LADOKE AKINTOLA UNIVERSITY OF TECHNOLOGY
P. M. B. 4000, OGBOMOSO.

INAUGURAL LECTURE SERIES

2nd Inaugural Lecture

BIOENGINEERING: ROADMAP TO NATIONAL GREATNESS.

By

Prof. J. K. Oloke

8th June 2004.

BIOENGINEERING

ROADMAP TO

NATIONAL GREATNESS

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Bioengineering: Roadmap to National Greatness.

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DESIGN & PRINTED BY:

LAUTECH PRESS,

OGBOMOSO.

CONTENT

The Vice-Chancellor, Distinguish Colleagues, Ladies and Gentlemen. To God be the glory, for great things He has done. It is with a heart of gratitude that I stand before you today to present to you an inaugural lecture on a subject that has been very dear to me. It is a well known fact that majority of the citizens of developing Nations like Nigeria are peasant farmers who work like elephant but eat like ant, lives in ghettos and die like pauper. Problems of man have been terribly compounded by the different incurable diseases like AIDS which on daily basis ravages the population like an unquenchable fire. The problems of AIDS is more devastating in the poor Nations.

Tackling the problem of poverty in the developing nations is one single achievement that has eluded several leaders. The different theories on econometrics that are working in more developed nations have no answer to the problems of poor nations. As a result, with only a small amount, it is possible to hire political thugs who are ready to serve as bouncers to the rich and mighty. The poor farmers in the developing nations are faced with the problem of poor yield due to low productivity in potential of his soil, difficulties in the transportation of his produce, storage losses, poor marketing of his produce, government policies that are unfavorable to his enterprise and unpredictable weather that constitutes major losses to his effort.

Yearly poor economic performance of less developed nations has always been based on budget deficit. On yearly basis, the politicians always promise to reduce budget deficit in the oncoming year. The truth of the matter is that for several nations especially the poor ones it has never been possible to get out of economic crisis. Associated with the different economic crisis of each of these nations are inflation, huge unemployment,

several cases of social unrest caused by retrenchment and its likes, lack of welfare programmes and political upheavals. The present terrible high tone of acts of terrorism in different nations may be deeply rooted in poverty with superfacial religious cover-up.

Since the time of old, the different philosophers have been asking: - Is there any hope for man to get out of his trouble? Indeed, several people have queried: who is man? He is so strong yet so fragile, so powerful yet so weak, so great yet so miserable. He is so curious to know and knows so much about the physical universe, yet he is so ignorant about himself. Many philosophers have concluded that man is a bundle of contradiction and that he is not only a problem to himself but also a mystery beyond his comprehension.

Does it mean that the poor nations will continue in the cycle of poverty? To answer that question, let us consider together the situation between Israel and Palestine. We all know that these two nations have been on each other's throats. They use every available opportunity not only to attack each other but cause life threatening calamities on each other's soil. Dangerous bombs are freely sent across the boarders on frequent basis while suicide bombers have never been tired of doing their work in prime places where many more lives could be terminated.

The United Nations in its own wisdom thought the only way to end the cycle of woes among the two nations is to evolve a pragmatic road-map to peace. As we all know, the road-map is an imaginary path which if the two nations can agree to pass through many bring the elusive peace. Very sadly, none of

the two nations is ready to follow the road map; so the circle of woes continues.

There is a road-map to national development which if the poor nations of the world will decide to follow will make a lot of different. The road-map is investment in bioengineering.

According to experts, the world economy was in as wretched a state in 2001 - 2002, 1991 – 1992 as in 1982 and 1975. In order to break the cycle, some analyst advocated both the revision of the mode of economic growth and the formulation of a plan for less destructive and more harmonious development. Furthermore, the Deputy Secretary General of the United Nation in charge of the Department of Economic and Social Development has indicated that priority needed to be given to investments so as to foster worldwide economic growth, rather than concentrating on reducing budget deficit. For example, Japan's financial power du ng the 1980's could be explained by the wealth produced by a vigorous economy. Japan's real wealth was based largely on investment ende vour (30% of the gross national product, compared to 17% of the GNP in the USA), resulting from significant saving, a trade surplus was accumulating, especially with the USA, whose intentional competitivity was decreasing due to overrated dollar (Meyer 1992). As a result, within a period from 1988 to 1989, Japan had a financial euphoria during which the Tokyo stock Exchange superseded the New York Stock Exchange as the leading world capitalization.

The greater percentage of investment done in many poor nations is on buying and selling. This has made several citizens of the poor nations to be traders

turning their countries to dumping grounds for disused materials. Each of these countries relies very heavily on importing with very little export value based mainly either on crude oil or unprocessed agricultural product. To be able to command respect in the committee of nations and to have an enhanced export value, among other things, emphasis should be placed on investment in bioengineering.

The word Engineer has meant different things to different people in our society. To some, he may be a craftsman, a technician, or a road building foreman on a worksite. To others the engineer is simply the outboard engine driver or mechanic who repairs cars or any person that deals with engines. Thus the question of who an Engineer is has continued to be an enigma, as anybody who has anything to do with the engineering industry or service calls himself an Engineer.

Engineering can be defined as the creative application of scientific principles to design or develop structures, machines, apparatus or manufacturing processes or to construct or operate the same with full cognizance of their design; or to forecast their behavior under specific operating conditions, all with respect to an intended function, economy of operation, safety of life and property and for the overall safety of mankind. So, the Engineer then is a creative and intellectually disciplined person, who by virtue of his training and experience is able to exploit the forces and materials of nature, be it the wind, water, soil, rock etc, for the improvement of and utilization by mankind. Since we all know that bio means life, bioengineering may be defined as the processing of biological materials and agents such as cells,

enzymes or antibodies for the improvement of and utilization of mankind. In bioengineering, cells are manipulated inside bioreactors to overproduce their metabolite which may be enzyme, amino acids, Vitamins etc, required by man both at industrial and domestic levels. It also involves cloning of DNA found in cells. While the civil Engineer handles gigantic structures on land, the Bioengineer handles complex structures in bioreactors and skyscraper DNA structures in test tubes. Indeed both the cell and the bioreactor are the two main basic tools of bioengineer.

