Biotechnology Advances 35 (2017) 458–465

Contents lists available at ScienceDirect

Biotechnology Advances

journal homepage: www.elsevier.com/locate/biotechadv

Research review paper

Very-large-scale production of antibodies in plants: The biologization of manufacturing

J.F. Buyel [a,b,*], R.M. Twyman [c], R. Fischer [a,b,1]

[a] Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany
[b] Fraunhofer Institute for Molecular Biology and Applied Ecology IME, Forckenbeckstraße 6, 52074 Aachen, Germany
[c] TRM Ltd, PO Box 463, York YO11 9FJ, United Kingdom

ARTICLE INFO

Article history:
Received 1 February 2017
Received in revised form 20 March 2017
Accepted 23 March 2017
Available online 25 March 2017

Keywords:
Biologization
Molecular farming
Monoclonal antibodies
Plant-based production
Process economy
Very-large-scale production

ABSTRACT

Gene technology has facilitated the biologization of manufacturing, i.e. the use and production of complex biological molecules and systems at an industrial scale. Monoclonal antibodies (mAbs) are currently the major class of biopharmaceutical products, but they are typically used to treat specific diseases which individually have comparably low incidences. The therapeutic potential of mAbs could also be used for more prevalent diseases, but this would require a massive increase in production capacity that could not be met by traditional fermenter systems. Here we outline the potential of plants to be used for the very-large-scale (VLS) production of biopharmaceutical proteins such as mAbs. We discuss the potential market sizes and their corresponding production capacities. We then consider available process technologies and scale-down models and how these can be used to develop VLS processes. Finally, we discuss which adaptations will likely be required for VLS production, lessons learned from existing cell culture-based processes and the food industry, and practical requirements for the implementation of a VLS process.

Contents

Abbreviations: ATPS, aqueous two-phase extraction; cGMP, current good manufacturing practice; CHO, Chinese hamster ovary; HCP, host cell protein; HIV/AIDS, human immunodeficiency virus/acquired immunodeficiency syndrome; mAb, monoclonal antibody; SMB, simulated moving bed; UF/DF, ultrafiltration/diafiltration; VFU, vertical farming unit; VLS, very large scale.

* Corresponding author at: Institute for Molecular Biotechnology, Worringerweg 1, RWTH Aachen University, 52074 Aachen, Germany.
E-mail addresses: johannes.buyel@rwth-aachen.de (J.F. Buyel), richard@twymanrm.com (R.M. Twyman), fischer@molbiotech.rwth-aachen.de (R. Fischer).
[1] Current address: Indiana Biosciences Research Institute, 1345 W. 16th St. Suite 300, Indianapolis, IN 46202, USA.

http://dx.doi.org/10.1016/j.biotechadv.2017.03.011

1. Introduction

The earliest manufacturing by humans was biological, e.g. animal skins as protection and tools made from wood. As technology developed so did the use of non-biological materials, beginning with stone tools and progressing to metals and eventually petrochemical derivatives such as plastics, even though the latter have an ultimate biological origin (Kolb and Kolb, 1979). This trend reversed during the last century and there is a renewed focus on biological manufacturing, mainly because biological products are often too complex for cost-effective total chemical synthesis. However, even simple biological products are beginning to replace synthetic counterparts because they are considered more sustainable, i.e. they originate from renewable sources such as plants. Arguably, the first complex biological products to have a major impact on society were the earliest antibiotics (Fleming, 1929). Nowadays, the pharmaceutical industry is dominated by complex small molecules as well as protein-based drugs, and we are increasingly reliant on biotechnology for manufacturing, which was foreseen almost 25 years ago (della Valle and Gambardella, 1993). The scope of biological manufacturing has recently expanded beyond the familiar territory of structurally-complex drugs and has begun to embrace spheres more traditionally dominated by chemical synthesis, particularly the replacement of petrochemical materials with 'biological' counterparts such as biodegradable plastics, biogas for energy and biofuels based on ethanol. Fermentation is another staple of biotechnology that has been used since ancient times as a form of food processing, but since the early 1970s it has evolved into a mainstream manufacturing technology for biological products (Jackson et al., 1972). Without fermentation, the development of recombinant DNA and gene cloning technology would have taken much longer, delaying the benefits brought about by gene transfer to living organisms including protein manufacturing and gene therapy (MedCap Advisors, 2016).

Today's protein-based biopharmaceuticals include vaccines, enzymes and especially monoclonal antibodies (mAbs) (PhRMA, 2015). Since the first therapeutic mAb was commercialized in 1986, the biologics industry has expanded to include diverse protein-based drugs, vaccines and technical reagents, and the market is now worth more than $US 70 billion (Ecker et al., 2015). Despite the intense competition for market share and a certain degree of bureaucratic inertia (della Valle and Gambardella, 1993), the major players have more recently begun to cooperate in areas of mutual benefit, such as regulatory compliance (The CMC Biotech Working Group, 2009) and clinical data analysis (Oo and Kalbag, 2016). The approval of the first plant-derived biopharmaceutical protein in 2012 ushered in an era where biological manufacturing has 'returned to its roots', in some cases quite literally, with plants now emerging as an alternative to production systems based on microbes and mammalian cells (Fischer et al., 2013; Stoger et al., 2014; Tschofen et al., 2016). The potential benefits of plants for the manufacture of biologicals include highly scalable and rapid production, a low burden of human pathogens, and the ability to carry out many of the post-translational modifications required for functional human proteins (Buyel, 2015). Here we discuss recent developments that presage a new era of biological manufacturing in which plants could be used to achieve an economy of scale that would have been unimaginable even 10 years ago.

