HUMAN RESOURCE DEVELOPMENT CENTRE

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BIOTECHNOLOGY AS A TOOL FOR ENVIRONMENTALLY FRIENDLY INDUSTRIAL PROCESSES

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1. Biotechnology at a glance

1.1. Introduction

Biotechnology is a multidisciplinary science which deals with the use of living organisms, their parts or by- products in industrial applications to improve quality of human life. It encompasses a group of related disciplines such as biology, biochemistry, microbiology, genetics, cell and molecular biology, genomics, proteomics and computer sciences in order to achieve technological application of microbes and cultured cells or cellular component. This integrated science offer new advances in understanding and controlling life processes through the development of exciting new technologies.

The word biotechnology is a cross between the Greek words “bios” (everything to do with life) and “technikos” (involving human knowledge and skills). As a branch of science, biotechnology is also not as young as some might think.

Biotechnology spans two main periods:

- Traditional biotechnology, based on empirical use rather than theoretical knowledge
- Modern biotechnology, based on theoretical knowledge and increasingly controlled applications

Fermentation technology, such as alcoholic fermentation and bread making, represent actually the earliest form of biotechnology, which originated in China and Egypt. Egyptians used yeasts to bake leavened bread, the Chinese developed fermentation techniques for brewing and cheese making, and the Aztecs used Spirulina algae to make cakes. This traditional or classical biotechnology does not reflect biotechnology as it is known today, because the processes were experimental and not really controlled. Natural strains of microorganisms has been exploited by humans for obtaining useful products such as curd, wine, vinegar, bread, cheese, etc.

Nowadays modern biotechnology is the application of genetically engineered microorganisms, plant and animal cells in developing production technologies that not only enhance their productivity but also results in new products and processes by that cells. Modern biotechnology`s current and potential applications include enhancing

nutritional quality of food crops, strengthening resistance to disease in economically important plants and animals and increasing crop and livestock productivity, preventing, diagnosing, treating and curing diseases, and replacing oil-based products and processes with bio based products and processes by recombinant DNA technology.

Based on the field of application biotechnology can be classified in five main groups, which have been identified by a color system.

Red biotechnology is also known as medical biotechnology brings together all those biotechnology uses devoted to medicine and human health. This field has changed enormously over the past decades after the discovery of the molecular structure of the genetic molecule DNA by Watson and Crick in 1953, which in the long run prompted the deciphering of the human genome in 2000.

The scope of its activity is the use of living cells and cell materials to research and produce pharmaceutical and diagnostic products that help treat and prevent human diseases. Knowledge about the genetic information is used by researchers to understand the mechanisms of life processes and diseases. The more the scientists know which genes are responsible for the production of certain proteins, the better able they are to develop targeted medicines. Using recombinant DNA technologies many therapeutic products include vaccines, monoclonal antibodies, antibiotics, human proteins and more effective but less toxic new drugs are made. Some relevant examples of red biotechnology are cell therapy and regenerative medicine (tissue engineering), gene therapy to cure disease that are previously incurable, stem cell research, cancer treatment. Red biotechnology also helps in reproductive technologies like in vitro fertilisation, DNA profiling, forensics and in transplantation technology. It is applied also in the manufacturing of drugs where microorganisms or animal cells are used to produce the desired medicines in specially developed bioreactors. This technique is especially applicable for protein-based medicines such as hormones and antibodies. Since these active biomolecules have three-dimensional structure and are targeted in their therapeutic mode of action, they can only be produced by living organisms or cells. Synthetically reproducing the required substance does not function in such cases.

Red biotechnology is not restricted to humans. It is primarily used in veterinary medicine for the development of better vaccines. It is used for the preparation of so-called marker vaccines for determining whether the immunity of immunised animals is disease or vaccine-induced. Biotechnology has also been used to produce toxin antigens and immunomodulatory agents.

White biotechnology comprises all the biotechnology uses related to industrial processes – that is why it is also called 'industrial biotechnology'. White biotechnology pays a special attention to design low resource-consuming processes and products, making them more energy efficient and less polluting than traditional ones. There can be found many examples of white biotechnology, such as the use of microorganisms in chemicals production, the design and production of new materials for daily use (plastics, textiles, food nutrient, washing powders and other products) and the development of new sustainable energy sources such as biofuels. To give an example of the benefits of white biotechnology over traditional production is the introduction of the white biotech-based fermentation technology for production of antibiotics on an industrial scale. This technology enables a complex 13-step chemical process to be replaced with a one-step fermentation, two-step enzyme process, with the result of energy savings of 65% and a halving of raw material costs. White biotechnology is already delivering considerable savings, both financially and environmentally, by reducing or eliminating human reliance on scarce resources and reducing greenhouse gas emissions from production.

Grey biotechnology also known as environmental biotechnology explores primarily problems concerning the environment such as recycling of potable water, cleaning sewage water, rehabilitation of contaminated ground, recycling of garbage or exhaust gas cleaning. The environmental benefits offered by biotechnology are enormous, particularly in the fields of waste treatment and bioremediation of contaminated sites water and air, pest control, treatment of industrial effluents and emissions and acid mine drainage. The most varied of analytical and modelling methods are employed for this purpose.

The grey biotechnology application can be split up into two main branches: biodiversity maintenance and contaminants removal. Regarding the first, it should be mentioned the application of molecular biology to genetic analysis of populations and

species that are part of ecosystems, their comparison and classification and also cloning techniques aimed to preserve species and genome storage technologies. Maintaining the biodiversity in natural habitats helps ecosystem functions over a wider area. Natural habitats afford sanctuary to breeding populations of birds and other predators which help control insect pests in agricultural areas, thus reducing the need for, and cost of natural artificial control measures.

The increasing amount of pollutants in the environment is an alarming concern to the ecosystem. A number of organic pollutants, such as polychlorinated biphenyls (PCBs), polyaromatic hydrocarbons (PAHs), and pesticides, are resistant to degradation, which represent toxological threat to wildlife as well as human beings. Various physiological and biological measures have been employed globally to degrade these hydrocarbons to improve environment quality. The most promising strategy for solving this problem is bioremediation where microorganisms are used to degrade the organic and inorganic pollutants with the added possibility of subsequently making use of these substances or by-products from this activity. There are many naturally existing microbes, which are routinely employed in bioremediation process, but in this era of industrialization the rate of xenobiotics discharges has crossed the tolerance limit of the nature. Therefore, various genetic engineering approaches are employed by biotechnologists to construct new microbial strains genetically engineered microorganisms (GEMs) with better bioremediation efficiency. Majorly biomolecular engineering approaches such as rational designing and directed evolution have been developed to genetically modify microorganisms and their enzymes for the degradation of persistent organic pollutants (POPs) like PAHs, PCBs, and pesticides. Recently, several developments in the field of recombinant DNA technologies such as development of “suicidal-GEMs” (S-GEMs) have also been carried out to achieve safe and efficient bioremediation of contaminated sites (Soccol, et al., 2003).

Green biotechnology also known as Plant or Agricultural Biotechnology is a rapidly increasing field within modern biotechnology. It is being used to address problems in all areas of agricultural production and processing. This includes plant breeding to raise and stabilize yields; to improve resistance to pests, diseases and abiotic stresses such as drought and cold; to enhance the nutritional content of foods; to improve the quality of crops in crop production; to develop low-cost disease-free planting materials

for crops such as cassava, banana and potato and is creating new tools for the diagnosis and treatment of plant and animal diseases

and for the measurement and conservation of genetic resources. These improvements are not possible with traditional crossing of related species alone.

The broad applications of biotechnology in agriculture, specifically in crops, include the development of disease diagnostic kits, biofertilizers and biopesticides, and the use of

molecular markers, tissue culture, and genetic engineering for varietal development (Teng, 2008; Ortiz, 2010).