1. The cell

1.1 Cell theory

A development critical to the understanding of living system started in 1838, when Schleiden and Schurann first proposed the cell theory. This theory state that all living systems are composed of cells and their products. Thus, the concept of a basic module, or building block, for life emerged. This notion of common denominator permits an important decomposition in the analysis of living systems for the component parts, the cells, can be studied, and then this knowledge is used to try to understand the completed organism.

The value of this decomposition rests on the fact that cells from a wide variety of organisms share many common features in their structure and function. In many instances this permits successful extrapolation of knowledge gained from experiments on cells from one organism to cells of other types. This existence of common cellular characteristics also simplifies our task of

learning how microorganisms behave. By concentrating on the apparently universal features of cellular function, a basic framework for understanding all living systems can be established.

1.2 Structures of Cells

Observations with the electron microscope have revealed two markedly different kinds of cells vi3 prokaryotic and Enearyotic cells. The prokaryotic cells or prokaryotes do not contain a membrane enclosed nucleus. Prokaryotes are relatively small and simple cells. Microorganisms of this type grow rapidly and are widespread in the biosphere. Typically, prokaryotes are biochemically versatile. that is they often can accept a wide variety of nutrients and further are capable of selecting the best nutrient from among several available in their environment.

Eucaryotic cells or eucaryotes are those cells which possess a membrane enclosed nucleus. All cells of higher organisms belong to this family. Many important microbial species like fungi and protozoan are also eucaryotic.

1.3 Important Cell Types

The different cell types include bacteria, yeast, molds, algae, protozoan animal and plant cells.

Bacteria are typically unicellular which may exist either as coci, bacilli or spiral shape. Based on the cell wall composition some are referred to as gram-positive while other are gram-negative. Manufacture of vinegar, some antibiotics and animal feed supplements are among the important microbial

products obtained when cultured inside a bioreactor. Some bacteria have been implicated in the different disease conditions.

Yeasts form one of the important subgroups of fungi. Fungi like bacteria are widespread in nature although they usually live in the soil and in regions of lower relative humidity than bacteria. Although most fungi have relatively complex morphology, yeasts are distinguished by their usual existence as single small cells. Yeasts have been used in the production of beer, wine, industrial alcohol and glycerol. Yeasts are also used for baking purposes and as protein supplements.

Molds are higher fungi with a vegetative structure called mycelium. Mycelium is a highly branched system of tubes. Different species produce different product e.g. Aspergillus niger produces oxalic acid.

Many vaccines and other useful biochemicals are produced by growth of animal cells in process reactors. That is, by cell propagation outside of the whole animal. The reactors in which tissue cultures may be propagated may be quite similar to microbial reactors. It is also possible to grow certain plant cells in culture, either as a callus or as aggregated cells in suspension. Cultured plant cells have several potential agricultural applications including whole plant generation.

1.4 Cell Manipulation

One of the primary duties of the Bioengineer is to manipulate the cell to over produce its metabolite. This is achieved either by genetic engineering or through fermentation technology.

1.4.1 Genetic Engineering

The tools of recombinant DNA, which have revolutionized the potential of biotechnology for mankind's benefit, permit precise construction of new genetic instructions which can be inserted into and utilized by living cells. The opportunity to introduce totally new DNA into cells has created unique industrial microorganisms able to synthesize valuable proteins. This is possible with the use of the enzyme restriction endonucleases which recognize and cut specific nucleotide sequences within the DNA molecule. Other enzymes use in genetic engineering include DNA ligase which allow different DNA molecules to anneal and DNA polymerize which catalyzes the synthesis of oligonucleotides. DNA fragments are introduced into the target cell by the use of vectors. Plasmids and bacteriophages are two important vectors used for cloning DNA in *Escherichia coli*

The capability for chemical synthesis of genes or gene fragments raises the fascinating possibility of protein engineering. It is possible to alter at will any amino acid in a protein with the possibility of increasing the biological activity and the process stability of that protein. Furthermore, cells can be made to synthesize novel amino acid sequences and produce new catalysts, drugs and food ingredients. It is common to alter or manipulate the promoter region of protein so that it can be over -expressed. Generally, it is desirable to use a controlled promoter that can be turned on by some environmental changes such as addition of inducer, depletion of repressor, or temperature shift. In a well-designed expression system, the cloned gene product may constitute up to 70 percent of the total cellular protein.

Cloning and gene expression in mammalian cells is mostly done using vectors derived from simian virus 40 (SV 40). The covalently closed circular DNA genome of this virus can replicate independently in mammalian cells or may be integrated into the host cell chromosomes. In addition to providing an origin of replication that function in mammalian cell, promoters from SV 40 may be used to obtain cloned gene expression.

1.4.2. Fermentation Technology

The use of fermentation technology to manipulate cell, which will as a result among other things over -express its metabolite, is very exciting. A typical fermentation process requires medium selection, medium preparation and sterilization, introduction of the medium into the fermentor, medium inoculation, fermentor operation and product recovery.

A variety of factors must be considered when formulating a fermentation medium. This is because to a very large extent, it is the type of medium used coupled with the other growth conditions used that determined whether or not a microbe will over-produce its metabolite.

