UNIVERSITY OF PORT HARCOURT

PROCESS AND PHENOMENAL MICROBIOLOGY: HOW MICROBES WERE CREATED TO CREATE JOBS FOR MANKIND

AN INAUGURAL LECTURE

By

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INAUGURAL LECTURE

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ORDER OF PROCEEDINGS

2:45 P.M. Guests are seated
3:00 P.M. Academic Procession Begins

The procession shall enter the Ebitimi Banigo Auditorium, University Park and the congregation shall stand as the procession enters the Hall in the following order:

ACADEMIC OFFICER
PROFESSORS
DEANS OF FACULTIES/SCHOOLS
DEAN, SCHOOL OF GRADUATE STUDIES
PROVOST, COLLEGE OF HEALTH SCIENCES
ORATOR
REGISTRAR
LECTURER
DEPUTY VICE-CHANCELLOR (ACADEMIC)
DEPUTY VICE-CHANCELLOR (ADMINISTRATION)
VICE – CHANCELLOR

After the Vice-Chancellor has ascended the dais, the congregation shall remain standing for the University of Port Harcourt Anthem. The congregation shall thereafter resume their seats.

THE VICE-CHANCELLOR'S OPENING REMARKS
The Registrar shall rise, cap and invite the Vice-Chancellor to make his opening remarks.

THE VICE-CHANCELLOR SHALL THEN RISE, CAP, MAKE HIS OPENING REMARKS AND RESUME HIS SEAT.

THE INAUGURAL LECTURE

The Registrar shall rise, cap and invite the Orator, Prof. Onyewuchi Akaranta to introduce the Lecturer.

The Orator shall then rise, cap, introduce the Lecturer and resume his seat. The Lecturer shall remain standing during the introduction. The Lecturer shall step on the rostrum, cap and deliver his Inaugural Lecture. After the Lecture, he shall step towards the Vice-Chancellor, cap and deliver a copy of the Inaugural Lecture to the Vice-Chancellor and return to his seat. The Vice-Chancellor shall present the document to the Registrar.

CLOSING

The Registrar shall then rise, cap and invite the Vice-Chancellor to make his closing remarks.

The Vice-Chancellor shall rise, cap and make his closing remarks. The Congregation shall rise for the University of Port Harcourt Anthem and remain standing as the Academic (Honour) Procession retreats in the following order:

VICE-CHANCELLOR
DEPUTY VICE-CHANCELLOR (ADMINISTRATION)
DEPUTY VICE-CHANCELLOR (ACADEMIC)
REGISTRAR
LECTURER
ORATOR
PROVOST, COLLEGE OF HEALTH SCIENCES
DEAN, SCHOOL OF GRADUATE STUDIES
DEANS OF FACULTIES
PROFESSORS

ACKNOWLEDGEMENTS

As part of my locus standi, I acknowledge the Almighty God as Creator of all things, visible and invisible, including microbes and man, and I acknowledge that I have received help from Him through the Lord Jesus Christ (as my saviour from the power of sin). The Lord used my parents Late Mrs Jato Ashanu Abu and Mr. Abu Ge to bring me into this world; I acknowledge their feeble efforts at giving me an education despite their limited understanding of what it was. I had an uncle in whom I saw what glory and honour education can bring to a people, late uncle David Gaadi Ature, with whom I grew up; his personal involvement in my education included fees payment and letters of encouragement and counsel, written to me in our language, right up to when I was in Graduate School, these were very precious to me; he helped me continue to think in our language; my late maternal grandmother Wanjoho Ade Ature actually enrolled me in school with her own shillings'.

I am very sincerely grateful to Professor Rita R. Colwell who accepted me for graduate work in her laboratory at the University of Maryland, College Park Campus. Her lab and home became an International consortium to many of us from all over the world. Professor Ron Weiner opened his lab to me for research and collaboration during Grad school. Professor Shoshana (Malis) Arad made space for me in her lab at the Institute of Applied Research, Ben Gurion University of the Negev, Beersheva, Israel for UNESCO and World Bank-NUC Post Doctoral Fellowships. I acknowledge a grant from the Petroleum Technology Development Fund (PTDF) for research in renewable bioenergy.

The Staff Development Award of the Federal Government of Nigeria administered by the University of Port Harcourt is gratefully acknowledged. Herein I do express my appreciation to my colleagues in the Department of Microbiology, Faculty of Science and other faculties for useful scientific interface and university-wide projects. Late Professor F. A. Onofeghara hired me into the School of Biological Sciences and he was my Scripture Union mentor too.

He and Professor B. E. Okoli encouraged me to pursue the pinnacle of academia in the face of challenges. My students, at the undergraduate and graduate levels, over the years, have enriched me academically and scientifically, I actually feel indebted to them all.

I have received great blessings in form of prayer, companionship, counsel, suggestions and discussions including *Sociomicrobiology*, from my dear wife, Dr (Mrs) Owapiriba P. Abu, I am most blessedly grateful.

I do sincerely thank our Eighth Vice Chancellor, Professor N.E.S. Lale for approving the date and presentation of this Inaugural Lecture.

PROTOCOL

The Vice Chancellor, Sir,

Members of the Governing Council here present,

Deputy Vice-Chancellors,

Registrar and other Principal Officers,

Provost, College of Health Sciences,

Dean, Graduate School,

Deans of Faculties,

My Fellow Professors and other Academic Colleagues,

Directors and Heads of Department,

Great Students of Unique Uniport,

Friends of the University,

Distinguished Ladies and Gentlemen

PREAMBLE

MY LOCUS STANDI, MY PERSPECTIVE

"The purpose of science is to read the mind of God". (Isaac Newton, 1643-1727)

Nothing is more agreeable to those devoted to a scientific career than to increase the number of discoveries, but when the results of these observations are demonstrated by practical utility, their joy is complete (Louis Pasteur, 1822-1895).

I may trace my exposure to learning and knowledge to when I was growing up with my maternal cousins and uncles. It was there that I also got introduced to the way of the Almighty God. We used to organize our own school after we came back from school. There, in our own school, we had all the trappings of a school with the Headmaster and all; even punishment was meted out as necessary. Reading materials included the Bible, but a lot of times, we used the Bible maps to play word games; here you were to spot a very obscure and hidden word on the map within a period of time (a minute or so). Arithmetic was a regular. All of these removed the fear of school and gave us confidence. By the time we were in primary 5, 6 or 7, we were competing among ourselves who would solve the toughest questions in the Lacombs, that was where we learned BODMAS. Of course soccer was a regular, trophies were made out of cardboard paper (carton), and the soccer ball was stitched rags (Kange bol = tie ball-nothing about rags; Dolly Portron knows what I am talking about; you could also "yam bol" = buy ball). This made school fun because "our" school was training us in very practical ways. We could take the Michael West Dictionary and learn words, especially such words as perambulate, tintinnabulation, cogitate. So when our regular primary school science teacher (Mr. Ajim), teaching on the ear, gave us the definition of sound as: "sound is a physiological sensation, caused by a vibration source, passed through a medium and received by the ears," it stuck with me. I have been teaching it; I taught it to my students at the University of Maryland College Park, USA and I have taught it at

UPH (I call it UP Here). In both places, the students were bashfully impressed, that stuff coming from a village school as far away as Timbuktu or Kauranamoda or Arondiuzogu or Ikpa Mbatierev. In High (Secondary) School, biology was a natural followed by chemistry. I don't know what had happened to my Lacombs prowess. But anyway, with Stone and Cozens, and Kimball and Vine and Rees, biology was ever exciting. I have kept on refining my understanding of the origin of species – the Darwin finches of the Galapagos Islands. Lab sessions were the biggest hit for me studying microbiology at the famous Ahmadu Bello University (ABU) Zaria, Nigeria; also the fat books – Microbiology by Pelczar and Reid was the low Priced Edition (Brown Paper Ed) we loved. I would end up attending Grad School at the University of Maryland, College Park, where Professor Michael J. Pelczar Jr. was Dean of Graduate School. At ABU, the lab sessions were great, but there was a severe limitation in equipment and instrumentation. Microbial ecology was introduced during our set, and Professor C.T. Odu came to teach us ecology and was talking about the use of microbes to rehabilitate crude oil impacted sites in the Niger Delta. Professor S.O. Emejuaiwe and Professor L. J. Egler were our HODs. Professor Emejuaiwe was buddies with Professor N. Okafor who was our external examiner. Alpha Diallo was our indefatigable lecturer together with, now, Professor A. Obayemi, and of course our ever pleasant Dr. Mrs Polebska, others included, now, Professor L. Ogbadu, Professor Lisa Holloway and Dr. T. West and Dr. J.A. Abalaka (Now Professor Abalaka). Late Professor (then, Dr.) Harrison taught us the Schroedinger equation using the chemistry text by Spratley. I remember vividly the anguish of a scientist when Professor Lisa Holloway of Biochemistry Department lost her priced Pipetman that could deliver liquids in microliter quantities as we did biochemistry labs. I wonder why someone would do a thing like that – but such are the woes of a scientist. We learned protein synthesis with now, Professor (Pastor) Duro Adegboye using the self instructional packages (SIPs). The Late, Saint, Professor F.A. Onofeghara was the Dean of the School of Biological Sciences when he asked Late Professor J. Ekundayo to interview me for the post of Graduate Assistant at the University of Port Harcourt. At the

interview Professor Ekundayo asked me if I was SU, and I said yes. Now, then, SU (for Scripture Union) stood for the dyed-in-the wool followers of Christ, of course Professor Onofeghara was silently loud SU.

I was hired for Staff Development to study Aquatic microbiology. I was eventually accepted in the lab of Professor Rita Rossi Colwell at the University of Maryland, College Park, Maryland, USA. This is the same lab, where, now, Professor G.S.C. Okpokwasili had been accepted a few years earlier. Professor Okpokwasili and his wife Nonye made things so fright-free for me when I finally got to College Park, MD, both in the lab and socially. We used to play soccer together too.

At College Park, UM Grad School, the phraseology was - higher degrees are earned, not given, and they are earned in the laboratory. Professor Colwell's Lab was always a beehive of activities with grad students from all over the world, it is/was an International Lab, it is an Institution. At Maryland, I got to another level of microbiology. The course offering was challenging but always interesting. In one virology class, the Professor, a guest lecturer of Professor Frank M. Hetrick, was teaching on retroviruses, and he said, "we all may just be a bunch of retroviruses". I went back to my room that day praying the Lord would deliver me so that I do not get swallowed up in my mind believing I came from retroviruses. I shall talk about viruses later on. In Professor Colwell's Lab, I would end up being co-advised by herself and Professor Ron Weiner, when I was placed on a SeaGrant funding to study the mechanisms of adhesion of a marine bacterium code named LST – for Lewes Spat Tank. The bacterium had been found in association with oyster spats in tanks at the University of Delaware Mariculture Center.

There was much discussion about carbon (oligotrophy, copoiotrophy) that my pronunciation of carbon (Kabon) was changing to (Korbon) as Professor Weiner was wont to call it. I was not only learning pronunciation, I was also learning many things, techniques, concepts and (Korbohydrate) Chemistry, more later on). I did not forget that I was SU, so I used to go to the shelter for the

homeless in SE Washington DC to share the gospel of our Lord Jesus with them there at the shelter. On one occasion, we were right in the mix of sharing out soup and other items to the needy. This was good and humbling. So, here I am learning about microbes and fellow human beings from different backgrounds and the reminder is that both mankind and microbes were designed by the same designer (God); they , both microbes and humans, could never create or make themselves, it is simply impossible in our human realm of existence. This is my locus standi, my perspective, and this is how I partly captured it in my acknowledgement in my Doctoral Thesis: "And because I cannot thank Him enough, therefore I worship the God whom I serve, the father of our Lord and saviour Jesus Christ, for His loving kindness and interest in my daily pursuits. May all this be to His glory".

INTRODUCTION

Process microbiology

The design of the microbe is a process. The study of particles at the size of atoms and molecules is nanoscience; the production of materials at that scale is nanotechnology. We may say that God is the greatest nanoscientist and nanotechnologist for that matter. The physicist, Professor Richard Feynman of the famed Feynman waves predicted the coming of nanotechnology in a lecture in December 1959. The talk was called "there is plenty of room at the bottom". In it, he imagined an advancement in science and technology where we would be manufacturing materials at the head of a pin, "tiny machines". At that scale he described it as nanotechnology (openculture.com Physics Technology April 23 2013). Today, nanotechnology is described as the manufacture of materials that are in the 1-100nm size range. Two main approaches to achieving this are: up-down approach-where you size down from big to the smallest. Special instrumentation is required to achieve this. The other approach is down-up approach, here you start from scratch – from atoms to molecules to the materials, still within the nanoscale. Dr. Feynman actually had enzymes in mind when he predicted nanotechnology. The microbe is the most amazing packaging of enzymes and enzyme systems. On their own, enzymes would be obliterated by prevailing environmental conditions. But when they are packaged in micrometer – size particles, enzymes can perform wonders. It took an astute microbial physiologist, Jacques Monod to come up with the Monod equation describing nutrient limiting conditions in the growth of microbes, the equivalent of the Michaelis-Menten equation for enzyme-substrate processes. In its basic form, the Monod equation is stated thus:

$$\mu = \frac{\mu_{max} * [S]}{K_S + [S]}$$

Where

μ = specific growth rate (g/g.h)

μmax = maximum specific growth rate

[S] = limiting substrate concentration

Ks = Monod constant – substrate saturation constant

The Michaelis-Menten equation is stated thus:

$$v = \frac{V_{max} * [S]}{K_s + [S]}$$

Where

ν = rate (velocity) of the enzyme catalysed reaction

Vmax = maximum rate of reaction

[S] = substrate concentration (M,)

Km = Michaelis constant, equal to ½ Vmax

So, now we have the second nanotechnologist, in that sense, and that is the microbe, the first being God. The microbe can actually perform up-down (top-down) and down-up (down-top) technologies. The biosynthetic capabilities of microbes represent the down-up (down-top) and the biodegradative capabilities of microbes represent the up-down (top-down) processes. The biosynthesis of the bacterial cell wall (the peptidoglycan) is a good example of a down-up process. The peptidoglucan is an amazing ultrastructure. The synthesis of this structure is a feat. What is the pin head that is in the nanometer range – the cytoplasmic membrane, it is 5-10nm thick, so thin it is also referred to as the plasma membrane.

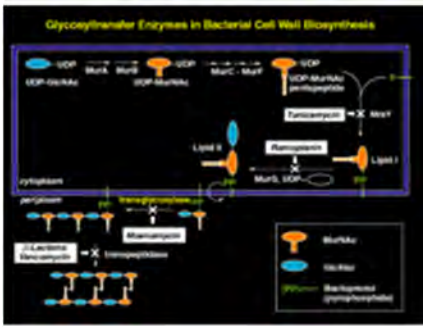


Fig. 1a: Peptidoglycan and its synthesis and cytoplasmic membrane

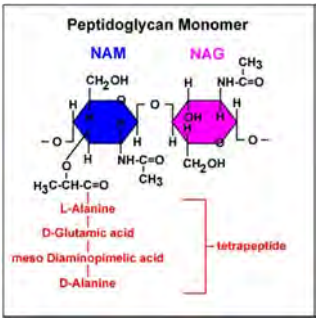


Fig. 1b: Peptidoglycan monomers

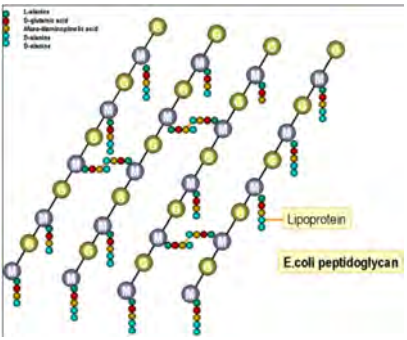


Fig. 1c: *E. coli* Peptidoglycan

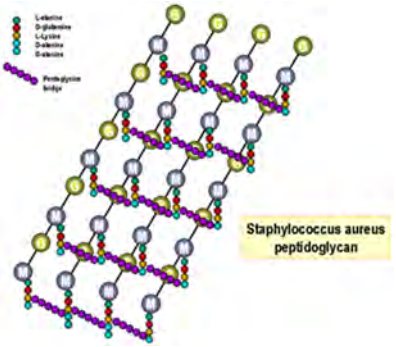


Fig.1d: *Staphylococcus aureus* Peptidoglycan

Where did the microbe learn this? May be the microbe was taught (by God), the overall designer. But can the microbe learn? The plasma membrane is the center of activity with the microbe, and it well may be described as the brain or the mind of the microbe. So, the plasma membrane determines which components of the cell wall (peptidoglycan) are synthesized on the outside of the membrane and which are synthesized on the inside of the membrane. All this forms the basis of synthesis and transport or excretion of metabolites, including secondary metabolites such as antibiotics. It is all a processing carried out at the plasma membrane-Process Microbiology.

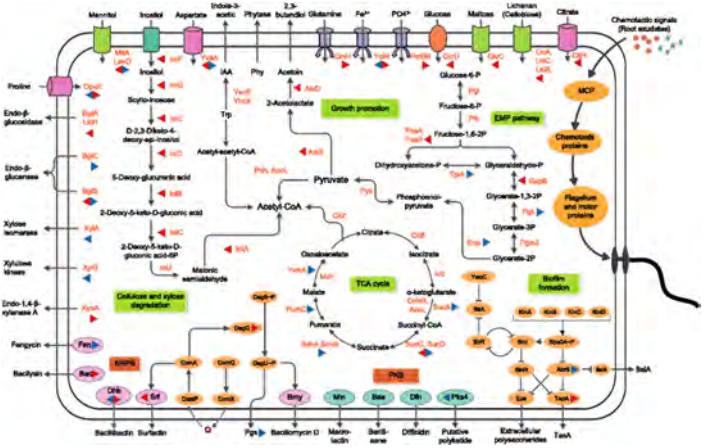


Fig. 2: The nanoprocess phenomenon machine – the microbe model (**Source**: www.google.com.ng/nature.com)

Phenomenal Microbiology

We have seen that the microbe is a phenomenon. It is the most sophisticated, complicated, smallest living thing. The microbial cell performs more complex tasks to stay alive than the plant cell or the animal cell. So astonishing that Professor Rita R. Colwell (Personal Communication) declared that – "microbes (bacteria) do not die, they may be killed, but they do not die of old age". At least four reproduction strategies have been identified in microbes; they are; (i) Binary fission (ii) Budding (iii) Sporulation and (iv) Baeocyte

formation. All these are strategically placed to actualize their immense numbers and survival. Take the binary fission exhibited by most bacteria. The basic growth equation (Pirt, 1975) is given as:

$$X_t = X_0 * 2^n ,$$

where X_t is the total number of cells after a given period of time t, X_0 is the number of cells at time t = 0, n is the number of generations and 2 is because the population of cells doubles, or each cell divides into two cells each time a cell completes a fission or division. This equation can also be written as:

$$X_t = X_0 * 2^{t/td}$$

Now, it means n = t/td, and td is the time it takes for a cell to complete a cycle of dividing into two, it is called the doubling time, or the generation time (g). It is estimated that, one (a) bacterium growing with a doubling time (td) of say 20min, in a period of 48h(t) would result into:

$$X_t = 1 \text{ x } 2^{48/0.3} = 1.61 \text{ x } 10^{43} \text{ cells}$$

Now, if 1 bacterium weighs on the average 10^{-12}g, that is 10^{-15}kg, then the weight of cells produced in 48h from 1 bacterium would be $1.61 \text{ x } 10^{43}$ (cells) x 10^{-15}kg = $1.61 \text{ x } 10^{28}$kg; it is estimated that this weight is about 4,000 times the weight of our planet Earth; that means the Earth could go off its orbit. Now, this is phenomenal and why it does not happen is inexplicably phenomenal. This is described as exponential growth. Laboratory experiments where we supply nutrients and provide other necessary environmental factors, reveal that there is a pattern of growth that is in phases, and one of those is the exponential growth phase. This phase is short, and is influenced by the carrying capacity of the system, according to the logistic growth model. This phenomenon is by design. I would like to state that a good appreciation of process and phenomenal microbiology would require a good understanding of microbial physiology. This is the backbone of Applied and Environmental Microbiology (and Biotechnology). This covers the science and the technology associated with microbes; can we call this Process and Phenomenal Microbiology? It is technology that creates jobs, so,

jobs would come from microbial technologies, microbial biotechnology. I would like to refer to MCB, which we use as a course code, that stands for Microbiology, as Microbial Chemistry and Biotechnology. Process microbiology is rooted in microbial chemistry and biochemistry. Phenomenal microbiology handles the mechanisms, the empirical and the explanatory theories, the ecological theories, the ecoforces of how the earth is influenced and maintained by the activities of microbes – this is ecophysiology. Biotechnology has done and is doing to many nations of the world what the electronics industry did for Japan many years back (Abu, 1992). Colwell (1989) stated that the aim of every technology is to bring about self-reliance and raise the standard of living.

So, I would like to describe my work as Process and Phenomenal Microbiology.

THE CONCEPT OF THE MICROBE AND MICROBIOLOGY

In critical thinking, everything is a concept (of course, except God). The microbe is a concept. The study of the microbe is the concept of microbiology which is a science based on size and tools; it is the study that covers living organisms too small they can only be observed with an aided eye. That aided eye signifies a device or a tool, and the phrase too small signifies size, especially in terms of length.

The SI (System Internationale) units of measurement of size has the metre rule where prefixes are attached to the metre e.g., millimetre (mm), centimetre (cm), micrometre (μm), nanometer (nm) etc. In numerics 1000mm =1m; 100cm = 1m; 1,000,000μm = 1m; 1,000,000,000nm =1m. In standard notation, $1m=10^{3}mm$; $1m= 10^{2}cm$; $1m = 10^{6}\mu m$ and $1m = 10^{9}nm$. Conversely $1mm = 10^{-3}m$; $1cm = 10^{-2}m$; $1\mu m = 10^{-6}m$ and $1nm = 10^{-9}m$; this means that placed on edge, you would cut 1000 lengths of 1mm each and 1,000,000 lengths of 1μm each. At this point, even the razor blade is thicker than the lengths you want to cut out. But there is actually the picometer which is $10^{-12}m$, and the femtometer which is $10^{-15}m$ and the zeptometer which is $10^{-18}m$ and the yoctometer which is $10^{-21}m$.

The smallest measure of distance or length is called the Planck length which is $1.6x10^{-35}$m. The most common microbes are classified (called) bacteria and archaea and fall within the size range of the micrometer (μm). We can conveniently refer to them on the basis of size as microorganisms (μorganisms). At this size, the human eye cannot distinguish two adjacent microbes as distinct, only the compound light microscope (an optical device, a tool) can resolve or distinguish two adjacent microbes as distinct. The human eye can resolve objects that are 0.2mm as distinct, that is 0.0002m, the light microscope can resolve objects that are 0.2μm, that is 0.0000002m and the electron microscope can resolve objects that are 0.5nm, that is 0.0000000005m.We also have size in terms of weight. The gram (g) is the SI unit of weight as the meter (m) is for length. An average bacterium has the weight of $1x10^{-12}$g, this is the femtogram (but there is the milligram 10^{-3}g, then the microgram 10^{-6}g and the nanogram 10^{-9}g before the femtogram 10^{-12}g.

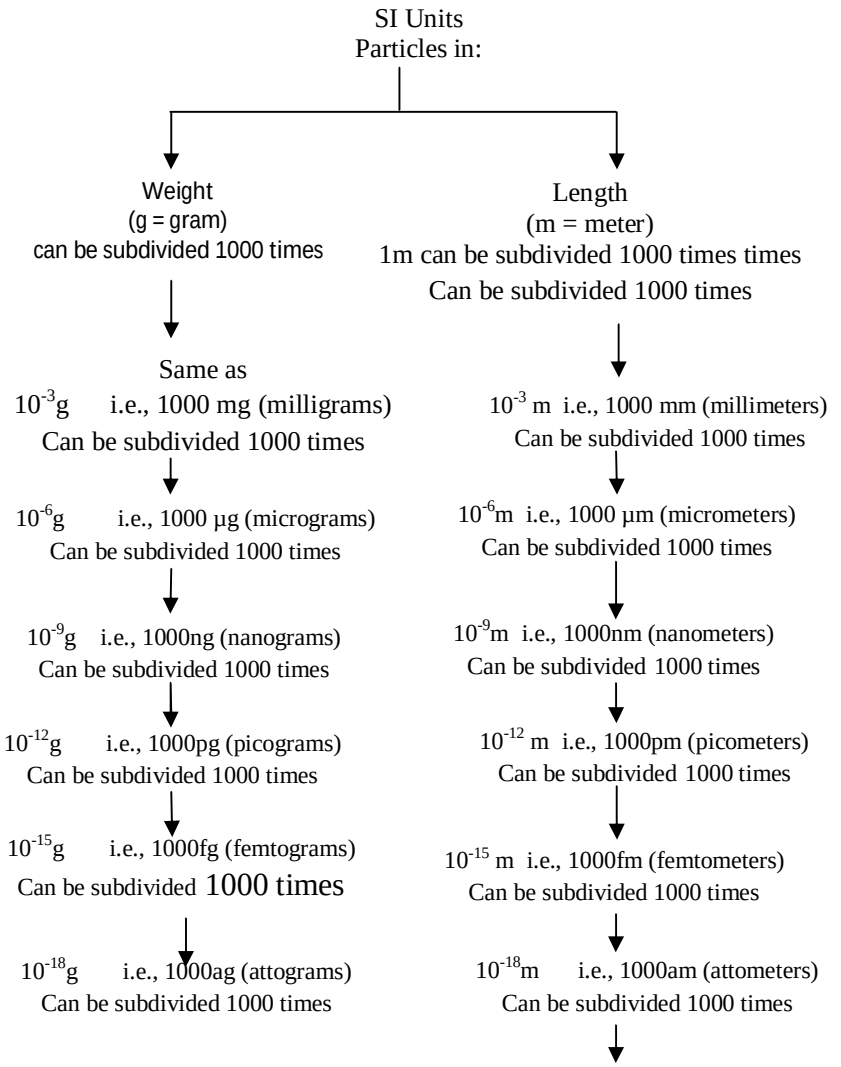


Fig. 3: Concept diagram of size of particles studied by microbiologists

The word microbe is the colloquium for microorganism. On the basis of arrangement of molecules within the cytoplasm, especially the organisation of the genetic molecules, microbes could be grouped as prokaryotic or eukaryotic, the karyon (Greek) indicating

a nut-like structure – the arrangement of the genetic material within the cell to form the nucleus, where there is a clear delineating nuclear membrane, the cell type is eukaryon (true Karyon) and where the nuclear material is not in a well enclosed membrane structure it is prokaryon (before or primordial karyon). In terms of size you have both eukaryotic and prokaryotic microbes. The prokaryotic microbe epitomizes the concept of the microbe and microbiology. With a size range in the micrometer, processes within and on the microbe are at the nanometer scale (nano scale). Manufacturing of molecules which can be by breaking down to size (degradation) or by building up to size (synthesis) are taking place at the nano scale (bionanoscience leading to bionanotechnology leading to bionanomaterials or nanobiomaterials; Nanotechnology deals with manufacture of meterials in the 1-100nm size range). All this would require energy production and utilization.

