

# Biotechnology for Wellness Industry: Concepts and Biofactories

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**Abstract:** One of the major issues in the 21<sup>st</sup>. century facing humankind is on how to stay healthy and delay the onset of chronic metabolic diseases. Chronic metabolic chronic diseases still afflict a substantial percentage of modern human population despite the advances in medical and health care technologies. They create a long-term financial burden to the nation as well as reducing the productivity and the quality of life. In the recent years, the wellness approach to healthy living by mean of health enhancement and disease prevention has been increasing in popularity. There is a tremendous global and local interest for wellness products. Wellness sector focuses on providing products and services to a wider community to improve appearance, slow down the effect of ageing and to reduce the risk of developing chronic metabolic diseases. The wellness products are intended for the promotion of health in soil, plants, animals and human. Soil health is the foundation of wellness as healthy and productive soil produce healthy plants and crops in turn produced healthy animals for human nutrition. It is a fact that human health is closely associated with the practice of healthy life style that include consuming wholesome nutrients, living in a non-toxic environment and enhancing physical and mental fitness. These factors in turn promote the attainment and maintenance of cellular homeostasis. Under cellular homeostasis the cellular metabolic activities are at their optimum. In this regard traditional and modern biotechnology offer comprehensive list of natural ingredients and metabolites essential for cellular metabolism. These natural ingredients and metabolites are derived from microbial, algal, plant, animals, and human sources. Most of these natural products are increasingly made available by using innovative bioprocess technologies as more of them are main components of functional foods, nutraceuticals, cosmeceuticals and therapeutics. Bioprocess industries are considered as source for both health and wealth. The new concept of bioprocess industries is based on using different types of cells as small micro-bio-factories. These small biofactories belong to different classes of living organisms ranging from the most primitive prokaryotic bacterial cells up to high eukaryotic human cells. In the present review, the concept of bioprocess design and cultivation of cells up to the industrial level will be presented.

**Keywords:** Wellness, homeostasis, natural ingredients, metabolites, wellness industry.

## 1. INTRODUCTION

Biotechnology at its simplest refers to the applications of biological system for the development of products or services. Biotechnology Industry Organization states that biotechnology is a know-how that used the cellular and bio-molecular processes to develop technologies and products that help improve our lives and the health of our planet [1]. The traditional biotechnology industry has been focusing on the development of food and industrial based products using fermentation processes. However, modern biotechnology involves molecular biology, cell biology, biochemistry and system biology for the applications in environment, agriculture, animal health and human health. Over the past 25 years, the contribution of biotechnology to these sectors has been tremendous. The greatest single impact has been in the field of human health, where the application of biotechnology techniques has allowed dramatic advances in the development of medical diagnosis for major the chronic diseases. Biotechnology is an intensive research based

sector. It is a multidisciplinary in nature drawn mainly from the life sciences with increasing role for the Information Technology IT, instrumentation and bioprocess engineering. Biotechnology is already changing the drug discovery process leading to the development of completely new biopharmaceutical and medical diagnostics. In addition genetically modified organisms, plants are engineered to improve their physical robustness, pest and herbicides tolerance. The possibilities offered by biotechnology appear to be almost endless: it is expected that in ten to fifteen years' time most of the bioactive molecules and chemicals will be produced using culture techniques.

Despite these exciting development in the healthcare products and services, humans are increasingly overwhelm by problems ranging from malnutrition, chronic metabolic diseases, iatropic in medical care [2], degradation of environment, contamination of water supply and increasing cost of living. Chronic metabolic diseases such as diabetic type 2 [3], cancer [4], cardiovascular disease, hypertension and autoimmune disorder are on the rise globally [5]. As a result, there is a major paradigm shift in the healthcare industry from sickness centered to wellness focus. Wellness is becoming an important

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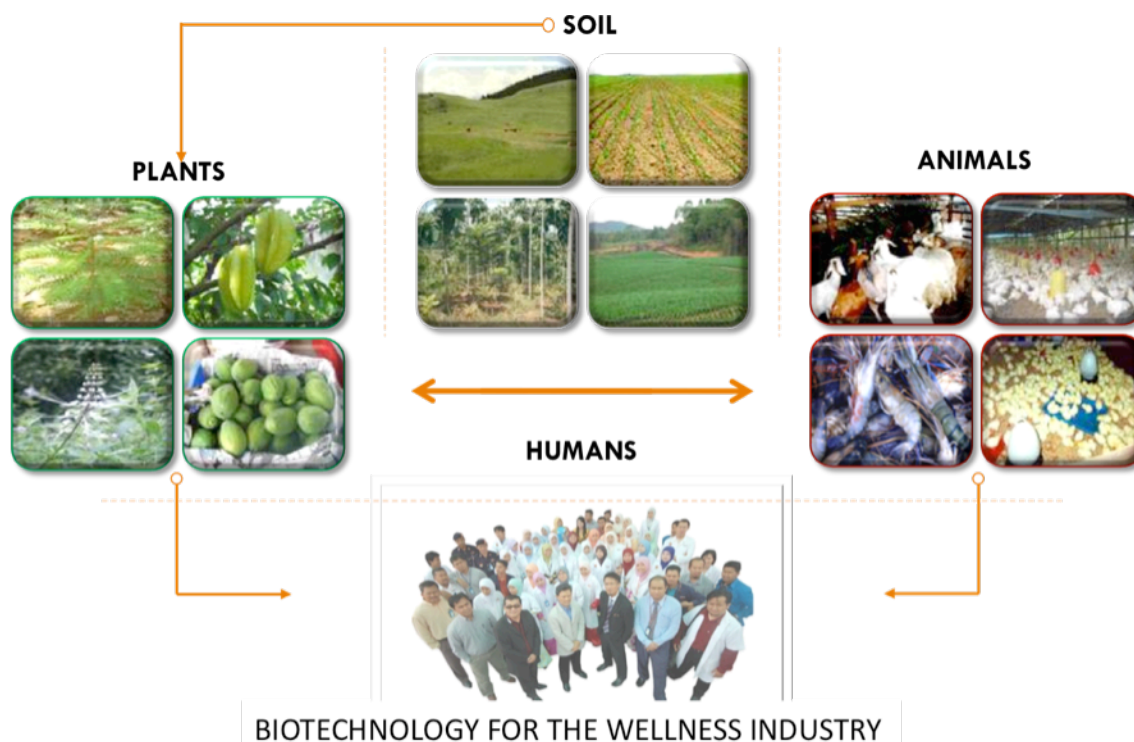
sector in the health industry. In the year 2010 it was reported that the wellness industry was worth USD 1.9 trillion per year and on the rise [6,7]. Wellness industry focuses on the enhancement of health and reduction of the risk of getting chronic disease in the first place. As such biotechnology has a lot to offer to the wellness industry especially in many areas of agro-biotechnology, food processing, nutraceuticals, cosmeceuticals, biotherapeutics and regenerative medicine [8,9]. At present, there is a tremendous global and local interest for wellness products. Wellness sector focus on providing products and services to a wider community to offer healthier life style, improve appearance, slow down the effect of ageing and to reduce the risk of developing chronic diseases. The wellness products are also intended for the promotion of health in soil, plants, animals and human. Soil health is the foundation of wellness as healthy soil produce healthy plants and crops in turn produce healthy animals for human nutrition (Figure 1).

## 2. HEALTH AND WELLNESS

World Health Organization defines human health as a state of complete physical, mental and social wellbeing [10]. It is an encompassing definition that advocate for systemic approach to health. The modern health care system is by geared toward therapy by

treating symptoms and disabilities that already established in a particular individual. The objective of the therapy is to restore the body function to its normal state of health and vigour. It is reactive in nature as the treatment is mostly given to the already sick and health's impaired individual. The common practice is that the individual seeks treatment after the symptom appeared or confirmation of the disease by specific diagnostic test. In addition to that there are occasional interactive activities in the form of regular periodic medical check-up. Any health issue detected during periodic medical check-up will result in some form of recommended treatment by the health care providers. In term of health management strategy, there is nothing wrong with this approach. It has benefitted the world community in taking good care of the sick and injured. However the reactive and interactive nature of this approach carries a higher risk. It also incurs a higher economic burden as the physiological harm is already done before any corrective action is made. Any metabolic or physiological disorder that is allowed to propagate beyond the body homeostatic control limit might inflict permanent physiological impairment.

On the other hand, the wellness approach is a proactive action taken by a relatively well inform individual to enhance their health by living a healthy lifestyle and addressing the root cause of metabolic



**Figure 1:** From soil health to human wellness.

disorder. Wellness approach in general is not intended to address any particular disease or impairment; it is rather designed to optimize the physiological function of the individual. Under optimum metabolic function, human body is essentially under a self-regulating and healing mode. In addition, wellness approach is increasingly used to enhance the performance of the physiological function beyond what is considered normal. The quest for healthier body, better and younger looking, superior physical strength, endurance and athletic performance, giving birth to a better offspring, creative mind and happier personality are some of the most desirable wellness attributes [11,12]. These promising areas are currently being addressed by the use of wellness biotechnology approach to enhance the health and wellbeing of the community.

