An Overview of Fermenter and the Design Considerations to Enhance Its Productivity

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The function of the fermenter or bioreactor is to provide a suitable environment in which an organism can efficiently produce a target product—the target product might be cell biomass, metabolite and bioconversion Product. It must be so designed that it is able to provide the optimum environments or conditions that will allow supporting the growth of the microorganisms. The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production.

The choice of microorganisms is diverse to be used in the fermentation studies. Bacteria, Unicellular fungi, Virus, Algal cells have all been cultivated in fermenters. Now more and more attempts are tried to cultivate single plant and animal cells in fermenters. It is very important for us to know the physical and physiological characteristics of the type of cells which we use in the fermentation.

Before designing the vessel, the fermentation vessel must fulfill certain requirements that is needed that will ensure the fermentation process will occur efficiently. Some of the actuated parameters are: the agitation speed, the aeration rate, the heating intensity or cooling rate, and the nutrients feeding rate, acid or base valve. Precise environmental control is of considerable interest in fermentations since oscillations may lower the system efficiency, increase the plasmid instability and produce undesirable end products. This review gives the complete information about fermenter and parameters to be considered to enhance its productivity.

Key words: Bioreactor, fermenter, impeller, sparger, and aspect ratio.

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1. Introduction: -

Fermentation technology: -

Fermentation Technology could be defined simply as the study of the fermentation process, techniques and its application. Fermentation should not be seen merely as a process that is entirely focused on the happenings occurring in the fermenter alone! There are many activities that occur upstream leading to the reactions that occur within the bioreactor or fermenter, despite the fermenter is regarded as the heart of the fermentation process.

Fermentation technology is the whole field of study which involves studying, controlling and optimization of the fermentation process right up from upstream activities, mid stream and downstream or post fermentation activities.

The study of fermentation technology requires essential inputs from various disciplines such as biochemistry, microbiology, genetics, chemical and bioprocess engineering and even a scatter of mathematics and physics.

Fermentation in terms of biochemistry and physiology:-

Fermentation is now defined as a process of energy generation by various organisms especially microorganisms. The fermentation process showed unique characteristics by which it generates energy in the absence of oxygen. The process of energy generation utilizes the use of substrate level phosphorylation (SLP) which do not involved the use of electron transport chain and free oxygen as the terminal electron acceptor.

Engineers definition of fermentation:-

It is only up to recently with the rise of industrial microbiology and biotechnology that the definition of fermentation took a less specific meaning. Fermentation is defined more from the point of view of engineers. They see fermentation as the cultivation of high amount of microorganisms and biotransformation being carried out in special vessels called fermenter or bioreactors.

Their definitions make no attempt to differentiate whether the process is aerobic or anaerobic. Neither are they bothered whether it involves microorganisms or single animal or plant cells.

They view bioreactors as a vessel which is designed and built to support high concentration of cells.

Bioreactor: -

Also known as **Fermenter.**

A bioreactor is a specially designed vessel which is built to support the growth of high concentration of microorganisms. It must be so designed that it is able to provide the optimum environments or conditions that will allow supporting the growth of the microorganisms.

Bioreactors are commonly cylindrical vessels with hemispherical top and/or bottom, ranging in size from some liter to cube meters, and are often made of stainless steel and glass.

The difference between a bioreactor and a typical composting system is that more parameters of the composting process can be measured and controlled in bioreactors.

The sizes of the bioreactor can vary over several orders of magnitudes. The microbial cell (few mm^3), shake flask (100-1000 ml), laboratory fermenter (1 – 50 L), pilot scale (0.3 – 10m^3) to plant scale (2 – 500 m^3) are all examples of bioreactors.

The design and mode of operation of a fermenter mainly depends on the production organism, the optimal operating condition required for target product formation, product value and scale of production. The design also takes into consideration the capital investment and running cost.

- Large volume and low value products like alcoholic beverages need simple fermenter and do not need aseptic condition.
- High value and low volume products require more elaborate system of operation and aseptic condition.

Bioreactors differ from conventional chemical reactors in that they support and control biological entities. As such, bioreactor systems must be designed to provide a higher degree of control over process upsets and contaminations, since the organisms are more sensitive and less stable than Chemicals. Biological organisms, by their nature, will mutate, which may alter the biochemistry of the bioreaction or the physical properties of the organism. Analogous to heterogeneous catalysis, deactivation or mortality occur and promoters or coenzymes influence the kinetics of the bioreaction. Although the majority of fundamental bioreactor engineering and design issues are similar, maintaining the desired biological activity and eliminating or minimizing undesired activities often presents a greater challenge than traditional chemical reactors typically require.

Other key differences between chemical reactors and bioreactors are selectivity and rate. In bioreactors, higher selectivity — that is, the measure of the system's capability for producing the preferred product (over other outcomes) — is of primary importance. In fact, selectivity is especially important in the production of relatively complex molecules such as antibiotics, steroids, vitamins, proteins and certain sugars and organic acids. Frequently, the activity and desired selectivity occur in a substantially smaller range of conditions than are present in

conventional chemical reactors. Further, deactivation of the biomass often poses more severe consequences than a chemical upset.

The Designing of a Bioreactor also has to take into Considerations the Unique Aspects of Biological Processes

a. The concentrations of starting materials (substrates) and products in the reaction mixture are frequently low; both the substrates and the products may inhibit the process. Cell growth, the structure of intracellular enzymes, and product formation depend on the nutritional needs of the cell (salts, oxygen) and on the maintenance of optimum biological conditions (temperature, concentration of reactants, and pH) within narrow limits.
b. Certain substances inhibitors effectors, precursors, metabolic products influence the rate and the mechanism of the reactions and intracellular regulation.
c. Microorganisms can metabolize unconventional or even contaminated raw materials (cellulose, molasses, mineral oil, starch, wastewater, exhaust air, biogenic waste), a process which is frequently carried out in highly viscous, non-Newtonian media.
d. In contrast to isolated enzymes or chemical catalysts, microorganisms adapt the structure and activity of their enzymes to the process conditions, whereby selectivity and productivity can change. Mutations of the microorganisms can occur under sub optimal biological conditions.
e. Microorganisms are frequently sensitive to strong shear stress and to thermal and chemical influences.
f. Reactions generally occur in gas-liquid -solid systems, the liquid phase usually being aqueous.
g. The microbial mass can increase as biochemical conversion progresses. Effects such as growth on the walls, flocculation, or autolysis of microorganisms can occur during the reaction.
h. Continuous bioreactors often exhibit complicated dynamic behaviour.

Requirements of Bioreactors

Due to above mentioned demands made by biological systems on their environment, there is no universal bioreactor. However, the general requirements of the bioreactor are as follows:

1. The vessel should be robust and strong enough to withstand the various treatments required such as exposure to high heat, pressure and strong chemicals and washings and cleanings.
2. The vessel should be able to be sterilized and to maintain stringent aseptic conditions over long periods of the actual fermentation process.
3. The vessel should be equipped with stirrers or mixers to ensure mass transfer processes occur efficiently.
4. It should have sensors to monitor and control the fermentation process.
5. It should be provided with inoculation point for aseptic transfer in inoculum.
6. Sampling valve for withdrawing a sample for different tests.

7. Baffles should be provided in case of stirred fermenter to prevent vertex formation.
8. It should be provided with facility for intermittent addition of an antifoam agent.
9. In case of aerobic submerged fermentation, the tank should be equipped with the aerating device.
10. Provision for controlling temperature and pH of fermentation medium.
11. Man hole should be provided at the top for access inside the fermenter for different purposes.

It is obvious that the design of the fermenter will involve co-operation between experts in microbiology, biochemistry, chemical engineering, mechanical engineering and costing.

Fermenter Design

What should be the basic points of consideration while designing a fermenter?

- Productivity and yield
- Fermenter operability and reliability
- Product purification
- Water management
- Energy requirements
- Waste treatment

Few Significant things of concern that should be taken into account while designing a fermenter:

- Design in features so that process control will be possible over reasonable ranges of process variables.
- Operation should be reliable
- Operation should be contamination free.
- Traditional design is open cylindrical or rectangular vessels made from wood or stone.
- Most fermentation is now performed in close system to avoid contamination.
- Since the fermenter has to withstand repeated sterilization and cleaning, it should be constructed from non-toxic, corrosion-resistant materials.
- Small fermentation vessels of a few liters capacity are constructed from glass and/or stainless steel.
- Pilot scale and many production vessels are normally made of stainless steel with polished internal surfaces.
- Very large fermenter is often constructed from mild steel lined with glass or plastic, in order to reduce the cost.
- If aseptic operation is required, all associated pipelines transporting air, inoculum and nutrients for the fermentation need to be sterilizable, usually by steam.

