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MINI-REVIEW

G. Kretzmer

Industrial processes with animal cells

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Abstract Industrial processes involving animal cells for the production of useful products still seem to be rather uncommon. Nevertheless, during the last four decades of the last century the number of relevant processes has increased from production of virus vaccines to monoclonal antibodies and finally complex structured glycoproteins. As soon as cell lines became permanent and culture medium changed from purely biological fluids to more or less defined chemical media, large-scale cultivation could begin. The developments of the 1970s – fusion of cells to form hybridomas, and genetic engineering – triggered a second wave of products. Monoclonal antibodies and recombinant proteins for diagnosis and therapy set new challenges for the inventors. Historically, there has been no straightforward process development since the product dictates the process operation. Therefore, the scale of production covers the whole range from small multiple-unit reactors (flasks or roller bottles) up to 10,000-l single-unit batch reactors. Products with high value and small demand can be produced in multiple-unit systems whereas "bulk" products for vaccination and therapy may need large-scale bioreactors to be cost effective. All the different systems have their advantages and disadvantages and significant challenges that curb the development of effective perfusion cultures still remain.

Historical outline

Nearly 100 years of progress and drawbacks have elapsed from the beginning of animal cell "cultivation" to the mass production of cells and products. Animal cell cultivation began in the last decade of the nineteenth century with the first tentative experiments to maintain pieces of tissue in plasma or ascites fluid over several days or even weeks. The success of those experiments was limited by the quality of the nutrient fluid and the sterility of the experimental set up. In 1907, Ross Harrison was the first to record the maintenance and growth of nerve cells in a hanging drop over a period of up to 30 days. These experiments (Harrison 1907) showed that normal cell functions can continue in vitro and therefore the year 1907 is commonly regarded as marking the beginning of cell cultivation. Harrison and his successors observed that strict aseptic conditions were crucial for the success of such experiments.

During the next four decades, the progress of cell cultivation was limited due to the stringent sterility controls necessary. The development of antibiotics in the late 1940s was another milestone. The addition of antibiotics eased the handling of complex undefined culture media. At the same time, the development of sterility techniques took place. During the following decade, great progress was made towards mass cultivation of animal cells and production processes. The world-famous HeLa cell line was isolated and found to grow very well in vitro. As early as 1928, Maitland and Maitland (1928) had developed a simple method for virus-propagation in tissue culture, but it took the findings of Enders and his co-workers (1949), showing that poliomyelitis virus could be cultured in HeLa cells and used as a vaccine, to indicate a great milestone towards production processes.

The most important breakthrough towards large scale cultivation was achieved by Earle and Eagle, who made an extensive analysis of the requirements of cells in vitro. In 1955, Eagle reported a chemically defined medium known as EMEM (Eagle's minimum essential medium; Eagle 1955) which could replace the biological fluids used thus far. The only handicap was the necessity for the addition of undefined blood serum.

This achievement, together with the development of permanent cell lines that can be sub-cultured indefinitely and which have the possibility to grow in suspension, had an enormous impact on large-scale cultivation of animal cells.

G. Kretzmer (✉)
Institut für Technische Chemie der Universität Hannover,
Callinstrasse 3, 30167 Hannover, Germany
e-mail: Kretzmer@iftc.uni-hannover.de
Tel.: +49-511-7622269, Fax: +49-511-7623004

Industrially relevant production processes

Five decades ago, the production of Salk polio virus vaccine in primary monkey kidney cells was the first process set up in industry (Griffiths 2000). The process was a multiple-unit process since these primary cells are anchorage-dependent. The success of Capstick and coworkers (1962) in culturing BHK (baby hamster kidney) cells in suspension like microorganisms meant the breakthrough to industrial usage of animal cells. This, together with the establishment of permanent cell lines, drove the development of large-scale processes in industry. Due to safety requirements, these new cell lines were used only for veterinary vaccines for a long period. Welcome was the first to produce the foot and mouth-disease virus vaccine for use in cattle in suspended BHK 21 cells in a 1,000-l agitated reactor (Finter et al. 1987). By 1975, over one million litres of vaccine were produced annually. The scale has since been enlarged up to 10,000-l reactors (Arathoon and Birch 1986).

