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Industrial Microbiology & Biotechnology

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ABSTRACT

Microbes are small living organisms, quite diverse and adapt to various environments. They live on our planet long before that markedly effects the human, animals and plants life. Biotechnologically, molecular genetics of microbes is systematically manipulated, enable them for the production of beneficial products. The large-scale production of insulin was made possible through transfer of human insulin gene to a bacterium to treat diabetes, a milestone in the history of biotechnology. The microbial cultures are managed, monitored and maintained for their genotype/phenotype stability. Bacteria are one of the biotechnologically important microbes for example Escherichia coli, some species of lactic acid bacteria, and some viruses such as bacteriophages etc. recognized as anti-cancer oncolytic viruses. Moreover, yeast has also been used with broad-spectrum applications, such as Saccharomyces cerevisiae. Using diverse genetic technologies, a number of beneficial products like microbial polymers, biologically active primary and secondary metabolites like ethanol and antibiotics have been produced so far and used. Here, it would be injustice not to comment on the importance of vaccines for several infectious diseases through their ability to trigger the host immune system. Furthermore, roles of microorganisms in the production of pharmaceutical proteins

like monoclonal antibodies and insulin, their broad-spectrum usage in industrial fermentation plus the vital control and biosafety measures are also focused here.

Keywords: Biotechnology; Industrial microbiology; Antimicrobial agents; Vaccines; Microbes; Large-scale microbial usage; Disease treatment and control

FUNDAMENTALS OF MICROORGANISMS

It is considered that microbes are one of the oldest forms of life on earth, with exceptionally diverse and adaptable nature; they are simple and small organisms to be seen with naked eye. As a negative impact, they are disease causing, or positively they aid in sustaining the atmosphere of earth [1] and endorsing plant growth. They synthesize number of products like enzymes, antimicrobial agents and antibiotics for research and health purposes.

A number of methods have been used effectively for the maintenance of microorganisms: oil overlay of slant-grown cultures[2], repeated sub-culturing, preservation on agar beads [3] use of silica gel and other sterile supports [4-6], lyophilization[7,8] and cryopreservation [6,9]. Lyophilization and cryopreservation are greatly utilized for culture collections and industry, with possible pros and cons of both methods. The fundamental selectivity of any particular medium and condition for incubation enforces limits on the diversity, nature and number of microbes recovered from natural samples. It follows the isolation procedures application, that better mimic of their environments from which the samples were collected that could increase the probability of retrieving organisms that were previously uncultured.

By using the following methods, recent efforts to accomplish maintenance of microorganisms have met with some success: (i) Relatively low concentrations of nutrients [10-14];(ii) Relatively lengthy periods of incubation [15-18], sometimes directly in the natural environment from which the inoculum was obtained [19] and (iii) non-traditional sources of nutrients, signaling molecules, or inhibitors (of undesired organisms) [20-22]. For microbes collected from soil, some of them may have adjusted with the elevated concentrations of Carbon dioxide (CO_2) and concentrations of oxygen(O_2) lower than the atmospheric oxygen concentrations [23], the incubation atmosphere composition might be main concern. High concentrations of carbon dioxide are used infrequently in the cultivation atmospheres for isolation of soil microbes; however, carbon dioxide could be essential for metabolic progressions other than pure autotrophy. Similarly, for soil microbes, the conversion to fully aerobic environment on plating in air may be a stressful event.

Genetic manipulations of microorganisms are practiced for the production of new organism with desirable characteristics. The classical approaches of microbial genetics cover a key part in the progression of cultures for industrial microbiology. Despite of development in genome sequencing and bioengineering, a big part remains unidentified about the prokaryotic functional organization. For example, approximately a third of the protein coding genes of the best-studied model bacterium, *Escherichia coli*, presently lack experimental interpretations.

Systems-level experimental approaches for examining the bacterial genes functional associations and genetic organizations are vital for defining the central molecular biology of microbes, avoiding the blowout of antimicrobial resistance in the clinic, and motivating the progression of biotechnological applications in future.