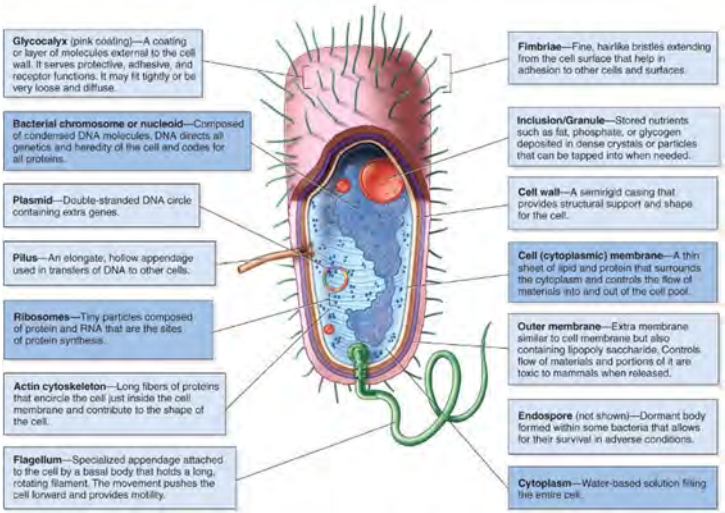


Fig. 4: A typical functional diagram of a microbe (**Source**: google: lacasamoret.com)

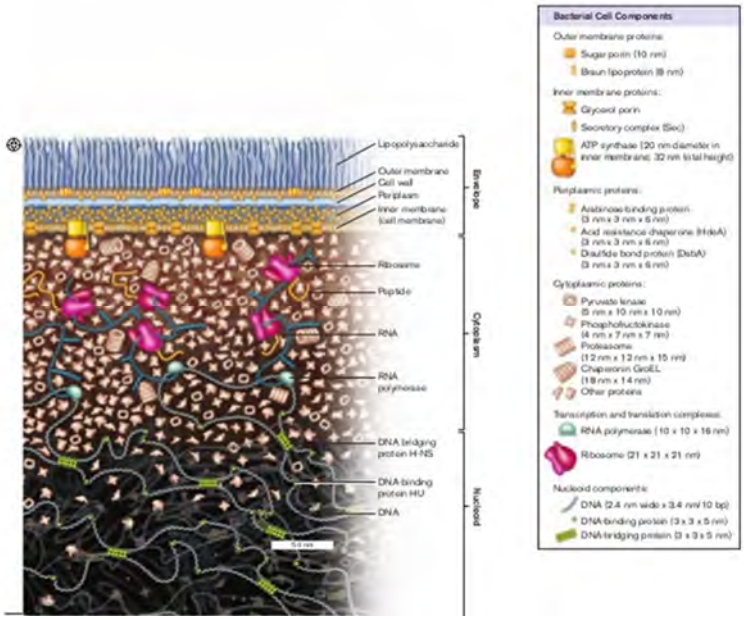


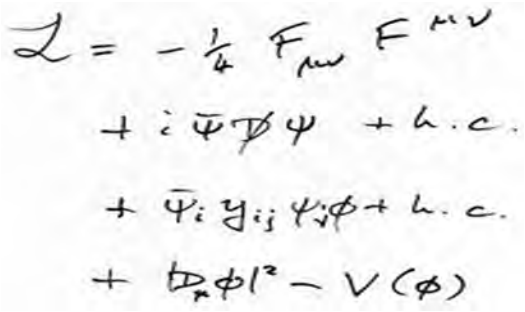
Fig. 5: Model of Distribution of molecules in the microbe (bacterial cell - *Escherichia coli)*. (**Source**: Microbiology by Slonczweski and Foster, 2011)

Energy in the Universe and Ecosystem, Self-Assembly, Intrinsic Design and the Vital force

Starting at the measureable level we say from the atoms to the molecules to the cell to the organelle to the tissue to the organ to the system to the organism to the population to the community to the ecosystem to the city to the society to the universe, energy is absolutely essential. If energy ceases, the universe ceases. In the physical world we have the Einstein's equation for how energy is created as:

$E = mc^2$

and now we have the Higgs-Boson energy equation as:



(Source: google:sacrit.blogspot.com.ng/2010/11)

Bioenergetics is the area of Microbial physiology that deals with the way energy is produced and used by the microbial cell and thus, in the ecosystem and universe. The energy could be from radiant energy or from reduction-oxidation (redox) reactions of chemical molecules. Biological energy is the best way to see design at work.

There are three types of energy systems in the biological world namely substrate level phosphorylation/fermentation, oxidative phosphorylation and photophosphorylation/chemosynthesis. The Nernst Equation is an oxidation-reduction (OR) equation; it is stated thus:

$$E = E_0 + \frac{R \cdot T}{n \cdot F} \cdot \ln \frac{[ox]}{[red]}$$

(**Source**: Lehninger, 1975)

Where R is gas constant, T, absolute temperature, n, number of electrons; F, Faraday constant. In all reactions involving protons the standard oxidation-reduction potential refers to pH = 0. Since most biological reactions proceed at pH values near 7 it is more practical

to calculate the standard oxidation-reduction potential of biological systems when the pH is 7.

In 1961 Peter Mitchell (working with microbes) proposed the chemiosmotic hypothesis of ATP formation by electron transport phosphorylation. He went on to win the Nobel prize in physiology and medicine (Gottschalk, G. 1986). This hypothesis is one of the most fundamental contributions made in the area of bioenergetics.

The chemiosmotic theory presumes that:

i. The cytoplasmic membrane is impermeable to OH^- and H^+.
ii. The respiratory chain is localized in the membrane in such a way that a pH gradient and a membrane potential are formed by vectorial extraction and excretion of protons during electron transport (proton translocation);
iii. The ATP synthase is so ingeniously constructed that it can take advantage of the pH gradient and the membrane potential for ATP synthesis from ADP and P_i. (Gottschalk, 1986)

The cytoplasmic (plasma) membrane is composed of a phospholipid bilayer interspersed with a number of proteins that are embedded in the bilayer. The membrane ordinarily is impermeable to charged and uncharged hydrophilic compounds. Only uncharged lipophilic or charged highly lipophilic compounds can pass the membrane without carrier systems. Acetic acid or butyric acid can pass the membrane but the acetate or butyrate anions cannot. Thus, the membrane is permeable to these compounds only at low pH values. One example of a charged highly lipophilic compound is, the tetraphenylphosphonium cation $[(Phenyl)_4 - P^+)]$. If there is a difference in pH between the cytoplasm and the cell outer surrounding, appropriate carrier systems would be needed to even out the charge difference across the membrane; the cytoplasmic membrane per se is impermeable to H^+ or OH^-; it has a very low conductance.

One way of explaining the workings of the chemiosmotic theory is the use of a scheme that depicts the functional organisation of the

redox carriers in the respiratory chain of E.coli, which comprises proton-translocating sites. Through proton translocation a protonmotive force (ΔP) is generated across the cytoplasmic membrane. It consists of two components:

(a) the membrane potential Δψ, protons are positively charged, and the inner surface of the membrane becomes negatively charged by proton extraction, (b) the proton gradient between outside and inside, it is usually expressed as ΔpH. The corresponding equation is:

$$\Delta P = \Delta\psi - Z \cdot \Delta pH$$

Where Z = 2.3 RT/F and is equal to 59 at 25^0C. ΔP can be related to the electrochemical gradient of H+ ($\Delta\mu_{H+}$) through the Faraday constant:

$$\Delta P \cdot F = \Delta\mu_{H+}$$

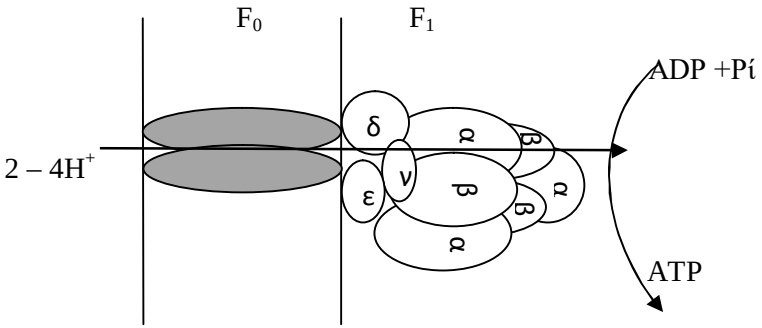


Fig. 6: Structure of ATP synthase. The α and β subunits alone exhibit activity of ATP hydrolysis. For ATP synthesis they have to be attached to F_0 via the γ, ε, and δ subunits (Gottschalk, 1986)

What we call self-assembly is actually the intrinsic design. The ATP synthase is ingeniously placed in the cytoplasmic membrane by design. By our human vocabulary we would understand that a molecule cannot design itself, just as a baby cannot design itself. So by vital force, it is meant the ultimate source of energy, which must

be provided from outside our universe. The only source of that vital force is God Almighty. This is the origin of life. The microbe epitomizes the best understandable form and operation of life. By human understanding, we describe life as characteristics: Ability to reproduce, excrete, move, grow, manufacture and use food. So there is energy life. But that is not the end; there is the creative life, it is beyond the molecules. This is the way the psychologists conceptualized it. But where did the energy come from?

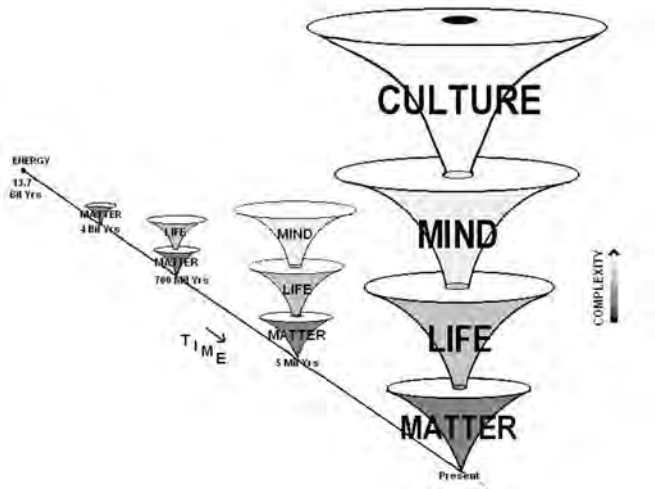


Fig. 7: The psychologist's view of energy, matter and life. (https//www.psychology today.com).

Design, Gene Expression and Energy Life

The energy life is intricately linked to the molecules of life as we call them. Do molecules think? Do microbes think? Gene expression is the most fascinating phenomenon known by man (as living/life carrying beings). Everything is everywhere, nature only selects. Microbes are ubiquitous. They modulate every habitat where they are found. Every microbe fits into the habitat where they are. What we may consider the lowliest of them is performing surprisingly very complex processes. From one extreme of habitat to another -

extreme cold to extreme heat, from complete absence of oxygen to well oxygen exchanged environments.

How did microbes arise? Philosophical molecular microbial ecology

The smallest particle discovered to date, that is measureable, is the boson. It has charge but no mass. So, it is sub-sub atomic. Anything that can be measured is real as far as humans are concerned. Could the boson grow into a microbe which is the smallest living form, the way we know it? The Boson is a supernano particle. It is a sub-subatomic (smallest measured) particle by humans to date. Did it arise from fragmentation or from synthesis. From human perspective, all the molecules in the microbial cell have a design to them. The design is evident in the language of energy, which is the ability or capacity to do work. The design and the working of the microbe epitomizes efficiency in manufacture and utilization of energy. This energy is in the molecules that arise from processes. The processes could be biosynthetic or biodegradative.

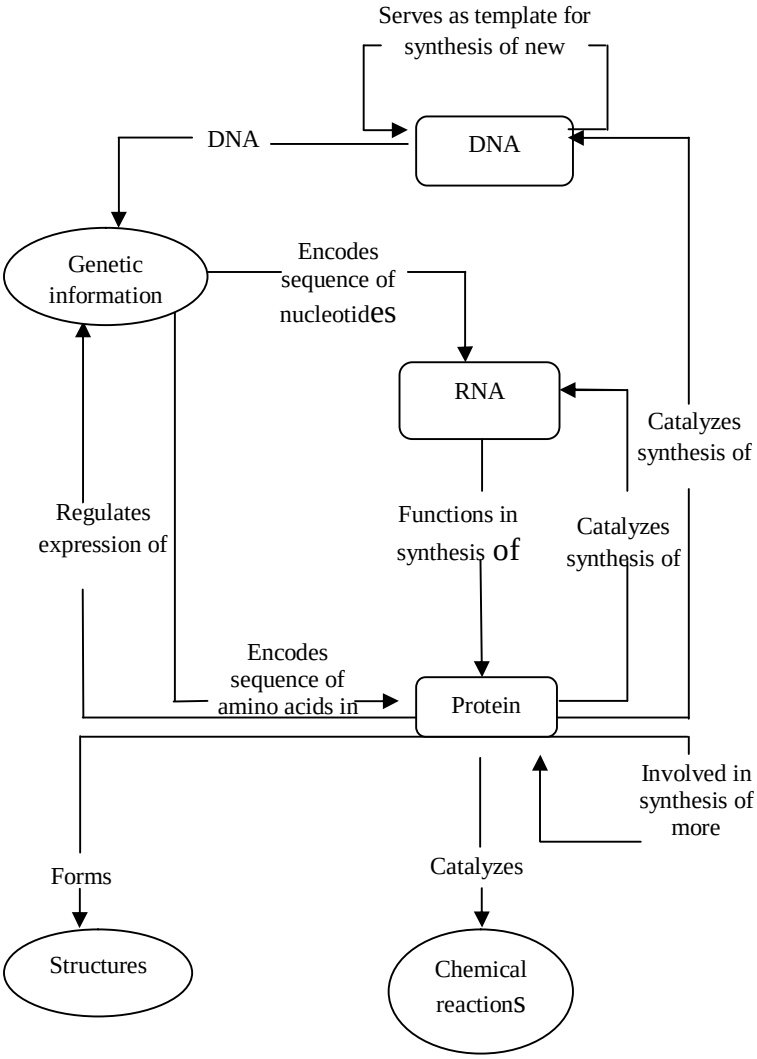


Fig. 8: Functions of DNA, RNA, and Protein, and Their Relationships to Each Other in Modern Cells. (Source: Prescott's Microbiology Ninth Edition (2014))

These molecules cannot arise on their own. For this reason, they cannot create an organism that will make use of all of them for different purposes.

So I want to interpret the famous microbial ecologist, Professor Martinus W. Beijerink (1851-1931) of Delft University in the Netherland, in what he said – "everything is everywhere, nature only selects." I want to argue that, that "is" was an event that took place, I want to imagine that the microbe was designed or created. The design took into consideration all the habitats imaginable. In the same way, I want to argue that all other forms of life were designed and or created.

The design concept looks plausible because, the molecules indicate a common designer. Look at RNA, a very intriguing molecule; the design of the RNA molecule is simply ingenious. It brings together elements of the periodic table, C, H, O, N and P. These are the major elements of organic matter. Six of them namely C, H, O, N, P and S are among what we call the "elements of life". Other elements of life are the monoatomic ions Na^{+}, K^{+}, Mg^{+2}, Ca^{+2} and Cl^{-}; then there are the trace elements Mn, Fe, Co, Cu, Zn, B, Al, V, Mo, I, Si, Sn, Ni, Cr, F and Se.

The earth crust harbours some of the major chemical elements that are also found in the human body and living cells.

Table 1. Relative abundance of the major chemical elements in the earth's crust and in the human body (as % of total number of atoms).

Earth's crust		Human body	
Element	%	Element	%
0	47	H	63
Si	28	O	25.5
Al	7.9	C	9.5
Fe	4.5	N	1.4
Ca	3.5	Ca	0.31
Na	2.5	P	0.22
K	2.5	Cl	0.08
Mg	2.2	K	0.06
Tl	0.46	S	0.05
H	0.22	Na	0.03
C	0.1g	Mg	0.01

(**Source:** Lehninger, 1975; Cowan and Talaro, 2009; Schaechter, Ingraham and Neidhart, 2006).

Only 27 of the 90 natural chemical elements in the earth's crust have been found to be essential components of various living organisms. These are the bioelements.

Table 2: Elements Essential in the nutrition of one or more species.

Elements of organic matter	Monoatomic ions	Trace Elements	
O	Na^+	Mn	Si
		Fe	
C	K^+	Co	Sn
N	mg^{2+}	Cu	Ni
H	Ca^{2+}	Zn	Cr
P	Cl^-	B	F
S		Al	Se
		V	I
		Mo	

Source: (Lehninger, 1975, Schachter *et al.*, 2006; Cowan and Talaro, 2009; Not all essential for every species)

The chemical elements in living organisms (bioelements) are not distributed in proportion to their occurrence in the earth's crust. The four most abundant elements in the earth's crust are oxygen, silicon, aluminum and iron. Conversely, the four elements that are the most abundant elements in living organisms are hydrogen, oxygen, carbon and nitrogen. These four elements make up about 95% of the total mass of most living cells. These elements were designed to function in biomolecules (life molecules or molecules of life). First, the bioelements are unique and then the molecules they form have unique molecular fitness as molecules of life (biomolecules). To form molecules, atoms must come together. Some of the unique features common with carbon, hydrogen, nitrogen and oxygen include:

(i) They can readily form covalent bonds by sharing a pair of electrons. To form covalent bonds, hydrogen needs one electron, oxygen needs two, nitrogen needs three and carbon needs four electrons to complete their outer electron shells. This would lead to stable covalent bonds.

(ii) The four elements (C, H, N, O) readily react with each other to form an amazingly large number of different covalent compounds.

(iii) Three of these elements namely C, N and O can share either one or two electron pairs to yield either single or double bonds. This feature bestows them with considerable versatility of chemical bond formation.

(iv) The elements C, N, H and O are the lightest elements capable of forming covalent bonds. We know that the strength of a covalent bond is inversely related to the atomic weights of the atoms involved in the bond. For this reason, the four elements (C, N, H and O) have the capacity to form very strong covalent bonds e.g., the peptide bond in proteins and the peptidoglycan.

(v) Yet another intriguing feature of the bioelement, C; carbon has the capacity to bond with each other. This is because a carbon atom may either accept or donate four electrons to complete an outer octet. It means that carbon can form covalent bonds with four other carbon atoms. For this reason, covalently linked carbon atoms can form linear or branched or cyclic backbones, leading to a great variety of different organic molecules.

(vi) A wide variety of functional groups can be introduced into the structure of organic molecules. This is because carbon atoms do form covalent bonds with oxygen, hydrogen, nitrogen and sulfur. No other chemical element can form molecules with such widely different sizes and shapes or such a variety of functional groups. Thus, the design of the atom (elements) is simply a marvel. The design of the bioelements and the biomolecules is ingenuous.

All of these are depicted in the design of the microbe (the microorganism). The microbe is the smallest unit that shows how bioelements and thus, biomolecules constitute a bio entity. The molecules have a design that enables energy budgeting. This energy budgeting is so clearly depicted in the design of the microbe.

The bioelements (carbon, hydrogen, oxygen and nitrogen) are considered to be organogens. They are considered to be the characteristic ingredients of an organic compound.

Order/Hierarchy in biomolecules

There is order and an increasing complexity in biomolecules. The microbial cell helps us to appreciate the complexity in living cells.

In terms of energy metabolism, the microbial cell can be said to be either autotrophic (can manufacture food from simple inorganic molecules) or heterotrophic (uses preformed organic molecules for food). There is a hierarchy in the complexity of molecules utilized by microbes and living things in our universe for the purposes of growth and reproduction. These groups can be recognized as precursors, intermediates, building blocks, macromolecules, supramolecular assemblies and cell structures e.g.,:

Table 3: Hierarchy in the molecular organization that leads to production of new cells

Precursors These are from the environment, they range from 18-44 mol. wt, they include cardon dioxide, water, ammonia, nitrogen.
Metabolic intermediates Most of these are from 50-250 mol wt and include pyruvate, citrate, malate, glyceraldehydes 3-phosphate, oxaloacetate, succinyl coenzyme A, 2-oxoglutarate, Acetyl coenzyme A, phosphoenolpyruvate, 3-phosphoglycerate,erythrose-4-phosphate, sedoheptulose-7-phosphate, pentose-5-phosphate, fructose-6-phosphate, glucose-6-phosphate
Building blocks Most of these are from 100-350 mol wt and include nucleotides, amino acids, monosaccharides, fatty acids, glycerol
Macromolecules These have mol wt from 10^3-10^9 and include nucleic acids (DNA, RNA), proteins, polysaccharides, lipids, glycogen, peptidoglycan
Supramolecular assemblies, cell structures These have mol wt of 10^6-10^9 and include ribosomes, enzyme complexes, inclusions, nucleoid, cytosol, flagella, pili, envelope.

Source: Lehninger, 1975; Schaechter *et al*., 2006.

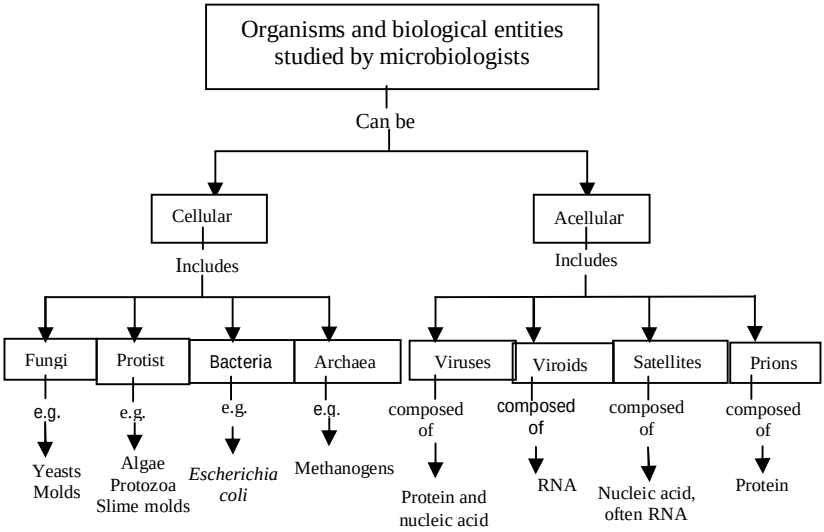


Fig. 9: Concept Map showing the Types of Biological entities studied by Microbiologists. (Source: Prescott's Microbiology 9th Ed. 2014)

The molecular composition of the bacterium *Escherichia coli* depicts how the molecules in their different levels of complexity are distributed in the microbe.

Table 4. Major molecular components of an *E.coli* cell during balanced exponential growth.[a]

Component	Percentage of total weight[b]	Approximate number of molecules/cell	Number of different kinds
Water	70	20,000,000,000	1
Proteins	16	2,400,000	2,000[c]
RNA: rRNA, tRNA, and other small RNA (sRNA) molecules,	6	250,000	200
mRNA	0.7	4,000	2,000[c]
Lipids: phospholipids (membrane)	3	25,000,000	50
Lipopolysaccharides (outer membrane)	1	1,400,000	1
DNA	1	2[d]	1
Metabolites and biosynthetic precursors	1.3	50,000,000	1,000
Peptidoglycan (murein sacculus)	0.8	1	1
Inorganic ions	0.1	250,000,000	20
Polyamines (mainly putrescine and spermine)	0.1	6,700,000	2

(Source: Slonczewski and Foster, 2009: Modified from Neidhardt, F., and H.E. Umbarger, 1998. Chemical composition of *Escherichia coli*, p. 14. In F.C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: Cellular and Molecular Biology, 2nd ed. ASM Press, Washington, DC).

[a]Values shown are for a hypothetical "average" cell cultured with aeration in glucose medium with minimal salts at 37^0C.

[b]The total weight of the cell (including water) is about 10^{-12} gram (g), or 1 picogram (pg).

[c]The number of kinds of mRNA and of proteins is difficult to estimate because some genes are transcribed at extremely low levels and because RNA and proteins include kinds that are rapidly degraded.

[d]In rapidly growing cells, cell fission typically lags approximately one generation behind DNA replication – hence, two identical DNA copies per cell.

The design and assembly of the microbial cell is a complex process. It involves the bioelements and biomolecules and energy. Many of the molecules are used to synthesize new molecules by supplying

energy. It takes about 100 proteins (including enzymes and cofactors) to synthesize one polypeptide chain.

Can we make life from nonlife?

Living things, we say, are improbable arrays of improbable molecules. It follows that the cell, considered the smallest unit of living things is an improbable array of improbable molecules. What that means is that neither the individual macromolecules – the proteins, carbohydrates, nucleic acids and lipids, nor yet, the complex cell structures made from these macromolecules are likely to accumulate spontaneously, moreso, under present Earth conditions (Schaechter *et al.*, 2006). By extension, we may say that molecules are improbable arrays of improbable atoms. And why could we not say that atoms are improbable arrays of improbable subatomic particles including bosons. Where does that lead us to? – Design and Create!! The thinking is that, in the present Earth conditions, if any of these macromolecules were to form spontaneously, the microbes, already present would rapidly consume them ever before they get to anywhere (Schaechter *et al.*, 2006).

It is rather believed that cells utilize information embedded in their existing structures to guide reactions of synthesis and assembly that result in the production of new cells. There are four principles that have been used to explain this phenomenon, and it is a phenomenon. The microbe is simply a phenomenon. The four principles:

a. Specific catalysts
   Specific catalysts are enzymes. This improbable assembly of molecules are specific and precision orientated, they can recognize differences in carbon atoms even in a ring structure like benzene. Enzymes are so specific they can accelerate otherwise extremely low reactions.
b. A design strategy designated reaction coupling:
   In the design, individual chemical processes that are necessary for life are made energetically favourable by coupling them with other favourable reactions, especially the hydrolysis of “high-energy” bonds (these bonds have high phosphoryl donor potential, or they can release large

amounts of free energy), such as adenosine triphosphate (ATP) to adenosine diphosphate (ADP) plus inorganic phosphare (Pi). ATP is thus considered as driving otherwise unfavourable reactions.

c. Harvesting energy to make or synthesize ATP:
This occurs by means of organic (fermentations), organic (respiration) or photochemical oxidation – reduction (redox) reactions.

d. The use of biological membranes to transduce energy into different forms; the energy harvested from oxidation can generate ion gradients which can be used either directly to perform work (including turning flagella) or to generate the biological energy currency, ATP, this is the chemiosmotic process that gives rise to the proton motive force across membranes.

The reasoning is that enzyme catalysis, energetically coupled reactions and acquisition of chemical energy and its transduction into ATP is what distinguishes life from nonlife and enables organisms to create order out of disorder (Schaechter *et al.*, 2006). Cowan and Talaro (2009) put it this way – as we proceed in this chemical survey from the level of simple molecules to increasingly complex levels of macromolecules, at some point, we cross a line from the realm of lifeless molecules and arrive at the fundamental unit of life called a cell. (The Word Cell was originally coined from an Old English term that means “small room”. This is because of the way especially plant cells looked to those early microscopists who were observing them under the microscope). That “small room” sounds like a designed structure to me; but designed by whom? This crossing of line and the necessary evolutionary step is the best way human beings can explain the origin of life so far. But really, can life evolve? Life is not matter, it is not material. Can life change?

The concept of protometabolism and protocells

According to Kee and Monnard (2016), protocells are envisaged as encapsulated networks of catalytic polymers, such as RNAs, which are thought to have existed on the prebiotic Earth as precursors to

contemporary biological cells. Such protocells were not “alive” in the way this word would apply to a contemporary unicellular organism. Rather, protocells represent a necessary evolutionary step toward those first forms of cellular life. In their review paper, they went on to explore how chemicals synthesized by minerals or delivered by meteorites could have contributed to the emergence of the first protocells and could have supported these protocell's evolution towards primitive cellular life.

