Linear Scale-Up of Cell Cultures

The Next Level in Disposable Bioreactor Design

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Biopharmaceutical companies and CMOs need to increase production capacity and flexibility in producing biological compounds. At the same time, regulatory agencies are increasing their demands and specifications for marketed products. Producing biologicals in stainless steel bioreactors is therefore becoming a tedious task, with the increasing cost factors of clean- and steam-in-place (CIP, SIP) and validation. These were the issues faced by CatchMabs B.V, a biotech company from Wageningen in The Netherlands. It develops low-cost affinity molecules (iMabs) for food-grade industrial affinity chromatography. With diverse targets and production volumes in the company's production portfolio, the need for a flexible, low-investment, medium-scale bioreactor was paramount. The major challenge was to decrease scale-up time and the ability to culture different cell types in parallel with minimal space. Other important demands were to reduce downtime, cleaning hassles, and validation costs.

Product Focus: All biologicals

Process Focus: Production, validation

Who Should Read: COOs, CFOs, plant managers, process engineers, and cell culture engineers

Keywords: Bioreactors, disposables, process optimization, cell culture fermentors

Level: Introduction

Instead of stirred bioreactors, we focused on wave-agitated bioreactors available on the market. With them, many topics on our wish list could be checked off — except for linear upscaling and low capital investment. Available equipment was not only expensive, but scale-up design required an increase of culture bag size in all three dimensions. Consequently, the changed wave hydrodynamics would influence gas exchange and growth conditions. As an alternative, we implemented a cell biologists' view on scaling up; once you have optimized, try not to change any critical parameter that affects cell growth. This culminated in a proprietary multilayer platform that uses wave-agitation in disposable bags as the mixing principle, but for which the wave hydrodynamics remain constant throughout all culture volumes. Early in 2004, a first version (16 × 35 L) was designed and named the Tsunami bioreactor (Photo 1). A miniature version (4 × 35 L) will be on display at Bio 2005 (Figure 1).

Photo 1: Detail of the Tsunami bioreactor: Because of the different angles of the rocking platforms, only two can be seen in detail. In this configuration, four bags per layer with a culture volume of 35 L each are placed on the platforms, all with air-in and air-out connectors and an inoculation/sampling connector in the middle. Additional connectors are optional. Bags are fixed in place by a slight overpressure; the top left bag is not yet fully inflated in this picture. Total capacity of this model is 16 × 35 L culture volume.

Bioreactor Types

Bioreactor selection and design is a key decision factor that affects commercial manufacturing and its corresponding capital investment for many years. Three basic reactor designs can be distinguished, all of which have advantages and disadvantages (Table 1).

The most common type of aerobic bioreactor in use today is the stirred-tank reactor (STR). Ideal for growth of high–cell-density cultures (HCDC) in large volume tanks, STR has emerged as the industry's technology of choice (1). However, HCDC have been successful for only a limited number of microorganisms or cell types that can survive in large-scale reactors (2). In addition, STR fermentations have been plagued by major disadvantages:

- high capital investments, energy requirements, and maintenance demands
- shear
- a need for intensive cleaning and sterilization
- the risk of cross-contamination
- scaling effects (3).

A second type of bioreactor uses a bubble-driven system such as an airlift bioreactor. Although these systems have overcome some barriers such as high shear, maintenance, and energy requirements, others such as high capital investment, validation of cleaning and sterilization, risk of cross-contamination, and upscaling are not addressed (3).

The third type of bioreactors are wave-agitated bioreactors, which take advantage of disposable sterile bags that do not require cleaning and sterilization, resulting in a minimum downtime. This breakthrough concept was developed by Wave Biotech (**4, 5**). Other advantages include reduced shear, closed-system containment, and easy operation and maintenance as well as lower cost due to elimination of mixers, sterilization piping, complex instrumentation, and stainless steel constructions (**6**). Disadvantages include reduced oxygen transfer, which may limit growth of HCDC. Therefore, wave agitated systems have thus far been most useful for low-volume and specialty applications.

However, the list of wave-cultured cells is growing rapidly, including many industrial favorites such as Chinese hamster ovary (CHO) cells, the PER.C6 cell line from Crucell NV (www.crucell.com), hybridomas, adenovirus, insect cells, and hairy root cells (**7**).

