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INVITED REVIEW PAPER

Recent advances in bioreactor engineering

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Abstract−A bioreactor is the core of biological processes. To design an appropriate bioreactor system for a particular bioprocess, intensive studies on the biological system, such as cell growth, metabolism, genetic manipulation, and protein or other product expression are needed to understand the cells' requirement on their physical and chemical environments. It is also necessary to control and optimize the bioreactor environment via operating variables in order to favor the desired functions of cells and achieve cost-effective large-scale manufacture. This article briefly describes fundamental design principles and new types of bioreactors such as centrifugal impeller and wave bioreactors. Bioreactor operation factors and modes including mixing, oxygen supply, shear force, fed-batch, and perfusion cultures are discussed. The trends in bioreactor engineering are also briefly shown.

Key words: Bioreactor Engineering, Bioprocess, Scale-up, Bioreactor Operation Strategy, Process Variables

INTRODUCTION

A bioreactor is a vessel in which a biological reaction or change takes place. The biological systems involved include enzymes, microorganisms, animal cells, plant cells, and tissues. To design an appropriate bioreactor for a particular bioprocess, intensive studies on the biological system, such as cell growth, metabolism, genetic manipulation, and protein or other product expression, are needed to understand the cells' requirement on their physical and chemical environments. Various bioreactor types and configurations have thus been exploited and developed along with the advances in the understanding of biological systems. In addition, it is necessary to control the bioreactor operating variables in order to favor the desired functions of the living cells or enzymes. Dissolved oxygen concentration, pH, temperature, mixing, and supplementation of nutrients all need to be controlled and optimized. Because two distinct bodies of knowledge, i.e., molecular biology and process engineering, are involved and bioreactor is the core of bioprocess, a systematic science-based approach to studying bioreactor is needed and the term "bioreactor engineering" becomes more appropriate than "bioreactor" or "fermentor."

Among the bioreactor types available for a certain bioprocess, it is important to have a balanced consideration of many factors, including oxygen transfer, mixing, shear, operational stability and reliability, scale-up, and cost. The chosen bioreactor should be further characterized and the operational mode should be optimized. The bioreactor characteristics and operational mode also greatly affect the biological performance. An efficient bioreactor system relies greatly on its control and support systems. No matter how important the bioreactor system is, it must be closely and efficiently integrated into the whole production system.

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DESIGN AND TYPES OF BIOREACTORS

Most biological reaction systems can be classified into two main groups including suspension systems and immobilization ones. Stirred tank, air-lift and bubble column bioreactors are mainly for the former case; membrane, packed bed, and fluidized bed bioreactors are mainly for cultivating attached cells or immobilized enzymatic reactions. There are some bioreactors that can be applied in both of these two categories. For example, the immobilized cells or enzymes on carriers can be suspended in stirred tank or air-lift/bubble column bioreactors.

1. Fundamental Design Principles

The design and selection of each type of bioreactors is unique but there are some fundamental principles. In general, the main criteria for designing bioreactor should consider adequate oxygen transfer, low shear stress and adequate mixing [1,2]. In considering the bioreactor design and selection, nutrients must be effectively provided to the cells, and waste products must be removed. Cell growth and product formation kinetics should be assessed so that an optimal environmental condition can be defined and an operational mode can be determined. Transport phenomena should be studied in order to define the criteria for bioreactor design and scale-up. Operating parameters, such as temperature, pH, dissolved oxygen and substrate concentrations should be easy to control and monitor. In addition, the bioreactor should be as simple and inexpensive as possible and it should easily operate free of contamination with microorganisms. In the biopharmaceutical industry, bioreactor design and selection should also consider current Good Manufacture Practices (cGMP) compliance. Most often it is impossible to meet all the requirements, and generally it is very important to give a balanced consideration between mixing and mass transfer requirements and the shear sen-