Fig. 10: The protometabolism and protocell concept
(**Sources**: google: mineralogical Society of America. 2016)

The protometabolism and protocell concept also assumes that there was a crossing of the line from geochemistry to biogeochemistry, and that is envisaged as the origin of life. But we would remember that life is not material. Is life therefore a force? That is the limit of we humans. We must therefore admit that we were made alive, we were created. So in the real sense, creation is bringing something out of nothing.

How did the microbe arise?

In human scientific reasoning, we recognize living and non living things (Life and Nonlife). So we can say life looks different from

nonlife. Nonliving things may be chemically active on a small scale or geologically active on a grand scale, but non-living things take no organized role in events; we may say they are inert.

On the other hand we say living things may – breathe, move, respond to changes in their environment, modify their surroundings, and reproduce themselves (this ability to reproduce is the hallmark and universal feature/attribute of living things).

We say that these attributes or characteristics of living organisms are the result of highly organized chemical reactions, which are collectively called metabolism.

A cell then is a huge aggregate of carbon, hydrogen, oxygen, nitrogen and many other atoms of the elements of the periodic table; and it follows the laws of chemistry and physics, and actually a lot more.

It is the combination of these atoms that produces characteristics, reactions and products that can only be described as living.

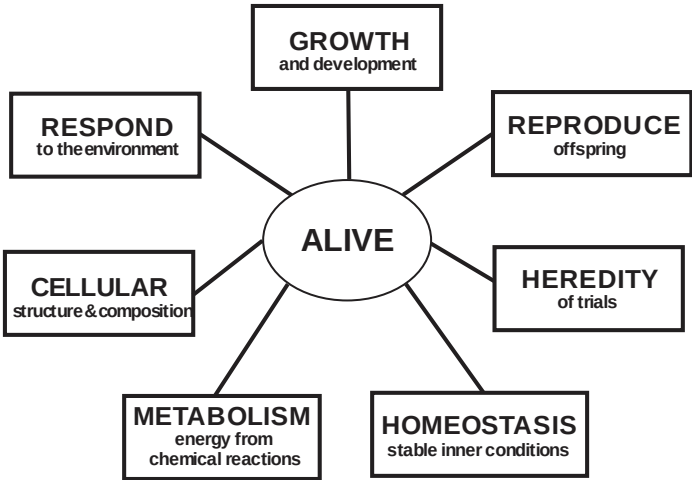


Fig. 11: The Fundamental Features of Cells (Source: google)

The Fundamental Features of Cells

Bacteria (eubacteria) and archaea are made of only a single cell (unicellular). Bodies of plants and animals (eucarya) are made up of trillions of cells. All cells have a few common characteristics. They tend to be spherical, polygonal, cubical or cylindrical, their protoplasm (internal contents of cell) is enclosed in a cytoplasmic membrane (also called the plasma membrane). Cells have chromosomes containing DNA and ribosomes for protein synthesis; cells are extremely complex in function.

Most cell types fall into one of two fundamentally different lines namely the prokaryotic cells-these are the small and seemingly simple types, and the eukaryotic cells – these are the larger and structurally more complicated cell types.

We say that eukaryotic cells are found in animals, plants, fungi and protists. The cells here contain a number of complex internal parts – the organelles, these perform vital functions for the cell, such as growth, nutrition or metabolism.

We may define organelles as cell components that perform specific functions and are enclosed by membranes. Organelles also partition the eukaryotic cell into smaller compartments. The most visible organelle is the nucleus, this is a circular-shaped mass surrounded by a double membrane that contains the DNA of the cell. In the plant and animal cells, other organelles apart from the nucleus include the Golgi apparatus, endoplasmic reticulum, vacuoles and mitochondria. Prokaryotic cells are a feature only of the bacteria and archaea. The prokaryotes have no nucleus or other organelles, making them look simple. But in reality prokaryotic cells can engage in nearly every activity that is performed by eukaryotic cells, and what more, many prokaryotic cells can function in ways that eukaryotic cells cannot.
Diagrammatically, we can show the arrangements of prokaryotic cells thus:

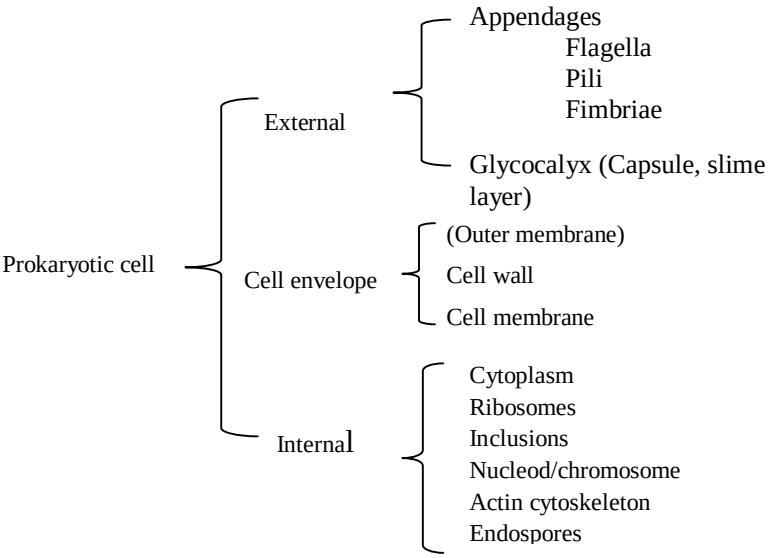


Fig. 12: Structure Flowchart of Prokaryotes

BRANCHES OF MICROBIOLOGY

One of the tenets in microbiology is that microbes are ubiquitous (found everywhere). The branches of microbiology attempt to deal with an ability to study microbes in their natural environments or under manipulated conditions.

Medical and Public Health Microbiology and Immunology:

Microbiomes in health, infections and disease. Study of antimicrobials; about 90% of diseases are infectious in nature. The science and technology of body immune defense systems against microbial infectious agents and other foreign substances. Microbes (normal flora) as agents of natural defense.

Food and Industrial Microbiology:

Microbiomes in modified conditions such as food. This is a waste-to-wealth technology where microbes get the energy they need and we take the waste, the metabolites. We design growth media and

equipment to harness the metabolites of interest. Among the oldest known biotechnologies practiced by man are in this field.

Applied and Environmental Microbiology:
This is niche science and technology. We study microbes under intrinsic and engineered conditions of the habitats of soil, water and air. This is where we learn the processes and phenomena associated with microbes before we can take them to the laboratory. Niche science includes microbial ecology and physiology; ecophysiological processes at the microenvironment/microhabitat level. This requires the science of the biology, molecular biology, genetics, the chemistry, the biochemistry and even biophysics of the microbes. We learn the mechanisms and theories (ecological theories) of the performance of the microbes in natural habitats, the modulation of the biosphere through the indispensable activities of microbes. We learn ecological economics and critical thinking from microbes. We develop technologies based on the science acquired with the microbes such as biosynthetic and biodegradative metabolism for energy and growth.

Agricultural microbiology
Deals with the impact of microbes on agriculture. The Nitrogen-fixing bacteria, growth promoting bacteria, soil fertility and crop yield. The use of bacterial and viral insect pathogens to control pests in the place of chemical pesticides.

Virology
Study of viruses and viral diseases. Viruses are accelular particles composed of nucleic acids and proteins. They have no membranes, no ribosomes, therefore they cannot carry out protein synthesis, but they have protein coatings. Viruses cannot be seen with the light microscope, that means their sizes of about 0.02μm are below the limit of resolution (0.2μm) of the light microscope. Under the electron microscope with a resolution of 0.5nm viruses can be resolved, but more readily with special techniques such as shadow casting. Viruses are therefore the smallest assemblage of nucleic acid and proteins studied by microbiologists. But the manner in

which they are assembled is absolutely amazing. Both eukaryotic and prokaryotic cells can assemble virus particles; so there are plant viruses, animal viruses and microbial viruses e.g., the bacteriophages. Viruses are by far the most abundant microbes in aquatic environments including the marine environment. Viruses comprise 94% of marine microbes. If everything is taken together, including virus-like particles (VLPs), viruses would be considered the most abundant microbes on Earth. Why so many on our planet? They are performing functions such as nutrient cycling. My position is that they were created for this and many other functions to create jobs for mankind.

Bosons are particles which obey Bose–Einstein statistics. Since bosons with the same energy can occupy the same place in space, bosons are often force carrier particles. Bosons are said to be the particles that transmit interactions (force carriers), or the constituents of radiation. Whereas the elementary particles that make up matter (i.e. leptons and quarks) are fermions, the elementary bosons are force carriers that function as the 'glue' holding matter together. An important characteristic of bosons is that their statistics do not restrict the number of them that occupy the same quantum state (https://en.wikipedia.org/wiki/Boson♯cite_note-11).

When viruses were first discovered physicists were excited thinking that finally we have found a particle that has life. Yes, the virus is made up of the bioelements and therefore the biomolecules, but no life on their own. From our Concept Map (Fig. 9), viruses are the molecules that did not cross the line from nonlife to life. But so, where did viruses originate from, are they next to bosons? There is no agreement on the origin of viruses. There is one line of thought that viruses arose early in the history of cells as loose pieces of genetic material that became dependent nomads, moving from cell to cell (Cowan and Talaro, 2009). What if these particles were actually designed and can be used as spare parts. They are there (everything is everywhere, nature only selects). That is why they are so many, accounting for about 270 million metric tons of organic matter.

When the boson was finally detected and measured as a matterless particle but rather as the glue that holds matter together, scientists exclaimed “now we don’t need God, we can figure out exactly how everything began”.

Why were microbes created and why so many and so small?
Microbes were created as model of life at the smallest scale, in this way we may appreciate how bioelements and biomolecules are not life itself, protometabolism could not give rise to microbes. So even the life of the microbe has to come from somewhere, from creation. I suppose that only creation can answer the question of the origin of life. Now, in human terminology and understanding, there is intelligent life and non intelligent life. We seem to indicate that microbes do not have intelligent life, that is, we think they (microbes) do not think. In physiological terms, microbes think. They can select one energy molecule ahead of another and come back later to the one rejected earlier, we call this diauxie, Jacques Monod was the first to describe the phenomenon (Gottschalk, 1986). Be fruitful, multiply and replenish the earth – microbes are ubiquitous – therefore fulfilling this injunction. Their numbers are astounding.

Differentiation, Phylogeny and Diversity
Differentiation is a process of directed change; it is controlled change. The process of differentiation is creative in the sense that life is creative (Swanson, 1969). Differentiation is the top secret of the biological world. The more modern terminology for differentiation is gene expression. Gene expression is the design secret. It is locked in there. Through molecular biology techniques we are privileged to have a peep into the mind of the designer, the mind of God (Isaac Newton, 1643-1727). Gene expression is one of the most mysterious and astounding phenomena observed. We know now that one cell of a multicellular organism like human, is all that is needed to reproduce a human being. So, why will all the cells not reproduce human beings? – gene expression – they are under control form an invisible designer. They must obey the laws of creation. The microbe is the epitome of gene expression. The microbe obeys the laws of creation. In fact we learn the laws of creation from the

microbe as a prototype. The microbe is not primitive, on the contrary, it is very complicated. The microbe did not evolve from the boson, both the boson and the microbe were created, and for their role. So, again I would like to say – everything is everywhere, nature only selects. I would say 'nature selecting' is the same as gene expression. It is gene expression that gives room for great biodiversity, and that great biodiversity is for resourcefulness (Chikere, Owolabi and Abu, 2004). Diversity is a metric for the number of populations in a community and the genetic relatedness among these populations (Moyer, Dobbs and Karl, 1994).

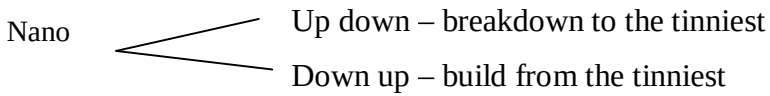
Why so small?

The volume of a representative bacterium is about $1m^3$ i.e., 1/1,000 that of human cells. But one is exceptionally large namely, *Epulopiscium fishelsoni* is about 0.5mm long and about $1/10mm^3$ in volume. It is visible with the naked eye since the human eye can resolve objects of down to 0.2mm diameter. It lives in the gut of the surgeon fish. I want you to estimate or imagine the size of a bacterium, how small a bacterium really is: Take a bacterium of average size 1.5µm in length and a human being of 1.5m. Now enlarge the two together 1million fold each. The bacterium would now be the size of a human. The human would now stretch from Port Harcourt to Sokoto, some 1,500 Km away. With respect to volume, it takes about 10^{17} bacteria or 100,000 terabacteria to occupy the volume of a non enlarged adult human. Smaller volume means greater surface-to-volume ratio (S/V). This ratio for a typical bacterium is about 1,000 times greater than that of a typical animal cell. This allows higher rates of uptake of nutrients from the environment.

Can they get sick/distressed?

E.coli may look like a well formed shelled groundnut with the brown covering, *Staphylococcus aureus* may look like small bunches of grapes "yiye". Under distressed conditions of *E.coli* may look like those groundnuts that have been soaked and sprinkled with salt ready for frying (Shrivelled). The microbiologist can tell.

If the microbe life forms are so small, can we therefore say we can measure life? The smallest measureable particle so far, by humans is the boson, which has also been referred to as the "God particle", that is to say it is the starting point of all matter. But now, it (boson) has to come from somewhere. The ultimate is that, in human terminology and understanding, it came from "nothing"; now, that, in human understanding, is creation. Therefore, the boson was created from nothing. So physiologically, we can demonstrate how life works. Work in terms of expenditure of energy. How the microbe is able to make and use energy is simply amazing. In the microbe, nanoscience and nanotechnology are exemplified. In nanotechnology you can up – down (break up a big molecule/material to the very smallest product) or down – up (build up a material from the smallest units).



Creation can do both, by design:

- the behemoth/whale was designed
- the microbe was designed
- the prion (Prusiner, 1982) was designed
- the boson (Dirac, 1945) was designed

In a virology class in Grad School a professor was so intrigued by the newly described retroviruses he remarked "we all may just be a bunch of retroviruses". But no, the viruses are only showing forth a design.

Ubiquity, Impact and Relative Abundance of Microbes

Microbes have been traditionally described as; free living organisms so small they are visible only under the microscope. Most living things are microbes. The organisms that are visible are a small minority. Microbes interact with all members of the living world as well as much of the inanimate world.

The study of microbes goes beyond just microbiology, rather it impinges on all of biology. It is not possible to study any branch of

biology or the earth sciences without giving serious consideration to the activities of microbes. Microbes have great impact on the lives of humans. Microbes are either prokaryotes or eukaryotes. Eukaryotic microbes other than algae and fungi are collectively called protists. Yeasts and mushrooms are fungi, yeasts are microbes but mushrooms are not microbes. The prokaryotes comprise bacteria and archeae. In terms of size microbes are small, the volume of a representative bacterium is about $1\mu m^{3,}$ this is roughly 1/1,000 that of a human cell. Some microbes, however, are large enough to be seen with the naked eye; for instance *Epulopiscium fishelsoni* is a monster of a microbe approximately 0.5mm long and a volume of about 1/10m3 . It was isolated from the gut of the brown surgeon fish. The total collective mass of microbes on earth is astounding or staggering. They are nearly ubiquitous, found practically anywhere there is free liquid water. In the ocean, there is about 1 million bacteria per millilitre of water. This would give a total of about 10^{29} cells in all the world's oceans. The estimate is that the total biomass of microbes on planet earth is nearly as large as that of all plant life, and may be even greater.

Similarly, the amount of carbon in the prokaryotic cells is nearly as large as if not greater than that of all plants on earth, both terrestrial and marine. In proportion prokaryotes contain more N and P than plants. Thus, microbes are the target reservoir of N and P in the biological world.

Microbes were created as control agents.

The microbes are under unnoticed control. Their numbers can be small or large and they would perform their task. Consider this:

$\mathbf{D} = \frac{nV}{R}$

R = host resistance

n = number of organisms

V = virulence (the severity of pathogenicity)

By this, a very virulent organism requires small numbers to bring about disease. You need few cells of *Klebsiella pneumoniae* to come down with the deadly pneumonia, but you need to ingest a large

number of *Vibrio cholerae* cells in order to come down with cholera. But bacterial dysentery can be acquired through ingesting just few bacterial cells. It is noteworthy that 1 trillion (one terabacterium) of these bacteria weigh scarcely 1 gram. That would be about the size of a sugar cube. This number of the dysentery bacilli is enough to infect not only all humans on Earth, it is also enough to infect all other susceptible vertebrates on Earth, and would still have plenty to spare.

And how about this?
The dry weight of vertebrate faeces in the lower intestines can be likened to a zoo of microbes. It is estimated that one third (1/3) to one half (½) of the dry weight is bacteria. How much would it take to pollute a large body of water (like the Port Harcourt Pleasure Park (PPK))? Actually, not much.

And this:
The space between teeth and gums – what the dentists call the gingival crevice, contains a carpet (wall to wall) of bacteria. There are more bacteria in and on our bodies than all our human cells.

The Microbe and the Earth
In a way we may say Earth is a planet of microbes. In talking about creating jobs for mankind, we are talking about the activities of microbes on Earth, where humans live.

Microbial populations are so widespread to the extent they can be said to determine the limits of the biosphere. For example, we may say where there is life, there are microbes, and where there are no microbes, there is no life.

The domination of Earth by microbes is clearly related to their ability to reproduce. This is definitely interesting in the sense that the creator said creation should multiply (reproduce) and fill the Earth and subdue it (Gen 1:28). Microbial reproduction is a biochemical process.

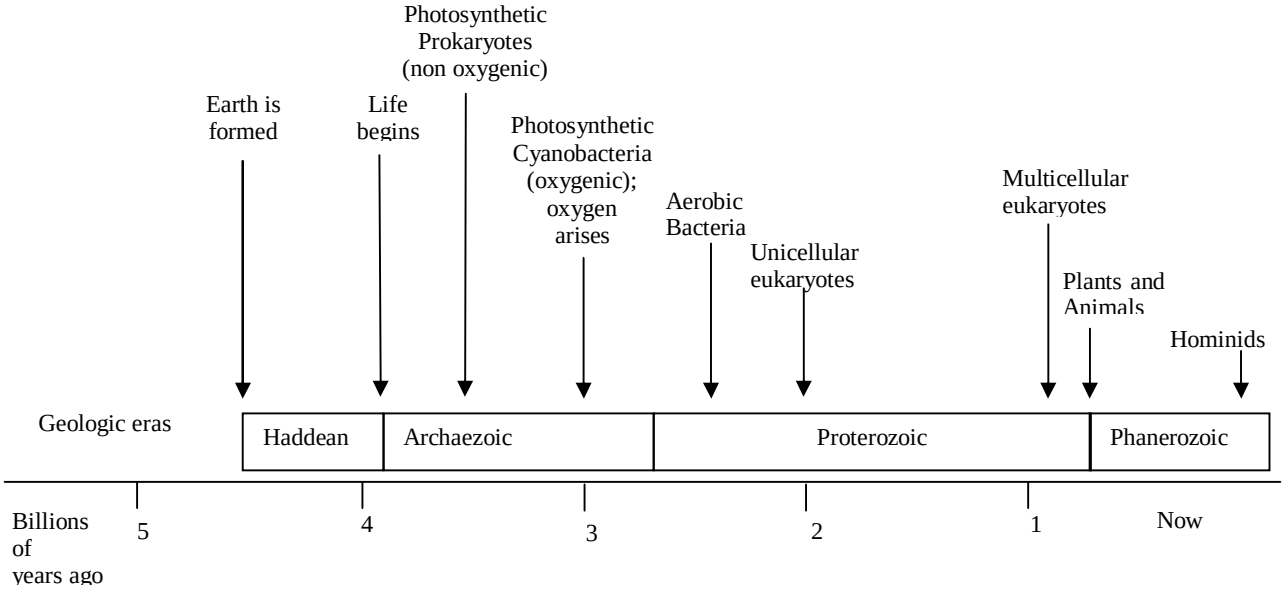


Fig. 13: Time scale of life on earth (**Source:** Schaechter *et al.*, 2006).

Science defines life by a set of characteristics. Science defines life by molecules and reactions. To what extent can we go with that? In this diagram (Fig. 13), when we say Earth is formed – what does it mean?

Geological Timelines and Life

Geological Timelines are given in periods by the geologist. The biologists now insert time as discrete events on that timeline. The only way it can be a discrete event is through creation. So, creation can account for the emergence of life, and in that way each life form

was created in its own complexity. In other words, the microbe was created a microbe and barracuda was created barracuda the mistletoe was created a mistletoe and the virus? Yes, the virus was created a virus. The "primitive" (cyanobacteria) plant cell engulfing a bacterium in order to form a plant cell with mitochondria would look like taking a complex and efficient system and making it less efficient. I tell my students in microbial physiology class that – a portion of the cytoplasmic membrane of the prokaryotic cell is the equivalent of a whole organelle in a plant and animal cell, i.e., the mitochondrion. Such is the level of complexity of the prokaryotic cell. It was created that way, just as the plant and animal cells were created that way. And the microbe and plant and animal cells could have been created on the same day or on different but discrete time.

What was responsible for the formation of Earth?

By the reasoning of science, we don't know, and it is as if we really don't want to know. This is where our problem as scientists lies. We do not know how to admit that we do not know! So, scientists said this when Boson, (the smallest particle observable and measured by man) was discovered, scientists declared; now we don't need God; we can figure out exactly how everything began.

Life beyond molecules can only be created

To create is to bring something out of nothing. Science recognizes that there is design. The architect designs – ideas – structures. The engineer designs – ideas – structures. The fashion designer designs ideas- structures. So who designed us? I think this is the crux of the matter. So I'd say in the science of creation, God designed, fashioned, formed the Earth. In the same way, God designed, fashioned created life.

So what is life?

The physicists research into energy as the binding force on all matter, hence the Higgs Boson of quantum mechanics in particle physics. The chemists research into energy in terms of chemical bonds, from atoms to elements to molecules, thus thermodynamics. The biochemists research into bioelements to understand the

peculiarities of elements, thus, the concept of energy is bioenergy or bioenergetics. The zoologists research into what we may call intelligent life; the concept of the cell as the smallest unit of organization of intelligent life. Now, the microbiologists, well, the microbiologists research into the interface between energy in particles and intelligent life, that is non-intelligent life. Looking at the entities studied by microbiologists, we see that there are the prions which are simply proteins, then we have the viroids which are just RNA, then the viruses comprising of proteins and nucleic acids, all of this comprises the acellular entities; then we have the cellular entities of archaea, bacteria, protists and fungi; all of these make up the microbes. We tend to group these microbes as non-intelligent life. By intelligence we human beings are talking about the ability to learn or understand from experience, ability to acquire and retain knowledge – mental ability, the ability to respond quickly and successfully to a new situation, use of the faculty of reason in solving problems, directing conduct etc effectively. Can we say then that microbes are non-intelligent life? But they can do virtually all that we described as intelligence. Microbes can move away from repellents (chemotaxis), microbes can preferentially use one substrate over another, and actually come back to the one earlier passed. This may even have happened with "new" cells. This is the concept of diauxie and cometabolism.

Crossing the line from non-life to life

Darwinian evolution depends on cellular organization. Selection through selective pressure simply means acting on an existing level of cellular organization. According to Harold (1986), "the heart of the abiding mystery of the origin of life is not the abiogenic origin of genes and proteins, it is the spontaneous generation of life".

A time for crossing that line from non-life to life: What is that length of time?

Franklin, M. Harold (2014) said "the history of life unfolds over a time span so vast it boggles the imagination; I, for one, can draw no meaning from a million years, l*et al*one a billion, and prefer a geographical metric. Let 1 millimetre, the thickness of a dime, stand

for 1 year. Then 1 meter makes a millennium, 1 kilometer a billion years and the age of the earth (about 4.5 billion years) spans 4,500 kilometers, a little more than the distance between Miami and Seattle". Even this reasoning by Harold would presuppose that life came into existence at a point in time. Therein lies the mystery. Creation is the unveiling of that mystery. Spontaneous generation of life; this is the creation of life. Pasteur disproved the theory of the spontaneous generation of putrefying agents; then he established that the broth got spoilt by life present in air, that life is microbes. But how microbes arose was not a point of debate. But, so, what is life?
Pope John Paul (1921-2005) said: "Life cannot be defined, it can only be appreciated and often, only in the absence of it". He went on – "I can do what I want, but can I want what I want? Hence, we are born without asking to be born and we die without intending to".
It is reasonable to think that life cannot evolve. In other words, a microbe may not imagine that it could become a paramecium. Even the cloning we are doing currently cannot bring forth life, because the cells used already are living.

So, a microbe is a microbe, an elephant is an elephant, an ape is an ape, a horse is a horse, a barracuda is a barracuda, a man is a man and a woman is a woman. From the design concept, there can be a lot of similarities. By creation, we may say man was created with a fully developed mind. Man was not an ape when he was created. Man did not arise from *E.coli.* All the knowledge we have acquired about ourselves (man) and our environment is just so that we would know that we could never make ourselves. The 16s rRNA genes of mitochondria and bacterial cells have revealed similarity. But bacterial cells are far more sophisticated than the mitochondria. In operational complexity, the bacterium is more complex than the mitochondrion. The cytoplasmic membrane (CM) of prokaryotes performs the functions of an organelle (the mitochondrion) in a Eukaryotic cell.

Ecologically, biodiversity is concerned about similarity and the pool of capabilities resident in a population. We can make use of those capabilities. This is the area of ecophysiology and ecological

economics. This is the basis of biotechnology. For this reason, I want to interpret, "Mr Ecology" himself – Professor Martinus W. Beijerink's famous ecological concept – "everything is everywhere, nature only selects". It is there. Everything "is".

Where there is life, there are microbes. We may say that, there is no habitat on Earth that supports life that is without microbes. Microbes are signature of design and creation. They are there in extremes of habitats as we may call it.

They can grow at extreme temperatures of 121^0C. This is the temperature of boiling water, but only at elevated pressures, such as is found at the depths of the sea where there is deadly hydrostatic pressure (at atmospheric pressure, water boils at 100^oC). They are the extremophiles, more bizarre than the thermophiles.

Table 5: The known extremes of life

There are microbes that survive:
5 megarads of gamma radiation (ca. 10,000 times what would kill a human being)
Very high pressures (ca. 8,000 atmospheres same as 117,000 psi)
There are some microbes that grow at:
Extremes of pH (0 to 11.4
Extreme temperatures (-15 to 121^0C)
Higher hydrostatic pressure (ca. 1,300 atm or 18,500 psi)
High osmotic pressure (5.2M NaCl)

Source: Schaechter *et al.,* 2006

What use? Job creation:
Industrial/Biotechnology/Molecular Biology Applications
Every feature displayed by microbes can be harnessed for use by mankind if we learn the way of the microbe. The microbe, we may say is the husbandman, caretaker, gardener over the earth. Jobs can be created out of simple microbial processes to complex phenomena. Meanwhile the microbe is just living its normal life. For example we can have heat – stable hydrolases for use in laundary detergents.

Forensic testing of DNA is done with a heat-stable enzyme from the microbe *Thermus aquaticus*. A foremost ecophysiologist, Professor Thomas D. Brock of University of Wisconsin, Madison, USA had isolated the organism from hot springs at the Yellowstone National Park.

