Many Considerations in Selecting Bioproduction Culture Media

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Not long ago, the ability to support efficient large-scale culture of cells was the main factor in choice and development of production media. However, a number of new performance demands have been imposed on production media (as listed in the "Key Factors" box). These new criteria arise from such sources as the demand for increased efficiency in a number of production operations, goals invoked by new quality initiatives, and a more science-based approach to process development.

Not only is the overall number of criteria growing, but in fact there are users who must consider many different features in specifying their production media. Here we outline and categorize many functions now desired from production media, emphasizing serum-free media (SFM) and feed supplements. Although some culturists continue to operate using animal sera, for most cell culture formats (e.g., Chinese hamster ovary or CHO cells) and production modes (e.g., fed batch) SFM has become standard (**1**, **2**).

Serum-free medium supports efficient bioproduction in a classically sparged 250-L single-use bioreactor using a pitched-blade impeller
(THERMO SCIENTIFIC HYCLONE, WWW.HYCLONE.COM)

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Process Focus: Production

Who Should Read: QA/QC, process development, manufacturing

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Level: Basic

Cell/Culture Quality and Performance

One of the first goals set for modern SFM was the **support of higher growth rates and culture densities** than are possible with serum-containing cultures. Serum-free culture was primarily accomplished through the specific replacement of serum components that provide identified functions, such as substituting particular chelating agents for transferrin as an iron transporter. However, advancements in nutritional and cofactor understanding have provided generally superior performance in SFM over serum-based culture (**3**). For most culture platforms currently available, optimized SFM can now support desired cell division rates yielding >10^6 viable cells/mL in batch culture and >20^6 viable cells/mL in fed-batch.

To increase culture lifespans, promotion of **culture longevity and suppression of apoptosis** at the plateau phase became more of an issue when serum was removed from media formulations. Though they are often cell-line and mode specific, optimized processes can now commonly maintain cell viability for four to five days at peak cell densities (even longer in some cases), which greatly increases volumetric productivity. Several factors, including those originating from process materials, must be considered in accomplishing this goal. For example, apoptosis can be

stimulated by nutrient deprivation, toxic metabolite accumulation, and growth factor loss (4).

Elimination of animal components and proteins has highlighted concerns regarding **clone stability in continued passages.** Early on, it was discovered that undesired selection, mutation, or alteration of gene expression can occur sooner in some SFM cultures (5). But optimization of cloning techniques and serum-free formulations has resulted in processes that demonstrate acceptable stability throughout scale-up and production.

Product Quantity and Quality

Commercial SFM are now designed to **promote high product yields.** Most on the market today boost yields to levels that are many-fold higher than obtainable using serum-containing media, largely because of cell-line or clone-specific customization of the components. This is accomplished through such means as supporting efficient expression, promoting and maintaining high culture densities, and directing production over cell mass generation. Whereas the removal of serum initially presented performance challenges, it should be noted that it also eliminated serum-derived factors that inhibit cell and quality product generation. Product yields exceeding 20 g/L have been reported, and depending on cloning strategies, 2–3g/L is now often expected.

Production of larger and/or highly processed proteins in animal cells establishes a risk of variability, which drives a number of activities to consistently **maintain critical product quality attributes** throughout bioreactor operation. Features of the culture medium used can influence process conditions that determine product quality, as when enzymatic activity cleaves a protein and renders it inactive and/or potentially immunogenic. Such factors must be reconsidered in specific applications because, as a process is optimized for other particular goals, product attributes can be affected (6).

A newer consideration of media potential is how SFM can help **reduce product microheterogeneities** in expression through harvesting. Several material factors can influence the generation of such molecular variants in a product as the type and degree of glycosylation or sialyation (7). The levels and types of product-related substances can be affected by the availability of donor substrates supporting posttranslational events, nutrition that inhibits ammonia or lactate build-up, and chemistries to maintain acceptable pH throughout culture.

The imperative to **reduce product- and process-related impurities** can apply to some otherwise desirable media components such as antibiotics, selection agents, peptide hormones, and proteins that behave similarly to the product during purification. Media formulations can help reduce such degradation products as oxidized or deaminated variants. Because this can be quite product and process specific, it must be reconsidered for each case and during the application of platform production approaches.

