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Strategies to enhance productivity and modify product quality in therapeutic proteins

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The production of commercially valuable biotherapeutic molecules in mammalian systems has expanded significantly in the last thirty years, but growing economic pressures within the industry are driving efforts to reduce costs and enhance process yields. At the upstream stage, two complementary approaches have evolved to increase productivity and maintain consistent product quality, that is either by altering the cell directly or by manipulating its environment. This review focuses on novel approaches to impact productivity and product quality by manipulating the environment through: (a) altering media composition; (b) modulating operating conditions such as pH and temperature; or (c) intensifying process operations by switching from fed-batch to continuous processes.

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Introduction

The biotechnology industry, a key driver for economic development, has undergone rapid growth since the commercialization of the first recombinant DNA product over 35 years ago [1]. Global sales for biotherapeutics exceeded $200 billion in 2016 [2] and are expected to account for a quarter of the projected $1.4 trillion global spending on medicines by 2020 [3]. As the industry matures, companies face increasing economic pressures stemming from expiring patents, competition from biosimilars, tighter regulations, and decreasing returns on research investments for drug development [4]. Thus, there is a growing need within the biopharmaceutical sector to innovate and improve productivity at each stage in the biopharmaceutical process—from increasing protein expression at the upstream stage, to debottlenecking purification trains and identifying innovative and modular solutions for manufacturing using single use technologies.

At the upstream stage, increasing the production of such complex biotherapeutic molecules comes with the associated challenge of maintaining consistent product quality, thereby ensuring the safety and efficacy of the drug product. The different techniques that have been used to enhance productivity and achieve consistent product quality can be broadly classified into two complementary tactics (Figure 1):

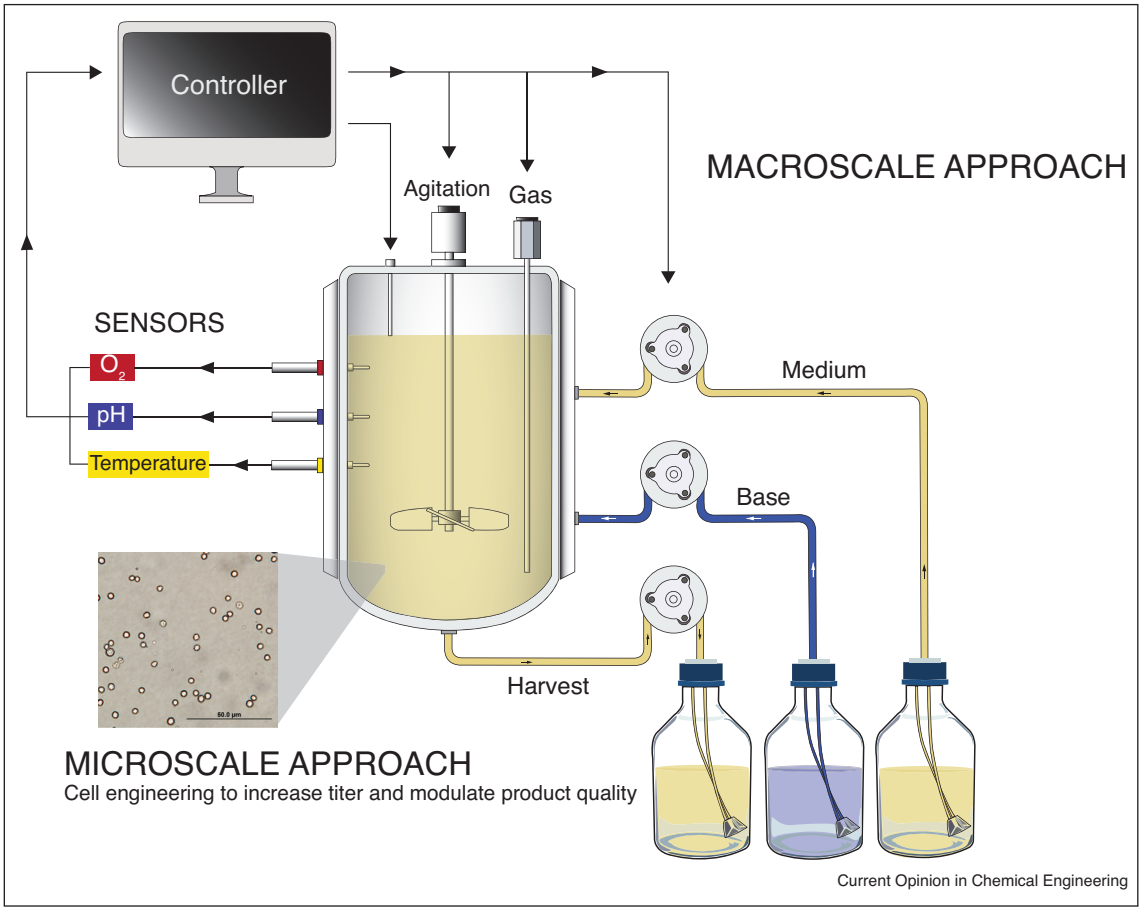
1 Microscale strategies—broadly, these strategies refer to manipulations that are performed at the cellular and subcellular level to identify bottlenecks to protein folding and secretion [5] and/or techniques to alter the final quality profile of the expressed protein [6].
2 Macroscale approaches—when cell line modifications are infeasible, a complementary approach to increase productivity and influence product quality uses factors available at the macroscopic scale by: (a) manipulating media compositions and formulating defined media via media additives and supplements; (b) using optimal process and operating conditions; or (c) altering the mode of operation by switching to perfusion or continuous production.