Once microbes are selected to serve a specific purpose, it is necessary to preserve them in original form for future study and usage. The principal approaches for culture preservation are drying, continuous growth and freezing. For those types of cultures that produce spores or other resting structures, drying is one of the most useful techniques of preservation. The cultures can be desiccated by elimination of water and using soil, sand, glass beads, grains, silica gel, paper strips and several other materials as carriers to prevent rehydration. In addition, drying approaches are technically simple and expensive equipment are not require. Continuous growth methods are simple as well as inexpensive, because specific equipment is not required. Besides persistence, an additional important necessity for effective preservation of microbial strains is the ability to preserve their features unchanged. It is extremely necessary to enhance the existing preservation techniques, unitingall the advantages and eliminate disadvantages of individual methods [24].

MICROORGANISM GROWTH IN CONTROLLED AND COMPLEX ENVIRONMENTS

The number of present microbial species are expected to be 10^5 to 10^6, but just few thousand of them have been isolated in pure culture, it is because only few microbes from environmental samples grow on nutrient media *in vitro*. Efforts to enhance the retrieval of microorganisms from environmental samples by controlling growth media have been observed with limited triumph, and the difficulty in non-cultivability remains a major challenge [17].The important knowledge available about the microbes, substrates, and process characterize the basis on which a coherent method for the design and preparation of media for microbial processes can be endeavored. In this regard, it is very important to critically examine the main objective to be optimized. The primary step for the development of medium is concerned with the assessment of using adequate components, trailed by the calculation of their concentrations. After medium formulation and experimentation, the implementation of the most accurate statistical optimization approach will make it potentially optimum medium.

However, some common strategies can be assumed for growth and production media, taken in account the experimental proofs available about the functions and effect of the medium constituents for endorsing growth and product formation. When a culture medium is developed, it is important to define the physical environment for microbial functioning in the mass culture system. The control of temperature, agitation, pH changes and oxygenation is taken into consideration. To control pH and phosphorus source, phosphate buffers can be used. The limitations of oxygen, especially, can be serious concern in aerobic growth processes. The oxygen concentration and fluctuation rate must be appropriately high to have excess oxygen within the cells so that it is not limiting. This is true especially when a thick microbial culture is growing[25].

Microbes inheritabsurd versatility, reduces the explosives as portion of the biogeochemical cycle. Various microorganisms catalyze mineralization and/or processing of non-specific explosive waste both by aerobic and anaerobic processes. It is likely that the genetic adaptation is underway to further increase the recruitment of silent sequences for the functional development of degradation pathways and the selection of substrates by mutations in structural genes, the reduction potential of bacteria in explosives direction and eventually contributing to environmental cleanup from these toxic and persistent chemicals. This can be useful in the development of safer and more economical microbiological process for the purification of soil and water contaminated with these compounds [26].

Working in both laboratory and fields, attempts are carried out to speed up the current microbiological progressions by the addition of identified active microorganisms to waters, soils, or other complex systems. The microorganisms utilized in these experiments are isolated from contaminated sites, taken from culture collections, or derivative of uncharacterized enrichment cultures. For instance, culture preparations at commercial level simplify materialization of silage andincrease septic tank performance [27].

MAJOR BACTERIA AND VIRUSES OF BIOTECHNOLOGICAL INTEREST

Microorganisms that are used for industrial production must meet certain requirements. The most important sources of microbial cultures for use in industrial microbiology were natural materials such as soil samples, waters, and spoiled bread and fruit. Cultures from all areas of the world were examined in an attempt to identify strains with desirable characteristics. These characteristics are important for a microorganism for considering it as an input in an industrial process[28,29].