Producing modified plant varieties is based almost exclusively on transgenesis, or introducing genes of interest from another variety or organism into the plant. This property makes the technique revolutionary in terms of the potential benefits that it may bring, but it has also caused concern regarding issues of safety, ethics, consumer choice and environmental impact.

There are four methods of delivering extra DNA into the nucleus of plant cells which have been successfully used to produce a transgenic plant.

The first one is the “cutting and pasting” approach in which bacterial enzymes are required to recognize, cut and join transgenic DNA at specific locations thereby acting as molecular scissors and tape. The selected gene of interest is transferred in the plant cell by using plasmid vectors containing marker genes such as genes for antibiotic resistance, which are used as a basis for selection.

The second approach is based on the use of *Agrobacterium tumefaciens* as a natural genetic engineer. In nature this soil bacteria invade plants through wounds in the stem and the root. Its extrachromosomal DNA known as a Ti plasmid enters the plant cell and inserts itself into the plant's chromosomes (Fig. 1). Upon insertion, the genes on the Ti plasmid are turned on. These genes encode enzymes that force the plant cell to make metabolites the bacteria need for growth. Genetic engineers have taken advantage of *Agrobacterium's* natural abilities. They have removed the genes from the Ti plasmid that alter metabolism and cause disease and in their place incorporated desired genes. These bacteria with their alter plasmids can be used to infect and transform plant cells growing in tissue culture.

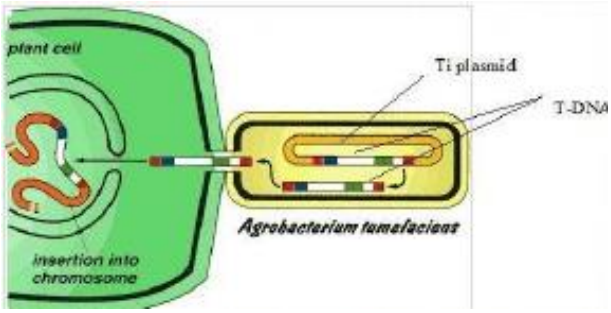

Fig. 1 Mechanism if invasion of *Agrobacterium tumefacians*

The third one is the gene gun approach also called particle acceleration or micro projectile bombardment. As the name implies, the plant cell is bombarding with microscopic gold or tungsten particles coated with hundreds of copies of the gene(s) of interest. Once inside the nucleus of a cell, the genes dissolve off of the gold particle and can potentially insert into a chromosome. The modified cell can then be used to regenerate a new organism.

The fourth approach is based on the use of microfibers to transform tissue culture cells. The microfibers known also as whiskers are coated with hundreds of copies of the gene(s). The fibers stab the plant cells potentially delivering the DNA into the nucleus of the cell without killing it.

Blue biotechnology (marine) is based on the exploitation of marine bioresources to create products and applications of industrial interest. It is fast developing and very important research field of the world biotechnology sector. The marine ecosystems are largely unexplored, understudied and underexploited in comparison with terrestrial ecosystems and organisms. They are characterized as unique habitats because of the extreme environmental conditions such as high salinity and hydrostatic pressures of the deep sea, extreme temperature and pH at the hydrothermal vent communities and coral reefs. The large diversity of species lead to an incredible chemical diversity of molecules, found in the oceans, including structures and bioactivities, which are not found anywhere (Burges, J., 2012). Their investigation and exploitation through the application of marine biotechnology approaches could satisfy the growing human demand for healthy food products, biomaterials, sustainable
alternative energy sources, novel drugs and personal care products and for dealing with the environmental problems.

The marine organisms are used as a source of biomaterials, gene pools, valuable ingredients such as pigments, bioactive compounds, fatty acids, enzymes, antioxidants. The field of marine natural products is already large and growing. Some of them are applied in industrial biotechnology, while others can support the development of new processes in food and pharmaceutical industries or in molecular biology and diagnostic kits.

Medicine and research are major beneficiaries of development in blue biotechnology. Bacteria and fungi as well as macroorganisms such as sponges, corals and algae are potent producers of biological active substances with prominent activities not only against pathogenic bacteria, fungi, and viruses but also against tumor cells. They may also effect of the cardiovascular, immune and nervous systems.

The commercial application of numerous discovered bioactive compounds is limited because of problems regarding reproduction and scale up. In addition, obtaining sufficient amounts of the pure substances also limited further progress in many cases. For example less than 1 g of substances such as halichondrin, ecteinascidin or bryostatin is obtained from a ton of sponges, ascidia or bryozoa, respectively (Molinski et al., 2009; Mayer et al., 2010). Alternative production processes solved these problems for several compounds (Battershill et al. 1998, Duckworth et al., 2004).

The application of genomic and metagenomic approaches in addition to culture-dependent studies offer new possibilities to investigate the immense diversity of microbes in marine environments and their capacity to produce new chemical structures with biological activities. The lack of a systematic approach often resulted in the frequent rediscovery of known compounds (Penesyan et al., 2010). The early detection of the known bioactive compounds is possible owing to the advances in the development of new analytical techniques, such as differential analysis of arrays of 2D NMR spectra (Schroeder et al., 2007). These techniques coupled with the establishment of large databases, are valuable tools for rapid identification of known and detection of probably new compounds.

Marker molecules from marine organisms are now commonly used in research. For example, the luminescent properties of the jellyfish *Aequorea victoria* led to the characterisation of the green fluorescent protein (GFP). GFP and the luciferase

enzyme from *Vibrio fischeri* have widespread applications in molecular biology as a marker protein.

Seeweeds are an abundant source of natural polysaccharides, many of which have already commercial uses due to their properties as gelling and stabilizing agents. The biopolymers agar and agarose have been used for research purposes for many years as nutrient media and in the preparation of gels for electrophoresis. The polysaccharides chitin, chitosan, (derived from shrimps and crabs) and carrageenan (derived from algae) are widely used in the food industry as thickeners and stabilizers.

The marine micro- and microalgae could be used as part of the solution for the increasing global need for bioenergy. There are promising results from pilot studies in utilizing these species as available resources for biogas production. The possible applications of large-scale microalgae cultivation are production of high-value commercial compounds, energy resource and removal of CO_2 from coal-fired power plants due to its ability to photosynthesize and grow rapidly.

Nanotechnology (sometimes refers as a nanotech) is a rapidly growing research area and is one of the 21st century`s most promising technology together with biotechnology. Nanoscience and nanotechnology deals with extremely small particles at dimensions between 1 and 100 nanometers and study their possible application at the molecular and cellular level. These dimensions are known as a nanoscale. Since its birth nanotechnology has never been a single field technology. It is more preferably called nanotechnologies, as refers to a set of methods and approaches in physichs, chemistry, engineering fields, biological and medical science. The nanobiotechnology occurs as a result of association of these two technologies. (Singh, et al., 2010).

Nanobiotechnology is a modern multidisciplinary field of activity and research that has emerged very recently and refers to the ways that nanotechnology is used to create devices to study biological systems on subcellular and molecular levels. The n**anoscale devices and materials help scientists to interact with a variety of biological processes on molecular level. These applications benefit life science research, clinical diagnostics, drug development, and many other areas.** The first application of nanobiotechnology is related to the advances in medicine where nanoparticles are used to bind or encapsulating drugs. Such a new class of nanotherapeutics could target specific sites in the body or even diseased tissue, thereby limiting side effects beyond the target.

Many metals formed metallic nanoparticles, which have a variety of useful properties. Some of them form crystal structures with unique optical characteristics and are used as nano-biosensors, while others show magnetic properties and are used in magnetic resonance imaging. The magnetic nanoparticles are widely used in molecular biology research as separation tools in developing methods of separation of macromolecules. Another important characteristics of nanostructures is their large surface area with functional groups which make them suitable magnetic sorbents in the process of immobilization of different biologically active molecules such as nucleic acids, and proteins.

