Biotechnology for Wellness Industry: Concepts and Biofactories

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Abstract: One of the major issues in the 21st. 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

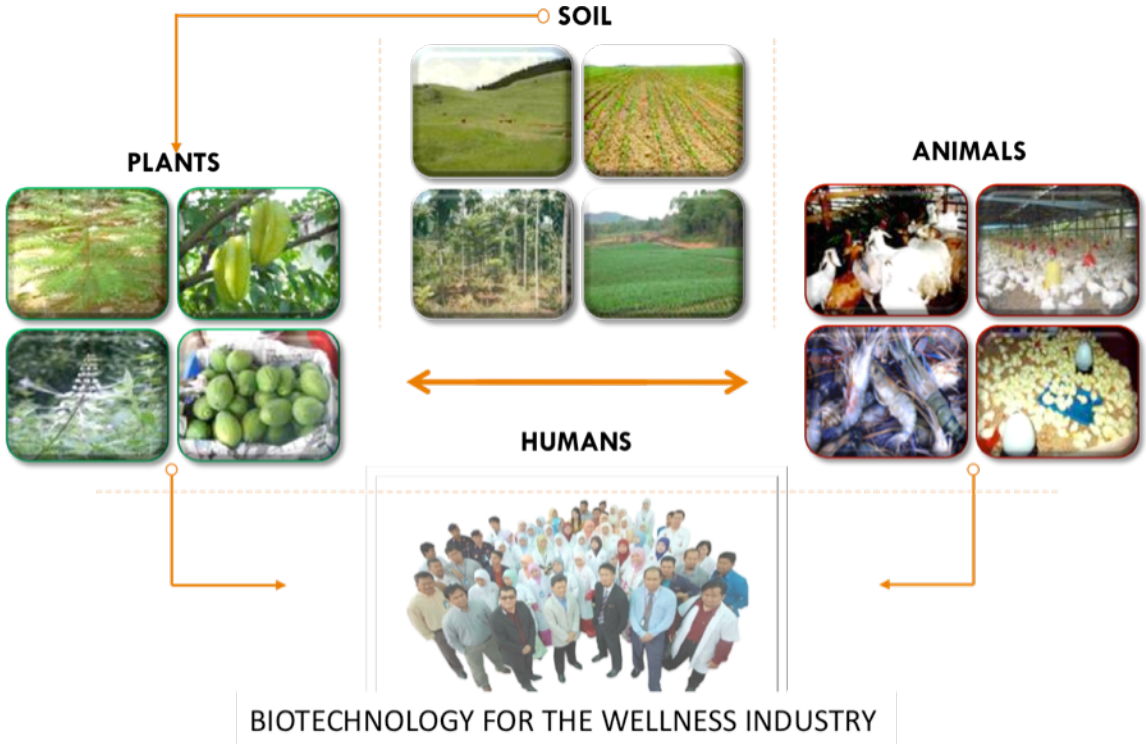

Figure 1: From soil health to human wellness.

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 used 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 cover for is book series "Biotechnology Annual Review". This paining 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, if 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, solid 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 plan 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, ω-3 fatty acids, yeast derived β-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
Probiotics	*Lactobacillus* (different species)	[17,18]
	Saccharomyces boulardii	[19,20]
	Kluyveromyces lactis	[21]
	Bifidobacterium (different species)	[22,23]
	Lactococcus (different species)	[24]
Starter Culture	*S. cerevisiae*	[25]
	Penicillium roqueforti	[26]
	Different Lactic acid bacteria (LAB)	[27,28]
Biometabolites		
Organic acids		
Citric acid	*Aspergillus niger*	[29,30]
Gluconic acid	*A. niger. Gluconobacter oxidans*	[31,32]
Oxalic acid	*A. niger*	[33,34]
Amino acids		
L-Arginine	*Brevibacterium glavum, Bacillus subtilis*	[35,36]
L-Aspartic acid	*Alcaligenes metacaligenes, E. coli*	[37,38]
L-Glutamic acid	*Corynebacterium glutamicum*	[39-41]
L-Lysine	*C. glutamicum*	[42,43]
DL-Methionine	*C. glutamicum, E. coli*	[44]
L-Phenylalanine	*C. glutamimcum,* r*E. coli*	[45,46]
L-Threonine	*E. coli*	[47,48]
L-Tryptophan	*C. glutamicum*	[49]
Fatty acids		
Vitamins / related products		
Vitamin B_2 (Riboflavin)	*Eremothecium ashbyii, Ashbya gossypii*	[50,51]
Vitamin B_7 (Biotin)	r*E. coli; Rhizopus nigricans*	[52,53]
Vitamin B_9 (Folic acid)	*Bifidobacterium* (different species)	[54,55]
Vitamin B_{12}	Different microorganisms	[56,57]
Vitamin C (Ascorbic acid)	*G. oxidans*	[58]
Vitamin K_2	*Geotrichum candidum, Flavobacterium sp.*	[58]
Enzymes		
Glucose isomerase	Different microorganisms	[59,60]
Glucose oxidase	*A. niger; G. oxidans*	[61,62]
Cholesterol oxidase	*Rhodococcus sp.;*	[63,64]
Amylases	Different Microorganisms	[65,66]
Xylanases	Different microorganisms	[67,68]
Phytases	Different microorganisms	[69,70]
Cellulases	Different microorganisms	[71,72]
Polysaccharides		
Alginate	*Azotobacter vinelandii*	[73,74]
Gellan	*Sphingomonas paucimobilis*	[75,76]
Pullulan	*Aureobasidium pullulans*	[77,78]
Kefiran	*Lactobacillus kefiranofaciens*	[79,80]
Lentinan	*Lentinula edodes*	[81,82]
Pleuran	*Pleurotus ostreatus*	[83,84]
Xanthan	*Xanthomonas capastris*	[85,86]
Bioplastics		
Polyhydroxyalkanoate (PHA)	Different microorganisms	[87,88]
Polylactate (PLA)	Different microorganisms	[89,90]

Table 1: continued

Living Cell		References
Antibiotics		
Penicillins	*Penicillium chrysogenum*	[91,92]
Cepaholsporins	*Penicillium chrysogenum*	[93,94]
Cyclosporins	*Tolypocladium inflatum*	[95,96]
Erythromycin	*Saccharopolyspora erythreae*	[97,98]
Rifamycins	*Amycolatopsis mediterranei*	[99,100]
Natamycin	*Streptomyces natalensis*	[101,102]
Recombinant proteins	Recombinant strains	
Insulin	r*E. coli,* r*P. pastoris,* r*S. cerevisiae*	[103,104]
Human growth hormones	r*E. coli*	[105,106]
Viral surface antigen	r*P. pastoris*	[107,108]
Hirudin	r*E. coli*	[109,110]

Saccharomyces cerevisiase, 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, β-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, β-glucanases, non-starch polysaccharidases (NSP'ases) and phytases are the most common functional enzymes applied to improve feed nutritional values. Xylanases and β-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, exo-polysaccharides, 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 metabolities 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, viatmins, 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 niether 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 homogenion and high pressure homogenion 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