- The organism should be able to grow vigorously and rapidly in the used medium.
- The end products should not include toxic and other undesirable materials, especially if these end products are for internal consumption.
- The organism should have a reasonable genetic, and hence physiological stability. An organism that mutates easily is an expensive risk. It could produce undesired products if an occurred mutation remained unobserved.
- An organism with optimum productivity at high temperatures, low pH values or which is able to elaborate agents inhibitory to competitors has a decided advantage over others.
- The organism should be reasonably resistant to predators such as bacteriophages. It should therefore be part of the fundamental research of an industrial establishment using a phage-susceptible organism to attempt to produce phage-resistant but high yielding strains of the organism.

The use of biotechnology to convert viruses into therapeutic agents by reprogramming viruses to treat diseases is known as virotherapy. Nowadays virotherapy has different usages for example in the treatment of cancer as anti-cancer oncolytic viruses, viral vectors for gene therapy and viral immunotherapy like phage display techniques. . In 1956, some of the earliest human clinical trials with oncolytic viruses were started to treat advanced oncolytic viruses in Cancer Therapy of cervical cancer. Herpes simplex virus (HSV) attack cancer cells [30], research in this field was limited due to technological limitations. Poliovirus,adenovirus,Coxsackie virus, ECHO enter virus RIGVIR and some others were used in cancer research during 1960s. [31,32].

There are several different effective mechanisms by which oncolytic viruses destroy malignant cells.Bacteriophages or phages are bacterial viruses that invade bacterial cells [33] by infecting bacteria; phage can proliferate in two ways lytic life cycle and lysogenic life cycle. When phages multiply vegetatively they kill their hosts and the life cycle is referred to as lytic life cycle. On the other hand some phages known as temperate phages can grow vegetatively and can integrate their genome into host chromosome replicating with the host for many generations [34]. Recently, bacteriophages have contributed more to the field of molecular biology and biotechnology. The existence of bacteriophages was marked by Ernest Hankin, a British bacteriologist in 1986 against *Vibrio cholerae* that he observed in waters of the Ganges and the Jumna Rivers in India, but bacteriophages were "officially" discovered by Felix d'Herelle, a French-Canadian microbiologist at the Pasteur Institute in Paris [35]. In humans, phages as therapeutic agents were first usedin 1919 [30,36]. Phage therapy has been used in animals, plants and humans with different degree of success. They have several advantages over antibiotics with some disadvantages as well. Among some advantages of bacteriophages in Phagotherapy are; (i)Their specificity for target bacteria which reduces the damage to normal flora of the host greatly, (ii) They are self-limiting i.e. they require their hosts to be constantly growing [37], (iii)Replication at the site of infection is another advantage of phages. They are safe with no or less side effects [38] and (iv)- when bacteria become resistant to phages, these phages do evolve naturally to infect the aforementioned resistant bacteria, hence minimizing the chances of bacterial escape, which scores another advantage of phage over antibiotics [39]. At the moment it seems a bit far that phagetherapy will replace antibiotics exclusively, but there is the hope that it will be used complementary to antibiotics especially for antibiotic resistant strains. Phage display is a molecular technique used for the study of protein-protein, protein-peptide and protein DNA interaction with novel characteristics, introduced by Smith et al., 1985 [40]. The DNA that encodes the polypeptide is fused with phage coat protein genes, and the desired protein is expressed on the surface of the phage particle that then can be used in drug design as reagents for understanding molecular recognition also minimizing mimics for receptors.

The most common bacteriophages used in phage display are the M13 and the fd filamentous phage, [41] though T4, T7 are also used for this purpose[42].

