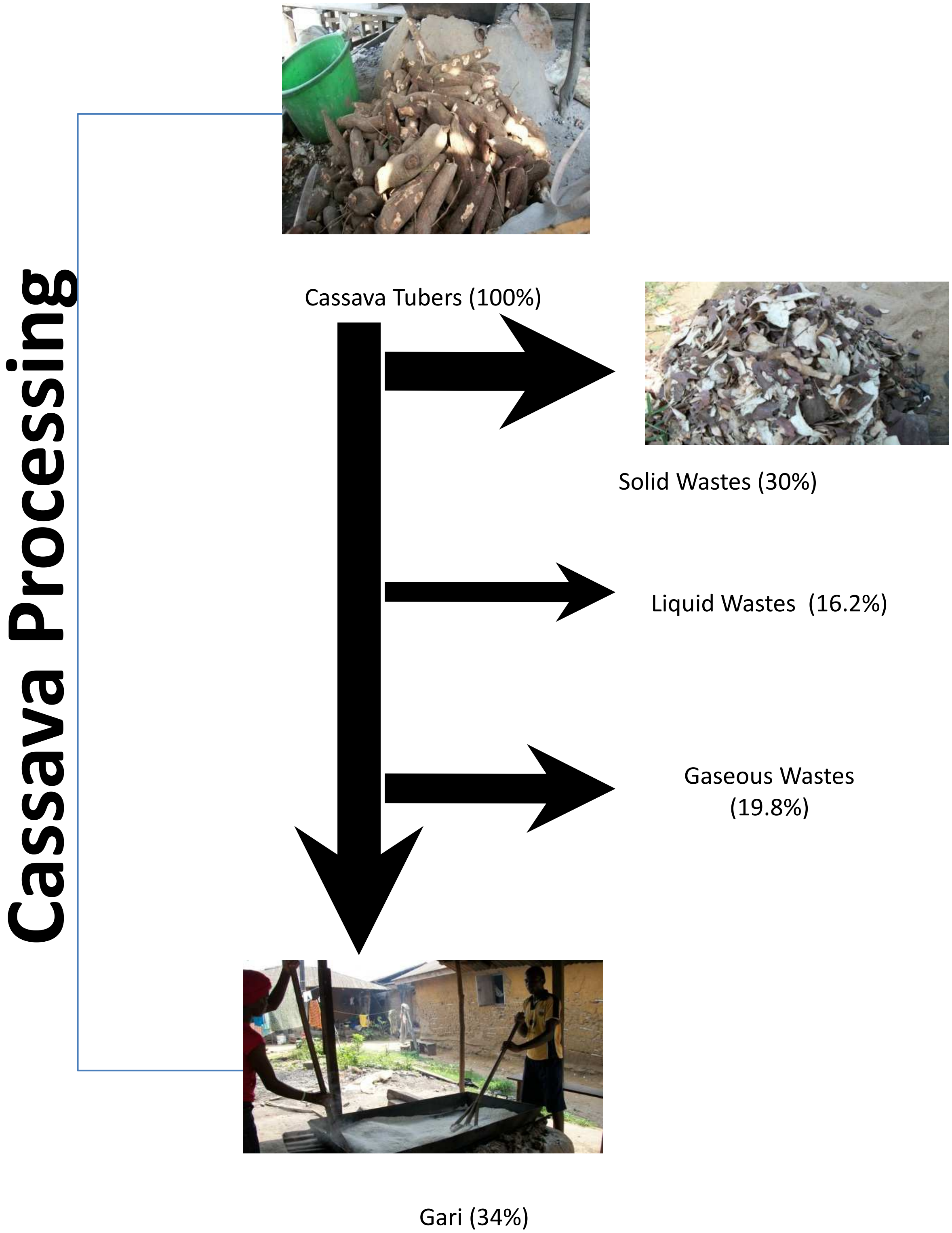


Fig. 20: Analysis of waste stream during the traditional processing of cassava to garri (**Ohimain** et al, 2013b)



(N=11, n=3, $p<0.05$)

Fig 21. Summary of waste streams generated during the conversion of Cassava tubers to garri (Ohimain et al, 2013)

Cassakero

During the President Obasanjo's regime, Nigeria attempted the replacement of kerosene with bioethanol produced from cassava feedstock in a project called 'cassakero'. The Nigerian national cassakero project that was designed to replace kerosene cooking fuel with bioethanol produced from cassava feedstock by smallholder agro-processors. The replacement of paraffin created an immediate demand of 3.75 billion litres of ethanol per annum. Potential environmental impact associated with the cassakero project is the production of liquid and solid waste streams, which could cause acidification, eutrophication, and aquatic toxicity and biodiversity impacts arising from the conversion of 400,000ha of forest to farmland. Notwithstanding, these waste streams could be converted to useful by-products including fertilizer, animal feeds, biogas and co-generated electricity using currently available technologies (**Ohimain** 2013, 2013; 2013; 2014; 2015, 2015).

The cassakero project target the installation of 10,000 units of small scale bio-ethanol refineries across the entire country to produce the daily ethanol cooking fuel requirement for 4 million families. The bio- ethanol refinery would be installed, owned and operated by private companies, and individuals in local cooperatives. About one billion US Dollars was been provided in a trust fund to kick start the project. The initial target would be to establish 1,000 units of small-scale bio-refineries to produce 400,000 litres of ethanol daily over the next one year, which would be increased to four million litres within four years. The refineries, which would be established in the rural areas, would each produce 400 litres of cassakero per day. The project also includes the establishment of an out growers-based feedstock supply system that will produce 8 million tonnes of cassava at an average yield 20 tonnes/ hectare/year from 400,000 hectares. To ensure steady supply of cassava for the feedstock, the Nigerian Cassava Growers Association has signed a $374 million contract for the supply of the 8 million tonnes of cassava tubers. The four-year renewable out grower contract would benefit over 250,000 cassava farmers across the country. The construction work of the first batch of ethanol small-scale refinery under this project has commenced in Edo State. The benefits of bio-energy are optimized when rural farmers are fully involved not only in the production of feedstock and employment in large-scale plantation, but in small scale cassava processing to flour/chips, ethanol conversion and other activities in the ethanol market chain. With small scale refineries, bio-energy could provide the basis for sustainable rural employment, income generation and boost the rural economies. This noble project, which involved the construction of 1000 mini-ethanol refineries, is now abandoned.

Cassava flour and bread

Bread is one of the most widely consumed foods in the world. In Nigeria, bread has become the second most widely consumed non-indigenous food after rice. The rapid urbanization, increasing population and changing food habits have resulted in the preference for convenient foods such as bread, biscuits, and other baked products. Bread is normally made from wheat (*Triticum* sp) flour dough that is cultured with yeast (*Saccharomyces cerevisiae*), allowed to rise, and baked. Bread-making is an age-long tradition in many countries. In biblical times, yeast is referred to as leaven. Its use in breadmaking determines the type of bread, whether leavened (conventional/food) bread or unleavened bread used for Holy Communion.

Despite the increasing consumption of bread in tropical countries, wheat performs poorly under tropical climates. Hence, huge amounts of money (foreign exchange) are spent on wheat importation annually in these countries. Nigeria spent ₦ 635 billion Naira (US $1 = ₦ 156) on the importation of wheat in 2010 alone. The country is therefore spending nearly ₦ 1.8 billion daily for wheat importation. Historical data of wheat importation by Nigeria is presented in Fig. 22. Nigeria now imports about 15 million metric tonnes of wheat flour yearly for the production of bread. The rippling effect of this high wheat importation in Nigeria includes loss of employment to foreign nations, food insecurity, increase in food miles, trade imbalance, overdependence on foreign foods, loss of foreign exchange, high domestic prices of bread, etc. Nigeria is planning to reduce these negative trends by partially substituting wheat with cassava for the production of composite bread.

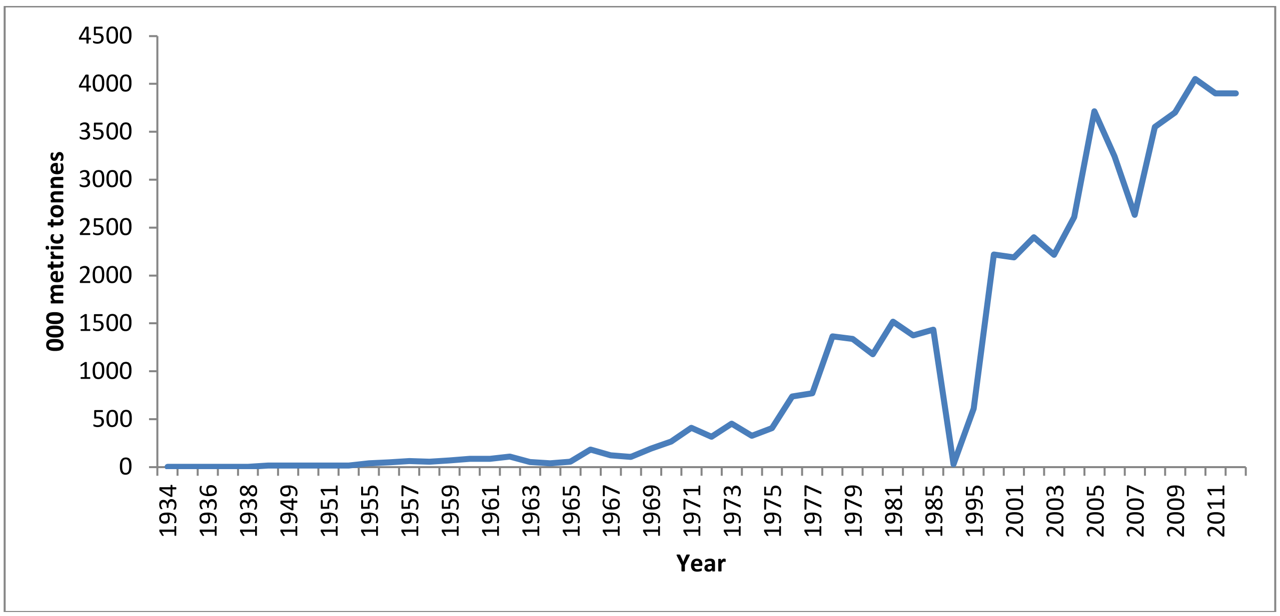


Fig. 22: Nigeria's wheat importation statistics (Source: Ohimain 2014)

Cassava inclusion policy

The Federal Government of Nigeria (FGN) released the 40% cassava inclusion in wheat flour policy which became effective 15 July 2012. The policy mandated flour mills and bakers to incorporate 40% cassava flour into wheat flour for the production of composite flour that will be used for bread making, pasta and other confectionaries. The policy is aimed at accelerating the pace of cassava utilization in the country and to make Nigeria both the largest producer and processor of cassava products. The benefits of the policy include increased domestic agricultural productivity, food security, foreign exchange savings, employment generation, and wealth creation. The 40% cassava inclusion in flour policy is expected to reduce wheat importation, spur rural development, create jobs, and help to reduce the trade imbalance between Nigeria and wheat producing countries. By implementing the policy, Nigeria planned to expand the current cassava production from 35 to 51 million tonnes per year. 40% inclusion could require 1.2 million tonnes of high quality cassava flour (HQCF)/year, an equivalent of 4.8 million tonnes of cassava tuber per year. Incentives included in the policy listed in Table 21:

Table 21: Nigeria wheat substitution policy incentives and potential benefits

Policy aspect	Content
Cassava bread policy	Nigeria is committed to the inclusion of 40% cassava in composite flour with effect from 15 July 2012
	The policy provided for a changeover period of 18 months for flour miller and bakers to switch to composite flour.
Policy incentives	Waivers on the importation of bread improvers, cassava processing and flour milling equipment
	12% tax reduction on cassava flour utilization for flour millers
	Provision of free starter packs of composite flours and bread improvers for 100 kg of bread for smallholder bakers
	Provision of 100kg fertilizer at 50% discount and 15 bundles of improved cassava varieties for free to smallholders' cassava farmers
	Additional 65% duty on wheat flour importation to the initial 35% duty (total duty 100%) and 15% duty to the initial 5% duty on wheat grain (total duty 20%)
	Creation of cassava bread development fund to be funded by the excess money realized from the importation of wheat, which shall be used for training, research, development and demonstration
	Training of about 400,000 master bakers in Nigeria
	Provision of loans to cassava processors for the purchase of equipment
	Ban on the importation of cassava flour
Potential benefits	Savings of the Nigeria's foreign exchange earning of N 254 billion per annum
	Reduction in the severity of coeliac disease via gluten dilution
	Utilization of locally available crops, thus eliminating glut
	Creation of massive employment in both farm operation and flour milling leading to an improved source of income and livelihood

Several challenges that made previous policies fail can threaten the current attempt including;

- Poor HQCF supply chains.
- Weak policy implementation.
- Reluctance of millers to use cassava flour.
- Technological challenges.
- Insufficient capacity of HQCF processors to meet the policy demand.
- Insufficient policy incentives.
- Processing challenges.
- Weath smuggling
- Quality challenges especially concerning flour fermentation resulting in low pH and high cyanide levels.
- Cassava bread policy instability.

Mister VC, permit me to expand on the issue of inconsistencies in the cassava bread policy. Policy inconsistencies between different governments are another challenge of the cassava policy. Over the past 20 years, the policy on cassava inclusion in bread has been unstable among successive governments. There was a total ban of wheat importation during the period of 1987 – 1990

(President Babangida), whereas from 2002 – 2007 (President Obasanjo), cassava inclusion policy in bread was 10%, which was subsequently reduced to 5% during 2007 – 2010 (President Yar'Adua). In fact, beyond 2007, most flour millers reverted to the use of 100% wheat flour, until the Jonathan administration released the 40% cassava inclusion policy in 2012. The current government of President Burahi has been silent on cassava bread. But the abandonment of cassava bread is another opportunity missed for cassava industrialization.

Bread improvers

Increasing substitution of wheat beyond 10% will require the use of bread improvers. Generally, improvers are preparations/additives intended to simplify the production of baked products to compensate for changes in the processing properties due to fluctuations or substitution of raw materials and to improve the quality of baking products. Recent technological advances that have been applied to increase the quality of highly substituted wheat bread (> 20% substitution), wheatless and gluten-free bread involves the use of additives and improvers such as modified starches, hydrocolloids and gums, proteins, enzymes, emulsifiers, chemicals and sourdough (Table 22). All these bread improvers are currently being imported. The big question is 'why should the government restrict wheat importation', only to approve the importation of bread improvers. Nevertheless, there is opportunity for the development of local bread improvers, which could also create jobs and boost the economy (**Ohimain** 2014a, 2014b, 2014c, 2014d, 2015a, 2015b, 2015c, 2015d).

Table 22: Improvers required for 40% CCW bread

Types	Examples of bread improvers
Enzymes	Lipase, amylase, hemicellulase, cellulase, glucanase, xylanases
Hydrocolloid and gums	Xanthan gum, guar gum, carrageenan, agar, carboxyl methyl cellulose
Emulsifiers	Glyceryl monostearate, sodium lauryl lactyylate, casinate, lecithin
Lipids	Margarine, vegetable oil
Animal proteins	Egg, milk
Oil seeds / proteins	Soya, peanut, cowpea, Bambara groundnut
Chemicals	Acids (ascorbic acid, lactic and acetic acids), oxidizing agents (potassium bromate, calcium peroxide), reducing agents (sorbic acid, sodium dioxide, sodium meta bisulphate)
Vitamins	Vitamin A, vitamin C
sourdough	Saccharomyces cerevisae and Lactobacillus

Mister VC sir, please permit me to throw more light on enzymes, since they are mostly of microbial origin. Enzymes are essential for the production of composite, wheatless and gluten-free bread. Enzymes are often added to improve dough- handling properties and bread quality and impact positively on shelf life, retro-degradation, water-holding capacity and crumb structure depending on the type of enzyme. For instance, xylanases make dough more tolerant of different flour qualities and variation in processing parameters, thus facilitating wheat substitution with other flour. Table 23 present a list of enzymes and their possible roles in bread making. Fungal α amylases and hemi-cellulase have been shown to increase bread volume and crumb structure. Emulsifiers can be produced from fatty acids by lipases. Modern enzyme preparations are capable of compensating for the loss in volume resulting from the composite flour as compared to wheat flour. Certain enzyme preparation based on protease and amylases allow the reduction or elimination of some expensive ingredients such as sugar, milk or whey powder, thus reducing the cost of production. Microbial transglutaminase (MTG) is increasingly being used to improve the quality of food products in the baking, meat, fish, soya bean and dairy industries. Addition of MTG to rice for instance, create a protein network through the formation of covalent cross-links between the polypeptide chains in the flour, thus enhancing the bread making properties of composite rice flour. Other enzymes useful in bread making are laccase, glucose oxidase, amylo-glucosidase, xylanases, pentosanases, proteases, cellulose, β-glucanases etc. Most of these enzymes are of microbial origin.

Table 23: Some Enzymes used in bread making

Substances	Roles
Amylases	Improving agent
	Antistaling and sweetening agent
Laccase	Improve Stability
Lipase	Flour improver, emulsifier
Glucose oxidase	Improving agent
	Dough conditioner
Transglutaminase	Improver
Protease	Improving agent
	Dough conditioner
Hemicellulose, xylanases	Increase bread volume
	Dough conditioner
cellulase	Improving agent
glucose oxidase	Used to replace potassium bromated as oxidizing agent
	Dough conditioner
B glucanases	Improving agent
Lipoxygenase	Oxidize fat, bleaches flour pigments
Lactase	Sweetening agent

Grassess

Grasses are becoming more useful than previously thought. No wonder that the minister of Agriculture planned to import grass from Brazil for cattle feed. Two grasses we worked on are elephant grass, *Pennisetum purpureum* Schumach (**Ohimain** et al 2014a) and wild sorghum, *Sorghum arundinacea* (**Ohimain** and Izah 2016). Both grasses, which are quite abundant in the wild in the Niger Delta especially in Bayelsa State, were studied for their bioenergy potentials. Their extracts were studied for bioethanol production and their bagasse as fuel for power generation.

Elephant Grass

Wild strains of elephant grass, *Pennisetum purpureum*, occur as invasive weed especially in disturbed freshwater swamps. Monospecific stands of wild elephant grass grew luxuriantly in Bayelsa and Rivers states even without irrigation, pesticides and fertilizer application. They grow especially in farms and other areas where the natural vegetation had been disturbed. The weed is typically cut down and burnt during land preparation at the beginning of the planting season. This practice, in addition to releasing smoke, carbon dioxide and other greenhouse gases into the atmosphere, results in the loss of useful energy. Meanwhile, there is an impending energy crisis in Nigeria due to fuel shortages and power instability. Only 40% of the population is connected to the national grid and electricity generation in Nigeria is of poor quality and very unstable with blackouts occurring frequently. Liquid transportation fuel (gasoline and diesel) and cooking fuel (kerosene and LPG) are in short supply (**Ohimain** 2012, 2013e).

A study was undertaken to assess the productivity and bioenergy potentials of the grass. Triplicate samples of the wild elephant grass were randomly collected at ten different locations from Wilberforce Island, Bayelsa State. Liquid extracts were recovered from the grass, while the resulting bagasse was dried. The grass was found to have a biomass productivity of 7-11t/ha. The liquid extract was analysed and was found to have the following characteristics; pH (5.55 – 5.98), electrical conductivity (14,610 - 48,214 µS/cm), specific gravity (1.56 – 1.60), sugars (2.59 – 4.47%), and ethanol (1.36 – 2.85%), while the gross calorific heating value of the bagasse ranged from 15.76 – 17.07 MJ/kg, which compares with 16.46 – 18.10MJ/kg for sugarcane bagasse and 14.65 – 21.63 MJ/kg for agro-forestry wood. Yeast population was 10^6 cfu/ml in the grass extract. With these properties, the liquid extracts of elephant grass could be used as alternative feedstock for sugar and ethanol production, while the bagasse could be used as fuel for power generation via conventional steam turbine cycle (Fig. 23).

Notwithstanding the numerous advantages of ethanol, there are several challenges such as food versus fuel conflicts, forest destruction and conversion, negative energy balance, large volume of water consumption and stillage generation, shortage of feedstock etc. Five crops have emerged as dominant feedstocks for ethanol production in different countries; sugarcane (Brazil), corn (USA), sugar beets (Europe), cassava (Nigeria, China, Thailand) and sorghum (India, Philippines) (**Ohimain**, 2010c). Most of these crops are food crops in the various countries; for instance, cassava is a staple food to more than 70% of Nigerians, while maize is a staple food in Africa generally. However, the utilization of cellulosic ethanol tends to minimize the negative aspects of biofuel while significantly increasing the benefits. Cellulosic ethanol, often referred to as a secondary biofuel, is produced from non-food sources such as municipal solid wastes, wood wastes, short rotation crops, grasses etc. Grasses, particularly the C_4 species are now increasingly being considered for cellulosic ethanol production due to their more efficient photosynthetic pathway, high water use efficiency and low nutrient requirements.

Elephant grass possesses several advantages that made it suitable as bioenergy crop; the grass is perennial, it can be vegetatively propagated and it can withstand repeated cutting/ harvesting and regenerates rapidly. Due to its highly efficient CO_2 fixation, elephant grass is capable of producing 60 tonnes/ha/yr of dry biomass under optimal condition and 30 tonnes/ha/year under sub-optimal conditions. . The ability of elephant grass to produce adequate biomass under limited nitrogen levels is linked to the occurrence of diazotrophic nitrogen fixing bacteria (e.g. *Rhizobium*, *Frankia* and *Azospirillum*) with the grass, which augment the nitrogen requirement of the plant by fixing atmospheric nitrogen. These symbiotic bacteria have been demonstrated to boost the yield of rice. Other features that made elephant grass suitable for bioenergy purposes include the possibility of multiple harvest per year, high levels of fibre and lignin and low levels of nitrogen and ash. When burned in a biomass power plant, elephant grass can generate 25 times as much energy as the amount of fossil fuel input, which is several orders higher than the energy ratios of US corn ethanol (1:1) and Brazilian sugar cane ethanol (8:1) but comparable to Miscanthus (22 – 50:1). Like other cellulosic feedstocks, elephant grass has high cellulose (28%), hemicelluloses and lignin (12%), low ash (2.6 – 3%) (de Morais et al., 2009) and is considered adequate for power generation. Like sugarcane bagasse, elephant grass bagasse can be practically combusted in furnaces and boilers to produce steam for a Rankine cycle (steam cycle) for power generation (Fig. 24). Because of the lower content of sulphur in biomass, they are considered easier to gasify than coal.