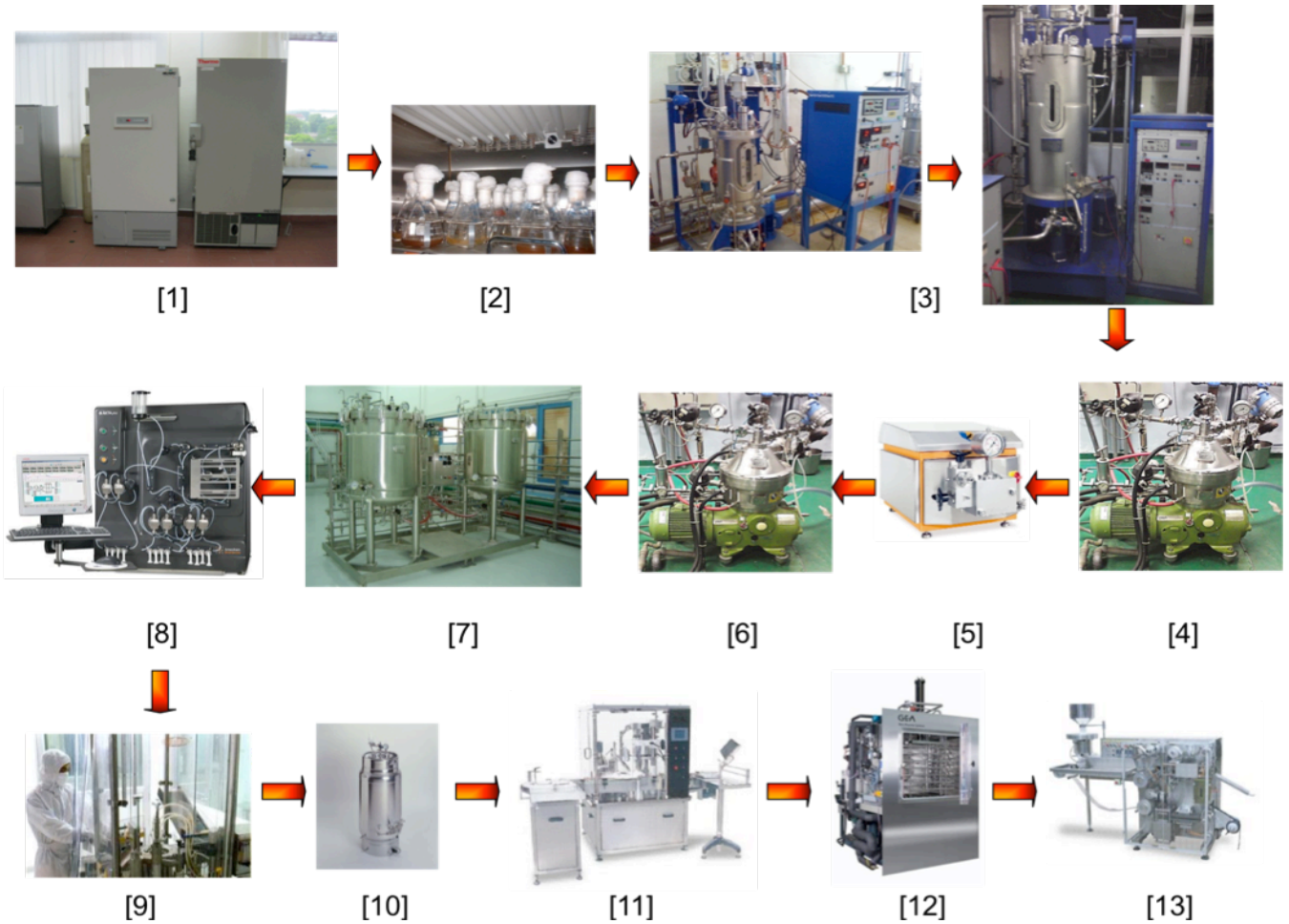

Figure 2: General bioprocess platform for biopharmaceutical protein production by recombinant *E. coli*. [1]: Ultra deep freezer for working cell bank preservation; [2] shake flask cultivation; [3] Bioreactor cultivation and scaling up; [4] Cell separation by self cleaning disk separator; [5] Cell break 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 physichochemical 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 primary 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 in active 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. Crystalization 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 macroaglae 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
Fatty acids		
Linoleic acid	*Chlorella* sp.	[162]
Palmitoleic acid	*Phormidium* sp.	[163]
Eicosapentaenoic acids	Different organisms	[164]
Omiga-3	*Nannochloropsis*	[165]
Sterols	*Karenia brevis*	[166]
	Pyramimonas	[167]
Pigments		
Astaxanthin	*Haematococcus pluvialis*	[168]
β-Carotene	*Dunaliells salina*	[169]
Marennin	*Haslea ostrearia*	[170]
Amino acids		
Glutamate	*Synechococcus* sp.	[171]
Phycolloids		
Agar	*Gracilaria chilensis*	[172]
Carrageenan	*Kappaphycus alvarezii*	[173]
Alginate	*Saragassum vulgare*	[174]
Pharmaceuticals		
Antitumor (Phycocyanin)	*Spirulina platensis*	[175]
Anti-viral (galactans)	*Grateloupia longifolia*	[176]
Anti-bacterial	*Euglena viridis*	[177]
Anti-fungal	*Sargassum filipendula*	[178]
Lactins	*Oscillatoria agardhii*	[179]
Single Cell Protein	*Scenedesmus acutus*	[180]
	Euglena gracilis	[181]
Biodiesel	Different strains	[158]
	Chlorella protothecoides	[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 μm or 250 times the cell diameter, vertical or horizontal displacement of 5×10^{-5} to 3×10^{-5} m/s, photon flux density (PFD) usually within light limited area, light supply subject to daytime rhythm, 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 60μm or 10 times the cell diameter, spatial displacements ranging from 0.3 to 1.2 m/s, turbulance-conditioned PFD variations with frequencies of 0.1-1000 s superseding the daytime rhythm, generally optimum or surplus nutrient and 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 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
Color		
Anthocyanins	*M. malabathricum*	[198]
Betalaines	*B. vulgaris*	[199]
Crocetins	*Gardenia jasminoides*	[200]
Anthraquinones	*Cinchon ledgeriana*	[201]
Flavours		
Vanillin	*Va. Planifolia*	[202]
Garlic	*Allium sativum*	[203]
Onion	*Allium cepa*	[204]
Basmati	*Oryza sativa*	[205]
Citrus	*Citrus* spp.	[197]
Cocoa flavour	*Theobromo cacao*	[206]
Sweeteners		
Stevioside	*Stevia rebaudiana*	[207]
Glycyrrhizin	*Glycyrrhiza glabra*	[208]
Thaumatin	*Thaumatococcus danielli*	[209]
Essential oils		
Mint oil	*Mentha piperata*	[210]
Chamomile oil	*Matricaria chamomilla*	[211]
Jasmine oil	*Jasmine officinale*	[212]
Anissed oil	*Pimpinella anisum*	[213]
Pharmaceuticals		
Hepato-protective	*Ligustrum robustum*	[214]
Anti-oxidant	*Artemisia judaica*	[215]
Anti-inflammatory	*Harpagophytum procumbens*	[216]
	Ficus racemosa	[217]
Anti-cancer	*Taxus chinensis*	[218]
	Catharanthus roseus	[219]
Anti-bacterial	*Ficus microcarpa*	[220]
Anti-fungal	*Backhousia citriodora*	[221]
Anti-ulcer	Different plants	[222]
Recombinant products		
Therapeutic proteins	Different plants	[223]
hGM-CSF	*Nicotiana tabacum*	[224]
HBsAg	Different plants	[225]
Interleukin-12	*N. tabacum*	[226]
h-lactoferrin	*Panax ginseng*	[227]
vaccine production	Different plants	[228]
MAb fragment	*N. tabacum*	[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 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$^{th.}$ 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 embroyonic 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 understudy. 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®	MAb	Graft rejection	Hybridoma	1986
Epogen®	Erythropoietin	Anemia	CHO	1989
Kogenate®	Factor VIII	Hemophilia A	BHK	1993
Avonex®	β-Interferon	Multiple sclerosis	CHO	1996
BeneFix®	Factor IX	Hemophilia B	CHO	1997
Herceptin®	MAb	Breast Cancer	CHO	1998
Simulect®	MAb	Kidney transplant	Hybridoma	1998
Campath®	hMAb	Leukemia	CHO	2001
Humira®	MAb	Rheumatoid arthritis	Hybridoma	2002
Xolair®	hMAb	Asthma	CHO	2003
Avastin®	hMAb	Colon and rectum carcinoma	CHO	2004
Vectibix®	MAb	Colorectal cancer	Hybridoma	2006
Cetuximab®	MAb	Colorectal cancer	Hybridoma	2006
XMAb 5871®	hMAb	Autoimmune disease	Hybridoma	2008
Ofatumumab®	MAb	Leukemia	Hybridoma	2009
Ipilimumab®	MAb	Melanoma	Hybridoma	2011

Notes; MAb: Monoclonal antibody) produced by hybridoma cells. hMAb: Huminized 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 neutrotoxins (BoNTs). In early 2008 the FDA had warned about the possible side effect of BoNTs as they can interfere with the release of neutrotransmitters 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.

REFERENCES

[1] Biotechnology Industry Organization (BIO). http://www.bio.org/. [cited 2012 Feb 12]

[2] Lazarou J, Pomeranz BH, Corey PN. Incidence of adverse drug reactions in hospitalized patients: a meta-analysis of prospective studies. JAMA 1998; 279: 1200-5. http://dx.doi.org/10.1001/jama.279.15.1200

[3] Chew YH, Shia YL, Lee CT, *et al*. Modeling of glucose regulation and insulin-signaling pathways. J Mol Cell Endocrinol 2009; 303: 13-24. http://dx.doi.org/10.1016/j.mce.2009.01.018

[4] Morgan G, Ward R, Barton M. The Contribution of Cytotoxic Chemotherapy to 5-year Survival in Adult Malignancies. Clin Oncol 2004; 16: 549-60. http://dx.doi.org/10.1016/j.clon.2004.06.007

[5] Moussavi S, Chatterji S, Verdes E, Tandon A, Patel V, Ustun B. Depression, chronic diseases, and decrements in health: results from the World Health Surveys. Lancet 2007; 370: 851-8. http://dx.doi.org/10.1016/S0140-6736(07)61415-9

[6] Pilzer PZ. Ed. The Wellness Revolution: How to Make a Fortune in the Next Trillion Dollar Industry. 1st ed. New York: Wiley 2003.

[7] Global Spa Summit, Spas and the Global Wellness Market: Synergies and *Opportunities*, prepared by SRI International, May 2010.

[8] Healing, Fueling, Feeding: How Biotechnology Is Enriching Your Life. Biotechnology Industry Organization (BIO). [cited 2012 Feb 15]: Available from: http://www.bio.org/articles/healing-fueling-feeding-how-biotechnology-enriching-your-life.

[9] Zhao J. Nutraceuticals, Nutritional Therapy, Phytonutrients, and Phytotherapy for Improvement of Human Health: A Perspective on Plant Biotechnology Application. Rec Pat Biotechnol 2007; 1: 175-97. http://dx.doi.org/10.2174/187220807779813893

[10] World Health Organization (WHO). [cited 2012 Feb 15]: available from http://www.who.int/en/

[11] Hallale N. Engineering a Healthy Body. Chem Eng Prog 2010; 106: 32-7.

[12] Beyond Therapy: *Biotechnology and the Pursuit of Happiness,* A Report by the President's Council on Bioethic 2003

[13] El Gewely MR. Biotechnology domain. Biotechnol Ann Rev 1995; 1: 5-68. http://dx.doi.org/10.1016/S1387-2656(08)70047-4

[14] Marquina D, Santos A, Peinado J. Biology of killer yeasts. Int Microbiol 2002; 5: 65-71. http://dx.doi.org/10.1007/s10123-002-0066-z

[15] Kjeldsen T. Yeast secretory expression of insulin precursors. Appl Microbiol Biotechnol 2000; 54: 277-87. http://dx.doi.org/10.1007/s002530000402

[16] Idiris A, Tohda H, Kumagai H, Takegawa K. Engineering of protein secretion in yeast: strategies and impact on protein production. Appl Microbiol Biotechnol 2010; 86: 403-17. http://dx.doi.org/10.1007/s00253-010-2447-0

[17] Pan DD, Zeng XQ, Yan YT. Characterisation of *Lactobacillus fermentum* SM-7 isolated from koumiss, a potential probiotic bacterium with cholesterol-lowering effects. J Sci Food Agric 2010; 91: 512-8. http://dx.doi.org/10.1002/jsfa.4214

[18] Ding WK, Shah NP. Acid, bile and heat tolerance of free and microencapsulated probiotic bacteria. J Food Sci 2007; 72: M446-50. http://dx.doi.org/10.1111/j.1750-3841.2007.00565.x

[19] Czerucka D, Rampal P. Experimental effects of *Saccharomyces boulardii* on diarrheal pathogens. Microbes Infect 2002; 4: 733-9. http://dx.doi.org/10.1016/S1286-4579(02)01592-7

[20] El Enshasy H, El Shereef A. Probiotic/biotherapeutic yeast *Saccharomyces boulardii* adapted to dryness stress: optimization of high cell density cultivation of yeast. Deut Lebensmittel Rundschau 2008; 104: 389-94.

