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BIOTECHNOLOGY ADVANCES

Conference Report

Biotechnology, bioprocessing and bioengineering: Who is doing what and why September 25–26, 1998; Ohio University, USA

This 2-day unique symposium–retreat was held for "brainstorming among representatives from industry, government, and academia." The rational for the meeting was given as follows: "In recent years, biotechnology has become increasingly multidisciplinary in its demands and incredibly rapid advances have been made. . . . There is a need for some introspective reflections." As the meeting title implied, a wide spectrum of topics was covered. Speakers, several international luminaries, were predominantly from the United States. Abstracts of the 25 presentation are given below.

1. Risk assessment/functional genomics

Michael L. Shuler. Department of Chemical Engineering, 270 Olin Hall, Cornell University, Ithaca, NY 1483-5201.

Biochemical engineering principles can be applied to emerging areas such as risk assessment and functional genomics. Advances in tissue engineering are providing the basis for in vitro assessment of toxicity of chemicals or pharmaceuticals. By combining physiologically based pharmacokinetic (PBPK) models with devices with interconnected "organ" compartments in vitro, cell culture analogs (CCA) of humans or animals can be constructed. When PBPK and CCA are used together, molecular-level mechanisms can be related to whole animal response. When cellular responses can be linked to the genome, one can link genetic sequenced information to whole animal response. Some of the challenges in relating linear sequence information to cell response will be described.

2. Inverse-growth-associated production (is better than growth-associated and non-growth-associated productions) for maximizing bioreactor productivity

Dhinakar S. Kompala. Department of Chemical Engineering, University of Colorado, Boulder, CO 80309.

Starting with the work of Leudeking and Piret, the production kinetics associated with microbial growth have been typically known as growth-associated and/or non-growth-associated. However, some of the more successful biotechnology products (e.g., antibiotics and

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monoclonal antibodies) are found to be synthesized in inverse-growth-associated production kinetics, which have been successfully exploited to maximize the bioreactor productivities in fed-batch and perfusion bioreactor cultures. Our recent work with recombinant mammalian cells shows that it may be possible to change the production kinetics from the undesirable growth-associated to the more desirable inverse-growth-associated pattern through simple molecular biology techniques. Thus, we advocate strong interaction between the bioprocess engineers and molecular biologists to maximize the large-scale production in high cell density fed-batch and perfusion bioreactors.

3. Extractive membrane bioreactors

Andrew Livingston. Department of Chemical Engineering, Imperial College, London SW7 2BY, England.

Extractive membrane bioreactors (EMB) comprise systems in which a selective extraction across a membrane is accompanied by a biological reaction on one side of the membrane. This concept has been developed for use in waste treatment and biotransformations for production of chiral intermediates. In the waste treatment area, the EMB allows application of specialised microbial cultures to degradation of toxic organics present in chemical industry wastes. The process has been scaled up in the United Kingdom to treatment of several thousands of tons per year of 30% $AlCl_3$ solution contaminated with benzene. For biotransformations, whole cell systems for production of R-citronellol and 1-epoxy-7-octene have been developed, where the EMB allows emulsion-free operation of biphasic reaction systems. These applications will be described and fundamental research issues will be highlighted.

4. Metabolic engineering—where are we going?

James C. Liao, Department of Chemical Engineering, University of California, Los Angeles, CA 90095-1592.

Since 1980s, metabolic engineering has become an increasingly popular subject of research in both academia and industry. The next challenges are (1) to redesign the regulatory systems to control the engineered pathway, and (2) to construct new pathways for production of novel compounds. In the first area, we employed DNA binding proteins that are modulated by specific metabolites to served as internal sensor and controllers. By use of these proteins, we can control the expression of key genes in the engineered pathway in response to physiological conditions. In the second area, we are using genomic techniques to search for new genes that are involved in isoprenoids biosynthesis. By overexpressing isopentenyl diphosphate isomerase and geranylgeranyl diphosphate (GGPP) synthase, we constructed an Escherichia coli host that can produce astaxanthan 50 times higher than literature value.

5. Photosynthetic bioreactors

Stanley M. Barnett. University of Rhode Island, Chemical Engineering Department, Kingston, RI 02881-0805.

The growth and metabolism of photosynthetic algae are strongly influenced by light. Scale-up of photobioreactors, particularly for pharmaceutical applications, present unique problems. A combination of light levels, energy, and wavelength of radiation, agitation, and aeration affect the growth rates. Blue and red wavelengths appear to have a combined effect on growth rates. Growth can be modeled using light as a limiting substrate. Deactivation energy has been used as a measure of inhibition at high light intensities. Agitation does not have a significant effect on the growth rates in the light-limited region, while an increased growth rate was observed in the light-saturated region.

