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Facility Design for Contract Manufacturing

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Mammalian Cell Culture Based Contract Manufacturing Demand

The number of mammalian cell-culture based products is increasing rapidly and has the potential to outpace the current production capacity. New facility construction for mammalian cell culture production is costly and time consuming. Planning for initial product launch and long-term commercial manufacturing can be daunting for promising products in late phase clinical trials. At such an early phase, market projections typically include a wide range of potential patients and dosage, and process development may be working through variability in product titer and yield. Additionally, there is still a substantial risk that the final clinical trials may not be successful, and the product may not receive final approval. Contract Manufacturing Organizations (CMOs) are increasingly relied on to provide a manufacturing capability for mammalian cell-culture based products to help deal with these issues. CMO services can avoid or defer the cost of constructing a production facility, and potentially allow faster production. The numbers of CMOs with plans or designs for new facilities to fulfill this growing demand are increasing.

However, contract manufacturing presents serious challenges. There is a number of questions that must be answered if you're a CMO interested in getting into the mammalian cell culture business. What capabilities do you need to provide in order to attract and retain customers? What are your capital resources? What market(s) do you target? Do you design for clinical trial production, initial product launch, or commercial manufacturing? What types of products will you manufacture? Will you design for purification of monoclonal antibodies (MAbs), or enzyme replacement therapies and growth factors (non-MAbs)? These questions and other related issues substantially impact production facility design.

Contract vs. Commercial Manufacturing Differences

CMOs differentiate their services based primarily on their ability to meet the market's technical demands, and their ultimate production costs. To successfully compete as a contract manufacturer, CMOs must balance their production capacity, manufacturing flexibility, and facility CGMP compliance features, with their fixed capital expenditure and life-cycle operating costs. A customer's selection decision will be based on the CMO's capability to produce a product at the lowest manufacturing cost, coupled with the customer's confidence that the product produced will meet quality expectations. Product quality issues can result in costly product recalls or the inability to supply the market. Such failures result in a customer's lost market share and revenue. As a result, issues of quality differentiate contract manufacturers and are key selling points.

A unique feature of CMOs is the need to provide facility tours for potential customers to survey manufacturing capabilities. As such, CMO facilities have basic "showcase" features that are not often included in commercial manufacturing facilities. These "showcase" features need not be expensive or interfere with plant operating efficiency. Along with attractive spaces for greeting customers and conducting contract discussions, there is a need to provide some concrete visual evidence of your capabilities. Such features commonly include unclassified viewing corridors and windows that offer key views into processing areas. This requires an understanding of the various production requirements and careful planning to provide an attractive and economically competitive facility. These features can often be provided with little significant cost if they are identified early and planned for prior to facility construction.

Contract manufacturing requirements also differ from those of traditional manufacturing in other, more basic process objectives. Traditional manufacturing typically deals with known products and processes. The potential range of contract manufacturing processes essentially includes all mammalian cell culture products. This is a fundamental difference, and selecting a range of manufacturing capability, matched to a market demand, is essential to success.

Manufacturing Capacities

Current commercial mammalian cell culture products employ bioreactor volumes that range from a few liters up to 25,000 liters and result in an even wider range of purification requirements. A facility that can meet this wide production range along with all of its processing requirements is unrealistic. Consequently, contract manufacturing facilities are designed around

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Table 1. Select Cell Culture Based Manufacturing Facility Information

Facility	Application	Biosafety Level	Manufacturing Trains		Production	Notes
			Cell Culture	Purification		
1	Clinical Trial & Initial Product Launch	BL1-LS	3	2	Multi-Product/ Concurrent	
2	Clinical Trial & Initial Product Launch	BL1-LS	1	1	Multi-Product/ Campaigned	
3	Clinical Trial & Initial Product Launch	BL1-LS	1	1	Multi-Product/ Campaigned	
4	Commercial Manufacturing	GLSP	1	1	Single-Product/ Dedicated	
5	Commercial Manufacturing	GLSP	2	1	Multi-Product/ Campaigned	Microbial Suite
6	Commercial Manufacturing	BL1-LS	1	1	Multi-Product/ Campaigned	
7	Commercial Manufacturing	GLSP	1	2	Multi-Product/ Campaigned	
8	Commercial Manufacturing	GLSP	2	2	Multi-Product/ Campaigned	

a given market segment with specifications that address the market's production requirements. Processing requirements that are unique or less common can then be provided on an as-needed basis to meet specific client requirements. Therefore, some facility space must be provided to allow adaptation to meet specific client needs. This strategy allows CMOs to target a wider spectrum of potential market with minimal initial capital expenditure, while providing the flexibility to accommodate unique requirements.