A variety of energy carriers can be produced from elephant grass biomass via gasification including syngas, Fischer Tropsch (FT) diesel, FT gasoline, kerosene, ethanol, methanol, methyl tertiary butyl ether (MTBE), other ethers and power via the gas cycle. Bio-oil can be produced from elephant grass via pyrolysis. Elephant grass has other bioenergy applications including the production of ethanol, pellets, and briquettes. The bagasse has been considered as possible replacement of coal for iron and steel processing).

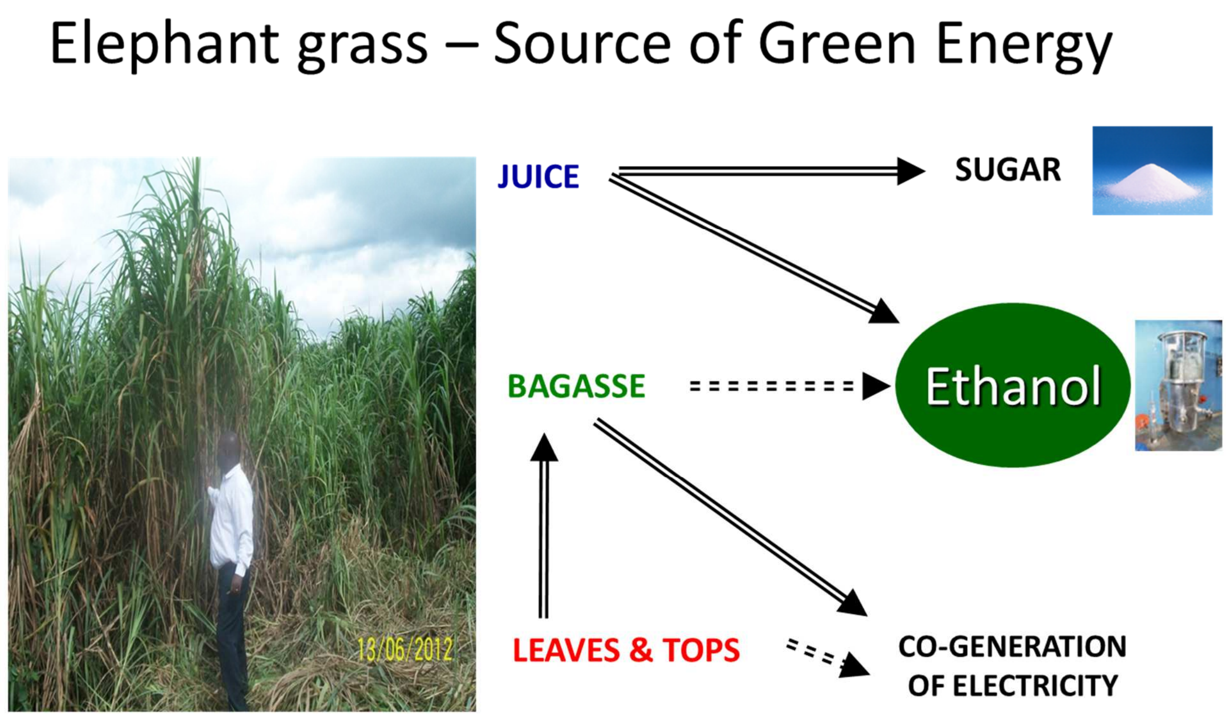


Figure 23: Elephant grass bioenergy

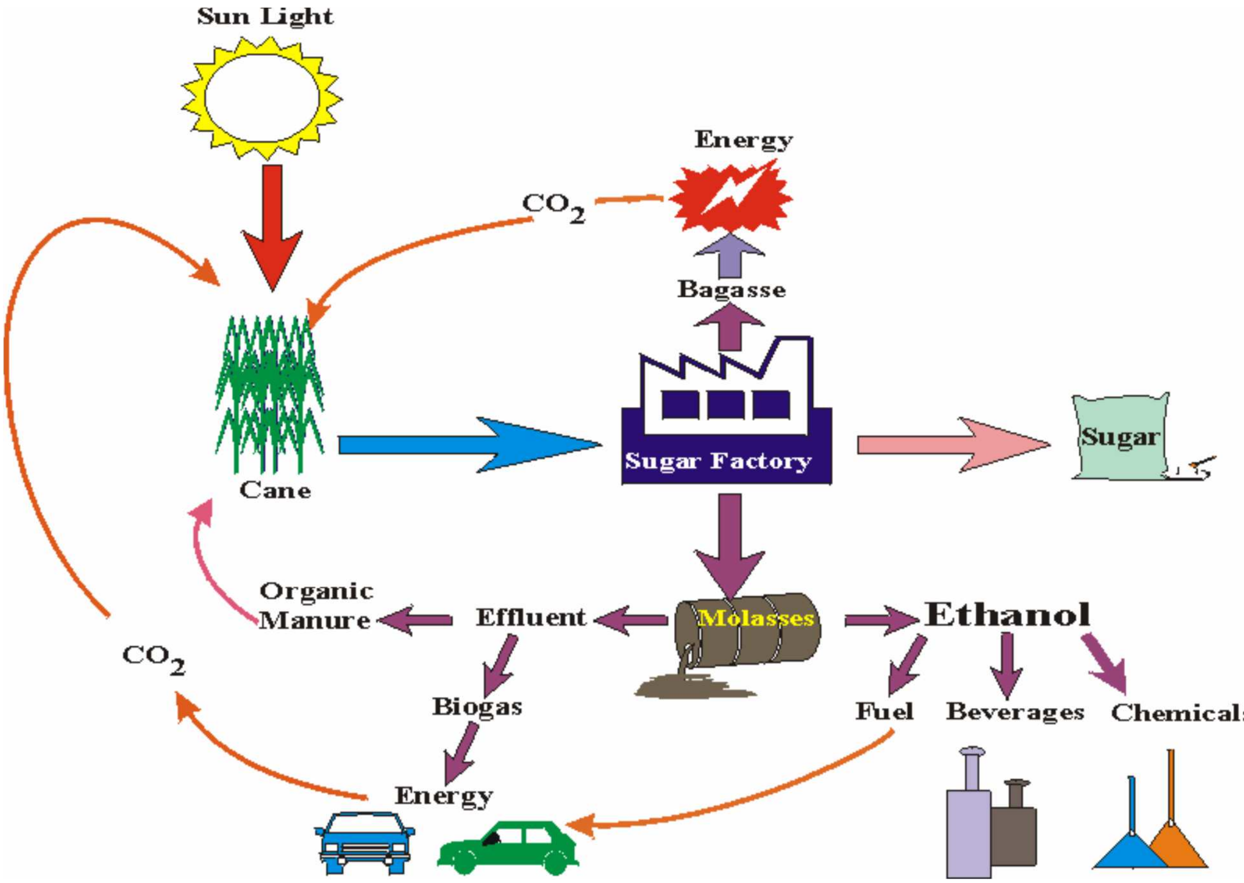


Figure 24: Sustainability of elephant grass bioenergy

WASTES AND WASTE WATER MICROBIOLOGY & MANAGEMENT

Waste management is a threat to sustainable development in Nigeria, where wastes are freely disposed-off into the environment without any form of treatment. Wastes emerging from industrial and domestic processing of biomass especially food, timber and other agricultural wastes abound in Nigeria. Such wastes are frequently dumped along road sides, street corners, gutters, rivers and other drainage channels causing environmental problems including water pollution, breeding of disease vectors, road accidents, blocking of drainage channels causing flooding, air pollution, nuisance smell and poor aesthetics. The indiscriminate disposal of wastes in Nigeria have led to many environmental impacts including the release of greenhouse gasses particularly carbon dioxide and methane into the atmosphere during degradation and combustion of biomass waste. Leachates containing heavy metals pollute the groundwater. Biomass waste dumpsite are now becoming breeding sites for insects and other disease-causing vectors including houseflies, mosquitoes, rodent, roaches etc (Abah and **Ohimain** 2010).

Diversity of wastes and treatment options

Mr VC sir, my team and I have worked on some of the waste stream found in the Niger Delta and our findings are summarized in Table 24. Generally, wastes could be reduced especially at source, recovered and reused.

Table 24: Waste sources, environmental effects and treatment options

Waste source	Environmental effects	Treatment/Applications	Reference
Cassava	Water pollution, toxic to domestic animals & fisheries	Production of microbial enzymes, biogas, animal feeds	Izah & **Ohimain** 2015; Ohimain et al., 2013b
Oil palm processing solid wastes	Poor aesthetics, environmental pollution,	Ethanol, biodiesel, briquettes, power generation	**Ohimain** and Izah, 2014, 2015
Oil palm processing liquid wastes	Environmental pollution,	Reuse, biogas, enzyme production	**Ohimain** et al, 2012c, 2013b; **Ohimain** & Izah 2016, 2017
Oil palm processing air emissions	Air pollution	Monitor, use scrubbers, recover waste heat	**Ohimain** et al 2013a; Ohimain & Izah, 2013, 2014
Plantain wastes	Poor aesthetics, environmental pollution,	Production of soap, biogas	**Ohimain** & Okezie (yet to be published)
Rice husk	Poor aesthetics, environmental pollution,	Production of microbial enzyme, mushroom cultivation	**Ohimain**, Ofongo (in press)
Saw dust and other wood wastes	Poor aesthetics, environmental pollution,	Production of microbial enzyme, mushroom cultivation, electricity	Mikiashvili, **Ohimain** et al 2011, **Ohimain** 2011a, 2011b, 2015e
Municipal solid wastes	Poor aesthetics, environmental pollution, breeding sites for disease vectors	Segregation, recovery, recycle and reuse; pyrolysis for bio-oil production	Abah & **Ohimain**, 2010, 2011
Cow dung and other animal solid wastes	Poor aesthetics, environmental pollution,	Biogas production	**Ohimain** & Agedah (yet to be published)
Scrap metal	Poor aesthetics, environmental pollution, breeding sites for disease vectors	Segregate and recycle	**Ohimain**, 2013c, **Ohimain** Jenakumo., 2013
Plastics	Poor aesthetics, environmental pollution, breeding sites for disease vectors	Segregate, recycle, biodeterioration using weevils	**Ohimain** et al 2015
Periwinkle shells	Poor aesthetics	Civil construction	**Ohimain** et al 2009b
Oyster shells	Poor aesthetics	Liming of acidic soils and fish ponds, chalk production	**Ohimain** & Ofiemya (yet to be published)
Medical wastes	Infectious	Incineration	Abah and **Ohimain**, 2011
Effluent	Sewage treatment plant	Monitor, treatment	Aghoghovwia, Ohimain et al 2015
Ballast water	Pollution and risk of alien invasive species	Monitor, treatment	Ojesanmi, **Ohimain** et al 2016
Heavy metal contaminated wastes	Environmental pollution	Biosorption, aerobic & anaerobic bio-precipitation	**Ohimain** 2001; **Ohimain** et al 2008a. 2008b

Plastic wastes

Plastic and polymeric materials constitute a major proportion of municipal solid wastes (MSW) both in developed and developing countries. In Nigeria, before the oil boom of the 1970s, plastic account for only 1% of the MSW, but it now accounts for 55-65%. The use of plastics is increasing globally. Plastics are now used by virtually all sectors of the economy. A very general estimate of worldwide plastic waste generation annually is about 57 million tons. Plastic wastes are mjor challenge to the environment (Plate 29).

MSW dumped in a river	Waste plastic recycling

Plate 29: Plastic waste; a menace to the environment

Nylon is a very important fibre and its market has grown greatly since its introduction. It is a thermoplastic polymer consisting of long chains of the monomer ethylene (ethene). The high strength, elasticity, luster, abrasion resistance, dyeability and shape-holding characteristics of nylon made it suitable for many applications. Polyethylene (Polythene) is one of the world's most popular plastics. It is an enormously versatile polymer which is suited to a wide range of applications from heavy-duty damp proof membrane to light, flexible bags and films. It is currently used in Nigeria in all forms and shades ranging from wrappers of biscuits, water, food etc. They retain their physical and chemical properties over a wide range of environmental conditions such as heat, cold and chemicals. They can resist mechanical stress for a very long period of time. Despite the importance of nylons, there are some environmental concerns (Plate 29). They are generally resistant to microbial degradation; hence, they tend to build up in the environment. They block water ways, pollute rivers, affect soil fertility and generally have poor aesthetic. The other option of disposal of plastic wastes, is by open air combustion, which pollutes the air and contributes to greenhouse gases. In our studies, we demonstrated the biodeterioration of nylon by three species of weevils, Tribolium, Sitophilus and Oryzaephilus (**Ohimain** et al 2015), thus, opening the polymer for microbial degradation ultimately. Plastic recycling is now increasingly being practiced at the artisanal level. It has also been demonstrated that plastics can be converted to crude oil by pyrolysis. At this stage of our national development, Nigeria require a definite policy on plastics to ensure environmental sustainability.

Metal wastes and recycling

Due to lack of better employment, waste pickers called scavengers have provided the service of recovering scrap metal from waste dump and selling them to scrap dealers, who will in turn sell them to metal recyclers. We investigated the scrap metal valorization value chain in Bayelsa State (Fig. 25). Scrap metal dealers and scavengers were interviewed while the weights of recovered scrap metals were measured. Results show that within 2 weeks, 32,209.65 kg of scrap metals were recovered by scavengers and sold to 4 major scrap dealers in Bayelsa State. Iron/ steel accounted for 82.84 – 86.19% of the total metal recovered, followed by aluminium which accounted for 6.09 – 9.00%, while copper accounted for 1.77 – 2.06%. The rates paid per kg of copper is N 500 -750 (US $ 1= N 156 exchange rate); brass is N 100 – 300; aluminium is N 70-90, while iron/steel is N 15 (Table 25). Based on the weight of the scrap metal received at the collation centre during the 14-day study period, the amount of money paid to the scavengers range from N 355,268.41 to N 452,830.97 from 102 - 133 transactions (Table 26. Hence, an average of N 25,376 - N 31,000 was spent daily on the scavengers. The scavenger makes N 2770 – 3565 per trip depending on the site. Meanwhile minimum wage in Nigeria is N 18,000/month, which some states are unable to pay due to lack of funds. Hence, the scrap metal recycling job appears to be more profitable than some formal employments. If these metal scraps are not recovered, there are microbes that specialize in the corrosion of metals, which will result in the leaching of heavy metals into the environment.

Table 25: Unit price (₦/kg) paid to scrap metal scavengers at the various dumps

Sites	Aluminum	Iron	Brass	Copper	Stainless Steel	Other metals
Sanni Abacha Express Way	80	15	100	500	30	200
Etegwe Primary School Road	90	15	300	500	30	180
Tombia road	80	15	250	650	40	200
Kalango/Amassoma	70	15	200	700	40	190
Average (N/kg)	80	15	212.5	587.5	35	192.5

1 US $ = ₦156 at the time of study

Ohimain and Jenakumo 2013

Table 26: Total amount (₦) paid to scavengers during the 14 day study period

	Site 1	Site 2	Site 3	Site 4
Aluminum	52,316.01	51,708.00	73,154.67	44,258.68
Iron	129,090.00	121,832.50	126,286.00	111,171.00
Brass	1,866.67	10,820.01	11,633.33	12,406.66
Copper	88,450.05	103,049.95	133,965.00	124,530.07
Stainless Steel	2,073.00	1,812.00	1,872.00	417.33
Other metals	92,013.34	79,247.95	105,919.98	62,484.67
Total sales (₦)	365,809.07	368,470.41	452,830.97	355,268.41
No. of cart trips	118	133	127	101
average amount (₦) paid/ trip	3,100.08	2,770.45	3,565.60	3,517.51
average amount (₦) paid/ day	26,129.22	26,319.32	32,345.07	25,376.31

1 US $ = ₦156 at the time of study. **Ohimain** and Jenakumo 2013

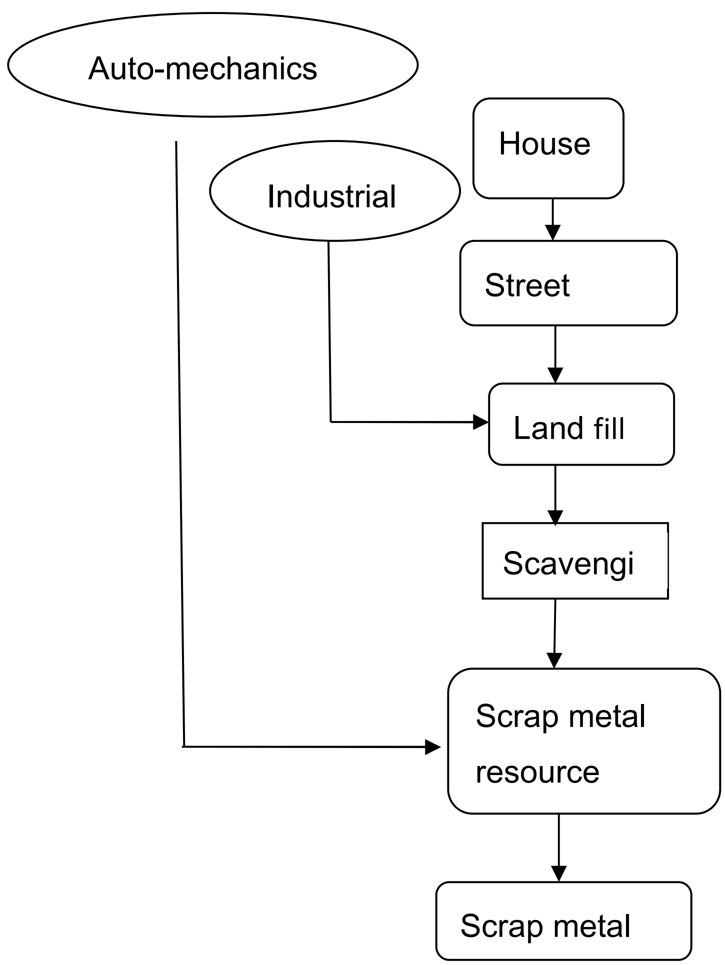


Fig. 25: Scrap metal recovery and recycling value chain in Bayelsa State

Roles of microbes in the iron and steel sector

Nigeria is blessed with abundant iron ore deposits of nearly 3 billion tonnes (Tables 27, 28) and other basic minerals for steel production including coal and limestone. The major ore deposits are located in six fields; Agbaja, Itakpe, Ajabanoko, Chokochoko, Agbade-Okudu and Nsude Hills. There are other deposits that are currently under investigation. The iron content of the proven ore reserves is in the range of 36 – 54%. Out of the six Nigerian iron ore field, only Itakpe, with a iron content of 36% was ever developed. The Nigerian Iron Ore Mining Company (NIOMCO) at Itakpe was designed to supply iron ore to both Ajaokuta (ASC)and Delta Steel Companies (DSC), which in turn ought to supply billets to rolling mills in the country. Nigeria has installed steel rolling capacity of 3.18 million tonnes per annum. Nigeria also installed 2 integrated steel plants, 13 rolling plants and 7 mini plants. Due to poor contracting strategy, Ajaokuta Steet Plant was never completed after 40 years of intermittent construction.

The Nigerian steel sector collapsed principally due to shortage of raw materials particularly iron ore for the integrated steel plants and billets for the rolling mills. The privatization of the steel sector that was done in 2004-2005 was unable to revive the sector. Our study found that the Nigeria steel sector is now being sustained through the recycling of scrap steel obtained mostly from municipal solid wastes (Plate 30). In many of the rolling mills, 100% scrap steel is recycled for the production of iron bars used for civil construction. Scrap steel recycling results in the generation of <7% and 7 – 15% slag for low and high carbon steel respectively (Fig. 26). There are energy and environmental benefits of scrap steel recycling for the production of new products and sustenance of the Nigerian steel sector.

Agbaja fields, which has the largest iron reserves (2 billion tonnes) and highest iron concentration (45 – 54%) is challenged by the presence of high phosphate in the range of 1.25% as P_2O_5. Phosphorus has been reported to cause deleterious problems in steel responsible for brittleness causing the steel to fracture even under low stress. Other problems associated with high phosphorous in steel include challenges of strong primary segregation during solidification of castings and formation of high brittle streaks between metal grains thereby impeding plastic deformation, hence the phosphorous level in steel should be less than 0.02%. Agbaja ore is very irreducible at 1100ºC because of sintering of the ore. There are also technical challenges during the beneficiation of Agbaja ore, which resulted in the abandonment of the field, until recently when an Australian firm started a fresh exploration and development of the field. This is one area that microbial technology invoving the removal of phosphorus can play a significant role in the processing of the iron ore. Anyakwo and Obot (2011) used the bacteria *Bacillus subtillis* to remove phosphorus from Agbaja ores. I had attempted to collect and work on these iron ore from Agbaja through the Raw Materials Research and Development Council, Abuja, but was unable to get samples thus far. Again, this type research require collaboration with geologist, chemist, metallurgical and materials engineers.