The replacement of the primary monkey kidney cells with safer cell lines (WI-38 and MRC-5) increased the number of licensed human vaccines: measles (1963), rabies (1964), mumps (1969) and rubella (1969). All these processes were batch type since the cells should grow to high density and then be infected with the virus. After virus propagation the cells are of no further interest and the virus is harvested.

It was no real surprise that the first wave of products produced in animal cells were vaccines and therefore "native" products. Other products of pharmaceutical interest naturally produced by animal cells were secreted in such low concentrations that economical production would not have been feasible.

The first native product other than a vaccine was human interferon, produced in Namalwa cells (human lymphoblastoid cells). The process was run in an 8,000-l agitated batch reactor making millions of litres of culture product per annum (Finter et al. 1987).

In the late 1970s, a second wave of industrially relevant products came to market when Köhler and Milstein (1975) succeeded in fusing lymphocytes with immortal myeloma cells. This hybridoma technique enabled companies to produce monoclonal antibodies against every known antigen by fusing antibody-producing lymphocytes with tumor-like myeloma cells. Again, these products are "native" although the producing cell line was created using modern techniques.

The production of monoclonal antibodies by hybridoma cells resulted in an increasing list of assays for diagnostic purposes. The biggest advantage of this method compared to in vivo methods is the absence of nonspecific mouse immunoglobulins and the minimising of unit variation. To obtain 1 kg of antibodies, 3,000 mice or ten culture runs of a 1,000-l reactor are necessary.

Although hybridoma cells are anchorage-independent, the production facilities were small flasks, roller bottles or hollow fibre reactors. The big advantage of these techniques was the high cell density and the high product titre, especially with hollow fibre reactors, and the reduction of protein content in crude preparations (Hirschel and Gruenberg 1987; Davis 1992, 1995). On the other hand, the small scale meant that a broad range of different monoclonal antibodies could be produced in parallel.

In 1985, Celltech (UK) used airlift reactors up to 1,000-l-scale for the production of monoclonal antibodies. With this system, air is pumped into the reactor at the bottom in order to maintain the cells in suspension. Those runs lasted for about 400 days, with antibody production being highest in the stationary and declining phase (Birch et al. 1985).

The high selectivity of monoclonal antibodies is particularly valuable in medical diagnosis. There is a broad range of applications, such as identifying viruses, bacteria or parasites, increase of certain enzyme levels (coronary disease) or as ABO blood-typing reagents.

Initial expectations that these antibodies could be used for therapy in humans were disappointed due to the severe immunogenity of murine antibodies in humans. Another problem is the high amount of antibodies necessary for therapy and the lack of economic large-scale production of those antibodies. Research in this area is ongoing with high speed and in some cases those problems were solved by using chimeric monoclonal antibodies, humanised mouse antibodies or even human antibodies.

Meanwhile, the optimisation of production processes continues. Examples of the success of this strategy are the, now licensed, monoclonal antibodies for human therapy, e.g. for immunosuppression in organ transplant operations (Zenapax, Simulect), against non-Hodgkin-lymphoma (Rituxan), against Morbus Crohn (Remicade), against breast cancer (Herceptin) and angioplastic surgery (Reopro). The end of the pipeline for the development of monoclonal antibodies for therapeutic use has not yet been reached by far. More than 100 new monoclonal antibodies are in clinical trials for treatment of different types of cancer as well as treatment against bacterial infections. Another idea is to shorten the antibody down to the variable site that recognises the antigen (e.g. the receptor on tumour cells) and modifying the rest to change the behaviour of the target cell.

Most of these antibodies are produced in stirred-tank reactors in batch or fed-batch cultures up to 10,000 l. Perfusion cultures, which would be the mode of choice for these large product volumes, still have some handicaps such as cell retention and cell separation (Birch 2000).