Production goals ultimately determine the total installed bioreactor capacity. Typical batch bioreactors operate on a nine to 14 day production cycle with a two to three day turnaround. Tables 2 and 3 provide production requirements for select MAb and non-MAb products. MAb product titers ranged from 0.45 to 1.79 grams per liter, while non-MAb product titers ranged from 0.0032 to 1.28 grams per liter. Purification yields for MAb products ranged from 51 percent to 75 percent, and for non-MAb products yields ranged from 12 percent to 50 percent. With the exception of one low titer non-MAb product, the product titers and purification yields provide very similar production volume based on installed bioreactor capacity. As a rule of thumb, each 100L installed bioreactor capacity is capable of producing approximately 1.0 kilogram of product per year assuming a 14-day bioreactor cycle time, a 1.0 g/L titer and a 50% purification yield.

A facility's production requirements will vary depending on whether commercial manufacturing is targeted, or capabilities for clinical trial and initial product launch. Facilities for clinical trial and initial product launch typically have production requirements in the range of five kilograms per year or less. This type of facility frequently manufactures multiple products and does not have extended production campaigns, as commercially successful products are moved to large-scale commercial manufacturing facilities. Consequently, clinical trial and initial product launch facilities typically require production bioreactors in the range of 100 to 2,500 liter volume, depending on the production campaign length.

Commercial manufacturing facilities typically have production requirements in excess of five kilograms per year. These facilities usually operate with a limited product number. Consequently, commercial manufacturing facilities typically require production bioreactors in the range of 2,500 liter volume and up. The actual production bioreactor size and number are frequently designed to optimize harvest and purification equipment utilization.

Thus, the intended target profile needs to be established in order to define the overall facility scale. Scale not only affects the size of equipment and the production building, but also the size of support facilities such as central utilities, warehouse, administrative areas, and laboratory space. Obviously, initial investment is relative to scale.

Production Requirements

Cell culture and purification are the two main components of cell-culture based manufacturing. The cell culture component typically consists of: the inoculum preparation laboratories, seed bioreactor train(s), production bioreactor(s), and harvest unit operations. The purification component, which typically has the greatest variability between products, is comprised of: chromatography, ultrafiltration, depth filtration, chemical reactors (viral inactivation, pegylation, etc.), column packing, and bulk fill.

The cell culture components typically require the least variability. The inoculum preparation laboratory tends to be similar for all cell-culture based projects, and usually employs disposable culture flasks or bags. Inoculum laboratories can handle volumes in excess of 100 liters in a single culture bag. These large, disposable culture bags represent an economical means for preparing large inoculum volumes before requiring costly seed bioreactors. The seed bioreactor train represents an intermediate-scale expansion to go from the inoculum laboratory scale to the production bioreactors. The challenges typically encountered are minimizing the operating and capital costs while maintaining high production bioreactor utilization. In a contract manufacturing setting, the larger seed bioreactors also present manufacturing flexibility by providing a wider bioreactor volume range available for production. Additionally, specifying bioreactors with a high turn-down ratio provides further flexibility.

In cell culture operation, equipment needs do not vary substantially from one product to another. However, operating conditions and yields can vary

substantially. These variations influence the downstream harvest and purification area design. The variability in culture duration, product titer, and bioreactor operating volume needs to be considered for effective facility design. A commonly employed method for reducing the impact of this variability is to decouple the cell culture and purification areas using an intermediate freeze or hold step. This intermediate step initially allows the purification to be gram-loaded rather than processing an entire cell culture lot. Thus, the harvest operation is the only unit operation that is subject to significant product loading fluctuations resulting from product or process changes. This strategy has been effectively used on projects with the addition of a hold tank or product pooling, and the use of equipment designed to handle a wide product loading range.