- Most vessel cleaning operations are now automated using spray jets, which are located within the vessels. They efficiently disperse cleaning fluids and this cleaning mechanism is referred to as cleaning-in-place CIP.
- Associated pipe work must also be designed to reduce the risk of microbial contamination. There should be no horizontal pipes or unnecessary joints and dead stagnant spaces where material can accumulate; otherwise this may lead to ineffective sterilization. Overlapping joints are unacceptable and flanged connections should be avoided as vibration and thermal expansion can result in loosening of the joints to allow ingress of microbial contaminants. Butt welded joints with polished inner surfaces are preferred.
- Normally, fermenters up to 1000 L capacity have an external jacket, and larger vessels have internal coils. Both provide a mechanism for vessel sterilization and temperature control during the fermentation.
- Other features that must be incorporated are pressure gauges and safety pressure valves, which are required during sterilization and operation. The safety valves prevent excess pressurization, thus reducing potential safety risks. They are usually in the form of a metal foil disc held in a holder set into the wall of the fermenter. These discs burst at a specified pressure and present a much lower contamination risk than spring-loaded valves.
- For transfer of media pumps are used. However pumps should be avoided if aseptic operation is required, as they can be a major source of contamination. Centrifugal pumps may be used, but their seals are potential routes for contamination. These pumps generate high shear forces and are not suitable for pumping suspensions of shear sensitive cells. Other pumps used include magnetically coupled, jet and peristaltic pumps.
- Alternate methods of liquid transfer are gravity feeding or vessel pressurization.
- In fermentations operating at high temperatures or containing volatile compounds, a sterilizable condenser may be required to prevent evaporation loss. For safety reasons, it is particularly important to contain any aerosols generated within the fermenter by filter-sterilizing the exhaust gases.
- Also, fermenters are often operated under positive pressure to prevent entry of contaminants.

2. Considerations that improve productivity of bioreactor: -

2.1 Material of construction: -

2.1.1 Laboratory scale bioreactor: -

In fermentation with strict aseptic requirements it is important to select materials that can withstand repeated sterilization cycles. On a small scale, it is possible to use glass and/or stainless steel.

Glass is useful because it gives smooth surfaces, is non-toxic, corrosion proof and it is usually easy to examine the interior of vessel. The glass should be 100% borosilicate, e.g. Pyrex® and Kimax®.

The following variants of the laboratory bioreactor can be made:

1. Glass bioreactor (without the jacket) with an upper stainless steel lid.
2. Glass bioreactor (with the jacket) with an upper stainless steel lid.
3. Glass bioreactor (without the jacket) with the upper and lower stainless steel lids.
4. Two-part bioreactor - glass/stainless steel.

   The stainless steel part has a jacket and ports for electrodes installation.

5. Stainless steel bioreactor with peepholes.

Vessels with two stainless steel plates cost approximately 50% more than those with just a top plate.

2.1.2 Pilot scale and large scale bioreactors: -

When all bioreactors are sterilized in situ, any materials use will have to assess on their ability to withstand pressure sterilization and corrosion and their potential toxicity and cost.

Pilot scale and large scale vessels are normally constructed of stainless steel or at least have a stainless steel cladding to limit corrosion. The American Iron and Steel Institute (AISI) states that steels containing less than 4% chromium are classified as steel alloys and those containing more than 4% are classified as stainless steel. Mild steel coated with glass or phenolic epoxy materials has occasionally been used. Wood, concrete and plastic have been used when contamination was not a problem in a process.

Although stainless steel is often quoted as the only satisfactory material, it has been reported that mild-steel vessels were very satisfactory after 12 years use for penicillin fermentations (Walker and Holdsworth, 1958) and mild steel clad with stainless steel has been used for at least 25 years for acetone-butanol production (Spivey, 1978).

The corrosion resistance of stainless steel is thought to depend on the existence of a thin hydrous oxide film on the surface of metal. The composition of this film varies with different steel alloys and different manufacturing process treatment. The film is stabilized by chromium and is considered to be continuous, non-porous, insoluble and self healing. If damaged, the film will repair itself when exposed to air or an oxidizing agent (cubberly et al., 1980).

The minimum amount of chromium needed to resist corrosion will depend on the corroding agent in a particular environment, such as acid, alkalis, gases, soil, salt or fresh water. Increasing the chromium concentration enhances the resistance to corrosion, but only grades of steel containing at least 10 to 13% chromium develop the effective film. The inclusion of nickel in high percent chromium steels enhances resistance and improves their engineering properties. The presence of molybdenum improves the resistance of stainless steels to solution of halogens salts and pitting by chloride ions in brine or sea water. Corrosion resistance can also be improved by tungsten, silicon and other elements.

AISI grade 316 steels which contains 18% chromium, 10% nickel, 2-2.5% molybdenum are now commonly use for fermenter or bioreactor construction.

In citric acid fermentation where pH may be 1 to 2, it will be necessary to use a stainless steel with 3-4% molybdenum (AISI grade 317) to prevent leaching of heavy metals from the steel which would interfere with the fermentation. AISI grade 304, which contains 18.5% chromium and 10% nickel, is used extensively for brewing equipment. Now also Stainless steels (e.g..: 1.4435, 1.4539, etc.), Hastelloy, Incolloy, Inconel, Monel, Titanium grades 1, 2, 7, 11 are used in construction of bioreactor.

With plant and animal cell tissue culture, a low-carbon version (type 316L) is often used.

The thickness of the construction material will increase with scale. At 300,000 to 400,000 dm^3 capacity, 7-mm plate may be used for the side of the vessel and 10-mm plate for the top and bottom, which should be hemispherical to withstand pressure.

It is also important to consider the ways in which a reliable aseptic seal is made between glass and glass, glass and metal, metal and metal joints such as between a fermenter vessel and a detachable top or base plate. With glass and metal a seal can be made with a compressible gasket, a lip seal or an ‘O’ ring. With metal to metal joints only ‘O’ ring is suitable.

A single ‘O’ seal is adequate for GILSP and levels 1 and B2; a double ‘O’ ring seal is required for levels 2 and b3; a double ‘O’ ring seal with steam between the seals (steam tracing) is necessary for levels 3 and B4 (Chapman 1989; Hambleton et al., 1991).

2.2 Vessel shape: -

Typical tanks are vertical cylinders with specialized top plates and bottom plates.In some cases, vessel design eliminates the need for a stirrer system especially in air lift fermenter. A tall, thin vessel is the best shape with aspect ratio (height to diameter ratio) around 10:1. Sometimes a conical section is used in the top part of the vessel to give the widest possible area for gas exchange.

2.2.1 Stainless steel top plates.

The top plates are of an elliptical or spherical dish shape. The top plates can be either removable or welded. A removable top plate provides best accessibility, but adds to cost and complexity. Various ports and standard nozzles are provided on the stainless plate for actuators and probes. These include pH, thermocouple, and dissolved oxygen probes ports, defaming, acid and base ports, inoculum port, pipe for sparging process air, agitator shaft and spare ports.

2.2.2 Bottom plates.

Tank bottom plates are also customized for specific applications. Almost most of the large vessels have a dish bottom, while the smaller vessels are often conical in shape or may have a smaller, sump type chamber located at the base of the main tank. These alternate bottom shapes aid in fluid management when the volume in the tank is low. One report states that a dish bottom requires less power than a flat one.

In all cases, it is imperative that tank should be fully drainable to recover product and to aid in cleaning of the vessel. Often this is accomplished by using a tank bottom valve positioned to eliminate any "dead section" that could arises from drain lines and to assure that all content will be removed from the tank upon draining.

If the bioreactor has a lower cover, then the following ports and elements should be placed and fastened there:

1. Discharge valve;
2. Sampling device;
3. Sparger;
4. Mixer's lower drive;
5. Heaters.

2.2.3 Height-to-diameter ratio (Aspect ratio).

The height-to-diameter ratio is also a critical factor in vessel design. Although a symmetrical vessel maximizes the volume per material used and results in a height-to-diameter ratio of one, most vessels are designed with higher ratio. The range of 2-3:1 is more appropriate and in some situation, where stratification of the tank content is not an issue or a mixer is used, will allow still higher ratio to be used in design.

The vessels for microbiological work should have an aspect ratio of 2.5-3:1, while vessels for animal cell culture tend to have an aspect ratio closer to 1. The basic configuration of stirred tank

bioreactors for mammalian cell culture is similar to that of microbial fermenter but the major difference is there in aspect ratio, which is usually smaller in mammalian cell culture bioreactor.

In stirred tank bioreactor (STR), height to diameter aspect ratio is 3:1 or 4:1 while in the case of CSTR, the aspect ratio is maintained more than 1, to ensure high residence time of gas phase, increase the transfer efficiency and to ensure less power input on introduction of gas, uniform power dissipation. In miniature bioreactor, aspect ratio is kept equal to large bioreactor in order to predict hydrostatic pressure and therefore oxygen solubility at the different scale of operation.

The tower fermenter is an elongated non-mechanically stirred fermenter with aspect ratio of at least 6:1 for tubular section or 10:1 overall, through which there is unidirectional flow of gases. The tower fermenter used for citric acid production on a laboratory scale having height: diameter ratio of 16:1. Cylindro-conical vessels used for the brewing of lagers and beers having aspect ratio usually 3:1,with fermenter heights around 10 to 20 m.

