INDUSTRIAL BIOTECHNOLOGY

J. M. Bruno-Bárcena
Department of Microbiology and Bio-manufacturing Training & Education Center, NC State University, USA

F. Siñeriz
Institute of Microbiology, University of Tucumán, PROIMI, Argentina

Keywords: Alcohol production, Biocatalyst, Biofilms, Biological agent, Biomaterial, Bioproduct, bioremediation, Biosensors, Bioaumengtation, Centrifugation, Chemostat, Citric acid , Cross flow microfiltration , downstream processing , Ethanol , Fed-batch cultures, Filtration, Food protection, GMP , Good Manufacturing Practices, Growth-linked products, Human growth hormone, Insulin, Land farming, Lactic acid, Metabolic engineering, Microarrays, Microbial community, Microbial enzymes, NIH guidelines, Non growth-linked products, Nutraceuticals, Organic acids, Pharmaceuticals, Postranslational modifications, Recombinant organisms, Saccharomyces cerevisiae, Scaling up, Separation steps, Validation, Viable but non culturable, Waste water treatment

Contents

Summary

The Organization for Economic Cooperation and Development (OECD) defined Modern biotechnology in 1982 as "the application of scientific and engineering principles to the processing of materials by biological agents, and the processing of

biological materials, to improve the quality of life." Industrial biotechnology takes over the rich inheritance of Industrial Microbiology, to which the modern tools of Molecular Biology are applied. Its language is both the language of the classical chemical engineer and of the biologist. Most of the processes involve microorganisms, though some advancement in the use of mammalian or plant cells are described. Industrial Microbiology covers the production of foods and beverages such as yogurt, soy sauce, beer, wine, vinegar to name the simpler; fuels such as ethanol or methane, to the more sophisticated bioactive molecules of human origin produced by recombinant microorganisms or cells as is the case of pharmaceuticals like human insulin or growth hormone. Wastewater purification plants and bioremediation of contaminated sites can also be included as industrial biotechnological processes. A biotechnological process consists of several interconnecting stages, ranging from the transforming agent to the conversion step and the downstream processing of the material.

1. Definition

Simply defined, biotechnology is the domestication of cells for the production of bioproducts or transformation of biomaterials. Biotransformation is probably one of the oldest uses of microorganisms made by man, for example, the preservation of various perishable foods by means of fermentation. It is interesting to note that many new applications, for example, in probiotics, are possible through the study of traditional food and beverage preparation in villages. It is probably true that the birth of biotechnology about 6000 years ago was associated with the birth of commercial practices like product trade. The influence on commerce persisted trade through ages and, in this context, biotechnology is not only a business explored by commercial enterprises, it is also an activity with a considerable influence on the development of the world economy. OECD defined modern biotechnology in 1982 as "the application of scientific and engineering principles to the processing of materials by biological agents, and the processing of biological materials, to improve the quality of life." However, it must be appreciated that biotechnology not only produces technological advances as a way to obtain direct economic benefits, but also has the weapons for an adequate control of public safety against disease. In this sense, biotechnology can also be used for the detection and characterization of pathogenic agents. The methods used to validate sterilization processes with biosensors or by inactivation of active molecules could be classed in this field. Biotechnology is also food safety, and the products obtained from these technologies can now improve the quality of foods, the final quality of pharmaceutical molecules and replace traditional empirical practices, introducing reproducibility and prediction with increasing confidence.

Even if biotechnology involves so many areas of application, as we have seen here, for the purpose of this article, biotechnology will be considered as the use of organisms or their components, in order to achieve technological (Industrial) applications and produce services and obtain useful and/or pure products, using methods from microbiology, biochemistry and engineering. Industrial biotechnological processes will be the final solutions to a specific problem, using the knowledge gathered on the biological systems, and the know how of engineering processes. Industrial Biotechnology comprises the application, mostly of microorganisms, at different production scales ranging from bench scale production for certain active biomolecules

to large plants with fermenters of several hundred cubic meters, as is the case of the production of beverages such as beer, alcohol for fuel or the classic wastewater treatment plants.