Biocontainment

The definition of a biocontainment level under which to operate is an important decision directly related to the cell culture component. Appendix K in the Guidelines for Research Involving Recombinant DNA Molecules (NIH Guidelines) defines biocontainment levels for large scale (>10 L) applications. These guidelines are voluntary, but represent good design practice and principles regarding biocontainment. The four large-scale biocontainment levels are:

GLSP (Good Large Scale Practices) — Recommended for work involving viable, non-pathogenic and non-toxigenic R-DNA with a history of safe large scale use (*Escherichia coli*, Chinese Hamster Ovary).

BL1-LS (Biosafety Level 1 Large Scale) — Recommended for work involving agents of no known or minimal hazard potential to personnel or the environment (*Escherichia coli*, Chinese Hamster Ovary).

BL2-LS (Biosafety Level 2 Large Scale) — Recommended for work involving agents of moderate risk that are spread primarily through ingestion or inoculation (*Bacillus subtilis, Salmonella cholerasuis*).

BL3-LS (Biosafety Level 3 Large Scale) — Recommended for work involving agents that cause lethal infections in man and for which effective treatment is unavailable (*Mycobacterium tuberculosis*).

Facility complexity and equipment requirements increase with higher biocontainment levels. After the facility is complete and operational, it is very difficult to raise the level. As shown in Table 1, mammalian cell-culture facilities typically operate in either the GLSP or BL1-LS mode. Considering the marginal costs for increasing the level of containment during the initial design and construction (as compared to renovation), facilities often choose to increase the level of containment for a space by one grade, say GLSP to BL2-L1, in order to plan for future flexibility in equipment. Biocontainment also tends to become more of an issue for high-profile facilities located near residential areas.

Table 2. Production Information for Select MAb Products

Type	Titer (g/L)	Yield (%)	Capacity (kg/year)	Bioreactor Type	Harvest			Pre-Viral			Post-Viral			Total Steps
					Cent	Chrom	MF/UF	React	Chrom	UF	React	Chrom	UF	
MAb "A"	0.80	51%	300	Batch	1	0	1	0	2	0	0	2	1	7
MAb "B"	1.00		10	Batch	1	1	1	2	1	0	0	0	0	6
MAb "C"	0.45		5	Batch	1	1	1	2	1	0	0	0	0	6
MAb "D"	0.45	75%	500	Fed-Batch	1	0	0	1	3	0	0	0	1	6
MAb "E"	1.79	76%	2,500	Fed-Batch	1	0	0	1	3	0	0	0	1	6
MAb "F"	1.50	66%	25	Fed-Batch	1	0	0	1	1	0	0	2	0	5
MAb "G"	1.50	66%	250	Fed-Batch	1	0	0	1	1	0	0	2	0	5
Median	1.00	66%	250		1	0	0	1	1	0	0	0	0	6

Abbreviations: Cent, centrifuge; Chrom, chromatography; MF, microfiltration; UF, ultrafiltration; React, reactor.

Table 3. Production Information for Select Non-MAb Products

Type	Titer (g/L)	Yield (%)	Capacity (kg/year)	Bioreactor Type	Harvest			Pre-Viral			Post-Viral			Total Steps
					Cent	Chrom	MF/UF	React	Chrom	UF	React	Chrom	UF	
Other "A"	0.50	50%	200	Perfusion	1	1	0	1	2	0	0	2	0	7
Other "B"			50	Batch	0	0	1	0	3	0	0	0	1	5
Other "C"			50	Batch	0	0	2	0	4	0	0	0	1	7
Other "D"	1.28	12%	1	Roller Bottle	0	0	1	2	6	3	0	0	0	12
Other "E"	0.78	35%	1	Roller Bottle	0	0	1	2	5	2	0	0	0	10
Other "F"	0.15	28%	10	Roller Bottle	0	0	1	0	4	0	0	0	0	5
Other "G"	0.0032	42%	1	Batch	1	3	1	1	1	1	0	0	0	8
Median	0.50	35%	10		0	0	1	1	4	0	0	0	0	7

Abbreviations: Cent, centrifuge; Chrom, chromatography; MF, microfiltration; UF, ultrafiltration; React, reactor.