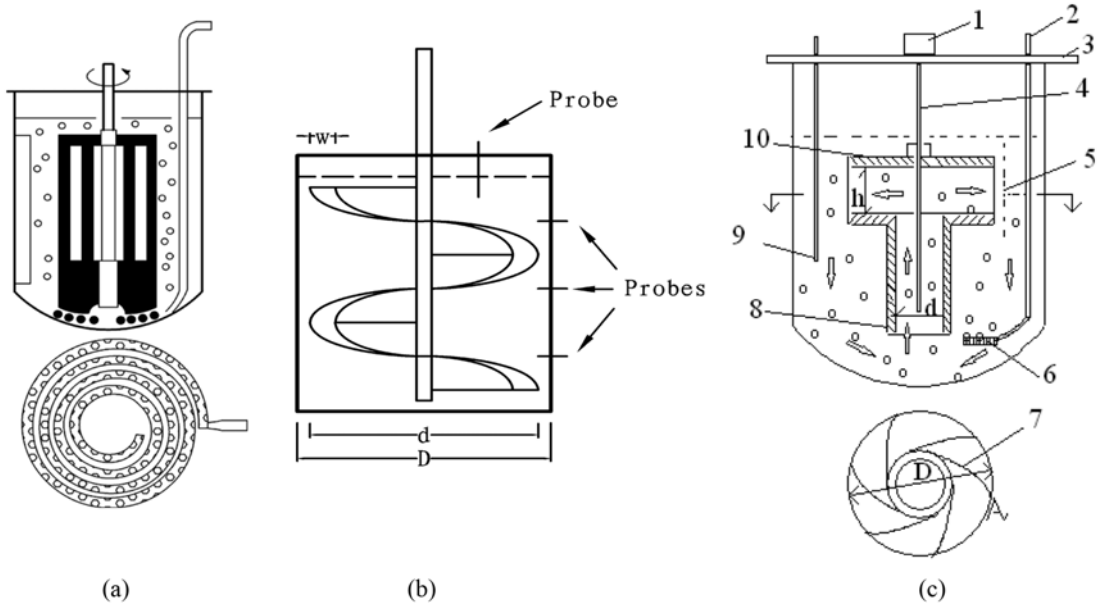

Fig. 1. Schematic diagram of stirred-tank bioreactors (STRs) with different impellers. (a) Gate paddle bioreactor with a spiral sparger; (b) helical ribbon impeller bioreactor; (c) centrifugal impeller bioreactor.

1. Stirrer
2. Gas in
3. Head plate
4. Shaft
5. Measuring points for liquid velocity
6. Sparger
7. Blade
8. Draft tube
9. DO probe
10. Rotating pan

Table 1. Large scale processes of animal cells (adapted from [10])

Cell line	Scale (L)	Reactor	Product
BHK 21	10,000	Agitated tank	Foot and mouth disease vaccine
CHO	10,000	Agitated tank	tPA
Namalwa cells	8,000	Agitated tank	Lymphoblastoid interferon
Bowes melanoma	7,000	Agitated tank/microcarrier	tPA
Murine hybridoma	2,000	Air-lift	mAbs
Vero cells	1,000	Agitated tank/microcarrriers	Killed polio vaccine
Murine hybridomas	1,000	Stirred-tank	mAbs against cell-surface antigens of Adenocarcinomas
BHK	500	Agitated tank/perfusion	Factor VIII

sitivity of cells in the design of large-scale bioreactors [3].

2. Stirred-tank Bioreactor (STRs)

STRs are one of the most conventional bioreactors. Due to its advantages such as easy scale-up, good fluid mixing and oxygen transfer ability, alternative impellers and easy compliance with cGMP requirements, STRs are commonly used. However, this type of bioreactor also has several limitations, such as high power consumption, high shear, and the concerns about sealing and stability of shafts in tall bioreactors. Compared to microorganisms, both animal cells and plant cells are shear-sensitive, which results in remarkable efforts to modify and optimize the impeller system in order to balance mixing and mass transfer requirement and potential damage by hydrodynamic force. Numerous modifications of conventional STRs have been made by developing new impeller designs such as a centrifugal impeller (Fig. 1) [4-8]. Several biopharmaceutical manufacturers have successfully implemented STRs at 10,000 to 20,000 liter scale for large-scale animal cell cultures (Table 1) [9,10].