In the early 1980s, a new kind of product was introduced to the pharmaceutical industry: recombinant proteins. Genetic engineering had progressed to the point that stable insertion of target DNA into mammalian cells was possible. The first non-native product produced by animal cells was tissue plasminogen activator (tPA), a protein necessary for dissolving blood clots for the treatment of cardiac infarction. Naturally secreted by mammalian cells in minimal concentrations (0.01 mg purified

Table 1 Milestones in the production of industrially relevant products

1949	Production of poliomyelitis virus in human embryonic tissues
1954	Salk polio vaccine in primary monkey kidney cells licensed for human use
1962	Foot and mouth disease vaccine in BHK21 cells for veterinary use
1980	Human interferon from Namalwa cells
1986	Production of monoclonal antibodies for diagnostics
1986	Production of recombinant tissue plasminogen activator (tPA) with genetically engineered CHO cells

tPA/uterus; Cartwright 1992), advances in genetic engineering made this important protein available for medical therapy. Inserting the human tPA gene into CHO (chinese hamster ovary) cells and growing the cells in agitated-tank reactors increased productivity to 50 mg 10^9 CHO cells^{-1} day^{-1} or even higher. The product was licensed in 1986/87 as Activased/Actilyse. Genentech and Dr. Karl Thomae began to use large-scale batch cultures of genetic engineered CHO cells (up to 10,000-l reactors; Lubiniecki et al. 1989). Milestones in the production of industrially relevant products are listed in Table 1.

The success of tPA paved the way for a significant number of other recombinant proteins. The next product to be licensed was erythropoietin (EPO), a hormone that controls the maturation of red blood cells. This product is also produced in genetically engineered CHO cells in a multi-stage roller-bottle process and was licensed in 1989 by Amgen (Kirin-Amgen 1986; Eridani 1990).

Development has progressed rapidly during the past decade, especially in the field of blood proteins used as pharmaceuticals in treatment of haemophilia. In the late 1980s, the spread of AIDS caused problems during recovery of those proteins from donated blood. This accelerated the development of processes for recombinant blood clotting factors (e.g. factor VIIb, factor VIII, factor IX, and antithrombin III). Those products are now produced at industrial scale, mostly in batch processes.

Recombinant factor VIII (Kogenate – produced by Bayer; licensed 1993) is one of the few products produced in perfusion culture. Factor VIII is produced by genetically engineered BHK 21 cells which grow in agitated perfusion reactors with up to a 30-fold increased cell density compared with batch cultures. Since productivity is not affected by density, this mode produces 30-fold more product. The perfusion mode allows smaller scale processes (100–500 l) to obtain even more product than 5,000–15,000-l batch reactors (Boedecker et al. 1994).

Industrially relevant process modes

To bring a product of pharmaceutical interest successfully produced in the laboratory to the market needs further process development. Laboratory scale processes, even if very productive, are seldom directly transferable to industrial scale. Production processes should be cost-effective and well-controlled to produce a consistent product (Lugo 1998). It depends on the product and its application at which scale the break-even point is reached.

Table 2 Process demands

Cost effective
Well-controlled
High reliability
High cell density and viability
High product quality
Easy recovery
High yield
High safety for personnel

Some high-value products may be sufficiently produced in small scale but most cell lines have relatively low productivity and/or the products are needed in high amounts. Therefore, the desired characteristics of the process have to be considered carefully since, especially for processes with genetically engineered material, the complete process has to be validated and approved by the authorities. The inventor has to show that variation of process parameters will fall within an acceptable range and produce the same product with the same quality. This process development is ongoing throughout the whole period of preclinical and clinical trials and has to be completed when the licence is applied for. The scale and mode chosen for the considered product depends on the amount required, the market price and the feasibility of the process (see Table 2). In the following section, the different types of reactors are considered, advantages and disadvantages listed and the demand of further developments and optimisation given (see Table 3).