### 3. BIOFACTORIES AND WELLNESS INDUSTRIES

Man used biotechnological applications of biosystems before he knew how to write. Hieroglyphics suggest that the ancient Egyptian civilizations were using living yeast and the process of fermentation to produce their bread over 6,000 years ago. Due in part to this application, there were more than 50 varieties of bread in Egypt at that time. Not only bread were made by bioprocess, they made also different wine varieties using fermentation techniques based on their understanding of that alcohol can be produced from sugars in the absence of oxygen. At that time, they didn't know what was responsible for the leavening process or alcohol production and probably assumed that chemical action of yeast is a mysterious and unreal phenomenon. A small portion of this dough was used to start or leaven each new lot of bread dough. It was believed that leavening mixtures for bread making were formed by natural contaminants in flour such as wild yeast and lactobacilli, organisms also present in milk. However, the oldest manufacturing ticket in human history with complete Standard Operation Procedure (SOP) have been written in details for bread and wine production from almost 4000 years ago on the wall of old ancient Egyptian house from Sakkara. This oldest manufacturing ticket was highlighted for the first time by Prof. El Gewely in his famous publication "Biotechnology Domain" [14], and he selected this photo as the cover for his book series "Biotechnology Annual Review". This painting from an old Egyptian temple is preserved in the Rijksmuseum van Oudheden (National Museum of Antiquities) in Leiden, The Netherlands. Therefore, food biotechnology industry is considered as one of the oldest industries in the world. The industrial bioprocess

deals with the use of living cells or part of it (enzymes or organelles) and cultivates them *in vitro* to produce either cell mass or certain metabolite(s). The modern form of the bioprocess industries was born in the 1910s as the main force for the production of ethanol, acetone and butanol. The production of citric acid as the first organic acid produced by fermentation in 1923 was also an important step for the growth of this industry. With the first discovery of antibiotics and the industrial production of penicillin in the mid of 1940s, significant growth of bioprocess industries was observed in parallel with other related industries (e.g. vessel manufacturing using high quality stainless steel, design of monitoring and control system for temperature, pH, DO and other cultivation parameters). This was very important step for the production of different microbial metabolites under well controlled and strict sterile conditions.

A leading milestone in biotechnology history was achieved in the early 1980s with the production of human insulin as the first recombinant biotherapeutic protein produced in large scale using recombinant strain of *Escherichia coli* carrying human insulin gene. Since that time, many other recombinant therapeutic proteins for medical applications such as streptokinase, hirudin and human growth hormones were commercialized. However, on parallel to the growth microbial fermentation industries, the use of animal cells and plant cells for the production of different metabolites was growing rapidly since the mid of 1970s. Plant cells and algae are now widely used in bioprocess industries for the production of many important metabolites in both wild type form or as recombinant cells. On the other hand, animal cells, both of mammalian and non-mammalian cells, are now the most important producer of different types of therapeutic proteins such as erythropoietin, tissue plasminogen activator (tPA). Moreover monoclonal antibodies (MAb) are widely produced nowadays not only for diagnostics but also for many therapeutic applications as immunosuppressive and anticancer drugs.

Different types of living organisms are currently involved in biotechnology based wellness industries. These includes: Microbial cells biofactories, Green cells biofactories (plant and algae), and Mammalian cells biofactories. The range of biometabolites, production capabilities and production processes for each living organism is varied and highly dependent on the nature and type of the cells applied. In general, to obtain bioactive metabolite from certain biofactory, both of

upstream and downstream processes should be considered. Upstream for some biofactories is carried out either in open field cultivation practice as in case of plant and algae or in closed system under sterile condition using different types of bioreactors. After cultivation step, extraction and purification of the targeted compound are carried out using different steps. The length and complexity of the downstream process is based on the nature of cells used, nature of bioactive product and byproducts, degree of purity required of the targeted molecule. Thus, bioprocess development and complete bioprocess design is required to establish industrial platform for any type of biofactories.

### 3.1. Microbial Cells: The Oldest and the well Established Biofactory

Microbial cells are the most important key players in wellness industries. They serve as important organisms with wide range of applications covering environmental, agricultural, industrial and biomedical fields. Microbial cell factories are highly diversified and include many members of both of Eukaryotic and Prokaryotic organisms and widely grew under different environments and cultivation conditions. Thus, their metabolites are diversified as well in terms of stability and ability to work under different conditions of pH, temperature, salinity and osmotic pressure.

In the microbial world, bacteria are widely used for the production of antibiotics, polysaccharides and different types of enzymes important for drug formulation. Fungal cells are famous for the production of bioactive metabolites such as antibiotics, enzymes, alkaloids and many products of biotransformation to transform less or non-bioactive compounds to more active form. However, the first known antibiotic (Penicillin) which produced by the filamentous fungi *Penicillium chrysogenum* saved the life of billions of people since its discovery in 1945 and commercial production in mid 1950s. Nowadays, different antibiotics derived from fungal cells are widely used beside the Penicillin G and its derivative forms. Beside the long history of yeasts in food and feed technologies, they find recently many new pharmaceutical applications based on their ability to produce some biotherapeutic killer toxins [14]. The role of yeast cells in wellness industries was increased significantly as yeasts such as *Saccharomyces cerevisiae* and *Pichia pastoris* are considered as the most important biofactories for the production of different types of recombinant proteins such as insulin,

albumin, hepatitis B surface antigen and different types of therapeutic proteins [15, 16]. Actinomycetes are very important source for the production of antibiotics. More than 70% of known antibiotics are produced by this type of microorganism. Moreover, actinomycetes are also important source for the production of different types of enzyme inhibitors used in the treatment of many diseases. In general, Table 1 gives some examples of different microbial products used in wellness industries.

#### 3.1.1. Microbial Cells in Environmental and Plant Wellness

It is well known that microbial cells play key role in many element cycles in our environment such as Carbon, Nitrogen, Oxygen and Sulphur. They act as important microbiocatalysts for many reactions in the environment based on their unique enzyme systems. For example, in water treatment plants, beside the physical and chemical treatments, biological treatments using nitrifying bacteria and phosphorus reducing bacteria are the main key players in waste water treatment process [111,112]. Moreover, bioremediation/biodegradation processes of biohazard and toxic compounds such as benzene, toluene, aromatic compounds and polycyclic aromatic hydrocarbons are mainly carried out using different types of microorganisms [113-115]. Moreover, microbial cells either in living or dead forms play many important roles in removal of heavy metals in agro-industrial wastes and act as bioadsorbant for toxic metals such as zinc, nickel, chromium and cadmium [116]. More recently, different microorganisms were used in the production of many environmental friendly and biodegradable biopolymers such as polyhydroxyalkanoates (PHAs) and polylactates (PLAs) for many industrial applications [87-90]. These two biopolymers are currently replacing, in small portion, the traditional non-biodegradable plastics such as polyethylene and polypropylene.

It is well known that type of soil and its nutritional contents are the most important factor for healthy plant growth. Beside the main three basic life elements C, H and O, which are supplied to plant through carbon dioxide, oxygen and water, plant requires six macro- and seven microelements for growth. The macroelements include nitrogen (N), phosphorus (P), potassium (K), calcium (Ca), magnesium (Mg), and sulfur (S); whereas, the microelements are boron (B), chlorine (Cl), copper (Cu), iron (Fe), manganese (Mn), molybdenum (Mo), and zinc (Zn). These nutrients are

added to soil in form of either inorganic or organic fertilizers. In parallel to industrial revolution and the growth of chemical industries, it was possible to produce many economically effective inorganic fertilizers which give fast plant growth with high yield. However, farmers now realized that uncontrolled and extensive use of this type of fertilizer cause many environmental and health problems. For example, extensive use of inorganic nitrogen fertilizers leads to eutrophication, soil acidification, heavy metal accumulation, blue baby syndrome (in case of extensive use of ammonium nitrate). On the other hand, continuous addition of inorganic phosphate in the forms of rock phosphate leads to radioactive compound accumulation. Moreover, extensive use of chemical fertilizers have negative effect on the viability and growth of beneficial microorganisms in soil which provide the plant with different essential nutrients such as growth hormones and inhibit the growth of different pathogens. Beside all these problems, most of inorganic fertilizers are water soluble, thus, small fraction will be only used by plant and large quantities diffuse in soil and contaminate ground water. Based on these problems, the growth of microbial fertilizers market grew extensively during the last few decades. This type of fertilizer is composed of different microbial consortiums which are able to survive in rhizosphere area and to support the plant growth by providing macro- and microelements in utilizable forms. The most famous group is the plant growth promoting rhizobacteria (PGPR) which includes various species like *Azotobacter*, *Alcaligenes*, *Arthrobacter*, *Acinetobacter*, *Bacillus*, *Pseudomonas*, *Enterobacter*, *Rhizobium*, *Bradyrhizobium*, *Serratia* [117-119]. In addition, other microorganisms such as mycorrhiza help in maintaining soil fertility and absorption of essential elements for better and healthier plant growth [120]. These types of beneficial soil microbes support plant growth through the conversion of soil natural elements from non-utilizable form to utilizable form. This carried out through different microbial processes such as nitrogen fixation (converting atmospheric nitrogen to ammonia), phosphate solubilization (converting insoluble organic or rock phosphorus to soluble form such as orthophosphate).

