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Interrelations of Chemistry and Biotechnology—IV†

BIOCHEMICAL ENGINEERING IN BIOTECHNOLOGY

(Technical Report)

Prepared for publication by

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†The Commission has chosen this series with a view to intensify the interrelations of chemistry and biotechnology. By improving the knowledge of chemists in the field of biotechnology it is hoped to initiate more ideas for applying biological methods in chemistry, inspire more use of chemical knowledge in the biological sciences and help scientists in both fields to work closer together. In the articles in the series, to be published in this journal, outstanding experts in their respective fields will give (a) an overview on topics related to the practical use of biotechnological methods in organic chemistry, (b) an outlook on upcoming research topics, their impact on existing areas and their potential for future developments.

The Commission solicits comments as well as suggestions for future topics, and will aim to help in providing answers to any questions in this field.

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Biochemical engineering in biotechnology (Technical Report)

Abstract. The role of biochemical engineering in biotechnology, as the technology of industrial exploitation of biochemical systems, is illustrated. The scientific disciplines underlying biotechnology are pointed out. Biochemical engineering is described as an established discipline, drawing upon chemical, biological and engineering sciences and concerned with design, development, implementation and operation of processes and process plant for handling biological materials and biocatalysts. The principal aspects of bioprocessing are outlined, including processing steps, biocatalysts, bioreactors and downstream processing considerations, to impart a flavour of the biochemical engineering activities and their interaction with chemical sciences. As shown by examples, applications of bioprocessing and biochemical engineering are wide ranging, covering such major facets of civilization as healthcare, agriculture and food, resource recovery, bulk and fine chemicals, energy and environmental pollution abatement.

INTRODUCTION

Biotechnology is considered variously as the science of genetic manipulation, or the technology of industrial exploitation of biochemical systems, genetically modified or not. For the purpose of this article, we subscribe to the second definition which predates modern developments in manipulation of genes, more popularly called "genetic engineering." The latter, combined with other aspects of molecular biology and biochemical sciences (*e.g.*, biomimetic chemistry, biochemistry, bioinorganic chemistry, molecular immunology, microchemical analysis and protein engineering), will remain the powerful fundamental scientific base[1,2] of industrial biotechnology in the foreseeable future. This multidisciplinary nature of biotechnology is recognized in Figure 1, where it is seen to be a subset composite of biological, chemical and engineering branches of knowledge.[3]

Biological and biochemical systems – microorganisms, animal and plant cells, living or dead, genetically modified, otherwise selected, or wild and material derived from these organisms (*e.g.*, enzymes, antibodies, cellular organelles, *etc.*) – are the workhorses of industrial biotechnology.[3-6] These systems, or biocatalysts, carry out a vast array of biochemical reactions which produce useful chemicals (*e.g.*, foods, pharmaceuticals, fuels, pesticides, fine chemicals, fertilizers, plastics, and industrial catalysts to name a few),[3-5,7-13] modify existing ones (*e.g.*, numerous biotransformations)[3,5,8,14] and degrade unwanted ones (*e.g.*, waste treatment);[3,15-17] hence, the impact of biotechnology on all facets of human civilization. Biotechnology, relying on renewable resources, is a sustainable technology which may eventually replace many of the current non-renewable-resource-dependent production methods. Depletion of finite resources, resulting changes in production economics, environmental factors and improvements in biotechnology-based production, will drive the industrial restructuring. The present, more defined niche of industrial biotechnology – generally in areas in which a biochemical product or service cannot be obtained by more traditional means, or when alternative technologies are clearly uneconomic – is bound to expand. Moreover, in contrast to many other high technology developments which have largely remained confined to the more advanced nations, biotechnology promises faster and relevant returns even in the lesser developed economies.[18] This realization has spurred noticeable activity

in the Third World countries which is already bearing fruit.

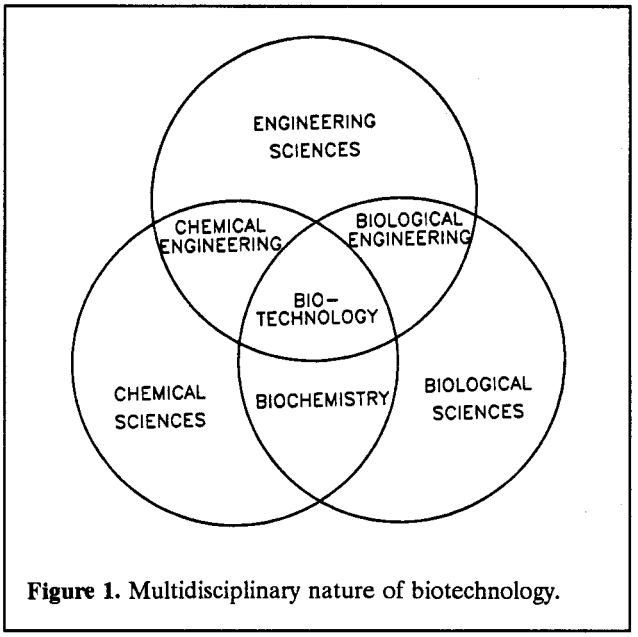


Figure 1. Multidisciplinary nature of biotechnology.

Just as the chemical industry before it, industrial biotechnology needed people capable of developing production processes with the bioscientists (biochemists, microbiologists, geneticist, *etc.*), and transforming these processes into economically viable large-scale plants for products and services. These processes and process plants had to be designed, built, operated and controlled. Thus arose the profession of biochemical or bioprocess engineer. Combining bioprocess-relevant chemical engineering knowhow (process analysis and control, bioreaction and reactor engineering,[19,20] transport phenomena,[21,22] fluid mechanics, thermodynamics, unit operations, bioseparations,[3,23,24] process design and economics[19]) with the specificities of biological systems (microbiology,[3,5] biochemistry,[4,19,25] biocatalysis,[19,25] sterile process engineering[26-29]), biochemical engineering is now a distinct discipline[30] proven through the 1940s development of the antibiotics industry[9] to the latest hybridoma and recombinant cell culture processes and enzyme bioreactors.[3,6,19,25,31] To better appreciate biochemical engineering and its interactions with other chemical disciplines, we will look at bioprocessing in some detail. General features of bioprocessing will be examined first, followed by bioreactors and biocatalysts; the basics of design of bioreactors will be seen, as well as some downstream supporting operations.

BIOPROCESSING

A bioprocess is typically made up of three steps shown in Figure 2. The raw material which may be of biological or non-biological origin, must be converted to a form suitable for processing. This is done in a pretreatment step which may involve such operations as sorting, size reduction, chemical or enzymatic hydrolysis, medium formulation and sterilization and other preparatory operations.[30] The pretreatment step is followed by one or more bioreaction stages where the aim may be the conversion of a substrate (raw material) to biomass (microbial or eucaryotic cells), or biomass and some biochemical including enzymes. Alternatively, the conversion may use dead or non-viable whole cells (immobilized or freely suspended) or purified enzymes as the biocatalytic agency. Bioreactors form the core of the bioreaction step.[30] The material produced in bioreactors must usually be processed further in the downstream section of the process to convert it to a useful form. Downstream processing consists of predominantly physical separation operations which are aimed at purification and concentration of the product.[23,24] Some commonly used operations are solid-liquid separation (centrifugation, filtration, sedimentation, floatation),[32-34] cell-disruption, precipitation, crystallization, adsorption, chromatographic separations, membrane separations (ultrafiltration,[35] microfiltration[36]), liquid-liquid extraction,[37] distillation, evaporation and drying (spray drying, freeze drying, drum and tray drying, *etc.*).[3,23,24,30,38-42] The purified product may have to be in different physical forms (liquid, slurry, powder, crystalline, emulsions) and additional steps for product stabilization and formulation may be needed.

The design of a bioprocess and the engineering of the process equipment require a careful consideration of the physical and chemical properties of the material being handled and a

delimitation of the maximum processing stresses (temperature, pH, shear forces, contamination, pressure, exposure to denaturing chemicals) that the material may safely tolerate.[30] Typically, a bioprocess must operate within the physiological ranges of pH and temperature (pH ~ 7.0; temperature ≤ 37 °C), the specific conditions being very process dependent. Sometimes during processing exposure of material to relatively severe environmental conditions is unavoidable. In such cases, the duration of exposure is minimised and other precautions (*e.g.*, low temperature; addition of chemicals to reduce oxidation, *etc.*) are taken to reduce the impact of exposure. Generally, microorganisms and enzymes are not damaged by pressures which would normally be encountered (≤ 2 MPa) in bioreactors but carbon dioxide associated toxicity effects due to increased solubility of CO_2 at higher pressures may become important.[30]

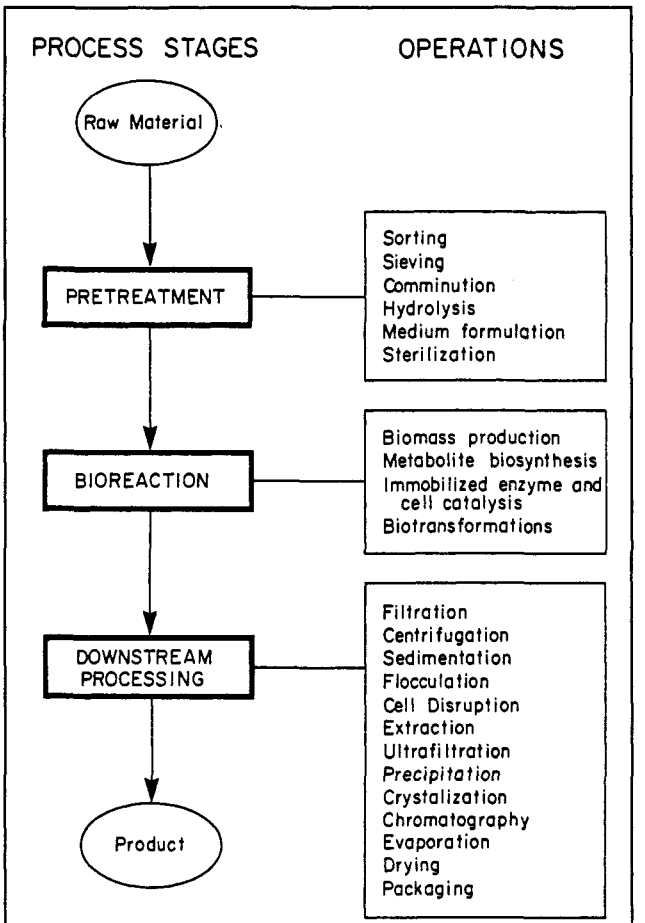


Figure 2. Bioprocess stages and unit operations.

