Chapter 10

Production Processes for Monoclonal Antibodies

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Additional information is available at the end of the chapter

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Abstract

Antibodies are glycoprotein structures with immune activity. They are able to identify or induce a neutralizing immune response when they identify foreign bodies such as bacteria, viruses, or tumor cells. Immunoglobulins are produced and secreted by B lymphocytes in response to the presence of antigens. The first monoclonal antibodies (mAbs) have emerged from a survey of hybridomas, and nowadays mAbs are produced mostly from cultivations of these cells. Additionally, there are studies and patents using a range of cells and microorganisms engineered for the production of mAbs at commercial scale. For some years, new methodologies have advanced with new production processes, allowing scale-up production and market introduction. Large-scale production has revolutionized the market for monoclonal antibodies by boosting its production and becoming a more practical method of production. Production techniques have only had a sizable breakthrough due to molecular techniques. Various systems of production are used, including animal cells, microorganisms, plants, and mammary glands. All of these require the technological development of production process such as a stirrer, a wave bioreactor, and roller bottles.

Keywords: monoclonal antibodies, bioprocess, bioreactors, antibodies, mAbs

1. Introduction

Monoclonal antibodies (mAbs) have been widely used as a way to successfully achieve a broad range of extracellular targets with high specificity [1]. mAbs have various applications in diagnosis and therapy for several diseases such as cancers, autoimmune diseases, sexually transmitted infections (STIs), and others [2, 3]. In recent years, the use of mAbs has been expanded due to significant advances in design. The effect of decreasing immunogenicity in humans, improvement in their bioavailability, optimizing the affinity and antigen-binding specificity, and other advances in protein engineering are improving therapeutic mAb profiles (**Figure 1**) [2].

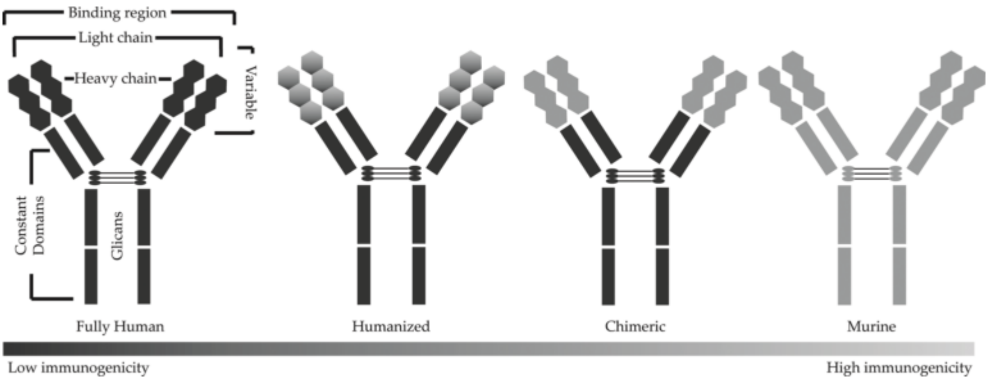

Figure 1. Schematic overview of a monoclonal antibody, showing their heavy and variable chain.

With the advent of genetic engineering, it has been possible to develop new methods to obtain monoclonal antibodies, both for improvement with regard to these humanized antibodies and for production models [4–6]. Advances in molecular and cell biology for the development of more efficient antibodies have allowed advances in diagnostic and therapeutic areas. Such advances have triggered improvements in production processes, allowing for the reduction of production costs and thus leading to an increase in the popularization of treatments with mAbs. All process improvements provide a consistent and reproducible production of large quantities of mAbs at a moderate cost [4–6].

Large-scale production has revolutionized the market for monoclonal antibodies by boosting its production, making this a more practical method of production. Production techniques have only had a sizable breakthrough due to molecular techniques [1, 7].

In general, a process of commercial production of mAb begins with the generation of an mAb by immunizing an animal or by molecular biology methods involving the identification and optimization of the coding DNA sequence and the construction and identification of a stable high-producing clone. Improvements in cultivation are similar to those applied in other bioproducts that rely on culturing microorganisms or cells, requiring the development of a well-designed culturing process comprising the full range of control and associated operations that will support technical evaluations [1, 8].

mAbs production processes in wave or single-use bioreactor (SUBs) are characterized by flexibility and low operating costs when compared to the production processes in fixed stainless steel vats. The development of bioprocesses involving these production platforms can reap greater acceptance by the industry [9–11].

Drugs based on mAbs have been controlled by regulatory agencies around the world. Therefore, it is necessary to elaborate regulatory protocols accompanying the increase in production and the nuances of the characteristics of this class of drugs [10, 11].

The proposed chapter covers the fundamental aspects of monoclonal antibody production methods, with emphasis on methodologies using immobilized cells, wave bioreactor systems, SUBs, and finally the roller bottles technique. Such techniques have been described in the most recent literature, both for murine monoclonal antibody production and for production of antibodies from modified microorganisms.

