Nano Plus: Sci. Tech. Nanomat. 2 (2021) 27 – 65
https://doi.org/10.48187/stnanomat.2021.2.003

Nano Plus: Science and Technology of Nanomaterials
Journal home page: https://www.stnanojournal.org/
ISSN: 2782-8166 (print) 2782-8174 (electronic)

The Next Big Thing is Very Small: The Paradox of Diminutive Microbes and Nanoparticles *

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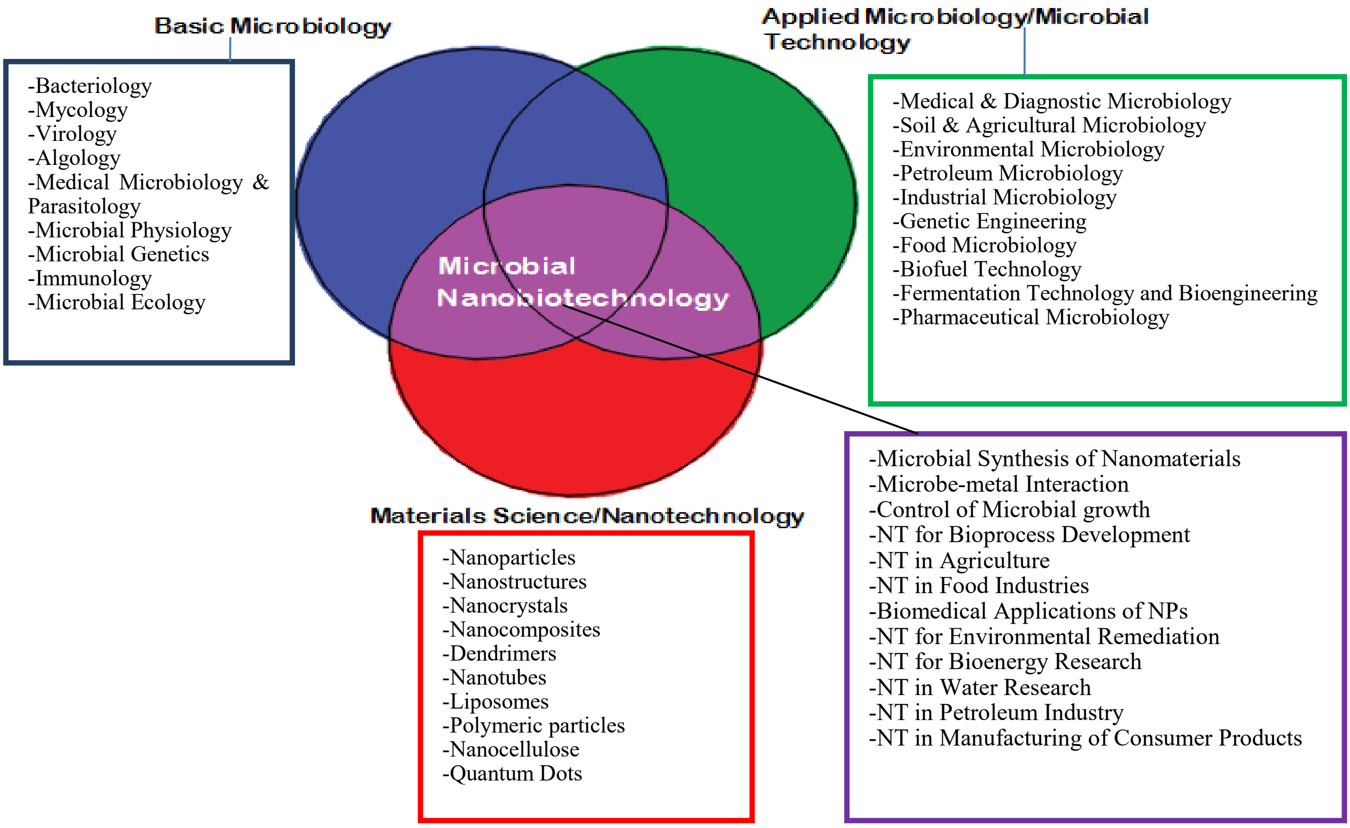
Highlights

- It chronicles research activities of the author covering microbiology, biotechnology and nanotechnology in a span of 22 years as presented in his inaugural lecture
- It documents the importance of microbes and nanoparticles for creating new range of products and wealth
- It establishes the fusion of life sciences with nanotechnology in the bourgeoning field of nanobiotechnology
- It addresses issues that can stimulate the interests of microbiologists in nanotechnology research

Graphical Abstract



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*The paper is an abridged version of the text of 38th inaugural lecture of Ladoke Akintola University of Technology, Ogbomoso, Nigeria that was delivered by the author (A.L) on 25 February, 2021.

Nano Plus: Sci. Tech. Nanomat. 2 (2021) 27 – 65
https://doi.org/10.48187/stnanomat.2021.2.003

Article info

Received: May 10, 2021
Revised: June 7, 2021
Accepted: June 8, 2021

Keywords:
Microbiology,
Biotechnology,
Nanotechnology,
Nanobiotechnology,
Bioeconomy

Abstract

This article chronicles the author's research activities in about twenty-two years, and a worthy enterprise of the intertwining patterns of microbiology, biotechnology and nanotechnology to document advances in this area. It details the pioneering roles at initiating researches in biotechnology to produce fructooligosaccharides, and microbial valorization of agrowastes. It also discusses nanobiotechnology where microbes, plants and animals have been used to produce nanoparticles for biomedical, environmental, agricultural and engineering applications. It was affirmed that although microorganisms consist of the good and the bad ones; the pathogenic microbes causing diseases in plants and animals are less than 1% of the hypothetical one trillion types of microorganisms that exist on earth. The contributions of microorganisms and nanoparticles in terms of their product formation were estimated at hundreds of billions of dollars. For instance, the contribution of biotechnology to the world's economy was put at $714.6 billion in 2021. Similarly, estimated cost of nano-based products was put at $2.6 trillion in 2015, with the expectation to reach 3 trillion in 2020. As an industrial microbiologist, the author has employed microbes to produce novel products that included biofertilizer, fructooligosaccharides, citric acid, biogas, industrial enzymes such as laccase, xylanase, keratinase, and fructosyltransferase. Other investigations included biofabrication of metal nanoparticles such as silver, gold, titanium oxide, calcium and silver-gold alloy for diverse applications, as well as fabrication and deployment of bioreactors for growing microorganisms. In a unique manner, a loop connecting basic microbiology, microbial technology and nanotechnology to herald the sub-discipline of microbial nanobiotechnology which is a gold mine was established. This represents a paradigm shift in the fusion of knowledge of immense benefits in developing self-reliant manpower in life sciences. The article underscored the importance of microbiology, noting that the field should be professionalized in Nigeria for the country to reap immense benefits inherent in the attributes and applications of microbes. It was concluded that microbes and nanoparticles although very small, will continue to define the development of man and contribute in no small measure to the world economy and development of creative products to improve the quality of life on earth.

1. Introduction

Microorganisms are living organisms that are very small in size, which cannot be seen with unaided eyes. They are therefore said to be microscopic, indicating a need for them to be magnified before they can be seen. This can be done using magnifying lenses or combinations of such. The study of these organisms was pioneered by a Dutchman, Anton Van Leeuwenhoek in 1667, the father of microbiology; when he used the microscopes that he invented to examine samples of living and non-living things, which could magnify up to 266 times [1]. He was surprised to have seen tiny objects in those samples [2], as he called them '*small animalcules*'. This subject is what is termed microbiology. Therefore, microbiology (Greek, *micros = small, bios = life, logos = science)* is the study of minute living organisms called microorganisms. Largely, it involves the study of bacteria, fungi, viruses, algae, actinomycetes and protozoans.

In the beginning, microbes were discovered to be responsible for causing diseases, putrefaction and fermentation. All these stemmed from the works of notable scientists such as Robert Koch and Louis Pasteur [3]. However, at the turn of 19th century, with the advent of sophisticated microscopes and general increase in the body of knowledge, more detailed studies about microbes revealed that they can serve useful purposes to mankind. These led to developments in industrial microbiology and biotechnology [4]. It is now clear that microbes have useful applications in all facets of human endeavour; despite the huge burden that they constitute to man. To this end, whole cells of microbes or their metabolites such as enzymes, organic acids, amino acids, polyhydroxylbutyrates, biosurfactants, volatile organic compounds, antibiotics, pigments among others have been applied in different areas to render goods and services for mankind [5]. Some microbes termed probiotics are deliberately consumed to improve the physiological well-being of the consumers. These include *Streptococcus thermophilus, Lactobacillus casei,* and *Bifidobacterium bifidus.* In 2016, the global market for probiotics was worth $36.6 billion, and Business Communications Company (BCC) Research [6] estimated that it would be valued at $57.2 billion by 2022. Other microbes that are very rich in proteins such as *Aureobasidium pullulans, Saccharomyces cerevisiae, Candida utilis* and *Spirulina platensis* are termed as single cell proteins (SCPs) and are also consumed as supplements as replacement for animal proteins with the market share predicted to reach $8.7 billion by 2023 [7]. There are also microbes that ordinarily should not affect us in any manner (except in cases of opportunistic infections and in immunologically compromised conditions); the commensals, such as *Staphylococcus epidermidis.*

Therefore, microbes are necessary evil that man must live with to advance his course on earth. In commerce, microbial-enhanced processes for the production of different products run into hundreds of billions of dollars. For instance, the world production of enzymes, organic acids and alcohols through microbial fermentation stood at $41.568 billion in 2012 [8] and expected to reach $63.371 billion in 2020 at annual growth rate of 5.4%. In India, $125 million was generated from sales of fermentation products in 2015 [8]. Thus, microbiology is a major participant in global industry and will be a major player in the new bioenergy industry, hopefully to replace petroleum within the next 30 years.

Today, microbiology is studied in different areas that seek to interrogate the roles of microbes in the environment with practical applications in agriculture, engineering, medicine, biotechnology and so on [9]. Such cosmopolitan investigations have led to sub-disciplines of soil, industrial, medical, petroleum, food, environmental, pharmaceutical, and agricultural microbiology as areas of practice. Its other branches include microbial physiology and metabolism, microbial genetics and molecular biology, microbial immunology, analytical and diagnostic microbiology, virology, mycology, bacteriology, algology, and parasitology.

Since the advent of early man on the earth, ways have been sought towards improving the quality of life; to fight diseases, increase life expectancy and create new range of products to meet human daily needs. In the quest for modernization, man has utilized many tools to achieve quality and healthy living; including the use of biological objects. Today, the use of biological objects, their parts or metabolites to render goods and services for mankind has given birth to a new branch of life sciences; which is biotechnology [4]. Therefore, growth and development of any nation and indeed the whole world cannot be discussed without reference to contributions from biological objects (plants, animals and microbes). In this connection, man has utilized several microorganisms in a positive manner (Table 1). Conversely, within the context of development, man has modified the ecosystem in different ways with its attendant consequences including the creation and dissemination of drug resistant microbes, pollution, environmental degradation, bioterrorism, desertification, and instigation of global warming that encourages multiplication of pathogenic microbes to cause conflict between man and the environment [10]. Thus, man stands on the brink of health cataclysm of transnational dimension due to the modification of the ecosystem over time. As a key component of biological resources, microbes must be handled with utmost care. Therefore, man, microbes and development are intertwined in such an intricate manner that must be well understood, for man to ensure healthy living on earth.

2. Biotechnology

Biotechnology though can be defined in

several ways; however, it can be simply put as the use of biological resources (whole cell, parts, metabolites or genetic resources) to render goods and services for mankind It is viewed as exploitation of bioresources by man. It can be divided into two components; traditional biotechnology and modern biotechnology (Table 2). While traditional biotechnology is as old as man, modern biotechnology that deals with the genetic manipulations of organisms is of recent advent (Okafor, 2007). Man has practiced biotechnology in the forms of plant and animal cross -breeding, grafting, fermentation (production) of foods, condiments and drinks such as *garri, fufu, iru, ogiri, lafun, tempeh,* yoghurt, *ogogoro, burukutu, nunu,* and bread.

Without microorganisms, fermentation processes are impossible, because microbes with the retinue of enzymes breakdown the complex organic molecules and convert them into the final products with new qualities and value addition [12]. Among several things, fermentation can be employed to achieve food safety and security through preservation (lowering of pH by producing organic acids that limit the proliferation of putrefactive organisms and antimicrobial factors like bacteriocin and nisin), improved nutritional quality (protein enrichment), detoxification (removal of anti-nutritional factors and toxic principles), improved consumer appeal (enhanced digestibility and flavouring) and production of nutraceuticals to promote physiological well-being to fight debilitating disorders including obesity, cancer and arteriosclerosis.