Velocity gradients within fluids, or close to moving surfaces (*e.g.*, agitators; solid particles, liquid droplets or gas bubbles), can potentially damage fragile biocatalyst. The sensitivity of catalysts to these shearing forces may vary widely. In general, bacteria and yeasts which grow as small individual cells are quite shear tolerant. Genetically engineered species and wall-less mutants (and protoplasts[43]) are frequently susceptible to shear damage due to their weaker cell wall/membrane structures or modified physical features.[1,44,45] Filamentous bacteria and mycelial fungi which have larger particle dimensions do show signs of mechanical damage in high shear fields.[1,7,11] Similarly, plant and animal cells are more sensitive to shear.[44] Enzymes, with the exception of multienzyme complexes and membrane-associated enzymes, are not damaged by shear in the absence of gas-liquid interfaces.[30,38] Many biofluids (*e.g.*, some fermentation broths, blood) show shear-dependent flow behaviour,[25,30] but quantification of shear fields in process equipment is not easy.[46]

Pretreatment considerations

Except in biological treatment of wastes, most productive fermentations requiring microbial or eucaryotic cell cultivation depend on maintenance in culture of the single species with which the fermenter was initially inoculated.[26,30] Contamination from other microorganisms and viruses is prevented by sterilization of the liquid and gaseous feeds to the fermenter,[5,28,30,47] sterile engineering of the bioprocess plant and use of sterile processing techniques.[5,6,26,28,29]

Liquids such as water and salt solutions can be inexpensively sterilized by filtration through absolute or properly designed depth filters which retain the contaminating microorganisms.[26,29,30] This method is applicable when the liquid is free of other suspended solids. Most industrial

fermentation media contain components with suspended solids (*e.g.*, molasses, corn steep liquor, yeast extract, soya bean meal, fish meal). These components must be heat sterilized either separately or with the medium.[5,28,30] Thermal denaturation of one or more enzymes in the contaminating microorganisms is used to render them non-viable. However, the temperatures necessary to cause effective microbial kill also cause reactions which destroy such essential thermolabile media constituents as vitamins and growth factors. Additionally, reactions occur between the components of the medium, *e.g.*, the Mailard reaction between carbohydrates and proteins, leading to formation of products which adversely affect microbial growth, lowering the fermentation yield.[30] Success of industrial fermentations depends on attaining an economic balance between insufficient sterilization with consequent frequent contamination-associated batch losses and yield reduction due to over-cooking. The rate constant for thermal denaturation of contaminating microbial spores (k_d) is a function of temperature

$$k_d = A e^{-\Delta E/RT}, \tag{1}$$

where ΔE is the activation energy for destruction of a particular microorganism, A is the Arrhenius parameter and T is the absolute temperature. Bacterial spores such as those of *Bacillus stearothermophilus* and *Clostridium botulinum* are some of the most heat resistant, and sterilization processes are designed to be effective against them.[30] The activation energy for the destruction of these spores is 2.5-2.9 $\times$ 10^8 J•kmol^{-1} and A is ~ 1.6 $\times$ 10^{36} s^{-1}. Similarly, the denaturation of the nutrients (k_n) also depends on temperature

$$k_n = A_n e^{-\Delta E_n/RT}. \tag{2}$$

From equations (1) and (2) it follows that

$$\frac{k_d}{k_n} = \frac{A}{A_n} e^{(\Delta E_n - \Delta E)/RT}. \tag{3}$$

The activation energy for the thermal deactivation of most nutrients (ΔE_n) is much lower than ΔE for microbial destruction.[19,28] For example, ΔE_n for thiamine hydrochloride (vitamin B_6) is only 9.2 $\times$ 10^7 J•kmol^{-1}. Thus, from this simple analysis of denaturation kinetics, we see that sterilization at high temperature for a shorter time is preferred for maximizing microbial destruction relative to nutrient loss (equation 3), *i.e.*, higher temperature operation would achieve a higher k_d/k_n.[30] This indeed is the basis of HTST (high-temperature-short-time) sterilization now being increasingly adopted in the bioprocessing industry.[29]

Medium formulation

Careful formulation of growth and production media is a prerequisite for successful fermentation.[4,5,30] Microbial nutritional requirements and environmental needs have to be met as well as several techno-economic constraints. Medium development aims to maximize product yield at minimal medium cost. The choice of the medium affects downstream processing and upstream (pretreatment) activities; therefore, medium design should be carried out in an overall process context.[30] The use of a purer carbon source such as glucose compared, say, with molasses may reduce purification problems and simplify pollution control and waste treatment.

The medium must provide sufficient carbon, nitrogen, minerals and other nutrients to yield the required amount of cell mass and product. Minimum requirements are estimated from the stoichiometry of growth and product formation.[19,30] In general,

C-source + N-source + minerals + specific nutrients (vitamins, hormones) + O_2 $\rightarrow$ cell mass + product + CO_2 + H_2O.

Most nutrients are supplied at levels well above the minimal needs.

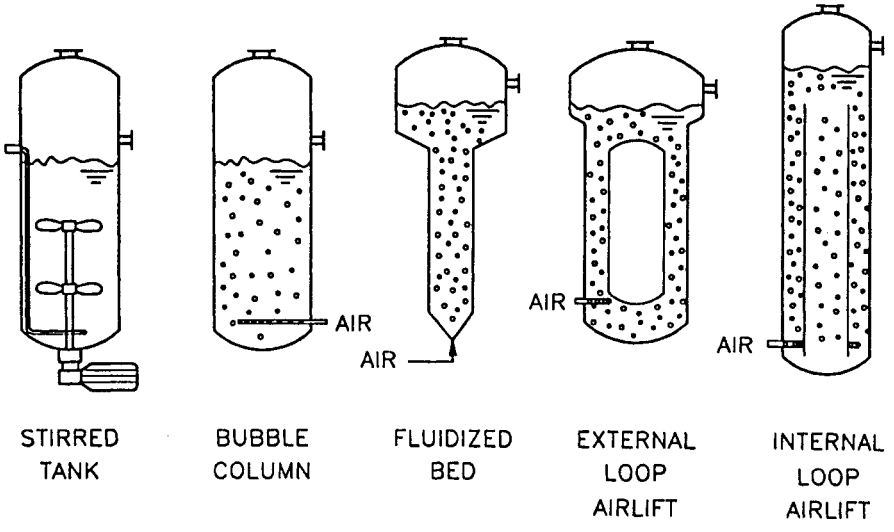


Figure 3. Some common fermentation bioreactors.

For the production of metabolites, understanding the biochemistry of product formation has an important role in medium formulation practice.[4,5,25] The course of a fermentation can be directed by precursor feeding (*e.g.*, in penicillin fermentations), limiting some nutrients and providing some others. Even trace amounts of some media components (*e.g.*, various metal ions in citric acid production by *Aspergillus niger*[5]) can have a major impact on the yield of the desired product or its degree of contamination with other unwanted byproducts. These yield-suppressory and stimulatory effects are usually related to inhibition or activation of enzyme action, or to enhancement or suppression of production of particular enzymes by the media components or by some product of metabolism of these components.[4,25,28] Frequently, knowledge of the biosynthetic pathways of fermentation products has lagged behind industrial practice of these fermentations; trial and error methods have been the basis of medium formulation and continue to play a dominant role.

In comparison with microbial culture media, the media used in animal cell culture are generally better defined, containing most of the 20 amino acids, several vitamins, simple sugars as carbon sources (*e.g.*, glucose), salts for osmotic pressure adjustment as well as for nutrients and trace elements. In addition, these media are usually supplemented with blood sera (*e.g.*, bovine serum, fetal calf serum, horse serum) at 5-20 % by volume. Serum provides essential hormones, lipids and other growth factors.[4,6]

Natural media components such as blood sera, molasses, corn steep liquor, often show tremendous variability between batches. Good chemical and biochemical quality control of these substances, relying on many of the techniques of modern analytical chemistry, is essential to maintaining consistency in fermentation performance.

BIOREACTORS AND BIOREACTION

Industrial bioreactors (fermenters) for sterile operation are usually designed as pressure vessels, irrespective of whether the stirred tank, bubble column, fluidized bed or the airlift configuration is employed.[27] These basic bioreactor configurations are shown in Figure 3.