Some scientists treat the microbe as a "black box", assuming one particular type of metabolic behavior. This approach ignores the metabolic versatility of microbes and that metabolic fluxes are highly dependent on the growth environment, and therefore can be manipulated by a careful choice of the culture conditions. For example a wild type strain of Escherichia coli grown under fully aerobic conditions in a batch culture in a medium containing glucose as the main carbon and energy source, will produce new

cells and usually some acetate. It comes as a surprise to many scientists that the same strain can be a reasonably good gluconate producer (63% of glucose converted into gluconate) when it is grown under special environmental conditions (Ruyter *et al;* 1991). The crucial point about this example is that this productivity has been achieved with a wild-type organism and without genetic engineering. Using the process of cometabolism (Conversion of a compound into a product without concomitant assimilation) a similar result has been obtained. Thus, Groeger and Sham (19870) fed the precursor and alpha -Ketoisocaproate to cultures of *Corynebacterium glutamicum* growing in glucose. The organism converted this compound into L- Lencine (yield 91%). When alpha - Ketobutyrate was added to a culture of this organism Isoleucine was formed (Eggeling *et al;* 1987).

The most important factor in all production process is the control of metabolic fluxes and the main problem to be solved in any production process is how to maximize the flux leading to the product and how to minimize fluxes leading to unwanted side reactions.

Kasser and Burns (1973), and Heinrich and Rapoport (1974) have made significant contribution to the theory of metabolic fluxes. The figure below shows a hypothetical pathway from substrate S to P catalyzed by four enzymes.

$$S \xrightarrow{E_1} I_1 \xrightarrow{E_2} I_2 \xrightarrow{E_3} I_3 \xrightarrow{E_4} P$$

Fig. I Hypothetical metabolic pathway for substrate S, via 3 intermediates.

Table I. Hypothetical metabolic pathway for substrates, via 3 intermediates.

Examples	S	control coefficient of			
		E_1	E_2	E_3	E_4
1	1	0.25	0.25	0.25	0.25
2	1	0.01	0.01	0.97	0.01
3	1	0.00	0.00	0.00	1.0
4	1	0.10	0.01	0.10	0.79

S, hypothetical substrate concentration

A measure of the influence of one particular enzyme on the flux is its control coefficient. By axiom the sum of the control coefficients of all enzymes taking part in the pathway is set at unity (this is called the summation theorem). Thus, in this hypothetical example, the four enzymes can have many different control coefficients. The first example shows that the influence of each enzyme of the pathway on the flux through the pathway is equal. The second case shows that one enzyme (E_2) has a great influence, whereas that of the others is almost negligible. The importance of this example lies in two consequences that have a great impact on the optimization product formation.

The first is that this theory predicts that all enzymes that are involved in a pathway will have an influence on the flux through this pathway. It is therefore usually wrong to consider a single particular reaction in a pathway as the "rate-limiting" step. This is only the case when the control coefficients of all other enzymes involved in the pathway are equal to zero, a very

exceptional situation. The third case in this example shows this: enzyme E_3 has a control coefficient of 1 and therefore controls the flux for 100%. One would be tempted to state that enzyme E_3 is catalyzing the rate-limiting step in the pathway from S to P, but one should not forget that the concentrations of S and P also play a role. Other values of these concentrations could give rise to a different situation.

The second consequence of this theory is that the longer the pathway (i.e. the more enzymes involved) the smaller the influence of a single enzyme is on the flux through the pathway.

The most efficient metabolism of the carbon and energy source in *Klebsiella pneumoniae* was observed (Neijssel and Tempest 1975, 1979) when the organism was grown in a chemostat (a type of bioreactor) under carbon-limited conditions, the sole products of glucose metabolism being carbon dioxide and new cell material. Under carbon excess conditions the specific rates of glucose and oxygen consumption were invariably higher. Interestingly, oxidized products of glucose metabolism were excreted into the culture fluid. For example under nitrogen limitation along with 1 kg of cells 4 M acetate, 3.5 M Pyruvate, 112 M Ketoglutarate and 1.9 kg extra cellular polysaccharide ware produced.

Table 2

Conversion of Glucose into products by Variously Limited Chemostat Cultures of *Klebsiella pneumoniae* (D= 0.17 h^{-1}, pH 6.8.35 °C

Carbon limitation

12 glucose + 26 O_2 + 9NH_3

1 kg cells + 31 CO_2 + 50H_2O

Nitrogen limitation

36 glucose + 44 O_2 + 9NH_3

1kg cells + 40CO_2 + 70H_2O + 4 acetate + 3.5 pyruvate + 11 2-ketoglutarate + 1.9 kg extacellular polysaccaride.

Nitrogen limitation plus 1 mM DNP

84 glucose + 76O_2 +9NH3-

1 kg cells + 35CO2 + 123H2O + 8 acetate + 19 pyruvate + 7-ketoglutatate + 4 gluconate + 48 2 – ketogluconate.

Potassium limitation

58 glucose + 106O_2 + 9NH3-

1 kg cells + 113 CO_2 + 165H_2O + 13 acetate + 7 pyruvate + 1 2-ketoglutarate + 11 gluconate + 7 2-ketogluconate + 0.4 kg extracellular protein.

All coefficients were adjusted to a production rate of 1 kg cells per hour. Substrates and products are given in moles. Data of NEUSSEL and TEMPEST (1975, 1979).

However under potassium limitation, along with 1 kg of cells 13 M acetate, 7 M pyruvate, 12 M Ketoglutarate, 11M gluconate, 72 M ketogluconate and 0.4 kg extracellular protein is formed. The same type of

experiment with other organisms, such as *Agrobacterium radiobacter* (Linton *et al;* 1987), *Candida utilis, Bacillus subsilis, Clostridium butyricum* (Crabbendam *et al;* 1985), *Escherichia coli* and *Pendomonas* species (Hardy 1992) have shown that all these organisms behave similarly in that carbon-excess condition led to a lowered energetic efficiency (higher specific rate of consumption) of the carbon energy source and, where applicable, of oxygen and overproduction of metabolites.

From all these, it is clear that the type of blind research done in many Microbiology Department of several institutions in developing countries (like Nigeria) in which what is happening to the growing medium is not known can never lead to any breakthrough. For example, in a typical Microbiology Department of several institutions of Developing countries, a researcher just inoculates his organism into the medium without the ability or the capacity either to supply oxygen to the medium, monitor the change in PH or assess the extent of utilization of substrate. The problem is further compounded by lack of information on medium formulation that may give the required output of a particular metabolite.