6. Computer-aided biomolecular modeling

Bhavin V. Mehta. Department of Mechanical Engineering, Ohio University, Athens, OH 45701.

The bovine growth hormone (bGH) and the human growth hormone (hGH) have been modeled using a three-dimensional protein modeling software package developed in-house. The graphical package was developed using the engineering modeling system and a C language interface on the CAD/CAM system and coupled with the Charmm21 molecular-modeling program to perform energy minimization and molecular dynamics. 12 different mutations in the third alpha helix of the bGH and hGH have been studied using computer modeling and the results have been compared to the experimental results obtained by the Edison Biotechnology Institute. The three-dimensional structure of the mutated sequence was predicted using statistical and neural network algorithms developed in-house. This research was performed in the biomolecular modeling facility at Ohio University, which utilizes a CAD system connected to the Ohio Supercomputer. The research is part of the ongoing developments in the field of biomolecular engineering and drug design at Ohio University in collaboration with the Chemical Engineering and the Biotechnology Departments. The interdisciplinary research efforts are focused on the design and production of recombinant molecules through a structure/function approach. The initial efforts have focused on the design, production, and biological evaluation of bGH and hGH analogs.

7. Solid phase fermentation

George T. Tsao. Department of Chemical Engineering, Purdue University, West Lafayette, IN 47907.

Fungal mycelia grow naturally on moist solid substrates. In the laboratory, several strains have been found to grow and to produce extracellular hydrolytic enzymes very well. Scale-up of solid phase fermentation, however, has not been easy. Heat dissipation and oxygen transfer in porous packed beds of moist solid substrates are limited. Heat released from the biological activities can increase the temperature of the porous beds to an elevated level detrimental to cell growth and can stop the fermentation prematurely. Mycelia often form large-sized aggregates with the solid substrates, and oxygen starvation in the interior of the aggregates is also a serious problem. A method of resolving such technical difficulties will be suggested and discussed.

8. Bioprocess control

M. Nazmul Karim. Colorado State University, Chemical and Bioresource Engineering Department, 100 Engineering South, Ft. Collins, CO 80523.

This presentation deals with recent developments in bioprocess control and modeling. Biological systems, because of their inherent nonlinear and time-variant behavior, are difficult to model, and as a consequence, appropriate control of state variables is difficult to achieve. Estimation of various unmeasured or infrequently measured variables, such as concentration of either excreted or intracellular recombinant protein, is key to optimize a cultivation process. Quite frequently, optimum-feeding policy of a fed-batch cultivation process may be a dual-feeding operation, where one needs information about unmeasured variables such as cell viability or glucose and glutamine (for mammalian cell culture) concentrations. In recent years data-based modeling such as Neural Networks and PCA have been used successfully to generate estimations of off-line measured variables, thus giving rise to accurate "soft-sensing." However, it is feasible to incorporate into the neural network existing knowledge about a given bioprocess, through mass and energy balances with appropriate reaction kinetics, to obtain a better predicting model. This presentation highlights some of the new developments in this area of bioprocessing.

9. Fibrous-bed bioreactor for fermentation and cell culture

Shang-Tian Yang. The Ohio State University, Chemical Engineering Department, 140 West 19th Ave., Columbus, OH 43210-1180.

A fibrous-bed bioreactor (FBB) has been developed for various bioprocessing and biotechnology applications. This new bioreactor overcomes many problems associated with conventional immobilized cell bioreactors, including membrane and hollow-fiber bioreactors. FBB has been successfully used to continuous microbial fermentations and animal cell cultures, including ethanol and recombinant GM-CSF production with yeasts, lactate, acetate, and propionate production with bacteria, monoclonal antibody production with hybridoma cells, and recombinant protein production with human osteosarcoma cells. Superior results—more than 10-fold increase in productivity and up to 1-year stable continuous operation—have been obtained with the FBB. FBB was also successfully used to adapt cells to tolerate high concentrations of inhibiting fermentation products or toxic substrates. In propionic and acetic acid fermentations, the final product concentration obtained in FBB was two times that of conventional fermentation processes. FBB also showed advantages in biotransformation for steroid drug production from sterols, viscous xanthan gum and mycelial mold fermentations, and bioremediation of hazardous chemicals. FBB also has been tested for cultivating primary luteal cells and showed sustained cell ability to produce progesterone at a higher level for a longer period than cultures in the conventional systems. This improvement is believed to be largely attributed to the unique three-dimensional fibrous matrix used in the reactor, which helps to maintain the morphological and physiological integrity of normal cells. FBB also was used successfully for long-term culturing of human trophoblast cells in our recent effort to develop an artificial placenta for drug screening and tox-

icology studies. Engineering fundamentals important to the design and scale-up of the FBB also will be discussed in this paper.