The presence of these microbial consortia in soil supports healthy plant growth using three main mechanisms. First, increase the availability of essential macro and micronutrients to promote healthy plant growth (biofertilizers). Second, produce different types

of plant growth hormones to increase the plant growth rate and yield (Biostimulants). Third, inhibiting the growth of other microbial plant pathogens in the rhizosphere area directly or indirectly through their different antimicrobial metabolites (Biocontrol).

### 3.1.2. Microbial Cells and Animal Wellness

Animal feed is usually plant based substance to support animal healthy growth. In many cases, the nutrient contents of the provided feed are not sufficient to provide the required nutrients for healthy growth. Thus, different additives are currently used to improve the feed quality. In general, feed additives are classified into the following groups:

1. Technological additives (preservatives, antioxidants, stabilizing agents and silage additives).
2. Sensory additives (flavors and colorants)
3. Nutritional additives (vitamins, minerals, amino acids, trace elements).
4. Zootechnical additives (digestibility enhancer, gut flora stabilizers).
5. Coccidiostats and histomonostats (control the health of animal through direct effect such as veterinary medicines).

The functional feed additives which have direct effect on animal health were classified recently by de Lange and his coworker [121] into four main groups as follows:

1. Immune system enhancers (immunoglobulins,  $\omega$ -3 fatty acids, yeast derived  $\beta$ -glucans).
2. Pathogen load reducers (organic acids, high level of zinc oxide, essential oils, spices, herbs, some types of probiotics, antibiotics).
3. Beneficial gut microbes stimulators (probiotics and some types of probiotics).
4. Digestive function stimulators (organic acids, amino acids and vitamins).

In almost all farm animals, the microbial environment of gastro intestinal tract (GIT) influences animal performance. Thus, in many animal feeding practice probiotics are used as an essential part of feed formula to facilitate the establishment and maintenance of the beneficial microorganisms in GIT. The most common types of microbes used in feed additives are

Table 1: Different Types of Microbial Cells and Microbial Metabolites Used in Wellness Industries

Living Cell		References
<b>Probiotics</b>	<i>Lactobacillus</i> (different species) <i>Saccharomyces boulardii</i> <i>Kluyveromyces lactis</i> <i>Bifidobacterium</i> (different species) <i>Lactococcus</i> (different species)	[17,18] [19,20] [21] [22,23] [24]
<b>Starter Culture</b>	<i>S. cerevisiae</i> <i>Penicillium roqueforti</i> Different Lactic acid bacteria (LAB)	[25] [26] [27,28]
<b>Biometabolites</b>		
<b>Organic acids</b>		
Citric acid	<i>Aspergillus niger</i>	[29,30]
Gluconic acid	<i>A. niger</i> , <i>Gluconobacter oxidans</i>	[31,32]
Oxalic acid	<i>A. niger</i>	[33,34]
<b>Amino acids</b>		
L-Arginine	<i>Brevibacterium glavum</i> , <i>Bacillus subtilis</i>	[35,36]
L-Aspartic acid	<i>Alcaligenes metacaligenes</i> , <i>E. coli</i>	[37,38]
L-Glutamic acid	<i>Corynebacterium glutamicum</i>	[39-41]
L-Lysine	<i>C. glutamicum</i>	[42,43]
DL-Methionine	<i>C. glutamicum</i> , <i>E. coli</i>	[44]
L-Phenylalanine	<i>C. glutamimcum</i> , <i>rE. coli</i>	[45,46]
L-Threonine	<i>E. coli</i>	[47,48]
L-Tryptophan	<i>C. glutamicum</i>	[49]
<b>Fatty acids</b>		
<b>Vitamins / related products</b>		
Vitamin B <sub>2</sub> (Riboflavin)	<i>Eremothecium ashbyii</i> , <i>Ashbya gossypii</i>	[50,51]
Vitamin B <sub>7</sub> (Biotin)	<i>rE. coli</i> ; <i>Rhizopus nigricans</i>	[52,53]
Vitamin B <sub>9</sub> (Folic acid)	<i>Bifidobacterium</i> (different species)	[54,55]
Vitamin B <sub>12</sub>	Different microorganisms	[56,57]
Vitamin C (Ascorbic acid)	<i>G. oxidans</i>	[58]
Vitamin K <sub>2</sub>	<i>Geotrichum candidum</i> , <i>Flavobacterium sp.</i>	[58]
<b>Enzymes</b>		
Glucose isomerase	Different microorganisms	[59,60]
Glucose oxidase	<i>A. niger</i> ; <i>G. oxidans</i>	[61,62]
Cholesterol oxidase	<i>Rhodococcus sp.</i> ;	[63,64]
Amylases	Different Microorganisms	[65,66]
Xylanases	Different microorganisms	[67,68]
Phytases	Different microorganisms	[69,70]
Cellulases	Different microorganisms	[71,72]
<b>Polysaccharides</b>		
Alginate	<i>Azotobacter vinelandii</i>	[73,74]
Gellan	<i>Sphingomonas paucimobilis</i>	[75,76]
Pullulan	<i>Aureobasidium pullulans</i>	[77,78]
Kefiran	<i>Lactobacillus kefiranofaciens</i>	[79,80]
Lentinan	<i>Lentinula edodes</i>	[81,82]
Pleuran	<i>Pleurotus ostreatus</i>	[83,84]
Xanthan	<i>Xanthomonas capastris</i>	[85,86]
<b>Bioplastics</b>		
Polyhydroxyalkanoate (PHA)	Different microorganisms	[87,88]
Poly lactate (PLA)	Different microorganisms	[89,90]

Table 1: continued

Living Cell		References
<b>Antibiotics</b>		
Penicillins	<i>Penicillium chrysogenum</i>	[91,92]
Cepaholsporins	<i>Penicillium chrysogenum</i>	[93,94]
Cyclosporins	<i>Tolypocladium inflatum</i>	[95,96]
Erythromycin	<i>Saccharopolyspora erythraea</i>	[97,98]
Rifamycins	<i>Amycolatopsis mediterranei</i>	[99,100]
Natamycin	<i>Streptomyces natalensis</i>	[101,102]
<b>Recombinant proteins</b>	Recombinant strains	
Insulin	<i>rE. coli</i> , <i>rP. pastoris</i> , <i>rS. cerevisiae</i>	[103,104]
Human growth hormones	<i>rE. coli</i>	[105,106]
Viral surface antigen	<i>rP. pastoris</i>	[107,108]
Hirudin	<i>rE. coli</i>	[109,110]

*Saccharomyces cerevisiae*, *Saccharomyces boulardii*, different strains belong to *Lactobacillus* sp. *Enterococcus* sp., and *Bifidobacterium* sp. [122,123]. These types of microbes play several roles to provide the animal with certain required nutrients such as amino acids and vitamins, decrease the production of ammonia, produce antimicrobial metabolites against pathogens, initiate non specific immunostimulation mechanism, and produce different types of digestive enzymes. However, probiotics applications are not limited to terrestrial farm animals but extended to aquaculture in fish and shrimp farms [124]. Beside bio-additives, different types of amino acids and vitamins are added to animal feed to balance the nutritional requirements for protein production. The most essential amino acids in animal feed formula are DL-methionine, L-lysine, L-threonine and L-tryptophan. In addition, vitamins A, B, C, D, E and K are commonly added to many new combined feed additives. Exogenous addition of amino acids and vitamins improves the efficiency of protein biosynthesis, supports many biosynthetic pathways and these two together improve animal health. Moreover, feed supplementation with amino acids has economic and environmental impact through decreasing the excessive protein in animal feed and thus become a cost effective solution to decrease nitrogen pollution problems associated with animal feed.