Purification Requirements

The facility purification component typically has the least variability in operating conditions and productivity for a given product or process. However, as shown in Tables 2 and 3, the purification component tends to have the greatest variability in actual equipment. The MAb products had five to seven recovery and purification steps while non-MAb products had five to 12 steps. In practice, the facility space requirements are more directly impacted by the number of steps than by the relative size of a given step. Therefore, it is more important to design around the number of purification unit operations than the type or size. This is especially true for contract manufacturing where the purification unit operations will surely change between products.

Purification areas pose the greatest challenges in providing the flexibility to deal with different chemistry. These challenges include not only a variable buffer solutions number and volume, but also issues dealing with flammable and corrosive materials, as well as processing at reduced temperatures (two to eight degrees Celsius). Table 4 shows select operational information for chromatography operations. The representative chromatography operations employed between three and nine different buffers with volumes ranging from one to 40 column volumes (CVs). Importantly, seven percent of the buffers employ solvent concentrations that are potentially flammable and another 20 percent of the buffers employ chloride concentrations that are potentially corrosive to 316L SS. Additionally, one-third of chromatography operations were performed at two to eight degrees Celsius. Consequently, processing at reduced temperatures is an important facility capability along with the capacity to handle flammable and corrosive materials.

Table 4. Operational Information for Select Chromatography Unit Operations

Column	Temperature	# Buffers	Corrosive	Flammable	Volume Range
1	2 - 8 C	6	No	No	5 - 10 CV
2	2 - 8 C	3	No	Yes	6 - 22 CV
3	2 - 8 C	4	Yes	No	10 - 40 CV
4	2 - 8 C	3	No	No	1 - 7 CV
5	2 - 8 C	5	No	Yes	4 - 35 CV
6	2 - 8 C	5	No	Yes	4 - 35 CV
7	4 - 8 C	5	No	Yes	2 - 8 CV
8	4 - 8 C	3	No	No	3 - 9 CV
9	4 - 8 C	4	No	Yes	2 - 6 CV
10	4 - 8 C	4	No	No	?
11	4 - 8 C	5	No	Yes	?
12	4 - 8 C	5	Yes	No	?
13	4 - 8 C	5	Yes	No	?
14	15 - 25 C	6	Yes	No	4 - 15 CV
15	15 - 25 C	6	Yes	No	3 - 35 CV
16	15 - 25 C	6	Yes	No	3 - 11 CV
17	15 - 25 C	6	Yes	No	3 - 19 CV
18	15 - 25 C	6	Yes	No	3 - 11 CV
19	15 - 25 C	6	Yes	No	3 - 11 CV
20	ambient	9	No	Yes	2 - 10 CV
21	ambient	7	Yes	Yes	3 - 10 CV
22	ambient	6	No	No	3 - 12 CV
23	ambient	7	Yes	No	2 - 16 CV
24	ambient	3	No	No	3 - 4 CV
25	ambient	5	No	No	3 - 15 CV
26	ambient	5	Yes	Yes	2 - 9 CV
27	ambient	6	Yes	No	2 - 11 CV
28	ambient	4	No	No	3 - 5 CV
29	ambient	8	Yes	Yes	3 - 7 CV
30	ambient	4	No	No	3 - 4 CV
31	ambient	5	Yes	No	5 - 30 CV
32	ambient	7	Yes	Yes	1 - 15 CV
33	ambient	6	Yes	No	6 - 20 CV
34	ambient	7	Yes	No	4 - 9 CV
35	ambient	3	Yes	No	3 - 10 CV
36	ambient	4	Yes	No	3 - 8 CV
37	ambient	4	Yes	No	3 - 8 CV
38	ambient	7	Yes	No	4 - 10 CV
39	ambient	7	Yes	No	4 - 10 CV
Median	33%	5	20%	7%	3 - 9 CV
Range	2 - 25 C	3 - 9			1-40 CV