In addition to applications in food production, fermentation can be employed to produce a number of products of immense use to mankind. For instance, citric acid production is the exclusive preserve of a fungus, *Aspergillus niger* [13] which has been used since

Table 1: Some of the applications of microorganisms

S/N	Sector/area	Applications
1	Agriculture	Biofertilizers, *Rhizobium* inoculants, nutrient cycling, microbial insecticides
2	Pharmaceutical	Antibiotics e.g. Penicillins; useful metabolites e.g. growth factors, amino acids, steroids
3	Environment	Biodegradation and bioremediation of organic matters, industrial effluents and xenobiotics
4	Waste conversion	Waste to wealth e.g. organic fertilizers; use of organic wastes in mushroom production
5	Fossil fuel	Microbial enhanced oil formation, recovery and uses
6	Industries	Enzymes as organic catalyst; organic acids e.g. citric acid; flavours
7	Food	Fermentation processes e.g. yoghurt, cheese, bakery products, *garri*, *fufu*, *iru*, *ogi* etc., starter cultures, sweeteners; food additives e.g. xanthan gum, biopreservatives e.g. nisin and bacteriocin; single-cell protein
8	Drinks	Fermentation to produce alcoholic and non-alcoholic drinks
9	Healthcare	Vaccine production, healthy foods (nutraceuticals), prebiotics, probiotics
10	Solid minerals	Recovery of metals, bioleaching and biomining
11	Renewable Energy	Renewable energy; biogas, bio-hydrogen, biodiesel and microbial fuel cells.
12	Diagnostics/ analytical	Biosensors, enzymes as analytical reagents e.g. GOD-POD for glucose determination
13	Forensics	Taq polymerase for DNA amplification; endonucleases, ligases etc.
14	Bioeconomy	Starter culture, enzymes, citric acid, biosurfactants, single cell proteins

Table 2: The types of biotechnology

Type	Other names	Nature	Examples
Traditional	Low-level	Old (since ancient times), low technology, simple, and low-cost	Production of enzymes, fermented foods (*garri, iru, fufu*), sewage treatment, biogas, mushroom cultivation, cross breeding of plants and animals for desirable characters, biofertilizers (*Rhizobium* inoculant, compost, mycorrhizae), biopesticides, SCP, algal technology (*Spirulina platensis*), tissue culture etc
New	Modern biotechnology, rDNA Technology, Cloning, Genetic engineering, molecular biology.	New (started in 70's), complex, capital intensive, involves manipulation of genetic material.	Creation of transgenic plants and animals, and recombinant microbes for diverse applications, gene therapy, GM foods, probes, markers, drug discovery, forensics, genomics, diagnostics, biochip etc.

1919 with world production that exceeded 2 million tons in 2018 and worth $2.545 billion [14]. Wide ranges of products that are listed in Table 1 are produced through fermentation by microbes for various applications, with enormous contributions to the world's economy. The global market for bioproducts should reach $714.6 billion by 2021 from $466.6 billion in 2016 at a compound annual growth rate (CAGR) of 8.9%, from 2016 to 2021 [15].

Modern biotechnology utilizes wide range of techniques to manipulate the genetic constituent of an organism in such a way that the genetically modified organisms (GMOs) depict new set of attributes that are not known to the natural forms (wild type) of the organisms. Through this technology, transgenic plants and animals and recombinant microbes have been created with unique properties [16]. For instance, golden rice with the ability to synthesize β-carotene, the precursor for the synthesis of vitamin A to forestall vitamin A deficiency among consumers of rice has been produced [17]. Also, *Bt*-cotton that has the ability to produce δ-endotoxin of entomopathogenic *Bacillus thuringiensis* has been produced with the ability to prevent insect infestation of the crop, thereby reducing the use of insecticides [18]. Through modern biotechnology, a bacterium, *Escherichia coli* can be used to produce human insulin for diabetic patients with the world production valued at $26.64 billion in 2016 [19]. Similarly, plants with vigour, tolerance to environmental stresses, improved yield, enhanced nutritional qualities, and shorter life cycle have been created to combat hunger and food insecurity[20]. Nigeria has recently licensed the commercial production of genetically modified pod borer-resistant cowpea (PBR Cowpea)-event AAT709A, genetically improved to resist *Maruca vitrata* (responsible for 70 -90 yield loss) [21]. Cultivation of the improved GM cowpea would reduce spraying with insecticides from eight to two times with yield expected to increase by 20%. Nigeria is projected to earn $132 million annually from the cultivation of the *Bt* cowpea which has been found to be safe for both human and animal consumption [21]. Biotechnology is a multidisciplinary field of study (Figure 1).

A biotechnologist can utilize techniques derived from chemistry, microbiology, biochemistry, chemical engineering and computer science. Chemical engineering and biochemistry are two well recognized examples of disciplines that have done much to clarify our understanding of chemical processes and the biochemical bases of biological systems, while advances in computer science are exploited in the monitoring and control of fermentation processes as well as computational analysis of data. Of course, microbiology is the bedrock of biotechnology with several microbes involved in the fermentation processes, and microbes being the sources of enzymes and vectors of gene transfer that are used in genetic engineering protocols [22]. The main objectives of biotechnology are innovation, development and optimal operation of processes in which biochemical

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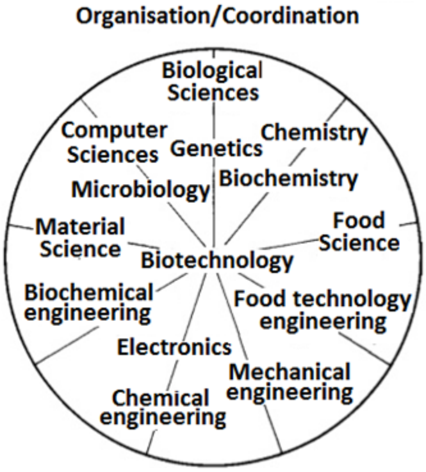

Fig. 1: The interdisciplinary nature of biotechnology

catalysis has a fundamental and irreplaceable role. Biotechnologists must also aim to achieve a close working cooperation with experts from other related fields; such as medicine, nutrition, the pharmaceutical and chemical industries, environmental protection and waste process technology. Biotechnology has two clear features: practical applications and interdisciplinary cooperation. Table 3 summarizes different applications of biotechnology using the colour coding system.

3. Nanotechnology

Nanotechnology is the art of creation, manipulation, investigations and applications of materials at the nanoscale (10^{-9} m or 1 billionth metre). It is in the core area of materials science, which had its origin from the lecture delivered by Prof. Richard Feynman in 1959 [23]. Feyman, a physicist and 1965 Nobel Laureate in his lecture titled "*There's Plenty of Room at the Bottom*", at the meeting of American Physical Society at California Institute of Technology, USA introduced the concept of manipulating matter at the atomic level [24]. He queried if it would be possible to put the 24 volumes of Encyclopedia Britannica on the head of a pin. This novel idea demonstrated new ways of thinking and Feynman's hypotheses have since been proven correct. It is for these reasons that he is considered the father of modern nanotechnology. However, it was a Japanese scholar, Prof. Norio Taniguchi of Tokyo Science University, Tokyo that first used the term

Table 3: Applications of biotechnology using the colour coding system

Colour	Applications
Red	Health, medical, gene therapy, regenerative medicine, vaccines and antibiotics, developing new drugs, molecular diagnostics techniques
Yellow	Food biotechnology, nutrition science
Blue	Aquaculture, coastal and marine biotechnology
Green	Agricultural, biofertilizers, biopesticides, transgenic plants and animals
Brown	Arid zone and desert biotechnology
Dark	Bioterrorism, biowarfare, biocrimes, anticrop warfare
Purple	Patents, publications, inventions, intellectual property rights
White	Gene-based bioindustries, biocatalysis, enzymes, chemicals, design and production of new materials for daily use
Grey	Environmental biotechnology, biofuels, bioremediation, geomicrobiology
Gold	Nanobiotechnology, bioinformatics, computational biology

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'nanotechnology' in 1974 to describe semiconductor processes occurring at nanometer [25]. Further, leveraging on the contributions of Feynman and Taniguchi, Prof. Eric Drexler of Massachusetts Institute of Technology (MIT), USA brought in the golden era of nanotechnology in 1986 in his book titled 'Engines of creation: The coming era of nanotechnology', where he proposed the idea of nanoscale 'assemblers' [26] that laid the foundation of molecular nanotechnology. These three scholars are recognized as champions of nanotechnology.

The fundamental issue about nanomaterials having dimensions in the range of few nanometers is how they differ from their bulk precursors. However, the changes in optical, electrical, biological, photothermal, physical and chemical properties have been linked to their improved surface area to volume ratio that enhances their reactivity. Nanomaterials are of various types; natural and man-made. The examples of natural materials of nanoscale dimensions include fine dust, volcanic ash, viruses and DNA. However, several nanomaterials can by synthesized via physical, chemical and biological methods. The engineered or synthetic nanomaterials can be inorganic (metal and metal oxide nanoparticles), organic (liposomes, dendrimer) and carbon-based (Graphene, fullerenes, Quantum-dot, carbon nanotubes). In the last four decades, several novel nanomaterials have been produced for applications in different areas of human endeavours (Table 4); ranging from agriculture, through medicine, engineering, environment to consumer services.

Nanotechnology is contributing to the world's economy (Table 5) and its adoption has led to production of novel materials, and rendering of specialized services, with the projection that by 2020, about 2 million workers in the US would have nanotechnology-related jobs, and the US market value of nano-products would be $1 trillion, or 5 % of the GDP of US [27]. It was projected that the world market of products containing nanomaterials would be $2.6 trillion in 2015 [28]. The beauty of nanotechnology as it is with biotechnology is its cosmopolitan nature in both practice and application. Everyone can have a bite of the cake as '*There's Plenty of Room at the Bottom*'. Series of data have shown steady rise in the number of articles published in nanotechnology, number of patents and investment by several countries (Figures 2-4). It can be deduced from these data that the major players in nanotechnology are advanced countries, notably US, Japan, Germany, South Korea, France, UK and Russia. Other countries like China, Taiwan, India, and Canada also featured prominently in patent filing and manufacturing of nano-based products. However, Africa lags behind with Egypt and South Africa having some sorts of investments in nanotechnology, policy on nanotechnology development and fair contributions to nanotechnology outputs (patents and publication). Nigeria's status in nanotechnology is abysmal, without any patent in USPTO, almost no budget heading or investment in nanotechnology, low-level of publications, low-level of public awareness about nanotechnology, deficiency of curricula of science and technology courses in nanotechnology, and dearth of experts in nanotechnology among others [42, 43]. For instance, nanotechnology–based article per million people in Nigeria in 2017 was just about one article, indicating contribution of less than 200 articles on nanotechnology and there was no patent in USPTO and EPO in the coverage period of 2015-2019 [44]. There is also dearth of equipment necessary to carryout nanotechnology research in Nigerian institutions as well as lack of centre of excellence in nanotechnology, and regulatory agency on nanotechnology. A comparative analysis of Nigeria and South Africa in nanotechnology R&D is presented in Table 6. Unlike Nigeria, where there is no dedicated fund for nanotechnology research, South Africa has streams of fund specifically for nanotechnology. These include nanotechnology flagships project (NFP) for emerging researchers, and national nanotechnology equipment pro-

Table 4: Some important applications of nanomaterials

S/N	Sector	Applications	Nanomaterials
1.	Agriculture	Pesticide, fertilizer, tissue culture	AgNPs, TiONPs, ZnONPs
2.	Environment	Degradation and adsorption of pollutants	AgNPs, TiONPs, ZnONPs
3.	Food	Antimicrobials, preservation, packaging, nutrient enhancement and bioavailability	AgNPs, TiONPs, ZnONPs, MgONPs, CaONPs
4.	Energy	Solar panel, super capacitors, fuel cells	AgNPs, TiONPs, ZnONPs, AuNPs, CNTs, graphene
5.	Healthcare	Antimicrobial, imaging, drug-delivery, tissue engineering, anticancer, antioxidant, antidiabetic, wound healing, dentistry	AgNPs, TiONPs, ZnONPs, AuNPs, graphene, CaONPs, dendrimer, liposomes, CNTs
6.	Engineering	Electronics, smart appliances, construction, novel material composites	AgNPs, TiONPs, ZnONPs, AuNPs, graphene, CNTs, SiONPs
7.	Water	Treatment and purification	AgNPs, TiONPs, ZnONPs, AuNPs, graphene, CNTs, Nanoclay
8.	Consumer products	Antimicrobial, anti-aging, sunscreen, UV-shielding, lightness and improved strength, fire-retardant, preservative	AgNPs, TiONPs, ZnONPs, AuNPs, graphene, CNTs
9.	Defense and security	Antimicrobial, water repellant and self-cleaning, exceptional strength	AgNPs, TiONPs, ZnONPs, AuNPs, graphene, CNTs
10.	Industries and analytics	Nanocatalysts, sensors, fuel-cell catalyst, anti-corrosion, oil-drilling, composites	PtNPs, TiONPs, ZnONPs, PdNPs, graphene, CNTs, AgNPs, AuNPs

gramme (NNEP) to support researches and procure state-of-the-art equipment [45].