Photosynthesis/ Chemosynthesis: Recycling of elements

About half of the oxygen in the air is the result of microbial photosynthesis; microbes are able to transduce radiant energy in the manufacture of energy for cellular use; the rest is from plant photosynthesis. In chemosynthesis, microbes can derive energy, not from light, but from chemical bonds in inorganic compounds. Thus, microbes can oxidize molecular hydrogen (H_2), reduced iron (Fe^{2+}), sulfides (eg, H_2S), and others. These microbes derive their cell carbon from CO_2 in about the same way CO_2 is fixed in photosynthesis. But these chemosynthetic microbes do not rely on the energy of the sun. They are the chemoautotrophs compared to the photoautotrophs. In this way, i.e., with such metabolic dexterity, the microbes can live in dark places that lack a supply of organic nutrients. These kind of bacteria constitute the ecoforce that sustains life in deep sea vents in the oceans and cracks and fissures in rocks. One of such *Desulforudis audaxviator* (for an audacious traveller representing a single species ecosystem) was discovered at a depth of about 3 km down a mine in South Africa (Dylan *et al*., 2008). Because of the very low levels of carbon present, less than 1mgC/l, these microbes are described as oligotrophs; they are thought to be cannibals among themselves – feeding on the carbon from other cells. There are thoughts that the subterranean microbes may outweigh all the oxygen-using-organisms above ground.

They shape and influence the Earth.

Their sheer numbers compensate for their extreme small size. The abundance of microbes on Earth has direct effects on the physical and chemical properties and quality of the environment. Through their intense biochemical activities, microbes bring about major changes in the biosphere. The elements of life including carbon, oxygen and nitrogen are actively recycled. Oxygen is recycled

through photosynthesis – microbes play a role in almost half of that. Carbon is recycled through the biodegradative capabilities of microbes – mineralization of dead organic material including plant material, to produce CO_2 (thus, CO_2 is mineral or inorganic carbon – Do you know why your coke is called mineral? Because it is fizzled with this mineral carbon, CO_2).

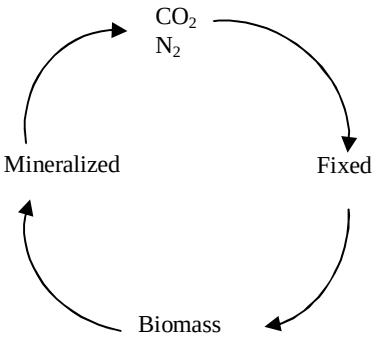


Fig. 14: Biogeochemical cycling of elements concepts

A plethora of microbes utilize a vast repertoire of organic substances for nutrition and growth (energy and biomass, about 50:50). It has been postulated that for nearly any naturally occurring organic compound, there is at least one kind of microbe that can break it down, as long as there is water available. No wonder they were created in such large numbers (about 10^{17} groups/species). This information is particularly useful in the management of our environment, the cleanup of environmental pollutants. What about non naturally occurring organic compounds? The microbes are fast learners, they are learning the ways of the spoilt child called man. And being such faithful stewards they can handle both the naturally occurring and the xenobiotics (Alexander, 1971; Atlas and Bartha, 1989; Okpokwasili, 2006; Odokuma, 2012). Certain species of *Pseudomonas* can metabolize hundreds of organic compounds. Many *Pseudomonas* species are found in abundance in soil, plant roots, bodies of water and animals. *Pseudomonas* species have lots of genes, they have some of the largest microbial genomes, the "super bug"

Table 6. Some functions of prokaryotic and eukaryotic cell membranes and organelles

	Structure	
Function	**Prokaryotes**	**Eukaryotes**[a]
Osmotic barrier	Cytoplasmic	Cytoplasmic membrane
Transport of solutes	Cytoplasmic membrane	Mainly cytoplasmic membrane
Respiratory electron transport	Cytoplasmic membrane	Mitochondrial membrane
Protein synthesis	Polyribosomes in cytoplasm	Polyribosomes on endoplasmic reticulum, Golgi apparatus in plants
Synthesis of lipids	Cytoplasmic membrane	Golgi apparatus in plants
Protein secretion	Cytoplasmic membrane	Endoplasmic reticulum and secretory vesicles
Photosynthesis	Various membrane types, some continuous with, others independent of the cytoplasmic membrane	Chloroplast membranes

Source: Schaechter, *et al.*, (2006). [a]Main sites only.

Microbes were created with biosynthetic and biodegradative capabilities. They were endowed with these capabilities, in a way they were taught. All these capabilities are geared toward getting energy for living. Virtually all that the microbe does is (how) to get energy. The acquiring and use of energy must be very efficient. So that the net benefit analysis is positive.

How are microbes creating jobs?
Ans. Through processes and phenomena

The jobs can be of negative impact or of positive impact – the four biotech – White, Blue, Green, Red.

(A) The Positive Impacts

- Products
  - Antibiotics
  - Biosurfactants
  - Biomaterials
    - Nanoscience – Build up / Break down
    - Nanomaterials – murein sacculus
    - Nanoscience – Nanotech – Fyneman had predicted it – 1960s at Inaugural Lecture

Energy

- Crude oil
  - Associated gas
    - Methane
    - Propane
    - Butane

    Microbes designed petroleum
    Bioprospecting of petroleum
    Methane utilizers – methylotrophs
- Coal
- Biofuels
  - Gas – H_2
  - Liquid
    - Ethanol
    - Biodiesel

- Food/beverages
- Other Industrial Products
- Env./marine biotech
- Waste treatment
  - Liquid waste
  - Industrialization
    - Sewage treatment

We may say that microbes were created before petroleum if they are to take part in the design of petroleum. Petroleum is from dead bodies of plants and animals (Paleontology). We may say that microbes were created when the earth was created and they were created to manage the earth including creating jobs for mankind. These are resource jobs. There are microbe-powered jobs where

microbiologists use microbes and the knowledge of microbes to create jobs.

On Saturday 5 August 2017 NASA announced they are hiring a "planetary protection officer". And what could be his job description? Help to protect us against invasion by foreign microbes from outer space. Interesting, even the fear of microbes creates jobs, how much more the love for microbes?

Even viruses add their "beauty" too.

Fig. 15: Picture of a tulip harbouring a tulip mosaic virus (Source: google)

This is a picture of a tulip – beautiful!! It contains the tulip mosaic virus. The virus alters the development of the plant cells and causes complex patterns of color in the petals. The virus does not cause severe harm to the plants.

Viruses are a significant determinant in the functioning of many ecosystems. For example – seawater can contain up to 10^6 per milliliter (ml). Since viruses are made up of the same element as living cells, it is estimated that the sum of viruses in the ocean represents some 270 million metric tons of organic matter (another

estimate has it that there are $4x10^{30}$ viruses in the oceans, this is equivalent in mass to 75 million blue whales at approximately 173 tons and 29.9m long. The blue whale is considered the largest animal known to have existed). This can contribute to the dynamic energy budget in the ecosystem.

Gene therapy using harmless viruses has been proposed. Here, the normal gene is inserted into a retrovirus, such as the mouse leukemia virus. It is hoped that the virus will introduce the needed gene into the cells and correct the defect. Trials have been on cystic fibrosis conditions.

Then, there is the use of bacteriophages. The phage typing therapy is on the basis that the bacterial viruses would seek out only their specific host bacteria and cause complete destruction of the bacterial cell. It is believed that this method can control infections just as well as chemotherapeutic drugs and antibiotics could. Viruses are used in sewage and wastewater treatment to eliminate pathogens.

B. Negative Impact

Diseases

About 80% of diseases are infectious caused by microbes. We may say these are inevitable jobs. These are consequences of living together on earth. The microbe has no intention of harming us, all it is concerned about is how to obtain energy for its living. Everything about the microbe is energy, for biosynthesis and for energy.

So what are the jobs? Physicians and medically oriented jobs. Some of the infectious diseases including cancer are so debilitating and vicious you would think it was a premeditated attack, but no, the microbe is simply living out its life, we just happen to be in their pathway. By their sheer number, they could easily wipe out the human race if it were to be an invasion; thank God it will not be so. Can anyone be allowed to practice real medicine without studying microbes? May be – very few, but I doubt it. So, we study and learn the way of the microbe and use the knowledge against the microbe in order to contain and control it. We study how it survives, grows

and multiplies. We study its energy metabolism, the intermediates and products that arise there from. The microbes create jobs for us when they produce metabolites and intermediates which we now use to control the establishment and growth of the microbes. We learn the secret of their strength in order to use it against them (sounds like Samson and Delilah).

Biodeterioration of materials

Man's use of materials is pervasive, from clothing including shoes, implements, to transportation including road, water, aviation and space travel, to industrial, to medical devices, to electronic and electrical devices, to pharmaceutical formulations, to scientific equipment to office and domestic utensils. All of these are subject to the elements of nature as we refer to it. One of those elements of nature is microbes. There is hardly any material, natural or manmade including the precious metals that are immune to microbial attack. The consequences of these attacks, take for example biocorrosion alone, is in billions of dollars of expenditure annually. The attacks of microbes on materials can either be induced (when microbes initiate it) or influenced (when microbes only aid it). From biofouling leading to loss of aesthetics and performance to biocorrosion leading to structural failure and loss of integrity, microbes are involved. The maintenance associated with biofouling (including biofilms, barnacles and diatoms) and biocorrosion of ships' hulls costs the U.S. Navy over **$6 billion** per year. Overall, the cost to industry has been estimated to be at least **$200 billion** per year in the United States alone (https//www.google.com.). Again, microbes are only living out their own life. For this reason, we have come to realize that what we call biodeterioration is actually the microbe-driven cycling of the elements of the periodic table. Our attempts to control them create jobs for us.

RESEARCH ACTIVITIES, IMPACTFULNESS AND COMMUNITY SERVICE

My graduate studies research was in Professor Rita R. Colwell's Lab at the University of Maryland, College Park Campus, Maryland, USA. In Colwell's Lab I was placed on a University of Maryland

Sea Grant funding to study the adhesion of a newly characterized marine bacterium. The organism had been code named LST for Lewes Spat Tank. It had been found in association with the oyster *Crassostrea virginica.* For the larvae to grow into adult oysters they first settle or set on a surface or substratum and then they can complete their growth. The bacterium had been shown to produce pigments likened to L-DOPA, a melanin precursor. Robert (Bob) Belas had worked in Professor Colwell's Lab on the adhesion of *Vibrio parahaemolyticus,* where he studied the role of flagella in adhesion and showed that kinetically the bacterium exhibited multilayer binding (Belas and Colwell,1982). I would now endeavour to elucidate the biochemical and biophysical aspects of the adhesion of the newly described bacterium. At UM College Park, the caption in the Grad School bulletin was – "higher degrees are earned, not given, and they are earned in the laboratory". So, course work was done which included courses in Microbial Physiology, Microbial Ecology and Biosystematics (including Computer Applications in Systematics), Genetic Engineering and Biochemistry (which included Nucleic Acids and Protein Chemistry); Biochemistry was also chosen as minor (in the course system, that meant that I would accumulate a specified number of credit units in Biochemistry). Partly course requirements and the need for necessary techniques took me to the, then state-of-the art Chesapeake Biological Laboratory at Solomon's Island. There, we learned marine scientific expeditions and electron microscopic techniques. I settled back in the lab at College Park, MD to elucidate the adhesion of LST to inanimate surfaces under laboratory conditions. We succeeded when we devised a method that led to a semi-solid culture. Polycarbonate nuclepore filters (0.4μm pore size) were placed onto marine agar in a plate, the set up was then seeded with a suspension of logarithmic cells. SEM photomicrographs revealed what we called the advancing front of a biofilm matrix on the nuclepore filters.

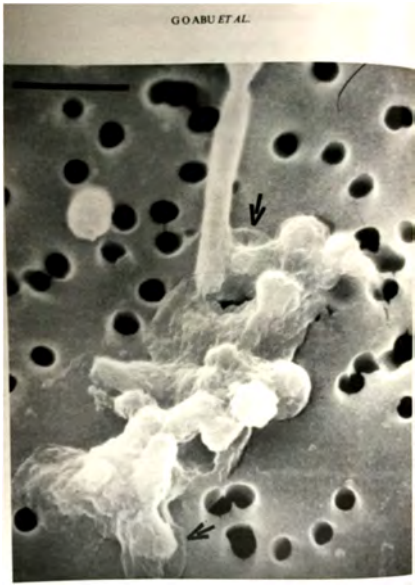


Fig.16: Biofilm of *Shewanella colwelliana* on Nuclepore filter. The bar is 1µm. The arrows indicate the anchoring advancing front. (Abu *et al*., 1991)

We demonstrated that the bacterium, now identified as *Shewanella colwelliana* could actually synthesize the exopolymeric substance (we called it PAVES for polysaccharide adhesive viscous exopolymeic substances), from non carbohydrate precursors i.e., amino acids. Using radiorespirometry, we showed that the organism could also metabolize glucose by a combination of either the Embden Meyer Hoff Parnas (EMP) or hexose monophosphate and the Entner Doudoroff pathways (Abu *et al*., 1994). Stoichiometric concentrations of yeast extract modulated the utilization of glucose.

Table 7: Major components of *S. colwelliana* exopolymer

Component	Percentage (w/w)
Carbohydrate	15 – 35
Protein	1 – 5
Nucleic acids	0.5 – 5
Lipid and residual moisture	5 – 10
Inorganics	40 – 45

(**Source:** Abu *et al*., 1991)

We had succeeded in isolating the adhesive polymer produced by *Shewanella colwelliana* under conditions approximating its natural environment. The inorganic content of the adhesive polymer was high with S, Ca, P, Cl and Si accounting for 95% of the inorganics.

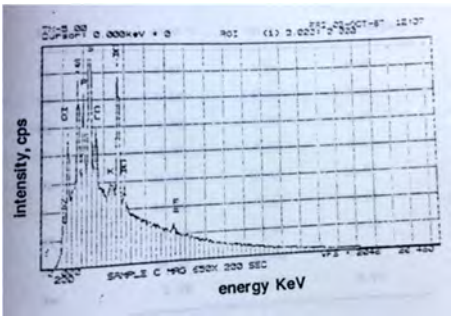


Fig. 17: X-ray microanalysis of the *S. S. colwelliana* exopolymer
(**Source:** Abu *et al*., 1991)

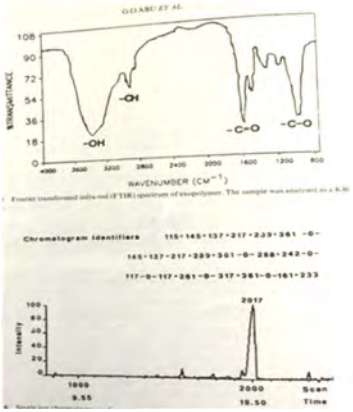


Fig. 18: FTIR spectrum and GC ion chromatogram of *S. colwelliana* exopolymer
(**Source:** Abu *et al*., 1991)

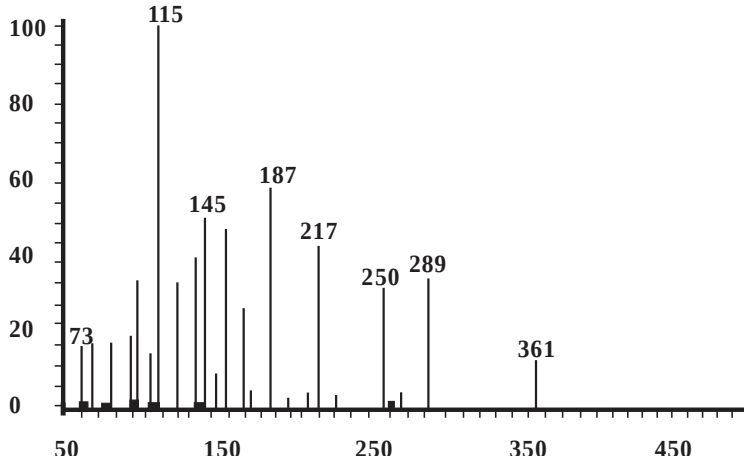


Fig. 19: Mass spectrum of the major component of *S. colwelliana* exopolymer, determined from a single ion chromatogram. (**Source:** Abu *et al*., 1991)

Table 8. Elemental (inorganic) composition of *S. colwelliana* exopolymer.

Element	Atom %	Wt %	Wt% in Polymer
Si	8.55	7.04	2.99
Na	<0.10	<0.10	<0.10
Mg	3.98	2.81	1.19
K	3.02	3.47	1.47
Ca	24.13	28.41	12.07
P	10.38	9.47	4.02
Cl	9.37	9.65	4.10

(**Source**: Abu, *et al*., 1991)

High concentration of sulfur and calcium, and almost complete absence of sodium is shown. We interpreted this finding as the elements being an integral part of the purified polymer. This conclusion was reached because the material analysed had been

subjected to extensive purification including alcohol precipitation, dialysis, gel filtration and ion exchange chromatography

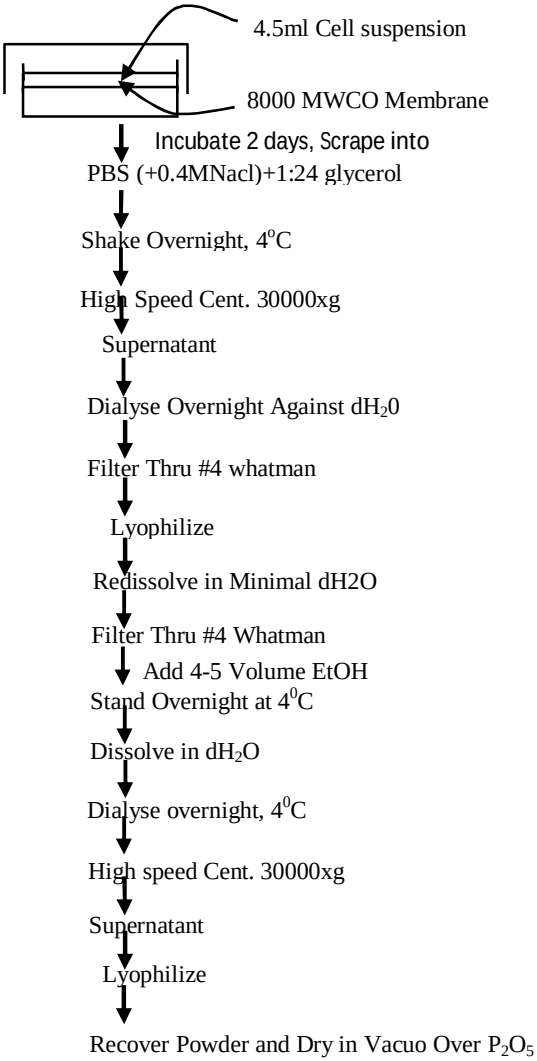


Fig. 20: Flowchart of PAVE purification. (Source: Abu, *et al*., 1991)

The atoms could be involved in tertiary structures of the polymer under biofilm conditions. Such tertiary structures could stabilize the adhesive polymers giving them properties of Stefan adhesives that can set under 100% humidity or under water. The advancing front (in Fig.12. Abu *et al.*, 1991) supported our deductions.

Impactfulness: The nature of forces involved in microbial adhesion and biofilm formation

The adhesion of microorganisms to surfaces is influenced by long-range, short-range, and hydrodynamic forces. In unambiguous terms two surfaces can be said to have adhered when work is done to separate them to their original positions (Rutter *et al.*, 1984). Therefore energy is involved in the formation of the adhesive junction, be it in the reversible phase or the irreversible phase of adhesion. Long range forces can be discussed in terms of the free energy as a function of separation between the microbe and the surface. The DLVO (Derjaguin and Landau, and Verwey and Overbek) theory of colloidal stability, has been used extensively in the analysis of long range forces of interaction between cells and surfaces (Rogers, 1979). The basic tenet in this theory is that total interaction between two particles is composed of two additive terms: (GA) due to the van der Waals forces of attraction and repulsive force (GE) due to the overlap of the electrical double layer associated with charged groups present on the particle and the macroscopic surface. Although these two forces are additive, they vary independently with the distance of separation between the interacting bodies (Rutter and Vincent, (1984). The expression connecting these forces is given as:

$G_i(= G_A+G_E)$

where G_i is the free energy of interaction, G_A is the force of attraction and G_E is the repulsive force between the interacting particles. The overall charge and shape of the interacting bodies is important and these make a significant contribution to the forces of attraction and repulsion. A plot of the computed interaction energies between cells and a plate, for example, gives a curve like that shown

in Fig. 21, below. At point A, a distance of about 1 nm from the surface, a very high potential energy barrier peak exists (a value of the order of 100 kT or 4 x 10^{-12} ergs), for cells the size of bacteria (Rogers, 1979). Because of this high energy barrier, long range forces are able only to bring a bacterium, for example, to the secondary minimum, point B. Cells at this point can easily desorb from the surfaces leading to the reversible phase of adhesion (Marshall *et al.*, 1971). However, having been brought to the secondary minimum by- long range forces, the bacterium can now go through an irreversible phase of adhesion for example by polymer bridging (Marshall *et al.*, l97l; Sutherland, 1983; Fletcher and Floodgate, l973). At short separations, short range forces, such as dipole-dipole interactions, H-bonds, etc, may become more significant. Short range forces are especially important in aqueous systems, where they may be repulsive or attractive, depending on the nature of the surfaces involved. Prominent in this class of short range forces are hydrophobic interactions. When the two surfaces involved are both hydrophobic, the short range interaction is a net attraction. This leads to a deepening of the primary minimum. The water molecules displaced into the bulk solution as the particle (cell) approaches the surface decrease their free energy, thus favoring adhesion (Rutter and Vincent, 1984, Absolom *et al.*, 1983). This decrease in free energy is due to the fact that there is a net increase in hydrogen bonding between the displaced water molecules and the bulk phase (Rutter and Vincent, 1984). The opposite effect will take place if both surfaces are hydrophilic.

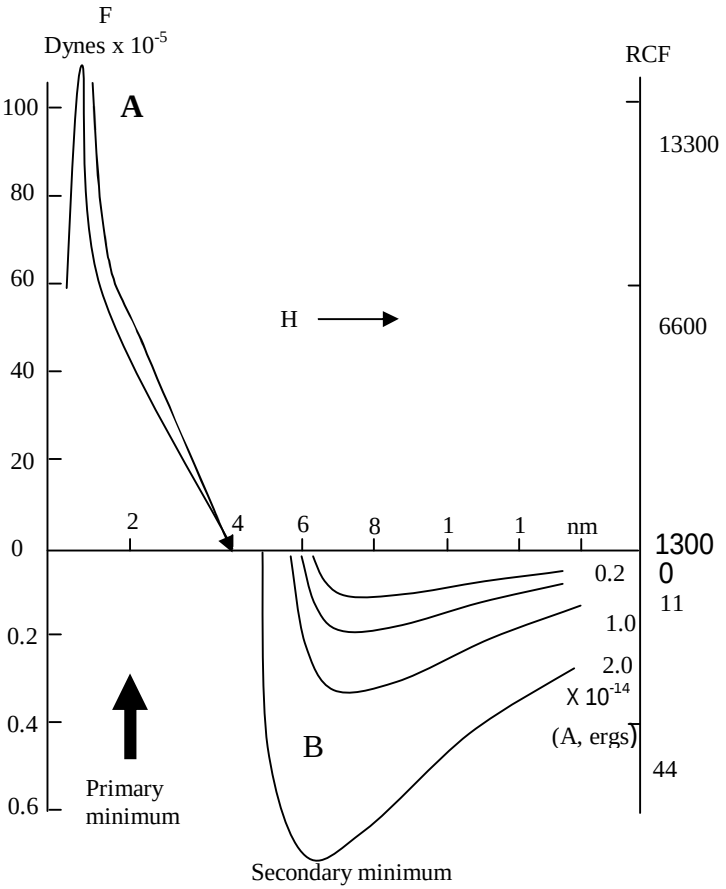


Fig. 21: The nature of forces involved in bacterial adhesion.
H is the distance (nm) separating the interacting bodies. On the right is given the relative centrifugal force required to overcome the forces of interaction and on the left the computed interaction energies. The high energy barrier at point A can be overcome by the presence of nanofibers such as fimbriae, flagella, and extracellular polysaccharides (Weiss, 1968; Rogers, 1979).

A thermodynamic model has been proposed to define further some of the parameters which determine the extent of the initial interaction of bacterial adhesion to surfaces (Absolom *et al.*, 1983). This model assumes that the free energy will be minimized at

equilibrium. Thus, the process under consideration, in this case, bacterial adhesion, will be favored if the process itself causes the thermodynamic function to decrease. Conversely, adhesion will not be favored if it causes the free energy function to increase. The mathematical expression of this thermodynamic model is:

$^{\Delta}Fadh = ŸBS - ŸBL - ŸSL$

where $^{\Delta}Fadh$ is the free energy of adhesion per unit surface area, ŸBS is the bacterium- substratum interfacial tension, ŸBL is the bacterium-liquid interfacial tension, and ŸSL is the substratum-liquid interfacial tension. The model also uses a combination of the Young's equation and a form of an equation of state, together with contact angle data and liquid- vapor interfacial tensions. These are connected thus:

$ŸSV-ŸSL = ŸLV \cos\theta$

where ŸSV is the interfacial tension between a solid substratum S and the vapor phase V, ŸSL is the interfacial tension between the substratum S and the liquid phase L, ŸLV is the interfacial tension between the liquid phase L and the vapor phase V, and θ represents the contact angle of the liquid on the surface of the solid. This model therefore attempts to define the parameters of the three components involved in adhesion viz: effects of the surface tension of the substratum (SV), that of the suspending medium (LV), and that of the bacterium (BV). As predicted by the model, adhesion to hydrophilic surfaces is more pronounced than to hydrophobic surfaces when the bacterial surface tension is larger than that of the bulk fluid. When the surface tension of the bulk fluid is higher than the surface tension of the bacterial surface (a similar situation to what exists in natural waters), bacterial adhesion to hydrophobic surfaces is greater than that to hydrophilic surfaces. Thus, hydrophobic interactions are important in the initial sorption of bacteria in aquatic environments (Marshall *et al.*, 1971; VanLoosdreht *et al*. 1987).

The thermodynamic model by Absolom *et al*. (1983), assumes negligible electric charge and absence of specific biochemical interactions between the phases involved.

It is evident that the DLVO theory and the thermodynamic model approach are not sufficient in explaining bacterial adhesion conclusively. Jucker *et al.* (1998) and Hermansson (1999) proposed an extension of the DLVO theory which includes the hydrophobic/hydrophilic interactions. We can therefore write the sum of the energy involved in adhesion as follows:

$$\Delta G\ adh = \Delta GvdW + \Delta Gdl + \Delta GAB$$

Here, ΔGvdW and ΔGdl represent the classical van der Waals (vdW) and double layer (dl) interactions, and ΔGAB is a function of acid-base interactions. This ΔGAB component reveals an aspect that can be said to describe the attractive hydrophobic interactions and forces of repulsion due to hydration effects, these are about 10-100 times the value of the vdW forces of interaction when particles or surfaces come into direct contact with each other.