Characteristics of laboratory and pilot scale vessels

Scale (nominal volume)	Working tank volume, VL (l)	Installed motor power, Po (hp)	Max. agitator speed, Nmax (rev/min)	Vessel tangent/tangent, HTT (m)	Vessel total height (with top and bottom dish), HT (m)	Vessel width, OD, DT (m)	Max. air flow rate, Qmax (l/min)
30 l	20	1	875	0.66	0.742	0.31	30
100 l	75	3	400	0.785	0.96	0.41 (ID)	120
280 l	180	7.5	460	1.12	1.34	0.56	300
800 l	600	7.5	330	1.63	2.08	0.81	600
1000 l	750	10	300	1.52	1.88	0.86 (ID)	1200
1200 l	900	15	282	1.83	2.185	0.92	1200

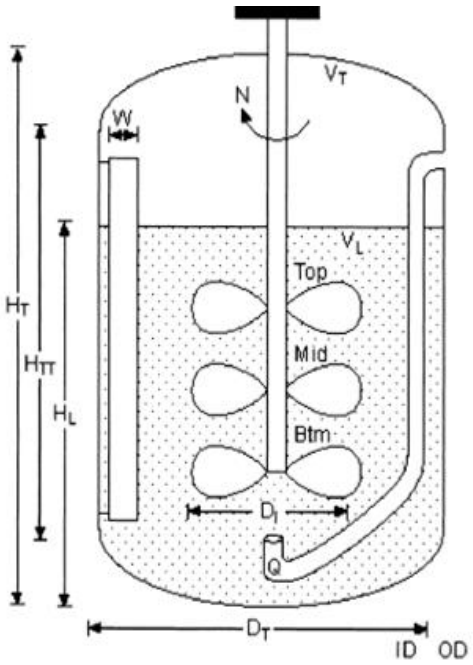
The 280 l scale fermenter was originally designed with a 3 hp motor which was enlarged to 7.5 hp for replacement convenience. OD, Outer diameter; ID, inner diameter

Geometric comparisons for laboratory and pilot scale fermenters

Scale (nominal volume)	HT/DT (HTT/DT)	DI/DT	DT/(VL)1/3 at VL (l), geometric similarity	Re ×106 (water)	VL/VT
30 l	2.4 (2.1)	0.33 (R)	1.14 at 20	0.48 (R)	0.57
100 l	2.4 (1.9)	0.45 (A)	0.97 at 75	0.71 (A)	0.75
280 l	2.4 (2.0)	0.36 (R) 0.5 (M)	0.99 at 180	1.0 (R) 1.9 (M)	0.60
800 l	2.6 (2.0)	0.38 (R) 0.5 (M) 0.5 (A)	0.96 at 600	1.6 (R) 2.9 (M) 2.9 (A)	0.704
1000 l	2.1 (1.7)	0.35 (R) 0.43 (A)	0.95 at 750	1.5 (R) 2.2 (A)	0.75

VT is total tank capacity not nominal volume. R, Rushton; M, Maxflo T; A, A315; Btm, bottom impeller.

The geometrical term of the bioreactors are shown in the figure on the next page.



Schematic of fermentor showing nomenclature.

2.3. Agitation: -

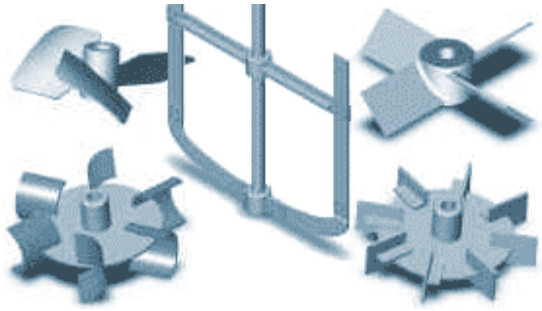
2.3.1 The agitator (impeller).

The agitator is required to achieve a number of mixing objective.

- Bulk fluid and gas-phase mixing,
- Air dispersion,
- Oxygen transfer,
- Heat transfer,
- Suspension of solid particles and maintain a uniform environment throughout the vessel contents.
- Enhancement of mass transfer between dispersed phases.

Bulk mixing and micro mixing both are influenced strongly by impeller type, broth rheology, and tank geometry and internals.

Impellers used bioreactors are:



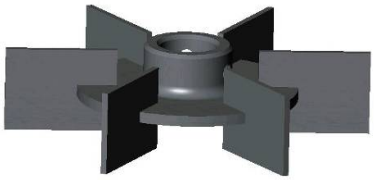
Rushton disc turbines, vaned discs, open turbines of variable pitch and propeller. The disc turbine consists of a disc with a series of rectangular vanes set in vertical plane around the circumstances and vaned disc has a series of rectangular vanes attached vertically to the underside. Air from the sparger hits the underside of the disc and is displaced towards the vanes where the air bubbles are broken up into smaller bubbles. The vanes of variable pitch open turbine and the blade of marine impellers are attached directly to a boss on the agitator shaft. In this case air bubbles do not initially hit any surface before dispersion by the vanes or blades.

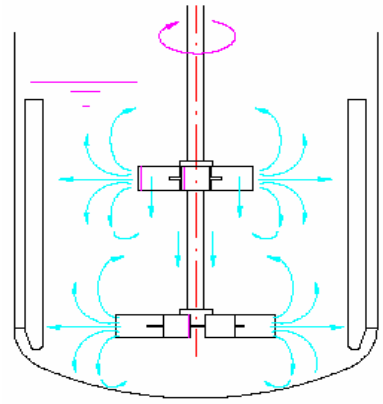
The Rushton disc turbine is the one used most often for highly aerobic fermentations, because it has among the highest power draws of any the commercially available impellers, and it is better characterized than others; hence, its behaviour is easier to predict. Rushton disc turbine of one third of the fermenter diameter has been considered the optimum design for use in fermentation processes. Disc turbine is most suitable in a fermenter since it can break up a fast air stream without itself becoming flooded in air bubbles. A marine propeller is an axial flow impeller which provides good top-to-bottom mixing. It is low power device does not provide large oxygen-transfer rates.

The propeller and the open turbine flood when superficial velocity (Vs) exceeds 21 m h^{-1} , whereas the flat blade turbine can tolerate Vs of 120 m h^{-1} before being flooded, when two sets are used on the same shaft. Besides this, propeller is also less efficient in breaking up the bubbles and the flow it produces is axial rather than the radial. One of major drawback of Rushton disc turbine is that it provides very axial flow, resulting in poor overall top-to-bottom mixing. In addition, agitation intensity decrease with distance from the impeller, and this decrease can become more pronounced for viscous, pseudoplastic broths.

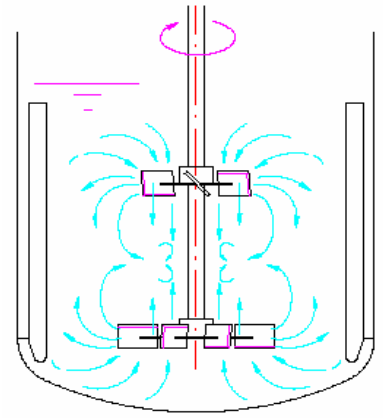
2.3.2 Various impellers use in bioreactors with their flow patterns.