Small-scale reactor

Developing a new product and studying the growth and production characteristics of the cell line used is carried out on the laboratory scale, normally starting with small flasks and then changing to spinner flasks or roller bottles depending on the growth characteristics of the cell line (anchorage dependent or not). For initial quality studies of the new product this scale is sufficient but a scale-up is necessary as soon as preclinical studies will follow. Depending on the product requirements there are two possibilities: keeping the scale and working in multiple units or transferring the process to larger reactors. The easiest way is to multiply the number of well-running small units each operating the same way, but this is uneconomical and time consuming. Furthermore, the risk of contamination is great and the process is not controllable as a feature of batch reactors is that the process parameters are continually changing throughout the process.

Table 3 Advantages and disadvantages of different process types

Process type	Advantage	Disadvantage
Small unit (small flasks, roller bottle, spinnerflasks, hollow fibre)	Easy to handle, direct transfer from laboratory to production, no scale-up procedure needed	Time-consuming because of multiple units, no monitoring of the process possible, inhomogenous
Batch reactor, fed-batch reactor	Homogenous, easy to scale up, partially controllable, depleted nutrients replaceable, plant is flexible for various products	Gradients during the run, accumulation of toxic metabolites, decrease of viability during the run
Perfusion reactor	High cell density, controllable, adjustment of culture conditions, no gradients, real steady state possible, small-scale core reactor	Long and complicated validation procedure, less flexibility, plant is designed for special product

Chiron Behring produces between 1 and 80 million doses of vaccine per year against FSME (Encepur), Rabies (Rabivac, Rabipur), and Polio (Oral-Virelon) with great batteries of roller bottles or trays. This is mainly because the cell lines used are anchorage-dependent but also because vaccine production is often linked to campaigns and small-scale processes make it easy to switch from one vaccine to another. On the other hand, it is only necessary to grow the cells to high density and then infect them with the virus. The cells will die and there is no need to keep them viable for a long production period.

Some recombinant products, like EPO, are also produced in roller bottles. As high value products (selling price US $0.012/U) the total estimated demand in the United States (1998) can be satisfied with only 1 kg/year. Amgen licensed a roller bottle process in 1989 (Kirin-Amgen 1986).

A small step towards scale-up and control are hollow fibre systems. They are also of small scale compared with stirred-tank reactors and scale-up is done by multiplying the number of units but the cells are trapped inside the hollow fibre reactor and the culture supernatant is continuously perfused through the hollow fibre. This type of reactor is widely used for small and medium-scale antibody production. The largest scale reported is a system with ten 1-l modules producing 2.5–6.5 kg/year of antibody (Jobsen et al. 1992). Hollow fibre reactors are high cell density reactors with high product concentration in the supernatant but the disadvantage is the gradient inside the hollow fibre and mass transfer limitations in these inhomogenous systems. An additional complication is the lack of possibility to monitor the cells during the process.

Batch reactor

Antibodies for research and some diagnostic applications may be produced in the small-scale reactors described above. Antibody doses for some therapeutic applications can range from 0.5 to more than 5 mg/kg and this can translate into a requirement for tens to hundreds of kilograms per year. Large scale (tens of grams upwards) needs systems with the potential for scale up.

The ideal reactor for optimal growth and/or production provides a homogenous environment and can be easily controlled. These conditions can be achieved in stirred-tank reactors, which are the preferred reactors for scaling up processes with suspension cells. The basic parameters for biotechnological processes, like temperature, pH value, dissolved oxygen, etc., are measured and controlled by standard devices implemented in every commercial stirred tank. For optimal mixing, various types of impellers are available as well as standard sampling probes for monitoring the environment and the cells during the process. A disadvantage of the stirred-tank reactor compared to the small-scale reactors is the oxygen supply. At small-scale, the ratio of surface area to culture volume is high enough to maintain the oxygen level in the supernatant. Using a stirred-tank reactor, this ratio is negligible with respect to aeration. Devices such as spargers, baffles and low shear impellers, have been developed to supply oxygen to cultures in stirred-tank reactors. Other reactors, such as bubble columns and airlift reactors, use direct gas sparging into the culture for oxygen supply and mixing. With microorganisms, direct aeration with air or oxygen bubbles is commonly used. This can cause problems when working with animal cells. Chalmers (2000) gives a comprehensive overview of the effects of hydrodynamic forces on cells and the methods used to prevent the adverse effects of these forces. Several studies have demonstrated that cells can withstand much higher shear forces generated by impellers than typically reached in a bioreactor (Oh et al. 1989; Kunas and Papoutsakis 1990). The interaction between gas bubbles and cells is of much greater importance. Many studies have shown that it is nearly impossible to grow suspended cells in sparged bioreactors, bubble columns or airlift reactors without using protective additives. The most common additive is Pluronic F-68, as described by several industrial researchers (Ozturk 1996; Zhou et al. 1996). For bioreactor scale-up there are still only “rules-of-thumb” approaches but research is still ongoing and answers begin to emerge.