To be able to monitor and exert a required control on a growing culture there is a need for Chemostat or a Bioreactor. Every Microbiology Department that will do a research with industrial relevance requires either of these equipment. However, both Chemostat and bioreactor are not only expensive but difficult to maintain in the developing countries. By the help of God, we have been able to assemble a computerized bioreactor adapted for the use of both developing and developed country in LAUTECH.

2. LAUTECH Computerized Bioreactor

Bioreactors offer a possibility to provide an optimally controlled environment for microbial fermentation processes-a condition required for optimal yield (William 2002). With this, one can specifically alter the metabolic fluxes and direct the cells resources to move desirable pathways while inhibiting unwanted ones. For instance, by imposing a given temperature profile on the culture, one can selectively denature certain enzymes, thus prioritizing some metabolic routes over others within the cell (Gueguim-Kana *et al;* 2002). Process control through bioreactor in submerged cultures is based on the measurement of physical, chemical and biochemical properties of the broth, such as PH, dissolved oxygen, temperature, agitation rate and others, using dedicated probes followed by the manipulation of the physicochemical properties of the culture with suitable actuators (Lim and Lee 1991). In many instances, overproduction of metabolites have been achieved in our laboratory using our Bioreactor by a simple medium manipulation (Lateef *et al;* 2001). The full description of constructional feature of our 4.5 litre and 15 litre bioreactor have been published (Gueguim kana *et al;* 2003a and Gueguim kana *et al;* 2003 b).

Automation is very important for effective control of different parameters in Bioreactor. Unlike simple chemical processes, bioprocesses are non-linear and have unpredictable dynamics. This is due to the intricacies and complexity of the network of dynamic biochemical reactions occurring within the cell, the cells and the cultural environments (Lim and Lee 1991). It

is therefore difficult to develop an efficient model or a set of mathematical equations which based on growth kinetics and reaction stoichiometry could comprehensively describe each process in time (Gueguim kana *et al;* 2002). Within these constraints, a precise control is required.

The decrease in cost of computer hardware and increase of performance of microprocessors offer the possibility of developing alternate superior control approaches (Richardson and Peacock 1995). This entails the design of artificially intelligent biosystems. They provide online information on the bioprocess; give accurate estimation of variables which cannot easily be measured online and detect process abnormalities by inference on the knowledge gained from previous fermentations, thus enhancing human supervision. These intelligent systems rely on some programming techniques such as the neural network, fuzzy logic and genetic algorithm. In our laboratory, we have developed a software package for the automation of our bioreactor (Gueguim Kana *et al;* 2003 c, and 2003 d).

Figure 3: LAUTECH Computerized bioreactor on dispby.

Figure 4: LAUTECH Computerized bioreactor

Figure 5: LAUTECH Computerized bioreactor

3. Biotechnology Potential of a Novel Local Strain of *Rhodotorula Glutinis*

Strains of a red yeast isolated by Ruinen (1956) from leaf surface of citrus plants in Indonesia were classified by Deinema (1961) as *Rhodotorula grutinis* di Menna CBS 3043. Since this early works other strain of *Rhodotorula* e.g. *Rhodotorula glutinis* have been isolated. (Ammers *et al;* 1964). A local novel isolate of *Rhodotorula glutinis* has been obtained in our laboratory.

The new isolate of *Rhodotorula glutinis* produces crystalliferous protein on potato dextrose agar. Each cell is made up of one crystal and one spore. When viewed under the electron microscope the isolate is completely different from the already known ones. Its surface is covered with slimy polysaccharide like projections, however, the organism is not slimy. The pigmenting gene has been cloned in *Escherichia coli* to obtained three different strains. Each strains is uniquely pigmented with unique shape but each has crystalliferous protein.

A special technique has been developed in our laboratory to develop teleomorphs of the isolate of *Rhodotorula glutinis.* As a result Rhodosporidium *toruloides* and *Rhodosporidium sphaerocarpum* have been developed from the local isolate of *Rhodotorula glutinis*.

One of the teleomorphs obtained i.e. *Rhodospiridium toruloides* has been cultured in our bioreactor. Using the theory of metabolic fluxes the organism has been made to overproduce its metabolite. The metabolite is being used as a probiotic (personal communication).

