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Biopharmaceutical Manufacturing: *Current Titers and Yields in Commercial-Scale Microbial Bioprocessing*

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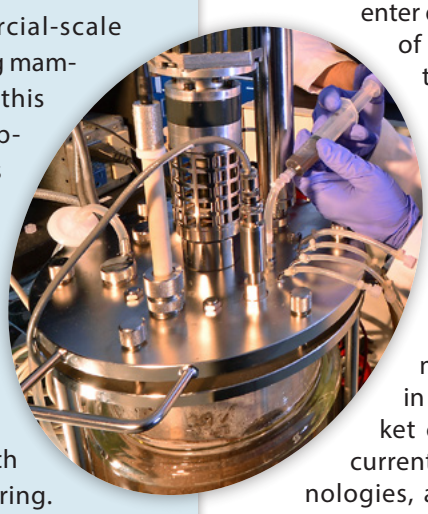
Abstract

This article reports the average titers and yields currently attained with commercially manufactured biopharmaceuticals expressed by microbial systems such as *E. coli* and yeasts. A recent *BioProcessing Journal* article comparably covered results from the first phase of this study concerning historical titers and yields attained for commercial-scale biopharmaceutical production using mammalian cells (*e.g.*, CHO).[1] As with this prior mammalian component, public domain data concerning titers and yields attained with microbially manufactured products were obtained using all available sources. These included a review of available literature and direct contact with over 200 bioprocessing professionals identified as involved in relevant product research and development, and manufacturing. Unexpectedly, current microbial titers with commercially manufactured products were found to be consistent with those previously determined for mammalian products. However, purification yields attained with microbial manufacturing averaged only about 15%. This is much lower than with mammalian products yielding approximately 69%.[1] Despite low downstream yields, microbial bioproduction continues to be considered less costly, simpler, faster, and generally more economical than mammalian manufacturing.

Introduction

Titer and yield are the primary quantitative measures indicative of bioprocess productivity and efficiency.[1] Titer is the ratio of expressed active agent/drug substance mass (weight) obtained at the end of upstream bioreactor processing relative to the volume of fluid withdrawn from the bioreactor (or bioreactor volume). This parameter, in grams/liter, is one of the key benchmarks used to determine operational efficiency and improvements in upstream bioprocessing. Titer, reflecting the concentration as products enter downstream operations, is a key measure of the efficiency of a product's manufacturing and related manufacturing costs. Yield is the ratio (percent) of the amount of active agent/drug substance mass (weight) at the end of downstream processing relative to the amount at the start of downstream processing. Yield is a key benchmark for purification productivity and efficiency.



A minority, about 30%, of biopharmaceutical products currently marketed in more affluent and high-tech major market countries (US, Europe, Japan, *etc.*) are currently manufactured using microbial technologies, all currently involving rather common microbial fermentation. The great majority of microbially manufactured biopharmaceuticals (microbial products) are manufactured using *Escherichia coli* (*E. coli*) bacteria, with a small portion using yeast such as *Saccharomyces cerevisiae* (*S. cerevisiae*) or *Pichia pastoris* (*P. pastoris*). The use of other microbial expression systems for commercial biopharmaceutical manufacture are rare outliers.

Microbial expression is generally considered simpler, cheaper, and faster than mammalian expression. However, mammalian-expressed products fully dominate commer-

PHOTO: A bioreactor being loaded with yeast. (*Courtesy of Wikipedia and USDA*)

cially manufactured biopharmaceuticals. This is because the market has come to be dominated largely by monoclonal antibodies (MAbs), with these generally requiring the use of mammalian cell lines. This is because microbial cells lack the ability to do most of the post-translational modifications, such as glycosylation, that mammalian cells can routinely perform. A few alternative microbial expression systems either use eukaryotic microbes, such as yeast, or genetically engineered cell lines (*e.g.*, enzymes spliced in) to attain some basic post-translational processing and glycosylation. So far, these have been unable to provide sufficiently human-like glycosylation. Many human and other proteins are innately glycosylated, often with rather complex carbohydrate structures, and microbial expression systems are not yet able to sufficiently reproduce human-like glycosylation. Thus, many mammalian glycoproteins, including antibodies, expressed in microbial systems behave differently and are lacking in structural and functional biosimilarity, therapeutic efficacy, and in the case of vaccines, immunogenicity.