Due consideration is usually given to the approach distance dependence, it is very important in calculating the total adhesion energy, this approach distance is given from the classical DLVO theory both for the vdW forces and the double layer charge interactions; the approach distance of the surface energy component ΔGAB shows exponential decay from its value at close range.. It is evident that acid-base interactions which are physicochemical in nature are not critical at the first stage of adhesion; therefore, what is observed as the time dependent strengthening of the cell-substratum interaction could actually be as a result of the cell approaching closer to the surface. The conclusion is that, the modified DLVO theory of lyophobic sols could be a promising way to further study bacterial adhesion, but that would require more rigorous testing of the model (Katsikogianni and Missirlis, 2004). The application of physicochemical theory, in some ways, has helped to explain some observations, but it has not been successful in fully predicting all the various types of attachment scenarios described in bacterial systems. Applying physical theories to explain biological systems may not

always tell the whole story. This is at the very root of the origin of life itself. There is the complexity of bacterial surface polymer composition, then there is the change in polymer composition and synthesis with changing environmental conditions or time, these can help explain to a great extent a lot of the differences in experimental results observed with bacterial attachment. It is obvious that the types of nutrients and their concentrations can influence the chemical composition of the cell surface polymers and thus cell surface characteristics (Nwanyanwu and Abu, 2012). Generally, it has been observed that, the process of biofilm formation commences with bacteria first been attached to a surface, then, within hours or days, hydrated amorphous polymers, the exopolymeric substances (EPS) accumulate, together with increasing numbers of attached cells (Abu, *et al*., 1991; Costerton et al., 1981). These polymers (EPS) form an intercellular matrix in which the cells are enmeshed; this is what constitutes the highly hydrated, slimy matrix that forms a major component of the seemingly highly structured bacterial biofilm. Since the polymers seem to accumulate after attachment has occurred, it is suggestive that attachment to a surface may actually signal the switching on or turning on genes for polymer synthesis in order to strengthen cell surface attachment, a sort of communication (Costerton *et al*., 1994). Attachment to surfaces induces expression of genes that result in the conversion of cells from single-cell, free swimming mode to a complex multicellular, surface associated or biofilm mode of existence (Heilmann *et al.*, 1996; Mack, 1999; Costerton *et al*., 1994).

It can be concluded that, cell surface proteins, polysaccharides, conditioning films on surfaces, co-adhesion and biological changes in attaching bacteria may all contribute in bacterial adhesion. To predict the adhesion process may require deciphering the roles of each of these components, this obviously is a daunting task. Our turning to the use of theories such as the DLVO theory, that predict adsorption of well-defined colloidal particles, to the field of bacterial adhesion, is very useful; this would help to form a framework into which we would be able to add biological factors in the overall understanding of the forces that bind matter together. This is

particularly important since bacteria are the smallest life-bearing particles, knowing the forces that influence their binding together and to inanimate surfaces would give us clues to the forces that bind matter together. Is the EPS the glue of life?

Exopolymer-mediated adhesion and biofilms

In the context used here, exopolymer refers to the extracellular polymeric material found on the outside of the peptidoglvcan-teichoic acid layer of Gram positive bacteria or on the outside of the lipopolysaccharide of Gram negative bacteria. Costerton *et al.* (1978) refers to this structure as the glycocalyx, a term used by Bennet (1963) to describe the predominantly polysaccharide, exocellular polymers found on the outside membranes of animal cells. In procaryotes, these polysaccharide exocellular polymers may be referred to as capsules or slime, depending on whether they remain attached to the cell surface when synthesized or are released into the medium (Sutherland, 1977; Geesey, 1982).

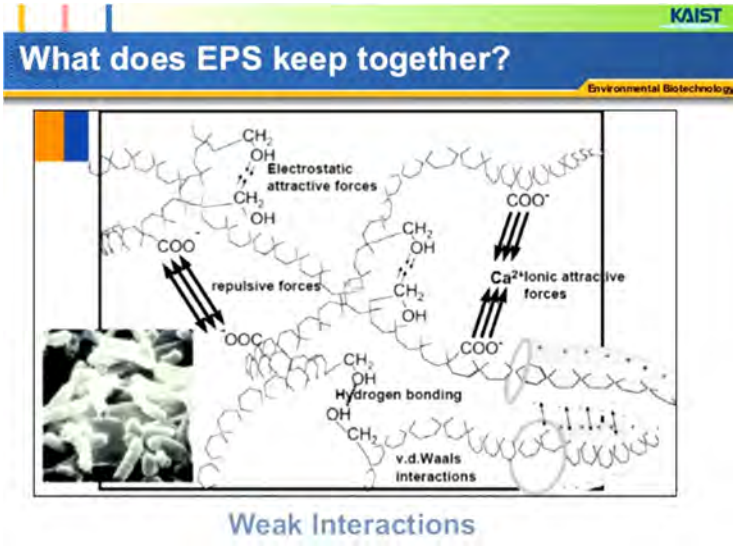


Fig. 22: Function of EPS in adhesion and biofilm formation (Source: Google: KAIST; Environmental Biotechnology)

Physical and Chemical Nature of Exopolymers

Microbial exopolymers are often seen as fibrillar structures under the electron microscope (Costerton *et al*., 1981; Brooker, 1979). This fibrillar nature, in most cases, may arise from excessive condensation of the polymers during the dehydration step in the electron microscopic preparation. According to Sutherland (1972), these exopolymers are invariably hydrated gels whose water content may be as high as 99%. These structures have little inert density and do not stain readily with conventional reagents (Roth, 1977).

Exocellular polymers have water-binding properties because of the presence of ionic species such as uronic acids or ketal-linked pyruvate and acetate, as well as hydrogen bonds, i.e., OH groups (Rees and Scott, 1971; Atkins *et al*., 1974; Avril *et al*., 1987). These exopolymers can therefore provide the organisms with a hydrophilic surface (Wilkinson. 1958). Exocellular polymers also have ion-exchange properties due to the presence of the ionic species (Mongar and Wassermann, 1952; Haug, 1959; Katchalsky *et al*., 1961). According to Dudman (1977), exocellular polymers can act as mechanical molecular sieves and adsorbents as well as diffusion barriers. Many exocellular polymers also have gelling properties (Sutherland, 1983; Boyle and Reade, 1983; Kang *et al*., (1982). For this reason exocellular polymers can exert excluded volume effects and decreased diffusion on solutes and other macromolecules such as proteins (Ogston and Phelps, 1961; Laurent *et al*., 1963).

The unique rheological and metal binding properties of exopolymers are areas of great industrial and environmental interest. The exopolymer of *Zooglea ramigera* behaves like a polyelectrolyte and shows a strong affinity for metal ions (Dugan and Pickrum, 1972), making it very suitable for secondary biological treatment of waste water (Butterfield, 1935; Krul, 1977). The exopolymers of *Klebsiella aerogenes* have been studied extensively and also have been employed in similar environmental biotechnologies (Lester *et al* ., 1984*)* . Such metal chelating properties of the exopolymers are believed to protect the producing organisms from the toxic effects of the metals (Bitton and Freihofer, 1978; Walch, l986; Mittleman and

Geesey, 1985). The rheology of xanthan gum, the exopolymer produced by *Xanthornonas campestris*, demonstrates a marked pseudoplastic effect (MacWilliams *et al.*, 1973; Kelco, 1977). Solutions of this exopolymer are very viscous and show sensitivity to increased shear stress, but when the shear stress is removed, they quickly recover their viscosity or thickening power. For this reason xanthan gum can be very conveniently employed in enhanced tertiary oil recovery (Gabriel, 1979). The shear thinning property may be important in the adhesive properties of aquatic bacteria (Sutherland, 1983). In the food, pharmaceutical, and related industries, extracellular polysaccharides have been employed for a variety of uses, e.g., paper coatings, textile pad dyeing, printing, flowable pesticides, liquid fertilizers, adhesives, gelling, and stabilizing of food (Sanford, 1977).

The Requirements for Exopolyrner-Mediated Adhesion.
For many microorganisms e.g., *Escherichia coli* and *Enterobacter aerogenes*, exopolymer synthesis is enhanced under conditions where growth is limited by nitrogen, sulfur or potassium content of the medium. A large number of exopolysaccharide-producing microorganisms utilize carbohydrates as their carbon and energy source and either an ammonium salt or amino acids as their source of nitrogen. Others use amino acids for energy and nitrogen, others use hydrocarbons such as propanol, methane e.g., *Methylococcus capsulatus* (Sutherland, 1982; Boyle and Reade, 1983).

From its anabolic nature, exopolysaccharide production is thermodynamically an uphill task (Harold, 1986; Jarman and Pace, 1984). Therefore, exopolymer-mediated adhesion is not simply a passive phenomenon. Genetically, biosynthsis of extracellular polysaccharides is a complex, but precise process, which requires a multitude of enzymes and regulatory controls (Sutherland, 1977).

A region consisting of a cluster of six genes has been shown to be involved in the biosynthesis of alginic acid by *Pseudomonas aeruginosa* (Darzins *et al*., 1985). The biosynthesis of xanthan gum by *Xanthomonas campestris* also involves a cluster of genes,

prominent among these is the gene encoding for pyruvylation (Harding *et al*., 1987). About eight enzymes may be involved in the biosynthesis of xanthan gum and these include specific transferases, acetylases, ketal transferases, and polymerases (Sutherland, 1977).

Exopolysaccharide Precursors

The change in free energy required to bring a molecule of glucose and a molecule of fructose together to form the disaccharide sucrose is enormous ($^{\Delta}G^{o}$ = 5kcal/mol or 23Kj/mol) and the Keq for this process is mere 1 x 10^{-4}, i.e.
glucose + fructose <---> sucrose + H_20.

Microorganisms and other polysaccharide-synthesizing systems, such as plants, are able to overcome this bottleneck through a sequence of phosphorylative reactions that involve nucleotides and sugar nucleotides. Thus:

ATP + glucose ----> glucose-6-P + ADP

glucose-6-P < ----> glucose-1-P

glucose-1-P + UTP ----> UDP-glucose + PPi

ATP + fructose ----> fructose-6-P + ADP

UDP-glucose + fructose-6-P ---> sucrose-6-P + UDP

PPi+H_20 --->2Pi

sucrose-6-P + H_20 ---> sucrose + Pi.

---

glucose + fructose + 2ATP + UTP ----> sucrose + 2ADP + UDP + 3Pi

$^{\Delta}G^{0}$ = -67 kJ/mol

and Keq 10^{11} (Harold, 1986). I describe this as “going from impossible to easy”.

The discovery of UDP-glucose (Cardini *et al*., 1950) has provided an invaluable step in the understanding of biochemical pathways in the synthesis of surface polysaccharides.

Today, nucleotide sugars are known to be intermediates in the biosynthesis of all heteropolysaccharides, as well as many homopolysaccharides such as glycogen, starch, and cellulose (Markovitz, 1977). The sugar nucleotides are usually carried on the isoprenoid (C_{55}) lipid intermediates as it is in peptidoglycan synthesis (Troy *et al*., 1971; Stoddart, 1984; Sutherland, 1982).

Biofilms

Bill Costerton (John William Costerton, 1934-2012) is recognized as the founding father of the field of biofilms; his multidisciplinary approach to the study of biofilms forged a common way of thinking about the ways in which microorganisms survive and function in the environment as well as in medical, dental, industrial, agricultural, engineering and other contexts (Lappin-Scott *et al*., 2014). A biofilm is a microbial consortium, adherent to a substratum that may be biological or non biological and enclosed in an extracellular polymeric matrix, synthesized by the consortium. Biofilms under industrial conditions may also contain significant amounts of inorganic material (sand, silt, scale, corrosion products) entrapped within the matrix (Sanders and Sturman, 2005). The applications of new techniques in the fields of molecular genetics, biochemical analysis instrumentation and microspcopic analysis, in recent time have helped a great deal in our understanding of the concepts and processes in biofilms (Fuqua *et al*., 1996). For instance, we can study the factors controlling the change from a planklonic mode of growth, the physiological differences between attached and planklonic cells, the detailed structure of biofilms under different environmental conditions, the interspecies and intraspecies interactions, all of which can result to an active and dynamic consortium– an observable structure (James *et al.*, 1995; O'Toole and Kolter, 1998). Biofilms develop as a response to system conditions or external stimuli and cell-produced chemical signals or

internal stimuli. The structure and activity of biofilms can be greatly influenced by both the external and internal stimuli.

Professor John William (Bill) Costerton (1934-2012): considered founding father of the field of Biofilms. At one time he and his wife were missionaries to India with the Episcopal Church.

The City of Microbes

Biofilms show that microbes would rather have a city of their own, a societal living. This is a form of sociomicrobiology. The work of Fuqua *et al.* (1996) led to the discovery of signaling compounds within biofilms. These compounds were first discovered in the marine bacterium *Vibrio fischeri*. The compounds are organic molecules that are produced by cells and secreted into the surrounding fluid. These molecules regulate gene expression when they are present in sufficient or threshold concentration. Biofilm cells have been shown to respond to chemical signals similar to what has been observed with bioluminescence; the phenomenon is termed quorum sensing. The chemical molecules are referred to as quorum sensing molecules, and include acyl homoserine lactones (HSLs). The molecules are also described as auto inducers (McLean *et al.*, 1997), Davies *et al.*, 1998). The molecules induce gene regulation when a sufficient concentration of the signal compound accumulates in close proximity to the cell.

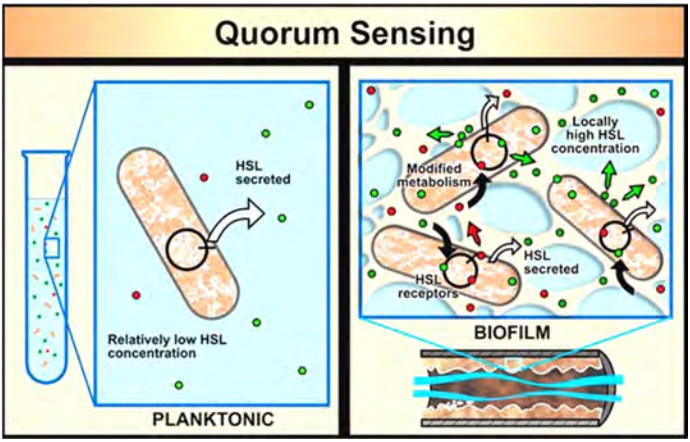


Fig. 23: Quorum Sensing. (Source: MSU biofilm engineering centre: google)

Though planktonic cells secrete chemical signals (HSLs, for homoserine lactones), the low concentration of signal molecules does not change genetic expression. Biofilm cells are held together in dense populations, so the secreted HSLs attain higher concentrations. HSL molecules then re-cross the cell membranes and trigger changes in genetic activity. (Source: MSU-CBE.)

The discovery that simple cells are capable of coordinated behavior has given us an entirely, new appreciation of their survival strategies. There is also good evidence that cell signaling can cause cells of the same variety to form sub-populations that carry out different activities. For example, in the late 1990s an investigation of a biofilm community of the marine bacterium *Pseudoalteromonas* revealed two physiologically distinct subpopulations. In effect there was a cellular division of labor: one group stayed attached to the surface and made nutrient available to the second group, which reproduced and released daughter cells to the surrounding water.
Biofilms reveal a structural arrangement that can lead to transport limitations (Lewandowski *et al*., 1991). Attached biofilm cells show that gene regulation and protein synthesis are altered compared to planktonic cells of the same species; this is almost as comparing

cells of different species (Stoodley *et al*., 2002). Genetic alterations include encoding for genes for synthesis of extracellular polymeric material as well as other changes that are characteristic of the biofilm phenotype (Sauer and Camper, 2011). Biofilms are not a haphazard accumulation of cells on a surface, but rather a fundamentally different condition of microbial growth (Costerton *et al*., 1994). Depending on their composition and associated activities, biofilms may drastically alter the physical as well as the chemical conditions in their immediate vicinity (Costerton *et al*., 1994). These changes can result to several of the common problems encountered with biofilm growth, such as biofouling, plugging, biologically influenced corrosion and petroleum product sourcing (Sanders, 2004). Medically, it is reported that there are an estimated 14,000,000 infections annually that are biofilms associated. About 300 different species of bacteria can inhabit the biofilms that form dental plague. About 1000x doses of antimicrobials are required in the treatment of biofilm infection compared to planktonic cells. About 80% of all infections are influenced by biofilms. The EPS, in biofilms serves as a force field or a shield around the bacteria, thus, protecting the bacteria from radiation and harmful chemicals. Current knowledge from research suggests that there are stages of biofilm formation, from the initial attachment and colonization, through the EPS production, to the structure development to the maturation stage and on to the dissolution or detachment and dispersal.

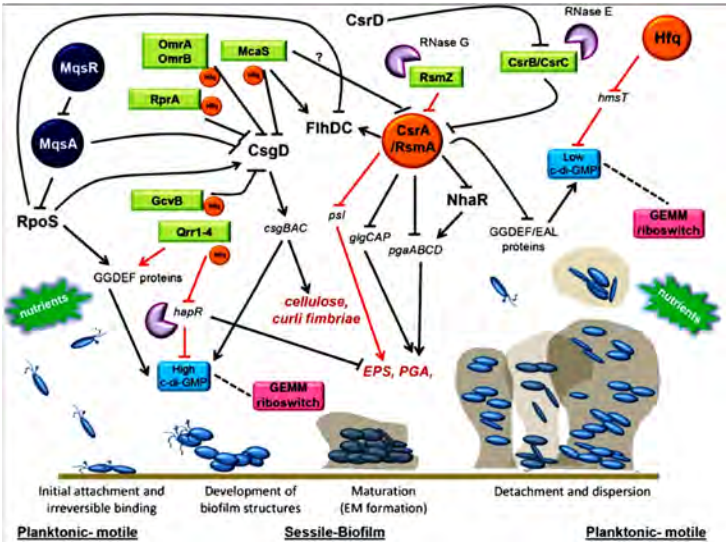


Fig. 24: Biofilm formation (**Source**: google: www.frontiersin.org)

In nature biofilms are typically highly structured multiscpecies microbial communities, encased in a biochemically complex matrix of self-produced extracellular polymeric substances (EPS) (Stoodley *et al*., 2002). In summary, the life of a simple, single-cell microorganism, such as a bacterium, is not so simple after all! And when these microorganisms are found in a biofilm colony their complexity increases tremendously. In order to treat and/or make beneficial use of biofilms, we must continue to identify and exploit the characteristics that are exhibited by microorganisms that form a biofilm. We (Abu *et al*., 1991) saw high levels of silicon in the purified EPS; silicon is a prized high tech metal in the nanotech or electronic and telecoms industry.

This could be a way (biotechnology by biosorption with the PAVES) of enriching silicon from the marine environment. No question, no doubt, the moment of reality in research is to see a problem solved and or jobs or potential of job creation. My doctoral research was funded from a grant of the University of Maryland Sea Grant Program, part of the State of Maryland development of Marine

Research including mariculture such as oyster hatcheries. So we were researching ways of improving oyster harvest by studying the adhesion of *Shewanella colwelliana* to surfaces. The surface energy of nuclepore filters measured at 65 ^{o}F and 65% humidity was 37.5 dynes/cm (Roger Shores, personal communication). Thus, the filters had a hydrophobic surface and would be considered a low energy surface in an aquatic environment and promoted the adhesion of the bacterium with a hydrophobic surface. Logarithmic cells of *S. colwelliana* showed more adhesion than late growth cells. This would also correspond with the synthesis of the EPS which promotes polymer-mediated adhesion. We can say that the predictions of the thermodynamic model of Absolom *et al*. (1983) held true for *S. colwelliana*. The offshoot to this was interest in the exopolymeric substances (EPS) produced by the bacterium which we called PAVES (for polysaccharide adhesive viscous exopolymeric substances). The PAVES were produced into liquid medium as well as on surfaces as biofilm. The US Navy was also interested in the work because of the adhesion studies. Following presentations at the American Society for Microbiology (ASM), a venture capital company, the Lord Corporation got interested, then the big one, Merck got interested because of the rheological properties of the EPS; Merck would have wanted me to relocate to their laboratories. Microbial adhesion is a process and it is of great interest to a wide range of researchers in different disciplines including microbial physiologists, ecologists and geneticists, industrial microbiologists, physical and polymer chemists, and bioengineers. Questions on aging (gerontology) are believed to have answers in adhesiology (I was actually contacted by a Center on Aging following my work). Ophthalmologists, Otolaryngologists (including ear) just as much as dentists are looking for answers to their questions from adhesiologists (I was offered a post Doc position at the Ohio State University, Department of Ophthalmology after they read my thesis). So, I may say I am a process microbiologist, an adhesiologist – actually a nano adhesiologist. Adhesion and aggregation of microbes involve interaction of microbes and some type of surface. Adhesion and aggregation are encountered in certain diseases of humans and animals (cystic fibrosis in lungs of humans), in dental

plaque formation (Anyiam, Ibe, Abu, Braimoh, and Nwaokorie, 2014) in medical treatment procedures such as implants, catheters, contact lenses, in industrial set ups, in microbial community interactions such as syntrophism, in the activity and survival of microbes in natural habitats and in fouling of man-made surfaces and structures in oil production facilities (Abu, 1992; Abu and Owate, 2004; Anichi and Abu, 2012; Immanuel, Abu, and Stanley, 2016). We showed the involvement of sulphate reducing bacteria and iron oxidizing bacteria as being responsible for the hydrogen sulfide and biofilms found in reservoir and production facilities. Working under anaerobic conditions we showed that ceramic materials were less susceptible to biocorrosion arising from biofilm formation.

Creating Jobs: Microbe-Powered jobs: how microbiologists can help build the bioeconomy.

The American Academy of Microbiology (AAM) is an arm of the American Society for Microbiology (ASM), the singular most populous association in the biochemical sciences in the world. This statement underscores the crucial role microbiologists with their microbes can play in creating jobs for building the economy of nations. Microbes were created to create jobs. The sheer number of microbes in terms of the diversity of species is a great gift to humanity. The study and the discipline of microbiology is a great gift to humanity (look at the list of Nobel Prizes related to microbiology; these have a direct implication on the well being of humans). In 1988 a British biotechnologist said this "By the end of this century, industrial processes based on biotechnology will be making a strikingly increased contribution to the wealth of nations. Economic performance may therefore suffer in countries where the opportunities in biotechnology are not pursued, developed and commercially exploited" (Lamorde, 1988). Every part of the microbe is useful (as they say of the palm tree and its fruits and fronds and all). From the flagella – antigens; to the LPS – endotoxin – hapten; to the cellwall-biomaterials for bionano technology; to the CM - research - vesicles - transport studies; to the cytoplasm – DNA, RNA, ribosomes – in vitro studies from where metabolites

both primary and secondary are synthesized including antibiotics; to capsules, EPS slimes – adhesives, bioadsorbents like biofilms, bioemulsifiers and biosurfactants. What of the virus? From the capsids – antigen; to the nucleic acids – recombinant DNA Technology; before the advent of PCR when cloning vectors were the bottleneck, the discovery of cosmids as cloning vectors compared to plasmids was like going from impossible to easy, in terms of the size of genetic material that could be packaged into the cosmid (much larger) than plasmids. According to a colloquium report of the American Academy of Microbiology (AAM) an arm of the American Society for Microbiology (ASM) "microbes can be highly efficient, versatile and sophisticated manufacturing tools, and have potential to form the basis of a vibrant economic sector. In order to take full advantage of the opportunity microbial-based industry can offer, though, educators need to rethink how future microbiologists are trained". The AAM report went on "educating and training the next generation of employees for the rapidly expanding microbe-based industry is critically important to their survival". This is a sustainable development statement i.e., when we are not compromising the survival of future generations. The potential of microbes to create jobs can be expressed in so many scenarios, e.g., the AAM report said "if there is a chemical you need to break down, there is probably a microbe that can do it. If there is a compound you wish to synthesize, a microbe can probably help". This is what I meant by saying the microbe is the second nanotechnologist, after God. Bioindustry products include bioenergy, biofuels, environmentally friendly industrial chemicals such as biosurfactants, and bioenzymes (currently the production of bioenzymes fuels a nearly $4 billion market).

Biopolymer research and marine biotechnology

In the early 1990s biotechnology had become a buzz-word in many developed nations. In Japan, for example, biotechnology had the promise of equaling if not surpassing the impact of electronics. Almost every major corporation in Japan had diversified into biotechnology. Soon it became clear that there was a severe shortage of trained research scientists, and there was also a poor appreciation

of the turnover rates of biotechnology ventures. Corporations all over the world started rethinking their investments in biotechnology; marine biotechnology was the exception. The natural marine resource pool for biotechnological manipulation is just vast. In the United States many states went on to set up Sea Grant programs with the intention of carrying out research in the marine environment; for instance the state of Maryland which borders the Chesapeake Bay, the World's richest estuary, set up the Marine Biotechnology Institute (MBI). I trained at the University of Maryland, College Park Campus, and my research was partly funded from a Grant of the University of Maryland Sea Grant. Having worked on the adhesion of a marine bacterium, my research would pass for marine microbiology and biotechnology, and I had fulfilled my obligation to train in aquatic microbiology from the University of Port Harcourt. So shortly after I had resumed work at Uniport, I was asked to contribute to a World Bank Project on Marine Biotechnology in Developing countries by giving a definition of Marine Biotechnology, this is what I sent in that was published: marine biotechnology is the integration of advances in marine microbiology, marine biochemistry (including cell biology, molecular biology and molecular genetics), marine biology and process engineering, for application in such areas as food and feed industry pharmaceutical industry, environmental pollution and energy, medical diagnostics, fermentation industry and chemical industries (World Bank Project, 1992). My first publication with Uniport address was titled: Marine Biotechnology: A viable and feasible bioindustry for Nigeria and other developing countries (Abu, 1992). It was a big hit, with requests from all over the world. In it I highlighted the feasibility of marine biotech and gave areas of possible research focus, of course biopolymers was among. We understood then, that the real initiation into academia was award of grants; it is the grants that would enable you do research in order to publish so you don't perish. I took a proposal to Shell, only to be told that it is contracts they give and not research grants. My first grant was with the famous International Foundation for Science (IFS), and it was on biopolymers. Getting a grant then was a milestone achievement, it was tedious and tasking, especially with the International granting bodies. The grant brought

you and your institution to world stage. Much of my work highlighted the University of Port Harcourt, Niger Delta and Nigeria. For example one publication from the IFS grant read: The occurrence of exopolymer producing microorganisms in the Port Harcourt marine environment in Nigeria (Abu and Jonathan, 1996.). We introduced the use of marine agar (Zobell's medium, Difco 2216) to the isolation and culturing of marine organisms in Nigeria. We formulated a new growth medium in line with marine microbiology principles. About 1.8% of the isolates screened, using marine agar and our new medium, had potential for exopolymer production, the isolates were putatively identified to genus level using phenetic characteristics as: *Acinetobacter* and *Alcaligenes*. We demonstrated that we could preserve the isolates in soft agar at room temperature and on ceramic beads using Revco at -70^{o}C for more than six months. We were introducing marine biotechnology to our society. Another work from the IFS grant was titled: Bacterial biodiversity of the Port Harcourt marine environment (Chikere, Owolabi, Abu, 2004). In this work we were introducing computer-based microbial biodiversity in the marine environment to the scientific community in Nigeria. The work highlighted the need for marine microbiology principles such as use of appropriate culture media; furthermore, with 51 isolates and 15 biotypes obtained from a shoreline of less than one kilometer, the work revealed the biodiversity and bioresourcefulness of the Port Harcourt marine environment as a pool for a marine biotechnological venture. We can go back now and do functional gene screening with high throughput sequencing (HTS).