Table 27: Iron ore proven reserves in Nigeria

	Percentage occurrence (%)									Reserved (tonnes)	Extent of development as at 2012
Location	Fe	Fe_2O_3	SiO_2	Al_2O_3	CaO	MgO	P_2O_5	MnO	TiO2		
Agbaja	45 – 54	62.64	8.55	9.60	0.72	0.38	4.16	0.14	0.37	2 billion	Exploration & development ongoing
Itakpe	38 – 45	53.10	44.80	1.00	0.30	0.20	0.05	0.05	0.10	200 – 300 Million	Operational but moribund
Ajabanoko	35.61	47.74	0.41				0.11	0.05	0.06	30 million	Exploration & development ongoing
Chokochoko	37.43	47.65	4.30				0.05	0.52		70 million	Yet to be developed
Agbade Okudu	37.43	29.41	0.62							70 million	
Nsude Hills	37.43									60 million	

Ohimain (2013f)

Table 28: Nigeria iron ore deposits under investigation

Locations	State	Iron content, %
Muro Hills	Nasarawa	25 – 35
Dakingari	Kebbi	22 – 52
Tajimi	Kaduna	22 – 52
Ayaba	Kaduna	27.5
Rishi	Bauchi	14 – 19
Gamawa	Bauchi	40 – 45
Karfa	Borno	34 – 45
Eginija (Egenerga)	Benue	34 – 45
Oko	Anambra	34.4
Gbege		42.7
Ajase		39.0

Source: **Ohimain** 2013f

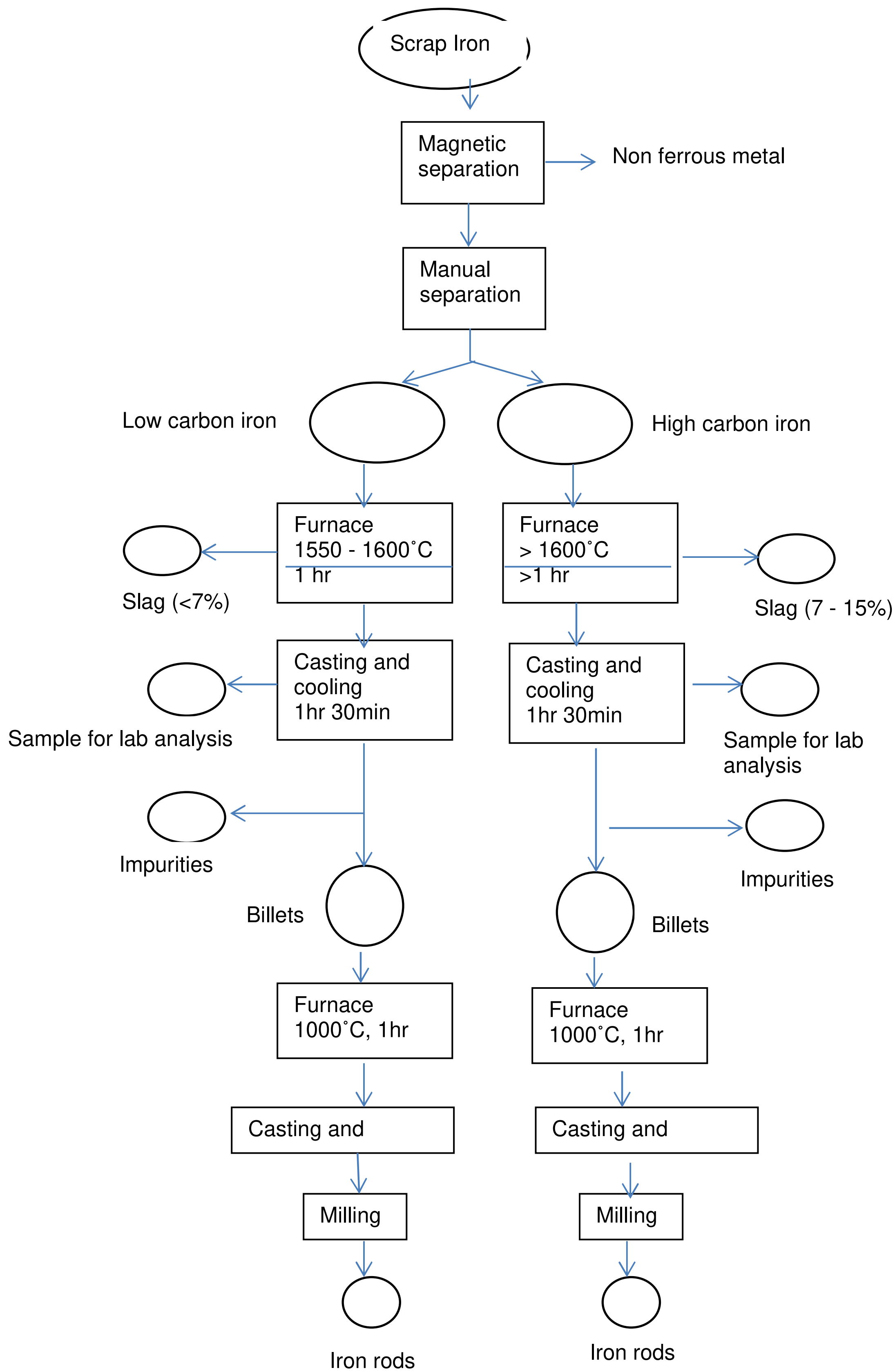


Fig. 26: Scrap iron and steel recycling in Nigeria (Ohimain and Jenakumo 2013)

Plate 30: Scrap metal recovery activities in Bayelsa State

Open Defecation

Due to lack or insufficient toilet facilities, sewage is often discharged into the rivers in most coastal communities in the Niger Delta (Plate 31). In most of these communities, they obtain water for domestic use upstream, bath at the middle stream and defecate at the downstream. This is quite scientific to some extent because the downstream of one community lead to the upstream of the next community. Hence, microbes particularly coliforms and *E. coli* are quite prevalent in most of our water bodies. It should be noted that coliforms and *E. coli* are indicators of faecal pollution, indicating that bad bugs could be present in the water such as *Salmonella*, *Shigella*, *Vibrio cholerae*, and enteroviruses. This practice can easily lead to the outbreak of *cholera* and other infectious diseases.

Statistics from the UN show that globally, 2.4 billion people lack access to basic sanitation services, such as toilets or latrines. Data from World Toilet Day Organization indicate that 4.5 billion people live without a household toilet that safely disposes of their waste. Over 80% wastewater resulting from human activities is discharged into rivers or sea without any form of treatment or removal of pollutant, resulting in about 1.8 billion people using a source of drinking water that is fecally contaminated. Each day, nearly 1,000 children die due to preventable water and sanitation-related diarrhoeal diseases. Nigeria was ranked third in open defecation after China and India. Over 122 million Nigerians lack access to basic sanitation. Only 58% of Nigerians have access to water, while 32% had access to sanitation. A recent study shows that about 70% of Bayelsans lack access to toilet facilities (Sample et al 2016). November 19 each year is celebrated as world toilet day. Sustainable Development Goal 6 is to ensure access to water and sanitation for all by 2030. Can this goal be achieved in Bayelsa State in the next 12 years?

Plate 31: floating/Over hanging toilets

Medical wastes

Medical wastes containing infectious microbes are freely dumped into the environment without treatment along with municipal wastes. In the course of scavenging, people including children are exposed to harmful bugs including HIV. Studies have shown that the microbiome of scavengers contained higher proportions of pathogens than that of non-scavengers.

Waste to energy (WTE)

Wastes contain energy that is lost to the environment when wastes are disposed indiscriminately or combusted openly. Instead of allowing waste biomass to constitute environmental problems, they could be converted to useful energy such as biogas, bio-liquids (methanol, ethanol, biodiesel) and biosolids (char, briquettes) and bioelectricity. Biogas produced through anaerobic digestion of plant residues, food wastes, human and animal dung can be used as household cooking fuel in place of LPG, for lighting, domestic and industrial heating, for boilers producing steam to run turbine to produce electricity. Biogas is also compressed and used to fuel cars. Apart from biogas production, the anaerobic digestion of biomass wastes produces bio fertilizer as by-products.

Akinbami et al (2001) reported that Nigeria produces 227,500 tonnes of fresh animal waste daily, which could be used to produce about 6.8 million m^3 of biogas daily (1kg fresh animal waste produces $0.03m^3$ biogas). About 20kg of MSW is produced per capita per annum in Nigeria. Since the population of Nigeria is over 270 million people, the biogas production potential from human sources is 162 million $m^{3.}$ However, on a dry weight basis, the total dung produced in the country is 19.5 million tonnes/year of which cow dung accounted for above 60% (Nwanchukwu and Lewis 1986). The authors also reported that gas yields of $340m^3$ can be produced per tonne of dry animal dung.

Crop residues, grasses and wood wastes especially sawdust are lignocellulosic biomass containing cellulose, hemi-cellulose and lignin (Table 29). There are conventional technologies for the hydrolysis of cellulose waste to sugar using enzymes, acid (dilute or concentrated) or alkaline prior to fermentation for the production of ethanol. There are also advanced technologies such as thermochemical for the conversion of lignocellulosic wastes to liquid fuels including methanol, ethanol, biodiesel and other biochemicals and for electricity generation (Zhang, 2010; Demirbas et al., 2001; Balat et al., 2009).

Some biomass waste streams such as MSW are heterogeneous consisting of biomass and non-biomass component. The challenge is therefore how to physically separate the waste stream to various components prior to biomass conversion. The commonest method is manual separation by scavengers who recover useful solid material from MSW including plastics, metals, wood, electrical and mechanical equipment part etc. In more advanced countries, other methods such as hydro-cyclone, sieves, magnetic method; etc are used.

Table 29: Component analysis of biomass (wt % on dry basis)

Biomass wastes	Calorific value (Kcal/kg)	Cellulose	Hemi-cellulose	Lignin	Ash
Bagasse	3406-4403.6	33.6-41.3	22.6-27.0	15.0-18.3	2.9
Coconut shell	3649	36..3	25.1	28.7	0.7
Corn cob		40.3	28.7	16.6	2.8
Corn stalks		42.7	23.6	17.5	6.8
Rice husk	3000-3618	31.3	24.3	14.3	23.5
Rice straw	3730	30.2-41.36	24.5-22.7	11.9-13.6	16.1-19.8
Municipal waste	1345-3376				27.5-70.0

Modified from Kaushik et al. (2007).

PUBLIC HEALTH MICROBIOLOGY

Bad bugs are can be a major threat to sustainable development on every scale, short-, medium- and long-term basis. They can cause disease that lead to death within a short time e.g. Ebola virus or long time e.g. HIV. In this era of heightened terrorism, bio-terrorism using harmful bugs is now a valid threat. Super bugs resisting all currently available antimicrobial agents are now in circulation. Weaponized microbes were used during the second world war and in the cold war that followed. Because of the self-replicating nature of microbes, bioweapons are difficult to control, and its effect could transcend generations.

Activities causing the spread of pathogenic microbes

Many activities contribute to the spread of microbes including pathogens:

- Open defecation
- Discharge of untreated animal slaughter waste into the environment
- Indiscriminate dumping of municipal wastes
- Dumping of healthcare waste into the environment without treatment
- Food processing under unsanitary conditions

Ebola virus disease outbreak in Nigeria

Vice Chancellor sir, Nigeria had a brief encounter with Ebolavirus in 2014. The sequence of events that followed involved 20 cases with 8 fatalities. News pertaining to Ebola virus disease (EVD) dominated the media in 2014. I published 4 papers in my study on ebolavirus disease.

Ebola virus was imported into Nigeria on 20 July 2014, when a 40 years old Liberian-American ECOWAS diplomat flew from Monrovia to Lagos via Togo. The diplomat was sick on arrival and was attended to in a private clinic in Obalande, Lagos. The diplomat (index case) claimed to have been sick of malaria; hence the medical staff that attended to him did not wear any special personal protective equipment (PPE). But when the diplomat began exhibiting EVD symptoms, samples were sent to the lab, which were positive for EVD (24 July 2014). The index case died of EVD on 25 July 2014 after infecting other people that triggered the spread of Ebola virus in Nigeria.

Figure 27 presents the chronology of Ebola virus infection and three-generational spread in Nigeria. Among the people that got infected were 2 ECOWAS associates of the index case and health workers at the hospital that attended to the index case. Of the 2 ECOWAS associates of the index case that became infected with EVD, one of them an ECOWAS protocol officer, age 36 died in Lagos on 12 August 2014 of EVD, while the other travelled to Port Harcourt (a distance of about 435 km from Lagos) for medical attention, which led to the spread of Ebola virus to Nigeria's largest oil city. About 4 persons contacted EVD in Port Harcourt including the doctor that attended to the diplomat, his wife and sister and an elderly woman. The Port Harcourt doctor and the elderly woman later died of EVD, while the others recovered. One of the nurses that contacted EVD in the hospital that attended to the index case in Lagos, travelled to Enugu, which raised fears of possible spread of the virus to the coal city. The nurse later recovered, and 25 persons were placed on surveillance at Enugu, none developed EVD. In all, including the index case, 20 persons were infected with EVD, while 12 persons recovered, 8 died, giving a case fatality rate of 40%, which is an improvement over the 90% fatality rate of the Zaire Ebola virus, the causative agent of the 2014 EVD outbreak in West Africa. Of the 20 cases reported in Nigeria, 8 were healthcare workers.

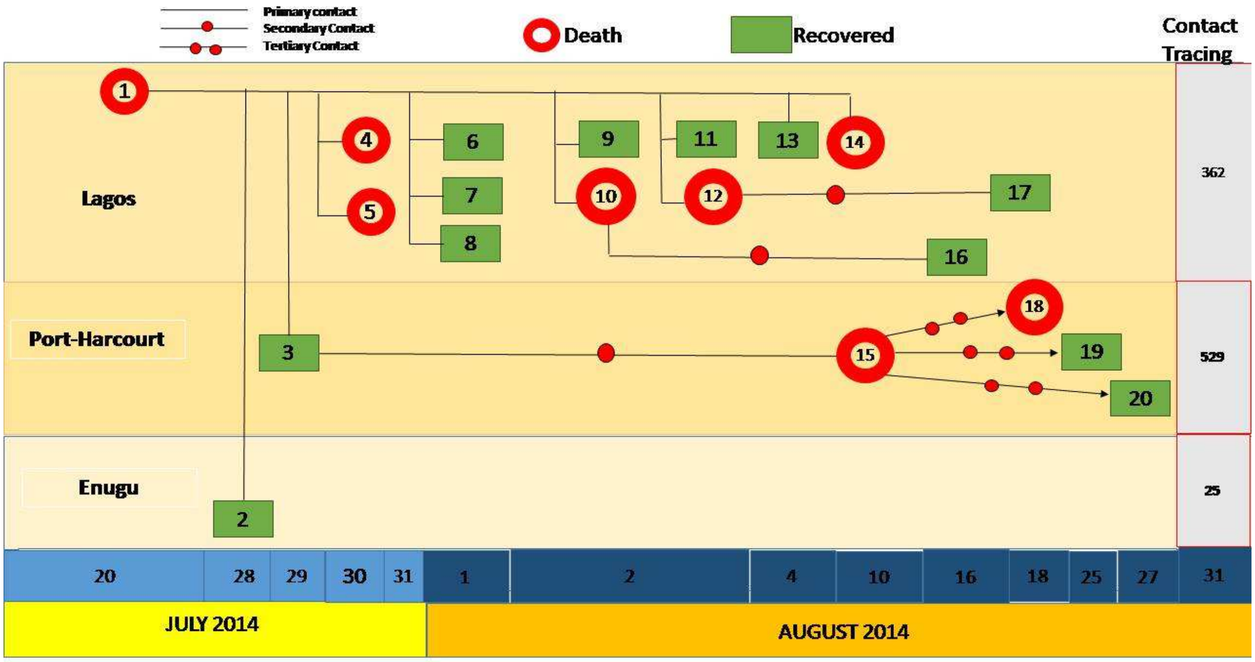


Figure 1: Chronology and spread of Ebola virus infection in Nigeria (x axis not drawn to scale)
Source: **Ohimain** 2015g

Ebola case #

1. Index case, a Liberian-American ECOWAS diplomat, brought Ebola virus to Nigeria via air travel, male, 40 years old, died 25/7/2014.
2. Nurse, female, travelled to Enugu by road, recovered.
3. ECOWAS diplomat, male, associate of index case, travelled to Port-Harcourt by air, recovered.
4. ECOWAS protocol officer, male, associate of index case, 36 years old, died 12/8/2014.
5. Female nurse, died 2/8/2014.
6. Patient that contracted the disease nosocomically, recovered.
7. Patient that contracted the disease nosocomically, recovered.
8. Patient that contracted the disease nosocomically, recovered.
9. Patient that contracted the disease nosocomically, recovered.
10. Female nurse, 25 years old, died 15/8/2014.
11. Health care worker that contracted the disease, recovered.
12. Female doctor that attended to the index case, died 19/08/2014.
13. Health care worker that contracted the disease, recovered.
14. Health care worker at the hospital where index case was treated, died 13/9/2014.
15. Port-Harcourt male doctor that treated case #3, died 22/8/2014.
16. Spouse to case #10 male recovered.
17. Sibling of case #12, recovered.
18. Elderly woman, died 31/8/2014, contracted EVD from #15
19. Spouse of case #15, Female medical doctor, not nosocomially transmitted, recovered.
20. Sister of case #15, recovered.

Nigeria's Response to EVD

Despite the devastating effects of EVD in neighbouring West African countries particularly Guinea, Liberia and Sierra Leone, Nigeria was not prepared. So when the index case brought EVD into Nigeria, the country was surprised. But Nigeria subsequently acted swiftly and appropriately to curtail the spread of the virus.

As soon as the Ebola disease outbreak was announced in Nigeria, the country began contact tracing (to break the chain of transmission) of all the people that had contact with the index case. As of 24 September 2014, a total of 814 contacts in 26,000 households, 18,500 face-to-face visits in 3 states (Lagos, Rivers and Enugu) have been monitored. Suspected cases were reclassified as confirmed case if the reverse transcriptase-polymerase chain reaction (RT-PCR) detect Ebola virus in a blood specimen. For PCR negative suspected cases, testing for anti-Ebola virus immunoglobulin G was also carried out using ELISA test kits. Positive EVD patients were isolated/quarantined and given intensive care, which involved the use of intravenous and/or oral rehydration therapy (ORT) and treatment of opportunistic infections, maintaining adequate oxygen level and blood pressure.

Then, there were no approved vaccines or drugs for the treatment of Ebola virus infections. Notwithstanding, some vaccines and drug for EVD were under development and some have entered clinical trials. During Ebola virus outbreak in Nigeria, the country attempted using nano-silver for treatment, but was discouraged by USA, despite the proven antiviral properties of nano-silver. But there are indications that some nano-silver especially the chemically synthesized ones can be toxic to humans, hence the Nigerian National Health Research Ethics Committee (NNHREC) did not approve the use of nano-silver, but on 9 August 2014, permitted the use of experimented drugs on humanitarian grounds. Nigeria subsequently requested for ZMapp, which US never supplied until the virus was controlled in Nigeria. In the absence of foreign drugs, the use of locally available antimicrobials of plant origin especially bitter kola (*Garcinia kola*), which have antiviral properties was considered, but was not used for EVD in Nigeria, perhaps because its effectiveness specifically against EVD have not been demonstrated. Hence, it will be unwise trying it out in the midst of an emergency. Nigeria also requested for the use of TKM-Ebola from Japan Again, it was not available for use in the country before EVD was eventually curtailed. TKM-Ebola has strong antiviral activity against influenza virus and has entered Phase 3 clinical trial for influenza virus and has commenced phase 1 trial for Ebola virus. During the outbreak, there were rumours of using table salt (NaCl) as protection against EVD. At least about 2 people died of excess salt consumption in Jos in an attempt to acquire protection against EVD. Some experimental drugs and vaccines used for the control of Ebola are listed in Tables 30 and 31 respectively. These drugs and vaccines were designed against come components of the ebolavirus include glycoprotein, nucleoprotein and viral protein (Fig. 28).

Table 30: Leading first generation experimental therapies for Ebola virus disease

Therapy	Manufacturer	Mode of action	Phase of development	Administration route	Remarks
ZMapp	Mapp Biopharmaceuticals Inc, USA	Cocktail of 3 monoclonal antibodies (c13c6, h-13F6, c6D8) binds and inactivates virus	Phase 1	Intravascular	Administered to 7 people of which 2 died. Completely protected monkeys from EVD
TKM Ebola	Tekmira Pharmaceuticals, Canada	si RNA which interferes with L, VP24, VP35. Gene silencing	Phase 1	Intravascular	Protect monkeys infected with Ebola virus. Used for humans under emergency in the 2014 EVD outbreak
Favipiravir (T-705 or Avigan)	Toyama Chemical/Fuji Film, Japan and Medi vector USA	Nucleotide analog that inhibits L. Broad spectrum antiviral agent. RNA chain terminator or lethal mutagenesis	Phase 1 (Ebola virus); Phase 3 (influenza)	Oral	Protect mice infected with Ebola virus
AVI 6002	Sarepta Therapeutics, USA	Antisense PMO which inhibits VP24. Gene silencing	Phase 1	Oral or intramuscular	
BCX 4430	Biocryst Pharmaceuticals, USA	Nucleoside which inhibits L. Broad spectrum antiviral agent. RNA chain terminator	Phase 1	Oral or intramuscular	Protect monkeys infected with Ebola virus
Brincidofovir (CMX-001)	ChimerixInc, USA	Ebola: unknown; CMV inhibits DNA synthesis	Phase 1 for Ebola virus, Phase 3 for CMV & ADV	Oral	Used for humans under emergency in the 2014 EVD outbreak

Sources: (**Ohimain** 2015h)

Table 31: Promising first generation Ebola virus vaccine candidates

Vaccine	Manufacturer	Mode of action	immunogen	Type of protection	Adm. Route*	Development status	Dose	Remarks
A. REPLICATING								
rVSV – ZAIV- GP	Newlink Genetics/ PHAC	Stimulates immune response to Ebola GP using rVSV	GP	Post exposure / Prophylactic	IM or oral	Phase 3 ongoing	Single	Replicating (Donated to WHO during outbreak)
Vesiculo Vax Ebola	Profectus Biosciences Inc	Viral expression vector (VSV)	GP	Post exposure / Prophylactic	IM or oral	Phase 1 ongoing		Trivalent vacccine
RABV vaccine	NIAID & Thomas Jefferson University	Viral expression vector	Live attenuated, GP		IM	Preclinical		
rCMV	Plymouth University	Viral expression vector	GP, NP, VP40, VP35	Post exposure / Prophylactic	IN, IM, IP	Preclinical	single	Disseminating vaccine, long lasting
rHPIV3		recipients exhibited high EBOV-specific IgG, IgA, and neutralizing antibody titers	GP, NP, GP/NP	Pre-exposure / Prophylactic	IN, IM	Preclinical	Single	Potential problems with pre-existing immunity
B. NON-REPLICATING								
cAd3 – EBOV	Glaxo SmithKline	Viral expression vector (Adenovirus)	GP, NP, GP/NP	Prophylactic	IM	Phase 3	Single dose	
Ebola rAd5	Johnson & Johnson (Crucell/ Bavarian Nordic)	Viral expression vector (Adenovirus)	GP, NP, GP/NP, GP/VP40	Pre-exposure / Prophylactic		Preclinical (Phase 1 ongoing)	Multiple dose	Not affected by pre-existing Ad immunity
Ebola GP Ad5	University of Texas at Austin	Viral expression vector (Adenovirus)	GP, NP, GP/NP	Prophylactic	IM	Phase 1		
VLPs			GP, NP, GP/NP, VP40/NP /GP	Pre-exposure / Prophylactic	SC	Preclinical	Multiple dose	
FiloVax (rVLP protein)	Integrated Bio-		GP, NP, GP/NP, VP40/NP	Pre-exposure /	SC	Preclinical		

Vaccine	Manufacturer	Mode of action	immunogen	Type of protection	Adm. Route*	Development status	Dose	Remarks
	Therapeutics Inc			Prophylactic				
SynCon	Inovio pharmaceuticals Inc	Plasmid expression vector	GP, NP	Prophylactic	IM	Preclinical	Multiple	DNA vaccines designed for multiple strains of Ebola
EBOV GP	Novavax Inc	nano particles	GP		IM	Phase 1 ongoing		
ArV (replocon)	Alpha vax Inc	Viral expression vector (VEEV)	GP, NP, GP/NP, VP			Preclinical	Multiple	
GOVX-E301, GOVX-E302	GeoVax Lab	MVA	GP		SC	Preclinical	Single	
EBOV Vaccinia	Bavarian Nordic	MVA, Immunostimulants	GP		SC	Phase 1		Protected immune-compromised population

Source; **Ohimain** 2016a

Abbreviations: HPIV3= Human para influenza virus type 3, RABV=Rabies virus; CMV=cytomegalovirus; r= recombinant; VEEV= Venezuelan Equine Encephalitis Virus; VLPs= Virus like particles; MVA=Modified Vaccinia Ankara. * vaccine administration routes; IM= intramuscular, IN =intranasal, IP =intra peritoneal, SC=subcutaneous

3' Leader	NP	VP35	VP40	GP	VP30	VP24	L	5' Trailer

Key
NP= Nucleoprotein
VP= Virus protein
GP= Glycoprotein
L= RNA dependent RNA polymerase

Figure 28: Simplified structure of Ebola virus genome

Nigeria therefore relied on using known best practices for EVD management including screening, hand washing, the use appropriate PPE (Plate 32), barrier nursing, training and information. The movement of dead bodies across states without approval or medical certificate was banned, while the country increased her healthcare infrastructure for Ebola virus disease detection and management. Burial of dead EVD patients was carried out under strict WHO guidelines of cremation or deep burial in leak proof body bags.