2. Potential future applications and market size for monoclonal antibodies

Nearly 10 t of diagnostic and therapeutic mAbs were manufactured in 2013, and the demand could double by 2020 assuming the annual growth rate remains at the current level of ~10% (Ecker et al., 2015). Much of the current demand centers on mAbs used for the diagnosis and treatment of comparably rare diseases (Kelley, 2007). Even the biggest selling therapeutic mAbs, with millions of patients each requiring gram quantities of product (such as rituximab for the treatment of lymphomas), can be provided by todays large-scale manufacturing processes based on fermenter capacities of up to 250,000 L (Ecker et al., 2015). However, if mAbs are to be used for the prevention or treatment of even more common diseases, e.g. Alzheimer's disease, HIV/AIDS or dental caries (Ma et al., 2015), then the demand could escalate dramatically, as observed when rituximab was approved for the treatment of rheumatoid arthritis a (Ecker et al., 2015; Singh et al., 2009). For the prevention of HIV infection, a typical dose of 30 mg mAb would be applied twice weekly by susceptible women in the form of a vaginal gel, equating to ~100 doses per year. Therefore, >3 t of the mAb would be required per year per million women. The HIV^+ population in sub-Saharan Africa is currently 26 million, and the at-risk population is therefore even higher. An effective prophylactic campaign scaled to entire countries or regions would therefore require 50–100 t of mAb per year, far outstripping the capacity of even the largest current processes and defining a new era of very-large-scale (VLS) production more akin to the food processing industry (Lokhorst et al., 2015).

Such demands are currently impossible to meet using mammalian cell cultures in stirred tank bioreactors, which are today's gold standard for production. Chinese hamster ovary (CHO) cells under optimal conditions produce titers of 5–10 g L^{-1}, and with the largest available fermenter volumes of 10,000–25,000 L and fermentation lasting ~12 days, the maximum output of a single fermenter would be 250 kg per campaign or ~7.5 t per year assuming continuous and perfect operation (Kelley, 2007). In reality, the yield would be much lower. Furthermore, such VLS reactor setups would require stainless steel equipment, thus attracting high investment costs but lacking the advantages of single-use technologies that have become popular for both upstream production and downstream processing in the pharmaceutical industry over the last decade (Shukla and Gottschalk, 2013).

Unlike fermentation platforms, plants are effectively living single-use bioreactors that can be scaled indefinitely simply by sowing more seeds. Biomass yields of 10,000 t km^{-2} y^{-1} have been reported for closely cropped tobacco in the open field (Stoger et al., 2002), and a vertical farming unit (VFU) has recently been built with a footprint of ~6500 m^2 including ~2000 m^2 used for plant growth, with an annual output of 182 t of biomass (Holtz et al., 2015). Accordingly, the annual space–biomass yield of the VFU was more than nine times higher than open field production (91,000 t km^{-2} y^{-1}). The yields of recombinant protein achieved in the VFU reached 0.65 g kg^{-1}, but titers of up to 2 g kg^{-1} have been reported for mAbs produced in plants after several rounds of optimization (Zischewski et al., 2015). Given these numbers and an average recovery of 70% for plant-derived mAbs in a process that handles 250 kg of biomass (our unpublished data), an annual demand of 50 t of pure mAb would require ~72 t y^{-1} of bulk mAb produced using 3.5–11.0 km^2 of open fields or 0.4–1.2 km^2 of VFU area. Importantly, VFUs can be designed as fully-automated plant-handling facilities that comply with current good manufacturing practice (cGMP) requirements (Wirz et al., 2012) whereas these requirements are never likely to be met byopen fields. Although VFUs offer the most promising approach, the production of mAbs in tonne amounts will be accompanied by significant investment costs. However, similar or even higher investments are necessary for CHO-based processes because the same output would require a total bioreactor volume of ~250,000 L. Nevertheless, the investments required for VFUs are likely to pay off due to the large quantities of product that will be manufactured, meaning that mAbs will become a "bulk product". However, this may require a paradigm shift, turning mAbs from "small quantity – high margin" to "large quantity – low margin" products.

The cost of CHO cell medium is $US 55–90 L^{-1}, corresponding to $US 14–22 million per batch for medium alone, which can be regarded as a high risk investment given recent issues with contamination (Lolas, 2013). In contrast, media costs for a plant-based expression system would be $US ~4.5 million, assuming recently reported biomass yields and fertilizer consumption (Buyel and Fischer, 2012). The VLS production of mAbs in plants therefore appears to have several advantages