A typical fermenter has the features shown in Figure 4. The reactor vessel is provided with a vertical sight glass, and side ports for pH, temperature and dissolved oxygen sensors are a

minimum requirement. A steam-sterilizable sample valve is provided. Connections for acid and alkali (for pH control), antifoam agents and inoculum are located above the liquid level in the reactor vessel. An air (or other gas mixture) sparger supplies oxygen (and sometimes CO_2 or ammonia for pH control) to the culture. A harvest nozzle is located at the lowest point on the reactor vessel. When mechanical agitation is used, either a top or bottom entering agitation system may be employed. The shorter agitator shaft on bottom entry design often eliminates the need for steady bearings within the vessel, hence an easier to clean and sterilize bioreactor configuration can be achieved.[27] The bottom placement of agitator and its drive allows the top of the bioreactor for a spinning or vibrating cell retention mesh which is sometimes used in animal cell culture bioreactors. The shaft of the high speed mechanical foam breaker (Figure 4) is provided with a single or double mechanical seal at the point of entry in the reactor vessel. Steam sterilizable double seals with sterile water lubrication are preferred, and may be a necessary requirement when biohazardous material must be contained (*e.g.*, during production of vaccines and other high bioactivity substances). Double seals are used also on the bottom mounted agitator shaft; however, sealless magnetically coupled designs are distinctly superior when torque limitations permit.[27]

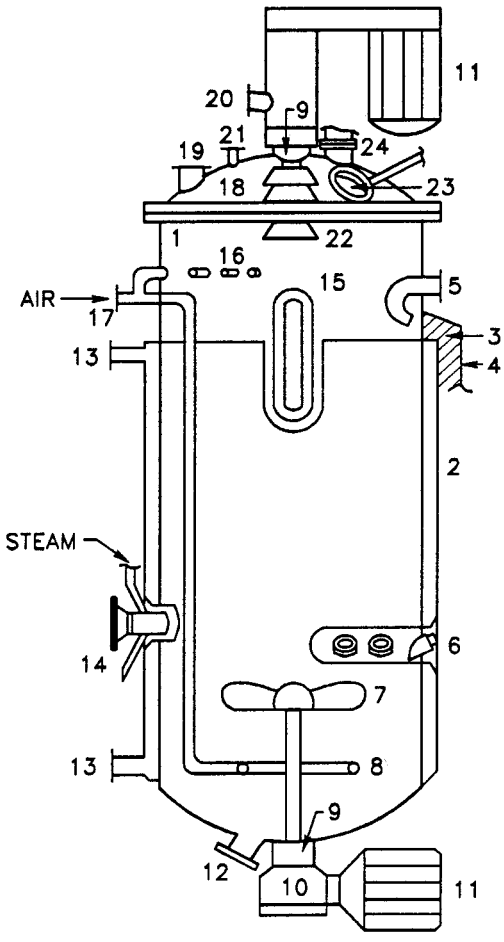


Figure 4. A typical fermenter: (1) reactor vessel; (2) jacket; (3) insulation; (4) shroud; (5) inoculum connection; (6) ports for pH, temperature and dissolved oxygen sensors; (7) agitator; (8) gas sparger; (9) mechanical seals; (10) reducing gearbox; (11) motor; (12) harvest nozzle; (13) jacket connections; (14) sample valve with steam connection; (15) sight glass; (16) connections for acid, alkali and antifoam chemicals; (17) air inlet; (18) removable top; (19) medium or feed nozzle; (20) air exhaust nozzle; (21) instrument ports (several); (22) foam breaker; (23) sight glass with light (not shown) and steam connection; (24) rupture disc nozzle.

The reactor is either provided with a manhole, or the top is removable. Flat headplates are commonly used on smaller vessels, but a domed or elliptical construction of the head is less expensive for larger units. The head plate supports a rupture disc, air exhaust ports, nozzles for media or feed addition, and for sensors (*e.g.*, foam electrode) and instruments (*e.g.*, pressure gauge); a foam breaker may also be located on the headplate. A sight glass with light is provided and can be internally cleaned by a jet of steam condensate. The vessels are invariably jacketed. Chloride-free fibreglass insulation, fully enclosed in a protective shroud (Figure 4), is applied to the jacket. The jacket and the reactor are provided with overpressure protection: a relief valve on the jacket or its associated piping, a bursting disc on the bioreactor.[27] Not all of the items shown in Figure 4 are required in every application.

The vessel should be fully draining, it should have a minimum number of ports, nozzles, connections and other attachments consistent with the current and anticipated future needs of the process. The bioreactor should be free of crevices, and stagnant areas where pockets of liquids and solids may accumulate.[27,28] The vessel should have few internals; the design should take into account the clean-in-place (CIP) needs.[26,27] Automated cleaning of process machinery by CIP techniques relies primarily on the action of cleaning chemicals on soil. Selection of cleaning and sanitizing agents with respect to soil-type, water quality and compatibility with the process equipment are important considerations. The cleaning action of chemicals is aided by heat, high velocity flow or jet sprays.[27]

All materials of construction must withstand the physical-chemical conditions encountered during cleaning (*e.g.*, highly alkaline detergents), and processing. The materials which come in contact with the process fluids should be non-reactive, non-additive, non-absorptive, and durable.[27] All product contact surfaces must be capable of being easily cleaned using cleaning-in-place techniques. Sterilization should be easy. For a great majority of applications, austenitic stainless steels are the preferred material of construction for bioreactors.[27]

As other important considerations, the bioreactor must be designed to satisfy the oxygen demand of the microorganisms;[3,21,22,48,49] there must be sufficient agitation for suspension of solids,[22,30] mixing of additives (nutrients, chemicals for pH and foam control) and mass transfer between gas-liquid,[22,50-52] solid-liquid,[53] or liquid-liquid (*e.g.*, hydrocarbon dispersions in water[54]) phases. Multiphase hydrodynamics must be given attention.[22,55,56] The heat generated during bioreaction must be evaluated using thermochemical and kinetic data[19,30,57,58] and the reactor must ensure adequate heat removal for temperature control; heat supply capability for sterilization must also be considered.[28,30] Some fermentations (*e.g.*, animal cell cultures) can be very sensitive to temperature fluctuations.

Fermentation, growth and product formation

A sterilized batch fermenter containing a properly developed medium, sufficient aeration (oxygen supply; CO_2 removal), supplies of pH control chemicals and antifoams must be inoculated with the desired microbial species to initiate the fermentation. The inoculum consists of a microbial suspension in rapid exponential growth added at a concentration of 5-10 % of working volume of the fermenter.[30] Animal and plant cell cultures, being slower growing, require larger inocula (up to 25 %) to avoid having long fermentation times (costs) in the production vessels. Because industrial fermenters tend to be quite large (up to 10 m^3 in animal cell culture; 100-300 m^3 in microbial fermentations), inoculum preparation from seed bank often requires several fermentation steps in series: shake flasks (T-flasks and roller bottles in animal cell culture), seed fermenter and secondary (or tertiary) seed fermentation.[28,30,59] For spore forming microorganisms, quantities of spores produced for inoculation in a seed stage may be blown into the production fermenter with the ingoing sterile air.

Unlike most microorganisms which are capable of growing in suspension unattached to a solid surface, certain animal cells are anchorage dependent.[6] For these, growth surface in large scale culture is provided most commonly by suspending solid (*e.g.*, glass, polystyrene) microspheres (150-300 μm diameter) in the culture medium.[6] When required, as during inoculation of the next larger fermenter in the production train, the cells can be dislodged from the microcarriers by addition of trypsin, a proteolytic enzyme. Following inoculation the growth of the microorganisms follows the typical pattern shown in Figure 5. An initial period of no cell growth, the lag period, is soon followed by the exponential growth phase.[5,28,30,60] The length of the lag phase is related to the growth history of the inoculum, the inoculum size and the composition of the medium.[25,30] The composition in the seed and the production fermenters should be identical to avoid excessive lag. Additionally, as pointed out earlier, rapidly growing cells (late exponential growth phase) should be used for inoculation and the volume of the inoculum (typically 5-10% of final working volume as mentioned earlier) should be such that possible osmotic shock effects on dilution in the larger vessel are minimal.[30] Also, the environmental conditions (pH, temperature, dissolved oxygen level) in the seed and production stages should be identical during inoculation. Some animal cell cultures

may not grow if the cell concentration at inoculation is less than 1-3 × 10^5 cells/mL. Consistency through the inoculum building stages is essential for reproducible behaviour of the production fermentation.[61]

Although the lag phase is preparatory to rapid exponential growth phase, it ties up the fermentation plant unproductively and fermentation optimization should attempt to reduce or eliminate the lag[30] (*e.g.*, by continuous culture methods). During exponential growth, cell mass (or number) increases exponentially. For a cell mass concentration X_o at the beginning of exponential growth (X_o usually equals the inoculum concentration in the fermenter) the concentration X at any time t can be described by the kinetic equation