Flat blade disk turbine

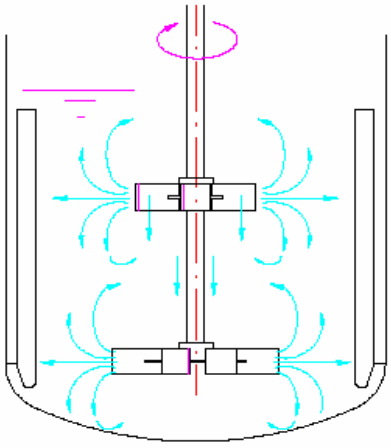




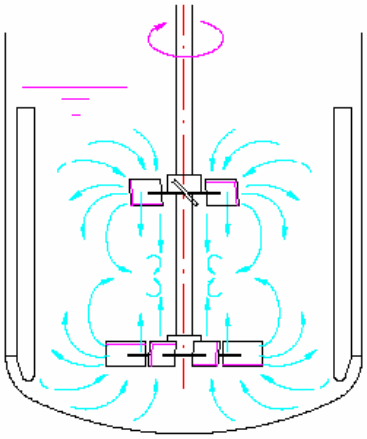
45° Flat blade disk turbine



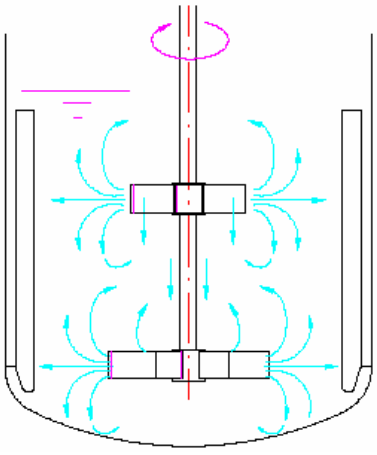
Curved blade disk turbine



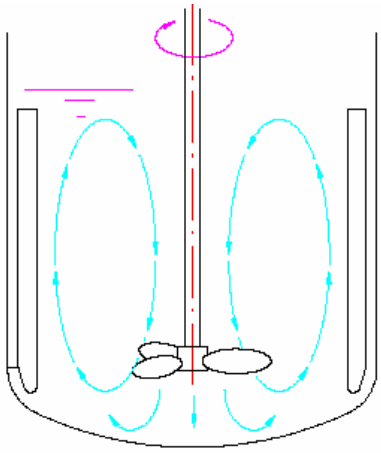
Pitched blade turbine



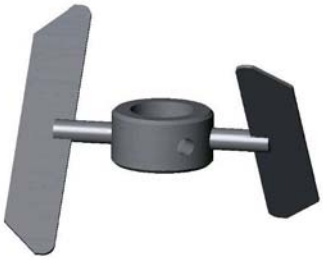
curved blade turbine

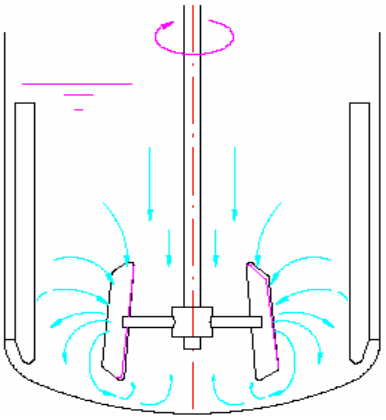


Marine propeller



Large pitch blade impeller





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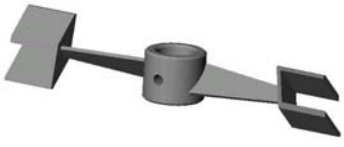
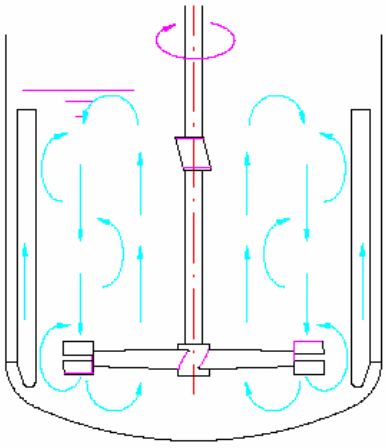
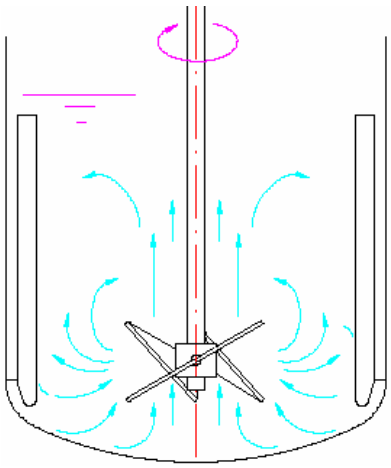
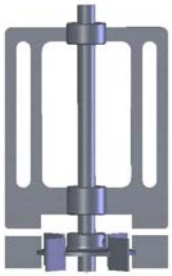


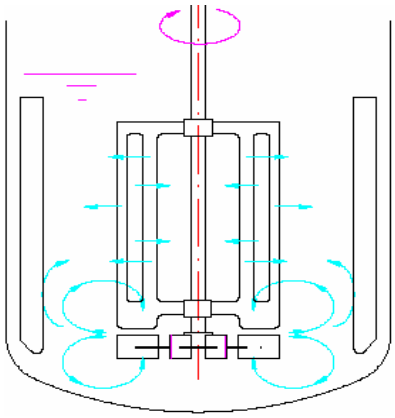


3 segment blade impeller

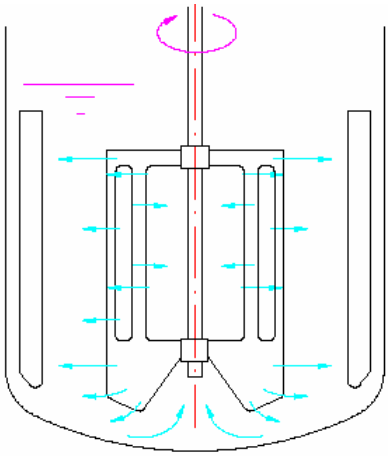


Gate with turbine

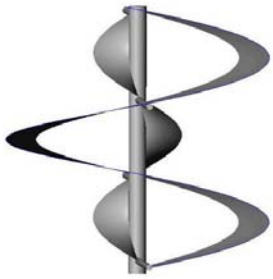


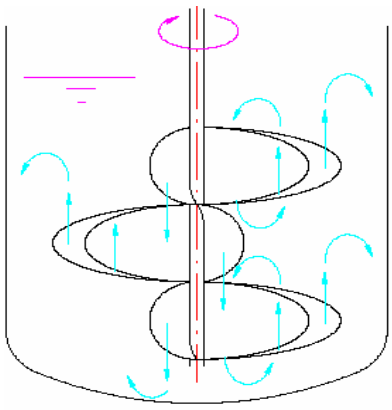


Maxblend



Helical Ribbon





The axial flow hydrofoil impellers have become increasingly popular. These axial flow systems can pump liquid either down or up. They have been shown to give superior performance (compare to Rushton radial flow impellers) with respect to lower energy demands for the same level oxygen transfer. Further, they show reduced maximum shear rates, making them usable with sensitive cultures such as animal cell culture, while still being capable of giving excellent performance with viscous mycelial fermentation. Combination of axial flow and radial flow impeller systems are sometimes used.

One way to improve bulk mixing while maintaining good oxygen transfer rate is to use Rushton turbine at lower position in tank and an axial flow impeller at the top. Marine propellers and A-215 work quite well in this scheme for many applications over a wide range of scales. The A-315 (hydrofoil design) will work even better because it has a higher power number than the others; hence it should contribute more to oxygen transfer than the others.

The designer can help to minimize top-to-bottom mixing problem by keeping the ratio of liquid height to tank diameter under 2, and by spacing the impeller properly. If the impellers are placed too closely, they interfere with each other, thereby decreasing OTR and mixing quality. If they are spaced too far apart, overall homogeneity suffers. Researchers have found that spacing between 1 and 1.5 impeller diameters gives good mixing in most practical cases, and oxygen transfer is not affected significantly by spacing within this range.

The effect of broth rheology on mixing are most pronounced for non-Newtonian broths. If Rushton turbine used in a pseudoplastic broth, the shear rate drops off rapidly and the viscosity increases rapidly with distance from the turbine tip; therefore, there is good mixing only in the immediate vicinity of the impeller, and air tends to channel around the impeller and rise up to shaft. One way to overcome this problem is to use very large diameter turbines.

There are some new impellers that have been designed to give adequate axial flow and very good OTR, particularly for non-newtonian broths. Also to overcome problems associated with efficient bulk blending in high-viscosity fermentation. Indeed, the Prochem Hydrofoil and the Mixco A-315 have been shown to do all this, and to require less power than Rushtons for the same OTR at commercial scale.

The Scaba 6SRGT agitator is one which at a given power input can handle a high air flow rate before flooding. This radial-flow agitator is also better for bulk blending than a Rushton turbine, but does not give top to bottom blending in a large fermenter which leads to lower concentrations of oxygen in broth away from the agitators and higher concentration of nutrients, acid or alkali or antifoam near to the feed points.

Prochem Maxflo agitator consists of four, five, or six hydrofoil blade set at a critical angle on a central hollow hub. A high hydrodynamic thrust is created during rotation, increasing the downward capacity of the blades. This design minimizes the drag forces associated with rotation of the agitator such that energy losses due to drag are low. This leads to low power number. The recommended agitator to vessel diameter ratio is greater than 0.4. When the agitator is used with 800-dm^3 *streptomyces* fermentation, the maximum power requirement at the most viscous stage is about 66% of that of Rushton turbines. The oxygen transfer efficiency is significantly improved.

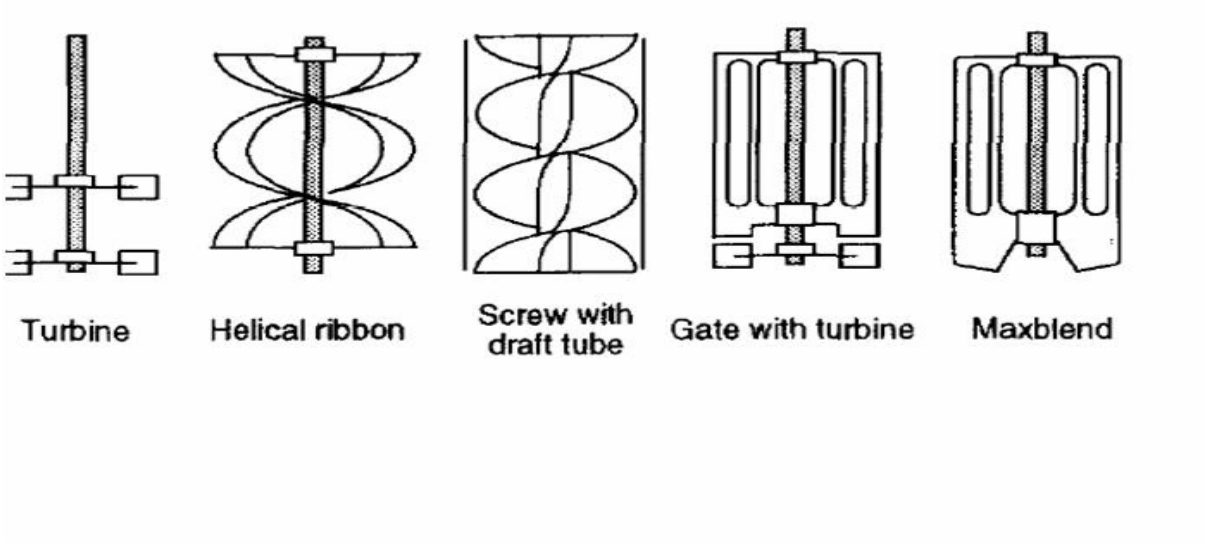
Intermig agitators are more complex in design. Two units are used instead of single Rushton turbine because their power number is so low. The large diameter sparger is used to optimize air dispersion. The loss in power is less than when aerating with a Rushton turbine. Here agitator diameter to vessel diameter ratio is 0.6 to 0.7.

This can be easily understood by the work done by TOHRU KOUDA, HISATO YANO, AND FUMIHIRO YOSHINAGA in Japan on “Effect of Agitator Configuration on Bacterial Cellulose Productivity in Aerated and Agitated Culture.”

