Green Factories

FOR PHARMACEUTICALS

BY RITA D'AQUINO

IMAGINE A VERY WEALTHY COUNTRY WITH unsurpassed expertise in discovering and successfully developing medications that cure immune system diseases. Imagine not enough people being able to get the drug because manufacturers can't make it fast enough. "You don't have to use your imagination because it is happening today," says Allan Felsot, environmental toxicologist, Food and Environmental Quality Laboratory, Washington State Univ. (Pullman, WA; www.wsu.edu). To increase capacity, drug companies have two costly options — add more fermentation equipment (if space permits) or build a new facility. But there may be an alternative solution to this problem.

A number of companies are honing techniques to produce a wide variety of pharmaceuticals, such as therapeutic proteins, monoclonal antibodies (a single antibody that can be produced synthetically in high concentrations) and edible vaccines, in plants. "A select elite of meticulous growers will be the new 'green' manufacturers of these plant-made pharmaceuticals (PMPs). And farming of these crops is going to be an 'industrial' process with scrupulous controls and regulatory oversight," Felsot points out.

PLANT-BASED ENZYMES AND ANTIBODIES

Crops such as corn, tobacco, rice and soy are already being genetically altered (*CEP,* Oct. 2002, p. 10) to yield proteins with purities and activities equivalent to those produced by mammalian cell culture (MCC) and other manufacturing systems. Advantages of this route include large-volume production capacity, reduced capital requirements, and freedom from potential viral and animal protein contamination.

In Feb. 2002, Prodigene, Inc. (College Station, TX; www.prodigene.com) began a commercial scale-up of trypsin, a pharmaceutical intermediate and a protein used in cell culture, marking the first kilogram-quantity production of a recombinant protein from transgenic corn. The company has arranged an agreement with Sigma-Aldrich Fine Chemicals (St. Louis, MO; www.sigma-aldrich.com) to make and distribute tens of kilograms of plant-based trypsin marketed under the TrypZean brand name, by the end of 2003, according to Zivko Nikolov, vice president of bioprocessing and development. In addition to trypsin, Prodigene is developing (in corn) transgenic versions of aprotinin, a protease inhibitor used to control blood loss, and oral vaccines for diseases such as hepatitis B. Preclinical trials for human use of aprotinin will begin this spring and commercialization is expected in 2006.

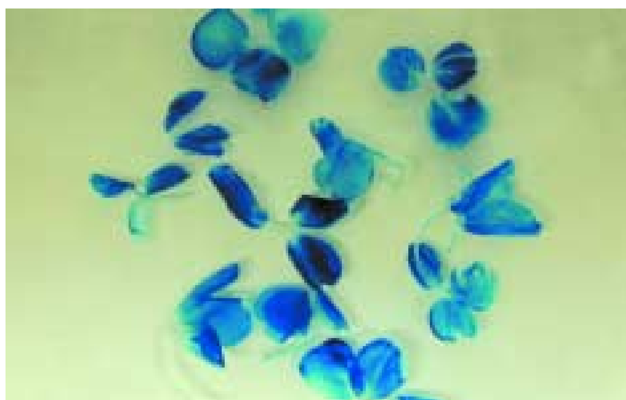
Approximately 200 antibody-based drugs are in various stages of development at biotechnology companies. Antibodies are valued because they can target a very specific antigen while causing few side effects, and companies are seeking cost-effective ways to produce them in large quantities. Epicyte Pharmaceutical, Inc. (San Diego, CA; www.epicyte.com) estimates that a single acre of genetically engineered corn can produce 1–5 kg of antibodies — about the same as a typical multimillion-dollar factory. Epicyte, using its Plantibodies technology, has five plant-based antibody products in development. The company's lead molecule, HX8, which is produced in corn, is designed to treat both oral and sexually transmitted strains of the Herpes Simplex virus.

In early 2002, Epicyte and Dow Chemical (Midland, MI; www.dow.com) through an alliance, increased the scale of production for HX8 for Phase I clinical trials, which are slated to begin in mid-2003. Epicyte also partnered with AAI International, Inc. (Wilmington, NC; www.aaipharma.com) to develop a topical gel formulation of HX8 that will be used during clinical studies.

Epicyte has granted Dow a license to use its proprietary technology to produce (in corn) Epicyte's respiratory syncytial virus (RSV) antibody product, R-19. RSV is a pulmonary infection that affects individuals with compromised immune systems. "Dow, the contract manufacturer, grows the crop, extracts and purifies the antibodies under cGMP conditions, and returns them to Epicyte," explains Kerr Anderson, technical director for Dow Plant-Based Biopharmaceuticals. Dow is now growing the first greenhouse plant lines that will generate seeds containing the genes for the RSV antibody. In the next stage of development, these seeds will be planted to grow a crop large enough to produce marketable amounts of R-19.

In Sept. 2001, Epicyte-Dow announced an agreement with drug developer Centocor (Malvern, PA; www.centocor.com) to research the production and characteristics of two monoclonal antibodies in transgenic plants. The agreement is the first licensing of Epicyte's Plantibodies technology to the pharmaceutical industry.

Medicago, Inc. (Sainte-Foy, Quebec; www.medicago.com) has developed methods that allow production of recombinant proteins in alfalfa (Figure 1), a perennial crop that yields 10–12 tons of dry matter per year for up to 6 consecutive years in the field, and for more than 10 yr when grown in greenhouses. Medicago's technology features a



■ Figure 1. Medicago, Inc.'s transgenic protein is produced in alfalfa leaves.

set of DNA fragments from the alfalfa plant that regulate the transcription of the foreign gene, and the translation of that gene into a recombinant protein expressed in the stems and leaves (Figure 2). "To obtain 200 mg of recombinant protein at a concentration of 0.5% in the total soluble protein, 100 alfalfa plants would need to be processed," explains Pierre Bilodeau, Medicago's team leader, transformation technologies.

To date, Medicago has established four prototype development projects with other companies to prove that various proteins could be produced in alfalfa: Hemosol, Inc. (Toronto, Ont.; www.hemosol.com), to produce hemoglobin in plants; CO2 Solution (Quebec City; www.co2solution.com) to produce carbonic anhydrase, a CO_2-degrading enzyme with potential applications in air filters; FibroGen, Inc. (South San Francisco, CA; www.fibrogen.com), to produce gelatin for cosmetic products; and a "major European-based pharmaceutical company" to produce a disease-curing drug. In all cases, Medicago is aiming for 0.1–1% of total soluble protein in the plant's extract. "The first products will not likely be on the market before 2005," says Bilodeau.

GLYCOSYLATION AND DRUG EFFICACY

Like other natural drug-producing organisms, such as E. coli, yeasts and MCCs, plant platforms for transgenic proteins have to comply with guidelines and regulations imposed by national food and drug administrations with regards to reproducibility, toxicity and allergenicity. To this end, glycosylation — the process by which carbohydrate units (glycans) are attached to the backbone of a protein during its formation — becomes a crucial factor. Patterns of glycosylation on proteins differ between species — thus, the pattern serves as a "signature" that allows an organism to identify and accept a protein as its own. "The presence of glycans on proteins is essential for full functionality of that protein in the human body, more specifically, in the bloodstream," says Kurt Hoeprich, director of market development for Dow Plant-Based Biopharmaceuticals.