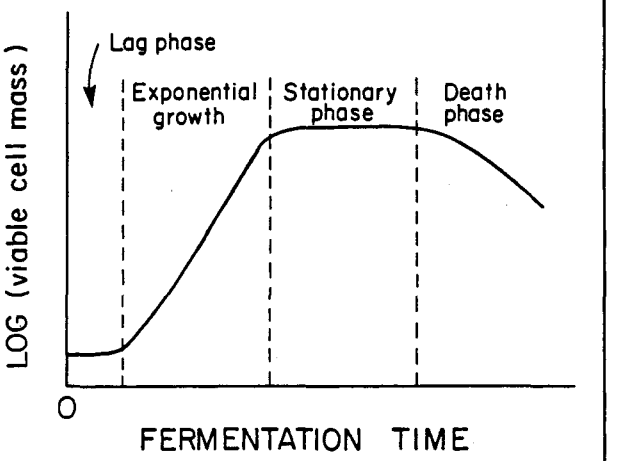


Figure 5. Progression of cell growth.

$$\ln \frac{X}{X_o} = (\mu - k_d)t, \tag{4}$$

where μ and k_d are the specific cell growth and death rates, respectively. During exponential growth k_d is usually much smaller than μ and a biomass doubling (t_d) can be shown to be

$$t_d = \frac{\ln 2}{\mu}. \tag{5}$$

In general, the doubling times (time to double cell mass) for various classes of microbial and eucaryotic cells increase in the order: bacteria, yeasts, moulds, protozoa, mammalian and insect cells, plant cells.[30] For any given species, the doubling time is dependent on the growth medium and environmental factors. In the limit, microbial growth is a reflection of biochemical reactions within the cell and like chemical reactions, growth is speeded by increase in temperature until the point where thermal denaturation of the enzyme catalysts causes precipitous decline in growth rate.[11] In nature, extremophilic microorganisms thrive at temperatures exceeding 100 °C (*e.g.*, in volcanic vents, hot springs).[2] With improved understanding of the molecular and other features which permit extremophilic enzyme action at such high temperatures, industrially useful enzymes may potentially be synthesized for enhanced rate bioreactors.

BIOREMEDIATION AND WASTE TREATMENT

Biological treatment of liquid, solid and gaseous effluents for environmental pollution control or abatement differs from most other industrial bioprocesses in two main ways: the scale of operation is generally very large and *mixed* microbial populations under non-sterile conditions are the biocatalytic entities responsible for degradation of waste.[19] Treatment of domestic and municipal wastewater by biological means (*e.g.*, activated sludge process, trickle bed biofilters, *etc.*) is a well established, predominant technology of treatment of these wastes.[3,62,63] Many industrial wastewaters contaminated with relatively recalcitrant organics are also amenable to biological treatment using microbial populations which have adapted (natural selection) to degrading these wastes for survival.[16,17,64,65] Bioremediation techniques are increasingly being applied to decontamination of soils polluted with wastes of petrochemical origin.[66,67] Both *in situ* treatment and treatment in

bioreactors such as the airlift devices are being implemented.[22,58,67,68] An important consideration in these processes is minimization of costs.

Gaseous streams polluted with low loadings of organics and odorous substances can be treated in biofilters now being developed.[15] Inorganic pollutants such as heavy metals, even in very dilute waste streams, can be adsorbed or otherwise accumulated by certain microbes (*e.g.*, some algae) thereby affording a method of concentration and treatment. Although biological waste treatment has a long and proven record of application, much greater contribution can yet be expected from bioprocessing in environmental quality improvement. Many traditional chemical industry processes as well as newer ones will depend on biotechnology for their waste management needs.[64,65] Elucidation of the degradative mechanisms and kinetics for the pollutants and design of reactors for their treatment would require innovative approaches from chemists, biochemists, microbiologists and biochemical engineers.

BIOCATALYSIS

Nearly all biochemical reactions taking place in plants, animals and microorganisms are catalysed by enzymes. Because of the vast array of reactions and the diverse life forms, an equally large number of enzymes exist in nature. Produced inside cells, the enzymes may be retained within (*i.e.*, intracellular enzymes) the cell or secreted (*i.e.*, extracellular enzymes) to the outside. Biochemical reactors or bioreactors use the enzyme systems of microbial and other cells to carry out the required biosyntheses, biodegradation or biotransformations.[8,14,31,60] Apart from cell growth and accompanying metabolite production in fermenters, non-viable or non-growing live cells and cell-free enzymes, either immobilized or freely suspended in their native state, are important forms of industrial biocatalysts.[8,20,25,30,31]

Cell-free enzymes

Soluble enzymes in their native state are used in industrial reactors as well as being commodity chemicals.[25,31,69] A soluble enzyme is not easily recovered after use and is lost with the product. Usually, inexpensive extracellular microbial enzymes are used in soluble form. These are mostly hydrolytic enzymes such as amylases (starch hydrolysis), cellulases (cellulose hydrolysis), pectinases and proteolytic enzymes.[31]

As commodity products, cellulases and proteinases are used in detergents,[69] pectinases in enhanced recovery of juices from fruits, proteinases in meat processing and cheese making, amylases in brewing, to cite but a few examples. In most applications the activity and the pH and temperature optima of the enzyme are dependent on its source and enzyme selection is often tailored to the process needs. For example, alkaline bacterial proteinases, such as subtilisin, are active over the pH range 7-11[31] and are used in detergents. Neutral bacterial proteinases are metaloproteins active over a pH range 6-9.[31] Similarly, bacterial amylases suitable for 70-85 °C or 90-105 °C temperature optima are available.[31]

Immobilized biocatalyst reactors

Immobilized enzyme (and immobilized cells,[70] protoplasts,[43] organelles) catalysts can be employed in a variety of reactor configurations.[19,25,30] Catalyst particles may be used in suspension as in stirred tank and fluidized bed reactors (Figure 6) or they may be held in place in fixed or packed bed devices. Hollow fibre reactors containing catalyst immobilized either throughout the thickness of the fibre wall or confined to one side of it (*e.g.*, perfusion systems) are possible.[3,30] Flat polymer membranes containing immobilized catalyst have been used in spirally wound configurations. Immobilized particulate biocatalyst can, of course, also be used in airlift and bubble column reactors so long as the solids loading and density are not excessive.[22,30,53,71] A packed-bed-airlift combination reactor, as shown in Figure 7, is another possibility.[72] In such reactors a compressed

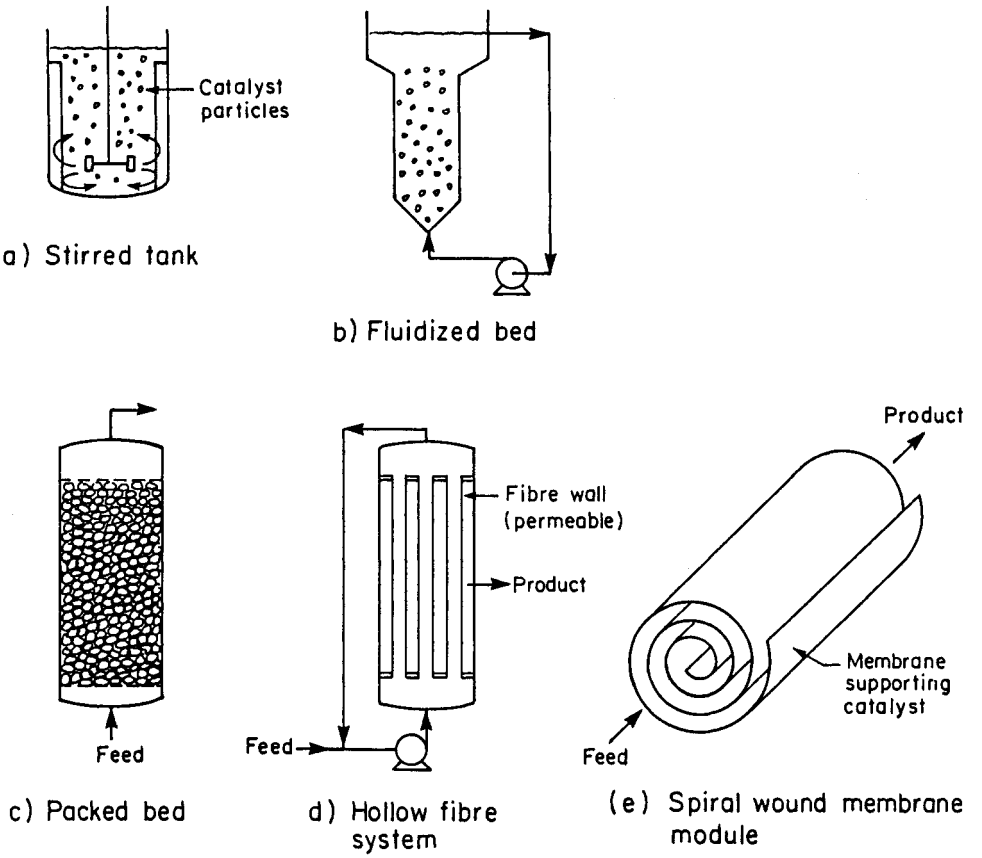


Figure 6. Deployment of immobilized enzymes and cells in bioreactors: Free suspension in stirred tank (a) or fluidized bed (b); fixed catalyst in packed bed (c), hollow fibre (d), or spiral wound polymer membrane module (e).

gas generates the necessary agitation and circulation of the fluid. In fact, airlift reactors using microbial films immobilized on sand particles have been described as the future of wastewater treatment where their low power requirements are an important advantage.[58,71]

Relative to soluble forms, immobilized biocatalysts are easily retained in reactors and can be reused. Often, immobilization[70] improves the stability of the catalyst and can be used to advantageously alter the apparent pH optima relative to the native form. However, immobilization represents an additional cost. Nevertheless, certain expensive intracellular enzymes (*e.g.*, glucose isomerase) could commercially be employed only because immobilization improved the overall process economics by permitting repeated long term use.[31]

Bioreactor efficiency is measured by the quantity of substrate (reactant) transformed per unit time per unit volume of the reactor.[20,30] Obviously, the efficiency is dependent on the activity per unit mass of the immobilized catalyst. For specified initial concentration of substrate (S_o) and its desired conversion $\chi = (S_o - S)/S_o$, the conversion characteristics of different reactor configurations can be calculated from a knowledge of the kinetics of reaction. Thus, for a reaction which obeys Michaelis-Menten kinetics

$$-\frac{dS}{dt} = \frac{k_r e S}{K_s + S}, \qquad (6)$$

where e is enzyme concentration, S is substrate concentration at time t, K_s is Michaelis constant and k_r is the rate constant, we can develop equations for predicting the conversion as explained below for a batch stirred tank reactor.