In addition to amino acids and vitamins, large numbers of microbial enzymes are also added to different types of animal feed formula [125]. These enzymes include amylases, xylanases, pectinases, lipases, invertases, celluloses,  $\beta$ -glucanases, phytases and non-starch polysaccharidases (NSPases). They are used to improve feed digestion, nutrient uptake,

and increase availability of certain minerals. The beneficial effects of enzymes are achieved through different mechanisms such as breakdown of anti-nutrient factors present in feed ingredients, elimination of nutrient encapsulation and chelation effects and thus increase availability, breakdown of specific bonds in raw material that are not cleaved naturally by animal endogenous enzymes to increase the feed nutritional value. Of different enzymes used, xylanases,  $\beta$ -glucanases, non-starch polysaccharidases (NSP'ases) and phytases are the most common functional enzymes applied to improve feed nutritional values. Xylanases and  $\beta$ -glucanases are added to cereal-based feed for monogastric animals which, contrary to ruminants, are unable to fully degrade plant based feeds rich of cellulose and hemicelluloses [126]. NSPases are used for degradation of non-starch polysaccharides (NSP) to improve nutrient utilization and to produce a variety of low molecular weight polysaccharides of prebiotic activities to support the growth of beneficial probiotic strains in animal intestine and also to minimize the proliferation of pathogens [127,128]. On the other hand, phytases are widely used as essential enzymes to improve phosphate consumption by monogastric animals. Phytic acid is the main storage form of phosphorus in plants. In addition, this compound is able to combine protein and vitamin through making insoluble complex and thus decrease their utilization efficiency and digestibility [129]. Therefore, phytase has been applied as one of the important ingredients of poultry and swine feeds as well as aquaculture [130]. Practically, these enzymes are used in combination to achieve their targeted roles.

Since many years, different antibiotics have been added to farm animal feed. The antibiotics were added

to prevent disease outbreaks in confined animal feeding operation (CAFO) as prophylactic agents. Moreover, antibiotics increase the growth rate by promoting feed efficiency. In general, the antibiotic concentration given to promote animal growth is usually less than the concentration given for therapy and prophylaxis [131]. The most commonly used antibiotics in feed are mainly belong to aminoglycosides, macrolides, beta lactams, and tetracyclines groups. Antibiotic addition to feed became general practice in farm animal and in aquaculture application as well, some even in daily basis to control bacterial diseases [132]. However, antibiotics used in feed are poorly adsorbed in the gut of the animal and thus as much as 30-90% of applied antibiotic are released to the environment. This gives great threat for development of new microbes of significant resistance to antibiotic due to continuous exposure to sub lethal doses. Thus, application of antibiotics in feed is under revision in many countries and the acceptance/rejection decisions for certain applications are dependent on their local authorities. For example, only few numbers of antibiotics are approved for animal feed application in Europe, North America, Australia and Japan. Other countries are still open and use many types of antibiotics in wider scale. Irrespective of these growing limitations, many types of antibiotics and vaccines are widely used in veterinary medicine for prophylaxis and treatment purposes.

However, the type and concentration of amino acid, vitamin, enzyme and antibiotic added to feed formula are highly dependent on type and age of animal, nutritional value of other feed components, and stress conditions. Most of above mentioned amino acids, vitamins, enzymes and antibiotics used in feed formula are industrially produced by different types of microorganisms.

### **3.1.3. Microbial Cells and Human Wellness**

Human wellness is the top of wellness pyramid. Microbial cells play important role in human wellness via direct and indirect ways. Once humans live in healthy non-contaminated environment and consuming nutrient rich and healthy food, this will reflect on human wellness. Moreover, microbial cells play direct role in human wellness as they are used in form of living cells or microbial metabolites in many food, nutraceutical, cosmetic, cosmeceutical and pharmaceutical industries.

Using microbial cells as part of human diet have long history to provide healthy life. Probiotic

microorganisms, belong to different species of *Lactobacillus*, *Lactococcus*, *Bifidobacterium*, *Saccharomyces*, *Kluyveromyces*, were used as major constituent in many fermented foods and drinks. This based on their *in vivo* application in human intestine in preventing the growth and colonization of pathogenic microorganisms. Moreover, they are able to produce some essential vitamins in human body such as vitamin K and folic acid. On the other hand, microbial metabolites such organic acids, amino acids, vitamins. and polysaccharides are extensively used in food industries as major ingredient or additives to enhance the nutritional value, increase shelf life and stability, improve taste and enhance food digestion and act as antimicrobial to prevent microbial contamination during storage process.

Beside food sector, microbial metabolites are important component in many cosmetic products either as major active ingredients or as filling, stabilizing, flavoring or coloring agents. These include peroxide inducible protective factors (from *S. cerevisiae*), chitosan, octadecandionic acid, rhizobium gum, exopolysaccharides, ex-foliation promoting enzymes, flavor and fragrance and natural pigments [133,134]. In addition, some new low molecular weight microbial metabolites such as ectoines are currently used as main component of lotions and creams based on their ability to prevent skin dehydration, protection against UV solar radiation and anti-aging activity [135,136].

Microbial metabolites are applied as pharmaceutical compound since many centuries. For example, mushrooms are traditionally consumed in many Asian countries as healthy food to prevent diseases and stimulate immune system. Mushrooms are rich source of different types of bioactive polysaccharides and many other low molecular weight compounds of immunomodulator, antioxidant, antimicrobial and anticancer activities [96]. However, antibiotic discovery in early 1940s was the most important event in the history of therapeutics microbial metabolites application in human health. Since that time, microbes received more attention and considered as the largest mine of therapeutics and anti-infective compounds in the earth. Based on the revolution in DNA technology, microorganisms were employed as cost effective biofactory for production of different human biotherapeutic proteins [137]. The first commercialization of bacterial insulin in 1982 by Eli Lilly Co. opened a new gate for multibillion dollar business gate to many companies. With completing the genome sequences of industrially important microorganisms



such as *E. coli* [138], *B. subtilis* [139], *S. cerevisiae* [140] and *P. pastoris* [141] in addition to completion of human genome project [142], the scientists were able to construct many microbial strains carrying human genes for large scale production of biopharmaceuticals proteins such as albumin [143], human growth hormones [144], interferons [145,146], and hirudin [109,110].

Another breakthrough was achieved by the construction of recombinant *E. coli* for antibody fragment production as alternative and cost effective process compared to the traditional method of production by hybridoma cells [147].

#### **3.1.4. Bioprocess Platform for Microbial Cell Cultivation**

To design a good bioprocess, it is necessary to consider both of biological and biochemical engineering parameters from the beginning of the design phase. This is necessary for high efficient production and for obtaining the highest possible yield from the biofactory applied. In general, the bioprocess involving microbial cells could be divided into upstream and downstream processes.

##### 3.1.4.1. Upstream Process

The upstream process is considering all steps necessary to optimize the productivity of the biosystem used which includes 4 main steps as follows:

- 1- *Primary Screening*: This step aims to the selection of the potent cells of the highest possible volumetric and specific production of the desired metabolite(s) in small scale. At this level, petri-dishes, microtiter-plates and shake flasks are usually applied.
- 2- *Bioprocess Optimization in Pre-Bioreactor Level*: Second, the process optimization of the cultivation medium and conditions should be studied. This step is carried out using small scale shake flask to optimize different process variables such as:
  - A) Physical process parameters: temperature, pH, mixing, etc...
  - B) Nutritional requirements: C-source, N-source, C/N ration, trace elements, complex organic sources, vitamins, growth factors, etc...
  - C) Biological parameters: type, age and size of inoculum, cells pretreatment before inoculation.

- D) *Adaptation parameters*: Design a smooth transfer process from vegetative growth medium to large scale industrial medium through medium formulation and fast cell adaptation.

However, this primary optimization, in some cases, is not reflecting the optimum condition especially for highly aerobic and pH sensitive organisms since the pH change during the cultivation process and aeration effect are neither measured nor controlled in this stage.

- 3- *Bioprocess Optimization in Bioreactor*: The main target in this step of process development is to study the cultivation process under fully controlled cultivation conditions using bioreactor. This step will allow full optimization of cultivation parameters under the same large scale production process (with fully controlled pH, aeration, and DO). During this step, the effects of different parameters such as pH, DO, aeration and agitation on the cultivation process are usually studied. The data sets obtained in this level can give more than 70% of the information required to identify the production process, to design process scaling up strategy, to calculate the system productivity, and to estimate the process economy. However, the transfer of process from small scale shake flask to bioreactor is often problematic in case of cultivation of shear sensitive organisms and this step is usually considered as the bottle neck for bioprocess development for these types of strains.

- 4- *Process Scaling Up*: The last step in upstream bioprocess development is to study the process scalability and to transfer the process from small laboratory scale bioreactor up to pilot scale and industrial scale bioreactor. Process scaling up is usually carried out using different biochemical engineering parameters.