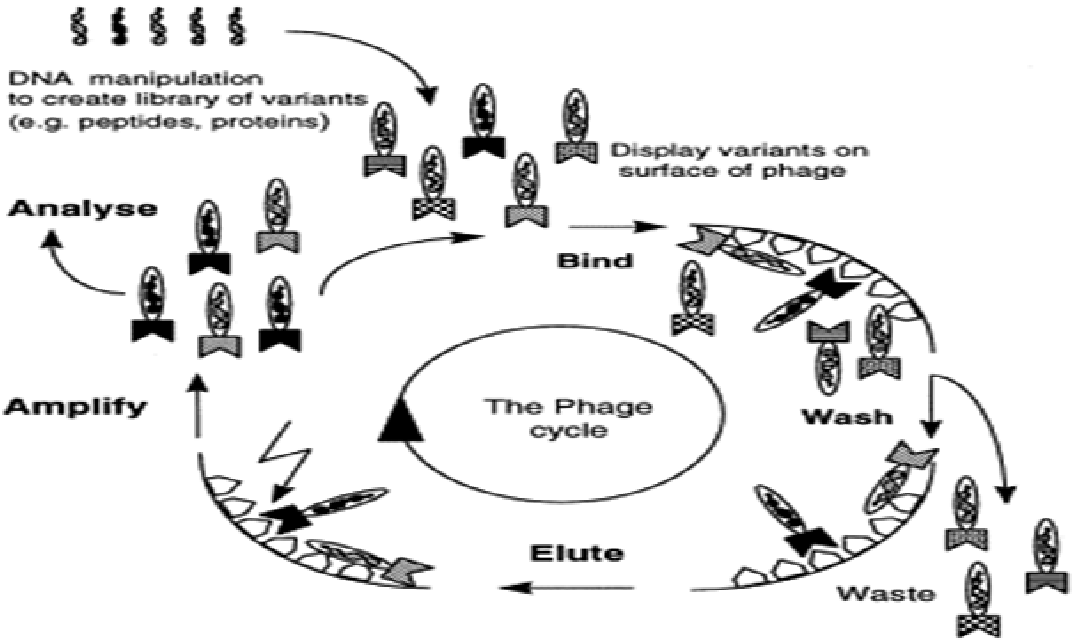

Figure 1: The phage display cycle**.** DNA encoding for millions of variants of certain ligands (e.g. peptides, proteins or fragments thereof) is batch-cloned into the phage genome as part of one of the phage coat proteins (pIII, pVI or pVIII). [43].

MAIN EUKARYOTIC ORGANISMS OF BIOTECHNOLOGICAL INTEREST

Fungi are important organisms from both the economic and as ecological point of view. They are diverse and widely distributed in different habitats (air, animals, soil, water and plants etc.). Most fungi are multicellular formed by a network of filaments called hyphae. Other forms fruiting structures called mushroom. In addition, there are single-celled yeast fungi, with typically spherical or oval shape. Fungi are also heterotrophic, do not perform photosynthesis, have no cell wall cellulose and reserves energy mainly as glycogen [74,75].

These organizations have broad and diverse applicability and importance in many branch of biotechnology [74-77]. Some of these features are scored below:

- *Aspergillus niger*:Produce citric acid for the food and beverage industry;
- *Saccharomyces cerevisiae*: It is important in the manufacture of bread and wine and alcohol industry. They can be genetically engineered to express proteins (antigens) for vaccines;
- *Trichoderma*: Cellulase enzyme production;
- *Taxomyces*:Taxol production, antitumor drugs;
- Biological pest control: *Coniothyrium minitans* (feeds on fungi that destroy soybean and bean crops), *Paecilomyces fumosoroseus* (kills termites present inside tree trunks) etc;

- *Penicillium chrysogenum*: Production of penicillin;

- *Ashbyagossypii and Pseudomonas, Propionibacterium, Acetobacter*species: Vitamin production by fermentation processes;

- *Penicillium* and *Cryptococcus* species: Causes opportunistic infections;

- *Aspergillus*: Generates aspergiliose - an infection that develops in immunocompromised patients with diabetes mellitus and leukemia etc;

- Compounds extracted from lichen: A dye used as clothing, paper and antimicrobial agent;among others.

In recent decades, the biotechnological potential of endophytic fungi is explored. They colonize the plant tissue within symbiotic way, that is, in a cooperative manner. These endophytic organisms has been used as a source of new and bioactive natural products. Recent data showed 2000 substances, with 51% unknown structures and 80% biological activity. Many of these substances have wide application as an antibiotic, antiparasitic, antifungal and antitumor etc. This shows the biotechnological potential of endophytes associated with plant species from different biomes. In particular, the gradual decrease of biomes is badly affected such as the Amazon and the risk of extinction of endophytic biodiversity and hence the loss of potential bioactive molecules with biotechnological applications [77].