2. mAbs production techniques

2.1. Hybridoma and phage display

Milstein and Köhler described the first technique developed for stable monoclonal antibody production in 1975. This technique consists of creating a hybridoma, a stable hybrid cell capable of producing a single type of antibody against a specific epitope present in an antigen. Hybridoma construction was initially produced from murine models. The technique consists of removing a pool of activated B lymphocytes from an immunized animal spleen and combining them with immortalized myeloma cells unable to produce the enzyme hypoxanthine-guanine-phosphoribosyltransferase (HGPRT), an important enzyme present in the *salvage* pathway, one of the pathways responsible for nucleotide production [1]. To select hybridoma cells, the pool of cells resulting from the fusion (a mix of hybridoma cells and non-fused B lymphocytes and myeloma cells) are cultivated in a selective medium containing aminopterin, which inhibits the nucleotide de novo synthesis. Myeloma cells lack the *salvage* pathway for nucleotide production. When they are exposed to aminopterin present in selective medium, the de novo synthesis is also blocked, and as a result, myeloma cells are no longer viable since all major pathways for nucleotide production are blocked. In contrast, non-fused, activated B lymphocytes can survive as their *salvage* pathway works perfectly and they can continue nucleotide production even if the de novo pathway is blocked by aminopterin. However, these cells are not immortalized and can replicate only a limited number of times after which they eventually die. With this in mind, only cells capable of replicating indefinitely and synthesizing nucleotides through the *salvage* pathway can survive through selection conditions, and these cells are the hybridomas.

In spite of the fact that the primary recombinant mAbs were delivered utilizing this innovation —including the first medication approved by the Food and Drug Administration (FDA) for therapeutic proposes (**Table 1**)—the great contribution of this technology was mostly to elucidate immune response mechanisms and control in vitro antibody production. Therefore, mAb hybridoma production from murine sources exhibits a genuine downside in human therapeutics (**Figure 1**).

Drug name	**Active ingredient**	**Description**	**Target**	**Therapeutic category**	**approval (FDA)**
ACTEMRA®	Tocilizumab	Humanized IgG1κ	IL-6 receptor	Immunological	2010
ADCETRIS®	Brentuximab vedotin	Chimeric IgG1	CD30	Cancer	2011
ARZERRA®	Ofatumumab	Human IgG1κ	CD20	Cancer	2009
AVASTIN®	Bevacizumab	Humanized IgG1	VEGF	Cancer	2004
BENLYSTA®	Belimumab	Human IgG1λ	BLyS	Immunological	2011
BEXXAR	Tositumomab; iodine I 131 tositumomab	IgG2αλ, I131	CD20	Cancer	2003
BLINCYTO	Blinatumomab	BiTE antibody-scFvs	CD19/CD3	Cancer	2014
CAMPATH (LEMTRADA™)	Alemtuzumab	Humanized IgG1κ	CD52	Immunological	2001
CEA-SCAN	Arcitumomab	Murine IgG1 Fab′	CEA	Diagnosys	1996
CIMZIA®	Certolizumab pegol	Humanized Fab′, PEG	TNFα	Immunological	2008
COSENTYX®	Secukinumab	Human IgG1κ	IL-17A	Immunological	2015
CYRAMZA	Ramucirumab	Human IgG1	VEGRF-2	Cancer	2014
DARZALEX	Daratumumab	Human IgG1κ	CD38	Cancer	2015
HERCEPTIN®	Trastuzumab	humanized IgG1κ	HER2	Cancer	1998
EMPLICITI™	Elotuzumab	Humanized IgG1	SLAMF7	Cancer	2015
ENTYVIO	Vedolizumab	Humanized IgG1	α4β7 Integrin	Immunological	2014
ERBITUX®	Cetuximab	Chimeric IgG1	EGFR	Cancer	2004
GAZYVA®	Obinutuzumab	Humanized IgG1	CD20	Cancer	2013
HUMIRA	Adalimumab	Human IgG1	TNF	Immunological	2002
ILARIS	Canakinumab	Human IgG1κ	uman-IL-1β	Immunological/ anti-inflammatory	2009
KADCYLA®	Ado-trastuzumab emtansine	Humanized IgG1; DM1	HER2	Cancer	2013
KEYTRUDA®	Pembrolizumab	Humanized IgG4κ	PD-1	Cancer	2014
LEMTRADA™	Alemtuzumab	Humanized IgG1κ	CD52	Immunological	2001
LUCENTIS	Ranibizumab	Humanized IgG1κ	VEGF-A	Ophthalmic	2006
Muromomab	Orthoclone	Murine IgG2α	CD3	Immunological	1992
Mylotarg®	Gemtuzumab ozogamicin	Humanized IgG4κ, calicheamicin	CD33	Cancer	2000