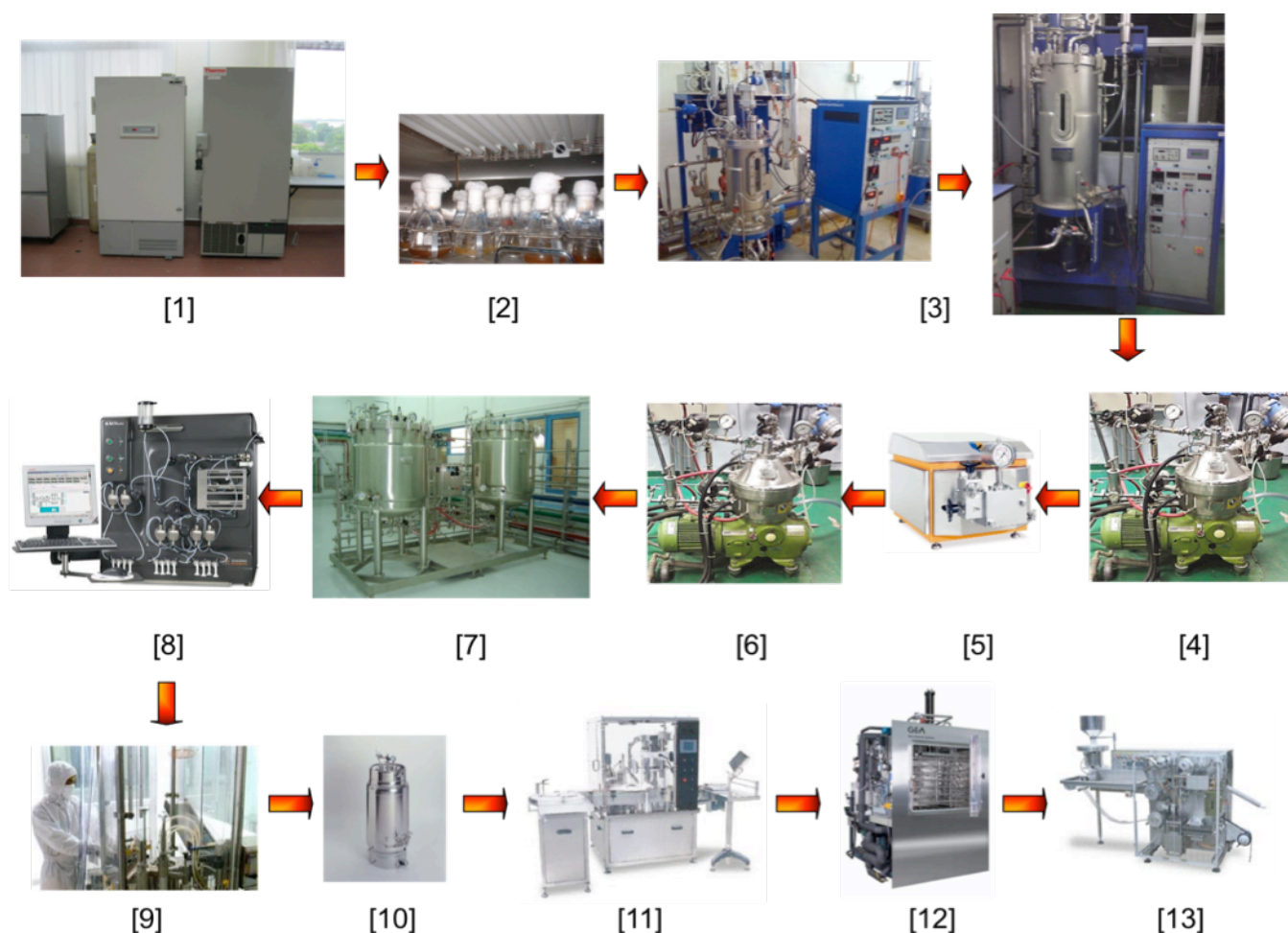
##### 3.1.4.2. Downstream Processing

The product in bioprocess industries may be in form of living cells such as baker yeast and probiotics or specific metabolite(s). The microbial products are either excreted to the cultivation medium or retain intracellular. However, the location of the product is very important for the downstream process design. The typical examples of extracellular products are: antibiotics, amino acids, organic acids and many bacterial and fungal enzymes. Whereas, the most famous examples of intracellular products are the recombinant (which produced in form of inclusion

bodies inside the bacterial cells) and many intracellular enzymes.

In most cases, the extracellular products are present in fermentation medium in relatively low concentration in mixture with other medium ingredients and byproducts. This makes a series complication to obtain the product in its pure form. On the other hand, if the biological product is intracellular, the first step in downstream process should include cell disintegration to obtain the desired product. In general, cell disruption is carried out either by physical or chemical method. Among the different chemical methods applied, using lysis buffer or permeabilizing agents are very common. On the other hand, for physical methods, freezing/thawing technique, cell rupture under high osmolarity, sonication, ball homogenization and high pressure homogenization are usually applied. However,

the method selected is based on the type of cells, concentration in broth, cell wall mechanical properties and the nature of the desired product. For industrial application, high pressure homogenizer is common to break all known types of microbial cells. This method is based on the cell exposure to high liquid shear rate by passing them through an orifice under high pressure. Full cell breakdown release large quantities of cell debris, organelles and many intracellular products, which subsequently complicate the purification process to obtain the desired product in pure form. In general, separation and purification steps involve different types of equipments and cause a significant loss in the final product concentration. Excessive cell debris breakage and micronization can increase the load on centrifugal operation and the passage of fine particle to packed chromatographic columns can lead to low productivity and high media replacement costs [148]. The number



**Figure 2:** General bioprocess platform for biopharmaceutical protein production by recombinant *E. coli*. [1]: Ultra deep freezer for working cell bank preservation; [2] shake flask cultivation; [3] Bioreactor cultivation and scaling up; [4] Cell separation by self cleaning disk separator; [5] Cell break with high pressure homogenizer; [6] inclusion bodies separation by self cleaning disk separator; [7] *in vitro* protein solubilization and refolding tanks; [8] Fast Protein Liquid Chromatography (FPLC) for protein separation; [9] Sterile filtration; [10] Bulk product; [11] Vial filling; [12] Freeze drying (lyophilization); [13] labeling and packaging of the finished product.

of steps involved depends on the original material used, the concentration, the physicochemical properties of the product, and the required quality/grade of the final product. For example, the different steps involved in recombinant protein downstream processing can be summarized as follows:

- 1- *Separation of Insoluble Substance:* Insoluble materials include cells, cell debris, pellets or protein aggregates, and insoluble substance in the cultivation medium. For this purpose many techniques are used in industries such as: sedimentation, centrifugation, filtration and membrane filtration.
- 2- *Isolation and Concentration:* This step is usually referred to the selective isolation of a desired product from large pool of impurities. During this step the product is primarily isolated with some other impurities in relatively concentrated form. The isolation of the desired product is based on its physical or chemical dissimilarities with other compounds in the liquid. For this step different equipment are used such as: extractor, adsorption column, ultrafiltration and precipitation techniques.
- 3- *Primary Purification:* This step is more selective step than isolation. This process includes the use of different selective purification techniques such as chromatography, electrophoresis and fractional precipitation.
- 4- *Refolding (this Step is Only for Intracellular Recombinant Products):* Recombinant bacteria produces many pharmaceutically important therapeutic proteins, but in biologically inactive form because of incorrect folding of the protein in its 3-D structure. The naturally produced proteins by its native producer frequently undergo post-translational modifications (e.g. proteolytic cleavage of precursor protein, macromolecular assembly and glycosylation). Biologically inactive recombinant protein are typically activated by in process called *in vitro* folding which include the activation in stirred tanks with buffer solution and denaturant agent that first unfold the proteins; then process conditions are changed to allow proper protein refolding.
- 5- *Final Purification:* This step is necessary for products produced in extra high purified form such as vaccines and recombinant proteins. Usually, after the primary purification, the

product is almost pure but may be not in the proper applied form. Partially pure solids may still contain discolored material or solvent. Crystallization and drying are typically employed to achieve final purity. The protein final processing may require also some step for chromatographic purification.

Thus, platform design in bioprocess industries is necessary to understand and to select the most appropriate industrialization strategy to produce the desired product economically in large scale. Figure 2 summarizes general bioprocess flow for recombinant protein production by genetically modified *E. coli*.

### 3.2. The Green Biofactories

#### 3.2.1. Algae: The Aquatic-Photosynthetic Biofactory

Both of microalgae and macroalgae have been used as fertilizer, fodder, food, and medicine since centuries. The first recorded use of algae as food was 500 B.C. in china and one thousand of years later in Europe. In parallel, there is also long history for utilization of microalgae in South America and Africa as food [149,150]. The migration wave of people from Asian countries such as China, Korea, Japan, Indonesia and Malaysia to other part of the world brought this custom with them to other continents such as US and Europe. Nowadays, algae, in form of crude cells or its extract, are common as shelf product almost in all supermarkets and pharmacies all over the world. Nowadays, there are 42 countries in the world with reports of commercial macroalgal activity. China holds first rank followed by North and South Korea, Japan, Philippines, Chile, Norway, Indonesia, US and India. These top ten countries contribute about 95% of the world's macroalgae market share. According to the Food and Agriculture Organization (FAO), the world total harvest of microalgae increased from 3 million tons to nearly 13 million tons in the period from 1981 to 2002. The macroalgae that are most exploited for culture are the brown algae with 6 million tons followed by the red algae with 3 million tons and small amount of green algae. East and Southeast Asian countries have more than 99% market share of macroalgae cultivation business, with almost 75% of this market is captured by China. Microalgae have also long history as human food and were produced traditionally as food source in aquaculture [151]. Nowadays, algae are considered as main source of many important metabolites such as polysaccharides, amino acids, vitamins, pigments, fatty acids and many other

metabolites. Thus, algae in form of whole cells, cell fraction or their metabolite have wide range of applications and used as fertilizer, aqua culture supplement, industrial pigment, food additives and food supplement (nutraceutical). More recently, algal products are used as main component in many cosmeceutical and pharmaceutical product [152-155].

