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Review

Prospectives on Mammalian Cell Culture Engineering

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동물세포의 대량배양에 관한 고찰

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Introduction

Mass cultivation of mammalian cells has intensively been investigated even though cell culture techniques have been developed for long times (1-16). There have been several difficulties in maintaining large quantities of live cells, especially more than millions of cells per m*l* in over five liter vessel. Fragility of animal cell, shopisticated culture conditions (including specific requirements of media) and less economical feasibility of being scaled-up were major bottle-necks in industrializing cell culture technologies to produce a large amount of pharmaceuticals, tissue Plasminogen Activator (tPA), Interferon, Factor VIII, Erythropoietin (EPO) and many other monoclonal antibodies (MAb).

This article is to introduce most recent biotechnological developments related to animal cell culture engineering. Essential biochemical engineering parameters will be discussed, and new methodology of cell culture will also be proposed. Table 1 indicates the trends of shifting traditional fermentation systems to animal cell cultures (4), because animal cell cultures have advantages in producing those products compared to bacterial and yeast fermentation (5-7). Table 2 contains the list of pharmaceuticals made from mammalain cell cultures (4).

Engineering considerations

First of all, the oxygen transfer into the media is the crucial factor in designing a "Bioreactor" (not to be confused with fermenter). Several methods have been applied to increase oxygen transfer rate and to keep certain levels of dissolved oxygen for fast growing cells. It would be said that the increasing gas flow rate could enhance gas(oxygen) transfer rates; however, it will cause to generate a lot of foams. The foams are very harmful especially in cultivating hybridomas. Therefore, conventional gas sparging system has never been used for the cell culture reactors. Gas permeable tubing is one of the methods to solve this problem, which is used by many biotechnology companies (8,9). The diffusivities of interested gases (oxygen, carbon dioxide and nitrogen) greatly vary depending on the composition of the tubing materials. Selection of

Table 1. Number of companies and other organizations that are involved in activities related to animal cell culture.

Country	Total Numbers
United States	579
Japan	100
United Kingdom	97
France	43
West Germany	37
Canada	23
Israel	14
Denmark	6
South Korea	3
India	2
Taiwan	2
Brazil	1

Table 2. Several mammalian cell culture products and the conditions for which they might be useful.

Product	Disease or Condition
Lymphokines	Viral infection
Erythropoietin	Anemia
	Anemic hemodialysis
Recombinant insulin	Insulin-dependent diabetes
Beta cells	Diabetes
Tissue Plasminogen Activator	Heart attacks
Protein C	Hip surgery
Factor VIII	Hemophilia
Alpha interferon	Hairy-cell leukemia

those tubings requires to understand conditional parameters of gas pressures, flow rates and vapor condensations. This method certainly has great advantages of not generating foams and never being contacted by media. The latter advantage is very important in analyzing oxygen uptake rates. However, water saturated gases in outlet stream may be a problem in controlling gas flow rates due to the condensation in the gas line.

New Brunswick Scientific (N.J., U.S.A.) developed a method to sparge gases into the reactor through an agitation shaft at the center (Patent no. 4680267, U.S.A.). The liquid flows into the opposite direction by centrifugal forces to contact with fed-in gases, whose method is similar to an air-lift fermenter. Air-lift fermenter is also used in L & H and Verax (U.S.A.) Co's. However, their systems require special type of carriers or holders for floating cells, and have physical limitations of oxygen transfer at certain portion of the reactor. The sedimentation of media through double settling bottles is developed for gentle supply of gases into the system (Kyowa Hakko Kogyo co., Japan). Modified spargers at the bottom of the reactor are used in rather small size vessels (200 m*l* to 1 *l*). The head gas sweep method has also been utilized in laboratory scales, from T-flask to 1-2 *l* spinner vessels.

It is apparent that the mixing should help increase the mass transfer rate when one of the above-mentioned methods is used. However, another factor to be considered is that the shear forces can damage mammalian cells much seriously than yeasts or bacteria due to the fragility of animal cell. Numerous reports on this subject have been published (10-13). Hybridomas are much susceptible to shear forces than anchorage-dependent cells. Cherry and his associates (14) found that low RPMs enhanced bead bridging, causing to decrease the growth rate of bovine embryonic cells. Dimension of microcarrier is another important factor. Generally speaking, the diameter of beads should be bigger than eddy effects, 200-250 μm, because of eddy velocities and collision of beads (13).