Plate 32: Ebolavirus responder, 100% coverage

Non-contact infrared thermometers were used to monitor the temperature of passengers at all Nigerian airports and all land and sea borders. Contacts are classified as suspect case if their temperature is >37.5ºC auxiliary or 38ºC core. However, there was a problem using temperature to detect EVD because other haemorrhagic fevers (yellow fever, Lassa and dengue), malaria, typhoid fever, and other types of fevers also result in elevated temperatures. Besides, asymptomatic Ebola carrier could beat the temperature screening.

Hand washing, good hygiene and sanitation were actively promoted. But these also faced with challenges. For instance, Lagos and Port Harcourt despite being coastal with abundant fresh water and brackish water resources, access to water and sanitation is poor. Many public buildings especially government institutions including schools and even hospitals lack water.

Many types of ethanol-based sanitizers of doubtful quality emerged, which is unlikely to be effective against Ebola virus. The sanitizers recommended for Ebola virus prevention should be anhydrous and contain at least 30% ethanol. Sodium hypochlorite was also used Ebola virus spread. Again, the concentration and purity of sodium hypochlorite used is uncertain. During the EVD outbreak, many other broad-spectrum disinfectants were freely used, which could affect non-target organisms in the ecosystem.

Several authors have reported that luck played a role in the successful curtailment of EVD in Nigeria. Nigeria, being a multi-religious country, it was therefore considered as divine intervention. Evidence suggest that the index case was exposed to Ebola virus and have developed EVD symptoms before leaving Liberia. Despite advice against travel, perhaps because of diplomatic passage or impunity, he was allowed to come to Nigeria. He came into Nigeria from Liberia with stops in Ghana and Togo and changed flight before arriving Lagos on 20 July 2014. Report also show that the index case was visibly ill and vomited during the flight, thus exposing the 72 persons on the aircraft.

Another element of luck was that the index case came into Nigeria through aircraft where it became possible to trace all the contacts. If the index case had come through the porous Nigerian borders and got ill in one of the numerous border villages, the scenario would have been different. The disease would have spread uncontrollably before it could have been detected. Also, as at the time EVD was imported into Nigeria, the Nigerian Medical Association (NMA) was on strike, hence the usually crowded public hospitals were not operational. Though, diplomats do not commonly patronize public hospitals in Nigeria. But if the index case was not a diplomat and was admitted into a Nigerian public hospital, it would have been a disaster. Also, diplomats are traditionally not restricted by the laws of the land. It was considered a feat when the index case was prevented from discharging himself despite the pressures from the Liberian Embassy in Nigeria. If the index case was released into Lagos, the largest mega city in Africa of over 21 million persons, it would have been a disaster. Lagos is industrialized, crowded with chaotic traffic situations, which could increase contacts that could enhance the spread of the disease.

There were 2 major flights that led to the spread of Ebola virus in Nigeria; the index case that flew from Monrovia via Accra and Lomé to Lagos and an associate of the index case (another ECOWAS Official) who flew to Port Harcourt from Lagos, bringing the virus to Port Harcourt. This diplomat was treated in a hotel not hospital or isolation centre without the use of Ebola virus response PPE. Again, by luck none of the hotel staff nor their guest was infected with Ebola virus. The Port Harcourt doctor that attended to other patient during the period, who also took part in other social activities, became infected. In all, only 3 persons contacted EVD from him, but only one died. Also, one of the infected nurses that treated the index case travelled by road to Enugu, South East Nigeria, a distance of about 500 km from Lagos. In all these cases, none of the passengers developed EVD including those that attended to the index case in the flight and clean up his vomits perhaps without appropriate PPE.

Before EVD was finally controlled in Nigeria, it caused several impacts. First the disease brought fear and panic into the country. Twenty persons became infected, while 12 recovered, 8 died. The fear of Ebola virus affected the educational sector next after the medical sector. Academic calendar was disrupted. Primary and secondary schools could not resume in August 2014 after the long (summer) holidays. The schools finally resumed on 22 September 2014 and 6 October 2014 after facilities to counter Ebola was installed and some teachers trained on EVD response. Foreign students from Ebola infected West African countries attending tertiary institutions in Nigeria were initially prevented from resuming. They later resumed after proper screening.

There were also fears concerning the large bat population (reservoir of Ebola virus) in Nigeria particularly at the Obafemi Awolowo University Campus and Aso Rock Vila (Presidential Villa). The fear of EVD also restricted public functions including religious worship, traditional festivals and other social gathering such as parties, ceremonies and burials. The usual Nigerian courtesy of hand shaking was minimized. Rumours fill the air waves many of non-medical ways of protection against EVD. Nigerians travelling abroad were stigmatized.

Responses to EVD are quite expensive. PPE alone cost about $75 a piece. Nigeria spent about N193.78 million monthly for the purchase of PPE alone. Huge amount of Money was also spent in training, sensitization, and campaigns and for the procurement of drugs, installation of water systems, laboratory facilities and maintenance of Ebola Centres. Money was also spent for contact tracing and for the payment of allowances and insurance for the Ebola virus response team. Ebola response became a high paying job.

Ecology of Ebola virus

Before EVD can be effectively controlled or eliminated, there is the need to understand ecology of the virus. By their very nature, viruses are mostly parasitic. Virus infections could be varied depending on the type of host. It could be innocuous i.e. asymptomatic in reservoir or primary host, amplified in secondary or intermediate host and fully parasitic/pathogenic in dead-end host (Fig. 29). This situation appears to be the case of Ebola virus where recent compelling evidence suggest that bats are the reservoir hosts, while great apes and duikers serve as the intermediate hosts and humans the end host. Hence, this subsection shall focus on the ecology of Ebola virus that could help to predict future outbreaks, direct monitoring efforts (location and target organisms) and focus research attention on risky or vulnerable ecosystems and our bish meat habit (Plate 33).

Plate 33: Does bush meat really play a role in Ebolavirus transmission?

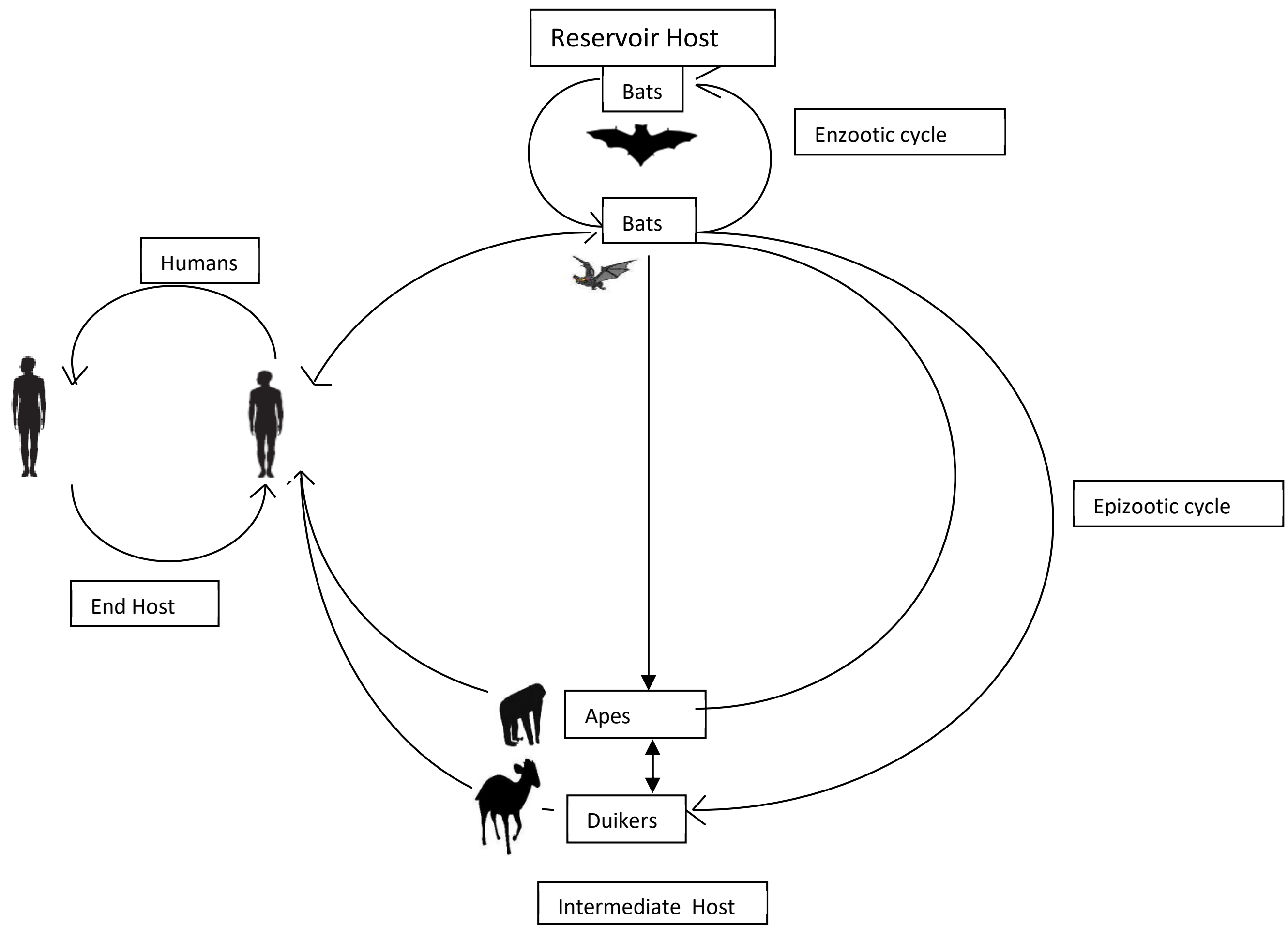


Plate 29: Ecology of Ebolavirus (**Ohimain** 2016b)

Apart from ebolavirus, bats are known to transmits several high impact disease pathogens, many of zoonotic origin (Table 32) with distinct enzootic (sylvantic) and epizootic (urban/human) cycles. Most of the viruses are maintained in nature by circulating among primary reservoirs (bats and rats), amplified by domestic animals (cattle, goats, horses, dogs, sheeps, hares, chicken) and wildlife (duikers, great apes i.e. Chimpanzees and gorilla). Bats are natural reservoirs of emerging and re-emerging zoonotic viruses including *Rhabdovirues, Coronaviruses, Paramyxoviruses, Lyssaviruses, Reoviruses, Flaviviruses, Adenoviruses*.

Table 32: Pathogenic microbes carried by some bats

Bat species	Disease	Causative pathogen
Hypsignathus monstrosus	EVD	Ebolavirus
Epomops franqueti	EVD	Ebolavirus
Myonycteris torquata	EVD	Ebolavirus
Mops condylurus	EVD	Ebolavirus
Eidolum helvum	EVD	Ebolavirus
Rousettus aegyptiacus	EVD	Ebolavirus
Epomophorus gambianus	EVD	Ebolavirus
Rousettus leschenaultii	EVD	Ebolavirus
Rousettus aegyptiacus	Marburg	Marburgvirus
Rhinolophus eloquens	Marburg	Marburgvirus
Miniopterus inflatus	Marburg	Marburgvirus
Hypsignathus monstrosus	Marburg	Marburgvirus
Micropteropus pusillus	RVF	RVFV
Hipposideros abae	RVF	RVFV
Taphozous perfortus	MERS	MERS-CoV
Tylopnecteris	MERS	HKU4
Pipistrellus	MERS	HKU5
Rhinolophus (horseshoe)	SARS	SARS-CoV
Pteropus vampyrus	Encephalitis	Nipah virus
P. hypomelanus	Encephalitis	Nipah virus
P. giganteus	Encephalitis	Nipah virus
P. lylei	Encephalitis	Nipah virus
Rousettus leschen	Encephalitis	Nipah virus
Cynoptera sphinx	Encephalitis	Nipah virus
Eidolum helvum	Encephalitis	Nipah virus

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Entomo-microbiology and the dilemma of mosquito eradication

Emerging and re-emerging pathogens of zoonotic origin have become a major global concern. Among the eleven-high priority emerging pathogens of global significance in 2016, three (Rift Valley fever virus-RVFV, chikungunya virus-CHINV, and Zika virus) are transmitted by mosquitoes, while 2 (severe acute respiratory syndrome virus-SFTSV and Nairavirus) are transmitted by ticks. It should be noted that mosquitoes also transmit other high impact emerging pathogens causing major diseases threatening humanity including malaria, Japanese encephalitis, dengue fever, yellow fever, and West Nile fever (Table 33). Mosquitoes have become a major challenge to humanity. Despite the discovery and use of many pesticides (some of which are dangerous to human health and the environment in general), mosquitoes have not been controlled worldwide.

Mosquitoes appear to be effective in transmitting not only Plasmodium, but members of flaviviridae family (Zika, Japanese encephalitis, West Nile, yellow fever and dengue viruses). About four species of *Plasmodium* causes malaria in humans including *Plasmodium falciparum, P. ovale, P. malariae* and *P. vivax* and in rare cases caused by *Plasmodium knowlesi*, of which *P. falciparum* is the most virulent. Over 30 species of mosquitoes from different genera including *Aedes*, *Culex* and *Anopheles* are involved in the transmission of RVFV.

Table 33: Some mosquito vector-borne diseases

Mosquito species	Disease	Causative pathogen
Aedes	Rift valley fever	Rift valley fever virus
Aedes	Chinkungiya fever	Chinkungiya fever
Aedes	West Nile	West Nile virus
Aedes aegypti	Dengue fever	Dengue fever virus
Aedes aegypti	Zika fever	Zika fever virus
Aedes aegypti	Yellow fever	Yellow fever virus
Aedes albopictus	Dengue fever	Dengue fever virus
Aedes albopictus	Zika fever	Zika fever virus
Aedes albopictus	Yellow fever	Yellow fever virus
Anopheles	Rift valley fever	Rift valley fever virus
Anopheles	Chinkungiya fever	Chinkungiya fever
Anopheles	West Nile	West Nile virus
Anopheles	Malaria	*Plasmodium falciparum*
Anopheles	Malaria	Plasmodium vivax
Culex	West Nile	West Nile virus
Culex	Rift valley fever	Rift valley fever virus
Culex	Chinkungiya fever	Chinkungiya fever virus
Culex tritaeniorhynchus	Japanese encephalitis	Japanese encephalitis virus virus
Cx. Pipiens	West Nile	West Nile virus

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Mosquitoes also transmit other encephalitis virus including western equine encephalitis, Eastern equine encephalitis, California encephalitis, Lacrosse encephalitis, St Louis encephalitis and Venezuela equine encephalitis. Japanese encephalitis, though relatively rare, causes viral encephalitis in over 24 countries in South East Asia with an estimated 68,000 annual cases and a case fatality rate of 30 – 50%. There are no cure or vaccine for the disease, thus putting over 3 billion people at risk (WHO, 2015). Yellow fever virus transmitted by mosquitoes causes an estimated 84000 – 170,000 infections per year, 90% of which occur in Africa with case fatality up to 50%. Several outbreaks have occurred in Africa and South America, putting a combined population of about 900 million people at risk. There are currently no known drugs, but vaccine has been the most effective preventive measure. Evidence suggests that yellow fever and dengue had appeared in western countries including USA, UK, and other European nations, but were controlled

There are about 3,500 species of mosquitoes worldwide, with a few hundreds sucking about 300 ml of blood daily (Fang 2010), but only about 60% of mosquitoes suck blood from humans. Mosquitoes also suck blood from other animals including non-human primates, domestic animals and several wild animals including snakes, hence their ease to serve as vector of many zoonotic infections.

Three genera namely *Aedes*, *Anopheles* and *Culex* are mostly implicated in the transmission of high impact emerging pathogens (Table 33). Hence, the scientific community is debating on the total eradication of mosquitoes. Though, some argue that eradication of mosquitoes could affect general ecology such as the food chain. Mosquitoes performed ecological functions such as pollination and their larva are food for fish species. Scientists are of the opinion that these ecological roles could be performed by other insect species. It should however be noted that mosquitoes have existed on earth for over 100 million years and are found in every continent and habitats (Fang, 2010). The three most notorious mosquitoes, which appears to be localized in the tropical world and being responsible for the transmission of many high impact emerging pathogens appears to be migrating northward and perhaps as far as the Scandinavia. *Aedes aegypti* and *A. albopictus* have expanded their range into Europe. These two species of Aedes mosquitoes are now found in all continents including North America and Europe. Karunamoorthi (2013) reported that climate change, which creates warmer temperature, could fasten/accelerate mosquito metamorphosis and expand the range/distribution of *A. aegypti* and other mosquitoes, while reducing/shorten the extrinsic incubation period of emerging pathogens. Environmental factors such as rainfall and flooding, which enhances mosquito breeding also facilitates the spread of mosquito-borne diseases.

Although, the WHO presented data showing a global decline (37% reduction) in malaria cases in the past 15 years (2002 – 2015), over 3.2 billion people are still at risk (WHO, 2016). Mosquitoes infect 247 million people worldwide killing about one million persons yearly, in about 109 countries (Fang 2010; Ndiok, **Ohimain** et al 2016). Malaria is widely transmitted by mosquitoes of the species *Anopheles gambiae*.

A recent study conducted by scientists at the Nigeria Institute of Medical Research (NIMR), Yaba, Lagos, revealed that mosquitoes have developed resistance to insecticide-treated nets in 20 states including Jigawa, Katsina, Kebbi, Sokoto, Zamfara, Benue, Kwara, Nasarawa, Niger, Plateau, Anambra, Enugu, Rivers, Lagos, Ogun, Ondo, Osun and Oyo. Data from 14 other states are not available. Nigeria had distributed over 182 million long-lasting insecticide-treated nets since 2003 till date in a bid to eradicate malaria by 2030. With the emergence of mosquito resistance to insecticide it is uncertain if this target can be met. The probable cause of the resistance might be due over-saturation of insecticide products. For instance, most of the insecticide products used in malaria control are also being used in agricultural sector. This over-saturation is a major source of resistance. The study concluded by recommending research to urgently explore alternative non-chemical-based control measures. This is an aspect where molecular and microbial application holds promise.

Various methods have been developed and new once emerging (Table 34) for the control of mosquitoes. The methods could be broadly classified into chemical, physical and biological, which could be further subdivided into genetically modified mosquitoes (GMM) and non-GMM techniques. Two approaches that have been traditionally used for the control of mosquitoes are the physical decontamination of the environment and water containers and application of pesticides (adulticides, larvicides, and repellents). Others are relatively new, except the use of some botanicals by indigenous people. The elimination of potential mosquito breeding sites and the application of larvicides have been quite successful in the control of mosquitoes (Karunamoorthi, 2013). For instance, the application of adulticide DDT led to the control of vector *A. gambiae* in the US in 1940. Also, the use of the larvicide Paris Green enabled Brazil to control the malaria vector (Fang, 2010). Different mosquitoes, which spread different diseases are active at different times of the day. Mosquitoes can therefore be targeted using their feed schedule and pattern. For instance, Culex species feed mostly at night i.e. from early evening to morning. Aedes aegypti and Aedes albopictus (vector of Zika and Dengue) mostly feed during the day but can also feed at night.

Application of pesticides led to the eradication of *A. aegypti* from 22 countries in the Americas post second world war. Despite the initial successes, there have been some challenges in the use of these traditional techniques. For instance, it is practically impossible to eliminate all standing water or mosquito breeding sites especially in coastal areas with heavy and frequent rainfalls. The proper management of disposed household containers such as bowls/buckets, jars, pots, plates, cups, bottles, vases and pans is a challenge especially in many developing countries with history of poor waste management. Similarly, it is also a challenge to manage industrial wastes in developing countries such as used tyres, wreckages (vehicles, trains, ship) and other structures that retain water serving as breeding sites for mosquitoes. Moreover, dichloro diphenyl trichloroethane (DDT) and similar pesticides that were effectively used to control mosquitoes post second world war, have either been restricted or out rightly banned in most countries due to environmental considerations. Hence, pyrethroids are now commonly used. Recently, mosquito resistance to pyrethroids has emerged in many countries (WHO, 2016). Furthermore, pesticides are less selective; they eliminate both beneficial and detrimental insects. Pesticides have been implicated in the contamination of surface and groundwater sources through run off. Hence, the scientific community is searching for alternative chemicals sourced from botanicals that could be effective against mosquitoes but with less environmental consequences. Cedar oil (Fang 2010) and *Hyptics suavolonse* (**Ohimain** *et al.*., 2015) are promising candidates.