The better investigated nanostructures are silver ones, which possess anti-microbial and antiflammatory properties. Their large surface area increasing the possibility for interactions, which determine the application of silver nanoparticles as effective anti-microbial agents. Gold nanoparticles are utilized in medical imaging for immunogold labelling of samples prepared to be viewed by transmission electron microscopy. Iron-based nanoparticles have been used in *in vitro* and *in vivo* experiments for tracking the way of stem cells implanted in wound site.

The current chemical and physical methods for nanoparticles production used by synthetic biology are expensive in respect to high temperature or pressure they require. As an alternative way for nanoparticle production is the biological synthesis using both prokaryotic and eukaryotic organisms. They produce them as part of their defence against toxic substances. Among the eukaryotes the fungi are well known producers of nanoparticles. For example the fungus *Phoma* produces silver nanoparticles as anti-microbial agents for medical application. Except silver nanostructure the fungus *Fusarium oxysporum* produces nanoparticles also from other metals: Pt, Ag, Au, Pb and Ti (Edmundson., M. et al., 2014).

Many species of bacteria are able to produce nanoparticles naturally and are preferable for this purpose because of their faster growth rates and ease of manipulation. Some of them such as aquatic bacteria *Magnetospirillum gryphyswaldense* forms magnetic iron nanostructures in their cells known as magnetosomes. Others such as *Shewanella* forms nanoparticles known as nanowire, which serve to transport electrons out of the cell in respiration under anaerobic conditions. These kind on nanoparticles are explored for their potentially conductive properties.

Nanotechnology is becoming increasingly important in different food sector. Promising results and applications are already being developed in the areas of nutrient delivery systems through bioactive nanoencapsulation, biosensors to detect and quantify pathogens organic compounds, other chemicals and food composition alteration, and even edible film to preserve fruit or vegetables.

1.2. Basic tools of biotechnology

Biotechnology deals with techniques of using live organisms or enzymes from organisms to produce products and processes useful to humans. There are a lot of potential tools, applied by researchers to manipulate millions of different species of plants, animals and microorganisms in the world, each having cells and molecules with unique characteristics. This is why biotechnology is so powerful and can be applied in many different ways.

1.2.1. Fermentation processes

Fermentation processes are the oldest of all biotechnological processes and are a very important discipline in modern society. This technology is applicable in many industries for the production of various foodstuffs and alcoholic beverages, baker's yeast, biofuels, organic acids, enzymes, vitamins, antibiotics, vaccines, monoclonal antibodies, steroids, hormones and fine chemicals. It is based on "microbial fermentations" and can be defined as traditional biotechnological technique. In this case, microorganisms serve as miniature factories, which convert raw materials into end products. The fermentation processes are carried out in bioreactors under strict control of insightful process parameters such as temperature, pH, aeration, mixing and some other process parameters. Because nearly all the products of biotechnology are manufactured by microorganisms, fermentation technology is an indispensable element of biotechnology`s support system.

Any industrial microbial process includes three main phases: upstream processing, fermentation process and downstream processing.

The upstream processing phase comprises selection of suitable microbial strain, formulation, optimization and sterilization of culture media and equipment preparation and sterilization, inoculum preparation and inoculation of the medium.

The screening (selection for the production of new metabolites with new isolates and/or new test methods) and isolation of suitable strain, producer of industrially valuable compound, which characterizes with high productivity is the most important step.

There are several strategies developed that aim to provide suitable strains of an industrial need. The simple, but yet powerful strategy is to exploit natural diversity by selecting a strain from natural samples that produces a desired product. The inherent production performance of microorganisms is sometimes poor, and therefore they should be rationally engineered to improve their metabolic and cellular characteristics to meet the demand for high yield and productivity. Metabolic engineering has become a vital approach for engineering microorganisms for the production of desired biochemicals (Chaudhary et al., 2015).

Other desirable strain characteristics are:

- Rapid growth
- Genetic stability
- Non-toxicity to humans
- Large cell size for easy removal from the culture liquid

Recent metagenomic investigations revealed that natural biodiversity is immense and largery unexplored and the current industrial strains represent only a small fraction of the natural biodiversity. There are multiple unknown species and strains in the nature that may prove superior for a certain industrial process. Some of them could not be proper for industrial implementation but may be used as genes resource. The metagenomic approach allows the genes of industrial interest to be isolated directly from nature samples and transferred to industrial strains, thereby creating novel strains with extra beneficial features (Steensels, J., 2014).

The real fermentation process phase involves the growth and propagation of the selected microbial strain and synthesis of the desired product. The cultivation is carried out in appropriate culture media which must provide the necessary amounts of

carbon, nitrogen, trace elements and growth factors. The formulation of the culture media affects the yield, rate and product profile. The scale-up process development is carried out in bioreactors under aerobic or anaerobic conditions in dependence on the microbe`s metabolism. The optimal physical conditions as temperature, pH, gaseous atmosphere and mixing are maintained and controlled constantly. More often, the strain productivity and growth is better on a small scale process because of the different conditions experienced in the large bioreactor.

There are two main approaches, which are applied for the realization of industrial microbiological process. In the first one (Fig. 2A**.**) the substrate is mixed with whole microbial cells and as a result of the substrate biotransformation the final product is achieved.

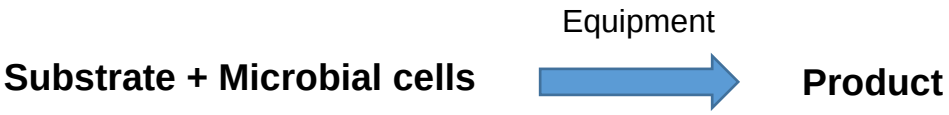

Fig. 2 A. The first approach in designing microbial process

In the second approach enzymes isolated from microorganisms are used as biocatalysts for substrate conversion into final products (Fig. 2 B)

Fig. 2 B. The second approach in designing microbial process

The downstream processing phase comprises product recovery and waste treatment.

The commercially important products of microbial fermentation can be divided into five main groups:

- Biomass (baker's yeast, starter cultures, animal feed, etc.)
- Primary metabolites (amino acids, organic acids, vitamins, polysaccharides, ethanol, etc.) and secondary metabolites (antibiotics, pigments, etc.)
- Bioconversion or biotransformation products (steroid biotransformation, L-sorbitol etc.)
- Intra- or extracellular enzymes (amylase, lipase, cellulase, etc.)
- Recombinant products (some vaccines, hormones such as insulin and growth hormones etc.)

Product recovery is carried out through a series of operations including cell separation by settling, centrifugation or filtration; product recovery by disruption of cells (if the product is produced intracellularly); extraction and purification of the product.

Usually, waste products from other industrial processes, such as molasses, lignocellulosic wastes, cheese whey and corn steep liquor, after some modifications are used as the substrate for many industrial fermentations.

Microbial cells have been most commonly used for industrial purposes because of their diversity and ease of handling. Rapid advances in the life sciences have greatly increased the availability of whole cell catalysts. The recombinant DNA technique is a good example.

More recently, fermentation technology has begun to use cells derived from higher plants and animals under growth conditions known as cell or tissue culture. The industrial application of these cell culture is still in progress although several high-value secondary metabolites have been achieved through plant cell cultivation in bioreactor. While plant cell cultures provide a viable system for the productions of these compound in the laboratory, their commercial scale application is limited due to the obtained low yields of the metabolites of interest or the feasibility of the process. The problem of producing low level of desired metabolite by plant cell cultures is still due to the insufficient knowledge of scientists how plants regulate metabolic biosynthesis (Stanbury, et al., 2003).

Biotechnological processes offers many advantages over conventional chemical methods of production because it uses living materials: lower temperature, pressure, and pH

are usually required; renewable resources such as raw materials can be used; and optimum quantities can be produced with minimum energy consumption.

