GOING HORIZONTAL FOR BIOREACTORS AND FERMENTORS

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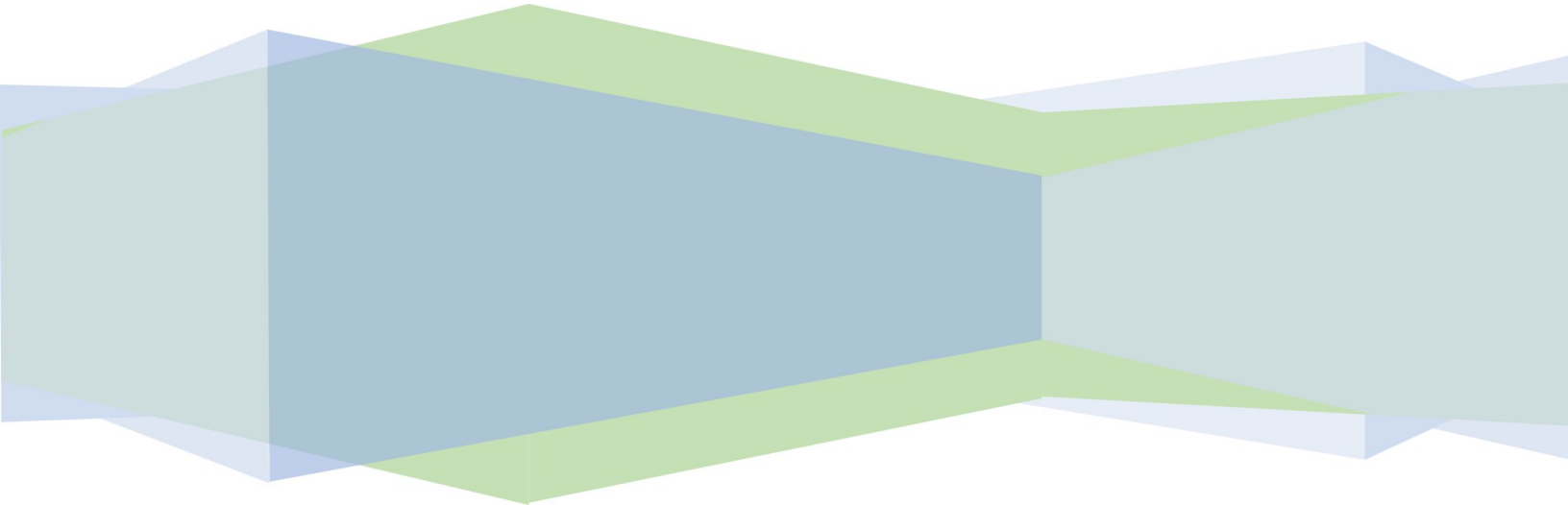
NEW HORIZON BIOTECH



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INTRODUCTION

Vertically oriented vessels have dominated fermentor and bioreactor design for well over 50 years. This is irrespective of stainless steel (SS) vessels or single-use (SU) bags. The single-use bag design in the range of 25 Liter (L) through 2000L has for good reason been an extension of existing SS vertical systems to replicate known geometry. However at large scale this has provided some challenges in the world of microbial fermentors. Let's take a look at the advantages / disadvantages of going to a horizontal form factor!

HISTORICAL PERSPECTIVE ON VERTICAL VESSELS

It has been long said that microbes and to a lesser extent mammalian cells do not care what is the shape the container they are cultivated in. One can readily translate this statement into a thought process that questions vertical tanks knowing that much work has been done to characterize mixing, heat transfer, oxygen transport capabilities, and overall homogeneity.