4. Microbiology, Biotechnology and Nanotechnology: The Nexus and a Worthy Enterprise

Nanomaterials are generally produced through two approaches; top-down and bottom-up (Figure 5), where larger molecules are broken down to nanomaterials and atoms are built-up to form nanomaterials, respectively. Both physical and chemical techniques have been used to fabricate nanomaterials and these include high energy ball milling, sintering, melt mixing, sol-gel, inverse micelles, laser ablation, sputter deposition, electric arc deposition, chemical vapour deposition, hydrothermal, sonication, and irradiation among others. However, these techniques are plagued with consumption of high energy, high cost of production, complexity in reaction, and non-ecofriendly and toxic procedures. These drawbacks can be avoided through greener synthesis using biological materials that are rich in biomolecules for the catalysis of formation of nanomaterials through bottom-up approach. The green process also termed biosynthesis, biomimetic, biogenic, bio-inspired or green synthesis is an economical process, simple, rapid, facile, eco-friendly, environmentally-benign and often yields more biocompatible nanomaterials that are devoid of toxic principles [48]. In this connection, several biological materials and biomolecules derived from plants, bacteria, fungi, algae, actinomycetes, insects, and other animals have been utilized to produce nanomaterials for diverse applications [49-53].

While the synergy between microbiology and biotechnology is well established, we may begin to wonder if such relationship exists between microbiology and nanotechnology. For ease of explanation, the relationship between microbiology and nanotechnology can be summarized as follows:

i. Several microbes, particularly bacteria, fungi and algae have immense abilities to tolerate, sequester, accumulate and detoxify metals through series of redox reactions. These attrib-

Table 5: Contributions of nanotechnology to the World's economy

S/N	Sector, product or application	Worth (USD billion) and year	References
1.	Energy	5.7 (2018)	[29]
2.	Medical	151.9 (2017)	[30]
3.	Printing technology	14.0 (2013)	[31]
4.	Environment	23.4 (2014)	[32]
5.	Nanomachines and devices	0.736 (2017)	[33]
6.	Drug development and delivery	29.6 (2014)	[34]
7.	Silver nanoparticles	1.3 (2017)	[35]
8.	Gold nanoparticles	1.3 (2014)	[36]
9.	Titanium dioxide nanoparticles	3.4 (2014)	[37]
10.	Graphene	0.0428 (2017)	[38]

utes have made them to be useful tools in the microbial remediation of metal-polluted soils and wastewaters, and for exploitation in the recovery of precious metals such as silver, copper, gold, lead and zinc via biomining and bioleaching in the fields of geomicrobiology and geobiotechnology. Thus, there is a special relationship between microbes and metals in terms of uptake, processing, utilization and cycling in nature. Economic viability of bioleaching of metals from polymetallic ore using bacteria has been demonstrated [54].

ii. In the bid to detoxify metals, microbes can use enzymes, proteins and pigments to reduce metal ions to metallic nanoparticles, which can then be accumulated within the cell or excreted out of the cell. Thus, either intra- or extracellularly, microbes can serve as cell factories to reduce various metals to zero valent species; which is the hallmark of the microbial synthesis of metallic nanoparticles [55].

iii. Conversely, metallic nanoparticles have been proven to have biocidal actions on bacteria, fungi, algae, protozoans and viruses. The biocidal activities of the particles are attributed to the generation of free radicals, reactive oxygen species, and denaturation of macromolecules such as proteins, enzymes and DNA amongst others [56]. The particles can also serve as carriers of drugs into the cell with improved surface area of activity. The use of nanoparticles in combating the scourge of multidrug resistant microbes is well documented in literature [57]. The multiple actions of nanoparticles have placed them at an advantage over antibiotics in suppressing resistance mechanisms among microbes. As

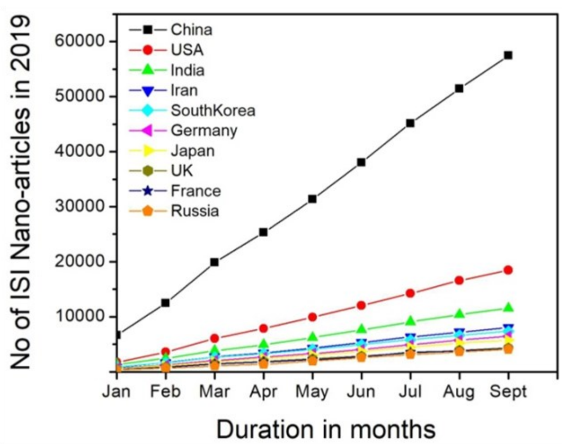

Fig. 2: Number of ISI nano-articles published in 2019 by 10 leading countries in nanotechnology [39]

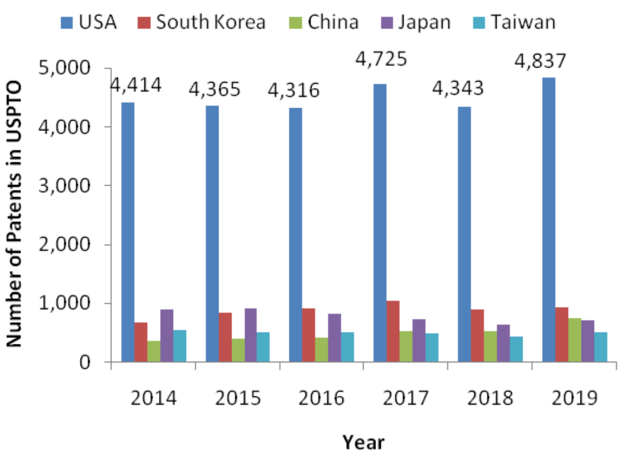

Fig. 3: Number of patents in nanotechnology in USPTO by five leading countries in nanotechnology [40]

such, nanoparticles have found diverse applications as coating materials to prevent microbial growth and survival (for instance in surgical instruments and textiles), filters for the purification of water, as additives in paints to prevent microbial deterioration and packaging materials in food industries. The global market of nanoparticles in biotechnology and pharmaceuticals was put at $25 billion in 2013, and projected to reach $79.8 billion in 2019 [58].

iv. In bioprocess development, it has been established that supplementation of growth media with nanoparticles (albeit at low concentrations) instead of the bulk form of metals (salts) can cause metabolic perturbation in microbes, thereby improving the performances at producing novel products [59]. Recently, we showed that nickel nanoparticles improved ethanol yield and protein accumulation in *Saccharomyces cerevisiae* [60], while other studies have shown positive impacts on biogas and biohydrogen yields by bacteria [61]. Some nanoparticles, particularly magnetic particles have also been applied as resin for the one-time purification of microbial enzymes [62]. Similarly, nanoparticles have been deployed for the immobilization of enzymes to enhance performance and reusability [63]. The impact of nanobiotechnology in bioprocess development is an evolving field whose results would not only be stimulating but a paradigm shift in fermentation process that would open new vista of research in microbial physiology and metabolism.

v. Products of microbial transformation can be enhanced in their activities through nanotechnology. For instance, the dispersion of oil in water and leaching of crude oil from soil matrix by microbial surfactants can be enhanced by surfactant-nanometal hybrid [64], which may lead to the development of novel nano-based biosurfactant for enhanced oil recovery, especially from marginal fields. Nanoparticles have also shown to enhance microbial transformation of xenobiotics [65] to control pollution.

From the foregoing, it can be established

Table 6: Comparative performance of South Africa and Nigeria in nanotechnology R&D

Indices	South Africa*	Nigeria**
Articles per million people	16.51	1.36
No. of nano-based products	14	0
No. of nano-companies	9	0
Patents in USPTO (2015-2019)	20	0
Nanopatents per 100 articles	1.06	0
Nanotechnology standards	11	0
ISI-indexed nano-articles in 2019	1151	408
Agency on public engagement	Yes, NPEP	No
Priority funding of nano-research	Yes; NRF#	No
National strategy on nanotechnology	YES, NNS	Yes, NIN but moribund

*[46];**[44]; NPEP, Nanotechnology public engagement programme (https://www.npep.co.za/about-npep/); #[45]; NNS, National nanotechnology strategy; NIN, National initiative on nanotechnology.

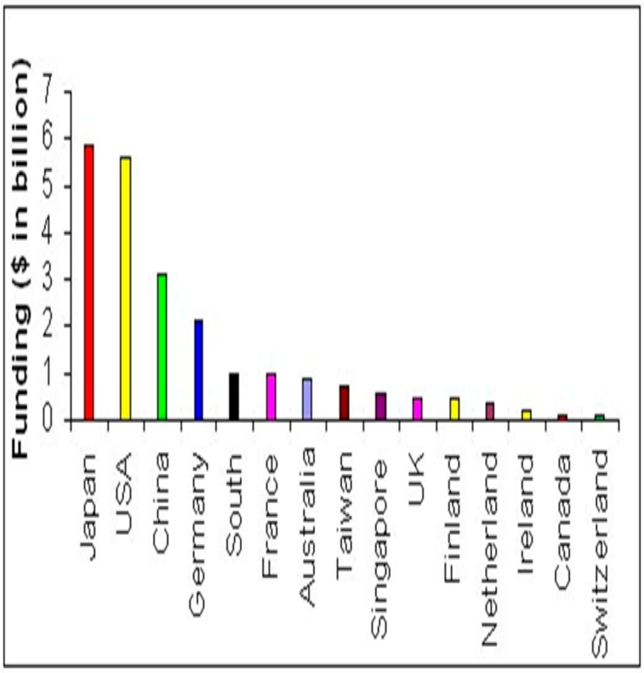

Fig. 4: Investment in nanotechnology by different countries from 2006-2010 [41]

that nanotechnology is of relevance to microbiology in the areas of microbial synthesis of nanoparticles, control of growth of microbes by nanoparticles and utilization of nanoparticles to improve the performance of microbes in fermentation processes to produce novel products. Nanomaterials are also important components of downstream process in fermentation for the recovery of products. In totality, there is concordance among the tripartite fields of microbiology, biotechnology and nanotechnology. Further, the union of biotechnology with nanotechnology birthed nanobiotechnology, which belongs to the section of 'gold biotechnology' as earlier presented in Table 2. This exposition lay to rest the argument about impropriety of foray of a microbiologist in the field of nanotechnology, as there is convergence between the two disciplines [66]. In actual fact, nanotechnology has been recommended as a novel tool for microbiologists to advance their research activities [67]. However, much is still needed to be done to elucidate the impacts of nanomaterials on microorganisms in terms of physiology, metabolism and genomics.

5. Is there any Gap in Knowledge between Microbiology and Nanotechnology?

The seemingly apathy of microbiologists to developments in nanotechnology is rooted in '*nanophobia*' which pervades several other disciplines in the sciences. Nanotechnology is often viewed as an area of research that is reserved exclusively for the experts in materials science, solid state physics and engineering fields. While nanotechnology has its roots in physics, it has extended its tentacles to allied fields. Today, it is at the interface of physics, chemistry, and materials science, thus necessitating that any serious investigation in the field would require some modicum of knowledge in the aforementioned areas. This requirement poses some constraints to many microbiologists; thereby limiting their engagements in nanotechnology research.

The challenges can be addressed through collaboration whereby nanotechnology-related researches could be executed by postgraduate students in order to train them and stimulate their interests in nanotechnology to produce new crops of microbiologists with proficiency in nanotechnology. Similarly, advanced studies on the interactions of microorganisms with nanomaterials should be vigorously pursued to open up new lines of researches.

Secondly, there is limited exposure of microbiologists to nanotechnology as both undergraduate and postgraduate curricula of microbiology lack coverage of materials science and nanotechnology [43]. To solve this problem, microbiology curriculum must be re-engineered to accommodate discourse on nanotechnology. Worldwide, there are limited textbooks on microbial nanotechnology and the concept of nanotechnology in microbiology [68, 69], although there are excellent reviews on specific aspects of nanotechnology in microbiology and vice-versa [65, 70-72]. Therefore, top researchers at the frontiers of microbiology and nanotechnology have the responsibility to evolve curriculum that would integrate principles and applications of nanotechnology into microbiology, and also produce reading texts for the budding microbiologists. These are parts of the vision of the author as a microbiologist, biotechnologist and nanobiotechnologist.

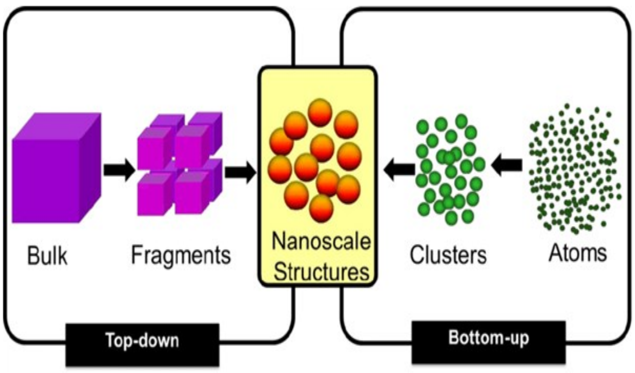

Fig. 5: Top-down and bottom-up approaches in the fabrication of nanomaterials [47]

6. Research Activities of the Author

An overview of the research activities of the author that cuts across microbiology, biotechnology and nanobiotechnology is hereby presented in fulfilment of the elucidation of the enormous importance of microbes and nanoparticles. Though, about 127 articles have been produced by the author in reputable journals and books; only a fraction of these publications are projected in this article.