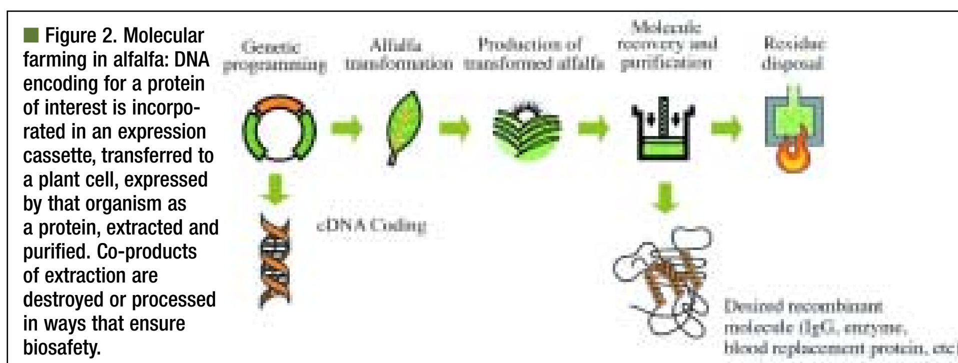
Last year, Dow secured exclusive rights to patents involving the production of "mammalian-like" glycans in plants. These patents, which were obtained from Osaka Univ. (Japan), cover all plants and describe the placement or addition of a terminal galactose unit onto the glycan. "The patents demonstrate that it is possible to modify the glycoprotein pattern typically produced by plants to more closely mimic that found on human proteins," explains Hoeprich. "Drug proteins with plant glycosylation patterns might not be as effective in humans and could cause an allergic reaction," he adds.

Last November, Dow Chemical and Plant Research International B.V. (PRI; Wageningen, The Netherlands; www.plant.dlo.nl) signed a cooperative agreement to develop and commercialize therapeutic proteins with mammalian-like glycan structures in transgenic plants. "By optimizing glycoprotein production in plants, we can greatly expand the applicability of this new production platform in the rapidly growing biopharmaceutical marketplace," remarks Hoeprich. This agreement dovetails Dow's proprietary expression technologies and biopharmaceutical protein processing expertise with PRI's R&D capabilities for biosynthetic pathway engineering and protein analysis," adds Dirk Bosch, group leader at PRI. Dow will lead commercialization of this technology for the therapeutic protein contract manufacturing market.

Alfalfa has been shown to produce recombinant C5-1 protein (an antibody) with only one type of glycan (referred to as homogeneous glycosylation), while other systems, such as Chinese hamster ovaries or transgenic tobacco, have been shown to places three and eight types of glycans, respectively (*i.e.*, heterogeneous glycosylation). "Alfalfa is the first reported system to provide homogeneous glycosylation of a foreign protein. "The biopharmaceutical is easier to humanize, since only one glycan has to be changed," says Medicago's chief scientific officer, Louis Philippe Vézina.

ECONOMICS ARE FAVORABLE

"Although the overall cost of producing an antibody in

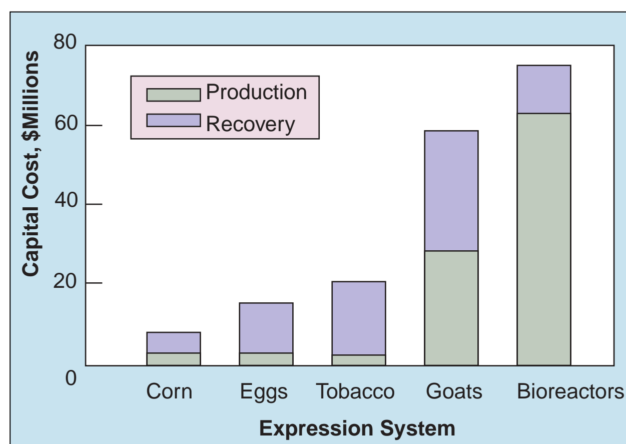


■ Figure 2. Molecular farming in alfalfa: DNA encoding for a protein of interest is incorporated in an expression cassette, transferred to a plant cell, expressed by that organism as a protein, extracted and purified. Co-products of extraction are destroyed or processed in ways that ensure biosafety.

crops is still uncertain, there are clear capital cost savings in using a 'green' factory (Figure 3)," says Mich Hein, Epicyte's president. A conventional manufacturing facility that produces 200 kg/yr might have a capital cost of $250–$500 million, while the capital cost required to establish a green manufacturing system could be 70% less. Heins explains,"The cost of an antibody in the harvested broth from a fermenter might be $100/g–$500/g, depending on the cell line used for culturing. The cost of antibody, after harvesting from the field and extraction into a liquid broth would be significantly less than $50/g, while the cost of purification, which can account for 30–60% that of the drug, would also be much lower."

Although the economics appear to be favorable, there are many legal issues to contend with. "The plant transgenic arena is an intellectual property minefield," cautions Brandon Price, president of Goodwin Biotechnologies, Inc. (Ft. Lauderdale, FL; www.goodwinbio.com). "Any company considering looking at a plant transgenic system as a means of manufacturing protein needs to fully understand the constraints on freedom to operate with that technology," he adds.

For example, last year, Epicyte was given exclusive licensing rights to a new patent awarded to Scripps Research Institute on a technique for producing therapeutic antibodies from corn and other farm crops. The patent, which expires in 2009, covers the use of DNA sequences (or molecular instructions) that direct the antibody production-process in plants. "Any other company seeking to use a plant to make a monoclonal antibody must negotiate an arrangement with Epicyte, Dow and Scripps. Otherwise, might be violating Epicyte's patent," Price concludes.



■ Figure 3. Cost comparison of different transgenic systems. Courtesy of BASF Plant Science GmbH.

PRECAUTIONARY MEASURES

As attractive as plants are a a vehicle for proteins production, their use raises concerns from industry critics about potential gene flow to food crops of the same species and worker exposure to plant material containing active pharmaceutical ingredients. Philip Eppard, regulatory affairs leader, Monsanto Protein Technologies (see sidebar) asserts that "PMPs are grown under a completely different paradigm than the biotechnology-derived agricultural crops designated for food and feed markets." Seeds for PMPs are not sold through conventional channels, but are only made available to pre-qualified contract growers who undergo extensive training. Producers of PMPs follow planting restrictions to keep these proteins from entering the food chain, including following standard operating procedures for confinement — *i.e.*, keeping the crop and its products on the land where it was grown until removed for processing. Furthermore, all processing, milling and extraction is conducted outside commercial food and feed channels to prevent co-mingling.

PREVENTING MEANDERING MEDICINES

Unquestionably, companies involved in the commercialization of PMPs as well as federal regulators are committed to ensuring the safety of these products during all stages of development and production. "The infrastructure of regulation is in place and has been legislatively placed in the hands of four federal agencies: the U.S. Dept. of Agriculture's (USDA; www.usda.gov) Animal and Plant Health Inspection Service (APHIS), the Food and Drug Administration (FDA; Rockville, MD; www.fda.gov), the Environmental Protection Agency (EPA; Washington, DC; www.epa.gov) and the Occupational Safety and Health Administration (OSHA; Washington, DC; www.osha.gov)," explains Lisa Dry from the Biotechnology Industry Organization (Washington, DC; www.bio.org).

Currently, test plots for crops modified to grow pharmaceuticals are regulated by the USDA permit system, which requires developers/growers to have clearly written procedures for production. The system also mandates the proper handling of wastes, as well as the maintenance of production and control records. Federal and state inspectors regularly visit the plots to ensure that these procedures are properly executed. "Unlike other genetically modified crops, pharmaceutical crops will need perpetual permitting from APHIS during both the R&D and the production phases," says James White, senior operations officer, biotechnology, USDA-APHIS. "Permits for growing small acreages of pharmaceutical crops for development purposes are already being issued," he adds.

The FDA monitors the manufacturing process, as well as the purity and consistency of the products under its "good manufacturing practices" guidelines. However, both USDA and FDA are in the process of coming up with new guidance specifically for pharmaceutical plants. EPA would be initially involved in the regulatory oversight of PMPs if the plants contain pest-protection characters (like the Bt protein) or herbicide-tolerance characters that might require a new use pattern for a herbicide. If worker safety becomes a concern owing to excessive exposure to PMPs, OSHA will dictate practices that minimize risk.

"One point to consider when pondering the ecological effects of PMPs is that for any one product, very limited acreage will be used," says Felsot. For example, about 1,000 acres may be required to produce enough immunoglobulins of any kind. That acreage will be isolated and devoid of the food crops subject to cross pollination, and the resulting crop will be the subject of extraordinary scrutiny throughout the whole production and postproduction process. Only elite growers who commit wholeheartedly to the principles of confinement and identity preservation need apply — and they will be duly rewarded for their technical skills, knowledge and infrastructure.