3. Wave Bioreactors

Wave reactors (Fig. 2) [11] can generate a wave motion by mechanically rocking of a culture-containing bag back and forth. These waves provide mixing and mass transfer, resulting in a suitable environment for suspension culture of both plant and animal cells. Several cell lines including CHO cells, NS0 cells, hybridoma cells, insect cells, as well as anchorage-dependant cells using microcarriers, have been cultivated in this reactor system. In addition, the reactor uses a pre-sterilized, plastic disposable chamber, providing the ultimate ease in operation and protection against cross-contamination. The chamber is placed on a special rocking platform and facilitates the compliance of cGMP regulations.

Based on similar principle, Terrier and coworkers [12] designed a wave and undertow (WU) bioreactor and a slug bubble (SB) bioreactor. The WU bioreactor provides agitation while offering con-

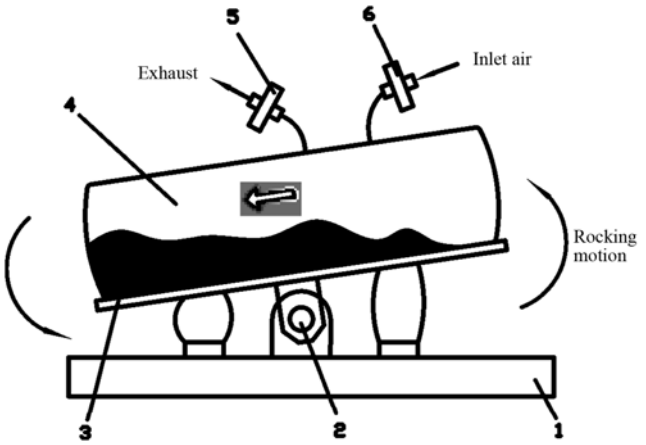

Fig. 2. Schematic of disposable wave bioreactor [11].

1. Base
2. Pivot
3. Cell culture media in bag
4. Inflated plastic bag forms a disposable cultivation chamber
5. Exhaust vent filter
6. Inlet air filter

venient mixing and aeration to the cell culture in the bioreactor. The SB bioreactor is a high aspect ratio bubble-column bioreactor, whose agitation and aeration are achieved through the intermittent generation of large diameter bubbles. Both bioreactors can have application in various cell cultures on small to medium scales.

Recently, such wave bioreactor systems have been scaled up to a capacity of 1,000 L, and a cell density as high as 10^7 cells/ml was achieved [11,13].

BIOREACTOR OPERATION FACTORS AND STRATEGIES

The main objective of bioreactor selection, design, and control is to provide the optimal environment for a biological reaction system, and the bioreactor should provide optimum conditions such as temperature, pH, oxygen transfer, mixing, and substrate concentration. In order to understand bioreactor operation, cellular metabolism must be considered together with the flow profile and the mass transfer characteristics of the bioreactor because they closely interact with each other.

1. Mixing

In bioreactors, adequate mixing is essential in order to ensure the adequate supply of nutrients and to prevent the accumulation of toxic metabolites. For a bioreactor designed for a suspension system, mixing time is a critical parameter to be studied and evaluated. The fluid hydrodynamics, fluid rheology, impeller type, power input, and vessel size can all influence the mixing conditions. Generally, the following equation can be used to describe the effects of different parameters on the mixing time in a stirred tank bioreactor [14]:

$$t_m = f\left(\frac{d}{D}, N, \eta, \rho, \frac{V_a}{V}, \frac{P}{P_a}, \varepsilon_T\right)$$

where t_m is the mixing time (s), d is the stirrer diameter (m), D is the bioreactor diameter (m), N is the impeller rotational speed (rpm), V is the medium volume (m^3), V_a is the volumetric air flow rate ($m^3\ s^{-1}$), P is the power consumption for mixing non-aerated broth (W), P_a is the power consumption for mixing aerated broth (W), η is the apparent viscosity (cP), ρ is the density (kg m^{-3}), and ε_T is the energy dissipated (Wm^{-3}).

It was found that the presence of biomass significantly reduced the mixing efficiency, even at low broth viscosity levels. The magnitude of this effect depends on the type of biomass and its concentration and morphology. The mixing time increases in the following order: fungal free mycelia>fungal pellets>yeasts>bacteria. At the same concentration and under the same operational conditions, the mixing time for fungal cell suspensions was significantly higher due to their high viscosity and non-Newtonian behavior.