6.1 Microbiology of Water

Water is a valuable resource for the survival of all forms of life in the ecosystem [73]. Its quality and availability are prime requirements in the society. Thus, efficient microbiological control is essential for the implementation of good management of this vital resource. In Nigeria, water is sought from different sources which include rainfall, streams, rivers, wells and boreholes for different use. Also, treated water is made available by water treatment plants (tap water) and packaged water (sachet and bottled). Irrespective of the source of water, there are basic microbiological requirements of potable water. It becomes imperative to evaluate the microbiological quality of water to ensure the safety of public health, as several pathogens can survive in water and instigate waterborne diseases such as cholera, dysentery, diarrhoea, typhoid fever, and shigellosis [74]. The microbiological standards of potable water in Nigeria stipulates maximum limit of 10 cfu/ml for total coliform count and complete absence of thermotolerant coliform, *E. coli,* faecal *Streptococcus* and *Enterococcus* in 100 ml of water [75].

In this connection, the author has conducted several studies, aiming at determining the microbiological safety of drinking water from different sources including; rain, tap, shallow wells, boreholes, ice and packaged water in Nigeria [76, 77]. These studies established contamination of many of the water and ice samples by microorganisms, and were adjudged not to be fit for human consumption. In an investigation spanning four months, forty samples of commercial ice used for the cooling of fish and drinks were obtained from small-scale producers of ice in Ogbomoso and examined microbiologically [76]. All the samples were contaminated with bacteria (Table 7), had microbial index of 10^4, which exceeded the limits of <500 and <10^2 cfu/ml for ice obtained from manufacturing plant and retail outlet respectively [78]. Many of the isolates obtained from the ice samples were pathogenic with public health concerns.

We have also probed the quality of one hundred water samples obtained from ten boreholes within Ogbomoso metropolis [77]. The physico-chemical attributes revealed that the ammonia, manganese, nitrate, nitrite, fluoride, chloride contents, conductivity and total dissolved solids were below the permissible levels. However, total alkalinity and total hardness values of some water samples were higher than the permissible levels, while all

Table 7: The microbiological attributes of commercial ice samples

Source	Type of ice	Microbial load (cfu/ml)*	Isolates**
A	Bar	1.88×10^4	*Pediococcus cerevisiae, Bacillus subtilis, Streptococcus s*p *pyogenes, Bacillus firmus,* and *Pseudomonas aeruginosa*
B	Shaved	2.19×10^4	*Streptococcus equi* and *Bacillus firmus*
C	Cube	3.10×10^4	*S. equi, Staphylococcus epidermidis, S. pyogenes,* and *Micrococcus luteus*
D	Shaved	3.20×10^4	*M. luteus* and *P. aeruginosa*

*, each value is an average of ten samples; **distinct isolates

the water samples had biochemical oxygen demand (BOD) and chemical oxygen demand (COD) values that were higher than the permissible levels. The microbial quality of the water samples indicates extensive microbial contamination involving heterotrophic bacteria, coliforms, yeasts/molds, staphylococci, and *Shigella.* The cumulative microbial loads of the water samples ranged from 3.14 to 8.60 $\times 10^3$ cfu/ml. Bacteria in the genera *Proteus, Escherichia, Shigella, Streptococcus, Staphylococcus, Bacillus, Pseudomonas, Enterobacter,* and *Klebsiella* were isolated in the study.

Similarly, studies have been conducted on natural bodies of water to determine the level of pollution due to human activities. Studies on Odo-Oba and Oyun rivers showed high level of microbial contamination. Odo-Oba river receives human-induced wastes and leachates from nearby refuse dumps and cassava flake (*garri*) processing centres, run-off from farms, deposition from air as a result of heavy vehicular traffic on Oyo-Ogbomoso road. Twenty-one samples analyzed over a period of seven months recorded microbial loads of 2.50×10^2 to 9.40×10^4 cfu/ml, with high MPN of ≥ 1800, presence of faecal coliforms and *E. coli* [79]. Similarly, Oyun river in Ilorin, Kwara State had mean microbial loads in the range of 3.83×10^5 to 7.84×10^5 cfu/ml with MPN of ≥ 1800, presence of faecal coliforms and *E. coli* [80]. Specifically, *E. coli, S. aureus, P. aeruginosa, Klebsiella* sp and *Enterobacter* sp were isolated from the eighteen water samples taken over a period of six months.

The results obtained in these studies with the analysis of two hundred and fifty-six samples of water from different sources indicate that 91.80% of the water samples were not fit for human consumption as they did not meet the required microbiological quality [75]. However, water obtained from these sources, including the rivers is being used for drinking and other activities by people. Thus, there is the need to prioritize the provision of potable water and popularize sanitation among populace as critical ways of preventing the scourge of water-borne diseases. Suffice to say that available clean water and sanitation is the goal 6 of sustainable development goals. Non -availability of clean water is a global problem. As of 2015, 2.3 billion people lacked basic sanitation and 844 million people did not have access to clean water [81].

6.2 Microbiology of Industrial Effluents and Genotoxic Studies

The rapid industrialization in the world is not without consequences. Among the consequences of industrial activities is the discharge of untreated or partially treated industrial wastewaters in the natural water bodies that lower the quality of such water bodies through pollution. Waterways have been shown to receive more than 80 % of wastewaters [81]. In the bid to look at microbiological impact of discharge of industrial effluents, industrial effluents from pharmaceutical and detergent industries have been studied with the view of determining their microbiological attributes; and possible genotoxic potentials in *Allium cepa.*

The pioneer study in this area was carried out in 2002, whereby the microbiology of a pharmaceutical effluent collected along its path of discharge was determined [82]. It had microbial load of 2.15×10^5 cfu/ml and there was evidence of faecal contamination with MPN of >1800. The organisms encountered included *S. aureus, E. coli, Proteus vulgaris, Serratia marcescens* and *P. aeruginosa.* We were able to establish that these effluents pose dangers to the environment, aquatic organisms and man. In a study by Lateef and Yekeen [83], wastewater from metronidazole production of a pharmaceutical had microbial load of 2.15×10^5 cfu/ml, MPN of ≥ 1800 and presence of *E. coli* and *S. aureus.* The effluent induced various types of chromosomal aberrations (sig. at $p < 0.05$), while the mitotic inhibition ranged from 35.33 to 69.76% at

Nano Plus: Sci. Tech. Nanomat. 2 (2021) 27 – 65
https://doi.org/10.48187/stnanomat.2021.2.003

tested concentrations of 0.1-10%. The EC_{50} of root growth inhibition was obtained as 9.3%, indicating its moderate toxicity.

In another study, samples of effluents from cotrimoxazole and piriton production of two pharmaceutical industries induced various types of chromosomal aberrations in *A. cepa* (Figure 6) and reduced the number of dividing cells by 38.6 to 67.2% at tested concentrations of 1 to 20% [84]. The effluents had microbial loads in the range of 1.85 to 3.50 × 10^7 cfu/ml, with the presence of *S. aureus*, *E. coli, Bacillus licheniformis, S. marcescens*, *Klebsiella* sp, *S. pyogenes*, *P. vulgaris*, *Yersinia* sp and *Bacillus subtilis*. The studies showed that exposure of fresh water to industrial wastes can adversely affect the quality of the water, thereby limiting its usefulness.

In a related study [85], we examined the microbiological quality of fish (*Clarias gariepinus*) that were exposed to effluent of a detergent industry. It was discovered that exposure to the effluent increased the level of microbial contamination of the fish in all the parts that were examined, namely; skin, gills and muscle. The bacterial load of fish surfaces ranged from 1.2 to 2.0 × 10^2 cfu/ml for the control, while values of 4.8 to 8.6 × 10^6 cfu/ml were obtained for the experimental fish exposed to the industrial effluent (0.025 ppm). The fungal count for the control ranged from 1.2 × 10^2 to 1.2 × 10^3 cfu/ml; while a range of 1.0 to 2.0 × 10^6 was obtained for the fish exposed to the industrial effluent. Several microbes were isolated from the parts of the exposed fish samples as opposed to limited isolation in the control fish samples. The study concluded that exposure to the effluent might have predisposed the fish to broad bacterial and fungal infections that limited their quality for consumption because of extensive microbial contamination. The industry from where the effluent was collected discharges its wastewater into a nearby river. Other studies have shown the capability of a bacterium, *Bacillus safensis* LAU 13 for biosorption of heavy metals in the effluents of steel processing facilities [86]. These studies underpinned the negative impact of industrial effluents in the environment from public health perspective and genotoxicology. Not only that these effluents stimulated the proliferation of several pathogenic organisms, they can also induce genetic damage in exposed organisms.

6.3 Microbiology of Drinks, Foods and Feeds

We have also carried out studies on orange juice products, *Akara Ogbomoso*, *Lafun* and poultry feeds with the view of determining their microbiological safety. In a study focusing on the microbiological assessment of sachet orange juice products, we analyzed forty samples of different brands with the incidence of bacteria (3.5 × 10^4 to 2.15 × 10^5 cfu/ml) and yeasts (7.5 × 10^4 to 1.25 × 10^5 cfu/ml) in all the samples [87]. The incidence of *E. coli, Micrococcus* sp, *Bacillus subtilis, Streptococcus, pyogenes, Bacillus cereus, Staphylococcus aureus, Saccharomyces* sp, *Saccharomyces cerevisiae* and *Rhodotorula* sp in the orange juice samples was considered a safety concern as many of the organisms are pathogens. In investigating the first study on the microbiology of the popular snack, *Akara Ogbomoso* (Figure 7), we recorded ample growth of aerobes, coliforms, staphylococci, *Shigella* and yeast/mold from the *Akara* samples, water and cowpea pastes, which were indicative of poor processing techniques. To remedy the situation, we established Hazard Analysis and Critical Control Points (HACCP) plan for its production (Table 8). *Akara* prepared in the laboratory through the implementation of HACCP was not contaminated and found to be microbiologically safe [88]. The work stressed the relevance of application of HACCP to ensure safety of indigenous foods.

We have also evaluated the microbiological and nutritional qualities of poultry feeds in Ogbomoso, Southwest Nigeria, and determined the incidence of aflatoxins in the samples [89]. Over a course of five months, one hundred and fifty samples of different types of poultry feeds were obtained from five feed mills with high incidence of bacteria and fungi. Further quality assessment showed that 36% of the feed samples were contaminated with aflatoxins. To improve the quality of the locally-produced poultry feeds, we evolved an effective HACCP plan. Efficient regulations for the production of feeds were recommended.

More recently, Lateef and Ojo [90] in a detailed investigation analyzed eight hundred samples of water, fermenting broth (24, 48 and 72 h) and final products in the processing of cassava tubers to produce *lafun* over a period of five months. All the dried *lafun* samples obtained from sixteen processors were contaminated with the cumulative microbial loads of 2.21 to 9.91 × 10^4 cfu/g. Microbes that included *S. aureus*, *E. coli*, *Salmonella enterica* serovar Typhimurium, *Lactobacillus* sp. *Bacillus cereus, Klebsiella oxytoca, Aspergillus fumigatus, A. flavus, A. niger, Absidia corymbifera* and *Rhizopus oryzae* were isolated from the samples. Aside, about 39 % of the fungal isolates produced aflatoxins ranging from 1 to 1600 µg/kg. The critical control points identified in the production were steeping, drying, packaging/storage, and the implementation of the corrective measures led to the production of laboratory-prepared *lafun* with improved microbiological safety.

6.4 Antibiotic Resistance Studies

A major issue confronting public health all over the world is the emergence and spread of antibiotic or drug resistance mechanisms among microbes. The declining effectiveness of antibiotics on microbes imposes dire health and economic burdens on the society [91]. Several mechanisms involved in antibiotic resistance have been unravelled and these include intrinsic factors such as membrane-bound action, efflux pump and resistance genes and their transfer [92], as well as extrinsic factors like abuse and widespread use of antibiotics in humans and animals, mis-diagnosis, wrong prescription, self-medication and non-compliance with treatment regimens [93].