Beside these important algal products and their applications, algae are considered also as potential source of the future biorenewable and clean fuels: biodiesel, biohydrogen and biogas. Biodiesel production from algae is a relatively novel concept. This based on the fact that, certain microalgae can accumulate more than 70% of their dry biomass as hydrocarbons and, therefore, are potential sources of biofuels [156-158]. Beside the important applications of microalgae as biofactory for the production of different metabolite, it is also widely used for bioremediation of toxic materials and heavy metals [159-160]. Based on the fact that, algae can grow in low quality water, it can also be used for waste water treatment. Algae remove

nitrogen and phosphate from industrial waste water and thus reduced the cost in water treatment plants. After algae separation, this water can be recycled again inside the factory. Algae, as photosynthetic microorganism, can also remove carbon dioxide from industrial out-gas [161]. Therefore, it is commonly used to reduce the carbon dioxide emission from power plant. This can work also as integrated production system for power generation and algal biomass productions. However, different applications of algae for metabolites and energy production are summarized in Table 2.

#### Algal Cell Cultivation

Algae grow in almost every habitat in all part all over the world. They can grow even on solid natural animal and plant substrate. They can also grow in different locations such as hot springs, rivers, open and closed sea. They have also unique capability to tolerate very harsh conditions in terms of high temperature and pH and grow under high osmotic stress of salt lakes.

**Table 2: Major Algal Products and their Corresponding Producer Organisms**

Product	Producer Organism	Reference
<b>Fatty acids</b>		
Linoleic acid	<i>Chlorella</i> sp.	[162]
Palmitoleic acid	<i>Phormidium</i> sp.	[163]
Eicosapentaenoic acids	Different organisms	[164]
Omiga-3	<i>Nannochloropsis</i>	[165]
<b>Sterols</b>		
	<i>Karenia brevis</i>	[166]
	<i>Pyramimonas</i>	[167]
<b>Pigments</b>		
Astaxanthin	<i>Haematococcus pluvialis</i>	[168]
$\beta$ -Carotene	<i>Dunaliella salina</i>	[169]
Marennin	<i>Haslea ostrearia</i>	[170]
<b>Amino acids</b>		
Glutamate	<i>Synechococcus</i> sp.	[171]
<b>Phycolloids</b>		
Agar	<i>Gracilaria chilensis</i>	[172]
Carrageenan	<i>Kappaphycus alvarezii</i>	[173]
Alginate	<i>Sargassum vulgare</i>	[174]
<b>Pharmaceuticals</b>		
Antitumor (Phycocyanin)	<i>Spirulina platensis</i>	[175]
Anti-viral (galactans)	<i>Grateloupia longifolia</i>	[176]
Anti-bacterial	<i>Euglena viridis</i>	[177]
Anti-fungal	<i>Sargassum filipendula</i>	[178]
Lactins	<i>Oscillatoria agardhii</i>	[179]
<b>Single Cell Protein</b>		
	<i>Scenedesmus acutus</i>	[180]
	<i>Euglena gracilis</i>	[181]
<b>Biodiesel</b>		
	Different strains	[158]
	<i>Chlorella protothecoides</i>	[182]

In natural life conditions, microalgal growth is characterized by the following: maximal cell density of  $10^3$  cells/ml, average distance between cells of 1350  $\mu\text{m}$  or 250 times the cell diameter, vertical or horizontal displacement of  $5 \times 10^{-5}$  to  $3 \times 10^{-5}$  m/s, photon flux density (PFD) usually within light limited area, light supply subject to daytime rhythm,  $\text{CO}_2$  and nutrient conditions, generally far from optimal, and prolonged stability of pH value, ion concentration and temperature. In contrast, for cultivation systems: cell densities can increase up to  $10^8$  cells/ml, average distance between cells reduced to 60  $\mu\text{m}$  or 10 times the cell diameter, spatial displacements ranging from 0.3 to 1.2 m/s, turbulence-conditioned PFD variations with frequencies of 0.1-1000 s superseding the daytime rhythm, generally optimum or surplus nutrient and  $\text{CO}_2$  supply, pH and temperature controlled condition and nearly continuous mechanical stress on the cell walls and the cells themselves [183,184].

However, to cultivate algal cells two main points should be considered:

- 1- Cultivation conditions and medium composition
- 2- Cultivation system

For autotrophic alga, the required conditions needed are: light, carbon dioxide, water, nutrients and trace elements. In general, by means of photosynthesis the alga will be able to synthesize all of the biochemical compounds required for growth and metabolite production. Small algal groups, entirely autotrophic, are not able to synthesize certain types of biochemical compounds and will require these to be added to the cultivation medium.

### 3.2.2. Plant Cells: The Traditional Green Biofactory

Plants and plant products play very important role in human life. As a food source, plants directly constitute 93% of the human diet, with remaining 7% being indirectly derived from plants *via* animal products [185]. Beside food application, plants have been utilized as medicines for thousands of years and still used as source of important medicines in both developed and developing countries [186,187]. In general, plants are a valuable source of a wide range of secondary metabolites, which are used as pharmaceuticals, agrochemicals, flavors, colors, food additives and bioinsecticides. However, more than 100,000 plant secondary metabolites have already been identified, which present only 10% of the actual total in nature and only half the structures have been fully elucidated [189,190]. In spite of large growth of chemical

industries, plants will continue to provide novel products as well as chemical models for new drugs in coming centuries. In the US, where chemical synthesis dominates the pharmaceutical industries, 25% of the pharmaceuticals are based on plant-derived chemicals [191]. Biotechnology offers an opportunity to get use of cells, tissues, and organ by growing them *in vitro* for high production of the desired metabolites. The micropropagation methods for a large number of medicinal plants has been already reported and needed to be adopted [192,193]. Cell banking by means of cell cryopreservation was also important to conserve medicinal important and rare plants. Many cell banks have now plant cell department for cell and callus preservation. Nowadays, plant cell culture is considered as potential alternative technique instead of using the whole plant for the production of many primary and secondary metabolites [194-196]. Plant cells are biosynthetically totipotent, which means that each cell in culture retains complete genetic information and hence is able to produce the range of chemicals found in parent plant. The advantages of cell culture technology and *in vitro* cultivation of plant in suspension culture over the conventional agricultural cultivation were summarized by Rao *et al.* [197] as follows:

- 1- It is independent of geographical and seasonal variations and various environmental factors.
- 2- It offers a defined production system, which ensure continuous supply of products, uniform quality and yield.
- 3- It is possible to produce novel compounds that are not normally found in parent plant.
- 4- It is independent of political interface.
- 5- Efficient downstream recovery with low cost and minimum number of steps.
- 6- High efficient production rate with significant short production time.

Therefore, on this, different types of food additives, secondary metabolites and pharmaceutically important compounds are currently produced in cell culture cultivation as summarized in Table 3.

Based on the industrial importance of plant cells as biofactory for the production of different metabolites, many cultivation strategies were developed during the last two decades. The recent achievements in plant cell *in vitro* cultivations have been recently reviewed by many authors [193,230-235]. To enhance the

Table 3: Different Metabolites Produced by Plant Cell Culture

Product type	Plant used	References
<b>Color</b>		
Anthocyanins	<i>M. malabathricum</i>	[198]
Betalaines	<i>B. vulgaris</i>	[199]
Crocetins	<i>Gardenia jasminoides</i>	[200]
Anthraquinones	<i>Cinchon ledgeriana</i>	[201]
<b>Flavours</b>		
Vanillin	<i>Va. Planifolia</i>	[202]
Garlic	<i>Allium sativum</i>	[203]
Onion	<i>Allium cepa</i>	[204]
Basmati	<i>Oryza sativa</i>	[205]
Citrus	<i>Citrus</i> spp.	[197]
Cocoa flavour	<i>Theobromo cacao</i>	[206]
<b>Sweeteners</b>		
Stevioside	<i>Stevia rebaudiana</i>	[207]
Glycyrrhizin	<i>Glycyrrhiza glabra</i>	[208]
Thaumatococcoside	<i>Thaumatococcus danielli</i>	[209]
<b>Essential oils</b>		
Mint oil	<i>Mentha piperata</i>	[210]
Chamomile oil	<i>Matricaria chamomilla</i>	[211]
Jasmine oil	<i>Jasmine officinale</i>	[212]
Anissed oil	<i>Pimpinella anisum</i>	[213]
<b>Pharmaceuticals</b>		
Hepato-protective	<i>Ligustrum robustum</i>	[214]
Anti-oxidant	<i>Artemisia judaica</i>	[215]
Anti-inflammatory	<i>Harpagophytum procumbens</i>	[216]
	<i>Ficus racemosa</i>	[217]
Anti-cancer	<i>Taxus chinensis</i>	[218]
	<i>Catharanthus roseus</i>	[219]
Anti-bacterial	<i>Ficus microcarpa</i>	[220]
Anti-fungal	<i>Backhousia citriodora</i>	[221]
Anti-ulcer	Different plants	[222]
<b>Recombinant products</b>		
Therapeutic proteins	Different plants	[223]
hGM-CSF	<i>Nicotiana tabacum</i>	[224]
HBsAg	Different plants	[225]
Interleukin-12	<i>N. tabacum</i>	[226]
h-lactoferrin	<i>Panax ginseng</i>	[227]
vaccine production	Different plants	[228]
MAb fragment	<i>N. tabacum</i>	[229]

production of different metabolites by plant cells, different strategies have been proposed. These includes: selection of proper cell line with high growth rate, cell mutation and genetic manipulation, medium and cultivation conditions optimizations, use of elicitors to enhance metabolite excretion, cell permeabilization, cell immobilization and optimization of scaling up process.