Because the change in the quantity of the substrate in a batch reactor equals substrate consumption by the reaction, we can write

$$-V_L \frac{dS}{dt} = \frac{k_r E S}{K_s + S}, \qquad (7)$$

where V_L is the volume of the reactor and E is the total amount of enzyme in it. Equation (7) may be integrated for $S = S_o$ at $t = 0$ and $S = S$ at $t = t$, to

$$\frac{k_r E t}{V_L} = S_o - S - K_s \ln \frac{S}{S_o}, \qquad (8)$$

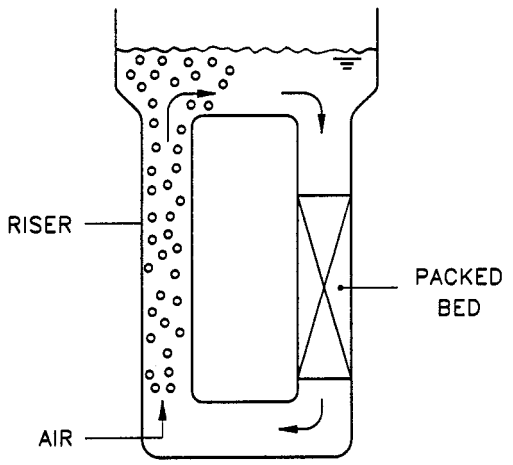


Figure 7. An external-loop airlift reactor with packed catalyst bed in downcomer.

which can be written in terms of the conversion χ as

$$\frac{k_r E t}{V_L} = \chi S_o - K_s \ln(1 - \chi). \qquad (9)$$

Hence, the batch time t for any degree of conversion may be calculated. We see from this example that the principles of analysis of biochemical reactors are the same as for chemical reactors;[20,60] of course, the exact nature of the rate equations may differ.

PRODUCTION OF 6-AMINOPENICILLANIC ACID

Here we illustrate, in the context of a current industrial bioprocess, some of the biochemical engineering considerations and their relationship to process chemistry. The production of 6-aminopenicillanic acid is used as an example.[30]

Penicillins are among the most widely used antibiotics. Benzylpenicillin (Penicillin G) obtained by fermentation with *Penicillium chrysogenum* is of limited use and most of it is converted to 6-aminopenicillanic acid (6-APA) which is a precursor for the manufacture of semi-synthetic penicillins. The chemical conversion of Penicillin G (PEN-G) to 6-APA, shown in Figure 8, has now been superseded by the one-step biochemical route (Figure 8). The chemical process required low temperatures (hence expensive low temperature steels for processing equipment) and strictly anhydrous conditions during parts of the process; moreover, several processing steps were needed. As a result, the process proved to be uncompetitive with the biochemical conversion. The enzyme used for the bioconversion is penicillin acylase (EC 3.5.1.11) produced by the bacterium *Escherichia coli*, in addition to other microorganisms. Several process schemes may be used: (1) *E. coli* cells grown separately and killed may be contacted with a solution of PEN-G in a batch stirred reactor. After one use, however, the cells lose most of their enzyme activity due to lysis and have to be replaced. (2) Live, immobilized *E. coli* cells may be brought in contact with a continuous flow of PEN-G solution. Either packed bed or suspension reactor modes may be used. Side reactions and contamination by metabolites and substances required to sustain the cells are possible problems. (3) The enzyme penicillin acylase may be extracted from *E. coli*, immobilized and used in continuous reactors. Scheme (1) has been used commercially, but scheme (3) seems to be the preferred industrial route. This route is examined further.

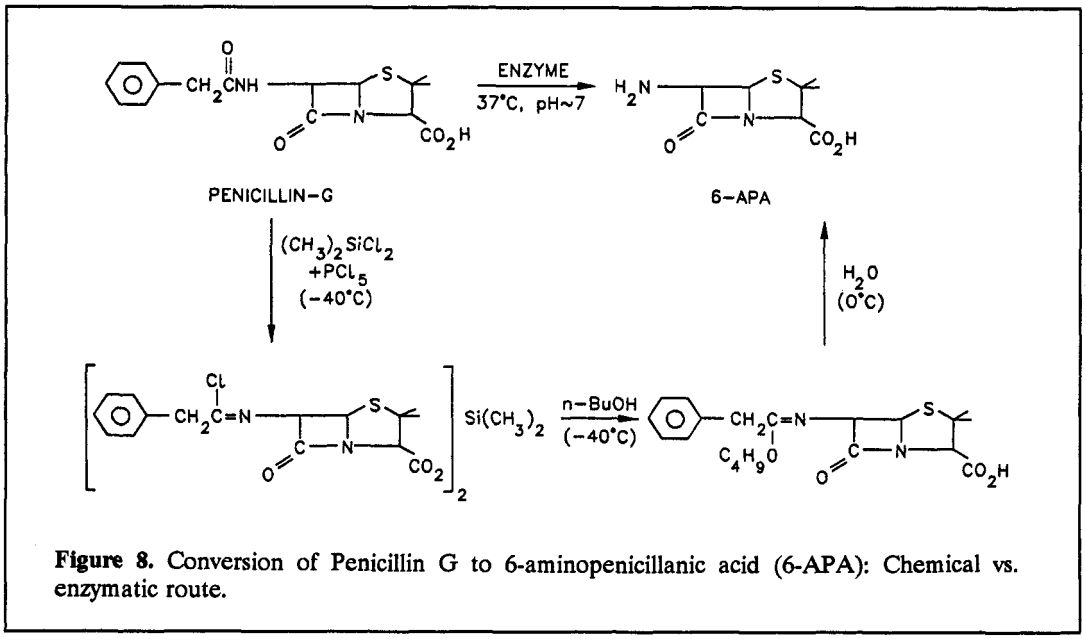


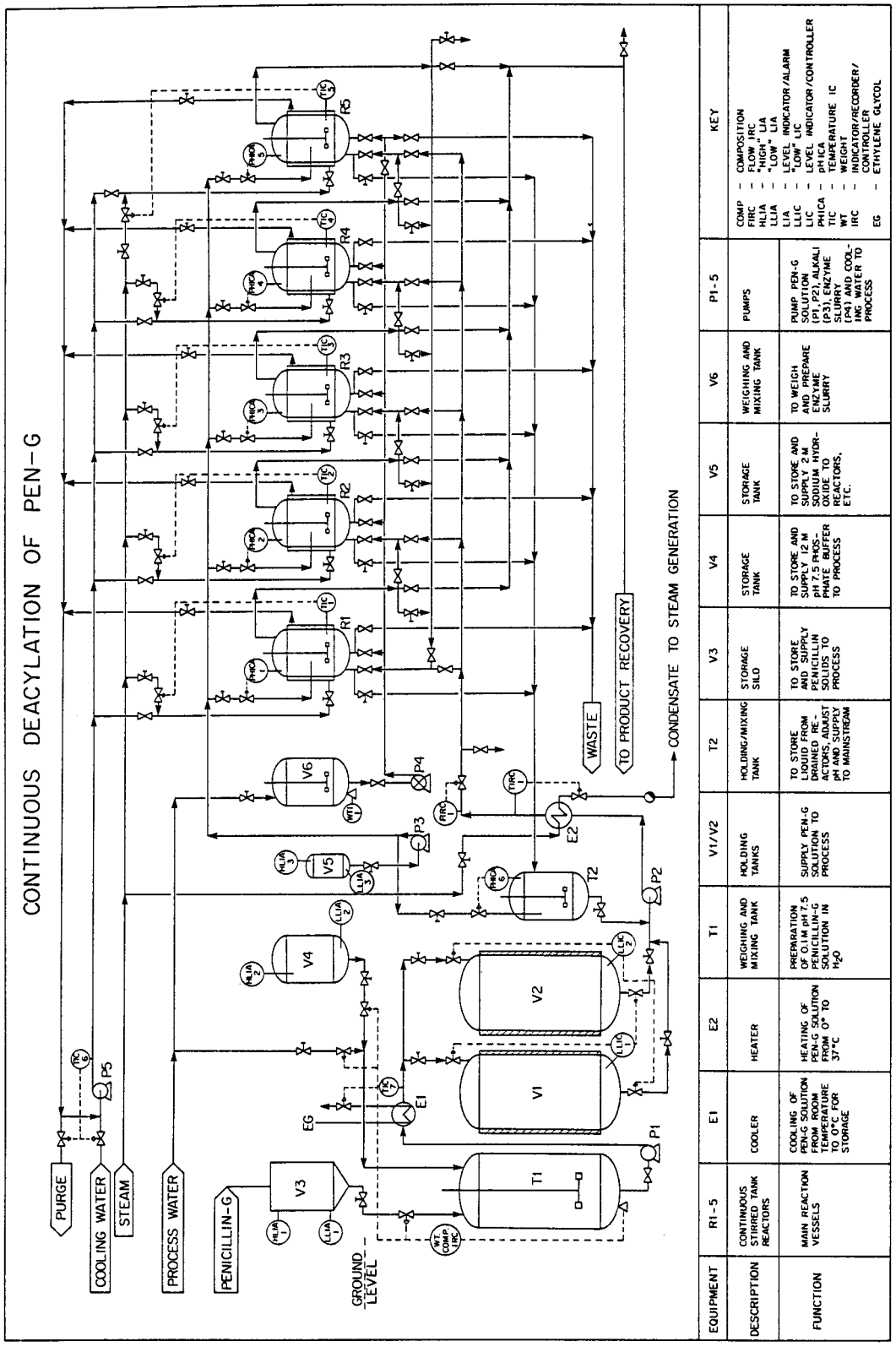
Figure 8. Conversion of Penicillin G to 6-aminopenicillanic acid (6-APA): Chemical vs. enzymatic route.