Quite a lot of our researches have studies on antibiotic resistance phenomenon incorporated in them, with the view of determining the public health implications [77, 82-84, 87-

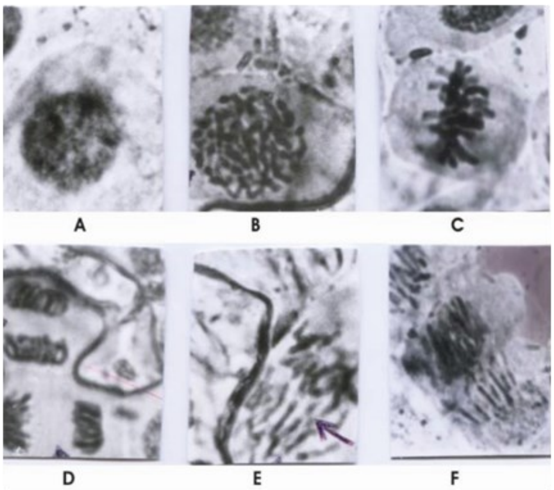

Fig. 6: Photomicrographs of some normal stages (A-D) and aberrations (E & F) in mitotic division observed in the cells of *Allium cepa* treated with

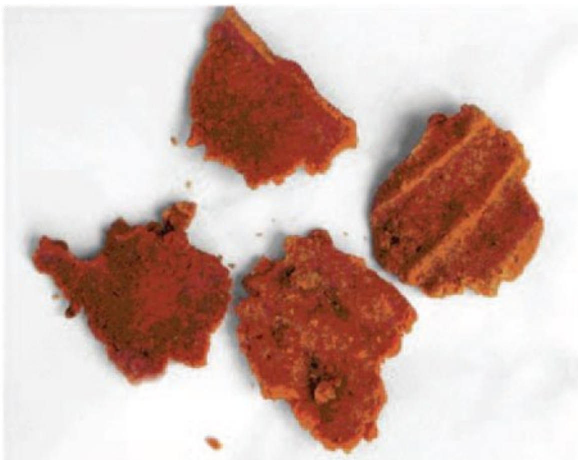

Fig. 7: Samples of *Akara Ogbomoso*

90, 94]. In these works, several bacterial isolates were obtained from diverse environments, namely; foods, feeds, water, clinical, industrial effluent, and soil. The isolates were evaluated for their resistance to commonly used antibiotics (Table 9). These studies concluded that there is widespread multi drug-resistance phenomena among bacterial isolates obtained from different samples in Southwest Nigeria. Also, several bacterial strains produced β-lactamase, an enzyme noted for the detoxification of penicillins [82, 87]. The studies also identified some of the practices that may encourage selection, enhancement and widespread of drug-resistance among bacteria and how to combat them.

6.5 Fabrication of Bioreactors, Control and Optimization of Fermentation Processes

Fermentation is the hallmark of industrial microbiology and biotechnology as several products can be produced by microorganisms on a large scale via fermentation; which is concerned with biotransformation of substrates to high-end products. The fermentation process, which is of two types; submerged (SmF) and solid substrate/state (SSF) is conducted in specialized facilities called bioreactors (Figure 8). The bioreactor provides optimal conditions such as pH, aeration, temperature, agitation, and dissolved oxygen for the growth of microorganisms and product formation which must be properly controlled [95]. Our research group has carried out a lot of studies in this area; ranging from construction of bioreactors [96, 97], through development of bioprocess software, Biopro_optimizer [98] for acidification process of yoghurt fermentation [99], fermentation of yeasts using novel feeding strategy [100], optimization of biogas and biohydrogen production [101, 102], optimization of citric acid production [103, 104], enzyme production

Table 8: Processing steps, sources of hazard and control measures in the production of *Akara* Ogbomoso

Processing steps	Sources of hazard	Hazard	Control measures
Procurement of beans/ sorting	Beans	Chemicals and stones	Use of high quality beans
Soaking	Water, container, soil and sewage	Pathogens, and metals	Use of potable water
Removal of seed coat	Hands	Pathogens	Personal hygiene
Grinding	Milling machine	Heavy metals, pathogens	Personal hygiene/GMP
Moulding of cakes	Hand	Pathogens	Use of moulds, personal hygiene/GMP
Cooling	Air and baskets	Vegetative pathogens and spores	Cooling under basket covered with muslin cloth; regular cleaning of baskets
Packaging	Hand (picking), and mouth (air-blowing to open nylon)	Pathogens	Use forceps or wearing of gloves; good personal hygiene
Storage	Vegetative cells and spores	Pathogens	GMP, storage in freezing bags at low temperature; microwave heated for 10 seconds to be ready for consumption
Hawking/selling	Hand (through repackaging) and air	Pathogens and particulates	Personal Hygiene; discouragement of repackaging

[105-107], and exopolysaccharide production in mushroom [108, 109]. Figure 9 shows the generation of bioreactors produced by our team, while some software interfaces are shown in Figure 10. Genetic algorithm software developed in-house has been used in these processes with commercial software on neural network to capture the non-linear relationship fermentation processes with outstanding outcomes, which are summarized in Table 10.

6.6 Production of Fructooligosaccharides

Oligosaccharides are intermediate sugars between disaccharides and polysaccharides and usually have 3-10 sugar moieties connected by glycosidic bonds [110] (Figure 11). Oligosaccharides are either (a) malto-oligosaccharides (α-glucans), principally occurring from the hydrolysis of starch and (b) non-α-glucan such as raffinose and stachyose (α-galactosides), fructo- and galacto-oligosaccharides or other oligosaccharides. Most oligosaccharides are non-digestible. They can be obtained by direct extraction from natural sources, or produced by chemical processes hydrolyzing polysaccharides, or by enzymatic and chemical synthesis from disaccharides. Oligosaccharides possess important physicochemical and physiological properties, and are claimed to behave as dietary fibers and prebiotics that support the growth of beneficial microbes [111]. As a result, oligosaccharides have been incorporated in to foods and drinks to produce functional foods. It has been projected that the probiotic market would reach $8.5 billion by 2024 [112].

Amongst oligosaccharides, fructooligosaccharides (FOS) also called oligofructose or

Table 9: Resistance patterns of some bacterial isolates obtained from *Akara Ogbomoso*

No of antibiotics	Resistance patterns	Isolates
1	Cxc	*S. epidermidis*
	Flx	*S. aureus*
2	Cxc Flx	*S. epidermidis*
	Cro Gen	*Shigella* sp
3	Aug Cro Tet	*S. marcescens*
	Aug Cro Amx	*S. marcescens*
	Aug Cro Cot	*S. aureus*
4	Tet Amx Aug Cro	*E. coli*
	Nit Aug Cot Cro	*E. coli*
	Cot Cld Cxc Flx	*P. vulgaris*
5	Cpx Cot Cld Cxc Flx	*B. cereus;*
	Aug Cro Cot Amx Nit	*S. marcescens*
6	Aug Cro Cot Amx Nit Tet	*E. coli*
	Aug Cro Cot Nit Tet Pfx	*E. coli*
7	Cip Gen Cpx Cot Cld Cxc Flx	*S. aureus*
	Aug Cro Cot Amx Nit Tet Pfx	*E. coli*
8	Aug Ery Gen Cpx Cot Cld Cxc Flx	*S. epidermidis*
	Aug Cro Nit Gen Cot Ofl Amx Tet	*C. freundii*
9	Aug Ofl Ery Gen Cpx Cot Cld Cxc Flx	*S. aureus*
	Aug Ery Cip Gen Cpx Cot Cld Cxc Flx	*S. epidermidis*

oligofructan (Figure 12) have been investigated for their physiological and rheological attributes, as well as their sweetness (~60% as sweet as sucrose), thereby making them useful in food applications to reduce glycemic index and diabetes. Their health benefits [113] are as summarized in Table 11. Although, FOS are present in plants such as onions, chicory, garlic, asparagus, wheat, banana, artichoke, tomatoes and other fruits, vegetables and grains where they occur in small amounts (0.15-0.75%), they are currently produced on large scale using enzymatic fructosylation of sucrose by fructosyltransferase (FTase) [114]. FTases obtained from microorganisms have been used to produce FOS with very high yield in excess of 60% and up to 98% [115]. Among the organisms that have been used industrially to produce FTase and FOS is the dimorphic black yeast, *Aureobasidium pullulans* [116].

The author's work on oligosaccharides was influenced by the work of Dr. Prapulla [118] and the opportunity to study under her guidance at Central Food Technological Research Institute (CFTRI), Mysore, India in 2004-2005 through a CSIR-TWAS postgraduate fellowship. In one of the studies, we examined the ability of a dimorphic fungus, *Aureobasidium pullulans* CFR 77 to produce FTase (Figure 13). It was established that application of ultrasound could be used to release intracellular FTase from the organism [119]. Ultrasonication at acoustic power of 20W for 9 minutes was found to be optimum to efficiently release intracellular FTase, which produced FOS yield of 57-59 % within a reaction time of 9h as against reaction times of 12-25 h reported in the literature. The study, which was first of its kind, demonstrated the potential role of ultrasonication in efficient release of the intracellular FTase which can be used for the production of FOS, an industrially important prebiotic. We carried out purification and partial characterization of intracellular FTase of *Aureobasidium pullulans*. The FTase obtained by wet-milling of the organism was purified, with two bands of molecular weights of 147 and 170 KD, having optimum pH and temperature of 5.0 and 55 ^{o}C respectively [120]. In another study [121], we reported the first reference to *Rhizopus stolonifer* LAU 07 as a producer of FTase. The local strain, which was isolated from spoilt orange fruit, produced FTase in submerged fermentation, which yielded 34% FOS.

Lateef *et al*. [106] reported a strain of *Aspergillus niger* which produced extracellular FTase in both submerged fermentation (SmF)

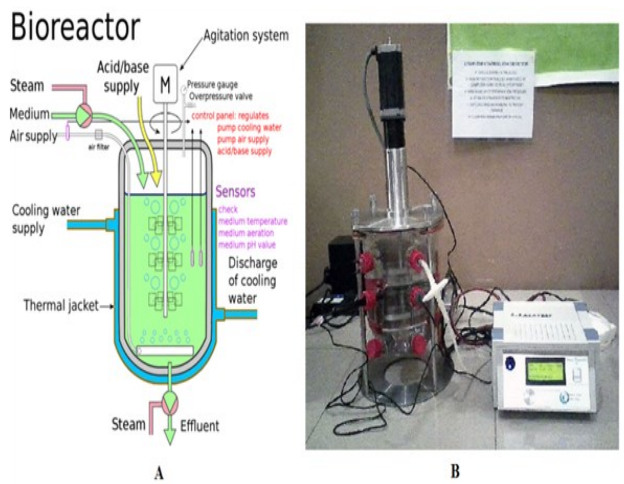

Fig. 8: Basic diagram of bioreactor (A) and a functional 5-litre bioreactor in the author's laboratory (B)

Fig. 9: Generation of bioreactors produced by our team

in chemically-defined medium, and solid state fermentation (SSF) using agricultural by-products such as kolanut pod and ripe plantain peel. Maximum enzyme activity of 24.49 U/ml was obtained in SmF after 48h of fermentation, while maximum enzyme activities of 20.77 and 27.77 U/g were obtained in SSF using ripe plantain peel and kolanut pod, respectively. The enzyme was used to prepare FOS, with the maximum yield of 33.24 %, consisting of kestose (GF2) and nystose (GF3). The safety of prepared FOS was investigated using albino rats. The study concluded that the prepared FOS may be considered safe for consumption as alternative sweetener to sucrose, as it did not produce any pathological effect in rats. In partnership with some international collaborators, we also documented an excellent review on the current trends in the microbial production of FOS [122]. Some milestones on our contributions to FOS research are as summarized in

Fig. 10: Control panels of Biopro_optimizer

Table 10: The impact of optimization on some investigated bioprocesses

Bioprocess	Result	Reference
Yoghurt production by *Lactobacillus bulgaricus* and *Streptococcus thermophilus*	Novel temperature profile reduced fermentation time from 6 h to 2 h	[99]
Biomass of *Saccharomyces cerevisiae*	Novel feeding strategy increased biomass yield from 3 g/l to 14.25 g/l in 24 h	[100]
Biogas	Increase production of biogas by 8.64%	[101]
Biohydrogen	Analysis of 15 published showed that ANN can be used for modeling of biohydrogen production	[102]
Citric acid by *Aspergillus niger* MCBN 297	GA-ANN predicted citric acid yield better than RSM	[104]
Citric acid by *Aspergillus niger* FUO I_{10}	Improved citric acid yield by 45.97 folds	[103]
Fructosyltransferase production by *Aspergillus niger*	Improved enzyme yield by 1-64-8.59 folds	[106]
Fructosyltransferase production by *R. Stolonifer* LAU 07	Improved enzyme yield by 3.80 folds	[105}
Xylanase production by *A. Niger* L3 and *Trichoderma longibrachiatum* L2	Improved enzyme yield by 192.59-208.09%	[107]
EPS production by *Lentinus edodes*	Improved yield by 20.70 folds	[108]
EPS production by *Pleurotus tuber-regium*	ANN modeled biomass and EPS production	[109]

GA, genetic algorithm; ANN, artificial neural network; RSM, response surface methodology; EPS, exopolysaccharide

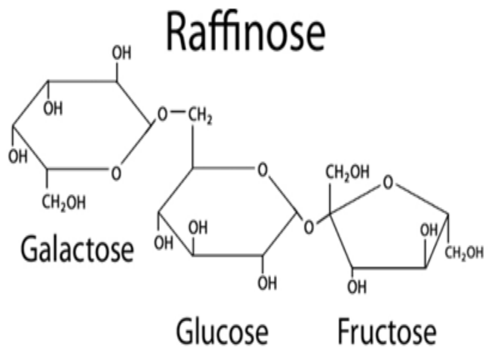

Fig11: An example of oligosaccharide, raffinose

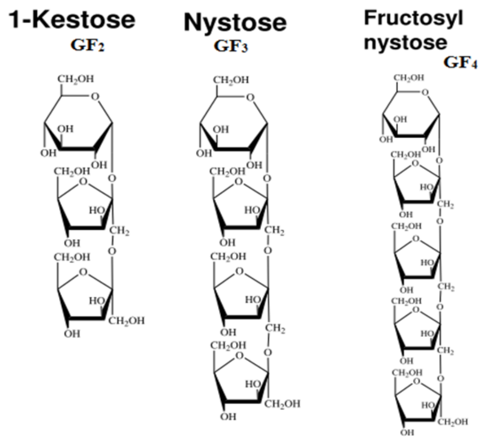

Fig 12: Structures of some fructooligosaccharides

Table 12.