Fermentation

GOES LARGE-SCALE

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Photo courtesy of Cargill Dow.

Designing an economically competitive and environmentally sustainable fermentation route means considering the downstream separation needed to capture the final product during the initial process design.

THE TREND TOWARD ENVIRONMENTAL sustainability and development of renewable resources has significantly increased interest in the recovery of fermentation products, such as organic acids, feed or food additives, and industrial chemicals. Consequently, the range of products produced by fermentation is expanding beyond the traditional high-value low-volume compounds, such as pharmaceuticals, and is beginning to compete with traditional synthetic production of commodity chemicals. As fermentation moves into lower-value higher-volume chemicals, it becomes necessary to maximize efficiency, and minimize costs and waste byproducts to compete effectively against traditional options. Achieving these goals means approaching the design of fermentation and downstream separations as a single, integrated process. As stated by Williams *(1)*: "The bioreactor should be regarded as an integrated unit operation with both upstream and downstream unit operations."

However, all too often, the design of fermentation and downstream separations are regarded separately in process development. Typically, a separation specialist takes on the challenge of designing steps to separate the various components of a complex fermentation broth that the fermentation-process designers included to maximize fermentation performance. Hence, if the required separation becomes complex and costly, the most efficient fermentation may not necessarily yield the optimum overall process.

Typically, 50–70% of the total production cost in classical processes is due to downstream processing, whereas in fermentation that employs recombinant DNA, the fraction can reach up to 80–90% *(2)*. This large percentage is often due to separation and purification of the fermentation product.

ECONOMY OF SCALE

Fermentation broths are complex aqueous mixtures of cells, soluble extracellular products, intracellular products, and converted substrate or unconvertible components. The particular separation techniques useful for any given bioprocess depend not only on the location of the product (intracellular vs. extracellular) and its size, charge and solubility, but also on the scale of the process itself and the product value. For example, chromatography is generally useful for high-value pharmaceuticals or biologicals, such as hormones, antibodies and enzymes, but is expensive and difficult to scale up.

As with other chemical processes, fermentation for producing commodity chemicals and products is also aimed at minimizing production costs. Due to the significant impact of raw substrate materials and down-

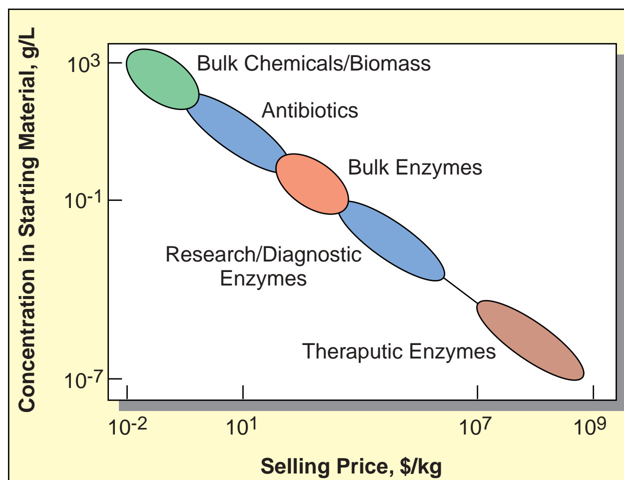

Figure 1. Economy of scale: correlation between achievable product concentration and selling price for common fermentation products.

stream processing on the total production cost, process optimization is primarily focused on finding new, competitive and, above all, sustainable production technologies.

In addition, many fermentation processes are hampered by the accumulation of products in the fermenter. The recovery cost as a percent of the total production cost can vary from as low as 5–10% in, for example, the production of single-cell protein (SCP) and extracellular enzymes such as proteases, amylases, etc., to as much as 90% for the bacterial production of poly(3-hydroxyalkanoates) used to produce biodegradable thermoplastics (Table). The high cost of recovery is due to the generally low product concentrations in aqueous fermentation broths; the complexity of the broth mixture, especially when liberating intracellular products by cell disruption; and the multiple, discrete separation and purification steps in purifying the product. The latter steps lead to high capital and operating costs, as well as multiple operations that each can result in significant product yield losses and generation of waste streams that must be disposed of or recycled.

Table. Recovery or purification cost as a percentage of total production cost for typical fermentation products.

Source	Product	Recovery cost as of percentage of total production cost
Whole-cell yeast biomass	Single-cell-protein, yeast extract	5%
Bulk chemicals	Lactic, citric and malic acids	10–50%
Extracellular enzymes	Amylases, proteases	10%
Antibiotics	Penicillin	20–50%
Intracellular enzymes/proteins	Human insulin, interferon	90%

Therefore, fermentation development is bound by the overall recovery strategy chosen for a certain product. This depends on the required product purity, which can be as high as 99.999% for diagnostic and therapeutic proteins. In addition, economic consideration must be given to the feasibility of minimizing unit-operation complexity and waste production, while maximizing yield and productivity.

The impact of fermentation on the overall production economics strongly depends on the type of product made, *i.e.,* industrial vs. therapeutic proteins; and specialty vs. bulk chemicals. This depends on the desired vs. achievable product concentration in the final broth. According to the economy of scale, the selling price of a product is inversely correlated with its achievable concentration at the end of fermentation, and can range by 9–10 orders of magnitude (Figure 1).

Regardless of the product, the highest purity in the final broth is the most desirable. Since, for bulk chemical and biomass routes, fermentation already represents a large fraction of the total production costs, it is here that so-called "clean" fermentation development (*i.e.,* integration of fermentation and separation to reduce the environmental 'footprint') can have a major impact on the overall economics. There is continual pressure for technical improvement on the upstream bioreactor section to yield a final broth of higher-product concentration to lower the cost of separation and recovery.

DEVELOPMENT OF LARGE-SCALE FERMENTATION TECHNOLOGY

The most obvious benefits that can be achieved from integrating fermentation and downstream processing are minimizing waste, raw materials, capital and energy. An often-forgotten and more-challenging-to-achieve benefit is environmental sustainability. As questioned in a recent article in *Time,* if there could ever be a system that's perfectly efficient, the author answered, "Yes, it already exists, and we call it nature. The same materials have been recycled for billions of years. The new industrial revolution is all about absorbing the lessons we should have learned from nature long ago. … Efficient use of energy and materials and a reduction in waste can help the bottom line." ("New War on Waste," *Time,* pp. A28-A31 (Aug. 26, 2002)).

Box. Roadmap for Integrated Process Development

- Analyze of economic and process constraints based on preliminary process design
- Identify opportunities for improvement, *e.g.,* reduced waste streams, energy use, impurity levels and raw material use
- Put together a wish list of physiological characteristics and downstream separation performance
- Evaluate feasibility of achieving the wish list based on technical difficulty and economics
- Define the best strategy for addressing each opportunity by taking into account both downstream and fermentation capabilities, such as high cell density, extractive fermentation, simplify broth, etc.
- Integrated fermentation and downstream process development

As fermentation is becoming an increasingly integral part of the development of many high-value products and is replacing conventional routes for commodity products, these processes will need to be integrated similarly to the way the petroleum industry has worked on this over the last few decades (Box).

In general, large-scale fermentation development comprises of the following steps:

1. Organism selection, with regard to:
   • substrate versatility
   • byproduct formation characteristics
   • robustness of the organism, *e.g.,* to process upsets
   • viability with regard to cell recycling
   • physiological characteristics (maximum growth rate, aeration requirements, etc.)
   • genetic accessibility.
2. Metabolic and cellular engineering:
   • improve existing properties of the organism
   • introduce novel functions, for example, by simplify-

ing product recovery, expanding substrate and product ranges, and enabling fermentation to occur under non-standard conditions

3. Fermentation process development:

• culture and media optimization (from complex to defined minimal media)

• optimization of cultivation parameters that take into account product recovery and purification (minimize byproduct formation, minimize chemical inputs, and develop high-cell-density cultivation)

• incorporation of cell retention/recycling

4. Introduction of downstream unit operations within a fermentation process:

• examples are extractive fermentation, electrodialysis and in-line membrane separation technologies.