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Drug name	Active ingredient	Description	Target	Therapeutic category	approval (FDA)
MYOSCINT®	Imciromab Penlelale	Murine IgG2/4κ Fab';DTPA	Heavy chain of human myosin	Detection of myocardial injury	1996
NUCALA®	Mepolizumab	Humanized IgG1κ	IL-5	Immunological	2015
OPDIVO	Nivolumab	Human IgG4κ	PD-1	Cancer	2014
PERJETA®	Pertuzumab	Humanized IgG	HER2/neu receptor	Cancer	2012
PORTRAZZA	Necitumumab	Human IgGκ	EGFR	Cancer	2015
PRALUENT™	Alirocumab	Human IgG1	PCSK9	Lipid-lowering	2015
PRAXBIND®	Idarucizumab	Humanized IgG1 Fab	Dabigatran (anticoagulant)	Hemostasis	2015
XGEVA®	Denosumab	Human IgG2	RANKL	Bone disorders	2010
ProstaScint®	Capromab pendetide	Murine IgG1κ, GYK-DTPA-HCl	PSMA	Cancer	1996
RAPTIVA®	Efalizumab	Humanized IgG1κ	CD11a	Immunological	2003
RAXIBACUMAB	Raxibacumab	Human IgG1λ	PA of B. Anthracis toxin	Anti-toxin	2012
REMICADE®	Infliximab	Chimeric IgG1κ	TNFα	Immunological	1998
ReoPro®	Abciximab	Chimeric IgG1κ Fab	GPIIb/IIIa	Hemostasis	1993
REPATHA	Evolocumab	Human IgG2	PCSK9	Lipid-lowering	2015
RITUXAN®	Rituximab	Chimeric IgG1κ	CD20	Cancer	1997
SIMPONI	Golimumab	Human IgG1κ	TNFα	Immunological	2009
SIMULECT®	Basiliximab	Chimeric IgG1κ	IL-2 receptor	Immunological	1998
SOLIRIS®	Eculizumab	Humanized IgG2/4κ	C5	Hemostasis	2007
STELARA®	Ustekinumab	Human IgG1κ	IL-12 and IL 23	Immunological	2009
SYLVANT	Siltuximab	Chimeric IgG	IL-6	Immunological	2014
SYNAGIS®	Palivizumab	Humanized IgG1κ	RSV F	Antiviral	1998
NeutroSpec™	FanolesomaB; technetium Tc 99m	Murine IgM	3-fucosyl-N-acetyllactosamine	Diagnosys	2004
TYSABRI	Natalizumab	Humanized IgG4κ	α4β1/α4β7 integrins	Immunological	2004
UNITUXIN™	Dinutuximab	Chimeric IgG1κ	Glycolipid GD2	Cancer	2015
VECTIBIX®	Panitumumab	Human IgG2κ	EGFR	Cancer	2006
VERLUMA™	Nofetumomab	Murine IgG2b Fab	Glycoprotein antigen expressed in a variety of cancers	Diagnosys	1996

Drug name	Active ingredient	Description	Target	Therapeutic category	approval (FDA)
XGEVA	Denosumab	Human IgG2	RANKL	Cancer	2010
XOLAIR®	Omalizumab	Humanized IgG1κ	Human IgE	Immunological	2003
YERVOY®	Ipilimumab	Human IgG1κ	CTLA-4	Cancer	2011
ZENAPAX®	Daclizumab	Humanized IgG1	IL-2 receptor	Immunological	1997
ZEVALIN®	Ibritumomab tiuxetan	murine IgG1κ, Yttrium-90	CD20	Cancer	2002

Table 1. Monoclonal antibody-based therapeutic drugs approved by FDA (Food and Drug Administration) until 2015.

After a few infusions, murine antibody molecules trigger the human anti-mouse antibody (HAMA) response of the human immune system [1, 12]. To work around this issue, new methodologies have been developed to deliver antibodies similar to human molecules, so the technology evolved to less immunogenic chimeric antibodies (constant regions of human antibodies linked to the variable region of the murine source), creating a new set of therapeutic possibilities (**Figure 1**). Subsequently, the need for an even less immunogenic alternative boosted the production of humanized antibodies (only the region that interacts with the antigen epitope is from mouse origin) (**Figure 1**). Even fully human antibodies (**Figure 1**) can be produced from genetically modified mice [13].

A great improvement in mAb production has come with the development of phage display libraries. This methodology helps to investigate interactions between molecules (protein-protein, protein-peptide, and protein-DNA) and consists, basically, in cloning Fab-region-coding genes amplified from B lymphocytes into bacteriophage plasmid vectors. Then the bacterium can be transformed with these vectors, going on to express the heterologous genes from a viral capsid. This capsid contains viral proteins and proteins encoded by the Fab sequence received by that specific cell. Once the library is complete, the affinity between proteins produced from different Fab regions can be tested against the antigen of interest and the cell transformed with the plasmid that contains those genes can be readily sequenced. The advantages of this methodology are the following: the same library has the potential to generate a great number of new antibodies, it is an in vitro process, so it does not require animal immunizations steps, and because of that, toxic antigens can be tested. Also, a greater variety of antigens can be tested, and antibody molecules can be rapidly obtained [13].