A very clear case for vertical can be extrapolated from early lab work using glass test tubes and flasks with magnetic stirrer bars. Early small-scale stainless steel (SS) fermentors were built routinely with a 3:1 vessel height to diameter aspect ratio. This results in a tall yet narrow diameter tank. As the bioprocess industry matured through the 80's and 90's, tanks were built with aspect ratios as low as 1:1. Some cell culture bioreactors were manufactured with hemispherical bottom heads rather than shallower dished heads. Most SS microbial fermentors were and still are built with 2:1 aspect ratios. Many engineers and scientists characterize this aspect ratio as being the tank diameter divided by the total inside height of the vessel from bottom to top crown in the dished heads.

But let's go back further in time to the early bench scale fermentors and bioreactors. The vessels were and still are glass, some with double walls for heat transfer purposes and they range in size from 0.5L to 10L working volume. Anything larger makes it difficult to handle and autoclave. The modern-day glass and now in some cases SU plastic vessels are outgrowths of early biostats that date to the 1950's. Following are examples of some of these vertical glass fermentors then and now:

Fig. 1 shows an early Warburg Apparatus circa 1950's by Bronwill Scientific for cultivating microbes with control of temperature and mixing by rocking motion while measuring the liberation or absorption of gases of living cells using manometers. It also served to study enzymatic reactions and metabolism as in a fermentation process. Here is an interesting video that shows a Warburg Apparatus in action:

https://youtu.be/CzCWvHI2qXQ

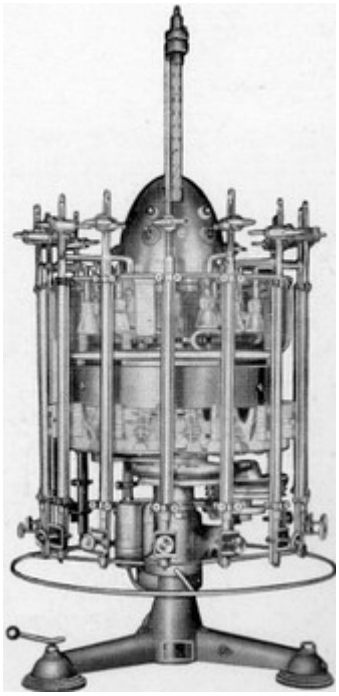

Figure 1: Warburg Apparatus

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The functionality in the Warburg Apparatus and more are still present and necessary in modern day fermentors as shown in Figure 2, a lab scale benchtop glass UniVessel® fermentor.

Figure 2: 5L Biostat Glass Fermentor

MODERN LAB AND PRODUCTION SCALE VERTICAL SYSTEMS

As the Biotechnology industry grew through the 1970's and 80's, the need arose for scaling up the glass benchtop fermentors to produce greater quantities of proteins of interest along with a variety of microbial and cell culture expression systems.

This was driven and still is by the need for biopharm and biotech companies to make sufficient quantities of their therapeutic protein to get through phase I, II, and III clinical trials as part of the path to gaining FDA (Federal Drug Agency) approval for a BLA or NDA (Biologic License Application or New Drug Approval). The quickest path forward for vertical systems was to scale-up the small-scale R&D systems using vertical ASME (American Society of Mechanical Engineers) pressure rated vessels.

These systems generally have either a bottom mounted or top mounted agitation system with a single vertical shaft that includes one or more mixing impellers of varying geometry. The vessels were and still are pressure rated for two reasons.

First, to accommodate operation under pressure to drive oxygen mass transfer for optimization of cell growth. Second, to permit the SS vessels to undergo SIP (Steam-In-Place) to prepare and sterilize the batch for receiving the host cell strain inoculum with no risk of contamination by adventitious organisms. It should be noted that these SS vessels are also commonly designed for CIP (Clean-In-Place) whereby all product contact surfaces can be chemically cleaned to remove residue in preparation of receiving a subsequent batch.

Figure 3: 1000L Fermentor System

Figure 3 shows a contemporary 1000L working volume vertical fermentor having a bottom center-mounted agitator drive. The system is complete with all support piping and fully functional control system.