[21] Tiago FCP, Martins FS, Rosa CA, Nardi RMD, Cara DC, Nicoli JR. Physiological characterization of non-*Saccharomyces* yeasts from agro-industrial and environmental origins with possible probiotic function. World J Microbiol Biotechnol 2009; 25: 657-66. http://dx.doi.org/10.1007/s11274-008-9934-9

[22] Sanders KME. Summary of probiotic activities of *Bifidobacterium lactis* HN019. J Clin Gastroenterol 2006; 40: 776-83.

[23] Yoshida Y, Seki T, Matsunaka H, *et al*. Clinical effects of probiotic *Bifidobacterium breve* supplementation in adult patients with atopic dermatitis. Yonago Acta Medica 2010; 53: 37-45.

[24] Ljungh Å, Wadström T. Lactic acid bacteria as probiotics. Curr Issues Intest Microbiol 2006; 7: 73-90.

[25] Moslehi-Jenabian S, Pedersen LL, Jespersen L. Beneficial effects of probiotic and food borne yeasts on human health. Nutrients 2010; 2: 449-73. http://dx.doi.org/10.3390/nu2040449

[26] Fairclough A, Cliffe D, Knapper S. Factors affecting *Penicillium roquefortii* (*Penicillium glaucum*) in internally mould ripened cheeses: implications for pre-packed blue cheeses. Int J Food Sci Technol 2011; 46: 1486-90. http://dx.doi.org/10.1111/j.1365-2621.2011.02658.x

[27] Liu DM, Li L, Yang XQ, Liang SX, Wang JS. Survivability of *Lactobacillus rhamnosus* during the preparation of soy cheese. Food Technol Biotechnol 2006; 44: 417-22.

[28] Rivera-Espinza Y, Gallardo-Navaro Y. Non-dairy probiotic products. Food Microbiol 2010; 27: 1-11. http://dx.doi.org/doi:10.1016/j.fm.2008.06.008

[29] Papagianni M. Advances in citric acid fermentation by *Aspergillus niger*: Biochemical aspects, membrane transport and modeling. Biotechnol Adv 2007; 25: 244-63. http://dx.doi.org/10.1016/j.biotechadv.2007.01.002

[30] Jernejc K, Cimerman A. Composition of Aspergillus niger mycelium during growth on productive and unproductive substrates. J Biotechnol 1992; 25: 341-8. http://dx.doi.org/10.1016/0168-1656(92)90166-7

[31] El Enshasy H. Optimization of gluconic acid production by recombinant *Aspergillus niger* carrying multiple copies of

glucose oxidase gene in batch and fed-batch cultures. Deutsche Lebensmittel Rundschau 2006; 102: 1-6.

[32] Dronawat SN, Scihla CK, Hanley TR. The effects of agitation and aeration on the production of gluconic acid by *Aspergillus niger*. Appl Biochem Biotechnol 1995; 51/52: 347-54. http://dx.doi.org/10.1007/BF02933438

[33] Bohmann JT, Cameselle C, Nunez MJ, Lema JM. Oxalic acid production by *Aspergillus niger*. Part II: optimization of fermentation with milk whey as carbon source. Bioproc Biosys Eng 1998; 19: 337-42. http://dx.doi.org/10.1007/PL00009022

[34] Strasser H, Burgstaller W, Schinner F. High-yield production of oxalic acid from metal leaching process by *Aspergillus niger*. FEMS Microbiol Lett 1994; 119: 365-70.

[35] Utagawa T. Production of arginine by fermentation. J Nut Suppl 2004; 2854S-7S.

[36] Momose H, Ishida M, Terabe M. Production of L-arginine by fermentation. Japanese Patent 1982; 57-5693.

[37] Sato T, Mori T, Tosa T, *et al*. Engineering analysis of continuous production of L-aspartic acid by immobilized *Escherichia coli* cells in fixed beds. Biotechnol Bioeng 1975; 17: 1797-804. http://dx.doi.org/10.1002/bit.260171209

[38] Plachy J, Sikyta B. Production of L-aspartic acid from fumaric acid by *Alcaligenes metalcaligenes* CCEB 312. Folia Microbiol 1977; 22: 410-4. http://dx.doi.org/10.1007/BF02877678

[39] Nakamura J, Hirano S, Ito H, Wachi M. Mutations of *Corynebacterium glutamicum* NCg11221 gene, encoding a mechanosensitive channel homolg, induce L-glutamic acid production. Appl Environ Microbiol 2007; 73: 4491-8. http://dx.doi.org/10.1128/AEM.02446-0

[40] Kurihara K. Glutamate: from discovery as a food flavor to role as basic taste (umami). Am J Clin Nutr 2009; 90: 719S-22S. http://dx.doi.org/10.3945/ajcn.2009.27462D

[41] Sano C. History of glutamate production. Am J Clin Nutr 2009; 90: 728S-32S. http://dx.doi.org/10.3945/ajcn.2009.27462F

[42] Leuchtenberger W, Huthmacher K. Biotechnological production of amino acids and derivatives: current status and prospects. Appl Microbiol Biotechnol 2005; 69: 1-8. http://dx.doi.org/10.1007/s00253-005-0155-y

[43] Anastassiadis S. L-Lysine fermentation. Rec Pat on Biotechnol 2007; 1: 11-24.

[44] Kumar D, Gomes J. Methionine production by fermentation. Biotechnol Adv 2005; 23: 41-61. http://dx.doi.org/10.1016/j.biotechadv.2004.08.005

[45] Shu C-H, Lia C-C. Optimization of L-phenylalanine production of *Corynebacterium glutamicum* under feedback inhibition by elevated oxygen transfer rate. Biotechnol Bioeng 2002; 77: 131-41. http://dx.doi.org/10.1002/bit.10125

[46] Förberg C, Häggström L. Phenylalanine production from a rec *Escherichia coli*-strain in fed-batch culture. J Biotechnol 1988; 8: 291-300. http://dx.doi.org/10.1016/0168-1656(88)90021-1

[47] Chen N, Huang J, Fend Z-b, Yu L, Xu Q-y, Wen T-y. Optimization of fermentation conditions for the biosynthesis of L-threonine by *Escherichia coli*. Appl Biochem Biotechnol 2009; 158: 595-604. http://dx.doi.org/10.1007/s12010-008-8385-y

[48] Lee, M-H, Lee, H-W, Park J-H, Ahn, J-O, Jung J-K, Hwang Y-I. Improved L-threonine production of *Escherichia coli* mutant by optimization of culture conditions. J Biosci Bioeng 2006; 101: 127-30. http://dx.doi.org/10.1263/jbb.101.127

[49] Ikeda M, Katsumata R. Hyperproduction of tryptophan by *Corynebacterium glutamicum* with the modified-pentose phosphate pathway. Appl Environ Microbiol 1999; 65: 2497-502.

[50] Kalingan AE, Liao CM. Influence of type and concentration of flavinogenic factors on production riboflavin by *Eremothecium ashbyii* NRRL 1363. Bioresour Technol 2002; 82: 219-24. http://dx.doi.org/10.1016/S0960-8524(01)00194-8

[51] Jiménez A, Santos MA, Pompejus M, Revuelta JL. Metabolic engineering of purine pathway for riboflavin production by *Ashbya gossypii*. Appl Environ Microbiol 2005; 71: 5743-51. http://dx.doi.org/10.1128/AEM.71.10.5743-5751.2005

[52] El-Refai HA, El-Helow E, Amin MA, Sallam LA, Salem H. Application of multi-factorial experimental designs for optimization of biotin production by a *Rhizopus nigricans* strain. J Am Sci 2010; 6: 179-87.

[53] Brown SW, Speck D, Sabatié J, Gloeckler R, O`Regan M, Viret JF, *et al*. The production of biotin by recombinant strains of *Escherichia coli*. J Chem Tech Biotechnol 1991; 50: 115-21. http://dx.doi.org/10.1002/jctb.280500114

[54] D`Aimmo MR, Mattarelli P, Biavati B, Carlsson NG, Andlid T. The potential of bifidobacteria as a source of natural folate. J Appl Microbiol 2012 (in press). http://dx.doi.org/10.1111/j.1365-2672.2012.05261.x

[55] Pompei A, Cordisco L, Amaretti A, Zanoni S, Matteuzzi D, Rossi M. Folate production by *Bifidobacteria* as potential probiotic property. Appl Environ Microbiol 2007; 73: 179-85. http://dx.doi.org/10.1128/AEM.01763-06

[56] Martens J-H, Barg H, Warren MJ, Jahn D. Microbial production of vitamin B_{12}. Appl Microbiol Biotechnol 2002; 58: 275-85. http://dx.doi.org/10.1007/s00253-001-0902-7

[57] El Enshasy H, Al Laboudy S, Abdel-Aal SS, Maroun BM, El Kelani TA. Production of vitamin B_{12} by methanol utilizing bacteria *Rubrobacter motiliticus* nov. var. *gelatinoliquificicans* in shake flask and bioreactor cultures under different cultivation conditions. Deutsche Lebensmittel Rundschau 2008; 104: 384-88.

[58] Vandamme EJ. Production of vitamins, Coenzymes and related biochemicals by biotechnological process. J Chem Tech Biotechnol 1992; 53: 313-27. http://dx.doi.org/10.1002/jctb.280530402

[59] Bhosale SH, Rao MB, Deshpande VV. Molecular and industrial aspects of glucose isomerase. Microbiol Rev 1996; 60: 280-300.

[60] Kovalenko GA, Perminova LV, Chuenko TV, Sapunova LI, Shlyakhotko EA, Lobanok AG. Immobilization of a recombinant strain producing glucose isomerase inside SiO_2-Xerogel and properties of prepared biocatalysts. Appl Biochem Microbiol 2011; 47: 151-7. http://dx.doi.org/10.1134/S0003683811020074

[61] El-Enshasy H, Hellmuth, K. and Rinas, U. *GpdA*-promoter controlled production of glucose oxidase by recombinant *Aspergillus niger* using non-glucose carbon sources. Appl Biochem Biotechnol 2001; 90: 1-10.