INTEGRATION TIPS

Integration can be approached from different angles. The following examples by no means comprise a comprehensive list, but relate to the steps involved in large-scale fermentation development.

Simplify the fermentation broth — In principle, any ingredient added to the broth that does not end up as product will have to be removed. It therefore behooves the fermentation-process designer to eliminate any unnecessary ingredients from the broth. For example, many fermentation processes employ complex media, such as yeast extract or corn steep liquor (initial waste stream of corn wet milling operation; generally used as a rich source for nitrogen in fermentation media). These media are inexpensive and ample in nutrients. Designing defined fermentation media from salts and vitamins requires a considerable development effort to provide a recipe capable of supporting microbial production at the desired levels. However, it is sometimes worth considering the tradeoff between slightly reduced fermentation performance and a greatly simplified downstream process. In addition, if a complex medium component must be used, the component may contain elements that do not directly benefit the fermentation, yet provide a separation challenge downstream. In some cases, it may be feasible to separate out these non-beneficial nutrients before they reach the broth.

Ease separation by altering the product form — In some cases, different forms of the product are easier to separate downstream than others. A common example of this is organic acids, where the free-acid form of the product may be easily extracted from a fermentation broth, while the salt is not easily removed. If fermentation is designed separately from downstream processes as is usually the case, an acidification step will be required downstream. However, this requires that a neutralizing base be added to the broth, which must later be removed by adding an acid, with both base and acid becoming a waste salt that must then be disposed of. This inefficiency can be avoided if the fermentation can be carried out at a low enough pH to provide the product predominantly as the acid. The resulting savings in acid, base and waste disposal costs may offset a considerable amount of decline in fermentation performance resulting from the more unfavorable acidic fermentation conditions.

Reuse the fermentation broth components — Significant improvements in fermentation raw-material yield and production rate can be effected by reusing components of the broth. For example, recycling cells, although a technical challenge, holds promise for improving fermentation efficiency. Separation of the biomass from the broth requires that the cell fraction be treated cleanly to prevent contamination of subsequent fermentations, and that the cells not be subjected to unnecessary stresses to maintain their viability. However, any live biomass that can be reused reduces the amount of new substrate that is required for biomass growth, as well as reducing the challenges of biomass disposal. Also, an increased concentration of cells can increase the production rate in the fermenter, which will reduce fermentation capital costs. Even if the biomass cannot be recycled viably, if it can at least be recycled cleanly, it can become a nutrient source for later biomass production.

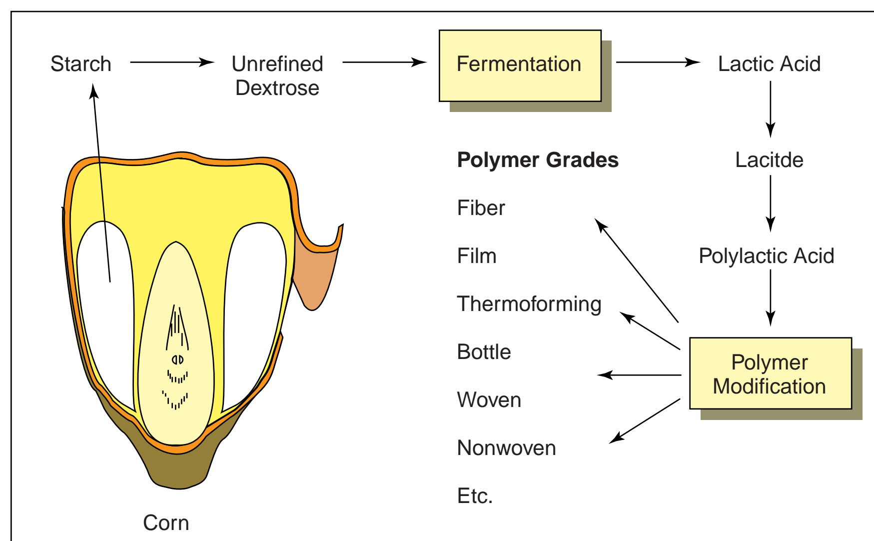

Figure 2. Simplified pathway for the production of polylactic acid (PLA) and derived consumer products.

Another strategy is reusing some or all of the broth after product separation. Often, optimum product synthesis and biomass growth take place when medium nutrients are present in excess. However, this results in nutrients being left over at the end of fermentation. Reuse of the broth allows a reduction of nutrient addition to the next batch, as well as avoiding the cost of treating a high biochemical-oxygen-demand (BOD) waste stream.

Remove the product during fermentation — In many fermentations, the product acts as an inhibitor to the production reactions. This can limit the

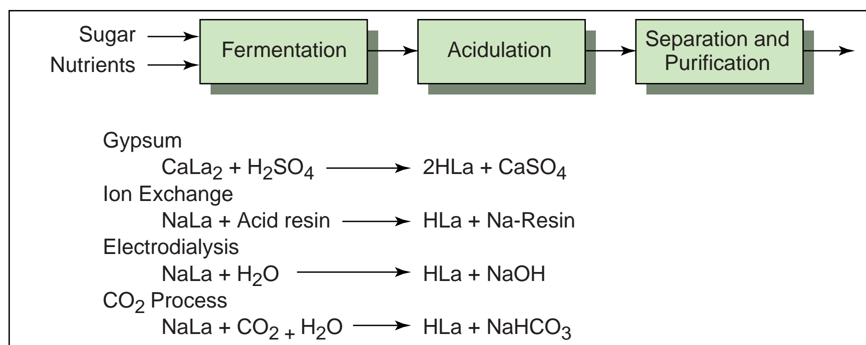

Figure 3. Various lactic acid acidification technologies are aimed at eliminating salt byproduct formation.

concentration that can be achieved in the fermenter. However, as said above, the product concentration in the finished fermentation broth is a clear inverse indicator of cost. Removing the product during fermentation increases the yield by allowing more to be produced from a given amount of biomass, plus increases the production rate by reducing the accumulation of an inhibitory product. Using continuous extraction, a side-stream can be pumped out of the unit and the extracted broth returned to it. Further, two-phase fermentations have been developed to extract the product from a biomass-containing aqueous phase into an organic phase, which can then be removed on-line.

Reduce the water content of the broth — Typically, as much as 90% or more of the broth is water, which must be removed. This is not only costly to separate, but also produces a large aqueous stream that must then be disposed of or recycled. Integrative approaches to water reduction include increasing the biomass concentration (*i.e.,* high-cell-density (HCD) fermentation), engineering the organism to tolerate higher product concentrations, and removing inhibitory elements from the fermentation recipe.

Several examples will now illustrate how costs can be kept down. The large-scale fermentation for the production of lactic acid *(3),* baker's yeast *(4, 5)* and recombinant human albumin *(6)* embody an integrated approach to process design that integrates the steps listed above to reduce waste-management and production costs.

LACTIC ACID

Lactic acid is a commodity chemical that is used in the production of medicines, foods and beverages, and polymers. In the U.S., a large portion of the lactic acid is used as a feedstock to make polylactic acid-based (PLA) polymers for the production of fibers, films, nonwovens, etc. (Figure 2). Lactic acid is generally produced by fermenting dextrose found in biomass via bacteria, yeast or fungi. PLA production is environmentally sustainable, with 20–50% more PLA being produced using the same amount of fossil fuel vs. petrochemical-based plastics *(7).* PLA-based plastics derive their carbon from plant carbohydrates which, in turn, are produced from carbon dioxide via photosynthesis. This ultimately results in lower net carbon dioxide emissions. In the near future, PLA is expected to be produced on multi-billion dollar/year scale. However, there are still challenges to be resolved to produce inexpensive pure lactic acid monomers in large quantities from renewable resources.

High production yields of lactic acid and a robust, simple production system with minimum byproduct formation are of key importance, since, generally, the sugar feedstock accounts for over 50% of the fermentation cost. Lactic acid is usually produced as a salt, such as NaLa or CaLa (where La = lactate), since the pH of the fermentation process has to be maintained far above the pKa of the acid species (= ~3.8; *i.e.,* at pH = pKa, the salt-form:free acid form is 50:50). A subsequent acidification obtains the undissociated form, HLa. Various acidification methods can retrieve undissociated lactic acid. These include using sulfuric acid, acid resins in ion-exchange chromatography or CO_2/triethylamine treatment.