2.2. Culture production factors

2.2.1. Cell lines

One of the most critical steps in developing an mAb production system is to choose the cell line. The cells must be stable and secrete the desired protein with the correct conformation at high levels. Based on these requirements, the mammalian cell is the most commonly chosen expression system for mAb production. The main advantage of a mammalian expression system is that the cellular machinery is adapted for the production, processing, and secretion of highly complex molecules. The great majority of commercial mAbs are produced in Chinese hamster ovary (CHO) and NS0 cells, originating from plasmacytoma cells that were modified until IgG generation in nonsecreting B cells. Genetic modifications in CHO cells have generated cell lines capable of producing a high quantity of humanized mAbs. These cell lines were able to secrete up to 100 pg/cell/day [14]. Other modifications led to a high production of a chimeric mAb, ranging from 80 to 110 pg/cell/day [15]. NS0 modifications also have been made, leading to higher mAb production rates, ranging from 20 to 50 pg/cell/day [16]. In smaller quantities, hybridoma cell lines are also used in industrial mAb production. Some hybridoma strains are reported to have a production rate up to 80 pg/cell/day [16]. In spite of this, different mammalian cell lines and even more peculiar expression systems such as genetically modified plant cells, genetically modified insect cells, and genetically modified microorganism cells have also been used in mAb production and have gained space in the biopharmaceutical industry [1, 8]

Microorganisms modified by genetic engineering techniques have attracted much focus in industry, because these cells are simpler to handle and to modify when compared to animal cells. Other advantages of production methods using genetically modified microorganisms are that these cells have well-defined expression systems, and the production methodology is reproducible and easy to validate. Modified yeast cells, such as *Pichia pastoris* have a great potential for usage since these cells are known to achieve high secretion levels of heterologous proteins. Yeast cultivation systems for mAb production are easier scale-up and are cheaper when compared to mammalian cell cultivation systems. They can be cultivated in regular stirred tank bioreactors, in batch, or in feed-batch modes of operation. Generally, microorganisms do not have physicochemical and biological characteristics for the appropriate expression and posttranslational processing of mAbs [4].

Modified plants have also gained attention since plants are easy to cultivate and propagate. Other cultivation advantages such as cheap medium, low maintenance cost, and high production yields make plant production a cheaper alternative when compared to mammalian cell cultures [17]. However, there are some limitations—different glycosylation patterns and post-translational processing can also make plant cell utilization difficult [17].

2.2.2. Culture medium

Cultivation media for mammalian cells must have a complex content of ingredients ranging from amino acids to trace elements. To supply the cellular demand of these nutrients, the culture medium uses serum in its composition, however, due to the emergence of diseases caused by defective prions, such as bovine spongiform encephalitis (BSE), there is a great incentive to remove any animal component of culture media composition, especially if the medium is used for industrial production of biopharmaceuticals products. This has led to the emergence of media free from any animal components, including well-defined media for CHO and NS0, the two most utilized cell types in mAb production. The development of a proper medium can be time consuming and very expensive. However, many companies prefer to develop their own production media to maintain the composition between production lots as well as develop an appropriate medium composition for the specific cell type that will be used and to achieve greater control over production. Added to this, the development of downstream processes that meet the requirement for high-purity products and tests to validate the final product quality raises the overall production cost of a drug based on monoclonal antibodies [1].

Despite the complexity of developing a culture medium, much progress has been made in this area, allowing for greater cell growth and increasing cell conservation time in suitable conditions for the growth and production of molecules of interest [8].

2.2.3. Culture conditions

Growing conditions can directly influence the cell growth and production levels of molecules of interest. Usually, mammalian cell culture conditions for mAb production are very well defined: 37 °C, pH 7.15, and dissolved O_2 (OD) levels at 30–60%. CO_2 level is monitored to mimic the physiological standard between 31 and 54 mmHg. However, changes in cellular conditions have shown great potential to change cellular metabolism toward cellular growth or molecule production and this can be used to increase mAb production. Bioprocesses can be designed to occur in two phases. First, cell growth is optimized to reach a certain cell density. Once this density is reached, the second phase begins and the bioreactor conditions are shifted so the cells continue to grow just at a maintenance rate and directing the metabolism toward monoclonal antibody production. Some CHO cell strains and hybridoma cells are sensitive to changes in temperature and pH. When subjected to temperature and pH values lower than those normally used, values between 30 and 35 °C and 6.7–7.0, respectively, cell growth metabolism is reduced and specific production increases. The growth metabolism reduction also contributes to lower production of some metabolic compounds which are toxic for cell cultures, allowing increased cell viability, which spend more time producing molecules of interest. A good way to monitor the growth stage of a cell culture for controlling changes in cultivation is watching the DO and pCO_2 levels, which can also be adjusted to maximize the production of proteins such as mAbs [1].