[62] El Enshasy H, Kleine J, Rinas, U. Agitation effects on morphology and protein productive fractions of filamentous and pelleted growth forms of recombinant *Aspergillus niger.* Process Biochem 2006; 41: 2103-12.

[63] Sojo M, Bru R, Loppez MD, Garcia CF, Arguelles JC. Cell-linked and extracellular cholesterol oxidase activities from *Rhodococcus erythropolis* isolation and physiological characterization. J Appl Microbiol Biotechnol 1997; 47: 583-9. http://dx.doi.org/10.1007/s002530050977

[64] Lashkarian H, Raheb J, Shahzamani K, Shahbani H, Shamsara M. Extracellular cholesterol oxidase from *Rhodococcus sp.*: Isolation and molecular characterization. Ir Biomed J 2010; 14: 49-57.

[65] El Enshasy, H. Bioprocess development for the production of α-amylase by *Bacillus amyloliquefaciens* in batch and fed-batch cultures. J Microbiol Res 2007; 7: 560-8.

[66] El Enshasy H, Abdel Fattah Y, Othman N.Z . Amylases. In: Yang S-T, El Enshasy HA, Thongchul N, Editors. Bioprocessing Technologes in Integrated Biorefinery from Production of Biofuels, Biochemicals, and Biopolymers from Biomass. New Yrok: John Wiley & Sons 2012; (in press).

[67] Beg QK, Kapoor M, Mahajan L, Hoondal GS. Microbial xylanases and their industrial applications: a review. Appl Microbiol Biotechnol 2001; 56: 326-38. http://dx.doi.org/10.1007/s002530100704

[68] Biely P. Microbial xylanolytic systems. Trends Biotehnol 1985; 3: 286-90. http://dx.doi.org/10.1016/0167-7799(85)90004-6

[69] Greiner R, Konielzny U. Phytase for food application. Food Technol Biotechnol 2006; 44: 125-40.

[70] Bajaj BK, Wani MA. Enhanced phytase production from *Nocardia* sp. MB 36 using agro-residues as substrates: Potential application for animal feed production. Eng Life Sci 2011; 11: 620-8. http://dx.doi.org/10.1002/elsc.201100039

[71] Maki ML, Broere M, Leung KT, Qin W. Characterization of some efficient cellulose producing bacteria from paper mill sludges and organic fertilizers. Int J Biochem Mol Biol 2011; 2: 146-54.

[72] Sukumaran RK, Singhania RR, Pandey A. Microbial cellulases-production, application and challenges. J Sci Ind Res 2005; 64: 832-44.

[73] Remminghorst U, Rehm BHA. Bacterial alginates: from biosynthesis to applications. Biotechnol Lett 2006; 28: 1701-12. http://dx.doi.org/10.1007/s10529-006-9156-x

[74] Sabra W, Zeng A-P, Deckwer W-D. Bacterial alginate: product quality and process aspects. Appl Microbiol Biotechnol 2001; 56: 315-25. http://dx.doi.org/10.1007/s002530100699

[75] Bajaj IB, Survase SA, Saudagar PS, Singhal RS. Gellan gum: Fermentative production, downstream processing and applications. Food Technol Biotechnol 2007; 45: 341-54.

[76] Arockiasamy S, Banik M. Optimization of gellan gum production by *Sphingomonas paucimobilis* ATCC 31461 with non ionic surfactants using central decomposite design. J Biosc Bioeng 2008; 105: 204-10. http://dx.doi.org/10.1263/jbb.105.204

[77] Leathers TD. Biotechnological production and application of pullulan. Appl Microbiol Biotechnol 2003; 62: 468-73.

http://dx.doi.org/10.1007/s00253-003-1386-4

[78] Cheng KC, Demirci A, Catchmark JM. Pullulan: Biosynthesis, production, and applications. Appl Microbiol Biotechnol 2011; 92: 29-44. http://dx.doi.org/10.1007/s00253-011-3477-y

[79] Cheirsilp B, Shimizu H, Shioya S. Enhanced kefiran production by mixed culture of *Lactobacillus kefiranofaciens* and *Saccharomyces cerevisiae*. J Biotechnol 2003; 100: 43-53. http://dx.doi.org/10.1016/S1389-1723(03)80194-9

[80] Zajšek K, Kolar M, Goršek A. Characterization of the exopolysaccharide kefiran produced by lactic acid bacteria entrapped within natural kefir grains. Int J Dairy Technol 2011; 64: 544-8. http://dx.doi.org/10.1111/j.1471-0307.2011.00704.x

[81] Nikitina VE, Tsivileva OM, Pankratov AN, Bychkov NA. *Lentinula edodes* biotechnology- from lentinan to lectins. Food Technol Biotechnol 2007; 45:230-7.

[82] Wasser SP. Medicinal mushrooms as a source of antitumor and immunomodulating polysaccharides. Appl Microbiol Biotechnol 2002; 60: 258-74. http://dx.doi.org/10.1007/s00253-002-1076-7

[83] El Enshasy H, Maftoun P, Abd Malek R. Pleuran: Immunomodulator polysaccharide from *Pleurotus ostreatus*: Structure, production and application. In: Andres S. and Baumann N Editors. Mushrooms: Type, properties and nutrition. New York, USA. Nova Publisher 2012; pp. 153-72.

[84] Gregori A, Švageli M, Pohleven J. Cultivation techniques and medicinal properties of *Pleurotus* sp. Food Technol Biotechnol 2007; 45: 238-49.

[85] Garcia-Ochoa F, Santos VE, Casas JA, Comez E. Xanthan gum: Production, Recovery and Properties. Biotechnol Adv 2000; 18: 549-79. http://dx.doi.org/10.1016/S0734-9750(00)00050-1

[86] El Enshasy H, Then C, Othman NZ, *et al*. Enhanced xanthan production process in shake flask and pilot scale bioreactors using industrial semi-defined medium. Afr J Biotechnol 2011; 10: 1029-38.

[87] Poirier Y, Mawrath C, Somerville C. Production of polyhydroxyalkanoates, a family of biodegradable plastics and elastomers in bacteria and plants. Nat Biotechnol 1995; 13: 142-50. http://dx.doi.org/10.1038/nbt0295-142

[88] Steinbüchel A. Perspectives for biotechnological production and utilization of biopolymers: Metabolic engineering of polyhydroxyalkanoate biosynthesis pathways as a successful example. Mol Biosci 2001; 1: 1-24.

[89] Weber CJ, Haugaard C, Festersen R, Bertelsen G. Production and application of biobased packaging materials for the food industry. Food Addit Contam 2002; 19: 172-7. http://dx.doi.org/10.1080/02652030110087483

[90] Obata S, Hiromi K, Masakazu I, Takashi S, Katsunori K. Method for production of polylactate using recombinant microorganisms. EU Patent 2011 (EP. 2377945).

[91] Elander RP. Industrial production of β-lactam antibiotics. Appl Microbiol Biotechnol 2003; 61: 385-92. http://dx.doi.org/10.1007/s00253-003-1274-y

[92] Demain AL, Elander RP. The β-lactam antibiotics: Past, present, and future. Ant Van Leeuw 1999; 75: 5-19. http://dx.doi.org/10.1023/A:1001738823146

[93] Martin JF, Casqueiro J, Kosalkova K, Marcos AT, Gutiérrez S. Penicillin and cephalosporin biosynthesis mechanism of carbon catabolite regulation of penicillin production. Ant Van Leeuw 1999; 75: 21-31. http://dx.doi.org/10.123/A:1001820109140

[94] Liras P. Biosynthesis and molecular genetics of cephalosporins. Ant Van Leeuw 1999; 75: 109-24. http://dx.doi.org/10.1023/A:1001804925843

[95] El Enshasy H, Abdel Fattah Y, Atta A, *et al*. Kinetics of cell growth and cyclosporine A production by *Tolypocladium inflatum* during process scaling up from shake flask to bioreactor. J Microbiol Biotechnol 2008; 18: 128-34.

[96] El Enshasy H. Immunomodulators. In: Hofrichter M, Ed. The Mycota (2nd. ed). Vol. X. Springer Verlag 2010; pp. 165-94. http://dx.doi.org/10.1007/978-3-642-11458-8_8

[97] El Enshasy HA, Mohamed NA, Farid MA, El Diwany AI. Improvement of erythromycin production by *Saccharopolyspora erythraea* in molasses based medium through cultivation medium optimization. Bioresour Technol 2008; 99: 4263-8. http://dx.doi.org/10.1016/j.biortech.2007.08.050

[98] Zou X, Hang HF, Chu J, Zhuang YP, Zhang SL. Oxygen uptake rate optimization with nitrogen regulation for erythromycin production and scale up from 50L to 372 m^3 scale. Bioresour Technol 2009; 100: 1406-12. http://dx.doi.org/10.1016/j.biortech.2008.09.017

[99] Abu-Shady MR, Farid MA, El Diwany AI, El Enshasy HA. Studies on rifamycins production by *Amycolatopsis mediterranei* cells immobilized on glass wool. J Basic Microbiol 1995; 35: 279-84. http://dx.doi.org/10.1002/jobm.3620350502

[100] El Enshasy H, El Baz A, Ammar E. Simultaneous production and decomposition of different rifamycins during *Amycolatopsis mediterranei* growth in shake flask and in stirred tank bioreactor. In: Communicating current research and educational. Topics and trends in applied microbiology. Vol. 1. Spain; Formatex Research Centre: Badajoz 2007; pp. 315-21.