MAJOR PRODUCTS OF INDUSTRIAL MICROBIOLOGY

The large scale production not only of the traditional goods, for example beer, alcohol, cheese, but also other new products including citric acid and pharmaceuticals of economics relevance, had become well established [44,45]. Growing economic relevance followed notably the success of penicillin manufacture and more antibiotics like streptomycin, become available followed by a new class of high value added products, mainly secondary metabolites e.g. steroids. Other major products of growing market relevance include amino acids, organic acids and enzymes of new applications [46,47].

Microorganisms produce antibiotics, mainly by actinomycetes in the genus *Streptomyces* and by filamentous fungi. Several hundreds of compounds with antibiotic activity have been isolated from microorganisms over the years, but only a few of them are clinically useful. *Streptomyces* is one of the largest antibiotics producing genus in the microbial world discovered so far [45].

Table 1: Major antibiotics Produced by Microbes.

Antibiotic	Producer Organism	Activity
Penicillin	*Penicillium chrysogenum*	Fungus
Polymyxin B	*Bacillus polymyxa*	Gram-negative bacteria
Erythromycin	*Streptomyces erythreus*	Gram-positive bacteria
Neomycin	*Streptomyces fradiae*	Broad spectrum
Streptomycin	*Streptomyces griseus*	Gram-negative bacteria
Tetracycline	*Streptomyces rimosus*	Broad spectrum
Rifamycin	*Streptomyces mediterranei*	Tuberculosis

Another important product obtain from microorganisms through industrial microbiology is organic acid. The production of organic acid demonstrates the effects of trace metal levels and balances on organic acid synthesis and excretion. The number of organic acid nowadays obtained from microorganism is described below.

Table 2: Major organic acids produced by microbial processes [29].

Product	Microorganism Used
Acetic acid	*Acetobacter* with ethanol solutions
Citric acid	*Aspergillus niger* in molasses-based medium
Fumaric acid	*Rhizopus nigricans* in sugar-based medium
Gluconic acid	*Aspergillus niger* in glucose-mineral salts medium
Itaconic acid	*Aspergillus terreus* in molasses-salts medium
Lactic acid	Homofermentative *Lactobacillus delbrueckii*

Biosurfactants are produced extracellularly or as part of the cell membrane of bacteria, yeasts and fungi that are structurally diverse group of surface-active molecules. These can be glycolipids, lipopeptides, phospholipids, fatty acids, neutral lipids, polymeric and particulate compounds[48-50].

Biosurfactants are used for emulsification and phase dispersion as well as for solubilization. These properties are especially important in bioremediation and oil spill dispersion.

Table 3: Type and microbial origin of biosurfactants[46].

Surfactants	Surfactant class microorganism
Glycolipids	
Rhamnolipids	*Pseudomonas aeruginosa*
Trehalose lipids	*Trehalose lipids Rhodococcus erithropolis, Arthobacter sp.*
Sophorolipids	*Candida bombicola, Candida apicola*
Lipopeptides	
Surfactin/iturin/fengycin	*Bacillus subtilis*
Viscosin	*Pseudomonas fluorescens*
Lichenysin	*Bacillus licheniformis*
Phospholipids	*Acinetobacter sp., Corynebacteriumlepus*

Fatty acids/neutral lipids	
Corynomicolic acids	*Corynebacterium insidibasseosum*
Polymeric surfactants	
Emulsan	*Acinetobacter calcoaceticus*
Alasan	*Acinetobacter radioresistens*
Liposan	*Candida lipolytica*
Lipomanan	*Candida tropicalis*

Many microorganisms have been reported to produce amino acids, mainly bacteria, but also include some molds and yeasts. The most widely reported bacteria belong to the following four genera, *Corynebacterium* spp. (*C. glutamicum; C. lilum), Brevibacterium spp. (B. divericartum: B. alanicum), Microbacterium spp. (M. flavum var. glutamicum) and Arthrobacter spp. (A. globiformis; A. aminofaciens)* [51,52].