#### Recombinant Protein Production by Plant Cells

As high Eukaryotes, plant cells are able to perform the complex post-translational modifications necessary

for active biological functions of the expressed heterologous proteins [236,237]. Thus, they considered as potential host for recombinant protein production. *In vitro* cultivation of plant cells also possess a number of advantages over transgenic plants. When cells cultivated in suspension they grew with faster rate compared to transgenic plant in the field. This type of cultivation also eliminates the worry of transgenic plant effect on the natural biodiversity and the GMO release associated problems. Cultivations in bioreactor are carried out under high aseptic and controlled

conditions. Moreover, the bioprocess industries are usually equipped with good waste management system which prevents any possibility of releasing biomaterials to the surrounding environment. In addition, cell culture are composed of dedifferentiated callus cells that lack fully functional plasmodesmata, and thus systemic post-transcriptional gene silencing (PTGS) may reduced since PTGS is generally believed to be transmitted *via* plasmodesmata and vascular system [235,238,239]. In general, production of high value therapeutic proteins in bioreactors using hairy roots has many advantages over using the commonly applied process which involves mammalian cells. The recombinant protein produced by plant cells required less purification steps, hairy roots can be made to store proteins in intracellular or extracellular spaces, from where they can be easily extracted [240]. Plant cells derived recombinant biopharmaceutical proteins are less likely to be contaminated with human pathogen than those from animal cells, because hairy roots do not act as hosts for human infectious agents [234]. Based on this technology, many recombinant proteins were expressed successfully in plant cells and produced in bioreactor cultures. These include: human granulocyte-macrophage colony stimulating factor (hGM-CSF) [241], Hepatitis B surface antigen (HBsAg) [243], Interleukins-2, 4 and 12 [226,242], human lactoferrin [227], green fluorescence protein [235], and many others. Beside these proteins, different types of antibodies derived from plant cells (Plantibodies) were produced in the recent years [243-245].

The expression of therapeutic proteins in plant cells opens the possibility of oral administration of some therapeutic antibodies without the need of expensive purification steps. The expression of antigen also opens new era for the development of oral vaccines as well. Oral vaccines are easy for application, administration and characterized by low cost. Therefore, oral vaccine is one of the future alternatives also to combating diseases that affects large populations in developing countries. Moreover, oral vaccines derived from plant cells are more thermostable and could be stored at room temperature [234].

### **3.3. Mammalian Cells: The Newest and the Most Wanted Biofactory in Healthcare and Medical Sector**

Mammalian cells are considered as the newest biofactory used for bioactive metabolite production. Unlike other cell types, mammalian cells are not used

to produce wide range of products related to wellness industries but they are mainly used for manufacturing of high-end products in medical and healthcare business sectors such as biopharmaceuticals, vaccines, and regenerative medicine products.

The history of animal cell culture began during the last few years of the 19<sup>th</sup> Century. During that time, many preliminary experiments were carried out to maintain piece of tissue in plasma or ascetic fluids for several days. This work faced two main challenges: first, the nutritional requirement of the tissue to sustain its viability during *in vitro* cultivation process and second the lack of high strict aseptic condition which required for animal cell cultivation. The first glimpse of light in the field of *in vitro* cell cultivation have been arisen in 1898 by Ljunggren and his group through their research on keeping a human skin tissue viable in ascetic fluid. In late 1900s, Harrison reported the maintenance and growth of nerve cells over long period up to 30 days. His experiments showed that normal cell function could continue outside the body of mammalian cell if supported by the necessary nutritional requirements under suitable cultivation conditions. These above scientific contributions were the early breakthrough in mammalian cell culture [246]. Because of the lack of highly controlled aseptic conditions requirements at that time, the process of mammalian cell cultivation was limited until the beginning of antibiotic era (mid 1940s). The development of antibiotic industry supported the development of highly aseptic cultivation techniques, which also was reflected on the progress of other fields as mammalian cell cultivation. Beside this, the addition of antibiotic to the cultivation medium facilitated handling of complex undefined culture media at that time [247]. The great milestone toward animal cell culture was the isolation of HeLa cells in 1953. This most famous cell line was isolated by Mary Kubicek from cervical cancer of Henrietta Lacks. At the end of 1940s, this cell line served as important biofactory for poliovirus cultivation and vaccine production. At that time, many researchers were working on medium development based on the high demand of mammalian cell cultivation in industrial scale. Based on this, the famous EMEM (Eagle's Minimum Essential Medium) was developed as the first known chemically defined culture medium for mammalian cell in 1955 [248]. However, the applications of this medium were also limited by the addition of undefined blood serum as main source of growth factors and low molecular weight key nutrients.

The period between the early 1950s until 1975 is considered to be the golden age of viral vaccine development that brought about effective cell culture based vaccines for human such as: measles, rabies and rubella and animal diseases such as: foot and mouth disease and many other vaccines. During the last 40 years big revolution was carried out in mammalian cell cultivation in many directions as follows:

- 1- *In Biological and Cell Factory:* many new cell lines were isolated, established, genetically manipulated and have successfully been used for the production of different biopharmaceuticals.
- 2- *In Medium Formulation:* different types of serum free media (SFM) and protein free media (PFM) have been formulated and successfully used in both of laboratory and industrial scale cultivations of mammalian cells.
- 3- *In Bioprocess:* New types of cultivation vessels and many novel bioreactor designs were developed for large scale cultivation of mammalian cells for biopharmaceuticals production. Moreover, new types of sensors and control systems for better on line monitoring of different cultivation parameters were developed.
- 4- *Tissue Engineering:* Different tissue engineering techniques have been established and used widely for soft and hard tissue repair as new trend of regenerative medicine.
- 5- *Stem Cell:* Different types of human stem cells were isolated and successfully applied for the production of biotherapeutic proteins as well as in regenerative medicine.
- 6- *In Regulation Issues:* Many new regulations from Food and Drug Administration (FDA) and other drug control bodies were established to ensure the safety mammalian cells derived products.

### 3.4. Biopharmaceuticals by Mammalian Cells

Despite the dominance of animal cell culture in the production of biopharmaceuticals in recent years, this technology was not considered into standardized large scale bioprocess until the mid 1990s. The range of culture flasks and reactors types used is quite wide for both of suspension and adherent culture. These ranged from small T-flasks to roller bottlers, Stirred tank

bioreactor, Air-lift bioreactors, fixed bed, fluidized-bed, and many new types of bioreactors with total volume up to 15,000 L. Such large capacity bioreactors are nowadays used for the production of biopharmaceuticals of large market demand such as therapeutic MAb [249]. However, the production of biopharmaceuticals by mammalian cells has long history since the first commercial production of poliomyelitis viral vaccines during the Second World War (1939-1945). A few years later, inactivated polio vaccine was produced and approved in the USA in 1955. This vaccine was initially produced in large scale using cell line derived from monkey kidney. In the mid of 1960s, this cell line was replaced by embryonic monkey tissue (WI-38) which showed high efficient productivity for up to 50 passages. This cell line was further used for human viral vaccine production against poliomyelitis and MMR (Measles, Mumps and Rubella). In parallel, another important cell line was isolated from baby hamster kidney (BHK) and used successfully for the production of many veterinary vaccines such as foot and mouth disease. Since that time, BHK cell line is one of the main biofactory applied for industrial production of biopharmaceuticals. After that time, many cell lines were developed such as: Vero cells (cells derived from monkey) and used for anti-rabies production, hybridoma cells for monoclonal antibodies (MAb) production, Chinese hamster ovary cells (CHO) for production of wide range of biopharmaceuticals such as tissue plasminogen activator (tPA), erythropoietin (EPO), human hormones and many humanized MAb, and human embryonic kidney cells (HEK-293) which have wide application for the production of recombinant proteins and vaccines.