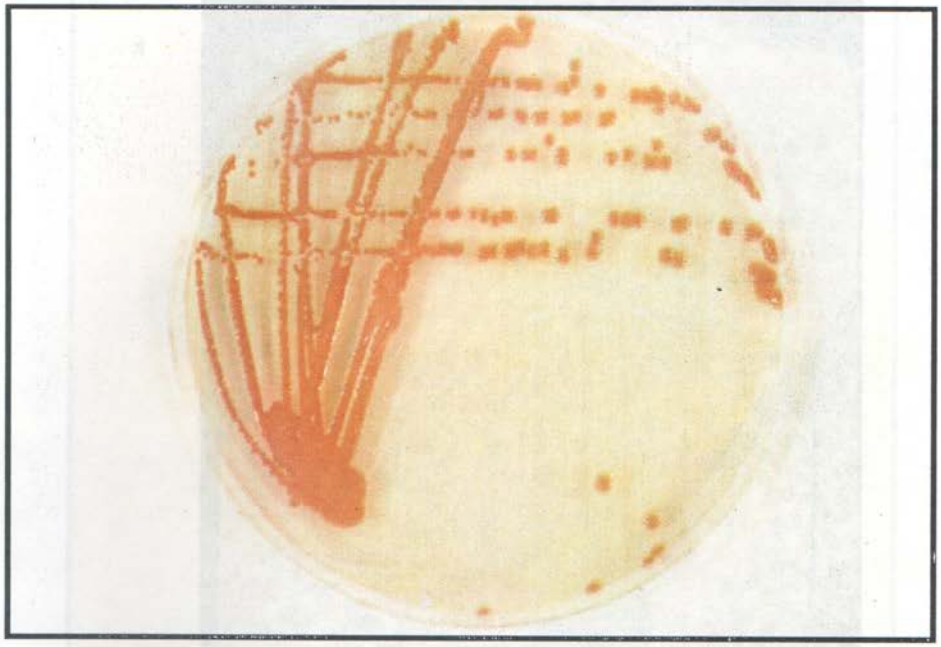


Figure 6: Rhodotorula glutinis on agar plate

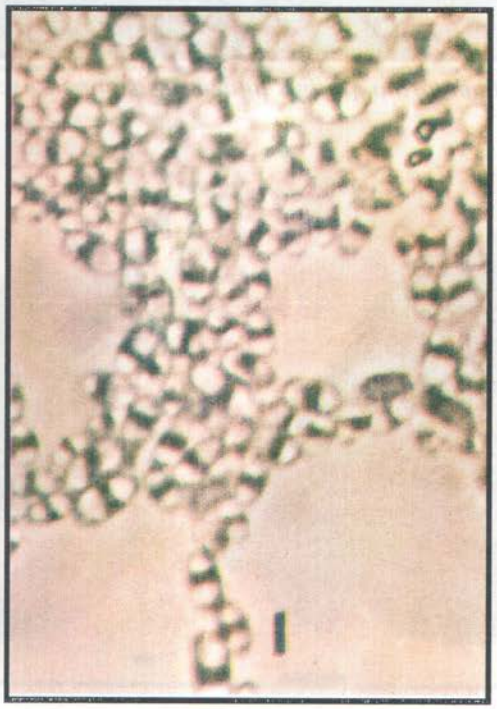


Figure 7: Cells of Rhodotorula glutinis showing spores and crystals

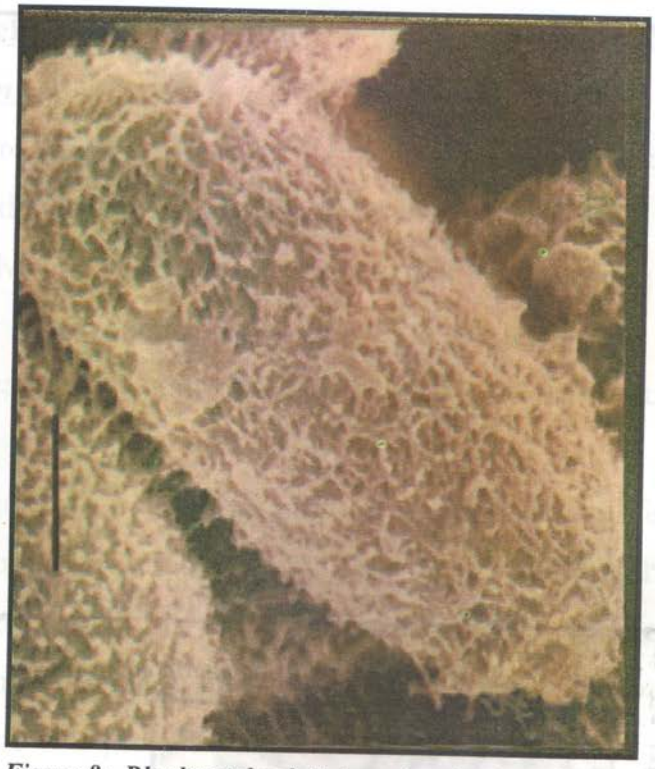


Figure 8: Rhodotorula glutinis under electron microscope

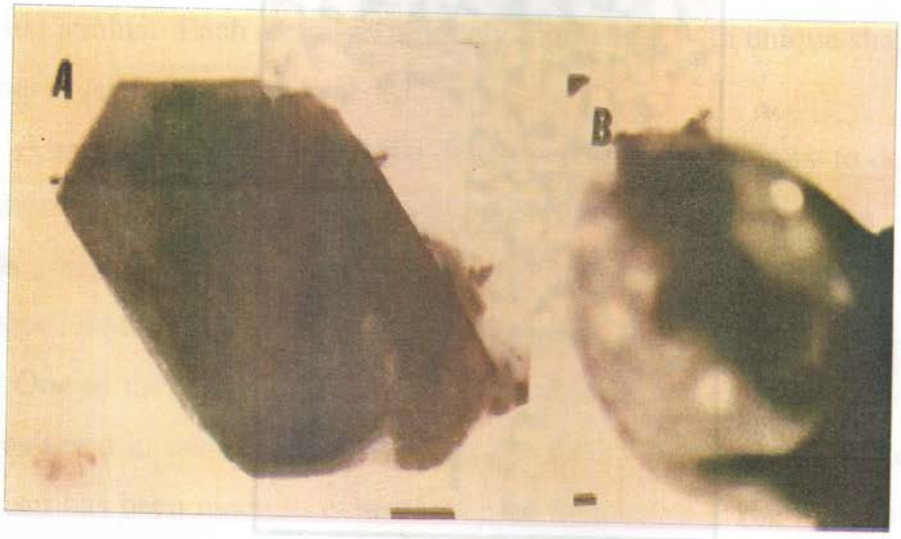


Figure 9: Crystal (A) and Spore (B) Rhodotorula glutinis