1.2.2. Genetic engineering

The basic principle of genetic engineering is gene transfer, achieved by various methods to produce recombinant DNA and respectively recombinant proteins, genetically modified microorganisms, transgenic plants and transgenic animals for commercial application. Recombinant DNA is any DNA molecule composed of sequences derived from different sources. Genetic engineering, thus ultimately influences the growth of biotech industry. The two significant feature of genetic engineering is production of beneficial proteins and enzymes

in surplus quantities and creation of transgenic plants, transgenic animals and genetically modified microorganisms with new characters using recombinant DNA technology.

The idea for occurrence of genetic engineering is given by two scientists Stanley Cohen and Herb Boyen in 1973, when they tried to insert genes from higher organisms in the genome of bacteria. Further developments in this area are due to the appearance of some important techniques such as PCR (polymerase chain reaction) introduced as an approach by Kary B. Mullis in 1983, sequence technology, methods of gene delivery (plasmids, transposons, gene gun). This revolutionary technique permits small quantities of DNA to be amplified and yield enough DNA for analytical analysis. It is still continuing to open up new applications of biotechnology. The polymerase chain reaction (PCR) permits exponential amplification of a specific segment of DNA from just a single initial template DNA molecule if the sequence flanking the DNA region to be amplified is known.

Before PCR could become a viable commercial technique, it was necessary to find a DNA polymerase that could survive temperatures cycling between 37 °C, where annealing and polymerization occur, and 95 °C, where DNA denaturing occurs. The DNA polymerase known as Taq polymerase from the thermophilic bacterium *Thermus aquaticus*, found in hot springs in Yellowstone National Park, was chosen.

Before a gene could be inserted into bacteria, it is necessary to know the sequence of nucleotides in that gene. At the beginning two independently developed methods are available

as a tool for sequencing the DNA nucleotide chains. The Sanger`s method, proposed by Frederick Sanger in 1977 and Gilbert`s method devised by Walter Gilbert at the same time.

In the first one, enzymatic approach is used for selective cleaving of DNA strands resulting in DNA chains at different length. The second method is based on chemical cleavage of DNA. In both of the methods the sequence of the bases in DNA is revealed by separating the resulted labelled DNA fragments by gel electrophoresis.

Except these sequencing methods, an automated method is developed by Leroy Hood and his coworkers at the California Institute of Technology through the invention of automated DNA sequencer in 1985. DNA fragments up to about 500

nucleotides long are most commonly sequenced in automated instruments based on the Sanger (dideoxy chain termination) method.

To clone a gene of interest have to link it to a vector molecule, which can replicate within a host cell. The vectors represent any DNA molecule that has the ability to replicate inside the host organism to which the desired gene has integrated for cloning. There are different kinds of vectors, that find application in cloning procedures - plasmids, bacteriophages, cosmids, BAC, yeast vectors, shuttle vectors. All of them should possess three important features: to have origin of replication, to contain at least two selectable markers (usually genes, determining antibiotic resistance), to have sites for specific restriction cleavage by restriction enzymes. When a single recombinant DNA molecule is introduced into a host cell, the inserted gene is replicated along with the vector, generating a large number of identical DNA molecules. The selection of recombinant cells is based on the ability of transformed cells to grow on a selective media. The complete characterization of any cloned DNA fragment requires determination of its nucleotide sequence.

The plasmids most commonly used in recombinant DNA technology are those that replicate in *E. coli.* The basic scheme of a transformation process can be summarized as follows:

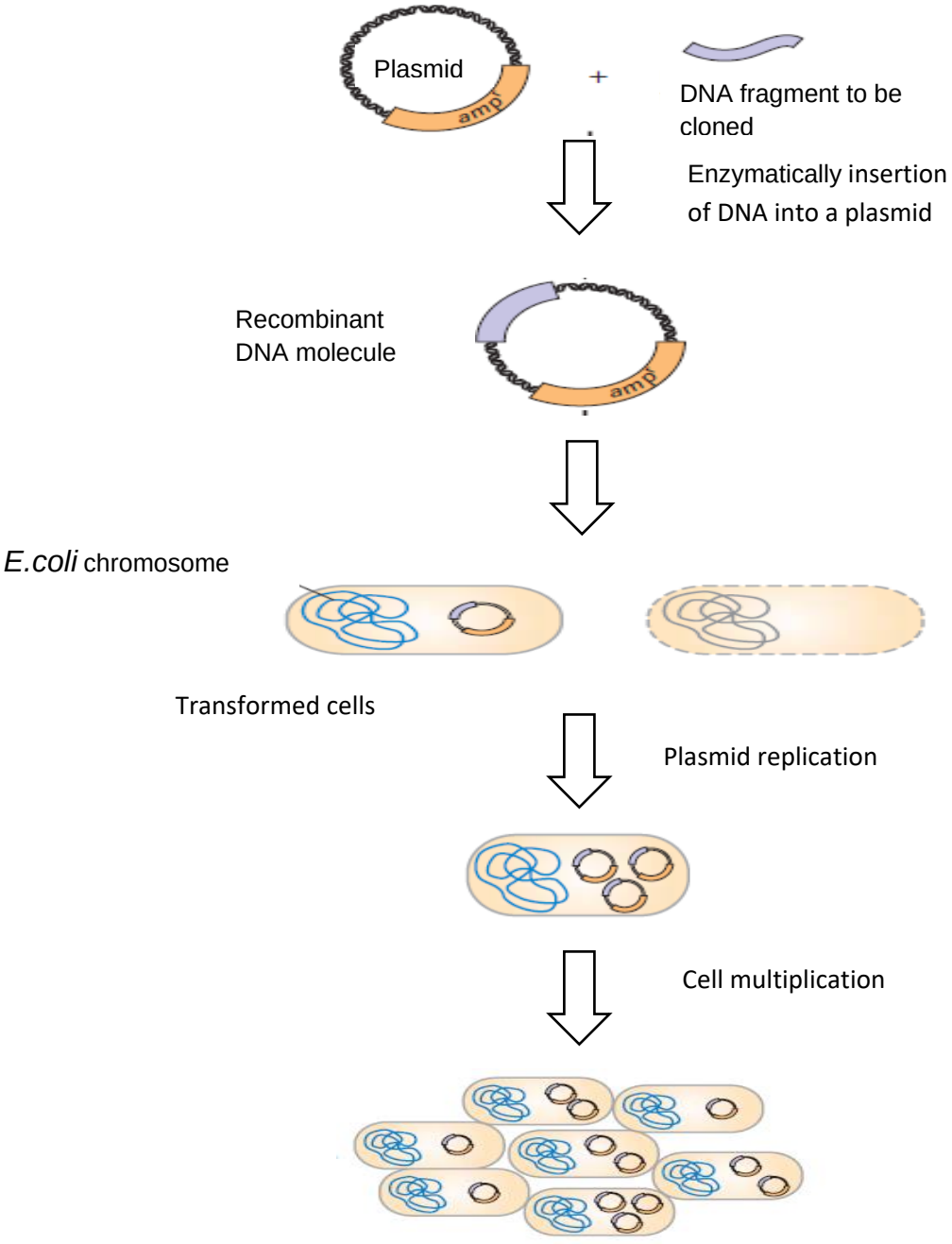

Fig. 2 Process of transformation of a plasmid vector in E.coli

1.2.3. Bioinformatics

Bioinformatics is a fast developing field offering a basic tool to the researchers, especially to the biologists, to accelerate the research, application and commercialization of biotechnology. in areas of medicine, agriculture and bio-energy. Using specialized recombinant DNA techniques, researchers have determined vast amounts of DNA and protein sequences including the entire genomic sequence of humans and many other organisms. Through computational tools the obtained raw data from sequencing and functional analysis are gathered, stored, classified, analyzied and distributed. The first complete sequencing was genome was of *Haemophilus influenzae* in 1995. Since then hundreds of genomes have been sequenced. The enormous volume of data, which is growing fast, has been stored and organized in data banks for example the GenBank /ftp://ftp.ncbi.nih.gov/genbank/ at the National Institutes of Health, Bethesda, Maryland, and the EMBL Sequence Data Base /http://www.ebi.ac.uk/ena/ at the European Molecular Biology, Laboratory in Heidelberg, Germany.