In large-scale bioreactors, poor mixing often leads to undesirable concentration gradients and a decrease in mass transfer efficiency. In animal and plant cell cultures, which are typical shear sensitive biological systems, mixing could be enhanced by increasing agitation speeds and/or aeration rates in agitated bioreactors, but it has to balance with the increase of shear stress because shear force caused by intense agitation or aeration can bring damage to living cells. How to find the maximum agitation speed without causing cell damage is important. Baffles are recommended to be installed to increase mixing performance and eliminate detrimental center vortex. Cell damage in baffle zone should not be a concern generally since its hydrodynamic force was lower than that at impeller zone [15].

2. Oxygen Transfer

Most nutrients required for cellular growth and metabolism are highly soluble in water, however, oxygen transfer often becomes a limiting step to the optimal performance of biological systems and also for scale-up because oxygen is only sparingly soluble in aqueous solutions. When the supply of oxygen is limited, both cell growth and product formation can be severely affected. For example, it was reported that ceasing aeration in the medium during penicillin fermentation for just a few minutes seriously impacted the ability of the cells to produce the antibiotic [16]. In a well-mixed suspension system, the oxygen mass balance is written as:

$$\frac{dC_o}{dt} = k_L a(C_o^* - C_o) - Q_o X$$

where $k_L a$, C_o^*, C_o and Q_o are the volumetric mass transfer coefficient, saturated oxygen concentration, the oxygen concentration in the liquid, and the specific oxygen uptake rate, respectively.

There are numerous reports on the effects of oxygen concentration or oxygen transfer on microbial fermentation. Although the oxygen consumption of plant and animal cells is lower than that of microorganisms, limitation in oxygen transfer is also often a constraining factor for cell cultures at high cell density. Maintaining a suitable oxygen concentration in the culture broth is equally important. The optimal dissolved oxygen concentration may be different for cell growth and product formation [17].

3. Shear Force

Compared to microorganisms, both plant and animal cells are much bigger and more sensitive to shear force. Shear has been mainly considered in the technical literature as a destructive element. A couple of parameters are related to the shear-induced cell death, such as shear stress, shear time, power dissipation and the growth phase of the cells. As aforementioned, sparging can also cause the shear damage, which can happen at different locations in bioreactor: bubble generation zone at the sparger; the rising zone through the bulk

liquid; and the surface of the suspension (either be covered with foam layer or free of foam). The mechanism of cell damage at bubble disengagement zone is as follows: cells attach to the bubble surface [18], and then bubble bursts at the suspension surface, which causes high shear [19]. Small bubbles cause more severe damage than large bubbles [20]. To analyze the effects of shear stress on plant cell cultures, a quantitative investigation of the influence of hydrodynamic shear on the cell growth and anthocyanin pigment production by *P. frutescens* was demonstrated by using a plant cell bioreactor with marine impeller of larger (85 mm) or smaller (65 mm) diameter [21]. There have been abundant reports on shear damage suffered by animal cells grown in suspension, especially damaged by disengaging bubbles [22-26].

For shear-sensitive cell cultures, therefore, reducing the shear stress intensity by decreasing the agitation speed of the impeller is a general solution. However, this can also bring inadequate mixing and may conflict with enhancing oxygen and heat transfer rates in a high-viscosity cell culture broth. Furthermore, at high biomass concentrations, low agitation rates can also enhance the clumping of cells into cell aggregates of various sizes. However, appropriate bioreactor design and control can minimize shear damage from agitation and aeration. Wang and Zhong successfully designed a novel centrifugal impeller bioreactor (CIB) (Fig. 1(c)) for shear-sensitive biological systems including animal and plant cell cultures [27,28]. This CIB has been demonstrated to be very successful in high cell density plant cell cultures [29,30-35].

Another possibility to overcome the problem of the rising air bubbles is bubble-free aeration using membranes for indirect aeration, in which the supply of oxygen is diffusion controlled and no bubbles arise. As the length of the membrane is limited, the average oxygen transfer rate is low. Also, the pressure inside the tubing has limitation. Therefore, this type of bioreactor is quite suitable for small scale cell cultures but limited for large scale [10].