2.2.4. Production platforms

The cell culture for mAb production can follow three different types of processes. The simplest of them is batch production, which consists of a closed system where a bioreactor is sterilized and prepared with a medium containing all the nutrients needed for cellular growth and product manufacturing and then, cells are inoculated. There is no feeding system with fresh medium or withdrawal of spent medium. As the process runs, nutrient concentration decreases and waste metabolites are produced, lowering cell viability. In spite of being a simple process, batch is not the most suitable type of production platform for mammalian cell cultures, as the environment inside the reactor quickly becomes unfavorable for cell growth and, at the same time, waste product concentration increases. Cultivation factors such as initial nutrient concentration and waste metabolite production directly determine the maximum concentration that cells can reach in a bath culture. Generally, this type of cultivation reaches a maximum density of 1–2 × 10^6 cells/mL, and then the cell viability drops rapidly [1]. The production process lasts for 4–7 days, when productivity reaches certain concentration of interest [1]. Supernatant is collected and the product is recovered by downstream processes. The time that each batch takes to finish also depends on the production kinetics. If the production is growth dependent (production occurs concomitantly with cellular growth), batch processes can be stopped as soon as cells reach the stationary phase. But if the product is not associated with growth (production only starts when the growth rate decreases), the culture needs to be carried for a longer period of time since production only starts at stationary phase.

In contrast to batch, a second type of production process utilized is continuous fermentation. There are two types of continuous production: chemostat cultures and perfusion cultures. Concerning chemostat cultures, fresh medium is added to the bioreactor and fermented medium is removed along with cells at a constant flow rate so that the culture volume remains unchanged. The flow rate (dilution rate) controls cellular growth and when these two variables are equal, the bioreactor reaches equilibrium—cell concentration, nutrient concentration, and product concentration are held constant. In this context, the culture can be kept in equilibrium for several months reaching a cell density of 10–30 × 10^6 cells/mL [1]. To avoid viable cell loss along with the constant outflow of the by-products of cell metabolism, many manufacturing plants have developed a cell-recycling system and thus, the perfusion culture method was developed where cells are kept inside the bioreactor. The disadvantages of continuous fermentation are the use of a large amount of expensive culture media and the difficulty in recovering the product, which comes out fairly diluted. These two disadvantages are consequences of the constant medium flow rate. To work around the product dilution problem, some production manufacturing plants have ultrafiltration systems which retain the product inside the bioreactor [18]. Another obstacle of this type of process is that the establishment of culture conditions for a stable industrial production plant can take months. For this to occur, the strain used must be very stable and have its physiological aspects clearly elucidated, such as growth rate, productivity, and response to certain stress conditions. It is not uncommon to hear that numerous attempts are made before the settlement of a stable production plant is achieved, but, once settled, this production process can bring many advantages, since it can be operated in smaller-volume bioreactors, and therefore have greater production flexibility.

The third type of process for producing monoclonal antibodies is by far the most utilized at industrial scale, which is fed-batch process. In this process, the cell density reaches 8–12 × 10^6 cells/mL, and cell viability in the bioreactor is enhanced by controlled nutrient addition at specified intervals [1]. The production process can take 12–20 days [1]. Usually, the same medium used in the initial culture is also used for feeding, but in a more concentrated version. The feeding solution composition can be designed to supply the cells based on their metabolic state at different culture phases by analyzing and identifying the spent medium nutrients that are being more consumed. Furthermore, the medium used in feeding can be modified to promote cell growth or to stimulate molecule production, since different components may modify the behavior of cells, changing the metabolism for different purposes. The feed solution can also be designed to minimize the production of waste metabolites that cause cell stress when in excess. However, their production is not completely avoidable as they eventually reach harmful concentrations. It is relatively easy to scale up and operate this system. More summarized data about the advantages and disadvantages of each process for mAb production can be seen in **Table 2**.

Production platform	Batch	Feed-batch	Perfusion culture
Advantages	• Simple to scale-up • Control by production lot	• Simple to scale-up • Control by production lot • Production facility is simple • Process is easy to perform and to validate • Higher volumetric production	• Cells are maintained in a relatively optimal biochemical environment • Culture reaches high cell density • Higher volumetric production
Disadvantages	• Difficult to define initial concentration of nutrients • Accumulation of waste metabolites • Degradation of more sensitive products	• Accumulation of waste metabolites • Degradation of more sensitive products	• Lack of homogeneity in the continuous reactor vessel • Challenges regarding long-term operability and maintenance • High cost and long times required for process development experiments • Genetic instability of cells

Adapted from [1, 21] (colocar referencias).

Table 2. Comparison between different operation modes that can be used for mAbs production.

A lot of effort has been made to increase cell longevity in batch and feed-batch modes of operation. It is expected that the longer the cells are maintained viable, the greater the antibodies' production will be. So, in order to maintain cell viability, some culture parameters can be optimized, such as culture media, feed solution, and mAb secretion rates and by-product production. To improve mAb titers in the batch platform, the start medium can be supplemented with glucose and amino acids, increasing mAb production up to eightfold when compared with regular media [9, 26]. Improvements for the fed-batch platform can be achieved by adjustments in feed solution, as mentioned before. Feed solutions containing glucose and aminoacids/glutamine have been reported to increase mAb titers from two to fourfold, reaching production of up to 2 g/L, when compared with the batch production platform [19].