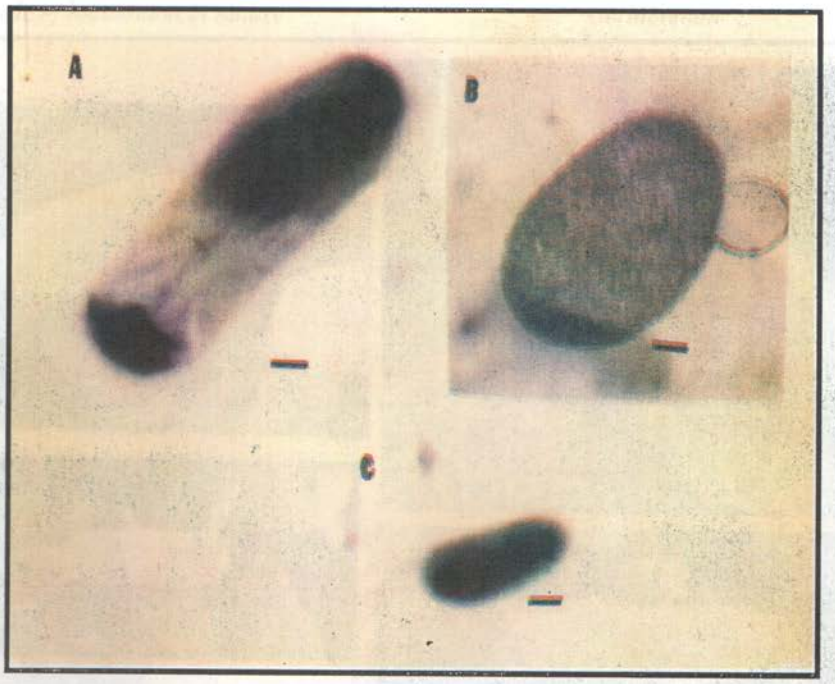


Figure 10: Escherichia Coli Cells. Cloned with pigmentins gene from Rhodotorula glutinis as seen under electron microscope

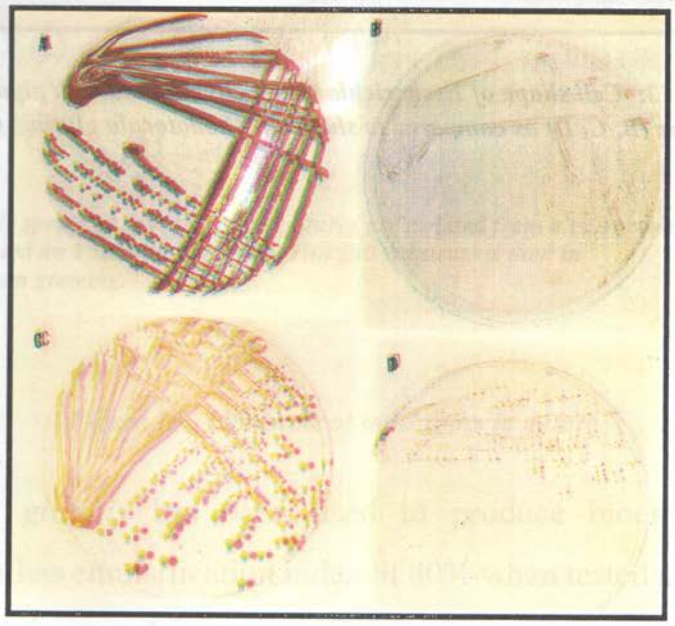


Figure 11: Morphology of Escherichia Coli Cells. Cloned with pigmentins gene from Rhodotorula glutinis

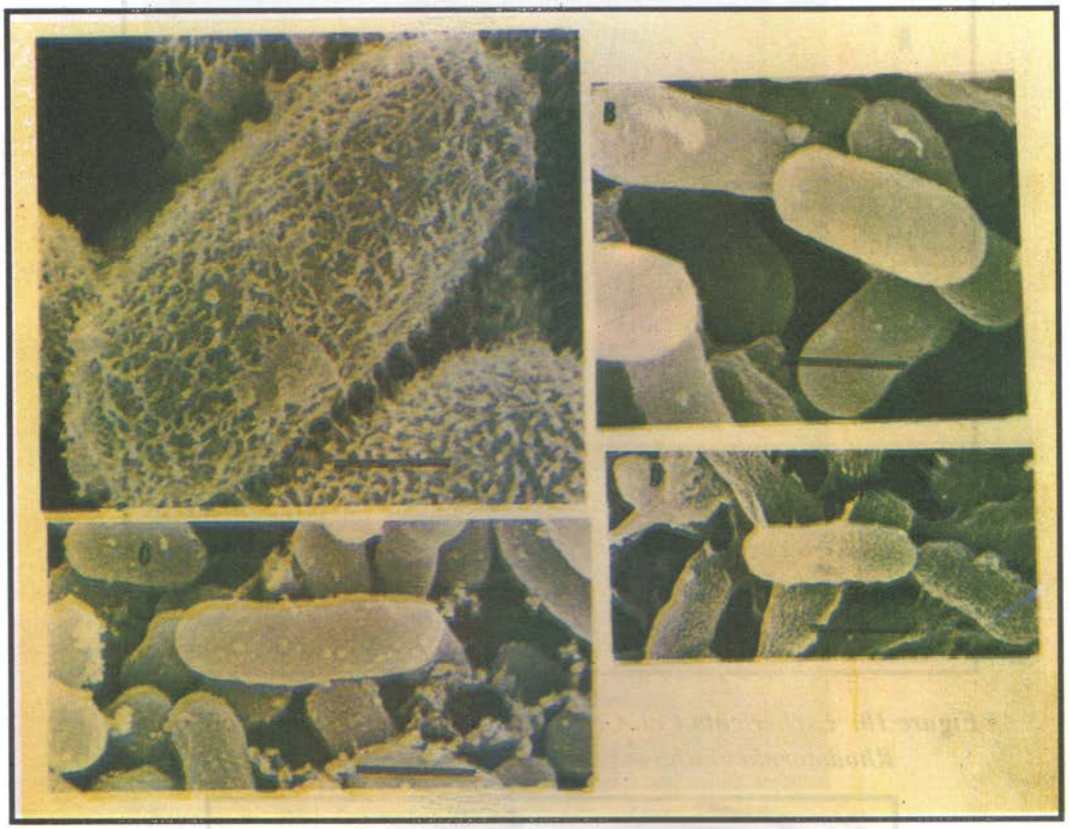
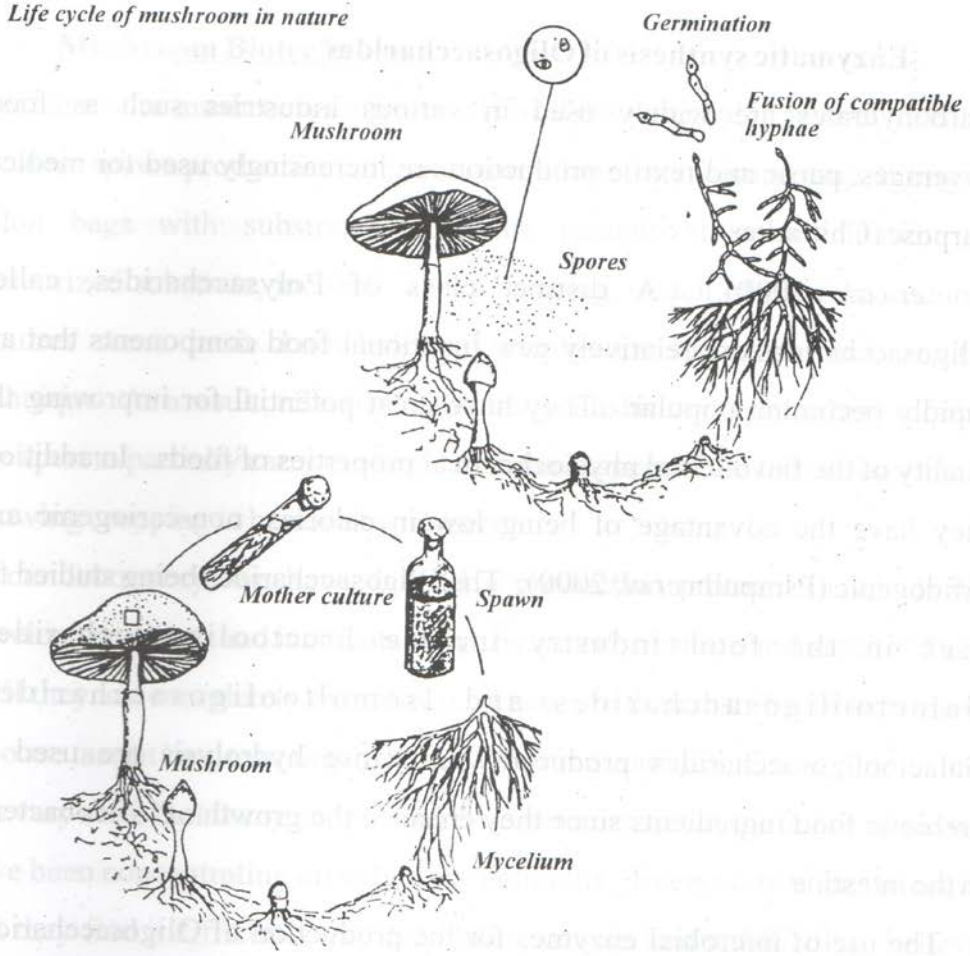