To improve the bacterial cellulose (BC) productivity in aerated and agitated culture, the agitator configuration should be improved. Agitators were selected by measuring mixing delay time and K_La of a 1% BC suspension and cultivating *Acetobacter xytinum* subsp. *sucrojermentuns* BPR3OOlA. They concluded that (i) the impellers such as Maxblend and gate with turbine were suitable for BC fermentation because they mixed culture broth well and had large &I, (ii) the production rate and yield of BC were dependent on *KS* and the oxygen consumption rate, and (iii) the static gassing-out method to measure K_La *was* useful for characterization of the agitation conditions and the agitator configuration.

Agitation impellers The vessel, 130 mm in diameter, had a working volume of 1.8 L in a 3 .O L nominal volume. Figure shows the agitator configuration used. Sizes and positions of the impeller and ring sparger are listed in Table. The vessel was fitted with a thermocouple and a pH electrode.

The temperature of the test fluid was maintained at 30°C in each experiment by circulating temperature-controlled water in a jacket. The impellers were driven by a variable-speed dc motor and the agitation speeds were measured by a tachometer generator indicating system.



Agitator configurations.

Sizes and positions of agitator and sparger

Agitator	*d*/*D* (—)	*h* (mm)	*L* or *L*′ (mm)	*d*s (mm)	*h*s (mm)
Turbine	0.5	15	80	30	5
Helical ribbon	0.9	20	240	30	5
Screw with draft tube	0.5	15	100	70	15
Gate with turbine	0.5	15	135	30	5
Maxblend	0.5	5	145	70	10

d/*D*, ratio of impeller diameter (*d*) to vessel diameter (*D*); *h*, distance between vessel bottom and impeller; *L*, height of impeller; *L*′, distance between two turbine impellers; *d*s, diameter of ring sparger; *h*s, distance between vessel bottom and ring sparger.

Effect of agitator configuration on BC production in aerated and agitated culture

Because of the non-Newtonian properties of the BC culture broth, homogeneous mixing was difficult in the culture broth furthest from the impeller where the viscosity was high. In case of the turbine impellers, a large proportion of the test fluid was difficult to mix because the distance between some areas of culture broth and the impellers was large. Even if the K_La of the culture broth around the impellers was high; the K_La was low in a large proportion where mixing was inhomogeneous. However for the Maxblend and the gate with turbine impellers, the distance between any area of the culture broth and the impeller is small. Thus, these impellers showed better mixing of the BC culture broth than did turbine impellers. Although the screw impeller with draft tube also showed good mixing, its K_La was low because most of the bubbles sparged to the culture broth passed out through the draft tube and were not dispersed. Thus we concluded that agitator configurations suitable for BC fermentation were the Maxblend and the gate with turbine impellers. They are both gate type impellers and their mixing and ***Kg*** properties are similar. The most distinctive difference between the Maxblend and the gate with turbine impellers is the configuration around the sparger. The Maxblend impeller dispersed bubbles with high shear stress between the bottom and tip of the impeller. In contrast, the gate with turbine impeller dispersed bubbles with high shear stress at the tip of the turbine blades.

The configuration characteristics suitable for BC fermentation are summarized as follows: the distance between any area of the culture broth and the impeller should be small. This is important for reducing high viscosity of the culture broth where the shear speed is low. An impeller should have a configuration which has a high shear region around the sparger. This is important for dispersion of bubbles and increase of the K_La**.** These characteristics should be adapted in scaling-up because mixing and oxygen transfer are important for BC production in aerated and agitated culture.

These new turbine designs make it possible to replace Rushton turbine by larger low power agitators which do not lose as much power when aerated, are able to handle higher air volumes without flooding and give better bulk blending and heat transfer characteristics in more viscous media.

Good mixing and aeration in high viscosity broths may also achieved by a dual impeller combination, where the lower impeller acts as the gas dispenser and upper impeller acts primarily as a device for aiding circulation of vessel contents.

This can be illustrated by taking one research work carried out by V.B. Shukla, U. Parasu Veera, P.R. Kulkarni and A.B. Pandit in India that is “Scale-up of biotransformation process in stirred tank reactor using dual impeller bioreactor”

The gas–liquid mass transfer coefficient K_La in the fermenter is a strong function of mode of energy dissipation and physico-chemical properties of the liquid media. A combination of disc turbine (DT) and pitched blade turbine down flow (PTD) impellers has been tested in laboratory

bioreactor for gas hold-up and gas–liquid mass transfer performance for the growth and biotransformation medium for an yeast isolate VS1 capable of biotransforming benzaldehyde to l-phenyl acetyl carbinol (l-PAC) and compared with those in water. Correlations have been developed for the prediction of the fractional gas hold-up and gas–liquid mass transfer coefficient for the above media. The mass transfer coefficient and respiration rate have been determined in the shake flask for the growth as well as for biotransformation medium. These results then have been used to optimize the operating parameters (impeller speed and aeration) for growth and biotransformation in a laboratory bioreactor. The comparison of cell mass production and l-PAC production in the bioreactor has been done with that obtained in shake flask studies.

Agitation and aeration requirements for mammalian cell cultures are very different from those for microbial culture. OTR requirements are very much lower, but the cells are much more easily damaged by fluid mechanical forces generated by impellers or collapsing gas bubbles. In most cases, the impeller must provide enough mixing to keep cells or microcarriers suspended homogenously while creating as little fluid force as possible. A few, including marine propellers, have worked well under specific practical conditions up to several thousand liters, but most simply are not suitable for commercial application. The impeller, called the elephant ear is satisfactorily used in tissue culture vessels up to 500 L, provides adequate mixing and OTR, with little or no cell damage.

For mixing of mechanically sensitive mycelial microorganisms, mixing systems are recommended, which generate dominating axial flows, thereby ensuring a more even mixing throughout the reactor's volume. Ekato Intermig mixing systems are among the most widespread ones. The so-called "Counter-flow mixing system" is known. The approach to the mixing of more sensitive cells (tissue culture, animal cell, etc.) should be different, since; in this case, the mixing regime should have a laminar character.

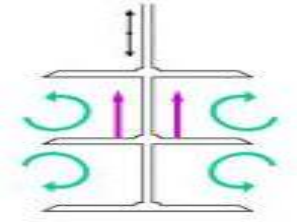
Characteristics of laboratory and pilot-plant scale impellers

Scale (nominal volume)	Impeller type (upper/lower)	Impeller diameter, DI (m)	Impeller tip speed, NmaxDI (m/s)	Number of impellers, NI
30 l	Rushton	0.102	4.7	4
100 l	Hydrofoil A315	0.184	3.8	2

280 l	Rushton	0.205	4.9	2
	Hydrofoil Maxflo T	0.28	6.7	3
800 l	Rushton	0.305	5.4	2
	Hydrofoil Maxflo T	0.406	7.2	2
	Hydrofoil A315	0.406	7.2	2
	Smith	Top, 0.343	Top, 6.1	2
	CD-6	Btm, 0.356	Btm, 6.3	
1000 l	Rushton	0.305	4.8	2
	Hydrofoil A315	0.37	5.8	2

Impeller data for top/bottom impellers. Units of length selected to minimize rounding errors. Top, Top impeller; Mid, middle impeller; Btm, bottom impeller

2.3.3 Novel non rotational mixing



Almost all laboratory fermenter-bioreactors use circular rotation to agitate the culture medium.

The major technical problem is that the axis of the stirrer (and the motors axis) rotates while the vessel is fixed. Thus, it is a physical necessity that a free space must exist between both, the moving axis and the immobile vessel, otherwise the rotation of the axis would not be possible. This free space allows viruses and microorganisms to get into the vessel. To limit the probability of contamination three ways are used:

The cheapest and less efficient solution is the use of so called lip-seals, which consist of elastic material with a central opening smaller than the axis diameter. This lip pushes onto the axis surface and should make the system tight. At the beginning, the closure can be satisfactory, but with time and especially at high rotation speed the lip is used up and the seal is no longer tight. Contaminating microorganisms can penetrate into the vessel. Therefore, such a system is not recommended for long time cultures or continuous cultures.

The second solution is the so-called mechanical seal or axial face seal. In this mechanically more advanced joint the stirrer axis is connected to the head plate by two discs, which glide on each other under a given pressure. The problem of this solution is that the system is mechanically stable only for certain time and if medium salts dry out between these discs their destruction is fast and contamination inevitable. Hence, they must be changed even though they are quite expensive. Much larger seals of this type are used in large, industrial scale fermenter. However, because of the knowledge of the mentioned problems, they are used in double sets with sterile water in between to protect the culture if the packing breaks during a run.

Today's best solution with respect to contamination problems connected with the rotational stirring is the magnetic coupling. The stirrer axis is completely separated from the motor axis and from the outside environment of the vessel and the driving force is transmitted by two sets of magnets. Since the magnetic force diminishes strongly with distance between poles, the slot separating the rotating cup and the stationary one is very narrow. Frequently, medium deposits and dries out in this space which leads to problems.

Because of the length of the axis and high transmitted force the magnetic coupling is technically quite complex and very expensive. For this reason, it is never proposed as standard equipment for laboratory fermenter. The client can sometimes buy it as an expensive option. In this way, the initial prices of many laboratory fermenters are kept lower despite of expensive consequences for the client at a later stage, when he is basically forced to buy the magnetic coupling option from the same producer.

LAMBDA has found a very simple, innovative solution for this mixing problem by selecting a non-rotational vertical up and down mixing solution. A simple elastic membrane allows the movement of the stirring axis and serves at the same time as a quality seal between the vessel and its central threaded cap. The membrane separates completely the interior of the vessel from the outside environment and this at low cost for the user.