The enzyme is produced by growing a high yielding strain of *E. coli* in a batch fermenter. The cells are harvested by centrifugation, resuspended in a smaller volume of buffer and disrupted by high pressure homogenization. A two stage salt precipitation follows, precipitated nucleic acids and cell debris are removed first (centrifugation) and then the enzyme. The enzyme precipitate is solubilized, further purified to a relatively pure form and immobilized on polymer beads. The immobilized catalyst, which can be purchased ready to use, has a half-life of the order of several months under the normal reactor conditions of 37 °C and pH 6-8, long enough for industrial purposes.

The kinetics of the deacylation reaction are such that it is strongly inhibited by 6-APA and is also susceptible to some substrate (PEN-G) inhibition. Based on detailed calculations, a packed bed catalytic reactor would be best for the conversion. However, as the deacylation reaction proceeds, the pH of the reaction medium drops and good pH control is essential to avoiding damage to the product, substrate and the enzyme catalyst. Control of pH is difficult in packed columns and in this reaction is achieved by addition of alkali to a buffered reaction medium. Rapid and uniform mixing is necessary to avoid high local pH values which lower the half-life of the enzyme. To satisfy the operational requirements of good reactor mixing and at the same time to approximate the reaction system to a plug-flow type, a battery of up to four continuous stirred tank reactors (CSTRs) in series is used for the reaction. Four CSTRs in series are about optimal, allowing a PEN-G conversion of more than 95 %. Addition of a fifth reaction stage would increase the conversion only slightly as the catalyst would be subject to severe product inhibition by 6-APA which would be at its maximum concentration in this last stage. A fifth stage would, of course, add to capital and operational costs.

After the reaction, 6-APA is precipitated from the product-containing solution by adjustment of pH to its isoelectric point; the precipitate is filtered, washed and dried. The product obtained is better than 98 % pure.

Although the 6-APA production process is relatively straightforward, the large scale production plant can be quite complex as revealed in the very basic piping and instrumentation diagram shown in Figure 9 which does not include the downstream plant. The need for continuous supply of PEN-G solution, automatic catalyst replacement, sequencing of flow through any combination of the four reactors (to allow for taking spent reactors off-line and replacing with one containing fresh catalyst), complicate the flow scheme. The requirements for cleaning-in-place and control (*e.g.*, temperature control) further add to the complexity, leading to extensive pipework, valves, pumps and other ancillaries, most of which must be built to hygienic standards.



EQUIPMENT	R1-5	E1	E2	T1	V1/V2	T2	V3	V4	V5	V6	P1-5	KEY
DESCRIPTION	CONTINUOUS STIRRED TANK REACTORS	COOLER	HEATER	WEIGHING AND MIXING TANK	HOLDING TANKS	HOLDING/MIXING TANK	STORAGE SILO	STORAGE TANK	STORAGE TANK	WEIGHING AND MIXING TANK	PUMPS	COMP - COMPOSITION FIRC - FLOW IRC HLIA - "HIGH" LIA LLIA - "LOW" LIA
FUNCTION	MAIN REACTION VESSELS	COOLING OF PEN-G SOLUTION FROM ROOM TEMPERATURE TO 0°C FOR STORAGE	HEATING OF PEN-G SOLUTION FROM 0° TO 37°C	PREPARATION OF 0.1 M pH 7.5 PENICILLIN-G SOLUTION IN H_2O	SUPPLY PEN-G SOLUTION TO PROCESS	TO STORE LIQUID FROM DRAINED REACTORS, ADJUST pH AND SUPPLY TO MAINSTREAM	TO STORE AND SUPPLY PENICILLIN SOLIDS TO PROCESS	TO STORE AND SUPPLY 12 M pH 7.5 PHOSPHATE BUFFER TO PROCESS	TO STORE AND SUPPLY 2 M SODIUM HYDROXIDE TO REACTORS, ETC.	TO WEIGH AND PREPARE ENZYME SLURRY	PUMP PEN-G SOLUTION (P1, P2), ALKALI (P3), ENZYME SLURRY (P4) AND COOLING WATER TO PROCESS	LIA - LEVEL INDICATOR/ALARM LLIC - "LOW" LIC LIC - LEVEL INDICATOR/CONTROLLER PHICA - pH ICA TIC - TEMPERATURE IC WT - WEIGHT IRC - INDICATOR/RECORDER/CONTROLLER EG - ETHYLENE GLYCOL

Figure 9. A simplified piping and instrumentation diagram for the bioreaction stage of 6-amino penicillanic acid manufacturing process.

DOWNSTREAM PROCESSING

As explained earlier in this article, downstream processing is essential to obtaining a saleable product from the raw product of the bioreaction step. This discussion is limited to factors which must be considered in developing any economically viable product purification and concentration scheme based on a few of the many downstream processing operations that are available. Individual operations have been described previously in one of the articles in this series,[39] as well as elsewhere.[3,23-25,30,33,35-38,40-42]

In general, a biological product is either secreted into the extracellular environment, or it is retained intracellularly. In comparison with the total amount of biochemicals produced by the cell, very little material is usually secreted to the outside; however, this selective secretion is itself a purification step which simplifies the task of the biochemical engineer. Extracellular products, being in a less complex mixture, are relatively easy to recover. On the other hand, because a greater quantity and variety of biochemicals are retained within cells, intracellular substances are bound to eventually become a major source of bioproducts. Among some of the newer intracellular products are recombinant proteins produced as dense inclusion bodies in bacteria and yeasts. Recovery of intracellular products is more expensive as it requires such additional processing as cell disruption,[38] lysis[73] or permeabilization.[74] In principle, selective release of the desired intracellular products is possible, but in practice it is neither easily achieved nor sufficiently selective. Hence, the desired product must be purified from a relatively complex mixture, complicating processing and adding to the cost. Nevertheless, an increasing number of intracellular products are in production. Economics of production may be improved by recovering several products (intracellular and extracellular) from the same fermentation batch.

Product recovery schemes commonly employ solid-liquid separations such as filtrations, centrifugation, sedimentation and floatation. Filtration and centrifugation are by far the more common. Solid-liquid separation is a means of concentration of cell mass prior to disruption, or a method for total removal of cells as product or waste. Some other concentration steps, applicable to products in solution, are precipitation, adsorption, chromatography, evaporation and ultrafiltration. Some of these operations are equally capable purification steps (*e.g.*, chromatographic separations). Typically, volume reduction of the fermentation broth should be one of the early downstream processing steps to reduce the capacity requirements (cost) of other processing equipment. Certain steps (*e.g.*, some chromatographic separations; membrane separations) may require a relatively clean process stream, free of debris, lipids or micelles which may cause fouling of the equipment. Such steps are often used downstream of steps which can handle cruder material.

As far as possible, the requisite purification and concentration should be achieved with the fewest processing steps; generally, no more than 6-7 steps are used, a situation quite different from that in chemistry and biochemistry laboratories, where the number of individual steps is often not a major consideration, purity of the product is usually more important than overall yield or costs. A train of only five steps, each with 90 % step yield, would reduce the overall recovery to less than 60 %.[75] To minimize reduction of the overall yield, a multi-step processing scheme should attempt to use steps with higher step yield earlier in the processing sequence than the lower-yielding operations.

The specifications on product purity and concentration should be carefully considered in developing a production protocol. Concentration or purification to levels beyond those dictated by needs is wasteful. When more than one processing options are technically feasible, evaluations of the economics of use in terms of capital expenditure on equipment and its operating costs (processing time, yields, labour, cleaning, maintenance, analytical support) is necessary for optimal process selection. Economic evaluations should be performed over the expected lifetime of the equipment. For example, for separation of solids from fermentation broth, centrifugation and microfiltration may be two competing alternatives.[32] In still other applications, for example when

very fragile cells are to be separated from suspending liquid, centrifugation may not at all be an option.

For many biological products, particularly pharmaceuticals, seemingly minor alterations in downstream processing can have important implications on the performance of the product. For example, penicillins may be recovered by liquid-liquid extraction of either the whole fermentation broth or solids-free broth. The latter scheme requires an additional solid-liquid separation than the whole broth process. However, the whole broth extracted product has been known to cause more frequent cases of allergenic reactions in comparison with the other processing alternative. In fact, some pharmaceutical companies now demand of contract suppliers that, in addition to meeting product specifications in terms of measurable contamination, the product they supply must conform to a certain production method, in this case extraction after removal of fungal solids. When raw penicillin is for bulk conversion to semi-synthetic penicillins, whole broth extraction may be acceptable in view of the security afforded by the additional steps involved in making and purifying 6-aminopenicillanic acid (6-APA) from raw penicillin.