The Tsunami bioreactor provides additional advantages over existing wave-agitated systems: flexible production volumes, parallel cell cultures, parallel culture of multiple cell-types, linear upscaling, and maximum flexibility.

Design Elements

The basic principle of the Tsunami design is a multilayer wave agitation in which the depth is kept at a fixed length. A series of holding platforms are placed on top of each other, each one rocking in counter phase to its adjacent platform to reduce engine power and provide maximum stability. The containers are made of stainless steel and closed with flat lids that have slit-like openings to enable access to tubings and filters connected to the bags. Containers can either hold just one segment or become segmented by fixed or removable vertical divider plates, enabling further containment. Depending on the segmentation configuration and the length of the machine, bags between five and 320 L in total volume (1–160 L of culture-medium volume) can be inserted into each layer. Many variations on this basic design can be applied, as illustrated in Figure 2.

A basic setup involves the following steps. Presterilized, disposable bags are placed inside the containers and filled with presterilized media. The media can be preheated, heated during transport to the bag, or heated after pumping into the bags by (for example) heated blankets. The bags are pressurized. As a result of minimal overpressure, each bag is secured in place and will assume an optimal shape dictated by the form of the container and lid. Because each cell type has its own optimal hydrodynamics, the platform's rocking angle can be adjusted (ranging ±2° and ±15°), and the rocking speed can be varied between two and 30 cycles per minute. All bags are connected to appropriate inlet and outlet air tubings, filters, inoculation, and sampling ports and can have as many ports and connectors for monitoring sensors as required.

At the end of the production cycle, cells and media are pumped through flexible, disposable tubing to a downstream processing (DSP) station or storage vessels directly adjacent to the device. Depending on needs (number of different cell types, pathogenicity, temperature requirements, and regulatory requirements), there are several options to control both temperature and containment. For example, an entire bioreactor can be built inside a gas-tight climate-controlled room to enable perfect climate control. In case of a calamity, electronic locks and valves seal off the entire system, and both the bioreactor and the room with all its contents can be gassed with ethylene oxide and neutralized before operators enter. With Class 2 and 3 hazardous pathogens, an entire system can be easily operated from outside the containment area.

Figure 1: Artist impression of the Mini-Tsunami bioreactor. This model has 25% (4 × 35 L) of the capacity of the basic model, but the bag dimensions are exactly the same as the machine in Figure 1.

Because of the very recent development of the Tsunami bioreactor concept and the production platforms we use, our experiences are still limited to several types of bacteria, bacteriophages, and a hybridoma cell line. For a particular *Listeria* strain, a host for *Listeria*-specific phages, it was shown that the total yield of bacteria and the number of infectious bacteriophages produced were exactly linear with the bag volume (measured in 5-, 10- and 35-L bags). In addition, parallel cultures in a 2-L fermentor and the Tsunami bioreactor indicated that the wave-agitated cultures gave two orders of magnitude better yield (10^{11} PFU/mL in disposable bags compared with 10^{9} PFU/mL in the fermentor). This difference in yield probably relates to reduced physical stress on host bacteria and severely reduced destruction of phage particles in wave agitation.

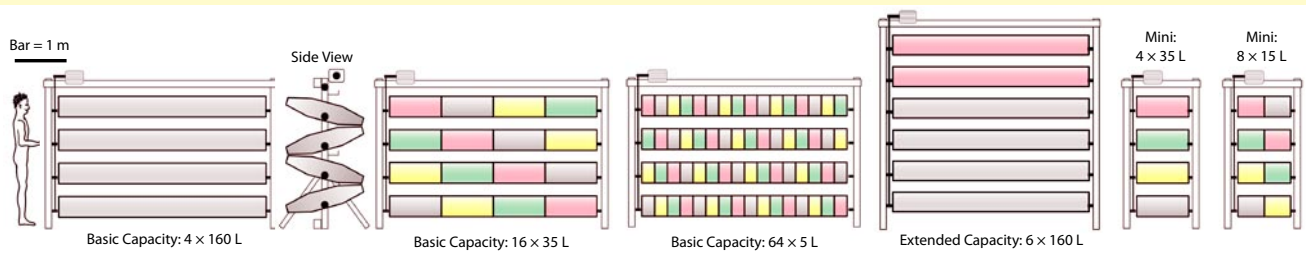

Figure 2: Overview of different capacity options (front view and lateral view).