[101] El Enshasy H, Farid M, El Sayed E. Influence of inoculum type and cultivation conditions on natamycin production by *Streptomyces natalensis*. J Basic Microbiol 2000; 40: 389-98. http://dx.doi.org/10.1002/1521-4028(200012)40:5

[102] Chen G-Q, Lu F-P, Du L-X. Natamycin production by *Streptomyces gilvosporeus* based on statistical optimization. Agr Food Chem 2008; 56: 5057-61. http://dx.doi.org/10.1021/jf800479u

[103] Schmidt M, Babu KR, Khanna N, Marten S, Rinas U. Temperature-induced production of recombinant human insulin in high-cell density cultures of recombinant *Escherichia coli*. J Biotechnol 1999; 68: 71-83. http://dx.doi.org/10.1016/S0168-1656(98)00189-8

[104] Gurramkonda C, Polez S, Skoko N, *et al.* Application of simple fed-batch technique to high-level secretory production of insulin precursor using *Pichia pastoris* with subsequent purification and conversion to human insulin. Microb Cell Fact 2010; 9: 31-42. http://dx.doi.org/10.1186/1475-2859-9-31

[105] Eitman MA, Altman E. Overcoming acetate in *Escherichia coli* recombinant protein fermentations. Trends Biotechnol 2006; 24: 530-6. http://dx.doi.org/10.1016/j.tibtech.2006.09.001

[106] Kamionka M. Engineering of therapeutic proteins production in *Escherichia coli*. Curr Pharm Biotechnol 2011; 12: 268-74. http://dx.doi.org/10.2174/138920111794295693

[107] Ottone S, Nguyen X, Baxin J, Bérad C, Jimenez S, Letourneur O. Expression of hepatitis B surface antigen major subtypes in *Pichia pastoris* and purification for *in vitro* diagnosis. Protein Expr Purific 2007; 66: 177-88. http://dx.doi.org/10.1016/j.pep.2007.07.008

[108] Hardy E, Martínez E, Diago D, Díaz R, González D, Horrera L. Large-scale production of recombinant hepatitis B surface antigen from *Pichia pastoris*. J Biotechnol 2000; 77: 157-67. http://dx.doi.org/10.1016/S0168-1656(99)00201-1

[109] Xiao A, Zhou X, Zhou L, Zhang Y. Improvement of cell viability and hirudin production by ascorbic acid in *Pichia pastoris* fermentation. Appl Microbiol Biotechnol 2006; 72: 837-44. http://dx.doi.org/10.1007/s00253-006-0338-1

[110] Yang J, Zhou X, Zhang Y. Improvement of recombinant hirudin production by controlling NH_4^+ concentration in *Pichia pastoris* fermentation. Biotechnol Lett 2004; 26: 1013-7. http://dx.doi.org/10.1023/B:BILE.0000030049.75092.95

[111] Wagner M, Loy A, Nogueira R, Purkhold U, Lee N, Daims H. Microbial community composition and function in wastewater treatment plants. Ant Van Leeuv 2002; 81: 665-80. http://dx.doi.org/10.1023/A:1020586312170

[112] Siripong S, Rittmann BE. Diversity study of nitrifying bacteria in full-scale municipal wastewater treatment plants. Water Res 2007; 41: 1110-20. http://dx.doi.org/10.1016/j.watres.2006.11.050

[113] Dua M, Singh A, Sethunathan N, Johri AK. Biotechnology and bioremediation: successes and limitations. Appl Microbiol Biotechnol 2001; 59: 143-52. http://dx.doi.org/10.1007/s00253-002-1024-6

[114] Ezezika OC, Singer PA. Genetically engineered oil-eating microbes for bioremediation: Prospects and regulatory challenges. Technol Soc 2010; 32: 331-5. http://dx.doi.org/10.1016/j.techsoc.2010.10.010

[115] Watanabe K. Microorganisms relevant to bioremediation. Curr Opin Biotechnol 2001; 12: 237-41. http://dx.doi.org/10.1016/S0958-1669(00)00205-6

[116] Fu F, Wang Q. Removal of heavy metal ions from wastewaters: A review. J Env Manag 2011; 92: 407-18. http://dx.doi.org/10.1016/j.jenvman.2010.11.011

[117] Kloepper JW, Lifshitz R, Zablotowicz RM. Free-living bacterial inocula for enhancing crop productivity. Trends Biotechnol 1989; 7: 39-44. http://dx.doi.org/10.1016/0167-7799(89)90057-7

[118] Rodriguez H, Fraga R. Phosphate solubilizing bacteria and their role in plant growth promotion. Biotechnol Adv 1999; 17: 319-39. http://dx.doi.org/10.1016/S0734-9750(99)00014-2

[119] Saharan BS, Nehra V. Plant growth promoting rhizobacteria: A critical review. Life Sci Med Res: LSMR 2011; 21: 1-30.

[120] Johansson JF, Paul LR, Finlay RD. Microbial interactions in the mycorrhizosphere and their significance for sustainable agriculture. FEMS Microb Ecol 2004; 48: 1-13. http://dx.doi.org/10.1016/j.femsec.2003.11.012

[121] De Lange CFM, Pluske J, Gong J, Nyachoti CM. Strategic use of feed ingredients and feed additives to stimulate gut health and development in young pigs. Livestock Sci 2010; 134: 124-34. http://dx.doi.org/10.1016/j.livsci.2010.06.117

[122] Anadón A, Martínez-Larrañaga MR, Aranzazu-Martínez M. Probiotics for animal nutrition in the European Union Regulation and safety assessment. Reg Toxicol Pharmacol 2006; 45: 91-5. doi:10.1016/j.yrtph.2006.02.004

[123] Coeuret V, Gueguen M, Vernoux JP. Numbers and strains of lactobacilli in some probiotic products. Int J Food Microbiol 2004; 97:147-56. http://dx.doi.org/10.1016/j.ijfoodmicro.2004.04.15

[124] Farzanfar A. The use of probiotics in shrimp aquaculture. FEMS Immunol Med Rev 2006; 48: 149-58.

http://dx.doi.org/10.1111/j.1574-695X.2006.00116.x

[125] Pariza MW, Cook M. Determining the safety of enzymes used in animal feed. Reg Toxicol Pharmacol 2010; 56: 332-42. http://dx.doi.org/10.1016/j.yrtph.2009.10.005

[126] Kirk O, Borchert TV, Fuglsang CC. Industrial enzyme applications. Curr Opin Biotechnol 2002; 13: 345-51. http://dx.doi.org/10.1016/S0958-1669(02)00328-2

[127] Simon O. The mode of action of NSP hydrolyzing enzymes in the gastrointestinal tract. J Anim Feed Sci 1998; 7: 115-23.

[128] Bedford MR, Cowieson AJ. Exogenous enzymes and their effects on intestinal microbiology. Anim Feed Sci Technol 2012; 173: 76-85. http://dx.doi.org/10.1016/j.anifeedsci.2011.12.018

[129] Kies AK, van Hemert KHF, Sauer WC. Effect of phytase on protein and amino acid digestibility and energy utilization. World Poul Sci J 2001; 57: 109-126.

[130] Cao L, Wang W, Yang C, *et al.* Application of microbial phytase in fish feed. Enz Microb Technol 2007; 40: 497-507. http://dx.doi.org/10.1016/j.enzmictec.2007.01.007

[131] Wegener H. Antibiotics in animal feed and their role in resistance development. Curr Opin Microbiol 2003; 6: 439-45. http://dx.doi.org/10.1016/j.mib.2003.09.009

[132] Defoirdt T, Sorgeloos P, Bossier P. Alternatives to antibiotics for the control of bacterial disease in aquaculture. Curr Opin Microbiol 2011; 14: 251-8. http://dx.doi.org/10.1016/j.mib2011.03.004

[133] Laughlin TJ, Ferrell TM. Biotechnology in the cosmetics industry. Nat Biotechnol 1987; 5: 1035-7. http://dx.doi.org/10.1038/nbt1087-1035

[134] Grabenhofer R. Biotechnology in cosmetics: Concepts, tools and techniques. Allured Publishing Corp. IL, USA 2007.

[135] Anzali S, Von Heydebreck A, Herget T. Elucidation of anti-aging effects of ectoine using cDNA microarray analysis and signaling pathway evaluation. Int J Cosm Sci 2010; 32: 319. http://dx.doi.org/10.1111/j.1468-2494.2010.00589_4.x

[136] Buenger J, Driller H. Ectoin: An effective natural substance to prevent UVA-induced premature photoaging. Skin Pharmacol Physiol 2004; 17: 232-7. http://dx.doi.org/10.1159/000080216

[137] Ferrer-Miralles N, Domingo-Espín J, Corchero JL, Vázquez E, Villaverde A. Microbial factories for recombinant pharmaceuticals. Microb Cell Factories 2009; 8: 17-27. http://dx.doi.org/10.1186/1475-2859-8-17

[138] Blattner FR, Plunkett III G, Bloch CA, *et al.* The complete genome sequence of *Escherichia coli* K-12. Science 1997; 277(5331): 1453-62. http://dx.doi.org/10.1126/science.277.5331.1453

[139] Kunst F, Ogasawara N, Moszer I, *et al.* The complete genome sequence of the Gram-positive bacterium *Bacillus subtilis*. Nature 1997; 390: 249-56. http://dx.doi.org/10.1038/36786

[140] Giaever G, Chu AM, Ni L, *et al.* Functional profiling of the *Saccharomyces cerevisiae* genome. Nature 2002; 418: 387-91. http://dx.doi.org/10.1038/nature00935

[141] De Schutter K, Lin Y-C, Tiels P, *et al.* Genome sequence of the recombinant protein production host *Pichia pastoris*. Nat Biotechnol 2009; 27: 561-6. http://dx.doi.org/10.1038/nbt.1544

[142] Venter JC, Adams MD, Myers EW, *et al.* The sequence of the human genome. Science 2001; 291(5507): 1304-51. http://dx.doi.org/10.1126/science.1058040

[143] Kobayashi K, Kuwae S, Ohya T, *et al.* High-level expression of recombinant human serum albumin in the methylotrophic yeast *Pichia pastoris* with minimal protease production and activation. J Biosci Bioeng 2000; 89: 55-61. http://dx.doi.org/10.1016/S1389-1723(00)80082-1