4. Fed-batch Culture

Generally, the final product concentration is primarily affected by the specific productivity of cells and integrated cell growth. To overcome nutrient limitation, fed-batch processes have been widely practiced which involves the addition of one or more nutrients continuously or intermittently to the initial medium after the start of cultivation, or from certain point during the batch process [36]. Fed-batch cultures are currently used for most cell culture processes, especially for intracellular products of cell cultures which are stored within the cells. For example, in *P. ginseng* cell culture for ginsenoside production [37] and *Taxus chinensis* [38] cell culture for taxoid production, fed-batch cultivation has been successfully demonstrated. During the cultivation of *Coptis japonica* to produce berberine in STR, the biomass concentration was reduced due to high osmotic pressure of the culture medium and accumulation of inhibitory products during cell growth, and this problem was solved by suitable fed-batch operation which enhanced both cell growth and berberine production at high cell density [39,40]. Hu et al. [41] performed fed-batch cultivation of *P. notoginseng* cells in a concentric-tube air-lift reactor to study the effects of bottom clearance on cell growth and the production of ginseng saponin and polysaccharide. A fed-batch operational mode was also developed, in which sucrose was fed just prior to a sharp decrease in the specific OUR (>0.20 mmol/g DW/h) [42]. By applying this feeding policy to *P. notoginseng* cell suspensions, cultivated in a 10-L air-lift bioreactor [42] and a 30-L CIB [30], very high biomass productivity of approximately 1.5 g/(L·d) was achieved, and both saponins and polysaccharide productivities were also higher than in a comparative batch process. In mycelia fermentation of *Ganoderma lucidum*, a famous Chinese traditional medicinal mushroom, Tang and Zhong developed a fed-batch process for simultaneous efficient production of bioactive ganoderic acids and *Ganoderma* polysaccharides [43]. The metabolites production was remarkably improved by pulse feeding of lactose in both shake flask and bioreactor.

Fed-batch processes are also common in animal cell cultures and have been operated at scales up to 25,000 L working volume for large-scale antibody production [44]. Huang et al. recently reported up to 13 g/L of final titer and high volumetric productivity of 800 mg/(L·d) in an 18-day fed batch process using a CHO cell line without anti-apoptosis gene [2]. It can be predicted that more companies can achieve this level in future, which will increase manufacture capability dramatically and put a positive pressure on downstream purification. In another case, due to the forecasted market demand is beyond the designed capability of the first-generation process using packed-bed perfusion technology, Meuwly et al. [45] converted it to a fed-batch model at 15 m^3 and increased product output by 18 folds while maintaining a comparable product quality.

A comparison of different feeding modes is presented in Table 2. Among these, the most popular and successfully used ones are fed-batch and perfusion.

5. Perfusion Culture

Perfusion model is normally chosen for the production of unstable proteins. This is because perfusion culture has low resident time in bioreactor and the product can be harvested and purified promptly. In addition, it can avoid the accumulation of toxic or inhibitory metabolites that might have negative impact on the productivity of fed-batch cultures. Perfusion cultivation is carried out by continuously feeding fresh medium to the bioreactor and constantly removing the cell-free spent medium while retaining the cells in the reactor; thus a much higher cell density can be obtained in perfusion cultures compared to continuous cultures, as cells are retained within

Table 2. Comparison of some commonly used modes of feeding based on product yield and process economics [2]

Culture mode	Product yield	Process manipulation	Cost (capital investment and labor)	Throughput
Batch	Low	Low	Low	Low
Repeated batch	Low	Medium	Medium	Medium
Fed-batch	High	Medium	Low	Low
Chemostat	Low	Medium	Low	Low
Perfusion	Medium	High	Medium	High

the reactor via a cell retention device. The perfusion rate depends on the demand of the cell line, the concentration of nutrients in the feed and the level of toxification.

The overall advantage of perfusion cultures is that it requires smaller scale compared to batch cultures in order to obtain the desired amount of product such as plant secondary metabolite [46], and perfusion culture has been used in mammalian cell processes to produce at least seven commercialized products [47]. However, it has low product titer compared to fed-batch culture, which brings challenges to downstream purification.