A detailed discussion of different microscopic approaches using cellular engineering to alter product quality and productivity can be found in other reviews [for e.g. see Refs. [6,7]]. Herein, we limit the scope of the current review to focus on macroscopic approaches to achieve higher titers and consistent product quality by manipulating process and operating conditions.

Media supplements

The advancement of process analytical tools has facilitated a comprehensive analysis of cell culture media and led to the identification of individual components that influence both the productivity and quality of different proteins expressed in mammalian cells (Table 1). For instance, productivity markers were identified in soy hydrolysate using LC–MS/MS and metabolomics, and mechanistic underpinnings for batch-to-batch variations in the process were then established from these insights [8]. Similarly, using NMR and 2D-DIGE, Blondeel *et al.* [9•]

Figure 1



Complementary approaches to modulating productivity and product quality in biomanufacturing. At the molecular level, cell line engineering can be performed to enhance cellular productivity and obtain a consistent or desired quality profile. Alternatively, manipulations at the macroscopic scale, such as changes to the media formulation, shifting process operating conditions, and employing different modes of operation can be utilized to improve productivity and alter product quality for proteins expressed in a specific cell line.

characterized cellular factors limiting growth and productivity and designed rational feeds to enhance productivities and increase cell densities by nearly 75%. By examining different amino acid supplements present in commercially available chemically defined (CD) cell culture media, feed and supplementation strategies have been successfully implemented to increase product titers in fed-batch conditions by minimizing lactate and ammonia accumulation [10,11]. The identification and optimization of media components have also led to strategies wherein media additives have been used to enhance titer. For instance, nucleoside sugars deoxyuridine and thymidine significantly increased the peak viable cell concentration, and consequently antibody titer, when supplemented singly into antibody-producing CHO cultures [12]. Supplementing with deoxyuridine, thymidine, and deoxycytidine together further improved final titers by 17% over cultures treated solely with deoxyuridine [12]. Although these individual approaches are promising and straightforward to test for any protein of interest, the successful implementation can vary in a product and/or process-specific manner, likely in part due to cell line instability and heterogeneity.

A detailed understanding of the effect of different media additives has also resulted in a new set of strategies to modulate product quality attributes. In one instance, researchers observed an increase in tryptophan oxidation in two biopharmaceutical products following a switch from hydrolysate containing media to chemically defined (CD) media [13]. By comparing the individual components of the two media types, they identified and modulated the concentrations of the amino acids, tryptophan and cysteine, and metal ions, copper and manganese to reduce tryptophan oxidation. Vijayasankaran *et al.* [14] have demonstrated that the addition of different media supplements such as hypotaurine, cystine, peptones, and hydrocortisone could be used to reduce coloration and alter the acidic charge variant levels in fed-batch cultures. Similarly, researchers have demonstrated that basic