This step should avoid producing large amounts of salts (calcium, ammonium or sodium sulfate), which have little added economic value, and are mainly discarded or sold as fertilizer. As an example, acidifying a lactic-acid fermentation-broth with sulfuric acid yields equal amounts of gypsum ($CaSO_4$) (Figure 3). Several separation and purification techniques can concentrate the acid, such as esterification with ethanol, and subsequent distillation and hydrolysis; direct distillation of HLa; or liquid/liquid extraction. Any optimization should have a high yield and meet the proper product quality.

With certain bacteria as biocatalysts, lactic acid can be made in high yields (> 90% of the sugar carbon is converted straight into lactic acid carbon) and high volumetric productivities (0.1–10 g/L/h). The biocatalysts used for lactic acid production are efficient, thus, small amounts are needed (< 5 g/L). This is favorable in terms of economics, since in bulk-chemical production, where the product is entirely made from sugar carbon, any loss of carbon (*e.g.,* to biomass production) is a loss of product yield.

Integration of the fermentation-process development for lactic acid focuses on reducing salt byproduct formation by developing new salt-regeneration strategies and lactic acid technologies at low pHs. Further reductions in capital and operating costs have been effected by: simplifying separation operations; developing clean, defined media; improving biocatalyst efficiency by metabolic engineering; using alternative feedstocks; and applying technologies that reduce lactic acid accumulation in the broth.

HIGH-CELL-DENSITY (HCD) BAKER'S YEAST PRODUCTION

HCD fermentations are defined by the presence of cell dry-weight biomass concentrations that exceed 100 g/L (vs.

5–20 g/L for other fermentations). The amount of protein derived per amount of feedstock, as well as the specific productivity, are generally two to three orders of magnitude lower compared to bulk chemical production. Therefore, high biomass concentrations are required to achieve high product concentrations in the final broth. HCD fermentations are being developed for the production of baker's yeast and for recombinant human serum albumin (rHSA), a major blood-plasma protein with the yeast *S. cerevisiae.*

In HCD fermentation, a substantial part (> 25%) of the culture volume is occupied by cell biomass. This has implications for downstream processing in protein production, since additional water and extracellular proteins tend to adhere to the cells at high concentrations, requiring washing of the biomass to liberate the proteins.

To make HCD fermentation for protein production successful, developments focus on media optimization with respect to use of carbon sources, inorganic nutrient packages, complex vs. defined media formulations, vitamins and foaming. Due to the large amounts of biomass generated, attention is being put on oxygen transfer and heat transfer. For HCD, large excess additions of nutrients should be avoided to eliminate osmotic and toxicity effects, reduce the cost of media, facilitate downstream processing of extracellular products, and manage waste-stream production. Therefore, development of balanced, "clean" media has high priority; requirements are species- and even strain-dependent.

The production of baker's yeast is about 2,500 kton/yr *(5).* Process optimization, focused on improving dough-leavening capacity, has been on strain selection and empirical optimization of environmental conditions during the fed-batch production process. The large demand for high-quality (drinking) water in downstream processing of commercial baker's yeast (> 15 m^3 washwater used per ton yeast produced) provides an environmental and economic incentive for novel fermentation processes on food-grade media. The final yeast produced can be used in food applications without the need for downstream processing.

A further incentive for sustainable production technology is that, due to improvements in beet and cane sugar refining and the quality of molasses, the conventional feedstock for baker's yeast production is declining. The major challenge is to reconcile the conditions necessary for HCD cultivation with the quality requirements for industrial baker's yeast (high dough-leavening power and high storage stability). Dough-leavening power can strongly depend on the growth-rate history of the biomass (Figure 4). Developing cleaner HCD production technology necessitates understanding how to improve the dough leavening capacity at low growth rates in baker's yeast cultures (gassing power is the selling trait in baker's yeast, not the biomass itself).

HCD HETEROLOGOUS PROTEIN PRODUCTION: RECOMBINANT HUMAN ALBUMIN (rHA)

Similar developments have been happening in the production of rHA. This protein is abundant in human blood (at about 40 g/L) and it serves various physiological functions. Therapeutically, it is used in multi-gram doses for the treatment of shock or burns, and also to compensate for blood loss *(6).* With an annual market of 400,000 kg, an estimated product concentration of 5 kg of rHA/m^3 (~ 5 g/L), and 80% recovery in downstream processing, roughly 1,000 HCD fermentations of 100 m^3 are run each year.

One of the major challenges is to find expression sys-

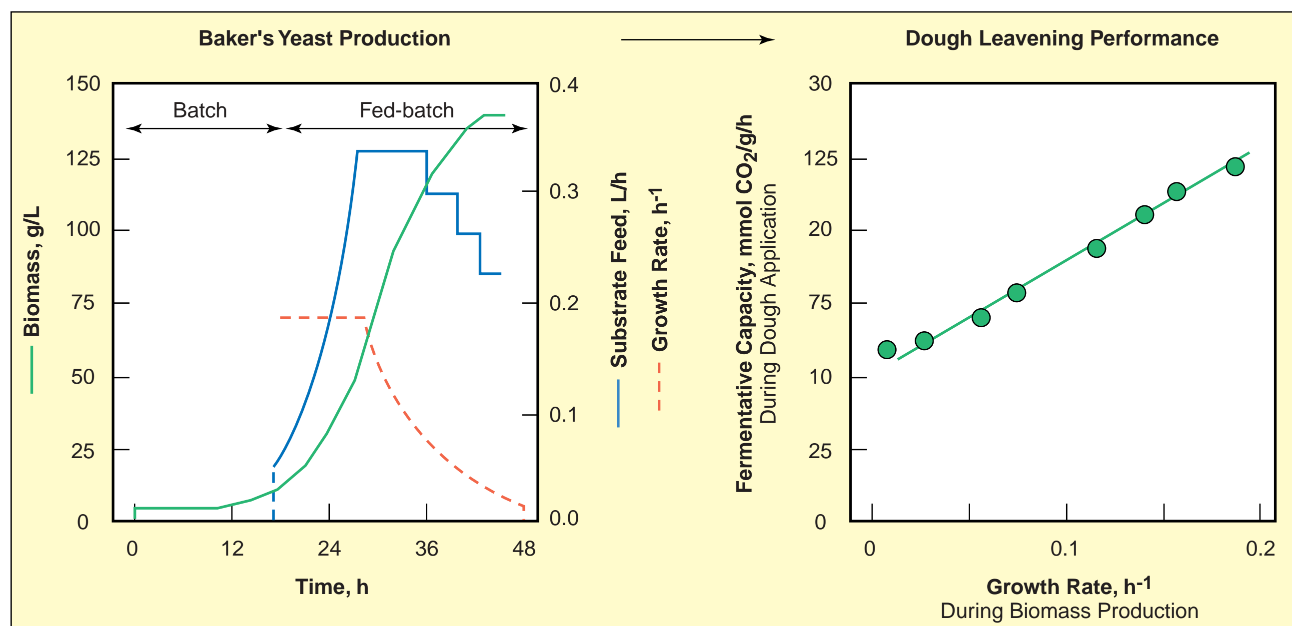

Figure 4. The fermentative capacity of baker's yeast, as manifested during the dough leavening application, is strongly dependent on the final specific growth rate during biomass production in high-cell-density fed-batch cultures.

tems for production of the protein under HCD cultivation conditions (i.e., under low growth rates) and to minimize downstream purification requirements.