Konstantinov et al. [48] proposed a "push-to-low" method, decreasing perfusion rate until it failed to maintain high viability, in order to reach comparable titer as batch or fed-batch culture. Using transient gene expression (TGE) to produce large amounts of recombinant proteins within a short period of time has recently attracted considerable interest [49]. Compared with the batch modes, which the transfections are conducted at a low cell density, high-density perfusion culture has been used in the production of recombinant adenovirus with a significant higher production [50,51]. The cell densities achieved in perfusion culture (10^7-10^8 cells/mL) are typically higher than most fed-batch processes ((1-30)×10^6 cells/mL). In specific cases it has been demonstrated that continuous perfusion offers an approximately 10-fold improvement in volumetric productivities compared to fed-batch culture [52,53]. Jardin et al. designed high cell density fed batch and continuous perfusion processes to maximize the productivity of stably transformed cell (Sf-9SEAP), with the total yield of 17.6 IU in batch process, of 46.1 IU in fed batch process, and of 394 IU in perfusion process [54]. Deo et al. [55] reported a 500 L scale spin filter perfusion hybridoma culture for antibody production that was run for 15-35 days. They estimated that the volumetric productivity of the perfusion process was approximately 10 times that of batch or fed-batch cultures. Coagulation factor VIII, licensed by Bayer, was the first biopharmaceutical produced by recombinant BHK cells using continuous perfusion culture. Bayer validated a 185-day production process. Compared to the batch culture, the advantage of Bayer's production process is a 30-fold increase in cell density that results in a 30-fold higher yield of factor VIII. This leads to significantly reduced requirements for plant capacity: a 100-500 L perfusion reactor vs. a 5,000-15,000 L batch reactor [56].

6. Bioreactor Scale-up

Bioreactors play an important role in many industries, including fermentation, food, pharmaceuticals, and wastewater treatment. Recently, the major challenge of bioreactor scale-up has been how to translate the laboratory-scale product designs into large-scale production. During the scale-up of cell culture processes, reduced productivity has often been reported, which can be attributable to any of several factors including shear stress, oxygen supply, and gas composition. The process characteristics which have been suggested to be maintained constant during scale-up include such criteria as volumetric oxygen transfer coefficient, k_La; maximum shear; power input per unit volume of liquid, P_g/V; volumetric gas flow rate per unit volume of liquid, Q/V or vvm; superficial gas velocity, v_s; and mixing time, which are commonly considered for scale-up of cell culture bioreactors.

7. Trends in Bioreactor Engineering

Bioreactor engineering science is experiencing rapid progress, and in recent years, microbioreactors have received great interest. With the tremendous progress in functional genomics, metabolic engineering and systems biology, there is a great potential for a single cell working as a super bioreactor. It is also very exciting to see more and more achievements using plants and animals as integrated bioreactor systems.

8. Microbioreactor

Low-cost microbioreactors or miniature bioreactors have been designed for use in high-throughput bioprocessing with applications ranging from media development and strain improvement to process optimization. An optical sensing system was used for continuous measurements of pH, dissolved oxygen, and optical density in a microbioreactor with 2-mL working volume [57]. When used for *Escherichia coli* fermentation, the microbioreactor showed similar pH, dissolved oxygen, and optical density profiles as those in a standard 1-L bioreactor. This work provided a basis for developing a multiple-bioreactor system for high-throughput bioprocess optimization, such as a low-cost noninvasive optical CO_2 sensing system for fermentation and cell culture [58], 24-well plate miniature bioreactor for microbial cultivations assessment [59], and as a scale-down model for cell culture process development [60].

Zanzotto et al. fabricated a microbioreactor, with microliter volume, out of poly(dimethylsiloxane) (PDMS) and glass [61]. Aeration was done through a gas-permeable PDMS membrane. Sensors were integrated for on-line measurement of optical density (OD), dissolved oxygen (DO), and pH, all of which were measured based on optical methods. Bacterial fermentations carried out in the microbioreactor under well-defined conditions were found to be comparable to the fermentation in a 500-mL bench-scale bioreactor. The behavior of the bacteria in the microbioreactor was similar to that in the larger bioreactor. Furthermore, it was demonstrated that the sensitivity and reproducibility of the microbioreactor system were such that statistically significant differences in the time evolution of the OD, DO, and pH could be used to distinguish between different physiological states.