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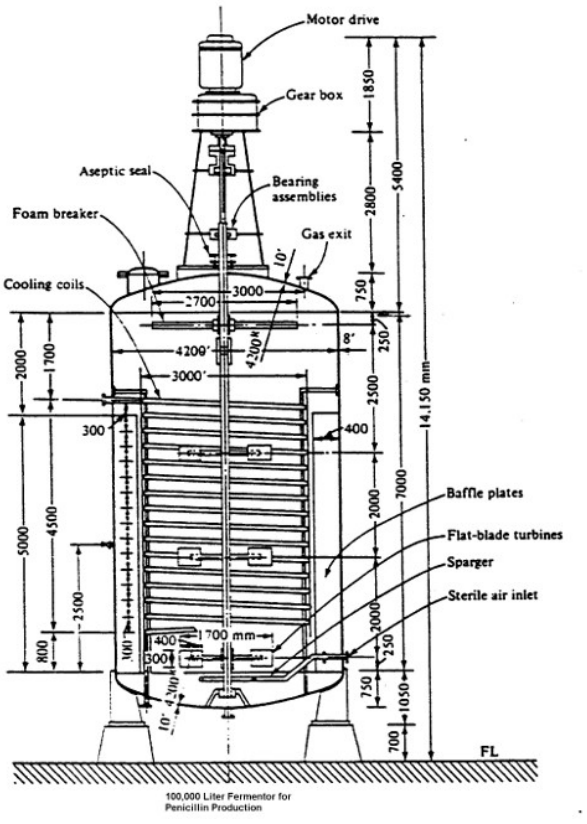

Figure 4: 100,000L Fermentor

Figure 4 is a cross-section sketch of a 100,000L working volume fermentor used in penicillin production to show the contrast and scope of fermentors in service. Note it has a top center-line agitator drive.

Generally, when speaking about large scale vessels, we can think about tanks or bags that have a working volume of 500L or greater. Suffice it to say, the larger the bioprocess vessel, the greater the need for elevated operating platforms, OSHA stairways and railing systems as well as overhead cranes to handle assembly and disassembly of various components.

Large scale vertical tanks also need operator access around the top head as well as at the lower-side instrument probe belt. Thus, operator safety going up and down steps is a concern as well as a productivity inhibitor. Keep in mind that operators in biologic production areas are gowned to varying degrees often making going up and down steps difficult, even fatiguing.

As the need for installed capacity grew in the 1990's and 2000's many large-scale bioprocess systems up to 25,000 Liters were installed in new facilities. These large-scale production systems all needed a seed train consisted of various sized vertically-oriented systems in the 500L to 3000L range. It is interesting to note that moving into 2010 and beyond, biologic yields from cell culture has achieved ever greater titers of protein and mammalian cell densities.

This new growth paradigm has meant the need for bioreactors greater than 10,000L has waned. The advent of SU systems trending towards use of multiple 1000L or 2000L bags in production will produce the protein quantities that once needed one or more 20,000L SS bioreactors.

The need for microbial fermented products can also be handled with multiple SU fermentor systems with the exception that certain limiting factors have not allowed vertical SU systems to become mainstream. These limiting factors include lack of pressure to support OTR (Oxygen Transfer Rate) driving force, limited power density input due to a single agitator drive, lack of necessary heat transfer surface area to remove metabolic heat, and lack of flexibility in adjusting production train size.

WHY CONSIDER HORIZONTAL FORM FACTOR

Consider the antiquated iron lung machine that kept Polio patients alive while thinking about horizontal form factor. These have all but been replaced by portable respirators that give patients more mobility. So, this analogy although imperfect serves to point out that horizontal systems can more readily fit into typical clean rooms with 9 ft. or 10 ft. ceiling height whereas vertically-oriented vessels of the same volume cannot.