6.7 Utilization of Agro-industrial Wastes and Microbial Upgrading

Agro-industrial wastes which are abundant all over the world are known to be rich in nutrients that can support microbial growth on one hand, or can be used to produce some important microbial metabolites on the other hand; thereby leading to the microbial upgrading of the fermented substrates (nutrient enhancement) and biotechnological utilization, respectively [123, 124]. In this connection, we have conducted some studies on the biotechnological utilization of agro-industrial wastes such as cocoa pod husk, palm kernel cake, kolanut pod, cassava peels, cassava wastewater, poultry feather, corn cob and sawdust[101, 103, 105, 106, 125, 126] for the production of biogas, citric acid and enzymes such as FTase, xylanase and keratinase. We have also published excellent reviews on valorization of poultry feather waste to produce keratinase for diverse biotechnological applications [127, 128]. In the fungal fermentation by *Rhizopus stolonifer* LAU 07, the protein contents of substrates (cocoa pod husk, palm kernel cake, cassava peels) increased tremendously, while crude fibre contents were lowered. The cyanide content of cassava peel was lowered by 90.6%, while the antioxidant activity was improved by 53-62% among the fermented substrates [129]. The study showed that scope exists for microbial upgrading of these low-quality agro-wastes for the development of healthy animal feed supplements.

We have also reported a strain of *Bacillus cereus* LAU 08 which completely degraded whole chicken feather (Figure 14) within a period of seven days at room temperature (30°C) [130]. It produced keratinase as in-

Table 11: Some health benefits of fructooligosaccharides

S/N	Benefits	Purpose
1.	Promote growth of colonic beneficial microbes	Supportive colon therapy, fight against pathogens
2.	Enhance mineral absorption e.g. calcium	Improves bone formation and prevent osteoporosis
3.	Lower cholesterol, triglycerides	Fight against obesity and cardiovascular diseases
4.	Lower glucose level	Control/prevention of diabetes
5.	Reduce calorie intake	Weight management in obese
6.	Sweet (60% as sweet as sucrose)	As sweetener in drinks/foods
7.	Lead to formation of lactate, acetate and butyrate	Anticancer activity by apoptosis

duced by hooves, horn and feather at growth temperature of 37 °C. Optimal keratinolytic activity was obtained at pH 7.0 and temperature of 50 °C. However more than 50% activities were displayed within the broad range of pH 7-9 and temperature of 40-70 °C. The isolate could be a promising strain for the management of chicken feather waste through novel biotechnological processes.

In 2013, we isolated a novel strain of *Bacillus safensis* LAU 13 [126]; the first report to produce keratinase with high titer of 108.5 U/ml. Its keratinase was investigated for dehairing and destaining activities with excellent performance (Figures 15 and 16). Our contributions on *Bacillus safensis* which was first isolated as a contaminant in USA in 2006 [131] is legendary. We published its second report of isolation in Africa and the first report on its ability to degrade feather and produce keratinase [126]. Also, till date, the only review paper on the biology and biotechnological applications of this novel bacterium was authored by us [132].

Furthermore, Elegbede and Lateef [125] utilized corncob to produce xylanase by local strains of fungi in both SmF and SSF. High titers of xylanase in SmF (10.38-50.55 U/ml) and SSF (12.30-48.63 U/g) were produced by the fungi. The fungal isolates, namely *Aspergillus fumigatus* SD5A, *A. flavus* SD4A, *A. fumigatus* L1, *Fusarium solani* SD3C, *A. niger* L3, *Trichoderma longibrachiatum* L2, *Botryodiplodia* sp. L5 and *A. flavus* L4 did not produce aflatoxin (Figure 17) on neutral red desiccated coconut agar [133], thereby enhancing their biotechnological relevance in food industries. The fungal xylanases improved dough-rising (1.87-2.20 folds) in bakery application (Figure 18) and also clarified orange juice with good performance (58.12-74.22%). These studies have shown that agro-industrial wastes can be valorized for enhanced nutritional quality and to produce novel bio-products that can drive our quest for the diversification of the economy to incorporate bioeconomy.

6.8 Author's Contributions to Nanobiotechnological Research

Research activities into the biosynthesis of nanoparticles started with experimentation using the crude keratinase of *B. safensis* LAU 13 to produce silver nanoparticles (AgNPs) (Figure 19) [134]. The colloidal AgNPs had maximum absorbance at 409 nm with spherical particles that inhibited the growth of strains of *E. coli.*

6.8.1 Research on Silver Nanoparticles (AgNPs)

We have carried out several investigations into biofabrication of AgNPs using diverse biomolecules of plants, bacteria, fungi, and arthropods for antimicrobial, antibiotics-nanoparticles synergistic, dye-degrading and adsorption, desulphurization, corrosion inhibition, larvicidal, osmotic, anti-deterioration, heavy-metal remediating, plant-growth promoting, antioxidant, anti-inflammatory, anticoagulant and thrombolytic activities. These activities are relevant in biomedical, food, environmental, agricultural and industrial applications. Our activities in this area are as presented in Table 13. Some of the fascinating results obtained in these studies are illustrated in Figures 20-37.

6.8.2 Research Activities on Gold (AuNPs), Silver-Gold alloy (Ag-AuNPs), Calcium (CaNPs) and TiO_2 NPs

Unlike silver, bulk gold is not acknowledged to have inherent antimicrobial properties. However, the properties of gold at nanoscale allow for robust particle functionalization, and researchers have explored the prospect of using AuNPs as antimicrobial agent. Also, the simultaneous reduction of Ag and Au ions in the mixed solution has led to the development of bimetallic Ag-Au nanoparticles with higher activities as antimicrobials compared to AgNPs and AuNPs and also biocompatible for biomedical applications. Bimetallic nanoparticles have gained attentions in their synthesis and applications, owning to the fact that they combine attributes of the monometallic components by altering the molar ratios of the two metals. Unlike Ag and AuNPs however, the reports on biomedical applications of green Ag-AuNPs are scanty [171].

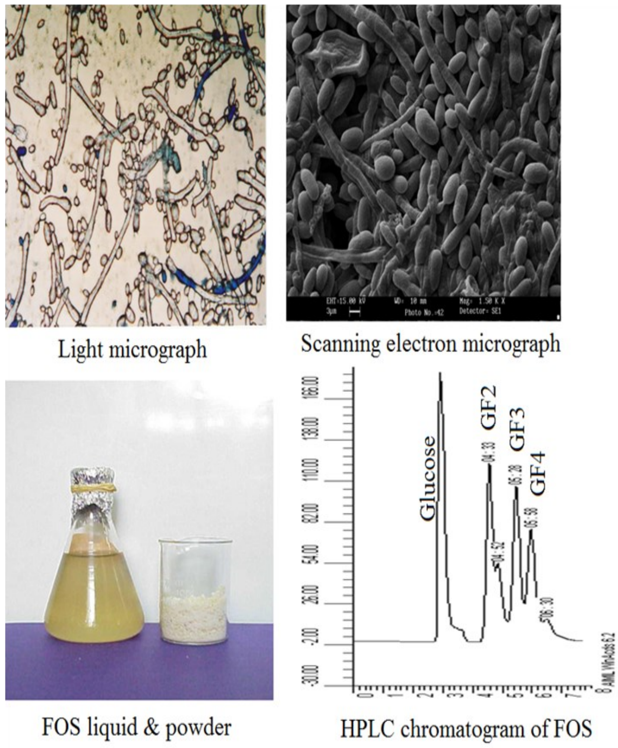

Fig. 13: *Aureobasidium pullulans* CFR 77 for the production of FOS [119, 120]

Calcium is a macronutrient essential to plants and animals for several physiological activities that include enzymatic activities, hormonal system signalling, antioxidant activity and bone development in vertebrates. The biofabrication of calcium nanoparticles has also be on a steady rise for different applications [172, 173]. We have carried out investigations on the biosynthesis and applications of AuNPs, Ag-AuNPs, CaNPs and TiO_2NPs in our laboratories for environmental, biomedical and agricultural applications over the years, which are summarized in Table 14 and some of the results presented in Figures 35-40.

6.8.3 Contributions of Review Articles

We have made enormous contributions to topical issues on nanotechnology by publishing excellent reviews as summarized in Table 15.

6.8.4 Bridging the Gap between Microbiology and Nanotechnology: The sub-discipline of Microbial Nanobiotechnology

We have made concerted efforts to situate nanotechnology in microbiology, and these efforts have come to fruition in producing the pioneering textbook in Microbial Nanobiotechnology. The author rallied scientists in seven countries in Asia, Africa, South America and Europe to produce the exciting textbook, 'Microbial Nanobiotechnology: Princi-

Table 12: Milestones on contributions to FOS research

S/N	Scope	Reference
1.	First report of ultrasonication to release intracellular FTase	[119]
2.	Reduction in reaction time to produce FOS from 12 to 25 h previously	[119]
3.	First report of *Rhizopus stolonifer* to produce FTase and FOS	[121]
4.	First report of kolanut pod and plantain peel as substrates to produce FTase in SSF	[106]
5.	First report of use of cassava steep liquor and cassava peel as substrates to produce FTase and FOS in SmF and SSF	[105]
6.	Excellent review of FOS production	[122]

ples and Applications' published by Springer-Nature in 2021 [69]. In this textbook, the contributions of microorganisms to developments in nanotechnology, in terms of microbial synthesis of nanoparticles, interactions and their applications in diverse areas were articulated in manners that will appeal to microbiologists and life scientists in general. In chapter one of the textbook, the author brought his experiences to bear in establishing the links between microbiology and nanotechnology (Figure 28), identify the gaps in knowledge and provided ways to address the challenges in the development of microbial nanobiotechnology, including a draft curriculum of an introductory course in nanobiotechnology

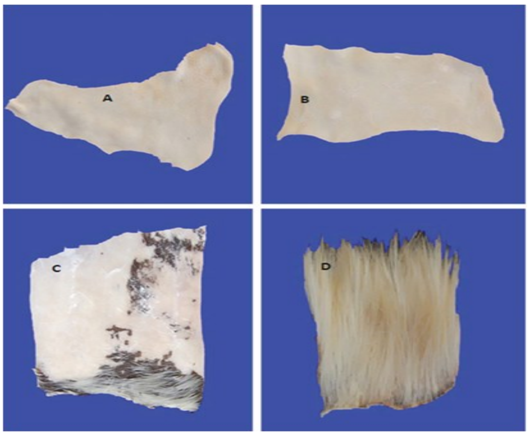

Fig. 16: Complete dehairing of goat skin by crude keratinase. Note: Dehairing by wild-type strain after 16 h (A) and by mutant strain after 12 h (B); incomplete dehairing by sodium sulphide and lime after 20 h (C) and control (D)

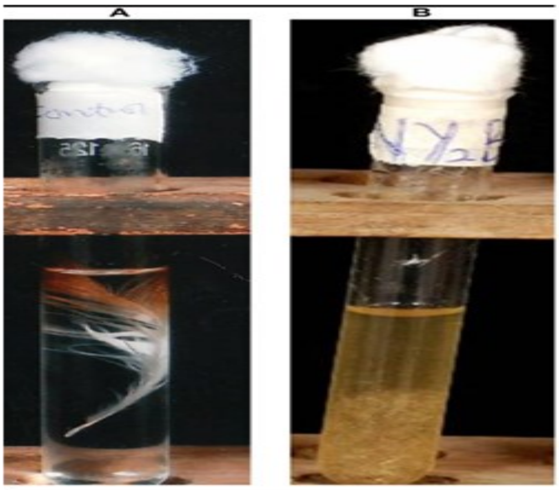

Fig. 14: Biodegradation of feather (A, control; B, digestion after 7 days) by *Bacillus cereus* LAU 08

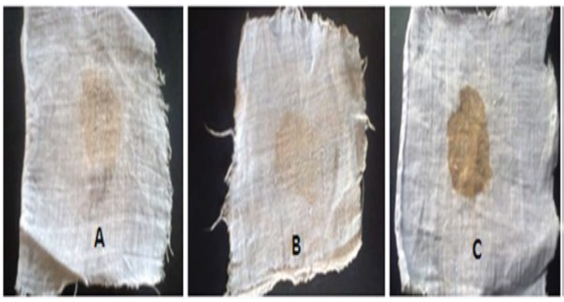

Fig. 15: Destaining of blood-stained cloth by crude keratinases. Note: Destaining of blood by the wild-type strain after 3 h of incubation (A), by the mutant strain after 2 h of incubation (B) vs. a control (C) incubated in water

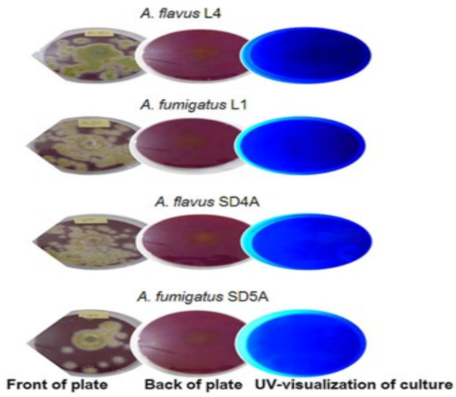

Fig. 17: Non-aflatoxigenic nature of the fungal isolates

Fig. 18: The effect of inclusion of xylanase on dough rising with good performance

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[189]. Other contributions to the book include nanozymes [190], algal nanobiotechnology [191], and beneficial microbes in the synthesis of nanoparticles and applications in nanomedicine [192].