Figure 12: Cell shape of Escherichia Coli Cells. Cloned with pigmentins gene (B, C, D) as compared to shape of Rhodotorula glutinis (A)



Life cycle spawn to spawn. Tissue cultures are isolated from a mushroom and propagated on a suitable substrate. This full substrate is then in mushroom growing.

Figure 13: Life circle of mushroom in nature

Rhodotorula glutinis has been used to produce bioemulsifier. The bioemulsifier has emulsification index of 80% when tested against crude oil and kerosene. (personal communication).

4. Enzymatic synthesis of Oligosaccharides

Carbohydrates are widely used in various industries such as food, beverages, paper and textile production are increasingly used for medical purpose (Chitradon

et al; 2000). A distinct class of Polysaccharides, called Oligosaccharides are relatively new functional food components that are rapidly becoming popular. They have great potential for improving the quality of the flavour and physiochemical properties of foods. In addition, they have the advantage of being low in calories, non-cariogenic and bifidogenic (Psrapulla *et al*; 2000). The Oligosaccharides being studied for uses in the food industry include Fructooligosaccharides, Galactooligosaccharides and Isomaltooligosaccharides. Galactooligosaccharides produced via lactose hydrolysis are used as prebiotic food ingredients since they enhance the growth of bifidobacteria in the intestine.

The use of microbial enzymes for the production of Oligosaccharides provides cost effective and convenient alternative to chemical synthesis that is laborious, expensive and often give low yields (Prapulla *et al;* 2000). In our laboratory, procedures have been developed to produce varieties of oligosaccharides under different reaction conditions (Lateef *et al;* 2003, Lateef and Oloke 2003). There is no doubt that this can form a basis for cottage industries in Nigeria.

5. Mushroom Biotechnology

Procedures for mushroom cultivation is well documented in several texts. It involves spawn production, substrate formulation and composting, filling of nylon bags with substrates, substrate pasteurization, inoculation of pasteurized substrate, incubation and cropping. Each of these procedures requires some basic knowledge of mushroom life cycle and aseptic technique. As a result, effective community mushroom farming may require an expert to partially carry out some technical aspect of the technology while allowing cropping to be done by farmers.

In our laboratory, we have defined a production protocol in which we develop the spawn and inoculate the pasteurized substrate and monitor them until they are completely overgrown. These are then distributed to farmers who are taught how to maintain humid cropping conditions.

It is very clear that many people find *Pleurotus ostreatus,* the major strain we have been concentrating on to be very delicious. Everybody wants to eat it. But they find it difficult to pay for. Many people find it difficult to believe that a kilogram of *Pleurotus* should be more expensive than a kilogram of fish and meat. They always forget that mushroom is a delicacy and are ignorant of the fact that the production protocol is very cumbersome. Until fresh mushroom is correctly prized in Nigeria, there is a little hope for the survival of mushroom production in Nigeria. Experience from our laboratory shows that emphasis should be placed on the production of Pleurotus *cystidiosis* and *Volvariella Volvaea* which could be dried and exported.