10. Biochemical engineering in China

Zhongyao Shen. Department of Chemical Engineering, Tsinghua University, Beijing 100084, China.

Biochemical engineering is a relatively new field in China and has been rapid development in the past decade. There are more than 30 universities that created a biochemical engineering major or similar majors, offering related courses. More than 1,000 undergraduate students and about 300 master- and doctoral-degree students enter universities and institutes in China with biochemical engineering majors every year. Academic research in biochemical engineering is supported through several channels, which are distributed over more than 100 universities and institutes of China. The major topics of research projects and main professors in the field of biochemical engineering are introduced in this talk. Finally, the future of biochemical engineering in China is discussed.

11. Bioprocessing in Europe

Spiros N. Agathos. Unit of Bioengineering, Catholic University of Louvain, Louvain-la-Neuve, Belgium.

Bioprocessing is alive and well in Europe, both as industrial practice and as R&D activity. This talk will address the progressive recognition of the discipline of biochemical engineering in European industry and academia. The place of biochemical engineering in the closing Framework IV Programme and the forthcoming Framework V Programme that funds transnational research of the European Union will be discussed. The concept of the cell as a factory, together with an increasing emphasis on an integrated multidisciplinary approach toward solving important problems of the modern bioprocess industries, will be highlighted through selected examples of precompetitive research and demonstration projects.

12. Biotechnology in Mexico

Mayra de la Torre. Department of Biotechnology, Cinvestav, P.O. Box 14-740, Mexico City, Mexico.

The four leading research groups are involved in health care, environment, agriculture, tissue plant culture, protein engineering, and biocatalysis (http://148.247.12.108/SIMBIOBAK.htm). Health care includes rDNA products, diagnostic kits, and cancer. Environmental biotechnology is focused on sewage treatment, gas biofiltration, and soil bioremediation. Research on agriculture includes development of improved crops and biological control of pest and fungi. Biocatalysis and protein engineering studies are dedicated to industrial enzymes. Bioremediation and biofiltration are promoted by the Mexican Institute of Oil, while research on improved vegetables and biocontrol is funded by farmers and companies. Emphasis is on iso-

lation of native strains, strain improvement through molecular biology, field testing, and bioprocess engineering. The most important achievements and bottlenecks will be discussed.

13. Bioprocess simulation in teaching and research

Charles L. Cooney. Department of Chemical Engineering, Massachusetts Institute of Technology, Cambridge, MA 02139.

An important activity in the process of commercialization of biotechnology is design of a process to manufacture high quality products at an appropriate cost. The challenge of process design is found in a combination of process synthesis (that is, the selection and sequencing of unit operations) and process analysis, which tells us how well the sequence is performing. An understanding of both activities is essential in process development and manufacturing. Furthermore, process design is especially important in teaching as a unifying framework for bioprocess engineering. Using case examples for production of biotherapeutics, the integration of process design, through process simulation, will be illustrated for research and teaching.

14. Growth hormone antagonists

John J. Kopchick. Edison Biotechnology Institute, Ohio University, Athens, OH 45701.

Employing a structure/function approach to the understanding of the molecular topology of growth hormone (GH), we discovered that glycine 119 of bovine GH (glycine 120 of human GH) was an amino acid required for GH activity. Substitution of this amino acid with a variety of amino acids resulted in molecules that lacked growth-promoting activity. Surprisingly, these molecules inhibited the action of GH in vitro and in vivo. These results were the basis for the discovery of GH antagonists. GH antagonists may have clinical significance, especially in situations where endogenous GH levels are elevated.

15. Process development for production of therapeutics

Sadettin S. Ozturk. Bayer Corporation, Biotechnology, 4th and Parker Streets, Berkeley, CA 94701.