Despite the post-translational limitations of microbial expression, given the choice, microbial manufacturing is sometimes preferable. Classic fermentation involves the growth of microbes to high cell concentrations in tanks, generally with maximally tolerated stirring, and higher temperatures and pressures — methods not tolerated by more sensitive mammalian cells. Microbial fermentation is an energetic process well-suited to the simple and rapid metabolism of bacteria and yeast. Microbial production processes typically wrap up, at most, a few days after inoculation, while mammalian bioprocesses can take several weeks. Microbial culture media are simpler than those required for mammalian cells, and are now almost universally chemically defined, free of animal-derived components. Also, most mammalian-expressed MAbs with any decent market share are generally manufactured in one ≥10,000 L bioreactor or more, with massive amounts required due to relatively high and repeated dosing. In contrast, most microbial products are often commercially manufactured in bioreactors less than half this size. Some microbial products, such as some hormones, are sufficiently potent enough that bioreactors in the low 100 L range can handle worldwide manufacturing needs — making microbial manufacturing lower scale and less costly.

However, mammalian cell culture using lower-cost, single-use technology now completely dominates development and clinical-scale manufacturing. Single-use bioreactor systems are now being incorporated at capacities sufficient for commercial manufacturing. In contrast, the first single-use microbial bioreactors are just starting to enter the market, with capacities currently limited to a maximum of ~300 L. Microbial cells expand rapidly, creating heat and pressure in the culture tank as well as unique design challenges. Current bag-based, single-use systems are not robust enough to handle the much higher temperatures, pressure increases, and energetic mixing commonly used with microbial production-scale bioprocessing. Rapid and energetic bioprocessing under extreme conditions are essentially what allow microbial commercial manufacturing to be more cost-effective and preferred over mammalian processes.

Microbial manufacturing is centuries-old and disseminated worldwide for use in industrial fermentation, ethanol production, and the manufacture of many industrial and other chemicals. In contrast, mammalian cell culture is relatively new, just decades old. Despite this and the cost advantages of microbial manufacturing, about 85–90% of currently marketed biopharmaceuticals — and an even higher proportion in the development pipeline — are now manufactured by mammalian cell culture processes. This is because the biopharmaceuticals now dominating the market are mostly MAbs and other more complex proteins. Alternative antibody scaffolds/frameworks suitable for microbial expression have been developed, but products on the market and in development using these will remain few, very much in the minority, with microbial post-translational processing (the lack of it) remaining problematic. Even if the product presents the choice of microbial expression, most developers now prefer to use mammalian expression for the manufacture of human and mammalian proteins, including antibodies. A comfortable familiarity exists, and this methodology is considered somewhat routine by regulatory agencies, while novel, custom molecule platforms and expression systems can be expected to receive more scrutiny.

Microbial products dominated the first recombinant proteins entering the market in the 1980s, with insulin the first product to receive US regulatory approval in 1981.[2] This was followed by recombinant microbially-expressed versions of various simpler, non-glycosylated human proteins such as interleukin-2, interferon, and tissue-type plasminogen activator (tPA); enzymes such as asparaginase; hormones such as insulin, glucagon, calcitonin, and somatropin; and glycoproteins such as G-CSF (granulocyte colony stimulating factor). Many of the earliest biopharmaceutical products are now prime candidates for biosimilar versions in major market countries, and many biogeneric versions of these manufactured in non- or lesser-regulated countries are now in international commerce.[3,4]

Methodology

The data collection methods used in our published study of mammalian titers and yields were replicated in our microbial data efforts. However, unlike with the mammalian study, our current analysis did not include examination of historical titer and yield data. This was partly because the prior annual surveys of biopharmaceutical bioprocessing professionals by the authors (BioPlan Associates, Inc., now in its 13th year), did not include questions regarding microbial titers obtained by survey respondents, while this data was available for most years for mammalian manufacturing.

Data collection for the microbial titers and yields consisted of published sources including the *BIOPHARMA* database[2], Top 1000 Global Biopharmaceutical Facilities[5], BioFacilities Newsletter[6], and searching online bibliographic databases including BIOSIS and Web of Science. Articles concerning development and bioprocessing with current commercially manufactured microbial biopharmaceuticals were reviewed, along with more generic articles concerning microbial titers, yields, and related trends.

Additional primary data collection included contacting hundreds of bioprocessing professionals by email and/or phone and requesting public domain (*e.g.*, published/presented) data concerning commercial-scale microbial product titers and yields. This included the 500+ members of the BioPlan Associates' Biotechnology Industry Council™ (BIC) and direct contact with over 200 other bioprocessing professionals. These individuals were identified as involved in microbial product R&D or bioprocessing using available sources including publications, patents, presentations, and LinkedIn records.

Results and Observations

The overall averages obtained for microbial titer and yield and previously-reported mammalian data are presented in **Table 1** (following page).

Table 2 (following page) presents the microbial products ($n = 44$) for which usable titer and yield data were obtained. Data were developed for more microbial products than in the prior mammalian products study.[1] In this respect, these data may be considered somewhat more robust. However, titer and yields attained with microbial manufacturing generally vary over a wider range than with mammalian, so it is harder to extrapolate these data to other specific products. Note, a few very late-stage clinical, biodefense and other non-mainstream marketed products, all currently being manufactured at commercial scale, are included in **Table 2**.