Another possibility to overcome the problem of the rising air bubbles is bubble-free aeration using membranes for indirect aeration. The supply of oxygen is diffusion-controlled and no bubbles arise. The length of the membrane is limited. The depletion of oxygen increases

Table 4 Large scale processes with animal cell lines (adapted from Palomares and Ramirez 2000)

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	monoclonal antibodies
Vero cells	1,000	Agitated tank/microcarrriers	Killed polio vaccine
Murine hybridomas	1,000	Stirred tank	Monoclonal antibodies against cell-surface antigens of adenocarcinomas
BHK	500	Agitated tank/perfusion	Factor VIII

with increasing length of the membrane, i.e. the average oxygen transfer rate is decreasing. On the other hand the pressure inside the tubing is limited. If the critical transmembrane pressure difference ("bubble point") is reached, bubbles are formed. A 2-l reactor needs a membrane length of at least 6 m to reach an oxygen transfer rate of 30–40 mg $(lh)^{-1}$. Several hundred metres of membrane for one reactor makes handling complicated and disturbances by rupture and contamination probable. Therefore, the scale-up of this system is limited to bioreactors of about 500 l but it works very well for small-scale tank reactors.

The largest stirred-tank bioreactor first reported was again for the production of vaccines. Welcome produced foot-and-mouth-disease vaccine in a 1,000-l batch reactor with suspended BHK cells and recently changed to a 10,000-l batch reactor (Finter et al. 1987; Smith 1994). Backer et al. (1988) described a stirred, air-sparged reactor up to 13,000 l scale for the production of monoclonal antibodies in hybridomas. Rhodes and Birch (1988) reported airlift reactors up to 2,000 l scale for the production of antibodies with hybridomas, human cell lines, and genetically engineered mouse myeloma cells. Large-scale single unit industrial processes for other products are also commonly performed in batch reactors (see Table 4).

Depending on the cell characteristics, either microcarriers are used as growth substrates or the cell line is adapted to suspension. The latter is preferable since the process is easier to handle with less complications such as clogging of microcarriers, transfer problems and so on.

Using batch reactors, the definition of a "lot" is straightforward and this was another reason why batch reactors were preferred by the authorities. However, problems and challenges still remain for the optimisation of this kind of process. The nature of a batch process generates a gradient of nutrients during the run. This leads not only to a depletion of the nutrients, and therefore a drop in viability and death of the cells, but also to a steady increase in toxic metabolites. Cell density and culture life-span can be increased by systematic modification of the culture medium with addition of the most important nutrients to keep the level constant. Fed-batch operation increases the product output of the process. Some products, for example tPA and a variety of monoclonal antibodies, are already produced by fed-batch operation (Noé et al. 1994).

Nevertheless, batch reactors ultimately result in a toxification of the process since the toxic metabolites are not removed. The main challenges to be addressed are the cell demands concerning nutrients (requiring study of cell metabolism), developing optimised feeding strategies, and detoxification of the culture supernatant.

Perfusion reactor

Stirred-tank reactors can also be operated in continuous mode. This technique is well-known from microorganisms, where large quantities of product are produced. Unfortunately, the technique is not directly transferable to animal cell cultures. In contrast to microorganisms, animal cells have very slow growth rates and this makes cell retention inevitable. This has led to the development of a variety of internal and external separation devices like spin filters, external tangential flow filtration units, acoustic resonance sedimentation, cell settlers and continuous centrifuges.