6.8.5 Activities of the LAUTECH Nanotechnology Research Group (*NANO*+)

The LAUTECH Nanotechnology Research Group which is multidisciplinary was formed on September 4, 2014 with the main

Table 13: Summary of research activities on AgNPs

S/N	Biomaterial	Highlight	Reference
1.	Second report of keratinase	Antimicrobial	[134]
2.	First report of seed and seed shell of *Cola nitida*	Antibacterial	[135]
3.	First report of extract of *B. safensis*	Antimicrobial, antioxidant and larvicidal	[136]
4.	Laccase of *L. edodes*	Antibacterial	[137]
5.	First report of cobweb	Antimicrobial, paint additive and antibiotic-AgNPs synergy	[138]
6.	First report of pod of *Cola nitida*	Antibacterial, antioxidant and paint additive	[139]
7.	First report of cocoa pod	Antimicrobial, antioxidant and larvicidal	[140]
8.	First report of nest of paper wasp	Antimicrobial, catalytic, anti-coagulant and thrombolytic	[141]
9.	First report of miracle fruit plant	Antimicrobial, catalytic, anti-coagulant and thrombolytic	[142]
10.	Cell-free extract of *B. safensis*	Anti-candida, anti-coagulant and thrombolytic	[143]
11.	First report of cocoa bean	Antimicrobial, larvicidal and anticoagulant	[144]
12.	Cell-free extract of *Enterococcus* sp	Antimicrobial	[145]
13.	Cobweb and Kola nut pod	Desulphurization of model oil	[146]
14.	Cobweb and Kola nut pod, seed and seed shell	Hydrogen peroxide scavenging, anticoagulant and thrombolytic	[147]
15.	Pod of *Cola nitida*	Enhanced antioxidants and phytochemicals in *Amaranthus caudatus*	[148]
16.	Kola nut pod, seed and seed shell	Cytogenotoxicity	[149]
17.	Cocoa pod and bean	Cytogenotoxicity	[150]
18.	First report of wonderful kola	Antimicrobial	[151]
19.	Cobweb	Adsorbent for Rhodamine B	[152]
20.	First report of xylanase	Catalytic and biomedical	[153]
21.	First report of *Petiveria alliacea*	Biomedical	[154]
22.	*Lentinus squarrosulus*	Antibacterial	[155]
23.	Cocoa pod	Antiphytopathogenic and hepatoprotection	[156]
24.	Cocoa bean	Osmotic dehydration of tomato	[157]
25.	Cocoa pod	Remediation of Cd and Pb polluted soil	[158]
26.	*Chasmanthera dependens*	Biomedical	[159]
27.	First report of *Persea americana*	Antimicrobial and antioxidant	[160]
28.	First report of *Opuntia ficus-indica*	Antimicrobial and antioxidant	[161]
29.	Cocoa bean	Adsorption of Rhodamine B	[162]
30.	*Hyptis suaveolens*	Biomedical	[163]
31.	First report of animal fur	Biomedical and cytogenotoxicity	[164]
32.	*Carica papaya*	Antibacterial and Larvicidal	[165]
33.	*Ehretia cymosa*	Anti-inflammatory	[166]
34.	Kola nut pod	Corrosion inhibition	[167]
35.	Kola nut pod	Anti-aging of bitumen	[168]
36.	*Annona muricata*	Biomedical	[169]
37.	Cobweb extract	Improved paint	[170]

purpose of conducting cutting-edge research in nanotechnology, training of manpower and dissemination of information on nanotechnology. As of now, the group has eleven members from the faculties of Pure and Applied Sciences, Basic Medical Sciences, and Engineering and Technology of the University. The group has consistently positioned LAUTECH as a centre of reference in nanotechnology research in the last six years. The modest achievements of the group are as stated in Table 16.

6.8.6 Other Activities to Promote Nanobiotechnology Research

The author has engaged in series of activities to promote nanobiotechnology research within and outside Nigeria. This include infusion of nanobiotechnology into the curriculum of ‘Introductory Biotechnology’ that is taught at 500 level in the Department of Pure and Applied Biology, LAUTECH, Ogbomoso which is not in existence in any University in Nigeria. This effort put our graduates at advantage with exposure to the cutting knowledge of nanotechnology. The promotion of nanobiotechnology research has also been extended through public lectures. In 2020, the author delivered two public lectures via webinar in two colleges in India [199, 200] to stimulate the interests of life scientists in nanotechnology. In 2021, the author took part as a panelist at a high powered policy workshop on ‘Nanotechnology for transfor-

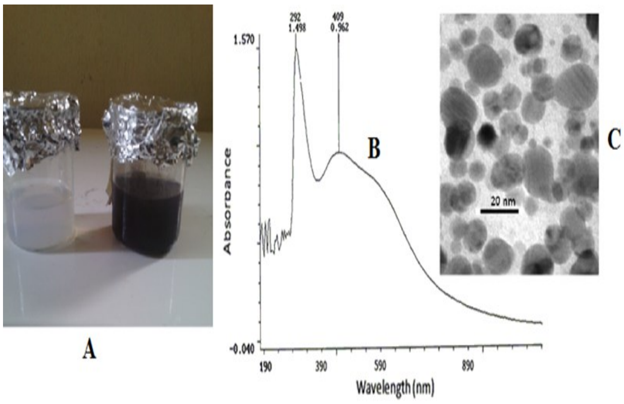

Fig. 19: A, formation of AgNPs ($AgNO_3$ (left) AgNPs (right); B, UV-vis spectrum; C, TEM micrograph

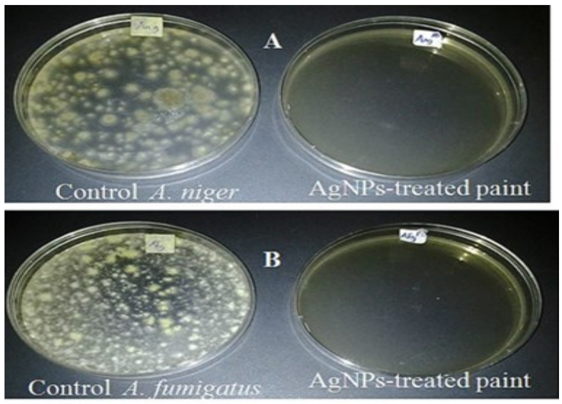

Fig. 21: Antifungal effect of AgNPs when used as additive in emulsion paint [139]

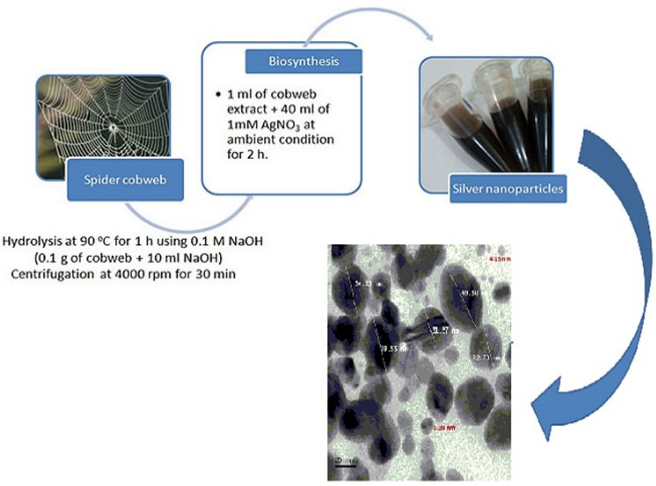

Fig. 20: Scheme for the synthesis of AgNPs using cobweb extract [138]

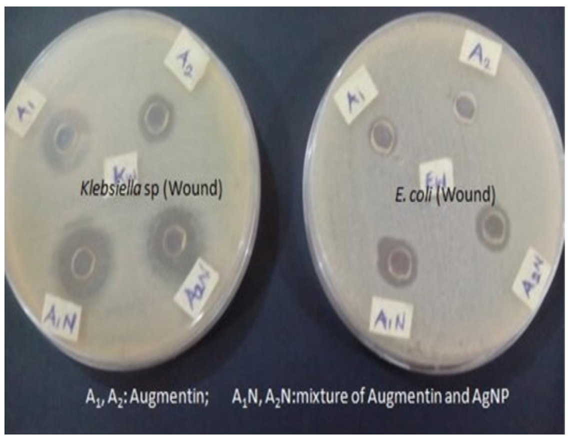

Fig. 22: Synergistic effect of AgNPs on Augmentin (upper, antibiotic alone; lower, antibiotic-AgNPs) against drug-resistant bacteria [138]

Table 14: Summary of research activities on AuNPs, Ag-AuNPs, CaNPs and TiO_2 NPs

S/N	Biomaterial used	Nanoparticles	Highlight	Reference
1.	First report extract of *B. safensis*	AuNPs and Ag-AuNPs	Antifungal, dye degradation, anti-coagulant and thrombolytic	[174]
2.	First report of Kola nut extracts	Ag-AuNPs	Antifungal, catalytic, larvicidal and thrombolytic	[175]
3.	First report of cell-free extract of *Enterococcus* sp	AuNPs	Antioxidant, larvicidal, anti-coagulant and thrombolytic	[176]
4.	First report of xylanase	Ag-AuNPs	Biomedical and catalytic	[177]
5.	First report of *Persea americana*	AuNPs and Ag-AuNPs	Antimicrobial and antioxidant	[160]
6.	First report of *Opuntia ficus -indica*	AuNPs and Ag-AuNPs	Antimicrobial and antioxidant	[161]
7.	First report of xylanase	AuNPs	Biomedical	[178]
8.	First report of *Datura stramonium* seed	AuNPs	Antidiabetic	[179]
9	*Datura stramonium* seed	AuNPs	Biomedical	[180]
10	First report of pod extract of *Cola nitida*	CaNPs	Enhanced plant growth and phytochemicals	[181]
11	First report of kola nut ex-	TiO_2NPs	Biomedical and catalytic	[182]

mation of African development: Looking towards a sustainable African future', organized by African Materials Research Society (AMRS) and United Nations Economic Commission for Africa (UNECA), Gaborone, Botswana to chart a course for nanotechnology R & D in Africa.