To improve primary adult rat hepatocyte cultures, two types of PDMS microbioreactors containing a membrane used as a scaffold for cell attachment were built: one with a commercially available polyester membrane, the other with a PDMS membrane (5×5 mm hole size) made in the laboratory. These new membrane-based PDMS microbioreactors, which closely mimic the *in vivo* liver architecture, revealed themselves to be very promising tools for future applications in drug screening and liver tissue engineering [62].

In an effort to develop microbioreactor device for animal cell culture processing, Hung et al. [63,64] recently designed a 10×10 microfluidic array for continuous perfusion culture. The 10×10 array was fabricated on a 2×2 cm device, consisting of a circular microfluidic chamber, a set of narrow perfusion channels surrounding the main chamber, and four ports for fluidic access. Human carcinoma (HeLa) cells were cultured inside the device, and the successful operation of the continuous perfusion culture was verified over 16 days. The device functioned well for repeated cell growth/passage cycles, reagent introduction, and real-time optical analysis [64].

9. Cell as a Super Bioreactor

Many different kinds of commercially important products are derived from the cell factory, and metabolic engineering serves as an integrated approach to design new cell factories by providing

rational design procedures and valuable mathematical and experimental tools [65]. For example, lactic acid bacteria were metabolically engineered to produce important compounds, including diacetyl, alanine, and exopolysaccharides [66]. As a consequence of large sequencing programs, the complete genomic sequence has become available for an increasing number of organisms. This has resulted in substantial research efforts in assigning functions to all identified open reading frames - referred to as functional genomics. In both metabolic engineering and functional genomics, there is a trend towards the application of a macroscopic view to cell function, leading to an expanded role for the classical approach in microbial physiology. With the increased understanding of molecular mechanisms, it will be possible to describe the interaction between all the components in a cellular system (the cell) at the quantitative level. This is the goal of systems biology, and would significantly facilitate studies on microbial physiology and metabolic engineering [67].

It is very interesting to engineer the plant cell factory for secondary metabolite production, because plants synthesize an extensive array of secondary metabolites that can be used as drugs, dyes, flavors, and fragrances. These plant metabolites often have highly complex structures. Currently, most pharmaceutically important secondary metabolites are isolated from wild or cultivated plants because their chemical synthesis is not economically feasible. To increase secondary metabolite production, different strategies may be adopted, such as overcoming rate limiting steps, reducing flux through competitive pathways, reducing catabolism, and overexpressing regulatory genes [68]. Our limited knowledge of secondary metabolic pathways and the genes involved is one of the main bottlenecks. However, advances in plant genomics and metabolite profiling offer unprecedented possibilities for exploring the extraordinary complexity of plant biochemical capacity. State-of-the-art genomics tools can be used to enhance the production of known target metabolites or to synthesize entire novel compounds by so-called combinatorial biochemistry in cultivated plant cells [69]. Plant cell cultures combine the merits of whole-plant systems with those of microbial and animal cell cultures and already have an established track record for the production of valuable therapeutic secondary metabolites. Although no recombinant proteins have yet been produced commercially using plant cell cultures, there have been many proof-of-principle studies and several companies are investigating the commercial feasibility of such production systems [70].

The heterogeneity of plant secondary metabolites is an extremely interesting and important issue because these structurally similar natural products have different biological activities. For example, Rg_1 stimulates the central nervous system, whereas Rb_1 tranquilizes it and Rc inhibits it. It is very advantageous to intentionally manipulate the heterogeneity of secondary metabolites in cell cultures by altering or stimulating their genome and/or the subsequent processes, resulting in the enzymatic biosynthesis of secondary metabolites and allowing the production of secondary metabolites with a high degree of chemical diversity from the existing plant cell culture library. The main strategy for manipulating the production of individual ginsenosides is to intentionally change external environmental factors in cell cultures [71]. It is expected that the dream of manipulating plant cells in order to directly produce various high-value-added secondary metabolites will come true with the advancement of functional genomics and plant metabolic engineering.

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