The optimization of the antibody secretion rate can be achieved by high-density cell cultivation. On a fed-batch platform, a high cell cultivation culture can reach an mAb productivity rate of 0.94 g/L/day and a final titration of 17 g/L, while a continuous culture performed at high density conditions can reach final titration and productivity rates of 0.8 and 1.6 g/L/day, respectively [20]. Optimizing mAb secretion highly depends on the cell line chosen for production. Each cell strain can be influenced by the manufacturing conditions and respond differently to increasing or decreasing mAb production and secretion [19]. The accumulation of toxic by-products is a great bottleneck in manufacturing processes since they can inhibit cell growth

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and then directly affect mAb production. Although a few strategies to minimize this by-product accumulation have shown to be promising, some are not applicable for a large-scale production. Optimizing medium composition and feed solutions with substrates that reduce toxic compound production is the most common strategy used at industrial scales of production [19].

Although most mAbs are produced by fed-batch process, there are tendencies indicating that in the future many bioprocesses will be operated in continuous platforms, especially for the production of biopharmaceuticals. On these platforms, the production system will be coupled to upstream and downstream processes [21]. However, for this to actually happen, a great improvement in technological development still needs to be achieved.

2.3. Production systems

The use of monoclonal antibodies as therapeutic drugs requires a large-scale production that far exceeds that of laboratory production (**Figure 2**). Various production systems have been developed and have evolved, while new alternatives are emerging. The production of mAbs at commercial scale can be performed with adherent cells or suspension cells, although the latter is by far the most used and is better established with more efficient production methods available for cells cultivation. Thus, scale-up using suspension cells is easier. Another advantage of the suspension production system is that a bioreactor with a large area for cell adhesion is not necessary since the cultivation of adherent cell productivity is directly linked to the bioreactor's area [22].

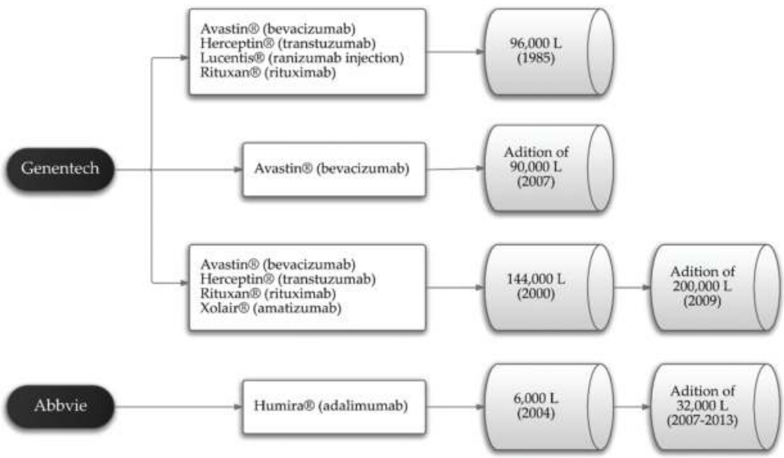

Figure 2. Work volumes used for industrial production of some commercial monoclonal antibodies [27, 28].

Some cultivation issues and worries have arisen regarding the production scale increase, maintenance of product quality, contamination control, demand for oxygen supply, and control over DO and CO_2 removal, among others. Regarding suspension cell cultures, aeration is in part dependent on the agitation of the culture inside the bioreactor, which can lead to cell shear stress. To work around cultivation problems, major advances have been made in the process itself by developing better culture control and conditions, as well as the improvement and development of new bioreactors [7, 23].

2.3.1. Production systems for cells in suspension cultures

The different types of bioreactors commonly used for mAb production in submerged mammalian cells are stainless steel stirred tank bioreactors (STR), air-lift reactors, and disposable bioreactors. More details on each of these bioreactors are discussed below.

2.3.2. Stainless steel stirred tank bioreactors

Stainless steel stirred tank bioreactors are the most consolidated type of bioreactor used for industrial mAb production and consist of baffle-stirred tanks linked to rotor systems (**Figure 3**). It is a consolidated system, and there is a lot of knowledge and experience surrounding this technology, acquired by its vast industrial use beyond production using mammalian cells.

The cultivation in this bioreactor allows for wide flexibility of working volumes, ranging from 1.0 to 25.0 L [1], since this system is easily scalable to larger volumes due to its high control over production conditions and extensive handling knowledge. The mechanisms and cleaning and sterilization protocols are well defined. Additionally, cultivation parameters for this system, such as gas transfer coefficient, agitation, aeration, temperature maintenance, pH, and

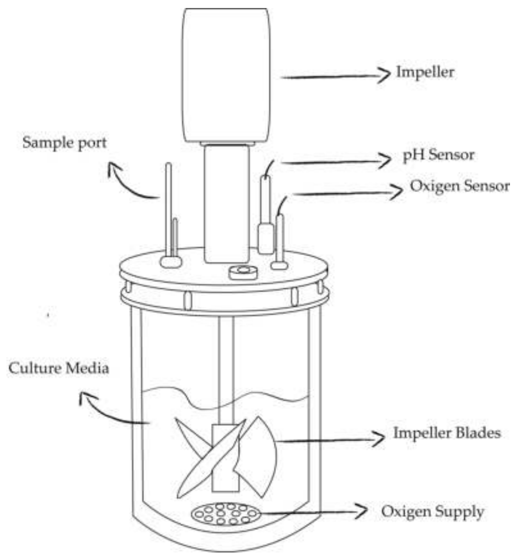

Figure 3. Schematic representation of a stainless steel stirred tank bioreactor. Showing the main components in a cell cultivation.