Economics

Although cost comparisons are difficult with so many different bioreactors on the market and the sensitive nature of CMO data, reviews and conference reports are published regularly from which current numbers can be extracted (8). The data for lifetime operating costs in Figure 3 are based on a recent article by Hazal Aranha (9) and extrapolated for the Tsunami bioreactor, using a production volume of 200 L and a 10-year depreciation period. Besides a decrease in total operating costs and an increase in production (facility) flexibility, the most notable shift in cost distribution is a relative increase in process costs. A portable bioreactor does require additional costs for setup and disassembly, but that is partly set off by a decrease in capital expenditure. For a Tsunami bioreactor, that results from a significantly lower capital expenditure but more important, from an almost complete elimination of cleaning and validations costs.

To date, such systems find economic preference in the 100–200-L market but are considered cost neutral at best in the 1000-L range when compared with stainless steel STRs. In the 10,000-L range the systems are too expensive an option (8). However, we believe that the Tsunami bioreactor concept allows entry in the 1000-L range — and possibly even larger when the costs of disposables will come down almost one order of magnitude. Currently high profit margins on disposables will attract a fierce competition between suppliers and should have a significant downward effect on the price of culture bags. Reliable, disposable sensors for pH and

Table 1: Advantages and disadvantages of basic bioreactor designs

STIRRED-TANK BIOREACTORS

Advantage	Disadvantage	Applications
High productivity (allows growing of high cell density cultures due to increased oxygen transfer)	Need for cleaning and sterilization	Batch mode
Large-volume tanks possible	High initial capital investment	Fed-batch mode
Well understood principles of scaling parameters and the ease of process control in homogeneous systems	More complex design (high maintenance, more risk of defects)	Continuous mode
Many existing variations on design (membrane dialysis reactor, plug flow reactor, membrane cyclone reactor)	Nonuniform and high shear (reactor not suited for sensitive cells)	Perfusion mode
	Linear upscaling impossible	Industry's technology of choice. At least 70% of licensed processes of large scale protein production use stirred tank reactors (3)
	High risk of contamination	
	Inefficient heat transfer (cooling is necessary)	
	Large footprint	

BUBBLE-DRIVEN (AIRLIFT) BIOREACTORS

Advantage	Disadvantage	Applications
High productivity (allows growing of high cell density cultures due to increased oxygen solubility when greater pressures are applied)	Need for cleaning and sterilization	Batch mode
Uniform and lower shear conditions (reactor allows for growing animal and plant cells)	Higher initial capital investments	Fed-batch mode
Large-volume tanks possible	Linear upscaling impossible	Continuous mode
Simple design (less maintenance, less risk of defects)	Higher risk of contamination	Perfusion mode
Lower energy requirements (stirring is not required)	Excessive foam formation (inefficient gas-liquid separation)	
Greater mass and heat transfer efficiencies	Large footprint	
Many existing variations on design (bubble column, internal- and external loop airlift reactors)	Impossible to maintain consistent levels of substrate, nutrients and oxygen	

WAVE-AGITATED BIOREACTORS

Advantage	Disadvantage	Applications
Simple design (less maintenance than other designs, fewer defects)	Lower oxygen transfer rate (lower productivity)	Batch mode
Minimal need for cleaning and sterilization	Large production volumes not yet realized	Perfusion mode
Maximum flexibility (production volume, different fermentation batches)	Limited existing variations on design (only batch and perfusion mode)	Low-volume and niche applications (vaccine production, pharmaceuticals)
Allows for growth of sensitive organisms (adherent cultures and suspension cell lines)		
Low shear; low risk of contamination		
Parallel fermentation		
Easy upscaling; easy operation		
Small footprint		

dissolved O_2 monitoring are currently being developed by several parties and will soon be introduced in the markets. Increasing competition and strong growth of the disposable market segment will inevitably drop prices and reduce total production costs. Furthermore, in our market-research interviews with long-time users of Wave systems, we came across several CMOs that positioned the Wave platform as very reliable and predictable. They had, in fact, abandoned in-the-bag monitoring completely, but instead were harvesting at exact time points and performing only routine quality checks on their products.