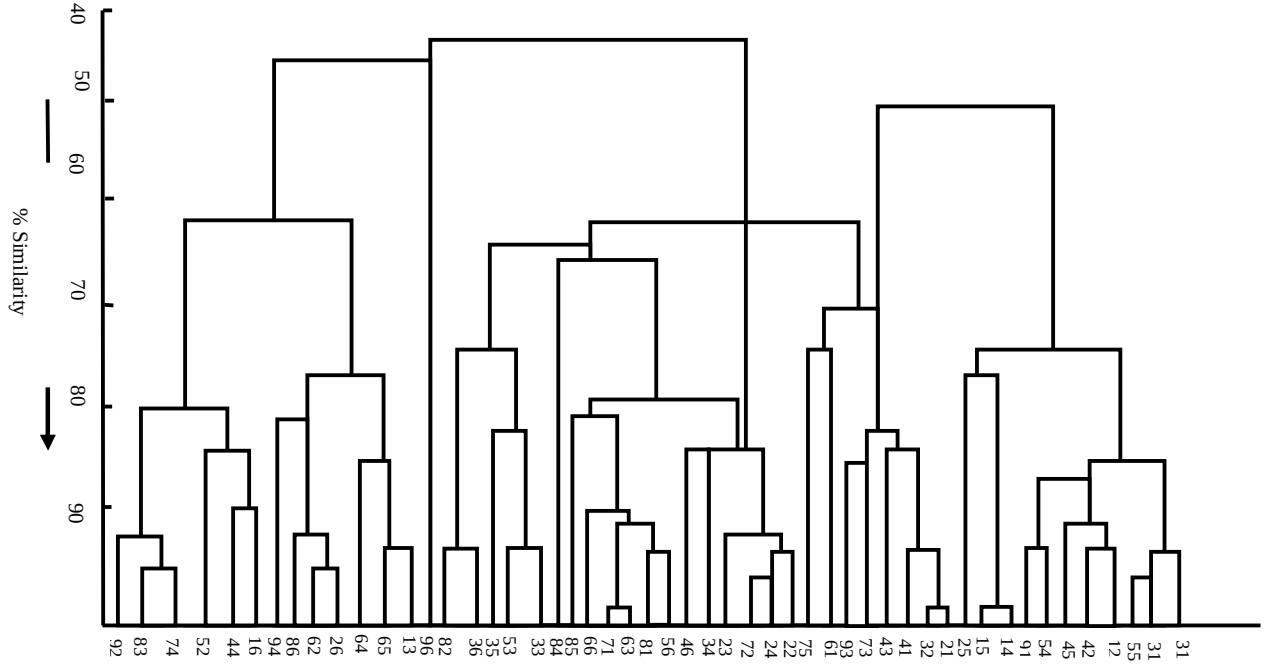


Fig. 25: Cluster analysis of all 51 isolates. Biotypes are defined as having 80 % similarity (**source**: Chikere, Owolabi and Abu, 2004)

Agal biotechnology

With a training in aquatic and marine science, I noticed that biotechnological potentials abound in the area of microalgae. There was not much applied research going in this area in the Niger delta. I had actually observed that the plant science/botany researchers were not looking towards that direction; most green and microalgae are actually eukaryotic microbes. Our first work was an undergraduate project on polymer production by microalgae isolated from the African Regional Aquaculture Center (ARAC) facilities at Aluu, Rivers state. The work was first published as an Abstract (Abu, and Epegu, 1992), at the Ninth International Biotechnology Symposium Subsection: Biotechnology in Developing countries in Crystal City, VA, USA. The work was later published as a journal research article (Abu and Epegu, 2006). Here we formulated growth media using animal wastes such as cow dung and effluent from a fertilizer company. This was meant to be a waste to wealth type of project knowing the added value of microalgal biomass including use as source of fine biochemicals and food supplements. A total of eleven microalgae were isolated. Some of the isolates were found to be intimately associated with a bacterium, a *Pseudomonas* sp. We now used the fertilizer company waste effluent to formulate culture media for the growth of *Chlorella* and *Spirulina*. This was to serve the dual purpose of producing algal biomass and treating the waste effluent from the fertilizer company. The work was funded from the IFS grant and a University of Port Harcourt Senate Development Research Grant and published in an Elsevier journal and it was a hit (Anaga and Abu, 1996). The microalgal work was sustained leading to the second PhD I supervised who is a Professor today; Humphrey K. Ogbonda worked on Protein biosynthesis by a putative *Spirulina platensis* isolated from a flame pit at an oil production facility. The work was published in reputable journals including Elsevier journals and it was a big hit. Through sequential elimination we identified the cardinal points for pH and temperature (Figs. 26, 27) for protein biosynthesis and biomass; we optimized other parameters such as SO_4^{-2}, PO_4^{-3}, HCO_3^{-}, NO_3^{-2}, Cl^{-1} and light intensity; we showed a full complement of all the essential amino acids in the protein hydrolysates (Tables 9 and 10), confirming the suitability of the microalga as a nutriceutical in feed and food for both animals and humans.

Table 9: Amino acid composition of *Spirulina* sp. Isolated from an oil-polluted flame pit grown at different temperatures and pH 9.0

Temp	Amino acid (g/16g N)																			
^{0}C	Ser	Met[b]	Ly	Asp	Try[a,b]	Tyr	Asn	Glu	L1e[b]	Cys[a]	Gln	His[b]	Arg[b]	Leu[b]	Phe	Ala	Thr[b]	Val[b]	Pro	Gly	Total
25	1.10	1.62	0.45	1.32	ND	5.95	0.78	7.94	2.20	ND	6.31	0.99	8.32	1.10	0.93	6.55	0.83	0.78	0.54	2.31	50.00
30	1.91	2.23	0.86	2.66	ND	8.11	1.14	9.26	2.70	ND	711	1,28	0.29	2.84	3.11	8.26	4.18	3.45	1.89	5.51	76.09
35	1.56	1.98	0.79	1.41	ND	6.64	1.86	8.88	2.61	ND	6.83	1.13	9.88	1.79	2.56	7.92	3.76	3.11	1.22	5.33	69.26
40	1.32	1.87	0.64	1.62	ND	2.73	0.83	6.59	1.15	ND	6.62	1.18	7.58	2.10	1.87	7.13	2.09	1.98	0.73	4.72	52.85

temp, Temperature,

[a] Not determined.

[b] Essential amino acid.

(**Source:** Ogbonda, Aminigo and Abu, 2007)

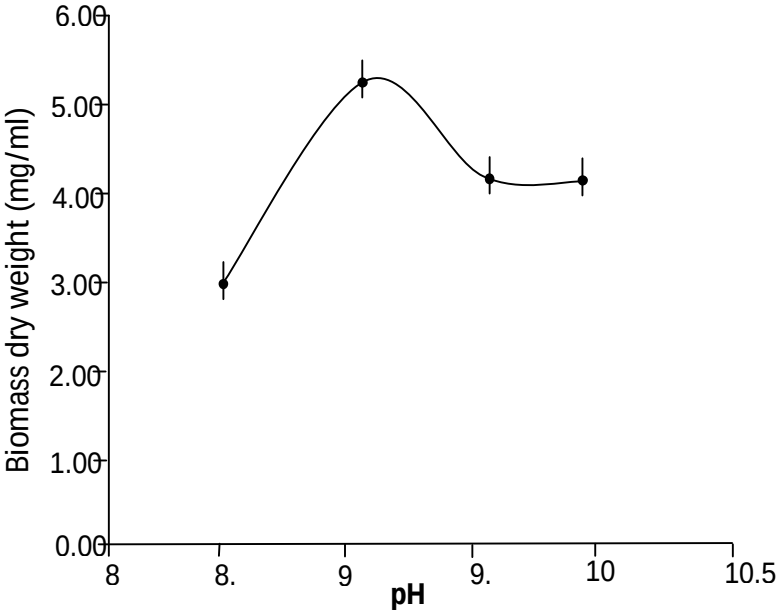


Fig. 26: Effect of pH on biomass production in a *Spirulina* sp. isolated from an oil-polluted flame pit. (**Source:** Ogbonda, Aminigo and Abu, 2007)

Table 10: Amino acid composition of *Spirulina* sp. isolated from an oil-polluted flame pit grown at different pH values and 30^0C

pH	Amino acid (g/16g N)																			
	Ser	Met[b]	Ly	Asp	Try[a,b]	Tyr	Asn	Glu	L1e[b]	Cys[a]	Gln	His[b]	Arg[b]	Leu[b]	Phe	Ala	Thr[b]	Val[b]	Pro	Gly	Total
8.5	2.23	0.99	3.28	1.88	ND	6.11	1.91	6.26	1.82	ND	4.18	0.45	7.13	0.98	0.84	5.39	2.18	3.41	0.88	3.44	53.36
9.0	5.58	1.73	4.13	2.89	ND	7.23	2.66	8.13	2.93	ND	5.23	1.81	9.28	2.11	2.11	7.21	3.44	5.28	2.10	4.89	78.74
9.5	4.64	1.33	3.86	2.27	ND	6.86	2.48	7.90	2.11	ND	5.10	1.22	8.16	1.93	1.66	6.99	3.12	4.11	1.88	4.56	69.22
10.0	3.11	1.10	2.38	1.68	ND	6.42	2.23	6.81	1.91	ND	4.63	0.92	7.94	1.24	1.13	5.86	2.81	3.68	1.12	3.48	58.45

[a] Not determined
[b] Not determined
(**Source:** Ogbonda, Aminigo and Abu, 2007)

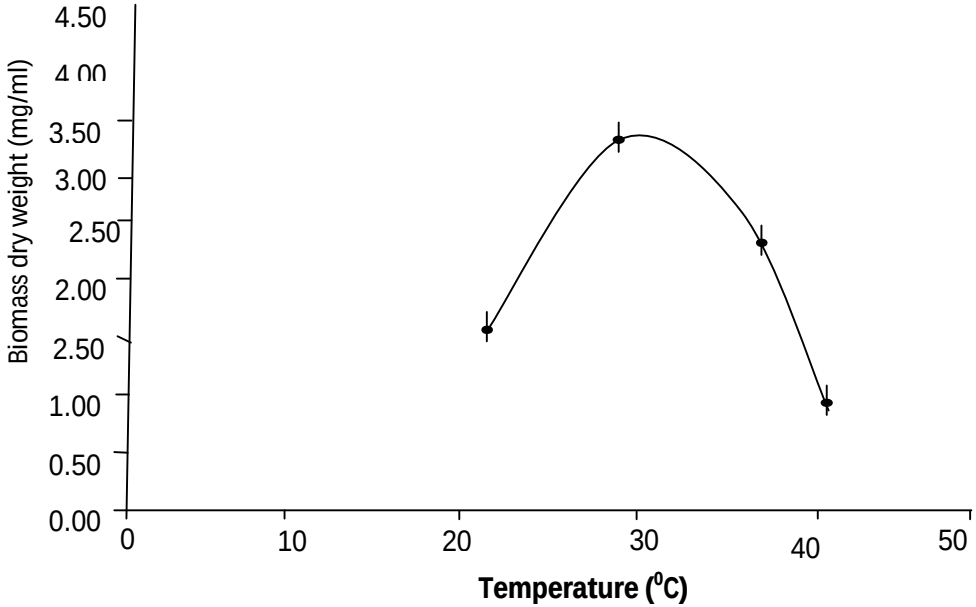


Fig 27: Effect of temperature on biomass production in a *Spirulina* sp. isolated from an oil-polluted pit. (**Source:** Ogbonda, Aminigo and Abu,2007)

Requests made from all over the world included asking for bulk supply of the microalga with price quotes. In the first PhD I co-supervised, who is also a Professor today, I had guided, N.R. Isu on adhesion studies involving her work where she was using cowpea granules to immobilize *Bacillus* cells and spores as starter cultures in the fermentation of African oil bean to ugba. This is food and Industrial biotechnology or white biotechnology. The immobilized cells and spores of *Bacillus* were superior in carrying out fermentation compared to the broth cultures; fermentation time was reduced by half, amino nitrogen was almost double and pH was brought to the preferred physiological range in less than half the time it took for broth cultures. This is the use of microbes in bioadhesion technology to improve the quality of our lives i.e., going from acidic foods to more neutral foods (Isu and Abu, 2000). For microbes to create jobs for us, we would need to do the science first and then we can develop the technology out of the science.

We have continued with algal biotechnology research. In the mid nineties I spent some time at the Institute of Applied Research, Ben Gurion University of the Negev, Beersheva in Israel on, first, a UNESCO Fellowship and then a World Bank-NUC Fellowship all in the Lab of Professor Shoshana (Malis) Arad, whom I had met at the American Society for Microbiology, Annual Conference, some years back, when she visited my presentation. I had set out to do a protoplast fusion between a polymer producing microalga and a high protein yielding species in her lab. In my search for a viscosity reducing system such as polysaccharidases, I ended up extracting a viscosity enhancing substance from a garden soil in Israel; I called it a rheology modulating extract

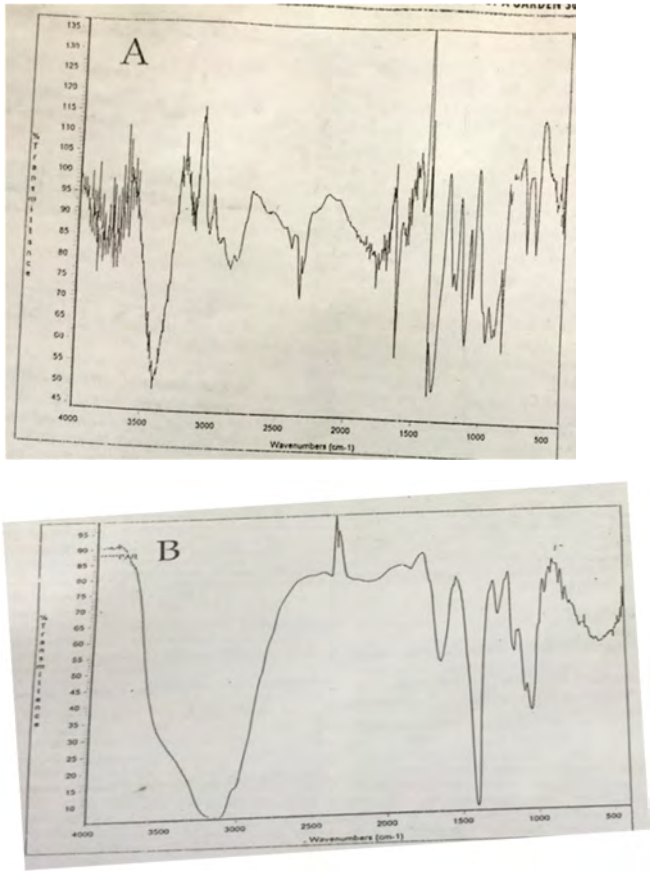


Fig. 28: FTIR spectra of new soil extract (A) and the *P. aerugineum* polysaccharide (B)
(**Source:** Abu, Sivan and Arad, 2004)

Table 11: Different treatments of the *P. aerugineum* polysaccharide and the outcome.

Treatment	SE in dH20 (0.2%)	SE+PSS (2:1 v/v)	PSSin dH20 (0.2%)	▲**n** (%)
Not autoclaved				
Viscosity	0.62(0.074)	37.8(1.2)	31.1(1.1)	23
pH	6.9(0.027)	7.5(0.11)	7.79(0.06)	
Autoclaved*				
Viscosity	0.61	28.7(1.4)	23.6(1.4)	21
pH	6.8	7.8(0.046)	8.1(0.053)	
▼**n** (%)	0	24(2.8)	24(1.9)	

(**Source:** Abu, Sivan and Arad, 2004)

* Autoclaving was done at 11 psi for 35 min, cooled for 24 h.
SE – is soil extract; PSS – is polysaccharide.
Standard deviation (sd) values are in bracket and are for five samples (n=5).

▲**n** is increase in viscosity as a result of the treatment of PSS with SE i.e., going left to right.

▼**n** is drop in viscosity resulting from autoclaving i.e., going top to bottom. Note that there is a drop in both the treated and the untreated PSS after autoclaving, but the resultant viscosity of the treated sample was still higher than the untreated.

The material was putatively identified as sulfated humic substances, possibly a combination of humic and tannic acid. The material showed synergistic effect with the polysaccharide of the red microalga *Porphiridium aerugeneum*. The new soil extract enhanced the thermal stability of the *P. aerugeneum* polysaccharide. This rheological synergism can be exploited in such areas as mucoadhesiion, formulations, thickeners and in flow control systems. Microalgal research has continued in the direction of carbon sequestration and biodiesel. Dr. O.K. Agwa obtained her PhD when she isolated *Chlorella* from the ARAC facilities and

using molecular methods identified it to the species level as *Chlorella vulgaris*, and optimized the conditions for its production of TAGs. The work led to several publications including a book published by Lambert Academic Publishing (Agwa, Ibe, Abu. 2012; Agwa, Abu, Ibe 2014. We (Neboh, Agwa, Abu, 2014.) have used the "scum bug" *Chlorella* to sequester CO_2 from flue gas as a model for mitigating global warming by reducing green house gases; to complete the cycle the *Chlorella* can be manipulated to synthesize large quantities of triacyglycerides (TAG) that can be transesterified into biodiesel. Biodiesel is considered a renewable energy and a sustainable development energy. Estimates (Wigmosta *et al*., 2011.) suggested that, at the prevailing technology, microalgae had the potential to generate 220×10^9 L yr^{-1} of oil; this amounted to 48% of prevailing US petroleum imports for transportation. It was also estimated that to replace 50% of US transport fuels, 1540 Mha (million hectares) of land would be needed for biodiesel from growing corn, 594 Mha from soyabean and just about the equivalent of 43 Mha for biodiesel from microalgae (1 ha is 10,000m2, approximately 1 football field; so you're looking at 43 mln football fields. Now for microbes, that space can be realized through engineering design and it could fit in a much smaller space, but look at the difference in terms of space requirement 13-35 times). Currently both private and corporate organizations are into algal cultivation. As part of its renewable energy drive, the Petroleum Technology Development Fund (PTDF) awarded us a grant in 2015 to research on biodiesel production with *Chlorella vulgaris* using locally formulated growth media. Based on radiant energy estimations for the Port Harcourt area (Kuye and Jagtap, 1992.), we have projected outdoor cultivation as a cost saving strategy in the production of *Chlorella* biomass for extraction of the (TAG) oil. This would be an avenue for job creation.

Table 12: Oil content of some microalgae

Microalga	Oil content (%dry weight)
Botryococcus braunii	25-80
Chlorella protothecoides	23-30
Chlorella vulgaris	14-40
Crypthecodinium cohnii	20
Cylindrotheca sp.	16-37
Dunaliella salina	14-20
Neochloris oleoabundans	35-65
Nitzshia sp.	45-47
Phaeodactylum tricornutum	20-30
Schizochytrium sp.	50-77
Spirulina maxima	4-9
Tetraselmis suecia	15-23

Source: Chisti, 2007; Gouveia and Oliveira, 2009. (Chisti, Y. 2007.

Biodiesel from microalgae. Biotechnology Advances 25: 294-306).
Biofuel from microalgae is considered the third generation range of renewable energy. Who knows about energy more than the microbe? As a matter of fact, one may say that microbes were used by God to design the fossil fuels. This is because the walls of microbes contain large quantities of such molecules as hopanes which are precursors of petroleum. Data from deep reservoirs indicate intimate involvement of bacteria with the reservoir conditions (Magot, 2005).

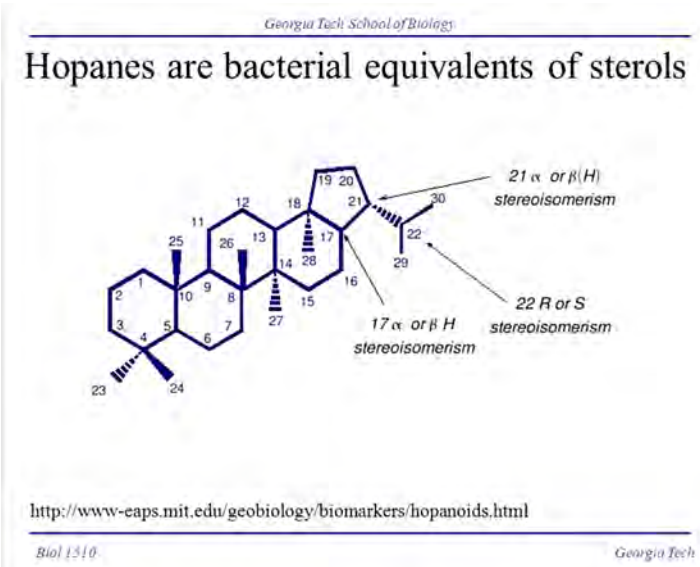


Fig. 29: Hopanoid (Source: http://www-caps.mit.edu/geobiology/biomarkers/hopanoids.html)

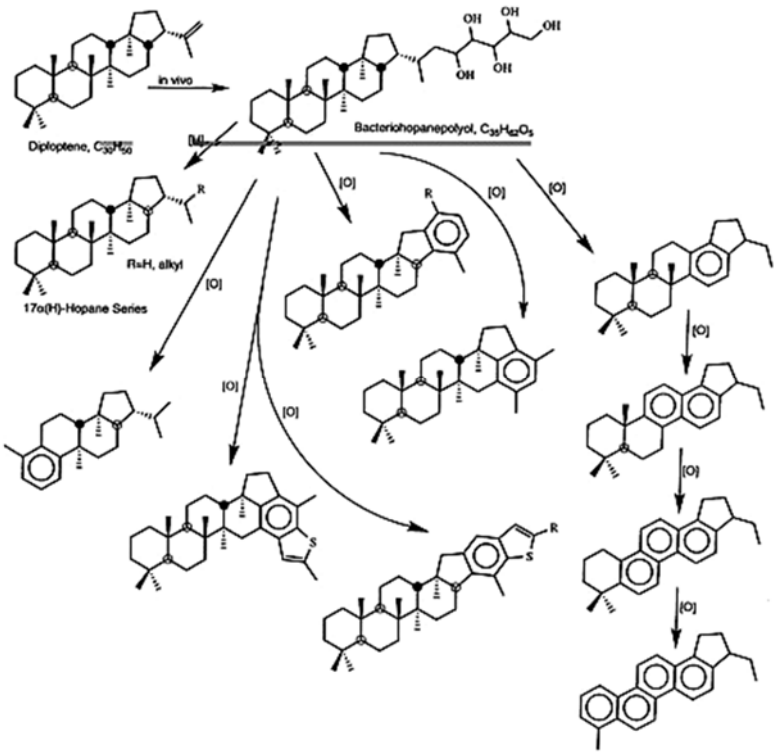


Fig. 30: Microbial biosynthesis of hopanes. (Source: Georgia Tech; http://www-caps.mit.edu/geobiology/biomarkers/hopanoids).

If microbes can synthesize hopanes, they can synthesize petroleum as well. It makes sense.

Environmental quality: Environmental biotechnology: Aquatic Microbiology

Bioremediation: Creating jobs for microbes. With a good background and training in microbial physiology, I recognized that we could apply ecophysiology to tackling our environmental problems including chemical water pollution. In 1990, Genetic Engineering News (GEN), in its Tenth Anniversary edition had projected bioremediation among the top ten biotechnologies that

would be making a great impact on society including commercialization (Gebhart, 1989).

Bioremediation is the pride of microbiologists. Bioremediation represents critical thinking in solving phenomenal problems. Bioremediation is a technology based on the science of biodegradation, which can be discussed as primary, secondary and ultimate biodegradation. In ultimate biodegradation, the organic molecule is mineralized (i.e., organic carbon is turned) to mineral carbon in the form of carbon dioxide (CO_2) and water (H_20) and biomass, plus heat. Biodegradation is one of several natural attenuation forces/processes that are working while we sleep, and they are crucial in maintaining and sustaining a healthy atmosphere of our planet earth i.e., keeping the earth alive. The theoretical framework for bioremediation is Monod kinetics which is based on nutrient limiting conditions, which can be traced back to Liebig's law of the minimum, which was first used to explain crop yield in an agricultural setting. Liebig's law can be stated as: the total yield or biomass of any organism will be determined by the nutrient that is present in the lowest or minimum concentration in relation to the overall requirements of that organism. Bioremediation and Monod kinetics are actually an application of Liebig's law which is one of the ecological theories. One of the practical applications of bioremediation is referred to as land farming; we actually farm microbes but this time on polluted environmental media that could be solid (e.g., soil, sediments), liquid (e.g., water including groundwater) and gaseous such as air. We grow the microbes on unwanted pollutants present in the environmental media. All the microbes want is energy; they will go anywhere to get energy including hell itself, that is why some microbes can grow at up to 121^{o}C (some have been found in association with tubeworms at kilometre depths in the sea and temperatures of around 350^{o}C, these are the hydrothermal vents, the source of energy here is the geothermal fluid; here it is chemosynthesis that takes place and not photosynthesis because it is total darkness). So, in bioremediation as a technology, we optimize the conditions for the microbes to carry out biodegradation of the pollutants. Part of the optimization

includes supply of nutrients and manipulation of the intrinsic parameters such as temperature, pH, moisture and we now supply nutrients for the microbes to grow on the pollutants. The growth of the microbes follows the basic growth equations, but the yield, the biomass follows Monod kinetics. The Monod equation is a kinetic model which describes microbial growth as a functional relationship between the specific growth rate and an essential substrate concentration (Liu, 2006); it can be stated thus:

$$\mu = \frac{\mu_{max} * [S]}{Ks + [S]}$$

Where:

μ = specific growth rate (g/g.h), h^{-1}

μ_{max} = max specific growth rate

[S] = limiting substrate (N, P, O_2) concentration (g/L)

Ks = Monod substrate saturation constant

To obtain high substrate removal, the Ks value of substrate has to be low. In batch systems such as that found in polluted ecosystems, where the pollutant is in excess, it is the limiting nutrient that drives the process. The limiting nutrients can also play the role of bioenergetic analogs where others (e.g., O_2, $NO^{=}_3$, $SO^{=}_4$ and Fe^{2+}) serve as electron acceptors while the pollutant serves as the electron donor; thus, the pollutant is used adventitiously as sole source of carbon for biomass formation and energy. That is how the pollutant is removed from the impacted medium. So, we have two modes of operation in bioremediation namely biostimulation and bioaugmentation. In biostimulation we take advantage of prevailing conditions that are favourable to the indigenous microbes which may be present in low numbers at the site. We stimulate the indigenous microbes through supply of nutrients of N, P, K, Mg and S. These can be supplied in solid or liquid form. We aerate the polluted site (could be by tilling or ploughing or direct release of air) in order to increase oxygen supply from air because most of the indigenous microbes in zones of impacted media that are easily accessible are

aerobes (grow best in the presence of oxygen). With the stimulation, the microbes are vitalized and grow and increase in numbers and in turn now degrade the pollutant in the impacted media. All of this is taking place quite fast. Biostimulation can also be referred to as engineered bioremediation; the manipulation of the environmental conditions is the engineering. In bioaugmentation, we can grow microbes with known capabilities such as ability to degrade a particular contaminant, in the laboratory, and then introduce them to the impacted site. The microbes brought in augment those that are present at the site, hence the term bioaugmentation. It is best practiced where known pollutants are generally difficult to degrade or are toxic to the indigenous population of microbes e.g., degradation of pentachlorophenol (PCP) and a mixture of aromatic hydrocarbons in soil (Crawford and Mohn. 1985; Moller and Ingvorsen. 1993).

We create jobs for ourselves indirectly by creating jobs for microbes.

Take for instance pollution. We are the ones polluting our environment – soil, water, groundwater, sediments and even air. In the US, about 300 million metric tons of wastes including pollutants, industrial wastes and garbage are deposited into the environment. There are burial grounds – dump sites for all types of wastes. This poses a threat to groundwater. In the US there is now the NIMBY (not in my back yard) protest (Cowan and Talaro, 2009). Environmentalists must join forces to create a stronger awareness about the well being of our planet Earth. Nevertheless, microbiologists are rising to the occasion using their techniques with microbes (Okpokwasili, 2006, Odokuma, 2012). I call this job creation by microbes. The on-going efforts to clean up Ogoniland is a huge project that would create thousands of jobs.