E. coli prokaryotic bacteria are used to manufacture 75% of the products, with 22% manufactured using yeast, *S. cerevisiae* or *P. pastoris*. No other expression systems are currently used for commercial microbial biopharmaceutical manufacturing. A large number of expression systems have been developed and are available for licensing, with most now off-patent and royalty-free.[7] However, despite commercial expression systems offering many competitive advantages, such as the Pfenex Expression Technology™ (based on *Pseudomonas fluorescens* [*P. fluorescens*] bacteria), Corynex™ (based on *Corynebacterium glutamicum*) from Althea/Ajinomoto, and the proprietary C1 filamentous fungi system from Dyadic, these systems have been largely ignored by industry in favor of familiar and regulatory-friendly classic *E. coli* and yeast systems.

Data were also collected and developed concerning the type of *E. coli* expression system used, whether involving inclusion body (IB) or soluble secretion (SS) expression systems. IB expression systems are older, more classic, and dominate all microbial manufacturing, not just *E. coli* manufacturing. IBs involve protein expression within *E. coli* as aggregates that form relatively large particles. SS expression involves protein secretion into the cell periplasm (cytoplasm).

In some respects, IBs can simplify downstream purification, such as their ready separation using centrifugation or filtration, but the proteins within IBs must be disaggregated and refolded by chemical reactions to their native or desired three-dimensional structures. This can complicate microbial process design and bioprocessing, and the extra steps, extra time, additional reagents required, *etc*. add to manufacturing costs.

SS expression involves its own, but less extreme, purification problems, with cells still needing to be broken up and the desired protein purified from this complex mix. The intricate purification process of *E. coli*-expressed products (and all microbially produced products, in general) are the primary driver for the rather low downstream yields reported, as compared to mammalian production processes. Despite low yields, microbial manufacture generally remains cheaper, much quicker, and simpler than mammalian (*e.g.*, MAb) manufacture. And with the costs associated with protein A resins and other aspects of MAb downstream processing, microbial products are still usually less expensive to purify than mammalian.

Surprisingly, microbial titers were essentially equivalent to those of mammalian products. It must be noted that most of the products surveyed are older, legacy products, with their bioprocessing often designed and originally implemented in prior decades. Retrospective, historical, legacy, and other data concerning microbial product titers and yields were not developed as part of this project, as was done with the prior mammalian titer/yield project.[1] However, it can be strongly presumed that microbial has followed much the same growth trajectory since the 1980s as portrayed in our prior mammalian titer/yield study, essentially nearly doubling every five years from a low baseline, a fraction of a gram/L. However, compared to mammalian expression, there is simply much less research and commercial development of improved microbial expression systems being done due to lower demands, fewer relevant products, and/or users being satisfied with current technologies. Thus, we expect microbial titers and yields to level off in their growth.

Several microbial products were identified as using fusion protein affinity purification "tags" such as His-tags. This involves initial expression of the desired protein as fusion protein with a short terminal peptide sequence or ligand with strong affinity for specific chromatography media, greatly simplifying initial protein concentration. However, the tags must be removed by enzymes, whether added or co-expressed with the fusion protein, and there are only a few self-cleaving microbial tag expression systems available.

TABLE 1. Commercial-scale manufacturing average titers and yields for microbial and mammalian products.

Class	Average Titer	Average Yield
Microbial	2.54 g/L	15.1%
Mammalian	2.56 g/L	70.0%

TABLE 2. The microbial products for which titer and yield data were developed.