Combined with the list of advantages for production of biologicals in disposable systems outlined in Table 1 and increasing pressure from regulatory bodies for validation aspects, those facts should be taken into account when considering the substantial investment involved in the setup of production facilities for biological compounds.

In the low-volume market, either for research or small-scale production, the flexible parallel capacity on a small footprint favors investment in a Tsunami bioreactor. Especially in high-containment areas, where laboratory floor space can be extraordinarily expensive, multilayer systems reduce the footprint to a minimum. Cross-contamination of cultures is abolished in disposable systems, and different cell types can be grown in parallel or in sequence with minimal downtime. In fact, operational costs for a Tsunami bioreactor are so low that even in the bulk market for starter cultures in the food and feed segments, this bioreactor will be an economically viable option.

Optimization Procedure

When a new cell line or clone is taken into production, often an extensive development program is required to optimize growth, expression and upscaling. Table 2 compares two types of production facility. In this example, the time to prepare for full-scale production in stirred fermentors is 111 days. The scale-up time can be reduced, but that will consequently require considerable additional investment in parallel systems.

A single Tsunami run allows a matrix of 64 parallel conditions in 5-L bags. The hydrodynamics in a 5-L bag are identical to those in one 160-L culture bag because of the fixed height and depth, which are the two parameters that influence the physics of the induced wave. From a 64-bag matrix, the optimal condition is chosen and can be used directly for the 160-L bag. A single confirmation run is included before production runs are started, reducing the optimization procedure from 111 to 16 days. Also time and money can be saved during full-scale production.

For the entire optimization and subsequent production cycle up to a total of 6000 L, the costs are reduced by over 70%, and the run time is down from 241 to 76 days. Media, seed cultures, and DSP were not included in these calculations because different cell types have very different cost effects on those parameters. However, the Tsunami bioreactor example in Table 2 shows a 40-L capacity in a production run free for seed cultures, whereas for the stirred-reactor types, several of the smaller machines would be required to seed the production batches.

Markets and Industries

Direct market opportunities for parallel disposable bioreactors range from low-profile fermentations to high-profile pharmaceutical-compliant cell cultures. The high containment level of disposable bag systems is not only very useful for production of biopharmaceuticals, but also for the production of starter cultures in the food, nonfood (e.g., paper, biorefineries, and biocatalytic conversions), and feed industries. The linear scalable and variable culture volume properties of such bioreactor systems can provide flexible cultures in seed trains for (multiple) large-volume production runs. The risk of contaminated seeds is minimized when using disposable bags together with decreasing the number of handling steps.

New and improved applications due to low shear forces in scalable wave-agitated culture systems involve thread-forming algae and fungi (which can seriously hamper STR mixing propellers), production of shear sensitive viruses, and solid-phase culture of cells attached to microcarrier beads or disks. In addition, we envision cultures of mixed cell types, tissues, organs, and even multicellular organisms such as nematodes, crustaceans, or fish.