Recent advances in molecular biology have opened a vista of opportunities for the development of biological methods for the control of mosquitoes. Several genetically modified mosquitoes (GMM) and non-GMM have been developed to counter wild species (Table 34). Two of the most advanced GMM that have entered pilot phase haven been field tested in many countries are based on sterile insect techniques (SIT) and the use of GMM containing the bacteria *Wolbachia* sp. The sterile insect techniques involve the release of GMM male *Aedes aegypti* that will seek and mate with wild females producing offspring that will die before maturity. Oxitec, a USA/British company have produced GMM *Aedes aegypti* that have been tested in many countries including Brazil, Cayman Island, Malaysia and Panama for the control of dengue fever and currently been tested for the control of zika in Brazil.

Table 34: Traditional and novel techniques for the control of mosquitoes

Method	Technology	Stage of development	Application
Chemical	Mosquito repellent	Current practice	Used globally
Chemical	Pesticides application including DDT, organophosphates, pyrethroids	Current practice	DDT used for the eradication of *A. aegypti* in 22 countries in South America; used globally
Physical	Sterile insect technique (irradiation)	Pilot field application	Used to eradicate screw worms (a pest of cattle) in the US in 1980s and control of tsetse fly in Tanzania
Physical	Mosquito traps	Pilot field application	Mosquito *A. taeniorhynchus* was eradicated from Florida US
Physical	Decontamination of containers and sanitation	Current practice	Have been used globally.
Biological	Botanicals	Experimental	*Hyptis suavolens* repels adult and kill mosquito larva, Cedar oil
Biological	RNA interference (gene silencing/ suicide) (Molecular)	Experimental	Female A. Aegypti control
Biological	Sterile insect technique (Molecular)	Pilot field application	Used for the control of dengue fever virus tested in some countries (Brazil, Cayman Islands, Malaysia, Panama), and currently being tested for Zika virus in Brazil
Biological	Cytoplasmic incompatibility (Molecular)	Pilot field application	Field applications in Brazil, Vietnam, Australia and Indonesia for the control of dengue carrying Aedes aegypti
Biological	Virus vectored RNA interference (Microbiological/ Molecular)	Experimental	Densovirus vector control *A. Albopictus, A. aegypti* larvae
Biological	Cytoplasmic incompatibility (Microbiological/ Molecular)	Experimental	Transfer of *Wolbachia* into Aedes aegypti
Biological	Cytoplasmic incompatibility (Microbiological)	Natural process	*Wolbachia*-mediated cytoplasmic incompatibility for the control of *Ae. albopictus*
Biological	Microbiological	Pilot field application	Entomopathogenic fungi *Metarhizium anisopliae* control of malaria parasite *Anopheles gambiae* tested in Tanzania
Biological	Microbiological	Pilot field application	*Bacillus thorugensis*

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Over 90% success rates have been recorded by this approach for the control of *Aedes aegypti*. The oxitec approach involved the insertion of two genes, a colour maker for monitoring and a self-limiting gene that will make the GMM not to be persistent in the environment. Unlike the chemical method, this approach is species specific, resulting in the elimination of*Aedes aegypti* alone and not all mosquitoes. Note that *Aedes aegypti* is involved in the transmission of yellow fever, dengue fever, Japanese encephalitis, and Zika. Incidentally, the use of interfering RNA for the control of insects by the application of dsRNA was first demonstrated in *Aedes aegypti* Linnaeus (Gu and Knipple, 2013). These and similar approaches can be developed for the two other problematic mosquitoes *Anopheles* and *Culex*.

Another approach that have also been field tested involves producing GMM containing the bacteria *Wolbachia* sp, which has antiviral properties. This approach has been used for the control of dengue viruses. *Wolbachia* prevent viral replication within *Aedes aegypti* and also prevents its transmission. Successful field tests were carried out in Brazil, Australia, Vietnam and Indonesia. Over 80% success rates have been recorded by this method. The entomo-pathogenic fungi *Metarhizium anisopliae* have been demonstrated to control the malaria parasites *Anopheles gambiae* (Cook *et al.*., 2007). Most of the biological control methods are species specific, which could potentially result in the elimination of key mosquito species particularly *Aedes aegypti*, *Anopheles gambiae* and *Culex* sp from mostly urban or human environment. However, the total elimination of these species in the forest (sylvatic cycle) by these methods could be challenging. Notwithstanding, the WHO has started issuing certifications to countries that have successfully eliminated malaria, which have recorded zero local cases of malaria for at least three consecutive years. Countries that have achieved this feat and have been certified include UAE (2007), Morocco (2010), Turkmenistan (2010), Amenia (2011), Maldaves (2015), while three other countries (Argentina, Sri Lanka, and Krygyzstan) have commenced the certification process (WHO, 2016). Can Nigeria attempt to join this league nations that have successfully eradicated malaria?

At the 5th NDU inaugural Lecture, Prof Epidi lectured on the importance of insect as both friends and foes to mankind. Other lecturers working on insects in NDU include Dr Bawo, Dr Commander and Rosemary Boate. There is therefore the need for research collaboration to develop the field of medical entomology and entomo-microbiology.

Cerebrospinal Meningitis

Since the discovery in 1805 of *Neisseria meningitidis* (Nm) as the causative agent of Cerebrospinal meningitis (CSM) also known as meningococcal meningitis, the bacteria have spread globally with different serogroups predominating in different parts of the World. An estimated 1.0 – 1.2 million cases are recorded each year killing 50-70% if no treatment is administered, which could reduce to 10% with the administration of antibiotics. And neurological complications often occur in over 10% of survivors, such as deafness, brain damage, paralysis, limb loss, learning disabilities etc.

Cerebrospinal meningitis (CSM) is caused mostly by microbial infections, but non-infectious CSM occur rarely due to cancer or the use of drugs that affects the meninges. Different types of microbes have been linked to CSM including bacteria, fungi, yeast, parasites and viruses. Among bacteria, three species have been commonly reported *Neisseria meningitidis*, *Streptococcus pneumonia* and *Haemophilus influenza*. *Neisseria meningitidis* account for over 80% cases of bacterial meningitis, hence the focus on this organism. This organism is a fastidious gram-negative diplococcus, aerobic and naturally inhabiting the nasopharynx of humans as normal flora. The bacterium is either capsulated or non-capsulated. Capsulation appears to have increased the virulence of capsulated strains over the non-capsulated ones. Based on the structure and chemical composition of the polysaccharide capsule and attendant immunological reactivity, *Neisseria meningitidis* (Nm) can be classified into 13 distinct serogroups (A, B, C, E29, H, I, K, W-135, X, Y, Z and Z') of which only six (A, B, C, W-135, X and Y) have been implicated in CSM. Different serogroups of Nm predominate in different parts of the World (Table 35) with serogroup A responsible for 80 – 85% of bacterial meningitis in African meningitis belt, with serogroups X, W135 and C accounting for the rest.

Table 35: Global spread of meningococcal strains

S/N	Serotype	Location recording outbreaks
1	A	Africa, Europe, Middle East
2	B	North and Central America, South America, Europe, Middle East, Australia, New Zealand
3	C	Africa, North and Central America, South America, Europe, Middle East, Australia
4	W-135	Africa, Middle East
5	X	Africa
6	Y	North and Central America

Frequent outbreaks of cerebrospinal meningitis occur in Northern Nigeria almost annually reaching epidemic proportions within 5-10 years. *Neisseria meningitides* serogroup A (NmA) is the major cause of CSM in Northern Nigeria and indeed most parts of the African Meningitis belt. Northern Nigeria is in the extended African meningitis belt, which comprises of 26 countries including Benin, Burkina Faso, Burundi, Cameroun, Central Africa Republic, Chad, Cote d'Ivoire, Democratic Republic of Congo, Eritrea, Ethiopia, Gambia, Ghana, Guinea, Guinea Bissau, Kenya, Mali, Mauritania, Niger Republic, Nigeria, Rwanda, Senegal, South Sudan, Sudan, Tanzania, Togo and Uganda. Thus, an estimated population of 430 million people could be at risk of cerebrospinal meningitis. About 60-65% of all meningitis cases worldwide, occur within the African meningitis belt. Though, the risk varies within and between countries, with about 65% of cases in Africa occurring in Burkina Faso, Ethiopia, Chad and Niger. Chad and Niger share common boundary with Northern Nigeria.

Historically, the first recorded case of meningitis occurred in Geneva Switzerland in 1805 and was reported in Massachusetts in the USA in 1806 and several outbreaks recorded in North America and Europe. It was first reported in Algiers in the African meningitis belt in 1840 and for the first time in Nigeria in 1905 i.e. about 100 years after it was first discovered. Thereafter, the disease spread rapidly westward and in 1906, it was detected in Ghana. Two major theories have emerged on the origin of invasive meningitis in Nigeria. It could either be due to acquisition of virulent genes by previously non-pathogenic Neisseria or probably imported from Mecca during the annual Hajj pilgrimage.

The first Nigerian case, which occurred in Zungeru in Northern Nigeria involved 32 persons. Other outbreaks soon followed, and the bacteria was detected in Yola, over 500 miles from Zungeru, resulting in over 20.000 cases. Ever since then, outbreaks of meningitis have been occurring in

Nigeria with increasing incidences and geographical spread, though mostly limited to Northern Nigeria. Among the worst cases was in 1996 when 109,580 cases with 11,717 fatalities, followed by 2008/2009 where 9086 cases with 562 deaths and in 2003 with 4130 cases and 401 deaths. In response to the frequent outbreaks of CSM caused by mostly Nm A in Northern Nigeria, reactive vaccination was carried out using meningococcal Polysaccharide ACYW135 vaccine in 2008 and 2009 and for the first time, MenAfricVac-A Serogroup A conjugate vaccine in 2010 – 2012, which led to the near elimination of Nm A from the region.

Recently, novel strains of Nm Serogroup C were reported in Kebbi and Sokoto states, North-West Nigeria in three sequential outbreaks from 2013 – 2015 (Table 36). On 13 December 2016, an outbreak of CSM started in neighbouring Zamfara state, which was caused by Nm C, spread almost throughout Nigeria, with cases reported in 23 out of the 36 states of the country. As of 9th May 2017, about 13,420 cases have been reported with Case Fatality Rates (CFR) reducing from 14.3% in March 2016 to 8.0% in May 2016, with children and adolescents in the age of 5-14 years old mostly affected. We reviewed the challenges of controlling repeated outbreaks of CSM and found several factors that contribute to the frequent outbreaks of CSM in Northern Nigeria. They can be broadly classified into demographics (location in African meningitis belt and transboundary effects), environmental (wind patterns, rainfall, humidity and dust), microbial diversity of the causative agents (bacteria, fungi, yeast, protozoa and viruses), bacterial diversity (*Neisseria meningitidis*, *Streptococcus pneumoniae* and *Haemophilus influenzae* type b), phenotypic and genetic diversity of Nm (serogroups, strains, sequence types, clonal complexes), antibiotic resistance, and conditions that creates over-crowding such as religious activities and camping caused by conflicts and other socio-cultural factors. Except these factors are addressed, CSM will continue to recur in Northern Nigeria.

Table 36: Recent emergence of NnC in Nigeria

Out-breaks	**Year (duration)**	**State affected**	**No. of communities affected**	**Size of spread, km^2**	**Population of Affected wards**	**Cases, #**	**Attack Rate (per 100,000)**	**Deaths, #**	**Case fatality rate (%)**
1st	2013 (19 Feb 2013 – 23 June /2013)	Sokoto	44	105	127,097	856	673	58	6.8
2nd	2014 (14 March 2014 – 3 May 2014)	Kebbi	57	150	201,457	333	165	35	10.5
3rd	2015 (10 Feb 2015-8 June 2015)	Sokoto	-	-	214,782	680	317	29	4.3
		Kebbi	-	-	2,049,883	5714	279	292	5.1
		2015 Total	1039	-	2,264,665	6394	282	321	5.0
2016-2017	13 Dec 2016-x 2017**	23*				13420		1069	14.3 – 8.0

*Zamfara, Sokoto, Kastina, Kebbi, Niger, Yobe, Kano, FCT, Nassarawa, Gombe, Taraba, Cross River, Osun, Lagos, Jigawa, Plateau, Kogi, Oyo, Delta, Adamawa, Benue and Kaduna states
** As of 9 May 2017

Typically, most infectious diseases are controlled using antibiotics and vaccines. Antibiotics resistance has been reported among Neisseria meningitidis. Recently, meningococcus has developed resistance to most of the antibiotics that were effective in the past including sulfonamides, penicillin, chloramphenicol and ciprofloxacin. However, ceftriaxone is being used for treatment in the current outbreak of NmC in Nigeria. Due to the combined problem of antibiotics resistance and the development of long-term sequelae/complications, the use of vaccines could provide the best options for the control of bacterial meningitis. There are at least three different types of vaccines that were developed against Neisseria meningitidis. They are polysaccharide vaccines (PS), Polysaccharide-protein conjugate vaccine (CV) and outer membrane vesicle vaccines (OMV). These vaccines are commercially available in various valence forms including monovalent, bivalent, trivalent, tetravalent and other polyvalent forms. The challenge of using vaccines to control meningococcus are many, including the presence of diverse serogroups, strains and clonal complexes of the predominant bacteria in different geographic locations, lack of cross protection among different vaccines for the different serogroups and high costs of vaccination. More research is therefore required to develop a common vaccine that will protect against all serogroups, strains and clonal complexes of Nm and possibly the other two species causing bacterial meningitis i.e. *Haemophilus influenzae* type b and *Streptococcus pneumoniae*. Polyvalent vaccine produced using both capsular-protein conjugate and outer membrane vesicle is likely going to be the ideal candidate. Only with research can this come to a reality.

Salmonella in turtles

From 1st March 2017 to 3rd August 2017, the US Centre for Disease Control (CDC) reported 37 cases of salmonellosis in 13 states, which have resulted in 16 hospitalizations but with no death. Twelve of the cases i.e. 32% are children 5 years of age or younger. Epidemiologic and laboratory findings link the outbreak of human *Salmonella agbeni* infections to contact with turtles or their environments, such as water from a turtle habitat. Salmonella infection symptoms include diarrhoea, fever and abdominal cramps. Reptiles are responsible for more than 70,000 cases of salmonellosis each year in the United States. Hence, the US Food and Drug Agency (FDA) in 1975, banned the distribution and sale of turtles with shells smaller than 4 inches.

In Nigeria, particularly Bayelsa and Delta states, turtles, tortoises and other reptiles are freely handled, sold and consumed without checking whether they contain Salmonella or other dangerous zoonotic microbes. We worked on turtles and tortoises (Plates 34, 35) sold in Bayelsa and Delta state and found that they are mostly used food and preparation of charms and to a lesser extent as pets (**Ohimain** et al 2014b). But could these herpetofauna contain any pathogenic strains of Salmonella or other pathogens like those found in the American turtle? Only research can tell.

Turtles

Tortoises

Plate 34: Turtle/tortoises business negatively impacts the ecosystem

Plate 35: Sliding trap used to capture freshwater turtles and tortoises made from raffia palm

Microbes in flood waters

We investigated the impacts on wildlife and vegetation of the 2012 water floods that occurred from mid-September to mid November 2012 in the Wilberforce Island. Forty-five (45) mammalian species belonging to 6 orders and 21 families were found in Wilberforce Island. Of these, 16 species were directly impacted, 7 indirectly impacted and 22 un-impacted. Seventy-eight (78) avian fauna were identified belonging to 27 families. Out of these, only 4 species were directly impacted, 17 indirectly impacted and 54 were un-impacted. A total of 56 plant species were identified belonging to 39 families. Of these, 31 species were directly impacted, s6 indirectly impacted, and 19 un-impacted. The plant species, which exhibited the greatest impact at the time of the study (December 2012) is *Musanga cecropoides*. The water level marks on *Musanga cecropoides* coincided with the physiological stress on the plant. The receding water level mark on *Musanga cecropoides* ranged from 32.67 to 59.33 cm for the dead plants, 12.00 to 32.67 cm for the dying plants, but the water was at ground level for intact vegetation. Analysis of variance showed that, there were significant differences in the receding water marks ($P < 0.05$) among the ten plots studied (**Ohimain** et al 2014c). *Musanga* fruits are consumed by monkeys, hence their death could affect wildlife (Plates 36-39). The flood also affected domestic animals particularly goats (Okpeku, **Ohimain** et al 2014).

Plate 36: Selective impacts of the water flooding on vegetation (notice dead mango on the left side, whereas orange on the right is healthy side; also observe the height of the receding water marks on the wall of the building at the background)

Plate 37: Buttress roots of Musanga, a wetland adaptation feature

Microbes appears to flourish during flooding. The origin of microbes in flood waters could be linked to leakage of sewage into the water and open defecation. In the 2017 Hurricane Harvey in Texas, both coliforms and E. coli were detected in the flood waters. Coliforms generally and *E. coli* particularly are normal microflora in the guts of warm blooded animals including humans. The presence of these enteric bacteria in the flood water, which confirmed that the water was faecally contaminated, also indicate the risk of outbreak of potentially dangerous microbes like *Shigella*, *Salmonella*, *Vibro cholerae* etc. For instance, in Haiti, the 2010 hurricane caused several thousand infections of cholera with over 10,000 deaths. But before the 2010 hurricane, there were no cholera in Haiti. The origin of the outbreak was linked to leakage from the UN camp, which has resulted in an intense legal battle between tne UN and Haiti. It now appears that Vibrio cholerae have now established in Haiti, with almost annual outbreaks since 2010. Since Nigeria now also experiencing flooding frequently, we also need to keep an eye on these pathogenic bugs during flooding events. Recall that cholera outbreak occurred in Kwara state in 2017. As of 9 July 2017, 17 persons have been confirmed dead in 1,617 reported suspected cases.

Plate. 38: Monkeys displayed for sale along the Wilberforce Island road

Plate 39: Notice dead Musanga species while other vegetation in the fore ground and background are healthy

Polio virus
Poliomyelitis (polio) is a highly contagious viral disease, which mainly affects young children. The virus is transmitted by person-to-person spread mainly through the faecal-oral route or, less frequently, by contaminated water or food. The virus multiplies in the intestine, from where it can invade the nervous system and can cause paralysis. Initial symptoms of polio include fever, fatigue, headache, vomiting, stiffness in the neck, and pain in the limbs and could lead to permanent disability/paralysis. There is no cure for polio, it can only be prevented by immunization by either inactivated poliovirus vaccine (IPV) or oral polio vaccine (OPV). The development of polio vaccines in 1953 and their subsequent deployment in 1957, have caused the disease to be wiped out round the world except in three countries (Afghanistan, Pakistan, and Nigeria), where insurgence is common.

Monkey virus
Monkey pox is a rare viral zoonosis (a virus transmitted to humans from animals) with symptoms in humans similar to those seen in the past in smallpox patients, although less severe. Smallpox was eradicated in 1980. Monkey pox occurs sporadically in remote parts of Central and West Africa, near tropical rainforests. The monkey pox virus is transmitted to people from various wild animals (monkey. rat, squirrel) but has limited secondary spread through human-to-human transmission. people could get Monkey pox if they are bitten or scratched by an animal or contact animal blood in preparing bushmeat or have contact with an infected animal's body fluids or sores.

Monkey pox may also be spread between people through prolonged face-to-face contact, or through contact with body fluids or sores of an infected person, or items that have been contaminated with fluids or sores (clothing, bedding, etc.). Transmission occurs primarily via droplet respiratory particles usually requiring prolonged face-to-face contact, which puts household members of active cases at greater risk of infection.

Typically, case fatality in monkey pox outbreaks has been between 1% and 10%, with most deaths occurring in younger age groups. There is no treatment or vaccine available although prior smallpox vaccination was highly effective in preventing monkey pox as well.

Bayelsa state was the ground zero of the 2017 monkey pox outbreak in Nigeria. The index case was alleged to come from Agbura, where somebody was purported to have killed and eaten a monkey and after which neighbours and family members started developing rashes. The NCDC was notified about monkey pox virus in Bayelsa State on 22 Sept 2017. Before the current outbreak, monkey pox was reported in Nigeria in the 1970s.

The pattern of spread was as follows:
- On 5 October 2017, 10 persons were infected, 49 others put under surveillance in Bayelsa State
- October 7, the virus spread to Rivers State
- October 9, the virus spread to Akwa Ibom
- By October 10, the virus had spread to 7 states; Bayelsa, Rivers, Ekiti, Akwa Ibom, Lagos, Ogun and Cross River
- 18 October 2017; Monkey Pox cases increase to 74 in 11 states; Akwa Ibom, Bayelsa, Cross River, Delta, Ekiti, Enugu, Imo, Lagos, Nasarawa and Rivers as well as the FCT

- 27 October; 94 suspected cases reported from 11 states, namely Akwa Ibom, Bayelsa, Cross River, Delta, Ekiti, Enugu, Imo, Lagos, Nasarawa, Niger, Rivers and the Federal Capital Territory.
- The virus was reported in Edo & Benue States on 1st November
- Thereafter, it fizzled out because of the self-limiting nature of the virus

Poxviruses are quite diverse and globally distributed. The Poxviridae family of viruses is divided into two sub-family groups based on the hosts they infect, vertebrates (Chordopoxvirinae) and invertebrates (Entomopoxvirinae). The Chordopoxvirinae are universally distributed and capable of infecting a wide range of avian, reptilian and mammalian species. Variola (smallpox) virus, vaccinia virus (VACV), cowpox virus and monkey pox virus (MPXV) remains a priority of biodefense preparedness research. Over 30 years after the eradication of smallpox, orthopox-viruses (OPVs) remain relevant public health threats. These viruses remain global emerging infectious disease threats. Monkey pox infection usually begins with fever, headache, muscle aches, and exhaustion. Monkey pox causes lymph nodes to swell and the incubation period (time from infection to symptoms) for monkey pox is usually 7-14 days but can range from 5-21 days.

The virus was first identified in the State Serum Institute in Copenhagen, Denmark, in 1958 during an investigation into a pox-like disease among monkeys. Hence, the name monkey pox. Human monkey pox was first identified in humans in 1970 in the Democratic Republic of Congo (then known as Zaire) in a 9-year-old boy in a region where smallpox had been eliminated in 1968.

Diagnosis of monkey virus is challenging, because of its similarity to other rashes. The differential diagnoses that must be considered will include other rash illnesses (Plate 40), such as, smallpox, chickenpox, measles, bacterial skin infections, scabies, syphilis, and medication-associated allergies. Lymphadenopathy during the prodromal stage of illness can be a clinical feature to distinguish it from smallpox. Currently, samples are sent to WHO reference lab in Dakar, Senegal for analysis.