However, depending on their applications and bio-business values, animal cells can be classified in four main groups:

- 1) Cells produce proteins employed in the production of complex therapeutics, sub-unit vaccines, and diagnostic product. This include CHO, BHK; HEK-293, NS0, WI-38 and hybridoma cells.
- 2) Cells produce viruses for gene therapy and viral vaccines applications such as VERO, HEK-293 cells.
- 3) Normal cells, tumor cells and stem cells are used in R&D. Different types of hepatic cells, cancer cells, nerve cells and skin cells are used in throughput screening to investigate the efficacy, toxicity and side effects of drug under study. Skin



cells are commonly used in the early phase of clinical trials as substitution for animal model to investigate the cytotoxicity and cutaneous irritancy studies of newly developed cosmetic and cosmeceutic products.

- 4) Human cells for subsequent use in cell therapy and regenerative medicine such as adult and embryonic stem cells.

The first two groups include the main biofactories in biopharmaceutical industries. The number of biopharmaceutical products obtained from mammalian cells increased significantly during the last 20 years. Table 4 demonstrates some examples of approved biopharmaceuticals produced by different cell lines.

### 3.5. Regenerative Medicine and Stem Cell

In addition to the well established use of mammalian cells for biotherapeutics production, they have also other important growing applications in regenerative medicine and cell therapy. During the last 40 years, beside the huge success of tissue engineering in skin repair [250], this technique was used successfully to repair human hard tissues such as bone and cartilage. In this technique, cells are cultivated *in vitro* using specific biocompatible matrix and grow in 3-D structure until reaching certain density and then re-transplanted to human body. Beside the

successful history of tissue engineering techniques to repair certain tissues such as skin, bone and cartilage, more interests were paid during the last few years by different research groups on soft-tissue engineering. This new approach will help to repair and regenerate soft tissues in different organs such as heart, lung and liver [251-253].

The progress in stem cells (SCs) research was also of great help and leads to dramatic progress in the field of regenerative medicine. SCs are characterized by their self renewal and potency. Self renewal implies the ability to reproduce in many cycles of cell division while maintaining the undifferentiated state. After cell division, each new cells may remain as undifferentiated stem cells or become another type of cells such as liver cells, red blood cells or brain cells [254]. Stem cells were first discovered in adult human cord blood in 1978 whereas the first embryonic human stem cells were isolated from inner cell mass of early embryos in 1998 by James Thomson in university of Wisconsin [255]. Nowadays, both of embryonic stem cells and adult stem cells are widely used in many regenerative medicine applications.

Hematopoietic stem cells (HSCs) were the first isolated type of adult stem cells and find many applications in research and cell therapy. Moreover, other five types of stem cells namely: Mesenchymal

**Table 4: Examples of FDA Approved Biopharmaceuticals Produced by Mammalian Cells**

Product	Protein	Application	Cell line	Approval year
Orthoclone OKT3 <sup>®</sup>	MAb	Graft rejection	Hybridoma	1986
Epogen <sup>®</sup>	Erythropoietin	Anemia	CHO	1989
Kogenate <sup>®</sup>	Factor VIII	Hemophilia A	BHK	1993
Avonex <sup>®</sup>	β-Interferon	Multiple sclerosis	CHO	1996
BeneFix <sup>®</sup>	Factor IX	Hemophilia B	CHO	1997
Herceptin <sup>®</sup>	MAb	Breast Cancer	CHO	1998
Simulect <sup>®</sup>	MAb	Kidney transplant	Hybridoma	1998
Campath <sup>®</sup>	hMAb	Leukemia	CHO	2001
Humira <sup>®</sup>	MAb	Rheumatoid arthritis	Hybridoma	2002
Xolair <sup>®</sup>	hMAb	Asthma	CHO	2003
Avastin <sup>®</sup>	hMAb	Colon and rectum carcinoma	CHO	2004
Vectibix <sup>®</sup>	MAb	Colorectal cancer	Hybridoma	2006
Cetuximab <sup>®</sup>	MAb	Colorectal cancer	Hybridoma	2006
XMAb 5871 <sup>®</sup>	hMAb	Autoimmune disease	Hybridoma	2008
Ofatumumab <sup>®</sup>	MAb	Leukemia	Hybridoma	2009
Ipilimumab <sup>®</sup>	MAb	Melanoma	Hybridoma	2011

Notes: MAb: Monoclonal antibody) produced by hybridoma cells. hMAb: Humanized monoclonal antibody.

(MSC), Bone marrow derived SC, Multipotent adult progenitor (MAPC), Endothelial progenitor (EPC) and Adipose derived (ADC) were used successfully as cell therapy in *in vitro* skin generation and to treat many diseases such as stroke, cardiovascular disease, myocardial disease, peripheral vascular disease, hypoxic ischemia, and Parkinson's disease [256-260].

On the other hand, the first clinical trial for spinal injuries treatment using human embryonic stem cells (hESCs) based medicines was achieved in 2009. More recently, hESCs-derived retinal pigment epithelium (RPM) were transplanted successfully and used in the treatment patients with Strgardt's macular dystrophy and dry age related macular degeneration [261].

In addition to the wide applications of mature cells and stem cells in regenerative medicine, they showed recently many new potential applications in cosmetic sector. Since 2008, many companies have introduced different product lines to the market using stem cell derived proteins as base active ingredient. For example, the company proteonomix (NJ, USA) launched a line of products including specific protein derived from stem cells to increase the collagen production of fibroblasts and keratinocytes and thus can be used as antiaging and in other dermatological conditions such as altered pigmentation, altered viscoelasticity and altered thickness [262,263]. At almost the same time, another set of antiaging products were launched by RNL Bio (Seoul, South Korea). The antiaging properties of this product are based on the integration of specific protein derived from cultured placenta stem cells [263,264].

These products may replace in part the widely used wrinkling remover cosmetics (Botox), which is based on the microbial botulinum neurotoxins (BoNTs). In early 2008 the FDA had warned about the possible side effect of BoNTs as they can interfere with the release of neurotransmitters leading to botulism toxicity if the toxin spread in the body beyond the injection site. Thus, the FDA gave instructions to all companies manufacturing botulinum toxin products to put warning label for the possible side effects if the product spread beyond the injection site [265].

The applications of stem cells in cosmetic industries are now beyond using the stem cells derived proteins and reached the level of direct injection of stem cells. In June 2012, FDA approved the first personalized intradermally applied cell therapy based cosmetics LAVIV™ (Azficel-T) for fine wrinkles or nasolabial folds elimination [266]. This cellular product is based on the

direct injection of collagen producing fibroblast cells originally isolated from the skin. This approval for sure will increase the interest of many companies to develop many cell therapy based cosmetics.

As shown, the field of regenerative medicine in human wellness is unlimited and demonstrates many promising biomedical and cosmeceutical applications. Stem cells will serve as potential solution for problems related to human tissue repair and will act as one of the main players in engineering the human health and wellness industries in the near future.

## CONCLUSIONS

Human cells are considered as the basic structural and functional unit of human body. They are as a matter of fact a microcosm of human life itself. The overall health and wellbeing of the whole organism depend on the normal function of each of the living cell. The cells assimilate nutrients, oxygen, water to grow, reproduce and excretes byproducts. The cellular machinery such as plasma membranes, mitochondria and ribosome that are involve in energy production, protein synthesis and metabolites production require specific nutrients, hormones and cofactors for their function. Any deficiency in a particular nutrients and the presence of metabolic inhibitors will render the cellular metabolic pathways compromised. A compromised metabolic pathway is known to be the prevailing cause of most of metabolic disorders. From bioprocess engineering perspectives, metabolic disorder can be regarded as a form of physiological adaptation for homeostasis maintenance as results of nutrients deficiency, physical stresses, hormonal imbalance and the toxic effect of metabolic inhibitors. The wellness strategy for the enhancement of human health and performance is mainly directed toward the optimizing metabolic activity of the cells. The use wholesome and functional foods, nutraceuticals, cosmeceuticals, prebiotics, probiotics, clean water and air and other non-toxic house-hold products help to provide the cells with the critical nutrients and reducing the exposure to metabolic inhibitors. Agriculture strategy that focuses on soil health by the use of biofertilizers, biopesticides, intelligent biotic farming, bi and bioprocessing will contribute to the development of higher nutrient dense products. Using biotechnology products such as amino acids, vitamins, enzymes and antibiotics in animal feed will improve animal health and reflect directly on meat quality. For human wellness, biotechnology products are diversified and not limited to the traditional use of plant and algae based bioactive molecules, microbial

metabolites such as probiotics, amino acids, vitamins, antibiotics, vaccines and biopharmaceutical proteins as important compounds with highly diversified applications in food, nutraceuticals, cosmetics, cosmeceuticals, pharmaceuticals and biopharmaceuticals industries. Moreover, mammalian and human cells are major components of high end products of wellness industries based on their new products and applications in biotherapeutics production, tissue engineering and cell therapy. As shown in this review, biotechnology for the wellness industry creates many opportunities for the development of products and services for the enhancement of soil, plant, animal and human health in sustainable manner.

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