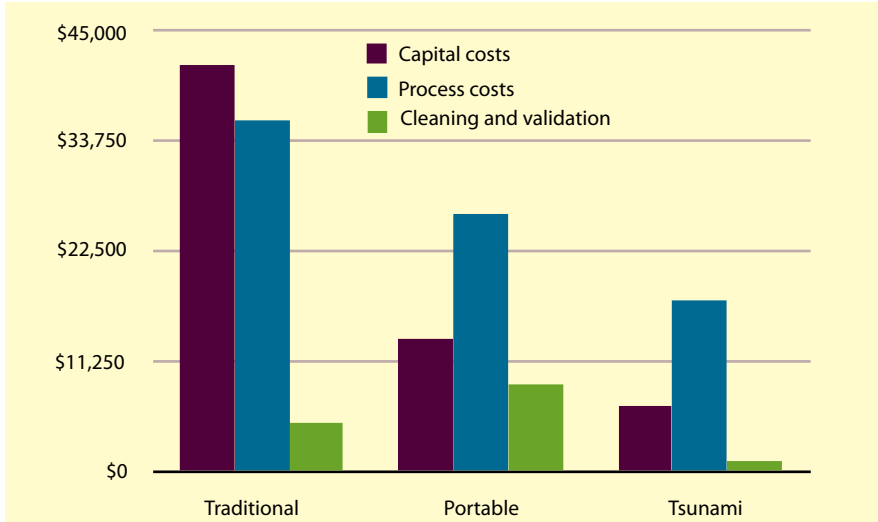

Figure 3: The graph combines both the cost type distribution and lifetime operating costs (200 L scale/yr) for a traditional bioreactor, a portable bioreactor, and the Tsunami bioreactor.

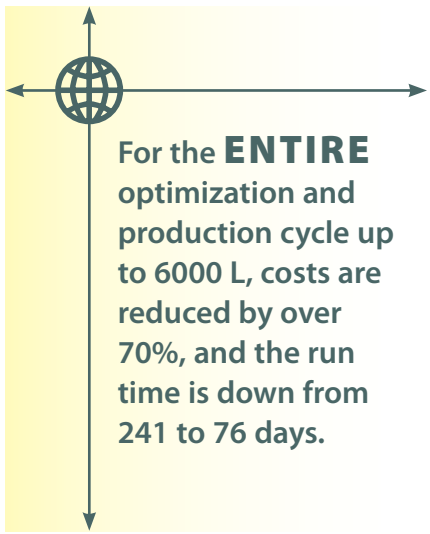

Table 2: Comparison of an optimization and production sequence between two sets of production facilities; (a) standard equipment comprises one stirred multi-bioreactor unit of 6 × 0.5-L, 4 × 20-L stirred bioreactors for upscaling, and one 600-L stirred bioreactor; (b) one standard size Tsunami bioreactor with a capacity that can be scaled between 64 × 5 and 4 × 160 L

	Available Equipment	Standard	Tsunami
Optimization and Scale-Up Time	**Step 1: Optimization with 6 x 0.5 or 60 x 5 L, Respectively**		
	Number of small-volume test runs	10	1
	Setup time for total number of runs	5	1
	Required run time	70	7
	Cleaning time (total)	5	0.5
	Step 2: Scale-up Using 4 x 20 or 4 x 160 L, Respectively		
	Number of test runs	1	1
	Setup time for total number of runs	2	0.4
	Required volume	7	7
	Cleaning time	2	0.1
	Step 3: Scale-up to 600 Resp. 640 L		
	Number of test runs	2	None
	Setup time for total number of runs	2	
	Required volume	14	
	Cleaning time and validation (total)***	4	
	First Production Run Possible After	111 Days	16 Days
Optimization and Scale-Up Costs	Process operators (180 $/day)	19,980	2,880
	Depreciation*	24,329	1,096
	Costs floorspace (300 $/m2)	1,825	92
	Overhead costs	3,996	576
	Disposables	0	1,500
	Total Operating Costs ($)**	$50,129	$6,144
Production Time	**Step 4: Production Runs for a Total of 6000 L**		
	Number of production runs	10	10
	Setup time for total number of runs	10	2
	Required volume	70	70
	Cleaning time and validation (total)***	50	2
	Total Time	130 Days	74 Days
Production Costs	Process operators (180 $/day)	23,400	13,320
	Depreciation*	28,493	5,068
	Costs floorspace (300 $/m2)	2,137	426
	Overhead costs	4,680	2,664
	Disposables	0	3000
	Total Operating Costs ($)**	$58,710	$24,478
	Total Costs (optimization, scale-up, and production)	$108,840	$30,622
	Total Time	241 Days	76 Days

Assumptions: 60 independent runs are required for growth optimization, four and two runs needed for upscaling to 600 L in stirred reactors, growth cycle zeven days. After optimization, the example calculates through for a total production of 6,000 L.

* Ten-year depreciation and floorspace costs are included for the actual rundays only, not for the period equipment is idle.
** Media and DSP costs are not included.
*** Cleaning and validation period includes outcome of sterility testing.

The economics and flexibility of parallel disposable bioreactors will serve different markets, ranging from CMOs to research labs, from starter cultures to vaccine productions, and will provide a next volume level for wave-agitated cell cultures.

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