Also, the modern concept of mobility has brought many advantages to enhance patient productivity. These same considerations apply to horizontal bioprocess vessel systems for culturing living microorganisms and mammalian cells. Microbes/animal cells do not care that they are mixing, respiring, doubling, and metabolizing product in a horizontal tank. In fact, a horizontal system that is modular and can scale up in tank or

bag diameter offers tremendous flexibility for biologic drugs makers.

The first and most obvious benefit is that a 3000L system can fit and operate quite nicely in a modular clean room with ceiling height, say, 9-10 ft. ceiling height. The horizontal form factor eliminates the need for fixed stairways, platforms, railings, or overhead cranes. In addition, multiple 3000L systems can be arranged side by side to achieve production capacities of 6000L to 12,000L or larger.

Using smaller horizontal seed trains for these large scale production systems, space utilization can be optimized within the same modular bioprocess suite. Now, like the iron lung doing the respiration, horizontal single use bags can be fully contained in a Bag Retention Vessel (BRV) with the major benefit of allowing the bag to operate under pressure, say 5 psig. For microbial systems, this brings about a step increase in driving force to increase oxygen mass transfer capability to improve process performance. Bag pressurization continues to be a limitation in current SU vertical systems.

Horizontal systems lend themselves nicely to modularization such that the system capacity for a fixed diameter vessel can be increased 2 or 3 fold (and reduced as well) by adding or removing intermediate BRV modules as show in Figure 5.

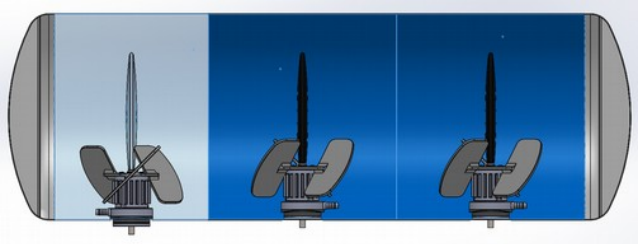

Figure 5: Three Module 3000L Bioreactor

Horizontal modular systems easily accommodate multiple simple agitators. The benefit of having multiple agitators, one per each module, is to keep power density constant on scale-up. Geometric similarity on scaling up to larger diameter bags also hold true in that power density in kW/Liter at 50L is the same as for a 3000L system. This assures equivalent cell densities and product formation rates in the large-scale production units as compared to smaller scale seed units.

The ability to achieve an operating range with a single horizontal train of 50L to 3000L operation in SU bags is currently not available with existing SU bioreactors and fermentors on the market. With various combinations of modular units in the train, multiple 3000L horizontal units can be installed side by side to increase total working volume to greater than 10,000 Liters and still fit the large scale equipment in a 10 ft ceiling height clean room.

To the extent possible, the base systems and expansion module mobility allows equipment to be rolled into and out of the production suite when different product production campaigns require different configurations.

With this in mind, it becomes apparent that the base system is fully equipped with process automation to facilitate operation of a one-, two-, or three-module system. A simple selection on the process control HMI (Human Machine Interface) will indicate which arrangement is active.

In a similar manner, all ports into and out of the system remain on the base unit along with all utility connections. Thus, the expansion modules serve only to increase capacity. They have quick connections for cooling water to the jackets and electrical power supply plugs for the agitators to tie them into the base system control cabinet. This methodology allows for quick turn-around on capacity change outs as well as improved operational efficiency.

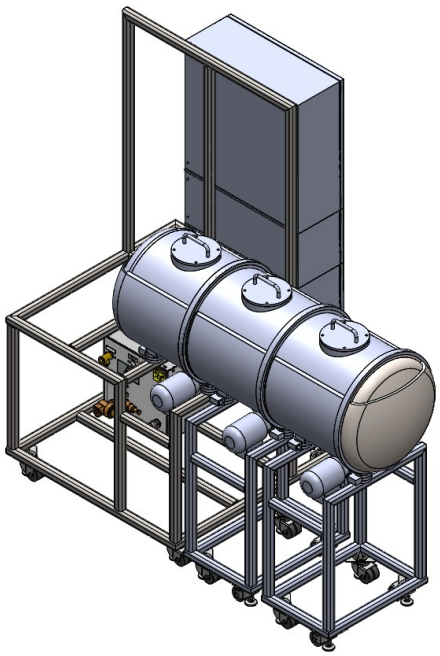

Figure 6: 150L Three Module Fermentor

Figure 6 shows a 50L base system with two expansion modules to increase the capacity to 150L.