Abbreviations: CV, column volumes

Table 5 shows select operational information for microfiltration (MF) and ultrafiltration (UF) operations. The representative MF/UF operations employed between four to five different buffers with volumes ranging from 0.5 to 100 retentate volumes (RVs; based on the target product volume and not the retentate tank volume). Importantly, none of the buffers employed potentially flammable solvent concentrations or corrosive chloride concentrations. Additionally, only nine percent of the MF/UF operations were performed at two to eight degrees Celsius.

Flammable buffers, including those containing low concentrations of ethanol, acetonitrile, etc., will require the use of electrically-classified areas. Such areas are classified either as Class I, Division 1 (vapor hazard normally present), or Division 2 (vapor hazard not normally present). Normally, the process equipment is isolated to discrete rooms within the facility in order to limit the area affected by these classifications. If quantities are large enough to exceed exemptions, then both flammable and corrosive processes will require that portions of facilities be classified as hazardous occupancy by the building code. A hazardous occupancy classification means that the building will be limited in size, or that expensive structural fireproofing must be provided. Also, additional costs are incurred

Table 5. Operational Information for Select Microfiltration/ Ultrafiltration Unit Operations

MF/UF	Buffers	Corrosive	Flammable	Temperature	Volume
1	4	No	No	2 - 8 C	1
2	4	No	No	ambient	4 - 65 RV
3	4	No	No	ambient	24 - 100 RV
4	4	No	No	ambient	2.5 - 10 RV
5	4	No	No	ambient	3 - 6 RV
6	4	No	No	ambient	4
7	4	No	No	ambient	12
8	5	No	No	ambient	0.5 - 7.5 RV
9	5	No	No	ambient	0.5 - 7.5 RV
10	4	No	No	ambient	10 RV
11	4	No	No	ambient	7 RV
Median	4	0%	0%		5 RV
Range	4-5			2 - 25 C	0.5 - 100 RV

Abbreviations: MF, microfiltration; UF, ultrafiltration; RV, retentate volumes (based upon product volume and not retentate vessel volume).

for an increase in the density of the fire protection (sprinkler) system and a spill/firewater containment system that typically must be sized to contain 30,000 gallons. Special operating procedures that ensure safety are also necessary. The weigh/dispense or buffer preparation area might need to provide deflagration (explosion) venting. Highly corrosive solutions, especially those with high chloride concentrations and low pHs, may be incompatible with 316L SS,

Figure 1. Purification Booth Concept showing utility panel.

and require the use of expensive alloys, including various types of Hasteloy™ or AL6XN™. The reduced temperature requirements may necessitate the use of cold rooms. These capabilities may or may not be needed for a given process, and represent expensive features if not required.

A more recent concern for cell-culture manufacturing facilities is viral inactivation and viral removal. Commonly employed viral inactivation methods include solvent detergent extraction and pH excursion. When employed, viral inactivation generally occurs early in the purification process and typically involves an agitated process vessel. Viral removal is more commonly employed, and involves filtration using a viral removal cartridge filter based on either size exclusion or viral affinity. Viral removal is frequently the last purification step prior to bulk filling. When purification suites are segregated based on pre- and post-viral areas, it generally occurs at the viral removal step rather than the viral inactivation step.

Portable Based Purification Suite Concept

A generic, portable based Purification Suite Concept leads to a highly flexible, minimal "first-cost" manufacturing solution. This approach provides multiple purification booths within a purification suite. Figure 1 shows a typical purification booth with a utility panel for connecting a portable purification skid. In a given campaign, a purification booth is dedicated to a single purification unit operation. This is feasible because the relative space requirements for typical purification operations (ultrafiltration and chromatography) tend to be comparable for similar manufacturing scales. Additionally, the actual production equipment tends to be skid-based and portable, which provides additional flexibility and facilitates product changeovers. This could allow a contract manufacturer the ability to defer procurement of skid-based purification equipment until needed. In a facility that supports variable purification unit operations, it is desirable to provide space for stored equipment within the clean environment.