CRITICAL ISSUES IN FERMENTATION DEVELOPMENT

Irrespective of producing proteins or biomass, HCD yeast biomass production requires aerobic conditions. Due to the low oxygen solubility in aqueous solutions and limited oxygen transfer/cooling capacity in large-scale fermenters, it is necessary to control the specific growth rate of biomass by controlling the carbohydrate feedstock addition-rate to maintain fully aerobic conditions in the fermenter. Concomitant with extremely low specific growth rates, a large amount of consumed sugar carbon is simply burned by the yeast biomass to carbon dioxide to provide energy for cell maintenance. This is a general observed phenomenon with many organisms that reduces the overall product yield in biomass and protein-production processes. In baker's yeast production, low, specific growth rates result in low dough-leavening capacity of the final product. Therefore, the focus in the baker's yeast production industry is to improve this parameter at low, specific growth rates.

In heterologous protein production, the specific rate of protein production, q_p, is a key parameter that is rarely reported in the scientific literature. Values of q_p generally range from 0.5–2.5 mg/g·h at a growth rate of 0.1/h and represents generally less than 10% of the overall cellular production rate *(8)*. Efforts are on understanding the relationship between the specific growth rate of the culture and the specific rate of protein production. This relationship is needed to further optimize the protein expression system with respect to factors such as the stability of expression constructs (over a large number of cell generations), selection markers (cost, stability), constitutive vs. inducible promoters, and the dependency on expression conditions, among other factors. As with baker's yeast production, work on HCD protein-production systems also focuses on improving expression levels in slowly growing cultures.

Over the past few years, research in this field has been directed towards innovative fermentation process design that can reduce downstream processing costs significantly or even eliminate them completely (as in baker's yeast production). Further, designing new-generation bioprocesses increasingly depend on engineering process-compatible microorganisms. The latter, whether through genetic or physiological manipulations, can be greatly assisted by metabolic engineering. To achieve these goals, more fundamental knowledge is needed about metabolic pathways, control mechanisms and process dynamics to optimally design integrated systems. This knowledge will enable industry to select the right biocatalyst in clean fermentation processes, as well as introduce and express new or improved properties of the biocatalyst via genetic engineering to facilitate and/or improve downstream processing. Chemical engineers, metabolic/genetic engineers and microbial physiologists will have to work together on this journey.

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INTEGRATING SINGLE-USE Filtration Systems IN BIOPHARMACEUTICAL APPLICATIONS

BY HOLLY HAUGHNEY
PALL CORP.

Disposable filtration systems are gaining popularity in the pharmaceutical arena. This article defines the equipment, specifications, applications and potential advantages of single-use filters and ancillary equipment, and draws comparisons to hard-piped systems.

SINGLE-USE PRODUCTS HAVE BECOME prevalent in almost every facet of life today, from cameras to contact lenses. They're cost-effective, convenient, and easy-to-use. However, when you apply the benefits of disposability to the biopharmaceutical world, the implications are far greater. Single-use filtration systems comprise the next-generation of disposable products (*CEP*, Nov. 2002, p. 10). Touting popularity mostly in biopharmaceutical applications, these products are improving the speed and safety of drug development and delivery. This article will explore the advantages of integrated, single-use filtration systems over stainless steel, hard-piped systems, and the applications in which they are used.

STANDARD COMPONENTS

Integrated, single-use filtration systems are comprised of disposable bags and capsule filters, and are coupled together with tubing, clamps, adaptors and connection devices. Each of these components is detailed below.

Single-use container. The flexible-container portion of the system, the bag, is used to contain the fluid that enters or exits the filter, and serves as a substitute for stainless steel and glass containers. In the pharmaceutical industry, the bag is used to hold fluids such as culture media (cell growth fluid which may contain serum or other growth promoters), buffers, reagents, sanitizing agents, cell-harvest fluid and intermediate and final products. For smaller volumes (50 mL to 50 L), single-use bioprocessing bags are typically a two-dimensional pillow-type bag, while in larger volume applications (100 L to 2,500 L) three-dimensional bags are used (Figure 1). The bags are made of advanced films that are ideally formulated to meet process requirements. For instance, the bags can be subjected to gamma irradiation for sterilization. In addition, the bag materials that contact the fluid are inert, and the bag also acts as a barrier to protect the fluid from contact with air. Since different films are used by different suppliers, specifications vary. Typically, bags can be used for temperatures as low as –70°C and as high as 45°C. The pressure rating depends on the connection, as opposed to the bag. Since the bags are made of polymeric materials, they need to be supported when they are filled with fluid. A tray can be used for smaller-sized bags, while square stainless steel or aluminum tanks can be used to support the larger, three-dimensional ones. As fluid fills a glass or stainless steel tank, vents are required to ensure that the tank fills completely. In contrast, disposable bag containers are designed to be self-venting, and thus do not require a vent. As the fluid fills the bag, the bag's walls expand and allow complete filling of the container.

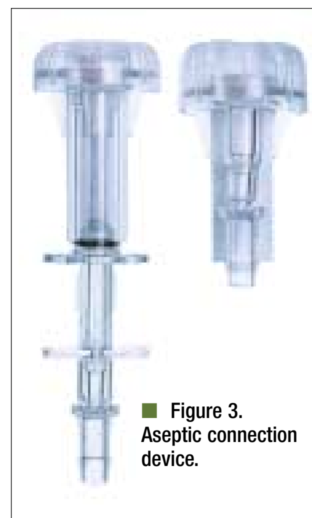
■ Figure 1. Single-use, 2-D bag system with a capacity of 1 L.

■ Figure 2. A variety of disposable capsule filters.

Single-use capsule filter. The single-use capsule filter serves as an effective substitute for stainless steel housings that require installation of filter elements (Figure 2). These filters are available in a variety of sizes that can be matched with the disposable bag container. In the development of a new drug product, the working volume at the R&D stage could be 1 L; at the pilot scale, the volume could be 100 L; and at full scale, the required volume could be 2,500 L. At each stage of the development process, the filter must be available in the appropriate size and with the same materials of construction to ensure an effective and valid scaleup.

Tubing. Although the disposable bag and filter are the main components of the system, there are a number of additional components required to connect the filter and bag, and to connect the system to other parts of the bioprocess. In many biopharmaceutical operations, platinum-cured silicone tubing is used. Despite its higher cost and shorter life than peroxide-cured silicone tubing, platinum-cured tubing is generally preferred by the biopharmaceuticals market. This is because a higher standard of cleanliness is required for biopharmaceutical fluids, and platinum-cured silicone tubing has fewer leachable constituents than peroxide-cured tubing. In addition, platinum-cured silicone tubing tends to have smoother internal surfaces, which reduces the chances of losing the proteins contained in many biopharmaceutical fluids by adhesion to the tubing surface.

Connections. Disposable systems are often connected to other systems. For example, a disposable system that has been sterilized by gamma irradiation may be connected to another part of the system that has been steam-sterilized; or, the upstream side of pre-sterilized system may be connected to a non-sterile operation. The most critical connections are the aseptic connections between two sterilized components (Figure 3). These connections are often performed in a laminar flow HEPA-filtered air hood. Laminar-flow hoods or cabinets are enclosed areas that are designed to keep air circulation in laminar flow to ensure that particles do not come into contact with the aseptic connectors while connection is being made. In many cases, components that need to be connected (for example two ends of a sanitary flange) are protected by a porous wrap.



■ Figure 3. Aseptic connection device.

During an aseptic connection, the wrap is removed from the two components that need to be connected, and each component may be passed through a flame to ensure that the components have not become contaminated after the protective wrap has been removed.

Newer techniques involve quick-connectors and tubing welders. Quick connectors have plastic caps used for protection and are also used in laminar flow hoods. Specialized tubing welders are also available for the performance of an aseptic connection; these devices use disposable wafers or blades to perform the weld. To use both quick connectors and tubing welders, capital equipment is required. This equipment must be maintained, and appropriate records on this equipment must be kept. Moreover, the equipment can be difficult to use in cramped environments.