2. History

Biotechnology began with artisan and empirical practices and the cultural traditions made them practically available for the community. Though the classical fermentations associated with food and beverage production were known since the dawn of civilization (see also *Production of Alcoholic Beverages*), it was not until the beginning of the nineteenth century that Antoine Laurent de Lavoisier and Louis Joseph Gay-Laussac described the stoichiometric conversion of sugar into alcohol and carbon dioxide. It was the work of Friedrich Traugott Kuetzing, Charles Cagniard de Latour and Theodor Schwamm in the 1830s that showed that the presence of yeast and the alcoholic fermentation were correlated, though it was Cagniard de Latour who proposed in 1837 that the alcoholic fermentation was produced by yeast. However, the results were not believed and it was not until Louis Pasteur, who repeated the experiments by Schwamm, concluded that the growth of yeast was responsible of ethanol formation ("La fermentation est la vie sans l'air"). He also described the lactic, the butyric and the acetic acid fermentations (see also *Production of Organic Acids*), other important food related fermentations, and so it was about that time, with the foundation of microbiology that the relation between agent and product was established. This gave birth to industrial microbiology, the predecessor of what is now called Industrial Biotechnology. The need to develop the industrial production of the few compounds obtained via fermentation led to the development in the engineering sciences to cope with the common phenomena such as mass transfer, aeration (in aerobic processes), product separation, drying, distillation, and so on. (see also *Methods in Biotechnology*). All this body of knowledge gave support to Industrial Microbiology. Until 1950 not many products were obtained at industrial scale via fermentation, e.g. the solvents acetone, butanol, and ethanol, the gas methane, and the organic acids acetic, lactic, gluconic, oxalic and citric acid. The large antibiotic industry started about that time with fungi and Streptomyces (see also *Production of Antibiotics*), and the pace of application accelerated until the more modern developments of transgenic human compounds production of which insulin was the first example in 1990.

Industrial biotechnology is the heir of the rich chapter of industrial microbiology to which the modern tools of biotechnology, that is, molecular biology and genetic engineering are incorporated (see also *Methods in Gene Engineering*). These tools, first developed in public academic laboratories, were rapidly taken by industry, either by the traditional pharmaceutical or veterinary related industries producing antibiotics or vaccines or newly created companies mostly of academic origin. At the same time the development of many service companies occurred, which introduced and are continuously introducing, a vast array of new tools in the form of ready to use kits and consumables, for the rapid development of industrial and academic research programs. Nowadays, applications developed in industrial R&D (Modern Biotechnology) are being utilized by an increasing number of large and small companies. The real power engine of biotechnology is the search of new applications that would allow the establishment of industrial processes for the development of new products arising from

the knowledge of biological systems and also, the search of new solutions to the older industries, enhancing quality and productivity. It must be taken into account that the marketing of new products in biotechnology will depend not only on scientific and technological advance, but will also be subject to considerable political and economic pressure, as well as other matters, such as public perception and acceptance. This latter aspect is particularly critical in the case of genetically modified plants for food production.

Traditionally, man learned to isolate organisms from their natural environments, control the environmental conditions and select new genetic variants able to ensure better industrial results. In the present times, man is able to do directed modifications to the genetic information pattern of the cells using the recombinant DNA technology and opening new frontiers for product development. It must be appreciated; in fact, that biotechnology developed itself through thousands of years and it is just now entering into a golden period where the commercial or industrial biotechnology could change inadequate cultural practices to greatly improve the quality of human life. But biotechnology itself cannot be the final answer to all world problems, though countries with climatic conditions suitable for rapid biomass growth could well have major energetic advantages over less climatically suitable parts of the world.

The successful occurrence of a biotechnological process involves a series of steps carried out to obtain the purest product from the overall biotechnological process, and, of course, must be commercially viable. A biotechnological industrial development (Figure 1) must go through three fundamental stages and their particular problems:

1. The first stage is the search for the best transforming agent or biological catalyst;
2. The second one is the development of the environmental conditions that allow the best performance of the transforming agent; and
3. The third, downstream processing, or the processing steps necessary to attain a useful process and/or a pure product.