Plate 40: Rashes caused by monkey pox virus (newspaper sources)

Microbes causing ulcers

Some microbes have been implicated as one of the causes of ulcers. Helicobacter pylori causes stomach ulcer, and in some cases stomach cancer, while *Haemophilus ducreyi* causes extensive ulcerative yaws and ulcers. But aonther type of ulcer called Buruli Buruli is in a class of its own (Plate 41). Buruli ulcer is caused by the flesh-eating bacteria called *Mycobacterium ulcerans.* The microbe belongs to the family of bacteria that causes tuberculosis and leprosy. Buruli ulcer is a chronic debilitating disease that affects mainly affects the skin and sometime bone and it can lead to permanent disability. The microbe releases the toxin mycolactone, which causes extensive abrasion and necrosis of skin and soft tissues. *Mycobacteria ulcerans* is an environmental pathogen, mostly found in water bodies in association with water insects and other organisms. The mode exact of transmission to humans is unknown, two potential mechanisms of transmission are being considered, environmental and vector-borne from aquatic sources. The environmental transmission hypothesis suggests that the bacteria, which is ubiquitous in endemic regions can be directly inoculated into the human dermis due to injuries by contaminated objects in the environment, leading to infection. Whereas, in the vector-borne transmission model, the bacteria are transmitted through the bites of aquatic (*Naucoris* and *Dyplonychus* species). About 70% of those infected with Buruli ulcer are children under 15 years old. Buruli ulcer has been reported in 33 countries in Africa, the Americas, Asia and the Western Pacific. Most of cases are reported from West and Central Africa, including Benin, Cameroon, Côte d'Ivoire, Democratic Republic of the Congo and Ghana. It should be noted that Cameroon and Benin share boundaries with Nigeria, while Ghana is closely linked to Nigeria. The southern parts of these neighbouring countries have similar ecosystem with the Niger Delta. Besides, there are some Bakassi returnees in Nigeria. Therefore, Nigeria could be exposed. The accepted current treatment for Buruli ulcer is usually surgery and the use of combined antibiotics such as rifampicin and moxifloxacin or streptomycin.

Plate 41: Flesh eating bacteria causing ulcer (source: internet)

Plagues

Plague is caused by infection with the bacteria, *Yersinia pestis* (a zoonotic bacteria) and is typically spread through the bite of infected fleas, frequently carried by rats. Human-to-human transmission can occur through contact with body fluid and inhalation of respiratory droplets from infected persons. Plagues occur in two clinical forms, bubonic plague and pneumatic plague. The symptoms of bbubonic plague include painful, swollen lymph nodes, called bubos, (Plate 42) as well as fever, chills and coughing. Pneumonic plague is more virulent and is characterized by a severe lung infection. People infected with *Y. pestis* often develop symptoms after an incubation period of one to seven days. Currently, the three most endemic countries are the Democratic Republic of the Congo, Madagascar, and Peru. Outbreaks occur in these countries quite frequently.

Plate 42: Bubonic plague caused by *Yersinia pestis*

Super bugs and bioterrorism

Incidence of antibiotic resistance is increasing globally. In 2017, the WHO listed 12 priority microbes that are resistant to many first line antibiotics (Table 37). The list present 12 separate families of antibiotic-resistant bacteria that pose the greatest threat to human health. Most of these organisms are gram-negative bacteria, which can cause severe, often deadly infections typically in hospitals and nursing homes and are more resistant to antibiotics due to the complexity of their cell walls. Apart from the microbes prioritized by WHO in 2017, there are other microbes of great concern. For instance, multi-drug resistant TB infections require a combination of at least three antibiotics to be sensitive.

The fungus, *Candida auris,* can cause severe illness with high mortality, especially among high-risk patients, including those in intensive care units, those with a central venous catheter and those who have received antibiotics or antifungal medications. Another concern is that yeast can be persistent in solid surfaces for weeks. It has been isolated from the rooms of patients in several places such as mattresses/ beds, windowsills, chairs, infusion pumps and countertops. This ability to linger on the surfaces of health care environments and spread between patients is unlike most of the other 20 species of Candida that cause infections in humans. The yeast is resistant to three groups of anti-fungi drugs, namely, azoles (e.g. fluconazole, itraconazole; voriconazole) amphotericin B (AMB), and echinocandins, and has globally emerged as a nosocomial pathogen. Although C. auris was just discovered in 2009, it has spread quickly and caused infections in more than a dozen countries including USA, Columbia, Venezuela, India, Pakistan, Spain, Norway, UK, Germany, Japan, South Korea, Israel, Kuwait, Kenya and South Africa

Table 37. WHO 2017 priority list of pathogens

PRIORITY 1: CRITICAL	PRIORITY 2: HIGH	PRIORITY 3: MEDIUM
**Acinetobacter baumanni (Carbapenem-resistant)	**Enterococcus faecium (Vancomycin-resistant)	Streptococcus pneumoniae (Penicillin-non-susceptible)
**Pseudomonas aeruginosa (Carbapenem-resistant)	**Staphylococcus aureus (Methicillin-resistant Vancomycin-intermediate and resistant)	Haemophilus influenza (Ampicillin-resistant)
**Enterobacteriaceae (Carbapenem-resistant, ***ESBL-Producing)	Helicobacter pylori (Clarithromycin-resistant)	*Shigella spp. (Fluoroquinolone-resistant)
	*Campylobacter spp. (Fluoroquinolone-resistant)	
	*Salmonellae (Fluoroquinolone-resistant)	
	Neisseria gonorrhoeae (cephalosporin-resistant fluoroquinolone-resistant)	

* common cause of food poisoning and diarrhea, ** common hospital-acquired infection
*** extended-spectrum beta-lactamases produced mostly Escherichia coli and Klebsiella species

The Komodo dragon (*Varanus komodoensis*), a large lizard found only in an Indonesian Island, have saliva containing deadly bacteria that they inject to poison and kill their prey. Of the 50 to 80 bacteria that cohabit the dragon's mouth two particularly are opportunistic pathogens, Pseudomonas aeruginosa and Pasteurella multocida. These microbes are innocuous to the dragon, but lethal to their hosts. The dragon uses the microbes as bioweapon to infect and kill their victims.

Some of these super bugs are now potential threat for the development of bio-weapons. The threat of bioterrorism is quite high. Biological hazards, also known as biohazards, refer to biological substances that pose a threat to the health of living organisms, particularly humans. Microbes are classified into four biohazard groups, biosafety level (BSL) 1-4, with BSL 4 being the most hazardous. BLS 4 microbes are potential agents for the development of bioweapons e.g., Ebolavirus, Marburgvirus. South Korea have started vaccination in preparation for anthrax attack from North Korea. The Centers for Disease Control and Prevention (CDC) classifies agents with recognized bioterrorism potential into three categories (A, B and C), *Anthrax*, which air borne and could persist in the environment for long period, is classified as a Category A agent. It should be noted that category A pose the greatest possible threat or cause major impact to public health.

The WHO study concluded that there are too few new antibiotics under development to combat the increasing threat of multidrug-resistant infections, according to them, it is likely that the speed of increasing resistance will outpace the slow drug development process. For instance, as of May 2017, a total of 51 antibiotics and 11 biologicals are under development. The idea is that biologicals could replace use of antibiotics, which could help in overcoming the resistance problem.

PHARMACEUTICAL MICROBIOLOGY & HERBAL MEDICINE

Mister VC sir, my research collaborators and I have carried out several studies on the potential use of plant extracts against insects particularly mosquito larva, ticks and other parasites and disease vectors (Table 38). Our findings show that *Jatropha curcas*, Neem plant i.e. *Azadirachta indica*, and mangrove plants (*Rhizophora mangle, R. racemosa, Avicennia germinans* and *Languncularia racemosa*) were effective in the control of schistosoma vectors (*Biomphalaria pfeifferi*, *Bulinus globosus and Bulinus rholfsi*) and mosquito larvae. *Hyptis suaveolens and Ocimum sanctum* were effective in the control of mosquito larvae and dog ticks.

We also studied antibacterial properties of selected plants and higher fungi (Table 39). Some of the plants we studied exhibited antimicrobial properties including *Vitex grandifolia*, *Alstonia Boonei* and *Moringa oleifera*. These plants were effective against several pathogenic microbes including *Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus, Salmonella* and *Proteus* species. We confirmed the efficacy of pawpaw and lemon grass used for the treatment of typhoid fever by indigenous people of the Niger Delta. Siam weed, *Chromolaena odorata* and moringa were equally effective against Salmonella typhi. We also studied eight mushrooms (*Fomes lignosus*, *Marasmius jodocodo*, *Pleurotus florida*, *Pleurotus tuberregium*, *Psathyrella atroumbonata*, *Polyporus giganteus*, *Termitomyces microcarpus* and *Termitomyces robustus*) that possess antibacterial properties, which were quite effective against *Bacillus cereus, Escherichia coli*, *Klebseilla pneumoniae Proteus vulgaris, Pseudomonas aeruginosa* and *Staplylococcus aureus*.

At the 8th and 9th NDU inaugural Lecture, Prof Omobuwajor and Prof Ajebisin lectured us on the benefits of several locally available medicinal plants. What is therefore left is research collaboration across disciplines for us to take the science of medicinal plants to the next level. We ned to extract and characterize the bioactive compounds in these herbs and using tissue culture tecniques, breed and domestic plant species with novel bioactive substances and turn them to products that can be marketed globally. India and China have done this. What is Nigeria waiting for. The herbal medicine sector is currently being dominated by traditionalists, who have little knowledge of the products that they are marketing.

Table 38: Activities of plant extracts against selected tropical disease vectors and parasites

Plant	Parasites/vectors	Research summary	Reference
Jatropha curcas	*Bulinus globosus* and *Bulinus rholfsi*	Demonstated in-vitro comparative molluscicidal activities of methanolic and aqueous extracts of *Jatropha curcas* leaves against *Bulinus globosus* and *Bulinus rholfsi,* vectors of urinary schistosomiasis	Bassey, **Ohimain** et al 2013
Jatropha curcas	Schistosoma vectors, *Biomphalaria pfeifferi*, *Bulinus globosus and Bulinus rholfsi*	Biomolluscicidal activities of Some Solvent Extracts of *Jatropha Curcas* Leaves against Vectors of Schistosomiasis"	Angaye, **Ohimain** et al 2015
Jatropha curcas	Mosquito	Comparative Larvicidal activities of the Leaves, Bark, Stem and Root of *Jatropha curcas* (Euphorbiaceae) against malaria vector *Anopheles gambiae*	**Ohimain** et al 2014
Hyptis suaveolens and Ocimum sanctum	Mosquito	Larvicidal activities of *Hyptis suaveolens and Ocimum sanctum* against *Anopheles gambiae.*	**Ohimain** et al 2015
Rhizophora mangle, R. racemosa, Avicennia germinans and *Languncularia racemosa*	Mosquito	Larvicidal activities of the leaves of Niger Delta mangrove plants against *Anopheles gambiae***.**	Angaye, **Ohimain** et al 2014
Rhizophora mangle, R. racemosa, Avicennia germinans and *Languncularia racemosa*	Schistosoma vector, *Biomphalaria pfeifferi*, *Bulinus globosus and Bulinus rholfsi*	Molluscicidal and Synergicidal Activities of the Leaves of Four Niger Delta Mangrove Plants against Schistosomiasis Vectors.	Angaye, **Ohimain** et al 2015
Azadirachta indica	*Anopheles gambiae and Bulinus globosus*	Biocidal activities of Solvent extracts of *Azadirachta indica* against Some Endemic Tropical Vector-borne diseases	Angaye, **Ohimain** et al 2014
Hyptis suaveolens and Ocimum sanctum	Dog Tick	Acaricidal activities of *Hyptis suaveolens and Ocimum sanctum* against African Dog Tick (*Rhipicephalus sanguinneus*)	**Ohimain** et al 2015

Table 39: Antibacterial activities of selected plants and higher fungi

Plant	Effective against (microbes)	Research summary	Reference
Pawpaw and lemon grass	*Salmonella tphi, S. paratyphi, S. typhimuirium*	Efficacy of *Cymbopogon citratus* and *Carica papaya* Used in the traditional treatment of enteric fever in Bayelsa State, Nigeria	Zige and **Ohimain** 2017; Zige and **Ohimain** et al 2017
Vitex grandifolia	*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus, Salmonella* and *Proteus* sp	Phytochemical, antibacterial and synergistic potency of tissues of *Vitex grandifolia*	Epidi, **Ohimain** et al 2016a
Alstonia Boonei	*Staphylococcus aureus, Pseudomonas aeruginosa, Bacillus, Salmonella* and *Proteus* species	Antibacterial and Synergistic Efficacy of Extracts of Alstonia Boonei Tissues	Epidi, **Ohimain** et al 2016b
Chromolaena odorata	Salmonella typhi and E. coli.	Antibacterial Activity of Ethanol, Crude and Water Extract of *Chromolaena odorata* leaves on S typhi and E. coli.	Zige, **Ohimain** et al (2013)
Moringa oleifera	*Pseudomonas aerogenosa*, *Escherichia coli*, *Klebsiella sp*, *Salmonella* typhi, *Proteus mirabilis*	Antibacterial activity of ethanol extract of the defatted seed and seed coat of *Moringa oleifera*	Alikwe, **Ohimain** et al (2013)
Fomes lignosus, *Marasmius jodocodo*, *Pleurotus florida*, *Pleurotus tuber-regium*, *Psathyrella atroumbonata*, *Polyporus giganteus*, *Termitomyces microcarpus* and *Termitomyces robustus*	*Bacillus cereus, Escherichia coli*, *Klebseilla pneumoniae* *Proteus vulgaris, Pseudomonas aeruginosa* and *Staplylococcus aureus*.	Evaluation of the inhibitory potentials of eight higher Nigerian fungi against pathogenic microorganisms	Gbolagade, **Ohimain,** et al., 2008
		Antagonistic effect of extracts of some Nigerian higher fungi against selected pathogenic microorganisms	Gbolagade **Ohimain,** et al., 2007

AGRICULTURAL AND VETERINARY MICROBIOLOGY

Vice Chancellor sir, between 2009 and 2010, I joined a team of Researchers at North Carolina A&T University (NCAT), working on the use of mushroom as alternative animal growth promoters in poultry. The research was quite promising because the probiotic activities of these fungi result in the increase of beneficial bacteria in the GIT including Bifidobacteria and Lactobacillus, while decreasing pathogens such as E. coli, Salmonella and Eimeria, resulting in increased performance of the chickens. On my return in 2010, I continued with the same line of research with our lecturers in the Faculty of Agriculture including Prof. Mathew Akusu, Dr. Ruth Ofongo, Dr Philip Alikwe, Dr. Austin Aghghovwia, Dr Medubari Nodu and Dr. Moses Okpeku. We worked extensively on the use of enzymes and plant extracts as alternative growth promoters, which boost performance in broilers.

Alternative feeds and growth promoters

We worked on using different plant species for the formulation of alternative feeds and growth promoters, which boost performance of chickens, rats, rabbits, goats, sheep and snail (Table 40). Plant with antibacterial properties were used for the control of harmful microbes (Ofongo et al 2016). Plant extracts that we tried for the control of harmful species in broilers such as E. coli, Salmonella and Clostridium include ginger (Ofongo and Ohimain 2015a, b), Ocimum (Ofongo and **Ohimain** 2015c; **Ohimain** et al 2015) and pawpaw and Alchornea leaves for rabbits (Nodu et al 2016). Some of the vegetation that we used particularly *Alchornea cordifolia* and *Ocimum* have been shown to have antimicrobial properties. Hence, this plant can be effectively used as alternative antibiotic growth promoters (AGP) in animal production, due to the ban of conventional AGP in many countries.

Enzymes have the capacity to breakdown agricultural wastes and convert them to useful feed sources (**Ohimain** and Ofongo 2014; Ofongo and **Ohimain** 2016; Ofongo et al 2016). Isikhuemhen, **Ohimain** et al. (2014) reported the use of selected white rot fungi (WRF) for the selective delignification of canola biomass via SSF, which resulted in the breakdown of the biomass, sugar release and improvement in digestability. Isikhuemhen, **Ohimain** et al. (2012) used the WRF *Lentinus sqarrosulus* Mont. for the degradation of corn stalk and sugar released under SSF. Selective degradation involves the selective breakdown of lignin, while hemicelluloses and cellulose were hydrolyzed resulting in the saccarification of the biomass, which can be used for the compunding animal feeds.

Table 40; Alternative animal feeds from selected plants and other diets

Plant	Animal	Research summary	Reference
Water Leaf	Chickens	Performance and growth rates of Broiler chickens fed Water Leaf (*Talinum triangulare*)	Nworgu, **Ohimain,** et al 2015a, 2015b
Fern	Chickens	Performance, carcass and organ characteristics of finisher broilers fed graded levels of Fern *(Asplenium barteri)* leaf meal	Alikwe, **Ohimain,** et al 2014
Alchornea cordifolia	Chickens	Tropical Christmas bush (*Alchornea cordifolia)* Leaf Meal as Unconventional Protein Supplement in the Diet of Broiler Chicks	Alikwe, **Ohimain,** et al 2014
rumen epithelial scrappings	Chickens	Performance, carcass quality and organ characteristics of broiler finishers fed rumen epithelial scrappings meal (RESM) as replacement for fish meal	Alikwe, **Ohimain,** et al 2014
Alchornea cordifolia	Rabbits	Haematological Characteristics and Organ Weights of Rabbits Fed *Alchornea cordifolia* Leaf Meal.	Timibitei, **Ohimain,** et al 2014
Alchornea cordifolia	Rabbits	Evaluating the impact of *Alchornea cordifolia* (christmas bush) root bark, seeds and pod husks on the gonads, serum level of testosterone, estrogen, serum enzymes and blood corpuscles of rabbits	Timibitei, **Ohimain,** et al 2014
Alchornea cordifolia	Rabbits	Effects of *Alchornea cordifolia* root bark, seeds and pod husks on the growth response and visceral organs of rabbits	Timibitei, **Ohimain,** et al 2013
Telfairia occidentalis and Centrosema pubescens	Rabbits	Growth performance of rabbit fed *Telfairia occidentalis* and *Centrosema pubescens* as protein	Alikwe, **Ohimain,** et al 2014
Alchornea cordifolia	Rabbits	Performance evaluation of new zealand white rabbits fed *Alchornea cordifolia* leaf meal as replacement for soya bean meal	Alikwe, **Ohimain,** et al 2014
Pawpaw and *Alchornea cordifolia*	Rabbits	Variation of microbial population in the gut (ileum and caecum) and faeces of rabbits fed with pawpaw and Alchonea leaf meals.	Nodu, **Ohimain,** et al 2016
Alchornea cordifolia	Rats	Performance charactristics of winstar rats gavaged with aqueous extract of *Alchornea cordifolia* leaf meal	Alikwe, **Ohimain,** et al 2014
maize stover, rice straw, malted sorghum sprout	Sheep	Comparative digestibility of maize stover, rice straw, malted sorghum sprout in West African Dwarf (WAD) sheep	Alikwe, **Ohimain,** et al 2014
Alchornea cordifolia	Snails	Performance and Carcass Characteristics of Giant African Land Snails fed *Alchornea cordifolia* Leaf Meal in Replacement for Soybean Meal.	Alikwe, **Ohimain,** et al 2014
Bidens pilosa	Non-ruminants	Evaluation of the Proximate, Mineral, Phytochemical and Amino Acid Composition of *Bidens pilosa* as Potential feed/feed additive for non-ruminant Livestock	Alikwe, **Ohimain,** et al 2014
Ocimum gratissimum and *Ocimum sanctum*	Non-ruminants	Proximate, mineral, phytochemical and amino acid composition of *Ocimum gratissimum* and *Ocimum sanctum* leaf meals as potential feed additive for Monogastric animals	Alikwe, **Ohimain,** et al 2013

Poultry Probiotics

Poultry is one of the most important sources of animal protein globally. Chicken is consumed by virtually every tribe, race or religion, almost without any forms of restriction. Unlike red meat, chicken is often considered as a healthier form of animal protein. Notwithstanding the importance of poultry in human nutrition, poultry farming is threatened by high cost of feeds and microbial infections. Some detrimental microbes causing infections in poultry include Salmonella, Escherichia coli, Clostridium and Eimeria, while others such as Lactobacillus, Saccharomyces and Bifidobacteria are considered as beneficial microbes. The use of antibiotic growth promoters (AGP) are either restricted or out rightly banned in many countries. Hence, the poultry farmers are at loss on how to effectively control infections and boost performance. Research is focused on the development of alternative feedstocks that are cheap and readily available and supplement for infections control. Several promising alternative feedstocks and infection control strategies have emerged including probiotics, prebiotics, enzymes, symbiotic, acidifiers, mushrooms and botanicals and combination of these and other growth promoters. Of recent, the importance of the gastrointestinal tract (GIT) health in overall performance of broiler is increasingly being recognized. The intestinal epithelium acts as a barrier protecting the animal against pathogenic microbes and toxic substances ingested by broilers. Probiotics acts by reinforcing the intestinal mucosal barrier against pathogenic microbes and toxic substances, while prebiotics selectively stimulate the activities of beneficial microbes in the mucosal surfaces.

In the USA, we used fungi myceliated grain to boost poultry through the control of Eimera infections (Willis, **Ohimain** et al., 2010a, 2011), stimulate the enteric colonization with beneficial *Bifidobacteria* while controlling detrimental Salmonella infection (Willis, **Ohimain** et al., 2010b), which result in the growth and enhancement of the health of broiler chickens (Hines, **Ohimain** et al., 2013). In Belgium, using broilers, Eeckhaut et al (2017) demonstrated that ingestion of the probiotic Butyricicocus pullicaecorum resulted in the significant reduction of detrimental bacteria including Campylobacter sp in the caecum, E. coli, Shigella and Enterococcus in the ileum and a decrease in necrotic enteritis/lesions caused by Clostridium perfringes, resulting in an improvement in the feed conversion ratio. In addition, Butyricicocus pullicaecorum can produce butyric acid in-situ during fermentation, which have been shown to reduce pro-inflammatory cytokines and induce antimicrobial host defence systems in broilers. The commonest probiotics used in poultry are members of the genera Lactobacillus, Bifidobacteria Bacillus and Saccharomyces. Similarly, Galarza-Seeber et al (2017) demonstrated the ability of three strains of Bacillus (B. subtilis, B. amyloliquifaciens & B. megaterium) to degrade aflatoxin in poultry feed caused by Aspergillus flavus.