New devices are available for performing aseptic connections. One such device is a disposable unit that allows the user to make a connection of two previously sterilized pathways in seconds, and without the use of a laminar flow hood or a tubing welder. The device consists of a male and female part, where each of the ports is protected with a hydrophobic strip. During a connection, the two ends are locked together, forming a joint that is effectively permanent. Then the two strips are peeled away simultaneously, bringing the two sterile faces together. The security offered by the system has been qualified by extensive studies using bacterial spores as a contaminant. The fluid path is then prepared for liquid flow by movement of an integral hollow plunger tube inside an outer tube. The inner plunger tube is pushed through the protected connection and into the female side of the device to complete the new sterile path-

way. The units are supplied suitable for gamma irradiation or autoclave sterilization, and thus can be incorporated into a range of equipment, including disposable containers and filters.

SYSTEM SET-UP

A typical single-use system is illustrated in Figure 4. For this system, a fluid needs to be pre-filtered for clarification and then filtered through a final 0.2-μm sterilizing-grade filter. The prefilter often does not need to be sterilized. The sterilizing-grade filter, as well as all of the components downstream of it, must also be sterilized. Once the fluid passes through the sterilizing-grade filter, and the filter is confirmed to be integral, it is considered to be sterile. The final filter and downstream components can be sterilized by gamma irradiation.

Fluids are typically sterilized by filtration because they cannot be sterilized by treatments that involve heat. Microorganisms can be destroyed by heated processes, such as steam sterilization, but the valuable products in the fluid are often destroyed at the same time. The filters serve to remove the microorganisms by a sieving mechanism. The final filter and components downstream need to be sterilized by steam or irradiation to ensure that there are no microorganisms after the fluid has passed through the sterilizing grade filter.

In order to perform the operation, (*i.e.,* sterile filtration of the fluid), the upstream side of the sterile system is connected to the non-sterile portion of the system, which includes the pre-filter and its fluid-delivery system. After the fluid passes through the sterilizing filter, it is collected into the bag, where it can be stored. The bag has a sterile outlet connection through which fluid may flow to another portion of the process. This allows the sterilized fluid to be transferred through the aseptic connection, without a risk to sterility of the fluid.

Clamps, supplied as slide or pinch types, allow the tubing to be closed either before or after the fluid has passed through a section of the disposable system. In some cases, multiple clamps are used to ensure closure of the tubing. In Figure 4, the clamps are closed after the bag has been filled with the filtered fluid, to prevent the fluid from leaving the bag. The clamp on the left is opened while the bag is filling with fluid, while the clamp on the right is opened while the fluid is leaving the bag.

For more-complex systems, such as those that require a series of small bags for sampling or that are used with a flushing procedure, straight runs of tubing may not be appropriate. Both T and Y connectors can be used to provide branched systems. Since tubing diameters can change, a variety of adaptors are available to accommodate different tubing sizes. When the silicone tubing is connected to a filter, an adaptor, a connector, or a T or Y, the tubing is stretched over a hose barb or a stepped hose barb. To ensure that fluid does not leak, one may use cable ties to hold the tubing in place. A common practice is to use two cable ties in opposite directions.

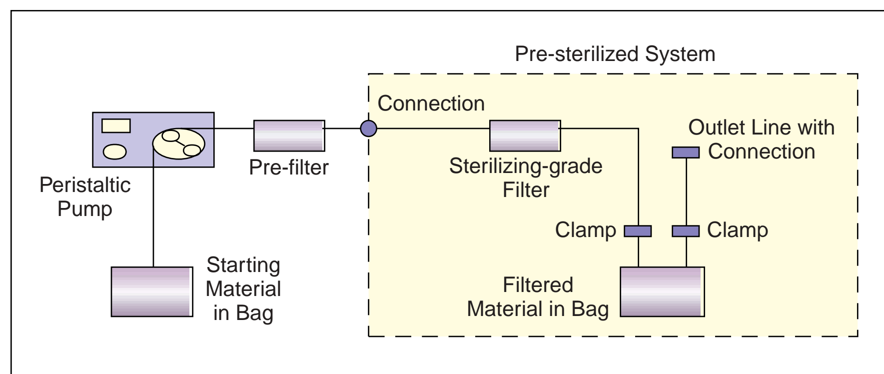
The single-use nature of this type of filtration system eliminates the need for system maintenance, cleaning and cleaning validation, and avoids any possibility of contamination from product to product or batch to batch. This not only provides economic benefits, but safety and time-to-market advantages as well.

As highlighted in the previous example, single-use filtration systems can be used to simultaneously filter and sterilize a variety of biopharmaceutical fluids. These fluids may also require, as a final step, 0.1-μm filtration for mycoplasma removal from culture media and even finer filtration for viral clearance subsequent in the final purification steps. The selection of the filter type is an important consideration. Guidelines are presented in Table 1.

SAFETY CONSIDERATIONS

Because a fully integrated, single-use system, including the filter, bag, tubing and other components, is manufactured and sterilized as one unit, the risks of contamination associated with aseptic connections during coupling are eliminated. Single-use filtration systems also ensure that operators do not come in contact with cleaning solutions, cytotoxic fluids or extremely acidic or alkaline buffers. Since these systems are completely enclosed and disposable, they can be sent to waste disposal after the operation is complete. When stainless steel or glass systems are used, the equipment must be disassembled and cleaned. In such cases, operators come in direct contact with surfaces where potentially dangerous fluids may remain.

Stainless steel systems are sterilized either by use of an autoclave or by steaming in place (SIP). While most SIP processes are well controlled, operation with steam does require proper caution. For example, after the steam



■ **Figure 4. Schematic of a typical single-use filtration system.**

Table 1. Guidelines for specifying filter capsules in single-use systems.

Fluid Type	Recommendation for Single-Use Capsule	Reason for Recommendation
Buffers	Polyethersulfone (PES) 0.2-µm sterilizing-grade capsule	PES is compatible with a wide pH range, which is typical of buffers. The use of a disposable filter that can be used with all buffers simplifies operations.
Biopharmaceuticals that require low adsorption (e.g., drugs, ophthalmics)	PES or polyvinylidiene fluoride (PVDF) 0.2-µm sterilizing-grade capsule	Both PES and PVDF have low affinity for proteins and preservatives, and thus can be used with many biopharmaceutical and ophthalmic products.
Serum	Polyvinylidiene fluoride (PVDF) 0.1-µm sterilizing grade capsule	For serum filtration, mycoplasma removal is needed. Filters used for mycoplasma removal have a 0.1-µm rating. In some cases, multiple 0.1-µm filters are used in series. PVDF membranes have a high flow rate, even with 0.1-µm rating.
Fluids that require viral clearance, such as blood products or derivatives, or biotechnology products such as recombinant proteins or monoclonal antibodies.	Disposable capsules for viral clearance	For applications that require viral clearance, capsule filters often a convenient method, especially when coupled with bags and appropriate pre-filters for clarification
Biotechnology fluids that require the removal of contaminants or capture large molecules in downstream processing.	Filter capsules with chromatographic ion-exchange capabilities	Disposable chromatography capsules are designed to remove contaminants or capture large molecules via ion exchange.
Gas	Hydrophobic PVDF 0.2-µm sterilizing-grade capsules	In some applications, a vent or and air filter may be required (*i.e.*, for long runs of tubing or for disposable bioreactors). Hydrophobic PVDF can be sterilized by gamma irradiation. PTFE, while used in many air or vent applications, is not suitable for gamma irradiation.

cycle is complete, it is necessary to introduce air or nitrogen to the filter to ensure that the pressure inside the system remains constant. This is because the steam in the upstream side of the filter can cool faster than the steam in downstream side of the filter, causing pressure to buildup on the downstream side. If the gas is not introduced, the excessive backpressure can damage the filter.

TIME AND COST SAVINGS

Disposable systems save time directly, since they do not need to be cleaned and sterilized. Furthermore, because the filter, bag and tubing are pre-assembled, the time required for aseptic connections is eliminated. For example, the initial set-up time to make an aseptic connection in a laminar flow hood can take approximately 10–15 min, and each connection thereafter can take at least three minutes.

The time required to complete validation documentation of cleaning, maintenance and sterilization is also eliminated. Moreover, when disposable systems are used during the initial stages of drug development, the validation process during scaleup becomes easier since the materials of construction are often the same. Stainless steel, hard-piped systems and glass containers are not only costly to clean and sterilize on a batch-by-batch basis, but they also require proper cleaning validation documentation for regulatory authorities.