Breaking News for University of Port Harcourt

In January 1997, the reputed foremost journal on water quality issues, Water Quality International (WQI) published in their R&D section an article with the caption **"Bioremediation hope for Nigerian oil pollution**". The opening line went like this: "Researchers at the Department of Microbiology, University of Port

Harcourt, Nigeria have been carrying out Laboratory-Based research to evaluate the potential for bioremediation of oil pollution in the Niger Delta by boosting activity of naturally-occurring microbial populations by addition of nutrients". Who were those researchers? The excerpt in WQI had been taken from our (Abu and Ogiji, 1996) publication in an Elsevier Journal. This publication with the word bioremediation was among the first of its kind in the Nigerian literature at that time. WQI made it prophetic, and what greater joy can there be for a researcher than to see his discovery being put into practical use for the benefit of all. The imminent Ogoniland clean up is relying heavily on bioremediation technology. Bioremediation technology is another solution that biology and biochemical biotechnologists have been able to come up with as a solution to address a global problem that is actually linked to the well being of our planet earth. I often talk of remediation including bioremediation as a proactive approach to resource control. Then I say of microbes that, they know how to clean up (do the dishes) after them, for they designed the natural molecules including petroleum hydrocarbons, and now we are using them to clean up our mess. It makes sense, so who says microbes have but just a non intelligent life, looks to me like they sure do have some intelligence, what do you think? We shall be farming microbes in their tons to bioremediate the impacted media of soil, sediment and groundwater impacted with, mainly, petroleum hydrocarbons. There is the potential of creating jobs for thousands of youths in the Niger Delta. These are microbe-powered jobs. I imagine that even microbes would be glad, fulfilling the purpose for which they were created i.e., creating jobs. There are now available commercial remediation services. We know how to treat contaminated soil, water, groundwater, sediments and even polluted air. Genetic engineering is being applied in order to create super bugs, but, in reality, we would intensify efforts to optimize the performance of naturally occurring biodegraders. It is estimated that there are over 3,000 toxic waste sites in the United States alone. Even for bioremediation alone this would mean a lot of jobs. A word of counsel to our younger generation of researchers, be patient and conscientious with your research. That our award winning paper had been rejected as a manuscript in another journal, incidentally of less

reputation nevertheless, I knew we were pushing a frontier research at that time, at least in Nigeria, so w braced up and sent it to another journal. At that time bioremediation was a relatively new technology world-wide, not to talk of Nigeria. One of the earliest reported cases of bioremediation of media impacted with hydrocarbons was in the early 70s. In July, 1971, approximately 510cm^3 of high octane gasoline were found to have seeped from a ruptured underground pipeline into a municipal ground water supply of drinking water. After exhaustive physical removal of the gasoline, it was estimated that about one-half of the gasoline remained in the system. An estimated time for removal of the residual gasoline by flushing and solubilisation was put at 100 years. Then entered bioremediation using nutrient enhancement (nitrate-nitrogen at 13ppm and phosphorus at 1.1ppm) and the indigenous organisms reduced the period to 18 months (Horowitz and Atlas, 1977. Again that looks like going from impossible to easy. Mr Chairman, we shall be deploying a lot of this technology to the restoration and recovery of impacted environmental media in Ogoniland and the Niger Delta. In 1998 Shell carried out their first bioremediation project on the Agbada II Borrow Pit. Mr. Chairman I was hired from the University of Port Harcourt by Shell to supervise that project. This was after they had read our research work on bioremediation. As a matter of fact our research findings at the University of Port Harcourt was one of the few data available at that time that was used to build up a case for the Risk Based Corrective Action (RBCA) approach for the cost effective remediation of impacted environmental media in the Niger Delta. The Remediation Department in SPDC was formed arising from that work and I was a pioneering member of the Department. This is clearly the desired Industry-Academia interfacing all over the world. I had been so excited about our work I went to show it to our ProChancellor then. Another reputable environmental company based in Canada was following the work and even sent a letter of commendation and interest. We (Neboh, Abu and Uyigue, 2016) produced biosurfactants using locally sourced microbes and raw materials (we used palm oil mill effluent POME); that product has been tested on Shell bioremediation projects with success and satisfaction. This is

sequel to earlier studies we (Abu and Eze, 2007) had carried out when we isolated a *Bacillus* sp from the New Calabar River and demonstrated its ability to overproduce a biopolymer with surfactant properties. The remediation of the famous Ejamah Ebubu historic spill site was accomplished majorly using bioremediation technology. That prophetic utterance concerning bioremediation is already a reality and still counting. Actually, what can you do without microbes? Very little.

Plate 1: Agbada II Borrow Pit Impacted with Petroleum Hydrocarbon sludge

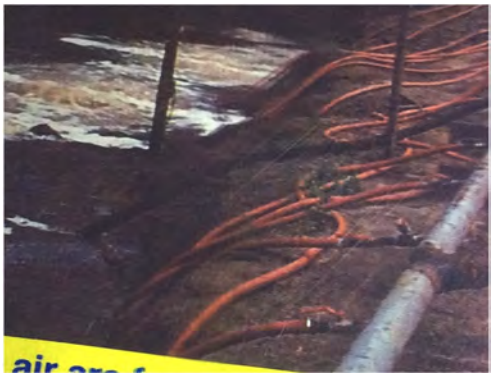


Plate 2: Air and Microbes from the Pit are delivered back into the Pit through a Manifold during bioremediation

Wetlands Research

Wetlands

It is estimated that about 9 million metric tons of oil are released into the world's waters on a yearly basis, close to 90% of this is directly related to human activities including deliberate waste disposal. About 90-99% of the total oil in the environment is associated with sediment, and this can greatly affect the sediment ecosystem. Up to 100,000mg/kg TPH had been entrained into sediments at an oil spill site in the Niger Delta after more than 30 years of the spill. Remediation of water and sediment impacted with non aqueous phase liquids (NAPL) such as petroleum hydrocarbons poses a great challenge of bioavailability. Our work (Abu and Onisiru, 2006) was another pioneering work in the Niger Delta on slow-release nutrient delivery in bioremediation. We were able to make essential nutrients for microbes slow-release both in the field and under laboratory conditions; there was about 10% difference in favour of the treated micro and mesocosms despite heavy rainfall. In another work, using microcosm models we showed how impacted wetland can be restored through monitored and enhanced natural attention under aerobic conditions (Abu and Dike, 2008). We showed, for a Niger Delta setting, that aerobic biodegradation alone accounted for 24.7% removal of hydrocarbon in the model sediments; photooxidation, evaporation and volatilization accounted for 15.6% removal. Forced aeration accounted for 13% removal of hydrocarbon. Aerobic biodegradation, thus, can lead to aerobic bioremediation. For the removal of phenol in sediments, we (Abu and Ofurum, 2006) reported that the combination of photooxidation, evaporation and volatilization collectively accounted for 55.8%, adsorption was responsible for 6% and biodegradation accounted for 38%. This explains the toxic nature of phenol on biological systems. Microbes adapted to high concentrations are potential candidates for treatment of environmental media impacted with phenol (Nwanyanwu and Abu, 2012). In other studies, we (Obiukwu and Abu, 2003; Okpara, Abu and Odey, 2007) isolated phenol degrading bacteria from the Port Harcourt Refinery and carried out kinetic studies on degradation of phenol. In both studies we showed that two isolates, a *Pseudomonas* sp and a *Citrobacter* sp. could grow well with up to

500 mg/L phenol in experimental set ups under laboratory conditions. Using the Monod kinetic model the two isolates had growth kinetic constants μmax = 0.144 h-1, Ks = 46.06 mg/L for *Pseudomonas* sp and μmax = 0.162 h-1 and Ks = 168.44 mg/L for *Citrobacter* sp. For the Niger Delta environment, we (Abu and Atu, 2008) have shown that aerobic biodegradation in a soil ecosystem was at least 30% more effective than anaerobic biodegradation consequently, aerobic bioremediation is about 30 percentage points above anaerobic bioremediation. Aerobic and facultative anaerobes were detected in the set ups.

In another work, Abu and Akomah, (2008) showed that biostimulating sediments (wetland) under anaerobic condition, using terminal electron acceptors such as nitrates had almost the same effect as under aerobic conditions. We recommended in-situ treatments against ex-situ treatments. The current drive in the cleanup of Ogoniland is giving preference to technologies with minimal disruption to the ecosystem both physically and chemically. We have done functional gene (16S rRNA, (ribosomal RNA) and dsrA, (sulphate reductase)) analysis on PAH impacted sediments under anaerobic conditions. High copy numbers of gene fragments were obtained for these functional genes in PAH impacted sediments. The conclusion was that sulphate be used for bioremediation of anoxic sites because sulphate does not pose a health risk like you have with nitrates, and there is less concern about corrosion of metal structures. We made a 2D Map with georeferenced points showing PAH concentrations in the wetland sediments; we referred to this as molecular georeferencing. This would be useful for trend analysis in the wetland sediments.

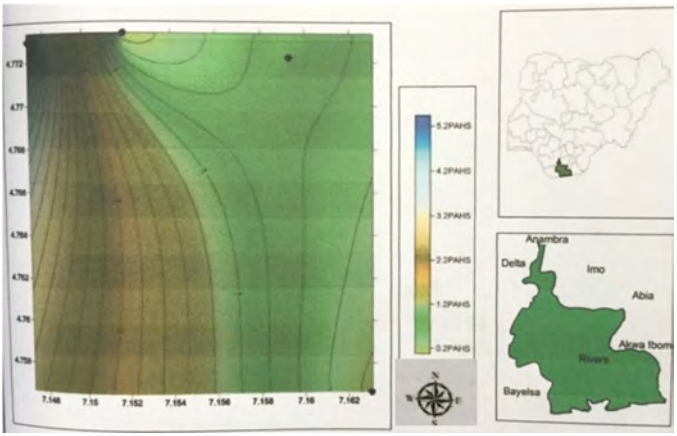


Fig. 31: Molecular georeferencing of polyaromatic hydrocarbons (PAH) in a wetland in the Niger Delta. (**Source**: Akomah and Abu, in press)

In another study, we (Ngerebara and Abu, 2014) using the bench top anaerobic jars carried out sediment augmentation studies under laboratory conditions where we showed that we could augment impacted anoxic sediments that are deficient in active microbial load. Again this is a promotion of in-situ treatment approaches to the restoration of impacted environmental media. We now have an anaerobic chamber (compare with anaerobic jars) where we can simulate these conditions almost to the mesocosm level under laboratory conditions. Using the anaerobic chamber, we (Immanuel, Abu, Stanley, 2016) have shown biocorrosion inhibition on carbon steel, using oils extracted from fungal sporocarps, under laboratory conditions. Values for ΔH_{ads} and ΔS_{ads} were negative suggesting that thermodynamically, the adsorption reactions of the oils were spontaneous at low temperature. The oils showed Langmuir adsorption isotherms on the carbon steel structures. This is part of the building up of research at the University of Port Harcourt.

Plate. 3: A double chamber Anaerobic Box in use at the Central Instruments Laboratory (CIL), Uniport.

Trends in water quality

Water education can be considered to be a conscious effort to enlighten the populace on the properties of water, the uses of water in relation to its properties, the threat to/on water and the risk associated with water. About 50,000 people die daily from water borne diseases. Children under five years suffer a very high mortality rate of about 4 million annually, in developing countries (Warner, 1998, USAID, 1990). The World Health Organization (WHO, 1997) reported 2.3 billion people worldwide with water-related ailments. Water borne diseases include cholera, typhoid fever, shigellosis and viral enteritis. Cholera can express in three modes namely epidemic – here a sudden occurrence is generally observed in regions with limited connection to coastal waters; endemic – when there is a persistent presence of human cholera cases with seasonal recurrent outbreaks and third, the mixed where you have the two. Cholera can kill up to 100,000 persons in a year, and can affect 3-5 million people annually (Zuckerman *et al*., 2007. The cholera cycle shows a link between zooplankton (copepods) and phytoplankton. First there is nutrient enrichment leading to phytoplankton bloom; the zooplankton carrying the pathogen *Vibrio cholerae* feed on the phytoplankton causing the phytoplankton numbers to go down and leading to the zooplankton bloom and thus

inundating the ecosystem with the pathogen; this is cyclical leading to endemicity (Jutla, Khan and Colwell, 2017). Professor Rita R. Colwell is a foremost vibrio ecologist; she pioneered the work that established this cyclical link of cholera outbreaks with the phytoplankton and zooplankton (copepod) blooms. She devised simple but elegant affordable techniques of water purification for low income countries like Bangladesh, where the women could use their sari cloth, folded over many times as filters for local water sources. The folded sari cloth can attain a 20μm mesh net; the plankton have sizes of up 200μm so they can be easily filtered out and since they harbour the cholera germs, that would reduce the incidence of cholera, it has worked. The microbes, how they get us to think, they were created to do that.

Professor Rita R. Colwell
(Distinguished Professor at the University of Maryland,
College Park Campus, MD USA).
She pioneered the research on the involvement of copepods
with cholera outbreaks and the Viable But Non Culturable (VBNC)
phenomenon in gram negative bacteria.

Human activities including industrialization and urbanization impact, to a great extent water resources. While human activities may give an impression of development, they may not stand the test of sustainable development. Of the three environmental media of soil, water and air, water poses arguably the greatest risk because we

interact with it, and depend on it for so much of our daily needs. Impact on soil may eventually get to us, albeit indirectly because we do not consume soil directly. As for air, we are limited by the activities we can carry out that would affect the air on a very large scale. But water, well that's a different thing altogether. We are completely dependent on water for our daily livelihood. We know that 70% of our human body is made of water. About 97% of earth's water is saline, only 3% is non saline. Water is the universal solvent – so things/chemical substances can easily dissolve in water. This is because the design of the water molecule makes it polar (the two hydrogen atoms form covalent bonds with oxygen at an angle, thus, creating a dipole – a polar molecule, this also leads to extensive hydrogen bonds). So, we can use water freely, but at the same time water can very freely get impacted and we may not know. Microbes love water. Because microbes (including viruses) are so small, they can go unnoticed in water just as colourless chemical substances that easily dissolve in water may go unnoticed by an untrained person. Our efforts at providing potable water to our people must include water education which could comprise water sources identification, source protection and conservation, water extraction and storage as well as water quality and uses. The provision of potable water has been a major problem in developing countries (Ashbolt, 2004), including Nigeria (FGN, 2000). The water sector has tended to rely on end-product standards to check water safety. The water sector has, however, made strides towards the risk based methodology/philosophy in addressing water issues. So, now you have risk assessment coupled with risk management as a more effective tool for the control of water safety in drinking water supplies; so, the WHO has the Guidelines for Drinking Water Quality (GDWQ) (Deere *et al*., 2001; Davidson *et al*., 2005; Howard *et al*., 2006). One tool for risk management is the Quantitative Microbial Risk Assessment (QMRA) which provides a tool for estimating the disease-burden from pathogenic microorganisms in water using information about the distribution and occurrence of the pathogen or an appropriate surrogate. The major tenets of QMRA are exposure assessment, dose-response and risk characterization (Haas and Eisenberg, 2001). In order to capture and compare various

outcomes from different pathogens, the use of disability adjusted life years (DALYs) has been recommended in risk assessment (Havelaar and Melse, 2003; WHO, 2004). In considering the outcomes, 1 DALY can be thought of as one lost year of healthy life and the burden of disease can be taken as a measure of the gap between current healthy status and an ideal situation where everyone lives into old age free of disease and disability. We (Abu and Egenonu, 2008) did a trend analysis on the pollution status of the New Calabar River in the Niger Delta region of Southern Nigeria, with respect to antibiotic resistance profiles of bacterial isolates.

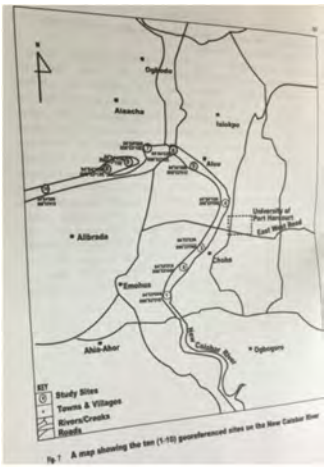


Fig. 32: Georeferenced sites on the New Calabar River (Source: Abu and Egenonu, 2008)

We established georeferenced points using the global positioning system (GPS). This formed the basis of GIS which is concerned with what, when and where? We came up with what we called biogeochemicographic information system (BGIS). A new pattern of antibiotic resistance, not strongly correlated to faecal pollution was detected among the bacterial isolates compared to previous studies on the river (Ogan and Nwiika, 1993) where faecal pollution was a strong factor. We observed high level of antibiotic resistance among bacterial isolates even in sites where there was low level of

pollution. The antibiotic resistance pattern became a focus of risk. Water resource protection education is crucial, because industrialization and urbanization are growing rapidly within the vicinity of the New Calabar River. We published the work with the title – "The Current Pollution Status of the New Calabar River in the Niger Delta Region of Southern Nigeria: A survey of antibiogram profiles of its bacterial isolates". This work became a hit. We ended up publishing a monograph from the work with Lambert Academic Publishers – now goes by name Omniscriptum GmbH, Germany. The Monograph was titled Microbiome antibiograms in pollution monitoring of water resources. It is available online with Amazon. Our (Dick, Abu and Ibe, 2015) work on a community's water sources in the Niger Delta, revealed presence of *Salmonella* sp., *Vibrio* sp and *Escherichia coli* pathogens which also had multiple and high level of antibiotic resistance to such top of the line antibiotics as augumentin, cotrimazole, amoxicillin and tetracycline. There was high plasmid carriage among the isolates even though this did not seem to influence the drug resistance. Enterotoxigenicity testing using ligated ileal loops fluid accumulation revealed only a questionable positive, but this is suspected to be influenced by environmental factors (Abu and Egenonu, 2010). The water sources were hand-dug wells and river water. We (Dick and Abu, 2015) further assessed the sanitary and microbial risk associated with the hand-dug wells in the rural community. The sanitary risk assessment of the hand-dug wells revealed very high risk (8-10) based on physical protection of the water point; distance to sources of contamination and open defecation (WHO, 2004). The quantitative microbial risk assessment (QMRA) of the hand-dug wells for *Escherichia coli* (2.21E03-9.69E03), *Vibrio* sp (1.53E09 – 3.14E09) and *Salmonella* sp. (1.59E09 – 2.83E09) far exceeded the risk level of 1.0E-06 (10^{-6}) suggested by the World Health Organisation and indicate a potential health hazard to the consumers. The study showed how sanitary inspection and QMRA can be used in areas with limited data, and that the outcome can provide valuable information for the management of water supplies.

Table 13: Mean Count of *Escherichia coli*, *Vibrio* sp. And *Salmonella* sp. (cfu/ml)

Organism	Station						
	1	2	3	4	5	6	7
Escherichia coli,	$7.1x10^1$	$4.8 x10^1$	$4.91 x10^1$	$3.4 x10^1$	$5.3 x10^1$	$6.16 x10^2$	$3.16 x10^2$
Vibrio sp.	$22. x10^1$	$2.8 x10^1$	$3.2 x10^1$	$2.3 x10^1$	$2.2 x10^1$	$1.5 x10^1$	$1.6 x10^1$
Salmonella sp.	$1.47 x10^2$	$1.06 x10^2$	$9.3 x10^1$	$9.6 x10^1$	$1.25 x10^2$	$8.9 x10^1$	$8.2 x10^1$

Table 14: Sanitary Risk Assessment

RISK		**ST. 1**	**ST. 2**	**ST. 3**	**ST. 4**	**ST. 5**	**ST. 6**	**ST. 7**
1.	Is there a latrine within 10m of the well? Y/N	Y	N	N	N	N	N	N
2.	Is there any other source of pollution within 10m of well? Y/N (e.g. animal breeding, cultivation, open defecation, footpath, waste dump)	Y	Y	Y	Y	Y	Y	Y
3.	Are the ropes and buckets exposed to contamination? Y/N	Y	N	Y	Y	Y	N	Y
4.	Is the height of the headwall (parapet) around the well absent? Y/N	Y	Y	Y	Y	Y	Y	Y
5.	Is the apron (cement floor) around the well less than 1m wide? Y/N	Y	Y	Y	Y	Y	Y	Y
6.	Is there poor drainage, allowing stagnant water within 2m of the well? Y/N	Y	Y	Y	Y	Y	Y	Y
7.	Is the drainage channel absent, cracked or broke? Y/N	Y	Y	Y	Y	Y	Y	Y
8.	Are the walls (well-lining/seal) absent? Y/N	Y	Y	Y	Y	Y	Y	Y
9.	Is the fence around the well absent? Y/N	Y	Y	Y	Y	Y	Y	Y
10.	Is the well-cover damage or open? Y/N	Y	Y	Y	Y	Y	Y	Y
Total Score of Risks …/10 (No. OF "YES" in the observations made)		10	8	9	9	9	9	9

(Source: Dick and Abu, 2015). Y: YES N: NO Risk score: 9-10 = Very high; 6-8 = High; 3-5 = Medium; 0-3 = Low

This is a very simple and direct approach to risk communication which is a major task in environmental quality management with regards to water resources.

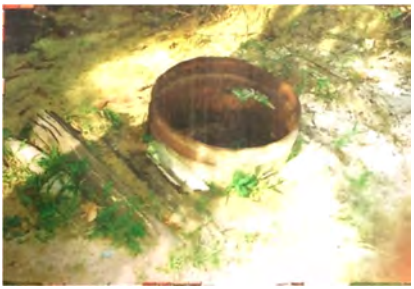


Plate 4a: A rural community hand-dug water well

Plate 4b: A rural community artisan water well

Plate 4c: A rural community hand-dug water well

Table 15: Disease burden for Pathogens

Pathogen	Outcomes	Disease burden per 100 symptomatic cases	Disease burden (DALY)
Escherichia coli	Watery diarrhea	1000 x 53% (watery diarrhoea) x 0.067 x 0.009	= 0.3
	Blood diarrhea	1000 x 47% (bloody diarrhoea) x 0.39 x 0.015	= 2.8
	Death from diarrhoea only	1000 x 0.7% (death) x 49.5	= 346.5
	Total diarrhoea only		= 349.6
Vibrio sp.	Mild diarrhea	1000 x 80% (mild diarrhoea) x 0.067 x 0.008	= 0.43
	Severe diarrhea	1000 x 20% (severe diarrhoea) x 0.23 x 0.013	= 0.59
	Death from diarrhea	1000 x 4.1% x 49.5	= 2029.5
	Death from diarrhea		= 2030.52
Salmonella sp.	Gastroenteritis	1000 x 64% x 0.23 x 0.013	= 1.93
	Death from gastroenteritis	1000 x 0.76% x 51	= 376.2
	Total from gastroenteritis		= 378.12
	Typhoid fever	1000 x 35.5% x 0.23 x 0.013	= 1.06
	Death from typhoid fever	1000 x 0.26% x 51	= 128.7
	Total from typhoid fever		= 129.76
	Total (gastroenteritis and typhoid fever)		= 507.89

(**Source:** Dick and Abu, 2015)

We (Mbah, Abu and Ibe, 2017) used a metagenomes-based approach with high throughput sequencing (HTS) on Illumina Miseq to investigate the impact of anthropogenic activities and run-offs on the microbial quality of a freshwater ecosystem in the Yenagoa

metropolis in Bayelsa state, Nigeria. The V_1-V_3 hyper variable regions of the 16S rRNA gene revealed high bacterial diversity, β-proteobacteria were dominant in water supplies from wells and river while $\propto -$proteobacteria were dominant in environmental soil supplies. The study revealed metagenomic indices of public health importance such as antibiotic resistance genes (ARG) and viral sequences. Unprotected water resources could be impacted by runoff discharges and anthropogenic activity in a metropolitan setting. There was an abundance of unclassified bacterial sequences, which may give rise to novel bacteria with unexploited microbial genetic diversity within the freshwater ecosystem. This work is an improvement on our earlier (Abu and Egenonu, 2008, Dick and Abu, 2014) work on water resources in the Niger Delta. With microbial metagenomic analysis we need to build more robust conceptual models that would lead to more stringent quantitative microbial risk assessment (QMRA) that would aid in establishing water quality for domestic and other uses in the Niger Delta. Again with metagenomic analysis we overcome the challenge of the viable but non culturable (VBNC) pathogens, a phenomenon that has been extensively studied in Professor Rita Colwell's Lab in connection with water quality. In this way we would be conscious to protect, preserve and conserve our precious water sources and resources in the Niger Delta.

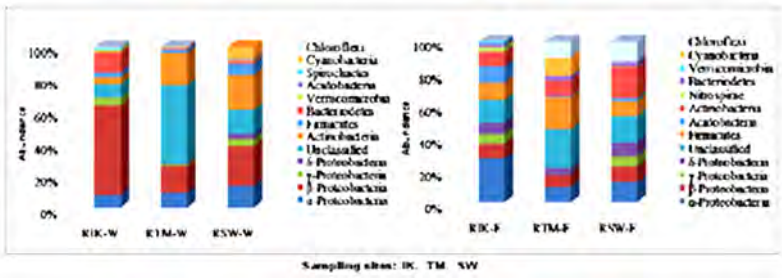


Fig. 33: Distribution of the most abundant bacterial communities at Phylum level in (a) water (W) and (b) Environmental soils (E) collected at communities' points of use. Notice the great diversity even at the Phylum level. (**Source:** Mbah, Abu and Ibe, 2017).

Creating Jobs

Ecological Theories: Describe, Explain, Predict and Control

For us to be able to use microbes to create jobs, we must be able to describe, explain, predict and control microbial processes. This is best exemplified in ecological theories. A theory is a hierarchical framework that contains clearly formulated postulates, based on a minimal set of assumptions, from which a set of predictions can be logically made. Theory is inherently deductive. New pieces of data stimulate a theory; and new theories refine, expand and replace old theories, leading to correcting of flaws, explaining and predicting phenomena in the purview in which they are operative. Ecological theories deal with the way organisms occupy and thrive in their habitats. Thus ecological theories can be empirical or explanatory. These theories give the impetus to describe, explain, predict and control microbes. The "control" component is exemplified in biotechnology. The science writer, Bernard Dixon captured it quite graphically – "the winning biotechnology teams I suppose, would be those that have a microbial ecologist on board". That is it, all the great diversity of microbes that we see (and as they say, we ain't seen nothing yet) is God's way of creating jobs for us through microbes. They can take part or carry out biogeochemical cycling or movement of matter in our universe. The matter includes elements such as carbon nitrogen, oxygen, sulphur and phosphorus, others are water, etc. These elements being cycled through the earth system atmosphere, hydrosphere, biosphere and lithosphere, are viewed as "the currency of the earth". These same elements are also the currency of all ecosystems on earth. They are moved from one organism to another and to the environment through ecoforces. The earth is ecophysiologicallly considered a closed system, so matter on earth is cycled. Some ecological theories include Liebig's Law of the minimum, Gaia hypothesis, the R/K selection theory, Shelford's law of tolerance etc. To create jobs, these laws have to be converted into technologies. Every technology has the science behind it. Biotechnology derives from scientific principles in Biology, Chemistry, Engineering so that you could have different spheres, and these spheres can be broken into subdisciplines referred to a Red, White, Green and Blue biotechnology.