7.0 The Intricate Cycle of Man, Microbes, Nanoparticles and Development: Personal Experience

From the research experiences of the author, it has been found that several microbes and nanoparticles can serve as tools to render goods and services for mankind, and to drive development agenda. In doing so however, there is need to exercise caution; particularly in the areas of water and food safety, and pollution of the environment by domestic, agricultural and industrial wastes. The recent event of COVID-19 has shown for instance how microbes can threaten the very existence of mankind, whereby social, religious, economic, political, educational sectors and well-being of man were disrupted by a microbe, a novel coronavirus. The abuse in the use of

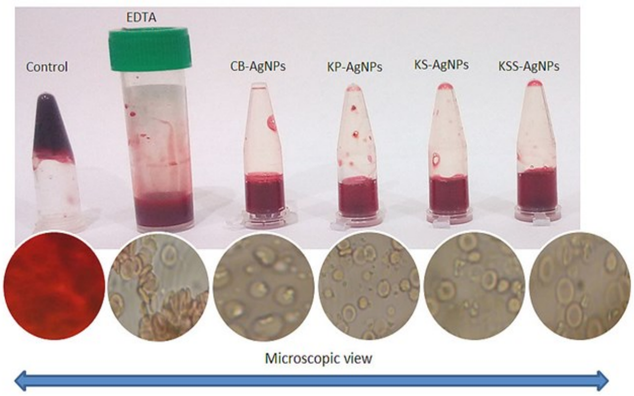

Fig. 23: Anticoagulant activities of biosynthesized AgNPs on human blood [147]

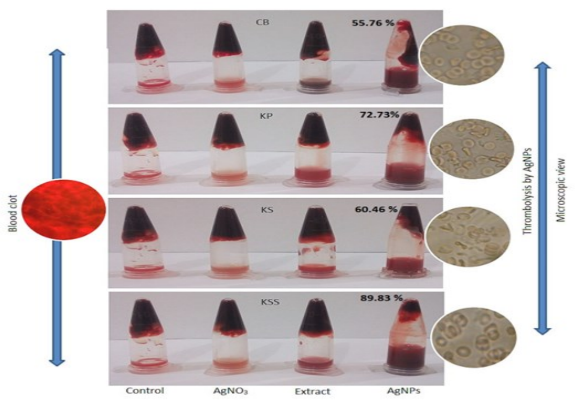

Fig. 24: The thrombolytic activities of some biosynthesized AgNPs [147]

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antibiotics and dissemination of drug-resistant isolates also contribute to the threats facing mankind, in terms of control of pathogens and treatment of diseases. As long as these precautions are not observed, microbes can portend serious danger to man; most especially practice that encourage creation, development and dissemination of resistant microbial strains. While nanoparticles that have been studied have shown potentials for applications in agriculture, healthcare, pollution control, food and water treatment; the very characteristics that enhance the performances of the particles can also enhance their toxicity in the other way round. Therefore, care should be taken in establishing the toxicological attributes of these particles to ascertain their safety on case by case basis and their subsequent applications.

Both microbes and nanoparticles belong to minute materials and objects that cannot be seen by man without magnifying equipment.

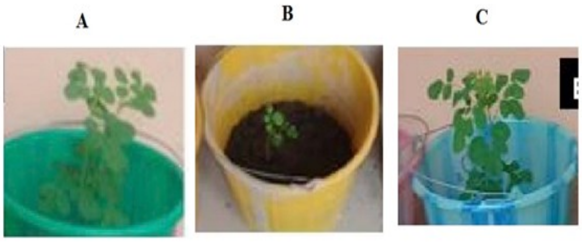

Fig. 25: The effect of biosynthesized AgNPs on amelioration of cadmium on the growth of *Moringa oleifera* (A, control; B, cadmium treated; C, amelioration with AgNPs) [158]

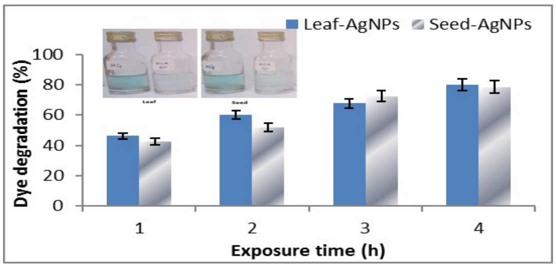

Fig. 26: Degradation of malachite green by biosynthesized AgNPs using leaf and seed extracts of *S. dulcificum* (inset, degradation at 2 h) [142]

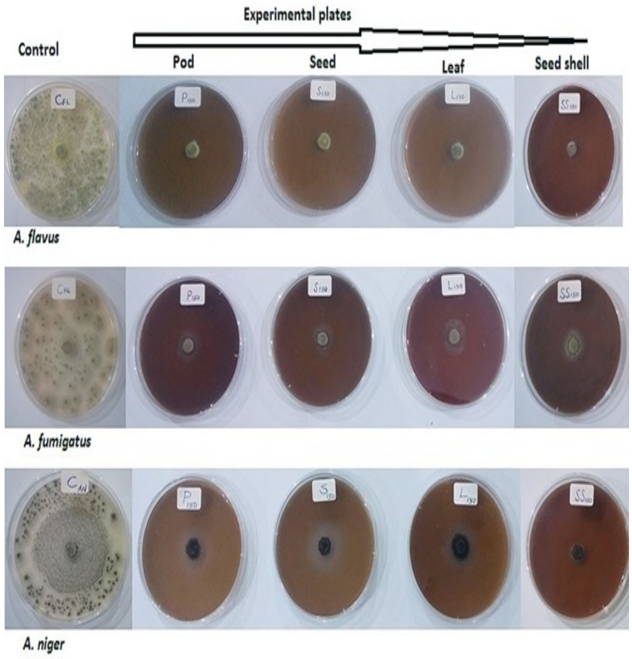

Fig. 27: Antifungal activities of biosynthesized Ag-AuNPs using kolanut extracts [175]

Table 15: Review articles on nanotechnology

S/N	Focus	Reference
1.	1st compendium on the use of agrowastes, enzymes and pigments to synthesize metal nanoparticles (MeNPs)	[183]
2.	1st compendium on the use of arthropods and their metabolites to synthesize MeNPs	[49]
3.	Applications of nanoparticles to manage blood coagulation disorders	[184]
4.	1st compendium on green nanotechnology research in Nigeria	[43]
5.	Biomedical applications of Ag, Au and Ag-AuNPs	[185]
6.	Biomedical applications of green synthesized MeNPs	[171]
7.	Nanotechnology in the built environment	[186]
8.	Application of Ag and AuNPs as anticoagulant and thrombolytic agents	[187]
9	Nanobiosensors in biomedical technology	[188]

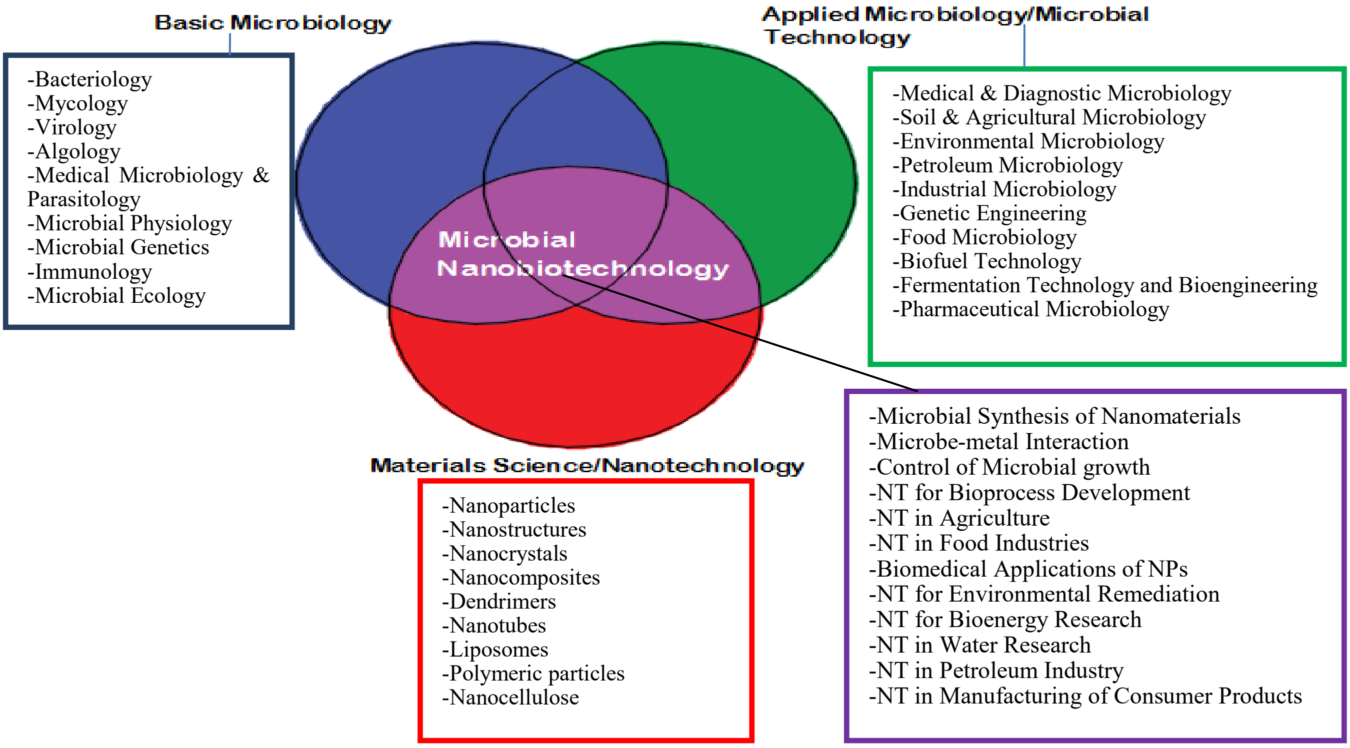

Fig. 28: The interrelatedness of microbiology, microbial technology and nanotechnology for the creation of microbial nanobiotechnology

Typically, their sizes range from 10^{-6} (1 millionth) to 10^{-9} (1 billionth) metre. They are very small, yet they have defined man in his activities in several ways as enunciated in this article. They contribute to the well-being of man, they dominate economic activities in the world, man has used them to produce products for his convenience, they have elevated the living conditions of man, and they put food on the table of man. Conversely, microbes and nanoparticles as small as they are can also subject man to unimaginable distress; can cause diseases, can harm/kill man, his animals and destroy cultivated crops, can cause economic crisis, can instil terror or fear in man, and they can simply put man on his knees, while he is helpless. Over ages, man has been assaulted by microbes especially, leading to the death of millions of people. Be it malaria, tuberculosis, polio, small pox, Ebola, Lassa fever, diarrhoea, COVID-19, cholera, tetanus, meningitis, syphilis, bubonic plagues, hepatitis, HIV-AIDS, food poisoning (through mycotoxins), man has suffered in no small measure from microbes. Thus, is it not a paradox that both good things of this life and the bad ones can be instigated by these diminutive living and non-living things? They are the foot soldiers of development and doom. It is left for man as a commander to responsibly deploy them for his common good and continuous existence in order to walk the intricate cycle with utmost care. So, the next big thing would be determined by small things…… whether positively or negatively.

8.0 Conclusion and Recommendations

In this article, the importance of microbes and nanoparticles to man has been established, to the extent that they can be exploited as tools for the much needed change to bring about sustainable development. This is the hallmark of research activities of the author since 1998 as an industrial microbiologist, biotechnologist and nanobiotechnologist. In pursuance of this, several students have been trained and junior colleagues have been mentored in several Universities. The author also established a laboratory of reference for research; the Laboratory of Industrial Microbiology and Nanobiotechnology. Among other things, it is necessary for Nigeria to professionalize the discipline of microbiology, establish microbial resources centres, and link the bioresources centres (BIOREC) of the National Biotechnology Development Agency (NABDA) with nearby Universities with clear mandate on research and development (R & D) to harness the vast potentials of indigenous microbial strains for the development of the nation. The country must as a matter of urgency formulate policy on nanotechnology for development of the nation, prioritize nanotechnology research, and establish centres of excellence in nanotechnology in institutions that are doing well in nanotechnology research.

Table 16: Achievements of LAUTECH Nanotechnology Research Group (*NANO*$^+$)

S/N	Achievements
1.	Publication of more than one hundred articles on nanotechnology since 2015
2.	Web presence; www.lautechnanotech.com with record of more than 111,500 visitors since its debut on June 24, 2016
3.	Successful organization of workshops and conferences on nanotechnology in 2017, 2018, 2019 and 2020
4.	Publication of a special issue of *Science Focus* **23** (2) dedicated to papers presented at LAUTECH NANO 2018 conference
5.	Publication of papers presented at LAUTECH NANO 2019 conference in Volume **805** of *IOP Conference Series: Materials Science and Engineering* (UK) in 2020
6.	Training of several undergraduate and postgraduate students
7.	Collaboration with researchers in South Africa, India, Saudi Arabia and Italy. At least a student has enjoined postgraduate fellowship with one of our partners
8.	Mentored several colleagues at different higher institutions and research institutes in Nigeria
9.	Sensitization of younger generation in primary and secondary schools on nanotechnology discourse, including organization of essay competition
10.	Massive dissemination of information on nanotechnology in international and national online and print media [193-198]
11.	Launching of the group's journal, '*Nano Plus: Science and Technology of Nanomaterials*', the first of its kind in the sub-Sahara Africa (https://stnanojournal.org/), of which volume 1 was dedicated to the publication of book of abstracts of LAUTECH NANO 2020

Acknowledgement

The author appreciates the authority of Ladoke Akintola University of Technology, Ogbomoso, Nigeria for providing support for his research activities. Gratefully acknowledged are the supports received from Council of Scientific and Industrial Research (CSIR) India, The World Academy of Sciences (TWAS), Italy and Tertiary Education Trust Fund (TETFund), Nigeria to carry out some of the research activities mentioned in this article.

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