These databases continuously exchange newly reported sequences and make them available to scientists throughout the world on the Internet. The stored sequence data are useful for scientist to plan and design their experiments and to analyze the obtained results.

Basic research in bioinformatics can be classified into:

- *Genomics* – sequencing and comparative study of genomes to identify gene and genome functionality,
- *Proteomics* – identification and characterization of protein related properties and reconstruction of metabolic and regulatory pathways,
- Cell visualization and simulation to study and model cell behavior
- Application to the development of drugs and anti-microbial agents.

Some of the approaches which are used in bioinformatics includes protein modeling, genomic analysis and comparison of entire genomes and proteomes, biomarker data analysis, expression profiles and pathway and disease modeling.

By comparing the amino acid sequence of the protein encoded by a newly cloned gene with the sequences of proteins of known function, an investigator can look for sequence similarities that provide clues to the function of the encoded protein.

Based on the entire sequence of macromolecules scientists could create model of their three-dimensional structure. Protein function can be predicted by matching the 3D structure of an unknown protein with the 3D structure of a known protein. However, 3D structures from X-ray crystallography and NMR spectroscopy are limited. Thus there is a need for alternate mechanism to match genes (Bansal, A., 2005).

There are two major approaches to model 3D structure of a protein:

-sequence homology based prediction

- *ab initio* (or *de novo*) method.

Several software tools have been developed like BLAST algorithm (basic local alignment search tool) that efficiently performs sequence-alignments against large databases of known sequence. Dynamic algorithms such as Smith-Waterman and other indexing schemes are more accurate for pair-wise gene alignment.

Bioinformatics researchers have compared extensively multiple microbial genomes to correlate and classify the genomes into various families and to study evolution. Comparison of related sequences from different species can give clues to evolutionary relationships among organisms. The 16S rRNA approach, based on the theory of slow mutation rate of conserved genes uses 16S rRNA database and multiple sequence alignment as a tool to compare multiple homologous genes (genes that have similar sequences) and to derive conserved segments. This approach is based also on Neighbor join algorithm to build an evolutionary tree based on the obtained conserved region

Microarray technology is one of the most promising tool for investigating gene expression developed in the last 15 years. DNA microarray analysis can reveal differences in gene expression in cells under different experimental conditions. It measures the relative change in the gene-expressions for a stressed (or a stimulated) cell and a change in cellular expression pattern – differentiation, cellular cycle, tissue remodeling, sporulation.

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2. More for Industrial /White / biotechnology

"The microbes will have the last word "

Louis Pasteur

2.1. Introduction

Among the major new technologies that have appeared since the 1970s, biotechnology

has perhaps attracted the most attention. It provides tools for adapting and modifying the biological organisms, products, processes and systems found in nature to develop processes that are eco-efficient and products that are not only more profitable but also more environment-friendly. In this way it has proved capable of generating enormous wealth and influencing every significant sector of the economy and this can contribute to sustainable industrial development.

Industrial biotechnology is one of the most promising new approaches to pollution prevention, resource conservation, and cost reduction. Recent scientific advances in the fields of genomics, molecular genetics, metabolic engineering and catalysis, coupled with advances in enzyme and fermentation technology as well as external factors such as soaring energy prices, renewed environmental concerns and energy security fears, have combined to make white biotechnology more important than ever. It is often referred to as the third wave in biotechnology (a term illustrating the chronology of development in which red and green biotechnology come first and second respectively). If developed to its full potential, industrial biotechnology may have a larger impact on the world than health care and agricultural biotechnology. It offers businesses a way to reduce costs and create new markets while protecting the environment.

Special attention is laid on enzymes from microorganisms, which are functioning as specialized catalysts for chemical reactions. These protein molecules contributed greatly to the traditional and modern chemical industry by improving existing processes. At present more than 4,000 different enzymes have been identified (Shuang Li et al., 2012) but only 200 microbial original types have found their way into various industrial sectors. The rest of enzymes are still not able to meet all requirements of the industry. The majority of enzymes used to date have been obtained from mesophilic microorganisms and despite their many

advantages their application is restricted because of their limited stability to extremes of temperature, pH, ionic strength and etc. (Joseph Gomes and Walter Steiner, 2004). As a result the characterization of microorganisms that thrive in extreme environment has received a great deal of attention. Thus, biocatalysis using extremophiles as well as extremozymes is rapidly being transformed from an academic science to an industrially viable technology.

Only a minor fraction of the microorganisms on Earth have been exploited. The great plate count anomaly cannot be overcome simply by improvements in culture techniques, but has come to rely more on "–omics" technologies. Novel developments in the cultivation and production of extremophiles but also developments related to the cloning and expression of their genes in heterologous hosts, will increase the number of enzyme-driven transformation in chemical, food, pharmaceutical and other industrial application (Kumar, L., et al., 2011).

Among all industrial sectors the chemical sector is the biggest user of white biotechnology products and processes. Already 16 % of the obtained raw materials are from renewable sources and this percent is constantly growing due to the fast development of white biotechnology. Other sectors that are experimenting with the new tools offered by biotechnology are food, textiles, paper, wood, pulp, pharmaceutical, agricultural, cosmetics, environmental and energy sectors.

By focussing attention to develop large-scale viable enzyme technologies, suited to applications in the industrial biotechnology, a glimmer of hope to save the environment for sustained future developments emerges (Srinivasan, M. C., et al., 1999).

2.2. Advantages of industrial biotechnology to traditional chemical industry

The advantage of using white biotechnology is that it improves the efficiency of chemical processes. There are several points which could be mentioned as advantages of biotechnological processes:

- Cleaner and ecological production since fewer disposals are generated and the harmful chemical processes products are eliminated.
- Low-cost raw materials feedstock as cellulose and biomass in comparison to petrochemical sources

- Biocatalysis with the participation of microbial cells or their enzymes. The biocatalyc reactions are characterized by high region and stereoselectivity.
- In the biotechnological process reaction steps are reduced and usually include one synthesis and one isolation step.
- Enable the synthesis of products that are not possible chemically. For example, among diacids with chain lengths ranging from 10 to 18 carbons, only a few (primarily diacid with a chain length of 12 carbons) can currently be produced economically through chemical processes, while a majority can be economically produced through bioprocesses. Polymers made from these longer-chain diacids have improved qualities such as greater flexibility and reduced moisture absorption, and can create new value-added downstream applications.
- Conventional chemical processes require high temperature and pressure, while microorganisms and enzymes work under pressure and normal temperatures, are biodegradable and can function in extreme conditions.

2.3. Developments of white biotechnology for environmentally friendly production of products

White biotechnology has its roots in ancient human history and its products are increasingly part of everyday life, from vitamins, medicines (fine chemicals), biofuel and bioplastics to enzymes in detergents or dairy and bakery products. The main long-term applications of white biotechnology will be focused on replacing fossil fuels with renewable resources (biomass conversion), replacing conventional processes with bioprocesses (including metabolic engineering) and creating new high-value bioproducts, including nutraceuticals, performance chemicals and bioactives (Patrick Lorenz and Jürgen Eck, 2005).

Many people consider green chemistry and industrial biotechnology to be synonyms of white biotechnology. The laudable aim of white biotechnology is to create a sustainable society (Gupta, M., et al., 2007). It refers to the use of living cells and/or their enzymes to create industrial products that are more easily degradable, require less energy, create less waste during production and sometimes perform better than

products created using traditional chemical processes. Such processes are more profitable, because they are cost-effective and less wasteful of materials and energy.