Beyond supporting the expression and secretion of high levels of good-quality protein products, it is necessary to **maintain product stability through harvest.** Product-related impurities can be generated after protein secretion by, for example, breakdown of the desired product by cellular or medium-derived proteases. Small-molecule antioxidants can be used to inhibit generation of detrimental inter- or extracellular reactive oxygen species — leading to, e.g., oxidation of the product. Even proper protein folding can be influenced by media formulations and supplements (8).

Risk and regulatory considerations for SFM include concerns about patient safety and support of current manufacturing regulations or guidances. Both the materials included and the performance of a formulation in an application are factors in the degree of compliance and potential for continuous improvement. Eliminating sera or animal-derived components alone can reduce or eliminate several risk factors (9). Media or feed strategies that are free of animal components and/or chemically defined address such concerns as raw material consistency. A medium's performance can contribute to development of robust, reproducible culture and downstream procedures as well as the ultimate yield and ratio of desired to degradation products.

Key Features to Consider in SFM Choice/Development

- Support higher growth rates and culture densities.
- Promote culture longevity and suppress apoptosis.
- Support clone stability in continued passage.
- Promote high product yield.
- Maintain critical product quality attributes.
- Reduce product micro-heterogeneities.
- Reduce product and process related impurities.
- Maintain product stability through harvest.
- Support advanced monitoring and analytics.
- Reduce regulatory and risk considerations.
- Reduce regulatory compliance costs.
- Support improved process control parameters.
- Support process variance identification.
- Increase process robustness.
- Be downstream process friendly.
- Control or reduce metabolic waste.
- Support the use of concentrated feeds.
- Support platform production approaches.
- Support of cryopreservation and recovery.
- Provide required physicochemical properties.
- Provide maximal batch-to-batch consistency.
- Be customization- or optimization-friendly.
- Support single-use application potential.
- Support novel and developing production formats.
- Support novel and developing production modes.
- Support small through large-scale processes.
- Be available in a variety of formats and packaging.

The appropriate SFM platform can **reduce regulatory compliance costs** by

• limiting the number of material components and reducing material qualification costs

• supporting efficient manufacturing operations, thereby reducing costs in corrective and preventive actions (CAPAs) throughout the product life cycle

• promoting manufacture of a consistent and high-quality product

• efficiently supporting new initiatives and guidances such as quality by design (QbQ), process analytical technology (PAT), and operational excellence (OpEx).

Process Friendly

Especially as driven by newer initiatives from the US Food and Drug Administration (www.fda.gov) and the International Conference on Harmonisation of Technical Requirements for Registration of Pharmaceuticals for Human Use (www.ich.org), the **support of advanced monitoring and analytics** has become one of the new selection criteria being applied to cell culture media. Of many emerging technologies, near-infrared (NIR) monitoring of media nutrients, metabolites, and products is showing great potential. Recent advances in on-line and simultaneous monitoring of bioreactor components ranging from glucose and lactate to cell density and the amount of product present demonstrate the possibilities afforded by this powerful technique (**10**). However, as NIR becomes accepted, such considerations as the absorption frequencies and coefficients of some media components must be considered in certain applications.

To promote yield and consistency in general, and to specifically enable implementation of QbD and PAT initiatives, there is a growing desire to **support improved process control parameters** (**11**). SFM can be designed to present reduced common-cause variation or accommodate special-cause variation in bioprocess applications. Simple illustrations of this include media that promote consistent cell and product yield or that contribute to either reducing or accommodating variation in process load, which could otherwise affect pH. Also, because the final variance in a system is the sum of variation from all steps involved, simply reducing lot-to-lot variability in the medium itself is valuable.

Media can be designed to specifically **support process variance identification,** and this aids in its reduction, elimination, or accommodation. Reduction of common-cause variation in a material input makes it easier to pinpoint variability in a process as it arises. For example, a chemically defined medium reduces the unknowns in material input and better supports required formulation adjustments. In other words, as variability and unknowns in the medium or its performance are reduced, it becomes easier to identify them in other aspects of bioreactor operation.