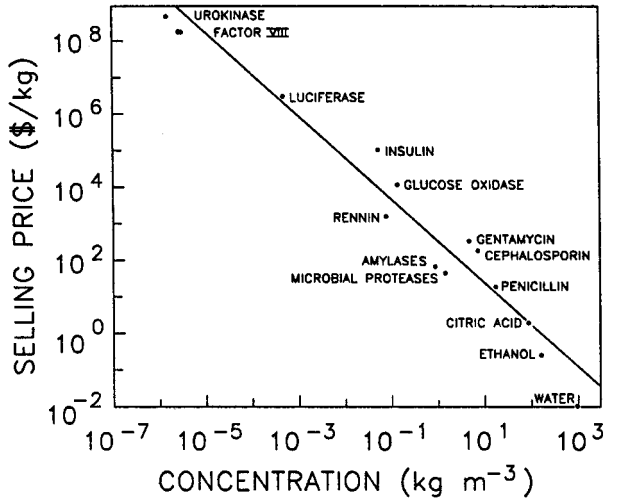


Figure 10. Cost of some bioproducts vs. concentration in the raw fermentation broth.

Downstream processing typically represents 60-80% of the cost of production of fermentation products. Thus, superficially it may appear that process improvement should focus on downstream. This is not so. Even small improvements in the yield or purity of the product in the bioreaction step can have a significant effect on downstream recovery costs. This is clearly shown in Figure 10, where the cost of production (reflected in selling price) of several biochemicals is plotted as a function of the product concentration in the fermentation broth or the starting material. The potential for yield improvement at the bioreaction stage is usually high. Major yield enhancements have been fairly commonly achieved by strain selection, medium development and environmental control. Process improvement or intensification should emphasize a global approach. Ascertaining the impact of process changes and monitoring the separation and purification steps require extensive chemical and biochemical analytical support.

THE PRODUCTION FACILITY

Biochemical engineers play an important role in the design of buildings which house bioprocessing plants.[76,77] Plant layout, containment and treatment of biohazardous materials,[78] controlled and directed movement of plant personnel, separation of processing areas according to cleanliness and other requirements, control of air flow and air pressure differentials in buildings, production, supply and distribution of utilities, and matters relating to finishings for ease of cleaning and sanitization are some of the aspects which must be considered.

CONCLUSIONS

Biochemical engineering is a flourishing discipline which makes industrial practice of biotechnology possible. Looking through the anatomy of bioprocessing, we saw how the biochemical engineer draws upon chemical sciences to research, design, develop and operate biochemical processes. Multidisciplinary skills in biological, chemical and engineering sciences are essential features of biochemical engineering. Advances in molecular genetics, biochemistry, protein chemistry, microchemical analyses, immunology and other biophysical sciences continue to pose new challenges and provide new tools to the biochemical engineer, hence ensuring new products and better production methods for pharmaceuticals, foods, fuels, bulk chemicals, fine chemicals, and environmental pollution control and resource recovery services.

NOMENCLATURE

A	Arrhenius parameter	(s^{-1})
A_n	Arrhenius parameter for nutrient denaturation	(s^{-1})
E	Total amount of enzyme in reactor	(kg)
ΔE	Activation energy	$(J \cdot kmol^{-1})$
ΔE_n	Activation energy for nutrient denaturation	$(J \cdot kmol^{-1})$
e	Enzyme concentration	$(kg \cdot m^{-3})$
e	Constant (= 2.7183)	(-)
K_s	Michaelis constant	$(kg \cdot m^{-3})$
k_d	Specific death rate	(s^{-1})
k_n	Specific nutrient denaturation rate	(s^{-1})
k_r	Rate constant	(as appropriate)
R	Gas constant	$(J \cdot K^{-1} \cdot kmol^{-1})$
S	Substrate concentration	$(kg \cdot m^{-3})$
S_o	Initial substrate concentration	$(kg \cdot m^{-3})$
T	Absolute temperature	(K)
t	Time	(s)
t_d	Mean doubling time	(s)
V_L	Liquid volume in reactor	(m^3)
X	Biomass concentration (dry)	$(kg \cdot m^{-3})$
X_o	Initial biomass concentration (dry)	$(kg \cdot m^{-3})$
μ	Specific growth rate	(s^{-1})
χ	Conversion	(-)
6-APA	6-Aminopenicillanic acid	
CIP	Cleaning-in-place	
CSTR	Continuous stirred tank reactor	
HTST	High temperature short time sterilization	
PEN-G	Penicillin G	

REFERENCES

1. **Dunnill, P.** (1987), *Chem. Eng. Res. Des.*, **65**, 211-217. Biochemical engineering and biotechnology.
2. **Stiefel, E. I.** (1987), *Chem. Eng. Prog.*, **83**(*10*), 21-34. The technological promise of the biological sciences.
3. **Moo-Young, M.**, editor (1985), *Comprehensive Biotechnology*, vol. **1-4**, Pergamon Press (Oxford).
4. **Neway, J. O.**, editor (1989), *Fermentation Process Development of Industrial Organisms*, Marcel Dekker (New York).
5. **Crueger, W. and Crueger, A.** (1990), *Biotechnology: A Textbook of Industrial Microbiology*, 2nd Edition, Science Tech Publishers (Madison).

6. **Lubiniecke, A. S.**, editor (1990), *Large-Scale Mammalian Cell Culture Technology*, Marcel Dekker (New York).
7. **Moo-Young, M., Chisti, Y. and Vlach, D.** (1993), *Biotechnol. Adv.*, **11**(*3*), in press. Fermentation of cellulosic residues to mycoprotein foods.
8. **Bowen, R. and Pugh, S.** (1985), *Chem. Ind. (Lond.)*, 20 May, 323-326. Redox enzymes in industrial fine chemicals synthesis.
9. **Elder, A. L.**, editor (1970), *Chem. Eng. Prog. Sym. Ser.*, **66**(*100*), 1-97. The history of penicillin production.
10. **Payne, C. C.** (1989), *Chem. Ind. (Lond.)*, 20 March, 182-186. Microbial control of insect pests: current and potential uses.
11. **Moo-Young, M., Chisti, Y. and Vlach, D.** (1992), *Biotechnol. Lett.*, **14**, 863-868. Fermentative conversion of cellulosic substrates to microbial protein by *Neurospora sitophila*.
12. **Lisansky, S.** (1989), *Chem. Ind. (Lond.)*, 7 August, 478-482. Biopesticides: The next revolution?
13. **McNaughton, K. J.** (1989), *Chemical Engineer (Lond.)*, **466**, 44-48. Twenty years of single cell protein ventures.
14. **Banerjee, U. C., Saxena, B. and Chisti, Y.** (1992), *Biotechnol. Adv.*, **10**, 577-595. Biotransformations of rifamycins: Process possibilities.
15. **Bohn, H.** (1992), *Chem. Eng. Prog.*, **88**(*4*), 34-40. Consider biofiltration for decontaminating gases.
16. **Thomas, D. R., Carswell, K. S., Georgiou, G.** (1992), *Biotechnol. Bioeng.*, **40**, 1395-1402. Mineralization of biphenyl and PCBs by the white rot fungus *Phanerochaete chrysosporium*.
17. **Allsop, P. J., Chisti, Y., Moo-Young, M. and Sullivan, G. R.** (1993), *Biotechnol. Bioeng.*, **41**, 572-580. Dynamics of phenol degradation by *Pseudomonas putida*.
18. **Chisti, Y.** (1984), *Daily Times (Nigeria)*, Wednesday, 22 February, 12-13. Biotechnology: Potential and role in Africa.
19. **Bailey, J. E. and Ollis, D. F.** (1986), *Biochemical Engineering Fundamentals*, 2nd edition, McGraw-Hill (New York).
20. **Moser, A.** (1988), *Bioprocess Technology: Kinetics and Reactors*, Springer-Verlag (New York).
21. **Moo-Young, M. and Blanch, H. W.** (1981), *Adv. Biochem. Eng.*, **19**, 1-69. Design of biochemical reactors: Mass transfer criteria for simple and complex systems.
22. **Chisti, Y.** (1989), *Airlift Bioreactors*, Elsevier Applied Science (London).
23. **Belter, P. A., Cussler, E. L. and Hu, W.-S.** (1988), *Bioseparations: Downstream Processing for Biotechnology*, John Wiley (New York).
24. **Wheelright, S. M.** (1991), *Protein Purification: Design and Scale up of Downstream Processing*, Hanser Publishers (New York).
25. **Wang, D. I. C., Cooney, C. L., Demain, A. L., Dunnill, P., Humphrey, A. E., Lilly, M. D.** (1979), *Fermentation and Enzyme Technology*, John Wiley (New York).
26. **Chisti, Y.** (1992), *Chem. Eng. Prog.*, **88**(*9*), 80-85 (1992). Assure bioreactor sterility.
27. **Chisti, Y.** (1992), *Chem. Eng. Prog.*, **88**(*1*), 55-58. Build better industrial bioreactors.
28. **Aiba, S., Humphrey, A. E. and Millis, N. F.** (1973), *Biochemical Engineering*, 2nd edition, University of Tokyo Press (Tokyo).
29. **Crueger, W.** (1990), In: Finn, R. K. and Präve, P., editors, *Biotechnology Focus 2*, Hanser Publishers (New York), pp. 391-422. Sterile techniques in biotechnology.
30. **Chisti, Y. and Moo-Young, M.** (1991), In: Moses, V. and Cape, R. E., editors, *Biotechnology: The Science and the Business*, Harwood Academic Publishers (New York), pp. 167-209. Fermentation technology, bioprocessing, scale-up and manufacture.
31. **Gerhartz, W.**, editor (1990), *Enzymes in Industry*, VCH Publishers (New York).
32. **Mackay, D. and Salusbury, T.** (1988), *Chemical Engineer (Lond.)*, **447**, 45-50. Choosing between centrifugation and cross flow microfiltration.
33. **Bowden, C.** (1985), *Chemical Engineer (Lond.)*, **415**, 50-54. Recovery of micro-organisms from fermented broth.
34. **Macdonald, D.** (1985), *Chemical Engineer (Lond.)*, **412**, 15-17. Evaluation of separation problems for disc bowl centrifuges.
35. **Cooper, A. R.** (1984), *Chemistry in Britain*, **20**(*9*), 814-818. Ultrafiltration.
36. **Butcher, C.** (1990), *Chemical Engineer (Lond.)*, **469**, 50-53. Microfiltration.
37. **Abbott, N. L. and Hatton, T. A.** (1988), *Chem. Eng. Prog.*, **84**(*8*), 31-41. Liquid-liquid extraction for protein separations.
38. **Chisti, Y. and Moo-Young, M.** (1986), *Enz. Microbial Technol.*, **8**, 194-204. Disruption of microbial cells for intracellular products.