The Organisation for economic and co-operation and development (OECD), has collected and analyzed case studies of the application of biotechnology in such diverse sectors as chemicals, plastics, food processing, textiles, pulp and paper, mining, metal refining and energy. The case studies show that biotechnology can not only reduce costs but also reduce the environmental footprint for a given level of production. In some cases, capital and operating costs decreased by 10-50%. In others, energy and water use decreased 10-80% while the use of petrochemical solvents was reduced by 90% or eliminated completely. In a number of cases, biotechnology enabled development of new products whose properties, cost and environmental performance could not be achieved using conventional chemical processes or petroleum as a feedstock (www.oecd.org/sti/biotechnology).

According to the reported information of World Wide Fund for Nature (WWF) the application of white biotechnology products and processes saves 33 million tones of CO_2 each year and thus help to be built a greener economy (WWF, 2009).

The use of white biotechnology is primarily related to fermentation and biocatalysis.

Fermentation involves the use of microorganisms such as bacteria, yeast and fungi which are cultivated in bioreactors, to convert efficiently sugars into useful materials such as bioethanol, lactic acid, succinic acid, citric acid, vitamins, amino acids, antibiotics, alkaloids, steroids, etc. It is envisaged that future plastics would come from sugars, starch, cellulose and vegetable oils (Carmichael H., 2006). The fermentation is the only method for obtaining some of this products and also in significant amounts. It may sometimes offer a much more eco-efficient route to an existing chemical synthesis. An example is the production of vitamin B2 (riboflavin). The conventional process is a sequence of eight steps, which combined chemical and biotechnological synthesis. The synthesis of D-ribose, the monomer of vitamin B2 is produced through fermentation with *Bacillus* sp., followed by chemical reactions to obtain vitamin B2. This complex process was replaced by the complete biotechnological synthesis of riboflavin using fermentation with the help of bacteria, yeast and fungi. At BASF (Ludwigshafen, Germany), more than 1,000 tonnes of vitamin B2 are now produced per year in a single fermentation. Using the fungus

Ashbya gossypii as a biocatalyst, BASF achieved an overall reduction in cost and environmental impact of 40%. Similarly, cephalexin, an antibiotic that is active against Gram-negative bacteria and is normally produced in a lengthy ten-step chemical synthesis, is now produced in a shorter fermentation-based process at DSM Life Sciences Products (Heerlen, The Netherlands) (Frazzeto, 2003).

The following are some of the key areas in which white biotechnology has shown considerable promise and wherein chemists and biochemists are expected to play an increasing role.

2.3.1. Biorefineries

Biorefineries are facilities that convert biomass or biological materials from living or recently living organisms into bio-based products. Examples of these products can include transportation fuels and heat, fine chemicals and materials such as plastics and polymers, fibers, food and feed. In comparison to the petroleum refineries that produce fuel and other basic chemicals from crude oil, the biorefinery produces the same but from renewable biomass. This system combines a variety of conversion technologies transforming renewable raw materials into final products (Fernando S., et al., 2006) (Fig. 1). The main goal is to optimize resources utilization and minimize wastes.

Biomass of different origin can be used as a raw material in the biorefinery. Whole crop (corn and cereal), wheat, rape, cotton, sorgo, cassava and lignocellulose (from wood or waste) can be used as feedstock. The application of available genetic modification (GM) and non-GM technologies permits the crops yield to be improved and the conversion processes to be easier. The feedstocks (or their rest products) can be used directly as raw materials for bioprocessing, or be used as cheap substrates for fermentation processes from which products can be extracted (Solaiman et al., 2006). There are two types of biorefinery depending on the feedstock used. In the simplest system only one type of feedstock is used (for example grains) and one final product is obtained. The flexible biorefineries utilizes a mix of feedstock and as a result different compounds are produced. In order to achieve efficient conversion of the raw material, a mixture of mechanical, biocatalytic and chemical treatments have to be combined.

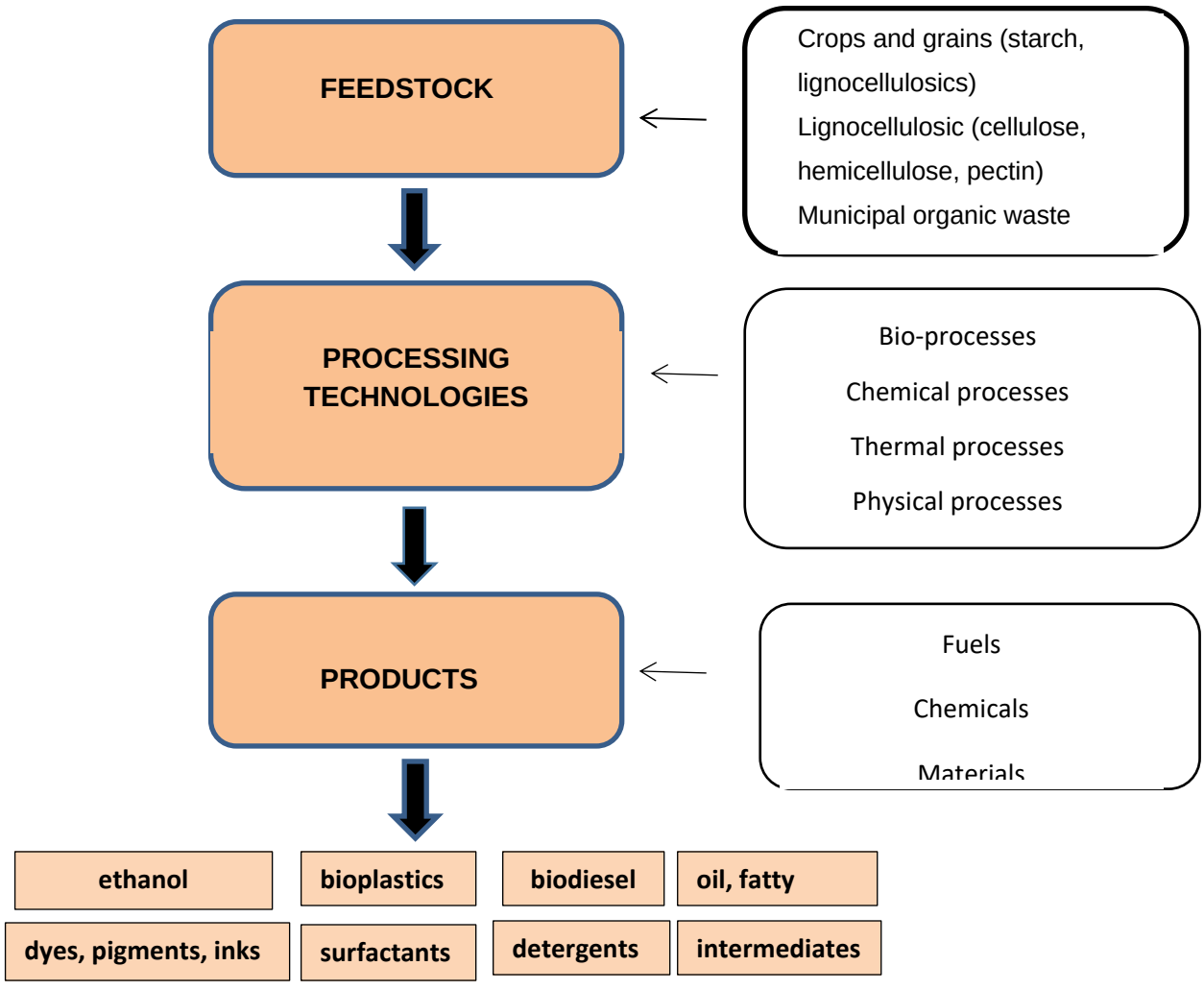

Fig.1 Schematic overview of the basic principle of a biorefinery, along with some product examples.