Process Support Requirements

Excluding the administration and laboratory support functions that are often housed in a separate structure, there are seven additional process components to a cell-culture facility: media prepa-

ration, buffer preparation, warehouse and weigh/dispense, wash, clean utilities, plant utilities, and waste handling. Media preparation and hold supports cell culture with food for cell growth. Buffer prep serves purification with solutions used in the chromatography and filtration processes. In addition to consumables (such as filters, hoses, gowning supplies, and perhaps office supplies), the warehouse is used to house materials used in the preparation of the media and buffer solutions. The weigh/dispense area provides the interface between the warehouse materials and dispenses these into batch lots for use in media and buffer preparation. The wash area is sometimes referred to as the "glass wash" area, but in addition to cleaning and sterilizing glassware, other mechanical parts are also handled, such as valves and hoses. The overall manufacturing facility is then supported by clean utilities that typically include water-for-injection (WFI), clean steam generation, clean gases (air, carbon dioxide, oxygen, and nitrogen), process chilled glycol, and clean-in-place (CIP) systems. Plant utilities typically include plant steam generation, plant water, compressed air, and plant chilled water, as well as waste handling systems, pH neutralization, and potentially biowaste inactivation.

Large-scale manufacturing brings complexities to the design of media and buffer systems. Media and buffer weigh/dispense and preparation operations are typically performed in separate areas for segregation according to differing process requirements. If desired physical adjacencies are not available, additional considerations may be required to renovate facilities. Fortunately, manufacturers have considerable flexibility with regard to the design and operation of media and buffer areas. Design issues normally focus on the selection of material handling equipment for given volumes of material, rather than on any real process-related issues. Material volume dictates the number and size distribution for containers. These in turn influence the material charging option and container wash requirements. Additionally, the design needs to account for operator protection, dust minimization, and cross contamination prevention.

Traditional fed-batch mammalian cell-culture bioreactors require media during inoculation and possibly one or two feeding operations over the course of the one to three-week fermentation. Media is typically made up in a media preparation vessel and filter- or heat-sterilized as it is transferred to a sterile hold container. Depending on the required volume, the hold container can be either a fixed or portable vessel, or a disposable bag. Disposable bags present an attractive means to provide both flexibility and the minimization process of initial capital costs, but tanks offer economy in the long term. On smaller scale operations, consideration should be given to outsource media preparation, and to the purchase of the prepared liquid culture media in disposable bags.

The buffer preparation and hold requirements for contract manufacturing tend to have the greatest variability of all the process requirements, as discussed above and shown in Tables 4 and 5. As a result, the design for the buffer area poses many similar design challenges as designing the cell culture and purification areas. The most common strategy used is to provide a reasonable range of buffer preparation capabilities while designing flexible buffer delivery systems. A number of proven options exist that provide flexibility in the buffer delivery systems ranging from using portable containers, to multiple fixed vessels dedicated to a specific purification booth. Flexibility and operational philosophy determine the choice between automated multi-port valves versus manual transfer panels, and is a basic decision that significantly affects a facility's design.

Conclusion

A sensible approach to designing a contract manufacturing facility involves first identifying the target facility and product types. A Clinical Trial and Initial Product Launch capability requires smaller, more flexible facilities as the process tends to have shorter production runs involving a larger number of products. Commercial-scale manufacturing facilities tend to be much larger and have less flexibility as they typically have longer production runs involving relatively fewer products. From a facility perspective, the cell culture design tends to have less variability between products, and requires approximately 100 liters of production bioreactor capacity for each annual 1 kilogram production. From a product perspective, purification has the greatest variability in both the number and types of operations and their supporting requirements. MAb products typically require five recovery and purification steps, while non-MAB products may require twice as many. A practical approach to purification design may be to provide two purification suites. This would provide the capability to concurrently produce two MAb products, or use both suites to produce one non-MAb product. The capability to purify products at reduced temperatures (two to eight degrees C) should also be considered. Finally, the capability to handle a limited number of potentially flammable and corrosive buffer solutions should be provided.