[144] Hoffmann F, Rinas U. Roles of heat-shock chaperones in the production of recombinant proteins in *Escherichia coli*. Adv Biochem Eng Biotechnol 2004; 89: 143-61. http://dx.doi.org/10.1007/b93996

[145] Babaejpour V, Shojaosadati SA, Robatjazi SM, Khalilzadeh R, Maghsoudi N. Over-production of human interferon-γ by HCDC of recombinant *Escherichia coli*. Process Biochem 2007; 42: 112-7. http://dx.doi.org/10.1016/j.procbio.2006.07.009

[146] Babu KR, Swaminathan S, Marten S, Khanna N, Rinas U. Production of interferon-α in high cell density cultures of recombinant *Escherichia coli* and its single step purification from refolded inclusion body proteins. Appl Microbiol Biotechnol 2000; 53: 655-60. http://dx.doi.org/10.1007/s002530000318

[147] Aldor IS, Krawitz DC, Forrest W, *et al.* Proteomic profiling of recombinant *Escherichia coli* in high-cell-density fermentations for improved production of an antibody fragment biopharmaceutical. Appl Env Microbiol 2005; 71: 1717-28. http://dx.doi.org/10.1128/AEM.71.4.1717-1728.2005

[148] Siddiqui SF, Bulmer M, Shamlou AP, Titchener-Hooker NJ. The effects of fermentation conditions on yeast cell debris particle size distribution during high pressure homogenization. Bioprocess Eng 1995; 14: 1-8. http://dx.doi.org/10.1007/BF00369846

[149] Gao K. Chinese studies on the edible blue-green alga, *Nostoc flagelliforme*: a review. J Appl Phycol 1998; 10: 37-49. http://dx.doi.org/10.1023/A:1008014424247

[150] Barsanti L, Gualtieri P. Algae anatomy, biochemistry and biotechnology. Boca Raton, London, New York: CRC Taylor & Francis 2006.

[151] Duerr EO, Molnar A, Sato V. Cultured microalgae as aquaculture feeds. J Mar Biotechnol 1998; 6: 65-70.

[152] Takeyama H, Kanamani A, Yoshino Y, Kakuta H, Kawamura Y, Matsunaga T. Production of antioxidant vitamins. β-carotene, vitamin C, vitamin E, by two-step culture of *Euglena gracilis* Z. Biotechnol Bioeng 1997; 53: 185-90. http://dx.doi.org/10.1002/(SICI)1097-0290(19970120)5

[153] Borowitzka MS. Commercial production of microalgae: ponds, tanks, tubes and fermentors. J Biotechnol 1999; 70: 313-21. http://dx.doi.org/10.1016/S0168-1656(99)00083-8

[154] Zemke-White WL, Ohno M. World seaweed utilisation: an end-of-century summary. J Appl Phycol 1999; 11: 369-76. http://dx.doi.org/10.1023/A:1008197610793

[155] Richmond A. Cell response to environmental factors. In: Richmond, A. Editor. Handbook of microalgal mass culture. Florida.CRC Press Inc. 1986; pp. 89-95.

[156] Banerjee A, Sharma R, Chisti Y, Banerjee UC. *Botryococcus braunii*: A renewable source of hydrocarbons and other chemicals. Crit Rev Biotechnol 2002; 22: 245-79. http://dx.doi.org/10.1080/07388550290789513

[157] Chisti Y. Microalgae as sustainable cell factories. Environ Eng Manag J 2006; 5: 261-74.

[158] Chisti Y. Biodiesel from microalgae. Biotechnol Adv 2007; 25: 294-306. http://dx.doi.org/10.1016/j.biotechadv.2007.02.001

[159] Mallick N. Biotechnological potential of immobilized algae for wastewater N, P and metal removal: a review. BioMetals 2002; 15: 377-90. http://dx.doi.org/10.1023/A:1020238520948

[160] Munoz R, Guieysse B. Algal-bacterial processes for the treatment of hazardous contaminants: a review. Water Res 2006; 40: 2799-815. http://dx.doi.org/10.1016/j.watres.2006.06.011

[161] Zeiler KG, Heacox DA, Toon ST, Kadam KL, Brown LM. The use of microalgae for assimilation and utilization of carbon dioxide from fossil fuel-fired power plant flue gas. Energy Convers Manag 1995; 36: 707-12.

[162] Miura Y, Sode K, Narasaki Y, Matsunaga T. Production of γ-linolenic acid from the marine green alga *Chlorella* sp. NKG 042401 FEMS Microbiol Lett 1993; 107: 163-8.

[163] Matsunga T, Takeyama H, Miura Y, Yamazaki T, Furuya H, Soda K. Screening of marine cy- anobacteria for high palmitoleic acid production, FEMS Microbiol Lett 1995; 133: 137-41.

[164] Cardozo KHM, Guaratini T, Barros MP, *et al.* Metabolites from algae with economical impact. Compart Biochem Physiol 2007; C146: 60-78. http://dx.doi.org/10.1016/j.cbpc.2006.05.007

[165] Mokady S, Sukenik A. A marine unicellular alga in diets of pregnant and lactating rats as a source of ω3 fatty acids for the developing brain of their progeny. J Sci Food Agric 1995; 68: 133-139.

[166] Leblond JD, Chapman PJ. A survey of the sterol composition of the marine dinoflagellates *Karenis brevis, Karena mikimotoi,* and *Karlodinium micrum*: distribution of sterols within other members of the class Dinophyceae. J Phycol 2002; 38: 670-82. http://dx.doi.org/10.1046/j.1529-8817.2002.01181.x

[167] Ponomarenko LP, Stonik IV, Aizdaicher NA, *et al*. Sterols of marine microalgae *Pyramimonas* cf. *cordata* (prasinophyta), *Atteya ussurensis* sp. nov. (Bacollariophyta) and a spring diatom bloom from lake Baikal. Compart Biochem Physiol 2004; B138: 65-70.

[168] Del Río E, Acién G, García-Malea MC, Rivas J, Molina-Grima E, Guerrero MG. Efficient one-step production of astaxanthin by microalga *Haematococcus pulvialis* in continuous culture. Biotechnol Bioeng 2005; 91: 808-15. http://dx.doi.org/10.1002/bit.20547

[169] Hejazi MA, Andrysiewicz E, Tramper J, Wijffels RH. Effect of mixing rate on β-carotene production and extraction by *Dunaliella salina* in two-phase bioreactors. Biotechnol Bioeng 2003; 84: 591-6. http://dx.doi.org/10.1002/bit.10791

[170] Lebeau T, Gaudin P, Junter G-A, Mignot L, Robert J-M. Continuous marennin production by agar-entrapped *Haslea ostrearia* using a tubular photobioreactor with internal illumination. Appl Microbiol Biotechnol 2000; 54: 634-40. http://dx.doi.org/10.1007/s002530000380

[171] Matsunga T, Takeyama H, Sudo H, *et al.* Glutamate production from CO_2 by marine *Cyanobacterium Synechococcus* sp using a novel biosolar reactor employing light-diffusing optical fibers. Appl Biochem Biotechnol 1991; 28/29: 157-67. http://dx.doi.org/10.1007/BF02922597

[172] Hammingson JA, Furneux RH, Murray-Brown HV. Biosynthesis of agar polysaccharides in *Gracilaria chilensis* Bird, McLachloan et Oliveira. Carbohyd Res 1996; 287: 101-15. http://dx.doi.org/10.1016/0008-6215(96)00057-2

[173] Hayashi L, de Paula EJ, Chow F. Growth rate and carrageenan analysis in four strains of *Kappaphycus alvarezii* (Rhodophyta, Gigartinales) farmed in the subtropical waters of São Paulo State, Brazil. J Appl Phycol 2007; 19: 393-9. http://dx.doi.org/10.1007/s10811-006-9135-6

[174] Torres MR, Sousa APA, Filho EATS, *et al.* Extraction and physicochemical characterization of *Sargassum vulgare* alginate from Brazil. Carbohyd Res 2007; 342: 2067-74. http://dx.doi.org/10.1016/j.carres.2007.05.022

[175] Liu Y, Xu L, Cheng N, Lin L, Zhang C. Inhibitory effect of phycocyanin from *Spirulina platensis* on the growth of human leukemia K562 cells. J Appl Phycol 2000; 12:125-30. http://dx.doi.org/10.1023/A:1008132210772

[176] Wang SC, Bligh SWA, Shi SS, *et al.* Structural features and anti-HIV-1 activity of novel polysaccharides from red algae *Grateloupia longifolia* and *Grateloupia filicina.* Int J Biol Macromol 2007; 41: 369-75. http://dx.doi.org/10.1016/j.ijbiomac.2007.05.008

[177] Das BK, Pradhan J, Pattnaik P, *et al.* Production of antibacterials from the freshwater alga *Euglena viridis* (Ehren). World J Microbiol Biotechnol 2005; 21: 45-50. http://dx.doi.org/10.1007/s11274-004-1555-3

[178] Martinez-Lozano SJ, Garcia S, Heredia N, Villareal-Rivera L, Garcia-Pailla CA. Antifungal activity of extract of *Sargassum filipendula.* Phyton 2000; 66: 179-182.

[179] Sato Y, Murakami M, Miyazawa K, Hori K. Purification and characterization of a novel lectin from a freshwater cyanobacterium *Oscillatoria agardhii.* Compar Biochem Physiol 2000; B125: 169-77. http://dx.doi.org/10.1016/S0305-0491(99)00164-9

[180] Venkataraman LV, Becker WE, Shamala TR. Studies on the cultivation and utilization of alga *Scenedesmus acutus* as a single cell protein. Life Sci 1977; 20: 223-33. http://dx.doi.org/10.1016/0024-3205(77)90316-2

[181] Chae SR, Hwang EJ, Shin HS. Single cell protein production of *Euglena gracilis* and carbon dioxide fixation in an innovative photo-bioreactor. Bioresour Technol 2006; 97: 322-9. http://dx.doi.org/10.1016/j.biortech.2005.02.037

[182] Xiong W, Li X, Xiang J, Wu Q. High-density fermentation of microalga *Chlorella protothecoides* in bioreactor for microbio-diesel production. Appl Microbiol Biotechnol 2008; 78: 29-36. http://dx.doi.org/10.1007/s00253-007-1285-1

[183] Tredici MR. Photobioreactors. In: Flickinger MC, Drew SW. editors. Encyclopedia of bioprocess technology: Fermentation, biocatalysis and bioseparation. John Wiley&Sons. New York, Chichester, Weinheim, Brisbane, Singapore, Torento 1999; pp. 395-419.