Biorefineries could be classified into three main groups according to the biomass used as a feedstock: starch and sugar (SSB), lignocellulosic (LCB) and triglycerides biorefineries.

The main steps which could be outlined in the SSB and LCB are:

- Pretreatment of the biomass by different methods in aim to remove lignin and hemicellulose and to disrupt the cellulose backbone. This will facilitate the enzyme hydrolysis on the next stage.
- Milling and following enzymatic hydrolysis of cellulose and starchy biomass with cellulases, amylases of microbial origin.
- Fermentation of the hexose-rich hydrolysate from starchy biomass by naturally occurring yeasts (usually Baker's yeast). The hydrolysate obtained from lignocellulosic feedstocks is more complex as it contains both pentose (xylose and arabinose) and hexose sugars (glucose, galactose and mannose). The fermentation of pentose sugars is challenging and costly as only a few yeast strains are available for fermentation to ethanol.

In the triglecyrides biorefinery vegetable oils and animal fats are used as feedstock for production of biodiesel. The production is based on transesterification of triglycerides with methanol and glycerol is obtained as a by-product.

2.3.2. Biomaterials

The demand for biodegradable polymers has grown at a rate of 20–30% per year (Carmichael H., 2006). Bioplastics have found uses in a variety of components of consumer electronics. They are used in connectors, PC housing, battery packages, chargers, mobile phones, portable music players and keyboards. The market segments include also gardening, packaging and flushable hygiene products.

A material is considered bio-based if it, or part of the raw materials used for its manufacture, is renewable. The bio-based versions of thermoplastics such as polyethylene and polypropylene have the greatest potential for market penetration.

Polyhydroxyalkanoate (PHA) belongs to the group of polyoxoesters, which together with other bio-green materials (polynucleotides, polyamides, polysaccharides, polyisoprenoids) are potential substitutes of synthetic plastics due to their similar physical characteristics. Poly (3-hydroxybutirate) most common known as PHA is a biologically –synthesized product and it is produced from sugars by various representatives of *Bacteria* and *Archaea*, which accumulate PHA in their cytoplasm as a storage material. It serves as carbon and energy source as well as a reservoirs of reducing equivalents for some microorganisms. It possess high thermal stability, as well as excellent biodegradability. Unlike synthetic plastics, PHA is degraded aerobically by microorganisms to CO_2 and H_2O. It has a very broad range of applications including molded products, films, foam, and fiber. PHA is synthesized by various representatives of *Bacteria* and *Archaea*, which accumulate PHA in their cytoplasm as a storage material. It serves as carbon and energy source as well as a reservoirs of reducing equivalents for some microorganisms.

1,3-Propanediol (PDO) is a bio-derived product which is obtained as a result of fermentation from corn sugar. The monomer is separated from the fermentation broth and then is available to be used in direct product formulations or as an ingredient in polymers. Its main application areas are cosmetics, personal care and home cleaning products. Efficient technology for the production of 1,3-PDO from crude glycerol is through a fermentation process with resting and immobilized cells of *Klebsiella* sp. (Wong, et al., 2011).

1,4-butanediol (BDO) is an important commodity chemical used to manufacture over 2.5 million tons annually of valuable polymers including polyesters, polyurethanes, co-polyester ethers, and other co-polymers. Traditionally it is produced exsclusively by feedstocks derived from oils and natural gas hydrocarbons. The need for bio-based BDO through a biotechnological process led to the development of technology in which the final product is produced from sustainable ingredients such as glucose, xylose, sucrose and biomass-derived mixed sugar streams. This technology is based on the utilization of genetically engineered *E.coli* strain that is capable of producing 18 g l^{-1} of this highly reduced, non-natural chemical. Genomatica's

technology also offers the potential to use a range of feedstocks, including conventional sugars, cellulosic biomass and syngas (Yim, et al., 2011).

Polylactic acid (PLA) is an aliphatic polyester which is built from the monomers of lactic acid (2-hydroxy propionic acid). At present this product has one of the highest potential due to its availability on the market and its low price. It is biodegradable and compostable thermoplastic derived from renewable plant sources, such as starch and sugar. This polymer is used in the production of food grade plastics – utensils, wrap, containers, packaging. According to reports, European demand for PLA is currently 25000 tonnes per year and could reach 650000 tonnes per year in 2025.

It is envisaged that future plastics would come from sugars, starch, cellulose and vegetable oils (Carmichael H., 2006).

Isobutanol is used as a solvent and as a feedstock for syntheses. Its various application includes: solvent for printers ink, extractant in the production of drugs and natural substances such as antibiotics, hormones, vitamines, alkaloids and camphor, additive in polishes and cleaners, e. g. floor cleaners and stain removers, solubilizer in the textile industry, e. g. additive in spinning baths or carrier for colouring plastics, additive in de-icing fluids etc.

The bacteria from genus *Clostridium* are recognized as natural and good butanol producers and are employed in the industrial-scale production of solvents. In recent years butanol pathways of *Clostridium* species are expressed in microorganisms such as *E.coli* and *Saccharomyces cerevisiae* due to difficulties in performing genetic manipulation with *Clostridia*. The butanol and isobutanol production in *Saccharomyces cerevisiae* is carried out by glycine conversion through the glyoxylate, β-ethylmalate and α-ketoacids intermediates. Using synthetic biology, scientists have engineered a yeast to concentrate on production of isobutanol by blocking production of ethanol and acetic acid (Atsumi et al., 2010).

Isoprene is a key organic compound in the chemical production of tires and rubber. It is produced almost entirely from petrochemical sources such as naphtha, oil or natural rubber decomposition. However these sources depleting progressively. A

reliable biological process for isoprene production utilizing renewable feedstocks will be an industry-redefining development. The enzyme isoprene synthase has only been identified in plants, but production strains of microorganisms are not efficient in expression of plant genes. Synthetic biology techniques allowed the construction of a gene that encodes the same amino acid sequence as the plant enzyme but is optimized for expression in engineered microorganisms. On this basis biotechnological company Genencore added to the *E. coli* a plant gene coding for isoprene synthase, an enzyme that converts the precursor directly into isoprene (Erickson et al., 2012).

Succinic acid is a key building block in the production of a large group secondary chemicals applied in the chemical, pharmaceutical food and agricultural industries. As an alternative to petroleum–based succinic acid is bio-based one, in which biotechnological production from raw materials metabolic engineering is used to construct organisms that make high-value, high-purity, renewable sugar-based succinic acid. It is produce through microbial fermentation of glucose and this product is cost competitive and offers superior functionality or performance with a better environmental footprint.

Acetic acid is used predominantly for the industrial production of film, bottles and fibers. The biotechnological company ZeaChem Inc., has produced bio-based acetic acid using naturally occurring organism. The bio-derived acetic acid is at the purity concentration level of the traditional product (Erickson et al., 2012).

2.3.3. Biofuels

Most energy demand is met by fossil fuels (oil, coal and natural gas). Current use of fossil fuels will not only deplete the world`s oil reservoirs but also have serious effect on the environment leading to increased health risk and global climate change (Panwar et al., 2010). The forecast for depletion of fossil fuels by the year 2100 and the problems with the global warming makes the need for alternative fuels solutions significant.

Interest in biofuel as an alternative fuel for transportation has increased hugely since 1980 (Balat, 2010). Biofuels are liquid fuels produced from biomass such as sugar cane, grains, agricultural residues, algae and household waste by the activity of microorganisms (Drapcho et al., 2008). They are used as substitutes of gasoline and diesel in the transportation and are the only existing liquid alternative to fossil fuels. They can be produced by different renewable materials such as plants and organic waste.