OTHER BENEFITS

So....what other somewhat intangible benefits are available with a horizontal system? Well, in microbial fermentors and to a lesser extent in cell culture bioreactors, generation of foam can become problematic.

This is particularly true if the antifoam addition system cannot prevent foam from reaching and going out the exhaust path, fouling the sterile exhaust gas filter. Based on pure geometry, a horizontal tank can have approximately 70% more freeboard liquid surface area compared to an equivalent vertical system of the same capacity.

This means that the sparge gas escape velocity at the boundary of the aerated and agitated liquid surface will be substantially lower. This is particularly true on SU microbial fermentors where the total sparge gas flow can be as high as 2 VVM (Vessel Volumes per Minute). The higher the gas escape velocity, the greater the formation of liquid fines and solid particles that will need to settle and disengage hopefully before going out the exhaust line.

Also at the point in the batch where maximum foaming is encountered, a lower escape velocity will give higher residence time of the foam until antifoam addition can successfully lower the foam level, based on foam sensor placement. A lower volume of foaming and fewer aerosols going out the exhaust also means there will be less liquid volume loss from the batch.

Of course, exhaust condensers can substantially control loss of batch liquid by refluxing broth back into the bag. Looking at overall mixing and oxygen transfer capability, a larger liquid surface area will also permit more gas flow into the batch without being limited by the superficial gas velocity constraints typically imposed on biological processes (relevant to microbial fermentors only).

In vertical systems, if superficial gas velocity is too high, the bottom turbine impeller can be rendered less effective due to a “flooding” of the impeller with high flow rate sparge gases. This occurrence can drastically affect the ability of the agitator to keep the power density high during the latter stages of the batch process.

Another important limitation of current large-scale vertical SU fermentors is the relatively small HTS (Heat Transfer Surface) area which along with the poor thermal heat transfer characteristics introduced by the bioreactor bags, can prevent adequate temperature control throughout the entire process duration.

Again, based solely on geometry, a horizontal tank can have nearly 70% more HTS area compared to an equivalent vertical system of the same volume. This is predicated on a horizontal system with internal baffles that provide additional HTS for cooling the batch having circulating cooling fluid in each baffles jacket cavity. Proper removal of metabolic heat in SU microbial fermentors allows larger scale (3000L) systems to achieve full cell density and product formation rates.

Oh, did I mention that your operators will be forever thankful for not having to trudge up and down stairways and platform to a second or third level in their production suite. The implication is that working in a single level production suite will improve operator safety and productivity.

CONCLUSION

It is not, at all, a stretch of the imagination to realize that horizontal systems can be the wave of the future in bioprocessing, particularly for SU bag-based systems. Bringing about a major paradigm shift from vertical to horizontal will come about as early adopters realize the full potential of building facilities, that cost less, are easier to operate, and can be put into service faster and with greater flexibility.

With time to market always a business driver in making decisions regarding new facilities, there will be no doubt that the horizontal form factor will eventually win out over the current vertical infatuation that has been the mainstay of the bioprocess industry for decades!

ABOUT THE AUTHOR

Ernest L. Stadler, P.E. is Founder and CEO of New Horizon Biotech, Inc., a start-up focused on innovative single use microbial fermentation and cell culture bioreactor technology.

Mr. Stadler has broad based expertise in automation, process and mechanical design for a wide range of Bioprocess equipment having served the biotechnology industry for over 30 years.

Photo Credit – Figure 2 shows a Sartorius UniVessel® glass benchtop fermentor.