The need for cell retention limits the duration of these perfusion processes. Due to the relatively high protein content of most cell culture media, as well as the cell size, all devices tend to foul and block the continuous flow of medium. Perfusion cultures, on the other hand, combine homogenous mixing with control of the environment and detoxification of the reactor. The continuous exchange of medium allows maintenance of very low concentrations of toxic metabolites. The perfusion rate depends on the demands of the cell line, the concentration of nutrients in the feed and the level of toxification. The overall advantage of perfusion cultures is the very small scale required compared with batch cultures in order to obtain the desired amount of product. There are few industrial perfusion processes described in the literature: Deo et al. (1996) reported a 500-l-scale spin filter perfusion hybridoma culture for antibody produc-

tion that ran for 15–35 days. They estimated that the volumetric productivity of the perfusion process is 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. The advantage compared to batch culture is the 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 (Boedeker et al. 1994).

Validation of the perfusion process is much more complicated and time-consuming than for batch cultures. The validation has to be done for the whole culture run. It has to be shown that the process is stable throughout the whole run (viability, product release and cell-related performance), the product quality is the same at different cultivation times and reactor scale, and finally that the cells are genetic stability for the entire process run. This disadvantage is compensated for by the advantages of perfusion cultures. They provide a high degree of control: conditions for optimal medium requirement can be kept constant and a real steady state is achieved. A high cell density perfusion culture can be a very effective and economic large-scale process.

Before more industrial processes can be transferred to perfusion mode, some challenges remain to be addressed. For example, cell retention has to be more sophisticated. The devices available so far are a major bottleneck because of fouling and blocking. Another important topic is the run-time of the perfusion. This depends on the cell line and culture medium. Therefore, cell metabolism under production conditions has to be studied carefully to gain more knowledge for developing better strategies for process control.

Outline of trends and prospects for the future

More than 200 monoclonal antibodies for diagnostic and therapeutic use, as well as more than 20 recombinant proteins, are licensed and on the market (see Table 5). The subject is evolving very fast, nearly the same number of products again is under development or already in the clinical phase (see Table 6).

An area of interest is cell adhesion molecules (CAMs). These mediate the cell-cell and cell-matrix interactions first found in the study of embryogenesis. There is hope that CAMs will be useful as drugs against inflammatory diseases and have some potential to treat metastatic diseases. Some of the investigated products undergoing clinical trials are Cylexin (reperfusion injury), Integretin (arterial thrombosis, angina) and Celadin (inflammatory diseases) (Griffiths 2000).

Although virus production in animal cells is the hub of industrial-scale production, there is a tremendous need for process development. The huge amount of influenza vaccine required is still produced in egg cells. For Begrivac (8 million doses per year) alone, 550,000 eggs/week are needed and vaccination campaigns last 23 weeks. There are efforts to develop this process in animal cells. Last year, Chiron Behring developed a process using suspension-adapted cells of the canine kidney cell line MDCK for influenza vaccine production.

With this increasing number of products, the need for development and optimisation of production and downstream processes is evident. The transfer of successful laboratory-scale reactor runs to large-scale industrial processes is still challenging. Many animal cell products are costly therapeutics or chemicals (US $\$10^4$-$10^9$/kg) and the demand is also small (10^{-1}–10^3 kg/year). This enables inventors to produce on a small scale and, if required, in multiple units. Multiple unit roller bottle or hollow fibre processes are not very economical. They run a rather large risk of contamination, and they are time- and cost-consuming. Additionally, the parameters during the process (e.g. pH, oxygen, nutrients and metabolites) are not constant and cannot be influenced. Products such as some vaccines, antibodies for therapeutic use and recombinant proteins are mainly produced in batch reactors up to 10,000-l scale. Batch reactors are easy to scale up but have the disadvantage of gradients and a low degree of control. For a large number of products, this type of production is sufficient, since worldwide demand is not high; the demand for EPO in the United States in 1998 was about 1 kg/year. Other products, like monoclonal antibodies and vaccines, are needed in much higher amounts to meet therapeutic or diagnostic demands. Those processes need ongoing development and optimisation of fed-batch and perfusion processes to meet the worldwide demand at an economically and socially acceptable price. Running the most cost-effective continuous perfusion processes is still challenging (see Table 7). Cell retention and separation is still ineffective; the devices undergo fouling very quickly, the cells are stressed to some extent by retention or separation and viability drops. The latter is also a problem for