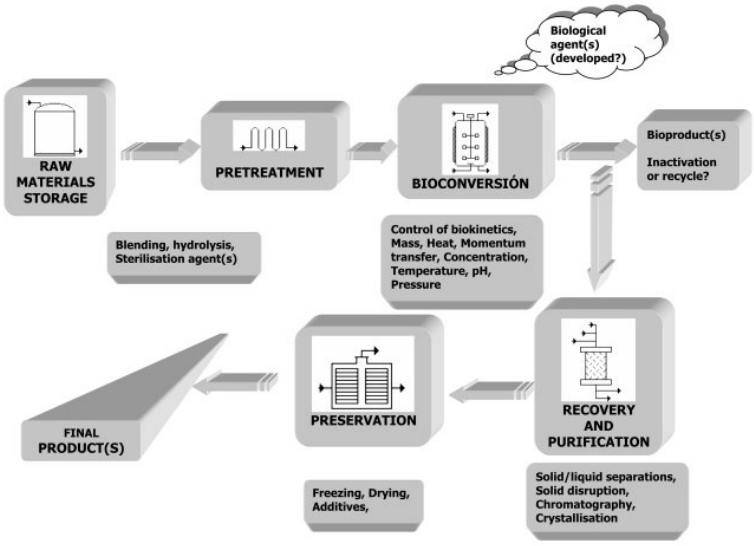

Figure 1: Schematic view of a generic biotechnological process

These stages are not independent but interact with one another, so the general picture of the whole possible process must be taken into account while developing each of the steps.

3. The Best Biological Agent

In industrial biotechnology, the most typical element utilized as a catalyst to obtain a biomolecule or to produce a transformation is a living organism or a community of microorganisms. In fact, microbial cells can be considered as bioreactors directed to their own perpetuation and/or to the production of biomolecules ranging from the most simple to complex polymers, for example, cells are organized factories controlled by their own genetic information. Some proteins cannot be produced in bacterial or yeast cells, mainly because some recombinant products (proteins) require postranslational modifications to result in an active molecule. In this case other cell types are used, mainly mammalian cells. The nature of the host cell both determines the nature of the recombinant product and the nature of the process itself (see also *Industrial Recombinant Protein Production*). There are constraints for the use of cell lines, which are different from those required for bacterial cells, especially in the nature of the culture media, the ability to withstand shearing and agitation, and the productivity. Though able to produce larger molecules, and to produce them with the appropriate postranslational modifications, the productivity is usually much lower than in the case of prokaryotes or yeast, mainly due to the small growth rate. So sometimes it is a better strategy to perform some steps for obtaining the active compound outside the cells, either chemically or enzymatically.

-
-
-

Bibliography

Barns S. M. and Nierzwicki-Bauer S. A. (1997). Microbial Diversity in Ocean, Surface and Subsurface Environments. (Banfield J. F. and Nealson K. H eds., Geo-microbiology: Interactions Between microbes and minerals). *Reviews in Mineralogy* 35, 35–79. Washington, DC: Mineralogical Society of America. [This is a very interesting paper on the capabilities of microbes for biotechnological purposes.]

DaSilva E. J. (1987). Microbial technology and the developing countries-an introduction. *Microbial Technology in the Developing World*. (DaSilva E. J., Dommergues Y. R., Nyns E. J., and Ratledge C. eds.). Oxford and New York: Oxford University Press. [An overall discussion, at an early stage, on the possibilities of the developing countries in biotechnology.]

DaSilva E. J. (1998). Review: Biotechnology: developing countries and globalization. *W.J. Microbiol. Biotech.* 14, 463–486. [Advantages and challenges offered by globalization to developing countries in the area of biotechnology.]

Demain A. L. and Davies J. (2000). *Manual of Industrial Microbiology and Biotechnology*. (Second

Edition). Washington, DC: ASM Press. [An excellent and up to date work all the various aspects of industrial biotechnology.]

Eking R. and Chu F. W. (1999). Microarrays: their origins and applications. *Trends in Biotechnology* 17, 217–218. [An approach to the use of microarrays.]

Hols P., Kleerebezen M., Schanck A. N., Ferain T., Hugenholtz J., Delcour J., and de Vos W. M. (1999). Conversion of *Lactococcus lactis* from homolactic to homoalanine fermentation through metabolic engineering. *Nature Biotechnology* 17, 588–592. [Example of the use of metabolic engineering for process development.]