39. **Hochuli, E.** (1992), *Pure & Appl. Chem.*, **64**(*1*), 169-184. Purification techniques for biological products.
40. **Fleming, H. L.** (1992), *Chem. Eng. Prog.*, **88**(*7*), 46-52. Consider membrane pervaporation.
41. **Redman, J.** (1990), *Chemical Engineer (Lond.)*, **469**, 46-49. Pervaporation heading for new horizons.
42. **Chisti, Y. and Moo-Young, M.** (1990), *Biotechnol. Adv.*, **8**, 699-708. Large scale protein separations: Engineering aspects of chromatography.
43. **Crueger, A.** (1990), In: Finn, R. K. and Präve, P., editors, *Biotechnology Focus 2*, Hanser Publishers (New York), pp. 365-390. Microbial protoplasts – their applications in biotechnology and genetics.
44. **Chisti, Y. and Moo-Young, M.** (1993), *Chimica Oggi (Chemistry Today)*, **11**(*3-4*), 25-27. Effectively use fragile biocatalysts in bioreactors.
45. **Moo-Young, M. and Chisti, Y.** (1988), *Biotechnology*, **6**(*11*), 1291-1296. Considerations for designing bioreactors for shear-sensitive culture.
46. **Chisti, Y. and Moo-Young, M.** (1989), *Biotechnol. Bioeng.*, **34**, 1391-1392. On the calculation of shear rate and apparent viscosity in airlift and bubble column bioreactors.
47. **Shuler, M. L. and Kargi, F.** (1992), *Bioprocess Engineering: Basic Concepts*, Prentice Hall (Englewood Cliffs).
48. **Chisti, Y.** (1989), *Chemical Engineer (Lond.)*, **457**, 41-43. Airlift reactors - design and diversity.
49. **Chisti, Y. and Moo-Young, M.** (1988), *Biotechnol. Bioeng.*, **31**, 487-494. Hydrodynamics and oxygen transfer in pneumatic bioreactor devices.
50. **Chisti, Y., Kasper, M. and Moo-Young, M.** (1990), *Canad. J. Chem. Eng.*, **68**, 45-50. Mass transfer in external-loop airlift bioreactors using static mixers.
51. **Chisti, Y. and Moo-Young, M.** (1988), *J. Chem. Technol. Biotechnol.*, **42**, 211-219. Prediction of liquid circulation velocity in airlift reactors with biological media.
52. **Chisti, Y., Halard, B. and Moo-Young, M.** (1988), *Chem. Eng. Sci.*, **43**, 451-457. Liquid circulation in airlift reactors.
53. **Mao, H. H., Chisti, Y. and Moo-Young, M.** (1992), *Chem. Eng. Commun.*, **113**, 1-13. Multiphase hydrodynamics and solid-liquid mass transport in an external-loop airlift reactor – A comparative study.
54. **Miura, Y.** (1978), *Adv. Biochem. Eng.*, **9**, 31-56. Mechanism of liquid hydrocarbon uptake by microorganisms and growth kinetics.
55. **Chisti, Y. and Moo-Young, M.** (1988), *Chem. Eng. J.*, **39**, B31-B36. Gas holdup behaviour in fermentation broths and other non-newtonian fluids in pneumatically agitated reactors.
56. **Chisti, Y. and Moo-Young, M.** (1988), *Chem. Eng. J.*, **38**, 149-152. Gas holdup in pneumatic reactors.
57. **Ouyoung, P. K., Chisti, Y. and Moo-Young, M.** (1989), *Chem. Eng. Res. Des.*, **67**, 451-456. Heat transfer in airlift reactors.
58. **Chisti, Y. and Moo-Young, M.** (1987), *Chem. Eng. Commun.*, **60**, 195-242. Airlift reactors: Characteristics, applications and design considerations.
59. **Chisti, Y.** (1993), *Bioproc. Eng.*, **9**, 191-196. Animal cell culture in stirred bioreactors: Observations on scale-up.
60. **Lee, J. M.** (1992), *Biochemical Engineering*, Prentice Hall (Englewood Cliffs).
61. **Webb, C. and Atkinson, B.** (1992), *Chem. Eng. J.*, **50**, B9-B16. The role of chemical engineering in biotechnology.
62. **Eckenfelder, W. W., Goodman, B. L. and Englande, A. J.** (1972), *Adv. Biochem. Eng.*, **2**, 145-180. Scale-up of biological wastewater treatment reactors.
63. **Hill, G. A., Tomusiak, M. E., Quail, B. and Van Cleave, K. M.** (1991), *Environ. Prog.*, **10**(*2*), 147-153. Bioreactor design effects on biodegradation capabilities of VOCs in wastewater.
64. **Choi, Y.-B., Lee, J.-Y., Kim, H.-S.** (1992), *Biotechnol. Bioeng.*, **40**, 1403-1411. A novel bioreactor for the degradation of inhibitory solvents: Experimental results and mathematical analysis.
65. **Eckenfelder, W. W., Argaman, Y. and Miller, E.** (1989), *Environ. Prog.*, **8**(*1*), 40-45. Process selection criteria for the biological treatment of industrial wastewaters.
66. **McDermott, J. B., Unterman, R., Brennan, M. J., Brooks, R. E., Mobley, D. P., Schwartz, C. C. and Dietrich, D. K.** (1989), *Environ. Prog.*, **8**(*1*), 46-51. Two strategies for PCB soil remediation: biodegradation and surfactant extraction.
67. **Halden, K.** (1991), *Trans. Inst. Chem. Eng.*, **69**(*B*), 173-179. Methanotrophic bacteria for the *in-situ* renovation of polluted aquifers.
68. **Redman, J.** (1987), *Chemical Engineer (Lond.)*, **441**, 12-13. Deep shaft treatment for sewage.
69. **Malmos, H.** (1990), *Chem. Ind. (Lond.)*, 19 March, 183-186. Enzymes for detergents.
70. **Kennedy, J. F., Melo, E. H. M. and Jumel, K.** (1990), *Chem. Eng. Prog.*, **86**(*7*), 81-89. Immobilized enzymes and cells.

71. **Varey, P.** (1992), *Chemical Engineer (Lond.)*, **529**, s37. Air lift for purity.
72. **Moo-Young, M. and Chisti, Y.** (1992), In: Ladisch, M. R. and Bose, A., editors, *Harnessing Biotechnology for the 21st Century*, American Chemical Society (Washington), pp. 174-177. Transport phenomena in novel bioreactors: Design of airlift-based devices.
73. **Dabora, R. L. and Cooney, C. L.** (1990), *Adv. Biochem. Eng./Biotechnol.*, **43**, 11-30. Intracellular lytic enzyme systems and their use for disruption of *Escherichia coli.*
74. **Dörnenburg, H. and Knorr, D.** (1992), *Process Biochem.*, **27**, 161-166. Release of intracellularly stored anthraquinones by enzymatic permeabilization of viable plant cells.
75. **Fish, N. M. and Lilly, M. D.** (1984), *Biotechnology*, **2**, 623-627. The interactions between fermentation and protein recovery.
76. **Nelson, K. L.** (1991), In: Prokop, A., Bajpai, R. K. and Ho, C. S., editors, *Recombinant DNA Technology and Applications*, McGraw-Hill (New York), pp. 509-565. Biopharmaceutical plant design.
77. **Hill, D. and Beatrice, M.** (1989), *BioPharm*, October, 20-26. Biotechnology facility requirements, Part I. Facility and systems.
78. **Lee, R. and Waltz, J.** (1990), *Chem. Eng. Prog.*, **86**(*12*), 44-48. Consider continuous sterilization of bioprocess wastes.