Because sources of variation cannot always be discovered, defined, or eliminated, it is therefore desirable to **increase process robustness.** Although the term is used in many contexts, for bioprocessing professionals a good definition of robustness is the "ability of a process to tolerate variability of materials and changes of the process and equipment without negative impact on quality" (**12**). Many bioproduction processes gain from robust design. An example SFM formulation that contributes to robust reactor operation is one that can accommodate variability in the reactor seed concentration or viability without producing significant lags in consequent culture division rate or reduction in productivity.

A SFM can be considered **downstream-process friendly** in certain distinct ways. Eliminating some media components (e.g., some proteins and unknowns in serum) can reduce purification difficulties (**13**). Substitution, reduction, or elimination of even some SFM ingredients (e.g., hydrolysates, antibiotics, and shear protectants) can both improve purification process efficiency and reduce process-related impurities. From a performance perspective, media formulated to promote cell integrity at later culture stages can reduce both process- and product-related impurities.

Control or reduction of metabolic waste is an important factor in maintaining both culture productivity and product quality. Waste generation can be controlled by both optimizing feeding profiles and the types and ratios of metabolites or precursors available to a cell line as influenced by the particular product or culture mode. One example of such control is directing relevant metabolisms to alternate pathways by replacing glucose with galactose and glutamine with glutamate (**14**).

Most cell culturists now prefer that production media formulations **support the use of concentrated feeds** because the addition of concentrated solutions in midculture (fed-batch mode) has become a common way of boosting productivity. Therefore, basal media must be selected to efficiently accept required production enhancers, antifoams, nutrients, or other midprocess supplements. When operating in this fed-batch mode, some culturists prefer to use a leaner basal media, allowing for increased production to be more efficiently controlled and optimized through addition of comprehensive feed formulations. Others prefer to use a richer media and more focused substrate feeds at later stages (**15**). Table 1 lists some commercially available feeds of varying composition and complexity.

Platform production approaches are generalized processes that apply to more than one protein of interest or production format, providing advantages in efficiency and economy. Examples in bioproduction include establishment of a production-optimized working stock of cells in one medium for use in the production of all new clones, or use of the same medium or process for more than one product or even cell line. Media and feeds are now being developed or customized to consider such latitude in application, which is providing other benefits that include allowing a production SFM to be used further upstream, even in product

development. Formulations that are more robust in this respect can accommodate various cell lines and process steps while simultaneously supporting design space initiatives (**16**).

Additional Features

The **support of cryopreservation and recovery** in a basal media formulation similar to that used through production provides such benefits as clone selection efficiency, documentation and regulatory economy, simplified processes, and gains in scale–up and technical transfer. When appropriately supplemented, some production SFM can support not only efficient cloning and selection, but effective cryopreservation as well. Another advantage of this development is the elimination of postcloning and freezing adaptation steps, which saves time and eliminates the risk of unintended selection (**17**).

Beyond direct nutritional support of a cell culture, it is necessary for a medium to **provide certain necessary physicochemical properties.** Examples include surfactants and detergents in a basal formulation interacting with added antifoams or the compatibility of dispersed amphophiles such as lipids with the product-contact surfaces of disposable systems and components (**18**). The type and level of component ions and osmolites should support both heavy feeding and downstream issues such as troublesome viscosity and medium component or product precipitation throughout a range of temperatures.

To **provide maximal batch-to-batch consistency,** a serum-free medium must be manufactured and tested to guarantee that its lot-to-lot variation is low — and the formulation itself must also support a highly consistent bioreactor production process. Elements of media design contributing to this goal range from the sourcing of ingredients to behavior of dry powdered media (DPM) in bulk preparation to the formulation's performance in a heavily fed high-density culture.

Customization- and optimization-friendly formulations allow end-users the choice of using a product "as is" or altering its formula in support of particular requirements. Optimization-friendly features include reduced, proprietary, or undefined ingredients and increased understanding of which specific media components drive each of the final media characteristics. Many biomanufacturers use commercially available SFM and feeds in process development without modification. However, such demands as clonal requirements or special processing steps cause some to develop a customized or optimized formulation.