Kieran P. M., Malone D., and MacLaughlin P. F. (2000). Effect of hydrodynamic and interfacial forces on plant cell suspension systems. *Advances in Biochemical Engineering/ Biotechnology*, 67 (Schügerl K. and Kretmer G. eds.), 139–177. Berlin, Heidelberg, New York, and Tokyo: Springer. [Comprehensive treatment of plant cell cultures in suspensions.]

Lydersen B.K., D'Elia N. A., and Nealson K.L. (1994). *Bioprocess Engineering, Systems, Equipment and Facilities*. New York, Chichester, Brisbane, Toronto, and Singapore: John Wiley and Sons. [A comprehensive treatise on equipment used for biotechnological processes, including validation of the facilities.]

Robinson C. (1999). The genetics of industrial microorganisms: the first half century. *Trends in Biotechnology* 17, 178–181 [An overview of the main advances in the genetics of industrial organisms.]

Schlegel H. G. (1999). Geschichte der Mikrobiologie. *Acta Historica Leopoldina*, Vol 28. Halle (Saale): Deutsche Akademie der Naturforscher Leopoldina. [A magnificent history of Microbiology written by one of the contemporary giants of European microbiology]

Rose A. H. (1978). Production and industrial importance of primary products of microbial metabolism. (Rose A. H., ed. Primary Products of Metabolism) *Economic Microbiology* 2, 1–30. New York: Academic Press. [Excellent work on the capabilities of microorganisms for the production of bulk products by fermentation.]

Smith J. E. (1996). An introduction to biotechnology. In Smith J. E. ed., *Biotechnology*. UK: Cambridge University Press. [A concise treatise on biotechnology.]

Spencer J. F. T. and Spencer D. M. (1994). Biodiversity and the Isolation of new industrially useful Microorganisms from nature. *Microbes For Better Living* (Sankaran R. and Manja K. S. eds.), Micon-94 and 35th AMI Conference, 9–12. India: Bangalore Printing and Publishing Company Ltd India [Use of the microbial biodiversity for new products and processes.]

Biographical Sketches

Faustino A. Siñeriz graduated at the University of Buenos Aires in 1965 and received his Ph.D. in 1973 at the same University. From 1974 to 1977 he did post doctoral studies at Queen Elizabeth College, University of London, with Prof. John Pirt and at the New York State Health Department in Albany. He was an Alexander von Humboldt fellow at the University of Konstanz, Germany, in 1984-1985. He held several positions at the University of Buenos Aires, University of Cordoba and University of Tucumán, where now is Professor of Microbiology. In 1978 entered the Research career in CONICET and since 1986 is Director of PROIMI, a research institute from CONICET specialized in fermentations and microbial biotechnology. His research interests include microbial physiology applied to biotechnological processes, continuous culture, bioremediation and wastewater treatment. He is actively advising pharmaceutical companies on growth and fermentation problems of both, recombinant and natural strains. He has participated as author or coauthor in more than 90 scientific publications in international journals. He is a fellow of the American Academy of Microbiology since 1998.

José M. Bruno-Bárcena graduated as a Biologist at University of Oviedo, Spain, in 1991. From 1994 to 1999, he worked at the Pilot Plant for Microbial Processes (CONICET-PROIMI) and successfully completed his Ph.D. in 1997 at the University of Tucumán, Argentina. From 1999 to 2002, he was a researcher at the Institute of Agrochemical and Food Technology (IATA) in Valencia, Spain. There, he assembled the fermentation and process development capabilities of the center, being also responsible for managing the technical operations of the laboratory, including resource and personnel management. He

developed processes for pharmaceutical companies and actively promoted IATA´s capabilities to produce custom fermentations for third parties in Europe. From 2002 to 2005, at North Carolina State University, he served as a researcher studying the molecular responses of microorganisms to reactive oxygen species. In 2005, NC State University appointed him as Research Assistant Professor to serve in the Biomanufacturing Training and Education Center. Since then, he has developed bioprocessing related courses and provided the expertise that led to establishment of the process development BTEC laboratory. Presently, he manages daily operations while actively serving in the Microbiology Department, his primary appointment. His research interests are microbial physiology, including process development and optimization of cell culture. He has written over 15 publications in peer-reviewed journals and books.