Secondary metabolites and organic compounds are produced from primary metabolites, which are formed during the end or at the stationary phase of microorganism's development. Thus, they are not related to a role during their growth, development or reproduction. Secondary metabolites may be involved with the microorganism's defense, acting as antibiotics and through the production of pigments. Furthermore, such compounds have an important effect on health, nutrition and economics in our society. Others examples of secondary metabolites are pesticides, toxins, pigments, effectors of ecological competition and symbiosis, pheromones, immunomodulation agents, enzyme inhibitors, receptor antagonists and agonists, antitumor agents, cholesterol-lowering agents, immunosuppressive, among others. Hundreds of antibiotics are available at the world market not only because of their large clinic usage but also due to their importance in chemotherapy during bacterial infection [53].

Table 4: Examples of microorganisms and their secondary metabolites produced[54-56].

Secondary Metabolites	Microorganism
Penicillin	*Penicillium griseofulvum* & *Penicillium verrucosum*
Erythromycin	*Saccharopolyspora erythraea*
Bacitracin	*Bacillus subtillis*
Lovastatin	*Aspergillus terreus*

Microbial biopolymers has proven to be very suitable for industrial and medical applications because of its very large chains and functional groups that can be blended with other low or high molecular weight materials to achieve new materials with various physicochemical properties [57]. Another feature is that microbial polymers are biodegradable and biocompatible, turning their use very interesting over the use of synthetic organic polymers derived from oil and rock that accumulate in the environment [58]. Moreover, toxicologically speaking, microbial polymers are harmless materials of low cost and relative abundant in Earth. Polysaccharides are among the polymers produced by microorganisms that are widely used in pharmaceutical formulations. Microbial polyesters, such as polyhydroxyalkanoates (PHA), produced by some *Lactococcus, Halomonas, Pseudomonas* and *Escherichia* species for instance, are used as excipients in medicaments as well (Kim, j. K 2013). Recently, for example, Konsoula and collaborators designed a strain where hyper-thermophilic alpha-amylase encoding gene from *Pyrococcus woesei* was transferred and expressed in a *Xanthomonas campestris,* which is a commercial strain. They observed that xanthan gum yield was enhanced by the recombinant bacterium at higher glucose or starch concentrations [59]. Currently, several works describe the cloning and expression of genes capable to increase the amount of xanthan gum, as well as others polymers, in different types of bacteria. Although, new efforts were made in this field, a better understanding of polymer biosynthesis and material properties is still needed as it may help to enhance the usage of bacterial biopolymers as valuable renewable products [60].

Use of biological processes or agents such as certain microorganism to convert organic materials, such as plant or animal waste, into usable products or energy sources known as bioconversion processes or biotransformation. Bacteria, actinomycetes, yeasts, and molds have been used in various bioconversions [58].

WHITE BIOTECHNOLOGY AND APPLICATION

For centuries, man has practiced the art of biotechnology without having proper knowledge of the biological processes for generating products such as bread, wine and milk products [61]. Advances in the areas of "omics", computational biology, systemic and synthetic biology and nanotechnology has contributed to the integration of biological and hence to the accumulation of knowledge and experience necessary to understand the cellular regulatory machinery and the generation of products of interest in industrial biotechnology.

Industrial biotechnology or white is modeled on the processes that occur in nature, using biotechnological tools to provide more efficiently products and lessen environmental impact to traditional industrial processes. This branch of biotechnology has a key role in solving some socio-economic challenges, such as: limiting the use of fossil fuels, generation of food that meets the growing demand of the world population, structural changes in industries to ensure sustainable economic growth [62]. This also comprises several industries, such as chemicals, biofuel, food and feed, paper and pulp and enzymes, etc[63].