Mammalian cell culture technology is now widely used as an established production method for the production of various biologicals of industrial importance. This talk will address the utilization of biochemical engineering fundamentals and novel engineering approaches for process development and process control for commercial cell culture production. Special attention will be given to high density cell culture systems as they offer cost-effective production based on high volumetric production rates. We discuss common techniques for cell line and medial development, cell cultivation and perfusion operation, process monitoring and control, scale-up, purification, validation, and product characterization.

16. Intelligent biomanufacturing at the University of Michigan

Henry Y. Wang, Mark A. Burns, Jennifer Linderman, and David Mooney. Department of Chemical Engineering, The University of Michigan, Ann Arbor, MI 48109.

A new graduate research and training focus in "Intelligent Biomanufacturing" has been proposed at The University of Michigan. This is based on modification and expansion of the traditional biochemical engineering curriculum. Intelligent biomanufacturing is defined as use of the fundamental understanding of biological and biochemical systems, transport processes, and reaction and separation systems to intelligently design and produce molecules, cells, tissues, biomaterials, and/or devices that are compatible to an environmentally sustainable society. Specifically, we will educate our students to intelligently design and evaluate: biomolecules and biomaterials to manipulate cells, tissues, and/or organisms; cells and tissues to produce biomolecules and biomaterials or to perform some other biological function(s); assays or sensors to quantify molecules, cells, and/or tissues and biomaterials; environmentally sustainable processes to produce all of the above.

The graduates of this program should possess the necessary skills and knowledge for an ever-changing "life science" industry that encompasses the traditional chemical and natural resource companies, as well as the new pharmaceutical and biomedical/biomedicine companies. The necessary skills include the use of modern cell and molecular biology in addition to engineering, automation, and information technologies.

17. Reversible aphrons in multiphase biocatalysis

Mark Worden. Department of Chemical Engineering, Michigan State University, 2527 Engineering Building, East Lansing, MI 48824-1226.

In biocatalytic processes involving two or more immiscible fluid phases, interphase mass transfer is often the rate-limiting step. Mass transfer can be enhanced by dispersing the second phase as aphrons (surfactant-stabilized microbubbles or droplets) in the continuous aqueous phase. However, the stability imparted by the surfactant hinders coalescence and phase separation after the reaction is complete. A new class of polymeric emulsifiers has been developed with emulsifying properties that can be switched on or off by a small change in pH. Dispersions formed using these emulsifiers can be stabilized as long as desired and then rapidly coalesced on demand. Biocatalytic applications of these reversible aphron dispersions will be discussed.

18. Recovery, concentration, and activity tradeoffs of protein foam fractionation

Robert D. Tanner. Chemical Engineering Department, Vanderbilt University, Nashville, TN 37235.

Our study in optimizing a protein foam fractionation process is focused on three measured responses: the separation ratio (SR), the protein recovery, and the activity if the protein is an enzyme. Two systems will be presented to illustrate the trade-offs in these responses to changes in pH, superficial air velocity (V_0), and initial protein concentration. It was observed that the SR and protein recovery are maximized near the isoelectric point (pI = 4) for egg albumin. On the other hand, the recovery, SR, and enzymatic activity are far from the pI of 4 (around pH 9–10) for the cellulase protein complex. It will also be shown that protein mixtures such as the storage protein sporamin and b-amylase in sweet potato pulp can be fractionated using step changes in pH and V_0.

19. Downstream processing centered on chromatography

Tingyue Gu. Department of Chemical Engineering, Ohio University, Athens, OH 45701.

The cost of downstream processing far exceeds that of upstream processing. Modern biopharmaceuticals require very high purity. The design of a downstream process should focus on one or two high resolution chromatographic steps. The steps before the high resolution chromatography are intended to reduce the feed volume and to remove the majority of the impurities. They enable the more expensive and fragile high resolution chromatography to achieve the desired purity with reasonable capital investment and operational costs. The steps after the high resolution chromatography are usually the polishing steps. An example of the purification of a growth hormone antagonist will be used to explain the design philosophy.

20. Training in FDA regulatory issues

Antonio R. Moreira. University of Maryland, Baltimore County Department of Chemical and Biochemical Engineering, 1000 Hilltop Circle, Baltimore, MD 21250.

Compliance with Good Manufacturing Practices (GMPs) requires that employers institute a comprehensive training program that focuses on both regulatory and technical issues. To assist companies in meeting these requirements and to provide our regular students with a working knowledge of these issues, we have developed a four-course certificate program that concentrates on regulatory issues, GMP requirements, quality control aspects, and facility design issues associated with biopharmaceutical manufacturing. Additionally, we have developed an interactive CD-ROM-based training program that companies can utilize for in-house self-paced training. This presentation will highlight our current FDA-related and GMP training programs. Plans for future offerings will be discussed as well.