The concept of controlling dissolved oxygen concentrations is not a new idea. However, the applications to animal cell culture processes have not been widely considered, since it requires absolutely fine control systems and highly reliable measuring devices. Commercially available electrode type dissolved oxygen probe is not autoclavable and easily drifts within a couple of months (needs regular calibrations). The sterile replacement of old probe is not an easy task, either. These problems should be resolved for on-line automatic control of dissolved oxygen concentration. Invitron (U.S.A.) adapts the novel idea to control dissolved oxygen level in the media by measuring the oxygen concentration in the gas phase equilibrated with liquid through gas permeable tubings. The absolute measurement error was $\pm 1\%$. Supply of required gases and removal of over-flow gases can easily be controlled by combination of solenoid valves and simple interfacing devices with host computer within the maximum error of $\pm 1.5\%$.

Bioreactor

Several types of bioreactors have been developed to maintain more than millions of cells per m*l*; modification of conventional fermenter, hollow-fiber reactor, air-lift type, glass-bead (ceramic) design and perfusion system. Advantages and disadvantages of these systems are discussed in this section.

Many biotechnology companies (Ely Lilly, Upjohn and Celltech, etc.) have made effort to convert

bacterial fermentation systems to bioreactors by using marine type impellers and special gas systems for anchorage-dependent cells. Generally, they are of large scales, up to 2000 liter vessels. However, the effectiveness of cell growth is relatively low, because most of them are batch systems and hydrostatic and shear forces are large along with less effective oxygen transfer. Air-lift bioreactor has been empolyed to overcome those problems by Verax Co. (N.J., U.S.A.). The company uses special types of beads to carry animal cells (for both anchorage dependent and independent cells) in a fluidized bed reactor. The supplying media flows in opposite direction to the gas flow to have better oxygen transfer and less shear stress, but it has high probability of collisions between beads.

Glass-bead and hollow-fiber reactors have favorite advantages for economical production of recombinant proteins and/or monocolnal antibodies by using serum-free media. However, these systems have concentration gradients of dissolved oxygen within the reactor because of poor uniformity of cell growth in the plug flow. They also have difficulties in measuring cell density. Current methods such as monitoring glucose uptake rate and correlating that value with cell density are highly inferential. A researcher in Colorado State University (U.S.A.) (15) developed to measure the electrical conductivity of whole cell suspension by using two electrodes inside the reactor. However, selective cellular products would have some effect on the conductivity measurements. His system had 30 percent of cell density of the reactor volume (typical cell densities in commercially available hollow-fiber bioreactor run at about 10 percent). This system can maintain cell densities up to 2×10^8 total cells per m*l*.

The perfusion system, where fresh media continuously flows into and wastes and products come out of the reactor, has also been developed (8). This has great advantages of continuous removal of lactic acid and other toxic wastes inside the reactor. Therefore, it turns out that the perfusion system can maintain cell density up to 10^9 total cells per m*l*. This also allows to make complete on-line processes of both up-and down-streams. This system can be highly competitive with most other methods that can not be operated over two months. Perfusion chemostat set-up has another merit in maintaining mechanically and biochemically constant conditions throughout the whole runs. But this system needs to improve cell recycle method to obtain the highest and most prolific growth. Current technique of recycling cells has problems of attaching cells to the filtration membrane and blocking the liquid flow. New ultra hollow-fiber system can be an alternative to resolve this problem. It must be remembered, however, that the prolific growth stage would not be the best condition to produce certain products. So a novel reactor (Static Maintenance Reactor) has been developed by a company in America (16). This process has an attractive merit of reducing serum concentrations and possibly running in serum-free media, even though there is a difficulty in maintaining constant flows of media. This causes the off-balance of pH control and difficulties in keeping certain levels of dissolved oxygen.

Conclusions

It is obvious that the commercialization of mammalian cell-drived products requires mass cultivations of cells by scaling up to production level. Current cell culture techniques and bioreactors must be further improved by considering essential biochemical engineering parameters, which are defined in this article, in conjunction with cell biology. The modified perfusion system will possibly lead one of areas in animal cell culture industries by economically producing highly purified and pharmaceutically active proteins. The improvement of the recycling process by ultra hollow-fiber catridge and off-line setting of measuring devices such as D.O., pH and temparature will greatly upgrade current perfusion systems. Especially, the off-line setting of these measurements has an excellent advantage of changing old or bad devices without contaminations, and not affecting on-going processes.

The automation of process controls must be utilized in cell culture techniques. New Brunswick Scientific Company has a patent on a method to

control growth parameters; however, the system needs to be more turned up for fine controls by carefully selecting interfacing devices and developing more accurate control algorithms. The utilization of commercially available personal computer is not difficult to automatize the bioreactor through on-line data acquisitions, parameter setting, process control (including feedback control), and recording and manipulating data. This will dramatically reduce man power and can run the system all through the day as well as remotely monitor and control the cell culture processes.

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