The biofuel sector, with increasing investments, comprises alternative source of energy generated from biological sources. In this context, we point out different biofuels: biodiesel (obtained from soy, corn, canalo, palm oil, sunflower and babassu oil etc.), the ethanol (obtained from sugarcane, corn and cellulosic materials etc.), biogas (from the decomposition of organic matter without oxygen), etc [64,65].

Bioethanol, is the biofuel more produced nowadays, an alternative liquid fuel obtained by fermentation via the most economical way over other methods, such as synthetically produced ethanol from unsaturated hydrocarbon [64]. The US is the world's largest producer of ethanol followed by Brazil.

In US, ethanol is obtained from corn while in Brazil it is produced from cane sugar [66]. But the energy productivity of cane sugar is much higher than maize, thus, the cost of production from corn is much higher. It is also possible to produce ethanol using algae, microalgae and mass of cellulosic matter as raw material. The three sources are still expensive and require too much cost [64,67,68]. For example, the cellulose hydrolysis process is complex and the sugar content is lower than the saccharide found in the raw materials[64], which is unfeasible for producing ethanol on a large scale by these method.

The use of biogas and biodiesel is growing rapidly around the world. The first is a mixture of esters from the transesterification of vegetable oils and the second is a mixture of methane and carbon dioxide from the decomposition of organic compounds in the absence of oxygen [69,70]. Biogas is a renewable source of energy that can be converted into electricity, their solid waste into composit materials and liquid waste can be used with bio-fertilizers for crops without groundwater contamination problems. It is therefore highly relevant to commercial establishments, industries and the environment [70].

The fine chemicals, an industry classified in the white biotechnology, is related to the production of amino acids, lipids, organic acids and vitamins etc. Below, we highlight the importance of some of these products to various industrial sectors:

- **Amino Acids:** great importance for human consumption and animal feed enrichment, in the production of aspartame (important in the soft drinks industry), manufacture of cosmetics, hyaluronic acid used in tanning (biotransformation of histidine amino acid) etc.

- **Vitamins:** Production of Riboflavin (Vitamin B2), and cobalamins (Vitamin B12), ascorbic acid (vitamin C), β-carotene (provitamin A), biotin (vitamin H), ergosterol (vitamin D2);

- **Lipids:** can be synthesized by prokaryote and eukaryote microorganisms. They are related to the production of sterols, wax esters, biosurfactants and edible oils etc.

Industry enzymes have a broad and diversified market and are used in medical, food and beverage industry, scientific, textile, paper, detergents and many others. It aims at improving a particular process, optimization of production costs, obtaining by-products (intermediates) of interest etc. Nowadays, an increasing number of researches are taking place for the discovery of new enzymes with a view to changing raw materials and/or for specific products [64]. A new area of knowledge, metagenomics, can contribute significantly to the discovery of new enzymes. This area explores the genetic material of microorganisms isolated from environmental samples. Thus, diverse habitats with different complexity can be exploited. Added to this, the use of next-generation sequencing techniques for metagenomas and the analysis of the functional dynamics of microbial communities through metaproteomics will be of great relevance in this exploratory process [63,71].

A new technology platform that comes as a promising one for various fields of biotechnology (white, gray, green and yellow etc.) has founded synthetic biology, which promises to make biological systems that perform tasks coded by man. For example, we could make simplified versions of genetic material from one microorganism to increase efficiency of biofuel generation process from cellulose. All this would be controlled by man, that is, it could enable or disable the synthetic model like a switch. The impact of synthetic biology in white biotechnology could be immense, with the generation of higher quality products and hence less costly [72].

It should be noted in this topic that the importance of environmental biotechnology or gray biotechnology in the treatment of waste from the white biotechnology. These wastes when released without proper treatment can have serious consequences for the environment, such as groundwater contamination, plant and animal slaughter etc. Thus, the environmental biotechnology is intended to treat hazardous wastes to the environment by biological systems [64,73].

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