21. Plant cell culture for the production of exudate gum glycoproteins

Marcia Kieliszewski. Department of Chemistry and Biochemistry, Ohio University, Athens, OH 45701.

Plants exude gum sealants as a wound response. One of the best characterized of these exudates is gum arabic, which has wide commercial use as an emulsifier, bulking agent, crystallization inhibitor, and stabilizer. Increasing demand for gum arabic tends to outpace an uncertain supply of the crop, which is inefficiently grown and harvested from thorny desert plants in an unfriendly African state. As such, there is much interest in alternative sources of the gum. Our approach uses tobacco and tomato cell suspension cultures for transgenic of gum arabic glycoprotein analogs.

22. Achieving economic feasibility with plant tissue culture technology

Wayne R. Curtis. Department of Chemical Engineering, The Pennsylvania State University, 108 Fenske Laboratory, University Park, PA 16802-4400.

The talk will cover three aspects of utilization of plant tissue culture as a biological catalyst. First, we are completing a series of design and scale-up studies that indicate feasibility of high density industrial-scale culture of plant roots. A few slides will summarize critical studies of fluid mixing, as well as liquid and gas phase flow resistance in high density root culture. Recent progress in utilizing green fluorescent protein to monitor gene regulation in sesquiterpene secondary metabolite pathway will also be presented. Finally the impetus for discussion will be our recent demonstrations of the utility of low-capital investment reactors for pilot to large-scale aseptic biological processing.

23. Transgenic animals as bioreactors

Kevin E. Van Cott. Pharmaceutical Engineering Institute, Department of Chemical Engineering, Virginia Tech, Blacksburg, VA 24061.

The utility of transgenic animal bioreactors for the production of complex therapeutic proteins is based on lower production costs, higher production capacities, and safer, specific pathogen-free products. Until gene therapy becomes broadly efficacious, transgenic-derived therapeutics are the most attractive alternative for prophylactic replacement therapy in genetic disorders such as hemophilia. The design process for making pharmaceuticals in transgenic animals is shown to be similar to that of using mammalian cell culture. This iterative sequence consists of biosynthesis within the transgenic tissue, characterization and purification of the target protein, amenability to formulation, and engineering of the second generation transgenic tissue to improve rate limitations in posttranslational processing. We have begun the commercialization of four recombinant protein therapeutics with industrial partners.

24. On-line monitoring of biological wastewater treatment by fluorescence

Lu-Kwang Ju. Department of Chemical Engineering, University of Akron, Akron, OH 44325.

Clean water is a priority in our industrialized society. Biological wastewater treatment has been largely responsible for addressing the issue. We have studied the biological nutrient removal (BNR) and aerobic sludge digestion processes using fluorescence techniques primarily with an on-line NAP(P)H fluorometer. The studies for BNR process (with sequential anaerobic, anoxic, and aerobic treatment) were made in three levels: (1) plant—long-term monitoring; (2) laboratory simulation—correlation of fluorescence change with treatment activity/operating conditions; and (3) pure or enriched culture—investigation of microbiological/biochemical fundamentals. The potential of on-line fluorescence in facilitating monitoring and management of wastewater treatment is clearly indicated.

25. Environmental biotechnology: Some current trends

Murray Moo-Young. Department of Chemical Engineering, University of Waterloo, Waterloo, Ontario, Canada N2L 3G1.

With a growing public ecoawareness and concomitant geopolitics regarding biohazards, environmental biotechnology has evolved as an active multidisciplinary area for scientific research and development. Of particular interest to engineers is the potential enhancement of bioremediation by various physical and physicochemical pretreatment strategies. Recent examples of these strategies involve photocatalytic and chemical preoxidation of offensive recalcitrant toxic compounds followed by complete biomineralization of the intermediates. These case studies seem to indicate technoeconomic attractiveness in terms of the risk assesment in the treatment of chemically contaminated soil, water, and air. In another application area, a better understanding of the basic biokinetic and transport phenomena in gas biofiltration is leading to improved design and operation. Compared to the bioproduction of new sellable products, the development of bioremediation service industries lack venture capital and rely heavily on government subsidies. Given current potential legislation on product cradle-to-grave responsibilities of industrial and agricultural manufacturers, this scenario could quickly change. Biotechnology and bioengineering should be prepared for new opportunities.

M. Moo-Young