The **support of single-use application potential** brings new factors to be considered in media selection. Previously, moving a procedure based on SFM into manufacturing involved such standard issues as scale factors and media lot sizes. New material contact surfaces in disposables present some additional considerations such as the possibility of different binding rates or specificities. That exists for all media, but in the past sera in serum-supplemented media generally masked the potential for component sorption (**18**). Much has now been published on this topic, and information may also be gathered from technical papers or reports by disposables manufacturers or from in-house testing at biopharmaceutical companies. Furthermore, the new reactor dimensions, impeller dynamics, and sparge apparatus of some single-use bioreactors have the potential to influence such media-relevant factors as foaming, hydrodynamics, and $K_L a$.

The need to **support novel and developing production formats** is driven by a number of new cell lines becoming more prevalent in bioproduction. From entirely new lines qualified for therapeutic production such as the PER.C6 human retinal cells (Crucell NV of Leiden, the Netherlands) to standard lines that have been reengineered to provide exogenous posttranslational processing or reduction in apoptosis, a number of new production cell formats are placing new demands on SFM (**19**). Others include cell lines selected to support novel cloning, amplification, and product purification techniques and those from new animal or tissue sources. Considerations here include cell-specific metabolic demands, growth-factor and tonicity requirements, and iron transporter specificities.

As new approaches and equipment become more commonly used in animal cell culture–based manufacturing, **support of novel and developing production modes** is becoming a priority. Disposable wave-action bioreactors and those based on other impeller-free mixing technologies are probably the most well known innovations in production modes. Larger-scale systems for adherent cultures (e.g., microcarriers and stacked chamber reactors) are proving successful in specialized applications. Unique SFM values can be revealed in such applications, such as allowing for more efficient cell detachment from solid substrates. High-density perfusion culture has returned as both culture needs have changed and the technology has improved — in for example, hollow-fiber perfusion reactors (**20**). Therapeutic production applications of three-dimensional culture might even create a new consideration in large-scale SFM applications in the near future.

Serum-free media that **support small- through large-scale processes** can assist in bioproduction several ways, such as in the generation of working stocks and the recovery and expansion of cryopreserved cultures throughout the seed train to large-scale bioreactor operation. Although not vitally necessary, it is valuable to have the same media parameters present for preparation of products used in early research and screening through clinical trials to full-scale manufacturing (**21**).

Especially when supporting large-scale manufacturing, **media availability in a variety of formats and packaging** becomes important. An ideal medium can be used from small-scale bench-top experiments through large-scale production runs. However, some otherwise very good

Table 1: Popular commercially available culture feeds (from Invitrogen, Irvine Scientific, Lonza, Sigma, and Thermo Scientific HyClone) organized by their ingredients

Brand*	Amino Acids	Vitamins	Trace Elements	Glucose	Growth Factor(s)	Plant Hydrolysates	Lipids	Cholesterol
Cell Boost 5								
CHO Feed							Undisclosed	Undisclosed
Cell Boost 4								
OptiMAb							Undisclosed	
CD EfficientFeed								
Cell Boost 2								
IS CHO Feed-CD								
GS-Max								
250X								
LS1000								
SyntheChol								Undisclosed

* Full brand names: Gibco 250X cholesterol lipid concentrate, Gibco CD EfficientFeed kit, Gibco OptiMAb, Irvine Scientific IS CHO Feed-CD, Lonza UltraCHO supplement, Sigma SyntheChol NS0 supplement, Sigma CHO Feed bioreactor supplement, Thermo Scientific HyClone Cell Boost 2, Thermo Scientific HyClone Cell Boost 4, Thermo Scientific Cell Boost 5, Thermo Scientific HyClone GS-Max, Thermo Scientific HyClone LS1000

research formulations cannot be produced as a single liquid without supplementation, and others cannot be produced as a dry powdered medium. Early research and screening (even in HTS formats) generally require media to be available in liter-bottle quantities or less, whereas for bench-top or pilot-scale bioreactor operations larger-volume liquid formats (even up to 500 L bags) are most convenient. But full-scale production requires consistent, large–lot-size DPM a manufacturer can prepare on-site for its own use.

A Good Place to Start

Our list of features to be considered in production media appears quite long and comprehensive. However, it does not begin to address many other factors required either in selection of commercially available media or development of in-house formulations — such as those of economics and geography or those demanded in material supplier audits.

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