This new type of stirring has several additional advantages*:*

The up and down stirring produces no vortex (eddy) and therefore eliminates the need for baffles. This spares costs, simplifies setting up and cleaning of the vessel and allows the creation new types of vessels (because the large vessel opening otherwise necessary for baffle insertion is no more needed).

The very expensive head plates which are heavy, complicated and long to set up and clean are no longer used. Because no special head plates are required anymore, the cost of passing to vessels of different volumes is much lower.

2.3.4 New softest agitation – the "Fish-Tail"

- A special "fish-tail" stirring disc based on the principle of the tail of a fish has been developed. The up and down movement of one or more „fish-tail" stirring discs provides gentle mixing both in horizontal and vertical direction. At the same time this type of stirring is more efficient and it eliminates cutting edges and micro-eddies formed on all common impellers used in bioreactors. As a consequence, the cell viability is increased.
- In analogy to a fish tail, this shape of the stirring disc produces a long range movement of the liquid (culture medium). No other stirrer type provides a similar positive combination of advantages for the agitation in cell cultures.

The agitation of cell cultures is problematic because the large mechanically sensitive eukaryotic cells can be destroyed by the cutting edges of any stirrer. And even completely round stirrers create eddies on their back side! Thus, the question arises: What is better?

1. either increase stirring speed and get good medium oxygenation and gas exchange and partially destroy cells or
2. To protect cells and work under suboptimal gas exchange in medium.

Nature has invented the right solution long before mankind appeared on earth! It is the shape of the fish tail, which has a perfect design which completely eliminates eddies (turbulences) in order to maximize the propulsion of the fish in water. When, in contrary, the fish tail is fixed then water will stream away with maximal efficiency.

It is clear that the very thin and soft rim of a fish tail has no cutting edge which would break cells. By mimicking the fish tail, LAMBDA created a new mixing disc, where high efficiency of stirring is provided without the destructive mechanical damaging and cutting of living cells. Thus, with the "Fish-Tail" stirring disks cell cultures with sensitive cells can be well aerated without destroying the cells.

2.3.5 Drive location: -

The stirrer shaft can enter the vessel from the top, side or bottom of the vessel. The top entry is most commonly used, but bottom entry may be advantageous if more space is needed on the top plate for entry ports, and the shorter shaft permits higher stirrer speeds to be used by eliminating the problem of the shaft whipping at the high speeds. Originally, bottom entry stirrers are considered undesirable as the bearings could be submerged. Mechanical seals can be used for bottom entry provided that they are routinely maintained and replaced at recommended intervals. The most common impeller mixer arrangement is centre mounted, top entering mixer.

Top entering, angle mounted mixers eliminate the need for baffles by using the axial flow of impeller to counteract swirl. Most angle mounted mixers are operated at less than 5 hp and speeds greater than 250 rpm.

Top-entering, off center mounted mixers also reduce the swirl sufficiently to eliminate the need for baffles. The elimination of baffles may avoid product hang up and make cleansing easier. Off center mounting creates large hydraulic loads may require a stronger shaft and drive, stiffer mounting and/or a bottom steady bearing. The centerline of tank is typically 1/6 to ¼ of diameter.

Side entering shaft are most often used I large, field-erected, storage tanks or concrete chests. The shaft is usually mounted near the bottom of the tank which provides the maximum variation in the liquid level.

Close-clearance impeller systems are a special case of the center mounted mixers. The center mounted mixers have impeller diameters that are between 15% to 75 % of the tank diameter. Close –clearance impellers are having diameter between 85% to 95% of tank diameter. Close – clearance impellers are typically used with viscous or non- Newtonian fluids.

2.3.6 Seals: -

Four basic types of seal assembly have been used: the stuffing box, the simple bush seal, the mechanical seal and the magnetic drive. Most modern fermenter stirrer mechanisms now incorporate mechanical seals instead of stuffing boxes and packed glands. Mechanical seals are most expensive but are more durable and less likely to be an entry point for organisms or contaminants or a leakage point for organisms or product which should be contained. Magnetic drives are also quite expensive and used in animal cell culture vessels.

The stuffing box or packed gland seal are only sufficient for the requirements of GILSP containment. Single mechanical seals are used with steam barrier in fermenter for the primary containment at level 1 or B2, whereas double mechanical seals are typically used in vessels with outer seal as back up and the inner seal for primary containment at level 2 or B3.

Magnetic drive in which the impeller shaft does not pierce the vessel, is satisfactorily used in microbial fermentation up to 1500 dm^3 when higher containment levels are specified. The stirring mechanism is ideal for animal cell culture to minimize the chances of potential contamination.

2.3.7 Baffles: -

To augment mixing and gas dispersion, baffles are employed. They are normally incorporated into agitated vessels of all sizes to prevent vortex and to improve aeration efficiency. Baffles are metal strips roughly one-tenth of vessel diameter and attached radially to the wall of bioreactor. The agitation effect is only slightly increased with increase in width of baffles, but drops sharply with narrower baffles. Generally four to eight baffles are incorporated. Baffles should be installed in such a way that a gap exist between them and vessel wall, so that there is scouring acting around and behind the baffles thus minimizing microbial growth on the baffles and fermenter walls. Extra cooling coils may be attached to baffles to improve the cooling capacity of the fermenter without affecting the geometry. With animal cell culture baffles causes shear damage, instead of baffles bottom drive axial impellers slightly off sight of centre is used.

2.4. The aeration system (sparger): -

Gas under pressure is supplied to the sparger (usually either a ring with holes or a tube with single orifice). It is defined as a device for introducing air into the liquid fermenter. Three basic types of sparger have been used and they are: the porous sparger, the orifice sparger (a perforated pipe), and he nozzle sparger (an open or partially closed pipe). A combined sparger-agitator may be used in laboratory fermenter.

Followings are adequate for good performance:

a) The sparge holes in the ring should be in line with the inner edges of the impeller blades.

b) The sparger holes should face downward to minimize medium retention in the sparger.

c) Hole diameter should be chosen such that each hole is a critical orifice at maximum glass flow.

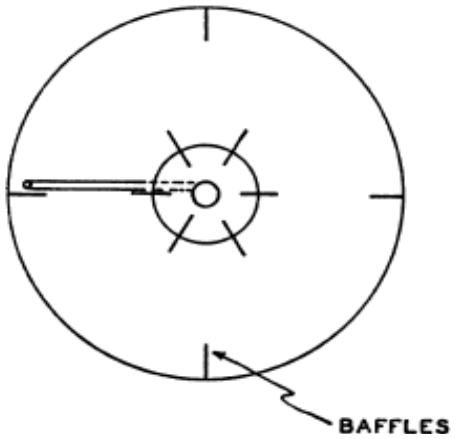
d) The sparger inlet pipe should be placed so as to allow free draining back into the vessel.

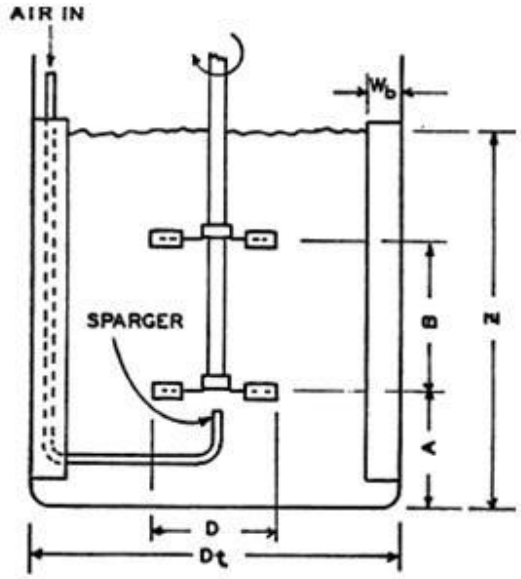
The porous sparger is made up of sintered glass, ceramics or metal and has been used primarily on a laboratory scale in non-agitated vessels. Here, throughput of air is low because of the pressure drop across the sparger and there is also problem of the fine holes becoming blocked by the growth of the microbial culture.

In small stirred fermenter the perforated piped (orifice sparger) are arranged below the impeller in the form of crosses or rings, approximately three quarters of the impeller diameter. Sparger holes should be at least 6 mm diameter because of the tendency of smaller holes to block and to minimize the pressure drop. In low viscosity fermentations sparged at 1 vvm with a power input of 1 W kg^{-1} , the power often falls below 50 % of its unaerated value when using a single Rushton disc turbine which is one third of the diameter of the vessel and a ring sparger smaller than the diameter of the agitator. If the ring sparger is placed close to disc turbine and its diameter is 1.2 times of that of disc turbine, a number of benefits can be obtained. A 50% higher aeration rate can be obtained before flooding occurs, the power drawn is 75% of the unaerated value, and a higher K_La can be obtained at same agitator speed and aeration rate. Orifice spargers without agitation have been used in limited extent in yeast manufacture, effluent treatment and in production of single cell protein in air lift fermenter.

In most modern mechanically stirred fermenter from laboratory to industrial scale have a single open or partially closed pipe (Nozzle sparger) as a sparger to provide the stream of air bubbles. Ideally the pipe should be positioned centrally below the impeller and as away as possible from it to ensure that impeller is not flooded by air stream. The single nozzle sparger causes a lower pressure loss than any other sparger and normally does not get block.

On a small scale ($1dm^3$), the combined sparger-agitator design, introducing air via a hollow agitator shaft and emitting it through holes drilled in the disc between the blades and connected to the base of the main shaft.