[184] Pulz O. Photobioreactors: production systems for phototrophic microorganisms. Appl Microbiol Biotechnol 2001; 57: 287-93. http://dx.doi.org/10.1007/s002530100702

[185] Bourlag NE. Contributions of Conventional Plant Breeding to Food Production. Science 1980; 219: 689-93. http://dx.doi.org/10.1126/science.219.4585.689

[186] Tyler VE. Phytomedicines: back to the future. J Nat Prod 1999; 62: 1589-92.

[187] Balunas MJ, Kinghorn DA. Drug discovery from medicinal plants. Life Sci 2005; 78: 431-41. http://dx.doi.org/10.1016/j.lfs.2005.09.012

[188] Verpoorte R. Exploration of nature's chemodiversity: the role of secondary metabolites as leads in drug development. Drug Disc Today 1988; 3: 232-8. http://dx.doi.org/10.1016/S1359-6446(97)01167-7

[189] De Luca V, St Pierre B. The cell and developmental biology of alkaloid biosynthesis. Trends Plant Sci 2000; 5: 168-73. http://dx.doi.org/10.1016/S1360-1385(00)01575-2

[190] Payne GF, Bringi V, Prince C, Shuler ML. Plant cell and tissue culture in liquid systems. Munich. Hanser Publications. 1991; pp. 1-10.

[191] Naik GR. Micropropagation studies in medicinal and aromatic plants. In: Khan I A, Khanun A. Eds. Role of biotechnology in medicinal and aromatic plants. Ukaz Publications, Hyderabad 1998; pp. 50-6.

[192] Rao AR, Dayananda C, Sarada R, Shamala TR, Ravishankar GA. Effect of salinity on growth of green alga *Botryococcus braunii* and its constituents. Bioresour Technol 2007; 98: 560-4. http://dx.doi.org/10.1016/j.biortech.2006.02.007

[193] Ravishankar GA, Bhyalakshmi N, Rao RS. Production of food additives. In: Ramawat KG, Merillon JM editors. Biotechnology: secondary metabolites 1999; pp. 89-110.

[194] Alfermann AW, Petersen M. Natural products formation by plant cell biotechnology. Plant Cell Tissue Organ Cult 1995; 43: 199-205. http://dx.doi.org/10.1007/BF00052176

[195] Stöckigt J, Obitz P, Flakenhagen H, Lutterbach R, Endress R. Natural products and enzymes from plant cell cultures. Plant Cell Tissue Organ Cult 1995: 43: 97-109. http://dx.doi.org/10.1007/BF00052163

[196] Rao SR, Ravishankar GA. Plant cell cultures: Chemical factories of secondary metabolites. Biotechnol Adv 2002; 20: 101-53. http://dx.doi.org/10.1016/S0734-9750(02)00007-1

[197] See KS, Bhatt A, Keng CL. Effect of sucrose and methyl jasmonate on biomass and anthocyaninproduction in cell suspension culture of *Melastoma malabathricum* (Melastomaceae). Rev Biol Trop 2011; 59: 597-606.

[198] Khlebnikov A, Dubuis B, Kut OM, Prenosil JE. Growth and productivity of *B. vulgaris* in culture in fluidized bed reactors. Bioproc Biosys Eng 1995; 14: 51-6. http://dx.doi.org/10.1007/BF00369852

[199] George PS, Ravishankar GA. Induction of crocin and crocetins in callus cultures of *gardenia jasminoides* ellis. Food Biotechnol 1995; 9: 29-38. http://dx.doi.org/10.1080/08905439509549883

[200] Robins RJ, Rhodes MJC. The stimulation of anthraquinone production by *Cinchona ledgeriana* cultures with polymeric adsorbents. Appl Microbiol Biotechnol 1986; 24: 35-41. http://dx.doi.org/10.1007/BF00266282

[201] Dorenburg H, Knorr D. Production of phenolic flavor compounds with cultured cells and tissues of *Vanilla* species. Food Biotechnol 1996; 10: 75-92. http://dx.doi.org/10.1002/1097-0010(200002)80:3

[202] Jones MG, Hughes J, Tregova A, Milne J, Tomsett AB, Collin HA.Biosynthesis of the flavour precursors of onion and garlic. J Exp Bot 2004; 55: 1903-18. http://dx.doi.org/10.1093/jxb/erh138

[203] Hrazdina G. Aroma production by tissue cultures. J Agric Food Chem 2006; 54:1116-23. http://dx.doi.org/10.1021/jf053146w

[204] Suvarnalatha G, Narayan MS, Ravishankar GA, Venkataraman LV. Flavour production in plant cell cultures of basmati rice (*Oryza sativa* L). J Sci Food Agr 1994; 66: 439-42. http://dx.doi.org/10.1002/jsfa.2740660403

[205] Jalal MA, Collin HA. Secondary metabolism in tissue culture of *Theobroma cacao*. New Phytologist 1979; 83: 343-9. http://dx.doi.org/10.1111/j.1469-8137.1979.tb07458.x

[206] Hwang SJ. Rapid *in Vitro* propagation and enhanced stevioside accumulation in *Stevia rebaudiana* Bert. J Plant Biol 2006; 49: 267-70. http://dx.doi.org/10.1007/BF03031153

[207] Hayashi H, Fukui H, Tabata M. Examination of triterpenoids produced by callus and cell suspension cultures of *Glycyrrhiza glabra*. Plant Cell Reports 1988; 7: 508-11. http://dx.doi.org/10.1007/BF00272743

[208] Van der Wel H, Ledeboer AM. The thaumatins. In: Stumpf PK, Conn EE. Ed. The Biochemisty of Plants: A Comprehensive Treatise, Vol 15. Academic Press, New York, 1989; pp. 379-91.

[209] Chung IS, Kang YM, Oh JH, Kim T, Lee HJ, Chae YA. Continuous suspended cell culture of Mentha piperita in cell-recycled air-lift bioreactor. Biotechnol Techniq 1994; 8: 789-92. http://dx.doi.org/10.1007/BF00152885

[210] Mulder-Krieger TH, Verpoorte R, Baerheim Svendsen A, Scheffer JJC. Production of essential oils and flavours in plant cell and tissue cultures. A review. Plant Cell, Tissue and Organ Cult 1988; 13: 85-154. http://dx.doi.org/10.1007/BF00034451

[211] Scragg AH. The production of aroma by plant cell cultures. Adv Biochem Eng Biotechnol 1997; 55: 239-63. http://dx.doi.org/10.1007/BFb0102068

[212] Salem KMSA, Charlwood BV. Accumulation of essential oils by Agrobacterium tumefaciens-transformed shoot cultures of *Pimpinella anisum*. Plant Cell, Tissue and Organ Cult 1995; 40: 209-15. http://dx.doi.org/10.1007/BF00048125

[213] Lau K-M, He Z-D, Dong H, Fung K-P, But PP-H. Anti-oxidative, anti-inflammatory and hepato-protective effects of *Ligustrum robustum*. J Ethnopharmacol 2002; 83: 63-71. http://dx.doi.org/10.1016/S0378-8741(02)00192-7

[214] Liu CZ, Murch SJ, El-Demerdash M, Saxena PK. *Artemisia judaica* L.: micropropagation and antioxidant activity. J Biotechnol 2004; 110: 63-71. http://dx.doi.org/10.1016/j.jbiotec.2004.01.011

[215] Ludwig-Müller J, Georgiev M, Bley T. Metabolite and hormonal status of hairy root cultures of Devil's claw (*Harpagophytum procumbens*) in flasks and in a bubble column bioreactor. Process Biochem 2008: 43: 15-23. http://dx.doi.org/10.1016/j.procbio.2007.10.006

[216] Li RW, Leach DN, Myers SP, Lin GD, Leach GJ, Waterman PG. A new anti-inflammatory glucoside from *Ficus racemosa*. L. Planta Medica 2004; 70: 421-6. http://dx.doi.org/10.1055/s-2004-818969

[217] Choi H-K, Kim SI, Son J-S, Hong S-S, Lee H-S, Lee H-J. Enhancement of paclitaxel production by temperature shift in suspension culture of *Taxus chinensis*. Enz Microb Technol 2000; 27: 593-8. http://dx.doi.org/10.1016/S0141-0229(00)00255-6

[218] Magnotta M, Murata J, Chen J, De Luca V. Identification of a low vindoline accumulating cultivar of *Catharanthus roseus* (L.) G. Don. by alkaloid and enzymatic profiling. Phytochem 2006; 67: 1758-64. http://dx.doi.org/10.1016/j.phytochem.2006.05.018

[219] Ao C, Li A, Elzaawely AA, Xuan TD, Tawata S. Evaluation of antioxidant and antibacterial activities of *Ficus microcarpa* L. fil. extract. Food Cont 2008; 19: 940-8. http://dx.doi.org/10.1016/j.foodcont.2007.09.007

[220] Wilkinson JM, Hipwell M, Ryan T, Cavanagh HMA. Bioactivity of *Backhousia citriodora:* Antibacterial and Antifungal Activity. J Agric Food Chem 2003; 51:76-81. http://dx.doi.org/10.1021/jf0258003

[221] Falcão HS, Mariath IR, Diniz MFFM, Batista LM, Barbosa-Filho JM. Plants of the american continent with antiulcer activity. Phytomed 2008; 15: 132-46. http://dx.doi.org/10.1016/j.phymed.2007.07.057

[222] Gomord V, Gaye L. Posttranslational modification of therapeutic proteins in plants. Curr Opin Plant Biol 2004; 7: 171-81. http://dx.doi.org/10.1016/j.pbi.2004.01.015

