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Research review paper

Upstream processes in antibody production: Evaluation of critical parameters

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Abstract

The demand for monoclonal antibody for therapeutic and diagnostic applications is rising constantly which puts up a need to bring down the cost of its production. In this context it becomes a prerequisite to improve the efficiency of the existing processes used for monoclonal antibody production. This review describes various upstream processes used for monoclonal antibody production and evaluates critical parameters and efforts which are being made to enhance the efficiency of the process. The upstream technology has tremendously been upgraded from host cells used for manufacturing to bioreactors type and capacity. The host cells used range from microbial, mammalian to plant cells with mammalian cells dominating the scenario. Disposable bioreactors are being promoted for small scale production due to easy adaptation to process validation and flexibility, though they are limited by the scale of production. In this respect Wave bioreactors for suspension culture have been introduced recently. A novel bioreactor for immobilized cells is described which permits an economical and easy alternative to hollow fiber bioreactor at lab scale production. Modification of the cellular machinery to alter their metabolic characteristics has further added to robustness of cells and perks up cell specific productivity. The process parameters including feeding strategies and environmental parameters are being improved and efforts to validate them to get reproducible results are becoming a trend. Online monitoring of the process and product characterization is increasingly gaining importance. In total the advancement of upstream processes have led to the increase in volumetric productivity by 100-fold over last decade and make the monoclonal antibody production more economical and realistic option for therapeutic applications.

Keywords: Monoclonal antibody production; Wave bioreactors; Cryogels as bioreactors; Disposable bioreactors; Process monitoring and control; Cell engineering

Contents

Abbreviations: HFBR, Hollow Fiber Bioreactor; mAbs, Monoclonal antibody; IgG1, Immunoglobulin gamma 1; ECS, Extracapillary Space; MWCO, Molecular weight cut off; CHO, Chinese hamster Ovary; DOT, Dissolved oxygen Tension; DO, Dissolved oxygen; OUR, oxygen uptake rate; q_{Mab}, specific antibody productivity; GS, Glutamine Synthetase; DHFR, Dihydro folate reductase; Q_p, Specific production rates; LDH, Lactate Dehydrogenase; CPS I, Carbamoyl phosphate synthetase I; HSP 70, Heat Shock protein 70.

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1. Introduction

Monoclonal antibody (mAb) based therapeutics have become an important class of drugs and diagnostic agents, specifically for treatment of human malignancies and other chronic inflammatory conditions. This is much due to their specificity and selectivity that their demands have risen in the last few years (Reichert et al., 2005; Chu and Robinson, 2001; Adams and Weiner, 2005). The original method for production of monoclonal antibodies involved use of murine hybridoma cells. Therapeutic murine antibodies were made commercially available since 1980, but could not gain much success as they instigated immunogenic reaction on administration and thus were rapidly cleared from the body. Attempts were made to improve the efficacy of the antibodies using two different strategies; one involving production of chimeric monoclonal antibodies (mAbs) in which the F_c portion of the murine mAbs was replaced by human F_c while the other was to produce fully human mAbs (Morrison et al., 1984, Boulianne et al., 1984; Cole et al., 1984). Reichert et al. (2005) have analysed the current trends in mAbs production and reports an inclination towards study of human mAbs and mAb fragments. The data collected by them indicates FDA approval rates of chimeric and human mAbs are in the range of 18 to 29%. Eighteen mAbs have been approved for therapeutic use out of which three are murine antibodies and produced in hybridoma cells, others are chimeric, human antibodies (Chu and Robinson, 2001). Hundreds of other mAbs are in different phases of clinical trials. The global market for mAbs is expanding rapidly and in a recent analysis by Tufts center for study and research for drug development, Tufts projects that by 2008, the U.S. Food and Drug Administration is likely to approve 11 of the current mAb products in the pipeline and total market is going to reach US$ 16.7 billion (Pavlou and Belsey, 2005). In effect, these approvals would come close to doubling the number of mAbs now on the market. According to the report, newer mAbs, which include humanized mAbs and human products, are of types that have had a much higher success rate in attaining FDA approval than original murine (mouse derived) products. Historically, chimeric mAbs (such as the recently approved cetuximab [Erbitux]) have had the greatest success with FDA approval, with 26% of products in development hitting the market (Reichert and Pavlou, 2004). The approval rate for murine products, by comparison, is 4.5%. The current global market share of modified and engineered antibodies is around 56% (in 2006) and which is likely to remain 54% by 2011 as estimated by BCC research reports. This rapid increase in the number of mAbs being regularly approved for therapeutic use puts up a need to produce sufficient quantity of these mAbs which require sophisticated but commercially simple process operation. This clearly indicates a requirement to develop such bioreactors or processes which can be scaled-up easily and also produce mAbs in grams.

Antibodies are amongst one of the most expensive therapeutics being used, this is mainly due to their use for chronic diseases in high dose ($\geq$100 mg) (due to their low potency). Consequently large scale processes are required to produce 10–100 kg/year to cope up with market demands. The upstream processes have been significantly improved (Birch and Racher, 2006; Farid,