Trade or Descriptive Product Names	Expression System	Manufacturers and Major Marketers
Recombumin® (albumin, rDNA [excipient])	*S. cerevisiae*	Novozymes Delta
BioThrax® (anthrax vaccine, rDNA, rPA)	*E. coli*	Emergent BioSolutions for national biodefense
Anthrax vaccine (rDNA, rPA102)	*E. coli*	Celltrion for national biodefense
Anthrax vaccine (rDNA, rPA)	*P. fluorescens*	Pfenex for national biodefense
SparVax® (anthrax vaccine, rPA)	*E. coli*	PharmAthene for national biodefense
Thraxine™ (anthrax vaccine, rPA)	*E. coli*	Avecia for national biodefense
Voraxaze® (carboxypeptidase, rDNA)	*E. coli*	Health Protection Authority (UK) for Protherics/BTG
Neulapeg® (G-CSF, rDNA)	*E. coli*	Green Cross for Celgene/Abraxis
Neupogen® (G-CSF, rDNA)	*E. coli* with IB	Amgen
Gattex® (GLP-2, rDNA)	*E. coli* with SS	Boehringer Ingelheim for NPS Pharma/Shire
Victoza® (GLP-1, rDNA)	*S. cerevisiae*	Novo Nordisk A/S
Trulicity® (GLP-1, rDNA)	[not disclosed]	Eli Lilly
GlucaGen® (glucagon, rDNA)	*S. cerevisiae*	Novo Nordisk
Glucagon Emergency Kit (glucagon, rDNA)	*E. coli*	Eli Lilly
Leukine® (GM-CSF, rDNA)	*S. cerevisiae*	Genzyme/Sanofi
HBvaxPRO® (hep B vaccine, rDNA)	*S. cerevisiae*	Merck & Co. for Sanofi Pasteur MSD
Recombivax HB® (hep B vaccine, rDNA)	*S. cerevisiae*	Merck & Co.
Iprivask® (hirudin, rDNA)	*S. cerevisiae*	Boehringer Ingelheim for Canyon Pharma
Refludan® (hirudin, rDNA)	*S. cerevisiae*	Sanofi for Bayer Pharmion
Gardasil® (HPV vaccine, rDNA)	*S. cerevisiae*	Merck & Co.
Lantus® (insulin glargine)	*E. coli* with IB and His-tags	Sanofi
Apidra® (insulin glulisine, rDNA)	*E. coli*	Sanofi
Increlex® (IGF-1, rDNA)	*E. coli*	Lonza for Ipsen
Intron A® (interferon alfa-2b)	*E. coli* with SS	Merck & Co.
Neumega® (interleukin-2, rDNA)	*E. coli*	Pfizer
Kineret® (interleukin-1ra, rDNA)	*E. coli* with SS	Amgen for Biovitrum AB/Pfizer and NPS Pharma
Kepivance® (KGF, rDNA)	*E. coli* with SS	Amgen for Biovitrum AB/Pfizer and NPS Pharma
Jetrea® (microplasmin, rDNA)	*P. pastoris*	Fujifilm Diosynth for ThromboGenics and Alcon/Novartis
Natrecor® (natriuretic peptide, rDNA)	*E. coli* with IB	Scios/Johnson & Johnson
Regranex® (PDGF, rDNA)	*S. cerevisiae*	Novartis for Smith & Nephew and Johnson & Johnson
Augment® (PDGF, rDNA)	*S. cerevisiae*	BioMimetic Pharma/Wright Med. Tech. for Luitpold Pharm/Sankyo
Somavert® (somatropin antagonist, rDNA)	*E. coli*	Fujifilm Diosynth for Pfizer
Accretropin™ (somatropin, rDNA)	*E. coli*	Cangene/Emergent Biosolutions (being acquired by Belrose Pharma)
Genotropin® (somatropin, rDNA)	*E. coli* with SS	Biovitrum/Pfizer
Humatrope® (somatropin, rDNA)	*E. coli* with SS	Eli Lilly
Norditropin® (somatropin, rDNA)	*E. coli* with DAP-tags	Novo Nordisk
Nutropin AQ® and Depot® (somatropin, rDNA)	*E. coli* with SS	Genentech/Roche
Omnitrope® (somatropin, rDNA)	*E. coli*	Sandoz AG/Novartis
Valtropin (somatropin, rDNA)	*S. cerevisiae*	Rentschler Biotech for LG Life Sci., BioPartners/Bioton and Nycomed Pharma
TEV-Tropin® (somatropin, rDNA)	*E. coli*	Ferring BV for itself and Gate Pharma
SciTropin A® (somatropin, rDNA)	*E. coli*	SciGen/Bioton
Somatropin, sustained-release (rDNA)	*E. coli*	Somatropin Biopartners; Bioton for LG Life Sci.
NPlate® (TPO peptibody, rDNA)	*E. coli* with IB	Amgen
Retavase® (tPA, rDNA)	*E. coli* with IB	Fujifilm Diosynth and Scil Proteins/Wacker for Cornerstone Ther./Chiesi and Actavis Group
Lucentis® (VEGF MAb FAb, rDNA)	*E. coli*	Genentech/Roche, including for Novartis

ABBREVIATIONS: rDNA (recombinant DNA), rPA (recombinant plasminogen activator), GLP (glucagon-like peptide), GM-CSF (granulocyte macrophage colony-stimulating factor), IGF (insulin-like growth factor), KGF (keratinocyte growth factor), PDGF (platelet-derived growth factor), TPO (thrombopoietin), VEGF (vascular endothelial growth factor), FAb (antibody fragment), His (histadine), DAP (death-associated protein 1)

Conclusions

Titers and yields currently attained with the commercial manufacturing or marketed (or very near market) microbial system-expressed proteins were determined by the collection and analysis of available public domain data. *E. coli* dominates commercial product microbial expression systems. Microbial manufacturing titers closely matched those now attained with commercial mammalian product manufacture.

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