Typical dimensions of a stirred fermenter: $Z/D_t = 1.0$; $D/D_t = 0.34$; $A/D = 0.8$ to 1.0; $B/D = 1.0$ to 1.2; $W_b/D = 0.08$ to 0.10.

Bubbles from a single orifice, bubble volume is directly proportional to orifice diameter and surface tension, and it is inversely proportional to the density of the liquid. Neither gas pressure, temperature, nor liquid viscosity is reported to have much effect on bubble size, but unfortunately no work has been done with systems exhibiting non-Newtonian viscosity (plastic flow) such as occurs in mold cultures. Bubble size is always larger than the pore size by a factor of 10 to 100 for porous spargers. Surface-active agents which are present in most complex media reduce the bubble size, and one might suppose that the resulting increase in interfacial area would cause higher values for K_La. On the other hand, tiny bubbles which are unable to create much turbulence in their passage through the broth would be surrounded by rather thick liquid films, and these in turn would adversely affect K_La.

Sparger design and its location also affect the aeration rate as well as productivity. This can be illustrated by taking research work of **D. BIRCH and N. AHMED** on **"THE INFLUENCE OF SPARGER DESIGN AND LOCATION ON GAS DISPERSION IN STIRRED VESSELS".**

A well designed agitated gas dispersion system should generate a large interfacial area between the gas and the liquid phases, exhibit minimal influence of gassing on the power draw, and not be prone to flooding. These characteristics are shown to be strongly affected by the location of the sparger. Significant performance improvements, in terms of improved power draw and delayed onset of flooding on aeration, are achieved through the use of `larger than impeller' ring spargers, positioned within the discharge stream from the impeller. There is little or no penalty in terms of the gas holdup generated. Results for the Rushton and upward and downward pumping pitched blade impellers are reported. The observed behavior may be explained in terms of the loading regime of the impeller and the cavity forms observed behind the blades, which are governed by the interaction between the sparger (gas discharge) position and the flow patterns generated by each impeller.

It has been demonstrated that the sparger dimensions and location with respect to the impeller has significant effect on the gas dispersion characteristics in an aerated stirred vessel. In broad terms, larger than impeller spargers which lead to indirect loading of the impeller offer superior operational alternatives for gas-liquid systems, with implication for three phase operations. This is because indirect loading, in turn, hinders the formation of large cavities behind the impeller blades, thus ensuring relatively low power loss with aeration. Clinging cavities may be preserved at progressively higher flow numbers by moving the sparger nearer to the vessel wall. However, a balance needs to be established, considering that beyond a certain point the influence of the impeller is insufficient to maintain good overall dispersion of the gas, thus reducing the gas holdup. Experimental results show that the most suitable location for introducing the gas appears to be in the output stream from the impeller, a finding supported by the observed flow patterns for the impellers studied. With the Pitched Blade Disc Turbine Pumping Upward (PDU) and Pitched Blade Disc Turbine Pumping Downward (PDD), larger than impeller ring spargers placed within the influence of the impeller discharge meet the above conditions, and thus provide the best gas dispersion characteristics in terms of improved gas holdup and good aerated power draw. The direction of flow from these impellers dictates that the sparger be placed above the impeller for the PDU, and below for the PDD. With the Rushton impeller (FDT), a sparger placed level to the impeller, and close enough to be influenced by the impeller discharge stream provide the best gas holdup. As expected, this arrangement also results in good aerated power consumption characteristics when measured against the more conventional arrangements of sparging from below. Of all the sparger impeller arrangements examined, the FDT generates the maximum holdup, most probably as it also draws the maximum power.

Conclusions made on the study are given:

(1) The combined effect of sparger design and column height on the fractional gas hold-up was investigated. With an increase in the height to diameter (*HD* /*D*) ratio, the holdup was found to decrease when multipoint spargers (with hole diameter <3 mm) were used. In contrast, for single point spargers, ε_G was found to increase. However, in both the cases, a limiting *HD* /*D* ratio was observed beyond which the values of ε_G remain practically constant.
(2) The above observations were found to hold for three gas-liquids systems:
(i) Air-water
(ii) Relatively less coalescing system air-aqueous solution of electrolyte and
(iii) Relatively more coalescing system air-aqueous solution of carboxymethyl cellulose (CMC).
(3) The limiting value of the *HD* /*D* ratio (beyond which ε_G is independent of *HD* /*D*) was found to be in the range of 4±5 for the air-water system, greater than 8 for the air-electrolyte system and 3 for the air-aqueous CMC system.
(4) The ε_G versus V_G data was analyzed using the Zuber and Findlay drift flux model. A predictive procedure has been developed for the drift constants on the basis of simulation using computational fluid dynamics. An excellent agreement has been shown between the CFD predictions and the experimental values of drift flux constants.

2.4.1 Self-cleaning micro-sparger

Many fermentation processes had to be terminated because the air sparger loop was blocked by salt deposits! This problem has now been solved with the self-cleaning microsparger.

When an air bubble forms and is released into the medium, a tiny portion of solution flows forth and back in the sparger orifice. This solution is partially dried up by the next bubble. When this happens many times then, especially in strongly mineralized media, a precipitate forms. This precipitate eventually completely closes the sparger openings. The deposit is sometimes so compact that it can be hardly removed. Some producers deliver special sparger end pieces to get rid of difficult cleaning procedures. A similar behavior is also observed with micro-spargers.

LAMBDA's innovative self-cleaning microsparger eliminates this problem. The sparger is made of special silicone with miniature openings. The elasticity of the material closes the openings when no air passes through. Under the air pressure, these pores open and let the air bubbles form. The medium deposits also form here. This is an inevitable physical process. However, when the deposit accumulates and starts obstructing the air flow the resulting higher air pressure forces the elastic pores to open and thereby the deposit is released into medium. The air passage is free again.

This microsparger can never be blocked even during very long continuous runs!

2.4.2 Novel economical sparger: -

Mott Corporation has introduced a new quick change sparger system that facilitates easier and economical replacement of sparger elements in bioreactors and fermenter. An adapter is available with the sparger system, which facilitates easy attachment with the mating sparger tip as well as easy removal in the event of replacement.

Porous metal sparger is used to introduce gases into the liquids used in bioreactors and fermenter, which in turn creates bubbles. Mott's sparger ensures that the size of the bubbles is small and thereby accommodating a large number of bubbles and allowing for optimal mass transfer of gases. This also ensures that the volume of process gases required for mass transfer is also low.

Applications of porous metal spargers include petroleum refining, commercial machine manufacturing, chemical manufacturing, food and beverage industry and environmental services. Mott's porous metal spargers are made from alloys of nickel, titanium, 316LSS and hastelloy, which provide resistance to corrosive cleaning agents.

2.5. Foam control: -

The problem often encounters in fermentation is foaming. It is very important to control foaming. When foaming becomes excessive, there is a danger that filters become wet resulting in contamination, increasing pressure drop and decreasing gas flow.

Foam can be controlled with mechanical foam breaker or the addition of surface active chemical agents, called anti foaming agents. Foam breaking chemicals usually lowers K_La values, reducing reactors capacity to supply to supply oxygen or other gases, and some cases they inhibit the cell growth.

Mechanical foam breaker available is “turbosep”, in which foam is directed over stationary turbine blades in a separator and the liquid is returned to fermenter.

Foam is also controlled by addition of oils. Researchers named H. R. Bungay, C. F. Simons, Hosler P , presented their work on **“Handling of antifoam oils for fermentations”.**

Control of foams by oil additions is of large economic importance to the fermentation industry. Excessive foaming causes loss of material and contamination, while excessive oil additions may decrease the product formation. Antifoam oils may be synthetic, such as silicones or polyglycols, or natural, such as lard oil or soybean oil. Either will substantially change the physical structure of foam, principally by reducing surface elasticity.
Industrial antifoam systems usually operate automatically from level-sensing devices. Methods for metering of oil under aseptic conditions are: timed delivery through a solenoid, two solenoids with an expansion chamber between, a motor-driven hypodermic syringe, and certain industrial pumps.

2.6 Temperature control: -

Normally in the design and construction of a fermenter there must be adequate provision for temperature control which will affect the design of the vessel body. Heat will be produced by microbial activity and mechanical agitation and if generated by these two processes is not ideal for the particular manufacturing process then heat may have to be added to or removed from, the system. On laboratory scale little heat is normally generated and extra heat has to be provided by placing fermenter in a thermostatically controlled bath, or by use of internal heating coils or by a heating jacket through which water is circulated or a silicone heating jacket.

When certain size has been exceeded, the surface area covered by the jacket becomes too small to remove the heat produced by the fermentation. When this situation occurs internal coils must used and cold water is circulated to achieve correct temperature.
Available heating/ cooling approaches are :

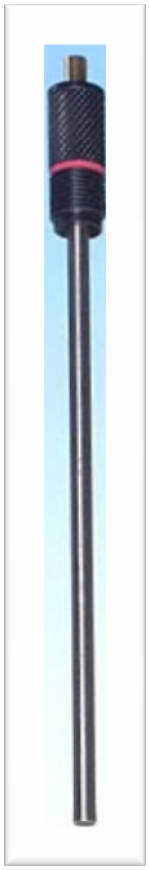
-Welded to the outside	-in the fermenter
• Jacket	Pillow plates,
• External coils	tube coils
• Pillow Plate	Thermo channels
	Tube bundles (vertical calandria).