The large and diverse group of micro-algae attracted much attention in recent years because they can provide between 10 and 100 times more oil per acre than other second-generation biofuel feedstock and the resulting oil content of some micro-algae exceeds 80% of the dry weight of algae biomass, almost 20 times that of traditional feedstock. This facts determine their potential value as a renewable energy source. The storage lipids in these unicellular heterotrophic organisms in the form of triacylglycerols, can be used to synthesize biodiesel via transesterification. The remaining carbohydrate content can also be converted to bioethanol via fermentation. The advantages of using algae-derived fuels as an alternative are numerous. The micro-algae are highly productive, safe and biodegradable, but additionally experiments requiring algal improvements through genetic and metabolic engineering, to achieve higher growth rate, higher lipid content and easier extraction.

The most common biofuels are biodiesel, bio-ethanol and biogas**.**

Biodiesel is produced primarily from vegetable oil by the process of transesterification. Two categories of oils are utilized in the biorefineries. The first one include pure plant oil extracted from palm, soybean, rapeseed and sunflower seeds and its production is limited only by the agricultural conditions in the country. The second category includes the waste vegetable oil, for example cooking oil or animal fat, as the main disadvantage in their use is that an additional processes of refinement and hydrogenation are nessesary in order to become usable biodiesel.

Obtaining biodiesel from the inedible oils, such as from Jatropha Curcas tree, is being looked at by developing countries to meet their energy requirements.

Bioethanol is a liquid transportation fuel. More than 50% of bio-ethanol produced today is from corn and more than one-third from sugarcane by fermentation.

The aim of the scientists is to develop cost effective biotechnological process, and as a result to make ethanol more practical and competitive to replace the crude oil imports. USA and Brazil produce 90% of the bio-ethanol produced in the world, which counts for about 34 and 24,5 billion m^3, respectively, in 2009 (Crago et al., 2010). Usually, yeast has been used for ethanol production. However, metabolically engineered bacterial strains such as *Zymomonas mobilis* and *Escherichia coli*

have been developed to efficiently produce bioethanol (Sang Yup Lee, 2006). The process combines cellulose hydrolysis and fermentation steps in one vessel to produce high yields

The composition of biomass used for the production of biofuels varies to a great extent. Sugar and starch-rich biomass like corn and sugarcane are examples of easily degradable biomass that, upon hydrolysis, yield mostly glucose and sucrose. Lignocellulosic biomass has a more complex structure and thus requires additional pretreatment in the form of heat, strong acids or bases, or enzymes such as cellulases and hemicellulases (Kosaric et al., 2001). Conversion of starch / lignocellulosic material to sugars for subsequent fermentation to produce ethanol is receiving a lot of attention in terms of investment by both governments and industry. Since there are vast quantities of waste materials such as straw, corn cobs or even waste paper available, research is underway to modify microorganisms to use these as an efficient substrate, or to make enzymes which can cost-effectively break down cellulose to easily-fermentable glucose. Success here would greatly improve the overall economics of ethanol production.

Methane and hydrogen Biomass can also be fermented to produce methane or hydrogen. Either of these could be a partial replacement for natural gas. Among the various hydrogen production technologies, anaerobic fermentative H_2 production from organic wastes is considered to be an environmentally friendly and energy-saving biological process (Holladay, et al., 2009). For this process to be economically competitive, renewable and low cost feedstock should be developed to provide a cost-effective energy supply

Biomass derived energy, based on biotechnology, is expected to cover an increasing amount of world energy consumption (Surinder, 2013).

2.3.4. Fine chemicals and pharmaceuticals

Antibiotics, their intermediates and vitamins are among the most important fine chemicals, which are produced exclusively by fermentation with genetically improved microorganisms. Vitamin B12, which is very complicated compound could be synthesized only by fermentation in comparison to vitamin B2 and vitamin C that can be produced by either a chemical route or either a biotechnological route or more often as a combination of both.

2.3.5. Food additives and food supplements

At present almost all 20 L-amino acids are synthesizes through a biotechnological process – fermentation or enzyme technology. Amino acids are applied as food additives in human food and animal feed. Among them glutamic acid, lysine and phenylalanine are produced by very large scale-up productions. Glutamic acids is used in the form of monosodium glutamate as a taste enhancer in many food. L-phenylalanine is taking part in the production of L-aspartame, which is added as an artificial sweetener that is 200 times sweeter than sugar. Currently the synthesis of both monomers phenylalanine and aspartic acid is based strongly on industrial biotechnology. The two amino acids are then linked to one another through an enzymatic process with the help of bacterial enzyme thermolysine.

Bio-colorants are another important group of compounds that is increasingly produced through biotechnological processes especially when they will be applied in food, pharmaceuticals and cosmetics products. Such compounds are the carotenoids astaxanthine and zeaxanthine which are mainly used in fish and animal feed. The pink pigment astaxanthine was initially synthetically produced by a combined chemical and enzymatic process. At present, the tendency is going to a fermentation production with the help of a red yeast *Xanthophyllomyces rhodochrous.* The orange and red pigments synthesized by the fungus from genus *Monascus, Monascus purpureus* is industrially produced only by a fermentation technology (Soetaert and Vandamme, 2006).

2.4. Microbial biocatalysis

Biocatalysis is very widely used in many industries and for many applications. It can be defined as the use of natural enzymes to speed up the chemical reactions. Consumers use products that have been manufactured using biocatalysts, such as food ingredients, or products that contain biocatalysts, such as fabric washing powders. Environmental concerns help drive the use of biotechnology in industry, to not only remove pollutants from the environment but prevent pollution in the first place.

Biocatalyst-based processes have major application in this context. The biocatalysts produces less toxic waste, fewer emissions and by-products compared to conventional chemical processes. They can act as non-toxic catalysts in aqueous medium, exhibit high substrate specificity and enantioselectivity and at the same time yielding high product purity. They operate under moderate reaction conditions at near ambient temperature, pressure and pH, thus resulting in reduced energy consumption. Biocatalysts have the potential to prevent high consumption of metals and organic solvents. Although biocatalytic processes are often greener than chemical ones, for industry, ecological reasons are not the only subjects to be addressed for the replacement of an existing process. On the other hand, sometimes there are no chemical alternatives to a biotechnological pathway.

New biocatalysts with improved selectivity and enhanced performance for use in diverse manufacturing and waste degrading processes are becoming available. In view of their selectivity, these biocatalysts reduce the need for purifying the product from byproducts, thus reducing energy demand and environmental impact. Unlike non-biological catalysts, biocatalysts can be self-replicating (Gavrilescu, M. et al., 2005).

The stability of the biocatalyst is an important issue since the enzyme ideally needs to be reused in several repeated biocatalytic cycles. Immobilisation of the enzyme can often increase its stability and allow it to be easily recovered for reuse (Littlechild, 2015). The enzyme immobilization can be defined as physically attaching an enzyme molecule to a solid career matrix over which a substrate is passed and converted to product. This is a very old practice and the first immobilized enzyme is amino acylase isolated from *Aspergillus oryzae* for the production of L-amino acids in Japan. There are several advantages, which can be outlined for the processes with immobilized enzymes. The most important are: increased functional efficiency, reuse

again after the product have been removed, high substrate enzyme ratio, minimum reaction time, improved process control, cost saving and investment of the process. As serious disadvantages of enzyme immobilization can be pointed: loss of stability for some enzyme after immobilization, high cost for the isolation, purification and recovery of the active enzyme, limited industrial application.

The enzyme cost is often the most expensive component of the industrial biotransformation and must be matched to the value of the end product. Higher value optically pure compounds which are important as drug intermediates for the pharmaceutical industries will allow a high enzyme price. Other enzymes that are required for the production of bulk chemicals, used as additives for domestic cleaning products, used in food production, or used to supplement biomass degradation processes, generally need to be marketed at a cheaper price and supplied in larger quantities.

Useful information on commercially available enzymes can be searched electronically in a variety of ways via the Enzyme Explorer (http://www.sigma-aldrich.com/enzyme explorer/.

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