To show the possible cost savings possible with single-use systems, we will compare the labor costs for installation of a pre-sterilized filter (without a bag and tubing assembly) with labor costs for a filter installed in a stainless steel housing. In the latter case, the filter must be sterilized.

During filter installation of a single-use, pre-sterilized capsule filter, the operator removes the filter from its packaging, which takes roughly 5 min, and aseptically connects the filter into the system, which takes about 15 min. The latter is performed in a laminar flow hood, as described earlier. (Note: If an aseptic connection device were used, the time required to make the aseptic connection would be less than 15 min).

As previously mentioned, filters installed in stainless steel housings need to be sterilized by either autoclave or SIP. An autoclave operation is performed before installation, while a SIP operation is performed after installation. The installation of the filter in the housing could take up to an hour, due to record-keeping and assembly. The entire process of autoclaving or steam sterilization of filters could take up to three hours. (The cycle time for the *insitu* steaming operation is longer, due to the time required to reach the sterilizing temperature). Each method of sterilization is performed at a temperature of at least 121°C.

Table 2. Assembly time and cost comparison of single-use system vs. stainless steel system.

Time required for each step, min	Disposable Filter or Filter and Bag	Autoclave	Steam Sterilization*
Remove filter from packaging	5	5	5
Gather components required and install filter into a housing		15	15
Perform sterilization		60	90
Allow assembly to cool to room temperature		60	90
Transport system from autoclave area to process area		5	NA
Aseptically connect the filter into the system	15	15	NA
Total time required	**20**	**160**	**200**
Time saved if disposable filter is used		**140**	**180**
Labor costs saved per filter, assuming rate of $75/h		**$200**	**$225**

* Assume the filter, tank and pipe in the hard-piped system are being sterilized.

After sterilization, time is required to allow the filter to cool down to ambient temperature. Records must be kept for these procedures and sterilization equipment must be maintained. Table 2 highlights the stages of sterilization of a filter cartridge via autoclaving and SIP, and the time required to perform each step. A comparison is drawn to the above example of using a disposable system, and the time and labor costs saved by using a disposable system are calculated.

The "time saved" is calculated by subtracting the time required to install the disposable system from the total time required to perform SIP or autoclaving and installation of the filter in the metal housing. For a facility that uses 1,000 filters/yr, the use of disposable products can save the owner $200,000–$225,000/yr in labor costs assigned to autoclaving or steam sterilization operations.

In Table 3, the labor costs to clean a pre-sterilized filter and bag system are compared with the labor costs for cleaning a filter installed in a stainless steel housing. Here, the cleaning of the filter alone is considered, since cleaning of a tank will vary depending on the size of the tank.

The savings in process time is 1 h and 40 min. If a labor rate of $75/h is assumed, the labor savings per filter is $125. For a facility that uses 1,000 filters/yr, labor savings of $125,000 can be achieved.

The savings in labor required for cleaning and sterilization when a combined filter and bag assembly are calculated as follows: For a filter alone, based on the autoclave sterilization operation detailed above, the overall savings in labor is estimated as $200/filter (for sterilization) + $125/filter (for cleaning) = $325/filter. The time required for cleaning and sterilizing a stainless steel assembly depends on the size of the system.

Additional cost savings can be realized by replacing stainless steel, hard-piped systems with single-use systems when scaling toward production. Single-use systems generally have a smaller footprint than hard-piped systems, since the tubing used is flexible and takes up less space. Moreover, since single-use systems are custom designed, the tubing arrangements are easier to modify.

The cost of a single use disposable system will depend on scale as well as on whether or not the bag system is used for a new facility of as a retrofit of an existing facility. In Ref. 1, Sinclair and Monge describe a process model for monoclonal antibody production in a 2,000-L-fed batch which fixed sterile vessels would be replaced with flexible bags. On a per-batch basis, the follow number of bags would be required: fifty-one 50-L bags, eighteen 100-L bags, twelve 20-L bags and twelve 500-L bags. For these capacity requirements, the capital cost of using a stainless steel system is $22.17 million, whereas the cost of using a disposable bag system is $17.46 million. The capital cost savings is 21.2%.

A cost-of-goods comparison was also performed for the model process — for a new-build as well as for a retrofit. As in the capital cost comparison, the entire pro-

Table 3. Time required for cleaning a single-use capsule vs. cleaning a filter in a stainless steel housing.

Time required for each step, min	Single-Use Capsule	Filter in Metal Housing
Post-use flush and integrity test	30	30
Remove filter from the housing	NA	5
Take housing to cleaning area	NA	5
Discard filter	5	5
Dismantle housing	NA	15
Put housing in washing equipment and run cleaning cycle; record and check water temperature, pressure, flowrate and chemical dosing rate	NA	60
Dry and store the housing	NA	15
Total time required	**35**	**135**

cess was considered in the model. Based on specific cases considered for the monoclonal application, the cost-of-goods savings on a per batch basis for a disposable system is approximately 9% for a new plant, while for a retrofit system, the saving is approximately 8%.

POINTS OF DIFFERENTIATION

Unlike stainless steel systems, single-use systems are designed with translucent housings that can show process fluids in the bag, tubing and capsule filters. Thus, operators can observe fluid levels and flow, as well as spot discoloration and air pockets.

The process by which single-use filtration systems are sterilized, gamma irradiation, provides another source of enhanced safety. Once the disposable system is assembled by the manufacturer, it is put into the final packaging and sent to a facility that performs gamma irradiation. Multiple systems can be irradiated at the same time.

The packaging includes a cardboard box and bubble wrap, which is used to protect the bags from damage by contact with other portions of the system. The system itself is usually placed in two plastic bags. This allows the end user to remove the outer bag in an inspection area, and to transport the system to a cleanroom for storage. The inner bag can be removed when the disposable system is needed.

During the gamma irradiation process, gamma rays from Cobalt 60 isotope provides the electromagnetic energy that penetrates materials of construction, destroying microorganisms that exist in the system's bioburden. The organisms included in a system's bioburden are those that are present in the location where the system is assembled. The radiation dose should not be too high, as it can degrade certain polymers, resulting in elevated extractables, and, in severe the cases, destruction of the polymers. The appropriate radiation dose for a system with a known bioburden level can be set and validated according to procedures for ISO11137, a standard that was developed by the Association for Advancement of Medical Instrumentation and has been in use for over 15 years. Examples of gamma-tolerant materials include hydrophilic and hydrophobic polyvinylidiene fluoride, hydrophilic polyethersulfone and hydrophilic nylon 6,6 filter membrane. As detailed above, most systems also use platinum-cured silicone tubing, which can sustain gamma irradiation.

MAKING THE SWITCH

For many facilities, switching from a completely stainless steel system to a completely disposable system may not be practical, due to the investment in equipment as well as the validation work that may have been performed on the hard-piped system. It is possible, however, to gradually make the change to a disposable process.

Some vendors manufacture capsule filters that contain the same filter cartridge that is used in stainless steel housings. This makes conversion easier. Even in areas where filters are reused routinely, such as in vent and gas lines, the advent of steamable capsule filters make it possible to use disposable products. In addition, the sizes of disposable capsules are increasing, making them more practical for use in larger systems. Today, 30-in. capsule filters with filter areas from 15 ft^2 to over 50 ft^2 are commercially available. Filters can also be coupled together, making larger filter areas possible (Figure 5).

■ Figure 5. Both small and large prefilters and final filters can be coupled together.

Stainless steel systems may be the method of choice when automation is required, such as in the production of large volumes of biopharmaceutical products. In these applications, the feed materials must be predictable and process conditions must be tightly controlled to produce identical product batches.

As single-use products gain wider acceptance in the biopharmaceutical industry, the integration of complementary disposable products, such as chromatography and cross-flow filtration devices, will increase to provide safer, more-efficient processing solutions. The introduction of fully integrated, single-use fluid filtration systems is an important milestone in helping biopharmaceutical companies realize these benefits.

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