A new-patented IR radiator with a glided parabolic reflector is used to warm the culture broth. The heat radiation (150 W) is concentrated on the bottom of the vessel where it is absorbed by the medium in a similar way to the sun heating water. There is no overheating of culture common with heater placed directly in the medium. Thanks to the low heat capacity of the IR source, overshooting of the temperature is reduced and the temperature can be controlled more precisely. The temperature sensor is placed directly in the pH sensor and is used at the same time for an automatic correction of pH and pO_2 electrodes.

2.7 pH control: -

Certain microorganisms grow in particular pH only. In fermentation it is very essential to control pH in order to grow the desired microorganisms for product formation. pH control sensors are used in fermenter for periodically checking of pH.

Here industrially used pH probe, temperature probe and pO_2 probe is shown.

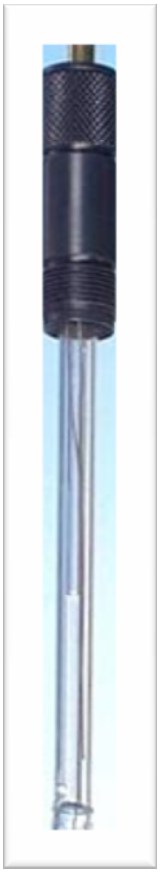


Temperature probe

1. Autoclavable to 130°C
2. Measurement range of up to 180°C
3. Deliverable into Pt/100 or Pt/1000
4. Electrode body made of stainless steel
5. High mentioning sensitiveness and short response time
6. The low diameters permit also the use in small bioreactors.
7. Adaptierungen to different screwing openings are possible

pO2 probe

1. Small diameter 6 and 12 mm, arbitrary lengths and adaptable to different screwing openings and systems. The small diameters permit the use in small reactors.
2. Function according to Clark principle
3. Electrode body made of stainless steel
4. Autoclaveable
5. High responsitivity and short response time
6. Simple exchange of the separating membrane
7. The separation



pH probe

1. Variable diameter 6 and 12 mm Ø in standard lengths
2. Measurement range 0 - 13
3. Has an integrated reference system
4. Electrode body made of glass
5. High mentioning sensitiveness and short response time
6. Autoclavable to 130° C
7. The low diameters permit also the application in small reactors
8. Adaptierungen to different one screwing

membrane is at a cap and the cap can be exchanged very simple.
8. Simple exchange of individual components.
9. Special developments for the installation into special equipments.
10. Electronic measuring rule circuit boards as an OEM installation or off-the-shelf instruments.

steam openings are possible

2.8. valves and steam traps: -

Valves attached to fermenter are used to controlling the flow of liquids and gases in a variety of ways. A wide range of valves are available, but not all of them are suitable for use in fermenter construction. These are also having a significant role in the fermenter productivity.

The different valves available are : gate valves, globe valves, piston valves, needle valves, plug valves, ball valves, butterfly valves, pinch valves, diaphragm valves, check valves, pressure control valves, safety valves and steam traps.

Depending upon fermentation type and requirements these valves are chosen in designing bioreactor with good productivity.

2.9 Sampling port: -

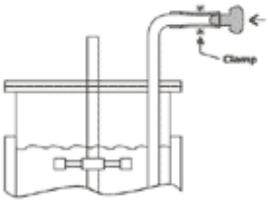
On the one hand, sampling may seem to be a simple procedure - just open the manual valve in the inlet of the bioreactor vessel, supply as much fermentation broth as required for the sample, and close the tap! In this sampling, we can quite easily guarantee that infection will not be avoided.

The sampling construction should be such so that measures for preventing non-sterility before and after the sampling be avoided. In the sites of the infection origin, sterilization should be performed promptly with alcohol or steam.

The essence of sampling is based on the following principle:

Simple sampling line.

A bladder made of silicone or a similar material is placed into the sampling pipe, and its end is stopped with a clamp. Thereby, it is sterilized together with the bioreactor vessel, and it remains in such a state until the sampling. When sampling, the clamp is removed, and the bladder is also pulled down. With the sample's discharge, the pipe's end is immediately washed with alcohol.



observed). This method is applicable if the given fermentation has not very high demands on sterility. Another drawback of this method is a hampered possibility of choosing the sample's amount.

Keofitt sampling device is available for sampling from bioreactor.

4. Newly available Bioreactors.

4.1 Disposable bioreactor.

Recently available **Disposable Bioreactors** having good productivity compare to classical multiple use bioreactors. Having following advantages.

- Pre sterilized bag, no cleaning or sterilization is needed.
- Powerful and flexible Deltav based bioNet control system.
- All bag contact parts are single use, class 4 tested and ready to use.
- Simplifies validation process.
- 50 through 1000 L working volumes.
- Scalable technology to support increasing volume demand.
- Minimizes investments and maximizes returns.
- A Single-Use Alternative to Conventional Stirred Tank Bioreactors
- Animal Derived Component Free (ADCF) Film

The Thermo Scientific HyClone S.U.B. (Single-Use Bioreactor) with BioNet® control provides all the advantages of single-use bioprocessing with the power and flexibility of DeltaV™ control.

The BioNet S.U.B. System is a turnkey product to replace the long lead time stainless steel bioreactor vessels used previously. The combination of BioNet® and HyClone products results in a flexible, rapid, and powerful option to quickly update or increase your bioreactor capacity.

The System consists of a reusable stainless steel outer support container and HyClone S.U.B. Bioprocess Container (BPC®) which integrate with the BioNet/ DeltaV Bioreactor Control System.

The another available disposable bioreactor is XDR single use bioreactor.

XDR Disposable Bioreactors from Xcellerex are fully-integrated systems that deliver proven stirred-tank performance that closely tracks conventional stainless steel systems. Every day, the

XDR is proven by Xcellerex's in-house process development and GMP contract manufacturing operations. Our production team has tested, optimized and run a wide range of cell lines including CHO, hybridoma, human, and insect cells.
Our process know-how and XDR's unique design make it the only disposables-based bioreactor that delivers process control better than conventional systems. But unlike conventional systems, new XDRs can be delivered in less than 12 weeks from order date and fully operational in hours.

Key XDR Features
• Jacketed kettle supporting 5-1 turndown
• Batch-to-Batch changeover in less than 1 hour
• Complete turn-key GMP-ready system
• Quality Certificate of Compliance
• Powerful magnetic drive agitation
• No rotating seals
• Highly optimized standard configuration
• Integrated temperature control
• Plug & Play hookup
• Small footprint
• No CIP/SIP required

.

Advanced Management of Networked Disposable Bioreactors with the BioNet® Bioreactor Control System.

4.2 Miniature bioreactors (MBRs): -

MBRs aim to achieve this acceleration as a result of their inherent high-throughput capability, which results from their ability to perform many cell cultivations in parallel. There are several applications for MBRs, ranging from media development and strain improvement to process optimization. The potential of MBRs for use in these applications will be explained in detail in this review. MBRs are currently based on several existing bioreactor platforms such as shaken devices, stirred-tank reactors and bubble columns. MBRs can be used in conjunction with automated

robotic systems and other miniature process units to deliver a fully-integrated, high-throughput (HT) solution for cell cultivation process development.

One novel approach to increase productivity of bioreactor: -

Free-radical induction has been employed as a novel strategy to improve bioreactor productivity and, more specifically, the quality and productivity of xanthan gum from *Xanthomonas campestris* cultures. A 210% increase in xanthan yield and a 20% increase in viscosity (quality) resulted from HOCl (oxidant) treatment. The acetate mass fraction in xanthan gum decreased by 42% and its pyruvate mass fraction increased by 63% as a result of HOCl treatment. The growth rate was almost unaffected by HOCl treatment. A hypothesis to explain the mechanism of xanthan gum overproduction by free-radical induction has been formulated. The significant aspects of the hypothesis, such as SoxS protein binding to the promoter region of the *gum* gene and the consequent increase in mRNA concentrations, have been experimentally verified.

One research work done by I. Gagné, T. Matsuura and Z. Duvnjak on "**PRODUCTIVITY OF BIOREACTOR INCREASED WHEN COUPLED WITH A PERVAPORATION SYSTEM"** is given below.

A mutant of *Saccharomyces cerevisiae* was used for the production of high fructose syrup from Sucrose. A considerable amount of ethanol, which inhibits the growth and the production capability Of the yeast, was also produced in this process. To decrease its inhibitory activity, a pervaporation Unit was coupled to the bioreactor to keep the ethanol concentration low by its continuous removal. The study showed that the time required to process a medium with 30% sucrose is more than 30% Shorter when the pervaporation unit was coupled to the bioreactor than in the same bioreactor, and Under the same conditions, without the pervaporation unit. The fructose yield was in the range of 96%-99%, while the ethanol yield was around 78% of the theoretical value. Coupling the pervaporation unit in the earlier stage of the process showed considerable advantage with respect to the length of process and the ethanol yield. It was also noticed that the yeast was able to convert the equivalent of a 40% sucrose feed in 24 hours in the fed batch mode, compared to over 40 hours without ethanol removal.

5. Conclusion: -

From the above studies it is observed that various bioreactor configurations affect the production rate of fermentation. Various factors like vessel shape, agitation, aeration, baffles, etc. play major role in productivity.

There is no bioreactor which can satisfy all the conditions but availability of disposable bioreactor may contribute to increase productivity as well as ease of fermentation process.

There are other so many new novel approaches are developed to increase productivity of spargers, agitators as well as various controlling probes in order to improve productivity of bioreactor.

Researchers are involved in developing new approaches to improve productivity of bioreactor in order to make process effective in economical means too.

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