Table 5 Products on the market produced by animal cells (adapted from ACTIP 1998; www.actip.org/manuals/products.htlm)

Human vaccines	Rabies, polio, rubella, mumps, measles, yellow fever, hepatitis A
Veterinary vaccines	Foot and mouth disease, pseudorabies, Marek's disease, dog parvovirus
Diagnostics	Diagnostic monoclonal antibodies (imaging agents for a variety of diseases)
Therapeutics	tPA, erythropoietin (EPO), interferon alpha, factor VII, factor VIII, factor IX, antithrombin III, monoclonal antibodies for reversal of organ transplant rejection and non-Hodgkins lymphoma and growth deficiency in children, granulocyte CSF, DNAse, glucocerebrosidase

Table 6 Products under development (list is not complete – adapted from ACTIP 1998; www.actip.org/manuals/products.htlm)

Product	Indication
Factor VII, factor IX	Haemophilia
Growth factors and hormones	Cancer, wound healing, infections, polycystic ovarian disease, growth disorders, bone marrow transplantation
Follicle stimulating hormone	Treatment of infertility
Interleukins	Cancer, blood cell disorders, inflammation
Fibrinolytic enzymes	Dissolution of blood clots
Vaccines	AIDS, herpes simplex
CD4 immunoadhesins	AIDS
Monoclonal antibodies including genetically engineered antibodies	Treatment and diagnosis of cancer, sepsis, rheumatoid arthritis, Crohn's and other diseases
Soluble receptors	Cancer, infections, inflammation, sepsis

Table 7 Challenges for the future

Reliable cell retention and separation devices and procedures
Defined protein-free media for industrial scale
Better understanding of cell metabolism for optimised control strategies
Optimised process strategies
Establishing of control strategies based on artificial intelligence

batch cultures when the product is harvested and separated from the cells. Dying cells release a variety of enzymes into the supernatant which might alter the product. In addition, DNA might also contaminate the product and has to be carefully removed.

Despite the long and complicated procedure for validation of perfusion cultures, this method will be desirable for most products in the future since it allows for very good control of the status. In particular, the evolution of control technology based on artificial intelligence will advance perfusion technology.

References

Arathoon WR, Birch JR (1986) Science 232:1390–1395

Backer MP, Metzger LS, Slaber PL (1988) Large scale production of monoclonal antibodies in suspension culture. Biotechnol Bioeng 32:993–1000

Birch JR (2000) Cell products – antibodies. In: Spier RE (ed) Encyclopedia of cell technology, vol 1. Wiley, New York, pp 411–423

Birch JR, Boraston R, Wood L (1985) Bulk production of monoclonal antibodies in fermenters. Trends Biotechnol 3:162–166

Boedecker BGD, Newcomb R, Yuan P, Braufman A, Kelsey W (1994) Production of recombinant factor VIII from perfusion cultures. I. Large-scale fermentation. In: Spier RE, Griffiths JB, Berthold W (eds) Animal cell technology: products of today, prospects for tomorrow. Butterworth Heinemann, Oxford, pp 580–583

Capstick PB, Telling RC, Chapman WG, Stewart DL (1962) Growth of a cloned strain of hamster kidney cells in suspended cultures and their susceptibility to virus of foot-and-mouth disease. Nature 195:1163–1164

Cartwright T (1992) Animal cell biotechnology, vol 5. Academic Press, London, pp 218–246

Chalmers J (2000) Animal cell culture, effects of agitation and aeration on cell adaptation. In: Spier RE (ed) Encyclopedia of cell technology, vol 1. Wiley, New York, pp 41–51