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others are well controlled and regulated when compared to other production systems. Another advantage of the STR is that it can be used for cultivation of various cell types and in addition, the products obtained from the cultivation in this type of bioreactor are easily approved for therapeutic use, as regulatory terms are well defined for this type of production [11].

However, the biggest disadvantage for the use of STR is the stress caused by shear. It can cause cell lysis and lead to loss in mAb productivity.

2.3.3. Air-lift reactors

Air-lift reactors are also broadly used for the industrial production of mAbs. The reactor consists of tanks with a bubble column inside, and air is injected into the column base (**Figure 4**). The air flows through the column's length to the top of the bioreactor as degassed culture medium flows in the opposite direction to the reactor bottom. This creates a constant gentle mixing of the medium as well as proper culture aeration, annulling part of the shear stress caused by other stirring systems. Other advantages of this operation system are that it is easier to scale-up, contamination problems are more unlikely to occur, and the equipment is simpler. In spite of these advantages, this system is less utilized than STR reactors because

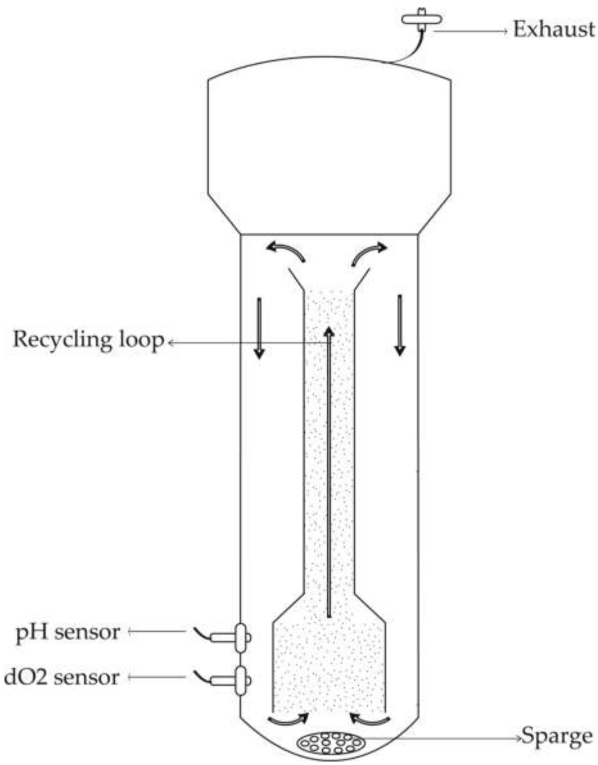

Figure 4. Schematic representation of an air-lift bioreactor. Showing the main components in a cultivation process.

the working volume ranges only from 2.0 to 5.0 L [1] and the air-lift reactor handling is not so well elucidated [11].

2.3.4. Disposable bioreactors

The first single-use bioreactors emerged in the late 1990s with the launch of a wave reactor system. After that, disposable stirred tank bioreactors were developed [11].

This method brought many advantages for mAb manufacturing. At the end of the process, the bioreactor is discarded and replaced by a new clean and sterile one. This eradicates cross contamination between batches and decreases the time consumed with the equipment preparation between batches. When all the advantages of this process are taken in account, the savings made regarding production and investment capital are highly significant when compared with other process methods. The great disadvantage of this production system is the small work volume supported, ranging from 50 to 2000 L [1].

The wave system consists of a sterile plastic bag (CellBag™) lying on a rocking platform (**Figure 5**). The bag is half filled with cultivation medium and half filled with a gas mix of interest. The platform motion creates an undulation movement in the culture, ensuring efficient aeration and culture mixing without causing shear damage [10, 11, 13]. The other available systems combine the convenience of a disposable system with the well-known stirred tank system and they are HyClone S.U.B®, Millipore® (CellReady™), or Xcellerex® (XDR™).