Microbial enzyme production and utilization in poultry

Microbes particularly fungi are good sources of industrial enzymes. We carried out a study on the solid-state fermentation of waste rice husk by three indigenous fungi (*Aspergillus niger, Trichoderma viride* and *Rhizopus oryzae*) both in pure and mixed culture for the production of enzymes. Enzymes were expressed including protease, xylanase, β- xylosidase, phytase, α -amylase, glucoamylase, protease, lignin peroxidase, pectinase, manganese peroxidase. *Aspergillus niger* expressed the highest levels of enzyme activities in most cases. The enzymes have the potential for the biodegradation of complex plant material leading to the modification, saccharification and nutrient release, which could be utilized beneficially in animal feed supplementation (Table 41). Thus, converting wastes to wealth (Ofongo, **Ohimain** et al 2016).

Table 41. Studies on poultry enzymes and probiotics

Enzyme & probiotic activities	Target microbe	Research summary/ findings	Reference
Enzyme modulation, & antimicrobial effects	Bifidobacteria, Lactobacillus, Salmonella and E. coli	*Azadirachta indica* (neem) and *Vernonia amygdalina* (bitter leaf) and Roxazyme G2 G ® enzyme were supplemented in the feeds of different groups of boilers. Results showed that the leaf extracts and enzymes significantly result in the decrease of intestinal microbes, both beneficial (Bifidobacteria and Lactobacillus) and detrimental (Salmonella and E. coli)	Ofongo & **Ohimain,** 2017 (in press)
Enzyme modulation	*Lactobacillus acidophilus*, *Escherichia coli* and *Clostridium* sp	Performance and molecular identification of bacteria isolated from the gut of broiler birds after antibiotic administration and enzyme supplementation.	Ofongo, **Ohimain,** et al 2016.
Enzyme modulation		Effect of diet type and enzyme supplementation on gut pH in broilers	Ofongo, **Ohimain,** et al 2016
Enzyme modulation		Enzyme supplemented wheat offal diet improves performance of broilers	Ofongo & **Ohimain,** 2016
Antimicrobial effect of ginger		Antimicrobial effect induced by fresh ginger root extracts in broilers	Ofongo & **Ohimain,** 2015
antimicrobial effect of *Ocimum gratissimum*		Proximate composition and antimicrobial effect of *Ocimum gratissimum* on broiler gut microflora	Ofongo & **Ohimain,** 2015
antimicrobial effect of *Ocimum gratissimum*		In-vitro antibacterial effect of *Ocimum gratissimum* on broiler gut microflora	Ohimain et al., 2015
Enzyme modulation		Effect of enzyme supplemented diet on gut microflora, digesta pH and performance of broiler chickens	**Ohimain** & Ofongo 2013
Lentinus edodes	Anti Eimeria parasite	Effect of Phase Feeding Supplemental Fungus Myceliated Grain on Oocyst Excretion and Performance of Broiler Chickens	Willis, **Ohimain,** et al 2011
Lentinus edodes	Anti-coccidiosis. Eimeria *acervelina, E. maxma* and *E. tenella*	Comparing the Feeding of Fungus Myceliated Grain with Other Anticoccidial Control Measures on Oocyst Excretion of *Eimeria* Challenged Broilers.	Willis, **Ohimain,** et al 2010a
Lentinus edodes	Boost beneficial *Bifidobacteria* and reduced *Salmonella*	Effect of Dietary Fungus Myceliated Grain on Broiler Performance and Enteric Colonization with *Bifidobacteria* and *Salmonella*	Willis, **Ohimain,** et al 2010b
*Lentinus edodes (*Shiitake), *Ganoderman lucidum* (Reishi), *Pleurotus ostreatus* (Oyster) and *Cordyceps sp*.	Eimeria and Bifidobacteria	Effect of Incubation Time and Level of Fungus Myceliated Grain Supplemented Diet on the Growth and Health of Broiler Chickens	Hines, **Ohimain,** et al 2013
rumen epithelial scrapings		Effects of processed rumen epithelial scrapings meal (RESM) based diet on gastro microbial load of broiler finishers	Alikwe, **Ohimain,** et al 2013

We used agricultural residues for the production of ligninolytic enzymes (Mikiashvili, **Ohimain** et al., 2011; Isikhuemhen, **Ohimain** et al., 2012, 2014). Enzyme expressed by these mushrooms include Laccase, carboxyl methyl cellulose, and β-glucosidase. **Ohimain** and Ofongo (2013) used enzyme supplemented wheat offal-based diet in broilers which resulted in increased performance and modulation of gut microorganisms.

One common challenge in poultry feed is the presence of anti-nutrient factors particularly non-starch polysaccharides (NSP) such as β-mannan, which decrease digestibility and negatively affect gut microbes, often resulting in decreased performance. Enzyme is able degrade NSP and reverse all their negative effects. Generally, enzymes are used in several industrial applications including textiles, food and feed processing, beverages and alcohol production. Most of the enzymes used industrially, which are currently imported, are produced by microbes. Hence, enzymes produced from waste biomass could create job and wealth.

Mushroom production

Mister VC sir, while in the US, we worked on mushroom breeding, domestication, cultivation and commercialization. Mushrooms are higher fungi mostly in the class Basidiomycota and Ascomycota. Unlike other microbes that are microscopic, mushrooms are quite visible to the naked eyes. We worked on several species including *Lentinus squarrosulus*, *Grifolia frondosa* (maitake), *Pleurotus* spp (oyster mushroom), *Truffle melanosporum*, *Lentinus edodes* (shiitake), among others. We utilized mushroom in several sectors including environmental remediation, production of enzymes, functional food and nutraceuticals, health supplements, veterinary medicine and agriculture. Mushroom expresses several enzymes for the biodegradation of wood and other complex polymers. Mushrooms are quite prolific in the production of lignin-cellulosic enzymes including laccase, peroxidase. Wood comprises of cellulose and hemi-cellulose protected by lignin. Lignin is generally resistant to biodegradation. But mushroom have the capacity to breakdown lignin, cellulose and hemi-cellulose. Current technology involves the selective breakdown of protective lignin (biodelignification), which now make the carbohydrates available for utilization in animal nutrition and biofuels (Isikhuemhen, **Ohimain** et al 2014). By this process, many agricultural wastes can become useful such as food and agricultural processing wastes including oil palm, corn, plantain, cassava, rice, cocoa pods, groundnut shells and wood wastes. These wastes can be used for the production of mushrooms fruit bodies, enzymes, sugars and feeds.

The biodegradative ability of mushroom can also utilized for the breakdown of spilled oil especially the PAHs that resist conventional biodegradation. Mushrooms are also useful in the breakdown of other recalcitrant organic compounds including pesticides. Fungi generally and mushroom can therefore be useful in oil spill bioremediation especially for the breakdown of PAHs, which also could create job especially for the clean-up of Ogoni and other contaminated sites in the Niger Delta.

Mushroom is quite delicious. It has been used as a source of protein especially for vegetarians. It used as a soup thickener. Mushroom is also used to increase the protein content of bread. For instance, the mushroom *Laetiporus* (Plate 43), commonly called chicken of the woods, tastes like chicken. Shiitake mushroom production is a big business (Plate 44). Today there are several commercially available nutraceuticals and health supplements produced from mushrooms.

Plate 43: *Laetiporus,* commonly called chicken of the woods & mushroom fruiting house

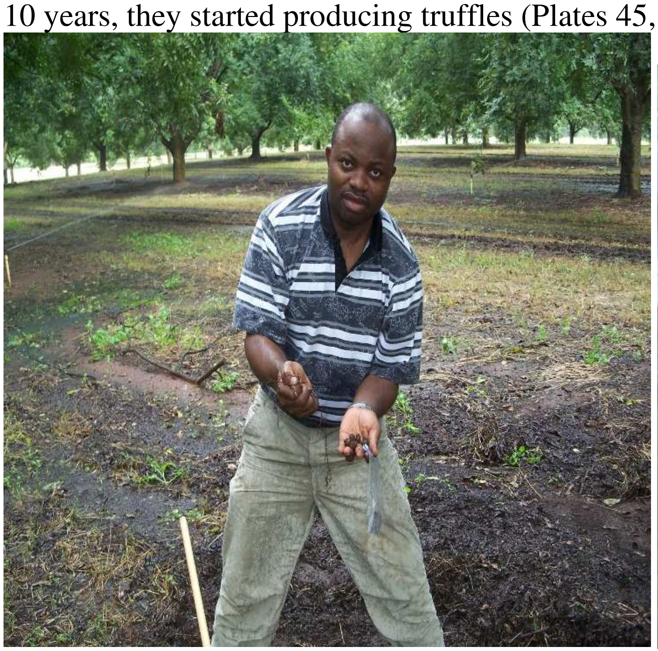
Plate 44: Shiitake mushrooms

Mushrooms produce beta glucan, which have been demonstrated in humans for immune system enhancement, lowering of blood cholesterol, normalization of blood pressure and sugar level (diabetes), prevention and treatment of cancers and many infectious diseases such as influenza, HIV/AIDS and tuberculosis (Friedman, 2016). This is another case of the good microbes against the bad ones.

Diabetes mellitus is a metabolic disorder caused by either defect in insulin secretion (type 1) or insulin action (type 2), or both. The disease has affected over 250 million people worldwide. Diabetes is increasing globally due to a combination of factors such as lifestyle and feed habits. Bioactive substances extracted from some mushroom have anti-diabetic (Table 41) and anticancer (Table 42) effects.

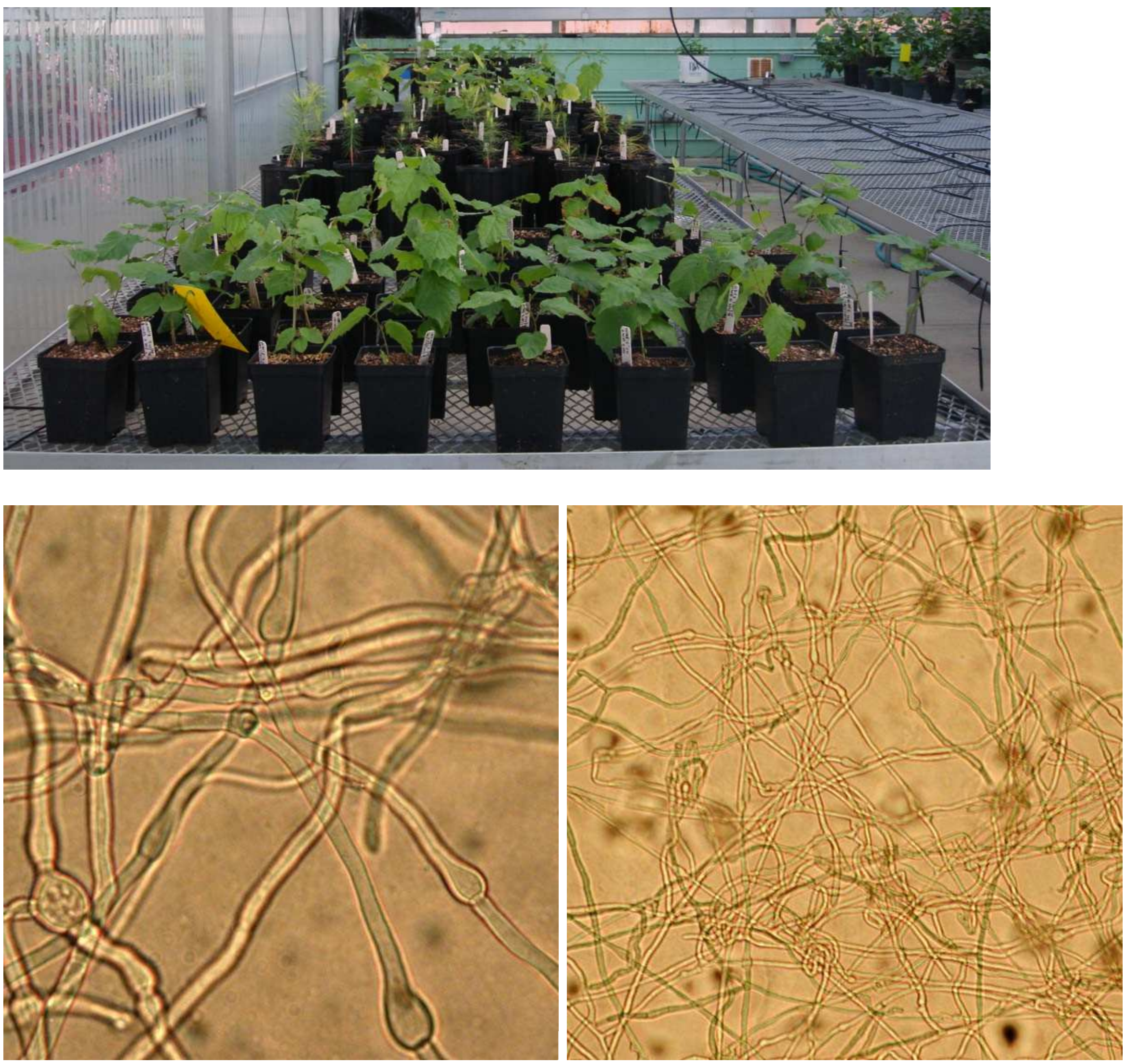
There are more than 2000 species of edible and/or medicinal mushrooms that have been identified worldwide, many of which are widely consumed, stimulating much research on their health-promoting properties, which are associated with bioactive compounds produced by the mushrooms, including polysaccharides.

While in the US, we worked on the domestication of truffles, Tuber species. There are different species of truffle, of which the most expensive is the French Perigod truffle, Truber melanosporum. This acosmycetous fungus is one of the most priced mushroom in the world costing over $1000 per kg. This mushroom exists naturally in the wild in mycorrhizal association with some plant species such as hazel nut, oak, pecans and pines. Truffles are used for culinary purposes and for the manufacturing of expensive perfumes. Truffles are typically harvested from the wild mostly in the forests. We went to the forest, harvested some truffles, cultured them in the lab and inoculated seedlings of oak tree with the spores of the mushroom and planted them in orchards and within 7-10 years, they started producing truffles (Plates 45, 46, 47).



Plates 45: Prof. Omon Isikhuemhen; the mushroom guru

Plate 46: Truffles



Plates 47: Domestication of Truffles

Table 42: Summary of our research on Mushrooms

Mushroom	Potential uses	Research summary	Reference
Coriolopsis caperata, Bjerkandera adusta, Fuscoporia gilva, Pleurotus tuberregium and *Pleurotus pulmonarius*	Sugar, enzyme & biofuel production, biodegradation & waste management, culinary	Biodegradation and Sugar Release from Canola Plant Biomass by Selected White Rot Fungi.	Isikhuemhen, **Ohimain,** et al., 2014
Lentinus squarrosulus	Sugar, enzyme & biofuel production, biodegradation & waste management, culinary	The tropical white rot fungus, *Lentinus squarrosulus* Mont.: Lignocellulolytic enzymes activities and sugar release from cornstalks under solid state fermentation.	Isikhuemhen, **Ohimain,** et al., 2012
Grifola frondosa	Sugar, enzyme & biofuel production, biodegradation & waste management, culinary	Lignin degradation, ligninolytic enzymes activities and exo-polysaccharide production by *Grifola frondosa* strains cultivated on oak sawdust.	Mikiashvili, **Ohimain,** et al., 2011
Lentinus edodes	Anti Eimeria parasite	Effect of Phase Feeding Supplemental Fungus Myceliated Grain on Oocyst Excretion and Performance of Broiler Chickens	Willis, **Ohimain,** et al 2011
Lentinus edodes	Anti-coccidiosis. Eimeria *acervelina, E. maxma* and *E. tenella*	Comparing the Feeding of Fungus Myceliated Grain with Other Anticoccidial Control Measures on Oocyst Excretion of *Eimeria* Challenged Broilers.	Willis, **Ohimain,** et al 2010a
Lentinus edodes	Boost beneficial *Bifidobacteria* and reduced *Salmonella*	Effect of Dietary Fungus Myceliated Grain on Broiler Performance and Enteric Colonization with *Bifidobacteria* and *Salmonella*	Willis, **Ohimain,** et al 2010b
Lentinus edodes (Shiitake), *Ganoderman lucidum* (Reishi), *Pleurotus ostreatus* (Oyster) and *Cordyceps sp.*	Eimeria and Bifidobacteria	Effect of Incubation Time and Level of Fungus Myceliated Grain Supplemented Diet on the Growth and Health of Broiler Chickens	Hines, **Ohimain,** et al 2013
Fomes lignosus, *Marasmius jodocodo*, *Pleurotus florida*, *Pleurotus tuber-regium*, *Psathyrella atroumbonata*, *Polyporus giganteus*, *Termitomyces microcarpus* and *Termitomyces robustus*	*Bacillus cereus, Escherichia coli*, *Klebseilla pneumoniae Proteus vulgaris, Pseudomonas aeruginosa* and *Staplylococcus aureus.*	Evaluation of the inhibitory potentials of eight higher Nigerian fungi against pathogenic microorganisms	Gbolagade, **Ohimain,** et al., 2008
		Antagonistic effect of extracts of some Nigerian higher fungi against selected pathogenic microorganisms	Gbolagade, **Ohimain,** et al., 2007

Table 43: Anti-diabetic properties of selected mushrooms

Biological name	Common name	Bioactive substance	Anti diabetic effects
Agaricus subrufescens	Almond mushroom	β-glucans and oligosaccharides	Anti-hyperglycemic
Agrocybe cylindracea	Chestnut mushroom, Poplar mushroom	A glucan and heteroglycan	Hyperglycemic activity
Auricularia auricular-judae	Ear mushroom	Polysaccharide	Lowering plasma glucose, insulin, urinary glucose, increased tolerance to intraperitoneal glucose loading and hepatic glycogen content
Agrocybe aegerita	-	Phenol	Hypocholesterolemic effect
		A glucan	Hypoglycemic effect
Agaricus campestris	Field mushroom, Meadow mushroom	Lectins	Hypoglycemic effect
Agaricus bisporus	-	Lectins	Hypoglycemic effect
Astralagalus campestris		Non-lectin-type component	Hypoglycemic effect
Coprinus comatus	Shaggy ink cap	4,5-Dihydroxy -2-methoxybenzaldehyde	Inhibition of the non-enzymatic glycosylation (NEG) reaction; decreased concentrations of fructosamine, triglycerides and total cholesterol. Maintained levels of blood glucose and improved glucose tolerance
Cordyceps militaris	Caterpillar Killer	Exo-polymer	Significantly decreased levels of plasma glucose, total cholesterol, triglyceride and plasma glutamate-pyruvate transaminase
Cordyceps sinensis	Caterpillar fungus	Polysaccharide	Significant drop in blood glucose levels and increased serum insulin levels, stimulation of pancreatic release of insulin and/or reduced insulin metabolism
Cordyceps. takaomontana	-	4- β- acetoxyscirpe-ndiol	Decreased blood sugar in the circulatory system as specific inhibitors of Na+/ glucose transporter-1
Ganoderma applanatum	Artist's Bracket	Exo-polymer	Reduced plasma glucose,
		Polysaccharide	Hypoglycemic effect
Ganoderma lucidum	Reishi or Lingzhi mushroom	Polysaccharide	Significant increase in body weights and serum insulin levels; Hypoglycemic effect
		Proteoglycan	Decrease in fasting plasma glucose and increase in insulin concentration

		Ganpderan A and B, glucans, Triterpenes	Hypoglycemic effect
Grifola frondosa	Hen of the woods, Maitake	MT-α-glucan	Antidiabetic activity, related to its effect on insulin receptors
		Ergosterol	Hypoglycemic effect
		Polysaccharide	Improved glycemic levels
Inonotus obliquus	Chaga mushroom	Polysaccharide	Hypoglycemic effect
Laetiporus sulphureus var. *miniatus*	Sulphur polypore	Polysaccharide	Decreased plasma glucose levels, increased insulin antigenesity via proliferation or regeneration of diabetic islet β-cells
Laetiporus sulphureus	-	Dehydrotrametenolic acid	*Laetiporus sulphureus*
Laricifomes officinalis	-	Dehydrotrametenolic acid	*Laetiporus sulphureus*
Lentinula edodes	Shiitake	Exo-polymers	Significant reduction in plasma glucose,
Lentinus strigosus F	Ruddy panus	Exo- Polysaccharides	Reduce plasma glucose
Phellinus baumii	-	Exo- Polysaccharides	Hypoglycemic effect with substantially reduced plasma glucose levels
Phellinus linteus	Meshimakobu, Song-Gen, Sang-Hwang	Exo-polymers	Reduce plasma glucose
		Exo- Polysaccharides	Hypoglycemic effect with reduced plasma glucose levels
Phellinus ribis	-	Polychlorinated	Therapeutic effects through the enhanced PPAR-γ agonistic activity
Pleurotus abalonus	Abalone mushroom	Polysaccharides	Hypoglycemic effect
Sparassis crispa	Cauliflower fungus	β-glucans	An effective promoter of wound healing in patients with diabetes. Increase in the migration of macrophages and fibroblasts, and directly increased synthesis of type I collagen
Stropharia rugosoannulata	Wine cap, Burgundy mushroom King stropharia	Polysaccharides	Decrease plasma glucose
Trametes gibbosa	Lumpy bracket	Polysaccharides	Decrease plasma glucose
Tremella aurantia	Golden ear	Polysaccharides	Decrease serum glucose level
Tremella fuciformis	Snow fungus, Silver ear	Glucuronoxylomannan	Hypoglycemic effect

	fungus, White jelly mushroom	Exo-polysaccharides	Hypoglycemic effects and improved insulin sensitivity possibly through regulating PPAR-γ mediated lipid metabolism
Tremella mesenterica	Yellow brain mushroom, Golden jelly fungus, Yellow trembler, Witches' butter	polysaccharide glucuronoxy-lomannan	reduction of intrinsic blood glucose levels
		Heteropolysaccharide	Reduction in blood glucose level
Tremella versicolor	-	Corinolan, a β-glucan-protein	Hypoglycemic effect
Wolfiporia extensa	Pine-tree rotting mushroom	Dehydro- tumulosic acid, dehydro-trametenolic acid and pachymic acid	Insulin sensitizer activity
		Dehydotrametenolic acid, dehydrotumulosic acid, pachymic acid	Reduce postprandial blood glucose level
Woliporia cocos	-	Dehydotrametenolic acid	Hypoglycemic effect

Table 44: Anti-cancer properties of selected mushrooms

Mushroom	Common name	Bioactive substance	Anti-cancer effect	Effective against
Agaricus blazei	Agaricus	B-D-glucan	Immune modulation	Hepatocellular carcinoma, Leukemia
Calvatia gigantean		Calvin	Anti-tumor	
Cordyceps militaris	Caterpillar Killer		Treatment of cancer	
Cordyceps sinesis	Cordyceps, Caterpillar mushroom	Adeonisne, Cordycepin	Immune modulation	Nonsamll-cell lung cancer, lymphoma
Elfringia applanata			Anti-cancer	
Flammulina velutipes			Anti-cancer	
Fomes formentarius		Polysaccharides	Treatment of cancer	
Fomitopsis pincicola			Anti-cancer	
Ganoderma lucidum	Reishi, lingzhi	Ganoderic acid, Danoderiol, Danderenic acid, Lucidenic acid, Ganoderma lucidum polysaccharide	Immune modulation, anti-tumor	Hepatocellular carcinoma, sarcoma Leukemia, breast, prostrate and colon cancer
Grifola umbellate			Anti-cancer	

Grifolia frondosa	Maitake, Hen of the woods	Grifolan, D-fraction, MD-fraction, (Grifron-D, GD)	Intestinal tumorigenesis, Immune modulation, anti-tumor	breast cancer, colon cancer
Helricum erinaceus		Galactoxloglucan-protein complex	Immune-modulating	
Inonotus hispidus				Cancer and stomach disease
Lentinus edodes	Maitake, Shiitake	β(1-6) glucan, Lentinan		
Lenzites betulina			Anti-cancer	
Phellinus igniarius			Anti-cancer	
Phellinus rimosus			Anti-tumor	
Phellinus yucatensis			Anti-cancer	
Pholiota nameko			Anti-cancer	
Pleurotus florida			Anti-tumor	
Pleurotus osteatus			Anti-cancer	
Pleurotus pulmonaris			Anti-tumor	
Schizopyllum commune		Polysaccharide	Anti-tumor	Sarcoma 180 tumor xenographs
Trametes versicolor (formerly Coriolus versicolor)	Turkey tail	Polysaccharide peptide, Polysaccharide K	Immune modulation	Gastric cancer, breast cancer, colon cancer
Tricholoma matsutake			Anti-cancer	

Fisheries and Aquatic Microbiology

Both as a researcher and environmental consultants, I have worked on several aquatic systems including surface and underground, inland and coastal, freshwater, brackish and saline water. I have worked virtually on all the rivers discharging into the Atlantic Ocean from Qua Ibo in Cross River State, through the Niger Delta, the Mahin coast, the Lagos lagoon and through Benin, Togo to up to Takoradi in Ghana. My work covered water physicochemical parameters and microbiology, fisheries and fish diseases. We also studied the toxicity of effluent to catfish (Aghoghovwia & **Ohimain,** 2015). Only a small fraction of my water quality data is published in peer-reviewed journals (Table 45).