6. Antibiosis

This is the process by which secondary metabolites inhibit growth of other microbial species even at low levels. Use for antibiotics are found in antimicrobials (human disease), antitumour agents, fungicides and pesticides for plants protection, animal disease and animal growth products and research. Although several antibiotics are produced commercially via fermentation, many others obtained from other sources like plants have been found to be very useful, especially in the control of development of resistant strains. In our laboratory, although we have found the prevalence of bacterial resistance in several environments (Lateef *et al;* 2004, Oloke 2000), it has been shown that many plants are reservoirs of several toxic agents which may find use in the control of resistant strains (Oloke *et al;* 1988 Oloke *et al;* 1989, Adebajo *et al;* 1989a, Adebajo *et al;* 1989b, Adebajo *et al;* 1991a, Adebajo *et al;* 1991b, Oloke 1992, Oloke 1997a, Oloke 1997b) Some synthetic compounds have also been demonstrated to possess antimicrobial properties against fungi and bacteria (Oloke *et al;* 1998, Akangbe *et al;* 1998). A small scale industry can evolve with speciality in the formulation of plant components into useful preparation For example, a formulation for treatment of scabies has been developed from the volatile oil of *Afromum melegueta.*

7. State of Research in Nigeria

The Vice-Chancellor Sir, please permit me to talk briefly on the state of research in Nigeria. Research has been relegated to the lowest minimum in Nigeria even by the Universities and Research Institutes. I remember the

other day when a Professor's former student was asking why the professor is still wasting his precious time on research when all his mates are chasing money in Abuja. Apart from the fact that the laboratories are becoming empty, the researchers have no interest in investing their lives in a business that has no immediate reward. Everyone knows that more than 99% of researches in Nigeria have no industrial value and will end up rotting away on library shelves. No wonder when a prospective research student ask the Supervisor for a topic the supervisor will tell the student to go and look for a topic. This shows the Supervisor is not a scholar because he is not following any topic. A supervisor who is a scholar will have a few topics he is following which he can use several students to develop.

For any student to do any meaningful work, the research topic should have been attempted by the Supervisor himself and he would have known the different areas he will want the student to concentrate on. Students can only benefit maximally from research topics the Supervisor is an expert in. But what do we see? Even in places where the supervisor gives the topic the student will go and carry out the work in a laboratory outside his institution in the complete absence of the Supervisor. The student will only bring the result to the Supervisor for approval.

Someone has said of research work that it cannot be research if you get it at the first attempt. So, the input of an expert is very important for any meaningful research.

Several research papers turned out in many developing countries are refused publications in places with better setting because such works lack

contribution from experts. There have been situations in which researchers in the developing nations not only plagiarize but also lie; pretending to have done what they did not do so that their work could be published in highly rated journals. It became so bad very recently when some developing countries were blacklisted on the internet from submitting their work to a journal. A blanket order was given that manuscripts submitted by researchers from those several countries will not be reviewed for publication in international journals.

The Vice-Chancellor Sir, before I end this lecturer, I want to attempt to propose a theory on why Jacob's rod of green poplar influenced the cattles' reproductive hormone to Jacob's favour as recorded in Genesis Chapter 30 verse 37 to verse 43. But before I do that let me give a brief background. Jacob, after serving Laban his inlaw for several years was about leaving the inlaw's place. To settle Jacob's wages"... The speckled ringstraked (cattle) shall be thy wages (Genesis 31:8a)". Jacob being the first person to carry out research in animal biotechnology decided to; and laid the rods (green poplar rods) before the eyes of the cattle in the gutters, that they might conceive among the rods (Genesis 30:41b). As a result, Jacob made several strong cattle to bring forth ringstraked ones as offspring and he therefore became very rich. My theorem to explain this is that maybe the green poplar rods with white materials on them probably contained certain volatile compounds which when inhaled caused mutation of reproductive genes. The mutated genes led to the production of ringstraked offsprings. But someone may ask:

why was the mutated gene in favour of the production of ringstraked offspring and not that of the other colours or even those with any other traits associated with mutated genes? I want to back out in formulating anything on that because it is too difficult for me to understand.

The question is, how did Jacob get the clue about this excellent work on the first genetic engineering work in animal husbandry? He told us in Genesis 31:11 that he got it in a dream.

Someone has sometime said that a breakthrough comes with 1% inspiration but 99% perspiration. If this is true, from my own experience; the 1% inspiration governs the 99% perspiration. Many people do not know what to do despite the fact that they are ready to pay the price of 99% perspiration. Others who are doing research are doing wrong things because of the lack of 1% inspiration. The only way to do a meaningful research in a correct manner especially in discouraging and hostile conditions is to have the 1% inspiration. The 1% inspiration is like a driving force which helps the researcher to keep on even when the going becomes very tough.

8. Conclusion

Many developing countries are still poor because they have no direct involvement in modern biotechnology. Although the development of Genetic Engineering Laboratory may be too expensive for the poor nations but investment in fermentation technology is within the reach of all. Priorities should be given to creating favourable conditions for the development of small-scale industries by reduction in import tariff for industrial equipment and different from of tax relief for upcoming Biotech

companies.

Research Institutes and Universities should constantly conduct seminars and workshops to showcase their technology for the Entrepreneurs. Since government has no business in business, the Biotech companies should be left to people in the private practice. However, government should adequately fund Biotech research institutions. Researchers should make adequate use of fund made available to them. They should also avail themselves with the 1% inspiration to be able to cope with frustrations associated with research work and to be relevant to the prevailing circumstances.

Acknowledgment

I wish to acknowledge my parents who took care of me. It is through them I am what I am. I thank the excellent research students for maximum cooperations. I want to appreciate the effort of different research colleagues and associate both within and outside Nigeria. I appreciate my wife and Children for their understanding and support.

I thank the Vice-Chancellor for allowing me to make this presentation. Finally, I want to strongly acknowledge the excellent contribution of the Holy Spirit at each stage of my life. He has never failed to provide the 1% inspiration. He is my greates mentor.

Thank you all.

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