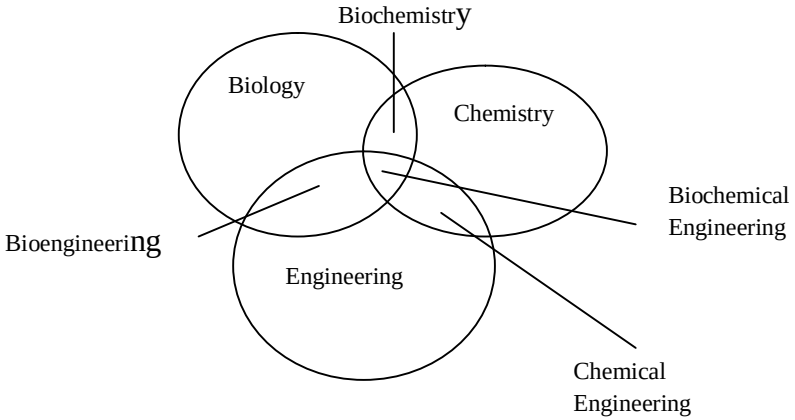


Fig. 34: The concept of biotechnology: Describe, Explain, Predict and Control

Through these, cellular and biomolecular processes are harnessed to develop technologies and products that help improve our lives and our planet- development. To maximize job creation using microbes, we need a good dose of biochemical engineers – look at the centre of our diagram. This calls also for interdisciplinary research. That brings us to another level of critical thinking in sustainable development - ecological economics. This is also called ecolonomy or bio economics. This is both a transdisciplinary and interdisciplinary endeavour of academic research that addresses the inter-dependence and convolution of human econometrics and natural ecosystems at inter-temporal and spatial levels. Ecological economics incorporates the principles of ecology into economics (Cunningham and Cunningham, 2008). Borrowing a leaf from microbes, ecological economics accounts for everything needed to come up with a product. It is a stoichiometric operation - ecostoichiometry. The environment is involved in the economy of the society.

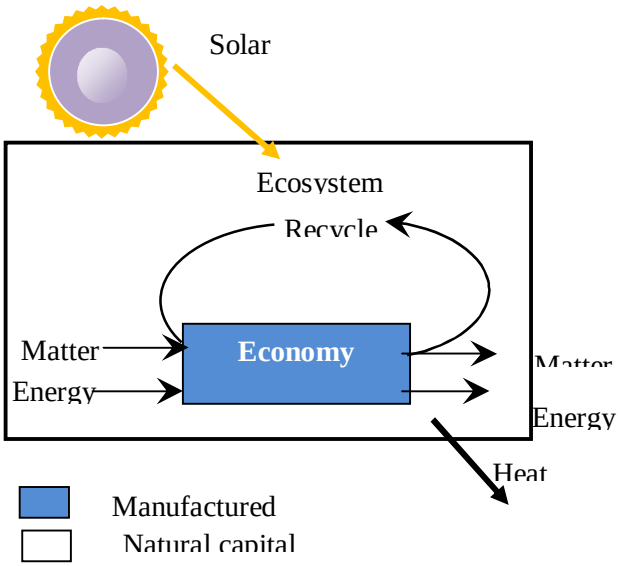


Fig. 35: Concept of natural resource economics (modified after Cunningham and Cunningham, 2008)

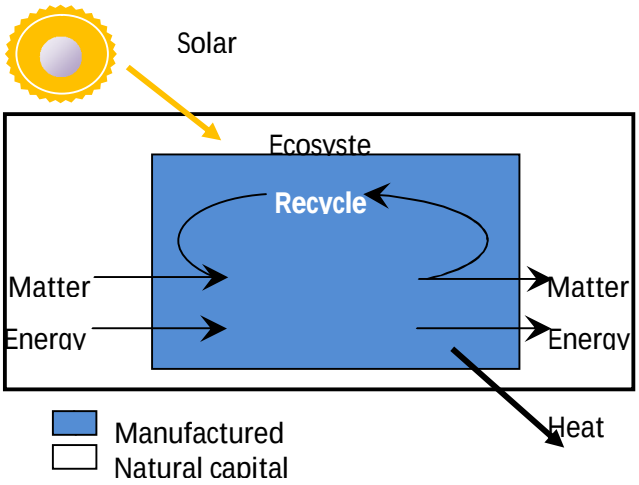


Fig. 35: Concept of ecological economics (modified after Cunningham and Cunningham, 2008)

Neoclassical Economics

This approach of economics relates supply and demand to an individual's rationality and his ability to maximize utility or profit. Resources can be classified as Natural Resource Capital and Manufactured Capital. In neoclassical economics, natural resource capital is deemed inexhaustible, such as you can fell a whole big tree just to make a wooden desk. Ecological economics states the reverse, that is, manufactured capital is what is considered inexhaustible. The capacity to come up with new products from the available natural resources is what should be tasked and considered limitless or inexhaustible. This is what we see in the microbe. The microbe is resourceful and has high capacity for manufactured capital. How can this be better? These are components of critical thinking. "Go to the microbe, ye lazy head, consider its processes and be wise, and not wasteful or prodigal or profligate". So ASM (AAM) states how microbiologists can help build the bioeconomy and it is by microbe – powered jobs (AAM of ASM, Feb. 2013 (Feb(Dallas, Texas, USA) (view: Microbe – Powered Jobs Infographic).

Creating jobs: See Moni (Money)!!

I was teaching my students PCR in a molecular biology course. I told them how the PCR is a bio/chemical, molecular biological, enzymatic *in vitro* procedure for amplifying a target sequence of DNA under laboratory conditions. Of course, I told them how the thermostable enzyme taq DNA polymerase is an ecophysiological, biotechnological product, that was purified from *Thermus aquaticus*, a heat loving organism (thermophile) which Professor Thomas D. Brock of University of Wisconsin, Madison, USA had isolated from hot springs at the Yellowstone National Park, USA. Then I now said we could apply the basic growth equations to this amplification thus: $N_t = N_o.2^n$

Where N_t is the total number of fragments after a given time t, N_o is the initial number of fragments, n is the number of cycles (equivalent of generations) and 2 because each double stranded

fragment would be duplicated (Rank Xeroxed like duplicating machine). A cycle may take less than five minutes. If 30 cycles had been performed, what would be the total number of double stranded DNA fragments that we would have if we started with just 1? After fiddling with the surds of exponents, they arrived at a staggering figure; then one of the students exclaimed – see Moni! (see money!). Apparently because of the use of N (N_t, N_o), to him this was Naira (₦ , Nigerian currency). Actually, it is money (moni) in form of jobs; sequencing of thousands of genomes is lots of money, and so the microbe *Thermus aquaticus* has created jobs and wealth for that matter; it makes sense !! Chairman Sir, right on the south bank of the New Calabar/Choba river, at NDU a molecular biology lab has become the rallying point for many of our graduate and even some undergraduate students who would be bold to do a decent, confidently publishable research in contemporary science which is often multidisciplinary. I imagine a situation where a number of researchers can pool their research grants together and set up a state-of-the art molecular biology lab. But I also would like to request Mr. Chairman to add a molecular biology unit to the Central Instruments Laboratory (CIL) as they move to their "most beautiful building on campus" facility. I wish I could take a vow that that lab would become fully operational in less than one year from now.

Future trends in using microbes in job creation

The lecture has as part of its theme job creation by microbes. We have accepted the key role of microbes in maintaining and sustaining our planet Earth. These invisible "creatures" have so much locked up in them. Please note that we, in everyday life, refer to these microbes as "critters" and that actually is the euphemism for creatures. Yes, they are creatures, they were created, they were created to create jobs. Work is the number one duty of creatures.

Biotechnology: Mining Biodiversity

The word biotechnology was coined by the Hungarian engineer Karoly Erecky, in 1919.

The UN defined biotechnology as – "any technological application that uses biological systems, living organisms, or that uses biological systems, living organisms, or derivatives thereof, to make or modify products or processes for specific use" (UN convention on Biological Diversity. Article 2). Biotechnology is interdisciplinary, so, depending on the tools and applications, there would often be overlaps with the related fields of bioengineering, biomedical/biochemical engineering and biomanufacturing, molecular/genetic engineering.

Terms used to identify some branches of biotechnology
Biotechnology has use and applications in several areas:
Bioinformatics:

This is an interdisciplinary area which addresses biological problems using computational techniques; it makes the rapid organization as well as analysis of biological data possible. Bioinformatics is also referred to as computational biology, which is defined as "conceptualizing biology in terms of molecules and then applying informatics techniques to understand and organize information associated with these molecules on a large scale. Bioinformatics is crucial in various areas, such as functional genomics, structural genomics, and proteomics; bioinformatics is a key component in biotechnology.

Red biotechnology
This is also referred to as medial biotechnology. This is applicable in such areas as monoclonal antibody production, stem cell technology to regenerate damaged tissues and possibly organs etc. the microbial component would be in processes such as getting microbes to produce new drugs like insulin, design and manufacture of pharmaceutical products like antibiotics, vaccines through genetic engineering procedures and principles.

White biotechnology (Industrial Biotechnology)
This is also referred to as commodity biotechnology; it refers to the use of living cells and/or their enzymes that are more easily degradable, require less energy, create less waste during production

and sometimes perform better than products created using traditional methods. Marine biotechnology products are a major contribution in commodity biotechnology (Abu, 1992).

Blue Biotechnology (environmental biotechnology): This encompasses processes in marine and aquatic environments and organisms that thrive here. These organisms are used for purposes of increasing seafood supplies and safety, controlling the reproduction of noxious water borne organisms and developing new drugs. Microbial bioremediation of impacted or polluted environmental media is a multimillion dollar industry.

Green Biotechnology (Plant biotechnology) applies to agriculture and involves such processes as the development of pest resistant grains or the accelerated evolution of disease resistant animals. It also involves the use of genetically altered plants or animals to produce more environmentally friendly farming solutions as alternatives to traditional agriculture, horticulture and animal breeding processes. Science has called for the advancement of small-scale agroecological farming systems and technology in order to achieve food security and the realization of the millennium developmental goals. Environmental biotechnology has been shown to play a significant role in agroecology in the form of zero waste agriculture and most significantly through the operation of over 15 million biogas digests worldwide. Phytoremediation is a fast developing technology in environmental quality management. Heavy metal pollution is currently best managed using phytoremediation.

Teaching at Uniport and job creation

I have taught Basic Biology when I was freshly hired and it was fun. I have taught Introductory Microbiology and that too has been fun, that was where I taught my primary school definition of sound, when I was teaching how sonication or sonic vibrations lead to cavitation as a means of disrupting microbial cells. And I have taught microbial systematics, biodiversity.

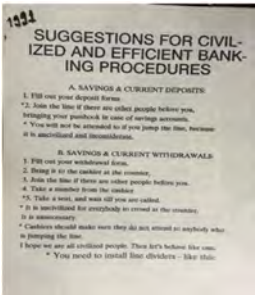
I have taught Microbial Physiology and Microbial Ecology to senior undergraduate students part of the fun here was teaching protein synthesis and having to line up students and give them names of amino acids and using their arms to illustrate peptide bond formation – transpeptidization. I have taught Introductory Biotechnology where I made the senior majors students to know what power there is in the Monod equation; then I made them to appreciate the biodiversity of the Port Harcourt marine environment as a veritable biotechnological resource base.

So, whereas we have known and used MCB as course code, I say it stands for Microbial Chemistry and Biotechnology (MCB). One of the things this should do is make the students feel comfortable and friendly with chemistry and biochemistry as microbiology majors. The treatment of Monod kinetics and growth dynamics is to help reduce the unease with quantitative skills. At the graduate level, I have taught Research methods, Fermentation Technology, Environmental microbiology and biotechnology, Biodeterioration of industry materials, Petroleum microbiology and Bioremediation.
I taught the first Principles of Biochemical Engineering in the Department of Chemical Engineering. I had an ITP Fellowship that took me to the GBF (Institute of Biotechnology) in Braunsweig, Germany where I was certified as a Biotechnologist. On return I introduced it to Professor A. Kuye. The following year he secured the fellowship and went to the GBF. When he came back, he was convinced that they needed a Biochemical Engineering Course in the Department of Chemical Engineering. Later on Late Professor C. Okpara with whom I published too, was hired as biochemical engineer. Point here is the beauty and benefits of interdisciplinary research.

I have endeavoured to promote Uniport and to impact the society. At Choba Park we planted hedges and created walkways to prevent lawn crossing – that was part of community service.

Teaching and Community Service

Now, look at this cartoon; it was created by Gabriel Agu (now Professor Agu) and I to introduce sanity and civilized behaviour in our public places, especially the banks. We actually went to discuss with the managers on how their banking halls could be more customer friendly. We discussed with them how they could computerize their operations. All this finally happened, but they could have happened long time ago too.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SUGGESTIONS FOR CIVILIZED AND EFFICIENT BANKING PROCEDURES

A. SAVINGS & CURRENT DEPOSITS:

1. Fill out your deposit forms.
*2. Join the line if there are other people before you, bringing your passbook in case of savings accounts.
* You will not be attended to if you jump the line, because it is uncivilized and inconsiderate.

B. SAVINGS & CURRENT WITHDRAWALS:

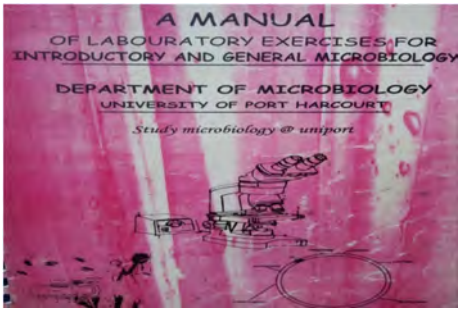
1. Fill out your withdrawal form.
2. Bring it to the cashier at the counter.
3. Join the line if there are other people before you.
4. Take a number from the cashier
*5. Take a seat, and wait till you are called.
* It is uncivilized for everybody in crowd at the counter, it is unnecessary.
* Cashiers should make sure they do not attend to anybody who is jumping the line.

I hope we are all civilized people. Then let's behave like one.

* You need to install line dividers – like this:

Back to Teaching

I have been passionate about microbiology. I felt that microbiology is a "show and tell"; there is no way the students would enjoy it without well organized laboratory exercises. As part my training, I had been deeply involved with undergraduate program during my grad school days at the University of Maryland, College Park Campus. So, here at Uniport, I pioneered the production of a well coordinated lab manual: "Laboratory Exercises for Introductory and General Microbiology". It is produced and used by the Department as a means of internally generated revenue/funds. It is so gratifying seeing neophyte aficionados trying to get into the



ways of microbiology with their safranin colored Lab Manual. I heard a senior microbiology majors student telling another student (junior majors), make sure you do the "Unknown" very well, because it will give you all the techniques you need for your project, in fact your project would be a walk over. "Unknown" is one of the exercises in the lab manual where each student is handed a bacterial culture with an undisclosed identity, the student should independently carry out some microbiological tests and come up with the correct identity of the bacterium, they are to write it up in form of a standard scientific paper where you have Title, Abstract, Introduction, Materials and Methods, Results and Discussion and References. With this done at the 200 level, the students have a sound foundation.

EPILOGUE, RECOMMENDATION AND ACTION POINTS

So where is life?

Is life a force?

Subatomic particles are held together by forces. Atoms are held together by forces to form molecules. Is that life? Now, we have ionic bonds, covalent bonds, hydrogen bonds etc – is that life? The microbes can break all the bonds and release the elements back to the atmosphere – mineralization. This job is given to microbes. It is obvious that life can only come to living (life forms) from an external source. In all our search as human beings, out of curiosity and out of desperation, we have not been able to find an answer. Only God is the source of life (Gen. 2:7). And that life is in Jesus. Now this is the order:- First God, then man, and after that microbes. This is because microbes are the smallest measurable living entity. We can see that life is not the assemblage of atoms and molecules. We can see the elegance of these and we must agree that there is a designer, the subatomic particles, the atoms, the molecules could not design

themselves. The forces, be they hydrophobic interactions, van der Waals, gravity, electromagnetic or the ecoforces could only be designed by someone.

So where is life? The Grand Unified Theorem

He (Professor Gabriel A. Oyibo) calls it the theory of "everything", and he says all forces both strong and weak in the whole universe are controlled in the theorem - God Almighty Grand Unified Theorem (GAGUT) statement thus: $G_{ij,j} = 0$

This is an equation of state (right? Math buffs)

Is this what would explain – "everything" is everywhere, nature only selects? Is that "selection" not gene expression? So Professor Gabriel A. Oyibo said the whole universe is a field filled with forces.

I agree with Professor Oyibo to the extent that God created all things (everything). We may differ when he describes God with an equation: $G_{ij}, j = 0$,

Where G_{ij}, is God – the Force unifying all (www.gagut.destee.com). God cannot be reduced to an equation!! God is not a force. God is a personality. God Almighty created and controls the forces. So, even the invisible forces were created and are under control. Microbes such as bacteria (not to talk of viruses) would have run us over. All the laws of ecology are part of the grand design. Both bacteria and viruses have growth cycles. This is a design. They obey the laws of ecology. Our problem is – who owns the laws of ecology? Our idea of Nature is the problem (Ogbonda, 2016). We want to accept nature, but not God. That is why scientists, when the boson was discovered, declared – "now we don't need God, we can figure out exactly how 'everything' began". But our minds may not understand what nothing is. We are finite. Only God is infinite. He is the only one who knows how "everything" began. My position is that He, God started what we all know and see today – He created "everything". This understanding should be taught in Theoretical Biology courses. Microbes were not created to create man (i.e.

evolve into man), rather they were created to create jobs for mankind!!

Recommendation and Action Points

According to a colloquium report of the American Academy of Microbiology (AAM) an arm of the American Society for Microbiology (ASM) "microbes can be highly efficient, versatile and sophisticated manufacturing tools, and have potential to form the basis of a vibrant economic sector. In order to take full advantage of the opportunity microbial-based industry can offer, though, educators need to rethink how future microbiologists are trained". The AAM report went on "educating and training the next generation of employees for the rapidly expanding microbe-based industry is critically important to their survival". This is a sustainable development statement i.e., when we are not compromising the survival of future generations. The potential of microbes to create jobs can be expressed in so many scenarios, e.g., the AAM report said "if there is a chemical you need to break down, there is probably a microbe that can do it. If there is a compound you wish to synthesize, a microbe can probably help". This is what I meant by saying the microbe is the second nanotechnologist, after God. Bioindustry products include bioenergy, biofuels, environmentally friendly industrial chemicals such as biosurfactants, and bioenzymes (currently the production of bioenzymes fuels a nearly $4 billion market). Academia needs to re-think how to take full advantage of the potential the bioeconomy offers; one way is to take a broader approach to the teaching of microbiology especially at the undergraduate level. It has become clear that the future growth of a microbial-based industry sector depends on two crucial components namely: expansion of the fundamental understanding of microbiology and translation of that understanding into viable products. When molecular biology emerged, life sciences programs including microbiology at many institutions made changes to their names to include molecular. Now we could direct our emphasis to translation based on a strong foundation in microbial physiology,

biodiversity or microbial ecology and good quantitative skills, and what is that? Process and Phenomenal Microbiology (you may also call it Industrial Microbial biotechnology). So, just as we introduced biochemical engineering to our chemical engineering department, this time we would introduce to them Process and Phenomenal Microbiology or Industrial Microbial Biotechnology. What we currently have in the School of Science Laboratory Technology is a good start. We shall re-design it in line with microbe-based ecological economics for job creation. We shall re-design microbiology programs to be job creation oriented in line with Process and Phenomenal Microbiology. There are more microbiology programs in our Nigerian universities than any other biological sciences program. Given the next 2-5 years, we shall come up with a new Microbiology Program that is process driven and job creation oriented. This is Process Microbiology.

Concluding thoughts

Scientists must learn to "stop at nothing"

We usually say we will stop at nothing, meaning we must get to the end of the matter. In human perspective we must have a limit. That limit is that we do not know what is nothing. We scientists must therefore be prepared to stop at nothing. Only God knows what nothing is. So, actually only God can create, can bring something out of nothing. It is not a disgrace if we scientists learn to stop at nothing. Curiosity and inquisitiveness can still lead us to discoveries and inventions that would be useful and beneficial to the wellbeing of humanity. Isaac Newton said "the purpose of science is to read the mind of God". This is what I have tried to do in this lecture, that the mind of God in creating microbes, the tinniest packages with "life" is so the microbes would create jobs. So, the microbe is the epitome of a designed life. It is the most complicated arrangement or assembly of life measurable by man. It is simply a marvel, a wonder, a spectacle. Louis Pasteur, the inventor, the great Chemist was so enamelled by microbes he uttered – "messieurs, c'est les microbes qui auront le dernier mot (=ladies and gentlemen, it is the microbes that have the final say). At the University of Port Harcourt, we added-After God (Apres Dieu). That means after God, it is microbes.

But since our humanness would not be comfortable with that notion, it means after God, it is the microbiologist, because he is the one that knows about and how to use/control these amazing critters (creatures) for the sustainable development of man. All microbes were created by the same creator, there is no stupid microbe, all of them know exactly what to do and they do it. *Pseudomonas aeruginosa*, with one of the biggest genomes, is not more intelligent than *Staphylococcus aureus* or *Mycobacterium tuberculosis* or the indomitable *Escherichia coli (E.coli)*. The same understanding is applicable to human beings; this is how the Bible puts it: "God that made the world and all things therein … He gives to all life and breath…. He made of one blood all nations of men for to dwell on all the face of the earth, and hath determined the times before appointed, and the bounds of their habitation: That they should seek the Lord…..though He be not far from every one of us (Acts 17:24-27. KJV). So, the founding fathers of the American Constitution (of the United States of America) were not stupid either when they inscribed in the Preamble to the Declaration of Independence: "we hold these truths to be self-evident, that all men are created equal, that they are endowed by their Creator with certain unalienable Rights, that among these are Life, Liberty and the pursuit of Happiness" (www.America's Founding Documents/archives.org). Speaking on the "genius principle" Professor Maxwell, one of only two professors of thinking in the world, demonstrated how that genius is not by race, it is not even genetic, rather genius is influenced by three components of age, environment and mentoring (Marxwell, W. 2014. The Genius Principle. Public Lecture at 40th Anniversary, University of Port Harcourt). Mr. Chairman, when I decided on this topic I had in mind to state my locus standi, my perspective that, the microbe was created by God Almighty in the same way that we human beings were created by God; that the microbe was created a microbe; it was created to create jobs; it was not created to create man or barracuda or hippopotamus, nor even to create the ant or the fly. Actually, it is said of the fly "God created the fly but did not tell us why" (Okiwelu, 2005). As for the microbe, I hope I have succeeded in telling you why it was created i.e. to create jobs.

Source: AAM of ASM, Feb. 2013, Dallas, Texas, USA) (view: Microbe – Powered Jobs Infographic).

When was the microbe created? When the earth was created. And when was the earth created? I may state that I am a creationist microbiologist, I hope a creationist geologist or geophysicist would give us an answer in our lifetime, a renewable sustainable answer. So, may I state again, this time as it is at UPHere "messieurs, c'est les microbes qui auront le dernier mot"; 'mais seulement, apres Dieu'. "(=Ladies and gentlemen, it is the microbes that have the final say, but, only after God)". God has the ultimate final say. Because after the microbes have mineralized our bodies back to the elements, the life, the soul returns to face the Almighty God. As for microbes, they were created to create jobs for us. This, according to Isaac Newton, I believe, is the mind of God who created all things, visible and invisible including the forces of bosons and the microbes. So, how do we use microbes to create jobs for mankind? Go to the Process and Phenomenal Microbiologists and God bless you.

Thank you for your patience and attention

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CITATION

PROFESSOR GIDEON ORKWAGH ABU

[B.Sc (ABU); Ph.D (Maryland, USA)]

Gideon Orkwagh Abu was born to Jato Ashanu Abu and Abu Imande Genyi (AbuGe) in Akpagher in Benue State. He attended Primary School at the Dutch Reformed Church Mission (DRCM) a.k.a Nongo u Kristu u Sudan ken Tiv (NKST), in Ikpa Mbatierev, in Benue State. He attended Secondary and Higher School (1970-1976) at the Government College, Keffi (GCK, the primodium of Keffi Old Boys Association, KOBA). He had his B.Sc. at the Ahmadu Bello University (ABU), Zaria from 1976 to 1979 where he studied Microbiology. After the National Youth Service Corps (NYSC) scheme in the old Anambra State; he was hired as Graduate Assistant at the University of Port Harcourt. Gideon went on Staff Development sponsorship to the University of Maryland, College Park, Maryland, USA, where he obtained the Doctor of Philosophy in Microbiology in 1988. His research focus was Interfacial and Process Microbiology. He used advanced microbiological techniques such as radiorespirometry, chemical techniques: gas

chromatography/mass spectrometry (GC/MS), HPLC, FTIR; nano imaging and analytical techniques such as scanning electron microscopy/energy dispersive x-ray microanalysis (SEM/EDX). With these he showed the exopolymeric substances (EPS)-mediated mechanism of adhesion of a newly characterized marine bacterium, *Shewanella colwelliana*. While at UM College Park, he served as Graduate Teaching Assistant and Instructor in the Department of Microbiology and Research Assistant at the University of Maryland Center for Engineering Research, BioScale-Up Unit, he was involved with media formulation for the mass culture of industrial microorganisms.

He returned to the University of Port Harcourt in 1989 as Lecturer II, he rose to the rank of Senior Lecturer in 1995, and finally attained the rank of Professor of Microbiology in 2009. At the University of Port Harcourt he received the International Biotechnology Program Fellowship at the GBF (Institute for Biotechnology Research), Braunschweig, Germany (1991) and was certified a biotechnologist. Part of his career development included research grants from the International Foundation for Science (IFS), University of Port Harcourt Senate, a UNESCO Fellowship and a World Bank-NUC Fellowship for Post Doctoral research that took him to the Institute of Research, Ben Gurion University of the Negev, Beersheva, Israel (1994-1995). He served as Senior Environmental Advisor/Engineer to Shell Nigeria (1998-2001). At Shell he helped form the present day Remediation Department; he was among the first team that was trained and certificated in the Risk-Based Corrective Action (RBCA) approach for contaminated media remediation, in Nigeria. At Shell, he assessed close to 1000 sites in both West and East, using the Risk Based Methodology (RBM). He was part of the team that introduced the Remediation by Enhanced Natural Attenuation, now tagged Accelerated Aerobic Bioremediation (AAB), in Shell, Nigeria. He supervised the first bioremediation project by Shell in Nigeria. Academically he has supervised more than 100 undergraduate majors' projects, more than 30 M.Sc dissertations and more than 10 PhD theses, some of whom are professors today. He has more than 70 published journal articles and two monographs from his research

activities. He was among the first to publish on bioremediation in Nigeria, in the 1990s and some of that work was on Ogoniland in the Niger Delta; the work brought much acclaim to the University of Port Harcourt. He served as Ag. HOD 2003-2006; Pioneer Director, Central Instruments Laboratory (CIL) 2007-2012; nominated ASM Ambassador to South Africa (2009); member NUC resource verification team to Kano State University (2012); Chairman Department of Microbiology, Uniport, Accreditation team (2016). He has assessed several persons for the professorial cadre, and has been external examiner for undergraduate programs on several occasions; he is a member of the Monday Prayer Group, Uniport; he also served on the Committee for Religious Activities and Harmony on Campus. He is a commissioned preacher of the gospel of our Lord Jesus Christ; he and his wife Dr (Mrs) Owapiriba P. Abu are labouring with the Redeemed Christian Church of God (RCCG), Peace House Revival Labours and the Scripture Union (SU).

Professor Onyewuchi Akaranta
Orator