Davis JM (1992) In: Spier RE, Griffiths JB, MacDonald C (eds) Animal cell technology: developments, processes and products. Butterworth-Heinemann, London, pp 130–133

Davis JM (1995) In: Beuvery EC, Griffiths JB, Zeijlemaker WP (eds) Animal cell technology: developments towards the 21st century. Kluwer, Dordrecht, pp 149–153

Deo YM, Mahadevan MD, Fuchs R (1996) Practical considerations in operation and scale-up of spin-filter based bioreactors for monoclonal antibody production. Biotechnol Prog 12: 57–64

Eagle H (1955) Nutrition needs of mammalian cells in tissue culture. Science 122:501–504

Enders JF, Weller TH, Robbins FC (1949) Cultivation of Lansing strain of poliomyelitis virus in cultures of various human embryonic tissues. Science 109:85

Eridani S (1990) Animal cell biotechnology, vol 4. Academic Press, London, pp 469–488

Finter NB, Johnston MD, Ball GD, Fantes K, Pullen K (1987) Mass mammalian cell culture. In: Umeda M, Koyama H, Minowada J, Oishi M (eds) Biotechnology of mammalian cells. Japanese Science Society Press, Tokyo/Springer, Berlin Heidelberg New York, pp 139–146

Griffiths JB (2000) Animal cell products, overview. In: Spier RE (ed) Encyclopedia of cell technology, vol 1. Wiley, New York, pp 71–76

Harrison RG (1907) Observations on the living developing nerve fiber. Proc Soc Exp Biol 4:140

Hirschel MD, Gruenberg ML (1987) In: Lydersen BK (ed) Large scale cell culture technology. Hanser-Gardiner, New York, pp 113–144

Jobsen I et al (1992) In: Spier RE, Griffiths JB, MacDonald C (eds) Animal cell technology: developments, processes and products. Butterworth-Heinemann, London, pp 130–133

Kirin-Amgen Inc (1986) Patent Application WO 85/02610

Köhler G, Milstein C (1975) Continuous culture of fused cell secreting antibody of predefined specificity. Nature 256: 495–497

Kunas KT, Papoutsakis ET (1990) The protective effect of serum against hydrodynamic damage of hybridoma cells in agitated and surface-aerated Bioreactors. J Biotechnol 15:57–70

Lubiniecki A. et al (1989) In: Spier RE, Griffiths JB, Stephenne J, Crooy PJ (eds) Advances in animal cell biology and technology for bioprocesses. Butterworth, Guilford, pp 442–451

Lugo NM (1998) Elements of downstream processing validation Biopharm 11(6):18–26

Maitland HB, Maitland MC (1928) Cultivation of vaccina virus without tissue culture. Lancet 215:596

Noé W, Schorn P, Bux R, Berthold W (1994) Fed-batch strategies for mammalian cell cultures. In: Spier RE, Griffiths JB, Berthold W (eds) Animal cell technology: products of today, prospects for tomorrow. Butterworth-Heinemann, Oxford, pp 580–583

Oh SKW, Nienow AW, Al-Rubeai M, Emery AN (1989) The effects of agitation intensity with and without continuous sparging on the growth and antibody production of hybridoma cells. J Biotechnol 12:45–62

Ozturk S (1996) Engineering challenges in high density cell culture systems. Cytotechnology 22:3–16

Palomares LA, Ramirez OT (2000) Bioreactor scale-up. In: Spier RE (ed) Encyclopedia of cell technology, vol 1. Wiley, New York, pp 183–201

Rhodes M, Birch J (1988) Large scale production of proteins from mammalian cells. Biotechnology 6:518–523

Smith GL (1994) Large scale animal cell culture. In: Lydersen BK, Dèlia NA, Nelson KL (eds) Bioprocess engineering: systems, equipment and facilities. Wiley, New York, pp 69–84

Zhou WC, Bibila T, Glazomitsky K (1996) Large scale production of recombinant mouse and rat growth hormone by fed-batch GS-NSO cell culture. Cytotechnology 22:239–250