We studied fish diseases especially of domesticated species. Most of their diseases are caused by microbes including bacteria, fungi, protozoa and viruses. Algae also produces toxins that are poisonous to fishes. There is need to study the diseases of fish in the wild especially those that are caught in communities where sewage is discharged into the river, to determine the risk of transmission of pathogens to humans.

Bayelsa State buy most of their potable water from neighbouring state due to the twin problem of acidity and high iron content of the under groundwater. The high acidity and iron content are due to the geology of the sedimentary basin containing pyrite, which become oxidized on exposure producing acidity and releasing iron. It should be noted that pyrite is usually present in coastal sediments. But during the delta formation, freshwater sediments might have overlain coastal sediments. And because surface water is sometimes polluted, most catfish farmers rely on under groundwater. So the problem of high acidity and iron affects catfish, causing poor performance, diseases and death. We carried out a study on using plantain trunk to treat groundwater prior to use in catfish farming (**Ohimain,** et al 2014). We also studied the suitability of groundwater for domestic and agricultural use (Okiongbo & **Ohimain,** 2014). Dr Okiongbo used electric properties (vertical electrical sounding) of the subsurface to locate sources of groundwater devoid of iron. We also designed a trickling filter that effectively removed coliforms, iron and acidity from shallow groundwater (**Ohimain,** et al 2014).

At the 21st NDU inaugural Lecture, Prof Adeyemo lectured us on fish parasites, while at the 27th inaugural lecture, Prof Abowei emphasized on the importance of fisheries to national development. What is therefore left is research collaboration to move fisheries to the next level. For instance, I was in Norway some years ago. I looked for stock fish in their restaurants in vain. Many restaurant operators do not know what stock fish is. I realized that Norway have been producing stock fish mostly for the export market and Nigeria is one of their major market destination. By the way, stock fish is mostly preserved by drying. Despite being as dry as stone, microbes particularly the xerophytes still grow on it.

Plate 48: The challenge of iron in underground water in Bayelsa State

Table 45: Fisheries and aquatic studies

Relevance	Research summary	Reference
Surface water quality - microbiological	Investigation of spatial variations in bacterial distribution in surface waters of Ikpoba River	Benka-Coker & **Ohimain**1995.
Surface water quality - physicochemical	. Spatial variations in water chemistry of Ikpoba River	**Ohimain** & Benka-Coker, 1997
Fisheries	Survey of Aquaculture Practices in Yenagoa, Bayelsa State, Nigeria	Aghoghovwia & **Ohimain,** 2015b.
Fisheries, toxicity studies, water quality	Toxicity of domestic effluents to *Clarias gariepinus* fingerlings	Aghoghovwia & **Ohimain,** 2015a.
Fisheries, water quality	Fish Species Composition and Diversity of Warri River, Niger Delta Nigeria	Aghoghovwia & **Ohimain,** 2015c.
Fisheries, water quality	Physicochemical Characteristics of Lower Kolo Creek, Otuogidi, Bayelsa State	Aghoghovwia & **Ohimain,** 2013.
Fisheries, toxicity studies, water quality	Heavy metal levels in water and sediment of Warri River, Niger Delta, Nigeria.	Aghoghovwia, **Ohimain,** et al 2015.
Groundwater quality, fisheries	Groundwater quality and its suitability for domestic and agricultural use in Wilberforce Island, Southern Nigeria	Okiongbo & **Ohimain,** 2014.
Fisheries, iron problems, toxicity studies, water quality	Iron Levels, Other Selected Physicochemical and Microbiological Properties of Earthen and Concrete Catfish Ponds in Central Niger Delt	**Ohimain** Angaye 2014
Fisheries, iron problems, toxicity studies, water quality	Toxicological assessment of groundwater containing high levels of iron against fresh water fish (*Clarias gariepinus)*	**Ohimain,** et al 2014
Biotreatment, Fisheries, toxicity studies, water quality	Biotreatment of acidic and high iron containing groundwater for aquaculture using plantain (*Musa paradisiaca*) tissues.	**Ohimain,** et al 2014
Biotreatment, Fisheries, toxicity studies, water quality	Removal of iron, coliforms and acidity from ground water obtained from shallow aquifer using trickling filter method	**Ohimain,** et al 2013
Flooding, water quality	Selective impacts of the 2012 water floods on the vegetation and wildlife of Wilberforce Island, Nigeria	**Ohimain,** et al 2014
Fish diseases	The challenge of non-infectious diseases in catfish farming	**Ohimain,** et al 2014
Microbial infections, fisheries	The challenge of microbial and parasitic infections in catfish farming	**Ohimain,** et al 2013
Catfish diseases	Prevalence of Catfish Diseases in Bayelsa State; A Case Study of Kolokuma/Opokuma Local Government Area (KOLGA).	**Ohimain,** et al 2013

Plate 49: Water-fisheries-education art work in NDU

MICROBIAL PHENOMENA IN BIBLICAL TIMES AND IMPLICATIONS FOR THE CHURCH OF TODAY

In the beginning, God created all things both visible and invisible including plants, animals, man and microbes (Genesis 1: 26-28). Microbes belong to the invisible world. But there are references in the Bible suggesting that the people in Biblical times have knowledge of microbiology. For instance, microbial infections such as leprosy and plagues are frequently mentioned in the Bible. These diseases are now known to be caused by *Mycobacteria leprae* and *Yersinia pestis* respectively. Also, the way they handle dead bodies suggest that they are aware that it contains dangerous microbes. Wine production using the yeast, Saccharomyces cerevisiae, is an age long tradition in the Bible. This same yeast has been used for bread production since Biblical times and up till today. Matthew 13:33- *The kingdom of heaven is like unto leaven, which a woman took, and hid in three measures of meal, till the whole was leavened*. Bread baked without yeast is referred to as unleavened bread, which is used specially for Holy Communion. There are some activities in the Church today that could possibly facilitate the transmission of microbes including hand shaking, anointing service, Holy Communion, baptism in fecally contaminated water, sharing of microbes etc. Hence, the pastor need knowledge of microbiology in order to protect his or her members.

SUSTAINABILITY AND INTERDISCIPLINARY NATURE OF MICROBIOLOGY

Many mega conferences have been held globally in an attempt to solve the twin problem of environment and development. (Table 46). The major environmental crises are climate change, biodiversity loss and poverty and the widening gap between the poor and the rich. The conferences recommend a transition to green economy involving the use of low carbon fuel and alternative energy sources among other recommendations, which are captured in the millennium development goals (MDG) and the sustainable development goals (SDG). Only through interdisciplinary collaboration can these goals and targets be achieved. Microbiology is at the center of the collaboration that will lead to the realization of the SDGs (Fig 30).

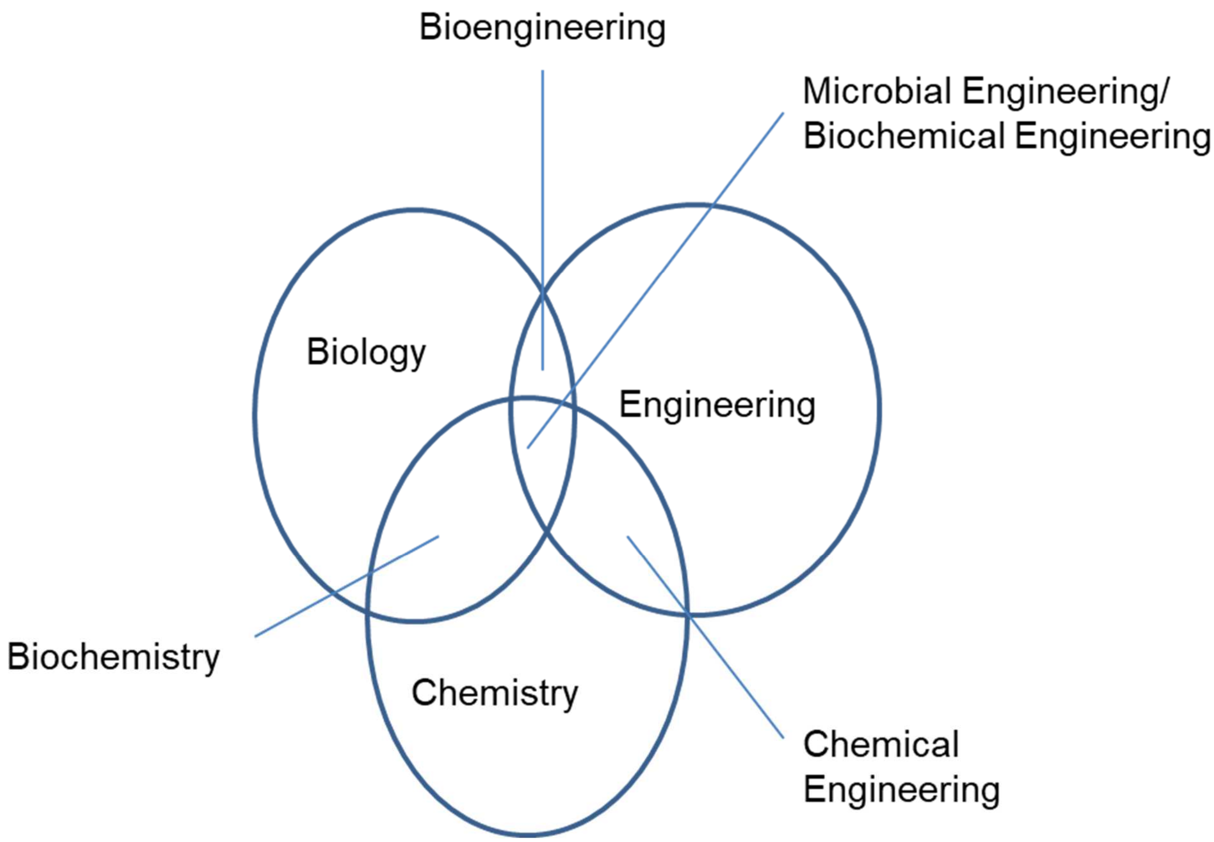


Figure 30: Interdisciplinary nature of microbiology and microbial engineering (modified from Abu (2017) 141st UNIPORT Inaugural Lecture)

Microbes produce sustainable alternatives to several products that are causing environmental challenges in modern times. For instance, conventional physicochemical processes which are used for the production of petrol, diesel, kerosene, LPG, electricity, plastics, pesticides, fertilizer and antibiotics are energy intensive and cause environmental impacts. Microbial processes can be used to produce green alternates with less energy input and environmental impacts. Such products include bioethanol/bio-gasoline, biodiesel/myco-diesel, ethanol gel, biogas, bioelectricity, bioplastics, biopesticides, biofertilizer and natural antimicrobial substances respectively and much more including green chemicals such as lactic acid, acetic acid, n- and iso-propanol, n- and iso-butanol. Rather than creating wastes, microbial processes make use of wastes from conventional physicochemical products and convert them to useful products. Due to the observed challenges of conventional processes, attention is now focused on green alternatives, which could create job, spur national development, with minimal impacts on the environment.

Table 46: Global mega conferences to tackle the problem of environment and development

Mega Conference	Major outcome
UN Conference on the Human Environment (1972). Held in Stockholm, 5-16 June 1972	Led to the establishment of the United Nations Environment Programme (UNEP)
World Commission on Environment and Development (1987). Entitled *Our Common Future*, also known as the Brundtland report.	Developed the theme of sustainable development
United Nations Conference on Environment and Development (1992). Held in Rio de Janeiro, 3-14 June 1992 AKA (Earth Summit, Rio Conference)	Three major agreements adopted (Rio Declaration on Environment and Development, Agenda 21, a global plan of action to promote sustainable development & Statement of Forest Principles, a set of principles to underpin the sustainable management of forests worldwide); Two multilateral treaties were opened for signature: United Nations Framework Convention on Climate Change UNFCCC) & Convention on Biological Diversity
Millennium Summit of the United Nations. Held 6-8 September 2000 at UN HQ, New York	The Millennium Development Goals (MDGs) were the 8 goals & 21 targets for the year 2010-2015.
World Summit on Sustainable Development (2002) AKA Rio +10. Held in Johannesburg, 26 August - 4 September 2002	Reviewed progress in the implementation of Agenda 21 since its adoption in 1992. Johannesburg Declaration on Sustainable Development
UN Conference on Sustainable Development (2012) AKA Rio+20 Held in Rio de Janeiro, 20-22 June 2012	The future we want
UN Resolution A/RES/70/1 of 25 September 2015. Transforming our World: the 2030 Agenda for Sustainable Development" or Agenda 2030	The Sustainable Development Goals (SDGs) replaced the MDGs in 2016. It consists of 17 goals with 169 targets
One Planet Summit held 12 December 2017 in Paris	Battle against climate change. Commitment for low carbon fuels

CONCLUSION

Microbes, which are the smallest living things are ubiquitous and have found applications in several fields and disciplines including medical sciences (medicine, dentistry, ophthalmology, nursing, pharmacy and medical laboratory sciences), agricultural sciences (animal science and veterinary medicine, fisheries and aquatic studies, crop production and soil science, food processing and storage) and engineering (petroleum and chemical, electrical and alternative energy, civil and water engineering) and other scientific fields including physics, geology and chemistry. Being ubiquitous, microbes are found in virtually all environments including air, soil surface and underground geological structures, surface and underground water, sediments and have also been detected in outer space. Microbes can strive in diverse ecosystems because they adapt to contrasting environmental conditions such as light and darkness, oxygenic and anaerobic, autotrophic and heterotrophic, phototrophic and chemotrophic, hot and cold, moist and dryness, salty and non-salty, high and low pressures and can transform metals and degrade organics. Microbes form relationship among themselves and with other living things including plants, animals and humans. Humans play host to diverse microbes that changes with age, from birth, through different life stages (infancy, childhood, teenage and youth, adult and the elderly) up till death and thereafter to return to organic matter.

Microbes mediate biological, physical and chemical processes. Some microbial phenomena are visible, others are quite small and majority invisible in the microscale, while nanobiology is emerging with even greater prospects. Many microbes are indeed beneficial, a few detrimental and the majority harmless or free-living. In a recessed and possibly depressed economy, this is the time to exploit the vast opportunities in the field of microbiology for national development. You can't possibly escape the microbial world. Microbes will continue to be relevant to us in several ways such as food, food supplements, drugs and antibiotics, normal resident of our bodies, source of fuel, electricity and green chemicals. Microbes are ubiquitous causing problems in many sectors, producing several industrial products and they are agents for the restoration of contaminated environments and treatment of the diverse waste generated by man, and in the process, create opportunity for jobs and business. However, it is only through research collaboration with other disciplines that these opportunities can become a reality. Such collaboration could lead to product development and commercialization. Microbes are quite busy 24/7 creating jobs for the microbiologists, agriculturists, lab scientists, doctors, pharmacists, nurses, engineers and environmentalists for sustainable national development. Can we kick start microbe-based economic revolution in the Niger Delta University?

RECOMMENDATIONS

The study of microbiology has gone beyond agar plating and detection of diseases. It has advanced with interdisciplinary applications. The study of microbiology and its interactions with other fields have opened a vista of opportunity for research. In NDU, microbiology is subsumed under Biological Sciences at inception of the university. It was later recognized as an option in Biological Sciences. By 2015, we got an interim approval for B.Sc. Microbiology program and the NUC will be coming in May 2018 for reaccreditation. It is therefore apt for the Department of Microbiology be created. This should subsequently metamorphose into an institute of Microbiology. From this a Faculty of Microbiological Sciences could be created with the following as departments: Bacteriology, Mycology, Virology, Phycology or Algology, Medical & Pharmaceutical Microbiology, Public Health and Environmental Microbiology, Food and Industrial Microbiology, Bioenergy and Petroleum Microbiology, Soil and Agricultural Microbiology, Immunology and Vaccinology, Molecular Microbiology and Genetic Engineering. This could spur interdisciplinary research collaboration with other faculties.

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To God be the Glory

CITATION OF PROFESSOR ELIJAH IGE OHIMAIN

Prof Elijah Ige Ohimain was born 13 June 1970 in former Bendel State now Edo State. He started his Primary Education at Izevbigie Primary School, Benin City in 1976 and continued in Ajama Primary School, Okpe in 1977, where he completed his primary school education in 1982. He immediately proceeded to Anglican Grammar School, Igarra, where he completed his WAEC with eight Credits in 1987. He was admitted for advanced level in School of Basic Studies, Rumuola Port Harcourt, where he completed the Interim Joint Matriculation Examination (IJMB) and proceeded to the University of Benin in 1988 from where He obtained B.Sc. degree second class upper in Microbiology in 1992, MSc in Environmental and Public Health Microbiology in 1995 and Ph.D. in Environmental Microbiology in 2001. He obtained Certificate in computer Operations and programming from the University of Port-Harcourt in 1994. Prof Ohimain completed a post-graduate diploma in Sustainable Development at Staffordshire University, UK in 2011. Prof Ohimain did his post-doctoral placements in the Laboratory of Soil Science and Geology, Wageningen University and Research, The Netherlands in 2002 and was visiting Scholar at the

Department of Natural Resources and Environmental Design, School of Agriculture and Environmental Sciences, North Carolina Agricultural and Technical State University, USA in 2009/2010. Prof. Ohimain worked in the petroleum and environmental sectors (National Fertilizer Company of Nigeria, Mail-In Oilfield Services Ltd, Environmental Resources Managers Ltd, Rohi Biotechnologies Ltd and Shell Petroleum Development Company Ltd) before joining the Niger Delta University in 2006 as Lecturer 1. He was promoted to Senior Lecturer in 2009, Reader in 2012 and Professor and in 2015. He was subsequently elected and appointed, dean Faculty of Science on 28 February 2017. He had previously held other positions in NDU including Head of Department (2010 – 2014), Deputy Director Consultancy in the Directorate of Consultancy, Entrepreneurship, Research and Development (DOCERAD) from17 April 2012 – Date, Deputy Director Entrepreneurship, Research and Development DOCERAD (01 September 2008 – 9 November 2009). He was a member of some university committees including flood control and University Research Committee. In 2013, He was the HOD that midwifed the full 5 years accreditation of the Biological Science Programme and as Dean, Faculty of Science midwifed the full five years accreditation of Mathematics, Computer Science and Pure & Applied Chemistry programmes.

Prof Ohimain has both industrial and academic experience, hence he is frequently invited to conferences both locally and internationally. In 2004, at the World Dredging Conference in Hamburg Germany, he was awarded the best presenter prize. Prof Ohimain is a member of many professional bodies including Nigerian Field Society, Society of Industrial Microbiology, American Society for Microbiology, International Society for Mangrove Ecosystems, Society of Wetland Scientists, Nigerian Environmental Society, Nigerian Society for Plant Protection, International Association for Impact Assessment and Renewable and Alternative Energy Society of Nigeria.

Professor Ohimain is prolific and has authored over 210 publications including 196 Journal publications, 6 book chapters, 6 published conference proceedings, 2 Magazine articles, 3 Poster presentations and 1 book. Professor Ohimain is an editorial board member for over 10 journals and reviewer for over 100 journal titles. Prof Ohimain is an environmental and energy consultant. He is the Pastor-In-Charge of RCCG Mount Zion Area, Rivers Province 13. He is a Special Marshal with the FRSC. He is married to Betty Ohimain.

▲ ***Dr. Ohimain (centre) is presented with his award by IADC secretary general Constantijn Dolmans (r.) and congratulated by CEDA Africa Section chairman Mohammed Bachiri***

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This is to certify that the Board of the International Association of Dredging Companies has awarded the paper

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