[223] Lee JH, Kim NS, Kwon TH, Jang YS, Yang MS. Increased production of human granulocyte-macrophage colony stimulating factor (hGM-CSF) by the addition of stabilizing polymer in plant suspension cultures. J. Biotechnol 2002; 96: 205-11. http://dx.doi.org/10.1016/S0168-1656(02)00044-5

[224] Guan Z-J, Guo B, Huo Y-L, Guan Z-P, Wei Y-H. Overview of expression of hepatitis B surface antigen in transgenic plants. Vaccine 2010; 28: 7351-62. http://dx.doi.org/10.1016/j.vaccine.2010.08.100

[225] Kwon TH, Seo JE, Kim J, Lee JH, Jang YS, Yang MS. Expression and secretion of heterodimeric protein interleukin-12 in plant cell suspension culture. Biotechnol Bioeng 2003; 81: 870-5. http://dx.doi.org/10.1002/bit.10528

[226] Kwon S, Jo S, Lee O, Choi S, Kwak S, Lee H. Transgenic ginseng cell lines that produce high levels of a human lactoferrin. Planta Medica 2002; 69: 1005-8. http://dx.doi.org/10.1055/s-2003-45146

[227] Daniell H, Singh ND, Mason H, Streatfield SJ. Plant-made vaccine antigens and biopharmaceuticals. Trends in Plant Sci 2009; 14: 669-79. http://dx.doi.org/10.1016/j.tplants.2009.09.009

[228] Sharp JM, Doran PM. Characterization of monoclonal antibody fragments produced by plant cells. Biotechnol Bioeng 2001; 73: 338-46. http://dx.doi.org/10.1002/bit.1067

[229] Zhong, J. Biochemical engineering of the production of plant-specific secondary metabolites by cell suspension cultures. Adv Biochem Biochem Eng Biotechnol 2001; 72: 1-26. http://dx.doi.org/10.1007/3-540-45302-4_1

[230] Eibl R, Eibl D. Bioreactors for plant cell and tissue cultures. In: Oksman-Caldentey KM, Barz WH Eds. Plant biotechnology and transgenic plants. Marcel Dekker, New York 2002; pp. 163-99.

[231] De Dobbeleer C, Cloutier M, Fouilland M, Legros R, Jolicoeur M. A high-rate perfusion bioreactor for plant cells. Biotechnol Bioeng 2006; 95: 1126-37. http://dx.doi.org/10.1002/bit.21077

[232] Choi YE, Kim YS, Paek KY. Types and design of bioreactors for hairy root culture. In: Dutta Gupta, S. and Ibaraki, Y. Editors. Plant tissue culture engineering. Series: Focus on biotechnology Vol. 6. Dorderecht, Springer Verlag 2006; pp. 161-71.

[233] Sivakumar G. Bioreactor technology: A novel industrial tool for high-tech production of bioactive molecules and biopharmaceuticals from plant roots. Biotechnol J 2006; 1: 1419-27. http://dx.doi.org/10.1002/biot.200600117

[234] Su WW, Lee K-T. Plant cell and hairy root cultures-process characteristics, products, and applications. In: Yang ST editor. Bioprocessing for value-added products from regenewable resources. Amsterdam; Elsevier B.V. 2007; pp. 263-92.

[235] Huang T-K, McDonald KA. Bioreactor systems for *in vitro* production of foreign proteins using plant cell cultures. Biotechnol Adv 2012; 30: 398-409. http://dx.doi.org/10.1016/j.biotechadv.2011.07.016

[236] Xu J, Ge X, Dolan MC. Towards high-yield production of pharmaceutical proteins with plant cell suspension cultures. Biotechnol Adv 2011; 29: 278-99. http://dx.doi.org/10.1016/j.biotechadv.2011.01.002

[237] Crawford KM, Zambryski PC. Plasmodesmata signaling: many roles, sophisticated status. Curr Opin Plant Biol 1999; 2: 382-7. http://dx.doi.org/10.1016/S1369-5266(99)00009-6

[238] Doran PM. Foreign protein production in plant tissue cultures. Curr Opin Biotechnol 2000; 11: 199-204. http://dx.doi.org/10.1016/S0958-1669(00)00086-0

[239] Conrad U, Fiedler U. Compartment-specific accumulation of recombinant immunoglobulins in plant cells: an essential tool for antibody production of physiological functions and pathogen activity. Plant Mol Biol 1998; 38: 101-9. http://dx.doi.org/10.1023/A:1006029617949

[240] Shin YJ, Hong SY, Kwon TH, Jang YS, Yang, MS. High level of expression of recombinant human granulocyte-macrophage colony stimulating factor in transgenic rice cell suspension culture. Biotechnol Bioeng 2003; 82: 778-83. http://dx.doi.org/10.1002/bit.10635

[241] Magnuson NS, Linzmaier PM, Reeves R, An G, HayGlass K, Lee JM. Secretion of biologically active human interleukin-2 and interleukin-4 from genetically modified tobacco cells in suspension culture. Protein Expr Pur 1998; 13:45-52. http://dx.doi.org/10.1006/prep.1998.0872

[242] Sharma AK, Sharma MK. Plants as bioreactors: Recent developments and emerging opportunities. Biotechnol Adv 2009; 27: 811-32. http://dx.doi.org/10.1016/j.biotechadv.2009.06.004

[243] Hood EE, Woodard SL, Horn ME. Monoclonal antibody manufacturing in transgenic plants-myths and realities. Curr Opin Biotechnol 2002; 13: 630-5. http://dx.doi.org/10.1016/S0958-1669(02)00351-8

[244] Drake PMW, Chargeleuge DM, Vine ND, van Dolleweerd CJ, Obregon B, Ma JKC. Rhizosecretion of a monoclonal antibody protein complex from transgenic tobacco roots. Plant Mol Biol 2003; 52: 233-41. http://dx.doi.org/10.1023/A:1023909331482

[245] Spier RE. History of animal cell technology. *In*. Spier RE Ed. Encyclopedia of cell technology, Vol. 2, New York. Wiley, 2000: pp. 853-72.

[246] Kretzmer G. Industrial processes with animal cells. Appl Microbiol Biotechnol 2002: 59: 135-42. http://dx.doi.org/10.1007/s00253-002-0991-y

[247] Eagle H. Nutrition needs of mammalian cell sin tissue culture. Science 1955; 122(3168): 501-4. http://dx.doi.org/10.1126/science.122.3168.501

[248] Adams GP, Weiner LM. Monoclonal antibody therapy of cancer. Nat Biotechnol 2005; 23:1147-57.

[249] Böttcher-Haberzeth S, Biedermann T, Reichmann E. Tissue engineering of skin. Burns 2010; 36: 450-60. http://dx.doi.org/10.1016/j.burns.2009.08.016

[250] Bär A, Haverich A, Hilfiker. Cardiac tissue engineering: "Reconstructing the mother of life". Scand J Surg 2007; 96: 154-8.

[251] Shigemura N, Okumura M, Mizuno S, *et al.* Lung tissue engineering technique with adipose stromal cells improves surgical outcome for pulmonary emphysema. Am J Respir Crit Care Med 2006; 1199-205. http://dx.doi.org/10.1164/rccm.200603-406OC

[252] Diekmann S, Bader A, Schmitmeier S. Present and Future Developments in Hepatic Tissue Engineering for Liver Support Systems: State of the art and future developments of hepatic cell culture techniques for the use in liver support systems. Cytotechnol 2006; 50: 163-79. http://dx.doi.org/10.1007/s10616-006-6336-4

[253] Kumar A, Pati NT, Sarin SK. Use of stem cells for liver diseases-Current scenario. J Clin Exp Hepatol 2011; 1: 17-26.

[254] Thomson JA, Itskovitz-Eldor J, Shapiro SS, *et al*. Blastocysts embryonic stem cell lines derived from human. Science 1998; 282(5391): 1145-7. http://dx.doi.org/10.1126/science.282.5391.1145

[255] Mayhall EA, Paffett-Lugassy N, Zon LI. The clinical potential of stem cells. Curr Opin Cell Biol 2004; 16: 713-20. http://dx.doi.org/10.1016/j.ceb.2004.09.007

[256] Goldman S. Stem and progenitor cell-based therapy of the human central nervous system. Nat Biotechnol 2005; 23: 862-71. http://dx.doi.org/10.1038/nbt1119

[257] Ting AE, Mays RW, Frey MR, Van`t Hof W, Medicetty S, Deans R. Therapeutic pathways of adult stem cell repair. Crit Rev Oncol Hematol 2008; 65: 81-93. http://dx.doi.org/10.1016/j.critrevonc.2007.09.007

[258] George JC. Stem cell therapy in acute myocardial infarction: a review of clinical trials. Transl Res 2010; 155: 10-9. http://dx.doi.org/10.1016/j.trsl.2009.06.009

[259] Haraguchi Y, Shimizu T, Yamato M, Okano T. Concise review: Cell therapy and tissue engineering for cardiovascular disease. Stem Cells Transl Med 2012; 1: 136-41. http://dx.doi.org/10.5966/stem.2011-0030

[260] Schwartz SD, Hubschman J-P, Heilwell G, *et al*. Embryonic stem cell trials for macular degeneration: a preliminary report. Lancet 2012; 379(9817): 713-20. http://dx.doi.org/10.1016/S0140-6736(12)60028-2

[261] Cohen M. Secreted Matrix™ and Matrix NC-138™. White paper. Mountainside, NJ, USA: Proteoderm Inc.

[262] Rinaldo A. Healing beauty?. EMPO reports 2008; 9: 1073-7. http://dx.doi.org/10.1038/embor.2008.200

[263] Ra JC, Kim BH, Lee HY, Woo SK, Park H, Kim H. Multipotent stem cells derived from placenta tissue and cellular therapeutic agents comprising the same. USP 20070243172.

[264] FDA Requires Boxed Warning for All Botulinum Toxin Products. 2009 Published April 20; cited 2012 Feb. 15]. Available from http://www.fda.gov/newsevents/newsroom/pressannouncements/ucm149574.htm

[265] Schmidt C. FDA approves first cell therapy for wrinkle-free visage. Nature Biotechnol 2011; 29: 674-5. http://dx.doi.org/10.1038/nbt0811-674

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