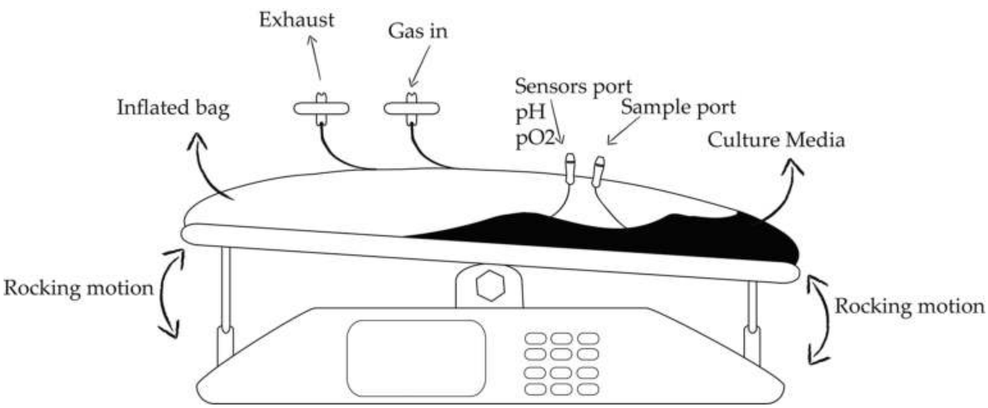

Figure 5. Schematic representation of a disposable wave bioreactor. Showing the main components in a cell cultivation process.

The main features of SUBs are related to their technical characteristics similar to those of stainless steel bioreactors, that is, aeration rate, agitation, reactor geometry, and ease of monitoring internal conditions, a process similar to stainless steel bioreactors [9].

SUBs are being widely used to replace many processes for the production of bioproducts. SUBs may be a cheaper and more efficient alternative from an industrial point of view, and its

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principle can easily replace any bioprocess to adapt the method to the platform of interest to be replaced, such as large tanks and stainless steel or the motion rocking platforms [9, 24].

SUBs have been used in bioprocesses for monoclonal antibody production involving several expression systems, including mammalian cells, microorganisms, plants, mammary glands, etc. Animal cell culture technology is one of the oldest techniques for the production of mAbs.

There is also the production of bottles known as roller bottles, consisting of mammalian cells growing in nutritional and physical conditions controlled in bottles which remain in rotational movement.

2.3.5. Roller bottles

Roller bottles are a rotary motion system for growing cells and for the production of some bioproducts. It has been an alternative to other monoclonal antibody production systems (**Figure 6**). Roller bottles provide conditions that favor the transfer of oxygen and temperature control without aeration, agitation propellers, or circulation pumps. The bottle is mounted on a turntable which gives homogeneity of growth and aeration of the culture medium [11, 25, 28].

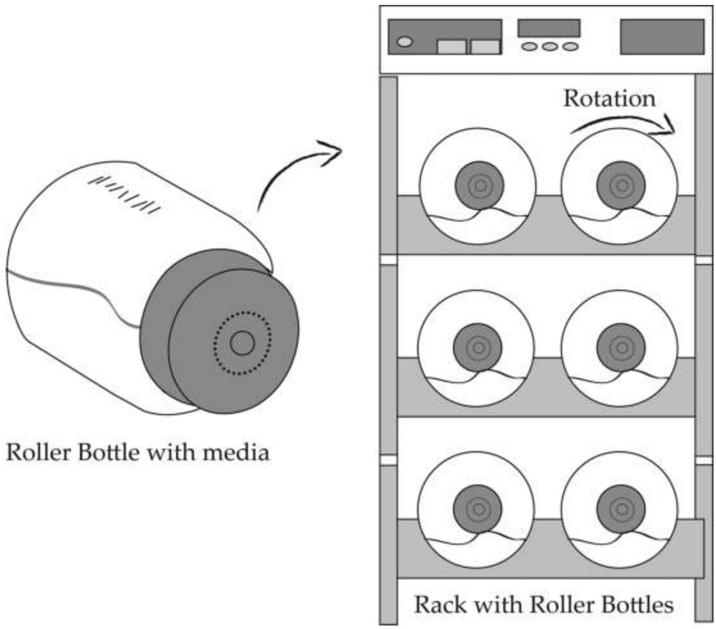

Figure 6. Schematic representation of roller bottles bioreactor and a rack with the rotational motion system in a cultivation for mAb production.

For the production of monoclonal antibodies at commercial scale, the roller bottle technique can be adapted to racks containing tens of bottle in a production line. The advantages of this technique is the high growth potential linked to ease of handling and monitoring of certain conditions such as temperature and rotation. However, the scale of view requires a large physical footprint, which can make the process less economical [11, 25].

3. Conclusions and perspectives

Actually, the trade of monoclonal antibodies makes up half of marketed biopharmaceuticals, reaching $ 75 billion. For some years, new development methodologies of antibodies have advanced with new production processes, allowing scale-up production and market introduction, and demands for high-quality biologics will continue to increase in the coming decades. Generally, processes are similar to those applied in the scheduling for other bioproducts/biosimilars that rely on culturing microorganisms or cells, requiring the development of a well-designed culturing process comprising the full range of control and associated operations that will support technical evaluations.

In combination with increasing pressure from regulatory agencies for enhanced quality and lower process costs from the health care systems, we are facing an important challenge. It will be necessary to make changes in plant design aiming for highly flexible multi-purpose facilities for small production volumes.

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